EFFECTS OF RAPAMYCIN AND CAFFEINE ON TOR SIGNALING PATHWAY IN *SACCHAROMYCES CEREVISIAE*

by

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ABSTRACT

EFFECTS OF RAPAMYCIN AND CAFFEINE ON TOR SIGNALING PATHWAY IN *SACCHAROMYCES CEREVISIAE*

The aim of this study is to understand the effect of caffeine and rapamycin on the growth of yeast cells and identify flux changes in the presence of these chemicals in yeast. The repression effect of different concentrations of caffeine and rapamycin on the growth of the yeast cells in both F1 and YPD media was observed. The higher the concentration of the chemicals, the more growth of the yeast cells was repressed. However the repression effects of caffeine and rapamycin were found to have different characteristics. Yeast cells treated with rapamycin or lower amount of caffeine for a longer time of period reached higher optical density values compared to the untreated cells, which may indicate extending life span effect of the chemicals. Well-controlled batch cultivations of *Saccharomyces cerevisiae* BY4743 were carried out inYPD media and samples were collected to obtain biomass and extracellular metabolite profiles in the absence or presence of chemicals. This data was used in flux balance analysis (FBA) to determine the distribution of metabolic fluxes under these conditions by using whole genome models. Ethanol production was successfully predicted by using FBA when the objective function was chosen as the maximization of ethanol production. The fluxes successfully predicted by FBA in the absence and presence of caffeine were analyzed by clustering via self-organizing maps methodology. A decrease in the magnitude of fluxes in glucose fermentation and glycerol biosynthesis pathways was observed, which may indicate that caffeine represses respiration and fermentation. Additionally, since it was reported that both caffeine and rapamycin affect the the TOR signalling pathway, a system based modular approach based on literature curated protein-protein interactions was developed within the framework for this thesis. TOR signalling network was found to have a scale-free property. Functional modules were identified using Bron-Kerbosch algorithm. Ras1p and Tor1p were the most common proteins among more than 3 member modules, indicating a possible interaction between TOR and RAS signalling. Tor1p and Gln3p were

found to have a central role in TOR signalling network. The proteins in modules are significantly annotated to gene ontology terms, indicating the organized structure of the TOR signalling network.

ÖZET

SACCHAROMYCES CEREVISIAE'DA RAPAMİSİN VE KAFEİNİN TOR SİNYAL İLETİ YOL İZİ ÜZERİNDEKİ ETKİLERİ

Bu tezin amacı rapamisin ve kafeinin maya hücrelerinin büyümesi üzeri üzerindeki etkilerini incelemek ve bu kimyasalların varlığında metabolik yollar üzerindeki akı değişimlerini incelenmektir F1 ve YPD ortamında gerçekleştirilen çalışmalarda kafein ve rapamisinin maya hücrelerinin büyümesini baskılayan etkileri gözlemlenmiş, fakat etkilerinin farklı olduğu bulunmuştur. Daha uzun zaman rapamisin ya da düşük miktarda kafein uygulanan hücreler uygulanmayanlara nazaran daha yüksek optik yoğunluk değerlerine ulaşmıştır; bu da kimyasalların ömür uzatma etkisine sahip olduklarını işaret edebilir. *Saccharomyces cerevisiae* BY4743 iyi kontrol edilen fermentörlerde kesikli üretim ile YPD ortamnda büyütülmüş, kimyasalların varlıklarında ve yokluklarındaki biyokütle ve metabolit profillerini elde etmek amacıyla örnekler toplanmıştır. Elde edilen bulgular, tüm genom modeli ve Akı Denge Analizi (AKD) kullanılarak bu kimyasalların varlığı ve yokluğundaki metabolik akı dağılımı belirlenmiştir. Etanol üretiminin en iyileştirilmesi objektif fonksiyon olarak kullanıldığında etanol üretimi AKD ile başarılı bir şekilde öngörülebimiştir. Kafeinin varlığında ve yokluğunda en iyi öngörülebilen koşullarda saptanan akılar kendi kendine düzenlenen şemalar yöntemi ile kümelere ayrılarak incelenmiştir. Kafein varlığında glikoz fermantasyon ve gliserol biyosentez yol izlerindeki akıların büyüklüklerinde azalma gözlemlenmesi, kafeinin solunumu ve fermantasyonu baskılayacı etkisi olduğunu işaret etmiştir. Bunlara ek olarak kafein ve rapamisinin TOR sinyal ileti yolu üzerinde etkileri bilindiği için, literatürde bu yol üzerinde işlevi olduğu bilinen proteinlerden oluşturulan bir protin-protein etkileşim ağını temel alan sistem bazlı bir modüler yaklaşım geliştirilmiştir. TOR sinyal ileti ağının ölçekten bağımsız özelliğe sahip olduğu görülmüştür. Bron-Kerbosch işlem yolu kullanılarak işlevsel birimler saptanmıştır. Üçten fazla üyeye sahip birimlerde en yaygın olarak Ras1p ve Tor1p proteinlerinin bulunması, TOR ve RAS sinyal ileti yolizlerinin olası bir etkileşimini sezindirmektedir. Tor1p ve Gln3p'nin TOR sinyal ağında merkez işleve

sahip oldukları bulunmuştur. Birimlerdeki proteinlerin anlamlı bir biçimde gen ontoloji terimleri ile ilişkilendirilmeleri TOR sinyal ağının düzenli bir yapıya sahip oluğu işaret etmektedir.

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LIST OF SYMBOLS / ABBREVIATIONS

<C>	Average clustering coefficient
<k>	Average degree
A	Absorbance
b	Betweenness
c	Concentration
C(k)	Clustering coefficient of node k
d	Light path
l	Interaction
n	Node
n(k)	Cumulative frequency of node k
P	Protein
p(k)	Frequency of node k
S	Substrate
t	Time
V	Flux
v	Volume
X	Metabolite
Γ	Number of the shortest paths
ε	Extinction coefficient
μ	Growth Rate
μ_{max}	Maximum growth rate
ADH	Alcohol dehydrogenase
ADP	Adenosine diphosphate
AlDH	Aldehyde dehydrogenase
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate

ChIP	Chromatin immunoprecipitation
CR	Calorie restriction
DAL	Degredation of urea and allantoin
deg	Degredation
DNA	Deoxyribonucleic nucleic acid
EC	European commission
EGO	Exit from growth arrest
FBA	Flux balance analysis
FDA	Food and drug administration
FRB	FKBP12-rapamycin binding
G6P-DH	Glucose-6-phosphate dehydrogenase
GIDH	Glutamate dehydrogenase
GK	Glycerolkinase
GTP	Glutamine triphosphate
HK	Hexokinase
HXT	Hexose transporters
L-LDH	L-lactate dehydrogenase
LS	Life span
MAT	Mating
MW	Molecular weight
NCI	National cancer institute
NCR	Nitrogen catabolite repression
NDP	Nitrogen discrimination pathway
OAA	Oxaloacetate
PDS	Post-diauxic shift
PIKK	Phosphatidylinositol kinase-related kinase
PK	Pyruvate kinase
PP2A	Protein phosphate 2A
rDNA	Ribosomal deoxyribonucleic nucleic acid
Ribi	Ribosome biogenesis
RNA	Ribonucleic nucleic acid
RP	Ribosomal protein
rRNA	Ribosomal ribonucleic nucleic acid

RTG	Retrograde
SF	Scale free
SOM	Self-organizing maps
syn	Synthesis
TCA	Tricarboxylic acid
TOR	Target of rapamycin
trans	Transformation
VPS	Vacuolar protein sorting
YPD	Yeast extract-peptone-dextrose

1. INTRODUCTION

1.1. Two Drugs: Rapamycin and Caffeine

Rapamycin (Rapamune) was approved as an immunosuppressant in 1999 by the Food and Drug Administration (FDA) and in 2000 by the European Commission (EC). Although antitumor activity of rapamycin has been found against several solid-tumor models in the National Cancer Institute (NCI) screening program, its development as a cancer therapy has been far slower yet. Formulation and stability concerns in producing a parenteral formulation are playing the main role in this slow progress (Bjornsti and Houghton, 2004).

In the 1970s, the bacterial strain, *Streptomyces hygroscopicus*, which produced a potent antifungal metabolite, was found in a soil sample from Easter Island (it is called "Rapa Nui" in the local language). After the purification of this metabolite, a macrocyclic lactone, which was named rapamycin after its place of discovery, was found. Recently, it has been found that rapamycin, which possesses immunosuppressive properties, inhibits the proliferation of mammalian cells (Wullschleger *et al.*, 2006)

Rapamycin targets the TOR (Target of rapamycin) kinase which is a member of the phosphatidylinositol 3-kinase-like kinase family of protein kinases (Reinke *et al.*, 2006). It binds to the FKBP12–rapamycin binding (FRB) domain of TOR located immediately N-terminal to its kinase domain in conjunction with the highly conserved prolyl-isomerase FK506 binding protein (FKBP). Many *in vitro* studies have found that the rapamycin-FKBP complex inhibit TOR kinase activity; and alternatively, it has been proposed that rapamycin impairs the interaction with a regulatory partner (Loewith *et al.*, 2002). Rapamycin in complex with Fpr1p (a 112-residue cytosolic FKBP) binds TOR complex 1 (TORC1), but not TOR complex 2 (TORC2). Rapamycin treatment, similar to inactivation of the TOR proteins, mimics nutrient deprivation in various organisms like yeast, Drosophila, and mammalian cells. (Raught *et al.*, 2001; Peng *et al.*, 2002; Boer *et al.*, 2008) The similar effects of rapamycin have been found in animal cells and yeast suggesting that biochemical steps affected by rapamycin are conserved (Peng *et al.*, 2002).

It has been found that TOR is affected by a number of other pharmacological agents in addition to rapamycin, and among those agents members of the methylxanthine family of compounds such as caffeine (1, 3, 7-trimethylxanthine), an analogue of purine bases, exist. Caffeine has been shown to involve in various cellular processes related to cell growth, DNA metabolism, and cell cycle progression, most likely by acting as a low affinity ATP analog, in different organisms including mammals, plants and fungi (Kuranda *et al.*, 2006; Reinke *et al.*, 2006). Earlier studies had suggested that caffeine targets cAMP phosphodiesterase. However, that TORC1, and not cAMP phosphodiesterase, is found as a major target of caffeine in recent studies in yeast (Kuranda *et al.*, 2006). Both *in vitro* as well as *in vivo*, phosphorylation of TOR-dependent substrates is inhibited by caffeine. Although caffeine has a huge potential to be used as a tool for probing TOR function due to its pleiotropic behavior and interaction with TOR displaying relatively low affinity, *i.e.* in the submillimolar range, it has not been used widely (Reinke *et al.*, 2006).

1.2. TOR, a Central Regulator

TOR (target of rapamycin) was first genetically identified in the budding yeast *Saccharomyces cerevisiae*. *S. cerevisiae* and other yeasts harbour two homologous genes of this atypical serine/threonine kinase, TOR1 and TOR2, whereas there is only one TOR gene in all other eukaryotes. (Martin *et al.*, 2004)

TOR is highly conserved from yeast to mouse, rat and mammals-human. 95% identity at the amino-acid level is shared by TOR proteins. TOR is a member of the phosphatidylinositol kinase-related kinase (PIKK) family and its carboxyl terminus is homologous to the catalytic domain of phosphatidylinositol 3-kinase (PI3K). Its homologues have also been identified in plants (*Arabidopsis thaliana*), flies (*Drosophila melanogaster*) and worms (*Caenorhabditis elegans*). TOR seems to be an essential protein. Disruption of TOR activity causes to embryonic lethality in *D. melanogaster* and *C. elegans*, severe forebrain defects are found in mouse TOR mutants and they die at 12.5 days post coitus (Bjornsti and Houghton, 2004).

TOR1 and TOR2 encode two closely related factors which have a role in regulation of cell growth with respect to nutrient availability and cellular stresses. They, directly or indirectly regulates many cellular processes including protein synthesis, ribosome biogenesis, autophagy, transcriptional activation, meiosis, cell cycling, nutrient permease sorting and turnover, and actin organization.

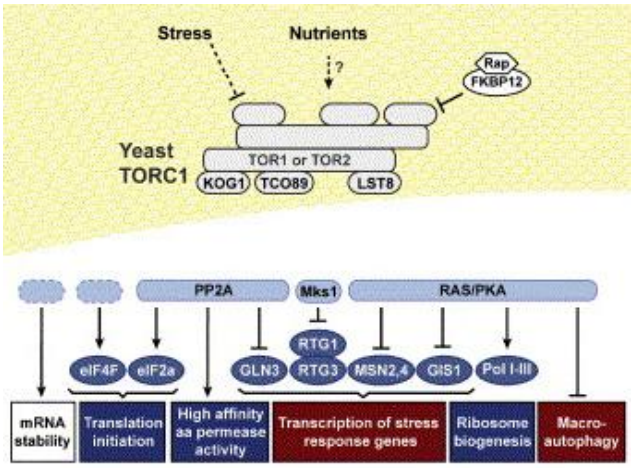

Figure 1.1. Schematic representation of TORC1 and its mode of action
(De Virgilio et al., 2006)

TOR1 and TOR2 are two main members of two functionally distinct TOR complexes in *S. cerevisiae*. The TOR complex 1 (TORC1) has been found to play a role in translation initiation, protein turnover inhibition, tetrad formation, and transcriptional repression of specific genes that are induced by nutrient starvation. It includes either Tor1p or Tor2p, together with Kog1p, Lst8p, and Tco89p (Figure 1.1). TOR complex 2 (TORC2) has a role in the regulation of actin cytoskeleton polarization during cell cycle progression, cell wall integrity, and receptor endocytosis. It consists of only Tor2p along with Avo1p, Avo2p, Tsc11p, Lst8p, Bit61p, Slm1p, and Slm2p. Although rapamycin inhibits TORC1, TORC2 is insensitive to rapamycin because the rapamycin-FKBP complex does not bind to Tor2p when it is present in TORC2. (Loewith *et al.*, 2002; Reinke *et al.*, 2006; Wullschleger *et al.*, 2006)

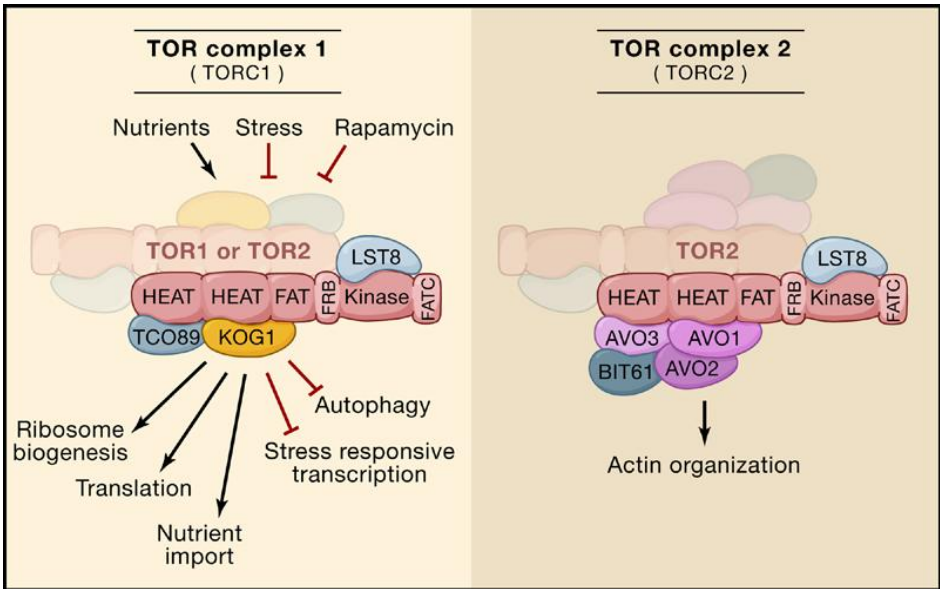

Figure 1.2. TOR Complex 1 (TORC1) and TOR Complex 2 (TORC2) of *S. cerevisiae* (Wullschleger *et al.*, 2006)

The TOR protein has a catalytic kinase domain, an FKBP12–rapamycin binding (FRB) domain, a putative auto-inhibitory domain which is the repressor domain near the C terminus and up to 20 tandemly repeated HEAT (an α-helical hairpin, HEAT is an acronym for four cytoplasmic proteins having this domain, Huntingtin, elongation factor 3 (EF3), protein phosphatase 2A (PP2A), and the yeast PI3-kinase Tor1p) motifs at the amino terminus, as well as FAT (FKBP-rapamycin-associated protein, FRAP-ATM-transformation/transcription domain-associated protein, TRRAP) and FATC (FAT C terminus) domains (Figure 1.2). HEAT motifs involve in protein–protein interaction domains; however FAT and FATC domains may have a role in the kinase activity of TOR. That TOR is conserved at the amino-acid level shows the significance of several domains in the cellular functions (Bjornsti and Houghton, 2004). It has found that binding of the rapamycin-FKBP complex to TOR is prevented by a single amino acid change at a highly conserved serine residue within the FRB domain both *in vivo* and *in vitro* (Loewith *et al.*, 2002).

1.3. TOR Signalling

For organisms, carbon and nitrogen are the two most basic nutrient sources. They are used in energy production and biomolecule synthesis. Energy metabolism and metabolic biosynthesis are carried out by several hundreds of individual chemical reactions, which are well organized along the centrally placed glycolysis and tricarboxylic acid (TCA) cycle. Regulation of those individual reactions is under the control of feedback, which adjusts the flux of metabolites through a particular pathway by temporarily increasing or decreasing the activity of crucial enzymes (Bertram *et al.*, 2002).

Therefore, regulation of cell growth in response to nutrients is crucial for the survival of all organisms. However, the questions, how nutrients are sensed, how nutrient-derived signals regulate cell growth and how these nutritional signals are integrated with growth factor pathways, remain to be answered. It is known that some kinases and signalling pathways are playing important roles in growth control and it is also known that those are well conserved in budding yeast, therefore this simple eukaryote offers a powerful opportunity to study mechanisms of nutrient sensing (De Virgilio *et al.*, 2006; Dechant *et al.*, 2008).

A complex change in the physiology of yeast cells occurs under the limitation of nutrients; they either reprogram their metabolism, so they can handle the results of the change in nutrient supply or activate a survival programme to outlive during the starvation (Drechant *et al.*, 2008). Studies with the whole-genome transcription profiling show that there is a remarkable similarity between shifting to low-quality carbon or nitrogen sources and with rapamycin treatment (Schamji *et al.*, 2000). Transcriptional profile observed under the rapamycin treatment is different from those observed under glucose, glutamine, or leucine deprivation but is most similar to that observed under amino acid deprivation. It was observed that TOR proteins are central sensors of the quality of carbon and nitrogen sources (Figure 1.3) (Schamji *et al.*, 2000; Peng *et al.*, 2002). Depending on the type of starvation, the TOR proteins can rearrrange a given pathway differentially (Schamji *et al.*, 2000). It has been suggested that rapamycin mimics a signal generated by the starvation of amino acids but that the signal is not likely to be the absence of amino acids themselves (Peng *et al.*, 2002).

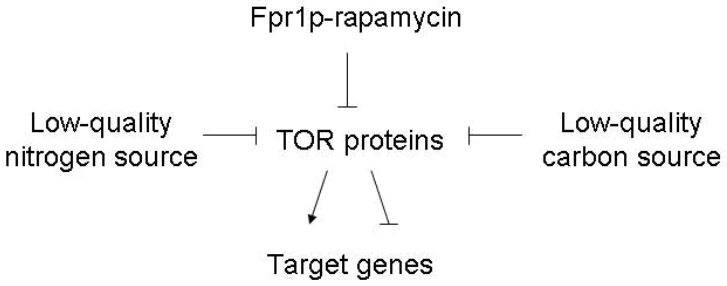

Figure 1.3. TOR proteins respond to low-quality of both carbon and nitrogen sources (Schamji *et al.*, 2000)

1.3.1. TOR Signalling as a Response to Nitrogen Sources

In response to different nitrogen nutrients, the expression of genes encoding proteins responsible for the transport and degradation of small nitrogenous compounds is regulated in the cell. For a yeast cell, glutamine is the preferred nitrogen source compared to glutamate; and in the presence of a preferred nitrogen source, the expression of genes associated with transport and degradation of poor nitrogen sources is decreased in the cell (Bertram *et al.*, 2000). In other words, when excess nitrogen is available, yeast cells prefer to utilize good nitrogen sources instead of poor ones, and they down-regulate transcription of genes encoding proteins that transport and degrade poor nitrogen sources. This regulation is designated nitrogen catabolite repression (NCR) (Cox *et al.*, 2004).

Regulation of NCR-sensitive genes is a complex mechanism and there are several important transcriptional factors such as four GATA type transcriptional factors Gln3p, Gat1p, Dal80p, and Gzf3p, and the Zinc finger transcriptional factors Dal81p and Dal82p. Each NCR-sensitive gene appears to be regulated in a combinatorial manner by several transcription factors; however, among those transcriptional factors, Gln3p is responsible for the expression of a majority of NCR-sensitive genes. Gln3p activity is inhibited by a yeast pro-prion protein, Ure2p, in the presence of preferred nitrogen sources (Bertram *et al.*, 2000).

When NCR genes are expressed, Gln3p is rapidly dephosphorylated and its nuclear accumulation is observed under nitrogen starvation. Inhibition of TOR by rapamycin also leads to Gln3p dephosphorylation. Therefore, TOR is considered to be responsible for Gln3p phosphorylation and dephosphorylation. In addition, rapamycin resistance is observed in the studies with *GLN3* deleted strains, which indicates that Gln3p plays an important role in rapamycin-sensitive TOR signalling (Carvalho *et al.*, 2003). Nevertheless, this mechanism has not been very well understood. The interaction between TOR and Gln3p is observed to happen via binding through the HEAT domain of TOR, which is crucial for phosphorylation of Gln3p, the inhibition of Gln3p nuclear translocation, and repression of Gln3p-dependent genes (Bertram *et al.*, 2000). The activation of Gln3p is also affected by the mutations in at least two phosphatase groups, Tap42p-Sit4p and Pph3p. Another demonstration is that, although Ure2p protects Gln3p from dephosphorylation, it can bind to both phosphorylated and dephosphorylated Gln3p. Thus, it is thought that there exists a tripartite regulatory mechanism for the repression and activation of Gln3p (Bertram *et al.*, 2000; Schamji *et al.*, 2000).

Nuclear accumulation of the nitrogen-regulated Gln3p is prevented by TOR via Tap42p-mediated inhibition of the phosphatase Sit4p (Figure 1.4). Phosphorylation of Gln3p is observed and it is hold in the cytoplasm by Ure2p under good nitrogen conditions. On the other hand; under nitrogen starvation or rapamycin treatment, Gln3p is dephosphorylated by Sit4p, which is activated as it releases from Tap42p. Dephosphorylated Gln3p dissociates from Ure2p and translocates into the nucleus, where it activates transcription of target genes.

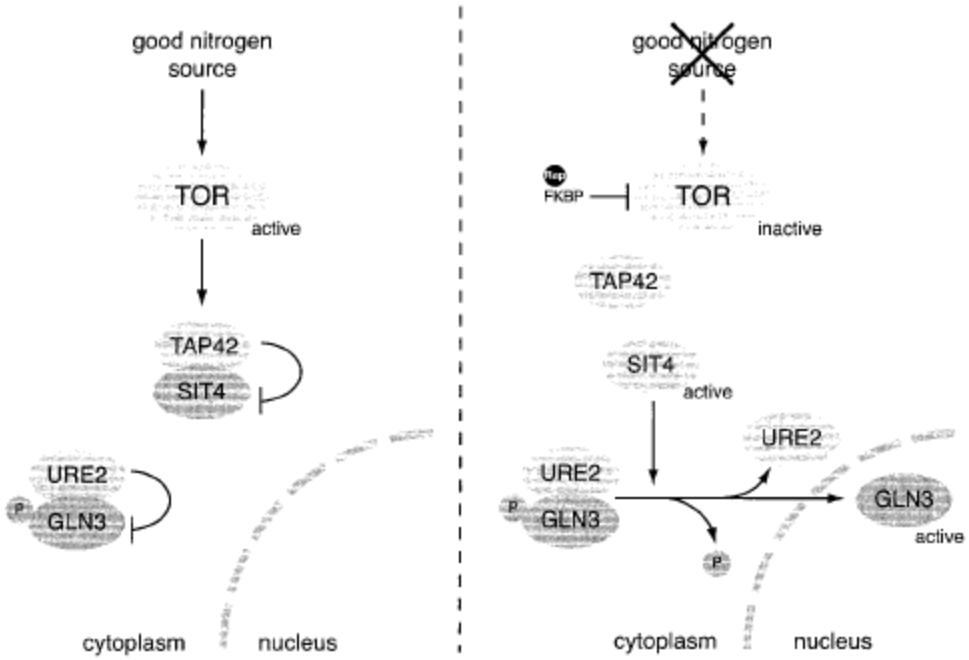

Figure 1.4. Regulation of Gln3p by TOR (Crespo *et al.*, 2002)

When yeast cells are shifted to a good nitrogen source from a poor one, Gln3p starts to re-accumulate in the cytoplasm, but interestingly, Gln3p is not uniformly distributed in the cytoplasm. In the study that was done to determine whether the actin cytoskeleton has a role in intracellular Gln3p movement, latrunculin, a drug that disrupts the actin cytoskeleton by inhibiting actin polymerization, was used, and it was observed that nuclear accumulation of Gln3p, in consequence NCR-sensitive transcription are prevented in the cells which are transferred from ammonia to proline medium under the latrunculin treatment, however, cytoplasmic accumulation of Gln3p is not prevented when the cells are transferred from proline to glutamine medium under the latrunculin treatment. On the contrary, it was observed that latrunculin treatment does not affect nuclear accumulation of Gln3p due to rapamycin treatment. As a result it can be suggested that the actin cytoskeleton is required for nuclear localization of Gln3p upon the nitrogen limitation but not rapamycin treatment. The actin cytoskeleton either has a role in intracellular localization of Gln3p under nitrogen limitation before TOR, or it can be concluded that TOR does not directly regulate the mechanism that is affected under the nitrogen limitation; inhibition of TOR only mimics its outcome (Cox *et al.*, 2004).

1.3.2. TOR Signalling as a Response to Mitochondrial Dysfunction

Mitochondrial retrograde signalling is a pathway of communication from mitochondria to the nucleus. It influences many cellular and organismal activities under both normal and pathophysiological conditions. The retrograde response has an important role in respiratory-deficient yeast cells, such as the maintenance of glutamate supplies. Otherwise, it would be compromised due to the respiratory-deficient state. At this time, metabolic readjustments become very significant, since a full TCA cycle cannot be operated, in other words succinate cannot be oxidized to fumarate in this cycle, which limits the production of oxaloacetate (OAA), and in turn α-ketoglutarate, the direct precursor to glutamate (Figure 1.5). In order to counterbalance this situation, cells induce the expression of many genes whose products function in anaplerotic pathways that are to resupply OAA and acetyl-CoA in mitochondria. As a result, for instance, acetyl-CoA production is increased via dramatic proliferation of peroxisomes and increase in fatty acid oxidation. Moreover, the activity of the citrate synthase isoform encoded by *CIT2* in glyoxylate cycle is increased and expression of genes encoding transporters that function to facilitate acetyl-CoA into mitochondria and glutamate and other nitrogen sources into cells is observed to be increased. It can be concluded that α-ketoglutarate which is the product of the first three steps of the TCA cycle is under the control of retrograde-specific regulatory genes in respiratory deficient yeast cells. These findings show the role of the retrograde pathway in the synthesis of α-ketoglutarate and the maintenance of glutamate supplies.

In yeast cells, retrograde signalling is used as a sensor of mitochondrial dysfunction that initiates readjustments of carbohydrate and nitrogen metabolism. Retrograde signalling is linked to TOR signalling in both yeast and animal cells, but the connection is not still very well understood.

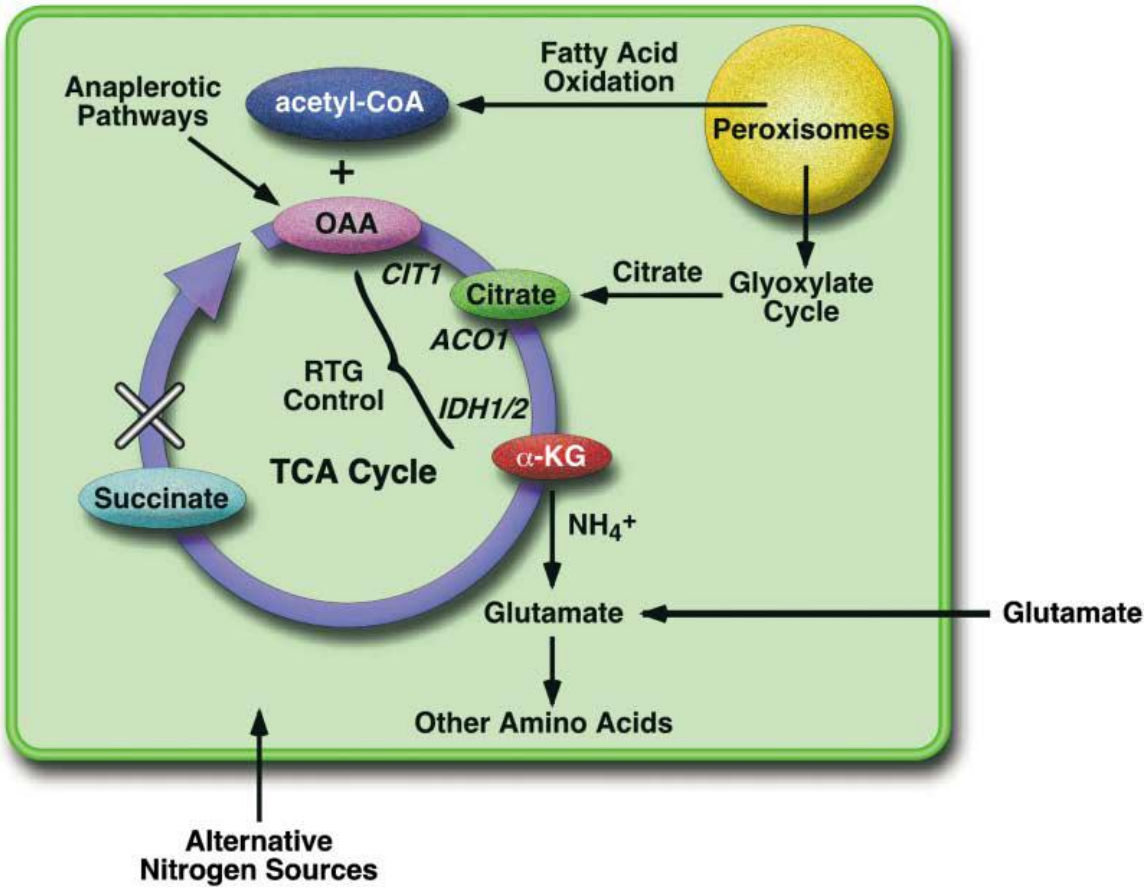

Figure 1.5. Retrograde control related to TCA cycle (Butow *et al.*, 2004)

The relationship between the retrograde (RTG) pathway and nutrient sensing was found out with the discovery of increased Rtg1/3p-dependent gene expression in cells in which TOR signalling is inhibited by rapamycin. In addition, it was found that activation of Rtg1/3p in the cells under the treatment of rapamycin is completely dependent on Rtg2p, novel regulator of the yeast retrograde response pathway (Komeili *et al.*, 2000; Butow *et al.*, 2004).

Rtg2p has a role in the upstream of the Rtg1p/3p complex. It passes the mitochondrial signals to Rtg1/3p complex which activates transcription of target genes and it is a sensor of mitochondrial dysfunction. When the RTG pathway is off, Rtg3p is phosphorylated at multiple sites and both Rtg1p and Rtg3p are maintained in the cytoplasm; and when the retrograde pathway is activated, Rtg3p is partially dephosphorylated and both Rtg1p and Rtg3p enter the nucleus, then they assemble at R box sites to activate transcription. In the absence of Rtg2p, Rtg3p is hyperphosphorylated

and Rtg1p/3p complex is sequestered in the cytoplasm, and no transcriptional response is observed to the retrograde signals. Therefore it can be concluded that the intracellular localization of the Rtg1/3p complex is a key point for the RTG-dependent retrograde response and Rtg2p appears to play an important role in this mechanism.

The finding that Lst8p an integral component of TORC1 and TORC2 proceeds further the understanding the relation between TOR signalling and the RTG pathway. The experiments with the cells lacking Lst8p show that Rtg1/3p-dependent transcription is activated and this effect is similar to the one of rapamycin treatment (Loewith *et al.*, 2002; Butow *et al.*, 2004).

What is the role of Rtg2p in controlling the location of the Rtg1/ 3p complex? An answer to this question could be obtained with the study of genetic screens designed to elucidate mutant strains that could bypass the requirement for Rtg2p. One of the protein revealed by this screen was Mks1p, which also identified by other studies as a negative regulator of *RTG*-dependent gene expression (Dilova *et al.*, 2002; Tate *et al.*, 2002). When *MKS1* is inactivated, like other negative regulators of the RTG pathway, the expression of *CIT2* is observed to be high, and this fact is independent of Rtg2p and insensitive to glutamate repression. The finding about the dynamical interaction of Rtg2p with Mks1p reveals that Rtg2p has a key role in regulation of the RTG pathway (Sekito *et al.*, 2002; Liu *et al.*, 2003). When the RTG pathway is active, the phosphoprotein Mks1p is dephosphorylated and forms a complex with Rtg2p; however, when the RTG pathway is off, Mks1p is more phosphorylated, is present in a complex with the 14-3-3 proteins Bmh1p and Bmh2p, instead of Rtg2p (Figure 1.6). It is thought that the complex that Mks1p forms with Bmh1p and Bmh2p act as a negative regulator of the RTG pathway via preventing nuclear accumulation of Rtg1/3p. In this respect, also Bmh1p and Bmh2p have a role in negative regulation of the pathway (Butow *et al.*, 2004).

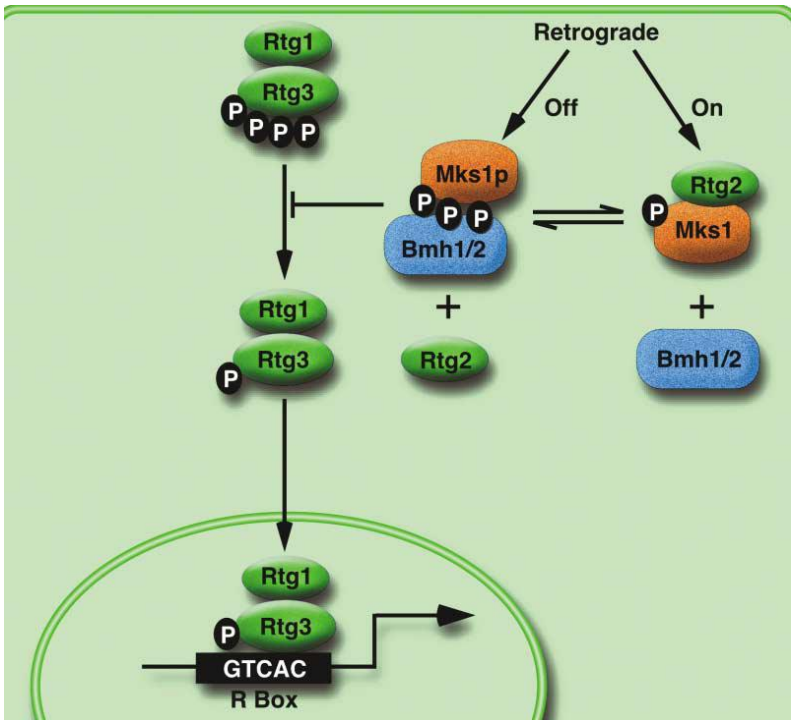

Figure 1.6. Mks1p and Bmh1p/2p act as a negative regulator of retrograde signalling (Butow *et al.*, 2004)

Research reveals that the phosphorylation state of Mks1p is influenced by different factors. Under different cellular conditions, distinct states of Mks1p phosphorylation can be observed (Figure 1.7). For instance, Mks1p is found in a hyperphosphorylated state, when glutamate is provided as the sole nitrogen source (Butow *et al.*, 2004). Partial dephosphorylation of Mks1p was observed under rapamycin treatment, suggesting that Mks1p is responsive to TOR under these conditions. Nevertheless, due to partial dephosphorylation, Mks1p is still sufficiently phosphorylated and associated with Bmh1p/2p. Thus, the pathway is kept in a repressed state. On the other hand, when glutamine is used as the sole nitrogen source, or when either ammonia or glutamine is additionally provided to the growth medium, Mks1p is found as intermediately phosphorylated that may weaken the interactions with Bmh1p/Bmh2p. Under such conditions, rapamycin treatment contributes to further dephosphorylation of the protein so the pathway is de-repressed (Figure 7C). As a result it can be concluded that different metabolic conditions give rise to distinct states of Mks1p phosphorylation and in this respect that the pathway responds at the transcriptional level to loss of TOR activity could be investigated. It can be understood from the partial phosphorylation of Mks1p under

rapamycin treatment of cells grown in glutamate that there are TOR-independent phosphorylation sites in this protein, or, alternatively, there is a stochastic process with a common set of phosphorylation sites.

It has been found that glutamine starvation acts as an inducer of *RTG* target gene expression. In particular, several studies reveal that this response correlates with dephosphorylation of Mks1p and is independent from Gcn4p-mediated general amino acid control. Nuclear accumulation of the Rtg1p/3p complex is seen under glutamine starvation and this response involves the same regulatory scheme outlined above (Figure 1.7).

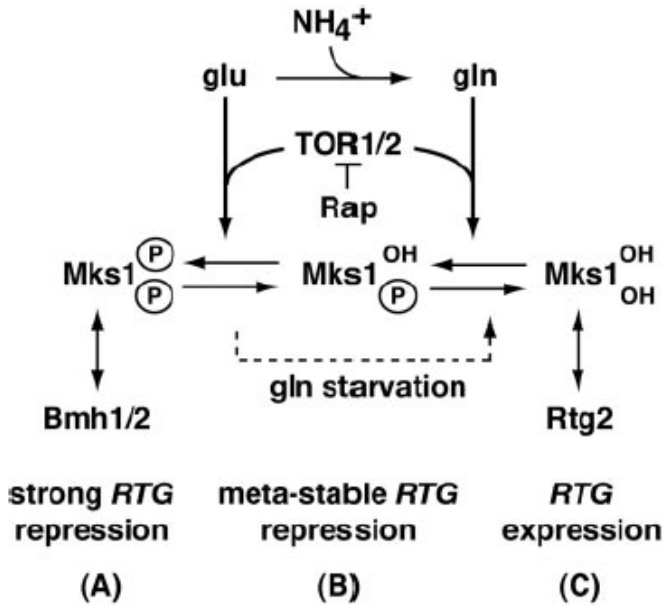

Figure 1.7. Phosphorylation of Mks1p under TOR control (Dilova *et al.*, 2004)

Unlike rapamycin treatment, however, glutamine starvation appears to be completely dominant with respect to the phosphorylation state of Mks1p that is established during growth in glutamate. Thus, it can be thought that the response to the glutamine starvation is, at least in part, independent from TOR (Figure 1.7, *dashed line*) (Dilova *et al.*, 2004).

Studies have shown that, Rtg1/3p-dependent gene expression, but not Gln3p target genes, is activated in cells lacking Lst8p; although both pathways are activated under rapamycin treatment. Moreover, activation of the RTG pathway in those mutant strains

was observed to be done independently from Rtg2p. One of the reasons for that fact may be that signalling by the Lst8p-TOR complexes has different outcomes according to interactions of targets with Lst8p. Another reason may be that some cellular Lst8p are not functionally associated with TOR, which has been advocated by some biochemical studies (Loewith *et al.*, 2002; Chen *et al.*, 2003; Butow *et al.*, 2004).

There is a debate on the issue whether the effects of TOR on the RTG pathway are direct or indirect. It is known that both TOR and RTG are involved in nutrient sensing; however, how these mechanisms are operated is not very well understood. First, the *RTG*-dependent retrograde response is very robust to mitochondrial dysfunction even when the cells are grown in rich medium, a condition in which the TOR kinases are active and the NCR genes are repressed. Second, rapamycin treatment of cells, which always leads to induction of NCR genes, sometimes fails to induce *CIT2* expression, an effect that is dependent on the nitrogen source in the medium (Tate *et al.*, 2003). Considering these results, it is difficult to conclude that TOR is a direct regulator of the RTG pathway. One explanation is that it affects the RTG pathway only indirectly, for instance, by regulating the activity of (*SSY1*, *PTR3*, *SSY5*) amino acid sensing system (Figure 1.8). The SPS sensor consists of Ssy1p, a plasma membrane amino acid sensor resembling amino acid permeases, and at least two peripheral membrane proteins, Ptr3p and Ssy5p, which probably associate with Ssy1p; together, these proteins function in a signal transduction cascade in response to external amino acids, such as glutamate. Inactivation of the SPS sensor system, or inactivation of *SHR3*, a gene that functions in the targeting of Ssy1p to the plasma membrane, results in elevated *CIT2* expression and a loss of glutamate repression. Nevertheless, *CIT2* expression in SPS-inactivated cells still requires Rtg2p. That regulation SPS sensor by TOR could be a function of the TOR-Lst8p complex (Butow *et al.*, 2004).

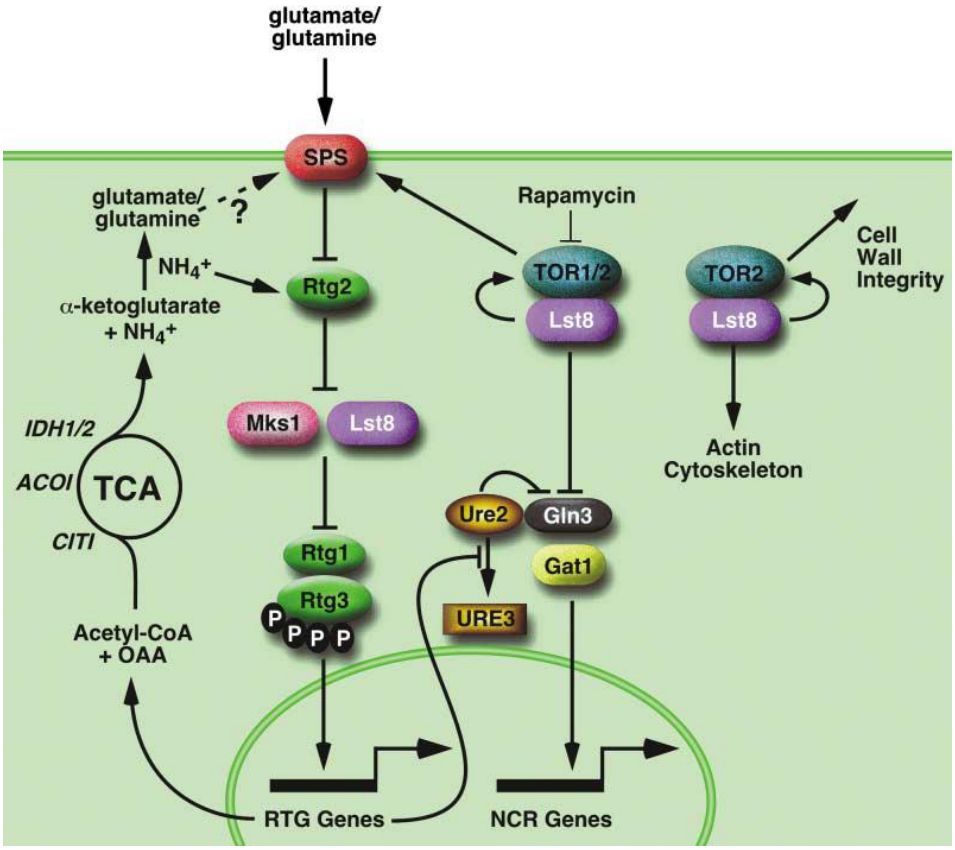

Figure 1.8. Regulation of *RTG*-Dependent Gene Expression (Butow *et al.*, 2004)

On the other hand, some studies suggest that retrograde gene expression is separable from TOR regulation of RTG- and NCR responsive genes. It was shown that expression of these two classes of genes is differentially regulated by glutamate starvation whether in the cells with defective mitochondria or under rapamycin treatment, as well by glutamine or histidine starvation (Figure 1.9). It was also found that the role of Lst8p, a component of the TOR1/2 complexes and a negative regulator of the RTG pathway, is complex in the regulation of RTG- and NCR-sensitive genes. The expression of *CIT2* and *GLN1* is negatively regulated by Lst8p, but it does not have a role in the regulation of *DAL5* expression, which depends on Gln3p and Gat1p; and Gat1p is translocated to the nucleus only upon TOR inhibition by rapamycin. Considering these data it can be concluded that Rtg1/3p, Gln3p, and Gat1p can be differentially regulated through different nutrient-sensing pathways, such as TOR and retrograde signalling, and by multiple factors, such as Lst8p, which is suggested to have a role in connecting the RTG and TOR pathways (Giannattasio *et al.*, 2005).

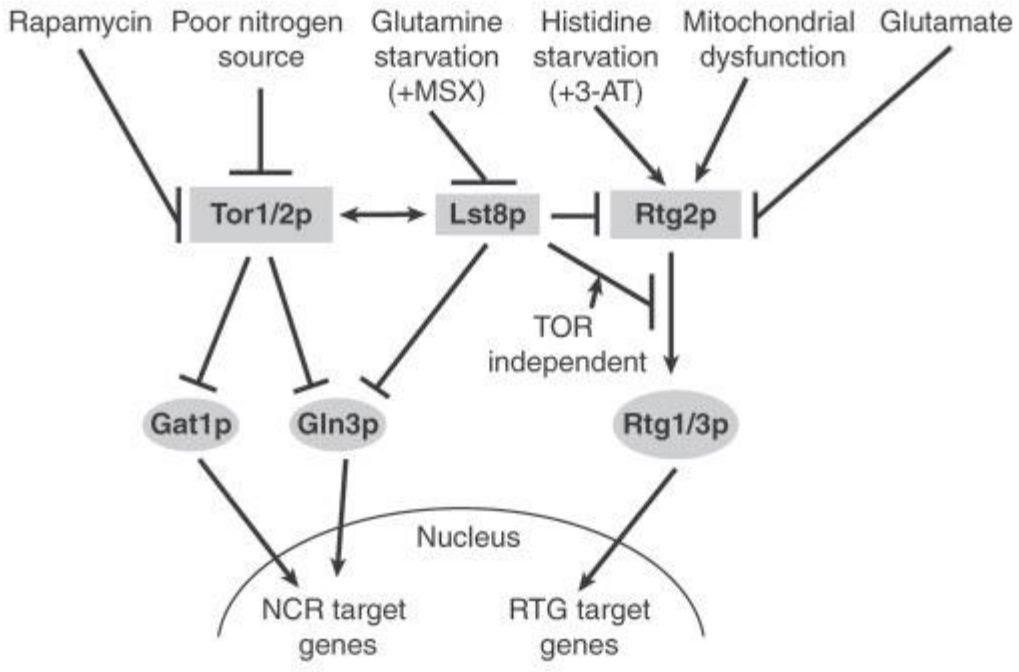

Figure 1.9. Regulation of NCR and RTG target genes by TOR and retrograde signalling (Giannattasio *et al.*, 2005)

It has been suggested that retrograde signalling genes, like NCR and starvation-specific genes, are controlled by TOR signalling via the interaction between Tap42p, the type 2A phosphatase (PP2A) and the type 2A-related phosphatase Sit4p (Figure 1.10) (Scmelzle *et al.*, 2000; Cherkasova *et al.*, 2003; Rohde *et al.*, 2004; Santhanam *et al.*, 2004; Inoki *et al.*, 2005; Rohde *et al.*, 2008). PP2A is a multimetric serine/threonine phosphatase and it is highly conserved in various organisms, from yeast to humans. In yeast, Pph21p and Pph22p are the homologs of mammalian PP2A catalytic subunits and are associated with an A subunit, Tpd3p, and one of two regulatory B subunits, Cdc55p or Rts1p. Sit4p is a yeast PP2A-related catalytic subunit that associates with one of its four regulatory proteins, Sit4p-associated proteins (SAPs): Sap4p, Sap155p, Sap185p, or Sap190p. Loss of three of the SAPs, *SAP155*, *SAP185*, and *SAP190*, results in a phenotype similar to that caused by *SIT4* deletion, which indicates that these regulatory SAPs are necessary for Sit4p function (Inoki *et al.*, 2005). Tap42p is also an essential, conserved protein and it independently associates with Sit4p and with the catalytic subunits of PP2A in response to nutrient availability and TOR activity (Crespo *et al.*, 2002).

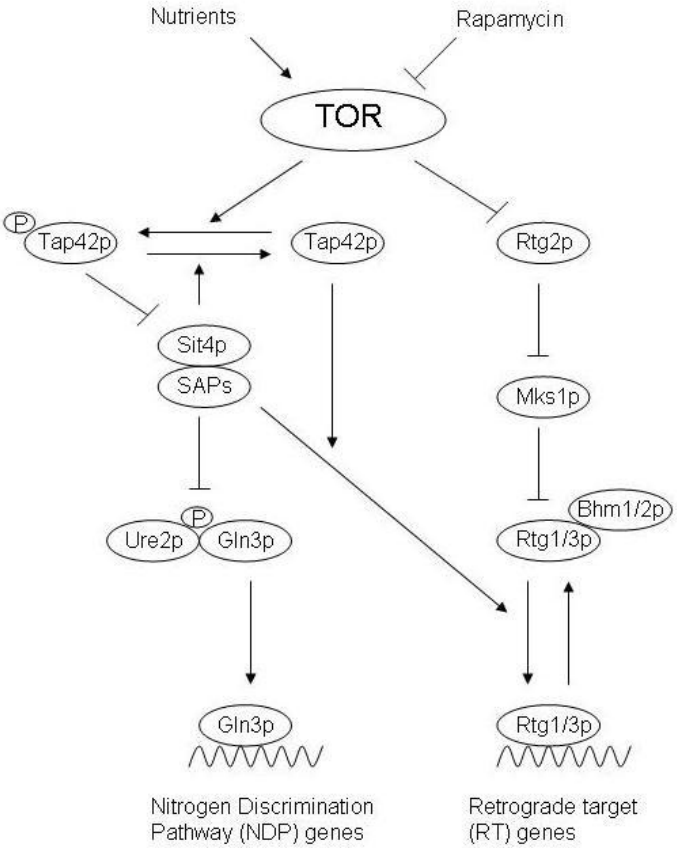

Figure 1.10. TOR signalling via the interaction of Tap42p-phosphatases (Inoki *et al.*, 2005)

Although the mechanism has not been understood in detail, two models are considered to explain how TOR regulates the Tap42p-Pphs/Sit4p complex. In the first model, TOR phosphorylates Tap42p and increases Tap42p binding to Sit4p and/or Pph21/22p via inactivating their phosphatase activities (Figure 1.11). This model is in parallel with the finding that TOR phosphorylates Tap42p both *in vivo* and *in vitro* in response to rapamycin treatment. In addition, it was observed in the study with mutation of Cdc55p and Tpd3p regulatory subunits that Pphs are inactivated via phosphorylation of Tap42p and, thus, binding between Tap42p and Pphs/Sit4p is increased, which leads to rapamycin resistance. Therefore, it is suggested that Tap42p leads to Pphs/Sit4p-dependent dephosphorylation. Considering these data, reciprocal regulation by both TOR and Pphs/Sit4p determines the phosphorylation status of Tap42p (Inoki *et al.*, 2005).

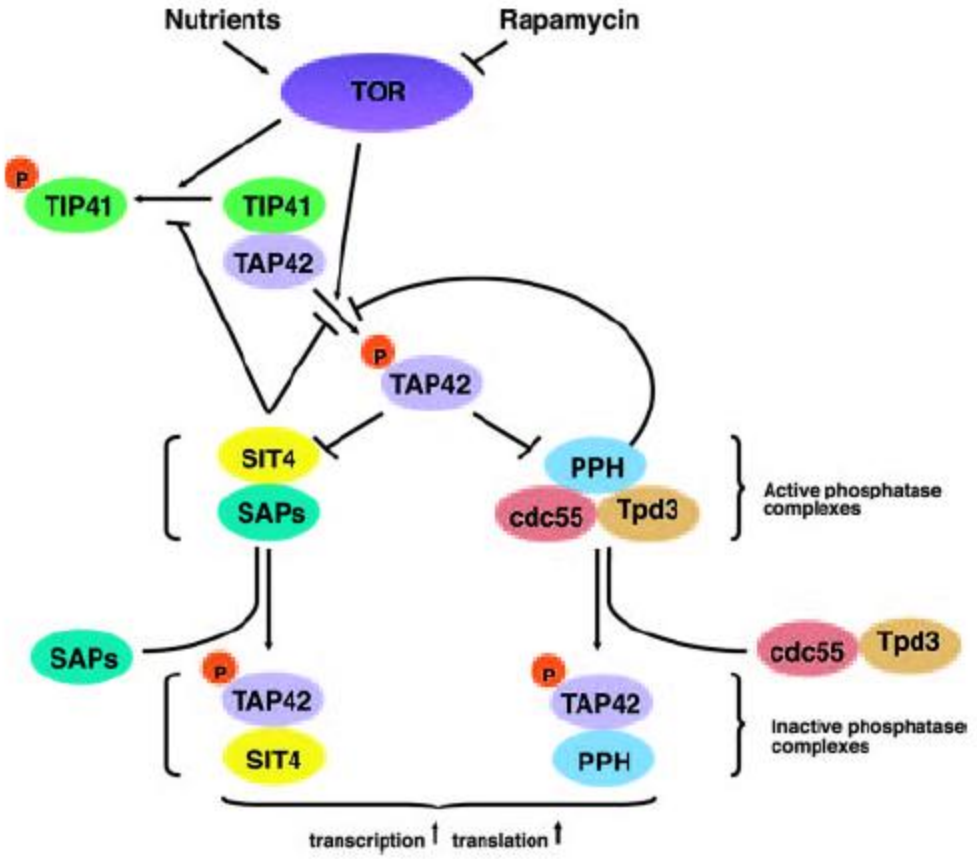

Figure 1.11. Possible interactions among Tip41p, Tap42p and Sit4p (Inoki *et al.*, 2005)

In the second model, TOR indirectly controls the binding of Tap42p to Sit4p via regulating an inhibitor of the interaction between Tap42p and Sit4p, Tip41p (Figure 1.11). Tip41p binds directly to and inhibits Tap42p, thereby negatively regulating the TOR pathway. Sit4p dephosphorylates Tip41p, which directly or indirectly associates with Tap42p. Tip41p dissociates from Tap42p, when it is phosphorylated under the control of TOR, and thereby Tap42p can bind to Sit4p. However, rapamycin leads to dephosphorylation of Tip41p via Sit4p, and, thus, to association of Tip41p and Tap42p; this suggests that Tip41p plays a role in the amplification of Sit4p phosphatase activity when TOR is inactive. These data indicate that TOR is responsible for the regulation of Tap42p both directly and indirectly through inhibiting Pphs/Sit4p phosphatase activity. It has been proposed that TOR inhibits the function of the phosphatase Pph21/22p and Sit4p via promoting the binding between Tap42p and Pphs/Sit4p (Jacinto *et al.*, 2001; Inoki *et al.*, 2005).

1.3.3. TOR Signalling as a Response to Carbon Sources

In a similar manner to nitrogen catabolite repression (NCR), there is a glucose repression mechanism in the cell, and the presence of glucose, the main carbon source of the cell, represses the expression of genes involved in transport and utilization of other carbon sources. The intracellular glucose sensor Snf1p, which is a yeast homolog of AMP-activated protein kinase (AMPK), mediates glucose repression and when the glucose level is low, it becomes activated. Snf1p phosphorylates and negatively regulates a transcriptional repressor involved in the utilization of alternative carbon sources, Mig1p. A second class of glucose sensors is involved in the glucose induction mechanism. Two plasma membrane proteins, Rgt2p and Snf3p, have a strong sequence similarity to hexose transporters (HXT). However, these proteins act as high- and low-glucose sensors, respectively, to activate transcription of HXT genes in response to extracellular glucose levels (Bertram *et al.*, 2000). Furthermore, it has been found that the TOR kinase pathway plays a role in the regulation of HXT1 expression by glucose, via affecting the activity of the Cdc55p–PP2A protein phosphatase complex (Tomas-Cobos *et al.*, 2005).

Although some researches show that TOR kinases are mainly activated by nitrogen and carbon sources activate PKA (cAMP-regulated protein kinase A) (Dechant *et al.*, 2008), several studies indicate that not only nitrogen, but also carbon nutrient, controls the expression of some NCR genes, as well as nitrogen availability regulates glucose repression genes. However, it is not very clear how cross talks between the NCR pathway and carbon signalling operate. It was found that Gln3p phosphorylation and, thus, subcellular localization via the Snf1p-AMPK pathway are regulated by glucose availability. TOR kinase has been shown to be responsible for negative regulation of Snf1p (Orlova *et al.*, 2006). Thus, nutrient signalling pathways can closely interact with each other. Such interplay between two key nutrient sensing and signalling pathways may be important for cells to rapidly adjust cellular metabolic activities as well as growth and proliferation in response to changing nutrient conditions (Bertram *et al.*, 2000; Orlova *et al.*, 2006).

1.3.4. TOR Signalling as a Response to Stress

It has been found that TOR signalling is responsible for the regulation of ribosomal protein, ribosomal biogenesis, retrograde signalling (RTG), degradation of urea and allantoin (DAL), nitrogen discrimination pathway (NDP), post-diauxic shift element (PDS) genes and amino acid synthesis genes, as well as stress-responsive element (STRE) genes (Figure 1.12). However, several lines of evidence suggest that TOR also promotes cell growth under stress conditions other than nutrient limitation (Crespo *et al.*, 2002; De Virgilio *et al.*, 2006; Bandhakavi *et al.*, 2008).

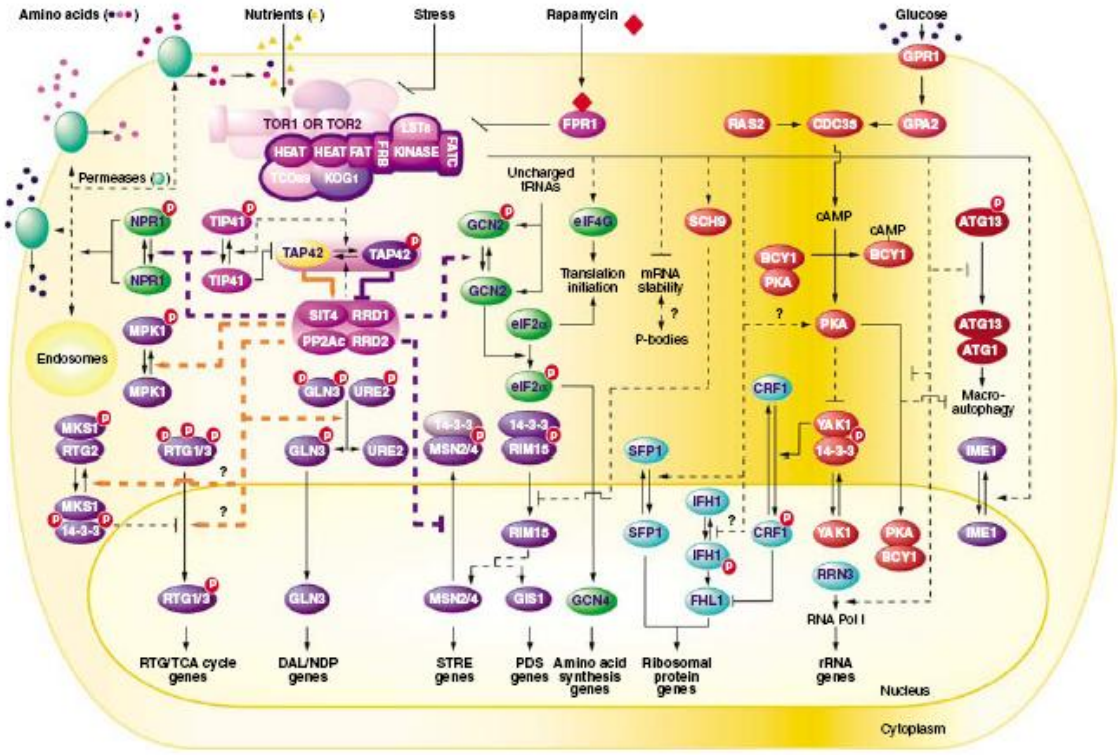

Figure 1.12. Diagram of TOR signaling in yeast (De Virgilio *et al.*, 2006b)

Almost 90% of genes that are significantly affected under rapamycin treatment show transcriptomic changes in similar direction under conditions of heat/oxidative stress. Specifically, it can be demonstrated that activation of regulator(s) of heat/oxidative stress responses phenocopied TOR inhibition and sought to identify these putative TOR inhibitor(s). It is found that constitutive activation of the conserved stress regulator Hsf1p confers rapamycin sensitivity and reduced TOR signaling via elevated expression of Hsf1p

target genes. Considering these findings, Hsf1p has been identified as a putative inhibitor of TOR signaling and new insights into the relationship between stress signals and the inhibition of cell growth has emerged (Bandhakavi *et al.*, 2008).

It has been found that transcription factors Msn2p and Msn4p, which activate expression of genes in response to several different environmental stress conditions, including heat shock and H_2O_2 treatment, are also controlled by TOR. Inactivation of TOR by rapamycin also partially activates Msnp2p/4p. Both Msn2p and Msn4p respond to different types of cellular stress, including carbon source limitation. Inhibition of Msns (Msn2p and Msn4p) by TOR in response to nutrients may be done via promoting the association of MSNs with the abundant 14-3-3 proteins Bmh1p and/or Bmh2p. In addition, Bmh1p and Bmh2p may have a role as a positive regulator of rapamycin-sensitive signalling. A release of Msns from Bmh2p was indicated in the studies upon glucose withdrawal or rapamycin treatment. It was found that this process is not related to interaction of Tap42p and Sit4p, suggesting that there may be another, unknown TOR pathway regulating Msns (Crespo *et al.*, 2002).

On the other hand, studies with Msn2p show that rapamycin affects Msn2p by inactivating Tap42p function. In response to stress and nutrient starvation, the *Saccharomyces cerevisiae* transcription factor Msn2p accumulates in the nucleus and activates expression of a broad array of genes. Moreover, in response to stresses, such as heat and osmotic shock, as well as nitrogen, but not glucose, starvation, PP2A function has also a role in nuclear accumulation of Msn2p. Thus, it can be concluded that PP2A and the TOR kinase pathway transduce stress and nitrogen starvation signals to Msn2p (Santhanam *et al.*, 2004).

Furthermore, research reveals that Msn2p/Msn4p could control stress-induced expression of Thiol peroxidase type II (*cTPx II*) (*YDR453C*) which is regulated in response to various stresses (*e.g.* oxidative stress, carbon starvation, and heatshock). Rapamycin induction greatly increases the transcriptional activity of the *cTPx II* promoter, and studies with deletion mutants of *TOR1, TOR2, RAS1*, and *RAS2* show that *cTPx II* is regulated by Msn2p/Msn4p transcription factors under negative control of the Ras-protein kinase A and TOR signaling pathways (Hong *et al.*, 2002).

Additionally it was observed that *TOR1* deleted mutants are sensitive to high concentrations of salt, which suggests that TOR1 plays a role in the cell in response to saline stress. This mechanism is not very clear. But, it is thought that there is a connection between the TOR-controlled transcription factors Gln3p and Gat1p and the lithium and sodium extrusion pump Ena1p, which is essential for survival under saline stress conditions. Gln3p and Gat1p are responsible for induction of *ENA1* transcription in response to rapamycin treatment and, like *TOR1*, are required for growth under salt stress (Crespr *et al.*, 2001). It is also found that under osmostress conditions, the protein on the downstream of TORC1, Sch9p, seems to activate stress defense genes. At least upon stress, Sch9 has a direct role in the transcriptional activation at the chromatin structure (Pascual-Ahuir *et al.*, 2007).

TOR signalling also responds to chemical stress. It was found that the ubiquitous toxic metalloid arsenic, mercury and nickel, efficiently inhibit the TORC1 protein kinase. This is also demonstrated *in vivo* by the dephosphorylation and inactivation of Sch9p. Arsenic, mercury, and nickel cause reduction of transcription of ribosome biogenesis genes, which are under the control of Sfp1p, a TORC1-regulated transcriptional activator. Researches have found that the transcription factor Sfp1, plays a role in the control of cell size, and regulates RP gene expression in response to nutrients and stress. It is reported that upon arsenic stress, Sfp1p is deactivated by dephosphorylation, and dissociates from chromatin, and then accumulates in the nucleus. Indeed, it is shown that arsenic treatment activates Msn2p and Msn4p (Marion *et al.*, 2004; Hoiner *et al.*, 2009).

Studies with of the yeast deletion collection show that 15 deletion strains which are leading to partial rapamycin resistance also exhibit elevated levels of superoxide anions, and it is found that elevated levels of reactive oxygen species specifically modify TORC1 such that it is no longer able to fully bind FKBP12: rapamycin. Therefore, it is concluded that elevated oxidative stress modifies TORC1 and prevents its binding to the FKBP12: rapamycin complex, ultimately leading to rapamycin resistance (Neklesa *et al.*, 2002).

What is the reason for that TOR responds to environmental stress? It might be considered as an explanation that TOR, as a central controller of cell growth, may respond to several different types of stress to ensure that growth occurs only when overall conditions are favourable.

1.4. TOR Signalling and Regulation of Important Cellular Events

TOR promotes cell growth in response to nutrient availability and under stress conditions other than nutrient limitation. In addition, TOR is responsible for regulation of amino acid permease activity, ribosomal biogenesis and cell proliferation. TOR also controls aging and autophagy in the cell.

1.4.1. TOR and Regulation of Amino Acid Permease Activity

The role of amino acid permeases is important in cell growth and viability, since some amino acids (such as glutamine, glutamate, and asparagine) are also important in *S. cerevisiae*, because, besides taking place in the constitution of the building blocks for protein synthesis which makes them essential for cell growth, they also serve as nitrogen sources (Crespo *et al.*, 2002; Dechant *et al.*, 2008).

Yeast amino acid permeases can be classified into two groups based on their function and regulation (Crespo *et al.*, 2002). In first class of amino acid permeases, including the general amino acid permease Gap1p, permeases are regulated in response to the available nitrogen source. In the presence of a good nitrogen source, such as ammonium or glutamine, the uptake activity of these permeases is low, whereas in medium containing a poor nitrogen source, such as proline or urea, transport activity is strongly induced. Permeases of second class consist of transporters that are specific for single amino acids or a small set of structurally related amino acids. The histidine permease Hip1p and the tryptophan permease Tat2p belong to this group of specific amino acid permeases.

Studies in *S. cerevisiae* have revealed that the TOR pathway plays a prominent role in regulation of amino acid permease activity (Crespo *et al.*, 2002; Dechant *et al.*, 2008). Rapamycin-inhibited TOR or nitrogen starvation induces ubiquitination and degradation of

Tat2p and, as a consequence, leads to a decrease in tryptophan import. Down-regulation of amino acid permeases due to starvation also applies to Hip1p and possibly to all specific amino acid permeases. In contrast to Tat2p and Hip1p, Gap1p protein is significantly increased under rapamycin treatment. Thus, the high-specificity permeases, such as Tat2p and Hip1p, and the broad-specificity permease Gap1p in response to nutrient availability are seem to be inversely regulated by TOR proteins.

Npr1p, the Ser/Thr nitrogen permease reactivator kinase, mediates upregulation of Gap1p upon nitrogen starvation. In the presence of a poor nitrogen source, Gap1p function is promoted by Npr1p. This is probably done via phosphorylation and it protects Gap1p from degradation. In agreement with the opposite regulation of Gap1p and Tat2p, Npr1p has been proposed to function as a negative regulator of Tat2p. Indeed, Npr1p overexpression causes a decrease in tryptophan import. How is Npr1p modulated to inversely regulate Gap1p and Tat2p according to the nitrogen source? Npr1p is a phosphoprotein and TOR signaling pathway controls its phosphorylation state in response to the nitrogen source. In response to a good nitrogen source, Npr1p is inactivated via phosphorylation by TOR and in this form; it is unable to protect Gap1p from ubiquitination. Under poor nitrogen conditions, Npr1p is activated via dephosphorylation by a Sit4p- and Tip41p-dependent manner. Activated Npr1p leads to Gap1p protection and ubiquitination and degradation of Tat2p. It is still unclear whether Npr1p directly phosphorylates Gap1p or Tat2p (Crespo *et al.*, 2002).

It has been suggested that nitrogen related regulation of TOR is activated by intracellular amino acid pools and this mechanism is controlled via EGO complex (Exit from growth arrest). Studies have identified a family of GTPases as potential upstream regulators of TORC1. A screen for genes required for recovery from rapamycin-induced growth arrest has identified the so-called EGO complex, which contains two small GTPases, Gtr1p and Gtr2p. Although the EGO complex is not necessary for the normal response to rapamycin, cells are unable to resume protein biosynthesis after relief from rapamycin treatment. Moreover, deletion of EGO components displays synthetic lethality with partial reduction of TORC1 activity, suggesting that the EGO complex acts upstream or parallel of TORC1; and it is conceivable that the EGO complex may activate TORC1 indirectly by increasing intracellular amino acid pools (Dechant *et al.*, 2008).

Although multiple pathways play a role in TORC1 activation in yeast and mammals, it is not very well understood which specific amino acids contribute to regulation of TORC1. Unlike in yeast cells, all amino acids cannot be synthesized *de novo* in mammalian cells, and thus, these amino acids may become limiting upon starvation. Indeed, a specific role for the essential amino acid leucine, and other branched chain amino acids have been shown in the activation of cell growth in mammalian cells by many studies. In humans, a rapid rise in intracellular concentration of Ca^{2+} is observed after amino acid stimulation of starved cells or stimulation with leucine alone. Thus, Ca^{2+} acts as a second messenger for amino acids, which directly links amino acid availability to TORC1 activity, and could therefore be used as a readout to study TORC1 activation in response to amino acid stimulation in mammalian cells. The related binding site in mammals has been mapped and is also found in the yeast protein, suggesting that Ca^{2+}-dependent activation might be conserved. In addition to that, deletion of *PMR1*, the Ca^{2+} ATPase which is required for Ca^{2+} storage in the Golgi, prevents efficient Gln3p activation in response to rapamycin treatment. It is possible that TORC1 may be hyperactivated due to higher cytosolic Ca^{2+} concentrations resulting from deletion of *PMR1*. Since both the vacuole and the Golgi are important storage sites for Ca^{2+} in yeast, some possible TORC1 regulators may be found at the endomembrane system (Dechant *et al.*, 2005).

Considering these data, it is possible to consider that Ca^{2+}-dependent activation of TORC1 also exists in yeast. It has, however, been found that TORC1 activation depends on glutamine, rather than leucine, in yeast cells (Dechant *et al.*, 2005). In the study with a specific inhibitor of glutamine synthase, l-methionine sulfoximine (MSX), it was observed that depletion of intracellular pools of glutamine leads to rapid inactivation of TORC1 and growth arrest. Similarly, it was found that addition of glutamate, the cellular precursor for glutamine, suppress the synthetic lethality of components of the EGO complex and TOR1. Therefore, it can be concluded that the intracellular concentration of glutamine, or a signal derived thereof, may play an important role in TORC1 activity (Figure 1.13).

Thus, TORC1 activation appears to depend on two structurally different amino acids, leucine and glutamine, in yeast and mammals (Dechant *et al.*, 2005). This discrepancy might indicate that TORC1 can be activated by multiple amino acids in both systems. Due to differences in the requirement or metabolism of different amino acids, depletion of a

specific amino acid can bring different quantitative effects in yeast and humans. Indeed, leucine is the most abundant amino acid found in proteins, but cannot be synthesized by mammalian cells. On the other hand, most other abundant amino acids are non-essential. Thus, starvation can affect intracellular leucine concentration more than any other, non-essential, amino acids.

Alternatively, there may be a functional linkage between leucine and glutamine. Intriguingly, leucine serves as a source for glutamine in mammalian cells by providing the amino group for transamination to glutamate, raising the possibility that leucine affects TORC1 activation indirectly via its effect on glutamine availability. In any case, the analysis of other essential amino acids as well as glutamine and leucine metabolism upon starvation will be crucial to identify the molecular mechanisms of TORC1 activation in yeast and mammalian cells (Dechant *et al.*, 2005).

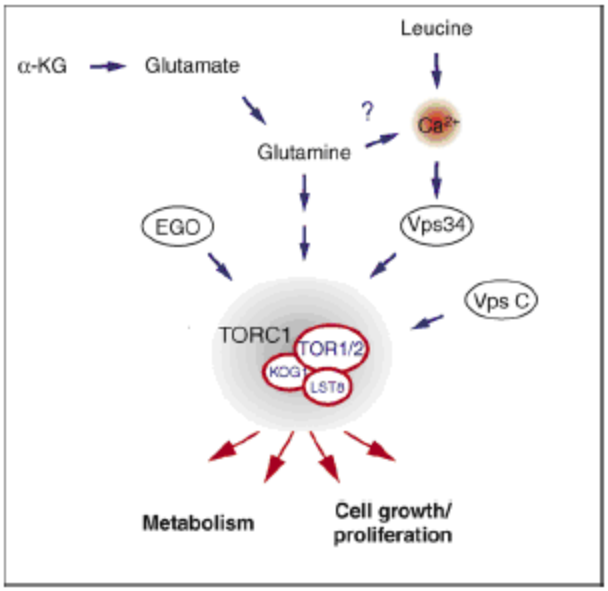

Figure 1.13. Regulation TOR signalling by amino acids, EGO and VPS components (Dechant *et al.*, 2008)

Vacuolar protein sorting (VPS) components are also required for proper TOR signalling (Figure 1.13). Class C and D VPS components mediate Golgi-to-endosome vesicle transport. Studies with mutations in class C and D VPS components show that TOR signalling to nitrogen-regulated transactivators may occur on endosomal membranes (Puria

et al., 2008). It was indicated that the class C VPS complex is required for proper regulation of Gln3p (Zurita-Martinez *et al.*, 2007; Dechant *et al.*, 2008).

1.4.2. TOR and Ribosomal Biogenesis

The regulation of ribosome biogenesis is a key aspect of cell growth control. In a growing cell, ribosome biogenesis is a major consumer of cellular energy and building blocks. Thus, as the availability of nutrient resources change, cells must rapidly and accurately rebalance ribosome production. Ribosome biogenesis on its own consumes up to 80% of a proliferating cell's energy and represents about 95% of total transcription. Recent evidence suggests that in addition to growth, regulation of cell growth is controlled via ribosome biogenesis. The regulation of ribosome biogenesis occurs primarily at the transcriptional level and involves all three nuclear RNA polymerases (Pol I thru III) (Figure 1.14). RNA polymerase I (Pol I) synthesize 25S, 18S, and 5.8S ribosomal RNAs, RNA Pol III transcribes the 5S rRNA, and RNA Pol II produces messenger RNAs encoding 79 ribosomal proteins (RP), expressed from 138 genes in budding yeast. In order to generate mature ribosomal subunits, ribosomal components have to be transported, processed, and assembled, under the control of 200 different transacting factors. The second group of Pol II-transcribed genes, called *Ribi*, is co-regulated with the RP genes. (Martin *et al.*, 2006; Berger *et al.*, 2007)

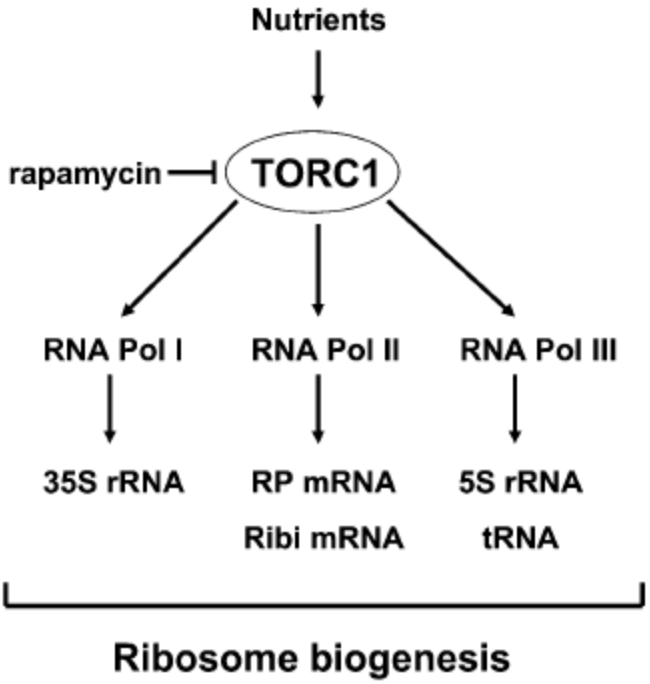

Figure 1.14. Transcriptional control of ribosome biogenesis by TORC1 (Martin *et al.*, 2006)

1.4.2.1. Regulation of Pol I- and Pol III-Dependent Transcription Pol I carries out the transcription of rDNA in the nucleolus. In both yeast and mammals, rDNA transcription is regulated by TOR pathway. Rrn3p (homolog to TIF-IA in mammalian cell), which is the conserved evolutionarily, is the key transcription activator of Pol I for rDNA transcription via recruiting Pol I to the rDNA promoter. Recent studies have demonstrated that TOR pathway promotes the association of Rrn3p with Pol I, which becomes competent for transcription initiation after forming complex with the initiation factors on promoter. The study with chromatin immunoprecipitation (ChIP) analysis has revealed that the level of Rrn3p-Pol I complex is significantly reduced and the binding level of Rrn3p at 35S rDNA promoter is decreased under rapamycin treatment. In addition, TOR pathway regulates rDNA chromatin structure in yeast via the recruitment of histone deacetylase Rpd3p (Tsang *et al.*, 2007).

TOR is intimately involved in gene regulation. It was observed that Tor1p nuclear localization is crucial for 35S rRNA synthesis. Indeed, the study with chromatin immunoprecipitation (ChIP) analysis shows the association of Tor1p with 35S rDNA promoter under favorable growth conditions when 35S rRNA synthesis is robust. Tor1p

dissociates from 35S rDNA promoter in response to starvation or rapamycin treatment. Due to the fact that Kog1p but not Avo2p/3p also interacts with 35S rDNA promoter, it can be indicated that TORC1 but not TORC2 is involved in this mechanism. A putative helix-turn-helix (HTH) motif of Tor1p plays a crucial role in association of Tor1p with 35S rDNA promoter. Deletion of HTH demolishes the ability of Tor1p to bind to 35S rDNA promoter and to regulate 35S rRNA synthesis. Considering these data, it can be demonstrated that physical association of TOR with 35S rDNA promoter is crucial for 35S rDNA transcription. That Tor1p is, interestingly, not detected by ChIP at the Pol II-dependent target genes such as RP genes, is consistent with the observation that cytoplasmic Tor1p is sufficient for their regulation. (Tsang *et al.*, 2007)

Studies have previously shown that Hmo1p is a genuine Pol I ranscription factor. Recently, it has been shown that Hmo1p is bound to both rDNA and RP gene promoters. Although Hmo1p is not required for global RP gene expression, the assembly of Fhl1p and Ifh1p onto RP promoters requires Hmo1p. Moreover, Hmo1p binding to RP promoters requires Rap1p (Berger *et al.*, 2007).

Studies have established that Hmo1p is genetically linked to Pol I, to specific RP genes, and to TORC1 *in vivo*. Hmo1p is hypersensitive to TORC1 inhibition. Although Ifh1p is still essential for RP gene expression in the absence of Hmo1p, some specific RP genes are de-regulated. In addition, it is found that in the absence of Hmo1p, the cross-regulation of Pol I-RP gene transcription is alleviated. Therefore, it can be concluded that Hmo1p is required for the TORC1-regulatable expression of RP genes. (Berger *et al.*, 2007)

Furthermore, studies show that the late stage of ribosome maturation in the nucleoplasm also requires TOR activity and this is done via the nuclear GTP-binding protein Nog1p. Upon nutrient starvation or rapamycin treatment, Nog1p-containing complex is tethered to the nucleolus, and intranuclear transport of pre-60S complexes is consequently inhibited. Therefore, it can be proposed that the Nog1p complex plays an important role in the regulation of ribosome maturation in response to nutrient availability.

TOR is also directly involved in the Pol III-dependent gene regulation. Tor1p binds to 5S rDNA region, and deletion of HTH also interferes with the ability of Tor1p to promote 5S rRNA synthesis (Tsang *et al.*, 2007). Due to co-regulation of 5S and 35S rRNAs synthesis, it is tempting to speculate that Tor1p association with their promoters appears to serve as a simple mechanism to coordinate their expression (Figure 1.15). It is proposed that Pol I has an intrinsic ability to communicate with Pol II which may further coordinate the expression of all ribosomal genes.

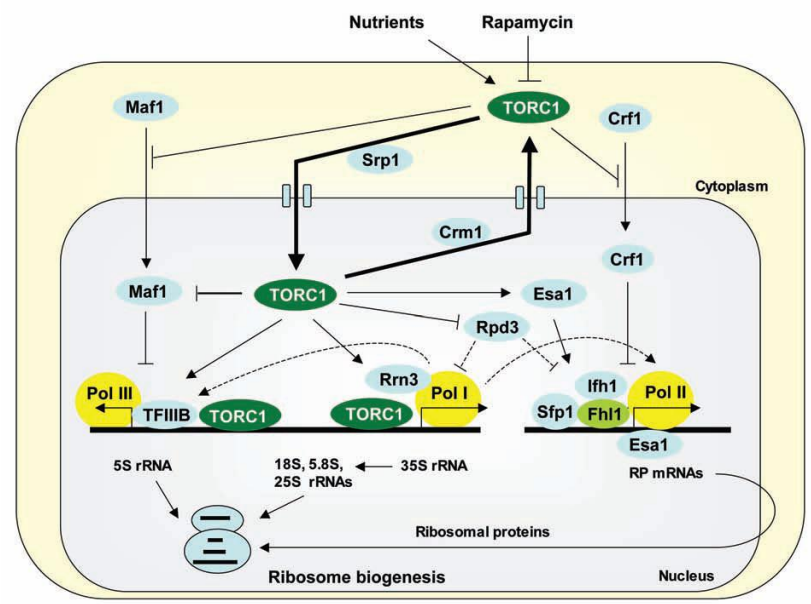

Figure 1.15. Transcriptional regulation of ribosome biogenesis by TOR (Tsang *et al.*, 2007)

1.4.2.2. Regulation of Pol II-Dependent Transcription TORC1 also regulates the nucleocytoplasmic transport of Pol II transcription factors in the cytoplasm (Figure 1.15). This demonstration is supported by the observation that Tor1 (nuclear export sequence deleted) or cytoplasmic Tor1 is sufficient to regulate Pol II-transcribed genes (Tsang *et al.*, 2007).

Studies show that TOR controls the subcellular localization of protein kinase A (PKA) and the PKA-regulated kinase Yak1p. It is shown that the Forkhead-like transcription factor Fhl1p and the two cofactors Ifh1p (a coactivator) and Crf1p (a corepressor) are involved in the regulation of RP gene transcription via TOR and PKA in

yeast. TOR, via PKA, negatively regulates Yak1p and maintains Crf1p in the cytoplasm. When TOR is inactivated, Yak1p is activated, and it activates Crf1p via phosphorylation. As Crf1p is phosphorylated, starts to accumulate in the nucleus and competes with Ifh1p for binding to Fhl1p at RP gene promoters, and thereby inhibits transcription of RP genes.

Studies have presented that TOR controls the expression of ribosomal protein genes via the Forkhead transcription factor Fhl1p (Figure 1.16). TOR controls Fhl1p via the RAS-PKA-Yak1p effector pathway and the two Fhl1p cofactors Ifh1p, a coactivator, and Crf1p, a corepressor. Under favourable growth conditions, TOR inhibits the kinase Yak1p via PKA. In the absence of active Yak1p, the corepressor Crf1p is cytoplasmic and inactive. Consequently, Ifh1p binds Fhl1p at RP gene promoters and activates transcription, possibly by recruiting other transcription factors such as the histone acetylase subunit Esa1p. It was also found that TOR signalling is required for the maintenance of Esa1p at RP gene promoters (Rohde *et al.*, 2003; Martin *et al.*, 2004). Targeting Ifh1p to a heterologous promoter is sufficient to activate the promoter; therefore the binding of Ifh1p to the promoter appears to be a key event in the expression of RP genes. On the other hand, upon unfavourable growth conditions, TOR and PKA are inactive and, consequently, Yak1p is active. Activated Yak1p phosphorylates Crf1p; and phosphorylated Crf1p accumulates in the nucleus and binds Fhl1p, thereby dislodging the coactivator Ifh1p from RP gene promoters. The binding of Crf1p to promoter bound Fhl1p may lead to repression of RP genes, firstly, by releasing the factors formerly recruited by Ifh1p and, secondly, by recruiting new factors such as the histone deacetylase subunit Rpd3p. Consistent with this notion, an *RPD3* deletion confers rapamycin resistance and, as shown by others, the binding of Esa1p and Rpd3p to RP gene promoters is inhibited and induced, respectively, by rapamycin. Thus, the findings provide a pathway from TOR to RP gene expression and, thereby, a molecular link between the growth environment and the regulation of ribosome biogenesis. (Martin *et al.*, 2004)

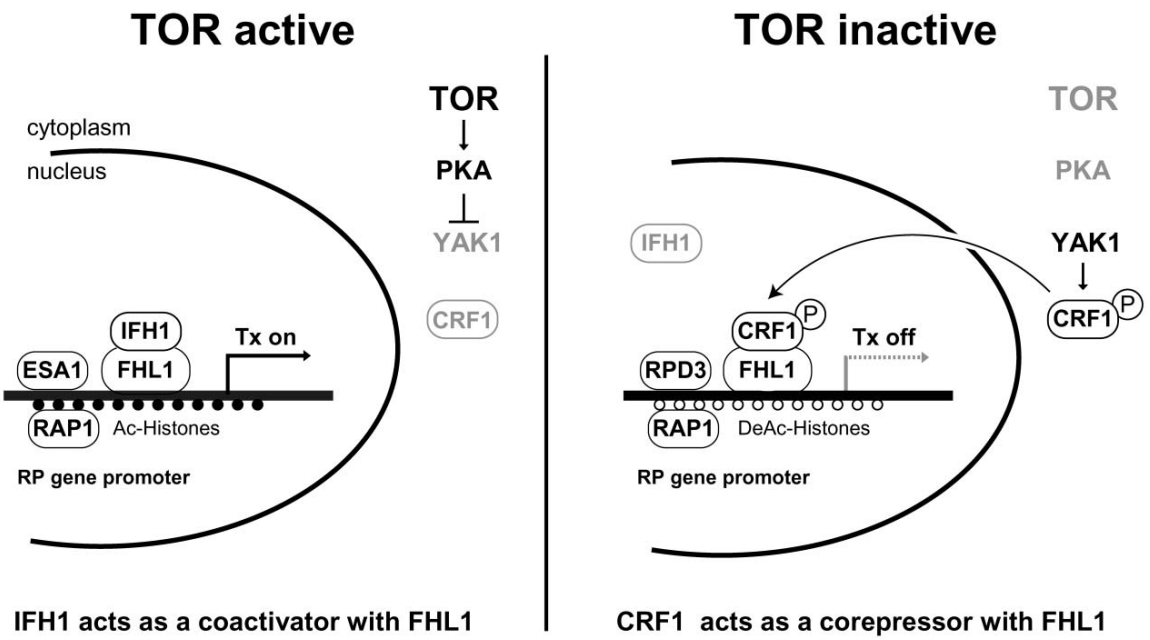

Figure 1.16. Regulation of RP genes by TOR via Fhl1p (Martin *et al.*, 2004)

Ifh1p and Crf1p, which are two Fhl1p cofactors, have an FHB (Forkhead binding) domain and they bind the FHA (Forkhead associated) domain of Fhl1p. Whether the Forkhead factor acts as a transcriptional activator or repressor is determined by the mutually exclusive binding of Ifh1p and Crf1p to Fhl1p. The yeast Forkhead factor Fkh2p plays a role in the activation of transcription of cyclin genes via the coactivator Ndd1p, but there is also a role for a corepressor which remains to be identified in this mechanism. (Martin *et al.*, 2004)

Yak1p has an important role in the nuclear localization of Crf1p. However, how the nuclear localization of Crf1p is controlled by Yak1p is not very well understood. Crf1p is phosphorylated by Yak1p and this phosphorylation coincides with nuclear accumulation of Crf1p. Furthermore, Yak1p phosphorylates the Crf1p FHB domain which is required by Crf1p for both Fhl1p binding and nuclear accumulation. Finally, both Yak1p and Crf1p accumulate in the nucleus upon rapamycin treatment. Therefore it can be suggested that Yak1p-mediated phosphorylation of Crf1p enhances Fhl1p binding and, thereby, nuclear retention of Crf1p. However, in the absence of Fhl1p nuclear accumulation of Crf1p is still observed. Furthermore, interaction of Crf1p with Fhl1p still occurs in the two-hybrid system without rapamycin treatment (i.e., without Yak1p activation); and this suggests that a heterologous nuclear localization signal is sufficient for an interaction of Crf1p and

Fhl1p. Thus, it can be concluded that Crf1p nuclear import rather than maintenance in the nucleus is controlled by Yak1p. It is still unclear whether the interaction of Crf1p with Fhl1p, with a factor mediating nuclear import, or both is stimulated via the phosphorylation of Crf1p. Although nuclear accumulation of Yak1p is significant for this mechanism but still not very well understood (Martin *et al.*, 2004)

Coordinated activity of all three RNA polymerases is required for ribosome biogenesis. *FHL1* was considered as a multicopy suppressor of a mutation affecting Pol III, and it was found that the Fhl1p interacts with Hmo1p which is a Pol I transcription factor. Furthermore, Rpd3p binds both Pol I-dependent promoters and Pol II-dependent RP gene promoters. Considering these data it can be suggeste that Fhl1p, Ifh1p, and Crf1p may play a role in the coordination of all three RNA polymerases by TOR-PKA pathway. (Martin *et al.*, 2004) Moreover, it has been proposed that Sch9p has a role in this mechanism (Pascual-Ahuir *et al.*, 2007).

TORC1, Yak1p, and FHA domain-containing Forkhead transcription factors (MNF and ILF1/2 in human) are conserved from yeast to human suggesting that this mechanism by which TORC1 regulates ribosome biogenesis via Yak1p may, at least in part, also be conserved (Martin *et al.*, 2004).

Moreover, some studies demonstrate that the transcription factor Sfp1p, which has a role in the control of cell size, regulates RP gene expression in response to nutrients and stress. Under optimal growth conditions, Sfp1p is localized to the nucleus, bound to the promoters of RP genes, and helps promote RP gene expression. In response to inhibition of TOR signalling, stress, or changes in nutrient availability, Sfp1p is released from RP gene promoters and leaves the nucleus, and RP gene transcription is down-regulated. Additionally, cells lacking Sfp1p fail to appropriately modulate RP gene expression in response to environmental cues. Therefore, it can be concluded that Sfp1p integrates information from nutrient- and stress-responsive signalling pathways to help control RP gene expression (Marion *et al.*, 2004)

In addition to those, it has been proposed that TOR regulation of the Tap42p-Pphs-Sit4p system in yeast is critical in ribosome biogenesis. It has been observed that polyribosome formation is inhibited in the *TAP42* deleted strains, suggesting that *TAP42* functions upstream of translation initiation. Moreover, *TPD3* (A subunit of PP2A) and *SIT4* have function upstream of RNA Pol III and Pol II, respectively. From these observations, it has been proposed that the Tap42p-Pphs-Sit4p system plays a major role in mediating TOR signalling to control ribosomal biogenesis. (Crespo *et al.*, 2002; Inoki *et al.*, 2005)

1.4.3. TOR and Regulation of Cell Proliferation

TOR signaling pathway also controls cell proliferation in response to nutrient availability in *Saccharomyces cerevisiae*. How the inhibition of TOR pathway regulates cell cycle progression and the mechanism is not completely understood. However, it has been found that rapamycin treatment causes cells to arrest growth in G1 phase due to the impairment of protein synthesis (Zinzalla *et al.*, 2007). In addition, Sch9p and Rim15p are shown to have a role in G0 entry (Wanke *et al.*, 2005; Urban *et al.*, 2007), and Sit4p involves in G1/S and G2/M transitions (Clotet *et al.*, 1999; Jiang *et al.*, 2006).

1.4.4. TOR and Aging

It has been known about 80 years that calorie restriction (CR) leads to life span extension. Although there are numerous suggestions about how CR affects aging, the underlying mechanism is still not very clear. Genetic studies with different organisms, from yeast to mammals points that nutrient-sensing/insulin/ insulin growth factor I (IGF-I) pathways have an important role of in life span modulation, which suggest that there is a common evolutionary origin of aging regulation. Furthermore, it has been implicated in the studies with yeast, flies, and mice that these signalling pathways are related with CR-induced life span extension (Wei *et al.*, 2008)

S. cerevisae has become one of the most valuable model organisms for ageing studies. In this uni-cellular eukaryote, longevity is measured by two distinct paradigms. The first paradigm is replicative life span (RLS), which is defined as the mean or

maximum number of daughter cells generated by individual mother cells. Alternatively, chronological life span (CLS) is a measure of the mean or maximum survival time of populations of non-dividing yeast. Yeast RLS has been proposed as a model for the ageing of dividing cells of higher eukaryotes, whereas CLS is believed to better model the ageing of post-mitotic cells. RLS was the first paradigm to be used for ageing studies. (Cheng *et al.*, 2007)

In order to determine RLS, about 50 genes have been implicated. On the other hand, it has been shown that the chronological ageing is regulated by fewer genes. Recent studies have indicated that three nutrient responsive yeast kinases: Sch9p, PKA, and TOR, act as major regulators of both types of ageing. Sch9p is a yeast kinase homologous to mammalian serine/threonine protein kinase Akt. RLS is increased by 30–40% and CLS is extended by nearly three folds as a result of inactivated Sch9p. Some researches show that induction of Gcn4p has a role in this mechanism (Steffen *et al.*, 2008). High-throughput screenings have also been performed in yeast to identify genes that determine RLS and CLS, respectively. One of these studies identified 10 gene deletions that increase RLS, and 6 of them (including the deletion of *TOR1*) correspond to genes encoding proteins in the TOR pathways. In addition, several TOR-related gene deletions that increase CLS were identified by another one. In yeast, as well as in higher eukaryotes, Sch9p, PKA, and TOR coordinate signals from nutrients to regulate ribosome biogenesis, stress response, cell size, autophagy, and other cellular processes. That life span extension has been observed in researches with mutations that decrease the activity of the orthologs of these proteins in higher eukaryotes suggests that the roles of these kinases in the regulation of life span are conserved along evolution. (Cheng *et al.*, 2007)

Furthermore several studies have proposed a complex and controversial relationships between mitochondrial respiration, reactive oxygen species (ROS), and life. It is shown that deletion of the TOR1 gene extends chronological life span in Saccharomyces cerevisiae, primarily by increasing mitochondrial respiration via enhanced translation of mtDNA-encoded oxidative phosphorylation complex subunits. It is demonstrated that deletion of *TOR1* delays aging independently of the antioxidant gene *SOD2*. Furthermore, a difference was observed in life span of wild type and *TOR1* deleted strains only when respiration competent and grown in normal level of oxygen in the presence of glucose. It is

proposed that inhibition of TOR signalling causes de-repression of respiration during growth in glucose and that the subsequent increase in mitochondrial oxygen consumption limits intracellular oxygen and ROS-mediated damage during glycolytic growth, leading to lower cellular ROS and extension of chronological life span. (Figure 1.17) (Bonawitz *et al.*, 2007; Scheike *et al.*, 2007)

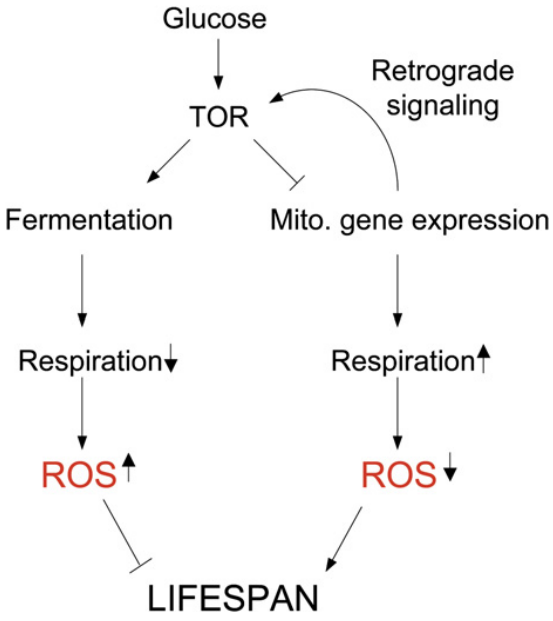

Figure 1.17. Effect of cellular ROS in life span (Scheike *et al.*, 2007)

TOR inhibition extends lifespan also by increasing Sir2p activity and stabilizing the rDNA locus, which is a similar mechanism as how CR does. Further, it is shown that the relocalization of the transcription factors Msn2p and Msn4p from the cytoplasm to the nucleus lead to rDNA stabilization and lifespan extension related to CR and TOR signalling. Msn2p/4p increases expression of the nicotinamidase gene *PNC1*. Considering these findings, it can be suggested that TOR and sirtuins may be part of the same longevity pathway in higher organisms, and that they may promote genomic stability during aging (Figure 1.18) (Medvedik *et al.*, 2007).

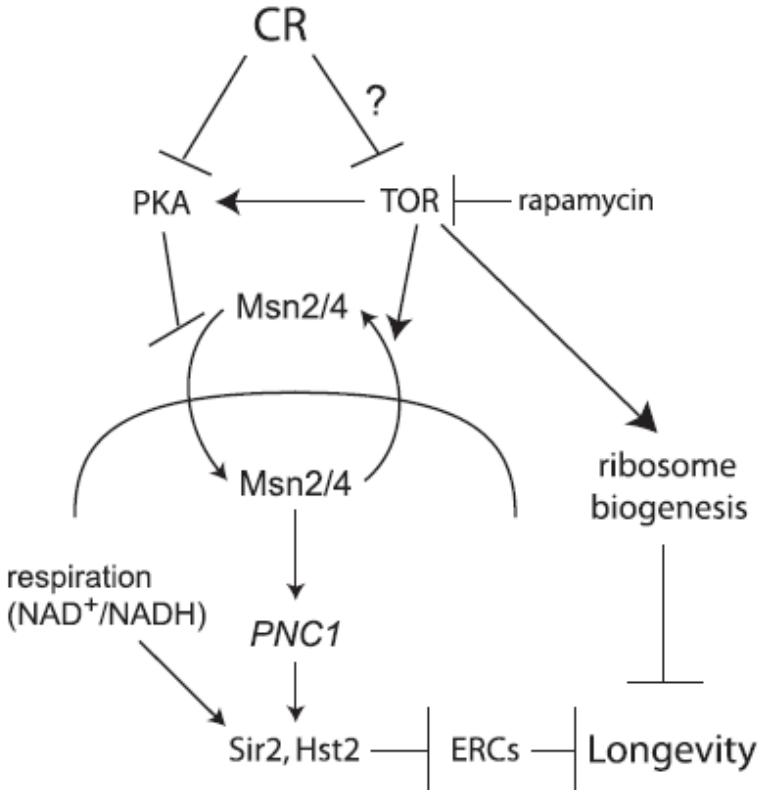

Figure 1.18. Role of Msn2p/4p in life span extension (Medvedik *et al.*, 2007)

1.4.5. TOR and Autophagy

One of the primary roles of autophagy is serving a response to stress, like nutrient limitation, in unicellular organisms. Autophagy is also considered to be involved in the extension of lifespan which is related to caloric restriction. TOR inhibits autophagy through RAS/PKA pathway under basal or nutrient-rich conditions, and it is one of the major regulatory components of autophagy (Klionsky *et al.*, 2005; De Virgilio *et al.*, 2006). TOR acts on autophagy in two ways. First, it directly or indirectly phosphorylates the autophagy protein Atg13p. This hyper-phosphorylated Atg13p has a lower affinity for the kinase Atg1p, which is a conserved component of the autophagic machinery, and autophagy may be inhibited due to this reduced interaction. Thus, TOR controls autophagy primarily at the induction step by activating the Atg1p kinase (Klionsky *et al.*, 2005; Diaz-Troya *et al.*, 2008). Partial dephosphorylation of Atg13p is observed in the inhibition of TOR through starvation or treatment with rapamycin which results in autophagic induction. Second, TOR acts in a signal transduction cascade and it controls phosphorylation of several effectors (e.g. Tap42p, Sit4p, Ure2p and Gln3p) that involve in

transcriptional and translational regulation of certain proteins, some of which are required for autophagy (Klionsky *et al.*, 2005). However, autophagy is also found to be regulated through different factors like protein kinase A, Gcn2p and Snf1p (Klionsky *et al.*, 2005; Orlova *et al.*, 2006).

1.5. Effect of Caffeine on TOR Signalling

Besides rapamycin, there are a number of other pharmacological agents that affect TOR, including caffeine, which is a member of the methylxanthine family of compounds. Caffeine affects a diverse array of cellular processes related to cell growth, DNA metabolism, and cell cycle progression, most likely by acting as a low affinity ATP analog. It has been shown that phosphorylation of TOR-dependent substrates is inhibited caffeine both *in vitro* and *in vivo*. Although caffeine has not been used widely as a tool for probing TOR function, it has a potential due to the pleiotropic behavior of this compound in addition to the fact that it interacts with TOR with relatively low affinity, *i.e.* in the submillimolar range (Reinke *et al.*, 2006).

It has been proposed that caffeine targets many cellular activities with cAMP phosphodiesterase. However, it is not very clear that caffeine really inhibits cAMP phosphodiesterase. Indeed, some studies in yeast (Kuranda *et al.*, 2006; Reinke *et al.*, 2006) have recently demonstrated that caffeine targets TORC1, but not cAMP phosphodiesterase. It has also been confirmed that TORC2 is not the growth-limiting target of caffeine in yeast. (Wanke *et al.*, 2008). Research reveals that high doses of caffeine (> 10 mM) have mutagenic effects in yeast cells; although similar effects have been observed, the effects of rapamycin and caffeine are not totally the same (Kuranda *et al.*, 2006). The figure below shows the comparison of expression data from *pde2*Δ mutant and wild type yeast cells treated with caffeine and rapamycin (data sets of rapamycin, Hardwick *et al.*, 1999; caffeine, Kuranda *et al.*, 2006; and *pde2Δ* mutant, Jones *et al.*, 2003) (Figure 1.19).

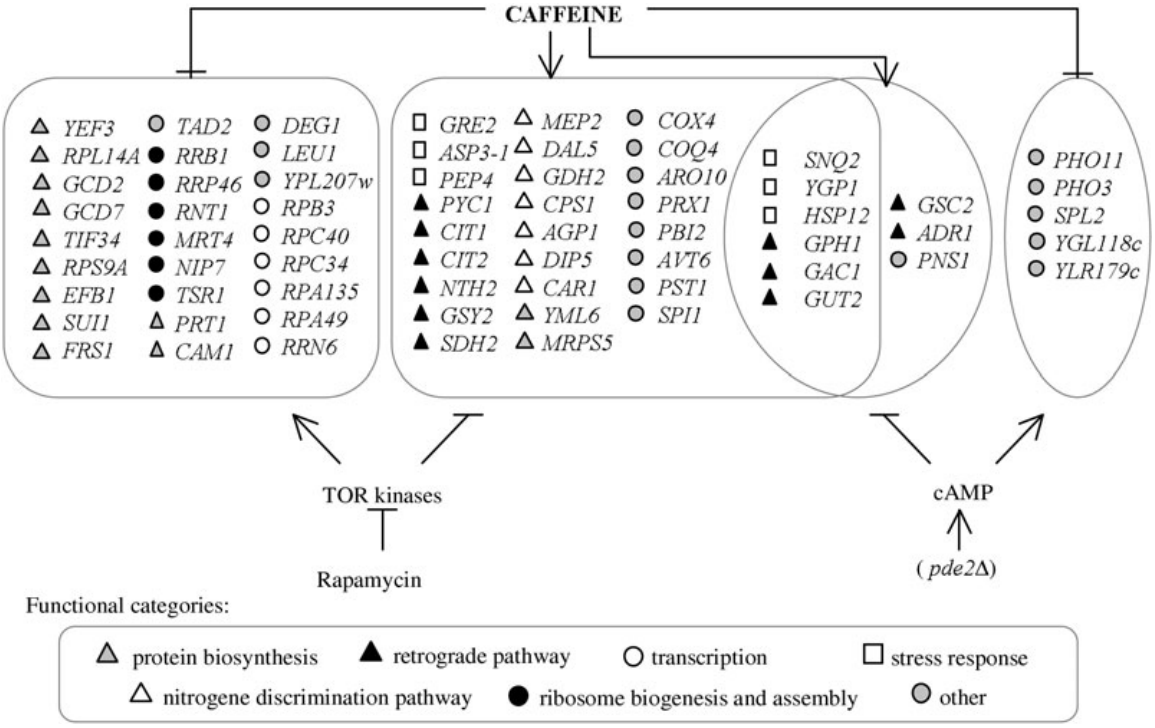

Figure 1.19. Comparison of expression data from cells treated with caffeine, rapamycin and *pde2Δ* mutant (Kuranda *et al.*, 2006)

Although several similarities between response to caffeine and rapamycin have been observed, some differences have been pointed out in the studies (Kuranda *et al.*, 2006). First, growth arrest in the early G1 phase was observed under rapamycin teatment; however, it was found that addition of caffeine did not cause growth arrest at any specific stage in cell division. Second, although genes in the Ras/cAMP signalling pathway responded to caffeine at the transcriptional level; it was observed that rapamycin did not cause significant change in expression of those genes. Finally, the expression data of caffeine overlapped 25% of the expression data of rapamycin; but it has to be considered that the experiments were not carried out under the same conditions.

1.6. Flux Balance Analysis

A cell is a complex system consisting of a numerous molecular components. Among those components, there are many complex interactions and the system works by the harmony of intricate cellular functions. This system could not be very well understood with the classical methods based solely on a description of the individual components;

therefore, in order to understand the complexity of cellular networks, an approach based on the integrated cellular systems is required (Edward *et al.*, 1999; Edward *et al.*, 2000).

Alternative to the classical methods, in order to analyze the biological system the engineering approach focuses on mathematical and computational models which consist of fundamental physicochemical laws and principles. In this approach, the ultimate goal is to simulating the complete cellular metabolism by dynamic models; however, in the lack of kinetic information on the reactions in the cell, there is a huge obstacle in front of this approach. Nevertheless, it is still possible to evaluate the capabilities of the cell without kinetic information on the dynamics and regulation of metabolism, and this approach is called flux balance analysis (Edward *et al.*, 2000).

Flux balance analysis (FBA) is based on the fundamental physicochemical constraints on metabolic networks. In order to analyze the metabolic networks, it only uses the stoichiometry of metabolic pathways and the metabolic demands of the cell. A closed solution space is defined by using physicochemical constraints and the most appropriate solution is found by linear optimization within this space. FBA can also use the annotated genome sequence information to define the stoichiometric parameters; thus, additional information can be obtained when it is available (Palsson *et al.*, 2001). FBA has been used in several studies. This approach has been successfully used to predict by-product secretion (Edward *et al.*, 2000; Forster *et al.*, 2003), time course of growth (Palsson *et al.*, 2001; Beard *et al.*, 2002; Lee *et al.*, 2006), and effect of knock-outs and mutants (Mahadevan *et al.*, 2002; Motter *et al.*, 2008).

1.7. Topological Study of Protein-Protein Interactions

In the twentieth-century, biological phenomena were studied by investigating the behaviour of molecules. However, most biological functions have been attributed to interactions among many components in the cell, rather than an individual molecule. For example, the cell reacts to a chemical via detection and amplification of signals arisen due to the chemical and shows a coordination of reactions (Hartwell *et al.*, 1999). That living cells are composed of a large number of different molecules interacting with each other has directed scientists from molecular to modular biology (Rodriguez-Caso *et al.*, 2005).

A functional module is a distinct entity whose function is distinguishable from those of other modules. Modules can be connected to or insulated from each other. Connectivity of modules produces interactions among reactions; whereas, insulation prevents the cell from possible harm arisen from cross-talk between networks. Types of molecules in a module can be discrete and the interactions among these molecules (proteins, DNA, RNA and small molecules) bring modules distinct functions. These functions cannot easily be predicted by studying the properties of individual components. Therefore, a linkage between biology and synthetic disciplines such as computer science and engineering is necessary (Hartwell *et al.*, 1999).

Mapping molecular interactions within metabolic and signalling pathways into a network is a starting point in this integrative approach, and biological databases provide a unique opportunity to characterize biological networks under the perspective of a system. In addition to topological studies of cellular networks by analyzing genomic and metabolic maps, protein networks have also been being studied after the progress in massive two-hybrid system screening methodology. Protein–protein interactions are very valuable to understand regulation, signalling and gene expression in the cell, since proteins form supramolecular activator or inhibitory complexes, depending on their components and possible combinations (Rodriguez-Caso *et al.*, 2005). High- throughput data together with network topology is also studied to identify functional modules (Ulitsky *et al.*, 2007, Mobini *et al.*, 2009)

1.8. The Aim of the Thesis

The aim of this study is to understand the effect of the caffeine on the growth of the cells and identify flux changes in the presence of this chemical in yeast. .

The wild type yeast cells were grown batch-wise in the absence or presence of rapamycin or caffeine. Samples were collected at different times during the cultivation. The impact of two chemicals on the yeast cells was investigated by observing the growth of cells under the chemical treatment.

Saccharomyces cerevisiae wild type strains were also grown in well-controlled batch cultures in the presence or absence of caffeine. Samples were collected at the mid-exponential phase. Biomass and extra-cellular metabolites were measured and this data was used in flux balance analysis to determine metabolic fluxes under these conditions by using the whole genome stoichiometric models. The fluxes in which significant changes were observed due to caffeine treatment were identified.

Additionally since it has been reported that both caffeine and rapamycin affect the TOR signalling pathway, a modular approach based on literature curated protein-protein interactions was developed within the framework of this thesis.

The materials and the experimental methods used are explained in the "Material and Methods" section. All findings that are obtained from this study are comprised in the "Results" section, and the obtained results are comprehensively argued in the "Discussion" section. Important key points and recommendation about possible future work are represented in the "Conclusions and Recommendations" section.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Microorganism

BY4743 type (MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/+ met15Δ/+ ura3Δ0/ura3Δ0) *Saccharomyces cerevisiae* strains which were kindly provided by Professor Stephen G. Oliver (Department of Biochemistry, University of Cambridge) were used in the experiments.

2.1.2. Chemicals and Disposable Materials

2.1.2.1. Culture Media Complex medium (YPD) and F1 medium were used as culture media in the experiments. The compositions were as follows:

YPD medium (per 1 litre culture) consists of 1 g Yeast Extract (Merck), 2 g Bacteriological Peptone (Merck), 2 g D-Glucose (Riedel de Haen), completed to 1 l with distilled H_2O. D-Glucose was added from previously sterilized stocks after the sterilization of the remaining of the medium.

F1 medium (per 1 litre culture) consists of 20 g D-Glucose (Riedel de Haen), 3.13 g $(NH_4)_2SO_4$ (Merck), 2 g KH_2PO_4 (Merck), 0.55 g $MgSO_4.7H_2O$ (Merck), 0.1 g NaCl (Merck), 0.02 g Uracil (Fluka), 0.02 g Histidine (Merck), 0.1 g Leucine (Merck), 100 µl Trace Element Solution I (per 100 µl solution: 70 µg $ZnSO_4.7H_2O$ (Merck), 10 µg $CuSO_4.5H_2O$ (Merck), 10 µg H_3BO_3 (Merck), 10 µg KI (Merck), completed to 100µl with distilled H_2O), 100 µl Trace element Solution II (per 100 µl solution: 50 µg $FeCl_3.6H_2O$ (BDH), completed to 100 µl with distilled H_2O), 1.67 µl Vitamin Stock Solution (per 100 ml solution: 3.72 g Inotisol (Merck), 0. 84 g Thiamine/HCl (Sigma), 0.24 g Ca-panthothenate (Fluka), 0.24 g Pyridoxine (Fluka), 0.018 g Biotine (Merck), completed to 100 ml with distilled H_2O), completed to 1 l with distilled H_2O. The medium was sterilized by filtration before use.

2.1.2.2. Kits Enzymatic kits to determine D-glucose, ammonia, ethanol and glycerol concentration were purchased from Boehringer Mannheim – Roche (Germany).

2.1.2.3. Miscellaneous Ethanol 96% (Laborteks), 0.20 µm sterile filter units (Sartopore), disposable tubes of 1.5 ml (USA Scientific Inc.), disposable sterile pipettes of 5 and 50 ml (Roth) and sterile centrifuge tubes of 10 ml (Roth) were also used in various applications and processes.

2.1.3. Laboratory Equipment

• Autoclave	ALP CL-40M (Japan)
• Balance	Sartorius AC211C (Germany)
• Blower	Nitto Kohki Co. Ltd. (Japan)
• Centrifuge	Sigma 1-15P (Germany)
• Dissolved Oxygen Sensor	Hamilton Oxyferm FDA 225 (Switzerland)
• Fermentor	Sartorius Biostat B plus (Germany)
• Freezer	New Brunswick Sci. U410 (England)
• Incubator Shaker	New Brunswick Sci. Innova 4340 (England)
• Laminar Flow Cabinet	Holten HBB 2460 LaminAir (Denmark)
• Microwave Oven	Arçelik MD553 (Turkey)
• Oven	MMM Group Incucell (Germany)
• Probe, pH	Hamilton Easyferm K8 200 (Switzerland)
• Refrigerator	Ariston +4^{o}C (Italy)
• Spectrophotometer	Beckman Coulter DU730 (USA)
• Stirrer	Heidolph 3001 Magnetic Stirrer (Germany)
• Vortex	Scientific Industries Vortex-Genie 2 (USA)
• Water Bath	HETO CB 8-30e (Denmark)
• Water Purification Systems	Millipore Milli Ro Plus (USA)
	Millipore Milli-Q UF Plus (USA)

2.2. Methods

2.2.1. Sterilization

Sterilization was performed in an effort to avoid contamination throughout this study. Two kinds of sterilization processes, steam and filter sterilization were applied for this purpose.

In the steam sterilization process, the materials or the solutions were kept under 15 psig pressure and 121^{o}C temperature in an autoclave. The duration of this process was varied according to the nature of the material to be sterilized. Due to the possibility of caramelization or the Maillard reaction of the glucose molecules, glucose solution was sterilized for 3 minutes. For all other chemical solutions and all materials that were used in the processes related to cultivation, 15 minute steam sterilization process was done.

Large amounts of cultivation medium were preferred to be sterilized by filtration. 0.20 μm sterile Sartorious disposable filters (Germany) were used for this process and the medium was fed into the sterile fermentor via passing through the filter with the help of a pump.

2.2.2. Cultivation

The precultures to be used to inoculate batch cultivations were prepared by growing yeast cells from frozen stocks in 50 ml YPD medium. They were incubated in orbital shakers at 30^{o}C and 180 rpm, and used for inoculation after yeast cells were grown two overnight periods.

In batch cultivations in flasks, yeast cells were grown in F1 or YPD medium. Inoculation was done from the precultures with the volume of one hundredth of the final working volume. Flasks were shaken at 180 rpm and kept at 30^{o}C in orbital shakers. Growth of the cells was followed via optical density at 600 nm. Yeast cells were first grown in a large volume and then aliquots were taken into separate flasks just before the chemical treatment. They were treated with rapamycin (200 and 400 ng/ml as the final

concentration) or caffeine (1, 3, 4, 5, 6, 8, 10, 12, 14, 15, 18, 20, 30, and 50 mM as the final concentration).

Yeast cells were grown in a total volume of 1.5 litre F1 or YPD medium throughout the controlled batch cultivations in fermentors. The temperature was kept constant at 30^{o}C with a PID controller and the agitation was set to 800 rpm. Inoculation was done from the precultures with the volume of one hundredth of the final working volume. The pH value of the culture was controlled by a PID controller with the automatic addition of 0.5 M NaOH or 0.5 M HCl, and was set to 5.5. Air was given into the cultures with a flow rate of 1 l/min by using a compressor. The dissolved oxygen concentration was monitored with the help of an oxygen probe.

Two different batch cultivations were done. They were carried out in the presence or absence of caffeine. In the controlled batch cultivation in F1 medium, samples were taken in 30 minutes intervals after yeast cells had an optical density of 0.2. In controlled batch cultivation in YPD medium, yeast cells were grown until the optical density reached 1.0, and then they were treated with caffeine. The batch cultures were treated with caffeine that the final concentration was 30 mM. To measure biomass and extracellular metabolite amounts, samples with the volume of 5 ml were collected every 30 minutes while the cells were growing in the exponential phase, and samples of 1ml were taken throughout the whole experiment in order to measure optical density.

2.2.3. Sample Preparation and Storage

Collected samples of 1 ml were taken in 1.5 ml Eppendorf tubes (Germany). They were centrifuged for 6 minutes at 8000 rpm in the Eppendorf (Germany) centrifuge with rotor 5415C. The supernatant was transferred into a new Eppendorf tube to be used in extracellular metabolite analyses and was stored at -20^{o}C. Prior to enzymatic analyses, they were incubated at 80^{o}C for 15 minutes to cease any possible enzymatic activity that would have remained. The cells precipitated via centrifugation were washed three times with 1 ml distilled water and finally dissolved in distilled water. They were stored at -4^{o}C before used in biomass measurement.

2.2.4. Enzymatic Analyses for the Measurement of Metabolite Concentrations

The concentrations of the metabolites, ammonia, D-glucose, ethanol and glycerol, were measured with the help of the enzymatic analysis kits (Boehringer – Mannheim, Germany). The procedures that were described by the manufacturers of the kits were followed in enzymatic analyses, and the necessary dilutions of supernatants were carried out as indicated in the protocols prior to the analyses.

2.2.4.1. D-Glucose Concentration Measurement D-Gluose is phosphorylated to D-glucose-6-phosphate (G-6-P) in the presence of the enzyme hexokinase (HK) and adenosine-5'-triphosphate (ATP) with the simultaneous formation of adenosine-5'-diphosphate (ADP).

$$D-Glucose+ATP \xrightarrow{HK} G-6-P+ADP \tag{2.1}$$

G-6-P is oxidized by nicotinamide-adenine dinucleotide phosphate (NADP) to D-gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH), in the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH). The amount of NADPH formed in the below reaction is stoichiometric to the amount of D-glucose. The increase in NADPH was measured by means of its light absorbance at 340 nm.

$$G-6-P+NADP^{+} \xrightarrow{G6P-DH} D-gluconate-6-phosphate+NADPH+H^{+} \tag{2.2}$$

Solution I of the kit was prepared by solving powder consisting of triethanolamine buffer, pH 7.6, NADP, ATP, magnesium sulfate in distilled water. Solution II of the kit, consisting of hexokinase and glucose-6-phosphate dehydrogenase was used as undiluted.

1.000 ml of solution I, 0.100 ml of sample solution and 1.900 ml of distilled water were pipetted into a cuvette for each sample and mixed. In addition, 1.000 ml of solution I and 2.000 ml of distilled water were pipetted into a cuvette to be used as a blank solution

and mixed. They were waited 3 minutes at room temperature, and then absorbances of the solutions were read and noted (A_1). The reaction was started with the addition of 0.020 ml of solution II to each cuvette. It was waited 15 minutes for the completion of the reaction and then absorbances of the solutions were read and noted again (A_2).

The absorbance difference for each cuvette was determined ($A_1 - A_2$), and the absorbance difference of the blank was subtracted from the absorbance difference of each sample.

$$\Delta A = (A_1 - A_2)_{sample} - (A_1 - A_2)_{blank} \tag{2.3}$$

The concentration was calculated via the following equation as described in the procedure of the kit.

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \left[g / l \right] \tag{2.4}$$

where V was the final volume [ml], v was the sample volume [ml], *MW* was the molecular weight of the substance to be assayed [g/mol], d was the light path [cm], and ε was the extinction coefficient of NADH at 340 nm, which was equal to 6.3 l/mmol.cm.

Then the equation is,

$$c = \frac{3.020\, ml \times 180.16\, g/ml}{6.3\, l/mmol.cm \times 1.00\, cm \times 0.100\, ml \times 1000} \times \Delta A = \frac{0.5441}{6.3} \left[g\ glucose / l\ solution \right] \tag{2.5}$$

2.2.4.2. Ethanol Concentration Measurement Ethanol is oxidized to acetaldehyde by nicotinamide-adenine dinucleotide (NAD) in the presence of the enzyme alcohol dehydrogenase (ADH).

$$Ethanol + NAD^+ \xleftrightarrow{ADH} acetaldehyde + NADH + H^+ \tag{2.6}$$

The equilibrium of this reaction lies on the side of ethanol and NAD. It can be completely displaced to the right side at alkaline conditions and by trapping of the acetaldehyde formed. Acetaldehyde is quantitatively oxidized to acetic acid in the presence of aldehyde dehydrogenase (Al-DH). NADH was determined by means of its light absorbance at 340 nm.

$$Acetaldehyde + NAD + + H_2O \xrightarrow{Al-DH} acetic\ acid + NADH + H^+ \quad (2.7)$$

Solution I of the kit, consisting of potassium diphosphate buffer, pH 9.0, was used as undiluted. One 4 mg tablet containing NAD and aldehyde dehydrogenase was dissolved with three ml solution I in a beaker for each assay depending on the number of determinations. Forceps were used for taking the tablets out of the bottle. Hereby reaction mixture was prepared. Solution III of the kit, consisting of ADH was also used as undiluted.

3.000 ml of reaction mixture and 0.100 ml of sample solution were pipetted into a cuvette for each sample and mixed. In addition, 3.000 ml of reaction mixture and 0.100 ml of distilled water were pipetted into a cuvette to be used as a blank solution and mixed. They were waited 3 minutes at room temperature, and then absorbances of the solutions were read and noted (A_1). The reaction was started with the addition of 0.050 ml of solution III to each cuvette. It was waited 10 minutes for the completion of the reaction and then absorbances of the solutions were read and noted again (A_2).

The absorbance differences for each cuvette were determined ($A_1 - A_2$), and the absorbance difference of the blank was subtracted from the absorbance difference of each sample.

$$\Delta A = (A_1 - A_2)_{sample} - (A_1 - A_2)_{blank} \quad (2.8)$$

The concentration was calculated via the following equation as described in the procedure of the kit.

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 2 \times 1000} \times \Delta A \left[g / l \right] \tag{2.9}$$

where V is the final volume [ml], v is the sample volume [ml], MW is the molecular weight of the substance to be assayed [g/mol], d is the light path [cm], and ε is the extinction coefficient of NADH at 340 nm, which is equal to 6.3 l/mmol.cm. Then the equation is,

$$c = \frac{3.020\, ml \times 46.07\, g/ml}{6.3\, l/mmol.cm \times 1.00\, cm \times 0.100\, ml \times 1000} \times \Delta A = \frac{0.7256}{6.3} \left[g\ ethanol/l\ solution \right] \tag{2.10}$$

2.2.4.3. Glycerol Concentration Measurement Glycerol is phosphorylated by adenosine-5'-triphosphate (ATP) to L-glycerol-3-phosphate in the reaction catalyzed by glycerokinase (GK).

$$Glycerol + ATP \xrightarrow{GK} L-glycerol-3-phosphate + ADP \tag{2.11}$$

The adenosine-5'-diphosphate (ADP) formed in the above reaction is reconverted into ATP by phosphoenolpyruvate (PEP) with the aid of pyruvate kinase (PK) with the formation of pyruvate.

$$ADP + PEP \xrightarrow{PK} ATP + pyruvate \tag{2.12}$$

Pyruvate is reduced to L-lactate by reduced nicotinamide-addenine dinucleotide (NADH) with the oxidation of NADH to NAD, in the presence of the enzyme L-lactate dehydrogenase (L-LDH).

$$Pyruvate + NADH + H^{+} \xrightarrow{L-LDH} L-lactate + NAD^{+} \tag{2.13}$$

The amount of NADH oxidized in the above reaction is stoichiometric to the amount of glycerol. NADH was determined by means of its light absorption at 340 nm. Solution I

of the kit was prepared by solving powder consisting of glycylglycine buffer, pH 7.4, NADH, ATP, PEP-CHA, magnesium sulfate in eleven ml of distilled water, and the solution was allowed to stand for 10 minutes at room temperature. Solution II of the kit, consisting of pyruvate kinase and L-lactate dehydrogenase, was used as undiluted. Solution III of the kit, consisting of glycerokinase suspension, was also used as undiluted.

1.000 ml of solution I, 0.100 ml of sample solution, 1.900 ml of distilled water and 0.010 ml of solution II were pipetted into a cuvette for each sample and mixed. In addition, 1.000 ml of solution I, 2.000 ml of distilled water and 0.010 ml of solution II were pipetted into a cuvette to be used as a blank solution and mixed. They were kept 7 minutes at room temperature, and then absorbances of the solutions were recorded (A_1). The reaction was started with the addition of 0.010 ml of solution III to each cuvette. 10 minutes were allowed for the completion of the reaction and then absorbances of the solutions were recoreded again (A_2).

The absorbance difference for each cuvette was determined ($A_1 - A_2$), and the absorbance difference of the blank was subtracted from the absorbance difference of each sample.

$$\Delta A = (A_1 - A_2)_{sample} - (A_1 - A_2)_{blank} \tag{2.14}$$

The concentration was calculated via the following equation as described in the procedure of the kit.

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \left[g / l \right] \tag{2.15}$$

where V was the final volume [ml], v was the sample volume [ml], MW was the molecular weight of the substance to be assayed [g/mol], d was the light path [cm], and ε was the extinction coefficient of NADH at 340 nm, which was equal to 6.3 l/mmol.cm.

Then the equation is,

$$c = \frac{3.020\ ml \times 92.1\ g/ml}{6.3\ l/mmol.cm \times 1.00\ cm \times 0.100\ ml \times 1000} \times \Delta A = \frac{2.781}{6.3} [g\ glycerol/l\ solution] \quad (2.16)$$

2.2.5. Biomass Measurement

Filter papers were placed into clean petri dishes of 50 mm diameter. They were dried twice in microwave oven (Arçelik MD553, Turkey) for 20 minutes at 350 watt. They were kept in the desicator overnight to cool petridishes to the ambient temperature and to dehumidify thoroughly. After measuring the weight of petri dishes using Sartorious balance AC 211S (Germany), cells which were previously washed and dissolved in distilled water were pipetted onto the filter paper in the petri dishes. They were dried in the microwave, dehumidified thoroughly in the desicator and weighed with the balance. The differences in two weight measurements were recorded as the biomass of the collected 1ml samples.

2.2.6. Determination of Growth Rate

Growth rate is the amount of biomass that that the cells has gained within a certain period. In this study, in order to calculate growth rate at time '*t*' was calculated with the biomass amounts at time '*t*' and one hour before the time '*t*' as in the following equation.

$$Growth\ Rate\ (t) = \frac{\frac{Biomass\ (t) - Biomass\ (t - 1\ hr)}{Biomass\ (t)}}{1\ hr} \quad (2.17)$$

2.2.7. Determination of Maximum Growth Rate

The Michaelis-Menten equation for the single limiting subsrate enzyme kinetics in the absence of substrate or product inhibition is given as follows.

$$r_x = x_v \frac{\mu_m \times S}{k_s + S} \tag{2.18}$$

where x_v represents viable cells (kg cell.m^{-3}), μ_m is maximum specific growth rate (hr^{-1}), S is the limiting substrate concentration (kg.m^{-3}), k_s represents the saturation constant (kg.m^{-3}) and r_x is the rate of reaction (kg S uptake.L^{-1}.hr^{-1}).

The rate of reaction equation can also be expressed in terms of growth rate, μ.

$$\frac{dx_v}{dt} = r_x = x_v \frac{\mu_m \times S}{k_s + S} = \mu \times x_v \tag{2.19}$$

Integrating both sides of the equation yields,

$$\ln(x_v) = \mu \times t \tag{2.20}$$

At the exponential phase of the batch processes, the plot for natural logarithm of the cells versus time has a constant slope and it gives the maximum growth rate.

2.2.8. Flux Balance Analysis

Flux balance analysis (FBA) is based on the fundamental physicochemical constraints on metabolic networks, and fundamentally it is based on the conservation of mass. In order to yield the dynamic mass balance equation in the network, a flux balance can be written for each metabolite (X_i) within the metabolic network.

$$\frac{dX_i}{dt} = V_{syn} - V_{\deg} - V_{use} \pm V_{trans} \tag{2.21}$$

where V is flux, the subscripts '*syn*' and '*deg*' refer to the synthesis and degradation reactions of the metabolite, the subscripts '*use*' corresponds to the requirements growth

and maintenance related to the metabolite, and the subscripts '*trans*' refers to exchange of the metabolite through the system boundary. This equation can be rearranged as follows.

$$\frac{dX_i}{dt} = V_{syn} - V_{\deg} - V_{use} - b_i \tag{2.22}$$

where b_i is the net transport of the external metabolite X_i into the system. Afterwards, all material balances can be written in matrix form as follows.

$$\frac{dX}{dt} = S \bullet v - b \tag{2.23}$$

where the vector X represents the quantity of the metabolites in the cell, S is the stoichiometric matrix, v refers to a vector including metabolic fluxes and b is the vector which represents metabolic demands. The time constants of cell growth and process dynamics are typically very slow compared to the time constants characterizing metabolic transients; thus, the transient mass balances can be simplified as in the following equation. This equation considers only the steady-state behaviour.

$$S \bullet v = b \bullet I \tag{2.24}$$

where I is the identity matrix. This equation states a presence of a balance among all the formation, degradation, utilization, and transport fluxes in the cell; otherwise, there will be an accumulation of metabolites inside the cell. This equation defines the mass constraints on the system. Additional constraints, such as the stoichiometric constraints and the capacity constraints on the exchange fluxes, should also defined on the network. Those constraints, which represent the maximum and minimum allowable flux through the corresponding reaction, should also be placed on the value of the fluxes through each of the reactions.

$$\alpha_i \le v_i \le \beta_i \tag{2.25}$$

In this study, FBA was used to obtain the flux distributions in the cell. A stoichiometric matrix consists of 822 metabolites participating in 1172 irreversible reactions was used (Forster *et al.*, 2003). Metabolites and reactions described in FBA are given in Appendix (Tables A.1 and A.2, respectively). FBA predicts successfully for steady-state system; therefore, batch cultivations were considered as a series of chemostat cultivations, and the change of metabolites were calculated with respect to the initial point.

In FBA, maximization of growth, oxygen uptake and ethanol production was chosen as the objective function. Experimentally measured growth rate, biomass and metabolite concentrations given were converted into flux measurements, and those flux measurements were used in prediction of ethanol production using FBA. Alternate optima were found (Mahadevan *et al.*, 2003) and similar fluxes were eliminated.

Metabolite concentrations were normalized by biomass measurements and multiplied with growth rate to find flux measurements in the unit of g/(gDW.hr). Using molecular weight fluxes were converted in the unit of mmole/(gDW.hr).

Since only a number of metabolites, such as glucose, ethanol, glycerol, were measured experimentally, linear optimization was used in FBA analysis to obtain fluxes in the cell. Linear optimization was done with the help of MATLAB Optimization – TOMLAB 7.1 toolbox. The code of the FBA programme is given in Appendix.

2.2.9. Topological Study of Protein-Protein Interactions

Topology of TOR signalling proteins that were in the literature was studied by investigating protein-protein interactions. Protein-protein interactions among the proteins were obtained from BioGRID database using Osprey 1.2.0. The degree distribution, betweenness centrality and clustering, which gave global information about the network, were obtained by using Networkx Module programming in Python v2.5. Bron-Kerbosch algorithm (Bron *et al.*, 1973) was used as the module enumeration algorithm, which identifies modules with Q=1, where Q is the modularity and described as,

$$Q = \frac{2l}{n(n-1)} \tag{2.26}$$

where l is the number of interactions between n nodes. In this manner, functional modules are regarded as highly condensed subgraphs having maximum number of interactions within its members, *i.e.* $Q = 1$.

Protein–protein interaction maps are complex networks which are defined as sets of N nodes (the proteins, P_i, i =1, . . ., N) linked together with interactions represented by l. The number of links of a node is defined as the degree (k_i) of a node and $<k>$ was the average degree. The number of neighbouring of l_i links between nodes divided by the total number allowed by its degree, k_i (k_i –1) is clustering coefficient (C_i) which shows the interconnection of the neighbours. The clustering coefficient of the whole network is,

$$< C > = \frac{1}{N} \sum_{i=1}^{N} \frac{2l_i}{k_i(k_i - 1)} \tag{2.27}$$

The number of short paths connecting each pair of nodes that contain the node P_i is indicated as betweenness centrality (b_i) for a node P_i. It is defined as,

$$b_i = \sum_{m \neq n} \frac{\Gamma(m, i, n)}{\Gamma(m, n)} \tag{2.28}$$

where $\Gamma(m, i, n)$ is the number of the shortest paths between proteins P_m and P_n, passing through P_i, whereas $\Gamma(m, n)$ is the total number of paths between those two proteins. The ratio $\Gamma(m, i, n) / \Gamma(m, n)$ shows the significance of the P_i role in connecting P_m and P_n.

In order to compare with the TOR signalling network and analyze if the network exhibits scale-free distribution, a random network was constructed by assigning the same number of interactions among the same number of nodes as in the TOR signalling network thousand times at random. Real networks also exhibit scale-free (SF) distributions of links,

where the cumulative frequency of nodes with degree k, $n(k)$, decays according to a power-law distribution,

$$n(k) = A.k^{-\gamma+1} \tag{2.29}$$

where A is a constant and $2<\gamma<3$ for a scale-free property. SF networks also exhibit power–law correlations in clustering and betweenness vs. degree plots (Rodriguez-Caso *et al.*, 2005).

2.2.10. Self-Organizing Maps

Self-organizing maps (SOM) methodology has been used in biological applications due to its various features that make it particularly well suited to clustering and analysis of biological patterns (Tamayo *et al.*, 1999). In this study it was used to cluster fluxes in the cell that were obtained in the absence or presence of caffeine. An SOM has a set of nodes with a simple topology and a distance of the the nodes are described as the function $d(N_1,N_2)$. Those nodes are iteratively mapped into k-dimensional space, where $f_i(N)$ shows the position of node N at iteration. The initial mapping f_0 is calculated as random. Subsequently, a data point, P, is selected and the nearest node, N_P is identified on the map. Then points are moved by the following formula toward P to adjust the mapping of nodes.

$$f_{i+1}(N) = f_i(N) + \tau(d(N, N_P), i)(P - f_i(N)) \tag{2.30}$$

As the distance of node N from N_P or iteration number, i, increases the learning rate, τ, decreases. At each iteration, the point, P, used is determined by random ordering of a certain number of data points generated once and recycled as needed. In this study, GeneCluster 2.0 was used to cluster fluxes in the cell that were obtained in the absence or presence of caffeine.

3. RESULTS

Batch cultivations were carried out in the absence and presence of caffeine or rapamycin in order to study the impact of those chemicals on yeast cells. *Saccharomyces cerevisiae* wild type BY4743 strains were grown in both F1 and YPD medium in flasks and fermentors and samples were collected to investigate the effects of the chemicals on the cells.

3.1. Effect of the Caffeine and Rapamycin on the Growth Characteristics of Yeast Cells in F1 Medium

Three preliminary experiments were carried out to observe growth characteristics of yeast cells in the presence or absence of caffeine and rapamycin in F1 medium. *S. cerevisiae* wild type BY4743 strains were grown in F1 medium in flasks that were shaken at 180 rpm at 30^{o}C. The growth of the yeast cells were followed spectrophotometrically at 600 nm. In all experiments, yeast cells were treated with caffeine at the early exponential phase, when the OD_{600} values reached 0.2-0.4.

In the first experiment, yeast cells were they were treated with caffeine at various concentrations (1, 3, 5, 8, and 12 mM as the final concentration) or rapamycin (200 ng/ml as the final concentration). Growth curves are shown in Figure 3.1 and arrow indicates time of the treatment. Cells were treated at the second hour and the experiment lasted 11 hours. Yeast cells treated with the lowest amount of caffeine showed similar growth behaviour with cells grown in the control flask, but had a higher optical density between 9th and 11th hours. The yeast cells grown in the presence of 3 mM caffeine had a lower optical density than the cells grown in the control flask. The growth curve of the caffeine treated cells had higher values than the growth curve of the cells grown in the control flask and had a higher optical density within the last 2 hour period. The growth behaviour of the cells grown in the presence of 200 ng/ml rapamycin was similar to that of the cells grown in the presence of 5 mM caffeine between the second and the sixth hours. Afterwards, it intersected with the growth curve of the cells grown in the presence of 8 mM caffeine.

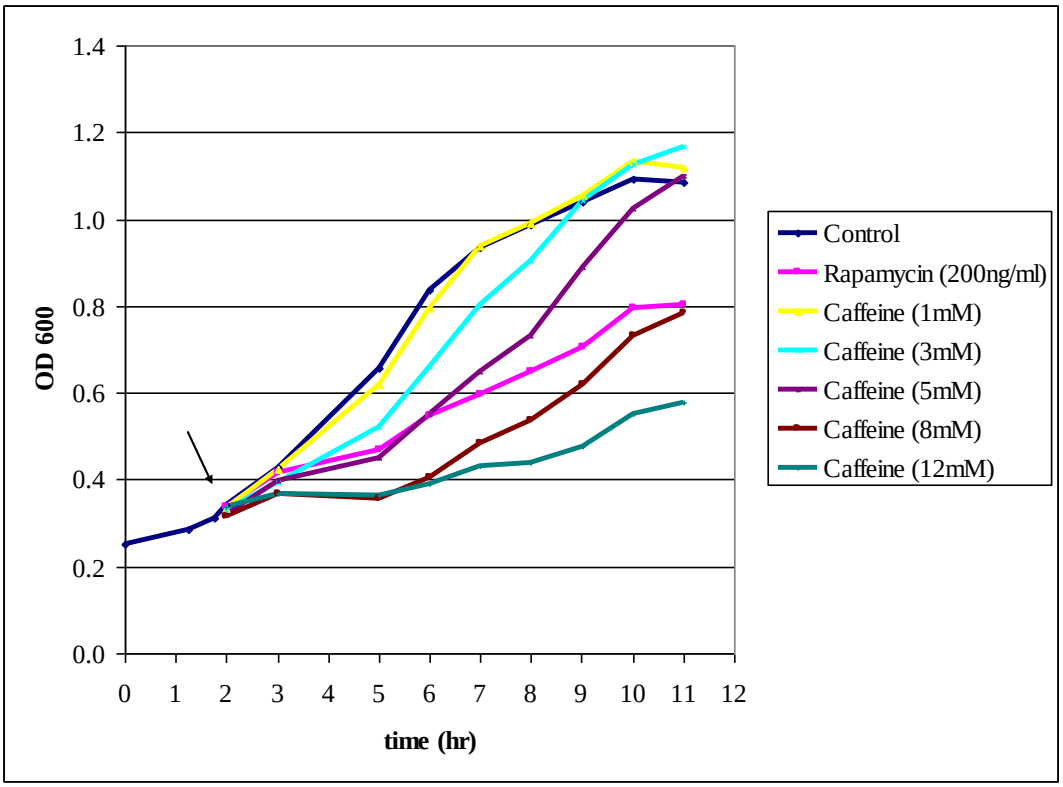

Figure 3.1. Growth curve of yeast cells under chemical treatment for 9 hours in F1 medium

In order to investigate the effect of these chemicals on the growth of cells for a longer period of time, the yeast cells were grown in different flasks and, they were treated with caffeine at various concentrations (4, 6, 8, 10, 12 and 14 mM as the final concentration) or rapamycin (200 ng/ml as the final concentration). Growth of the cells in the presence of chemicals was followed for 27 hours. Arrow indicates time of the treatment. The growth curve of the cells grown in the presence of 200 ng/ml rapamycin reached higher values than the growth curve of the cells grown in the presence of 4 mM caffeine at the seventh hour, of the cells in the presence of 6 mM at the eighth hour and of the cells in the presence of 8 mM at the twelfth hour. Afterwards, it intersected with the growth curve of the cells grown in the presence of 10 mM caffeine. 200 ng/ml rapamycin and 10 mM caffeine had similar repression effect on the growth of yeast cells twenty hours after the treatment; however 12 and 14 mM caffeine had more impact on the cells compared to 200 ng/ml rapamycin. Optical density of the cells grown in the presence of 4, 6 and 8 mM caffeine had a higher value than the optical density of the cells in the control flask after the 15^{th}, 17^{th} and 22^{nd} hour, respectively. Growth curves are given in Figure 3.2.

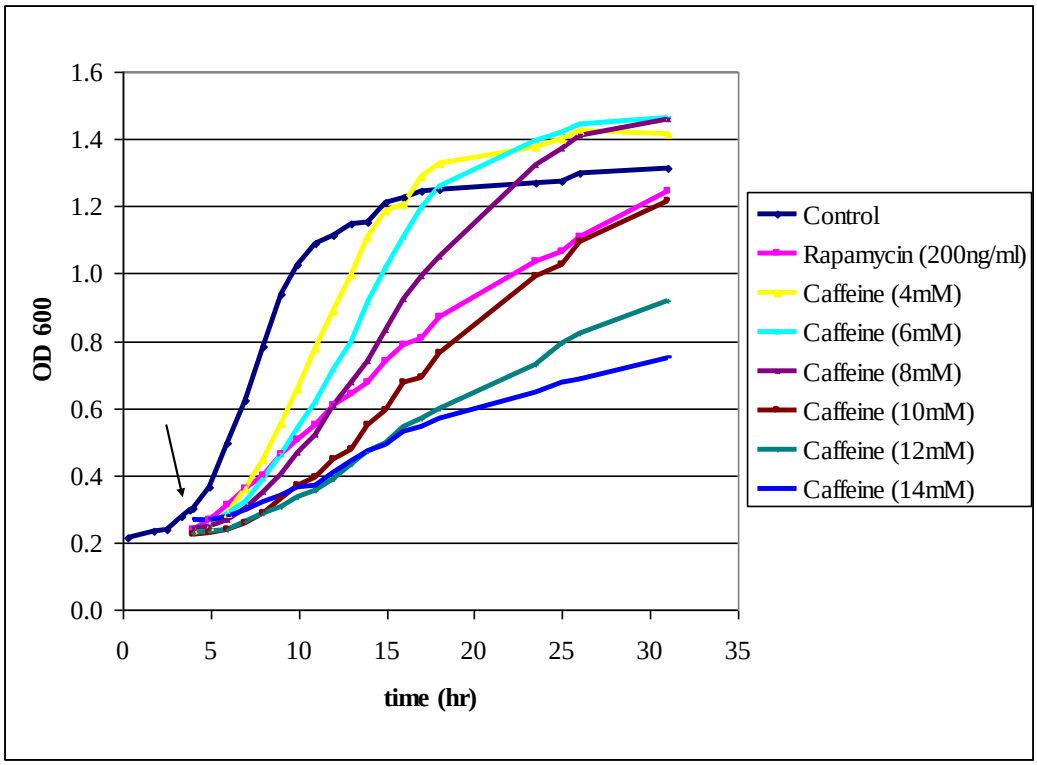

Figure 3.2. Growth curve of yeast cells under chemical treatment in F1 medium for 27 hours

In the third experiment, yeast cells were treated with caffeine at various concentrations (6, 8, 10, 12 and 14 mM as the final concentration) or rapamycin (200 ng/ml as the final concentration). Growth curves are shown in Figure 3.3 and arrow indicates time of the treatment. The cells were treated at the ninth hour and the experiment lasted 169 hours. The growth curve of the cells grown in the presence of 200 ng/ml rapamycin reached higher values than the growth curve of the cells grown in the presence of 8 mM caffeine 3.5 hours after the treatment and the cells displayed very similar growth characteristics with the cells grown in the presence of 10 mM caffeine within a period between 20^{th} and 40^{th} hours. Growth curve of the rapamycin treated cells reached higher values than the growth curve of the cells in control flask at the 45^{th} hour and after the 80^{th} hour optical density of the cells grown in the presence of rapamycin was very close to the optical density of the cells grown in the presence of 6mM and 8mM caffeine. Yeast cells grown in the presence of lower concentrations of caffeine, 6, 8 and 10 mM, had a higher optical density than the cells grown in the control flask. Yeast cells grown in the presence

of higher concentrations of caffeine, 12 and 14 mM, never had an optical density value as high as the cells grown in the control flask.

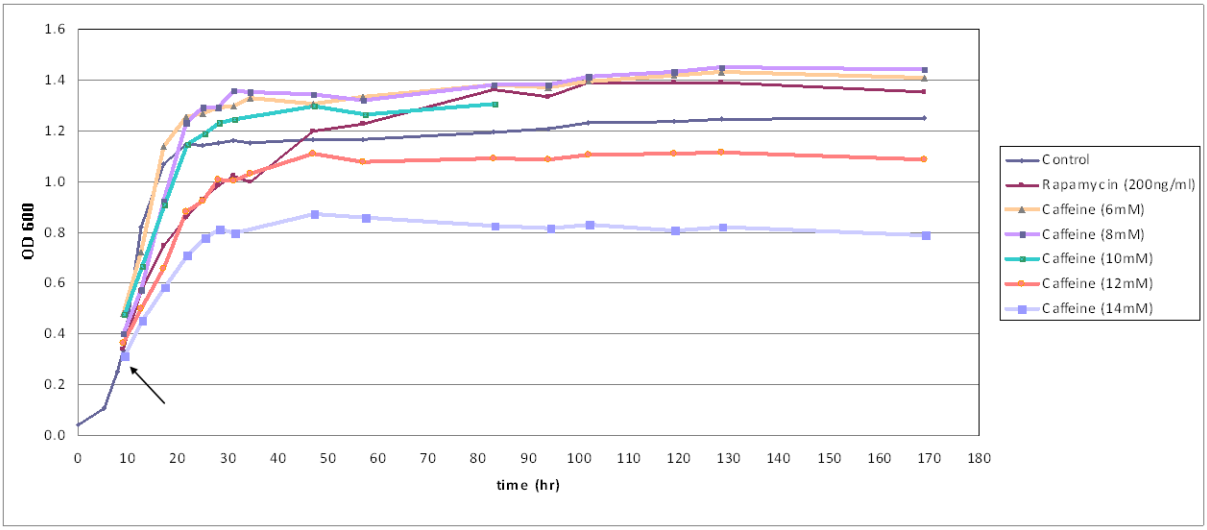

Figure 3.3. Growth curve of yeast cells under chemical treatment for 160 hours in F1 medium

3.2. Effect of Caffeine and Rapamycin on Growth Characteristics of Yeast Cells in YPD Medium

Two preliminary experiments were carried out to observe the effect of caffeine and rapamycin on the growth characteristics of yeast cells in YPD medium. *S. cerevisiae* wild type BY4743 strains were grown in YPD medium in flasks, which were shaken at 180 rpm and the temperature were kept constant at 30^{o}C. Samples were taken periodically to follow the growth of the yeast cells spectrophotometrically at 600 nm. Arrows indicate time of the treatment.

In the first experiment, yeast cells were grown to optical density of 1.8 in different flasks, and they were treated with caffeine at various concentrations (4, 8, 10, 12, 14, 18 mM as the final concentration) or rapamycin (200 ng/ml as the final concentration). Growth curves are given in Figure 3.4. Chemical treatment was done at 10th hour and optical density of yeast cells grown in the control flask or treated with low amounts of caffeine (4mM as the final concentration) or rapamycin was measured higher than 2.1 at the end of 70 hours. Yeast cells treated with higher amount of caffeine had an optical density lower than 2.1.

These results indicated that rapamycin (200ng/ml) has almost no effect on the growth of yeast cells in YPD. However caffeine has an inhibitory effect and this effect on the growth becomes more pronounced with the increasing caffeine concentrations under these experimental conditions.

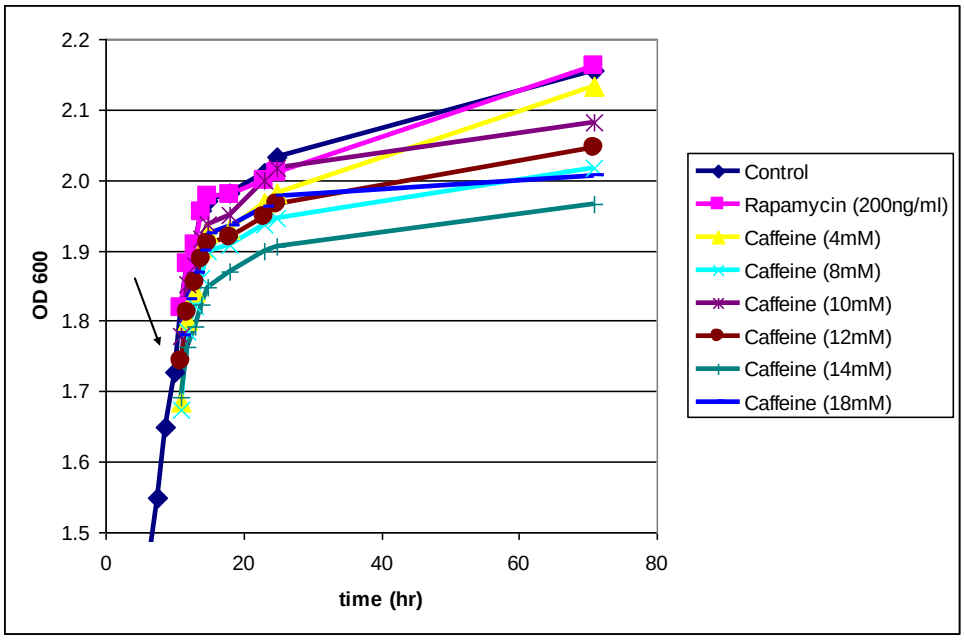

Figure 3.4. Growth curves of yeast cells in the presence of rapamycin and caffeine in YPD

In the second experiment, yeast cells were treated with chemicals when the optical density values reached to 1.8. 35 hours after the treatment, caffeine effect on yeast cells could be seen easily. Although yeast cells in the control flask had an optical density higher than 2.0, optical density of caffeine treated cells were below 2.0. In addition, 50 hours after the treatment, although yeast cells in the control flask had an optical density higher than 2.1, the optical density of caffeine treated cells were still below 2.0. Moreover, a slight repression effect of rapamycin was observed on the cells grown under 400 ng/ml rapamycin. While an increase of 0.20 in optical density was observed for caffeine treated cells in 35 hours after the treatment, the optical density of cells in the control flask had increased 0.25 within this period. Growth curves of the cells are shown in Figure 3.5.

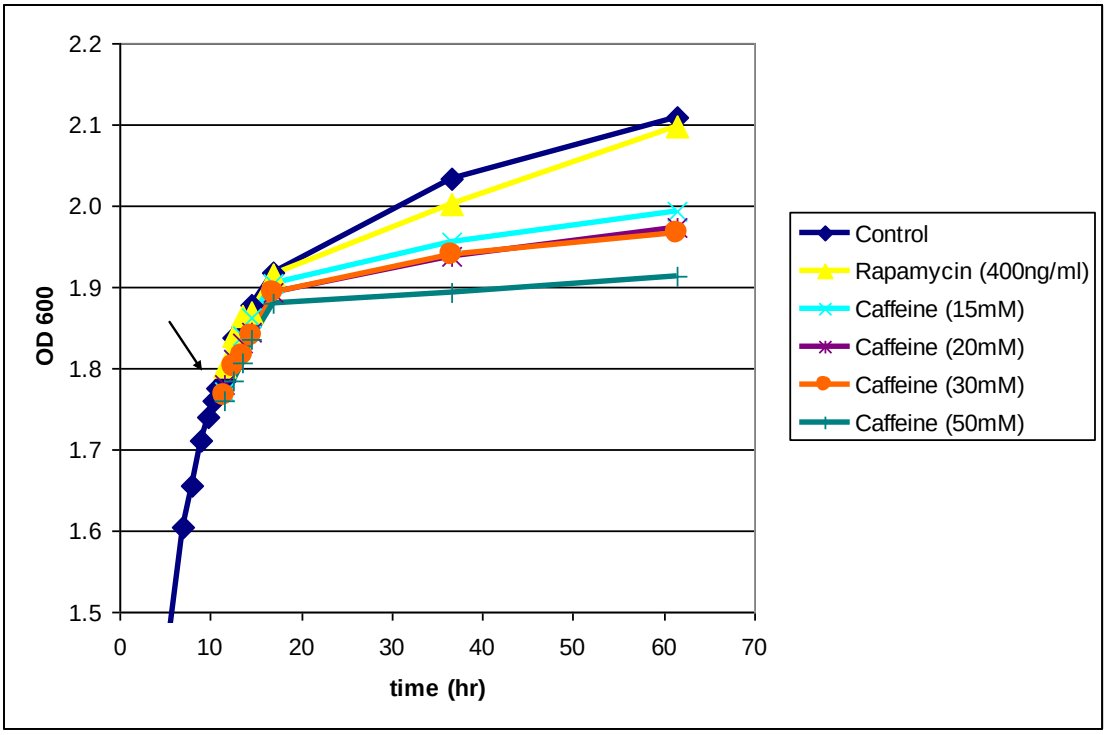

Figure 3.5. Growth curve of yeast cells under rapamycin (400 ng/ml as the final concentration) and caffeine (15-50 mM as the final concentration) treatment in YPD medium (treatment at OD 1.8)

3.3. Controlled Batch Cultivations of Yeast Cells in YPD Medium

In order to investigate the effect of caffeine on the yeast cells, controlled batch cultivations of *Saccharomyces cerevisiae* wild type BY4743 strains were carried out in the presence and absence of caffeine. In this study, growth of yeast cells was followed spectrophotometrically at 600 nm. Biomass was measured and extracellular metabolite profiles of ammonia, ethanol, glucose and glycerol were obtained. Metabolic fluxes in the presence and absence of the caffeine were calculated by using whole genome stoichiometric models and flux balance analysis.

3.3.1. Growth Characteristics of Yeast Cells in the Absence of Caffeine

Controlled batch cultivation of *S. cerevisiae* wild type BY4743 strain was carried out in YPD medium in the absence of caffeine. As described in the "Materials and Methods" section, the temperature of the culture was kept constant at 30^{o}C and the agitation was set

to 800 rpm. Air was given into the cultures with a flow rate of 1l/min and the pH value of the culture was set to 5.5. The growth of the yeast cells were followed spectrophotometrically at 600 nm throughout the whole experiment. Samples for biomass and metabolite measurements were taken at 30 minute intervals when OD_{600} values reached 0.4.

Growth curve of yeast cells are given in Figure 3.6, where the first and the last sampling in the exponential phase and the sampling at the stationary phase are indicated by straight arrows. At the 3rd hour, the first sample was taken when the optical density was about 0.4. Afterwards, samples were taken at 30 minute intervals in the exponential phase and the last sample in this phase was taken 5.5 hours after the first sampling. In addition, one more sample was taken at the stationary phase, at the 28th hour of the experiment.

The plot for natural logarithm of biomass versus time for sampling period shows linearity with a R^2 value of 0.986 within a 6.5 hour period after the second hour. The maximum growth rate, which is the slope in Figure 3.7, was 0.37 hr^{-1}.

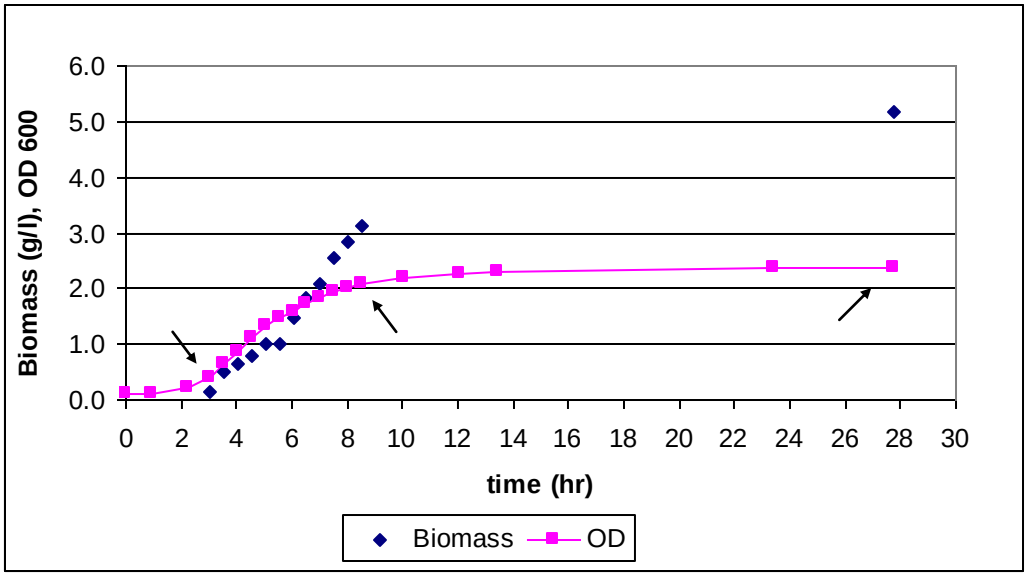

Figure 3.6. Growth curve of *S. cerevisiae* BY4743 in the absence of caffeine

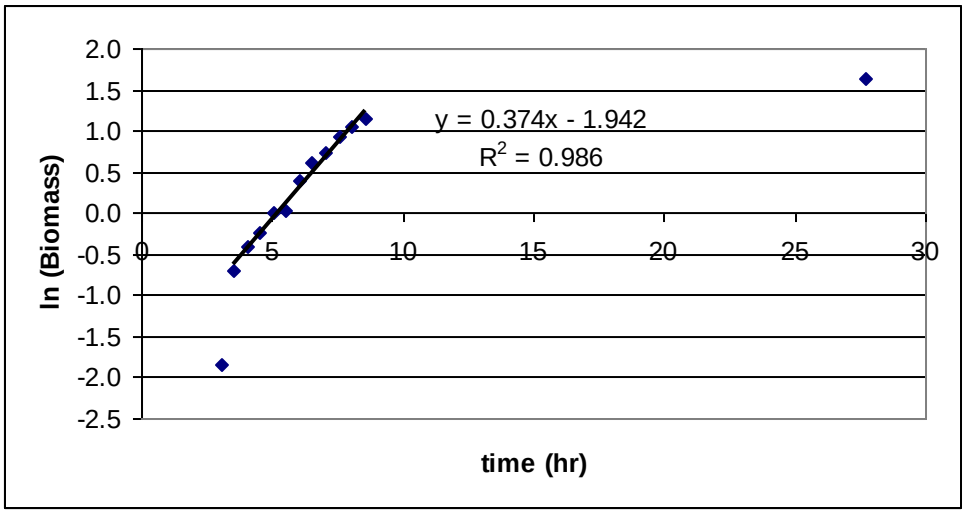

Figure 3.7. Natural logarithmic values of biomass of yeast cells grown in the absence of caffeine

Biomass, growth rate and extracellular metabolite measurements of *S. cerevisiae* wild type BY4743 strain in the absence of caffeine were given in Figures 3.6 and 3.8. The biomass of the first sample was measured as 0.160 g/l. Biomass increased to 1 g/l in 2 hours after the first sampling, and continued to increase along the sampling time. It was measured as 3.125 g/l at the end of exponential phase and finally, it was 5.160 g/l at the stationary phase. Glucose concentration was 20 g/l in the fresh YPD medium and decreased to 19.69 g/l in 3 hours. Then it gradually decreased to 15.20 g/l in 5 hours and at the end of exponential phase it was measured as 10.71 g/l. Glucose concentration was 0.03 g/l at the stationary phase. Ethanol concentration increased to 0.45 g/l in the first three hours and steadily rose up to 5.12 g/l at the end of exponential phase. It was 8.18 g/l at the stationary phase. Glycerol concentration was found as 0.11 g/l in the first sample and it was measured between 0.06 and 0.15 g/l along the exponential phase. Glycerol concentration was 0.03 g/l at the stationary phase.

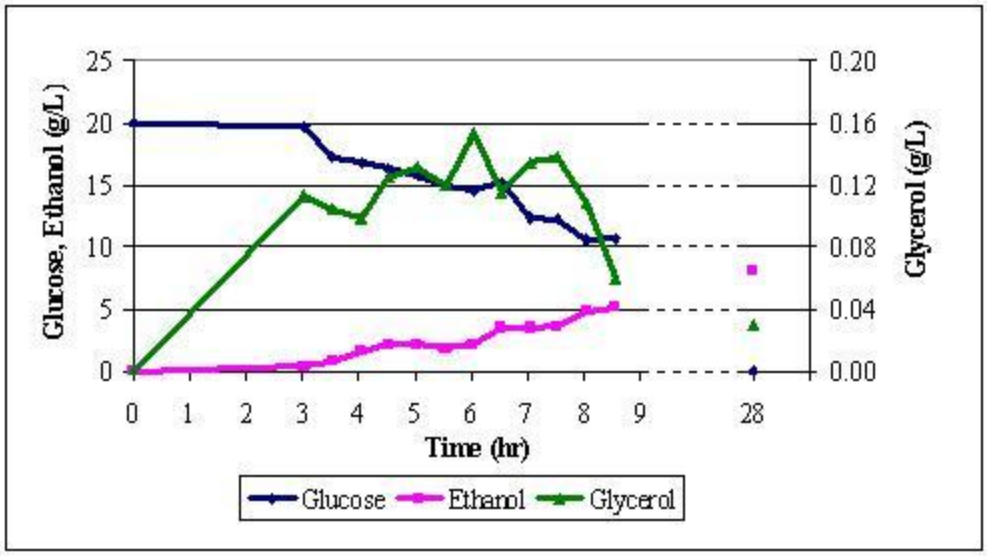

Figure 3.8. Extracellular metabolite profile of *S. cerevisiae* BY4743 in the absence of caffeine

3.3.2. Growth Characteristics of Yeast Cells in the Presence of Caffeine

Controlled batch cultivation of yeast cells treated with caffeine was also carried out in YPD medium. During the experiment, the temperature was kept constant at 30^{o}C and the agitation was set to 800 rpm. Air was supplied to the cultures with a flow rate of 1l/min and the pH value was set to 5.5. The growth of the yeast cells was followed via optical density at 600 nm throughout the whole experiment. Yeast cells were treated with caffeine as the final concentration was 30 mM when the optical density was 1.3. Samples for biomass and metabolite measurements were taken at 30 minute intervals after reaching an optical density close to 0.4.

Growth curve of yeast cells are given in Figure 3.9, where the first and the last sampling in the exponential phase and the sampling at the stationary phase are indicated by straight arrows and time of caffeine treatment is indicated by a dashed arrow. At the 3rd hour, the first sample was taken when the optical density was higher than 0.4. Afterwards, samples were taken at 30 minute intervals for 7 hours in the exponential phase and a sample was taken in the stationary phase, at the 24th hour of the experiment.

The plot for natural logarithm of biomass versus time for sampling period shows linearity with a R^2 value of 0.965 within a 6.5 hour period after the second hour. The maximum growth rate, which is the slope in Figure 3.10, was 0.28 hr^{-1}.

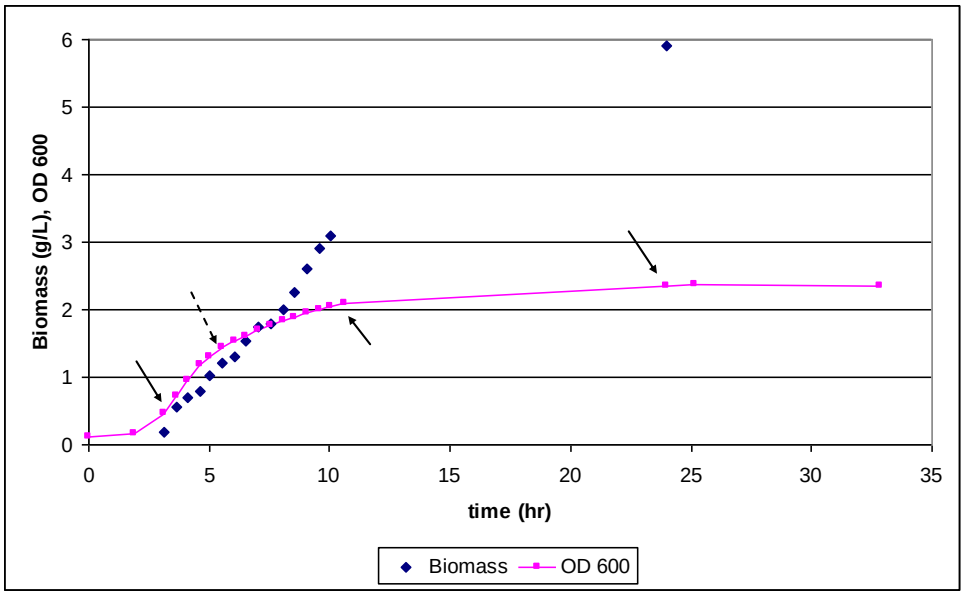

Figure 3.9. Growth curve of *S. cerevisiae* BY4743 in the presence of caffeine

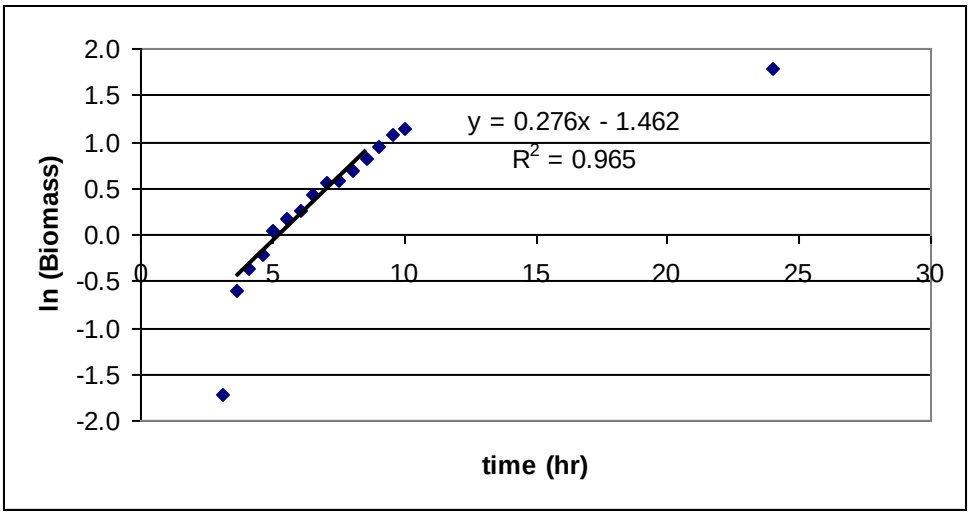

Figure 3.10. Natural logarithmic values of biomass of yeast cells grown in the presence of caffeine

Biomass, growth rate and extracellular metabolite measurements of yeast cells in the presence of caffeine are given in Figures 3.9 and 3.11. Arrows in Figure 3.11 indicate the the time of caffeine treatment. At the 3rd hour of the experiment the biomass was measured as 0.180 g/l. It was 1.033 g/l at the treatment time, and increased to 3.100 g/l along the exponential phase. Biomass was 5.900 g/l at the stationary phase. Glucose concentration was 20 g/l in the fresh YPD medium and decreased to 18.80 g/l in 3 hours. Then it gradually decreased to 14.16g/l until the treatment time and at the end of exponential phase it was measured as 10.60 g/l. Glucose concentration was measured as 0.51 g/l at the stationary phase. Ethanol concentration was measured as 0.46 g/l in the first sample and it was 2.12 g/l at the treatment time. It increased to 3.29 g/l along the exponential phase and measured as 5.67 g/l at the stationary phase. Glycerol concentration was found as 0.12 g/l in the first sample and it was again 0.12 g/l at the treatment time. It measured between 0.05 and 0.12 g/l along the exponential phase. Glycerol concentration was 0.02 g/l at the stationary phase.

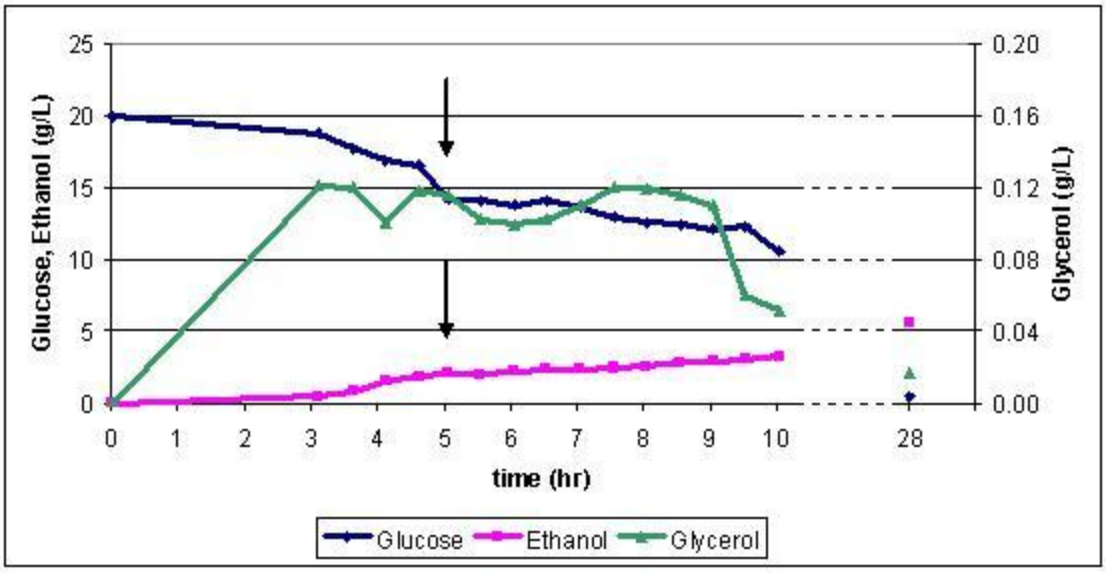

Figure 3.11. Extracellular metabolite profile of *S. cerevisiae* BY4743 in the presence of caffeine

3.3.3. Comparison of Growth Characteristics of Yeast Cells in the Absence and the Presence of Caffeine

Growth curves of the cells in the absence and presence of caffeine were compared in Figure 3.12. In this figure, caffeine treatment is indicated by an arrow. Growth is initially repressed after treatment; however cells reach to the same stationary phase within the same amount of period.

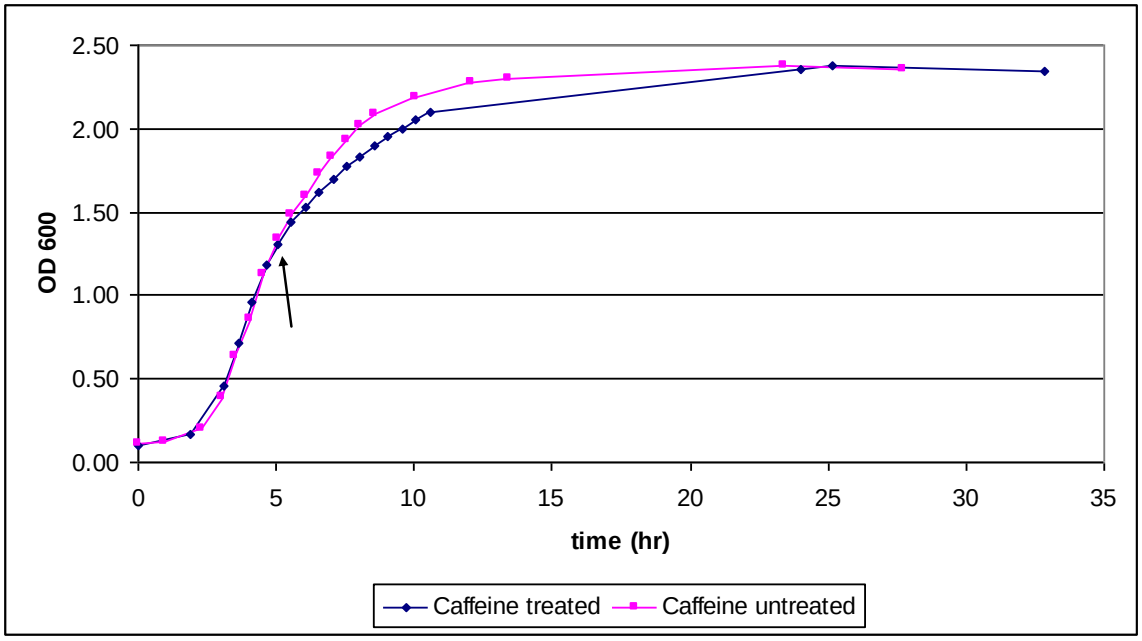

Figure 3.12. Growth curves of yeast cells in the absence and presence of caffeine

The plots of extracellular metabolite and biomass profiles versus optical density values for the cells grown in the absence and presence of caffeine are given in Figures 3.13 and 3.14. Arrows in the figures indicate the time of caffeine treatment. A decrease in ethanol and glycerol production was observed after treatment, while no significant change was observed in glucose. Biomass measurements were very close during the experiment in the untreated and treated cells.

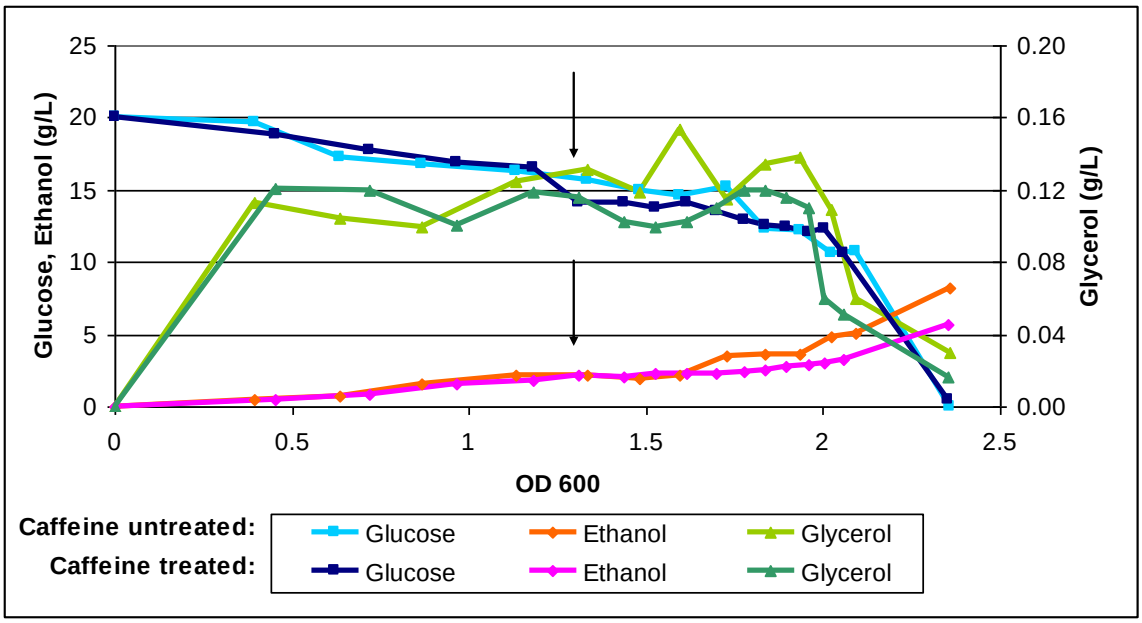

Figure 3.13. Comparison of extracellular metabolite profiles in the absence and presence of caffeine

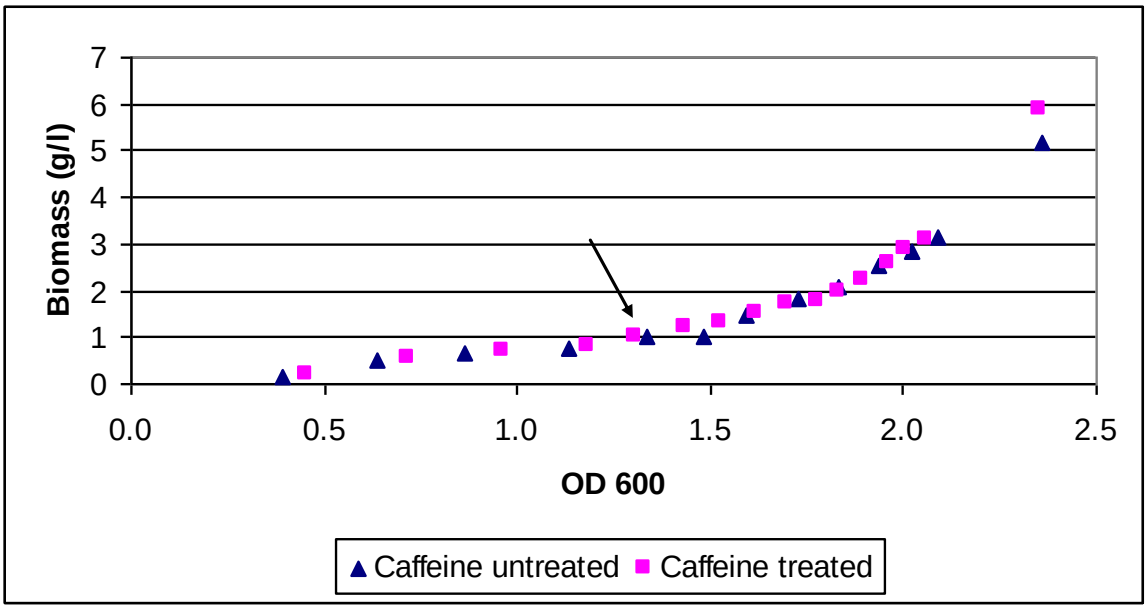

Figure 3.14. Comparison of biomass profiles in the absence and presence of caffeine

3.4. Flux Balance Analysis

Flux balance analysis (FBA) was performed for yeast cells grown in YPD medium in the absence or presence of caffeine. The stoichiometric model consists of 822 metabolites participating in 1172 irreversible reactions (Forster *et al.*, 2003). Objective function was chosen as the maximization of growth, oxygen uptake or ethanol production and the results obtained from FBA were compared with experimental results. FBA predicts the steady-state growth of the cells; it was used for the prediction of biomass or ethanol production within a period when the growth of the cells was linear. This period is indicated by straight arrows and the time of caffeine treatment is indicated by a dashed arrow in Figure 3.15. No biomass production (0 g/l) was predicted when the objective function was chosen as the maximization of oxygen uptake. Comparison of experimental and predicted biomass or ethanol production in the absence or presence of caffeine is given in Tables 3.1, 3.2, 3.3 and 3.4 when the objective function was chosen as the maximization of growth or ethanol production, respectively.

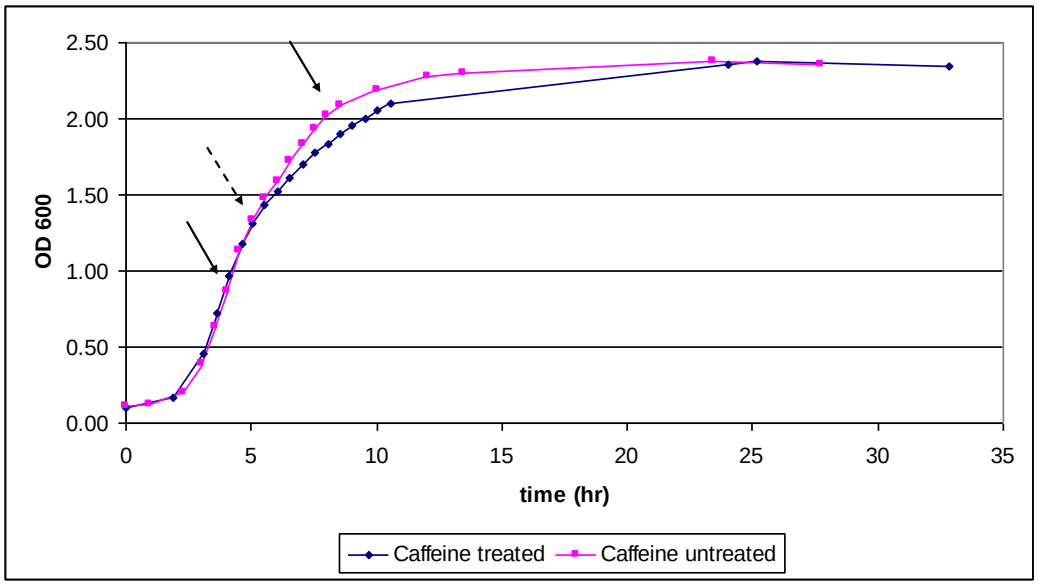

Figure 3.15. The period for which the growth of the cells were predicted by FBA

Table 3.1. Comparison of experimental and predicted biomass production in the absence of caffeine when the maximization of growth was objected

OD_{600}	time (hr)	Experimental biomass production	Prediction of biomass production by FBA	per cent error
1.34	5.0	0.34	-0.11	132.35
1.48	5.5	0.24	0.19	19.25
1.59	6.0	0.32	0.17	47.58
1.73	6.5	0.44	0.02	95.47
1.83	7.0	0.30	0.06	79.68
1.94	7.5	0.28	0.06	78.69
2.02	8.0	0.26	-0.04	115.51
2.09	8.5	0.19	-0.09	148.08

Table 3.2. Comparison of experimental and predicted biomass production in the presence of caffeine when the maximization of growth was objected

OD_{600}	time (hr)	Experimental biomass production	Prediction of biomass production by FBA	per cent error
1.31	5.0	0.32	0.17	37.53
1.44	5.5	0.33	0.35	48.38
1.52	6.0	0.21	0.19	40.56
1.62	6.5	0.22	0.13	27.41
1.7	7.0	0.25	0.17	37.11
1.78	7.5	0.15	0.11	43.59
1.84	8.0	0.13	0.10	45.36
1.90	8.5	0.20	0.12	37.33

Table 3.3. Comparison of experimental and predicted ethanol production in the absence of caffeine when the maximization of ethanol production was objected

OD_{600}	time (hr)	Experimental ethanol production	Prediction of ethanol production by FBA	per cent error
1.34	5.0	16.34	15.61	4.5
1.48	5.5	9.82	12.62	28.53
1.59	6.0	10.21	12.73	24.74
1.73	6.5	18.22	18.45	1.29
1.83	7.0	10.98	11.71	6.62
1.94	7.5	8.69	9.44	8.69
2.02	8.0	9.61	9.37	2.48
2.09	8.5	6.67	6.14	7.98

Table 3.4. Comparison of experimental and predicted ethanol production in the presence of caffeine when the maximization of ethanol production was objected

OD_{600}	time (hr)	Experimental ethanol production	Prediction of ethanol production by FBA	per cent error
1.31	5.0	19.78	14.38	37.53
1.44	5.5	17.92	12.08	48.38
1.52	6.0	10.80	7.68	40.56
1.62	6.5	9.14	7.17	27.41
1.70	7.0	10.12	7.38	37.11
1.78	7.5	6.33	4.41	43.59
1.84	8.0	5.44	3.74	45.36
1.90	8.5	7.34	5.34	37.33

Flux measurements used in FBA are given in Appendix (Table A.3). When FBA was used to predict biomass production, negative biomass values were found or biomass was predicted with relatively high errors. However, FBA predicted ethanol production in the absence of caffeine with less than 30 per cent error at the steady-state growth. It was also used to predict ethanol production in the cultivation treated with caffeine. Ethanol production was predicted with less than 50 percent error at the steady-state growth after the treatment. It was observed that prediction of ethanol production in the presence of caffeine was not as successful as in the absence of caffeine. Predicted and experimental ethanol fluxes are also shown in Figure 3.16 and 3.17.

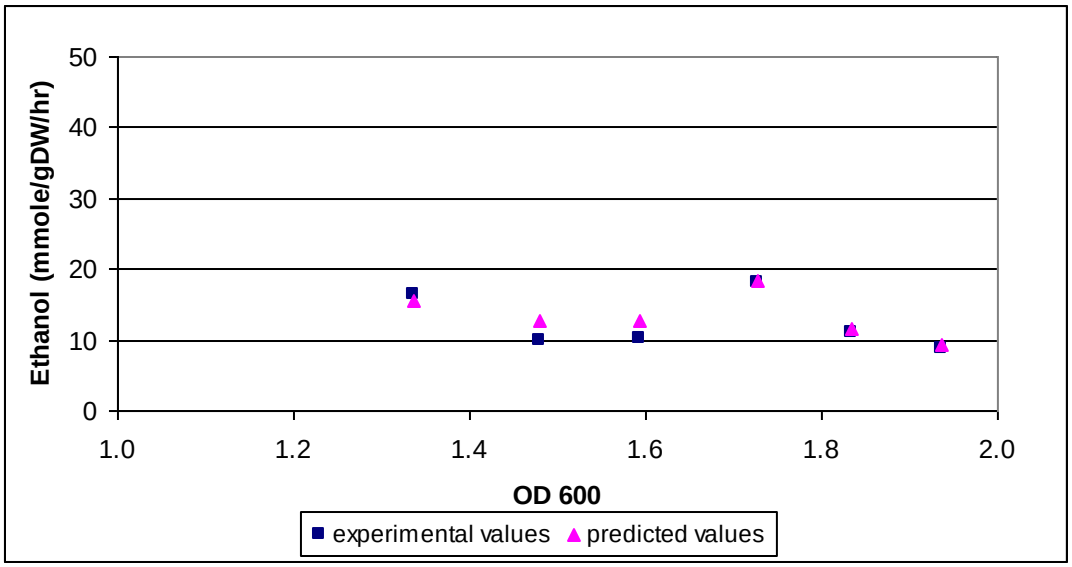

Figure 3.16. Comparison of experimental and predicted ethanol production in the absence of caffeine

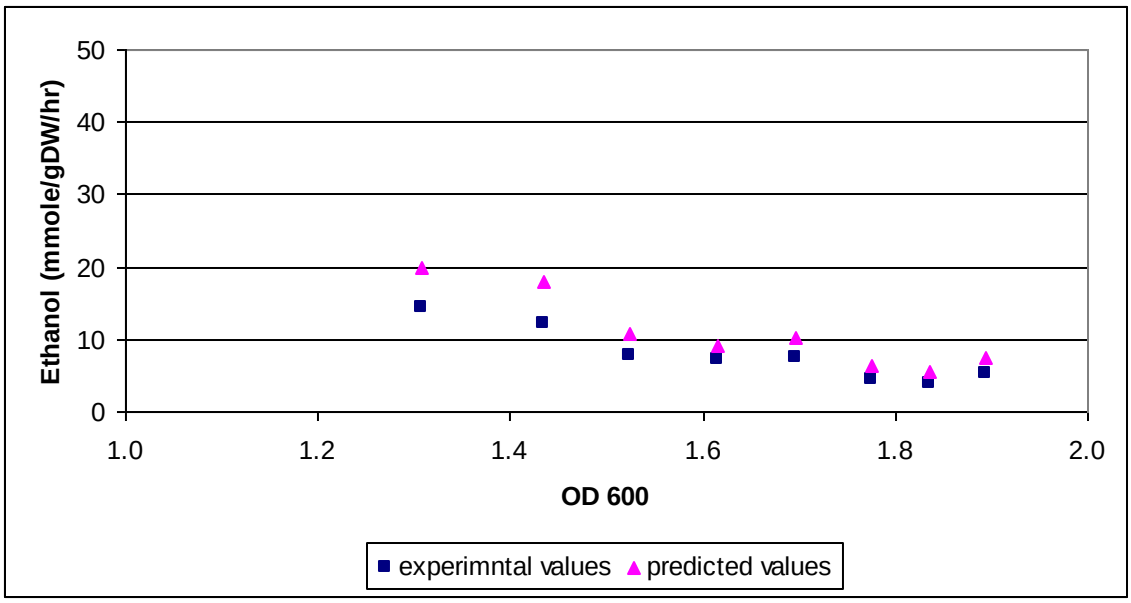

Figure 3.17. Comparison of experimental and predicted ethanol production in caffeine treated cells

3.5. Flux Analysis via Clustering by Self-Organizing Maps

In order to investigate the effect of caffeine on the fluxes, fluxes predicted by FBA were clustered using self-organizing maps (SOM) clustering methodology. GeneCluster 2.0 version 2.1.7 was used for clustering. Since the ethanol production at 1.5 hours after

the treatment was most successfully predicted both in the absence and presence of the caffeine, the fluxes at that time which were predicted by FBA were compared by clustering via SOM. Alternate optima were eliminated before clustering.

Absolute magnitudes of fluxes were normalized and clustered in 4 groups (2*x*2) and the clustering is shown in Figure 3.18. In the figure, cluster numbers are shown as from c0 to c3, and the numbers in boxes indicate fluxes which were clustered together. Dots represent the magnitude of fluxes. In each box, first dots stand for the fluxes of untreated cells and the next dots are of the caffeine treated cells.

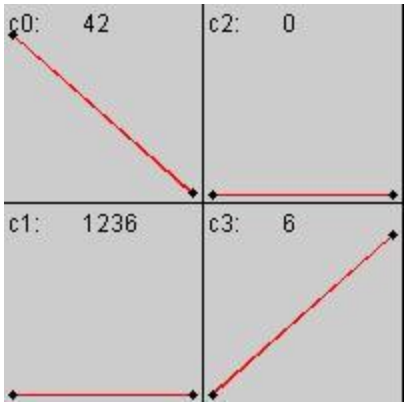

Figure 3.18. Clusters of the fluxes obtained via SOM

A change was observed in totally 48 fluxes after the treatment and they were grouped in clusters c0 and c3. 42 fluxes which were repressed with the caffeine treatment were grouped in the cluster c0, 6 fluxes which were activated under the caffeine treatment were grouped in the cluster c3. No change was observed in 1236 fluxes which were grouped in the cluster c1. Significantly enriched biological process terms of the fluxes were found from Saccharomyces Genome Database. 13 members of the cluster c0 have a role in glucose catabolic process ($p = 1.46x10^{-20}$). 7 of them are significantly annotated to glycolysis ($p = 3.29x10^{-10}$) and 4 of them to gluconeogenesis ($p = 8.30x10^{-4}$). 12 members of the cluster c0 have a role in generation of precursor metabolites and energy ($p = 1.23x10^{-9}$) and 8 members are associated with nucleotide metabolic process ($p = 5.14x10^{-6}$). In addition, 4 members of the cluster c0 were found to be significantly annotated to nitrogen compound metabolic process ($p = 6.20x10^{-4}$) and 3 of them are significantly annotated to proline biosynthetic process ($p = 9.31x10^{-6}$). Moreover, 5 members of the

cluster c3 play a role in glucose metabolic process ($p = 3.73x10^{-9}$) and 4 of them are related to pentose-phosphate shunt ($p = 6.76x10^{-10}$) (Table 3.5).

Table 3.5. Gene ontology terms annotated to the members of the clusters c0 and c3

cluster c0						
GO term	*p* value	members				
glucose catabolic process	1.46E-20	Adh5p	Glk1p	Pgk1p	Tpi1p	Tdh3p
		Pfk1p	Sol4p	Gnd2p	Eno2p	Rpe1p
		Fba1p	Zwf1p	Rki1p		
glycolysis	3.29E-10	Glk1p	Pgk1p	Tpi1p	Tdh3p	Pfk1p
		Eno2p	Fba1p			
generation of precursor metabolites and energy	1.23E-09	Atp1p	Adh5p	Glk1p	Pgk1p	Tpi1p
		Hor2p	Tdh3p	Pfk1p	Eno2p	Fba1p
		Mdh1p	Aac1p			
nucleotide metabolic process	5.14E-06	Atp1p	Adh5p	Sol4p	Gnd2p	Rpe1p
		Zwf1p	Gpd2p	Rki1p		
proline biosynthetic process	9.31E-06	Pro1p	Pro2p	Pro3p		
glutamine family amino acid biosynthetic process	6.20E-04	Pro1p	Pro3p	Put1p	Pro2p	
gluconeogenesis	8.30E-04	Pgk1p	Tdh3p	Eno2p	Fba1p	
cluster c3						
GO term	*p* value	members				
pentose-phosphate shunt	6.76E-10	Tkl2p	Tkl1p	Tal1p	Pgi1p	
glucose metabolic process	3.73E-09	Pgi1p	Mdh2p	Tkl2p	Tkl1p	Tal1p

Members of clusters c0 and c3 and the all fluxes described by Forster *et al.* (Forster *et al.*, 2003) are tabulated in the Appendix.

3.6. Topological study of TOR signalling proteins

The protein interaction network of TOR signalling proteins was constructed with the protein-protein interactions among proteins implicated in TOR signalling. Protein-protein interactions among 194 proteins which were reported to be related to TOR pathway in literature (Table B.1) were obtained using Osprey 1.2.0 (Figure 3.19). The topological properties of this protein interaction network were investigated.

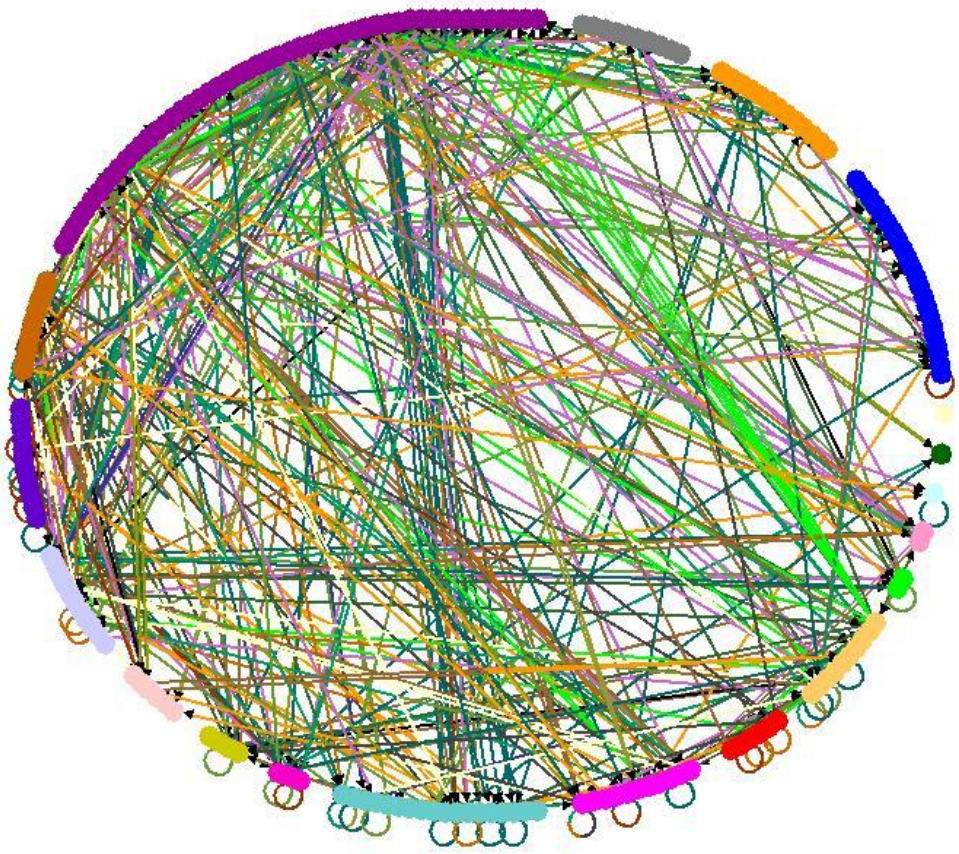

Figure 3.19. Protein-protein interaction of TOR signalling network displayed by Osprey 1.2.0

Initially, Osprey resulted in 620 interactions for 162 proteins including self-interactions. (Table B.2.). When self-interactions were excluded, 561 interactions were found among 162 proteins. The topological features of this network were analyzed by investigating degree, (k), clustering coefficient (C), and betweenness (b), which are the global measures that are used to determine the general characteristics of a biological network. Thus, for the complete network, average degree, which is the ratio of all interactions to the total number of nodes ($<k>$), average clustering coefficient ($<C>$) and average betweenness ($<b>$) were calculated as 3.46, 0.344 and 329.08, respectively. The average clustering coefficient for 10^3 random networks, generated by keeping the number of nodes and interactions constant, was calculated as 0.0409 (Table 3.6). This remarkable difference in the clustering coefficient for constructed TOR signalling protein interaction network and random network implies that the network of interest is scale free.

Table 3.6. Topological parameters of the TOR signalling and random networks

Topological parameters	TOR signalling network	Random network
N	162	162
l	561	561
<k>	3.46	3.46
<C>	0.34	0.04

Degree is the number of existing interactions that this particular node has. Degree distribution of the network is accepted as a primary measure that is frequently used to test scale-freeness. Hence, to determine whether TOR signalling protein interaction network is scale-free, degree for each of the nodes in the network was calculated. For this network, the degree of proteins in the network ranges from one to 36. It should be noted that, for the random network, the maximum degree was calculated as 19. In the TOR signalling protein interaction network, 26 out of 162 nodes had a degree of 3. Nodes with *k* value greater than 20 were Tor1p (k = 36), Esa1p (k = 22) and Ras2p (k = 21). Complete list of individual degree of nodes is given in Appendix (Table B.4). The frequency of nodes with degree *k*, $p(k)$, and cumulative distribution of nodes with degree k, $n(k)$ were presented in Table 3.7. As shown in Figure 3.20, the cumulative degree distribution follows power law with a slope of -1.53 with R^2 = 0.8. Hence the degree exponent for the network is γ = 2.53 as indicated with the equation $n(k) = A.k^{-\gamma+1}$ (Rodriguez-Caos *et al.*, 2005). This linear trend in the cumulative degree distribution indicate that many proteins are linked to few proteins, on the other hand there are few proteins that are connected to many proteins, which provides additional evidence that TOR signalling protein interaction network is scale-free and biologically significant.

Table 3.7. The frequency, $p(k)$, and cumulative frequency, $n(k)$, of nodes and their natural logarithmic values

k	p(k)	n(k)	ln(k)	ln(n(k))
36	1	1	1.56	0.00
22	1	2	1.34	0.30
21	1	3	1.32	0.48
20	1	4	1.30	0.60
19	2	6	1.28	0.78
18	1	7	1.26	0.85
17	4	11	1.23	1.04
16	3	14	1.20	1.15
15	2	16	1.18	1.20
14	1	17	1.15	1.23
13	3	20	1.11	1.30
12	4	24	1.08	1.38
11	7	31	1.04	1.49
10	11	42	1.00	1.62
9	14	56	0.95	1.75
8	2	58	0.90	1.76
7	11	69	0.85	1.84
6	9	78	0.78	1.89
5	16	94	0.70	1.97
4	15	109	0.60	2.04
3	26	135	0.48	2.13
2	15	150	0.30	2.18
1	12	162	0.00	2.21

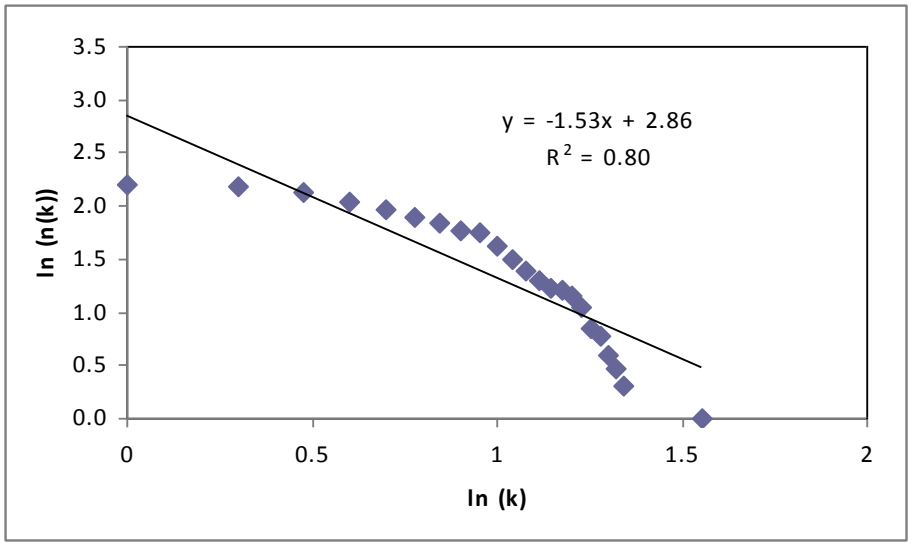

Figure 3.20. Cumulative degree distribution of the TOR signalling proteins

Clustering coefficient (C) is the fraction of the number of existing interactions among the neighbours of a particular node to the maximum allowable interactions among them. Clustering coefficients ($C(k)$) of all nodes are given in Appendix (Table B.4). For each degree, average clustering coefficient values (<C>), were also calculated by taking the average of clustering coefficient values for its corresponding degree, as shown in Table 3.8. The distribution of clustering coefficients and average clustering coefficients is shown on log-log scale in Figures 3.21 and 3.22. The clustering coefficient distribution with respect to degree shows a heterogeneous, skewed shape, indicating power–law behaviour. The average clustering coefficients on log-log scale shows linearity with $R^2 = 0.71$.

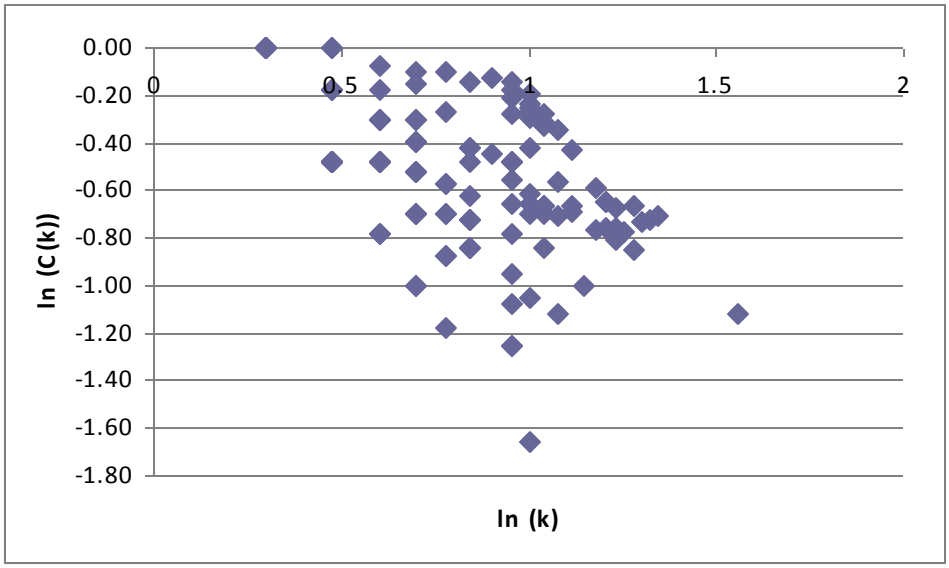

Figure 3.21. The distribution of clustering coefficients

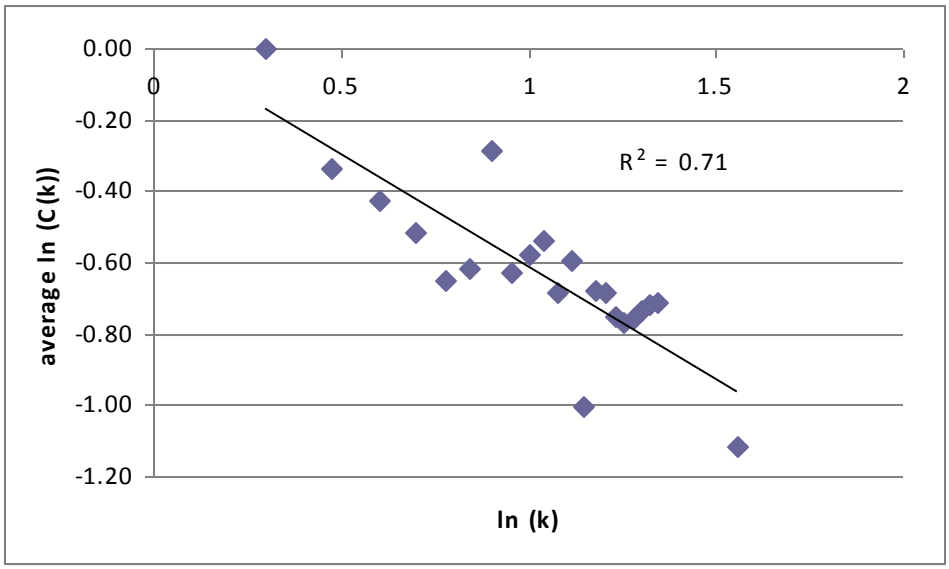

Figure 3.22. The distribution of average clustering coefficients

Betweenness (b) for a node is the fraction of the number of shortest paths between two nodes that passes through a particular node to the total number of shortest paths between these two. The property is also accepted as another measure for scale-free behaviour, since the highest betweenness nodes act like a bridge in the network, and a few high betweenness nodes are present in a scale-free network. Betweenness ($b(k)$) for each of the nodes in the network, was calculated as given in Appendix (Table B.4). For each degree, average betweenness values ($<b>$), which are given in Table 3.8, were calculated by averaging the betweenness values for their corresponding degree. The distribution of betweenness and average betweenness is shown on log-log scale in Figures 3.23 and 3.24. The average betweenness on log-log scale shows linearity with R^2 of 0.95, indicating that there are few proteins located at the centre of the network, hence responsible for communication, and many proteins are located at the periphery. Tor1p had the highest betweenness value (b = 4754.2) implying that the most number of shortest paths passed through Tor1p in the network; thus, it provides evidence that Tor1p has a central role in the network.

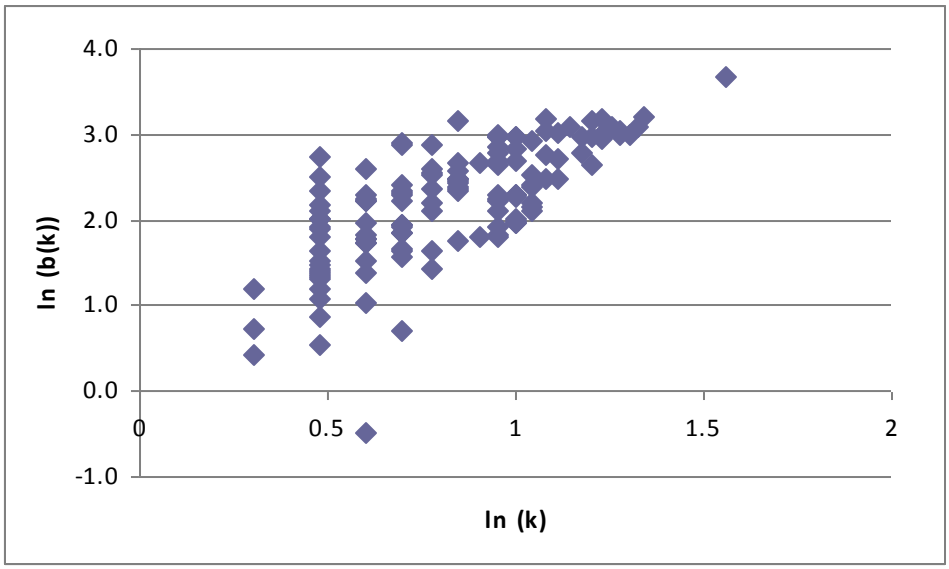

Figure 3.23. The distribution of betweenness

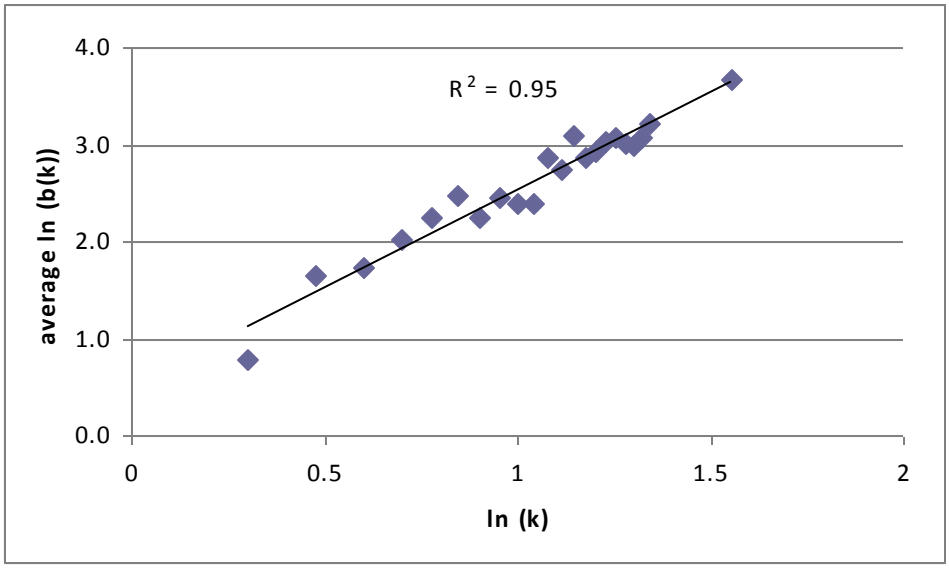

Figure 3.24. The distribution of average betweenness

Table 3.8. Average betweenness centralities and average clustering coefficient of each degree values

k	<*b*>	<*C*>		k	<*b*>	<*C*>
2	6.18	1.00		13	549.86	0.26
3	45.16	0.46		14	1248.45	0.10
4	53.08	0.38		15	750.45	0.21
5	106.90	0.31		16	847.99	0.21
6	180.98	0.22		17	1079.91	0.18
7	291.97	0.24		18	1207.81	0.17
8	175.77	0.52		19	1026.58	0.17
9	281.66	0.24		20	992.33	0.18
10	244.54	0.27		21	1195.46	0.19
11	244.01	0.29		22	1647.01	0.19
12	734.22	0.21		36	4754.24	0.08

To sum, the cumulative degree distribution, clustering coefficient and betweenness (γ = 2.53, R^2=0.71, R^2=0.95) show that protein interaction network constructed with the TOR signalling proteins exhibit scale-free behaviour. This indicates that most proteins are linked to only a few others, whereas most of them have many connections.

The scale-free behaviour of allows a more organized structure in the network. Proteins participate in similar functions tend to cluster in functional modules. These modules are regarded as highly condensed subgraphs within a network. The functional modules in the TOR signalling protein interaction networks was determined based on modularity measure, *Q*, with the Bron-Kerbosch (BK) algorithm (Bron *et al.*, 1973). This measure is the fraction of the number of existing interactions to the maximum number of possible interactions within a set of particular nodes. 289 functional modules with $Q = 1$ were identified within the TOR signalling protein interaction network using this algorithm implemented in Python scripting language. The complete list of functional modules is presented in Appendix (Table B.3). The size of the functional modules ranges from two to six, with a weighted average of 2.95. Three per cent (10 out of 289) of the functional modules contains six members. Eight per cent (23 out of 289) of the cliques consists of 5 or more nodes and 60 cliques (21 per cent of the total modules) include 4 or more nodes. Modules with respect to number of members present are given in Table 3.9. The diversity in the functional module size of TOR signalling protein interaction network implies that the network exhibit an organized structure. On the other hand, for 10^3 random networks, where the number of nodes and interactions were kept constant, the same algorithm yielded

475,745 functional modules, sizes ranging from two to four, with a weighted average module size 2. The comparison for the distribution of module size for TOR signalling protein interaction network and random network is given in Figure 3.25. Since the average size of the modules identified is 2.95. As indicated in the previous studies, modules having size four and above are biologically meaningful (Spirin and Mirny, 2003), therefore 60 modules having four or more members were considered for further investigation.

Table 3.9. Number of cliques with respect to number of members

Number of clique members	Number of cliques
2	110
3	119
4	37
5	13
6	10

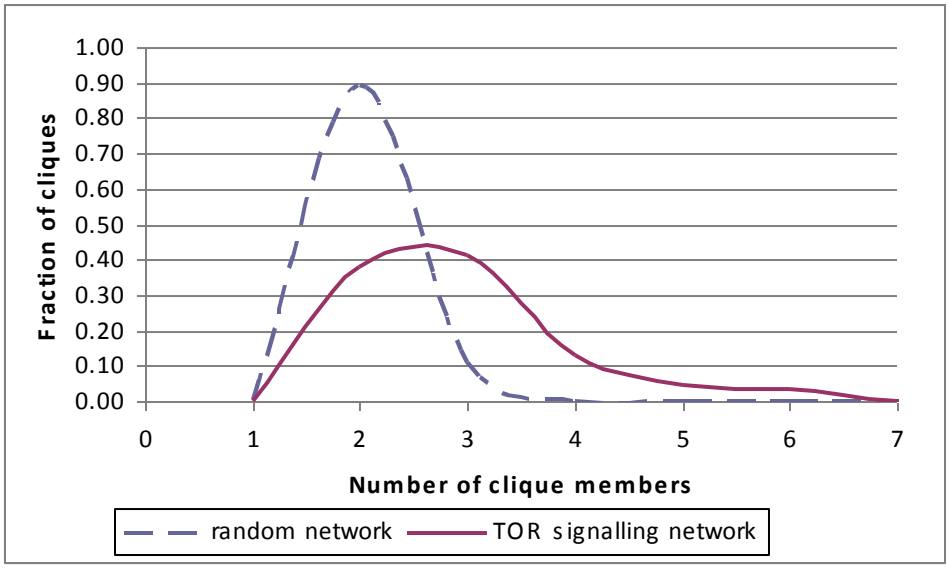

Figure 3.25. Comparison of clique member distributions of TOR signalling and random networks

60 out of 289 functional modules comprise more than three members. Out of 162 proteins in the network, 79 (48.76 per cent) proteins are represented in these functional modules. Most frequent nodes, which were present in six and more functional modules, are shown in Table 3.10. The distribution of these particular nodes according to the size of the module is presented in Figure 3.26. The proteins showing frequent presence in functional modules form also functional modules. Five modules of which members are among those

most common nodes are Tor1p-Tor2p-Gln3p-Ure2p, Yak1p-Sch9p-Msn2p-Ras2p, Yak1p-Sch9p-Tpk1p-Ras2p, Yak1p-Tpk2p-Tpk1p-Ras2p, Yak1p-Tpk2p-Msn2p-Ras2p. Due to high connectivity of all members with the other cliques, those modules might have central roles in the network.

Table 3.10. Most common nodes of more than 3 member cliques

Protein name	Number of cliques		Protein name	Number of cliques
Ras2p	12		Pph22p	7
Tor1p	11		Tor2p	7
Gln3p	10		Pep5p	6
Esa1p	9		Pph21p	6
Tpk1p	9		Rim15p	6
Bmh2p	8		Sit4p	6
Msn2p	8		Tpk2p	6
Pep3p	8		Ure2p	6
Sch9p	8		Vps33p	6
Tap42p	8		Yak1p	6

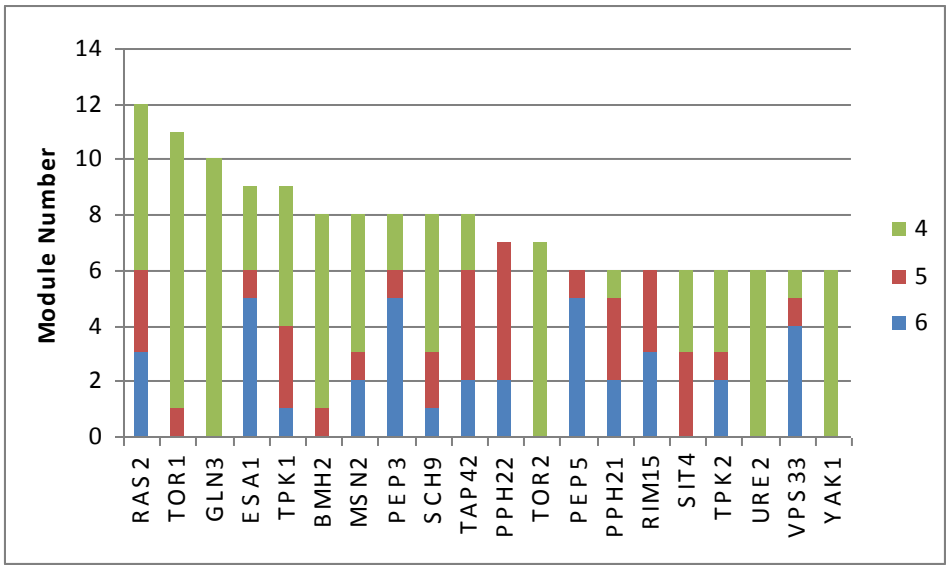

Figure 3.26. Distribution of particular nodes present in more than three member modules

To determine how the functional modules are closely related with the rest of the network, each functional module was scored according to the interactions within the module and the modules' interactions to the rest of the network. The score is the fraction of the outer degrees of the module to the total number of degrees in the module. This score would be high, if the functional module is highly connected to the other nodes in the

network, and a low score indicates an isolated fragment. The score table is given in Appendix (Table B.5). The top highest scores corresponds to modules 24, 25, 27, 28, and 33, all of which include Tor1p (Table 3.11). Additionally, among these five top scored modules Gln3p is present in four of the modules. These indicate the central roles of Tor1p and Gln3p in the TOR signalling pathway. The list of the modules and their corresponding scores is given in the table below:

Table 3.11. Top scored more than three member modules

Module number	Module members				total-degree	number of members	in-degree	out-degree	score
24	Tor1p	Tor2p	Gln3p	Ure2p	83	4	12	71	5.92
25	Tor1p	Tor2p	Gln3p	Lst8p	77	4	12	65	5.42
27	Tor1p	Tor2p	Tpd3p	Tap42p	74	4	12	62	5.17
28	Tor1p	Gat1p	Ure2p	Gln3p	72	4	12	60	5.00
33	Tor1p	Gcn4p	Ure2p	Gln3p	71	4	12	59	4.92

On the other hand, the lowest scores belong to modules 3, 4, 5, 16 and 57 (Table 3.12). The members on modules 3, 4 and 5 are related with vacuolar transport. Members in module 16 are function in translation initiation. Module 57, where the members are Hap2p, Hap3p, Hap4p and Hap5p, functions in respiration. Its score 0.33 indicates that this module is tightly bound within but is an isolated module.

Table 3.12. Bottom scored more than three member modules

Module number	Module members						total-degree	number of members	in-degree	out-degree	score
3	Esa1p	Vps16p	Pep5p	Pep3p	Vps33p	Pep7p	67	6	30	37	1.23
4	Esa1p	Vps16p	Pep5p	Pep3p	Vps33p	Vps3p	65	6	30	35	1.17
5	Esa1p	Vps16p	Pep5p	Pep3p	Vps45p	Pep7p	64	6	30	34	1.13
16	Tif4632p	Tif1p	Tif2p	Tif4631p	Cdc33p		39	5	20	19	0.95
57	Hap5p	Hap4p	Hap2p	Hap3p			16	4	12	4	0.33

To determine the significance of the functional modules, AmiGO Gene Ontology term enrichment tool (Harris *et al.*, 2008) was employed to find significantly enriched biological process terms. The terms are listed in Appendix (Table B.6).

The members in modules 1 – 5 are significantly enriched with vacuole organization ($p < 9.15x10^{-4}$). Esa1, Pep3 and Pep5 are co-located in all of six member functional modules. Six proteins, namely Esa1p, Rad27p, Vps33p, Pep5p, Pep3p and Pep7p, in

module 1 are related with post-Golgi vesicle-mediated transport ($p = 5.79x10^{-5}$) and vacuolar transport ($p = 5.56x10^{-4}$). Esa1 is the yeast histone acetylase that is required for cell growth (Reid *et al.*, 2000). This protein has found to function in the activation and repression of ribosomal proteins (RP) in yeast (Martin *et al.*, 2005). TOR pathway responds to nutrients by controlling expression of RP genes. The coordinated expression of RP genes is associated with recruitment of Esa1p to the RP gene promoters. It was shown that the association of Esa1p with the RP gene promoters is rapamycin sensitive and that the loss of Esa1p from these promoters resembles the kinetics of RP gene repression upon addition of rapamycin (Rohde *et al.*, 2003). Key components of the transport into the vacuole are the vesicle-mediated transport class that is essentially composed by vacuolar sorting proteins (VPS) that comprise multimeric complexes involved in the formation of vesicles responsible for intracellular trafficking between Golgi compartments, the endosome and the vacuole (Vps16p, Vps33p, Pep3p and Pep5p) (Mira *et al.*, 2008). The systematic deletion of class VPS strains showed as decreased chronological life span compared to wild type (Schauer *et al.*, 2009).

Six members in module 6, namely, Cdc55p, Tpd3p, Rrd2p, Tap42p, Pph22p and Pph21p are significantly associated with mitotic cell cycle spindle checkpoint assembly ($p = 1.81x10^{-7}$). Through TORC1, rapamycin affects many nutrient-responsive gene expressions at several levels, including transcription, translation and protein trafficking and stability (Rohde *et al.*, 2003). Many rapamycin sensitive events are mediated by several phosphatases, including protein phosphatase 2A (PP2A). In this module, *PPH21* and *PPH22* encode PP2A catalytic subunit. PP2A is one of the major protein serine/threonine phosphatases in the cell, which plays a role in several cellular processes, including metabolism, transcription, RNA splicing, translation, cell cycle progression, morphogenesis, signal transduction, development, and transformation. *TPD3* encodes the only A subunit, whereas two other B subunits are encoded by *CDC55* and *RTS1* (Shu *et al.*, 1997). Mutations of *CDC55* are viable, but lead to defects in cytokinesis, spindle checkpoint and result in abnormal cell morphology (Koren *et al.*, 2004). All of the units in PP2A interact with the Tap42p regulatory subunit (Jiang and Broach, 1999). In particular, these proteins have been shown to regulate the phosphorylation state and activity of distinct transcriptional regulators whose target genes are controlled by TORC1 (Jiang *et al.*, 2008).

Co-location of Gis1p-Rim15p-Msn2p-Msn4p and Ras2p in two six member functional modules, modules 7 and 8, supports the evidence that TOR signalling pathway is related with cellular aging. The GO term enriched in these functional modules are age-dependent general metabolic decline during chronological cell aging ($p = 9.15x10^{-10}$ and $p = 2.45x10^{-6}$, respectively). Msn2p/4p and Gis1p are stress response transcription factors, which are positively regulated with Rim15p. It was also reported that, longevity regulation controlled by Tor1p, Sch9p and Ras2p converges on the protein kinase Rim15p (Wei *et al.*, 2009). Deletion of *MSN2/4* in *ras2Δ* and of *RIM15* in *sch9Δ* mutants reduces life span extension. TORC1 signaling is negatively regulated with Msn2p/4p and Rim15p. Deficiency in Gis1p led to a reversion of life span extension of the *sch9Δ* and, to a lesser extent, *RAS2* deleted mutants (Wei *et al.*, 2008). Recently, Gis1p and Rim15p are required for the upregulation of many of the genes that function upon starvation for different macronutrients (Zhang *et al.*, 2009).

Six members in module 9, namely Bcy1p, Tpk1p, Ras2p, Tpk2p, Msn4p and Rim15p, are significantly associated with RAS protein signal transduction ($p = 5.39x10^{-6}$). The RAS/PKA pathway plays an important role in regulation of growth in response to extracellular nutrients. In rich nutrient conditions, Ras1p and Ras2p are activated, in turn they activate adenylate cyclase (encoded by *CDC35* gene) to produce cAMP. PKA consists of three catalytic subunits, Tpk1p, Tpk2p, and Tpk3p, and a regulatory subunit, Bcy1p. Binding to cAMP allows the dissociation of Bcy1 from the catalytic subunits and activation of PKA. The RAS/PKA pathway is also associated with some TORC1-regulated responses other than autophagy (Schmelzle *et al.*, 2004). The activation of the RAS/cAMP pathway suppresses a TOR deficiency. Also, the regulation of PKA is achieved by TOR. These findings suggest that the RAS/cAMP pathway is a TOR effector pathway (Yorimitsu *et al.*, 2007).

Members in module 10 are involved in cytoskeleton organization ($p = 5.37x10^{-5}$). The members *PPH21* and *PPH22* encode protein phosphatase 2A (PP2A). On the other hand, *YPA1* and *YPA2* (also named *RRD1* and *RRD2* for Rapamycin-resistant deletion) encode Phosphotyrosyl Phosphatase Activator (PTPA). Inactivation of either gene renders yeast cells rapamycin resistant (Zheng *et al.*, 2005). Individual deletion of *YPA1* produces a more severe phenotype than deletion of *YPA2*. Deletion of *YPA1* leads to an abnormal

actin distribution, growth defects, and rapamycin resistance (Van Hoof *et al.*, 2000; Van Hoof *et al.*, 2001). *YPA1* and *YPA2* deletions confer rapamycin resistance probably by indirect regulation of TOR (Leulliot *et al.*, 2006). Rrd1p and Rrd2p are shown to interact with TAP42-containing complexes (Zheng *et al.*, 2005).

The members in module 12 (Sap185p, Sap155p, Sap4p, Sap190p, Sit4p) are significantly associated with G1/S transition of mitotic cell cycle ($p = 1.59x10^{-9}$). It was known that Sit4p associates with the other four proteins and those are called Sit4p-associated proteins (SAPs) (Inoki *et al.*, 2005). In addition to cell cycle regulation (Luke *et al.*, 1996),Sit4p together with SAPs were found to be related with transcriptional and translational programs that couple cell growth to amino acid availability (Rohde *et al.*, 2004).

5 members of module 16 are involved in translational initiation ($p = 2.81x10^{-9}$). *Saccharomyces cerevisiae* encodes the eukaryotic initiation factor 4E (eIF4E) protein by the *CDC33* gene, the eIF4A protein by *TIF1* and *TIF2*, and the two eIF4G proteins (eIF4G1 and eIF4G2) by the *TIF4631* and *TIF4632* genes, respectively. All these proteins physically associate and that this association performs an essential function for translational initiation (Neff *et al.*, 1999).

The members in module 24 (Tor1p, Tor2p, Ure2p, Gln3p), module 28 (Tor1p, Gat1p, Ure2p, Gln3p) and module 29 (Tor1p, Dal80p, Ure2p, Gzf3p) are significantly associated with the regulation of nitrogen utilization ($p = 2.55x10^{-3}$, $1.87x10^{-6}$, $1.71x10^{-6}$ respectively). Gat1p, Dal80p, and Gzf3p are four GATA type transcriptional factors which have roles in the regulation of nitrogen catabolite repression sensitive genes and it was found that Gln3p activity is inhibited by Ure2p in the presence of preferred nitrogen sources (Bertram *et al.*, 2000). Moreover, TOR complex 1, which includes Tor1p and Tor2p, is considered to be responsible for Gln3p phosphorylation and dephosphorylation (Carvalho *et al.*, 2003).

4 members of module 57 constitute Hap2p/3p/4p/5p complex in *Saccharomyces cerevisiae* (McNapp *et al.*, 2005). Four proteins are members of the CCAAT-binding factor, is an evolutionarily conserved multimeric transcriptional activator responsible for

the activation of many of the genes involved in respiratory metabolism. They are significantly associated with the regulation of carbohydrate metabolic process ($p = 5.48x10^{-8}$).

4 members of module 58 are subunits of RNA polymerase I and/or III. Rpc40p is a subunit of RNA polymerase I and III, Rpa135p and Rpa49p are two subunits of RNA polymerase I, and Rpc34p is a subunit of RNA polymerase III. They are significantly associated with DNA dependent-transcription ($p = 5.76x10^{-3}$).

4. DISCUSSION

In this study, the aim is to have a better understanding of the TOR signalling pathway and for this purpose; the impact of caffeine and rapamycin on the growth of yeast cells was investigated. *Saccharomyces cerevisiae* wild type BY4743 strains were grown in different type of media in both flasks and fermentors and samples were collected from these experiments to obtain biomass and extracellular metabolite profiles in the absence and the presence of the chemicals. This data was used in flux balance analysis (FBA) to determine metabolic fluxes under these conditions by using the whole genome stoichiometric models. The fluxes predicted by FBA were analyzed via self-organizing maps (SOM) methodology. Moreover, topology of TOR signalling proteins and the organization of functional modules were identified.

Saccharomyces cerevisiae wild type BY4743 strains were cultivated in F1 or YPD medium in both flasks and controlled fermentors. Growth characteristics of yeast cells were observed in the absence and presence of caffeine or rapamycin.

The effect of the caffeine and rapamycin on growth profiles of yeast cells in F1 medium was investigated and these chemicals were found to inhibit the growth of the yeast cells after the treatment. This observation is consistent with several studies (Kuranda *et al.*, 2006; Reinke *et al.*, 2006; Wullschleger *et al.*, 2006). The repression of growth by caffeine has increased with the increasing concentrations of. It had also been reported that (Reinke *et al.*, 2006; Wanke *et al.*, 2008) that the higher caffeine concentrations result in higher degrees of inhibition of the growth of yeast cells. Moreover, yeast cells treated with lower amount of caffeine (less than or equal to 10 mM as the final concentration) reached to higher optical density values compared to the cells grown in the control flask at the stationary phase. However yeast cells treated with higher amount of caffeine (more than 10 mM as the final concentration) could never reach to optical density level of the untreated cells. These results are consistent with the findings that low amount of caffeine extended life span of the cells grown on the plates (Wanke *et al.*, 2008). Wanke *et al.* counted more colonies of yeast cells living on the caffeine treated plates compared to untreated ones. In addition, it rapamycin treated cells also reached to higher optical density values than the

untreated cells indicating that a certain concentration of rapamycin may extend the life span as low concentrations of caffeine do

It was also observed that growth curve of the rapamycin treated cells reached higher values than the growth curves of the cells treated with different concentrations of caffeine at different time points. First, the growth curve of the rapamycin treated cells reached higher values than the growth curves of the cells treated with lower amounts of caffeine, as time passed the growth curve of the rapamycin treated cells reached higher values than the growth curves of the cells treated with lower amounts of caffeine, and at the end of exponential phase or at the stationary phase it had similar optical density values as high as the growth curves of the cells treated with lower amounts of caffeine. Furthermore, rapamycin treated cells reached to the stationary phase later than the caffeine treated or untreated cells. This shows that repression effects of caffeine and rapamycin were different on yeast cells, which was claimed by previous studies (Kuranda *et al.*, 2006; Reinke *et al.*, 2006).

Investigation of the effect of these chemicals on growth of yeast cells in YPD indicated that an increasing concentration of caffeine has an increasing inhibitory effect on the growth properties of the cells. However, growth behaviour of the cells was not affected when cells were treated with rapamycin at the final concentration of 200 ng/ml and only a slight inhibitory effect was observed when the concentration was doubled .The effect of rapamycin and caffeine on the growth was more pronounced on cells grown in F1 medium when compared to YPD which is a rich medium.

The effect of caffeine on the fermentation characteristics on *S. cerevisiae* BY4743 was comparatively investigated under controlled batch fermentation conditions in the present study and flux distribution was determined in both caffeine treated and control cultures

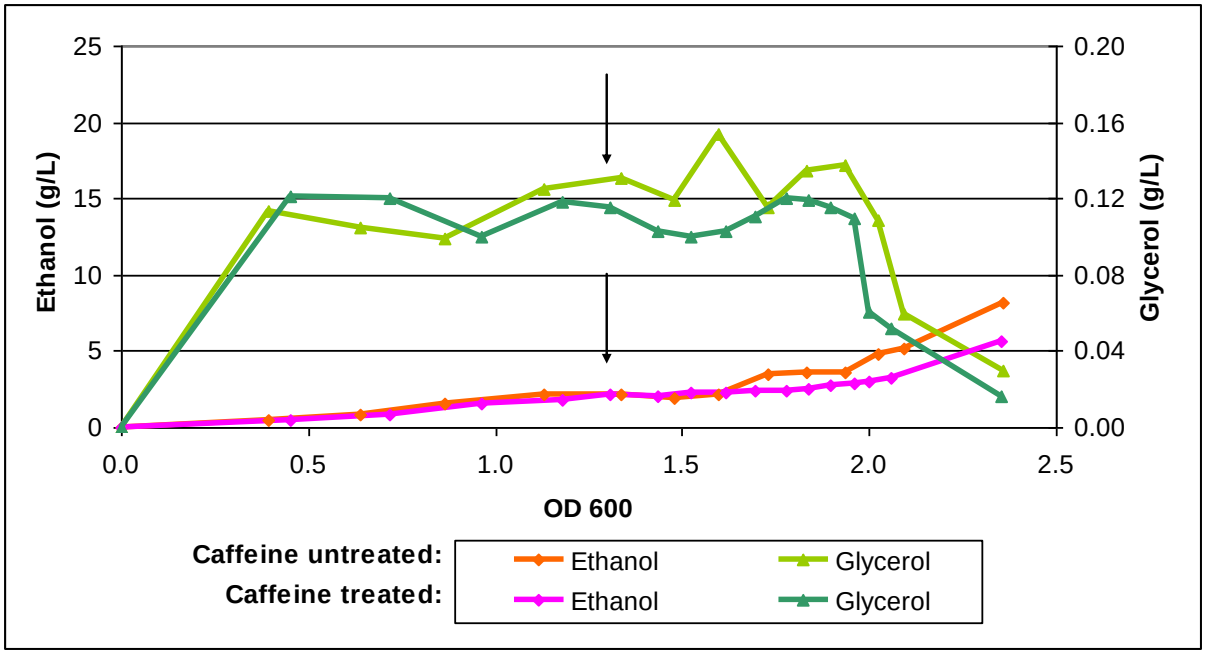

Figure 4.1. Comparison of ethanol and glycerol production in the absence and presence of caffeine

Decrease in the ethanol and glycerol production after caffeine treatment is shown in Figure 4.1. The decrease may be a result of caffeine repressing growth. Decreased ethanol and glycerol production indicates decrease in fermentation and respiration, respectively; and decrease in fermentation and respiration results in decrease in the energy production and consequently repression in the growth.

Although biomass production in YPD medium could not be successfully predicted by flux balance analysis (FBA) when the maximization of growth or oxygen uptake was used as objective function, ethanol production in YPD medium could be successfully predicted when the maximization of ethanol production was used as the objective function. Moreover, it was observed that prediction of ethanol production in the presence of caffeine was not as successful as in the absence of caffeine.

In order to investigate the effect of caffeine on the fluxes, fluxes predicted by FBA both in the absence and the presence of caffeine were clustered. FBA predicts the growth of the cells successfully in the steady-state growth period and since the ethanol production in that period was most successfully predicted 1.5 hours after the treatment both in the absence and presence of the caffeine, the fluxes at that time which were predicted by FBA

were compared via clustering. In order to consider the fluxes independent of their direction, absolute magnitudes of fluxes were normalized and clustered. Although the magnitude of 42 fluxes was observed to decrease, the magnitude of 6 fluxes increased after caffeine treatment. The repressed fluxes are significantly associated with glucose catabolic process, glycolysis, generation of precursor metabolites and energy, nucleotide metabolic process and glutamine family amino acid biosynthetic process; whereas, the activated fluxes are significantly associated with glucose metabolic process and pentose-phosphate shunt.

The repressed fluxes in glycerol synthesis and glucose fermentation pathways, which are indicated by green arrows, are given in Figures 4.2 and 4.3. This data is consistent with the decreased glycerol and ethanol production in response to caffeine treatment, which may indicate the repression of growth due to caffeine. Moreover, almost all fluxes in glycolysis were repressed under caffeine treatment, as is shown in Figure 4.3. In addition, fluxes playing role in glucose (Hxt4p), ethanol, glycerol (Fps1p), acetic acid (Fps1p), proton (Atp1p) and carbon dioxide transport, and transportation through mitochondrial membrane (Oac1p, Aac1p) as well as mitochondrial carriers (Mir1p, Dic1p) were repressed in response to caffeine treatment. The flux under the control of Mdh1p, which is in TCA cycle were also decreased under the caffeine treatment.

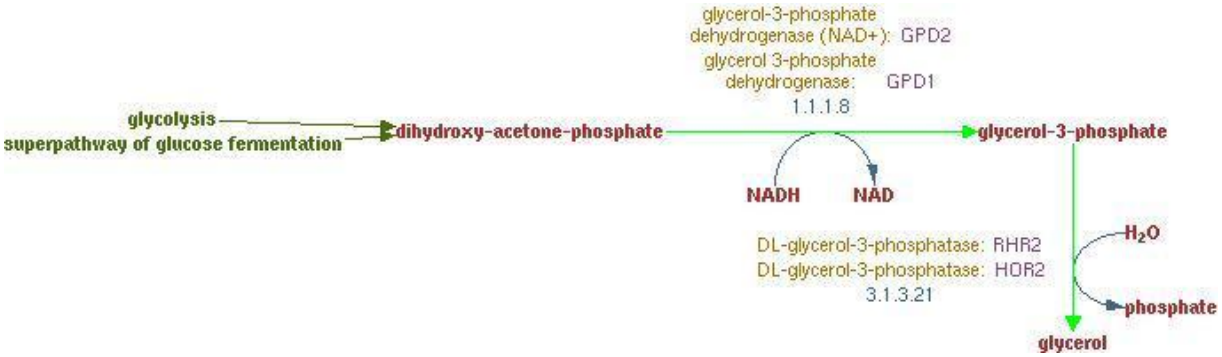

Figure 4.2. Glycerol synthesis pathway under caffeine treatment

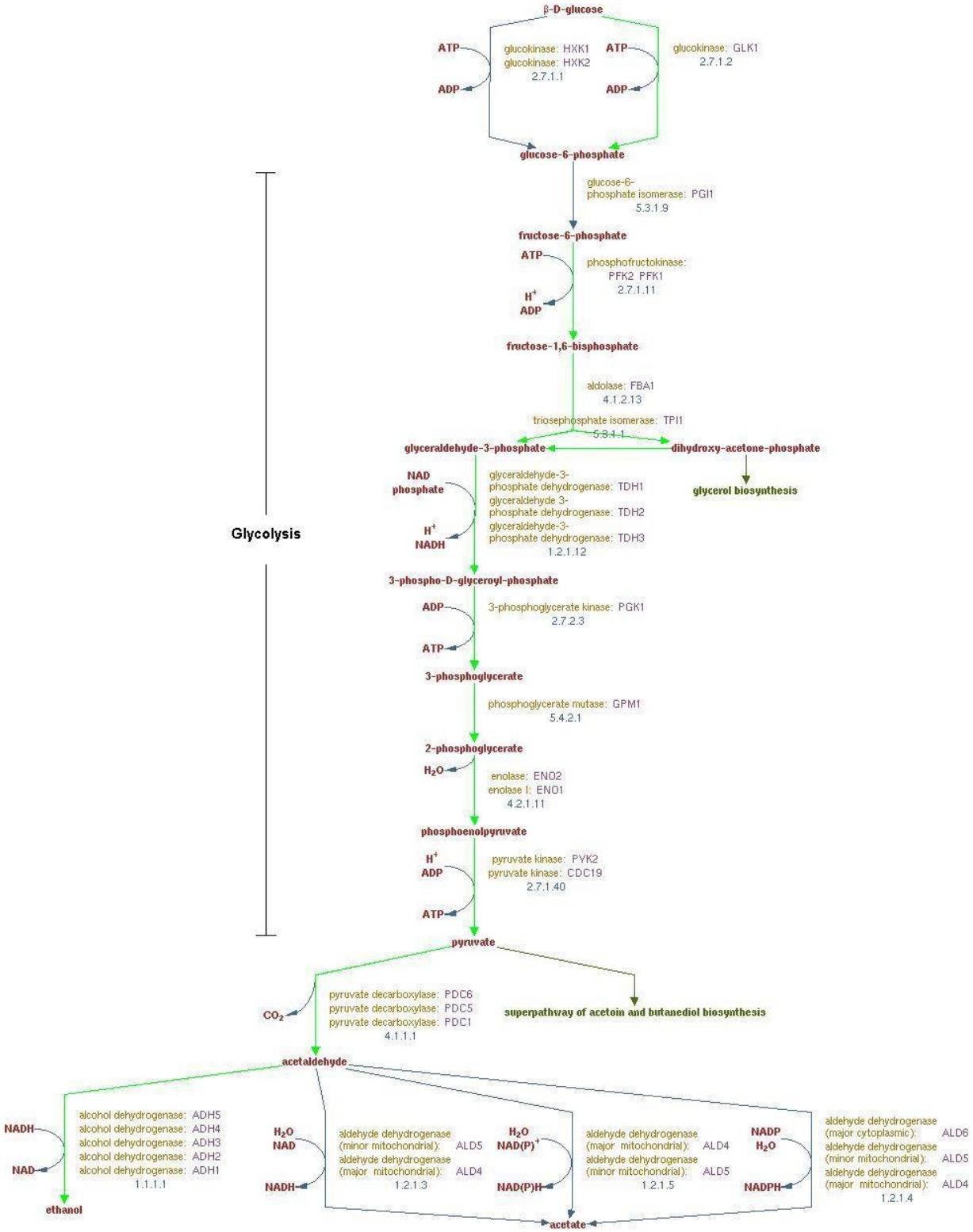

Figure 4.3. Glucose fermentation pathway under caffeine treatment

Moreover, the fluxes in proline biosynthesis were repressed in response to caffeine, which are indicated by green arrows in Figures 4.4. The flux under the control of Put1p which has a role in proline utilization pathway was also repressed under the caffeine treatment. It is known that TOR signalling regulates protein synthesis (Hinnebusch *et al.*, 2005) and inhibition of TOR signalling by caffeine may repress proline biosynthesis and utilization.

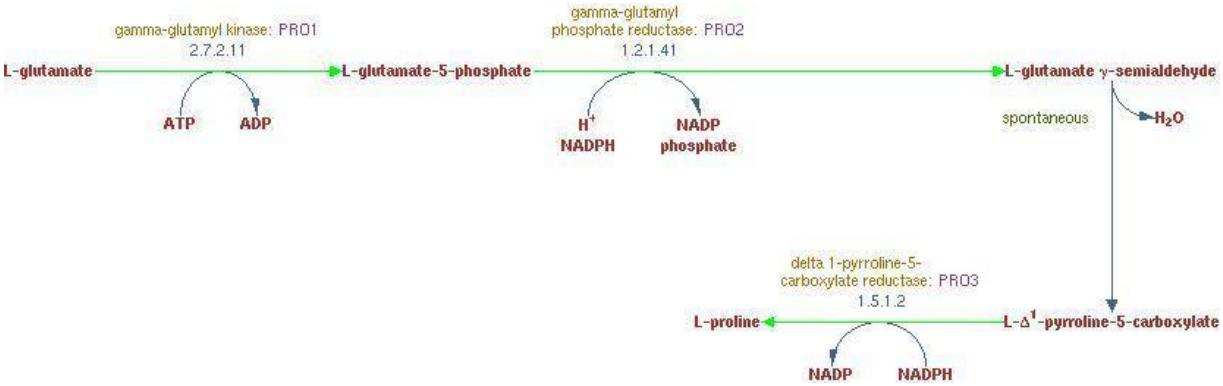

Figure 4.4. Proline biosynthesis pathway under caffeine treatment

The repressed and activated fluxes in pentose phosphate shunt pathway, which are indicated by green and red arrows, respectively, are given in Figure 4.5. The pentose phosphate pathway is important for generating NADPH, which is a source of reducing energy, as well as a variety of sugar molecules that are required for the biosynthesis of nucleic acids and amino acids. This pathway is also important for protecting yeast from oxidative stress. It was observed that fluxes having a role in the synthesis of glyceraldehyde-3-phosphate and sedoheptulose-7-phosphate from xylulose-5-phosphate and ribose-5-phosphate were decreased; however, fluxes after this point in the pentose phosphate pathway were activated. Activation of those fluxes may derive from the alternative pathways in which glyceraldehyde-3-phosphate and sedoheptulose-7-phosphate are synthesized from degradation of deoxyribose-5-phosphate and D-sedoheptulose-1,7-bisphosphate. It was found that TOR signalling regulates nucleotide biosynthesis (Hinnebusch *et al.*, 2005) and inhibition of TOR signalling by caffeine may result in degradation of nucleotides.

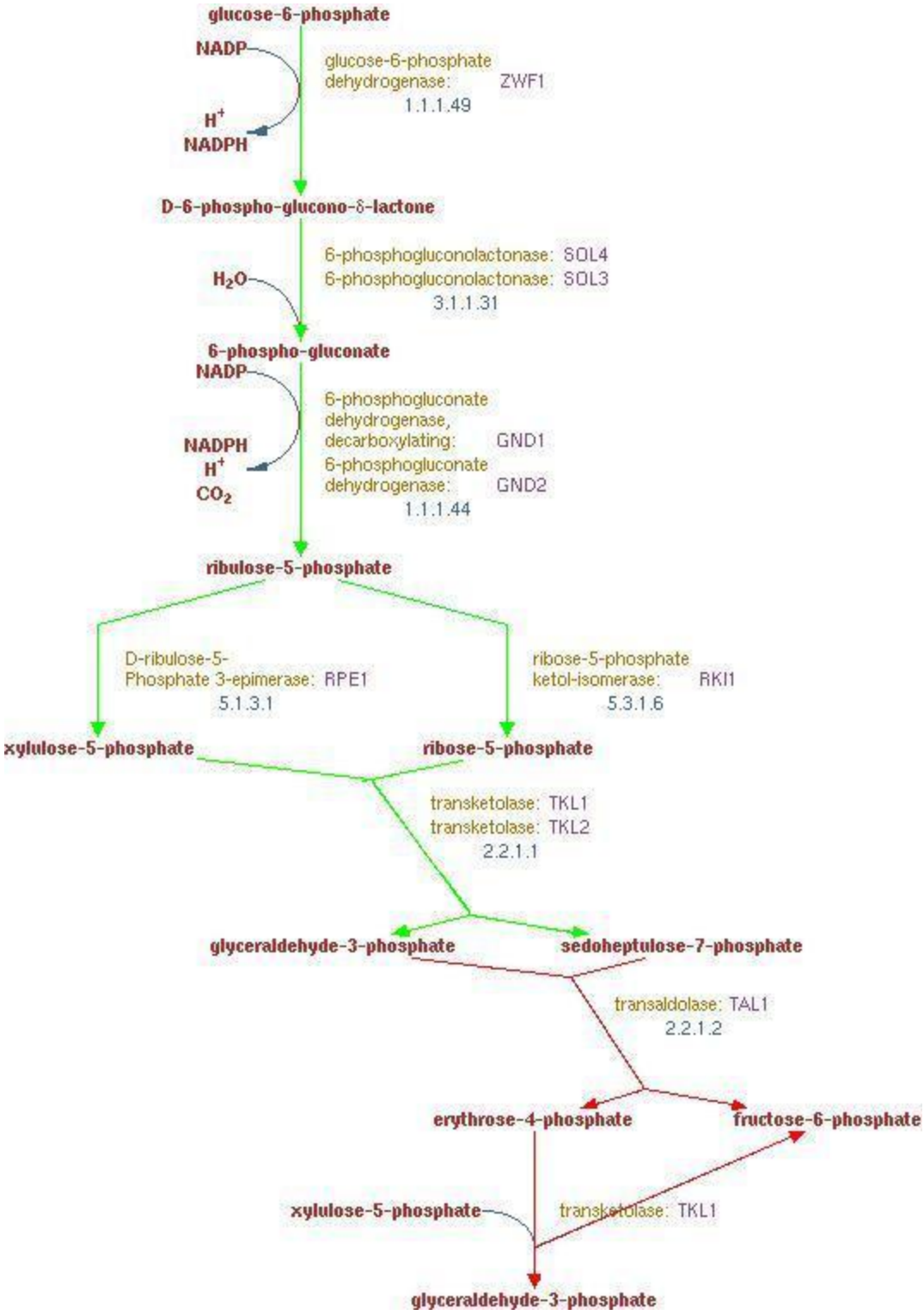

Figure 4.5. Pentose phosphate shunt pathway under caffeine treatment

Topological analysis of the protein-protein interaction network related to TOR signalling is scale-free (SF) as expected for biological networks. The degree distribution and clustering are heterogeneous, and skewed shapes of clustering and betweenness centrality indicate power–law behaviour. Therefore, TOR signalling network shares the small-world behaviour of real networks. Compared to a random network, it also had a higher average clustering coefficient and higher maximum degree value. Most proteins in a scale-free network are linked to only a few others, whereas most of them have many connections. In addition, when nodes are removed at random, internal stability is maintained in the network. Otherwise, eliminating the most connected nodes fragments the network. However, a similar fragility is observed both if the nodes are removed at random or in order of increasing degree in random webs (Rodriguez-Caso *et al.*, 05).

Due to scale-free behaviour TOR signalling network had an organized structure. Proteins that participate in similar functions tended to cluster in functional modules. These modules constructed highly condensed subgraphs within the network The weighted average of functional module sizes were found as 2.95; however, modules of size four and above were considered as biologically meaningful, as indicated in previous studies (Spirin and Mirny, 2003). Most modules were found to be biologically meaningful; the members have a role in the same biological function. When the functions of the proteins in modules were investigated, it was observed that proteins have a role in vacuolar organization, mitotic cell cycle spindle check point assembly, cellular aging, RAS protein signal transduction, cytoskeleton organization, G1/S transition of mitotic cell cycle, translational initiation, regulation of nitrogen utilization, regulation of carbohydrate metabolic process and DNA dependent-transcription, were grouped in modules. This may indicate the organized structure of the TOR signalling network.

Ras2p and Tor1p were the most common proteins among those modules. This is consistent with the data that there is a functional interaction between TOR and the RAS/cAMP pathway (Schmelzle *et al.*, 2004), although it was observed that rapamycin did not cause significant change in expression of RAS signalling pathway genes (Kuranda *et al.*, 2005). TOR and RAS signalling were thought to work together in response to stress (Hong *et al.*, 2002), in transcription process of ribosomal protein genes (Martin *et al.*, 2004) and in autophagy (Klionsky *et al.*, 2005; De Virgilio *et al.*, 2006).

When the ratio of out-degree to the in-degree of the modules was compared, Tor1p and Gln3p were found in the highest scored modules and this may indicate the central roles of Tor1p and Gln3p in the TOR signalling pathway. On the other hand, the lowest scored modules were HAP complex and related to vacuolar transport. These modules may be considered as isolated modules due to low interaction with the other proteins.

5. CONCLUSIONS AND RECOMMENDATONS

5.1. Conclusions

In this thesis, the impact of caffeine and rapamycin on yeast cells was investigated in order to understand the TOR signalling pathway in deep. For this purpose, *Saccharomyces cerevisiae* wild type strain was cultivated in F1 and YPD medium in both flask and controlled fermentor and samples were collected to obtain biomass and extracellular metabolite profiles in the absence or presence of chemicals. This data was used in flux balance analysis (FBA) to determine metabolic fluxes under these conditions by using the whole genome stoichiometric models. Fluxes successfully predicted by (FBA) in the absence and presence of caffeine were clustered via self-organizing maps methodology in order to analyze caffeine effect on the yeast cells. Moreover, topology of TOR signalling proteins was studied.

Caffeine and rapamycin repressed the growth of the yeast cells in both F1 and YPD mediums. The higher the concentration of the chemicals, the more the growth of the yeast cells was repressed. However, the repression effects of caffeine and rapamycin were found to have different characteristics. Furthermore, the repression effect of the chemicals were more effective in F1 medium, indicating that richness of the YPD medium in nutrients might decrease the repression effect of chemicals.

Moreover, yeast cells treated with lower amount of caffeine (less than or equal to 10 mM as the final concentration) were observed to reach to higher optical density values compared to the cells grown in the control flask, consistent with the effect of caffeine that extends the life span of the yeast cells. Rapamycin treated cells were also observed to reach to higher optical density values than the untreated cells, which may indicate a similar effect of rapamycin.

Ethanol and glycerol production was decreased under the caffeine treatment, which indicates respiration and fermentation were repressed in response to caffeine. This might be a result of repression effect of caffeine on the growth. Compared to the prediction of

biomass production as the maximization of growth or oxygen uptake was objected, ethanol production in YPD medium was successfully predicted by using flux balance analysis when the objective function was chosen as the maximization of ethanol production. The fluxes successfully predicted by FBA in the absence and presence of caffeine were analyzed by clustering via self-organizing maps methodology. Clustering results showed that caffeine affected the glucose metabolism and therefore the generation of energy. Moreover, repression effect of caffeine was observed in glucose fermentation, glycerol biosynthesis and pentose phosphate and proline biosynthesis pathways.

Topology of TOR signalling proteins was also analyzed and it was found that TOR signalling network had a scale-free property. Like real networks, it exhibits high homeostasis when nodes are removed at random. It was found that most proteins are linked to only a few others, whereas most of them have many connections. Ras2p and Tor1p were the most common proteins among more than 3 member modules, indicating a possible interaction between TOR and RAS signalling. Moreover, Tor1p and Gln3p were found to have a central role in TOR signalling network. When the functions of the proteins in modules were investigated, it was found that they are significantly annotated to gene ontology terms, indicating the organized structure of the TOR signalling network.

5.2. Recommendations

In this study, fluxes that were significantly changed under caffeine treatment were determined with the help of flux balance analysis using whole genome metabolic models of *Saccharomyces cerevisiae*.

For further analysis of the genes that catalyze the reactions through which the fluxes have changed samples need to be collected for RNA extraction and extracellular metabolite analyses in similar experiment. Gene expression profiles can be obtained with the help of quantitative PCR methodology. Relative quantification of gene expression would provide information about transcriptional response of the genes under caffeine treatment. Moreover, the experiment can be carried out using mutant strains in which the genes implicated in TOR signalling are deleted. The role of those genes and their relationship

with other genes may be investigated by studying significantly changed fluxes or gene expressions in the absence of those genes.

Genes significantly responding to the caffeine treatment can be identified using microarray technology to monitor transcriptional response of all genes.

It has been shown that both caffeine and rapamycin affect TOR signalling in the cell. A comparative study at all omics levels will be very helpful in elucidating the differences in response of organism to these drugs. .

In the present study, the effect of caffeine on growth of yeast cells was investigated in rich medium. However, since our results showed that the repressive effect of rapamycin is not observable in this rich medium, controlled batch cultivations in the presence of these two chemicals should be carried out in a different medium such as chemically defined F1 medium.

Chemostat cultivations in the presence of these drugs may give better estimates for flux distribution and further information on the effect of these chemicals on the metabolism of the cells.

Analysis of the functional modules of the protein-protein interaction network constructed in the present study provided information about previously known functional relationships in the TOR signalling pathway Therefore extension of this preliminary network using the first neighbours of the genes in the network or functionally unknown genes may provide additional information about this important signalling pathway and enhance our understanding of the molecular mechanisms involved in the response to drugs.

APPENDIX A: METABOLITES AND REACTIONS DESCRIBED IN FBA AND FLUX VALUES PREDICTED BY FBA

Metabolites and reactions described in flux balance analysis (FBA) by Forster *et al.*, flux magnitudes used in self-organizing maps clustering (SOM) and SOM cluster members are included in this section.

Table A.1. Metabolites described in FBA

Abbreviation	Metabolite
13GLUCAN	1,3-beta-D-Glucan
13PDG	3-Phospho-D-glyceroyl phosphate
23DAACP	2,3-Dehydroacyl-[acyl-carrier-protein]
23PDG	2,3-Bisphospho-D-glycerate
2HDACP	Hexadecenoyl-[acp]
2MANPD	("alpha"-D-mannosyl)(,2)-"beta"-D-mannosyl-diacetylchitobiosyldiphosphodolichol
2N6H	2-Nonaprenyl-6-hydroxyphenol
2NMHMBm	3-Demethylubiquinone-9M
2NPMBm	2-Nonaprenyl-6-methoxy-1,4-benzoquinoneM
2NPMMBm	2-Nonaprenyl-3-methyl-6-methoxy-1,4-benzoquinoneM
2NPMP	2-Nonaprenyl-6-methoxyphenol
2NPMPm	2-Nonaprenyl-6-methoxyphenolM
2NPPP	2-Nonaprenylphenol
2PG	2-Phospho-D-glycerate
3DDAH7P	2-Dehydro-3-deoxy-D-arabino-heptonate 7-phosphate
3HPACP	(3R)-3-Hydroxypalmitoyl-[acyl-carrier protein]
3PG	3-Phospho-D-glycerate
3PSER	3-Phosphoserine
3PSME	5-O-(1-Carboxyvinyl)-3-phosphoshikimate
4HBZ	4-Hydroxybenzoate
4HLT	4-Hydroxy-L-threonine
4HPP	3-(4-Hydroxyphenyl)pyruvate
4PPNCYS	(R)-4'-Phosphopantothenoyl-L-cysteine
4PPNTE	Pantetheine 4'-phosphate
4PPNTEm	Pantetheine 4'-phosphateM
4PPNTO	D-4'-Phosphopantothenate
5MTA	5'-Methylthioadenosine
6DGLC	D-Gal alpha 1->6D-Glucose
A6RP	5-Amino-6-ribitylamino-2,4 (1H, 3H)-pyrimidinedione

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
A6RP5P	5-Amino-6-(5'-phosphoribosylamino)uracil
A6RP5P2	5-Amino-6-(5'-phosphoribitylamino)uracil
AACCOA	Acetoacetyl-CoA
AACP	Acyl-[acyl-carrier-protein]
ABUTm	2-Aceto-2-hydroxy butyrateM
AC	Acetate
ACACP	Acyl-[acyl-carrier protein]
ACACPm	Acyl-[acyl-carrier protein]M
ACAL	Acetaldehyde
ACALm	AcetaldehydeM
ACAR	O-Acetylcarnitine
ACARm	O-AcetylcarnitineM
ACCOA	Acetyl-CoA
ACCOAm	Acetyl-CoAM
ACLAC	2-Acetolactate
ACLACm	2-AcetolactateM
ACm	AcetateM
ACNL	3-Indoleacetonitrile
ACOA	Acyl-CoA
ACP	Acyl-carrier protein
ACPm	Acyl-carrier proteinM
ACTAC	Acetoacetate
ACTACm	AcetoacetateM
ACYBUT	gamma-Amino-gamma-cyanobutanoate
AD	Adenine
ADCHOR	4-amino-4-deoxychorismate
ADm	AdenineM
ADN	Adenosine
ADNm	AdenonsineM
ADP	ADP
ADPm	ADPM
ADPRIB	ADPribose
ADPRIBm	ADPriboseM
AGL3P	Acyl-sn-glycerol 3-phosphate
AHHMD	2-Amino-7,8-dihydro-4-hydroxy-6-(diphosphooxymethyl)pteridine
AHHMP	2-Amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine
AHM	4-Amino-5-hydroxymethyl-2-methylpyrimidine
AHMP	4-Amino-2-methyl-5-phosphomethylpyrimidine
AHMPP	2-Methyl-4-amino-5-hydroxymethylpyrimidine diphosphate
AHTD	2-Amino-4-hydroxy-6-(erythro-1,2,3-trihydroxypropyl)-dihydropteridine triphosphate
AICAR	1-(5'-Phosphoribosyl)-5-amino-4-imidazolecarboxamide
AIR	Aminoimidazole ribotide
AKA	2-Oxoadipate
AKAm	2-OxoadipateM

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
AKG	2-Oxoglutarate
AKGm	2-OxoglutarateM
AKP	2-Dehydropantoate
AKPm	2-DehydropantoateM
ALA	L-Alanine
ALAGLY	R-S-Alanylglycine
ALAm	L-AlanineM
ALAV	5-Aminolevulinate
ALAVm	5-AminolevulinateM
ALTRNA	L-Arginyl-tRNA(Arg)
AM6SA	2-Aminomuconate 6-semialdehyde
AMA	L-2-Aminoadipate
AMASA	L-2-Aminoadipate 6-semialdehyde
AMG	Methyl-D-glucoside
AMP	AMP
AMPm	AMPM
AMUCO	2-Aminomuconate
AN	Anthranilate
AONA	8-Amino-7-oxononanoate
APEP	Nalpha-Acetylpeptide
APROA	3-Aminopropanal
APROP	alpha-Aminopropiononitrile
APRUT	N-Acetylputrescine
APS	Adenylylsulfate
ARAB	D-Arabinose
ARABLAC	D-Arabinono-1,4-lactone
ARG	L-Arginine
ARGSUCC	N-(L-Arginino)succinate
ASER	O-Acetyl-L-serine
ASN	L-Asparagine
ASP	L-Aspartate
ASPERMD	N1-Acetylspermidine
ASPm	L-AspartateM
ASPRM	N1-Acetylspermine
ASPSA	L-Aspartate 4-semialdehyde
ASPTRNA	L-Asparaginyl-tRNA(Asn)
ASPTRNAm	L-Asparaginyl-tRNA(Asn)M
ASUC	N6-(1,2-Dicarboxyethyl)-AMP
AT3P2	Acyldihydroxyacetone phosphate
ATN	Allantoin
ATP	ATP
ATPm	ATPM
ATRNA	tRNA(Arg)
ATRP	P1,P4-Bis(5'-adenosyl) tetraphosphate

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
ATT	Allantoate
bALA	beta-Alanine
BASP	4-Phospho-L-aspartate
bDG6P	beta-D-Glucose 6-phosphate
bDGLC	beta-D-Glucose
BIO	Biotin
BT	Biotin
C100ACP	Decanoyl-[acp]
C120ACP	Dodecanoyl-[acyl-carrier protein]
C120ACPm	Dodecanoyl-[acyl-carrier protein]M
C140	Myristic acid
C140ACP	Myristoyl-[acyl-carrier protein]
C140ACPm	Myristoyl-[acyl-carrier protein]M
C141ACP	Tetradecenoyl-[acyl-carrier protein]
C141ACPm	Tetradecenoyl-[acyl-carrier protein]M
C160	Palmitate
C160ACP	Hexadecanoyl-[acp]
C160ACPm	Hexadecanoyl-[acp]M
C161	1-Hexadecene
C161ACP	Palmitoyl-[acyl-carrier protein]
C161ACPm	Palmitoyl-[acyl-carrier protein]M
C16A	C16_aldehydes
C180	Stearate
C180ACP	Stearoyl-[acyl-carrier protein]
C180ACPm	Stearoyl-[acyl-carrier protein]M
C181	1-Octadecene
C181ACP	Oleoyl-[acyl-carrier protein]
C181ACPm	Oleoyl-[acyl-carrier protein]M
C182ACP	Linolenoyl-[acyl-carrier protein]
CAASP	N-Carbamoyl-L-aspartate
CAIR	1-(5-Phospho-D-ribosyl)-5-amino-4-imidazolecarboxylate
CALH	2-(3-Carboxy-3-aminopropyl)-L-histidine
cAMP	3',5'-Cyclic AMP
CAP	Carbamoyl phosphate
CAR	Carnitine
CARm	CarnitineM
CBHCAP	3-Isopropylmalate
CBHCAPm	3-IsopropylmalateM
cCMP	3',5'-Cyclic CMP
cdAMP	3',5'-Cyclic dAMP
CDP	CDP
CDPCHO	CDPcholine
CDPDG	CDPdiacylglycerol

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
CDPDGm	CDPdiacylglycerolM
CDPETN	CDPethanolamine
CER2	Ceramide-2
CER3	Ceramide-3
CGLY	Cys-Gly
cGMP	3',5'-Cyclic GMP
CHCOA	6-Carboxyhexanoyl-CoA
CHIT	Chitin
CHITO	Chitosan
CHO	Choline
CHOR	Chorismate
cIMP	3',5'-Cyclic IMP
CIT	Citrate
CITm	CitrateM
CITR	L-Citrulline
CLm	CardiolipinM
CMP	CMP
CMPm	CMPM
CMUSA	2-Amino-3-carboxymuconate semialdehyde
CO2	CO2
CO2m	CO2M
COA	CoA
COAm	CoAM
CPAD5P	1-(2-Carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate
CPP	Coproporphyrinogen
CTP	CTP
CTPm	CTPM
CYS	L-Cysteine
CYTD	Cytidine
D45PI	1-Phosphatidyl-D-myo-inositol 4,5-bisphosphate
D6PGC	6-Phospho-D-gluconate
D6PGL	D-Glucono-1,5-lactone 6-phosphate
D6RP5P	2,5-Diamino-6-hydroxy-4-(5'-phosphoribosylamino)-pyrimidine
D8RL	6,7-Dimethyl-8-(1-D-ribityl)lumazine
DA	Deoxyadenosine
DADP	dADP
DAGLY	Diacylglycerol
DAMP	dAMP
DANNA	7,8-Diaminononanoate
DAPRP	1,3-Diaminopropane
DATP	dATP
DB4P	L-3,4-Dihydroxy-2-butanone 4-phosphate
DC	Deoxycytidine

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
DCDP	dCDP
DCMP	dCMP
DCTP	dCTP
DFUC	alpha-D-Fucoside
DG	Deoxyguanosine
DGDP	dGDP
DGMP	dGMP
DGPP	Diacylglycerol pyrophosphate
DGTP	dGTP
DHF	Dihydrofolate
DHFm	DihydrofolateM
DHMVAm	(R)-2,3-dihydroxy-3-methylbutanoateM
DHP	2-Amino-4-hydroxy-6-(D-erythro-1,2,3-trihydroxypropyl)-7,8-dihydropteridine
DHPP	Dihydroneopterin phosphate
DHPT	Dihydropteroate
DHSK	3-Dehydroshikimate
DHSP	Sphinganine 1-phosphate
DHSPH	3-Dehydrosphinganine
DHVALm	(R)-3-Hydroxy-3-methyl-2-oxobutanoateM
DIMGP	D-erythro-1-(Imidazol-4-yl)glycerol 3-phosphate
DIN	Deoxyinosine
DIPEP	Dipeptide
DMPP	Dimethylallyl diphosphate
DMZYMST	4,4-Dimethylzymosterol
DOL	Dolichol
DOLMANP	Dolichyl beta-D-mannosyl phosphate
DOLP	Dolichyl phosphate
DOROA	(S)-Dihydroorotate
DPCOA	Dephospho-CoA
DPTH	2-[3-Carboxy-3-(methylammonio)propyl]-L-histidine
DQT	3-Dehydroquinate
DR1P	Deoxy-ribose 1-phosphate
DR5P	2-Deoxy-D-ribose 5-phosphate
DRIB	Deoxyribose
DSAM	S-Adenosylmethioninamine
DT	Thymidine
DTB	Dethiobiotin
DTBm	DethiobiotinM
DTDP	dTDP
DTMP	dTMP
DTP	1-Deoxy-d-threo-2-pentulose
DTTP	dTTP
DU	Deoxyuridine

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
DUDP	dUDP
DUMP	dUMP
DUTP	dUTP
E4P	D-Erythrose 4-phosphate
EPM	Epimelibiose
EPST	Episterol
ERGOST	Ergosterol
ERTEOL	Ergosta-5,7,22,24(28)-tetraenol
ERTROL	Ergosta-5,7,24(28)-trienol
ETH	Ethanol
ETHm	EthanolM
ETHM	Ethanolamine
F1P	D-Fructose 1-phosphate
F26P	D-Fructose 2,6-bisphosphate
F6P	beta-D-Fructose 6-phosphate
FAD	FAD
FADH2m	FADH2M
FADm	FADM
FALD	Formaldehyde
FDP	beta-D-Fructose 1,6-bisphosphate
FERIm	Ferricytochrome cM
FEROm	Ferrocytochrome cM
FEST	Fecosterol
FGAM	2-(Formamido)-N1-(5'-phosphoribosyl)acetamidine
FGAR	5'-Phosphoribosyl-N-formylglycinamide
FGT	S-Formylglutathione
FKYN	L-Formylkynurenine
FMN	FMN
FMNm	FMNM
FOR	Formate
FORm	FormateM
FPP	trans,trans-Farnesyl diphosphate
FRU	D-Fructose
FTHF	10-Formyltetrahydrofolate
FTHFm	10-FormyltetrahydrofolateM
FUACAC	4-Fumarylacetoacetate
FUC	beta-D-Fucose
FUM	Fumarate
FUMm	FumarateM
G1P	D-Glucose 1-phosphate
G6P	alpha-D-Glucose 6-phosphate
GA1P	D-Glucosamine 1-phosphate
GA6P	D-Glucosamine 6-phosphate

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
GABA	4-Aminobutanoate
GABAL	4-Aminobutyraldehyde
GABALm	4-AminobutyraldehydeM
GABAm	4-AminobutanoateM
GAL1P	D-Galactose 1-phosphate
GAR	5'-Phosphoribosylglycinamide
GBAD	4-Guanidino-butanamide
GBAT	4-Guanidino-butanoate
GC	gamma-L-Glutamyl-L-cysteine
GDP	GDP
GDPm	GDPM
GDPMAN	GDPmannose
GGL	Galactosylglycerol
GL	Glycerol
GL3P	sn-Glycerol 3-phosphate
GL3Pm	sn-Glycerol 3-phosphateM
GLAC	D-Galactose
GLACL	1-alpha-D-Galactosyl-myo-inositol
GLAL	Glycolaldehyde
GLAM	Glucosamine
GLC	alpha-D-Glucose
GLN	L-Glutamine
GLP	Glycylpeptide
GLT	L-Glucitol
GLU	L-Glutamate
GLUGSAL	L-Glutamate 5-semialdehyde
GLUGSALm	L-Glutamate 5-semialdehydeM
GLUm	GlutamateM
GLUP	alpha-D-Glutamyl phosphate
GLY	Glycine
GLYCOGEN	Glycogen
GLYm	GlycineM
GLYN	Glycerone
GMP	GMP
GN	Guanine
GNm	GuanineM
GPP	Geranyl diphosphate
GSN	Guanosine
GSNm	GuanosineM
GTP	GTP
GTPm	GTPM
GTRNA	L-Glutamyl-tRNA(Glu)
GTRNAm	L-Glutamyl-tRNA(Glu)M

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
GTRP	P1,P4-Bis(5'-guanosyl) tetraphosphate
H2O2	H2O2
H2S	Hydrogen sulfide
H2SO3	Sulfite
H3MCOA	(S)-3-Hydroxy-3-methylglutaryl-CoA
H3MCOAm	(S)-3-Hydroxy-3-methylglutaryl-CoAM
HACNm	But-1-ene-1,2,4-tricarboxylateM
HACOA	(3S)-3-Hydroxyacyl-CoA
HAN	3-Hydroxyanthranilate
HBA	4-Hydroxy-benzyl alcohol
HCIT	2-Hydroxybutane-1,2,4-tricarboxylate
HCITm	2-Hydroxybutane-1,2,4-tricarboxylateM
HCYS	Homocysteine
HEXT	H+EXT
HHTRNA	L-Histidyl-tRNA(His)
HICITm	HomoisocitrateM
HIS	L-Histidine
HISOL	L-Histidinol
HISOLP	L-Histidinol phosphate
HKYN	3-Hydroxykynurenine
Hm	H+M
HMB	Hydroxymethylbilane
HOMOGEN	Homogentisate
HPRO	trans-4-Hydroxy-L-proline
HSER	L-Homoserine
HTRNA	tRNA(His)
HYXN	Hypoxanthine
IAC	Indole-3-acetate
IAD	Indole-3-acetamide
ICITm	IsocitrateM
IDP	IDP
IDPm	IDPM
IGP	Indoleglycerol phosphate
IGST	4,4-Dimethylcholesta-8,14,24-trienol
IIMZYMST	Intermediate_Methylzymosterol_II
IIZYMST	Intermediate_Zymosterol_II
ILE	L-Isoleucine
ILEm	L-IsoleucineM
IMACP	3-(Imidazol-4-yl)-2-oxopropyl phosphate
IMP	IMP
IMZYMST	Intermediate_Methylzymosterol_I
INAC	Indoleacetate
INS	Inosine

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
IPC	Inositol phosphorylceramide
IPPMAL	2-Isopropylmalate
IPPMALm	2-IsopropylmalateM
IPPP	Isopentenyl diphosphate
ISUCC	a-Iminosuccinate
ITCCOAm	Itaconyl-CoAM
ITCm	ItaconateM
ITP	ITP
ITPm	ITPM
IZYMST	Intermediate_Zymosterol_I
K	Potassium
KYN	L-Kynurenine
LAC	(R)-Lactate
LACALm	(S)-LactaldehydeM
LACm	(R)-LactateM
LCCA	a Long-chain carboxylic acid
LEU	L-Leucine
LEUm	L-LeucineM
LGT	(R)-S-Lactoylglutathione
LGTm	(R)-S-LactoylglutathioneM
LIPOm	LipoamideM
LLACm	(S)-LactateM
LLCT	L-Cystathionine
LLTRNA	L-lysyl-tRNA(Lys)
LLTRNAm	L-lysyl-tRNA(Lys)M
LNST	Lanosterol
LTRNA	tRNA(Lys)
LTRNAm	tRNA(Lys)M
LYS	L-Lysine
MACOA	2-Methylprop-2-enoyl-CoA
MAL	Malate
MALACP	Malonyl-[acyl-carrier protein]
MALACPm	Malonyl-[acyl-carrier protein]M
MALCOA	Malonyl-CoA
MALm	MalateM
MALT	Malonate
MALTm	MalonateM
MAN	alpha-D-Mannose
MAN1P	alpha-D-Mannose 1-phosphate
MAN2PD	beta-D-Mannosyldiacetylchitobiosyldiphosphodolichol
MAN6P	D-Mannose 6-phosphate
MANNAN	Mannan
MELI	Melibiose

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
MELT	Melibiitol
MET	L-Methionine
METH	Methanethiol
METHF	5,10-Methenyltetrahydrofolate
METHFm	5,10-MethenyltetrahydrofolateM
METTHF	5,10-Methylenetetrahydrofolate
METTHFm	5,10-MethylenetetrahydrofolateM
MHIS	N(pai)-Methyl-L-histidine
MI	myo-Inositol
MI1P	1L-myo-Inositol 1-phosphate
MIP2C	Inositol-mannose-P-inositol-P-ceramide
MIPC	Mannose-inositol-P-ceramide
MLT	Maltose
MMET	S-Methylmethionine
MNT	D-Mannitol
MNT6P	D-Mannitol 1-phosphate
MTHF	5-Methyltetrahydrofolate
MTHFm	5-MethyltetrahydrofolateM
MTHGXL	Methylglyoxal
MTHN	Methane
MTHNm	MethaneM
MTHPTGLU	5-Methyltetrahydropteroyltri-L-glutamate
MTRNAm	L-Methionyl-tRNAM
MVL	(R)-Mevalonate
MVLm	(R)-MevalonateM
MYOI	myo-Inositol
MZYMST	4-Methylzymsterol
N4HBZ	3-Nonaprenyl-4-hydroxybenzoate
NA	Sodium
NAADm	Deamino-NAD+M
NAC	Nicotinate
NACm	NicotinateM
NAD	NAD+
NADH	NADH
NADHm	NADHM
NADm	NAD+M
NADP	NADP+
NADPH	NADPH
NADPHm	NADPHM
NADPm	NADP+M
NAG	N-Acetylglucosamine
NAGA1P	N-Acetyl-D-glucosamine 1-phosphate
NAGA6P	N-Acetyl-D-glucosamine 6-phosphate

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
NAGLUm	N-Acetyl-L-glutamateM
NAGLUPm	N-Acetyl-L-glutamate 5-phosphateM
NAGLUSm	N-Acetyl-L-glutamate 5-semialdehydeM
NAM	Nicotinamide
NAMm	NicotinamideM
NAMN	Nicotinate D-ribonucleotide
NAMNm	Nicotinate D-ribonucleotideM
NAORNm	N2-Acetyl-L-ornithineM
NH3	NH3
NH3m	NH3M
NPP	all-trans-Nonaprenyl diphosphate
NPRAN	N-(5-Phospho-D-ribosyl)anthranilate
O2	Oxygen
O2m	OxygenM
OA	Oxaloacetate
OACOA	3-Oxoacyl-CoA
OAHSER	O-Acetyl-L-homoserine
OAm	OxaloacetateM
OBUT	2-Oxobutanoate
OBUTm	2-OxobutanoateM
OFP	Oxidized flavoprotein
OGT	Oxidized glutathione
OHB	2-Oxo-3-hydroxy-4-phosphobutanoate
OHm	HO-M
OICAP	3-Carboxy-4-methyl-2-oxopentanoate
OICAPm	3-Carboxy-4-methyl-2-oxopentanoateM
OIVAL	(R)-2-Oxoisovalerate
OIVALm	(R)-2-OxoisovalerateM
OMP	Orotidine 5'-phosphate
OMVALm	3-Methyl-2-oxobutanoateM
OPEP	Oligopeptide
ORN	L-Ornithine
ORNm	L-OrnithineM
OROA	Orotate
OSLHSER	O-Succinyl-L-homoserine
OSUC	Oxalosuccinate
OSUCm	OxalosuccinateM
OTHIO	Oxidized thioredoxin
OTHIOm	Oxidized thioredoxinM
OXA	Oxaloglutarate
OXAm	OxaloglutarateM
P5C	(S)-1-Pyrroline-5-carboxylate
P5Cm	(S)-1-Pyrroline-5-carboxylateM

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
P5P	Pyridoxine phosphate
PA	Phosphatidate
PABA	4-Aminobenzoate
PAC	Phenylacetic acid
PAD	2-Phenylacetamide
PALCOA	Palmitoyl-CoA
PAm	PhosphatidateM
PANT	(R)-Pantoate
PANTm	(R)-PantoateM
PAP	Adenosine 3',5'-bisphosphate
PAPS	3'-Phosphoadenylylsulfate
PBG	Porphobilinogen
PC	Phosphatidylcholine
PC2	Sirohydrochlorin
PCHO	Choline phosphate
PDLA	Pyridoxamine
PDLA5P	Pyridoxamine phosphate
PDME	Phosphatidyl-N-dimethylethanolamine
PE	Phosphatidylethanolamine
PEm	PhosphatidylethanolamineM
PEP	Phosphoenolpyruvate
PEPD	Peptide
PEPm	PhosphoenolpyruvateM
PEPT	Peptide
PETHM	Ethanolamine phosphate
PGm	PhosphatidylglycerolM
PGPm	PhosphatidylglycerophosphateM
PHC	L-1-Pyrroline-3-hydroxy-5-carboxylate
PHE	L-Phenylalanine
PHP	3-Phosphonooxypyruvate
PHPYR	Phenylpyruvate
PHSER	O-Phospho-L-homoserine
PHSP	Phytosphingosine 1-phosphate
PHT	O-Phospho-4-hydroxy-L-threonine
PI	Orthophosphate
PIm	OrthophosphateM
PIME	Pimelic Acid
PINS	1-Phosphatidyl-D-myo-inositol
PINS4P	1-Phosphatidyl-1D-myo-inositol 4-phosphate
PINSP	1-Phosphatidyl-1D-myo-inositol 3-phosphate
PL	Pyridoxal
PL5P	Pyridoxal phosphate
PMME	Phosphatidyl-N-methylethanolamine

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
PMVL	(R)-5-Phosphomevalonate
PNTO	(R)-Pantothenate
PPHG	Protoporphyrinogen IX
PPHGm	Protoporphyrinogen IXM
PPI	Pyrophosphate
PPIm	PyrophosphateM
PPIXm	ProtoporphyrinM
PPMAL	2-Isopropylmaleate
PPMVL	(R)-5-Diphosphomevalonate
PRAM	5-Phosphoribosylamine
PRBAMP	N1-(5-Phospho-D-ribosyl)-AMP
PRBATP	N1-(5-Phospho-D-ribosyl)-ATP
PRFICA	1-(5'-Phosphoribosyl)-5-formamido-4-imidazolecarboxamide
PRFP	5-(5-Phospho-D-ribosylaminoformimino)-1-(5-phosphoribosyl)-imidazole-4-carboxamide
PRLP	N-(5'-Phospho-D-1'-ribulosylformimino)-5-amino-1-(5''-phospho-D-ribosyl)-4-imidazolecarboxamide
PRO	L-Proline
PROm	L-ProlineM
PRPP	5-Phospho-alpha-D-ribose 1-diphosphate
PRPPm	5-Phospho-alpha-D-ribose 1-diphosphateM
PS	Phosphatidylserine
PSm	PhosphatidylserineM
PSPH	Phytosphingosine
PTHm	HemeM
PTRSC	Putrescine
PURI5P	Pseudouridine 5'-phosphate
PYR	Pyruvate
PYRDX	Pyridoxine
PYRm	PyruvateM
QA	Pyridine-2,3-dicarboxylate
QH2m	UbiquinolM
Qm	Ubiquinone-9M
R1P	D-Ribose 1-phosphate
R5P	D-Ribose 5-phosphate
RAF	Raffinose
RFP	Reduced flavoprotein
RGT	Glutathione
RGTm	GlutathioneM
RIB	D-Ribose
RIBFLAV	Riboflavin
RIBFLAVm	RiboflavinM
RIPm	alpha-D-Ribose 1-phosphateM
RL5P	D-Ribulose 5-phosphate
RMN	D-Rhamnose

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
RTHIO	Reduced thioredoxin
RTHIOm	Reduced thioredoxinM
S17P	Sedoheptulose 1,7-bisphosphate
S23E	(S)-2,3-Epoxysqualene
S7P	Sedoheptulose 7-phosphate
SACP	N6-(L-1,3-Dicarboxypropyl)-L-lysine
SAH	S-Adenosyl-L-homocysteine
SAHm	S-Adenosyl-L-homocysteineM
SAICAR	1-(5'-Phosphoribosyl)-5-amino-4-(N-succinocarboxamide)-imidazole
SAM	S-Adenosyl-L-methionine
SAMm	S-Adenosyl-L-methionineM
SAMOB	S-Adenosyl-4-methylthio-2-oxobutanoate
SAPm	S-AminomethyldihydrolipoylproteinM
SER	L-Serine
SERm	L-SerineM
SLF	Sulfate
SLFm	SulfateM
SME	Shikimate
SME5P	Shikimate 3-phosphate
SOR	Sorbose
SOT	D-Sorbitol
SPH	Sphinganine
SPRM	Spermine
SPRMD	Spermidine
SQL	Squalene
SUC	Sucrose
SUCC	Succinate
SUCCm	SuccinateM
SUCCOAm	Succinyl-CoAM
T3P1	D-Glyceraldehyde 3-phosphate
T3P2	Glycerone phosphate
T3P2m	Glycerone phosphateM
TAG16P	D-Tagatose 1,6-bisphosphate
TAG6P	D-Tagatose 6-phosphate
TAGLY	Triacylglycerol
TCOA	Tetradecanoyl-CoA
TGLP	N-Tetradecanoylglycylpeptide
THF	Tetrahydrofolate
THFG	Tetrahydrofolyl-[Glu](n)
THFm	TetrahydrofolateM
THIAMIN	Thiamin
THMP	Thiamin monophosphate
THPTGLU	Tetrahydropteroyltri-L-glutamate

Table A.1. Metabolites described in FBA (continued)

Abbreviation	Metabolite
THR	L-Threonine
THRm	L-ThreonineM
THY	Thymine
THZ	5-(2-Hydroxyethyl)-4-methylthiazole
THZP	4-Methyl-5-(2-phosphoethyl)-thiazole
TPI	D-myo-inositol 1,4,5-trisphosphate
TPP	Thiamin diphosphate
TPPP	Thiamin triphosphate
TRE	alpha,alpha-Trehalose
TRE6P	alpha,alpha'-Trehalose 6-phosphate
TRNA	tRNA
TRNAm	tRNAM
TRP	L-Tryptophan
TRPm	L-TryptophanM
TRPTRNAm	L-Tryptophanyl-tRNA(Trp)M
TYR	L-Tyrosine
UDP	UDP
UDPG	UDPglucose
UDPGAL	UDP-D-galactose
UDPNAG	UDP-N-acetyl-D-galactosamine
UDPP	Undecaprenyl diphosphate
UGC	(-)-Ureidoglycolate
UMP	UMP
UPRG	Uroporphyrinogen III
URA	Uracil
UREA	Urea
UREAC	Urea-1-carboxylate
URI	Uridine
UTP	UTP
X5P	D-Xylose-5-phosphate
XAN	Xanthine
XMP	Xanthosine 5'-phosphate
XTSINE	Xanthosine
XUL	D-Xylulose
XYL	D-Xylose
ZYMST	Zymosterol

Table A.2. Reactions described in FBA

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
# CARBOHYDRATE METABOLISM				
# GLYCOLYSIS/GLUCONEOGENESIS				
YCL040W	2.7.1.2	*GLK1*	GLUCOKINASE	GLC + ATP -> G6P + ADP
YCL040W	2.7.1.2	*GLK1*	GLUCOKINASE	MAN + ATP -> MAN6P + ADP
YCL040W	2.7.1.2	*GLK1*	GLUCOKINASE	bDGLC + ATP -> bDG6P + ADP
YFR053C	2.7.1.1	*HXK1*	HEXOKINASE I (PI) (ALSO CALLED HEXOKINASE A)	bDGLC + ATP -> G6P + ADP
YFR053C	2.7.1.1	*HXK1*	HEXOKINASE I (PI) (ALSO CALLED HEXOKINASE A)	GLC + ATP -> G6P + ADP
YFR053C	2.7.1.1	*HXK1*	HEXOKINASE I (PI) (ALSO CALLED HEXOKINASE A)	MAN + ATP -> MAN6P + ADP
YFR053C	2.7.1.1	*HXK1*	HEXOKINASE I (PI) (ALSO CALLED HEXOKINASE A)	ATP + FRU -> ADP + F6P
YGL253W	2.7.1.1	*HXK2*	HEXOKINASE II (PII) (ALSO CALLED HEXOKINASE B)	bDGLC + ATP -> G6P + ADP
YGL253W	2.7.1.1	*HXK2*	HEXOKINASE II (PII) (ALSO CALLED HEXOKINASE B)	GLC + ATP -> G6P + ADP
YGL253W	2.7.1.1	*HXK2*	HEXOKINASE II (PII) (ALSO CALLED HEXOKINASE B)	MAN + ATP -> MAN6P + ADP
YGL253W	2.7.1.1	*HXK2*	HEXOKINASE II (PII) (ALSO CALLED HEXOKINASE B)	ATP + FRU -> ADP + F6P
YBR196C	5.3.1.9	*PGI1*	GLUCOSE-6-PHOSPHATE ISOMERASE	G6P <-> F6P
YBR196C	5.3.1.9	*PGI1*	GLUCOSE-6-PHOSPHATE ISOMERASE	G6P <-> bDG6P
YBR196C	5.3.1.9	*PGI1*	GLUCOSE-6-PHOSPHATE ISOMERASE	bDG6P <-> F6P
YMR205C	2.7.1.11	*PFK2*	PHOSPHOFRUCTOKINASE BETA SUBUNIT	F6P + ATP -> FDP + ADP
YGR240C	2.7.1.11	*PFK1*	PHOSPHOFRUCTOKINASE ALPHA SUBUNIT	F6P + ATP -> FDP + ADP
YGR240C	2.7.1.11	*PFK1*	PHOSPHOFRUCTOKINASE ALPHA SUBUNIT	ATP + TAG6P -> ADP + TAG16P
YGR240C	2.7.1.11	*PFK1*	PHOSPHOFRUCTOKINASE ALPHA SUBUNIT	ATP + S7P -> ADP + S17P
YKL060C	4.1.2.13	*FBA1*	FRUCTOSE-BISPHOSPHATE ALDOLASE	FDP <-> T3P2 + T3P1
YDR050C	5.3.1.1	*TPI1*	TRIOSEPHOSPHATE ISOMERASE	T3P2 <-> T3P1
YJL052W	1.2.1.12	*TDH1*	GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE 1	T3P1 + PI + NAD <-> NADH + 13PDG
YJR009C	1.2.1.12	*TDH2*	GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE	T3P1 + PI + NAD <-> NADH + 13PDG
YGR192C	1.2.1.12	*TDH3*	GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE 3	T3P1 + PI + NAD <-> NADH + 13PDG
YCR012W	2.7.2.3	*PGK1*	PHOSPHOGLYCERATE KINASE	13PDG + ADP <-> 3PG + ATP
YKL152C	5.4.2.1	*GPM1*	PHOSPHOGLYCERATE MUTASE	13PDG <-> 23PDG
YKL152C	5.4.2.1	*GPM1*	PHOSPHOGLYCERATE MUTASE	3PG <-> 2PG

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YDL021W	5.4.2.1	*GPM2*	SIMILAR TO GPM1 (PHOSPHOGLYCERATE MUTASE)	3PG <-> 2PG
YOL056W	5.4.2.1	*GPM3*	PHOSPHOGLYCERATE MUTASE	3PG <-> 2PG
YGR254W	4.2.1.11	*ENO1*	ENOLASE I	2PG <-> PEP
YHR174W	4.2.1.11	*ENO2*	ENOLASE	2PG <-> PEP
YMR323W	4.2.1.11	*ERR1*	PROTEIN WITH SIMILARITY TO ENOLASES	2PG <-> PEP
YPL281C	4.2.1.11	*ERR2*	ENOLASE RELATED PROTEIN	2PG <-> PEP
YOR393W	4.2.1.11	*ERR1*	ENOLASE RELATED PROTEIN	2PG <-> PEP
YAL038W	2.7.1.40	*CDC19*	PYRUVATE KINASE	PEP + ADP -> PYR + ATP
YOR347C	2.7.1.40	*PYK2*	PYRUVATE KINASE, GLUCOSE-REPRESSED ISOFORM	PEP + ADP -> PYR + ATP
YER178W	1.2.4.1	*PDA1*	PYRUVATE DEHYDROGENASE (LIPOAMIDE) ALPHA CHAIN PRECURSOR, E1 COMPONENT, ALPHA UNIT	PYRm + COAm + NADm -> NADHm + CO2m + ACCOAm
YBR221C	1.2.4.1	*PDB1*	PYRUVATE DEHYDROGENASE (LIPOAMIDE) BETA CHAIN PRECURSOR, E1 COMPONENT, BETA UNIT	
YNL071W	2.3.1.12	*LAT1*	DIHYDROLIPOAMIDE S-ACETYLTRANSFERASE, E2 COMPONENT	
# CITRATE CYCLE (TCA CYCLE)				
YNR001C	4.1.3.7	*CIT1*	CITRATE SYNTHASE, NUCLEAR ENCODED MITOCHONDRIAL PROTEIN.	ACCOAm + OAm -> COAm + CITm
YCR005C	4.1.3.7	*CIT2*	CITRATE SYNTHASE, NON-MITOCHONDRIAL CITRATE SYNTHASE	ACCOA + OA -> COA + CIT
YPR001W	4.1.3.7	*CIT3*	CITRATE SYNTHASE, MITOCHONDRIAL ISOFORM OF CITRATE SYNTHASE	ACCOAm + OAm -> COAm + CITm
YLR304C	4.2.1.3	*ACO1*	ACONITASE, MITOCHONDRIAL	CITm <-> ICITm
YJL200C	4.2.1.3	*YJL200C*	ACONITATE HYDRATASE HOMOLOG	CITm <-> ICITm
YNL037C	1.1.1.41	*IDH1*	ISOCITRATE DEHYDROGENASE (NAD+) MITO, SUBUINT1	ICITm + NADm -> CO2m + NADHm + AKGm
YOR136W	1.1.1.41	*IDH2*	ISOCITRATE DEHYDROGENASE (NAD+) MITO, SUBUNIT2	
YDL066W	1.1.1.42	*IDP1*	ISOCITRATE DEHYDROGENASE (NADP+)	ICITm + NADPm -> NADPHm + OSUCm
YLR174W	1.1.1.42	*IDP2*	ISOCITRATE DEHYDROGENASE (NADP+)	ICIT + NADP -> NADPH + OSUC
YNL009W	1.1.1.42	*IDP3*	ISOCITRATE DEHYDROGENASE (NADP+)	ICIT + NADP -> NADPH + OSUC
YDL066W	1.1.1.42	*IDP1*	ISOCITRATE DEHYDROGENASE (NADP+)	OSUCm -> CO2m + AKGm
YLR174W	1.1.1.42	*IDP2*	ISOCITRATE DEHYDROGENASE (NADP+)	OSUC -> CO2 + AKG
YNL009W	1.1.1.42	*IDP3*	ISOCITRATE DEHYDROGENASE (NADP+)	OSUC -> CO2 + AKG
YIL125W	1.2.4.2	*KGD1*	ALPHA-KETOGLUTARATE DEHYDROGENASE COMPLEX, E1 COMPONENT	AKGm + NADm + COAm -> CO2m + NADHm + SUCCOAm

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C.#	GENE	GENE DESCRIPTION	REACTION
YDR148C	2.3.1.61	*KGD2*	DIHYDROLIPOAMIDE S-SUCCINYLTRANSFERASE, E2 COMPONENT	
YGR244C	6.2.1.4/6.2.1.5	*LSC2*	SUCCINATE--COA LIGASE (GDP-FORMING)	ATPm + SUCCm + COAm <-> ADPm + PIm + SUCCOAm
YOR142W	6.2.1.4/6.2.1.5	*LSC1*	SUCCINATE-COA LIGASE ALPHA SUBUNIT	ATPm + ITCm + COAm <-> ADPm + PIm + ITCCOAm
# ELECTRON TRANSPORT SYSTEM, COMPLEX II				
YKL141W	1.3.5.1	*SDH3*	SUCCINATE DEHYDROGENASE CYTOCHROME B	SUCCm + FADm <-> FUMm + FADH2m
YKL148C	1.3.5.1	*SDH1*	SUCCINATE DEHYDROGENASE CYTOCHROME B	
YLL041C	1.3.5.1	*SDH2*	SUCCINATE DEHYDROGENASE (UBIQUINONE) IRON-SULFUR PROTEIN SUBUNIT	
YDR178W	1.3.5.1	*SDH4*	SUCCINATE DEHYDROGENASE MEMBRANE ANCHOR SUBUNIT	
YLR164W	1.3.5.1	*YLR164W*	STRONG SIMILARITY TO SDH4P	
YMR118C	1.3.5.1	*YMR118C*	STRONG SIMILARITY TO SUCCINATE DEHYDROGENASE	
YJL045W	1.3.5.1	*YJL045W*	STRONG SIMILARITY TO SUCCINATE DEHYDROGENASE FLAVOPROTEIN	
YEL047C	1.3.99.1	*YEL047C*	SOLUBLE FUMARATE REDUCTASE, CYTOPLASMIC	FADH2m + FUM -> SUCC + FADm
YJR051W	1.3.99.1	*OSM1*	MITOCHONDRIAL SOLUBLE FUMARATE REDUCTASE INVOLVED IN OSMOTIC REGULATION	FADH2m + FUMm -> SUCCm + FADm
YPL262W	4.2.1.2	*FUM1*	FUMARATASE	FUMm <-> MALm
YPL262W	4.2.1.2	*FUM1*	FUMARATASE	FUM <-> MAL
YKL085W	1.1.1.37	*MDH1*	MITOCHONDRIAL MALATE DEHYDROGENASE	MALm + NADm <-> NADHm + OAm
YDL078C	1.1.1.37	*MDH3*	MALATE DEHYDROGENASE, PEROXISOMAL	MAL + NAD <-> NADH + OA
YOL126C	1.1.1.37	*MDH2*	MALATE DEHYDROGENASE, CYTOPLASMIC	MAL + NAD <-> NADH + OA
# ANAPLEROTIC REACTIONS				
YER065C	4.1.3.1	*ICL1*	ISOCITRATE LYASE	ICIT -> GLX + SUCC
YPR006C	4.1.3.1	*ICL2*	ISOCITRATE LYASE, MAY BE NONFUNCTIONAL	ICIT -> GLX + SUCC
YIR031C	4.1.3.2	*DAL7*	MALATE SYNTHASE	ACCOA + GLX -> COA + MAL
YNL117W	4.1.3.2	*MLS1*	MALATE SYNTHASE	ACCOA + GLX -> COA + MAL
YKR097W	4.1.1.49	*PCK1*	PHOSPHOENOLPYRUVATE CARBOXYLKINASE	OA + ATP -> PEP + CO2 + ADP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YLR377C	3.1.3.11	*FBP1*	FRUCTOSE-1,6-BISPHOSPHATASE	FDP -> F6P + PI
YGL062W	6.4.1.1	*PYC1*	PYRUVATE CARBOXYLASE	PYR + ATP + CO2 -> ADP + OA + PI
YBR218C	6.4.1.1	*PYC2*	PYRUVATE CARBOXYLASE	PYR + ATP + CO2 -> ADP + OA + PI
YKL029C	1.1.1.38	*MAE1*	MITOCHONDRIAL MALIC ENZYME	MALm + NADPm -> CO2m + NADPHm + PYRm
# PENTOSE PHOSPHATE CYCLE				
YNL241C	1.1.1.49	*ZWF1*	GLUCOSE-6-PHOSPHATE-1-DEHYDROGENASE	G6P + NADP <-> D6PGL + NADPH
YNR034W	3.1.1.31	*SOL1*	POSSIBLE 6-PHOSPHOGLUCONOLACTONASE	D6PGL -> D6PGC
YCR073W-A	3.1.1.31	*SOL2*	POSSIBLE 6-PHOSPHOGLUCONOLACTONASE	D6PGL -> D6PGC
YHR163W	3.1.1.31	*SOL3*	POSSIBLE 6-PHOSPHOGLUCONOLACTONASE	D6PGL -> D6PGC
YGR248W	3.1.1.31	*SOL4*	POSSIBLE 6-PHOSPHOGLUCONOLACTONASE	D6PGL -> D6PGC
YGR256W	1.1.1.44	*GND2*	6-PHOPHOGLUCONATE DEHYDROGENASE	D6PGC + NADP -> NADPH + CO2 + RL5P
YHR183W	1.1.1.44	*GND1*	6-PHOPHOGLUCONATE DEHYDROGENASE	D6PGC + NADP -> NADPH + CO2 + RL5P
YJL121C	5.1.3.1	*RPE1*	RIBULOSE-5-P 3-EPIMERASE	RL5P <-> X5P
YOR095C	5.3.1.6	*RKI1*	RIBOSE-5-P ISOMERASE	RL5P <-> R5P
YBR117C	2.2.1.1	*TKL2*	TRANSKETOLASE	R5P + X5P <-> T3P1 + S7P
YBR117C	2.2.1.1	*TKL2*	TRANSKETOLASE	X5P + E4P <-> F6P + T3P1
YPR074C	2.2.1.1	*TKL1*	TRANSKETOLASE	R5P + X5P <-> T3P1 + S7P
YPR074C	2.2.1.1	*TKL1*	TRANSKETOLASE	X5P + E4P <-> F6P + T3P1
YLR354C	2.2.1.2	*TAL1*	TRANSALDOLASE	T3P1 + S7P <-> E4P + F6P
YGR043C	2.2.1.2	*YGR043C*	TRANSALDOLASE	T3P1 + S7P <-> E4P + F6P
YCR036W	2.7.1.15	*RBK1*	RIBOKINASE	RIB + ATP -> R5P + ADP
YCR036W	2.7.1.15	*RBK1*	RIBOKINASE	DRIB + ATP -> DR5P + ADP
YKL127W	5.4.2.2	*PGM1*	PHOSPHOGLUCOMUTASE	R1P <-> R5P
YKL127W	5.4.2.2	*PGM1*	PHOSPHOGLUCOMUTASE 1	G1P <-> G6P
YMR105C	5.4.2.2	*PGM2*	PHOSPHOGLUCOMUTASE	R1P <-> R5P
YMR105C	5.4.2.2	*PGM2*	PHOSPHOGLUCOMUTASE	G1P <-> G6P
# MANNOSE				
YER003C	5.3.1.8	*PMI40*	MANNOSE-6-PHOSPHATE ISOMERASE	MAN6P <-> F6P
YFL045C	5.4.2.8	*SEC53*	PHOSPHOMANNOMUTASE	MAN6P <-> MAN1P
YDL055C	2.7.7.13	*PSA1*	MANNOSE-1-PHOSPHATE GUANYLTRANSFERASE, GDP-MANNOSE PYROPHOSPHORYLASE	GTP + MAN1P -> PPI + GDPMAN

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
# FRUCTOSE				
YIL107C	2.7.1.105	*PFK26*	6-PHOSPHOFRUCTOSE-2-KINASE	ATP + F6P -> ADP + F26P
YOL136C	2.7.1.105	*PFK27*	6-PHOSPHOFRUCTO-2-KINASE	ATP + F6P -> ADP + F26P
YJL155C	3.1.3.46	*FBP26*	FRUCTOSE-2,6-BIPHOSPHATASE	F26P -> F6P + PI
*U1_*	2.7.1.56	*U1_*	1-PHOSPHOFRUCTOKINASE (FRUCTOSE 1-PHOSPHATE KINASE)	F1P + ATP -> FDP + ADP
# SORBOSE				
YJR159W	1.1.1.14	*SOR1*	SORBITOL DEHYDROGENASE (L-IDITOL 2-DEHYDROGENASE)	SOT + NAD -> FRU + NADH
# GALACTOSE METABOLISM				
YBR020W	2.7.1.6	*GAL1*	GALACTOKINASE	GLAC + ATP -> GAL1P + ADP
YBR018C	2.7.7.10	*GAL7*	GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE	UTP + GAL1P <-> PPI + UDPGAL
YBR019C	5.1.3.2	*GAL10*	UDP-GLUCOSE 4-EPIMERASE	UDPGAL <-> UDPG
YHL012W	2.7.7.9	*YHL012W*	UTP--GLUCOSE 1-PHOSPHATE URIDYLYLTRANSFERASE	G1P + UTP <-> UDPG + PPI
YKL035W	2.7.7.9	*UGP1*	URIDINEPHOSPHOGLUCOSE PYROPHOSPHORYLASE	G1P + UTP <-> UDPG + PPI
YBR184W	3.2.1.22	*YBR184W*	ALPHA-GALACTOSIDASE (MELIBIASE)	MELI -> GLC + GLAC
YBR184W	3.2.1.22	*YBR184W*	ALPHA-GALACTOSIDASE (MELIBIASE)	DFUC -> GLC + GLAC
YBR184W	3.2.1.22	*YBR184W*	ALPHA-GALACTOSIDASE (MELIBIASE)	RAF -> GLAC + SUC
YBR184W	3.2.1.22	*YBR184W*	ALPHA-GALACTOSIDASE (MELIBIASE)	GLACL <-> MYOI + GLAC
YBR184W	3.2.1.22	*YBR184W*	ALPHA-GALACTOSIDASE (MELIBIASE)	EPM <-> MAN + GLAC
YBR184W	3.2.1.22	*YBR184W*	ALPHA-GALACTOSIDASE (MELIBIASE)	GGL <-> GL + GLAC
YBR184W	3.2.1.22	*YBR184W*	ALPHA-GALACTOSIDASE (MELIBIASE)	MELT <-> SOT + GLAC
YBR299W	3.2.1.20	*MAL32*	MALTASE	MLT -> 2 GLC
YGR287C	3.2.1.20	*YGR287C*	PUTATIVE ALPHA GLUCOSIDASE	MLT -> 2 GLC
YGR292W	3.2.1.20	*MAL12*	MALTASE	MLT -> 2 GLC
YIL172C	3.2.1.20	*YIL172C*	PUTATIVE ALPHA GLUCOSIDASE	MLT -> 2 GLC
YJL216C	3.2.1.20	*YJL216C*	PROBABLE ALPHA-GLUCOSIDASE (MALTASE)	MLT -> 2 GLC
YJL221C	3.2.1.20	*FSP2*	HOMOLOGY TO MALTASE(ALPHA-D-GLUCOSIDASE)	MLT -> 2 GLC
YJL221C	3.2.1.20	*FSP2*	HOMOLOGY TO MALTASE(ALPHA-D-GLUCOSIDASE)	6DGLC -> GLAC + GLC
YBR018C	2.7.7.12	*GAL7*	UDPGLUCOSE--HEXOSE-1-PHOSPHATE URIDYLYLTRANSFERASE	UDPG + GAL1P <-> G1P + UDPGAL
# TREHALOSE				
YBR126C	2.4.1.15	*TPS1*	TREHALOSE-6-P SYNTHETASE, 56 KD SYNTHASE SUBUNIT OF TREHALOSE-6-PHOSPHATE SYNTHASE\PHOSPHATASE COMPLEX	UDPG + G6P -> UDP + TRE6P

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YML100W	2.4.1.15	*TSL1*	TREHALOSE-6-P SYNTHETASE, 123 KD REGULATORY SUBUNIT OF TREHALOSE-6-PHOSPHATE SYNTHASE\PHOSPHATASE COMPLEX\; HOMOLOGOUS TO TPS3 GENE PRODUCT	UDPG + G6P -> UDP + TRE6P
YMR261C	2.4.1.15	*TPS3*	TREHALOSE-6-P SYNTHETASE, 115 KD REGULATORY SUBUNIT OF TREHALOSE-6-PHOSPHATE SYNTHASE\PHOSPHATASE COMPLEX	UDPG + G6P -> UDP + TRE6P
YDR074W	3.1.3.12	*TPS2*	TREHALOSE-6-PHOSPHATE PHOSPHATASE	TRE6P -> TRE + PI
YPR026W	3.2.1.28	*ATH1*	ACID TREHALASE	TRE -> 2 GLC
YBR001C	3.2.1.28	*NTH2*	NEUTRAL TREHALASE, HIGHLY HOMOLOGOUS TO NTH1P	TRE -> 2 GLC
YDR001C	3.2.1.28	*NTH1*	NEUTRAL TREHALASE	TRE -> 2 GLC
# GLYCOGEN METABOLISM (SUCOROSE AND SUGAR METABOLISM)				
YEL011W	2.4.1.18	*GLC3*	BRANCHING ENZYME, 1,4-GLUCAN-6-(1,4-GLUCANO)-TRANSFERASE	GLYCOGEN + PI -> G1P
YPR160W	2.4.1.1	*GPH1*	GLYCOGEN PHOSPHORYLASE	GLYCOGEN + PI -> G1P
YFR015C	2.4.1.11	*GSY1*	GLYCOGEN SYNTHASE (UDP-GLUOCSE--STARCH GLUCOSYLTRANSFERASE)	UDPG -> UDP + GLYCOGEN
YLR258W	2.4.1.11	*GSY2*	GLYCOGEN SYNTHASE (UDP-GLUOCSE--STARCH GLUCOSYLTRANSFERASE)	UDPG -> UDP + GLYCOGEN
# PYRUVATE METABOLISM				
YAL054C	6.2.1.1	*ACS1*	ACETYL-COENZYME A SYNTHETASE	ATP + AC + COA -> AMP + PPI + ACCOA
YLR153C	6.2.1.1	*ACS2*	ACETYL-COENZYME A SYNTHETASE	ATP + AC + COA -> AMP + PPI + ACCOA
YDL168W	1.2.1.1	*SFA1*	FORMALDEHYDE DEHYDROGENASE/LONG-CHAIN ALCOHOL DEHYDROGENASE	FALD + RGT + NAD <-> FGT + NADH
YJL068C	3.1.2.12	*YJL068C*	S-FORMYLGLUTATHIONE HYDROLASE	FGT <-> RGT + FOR
YGR087C	4.1.1.1	*PDC6*	PYRUVATE DECARBOXYLASE	PYR -> CO2 + ACAL
YLR134W	4.1.1.1	*PDC5*	PYRUVATE DECARBOXYLASE	PYR -> CO2 + ACAL
YLR044C	4.1.1.1	*PDC1*	PYRUVATE DECARBOXYLASE	PYR -> CO2 + ACAL
YBL015W	3.1.2.1	*ACH1*	ACETYL COA HYDROLASE	ACCOA -> COA + AC
YDL131W	4.1.3.21	*LYS21*	PROBABLE HOMOCITRATE SYNTHASE, MITOCHONDRIAL ISOZYME PRECURSOR	ACCOA + AKG -> HCIT + COA
YDL182W	4.1.3.21	*LYS20*	HOMOCITRATE SYNTHASE, CYTOSOLIC ISOZYME	ACCOA + AKG -> HCIT + COA
YDL182W	4.1.3.21	*LYS20*	HOMOCITRATE SYNTHASE	ACCOAm + AKGm -> HCITm + COAm

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YGL256W	1.1.1.1	*ADH4*	ALCOHOL DEHYDROGENASE ISOENZYME IV	ETH + NAD <-> ACAL + NADH
YMR083W	1.1.1.1	*ADH3*	ALCOHOL DEHYDROGENASE ISOENZYME III	ETHm + NADm <-> ACALm + NADHm
YMR303C	1.1.1.1	*ADH2*	ALCOHOL DEHYDROGENASE II	ETH + NAD <-> ACAL + NADH
YBR145W	1.1.1.1	*ADH5*	ALCOHOL DEHYDROGENASE ISOENZYME V	ETH + NAD <-> ACAL + NADH
YOL086C	1.1.1.1	*ADH1*	ALCOHOL DEHYDROGENASE I	ETH + NAD <-> ACAL + NADH
YDL168W	1.1.1.1	*SFA1*	ALCOHOL DEHYDROGENASE I	ETH + NAD <-> ACAL + NADH
# GLYOXYLATE AND DICARBOXYLATE METABOLISM				
# GLYOXAL PATHWAY				
YML004C	4.4.1.5	*GLO1*	LACTOYLGLUTATHIONE LYASE, GLYOXALASE I	RGT + MTHGXL <-> LGT
YDR272W	3.1.2.6	*GLO2*	HYDROXYACYLGLUTATHIONE HYDROLASE	LGT -> RGT + LAC
YOR040W	3.1.2.6	*GLO4*	GLYOXALASE II (HYDROXYACYLGLUTATHIONE HYDROLASE)	LGTm -> RGTm + LACm
# ENERGY METABOLISM				
# OXIDATIVE PHOSPHORYLATION				
YBR011C	3.6.1.1	*IPP1*	INORGANIC PYROPHOSPHATASE	PPI -> 2 PI
YMR267W	3.6.1.1	*PPA2*	MITOCHONDRIAL INORGANIC PYROPHOSPHATASE	PPIm -> 2 PIm
*U2_*	1.2.2.1	*U2_*	FORMATE DEHYDROGENASE	FOR + Qm -> QH2m + CO2 +2 HEXT
YML120C	1.6.5.3	*NDI1*	NADH DEHYDROGENASE (UBIQUINONE)	NADHm + Qm -> QH2m + NADm
YDL085W	1.6.5.3	*NDH2*	MITOCHONDRIAL NADH DEHYDROGENASE THAT CATALYZES THE OXIDATION OF CYTOSOLIC NADH	NADH + Qm -> QH2m + NAD
YMR145C	1.6.5.3	*NDH1*	MITOCHONDRIAL NADH DEHYDROGENASE THAT CATALYZES THE OXIDATION OF CYTOSOLIC NADH	NADH + Qm -> QH2m + NAD
YHR042W	1.6.2.4	*NCP1*	NADPH--FERRIHEMOPROTEIN REDUCTASE	NADPH + 2 FERIm -> NADP + 2 FEROm
YKL141W	1.3.5.1	*SDH3*	SUCCINATE DEHYDROGENASE CYTOCHROME B	FADH2m + Qm <-> FADm + QH2m
YKL148C	1.3.5.1	*SDH1*	SUCCINATE DEHYDROGENASE CYTOCHROME B	
YLL041C	1.3.5.1	*SDH2*	SUCCINATE DEHYDROGENASE CYTOCHROME B	
YDR178W	1.3.5.1	*SDH4*	SUCCINATE DEHYDROGENASE CYTOCHROME B	

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
# ELECTRON TRANSPORT SYSTEM, COMPLEX III				
YEL024W	1.10.2.2	*RIP1*	UBIQUINOL-CYTOCHROME C REDUCTASE IRON-SULFUR SUBUNIT	QH2m + 2 FERIm + 1.5 Hm -> Qm + 2 FEROm
Q0105	1.10.2.2	*CYTB*	UBIQUINOL-CYTOCHROME C REDUCTASE CYTOCHROME B SUBUNIT	
YOR065W	1.10.2.2	*CYT1*	UBIQUINOL-CYTOCHROME C REDUCTASE CYTOCHROME C1 SUBUNIT	
YBL045C	1.10.2.2	*COR1*	UBIQUINOL-CYTOCHROME C REDUCTASE CORE SUBUNIT 1	
YPR191W	1.10.2.2	*QCR1*	UBIQUINOL-CYTOCHROME C REDUCTASE CORE SUBUNIT 2	
YPR191W	1.10.2.2	*QCR2*	UBIQUINOL-CYTOCHROME C REDUCTASE	
YFR033C	1.10.2.2	*QCR6*	UBIQUINOL-CYTOCHROME C REDUCTASE SUBUNIT 6	
YDR529C	1.10.2.2	*QCR7*	UBIQUINOL-CYTOCHROME C REDUCTASE SUBUNIT 7	
YJL166W	1.10.2.2	*QCR8*	UBIQUINOL-CYTOCHROME C REDUCTASE SUBUNIT 8	
YGR183C	1.10.2.2	*QCR9*	UBIQUINOL-CYTOCHROME C REDUCTASE SUBUNIT 9	
YHR001W-A	1.10.2.2	*QCR10*	UBIQUINOL-CYTOCHROME C REDUCTASE SUBUNIT 10	
# ELECTRON TRANSPORT SYSTEM, COMPLEX IV				
Q0045	1.9.3.1	*COX1*	CYTOCHROME C OXIDASE SUBUNIT I	4 FEROm + O2m + 6 Hm -> 4 FERIm
Q0250	1.9.3.1	*COX2*	CYTOCHROME C OXIDASE SUBUNIT I	
Q0275	1.9.3.1	*COX3*	CYTOCHROME C OXIDASE SUBUNIT I	
YDL067C	1.9.3.1	*COX9*	CYTOCHROME C OXIDASE SUBUNIT I	
YGL187C	1.9.3.1	*COX4*	CYTOCHROME C OXIDASE SUBUNIT I	
YGL191W	1.9.3.1	*COX13*	CYTOCHROME C OXIDASE SUBUNIT I	
YHR051W	1.9.3.1	*COX6*	CYTOCHROME C OXIDASE SUBUNIT I	
YIL111W	1.9.3.1	*COX5B*	CYTOCHROME C OXIDASE SUBUNIT I	
YLR038C	1.9.3.1	*COX12*	CYTOCHROME C OXIDASE SUBUNIT I	
YLR395C	1.9.3.1	*COX8*	CYTOCHROME C OXIDASE SUBUNIT I	
YMR256C	1.9.3.1	*COX7*	CYTOCHROME C OXIDASE SUBUNIT I	
YNL052W	1.9.3.1	*COX5A*	CYTOCHROME C OXIDASE SUBUNIT I	
# ATP SYNTHASE				
YBL099W	3.6.1.34	*ATP1*	F1F0-ATPASE COMPLEX, F1 ALPHA SUBUNIT	ADPm + PIm -> ATPm + 3 Hm
YPL271W	3.6.1.34	*ATP15*	F1F0-ATPASE COMPLEX, F1 EPSILON SUBUNIT	

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YDL004W	3.6.1.34	*ATP16*	F-TYPE H+-TRANSPORTING ATPASE DELTA CHAIN	
Q0085	3.6.1.34	*ATP6*	F1F0-ATPASE COMPLEX, FO A SUBUNIT	
YBR039W	3.6.1.34	*ATP3*	F1F0-ATPASE COMPLEX, F1 GAMMA SUBUNIT	
YBR127C	3.6.1.34	*VMA2*	H+-ATPASE V1 DOMAIN 60 KD SUBUNIT, VACUOLAR	
YPL078C	3.6.1.34	*ATP4*	F1F0-ATPASE COMPLEX, F1 DELTA SUBUNIT	
YDR298C	3.6.1.34	*ATP5*	F1F0-ATPASE COMPLEX, OSCP SUBUNIT	
YDR377W	3.6.1.34	*ATP17*	ATP SYNTHASE COMPLEX, SUBUNIT F	
YJR121W	3.6.1.34	*ATP2*	F1F0-ATPASE COMPLEX, F1 BETA SUBUNIT	
YKL016C	3.6.1.34	*ATP7*	F1F0-ATPASE COMPLEX, FO D SUBUNIT	
YLR295C	3.6.1.34	*ATP14*	ATP SYNTHASE SUBUNIT H	
Q0080	3.6.1.34	*ATP8*	F-TYPE H+-TRANSPORTING ATPASE SUBUNIT 8	
Q0130	3.6.1.34	*ATP9*	F-TYPE H+-TRANSPORTING ATPASE SUBUNIT C	
YOL077W-A	3.6.1.34	*ATP19*	ATP SYNTHASE K CHAIN, MITOCHONDRIAL	
YPR020W	3.6.1.34	*ATP20*	SUBUNIT G OF THE DIMERIC FORM OF MITOCHONDRIAL F1F0-ATP SYNTHASE	
YML054C	1.1.2.3	*CYB2*	LACTIC ACID DEHYDROGENASE	2 FERIm + LLACm -> PYRm + 2 FEROm
YDL174C	1.1.2.4	*DLD1*	MITOCHONDRIAL ENZYME D-LACTATE FERRICYTOCHROME C OXIDOREDUCTASE	2 FERIm + LACm -> PYRm + 2 FEROm
# METHANE METABOLISM				
YPL275W	1.2.1.2	*YPL275W*	PUTATIVE FORMATE DEHYDROGENASE/PUTATIVE PSEUDOGENE	FOR + NAD -> CO2 + NADH
YPL276W	1.2.1.2	*YPL276W*	PUTATIVE FORMATE DEHYDROGENASE/PUTATIVE PSEUDOGENE	FOR + NAD -> CO2 + NADH
YOR388C	1.2.1.2	*FDH1*	PROTEIN WITH SIMILARITY TO FORMATE DEHYDROGENASES	FOR + NAD -> CO2 + NADH
# NITROGEN METABOLISM				
YBR208C	6.3.4.6	*DUR1*	UREA AMIDOLYASE CONTAINING UREA CARBOXYLASE / ALLOPHANATE HYDROLASE	ATP + UREA + CO2 <-> ADP + PI + UREAC
YBR208C	3.5.1.54	*DUR1*	ALLOPHANATE HYDROLASE	UREAC -> 2 NH3 + 2 CO2

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YJL126W	3.5.5.1	*NIT2*	NITRILASE	ACNL -> INAC + NH3
# SULFUR METABOLISM				
YJR137C	1.8.7.1	*ECM17*	SULFITE REDUCTASE	H2SO3 + 3 NADPH <-> H2S + 3 NADP
# LIPID METABOLISM				
# FATTY ACID BIOSYNTHESIS				
YER015W	6.2.1.3	*FAA2*	LONG-CHAIN-FATTY-ACID--COA LIGASE, ACYL-COA SYNTHETASE	ATP + LCCA + COA <-> AMP + PPI + ACOA
YIL009W	6.2.1.3	*FAA3*	LONG-CHAIN-FATTY-ACID--COA LIGASE, ACYL-COA SYNTHETASE	ATP + LCCA + COA <-> AMP + PPI + ACOA
YOR317W	6.2.1.3	*FAA1*	LONG-CHAIN-FATTY-ACID--COA LIGASE, ACYL-COA SYNTHETASE	ATP + LCCA + COA <-> AMP + PPI + ACOA
YMR246W	6.2.1.3	*FAA4*	ACYL-COA SYNTHASE (LONG-CHAIN FATTY ACID COA LIGASE); CONTRIBUTES TO ACTIVATION OF IMPORTED MYRISTATE	ATP + LCCA + COA <-> AMP + PPI + ACOA
YKR009C	1.1.1.-	*FOX2*	3-HYDROXYACYL-COA DEHYDROGENASE	HACOA + NAD <-> OACOA + NADH
YIL160C	2.3.1.16	*POT1*	3-KETOACYL-COA THIOLASE	OACOA + COA -> ACOA + ACCOA
YPL028W	2.3.1.9	*ERG10*	ACETYL-COA C-ACETYLTRANSFERASE, ACETOACETYL-COA THIOLASE	2 ACCOA <-> COA + AACCOA
YPL028W	2.3.1.9	*ERG10*	ACETYL-COA C-ACETYLTRANSFERASE, ACETOACETYL-COA THIOLASE (MITOCH)	2 ACCOAm <-> COAm + AACCOAm
# FATTY ACIDS METABOLISM				
# MITOCHONDRIAL TYPE II FATTY ACID SYNTHASE				
YKL192C	1.6.5.3	*ACP1*	ACYL CARRIER PROTEIN, COMPONENT OF MITOCHONDRIAL TYPE II FATTY ACID SYNTHASE	NADHm + Qm -> NADm + QH2m
YER061C	-	*CEM1*	BETA-KETOACYL-ACP SYNTHASE, MITOCHONDRIAL (3-OXOACYL-[ACYL-CARRIER-PROTEIN] SYNTHASE)	
YOR221C	-	*MCT1*	MALONYL COA:ACYL CARRIER PROTEIN TRANSFERASE	
YKL055C	-	*OAR1*	3-OXOACYL-[ACYL-CARRIER-PROTEIN] REDUCTASE	
YKL192C	1.6.5.3/-/-/-	*ACP1/CEM1/MCT1/OAR1*	TYPE II FATTY ACID SYNTHASE	ACACPm + 4 MALACPm + 8 NADPHm -> 8 NADPm + C100ACPm + 4 CO2m + 4 ACPm
YKL192C	1.6.5.3/-/-/-	*ACP1/CEM1/MCT1/OAR1*	TYPE II FATTY ACID SYNTHASE	ACACPm + 5 MALACPm + 10 NADPHm -> 10 NADPm + C120ACPm + 5 CO2m + 5 ACPm
YKL192C	1.6.5.3/-/-/-	*ACP1/CEM1/MCT1/OAR1*	TYPE II FATTY ACID SYNTHASE	ACACPm + 6 MALACPm + 12 NADPHm -> 12 NADPm + C140ACPm + 6 CO2m + 6 ACPm

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YKL192C	1.6.5.3/-/-/-	*ACP1*	TYPE II FATTY ACID SYNTHASE	ACACPm + 6 MALACPm + 11 NADPHm -> 11 NADPm + C141ACPm+ 6 CO2m + 6 ACPm
YKL192C	1.6.5.3/-/-/-	*ACP2*	TYPE II FATTY ACID SYNTHASE	ACACPm + 7 MALACPm + 14 NADPHm -> 14 NADPm + C160ACPm + 7 CO2m + 7 ACPm
YKL192C	1.6.5.3/-/-/-	*ACP3*	TYPE II FATTY ACID SYNTHASE	ACACPm + 7 MALACPm + 13 NADPHm -> 13 NADPm + C161ACPm + 7 CO2m + 7 ACPm
YKL192C	1.6.5.3/-/-/-	*ACP4*	TYPE II FATTY ACID SYNTHASE	ACACPm + 8 MALACPm + 16 NADPHm -> 16 NADPm + C180ACPm + 8 CO2m + 8 ACPm
YKL192C	1.6.5.3/-/-/-	*ACP5*	TYPE II FATTY ACID SYNTHASE	ACACPm + 8 MALACPm + 15 NADPHm -> 15 NADPm + C181ACPm + 8 CO2m + 8 ACPm
YKL192C	1.6.5.3/-/-/-	*ACP6*	TYPE II FATTY ACID SYNTHASE	ACACPm + 8 MALACPm + 14 NADPHm -> 14 NADPm + C182ACPm + 8 CO2m + 8 ACPm
# CYTOSOLIC FATTY ACID SYNTHESIS				
YNR016C	6.4.1.2 6.3.4.14	*ACC1*	ACETYL-COA CARBOXYLASE (ACC) / BIOTIN CARBOXYLASE	ACCOA + ATP + CO2 <-> MALCOA + ADP + PI
YKL182W	4.2.1.61	*FAS1*	FATTY-ACYL-COA SYNTHASE, BETA CHAIN	MALCOA + ACP <-> MALACP + COA
YPL231W	2.3.1.85;1.1.1.100;2.3.1.41	*FAS2*	FATTY-ACYL-COA SYNTHASE, ALPHA CHAIN	
YKL182W	4.2.1.61	*FAS1*	FATTY-ACYL-COA SYNTHASE, BETA CHAIN	ACCOA + ACP <-> ACACP + COA
YER061C	2.3.1.41	*CEM1*	3-OXOACYL-[ACYL-CARRIER-PROTEIN] SYNTHASE	MALACPm + ACACPm -> ACPm + CO2m + 3OACPm
YGR037C	6.4.1.2	*ACB1*	B-KETOACYL-ACP SYNTHASE (C10,0), FATTY ACYL COA SYNTHASE	ACACP + 4 MALACP + 8 NADPH -> 8 NADP + C100ACP + 4 CO2 + 4 ACP
YGR037C	6.4.1.3	*ACB2*	B-KETOACYL-ACP SYNTHASE (C12,0), FATTY ACYL COA SYNTHASE	ACACP + 5 MALACP + 10 NADPH -> 10 NADP + C120ACP + 5 CO2 + 5 ACP
YGR037C	6.4.1.4	*ACB3*	B-KETOACYL-ACP SYNTHASE (C14,0)	ACACP + 6 MALACP + 12 NADPH -> 12 NADP + C140ACP + 6 CO2 + 6 ACP
YGR037C	6.4.1.5	*ACB4*	B-KETOACYL-ACP SYNTHASE I (C14,1)	ACACP + 6 MALACP + 11 NADPH -> 11 NADP + C141ACP + 6 CO2 + 6 ACP
YGR037C	6.4.1.6	*ACB5*	B-KETOACYL-ACP SYNTHASE I (C16,0)	ACACP + 7 MALACP + 14 NADPH -> 14 NADP + C160ACP + 7 CO2 + 7 ACP
YGR037C	6.4.1.7	*ACB6*	B-KETOACYL-ACP SYNTHASE I (C16,1)	ACACP + 7 MALACP + 13 NADPH -> 13 NADP + C161ACP + 7 CO2 + 7 ACP
YGR037C	6.4.1.8	*ACB7*	B-KETOACYL-ACP SYNTHASE I (C18,0)	ACACP + 8 MALACP + 16 NADPH -> 16 NADP + C180ACP + 8 CO2 + 8 ACP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YGR037C	6.4.1.2	*ACB7*	B-KETOACYL-ACP SYNTHASE I (C18,1)	ACACP + 8 MALACP + 15 NADPH -> 15 NADP + C181ACP + 8 CO2 + 8 ACP
YGR037C	6.4.1.2	*ACB7*	B-KETOACYL-ACP SYNTHASE I (C18,2)	ACACP + 8 MALACP + 14 NADPH -> 14 NADP + C182ACP + 8 CO2 + 8 ACP
YKL182W	4.2.1.61	*FAS1*	3-HYDROXYPALMITOYL-[ACYL-CARRIER PROTEIN] DEHYDRATASE	3HPACP <-> 2HDACP
YKL182W	1.3.1.9	*FAS1*	ENOYL-ACP REDUCTASE	AACP + NAD <-> 23DAACP + NADH
# FATTY ACID DEGRADATION				
YGL205W	1.3.3.6	*POX1*	FATTY ACID DEGRADATION	C140 + ATP + 7 COA + 7 FADm + 7 NAD -> AMP + PPI + 7 FADH2m + 7 NADH + 7 ACCOA
YGL205W	1.3.3.6	*POX2*	FATTY ACID DEGRADATION	C160 + ATP + 8 COA + 8 FADm + 8 NAD -> AMP + PPI + 8 FADH2m + 8 NADH + 8 ACCOA
YGL205W	1.3.3.6	*POX3*	FATTY ACID DEGRADATION	C180 + ATP + 9 COA + 9 FADm + 9 NAD -> AMP + PPI + 9 FADH2m + 9 NADH + 9 ACCOA
# PHOSPHOLIPID BIOSYNTHESIS				
*U3_*		*U3_*	GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE	GL3P + 0.017 C100ACP + 0.062 C120ACP + 0.1 C140ACP + 0.27 C160ACP + 0.169 C161ACP + 0.055 C180ACP + 0.235 C181ACP + 0.093 C182ACP -> AGL3P + ACP
*U4_*		*U4_*	GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE	GL3P + 0.017 C100ACP + 0.062 C120ACP + 0.1 C140ACP + 0.27 C160ACP + 0.169 C161ACP + 0.055 C180ACP + 0.235 C181ACP + 0.093 C182ACP -> AGL3P + ACP
*U5_*		*U5_*	GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE	T3P2 + 0.017 C100ACP + 0.062 C120ACP + 0.1 C140ACP + 0.27 C160ACP + 0.169 C161ACP + 0.055 C180ACP + 0.235 C181ACP + 0.093 C182ACP -> AT3P2 + ACP
*U6_*		*U6_*	GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE	T3P2 + 0.017 C100ACP + 0.062 C120ACP + 0.1 C140ACP + 0.27 C160ACP + 0.169 C161ACP + 0.055 C180ACP + 0.235 C181ACP + 0.093 C182ACP -> AT3P2 + ACP
*U7_*		*U7_*	ACYLDIHYDROXYACETONEPHOSPHATE REDUCTASE	AT3P2 + NADPH -> AGL3P + NADP
YDL052C	2.3.1.51	*SLC1*	1-ACYLGLYCEROL-3-PHOSPHATE ACYLTRANSFERASE	AGL3P + 0.017 C100ACP + 0.062 C120ACP + 0.100 C140ACP + 0.270 C160ACP + 0.169 C161ACP + 0.055 C180ACP + 0.235 C181ACP + 0.093 C182ACP -> PA + ACP
*U8_*	2.3.1.51	*U8_*	1-ACYLGLYCEROL-3-PHOSPHATE ACYLTRANSFERASE	AGL3P + 0.017 C100ACP + 0.062 C120ACP + 0.100 C140ACP + 0.270 C160ACP + 0.169 C161ACP + 0.055 C180ACP + 0.235 C181ACP + 0.093 C182ACP -> PA + ACP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YBR029C	2.7.7.41	*CDS1*	CDP-DIACYLGLYCEROL SYNTHETASE	PAm + CTPm <-> CDPDGm + PPIm

YBR029C	2.7.7.41	*CDS1*	CDP-DIACYLGLYCEROL SYNTHETASE	PA + CTP <-> CDPDG + PPI
YER026C	2.7.8.8	*CHO1*	PHOSPHATIDYLSERINE SYNTHASE	CDPDG + SER <-> CMP + PS
YER026C	2.7.8.8	*CHO1*	PHOSPHATIDYLSERINE SYNTHASE	CDPDGm + SERm <-> CMPm + PSm
YGR170W	4.1.1.65	*PSD2*	PHOSPHATIDYLSERINE DECARBOXYLASE LOCATED IN VACUOLE OR GOLGI	PS -> PE + CO2
YNL169C	4.1.1.65	*PSD1*	PHOSPHATIDYLSERINE DECARBOXYLASE 1	PSm -> PEm + CO2m
YGR157W	2.1.1.17	*CHO2*	PHOSPHATIDYLETHANOLAMINE N-METHYLTRANSFERASE	SAM + PE -> SAH + PMME
YJR073C	2.1.1.16	*OPI3*	METHYLENE-FATTY-ACYL-PHOSPHOLIPID SYNTHASE.	SAM + PMME -> SAH + PDME
YJR073C	2.1.1.16	*OPI3*	PHOSPHATIDYL-N-METHYLETHANOLAMINE N-METHYLTRANSFERASE	PDME + SAM -> PC + SAH
YLR133W	2.7.1.32	*CKI1*	CHOLINE KINASE	ATP + CHO -> ADP + PCHO
YGR202C	2.7.7.15	*PCT1*	CHOLINEPHOSPHATE CYTIDYLYLTRANSFERASE	PCHO + CTP -> CDPCHO + PPI
YNL130C	2.7.8.2	*CPT1*	DIACYLGLYCEROL CHOLINEPHOSPHOTRANSFERASE	CDPCHO + DAGLY -> PC + CMP
YDR147W	2.7.1.82	*EKI1*	ETHANOLAMINE KINASE	ATP + ETHM -> ADP + PETHM
YGR007W	2.7.7.14	*MUQ1*	PHOSPHOETHANOLAMINE CYTIDYLYLTRANSFERASE	PETHM + CTP -> CDPETN + PPI
YHR123W	2.7.8.1	*EPT1*	ETHANOLAMINEPHOSPHOTRANSFERASE.	CDPETN + DAGLY <-> CMP + PE
YJL153C	5.5.1.4	*INO1*	MYO-INOSITOL-1-PHOSPHATE SYNTHASE	G6P -> MI1P
YHR046C	3.1.3.25	*INM1*	MYO-INOSITOL-1(OR 4)-MONOPHOSPHATASE	MI1P -> MYOI + PI
YPR113W	2.7.8.11	*PIS1*	PHOSPHATIDYLINOSITOL SYNTHASE	CDPDG + MYOI -> CMP + PINS
YJR066W	2.7.1.137	*TOR1*	1-PHOSPHATIDYLINOSITOL 3-KINASE	ATP + PINS -> ADP + PINSP
YKL203C	2.7.1.137	*TOR2*	1-PHOSPHATIDYLINOSITOL 3-KINASE	ATP + PINS -> ADP + PINSP
YLR240W	2.7.1.137	*VPS34*	1-PHOSPHATIDYLINOSITOL 3-KINASE	ATP + PINS -> ADP + PINSP
YNL267W	2.7.1.67	*PIK1*	PHOSPHATIDYLINOSITOL 4-KINASE (PI 4-KINASE), GENERATES PTDINS 4-P	ATP + PINS -> ADP + PINS4P
YLR305C	2.7.1.67	*STT4*	PHOSPHATIDYLINOSITOL 4-KINASE	ATP + PINS -> ADP + PINS4P
YFR019W	2.7.1.68	*FAB1*	PROBABLE PHOSPHATIDYLINOSITOL-4-PHOSPHATE 5-KINASE, 1-PHOSPHATIDYLINOSITOL-4-PHOSPHATE KINASE	PINS4P + ATP -> D45PI + ADP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YDR208W	2.7.1.68	*MSS4*	PHOSPHATIDYLINOSITOL-4-PHOSPHATE 5-KINASE; REQUIRED FOR PROPER ORGANIZATION OF THE ACTIN CYTOSKELETON	PINS4P + ATP -> D45PI + ADP
YPL268W	3.1.4.11	*PLC1*	1-PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE PHOSPHODIESTERASE	D45PI -> TPI + DAGLY
YCL004W	2.7.8.8	*PGS1*	CDP-DIACYLGLYCEROL--SERINE O-PHOSPHATIDYLTRANSFERASE	CDPDGm + GL3Pm <-> CMPm + PGPm
*U9_*	3.1.3.27	*U9_*	PHOSPHATIDYLGLYCEROL PHOSPHATE PHOSPHATASE A	PGPm -> PIm + PGm
YDL142C	2.7.8.5	*CRD1*	CARDIOLIPIN SYNTHASE	CDPDGm + PGm -> CMPm + CLm
YDR284C		*DPP1*	DIACYLGLYCEROL PYROPHOSPHATE PHOSPHATASE	PA -> DAGLY + PI
YDR503C		*LPP1*	LIPID PHOSPHATE PHOSPHATASE	DGPP -> PA + PI
# SPHINGOGLYCOLIPID METABOLISM				
YDR062W	2.3.1.50	*LCB2*	SERINE C-PALMITOYLTRANSFERASE	PALCOA + SER -> COA + DHSPH + CO2
YMR296C	2.3.1.50	*LCB1*	SERINE C-PALMITOYLTRANSFERASE	PALCOA + SER -> COA + DHSPH + CO2
YBR265W	1.1.1.102	*TSC10*	3-DEHYDROSPHINGANINE REDUCTASE	DHSPH + NADPH -> SPH + NADP
YDR297W		*SUR2*	SYRINGOMYCIN RESPONSE PROTEIN 2	SPH + O2 + NADPH -> PSPH + NADP
*U10_*		*U10_*	CERAMIDE SYNTHASE	PSPH + C260COA -> CER2 + COA
*U11_*		*U11_*	CERAMIDE SYNTHASE	PSPH + C240COA -> CER2 + COA
YMR272C		*SCS7*	CERAMIDE HYDROXYLASE THAT HYDROXYLATES THE C-26 FATTY-ACYL MOIETY OF INOSITOL-PHOSPHORYLCERAMIDE	CER2 + NADPH + O2 -> CER3 + NADP
YKL004W		*AUR1*	IPS SYNTHASE, AUREOBASIDIN A RESISTANCE PROTEIN	CER3 + PINS -> IPC
YBR036C		*CSG2*	PROTEIN REQUIRED FOR SYNTHESIS OF THE MANNOSYLATED SPHINGOLIPIDS	IPC + GDPMAN -> MIPC
YPL057C		*SUR1*	PROTEIN REQUIRED FOR SYNTHESIS OF THE MANNOSYLATED SPHINGOLIPIDS	IPC + GDPMAN -> MIPC
YDR072C	2.-.-.-	*IPT1*	MIP2C SYNTHASE, MANNOSYL DIPHOSPHORYLINOSITOL CERAMIDE SYNTHASE	MIPC + PINS -> MIP2C
YOR171C		*LCB4*	LONG CHAIN BASE KINASE, INVOLVED IN SPHINGOLIPID METABOLISM	SPH + ATP -> DHSP + ADP
YLR260W		*LCB5*	LONG CHAIN BASE KINASE, INVOLVED IN SPHINGOLIPID METABOLISM	SPH + ATP -> DHSP + ADP
YOR171C		*LCB4*	LONG CHAIN BASE KINASE, INVOLVED IN SPHINGOLIPID METABOLISM	PSPH + ATP -> PHSP + ADP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YLR260W		*LCB5*	LONG CHAIN BASE KINASE, INVOLVED IN SPHINGOLIPID METABOLISM	PSPH + ATP -> PHSP + ADP
YJL134W		*LCB3*	SPHINGOID BASE-PHOSPHATE PHOSPHATASE, PUTATIVE REGULATOR OF SPHINGOLIPID METABOLISM AND STRESS RESPONSE	DHSP -> SPH + PI
YKR053C		*YSR3*	SPHINGOID BASE-PHOSPHATE PHOSPHATASE, PUTATIVE REGULATOR OF SPHINGOLIPID METABOLISM AND STRESS RESPONSE	DHSP -> SPH + PI
YDR294C		*DPL1*	DIHYDROSPHINGOSINE-1-PHOSPHATE LYASE	DHSP -> PETHM + C16A
# STEROL BIOSYNTHESIS				
YML126C	4.1.3.5	*HMGS*	3-HYDROXY-3-METHYLGLUTARYL COENZYME A SYNTHASE	H3MCOA + COA <-> ACCOA + AACCOA
YLR450W	1.1.1.34	*HMG2*	3-HYDROXY-3-METHYLGLUTARYL-COENZYME A (HMG-COA) REDUCTASE ISOZYME	MVL + COA + 2 NADP <-> H3MCOA + 2 NADPH
YML075C	1.1.1.34	*HMG1*	3-HYDROXY-3-METHYLGLUTARYL-COENZYME A (HMG-COA) REDUCTASE ISOZYME	MVL + COA + 2 NADP <-> H3MCOA + 2 NADPH
YMR208W	2.7.1.36	*ERG12*	MEVALONATE KINASE	ATP + MVL -> ADP + PMVL
YMR208W	2.7.1.36	*ERG12*	MEVALONATE KINASE	CTP + MVL -> CDP + PMVL
YMR208W	2.7.1.36	*ERG12*	MEVALONATE KINASE	GTP + MVL -> GDP + PMVL
YMR208W	2.7.1.36	*ERG12*	MEVALONATE KINASE	UTP + MVL -> UDP + PMVL
YMR220W	2.7.4.2	*ERG8*	48 KDA PHOSPHOMEVALONATE KINASE	ATP + PMVL -> ADP + PPMVL
YNR043W	4.1.1.33	*MVD1*	DIPHOSPHOMEVALONATE DECARBOXYLASE	ATP + PPMVL -> ADP + PI + IPPP + CO2
YPL117C	5.3.3.2	*IDI1*	ISOPENTENYL DIPHOSPHATE:DIMETHYLALLYL DIPHOSPHATE ISOMERASE (IPP ISOMERASE)	IPPP <-> DMPP
YJL167W	2.5.1.1	*ERG20*	PRENYLTRANSFERASE	DMPP + IPPP -> GPP + PPI
YJL167W	2.5.1.10	*ERG20*	FARNESYL DIPHOSPHATE SYNTHETASE (FPP SYNTHETASE)	GPP + IPPP -> FPP + PPI
YHR190W	2.5.1.21	*ERG9*	SQUALENE SYNTHASE.	2 FPP + NADPH -> NADP + SQL
YGR175C	1.14.99.7	*ERG1*	SQUALENE MONOOXYGENASE	SQL + O2 + NADP -> S23E + NADPH
YHR072W	5.4.99.7	*ERG7*	2,3-OXIDOSQUALENE-LANOSTEROL CYCLASE	S23E -> LNST
YHR007C	1.14.14.1	*ERG11*	CYTOCHROME P450 LANOSTEROL 14A-DEMETHYLASE	LNST + RFP + O2 -> IGST + OFP
YNL280C	1.-.-.-	*ERG24*	C-14 STEROL REDUCTASE	IGST + NADPH -> DMZYMST + NADP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YGR060W	1.-.-.-	*ERG25*	C-4 STEROL METHYL OXIDASE	3 O2 + DMZYMST -> IMZYMST
YGL001C	5.3.3.1	*ERG26*	C-3 STEROL DEHYDROGENASE (C-4 DECARBOXYLASE)	IMZYMST -> IIMZYMST + CO2
YLR100C		*YLR100C*	C-3 STEROL KETO REDUCTASE	IIMZYMST + NADPH -> MZYMST + NADP
YGR060W	1.-.-.-	*ERG25*	C-4 STEROL METHYL OXIDASE	3 O2 + MZYMST -> IZYMST
YGL001C	5.3.3.1	*ERG26*	C-3 STEROL DEHYDROGENASE (C-4 DECARBOXYLASE)	IZYMST -> IIZYMST + CO2
YLR100C		*YLR100C*	C-3 STEROL KETO REDUCTASE	IIZYMST + NADPH -> ZYMST + NADP
YML008C	2.1.1.41	*ERG6*	S-ADENOSYL-METHIONINE DELTA-24-STEROL-C-METHYLTRANSFERASE	ZYMST + SAM -> FEST + SAH
YMR202W		*ERG2*	C-8 STEROL ISOMERASE	FEST -> EPST
YLR056W	1.-.-.-	*ERG3*	C-5 STEROL DESATURASE	EPST + O2 + NADPH -> NADP + ERTROL
YMR015C	1.14.14.-	*ERG5*	C-22 STEROL DESATURASE	ERTROL + O2 + NADPH -> NADP + ERTEOL
YGL012W	1.-.-.-	*ERG4*	STEROL C-24 REDUCTASE	ERTEOL + NADPH -> ERGOST + NADP
*U12_*		*U12_*		LNST + 3 O2 + 4 NADPH + NAD -> MZYMST + CO2 + 4 NADP + NADH
*U13_*		*U13_*		MZYMST + 3 O2 + 4 NADPH + NAD -> ZYMST + CO2 + 4 NADP + NADH
*U14_*	5.3.3.5	*U14_*	CHOLESTENOL DELTA-ISOMERASE	ZYMST + SAM -> ERGOST + SAH
# NUCLEOTIDE METABOLISM				
# HISTIDINE BIOSYNTHESIS				
YOL061W	2.7.6.1	*PRS5*	RIBOSE-PHOSPHATE PYROPHOSPHOKINASE	R5P + ATP <-> PRPP + AMP
YBL068W	2.7.6.1	*PRS4*	RIBOSE-PHOSPHATE PYROPHOSPHOKINASE 4	R5P + ATP <-> PRPP + AMP
YER099C	2.7.6.1	*PRS2*	RIBOSE-PHOSPHATE PYROPHOSPHOKINASE 2	R5P + ATP <-> PRPP + AMP
YHL011C	2.7.6.1	*PRS3*	RIBOSE-PHOSPHATE PYROPHOSPHOKINASE 3	R5P + ATP <-> PRPP + AMP
YKL181W	2.7.6.1	*PRS1*	RIBOSE-PHOSPHATE PYROPHOSPHOKINASE	R5P + ATP <-> PRPP + AMP
YIR027C	3.5.2.5	*DAL1*	ALLANTOINASE	ATN <-> ATT
YIR029W	3.5.3.4	*DAL2*	ALLANTOICASE	ATT <-> UGC + UREA
YIR032C	3.5.3.19	*DAL3*	UREIDOGLYCOLATE HYDROLASE	UGC <-> GLX + 2 NH3 + CO2
# PURINE METABOLISM				
YJL005W	4.6.1.1	*CYR1*	ADENYLATE CYCLASE	ATP -> cAMP + PPI
YDR454C	2.7.4.8	*GUK1*	GUANYLATE KINASE	GMP + ATP <-> GDP + ADP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YDR454C	2.7.4.8	*GUK1*	GUANYLATE KINASE	DGMP + ATP <-> DGDP + ADP
YDR454C	2.7.4.8	*GUK1*	GUANYLATE KINASE	GMP + DATP <-> GDP + DADP
YMR300C	2.4.2.14	*ADE4*	PHOSPHORIBOSYLPYROPHOSPHATE AMIDOTRANSFERASE	PRPP + GLN -> PPI + GLU + PRAM
YGL234W	6.3.4.13	*ADE5,7*	GLYCINAMIDE RIBOTIDE SYNTHETASE AND AMINOIMIDAZOLE RIBOTIDE SYNTHETASE	PRAM + ATP + GLY <-> ADP + PI + GAR
YDR408C	2.1.2.2	*ADE8*	GLYCINAMIDE RIBOTIDE TRANSFORMYLASE	GAR + FTHF -> THF + FGAR
YGR061C	6.3.5.3	*ADE6*	5'-PHOSPHORIBOSYLFORMYL GLYCINAMIDINE SYNTHETASE	FGAR + ATP + GLN -> GLU + ADP + PI + FGAM
YGL234W	6.3.3.1	*ADE5,7*	PHOSPHORIBOSYLFORMYLGLYCINAMIDE CYCLO-LIGASE	FGAM + ATP -> ADP + PI + AIR
YOR128C	4.1.1.21	*ADE2*	PHOSPHORIBOSYLAMINO-IMIDAZOLE-CARBOXYLASE	CAIR <-> AIR + CO2
YAR015W	6.3.2.6	*ADE1*	PHOSPHORIBOSYL AMINO IMIDAZOLESUCCINOCARBOZAMIDE SYNTHETASE	CAIR + ATP + ASP <-> ADP + PI + SAICAR
YLR359W	4.3.2.2	*ADE13*	5'-PHOSPHORIBOSYL-4-(N-SUCCINOCARBOXAMIDE)-5-AMINOIMIDAZOLE LYASE	SAICAR <-> FUM + AICAR
YLR028C	2.1.2.3	*ADE16*	5-AMINOIMIDAZOLE-4-CARBOXAMIDE RIBONUCLEOTIDE (AICAR) TRANSFORMYLASE\/IMP CYCLOHYDROLASE	AICAR + FTHF <-> THF + PRFICA
YMR120C	2.1.2.3	*ADE17*	5-AMINOIMIDAZOLE-4-CARBOXAMIDE RIBONUCLEOTIDE (AICAR) TRANSFORMYLASE\/IMP CYCLOHYDROLASE	AICAR + FTHF <-> THF + PRFICA
YLR028C	3.5.4.10	*ADE16*	5-AMINOIMIDAZOLE-4-CARBOXAMIDE RIBONUCLEOTIDE (AICAR) TRANSFORMYLASE\/IMP CYCLOHYDROLASE	PRFICA <-> IMP
YMR120C	2.1.2.3	*ADE17*	IMP CYCLOHYDROLASE	PRFICA <-> IMP
YNL220W	6.3.4.4	*ADE12*	ADENYLOSUCCINATE SYNTHETASE	IMP + GTP + ASP -> GDP + PI + ASUC
YLR359W	4.3.2.2	*ADE13*	ADENYLOSUCCINATE LYASE	ASUC <-> FUM + AMP
YAR073W	1.1.1.205	*FUN63*	PUTATIVE INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE	IMP + NAD -> NADH + XMP
YHR216W	1.1.1.205	*PUR5*	PURINE EXCRETION	IMP + NAD -> NADH + XMP
YML056C	1.1.1.205	*IMD4*	PROBABLE INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE (IMP	IMP + NAD -> NADH + XMP
YLR432W	1.1.1.205	*IMD3*	PROBABLE INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE (IMP	IMP + NAD -> NADH + XMP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YAR075W	1.1.1.205	*YAR075W*	PROTEIN WITH STRONG SIMILARITY TO INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE, FRAMESHIFTED FROM YAR073W, POSSIBLE PSEUDOGENE	IMP + NAD -> NADH + XMP
YMR217W	6.3.5.2, 6.3.4.1	*GUA1*	GMP SYNTHASE	XMP + ATP + GLN -> GLU + AMP + PPI + GMP
YML035C	3.5.4.6	*AMD1*	AMP DEAMINASE	AMP -> IMP + NH3
YGL248W	3.1.4.17	*PDE1*	3',5'-CYCLIC-NUCLEOTIDE PHOSPHODIESTERASE, LOW AFFINITY	cAMP -> AMP
YOR360C	3.1.4.17	*PDE2*	3',5'-CYCLIC-NUCLEOTIDE PHOSPHODIESTERASE, HIGH AFFINITY	cAMP -> AMP
YOR360C	3.1.4.17	*PDE2*		cdAMP -> DAMP
YOR360C	3.1.4.17	*PDE2*		cIMP -> IMP
YOR360C	3.1.4.17	*PDE2*		cGMP -> GMP
YOR360C	3.1.4.17	*PDE2*		cCMP -> CMP
YDR530C	2.7.7.53	*APA2*	5',5'''-P-1,P-4-TETRAPHOSPHATE PHOSPHORYLASE II	ADP + ATP -> PI + ATRP
YCL050C	2.7.7.53	*APA1*	5',5'''-P-1,P-4-TETRAPHOSPHATE PHOSPHORYLASE II	ADP + GTP -> PI + ATRP
YCL050C	2.7.7.53	*APA1*	5',5'''-P-1,P-4-TETRAPHOSPHATE PHOSPHORYLASE II	GDP + GTP -> PI + GTRP
# PYRIMIDINE METABOLISM				
YJL130C	2.1.3.2	*URA2*	ASPARTATE-CARBAMOYLTRANSFERASE	CAP + ASP -> CAASP + PI
YLR420W	3.5.2.3	*URA4*	DIHYDROORATASE	CAASP <-> DOROA
YKL216W	1.3.3.1	*URA1*	DIHYDROOROTATE DEHYDROGENASE	DOROA + O2 <-> H2O2 + OROA
YKL216W	1.3.3.1	*PYRD*	DIHYDROOROTATE DEHYDROGENASE	DOROA + Qm <-> QH2m + OROA
YML106W	2.4.2.10	*URA5*	OROTATE PHOSPHORIBOSYLTRANSFERASE 1	OROA + PRPP <-> PPI + OMP
YMR271C	2.4.2.10	*URA10*	OROTATE PHOSPHORIBOSYLTRANSFERASE 2	OROA + PRPP <-> PPI + OMP
YEL021W	4.1.1.23	*URA3*	OROTIDINE-5'-PHOSPHATE DECARBOXYLASE	OMP -> CO2 + UMP
YKL024C	2.7.4.14	*URA6*	NUCLEOSIDE-PHOSPHATE KINASE	ATP + UMP <-> ADP + UDP
YHR128W	2.4.2.9	*FUR1*	UPRTASE, URACIL PHOSPHORIBOSYLTRANSFERASE	URA + PRPP -> UMP + PPI
YPR062W	3.5.4.1	*FCY1*	CYTOSINE DEAMINASE	CYTS -> URA + NH3
*U15_*	2.7.1.21	*U15_*	THYMIDINE (DEOXYURIDINE) KINASE	DU + ATP -> DUMP + ADP
*U16_*	2.7.1.21	*U16_*	THYMIDINE (DEOXYURIDINE) KINASE	DT + ATP -> ADP + DTMP
YNR012W	2.7.1.48	*URK1*	URIDINE KINASE	URI + GTP -> UMP + GDP
YNR012W	2.7.1.48	*URK1*	CYTODINE KINASE	CYTD + GTP -> GDP + CMP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YNR012W	2.7.1.48	*URK1*	URIDINE KINASE, CONVERTS ATP AND URIDINE TO ADP AND UMP	URI + ATP -> ADP + UMP
YLR209C	2.4.2.4	*PNP1*	PROTEIN WITH SIMILARITY TO HUMAN PURINE NUCLEOSIDE PHOSPHORYLASE, THYMIDINE (DEOXYURIDINE) PHOSPHORYLASE, PURINE NUCLEOTIDE PHOSPHORYLASE	DU + PI <-> URA + DR1P
YLR209C	2.4.2.4	*PNP1*	PROTEIN WITH SIMILARITY TO HUMAN PURINE NUCLEOSIDE PHOSPHORYLASE, THYMIDINE (DEOXYURIDINE) PHOSPHORYLASE	DT + PI <-> THY + DR1P
YLR245C	3.5.4.5	*CDD1*	CYTIDINE DEAMINASE	CYTD -> URI + NH3
YLR245C	3.5.4.5	*CDD1*	CYTIDINE DEAMINASE	DC -> NH3 + DU
YJR057W	2.7.4.9	*CDC8*	DTMP KINASE	DTMP + ATP <-> ADP + DTDP
YDR353W	1.6.4.5	*TRR1*	THIOREDOXIN REDUCTASE	OTHIO + NADPH -> NADP + RTHIO
YHR106W	1.6.4.5	*TRR2*	MITOCHONDRIAL THIOREDOXIN REDUCTASE	OTHIOm + NADPHm -> NADPm + RTHIOm
YBR252W	3.6.1.23	*DUT1*	DUTP PYROPHOSPHATASE (DUTPASE)	DUTP -> PPI + DUMP
YOR074C	2.1.1.45	*CDC21*	THYMIDYLATE SYNTHASE	DUMP + METTHF -> DHF + DTMP
*U17_*	2.7.4.14	*U17_*	CYTIDYLATE KINASE	DCMP + ATP <-> ADP + DCDP
*U18_*	2.7.4.14	*U18_*	CYTIDYLATE KINASE	CMP + ATP <-> ADP + CDP
YHR144C	3.5.4.12	*DCD1*	DCMP DEAMINASE	DCMP <-> DUMP + NH3
YBL039C	6.3.4.2	*URA7*	CTP SYNTHASE, HIGHLY HOMOLOGUS TO URA8 CTP SYNTHASE	UTP + GLN + ATP -> GLU + CTP + ADP + PI
YJR103W	6.3.4.2	*URA8*	CTP SYNTHASE	UTP + GLN + ATP -> GLU + CTP + ADP + PI
YBL039C	6.3.4.2	*URA7*	CTP SYNTHASE, HIGHLY HOMOLOGUS TO URA8 CTP SYNTHASE	ATP + UTP + NH3 -> ADP + PI + CTP
YJR103W	6.3.4.2	*URA8*	CTP SYNTHASE	ATP + UTP + NH3 -> ADP + PI + CTP
YNL292W	4.2.1.70	*PUS4*	PSEUDOURIDINE SYNTHASE	URA + R5P <-> PURI5P
YPL212C	4.2.1.70	*PUS1*	INTRANUCLEAR PROTEIN WHICH EXHIBITS A NUCLEOTIDE-SPECIFIC INTRON-DEPENDENT TRNA PSEUDOURIDINE SYNTHASE ACTIVITY	URA + R5P <-> PURI5P
YGL063W	4.2.1.70	*PUS2*	PSEUDOURIDINE SYNTHASE 2	URA + R5P <-> PURI5P
YFL001W	4.2.1.70	*DEG1*	SIMILAR TO RRNA METHYLTRANSFERASE (CAENORHABDITIS ELEGANS) AND HYPOTHETICAL 28K PROTEIN (ALKALINE ENDOGLUCANASE GENE 5' REGION) FROM BACILLUS SP.	URA + R5P <-> PURI5P

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
# SALVAGE PATHWAYS				
YML022W	2.4.2.7	*APT1*	ADENINE PHOSPHORIBOSYLTRANSFERASE	AD + PRPP -> PPI + AMP
YDR441C	2.4.2.7	*APT2*	SIMILAR TO ADENINE PHOSPHORIBOSYLTRANSFERASE	AD + PRPP -> PPI + AMP
YNL141W	3.5.4.4	*AAH1*	ADENINE AMINOHYDROLASE (ADENINE DEAMINASE)	ADN -> INS + NH3
YNL141W	3.5.4.4	*AAH1*	ADENINE AMINOHYDROLASE (ADENINE DEAMINASE)	DA -> DIN + NH3
YLR209C	2.4.2.1	*PNP1*	PURINE NUCLEOTIDE PHOSPHORYLASE, XANTHOSINE PHOSPHORYLASE	DIN + PI <-> HYXN + DR1P
YLR209C	2.4.2.1	*PNP1*	XANTHOSINE PHOSPHORYLASE, PURINE NUCLEOTIDE PHOSPHORYLASE	DA + PI <-> AD + DR1P
YLR209C	2.4.2.1	*PNP1*	XANTHOSINE PHOSPHORYLASE	DG + PI <-> GN + DR1P
YLR209C	2.4.2.1	*PNP1*	XANTHOSINE PHOSPHORYLASE, PURINE NUCLEOTIDE PHOSPHORYLASE	HYXN + R1P <-> INS + PI
YLR209C	2.4.2.1	*PNP1*	XANTHOSINE PHOSPHORYLASE, PURINE NUCLEOTIDE PHOSPHORYLASE	AD + R1P <-> PI + ADN
YLR209C	2.4.2.1	*PNP1*	XANTHOSINE PHOSPHORYLASE, PURINE NUCLEOTIDE PHOSPHORYLASE	GN + R1P <-> PI + GSN
YLR209C	2.4.2.1	*PNP1*	XANTHOSINE PHOSPHORYLASE, PURINE NUCLEOTIDE PHOSPHORYLASE	XAN + R1P <-> PI + XTSINE
YJR133W	2.4.2.22	*XPT1*	XANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE	XAN + PRPP -> XMP + PPI
YDR400W	3.2.2.1	*URH1*	PURINE NUCLEOSIDASE	GSN -> GN + RIB
YDR400W	3.2.2.1	*URH1*	PURINE NUCLEOSIDASE	ADN -> AD + RIB
YJR105W	2.7.1.20	*YJR105W*	ADENOSINE KINASE	ADN + ATP -> AMP + ADP
YDR226W	2.7.4.3	*ADK1*	CYTOSOLIC ADENYLATE KINASE	ATP + AMP <-> 2 ADP
YDR226W	2.7.4.3	*ADK1*	CYTOSOLIC ADENYLATE KINASE	GTP + AMP <-> ADP + GDP
YDR226W	2.7.4.3	*ADK1*	CYTOSOLIC ADENYLATE KINASE	ITP + AMP <-> ADP + IDP
YER170W	2.7.4.3	*ADK2*	ADENYLATE KINASE (MITOCHONDRIAL GTP:AMP PHOSPHOTRANSFERASE)	ATPm + AMPm <-> 2 ADPm
YER170W	2.7.4.3	*ADK2*	ADENYLATE KINASE (MITOCHONDRIAL GTP:AMP PHOSPHOTRANSFERASE)	GTPm + AMPm <-> ADPm + GDPm
YER170W	2.7.4.3	*ADK2*	ADENYLATE KINASE (MITOCHONDRIAL GTP:AMP PHOSPHOTRANSFERASE)	ITPm + AMPm <-> ADPm + IDPm
YGR180C	1.17.4.1	*RNR4*	RIBONUCLEOTIDE REDUCTASE, SMALL SUBUNIT (ALT), BETA CHAIN	
YIL066C	1.17.4.1	*RNR3*	RIBONUCLEOTIDE REDUCTASE (RIBONUCLEOSIDE-DIPHOSPHATE REDUCTASE) LARGE SUBUNIT, ALPHA CHAIN	ADP + RTHIO -> DADP + OTHIO

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YJL026W	1.17.4.1	*RNR2*	SMALL SUBUNIT OF RIBONUCLEOTIDE REDUCTASE, BETA CHAIN	
YKL067W	2.7.4.6	*YNK1*	NUCLEOSIDE-DIPHOSPHATE KINASE	UDP + ATP <-> UTP + ADP
YKL067W	2.7.4.6	*YNK1*	NUCLEOSIDE-DIPHOSPHATE KINASE	CDP + ATP <-> CTP + ADP
YKL067W	2.7.4.6	*YNK1*	NUCLEOSIDE-DIPHOSPHATE KINASE	DGDP + ATP <-> DGTP + ADP
YKL067W	2.7.4.6	*YNK1*	NUCLEOSIDE-DIPHOSPHATE KINASE	DUDP + ATP <-> DUTP + ADP
YKL067W	2.7.4.6	*YNK1*	NUCLEOSIDE-DIPHOSPHATE KINASE	DCDP + ATP <-> DCTP + ADP
YKL067W	2.7.4.6	*YNK1*	NUCLEOSIDE-DIPHOSPHATE KINASE	DTDP + ATP <-> DTTP + ADP
YKL067W	2.7.4.6	*YNK1*	NUCLEOSIDE-DIPHOSPHATE KINASE	DADP + ATP <-> DATP + ADP
YKL067W	2.7.4.6	*YNK1*	NUCLEOSIDE DIPHOSPHATE KINASE	GDP + ATP <-> GTP + ADP
YKL067W	2.7.4.6	*YNK1*	NUCLEOSIDE DIPHOSPHATE KINASE	IDP + ATP <-> ITP + IDP
*U19_*	2.7.4.11	*U19_*	ADENYLATE KINASE, DAMP KINASE	DAMP + ATP <-> DADP + ADP
YNL141W	3.5.4.2	*AAH1*	ADENINE DEAMINASE	AD -> NH3 + HYXN
*U20_*	2.7.1.73	*U20_*	INOSINE KINASE	INS + ATP -> IMP + ADP
*U21_*	2.7.1.73	*U21_*	GUANOSINE KINASE	GSN + ATP -> GMP + ADP
YDR399W	2.4.2.8	*HPT1*	HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE	HYXN + PRPP -> PPI + IMP
YDR399W	2.4.2.8	*HPT1*	HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE	GN + PRPP -> PPI + GMP
*U22_*	2.4.2.3	*U22_*	URIDINE PHOSPHORYLASE	URI + PI <-> URA + R1P
YKL024C	2.1.4.-	*URA6*	URIDYLATE KINASE	UMP + ATP <-> UDP + ADP
YKL024C	2.1.4.-	*URA6*	URIDYLATE KINASE	DUMP + ATP <-> DUDP + ADP
*U23_*	3.2.2.10	*U23_*	CMP GLYCOSYLASE	CMP -> CYTS + R5P
YHR144C	3.5.4.13	*DCD1*	DCTP DEAMINASE	DCTP -> DUTP + NH3
*U24_*	3.1.3.5	*U24_*	5'-NUCLEOTIDASE	DUMP -> DU + PI
*U25_*	3.1.3.5	*U25_*	5'-NUCLEOTIDASE	DTMP -> DT + PI
*U26_*	3.1.3.5	*U26_*	5'-NUCLEOTIDASE	DAMP -> DA + PI
*U27_*	3.1.3.5	*U27_*	5'-NUCLEOTIDASE	DGMP -> DG + PI
*U28_*	3.1.3.5	*U28_*	5'-NUCLEOTIDASE	DCMP -> DC + PI
*U29_*	3.1.3.5	*U29_*	5'-NUCLEOTIDASE	CMP -> CYTD + PI
*U30_*	3.1.3.5	*U30_*	5'-NUCLEOTIDASE	AMP -> PI + ADN
*U31_*	3.1.3.5	*U31_*	5'-NUCLEOTIDASE	GMP -> PI + GSN
*U32_*	3.1.3.5	*U32_*	5'-NUCLEOTIDASE	IMP -> PI + INS
*U33_*	3.1.3.5	*U33_*	5'-NUCLEOTIDASE	XMP -> PI + XTSINE
*U34_*	3.1.3.5	*U34_*	5'-NUCLEOTIDASE	UMP -> PI + URI
YER070W	1.17.4.1	*RNR1*	RIBONUCLEOSIDE-DIPHOSPHATE REDUCTASE	ADP + RTHIO -> DADP + OTHIO
YER070W	1.17.4.1	*RNR1*	RIBONUCLEOSIDE-DIPHOSPHATE REDUCTASE	GDP + RTHIO -> DGDP + OTHIO

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YER070W	1.17.4.1	*RNR1*	RIBONUCLEOSIDE-DIPHOSPHATE REDUCTASE	CDP + RTHIO -> DCDP + OTHIO
YER070W	1.17.4.1	*RNR1*	RIBONUCLEOSIDE-DIPHOSPHATE REDUCTASE	UDP + RTHIO -> OTHIO + DUDP
*U35_*	1.17.4.2	*U35_*	RIBONUCLEOSIDE-TRIPHOSPHATE REDUCTASE	ATP + RTHIO -> DATP + OTHIO
*U36_*	1.17.4.2	*U36_*	RIBONUCLEOSIDE-TRIPHOSPHATE REDUCTASE	GTP + RTHIO -> DGTP + OTHIO
*U37_*	1.17.4.2	*U37_*	RIBONUCLEOSIDE-TRIPHOSPHATE REDUCTASE	CTP + RTHIO -> DCTP + OTHIO
*U38_*	1.17.4.2	*U38_*	RIBONUCLEOSIDE-TRIPHOSPHATE REDUCTASE	UTP + RTHIO -> OTHIO + DUTP
*U39_*	3.6.1.-	*U39_*	NUCLEOSIDE TRIPHOSPHATASE	GTP -> GSN + 3 PI
*U40_*	3.6.1.-	*U40_*	NUCLEOSIDE TRIPHOSPHATASE	DGTP -> DG + 3 PI
YML035C	3.2.2.4	*AMD1*	AMP DEAMINASE	AMP -> AD + R5P
YBR284W	3.2.2.4	*YBR284W*	PROTEIN WITH SIMILARITY TO AMP DEAMINASE	AMP -> AD + R5P
YJL070C	3.2.2.4	*YJL070C*	PROTEIN WITH SIMILARITY TO AMP DEAMINASE	AMP -> AD + R5P
# AMINO ACID METABOLISM				
# GLUTAMATE METABOLISM (AMINOSUGARS METABOLISM)				
YMR250W	4.1.1.15	*GAD1*	GLUTAMATE DECARBOXYLASE B	GLU -> GABA + CO2
YGR019W	2.6.1.19	*UGA1*	AMINOBUTYRATE AMINOTRANSAMINASE 2	GABA + AKG -> SUCCSAL + GLU
YBR006W	1.2.1.16	*YBR006W*	SUCCINATE SEMIALDEHYDE DEHYDROGENASE –NADP	SUCCSAL + NADP -> SUCC + NADPH
YKL104C	2.6.1.16	*GFA1*	GLUTAMINE_FRUCTOSE-6-PHOSPHATE AMIDOTRANSFERASE (GLUCOSEAMINE-6-PHOSPHATE SYNTHASE)	F6P + GLN -> GLU + GA6P
YFL017C	2.3.1.4	*GNA1*	GLUCOSAMINE-PHOSPHATE N-ACETYLTRANSFERASE	ACCOA + GA6P <-> COA + NAGA6P
YEL058W	5.4.2.3	*PCM1*	PHOSPHOACETYLGLUCOSAMINE MUTASE	NAGA1P <-> NAGA6P
YDL103C	2.7.7.23	*QRI1*	N-ACETYLGLUCOSAMINE-1-PHOSPHATE-URIDYLTRANSFERASE	UTP + NAGA1P <-> UDPNAG + PPI
YBR023C	2.4.1.16	*CHS3*	CHITIN SYNTHASE 3	UDPNAG -> CHIT + UDP
YBR038W	2.4.1.16	*CHS2*	CHITIN SYNTHASE 2	UDPNAG -> CHIT + UDP
YNL192W	2.4.1.16	*CHS1*	CHITIN SYNTHASE 2	UDPNAG -> CHIT + UDP
YHR037W	1.5.1.12	*PUT2*	DELTA-1-PYRROLINE-5-CARBOXYLATE DEHYDROGENASE	GLUGSALm + NADPm -> NADPHm + GLUm
*U41_*		*U41_*		P5Cm + NADm -> NADHm + GLUm
YDL171C	1.4.1.14	*GLT1*	GLUTAMATE SYNTHASE (NADH)	AKG + GLN + NADH -> NAD + 2 GLU

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YDL215C	1.4.1.4	*GDH2*	GLUTAMATE DEHYDROGENASE	GLU + NAD -> AKG + NH3 + NADH
YAL062W	1.4.1.4	*GDH3*	NADP-LINKED GLUTAMATE DEHYDROGENASE	AKG + NH3 + NADPH -> GLU + NADP
YOR375C	1.4.1.4	*GDH1*	NADP-SPECIFIC GLUTAMATE DEHYDROGENASE	AKG + NH3 + NADPH -> GLU + NADP
YPR035W	6.3.1.2	*GLN1*	GLUTAMINE SYNTHETASE	GLU + NH3 + ATP -> GLN + ADP + PI
YEL058W	5.4.2.3	*PCM1*	PHOSPHOGLUCOSAMINE MUTASE	GA6P <-> GA1P
*U42_*	3.5.1.2	*U42_*	GLUTAMINASE A	GLN -> GLU + NH3
*U43_*	3.5.1.2	*U43_*	GLUTAMINASE B	GLN -> GLU + NH3
# GLUCOSAMINE				
U44_	5.3.1.10	*U44_*	GLUCOSAMINE-6-PHOSPHATE DEAMINASE	GA6P -> F6P + NH3
# ARABINOSE				
YBR149W	1.1.1.117	*ARA1*	D-ARABINOSE 1-DEHYDROGENASE (NAD(P)+).	ARAB + NAD -> ARABLAC + NADH
YBR149W	1.1.1.117	*ARA1*	D-ARABINOSE 1-DEHYDROGENASE (NAD(P)+).	ARAB + NADP -> ARABLAC + NADPH
# XYLOSE				
YGR194C	2.7.1.17	*XKS1*	XYLULOKINASE	XUL + ATP -> X5P + ADP
# MANNITOL				
*U45_*	1.1.1.17	*U45_*	MANNITOL-1-PHOSPHATE 5-DEHYDROGENASE	MNT6P + NAD <-> F6P + NADH
# ALANINE AND ASPARTATE METABOLISM				
YKL106W	2.6.1.1	*AAT1*	ASPARATE TRANSAMINASE	OAm + GLUm <-> ASPm + AKGm
YLR027C	2.6.1.1	*AAT2*	ASPARATE TRANSAMINASE	OA + GLU <-> ASP + AKG
YAR035W	2.3.1.7	*YAT1*	CARNITINE O-ACETYLTRANSFERASE	COAm + ACARm -> ACCOAm + CARm
YML042W	2.3.1.7	*CAT2*	CARNITINE O-ACETYLTRANSFERASE	ACCOA + CAR -> COA + ACAR
YDR111C	2.6.1.2	*YDR111C*	PUTATIVE ALANINE TRANSAMINASE	PYR + GLU <-> AKG + ALA
YLR089C	2.6.1.2	*YLR089C*	ALANINE AMINOTRANSFERASE, MITOCHONDRIAL PRECURSOR (GLUTAMIC--	PYRm + GLUm <-> AKGm + ALAm
YPR145W	6.3.5.4	*ASN1*	ASPARAGINE SYNTHETASE	ASP + ATP + GLN -> GLU + ASN + AMP + PPI
YGR124W	6.3.5.4	*ASN2*	ASPARAGINE SYNTHETASE	ASP + ATP + GLN -> GLU + ASN + AMP + PPI
YLL062C	2.1.1.10	*MHT1*	PUTATIVE COBALAMIN-DEPENDENT HOMOCYSTEINE S-METHYLTRANSFERASE, HOMOCYSTEINE S-METHYLTRANSFERASE	SAM + HCYS -> SAH + MET
YPL273W	2.1.1.10	*SAM4*	PUTATIVE COBALAMIN-DEPENDENT HOMOCYSTEINE S-METHYLTRANSFERASE	SAM + HCYS -> SAH + MET

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
# ASPARAGINE				
YCR024C	6.1.1.22	*YCR024C*	ASN-TRNA SYNTHETASE, MITOCHONDRIAL	ATPm + ASPm + TRNAm -> AMPm + PPIm + ASPTRNAm
YHR019C	6.1.1.23	*DED81*	ASN-TRNA SYNTHETASE	ATP + ASP + TRNA -> AMP + PPI + ASPTRNA
YLR155C	3.5.1.1	*ASP3-1*	ASPARAGINASE, EXTRACELLULAR	ASN -> ASP + NH3
YLR157C	3.5.1.1	*ASP3-2*	ASPARAGINASE, EXTRACELLULAR	ASN -> ASP + NH3
YLR158C	3.5.1.1	*ASP3-3*	ASPARAGINASE, EXTRACELLULAR	ASN -> ASP + NH3
YLR160C	3.5.1.1	*ASP3-4*	ASPARAGINASE, EXTRACELLULAR	ASN -> ASP + NH3
YDR321W	3.5.1.1	*ASP1*	ASPARAGINASE	ASN -> ASP + NH3
# GLYCINE, SERINE AND THREONINE METABOLISM				
YER081W	1.1.1.95	*SER3*	PHOSPHOGLYCERATE DEHYDROGENASE	3PG + NAD -> NADH + PHP
YIL074C	1.1.1.95	*SER33*	PHOSPHOGLYCERATE DEHYDROGENASE	3PG + NAD -> NADH + PHP
YOR184W	2.6.1.52	*SER1*	PHOSPHOSERINE TRANSAMINASE	PHP + GLU -> AKG + 3PSER
YGR208W	3.1.3.3	*SER2*	PHOSPHOSERINE PHOSPHATASE	3PSER -> PI + SER
YBR263W	2.1.2.1	*SHM1*	GLYCINE HYDROXYMETHYLTRANSFERASE	THFm + SERm <-> GLYm + METTHFm
YLR058C	2.1.2.1	*SHM2*	GLYCINE HYDROXYMETHYLTRANSFERASE	THF + SER <-> GLY + METTHF
YFL030W	2.6.1.44	*YFL030W*	PUTATIVE ALANINE GLYOXYLATE AMINOTRANSFERASE (SERINE PYRUVATE AMINOTRANSFERASE)	ALA + GLX <-> PYR + GLY
YDR019C	2.1.2.10	*GCV1*	GLYCINE CLEAVAGE T PROTEIN (T SUBUNIT OF GLYCINE DECARBOXYLASE COMPLEX	GLYm + THFm + NADm -> METTHFm + NADHm + CO2 + NH3
YDR019C	2.1.2.10	*GCV1*	GLYCINE CLEAVAGE T PROTEIN (T SUBUNIT OF GLYCINE DECARBOXYLASE COMPLEX	GLY + THF + NAD -> METTHF + NADH + CO2 + NH3
YER052C	2.7.2.4	*HOM3*	ASPARTATE KINASE, ASPARTATE KINASE I, II, III	ASP + ATP -> ADP + BASP
YDR158W	1.2.1.11	*HOM2*	ASPARTIC BETA SEMI-ALDEHYDE DEHYDROGENASE, ASPARTATE SEMIALDEHYDE DEHYDROGENASE	BASP + NADPH -> NADP + PI + ASPSA
YJR139C	1.1.1.3	*HOM6*	HOMOSERINE DEHYDROGENASE I	ASPSA + NADH -> NAD + HSER
YJR139C	1.1.1.3	*HOM6*	HOMOSERINE DEHYDROGENASE I	ASPSA + NADPH -> NADP + HSER
YHR025W	2.7.1.39	*THR1*	HOMOSERINE KINASE	HSER + ATP -> ADP + PHSER
YCR053W	4.2.99.2	*THR4*	THREONINE SYNTHASE	PHSER -> PI + THR
YGR155W	4.2.1.22	*CYS4*	CYSTATHIONINE BETA-SYNTHASE	SER + HCYS -> LLCT
YEL046C	4.1.2.5	*GLY1*	THREONINE ALDOLASE	GLY + ACAL ->THR

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YMR189W	1.4.4.2	*GCV2*	GLYCINE DECARBOXYLASE COMPLEX (P-SUBUNIT), GLYCINE SYNTHASE (P-SUBUNIT), GLYCINE CLEAVAGE SYSTEM (P-SUBUNIT)	GLYm + LIPOm <-> SAPm + CO2m
YCL064C	4.2.1.16	*CHA1*	THREONINE DEAMINASE	THR -> NH3 + OBUT
YER086W	4.2.1.16	*ILV1*	L-SERINE DEHYDRATASE	THRm -> NH3m + OBUTm
YCL064C	4.2.1.13	*CHA1*	CATABOLIC SERINE (THREONINE) DEHYDRATASE	SER -> PYR + NH3
YIL167W	4.2.1.13	*YIL167W*	CATABOLIC SERINE (THREONINE) DEHYDRATASE	SER -> PYR + NH3
*U46_*	1.1.1.103	*U46_*	THREONINE DEHYDROGENASE	THR + NAD -> GLY + AC + NADH
# METHIONINE METABOLISM				
YFR055W	4.4.1.8	*YFR055W*	CYSTATHIONINE-B-LYASE	LLCT -> HCYS + PYR + NH3
YER043C	3.3.1.1	*SAH1*	PUTATIVE S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE	SAH -> HCYS + ADN
YER091C	2.1.1.14	*MET6*	VITAMIN B12-(COBALAMIN)-INDEPENDENT ISOZYME OF METHIONINE SYNTHASE (ALSO CALLED N5-METHYLTETRAHYDROFOLATE HOMOCYSTEINE METHYLTRANSFERASE OR 5-METHYLTETRAHYDROPTEROYL TRIGLUTAMATE HOMOCYSTEINE METHYLTRANSFERASE)	HCYS + MTHPTGLU -> THPTGLU + MET
*U47_*	2.1.1.13	*U47_*	METHIONINE SYNTHASE	HCYS + MTHF -> THF + MET
YAL012W	4.4.1.1	*CYS3*	CYSTATHIONINE GAMMA-LYASE	LLCT -> CYS + NH3 + OBUT
YNL277W	2.3.1.31	*MET2*	HOMOSERINE O-TRANS-ACETYLASE	ACCOA + HSER <-> COA + OAHSER
YLR303W	4.2.99.10	*MET17*	O-ACETYLHOMOSERINE (THIOL)-LYASE	OAHSER + METH -> MET + AC
YLR303W	4.2.99.8	*MET17*	O-ACETYLHOMOSERINE (THIOL)-LYASE	OAHSER + H2S -> AC + HCYS
YLR303W	4.2.99.8, 4.2.99.10	*MET17*	O-ACETYLHOMOSERINE SULFHYDRYLASE (OAH SHLASE); CONVERTS O-ACETYLHOMOSERINE INTO HOMOCYSTEINE	OAHSER + H2S -> AC + HCYS
YML082W	4.2.99.9	*YML082W*	PUTATIVE CYSTATHIONINE GAMMA-SYNTHASE	OSLHSER <-> SUCC + OBUT + NH3
YDR502C	2.5.1.6	*SAM2*	S-ADENOSYLMETHIONINE SYNTHETASE	MET + ATP -> PPI + PI + SAM
YLR180W	2.5.1.6	*SAM1*	S-ADENOSYLMETHIONINE SYNTHETASE	MET + ATP -> PPI + PI + SAM
YLR172C	2.1.1.98	*DPH5*	DIPHTHINE SYNTHASE	SAM + CALH -> SAH + DPTH
# CYSTEINE BIOSYNTHESIS				
YJR010W	2.7.7.4	*MET3*	ATP SULFURYLASE	SLF + ATP -> PPI + APS
YKL001C	2.7.1.25	*MET14*	ADENYLYLSULFATE KINASE	APS + ATP -> ADP + PAPS
YFR030W	1.8.1.2	*MET10*	SULFITE REDUCTASE	H2SO3 + 3 NADPH <-> H2S + 3 NADP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
*U48_*	2.3.1.30	*U48_*	SERINE TRANSACETYLASE	SER + ACCOA -> COA + ASER
YGR012W	4.2.99.8	*YGR012W*	PUTATIVE CYSTEINE SYNTHASE (O-ACETYLSERINE SULFHYDRYLASE) (O-	ASER + H2S -> AC + CYS
YOL064C	3.1.3.7	*MET22*	3' - 5' BISPHOSPHATE NUCLEOTIDASE	PAP -> AMP + PI
YPR167C	1.8.99.4	*MET16*	PAPS REDUCTASE	PAPS + RTHIO -> OTHIO + H2SO3 + PAP
YCL050C	2.7.7.5	*APA1*	DIADENOSINE 5',5'''-P1,P4-TETRAPHOSPHATE PHOSPHORYLASE I	ADP + SLF <-> PI + APS
# BRANCHED CHAIN AMINO ACID METABOLISM (VALINE, LEUCINE AND ISOLEUCINE)				
YHR208W	2.6.1.42	*BAT1*	BRANCHED CHAIN AMINO ACID AMINOTRANSFERASE	OICAPm + GLUm <-> AKGm + LEUm
YHR208W	2.6.1.42	*BAT1*	BRANCHED CHAIN AMINO ACID AMINOTRANSFERASE	OMVALm + GLUm <-> AKGm + ILEm
YJR148W	2.6.1.42	*BAT2*	BRANCHED-CHAIN AMINO ACID TRANSAMINASE, HIGHLY SIMILAR TO MAMMALIAN ECA39, WHICH IS REGULATED BY THE ONCOGENE MYC	OMVAL + GLU <-> AKG + ILE
YJR148W	2.6.1.42	*BAT2*	BRANCHED CHAIN AMINO ACID AMINOTRANSFERASE	OIVAL + GLU <-> AKG + VAL
YJR148W	2.6.1.42	*BAT2*	BRANCHED-CHAIN AMINO ACID TRANSAMINASE, HIGHLY SIMILAR TO MAMMALIAN ECA39, WHICH IS REGULATED BY THE ONCOGENE MYC	OICAP + GLU <-> AKG + LEU
YMR108W	4.1.3.18	*ILV2*	ACETOLACTATE SYNTHASE, LARGE SUBUNIT	OBUTm + PYRm -> ABUTm + CO2m
YCL009C	4.1.3.18	*ILV6*	ACETOLACTATE SYNTHASE, SMALL SUBUNIT	
YMR108W	4.1.3.18	*ILV2*	ACETOLACTATE SYNTHASE, LARGE SUBUNIT	2 PYRm -> CO2m + ACLACm
YCL009C	4.1.3.18	*ILV6*	ACETOLACTATE SYNTHASE, SMALL SUBUNIT	
YLR355C	1.1.1.86	*ILV5*	KETO-ACID REDUCTOISOMERASE	ACLACm + NADPHm -> NADPm + DHVALm
YLR355C	1.1.1.86	*ILV5*	KETO-ACID REDUCTOISOMERASE	ABUTm + NADPHm -> NADPm + DHMVAm
YJR016C	4.2.1.9	*ILV3*	DIHYDROXY ACID DEHYDRATASE	DHVALm -> OIVALm
YJR016C	4.2.1.9	*ILV3*	DIHYDROXY ACID DEHYDRATASE	DHMVAm -> OMVALm
YNL104C	4.1.3.12	*LEU4*	ALPHA-ISOPROPYLMALATE SYNTHASE (2-ISOPROPYLMALATE SYNTHASE)	ACCOAm + OIVALm -> COAm + IPPMALm
YGL009C	4.2.1.33	*LEU1*	ISOPROPYLMALATE ISOMERASE	CBHCAP <-> IPPMAL
YGL009C	4.2.1.33	*LEU1*	ISOPROPYLMALATE ISOMERASE	PPMAL <-> IPPMAL

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C.#	GENE	GENE DESCRIPTION	REACTION
YCL018W	1.1.1.85	*LEU2*	BETA-IPM (ISOPROPYLMALATE) DEHYDROGENASE	IPPMAL + NAD -> NADH + OICAP + CO2
# LYSINE BIOSYNTHESIS/DEGRADATION				
*U49_*	4.2.1.79	*U49_*	2-METHYLCITRATE DEHYDRATASE	HCITm <-> HACNm
YDR234W	4.2.1.36	*LYS4*	HOMOACONITATE HYDRATASE	HICITm <-> HACNm
YIL094C	1.1.1.155	*LYS12*	HOMOISOCITRATE DEHYDROGENASE (STRATHERN:1.1.1.87)	HICITm + NADm <-> OXAm + CO2m + NADHm
*U50_*		*U50_*	NON-ENZYMATIC	OXAm <-> CO2m + AKAm
*U51_*	2.6.1.39	*U51_*	2-AMINOADIPATETRANSAMINASE	AKA + GLU <-> AMA + AKG
YBR115C	1.2.1.31	*LYS2*	L-AMINOADIPATE-SEMIALDEHYDE DEHYDROGENASE, LARGE SUBUNIT	AMA + NADPH + ATP -> AMASA + NADP + AMP + PPI
YGL154C	1.2.1.31	*LYS5*	L-AMINOADIPATE-SEMIALDEHYDE DEHYDROGENASE, SMALL SUBUNIT	
YBR115C	1.2.1.31	*LYS2*	L-AMINOADIPATE-SEMIALDEHYDE DEHYDROGENASE, LARGE SUBUNIT	AMA + NADH + ATP -> AMASA + NAD + AMP + PPI
YGL154C	1.2.1.31	*LYS5*	L-AMINOADIPATE-SEMIALDEHYDE DEHYDROGENASE, SMALL SUBUNIT	
YNR050C	1.5.1.10	*LYS9*	SACCHAROPINE DEHYDROGENASE (NADP+, L-GLUTAMATE FORMING)	GLU + AMASA + NADPH <-> SACP + NADP
YIR034C	1.5.1.7	*LYS1*	SACCHAROPINE DEHYDROGENASE (NAD+, L-LYSINE FORMING)	SACP + NAD <-> LYS + AKG + NADH
YDR037W	6.1.1.6	*KRS1*	LYSYL-TRNA SYNTHETASE, CYTOSOLIC	ATP + LYS + LTRNA -> AMP + PPI + LLTRNA
YNL073W	6.1.1.6	*MSK1*	LYSYL-TRNA SYNTHETASE, MITOCHONDRIAL	ATPm + LYSm + LTRNAm -> AMPm + PPIm + LLTRNAm
YDR368W	1.1.1.-	*YPR1*	SIMILAR TO ALDO-KETO REDUCTASE	
# ARGININE METABOLISM				
YMR062C	2.3.1.1	*ECM40*	AMINO-ACID N-ACETYLTRANSFERASE	GLUm + ACCOAm -> COAm + NAGLUm
YER069W	2.7.2.8	*ARG5*	ACETYLGLUTAMATE KINASE	NAGLUm + ATPm -> ADPm + NAGLUPm
YER069W	1.2.1.38	*ARG5*	N-ACETYL-GAMMA-GLUTAMYL-PHOSPHATE REDUCTASE AND ACETYLGLUTAMATE KINASE	NAGLUPm + NADPHm -> NADPm + PIm + NAGLUSm
YOL140W	2.6.1.11	*ARG8*	ACETYLORNITHINE AMINOTRANSFERASE	NAGLUSm + GLUm -> AKGm + NAORNm
YMR062C	2.3.1.35	*ECM40*	GLUTAMATE N-ACETYLTRANSFERASE	NAORNm + GLUm -> ORNm + NAGLUm
YJL130C	6.3.5.5	*URA2*	CARBAMOYL-PHOPHATE SYNTHETASE, ASPARTATETRANSCARBAMYLASE, AND GLUTAMINE AMIDOTRANSFERASE	GLN + 2 ATP + CO2 -> GLU + CAP + 2 ADP + PI

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YJR109C	6.3.5.5	*CPA2*	CARBAMYL PHOSPHATE SYNTHETASE, LARGE CHAIN	GLN + 2 ATP + CO2 -> GLU + CAP + 2 ADP + PI
YOR303W	6.3.5.5	*CPA1*	CARBAMOYL PHOSPHATE SYNTHETASE, SAMLL CHAIN, ARGININE SPECIFIC	
YJL088W	2.1.3.3	*ARG3*	ORNITHINE CARBAMOYLTRANSFERASE	ORN + CAP -> CITR + PI
YLR438W	2.6.1.13	*CAR2*	ORNITHINE TRANSAMINASE	ORN + AKG -> GLUGSAL + GLU
YOL058W	6.3.4.5	*ARG1*	ARGINOSUCCINATE SYNTHETASE	CITR + ASP + ATP <-> AMP + PPI + ARGSUCC
YHR018C	4.3.2.1	*ARG4*	ARGININOSUCCINATE LYASE	ARGSUCC <-> FUM + ARG
YKL184W	4.1.1.17	*SPE1*	ORNITHINE DECARBOXYLASE	ORN -> PTRSC + CO2
YOL052C	4.1.1.50	*SPE2*	S-ADENOSYLMETHIONINE DECARBOXYLASE	SAM <-> DSAM + CO2
YPR069C	2.5.1.16	*SPE3*	PUTRESCINE AMINOPROPYLTRANSFERASE (SPERMIDINE SYNTHASE)	PTRSC + SAM -> SPRMD + 5MTA
YLR146C	2.5.1.22	*SPE4*	SPERMINE SYNTHASE	DSAM + SPRMD -> 5MTA + SPRM
YDR242W	3.5.1.4	*AMD2*	AMIDASE	GBAD -> GBAT + NH3
YMR293C	3.5.1.4	*YMR293C*	PROBABLE AMIDASE	GBAD -> GBAT + NH3
YPL111W	3.5.3.1	*CAR1*	ARGINASE	ARG -> ORN + UREA
YDR341C	6.1.1.19	*YDR341C*	ARGINYL-TRNA SYNTHETASE	ATP + ARG + ATRNA -> AMP + PPI + ALTRNA
YHR091C	6.1.1.19	*MSR1*	ARGINYL-TRNA SYNTHETASE	ATP + ARG + ATRNA -> AMP + PPI + ALTRNA
YHR068W	1.5.99.6	*DYS1*	DEOXYHYPUSINE SYNTHASE	SPRMD + Qm -> DAPRP + QH2m
# HISTIDINE METABOLISM				
YER055C	2.4.2.17	*HIS1*	ATP PHOSPHORIBOSYLTRANSFERASE	PRPP + ATP -> PPI + PRBATP
YCL030C	3.6.1.31	*HIS4*	PHOSPHORIBOSYL-AMP CYCLOHYDROLASE / PHOSPHORIBOSYL-ATP PYROPHOSPHOHYDROLASE / HISTIDINOL DEHYDROGENASE	PRBATP -> PPI + PRBAMP
YCL030C	3.5.4.19	*HIS4*	HISTIDINOL DEHYDROGENASE	PRBAMP -> PRFP
YIL020C	5.3.1.16	*HIS6*	PHOSPHORIBOSYL-5-AMINO-1-PHOSPHORIBOSYL-4-IMIDAZOLECARBOXIAMIDE ISOMERASE	PRFP -> PRLP
YOR202W	4.2.1.19	*HIS3*	IMIDAZOLEGLYCEROL-PHOSPHATE DEHYDRATASE	DIMGP -> IMACP
YIL116W	2.6.1.9	*HIS5*	HISTIDINOL-PHOSPHATE AMINOTRANSFERASE	IMACP + GLU -> AKG + HISOLP
YFR025C	3.1.3.15	*HIS2*	HISTIDINOLPHOSPHATASE	HISOLP -> PI + HISOL

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YCL030C	1.1.1.23	*HIS4*	PHOSPHORIBOSYL-AMP CYCLOHYDROLASE / PHOSPHORIBOSYL-ATP PYROPHOSPHOHYDROLASE / HISTIDINOL DEHYDROGENASE	HISOL + 2 NAD -> HIS + 2 NADH
YBR248C	2.4.2.-	*HIS7*	GLUTAMINE AMIDOTRANSFERASE:CYCLASE	PRLP + GLN -> GLU + AICAR + DIMGP
YPR033C	6.1.1.21	*HTS1*	HISTIDYL-TRNA SYNTHETASE	ATP + HIS + HTRNA -> AMP + PPI + HHTRNA
YBR034C	2.1.1.-	*HMT1*	HNRNP ARGININE N-METHYLTRANSFERASE	SAM + HIS -> SAH + MHIS
YCL054W	2.1.1.-	*SPB1*	PUTATIVE RNA METHYLTRANSFERASE	
YML110C	2.1.1.-	*COQ5*	UBIQUINONE BIOSYNTHESIS METHLYTRANSFERASE COQ5	
YOR201C	2.1.1.-	*PET56*	RRNA (GUANOSINE-2'-O-)-METHYLTRANSFERASE	
YPL266W	2.1.1.-	*DIM1*	DIMETHYLADENOSINE TRANSFERASE	
# PHENYLALANINE, TYROSINE AND TRYPTOPHAN BIOSYNTHESIS (AROMATIC AMINO ACIDS)				
YBR249C	4.1.2.15	*ARO4*	3-DEOXY-D-ARABINO-HEPTULOSONATE 7-PHOSPHATE (DAHP) SYNTHASE ISOENZYME	E4P + PEP -> PI + 3DDAH7P
YDR035W	4.1.2.15	*ARO3*	DAHP SYNTHASE\; A.K.A. PHOSPHO-2-DEHYDRO-3-DEOXYHEPTONATE ALDOLASE, PHENYLALANINE-INHIBITED\; PHOSPHO-2-KETO-3-DEOXYHEPTONATE ALDOLASE\; 2-DEHYDRO-3-DEOXYPHOSPHOHEPTONATE ALDOLASE\; 3-DEOXY-D-ARABINE-HEPTULOSONATE-7-PHOSPHATE SYNTHASE	E4P + PEP -> PI + 3DDAH7P
YDR127W	4.6.1.3	*ARO1*	PENTAFUNCTIONAL AROM POLYPEPTIDE (CONTAINS: 3-DEHYDROQUINATE SYNTHASE, 3-DEHYDROQUINATE DEHYDRATASE (3-DEHYDROQUINASE), SHIKIMATE 5-DEHYDROGENASE, SHIKIMATE KINASE, AND EPSP SYNTHASE)	3DDAH7P -> DQT + PI
YDR127W	4.2.1.10	*ARO1*	3-DEHYDROQUINATE DEHYDRATASE	DQT -> DHSK
YDR127W	1.1.1.25	*ARO1*	SHIKIMATE DEHYDROGENASE	DHSK + NADPH -> SME + NADP
YDR127W	2.7.1.71	*ARO1*	SHIKIMATE KINASE I, II	SME + ATP -> ADP + SME5P
YDR127W	2.5.1.19	*ARO1*	3-PHOSPHOSHIKIMATE-1-CARBOXYVINYLTRANSFERASE	SME5P + PEP -> 3PSME + PI
YGL148W	4.6.1.4	*ARO2*	CHORISMATE SYNTHASE	3PSME -> PI + CHOR

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YPR060C	5.4.99.5	*ARO7*	CHORISMATE MUTASE	CHOR -> PHEN
YNL316C	4.2.1.51	*PHA2*	PREPHENATE DEHYDRATASE	PHEN -> CO2 + PHPYR
YHR137W	2.6.1.-	*ARO9*	PUTATIVE AROMATIC AMINO ACID AMINOTRANSFERASE II	PHPYR + GLU <-> AKG + PHE
YBR166C	1.3.1.13	*TYR1*	PREPHENATE DEHYDROGENASE (NADP+)	PHEN + NADP -> 4HPP + CO2 + NADPH
YGL202W	2.6.1.-	*ARO8*	AROMATIC AMINO ACID AMINOTRANSFERASE I	4HPP + GLU -> AKG + TYR
YHR137W	2.6.1.-	*ARO9*	AROMATIC AMINO ACID AMINOTRANSFERASE II	4HPP + GLU -> AKG + TYR
*U52_*	1.3.1.12	*U52_*	PREPHANATE DEHYDROGENASE	PHEN + NAD -> 4HPP + CO2 + NADH
YER090W	4.1.3.27	*TRP2*	ANTHRANILATE SYNTHASE	CHOR + GLN -> GLU + PYR + AN
YKL211C	4.1.3.27	*TRP3*	ANTHRANILATE SYNTHASE	CHOR + GLN -> GLU + PYR + AN
YDR354W	2.4.2.18	*TRP4*	ANTHRANILATE PHOSPHORIBOSYL TRANSFERASE	AN + PRPP -> PPI + NPRAN
YDR007W	5.3.1.24	*TRP1*	N-(5'-PHOSPHORIBOSYL)-ANTHRANILATE ISOMERASE	NPRAN -> CPAD5P
YKL211C	4.1.1.48	*TRP3*	INDOLEGLYCEROL PHOSPHATE SYNTHASE	CPAD5P -> CO2 + IGP
YGL026C	4.2.1.20	*TRP5*	TRYPTOPHAN SYNTHETASE	IGP + SER -> T3P1 + TRP
YDR256C	1.11.1.6	*CTA1*	CATALASE A	2 H2O2 -> O2
YGR088W	1.11.1.6	*CTT1*	CYTOPLASMIC CATALASE T	2 H2O2 -> O2
YKL106W	2.6.1.1	*AAT1*	ASPARATE AMINOTRANSFERASE	4HPP + GLU <-> AKG + TYR
YLR027C	2.6.1.1	*AAT2*	ASPARATE AMINOTRANSFERASE	4HPP + GLU <-> AKG + TYR
YMR170C	1.2.1.5	*ALD2*	CYTOSOLIC ALDEYHDE DEHYDROGENASE	ACAL + NAD -> NADH + AC
YMR169C	1.2.1.5	*ALD3*	STRONG SIMILARITY TO ALDEHYDE DEHYDROGENASE	ACAL + NAD -> NADH + AC
YOR374W	1.2.1.3	*ALD4*	MITOCHONDRIAL ALDEHYDE DEHYDROGENASE	ACALm + NADm -> NADHm + ACm
YOR374W	1.2.1.3	*ALD4*	MITOCHONDRIAL ALDEHYDE DEHYDROGENASE	ACALm + NADPm -> NADPHm + ACm
YER073W	1.2.1.3	*ALD5*	MITOCHONDRIAL ALDEHYDE DEHYDROGENASE	ACALm + NADPm -> NADPHm + ACm
YPL061W	1.2.1.3	*ALD6*	CYTOSOLIC ALDEHYDE DEHYDROGENASE	ACAL + NADP -> NADPH + AC
YJR078W	1.13.11.11	*YJR078W*	PROTEIN WITH SIMILARITY TO INDOLEAMINE 2,3-DIOXYGENASES, WHICH CATALYZE CONVERSION OF TRYPTOPHAN AND OTHER INDOLE DERIVATIVES INTO KYNURENINES, TRYPTOPHAN 2,3-DIOXYGENASE	TRP + O2 -> FKYN

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
*U53_*	3.5.1.9	*U53_*	KYNURENINE FORMAMIDASE	FKYN -> FOR + KYN
YLR231C	3.7.1.3	*YLR231C*	PROBABLE KYNURENINASE (L-KYNURENINE HYDROLASE)	KYN -> ALA + AN
YBL098W	1.14.13.9	*YBL098W*	KYNURENINE 3-HYDROXYLASE, NADPH-DEPENDENT FLAVIN MONOOXYGENASE THAT CATALYZES THE HYDROXYLATION OF KYNURENINE TO 3-HYDROXYKYNURENINE IN TRYPTOPHAN DEGRADATION AND NICOTINIC ACID SYNTHESIS, KYNURENINE 3-MONOOXYGENASE	KYN + NADPH + O2 -> HKYN + NADP
YLR231C	3.7.1.3	*YLR231C*	PROBABLE KYNURENINASE (L-KYNURENINE HYDROLASE)	HKYN -> HAN + ALA
YJR025C	1.13.11.6	*BNA1*	3-HYDROXYANTHRANILATE 3,4-DIOXYGENASE (3-HAO) (3- HYDROXYANTHRANILIC ACID DIOXYGENASE) (3-HYDROXYANTHRANILATE HYDROXYANTHRANILIC ACID DIOXYGENASE) (3-HYDROXYANTHRANILATE OXYGENASE)	HAN + O2 -> CMUSA
*U54_*	4.1.1.45	*U54_*	PICOLINIC ACID DECARBOXYLASE	CMUSA -> CO2 + AM6SA
*U55_*	1.2.1.32	*U55_*		AM6SA + NAD -> AMUCO + NADH
*U56_*	1.5.1.-	*U56_*		AMUCO + NADPH -> AKA + NADP + NH3
*U57_*	1.3.11.27	*U57_*	4-HYDROXYPHENYLPYRUVATE DIOXYGENASE	4HPP + O2 -> HOMOGEN + CO2
*U58_*	1.13.11.5	*U58_*	HOMOGENTISATE 1,2-DIOXYGENASE	HOMOGEN + O2 -> MACAC
*U59_*	5.2.1.2	*U59_*	MALEYL-ACETOACETATE ISOMERASE	MACAC -> FUACAC
*U60_*	3.7.1.2	*U60_*	FUMARYLACETOACETASE	FUACAC -> FUM + ACTAC
YDR268W	6.1.1.2	*MSW1*	TRYPTOPHANYL-TRNA SYNTHETASE, MITOCHONDRIAL	ATPm + TRPm + TRNAm -> AMPm + PPIm + TRPTRNAm
YDR242W	3.5.1.4	*AMD2*	PUTATIVE AMIDASE	PAD -> PAC + NH3
YDR242W	3.5.1.4	*AMD2*	PUTATIVE AMIDASE	IAD -> IAC + NH3
*U61_*	2.6.1.29	*U61_*	DIAMINE TRANSAMINASE	SPRMD + ACCOA -> ASPERMD + COA
*U62_*	1.5.3.11	*U62_*	POLYAMINE OXIDASE	ASPERMD + O2 -> APRUT + APROA + H2O2
*U63_*	1.5.3.11	*U63_*	POLYAMINE OXIDASE	APRUT + O2 -> GABAL + APROA + H2O2
*U64_*	2.6.1.29	*U64_*	DIAMINE TRANSAMINASE	SPRM + ACCOA -> ASPRM + COA
*U65_*	1.5.3.11	*U65_*	POLYAMINE OXIDASE	ASPRM + O2 -> ASPERMD + APROA + H2O2

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
# PROLINE BIOSYNTHESIS				
YDR300C	2.7.2.11	*PRO1*	GAMMA-GLUTAMYL KINASE, GLUTAMATE KINASE	GLU + ATP -> ADP + GLUP
YOR323C	1.2.1.41	*PRO2*	GAMMA-GLUTAMYL PHOSPHATE REDUCTASE	GLUP + NADH -> NAD + PI + GLUGSAL
YOR323C	1.2.1.41	*PRO2*	GAMMA-GLUTAMYL PHOSPHATE REDUCTASE	GLUP + NADPH -> NADP + PI + GLUGSAL
*U66_*		*U66_*	SPONTANEOUS CONVERSION (STRATHERN)	GLUGSAL <-> P5C
*U67_*		*U67_*	SPONTANEOUS CONVERSION (STRATHERN)	GLUGSALm <-> P5Cm
YER023W	1.5.1.2	*PRO3*	PYRROLINE-5-CARBOXYLATE REDUCTASE	P5C + NADPH -> PRO + NADP
YER023W	1.5.1.2	*PRO3*	PYRROLINE-5-CARBOXYLATE REDUCTASE	PHC + NADPH -> HPRO + NADP
YER023W	1.5.1.2	*PRO3*	PYRROLINE-5-CARBOXYLATE REDUCTASE	PHC + NADH -> HPRO + NAD
YLR142W	1.5.3.-	*PUT1*	PROLINE DEHYDROGENASE	PROm + NADm -> P5Cm + NADHm
# METABOLISM OF OTHER AMINO ACID				
# BETA-ALANINE METABOLISM				
*U68_*	1.2.1.3	*U68_*	ALDEHYDE DEHYDROGENASE, MITOCHONDRIAL 1	GABALm + NADm -> GABAm + NADHm
YER073W	1.2.1.3	*ALD5*	MITOCHONDRIAL ALDEHYDE DEHYDROGENASE	LACALm + NADm <-> LLACm + NADHm
# CYANOAMINO ACID METABOLISM				
YJL126W	3.5.5.1	*NIT2*	NITRILASE	APROP -> ALA + NH3
YJL126W	3.5.5.1	*NIT2*	NITRILASE	ACYBUT -> GLU + NH3
# PROTEINS, PEPTIDES AND AMINO ACIDS METABOLISM				
YLR195C	2.3.1.97	*NMT1*	GLYCYLPEPTIDE N-TETRADECANOYLTRANSFERASE	TCOA + GLP -> COA + TGLP
YDL040C	2.3.1.88	*NAT1*	PEPTIDE ALPHA-N-ACETYLTRANSFERASE	ACCOA + PEPD -> COA + APEP
YGR147C	2.3.1.88	*NAT2*	PEPTIDE ALPHA-N-ACETYLTRANSFERASE	ACCOA + PEPD -> COA + APEP
# GLUTATHIONE BIOSYNTHESIS				
YJL101C	6.3.2.2	*GSH1*	GAMMA-GLUTAMYLCYSTEINE SYNTHETASE	CYS + GLU + ATP -> GC + PI + ADP
YOL049W	6.3.2.3	*GSH2*	GLUTATHIONE SYNTHETASE	GLY + GC + ATP -> RGT + PI + ADP
YBR244W	1.11.1.9	*GPX2*	GLUTATHIONE PEROXIDASE	2 RGT + H2O2 <-> OGT
YIR037W	1.11.1.9	*HYR1*	GLUTATHIONE PEROXIDASE	2 RGT + H2O2 <-> OGT
YKL026C	1.11.1.9	*GPX1*	GLUTATHIONE PEROXIDASE	2 RGT + H2O2 <-> OGT
YPL091W	1.6.4.2	*GLR1*	GLUTATHIONE OXIDOREDUCTASE	NADPH + OGT -> NADP + RGT

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YLR299W	2.3.2.2	*ECM38*	GAMMA-GLUTAMYLTRANSPEPTIDASE	RGT + ALA -> CGLY + ALAGLY
# METABOLISM OF COMPLEX CARBOHYDRATES				
# STARCH AND SUCROSE METABOLISM				
YGR032W	2.4.1.34	*GSC2*	1,3-BETA-GLUCAN SYNTHASE	UDPG -> 13GLUCAN + UDP
YLR342W	2.4.1.34	*FKS1*	1,3-BETA-GLUCAN SYNTHASE	UDPG -> 13GLUCAN + UDP
YMR306W	2.4.1.34	*FKS3*	PROTEIN WITH SIMILARITY TO FKS1P AND GSC2P	UDPG -> 13GLUCAN + UDP
YDR261C	3.2.1.58	*EXG2*	EXO-1,3-B-GLUCANASE	13GLUCAN -> GLC
YGR282C	3.2.1.58	*BGL2*	CELL WALL ENDO-BETA-1,3-GLUCANASE	13GLUCAN -> GLC
YLR300W	3.2.1.58	*EXG1*	EXO-1,3-BETA-GLUCANASE	13GLUCAN -> GLC
YOR190W	3.2.1.58	*SPR1*	SPORULATION-SPECIFIC EXO-1,3-BETA-GLUCANASE	13GLUCAN -> GLC
# GLYCOPROTEIN BIOSYNTHESIS / DEGRADATION				
YMR013C	2.7.1.108	*SEC59*	DOLICHOL KINASE	CTP + DOL -> CDP + DOLP
YPR183W	2.4.1.83	*DPM1*	DOLICHYL-PHOSPHATE BETA-D-MANNOSYLTRANSFERASE	GDPMAN + DOLP -> GDP + DOLMANP
YAL023C	2.4.1.109	*PMT2*	DOLICHYL-PHOSPHATE-MANNOSE--PROTEIN MANNOSYLTRANSFERASE	DOLMANP -> DOLP + MANNAN
YDL093W	2.4.1.109	*PMT5*	DOLICHYL-PHOSPHATE-MANNOSE--PROTEIN MANNOSYLTRANSFERASE	DOLMANP -> DOLP + MANNAN
YDL095W	2.4.1.109	*PMT1*	DOLICHYL-PHOSPHATE-MANNOSE--PROTEIN MANNOSYLTRANSFERASE	DOLMANP -> DOLP + MANNAN
YGR199W	2.4.1.109	*PMT6*	DOLICHYL-PHOSPHATE-MANNOSE--PROTEIN MANNOSYLTRANSFERASE	DOLMANP -> DOLP + MANNAN
YJR143C	2.4.1.109	*PMT4*	DOLICHYL-PHOSPHATE-MANNOSE--PROTEIN MANNOSYLTRANSFERASE	DOLMANP -> DOLP + MANNAN
YOR321W	2.4.1.109	*PMT3*	DOLICHYL-PHOSPHATE-MANNOSE--PROTEIN MANNOSYLTRANSFERASE	DOLMANP -> DOLP + MANNAN
YBR199W	2.4.1.131	*KTR4*	GLYCOLIPID 2-ALPHA-MANNOSYLTRANSFERASE	MAN2PD + 2 GDPMAN -> 2 GDP + 2MANPD
YBR205W	2.4.1.131	*KTR3*	GLYCOLIPID 2-ALPHA-MANNOSYLTRANSFERASE	MAN2PD + 2 GDPMAN -> 2 GDP + 2MANPD
YDR483W	2.4.1.131	*KRE2*	GLYCOLIPID 2-ALPHA-MANNOSYLTRANSFERASE	MAN2PD + 2 GDPMAN -> 2 GDP + 2MANPD
YJL139C	2.4.1.131	*YUR1*	GLYCOLIPID 2-ALPHA-MANNOSYLTRANSFERASE	MAN2PD + 2 GDPMAN -> 2 GDP + 2MANPD
YKR061W	2.4.1.131	*KTR2*	GLYCOLIPID 2-ALPHA-MANNOSYLTRANSFERASE	MAN2PD + 2 GDPMAN -> 2 GDP + 2MANPD
YOR099W	2.4.1.131	*KTR1*	GLYCOLIPID 2-ALPHA-MANNOSYLTRANSFERASE	MAN2PD + 2 GDPMAN -> 2 GDP + 2MANPD
YPL053C	2.4.1.131	*KTR6*	GLYCOLIPID 2-ALPHA-MANNOSYLTRANSFERASE	MAN2PD + 2 GDPMAN -> 2 GDP + 2MANPD

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
# AMINOSUGARS METABOLISM				
YER062C	3.1.3.21	*HOR2*	DL-GLYCEROL-3-PHOSPHATASE	GL3P -> GL + PI
YIL053W	3.1.3.21	*RHR2*	DL-GLYCEROL-3-PHOSPHATASE	GL3P -> GL + PI
YLR307W	3.5.1.41	*CDA1*	CHITIN DEACETYLASE	CHIT -> CHITO + AC
YLR308W	3.5.1.41	*CDA2*	CHITIN DEACETYLASE	CHIT -> CHITO + AC
# METABOLSIM OF COMPLEX LIPIDS				
# GLYCEROL (GLYCEROLIPID METABOLISM)				
YFL053W	2.7.1.29	*DAK2*	DIHYDROXYACETONE KINASE	GLYN + ATP -> T3P2 + ADP
YML070W	2.7.1.29	*DAK1*	PUTATIVE DIHYDROXYACETONE KINASE	GLYN + ATP -> T3P2 + ADP
YDL022W	1.1.1.8	*GPD1*	GLYCEROL-3-PHOSPHATE DEHYDROGENASE (NAD)	T3P2 + NADH -> GL3P + NAD
YOL059W	1.1.1.8	*GPD2*	GLYCEROL-3-PHOSPHATE DEHYDROGENASE (NAD)	T3P2 + NADH -> GL3P + NAD
YHL032C	2.7.1.30	*GUT1*	GLYCEROL KINASE	GL + ATP -> GL3P + ADP
YIL155C	1.1.99.5	*GUT2*	GLYCEROL-3-PHOSPHATE DEHYDROGENASE	GL3P + FADm ->T3P2 + FADH2m
*U69_*		*U69_*		DAGLY + 0.017 C100ACP + 0.062 C120ACP + 0.100 C140ACP + 0.270 C160ACP + 0.169 C161ACP + 0.055 C180ACP + 0.235 C181ACP + 0.093 C182ACP -> TAGLY + ACP
# METABOLISM OF COFACTORS, VITAMINS, AND OTHER SUBSTANCES				
# THIAMINE (VITAMIN B1) METABOLISM				
YOR143C	2.7.6.2	*THI80*	THIAMIN PYROPHOSPHOKINASE	ATP + THIAMIN -> AMP + TPP
YOR143C	2.7.6.2	*THI80*	THIAMIN PYROPHOSPHOKINASE	ATP + TPP -> AMP +TPPP
*U70_*		*U70_*	THIC PROTEIN	AIR -> AHM
YOL055C	2.7.1.49	*THI20*	BIPARTITE PROTEIN CONSISTING OF N-TERMINAL HYDROXYMETHYLPYRIMIDINE PHOSPHATE (HMP-P) KINASE DOMAIN, NEEDED FOR THIAMINE BIOSYNTHESIS, FUSED TO C-TERMINAL PET18P-LIKE DOMAIN OF INDETERMINANT FUNCTION	AHM + ATP -> AHMP + ADP
YPL258C	2.7.1.49	*THI21*	BIPARTITE PROTEIN CONSISTING OF N-TERMINAL HYDROXYMETHYLPYRIMIDINE PHOSPHATE (HMP-P) KINASE DOMAIN, NEEDED FOR THIAMINE BIOSYNTHESIS, FUSED TO C-TERMINAL PET18P-LIKE DOMAIN OF INDETERMINANT FUNCTION	AHM + ATP -> AHMP + ADP

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YPR121W	2.7.1.49	*THI22*	BIPARTITE PROTEIN CONSISTING OF N-TERMINAL HYDROXYMETHYLPYRIMIDINE PHOSPHATE (HMP-P) KINASE DOMAIN, NEEDED FOR THIAMINE BIOSYNTHESIS, FUSED TO C-TERMINAL PET18P-LIKE DOMAIN OF INDETERMINANT FUNCTION	AHM + ATP -> AHMP + ADP
YOL055C	2.7.4.7	*THI20*	HMP-PHOSPHATE KINASE	AHMP + ATP -> AHMPP + ADP
*U71_*		*U71_*	HYPOTHETICAL	T3P1 + PYR -> DTP
*U72_*		*U72_*	THIG PROTEIN	DTP + TYR + CYS -> THZ + HBA + CO2
*U73_*		*U73_*	THIE PROTEIN	DTP + TYR + CYS -> THZ + HBA + CO2
*U74_*		*U74_*	THIF PROTEIN	DTP + TYR + CYS -> THZ + HBA + CO2
*U75_*		*U75_*	THIH PROTEIN	DTP + TYR + CYS -> THZ + HBA + CO2
YPL214C	2.7.1.50	*THI6*	HYDROXYETHYLTHIAZOLE KINASE	THZ + ATP -> THZP + ADP
YPL214C	2.5.1.3	*THI6*	TMP PYROPHOSPHORYLASE, HYDROXYETHYLTHIAZOLE KINASE	THZP + AHMPP -> THMP + PPI
*U76_*	2.7.4.16	*U76_*	THIAMIN PHOSPHATE KINASE	THMP + ATP <-> TPP + ADP
*U77_*	3.1.3.-	*U77_*	(DL)-GLYCEROL-3-PHOSPHATASE 2	THMP -> THIAMIN + PI
# RIBOFLAVIN METABOLISM				
YBL033C	3.5.4.25	*RIB1*	GTP CYCLOHYDROLASE II	GTP -> D6RP5P + FOR + PPI
YBR153W	3.5.4.26	*RIB7*	HTP REDUCTASE, SECOND STEP IN THE RIBOFLAVIN BIOSYNTHESIS PATHWAY	D6RP5P -> A6RP5P + NH3
YBR153W	1.1.1.193	*RIB7*	PYRIMIDINE REDUCTASE	A6RP5P + NADPH -> A6RP5P2 + NADP
*U78_*		*U78_*	PYRIMIDINE PHOSPHATASE	A6RP5P2 -> A6RP + PI
*U79_*		*U79_*	3,4 DIHYDROXY-2-BUTANONE-4-PHOSPHATE SYNTHASE	RL5P -> DB4P + FOR
YBR256C	2.5.1.9	*RIB5*	RIBOFLAVIN BIOSYNTHESIS PATHWAY ENZYME, 6,7-DIMETHYL-8-RIBITYLLUMAZINE SYNTHASE, APHA CHAIN	DB4P + A6RP -> D8RL + PI
YOL143C	2.5.1.9	*RIB4*	RIBOFLAVIN BIOSYNTHESIS PATHWAY ENZYME, 6,7-DIMETHYL-8-RIBITYLLUMAZINE SYNTHASE, BETA CHAIN	
YAR071W	3.1.3.2	*PHO11*	ACID PHOSPHATASE	FMN -> RIBFLAV + PI
YDR236C	2.7.1.26	*FMN1*	RIBOFLAVIN KINASE	RIBFLAV + ATP -> FMN + ADP
YDR236C	2.7.1.26	*FMN1*	RIBOFLAVIN KINASE	RIBFLAVm + ATPm -> FMNm + ADPm

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YDL045C	2.7.7.2	*FAD1*	FAD SYNTHETASE	FMN + ATP -> FAD + PPI
*U80_*	2.7.7.2	*U80_*	FAD SYNTHETASE	FMNm + ATPm -> FADm + PPIm
# VITAMIN B6 (PYRIDOXINE) BIOSYNTHESIS METABOLISM				
*U81_*	2.7.1.35	*U81_*	PYRIDOXINE KINASE	PYRDX + ATP -> P5P + ADP
*U82_*	2.7.1.35	*U82_*	PYRIDOXINE KINASE	PDLA + ATP -> PDLA5P + ADP
*U83_*	2.7.1.35	*U83_*	PYRIDOXINE KINASE	PL + ATP -> PL5P + ADP
YBR035C	1.4.3.5	*PDX3*	PYRIDOXINE 5'-PHOSPHATE OXIDASE	PDLA5P + O2 -> PL5P + H2O2 + NH3
YBR035C	1.4.3.5	*PDX3*	PYRIDOXINE 5'-PHOSPHATE OXIDASE	P5P + O2 <-> PL5P + H2O2
YBR035C	1.4.3.5	*PDX3*	PYRIDOXINE 5'-PHOSPHATE OXIDASE	PYRDX + O2 <-> PL + H2O2
YBR035C	1.4.3.5	*PDX3*	PYRIDOXINE 5'-PHOSPHATE OXIDASE	PL + O2 + NH3 <-> PDLA + H2O2
YBR035C	1.4.3.5	*PDX3*	PYRIDOXINE 5'-PHOSPHATE OXIDASE	PDLA5P + O2 -> PL5P + H2O2 + NH3
YOR184W	2.6.1.52	*SER1*	HYPOTHETICAL TRANSAMINASE/PHOSPHOSERINE TRANSAMINASE	OHB + GLU <-> PHT + AKG
YCR053W	4.2.99.2	*THR4*	THREONINE SYNTHASE	PHT -> 4HLT + PI
*U84_*	3.1.3.-	*U84_*	HYPOTHETICAL ENZYME	PDLA5P -> PDLA + PI
# PANTOTHENATE AND COA BIOSYNTHESIS				
*U85_*		*U85_*		3 MALCOA -> CHCOA + 2 COA + 2 CO2
*U86_*	2.3.1.47	*U86_*	8-AMINO-7-OXONONANOATE SYNTHASE	ALA + CHCOA <-> CO2 + COA + AONA
YNR058W	2.6.1.62	*BIO3*	7,8-DIAMINO-PELARGONIC ACID AMINOTRANSFERASE (DAPA) AMINOTRANSFERASE	SAM + AONA <-> SAMOB + DANNA
YNR057C	6.3.3.3	*BIO4*	DETHIOBIOTIN SYNTHETASE	CO2 + DANNA + ATP <-> DTB + PI + ADP
YGR286C	2.8.1.6	*BIO2*	BIOTIN SYNTHASE	DTB + CYS <-> BT
# FOLATE BIOSYNTHESIS				
YGR267C	3.5.4.16	*FOL2*	GTP CYCLOHYDROLASE I	GTP -> FOR + AHTD
*U87_*	3.6.1.-	*U87_*	DIHYDRONEOPTERIN TRIPHOSPHATE PYROPHOSPHORYLASE	AHTD -> PPI + DHPP
YDR481C	3.1.3.1	*PHO8*	GLYCEROPHOSPHATASE, ALKALINE PHOSPHATASE; NUCLEOSIDE TRIPHOSPHATASE	AHTD -> DHP + 3 PI
YDL100C	3.6.1.-	*YDL100C*	DIHYDRONEOPTERIN MONOPHOSPHATE DEPHOSPHORYLASE	DHPP -> DHP + PI
YNL256W	4.1.2.25	*FOL1*	DIHYDRONEOPTERIN ALDOLASE	DHP -> AHHMP + GLAL
YNL256W	2.7.6.3	*FOL1*	6-HYDROXYMETHYL-7,8 DIHYDROPTERIN PYROPHOSPHOKINASE	AHHMP + ATP -> AMP + AHHMD
YNR033W	4.1.3.-	*ABZ1*	AMINODEOXYCHORISMATE SYNTHASE	CHOR + GLN -> ADCHOR + GLU
*U88_*	4.-.-.-	*U88_*	AMINODEOXYCHORISMATE LYASE	ADCHOR -> PYR + PABA

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YNL256W	2.5.1.15	*FOL1*	DIHYDROPTEROATE SYNTHASE	PABA + AHHMD -> PPI + DHPT
YNL256W	2.5.1.15	*FOL1*	DIHYDROPTEROATE SYNTHASE	PABA + AHHMP -> DHPT
*U89_*	6.3.2.12	*U89_*	DIHYDROFOLATE SYNTHASE	DHPT + ATP + GLU -> ADP + PI + DHF
YOR236W	1.5.1.3	*DFR1*	DIHYDROFOLATE REDUCTASE	DHFm + NADPHm -> NADPm + THFm
YOR236W	1.5.1.3	*DFR1*	DIHYDROFOLATE REDUCTASE	DHF + NADPH -> NADP + THF
*U90_*	6.3.3.2	*U90_*	5-FORMYLTETRAHYDROFOLATE CYCLO-LIGASE	ATPm + FTHFm -> ADPm + PIm + MTHFm
*U91_*	6.3.3.2	*U91_*	5-FORMYLTETRAHYDROFOLATE CYCLO-LIGASE	ATP + FTHF -> ADP + PI + MTHF
YKL132C	6.3.2.17	*RMA1*	PROTEIN WITH SIMILARITY TO FOLYLPOLYGLUTAMATE SYNTHASE; CONVERTS TETRAHYDROFOLYL-[GLU(N)] + GLUTAMATE TO TETRAHYDROFOLYL-[GLU(N+1)]	THF + ATP + GLU <-> ADP + PI + THFG
YMR113W	6.3.2.17	*FOL3*	DIHYDROFOLATE SYNTHETASE	THF + ATP + GLU <-> ADP + PI + THFG
YOR241W	6.3.2.17	*MET7*	FOLYLPOLYGLUTAMATE SYNTHETASE, INVOLVED IN METHIONINE BIOSYNTHESIS AND MAINTENANCE OF MITOCHONDRIAL GENOME	THF + ATP + GLU <-> ADP + PI + THFG
# ONE CARBON POOL BY FOLATE				
YPL023C	1.5.1.20	*MET12*	METHYLENE TETRAHYDROFOLATE REDUCTASE	METTHFm + NADPHm -> NADPm + MTHFm
YGL125W	1.5.1.20	*MET13*	METHYLENE TETRAHYDROFOLATE REDUCTASE	METTHFm + NADPHm -> NADPm + MTHFm
YBR084W	1.5.1.5	*MIS1*	THE MITOCHONDRIAL TRIFUNCTIONAL ENZYME C1-TETRAHYDROFLATE SYNTHASE	METTHFm + NADPm <-> METHFm + NADPHm
YGR204W	1.5.1.5	*ADE3*	THE CYTOPLASMIC TRIFUNCTIONAL ENZYME C1-TETRAHYDROFOLATE SYNTHASE	METTHF + NADP <-> METHF + NADPH
YBR084W	6.3.4.3	*MIS1*	THE MITOCHONDRIAL TRIFUNCTIONAL ENZYME C1-TETRAHYDROFLATE SYNTHASE	THFm + FORm + ATPm -> ADPm + PIm + FTHFm
YGR204W	6.3.4.3	*ADE3*	THE CYTOPLASMIC TRIFUNCTIONAL ENZYME C1-TETRAHYDROFOLATE SYNTHASE	THF + FOR + ATP -> ADP + PI + FTHF
YBR084W	3.5.4.9	*MIS1*	THE MITOCHONDRIAL TRIFUNCTIONAL ENZYME C1-TETRAHYDROFLATE SYNTHASE	METHFm <-> FTHFm

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YGR204W	3.5.4.9	*ADE3*	THE CYTOPLASMIC TRIFUNCTIONAL ENZYME C1-TETRAHYDROFOLATE SYNTHASE	METHF <-> FTHF
YKR080W	1.5.1.15	*MTD1*	NAD-DEPENDENT 5,10-METHYLENETETRAHYDRAFOLATE DEHYDROGENASE	METTHF + NAD -> METHF + NADH
YBL013W	2.1.2.9	*FMT1*	METHIONYL-TRNA TRANSFORMYLASE	FTHFm + MTRNAm -> THFm + FMRNAm
# COENZYME A BIOSYNTHESIS				
YBR176W	2.1.2.11	*ECM31*	KETOPENTOATE HYDROXYMETHYL TRANSFERASE	OIVAL + METTHF -> AKP + THF
YHR063C	1.1.1.169	*PAN5*	PUTATIVE KETOPANTOATE REDUCTASE (2-DEHYDROPANTOATE 2-REDUCTASE) INVOLVED IN COENZYME A SYNTHESIS, HAS SIMILARITY TO CBS2P, KETOPANTOATE REDUCTASE	AKP + NADPH -> NADP + PANT
YLR355C	1.1.1.86	*ILV5*	KETOL-ACID REDUCTOISOMERASE	AKPm + NADPHm -> NADPm + PANTm
YIL145C	6.3.2.1	*YIL145C*	PANTOATE-B-ALANINE LIGASE	PANT + bALA + ATP -> AMP + PPI + PNTO
YDR531W	2.7.1.33	*YDR531W*	PUTATIVE PANTOTHENATE KINASE INVOLVED IN COENZYME A BIOSYNTHESIS, PANTOTHENATE KINASE	PNTO + ATP -> ADP + 4PPNTO
*U92_*	6.3.2.5	*U92_*	PHOSPHOPANTOTHENATE-CYSTEINE LIGASE	4PPNTO + CTP + CYS -> CMP + PPI + 4PPNCYS
*U93_*	4.1.1.36	*U93_*	PHOSPHOPANTOTHENATE-CYSTEINE DECARBOXYLASE	4PPNCYS -> CO2 + 4PPNTE
*U94_*	2.7.7.3	*U94_*	PHOSPHO-PANTETHIENE ADENYLYLTRANSFERASE	4PPNTE + ATP -> PPI + DPCOA
*U95_*	2.7.7.3	*U95_*	PHOSPHO-PANTETHIENE ADENYLYLTRANSFERASE	4PPNTEm + ATPm -> PPIm + DPCOAm
*U96_*	2.7.1.24	*U96_*	DEPHOSPHOCOA KINASE	DPCOA + ATP -> ADP + COA
*U97_*	2.7.1.24	*U97_*	DEPHOSPHOCOA KINASE	DPCOAm + ATPm -> ADPm + COAm
*U98_*	4.1.1.11	*U98_*	ASPARTATE ALPHA-DECARBOXYLASE	ASP -> CO2 + bALA
YPL148C	2.7.8.7	*PPT2*	ACYL CARRIER-PROTEIN SYNTHASE, PHOSPHOPANTETHEINE PROTEIN TRANSFERASE FOR ACP1P	COA -> PAP + ACP
# NAD BIOSYNTHESIS				
YGL037C	3.5.1.19	*PNC1*	NICOTINAMIDASE	NAM <-> NAC + NH3
YOR209C	2.4.2.11	*NPT1*	NAPRTASE	NAC + PRPP -> NAMN + PPI
*U99_*	1.4.3.-	*U99_*	ASPARTATE OXIDASE	ASP + FADm -> FADH2m + ISUCC
*U100_*	1.4.3.16	*U100_*	QUINOLATE SYNTHASE	ISUCC + T3P2 -> PI + QA

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YFR047C	2.4.2.19	*QPT1*	QUINOLATE PHOSPHORIBOSYL TRANSFERASE	QA + PRPP -> NAMN + CO2 + PPI
YLR328W	2.7.7.18	*YLR328W*	NICOTINAMIDE MONONUCLEOTIDE (NMN) ADENYLYLTRANSFERASE	NAMN + ATP -> PPI + NAAD
YHR074W	6.3.5.1	*QNS1*	DEAMIDO-NAD AMMONIA LIGASE	NAAD + ATP + NH3 -> NAD + AMP + PPI
YJR049C	2.7.1.23	*UTR1*	NAD KINASE, POLYPHOSPHATE KINASE (EC 2.7.4.1) / NAD+ KINASE (EC 2.7.1.23)	NAD + ATP -> NADP + ADP
YEL041W	2.7.1.23	*YEL041W*	NAD KINASE, POLYPHOSPHATE KINASE (EC 2.7.4.1) / NAD+ KINASE (EC 2.7.1.23)	NAD + ATP -> NADP + ADP
YPL188W	2.7.1.23	*POS5*	NAD KINASE, POLYPHOSPHATE KINASE (EC 2.7.4.1) / NAD+ KINASE (EC 2.7.1.23)	NAD + ATP -> NADP + ADP
*U101_*	3.1.2.-	*U101_*	NADP PHOSPHATASE	NADP -> NAD + PI
*U102_*	3.2.2.5	*U102_*		NAD -> NAM + ADPRIB
*U103_*	2.4.2.1	*U103_*	STRONG SIMILARITY TO PURINE-NUCLEOSIDE PHOSPHORYLASES	ADN + PI <-> AD + RIP
*U104_*	2.4.2.1	*U104_*	STRONG SIMILARITY TO PURINE-NUCLEOSIDE PHOSPHORYLASES	GSN + PI <-> GN + RIP
# NICOTINIC ACID SYNTHESIS FROM TRP				
YFR047C	2.4.2.19	*QPT1*	QUINOLATE PHOSPHORIBOSYL TRANSFERASE	QAm + PRPPm -> NAMNm + CO2m + PPIm
YLR328W	2.7.7.18	*YLR328W*	NAMN ADENYLYL TRANSFERASE	NAMNm + ATPm -> PPIm + NAADm
YLR328W	2.7.7.18	*YLR328W*	NAMN ADENYLYL TRANSFERASE	NMNm + ATPm -> NADm + PPIm
YHR074W	6.3.5.1	*QNS1*	DEAMIDO-NAD AMMONIA LIGASE	NAADm + ATPm + NH3m -> NADm + AMPm + PPIm
YJR049C	2.7.1.23	*UTR1*	NAD KINASE, POLYPHOSPHATE KINASE (EC 2.7.4.1) / NAD+ KINASE (EC 2.7.1.23)	NADm + ATPm -> NADPm + ADPm
YPL188W	2.7.1.23	*POS5*	NAD KINASE, POLYPHOSPHATE KINASE (EC 2.7.4.1) / NAD+ KINASE (EC 2.7.1.23)	NADm + ATPm -> NADPm + ADPm
YEL041W	2.7.1.23	*YEL041W*	NAD KINASE, POLYPHOSPHATE KINASE (EC 2.7.4.1) / NAD+ KINASE (EC 2.7.1.23)	NADm + ATPm -> NADPm + ADPm
*U105_*	3.1.2.-	*U105_*	NADP PHOSPHATASE	NADPm -> NADm + PIm
YLR209C	2.4.2.1	*PNP1*	STRONG SIMILARITY TO PURINE-NUCLEOSIDE PHOSPHORYLASES	ADNm + PIm <-> ADm + RIPm
YLR209C	2.4.2.1	*PNP1*	STRONG SIMILARITY TO PURINE-NUCLEOSIDE PHOSPHORYLASES	GSNm + PIm <-> GNm + RIPm
YGL037C	3.5.1.19	*PNC1*	NICOTINAMIDASE	NAMm <-> NACm + NH3m
YOR209C	2.4.2.11	*NPT1*	NAPRTASE	NACm + PRPPm -> NAMNm + PPIm
*U106_*	3.2.2.5	*U106_*		NADm -> NAMm + ADPRIBm
# UPTAKE PATHWAYS				
# PORPHYRIN METABOLISM				
YDR232W	2.3.1.37	*HEM1*	5-AMINOLEVULINATE SYNTHASE	SUCCOAm + GLYm -> ALAVm + COAm + CO2m

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YGL040C	4.2.1.24	*HEM2*	AMINOLEVULINATE DEHYDRATASE	2 ALAV -> PBG
YDL205C	4.3.1.8	*HEM3*	HYDROXYMETHYLBILANE SYNTHASE	4 PBG -> HMB + 4 NH3
YOR278W	4.2.1.75	*HEM4*	UROPORPHYRINOGEN-III SYNTHASE	HMB -> UPRG
YDR047W	4.1.1.37	*HEM12*	UROPORPHYRINOGEN DECARBOXYLASE	UPRG -> 4 CO2 + CPP
YDR044W	1.3.3.3	*HEM13*	COPROPORPHYRINOGEN OXIDASE, AEROBIC	O2 + CPP -> 2 CO2 + PPHG
YER014W	1.3.3.4	*HEM14*	PROTOPORPHYRINOGEN OXIDASE	O2 + PPHGm -> PPIXm
YOR176W	4.99.1.1	*HEM15*	FERROCHELATASE	PPIXm -> PTHm
YGL245W	6.1.1.17	*YGL245W*	GLUTAMYL-TRNA SYNTHETASE, CYTOPLASMIC	GLU + ATP -> GTRNA + AMP + PPI
YOL033W	6.1.1.17	*MSE1*		GLUm + ATPm -> GTRNAm + AMPm + PPIm
YKR069W	2.1.1.107	*MET1*	UROPORPHYRIN-III C-METHYLTRANSFERASE	SAM + UPRG -> SAH + PC2
# QUINONE BIOSYNTHESIS				
YKL211C	4.1.3.27	*TRP3*	ANTHRANILATE SYNTHASE COMPONENT II AND INDOLE-3-PHOSPHATE (MULTIFUNCTIONAL ENZYME)	CHOR -> 4HBZ + PYR
YER090W	4.1.3.27	*TRP2*	ANTHRANILATE SYNTHASE COMPONENT I	CHOR -> 4HBZ + PYR
YPR176C	2.5.1.-	*BET2*	GERANYLGERANYLTRANSFERASE TYPE II BETA SUBUNIT	4HBZ + NPP -> N4HBZ + PPI
YJL031C	2.5.1.-	*BET4*	GERANYLGERANYLTRANSFERASE TYPE II ALPHA SUBUNIT	
YGL155W	2.5.1.-	*CDC43*	GERANYLGERANYLTRANSFERASE TYPE I BETA SUBUNIT	
YBR003W	2.5.1.-	*COQ1*	HEXAPRENYL PYROPHOSPHATE SYNTHETASE, CATALYZES THE FIRST STEP IN COENZYME Q (UBIQUINONE) BIOSYNTHESIS PATHWAY	4HBZ + NPP -> N4HBZ + PPI
YNR041C	2.5.1.-	*COQ2*	PARA-HYDROXYBENZOATE--POLYPRENYLTRANSFERASE	4HBZ + NPP -> N4HBZ + PPI
YPL172C	2.5.1.-	*COX10*	PROTOHEME IX FARNESYLTRANSFERASE, MITOCHONDRIAL PRECURSOR	4HBZ + NPP -> N4HBZ + PPI
YDL090C	2.5.1.-	*RAM1*	PROTEIN FARNESYLTRANSFERASE BETA SUBUNIT	4HBZ + NPP -> N4HBZ + PPI
YKL019W	2.5.1.-	*RAM2*	PROTEIN FARNESYLTRANSFERASE ALPHA SUBUNIT	
YBR002C	2.5.1.-	*RER2*	PUTATIVE DEHYDRODOLICHYL DIPHOSPATE SYNTHETASE	4HBZ + NPP -> N4HBZ + PPI

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YMR101C	2.5.1.-	*SRT1*	PUTATIVE DEHYDRODOLICHYL DIPHOSPATE SYNTHETASE	4HBZ + NPP -> N4HBZ + PPI
YDR538W	4.1.1.-	*PAD1*	OCTAPRENYL-HYDROXYBENZOATE DECARBOXYLASE	N4HBZ -> CO2 + 2NPPP
*U107_*	1.13.14.-	*U107_*	2-OCTAPRENYLPHENOL HYDROXYLASE	2NPPP + O2 -> 2N6H
YPL266W	2.1.1.-	*DIM1*		2N6H + SAM -> 2NPMP + SAH
*U108_*	1.14.13.-	*U108_*	2-OCTAPRENYL-6-METHOXYPHENOL HYDROXYLASE	2NPMPm + O2m -> 2NPMBm
YML110C	2.1.1.-	*COQ5*	2-OCTAPRENYL-6-METHOXY-1,4-BENZOQUINONE METHYLASE	2NPMBm + SAMm -> 2NPMMBm + SAHm
YGR255C	1.14.13.-	*COQ6*	COQ6 MONOOXYGENASE	2NPMMBm + O2m -> 2NMHMBm
YOL096C	2.1.1.64	*COQ3*	3-DIMETHYLUBIQUINONE 3-METHYLTRANSFERASE	2NMHMBm + SAMm -> QH2m + SAHm
# MEMBRANE TRANSPORT				
# MITOCHONDRIAL MEMBRANE TRANSPORT				
# THE FOLLOWINGS DIFFUSE THROUGH THE INNER MITOCHONDRIAL MEMBRANE IN A NON-CARRIER-MEDIATED MANNER:				
*U109_*		*U109_*		O2 <-> O2m
*U110_*		*U110_*		CO2 <-> CO2m
*U111_*		*U111_*		ETH <-> ETHm
*U112_*		*U112_*		NH3 <-> NH3m
*U113_*		*U113_*		MTHN <-> MTHNm
*U114_*		*U114_*		THFm <-> THF
*U115_*		*U115_*		METTHFm <-> METTHF
*U116_*		*U116_*		SERm <-> SER
*U117_*		*U117_*		GLYm <-> GLY
*U118_*		*U118_*		CBHCAPm <-> CBHCAP
*U119_*		*U119_*		OICAPm <-> OICAP
*U120_*		*U120_*		PROm <-> PRO
*U121_*		*U121_*		CMPm <-> CMP
*U122_*		*U122_*		ACm <-> AC
*U123_*		*U123_*		ACAR -> ACARm
*U124_*		*U124_*		CARm -> CAR
*U125_*		*U125_*		ACLAC <-> ACLACm
*U126_*		*U126_*		ACTAC <-> ACTACm
*U127_*		*U127_*		SLF -> SLFm + Hm
*U128_*		*U128_*		THRm <-> THR
*U129_*		*U129_*		AKAm -> AKA
YMR056C		*AAC1*	ADP/ATP CARRIER PROTEIN (MCF)	ADP + ATPm + PI -> Hm + ADPm + ATP + PIm

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YBL030C		*PET9*	ADP/ATP CARRIER PROTEIN (MCF)	ADP + ATPm + PI -> Hm + ADPm + ATP + PIm
YBR085W		*AAC3*	ADP/ATP CARRIER PROTEIN (MCF)	ADP + ATPm + PI -> Hm + ADPm + ATP + PIm
YJR077C		*MIR1*	PHOSPHATE CARRIER	PI <-> Hm + PIm
YER053C		*YER053C*	SIMILARITY TO C.ELEGANS MITOCHONDRIAL PHOSPHATE CARRIER	PI + OHm <-> PIm
YLR348C		*DIC1*	DICARBOXYLATE CARRIER	MAL + SUCCm <-> MALm + SUCC
YLR348C		*DIC1*	DICARBOXYLATE CARRIER	MAL + PIm <-> MALm + PI
YLR348C		*DIC1*	DICARBOXYLATE CARRIER	SUCC + PIm -> SUCCm + PI
*U130_*		*U130_*		MALT + PIm <-> MALTm + PI
YKL120W		*OAC1*	MITOCHONDRIAL OXALOACETATE CARRIER	OA <-> OAm + Hm
YBR291C		*CTP1*	CITRATE TRANSPORT PROTEIN	CIT + MALm <-> CITm + MAL
YBR291C		*CTP1*	CITRATE TRANSPORT PROTEIN	CIT + PEPm <-> CITm + PEP
YBR291C		*CTP1*	CITRATE TRANSPORT PROTEIN	CIT + ICITm <-> CITm + ICIT
*U131_*		*U131_*		IPPMAL <-> IPPMALm
*U132_*		*U132_*		LAC <-> LACm + Hm
*U133_*		*U133_*	PYRUVATE CARRIER	PYR <-> PYRm + Hm
*U134_*		*U134_*	GLUTAMATE CARRIER	GLU <-> GLUm + Hm
*U135_*		*U135_*		GLU + OHm -> GLUm
YOR130C		*ORT1*	ORNITHINE CARRIER	ORN + Hm <-> ORNm
YOR100C		*CRC1*	CARNITINE CARRIER	CARm + ACAR -> CAR + ACARm
*U136_*		*U136_*		OIVAL <-> OIVALm
*U137_*		*U137_*		OMVAL <-> OMVALm
YIL134W		*FLX1*	PROTEIN INVOLVED IN TRANSPORT OF FAD FROM CYTOSOL INTO THE MITOCHONDRIAL MATRIX	FAD + FMNm -> FADm + FMN
*U138_*		*U138_*		RIBFLAV <-> RIBFLAVm
*U139_*		*U139_*		DTB <-> DTBm
*U140_*		*U140_*		H3MCOA <-> H3MCOAm
*U141_*		*U141_*		MVL <-> MVLm
*U142_*		*U142_*		PA <-> PAm
*U143_*		*U143_*		4PPNTE <-> 4PPNTEm
*U144_*		*U144_*		AD <-> ADm
*U145_*		*U145_*		PRPP <-> PRPPm
*U146_*		*U146_*		DHF <-> DHFm
*U147_*		*U147_*		QA <-> QAm
*U148_*		*U148_*		OPP <-> OPPm
*U149_*		*U149_*		SAM <-> SAMm

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
*U150_*		*U150_*		SAH <-> SAHm
YJR095W		*SFC1*	MITOCHONDRIAL MEMBRANE SUCCINATE-FUMARATE TRANSPORTER, MEMBER OF THE MITOCHONDRIAL CARRIER FAMILY (MCF) OF MEMBRANE TRANSPORTERS	SUCC + FUMm -> SUCCm + FUM
YPL134C		*ODC1*	2-OXODICARBOYLATE TRANSPORTER	AKGm + OXA <-> AKG + OXAm
YOR222W		*ODC2*	2-OXODICARBOYLATE TRANSPORTER	AKGm + OXA <-> AKG + OXAm
# MALATE ASPARTATE SHUTTLE				
# INCLUDED ELSEWHERE				
# GLYCEROL PHOSPHATE SHUTTLE				
*U151_*		*U151_*		T3P2m -> T3P2
*U152_*		*U152_*		GL3P -> GL3Pm
# PLASMA MEMBRANE TRANSPORT				
# CARBOHYDRATES				
YHR092C		*HXT4*	MODERATE- TO LOW-AFFINITY GLUCOSE TRANSPORTER	GLCxt -> GLC
YLR081W		*GAL2*	GALACTOSE (AND GLUCOSE) PERMEASE	GLCxt -> GLC
YOL156W		*HXT11*	LOW AFFINITY GLUCOSE TRANSPORT PROTEIN	GLCxt -> GLC
YDR536W		*STL1*	PROTEIN MEMBER OF THE HEXOSE TRANSPORTER FAMILY	GLCxt -> GLC
YHR094C		*HXT1*	HIGH-AFFINITY HEXOSE (GLUCOSE) TRANSPORTER	GLCxt -> GLC
YOL156W		*HXT11*	GLUCOSE PERMEASE	GLCxt -> GLC
YEL069C		*HXT13*	HIGH-AFFINITY HEXOSE TRANSPORTER	GLCxt -> GLC
YDL245C		*HXT15*	HEXOSE TRANSPORTER	GLCxt -> GLC
YJR158W		*HXT16*	HEXOSE PERMEASE	GLCxt -> GLC
YFL011W		*HXT10*	HIGH-AFFINITY HEXOSE TRANSPORTER	GLCxt -> GLC
YNR072W		*HXT17*	PUTATIVE HEXOSE TRANSPORTER	GLCxt -> GLC
YMR011W		*HXT2*	HIGH AFFINITY HEXOSE TRANSPORTER-2	GLCxt -> GLC
YHR092C		*HXT4*	HIGH-AFFINITY GLUCOSE TRANSPORTER	GLCxt -> GLC
YDR345C		*HXT3*	LOW-AFFINITY GLUCOSE TRANSPORTER	GLCxt -> GLC
YHR096C		*HXT5*	HEXOSE TRANSPORTER	GLCxt -> GLC
YDR343C		*HXT6*	HEXOSE TRANSPORTER	GLCxt -> GLC
YDR342C		*HXT7*	HEXOSE TRANSPORTER	GLCxt -> GLC
YJL214W		*HXT8*	HEXOSE PERMEASE	GLCxt -> GLC
YJL219W		*HXT9*	HEXOSE PERMEASE	GLCxt -> GLC
YLR081W		*GAL2*	GALACTOSE PERMEASE	GLACxt -> GLAC
YFL011W		*HXT10*	HIGH-AFFINITY HEXOSE TRANSPORTER	GLACxt -> GLAC

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YOL156W		*HXT11*	GLUCOSE PERMEASE	GLACxt -> GLAC
YNL318C		*HXT14*	MEMBER OF THE HEXOSE TRANSPORTER FAMILY	GLACxt -> GLAC
YJL219W		*HXT9*	HEXOSE PERMEASE	GLACxt -> GLAC
YDR536W		*STL1*	PROTEIN MEMBER OF THE HEXOSE TRANSPORTER FAMILY	GLACxt -> GLAC
YFL055W		*AGP3*	AMINO ACID PERMEASE FOR SERINE, ASPARTATE, AND GLUTAMATE	GLUxt <-> GLU
YDR536W		*STL1*	PROTEIN MEMBER OF THE HEXOSE TRANSPORTER FAMILY	GLUxt <-> GLU
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	GLUxt <-> GLU
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	GLUxt <-> GLU
YPL265W		*DIP5*	DICARBOXYLIC AMINO ACID PERMEASE	GLUxt <-> GLU
YDR536W		*STL1*	PROTEIN MEMBER OF THE HEXOSE TRANSPORTER FAMILY	GLUxt <-> GLU
YHR094C		*HXT1*	HIGH-AFFINITY HEXOSE (GLUCOSE) TRANSPORTER	FRUxt -> FRU
YFL011W		*HXT10*	HIGH-AFFINITY HEXOSE TRANSPORTER	FRUxt -> FRU
YOL156W		*HXT11*	GLUCOSE PERMEASE	FRUxt -> FRU
YEL069C		*HXT13*	HIGH-AFFINITY HEXOSE TRANSPORTER	FRUxt -> FRU
YDL245C		*HXT15*	HEXOSE TRANSPORTER	FRUxt -> FRU
YJR158W		*HXT16*	HEXOSE PERMEASE	FRUxt -> FRU
YNR072W		*HXT17*	PUTATIVE HEXOSE TRANSPORTER	FRUxt -> FRU
YMR011W		*HXT2*	HIGH AFFINITY HEXOSE TRANSPORTER-2	FRUxt -> FRU
YDR345C		*HXT3*	LOW-AFFINITY GLUCOSE TRANSPORTER	FRUxt -> FRU
YHR092C		*HXT4*	HIGH-AFFINITY GLUCOSE TRANSPORTER	FRUxt -> FRU
YHR096C		*HXT5*	HEXOSE TRANSPORTER	FRUxt -> FRU
YDR343C		*HXT6*	HEXOSE TRANSPORTER	FRUxt -> FRU
YDR342C		*HXT7*	HEXOSE TRANSPORTER	FRUxt -> FRU
YJL214W		*HXT8*	HEXOSE PERMEASE	FRUxt -> FRU
YJL219W		*HXT9*	HEXOSE PERMEASE	FRUxt -> FRU
YHR094C		*HXT1*	HIGH-AFFINITY HEXOSE (GLUCOSE) TRANSPORTER	MANxt -> MAN
YFL011W		*HXT10*	HIGH-AFFINITY HEXOSE TRANSPORTER	MANxt -> MAN
YOL156W		*HXT11*	GLUCOSE PERMEASE	MANxt -> MAN
YEL069C		*HXT13*	HIGH-AFFINITY HEXOSE TRANSPORTER	MANxt -> MAN
YDL245C		*HXT15*	HEXOSE TRANSPORTER	MANxt -> MAN
YJR158W		*HXT16*	HEXOSE PERMEASE	MANxt -> MAN
YNR072W		*HXT17*	PUTATIVE HEXOSE TRANSPORTER	MANxt -> MAN

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YMR011W		*HXT2*	HIGH AFFINITY HEXOSE TRANSPORTER-2	MANxt -> MAN
YDR345C		*HXT3*	LOW-AFFINITY GLUCOSE TRANSPORTER	MANxt -> MAN
YHR092C		*HXT4*	HIGH-AFFINITY GLUCOSE TRANSPORTER	MANxt -> MAN
YHR096C		*HXT5*	HEXOSE TRANSPORTER	MANxt -> MAN
YDR343C		*HXT6*	HEXOSE TRANSPORTER	MANxt -> MAN
YDR342C		*HXT7*	HEXOSE TRANSPORTER	MANxt -> MAN
YJL214W		*HXT8*	HEXOSE PERMEASE	MANxt -> MAN
YJL219W		*HXT9*	HEXOSE PERMEASE	MANxt -> MAN
YDR497C		*ITR1*	MYO-INOSITOL TRANSPORTER	MIxt + HEXT -> MI
YOL103W		*ITR2*	MYO-INOSITOL TRANSPORTER	MIxt + HEXT -> MI
*U153_*		*U153_*	MALTASE PERMEASE	MLTxt + HEXT -> MLT
YIL162W	3.2.1.26	*SUC2*	INVERTASE (SUCROSE HYDROLYZING ENZYME)	SUCxt -> GLCxt + FRUxt
*U154_*		*U154_*	SUCROSE	SUCxt + HEXT -> SUC
YBR298C		*MAL31*	DICARBOXYLATES	MALxt + HEXT <-> MAL
*U155_*		*U155_*	A-KETOGLUTARATE/MALATE TRANSLOCATOR	MALxt + AKG <-> MAL + AKGxt
*U156_*		*U156_*	A-METHYLGLUCOSIDE	AMGxt <-> AMG
*U157_*		*U157_*	SORBOSE	SORxt <-> SOR
*U158_*		*U158_*	ARABINOSE (LOW AFFINITY)	ARABxt <-> ARAB
*U159_*		*U159_*	FUCOSE	FUCxt + HEXT <-> FUC
*U160_*		*U160_*		GLTLxt + HEXT -> GLTL
*U161_*		*U161_*	GLUCITOL	GLTxt + HEXT -> GLT
*U162_*		*U162_*	GLUCOSAMINE	GLAMxt + HEXT <-> GLAM
YLL043W		*FPS1*	GLYCEROL	GLxt <-> GL
YKL217W		*JEN1*	LACTATE TRANSPORT	LACxt + HEXT <-> LAC
*U163_*		*U163_*	MANNITOL	MNTxt + HEXT -> MNT
*U164_*		*U164_*	MELIBIOSE	MELIxt + HEXT -> MELI
*U165_*		*U165_*	N-ACETYLGLUCOSAMINE	NAGxt + HEXT -> NAG
*U166_*		*U166_*	RHAMNOSE	RMNxt + HEXT -> RMN
*U167_*		*U167_*	RIBOSE	RIBxt + HEXT -> RIB
*U168_*		*U168_*	TREHALOSE	TRExt + HEXT -> TRE
*U170_*		*U170_*		XYLxt <-> XYL
# AMINO ACIDS				
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	ALAxt + HEXT <-> ALA
YPL265W		*DIP5*	DICARBOXYLIC AMINO ACID PERMEASE	ALAxt + HEXT <-> ALA
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	ALAxt + HEXT <-> ALA
YOL020W		*TAT2*	TRYPTOPHAN PERMEASE	ALAxt + HEXT <-> ALA

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YOR348C		*PUT4*	PROLINE PERMEASE	ALAxt + HEXT <-> ALA
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	ARGxt + HEXT <-> ARG
YEL063C		*CAN1*	PERMEASE FOR BASIC AMINO ACIDS	ARGxt + HEXT <-> ARG
YNL270C		*ALP1*	PROTEIN WITH STRONG SIMILARITY TO PERMEASES	ARGxt + HEXT <-> ARG
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	ASNxt + HEXT <-> ASN
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	ASNxt + HEXT <-> ASN
YDR508C		*GNP1*	GLUTAMINE PERMEASE (HIGH AFFINITY)	ASNxt + HEXT <-> ASN
YPL265W		*DIP5*	DICARBOXYLIC AMINO ACID PERMEASE	ASNxt + HEXT <-> ASN
YFL055W		*AGP3*	AMINO ACID PERMEASE FOR SERINE, ASPARTATE, AND GLUTAMATE	ASPxt + HEXT <-> ASP
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	ASPxt + HEXT <-> ASP
YPL265W		*DIP5*	DICARBOXYLIC AMINO ACID PERMEASE	ASPxt + HEXT <-> ASP
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	CYSxt + HEXT <-> CYS
YDR508C		*GNP1*	GLUTAMINE PERMEASE (HIGH AFFINITY)	CYSxt + HEXT <-> CYS
YBR068C		*BAP2*	BRANCHED CHAIN AMINO ACID PERMEASE	CYSxt + HEXT <-> CYS
YDR046C		*BAP3*	BRANCHED CHAIN AMINO ACID PERMEASE	CYSxt + HEXT <-> CYS
YBR069C		*VAP1*	AMINO ACID PERMEASE	CYSxt + HEXT <-> CYS
YOL020W		*TAT2*	TRYPTOPHAN PERMEASE	CYSxt + HEXT <-> CYS
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	GLYxt + HEXT <-> GLY
YOL020W		*TAT2*	TRYPTOPHAN PERMEASE	GLYxt + HEXT <-> GLY
YPL265W		*DIP5*	DICARBOXYLIC AMINO ACID PERMEASE	GLYxt + HEXT <-> GLY
YOR348C		*PUT4*	PROLINE PERMEASE	GLYxt + HEXT <-> GLY
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	GLNxt + HEXT <-> GLN
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	GLNxt + HEXT <-> GLN
YDR508C		*GNP1*	GLUTAMINE PERMEASE (HIGH AFFINITY)	GLNxt + HEXT <-> GLN
YPL265W		*DIP5*	DICARBOXYLIC AMINO ACID PERMEASE	GLNxt + HEXT <-> GLN
YGR191W		*HIP1*	HISTIDINE PERMEASE	HISxt + HEXT <-> HIS
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	HISxt + HEXT <-> HIS
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	HISxt + HEXT <-> HIS
YBR069C		*VAP1*	AMINO ACID PERMEASE	HISxt + HEXT <-> HIS
YBR069C		*TAT1*	AMINO ACID PERMEASE THAT TRANSPORTS VALINE, LEUCINE, ISLEUCINE, TYROSINE, TRYPTOPHAN, AND THREONINE	ILExt + HEXT <-> ILE

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	ILExt + HEXT <-> ILE
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	ILExt + HEXT <-> ILE
YBR068C		*BAP2*	BRANCHED CHAIN AMINO ACID PERMEASE	ILExt + HEXT <-> ILE
YDR046C		*BAP3*	BRANCHED CHAIN AMINO ACID PERMEASE	ILExt + HEXT <-> ILE
YBR069C		*VAP1*	AMINO ACID PERMEASE	ILExt + HEXT <-> ILE
YBR069C		*TAT1*	AMINO ACID PERMEASE THAT TRANSPORTS VALINE, LEUCINE, ISLEUCINE, TYROSINE, TRYPTOPHAN, AND THREONINE	LEUxt + HEXT <-> LEU
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	LEUxt + HEXT <-> LEU
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	LEUxt + HEXT <-> LEU
YBR068C		*BAP2*	BRANCHED CHAIN AMINO ACID PERMEASE	LEUxt + HEXT <-> LEU
YDR046C		*BAP3*	BRANCHED CHAIN AMINO ACID PERMEASE	LEUxt + HEXT <-> LEU
YBR069C		*VAP1*	AMINO ACID PERMEASE	LEUxt + HEXT <-> LEU
YDR508C		*GNP1*	GLUTAMINE PERMEASE (HIGH AFFINITY)	LEUxt + HEXT <-> LEU
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	METxt + HEXT <-> MET
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	METxt + HEXT <-> MET
YDR508C		*GNP1*	GLUTAMINE PERMEASE (HIGH AFFINITY)	METxt + HEXT <-> MET
YBR068C		*BAP2*	BRANCHED CHAIN AMINO ACID PERMEASE	METxt + HEXT <-> MET
YDR046C		*BAP3*	BRANCHED CHAIN AMINO ACID PERMEASE	METxt + HEXT <-> MET
YGR055W		*MUP1*	HIGH-AFFINITY METHIONINE PERMEASE	METxt + HEXT <-> MET
YHL036W		*MUP3*	LOW-AFFINITY METHIONINE PERMEASE	METxt + HEXT <-> MET
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	PHExt + HEXT <-> PHE
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	PHExt + HEXT <-> PHE
YOL020W		*TAT2*	TRYPTOPHAN PERMEASE	PHExt + HEXT <-> PHE
YBR068C		*BAP2*	BRANCHED CHAIN AMINO ACID PERMEASE	PHExt + HEXT <-> PHE
YDR046C		*BAP3*	BRANCHED CHAIN AMINO ACID PERMEASE	PHExt + HEXT <-> PHE
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	PROxt + HEXT <-> PRO
YOR348C		*PUT4*	PROLINE PERMEASE	PROxt + HEXT <-> PRO

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YBR069C		*TAT1*	AMINO ACID PERMEASE THAT TRANSPORTS VALINE, LEUCINE, ISLEUCINE, TYROSINE, TRYPTOPHAN, AND THREONINE	TRPxt + HEXT <-> TRP
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	TRPxt + HEXT <-> TRP
YBR069C		*VAP1*	AMINO ACID PERMEASE	TRPxt + HEXT <-> TRP
YOL020W		*TAT2*	TRYPTOPHAN PERMEASE	TRPxt + HEXT <-> TRP
YBR068C		*BAP2*	BRANCHED CHAIN AMINO ACID PERMEASE	TRPxt + HEXT <-> TRP
YDR046C		*BAP3*	BRANCHED CHAIN AMINO ACID PERMEASE	TRPxt + HEXT <-> TRP
YBR069C		*TAT1*	AMINO ACID PERMEASE THAT TRANSPORTS VALINE, LEUCINE, ISLEUCINE, TYROSINE, TRYPTOPHAN, AND THREONINE	TYRxt + HEXT <->TYR
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	TYRxt + HEXT <->TYR
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	TYRxt + HEXT <->TYR
YBR068C		*BAP2*	BRANCHED CHAIN AMINO ACID PERMEASE	TYRxt + HEXT <->TYR
YBR069C		*VAP1*	AMINO ACID PERMEASE	TYRxt + HEXT <->TYR
YOL020W		*TAT2*	TRYPTOPHAN PERMEASE	TYRxt + HEXT <->TYR
YDR046C		*BAP3*	BRANCHED CHAIN AMINO ACID PERMEASE	TYRxt + HEXT <->TYR
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	VALxt + HEXT <-> VAL
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	VALxt + HEXT <-> VAL
YDR046C		*BAP3*	BRANCHED CHAIN AMINO ACID PERMEASE	VALxt + HEXT <-> VAL
YBR069C		*VAP1*	AMINO ACID PERMEASE	VALxt + HEXT <-> VAL
YBR068C		*BAP2*	BRANCHED CHAIN AMINO ACID PERMEASE	VALxt + HEXT <-> VAL
YFL055W		*AGP3*	AMINO ACID PERMEASE FOR SERINE, ASPARTATE, AND GLUTAMATE	SERxt + HEXT <-> SER
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	SERxt + HEXT <-> SER
YDR508C		*GNP1*	GLUTAMINE PERMEASE (HIGH AFFINITY)	SERxt + HEXT <-> SER
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	SERxt + HEXT <-> SER
YPL265W		*DIP5*	DICARBOXYLIC AMINO ACID PERMEASE	SERxt + HEXT <-> SER

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YBR069C		*TAT1*	AMINO ACID PERMEASE THAT TRANSPORTS VALINE, LEUCINE, ISLEUCINE, TYROSINE, TRYPTOPHAN, AND THREONINE	THRxt + HEXT <-> THR
YCL025C		*AGP1*	AMINO ACID PERMEASE FOR MOST NEUTRAL AMINO ACIDS	THRxt + HEXT <-> THR
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	THRxt + HEXT <-> THR
YDR508C		*GNP1*	GLUTAMINE PERMEASE (HIGH AFFINITY)	THRxt + HEXT <-> THR
YNL268W		*LYP1*	LYSINE SPECIFIC PERMEASE (HIGH AFFINITY)	LYSxt + HEXT <-> LYS
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	LYSxt + HEXT <-> LYS
YLL061W		*MMP1*	HIGH AFFINITY S-METHYLMETHIONINE PERMEASE	MMETxt + HEXT -> MMET
YPL274W		*SAM3*	HIGH AFFINITY S-ADENOSYLMETHIONINE PERMEASE	SAMxt + HEXT -> SAM
YOR348C		*PUT4*	PROLINE PERMEASE	GABAxt + HEXT -> GABA
YDL210W		*UGA4*	AMINO ACID PERMEASE WITH HIGH SPECIFICITY FOR GABA	GABAxt + HEXT -> GABA
YBR132C		*AGP2*	PLASMA MEMBRANE CARNITINE TRANSPORTER	CARxt <-> CAR
YGL077C		*HNM1*	CHOLINE PERMEASE	CHOxt + HEXT -> CHO
YNR056C		*BIO5*	TRANSMEMBRANE REGULATOR OF KAPA/DAPA TRANSPORT	BIOxt + HEXT -> BIO
YDL210W		*UGA4*	AMINO ACID PERMEASE WITH HIGH SPECIFICITY FOR GABA	ALAVxt + HEXT -> ALAV
YKR039W		*GAP1*	GENERAL AMINO ACID PERMEASE	ORNxt + HEXT <-> ORN
YEL063C		*CAN1*	PERMEASE FOR BASIC AMINO ACIDS	ORNxt + HEXT <-> ORN
*U171_*		*U171_*	PUTRESCINE	PTRSCxt + HEXT -> PTRSC
*U172_*		*U172_*	SPERMIDINE & PUTRESCINE	SPRMDxt + HEXT -> SPRMD
YKR093W		*PTR2*	DIPEPTIDE	DIPEPxt + HEXT -> DIPEP
YKR093W		*PTR2*	OLIGOPEPTIDE	OPEPxt + HEXT -> OPEP
YKR093W		*PTR2*	PEPTIDE	PEPTxt + HEXT -> PEPT
YBR021W		*FUR4*	URACIL	URAxt + HEXT -> URA
*U173_*		*U173_*	NICOTINAMIDE MONONUCLEOTIDE TRANSPORTER	NMNxt + HEXT -> NMN
YER056C		*FCY2*	CYTOSINE PURINE PERMEASE	CYTSxt + HEXT -> CYTS
YER056C		*FCY2*	ADENINE	ADxt + HEXT -> AD
YER056C		*FCY2*	GUANINE	GNxt + HEXT <-> GN
YER060W		*FCY21*	CYTOSINE PURINE PERMEASE	CYTSxt + HEXT -> CYTS
YER060W		*FCY21*	ADENINE	ADxt + HEXT -> AD
YER060W		*FCY21*	GUANINE	GNxt + HEXT <-> GN

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YER060W-A		*FCY22*	CYTOSINE PURINE PERMEASE	CYTSxt + HEXT -> CYTS
YER060W-A		*FCY22*	ADENINE	ADxt + HEXT -> AD
YER060W-A		*FCY22*	GUANINE	GNxt + HEXT <-> GN
YGL186C		*YGL186C*	CYTOSINE PURINE PERMEASE	CYTSxt + HEXT -> CYTS
YGL186C		*YGL186C*	ADENINE	ADxt + HEXT -> AD
YGL186C		*YGL186C*	GUANINE	GNxt + HEXT <-> GN
*U174_*		*U174_*	G-SYSTEM	ADNxt + HEXT -> ADN
*U175_*		*U175_*	G-SYSTEM	GSNxt + HEXT -> GSN
YBL042C		*FUI1*	URIDINE PERMEASE, G-SYSTEM	URIxt + HEXT -> URI
*U176_*		*U176_*	G-SYSTEM	CYTDxt + HEXT -> CYTD
*U177_*		*U177_*	G-SYSTEM (TRANSPORTS ALL NUCLEOSIDES)	INSxt + HEXT -> INS
*U178_*		*U178_*	G-SYSTEM	XTSINExt + HEXT -> XTSINE
*U179_*		*U179_*	G-SYSTEM	DTxt + HEXT -> DT
*U180_*		*U180_*	G-SYSTEM	DINxt + HEXT -> DIN
*U181_*		*U181_*	G-SYSTEM	DGxt + HEXT -> DG
*U182_*		*U182_*	G-SYSTEM	DAxt + HEXT -> DA
*U183_*		*U183_*	G-SYSTEM	DCxt + HEXT -> DC
*U184_*		*U184_*	G-SYSTEM	DUxt + HEXT -> DU
*U185_*		*U185_*	C-SYSTEM	ADNxt + HEXT -> ADN
YBL042C		*FUI1*	URIDINE PERMEASE, C-SYSTEM	URIxt + HEXT -> URI
*U186_*		*U186_*	C-SYSTEM	CYTDxt + HEXT -> CYTD
*U187_*		*U187_*	C-SYSTEM	DTxt + HEXT -> DT
*U188_*		*U188_*	C-SYSTEM	DAxt + HEXT -> DA
*U189_*		*U189_*	C-SYSTEM	DCxt + HEXT -> DC
*U190_*		*U190_*	C-SYSTEM	DUxt + HEXT -> DU
*U191_*		*U191_*	NUCLEOSIDES AND DEOXYNUCLEOSIDE	ADNxt + HEXT -> ADN
*U192_*		*U192_*	NUCLEOSIDES AND DEOXYNUCLEOSIDE	GSNxt + HEXT -> GSN
YBL042C		*FUI1*	URIDINE PERMEASE, NUCLEOSIDES AND DEOXYNUCLEOSIDE	URIxt + HEXT -> URI
*U193_*		*U193_*	NUCLEOSIDES AND DEOXYNUCLEOSIDE	CYTDxt + HEXT -> CYTD
*U194_*		*U194_*	NUCLEOSIDES AND DEOXYNUCLEOSIDE	INSxt + HEXT -> INS
*U195_*		*U195_*	NUCLEOSIDES AND DEOXYNUCLEOSIDE	DTxt + HEXT -> DT
*U196_*		*U196_*	NUCLEOSIDES AND DEOXYNUCLEOSIDE	DINxt + HEXT -> DIN
*U197_*		*U197_*	NUCLEOSIDES AND DEOXYNUCLEOSIDE	DGxt + HEXT -> DG
*U198_*		*U198_*	NUCLEOSIDES AND DEOXYNUCLEOSIDE	DAxt + HEXT -> DA
*U199_*		*U199_*	NUCLEOSIDES AND DEOXYNUCLEOSIDE	DCxt + HEXT -> DC
*U200_*		*U200_*	NUCLEOSIDES AND DEOXYNUCLEOSIDE	DUxt + HEXT -> DU
*U201_*		*U201_*	HYPOXANTHINE	HYXNxt + HEXT <-> HYXN

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
*U202_*		*U202_*	XANTHINE	XANxt <-> XAN
# METABOLIC BY-PRODUCTS				
YCR032W		*BPH1*	PROBABLE ACETIC ACID EXPORT PUMP, ACETATE TRANSPORT	ACxt + HEXT <-> AC
*U203_*		*U203_*	FORMATE TRANSPORT	FORxt <-> FOR
*U204_*		*U204_*	ETHANOL TRANSPORT	ETHxt <-> ETH
*U205_*		*U205_*	SUCCINATE TRANSPORT	SUCCxt + HEXT <-> SUCC
YKL217W		*JEN1*	PYRUVATE LACTATE PROTON SYMPORT	PYRxt + HEXT -> PYR
# OTHER COMPOUNDS				
YHL016C		*DUR3*	UREA ACTIVE TRANSPORT	UREAxt + 2 HEXT <-> UREA
YGR121C		*MEP1*	AMMONIA TRANSPORT	NH3xt <-> NH3
YNL142W		*MEP2*	AMMONIA TRANSPORT, LOW CAPACITY HIGH AFFINITY	NH3xt <-> NH3
YPR138C		*MEP3*	AMMONIA TRANSPORT, HIGH CAPACITY LOW AFFINITY	NH3xt <-> NH3
YJL129C		*TRK1*	POTASSIUM TRANSPORTER OF THE PLASMA MEMBRANE, HIGH AFFINITY, MEMBER OF THE POTASSIUM TRANSPORTER (TRK) FAMILY OF MEMBRANE TRANSPORTERS	Kxt + HEXT <-> K
YBR294W		*SUL1*	SULFATE PERMEASE	SLFxt -> SLF
YLR092W		*SUL2*	SULFATE PERMEASE	SLFxt -> SLF
YGR125W		*YGR125W*	SULFATE PERMEASE	SLFxt -> SLF
YML123C		*PHO84*	INORGANIC PHOSPHATE TRANSPORTER, TRANSMEMBRANE PROTEIN	PIxt + HEXT <-> PI
*U206_*		*U206_*	CITRATE	CITxt + HEXT <-> CIT
*U207_*		*U207_*	DICARBOXYLATES	FUMxt + HEXT <-> FUM
*U208_*		*U208_*	FATTY ACID TRANSPORT	C140xt -> C140
*U209_*		*U209_*	FATTY ACID TRANSPORT	C160xt -> C160
*U210_*		*U210_*	FATTY ACID TRANSPORT	C161xt -> C161
*U211_*		*U211_*	FATTY ACID TRANSPORT	C180xt -> C180
*U212_*		*U212_*	FATTY ACID TRANSPORT	C181xt -> C181
*U213_*		*U213_*	A-KETOGLUTARATE	AKGxt + HEXT <-> AKG
YLR138W		*NHA1*	PUTATIVE NA+/H+ ANTIPORTER	NAxt <-> NA + HEXT
YCR028C		*FEN2*	PANTOTHENATE	PNTOxt + HEXT <-> PNTO
*U214_*		*U214_*	ATP DRAIN FLUX FOR CONSTANT MAINTANENCE REQUIREMENTS	ATP -> ADP + PI

Table A.2. Reactions described in FBA (contiuned)

ORF	E.C. #	GENE	GENE DESCRIPTION	REACTION
YCR024C-A		*PMP1*	H+-ATPASE SUBUNIT, PLASMA MEMBRANE	ATP -> ADP + PI + HEXT
YEL017C-A		*PMP2*	H+-ATPASE SUBUNIT, PLASMA MEMBRANE	ATP -> ADP + PI + HEXT
YGL008C		*PMA1*	H+-TRANSPORTING P-TYPE ATPASE, MAJOR ISOFORM, PLASMA MEMBRANE	ATP -> ADP + PI + HEXT
YPL036W		*PMA2*	H+-TRANSPORTING P-TYPE ATPASE, MINOR ISOFORM, PLASMA MEMBRANE	ATP -> ADP + PI + HEXT
*U215_*		*U215_*	GLYCERALDEHYDE TRANSPORT	GLALxt <-> GLAL
*U216_*		*U216_*	ACETALDEHYDE TRANSPORT	ACALxt <-> ACAL
YLR237W		*THI7*	THIAMINE TRANSPORT PROTEIN	THMxt + HEXT -> THIAMIN
YOR071C		*YOR071C*	PROBABLE LOW AFFINITY THIAMINE TRANSPORTER	THMxt + HEXT -> THIAMIN
YOR192C		*YOR192C*	PROBABLE LOW AFFINITY THIAMINE TRANSPORTER	THMxt + HEXT -> THIAMIN
YIR028W		*DAL4*		ATNxt -> ATN
YJR152W		*DAL5*		ATTxt -> ATT
*U217_*		*U217_*		MTHNxt <-> MTHN
*U218_*		*U218_*		PAPxt <-> PAP
*U219_*		*U219_*		DTTPxt <-> DTTP
*U220_*		*U220_*		THYxt <-> THY + HEXT
*U221_*		*U221_*		GA6Pxt <-> GA6P
YGR065C		*VHT1*	H+/BIOTIN SYMPORTER AND MEMBER OF THE ALLANTOATE PERMEASE FAMILY OF THE MAJOR FACILITATOR SUPERFAMILY	BTxt + HEXT <-> BT
*U222_*		*U222_*		AONAxt + HEXT <-> AONA
*U223_*		*U223_*		DANNAxt + HEXT <-> DANNA
*U224_*		*U224_*		OGTxt -> OGT
*U225_*		*U225_*		SPRMxt -> SPRM
*U226_*		*U226_*		PIMExt -> PIME
*U227_*		*U227_*	OXYGEN TRANSPORT	O2xt <-> O2
*U228_*		*U228_*	CARBON DIOXIDE TRANSPORT	CO2xt <-> CO2
YOR011W		*AUS1*		ERGOSTxt <-> ERGOST
YOR011W		*AUS1*	PUTATIVE STEROL TRANSPORTER	ZYMSTxt <-> ZYMST
*U229_*		*U229_*		RFLAVxt + HEXT -> RIBFLAV

Table A.3. Flux measurements of the cells grown in YPD medium

Fluxes in g/(gDW.hr)					
Cultivation in the absence of caffeine			Cultivation in the presence of caffeine		
OD 600	Remaining Glucose	Glycerol	OD 600	Remaining Glucose	Glycerol
0.86	3.63	0.1140	0.96	3.29	0.1064
1.13	1.69	0.0575	1.18	1.34	0.0464
1.34	1.46	0.0446	1.31	1.82	0.0361
1.48	1.17	0.0275	1.44	1.65	0.0285
1.59	1.18	0.0337	1.52	0.99	0.0158
1.73	1.69	0.0278	1.62	0.84	0.0145
1.83	1.08	0.0189	1.70	0.93	0.0159
1.94	0.87	0.0153	1.78	0.58	0.0099
2.02	0.85	0.0099	1.84	0.50	0.0080
2.09	0.56	0.0036	1.90	0.67	0.0103
Fluxes in mmole/(gDW.hr)					
Cultivation in the absence of caffeine			Cultivation in the presence of caffeine		
OD 600	Remaining Glucose	Glycerol	OD 600	Remaining Glucose	Glycerol
0.86	20.14	1.239	0.96	18.28	1.1563
1.13	9.40	0.625	1.18	7.42	0.5039
1.34	8.09	0.485	1.31	10.12	0.3924
1.48	6.48	0.299	1.44	9.14	0.3099
1.59	6.58	0.366	1.52	5.50	0.1715
1.73	9.40	0.303	1.62	4.66	0.1581
1.83	5.97	0.206	1.70	5.16	0.1726
1.94	4.82	0.166	1.78	3.23	0.1077
2.02	4.75	0.108	1.84	2.77	0.0866
2.09	3.09	0.039	1.90	3.74	0.1117

Table A.4. Members of the SOM clusters

Clusters	Members						
c0	PGI1	PGI1	MDH2	TKL2	TKL1	TAL1	
c3	GLK1	PFK1	FBA1	TPI1	TDH3	PGK1	GPM2
	ENO2	PYK2	MDH1	ZWF1	SOL4	GND2	RPE1
	RKI1	PDC6	ADH5	ATP1	LCB5	LCB3	U41_
	PRO1	PRO2	U66_	PRO3	PUT1	HOR2	GPD2
	U120_	AAC1	MIR1	DIC1	OAC1	U134_	HXT4
	FPS1	U204_	U228_	CO2xt	ETHxt	GLxt	GLCxt

APPENDIX B: PROTEINS, PROTEIN INTERACTIONS, MODULES AND TOPOLOGICAL STUDY OF TOR SIGNALLING NETWORK

Proteins, protein interactions (as binary interaction) and modules of the TOR signalling network, gene ontology terms annotated to module members and topological parameters are included in this section.

Table B.1. ORF of proteins found related to TOR signalling in the literature

YAL003W	YDR040C	YFL001W	YGR162W	YJR090C	YLR291C	YNL189W	YPL015C
YAL016W	YDR055W	YFL014W	YGR163W	YJR094C	YLR304C	YNL216W	YPL045W
YAR071W	YDR075W	YFL021W	YGR191W	YJR152W	YLR396C	YNL229C	YPL048W
YBL014C	YDR096W	YFL033C	YGR218W	YKL006W	YLR403W	YNL244C	YPL081W
YBL021C	YDR099W	YFR029W	YGR246C	YKL009W	YML025C	YNL248C	YPL093W
YBL064C	YDR160W	YFR040W	YHR008C	YKL062W	YML097C	YNL262W	YPL111W
YBL103C	YDR174W	YGL009C	YHR030C	YKL092C	YMR028W	YNL298W	YPL152W
YBR001C	YDR204W	YGL035C	YHR094C	YKL109W	YMR037C	YNL314W	YPL154C
YBR077C	YDR216W	YGL037C	YHR136C	YKL113C	YMR068W	YNL330C	YPL180W
YBR092C	YDR223W	YGL049C	YHR186C	YKL125W	YMR131C	YNR001C	YPL203W
YBR097W	YDR283C	YGL062W	YHR205W	YKL166C	YMR146C	YNR003C	YPL207W
YBR251W	YDR323C	YGL073W	YIL021W	YKL203C	YMR231W	YOL020W	YPL211W
YCL025C	YDR380W	YGL095C	YIL033C	YKR007W	YMR239C	YOL067C	YPL265W
YCR005C	YDR453C	YGL118C	YIL153W	YKR028W	YNL006W	YOL139C	YPR010C
YCR027C	YDR477W	YGL167C	YIL155C	YKR034W	YNL015W	YOL151W	YPR040W
YDL042C	YDR495C	YGL180W	YIR023W	YKR059W	YNL037C	YOR014W	YPR104C
YDL047W	YDR507C	YGL187C	YJL035C	YLL041C	YNL039W	YOR036W	YPR110C
YDL060W	YEL009C	YGL190C	YJL098W	YLR060W	YNL068C	YOR101W	YPR160W
YDL102W	YER020W	YGL229C	YJL110C	YLR148W	YNL076W	YOR136W	YPR185W
YDL134C	YER040W	YGL237C	YJL138C	YLR155C	YNL098C	YOR161C	
YDL188C	YER093C	YGL252C	YJL141C	YLR179C	YNL102W	YOR178C	
YDL212W	YER119C	YGR032W	YJL156C	YLR223C	YNL135C	YOR244W	
YDL215C	YER148W	YGR054W	YJL164C	YLR240W	YNL142W	YOR358W	
YDR005C	YER150W	YGR083C	YJL172W	YLR249W	YNL160W	YOR361C	
YDR011W	YER177W	YGR095C	YJR066W	YLR258W	YNL183C	YOR372C	

Table B.2. Protein-protein interactions found using Osprey 1.2.0

YAL003W	YLR249W	YDL188C	YPR010C	YDR477W	YER177W	YER177W	YNL076W
YAL016W	YDL134C	YDL212W	YDL212W	YDR477W	YGL180W	YER177W	YNL102W
YAL016W	YDL188C	YDL212W	YPL265W	YDR477W	YHR205W	YER177W	YNL330C
YAL016W	YMR028W	YDR005C	YNR003C	YDR477W	YJL110C	YFL001W	YKL009W
YAL016W	YPL152W	YDR005C	YPR110C	YDR477W	YLR258W	YFL001W	YKR059W
YBL014C	YBL014C	YDR011W	YDR011W	YDR477W	YMR037C	YFL001W	YNL135C
YBL014C	YDL042C	YDR011W	YHR094C	YDR477W	YNL330C	YFL021W	YDR099W
YBR077C	YGR163W	YDR011W	YPL265W	YDR477W	YOR178C	YFL021W	YJL164C
YBR077C	YJR066W	YDR075W	YBL103C	YDR477W	YPL180W	YFL021W	YNL098C
YBR092C	YDL047W	YDR075W	YDR099W	YDR477W	YPR185W	YFL021W	YNL229C
YBR092C	YPL152W	YDR075W	YKL113C	YDR495C	YLR148W	YFL033C	YDR096W
YBR251W	YGR162W	YDR096W	YKL062W	YDR495C	YLR396C	YFL033C	YFL033C
YCL025C	YCL025C	YDR096W	YMR037C	YDR495C	YMR231W	YFL033C	YMR037C
YCL025C	YDL212W	YDR099W	YBL103C	YDR495C	YPL045W	YFR029W	YFR029W
YCL025C	YPL265W	YDR099W	YBR001C	YDR507C	YDR507C	YFR029W	YJL156C
YCR005C	YCR005C	YDR099W	YDR099W	YEL009C	YEL009C	YFR040W	YGL229C
YCR005C	YOR101W	YDR099W	YER177W	YER020W	YDL042C	YFR040W	YIL153W
YDL042C	YDL042C	YDR099W	YGL252C	YER020W	YHR205W	YFR040W	YJL098W
YDL042C	YLR249W	YDR099W	YJL164C	YER020W	YNL037C	YFR040W	YJL141C
YDL047W	YDL047W	YDR099W	YNL330C	YER020W	YOR101W	YFR040W	YKR028W
YDL047W	YDL188C	YDR160W	YFR029W	YER020W	YPL203W	YFR040W	YPR040W
YDL047W	YDR075W	YDR160W	YJL156C	YER040W	YDL047W	YGL035C	YDR096W
YDL047W	YDR099W	YDR174W	YDR174W	YER040W	YDR099W	YGL035C	YDR099W
YDL047W	YDR283C	YDR174W	YER148W	YER040W	YDR477W	YGL035C	YDR174W
YDL047W	YFR040W	YDR174W	YJR066W	YER040W	YEL009C	YGL035C	YDR477W
YDL047W	YGL229C	YDR174W	YNL076W	YER040W	YFL021W	YGL037C	YGL037C
YDL047W	YIL153W	YDR174W	YPL180W	YER040W	YGR218W	YGL049C	YGR162W
YDL047W	YJL141C	YDR216W	YDR477W	YER040W	YJL164C	YGL073W	YDR477W
YDL047W	YKR028W	YDR216W	YDR507C	YER040W	YJR066W	YGL073W	YER040W
YDL047W	YMR028W	YDR216W	YER148W	YER040W	YNL098C	YGL073W	YFL033C
YDL047W	YNL229C	YDR216W	YNL330C	YER093C	YER177W	YGL073W	YHR030C
YDL047W	YNL298W	YDR283C	YDR174W	YER093C	YHR030C	YGL073W	YHR205W
YDL047W	YPL152W	YDR283C	YDR283C	YER093C	YKL203C	YGL073W	YKL062W
YDL060W	YGR054W	YDR283C	YEL009C	YER093C	YMR068W	YGL073W	YMR037C
YDL060W	YGR162W	YDR283C	YGR083C	YER093C	YNL006W	YGL073W	YNL135C
YDL102W	YKL113C	YDR283C	YLR291C	YER148W	YBL014C	YGL073W	YOR014W
YDL134C	YDL188C	YDR283C	YNL298W	YER148W	YEL009C	YGL073W	YOR178C
YDL134C	YDR075W	YDR323C	YLR148W	YER148W	YER148W	YGL095C	YDR323C
YDL134C	YKL203C	YDR323C	YLR240W	YER148W	YGR246C	YGL167C	YER040W
YDL134C	YMR028W	YDR323C	YMR231W	YER148W	YLR249W	YGL167C	YJR066W
YDL134C	YPR110C	YDR477W	YDL047W	YER148W	YOL067C	YGL167C	YNL006W
YDL188C	YDR075W	YDR477W	YDR040C	YER177W	YBL103C	YGL167C	YNL183C
YDL188C	YHR205W	YDR477W	YDR096W	YER177W	YDR216W	YGL180W	YDR099W
YDL188C	YJL141C	YDR477W	YDR099W	YER177W	YJL164C	YGL180W	YFL001W
YDL188C	YMR028W	YDR477W	YDR477W	YER177W	YLR258W	YGL180W	YFR029W

Table B.2. Protein-protein interactions found using Osprey 1.2.0 (continued)

YGL180W	YGL180W	YGR163W	YOL067C	YIL153W	YKL113C	YJR066W	YFL033C
YGL180W	YGL190C	YGR191W	YDL212W	YIL153W	YLR258W	YJR066W	YGL252C
YGL180W	YNL314W	YGR218W	YKR059W	YIL153W	YMR028W	YJR066W	YGR032W
YGL180W	YPR160W	YGR218W	YNL189W	YIL153W	YNL098C	YJR066W	YGR163W
YGL190C	YAL016W	YGR246C	YDR005C	YIL153W	YNL229C	YJR066W	YGR218W
YGL190C	YDL134C	YGR246C	YNR003C	YIL153W	YNL314W	YJR066W	YHR094C
YGL190C	YDL188C	YHR008C	YGL167C	YIL153W	YPL152W	YJR066W	YHR205W
YGL190C	YDR507C	YHR030C	YHR030C	YJL098W	YDL047W	YJR066W	YIR023W
YGL190C	YER177W	YHR030C	YJL138C	YJL098W	YGL229C	YJR066W	YJL110C
YGL190C	YHR030C	YHR030C	YJR066W	YJL098W	YJL098W	YJR066W	YKR034W
YGL190C	YKL113C	YHR030C	YNL037C	YJL098W	YKR028W	YJR066W	YLR148W
YGL190C	YLR249W	YHR094C	YDL212W	YJL098W	YLR249W	YJR066W	YLR240W
YGL190C	YMR028W	YHR094C	YER020W	YJL098W	YOL139C	YJR066W	YLR396C
YGL190C	YNL098C	YHR094C	YHR094C	YJL098W	YOR244W	YJR066W	YMR231W
YGL190C	YNL298W	YHR094C	YPL265W	YJL098W	YPR040W	YJR066W	YNL189W
YGL190C	YPL180W	YHR186C	YFL033C	YJL110C	YJL110C	YJR066W	YNL229C
YGL229C	YPR040W	YHR186C	YJR066W	YJL138C	YGL049C	YJR066W	YNL314W
YGL237C	YBL021C	YHR186C	YNL006W	YJL138C	YLR249W	YJR066W	YPL045W
YGL237C	YJL138C	YHR186C	YPL180W	YJL138C	YOL139C	YJR066W	YPL180W
YGL237C	YKR028W	YHR186C	YPR040W	YJL141C	YDR099W	YJR066W	YPR160W
YGL237C	YOL139C	YHR205W	YDR096W	YJL141C	YDR223W	YJR090C	YDR099W
YGL237C	YOR358W	YHR205W	YFL033C	YJL141C	YDR453C	YJR090C	YGL190C
YGL252C	YCR005C	YHR205W	YHR008C	YJL141C	YER177W	YJR090C	YHR094C
YGL252C	YER177W	YHR205W	YJL141C	YJL141C	YGL073W	YJR090C	YJL156C
YGL252C	YGL252C	YHR205W	YJL164C	YJL141C	YMR037C	YJR090C	YNL076W
YGL252C	YJR090C	YHR205W	YKL109W	YJL164C	YFL001W	YJR094C	YJR094C
YGL252C	YNL006W	YHR205W	YLR223C	YJL164C	YFL033C	YKL009W	YLR249W
YGL252C	YNL076W	YHR205W	YNL098C	YJL164C	YGL167C	YKL009W	YPL211W
YGL252C	YNR001C	YIL021W	YER148W	YJL164C	YIL033C	YKL062W	YFL033C
YGL252C	YOL067C	YIL021W	YFL001W	YJL164C	YJL110C	YKL062W	YGL037C
YGR054W	YGR054W	YIL021W	YIL021W	YJL164C	YJL141C	YKL062W	YHR205W
YGR054W	YJL138C	YIL033C	YFL033C	YJL164C	YJL164C	YKL109W	YBL021C
YGR054W	YKR059W	YIL033C	YIL033C	YJL164C	YKL062W	YKL109W	YGL237C
YGR054W	YOL139C	YIL033C	YJR094C	YJL164C	YKL166C	YKL109W	YOR358W
YGR095C	YGR095C	YIL033C	YKL062W	YJL164C	YLR258W	YKL113C	YDR323C
YGR095C	YNL189W	YIL033C	YMR239C	YJL164C	YNL189W	YKL113C	YDR495C
YGR162W	YGR162W	YIL033C	YOL139C	YJL164C	YPL203W	YKL113C	YGL167C
YGR162W	YJL138C	YIL033C	YPL048W	YJL164C	YPR040W	YKL113C	YJR090C
YGR162W	YKR059W	YIL033C	YPR040W	YJL164C	YPR160W	YKL113C	YLR148W
YGR162W	YLR249W	YIL153W	YAL016W	YJR066W	YAL016W	YKL113C	YLR240W
YGR162W	YNL244C	YIL153W	YDL134C	YJR066W	YBR097W	YKL113C	YLR396C
YGR162W	YNL262W	YIL153W	YDL188C	YJR066W	YDR011W	YKL113C	YLR403W
YGR162W	YOL139C	YIL153W	YDR075W	YJR066W	YDR040C	YKL113C	YMR231W
YGR163W	YGL252C	YIL153W	YDR477W	YJR066W	YEL009C	YKL113C	YNL298W
YGR163W	YGR163W	YIL153W	YHR030C	YJR066W	YFL021W	YKL125W	YBL014C

Table B.2. Protein-protein interactions found using Osprey 1.2.0 (continued)

YKL166C	YDL047W	YLR291C	YGR083C	YNL098C	YFL033C	YNL248C	YPR010C
YKL166C	YFL033C	YLR291C	YKR034W	YNL098C	YIL033C	YNL248C	YPR110C
YKL166C	YGL180W	YLR291C	YLR258W	YNL098C	YJL141C	YNL262W	YDL102W
YKL166C	YGL190C	YLR291C	YLR291C	YNL098C	YJL164C	YNL262W	YKL113C
YKL166C	YIL033C	YLR291C	YNL229C	YNL098C	YJR094C	YNL262W	YNL262W
YKL166C	YKL166C	YLR291C	YNL244C	YNL098C	YKL062W	YNL298W	YDR099W
YKL166C	YNL298W	YLR291C	YNL314W	YNL098C	YKL166C	YNL298W	YDR507C
YKL166C	YPR160W	YLR291C	YPR010C	YNL098C	YMR037C	YNL298W	YER177W
YKL203C	YAL016W	YLR304C	YNR001C	YNL098C	YNL098C	YNL298W	YHR030C
YKL203C	YDL047W	YLR396C	YDR323C	YNL098C	YNL183C	YNL298W	YIL021W
YKL203C	YDR011W	YLR396C	YLR396C	YNL098C	YOR101W	YNL298W	YIL153W
YKL203C	YDR040C	YLR396C	YOR036W	YNL098C	YPL203W	YNL298W	YJL098W
YKL203C	YER040W	YLR403W	YNL098C	YNL102W	YDL102W	YNL298W	YJR094C
YKL203C	YGR032W	YMR028W	YDR075W	YNL102W	YKL125W	YNL298W	YNL068C
YKL203C	YHR030C	YMR028W	YJR066W	YNL102W	YNL189W	YNL298W	YNL229C
YKL203C	YHR094C	YMR028W	YPR040W	YNL102W	YNL262W	YNL314W	YIR023W
YKL203C	YJR066W	YMR037C	YGL037C	YNL135C	YDR174W	YNL314W	YNL314W
YKL203C	YKL203C	YMR037C	YHR205W	YNL135C	YJR066W	YNL330C	YDL042C
YKL203C	YMR028W	YMR037C	YKL062W	YNL142W	YKR034W	YNL330C	YFL001W
YKL203C	YNL135C	YMR068W	YKL203C	YNL142W	YNL098C	YNL330C	YGR162W
YKL203C	YNL183C	YMR068W	YNL006W	YNL142W	YNL142W	YNL330C	YJR090C
YKL203C	YNL229C	YMR131C	YMR131C	YNL142W	YNL229C	YNL330C	YNL248C
YKR007W	YBR077C	YMR131C	YPL207W	YNL189W	YDR174W	YNL330C	YNL298W
YKR007W	YGR163W	YMR146C	YMR146C	YNL189W	YDR453C	YNL330C	YNL330C
YKR007W	YKR007W	YMR146C	YPL081W	YNL189W	YER040W	YNL330C	YPR104C
YKR028W	YDR283C	YMR231W	YGL095C	YNL189W	YGL037C	YNR001C	YCR005C
YKR028W	YER177W	YMR231W	YLR396C	YNL189W	YKL009W	YNR003C	YNL248C
YKR028W	YGL229C	YMR231W	YMR231W	YNL189W	YMR239C	YNR003C	YNR003C
YKR034W	YJL110C	YMR231W	YPL045W	YNL189W	YNL189W	YNR003C	YPR010C
YKR034W	YKR034W	YMR239C	YMR239C	YNL189W	YPL111W	YNR003C	YPR110C
YKR034W	YNL229C	YNL006W	YER040W	YNL216W	YER148W	YOL020W	YDL212W
YKR059W	YGL049C	YNL006W	YJR066W	YNL216W	YLR223C	YOL067C	YBL103C
YKR059W	YJL138C	YNL006W	YKL203C	YNL216W	YNL330C	YOL067C	YNR001C
YKR059W	YOL139C	YNL006W	YPL180W	YNL216W	YPR104C	YOL139C	YGL049C
YLR148W	YGL095C	YNL037C	YNR001C	YNL229C	YEL009C	YOL139C	YNL262W
YLR148W	YLR148W	YNL037C	YOR136W	YNL229C	YER040W	YOR014W	YAL016W
YLR148W	YLR396C	YNL039W	YER148W	YNL229C	YJL110C	YOR014W	YDL134C
YLR148W	YMR231W	YNL039W	YGR246C	YNL229C	YNL229C	YOR014W	YDL188C
YLR148W	YOR036W	YNL068C	YOR361C	YNL244C	YGL049C	YOR014W	YER177W
YLR148W	YPL045W	YNL068C	YOR372C	YNL244C	YMR146C	YOR014W	YFR040W
YLR240W	YBR097W	YNL076W	YDL102W	YNL248C	YDR174W	YOR014W	YKL113C
YLR249W	YPL048W	YNL076W	YNL229C	YNL248C	YFL001W	YOR014W	YPL152W
YLR258W	YLR258W	YNL098C	YDL060W	YNL248C	YKL125W	YOR036W	YDR323C
YLR258W	YOR178C	YNL098C	YDR096W	YNL248C	YMR239C	YOR036W	YGL095C
YLR291C	YGL252C	YNL098C	YER020W	YNL248C	YNL248C	YOR101W	YIL033C

Table B.2. Protein-protein interactions found using Osprey 1.2.0 (continued)

YOR101W	YJL164C	YOR244W	YNL229C	YPL152W	YGL190C	YPR040W	YDL134C
YOR101W	YOL067C	YOR244W	YNL298W	YPL152W	YHR030C	YPR040W	YDL188C
YOR101W	YOR101W	YOR244W	YNL330C	YPL152W	YMR028W	YPR040W	YDR075W
YOR101W	YPL203W	YOR244W	YOL067C	YPL180W	YAL016W	YPR104C	YDR174W
YOR136W	YNR001C	YOR244W	YOR014W	YPL180W	YDR096W	YPR104C	YDR223W
YOR244W	YDL042C	YOR244W	YPL045W	YPL180W	YHR030C	YPR104C	YHR205W
YOR244W	YDR099W	YOR358W	YBL021C	YPL180W	YNL248C	YPR104C	YJL098W
YOR244W	YDR323C	YOR361C	YMR146C	YPL180W	YOR014W	YPR104C	YLR223C
YOR244W	YDR495C	YOR361C	YNL244C	YPL203W	YDR096W	YPR110C	YDR075W
YOR244W	YER177W	YOR361C	YOR361C	YPL203W	YFL033C	YPR110C	YDR216W
YOR244W	YFL001W	YPL045W	YDR323C	YPL203W	YGL035C	YPR110C	YDR453C
YOR244W	YFR040W	YPL045W	YGL095C	YPL203W	YIL033C	YPR110C	YDR495C
YOR244W	YGL095C	YPL045W	YLR396C	YPL203W	YJL141C	YPR110C	YGL062W
YOR244W	YGL252C	YPL048W	YAL003W	YPL203W	YKL062W	YPR110C	YKL113C
YOR244W	YIR023W	YPL048W	YKL009W	YPL203W	YMR037C	YPR110C	YNL229C
YOR244W	YJR090C	YPL093W	YKL009W	YPL203W	YPL203W	YPR110C	YOR014W
YOR244W	YKL113C	YPL093W	YPL093W	YPL211W	YPL093W	YPR110C	YOR136W
YOR244W	YLR148W	YPL111W	YPL111W	YPL265W	YPL265W	YPR110C	YPR010C
YOR244W	YLR396C	YPL152W	YDL134C	YPR010C	YDL042C	YPR110C	YPR110C
YOR244W	YMR231W	YPL152W	YDL188C	YPR010C	YKL125W	YPR185W	YGL180W

Table B.3. Modules found in the TOR signalling network

Module number	Module members					
1	YOR244W	YKL113C	YLR396C	YMR231W	YLR148W	YDR323C
2	YOR244W	YKL113C	YLR396C	YMR231W	YLR148W	YDR495C
3	YOR244W	YPL045W	YMR231W	YLR148W	YLR396C	YDR323C
4	YOR244W	YPL045W	YMR231W	YLR148W	YLR396C	YDR495C
5	YOR244W	YPL045W	YMR231W	YLR148W	YGL095C	YDR323C
6	YGL190C	YAL016W	YPL152W	YMR028W	YDL188C	YDL134C
7	YDR096W	YKL062W	YMR037C	YNL098C	YFL033C	YHR205W
8	YDR096W	YKL062W	YMR037C	YNL098C	YFL033C	YPL203W
9	YIL033C	YJL164C	YNL098C	YPL203W	YKL062W	YFL033C
10	YPL152W	YAL016W	YDL188C	YDL134C	YMR028W	YIL153W
11	YJR066W	YLR396C	YMR231W	YPL045W	YLR148W	
12	YFR040W	YJL098W	YGL229C	YDL047W	YKR028W	
13	YOR244W	YDR099W	YER177W	YNL298W	YNL330C	
14	YDL047W	YIL153W	YMR028W	YDL188C	YDR075W	
15	YDL047W	YIL153W	YMR028W	YDL188C	YPL152W	
16	YGL049C	YKR059W	YJL138C	YGR162W	YOL139C	
17	YPR040W	YDR075W	YMR028W	YDL188C	YDL134C	
18	YGL073W	YHR205W	YKL062W	YMR037C	YFL033C	
19	YIL033C	YJL164C	YNL098C	YPL203W	YOR101W	
20	YIL033C	YJL164C	YNL098C	YKL166C	YFL033C	
21	YDR075W	YDL134C	YMR028W	YDL188C	YIL153W	
22	YPL152W	YAL016W	YDL188C	YDL134C	YOR014W	
23	YKL062W	YNL098C	YFL033C	YHR205W	YJL164C	

Table B.3. Modules found in the TOR signalling network (continued)

Module number	Module members			
24	YJR066W	YKL203C	YER040W	YNL229C
25	YJR066W	YKL203C	YER040W	YNL006W
26	YJR066W	YKL203C	YHR094C	YDR011W
27	YJR066W	YKL203C	YAL016W	YMR028W
28	YJR066W	YFL021W	YNL229C	YER040W
29	YJR066W	YKR034W	YNL229C	YJL110C
30	YJR066W	YNL189W	YGR218W	YER040W
31	YJR066W	YHR186C	YPL180W	YNL006W
32	YJR066W	YGL167C	YER040W	YNL006W
33	YJR066W	YEL009C	YNL229C	YER040W
34	YFR040W	YJL098W	YGL229C	YPR040W
35	YOR244W	YDR099W	YER177W	YGL252C
36	YOR244W	YDR099W	YJR090C	YGL252C
37	YOR244W	YDR099W	YJR090C	YNL330C
38	YGR054W	YKR059W	YJL138C	YOL139C
39	YDR323C	YLR148W	YLR396C	YOR036W
40	YDR323C	YLR148W	YGL095C	YOR036W
41	YDR099W	YER177W	YNL330C	YDR477W
42	YDR099W	YER177W	YJL141C	YJL164C
43	YDR099W	YDL047W	YDR477W	YER040W
44	YDR099W	YFL021W	YJL164C	YER040W
45	YDL047W	YIL153W	YNL298W	YNL229C
46	YDL047W	YKL203C	YNL229C	YER040W
47	YJL141C	YHR205W	YMR037C	YGL073W
48	YJL141C	YHR205W	YMR037C	YNL098C
49	YJL141C	YHR205W	YJL164C	YNL098C
50	YJL141C	YPL203W	YNL098C	YJL164C
51	YJL141C	YPL203W	YNL098C	YMR037C
52	YDR477W	YDR096W	YHR205W	YMR037C
53	YDR477W	YGL073W	YHR205W	YMR037C
54	YDR477W	YER177W	YNL330C	YDR216W
55	YER020W	YNL098C	YOR101W	YPL203W
56	YMR068W	YKL203C	YER093C	YNL006W
57	YOR358W	YKL109W	YGL237C	YBL021C
58	YNR003C	YPR110C	YPR010C	YNL248C
59	YDL134C	YAL016W	YMR028W	YKL203C
60	YJL164C	YNL098C	YFL021W	YER040W

Table B.3. Modules found in the TOR signalling network (continued)

Module number	Module members			Module number	Module members		
61	YJR066W	YKL203C	YHR030C	105	YDR323C	YLR240W	YKL113C
62	YJR066W	YKL203C	YDR040C	106	YLR223C	YPR104C	YNL216W
63	YJR066W	YKL203C	YNL135C	107	YLR223C	YPR104C	YHR205W
64	YJR066W	YKL203C	YGR032W	108	YDR099W	YER177W	YBL103C
65	YJR066W	YBR097W	YLR240W	109	YDR099W	YDL047W	YJL141C
66	YJR066W	YGL252C	YGR163W	110	YDR099W	YDL047W	YDR075W
67	YJR066W	YGL252C	YNL006W	111	YDR099W	YDL047W	YNL298W
68	YJR066W	YNL189W	YDR174W	112	YDR099W	YDR075W	YBL103C
69	YJR066W	YHR205W	YFL033C	113	YDR099W	YGL035C	YDR477W
70	YJR066W	YHR186C	YFL033C	114	YKL113C	YDR495C	YPR110C
71	YJR066W	YIR023W	YNL314W	115	YKL113C	YGL190C	YNL298W
72	YJR066W	YDR174W	YNL135C	116	YKL113C	YGL190C	YJR090C
73	YJR066W	YDR174W	YPL180W	117	YKL113C	YDR075W	YPR110C
74	YJR066W	YBR077C	YGR163W	118	YKL113C	YDR075W	YIL153W
75	YJR066W	YPL180W	YHR030C	119	YKL113C	YNL298W	YIL153W
76	YJR066W	YPL180W	YAL016W	120	YKL113C	YOR014W	YPR110C
77	YFR040W	YJL098W	YOR244W	121	YDL047W	YIL153W	YDR477W
78	YFR040W	YJL141C	YDL047W	122	YDL047W	YKL203C	YMR028W
79	YFR040W	YIL153W	YDL047W	123	YDL047W	YJL141C	YDL188C
80	YFR040W	YOR014W	YOR244W	124	YDL047W	YKR028W	YDR283C
81	YJL098W	YDL047W	YNL298W	125	YDL047W	YBR092C	YPL152W
82	YJL098W	YOR244W	YNL298W	126	YDL047W	YDR283C	YNL298W
83	YPL211W	YKL009W	YPL093W	127	YDL047W	YKL166C	YNL298W
84	YCR005C	YGL252C	YNR001C	128	YJL156C	YDR160W	YFR029W
85	YGL180W	YDR477W	YDR099W	129	YNL244C	YGL049C	YGR162W
86	YGL180W	YDR477W	YPR185W	130	YNL244C	YOR361C	YMR146C
87	YGL180W	YGL190C	YKL166C	131	YJL141C	YHR205W	YDL188C
88	YGL180W	YPR160W	YKL166C	132	YGL190C	YAL016W	YPL180W
89	YER148W	YNL039W	YGR246C	133	YGL190C	YHR030C	YPL152W
90	YOR244W	YKL113C	YNL298W	134	YGL190C	YHR030C	YNL298W
91	YOR244W	YKL113C	YJR090C	135	YGL190C	YHR030C	YPL180W
92	YOR244W	YKL113C	YOR014W	136	YGL190C	YER177W	YNL298W
93	YOR244W	YGL252C	YOL067C	137	YGL190C	YDR507C	YNL298W
94	YOR244W	YER177W	YOR014W	138	YGL190C	YNL298W	YKL166C
95	YOR244W	YFL001W	YNL330C	139	YGL190C	YKL166C	YNL098C
96	YOR244W	YNL330C	YDL042C	140	YDR477W	YDR096W	YGL035C
97	YOR244W	YNL229C	YNL298W	141	YDR477W	YDR096W	YPL180W
98	YPL048W	YLR249W	YKL009W	142	YDR477W	YGL073W	YOR178C
99	YPL048W	YLR249W	YAL003W	143	YDR477W	YGL073W	YER040W
100	YKR007W	YBR077C	YGR163W	144	YDR477W	YER177W	YLR258W
101	YNL262W	YDL102W	YKL113C	145	YDR477W	YOR178C	YLR258W
102	YNL262W	YDL102W	YNL102W	146	YDR477W	YLR258W	YIL153W
103	YNL262W	YGR162W	YOL139C	147	YER020W	YNL098C	YHR205W
104	YGL037C	YKL062W	YMR037C	148	YOL067C	YGL252C	YNR001C

Table B.3. Modules found in the TOR signalling network (continued)

Module number	Module members			Module number	Module members	
149	YOL067C	YGL252C	YGR163W	193	YER148W	YIL021W
150	YDL212W	YPL265W	YHR094C	194	YER148W	YNL216W
151	YDL212W	YPL265W	YCL025C	195	YER148W	YDR174W
152	YDR096W	YGL035C	YPL203W	196	YER148W	YEL009C
153	YJR094C	YIL033C	YNL098C	197	YER148W	YDR216W
154	YNL216W	YNL330C	YPR104C	198	YER148W	YLR249W
155	YNL076W	YGL252C	YER177W	199	YOR244W	YIR023W
156	YNL076W	YGL252C	YJR090C	200	YPL048W	YIL033C
157	YJL138C	YGR162W	YLR249W	201	YGL037C	YNL189W
158	YJL138C	YGL237C	YOL139C	202	YGR054W	YDL060W
159	YDR005C	YNR003C	YPR110C	203	YNL068C	YOR372C
160	YDR005C	YNR003C	YGR246C	204	YNL068C	YNL298W
161	YPR040W	YIL033C	YJL164C	205	YNL068C	YOR361C
162	YER093C	YKL203C	YHR030C	206	YDR099W	YBR001C
163	YGR083C	YDR283C	YLR291C	207	YBL014C	YKL125W
164	YER177W	YJL164C	YLR258W	208	YBL014C	YDL042C
165	YFL001W	YNL330C	YNL248C	209	YKL113C	YLR403W
166	YDR075W	YDL134C	YPR110C	210	YKL113C	YGL167C
167	YPL152W	YHR030C	YIL153W	211	YJL156C	YJR090C
168	YKL125W	YPR010C	YNL248C	212	YNL244C	YLR291C
169	YPL265W	YHR094C	YDR011W	213	YJL141C	YDR223W
170	YNL298W	YIL153W	YHR030C	214	YJL141C	YDR453C
171	YPR110C	YDL134C	YOR014W	215	YNL102W	YNL189W
172	YNL248C	YDR174W	YPL180W	216	YNL102W	YER177W
173	YNL142W	YKR034W	YNL229C	217	YNL102W	YKL125W
174	YJL164C	YNL189W	YER040W	218	YGL190C	YLR249W
175	YJL164C	YPR160W	YKL166C	219	YDR477W	YDR040C
176	YJL164C	YGL167C	YER040W	220	YDR477W	YJL110C
177	YNR001C	YOR136W	YNL037C	221	YER020W	YHR094C
178	YOR014W	YAL016W	YPL180W	222	YER020W	YDL042C
179	YLR291C	YKR034W	YNL229C	223	YER020W	YNL037C
180	YJR066W	YPR160W		224	YOL067C	YBL103C
181	YJL098W	YLR249W		225	YOL067C	YOR101W
182	YJL098W	YOL139C		226	YKR028W	YER177W
183	YJL098W	YPR104C		227	YKR028W	YGL237C
184	YMR131C	YPL207W		228	YIL021W	YFL001W
185	YOL020W	YDL212W		229	YIL021W	YNL298W
186	YGR191W	YDL212W		230	YJR094C	YNL298W
187	YCR005C	YOR101W		231	YDL102W	YNL076W
188	YGL180W	YFR029W		232	YKR059W	YFL001W
189	YGL180W	YFL001W		233	YKR059W	YGR218W
190	YGL180W	YNL314W		234	YPL111W	YNL189W
191	YER148W	YBL014C		235	YNL076W	YDR174W
192	YER148W	YOL067C		236	YNL076W	YNL229C

Table B.3. Modules found in the TOR signalling network (continued)

Module number	Module members	Module number	Module members
237	YLR403W YNL098C	264	YPR010C YDL188C
238	YJL138C YHR030C	265	YPR010C YLR291C
239	YPR040W YHR186C	266	YDL060W YGR162W
240	YDR223W YPR104C	267	YDL060W YNL098C
241	YER093C YER177W	268	YMR146C YPL081W
242	YGL073W YHR030C	269	YNL248C YMR239C
243	YGL073W YNL135C	270	YGR095C YNL189W
244	YGL073W YOR014W	271	YGL035C YDR174W
245	YBR251W YGR162W	272	YNL142W YNL098C
246	YKL009W YNL189W	273	YJR090C YHR094C
247	YKL009W YFL001W	274	YHR008C YHR205W
248	YIL033C YOL139C	275	YHR008C YGL167C
249	YIL033C YMR239C	276	YJL164C YJL110C
250	YFL001W YNL135C	277	YLR258W YLR291C
251	YFL001W YJL164C	278	YNR001C YLR304C
252	YDR283C YDR174W	279	YIL153W YNL314W
253	YDR283C YEL009C	280	YIL153W YNL098C
254	YDR507C YDR216W	281	YMR239C YNL189W
255	YKL109W YHR205W	282	YNL183C YKL203C
256	YNL330C YGR162W	283	YNL183C YGL167C
257	YPR110C YDR216W	284	YNL183C YNL098C
258	YPR110C YGL062W	285	YPR104C YDR174W
259	YPR110C YNL229C	286	YDR453C YNL189W
260	YPR110C YOR136W	287	YNL037C YHR030C
261	YPR110C YDR453C	288	YLR291C YGL252C
262	YDL042C YPR010C	289	YLR291C YNL314W
263	YDL042C YLR249W		

Table B.4. Topology parameters of the TOR signalling network

ORF	k	b	C	ORF	k	b	C
YAL003W	2	0.00	1.00	YFL033C	12	296.20	0.45
YAL016W	10	98.20	0.53	YFR029W	3	148.81	0.33
YBL014C	3	33.61	0.00	YFR040W	9	126.63	0.33
YBL021C	3	0.00	1.00	YGL035C	5	45.01	0.30
YBL103C	4	24.03	0.33	YGL037C	3	22.22	0.33
YBR001C	1	0.00	0.00	YGL049C	5	70.56	0.70
YBR077C	3	103.89	0.67	YGL062W	1	0.00	0.00
YBR092C	2	0.00	1.00	YGL073W	11	245.09	0.22
YBR097W	2	0.00	1.00	YGL095C	6	27.06	0.80
YBR251W	1	0.00	0.00	YGL167C	7	235.86	0.19
YCL025C	2	0.00	1.00	YGL180W	9	595.20	0.11
YCR005C	3	26.31	0.33	YGL190C	16	939.58	0.18
YDL042C	6	356.39	0.07	YGL229C	5	4.96	0.80
YDL047W	19	975.45	0.22	YGL237C	6	341.60	0.27
YDL060W	3	131.46	0.00	YGL252C	12	1082.24	0.20
YDL102W	4	32.70	0.33	YGR032W	2	0.00	1.00
YDL134C	11	155.31	0.49	YGR054W	4	10.99	0.50
YDL188C	13	309.92	0.37	YGR083C	2	0.00	1.00
YDL212W	5	750.71	0.20	YGR095C	1	0.00	0.00
YDR005C	3	30.55	0.67	YGR162W	10	923.81	0.20
YDR011W	4	169.74	0.67	YGR163W	5	255.53	0.40
YDR040C	3	22.50	0.33	YGR191W	1	0.00	0.00
YDR075W	10	184.99	0.38	YGR218W	4	175.13	0.50
YDR096W	9	62.40	0.53	YGR246C	4	61.95	0.33
YDR099W	17	991.90	0.21	YHR008C	2	5.47	0.00
YDR160W	2	0.00	1.00	YHR030C	11	833.83	0.15
YDR174W	10	667.23	0.09	YHR094C	7	1469.23	0.24
YDR216W	6	237.33	0.20	YHR186C	5	38.13	0.40
YDR223W	2	2.73	0.00	YHR205W	16	1450.15	0.23
YDR283C	7	298.35	0.14	YIL021W	3	63.87	0.00
YDR323C	9	68.40	0.61	YIL033C	12	592.53	0.27
YDR453C	3	104.07	0.00	YIL153W	16	447.53	0.23
YDR477W	19	1080.40	0.14	YIR023W	3	11.71	0.33
YDR495C	7	57.93	0.71	YJL098W	10	495.04	0.24
YDR507C	3	7.21	0.33	YJL110C	5	44.74	0.30
YEL009C	5	200.23	0.30	YJL138C	8	474.94	0.36
YER020W	7	467.74	0.19	YJL141C	13	509.18	0.22
YER040W	15	600.30	0.26	YJL156C	3	221.00	0.33
YER093C	5	70.21	0.40	YJL164C	20	992.33	0.18
YER148W	10	938.78	0.02	YJR066W	36	4754.24	0.08
YER177W	17	990.95	0.15	YJR090C	9	937.77	0.22
YFL001W	9	706.66	0.06	YJR094C	3	25.48	0.33
YFL021W	6	43.09	0.53	YKL009W	6	740.76	0.13

Table B.4. Topology parameters of the TOR signalling network (continued)

ORF	k	b	C	ORF	k	b	C
YKL062W	10	93.22	0.58	YNL229C	15	938.15	0.17
YKL109W	4	400.57	0.50	YNL244C	5	781.40	0.20
YKL113C	18	1207.81	0.17	YNL248C	9	480.75	0.17
YKL125W	4	54.76	0.17	YNL262W	5	222.07	0.30
YKL166C	9	200.62	0.33	YNL298W	17	1530.83	0.17
YKL203C	17	903.86	0.18	YNL314W	5	164.93	0.10
YKR007W	2	0.00	1.00	YNL330C	13	1053.49	0.21
YKR028W	7	281.53	0.33	YNR001C	6	400.90	0.20
YKR034W	5	90.12	0.50	YNR003C	5	89.69	0.50
YKR059W	7	242.70	0.38	YOL020W	1	0.00	0.00
YLR148W	10	200.46	0.64	YOL067C	7	376.87	0.14
YLR223C	3	23.73	0.67	YOL139C	9	445.12	0.28
YLR240W	4	53.93	0.33	YOR014W	11	258.80	0.22
YLR249W	9	978.59	0.08	YOR036W	4	0.33	0.83
YLR258W	6	159.56	0.27	YOR101W	7	218.16	0.38
YLR291C	9	913.37	0.06	YOR136W	3	76.99	0.33
YLR304C	1	0.00	0.00	YOR178C	3	3.46	0.67
YLR396C	9	167.03	0.67	YOR244W	22	1647.01	0.19
YLR403W	2	15.80	0.00	YOR358W	3	0.00	1.00
YMR028W	11	145.07	0.53	YOR361C	3	83.97	0.33
YMR037C	10	104.81	0.56	YOR372C	1	0.00	0.00
YMR068W	3	0.00	1.00	YPL045W	8	65.05	0.75
YMR131C	1	0.00	0.00	YPL048W	4	94.53	0.33
YMR146C	3	316.00	0.33	YPL081W	1	0.00	0.00
YMR231W	9	83.13	0.72	YPL093W	2	0.00	1.00
YMR239C	3	43.97	0.00	YPL111W	1	0.00	0.00
YNL006W	9	174.10	0.33	YPL152W	10	92.16	0.51
YNL037C	4	166.49	0.17	YPL180W	11	343.84	0.20
YNL039W	2	0.00	1.00	YPL203W	11	125.70	0.47
YNL068C	3	552.14	0.00	YPL207W	1	0.00	0.00
YNL076W	6	130.47	0.13	YPL211W	2	0.00	1.00
YNL098C	21	1195.46	0.19	YPL265W	4	192.29	0.50
YNL102W	5	204.52	0.10	YPR010C	7	309.54	0.19
YNL135C	5	84.73	0.20	YPR040W	10	199.06	0.22
YNL142W	3	20.70	0.33	YPR104C	7	269.83	0.14
YNL183C	3	15.70	0.00	YPR110C	14	1248.45	0.10
YNL189W	12	1529.99	0.08	YPR160W	4	59.97	0.33
YNL216W	4	65.67	0.33	YPR185W	2	0.00	1.00

Table B.5. Score table of more than three member modules

Module number	Module members						total-degree	in-degree	out-degree	score
24	TOR1	TOR2	GLN3	URE2			83	12	71	5.92
25	TOR1	TOR2	GLN3	LST8			77	12	65	5.42
27	TOR1	TOR2	TPD3	TAP42			74	12	62	5.17
28	TOR1	GAT1	URE2	GLN3			72	12	60	5.00
33	TOR1	GCN4	URE2	GLN3			71	12	59	4.92
43	BMH2	SIT4	SNF1	GLN3			70	12	58	4.83
49	YAK1	SCH9	TPK1	RAS2			70	12	58	4.83
35	ESA1	BMH2	BMH1	RTG2			68	12	56	4.67
30	TOR1	SRP1	CRM1	GLN3			67	12	55	4.58
32	TOR1	PMR1	GLN3	LST8			67	12	55	4.58
42	BMH2	BMH1	YAK1	TPK1			67	12	55	4.58
45	SIT4	RRD1	CLA4	URE2			67	12	55	4.58
41	BMH2	BMH1	RPD3	SNF1			66	12	54	4.50
46	SIT4	TOR2	URE2	GLN3			66	12	54	4.50
50	YAK1	TPK2	RAS2	TPK1			65	12	53	4.42
26	TOR1	TOR2	HXT1	SNQ2			64	12	52	4.33
60	TPK1	RAS2	GAT1	GLN3			62	12	50	4.17
29	TOR1	DAL80	URE2	GZF3			61	12	49	4.08
31	TOR1	KOG1	TCO89	LST8			61	12	49	4.08
37	ESA1	BMH2	GRR1	RPD3			61	12	49	4.08
36	ESA1	BMH2	GRR1	RTG2			60	12	48	4.00
48	YAK1	SCH9	MSN2	RAS2			60	12	48	4.00
44	BMH2	GAT1	TPK1	GLN3			58	12	46	3.83
53	SNF1	HSF1	SCH9	MSN2			56	12	44	3.67
51	YAK1	TPK2	RAS2	MSN2			55	12	43	3.58
54	SNF1	BMH1	RPD3	ADR1			55	12	43	3.58
52	SNF1	GIS1	SCH9	MSN2			54	12	42	3.50
13	ESA1	BMH2	BMH1	CLA4	RPD3		86	20	66	3.30
47	YAK1	SCH9	MSN2	HSF1			50	12	38	3.17
59	PPH21	TPD3	TAP42	TOR2			49	12	37	3.08
23	MSN4	RAS2	RIM15	SCH9	TPK1		79	20	59	2.95
55	GPA2	RAS2	RAS1	TPK2			46	12	34	2.83
20	BCY1	TPK1	RAS2	TPK3	RIM15		74	20	54	2.70
11	TOR1	VPS33	PEP5	VPS16	PEP3		72	20	52	2.60
19	BCY1	TPK1	RAS2	TPK2	RAS1		71	20	51	2.55
14	SIT4	RRD1	TAP42	PPH22	PPH3		69	20	49	2.45
15	SIT4	RRD1	TAP42	PPH22	RRD2		69	20	49	2.45
21	PPH3	PPH21	TAP42	PPH22	RRD1		61	20	41	2.05
18	HSF1	SCH9	MSN4	MSN2	RIM15		59	20	39	1.95
58	RPC34	RPC40	RPA135	RPA49			35	12	23	1.92
9	BCY1	TPK1	RAS2	TPK2	MSN4	RIM15	86	30	56	1.87
34	SAP155	SAP185	SAP4	TIP41			34	12	22	1.83
56	AVO2	TOR2	TSC11	LST8			34	12	22	1.83

Table B.5. Score table of more than three member modules (contiuned)

Module number	Module members						total-degree	in-degree	out-degree	score
17	TIP41	PPH3	TAP42	PPH22	PPH21		55	20	35	1.75
22	RRD2	TPD3	PPH22	PPH21	RTS1		55	20	35	1.75
39	PEP7	PEP3	VPS33	PEP12			32	12	20	1.67
7	GIS1	MSN4	MSN2	RAS2	RIM15	SCH9	78	30	48	1.60
1	ESA1	RAD27	VPS33	PEP5	PEP3	PEP7	77	30	47	1.57
2	ESA1	RAD27	VPS33	PEP5	PEP3	VPS3	75	30	45	1.50
12	SAP155	SAP185	SAP4	SIT4	SAP190		50	20	30	1.50
8	GIS1	MSN4	MSN2	RAS2	RIM15	TPK2	73	30	43	1.43
40	PEP7	PEP3	VPS45	PEP12			29	12	17	1.42
6	CDC55	TPD3	RRD2	TAP42	PPH22	PPH21	71	30	41	1.37
10	RRD2	TPD3	PPH22	PPH21	TAP42	RRD1	71	30	41	1.37
38	YGR054W	TIF1	TIF2	CDC33			28	12	16	1.33
3	ESA1	VPS16	PEP5	PEP3	VPS33	PEP7	67	30	37	1.23
4	ESA1	VPS16	PEP5	PEP3	VPS33	VPS3	65	30	35	1.17
5	ESA1	VPS16	PEP5	PEP3	VPS45	PEP7	64	30	34	1.13
16	TIF4632	TIF1	TIF2	TIF4631	CDC33		39	20	19	0.95
57	HAP5	HAP4	HAP2	HAP3			16	12	4	0.33

Table B.6. Gene ontology terms annotated to members of the modules

Module1				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006904 vesicle docking during exocytosis	3.46E-05	3/6 (50.0%)	13/6353 (0.2%)	PEP5 PEP3 PEP7
GO:0006895 Golgi to endosome transport	3.46E-05	3/6 (50.0%)	13/6353 (0.2%)	PEP5 VPS33 PEP3
GO:0006892 post-Golgi vesicle-mediated transport	5.79E-05	4/6 (66.7%)	72/6353 (1.1%)	PEP5 VPS33 PEP3 PEP7
GO:0006944 membrane fusion	7.22E-05	4/6 (66.7%)	76/6353 (1.2%)	PEP5 VPS33 PEP3 PEP7
GO:0007034 vacuolar transport	5.56E-04	4/6 (66.7%)	126/6353 (2.0%)	PEP5 VPS33 PEP3 PEP7
Module2				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0007033 vacuole organization	1.08E-05	4/6 (66.7%)	47/6353 (0.7%)	PEP5 VPS33 PEP3 VPS3
GO:0006895 Golgi to endosome transport	3.70E-05	3/6 (50.0%)	13/6353 (0.2%)	PEP5 VPS33 PEP3
GO:0042144 vacuole fusion, non-autophagic	4.70E-04	3/6 (50.0%)	29/6353 (0.5%)	PEP5 VPS33 PEP3
GO:0007034 vacuolar transport	5.95E-04	4/6 (66.7%)	126/6353 (2.0%)	PEP5 VPS33 PEP3 VPS3
GO:0045324 late endosome to vacuole transport	9.15E-04	3/6 (50.0%)	36/6353 (0.6%)	PEP5 VPS33 PEP3
Module3				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006895 Golgi to endosome transport	3.39E-08	4/6 (66.7%)	13/6353 (0.2%)	PEP5 VPS33 VPS16 PEP3
GO:0006892 post-Golgi vesicle-mediated transport	2.08E-07	5/6 (83.3%)	72/6353 (1.1%)	PEP5 VPS33 VPS16 PEP3 PEP7
GO:0006944 membrane fusion	2.74E-07	5/6 (83.3%)	76/6353 (1.2%)	PEP5 VPS33 VPS16 PEP3 PEP7
GO:0042144 vacuole fusion, non-autophagic	1.12E-06	4/6 (66.7%)	29/6353 (0.5%)	PEP5 VPS33 VPS16 PEP3
GO:0045324 late endosome to vacuole transport	2.78E-06	4/6 (66.7%)	36/6353 (0.6%)	PEP5 VPS33 VPS16 PEP3
Module4				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0007033 vacuole organization	2.40E-08	5/6 (83.3%)	47/6353 (0.7%)	PEP5 VPS33 VPS16 PEP3 VPS3
GO:0006895 Golgi to endosome transport	3.57E-08	4/6 (66.7%)	13/6353 (0.2%)	PEP5 VPS33 VPS16 PEP3
GO:0042144 vacuole fusion, non-autophagic	1.18E-06	4/6 (66.7%)	29/6353 (0.5%)	PEP5 VPS33 VPS16 PEP3
GO:0045324 late endosome to vacuole transport	2.92E-06	4/6 (66.7%)	36/6353 (0.6%)	PEP5 VPS33 VPS16 PEP3
GO:0007034 vacuolar transport	3.78E-06	5/6 (83.3%)	126/6353 (2.0%)	PEP5 VPS33 VPS16 PEP3 VPS3

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module5				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006892 post-Golgi vesicle-mediated transport	2.07E-07	5/6 (83.3%)	72/6353 (1.1%)	PEP5 VPS16 VPS45 PEP3 PEP7
GO:0006944 membrane fusion	2.73E-07	5/6 (83.3%)	76/6353 (1.2%)	PEP5 VPS16 VPS45 PEP3 PEP7
GO:0007034 vacuolar transport	3.58E-06	5/6 (83.3%)	126/6353 (2.0%)	PEP5 VPS16 VPS45 PEP3 PEP7
GO:0007033 vacuole organization	8.35E-06	4/6 (66.7%)	47/6353 (0.7%)	PEP5 VPS16 VPS45 PEP3
GO:0048193 Golgi vesicle transport	2.29E-05	5/6 (83.3%)	182/6353 (2.9%)	PEP5 VPS16 VPS45 PEP3 PEP7
Module6				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0007094 mitotic cell cycle spindle assembly checkpoint	1.81E-07	4/6 (66.7%)	20/6353 (0.3%)	CDC55 PPH22 PPH21 TPD3
GO:0045841 negative regulation of mitotic metaphase/anaphase transition	1.81E-07	4/6 (66.7%)	20/6353 (0.3%)	CDC55 PPH22 PPH21 TPD3
GO:0051784 negative regulation of nuclear division	3.31E-07	4/6 (66.7%)	23/6353 (0.4%)	CDC55 PPH22 PPH21 TPD3
GO:0045839 negative regulation of mitosis	3.31E-07	4/6 (66.7%)	23/6353 (0.4%)	CDC55 PPH22 PPH21 TPD3
GO:0030071 regulation of mitotic metaphase/anaphase transition	3.98E-07	4/6 (66.7%)	24/6353 (0.4%)	CDC55 PPH22 PPH21 TPD3
Module7				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001323 age-dependent general metabolic decline during chronological cell aging	9.15E-10	4/6 (66.7%)	6/6353 (0.1%)	MSN2 RIM15 SCH9 MSN4
GO:0001324 age-dependent response to oxidative stress during chronological cell aging	9.15E-10	4/6 (66.7%)	6/6353 (0.1%)	MSN2 RIM15 SCH9 MSN4
GO:0007571 age-dependent general metabolic decline	2.14E-09	4/6 (66.7%)	7/6353 (0.1%)	MSN2 RIM15 SCH9 MSN4
GO:0001306 age-dependent response to oxidative stress	2.14E-09	4/6 (66.7%)	7/6353 (0.1%)	MSN2 RIM15 SCH9 MSN4
GO:0007569 cell aging	1.14E-07	5/6 (83.3%)	61/6353 (1.0%)	MSN2 RIM15 SCH9 MSN4 RAS2
GO:0007568 aging	1.84E-07	5/6 (83.3%)	67/6353 (1.1%)	MSN2 RIM15 SCH9 MSN4 RAS2
Module8				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001323 age-dependent general metabolic decline during chronological cell aging	2.45E-06	3/6 (50.0%)	6/6353 (0.1%)	MSN2 RIM15 MSN4
GO:0001324 age-dependent response to oxidative stress during chronological cell aging	2.45E-06	3/6 (50.0%)	6/6353 (0.1%)	MSN2 RIM15 MSN4
GO:0007571 age-dependent general metabolic decline	4.29E-06	3/6 (50.0%)	7/6353 (0.1%)	MSN2 RIM15 MSN4
GO:0001306 age-dependent response to oxidative stress	4.29E-06	3/6 (50.0%)	7/6353 (0.1%)	MSN2 RIM15 MSN4
GO:0007569 cell aging	2.98E-05	4/6 (66.7%)	61/6353 (1.0%)	MSN2 RIM15 MSN4 RAS2
GO:0007568 aging	4.37E-05	4/6 (66.7%)	67/6353 (1.1%)	MSN2 RIM15 MSN4 RAS2

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module9				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0007265 Ras protein signal transduction	5.39E-06	4/6 (66.7%)	41/6353 (0.6%)	TPK2 BCY1 TPK1 RAS2
GO:0007264 small GTPase mediated signal transduction	5.14E-05	4/6 (66.7%)	71/6353 (1.1%)	TPK2 BCY1 TPK1 RAS2
GO:0007154 cell communication	2.83E-04	5/6 (83.3%)	293/6353 (4.6%)	TPK2 BCY1 TPK1 MSN4 RAS2
GO:0007242 intracellular signaling cascade	1.65E-03	4/6 (66.7%)	168/6353 (2.6%)	TPK2 BCY1 TPK1 RAS2
GO:0007165 signal transduction	6.60E-03	4/6 (66.7%)	238/6353 (3.7%)	TPK2 BCY1 TPK1 RAS2
Module10				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0007010 cytoskeleton organization	5.37E-05	5/6 (83.3%)	226/6353 (3.6%)	PPH22 PPH21 RRD2 RRD1 TPD3
GO:0007094 mitotic cell cycle spindle assembly checkpoint	8.97E-05	3/6 (50.0%)	20/6353 (0.3%)	PPH22 PPH21 TPD3
GO:0045841 negative regulation of mitotic metaphase/anaphase transition	8.97E-05	3/6 (50.0%)	20/6353 (0.3%)	PPH22 PPH21 TPD3
GO:0051784 negative regulation of nuclear division	1.39E-04	3/6 (50.0%)	23/6353 (0.4%)	PPH22 PPH21 TPD3
GO:0045839 negative regulation of mitosis	1.39E-04	3/6 (50.0%)	23/6353 (0.4%)	PPH22 PPH21 TPD3
GO:0030071 regulation of mitotic metaphase/anaphase transition	1.59E-04	3/6 (50.0%)	24/6353 (0.4%)	PPH22 PPH21 TPD3
GO:0000279 M phase	1.78E-04	5/6 (83.3%)	287/6353 (4.5%)	PPH22 PPH21 RRD2 RRD1 TPD3
Module11				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006895 Golgi to endosome transport	9.22E-09	4/5 (80.0%)	13/6353 (0.2%)	PEP5 VPS33 VPS16 PEP3
GO:0042144 vacuole fusion, non-autophagic	3.06E-07	4/5 (80.0%)	29/6353 (0.5%)	PEP5 VPS33 VPS16 PEP3
GO:0045324 late endosome to vacuole transport	7.57E-07	4/5 (80.0%)	36/6353 (0.6%)	PEP5 VPS33 VPS16 PEP3
GO:0007033 vacuole organization	2.29E-06	4/5 (80.0%)	47/6353 (0.7%)	PEP5 VPS33 VPS16 PEP3
GO:0016197 endosome transport	7.63E-06	4/5 (80.0%)	63/6353 (1.0%)	PEP5 VPS33 VPS16 PEP3
Module12				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0000082 G1/S transition of mitotic cell cycle	1.59E-09	5/5 (100.0%)	45/6353 (0.7%)	SAP185 SAP155 SAP4 SAP190 SIT4
GO:0051329 interphase of mitotic cell cycle	1.67E-07	5/5 (100.0%)	111/6353 (1.7%)	SAP185 SAP155 SAP4 SAP190 SIT4
GO:0051325 interphase	2.09E-07	5/5 (100.0%)	116/6353 (1.8%)	SAP185 SAP155 SAP4 SAP190 SIT4
GO:0000278 mitotic cell cycle	2.55E-05	5/5 (100.0%)	300/6353 (4.7%)	SAP185 SAP155 SAP4 SAP190 SIT4
GO:0002098 tRNA wobble uridine modification	5.28E-05	3/5 (60.0%)	24/6353 (0.4%)	SAP185 SAP190 SIT4

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module13				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0051436 negative regulation of ubiquitin-protein ligase activity during mitotic cell cycle	5.72E-04	2/5 (40.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051444 negative regulation of ubiquitin-protein ligase activity	5.72E-04	2/5 (40.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051352 negative regulation of ligase activity	5.72E-04	2/5 (40.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051726 regulation of cell cycle	8.69E-04	4/5 (80.0%)	167/6353 (2.6%)	BMH1 BMH2 ESA1 CLA4
GO:0034984 cellular response to DNA damage stimulus	2.62E-03	4/5 (80.0%)	220/6353 (3.5%)	BMH1 RPD3 BMH2 ESA1
Module14				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0000082 G1/S transition of mitotic cell cycle	6.22E-04	3/5 (60.0%)	45/6353 (0.7%)	PPH22 RRD1 SIT4
GO:0016311 dephosphorylation	8.58E-04	3/5 (60.0%)	50/6353 (0.8%)	PPH22 PPH3 SIT4
GO:0051329 interphase of mitotic cell cycle	9.57E-03	3/5 (60.0%)	111/6353 (1.7%)	PPH22 RRD1 SIT4
Module15				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0000082 G1/S transition of mitotic cell cycle	6.12E-04	3/5 (60.0%)	45/6353 (0.7%)	PPH22 RRD1 SIT4
GO:0007010 cytoskeleton organization	1.41E-03	4/5 (80.0%)	226/6353 (3.6%)	PPH22 RRD2 RRD1 SIT4
GO:0000278 mitotic cell cycle	4.37E-03	4/5 (80.0%)	300/6353 (4.7%)	PPH22 RRD2 RRD1 SIT4
GO:0051329 interphase of mitotic cell cycle	9.41E-03	3/5 (60.0%)	111/6353 (1.7%)	PPH22 RRD1 SIT4
Module16				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006413 translational initiation	2.81E-09	5/5 (100.0%)	50/6353 (0.8%)	TIF4631 TIF4632 TIF1 TIF2 CDC33
GO:0034063 stress granule assembly	5.64E-04	2/5 (40.0%)	5/6353 (0.1%)	TIF4631 TIF4632
Module17				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006470 protein amino acid dephosphorylation	1.28E-04	3/5 (60.0%)	30/6353 (0.5%)	PPH22 PPH21 PPH3
GO:0016311 dephosphorylation	6.17E-04	3/5 (60.0%)	50/6353 (0.8%)	PPH22 PPH21 PPH3

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module18				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001323 age-dependent general metabolic decline during chronological cell aging	1.97E-10	4/5 (80.0%)	6/6353 (0.1%)	MSN2 RIM15 SCH9 MSN4
GO:0001324 age-dependent response to oxidative stress during chronological cell aging	1.97E-10	4/5 (80.0%)	6/6353 (0.1%)	MSN2 RIM15 SCH9 MSN4
GO:0007571 age-dependent general metabolic decline	4.59E-10	4/5 (80.0%)	7/6353 (0.1%)	MSN2 RIM15 SCH9 MSN4
GO:0001306 age-dependent response to oxidative stress	4.59E-10	4/5 (80.0%)	7/6353 (0.1%)	MSN2 RIM15 SCH9 MSN4
GO:0001300 chronological cell aging	6.35E-08	4/5 (80.0%)	20/6353 (0.3%)	MSN2 RIM15 SCH9 MSN4
GO:0006950 response to stress	3.31E-03	5/5 (100.0%)	721/6353 (11.3%)	MSN2 RIM15 SCH9 MSN4 HSF1
Module19				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0007265 Ras protein signal transduction	1.76E-09	5/5 (100.0%)	41/6353 (0.6%)	TPK2 BCY1 RAS1 TPK1 RAS2
GO:0007264 small GTPase mediated signal transduction	3.05E-08	5/5 (100.0%)	71/6353 (1.1%)	TPK2 BCY1 RAS1 TPK1 RAS2
GO:0007242 intracellular signaling cascade	2.46E-06	5/5 (100.0%)	168/6353 (2.6%)	TPK2 BCY1 RAS1 TPK1 RAS2
GO:0007165 signal transduction	1.43E-05	5/5 (100.0%)	238/6353 (3.7%)	TPK2 BCY1 RAS1 TPK1 RAS2
GO:0007154 cell communication	4.08E-05	5/5 (100.0%)	293/6353 (4.6%)	TPK2 BCY1 RAS1 TPK1 RAS2
Module20				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0007265 Ras protein signal transduction	1.69E-06	4/5 (80.0%)	41/6353 (0.6%)	BCY1 TPK1 TPK3 RAS2
GO:0007264 small GTPase mediated signal transduction	1.61E-05	4/5 (80.0%)	71/6353 (1.1%)	BCY1 TPK1 TPK3 RAS2
GO:0007242 intracellular signaling cascade	5.25E-04	4/5 (80.0%)	168/6353 (2.6%)	BCY1 TPK1 TPK3 RAS2
GO:0007165 signal transduction	2.12E-03	4/5 (80.0%)	238/6353 (3.7%)	BCY1 TPK1 TPK3 RAS2
GO:0007154 cell communication	4.85E-03	4/5 (80.0%)	293/6353 (4.6%)	BCY1 TPK1 TPK3 RAS2
Module21				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006470 protein amino acid dephosphorylation	1.49E-04	3/5 (60.0%)	30/6353 (0.5%)	PPH22 PPH21 PPH3
GO:0000082 G1/S transition of mitotic cell cycle	5.20E-04	3/5 (60.0%)	45/6353 (0.7%)	PPH22 PPH21 RRD1
GO:0016311 dephosphorylation	7.17E-04	3/5 (60.0%)	50/6353 (0.8%)	PPH22 PPH21 PPH3
GO:0051329 interphase of mitotic cell cycle	8.00E-03	3/5 (60.0%)	111/6353 (1.7%)	PPH22 PPH21 RRD1
GO:0051325 interphase	9.13E-03	3/5 (60.0%)	116/6353 (1.8%)	PPH22 PPH21 RRD1

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module22				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006470 protein amino acid dephosphorylation	3.26E-07	4/5 (80.0%)	30/6353 (0.5%)	PPH22 PPH21 RTS1 TPD3
GO:0016311 dephosphorylation	2.74E-06	4/5 (80.0%)	50/6353 (0.8%)	PPH22 PPH21 RTS1 TPD3
GO:0000279 M phase	2.95E-05	5/5 (100.0%)	287/6353 (4.5%)	PPH22 PPH21 RRD2 RTS1 TPD3
GO:0007094 mitotic cell cycle spindle assembly checkpoint	4.31E-05	3/5 (60.0%)	20/6353 (0.3%)	PPH22 PPH21 TPD3
GO:0045841 negative regulation of mitotic metaphase/anaphase transition	4.31E-05	3/5 (60.0%)	20/6353 (0.3%)	PPH22 PPH21 TPD3
Module23				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001323 age-dependent general metabolic decline during chronological cell aging	1.15E-06	3/5 (60.0%)	6/6353 (0.1%)	RIM15 SCH9 MSN4
GO:0001324 age-dependent response to oxidative stress during chronological cell aging	1.15E-06	3/5 (60.0%)	6/6353 (0.1%)	RIM15 SCH9 MSN4
GO:0007571 age-dependent general metabolic decline	2.01E-06	3/5 (60.0%)	7/6353 (0.1%)	RIM15 SCH9 MSN4
GO:0001306 age-dependent response to oxidative stress	2.01E-06	3/5 (60.0%)	7/6353 (0.1%)	RIM15 SCH9 MSN4
GO:0007569 cell aging	9.36E-06	4/5 (80.0%)	61/6353 (1.0%)	RIM15 SCH9 MSN4 RAS2
Module24				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006808 regulation of nitrogen utilization	2.55E-03	2/4 (50.0%)	9/6353 (0.1%)	GLN3 URE2
GO:0001558 regulation of cell growth	3.90E-03	2/4 (50.0%)	11/6353 (0.2%)	TOR1 TOR2
GO:0051171 regulation of nitrogen compound metabolic process	3.90E-03	2/4 (50.0%)	11/6353 (0.2%)	GLN3 URE2
GO:0040008 regulation of growth	6.45E-03	2/4 (50.0%)	14/6353 (0.2%)	TOR1 TOR2
GO:0031929 TOR signaling pathway	8.50E-03	2/4 (50.0%)	16/6353 (0.3%)	TOR1 TOR2
Module25				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001558 regulation of cell growth	2.84E-06	3/4 (75.0%)	11/6353 (0.2%)	LST8 TOR1 TOR2
GO:0040008 regulation of growth	6.26E-06	3/4 (75.0%)	14/6353 (0.2%)	LST8 TOR1 TOR2
GO:0031929 TOR signaling pathway	9.63E-06	3/4 (75.0%)	16/6353 (0.3%)	LST8 TOR1 TOR2
GO:0031930 mitochondrial signaling pathway	8.20E-04	2/4 (50.0%)	6/6353 (0.1%)	LST8 TOR1
GO:0016049 cell growth	1.94E-03	3/4 (75.0%)	89/6353 (1.4%)	LST8 TOR1 TOR2

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module26				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001558 regulation of cell growth	2.81E-03	2/4 (50.0%)	11/6353 (0.2%)	TOR1 TOR2
GO:0040008 regulation of growth	4.64E-03	2/4 (50.0%)	14/6353 (0.2%)	TOR1 TOR2
GO:0031929 TOR signaling pathway	6.12E-03	2/4 (50.0%)	16/6353 (0.3%)	TOR1 TOR2
Module27				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0031929 TOR signaling pathway	1.06E-05	3/4 (75.0%)	16/6353 (0.3%)	TOR1 TAP42 TOR2
GO:0001558 regulation of cell growth	3.31E-03	2/4 (50.0%)	11/6353 (0.2%)	TOR1 TOR2
GO:0040008 regulation of growth	5.48E-03	2/4 (50.0%)	14/6353 (0.2%)	TOR1 TOR2
Module28				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006808 regulation of nitrogen utilization	1.87E-06	3/4 (75.0%)	9/6353 (0.1%)	GAT1 GLN3 URE2
GO:0051171 regulation of nitrogen compound metabolic process	3.67E-06	3/4 (75.0%)	11/6353 (0.2%)	GAT1 GLN3 URE2
GO:0019740 nitrogen utilization	1.82E-05	3/4 (75.0%)	18/6353 (0.3%)	GAT1 GLN3 URE2
Module29				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006808 regulation of nitrogen utilization	1.71E-06	3/4 (75.0%)	9/6353 (0.1%)	DAL80 URE2 GZF3
GO:0051171 regulation of nitrogen compound metabolic process	3.35E-06	3/4 (75.0%)	11/6353 (0.2%)	DAL80 URE2 GZF3
GO:0019740 nitrogen utilization	1.66E-05	3/4 (75.0%)	18/6353 (0.3%)	DAL80 URE2 GZF3
Module31				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001558 regulation of cell growth	7.54E-10	4/4 (100.0%)	11/6353 (0.2%)	LST8 TCO89 TOR1 KOG1
GO:0040008 regulation of growth	2.29E-09	4/4 (100.0%)	14/6353 (0.2%)	LST8 TCO89 TOR1 KOG1
GO:0031929 TOR signaling pathway	4.16E-09	4/4 (100.0%)	16/6353 (0.3%)	LST8 TCO89 TOR1 KOG1
GO:0016049 cell growth	5.58E-06	4/4 (100.0%)	89/6353 (1.4%)	LST8 TCO89 TOR1 KOG1
GO:0031505 fungal-type cell wall organization	1.40E-05	3/4 (75.0%)	19/6353 (0.3%)	LST8 TCO89 TOR1

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module32				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0031930 mitochondrial signaling pathway	1.01E-03	2/4 (50.0%)	6/6353 (0.1%)	LST8 TOR1
GO:0001558 regulation of cell growth	3.71E-03	2/4 (50.0%)	11/6353 (0.2%)	LST8 TOR1
GO:0040008 regulation of growth	6.13E-03	2/4 (50.0%)	14/6353 (0.2%)	LST8 TOR1
GO:0031929 TOR signaling pathway	8.08E-03	2/4 (50.0%)	16/6353 (0.3%)	LST8 TOR1
Module33				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006808 regulation of nitrogen utilization	2.78E-03	2/4 (50.0%)	9/6353 (0.1%)	GLN3 URE2
GO:0051171 regulation of nitrogen compound metabolic process	4.24E-03	2/4 (50.0%)	11/6353 (0.2%)	GLN3 URE2
Module34				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0000082 G1/S transition of mitotic cell cycle	8.20E-05	3/4 (75.0%)	45/6353 (0.7%)	SAP185 SAP155 SAP4
GO:0051329 interphase of mitotic cell cycle	1.27E-03	3/4 (75.0%)	111/6353 (1.7%)	SAP185 SAP155 SAP4
GO:0051325 interphase	1.45E-03	3/4 (75.0%)	116/6353 (1.8%)	SAP185 SAP155 SAP4
Module35				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0051436 negative regulation of ubiquitin-protein ligase activity during mitotic cell cycle	2.09E-04	2/4 (50.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051444 negative regulation of ubiquitin-protein ligase activity	2.09E-04	2/4 (50.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051352 negative regulation of ligase activity	2.09E-04	2/4 (50.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051439 regulation of ubiquitin-protein ligase activity during mitotic cell cycle	6.95E-04	2/4 (50.0%)	5/6353 (0.1%)	BMH1 BMH2
GO:0051438 regulation of ubiquitin-protein ligase activity	1.04E-03	2/4 (50.0%)	6/6353 (0.1%)	BMH1 BMH2
Module37				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006974 response to DNA damage stimulus	9.50E-04	4/4 (100.0%)	257/6353 (4.0%)	GRR1 RPD3 BMH2 ESA1
GO:0034401 establishment or maintenance of chromatin architecture during transcription	1.08E-03	2/4 (50.0%)	5/6353 (0.1%)	RPD3 ESA1
Module38				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006413 translational initiation	3.84E-07	4/4 (100.0%)	50/6353 (0.8%)	YGR054W TIF1 TIF2 CDC33

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module39				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006892 post-Golgi vesicle-mediated transport	1.47E-06	4/4 (100.0%)	72/6353 (1.1%)	VPS33 PEP12 PEP3 PEP7
GO:0006944 membrane fusion	1.84E-06	4/4 (100.0%)	76/6353 (1.2%)	VPS33 PEP12 PEP3 PEP7
GO:0007034 vacuolar transport	1.43E-05	4/4 (100.0%)	126/6353 (2.0%)	VPS33 PEP12 PEP3 PEP7
GO:0048193 Golgi vesicle transport	6.33E-05	4/4 (100.0%)	182/6353 (2.9%)	VPS33 PEP12 PEP3 PEP7
GO:0016044 membrane organization	2.46E-04	4/4 (100.0%)	255/6353 (4.0%)	VPS33 PEP12 PEP3 PEP7
Module40				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006892 post-Golgi vesicle-mediated transport	1.44E-06	4/4 (100.0%)	72/6353 (1.1%)	VPS45 PEP12 PEP3 PEP7
GO:0006944 membrane fusion	1.80E-06	4/4 (100.0%)	76/6353 (1.2%)	VPS45 PEP12 PEP3 PEP7
GO:0007034 vacuolar transport	1.40E-05	4/4 (100.0%)	126/6353 (2.0%)	VPS45 PEP12 PEP3 PEP7
GO:0006896 Golgi to vacuole transport	2.04E-05	3/4 (75.0%)	25/6353 (0.4%)	VPS45 PEP12 PEP7
GO:0048193 Golgi vesicle transport	6.20E-05	4/4 (100.0%)	182/6353 (2.9%)	VPS45 PEP12 PEP3 PEP7
Module41				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0051436 negative regulation of ubiquitin-protein ligase activity during mitotic cell cycle	3.20E-04	2/4 (50.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051444 negative regulation of ubiquitin-protein ligase activity	3.20E-04	2/4 (50.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051352 negative regulation of ligase activity	3.20E-04	2/4 (50.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051439 regulation of ubiquitin-protein ligase activity during mitotic cell cycle	1.07E-03	2/4 (50.0%)	5/6353 (0.1%)	BMH1 BMH2
GO:0007124 pseudohyphal growth	1.53E-03	3/4 (75.0%)	66/6353 (1.0%)	SNF1 BMH1 BMH2
Module42				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0051436 negative regulation of ubiquitin-protein ligase activity during mitotic cell cycle	1.34E-04	2/4 (50.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051444 negative regulation of ubiquitin-protein ligase activity	1.34E-04	2/4 (50.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0051352 negative regulation of ligase activity	1.34E-04	2/4 (50.0%)	3/6353 (0.0%)	BMH1 BMH2
GO:0007265 Ras protein signal transduction	1.49E-04	3/4 (75.0%)	41/6353 (0.6%)	BMH1 TPK1 BMH2
GO:0051439 regulation of ubiquitin-protein ligase activity during mitotic cell cycle	4.46E-04	2/4 (50.0%)	5/6353 (0.1%)	BMH1 BMH2

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module44				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006808 regulation of nitrogen utilization	2.28E-03	2/4 (50.0%)	9/6353 (0.1%)	GAT1 GLN3
GO:0051171 regulation of nitrogen compound metabolic process	3.48E-03	2/4 (50.0%)	11/6353 (0.2%)	GAT1 GLN3
GO:0019740 nitrogen utilization	9.66E-03	2/4 (50.0%)	18/6353 (0.3%)	GAT1 GLN3
Module46				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006808 regulation of nitrogen utilization	2.77E-03	2/4 (50.0%)	9/6353 (0.1%)	GLN3 URE2
GO:0051171 regulation of nitrogen compound metabolic process	4.23E-03	2/4 (50.0%)	11/6353 (0.2%)	GLN3 URE2
GO:0031929 TOR signaling pathway	9.22E-03	2/4 (50.0%)	16/6353 (0.3%)	TOR2 SIT4
Module47				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001323 age-dependent general metabolic decline during chronological cell aging	7.00E-04	2/4 (50.0%)	6/6353 (0.1%)	MSN2 SCH9
GO:0001324 age-dependent response to oxidative stress during chronological cell aging	7.00E-04	2/4 (50.0%)	6/6353 (0.1%)	MSN2 SCH9
GO:0007571 age-dependent general metabolic decline	9.79E-04	2/4 (50.0%)	7/6353 (0.1%)	MSN2 SCH9
GO:0043620 regulation of transcription in response to stress	9.79E-04	2/4 (50.0%)	7/6353 (0.1%)	MSN2 SCH9
GO:0043618 regulation of transcription from RNA polymerase II promoter in response to stress	9.79E-04	2/4 (50.0%)	7/6353 (0.1%)	MSN2 SCH9
Module48				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001302 replicative cell aging	3.10E-04	3/4 (75.0%)	46/6353 (0.7%)	MSN2 SCH9 RAS2
GO:0007569 cell aging	7.33E-04	3/4 (75.0%)	61/6353 (1.0%)	MSN2 SCH9 RAS2
GO:0007568 aging	9.75E-04	3/4 (75.0%)	67/6353 (1.1%)	MSN2 SCH9 RAS2
GO:0001323 age-dependent general metabolic decline during chronological cell aging	9.76E-04	2/4 (50.0%)	6/6353 (0.1%)	MSN2 SCH9
GO:0001324 age-dependent response to oxidative stress during chronological cell aging	9.76E-04	2/4 (50.0%)	6/6353 (0.1%)	MSN2 SCH9
Module49				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006468 protein amino acid phosphorylation	4.02E-03	3/4 (75.0%)	109/6353 (1.7%)	TPK1 SCH9 YAK1

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module50				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0007265 Ras protein signal transduction	1.72E-04	3/4 (75.0%)	41/6353 (0.6%)	TPK2 TPK1 RAS2
GO:0007264 small GTPase mediated signal transduction	9.18E-04	3/4 (75.0%)	71/6353 (1.1%)	TPK2 TPK1 RAS2
GO:0006468 protein amino acid phosphorylation	3.36E-03	3/4 (75.0%)	109/6353 (1.7%)	TPK2 TPK1 YAK1
Module52				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001302 replicative cell aging	3.65E-04	3/4 (75.0%)	46/6353 (0.7%)	MSN2 SNF1 SCH9
GO:0007569 cell aging	8.64E-04	3/4 (75.0%)	61/6353 (1.0%)	MSN2 SNF1 SCH9
GO:0007568 aging	1.15E-03	3/4 (75.0%)	67/6353 (1.1%)	MSN2 SNF1 SCH9
GO:0001323 age-dependent general metabolic decline during chronological cell aging	1.15E-03	2/4 (50.0%)	6/6353 (0.1%)	MSN2 SCH9
GO:0001324 age-dependent response to oxidative stress during chronological cell aging	1.15E-03	2/4 (50.0%)	6/6353 (0.1%)	MSN2 SCH9
Module53				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0001302 replicative cell aging	3.25E-04	3/4 (75.0%)	46/6353 (0.7%)	MSN2 SNF1 SCH9
GO:0007569 cell aging	7.70E-04	3/4 (75.0%)	61/6353 (1.0%)	MSN2 SNF1 SCH9
GO:0007568 aging	1.02E-03	3/4 (75.0%)	67/6353 (1.1%)	MSN2 SNF1 SCH9
GO:0001323 age-dependent general metabolic decline during chronological cell aging	1.03E-03	2/4 (50.0%)	6/6353 (0.1%)	MSN2 SCH9
GO:0001324 age-dependent response to oxidative stress during chronological cell aging	1.03E-03	2/4 (50.0%)	6/6353 (0.1%)	MSN2 SCH9
Module55				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0051349 positive regulation of lyase activity	1.81E-08	3/4 (75.0%)	3/6353 (0.0%)	GPA2 RAS1 RAS2
GO:0045762 positive regulation of adenylate cyclase activity	1.81E-08	3/4 (75.0%)	3/6353 (0.0%)	GPA2 RAS1 RAS2
GO:0031281 positive regulation of cyclase activity	1.81E-08	3/4 (75.0%)	3/6353 (0.0%)	GPA2 RAS1 RAS2
GO:0007190 activation of adenylate cyclase activity	1.81E-08	3/4 (75.0%)	3/6353 (0.0%)	GPA2 RAS1 RAS2
GO:0051339 regulation of lyase activity	7.23E-08	3/4 (75.0%)	4/6353 (0.1%)	GPA2 RAS1 RAS2
Module56				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0030950 establishment or maintenance of actin cytoskeleton polarity	4.52E-10	4/4 (100.0%)	10/6353 (0.2%)	LST8 AVO2 TSC11 TOR2
GO:0030952 establishment or maintenance of cytoskeleton polarity	4.52E-10	4/4 (100.0%)	10/6353 (0.2%)	LST8 AVO2 TSC11 TOR2
GO:0001558 regulation of cell growth	7.11E-10	4/4 (100.0%)	11/6353 (0.2%)	LST8 AVO2 TSC11 TOR2
GO:0040008 regulation of growth	2.16E-09	4/4 (100.0%)	14/6353 (0.2%)	LST8 AVO2 TSC11 TOR2
GO:0031929 TOR signaling pathway	3.92E-09	4/4 (100.0%)	16/6353 (0.3%)	LST8 AVO2 TSC11 TOR2

Table B.6. Gene ontology terms annotated to members of the modules (continued)

Module57				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006109 regulation of carbohydrate metabolic process	5.48E-08	4/4 (100.0%)	35/6353 (0.6%)	HAP5 HAP2 HAP3 HAP4
GO:0005975 carbohydrate metabolic process	3.42E-04	4/4 (100.0%)	299/6353 (4.7%)	HAP5 HAP2 HAP3 HAP4
GO:0006350 transcription	7.86E-03	4/4 (100.0%)	653/6353 (10.3%)	HAP5 HAP2 HAP3 HAP4
Module58				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006360 transcription from RNA polymerase I promoter	7.30E-04	3/4 (75.0%)	90/6353 (1.4%)	RPC40 RPA135 RPA49
GO:0006351 transcription, DNA-dependent	5.76E-03	4/4 (100.0%)	613/6353 (9.6%)	RPC40 RPA135 RPC34 RPA49
GO:0032774 RNA biosynthetic process	5.83E-03	4/4 (100.0%)	615/6353 (9.7%)	RPC40 RPA135 RPC34 RPA49
GO:0006350 transcription	7.42E-03	4/4 (100.0%)	653/6353 (10.3%)	RPC40 RPA135 RPC34 RPA49
Module59				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0007015 actin filament organization	7.01E-04	3/4 (75.0%)	64/6353 (1.0%)	PPH21 TOR2 TPD3
GO:0030036 actin cytoskeleton organization	3.05E-03	3/4 (75.0%)	104/6353 (1.6%)	PPH21 TOR2 TPD3
GO:0030029 actin filament-based process	3.71E-03	3/4 (75.0%)	111/6353 (1.7%)	PPH21 TOR2 TPD3
GO:0031929 TOR signaling pathway	6.44E-03	2/4 (50.0%)	16/6353 (0.3%)	TAP42 TOR2
GO:0040007 growth	9.55E-03	3/4 (75.0%)	152/6353 (2.4%)	PPH21 TOR2 TPD3
Module60				
GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0006808 regulation of nitrogen utilization	2.24E-03	2/4 (50.0%)	9/6353 (0.1%)	GAT1 GLN3
GO:0051171 regulation of nitrogen compound metabolic process	3.43E-03	2/4 (50.0%)	11/6353 (0.2%)	GAT1 GLN3
GO:0045941 positive regulation of transcription	7.59E-03	3/4 (75.0%)	134/6353 (2.1%)	GAT1 GLN3 RAS2
GO:0010628 positive regulation of gene expression	7.76E-03	3/4 (75.0%)	135/6353 (2.1%)	GAT1 GLN3 RAS2
GO:0045935 positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	9.04E-03	3/4 (75.0%)	142/6353 (2.2%)	GAT1 GLN3 RAS2

APPENDIX C: CODE OF FLUX BALANCE ANALYSIS PROGRAMME

Code of the flux balance analysis programme is included in this section.

Code of the programme is,

```
clear all
load stoich_sparse822_1172
load gene
load metname
load revirrev
extmet = textread('extmet.txt','%s','delimiter',',');
 for i = 1:length(extmet)
    extmet2{i} = [extmet{i} 'xt'];
 end
[metnum rxnnum] = size(stoich_sparse);
for i = 1:length(extmet2) % secreted
   a= strmatch(extmet2{i},metname,'exact');
   rxnnum = rxnnum + 1;
   stoich_sparse(a,rxnnum) = -1;
   lb(rxnnum) = 0; ub(rxnnum) = 1000;
end
upmet = {'SLF' 'ZYMST' 'ERGOST' 'O2' 'NH3' 'PI' 'GLC'};
upmet2 = {'SLFxt' 'ZYMSTxt' 'ERGOSTxt' 'O2xt' 'NH3xt' 'PIxt' 'GLCxt'};
for i = 1:length(upmet2)
   a= strmatch(upmet2{i},metname,'exact');
   rxnnum = rxnnum + 1;
   stoich_sparse(a,rxnnum) = -1;
   lb(rxnnum) = -1000; ub(rxnnum) = 0;
   end
revirrev = cellstr(revirrev');
for i = 1:length(revirrev)
```

```
    if length(strfind(revirrev{i},'I') ) ~= 0
        lb(i) = 0; ub(i) = 1000;
    else
        lb(i) = -1000; ub(i) = 1000;
    end
end
allrxnname = [gene; {'Growth'}; extmet2'; upmet2'];
 lb(1125) = -1000; % JEN1- to allow PYR secretion
 ub(1125) = 0;
f = zeros(1,length(allrxnname));
b = zeros(metnum,1);
% meas = [GLC  BIOM   GOH   ETC]
Rm = [-5.5014 0.2053  0.1715 -2.3609];
% metaboite units:mmole/gDW/hr - biomass unit:g/gDW/hr%
lb(strmatch('GLCxt',allrxnname,'exact')) = Rm(1);
ub(strmatch('GLCxt',allrxnname,'exact')) = Rm(1);
lb(strmatch('Growth',allrxnname,'exact')) = Rm(2);
ub(strmatch('Growth',allrxnname,'exact')) = Rm(2);
lb(strmatch('ETHxt',allrxnname,'exact')) = Rm(4);
ub(strmatch('ETHxt',allrxnname,'exact')) = Rm(4);
lb(strmatch('GLxt',allrxnname,'exact')) = Rm(3);
ub(strmatch('GLxt',allrxnname,'exact')) = Rm(3);
lb(strmatch('ZYMSTxt',allrxnname,'exact')) = 0;
ub(strmatch('ZYMSTxt',allrxnname,'exact')) = 0;
lb(strmatch('ERGOSTxt',allrxnname,'exact')) = 0;
ub(strmatch('ERGOSTxt',allrxnname,'exact')) = 0;
 lb(strmatch('Growth',allrxnname,'exact')) = 0;
 ub(strmatch('Growth',allrxnname,'exact')) = 100;
lb(strmatch('PIxt',allrxnname,'exact')) = -1000;
ub(strmatch('PIxt',allrxnname,'exact')) = 1000;
lb(strmatch('U214_',allrxnname,'exact')) = 1;
ub(strmatch('U214_',allrxnname,'exact')) = 1;
  e = zeros(length(allrxnname),1);
```

```
[X, objfun] = linprog(f,[],[],stoich_sparse,b,lb',ub'); %MOSEK- TOMLAB OR MATLAB
[Xcp,slack,vv,rc,f_k,ninf, sinf, Inform] = ...
    cplex(f,stoich_sparse,lb,ub,b,[],cpxControl); % TOMLAB/CPLEX
Inform
display('------------'); display('FBA Solution'); display('------------');
sim_out(Xcp,allrxnname);
return
```

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