Chemical-genetics profile and genetic interaction analyses for identification of novel genes involved in protein synthesis in yeast

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by

Md Alamgir

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Einstein, Albert (1879-1955)

ABSTRACT

Chemical-genetics profile and genetic interaction analyses for identification of novel genes involved in protein synthesis in yeast

As a major step in the gene expression pathway, protein synthesis, also known as translation plays an indispensable role in the survival of a cell. Much has been discovered about protein synthesis over the last few decades. However the list of novel factors that affect this process continues to grow, indicating the presence of other elements that are associated with protein synthesis. This thesis aimed to identify novel genes that affect the process of protein synthesis. For this purpose we employed large-scale screening methods developed for genetic studies in the yeast, *Saccharomyces cerevisiae*. Specifically, we used increased sensitivity to translation drugs (paromomycin, cycloheximide, 3-amino-1, 2, 4 triazole, streptomycin, neomycin) as a method to screen the entire set of yeast non-essential gene knockout collection (~4700 strains). The chemical-genetics profiles identified in this manner were used to identify genes with possible links to protein synthesis. We further studied the activity of four genes that here we termed *TAE1*-4 for translation associated elements 1-4, respectively; using various genetic assays including synthetic genetic interaction and phenotypic suppression analyses.

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LIST OF ABBREVIATIONS

µCi/mL	Micro-curies per milliliters
µg	Micro-Grams
µL	Micro-Liters
^{0}C	Degrees Celsius
3-AT	3-Amino-1, 2, 4 Triazole
ATP	Adenosine Triphosphate
cDNA	Complementary DNA
CS	Colony Size
CSR	Colony Size Reduction
CW	Calcofluor White
dATP	Deoxy-Adenine Triphosphate
dCTP	Deoxy-Cytosine Triphosphate
dGTP	Deoxy-Guanine Triphosphate
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
dNTP	Deoxy-Nucleoside Triphosphate
dTTP	Deoxy-Thymine Triphosphate
EDTA	Ethylene Diamine Tetra Acetic Acid
eEF	Eukaryotic Elongation Factor
eIF	Eukaryotic Initiation Factor

GD	Growth Detector
g	Gram
HIP	Haploinsufficency Profiling
hr	Hour
HTT	High Throughput Techniques
HU	Hydroxy Urea
MAT	Mating-type Locus
MDR	Multi Drug Resistance
mg	Milligrams
MIC	Minimum Inhibitory Concentration
min	Minute
MIPS	Munich Information Centre for Protein Sequences
mL	Milli-Liters
mM	Milli-Molar
MOA	Mode of Activity
mRNA	Messenger Ribonucleic acid
NaOH	Sodium Hydroxide
OD	Optical Density
ONPG	O-Nitro-Phenyl-β-D-Galactosidase
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
Q-RT-PCR	Quantitative RT-PCR

RF	Release Factor
RNA	Ribonucleic Acid
RPM	Revolutions Per Minute
RT	Room Temperature
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SC Medium	Synthetic Complete Medium
SDS	Sodium Dodecyl Sulfate
SGA	Synthetic Genetic Array
SGD	Saccharomyces Genome Database
ST	Spot Test
TAE	Translation Associate Element
TAP	Tandem Affinity Purification
TOR	Target of Rapamycin
UTR	Untranslated Region
WT	Wild Type
yGDA	Yeast Non-essential Gene Deletion Array
YPD Medium	Yeast Peptone Dextrose Medium

This thesis is dedicated to my beloved father, mother and brother,
in honor of their love

1.0 INTRODUCTION

1.1 Systems Molecular Biology

For more than a century, researchers have been accumulating genetic and biochemical data using different model organisms such as *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans* (Castrillo and Oliver, 2004) to develop a better understanding of cellular physiology and processes, and to model complex human diseases. In classical genetic studies, researchers often focus on a single gene, a gene family or a cellular pathway. And by combining such isolated data points, they try to better understand the biology of a cell/organism as a whole. This approach is resource consuming and often does not accurately represent the biology of the system, mainly because it lacks a comprehensive coverage of the global interactions between cellular components. This limitation has lead to the development of new techniques that enable researchers to study a number of molecules, genes, proteins and pathways at a time. Consequently a growing number of high throughout techniques are being developed to address this need (Hillenmeyer *et al.* 2008; Parsons *et al.* 2004; Tong *et al.* 2001; Ito *et al.* 2001; Rigaut *et al.* 1999).

For the past 15 years, the increase in the availability of genome-wide nucleotide sequence for various model organisms has significantly impacted the field of experimental biology. During this period, various large-scale analyses coupled with improved high-throughput techniques have been developed and applied to study complex cellular pathways and processes at a systems level. These methods and strategies mainly focus on the elucidation of new genes, their functions, and regulatory networks for different cellular

phenotypes at the levels of transcriptome, proteome and metabolome (Oliver *et al.* 2002). In the context of large-scale studies, the yeast *S. cerevisiae* emerged as a key organism for studying eukaryotic cells at a systems level. For long, *S. cerevisiae* has been the target of various large-scale experimentations including genome sequencing (Goffeau *et al.* 1996), expression profiling (Ghaemmaghami *et al.* 2003) and interaction mapping (Costanzo *et al.* 2010; Krogan *et al.* 2006). A list of available yeast resources derived from such large-scale studies are presented in Table 1-1.

The systematic study of complex interactions and cellular processes at the molecular and genetics level is often referred as systems molecular biology. It represents an integrated approach of genetic, molecular and functional genomics that explains functional relationships between genes and cellular molecules, from a sub-cellular organelles level to the organism as a whole. For the most part, systems molecular biology is carried out by an interdisciplinary team of molecular and computational biologists and provides insight into how a cell and an organism function as an integrated system of molecules. It focuses not only on the importance of a global and integrative view of modern biological research, but also in the development of novel and improved approaches to study the complexity of cells (Pitre *et al.* 2008; Sopko *et al.* 2006; Aderem, 2005). One goal of systems molecular biology is to develop testable hypothesis to better understand the network(s) of life (Castrillo and Oliver, 2004) (Figure 1-1). Some computational investigations in systems molecular biology aim to predict interaction networks within a system. Others focus on integrating the available data obtained from different high

throughput techniques (HTT), to computationally simulate the system and to make further predictions.

1.1.1 Functional Genomics

The term functional genomics emerged around 1997 to describe investigations which focused on the understanding of gene(s) and its function(s) (Hieter and Boguski, 1997). Today it also covers comprehensive genome-wide approaches and analytical tools to identify and characterize novel genes, to study the control of gene expressions at molecular level and often to identify drug targets and their mode of activity (MOA) (Batova *et al.* 2010; Parsons *et al.* 2006). Other studies under the same umbrella aim to understand and compare the characteristics of coding and non-coding regions of genomes.

In recent years, the emergence of a number of high-throughput techniques such as RNAi-based approaches (Tischler *et al.* 2008), cell-based assays using small inhibitory RNA (siRNA) libraries or small hairpin RNA (shRNA) expression vectors ((Moffat and Sabatini, 2006) and DNA microarrays (Moffat and Sabatini, 2006), protein arrays (Ruffner *et al.* 2007), etc, have made a significant contribution to the advancement of this field. These techniques attempted to correlate data from genomics and proteomics to reveal the function of novel genes, identify drug targets and determine bioactive compounds' MOA. Novel MOAs can be predicted for compounds and natural products with interesting antimicrobial activities (Freiberg and Brötz-Oesterhelt, 2005). Similarly, bioactive compounds with known MOAs have been recently used in genome wide

chemical-genetic profile analyses to identify novel gene functions in different organisms (Pukkila-Worley *et al.* 2009; Hoon *et al.* 2008).

One challenge for these high-throughput techniques is the analysis and validation of the complex data derived from such large-scale studies. This makes their interpretation and analyses a difficult task for molecular biologists (Venancio *et al.* 2010; Urbanczyk-Wochniak *et al.* 2003; Oliver *et al.* 2002; Phelps *et al.* 2002; Delneri *et al.* 2001). Certain computational approaches have been developed to help and interpret such complicated data sets with high occurrence of false-positive/negative data points (Dumontier *et al.* 2008)

1.1.2 Yeast and Functional Genomics

In the post genomic era, the use of the budding yeast *S. cerevisiae* has emerged as an important tool for large-scale functional genomics studies. Traditionally, *S. cerevisiae* has been the first eukaryotic organism for which novel system-wide molecular and genetic methodologies were described (Suter *et al.* 2006). Genome-wide methods such as transcriptional array studies (DeRisi *et al.* 1997), mass-spectrometry analysis of protein–protein interactions (Gavin *et al.* 2002; Ho *et al.* 2002), two-hybrid analysis of protein–protein interactions (Uetz *et al.* 2000; Ito *et al.* 2001), protein arrays (Zhu *et al.* 2001), synthetic lethality screens (Tong *et al.* 2001) and chemical genetic analysis (Hillenmeyer *et al.* 2008; Alamgir *et al.* 2008) are first validated in yeast, using well-controlled reproducible conditions. Today in addition to yeast, many other organisms such as fly and worm to complex systems such as mouse and human are used to elucidate genetic information. However, yeast continues to offer a cost and time effective way to evaluate

the function of genes. The vast knowledge gathered for the past 13 years from the completely sequenced genome of yeast combined with the availability of a number of high-throughput techniques (Tong *et al.* 2001; Ito *et al.* 2001, Rigaut *et al.* 1999), the set of yeast deletion strains (Winzeler *et al.* 1999; Giaever *et al.* 2002; Suter *et al.* 2006), the overexpression array for yeast genes (Sopko *et al.* 2006), etc are rapidly expanding the range of applications for functional genomics. These techniques help explain the regulatory mechanism of genes, proteins and metabolites, networks, and pathways with respect to cell function and behaviour. To date, the collection and combination of these large-scale functional genomics data in yeast has offered fundamental insights into the complexity of biological networks and pathways (Jessulat *et al.* 2008; Lesage *et al.* 2004).

Evolutionary analysis of related organisms can provide valuable information about the conserved and non-conserved sequences of the genome and its structures and evolution (Glaser and Boone, 2004). These comparative studies may lead to the development and understanding of proteins and their functional regulation in different organisms. In this context, yeast orthologs have been identified for various human genes involved in different genetic disorders including neurodegenerative diseases such as Parkinson's (PKD) (Outeiro and Giorgini, 2006), and Alzheimer diseases (Bagriantsev and Liebman, 2006) and in ageing (Petranovic and Neilsen, 2008). Though the molecular mechanisms between yeast and higher eukaryotes have significant differences, experimental evidence suggests that certain fundamental processes are conserved between these organisms (Boube *et al.* 2002). This has lead to the use of yeast genetics as a functional platform

for studying human genes, pathways and conditions (Steinmetz *et al.* 2002) as well as for high-throughput drug discovery (Neef *et al.* 2010; Hoon *et al.* 2008; Sturgeon *et al.* 2006).

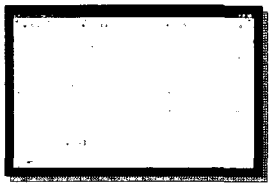
Despite the availability of a variety of large-scale methodologies developed for yeast genetics, there remains a demand for improved and more reliable methodologies as well as new algorithms that can help interpret the results.

Table 1-1: List of yeast resources.

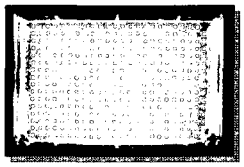
	Web addresses
Saccharomyces Genome Database (SGD)	http://www.yeastgenome.org
Yeast Proteome Database (YPD)	http://www.proteome.com/
Munich Information Centre for Protein Sequences (MIPS)	http://mips.gsf.de/genre/proj/yeast/
Triples database	http://ygac.med.yale.edu
Yeast deletion project and mitochondrial proteomics database	http://www-deletion.stanford.edu/YDPM/YDPM_index.html
Microarray data sets	http://web.wi.mit.edu/young/pub/regulation.html
Yeast protein function assignment	http://www.doe-mbi.ucla.edu
European *S. cerevisiae* archive for functional analysis (EUROSCARF)	http://www.uni-frankfurt.de/fb15/mikro/euroscarf/complete.html
Stanford Microarray Database	http://genome-www5.stanford.edu
Yeast transcription factors and related components (YTF)	http://biochemie.web.med.uni-muenchen.de/YTFD/
Yeast protein function assignment	http://www.doe-mbi.ucla.edu
Yeast Resource Centre (YRC)	http://depts.washington.edu/yeastrc/
ArrayExpress	http://www.ebi.ac.uk/arrayexpress
GeneOntology; FatiGO	http://www.geneontology.org
Yeast GFP fusion localization database	http://yeastgfp.ucsf.edu/
Yeast Protein Localization Database	http://ypl.uni-graz.at/
Yeast GRID	http://biodata.mshri.on.ca/yeast_grid/
Saccharomyces cerevisiae Morphology Database SCMD	http://yeast.gi.k.u-tokyo.ac.jp/
PROPHECY	http://prophecy.lundberg.gu.se/

Figure 1-1: An overview of system molecular biology.

System molecular biology mediates the development of hypotheses using novel approaches and by integrating frameworks of pathways, interactions and networks.



Data Generation
and Analysis



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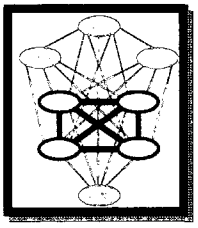
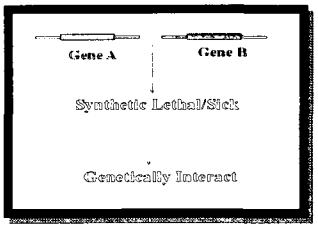
Techniques

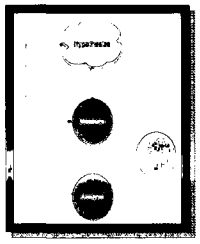


Computer Simulation
for predictions

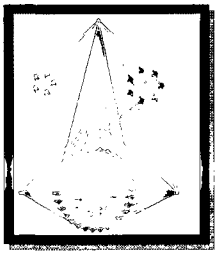
Global Analysis



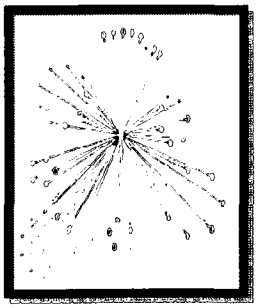
Genetic interaction
analysis



Development of
Hypothesis



Optimization and
development of
visualization model



Network of life

1.2 Yeast

Yeasts are single celled fungi of the phylum *Ascomycetes*, class *Saccharomycetes*. *S. cerevisiae*, commonly referred to as bakers yeast, or simply "yeast", has been widely used as a model organism to understand the biology of a cell/organism at the molecular and physiological levels and to elucidate the cellular processes and pathways.

The colony morphologies of yeasts are diverse. Yeast cells can vary in size from 3 to 40 μm. Yeasts can multiply as single cells that divide by budding (e.g. *Saccharomyces*) or fission (e.g. *Schizosaccharomyces*), or they may grow as simple irregular filaments (mycelium). Yeast is a simple and genetically well defined organism that can be easily manipulated. It has a versatile DNA transformation system, linear and circular plasmids with high and low copy numbers and regulated promoters. This allows researchers to readily manipulate the genes and genetics of yeast. *S. cerevisiae* was the first eukaryotic organism whose genome was sequenced by the international consortium (Goffeau *et al.* 1996; Dujon, 1996). This was one of the cornerstones of modern molecular biology and genomics research in 20^{th} century.

Yeast has 16 well characterized chromosomes ranging from 200-2200 kb containing a total of approximately 6200 open reading frames (ORF) (Luesch *et al.* 2005). Approximately 20% (~1100) of these genes are essential (Stelzl and Wanker, 2006). Of the 6200 ORFs, ~1000 genes are considered functionally characterized and 20-30% genes

are considered uncharacterized; the rest are partially characterized (Peña-Castillo and Hughes, 2007; Ashburner *et al.* 2000). Relative to other eukaryotes, *S. cerevisiae* has a compact genome. It has very short non-coding regions and ~70% of its total (non-ribosomal DNA) genetic material is protein-coding sequence.

The development of the yeast gene deletion array (Suter *et al.* 2006; Giaever *et al.* 2002; Winzeler *et al.* 1999) was another major breakthrough in biological research. In this array, each of the ORFs was systematically deleted and replaced with *kanMX4,* a kanamycin resistance cassette (Figure 1-2). Today four different types of yeast deletion sets are available consisting ~21000 strains: a) *MATa* and *MATα* heterozygous diploid; b) *MATα* haploid; c) *MATa* haploid; and d) *MATa* and *MATα* homozygous diploid (Hoon *et al.* 2008). These arrays are used in a wide range of analyses including functional profiling of novel genes, chemical sensitivity, synthetic lethality, genetic interactions, etc. To date, many novel and fundamental genetic interactions have been identified using the *S. cerevisiae* gene deletion array. These interactions have provided important functional information about higher eukaryotic organisms such as human (Lehner, 2007), fly (Giot *et al.* 2003), and worm (Tischler *et al.* 2008). Similarly the yeast gene overexpression array has opened another era of functional genomics to determine functional pathways and processes and to identify new drug targets within a cell (Boone *et al.* 2007).

After the sequencing of the human genome, it was observed that approximately 30% of the yeast genome shares homology to human genes (Suter *et al.* 2006) and nearly 50% of genes related to human genetic disorders have yeast orthologs (Hartwell, 2004). They

also share common biochemical pathways at the molecular level, as well as various protein complex networks and signaling pathways. The presence of such common features between human and yeast further highlights the importance of studying yeast as a model organism for studying human diseases.

1.3 Genetic and Protein-Protein Interactions (PPIs)

In biological systems, characterizing the details of biochemical pathways and elucidating the multifaceted interactions among them are an important and challenging task. This task is further complicated by the presence of transient interactions that take place under unique physiological conditions. To certain an extent, however, with the advent of improved large-scale techniques, presently it is possible to partially analyze and investigate the fundamentals of certain pathways using protein-protein and genetic interaction analyses. Investigating protein-protein and genetic interactions is a powerful approach to study the relationship between genes and/or proteins, various pathways and processes (Figure 1-3). A gene can produce more than one protein in different cell types within an organism, at various times and in response to developmental and/or environmental stimuli. Similarly different proteins can express disparate functions in various biological contexts and in association with different interacting and non-interacting partners. In the post-genomic era, one of the major emerging challenges is to define and develop the functional relationship of genes and characterisation of their products in relations to each other.

Figure 1-2: Schematic representation for construction of gene deletion in yeast.

Each of the ORFs was systematically deleted and replaced with *KanMX4* in a *S. cerevisiae* background. The cassette consists of Kanamycin-resistance gene and two flanked regions, uptag and downtag. The 5' and 3' of the yeast DNA is homologous to the flanking region of the yeast ORF that is to be deleted. After homologous recombination, the ORF is replaced with *KanMX4*. The presence of a KanMX4 gene in each of these strains was confirmed by plating onto both YEPD and YEPD with geneticin plates as well as by PCR.

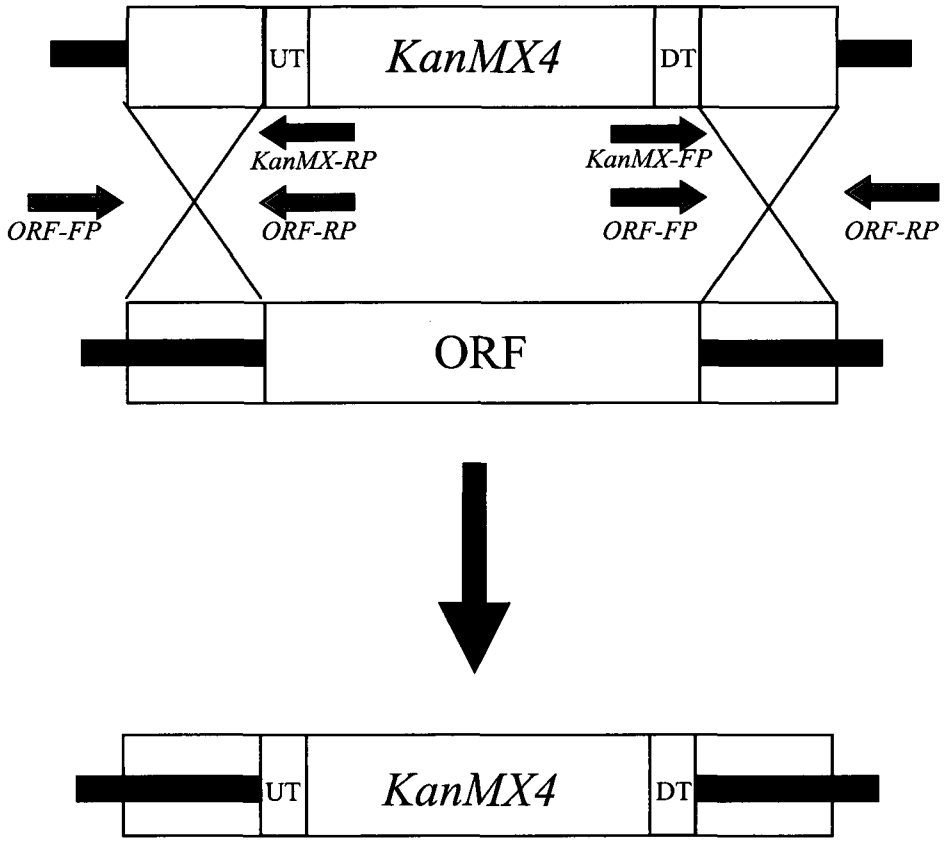
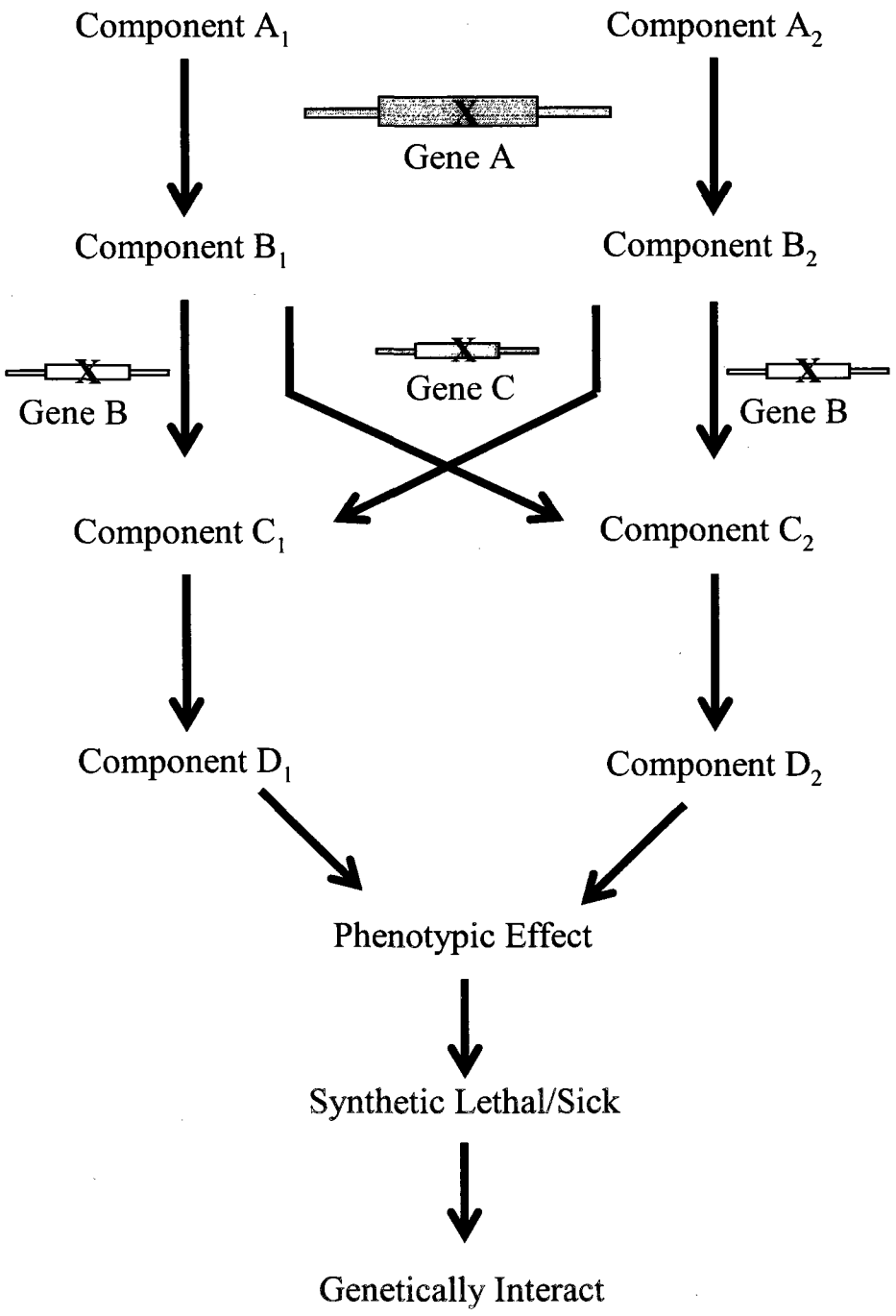

UT
KanMX4
DT
KanMX-RP
KanMX-FP
ORF-FP
ORF-RP
ORF-FP
ORF-RP
ORF
UT
KanMX4
DT

Figure 1-3: Genetic interactions of non-essential genes.

Two biochemical pathways convert Compound A_1 to D_1 (left) and Compound A_2 to D_2 (right) mediated by Gene A, Gene B and Gene C. Mutation in Gene A will affect both pathways and cause a phenotypic effect. On the other hand, mutation in either Gene B or Gene C alone will not have any phenotypic effect, because Gene B and Gene C are involved in the redundant/parallel pathway. Gene C will show phenotypic effect only when Gene B are mutated. So, Gene C will genetically interact with Gene B, but not with Gene A.


Component A_1
Component A_2
Gene A
Component B_1
Component B_2
Gene B
Gene C
Gene B
Component C_1
Component C_2
Component D_1
Component D_2
Phenotypic Effect
Synthetic Lethal/Sick
Genetically Interact

Yeast two **H**ybrid (Y2H) (Fields and Song, 1989) and **T**andem **A**ffinity **P**urification (TAP) coupled with mass spectrometry (Rigaut *et al.* 1999) are two established approaches to identify genome-wide **P**rotein-**P**rotein **I**nteractions (PPIs). Application of these methodologies has generated thousands of functional association between proteins in various cells (Stelzl and Wanker, 2006). These assays provide a general platform for analyzing protein functions, finding novel partners and discovering novel protein complexes.

Briefly, Y2H relies on transcriptional activator proteins. These proteins contain two functional domains: a) a DNA **B**inding **D**omain (BD) that binds at the **U**pstream **A**ctivation **S**equence (UAS) and b) a transcriptional **A**ctivator **D**omain (AD) which is responsible for recruiting the transcription machinery. In Y2H, BD (Bait) and AD (Prey) regions with lost affinity for each other are fused with protein of interest to form BD-X and AD-Y hybrid proteins, respectively. Initially, Fields and Song (1989) used the BD and AD of the GAL4 transcription factor, and the *lacZ* reporter gene that encodes β-galactosidase. After the formation of a physical complex UAS-BD-AD, the *LacZ* reporter gene is activated and produces blue colonies in X-gal medium. The intensity of the color indicated the level of interaction among the protein of interest.

TAP-**M**ass **S**pectroscopy (MS) tagging is based on a double affinity tagging of a target protein followed by MS identification of co-purified protein partners. To purify and characterize a complex of a protein of interest (the bait), the protein is fused to a double tag at a chromosomal locus. TAP-tag consists of **C**almodulin **B**inding **P**rotein (CBP), a TEV protease cleavage site, and *Staphylococcus* Protein A which binds to the IgG

domain. After lysis, the tagged protein and its corresponding complex is purified through two step-affinity purification. In the first step of purification, the tagged protein binds to the IgG coated beads. The protein is released from the beads via TEV protease digestion. The eluant is subjected to a second purification step using calmodulin beads. Proteins are released from these beads by **E**thylene **G**lycol **T**etra-acetic **A**cid (EGTA). This eluant consisting of the target protein and its interacting partners are subjected to **S**odium **D**odecyl **S**ulfate **P**oly-**A**cryl amide **G**el **E**lectrophoresis (SDS-PAGE). Each of the interacting partners is identified by MS.

Both Y2H and TAP tagging have several advantages and disadvantages. Among them, Y2H has the advantage of being an *in vivo* technique which works with the binary protein interactions. In contrast, TAP tag identifies interactions under natural physiological conditions at native expression levels.

The first comprehensive Y2H analysis of the predicted yeast ORF's was done in 2000 by two groups: Uetz *et al.* (2000) and Ito *et al.* (2000). In 2002, Gavin *et al.* (2002) reported the first high-throughput use of TAP-tag method to analyze the yeast interactome. In 2006, TAP-tagging of yeast proteome was further expanded to cover the majority of yeast proteins (Krogan *et al.* 2006; Gavin *et al.* 2006). Recently, Pitre *et al.* (2006, 2008) developed and used a novel computational tool called **P**rotein-protein **I**nteraction **P**rediction **E**ngine (PIPE), to predict a global interaction map of yeast proteins. They predicted thousands of novel PPIs using a pair wise analysis of the entire yeast proteome. This approach is based on identifying re-occurring short polypeptide sequences between

known interacting partners. The yeast TAP-tagging project is currently being expanded to include membrane proteins (Dr. Greenblatt personal communication). Aside from yeast, the list of organisms that are subjected to global PPI investigation analysis continues to grow (Zanzoni A, 2009; LaCount *et al.* 2005; Li *et al.* 2004; Rain *et al.* 2001).

In yeast, only ~20% of genes are essential (Hillenmeyer *et al.* 2008; Davierwala *et al.* 2005) for cell viability, under normal experimental conditions. The remaining 80% of genes have at least one other genetic partner with an overlapping function. When one of these genes is deleted, no specific phenotype is observed due to functional compensation by the activity of the other genetic partner. However, inactivating of both genetic partners renders a phenotypic consequence that is mostly characterized by lethality or sickness (Tong *et al.* 2004). Synthetic lethality and sickness represent an aggravating interaction which is the simplest case of genetic interactions. Genetic interactions are enriched with the components of associated pathways and complexes which share similar functions. Therefore they can be used as a tool to study novel association and function for various genes.

Other types of genetic interactions include synthetic rescue, dosage lethality and dosage rescue (Boone *et al.* 2007). In synthetic rescue interactions, deletion of a gene reverses the inhibitory effect, or mutant phenotype, of a second gene. In other cases overexpression of some gene products may cause lethal phenotypes only when expressed in a gene deletion mutant strain, but not in the wild type strain. This is often referred to as dosage lethality and is often thought to provide some information about the identity of

functionally related interacting proteins. In dosage rescue, overexpression of one gene compensates for the phenotype caused by the deletion of another gene.

In recent years, genetic interaction analysis has received considerable attention for systematic studying of genes that were not well-characterized or were uncharacterized. In 2001, Tong *et al.* (2001) developed a novel large-scale approach to identify genetic interaction in *S. cerevisiae*, termed SGA analysis. In this technique, a query strain carrying a mutation in a gene of interest is mated with a nonessential haploid deletion set of the opposite mating type. After a few rounds of selections, a progeny of double mutants is obtained, each strain carrying the query mutation together with a second gene deletion. The fitness of the double mutant is then used to identify synthetic sick/lethal phenotypes. To date, using SGA analysis, a number of previously uncharacterized genes have been studied and various interactions have been identified in different cellular pathways such as, phospholipid biosynthesis (Morii *et al.* 2009), signal transduction (Krause *et al.* 2008), and dynein-dynactin spindle orientation pathway (Tong *et al.* 2004).

1.4 Chemical-Genetics

Chemical-genetics is a term often used to indicate the use of small inhibitory molecules to perturb different genetic and cellular networks within a cell. It combines our knowledge of the chemistry of a compound, with the genetic pathways that are targeted by that compound. Chemical-genetics is often used in drug discovery but it can also be used to study the molecular mechanisms underlying various biological pathways and diseases. Large-scale chemical-genetics analysis is also called chemical genomics where the inhibitory effects of a compound on different genes, proteins or genetic/biochemical pathways is investigated at a high-throughput level (Wuster and Madan Babu, 2008).

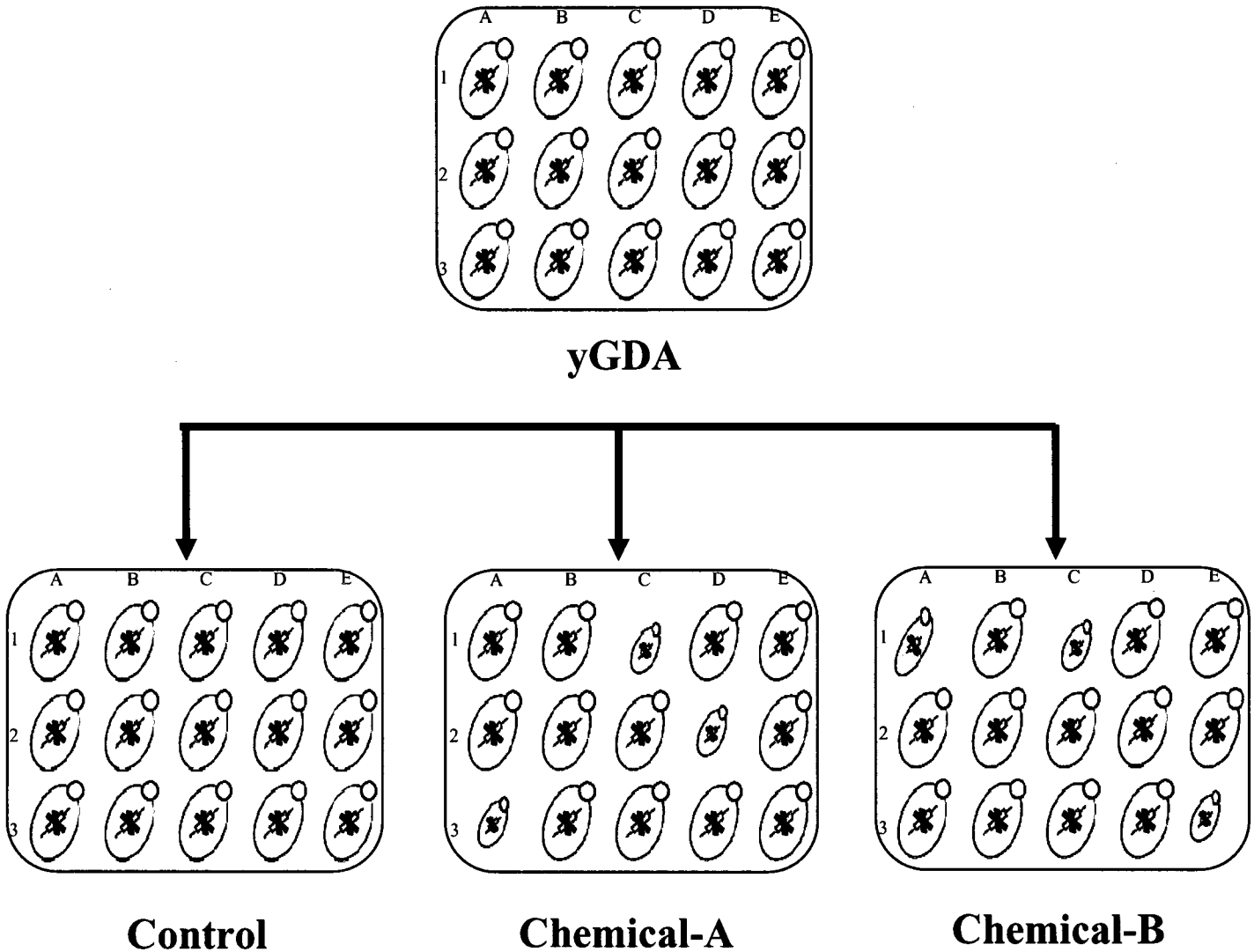
The identification of cellular drug targets is one of the early steps of drug development processes. It is a key element in chemical genomic screenings. In a classical fashion, drug target identification is either done using an array of reconstituted chemical pathways in test tubes, or *in vivo* using a series of deletion mutants for genes involved in specific pathways. In this way the profiles of chemical reactions or the cell growths, in the presence or absence of the target compounds are compared and analyzed. In general, the target biomolecules bind to their associated targets or group of proteins interfering with a chemical reaction resulting in a measurable consequence (Schreiber, 2005). This approach has been utilized systematically to identify various drug targets such as HDAC (histone deacetylase) in cancer research (Bernstein *et al.* 2000), and in angiogenesis (Baek *et al.* 2008).

Recent approaches use the available library of yeast gene deletion strains to study chemical-genetics profiles of various inhibitory compounds (Figure 1-4). For example, in 2000, Chan and colleagues examined the set of yeast deletion strains for sensitivity to rapamycin on a genome-wide scale to determine their sensitivity. Later, Lum *et al.* (2004) used 78 chemicals in a heterozygous set of *S. cerevisiae* deletion strains and Parsons *et al.* (2006) used 82 chemicals in yeast haploid deletion array to determine their chemical-genetics profiles.

Chemical-genetics can also be used for functional genomics investigations. The function of genes and the multifaceted pathways and cellular processes that they affect may be studied in a systematic way by elucidating the profiles of sensitive mutants against the inhibitory effect of a compound with known mode of activity (Giaever *et al.* 2004; Lum *et al.* 2004; Parsons *et al.* 2004). For example, Hughes *et al.* (2000) used an integrated chemical genetic approach to cluster functionally related genes and compared their drug-induced profiles with the existing gene expression databases to identify relevant biological pathway(s), and gene functions.

Figure 1-4: Chemical-genetic profile of bioactive compounds.

Chemical-genetic profiling of two similar bioactive compounds with similar mode of activity. Chemical-genetic analysis is a non-target based screening that is used for studying novel drug targets. It is based on phenotyping the non-essential yeast gene deletion array (yGDA) for hypersensitivity to inhibitory compounds (for example, chemical A and chemical B). In this diagram, compared to the control, yeast deletion mutant A3 and D2 are sensitive to chemical A; E3 is sensitive to chemical B. C1 is hypersensitive to both chemical A and B. It is hypothesized that the pathway in which gene C1 participates could be the target of the both compounds.


A B C D E
1
2
3
yGDA
Control
Chemical-A
Chemical-B

1.5 Ribosome

Ribosomes are megadalton molecules of ~20 nm in diameter, which are made up of ~60% ribosomal RNAs and ~40% proteins. They are the macromolecules responsible for synthesizing proteins by reading the genetic codes embedded in mRNA molecules. This process is called protein synthesis or translation. In the mid 50's, George Palade observed ribosomes for the first time using an electron microscope (Palade, 1955). Approximately 2000 ribosome are synthesized per minute in eukaryotes to maintain the growth requirement of cells (Warner, 1999).

1.5.1 Ribosome Biogenesis

Ribosome biogenesis is a highly complex and coordinated process that includes rRNA transcription, modification, processing, folding, ribonucleoprotein (RNP) assembly and export. The biogenesis of ribosomes occurs throughout the cell from nucleolus through the nucleoplasm to cytoplasm. Most of our current knowledge of ribosome biogenesis comes from studying yeast, where a large number of ribosome precursors and their components have been identified and characterized (Granneman and Baserga, 2004).

Ribosomes are comprised of two subunits: small and large. In bacteria, they are 30S (small) and 50S (large) free subunits; the 70S ribosome refers to a joined state of ribosome (whole ribosome). Ribosomes in higher eukaryotes such as yeast consist of 40S and 60S free ribosomal subunits and the whole ribosome is called 80S. The small subunit

of yeast contains an 18S rRNA and 30 to 50 small ribosomal subunit proteins (Woolford and Warner, 1991; Planta and Mager, 1998). The large subunit contains a 25S, a 5.8S, and a 5S rRNAs, and 40 to 50 large ribosomal subunit proteins (Fromont-Racine *et al.* 2003; Nazar, 2004). It has been suggested that ~200 accessory factors and ribosomal proteins are involved in pre-rRNA processing (Krogan *et al.* 2004). In yeast, ribosomal biogenesis requires the involvement of three RNA polymerases: RNA Pol I transcribe large rRNA precursors in the nucleolus that get processed into 5.8S, 18S and 25S RNAs, RNA Pol II synthesizes the pre-mRNAs of ribosomal proteins and accessory factors; and Pol III synthesizes 5S rRNA (Venema and Tollervey, 1999).

In eukaryotes, rRNA processing is generally associated with nucleolus where RNA Pol I transcribes rDNA as a polycystronic transcript known as pre-rRNAs. These pre-rRNAs contain external and internal transcribed spacers along with the mature rRNA (18, 5.8S, 25-28S rRNA). The pre-rRNA undergoes exo- and endo-nucleolytic cleavages, with concomitant modification of the rRNA by methylation and pseudouridylation (Venema and Tollervey, 1999). These chemical modifications remove the transcribed spacers and thus release the individual rRNA species. The fourth rRNA, 5S rRNA is independently transcribed as a precursor by RNA polymerase III in the nucleolus (Kressler *et al.* 1999).

In yeast, the 35S pre-rRNA is cleaved at A_0-site in the 5'-**E**xternal **T**ranscribed **S**pacer (ETS) to form 33S pre-rRNA. This is followed by a cleavage at A_1-site to yield 18S rRNA. In the subsequent cleavage at A_2-site in the **I**nternal **T**ranscribed **S**pacer 1 (ITS1) region produces 27S pre-rRNA which contains 25S rRNA and 5.8S precursors (Figure 1-

5). The processing and assembling of pre-rRNA requires various ribosomal, non-ribosomal and small nucleolar ribonucleoprotein particles (snoRNPs) to yield 90S pre-ribosomal particles. These 90S pre-ribosomes are the precursor of 43S and 66S complexes. 43S and 66S pre-ribosomes species eventually form 40S and 60S ribosomal subunits, respectively. The 43S pre-ribosome is exported from the nucleus to the cytoplasm through the nuclear pore complexes (NPCs) where its final assembly and maturation occurs to yield mature, functional ribosomes. Maturation of 60S pre-ribosomes (66S) continues at the nucleus. This 60S pre-ribosomal protein contains about 50 accessory proteins (Nissan *et al.* 2002) which are later transported to the nucleoplasm (Milkereit *et al.* 2001). Only 5 factors are present when 60S pre-ribosomes are exported to the cytoplasm (Tschochner and Hurt, 2003). It contains 27S and 7S rRNA precursors of the mature 25S and 5.8S rRNAs. It is thought that 5.8S rRNAs can also be generated by a different pathway via an unknown mechanism (Fatica and Tollervey, 2002). The 5.8S rRNAs formed in this way differ slightly at their 5'-ends. The outlines of the rRNA processing pathway are described in the Figure 1-6.

1.5.2 Regulation of Ribosome Biogenesis

Ribosome biosynthesis is an essential and highly regulated cellular process which influences cellular growth, proliferation and differentiation (Jorgensen *et al.* 2002). During the last decade, development of various novel techniques such as affinity purification followed by mass-spectrometry for protein identification has revealed numerous cellular factors including novel genes, proteins, and RNA components which are involved in regulating ribosome biogenesis (Merl *et al.* 2010; Takahashi *et al.* 2003).

Figure 1-5: Schematic diagram of the pre-RNA processing of yeast *S. cerevisiae.*

The mature RNA indicated by black boxes (18S, 5.8S and 25S rRNAs) and flanked by the 5′ and 3′ ETS and separated by ITS 1 and ITS 2. rRNA is transcribed initially by cleavage at B_0 in the 3′-ETS by an endonuclease and generates 35S pre-rRNA. Followed by three successive cleavages at sites A_0, A_1 and A_2, it yields 20S and 27SA2 pre-RNAs, the precursor of 40S and 60S subunits, respectively. The 20S pre-rRNA cleaved at site D to generate mature 18S rRNA and then exported to the cytoplasm. Alternatively, the $27SA_2$ pre-rRNA can be processed and cleaved either (major or minor pathway) by B_{1L} or A_3 site. Cleavage at the 3′ end yields $27SA_3$ which leads to generation of 25S rRNA by exonucleolytic digestion at B_0 to B_2. The precursors to the 5.8S and 25S rRNAs are separated by cleavage at site C_2 in ITS 2. In the minor pathway, 27SA2 is cleaved at B_{1L} to yield 27SBL, which is then cleaved at C2 and C1 to release mature 25S and either 7SS or 7SL. Finally, 5.8S is generated from exonucleolytic digestion at C2 end.

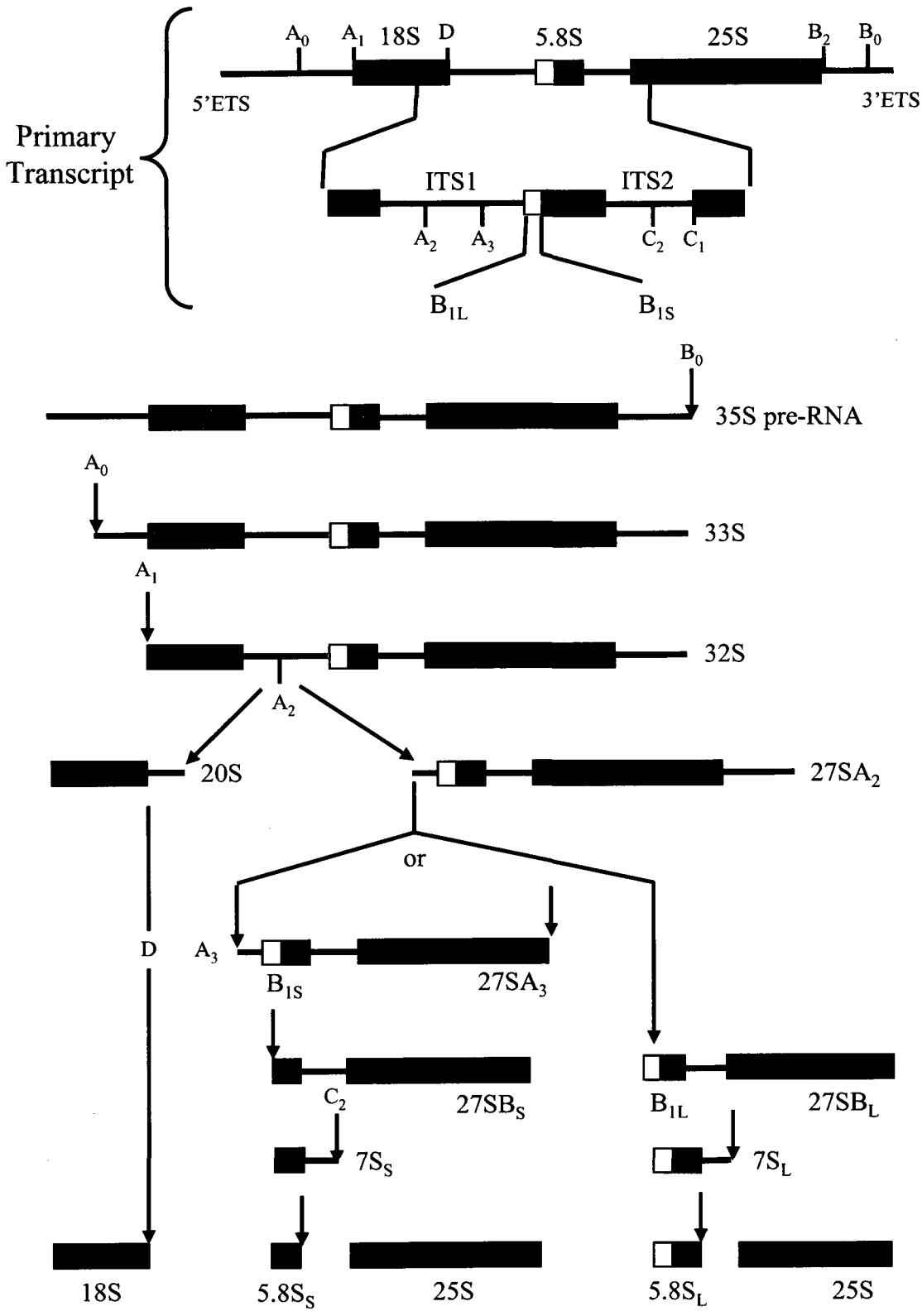
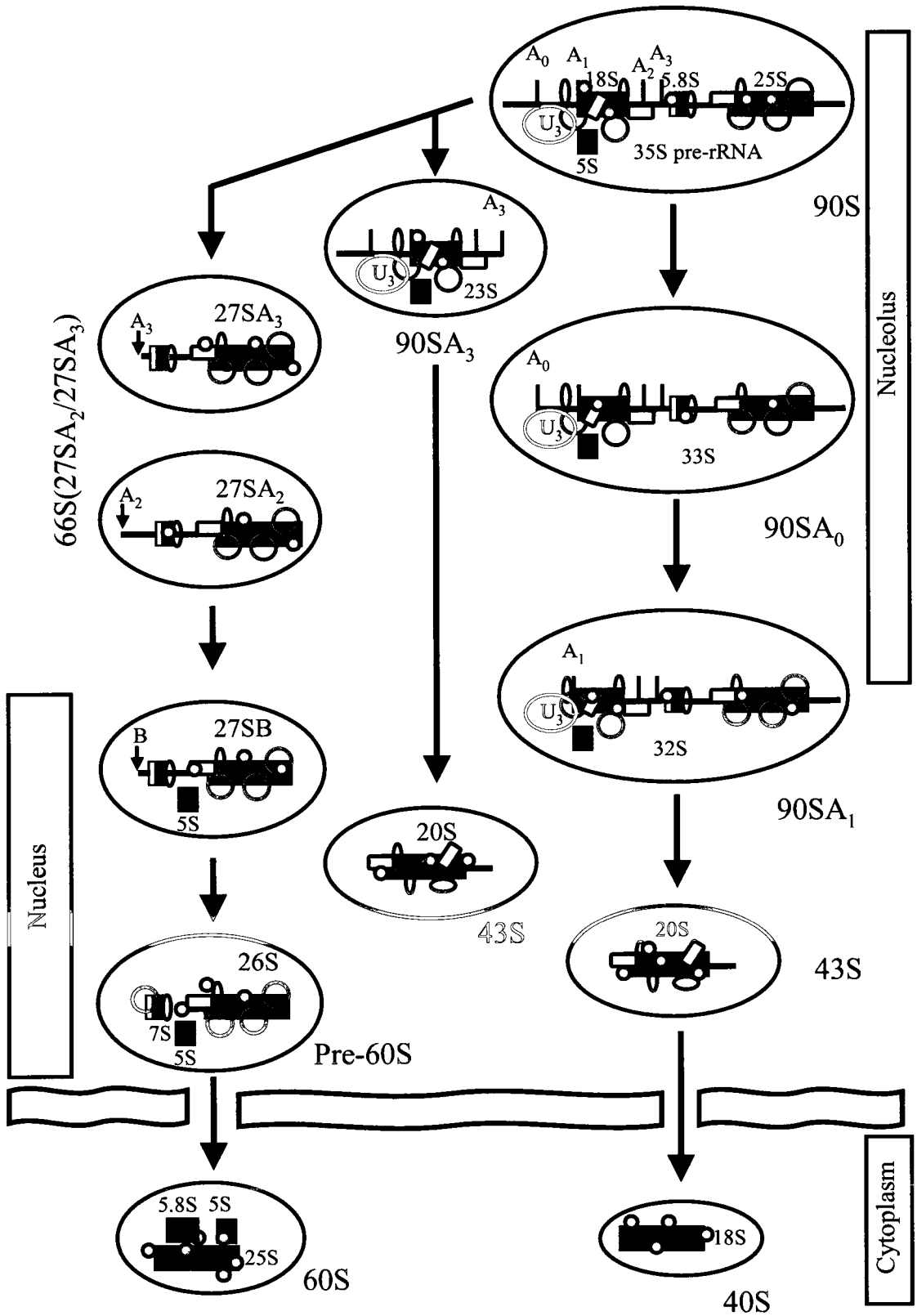

Primary Transcript
A0 A1 18S D 5.8S 25S B2 B0
5'ETS
3'ETS
ITS1 ITS2
A2 A3 C2 C1
B1L B1S
B0
35S pre-RNA
A0
33S
A1
32S
A2
20S
27SA2
or
D
A3
B1S
27SA3
C2
27SBS
B1L
27SBL
7SS
7SL
18S
5.8SS
25S
5.8SL
25S

Figure 1-6: Outline of the processing steps of ribosome biogenesis.

The 90S pre-ribosomal complex contains the 35S rRNA and the U_3 snoRNA. This 90S is processed to form 66S and 43S which are the precursor of 60S and 40S particles respectively. Initially, 90S pre-rRNA is cleaved either at A_0 or A_3 to yield 43S or pre-60S. Alternatively, the 90S pre-rRNA is cleaved at sites A_1 and A_2 to yield 43S and 66S pre-ribosome complexes. After processing in the nucleoplasm, 43S and pre-60S are exported to the cytoplasm where they are matured to form 40S and 60S ribosomal subunits.


A0 A1 A2 A3
18S
5.8S
25S
U3
5S
35S pre-rRNA
90S
Nucleolus
A3
U3
23S
90SA3
27SA3
A3
66S(27SA2/27SA3)
A0
U3
33S
90SA0
27SA2
A2
A1
U3
32S
90SA1
B
27SB
5S
Nucleus
20S
43S
20S
43S
26S
7S
5S
Pre-60S
5.8S 5S
25S
60S
18S
40S
Cytoplasm

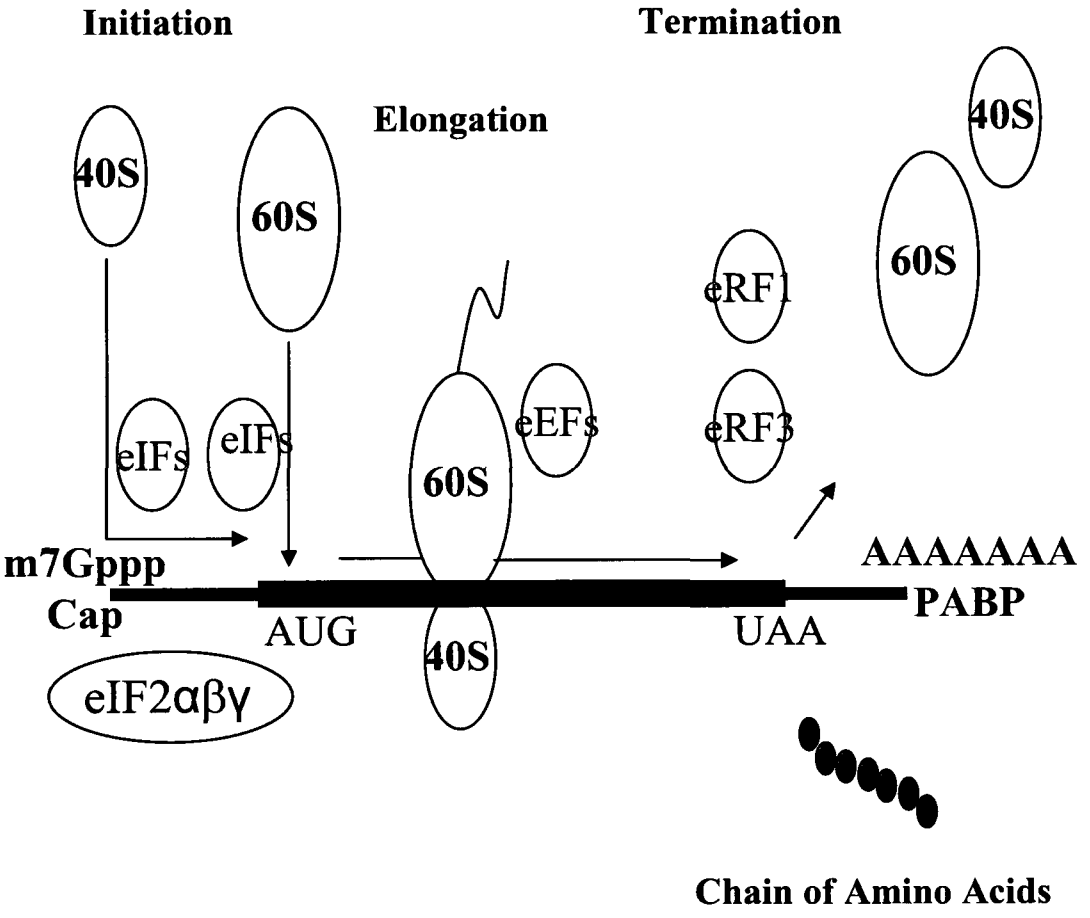
Discovery of these factors has enhanced our understanding of the cellular processes of pre-mRNA splicing, mRNA/tRNA turnover and cell cycle progression and most importantly, the molecular mechanism of ribosomal assembly processes (for recent reviews see Kressler *et al.* 2010; Fatica and Tollervey, 2002; Tschochner and Hurt, 2003; Fromont-Racine *et al.* 2003; Gavin *et al.* 2002). The processing and export of ribosomal subunits from nucleolus to cytoplasm depends on many factors including ~80 ribosomal proteins that are assembled on rRNAs and a large number of non-ribosomal proteins and snoRNPs (Warner, 1999; Warner, 1989). Assembly of these factors occurs in distinct stages of pre-ribosome processing from nucleus to cytoplasm.

The biogenesis of ribosomes in yeast is primarily regulated at the transcription level. It requires the coordination between a series of factors including the active **T**arget **O**f **R**apamycin (TOR) and **P**rotein **K**inase **A** (PKA) pathway; as well as availability of nutrients. Ribosome biosynthesis requires coordination between the activity of three RNA polymerases to maintain a balance between small and large ribosomal subunits. Though the molecular mechanisms that balance the three RNA polymerases are still unclear, it has been reported that TOR may play a role in this process (Li *et al.* 2006). It is well documented that TOR also regulates the expression of various transcription factors (Martin and Hall, 2005) and translational initiation factors in yeast. But the role of TOR in the synthesis of ribosomal protein components is not well understood. A previous study shows that when cells grow in either glucose depleted medium or in anticancer drug rapamycin containing medium, the level of 35S rRNA synthesis decreases (Powers

and Walter, 1999). TOR also regulates the activity and expression of Pol II-dependent ribosomal protein genes, **Ri**bosome **Bi**ogenesis (Ribi) regulon (Jorgensen *et al.* 2004) and Pol III-dependent 5S rRNA synthesis (Chédin *et al.* 2007), as well as decreases the initiation of translation in the yeast cell (Barbet *et al.* 1996). These results suggest an activity of TOR that may coordinate the action of three RNA polymerases. This might be done via the activity of Pol I, since the deregulation of RNA Pol I causes an accumulation of large ribosomal RNAs and leads to the buildup of large ribosomal RNAs, 5S rRNA and mRNAs encoding RPs (Laferté *et al.* 2006).

Figure 1-7: Steps of translation.

Three steps of translation: initiation, elongation and termination.


Initiation
Termination
Elongation
40S
60S
40S
60S
eRF1
eIFs
eIFs
eEFs
eRF3
60S
AAAAAAA
m7Gppp
Cap
PABP
AUG
UAA
40S
eIF2αβγ
Chain of Amino Acids

1.6 Translation

During translation, the genetic information of mRNA directs protein synthesis by generating polypeptide chains. This is one of the most conserved and regulated steps of gene expression. The rate of translation in prokaryotes is about 20 amino acids per second, that is, 60 nucleotides of mRNA are read per second. This is in comparison to the rate of transcription of duplex DNA by RNA polymerase II which is ~100-300 nucleotides per min (Ali *et al.* 2003). In eukaryotes, such as the yeast *S. cerevisiae*, translation occurs at about 2-4 aa per second (von der Haar, 2008).

An mRNA bearing multiple ribosomes is known as polyribosome or polysome. Generally, a single ribosome makes contact with about 30 nucleotides on an mRNA (Wolin and Walter, 1988), but the large size of the ribosome allows a density of 1 ribosome for about 50-80 nucleotides of mRNA (Legon *et al.* 1977). This ability of multiple ribosomes to function on a single mRNA explains the relatively limited abundance of mRNA in the cell (about 1-5% of total RNA) and a relative abundance of the ribosomes.

In general, translation is divided into three steps: i) initiation, ii) elongation, and iii) termination (Figure 1-7) (Linder, 1992). The regulation of translation is an important part of cellular growth, differentiation, and apoptosis (Holcik and Sonenberg, 2005), and significantly contributes to the regulation of gene expression in cells. It was found that,

defects in the process of translation can cause certain types of cancers in humans (Ruggero and Pandolfi, 2003).

1.6.1 Initiation

Initiation is the most complex step of translation (Figure 1-8). Its efficiency also defines the rate of protein synthesis. This multistep process requires numerous factors and several pre-initiation stages that are mediated by eukaryotic initiation factors (eIFs) (Pestova *et al.* 2001; Preiss *et al.* 2003). During initiation, ribosomal subunits along with translation initiation factor complex eIF4F (composed of three translation initiation factor eIF4E: cap binding protein, eIF4A: RNA helicase and eIF4G: bridges between mRNA and ribosome) binds to the 5'-cap structure of the mRNA with the eIF4B. This 5'-cap structure of mRNA serves as an assembly site for translation 43S pre-initiation factor complex formation (Goodfellow and Roberts, 2008). On the other end of mRNA, **P**oly (**A**)-**B**inding **P**rotein (PABP) binds to the poly (A) tail of the 3'-end. Its physical association with the initiation complex circularizes the mRNA (Sachs and Varani, 2000).

Eukaryotic mRNAs are mostly monocistronic, capped at the 5'-end and contain 5'-**U**n**T**ranslated **R**egions (UTR) of approximately 20-100 nt long (Houdebine and Attal, 1999). Their 3'-end is polyadenylated. Prior to the onset of translation initiation, 80S ribosomes dissociate into 40S and 60S subunits. The dissociated 40S subunits form 43S pre-initiation complex by binding with a protein complex containing eIF1, eIF3, eIF5 and the **T**ernary **C**omplex eIF2-GTP-Met-tRNAi (TC) (Lamphear *et al.* 1995; Imataka and Sonenberg, 1997). The resulting 43S complex starts scanning the mRNA from the cap structure towards the 3'-direction to locate the first initiation codon, which is generally an

AUG. During the scanning of the mRNA-bound ribosomal complex along the 5'-UTR, the 48S initiation complex is formed. The initiation codon is base paired to this 48S complex with the anticodon of tRNA initiator. It is generally accepted that the efficiency of the scanning and start codon recognition depends on the length and the structure of the UTR of mRNAs. mRNAs with short unstructured 5'-UTRs are more readily translated than mRNAs harboring lengthy, highly structured 5'-UTRs. After recognizing the initiation codon, Met-tRNAi binds at the P-site to the initiation codon and form AUG-Met-tRNAi. At this stage, eIF2 and eIF3 are released. This triggers the binding of large 60S ribosomal subunit at the initiation codon with the 40S initiation complex to form 80S ribosome, leaving Met-$tRNA_i$ in the ribosomal P-site, competent for translation elongation (Scheper *et al.* 2007).

1.6.2 Elongation

During the elongation phase of translation, ribosomes move along the mRNA from a 5' to 3' direction to incorporate new amino acids sequentially and form polypeptide chains (Figure 1-9). Unlike translation initiation, the mechanism of elongation is highly conserved between prokaryotic and eukaryotic organisms. In most eukaryotic organisms, the elongation step requires two soluble protein factors. The first one is **e**ukaryotic **E**longation **F**actor **1A** (eEF1A), which catalyzes the binding of aminoacyl-tRNA to the A-site of the ribosome (Merrick and Nyborg, 2000). Once the A-site is occupied by a charged tRNA, the peptidyl transferase activity of the large subunit promotes a peptide bond between the 2 amino acids. **e**ukaryotic **E**longation **F**actor-**2** (eEF-2) facilitates the translocation of peptidyl-tRNA from the ribosomal A-site to the P-site. At the same time,

the next codon on the mRNA is moved into the A-site. The activities of these protein factors are GTP dependent. In addition, yeast requires a third initiation factor, **E**longation **F**actor-**3** (EF-3), an ATPase, for translation of natural and synthetic messages (Herrera *et al.* 1984; Sandbaken *et al.* 1990). EF-3 stimulates binding of aminoacyl-tRNA-eEF1A-GTP to the ribosomal A-site by facilitating release of deacylated tRNA from the **E**xit-site (E-site) (Andersen *et al.* 2006). In agreement with this, an antibody against EF-3 is shown to block natural mRNA translation *in vitro* (Tariana-Alonso *et al.* 1995).

1.6.3 Termination

Termination of protein synthesis occurs when the elongation machinery encounters one of the three in-frame termination codons UAG, UAA or UGA on the A-site of mRNA (Figure 1-9). The stop codons are recognized by the **e**ukaryotic **R**elease **F**actor eRF1 in eukaryotes and in bacteria by **R**elease **F**actors, RF1 and RF2. These release factors activate the hydrolysis of an ester bond in peptidyl-tRNA to release the polypeptide chain from the ribosome. A third release factor, eRF3 binds to the ribosome in complex with GTP. The hydrolysis of GTP produces energy which facilitates the release of eRF1 and eRF2 from ribosomes (Grentzmann *et al.* 1998). The eRF3·GDP complex is released when **R**ibosome **R**ecycling **F**actor (RRF) and EF-G·GTP bind to the ribosomal A-site. Afterwards, the hydrolysis of GTP causes the RRF to move to the ribosomal P-site and kicks out the tRNA. Finally, the RRF, EF-G·GDP and mRNA dissociate, yielding an inactive 80S ribosome ready for reinitiation (Voet, 2008).

Figure 1-8: Eukaryotic translation initiation.

ORF is indicated by black line containing AUG start and UAA stop codon. The 3'-end contains poly A-tail (AAA) and the 5'-end carries a cap structure indicated by a blue circle. eIF3, eIF1, eIF1A and ternary complex (eIF2 and tRNAmet) bind to the 40S ribosome, forming 43S pre-initiation complex. eIF4F multimer (eIF4E, eIF4G, eIF4A, and eIF4B) and PABP bind to the upstream region of the AUG initiator codon and the poly A tail, and circularize the mRNA. The 43S pre-initiation complex binds to the mRNA molecule forming 48S pre-initiation complex and scans in 5' to 3' direction to recognize the initiation codon, AUG. eIFs dissociate from the ribosome leaving the tRNAmet bound to the initiator AUG codon which allows the binding of the 60S ribosomal subunit to form 80S initiation complex for the elongation steps. E site is the exit site, P site is the peptidyl-tRNA site, and A site is for the binding of incoming aminoacyl-tRNAs.

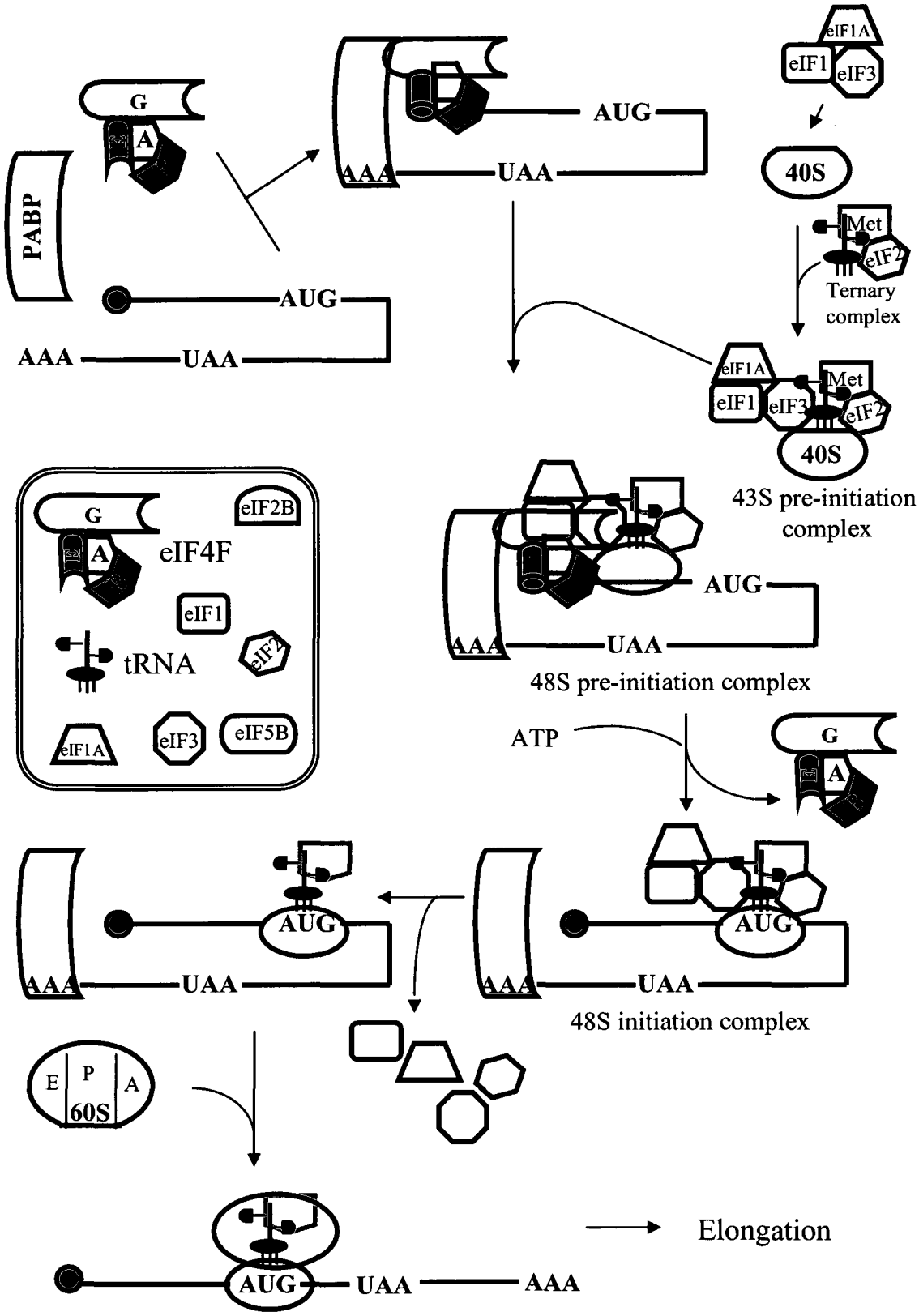
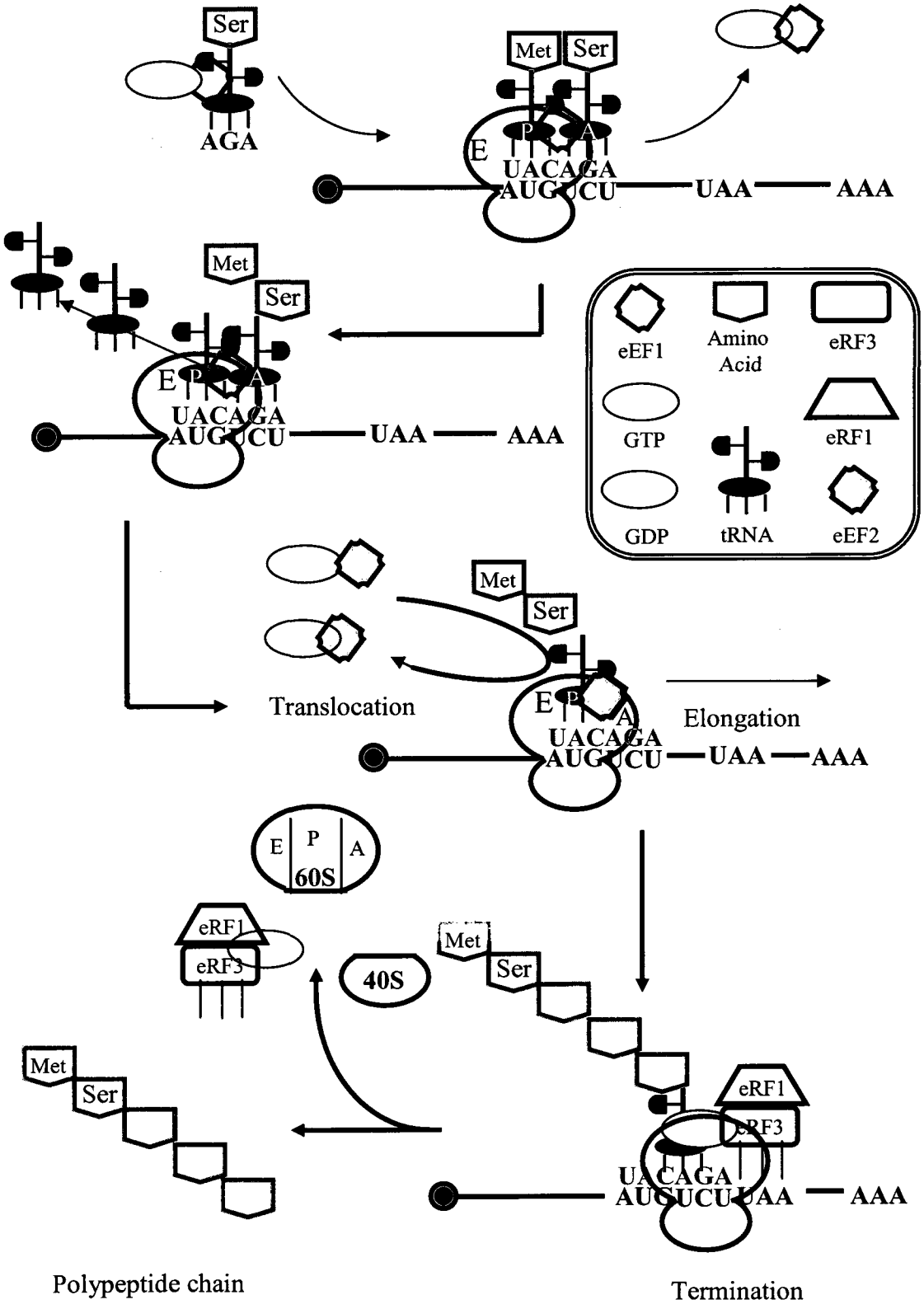

G
A
PABP
AUG
AAA
UAA
eIF1A
eIF1
eIF3
40S
Met
eIF2
Ternary complex
43S pre-initiation complex
eIF2B
eIF4F
tRNA
48S pre-initiation complex
ATP
48S initiation complex
E
P
A
60S
Elongation

Figure 1-9: Steps of translation elongation and termination.

During the elongation step, polypeptide chain assembled as directed by the ORF of the mRNA. eEF1A-GTP recruits an amino-acylated tRNA to the A site of the ribosome. When codon-anticodon recognition occurs, GTP hydrolyzed to GDP, allows eEF1A to dissociate from the ribosome and then interact with eEF1B. eEF1B is then exchanges GDP for GTP on eEF1A. eEF1A is free to recruit a new amino-acylated tRNA to the ribosomal A site. A peptide bond forms between the amino acids in the A and P sites. eEF2 transiently binds near the ribosomal A site. GTP hydrolysis by eEF2 causes the ribosome to translocate amino acids down the mRNA and leads to dissociation of eEF2 from the ribosome. The A site is left free for eEF1A to bring in a new amino-acylated tRNA. When the ribosome encounters a 'stop' codon, termination occurs, resulting in release of the completed polypeptide and the ribosomal subunits.


Ser
AGA
Met
Ser
E
P
A
UACAGA
AUGUCU
UAA
AAA
eEF1
Amino Acid
eRF3
GTP
eRF1
GDP
tRNA
eEF2
Translocation
Elongation
60S
40S
eRF1
eRF3
Polypeptide chain
Termination

1.6.4 Internal Ribosome Entry Sites (IRES)

Aside from the cap dependent initiation, translation of some mRNAs can be initiated by direct binding of the ribosome to specific regions of mRNA termed **I**nternal **R**ibosome **E**ntry **S**ites (IRES) (Le Quesne *et al.* 2010, Schüler *et al.* 2006). This is a cap-independent process and may constitute approximately 10% of the cellular mRNA translations (Stoneley and Willis, 2004). IRESs usually facilitate alternative initiation of translation when the cap-dependent mechanism is blocked, and under different cellular stress conditions. IRESs are also utilized to control translation during cell cycle and apoptosis (Holcik and Sonenberg, 2005). Originally identified in picornavirus RNAs (Pelletier and Sonenberg, 1998), they contain extremely long and structured UTR sequences. Most natural IRESs are located at the 5'-UTRs of mRNAs (Holcik and Sonenberg, 2005), but some mediate internal initiation of bicistronic mRNAs (Hellen and Sarnow, 2001). During IRES mediated initiation, IRESes directly recruit ribosomes by an unknown mechanism and scans the mRNA sequence from the ribosome binding site to the initiator codon (Kieft, 2008; Houdebine and Attal, 1999). The process require different IRES trans-acting factors, such as polypyrimidine tract-binding protein (PTB) that binds polypyrimidine containing sequences in the mRNA, upstream of N-ras and embryonic-lethal abnormal vision (ELAV/Hu) (Holcik and Sonenberg, 2005). Examples of natural mRNAs that contain IRESs are certain cellular growth promoting genes such as FGF-2, PDGF and c-myc mRNAs (Willis, 1999).

1.6.5 Translation Regulation and Diseases

The process of protein synthesis is of central importance for all biological system. It is the last step of transmission of genetic information that is stored in DNA, to proteins that maintain specific biological functions. During the last 15 years, the involvement of protein synthesis in various neurological diseases (Le Quesne *et al.* 2010), cellular function, growth, proliferation and tumorigenesis (Clemens, 2004), has been critically investigated. It is well-documented that the uncontrolled growth of cells requires certain alteration in translation control, clearly connecting cancer to the process of translation (Tenesa *et al.* 2008). Translation can also be regulated by numerous physiological stimuli, including changes in the availability of growth factors, essential cell nutrients, cytokines and a wide range of stresses such as heat shock, virus infection, DNA damage, and exposure to pro-apoptotic conditions (Clemens, 2001).

The initiation phase of translation is the most complex and regulated step of protein synthesis (Le Quesne *et al.* 2010). There is a growing body of evidence that suggests the factors involved in the initiation of protein synthesis, also regulate cell cycle progression, proliferation, transformation, apoptosis and play a role in different neurological diseases. It was found that overexpression of initiation factor eIF4E results in the rapid proliferation of cells (De Benedetti *et al.* 1999; Anthony *et al.* 1996; West *et al.* 1995) and might lead into tumor development (Graff *et al.* 2008; Zimmer *et al.* 2000). It is also shown that the overexpression of eIF4E and eIF4G also cause oncogenic transformation in different mammalian cell lines (Fukuchi-Shimogori *et al.* 1997; Raught *et al.* 1996; Anthony *et al.* 1996; West *et al.* 1995). Recent evidence suggests that the high-level

expression of eIF4E-binding protein, 4E-BP1, inhibits cell growth and promotes apoptosis (Polunovsky *et al.* 2000). Previous findings suggested that the overexpression of eIF4E gene might be responsible for a variety of human carcinomas including Hodgkins lymphomas and breast cancer (Graff *et al.* 2008; De Benedetti *et al.* 1999). In addition, the overexpression of eIF4G might be linked to breast (Silvera *et al.* 2009) and squamous cell lung carcinoma (Brass *et al.* 1997).

eukaryotic **I**nitiation **F**actor **5A** (eIF5A) is also reported to be involved in the regulation of cell growth, differentiation and apoptosis. It plays a key role in the formation of the unusual amino acid hypusine which is involved in the regulation of cell proliferation and transformation (Caraglia *et al.* 1997). Previously, Schiffmann and van der Knaap (2004) described the role of eIF2B in vanishing white matter, an autosomal recessive neurodegenerative disease in young children, which may arise by mutation in one of the five subunits of eIF2B.

A defect in eEF also plays a role in cytoskeletal regulation (Thornton *et al.* 2003), cellular growth (Thornton *et al.* 2003) and neurological diseases (Le Quesne *et al.* 2010). It is also reported that higher levels of elongation factor eEF1 may elevate the rate of protein synthesis and cause an increase in missense errors (Carr-Schmid *et al.* 1999) or premature termination of translation (Browne and Proud, 2002). In an earlier study, a direct relationship between elevated levels of eEF1A (formerly known as eEF1α) and eEF1Bγ (known as eEF1γ), and acceleration in apoptosis was reported (Duttaroy *et al.* 1998). Inhibition of the **e**ukaryotic **R**elease **F**actors (eRFs) that recognize the stop codons

at the termination step of protein synthesis might lead to ribosome stalling and reduce the rate of protein synthesis in cells. Previous evidence also suggests that eRF3 controls the chromosome segregation and also regulates apoptosis (Malta-Vacas *et al.* 2005).

1.7 Purpose and Objectives

Due to its crucial role in the survival of the cell, the process of translation has been extensively studied over the past few decades. Although much has been learned about translation, genome-wide investigations (Peng *et al.* 2003; Krogan *et al.* 2004) suggest that there may exist other uncharacterized factors that can affect different stages of this process. Similarly, the cross-communication of translation with other cellular processes has long been hypothesized. However, the detailed mechanisms of the factors that affect these communications are still not understood. In addition, the rate of *in vitro* reconstituted translation is known to be significantly slower than *in vivo*, further suggesting the existence of uncharacterized factors that affect translation *in vivo*.

This thesis aims to identify novel genes that affect the process of translation. Due to recent development of novel genome–wide techniques in *S. cerevisiae*, we used this yeast as a model for our investigations. One of our assumptions has been that deletion of genes that affect translation may cause mutant cells to be more sensitive to bioactive compounds that target translation. We therefore used chemical-genomics profiling as a tool to identify potential candidate genes that may play a role in translation. Once novel gene candidates are identified we aim to confirm their involvement in translation. We

also intend to characterize their activities in protein synthesis using different genetic approaches.

2.0 METHODS AND MATERIALS

2.1 Buffers and Reagents

Analytical grade chemicals were used to prepare all buffers and solutions unless otherwise stated. All buffers and solutions were prepared using distilled and de-ionised water (Milli-Q Plus Ultra Pure Water System, Millipore, Billerica, MA, USA). Buffers and solutions were sterilized by autoclaving at 121°C for a minimum of 20 minutes or, where indicated, filter sterilized using a 0.2 μm or 0.45 μm Millipore membrane filter. Buffers for RNA work were prepared in RNase-free glassware using diethyl pyrocarbonate (DEPC)-treated water. DEPC-treated water (0.2%) was prepared with distilled and de-ionized Milli-Q water. Chemicals used were supplied by Bioshop Canada, Sigma-Aldrich, or Fluka Chemicals.

2.2 Medium, Strains, Plasmids and Yeast Manipulations

Standard organic (YEPD) and synthetic complete (SC) medium was used for yeast deletion mutant strains hypersensitivity experiments (Sherman *et al.* 1986) (Table 2-1). YEPD was used to grow the strains in all other experiments except where indicated. Yeast cells were grown at 30°C for 1-2 days. The YPD medium with Geneticin (G418; 200 μg/mL) was used for the maintenance of deletion strains carrying the $G418^r$ marker. To find the effect of drug on the growth of yeast deletion mutant, paromomycin (13 mg/mL), streptomycin (40 mg/mL), neomycin (5.5 mg/mL) and 3-Amino-1,2,4-triazole (3-AT) (22 mg/mL) was added in the SC medium and cycloheximide (45 ng/mL) was added in YPD medium. Haploid *S. cerevisiae* strains BY4742 (*MATa, ura3Δ0 his3Δ1*

leu2Δ0 lys2Δ0) were used from the non-essential gene deletion array (yGDA) for the work described throughout this thesis. Five knockout strains of *S. cerevisiae* were constructed by using strain Y7092 mating type *MATa* (*can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δo met15Δ0*). Each had a target gene (*TAE1*, *TAE2*, *TAE3*, *TAE4* or *YKL075C*) replaced by the *NatMX4* module to generate five knockout strains (Table 2-2). Yeast cells were grown at 30°C for 1-2 days. Plasmids p416*GAL1-lacZ*, which contains the *Escherichia coli lacZ* gene fused to a *GAL1* promoter (Krogan *et al.* 2003), was used for the determination of translation efficiency. pUKC816, pUKC817, pUKC818 contains *lacZ* gene with premature stop codons UAA, UAG and UGA respectively, and pUKC815, without any stop codon (Urakov *et al.* 2001) were used for the translation fidelity analysis. Yeast transformation was performed according to Ito *et al* (1983) using the lithium acetate approach.

Table 2-1: List of bioactive compounds and their targets.

Compounds	Description of activity	Media Used	MIC	References
Paromomycin	Alters translation fidelity by affecting aminoacyl-tRNA binding to the A-site of the ribosome and causes misreading.	SC	13 mg/mL	Alamgir *et al.* 2008
Cycloheximide	Binds to the 60S ribosomal subunit and inhibits translation elongation	YEPD	0.045 µg/mL	Alamgir *et al.* 2008
3-AT	Inhibitor of imidazoleglycerol-phosphate dehydratase, a key enzyme in histidine biosynthetic pathway.	SC	22 mg/mL	Alamgir *et al.* 2008
Streptomycin	Binds to the 16S rRNA of the bacterial ribosome and interfere with the binding of formyl-methionyl-tRNA to the 30S subunit, inhibit initiation of protein synthesis.	SC	40 mg/mL	Tuite and McLaughlin, 1984
Neomycin	Binds small ribosomal subunit of eukaryotic cells and inhibit ribosomal translocation and compromise translation fidelity	SC	5.5 mg/mL	Tuite and McLaughlin, 1984
Calcofluor White (CW)	Binds with chitin and interferes with cell wall function	YEPD	40 µg/mL	Hughes *et al.* 2000

Table 2-2: List of *S. cerevisiae* strains used in this study.

Strains	Description	Genotype
BY4742	Wild type	BY4742 (*MATα ura3Δ0 leu2Δ0 his3Δ1met15Δ0*)
S288C	Wild type	*MATα SUC2 gal2 mal mel flo1 flo8-1 hap1 ho bio1 bio6*
Y7092		*MATα can1Δ::STE2pr-SpΔhis5 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lys2*
Y7092 ybr261c**Δ**::*natR*	Knockout	*Y7092 (MATα can1Δ::STE2pr-SpΔhis5 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lys2)* ybr261c**Δ** ::*natR*
Y7092 ypl183W**Δ**::*natR*	Knockout	*Y7092 (MATα can1Δ::STE2pr-SpΔhis5 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lys2)* ypl183w**Δ** ::*natR*
Y7092 ykl075c**Δ**::*natR*	Knockout	*Y7092 (MATα can1Δ::STE2pr-SpΔhis5 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lys2)* ykl075c**Δ** ::*natR*
Y7092 ypl009c**Δ**::*natR*	Knockout	*Y7092 (MATα can1Δ::STE2pr-SpΔhis5 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lys2)* ypl009c**Δ** ::*natR*
Y7092 yil137c**Δ**::*natR*	Knockout	*Y7092 (MATα can1Δ::STE2pr-SpΔhis5 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lys2)* yil137c**Δ** ::*natR*

2.3 Minimum Inhibitory Concentration (MIC)

The MIC of a compound is described as the lowest drug concentration that resulted in full inhibition of cell growth. Standard 96 well-plate protocol was used to determine MIC for our yeast strains (Galván *et al.* 2008, NCCLS 2002). Briefly, yeast cells were grown overnight in YEPD at 30^0C with shaking at 200 rpm to an OD A_{600} of 0.8-1.0. Yeast cell cultures were diluted 1000X in YEPD medium and 100 µl aliquots were dispersed in each of the wells of a 96 well-microtitre plate (Costar, round bottom, ULTIDENT). Serial dilutions of the compounds were added to the microtitre plates and the control was created with no compound. The plates were incubated at 30^0C for 24-48 h. Inhibition of growth was compared visually with the control wells. The observed level of variation was consistently within the range of variation commonly observed in MIC tests.

2.4 Drug Resistance Screening With Yeast Gene Deletion Array (yGDA)

Approximately, 4700 *MAT***a** haploid deletion strains of *S. cerevisiae* (Tong *et al.* 2001), BY4742 (*MATa, ura3Δ0 his3Δ1 leu2Δ0 lys2Δ0*) parental strain (Open biosystems, Huntsville, AL), were maintained in an ordered array of about 384 individual strains in 16 plates. For high throughput phenotypic screening, yGDA was arrayed using a BioRAD colony arrayer robot or a hand held VP arrayer, onto YEPD agar plates as described (Ideker and Sharan 2008, Galván *et al.* 2008, Parsons *et al.* 2004). All plates were incubated at 30°C for 1-2 days. The sensitivity of the gene deletion array to different

drugs was investigated as before (Ideker and Sharan 2008). The chemical structures of drugs are listed in Figure 2-1. Briefly, different strains are pinned on two plates, one containing a subinhibitory concentration of a target drug (Table 2-1) and the other without a drug (control) (Figure 2-2). Sensitivity of the deletion strains to drugs were analyzed by using growth detector software (Memarian *et al.* 2007) where digital images of plates were taken to analyze the growth of individual colonies. In brief, the images were converted to black (medium) and white (colonies) and were segmented using threshold values derived from Otsu's approach (Otsu, 1979). Objects empirically determined to be smaller than 0.00025 of the total white pixels in a plate were considered artifacts and were eliminated. Colonies were ordered based on local centers and area maps. The average value of white pixels S_{ave} (average colony size) for each plate Pn was calculated from equation [1].

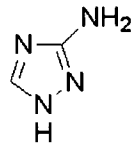
$$S_{ave}Pn = 1/N \sum_{i=1}^{N} S_i Pn \qquad [1]$$

where N is the total number of colonies present in a given plate and S_i is the area of object i. The deviation of area for each colony from the plate's average area was used for further analysis (relative growth) and was calculated by subtracting the scalar S_{ave} from the plate's ordered area array explained in equation [2].

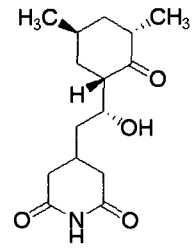
$$\Delta S_i = S_i - S_{ave} \; ; \; i = 1, \ldots, 384 \; (16 \times 24 = 384) \qquad [2]$$

The relative size of colonies calculated in this way was used to determine relative growth differences for each colony under different experimental conditions (that is treated versus control). Each experiment was repeated three times. The Relative colony size (CS) difference of 30% or more in two replicates, or those with the average reduction of more than 20% (with internal variation of 20% or less) in all three experiments were classified as "hits" or sensitive colonies. Colonies with the highest two average reductions of 61% or more and 30-60% were defined supersensitives and sensitives, respectively.

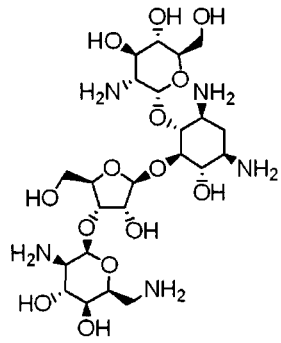
Figure 2-1: The chemical structure of bioactive molecules.



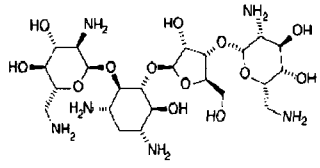
3-Amino 1,2,4-Triazole (3-AT)



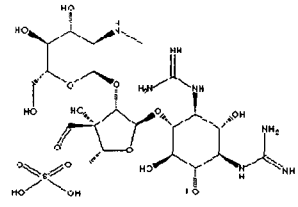
Cycloheximide



Paromomycin



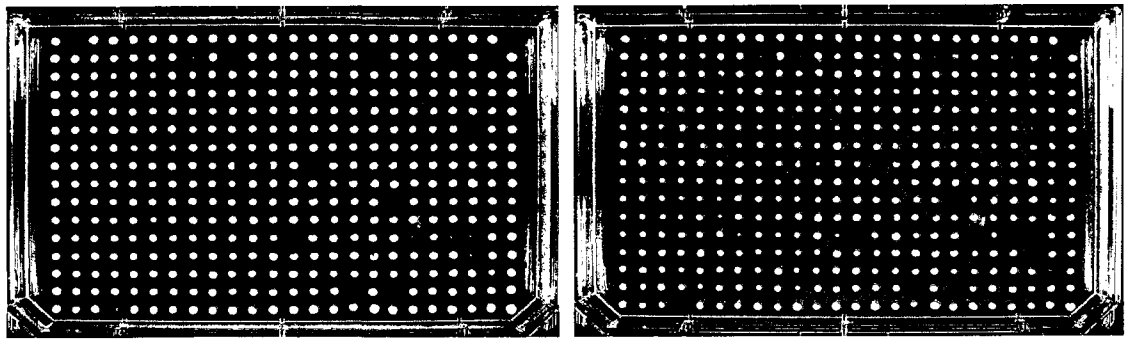
Neomycin



Streptomycin

Figure 2-2: Example of the effect of biomolecules on yGDA.

Two different strains marked by square and circle reduce their growth compared to the control plate (non-treated plate) in the presence of chemicals (treated plate).



Non-treated plate **Treated plate**

2.5 Spot Test Analysis

The mutant strains identified in the primary screens were further confirmed by spot test analysis. The spot test analysis was performed by growing yeast cell cultures in either YEPD or SC liquid medium to mid-log, following 10^{-3} to 10^{-6} fold dilutions. Ten to twenty microliters of each dilution (gradually decreasing) was then spotted onto medium containing sub-inhibitory concentrations of the target drugs as above, and without drug (control). The growth patterns were compared after 1-2 days at 30°C as in (Jessulat *et al.* 2008, Anderson *et al.* 2003).

2.6 Gene Expression Analysis

Alteration in translation fidelity was measured using plasmids pUKC817, pUKC818 and 819 which carry premature stop mutations UAA, UAG and UGA respectively, in a β-galactosidase expression cassette. pUKC815 contains no premature stop codon and was used as a control. β-galactosidase was assayed by using O-nitrophenyl-α-D-galactopyranoside (ONPG) as described previously (Lucchini *et al.* 1984) with some modification. Briefly, the mutant cells were grown overnight in repressed condition in minimal medium lacking uracil to OD_{600} 0.8-1.0. Subcultures (OD_{600} ~0.2-0.3) were grown at 30^0C with 200 rpm until OD_{600} ~1.0 is reached. Cells were collected and then resuspended in 3 ml Z-buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4.7H_2O$). Cells were collected from the 3 mL Z-buffer and resuspended in 0.5 ml Z-buffer. 100 µl of cells were then lysed by vortexing 15 sec after adding 20 µl of 0·1%

SDS and 50 µl of $CHCl_3$. The mixture was incubated for 15 min at 30^0C. 200 µl of pre-warmed (at 30^0C) OPNG (4 mg/mL) was added to the cell lysate, vortexed immediately for 5 sec and incubated at 30^0C until some yellow color was apparent. The OD_{550} (for the cell debris) and OD_{420} to monitor the hydrolysis of OPNG were recorded after the reaction was stopped. The units of enzyme activity were calculated as nanomoles of OPNG hydrolyzed per microgram of total protein (Shenton *et al.* 2006). For induced condition, β-galactosidase activity was measured using an inducible reporter gene in p416 plasmid under the transcriptional control of Gal1 promoter, after 4 hours of induction as before (Krogan *et al.* 2003).

2.7 Ribosome Profile Analysis

Ribosome profiling was performed as described by Foiani *et al* (1991) with the following modifications. Haploid wild type and yeast gene deletion mutant strains were grown overnight in YEPD at 30°C to an OD_{600} of 0.8-1.0 (~$2x10^7$ cells/mL). Immediately, 200 µl of cycloheximide (50 µg/mL) was added to each culture and the cultures were quickly chilled in an ice water bath. Cells were harvested, washed then centrifuged by Sorvall SLA-1500 rotor at 4000 rpm for 4 min at 4°C to remove the supernatant. Cell pellets were resuspended in 10 ml of ice-cold breaking YA buffer (breaking buffer A: 10 mM Tris-HCl [pH 7.4], 100 mM NaCl, 30 mM $MgCl_2$, cycloheximide 50 µg/mL, heparin 200 µg/mL) and centrifuged twice at 4000 rpm for 4 min, at 4°C (Sorvall SS34 rotor). The pellets were then resuspended in 0.5 ml of YA buffer, lysed by vortexing with glass beads and stored at -80°C for later use. Cells were then thawed in an ice water bath. Glass

beads were added and vortex for 20 sec at maximum speed, 10 times with 30 sec interval. The supernatants were centrifuged at 8,000X g and 10,000X g for 10 min and 30 min, respectively. Twenty OD_{260} units of each supernatant were fractionated on 8-48% sucrose gradients containing 50 mM Tris-acetate (pH 7.0), 50 mM NH_4Cl, 12 mM $MgCl_2$, and 1 mM dithiothreitol. The extracts were then centrifuged for 2.5 hrs at 39,000 rpm using a SW40-Ti rotor in a Beckman LE-80K centrifuge at 4°C. The ribosome profiles were analyzed from the collected gradient solutions by monitoring the absorbance at 254 nm in a Beckman spectrophotometer.

2.8 Total Protein Synthesis Measurement

The rate of total protein synthesis was measured *in vivo* by measuring the incorporation of [^{35}S] methionine into the cellular proteins as previously described (Schwartz and Parker, 1999) with modifications. Yeast strains were grown to mid-log phase at 30°C in YEPD. The cells were harvested, resuspended in pre-warmed minimal medium lacking methionine, and supplemented with 10 μCi/mL of [^{35}S] methionine. The cells were incubated for 1h at 30°C and harvested by centrifugation. The samples were then washed with distilled water six times and collected 1 or 2 μl aliquots of the final cell suspensions were dotted on Whatman paper. The paper was then air dried and exposed to a storage phosphor screen for 1h. The amount of [^{35}S] methionine incorporated into total cellular proteins measured by a Cyclon phosphor imager. Each experiment was repeated at least four times. Induced translation was measured using an inducible β-galactosidase reporter gene in p416 (Stansfield *et al.* 1995) plasmid after 4 hours of induction, as above.

2.9 Yeast DNA Extraction

Yeast cells were grown overnight at 30°C to an OD_{600} of 0.8-1.0. Five (5) OD_{600} units of cells were then collected and resuspend in 100 µl STET buffer (8% Sucrose, 50 mM Tris pH 8.0, 5% Triton X-100, 50 mM EDTA) and mixed. About 0.3 g acid washed glass beads (0.4 µm) (Sigma) were added and vortex for 5 min. Another 100 µl STET buffer was added and mixed. Samples were then heated in a boiling water bath (100°C) for 3 min, cooled briefly on ice and were transferred to a microfuge tube. Samples were centrifuged for 10 min at 4°C and supernatants were collected. 50 µl of 7.5 M ammonium acetate was added and mixed briefly, and 100 µl of supernatant was transferred in another microfuge containing 200 µl of ice-cold 100% ethanol. Samples were then centrifuged at 12000 g for 10 min at RT, supernatant was discarded, and the pellet was washed with 200 µl of 70% ethanol, mixed, and centrifuged as previously. The supernatant was removed and the DNA pellet air-dried for 10–15 min. DNA was resuspended in 20 µl of TE or distilled water. DNA was subsequently visualized on a 0.8% agarose gel (80V for 40 mins), stained with ethidium bromide and photographed with UV transillumination. The samples were also analysed spectrophotometrically at 260 and 280 nm using an Ultraspec III UV/Vis spectrophotometer (Pharmacia). The reading at 260 nm allowed for calculation of nucleic acid concentration in the sample and the ratio of OD at 260 and 280 nm was used to assess quality.

2.10 RT-PCR

Yeast gene deletion and wild type strains were transformed with pUKC815, pUKC817and pUKC818 plasmids (Stansfield *et al.* 1995) individually using lithium acetate method (Ito *et al.* 2001). These transformed strains were grown overnight in – URA medium. The overnight cultures were then harvested, transferred into the fresh medium, and grown for 3 h. Yeast total RNA was isolated using a Bio-Rad total RNA extraction kit. The amount of total RNA was quantified by monitoring absorbance at 260 nm (Sambrook *et al.* 1989). cDNA was synthesized using 0.5 µg of total extracted RNA from each of the strains by using iScript cDNA synthesis kit with SYBR green supermix (Bio-Rad Canada) according to the manufacturer instructions at 42°C for 45 min, and the reaction was stopped after 5 min by incubation at 85°C.

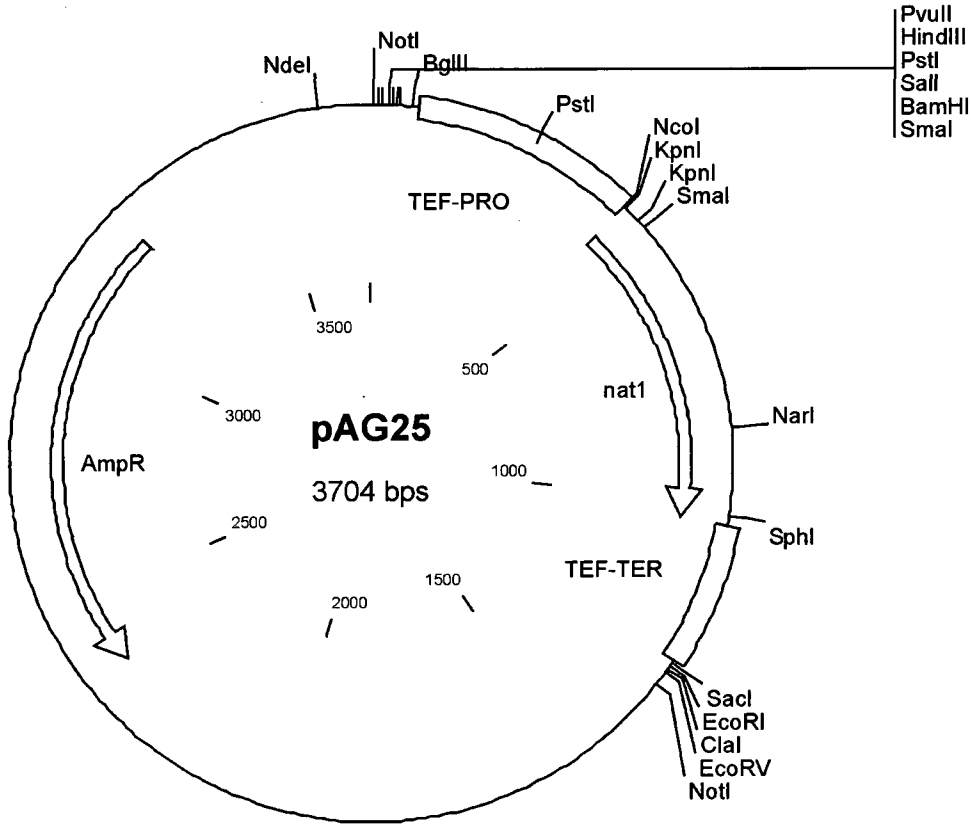
To quantify the amount of mRNA, real-time PCR analysis was performed using the Rotor Gene RG-300 from Corbett research. The forward (5'-ACTATCCCGACCGCCTTACT) and reverse (5'-TAGCGGCTGATGTTGAACTG) primers of *lacZ* gene was used to detect the expression level of *LacZ* mRNA in yeast strains. The PCR quantification and melting curves were generated using the Rotor gene 6 software. The amplification was performed with initial denaturation at 95°C for 10 min followed by 40 cycles at 95°C for 30 sec, 55°C for 20 sec and 72°C for 20 sec. The C_t value, the cycle when the sample fluorescence exceeded the threshold above background fluorescence, was determined using the Rotor gene 6 software. Quantification of mRNA were achieved by comparing the threshold cycle (C_t) value of the sample RNA from deletion strain with the C_t value of

WT strain's standard RNA (Yu *et al.* 2007). A negative control reaction in the absence of template was also performed to eliminate non-specific reactions.

2.11 Construction of Query Gene Deletion Strains

Query gene deletion strains were constructed by PCR-mediate gene deletion method. The target genes within *MATα* Y7092 strains were replaced with *NatMX4* using PCR-based transformation (Gola *et al.* 2003). Two gene-deletion primers were designed to contain 55 bp complementary to regions upstream (forward primer) and downstream (reverse primer) of the gene of interest. The lists of primers used in this study are listed in Table 2-3. These primers also include 22 bp complementary to upstream or downstream of *NatMX4* cassette. The *NatMX4* cassette with flanking sequences is amplified by PCR. Briefly, 0.5 μL forward and reverse primers of the gene of interest along with 0.5 μL of DNA template (pAG25 plasmid, Figure 2-3) (Goldstein and McCusker, 1999) were mixed in a PCR tube containing 14.5 μL of sterile H_2O (Table 2-4). A PCR mixture reagent (10X-PCR buffer: 2.0 μL, 10 mm $MgCl_2$: 0.6 μL, 10 mm dNTP: 0.4 μL and Taq polymerase: 0.3 μL) was added to each tube. The PCR amplification protocol consisted of 5 minutes at 95°C, followed by 35 cycles of 60 sec at 94°C, 60 sec at 48°C and 180 sec at 72°C and finally pause at 11°C. PCR products were visualized using gel electrophoresis on 0.8% agarose gel.

Figure 2-3: Map of plasmid vector pAG25.


PvuII
HindIII
PstI
SalI
BamHI
SmaI
NotI
NdeI
BglII
PstI
NcoI
KpnI
KpnI
SmaI
TEF-PRO
3500
500
nat1
NarI
3000
pAG25
AmpR
1000
3704 bps
2500
SphI
TEF-TER
1500
2000
SacI
EcoRI
ClaI
EcoRV
NotI

Parental yeast strain Y7092 that is suitable for SGA analysis was then transformed with the amplified DNA as described earlier by Ito *et al* (1983). Gene deletion is mediated by homologous recombination between the end target sequences of the PCR product and the corresponding genomic DNA sequences. Transformants carrying the target gene deletion (*gene xΔ::natR*) are selected on YEPD + clonNAT.

The replacement of target ORF with the *NatMX4* module was confirmed by colony PCR. Proper integration of *NatMX4* was tested using a combination of two confirmation primers specific to the yeast genome (at the target loci) and *NatMX4* gene. PCR reaction was prepared and amplified as above. PCR products were resolved on 0.8% agarose gel by electrophoresis at 80V for 45-60 mins.

Table 2-3: The list of primers used in this study.

Gene	ORF	Primer pairs
TAE1	YBR261C	F 5'-ACTCTGGTAATGTGCATCGGTGTCAAGAGCATCTCCAAGAAAGAATACATAAACAACAACG-3'
		R 5'-GATCAAGGATGCATAGATTTATATAGATACGCCCCTTTTCTTGTGCTGGCATTCACCATTT-3'
		Con-GGGAGAGCATAAAAGATATTTGG
TAE2	YPL009C	F 5'-AAGAAAGTTGCTACTTATTATCCGGTCTAAGAAGTCAGGCAGGCAAGAGATTAATAGCG-3'
		R 5'-ATCATATGAGATGAAAAGAAAATAGACAGCAATTATAATTTTTTCATTATTTTTCATCACAGA-3'
		Con-GACTTTCGATAAGTGTCAATAACCC
TAE3	YIL137C	F 5'-AAAAAAAGGTCCGAAGACGTGTAAAGGATATATAACGCCAGGTTGATCATCACTAACCATC-3'
		R 5'-TTTTTTACAAGTATTTCAACACACTATCGTACGTAGATTTAGTAGTCTTCTAAAATAGTAC-3'
		Con-GAAGCAGGACTTTAGCAAGAACCG
TAE4	YPL183W-A	F 5'-AACGGCTACGGTTCATAGTACAAATCTCAGAATTTTTCAGCAAGCGAAAGGATTTCAAAGA-3'
		R 5'-AAGATGTAAATCTCTTCGCTGTTCTCCGTTCGGGTGTTTACAAAATATTCTCATTTTCTAA-3'
		Con-TAGATATAGCGCTGTGCTGAACC
Nat-Conf		GTCACCCTTCCCCTTTCGT
Lac-Z		F 5'-ACTATCCCGACCGCCTTACT
		R 5'-TAGCGGCTGATGTTGAACTG

Table 2-4: Table for the mixture of PCR reaction.

	PCR amplification	Colony PCR
Primer F (Forward)	0.5 µL (F-Gene of interest)	0.5 µL (Confirmed primer of the gene of interest)
Primer R (Reverse)	0.5 µL (R-Gene of interest)	0.5 µL (Nat Confirm primer)
Template	0.5 µL (pAG25 plsmid)	0.5 µL (Deleted mutant cell)
Sterile H2O	14.5 µL	14.5 µL
10X PCR buffer	2.0 µL	2.0 µL
10 mm MGCl2	0.6 µL	0.6 µL
10 mm dNTP	0.4 µL	0.4 µL
Taq polymerase	0.3 µL	1.0 µL

2.12 Genetic Interaction Analysis

Deletion mutant of the query strains Y7092 (*MATα* strains) was constructed by replacing the gene with a dominant selectable marker *nat*MX as above. Deletion mutant strains were grown in YEPD medium overnight and transferred manually in omnitray at a density of 384 colonies arrayed onto each Omni plate. The query strains are crossed with a set of 384 opposite mating type *MATa* yeast gene deletion strains (*xxxΔ::kanR*). This set of genes are known or thought to be involved in translation. The mating step was carried out using a previously described **S**ynthetic **G**enetic **A**rray (SGA) analysis technique (Tong *et al.* 2001) (Figure 2-4). After manually mating the strains using a 384 handheld arraying device, the growths of the diploids were selected on YEPD medium containing G418 (200 μg/mL) and clonNAT (100 μg/mL). The heterozygous diploids were then transferred to a medium with reduced levels of carbon and nitrogen (1% potassium acetate, 0.1% yeast extract, 0.05% glucose, 2% agar, supplemented with uracil, histidine and leucine) for 5 days at 25^0C to induce sporulation and the formation of haploid meiotic spore progeny. Spores are transferred to synthetic medium (SD-His/Arg + canavanine (50 μg/mL), which allows for selective germination of *MATa* meiotic progeny (*MATa can1Δ::MFA1pr-HIS3::MFα1pr-LEU2*). The spores are then germinated on SC-His-Arg medium containing L-canavanine for 2 days at 30^0C and transferred to the same medium for another day under the same condition. The *MATa* meiotic progeny are then transferred to SC/MSG-His-Arg medium that contains G418 and L-canavanine, which selects for growth of meiotic progeny that carry the gene deletion mutation

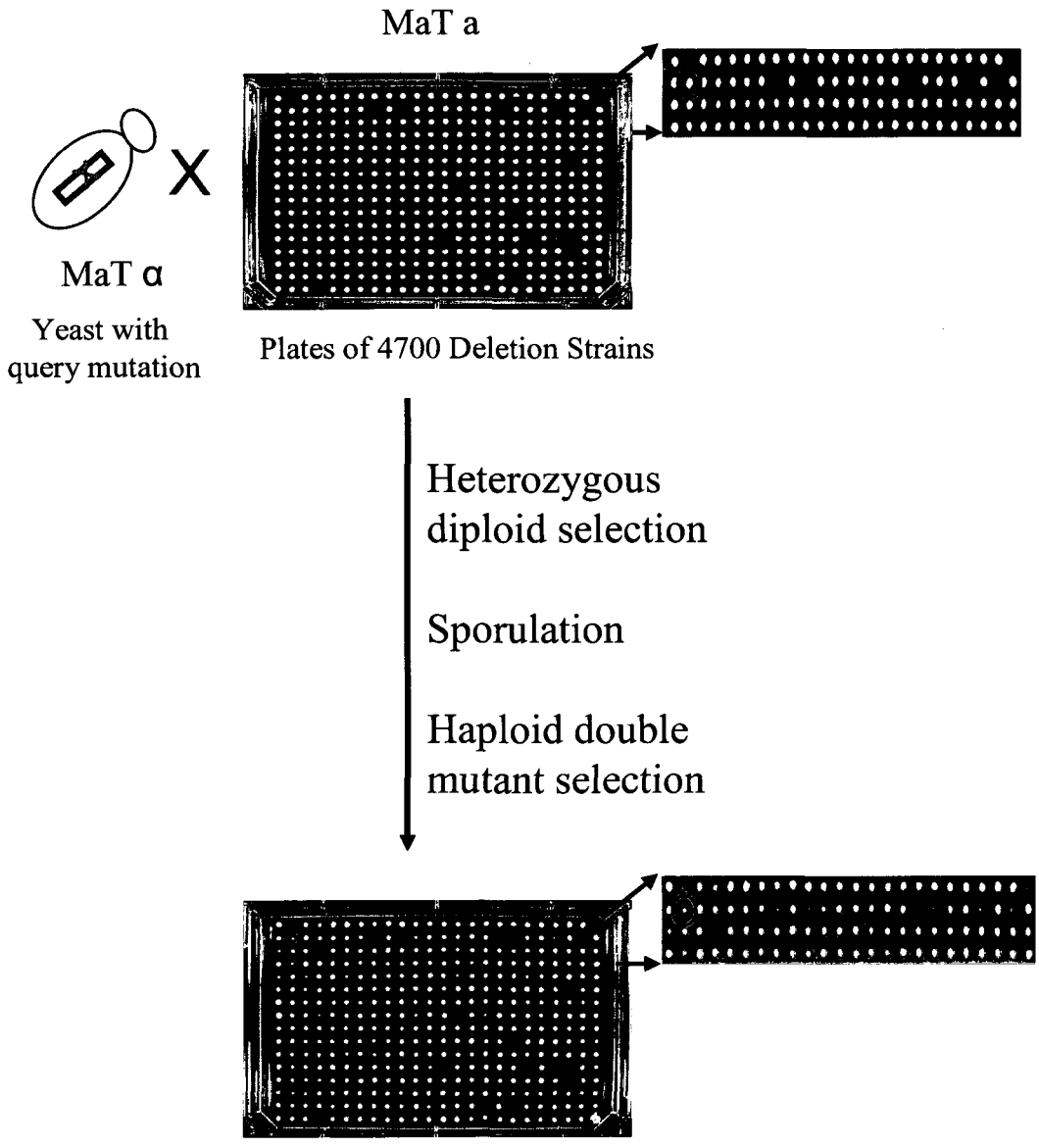
(*xxxΔ::kanR*). Finally, the *MATa* meiotic progeny are transferred to medium that contains both clonNAT and G418 (SD/MSG-His/Arg + canavanine + G418 + clonNAT) to select for growth of double mutant (*yyyΔ::natR xxxΔ::kanR*). We compared our analysis with a control set and scored the resultant double mutants for genetic sick interactions by both image analysis (Memarian *et al.* 2007) and visual inspection. Possible synthetic sick or synthetic lethal interactions were confirmed by **R**andom **S**pore **A**nalysis (RSA).

2.13 Random Spore Analysis (RSA)

Random spore analysis was performed as described previously (Tong *et al.* 2004). Strains carrying kanR-marker genes were mated with the yeast deletion query strains (*yyyΔ::natR*). The mated heterozygotes were then sporulated 7-9 days at room temperature. The spores were inoculated in 3ml of liquid haploid selection medium [synthetic dextrose (SD) medium lacking histidine and arginine but containing canavanine: SD-His/Arg + canavanine] and incubated at 30^0C for 2 days. The germinated *MATa* spore progeny were diluted in sterile ddH_2O and plated out on medium which selects for the query-gene mutation [(SD/MSG)–His/Arg + canavanine/clonNAT], the DMA mutation [(SD/MSG) – His/Arg + canavanine/G418], or both the query-gene and DMA mutations [(SD/MSG) – His/Arg + canavanine/clonNAT/G418], then incubated at 30^oC for ~2 days. Colony growth under the three conditions was compared and the double mutants were scored as synthetic sick (SS), synthetic lethal (SL) or no interaction (No).

Figure 2-4: Synthetic Genetic Array (SGA) analysis outline.

The query strain was grown in –URA liquid culture. *MAT***a**/α zygotes were pinned onto –URA+G418/clonNAT and incubated at 25°C for 9 days for sporulation. For both haploid progeny selections, thialysine was not used in selection plates.


MaT a
X
MaT α
Yeast with
query mutation
Plates of 4700 Deletion Strains
Heterozygous
diploid selection
Sporulation
Haploid double
mutant selection
Visualize growth on plate

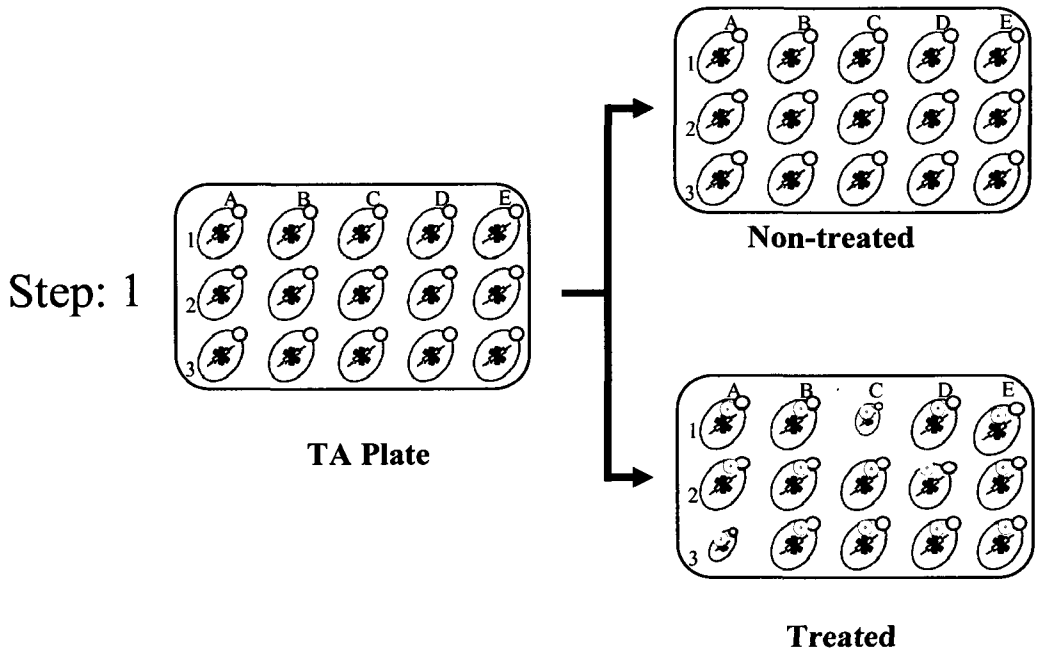
Alternatively, spores were resuspended in sterile dH2O and plated out on the haploid selection medium [SD-His/Arg + canavanine] and medium selecting for the query gene mutation, the deletion mutant array mutation, and both the query gene and DMA mutations, then incubated at 30^0C for 2 days. Colony growth under the four conditions was compared and double mutants were scored as synthetic sick (SS), synthetic lethal (SL) or no interaction (No).

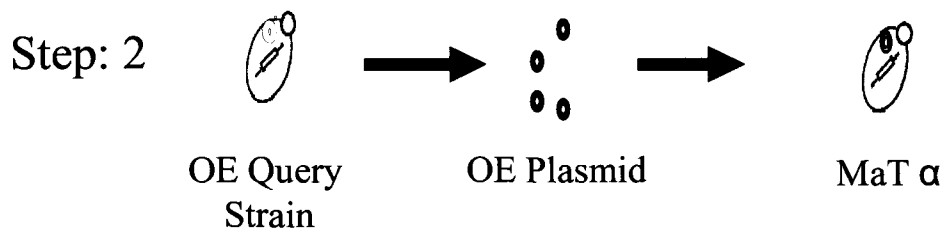
2.14 Phenotypic Suppression Analysis (PSA)

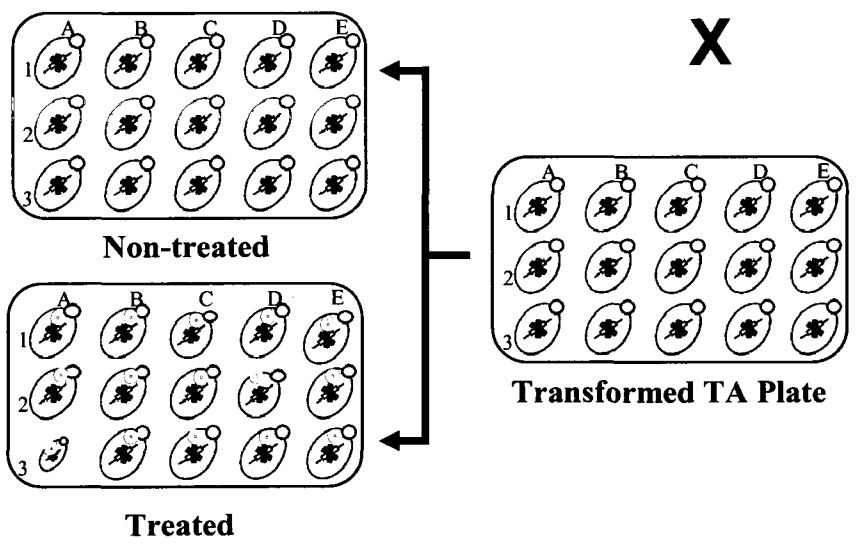
A yeast gene deletion array containing a plasmid with an inducible copy of an ORF tagged at amino terminus with GST and expressed from GAL1/10 promoter has been previously described (Sopko *et al.* 2001). In this array, the expression of the target gene is repressed by glucose and induced using galactose. The overview of the PSA is described in Figure 2-5. Target plasmids were isolated from corresponding overexpression strains and introduced into Y7092 (*MATα*) strain. The transformants were crossed with a target set of 384 *MATa* yeast gene deletion strains (*xxxΔ::kanR*). These deleted genes are known to be involved in translation. Sensitivity of yeast strains with or without overexpression construct against neomycin and streptomycin was observed and assessed using CS measurements (Memarian *et al.* 2007). Phenotypic complementations were divided into two categories of complete and partial suppressions. Partial complementation against both drugs was assigned as partial. Complete complementation against one drug, regardless of the other, was assigned complete.

Figure 2-5: Overview of **P**henotypic **S**uppression **A**nalysis (PSA).

Step1) A subset of yGDA, known to be involved in translation (translation array: TA) is exposed to aminoglycosides; the control plate has no antibiotics. Step 2) The overexpression plasmid for the query strain is transferred into a MaTα strain. The transformed MaTα strain is then crossed with the TA set. The mated set of strains carrying the target overexpression plasmid along with specific gene deletions in MATa background are further screened and compared for their sensitivity to aminoglycosides. Results are compared from both steps to determine those strains that suppress the inhibitory effects of aminoglycosides.


Step: 1
A B C D E
1
2
3
TA Plate
Non-treated
Treated


Step: 2
OE Query Strain
OE Plasmid
MaT α


X
A B C D E
1
2
3
Non-treated
Treated
Transformed TA Plate

3.0 CHEMICAL-GENETIC PROFILE ANALYSIS IN YEAST SUGGESTS THAT A PREVIOUSLY UNCHARACTERIZED OPEN READING FRAME, *YBR261C*, AFFECTS PROTEIN SYNTHESIS

3.1 Abstract

3.1.1 Background

Functional genomics has received considerable attention in the post-genomic era, as it aims to identify function(s) for different genes. One way to study gene function is to investigate the alterations in the responses of deletion mutants to different stimuli. Here we investigate the genetic profile of non-essential yeast **G**ene **D**eletion **A**rray (yGDA, ~4700 strains) for increased sensitivity to paromomycin, which targets the process of protein synthesis.

3.1.2 Results

As expected, our analysis indicated that the majority of deletion strains (134) with increased sensitivity to paromomycin are involved in protein biosynthesis. The remaining strains can be divided into smaller functional categories: metabolism (45), cellular component biogenesis and organization (28), DNA maintenance (21), transport (20), others (38) and unknown (39). These may represent minor cellular target sites (side-effects) for paromomycin. They may also represent novel links to protein synthesis. One of these strains carries a deletion for a previously uncharacterized ORF, *YBR261C*, that we term *TAE1* for **T**ranslation **A**ssociated **E**lement **1**. Our focused follow-up experiments indicated that deletion of *TAE1* alters the ribosomal profile of the mutant cells. Also, gene deletion strain for *TAE1* has defects in both translation efficiency and fidelity. Miniaturized synthetic genetic array analysis further indicates that *TAE1* genetically

interacts with 16 ribosomal protein genes. Phenotypic suppression analysis using *TAE1* overexpression also links *TAE1* to protein synthesis.

3.1.3 Conclusion

We show that a previously uncharacterized ORF, *YBR261C*, affects the process of protein synthesis and reaffirm that large-scale genetic profile analysis can be a useful tool to study novel gene function(s).

3.2 Introduction

The number of available sequenced genomes has provided the biologists with a wealth of sequence information containing thousands of genes. Many of these genes code for proteins with multiple functions, some of which are not known. Others code for proteins of completely unknown function(s). To tackle this challenge, several large-scale methodologies, under the term functional genomics, have been developed which aim at revealing putative gene functions (Gavin *et al.* 2002; Ito *et al.* 2001; Tong *et al.* 2001). Due to its simple genetics, ease of manipulation, and conserved pathways, the yeast *Saccharomyces cerevisiae*, emerged as a model organism of choice for functional genomics (Peña-Castillo *et al.* 2007). While significant knowledge has been gained from various large-scale investigations, more experiments are needed to uncover the details of the functions of genes involved in different cellular processes. Exploring the function of individual proteins can greatly advance our understanding of the biology of a cell as a system.

Genes, which are involved in similar pathways often genetically, interact with each other. Therefore, one way to study gene functions is to investigate the interactions they make with each other (Tong *et al.* 2001). This is based on the assumption that many eukaryotic pathways are functionally redundant. Thus, deletion of a gene may be tolerated with no phenotypic consequences. Inactivation of a second functionally related gene however, can cause sickness or even lethality. Therefore sickness of double mutants or "synthetic lethality" has been used to reveal novel gene functions. In simple terms, synthetic genetic array (SGA) analysis refers to large-scale investigation aimed at examining gene functions using double gene knockouts (Tong *et al.* 2001).

In addition to its role in functional genomics, availability of the yeast non-essential gene deletion array (yGDA, approximately 4700 strains) also provided the opportunity to investigate the cellular target sites of inhibitory compounds (Lopez *et al.* 2008; Parsons *et al.* 2006; Parsons *et al.* 2004). In this way, compounds with unknown cellular target sites are examined for their inhibitory effects on yGDA. The hypersensitive strains for genes with known functions are used to form a genetic profile for the activity of the target compound. This provides a fast and effective way to investigate cellular target sites of inhibitory compounds.

Similarly, inhibitory compounds with known modes of activity could be used to detect novel gene functions. This is not a novel concept and in various small-scale studies, numerous gene functions have been examined based on the increased sensitivity of their

gene deletion strains to different compounds (Lesage *et al.* 2004; Chloupková *et al.* 2003).

As a final step in the gene expression pathway, the regulation of protein synthesis (translation) is used to control the expression of a variety of genes under different physiological conditions. For example, during cell division in the early steps of embryonic development (Kuersten *et al.* 2003; Johnstone *et al.* 2001), or during cellular transformation and cancer development (Ruggero *et al.* 2003), and as well, in stress conditions and apoptosis (Holcik *et al.* 2005).

Even though the underlying principles of translation machinery have been the subject of vigorous investigations over the last few decades, details of all translation related proteins, protein complexes and pathways, as well as their communications and cross-talks with other cellular processes, have not been fully elucidated. Recently, several large-scale genomic investigations have uncovered numerous novel proteins thought to be functionally related to protein synthesis in *S. cerevisiae* (Fleischer *et al.* 2006; Krogan *et al.* 2004; Peng *et al.* 2003), suggesting that there remain other undiscovered translation proteins.

Here, we applied a large-scale chemical-genetic profile analysis to identify yeast deletion strains that show increased sensitivity to the aminoglycoside antibiotic paromomycin. This compound exerts its activity by targeting the process of protein synthesis. Focused follow-up experiments provided evidence that *YBR261C*, a previously uncharacterized

open reading frame (ORF) which is identified by this screen, may affects the process of protein synthesis in yeast.

3.3 Results

3.3.1 The Initial Screening and Identification of *TAE1*

In order to identify genes that affect protein synthesis, we screened the entire set of yGDA (~4700) for increased sensitivity to the aminoglycoside paromomycin. Paromomycin binds to the small ribosomal subunit of eukaryotic cells and compromises the translation fidelity (Fan-Minogue *et al.* 2008). Previously, it was shown that deletion of certain translation related genes caused increased sensitivity to paromomycin (Frigieri *et al.* 2008; Kressler *et al.* 1997). Therefore, we employed hypersensitivity to this drug as a way to detect novel gene candidates involved in translation. It should be noted that the deletion of certain translation related genes would cause increased resistance to paromomycin. This however, has not been investigated in our analysis.

We used yeast colony size reduction (CSR) as a tool to detect sensitivity to drug treatments. We have previously shown, that based on the parameters used by us, CSR analysis can detect approximately 63% of the sensitive strains that are detected by a large-scale spot test (ST) analysis with no repeats (Memarian *et al.* 2007). Hence, there are a number of sensitive strains that would be missed by our analysis that might otherwise be detected by ST. Similarly, the same large-scale ST analysis failed to detect

59% of the strains detected by CSR, which may represent novel/false-positives associated with CSR.

Here, we use sensitivity to paromomycin as a tool to detect protein synthesis related genes. A draw back for this, as well as other similar drug-based screening tests, is the detection of sensitive strains for those genes with no direct relation to the activity of the target drugs. A major source of such false-positives in our experiments may stem from those genes that play a role in general stress conditions. Multi-drug resistant genes represent some of these examples. For instance, it has been shown that the deletion of *QDR1*, a transporter gene and a member of efflux pumps, confers sensitivity to several unrelated drugs (Nunes *et al.* 2001). To increase the specificity of our selection procedure, we coupled our initial screen with a secondary search (based on the same parameters) for increased sensitivity to a second drug, which has no reported activity on the process of translation. For this purpose, we selected **C**alcofluor **W**hite (CW), which is known to inhibit cell wall function by binding with chitin (Hughes *et al.* 2000). In this way, only those gene deletions that conferred sensitivity to paromomycin alone may represent meaningful positives.

As expected, our large-scale approach identified numerous translation genes such as *TEF4* (translation elongation factor EF-1 gamma), *HCR1* (a component of translation initiation factor 3), *RPS18B* (a ribosomal protein of small subunit), etc, which are sensitive to paromomycin and not to CW. The complete list of these genes is found in Supplemental Table 8-1. The list of the genes that were sensitive to both paromomycin

and CW is found in Supplemental Table 8-2. Of the 325 gene deletions sensitive to paromomycin alone, we found 42 genes that often appeared in our similar drug screenings using different bioactive compounds. These may represent false-positives and should be treated with caution. From the 325 total reported genes, 134 have been previously linked to the overall process of protein biosynthesis. 191 of them however, have never been connected in any way to this process, and therefore, may also represent novel/false-positive genes. These genes can be further classified into 5 smaller categories based on the cellular processes in which they participate, plus those which are unknown. As indicated in Figure 3-1, the minor categories are: metabolism with 45 genes, cellular component biogenesis and organization with 28, DNA maintenance with 21, transport with 20 and others with 38 genes. There were also 39 genes, which have unknown functions. An explanation for these smaller categories is that they may represent minor cellular target sites (side-effects) for paromomycin. Alternatively, they may simply represent false-positives (see above). It is also possible that some of these genes might have novel roles during translation. In this case, they may represent communication bridges between different cellular processes and protein synthesis.

One of the previously uncharacterized ORFs identified in this screen is *YBR261C*. There is no reported information about this ORF, except that it is computationally predicted to have a methyltransferases domain (Katz *et al.* 2003). We therefore hypothesized that this might be a novel translation related gene. We termed this ORF, *TAE1*, for **T**ranslation **A**ssociated **E**lement 1, and subjected it to further analysis for its potential involvement in protein synthesis. ST analysis was used to confirm our large-scale observations (Figure 3-

2). When the growth medium was supplemented with a sub-inhibitory concentration of paromomycin (13 mg/ml), a deletion strain for *TAE1* (*tae1*Δ) showed a reduction in its growth pattern (Figure 3-2).

We then examined *tae1*Δ strain for its increased sensitivity to 3-amino-1,2,4-triazole (3-AT) and cycloheximide. 3-AT can affect translation by altering the pool of amino acids in the cell (Zhou *et al.* 2003). Cycloheximide binds to large ribosomal subunit (Leeds *et al.* 1992), and inhibits translation elongation by interfering with tRNA translocation (Obrig *et al.* 1971). It has previously been reported that deletion of certain genes that affect translation may confer sensitivity to multiple drugs that target translation. For example, deletion of Sfp1, which regulates ribosome biogenesis, confers increased sensitivity to both cycloheximide and paromomycin (Fingerman *et al.* 2003). Shown in Figure 3.2, our spot test analysis indicated that in addition to paromomycin, *tae1*Δ also showed increased sensitivity to 3-AT. Also some sensitivity for *tae1*Δ to cycloheximide was occasionally observed. However, due to irreproducibility of these observations, this sensitivity was presumed to be an artifact.

Figure 3-1: Distribution of gene deletion strains with sensitivity to paromomycin.

Distribution of paromomycin hypersensitive yeast deletion strains in percentages according to their cellular functions.

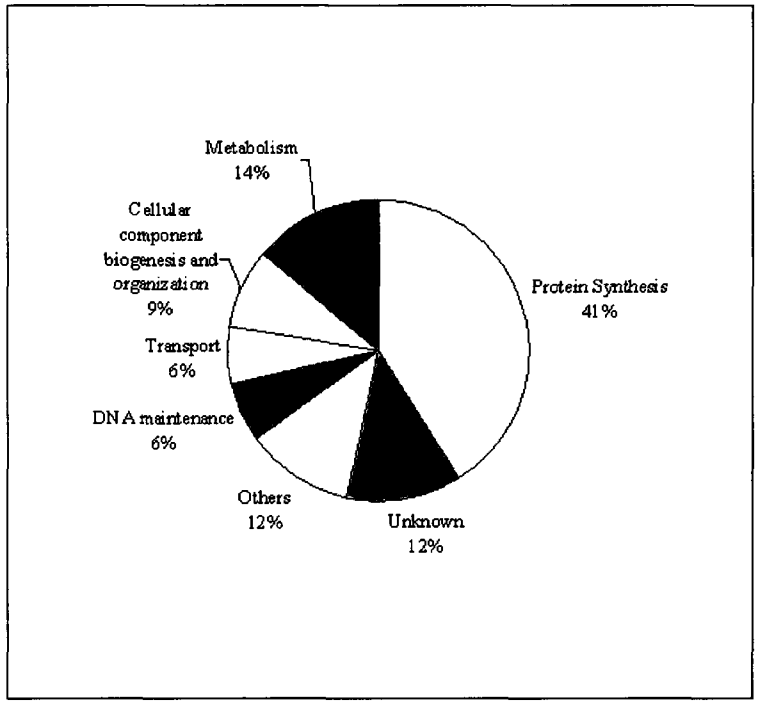
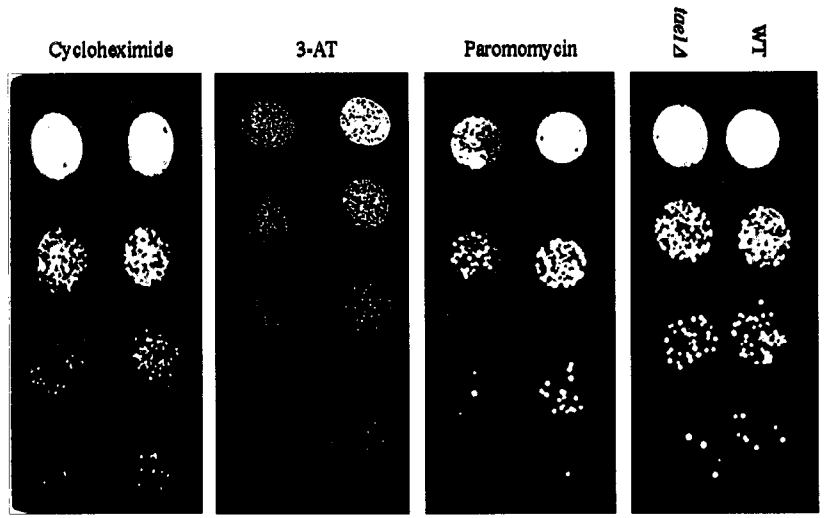

Metabolism
14%
Cellular
component
biogenesis and
organization
9%
Transport
6%
DNA maintenance
6%
Others
12%
Unknown
12%
Protein Synthesis
41%

Figure 3-2: Increased sensitivity of *tae1Δ* to different translation inhibitory drugs.

Deletion of TAE1 confers increased sensitivity to different drugs that target translation. Decreasing numbers of wild type and mutant (*tae1Δ*) yeast cells were spotted on solid medium. The medium was supplemented with sub-inhibitory concentrations of paromomycin (13 mg/ml), 3-AT (22 mg/ml), cycloheximide (45 ng/ml), or none (used as a control and shown in the top panel). Standard organic (YEPD) medium was used for cycloheximide and synthetic complete (SC) medium was used for paromomycin and 3-AT plates, and for the control plate shown here. Yeast cells were grown to mid-log phase and diluted 10^{-3} to 10^{-6} folds. 20 μL of each dilution (gradually decreasing) was spotted onto the medium and grown at 30°C for 1–2 days. Deletion of *TAE1* conferred increased sensitivity to paromomycin and 3-AT. Occasional sensitivity to cycloheximide was assumed to be an artifact.


Cycloheximide
3-AT
Paromomycin
tad1 Δ
WT

During the preparation of this manuscript, a new study was reported in which sensitivity of yeast gene deletion mutants to paromomycin were investigated using a heterozygous diploid yeast gene deletion mutant array (Hillenmeyer *et al.* 2008). This system is different from ours (haploid based), as its diploid mutant strains always carry a copy of the wild type genes and consequently show less obvious growth defects (Deutschbauer *et al.* 2005). Of the 51 mutant strains that were identified by the authors as sensitive to paromomycin, 39 are shared between the two systems. Of these, 16 mutants were also identified by our haploid system. It is worth mentioning that *tae1*Δ was not detected to have increased sensitivity to paromomycin in the heterozygous system, further highlighting the difference between the two systems (Hillenmeyer *et al.* 2008).

3.3.2 The Effect of *TAE1* Gene Deletion on Protein Synthesis

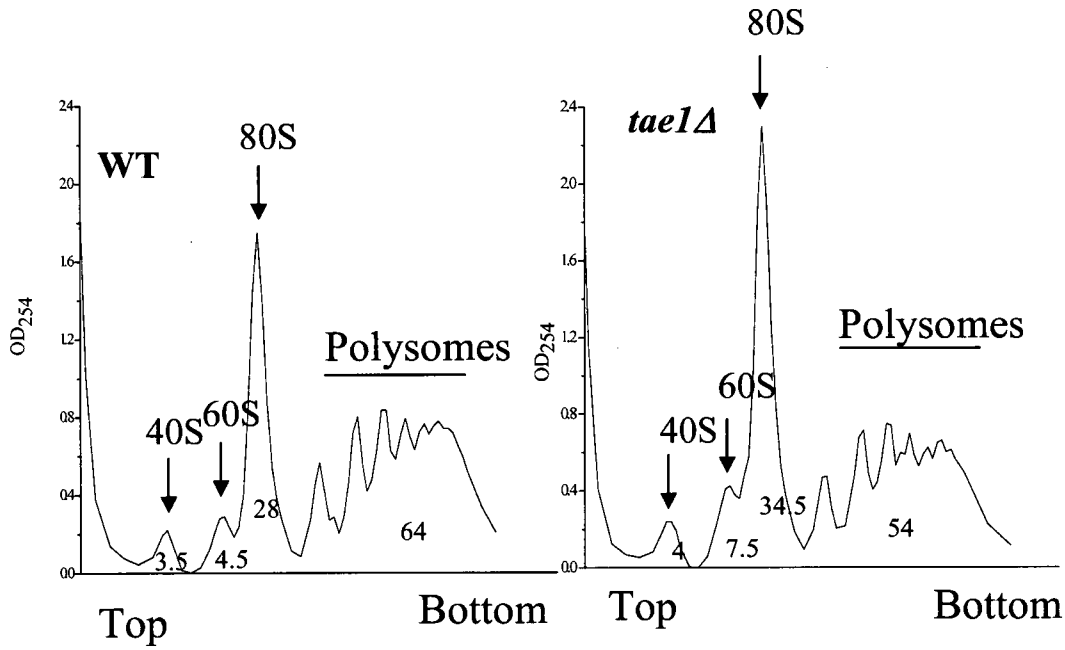
Translation genes can be involved in different aspects of translation. To examine the involvement of novel genes in protein synthesis, we divided translation into three general categories. Group one includes those genes that are associated with ribosome biogenesis, group two contains genes that alter translation efficiency, and group three is composed of the genes that affect translation fidelity. Depending on their molecular function(s), some translation genes may fall into none (Walter *et al.* 1999), one (Fingerman *et al.* 2003; Iouk *et al.* 2001) or more (Dinman *et al.* 1997) of these three categories. If *TAE1* is involved in the process of translation, it might fall into one or more of these categories.

3.3.2.1 Involvement of *TAE1* in Ribosome Biogenesis

Ribosome biogenesis and assembly is one of the most important processes in the protein synthesis pathway (Venema *et al.* 1999). This process might be explained as the overall step that leads to the formation and assembly of functional ribosomes and includes regulation of pre-rRNA transcription, and its corresponding mechanisms, pre-rRNA processing, rRNA transport, rRNA maturation, ribosome assembly, etc. (Rosado *et al.* 2007). We therefore reasoned that depending on the molecular function(s) of *TAE1*, if this gene is involved in ribosome biogenesis or assembly, its deletion might result in the alteration of the profile of ribosomal subunits. To examine this possibility, ribosome profile analysis was performed. As expected, distinct 40S and 60S subunit peaks, as well as 80S monosome and polysome peaks (Figure 3-3) were detected in both the wild type and *tae1Δ* strains. In addition, *tae1Δ* strain showed a reduction in polysomes and corresponding increases in 80S monosomes and 60S subunits (Figure 3-3). These alterations in ribosomal profile further indicate the involvement of *TAE1* in protein synthesis.

Figure 3-3: Ribosome profile analysis of yeast strains.

Deletion of *TAE1* results in an overall decrease in polysomes and a corresponding increase in 80S monosome formations. There is also an increase in 60S subunit in *tae1Δ* cells. The large polysomes were collected at the lower half of the gradients ("Bottom", x-axis). Each experiment was repeated three times with similar outcomes.

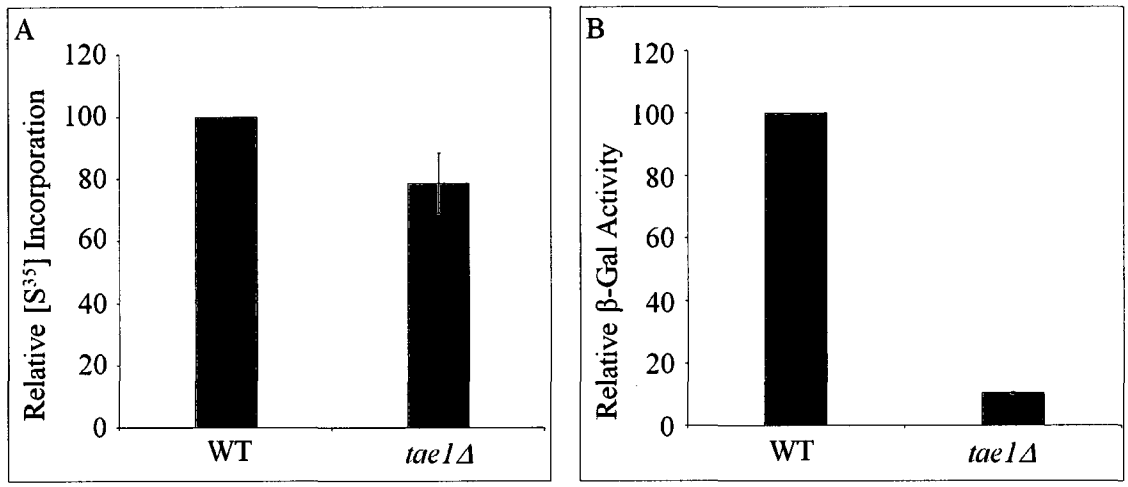

WT
80S
Polysomes
40S
60S
28
64
3.5
4.5
OD254
Top
Bottom
tae1Δ
80S
Polysomes
60S
40S
34.5
54
4
7.5
OD254
Top
Bottom

3.3.2.2 Involvement of *TAE1* in Translation Efficiency

If *TAE1* is involved in protein synthesis, then based on its molecular function(s), its deletion may alter the cell's efficiency to synthesize proteins. To investigate this possibility, we used (^{35}S) methionine incorporations to measure the rate of total protein synthesis in different strains. As indicated in Figure 3-4: A, it was observed that on average, *tae1Δ* had a reduced level of (^{35}S) methionine incorporation (approximately 22%) compared to the control strain. To confirm this observation, we used an inducible β-galactosidase reporter construct (Figure 3-4: B). It was observed that the deletion of *TAE1* reduced the level of β-galactosidase synthesis from an inducible expression plasmid by approximately ten-fold (Figure 3-4: B). These observations suggest that deletion of *TAE1* reduces the efficiency of protein synthesis in a cell, and provides further support that *TAE1* affects protein synthesis.

Figure 3-4: The efficiency of protein synthesis in the presence and absence of Tae1.

In (A) total protein synthesis is measured using [^{35}S] methionine incorporation. The average unit of [^{35}S] methionine incorporation for wild type is 11356073 (±1300000) units which is correspondent to 100% and for *tae1Δ* is 8864201 (±6000000) units. In the absence of Tae1, total protein production is reduced by approximately 22%. In (B) the efficiency of protein synthesis is measured using an inducible β-galactosidase reporter gene. The average β-galactosidase activity for wild type is 7.51 (±0.6) units which is set to 100% and for *tae1Δ* is 0.78 (±0.4) units. In the absence of Tae1, an approximately ten-fold reduction in β-galactosidase production is observed.


A
Relative [S35] Incorporation
120
100
80
60
40
20
0
WT
tae1Δ
B
Relative β-Gal Activity
120
100
80
60
40
20
0
WT
tae1Δ

3.3.2.3 *TAE1* and Translation Fidelity

To make a functional protein, the fidelity of protein synthesis is maintained at almost all stages of translation. This fidelity is controlled during the start site selection and elongation when the mis-incorporation of the wrong amino acid may alter the integrity of the final product, as well as during termination, when a stop codon might be read through. If *TAE1* is involved in translation, then it might be expected that based on its molecular function(s), the deletion of this gene may alter the fidelity of translation. Translation fidelity can be studied using specialized expression systems such as those that contain β-galactosidase expression cassettes, with premature stop codons (Stansfield *et al.* 1995). In this investigation, we used plasmids pUKC817 and pUKC818 that contain *lacZ* genes carrying the nonsense codons UAA and UAG, respectively (Stansfield *et al.* 1995). It was observed that the deletion of *TAE1* resulted in an increased level of read through for the nonsense codons investigated, suggesting that in this deletion strain, the translation fidelity seem to be compromised (Figure 3-5: A). For unknown reasons, the average values for β-galactosidase activities in the wild type strain, were systematically higher than expected.

Since differential levels of β-galactosidase activity in the above experiments may also stem from altered levels of mRNAs, the content of β-galactosidase mRNAs of WT and *tae1*Δ were investigated using Q-RT-PCR. We observed no noticeable variation in the amounts of these mRNAs (Figure 3-5: B), which could explain the observed difference for the β-galactosidase activities. It was therefore concluded that the observed differences

likely stem from translation read through. Altogether, the results indicate that the deletion of *TAE1* seems to compromise translation fidelity, providing further evidence that *TAE1* affects translation.

3.3.3 *TAE1* Genetically Interacts With Translation Related Genes

The genes that are functionally related and are involved in similar pathways, often genetically interact with each other. Consequently, studying the genetic interactions of a novel gene is often used as a method to infer the function of that gene (Ideker *et al.* 2008; Boone *et al.* 2007). If *TAE1* is a true translation gene, then it might be expected that *TAE1* would genetically interact with certain known translation associated genes. To investigate this possibility, we examined the genetic interactions of *TAE1* with a set of 384 genes, which are known or thought to be involved in translation. As indicated in Figure 3-6, it was observed that *TAE1* genetically interacted with numerous translation related genes to produce sick phenotypes, which are classified as i) very sick, ii) sick, and iii) moderate. Lethal interactions were not considered. The interactions between *TAE1* and translation related genes were further divided into three categories with ribosomal proteins forming the largest cluster (16 genes), followed by those involved in amino acids and protein production (7 genes), and those involved in rRNA synthesis (2 genes). Descriptions of these genes are listed in Supplemental Table 8-3. The fact that *TAE1* genetically interacts with different translation associated genes provides further evidence for the involvement of *TAE1* in the process of translation.

Figure 3-5: Effect of TAE1 deletion on translation fidelity.

(A) Deletion of *TAE1* resulted in increased levels of β-galactosidase from *lacZ* reporters with premature stop codons (pUKC817 and pUKC818). The average β-galactosidase unit for wild type is 19.05 (±1.1) and for *tae1Δ* is 16.82 ((±2.5) units, which is set to 100%. (B) Q-RT-PCR analysis indicates that alterations for the relative contents of LacZ mRNAs do not explain the difference in β-galactosidase productions observed in (A). The C_t for the control and experimental samples were calculated from the threshold cycles. pUKC815 is the background construct without a premature stop codon and used as a control. All assays were done in triplicate.

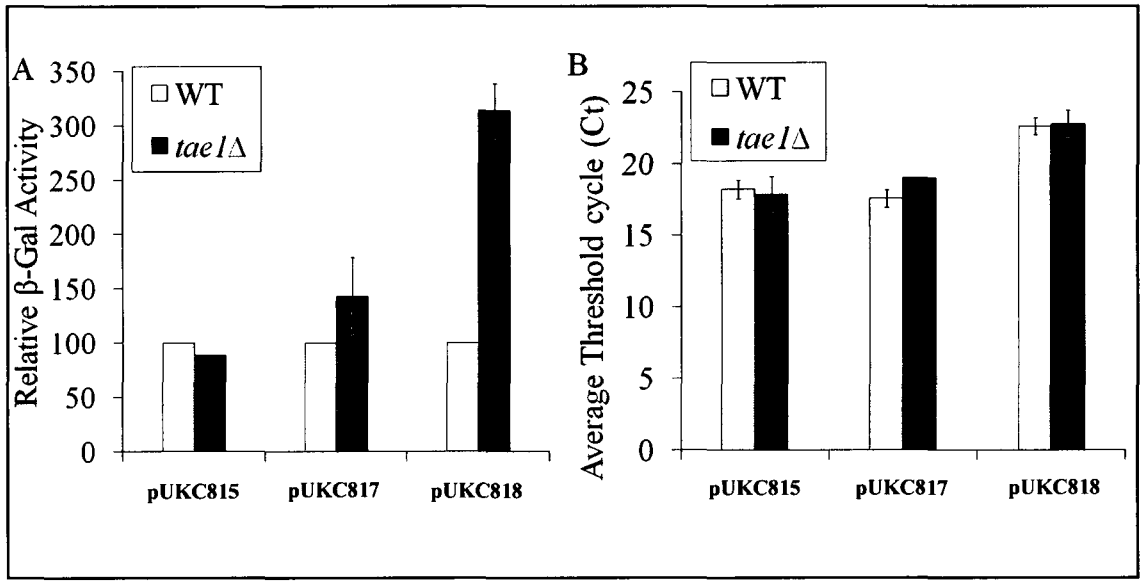
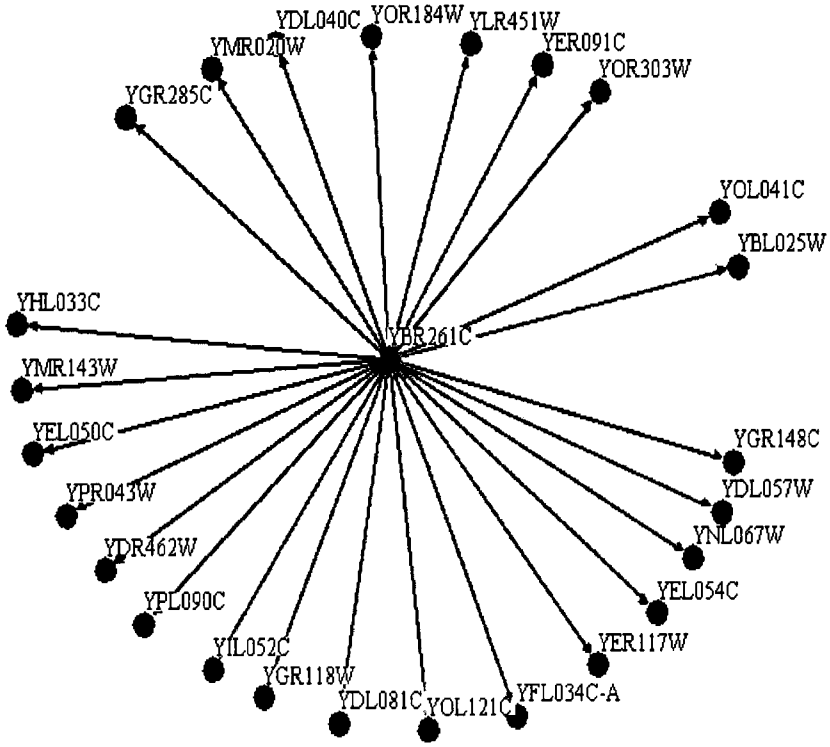

A
Relative β-Gal Activity
350
300
250
200
150
100
50
0
WT
tae1Δ
pUKC815
pUKC817
pUKC818
B
Average Threshold cycle (Ct)
25
20
15
10
5
0
WT
tae1Δ
pUKC815
pUKC817
pUKC818

Figure 3-6: Genetic interaction of *TAE1* with translation genes.

TAE1 genetically interacts with numerous translations related genes. These interacting genes could be further divided into two major categories of ribosomal subunits (16 genes; blue circles) and amino acid and protein biosynthesis (7 genes; black circles). Very sick, sick and moderate interactions are shown by red, green and blue edges, respectively.


YDL040C
YOR184W
YLR451W
YMR020W
YER091C
YOR303W
YGR285C
YOL041C
YBL025W
YHL033C
YBR261C
YMR143W
YEL050C
YGR148C
YPR043W
YDL057W
YDR462W
YNL067W
YPL090C
YEL054C
YIL052C
YER117W
YGR118W
YDL081C
YOL121C
YFL034C-A

3.3.4 Phenotypic Suppression by the Overexpression of *TAE1*

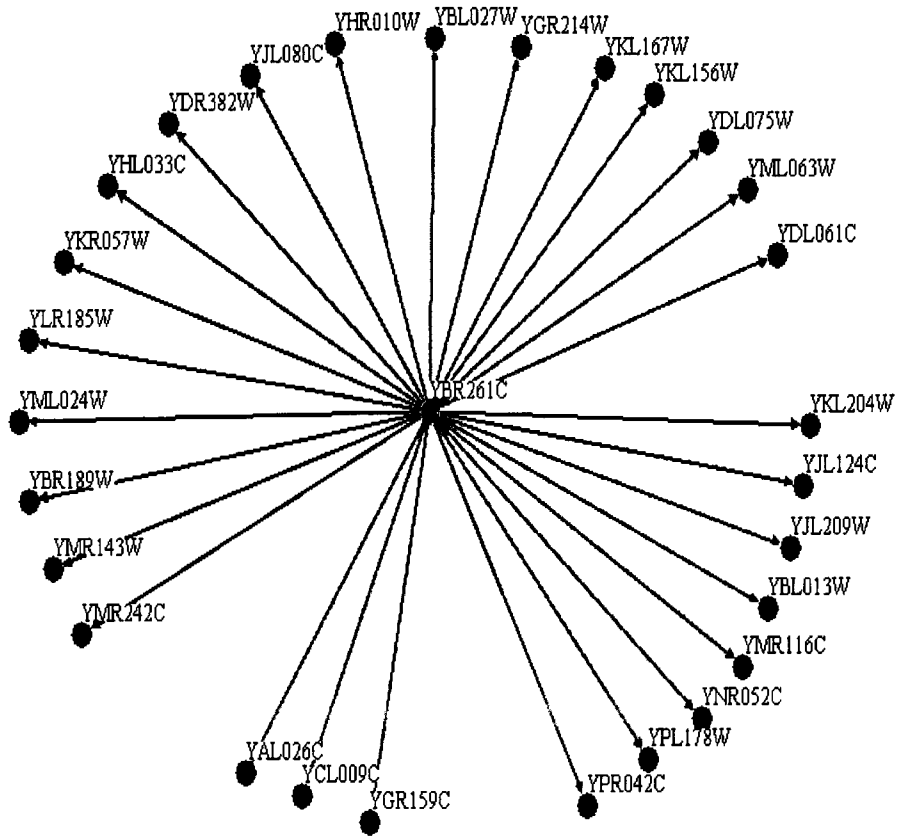
To examine the cellular activity of Tae1 protein (Tae1p), we employed a high throughput approach, based on the phenotypic suppression of the gene deletion mutants that have known functions. Deletion of genes, which are involved in a specific pathway, may cause increased sensitivity to treatments that target the same process. Such hypersensitivities can be compensated by the overexpression of other genes with similar cellular functions. For example, it has previously been reported that the absence of Yku80, involved in telomere maintenance, causes increased sensitivity to elevated temperature. Overexpression of either Est2, a catalytic subunit of telomerase, or Tlc1, the RNA template component of telomerase, compensated for the absence of Yku80 and reversed the heat hypersensitivity of *yku80Δ* (Teo *et al.* 2001).

Here, we investigated the activity of Tae1p by examining the effect of its overexpression in suppressing hypersensitivities to antibiotics neomycin and streptomycin for the above 384 translation related gene deletion yeast strains. Like paromomycin, the antibiotics neomycin and streptomycin, belong to the aminoglycoside family, which binds to ribosomes and disrupts translation (Walter *et al.* 1999). We observed that overexpression of Tae1p, suppressed the drug sensitivity phenotypes for 28 deletion mutants of known translation genes (Figure 3-7). These 28 mutants showed sensitivity to treatment with neomycin and/or streptomycin. Tae1 overexpression however, reversed the observed drug sensitivities. These 28 gene deletion mutants can be categorized into two main groups of gene deletions for ribosomal proteins (17 genes), and those for translation control proteins (8 genes). Descriptions of the deleted genes are listed in Supplemental

Table 8-4. The fact that overexpression of Tae1 suppresses the phenotypes of deletion mutants for translation genes, further confirms an involvement for *TAE1* in protein synthesis.

Figure 3-7: Overexpression of *TAE1*.

Overexpression of *TAE1* phenotypically suppresses the hypersensitivity of numerous translation genes against drug treatments. Overexpression of *TAE1* suppressed the inhibitory effects of either or both neomycin and/or streptomycin for 28 yeast gene deletion strains. Among them, 17 genes belong to ribosomal proteins (blue circles) and 8 genes are involved in translation control (black circles). Red circles represent other genes. Complete and partial suppressions are shown by green and blue edges, respectively.


YHR010W
YBL027W
YGR214W
YJL080C
YKL167W
YKL156W
YDR382W
YDL075W
YHL033C
YML063W
YKR057W
YDL061C
YLR185W
YBR261C
YML024W
YKL204W
YBR189W
YJL124C
YMR143W
YJL209W
YMR242C
YBL013W
YMR116C
YNR052C
YAL026C
YPL178W
YCL009C
YPR042C
YGR159C

3.4 Discussion

Annotating gene functions has been an important aspect of post-genomic era. Identifying "which gene does what" is one of the fundamental tasks of systems biology, and sets the basis for understanding the biology of a cell. There are currently numerous uncharacterized genes with no known functions (Peña-Castillo *et al.* 2007). Moreover, there are numerous genes with multiple functions, some of which are not yet elucidated.

One approach to study gene function is to make gene knockouts, and observe the mutant cells' behavior to internal and/or external stimuli. Here, we screened the set of yGDA for sensitivity to paromomycin, which is known to target protein synthesis machinery. Due to an increased sensitivity, we hypothesized that a yeast gene deletion strain for *TAE1* might affect the process of protein synthesis. We observed that the deletion of *TAE1* reduced translation fidelity. This observation might be expected, since paromomycin is known to decrease the fidelity of translation. We also observed that deletion of *TAE1* reduced the efficiency of translation, which is not necessarily coupled with translation fidelity, suggesting a wide-range effect for *TAE1* on translation. The follow-up experiments were done atleast triplicate. For statistical analysis, we use standard error bars to estimate the standard deviation of the sample.

Tae1p is found in the cytoplasm and is bioinformatically predicted to contain an S-adenosylmethionine-dependent methyltransferase activity. Certain members of this

family of proteins have been shown to methylate different components of the translation machinery. For example, DIM1 and SPB1, which are nucleolar proteins involved in rRNA methylation; TRM proteins (such as TRM1 and TRM2), which are tRNA methyltransferases found in both cytoplasm and nucleus; and Mtq2, which methylates translation release factor SUP45 and is found in both cytoplasm and nucleus. Presuming that Tae1 has a methyltransferase activity, we can assume that Tae1 affects translation by methylating a component of translation machinery.

In agreement with the observed reduction in translation efficiency for *tae1Δ*, our ribosomal profile analysis indicated an overall decrease in polysomes when *TAE1* was deleted. In contrast, the 60S free subunits were specifically accumulated in *tae1Δ* cells. Since 40S and 60S subunits are in equilibrium with 80S monosomes, the increase in 60S subunit may indicate a defect in 40S biogenesis (Vlásek *et al.* 2001). Assuming that *TAE1* is a methyltransferase, a possible explanation is that *TAE1* may affect 40S biogenesis by either methylating 18S rRNA directly, or by methylating a factor which might affects 40S biogenesis. In agreement with a role for *TAE1* in ribosome biogenesis, *TAE1* is found to be co-regulated with a number of ribosome processing factors in at least five different microarray analyses (*Saccharomyces* Genome Database. http://www.yeastgenome.org/).

There is also an accumulation of 80S monosomes in *tae1Δ* cells, which may indicate a defect in translation initiation. Formation of defective ribosomes in the absence of *TAE1* that cannot readily start elongation, may explain this accumulation of 80S monosomes.

Alternatively, it is possible that *TAE1* might affect translation initiation by modifying a translation initiation protein. The latter explanation however, cannot explain the accumulation of 60S subunits. Regardless, further experiments are required to investigate details of the molecular activity of *TAE1*, and to identify its potential substrate(s).

Our genetic analysis revealed that *TAE1* genetically interacts with a number of translation genes. In accord with the above-suggested function for *TAE1* in ribosome subunit biogenesis, the majority of the observed interactions were found to be with ribosomal protein genes. Similarly, our phenotypic suppression analysis indicated a functional compensation by *TAE1* overexpression, for the absence of 17 different ribosomal proteins against drug treatments. It should be noted that our genetic and phenotypic suppression analysis resulted in two different sets of proteins. This is expected, as genetic interaction analyses generally target the genes involved in different pathways within a process (redundant pathways), whereas phenotypic suppression analyses generally target genes within the same pathway. This data can be further used to study the detailed mechanism of *TAE1* activity.

4.0 CHEMICAL-GENETICS PROFILE ANALYSIS OF FIVE INHIBITORY COMPOUNDS IN YEAST

4.1 Abstract

4.1.1 Background

Chemical genetics profile of inhibitory compounds can lead to identification of their mode of actions. These profiles can also help elucidate the complex interactions between the small bioactive compounds and the cell machinery.

4.1.2 Results

Here, we use CSR to investigate the chemical genetic profile of cycloheximide, 3-amino-1,2,4-triazole (3-AT), paromomycin, streptomycin and neomycin, in the yeast, *Saccharomyces cerevisiae*. The array of gene deletion mutant yeast strains were used for a total of more than 70,000 strains analyses. The overall profiles of the tested compounds were very similar to each other with deletions for genes involved in protein biosynthesis as the major category followed by metabolism. We followed up our results by investigating the activity of three profiled genes using relative fitness of double mutants and other genetic assays.

4.1.3 Conclusion

Our chemical genetics profiles provide further insight into the molecular mechanism of the examined compounds by elucidating clues into their primary and secondary cellular target sites. Our investigations in the activity of the profiled genes provide further evidence for the usefulness of chemical genetics analyses in annotating gene functions.

4.2 Introduction

Predicting gene function is one of the major goals of systems molecular biology in the post genome sequencing eras. In this context, the yeast *Saccharomyces cerevisiae* has emerged as the eukaryotic model organism of choice for large-scale functional genomics investigations. Yeast cells have been subjected to a number of high throughput investigations such as gene expression analysis (Ghaemmaghami *et al.* 2003) protein-protein interaction mapping (Ito *et al.* 2001; Uetz *et al.* 2000) and synthetic genetic interaction analysis (Tong *et al.* 2001). Although much has been learned about the functions of yeast genes, there remains a significant portion of the genes that are still uncharacterized in this model organism (Peña-Castillo and Hughes, 2007). Consequently more studies are needed to examine the function(s) of uncharacterized genes, and to investigate novel function(s) for genes that are not fully characterized.

Increased sensitivity of gene deletion mutant strains to inhibitory compounds has been extensively used to study gene functions (Chloupková *et al.* 2003; Lesage *et al.* 2004). This approach is partly based on the theory that in general the presence of redundant pathways compensates for the genetic inactivation of a single pathway, with no phenotypic consequence (Hartman *et al.* 2001). However, the inactivity of a second functionally overlapping pathway, in this case using a chemical treatment, can cause a "double hit" effect and result in a phenotypic consequence that can be scored as a reduction in the rate of growth, or a sick/sensitive phenotype (Tong *et al.* 2001; Parsons

et al. 2004). Similarly, such chemical genetics profile analyses can also be used to study cellular target sites of various bioactive compounds (Parsons *et al.* 2004), pharmaceuticals (Baetz *et al.* 2004) and herbal extracts (Galván *et al.* 2008) with unknown mechanisms of activities.

In general, chemical sensitivity profile of yeast gene knockout can be studied using three complementary high throughput approaches. In the first case, deletion mutants can be individually grown in liquid cultures and their growth rates can be monitored spectrophotometrically using a microplate reader. In this case the growth curve of micro-cultivated mutant strains in the presence and absence of a bioactive compound is used to determine strain sensitivity (Warringer *et al.* 2008; Warringer *et al.* 2003; Engler *et al.* 1999). The second approach is based on **S**ynthetic **L**ethality **A**nalysis on **M**icroarray (SLAM) (Ooi *et al.* 2003). A pool of tagged deletion strains is first grown in the presence and absence of the target compounds. Then, due to the presence of a specific barcode in each mutant strain, the relative growth of each strain can be determined using microarray methodology. In this case, sensitivity is measured based on the relative growth of a specific mutant strain in the presence of other strains. In the third approach, colonies of yeast gene deletion mutant strains can be arrayed on solid medium in the presence and the absence of the target compounds (Parsons *et al.* 2004; Davis-Kaplan *et al.* 2004). The growth rates of individual colonies are estimated by their relative CS and compared to a control. Each of these techniques has inherent advantages and disadvantages. The results obtained from these methodologies are thought to be complementary (Pan *et al.* 2004).

Here, we used CSR to screen and analyze the yeast gene knockout collection for their sensitivity to five bioactive compounds. We followed up by studying the activity of three profiled genes.

4.3 Results

4.3.1 Drug Sensitivity Screens

We screened the entire collection of the haploid yeast gene deletion array (yGDA) (~4700) for their increased sensitivity to bioactive compounds cycloheximide, 3-AT, paromomycin, streptomycin and neomycin. These drugs all have reported activities on the process of protein biosynthesis or translation. Cycloheximide is a glutarimide antibiotic that binds to the 60S ribosomal subunit and inhibits translation elongation (Schneider-Poetsch *et al.* 2010). 3-AT is a competitive inhibitor of imidazole glycerol phosphate dehydratase, an enzyme involved in the amino acid histidine biosynthesis (Hinnebusch, 2005) and causes amino acid starvation (Zhou *et al.* 2003). Paromomycin, neomycin and streptomycin are known to bind small ribosomal subunit of eukaryotic cells and inhibit ribosomal translocation and compromise translation fidelity (Tuite and McLaughlin, 1984). We reasoned that sensitivity to these drugs might be used as a method to identify new genes that are associated with the process of protein biosynthesis. In this study, sub-inhibitory concentrations of the drugs were used. Under such growth conditions only the strains with increased sensitivity would show growth reductions and the growth of remaining strains would be largely unaffected. Each experiment was

repeated three times bringing our total number of analyses to more than 70,000. We have previously reported our analysis of sensitivity to paromomycin alone (Alamgir *et al.* 2008). Here, we present our collective analysis of the total data from the entire collection of sensitive strains.

Colony **S**ize (CS) measurement was used to determine sensitivity. This was done by analyzing the relative colony growths (normalized to the average growth on the plate) in the treated plates, compared to those grown under control condition (untreated) as described in (Memarian *et al.* 2007). CS measurement has been repeatedly used to identify drug sensitive strains (Davis-Kaplan *et al.* 2004; Parsons *et al.* 2006; Parsons *et al.* 2004) and is reported to identify approximately 63% of the sensitive strains that are detected by standard large-scale **S**pot **T**est (ST) analysis (Memarian *et al.* 2007). Therefore, there are a number of sensitive strains that would be missed by CS that might be detected by ST analysis, indicating that our current analysis using CS to identify sensitivity is not exhaustive. Similarly, it is reported that 59% of the sensitive strains detected by CS are not detected by ST and hence may represent novel/false positives.

To reduce false positives, gene deletion strains with sensitivity to different unrelated bioactive compounds that we continuously observed in our previous independent screens, as well as those reported by others (Hillenmeyer *et al.* 2008) were eliminated from the list of the sensitive strains. These genes typically represent multiple-drug resistant genes and are generally not linked to the cellular target sites of the drugs of interest. Inclusion of these genes can complicate the analysis of the molecular activity of the target compound.

The final list of the genes that when deleted increased drug sensitivity to the tested bioactive compounds is reported in Supplementary Table 8-5. There are 384, 320, 205, 99 and 89 genes that when deleted confer increased sensitivity to cycloheximide, 3-AT, streptomycin, neomycin and paromomycin, respectively. These are non-essential genes that ordinarily are not required for growth of yeast cells under typical laboratory conditions, suggesting that the slow growth phenotype of their deletion strains is a direct result of the inhibitory effects of the target drugs. Cycloheximide, streptomycin, neomycin and paromomycin are known to bind to ribosomes and cause defects in protein synthesis (Wang *et al.* 2008; Chernoff *et al.* 1994). Therefore, as expected, among gene deletion strains with increased sensitivity to these drugs, we find numerous previously characterized protein synthesis related genes such as ribosomal protein L27A gene *YHR010W* (*RPL27A*), translation initiation factor *YJL138C* (*TIF2*), tRNA methyltransferase *YDL201W* (*TRM8*), mitochondrial translation initiation factor *YOL023W* (*IFM1*), eIF4E-associated gene *EAP1* (*YKL204W*), etc.

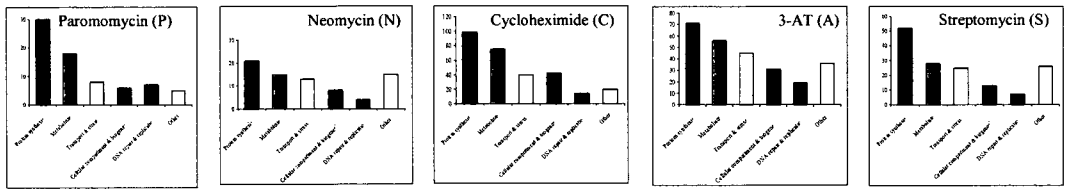
Figure 4-1: Clustering of drug sensitive gene deletion mutants.

The haploid non-essential yeast gene deletion array was subjected to sub-inhibitory concentrations of five inhibitory compounds. Colony size reduction (CSR) was used to detect sensitivity. (A) Drug sensitive yeast gene deletion mutants are clustered according to the cellular processes in which their deleted genes participate. The overall distributions of gene functions are very similar for different treatments with protein biosynthesis as a major group for all treatments. (B) Chemical profiles are clustered according to drug sensitivities to two or more drugs. Hierarchical clustering of mutants is illustrated using complete linkage. Absolute correlation coefficient (centered) is used for similarity measures and displayed in Java TreeView. Several regions of interest (a-e) are enlarged. The cellular processes of the deleted genes are color-coded. Based on sensitivity profiles paromomycin is grouped with neomycin. Cycloheximide is grouped with 3-AT which then merges with streptomycin. Sensitivity indices of the gene deletion mutants are shown in high to low as light to dark red. (C) Sensitivity overlaps for gene deletion mutants to different drug treatments. The numbers of gene deletion mutants with a particular sensitivity, for example paromomycin (P) alone (89), paromomycin and 3-AT (17) and paromomycin, 3-AT and neomycin (3), are indicated. (D) The overlapping drug sensitive yeast gene deletion mutants are clustered according to the cellular processes in which their deleted genes participate. No significant enrichment for protein biosynthesis genes among overlapping sensitive strains is observed. The numbers of sensitive strains are shown in z-axis.

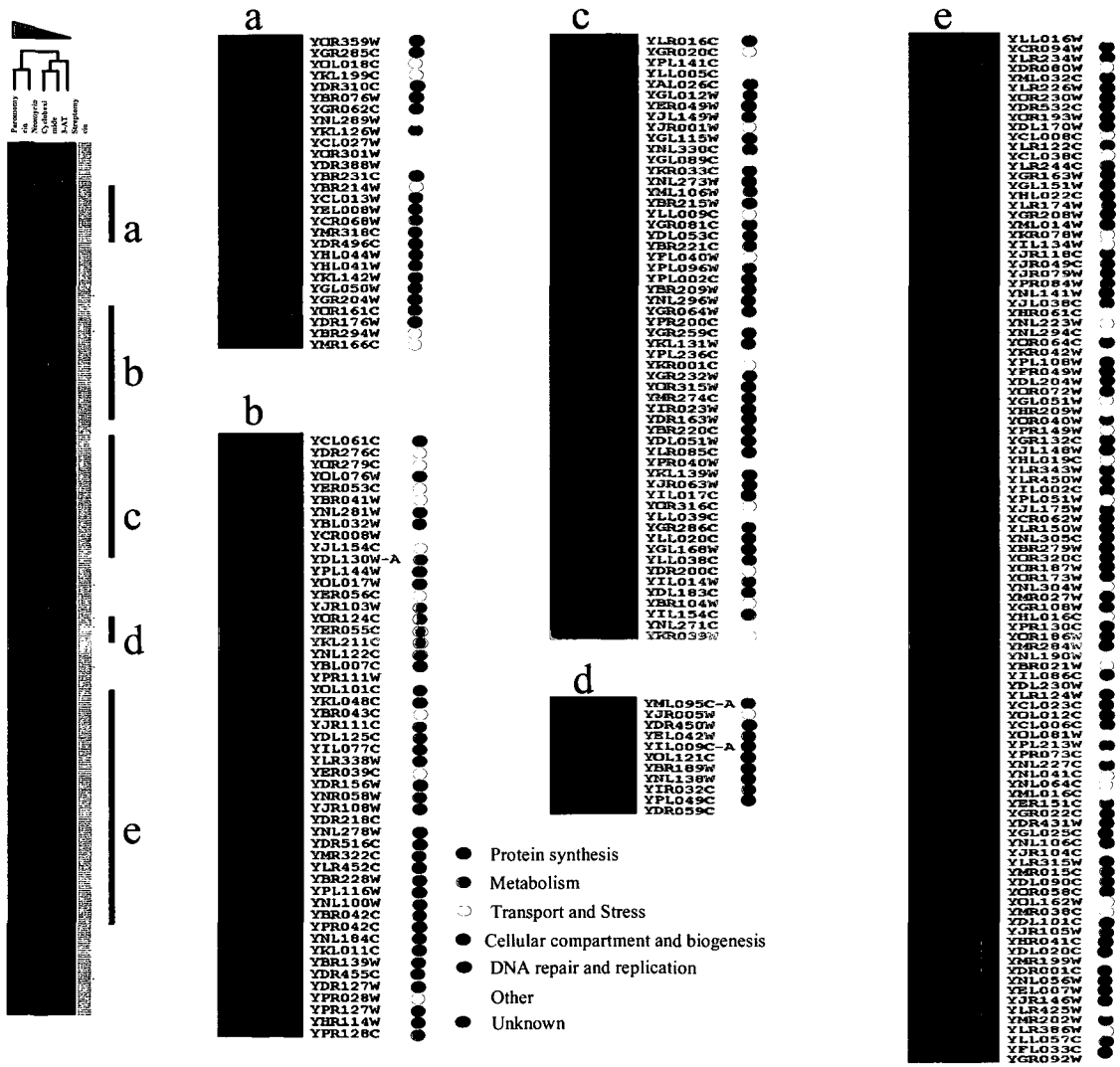
C: cycloheximide; P: paromomycin; A: 3-AT; N: neomycin; and S: streptomycin.

The sensitivity overlaps between P and N, C and 3-AT, C and S, and 3-AT and S were significant with P-values $\leq 5 \times 10^{-14}$. Other overlaps are significant with P-values of $\leq$ 0.029.

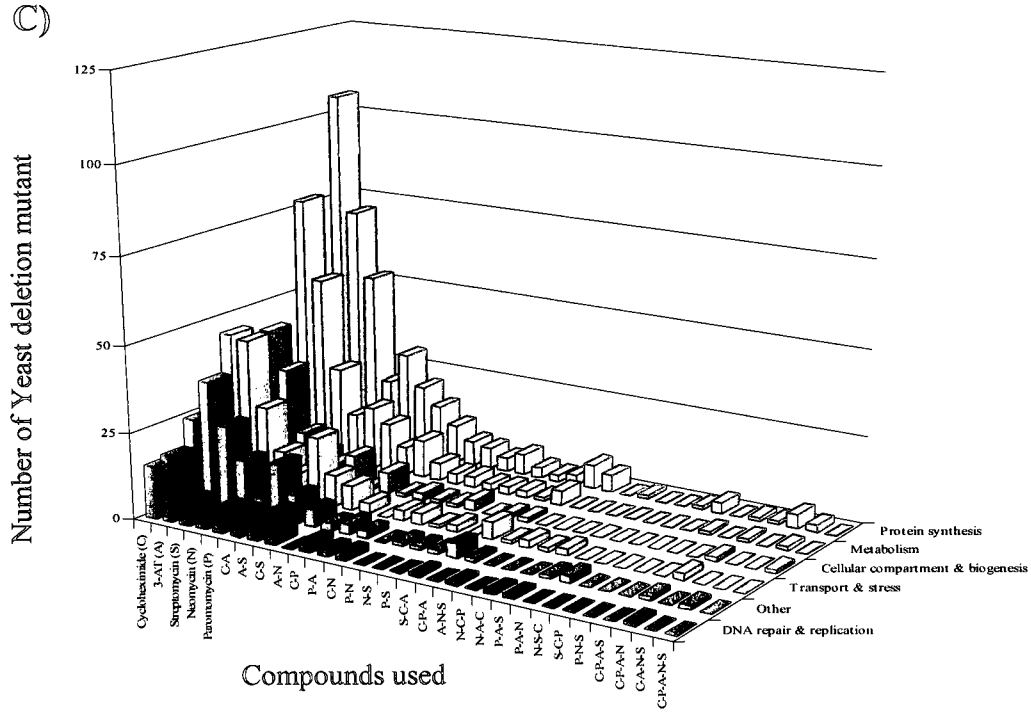
A)


Paromomycin (P)
Neomycin (N)
Cycloheximide (C)
3-AT (A)
Streptomycin (S)

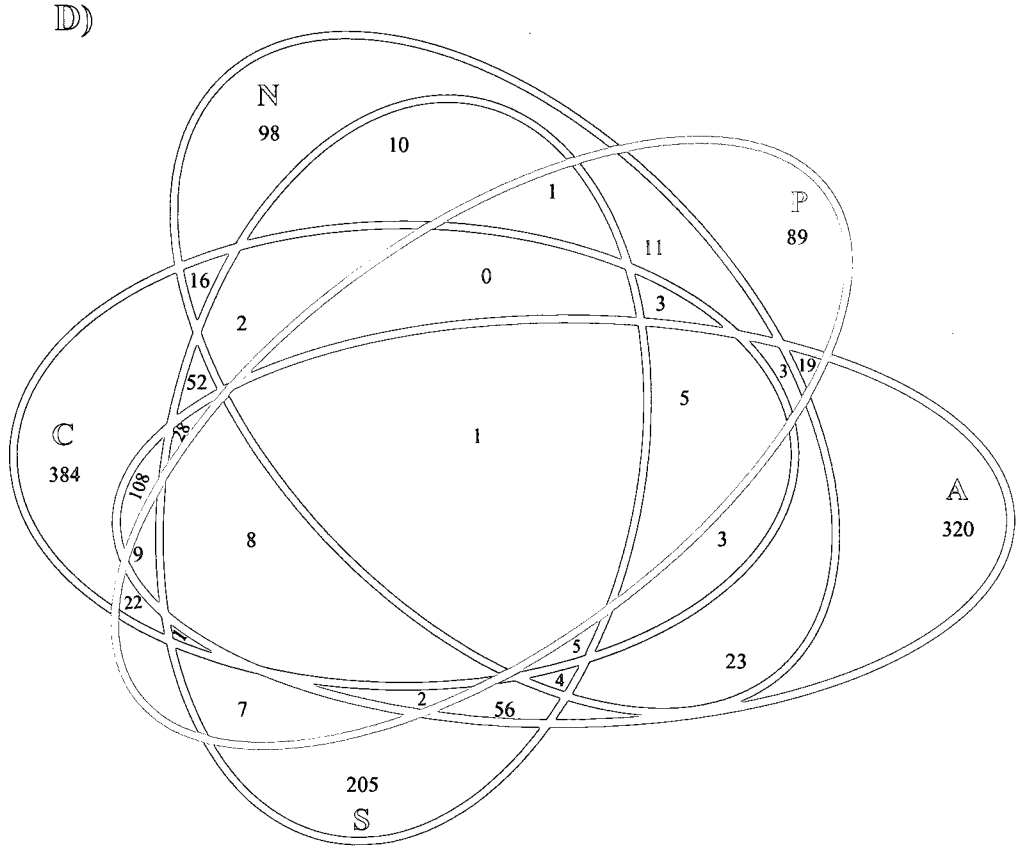
B)


a
b
c
d
e
Protein synthesis
Metabolism
Transport and Stress
Cellular compartment and biogenesis
DNA repair and replication
Other
Unknown

C)


Number of Yeast deletion mutant
125
100
75
50
25
0
Cycloheximide (C)
3-AT (A)
Streptomycin (S)
Neomycin (N)
Paromomycin (P)
C-A
A-S
C-S
A-N
C-P
P-A
C-N
P-N
N-S
P-S
S-C-A
C-P-A
A-N-S
N-C-P
N-A-C
P-A-S
P-A-N
N-S-C
S-C-P
P-N-S
C-P-A-S
C-P-A-N
C-A-N-S
C-P-A-N-S
Compounds used
Protein synthesis
Metabolism
Cellular compartment & biogenesis
Transport & stress
Other
DNA repair & replication

D)


N
98
10
1
P
89
11
16
0
3
2
52
3
19
5
C
384
28
1
108
A
320
9
8
3
22
4
5
23
4
7
2
56
205
S

Excluding the genes with unknown functions, the clustering of the identified genes based on the cellular processes in which they participate is shown in Figure 4-1: B (also see Supplementary Table 8-5). As indicated the dominant clusters are protein biosynthesis related genes. For example approximately 33% of sensitive strains to cycloheximide are linked to protein biosynthesis, followed by approximately 24%, 15%, 13%, 5% and 10% which are associated with metabolism, cellular compartment and biogenesis, transport and stress, DNA repair and replication, and others, respectively. This might be expected as cycloheximide, streptomycin, neomycin and paromomycin are known to directly interact with ribosomal subunits. 3-AT also affects protein biosynthesis by altering the pool of amino acids (Hinnebusch, 2005; Zhou *et al.* 2003).

The smaller clusters may represent additional target sites (side effects) of the drugs. For example, neomycin is also known to inhibit phospholipase C pathway and thus interfere with signal transduction in eukaryotic cells (Hildebrandt *et al.* 1997). This may explain our observation that deletion of *YIL050W* (*PCL7*), which codes for a member of a metabolism associated Pho85c kinase complex, confers cell sensitivity to neomycin. The smaller clusters may also represent novel secondary functions for certain genes, some of which may link translation to other cellular processes. For example, we observe that deletion of *YER095W* (*RAD51*) or *YOL090W* (*MSH2*) increased sensitivity to cycloheximide. *YER095W* and *YOL090W* are involved in repair of DNA strand breaks. Interestingly, *YER095W* is reported to have a genetic interaction (positive genetic) with translation termination factor eRF3 gene *YDR172W* (*SUP35*) and translation elongation factor *YLR249W* (*YEF3*) gene (Wilmes *et al.* 2008), and its gene product is reported to

physically interact with glutamyl tRNA synthetase protein, YGL245Wp (Gus1p) (Gavin *et al.* 2006). Similarly, the gene product for *YOL090W* is reported to physically interact with translation initiation factor eIF4A, *YJL138C* (*TIF2*) (Gavin *et al.* 2002). This is in agreement with a linkage between DNA damage response and translation, which has been recently reported (Begley *et al.* 2007). Alternately, the smaller clusters may simply represent false positives. However, the most likely scenario is that each of the above mentioned cases, represent different integrated parts of the data. For example, secondary target sites of a drug can be investigated with the prior knowledge that the smaller clusters may also contain genes with novel secondary functions as well as a number of false positives. An interesting observation is that the overall distribution of genes within each functional cluster is very similar for all five drugs (Figure 4-1: A). This may further highlight the cross-talk between protein synthesis and the other four cellular processes. It is worth mentioning that based on our previous observations with chemical genomics profiles of other inhibitory compounds such as calcofluor white (CW), methyl methane sulfate (MMS), sodium dodecyl sulfate (SDS), etc with diverse modes of activities, the profiles in Figure 4-1: A are distinct.

To analyze the chemical profiles presented here, we applied a hierarchical clustering approach to drug sensitivity (Figure 4-1: B) as in (Eisen *et al.* 1998). It is expected that compounds with similar modes of activity show similar patterns and cluster together. As expected, paromomycin and neomycin clustered together. These aminoglycosides bind the small ribosomal subunit, and compromise translation fidelity and translocation. Cycloheximide and 3-AT also clustered. Again this might be expected as both

cycloheximide and 3-AT can affect the elongation phase of translation. Cycloheximide does so by binding 60S ribosomal subunit (Schneider-Poetsch *et al.* 2010) whereas 3-AT causes starvation of amino acids needed for successful elongation. An interesting observation was that streptomycin, an aminoglycoside, appeared more closely associated with cycloheximide and 3-AT. Unlike other aminoglycosides, streptomycin does not bind ribosomal A-site (Fourmy *et al.* 1998). Therefore, it is possible that streptomycin's binding to ribosome may cause an alternative ribosomal conformation that resembles the action of cycloheximide and 3-AT. The effect of streptomycin on prokaryotic translation elongation, which is different from other aminoglycosides, has long been known (Laughrea, 1981).

In our analysis a total of 1519 gene deletion mutants were identified to have increased sensitivity to at least one drug (Figure 4-1: C). 408 of these were sensitive to two or more drugs. A mutant for a vacuole gene *YDR495C*Δ (*vps3*Δ), was sensitive to all five treatments. We often see this mutant in our other screens suggesting a non-specific involvement with multiple drug resistance. We observed that the ratio of protein synthesis related genes did not significantly increase when sensitivities to 2 or more drugs were analyzed (Figure 4-1: C and 4-1: D; and Supplementary Table 8-5). Some enrichment in the category of transport and stress related genes, which multiple drug resistant genes generally fall in, is observed for some multiple drug sensitive groups highlighting that selection based on several drugs may partially target multiple drug resistant genes.

To examine the accuracy of our large-scale approach to detect drug sensitive mutants, we selected 5 deletion strains and applied them to spot test analysis. Shown in Figure 4-2, our spot test experiments confirmed that deletion of *YPL009C* confers increased sensitive to cycloheximide, *YDR056C* to streptomycin and neomycin, *YJR111C* to streptomycin, and *YIL137C* and *YPL183W*-A to 3-AT. This is in agreement with our large-scale analysis and reaffirms the ability of our approach to identify strains which are sensitive to the tested drugs.

4.3.2 Synthetic Genetic Array (SGA) Analysis for *TAE2*, *TAE3* and *TAE4*

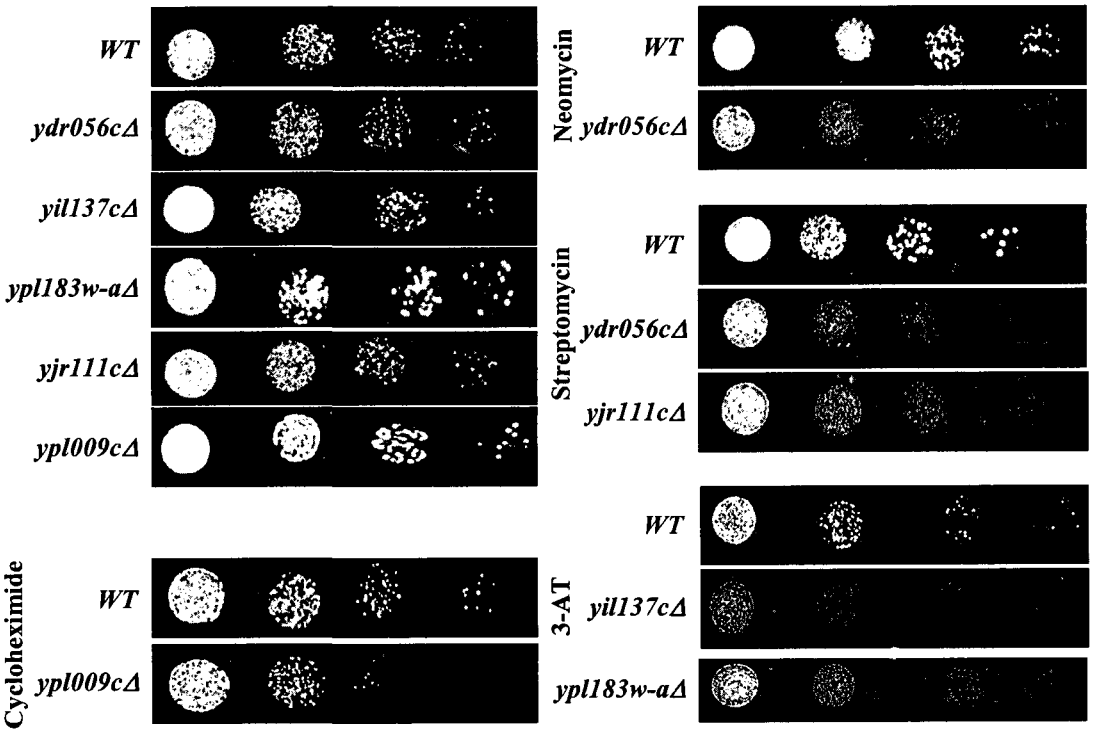
As indicated above, the majority of mutants with increased sensitivity to our target drugs had deletions of genes with known functions in protein biosynthesis. We therefore examined the activity of three mutants for genes which are not well characterized, *YPL009C*, *YIL137C* and *YPL183W*-A, by studying the genetic interactions they made with previously reported protein biosynthesis related genes. Though these genes are not well studied, nor characterized, the available literature and our data suggest a possible association for them with certain disease related-genes and phenotypes (see Discussion).

It is generally accepted that many genes/pathways in eukaryotic cells such as *S. cerevisiae* are functionally redundant and in the absence of one, there will be another that compensates for its loss of activity (Hartman *et al.* 2001). However, the deletion of the second functionally related gene/pathway may result in sickness or lethality, indicating an aggravating interaction. Consequently, the sickness of double mutants is one way to investigate genetic interaction, and hence functional relationships between genes (SGA

analysis) (Tong *et al.* 2001). We investigated the synthetic genetic interactions of *YPL009C*, *YIL137C* and *YPL183W*-A with other protein biosynthesis genes by systematically investigating double gene deletions for alterations in CS (Tong *et al.* 2001). If our targeted genes are involved in protein biosynthesis, it is expected that based on their molecular function they will genetically interact with other translation genes with related functions. As indicated in Figure 4-3 and Supplementary Table 8-6, it was observed that *YPL009C*, *YIL137C* and *YPL183W*-A genetically interacted with a number of translation genes as evident by the sick phenotype of the double mutants. This suggests a functional association for our target genes with the process of protein biosynthesis. Therefore, we called the studied genes *TAE2* (*YPL009C*), *TAE3* (*YIL137C*) and *TAE4* (*YPL183W-A*) for translation associated elements 2-4, respectively. The largest group of interacting genes for *TAE3* and *TAE4* were those involved in translation associated RNA processing, with 3 and 7 interactions, respectively. This group includes genes such RNA exonuclease *YLR059C* (*REX2*) involved in rRNA maturation and processing, rRNA binding proteins YHR066Wp (Ssf1p) which is a constituent of 66S pre-ribosomal subunit, nuclear pore complex protein YKL068Wp (Nup100p) involved in mRNA and rRNA export and ribosomal protein import to nucleus, etc. *TAE4* also interacted with 5 genes for different small ribosomal subunit proteins including *YLR441C* that codes for S1A and *YJL190C* that code for S22A. *TAE2* had a general pattern of interactions with genes of different functions. The largest groups of genes (three) that interacted with *TAE2* had five members each, with functions in amino acid biosynthesis, small ribosomal subunit proteins, and regulation of translation.

Figure 4-2: Strain sensitivity to different translation inhibitory drugs.

Wild type (WT) or gene deletion mutant strains (*yploo9cΔ*, *yil137cΔ*, *ypl183w-aΔ*, *ydr056cCΔ* and *yjr111cΔ*) were serially diluted to 10^{-3} to 10^{-6} and spotted on solid medium with sub-inhibitory concentrations of cycloheximide, paromomycin, 3-AT, streptomycin and neomycin as indicated, or without, used as a control. The plates were incubated at 30°C for 1-2 days. Deletion of *ypl009c* confers increased sensitivity to cycloheximide; *yil137c* and *ypl183w-a* to 3-AT, *ydr056c* to streptomycin and neomycin, and *yjr111c* to streptomycin.


WT
ydr056cΔ
yil137cΔ
ypl183w-aΔ
yjr111cΔ
ypl009cΔ
Neomycin
WT
ydr056cΔ
Streptomycin
WT
ydr056cΔ
yjr111cΔ
Cycloheximide
WT
ypl009cΔ
3-AT
WT
yil137cΔ
ypl183w-aΔ

In addition, some of the identified genetic partners were shared between the query genes (Figure 4-3). For example, *YDR025W*, which codes for the small ribosomal subunit protein S11A, genetically interacted with both *TAE2* and *TAE4*, and *YFR009W* (*GCN20*) which is involved in positive activation of GCN2 kinase, interacts with both *TAE3* and *TAE4*. A synthetic genetic interaction between *TAE2* and *TAE4* was also observed.

In contrast to the above aggravating interactions where sickness of double mutants was investigated, we also examined the interactions where double mutants had higher fitness than expected. Such alleviating interactions, also called synthetic rescue, are thought to exist between genes in a same pathway (Schuldiner *et al.* 2005). Here, we observed a total of 6 such interactions (Figure 4-3). In agreement with the above synthetic sickness interactions where the largest functional interaction partners for *TAE3* and *TAE4* were involved in translation associated RNA processing, we further observed that *TAE3* interacted with an RNA processing gene *YLR107W* (*REX3*) and with a second gene *YKL068W* (*NUP100*) involved in RNA transport from nucleus, also known to be associated with rRNA and tRNA export. Similarly, *TAE4* interacted with an RNA processing gene, *YNL001W* (*DOM34*). *TAE2* had alleviating interactions with three genes with three different functions: in translation initiation *YKR059W* (*TIF1*), mitochondrial translation *YDR494W* (*RSM28*) and structure of small ribosomal subunit *YDR450W* (*RPS18A*). The diversity of the interactions for *TAE2* is also in accord with the synthetic sick interactions observed above without a clear major functional group.

In agreement with above, in a recent genome-wide synthetic genetic interaction study (Costanzo *et al.* 2010), when *TAE3* and *TAE4* were used as query genes, the major category of genes with which they formed synthetic sick and lethal interactions, were involved in protein biosynthesis. Similarly the synthetic sick and lethal interactions reported for *TAE2* as a hit also pointed an enrichment of protein biosynthesis genes.

4.3.3 Functional Correlations for *TAE2* and *TAE4* with Other Protein Synthesis Related Genes

It is well documented that the overexpression of a gene can often compensate for a phenotypic consequence caused by the absence of a functionally related gene (Kim *et al.* 2009, Mathur *et al.* 2009). Consequently, one way to study protein function might be to investigate the ability of its overexpression to compensate for the absence of others with known functions. To further investigate the biological activity of the gene products for *TAE2* and *TAE4*, we systematically studied the ability of their overexpression to reverse the phenotypic consequences caused by the absence of other translation genes (phenotypic suppression analysis). For an unknown reason our multiple attempts to isolate an overexpression plasmid for *TAE3* from the yeast gene overexpression library was unsuccessful. Consequently, *TAE3* was omitted from this part of our investigation. Reduced growth for gene deletion strains in the presence of neomycin and streptomycin was used as the target phenotypic consequence. As indicated in Figure 4-4 (and Supplementary Table 8-7), we observed that the growth defects in the presence of neomycin and/or streptomycin for a number of deletion strains for translation genes was compensated when *TAE2* (Figure 4-4: A) or *TAE4* (Figure 4-4: B) were overexpressed.

In strong agreement with the synthetic genetic interactions above, the two main functional categories that *TAE4* overexpression rescued contain genes involved in translation related RNA processing and 40S ribosomal structure maintenance. For example, *TAE4* overexpression rescued drug sensitivity of deletion strains for pre rRNA processing gene *YGR159C* (*NSR1)* and 40S ribosomal subunit protein S28 gene *YGR118W* (*RPS23A*). A role for *TAE4* in 40S biogenesis can explain these observations. This is also in agreement with the synthetic sick and synthetic rescue interactions observed for *TAE4*.

Similar to the above synthetic genetic interactions for *TAE2*, our phenotypic suppression analyses also suggest a general role for *TAE2* in translation. Overexpression of *TAE2* compensated for the deletion of a number of genes with diverse roles in translation. For example, *YMR242C* (*RPL20A*) codes for a 60S ribosomal subunit protein, *YDR462W* (*MRPL28*) codes for a mitochondrial ribosome protein, and *YKR059W* (*TIF1*) codes for translation initiation factor eIF4A.

Three of the rescued gene deletion strains *YDL083C*Δ (*rps16B*Δ), *YPL081W*Δ (*rps9A*Δ) and *YIL052C*Δ (*rpl34B*Δ) were also shared between *TAE2* and *TAE4*. This is in accord with the above observed synthetic genetic interaction between these two genes (Figure 4.3). Such interactions further highlight the interconnectivity of a genetic interaction map for translation genes.

Figure 4-3: Synthetic genetic interaction analysis for *TAE2*, *TAE3* and *TAE4* with translation related genes.

There are 72 interactions that represent synthetic genetic interactions for three query genes *TAE2*, *TAE3* and *TAE4*, with 59 different translation genes. Genes are represented as nodes (circles) and interactions are represented as edges (lines). The interacting genes are further divided into eight functional categories. There are a number of shared interactions that highlight the interconnectivity of the network. The nodes are colored according to functional groups. Black edges represent synthetic sick (aggravating) interactions, and the 6 pink thick edges represent synthetically rescue (alleviating) interactions.

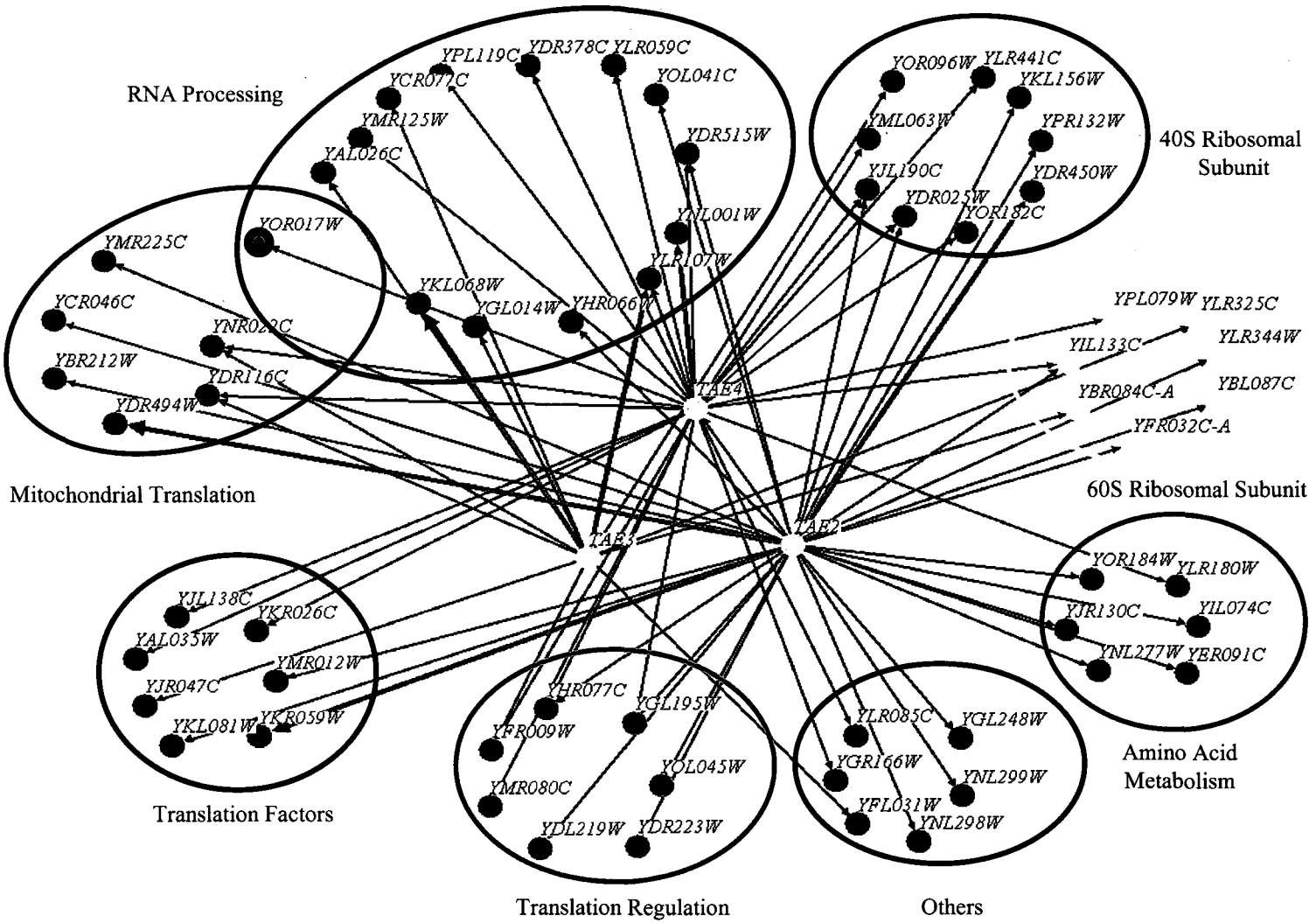
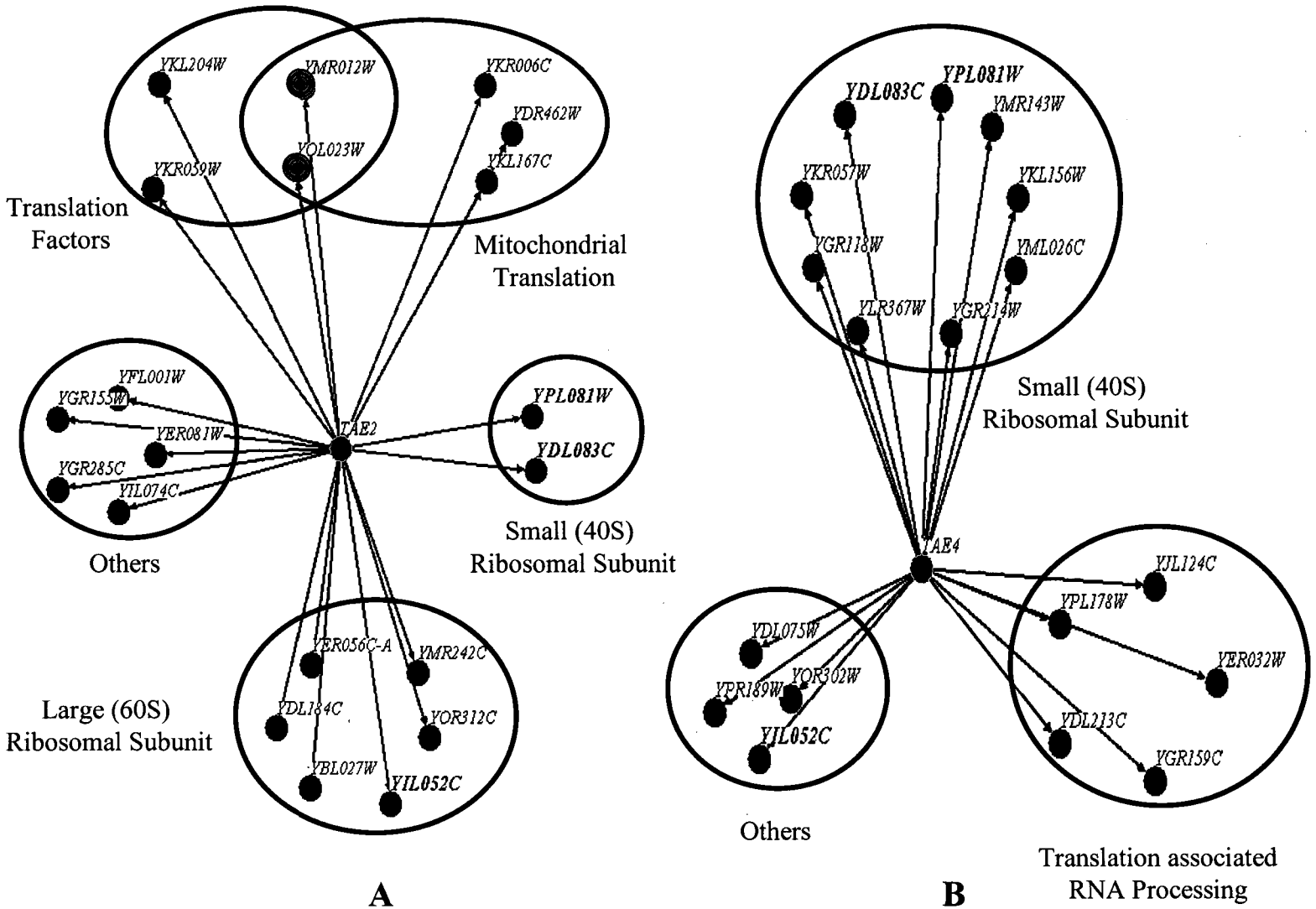

RNA Processing
YPL119C
YDR378C
YLR059C
YCR077C
YOL041C
YMR125W
YDR515W
YAL026C
YJL001W
YLR107W
YOR017W
YMR225C
YKL068W
YCR046C
YGL014W
YHR066W
YNR022C
YBR212W
YDR116C
YDR494W
Mitochondrial Translation
YOR096W
YLR441C
YKL156W
YML063W
YPR132W
YJL190C
YDR450W
YDR025W
YOR182C
40S Ribosomal Subunit
YPL079W
YLR325C
YIL133C
YLR344W
YBR084C-A
YBL087C
YFR032C-A
60S Ribosomal Subunit
TAE4
TAE1
TAE2
YOR184W
YLR180W
YJR130C
YIL074C
YNL277W
YER091C
Amino Acid Metabolism
YJL138C
YKR026C
YAL035W
YMR012W
YJR047C
YKL081W
YKR059W
Translation Factors
YHR077C
YGL195W
YFR009W
YOL045W
YMR080C
YDL219W
YDR223W
Translation Regulation
YLR085C
YGL248W
YGR166W
YNL299W
YFL031W
YNL298W
Others

Figure 4-4: Overexpression of *TAE2* and *TAE4*.

Overexpression of *TAE2* and *TAE4* suppresses the sensitivity of numerous translation genes to drug treatments. Overexpression of *TAE2* and *TAE4* suppresses the phenotype of a number of translation gene deletion strains against neomycin and/or streptomycin treatments. Genes are represented as nodes (circles) and interactions are represented as edges (lines). The interacting genes are divided into functional categories and colored accordingly. (A) *TAE2* overexpression rescues 20 gene deletions with variety of functions. (B) *TAE4* overexpression rescues 18 gene deletions, the majority of which are 40S subunit proteins (9 genes) or function as translation-associated RNA processing proteins (5 genes). Blue letters represent genes that are rescued by the overexpression of both *TAE2* and *TAE4*.


YKL204W
YMR012W
YKR006C
YDR462W
YOL023W
YKR059W
YKL167C
Translation
Factors
Mitochondrial
Translation
YFL001W
YGR155W
YPL081W
TAE2
YER081W
YDL083C
YGR285C
YIL074C
Others
Small (40S)
Ribosomal Subunit
YER056C-A
YMR242C
Large (60S)
Ribosomal Subunit
YDL184C
YOR312C
YBL027W
YIL052C
A
YDL083C
YPL081W
YMR143W
YKR057W
YKL156W
YGR118W
YML026C
YLR367W
YGR214W
Small (40S)
Ribosomal Subunit
TAE4
YJL124C
YPL178W
YDL075W
YER032W
YOR302W
YPR189W
YDL213C
YJL052C
YGR159C
Others
Translation associated
RNA Processing
B

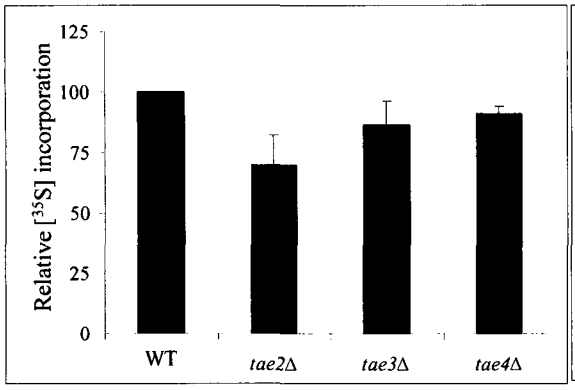
4.3.4 Deletions of *TAE2*, *TAE3* and *TAE4* Affect the Process of Protein Synthesis

The genetic interaction analyses above provide a direct link between *TAE2*, *TAE3* and *TAE4,* and the process of protein biosynthesis. To further study this link we examined the effect of deletion of the target genes in translation efficiency, stop codon read-through and ribosome biogenesis. The differences, if any, that we expect to observe are likely to be subtle. This is because of the importance of protein biosynthesis in the survival of a cell and the fact that the deletion of the target genes do not appear to change the growth rate of the mutants under standard laboratory conditions.

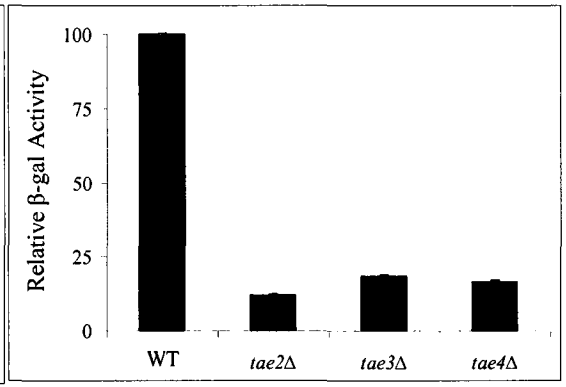
We first investigated the involvement of *TAE2*, *TAE3* and *TAE4* in translation efficiency. For this we subjected the deletion mutants *tae2*Δ, *tae3*Δ and *tae4*Δ to [^{35}S] methionine incorporation analysis. As indicated in Figure 4-5: A, it was observed that *tae2*Δ, *tae3*Δ and *tae4*Δ mutant strains had reduced levels of [^{35}S] methionine incorporation by approximately 30%, 14% and 10%, respectively. To complement these findings, we investigated the rate of protein synthesis using an inducible β-galactosidase reporter construct (p416) under the control of a GAL1 promoter (Krogan *et al.* 2003), which better highlights the differences in translation efficiencies (Alamgir *et al.* 2008). It was found that after four hours of induction, the levels of β-galactosidase activity were six fold lower for *tae2*Δ and *tae3*Δ,and five fold lower for *tae4*Δ (Figure 4-5: B) while their mRNA content remained relatively unchanged.

Figure 4-5: Characterization of *TAE2*, *TAE3* and *TAE4* deletions.

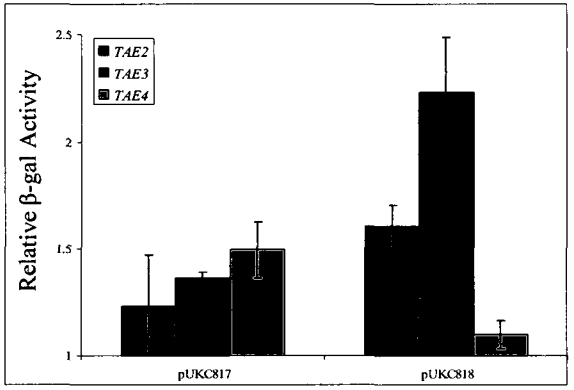
(A) Total protein synthesis is measured using [^{35}S] methionine incorporation in wild type, *tae2Δ*, *tae3Δ* and *tae4Δ* strains. The average count for [^{35}S] methionine incorporation for wild type is 11,356,073 (±1,400,000) counts, which is set to 100%. In average, in the absence of Tae2p, Tae3p and Tae4p, [^{35}S] methionine incorporation is reduced by approximately 30, 14 and 10%, respectively. (B) The efficiency of protein synthesis is measured using an inducible β-galactosidase reporter construct (p416). The average β-galactosidase activity for wild type is 7.5 (±0.6) units, which is set to 100%. The β-galactosidase activity was measure after 4 hrs of induction. Deletion of *TAE2*, *TAE3* and *TAE4* limits the expression of β-galactosidase to 13, 21 and 17% of that in wild type, respectively. (C) Deletion of *TAE2*, *TAE3* and *TAE4* resulted in increased levels of β-galactosidase from *lacZ* reporters with different premature stop codons (pUKC817 and pUKC818). The activity of β-galactosidase is determining by normalizing the activity of the mutant (pUKC817 and pUKC818) to the control (pUKC815). pUKC815 is the background construct without a premature stop codon and is used as a control. Bars represent standard deviation for the means. (D) Ribosome profile analysis of yeast deletion strains *tae3Δ* and *tae4Δ* compared to wild type. Deletion of *TAE3* decreases the levels of polysomes along with an increase in free 60S subunits. Deletion of *TAE4* causes an increase in free 60S subunits and a mild decrease in larger polysomes. Each experiment was repeated three times or more. Ratios for free 60S:40S is calculated from the areas under the curves.


Relative [35S] incorporation
125
100
75
50
25
0
WT
tae2Δ
tae3Δ
tae4Δ

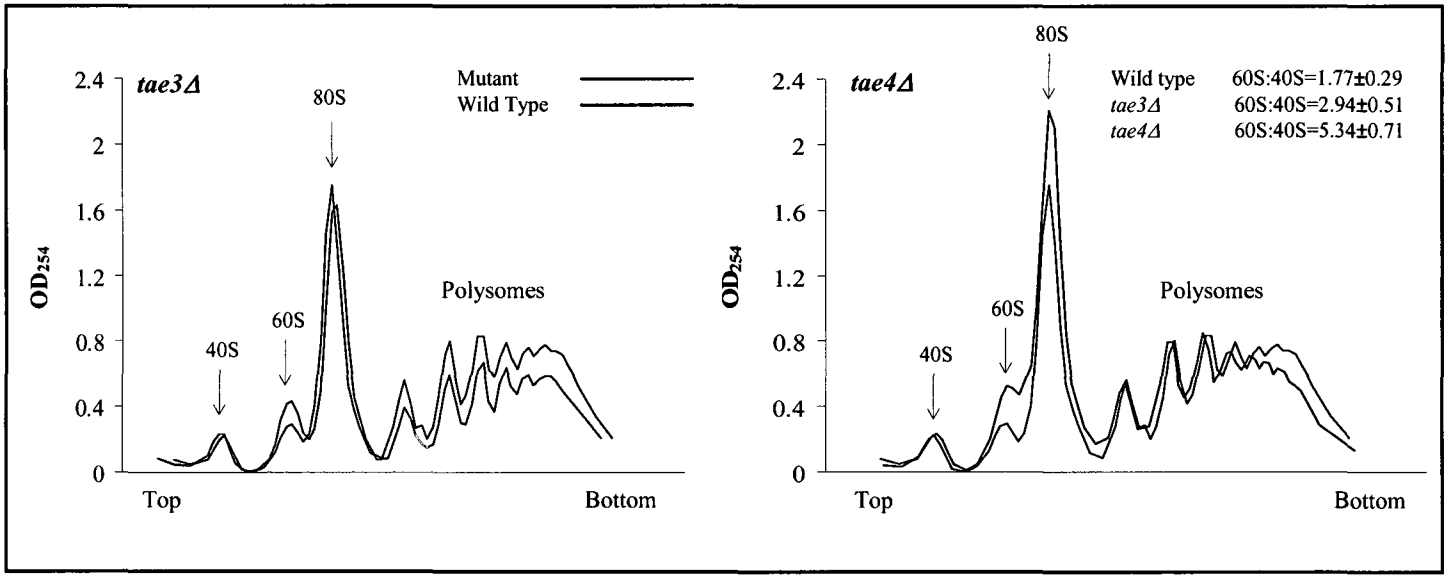
A


Relative β-gal Activity
100
75
50
25
0
WT
tae2Δ
tae3Δ
tae4Δ

B


Relative β-gal Activity
2.5
2
1.5
1
TAE2
TAE3
TAE4
pUKC817
pUKC818

C


tae3Δ
Mutant
Wild Type
80S
60S
40S
Polysomes
OD254
2.4
2
1.6
1.2
0.8
0.4
0
Top
Bottom
tae4Δ
80S
60S
40S
Polysomes
Wild type 60S:40S=1.77±0.29
tae3Δ 60S:40S=2.94±0.51
tae4Δ 60S:40S=5.34±0.71

D

To study stop codon read-through we used a plasmid-based β-galactosidase system with different premature termination codons. In this way alterations in translation fidelity will lead to an increase in termination codon read-through, and thus will elevate the production of full length functional β-galactosidase. To this end, we transformed the target deletion strains with three different plasmid pUKC815, pUKC817and pUKC818 (Stansfield *et al.* 1995) and quantified the expression of β-galactosidase in each mutant. pUKC815 contains no in-frame premature termination codon and is used as a control. pUKC817 and pUKC818 contain in-frame termination codons UAA and UAG, respectively.

Apparent from the increased relative productions of β-galactosidase shown in Figure 4-5: C, deletion of *TAE2*, *TAE3* and *TAE4* resulted in higher levels of termination codon read-through. We also observed nearly identical β-galactosidase mRNA contents for each of the tested strains indicating that the observed increases in β-galactosidase activities are not due to altered levels of mRNAs.

A surprising observation was that deletion of *TAE4* resulted in higher read-through for UAA (pUKC817) stop codon and not UAG (pUKC818). Generally, it is expected that alterations in translation fidelity result in more read-through for a less stringent stop codon, in this case UAG. This is observed for *tae2*Δ and *tae3*Δ, but not for *tae4*Δ. A possible explanation is that deletion of *TAE4* causes an alteration which is stop codon specific. For example, it may reduce the affinity of ribosomes to a specific translation release factor (RF), but not others.

Next, we investigated the ribosome profiles for *tae2*Δ, *tae3*Δ and *tae4*Δ gene deletion strains. As expected all profiles had three peaks associated with free 40S and 60S subunits, and 80S monosomes, followed by a series of peaks representing polysomes (Figure 4-5: D). The ribosome profile for *tae2*Δ was nearly identical to the wild type strain. For *tae3*Δ, however, a reduction in polysomes was observed along with an increase in free 60S subunit (Figure 4-5: D). From the area under the curve, the free 60S:40S subunit ratio for this mutant was 2.94 ±0.51 in comparison to 1.77 ±0.29 for wild type. Similarly, the profile for *tae4*Δ had a significant increase in free 60S subunits along with a mild increase in 80S monosomes and a mild reduction in larger polysomes, found at the right end of the x-axis in Figure 4-5: D. The free 60S:40S subunit ratio for *tae4*Δ is 5.34 ±0.71. The experiments were repeated three times. Reduction of polysomes may explain the observed reductions in the efficiency of protein synthesis for *tae3*Δ and *tae4*Δ. Alterations in the pool of free ribosomal subunits may suggest deficits in subunit biogenesis, suggesting that *TAE3* and *TAE4* may be involved in the process of ribosome biogenesis. Since the 40S and 60S subunits are in equilibrium with 80S monosomes, an increase in 60S free subunits may suggest a defect in 40S biogenesis (Vlásek *et al.* 2001) as observed for *tae3*Δ and *tae4*Δ mutants. A more precise calculation for free 60S:40S can be accomplished by measuring 40S and 60S subunits separated on a sucrose gradient with low concentrations of Mg^{2+}; this however, was not investigated in the current study.

4.4 Discussion

Gene deletions that cause increased sensitivity to a bioactive compound can help identify pathways that buffer the cell against the activity of that compound (Kemmer *et al.* 2009). Consequently, chemical genetic profile of inhibitory compounds can lead to identification of their overall mode of action, as well as the side effects associated with the toxicity of the drug. Similarly, these profiles can also help identify novel genes that are involved in specific cellular pathways that are targeted by compounds (Parsons *et al.* 2004). Here, we investigated the sensitivity of yeast gene deletion array to five different bioactive compounds using CSR. The overall profile of these compounds showed significant resemblance to each other, with the deletion of genes involved in protein biosynthesis as the dominant cluster. We followed up our investigations with three sensitive deletion strains for genes that are not well characterized, that here we call *TAE2*, *TAE3* and *TAE4*. Our genetic analyses provided further support for the involvement of these genes in protein biosynthesis.

There is limited information about the molecular activity of Tae4p. In a large-scale investigation it was reported that deletion of *TAE4* rescued the temperature sensitivity of *cdc13-1*, and hence was thought to be a restrictor of telomere capping. Cdc13p is an essential protein involved in checkpoint and telomere capping. Also, the C-terminal domain of Tae4p, amino acids 56-93, has sequence homology to prokaryotic ribosomal protein L36. Here we observed that *TAE4* formed synthetic sick interactions with two predominant categories of genes with functions in RNA processing associated with

protein biosynthesis and genes that code for 40S ribosomal subunit associated proteins. Similarly, it formed phenotypic suppression interactions with the same two categories of genes connecting the activity of *TAE4* to RNA processing and the 40S subunit. The synthetic rescue interaction for *TAE4* also supported this activity. Involvement of TAE4 in 40S biogenesis was confirmed by ribosome profile analysis and can explain the observed affect that the deletion of *TAE4* had on stop codon read-through and the efficiency of translation. In agreement with this, *TAE4* is found to be co-regulated with several rRNA processing proteins such as an LSM protein YJR022Wp (Lsm8p) implicated in pre-rRNA and pre-tRNA processing (Roberts and Hudson, 2006) and a pre-ribosome processing protein YLR409Cp (Utp21p) involved in 18S rRNA processing (Cullen *et al.* 2004) further connecting the activity of *TAE4* to RNA processing and ribosome biogenesis. The C-terminal domain of Tae4p contains two RNA binding domains. Therefore, Tae4p may affect rRNA processing by directly binding to rRNA or by recruiting other factors to rRNA, which in turn can affect ribosome biogenesis.

TAE2 (*YPL009C*) has no previously reported cellular function. Its protein product shares a domain similarity to a human colon cancer antigen 1, *SDCCAG1* and has sequence similarity to a putative RNA binding protein in *Drosophila melanogaster.* Our synthetic sick and synthetic rescue genetic interaction analyses indicated a very diverse interaction pattern for *TAE2* with different translation genes. *TAE2* overexpression also rescued the phenotype of deletion strains for genes with different functions in various steps of translation, further hinting a general involvement for *TAE2* in protein biosynthesis that is not targeted to a specific pathway. Deletion of *TAE2* also caused as increase in stop

codon read-through and a decrease in translation efficiency. One possible explanation is that *TAE2* may transiently help in mediating the overall activity of ribosomes and hence translation efficiency and fidelity. It is known that translation *in vivo* is more efficient than in reconstituted *in vitro* experiments indicating the presence of uncharacterized translation elements *in vivo* (Ganoza *et al.* 2002). This activity of *TAE2* can be supported by the observation that in a large-scale affinity purification experiments Tae2p co-purified with several ribosomal subunit proteins (Gavin *et al.* 2006). However, a very mild concentration of salt was shown to destabilize this interaction (Fleischer *et al.* 2006) suggesting that perhaps Tae2p has only a transient interaction with ribosomes. In addition, very recently, Tae2p was computationally predicted to directly interact with ribosomal subunit protein YDR418Wp (Rpl2Bp) (Pitre *et al.* 2008).

We found that *TAE3* predominantly formed synthetic sick interactions with RNA processing genes involved in translation. It also formed synthetic rescue interactions with genes with similar RNA processing functions. Alterations in the ribosomal profile in *tae3*Δ strain suggest a deficiency in 40S subunit biogenesis. A role for *TAE3* in RNA processing associated with 40S biogenesis can explain these observations. It can also explain the observation that deletion of *TAE3* caused increased stop codon read-through and a reduction in the efficiency of protein synthesis. In agreement with this role for *TAE3*, the expression of *TAE3* is reported to be strongly co-regulated with different essential translation genes including *YAL003W* (*FUN53*), which codes for a subunit of RNase MRP involved in pre-rRNA cleavage and *YBL004W* (*UTP20*), which is involved in 18S rRNA processing (Mnaimneh *et al.* 2004). Tae3p does not appear to contain an

RNA binding domain. Therefore, it is possible that Tae3p may interact with an intermediate RNA binding protein(s) to exert its activity. In agreement with this, Tae3p was reported to co-purify with YOR272Wp (Ytm1p), a constituent of 66S pre-ribosomal particle (Ho *et al.* 2002), which provides further support for involvement of Tae3p in ribosome biogenesis. In addition, we previously observed that deletion of *TAE3* reduced the efficiency of double stranded DNA break repair which appears to be an independent activity for *TAE3*.

The effect of *TAE3* deletion on stop codon read-through is in agreement with a previous observation (Fleischer *et al.* 2006). However, in the same investigation, no apparent alteration in translation efficiency was detected. A possible explanation for this difference might be that in the previous study diploid homozygous gene deletion cells were used, whereas here we used haploid cells. Differences between the experimental observations in the two haploid and diploid systems are reported (Deutschbauer *et al.* 2005). In addition, the efficiency of induced translation was not measured in the former case.

5.0 IDENTIFICATION OF NOVEL TRANSLATION FIDELITY RELATED NON-ESSENTIAL GENES IN *Saccharomyces cerevisiae*

5.1 Abstract

5.1.1 Background

Translation fidelity is crucial to the survival of a cell as it ensures the production of full length functional proteins. There are a number of pathways that are thought to affect the fidelity of translation. Identification of novel genes that affect this process is an important step to better understand the process of translation. Here, we used large-scale high throughput analysis coupled with follow up analysis to broaden our knowledge on translation fidelity by identifying and characterizing novel genes in yeast.

5.1.2 Results

The entire yGDA (~4700 *S. cerevisiae* deletion mutants) were transformed with plasmids pUKC815, pUKC817, pUKC818 and pUKC819. With the exception of pUKC815 which is used as a control, these plasmids contain in-frame nonsense codons in a reporter *LacZ* gene. To this end, more than 20,000 analyses were performed. Using large-scale β-gal filter assays, increased level of β-gal expression was observed in 106 non-essential gene deletion strains. Forty-six deletion strains were further confirmed using β-gal liquid assay. Among them, 9 genes were confirmed in triplicate. Seven of the 9 (nine) studied genes do not have a reported association with translation fidelity and therefore may represent novel genes that affect translation fidelity.

5.1.3 Conclusion

We have identified 106 genes with possible link to translation fidelity using large-scale analysis. We have confirmed our results for 7 novel genes using small scale β-gal liquid assay.

5.2 Introduction

Cell function is controlled at various stages via genetically regulated pathways, such as transcription, post-transcriptional processing, mRNA stability, translation, post-translational modifications and protein degradation (Mata *et al.* 2005; Harger and Dinman, 2004). Among them, regulation of translation or protein synthesis mediates a number of cellular responses by external and internal stimuli.

The mechanism of translation is conserved in both eukaryotes and prokaryotes. Numerous accessory factors are required to assist in the various stages of protein synthesis such as initiation, elongation and termination to ensure the proper production of full length functional protein products (McCarthy, 1998; Linder, 1992). Alteration of any of these factors can compromises the fidelity of translation and hence may result in production of truncated or non functional proteins. Initiation of translation begins with the formation of pre-initiation complex. In eukaryotes, **e**ukaryotic **I**nitiation **F**actors (eIF) play a key role in the initiation of translation. The proper selection of the AUG start codon during the initiation of translation may be considered as the first step in maintaining the fidelity of translation. During initiation, the 40S moves along the mRNA

chain towards its 3'-end, scanning for a start codon (Kozak, 1999). After reaching the first start codon, the initiator tRNA charged with MET is brought to the P-site of the small ribosomal subunit with eIF2 and GTP. Hydrolysis of this eIF2 bound GTP triggers signals for the dissociation of several factors from the small ribosomal subunit which leads to its association with the large subunit (60S). The initiation step ends with the formation of the complete ribosome (80S) (Müller and Trachsel, 1990).

The elongation step requires binding of aminoacyl-tRNA to the ribosomal A-site, forming peptide bond, followed by its translocation to the P-site. Elongation continues until the ribosome reaches a stop codon where translation is terminated (Valouev *et al.* 2009). During elongation a +1 or -1 shift in reading frame may lead to a premature termination. This event may trigger the nonsense-mediated mRNA decay or NMD pathway, which degrades the mRNA associated with premature termination. The background frequency of frame shift is estimated to be around 3×10^{-5} to 10^{-4} (Atkins *et al.* 1991). Furthermore, error or mistakes during the termination process can also affect the recognition of stop codon (UAA, UAG or UGA) by nonsense suppression. Similarly read-through of the stop codons is another factor that leads to compromised translation fidelity, by allowing the incorporation of additional amino acids during the extension of the polypeptide chain. Therefore, translation fidelity not only depends on the accurate selection of correct reading frame during initiation (Alone *et al.* 2008), but also in the maintaining and incorporating of correct base pair in elongation (Ortiz *et al.* 2006) and accurately recognizing stop codons in termination (Valente and Kinzy, 2003). In addition, alteration in a number of different cellular components, such as rRNA, tRNA, ribosomal

proteins and different translational factors are also known to affect the accuracy of protein synthesis.

Here, we use a β-gal based expression system that contains premature stop codons to detect novel non-essential genes that affect translation fidelity. To this end, we transformed the collection of non-essential yeast gene deletion array ~4,700 strains with this system. We provide evidence that deletion of 7 novel ORFs affect the process of translation fidelity in *S. cerevisiae*.

5.3 Results: Screening for Novel Genes Involved in Translation Fidelity

5.3.1 Large-scale Transformation of yGDA Collection

The accuracy of translation was investigated in the absence of the candidate genes in the entire yGDA collection. We transformed the entire yGDA collection with plasmids pUKC815, pUKC817, pUKC818 and pUKC819. Plasmid pUKC817, pUKC818 and pUKC819 contain *LacZ* gene carrying premature stop codon UAA, UAG and UGA respectively (Stansfield *et al.* 1995). pUKC815 was used as a control and does not contain a premature stop codon. We used a modified **S**ynthetic **G**enetic **A**rray (SGA) analysis method to transform the entire yGDA. SGA is a large-scale method previously used to make double gene mutant strains from yGDA for genetic interaction analysis (Tong *et al.* 2001). We did this by transforming an opposite mating type strain (α) with target plasmids and then mating the transformants with the entire yGDA collection (a-mating type). Enriched sporulation medium was used to facilitate the sporulation of the

yeast diploid cells. The haploid cells containing plasmids are selected and scored on SD/MSG-His/Arg/Lys +canavanine+G418 plates.

5.3.2 β-galactosidase (β-gal) Analysis

Large-scale β-gal colony lift filter assay followed by small-scale β-gal liquid assay were used to determine the effect of different gene deletions on translation fidelity. 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) was used as a substrate for β-gal lift filter assay to screen the transformed deletion strains with pUKC817, pUKC818 and pUKC819. Yeast deletion strains with compromised translation fidelity produce higher β-gal activity compared to the control by suppressing the premature stop codons in the *LacZ* gene. In the presence of β-gal, X-gal is converted into 5-bromo-4-chloro-indigo which has a blue color.

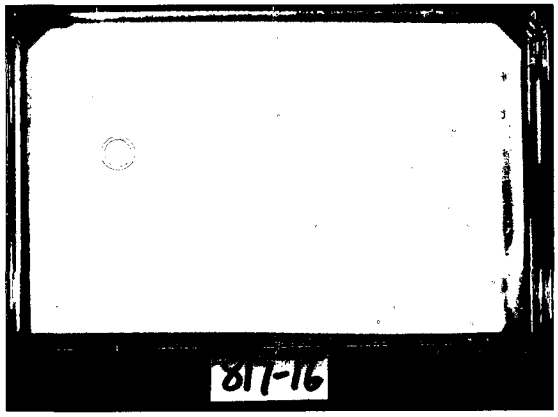
Figure 5-1 represents an example of a typical filter which is subjected to β-gal colony lift filter assay. The blue colonies represent the strains that have produced higher β-gal in comparison to others and therefore might have suppressed the premature stop codons. We observed that 106 yeast deletion mutant strains show increased level of β-gal expression. The complete list of the genes that when deleted increased the activity of β-gal expression are listed in Supplemental Table 8-8.

Colony lift filter assay is a non-quantitative approach to detect β–gal positive colonies. It is a large-scale approach which is suitable to identify candidate colonies for further screenings and confirmation. To confirm that our large-scale screening provided meaningful results we subjected 9 of 106 (YJR137C, YLL060C, YPL220W, YMR183C,

YAL027W, YDL076C, YFR044C, YNL122C, and YJR014W) yeast deletion strains that were identified as positives in the lift assay, to a more quantitative liquid culture-based β-gal analysis (Table 5-1). Figure 5-2 summarizes our findings for these analyses providing support for the validity of our screen. With the exception of *YLL060C*, all other examined gene deletion strains had a higher level of β-gal activity suggesting an involvement for these genes in translation fidelity. Seven of these genes have never been linked to translation and hence may represent novel translation fidelity associated genes. One of them, *YNL122C* is an uncharacterized gene with no reported molecular or biological function. Our analysis indicated that the relative β-gal activity for *YNL122C*Δ strain is ~5-6 times more than the background (Table 5-1).

Generally, aminoglycosides affect translation fidelity by binding to the small ribosomal subunit of eukaryotic cells and inhibiting ribosomal translocation (Tuite *et al.* 1984). Interestingly, we found that when deleted, 86 of the 106 genes identified in this study to affect translation fidelity, also cause increased sensitivity to at least one of the three aminogylcosides investigated in our previous studies (Supplemental Table 8-8). Among them the previously uncharacterized gene *YNL122C*, had increased sensitivity to paromomycin and neomycin. This overlap serves as cross validation and increases our confidence on the biological relevance of our data.

Figure 5-1: A colony filter subjected to β-gal analysis. Blue colonies indicate higher level of β-gal activity.


817-16

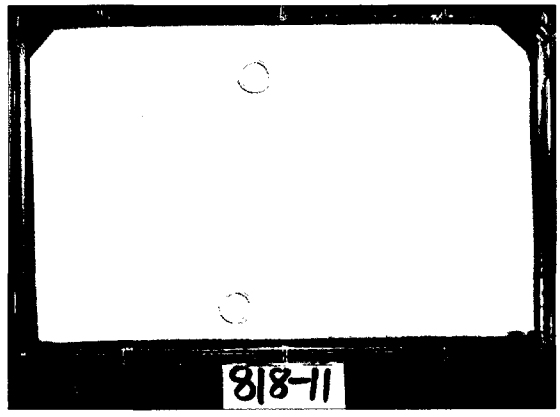

818-11

Figure 5-2: Ratio of β-gal units of 9 deletion mutant strains in comparison to the negative control (YKL205W). Plasmid pUKC817, pUKC818 and pUKC819 contain in-frame premature stop codon UAA, UAG and UGA respectively.

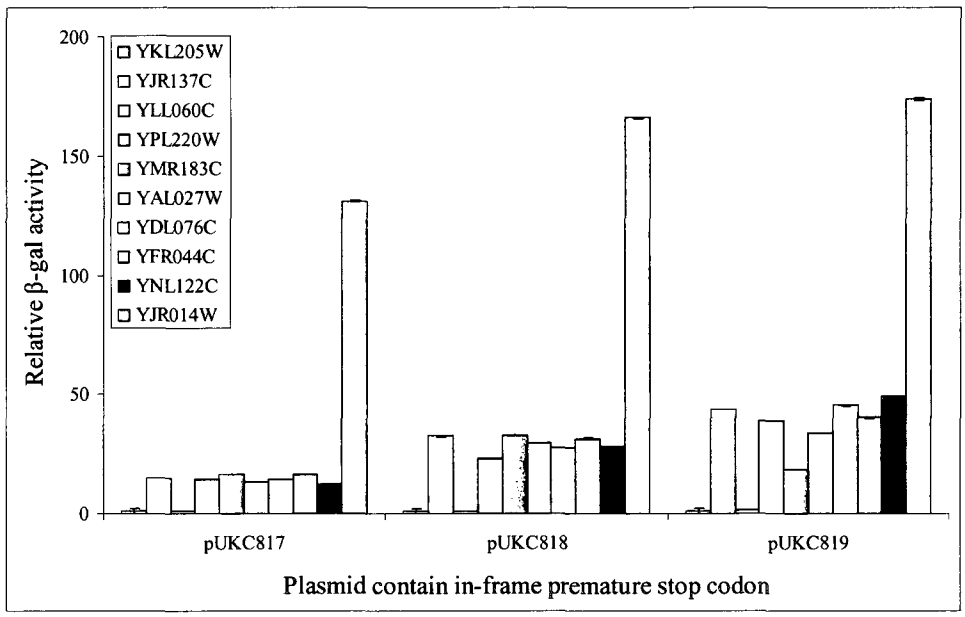

200
150
100
50
0
Relative β-gal activity
YKL205W
YJR137C
YLL060C
YPL220W
YMR183C
YAL027W
YDL076C
YFR044C
YNL122C
YJR014W
pUKC817
pUKC818
pUKC819
Plasmid contain in-frame premature stop codon

Table 5-1: Quantitative liquid culture-based β-gal analysis of the yeast deletion strains.

Systematic name	Standard Name	pUKC817	pUKC818	pUKC819
YKL205W	LOS1	1.00	1.00	1.00
YNL145W	MFA2	3.13	3.90	0.18
YDR503C	LPP1	3.98	22.92	20.82
YNL281W	HCH1	3.50	3.06	3.97
YLL060C	GTT2	1.28	0.99	1.42
YOR381W	FRE3	1.02	6.24	0.12
YMR031W-A	YMR031W-A	16.86	14.11	22.60
YPL220W	RPL1A	2.76	6.29	9.12
YLR219W	MSC3	1.80	2.35	0.26
YBR025C	OLA1	1.33	0.91	2.30
YLR214W	FRE1	0.03	4.12	9.89
YMR183C	SSO2	4.22	19.38	3.46
YLL019C	KNS1	0.31	23.87	0.47
YJR062C	NTA1	4.24	7.59	13.81
YER167W	BCK2	2.93	6.41	10.19
YDR300C	PRO1	1.14	0.41	1.59
YOR309C	YOR309C	4.53	5.35	5.24
YAL015C	NTG1	3.44	0.65	0.90
YDR455C	Dubious	3.36	8.73	8.32
YPR152C	URN1	2.08	2.08	2.73
YJR014W	TMA22	79.39	117.53	156.76
YGR118W	RPS23A	2.09	5.07	5.87
YMR080C	NAM7	101.05	99.06	137.34
YBR212W	NGR1	0.97	0.36	4.70
YDL076C	RXT3	8.38	18.56	4.43
YDR467C	Dubious	7.48	7.74	2.90
YER156C	YER156C	2.68	3.76	5.29
YAL027W	SAW1	4.86	3.43	24.17
YGR117C	YGR117C	3.37	6.21	6.29
YFR044C	DUG1	7.79	15.53	11.18
YGR072W	UPF3	71.89	87.10	95.53
YGL197W	MDS3	1.62	2.26	0.76
YDR099W	BMH2	1.48	1.66	1.80

Systematic name	Standard Name	pUKC817	pUKC818	pUKC819
YNL128W	TEP1	1.51	1.16	2.65
YFL043C	YFL043C	2.40	2.93	9.12
YOR365C	YOR365C	2.70	11.23	6.60
YNL122C	YNL122C	5.31	5.92	5.60
YHR077C	NMD2	74.84	68.53	79.10
YCL025C	AGP1	3.98	5.69	0.48
YOL049W	GSH2	164.79	2.41	67.05
YFR030W	MET10	2.13	5.28	3.73
YNR069C	BSC5	2.43	1.48	15.09
YJR137C	ECM17	8.60	13.83	19.58
YDR063W	AIM7	3.66	10.64	35.46
YDR275W	BSC2	4.08	1.67	10.33
YER007C-A	TMA20	53.37	51.90	56.57
YNR047W	FPK1	0.14	0.19	0.31

Red indicates the strains that used for quantitative liquid culture-based β-gal analysis

5.4 Discussion

S. cerevisiae is a widely used model system to study translation and other related processes and a number of translation factors have been characterized in this yeast. However there still remain a number of uncharacterized genes that can potentially affect translation in this organism. It is reported that about a quarter of the yeast non-essential genes are either ill or uncharacterized.

An important feature of translation is the maintenance of its accuracy. Mutation of key elements of translation is shown to affect its fidelity. In order to better understand the process of translation and its cross-talk with other related processes, it is important to identify the genes that can affect the accuracy of translation. Here, we investigated the entire yGDA collection to identify genes that are involved in stop codon read-through. We identified 106 genes that when deleted putatively affect the fidelity of translation. Of nine of these 106 deletion mutants tested, 8 were confirmed to reduce fidelity of translation.

Our approach was made possible via a modified SGA method that allowed us to transform ~4,700 yeast strains by 4 different plasmids for a total of more than 20,000 transformations. Using conventional transformation technology this approach would have taken approximately 3 years to complete at a rate of ~25 transformations per day. Our measurement of translation fidelity is based on the assumption that compromised

translation fidelity can result in stop codon read-through. We therefore used expression plasmids containing *LacZ* gene that carry premature stop codons. Initially large-scale colony lift β-gal assay was performed to identify potential positives. The validity of these candidates was then confirmed using a small-scale liquid culture β-gal assay.

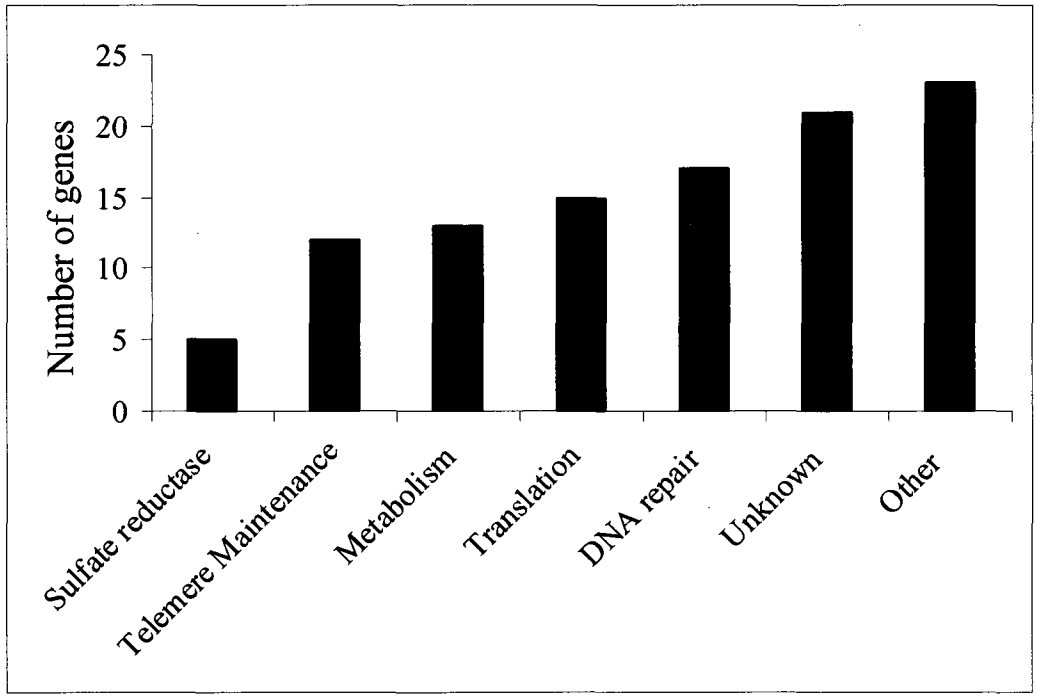
The genes identified in our screen had very diverse functions (Figure 5-3). Some of them are directly involved in translation, some are likely to affect telomere length indirectly either by altering the activity of proteins involved in telomere maintenance or DNA maintenance, and some are involved in metabolism. It appears that approximately 50% of the identified genes have a known relationship with translation and hence its accuracy. The other 50% however appear to be novel in their association with translation. For example, it has been previously reported that mutations in YGR118W, a subunit of small (40S) ribosomal subunit both altered translation accuracy and telomere length maintenance (Synetos *et al.* 1996). Here we observed that *YGR118WΔ* strain had approximately 2-6 times higher level of β-gal expression compared to a control (Table 5-1). Though there is no evidence for a direct link between telomere maintenance and translation fidelity, these findings suggests that there might be a relation between these two processes (Askree *et al.* 2004.). Other identified genes previously reported to be involved in telomere length maintenance include YIL036W, YNL281W, YMR080C, YPR049C, YJL212C, and YGR118W. In addition, our screen identified several components of sulfite reductase complex (5 genes) (Figure 5-3). Genes that are involved in sulfite reductase complex, participate in both selenoamino acid metabolism and sulfur metabolism (Jacob *et al.* 2003, Barreto *et al.* 2006). A connection between translation and

the sulfur biosynthesis pathway may stem from sulfur-based tRNA modification. It is known that sulfur is used to modify MET-tRNA at its active site (Kim *et al.* 1993). Alterations in tRNA modification can explain defects in translation fidelity (Ling *et al.* 2007).

We observed that most of the deletion strains transformed with pUCK818 and pUCK819 had significantly higher activity than those transformed with pUKC817 (Table 5-1). pUCK817 contains an in-frame stop codon UAA which is recognized by Release Factor 1 (RF1) and Release Factor 2 (RF2) and is believe to be a more stringent stop codon (Linder, 1992). It is therefore not surprising to see a higher level of read-through for pUCK818 and pUCK819, which carry UAG and UGA premature stop codons. UAG and UGA stop codons are recognized by RF1 and RF2 respectively.

In conclusion, we have used a large-scale approach to identify novel genes involved in translation fidelity. This investigation has provided a more comprehensive framework for understanding different factors that can affect the accuracy of translation. Our data has also revealed unexpected links for translation to different cellular processes such as telomere length maintenance and sulfur metabolism. Understanding of the cross-communications between different cellular processes can help better realize the activity of a cell as a system.

Figure 5-3: Functional distribution of yeast deletion strains that affect translation fidelity according to their cellular functions.


Number of genes
25
20
15
10
5
0
Sulfate reductase
Telemere Maintenance
Metabolism
Translation
DNA repair
Unknown
Other

6.0 CONCLUSION

6.1 Concluding Remarks:

S. cerevisiae has been traditionally used as a model organism of choice to study various conserved cellular pathways and processes as well as protein complexes and gene functions in higher eukaryotes. There are different ways to study gene function. Among them, removing (deleting) or repressing the expression of a target gene and observing the corresponding phenotypic changes that have been used as a standard tool to obtain clues into the activity of the target genes.

Currently, four types of gene deletion arrays are used for genome wide functional genomics analyses: i) *MATa* and *MATα* heterozygous diploid, ii) *MATa* and *MATα* homozygous haploid, iii) *MATa* haploid, and iv) *MATα* diploid. In our study, we employed *MATa* haploid yeast gene deletion library of non-essential genes, to systematically investigate increased sensitivity of deletion strains to five different bioactive compounds that exert their activity on the process of translation. The diploid mutant strains always carry a copy of the wild type genes and consequently show less growth difference compared to the heterozygous haploid system. In comparison to the diploid yeast deletion array, in haploid yeast deletion strains the expression level of gene is reduced to none. Though there is no significant discrepancy found between the results of these two sets of array for chemical sensitivity and the results are generally thought to be more complementary rather than redundant.

In our study we identified a number of previously uncharacterized genes than when deleted caused sensitivity to translation drugs. The underlying assumption is that deletion of genes that affect translation may increase the sensitivity of the deletion mutants to different translation inhibitory drugs. The chemical-genetic profiles that are obtained in this fashion can be used to better understand the activity of the tested bioactive compounds on a cell. Colony size measurement is used to detect growth differences and hence sensitivity. This approach is partly based on the theory that in general the presence of redundant pathways compensate for the genetic inactivation of a single pathway, with no phenotypic consequence (Hartman *et al.* 2001). However, the inactivity of a second functionally overlapping pathway, in this case using a chemical treatment, can cause a "double hit" effect and result in a phenotypic consequence that can be scored as a reduction in the rate of growth, or a sick/sensitive phenotype. Because there is no absolute threshold level for sick/sensitive phenotype, determining the phenotype of the mutant strains remains an arbitrary with respect to the fitness profile. So, there may remain a number of gene deletions which may be considered sensitive by certain accounts. But based on the arbitrary cutoff in our investigations, we were not able to select as sensitive. One way to identify such candidates is through cell culture doubling time analysis which may be considered as more accurate than CS used here.

One limitation of large-scale analysis is the generation of large false positive and negative results. Here, we expect that we have detected many sensitive strains that are not really sensitive to the tested compounds (false positives). On the other hand, there might be some strains that did not show sensitivity to the tested compound but are in fact true

sensitive (false negative). Generally a false positive/negative strains can be detected due to i) differences in the chemical and physical properties of media from one experiment to next ii) number of cells lifted during the pinning procedure iii) differences in incubation period of the plate, iv) presence of unexpected stress conditions and v) presence of multi-drug resistant genes.

Furthermore, we used compounds at subinhibitory concentrations. This subinhibitory amount could be another reason for the false positive or negative results. If we increase the concentration of the compound, the number of sensitive strains will rise. Similarly lower concentration of a drug may miss a number of true positives. Since there is no standard way to select subinhibitory concentrations, we expect that a similar investigation with a different subinhibitory concentration may result in a very different set of sensitive strains.

Here we used CSR to compare the growth of control plate and experimental plate (Memarian *et al.* 2007). There are numerous advantages and disadvantages of using CSR which uses solid medium. One advantage is the ease and the speed of analysis. A disadvantage of using CSR is that the number of cells those are transferred during the pinning procedure cannot be readily regulated and might produce misleading results. To validate and avoid large amount of false positive/negative results in our analysis, we perform i) spot test analysis, ii) cross validate our dataset with other published dataset, and iii) eliminated genes those are sensitive to compound other than translation inhibitory compound, such as CW.

We also hypothesized that using sensitivity to multiple compounds that target the same process may be a better way to detect genes that specifically target that corresponding pathway. Therefore, we assumed that using five inhibitory compounds that target translation might be a suitable approach to target translation genes. However to our surprise we observed that as we increased sensitivity to more translation drugs, the ratio of known protein synthesis related gene deletions were reduced. This clearly indicated that contrary to our prediction, detection of sensitive strains to more translation drugs in fact may reduce our specificity to detect translation genes. An explanation for this is the presence of multiple drug resistance genes. For some of phenotypic clusters and enrichment of stress related genes were observed.

Generally, drugs with similar mode of activity are thought to cluster together in chemical profile analyses. Here we used, aminoglycosides paromomycin, neomycin and streptomycin, which are known to induces codon misreading, inhibit translocation and affect protein synthesis by binding with small ribosomal subunit (Tuite and McLaughlin, 1984); cycloheximide that binds to large ribosomal subunit and inhibits translation elongation by interfering with tRNA translocation (Schneider-Poetsch *et al.* 2010), and 3-AT that alters the pool of amino acids in a cell. However our chemical profile analysis which clusters the sensitive genes according to their sensitivity and function, suggested that paromomycin clustered with neomycin; and cycloheximide grouped with 3-AT and eventually merges with streptomycin. This discrepancy of chemical clustering could be due to the structural differences between paromomycin, neomycin and streptomycin.

Neomycin and paromomycin contain conserved functional groups on rings I and II that cause a characteristic miscoding pattern. On the other hand, streptomycin does not contain those conserved functional groups. So, it is possible that streptomycin binding with ribosomes, might change the confirmation of ribosome causing similar effect to those made by cycloheximide and 3-AT.

From the list of profiled genes, we focused on 4 previously ill characterized genes and hypothesized that they may be novel translation related genes. We followed up our analysis by investigating the effect of the deletion of these genes on: **1.** translation fidelity, measured by premature stop codon read-through analysis; **2.** translation efficiency, analyzed by incorporation of [^{35}S]-Met into newly synthesized proteins as well as induced production of a reporter gene; and **3.** ribosome biogenesis, studied by ribosomal subunit profiling. Involvement of candidate genes in translation was further studied by a systematic genetic interaction analysis to investigate aggravating and alleviating interactions between target genes and genes which have been previously linked to translation. We further investigated the activity of the target genes in translation by designing a novel systematic genetic approach in which the overexpression of a target gene compensated for a phenotypic consequence (drug sensitivity in this case) of a deletion strain for a previously reported translation gene.

Our chemical-genetics profiles suggest that all the examined anti-translation compounds seem to genetically interact with at least a few different cellular processes. Additionally, all the profiles were almost identical to each other suggesting a genetic connection

between these drugs and the same cellular process with translation as a major category followed by metabolism, cellular compartments and biogenesis, transport and stress, DNA repair and replication. One way to explain these data is through the hypothesized cross-talk between translation and other cellular processes. Due to its central importance to the survival of the cell, along with the massive energy requirement for protein synthesis, the process of translation is thought to be in communication with different cellular processes such as metabolism and stress response (Begley *et al.* 2007, Hildebrandt *et al.* 1997). In this context some of the identified gene candidates in our chemical-genetics profiles may serve as bridges for such communications between translation and other processes. The smaller clusters may also represent alternative modes of activities for these compounds.

Of interest is the use of 3-AT in identifying translation genes. 3-AT is known to affect the pool of amino acids inside the cell. Our analysis clearly indicates that the main category of genes that once deleted confirms sensitivity to 3-AT belongs to translation. Since altering the pool of amino acids might be expected to affect the elongation phase of translation, sensitivity to 3-AT may be used to detect alteration in translation elongation.

Our genetic assays to study translation fidelity, efficiency and ribosome biogenesis all confirmed an involvement for our 4 target genes *YBR261C*, *YPL009C*, *YIL137C* and *YPL183W-A* that here we termed *TAE1-4*, respectively, in the process of translation. Deletions of these genes affect translation to a different extent; some seem to cause modest variations whereas others appear to have higher effects. In general however, the

observed alterations in translation in the absence of these genes do not appear to be extreme. Due to the importance of translation in cell survival, this might be expected as any severe defect in translations could be lethal to the cell. The targeted genes in our studies are all non-essential and the growth rates of their gene deletion mutant strains do not seem to be significantly different from wild type.

Our genetic interaction analyses further reaffirmed the involvements of the target genes in translation. *YBR261C*, *YPL009C* and *YIL137C* seem to have a role in ribosome biogenesis. This was observed by alteration in their ribosome profiles and consequence effects that their deletions had on translation fidelity and efficiency. Similarly when systematic double mutants were produced between these query genes and 384 genes thought to be involved in translation, these genes formed genetic aggravating and alleviating interactions with a number of genes with roles in RNA processing associated with translation. Their overexpression also compensated for the sensitivity of deletion strains for a number of RNA processing genes associated with translation to streptomycin and neomycin. *YPL183W-A*, on the other hand, appeared to have a general affect on translations and its ribosome profile appeared nearly identical to the wild type. It should be mentioned that our current analysis is considered preliminary. Further work is needed to elucidate the molecular activity of these novel factors in translation.

Our genetic interaction analysis using the target genes as a query represented a dense interaction map for translation genes. It appears that the genetic distance between different genes might be small and that many genes or gene products interact with each

other. Additional evidence for this observation came from a recent publication in which a number of translation genes were found to form dense interactions with each other (Costanzo *et al.* 2010). The appearance of a small-world network for translation genes suggests the presence of genetic hubs which form local or regional genetic centers to which many genes interact. These genetic hubs may prove to be useful target sites for development of novel and improved drugs.

6.2 Future directions

Translation represents one of the most fundamental aspects of a living cell. Identification of 4 novel genes in the process of translation suggests the presence of other undiscovered genes that affect this process. Consequently, this study sets path for similar investigations to discover other novel genes involved in translation. These investigations also reaffirm the usages of large-scale chemical-genetic profile analysis in functional genomics. This type of screening is of course not limited to the process of translation. In theory, any inhibitory compound could potentially be used to identify novel genes involved in a target pathway.

In accord with the presence of other novel genes that affect translation, our preliminary large-scale attempt (Chapter 5) to identify genes that affect translation fidelity has indicated the existence of a number of novel genes that can affect translation. Further work is needed to confirm the involvement and the activity of these genes. A possible experiment is the use of reconstituted translation system in vitro. In this system, the minimum necessary components of translation are added to a test tube and translation reaction programmed by a synthetic mRNA is used to study the kinetics of translation in the presence and absence of the target elements. In this way incorporation of labeled amino acids can be monitored to measure translation fidelity. Similarly the rate of amino acid incorporation can be used to calculate translation efficiency. The binding of the target proteins to rRNA can also be investigated using RNA protection analysis. The

rRNA region to which a target protein binds can give solid clues to the activity of that protein at a mechanistic level.

Also involvement of target genes in ribosome biogenesis can be further investigated. Northern blot analysis of rRNA precursors is a common approach to study the effect of target genes on rRNA biogenesis at a mechanistic level. Increase and decreases in different intermediate steps provide insights into the activity of target genes. Similarly modified ribosome profile analysis can be performed to better measure the differences between different rRNA species.

Homologs of the identified genes can also be examined for their translational activities in higher eukaryotes. Investigating novel translation genes in higher eukaryotes on the basis of the activity of their homologs in yeast provides a suitable opportunity to better understand the fundamentals of this important process in higher eukaryotes such as human.

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8.0 APPENDIX

8.1 Supplemental Table 8-1: List of genes deletions which are sensitive to paromomycin.

Standard Gene Name	Systematic Gene Name	Standard Gene Name	Systematic Gene Name	Standard Gene Name	Systematic Gene Name
Supersensitive					
CCR4	YAL021C*	CKB1	YGL019W*	CDA1	YLR307W
LDB7	YBL006C*	PIB2	YGL023C	EST2	YLR318W
GAL1	YBR020W*	PGD1	YGL025C	MID2	YLR332W
SEC66	YBR171W	SKN1	YGR143W*	ROM2	YLR371W
RIM1	YCR028C-A*	LAS21	YJL062W	YLR381W	YLR381W
YDR067C	YDR067C	ASF1	YJL115W	REC114	YMR133W
MHR1	YDR296W	OPI3	YJR073C*	UBP15	YMR304W
YDR307W	YDR307W	RPL43B	YJR094W-A	COG6	YNL041C*
TRP4	YDR354W*	UBP11	YKR098C	RPL20B	YOR312C
GIM4	YEL003W	BAS1	YKR099W		
YER175C	YER175C	YLR190W	YLR190W		
Sensitive					
ATS1	YAL020C	LYS2	YBR115C	PMP1	YCR024C-A*
DRS2	YAL026C*	GRS1	YBR121C	THR4	YCR053W*
CYC3	YAL039C	HSL7	YBR133C	BUD31	YCR063W
GCV3	YAL044C	UMP1	YBR173C	RAD18	YCR066W
YAL045C	YAL045C	ECM31	YBR176W	YCR095C	YCR095C*
SPC72	YAL047C	RPS6B	YBR181C	THI3	YDL080C
PEX22	YAL055W	SMP1	YBR182C	PHO2	YDL106C
GPE2	YAL056W	RPS9B	YBR189W	CYK3	YDL117W
FUI1	YBL042C	KTR4	YBR199W	YDL118W	YDL118W
EDE1	YBL047C*	BEM1	YBR200W	AAD4	YDL243C
YBL051C	YBL051C*	MET8	YBR213W	GCV1	YDR019C
YBL057C	YBL057C	APG12	YBR217W	DOS2	YDR068W
YEL1	YBL060W	YBR238C	YBR238C	IPT1	YDR072C*
YBL064C	YBL064C	YBR261C	YBR261C	PPH3	YDR075W*
UBP13	YBL067C*	MAL32	YBR299W	RAD55	YDR076W
RPS8A	YBL072C	STP22	YCL008C*	RRP8	YDR083W
NUP170	YBL079W	GBP2	YCL011C	YDR084C	YDR084C
PDX3	YBR035C	KCC4	YCL024W	AFR1	YDR085C
CSG2	YBR036C*	RNQ1	YCL028W	YDR089W	YDR089W
FAT1	YBR041W	BIK1	YCL029C	GIS1	YDR096W

Standard Gene Name	Systematic Gene Name	Standard Gene Name	Systematic Gene Name	Standard Gene Name	Systematic Gene Name
Sensitive					
RPS11B	YBR048W	HIS4	YCL030C	MSH6	YDR097C
YBR077C	YBR077C*	YCL044C	YCL044C	YDR101C	YDR101C
SIF2	YBR103W*	RVS161	YCR009C	YDR116C	YDR116C
YDR185C	YDR185C	GAL83	YER027C	PRS3	YHL011C
REF2	YDR195W	YER028C	YER028C	YHL012W	YHL012W
EBS1	YDR206W	PHM8	YER037W	SNF6	YHL025W
YDR210W	YDR210W	MXR1	YER042W	NEM1	YHR004C
YDR222W	YDR222W	BUD18	YER044C	ESC4	YHR154W
YDR248C	YDR248C	MEI4	YER044C-A	RPL34B	YIL052C
HNT2	YDR305C	YER045C	YER045C	YIL092W	YIL092W
GIC2	YDR309C	ILV1	YER086W*	PRK1	YIL095W
SSF2	YDR312W	SBH1	YER087C-A	YIL110W	YIL110W
YDR326C	YDR326C	DEG1	YFL001W	COX5B	YIL111W
PEX3	YDR329C	BLM3	YFL007W	YIL122W	YIL122W
YDR333C	YDR333C	RIM15	YFL033C	YIL130W	YIL130W
YDR352W	YDR352W	LOC1	YFR001W	YIL132C	YIL132C
YDR387C	YDR387C	YFR008W	YFR008W	FLX1	YIL134W
SAC7	YDR389W*	IOC3	YFR013W	YIL161W	YIL161W
SHE9	YDR393W	CMK1	YFR014C	NIT1	YIL164C
YDR444W	YDR444W	COG7	YGL005C*	YIL166C	YIL166C
RPS17B	YDR447C	PMC1	YGL006W	IST3	YIR005W
RPS18A	YDR450W	YGL010W	YGL010W	YIR007W	YIR007W
MFA1	YDR461W	YGL020C	YGL020C	MGA2	YIR033W*
VPS3	YDR495C	TRP5	YGL026C*	LYS1	YIR034C
ITR1	YDR497C	SCY1	YGL083W	YJL065C	YJL065C
RPL37B	YDR500C	GUP1	YGL084C	SCP160	YJL080C
KRE28	YDR532C	YGL101W	YGL101W	ARG3	YJL088W
YEL006W	YEL006W	ARC1	YGL105W	YJL123C	YJL123C
TOS9	YEL007W	YGL232W	YGL232W	CPS1	YJL172W
ECM10	YEL030W	MTO1	YGL236C	SWI3	YJL176C
RAD23	YEL037C*	HAP2	YGL237C	YJL178C	YJL178C
PAU2	YEL049W	YGR004W	YGR004W	TOR1	YJR066W
RML2	YEL050C	YGR125W	YGR125W	YJR082C	YJR082C
CIN8	YEL061C	NSR1	YGR159C	JSN1	YJR091C

Standard Gene Name	Systematic Gene Name	Standard Gene Name	Systematic Gene Name	Standard Gene Name	Systematic Gene Name
Sensitive					
CAN1	YEL063C	RSM27	YGR215W	BUD4	YJR092W
AVT2	YEL064C	PFK1	YGR240C	IME1	YJR094C
SIT1	YEL065W	YGR273C	YGR273C	SFC1	YJR095W
DLD3	YEL071W	BGL2	YGR282C	VPS25	YJR102C*
MNN1	YER001W	ZUO1	YGR285C	ADO1	YJR105W
BIM1	YER016W	MAL12	YGR292W	YJR149W	YJR149W
TEF4	YKL081W	CPR3	YML078W	MDH2	YOL126C
LTV1	YKL143W	DUS1	YML080W	WHI2	YOR043W*
YSR3	YKR053C	ERG5	YMR015C*	ATX2	YOR079C
YKR100C	YKR100C	ASC1	YMR116C	KTR1	YOR099W
RPL8B	YLL045C	YMR130W	YMR130W	YOR138C	YOR138C
RIC1	YLR039C	YMR144W	YMR144W	YOR173W	YOR173W
IES3	YLR052W	YMR147W	YMR147W	YOR245C	YOR245C
CHA4	YLR098C	MSS11	YMR164C	SNF2	YOR290C
ICT1	YLR099C	YMR187C	YMR187C*	SNF2	YOR290C
YLR118C	YLR118C	ICY1	YMR195W	YOR291W	YOR291W
PDC5	YLR134W	YMR196W	YMR196W	RPS10A	YOR293W
PUS5	YLR165C	HFA1	YMR207C	YOR295W	YOR295W
RFX1	YLR176C*	YMR215W	YMR215W	YOR304C-A	YOR304C-A
SWI6	YLR182W	RPS10B	YMR230W	PUT4	YOR348C
HCR1	YLR192C	GFD1	YMR255W	MNE1	YOR350C
YLR193C	YLR193C	PET111	YMR257C	YOR352W	YOR352W
YKE2	YLR200W	ZDS1	YMR273C*	GRD19	YOR357C
YLR221C	YLR221C	YMR313C	YMR313C	HAP5	YOR358W
RCK2	YLR248W	SFB2	YNL049C	GDH1	YOR375C
SSP120	YLR250W	YNL144C	YNL144C	FIT3	YOR383C
YLR285W	YLR285W	ALF1	YNL148C*	ULA1	YPL003W
REC102	YLR329W	YNL211C	YNL211C	MET12	YPL023C
CHS5	YLR330W	YNL224C	YNL224C	NCE4	YPL024W
NUP2	YLR335W	SIP3	YNL257C*	ELC1	YPL046C
YLR350W	YLR350W	PUS4	YNL292W	YPL056C	YPL056C
YLR352W	YLR352W	MON2	YNL297C	BTS1	YPL069C
YLR392C	YLR392C	CLA4	YNL298W	RNY1	YPL123C

Standard Gene Name	Systematic Gene Name	Standard Gene Name	Systematic Gene Name	Standard Gene Name	Systematic Gene Name
Sensitive					
YPT7	YML001W*	ATP11	YNL315C	KAP120	YPL125W
YML003W	YML003W	YNR048W	YNR048W	UME1	YPL139C
GLO1	YML004C	YOL029C	YOL029C	YPL144W	YPL144W*
YML014W	YML014W	PEX15	YOL044W	APG5	YPL149W
OST6	YML019W*	YOL053W	YOL053W	MLH3	YPL164C
RPS17A	YML024W	YOL054W	YOL054W*	PCL8	YPL219W
RPS18B	YML026C	YOL063C	YOL063C	ALG5	YPL227C*
TSA1	YML028W*	INP54	YOL065C	CLN2	YPL256C
YML072C	YML072C	RPS19A	YOL121C	MDL2	YPL270W
YPR015C	YPR015C	CVT9	YPR049C	CTF4	YPR135W
YPR027C	YPR027C	SPE3	YPR069C	KIM3	YPR164W

Red indicates genes those shared with paromomycin sensitivity test using a heterozygous deletion array.

* Represents those genes that are repeatedly seen in our similar screenings using different drugs. Therefore they may represent false-positives and should be treated with caution.

8.2 Supplemental Table 8-2: List of genes deletions which are sensitive to both Paromomycin and Calcofluor White (CW).

Standard Gene Name	Systematic Gene Name	Standard Gene Name	Systematic Gene Name	Standard Gene Name	Systematic Gene Name
POP2	YNR052C	YML030W	YML030W	HOF1	YMR032W
SPT3	YDR392W	KRE20	YAL058C-A*	ESC2	YDR363W
RPS1B	YML063W	YLR374C	YLR374C	YOR161C	YOR161C
RTF1	YGL244W	YDL206W	YDL206W	KHA1	YJL094C
NUP84	YDL116W	YAL004W	YAL004W	SNQ2	YDR011W
IST1	YNL265C	ERG4	YGL012W*	YOR383C	YOR383C
YOR285W	YOR285W	MTH1	YDR277C	VMA11	YPL234C
RPL11B	YGR085C	MAP1	YLR244C	SPC72	YAL047C
YPT6	YLR262C	BCK2	YER167W	VPS29	YHR012W*
YPR087W	YPR087W	SUR4	YLR372W	MNN10	YDR245W
FYV5	YCL058C	YHR151C	YHR151C	YLR254C	YLR254C
YLR322W	YLR322W	SHP1	YBL058W	MDM10	YAL010C
YJR087W	YJR087W	YAL008W	YAL008W	GLC8	YMR311C*
YEL045C	YEL045C	SRO7	YPR032W*	AHP1	YLR109W
YLR112W	YLR112W	IRA2	YOL081W	SPF1	YEL031W*
YDR136C	YDR136C	XRS2	YDR369C	ATP4	YPL078C
YLR123C	YLR123C	YDR279W	YDR279W	YMR145C	YMR145C

* Represents genes that are repeatedly seen in our similar screenings using different drugs. Therefore they may represent false-positives and should be treated with caution.

8.3 Supplemental Table 8-3: Descriptions of translation related genes that genetically interact with *TAE1*.

Standard Gene Name	Systematic Gene Name	Phenotype	Description and Cellular Function
Ribosomal Proteins			
YDL075W	RPL31A	Sick	Component of large (60S) subunit, similar to Rpl31Bp and rat L31 ribosomal protein
YDL081C	RPLA1	Sick	Involved in the interaction between ribosome and translational elongation factors
YDR462W	MRPL28	Very sick	Mitochondrial large ribosomal subunit of yeast
YEL050C	RML2	Very sick	Large subunit of Mitochondrial ribosome, similar to *E. coli* L2 ribosomal protein
YEL054C	RPL12A	Sick	Large (60S) ribosomal subunit which is similar to Rpl12Bp; rpl12a rpl12b, *E. coli* L11 and rat L12 ribosomal proteins
YER117W	RPL23B	Sick	Large (60S) ribosomal subunit, similar to Rpl23Ap, *E. coli* L14 and rat L23 ribosomal proteins
YFL034C-A	RPL22B	Sick	Large (60S) ribosomal subunit, identical to Rpl22Ap and rat L22 ribosomal protein
YGR118W	RPS23A	Sick	Ribosomal protein 28 (rp28) of the small (40S) ribosomal subunit involved in translational accuracy; and similar to *E. coli* S12 and rat S23 ribosomal proteins
YGR148C	RPL24B RPL30B	Moderate	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl24Ap and similar to rat L24
YHL033C	RPL8A	Very sick	Component of large (60S) ribosomal subunit, similar to rat L7a ribosomal protein
YIL052C	RPL34B	Sick	Protein component of the large (60S) ribosomal subunit, similar to rat L34 ribosomal protein
YMR143W	RPS16A	Very sick	Protein component of the small (40S) ribosomal subunit; involved in translation
YNL067W	RPL9B	Sick	Large (60S) ribosomal subunit component, similar to *E. coli* L6 and rat L9 ribosomal proteins
YOL121C	RPS19A	Sick	Protein component of the small (40S) ribosomal subunit, involved in small ribosomal subunit biogenesis and organization
YPL090C	RPS6A	Very sick	Component of Small (40S) ribosomal subunit; involved in translation
YPR043W	RPL43A	Very sick	Large (60S) ribosomal subunit, similar to rat L37a ribosomal protein; involved in translation

Standard Gene Name	Systematic Gene Name	Phenotype	Description and Cellular Function
Amino Acids and Protein Production			
YDL040C	NAT1 AAA1	Moderate	Involved in N-terminal protein amino acid acetylation, which affects different cellular processes such as the cell cycle, heat-shock resistance, mating, sporulation, and telomeric silencing
YER091C	MET6	Sick	Involved in amino acid biosynthesis; similar to bacterial metE homologs
YGR285C	ZUO1	Moderate	Involved in protein folding, translation fidelity.
YLR451W	LEU3	Sick	Regulate transcription from RNA polymerase II promoter that regulates branched chain amino acid biosynthesis
YMR020W	FMS1	Moderate	Involved in the modification of translation factor eIF-5A; pantothenic acid biosynthesis
YOR184W	SER1 ADE9	Sick	Regulate serine and glycine biosynthesis and the general control of amino acid biosynthesis mediated by Gcn4p
YOR303W	CPA1	Very sick	Small subunit of arginine specific carbamoyl phosphate synthetase, which is involved in the ariginine biosynthetic pathway
rRNA Synthesis			
YBL025W	RRN10	Sick	Protein involved in promoting high level transcription of rDNA and RNA polymerase I promoter transcription factor activity
YOL041C	NOP12	Moderate	Nucleolar protein involved in pre-25S rRNA processing, RNA binding. It is similar to Nop13p, Nsr1p

8.4 Supplemental Table 8-4: Descriptions of translation related genes that are phenotypically suppressed by overexpression of *TAE1*, against treatment with neomycin and/or streptomycin

Deletion strains which are hypersensitive to:		Recovery		Description and Cellular Function
Neomycin	Streptomycin	Neomycin	Streptomycin	
Ribosomal Proteins				
YBL027W		Complete		Large (60S) ribosomal subunit component, involved in translation. Similar to rat L19 ribosomal protein; rpl19a and rpl19b
YBR189W	YBR189W	Partial	Partial	Small (40S) ribosomal subunit component; Similar to *E. coli* S4 and rat S9 ribosomal proteins; involved in translation and regulate translation fidelity
YDL061C		Complete		Cytosolic small (40S) ribosomal subunit involved in translation; nearly identical to Rps29Ap and similar to rat S29 and *E. coli* S14 ribosomal proteins.
YDL075W	YDL075W	Complete	Complete	Ribosomal protein of the large (60S) subunit, similar to rat L31
YDR382W	YDR382W	Partial	Partial	Proteins involved in the interaction between ribosome and elongation factors
YGR214W		Complete		Component of the small (40S) ribosomal subunit, involved in translation; assembly and maintenance of small ribosomal subunit
	YHL033C		Partial	Large (60S) ribosomal protein subunit, similar to rat L7
	YHR010W		Complete	Large (60S) ribosomal subunit component, involved in translation
YJL080C		Partial		RNA-binding protein, translate ribosomes via multiple KH domains, demonstrates significant sequence homology to vertebrate vigilins
YKL156W		Complete		Small (40S) ribosomal subunit; similar to rat S27 ribosomal protein
YKL167C		Complete		Mitochondrial large ribosomal subunit involved in translation
YKR057W		Partial		Small (40S) ribosomal subunit protein component involved in translation and telomere maintenance
YLR185W		Partial		Cytosolic protein component of the large (60S) ribosomal subunit, similar to Rpl37Bp and to rat L37

Deletion strains which are hypersensitive to:		Recovery		Description and Cellular Function
Neomycin	**Streptomycin**	**Neomycin**	**Streptomycin**	
YML024W		Partial		Ribosomal protein 51 (rp51) of the small (40s) subunit; nearly identical to Rps17Bp and has similarity to rat S17 ribosomal protein
YML063W	YML063W	Partial	Complete	Small (40S) ribosomal protein subunit involved in translation; maintenance and assembly of small ribosomal subunit
	YMR143W		Partial	Small (40S) ribosomal subunit component; identical to Rps16Bp and similar to *E. coli* S9 and rat S16 ribosomal proteins
YMR242C		Partial		Protein component of the large (60S) ribosomal subunit which is nearly identical to Rpl20Bp and comparable to rat L18a ribosomal protein
Translational Control				
	YBL013W		Complete	Formyl-Met-tRNA formyltransferase, involved in translation initiation
YJL124C		Partial		Lsm (Like Sm) protein; involved in degradation of cytoplasmic mRNAs, RNA binding
YJL209W		Complete		Mitochondrial protein which interacts with the 5'-UTR of mRNA and has a role mRNA catabolic process
	YKL204W		Partial	eIF4E-associated protein, involved in the negative regulation of translation and inhibits cap-dependent translation
YMR116C	YMR116C	Complete	Complete	G-beta protein involved in the negative regulation of translation, transposition; ortholog of mammalian RACK1
YNR052C		Complete		Involved in RNA elongation and regulation of transcription from RNA polymerase II promoter
	YPL178W		Complete	Small subunit of the heterodimeric cap binding complex involved in RNA cap binding, mRNA splicing
YPR042C		Complete		PUF protein family member involved in mRNA binding
Others				
YAL026C	YAL026C	Partial	Complete	Aminophospholipid translocase (flippase) involved in the maintenance of lipid asymmetry; ribosomal small subunit assemble and maintenance
YCL009C		Complete		Subunit of acetolactate synthase which involves in the biosynthesis of branched-chain amino acid
YGR159C		Complete		Nucleolar protein involved in pre-rRNA processing and ribosome biogenesis

8.5 Supplementary Table 8-5: List of genes deletions which are sensitive to translation inhibitory drugs.

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
VPS3	YDR495C	38.56	27.53	48.13	54.08	58.96
NTC20	YBR188C	39.40	32.86	40.93	47.37	
EST2	YLR318W	49.80	46.83	39.56	36.28	
YLR435W	YLR435W	53.67	32.55	56.00	41.99	
SNF2	YOR290C	63.41	30.91	52.28	47.28	
PRO2	YOR323C	67.75	58.81	39.23	45.68	
RVS161	YCR009C	60.83	27.79	35.55		85.05
YGL072C	YGL072C	58.71	49.62	65.42		32.19
GUP1	YGL084C	49.35	31.59	45.68		33.66
OPI3	YJR073C	57.57	73.09	64.28		41.87
YLR322W	YLR322W	90.48	54.29	44.25		26.16
CLA4	YNL298W	41.22	26.50	56.85		57.30
YOL163W	YOL163W	64.39	41.39	29.92		32.18
YOR295W	YOR295W	26.24	39.24	43.20		58.45
UBC4	YBR082C	53.77		55.96	44.35	58.50
LYS20	YDL182W	54.43		35.39	24.19	45.22
SOH1	YGL127C	43.59		79.00	40.04	26.20
YGL214W	**YGL214W**	26.75		34.20	39.39	40.96
MLC2	YPR188C	58.40		28.60	46.65	51.77
ECM8	YBR076W	47.78		68.77		50.85
SDS24	YBR214W	47.77		41.34		25.67
AOR1	YBR231C	42.08		38.76		36.34
SUL1	YBR294W	46.09		53.46		34.07
#N/A	YCL013W	60.22		34.87		87.79
FUS1	YCL027W	33.79		28.35		23.12
CVT17	YCR068W	38.19		42.65		34.70
NGG1	YDR176W	46.60		51.10		36.07
SUM1	YDR310C	49.82		59.57		57.45
RVS167	YDR388W	62.75		37.73		34.96
YDR496C	YDR496C	33.36		43.89		39.70
YEL008W	YEL008W	45.98		41.73		47.74
ERG4	**YGL012W**	87.32		59.16		49.34
YGL050W	YGL050W	37.26		50.01		50.26

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
ADE3	YGR204W	46.55		50.79		28.35
ZUO1	YGR285C	39.23		57.67		66.35
YHL041W	YHL041W	37.32		48.04		53.78
YHL044W	YHL044W	27.73		47.99		50.68
YPK1	YKL126W	53.09		27.96		48.36
MRP8	YKL142W	60.05		49.43		30.13
YKT9	YKL199C	32.78		59.24		59.83
YMR166C	YMR166C	25.28		54.29		33.89
YMR318C	YMR318C	45.51		43.66		48.58
PCL1	YNL289W	55.65		27.41		46.50
TLG2	YOL018C	54.26		58.77		35.17
RAX1	YOR301W	44.99		36.88		32.90
YOR359W	YOR359W	22.77		27.22		81.02
YBL083C	YBL083C	26.06	29.73	29.94		
YBR030W	YBR030W	51.91	27.97	45.35		
YBR077C	**YBR077C**	70.51	37.53	44.42		
HTL1	YCR020W-B	65.97	31.08	44.46		
YDR442W	YDR442W	57.98	43.58	30.56		
SNF1	YDR477W	62.89	53.42	37.30		
KEM1	YGL173C	24.11	25.64	38.81		
SNF6	YHL025W	58.11	37.39	28.19		
YLR287C	YLR287C	76.17	33.48	49.48		
CHS5	**YLR330W**	40.08	29.23	36.99		
ASC1	YMR116C	51.46	44.21	43.08		
YKL202W	YKL202W			30.69	23.29	38.97
RSN1	YMR266W			48.06	24.15	38.72
PMS1	YNL082W			52.43	39.83	41.22
VMA11	YPL234C			31.19	25.13	36.38
FYV5	YCL058C	27.30	54.33		44.75	
SSN3	YPL042C	56.80	49.46		32.14	
YPR139C	YPR139C	43.89	48.47		55.94	
RAD4	YER162C	48.72		54.94	58.76	
ZAP1	YJL056C	65.73		41.67	40.13	
YJR149W	YJR149W	29.79		24.31	30.24	
YDL225W	YDL225W		51.80	45.94		27.99
CBF1	YJR060W		26.31	37.77		41.85
BUD23	YCR047C		71.02	60.19	58.67	

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
SIP3	YNL257C		32.56	56.11	31.70	
PDR13	YHR064C	33.77			27.59	44.81
LSM1	**YJL124C**	61.49			35.21	35.71
RTS1	YOR014W	50.28			66.41	27.58
BUD18	YER044C	44.42	34.37			37.96
RPS10A	YOR293W		45.91		67.99	62.41
FUR4	YBR021W	27.53		57.78		
PAF1	YBR279W	30.36		43.57		
#N/A	YCL006C	39.85		53.11		
STP22	YCL008C	43.64		48.02		
YCL023C	YCL023C	39.41		57.37		
YCL038C	YCL038C	42.59		39.38		
#N/A	YCR062W	30.87		31.11		
CDC50	YCR094W	45.22		49.56		
RPN4	YDL020C	73.12		25.38		
RAM1	YDL090C	60.05		41.12		
DUN1	YDL101C	62.72		42.16		
UGA3	YDL170W	43.15		60.97		
YDL204W	YDL204W	25.33		40.86		
PTP1	YDL230W	27.65		28.23		
NTH1	YDR001C	68.54		21.79		
VPS41	YDR080W	45.83		26.84		
YDR431W	YDR431W	54.67		44.64		
KRE28	YDR532C	44.61		42.88		
TOS9	YEL007W	70.36		52.03		
UBP3	YER151C	51.54		53.13		
RIM15	YFL033C	40.49		36.48		
YMR31	YFR049W	25.60		32.75		
PGD1	YGL025C	56.38		26.32		
YGL051W	YGL051W	33.81		74.05		
NUT1	YGL151W	38.23		34.10		
YGR022C	YGR022C	53.34		27.44		
DBF2	YGR092W	49.18		38.57		
CLB1	YGR108W	23.75		38.72		
PHB1	YGR132C	35.26		64.42		
GTR2	YGR163W	41.74		49.96		
SER2	YGR208W	39.20		82.50		

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
APM2	YHL019C	35.35		32.38		
SPO11	YHL022C	38.37		53.18		
SRB2	YHR041C	75.90		26.06		
GIC1	YHR061C	35.63		38.52		
YHR209W	YHR209W	34.59		41.43		
INP51	YIL002C	31.05		45.05		
YIL086C	YIL086C	27.57		44.46		
FLX1	YIL134W	37.12		33.78		
YJL038C	YJL038C	36.34		54.21		
RPA34	YJL148W	35.35		72.22		
YJL175W	YJL175W	31.25		33.92		
RAV1	**YJR033C**	30.61		40.11		
UTR1	YJR049C	36.89		26.50		
YJR079W	YJR079W	36.54		32.57		
SOD1	YJR104C	57.61		36.24		
ADO1	YJR105W	63.45		35.97		
ILM1	YJR118C	36.89		48.70		
ECM17	**YJR137C**	50.46		45.12		
YJR146W	YJR146W	77.05		25.89		
UTH1	YKR042W	31.80		54.03		
YKR078W	YKR078W	37.02		57.75		
#N/A	YLL016W	28.00		37.70		
YLL057C	YLL057C	85.45		56.86		
YLR122C	YLR122C	42.51		21.84		
YLR124W	YLR124W	24.99		31.93		
STM1	YLR150W	30.72		40.64		
IDP2	YLR174W	38.81		40.69		
BUR2	YLR226W	42.51		48.17		
TOP3	YLR234W	47.71		36.61		
MAP1	YLR244C	42.60		39.16		
YLR315W	YLR315W	59.49		36.04		
YLR343W	YLR343W	35.46		28.01		
YLR386W	YLR386W	81.83		38.74		
TUS1	YLR425W	78.25		75.95		
HMG2	YLR450W	30.99		34.85		
YML014W	YML014W	37.48		38.82		
PPZ1	YML016C	51.50		42.70		

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
GTR1	**YML121W**	29.67		59.55		
ERG5	YMR015C	60.36		59.71		
HRT2	YMR027W	23.43		40.89		
LYS7	YMR038C	62.67		45.18		
CLN1	YMR199W	68.30		28.67		
ERG2	YMR202W	80.17		37.52		
YKU70	YMR284W	27.06		24.62		
YNL041C	YNL041C	50.25		38.54		
YNL056W	YNL056W	70.27		45.19		
YDJ1	YNL064C	52.29		57.30		
INP52	YNL106C	57.61		42.24		
AAH1	YNL141W	36.02		33.10		
YNL190W	YNL190W	27.51		32.66		
AUT2	YNL223W	35.66		53.74		
YNL227C	YNL227C	50.06		26.33		
CAF40	YNL288W	69.85				
YNL294C	YNL294C	35.86		56.93		
YPT11	YNL304W	23.23		34.79		
YNL305C	YNL305C	30.73		29.24		
YNR047W	YNR047W	50.18				
HTZ1	YOL012C	39.48		36.54		
IRA2	YOL081W	40.13		62.78		
YOL162W	YOL162W	62.01		31.99		
GLO4	YOR040W	34.66		31.90		
ASE1	YOR058C	61.04		27.97		
YNG1	YOR064C	32.04		59.00		
YOR072W	YOR072W	25.54		35.33		
YOR161C	YOR161C	41.18		50.87		
YOR173W	YOR173W	47.59		58.28		
YOR186W	YOR186W	26.53		39.04		
TUF1	YOR187W	23.13		29.13		
YOR193W	YOR193W	44.70		58.82		
WTM1	YOR230W	44.50		39.77		
YOR320C	YOR320C	28.65		33.49		
ARL3	YPL051W	31.16		31.80		
YPL108W	YPL108W	31.84		58.33		
LEA1	YPL213W	50.36		30.38		

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
YPR084W	YPR084W	36.59		56.35		
YPR130C	YPR130C	26.51		30.59		
NCE102	YPR149W	34.71		26.39		
SKI3	**YPR189W**	54.88		70.65		
DRS2	YAL026C			56.48		52.16
YMC2	YBR104W			28.22		27.84
YBR209W	YBR209W			48.38		62.26
HPC2	YBR215W			42.84		30.14
YBR220C	YBR220C			38.67		37.19
PDB1	YBR221C			44.92		41.48
LHP1	YDL051W			39.08		49.00
YDL053C	YDL053C			44.17		26.76
YDL183C	YDL183C			27.94		46.87
YDR163W	YDR163W			38.56		66.90
YDR200C	YDR200C			26.54		56.24
YER049W	YER049W			59.38		37.22
YFL040W	YFL040W			45.59		44.95
MFALPHA2	YGL089C			68.23		29.35
SNF4	YGL115W			65.63		41.35
YGL168W	YGL168W			24.94		43.56
VMA7	YGR020C			50.89		26.69
YGR064W	YGR064W			49.30		30.72
YGR081C	YGR081C			44.10		35.51
NAS6	YGR232W			35.91		28.14
YGR259C	YGR259C			33.38		33.85
BIO2	YGR286C			29.92		31.44
MNT3	YIL014W			27.47		44.95
VID28	YIL017C			37.83		44.91
YIL154C	YIL154C			28.43		33.75
DAL81	YIR023W			38.26		34.11
YJL149W	YJL149W			61.34		52.21
YJR001W	YJR001W			61.51		58.72
RPA12	YJR063W			37.12		35.98
YKL131W	YKL131W			33.52		32.56
CTK1	YKL139W			41.46		42.60
VPS1	YKR001C			35.59		36.25
YKR033C	YKR033C			69.82		58.55

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
YLL005C	YLL005C			56.07		23.20
COX17	YLL009C			43.54		29.89
YLL020C	YLL020C			23.68		37.29
ENT4	YLL038C			26.34		28.31
UBI4	YLL039C			30.77		50.66
YLR016C	YLR016C			38.08		45.61
ARP6	YLR085C			39.60		40.70
URA5	YML106W			32.10		43.80
RCE1	YMR274C			36.02		30.03
BNI1	YNL271C			29.24		33.66
TOF1	YNL273W			73.55		24.04
KRE25	YNL296W			48.99		57.99
RPD3	YNL330C			67.98		27.06
YOR315W	YOR315W			36.01		45.28
COT1	YOR316C			37.27		39.67
SNF8	YPL002C			48.04		31.44
PNG1	YPL096W			47.56		40.94
YPL141C	YPL141C			52.15		45.16
YPL236C	YPL236C			35.46		61.65
YPR040W	YPR040W			40.39		27.20
ARR2	YPR200C			49.60		34.05
SLA1	YBL007C	42.19				39.02
YBL032W	YBL032W	54.38				51.34
FAT1	YBR041W	67.30				31.15
YBR042C	YBR042C	25.05				23.14
YBR043C	YBR043C	40.86				32.28
YBR056W	**YBR056W**	51.48				41.54
YBR139W	YBR139W	26.27				33.93
SLX1	YBR228W	32.55				42.11
#N/A	YCL061C	55.78				35.87
SAT4	YCR008W	47.44				48.66
HNT1	YDL125C	27.40				66.41
STF1	YDL130W-A	52.61				23.91
ARO1	YDR127W	28.63				48.23
RPA14	YDR156W	35.89				51.78
SPR28	YDR218C	30.20				26.09
PMP3	YDR276C	72.77				61.56

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
YDR516C	YDR516C	30.94				41.59
HVG1	YER039C	38.10				33.93
YER053C	YER053C	62.88				25.02
HIS1	YER055C	45.82				29.62
FCY2	YER056C	47.57				29.76
PDR1	**YGL013C**	60.21				29.93
BZZ1	YHR114W	49.45				27.78
YIL077C	YIL077C	37.56				26.98
VPS35	YJL154C	52.01				37.40
URA8	***YJR103W***	22.47				52.57
ABM1	YJR108W	36.90				24.73
YJR111C	YJR111C	41.03				47.25
CCE1	YKL011C	25.60				65.64
ELM1	YKL048C	40.57				31.93
TRP3	YKL211C	44.30				31.29
KRE21	YLR338W	37.92				38.00
SKI2	**YLR398C**	29.60				25.70
SST2	YLR452C	32.33				23.84
YMR322C	YMR322C	31.62				41.65
YNL100W	YNL100W	32.75				37.98
YNL122C	YNL122C	42.11				46.43
YNL184C	YNL184C	25.56				23.17
CAF120	YNL278W	30.34				32.50
HCH1	YNL281W	59.73				62.35
CIT1	**YNR001C**	68.12				50.52
BIO3	YNR058W	36.47				29.51
YOL017W	YOL017W	51.41				23.90
MDM20	YOL076W	75.22				44.90
YOL101C	YOL101C	42.01				22.96
SKI7	**YOR076C**	31.23				50.23
UBP2	YOR124C	46.16				44.24
YOR279C	YOR279C	74.41				49.23
HOS3	YPL116W	32.61				29.58
YPL144W	YPL144W	50.67				60.72
YIP2	YPR028W	28.82				24.34
PUF2	YPR042C	25.15				35.08
DBF20	YPR111W	41.65				32.54

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
YPR128C	YPR128C	29.23				30.47
YAL034C	YAL034C			33.55	52.80	
MAP2	YBL091C			31.28	27.51	
ECM31	YBR176W			41.95	27.40	
YDR157W	YDR157W			63.51	30.49	
ARO10	YDR380W			63.86	31.61	
FCY22	YER060W-A			37.93	46.34	
YCK3	YER123W			50.11	44.84	
YGL260W	YGL260W			45.70	31.27	
YHR029C	YHR029C			23.77	33.84	
TOK1	YJL093C			55.83	23.11	
STE24	YJR117W			23.76	34.45	
YKR027W	YKR027W			55.03	23.85	
YLL044W	YLL044W			32.14	42.71	
YLR089C	YLR089C			37.63	43.95	
YML066C	YML066C			35.65	34.83	
YIM1	YMR152W			32.21	57.44	
CRZ1	YNL027W			34.88	52.34	
ESBP6	YNL125C			33.23	28.79	
MPA43	YNL249C			29.63	25.87	
YOL150C	YOL150C			23.39	32.80	
YOR302W	YOR302W			34.55	28.73	
CPA1	YOR303W			42.77	41.02	
SNU66	YOR308C			36.48	28.69	
CCR4	YAL021C	48.80	49.90			
GRS1	YBR121C	47.28	56.57			
BRE1	YDL074C	39.33	38.78			
MRK1	YDL079C	48.63	26.98			
YDL096C	YDL096C	32.34	24.12			
MHR1	YDR296W	58.22	33.35			
HNT2	YDR305C	40.55	24.03			
ECM10	YEL030W	27.92	31.33			
YFL010C	YFL010C	37.65	33.11			
YGL110C	YGL110C	25.90	28.28			
FIS1	YIL065C	34.44	32.77			
DBP7	YKR024C	31.61	49.92			
MID2	YLR332W	42.84	28.94			

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
YLR350W	YLR350W	56.04	24.19			
YML013C-A	YML013C-A	31.83	32.34			
ALF1	YNL148C	50.27	44.12			
YNL157W	YNL157W	28.17	53.08			
SPT20	YOL148C	44.33	43.74			
YOR108W	YOR108W	24.76	26.56			
YOR343C	YOR343C	23.07	25.91			
CTF4	YPR135W	45.28	30.68			
YBL094C	YBL094C		42.49	60.99		
YDR372C	YDR372C		97.13	27.86		
SPI1	YER150W		24.59	54.16		
YGL005C	YGL005C		49.37	23.14		
FYV13	YGR160W		45.09	49.33		
MGA2	YIR033W		35.61	31.34		
ASF1	YJL115W		37.47	33.34		
SRY1	YKL218C		23.84	75.69		
UBP11	YKR098C		37.78	30.79		
RIC1	YLR039C		31.75	42.56		
ACE2	YLR131C		36.18	31.52		
SWI6	YLR182W		28.98	51.01		
NPL6	YMR091C		24.03	39.53		
ZDS1	YMR273C		34.57	32.80		
UBP15	YMR304W		79.04	42.93		
YNL051W	YNL051W		28.18	36.58		
RNY1	YPL123C		21.94	48.72		
ROX3	YBL093C	54.93			43.86	
YDR112W	YDR112W	31.87			42.29	
MEI4	YER044C-A	42.72			52.89	
MIG2	YGL209W	36.37			26.40	
YGR271W	**YGR271W**	38.83			41.87	
RPL27A	YHR010W	47.89			44.63	
SSF1	YHR066W	33.78			33.56	
YHR130C	YHR130C	30.68			29.40	
YHR207C	YHR207C	33.16			33.43	
MCM22	YJR135C	22.79			43.96	
VPS9	YML097C	36.35			22.34	

Standard Gene name	Systematic Gene name	Cyclo-hexomide (C)	Paro-momycin (P)	3-AT (A)	Neomycin (N)	Strepto-mycin (S)
GCV2	YMR189W	30.85			34.70	
CUS2	YNL286W	47.19			57.98	
VPS28	YPL065W	24.75			61.73	
YPR031W	YPR031W	40.13			41.36	
RPL43A	YPR043W	42.78			29.12	
RPS9B	YBR189W		41.44		61.79	
UBC5	YDR059C		31.59		29.91	
RPS18A	YDR450W		37.86		28.91	
GDA1	YEL042W		39.38		32.29	
EST3	YIL009C-A		39.72		41.95	
DAL3	YIR032C		34.96		37.92	
APL1	YJR005W		37.83		30.14	
YML095C-A	YML095C-A		54.69		70.23	
SRV2	YNL138W		23.04		29.73	
RPS19A	YOL121C		46.77		33.92	
DIG1	YPL049C		25.28		37.23	
YCL005W	YCL005W				24.26	45.13
YDR056C	YDR056C				29.18	41.75
SSF2	YDR312W				32.27	48.71
YER188W	YER188W				25.06	46.47
PEP8	YJL053W				39.06	46.93
LCB3	YJL134W				35.20	24.38
YKL030W	YKL030W				35.47	56.22
FAA4	YMR246W				46.00	33.97
ATP11	YNL315C				57.43	60.44
YBR071W	YBR071W		24.90			30.97
YGR283C	YGR283C		24.72			58.59
VTC4	YJL012C		27.89			24.90
DYN1	YKR054C		24.99			67.06
ICT1	YLR099C		47.54			30.60
HCR1	YLR192C		36.66			37.21
ZEO1	YOL109W		32.97			23.62

3-AT					
SPC72	YAL047C	33.01	RPS16B	YDL083C	33.20
FLO1	YAR050W	28.13	YDL085W	YDL085W	32.20
YBL055C	YBL055C	27.71	YDL099W	YDL099W	41.80
RPS8A	YBL072C	41.81	CYK3	YDL117W	50.96
SRO77	YBL106C	39.31	APG9	YDL149W	48.11
YBR016W	YBR016W	31.35	YDL173W	YDL173W	50.16
GAL10	YBR019C	62.89	SNF3	YDL194W	33.59
GAL1	YBR020W	33.46	UGA4	YDL210W	23.63
CSG2	YBR036C	23.38	LRG1	YDL240W	47.83
BAP2	YBR068C	34.65	RGP1	YDR137W	47.42
HSP26	YBR072W	34.84	YDR179W-A	YDR179W-A	36.54
RDH54	YBR073W	31.59	UME6	YDR207C	56.66
TEC1	YBR083W	32.37	YDR215C	YDR215C	23.37
MIS1	YBR084W	27.48	YDR219C	YDR219C	25.54
YBR090C	YBR090C	62.47	EXG2	YDR261C	24.08
KTR4	YBR199W	36.35	SSD1	YDR293C	35.86
MET8	YBR213W	31.87	SWA2	YDR320C	28.71
YBR219C	YBR219C	33.97	PEX3	YDR329C	65.79
YBR235W	YBR235W	30.85	YDR458C	YDR458C	59.61
ISW1	YBR245C	24.49	YDR459C	YDR459C	31.92
YBR261C	YBR261C	42.43	SAC2	YDR484W	66.83
MAL32	YBR299W	35.49	PKH1	YDR490C	44.79
YCL010C	YCL010C	63.73	YDR491C	YDR491C	29.53
YCL016C	YCL016C	26.76	YDR492W	YDR492W	49.76
#N/A	YCL026C	61.72	ITR1	YDR497C	28.94
APA1	YCL050C	28.57	HLR1	YDR528W	33.00
YCL056C	YCL056C	32.46	VAC8	YEL013W	44.38
HSP30	YCR021C	28.84	YEL020C	YEL020C	40.68
YCR033W	YCR033W	32.29	YEL023C	YEL023C	35.59
YCR082W	YCR082W	49.29	RIP1	YEL024W	39.90
YDL023C	YDL023C	29.58	UTR2	YEL040W	45.38
YDL033C	YDL033C	27.60	YPT31	YER031C	42.57
STP4	YDL048C	36.26	GLN3	YER040W	52.38
YDL050C	YDL050C	48.88	ILV1	YER086W	45.07
YDL072C	YDL072C	48.30	SHC1	YER096W	56.90
MAM1	YER106W	25.04	TDH3	YGR192C	34.01
SPR6	YER115C	26.44	PDX1	YGR193C	37.92

3-AT					
RPL23B	YER117W	27.73	YGR212W	YGR212W	33.60
YER134C	YER134C	29.30	GND2	YGR256W	46.23
YER135C	YER135C	27.59	TNA1	YGR260W	26.68
MAG1	YER142C	38.11	APL6	YGR261C	68.35
BCK2	YER167W	46.38	YGR268C	YGR268C	32.67
ADK2	YER170W	29.80	YOR1	YGR281W	67.83
GRX4	YER174C	34.03	BGL2	YGR282C	49.95
PDA1	YER178W	35.01	YHL013C	YHL013C	46.24
YFL042C	YFL042C	28.85	WSC4	YHL028W	53.16
YFL043C	YFL043C	30.96	GOS1	YHL031C	43.26
YFR011C	YFR011C	57.26	YHR035W	YHR035W	49.75
CDH1	YGL003C	37.58	MSC7	YHR039C	37.64
TRP5	YGL026C	45.18	YHR049C-A	YHR049C-A	27.19
YGL045W	YGL045W	36.76	SBE22	YHR103W	26.34
YGL046W	YGL046W	61.11	YHR121W	YHR121W	40.34
HNM1	YGL077C	30.21	YCK1	YHR135C	30.98
YGL131C	YGL131C	43.50	SPL2	YHR136C	31.65
YGL140C	YGL140C	46.99	ESC4	YHR154W	53.33
SAE2	YGL175C	34.48	REC104	YHR157W	44.20
SHE10	YGL228W	36.95	STB5	YHR178W	44.39
HXK2	YGL253W	48.81	YIL001W	YIL001W	45.45
ZRT1	YGL255W	36.80	YIL023C	YIL023C	47.58
YGR001C	YGR001C	23.49	YIL029C	YIL029C	43.03
YGR012W	YGR012W	37.46	YIL032C	YIL032C	25.87
UGA1	YGR019W	30.18	YIL058W	YIL058W	29.42
MTL1	YGR023W	43.64	YIL064W	YIL064W	39.04
SPR3	YGR059W	43.84	YIL103W	YIL103W	49.87
SPT4	YGR063C	61.65	YIL121W	YIL121W	30.64
YGR125W	YGR125W	24.09	YIL137C	YIL137C	28.80
YGR127W	YGR127W	26.90	UBP7	YIL156W	25.63
SYF2	YGR129W	43.54	YIL157C	YIL157C	38.68
CHO2	YGR157W	37.74	BNR1	YIL159W	40.94
SDL1	YIL168W	35.92	MUD2	YKL074C	26.31
SGN1	YIR001C	43.98	SMY1	YKL079W	35.05
YIR003W	YIR003W	35.27	YKL084W	YKL084W	64.29
IST3	YIR005W	45.13	YKL118W	YKL118W	56.93
YIR016W	YIR016W	35.52	RMA1	YKL132C	56.15
MUC1	YIR019C	28.71	LST4	YKL176C	24.96

3-AT					
LYS1	YIR034C	34.12	EAP1	YKL204W	67.72
YJL020C	YJL020C	25.37	YKL215C	YKL215C	33.64
YJL043W	YJL043W	32.80	TOF2	YKR010C	54.80
YJL051W	YJL051W	22.10	IRS4	YKR019C	47.67
IKS1	YJL057C	31.12	SAP190	YKR028W	36.06
YHC3	YJL059W	34.35	YKR040C	YKR040C	26.92
BCK1	YJL095W	40.35	YKR043C	YKR043C	23.40
YJL105W	YJL105W	28.67	SRL3	YKR091W	45.70
YJL144W	YJL144W	22.74	PTR2	YKR093W	28.68
YJL193W	YJL193W	56.13	YLL013C	YLL013C	52.33
YJL213W	YJL213W	49.64	YLL023C	YLL023C	23.58
YJR018W	YJR018W	33.57	AAT2	YLR027C	48.53
BNA1	YJR025C	64.21	REX2	YLR059C	37.93
BFA1	YJR053W	27.24	RPL22A	YLR061W	22.51
PTK2	YJR059W	52.62	YLR205C	YLR205C	24.04
YJR083C	YJR083C	28.26	YLR225C	YLR225C	24.86
YJR087W	YJR087W	43.77	YLR231C	YLR231C	25.62
VPS25	YJR102C	51.82	THI7	YLR237W	54.89
YJR120W	YJR120W	24.10	YLR262C-A	YLR262C-A	52.27
ENT3	YJR125C	26.11	BOP2	YLR267W	29.85
YJR129C	YJR129C	29.72	SEC22	YLR268W	41.45
MNS1	YJR131W	27.78	BUD6	YLR319C	55.40
HOM6	YJR139C	55.15	IKI3	YLR384C	39.75
YJR142W	YJR142W	42.84	IMD3	YLR432W	25.47
VPS24	YKL041W	29.21	YLR434C	YLR434C	45.00
MSN4	YKL062W	25.10	ECM30	YLR436C	62.39
YKL069W	YKL069W	57.00	ERG6	YML008C	40.31
YML013W	YML013W	29.43	YNL253W	YNL253W	46.93
UNG1	YML021C	27.44	MID1	YNL291C	54.80
YML036W	YML036W	29.01	KRE25	YNL296W	30.64
SUR7	YML052W	57.40	PHA2	YNL316C	38.56
YML053C	YML053C	42.77	HXT14	YNL318C	41.19
YML071C	YML071C	57.49	THI12	YNL332W	36.82
DUS1	YML080W	27.45	YNR002C	YNR002C	40.89
BUD22	YMR014W	32.94	CSE2	YNR010W	32.60
UBC7	YMR022W	37.46	SSK2	YNR031C	28.94
ABF2	YMR072W	53.34	YNR062C	YNR062C	46.83
PKR1	YMR123W	26.32	YNR073C	YNR073C	27.92

3-AT					
PSO2	YMR137C	30.61	YOL002C	YOL002C	33.50
MSS11	YMR164C	24.17	YOL003C	YOL003C	26.44
PAI3	YMR174c	22.82	TOP1	YOL006C	46.14
MMT1	YMR177W	35.51	IFM1	YOL023W	32.63
RGM1	YMR182C	40.99	YOL063C	YOL063C	36.70
YMR188C	YMR188C	29.82	YOL071W	YOL071W	53.34
YMR251W	YMR251W	27.55	SPO21	YOL091W	27.63
COX7	YMR256C	25.62	SHR5	YOL110W	43.58
PET111	YMR257C	23.16	YOL111C	YOL111C	59.03
CUE1	YMR264W	29.22	YOL114C	YOL114C	30.70
SCS7	YMR272C	22.67	YOR006C	YOR006C	46.45
JNM1	YMR294W	32.60	KIM1	YOR008C-A	32.98
YMR304C-A	YMR304C-A	29.23	YOR009W	YOR009W	44.22
FKS3	YMR306W	38.63	YOR013W	YOR013W	26.05
YMR312W	YMR312W	27.35	YOR015W	YOR015W	24.62
ARK1	YNL020C	34.96	PET127	YOR017W	26.72
SUN4	YNL066W	40.58	YOR019W	YOR019W	30.65
RPL9B	YNL067W	37.62	DFG16	YOR030W	42.90
LAT1	YNL071W	44.50	EXO1	YOR033C	41.20
YNL094W	YNL094W	50.89	YOR044W	YOR044W	42.11
YNL136W	YNL136W	60.27	YOR052C	YOR052C	27.61
CYT1	YOR065W	42.34	GLR1	YPL091W	26.15
TRS33	YOR115C	23.03	SSU1	YPL092W	24.61
YOR164C	YOR164C	26.54	YPL095C	YPL095C	49.15
FYV12	YOR183W	24.20	MEI5	YPL121C	53.92
BFR1	YOR198C	41.01	BEM4	YPL161C	48.01
HIS3	YOR202W	26.18	YPL202C	YPL202C	26.76
NPT1	YOR209C	28.92	ALG5	YPL227C	42.23
YOR214C	YOR214C	38.41	VIK1	YPL253C	56.54
YOR275C	YOR275C	67.51	YPR004C	YPR004C	29.79
YOR289W	YOR289W	33.87	YPR008W	YPR008W	30.72
YOR292C	YOR292C	38.99	YPR012W	YPR012W	25.70
TIM18	YOR297C	36.77	CSR2	YPR030W	46.69
ISW2	YOR304W	24.00	YPR059C	YPR059C	25.35
SPS4	YOR313C	46.18	YPR061C	YPR061C	25.27
YOR338W	YOR338W	43.49	UBA3	YPR066W	38.90
YOR364W	YOR364W	28.74	YPR078C	YPR078C	47.14
RPS12	YOR369C	32.38	YPR147C	YPR147C	32.67

3-AT					
YPL004C	YPL004C	55.86	NCA2	YPR155C	65.34
SRD2	YPL021W	61.32	KRE6	YPR159W	33.03
YPL034W	YPL034W	67.76	GPH1	YPR160W	47.38
PMA2	YPL036W	42.69	YPR170C	YPR170C	47.67
EGD1	YPL037C	68.87	PLO1	YPR179C	63.06
YPL064C	YPL064C	39.82	APG13	YPR185W	65.46
BTS1	YPL069C	47.92	QCR2	YPR191W	39.11

Paromomycin					
YAL008W	YAL008W	22.20	#N/A	YFL010W-A	29.55
PEX22	YAL055W	22.43	FET5	YFL041W	29.79
YAL065C	YAL065C	22.94	YFL044C	YFL044C	30.02
YBL053W	YBL053W	24.45	CKB1	YGL019W	30.16
UBP13	YBL067C	24.52	ARC1	YGL105W	30.53
YBL071C	YBL071C	24.58	YGL107C	YGL107C	30.59
GIP1	YBR045C	34.29	YGR169C	YGR169C	30.65
RPS11B	YBR048W	38.86	BNS1	YGR230W	30.98
YBR134W	YBR134W	38.27	ECM29	YHL030W	31.19
#N/A	YCL074W	36.69	YHR003C	YHR003C	31.24
RAD18	YCR066W	26.50	ARO9	YHR137W	31.55
AAD3	YCR107W	25.58	GND1	YHR183W	28.01
FYV3	YDL151C	51.39	YIL057C	YIL057C	28.15
AAD4	YDL243C	28.47	YIL161W	YIL161W	28.67
TRP1	YDR007W	35.07	DAL7	YIR031C	28.81
KCS1	YDR017C	56.25	YJL064W	YJL064W	28.97
IPT1	YDR072C	55.60	KHA1	YJL094C	58.45
YDR084C	YDR084C	25.34	GLG2	YJL137C	61.48
RAV2	YDR202C	28.85	YUR1	YJL139C	61.80
YDR210W	YDR210W	24.09	CPR7	YJR032W	49.93
YDR222W	YDR222W	27.54	RPL43B	YJR094W-A	55.86
YDR223W	YDR223W	32.40	MET14	YKL001C	51.03
YDR326C	YDR326C	34.58	YJU3	YKL094W	51.92
YDR333C	YDR333C	25.21	HSL1	YKL101W	39.04
SWR1	YDR334W	30.98	YKL162C-A	YKL162C-A	39.27
YDR352W	YDR352W	35.05	YKR100C	YKR100C	40.54
TRP4	YDR354W	35.56	SIR1	YKR101W	40.54
XRS2	YDR369C	26.75	SPA2	YLL021W	40.85
YDR444W	YDR444W	33.16	YLR054C	YLR054C	41.28

Paromomycin					
YDR479C	YDR479C	29.33	RFX1	YLR176C	41.40
SPF1	YEL031W	29.44	RPS28B	YLR264W	41.90
#N/A	YER007C-A	38.57	YLR352W	YLR352W	43.66
#N/A	YER108C	31.60	RPS17A	YML024W	45.17
SHO1	YER118C	40.35	RPS18B	YML026C	45.95
SCS2	YER120W	29.53	ICY1	YMR195W	46.07
RPS10B	YMR230W	48.32	PUT4	YOR348C	25.19
GFD1	YMR255W	48.43	MET12	YPL023C	25.26
PPA2	YMR267W	32.43	NCE4	YPL024W	25.29
YNL224C	YNL224C	31.88	ELC1	YPL046C	26.07
PUS4	YNL292W	32.03	UME1	YPL139C	25.69
PPG1	YNR032W	34.14	CLN2	YPL256C	25.74
PEX11	YOL147C	35.15	YPL261C	YPL261C	27.71
ATX2	YOR079C	36.34	ICL2	YPR006C	27.71
KTR1	YOR099W	24.88	YPR064W	YPR064W	23.85
FAA1	YOR317W	26.66			

Cycloheximide					
KRE20	YAL058C-A	27.19	YBR269C	YBR269C	50.46
HIR1	YBL008W	46.55	YBR271W	YBR271W	31.71
YBL046W	YBL046W	40.23	RIF1	YBR275C	40.79
YBL051C	YBL051C	37.81	YBR277C	YBR277C	54.24
SKT5	YBL061C	38.87	YBR284W	YBR284W	37.54
YBL094C	YBL094C	43.41	YBR285W	YBR285W	33.00
RTG3	YBL103c	36.33	BSD2	YBR290W	48.16
FLR1	YBR008C	55.91	YBR292C	YBR292C	38.33
YBR014C	YBR014C	28.58	YCL002C	YCL002C	37.55
YBR022W	YBR022W	45.93	YCL047C	YCL047C	45.91
CHS3	YBR023C	38.95	YCL063W	YCL062W	39.75
SCO2	YBR024W	31.56	YCR045C	YCR045C	51.36
YBR026C	YBR026C	42.49	YCR050C	YCR050C	60.35
YBR033W	YBR033W	24.03	YCR062W	YCR061W	52.58
PDX3	YBR035C	58.66	SRB8	YCR081W	38.49
AKL1	YBR059C	53.13	GIT1	YCR098C	39.28
YBR095C	**YBR095C**	65.01	NHP10	YDL002C	35.92
YBR116C	YBR116C	28.35	PTC1	YDL006W	29.51
TPS1	YBR126C	39.00	ERP3	YDL018C	42.59
OPY1	YBR129C	39.19	SIT4	YDL047W	32.68

Cycloheximide					
MRPS9	YBR146W	49.47	THI3	YDL080C	49.31
YSW1	YBR148W	27.30	YDL091C	YDL091C	28.75
ARA1	YBR149W	57.55	YDL094C	YDL094C	39.94
SLI15	YBR156C	30.47	PMT1	YDL095W	47.69
TYR1	YBR166C	42.41	UBP1	YDL122W	46.32
EHT1	YBR177C	38.61	YDL172C	YDL172C	31.99
YBR178W	YBR178W	64.99	DLD1	YDL174C	49.18
FZO1	**YBR179C**	35.42	YDL176W	YDL176W	43.90
SMP1	YBR182C	68.11	INH1	YDL181W	29.41
YBR184W	YBR184W	32.06	YDL187C	YDL187C	37.26
YBR187W	YBR187W	48.46	PPH22	YDL188C	77.08
YBR197C	YBR197C	49.88	YDL189W	YDL189W	60.94
BEM1	YBR200W	45.09	RPL35A	YDL191W	27.19
DER1	YBR201W	32.46	YDL201W	YDL201W	34.60
APG12	YBR217W	22.17	YDL218W	YDL218W	34.86
YPT10	YBR264C	38.54	BRE4	YDL231C	42.30
OST4	YDL232W	73.33	YER175C	YER175C	89.86
YDR003W	YDR003W	26.59	ECM32	YER176W	60.23
YDR048C	YDR048C	52.65	BMH1	YER177W	29.69
YDR049W	**YDR049W**	77.30	DMC1	YER179W	44.25
TPS2	YDR074W	31.55	YER181C	YER181C	33.86
YDR117C	YDR117C	33.10	YER185W	YER185W	49.07
ECM18	YDR125C	28.05	LPD1	YFL018C	48.67
YDR152W	YDR152W	35.52	CAF16	YFL028C	43.32
HOM2	YDR158W	31.33	YFL032W	YFL032W	31.94
NUP42	YDR192C	38.93	YFR024C-A	YFR024C-A	46.22
YDR198C	YDR198C	28.88	PHO4	YFR034C	73.84
YDR287W	YDR287W	51.72	SAP155	YFR040W	58.47
SUR2	YDR297W	63.87	PIB2	YGL023C	36.54
YDR307W	YDR307W	63.42	YGL024W	YGL024W	62.47
YDR319C	YDR319C	31.16	MIG1	YGL035C	42.62
MSN5	**YDR335W**	43.26	YGL059W	YGL059W	36.46
EFT2	YDR385W	32.60	YGL066W	YGL066W	53.24
SRB9	YDR443C	48.10	YGL082W	YGL082W	42.09
YDR445C	YDR445C	39.76	MAD1	YGL086W	31.33
MRPL28	YDR462W	64.02	YGL117W	YGL117W	29.90
YDR466W	YDR466W	32.78	YGL118C	YGL118C	44.90
YDR467C	YDR467C	38.06	YGL144C	YGL144C	34.63

Cycloheximide					
YDR469W	YDR469W	35.65	YGL161C	YGL161C	54.00
YDR485C	YDR485C	41.11	TOS3	YGL179C	43.12
PLM2	YDR501W	29.56	GTS1	YGL181W	31.89
GIM4	YEL003W	28.42	**HOS2**	**YGL194C**	45.51
YEL044W	YEL044W	27.86	**MDS3**	**YGL197W**	37.67
BUD25	YER014C-A	33.59	YGL198W	YGL198W	26.00
YER028C	YER028C	28.65	YGL199C	YGL199C	26.55
RSM18	YER050C	34.95	SIP2	YGL208W	37.20
GIP2	YER054C	29.01	YPT32	YGL210W	47.94
THO1	YER063W	22.97	YGL211W	YGL211W	64.41
PTC2	YER089C	29.10	VAM7	YGL212W	45.23
RAD51	YER095W	37.16	MUQ1	YGR007W	25.83
YGR071C	YGR071C	25.91	YIL105C	YIL105C	35.59
PAC10	YGR078C	44.17	PFK26	YIL107C	29.45
YGR093W	YGR093W	39.03	YIL110W	YIL110W	40.26
ASN2	YGR124W	31.87	YIL112W	YIL112W	55.65
PRE9	YGR135W	28.08	YIL113W	YIL113W	33.89
KRE11	YGR166W	40.60	YIL132C	YIL132C	35.78
ATF2	YGR177C	23.19	RPL16A	YIL133C	44.23
MRPL9	YGR220C	34.69	MLP2	YIL149C	46.18
YAP3	YHL009C	32.12	YIL152W	YIL152W	43.05
YHL023C	YHL023C	22.19	**RRD1**	**YIL153W**	26.22
RIM101	YHL027W	33.60	YIR036C	YIR036C	34.56
SBP1	YHL034C	31.80	MHP1	YJL042W	52.69
YHL037C	YHL037C	26.68	IML2	YJL082W	38.68
TAF1	YHL047C	45.13	**YJL083W**	**YJL083W**	50.54
NEM1	YHR004C	76.71	YJL100W	YJL100W	34.19
GPA1	YHR005C	33.88	YJL118W	YJL118W	28.24
ARD1	YHR013C	33.28	RPE1	YJL121C	62.16
SLT2	YHR030C	66.59	NIT2	YJL126W	42.63
DOG2	YHR043C	41.61	**YJL163C**	**YJL163C**	47.10
BIG1	YHR101C	62.14	TPK1	YJL164C	34.67
ERP5	YHR110W	64.83	YJL185C	YJL185C	40.51
YHR115C	YHR115C	38.10	ECM25	YJL201W	47.16
YHR156C	YHR156C	34.49	YJL216C	YJL216C	33.73
RPN10	**YHR200W**	37.46	GEF1	YJR040W	37.62
YIL007C	YIL007C	45.77	CYC1	YJR048W	43.13
RPL2B	YIL018W	39.76	ISY1	YJR050W	57.36

Cycloheximide					
YIL040W	YIL040W	65.17	APS2	YJR058C	29.59
YIL041W	YIL041W	42.82	TOR1	YJR066W	31.81
YIL042C	YIL042C	38.70	HAM1	YJR069C	37.35
DFG10	YIL049W	35.34	MOG1	YJR074W	83.65
FYV2	YIL054W	49.32	YJR084W	YJR084W	45.31
SDS3	YIL084C	34.69	YJR088C	YJR088C	53.71
FMC1	YIL098C	45.65	SFC1	YJR095W	50.65
YUH1	YJR099W	58.68	FRE1	YLR214W	44.00
YJR100C	YJR100C	63.67	MSC3	YLR219W	54.29
YJR110W	YJR110W	57.75	CCC1	YLR220W	47.91
RPS4A	YJR145C	52.55	ECM22	YLR228C	22.63
YJR154W	YJR154W	29.04	YLR235C	YLR235C	23.23
RPL14A	***YKL006W***	32.13	ARV1	YLR242C	25.75
CAP1	**YKL007W**	42.00	RPS30A	YLR287C-A	35.52
YKL051W	YKL051W	31.31	MET17	YLR303W	34.54
LHS1	YKL073W	52.81	#N/A	YLR337C	59.45
KTI12	YKL110C	47.93	DIC1	YLR348C	40.10
DOA1	YKL213C	23.40	VPS38	YLR360W	39.04
YKR023W	**YKR023W**	41.80	YLR361C	YLR361C	55.63
DID2	YKR035W-A	36.24	RPS29A	YLR388W	26.00
YKR070W	YKR070W	57.89	**YLR391W**	**YLR391W**	59.42
SIS2	YKR072C	56.97	YLR413W	YLR413W	28.60
YKR077W	YKR077W	25.33	YLR416C	YLR416C	48.09
NUP133	YKR082W	38.59	CDC73	YLR418C	49.08
YKR088C	YKR088C	38.67	CRN1	YLR429W	41.74
BAS1	YKR099W	41.78	YLR445W	YLR445W	50.71
YLL007C	**YLL007C**	47.10	YLR456W	YLR456W	30.98
YLL014W	YLL014W	41.30	YML005W	YML005W	37.97
KNS1	YLL019C	32.72	GIS4	YML006C	59.09
YLR036C	YLR036C	40.87	YML011C	YML011C	42.47
FYV7	YLR068W	55.85	PSP2	YML017W	47.13
BUD20	YLR074C	40.73	TSA1	YML028W	37.71
SIC1	YLR079W	39.48	PRM6	YML047C	25.50
AHP1	YLR109W	23.09	YML048W-A	YML048W-A	29.51
YLR111W	YLR111W	60.35	YML058C-A	YML058C-A	46.60
SRN2	YLR119W	51.39	OGG1	YML060W	48.86
PDC5	YLR134W	38.13	MFT1	YML062C	46.29

Cycloheximide					
YLR184W	YLR184W	31.60	**NUP188**	**YML103C**	57.54
UGT51	YLR189C	31.46	VAN1	YML115C	35.82
YKE2	YLR200W	38.38	YMR031C	YMR031C	35.02
FET3	YMR058W	27.37	ALG9	YNL219C	43.95
YMR075C-A	YMR075C-A	25.70	ATX1	YNL259C	53.39
YMR075W	YMR075W	29.34	WSC2	YNL283C	36.26
CTF18	YMR078C	42.23	YNL285W	YNL285W	42.57
NAM7	YMR080C	38.07	MID1	YNL291C	36.62
MYO5	YMR109W	45.12	TRF5	YNL299W	51.79
STO1	YMR125W	46.36	TOS6	YNL300W	33.43
YMR132C	**YMR132C**	26.79	RPL18B	YNL301C	39.36
RPS16A	YMR143W	46.53	YRF1-6	YNL339C	28.67
YMR155W	YMR155W	35.66	LRO1	YNR008W	63.16
YMR187C	**YMR187C**	43.32	YNR009W	YNR009W	36.54
HFA1	YMR207C	46.59	ARE2	YNR019W	45.03
SCJ1	YMR214W	32.47	YNR021W	YNR021W	49.43
MRE11	YMR224C	30.67	YNR022C	YNR022C	41.65
RPL20A	YMR242C	34.65	YNR025C	YNR025C	81.86
AEP2	YMR282C	43.00	ECM39	YNR030W	37.94
RIT1	YMR283C	41.74	BRE5	YNR051C	33.30
FET4	YMR319C	56.49	MNT4	YNR059W	37.39
HAD1	YNL021W	61.43	YNR062C	YNR062C	36.74
FAP1	YNL023C	40.03	YNR063W	YNR063W	37.11
HHF2	YNL030W	38.16	YNR064C	YNR064C	45.68
YNL043C	YNL043C	45.64	YNR069C	YNR069C	29.86
SFB2	***YNL049C***	31.77	SIN3	YOL004W	82.72
TPM1	YNL079C	38.01	HRD1	YOL013C	39.16
YNL099C	YNL099C	43.86	YOL015W	YOL015W	38.50
YNL140C	YNL140C	38.08	GAL11	YOL051W	65.01
YNL146W	YNL146W	51.17	MET22	YOL064C	40.76
YNL177C	YNL177C	43.05	MSH2	YOL090W	37.36
CHS1	YNL192W	27.25	ITR2	YOL103W	53.06
YNL193W	YNL193W	36.28	NDJ1	YOL104C	68.48
YNL201C	YNL201C	59.82	WSC3	YOL105C	58.30
YNL213C	YNL213C	56.22	GRE2	YOL151W	36.25
PEX17	YNL214W	38.26	ENB1	YOL158C	48.79
YNL217W	YNL217W	40.46	ERP4	YOR016C	24.68

Cycloheximide					
YOR042W	YOR042W	35.99	KES1	YPL145C	36.25
YOR050C	***YOR050C***	44.89	PEP4	YPL154C	33.25
YOR055W	YOR055W	58.56	YPL162C	YPL162C	48.86
YOR059C	YOR059C	51.63	YPL170W	YPL170W	37.94
OST3	YOR085W	54.94	NIP100	YPL174C	29.04
YOR086C	YOR086C	47.76	YPL181W	YPL181W	38.79
YOR088W	YOR088W	50.81	YPL182C	YPL182C	30.19
YOR105W	YOR105W	33.79	**APL5**	**YPL195W**	37.28
YOR118W	YOR118W	43.58	**YPL196W**	**YPL196W**	32.94
GCY1	YOR120W	52.31	RPL7B	YPL198W	48.15
RGA1	**YOR127W**	33.37	YPL205C	YPL205C	67.11
YOR172W	YOR172W	62.44	YPL206C	YPL206C	55.73
YOR199W	YOR199W	64.70	YPL207W	YPL207W	54.01
ODC2	YOR222W	27.34	MMT2	YPL224C	27.64
YOR227W	YOR227W	30.41	USV1	YPL230W	23.33
KIN4	YOR233W	53.10	YPL245W	YPL245W	31.49
RPL33B	YOR234C	29.79	KAR9	YPL269W	48.03
YOR251C	YOR251C	39.90	YPR050C	YPR050C	32.55
YOR264W	YOR264W	59.15	BRR1	YPR057W	30.62
YOR277C	YOR277C	55.52	HOS1	YPR068C	34.05
MBF1	**YOR298C-A**	65.79	**MED1**	**YPR070W**	45.53
MBF1	**YOR298C-A**	65.79	OPY2	YPR075C	24.11
RAD17	YOR368W	34.35	YPR076W	YPR076W	44.11
YPL009C	**YPL009C**	25.83	YPR077C	YPR077C	27.47
YPL055C	**YPL055C**	24.65	YPR089W	YPR089W	27.08
YDC1	YPL087W	46.09	YPR090W	YPR090W	44.46
RLM1	YPL089C	27.41	YPR093C	YPR093C	42.15
YPL101W	YPL101W	30.71	SYT1	YPR095C	31.18
KRE24	YPL102C	47.60	YPR100W	YPR100W	38.67
YPL105C	YPL105C	37.22	YPR114W	YPR114W	66.49
YPL110C	YPL110C	27.58	ASN1	YPR145W	51.98
CAR1	YPL111W	38.23	YPR154W	YPR154W	39.94
YPL114W	YPL114W	38.12	APG13	YPR185W	35.95
BEM3	YPL115C	53.17	YPR195C	YPR195C	27.88
YPL133C	YPL133C	48.24			

Streptomycin					
SNC1	YAL030W	39.43	SLC1	YDL052C	50.56
GCV3	YAL044C	60.46	YDL118W	YDL118W	29.53
YAR029W	YAR029W	45.12	YDL129W	YDL129W	37.71
YAR030C	YAR030C	36.33	RPP1B	YDL130W	45.00
ECM13	YBL043W	78.33	ARF1	YDL192W	47.16
YBL064C	YBL064C	30.08	YDL203C	YDL203C	33.07
HHT1	YBR010W	47.66	RRI1	YDL216C	30.14
YBR025C	YBR025C	32.21	SSB1	YDL229W	25.23
FIG1	YBR040W	28.51	RPS11A	YDR025W	27.07
REG2	YBR050C	27.43	YDR057W	YDR057W	67.45
YBR052C	YBR052C	33.34	YDR065W	YDR065W	52.06
YRO2	YBR054W	32.76	PPH3	YDR075W	34.29
YBR061C	YBR061C	63.57	YDR105C	YDR105C	51.29
ECM2	YBR065C	40.19	CPH1	YDR155C	24.34
YBR100W	YBR100W	26.57	TCI1	YDR161W	64.78
YBR108W	YBR108W	24.61	NBP2	YDR162C	34.48
CCZ1	YBR131W	41.07	STB3	YDR169C	42.40
YBR144C	YBR144C	25.82	HSP42	YDR171W	39.48
ADH5	YBR145W	25.88	ATP5	YDR298C	22.30
TBS1	YBR150C	31.42	RPL12B	YDR418W	25.60
APD1	YBR151W	32.05	LPP1	YDR503C	25.53
UBS1	YBR165W	26.32	AGE1	YDR524C	38.28
EHT1	YBR177C	58.86	PAD1	YDR538W	27.18
DTR1	YBR180W	26.15	YDR539W	YDR539W	25.67
PYC2	YBR218C	55.83	YEL001C	YEL001C	23.22
YBR224W	YBR224W	30.58	VAB2	YEL005C	40.14
YBR226C	YBR226C	46.68	CAJ1	YER048C	29.27
MCX1	YBR227C	34.59	YER091C-A	YER091C-A	53.77
YBR230C	YBR230C	53.46	YER092W	YER092W	34.26
YBR287W	YBR287W	25.47	YER097W	YER097W	24.03
YCL048W	YCL048W	36.73	FLO8	YER109C	30.18
CDC10	YCR002C	57.94	YER152C	YER152C	24.25
FEN2	YCR028C	33.55	RPO41	YFL036W	30.62
HCM1	YCR065W	50.23	QCR6	YFR033C	32.71
YCR105W	YCR105W	55.76	YFR038W	YFR038W	34.77
DIA3	YDL024C	25.80	YFR055W	YFR055W	31.58
YDL037C	YDL037C	33.75	RPB9	YGL070C	40.70

Streptomycin					
YJR124C	YJR124C	27.37	TWF1	YGR080W	47.87
DAN1	YJR150C	38.16	YGR130C	YGR130C	31.03
MRT4	YKL009W	23.18	AMA1	YGR225W	47.97
YKL090W	YKL090W	39.75	YGR266W	YGR266W	27.60
RCN1	YKL159C	50.98	ERV29	YGR284C	46.84
YKL162C	YKL162C	42.86	YGR287C	YGR287C	62.52
YKR007W	YKR007W	34.10	MAL13	YGR288W	34.67
YKR065C	YKR065C	28.22	YHL010C	YHL010C	52.77
MET1	YKR069W	22.29	YHL042W	YHL042W	51.39
YKR074W	YKR074W	26.91	YHL046C	YHL046C	55.81
YKR096W	YKR096W	27.56	RRM3	YHR031C	23.02
YBT1	YLL048C	37.13	VMA10	YHR039C-B	52.10
MEU1	YLR017W	32.12	SAE3	YHR079C-B	22.48
YLR021W	YLR021W	27.46	KSP1	YHR082C	43.14
YLR031W	YLR031W	39.88	TRR2	YHR106W	51.21
RPS0B	YLR048W	39.02	YHR111W	YHR111W	36.50
EMP70	YLR083C	37.26	YHR140W	YHR140W	52.80
YLR087C	YLR087C	49.89	PDR11	YIL013C	36.61
YLR358C	YLR358C	39.06	FIS1	YIL065C	28.92
BDF1	YLR399C	39.72	YIL090W	YIL090W	74.93
APG17	YLR423C	26.46	RSM25	YIL093C	39.33
YLR444C	YLR444C	29.98	GIF1	YIR024C	43.17
YLR446W	YLR446W	33.93	SYS1	YJL004C	37.56
HMG1	YML075C	29.88	YJL027C	YJL027C	40.64
YML079W	YML079W	32.26	VPS53	YJL029C	22.96
GAT2	YMR136W	45.44	YJL038C	YJL038C	32.25
RPL13B	YMR142C	57.78	YJL084C	YJL084C	28.52
HSC82	YMR186W	21.32	GSH1	YJL101C	23.47
YMR191W	YMR191W	27.17	TRK1	YJL129C	51.86
YMR226C	YMR226C	30.64	TIF2	YJL138C	43.00
YMR244C-A	YMR244C-A	33.44	FBP26	YJL155C	28.22
YMR316C-B	YMR316C-B	27.65	FAR1	YJL157C	36.40
YNL058C	YNL058C	28.27	PFD1	YJL179W	33.82
LEU4	YNL104C	24.64	NUC1	YJL208C	73.78
SLZ1	YNL196C	34.66	YJL215C	YJL215C	70.29
MMS2	YGL087C	46.49	POL32	YJR043C	30.92
ARO2	YGL148W	40.38	ECM27	YJR106W	22.13
POX1	YGL205W	31.93	HAP2	YGL237C	27.88

Streptomycin					
YNL211C	YNL211C	28.73	VPS21	YOR089C	27.49
YNL254C	YNL254C	48.35	RPS7A	YOR096W	28.37
YNL266W	YNL266W	33.66	EFT1	YOR133W	34.08
YNL275W	YNL275W	29.21	BAG7	YOR134W	28.93
MID1	YNL291C	27.09	YOR135C	YOR135C	39.41
MSB3	YNL293W	39.11	YOR137C	YOR137C	29.68
YNL305C	YNL305C	40.07	YOR267C	YOR267C	26.17
KRE1	YNL322C	31.14	YOR291W	YOR291W	33.62
YNR014W	YNR014W	31.92	YOR314W	YOR314W	33.95
CMK2	YOL016C	61.38	YOR378W	YOR378W	52.74
YOL035C	YOL035C	66.58	PHR1	YOR386W	28.66
YOL046C	YOL046C	53.77	YPL099C	YPL099C	26.91
GSH2	YOL049W	59.27	YPL103C	YPL103C	26.21
TRF4	YOL115W	33.33	ODC1	YPL134C	26.79
YOL137W	YOL137W	24.41	POS5	YPL188W	59.84
SGT2	YOR007C	42.45	YPL216W	YPL216W	28.52
SLG1	YOR008C	24.38	PCL8	YPL219W	24.76
WHI2	YOR043W	35.83	NEW1	YPL226W	37.56
YOR044W	YOR044W	51.74	YAR1	YPL239W	40.99
YPR148C	YPR148C	74.69	SNT309	YPR101W	33.32
YPR157W	YPR157W	29.84	ISR1	YPR106W	60.69
OPT2	YPR194C	55.64	THI22	YPR121W	31.88

Neomycin					
APN2	YBL019W	41.72	RNR4	YGR180C	40.28
NHP6B	YBR090C-A	33.71	PCT1	YGR202C	27.59
YBR203W	YBR203W	31.38	YGR203W	YGR203W	54.50
ADP1	YCR011C	25.97	RTA1	YGR213C	37.35
FIG2	YCR089W	30.87	RAD2	YGR258C	59.86
YDL072C	YDL072C	32.85	MIP6	YHR015W	66.70
YDR185C	YDR185C	37.13	YHR048W	YHR048W	50.56
MRPL7	YDR237W	32.45	YHR113W	YHR113W	56.29
BUD26	YDR241W	70.56	YHR139C-A	YHR139C-A	34.74
ZIP1	YDR285W	32.63	YIL005W	YIL005W	33.76
PLO2	YDR295C	27.09	PCL7	YIL050W	47.35
BCS1	YDR375C	64.76	YIL088C	YIL088C	63.15
ERD1	YDR414C	74.59	YIL092W	YIL092W	26.78
CAD1	YDR423C	38.55	FYV10	YIL097W	35.84

Neomycin					
YDR430C	YDR430C	38.79	YJL055W	YJL055W	27.71
YEL010W	YEL010W	58.15	YJL200C	YJL200C	48.85
YEL016C	YEL016C	28.50	OPT1	YJL212C	43.49
PRB1	YEL060C	33.85	YJR038C	YJR038C	42.86
YEL072W	YEL072W	37.40	YJR056C	YJR056C	30.28
GAL83	YER027C	24.23	NUP100	YKL068W	55.68
#N/A	YER067C-A	32.76	TEF4	YKL081W	52.69
RPS24A	YER074W	51.39	YKL086W	YKL086W	46.94
YER083C	YER083C	52.56	YKL097C	YKL097C	57.99
YER187W	YER187W	56.00	PMU1	YKL128C	31.85
AGP3	YFL055W	43.56	MRP49	YKL167C	48.28
YGL020C	YGL020C	27.61	YKL187C	YKL187C	22.40
YGL057C	YGL057C	51.02	#N/A	YKL200C	74.51
FZF1	YGL254W	39.77	URA1	YKL216W	53.33
CTT1	YGR088W	45.03	CAF4	YKR036C	30.03
YGR131W	YGR131W	34.30	GLG1	YKR058W	40.77
TFS1	YLR178C	53.18	YLL049W	YLL049W	25.92
YLR194C	YLR194C	42.44	FRE6	YLL051C	29.68
EXG1	YLR300W	38.01	MMP1	YLL061W	33.40
YLR331C	YLR331C	28.35	CHA4	YLR098C	44.22
YLR385C	YLR385C	24.98	YLR108C	YLR108C	48.30
COX8	YLR395C	39.06	YLR122C	YLR122C	31.90
YML029W	YML029W	54.13	YOR114W	YOR114W	35.69
NDI1	YML120C	28.90	ABP140	YOR239W	30.64
DFG5	YMR238W	25.34	SSP2	YOR242C	50.36
YNL087W	YNL087W	34.55	YOR324C	YOR324C	30.20
YNL105W	YNL105W	42.30	ARL3	YPL051W	66.74
YNR020C	YNR020C	27.79	SUR1	YPL057C	35.63
HUB1	YNR032C-A	26.63	ATP4	YPL078C	29.37
BRE5	YNR051C	28.68	VPS30	YPL120W	49.25
YOL036W	YOL036W	32.01	GUP2	YPL189W	26.91
YOL138C	YOL138C	39.08	YPL205C	YPL205C	29.03
HIR2	YOR038C	44.14	YPL207W	YPL207W	28.55
CKB2	YOR039W	62.13	ECM3	YOR092W	52.21

8.6 Supplemental Table 8-6: Descriptions of translation related genes that genetically interact with *TAE2*, *TAE3* and *TAE4* and produce a synthetic sick (SS: Super sick, VS: Very sick, MS: Moderately sick) or synthetic rescue (R) interaction.

Systematic Gene Name	Standard Gene Name	Interacting gene	Phenotype	Description and Cellular Function
RNA Processing				
YAL026C	*FUN38*	*TAE3*	MS	Aminophospholipid translocase protein homolog to human ATP8B1, involved in RNA processing
YCR077C	*PAT1* *MRT1*	*TAE3*	MS	Small cytosolic ribosomal protein, involved in translation initiation and metabolic processes
YDR378C	*LSM6*	*TAE4*	SS	Component of heteroheptameric complexes, involved in mRNA processing
YDR515W	*SLF1* *SRO99*	*TAE2* *TAE4*	VS	Ribonucleoprotein involved in regulating mRNA translation, transport, processing
YGL014W	*PUF4*	*TAE3*	SS	PUF protein family member, involved in DNA metabolism and transcription
YHR066W	*SSF1*	*TAE2*	VS	Part of 66S ribosomal particle, participate in ribosome assembly and maintenance
YKL068W	*NUP100*	*TAE3*	R	Nuclear pore subunit, interact with Mex67p and involved in RNA processing
YLR059C	*REX2* *YNT20*	*TAE4*	MS	RNA exonuclease, involved in rRNA maturation and RNA processing
YLR107W	*REX3*	*TAE3* *TAE4*	R (*TAE3*) SS (*TAE4*)	RNA exonuclease activity, required for the maturation of RNA
YMR125W	*STO1* *CBC1*	*TAE4*	VS	Component of the large subunit of mRNA cap protein complex, maintain RNA component in the nucleus, orthologous to mammalian CBP80

Systematic Gene Name	Standard Gene Name	Interacting gene	Phenotype	Description and Cellular Function
YNL001W	*DOM34*	*TAE4*	R	Endoribonuclease activity, involved in mRNA catabolic process, translation
YOL041C	*NOP12*	*TAE2*	VS	This nucleolar protein required for 25S rRNA processing, similar to Nop13p
YOR017W	*REX3*	*TAE4*	MS	RNA exonuclease, involved in RNA processing
YPL119C	*DBP1* *LPH8*	*TAE4*	SS	ATP dependent RNA helicase, involved in translation initiation, mRNA processing
Mitochondrial Translation				
YBR212W	*NGR1* *RBP1*	*TAE2*	VS	RNA binding protein, regulates mitochondrion organization and biogenesis
YCR046C	*IMG1*	*TAE2*	MS	Structural constituent of mitochondrial ribosome, maintain mitochondrial genome
YDR116C	*MRPL1*	*TAE3* *TAE4*	MS (*TAE3*) SS (*TAE4*)	Large mitochondrial subunit involved in translation
YDR494W	*RSM28*	*TAE2*	R	Small subunit of mitochondrial ribosome involved in translation
YMR225C	*MRPL44* *YMR44*	*TAE2*	VS	Mitochondrial large ribosomal subunit
YNR022C	*MRPL50*	*TAE3* *TAE4*	VS (*TAE3*) MS (*TAE4*)	Large subunit of mitochondrial ribosomal protein
YOR017W	*PET127*	*TAE4*	MS	Mitochondrial membrane protein, involved in RNA processing
Translation Regulation				
YDL219W	*DTD1*	*TAE2*	SS	D-tyrosyl-tRNA(Tyr) deacylase activity, involved in translation
YDR223W	*CRF1*	*TAE2*	SS	Transcriptional corepressor, regulate ribosomal protein gene transcription
YFR009W	*GCN20*	*TAE3* *TAE4*	VS (*TAE3*) SS (*TAE4*)	Gcn20p regulate Gcn2p kinase, translation elongation activity

Systematic Gene Name	Standard Gene Name	Interacting gene	Phenotype	Description and Cellular Function
YGL195W	*GCN1*	*TAE2* *TAE4*	MS (*TAE2*) VS (*TAE4*)	Cytosolic protein which regulates translation elongation, Gcn2p kinase activity
YHR077C	*NMD2* *IFS1*	*TAE2* *TAE4*	MS (*TAE2*) SS (*TAE4*)	Involved in the nonsense mediated decay pathway, maintain telomere
YMR080C	*NAM7* *IFS2*	*TAE4*	MS	ATP-dependent RNA helicase of the SFI superfamily, involved in mRNA metabolism, regulate translation termination
YOL045W	*PSK2*	*TAE2*	MS	Member of the PAS domain S/T protein kinase, regulate sugar flux and translation
Translation Factor				
YAL035W	*FUN12*	*TAE4*	VS	Cytosolic small ribosomal subunit, GTPase activity, involved in translation initiation
YJL138C	*TIF2*	*TAE4*	VS	Member of eukaryotic translation initiation factor 4F complex
YJR047C	*ANB1* *HYP1*	*TAE3*	SS	Translation initiation factor, involved in the formation of peptide bond and metabolic processes
YKL081W	*TEF4* *EFC1*	*TAE2*	SS	Component of translation elongation factor 1 complex
YKR026C	*GCN3* *AAS2*	*TAE4*	SS	Alpha subunit of the eukaryotic translation initiation factor eIF2B
YKR059W	*TIF1*	*TAE2*	R	Translation initiation factor eIF4A, identical to Tif2p
YMR012W	*CLU1* *TIF31*	*TAE2*	VS	Eukaryotic translation initiation factor 3 complex

Systematic Gene Name	Standard Gene Name	Interacting gene	Phenotype	Description and Cellular Function
60S Ribosomal Subunit				
YBL087C	*RPL23A*	*TAE2*	SS	Cytosolic large ribosomal subunit, similar to *E. coli* L14 and rat L23
YBR084C-A	*RPL19A*	*TAE3*	MS	Large ribosomal (60S) subunit, identical to Rp119Bp and similar to rat L19 ribosomal protein
YFR032C-A	*RPL29*	*TAE2*	MS	Structural constituent of ribosome, has similarity to rat L29 ribosomal protein
YIL133C	*RPL16A* *RPL13*	*TAE2* *TAE4*	VS	Large ribosomal subunit, binds to 5.8 S rRNA, similar to *E. coli* L13 and rat L13a
YLR325C	*RPL38*	*TAE3*	SS	Large (60S) ribosomal subunit, identical to rat L38 ribosomal protein
YLR344W	*RPL26A*	*TAE2*	MS	Large ribosomal subunit, nearly identical to Rpl26Bp, similar to *E. coli* L24 and rat L26
YPL079W	*RPL21B*	*TAE4*	SS	Large ribosomal subunit, similar to rat L21 ribosomal protein
40S Ribosomal Subunit				
YDR025W	*RPS11A*	*TAE2* *TAE4*	MS	Protein component of the small (40S) ribosomal subunit, identical to Rps11Bp, similar to *E. coli* S17 and rat S11
YDR450W	*RPS18A*	*TAE2*	R	Cytosolic small ribosomal subunit, similar to *E. coli* S13 and rat S18
YJL190C	*RPS22A* *RPS24*	*TAE2* *TAE4*	SS (*TAE2*) MS (*TAE4*)	Structural constituent of ribosome, has similarity with *E. coli* S8 and rat S15a ribosomal proteins
YKL156W	*RPS27A*	*TAE2*	VS	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps27Bp and has similarity to rat S27 ribosomal protein

Systematic Gene Name	Standard Gene Name	Interacting gene	Phenotype	Description and Cellular Function
YLR441C	*RPS1A RP10A*	*TAE4*	SS	Ribosomal protein 10 (rp10) of the small (40S) subunit; nearly identical to Rps1Bp and has similarity to rat S3a ribosomal protein
YML063W	*RPS1B RP10B*	*TAE4*	VS	Ribosomal protein 10 (rp10) of the small (40S) subunit; nearly identical to Rps1Ap and has similarity to rat S3a ribosomal protein
YOR096W	*RPS30 RPS7A*	*TAE3*	MS	Ribosomal protein component of the small (40S) subunit, similar to Rps7Bp and rat S7
YOR182C	*RPS30B*	*TAE4*	VS	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps30Ap and has similarity to rat S30 ribosomal protein
YPR132W	*RPS23B*	*TAE2*	VS	Cytosolic small ribosomal subunit, identical to Rps23Ap, similar to *E. coli* S12 and rat S23
Amino Acid Metabolism				
YER091C	*MET6*	*TAE2*	VS	Methyl transferase activity, involved in amino acid metabolic process
YIL074C	*SER33*	*TAE2*	SS	Phosphoglycerate dehydrogenase activity, involved in the biosynthesis of serine family amino acids
YJR130C	*STR2*	*TAE2*	VS	Cystathionine gamma-synthase activity, involved in amino acid metabolism
YLR180W	*SAM1 ETH10*	*TAE4*	MS	S-adenosinemethionine synthetase, involved in methionine metabolic processes
YNL277W	*MET2*	*TAE2*	SS	L-homoserine-O-acetyltransferase, catalyzes the conversion of homoserine to O-acetyl homoserine which is the first step of the methionine biosynthetic pathway
YOR184W	*SER1 ADE9*	*TAE2*	VS	3-phosphoserine aminotransferase, catalyzes the formation of phosphoserine from 3-phosphohydroxypyruvate, required for serine and glycine biosynthesis

Systematic Gene Name	Standard Gene Name	Interacting gene	Phenotype	Description and Cellular Function
Others				
YFL031W	*SNF4* *CAT3*	*TAE3*	SS	AMP-activated Snf1p protein kinase complex, involved in sporulation, and peroxisome biogenesis
YGL248W	*PDE1*	*TAE2*	SS	3',5'-cyclic-AMP phosphodiesterase activity, induce transcription
YGR166W	*KRE11* *TRS65*	*TAE4*	MS	Protein involved in the biosynthesis of cell wall component
YLR085C	*APR6*	*TAE4*	MS	Nucleosome binding protein, involved in DNA metabolic processes
YNL298W	*CLA4* *ERC10*	*TAE2*	SS	Cdc42p activated signal transducing kinase of the p21-activated kinase family, involved in septin ring assembly and cytokinesis
YNL299W	*TRF5*	*TAE2*	MS	Poly (A) polymerase involved in nuclear RNA quality control based on: homology with Trf4p

8.7 Supplemental Table 8-7: Descriptions of translation related genes that are phenotypically suppressed by overexpression of *TAE2* and *TAE4* against treatment with neomycin and/or streptomycin.

Gene deletion strains		Treatment		Description and Cellular Function
Systematic gene name	**Standard gene name**	**Neomycin**	**Streptomycin**	
TAE2 OVEREXPRESSION				
Translation Factor				
YKL204W	*EAP1*		Complete	eIF4E-associated protein, maintain genetic stability
YKR059W	*TIF1*	Complete		Eukaryotic translation initiation factor eIF4A
YMR012W	*CLU1* *TIF31*		Complete	Translation initiation factor, eIF3 component of unknown function
YOL023W	*IFM1*	Partial		Mitochondrial translation initiation factor 2
Mitochondrial Translation				
YDR462W	*MRPL28*		Partial	Mitochondrial ribosomal protein of the large subunit|Mrpl28p
YKL167C	*MRP49*	Partial		Structural constituent of mitochondrial ribosomal protein
YKR006C	*MRPL13*	Partial		mitochondrial large ribosomal subunit
YMR012W	*CLU1* *TIF31*		Complete	Translation initiation factor, eIF3 component of unknown function
YOL023W	*IFM1*	Partial		Mitochondrial translation initiation factor 2
Large (60S) ribosomal subunit				
YBL027W	*RPL19B*	Complete	Complete	Large (60S) ribosomal subunit, identical to Rpl19Ap and similar to rat L19 ribosomal protein
YDL184C	*RPL41A* *RPL47A*	Complete		Ribosomal protein L47 of the large (60S) ribosomal subunit, identical to Rpl41Bp and has similarity to rat L41 ribosomal protein

Gene deletion strains		Treatment		Description and Cellular Function
Systematic gene name	**Standard gene name**	**Neomycin**	**Streptomycin**	
YER056C-A	*RPL34A*	Partial		Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl34Bp and has similarity to rat L34 ribosomal protein
YIL052C	*RPL34B*		Complete	Cytosolic large (60S) ribosomal subunit, nearly identical to Rpl34Ap and has similarity to rat L34 ribosomal protein
YMR242C	*RPL20A RPL18A2*	Complete	Partial	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl20Bp and has similarity to rat L18a ribosomal protein
YOR312C	*RPL20B RPL18A1*	Complete		the large (60S) ribosomal subunit, nearly identical to Rpl20Ap and has similarity to rat L18a ribosomal protein
Small (40S) ribosomal subunit				
YDL083C	*RPS16B*	Complete		The small (40S) ribosomal subunit; identical to Rps16Ap and has similarity to E. coli S9 and rat S16 ribosomal protein
YPL081W	*RPS9A*		Complete	Cytosolic small (40S) ribosomal subunit; nearly identical to Rps9Bp and has similarity to E. coli S4 and rat S9 ribosomal proteins
Others				
YER081W	*SER3*		Complete	3-phosphoglycerate dehydrogenase, involved in serine and glycine biosynthesis
YFL001W	*DUG1 PUS3*	Partial	Partial	tRNA-pseudouridine synthase activity
YGR155W	*CYS4 NHS5*		Complete	Cystathionine beta-synthase, catalyzes the synthesis of cystathionine from serine and homocysteine
YGR285C	*ZUO1*	Complete		Cytosolic ribosome-associated chaperone
YIL074C	*SER33*		Complete	3-phosphoglycerate dehydrogenase, catalyzes the first step in serine and glycine biosynthesis

Gene deletion strains		Treatment		Description and Cellular Function
Systematic gene name	**Standard gene name**	**Neomycin**	**Streptomycin**	
			***TAE4* OVEREXPRESSION**	
RNA processing				
YDL213C	*NOP6*		Partial	Putative RNA-binding protein implicated in ribosome biogenesis
YER032W	*FIR1*		Complete	Protein involved in 3' mRNA processing
YGR159C	*NSR1*	Complete		Nuclear protein, required for pre-rRNA processing and ribosome biogenesis
YJL124C	*LSM1 SPB8*	Complete		small nucleolar ribonucleoprotein forms heteroheptameric complex
YPL178W	*CBC2 CBP20*		Partial	Small subunit of the heterodimeric cap binding complex
Small (40S) ribosomal subunit				
YDL083C	*RPS16B*		Partial	Small (40S) ribosomal subunit; identical to Rps16Ap and has similarity to E. coli S9 and rat S16 ribosomal proteins
YGR118W	*RPS23A*		Complete	Ribosomal protein 28 (rp28) of the small (40S) ribosomal subunit, required for translational accuracy
YGR214W	*RPSOA NAB1*	Complete		Cytosolic protein component of the small (40S) ribosomal subunit, nearly identical to Rps0Bp; required for maturation of 18S rRNA
YKL156W	*RPS27A*	Partial		Protein component of the small (40S) ribosomal subunit; nearly identical to Rps27Bp and has similarity to rat S27 ribosomal protein
YKR057W	*RPS21A RPS25*	Complete		Small (40S) ribosomal subunit; nearly identical to Rps21Bp and has similarity to rat S21 ribosomal protein
YLR367W	*RPS22B*	Complete		Protein component of the 40S ribosomal subunit; nearly identical to Rps22Ap and has similarity to E. coli S8 and rat S15a ribosomal proteins
YML026C	*RPS18B*	Partial		Small ribosomal subunit, nearly identical to Rps27Bp and similar to rat S27 ribosomal protein

Gene deletion strains		Treatment		Description and Cellular Function
Systematic gene name	**Standard gene name**	**Neomycin**	**Streptomycin**	
YMR143W	*RPS16A*	Partial	Complete	Protein component of the small (40S) ribosomal subunit; identical to Rps16Bp and has similarity to E. coli S9 and rat S16 ribosomal proteins
YPL081W	*RPS9A*		Partial	Small (40S) ribosomal subunit; nearly identical to Rps9Bp and has similarity to E. coli S4 and rat S9 ribosomal proteins
Others				
YDL075W	*RPL31A RPL34*	Complete		Large (60S) ribosomal subunit, nearly identical to Rpl31Bp and has similarity to rat L31 ribosomal protein
YIL052C	*RPL34B*		Partial	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl34Ap and has similarity to rat L34 ribosomal protein
YOR302W	None	Complete		Arginine attenuator peptide, regulates translation of the CPA1 mRNA
YPR189W	*SKI3*		Complete	Protein involved in exosome mediated 3' to 5' mRNA degradation and translation inhibition of non-poly(A) mRNAs

8.8 Supplemental Table 8-8: List of genes which showed increased activity for β-gal activity using large-scale approach (filter assay) and their sensitivity to amino glycosides (P-Paromomycin, S-Streptomycin, N-Neomycin).

Systematic Name	Standard Name	Gene Description	P	S	N
YDL075W	RPL31A	Regulation of translation fidelity	14.49		
YFR044C	DUG1	Protein catabolic process	14.61	41.89	25.43
YJL212C	OPI1	Sulfur metabolic processes/telomere maintenance	15.09		40.68
YDL076C	RXT3	DNA metabolic process	15.33	15.13	22.71
YLR120C	YPS1	Protein metabolic process	15.48		
YLR192C	HCR1	Translation initiation	16.22		28.14
YBR025C	OLA1	ATP catabolic process	17.07	13.18	51.02
YER044C	BUD18	Biosynthesis	17.83	57.99	
YER056C	FCY2	Transport	18.12		
YER113C	TMN3	Transport	18.19		
YIL136W	OM45	Unknown	18.29		15.21
YIL056W	VHR1	DNA Transcription factor	18.60		33.53
YNL029C	KTR5	Protein metabolic process	18.78		34.23
YNL128W	TEP1	Sporulation	19.29		
YMR080C	NAM7	Translation termination, telomere maintenance	20.25		
YPL220W	RPL1A	Ribosomal Protein of the Large subunit	20.40		
YLR445W	YLR445W	Transcription regulation	21.15	16.22	20.36
YNL040W	YNL040W	Unknown	21.15		
YOR309C	YOR309C	Unknown	21.98		18.38
YMR031W-A	YMR031W-A	Telomere maintenance	22.49		50.36
YNL122C	YNL122C	Unknown	23.04		22.25
YAL027W	SAW1	DNA repair	23.33		24.18
YHR021W-A	ECM12	Cell wall organisation	23.85		35.34
YHR077C	NMD2	Telomere maintenance	23.89	63.41	19.42
YGR078C	PAC10	Metabolic process	24.24	26.36	
YDR193W	Dubious	Unknown	25.41	58.56	
YOR324C	FRT1	Response to stress	26.18	43.95	46.34
YER028C	MIG3	Transcription initiation	26.72	17.23	
YJR069C	HAM1	DNA repair	27.19	29.14	
YIL032C	YIL032C	Unknown	27.26	32.60	
YKL148C	SDH1	Transport	27.38	26.00	63.16
YKL011C	CCE1	DNA recombination	27.99		27.24
YLR219W	MSC3	DNA recombination	28.90		
YDR198C	RKM2	Ribosomal Protein	30.62	25.34	66.83

Systematic Name	Standard Name	Gene Description	P	S	N
YPR095C	SYT1	Transport	31.12	56.60	31.83
YDL170W	UGA3	Transcription factor	32.32		
YML052W	SUR7	Sporulation	32.70		16.77
YOR365C	YOR365C	Unknown	33.96		
YDR300C	PRO1	Proline biosynthesis, telomere maintenance	34.37	30.61	
YJR105W	ADO1	Utilize S-adenosylmethionine , telomere maintenance	34.73	22.46	
YNL281W	HCH1	Telomere ***	35.22	37.51	
YPR049C	ATG11	Protein transport, telomere maintenance	35.53		
YBR261C	TAE1	Translation	37.75		22.59
YDR467C	Dubious	Unknown	39.76		25.08
YDR503C	LPP1	Phospholipid metabolism	40.05	29.54	15.67
YMR231W	PEP5	Transport	42.17	29.91	44.35
YGL072C	YGL072C	Unknown	42.85		
YOR378W	YOR378W	Unknown	44.62	18.25	18.40
YDR099W	BMH2	DNA replication	46.53		
YOR230W	WTM1	DNA Transcription factor	47.25	23.15	33.38
YNR047W	FPK1	transport	47.33		
YNR069C	BSC5	Translation read-through	47.54		
YOR064C	YNG1	DNA metabolic process	51.47	22.87	19.41
YBR277C	Dubious	Unknown	52.07	21.88	37.13
YDR477W	SNF1	Carbohydrate metabolism	58.81	16.00	
YPR152C	URN1	Unknown		16.92	
YJR062C	NTA1	Protein metabolic process		21.56	
YER087C-B	SBH1	Protein transport		23.38	
YDR063W	AIM7	Unknown		24.62	
YGR118W	RPS23A	Regulation of translation fidelity, telomere maintenance		25.93	18.84
YGR072W	UPF3	Telomere maintenance		26.05	
YDR107C	TMN2	transport		27.37	
YLR094C	GIS3	Unknown		28.14	24.27
YFL043C	YFL043C	Unknown		29.42	
YGL197W	MDS3	Sporulation		29.71	
YIL036W	CST6	Chromosome stability, telomere maintenance		30.47	
YBR212W	NGR1	Translation regulation		30.59	98.02
YFL001W	DEG1	Translation		31.25	58.15
YML094W	GIM5	Protein transport		31.85	25.58
YIR025W	MND2	DNA recombination		32.02	
YOL006C	TOP1	DNA Transcription		34.40	
YOL049W	GSH2	Glutathione synthesis		35.69	
YLL060C	GTT2	Glutathione S-transferase		35.74	22.56

Systematic Name	Standard Name	Gene Description	P	S	N
YCL025C	AGP1	Protein transport		52.02	32.26
YLR037C	PAU23	Response to stress			15.18
YLL048C	YBT1	Transport			16.03
YAL015C	NTG1	DNA repair			17.05
YLL019C	KNS1	protein amino acid phosphorylation			17.61
YGL237C	HAP2	DNA Transcription			20.07
YHR207C	SET5	Unknown			20.32
YJR137C	ECM17	Sulfate reductase complex			27.79
YHR028C	DAP2	Protein metabolic process			33.25
YJR148W	BAT2	Amino acid metabolism			35.59
YER156C	YER156C	Transcription factor/Unknown			83.52