APPROVAL SHEET

Title of Dissertation: Differential accumulation of extra-ribosomal L5(uL18) in response to various ribosomal stresses in a p53-free system

Name of Candidate: Nusrat Rahman
Doctorate in Philosophy, 2017

Dissertation and Abstract Approved: Lasse Lindahl

Lasse Lindahl
Professor
Department of Biological Sciences

Date Approved: 9/13/17

NOTE: *The Approval Sheet with the original signature must accompany the thesis or dissertation. No terminal punctuation is to be used.

ABSTRACT

Title of Document: DIFFERENTIAL ACCUMULATION OF RIBOSOMAL PROTEIN L5(UL18) IN RIBOSOMAL STRESSES IN A P53-FREE SYSTEM

Nusrat Rahman, PhD, 2017

Directed By: Professor Lasse Lindahl, Biology

Ribosome biogenesis is a highly regulated high-energy requiring process; it is coordinated with cell cycle and proliferation. Defects in ribosome assembly are connected with abnormal growth-related diseases. Ribosomal protein L5 is reported to involve regulating level of tumor-suppressor protein p53 in metazoans. Although the presence of extra-ribosomal L5 is reported in multiple organisms, systematic characterization of its fate in ribosome biogenesis stresses remains to be explored. Here we checked the fate of L5 in various nucleolar/ribosome stresses and commented on possible source of the accumulation. The variation in L5 accumulation may provide further understanding of different cell surveillance mechanisms.

DIFFERENTIAL ACCUMULATION OF RIBOSOMAL PROTEIN L5(uL18) IN RIBOSOMAL STRESSES IN A P53-FREE SYSTEM.

By

Nusrat Rahmann

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore County, in partial fulfillment
of the requirements for the degree of
Doctorate in Philosopy
2017

© Copyright by
Nusrat Rahman
2017

Dedication

My mother, Laila Noor Begum

Acknowledgements

First I want to send my gratitude to my parents, Laila Noor Begum and Lutfor Rahman, for their life-long dedication, encouragement and trust on me. To my parents, I am still a wonder-girl; it was hard leaving them 8000 miles away and keep trying to live up to their expectations last eight years. My grandmother – a true iron lady, my brother - my first ever math teacher, and my sister - love of my life: you three have inspired me in many unspoken ways.

I feel acknowledgments should be written multiple times, as every time I learned, whether in a formal or in an informal way, through ease or through woes, I felt grateful. I was often surprised by the beautiful grace and patience my academic adviser, Dr. Lindahl showed towards me. He truly cares about his students' growth, and often selflessly went beyond his means to accommodate such opportunities. Thank you for all your generosity and trust. Respectable committee members, Drs. Farabaugh, Zengel, Marten, Erill and Li, thank you for your valuable contributions. My lab members and alumni, Marlon, Mamata, Jesse, Brian, Shams and Rob, you all have helped me grow as a thinker.

I want to express my love and gratitude towards my friends Marlon, Uzma, Alimatou, members of Bangladeshi Student Association – you have been there to celebrate every milestone of my life. My colleagues, Ling, Preethi, TJ, Afsoon, Neus, Ariya – thank you for all your support, I love you all.

My words fall short when it comes to thanking my partner, Md. Shamsuzzaman. Since our first meeting at undergrad back in 2003, I have been inspired by your merit, persistence and hard-earned accomplishments. Grad life has been stressful, raising a toddler without support was a challenge, but over and over we found ways to help each other grow. I love you. Thank you! Lastly, I want to thank my son, Reyhan Noor Shams for being the coolness of my eyes. I want to grow with you, learn from you how to be a curious explorer just like you. Thank you!

Table of Contents

Dedication…….. ii

Acknowledgements……iii

Table of Contents……...ivv

List of Tables…… vi

List of Figures…….. vii

Chapter 1: Introduction…….....1

1.1 Ribosomal components 1
1.1.1 Nomenclature of ribosomal components 2
1.1.2 Functions of ribosomal components 3
1.1.3 Conservation of ribosomal components..... 6
1.1.4 Modification of ribosomal components 13

1.2 Ribosome Biogenesis..... 15

1.3 Ribosome biogenesis stress/nucleolar stress and cell cycle..... 17
1.3.1 Ribosome-mediated cell cycle arrest by p53-dependent mechanism (metazoans and higher Eukaryotes) 18
1.3.2 Ribosome-mediated cell cycle arrest by p53-independent mechanism (p53 mutant metazoans and yeast) 19

1.4 Balanced production of ribosomal components..... 21

1.5 Disease, drug discovery and current research..... 23

Chapter 2: Differential accumulation of ribosomal protein L5(uL18) in ribosomal stresses in a p53-free system…….. 26

Introduction..... 26
Results..... 29
Discussion 47

Chapter 3: On the functional importance of ribosomal protein phosphorylation….. 52

Introduction 52
Results 53
Conclusion 70
Supplementary 71

Chapter 4: Materials and Methods……. 73

Chapter 5: Summary and Future Direction…...…… 75

5.1 Differential accumulation of ribosomal protein L5…… 76
5.2 Phosphorylation of ribosomal proteins……... 80

Appendices…… 82

Bibliography……. 123

List of Tables

1.1 Components of a mature cytosolic yeast ribosome……...…… 3

1.2 List of chemicals/biologics targeting ribosome biogenesis currently being used/explored as anti-cancer therapeutic……..…… 24

2.1 T-test comparison among different ribosomal protein depletions…….. 42

2.2 Ribosomal proteins from small and large ribosomal subunit and their position in the assembly…….…..… 49

3.1 Databases used for compiling phosphorylation data……..…..… 53

3.2 Phosphorylation in yeast ribosomal proteins……..…… 54

3.3 Expected and actual phosphorylation outcomes among each group of ribosomal proteins…….……... 55

3.4 Frequency of phosphorylation among STY in each group……..……57

3.5 Expected and observed number of phosphorylation in the two subunits…… 58

3.6 Conserved phosphorylation sites and their location on the ribosome……..59

3.7 Criteria for classification of phosphomutant colonies grown in glucose …....62

3.8 Growth data for phosphomutants at different temperatures……...…. 63

3.9 Growth chart of S11 mutants……..… 67

3.10 Databases of different numbers of pfam domain ……..…68

4.1 Genotype of the strains used……..…. 73

List of Figures

1.1 Crystal structure of a mature cytosolic yeast ribosome ……... 1

1.2 Crystal structure of *T. thermophiles* ribosome……. 4

1.3 Core and Shell of ribosome…….. 7

1.4 A hierarchy of evolution of life……... 9

1.5 Model for rRNA evolution……..… 10

1.6 Sites in rRNA to which nuclear localization signals bind……..…. 13

1.7 Yeast rRNA modification repertoire……..…. 14

1.8 Maturation of rRNA in *S.cerevisiae*……..….. 17

1.9 Schematic diagram of molecular mechanism of ribosomal components expression through Ifh1……...…… 22

2.1 Ribosome profiles of different strains grown in galactose…….. 31

2.2 Ribosome profiles of different strains grown in glucose for 4-21 hours…… 34

2.3 Extra-ribosomal accumulation of L5(uL18) is significantly higher compared to other ribosomal proteins (L4, S9, L11) in case of ribosomal stress caused by a small subunit protein depletion……. 35

2.4 The upper band (star-band) reacting with anti-L5 is due to a cross reaction of a protein not related to L5…… 38

2.5 Extra-ribosomal accumulation of L5 in ribosomal stresses…… 40

2.6 Lack of significant extra-ribosomal accumulation of L5(uL18) in S31(eS31) depletion over time……... 43

2.7 Cycloheximide eliminates accumulation of extra-ribosomal L5 during ribosomal stress……. 45

2.8 Extra-ribosomal accumulation of L11 in ribosomal stresses…….. 47

2.9 Proposed mechanism for L5 accumulation……. 51

3.1 Percent of phosphorylations among BAE, AE and E groups…….. 54

3.2 Percent frequency of reported phosphorylation among Serine Threonine and Tyrosine residues……...……... 57

3.3 Criteria for choosing candidate site……...…….. 59

3.4 Genetic construction of the strain used for functional analysis…….. 61

3.5 Example of classification of growth of mutants by spot dilution essay...…... 62

3.6 Growth impairment of mutant S112E and Y90F strains are due to instability of the protein……...……... 65

3.7 Secondary structure of the region of 90th and 112th amino acid……... 66

3.8 Occupancy of amino acids at the position corresponding to Y90 of S11 in yeast……69

3.9 Occupancy of amino acids at the position corresponding to S112 of S11 in yeast ……69

Chapter 1: Introduction

Ribosome is the protein synthesis machinery of all living cells. A ribosome carries out the translation step of the central dogma (conversion of messenger RNA into protein). This chapter describes the structural components of the ribosome and recent understanding on the coordination between ribosome biogenesis, cell cycle and drug targeting ribosome biogenesis pathway.

1.1 Ribosomal Components

A mature ribosome is a large complex made of two subunits each of which is composed of one or more ribosomal RNAs and a number of ribosomal proteins. The ribosomal RNAs and ribosomal proteins are referred here as 'ribosomal components' (Fig 1.1).

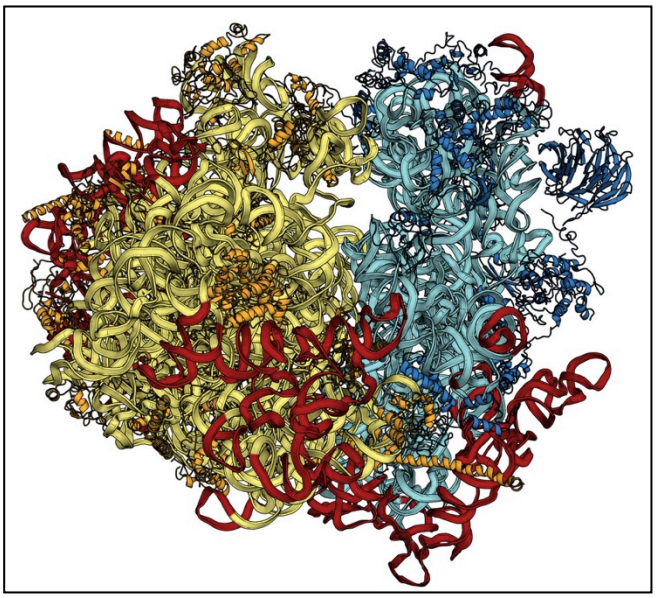


Fig 1.1: Crystal structure of a mature cytosolic yeast ribosome (Source: M. Yusupov. IGBMC). Small subunit components are depicted in light blue (rRNA) and dark blue (ribosomal proteins) and large subunit components are depicted in yellow (25S rRNA), orange (5S rRNA and 5.8 rRNA) and red (ribosomal proteins).

1.1.1 Nomenclature of ribosomal components

Subunits and RNAs of a ribosome are named by their sedimentation coefficient (Svedberg unit); however, ribosomal proteins were originally named by their ranks/location on a 2D gel electrophoresis system. Due to variations in length and non-conserved parts of orthologues, the same protein was given different names in different species (for a history on the ribosomal protein nomenclature, see (Ban et al., 2014)). These differences in naming created much ambiguity in the studies of conservation of structure/functions of ribosomal proteins. For example, ribosomal protein L5 was called L1a or YL3 in yeast, L5 in rats, while the bacterial homolog was named as L18 (Mager et al., 1997). Multiple attempts have been taken for coming to a universal nomenclature system. Wittmann-Liebold in 1989 suggested that, for Archaeal ribosome, specifically for *H. marismortui,* some of r-proteins be named according to their Eubacterial homologs (Wittmann-Liebold et al., 1990). In 1997, new nomenclature for yeast r-proteins was adapted (Mager et al., 1997) which basically tried to keep same name for homologs of r-proteins between yeast and rat ribosomes, according to the structure and evolution information known around that time (Wool et al., 1995). Although it partially solved different naming for some species, assigning consistent names across all species was still a challenge. Finally, a universal nomenclature system for ribosomal proteins was suggested in 2014 (Ban et al., 2014). The new structure of the name adds conservation information of the proteins as prefix (for example, ‘u’ for universally conserved, ‘e’ for eukaryote specific ones) and assigns the name after the prefix according the name used for the evolutionary domain that is closest to the root of the evolutionary tree. This thesis will

mention the conventional names (Mager et al., 1997) with the new name (Ban et al., 2014) in parenthesis.

The ribosomal components in *S. cerevisiae* are listed in Table 1.1 (Appendix 1a lists the new nomenclature of all yeast ribosomal proteins) –

Table 1.1: Components of a mature cytosolic yeast ribosome

Subunits	Ribosomal RNA(s)	Ribosomal proteins (traditional names)
40S (small)	18S	RPS0-RPS31
60S (large)	5S, 5.8S, 25S	RPL3-RPL41, RPP0-RPP2

1.1.2 Functions of ribosomal components

As the overall function of a ribosome is translating the mRNA sequences into amino acid sequences, individual components (or each group of components) have distinct roles in the process. Historically, structural and biochemical analysis of ribosome helped in identifying roles of these components.

1.1.2.1 Functions of rRNA

The translation process is a stepwise process of recognition of mRNA codon, incorporation of amino-acyl tRNAs, peptide bond formation, and translocation of ribosomal subunits. Biochemical and genetic experiments have mapped the decoding function to the small subunit and the peptide formation function to the large subunit. Structure analysis of *in vitro* formed complexes helped define a "Decoding Center" (matching of aminoacyl tRNA properly with mRNA codons) and a "Peptidyl Transferase Center" (forming peptide bonds between amino acids

properly aligned through by the Decoding Center) (Schlünzen et al., 2001; Schmeing et al., 2009). According to the crystal structure of ribosomes, these centers are heavily surrounded by rRNAs (Moore and Steitz, 2002) (Fig 1.2) with the closest ribosomal protein at some distance, leading to the concept that "the ribosome is a ribozyme".

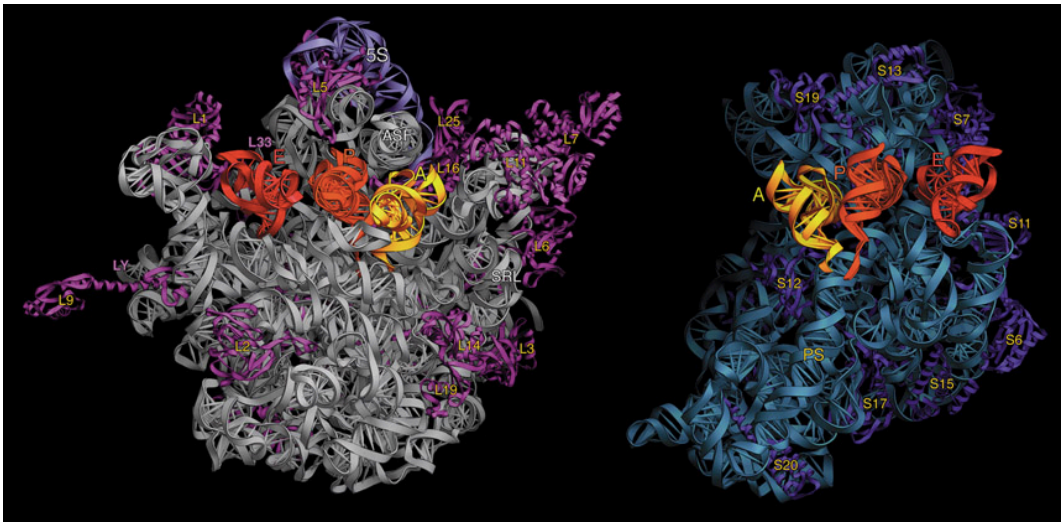


Fig 1.2: Crystal structure of *T. thermophilus* ribosome (large subunit on the left and small subunit on the right) bound to tRNAs (red and yellow) in A, P and E sites of the subunits. Note that the tRNA binding sites are heavily surrounded by rRNAs (grey in large subunit and blue in small subunit). (Noller lab webpage)

In a recent issue of Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences termed 'Perspective of the ribosome', Harry F Noller and Thomas Steitz compiled the claims supportive of rRNA being the 'doer' of translation process with r-proteins only providing a 'supporting' role for the rRNAs (Noller, 2017; Steitz and Moore, 2017). However, roles of r-proteins have also been reported in ribosomal and extra-ribosomal functions (elaborated in next section)

1.1.2.2 Functions of r-proteins

Despite the popularity of the RNA centric view, importance of ribosomal proteins in ribosome's function is astounding. In yeast, many of the ribosomal protein genes have two copies (termed as RPXA and RPXB). To determine which ribosomal proteins are essential for cell viability, a library of haploid ribosomal protein gene deletion strains was constructed. After that, double deletion mutants were created through mating and checked for viability of the strains. Sixty-four out of the 79 proteins were identified as essential, since the double mutants of the genes were not viable (Raué et al., 1991; Winzeler et al., 1999; Wolfe and Shields, 1997). The remaining 15 proteins were not required for yeast viability, but were nevertheless found to have important roles in ribosome biogenesis and assembly (Briones et al., 1998; DeLabre et al., 2002). The effects of depletion of ribosomal proteins on ribosome functioning have been systematically characterized (Andersson et al., 1982; Dresios et al., 2000; Herbst et al., 1994; Peltz et al., 1999; Piepersberg et al., 1975; Randall-Hazelbauer and Kuland, 1972; Tait-Kamradt et al., 2000; Zhou et al., 2010). Recently the crystal structure of yeast ribosome carrying a point mutation (W255C) in the ribosomal protein L3 (uL3) was resolved; it shows that the mutation deformed/ disrupted orientation in the A site of the peptidyl transferase center, resulting in increased affinities for transfer RNAs, decreased rates of peptidyl-transfer (Mailliot et al., 2016). Taken all the information together, it is fair to say ribosomal proteins are essential for proper ribosome biogenesis and function as well as for cell viability, even though they may exercise much of their function by molding the rRNA structure.

Furthermore, some ribosomal proteins appear to have other prominent roles outside the ribosome. Their extra-ribosomal roles have been well documented in regulation of transcription and translation of genes of ribosomal components, coordination of the steps of ribosome biogenesis and cell cycle regulation (detail in 1.3).

1.1.3 Conservation of ribosomal components

Despite substantial conservation of overall structure and function, ribosomes have drastically different molecular weights across the different branches of life. A bacterial ribosome is 2.3MDa while a ribosome in higher eukaryote weighs about 4.3MDa. A comparison of the structure of ribosomes from bacteria, Archaea, and eukaryotic ribosomes revealed conserved ribosomal 'core' consisting parts of rRNA and a subset of ribosomal proteins. The core is surrounded by a 'shell', which is unique to each domain of life and consists of insertion elements of rRNAs and expansion elements in r-proteins along with a set of proteins that evolved after the lineages separated (Fig 1.3). (Melnikov et al., 2012)

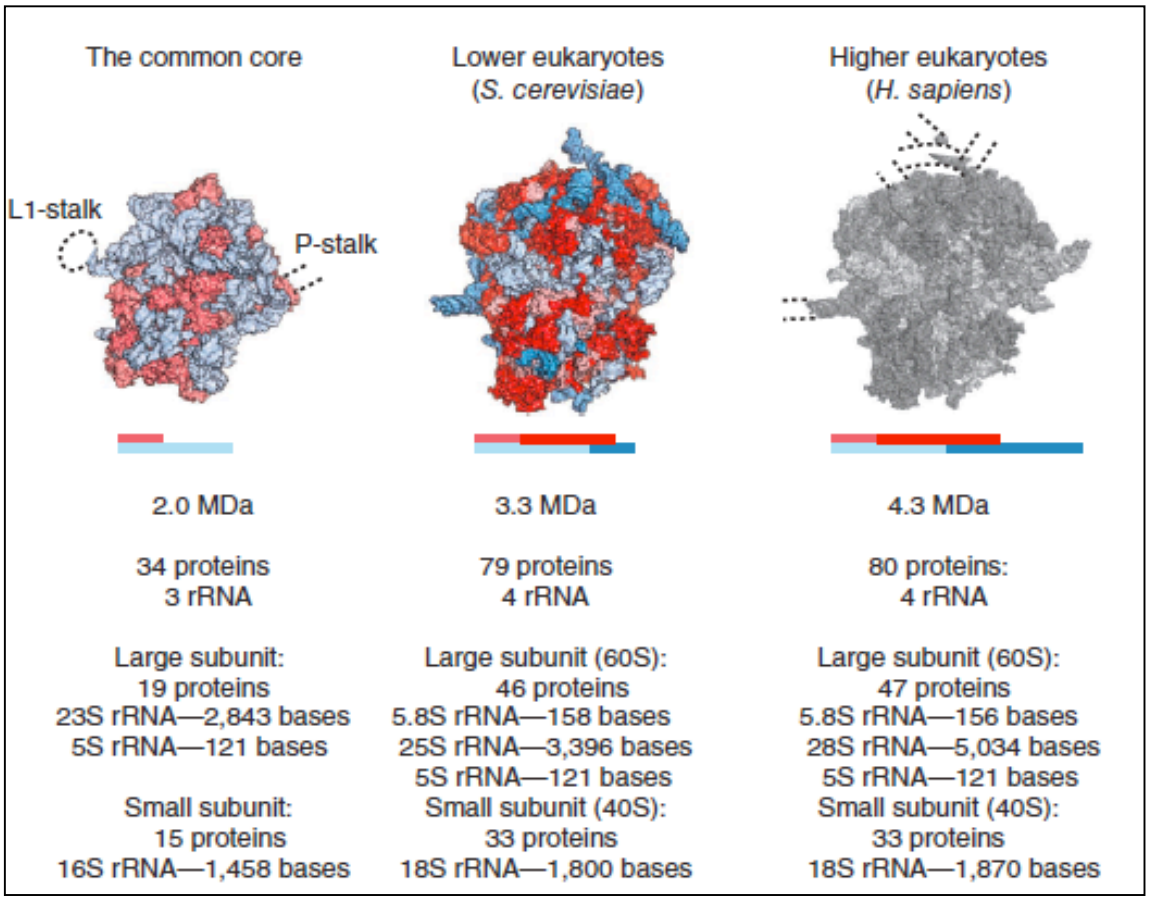


Fig 1.3: Core and shell of ribosome (copied from (Melnikov et al., 2012)).

The common core depicts the structurally conserved rRNA in light blue and structurally conserved ribosomal proteins in light red. Expansions in rRNAs and proteins are marked in dark blue and dark red respectively. Notice the large expansion of rRNA in H. sapiens (dark blue).

1.1.3.1 Evolution of rRNA

Evolution of RNAs and rRNAs is very much tied up with studies of evolution of life. With the premise that life evolved from nonliving chemicals into a membrane-bound living cell, a ‘precellular life’ would be an intermediate prior to forming a cell.

RNA molecules were given that attribute of having a ‘precellular life’, suggesting RNA molecules were stable and carried important functional role for evolutionary progress. The discovery of ribozyme, i.e. RNAs carrying catalytic properties, strengthened the hypothesis (Guerrier-Takada et al., 1983; Kruger et al., 1982; Müller, 2009). According to this hypothesis, the very initial self-replicating ribozyme had decoding and peptide synthesis sites, which remain functionally conserved till today. Ribosomal proteins were added to it later (Moore and Steitz, 2002).

Ribosome was also proposed to be the ‘missing link’ between the RNA world (precellular life) and the Last Universal Common Ancestor (LUCA) - the last organism prior to the separation of known lineages (Fig 1.4). Root-Bernstein showed that the nucleotide sequence of 16S and 23S rRNAs contain genetic information for tRNAs of all 20 amino acids and a several actives sites of key ribosomal proteins (Root-Bernstein and Root-Bernstein, 2015), suggesting that the rRNA sequences carrying necessary information to lead to the formation of an active ribosome.

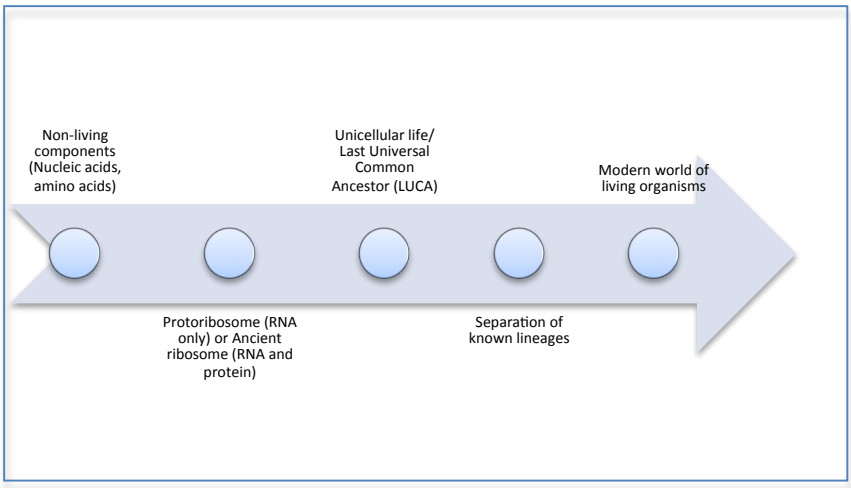


Fig 1.4: A hierarchy of evolution of life

There is a difference in opinion as to whether the step leading to a functional ribosome is really an 'RNA-only' complex, or is it a mix of RNA and protein (ribonucleoprotein) complex. Different groups considered evolution of only rRNAs or coevolution of rRNAs and r-proteins. For example, Petrov et al. modeled the evolution of ribozymes by comparing 3D structures of rRNAs from bacteria, yeast and metazoans. They showed that the expansion from the core functional ancestor happened in a 6-step process, mainly by adding branched helices to the existing helices (Petrov et al., 2014). Starting from the formation of a catalytic core, they proposed how the RNA could have evolved independently to incorporate more functionally important regions, e.g. acquisition of SSU interface or late tunnel extension etc. (Fig 1.5).

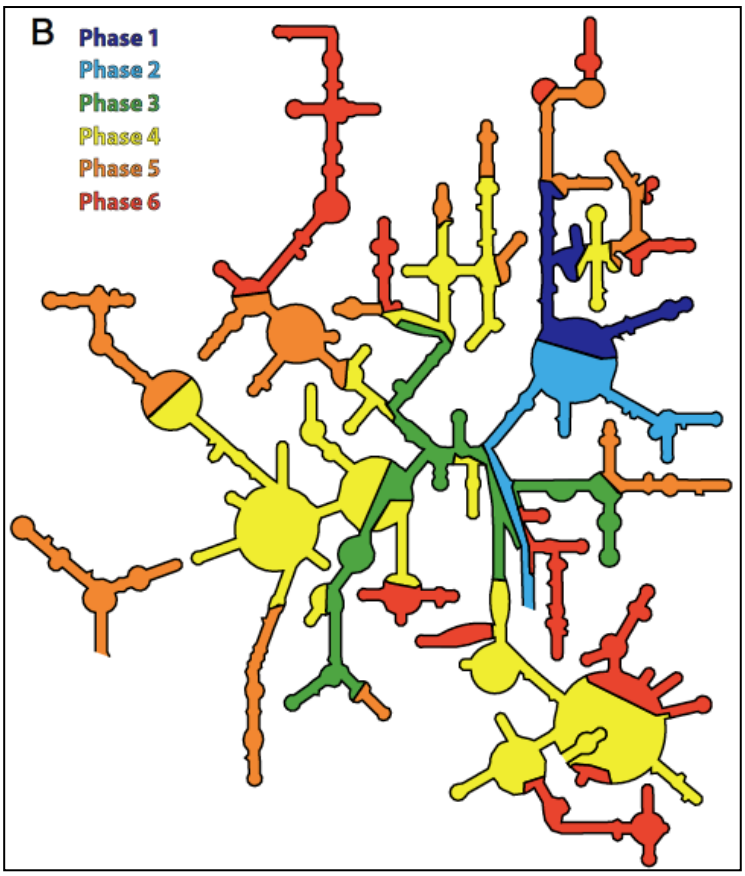


Fig 1.5: Model for rRNA evolution proposed by (Petrov et al., 2014) using comparison of 3D structures of rRNAs from prokaryotes, archaea and eukaryotes. The large subunit ribosomal RNA is proposed to have gone through six distinct expansion steps. Step 1 (dark blue): Formation of catalytic core; Step 2 (light blue): Maturation of Peptidyl Transferase Center; Step 3 (green): Early tunnel extension; Step 4 (yellow): Acquisition of small subunit interface; Step 5 (orange): Acquisition of translocation function; Step 6 (red): Late tunnel extension.

A rather different view was proposed by Caetano-Anolles (Harish and Caetano-Anollés, 2012). This group took functionally conserved regions from both rRNAs and r-proteins. Then they made independent phylogenetic trees both for rRNAs

and for r-proteins and finally merged rRNA and r-proteins to form a structure that they call 'ancient ribonuceoprotein complex'. They further claimed the most ancient region in the ribosome is small subunit's tRNA binding sites; and instead of a single self-replicating rRNA, multiple rRNA and r-proteins from both subunits were present in the primordial mix.

To conclude, both the RNA-only hypothesis and the ribonucleoprotein hypothesis are still developing. A unified conclusion regarding components of 'protoribosome' is still ahead of us.

1.1.3.2. Evolution of ribosomal proteins

Like the studies in rRNAs, studies on evolution of r-proteins also stemmed from two approaches, one through phylogenetic analysis of numerous primary sequences of ribosomal proteins (or genes) available in the database and another through comparison of 3D structure of the entire ribosome and finding common core. This section will shed light on both approaches.

(Lecompte et al., 2002) compared ribosomal protein genes in complete genomes of 66 species and showed that 15% of the ribosomal protein genes seem to be lost in Archaea. Another study on comparison of r-proteins from three domains of life shows that there are 46 ribosomal proteins that are eukaryote specific (Ban et al., 2000). There have also been a number of expansions of the core proteins. About half of the ribosomal proteins contain a globular part and an extended loop part that penetrates to the functional core of the ribosome, but most of these expansions are located in the outskirt of the ribosome. Evolutionary conservation

and functions of those extensions have been studied in uL4 (L4) and in uL22 (L17) (Calidas et al., 2014; Gamalinda and Woolford, 2014; Guillier et al., 2005; Lawrence et al., 2016; Stelzl and Nierhaus, 2001).

Melnikov et al. studied the evolution of ribosomal proteins by comparing structures of individual ribosomal proteins in the crystal structures of ribosome from *S. cerevisiae* and bacteria (Melnikov et al., 2015). They focused on the currently known nuclear localization signals in conserved ribosomal proteins. In order to be transported to the nucleolus for ribosome biogenesis, eukaryotic ribosomal proteins had to evolve nuclear localization signals (Fig 1.6). Although all these conserved proteins carry eukaryote specific expansion segments, the nuclear localization signals developed in the existing RNA-binding segments. This may indicate that nuclear localization signal predated the expansion of eukaryotic specific segments in the ribosomal protein.

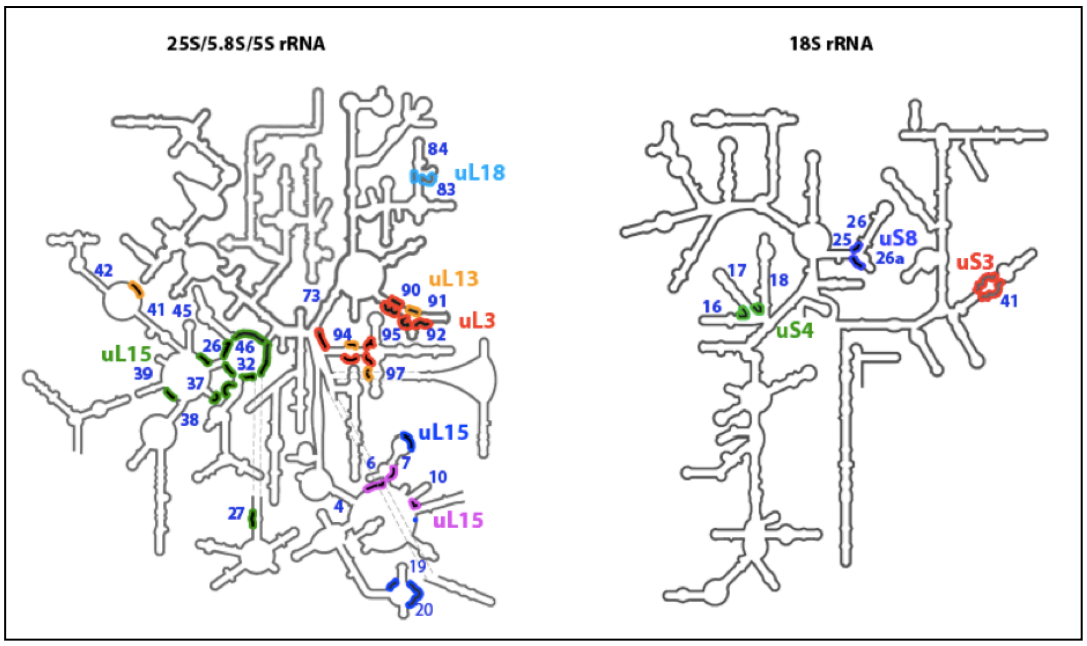


Fig 1.6: Sites in rRNA to which ribosomal protein nuclear localization signals bind (colored). The binding sites mostly comprise internal loops and helical junctions of the rRNAs (Melnikov et al., 2015).

1.1.4 Modification of ribosomal components

rRNAs and r-proteins, like any other nucleic acid or proteins, go through co/post-transcriptional and post-translational chemical modifications. Functions of these modifications have been studied through mutational analysis, deletion of the enzyme causing the mutation etc.

1.1.4.1 Modification of ribosomal RNA

rRNA is modified post- or cotranscriptionally**.** Among the possible 112 different chemical modifications on the RNA, only 12 types of modification repertoire have been identified in budding yeast (Sharma and Lafontaine, 2015); one in the sugar ring, and the rest in the purine or pyrimidine ring (Fig 1.7).

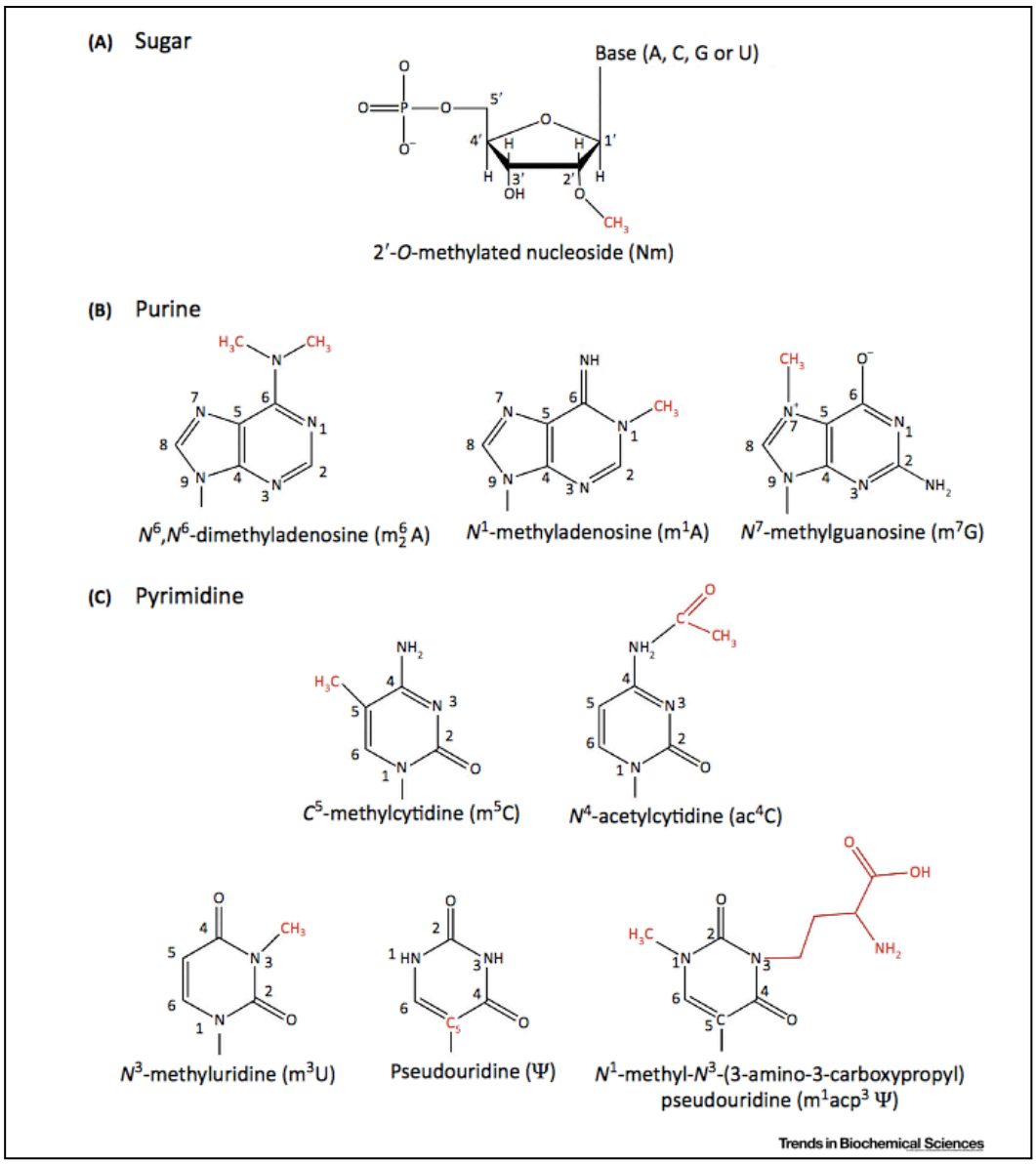


Fig 1.7: Yeast rRNA modification repertoire (Sharma and Lafontaine, 2015)

Their presence was necessary for ribosome biogenesis. Their roles were found to be important in subcellular localization of rRNA, binding of r-proteins and absence of these modifications led to growth impairment as a by-product of impaired ribosome biogenesis (Sharma and Lafontaine, 2015; Sloan et al., 2016).

1.1.4.2 Ribosomal protein modification

Over the years, thanks to advancement of proteome wide quantitative mass spectrometry, the number of entries on different types of post-translational modifications of ribosomal proteins has increased tremendously. However, a comprehensive report focusing specifically on ribosomal protein modifications is missing, possibly due to reversible nature of the modifications. In general, reports on presence of modification far outweighed research on the enzymes responsible for the modification. Cross talk between modifications, multiple enzymes acting on one side chain made the causal/correlational study on effects of the modifications harder to define. A few ribosomal protein modification gained enough attention, for example, ribosomal protein S6 phosphorylation (Biever et al., 2015), or ribosomal protein methylations (Bachand and Silver, 2004).

1.2 Ribosome Biogenesis

Eukaryotic ribosome biogenesis is an intricate process involving three cellular compartments (cytosol, nucleoplasm, nucleolus). All three RNA polymerases and >200 assembly factors are required to complete the process. In *S. cerevisiae,* the initial 35S rRNA transcript is a long chain (6.6kb) consisting of 5.8S, 25S, 18S along with external (ETS) and internal transcribed spacers (ITS). The transcribed spacers are cleaved from the 35S pre-rRNA by endonucleases and exonucleases during the maturation of the rRNAs (Fig 1.8).

The ribosome biogenesis pathway was initially elucidated by *in vitro* reconstitution of bacterial ribosomal subunits (Held et al., 1973; Nierhaus and Dohme, 1974). Since then, significant progress has been made towards understanding of prokaryotic and eukaryotic ribosome biogenesis pathway

through depletion of different ribosomal components. The depletion led to accumulation of intermediate ribosome complexes which could be characterized by quantitative mass spectrometry, single-particle cryo-electron microscopy etc. (Talkish et al., 2016). This also helped categorizing ribosomal proteins into 'early', 'late' and 'intermediate' components, depending on when they were incorporated into the maturing ribosome. However, point to note that, parallel or alternative assembly pathways exist, if one assembly pathway is blocked, the process can be 'rerouted' as needed (Davis et al., 2016). Recent effort was made towards inducing tagged ribosomal components and immunoprecipitating early complexes (current work, Lindahl lab). Tagging transcribed spacers within rRNAs and then immunoprecipitating with the tag helps specifically find the newly synthesized rRNA along with the proteins associated with it (Gupta and Culver, 2014(Chen et al., 2017). For a detailed review on rRNA maturation and ribosome assembly factors in *S. cerevisiae*, see (Woolford and Baserga, 2013).

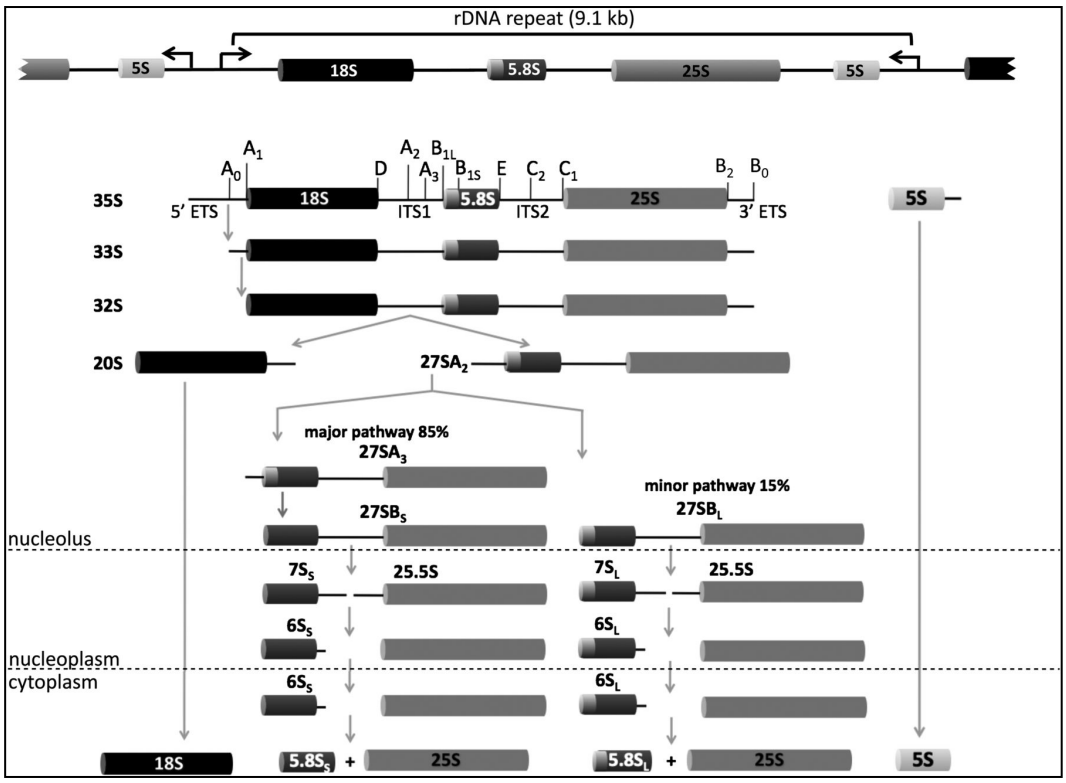


Fig 1.8: Maturation of rRNA in *S.cerevisiae* (Woolford and Baserga, 2013). The gene for ribosomal DNA is transcribed by RNA polI into a 35S rRNA, which undergoes subsequent maturation by cleavage and transportation. Final maturation happens at the cytoplasm.

1.3 Ribosome biogenesis stress/nucleolar stress and cell cycle

In eukaryotes, the nucleolus is defined as a dense fibrillar structure formed during interphase. This structure is formed as a result of unwinding of rDNA at the beginning of transcription and subsequent binding of ribosomal proteins to the newly transcribed rRNA. When an environmental or cellular stress is encountered, such as addition of Actinomycin D or depletion of a ribosomal protein, the nucleolar structure gets disrupted. The connection between ribosomal proteins and

cell cycle arrest in p53-dependent and p53-independent system is discussed below.

1.3.1 Ribosome-mediated cell cycle arrest by p53-dependent mechanism (metazoans)

Several lines of evidence have shown that the cell cycle is arrested during disruption of ribosome biogenesis (here referred to as 'ribosomal stress'). The first observation of cell cycle arrest in response to ribosomal stress was reported around late 90's. Pestov searched for mutants in mammalian cells that effectively cause irreversible cell cycle arrest (Pestov et al., 1998). They found that a conditional mutation in a novel nuclear protein BOP1, which is involved in ribosome assembly, leads to cell cycle arrest upon mutation. In 2001, they proposed a p53-dependent mechanism of cell cycle arrest in BOP1 depletion state and compared regulatory mechanisms between mammalian system and yeast system (Pestov et al., 2001). The yeast homolog of BOP1 (ERB1) does not show similar growth impediment despite similar effect on ribosome biogenesis. The group then suggested that cell cycle arrest upon ribosome biogenesis defects might be a mechanism that evolved in multicellular organisms. Studies since then showed that a fully formed p53 pathway is available in metazoans.

In the year 2003, two independent groups reported p53-dependent cell cycle arrest upon ribosomal stress (Lohrum et al., 2003; Zhang et al., 2003). They both suggested that ribosomal protein uL5 (traditional name L11) sequesters HDM2, and in the absence of HDM2, the tumor suppressor protein p53 is stabilized. Since then, multiple groups administered ribosome biogenesis disruption by blocking rRNA maturation and tested for ribosomal proteins that coprecipitate with HDM2

(He et al., 2016; Zhang et al., 2010). Finally, L5 and L11 and 5S rRNA were established/proposed as the key regulators of p53 in ribosome-mediated cell cycle arrest pathway (Donati et al., 2013; Nicolas et al., 2016; Sun et al., 2010). A systematic depletion of ribosomal protein mRNAs using siRNA for 2.5 doublings in mammalian cells revealed that depletion of most of the small subunit proteins and more than two-thirds of the large subunit proteins had no significant impact on nucleolar structure (Nicolas et al., 2016). A small set of ribosomal proteins, however, when depleted led to almost 5-fold increase in p53 level, indicating direct relationship between ribosomal proteins and p53 stability in mammalian system.

1.3.2 Ribosome-mediated cell cycle arrest by p53-independent mechanism (p53 mutant metazoans and yeast)

Studies on p53-/- mammalian cells show cell cycle arrest still happens in response to ribosome biogenesis alterations. Pagliara showed when colon cancer cells (devoid of p53) are treated with 5-Fluorouracil, extra-ribosomal protein uL3 (traditional name L3) accumulates and acts as a proapoptotic factor (Pagliara et al., 2016). In other cases, c-Myc was found as a key player, which seems to be affected by L5 or L11 depletion (Liao, J.M, 2014). These two studies provide two important observations regarding p53-free system: 1) Extra-ribosomal accumulation of ribosomal protein plays a role in cell cycle regulation; and 2) L5-L11 combination is involved in regulation even in a p53-free system. Since p53 dependent mechanisms are missing in yeast and lower eukaryotes (Belyi et al.,

2010), yeast is an appropriate model for studying p53-free/mutated system (Donati et al., 2012). Studying stress in a single cell eukaryote offers advantages in experimental designs and genetic studies, especially because many of the stress pathways/response mechanisms have been conserved in higher organisms, although the molecules involved in the expression may differ (Hohmann and Mager, 2007).

In agreement with the observation of ribosomal stress in p53 free cells, cell cycle regulation mechanism upon ribosome biogenesis impediments has also been observed in yeast. Thapa and Soifer systematically characterized cell cycle arrest in G1 or G2/M upon repression of ribosomal protein genes (Soifer and Barkai, 2014; Thapa et al., 2013). M. Shamuzzaman, A. Bommakanti, A. Zapinsky, N. Rahman, C. Pascual, and L. Lindahl (submitted) showed that membrane ingression was completed even though cells have not separated. This suggests that cells are arrested in G1 even though flow cytometry suggests arrest in G2/M for depletion of some r-proteins. They also showed that the cell cycle was arrested in G1 in ways that are very similar to the arrest during disruption of ribosome biogenesis. In a related study, M. Shamsuzzaman, B. Gregory, V. Bruno, and L. Lindahl performed a global analysis of the level of mRNA (RNA-seq) during ribosomal stress. This has led to an elaboration on mechanisms of cell cycle in different types of stresses.

1.4 Balanced production of ribosomal components

Cells maintain equimolar amounts of ribosomal components through different regulatory processes at the transcriptional and translational levels. In Eukaryotes, ribosomal RNAs are transcribed by RNA polymerase I, except for the 5S rRNA, which is transcribed by RNA polymerase III. Ribosomal protein genes are transcribed by RNA polymerase II. The requirement of the TATA binding proteins to bind to all three polymerases for proper functioning provides opportunities for coordinated expression of ribosomal components (Cormack and Struhl, 1992). It was found early in 1980s that nutritional shifts up regulate expression of ribosomal components (Kief and Warner, 1981). Later, the signal transduction pathway involved in the nutrient signaling was discovered as the Target of Rapamycin (TOR). And this pathway was found to regulate activities of all RNA polymerases, which means production of all ribosomal components would be directly affected (Mayer and Grummt, 2006). Although a detailed mechanism is not yet known, Ifh1, a transcriptional activator of r-protein genes, was proposed as a regulator, which by binding with UTP22, sequesters RNAPI activating complex (Albert et al., 2016) (Fig 1.9). Ribosomal protein S6 kinases are also found to affect transcription of ribosomal components (Chauvin et al., 2014). In mammalian system, c-Myc is found to affect transcription of all RNA polymerases (van Riggelen et al., 2010). For a detailed review, see (Lempiäinen and Shore, 2009) (Donati et al., 2012).

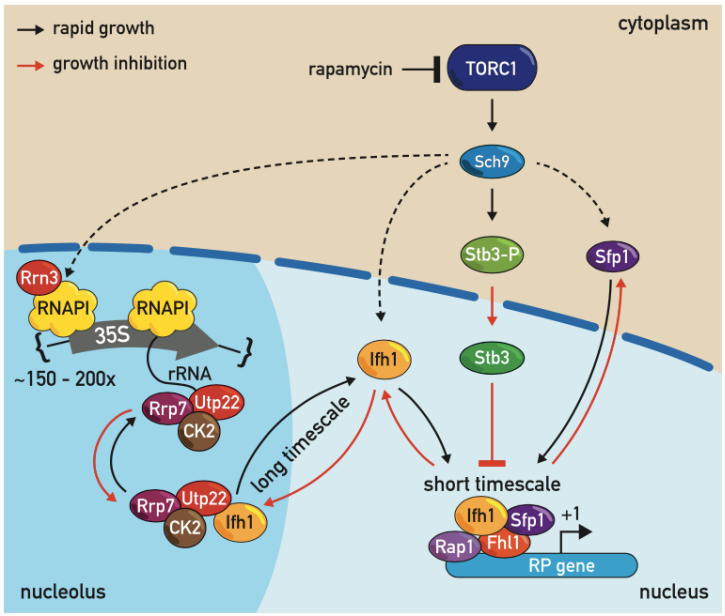


Fig 1.9: Schematic diagram of molecular mechanism of ribosomal components expression through Ifh1. In the TORC1 suppression (nutrient stress), Ifh1 is released from RP gene activation compound and binds to Utp22 complex, hindering RNA polymerase I activity (Albert et al., 2016).

The production of near-equimolar amounts of individual ribosomal proteins is coordinated by regulation of transcription, splicing and translation efficiency (Warner, 1999). However, this does not result in exact matching amount, so excess proteins are rapidly turned over. Along with coordinated expressions of ribosomal components, excess ribosomal proteins are prone to proteasomal degradation in the nucleolus. In a time course study on nucleolar proteome of mammalian cells, Lam et al. showed that ribosomal proteins accumulate quicker in the nucleolus, and inhibition of proteasome lead to increased accumulation of ribosomal proteins in the nucleolus, but not in the cytoplasm (Lam et al., 2007; Sung et al., 2016). In *S.cerevisiae,* studies on overexpressed ribosomal proteins and on rRNA depletion showed in either case, excessive ribosomal proteins

degrade quickly (Gorenstein and Warner, 1976; Tsay et al., 1988; Warner et al., 1985). All these data indicate that a balanced production of ribosomal components is maintained in the ribosome through control of expressions as well as through quick degradation of free components. Excessive r-proteins are quickly degraded with a half-life of 1-10 minutes where average half-life of *S. cerevisiae* cytosolic ribosomal proteins is ~10 hours (Christiano et al., 2014).

1.5 Disease, drug discovery and current research

The discovery of ribosomal protein-mediated p53 regulation opened up opportunities to explore anticancer therapeutics through the administration of ribosomal stress leading to cell cycle arrest or apoptosis via p53 (Derenzini et al., 2017). Cell cycle progression relies on timely progression of ribosome biogenesis. If ribosome biogenesis is slowed down, faster growing cells will be affected more, this is the underlying mechanism, based on which tumor therapies have been used. Only recently it came into light that genotoxic chemotherapeutic agents are also relying on RNA metabolism (Zhou et al., 2015). Currently several anti-tumor drugs targeting ribosome biogenesis are FDA approved and a few are in clinical trials (Table 1.2).

Table 1.2: List of chemicals/biologics targeting ribosome biogenesis currently being used/explored as anti-cancer therapeutic

Drug Ingredient	Mode of Action	Type of Cancer	Status
Methotrexate	Anti purine metabolite (disrupts nucleotide formation, rRNA biogenesis)	Acute Lymphoblastic Leukemia, Breast Cancer, Lung Cancer, Non-Hodgkin Lymphoma	FDA approved
Actinomycin D	Transcription blocker	Rhabdomyosarcoma, Ewing Sarcoma, Testicular Cancer	FDA approved
5-FluoroUracil	Competes with Uracil and inhibits RNA processing	Breast cancer, Colorectal cancer, Stomach cancer, Pancreatic cancer	FDA approved
Amicoumacin A	Traditional antibiotic, now found to bind yeast and mammalian ribosomes. Cancer cell lines more susceptible to inhibition		Experi-mental
CY-503	A ribosome-inactivating protein, shown to activate natural killer cell and stimulate release of cytokines	Metastatic Colorectal Cancer	Alternative medicine (Mistletoe extract)
BMH-21	RNA polymerase I inhibitor		Experimental
CX-5461	rRNA synthesis inhibitor		Clinical Trial (Phase 2)
Cisplatin, oxaliplatin, doxorubicin, and mitoxantrone	Intercalates between DNA strands, resulting in DNA replication and RNA transcription block	Multiple cancers	FDA approved
DRB (5, 6-dichloro-1-β-d-ribofuranosylbenzimidazole)	Chematherapeutic drus, inhibits transcription elongation	Colon Carcinoma cell	Experimental
homoharringtonine	Alkaloid, translation blocker	Chronic Myelogenous Leukemia	FDA approved

However, whether such treatments will be effective in p53 mutant cancerous cell (which constitutes >50% of all the cancer types) is still to be explored. To sum up, the mechanism of ribosomal stress induced cell cycle arrest is explored in multiple model systems will provide important insight into addressing growth and development related diseases.

Chapter 2: Differential accumulation of ribosomal protein L5(uL18) in ribosomal stresses in a p53-free system

Introduction

The mechanism of communication between ribosome biogenesis and cell cycle regulation has begun to unfold in recent years. The first report of ribosome biogenesis and cell cycle interconnection was based on a study of a mutant of nuclear protein BOP1 in humans (Pestov et al., 1998). Since then multiple studies have been dedicated to explore ribosomal protein-mediated cell cycle arrest through p53-dependent mechanism (Donati et al., 2013; Lohrum et al., 2003; Sun et al., 2010; Zhang et al., 2003). During normal cell growth, balanced production of ribosomal components is ensured through transcriptional and translational controls as well as protein degradations (Tsay et al., 1988; (Gasch; Lam et al., 2007). However, when ribosome biogenesis is disrupted, certain ribosomal proteins, L5 (uL18) and L11 (uL5), evade proteolysis through mutual protection (Bursać et al., 2012). These proteins accumulate extraribosomally and stabilize p53 through binding with MDM2, an ubiquitin ligase enzyme. When MDM2 is not bound with L5 (uL18) and L11(uL5), it ubiquitinates p53 and leads to proteolysis of p53 (Dai, M.S., 2004), which is constantly synthesized and degraded during normal growth.

But what happens in a p53-mutant system (which is >50% of cancerous cells)? What happens when their ribosome biogenesis is disrupted? Do ribosomal proteins accumulate? If so, do they signal cell cycle arrest? Pagliara showed that when p53-/-colon cancer cells are treated with 5-Fluorouracil (a transcription blocker), extra-ribosomal L3 (uL3) accumulates and acts as a proapoptotic factor (Pagliara et al., 2016). In another case, c-Myc seems to be affected by L5 (uL18) or L11 (uL5) depletion in a p53-independent manner (Liao et al., 2014). These two studies provide two important observations regarding p53-free system: 1) Extra-ribosomal accumulation of ribosomal protein plays a role in cell cycle regulation; and 2) the L5-L11 (uL18-uL5) complex is involved in regulation even in a p53-free system. Here we explore L5 (uL18) accumulation scenario in a p53-free system.

Yeast is an appropriate model for studying p53-free system, since no p53 or p53 analogue is found in yeast and lower eukaryotes (Belyi et al., 2010). Studying stress in a single cell eukaryote offers advantages to experimental designs and genetic studies. Furthermore many of the stress pathways/response mechanisms are conserved in higher organisms, although the molecules involved in the expression may differ (Hohmann and Mager, 2007), suggesting the yeast can be a useful model for p53-independent interactions between ribosome biogenesis and cell proliferation.

It has been shown that mild dosage of one stress may induce resistance to another stress; for example, heat shock induced ionizing radiation resistance in yeast (Mitchel and Morrison, 1982). Also, mild chronic stress (mutation causing delays in cell cycle progression) was found to help develop resistance to higher levels of

stress (Markiewicz-Potoczny and Lydall, 2016). Such interactions between different stresses suggest that different stress types share mechanistic pathways, a point that is further underpinned by the fact that different environmental stresses (e.g. heat shock, ethanol shock, pH extremes, oxidative and reductive stress, osmotic shock, starvation, diverse drug treatments) instigate similar stress responses. It has thus been proposed that there is a general stress-response mechanism in yeast (Hohmann and Mager, 2007).

Ribosomal component depletion led to specific cell cycle arrest. Systematic depletion of different ribosomal proteins showed mostly G1/S arrest (Bernstein et al., 2007; Soifer and Barkai, 2014; Thapa et al., 2013) and Shamsuzzaman et al. (manuscript submitted). Md Shamsuzzaman has looked further into differences between mechanisms of cell cycle arrest in stresses caused by inhibition of ribosome synthesis and inhibition of ribosome function (Shamsuzzaman et al., submitted). While the difference between ribosomal stress response and environmental stress response is an intriguing question, we focused on exploring how much of a parallel can be drawn between p53 equipped metazoan system and p53-free earlier eukaryotic systems in case of ribosomal stress induced by ribosomal component depletion. To answer this question, we have depleted multiple ribosomal proteins from different stages of ribosome biogenesis pathway and measured the level of extra-ribosomal L5 (uL18) accumulation.

Results

Extra-ribosomal L5 (uL18) accumulates during ribosomal stress, but several other ribosomal proteins do not

We first checked whether the phenomenon of extra-ribosomal accumulation is unique to ribosomal protein L5 (uL18) or if this is also observed for other ribosomal proteins. We imposed ribosomal stress by repressing the synthesis of individual ribosomal proteins. This was achieved by using strains derived from BY4741 in which the only gene for a given r-protein is expressed from the GAL1/10 promoter. These strains are referred to as Pgal-r-protein name (Pgal-S4, Pgal-L4, etc.). Cells were harvested before and at the indicated times after shifting the growth medium from galactose to glucose, which repressed transcription of the r-protein gene under GAL control. All the strains had a doubling time of 1.5hr-2.0hr in galactose (data not shown).

Whole cell extracts were analyzed on sucrose gradients (Fig 2.1). The A260 trace from cells grown in galactose looked like the trace from the parent strain (BY4741). This is compatible with the assumption that ribosome assembly is normal (or unperturbed) when these strains are grown in galactose. However, when S6 (eS6), S11(uS17), S19(eS19), S31(eS31), L5(uL18), or L40(eL40) were expressed from the GAL promoter a small infliction, or minor peak (here called "blip") was observed in between the 40S and 60S peak (marked by black arrow). Moreover, these blips sedimented at the same position between the free subunits peaks. This suggests that assembly in these strains was near-normal, but that a minor defect in assembly results in accumulation of small amounts of assembly intermediates not seen in the parent strain. This blip was most prominent in the

S19 strain, however S19 was taking significantly longer to grow both in galactose and in glucose indicating an unhealthy/not-so-optimum state of the strain. Alternatively, a slight instability of the mature subunits could be the result of assembly of abnormal subunits. The reason for either of these events may be that the GAL promoter does not provide sufficient transcript for synthesis of the indicated r-protein in amounts that are equimolar with r-proteins expressed from their native promoter.

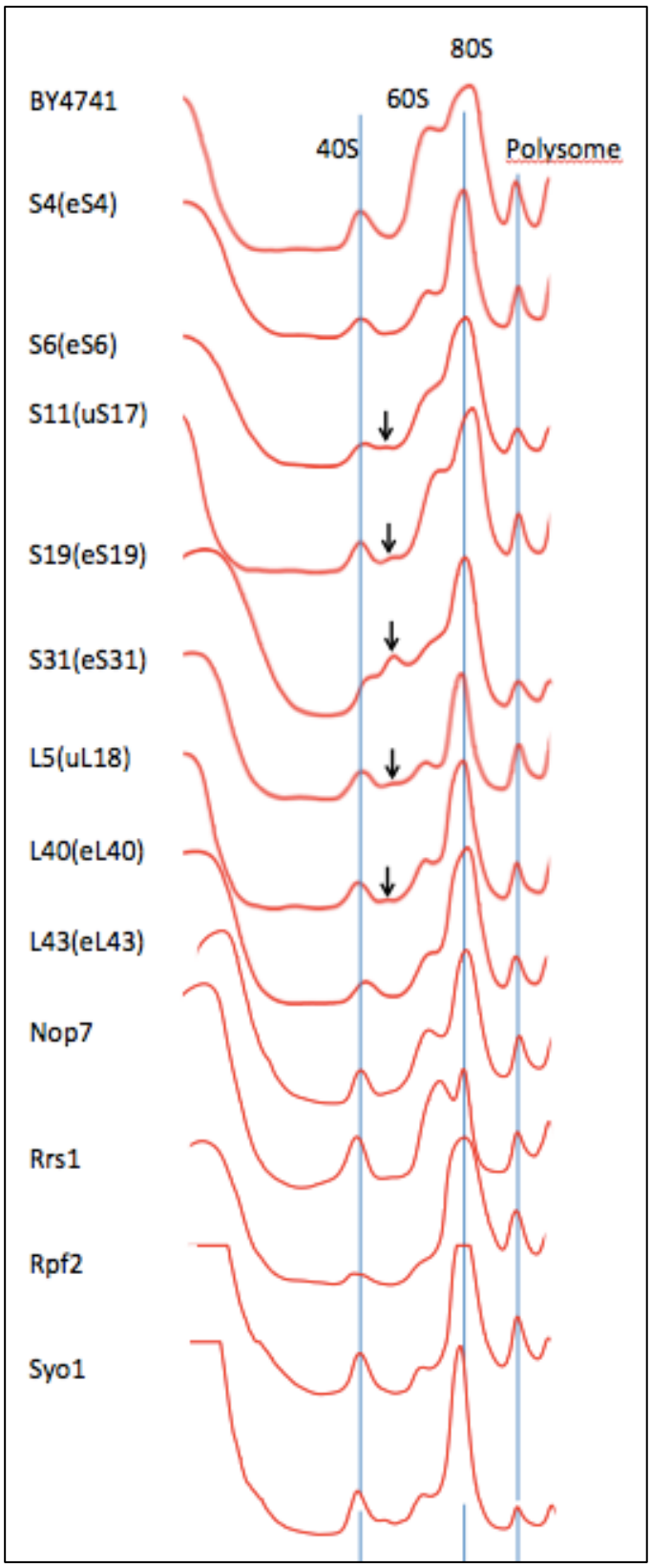


Fig 2.1: Ribosome profiles of different strains grown in galactose. Cells carrying double deletions of a single ribosomal protein gene from the genome were grown in YEP media in the presence of 20% galactose, which ensures supply of the

essential ribosomal protein from a galactose-inducible promoter from a plasmid. Cells growing in exponential phase (OD 600 0.6 – 1.0) were harvested and 20A260 crude cell lysates were applied to 10%-50% sucrose density gradient centrifugation. Each strain was biologically replicated for at least twice.

Once shifted to glucose, the cells were deprived of new synthesis of a specific ribosomal protein or ribosome assembly factor. This significantly altered the ribosome profile in ways specific to whether proteins from the small or large subunits were depleted. When the synthesis of a 40S protein was repressed the peak of free 40S virtually disappeared and a new peak between 40S and 60S appeared (Fig 2.2). When the synthesis of a 60S protein was repressed, the 60S peak diminished relative to the 40S peak. These effects were analyzed in detail by Brian Gregory (UMBC Thesis Brian Gregory). In my work I focused on determining accumulation of extra-ribosomal proteins. Such extra-ribosomal proteins could be free proteins or, probably more likely, part of an extra-ribosomal complex – since unused/free ribosomal proteins are found to degrade rapidly in the proteasome(Sung et al., 2016). In either case, they would be expected to sediment slower than ribosomal particles. Accordingly, I performed western analysis for several ribosomal proteins in fractions sedimenting at 80S or slower. Fig 2.3 shows western blots probed with antisera against L5(uL18), L4(uL4), L11(uL5) and S9(uS4). After repression of the synthesis of ribosomal S4(eS4) bands reacting with anti-L5(uL18) was detected at the top of the sucrose gradient in fractions 1,2 and 3. But no significant amount of L4, L11 or S9 was found outside of the ribosome peaks. Similar patterns were seen after repression of other r-proteins (elaborated in next section).

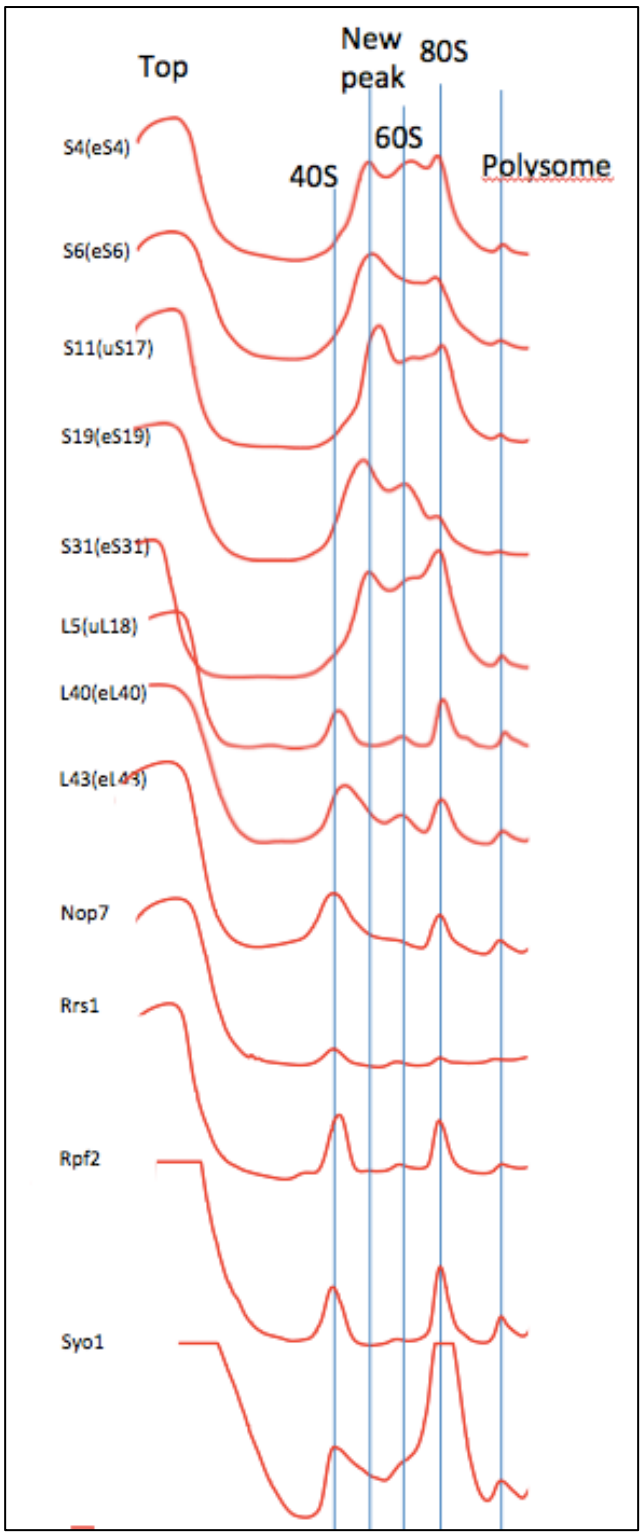


Fig 2.2: Ribosome profile of different strains grown in glucose. Cells with genomic deletion of a single ribosomal protein gene were supplied with one copy of the gene from a galactose inducible promoter in a plasmid. Cells were grown in 20% galactose containing YEP media till OD600 1.0 then diluted 1:10 to OD600

0.1 in 20% glucose containing pre-warmed YEP media. Cells were grown in YPD for a minimum of 6 hours. A total of 200 – 250 OD600 cells were harvested, lysed and ~20A260 was applied on density gradient centrifugation. All of the strains (except for SYO1), had 2 or more biological replicates.

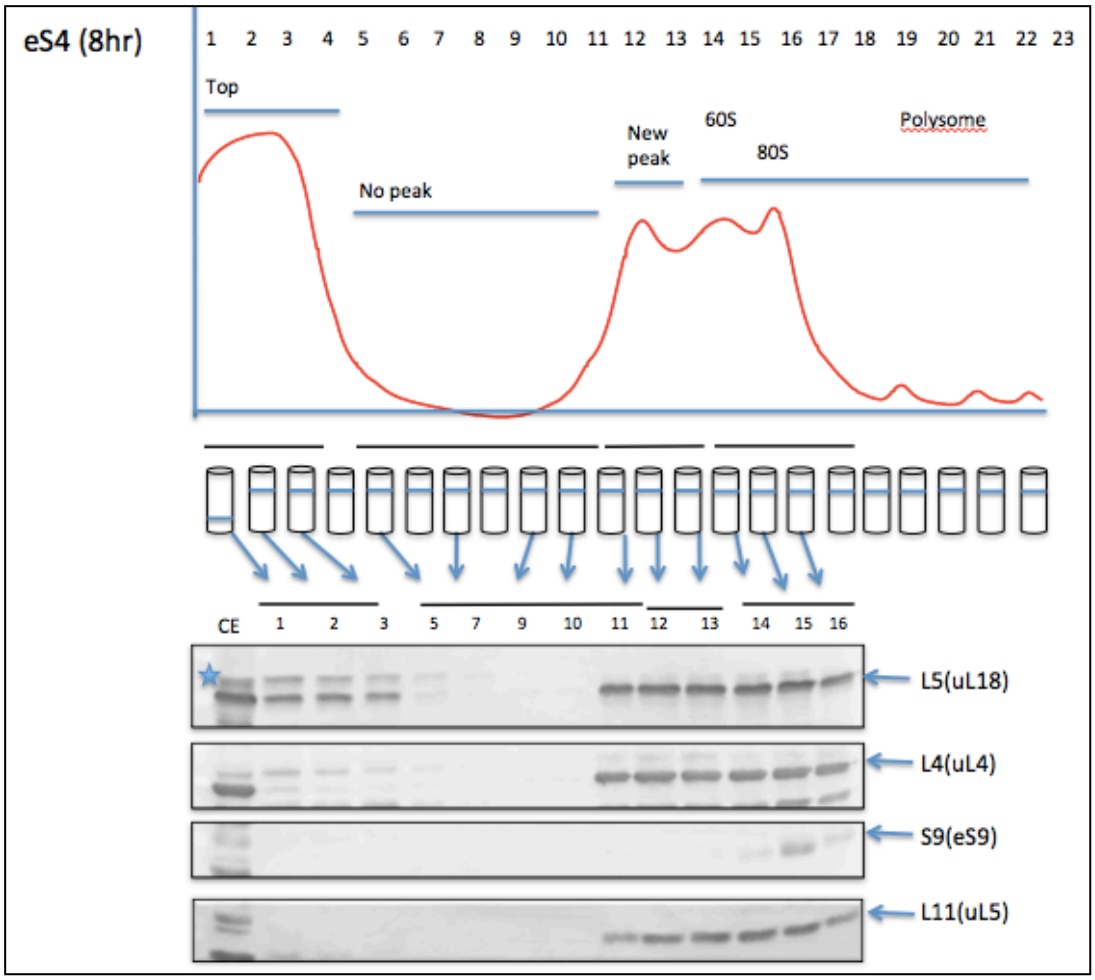


Fig 2.3: Extra-ribosomal accumulation of L5(uL18) is significantly higher compared to other ribosomal proteins (L4, S9 and L11) in case of ribosomal stress caused by a small subunit protein depletion. The blue asterisk indicates a non-specific band that possibly cross reacts with heavy chain of L5 and L4 antibodies and is not correlated with ribosomal stress (see text).

A band of non-specific protein cross-reacting with L5 antibody that runs close to the L5 band, but is not correlated with ribosomal stress condition.

Probing with anti-L5(uL18) generated a single distinct band with fractions from the 60S-80S region, but fractions from the top part of the gradient generated two bands with anti-L5 prepared for our lab by Covance, Inc. using synthetic peptides as antigens. The sequence of these peptides correspond to the 20 or 21 amino acids of the N-terminal end of the respective proteins (Fig. 2.3). One of the two bands migrate with the L5 band in the 60S-80S fractions closer to 35kDa and one runs slightly slower in gel electrophoresis, almost in the middle of 35kDa and 40kDa protein in the ladder of mw makers (marked by blue asterisk). This phenomenon was also observed in (Sun et al., 2010)(Fig 4D), (Yu et al., 2006) (Fig 5C&6) and (Dai and Lu, 2004)(Fig 1D), even though it was not addressed in these papers.

We first hypothesized that this is a modified form of free L5 accumulating at the top, as free ribosomal proteins get NEDDYLATED/Ubiquitinated for degradation (Xirodimas et al., 2008). To explore this, we transformed a L43 genomic deletion strain with a plasmid carrying a FLAG-L5 gene expressed under the native promoter of RPS28 promoter (obtained from Philipp Milkereit, Regensburg, Germany). This presumably results in oversynthesis of L5 proteins (native L5 and FLAG-L5) when the strain is grown in galactose, but it is known that r-proteins that are not incorporated into ribosomal particles are rapidly degraded (Tsay et al., 1988). Supporting this notion, we saw neither native L5 nor FLAG-L5 at the top of the gradient loaded with a lysate from cells grown in galactose. However, lysates of cells depleted of L43 for 16 hrs, contained a significant accumulation of

a protein reacting with anti-FLAG in the fractions at the top of the gradient. This band comigrated in gel electrophoresis with the FLAG-L5 band from the 60S-80S fractions, and was the only FLAG-L5 band at the top of the gradient. We conclude from this that unless the FLAG tag interferes with protein degradation, the upper band (starred in Fig. 2.3, called "star-band" below) is not a modified version of L5, but rather a protein unrelated to L5 that happens to cross cross-react with our L5 antibody. This conclusion was borne out by checking for L5 and "star-band" at the top of the gradient in two additional control experiments. The star-band appeared at the top of sucrose gradients of lysates from the parent strain BY4741 both before and 16 hours after a shift to glucose, but neither L5 nor any other ribosomal proteins we probed for were seen at the top of the gradient (Fig 2.4E). Thus the star-band is not associated with cessation of ribosomal protein synthesis. Furthermore, the star-band, but not L5 was observed at the top of the gradient after abrogating synthesis of L5 using a Pgal-L5 strain (Fig 2.4C). Thus the L5 band at the top of the sucrose gradient depends both on imposition of ribosomal stress and expression of the L5 gene. Finally, neither of the bands was seen by probing only with the secondary antibody, suggesting that the star-band is cross-reacting with our L5 antiserum. Overall, we conclude that extra-ribosomal L5, or complexes containing L5, is the result of inhibiting r-protein synthesis, but the star-band is not.

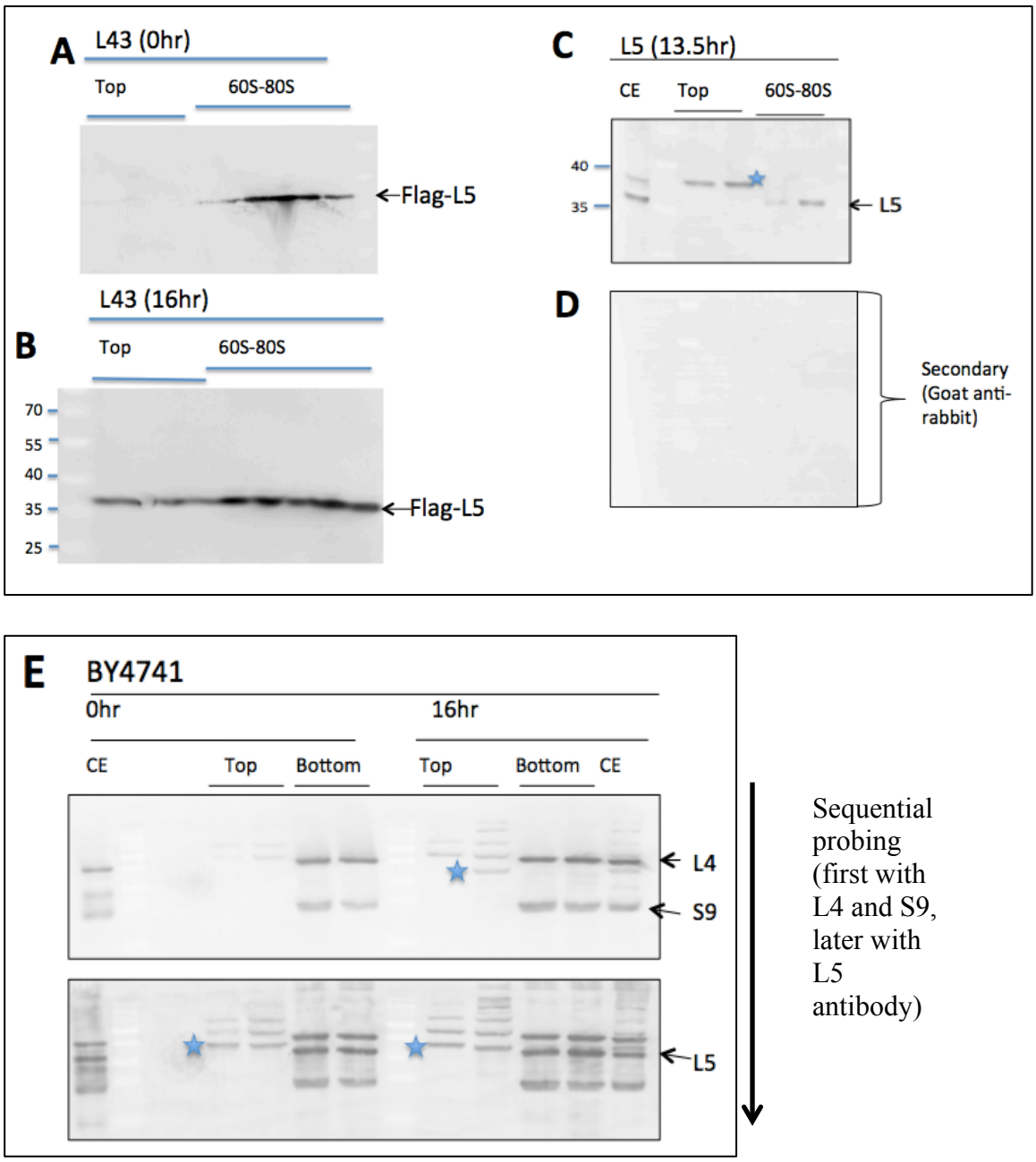


Fig 2.4: The upper band (star-band) reacting with anti-L5 is due to a cross reaction of a protein nor related to L5. Top and bottom fractions of an L43 strain with a plasmid carrying a FLAG-L5 gene expressed from the natural S28 promoter grown in galactose (A) and in glucose (B) and probed with anti FLAG antibody. Top and bottom fractions of Pgal-L5 strain grown in glucose and probed with L5 antibody (C) and secondary goat rabbit antibody (D). Top and bottom

fractions of BY4741 in galactose (0hr) and in glucose (16hrs) and probed sequentially (first with L4 and S9) and later with L5 (E). Note the emergence of star band even before probing with anti-L5 antibody

Extra-ribosomal accumulation of L5(uL18) varies significantly depending on which ribosomal protein is depleted

Once the band of extra-ribosomal L5 at the top of the gradient was identified, we determined if the amount of extra-ribosomal L5 varied with the repressed ribosomal protein gene. The extra-ribosomal L5 band at the top of the gradient was as intense as the L5 band in the 80S fractions after depleting L40 and L43 for ~16 hrs (Fig 2.5A). Surprisingly, extra-ribosomal L5 also accumulated after depleting small subunits proteins S4 and S6 (Fig 2.5A). This suggests that assembly of the small subunit interferes with the synthesis or stability of the large subunits. However, the accumulation of extra-ribosomal L5 responds differently depending on which protein is being depleted. Thus, no extra-ribosomal L5 band was found after depleting S31 (Fig 2.5A) and the extra-ribosomal L5 band was weaker after depleting S19 than depleting S4 and S6. We quantified L5 extra-ribosomal and ribosomal L5 (in 60S and 80S ribosomal particles) and found that compared to BY4741 grown in glucose, extra-ribosomal L5 accumulation was statistically significant for depletion of S4, S6, S19, L40, and L43, but not for S11 and S31 (Fig 2.5B).

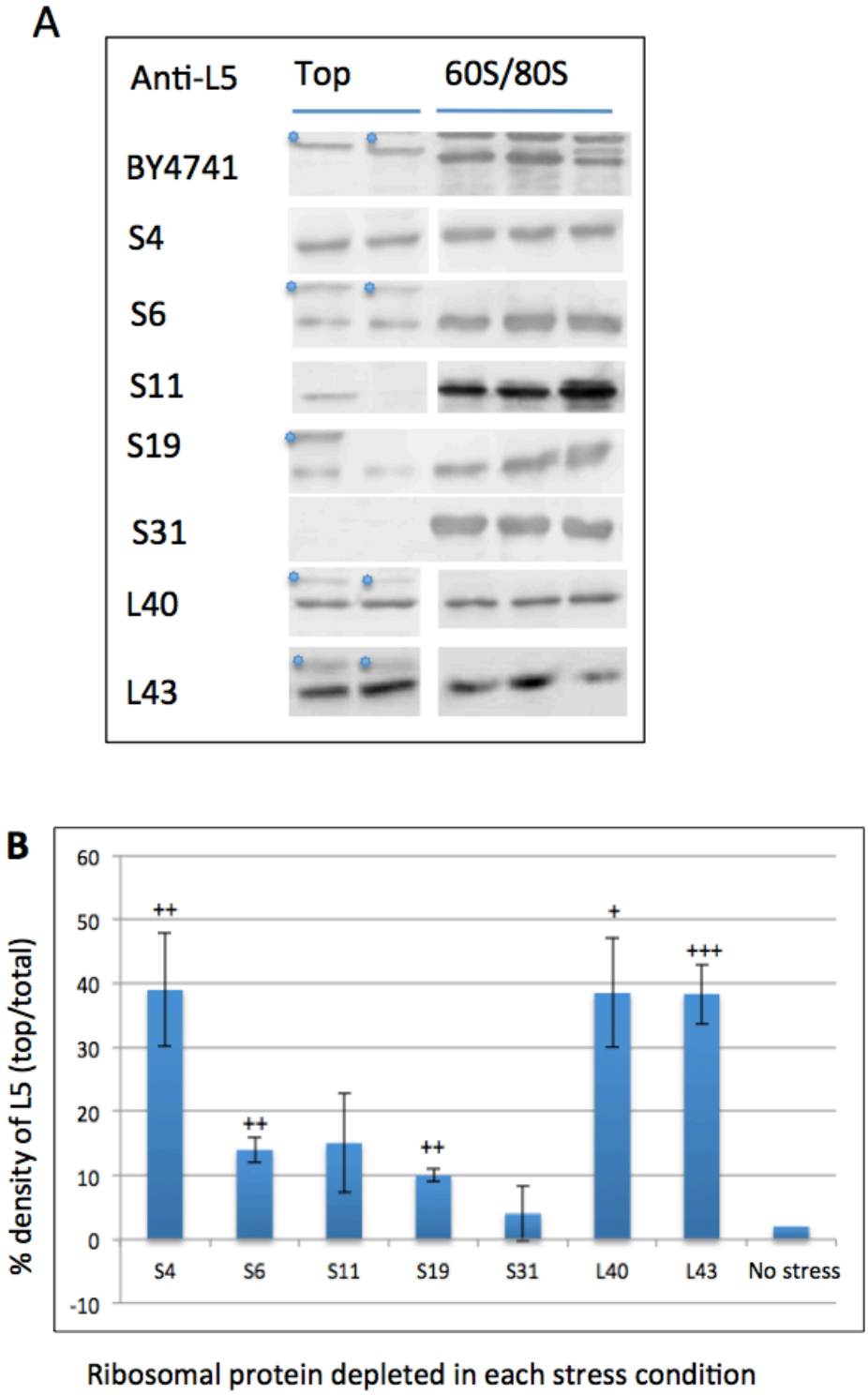


Fig 2.5 A: Extra-ribosomal accumulation of L5 in ribosomal stresses (depletion of a ribosomal protein for 4-21 hrs). B: Quantification of the extra-ribosomal L5 band. The indicated ribosomal proteins were depleted for a minimum of 6 hours around which the L5 level reaches a steady level. Fractions representing extra-

ribosomal and 60S and 80S (denoted as ‘top’ and ‘bottom’ of the gradient) were analyzed by western blot probed with L5 antibody. The pixel number in the extra-ribosomal L5 and ribosomal (60S and 80S) bands was determined using ImageJ and extra-ribosomal L5 was calculated as a fraction of the total intensity of the sum of the extraribosomal and ribosomal L5 bands. For example, while in the host strain BY4741, ~1-2% can be attributed to background noise, in case of L43 repression, the top part represents ~38% of total L5 intensity. Error bars indicate standard deviation. Statistical significance determined by t-test (+ indicates significant difference compared to wild type), +++$p<0.001$, ++$p<0.05$, +$p<0.01$. n=3 biological replicates for S4, S11, S31, L43 and n=2 biological replicates for (S6, S19, L40).

We further did pairwise T-test comparison, where we see that S4, L40 and L43 could fall in one category where all of the strains fall around 40% accumulation, while S31 can be put in a different category showing ~5% accumulation. There is another group (S6, S11, S19) whose level of L5 accumulation was distinct from both categories, but the difference could not be established with statistical significance (Table 2.1).

Table 2.1: T-test comparison among different ribosomal protein depletions

Strain 1	**Strain 2**	**P Value**
S4	L40	0.96
L43	L40	0.76
S4	S31	0.007
L43	S31	0.004
S6	S31	0.10
S6	L40	0.10

Accumulation of L5 (or lack thereof) is consistent over time

The differences in the extra-ribosomal L5 (uL18) accumulation among strains led us to question whether the accumulation pattern changes over time. We depleted ribosomal protein S31 (eS31) for different time points and checked for the presence of L5 accumulation over time. While there was a small (~10%) accumulation at 8hrs depletion; 9.5hrs or 17hrs did not show any accumulation (Fig 2.6). Compared to that, L43 had consistent accumulation (~40% of total L5 is seen at the top) between 6hrs and (Fig 2.7A) and 16 hrs. (Fig 2.4B).

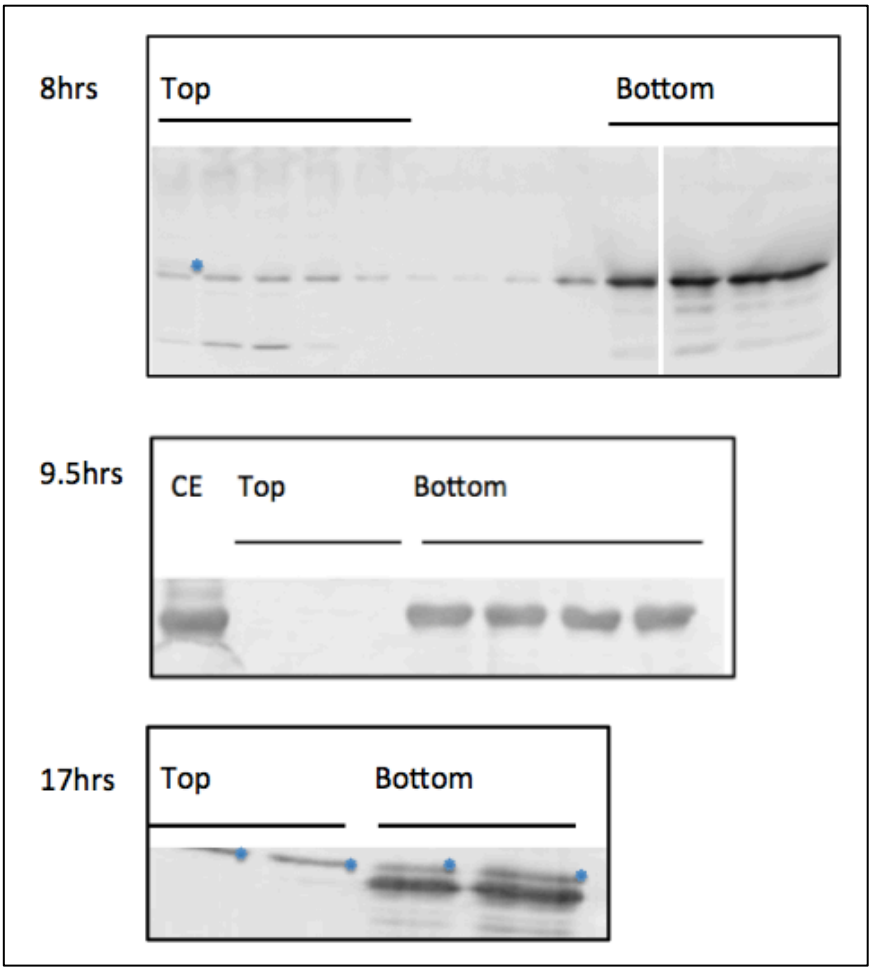
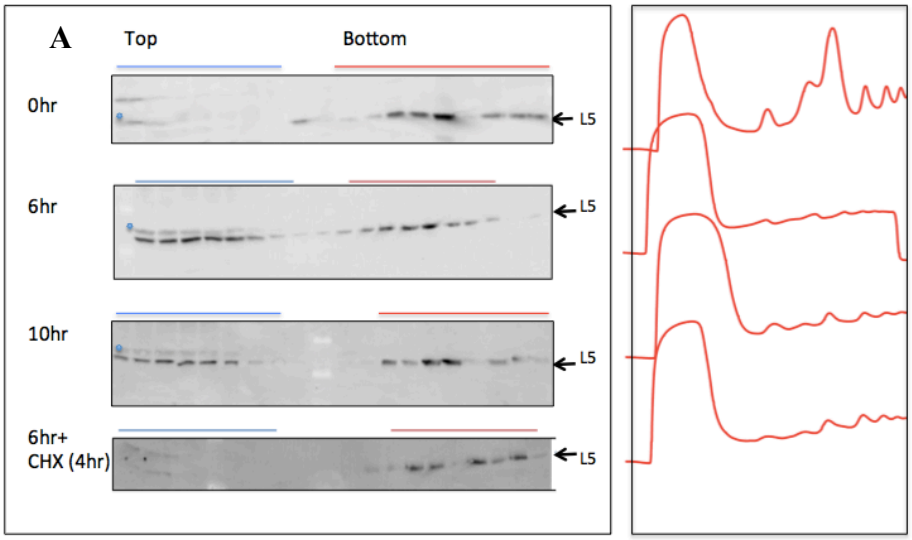


Fig 2.6: Lack of significant extra-ribosomal accumulation of L5(uL18) in S31(eS31) depletion over time. S31 ribosomal protein gene was depleted from a strain carrying double genomic deletion of the S31 gene and one copy was expressed under a galactose inducible promoter from a plasmid. Cells were grown at galactose-containing liquid media (YEP-gal) until OD 600 of 1.0 then diluted to OD 0.1 using pre-warmed glucose containing liquid media (YPD) and let grow for 8, 9.5 and 17 hours. Pictures here are biological replicates, staring from new aliquote from the freezer stock.

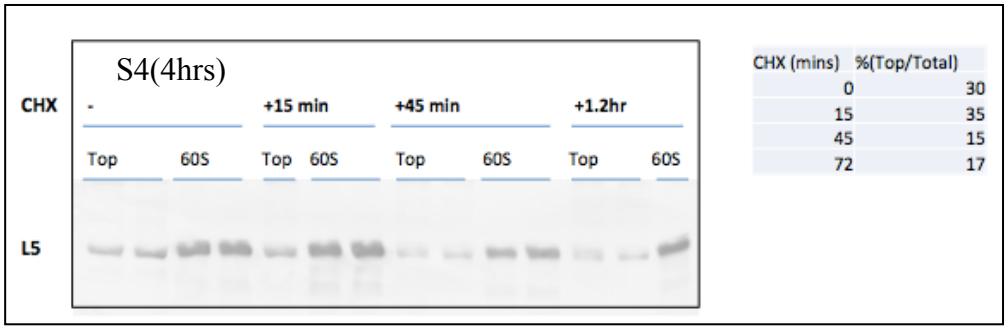
Extra-ribosomal L5 is due to new synthesis of L5 rather than from breakdown of existing ribosomes.

We next wanted to determine if the extra-ribosomal L5 stems from new synthesis of L5 protein or from breakdown of existing ribosomes. To that end, we blocked synthesis of new protein by adding cycloheximide after inducing ribosomal stress by depleting L43 for 6 hrs, at which time the accumulation of extra-ribosomal L5 at the top of the gradient was approximately 50% of the total L5 (Fig 2.7 6hr). However, 4 hours after adding cycloheximide the L5 band at the top of the gradient disappeared (Fig 2.7A 6hr+CHX(4hr)), while the L5 band at the top of the gradient persisted if incubation was continued without cycloheximide for an additional four hrs (Fig 2.7A 10hr). Similarly, extra-ribosomal L5 accumulated in response to abrogation of the small subunit protein S4 was diminished by about 50% during incubation with cycloheximide for 45 minutes (Fig 2.7B). This suggests that the extra-ribosomal L5 turns over and is depleted, if it is not resupplied by new synthesis.

We also blocked docking of L5/L11 to the large subunit by depleting Rpf2 and Rrs1, two assembly factors that help the L5/L11/5SrRNA complex to assemble to the ribosome (Zhang et al., 2007). Our Rpf2 and Rrs1 depletion data shows that despite the absence of docking material (Rpf2 or Rrs1), L5 accumulation increases in the depletion condition, indicating that activity of Rpf2 and Rrs1 (docking of 5S-L5 complex to the pre-60S) is not required for L5 to accumulate.

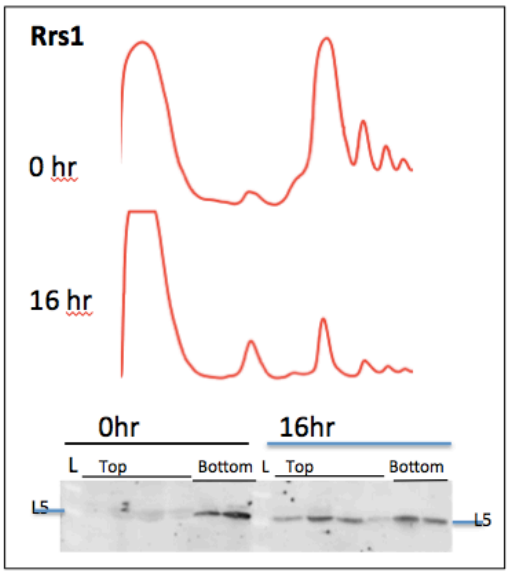


B



CHX (mins)	%(Top/Total)
0	30
15	35
45	15
72	17

C



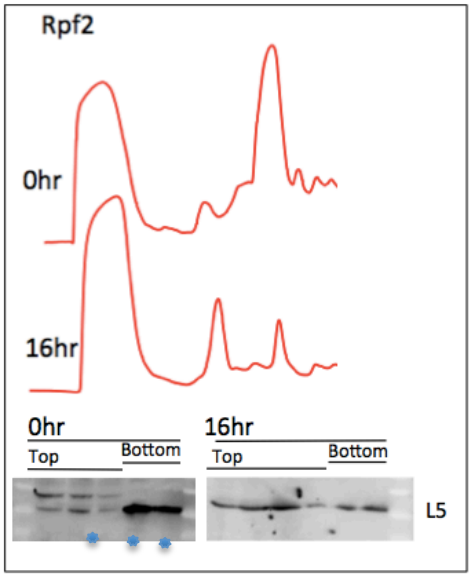


Fig 2.7 Cycloheximide eliminates accumulation of extra-ribosomal L5 during ribosomal stress (L43 depletion). A. L43 ribosomal protein gene was depleted for 6 hours, and then the cells grown with and without cycloheximide for 4 hours. The extra-ribosomal L5 band faded during the cycloheximide treatment (Biological replicate n=2), but not upon further incubation in glucose medium. B: Small subunit protein S4 was depleted for 6 hrs., after which cycloheximide was added for 15, 45 or 72 minutes. The L5 bands were quantified and extra-ribosomal L5 was expressed as a fraction of the total L5 in or outside ribosomes. C: Increased accumulation of extra-ribosomal L5 after depletion of Rpf2 and Rrs1 for 16 hrs.

L11(uL5) accumulation does not follow the same trend as L5(uL18) in yeast

Since our initial data showed slight accumulation of extra-ribosomal L11, we went ahead to check if L11 accumulation matches with the trend of L5 accumulation. Fig 2.8 shows that there is slight (~5-10%) accumulation of L5 in S4 and L43 depletion. However, somewhat higher (~25%) accumulation of L11 is seen in case of L40 depletion. No quantification was done on L11 data, as majority of the strains did not show significant accumulation.

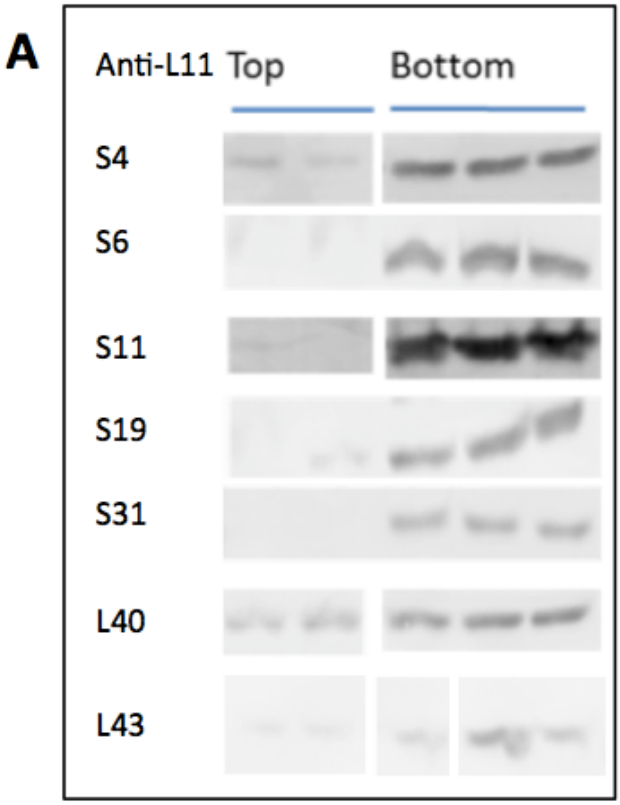


Fig 2.8 Extra-ribosomal accumulation of L11(uL5) in ribosomal stresses (depletion of a ribosomal protein for 6-21 hrs. Western blots with material from the fractions from top of the gradient and the 60S and 80S peaks was probed with anti L11(uL5) antibody.

Discussion

We depleted multiple ribosomal proteins both from small subunit and from large subunit and checked for extra-ribosomal L5 (uL18) and L11 (uL5) accumulation. The results showed 1. Extra-ribosomal accumulation of L5 and L11 but not L4 and S9. 2. A significant L5 accumulation is seen after depleting several, but not all r-proteins from both subunits. 3. Extra-ribosomal L5 also accumulated after depletion of the two ribosomal assembly factors found in the L5/L11/5S rRNA prior to docking into the pre-60S particle. 4. L5(uL18) is likely to come from new protein synthesis rather than from breakdown of existing ribosomes and 5. Despite

showing some accumulation, extra-ribosomal L11(uL5) accumulation does not follow the same pattern as L5.

Ribosomal stress has previously been investigated by inhibiting the synthesis of rRNA, ribosomal protein and assembly factor depletion. (Deshmukh et al., 1993) first reported on an extra-ribosomal pool of L5-5S ribonucleoprotein particle (L5 was at the time named as L1, (Mager et al., 1997)). However, they saw equal amount of extra-ribosomal L5-5S RNP both in non-stress and during L16 (L11 in the nomenclature of Mager et al.) depletion. Our data differs in the sense that we see significantly higher amount of extra-ribosomal L5 upon ribosomal stress. (Gómez-Herreros et al., 2013) administered ribosomal stress through nucleotide depletion and through a ribosome assembly factor depletion (Rrs1), in both cases, significant accumulation of extra-ribosomal L5(uL18) was observed only after blocking of proteasome degradation. So far, extra-ribosomal accumulation of L5 (uL18) upon ribosomal protein depletion has not been explored in detail in yeast system. Our data is the first to show that extra-ribosomal L5 accumulation also occurs when small subunit r-protein are depleted, although the accumulation of extra-ribosomal L5 varies with which 40S protein is depleted.

It is understandable that depletion of a large subunit protein will affect the maturation of large subunit, therefore extra-ribosomal L5, which is more stable compared to other extra-ribosomal proteins will accumulate. To explain the occurrence of extra-ribosomal accumulation in small subunit depletion, it is useful to structure the thinking around the stepwise hierarchical attachment of ribosomal protein during ribosome assembly (Fig 1.8). Prior to A2 cleavage in rRNA

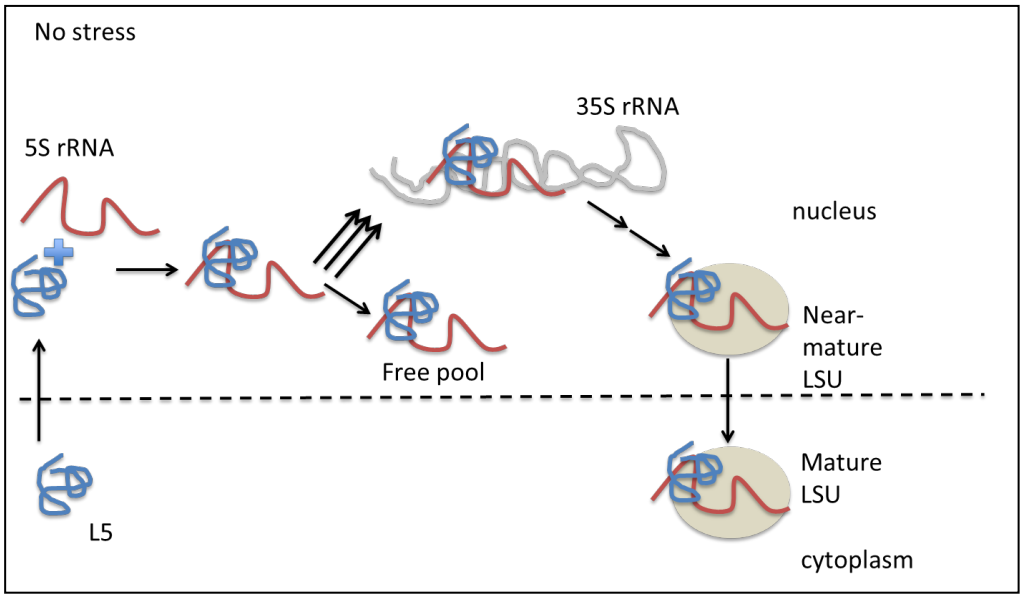
biogenesis, large subunit and small subunit were part of the same particle. Any small subunit protein that binds to the nascent rRNA (prior to A2 cleavage) is termed 'early' in the sense that although in the mature ribosome those proteins were found in small subunit particle, their binding in the rRNA came in before formation of pre-40S particle. Accordingly, a late ribosomal protein was depicted as one that binds to the pre-ribosome in the cytoplasm. Based on the ribosome assembly hierarchy known so far, we categorized the ribosomal proteins into early and late binding proteins (Table 2.2).

Table 2.2: Ribosomal proteins from small and large ribosomal subunit and their position in the assembly

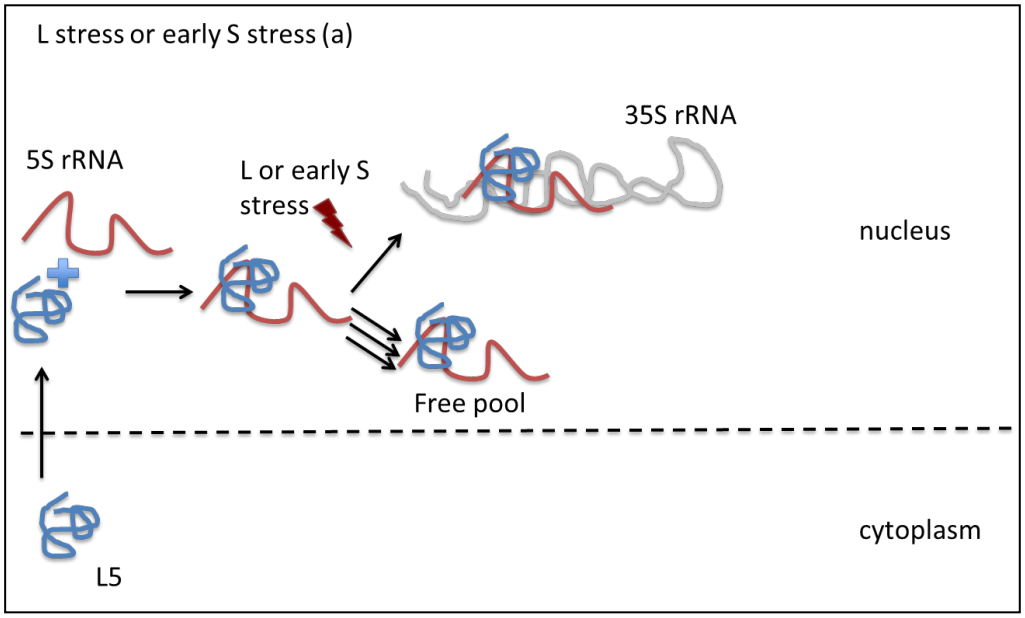
Ribosomal protein	Position in assembly	Reference
S4 (eS4)	Early	(Zhang et al., 2016)
S6 (eS6)	Early	(Zhang et al., 2016)
S11 (uS17)	Early	(Zhang et al., 2016)
S19 (eS19)	Early	(Zhang et al., 2016)
S31 (eS31)	Late	(Fernández-Pevida et al., 2016)
L40 (eL40)	Late	(Fernández-Pevida et al., 2012)
L43 (eL43)	?	

We propose any ribosomal protein that is necessary for large subunit maturation will result in L5 accumulation when depleted. All large ribosomal proteins and only early small ribosomal proteins fall under this category. However, if the

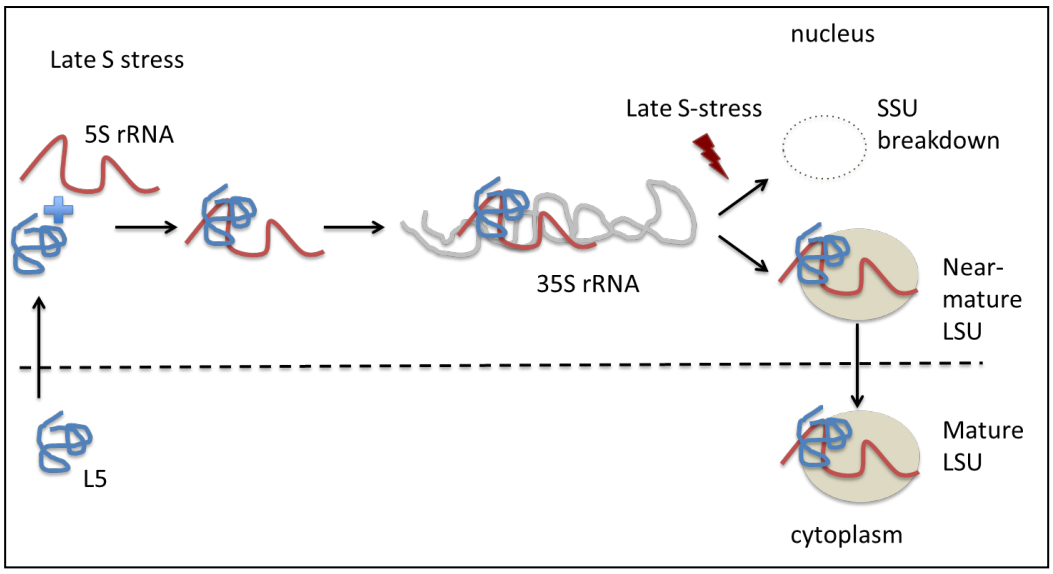
protein depletion does not hurt large subunit maturation, L5 may not accumulate and stay as part of the mature 60S (Fig 2.9C).



A



B



C

Fig 2.9: Proposed mechanism for L5 accumulation. (A) In normal cell condition, L5 is exported to cytoplasm as part of the mature ribosome and does not accumulate. (B) When other ribosomal proteins are depleted, excessive L5 remains as a stable 5S RNP complex in the nucleus. (C) In case of late binding proteins that do not affect large subunit maturation, mature large subunit is exported to ribosome and L5 does not accumulate.

Chapter 3: On the functional importance of ribosomal protein phosphorylation

Introduction

Post-translational modification of cellular proteins is an effective reversible mechanism for regulating signaling cascades, binding/release of proteins, intracellular trafficking, and protein degradation etc. Ribosomal proteins are the most abundant proteins in the cell, and they are involved in extra-ribosomal cellular functions in addition to ribosome biogenesis, function, and stability of ribosome. Thanks to the advancement of quantitative proteomic mass spectrometry, the number of entries of different types of post-translational modifications of ribosomal proteins has increased tremendously in recent years. However, a comprehensive report focusing specifically on conservation and function of such ribosomal protein modifications has not yet appeared. To begin evaluating the potential roles of ribosomal phosphorylation, on a number of post-translational modifications, we analyzed the effect of phosphorylation of yeast ribosomal proteins. To that end we correlated phosphorylation density with evolutionary origin of ribosomal proteins. For one protein (S11/uS17) we did phylogenetic analysis of two residues reported to be phosphorylated in available databases. We hypothesized that phosphorylations that are conserved among multiple species have an important functional role in ribosome biogenesis function and experimented on this idea by analyzing the phenotypes of mutations in the S11(uS17) gene in *Saccharomyces cerevisiae* (yeast). The results suggest

that phenotypes caused by mutations in single phosphosite are rare, despite their strong phylogenics.

Results:

Phosphorylation is not a random event and evolutionarily conserved ribosomal proteins are more densely phosphorylated than eukaryote-specific ones

Queries of six databases (Table 3.1) yielded a total of 233 phosphorylation sites in ribosomal proteins in *S. cerevisiase*. Seventy-two ribosomal proteins for which phosphorylation data were available at the time of query were classified into 3 groups based on their conservation on the phylogeny (Melnikov et al., 2012). Ribosomal proteins that have homologs in Bacteria, Archaea and Eukarya (abbreviated BAE) are marked with a prefix 'u' in the recent revision of ribosomal protein nomenclature (Ban et al., 2014). Proteins that have homologs in the evolution branch of Archaea and eukaryotes (AE), or specifically in the eukaryotic branch (E), have the prefix 'e'. Proteins found only in bacterial ribosomes have the prefix 'b'. No ribosomal proteins are found only in Archaea. A total of 28 BAE proteins, 32 AE proteins and 12 E proteins carrying 116, 94 and 23 phosphorylation sites in yeast (Table 3.1) were further analyzed.

Table 3.1: Databases used for compiling phosphorylation data

Database Name	Link
PHOSIDA	http://141.61.102.18/phosida/index.aspx
PhosphoSitePlus	http://www.phosphosite.org/homeAction.action
Phosphogrid	http://phosphogrid.org/
Phosphopep	www.phosphopep.org
Uniprot	www.uniprot.org
PhosphoELM	http://phospho.elm.eu.org/

Table 3.2: Phosphorylation in yeast ribosomal proteins

	# of proteins	LSU	SSU	Total	%Phosphorylation (%P)
BAE	28	62	54	116	49.8%
AE	32	35	59	94	40.3%
E	12	9	14	23	9.9%
Total	72	106	127	233	100

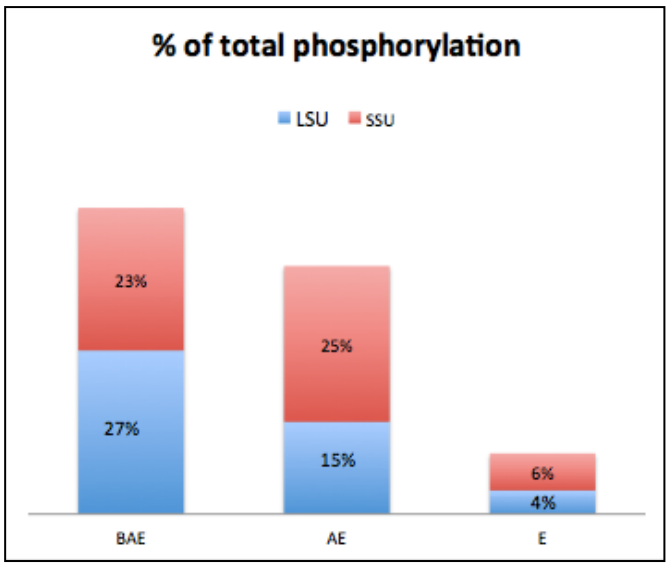


Fig 3.1: % of total phosphorylation among BAE, AE and E groups (Blue: Large subunit proteins, Red: Small subunit proteins)

Fifty per cent % of the total phosphorylated proteins were classified as BAE, 40% as AE and 10% as E (Table 3.2, Fig 3.1). Although AE had a higher number of phosphorylated proteins (32) compared to BAE (28), this number should be corrected for differences in the length of proteins, which tends to get longer through evolution. BAE had higher number of amino acids (5427) compared to AE (5156). Therefore the %of phosphorylation/amino acid was BAE (2.1%), AE (1.8%) and E (0.5%).

We further normalized to the total number of amino acids with an alcohol group, i.e. the amino acids that can form a phosphoester upon phosphorylation on the side chain. Only three out of 20 amino acids (Serine, Threonine and Tyrosine) carry hydroxyl group in their side chains, therefore it was important to normalize on the numbers of STY in each category. If phosphorylation occurrences are a matter of random events, any group that carries higher number of phosphorylatable sites, will carry higher number of phosphorylation as well. The frequency of phosphorylatable amino acids (Serine, Threonine and Tyrosine) is ~14% among all the groups (Table 3.3) and frequency of phosphorylation out of phosphorylatable site was 15% for BAE, 13.4% for AE and 3.7% for E protein groups. This means old proteins that evolved early (BAE) are more phosphorylated.

Table 3.3 Expected and actual phosphorylation outcomes among each group of ribosomal proteins

	STY (Ser, Thr, Tyr)	AA (Amino acids)	% STY/AA	% P/STY	Expected P (with 11% frequency)	Actual P events
BAE	773	5427	14.2	15	86	116
AE	701	5156	13.6	13.4	78	94
E	618	4470	13.8	3.7	69	23
Total	2092	15053			233	233

To test if the phosphorylation data were different from a random distribution we performed a chi-square test considering phosphorylation as a random event with a frequency of 11% (233 total phosphorylation out of 2092 STY) across the ribosomal proteins. The expected outcome from each group is listed in Table 3.3. The chi-square value (with a null hypothesis that the distribution of observed

phosphorylation is not different than random) with (alpha = 0.005), the difference turned out significant; chi-square (2, N= 2) = 44.41, $p<0.001$. Based on this, we deduce that phosphorylation in yeast is not a random event, and evolutionary conservation of the protein likely correlates with the frequency of the phosphorylation.

Phosphorylation event is not equally distributed between subunits

Given that the proteins from each group of organisms had a different phosphorylation frequency, we asked whether the two subunits were equally phosphorylated, i.e. #Phosphorylation/#STY in each group and subunit were the same. For that, we further categorized phosphorylation data of each group into large and small subunits and calculated frequency of phosphorylation. BAE large and small subunit showed similar distribution (~15%) while the small subunit proteins from AE and E showed significantly higher density of phosphorylation (22.7% and 15.9%) comparing to their large subunit counterparts (9.8% and 7%) (Fig 3.2). It should be noted that eukaryote-only (E) large subunit proteins had higher frequency of STY amino acids (16.8% compared to the average 14% in other categories) (Table 3.3). But the frequency of phosphorylation event did not follow the same pattern.

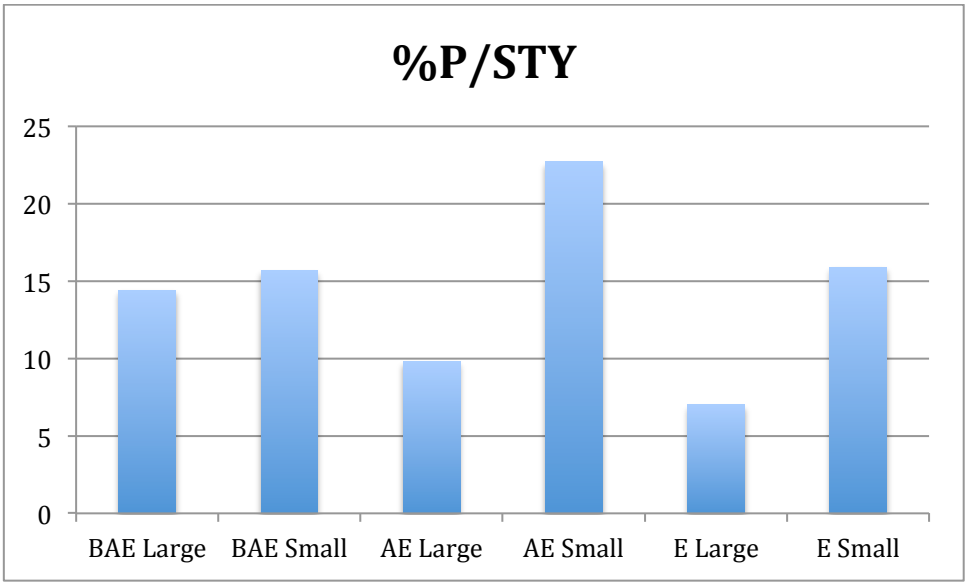


Fig 3.2: %frequency of reported phosphorylation among Serine Threonine and Tyrosine residues

To test if the phosphorylation frequency was different for large and small subunit in each group, we performed a chi-square test considering phosphorylation as an unbiased event for both subunits with a frequency of 15% for BAE and AE proteins and 10% for E proteins (Table 3.4).

Table 3.4: Frequency of phosphorylation among STY in each group

	STY (Ser, Thr, Tyr)	Actual P events	% P/STY
BAE	773	116	15
AE	618	94	15
E	216	23	10
Total	1607	233	

The expected outcome from each group is listed in Table 3.5. The chi-square value (with a null hypothesis that the distribution of observed phosphorylation is not different between subunits) with (alpha = 0.005), the difference turned out

significant; chi-square (2, N= 2) = 20.60, $p<0.005$. Based on this, we deduce that phosphorylation event is not equally distributed between large subunit and small subunit.

Table 3.5 Expected and observed number of phosphorylation in large and small subunits

	# STY	Expected #P	Observed #P
BAE Large	430	65	62
BAE Small	343	51	54
AE Large	358	54	35
AE Small	260	40	59
E Large	128	14	9
E Small	88	9	14
Total	1607	233	233

Most of the conserved phosphorylation sites were surface accessible

Once we deduced that phosphorylation events are not random events, and the frequency differs between the subunits, we narrowed down candidate sites from both subunits for further functional analysis. We chose the sites that were structurally conserved among eukaryotes and for which phosphorylation has been reported in multiple species. Therefore, we looked for sites that 1) had the same/ interchangeable phosphorylatable sites conserved among 5 eukaryotic homologs and 2) Phosphorylation has been reported in more than two homologs among 5 eukaryote model species (Fig 3.3).

```
S24 a Homo                  MN-DTVTIRTRKFMTNRLLQRKQMVIDVLHPGKATVPKTEIREKLAKMYK 49
S24_3_Mus                   MN-DTVTIRTRKFMTNRLLQRKQMVIDVLHPGKATVPKTEIREKLAKMYK 49
S24 Drosophila              MSGTTATIRTRKFMTNRLLARKQMVCDVLHPGLSSVNKTEIREKLAAMYK 50
RPS-24_Caenorhabditis       MG-DVVTIRTRKVLTNKLLYRKQMVVEVIHPGRPTVPKADIREKIAKLYK 49
S24-1_Arabidopsis           MAEKAVTIRTRKFMTNRLLSRKQFVIDVLHPGRANVSKAELKEKLARMYE 50
S24B_Saccharomyces          MS-DAVTIRTRKVISNPLLARKQFVVDVLHPNRANVSKDELREKLAEVYK 49
                            *   ..******.::: ** ***:* :*:**. ..* * :::**:* :*:
```

Fig 3.3 Criteria for choosing candidate site: The Serine marked in orange box has

1) the same/interchangeable phosphorylatable site conserved among 5 eukaryotes

2) Is reported to be phosphorylated (marked as green) in at least two organisms.

We excluded the sites that could not be located through X-ray crystallographic structure with a resolution ≤3Å (Ben-Shem et al., 2011). Eighteen sites out of the total 233 phosphorylation sites met the selection criteria (Table 3.4). Among those 18 sites, 12 were from small subunit proteins, and 6 were from large subunit proteins.

Table 3.6: Conserved phosphorylation sites and their location on the ribosome

Name	New Name	Site	Position on ribosome
S2	uS5	S238	Surface
S3	uS3	T70	Surface
S3	uS3	T104	Surface
S6	eS6	T180	Surface
S9	uS4	S145	Internal
S9	uS4	S152	Surface
S9	uS4	S162	Surface
S11	uS17	Y90	Internal
S11	uS17	S112	Internal
S13	uS15	S48	Surface
S23	uS12	S131	Surface
S24	uS8	S14	Surface
L2	uL2	S159	Surface
L9	uL6	S182	Surface
L13	uL13	T76	Surface
L17	uL22	S141	Internal
L27	eL27	Y77	Surface
L28	uL15	Y52	Internal

However, despite high density of phosphorylation among AE small subunit proteins, only one candidate (S6/eS6) was found structurally conserved, while majority of the sample were from BAE category, one from AE large category (L27/eL27). Next we mapped those sites on the yeast X-ray crystal structure (Ben-Shem et al., 2011) in order to locate the sites from a spherical view of the crystal structure. Most of the sites were solvent-accessible of the surface of the ribosome and termed 'surface accessible'. The sites that were not exposed to the solvent were termed as 'surface inaccessible'. Since the ribosome landscape is very dense, as evidenced by x-ray crystallography of cytosolic ribosome, we assumed that surface inaccessible sites in a mature ribosome would be inaccessible for kinases/phosphatase enzymes as well. The internal sites are thus more likely to be phosphorylated during ribosome biogenesis step and be static after ribosome maturation. Solvent accessible site, on the other hand, could be dynamic after ribosome maturation. Five out of 18 sites were inaccessible from the surface, and the rest were surface accessible.

Functional analysis of the mutation of the conserved phosphosites

Next we did mutational analysis on a few representative sites. Mutating the phosphosite to an amino acid that is structurally similar, but lacks OH group (e.g. Alanine for Ser/Thr or Phenylalanine for Tyr) will mimic the dephosphorylated state of the site. This is referred as 'phospho-null mutant'. On the other hand, if the site is mutated to a negatively charged amino acid, (e.g. Glutamic acid), this will mimic/resemble the permanently phosphorylated state of the site (referred as phosphomimetic mutation). We wanted to check if absence of phosphorylation

(phosphonull) or persistent phosphorylation (phosphomimetic) has any effect on cell viability along with resistance to stress.

In order to study a particular protein (x) both of the chromosomal copies of that gene (rpx a and rpx b) were deleted, and a copy of rpxa or rpxb was expressed from galactose promoter from a plasmid (pGal rpx a/b). A Flag-tagged gene encoding phosphosite mutation of that protein (Flag-rpx* a/b) was expressed from a constitutive promoter (rps28) and located on a second plasmid. Therefore, the cells will synthesize both the wild type and the mutated proteins when cells were grown in galactose medium, but in glucose medium only the mutated protein will be produced, since the native protein gene will be repressed by the presence of glucose (Fig 3.4).

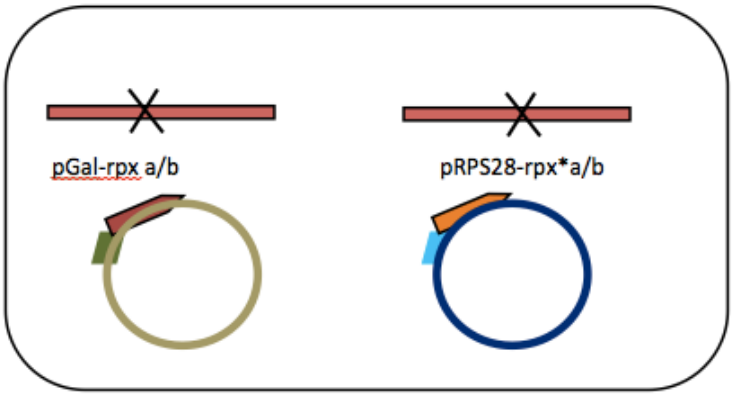


Fig 3.4: Genetic construction of the strain used for functional analysis

Representatives from surface accessible and surface inaccessible sites were selected for functional analysis. A total of 7 (4 inaccessible and 3 accessible) phosphorylation sites were mutated to phosphonull (A/Y) and phosphomimic (E) residues and checked for their ability to support cell viability through streaking

and spot dilution assay. Strains were grown in galactose and in glucose. Then the colony sizes in glucose plate were compared with the ones in galactose and categorized according to colony sizes (Table 3.7, Fig 3.5).

Table 3.7 Criteria for classification of phosphomutant colonies grown in glucose media

Notation	Description	Conclusion
+++	Colony in galactose is equal size/smaller than colony in glucose	Mutation has no visible effect on growth
++	Colony in galactose is larger than the colony in glucose	Mutation impaired growth of the strain
+	Cells in glucose are non-viable/colonies barely visible	Mutation had deleterious effect on the strain

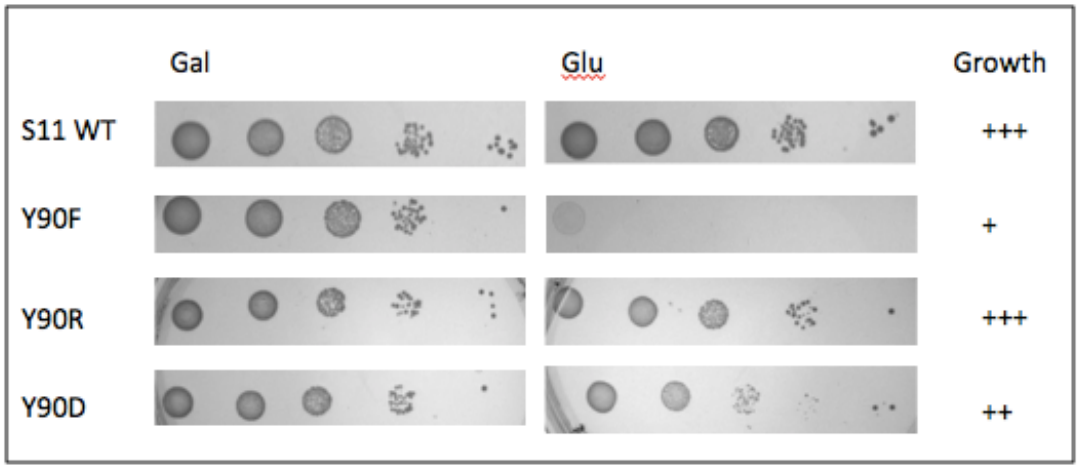


Fig 3.5 Example of classification of growth of mutants by spot dilution essay

Among 12 mutants, 2 mutants from ribosomal proteins S11(uS17), Y90F and S112E, showed minimal/no growth. Both of those sites from S11(uS17) were surface inaccessible, meaning once incorporated to the ribosome, they may very well be inaccessible for kinases/phosphatases as well. The remaining 10 mutants

grew equally well in galactose and glucose (Table 3.6). We also tested the cold and heat sensitivity of these mutants by growing the cells in room temperature (20°C) and in a 37°C incubator. In addition to the aforementioned S11(uS17) mutants, one surface-accessible mutant from S9(uS4) showed moderate growth defect and another surface-inaccessible mutant from L28(uL15) showed severe growth defects in 37°C. None of the mutants showed cold sensitivity.

Table 3.8: Growth data for phosphomutants at different temperatures

Protein	Site	Location type	20°	30°	37°
S9	S145A	Inside	+++	+++	+++
S9	S145E	Inside	+++	+++	+++
S9	S152E	Surface	+++	+++	+++
S9	S162A	Surface	+++	+++	++
S9	S162E	Surface	+++	+++	+++
S11	Y90F	Inside	+	+	+
S11	Y90E	Inside	+++	+++	+++
S11	S112A	Inside	+++	+++	+++
S11	S112E	Inside	+	+	+
L17	S141A	Inside	+++	+++	+++
L17	S141E	Inside	+++	+++	+++
L28	Y52	Inside	+++	+++	+

+++ Growth same as wild type, ++ Moderate growth, + Minimal/No growth

<u>Growth impairment from S11 mutants was due to instability of the mutant protein</u>

The two S11 mutants (S11E and Y90F) that showed extreme growth impairments in all temperatures were further analyzed. In culture plate, it typically takes ~60-65 hours (2.5 days) to reach the maximum colony size (Kamath and Bungay, 1988). To monitor their growth impediment in early

time points and compare with the growth curve of S11 depletion, we checked liquid culture growth in flask (Fig 3.6A). Both of the mutants, S112E and Y90F began to diverge from the wild type (S11WT in galactose) within 3 hours (1 doubling) of beginning of the culture and no significant difference was found between S11 depletion and the mutants, which suggests that the mutanted S11 proteins do not support growth.

Most likely this phenotype means that the protein is not incorporated into ribosomes, or it is incorporated into non-functional ribosomes. If the protein is not incorporated into ribosomes, it will most likely be degraded, because 'excess' r-proteins, i.e. protein not incorporated into a stable ribosomal particle, are targeted to proteasomes (Sung et al., 2016)e. Accordingly, we checked the presence of the mutant proteins in the cell extract (Fig 3.6B). Cleared whole cell lysates from the wild type and two mutants, S112A and Y90E, both of which grew about as well as wildtype, showed solid bands of the proper molecular weight in a western probed with anti-FLAG. However, lysates of cells carrying of Y90F or S112E, neither of which grew, failed to produce a band with anti FLAG, just like the S11 delta strain. However, once we checked multiple colonies from S112E, two S112E variants showed trace amount of Flag-tagged mutant, indicating the protein was being expressed but due to the non-functional mutation, it is quickly degraded. Hence we tracked location of S112E protein in sucrose density gradient centrifugation, with a premise that if loaded in high amount, it may be traced in the fractions. Probing with Flag antibody, the mutant S11 could be detected at the ribosomal fractions but a majority was detected at the non-ribosomal

fractions (Fig 3.6 D). We did not pursue the reason for the slight accumulation of the mutant protein in these two mutants. Potentially they could accumulate suppressor mutants (e.g. affecting the proteasome pathway, or mutant gene in a fraction of the cells may have recombined with the wildtype gene on the gal plasmid to make a gene a wildtype FLAG-tagged protein).

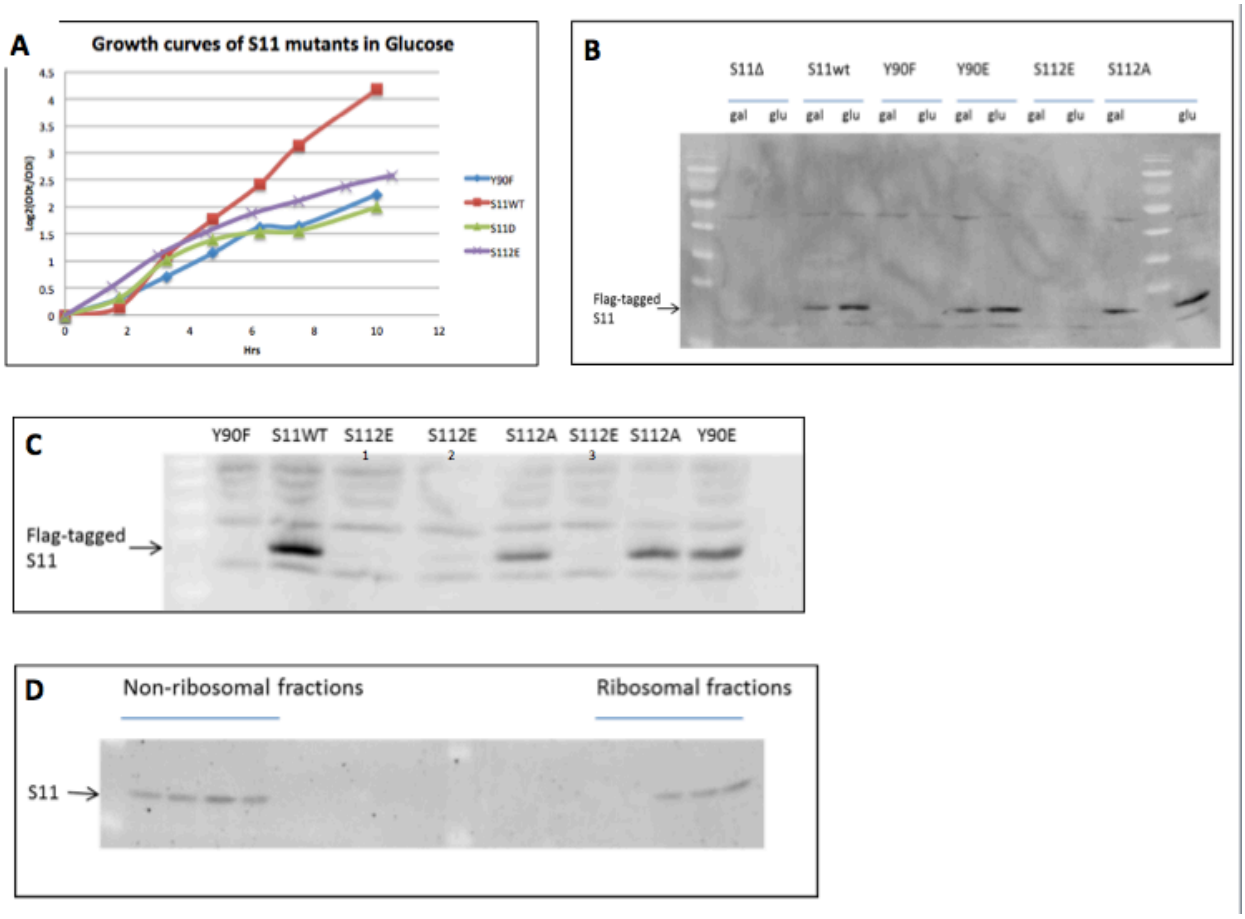


Fig 3.6: Growth impairment of mutant S112E and Y90F strains are due to instability of the protein. Growth curve of S112E, Y90F, S11 wild type (S11WT) and S11 depletion (S11Δ) (A). Cell extracts of S11 mutants grown in galactose and in glucose, probed with alpha Flag antibody (B). Cell extracts of multiple colonies of S112E mutants along with S11 wild type and other S11 mutants,

probed with alpha Flag antibody (C). : Flag-tagged mutant S112 is tracked in fractions collected from density gradient centrifugation of the lysate of the cell carrying Y90F mutant S11 protein as the only source (D).

Next we checked whether the instability of the protein was due to presence/ absence of the phosphorylation or due to a change in the structure of the protein backbone. The Y90 falls in a beta-strand while S112 falls in an unstructured link between two alpha helices (Fig 3.7).

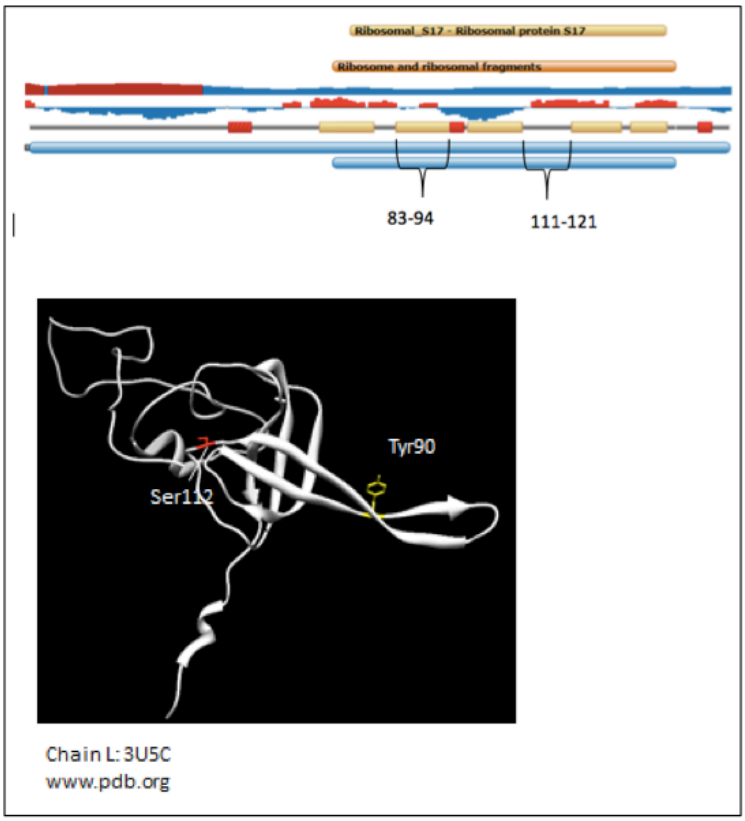


Fig 3.7: Secondary structure of the region of 90th and 112th amino acid

We mutated Y90 and S112 to non-aromatic amino acids (Arginine) and Proline, and checked for growth, Proline had a drastic effect (no growth) at 90th position, and moderate effect (moderate growth) at S112 position.

Table 3.9: Growth chart of S11 mutants

Mutant	Gal	Glu	Location in the structure
Y90F	+++	+	Beta strand
Y90E	+++	+++	
Y90D	+++	++	
Y90P	+++	+	
Y90R	+++	+++	
S112A	+++	+++	Coil
S112E	+++	+	
S112P	+++	++	

Frequency analysis of the mutant amino acids across phylogeny:

Finally we did frequency analysis of presence of these phosphomutations in the S11 across phylogeny. (Supplementary Table 2 & 3). We hypothesized that Phenylalanine in 90th position and Glutamic acid in S112th position are deleterious for the protein stability therefore they will be evolutionarily selected against and the frequency of these two amino acids in these positions will be low.

Amino acid 90 and 112 in yeast RPS11(uS17) falls within a domain that stayed conserved across phylogeny, therefore, it was possible to look at the frequency of amino acids in these two positions. We took representative groups of pfam domains and calculated frequency of desired amino acids in each of the groups (Table 3.10). Briefly, pfam domain makes representative protein groups of

different sizes for analysis purposes, RP15 being the smallest (595 entries) and Full being the largest (~20.6k entries).

Table 3.10: Databases of different numbers of pfam domain used to calculate frequency

Database Name	**# of entries**
RP15	595
RP35	1460
Full	20653

RP: Reference Proteome

Phenylalanine (F) is rare (~2%) in 90th position, while Arginine is highly frequent among other species; and our functional analysis shows that this mutant is equally viable in yeast too (Table 3.7). The same, however, is not true for Glutamic acid in 90th position. Although Y90E is equally viable in yeast, it is not frequent among homologs. There is also a high variability in Threonine (T) representation among databases. Threonine frequency rose from ~14% in RP15 and RP35 to 35% in Full (Fig 3.8) and Tyrosine went down from 22% to 5%. Therefore, Threonine and Tyrosine occupy the position 90 in yeast S11 interchangeably.

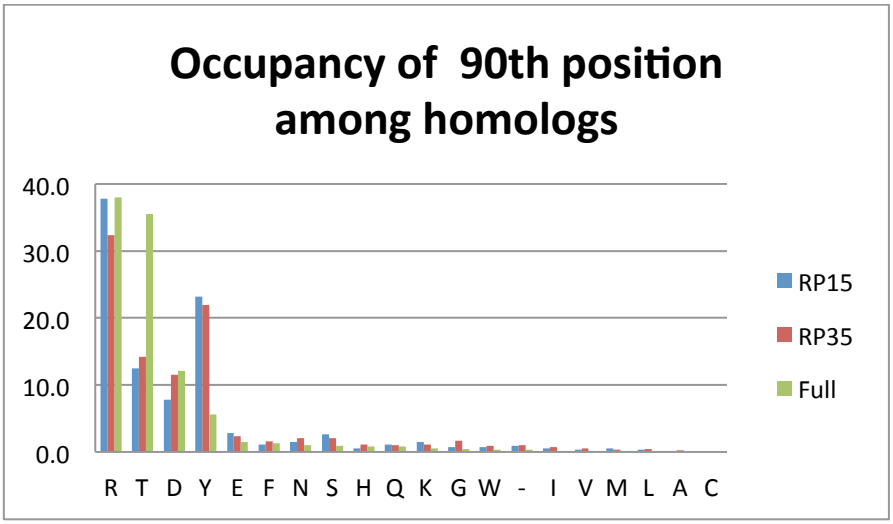


Fig 3.8: Occupancy of amino acids at the position corresponding to Y90 of S11 in yeast

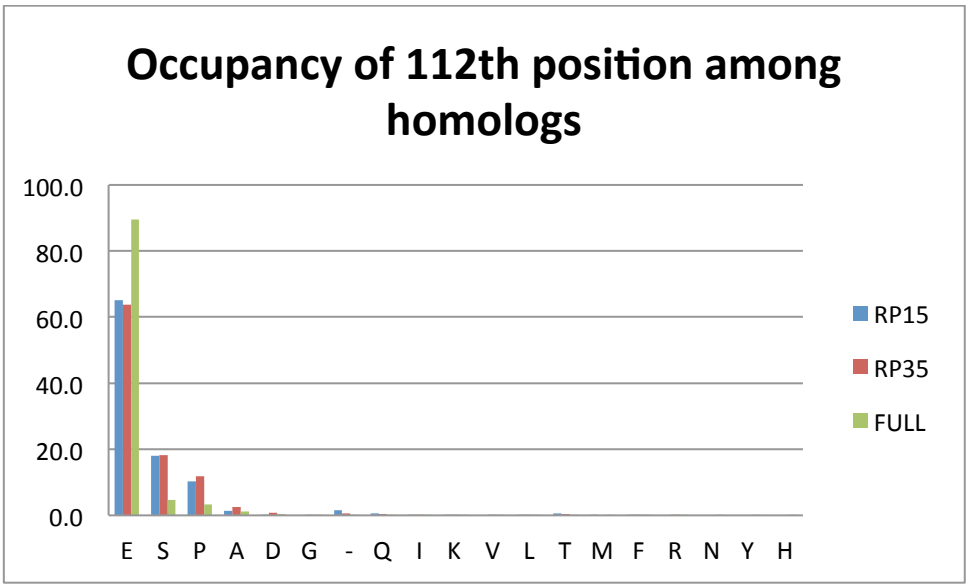


Fig 3.9: Occupancy of amino acids at the position corresponding to S112 of S11 in yeast

Checking the same for S112E, it is surprising to see high representation of E (Glutamic acid) at S112 position. S112E was deleterious for yeast (Fig 3.9).

S112E mutant failed to grow in the agar plate but did show slower growth in liquid culture. And the ribosome profile analysis shows that S112E does make mature ribosome. It is possible, however, that S112E mutant changes the ribosome fidelity/translation rate, which is useful for some species while detrimental for others. It may also be likely that a compensatory/complementary mutation arises to suppress the deleterious effect of S112E mutation. A frequency analysis on all the amino acids located in the vicinity of S112 position may reveal correlational changes.

Conclusion:

Ribosomal proteins are reported as substrates of various kinases. The distribution of phosphorylated ribosomal proteins between phylogenetic types of organisms and subunit is significantly different from a model of random distribution. This suggests that the phosphorylations have a role in ribosome formation, function, and/or stability. However, the majority of phosphonull or phosphomimetic mutations did not show a growth related phenotype. The two mutants that showed a slow or no growth phenotype were both from RPS11, but their distribution across the phylogeny was much different from each other. Given that the distribution of phosphorylation sites are not random, and that some of the phosphosite mutants do portray growth related phenotypes, further studies on phosphorylation site mutants are required to have better understanding on their role in biogenesis and translation.

Supplementary

Supplementary Table 1: Frequency of amino acid occupations in 90th position of RPS11

90	RP15	RP35	Full
R	37.8	32.4	38.0
T	12.5	14.2	35.5
D	7.8	11.5	12.1
Y	23.1	21.9	5.6
E	2.8	2.3	1.5
F	1.1	1.6	1.3
N	1.5	2.1	1.1
S	2.7	2.1	1.0
H	0.6	1.1	0.8
Q	1.1	1.0	0.8
K	1.5	1.1	0.6
G	0.8	1.6	0.4
W	0.8	1.0	0.4
-	0.9	1.0	0.3
I	0.6	0.7	0.2
V	0.4	0.5	0.2
M	0.6	0.3	0.1
L	0.4	0.5	0.1
A	0.2	0.2	0.1
C	0.0	0.0	0.0

Supplementary Table 2: Frequency of amino acid occupations in 112nd position of RPS11

S112	RP15	RP35	FULL
E	65.2	63.7	89.5
S	18.1	18.2	4.6
P	10.3	11.8	3.3
A	1.3	2.5	1.2
D	0.1	0.8	0.4
G	0	0.3	0.2
-	1.5	0.7	0.2
Q	0.6	0.5	0.1
I	0.2	0.1	0.1
K	0.2	0.2	0.1
V	0	0.1	0.1
L	0.2	0.2	0.0
T	0.6	0.3	0.0
M	0.2	0.0	0.0
F	0.2	0.2	0.0
R	0	0.1	0.0
N	0	0.0	0.0
Y	0	0.0	0.0
H	0	0.0	0.0

Chapter 4: Materials and methods

Strains, cell culture

All strains, except for BY4741, are galactose repressible strains. Gene(s) coding for a specific ribosomal protein are deleted from the chromosome and one paralog of the genes is expressed from plasmids containing galactose inducible promoter. Strains were kindly provided by Philipp Milkereit (University of Regensburg, Regensburg, Germany) and John Woolford (Carnegie Mellon University, Pittsburgh, PA; Ferreira-Cerca et al., 2005; Pöll et al., 2009; J. Woolford, personal communication).

Table 4.1 Genotype of the strains used

Strain Name (Original)	Strain Name (pGal)	Chromosomal Genotype	Plasmid	Reference
BY4741		Matα; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0		(FerreiraCerca et al., 2005)
Y800	RpS4	his3Δ1; leu2Δ0; ura3Δ0; YJR145c::KanMX4; YHR203c::KanMX4	Ycplac111-pGAL-RPS4A	(Jakob, 2010)
Y700	RpS6	his3Δ1; leu2Δ0; ura3Δ0; YBR181c::KanMX4; YPL090c::HIS3	Ycplac111-pGAL-RPS6A	(FerreiraCerca et al., 2005)
Y325	RpS11	his3Δ1; leu2Δ0; ura3Δ0; YBR048w::KanMX4; YDR025w::HIS3	Ycplac111-pGALRPS11A	(FerreiraCerca et al., 2005)
Y567	RpS19	his3Δ1; leu2Δ0; ura3Δ0; YOL121c::KanMX4; YNL302c:KanMX4	Ycplac111-pGAL-RPS19	(FerreiraCerca et al., 2005)
Y1101	RpL40	his3-1/leu2-0/ura3-0/YIL148w::HIS3MX6/YKR094c::KANMX4	Ycplac111-pGALRPL40A*	(Pöll et al., 2009)
Y1103	RpL43	his3-1/leu2-0/ura3-0/YJR094wa::HIS3MX6/YPR043w::KANMX4	Ycplac111-pGALRPL43A	(Pöll et al., 2009)

Cells were grown in YEP-galactose media up to OD600 of 1.0, then shifted to YPD media for repressing particular protein gene for different times (6-21hrs). Cells were

harvested at 8000rpm for 10 minutes and washed once with 10mL ice cold RNAse free water and stored at -20c until further use.

Cell lysis and polysome preparation

Frozen yeast cells were resuspended in gradient lysis buffer (1 mL/ 100 OD600), and lysed by vortexing for 4minutes at high speed with 2g 0.5mm glass beads (30 sec vortex, 30 sec incubation in ice, repeating 8 times). Cell debris was pelleted by 6000 rpm, for 6 minutes. The supernatant was poured into a microfuge tube and was further purified by an additional spinning of 10' at 10000 rpm. The supernatant was transferred to a fresh tube, and RNA concentration was measured by nanodrop lite (thermo).

Cell lysates (20 A260 unit of RNA) were loaded on a 10-50% gradient of 60% sucrose. Gradient was spun for 4hr at 40000 rpm. Fractions were collected in 26 aliquotes which were saved in -20 for western blotting.

Western blotting

45uL of each gradient fraction was mixed with 15uL 4X SDS sample buffer and loaded on a 12% polyacrylamide gel. For cell extract, appropriate volume containing 0.3A260 of RNA was used as the positive control. Proteins were transferred to nitrocellulose membrane (200V, 1.2hr) and later probed with different antibodies.

Quantification of western blots

Bands of interest were quantified with imageJ software. Same area around each of the bands were selected and a peak corresponding to the pixel density of that area was automatically generated with the tool Analyze -> Gel -> Plot Lanes. This gives a

plot/histogram where the height of the peak will be correlated to pixel density at that point. Then area under that curve was selected. The area will be correlated with the intensity of a band, and larger area with high peak is due to darker/stronger band to the corresponding band. This generates a two-column data consisting of lane number and area.

Chapter 5: Summary and future direction

5.1 Differential accumulation of ribosomal protein L5

Ribosome synthesis is connected to the progression of cell cycle and proliferation. Mutations in gene for ribosomal proteins or ribosome assembly factors typically result in cell cycle arrest or apoptosis. Moreover, diseases resulting from growth related disorders, typically known as ribosomopathies, were reported as a byproduct of ribosomal dysfunctions. For example, RPS19 mutation is found in approximately 25% of Diamond Blackfan Anemia (DBA) disease cases, and overall, ~70% of the DBA causes were attributed to mutations in ribosomal proteins (Gazda et al., 2004). The fact that mutations in multiple ribosomal components result in the same phenotype indicates that there may be a set of general response pathways rising from ribosomal component dysfunctions. Current information suggests that in higher eukaryotes, there are at least two ribosomal stress response mechanisms - one that involves p53 (p53-dependent), and one that does not involve p53 (p53-independent). In chapter one I provided a comprehensive summary of the works done on these two ribosomal stress response pathways. So far, most attention has been given to the p53-dependent pathway; a number of cancer therapeutics were developed targeting this pathway – a non-exhaustive list of FDA-approved drugs is provided in chapter one. But somatic mutations on P53 protein are extremely common (~38-50%) among all types of cancers, making these drugs useless for half of the cancer population (Olivier et al., 2010). Unfortunately, studies on p53-independent pathway are still

scarce; reasons may be scarcity of appropriate model system, dominance of p53 pathway in the mammalian cell lines etc.

In chapter 2, in addition to making a case for the appropriateness of yeast as a model for p53-mutated system, I explored cells' response mechanism in case of ribosomal dysfunctions. Although the signaling pathways connecting ribosome biogenesis to cell proliferation have been studied for over a decade now, the mechanisms remain poorly understood. My results show that there have been at least two mechanisms, one that results in significant L5 accumulation, and one that does not. This goes against the 'general stress response pathway in yeast' proposed by Audrey P. Gasch (Chapter 2, Hohman and Mager), as Gasch indicated that multiple stresses result in a general stress response mechanism, evidenced by upregulation and downregulation of a common set of genes. Our data indicates that even for ribosomal stress, there is variation in the response, let alone all other ribosome-related stress, e.g. translational stress, balanced and imbalanced ribosomal component depletions etc. A fellow lab member Brian Gregory assessed relative abundance of r-proteins during ribosomal stresses and concluded that L5 is notably stable compared to other ribosomal proteins in ribosomal stress condition. Similar observation was reported in (Gómez-Herreros et al., 2013). However, my study is the first to show that the stability or accumulation pattern of L5 varies depending on which component is depleted. If accumulation of L5 was merely a by-product of greater stability due to the protection it enjoys as being part of pre-assembly 5S RNP complex (L5/L11/5S rRNA), one would expect similar accumulation/stability for all of the cases. My result is the first to point out the statistically significant variation among these L5 accumulation trends. Another important observation is that L11 did not show

accumulation pattern similar to L5. Current understanding regarding the L5 stability is that it forms a stable 5S RNP complex which comprises L5, L11 and 5S RNA. Although further exploration will be required to measure 5S accumulation pattern, so far my study shows that in yeast, L11 and L5 are not equally stable. This questions our current understanding regarding 5S RNP stability in ribosomal stress condition. During my doctoral research, I took several attempts to isolate and characterize the accumulated L5 after the administration of ribosomal stress; but they were not pursued due to technical difficulties associated with chemical cross linking followed by density gradient centrifugation and mass spectrometry of the free particle.

The current knowledge regarding the mechanism of ribosome-mediated cell cycle arrest has been confined to a few causal studies where blocking of rRNA synthesis is implicitly considered as blocking of large subunit rRNA synthesis without specific attention to outcome of small subunit component depletion. So far, the dogma is that assembly of the two ribosomal subunits is largely independent of each other. If this were true, repression of small subunits proteins should not cause L5 accumulation. My thesis work has contributed to understanding that the dogma is false, i.e. assembly of the small subunit affects metabolism of the large subunit, which in turn causes accumulation of extra-ribosomal L5. With my study, the phenomenon of differential accumulation is also tied up with the stage of ribosome biogenesis of each of the subunits; we depleted early and late proteins for each subunit. Both early and late 60S proteins have an effect on L5 accumulation, but only early 40S proteins affect L5 accumulation. We have integrated this into a model suggesting 40S proteins

binding to the assembly complexes prior to separation of small and large subunit precursors (A2 cleavage and subsequent separation) lead to ribosomal protein L5 accumulation. As long as the pre-40S and pre-60S have not separated physically, the two precursor particles can affect the assembly of each other.

Up until this point, there is also no clear definition of ribosomal stress - is this merely an outcome of single component depletion resulting in an imbalance of rest of the ribosomal components? Would a balanced depletion of components be called a ribosomal stress as well? No work has looked up balanced vs. imbalanced ribosomal component depletion; although Gomez-Herreros proposed the necessity of 'balanced production of ribosomal components' in order to ensure proper cell cycle transition (Gómez-Herreros et al., 2013). My work is the first to directly compare these two types of stresses and differentiate between L5 outcomes in these two conditions.

Lastly, we establish that L5 accumulation and effects of ribosome biogenesis on the cell cycle is not specific to higher eukaryotes. This makes yeast a good model system for studying ribosome-mediated cell cycle arrest and L5 a likely candidate as a surveillance component detecting ribosomal stress in the p53-free system. How does L5 play role in cell cycle arrest? At this point not enough data is available to speculate on binding partners of free L5. As my data shows that newly synthesized L5 is likely to accumulate, one of the earliest binding partners of L5 known so far (e.g. Syo1, Kapβ2) may recruit/dock cell cycle regulatory proteins. Current literature and database search did not show any relevant binding

partner of these two proteins. In order to speculate further on the mechanism, more information is required regarding free L5 binding partners.

5.2 Phosphorylation of ribosomal proteins:

Over the past couple years, thanks to the advancement of whole cell proteomics using mass spectrometry, phosphorylation information in different treatment conditions has increased exponentially. While it is tempting to find correlation between treatment and phosphorylation pattern, meaningful information regarding the function of each of the phosphorylations is still far and behind. While embarking on the question of 'what do these phosphorylations do in ribosomal proteins' – I attempted taking a big data approach, categorize all the available data based on conservation, location etc. A fellow lab member looked into each of the 13 phosphorylation sites of ribosomal protein L4 by creating single mutation as well as serial mutations to look for functional phenotypes. I attempted to ask a bigger question instead, why are ribosomal proteins phosphorylated? My statistical analysis in chapter 3 shows that phosphorylations are actually more than a random event, and small subunit ribosomal proteins seem to have denser phosphorylation compared to large subunit ribosomal proteins. I also attempted to categorize based on evolution of the ribosomal proteins; however, the presence of extensions and expansions among older ribosomal proteins made the task complicated and out of the time scale of the thesis work. However, alignment of the phosphorylation (provided as appendix) of yeast ribosomal proteins with that of four other model organisms definitely adds more confidence in terms of picking stronger candidates for further functional analysis. With newer phosphorylation information, it may be possible to build further on the effort and

possibly maintain a website/database on specifically just the alignment of phosphorylation.

The observation that despite strong conservation, only two out of 18 phosphosites showed any visible growth defect when mutated was intriguing to side on Jonathon Warner's famous claim "phosphorylation of ribosomal protein S6 does not have any function" – although later multiple functions in ribosome biogenesis and cell signaling were attributed to those phosphorylations (Johnson and Warner, 1987). Based on our learning from coworker Jesse Fox and my work, I want to conclude that single mutation on phosphorylation sites are mostly not strong enough to disrupt cell's overall function. This makes sense from the evolutionary concept of housekeeping genes, since ribosomal components play a crucial role in ribosomal function along with cell cycle regulation – compensatory mechanisms likely to be present for any mutation.

Appendices

Sequences of phosphorylated ribosomal proteins, aligned with their homologs

from other species.

Species used:

Mus – *Mus musculus*
Homo – *Homo sapiens*
Arabidopsis – *Arabidopsis thaliana*
Caenorhabditis – *Caenorhabditis elegans*
Bacillus – *Bacillus subtilis*
Ecoli – *Escherichia coli*

Color codes

Green – phosphorylation reported in one of the 6 databases
Yellow box over one row – phosphorylated in more than two species, therefore a candidate for functional analysis
Blue arrows along with text – information collected through literature search
Red – phosphorylations reported with low confidence in mass spec result
Underlines – nuclear localization signals according to literature

S0-A YGR214W

```
SA_Mus                  -----------------LAAGTHLGGTNLDFQMEQYIYKRKSDGIYIINLKRTWEKLLLA 43
SA_Homo                 -----------------LAAGTHLGGTNLDFQMEQYIYKRKSDGIYIINLKRTWEKLLLA 43
S0A_Saccharomyces       MSLPATFDLTPEDAQLLLAANTHLGARNVQVHQEPYVFNARPDGVHVINVGKTWEKLVLA 60
Sa-1_Arabidopsis        ------------------AAEVHLGTKNCNYQMERYVFKRRNDGIYIFNLGKTWEKLQMA 42
RPS-0_Caenorhabditis    --------LTEEDVMKLLATQAHLGSTNLNFQMQQYVYKRRFDGPNIINVKKTWEKLLLA 52
                                         *: .***  * : : : *::: : **  ::*: :***** :*

SA_Mus                  ARAIVAIENPADVSVISSRNTGQRAVLKFAAATGATPIAGRFTPGTFTNQIQAAFREPRL 103
SA_Homo                 ARAIVAIENPADVSVISSRNTGQRAVLKFAAATGATPIAGRFTPGTFTNQIQAAFREPRL 103
S0A_Saccharomyces       ARIIAAIPNPEDVVAISSRTFGQRAVLKFAAHTGATPIAGRFTPGSFTNYITRSFKEPRL 120
Sa-1_Arabidopsis        ARVIVAIENPQDIIVQSARPYGQRAVLKFAQYTGANAIAGRHTPGTFTNQMQTSFSEPRL 102
RPS-0_Caenorhabditis    ARAIAAVENPADVVVVSARPYAQRALLKFAAHTGATAIFGRFSPGCLTNQIQKTFKEPRL 112
                        ** *.*: ** *: . *:*  .***:****  ***..* **.:** :** :  :* ****

SA_Mus                  LVVTDPRADHQPLTEASYVNLPTIALCNTDSPLRYVDIAIPCNNKGAHSVGLMWWMLARE 163
SA_Homo                 LVVTDPRADHQPLTEASYVNLPTIALCNTDSPLRYVDIAIPCNNKGAHSVGLMWWMLARE 163
S0A_Saccharomyces       VIVTDPRSDAQAIKEASYVNIPVIALTDLDSPSEFVDVAIPCNNRGKHSIGLIWYLLARE 180
Sa-1_Arabidopsis        LILTDPRTDHQPIKEGALGNIPIIAFCDTDSPMRFVDIGIPANNKGKHSIGCLFWLLARM 162
RPS-0_Caenorhabditis    LVISDPRIDHQAVTEASYVGVPVISFVNTESPLKLIDIGVPCNNKGERSIGLMWWMLARE 172
                        ::::*** * *.:.*.:  .:* *:: : :** . :*:.:*.**:* :*:* ::::***

SA_Mus                  VLRMRGTISRE-----HPWEVMPDLYFYRDPEEIEKE-EQAAAEKAVTKEEFQG----EW 213
SA_Homo                 VLRMRGTISRE-----HPWEVMPDLYFYRDPEEIEKE-EQAAAEKAVTKEEFQG----EW 213
S0A_Saccharomyces       VLRLRGALVDRT----QPWSIMPDLYFYRDPEEVEQQVAEEATTEEAGEEEAKE----EV 232
Sa-1_Arabidopsis        VLQMRGTIAAG-----QKWDVMVDLFFYREPEETKPEDEDEAG----------------- 200
RPS-0_Caenorhabditis    ILILRGKISRQTGFVLEGKEIMPDLYFYRDPTETEKE-ETGAHADVAEAQEYQQPTDIDF 231
                        :* :** :        .  .:* **:***:* * : :    *

SA_Mus                  TAPAPEFTAAQPEVADWSE- 232
SA_Homo                 TAPAPEFTATQPEVADWSE- 232
S0A_Saccharomyces       TEEQAEATEWAEENADNVEW 252
Sa-1_Arabidopsis        --------------------
RPS-0_Caenorhabditis    TTQGGKVDDWAAETA----- 246
```

S1-A YLR441C

```
S3a_Homo                MAVGKNKRLTKGGKKGAKKKVVDPFSKKDWYDVKAPAMFNIRNIGKTLVTRTQGTKIASD 60
S3a_Mus                 MAVGKNKRLTKGGKKGAKKKVVDPFSKKDWYDVKAPAMFNIRNIGKTLVTRTQGTKIASD 60
S3A_Drosophila          MAVGKNKGLSKGGKKGGKKKVVDPFSRKDWYDVKAPNMFQTRQIGKTLVNRTQGQRIASD 60
RPS-1_Caenorhabditis    MAVGKNN--NKMGKKGGKKKAVDPFSRKEWYDIKAPNMFNTRQVGKTLINRTQGTKIASE 58
S3a-2_Arabidopsis       MAVGKNKRISK-GRKGGKKKAVDPFSKKDWYDVKAPGSFTNRNVGKTLVSRTQGTKIASE 59
S1A_Saccharomyces       MAVGKNKRLSK-GKKGQKKRVVDPFTRKEWFDIKAPSTFENRNVGKTLVNKSTGLKSASD 59
                        ******:  .* *:** **:.****::*:*:*:***  *  *::****:.:: * : **:

S3a_Homo                GLKGRVFEVSLADLQND---EVAFRKFKLITEDVQGKNCLTNFHGMDLTRDKMCSMVKKW 117
S3a_Mus                 GLKGRVFEVSLADLQND---EVAFRKFKLITEDVQGKNCLTNFHGMDLTRDKMCSMVKKW 117
S3A_Drosophila          YLKGRVFEVSLADLQKDIDPERSFRKFRLIAEDVQDRNVLCNFHGMDLTTDKYRSMVKKW 120
RPS-1_Caenorhabditis    GLKGRVFEVSLGDLNNS---EADFRKFKLIAEDVQGKNVLTNFHAMSMTHDKLCSIVKKW 115
S3a-2_Arabidopsis       GLKHRVFEVSLADLQND--EDNAYRKIRLRAEDVQGRNVLTQFWGMDFTTDKLRSLVKKW 117
S1A_Saccharomyces       ALKGRVVEVCLADLQGS--EDHSFRKIKLRVDEVQGKNLLTNFHGMDFTTDKLRSMVRKW 117
                         ** **.**.*.**: .   :  :**::* .::**.:* * :* .*.:* **  *:*:**

S3a_Homo                QTMIEAHVDVKTTDGYLLRLFCVGFTKKRNNQIRKTSYAQHQQVRQIRKKMMEIMTREVQ 177
S3a_Mus                 QTMIEAHVDVKTTDGYLLRLFCVGFTKKRNNQIRKTSYAQHQQVRQIRKKMMEIMTREVQ 177
S3A_Drosophila          QTLIEAIVEAKTVDGYLLRVFCIGFTAKDQQSQRKTCYAQQSQVRKIRARMTDIITNEVS 180
RPS-1_Caenorhabditis    HTLIEANTAVKTTDGYTLRVFVIAFTKKSVNQVKKTSYTKTSKIRKIRSEMIGCIEKEVT 175
S3a-2_Arabidopsis       QTLIEAHVDVKTTDGYTLRMFCIAFTKRRANQVKRTCYAQSSQIRQIRRKMSEIMVKEAS 177
S1A_Saccharomyces       QTLIEANVTVKTSDDYVLRIFAIAFTRKQANQVKRHSYAQSSHIRAIRKVISEILTKEVQ 177
                        :*:*** . .** *.* **:* :.** :  :. :: .*:: .::* **  :   : .*.

S3a_Homo                TNDLKEVVNKLIPDSIGKDIEKACQSIYPLHDVFVRKVKMLKKPKFELGKLMELHGEGSS 237
S3a_Mus                 TNDLKEVVNKLIPDSIGKDIEKACQSIYPLHDVFVRKVKMLKKPKFELGKLMELHGEGGS 237
S3A_Drosophila          GADLKQLVNKLALDSIAKDIEKSCQRIYPLHDVYIRKVKVLKKPRFDVSKLLELHGDGGG 240
RPS-1_Caenorhabditis    GCDLKEVVSKLIPDSIGKDIEKTCSKLYPLQEVYIRKVKIIKRPKVDLGRLHDLHGDSIT 235
S3a-2_Arabidopsis       SCDLKELVAKFIPEAIGREIEKATQGIYPLQNVFIRKVKILKAPKFDLGKLMEVHGD--- 234
S1A_Saccharomyces       GSTLAQLTSKLIPEVINKEIENATKDIFPLQNIHVRKVKLLKQPKFDVGALMALHGEGSG 237
```

```
                                  * ::. *:  : * ::**:: . ::**::::.:****::* *:.::. *  :**:

S3a_Homo                  SGKATGDETGAKV----- 250
S3a_Mus                   SGKAAGDETGAKV----- 250
S3A_Drosophila            ------------------
RPS-1_Caenorhabditis      VG-ADGEK---------- 242
S3a-2_Arabidopsis         ------------------
S1A_Saccharomyces         EEKGKKVTGFKDEVLETV 255
```

S2 YGL123W

```
HumanS2          MADDAGAAGGPGGPGGPGMGNRGGFRGGFGSGIRGRGRGRGRGRGRGRGARG-GKAEDKE 59
MusS2            MADDAGAAGGPGGPGGPGLGGRGGFRGGFGSGLRGRGRGRGRGRGRGRGARG-GKAEDKE 59
DrosoS2          MADEAPARSG--------------FRGGFGS--RG-GRG-GRGRGRGRWARGRGKEDSKE 42
CelegansS2       MADRGGFQSGFGGRGGGRGGARPAGDRPAGRGGRG-GRG-GRGGRGGRAGRG--GEKETE 56
ArabiS2          MAERGGEGGAERG------GDRGDFGRGFGGG-RGGGRGRDRGPR-GRGRRGGRASEETK 52
YeastS2          MSAPEAQQQK---------------RGGFGGR--------NRGRPNRRGPRN---TEEKG 34
Bacillus         ------------------------------------------------------------
EcoliS5          ------------------------------------------------------------


HumanS2          WMPVTKLGRLVKDMKIKSLEEIYLFSLPIKESEIIDFFLGASLKDEVLKIMPVQKQTRAG 119
MusS2            WIPVTKLGRLVKDMKIKSLEEIYLFSLPIKESEIIDFFLGASLKDEVLKIMPVQKQTRAG 119
DrosoS2          WVPVTKLGRLVREGKIKSLEEIYLYSLPIKEFEIIDFFLGSSLKDEVLKIMPVQKQTRAG 102
CelegansS2       WTPVTKLGRLVKEKKITTLEEIYLNSLPIKEFEIIDALC-SNLKDEVLKISPVQKQTTAG 115
ArabiS2          WVPVTKLGRLVADNKITKLEQIYLHSLPVKEYQIIDHLVGPTLKDEVMKIMPVQKQTRAG 112
YeastS2          WVPVTKLGRLVKAGKITTIEEIFLHSLPVKEFQIIDTLL-PGLQDEVMNIKPVQKQTRAG 93
Bacillus         ------MRRIDPSK--------------------------LELEERLVTVNRVAKVVKGG 28
EcoliS5          ------MAHIEKQA--------------------------GELQEKLIAVNRVSKTVKGG 28
                       : ::                                *::.:: :  * * . .*

HumanS2          QRTRFKAFVAIGDYNGHVGLGVKCSKEVATAIRGAIILAKLSIVPVRRGYWGNKIGKPHT 179
MusS2            QRTRFKAFVAIGDYNGHVGLGVKCSKEVATAIRGAIILAKLSIVPVRRGYWGNKIGKPHT 179
DrosoS2          QRTRFKAFVAIGDNNGHIGLGVKCSKEVATAIRGAIILAKLSVVPVRRGYWGNKIGKPHT 162
CelegansS2       QRTRFKAFVAIGDHAGHVGLGVKCSKEVATAIRGAIVAAKLAVVPVRRGYWGNKIGLPHT 175
ArabiS2          QRTRFKAFVVVGDGNGHVGLGVKCSKEVATAIRGAIILAKLSVVPVRRGYWGNKIGKPHT 172
YeastS2          QRTRFKAVVVVGDSNGHVGLGIKTAKEVAGAIRAGIIIAKLSVIPIRRGYWGTNLGQPHS 153
Bacillus         RRFRFAALVVVGDKNGHVGFGTGKAQEVPEAIRKAVEDAKKNLIEVPMVGT--------T 80
EcoliS5          RIFSFTALTVVGDGNGRVGFGYGKAREVPAAIQKAMEKARRNMINVALNNG--------T 80
                 :   * *...:**  *::*:*   ::**. **: .:  *:  :: :             :

HumanS2          VPCKVTGRCGSVLVRLIPAPRGTGIVSAPVPKKLLMMAGIDDCYTSARGCTATLGNFAKA 239
MusS2            VPCKVTGRCGSVLVRLIPAPRGTGIVSAPVPKKLLMMAGIDDCYTSARGCTATLGNFAKA 239
DrosoS2          VPCKVTGKCGSVSVRLIPAPRGTGIVSAPVPKKLLTMAGIEDCYTSARGSTGTLGNFAKA 222
CelegansS2       VPCKVTGKCASVMVRLIPAPRGTGIVSAPVPKKLLHMAGIEDCYTAAKGSTATLGNFAKA 235
ArabiS2          VPCKVTGKCGSVTVRMVPAPRGSGIVAARVPKKVLQFAGIDDVFTSSRGSTKTLGNFVKA 232
YeastS2          LATKTTGKCGSVTVRLIPAPRGSGIVASPAVKKLLQLAGVEDVYTQSNGKTRTLENTLKA 213
Bacillus         IPHEIIGRFGAGNILLKPASEGTGVIAGGPVRAVLELAGVADILSKSLGSNTPIN-MIRA 139
EcoliS5          LQHPVKGVHTGSRVFMQPASEGTGIIAGGAMRAVLEVAGVHNVLAKAYGSTNPIN-VVRA 139
                 :     *   .  : : **..*:*:::.   : :* .**: :  : : * . .:    :*

HumanS2          TFDAISKTYSYLTPDLWKETVFTKSPYQEFTDHLVKTHTRVSVQRTQAPAVATT 293
MusS2            TFDAISKTYSYLTPDLWKETVFTKSPYQEFTDHLVKTHTRVSVQRTQAPAVATT 293
DrosoS2          TYAAIAKTYAYLTPDLWKEMPLGSTPYQAYSDFLSKPTPRLHADA--------- 267
CelegansS2       TYAALQRTYSYLTPDLWKEEALEKSPYQRHHEYLARN----------------- 272
ArabiS2          TFDCLQKTYGFLTPEFWKETRFSRSPYQEHTDFLSTKAVSATKVITEGEDQA-- 284
YeastS2          AFVAIGNTYGFLTPNLWAEQPLPVSPLDIYSDEASAQKKRF------------- 254
Bacillus         TLQGLSELK--------RAEDVAKLRGKSVEELLG------------------- 166
EcoliS5          TIDGLENMN--------SPEMVAAKRGKSVEEILGK------------------ 167
                 :   : .              .     .   :
```

S3

```
HumanS3            ------------------MAVQISKKRKFVADGIFKAELNEFLTRELAEDGYSGVEVRVT 42
MusS3              ------------------MAVQISKKRKFVADGIFKAELNEFLTRELAEDGYSGVEVRVT 42
DrosophilaS3       -----------------MNANLPISKKRKFVSDGIFKAELNEFLTRELAEDGYSGVEVRVT 44
CelegansS3         ----------------MAANQNVTKKKKAVIGGIFKAELNNFLMKELAEDGYSGVEVRST 44
ArabidopsisS3      ------------------MTTQISKKRKFVADGVFYAELNEVLTRELAEDGYSGVEVRVT 42
YeastS3            ------------------MVALISKKRKLVADGVFYAELNEFFTRELAEEGYSGVEVRVT 42
BacillusS3         VGQKVNPVGLRIGVIRDWESKWYAG-KDYADFLHEDLKIREYISKRLSDASVSKVEIERA 59
EcoliS3            MGQKVHPNGIRLGIVKPWNSTWFANTKEFADNLDSDFKVRQYLTKELAKASVSRIVIERP 60
                                          :  :. .       ::.: : :.*:. . * : :. .

HumanS3            PTRTEIIILATRTQNVLGEKGRRIREITAVVQKRFGFPEGSVELYAEKVATRGLCAIAQA 102
MusS3              PTRTEIIILATRTQNVLGEKGRRIREITAVVQKRFGFPEGSVELYAEKVATRGLCAIAQA 102
DrosophilaS3       PSRTEIIIMATKTQQVLGEKGRRIREITAMVQKRFNFETGRIELYAEKVAARGLCAIAQA 104
CelegansS3         PARAEVIIMATRTQNVLGERGRRIKEITSVVQKRFGFEEGSVELYAEKVSNRGLCAVAQC 104
ArabidopsisS3      PMRTEIIIRATRTQNVLGEKGRRIREITSLVQKRFKFPVDSVELYAEKVNNRGLCAIAQA 102
YeastS3            PTKTEVIIRATRTQDVLGENGRRINEITLLVQKRFKYAPGTIVLYAERVQDRGLSAVAQA 102
BacillusS3         ANRVNITIHTAKPGMVIGKGGSEVEALRKALNSLTGKR---VHINILEIKRADLDAQLVA 116
EcoliS3            AKSIRVTIHTARPGIVIGKKGEDVEKLRKVVADIAGVP---AQINIAEVRKPELDAKLVA 117
                   .   .: * :::.  *:*: *  :. *   : .          :   .:    * *   .

HumanS3            ESLRYKLLGGLAVRRACYGVLRFIMESGAKGCEVVVSGKLRGQRAKSMKFVDGLMIHSGD 162
MusS3              ESLRYKLLGGLAVRRACYGVLRFIMESGAKGCEVVVSGKLRGQRAKSMKFVDGLMIHSGD 162
DrosophilaS3       ESLRYKLTGGLAVRRACYGVLRYIMESGAKGCEVVVSGKLRGQRAKSMKFVDGLMIHSGD 164
CelegansS3         ESLRYKLVGGLAVRRACYGVLRFIMESGAQGVEVIVSGKLRGQRAKAMKFVDGLMIHSGH 164
ArabidopsisS3      ESLRYKLLGGLAVRRACYGVLRFVMESGAKGCEVIVSGKLRAARAKSMKFKDGYMVSSGQ 162
YeastS3            ESMKFKLLNGLAIRRAAYGVVRYVMESGAKGCEVVVSGKLRAARAKAMKFADGFLIHSGQ 162
BacillusS3         DNIARQLENRVSFRRAQKQQIQRTMRAGAQGVKTMVSGRLGGADIARPEYYSEGTVP-LH 175
EcoliS3            DSITSQLERRVMFRRAMKRAVQNAMRLGAKGIKVEVSGRLGGAEIARTEWYREGRVP-LH 176
                   :.:  :*   : .***    ::  *. **:* :. ***:* .      ::     :   .

HumanS3            PVNYYVDTAVRHVLLRQGVLGIKVKIMLPWDPTGKIGPKKPLPDHVSIVEPKDE-ILPTT 221
MusS3              PVNYYVDTAVRHVLLRQGVLGIKVKIMLPWDPSGKIGPKKPLPDHVSIVEPKDE-ILPTT 221
DrosophilaS3       PCNDYVETATRHVLLRQGVLGIKVKVMLPYDPKNKIGPKKPLPDNVSVVEPKEE-KIYET 223
CelegansS3         PVNDYIQQAVRHVQLRQGVIGIKVKIMLPYDPRGQNGPRNALPDHVQIVEPQEE-VLPKE 223
ArabidopsisS3      PTKEYIDSAVRHVLLRQGVLGIKVKVMLDWDPKGISGPKTPLPDVVIIHSPKEEEAIYAP 222
YeastS3            PVNDFIDTATRHVLMRQGVLGIKVKIMRDP-AKSRTGPK-ALPDAVTIIEPKEEEPILAP 220
BacillusS3         TLRADIDYATSEADTTYGKLGVKVWIYRGEVLP--------------TKKKNEEGGK--- 218
EcoliS3            TLRADIDYNTSEAHTTYGVIGVKVWIFKGEILGGMAAVEQPEKPAAQPKKQQRKGRK--- 233
                   . .  ::  . ..    * :*:** :                        . : :

HumanS3            PISEQKGGKP-EPPAMPQPVPTA---- 243
MusS3              PISEQKGGKP-EPPAMPQPVPTA---- 243
DrosophilaS3       PETEYKIPPP-SKPLDDLSEAKVL--- 246
CelegansS3         PHSQHKEEKKDVQVPAAQPVAPVQ--- 247
ArabidopsisS3      AQVAAPAALVADAPLTAVDYPAMIPVA 249
YeastS3            SVKDYRPAEETEAQAEPVEA------- 240
BacillusS3         ---------------------------
EcoliS3            ---------------------------
```

S4-A YJR145C

```
S4B_Saccharomyces        MARGPKKHLKRLAAPHHWLLDKLSGCYAPRPSAGPHKLRESLPLIVFLRNRLKYALNGRE 60
S4A_Drosophila           MARGPKKHLKRLAAPKAWMLDKLGGVFAPRPSTGPHKLRESLPLLIFLRNRLKYALNGAE 60
S4_Homo                  MARGPKKHLKRVAAPKHWMLDKLTGVFAPRPSTGPHKLRECLPLIIFLRNRLKYALTGDE 60
S4-1_Arabidopsis         MARGLKKHLKRLNAPKHWMLDKLGGAFAPKPSSGPHKSRECLPLVLIIRNRLKYALTYRE 60
RPS-4_Caenorhabditis     -MRGPKKHLKRLAAPSHWMLDKLGGVFAVRPNPGPHKLRESLPLSLFLRNRLKYALNYTE 59
                           ** ******: **  *:**** * :* :*..**** **.*** :::********.  *
```

```
S4B_Saccharomyces         VKAILMQRHVKVDGKVRTDTTYPAGFMDVITLDATNENFRLVYDVKGRFAVHRITDEEAS 120
S4A_Drosophila            VTKIVMQRLVKVDGKVRTDPTYPAGYMDVITLEKTGEFFRLVYDVKGRFVIHRISAEEAK 120
S4_Homo                   VKKICMQRFIKIDGKVRTDITYPAGFMDVISIDKTGENFRLIYDTKGRFAVHRITPEEAK 120
S4-1_Arabidopsis          VISILMQRHIQVDGKVRTDKTYPAGFMDVVSIPKTNENFRLLYDTKGRFRLHSIKDEEAK 120
RPS-4_Caenorhabditis      AKKILTQRVVRVDGKVRTCHKFPTGFMDVVAIERTNEYFRMLYDTKGRYVVHRIQAAEAD 119
                          .  *  ** :::******  .:*:*:***:::  *.* **::**.***: :* *   **.

S4B_Saccharomyces         YKLGKVKKVQLGKKGVPYVVTHDGRTIRYPDPNIKVNDTVKIDLASGKITDFIKFDAGKL 180
S4A_Drosophila            YKLCKVKKTQLGAKGVPFLVTHDGRTIRYPDPLIHANDSVQVDIASGKITDYIKFDSGNL 180
S4_Homo                   YKLCKVRKIFVGTKGIPHLVTHDARTIRYPDPLIKVNDTIQIDLETGKITDFIKFDTGNL 180
S4-1_Arabidopsis          FKLCKVRSIQFGQKGIPYLNTYDGRTIRYPDPLIKPNDTIKLDLEENKIVEFIKFDVGNV 180
RPS-4_Caenorhabditis      FKLCKVKSVRTVNKGVPVLTTTDGRTIRYPDPHVKVNDTIVFNISTQKITDSVKFEPGNL 179
                          :** **:.     **:* : * *.******** :: **:: .::   **.: :**: *::

S4B_Saccharomyces         VYVTGGRNLGRIGTIVHKERHDGGFDLVHIKDSLDNTFVTRLNNVFVIGEQGKPYISLPK 240
S4A_Drosophila            CMITGGRNLGRVGTVVNRERHPGSFDIVHIKDSQGHVFATRLTNVFIIGKGNKPYISLPK 240
S4_Homo                   CMVTGGANLGRIGVITNRERHPGSFDVVHVKDANGNSFATRLSNIFVIGKGNKPWISLPR 240
S4-1_Arabidopsis          VMVTGGRNRGRVGVIKNREKHKGSFETIHIQDSTGHEFATRLGNVYTIGKGTKPWVSLPK 240
RPS-4_Caenorhabditis      AYVTGGRNVGRVGIIGHRERLPGASDIIHIKDSAGHSFATRISNVFVIGKGNKALVSLPT 239
                            :*** * **:* : ::*:  *. : :*::*: .: *.**: *:: **:  *. :***

S4B_Saccharomyces         GKGIKLSIAEERDRRRAQQGL 261
S4A_Drosophila            GKGVKLSIAEERDKR------ 255
S4_Homo                   GKGIRLTIAEERDKR------ 255
S4-1_Arabidopsis          GKGIKLTIIEEARKRLSAQQ- 260
RPS-4_Caenorhabditis      GAGIRLSIAEERDKRMAQ--- 257
                          * *::*:* **  :*
```

S5 YJR123W

```
HumanS5          -------------------------MTEWETAAPAVAETPDIKLFGKWSTDDVQINDISLQ
MusS5            -------------------------MTEWEAATPAVAETPDIKLFGKWSTDDVQINDISLQ
DrosoS5          MAEVAENVVETFEEPAAPMEAEVAETILETNVVSTTELPEIKLFGRWSCDDVTVNDISLQ
CelegansS5       ------------------MADNWGSENVVADAAPATEAPEVALFGKWSLQSVNVSDISLV
ArabiS5          ----------------------MAASAEIDAEIQQQLTNEVKLFNRWSFDDVSVTDISLV
YeastS5          -MSDTEAPVEVQEDFEVVEEFTPVVLATPIPEEVQQAQTEIKLFNKWSFEEVEVKDASLV
BacillusS5       ----------------------------------------------------------
EcoliS5          ----------------------------------------------------------
                                       PKA
                                 NEK6
HumanS5          DYIAVK-EKYAKYLPHSAGRYAAKRFRKAQCPIVERLTNSMMMHGRNNGKKLMTVRIVKH
MusS5            DYIAVK-EKYAKYLPHSAGRYAAKRFRKAQCPIVERLTNSMMMHGRNNGKKLMTVRIVKH
DrosoS5          DYISVK-EKFARYLPHSAGRYAAKRFRKAQCPIVERLTCSLMMKGRNNGKKLMACRIVKH
CelegansS5       DYIPVK-EKSAKYLPHSAGRFQVRRFRKAACPIVERLANSLMMHGRNNGKKLMTVRIVKH
ArabiS5          DYIGVQPSKHATFVPHTAGRYSVKRFRKAQCPIVERLTNSLMMHGRNNGKKLMAVRIVKH
YeastS5          DYVQVR---QPIFVAHTAGRYANKRFRKAQCPIIERLTNSLMMNGRNNGKKLKAVRIIKH
BacillusS5       ---------------MPRKGPVAKRDVLPDPIYNSKLVSRLINKMMIDGKKGKPQTILYK
EcoliS5          ---------------MPRRRVIGQRKILPDPKFGSELLAKFVNILMVDGKKSTAESIVYS
                                  .      :*   .     ..*   ::     :***  .  *:


HumanS5          AFEIIHLLTGENPLQVLVNAIINSGPREDSTRIGRAGTVRRQAVDVSPLRRVNQAIWLLC
MusS5            AFEIIHLLTGENPLQVLVNAIINSGPREDSTRIGRAGTVRRQAVDVSPLRRVNQAIWLLC
DrosoS5          SFEIIHLLTGENPLQILVSAIINSGPREDSTRIGRAGTVRRQAVDVSPLRRVNQAIWLLC
CelegansS5       AFEIIYLLTGENPVQVLVNAVINSGPREDSTRIGRAGTVRRQAVDVAPLRRVNQAIWLLC
ArabiS5          AMEIIHLLSDLNPIQVIIDAIVNSGPREDATRIGSAGVVRRQAVDISPLRRVNQAIFLLT
YeastS5          TLDIINVLTDQNPIQVVVDAITNTGPREDTTRVGGGGAARRQAVDVSPLRRVNQAIALLT
BacillusS5       SFDIIKERTGNDAMEVFEQALKNIMPVLEVKARRVGGANYQVPVEVRPERRTTLGLRWLV
EcoliS5          ALETLAQRSGKSELEAFEVALENVRPTVEVKSRRVGGSTYQVPVEVRPVRRNALAMRWIV
                 ::: :   :. . :: .  *: *  *  : .    .*   : .*:: * **   .:  :
```

```
HumanS5         TGAREAAFRNIKTIAECLADELINAAKGSSNSYAIKKKDELERVAKSNR-----------
MusS5           TGAREAAFRNIKTIAECLADELINAAKGSSNSYAIKKKDELERVAKSNR-----------
DrosoS5         TGAREAAFRNIKTIAECLADELINAAKGSSNSYAIKKKDELERVAKSNR-----------
CelegansS5      TGAREAAFRNVKTIAECLADELINAAKGSSNSYAIKKKDELERVAKSNR-----------
ArabiS5         TGAREAAFRNIKTIAECLADELINAAKGSSNSYAIKKKDEIERVAKANR-----------
YeastS5         IGAREAAFRNIKTIAETLAEELINAAKGSSTSYAIKKKDELERVAKSNR-----------
BacillusS5      NYARL---RGEKTMEERLANEILDAAN--NTGAAVKKREDTHKMAEANKAFAHYRW----
EcoliS5         EAARK---RGDKSMALRLANELSDAAE--NKGTAVKKREDVHRMAEANKAFAHYRWLSLR
                  **    *. *::   **:*: :**:  ... *:**::: .::*::*:

HumanS5         -------------------
MusS5           -------------------
DrosoS5         -------------------
CelegansS5      -------------------
ArabiS5         -------------------
YeastS5         -------------------
BacillusS5      -------------------
EcoliS5         SFSHQAGASSKQPALGYLN
```

S6-A YPL090C

```
S6A_Saccharomyces          MKLNISYPVNGSQKTFEIDDEHRIRVFFDKRIGQEVDGEAVGDEFKGYVF 50
S6-1_Arabidopsis           MKFNVANPTTGCQKKLEIDDDQKLRAFYDKRISQEVSGDALGEEFKGYVF 50
S6_Mus                     MKLNISFPATGCQKLIEVDDERKLRTFYEKRMATEVAADALGEEWKGYVV 50
RPS6_homo                  MKLNISFPATGCQKLIEVDDERKLRTFYEKRMATEVAADALGEEWKGYVV 50
S6_B_Drosophila            MKLNVSYPATGCQKLFEVVDEHKLRVFYEKRMGQVVEADILGDEWKGYQL 50
RPS-6a_Caenorhabditis      MRLNFAYPATGLQKSFEVDEEKKLRLFFEKRMSQEVAIDALGDEWKGYVV 50
                           *::*.: *..* ** :*: :::::* *::**:.  *  : :*:*:*** .

S6A_Saccharomyces          KISGGNDKQGFPMKQGVLLPTRIKLLLTKNVSCYR--PRRDGERKRKSVR 98
S6-1_Arabidopsis           KIKGGCDKQGFPMKQGVLTPGRVRLLLHRGTPCFRGHGRRTGERRRKSVR 100
S6_Mus                     RISGGNDKQGFPMKQGVLTHGRVRLLLSKGHSCYR--PRRTGERKRKSVR 98
RPS6_homo                  RISGGNDKQGFPMKQGVLTHGRVRLLLSKGHSCYR--PRRTGERKRKSVR 98
S6_B_Drosophila            RIAGGNDKQGFPMKQGVLTHGRVRLLLKKGHSCYR--PRRTGERKRKSVR 98
RPS-6a_Caenorhabditis      RIGGGNDKQGFPMKQGILTNGRVRLLLKKGQSCYR--ERKNGERKRKSVR 98
                           :* ** **********:*   *::*** :. .*:*   *: ***:*****

S6A_Saccharomyces          GAIVGPDLAVLALVIVKKGEQELEGLTDTTVPKRLGPKRANNIRKFFGLS 148
S6-1_Arabidopsis           GCIVSPDLSVLNLVIVKKGENDLPGLTDTEKPRMRGPKRASKIRKLFNLK 150
S6_Mus                     GCIVDANLSVLNLVIVKKGEKDIPGLTDTTVPRRLGPKRASRIRKLFNLS 148
RPS6_homo                  GCIVDANLSVLNLVIVKKGEKDIPGLTDTTVPRRLGPKRASRIRKLFNLS 148
S6_B_Drosophila            GCIVDANMSVLALVVLKKGEKDIPGLTDTTIPRRLGPKRASKIRKLYNLS 148
RPS-6a_Caenorhabditis      GCIVDANMSALSLVIVKKGDGEIEGLTDSVLPRKLGPKRASKIRKLFNLT 148
                           *.**..::::.* **::***: :: ****:  *:  *****..***::.*.

S6A_Saccharomyces          KEDDVRDFV--IRREVT-KGEKTY-TKAPKIQRLVTPQRLQRKRHQRALK 194
S6-1_Arabidopsis           KEDDVRTYVNTYRRKFTNKKGKEV-SKAPKIQRLVTPLTLQRKRARIADK 199
S6_Mus                     KEDDVRQYV--VRKPLN-KEGKKPRTKAPKIQRLVTPRVLQHKRRRIALK 195
RPS6_homo                  KEDDVRQYV--VRKPLN-KEGKKPRTKAPKIQRLVTPRVLQHKRRRIALK 195
S6_B_Drosophila            KEDDVRRFV--VRRPLPAKDNKKATSKAPKIQRLITPVVLQRKHRRIALK 196
RPS-6a_Caenorhabditis      KHDDVTKYV--ITHDKTFPDGVTK-TIAPKIQRLITPARIARKKYLLRQK 195
                           *.***  :*    :           : *******:**  : :*:     *

S6A_Saccharomyces          VRNAQAQREAAAEYAQLLAKRLSERKAEKAE--IRKRRASSLKA 236
S6-1_Arabidopsis           KKKIAKANSDAADYQKLLASRLKEQ---------RDRRSESL-- 232
S6_Mus                     KQRTKKNKEEAAEYAKLLAKRMKEAKEKRQEQIAKRRRLSSLRA 239
RPS6_homo                  KQRTKKNKEEAAEYAKLLAKRMKEAKEKRQEQIAKRRRLSSLRA 245
S6_B_Drosophila            KKRQIASKEASADYAKLLVQRKKESKAKREE--AKRRRSASS--- 235
RPS-6a_Caenorhabditis      RNQKIKMRDDAAAYHKLLAKYSKEEHDAK----IARRRSS---- 231
                            ..    .. :* * :**..  .*            **
```

S7-A YOR3177W

```
S7_Homo                  ---------------PDEFESGISQALLELEMNS-DLKAQLRELNITAAKEIEVGGGRKA 44
S7_A_Drosophila          ----------------------IAQALVELEANS-DLKPYLRDLHITRAREIEFGS-KKA 36
RPS-7_Caenorhabditis     -----------------EIEKQVSQALIDLETND-DVQSQLKELYIVGVKEVELGN-KSA 41
S7A_Saccharomyces        MSAPQAKILSQ---APTELELQVAQAFVELENSSPELKAELRPLQFKSIREIDVAGGKKA 57
S7-1_Arabidopsis         MFSAQHKIHKEKGVELSELDEQVAQAFFDLENTNQELKSELKDLYVNSAVQVDISGGRKA 60
                                                ::**:.:** .. :::. *: * .    ::...  :.*

S7_Homo                  IIIFVPVPQLKSFQKIQVRLVRELEKKFSGKHVVFIAQRRILPKPTRKSRT-KNKQKRPR 103
S7_A_Drosophila          VIIYVPIPQQKVFQKIQIILVRELEKKFSGKHVVVIAERKILPKPTRKARN-PLKQKRPR 95
RPS-7_Caenorhabditis     IIIYVPVPQLKAFHKIHPALVRELEKKFGGRDILILAKRRILPKPQRGSKARPQKQKRPR 101
S7A_Saccharomyces        LAIFVPVPSLAGFHKVQTKLTRELEKKFQDRHVIFLAERRILPKPSRTSRQ---VQKRPR 114
S7-1_Arabidopsis         IVVNVPYRLRKAYRKIHVRLVRELEKKFSGKDVILIATRRIVRPPKKGSAA-----KRPR 115
                         : : **      ::*::  *.******* .:.::.:* *:*:  * : :       ****

S7_Homo                  SFTLLTAVHDAILEDLVFPSEIVGKRIRVKLDGSRLIKVHLDKAQQNNVEHKVETFSGVYK 163
S7_A_Drosophila          SFTLLTAVYDAILEDLVFPAEIVGKRIRVKLDGSQLVKVHLDKNQQTTIEHKVDTFTSVYK 155
RPS-7_Caenorhabditis     SFTLLTAVHDAWLDELVYPAEVVGRRIRVKLDGKKVYKVHLDKSHQTNVGHKIGVFASVYR 161
S7A_Saccharomyces        SFTLLTAVHDKILEDLVFPTEIVGKRVRYLVGGNKIQKVLLDSKDVQQIDYKLESFQAVYN 174
S7-1_Arabidopsis         NFTLLTSVHEAILDDVVLPAEIVGKRTRYRLDGTKIMKVFLDPKERNNTEYKVEAFSAVYK 175
                         .*****:*::  *:::* *:*:**:* *  :.*.:: ** **  .     :*:  * .**.

S7_Homo                  KLTGKDVNFEFP---- 175
S7_A_Drosophila          KLTGRDVTFEFP---- 167
RPS-7_Caenorhabditis     KLTGKDVTFEFP---- 173
S7A_Saccharomyces        KLTGKQIVFEIPSETH 190
S7-1_Arabidopsis         KLTGKDVVFEFP---- 187
                         ****::: **:*
```

S8-A YBL072C

```
S8_Homo                  MGISRDNWHKRRKTGGKRKPYHKKRKYELGRPAANTKIGPRR-IHTVRVRGGNKKYRALR 59
S8-like_Mus              LGIFQDNWHKRRKTWGKRKPYHKKRKYELGRPAANTKIGPRR-IHTVRVRGGNKKYRALR 59
RPS-8_Caenorhabditis     MGISRDSWHKRYKTGATQPVPHKKRKFELGRPAANTKIGAHR-VRLVRTRGGNEKYRALR 59
S8_C_Drosophila          MGISRDSAHKRRATGGKRKSLRKKRKFELGRPAANTKLGSGR-VHKVRTRGGNTKLRALR 59
S8A_Saccharomyces        MGISRDSRHKRSATGAKRAQFRKKRKFELGRQPANTKIGAKR-IHSVRTRGGNKKYRALR 59
S8-1_Arabidopsis         MGISRDSIHKRRATGGKQKQWRKKRKYEMGRQPANTKLSSNKTVRRIRVRGGNVKWRALR 60
                         :** :*. ***  * ..:   :****:*:** .****:.. : :: :*.**** * ****

S8_Homo                  LDVGNFSWGSECCTRKTRIIDVVYNASNNELVRTKTLVKNCIVLIDSTPYRQWYESHYAL 119
S8-like_Mus              LDVGNFSWGSECCTRKTRIIDVVYNASNNELVRTKTLVKNCIVLIDSTPYRQWYESHCAL 119
RPS-8_Caenorhabditis     LDSGNFSWASEQTTRKTRIVDTMYNATNNELVRTKTLVKGAIISVDAAPFRQWYEAHYAL 119
S8_C_Drosophila          LETGNFAWASEGVARKTRIADVVYNASNNELVRTKTLVKNSIVVIDATPFRQWYEAHYVL 119
S8A_Saccharomyces        IETGNFSWASEGISKKTRIAGVVYHPSNNELVRTNTLTKAAIVQIDATPFRQWFEAHYGQ 119
S8-1_Arabidopsis         LDTGNYSWGSEATTRKTRVLDVVYNASNNELVRTKTLVKSAIVQVDAAPFKQWYLSHYGV 120
                         :: **::*.**  ::***: ..:*:.:*******:**.* .*: :*::*::**: :*

S8_Homo                  PLGRKK----GAKLT--PEEEEILNK----KRSKKIQKKYDERKKNAKISSLLEEQFQQG 169
S8-like_Mus              PLGRKK----GAKLT--PEEEEILNK----KRSKKIQKKYDERKKNAKISSLLEEQFQQG 169
RPS-8_Caenorhabditis     PLARKK----NAKLS--EEDNAILNK----KRSHHTMKKYTERQKTAAVDALLIEQFNTG 169
S8_C_Drosophila          PLGRKR----NPKHAQKEDENDVLTK----KRSEKVMKKYLERQKYGKVEQALEDQFTSG 171
S8A_Saccharomyces        TLGKKK----NVKEE------ETVAK------SKNAERKWAARAASAKIESSVESQFSAG 163
S8-1_Arabidopsis         ELGRKKKSASSTKKDGEEGEEAAVAAPEEVKKSNHLLRKIASRQEGRSLDSHIEDQFASG 180
                          *.:*:    . *          :        *.:  :*   *     :.  : .**  *

S8_Homo                  KLLACIASRPGQCGRADGYVLEGKELEFYLRKIKARK 206
S8-like_Mus              KLLACIASRPGQCGRADGYVLEGKELEFYLWKIKARK 206
RPS-8_Caenorhabditis     RLLARISSSPGQVGQANGYILEGKELDFYLRKIRAKK 206
S8_C_Drosophila          RILACISSRPGQCGRSDGYILEGKELEFYLKKIKSKK 208
S8A_Saccharomyces        RLYACISSRPGQSGRCDGYILEGEELAFYLRRLTAKK 200
S8-1_Arabidopsis         RLLACISSRPGQCGRADGYILEGKELEFYMKKIQKKK 217
                         :: * *:* *** *:.:**:***:** **: ::  :*
```

S9A

```
HumanS9         MPVARS-WVCRKTYVTPRRPFEKSRLDQELKLIG---EYGLRNKREVWRVKFTLAKIRKA 56
MusS9           MPVARS-WVCRKTYVTPRRPFEKSRLDQELKLIG---EYGLRNKREVWRVKFTLAKIRKA 56
DrosoS9         MVNGRIPSVFSKTYVTPRRPYEKARLDQELKIIG---EYGLRNKREVWRVKYALAKIRKA 57
CelegansS9      --MARLKTVQSKVTKSPRRPFEKERLDQELKLIG---TFGLKNKREVWRVKYTLAKVRKA 55
ArabiS9         MVHVCYYRNYGKTFKGPRRPYEKERLDSELKLVG---EYGLRNKRELWRVQYSLSRIRNA 57
YeastS9         --MPRAPRTYSKTYSTPKRPYESSRLDAELKLAG---EFGLKNKKEIYRISFQLSKIRRA 55
BacillusS9      ----------MARYTGPSWKLSR-RLGISLSGTG--KELE---KRPYAPGPHGPGQRKKL 44
EcoliS4         ----------MARYLGPKLKLSR-REGTDLFLKSGVRAIDTKCKIEQAPGQHG-ARKPRL 48
                               *    .  * . .*   .         *       .  .:  .

HumanS9         ARELLTLDEKDPRRLFEGNALLRRLVRIGVLDEGKMKLDYILGLKIEDFLERRLQTQVFK 116
MusS9           ARELLTLDEKDPRRLFEGNALLRRLVRIGVLDEGKMKLDYILGLKIEDFLERRLQTQVFK 116
DrosoS9         ARELLTLDEKDEKRLFQGNALLRRLVRIGVLDESRMKLDYVLGLKIEDFLERRLQTQVFK 117
CelegansS9      ARELLTLEDKDPKRLFEGNALLRRLVKIGVLDETKMKLDYVLGLKVEDFLERRLQTQVFK 115
ArabiS9         ARDLLTLDEKSPRRIFEGEALLRRMNRYGLLDESQNKLDYVLALTVENFLERRLQTIVFK 117
YeastS9         ARDLLTRDEKDPKRLFEGNALIRRLVRVGVLSEDKKKLDYVLALKVEDFLERRLQTQVYK 115
BacillusS9      SEYGLQLQEKQKLRHMYG--VNERQFRT--LFDKAGKLAGKHGENFMILLDSRLDNVVYK 100
EcoliS4         SDYGVQLREKQKVRRIYG--VLERQFRN--YYKEAARLKGNTGENLLALLEGRLDNVVYR 104
                :   :   :*.  * : *  : .*  :     .   :*    . ..  :*: **:. *::

HumanS9         LGLAKSIHHARVLIRQRHIRVRKQVVNIPSFIVRLD-----SQKHIDFSLRSPYGGGRPG 171
MusS9           LGLAKSIHHARVLIRQRHIRVRKQVVNIPSFIVRLD-----SQKHIDFSLRSPYGGGRPG 171
DrosoS9         LGLAKSIHHARVLIRQRHIRVRKQVVNIPSFVVRLD-----SQKHIDFSLKSPFGGGRPG 172
CelegansS9      LGLAKSIHHARILIKQHHIRVRRQVVDVPSFIVRLD-----SQKHIDFSLQSPYGGGRPG 170
ArabiS9         SGMAKSIHHSRVLIRQRHIRVGKQLVNIPSFMVRLD-----SQKHIDFALTSPFGGGRPG 172
YeastS9         LGLAKSVHHARVLITQRHIAVGKQIVNIPSFMVRLD-----SEKHIDFAPTSPFGGARPG 170
BacillusS9      LGLARTRRQARQLVNHGHILVDGSRVDIPSYLVKPGQTIGVREKSRNLSIIKESVEVNN- 159
EcoliS4         MGFGATRAEARQLVSHKAIMVNGRVVNIASYQVSPNDVVSIREKAKKQSRVKAALELAEQ 164
                 *:. :  .:* *: :  * *    *::.*: *  .      :*  . :  .

HumanS9         RVKRKN----AKKGQGGAGAGDDEEED--------------- 194
MusS9           RVKRKN----AKKGQGGAGAGDDEEED--------------- 194
DrosoS9         RVKRKN----LKKNQGGGGGAAEEEED--------------- 195
CelegansS9      RVKRRT----LRKGDGAGG--DDEE----------------- 189
ArabiS9         RVKRRNEKSASKKASGGGDADGDDEE---------------- 198
YeastS9         RVARRN---AARKAEASGEAADEADEADEE------------ 197
BacillusS9      -FVPEYLTFDAEKLEGTFTRLPERSELAPEINEALIVEFYSR 200
EcoliS4         REKPTWLEVDAGKMEGTFKRKPERSDLSADINEHLIVELYSK 206
```

S10-A YOR293W

```
S10_Homo                MLMPKKNRIAIYELLFKEGVMVAKKDVHMPKHPELADKNVPNLHVMKAMQ 50
S10_Mus                 MLMPKKNRIAIYELLFKEGVMVAKKDVHMPKHPELADKNVPNLHVMKAMQ 50
S10b_Drosophila         MFMPKAHRVAIYEYLFKEGVIVAKKDFHAQKHPELES--IPNLHVIKAMQ 48
RPS-10_Caenorhabditis   MFIPKSHTKLIYEYLFNEGVTVAKKDFNAKTHPNIEG--VSNLEVIKTLK 48
S10-3_Arabidopsis       MIISEANRKEICKYLFKEGVCFAKKDFNLAKHPLIDV---PNLQVIKLMQ 47
S10A_Saccharomyces      MLMPKEDRNKIHQYLFQEGVVVAKKDFNQAKHEEIDT---KNLYVIKALQ 47
                        *::.: .   * : **:*** .****.:  .*  :      ** *:* ::

S10_Homo                SLKSRGYVKEQFAWRHFYWYLTNEGIQYLRDYLHLPPEIVPAT------- 93
S10_Mus                 SLKSRGYVKEQFAWRHFYWYLTNEGIQYLRDYLHLPPEIVPAT------- 93
S10b_Drosophila         SLHSRGLVKEQFAWRHYYWYLTNEGIEELRSYLHLPPEIVPST------- 91
RPS-10_Caenorhabditis   SLASRELVKEQFAWRHYYWYLTDAGILYLREYLALPAEIVPAT------- 91
S10-3_Arabidopsis       SFKSKEYVRETFAWMHYYWFLTNEGIEFLRTYLNLPSDVVPAT------- 90
S10A_Saccharomyces      SLTSKGYVKTQFSWQYYYYTLTEEGVEYLREYLNLPEHIVPGTYIQERNP 97
                        *: *:  *:  *:* ::*: **: *:  ** ** ** .:**.*

S10_Homo                --------
S10_Mus                 --------
S10b_Drosophila         --------
RPS-10_Caenorhabditis   --------
S10-3_Arabidopsis       --------
S10A_Saccharomyces      TQRPQRRY 105
```

S11A

```
HumanS11         MADI---QTERAYQKQPTIFQNKKRVLLGETGKEKLPRYYKNIGLGFKTPKEAIEGTYID 57
MusS11           MADI---QTERAYQKQPTIFQNKKRVLLGETGKEKLPRYYKNIGLGFKTPKEAIEGTYID 57
DrosoS11         MAD----QNERAFQKQFGVNLNRK-VKPGIT-KKKLLRRSRDVGLGFKTPREAIDGTYID 54
CelegansS11      MSE----QTERAFLKQPTVNLNNKARILAGS--KKTPRYIREVGLGFKAPRDAVEGTYID 54
ArabiS11         MAE----QTEKAFLKQPKVFLSSKKSGKGKRPGKGGNRFWKNIGLGFKTPREAIDGAYVD 56
YeastS11         MSTELTVQSERAFQKQPHIFNNPK-----VKTSKRTKRWYKNAGLGFKTPKTAIEGSYID 55
BacillusS17      ------------------------------------------------------------
EcoliS17         ------------------------------------------------------------

HumanS11         KKCPFTGNVSIRGRILSGVVTKMKMQRTIVIRRDYLHYIRKYNRFEKRHKNMSVHLSPCF 117
MusS11           KKCPFTGNVSIRGRILSGVVTKMKMQRTIVIRRDYLHYIRKYNRFEKRHKNMSVHLSPCF 117
DrosoS11         KKCPWTGDVRIRGRILTGVVRKAKMQRTIVIRRDYLHFVRKYSRFEKRHRNMSVHCSPVF 114
CelegansS11      KKCPWAGNVPIRGMILTGVVLKNKMTRTIVVRRDYLHYIKKYRRYEKRHKNVPAHCSPAF 114
ArabiS11         KKCPFTGTVSIRGRILAGTCHSAKMQRTIIVRRDYLHFVKKYQRYEKRHSNIPAHVSPCF 116
YeastS11         KKCPFTGLVSIRGKILTGTVVSTKMHRTIVIRRAYLHYIPKYNRYEKRHKNVPVHVSPAF 115
BacillusS17      ------MSERNQRKVYQGRVVSDKMDKTITVVVETYKKHTLYGKRVKYSKKFKAHD--EN 52
EcoliS17         ------MTDKIR--TLQGRVVSDKMEKSIVVAIERFVKHPIYGKFIKRTTKLHVHD--EN 50
                            :     *   . ** ::* :          * :  *   :. .*

HumanS11         RDVQIGDIVTVGECRPLSKTVRFNVLKVTKAAGT----KKQFQKF 158
MusS11           RDVQIGDIVTVGECRPLSKTVRFNVLKVTKAAGT----KKQFQKF 158
DrosoS11         RDVEHGDIVTIGECRPLSKTVRFNVLKVSKGQGA----KKSFKKY 155
CelegansS11      RDIHPGDLVTIGECRPLSKTVRFNVLKVNKSGTS----KKGFSKF 155
ArabiS11         R-VKEGDHIIIGQCRPLSKTVRFNVLKVIPAGSSSSFGKKAFTGM 160
YeastS11         R-VQVGDIVTVGQCRPISKTVRFNVVKVSAAAGK---ANKQFAKF 156
BacillusS17      NQAKIGDIVKIMETRPLSATKRFRLVEVVEEAVII---------- 87
EcoliS17         NECGIGDVVEIRECRPLSKTKSWTLVRVVEKAVL----------- 84
                 .    ** : : : **:* *  : ::.*
```

S12 YOR369C

```
S12_Saccharomyces          MSDVEEVVEVQEETVVEQTAEVTIEDALKVVLRTALVHDGLARGLRESTK 50
RPS-12_Caenorhabditis      ------------------------EGALRAVLRAAHHADGLAKGLHETCK 26
S12_Homo                   --------------------------ALQEVLKTALIHDGLARGIREAAK 24
S12-like_Mus               --------------------------ALQEVLKTALVHDGLARGIHEAAK 24
S12_Drosophila             -----------------------INTALQEVLKKSLIADGLVHGIHQACK 27
S12-1_Arabidopsis          ---------VAEPAAIP--EDMDLMTALELTLRKARAYGGVVRGLHECAK 39
                                                  **. .*: :   .*:.:*:::  *

S12_Saccharomyces          ALTRGEALLVVLVSSVTEANIIKLVEGLANDPENKVPLIKVADAKQLGEW 100
RPS-12_Caenorhabditis      ALDKREAHFCVLAENCDEPQYVKLVETLC--AEHQIPLIKVADKKIIGEY 74
S12_Homo                   ALDKRQAHLCVLASNCDEPMYVKLVEALC--AEHQINLIKVDDNKKLGEW 72
S12-like_Mus               ALGKRQAHLCVLASNCDKPMYIKLVETLC--AEHQINLIKVDDNKKLGEW 72
S12_Drosophila             ALDKRQAVLCILAESFDEPNYKKLVTALC--NEHQIPLIRVDSHKKLGEW 75
S12-1_Arabidopsis          LIEKRVAQLVVLAEDCNQPDYVKLVKALC--ADHEVRLLTVPSAKTLGEW 87
                            : :  * : :*...  :.   ***  *.   :::: *: * . * :**:

S12_Saccharomyces          AGLGKIDREGNARKVVGASVVVVKNWGAETDELSMIMEHFSQQ 143
RPS-12_Caenorhabditis      CGLCKYDKEGKARKVVGCSSAVVTNWGNE-------------- 103
S12_Homo                   VGLCKIDREGKPRKVVGCSCVVVKDYGKE-------------- 101
S12-like_Mus               VGLCKIDRKGKPRKVIGCSCVVVKDFGQE-------------- 101
S12_Drosophila             SGLCKIDKEGKPRKVCGCSVVVIKDFGEETPALDVVKDHLRQ- 117
S12-1_Arabidopsis          AGLCKIDSEGNARKVVGCSCLVVKDFGEETTALSIV------- 123
                            ** * * :*:.*** *.*  *:.::* *
```

S13

DNA damage response kinase Aurora kinase Not sure (phosida)

```
HumanS13        MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGV
MusS13          MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGV
DrosoS13        MGRMHAPGKGISQSALPYRRTVPSWLKLNADDVKEQIKKLGKKGLTPSKIGIILRDSHGV
ArabiS13        MGRMHSRGKGISASALPYKRSSPSWLKTTPQDVDESICKFAKKGLTPSQIGVILRDSHGI
CelegansS13     MGRMHNPGKGMAKSAIPYRRSVPSWQKMTAEEVQDQIVKMAKKGLRPSQIGVILRDSHGV
YeastS13        MGRMHSAGKGISSSAIPYSRNAPAWFKLSSESVIEQIVKYARKGLTPSQIGVLLRDAHGV
EcoliS15        -------------------------MSLSTEATAKIVSEFGR-----------DANDTGS
BacillusS15     -------------------------MAITQERKNQLINEFKT-----------HESDTGS
                                             . :   . : :                .  *

                                         Casein Kinase2

HumanS13        AQVRFVTGNKILRILKSKGLAPDLPEDLYHLIKKAVAVRKHLERNRKDKDAKFRLILIES
MusS13          AQVRFVTGNKILRILKSKGLAPDLPEDLYHLIKKAVAVRKHLERNRKDKDAKFRLILIES
DrosoS13        AQVRFVNGNKILRIMKSVGLKPDIPEDLYHMIKKAVAIRKHLERNRKDKDGKFRLILVES
ArabiS13        PQVKSVTGSKILRILKAHGLAPEIPEDLYHLIKKAVAIRKHLERNRKDKDSKFRLILVES
CelegansS13     GQVRRLAGNKIFRILKSKGMAPELPEDLYHLVKKAVAIRKHLERSRKDIDSKYRLILVES
YeastS13        TQARVITGNKIMRILKSNGLAPEIPEDLYYLIKKAVSVRKHLERNRKDKDAKFRLILIES
EcoliS15        TEVQVALLTAQINHLQGHFAEHKKDHHSRRGLLRMVSQRRKLLDYLKRKDVARYTQLIER
BacillusS15     PEVQIAILTDSINNLNEHLRTHKKDHHSRRGLLKMVGKRRNLLTYLRNKDVTRYRELINK
                 :.:    .  :. ::      .  ..    : : *. *::*    :  *      *::

HumanS13        RIHRLARYYKTKRVLPPNWKYESSTASALVA
MusS13          RIHRLARYYKTKRVLPPNWKYESSTASALVA
DrosoS13        RIHRLARYYKTKSVLPPNWKYESSTASALVA
ArabiS13        RIHRLARYYKKTKKLPPVWKYESTTASTLVA
CelegansS13     RIHRLARYYKTKRQLPPTWKYESGTAASLVS
YeastS13        RIHRLARYYRTVAVLPPNWKYESATASALVN
EcoliS15        LGLRR--------------------------
BacillusS15     LGLRR--------------------------
                   *
```

S14-A

```
HumanS14            -MAPRKGKEKKEEQVISLGPQVAEGENVFGVCHIFASFNDTFVHVTDLSGKETICRVTGG
MusS14              -MAPRKGKEKKEEQVISLGPQVAEGENVFGVCHIFASFNDTFVHVTDLSGKETICRVTGG
DrosophilaS14       -MAPRKAKVQKEEVQVQLGPQVRDGEIVFGVAHIYASFNDTFVHVTDLSGRETIARVTGG
ArabiS14            --MSKRKTKEPKVDVVTLGPSVREGEQVFGVVHIFASFNDTFIHVTDLSGRETLVRITGG
YeastS14            ---------------MSNVVQARDNSQVFGVARIYASFNDTFVHVTDLSGKETIARVTGG
CelegansS14         MAPARKGKAKEEQAVVSLGPQAKEGELIFGVAHIFASFNDTFVHITDISGRETIVRVTGG
BacillusS14         ---------MAAARKSNTRKRRVKKNIESGIAHIRSTFNNTIVTITDTHGNAISWSSAGA
EcoliS11            -----------MAKAPIRARKRVRKQVSDGVAHIHASFNNTIVTITDRQGNALGWATAGG
                                                .   *: :* ::**:*:: :**  *.      :*.

HumanS14            MKVKADRDESSPYAAMLAAQDVAQRCKELGITALHIKLRATGGNRTKTPGPGAQSALRAL
MusS14              MKVKADRDESSPYAAMLAAQDVAQRCKELGITALHIKLRATGGNRTKTPGPGAQSALRAL
DrosophilaS14       MKVKADRDEASPYAAMLAAQDVAEKCKTLGITALHIKLRATGGNKTKTPGPGAQSALRAL
ArabiS14            MKVKADRDESSPYAAMLAAQDVAQRCKELGITAMHVKLRATGGNKTKTPGPGAQSALRAL
YeastS14            MKVKADRDESSPYAAMLAAQDVAAKCKEVGITAVHVKIRATGGTRTKTPGPGGQAALRAL
CelegansS14         MKVKADRDESSPYAAMLAAQDVADRCKQLGINALHIKLRATGGTRTKTPGPGAQSALRAL
BacillusS14         LGFRGSR-KSTPFAAQMAAETAAKGSIEHGLKTLEVTVKG--------PGSGREAAIRAL
EcoliS11            SGFRGSR-KSTPFAAQVAAERCADAVKEYGIKNLEVMVKG--------PGPGRESTIRAL
                      .:..* :::*:** :**:  *      *:. :.: ::.        **.* ::::***
```

```
HumanS14          ARSGMKIGRIEDVTPIPSDSTRRKGGRRGRRL
MusS14            ARSGMKIGRIEDVTPIPSDSTRRKGGRRGRRL
DrosophilaS14     ARSSMKIGRIEDVTPIPSDSTRRKGGRRGRRL
ArabiS14          ARSGMKIGRIEDVTPIPTDSTRRKGGRRGRRL
YeastS14          ARSGLRIGRIEDVTPVPSDSTRKKGGRRGRRL
CeleganssS14      ARAGMKIGRIEDVTPIPSDCTRRKGGRRGRRL
BacillusS14       QAAGLEVTAIRDVTPVPHNGCRPPKRRRV---
EcoliS11          NAAGFRITNITDVTPIPHNGCRPPKKRRV---
                    :.:.:  * ****:* :  *    **
```

S15

```
HumanS15        -------MAEVEQKKKRTFRKFTYRGVDLDQLLDMSYEQLMQLYSARQRRRLNRGLRRKQ
MusS15          -------MAEVEQKKKRTFRKFTYRGVDLDQLLDMSYEQLMQLYSARQRRRLNRGLRRKQ
CeleganssS15    -MATQDDAHLAELKKKRTFRKFMYRGVDLDQLLDMSREQFTKLLPCRMRRRLDRGLKRKH
DrosoS15        ----MADQVDENLKKKRTFKKFTYRGVDLDQLLDMPNNQLVELMHSRARRRFSRGLKRKP
ArabiS15        MAEVEPDVSAAALAKKRTFKKFSFKGVDLDALLDMPTDDLVELFPSRIRRRMSRGLTRKP
YeastS15        -------MSQAVNAKKRVFKTHSYRGVDLEKLLEMSTEDFVKLAPARVRRRFARGMTSKP
BacillusS19     ----------------------------------------------------MARSLKKGP
EcoliS19        ----------------------------------------------------MPRSLKKGP
                                                                    : *.:

HumanS15        HSLLKRLRKAKKEAPPMEKPEVVKTHLRDMIILPEMVGSMVGVYNGKTFNQVEIKPEMIG
MusS15          HSLLKRLRKAKKEAPPMEKPEVVKTHLRDMIILPEMVGSMVGVYNGKTFNQVEIKPEMIG
CeleganssS15    LALIAKVQKAKKAAGVLEKPATVKTHLRDMIILPELVGGVIGIYNGKVFNQTEIKPEMIG
DrosoS15        MALIKKLRKAKKEAPPNEKPEIVKTHLRNMIIVPEMTGSIIGVYNGKDFGQVEVKPEMIG
ArabiS15        MALIKKLRKAKLDAPAGEKPEVVRTHLRNMVIMPEMIGSIIGVYNGKTFNQIEIKPEMIG
YeastS15        AGFMKKLRAAKLAAPENEKPAPVRTHMRNMIIVPEMIGSVVGIYNGKAFNQVEIRPEMLG
BacillusS19     FVDGHLMTKIEKLNETDKK-QVVKTWSRRSTIFPQFIGHTIAVYDGRKHVPVFISEDMVG
EcoliS19        FIDLHLLKKVEKAVESGDK-KPLRTWSRRSTIFPNMIGLTIAVHNGRQHVPVFVTDEMVG
                      :   :      .*   ::*  *   *.*:: *  :.:::*: .    :  :*:*

HumanS15        HYLGEFSITYKPVKHGRPGIGATHSSRFIPLK
MusS15          HYLGEFSITYKPVKHGRPGIGATHSSRFIPLK
CeleganssS15    FYLGEFAISYKPVKHGRPGIGATHSSRFIPLK
DrosoS15        HYLGEFALTYKPVKHGRPGIGATHSSRFIPLK
ArabiS15        HYLAEFSITYKPVRHGKPGHGATHSSRFIPLK
YeastS15        HYLGEFSITYTPVRHGR---AGATTSRFIPLK
BacillusS19     HKLGEFAPTRTYKGHASDDKKTRR--------
EcoliS19        HKLGEFAPTRTYRGHAADKKAKKK--------
                . *.**: : .   *.
```

S16

```
HumanS16          MPSKG--PLQSVQVFGRKKTATAVAHCKRGNGLIKVNGRPLEMIEP-RTLQYKLLEPVLL 57
MusS16            MPSKG--PLQSVQVFGRKKTATAVAHCKRGNGLIKVNGRPLEMIEP-RTLQYKLLEPVLL 57
DrosoS16          MQQKRREPVQAVQVFGRKKTATAVAYCKRGNGLLKVNGRPLEQIEP-KVLQYKLQEPLLL 59
CelegansS16       MTSTV----QSVQTFGRKKTATAVAHCKKGQGLIKVNGRPLEFLEP-QILRIKLQEPLLL 55
ArabiS16          MATQP--ATESVQCFGRKKTAVAVTHCKRGSGLIKLNGCPIELFQP-EILRFKIFEPILL 57
YeastS16          MSAVP-----SVQTFGKKKSATAVAHVKAGKGLIKVNGSPITLVEP-EILRFKVYEPLLL 54
BacillusS9        LAQVQ------YYGTGRRKSSVARVRLVPGEGRIVVNNREISEHIPSAALIEDIKQPLTL 54
EcoliS9           MAENQ------YYGTGRRKSSAARVFIKPGNGKIVINQRSLEQYFGRETARMVVRQPLEL 54
                  :              *::*::.* .    *.* : :*   :            : :*: *

HumanS16          LGKERFAGVDIRVRVKGGGHVAQIYAIRQSISKALVAYYQKYVDEASKKEIKDILIQYDR 117
MusS16            LGKERFAGVDIRVRVKGGGHVAQIYAIRQSISKALVAYYQKYVDEASKKEIKDILIQYDR 117
DrosoS16          LGKEKFAGVDIRVRVSGGGHVAQIYAIRQAISKALVAFYQKYVDEASKKEIKDILVQYDR 119
CelegansS16       VGKERFQDVDIRIRVSGGGHVAQIYAVRQALAKALVAYYHKYVDEQSKRELKNIFAAYDK 115
ArabiS16          LGKHRFAGVNMRIRVNGGGHTSQVYAIRQSIAKALVAYYQKYVDEQSKKEIKDILVRYDR 117
YeastS16          VGLDKFSNIDIRVRVTGGGHVSQVYAIRQAIAKGLVAYHQKYVDEQSKNELKKAFTSYDR 114
BacillusS9        T--ETAGTYDVLVNVHGGGLSGQAGAIRHGIARALLEADPEYRTTLKR-----------A 101
EcoliS9           V--DMVEKLDLYITVKGGGISGQAGAIRHGITRALMEYDESLRSELRK-----------A 101
                     .     :: : * ***  .*  *:*:.:::.*:    .      :

HumanS16          TLLVADPRRCESKKFGGPGARARYQKSYR 146
MusS16            TLLVADPRRCESKKFGGPGARARYQKSYR 146
DrosoS16          TLLVGDPRRCEPKKFGGPGARARYQKSYR 148
CelegansS16       SLLVADPRRRESKKFGGPGARARYQKSYR 144
ArabiS16          TLLVADPRRCEPKKFGGRGARSRYQKSYR 146
YeastS16          TLLIADSRRPEPKKFGGKGARSRFQKSYR 143
BacillusS9        GLLTRDARMKERKKYGLKGARRAPQFSKR 130
EcoliS9           GFVTRDARQVERKKVGLRKARRRPQFSKR 130
```

S17-A YML024W

```
S17_Homo                  MGRVRTKTVKKAARVIIEKYYTRLGNDFHTNKRVCEEIAIIPSKKLRNKI 50
S17_Mus                   MGRVRTKTVKKAARVIIEKYYTRLGNDFHTNKRVCEEIAIIPSKKLRNKI 50
S17_Drosophila            MGRVRTKTVKKAAKVIIEKYYTRLTLDFHTNKRICEEVAIIPTKPLRNKI 50
RPS-17_Caenorhabditis     MSRVRTKTVKKASRVLIEKYYTRMTNDFHNNKRVCDEVAIIGSKPLRNKI 50
S17-3_Arabidopsis         MGRVRTKTVKKSSRQVIEKYYSRMTLDFHTNKKILEEVAIIPSKRLRNKI 50
S17A_Saccharomyces        MGRVRTKTVKRASKALIERYYPKLTLDFQTNKRLCDEIATIQSKRLRNKI 50
                          *.********:::: :**:**.::  **:.**:: :*:* * :* *****

S17_Homo                  AGYVTHLMKRIQRGPVRGISIKLQEEERERRDNYVPEVSALD-------- 92
S17_Mus                   AGYVTHLMKRIQRGPVRGISIKLQEEERERRDNYVPEVSALD-------- 92
S17_Drosophila            AGYVTHLMGRLRHSQVRGISIKLQEEERERRDNYVPAVSALEQD----II 96
RPS-17_Caenorhabditis     AGYITHLMRRIERGPVRGISIKLQEEERERRDNYMPEISTVDPSQLT-SI 99
S17-3_Arabidopsis         AGFSTHLMKRIQKGPVRGISLKLQEEERERRMDFVPDESAIKID----DV 96
S17A_Saccharomyces        AGYTTHLMKRIQKGPVRGISFKLQEEERERKDQYVPEVSALDLSRSNGVL 100
                          **: **** *:.:. *****:*********: :::*  *::.

S17_Homo                  ------------------------------------
S17_Mus                   ------------------------------------
S17_Drosophila            EVDADTKEMLKL------------------------- 108
RPS-17_Caenorhabditis     KVDTDTSDMLKAAGFNLPNV---------------- 119
S17-3_Arabidopsis         KVDKETLEMLASLGM--------------------- 111
S17A_Saccharomyces        NVDNQTSDLVKSLGLKLPLSVINVSAQRDRRYRKRV 136
```

S18

```
S18                 --MSLVIPEKFQHILRVLNTNIDGRRKIAFAITAIKGVGRRYAHVVLRKADIDLTKRAGE
MusS18              --MSLVIPEKFQHILRVLNTNIDGRRKIAFAITAIKGVGRRYAHVVLRKADIDLTKRAGE
DrosophilaS18       --MSLVIPEKFQHILRIMNTNIDGKRKVGIAMTAIKGVGRRYSNIVLKKADVDLTKRAGE
CelegansS18         --MSLIIPEKFQHIHRVMNTNIDGNRKVPYALTAIKGVGRRFAFVCCRKADVDVNKRAGE
ArabiS18            --MSLVANEEFQHILRVLNTNVDGKQKIMFALTSIKGIGRRLANIVCKKADVDMNKRAGE
YeastS18            MSLVVQEQGSFQHILRLLNTNVDGNIKIVYALTTIKGVGRRYSNLVCKKADVDLHKRAGE
BacillsuS13         -------------MARIAGVDIPRDKRVVISLTYIFGIGRTTRQQVLKEAGVSEDTRVRD
EcoliS13            -------------VARIAGINIPDHKHAVIALTSIYGVGKTRSKAILAAAGIAEDVKISE
                                 : *: . ::    :   ::* * *:*:  :      *.:    :  :
                                                 cAMP dependant kinase (PKA)

S18                 LTEDEVERVITIMQNPRQYKIPDWFLNRQKDVKDGKYSQVLANGLDNKLREDLERLKKIR
MusS18              LTEDEVERVITIMQNPRQYKIPDWFLNRQKDVKDGKYSQVLANGLDNKLREDLERLKKIR
DrosophilaS18       CTEEEVDKVVTIISNPLQYKVPNWFLNRQKDIIDGKYWQLTSSNLDSKLRDDLERLKKIR
CelegansS18         LTEEDFDKIVTIMQNPSQYKIPNWFLNRQKDIKDGKTGQLLSTAVDNKLREDLERMKKIR
ArabiS18            LSAAEIDNLMTIVANPRQFKIPDWFLNRQKDYKDGKYSQVVSNALDMKLRDDLERLKKIR
YeastS18            LTQEELERIVQIMQNPTHYKIPAWFLNRQNDITDGKDYHTLANNVESKLRDDLERLKKIR
BacillsuS13         LTEEELGKIRDIIDK-----------------------LKVEGDLRREVSLNIKRLIEIG
EcoliS13            LSEGQIDTLRDEVAK-----------------------FVVEGDLRREISMSIKRLMDLG
                     :  :.  :   : :                            :  ::  .::*: .:


S18                 AHRGLRHFWGLRVRGQHTKTTGRRGRTVGVSKKK---
MusS18              AHRGLRHFWGLRVRGQHTKTTGRRGRTVGVSKKK---
DrosophilaS18       SHRGLRHYWGLRVRGQHTKTTGRRGRTVGVSKKK---
CelegansS18         LHRGLRHYWGLRVRGQHTKTTGRKGRTVGVSKKKGG-
ArabiS18            NHRGLRHYWGLRVRGQHTKTTGRRGKTVGVSKKR---
YeastS18            AHRGIRHFWGLRVRGQHTKTTGRRRA-----------
BacillsuS13         TYRGIRHRRGLPVRGQNSKNNARTRKGPRRTVANKKK
EcoliS13            CYRGLRHRRGLPVRGQRTKTNARTRKGPRKPIKK---
                     :**:**  ** ****.:*...*
```

S19-A YOL121C

```
S19_Mus                     MPGVTVKDVNQQEFVRALAAFLKKSGKLKVPEWVDTVKLAKHKELAPYDE 50
S19_Homo                    MPGVTVKDVNQQEFVRALAAFLKKSGKLKVPEWVDTVKLAKHKELAPYDE 50
S19a_A_Drosophila           MPGVTVKDIDQHAVTKAVAVFLKKTGKLKVPDQMDIVKTAKFKELAPYDP 50
RPS-19_Caenorhabditis       ----SIKDVDQHEATKSIAHFLKKSGKVKVPEWSDLVKLGVNKELAPVDP 46
S19-2_Arabidopsis           -----VKDVSPHDFVKAYASHLKRSGKIELPLWTDIVKTGRLKELAPYDP 45
S19A_Saccharomyces          MPGVSVRDVAAQDFINAYASFLQRQGKLEVPGYVDIVKTSSGNEMPPQDA 50
                                 ::*:  :   .: * .*:: **:::*   * ** .  :*:.* *

S19_Mus                     N-WFYTRAASTARHLYLRGGAGVGSMTKIYGGRQRNGVRPSHFSRGSKSV 99
S19_Homo                    N-WFYTRAASTARHLYLRGGAGVGSMTKIYGGRQRNGVMPSHFSRGSKSV 99
S19a_A_Drosophila           D-WFYVRCASILRHLYHRSPAGVGSITKIYGGRKRNGVHPSHFCRAADGA 99
RPS-19_Caenorhabditis       D-WFYTRAASLARHLYFR-PAGIGAFKKVYGGNKRRGVAPNHFQTSAGNC 94
S19-2_Arabidopsis           D-WYYIRAASMARKIYLRGGLGVGAFRRIYGGSKRNGSRPPHFCKSSGGI 94
S19A_Saccharomyces          EGWFYKRAASVARHIYMRKQVGVGKLNKLYGGAKSRGVRPYKHIDASGSI 100
                            : *:* *.**  *::* *   *:* : ::*** : .*  * :.  .: .

S19_Mus                     ARRVLQALEGLKMVEKDQDG-GRKLTPQGQRDLDRIAGQ------ 137
S19_Homo                    ARRVLQALEGLKMVEKDQDG-GRKLTPQGQRDLDRIAGQ------ 137
S19a_A_Drosophila           ARKALQALEHARLVEKHPDG-GRKLSSIGQRDLDRIANQ------ 137
RPS-19_Caenorhabditis       LRKAVQQLEKIKWVEKHPDGKGRILSKQGRKDLDRIA-------- 131
S19-2_Arabidopsis           ARHILQQLETMSIVELDTKG-GRRITSSGQRDLDQVAGRIAAE-- 136
S19A_Saccharomyces          NRKVLQALEKIGIVEISPKG-GRRISENGQRDLDRIAAQTLEEDE 144
                             *: :* **    **   .* ** ::  *::***::*
```

S20

```
HumanS20            -----MAFKDTGKTPVEPEVAIHRIRITLTSRNVKSLEKVCADLIRGAKEKNLKVKGPVR
MusS20              -----MAFKDTGKTPVEPEVAIHRIRITLTSRNVKSLEKVCADLIRGAKEKNLKVKGPVR
DrosophilaS20       ---MAAAPKDIEKPHVGDSASVHRIRITLTSRNVRSLENVCRDLINGAKNQNLRVKGPVR
CelegansS20         ------MAVAFKNEKAITDNSEHRIRLTLTSQNVKPLEKVCAQLIDGAKNEHLIVKGPIR
ArabiS20            MATAYQPMKPGKAGLEEPLEQIHKIRITLSSKNVKNLEKVCTDLVRGAKDKRLRVKGPVR
YeastS20            ---MSDFQKEKVEEQEQQQQQIIKIRITLTSTKVKQLENVSSNIVKNAEQHNLVKKGPVR
```

```
BacillusS20        -------------------MAKQKIRIRLKAYDHRILDQSAEKIVETAKRSGASVSGPIP
EcoliS10           -------------------MQNQRIRIRLKAFDHRLIDQATAEIVETAKRTGAQVRGPIP
                                          :**: *.: . : :::   .::  *:       **:

HumanS20           MPTKTLRITTRKTPCGEGSKTWDRFQMRIHKRLIDLHSP-SEIVKQITSISIEPGVEVEV
MusS20             MPTKTLRITTRKTPCGEGSKTWDRFQMRIHKRLIDLHSP-SEIVKQITSISIEPGVEVEV
DrosophilaS20      MPTKTLRITTRKTPCGEGSKTWDRFQMRIHKRIIDLHSP-SEIVKKITSINIEPGVEVEV
CeleganssS20       MPTKVLRITTRKTPCGEGSKTWDRFQMRIHKRLINLHAP-AEVLRQITSISIEPGVDIEV
ArabiS20           MPTKVLKITTRKAPCGEGTNTWDRFELRVHKRVIDLFSS-PDVVKQITSITIEPGVEVEV
YeastS20           LPTKVLKISTRKTPNGEGSKTWETYEMRIHKRYIDLEAP-VQIVKRITQITIEPGVDVEV
BacillusS20        LPTEKSVYTILRAVHKYK-DSREQFEMRTHKRLIDIVNPTPQTVDALMRLDLPSGVDIEI
EcoliS10           LPTRKERFTVLISPHVNK-DARDQYEIRTHLRLVDIVEPTEKTVDALMRLDLAAGVDVQI
                   :**.    :   :      .: : :::* * * :::  .  . :  :  : : .**::::

HumanS20           TIADA
MusS20             TIADA
DrosophilaS20      TIAN-
CeleganssS20       TRAD-
ArabiS20           TIADS
YeastS20           VVASN
BacillusS20        KL---
EcoliS10           SLG--
```

S21-A YKR057W

```
S21_Homo                   MQNDAGEFVDLYVPRKCSASNRIIGAKDHASIQMNVAEVDKVTGRFN-GQ 49
S21_Mus                    MQNDAGEFVDLYVPRKCSASNRIIAAKDHASIQMNVAEVDRTTGRFN-GQ 49
S21_A_Drosophila           MENDAGENVDLYVPRKCSASNRIIHAKDHASVQLSIVDVDPETGRQT-DG 49
RPS-21_Caenorhabditis      MQNDAGQTVELYVPRKCSSSNRIIGPKDHASVQIDFVDVDPETGRMIPGK 50
S21A_Saccharomyces         MENDKGQLVELYVPRKCSATNRIIKADDHASVQINVAKVD-EEGRAIPGE 49
S21-1_Arabidopsis          MENDAGQVTELYIPRKCSATNRMITSKDHASVQLNIGHLD-ANGLYT-GQ 48
                           *:** *: .:**:*****::**:* ..****:*:.. .:*   *    .

S21_Homo                   FKTYAICGAIRRMGESDDSILRLAKADGIVSKN----- 82
S21_Mus                    FKTYGICGAIRRMGESDDSILRLAKADGIVSKN----- 82
S21_A_Drosophila           SKTYAICGEIRRMGESDDCIVRLAKKDGIITKN----- 82
RPS-21_Caenorhabditis      STRYAICGAIRRMGESDDAILRLAQKDGL--------- 79
S21A_Saccharomyces         YVTYALSGYVRSRGESDDSLNRLAQNDGLLKNVWSYSR 87
S21-1_Arabidopsis          FTTFALCGFVRAQGDADSGVDRL--------------- 71
                              :.:.* :*  *::*. : **
```

S22a

CDK

```
YeastS22A          MTRSSVLADALNAINNAEKTGKRQVLIRPSSKVIIKFLQVMQKHGYIGEFEYIDDHRSGK
ArabiS15a          MVRISVLNDALKSMYNAEKRGKRQVMIRPSSKVIIKFLIVMQKHGYIGEFEYVDDHRSGK
humanS15A          MVRMNVLADALKSINNAEKRGKRQVLIRCSKVIVRFLTVMMKHGYIGEFEIIDDHRAGK
MusS15a            MVRMNVLADALKSINNAEKRGKRQVLIRPCSKVIVRFLTVMMKHGYIGEFEIIDDHRAGK
DrosophilaS15      MVRMNVLADALKCINNAEKRGKRQVLLRPCSKVIIKFLTVMMKHGYIGEFEIVEDHRAGK
CeleganssS15       MVRMNVLADALNAINNAEKRGKRQVLIRPASKVIVRFLTVMMKHGYIGEFEIVDDHRAGK
Bacillus           MVMTDPIADMLTRIRNAN-MVRHEKLEIPASKLKREIPEILKREGFIRDVEFVEDSKQGI
EcoliS8            MSMQDPIADMLTRIRNGQ-AANKAAVTMPSSKLKVAIANVLKEEGFIEDFKVEGDTKP-E
                   *   . : * *. : *.:   .:  :  *.**:   :  :: ..*:* :.:   * :

YeastS22A          IVVQLNGRLNKCGVISPRFNVKIGDIEKWTANLLPARQFG---YVILTTSAGIMDHEEAR
ArabiS15a          IVVELNGRLNKCGVISPRFDVGVKEIEGWTARLLPSRQFG---YIVLTTSAGIMDHEEAR
humanS15A          IVVNLTGRLNKCGVISPRFDVQLKDLEKWQNNLLPSRQFG---FIVLTTSAGIMDHEEAR
MusS15a            IVVNLTGRLNKCGVISPRFDVQLKDLEKWQNNLLPSRQFG---FIVLTTSAGIMDHEEAR
DrosophilaS15      IVVNLTGRLNKCGVISPRFDAPINDIEKWTNNLLPSRQFG---YVVLTTSGGIMDHEEAR
CeleganssS15       IVVNLTGRLNKASVISPRLNIRLNDLEKYTNTLLPSRQFG---YLILTTSAGIMDHEEAR
Bacillus           IRVSLKYGQNNERVITGLKRISKPGLRVYAKSNEVPRVLNGLGIAIISTSQGVLTDKEAR
EcoliS8            LELTLKYFQG-KAVVESIQRVSRPGLRIYKRKDELPKVMAGLGIAVVSTSKGVMTDRAAR
                   : : *.   .   *:          :. :      .: :      :::** *:: .. **

YeastS22A          RKHVSGKILGFVY
```

```
ArabiS15a          RKNVGGKVLGFFY
humanS15A          RKHTGGKILGFFF
MusS15a            RKHTGGKILGFFF
DrosophilaS15      RKHLGGKILGFFF
CelegansS15        RKHLGGKILGFFF
Bacillus           AKQAGGEVLAYVW
EcoliS8            QAGLGGEIICYVA
                       .*::: :.
```

S23

```
HumanS23           --MGKCRGLRTARKLRSHRRDQKWHDKQYKKAHLGTALKANPFGGASHAKGIVLEKVGVE
MusS23             --MGKCRGLRTARKLRSHRRDQKWHDKQYKKAHLGTALKANPFGGASHAKGIVLEKVGVE
DrosophilaS23      --MGKPRGLRTARKHVNHRRDQRWADKDYKKAHLGTRWKANPFGGASHAKGIVLEKVGVE
CelegansS23        --MGKPKGLCTARKLKTHRQEQRWNDKRYKKAHIGTRWKSNPFGGASHAKGIVLEKIGVE
ArabiS23           --MGKTRGMGAGRKLKQLRITQRWADKHYKKSNRGNEWK-KPFACSSHAKGIVLEKIGIE
YeastS23           MGKGKPRGLNSARKLRVHRRNNRWAENNYKKRLLGTAFKSSPFGGSSHAKGIVLEKLGIE
BacillusS12        ----MPTINQLIRKGRVSKVEN--SKSPALNKGYNSFKKEHTNVSSPQKRGVCTRVGTMT
EcoliS12           ----MATVNQLVRKPRARKVAK--SNVPALEA-------------CPQKRGVCTRVYTTT
                               **    :  :   .    :              ..: :*:  .

HumanS23           AKQPNSAIRKCVRVQLIKNGKKITAFVPNDGCLNFIEENDEVLVAGFGRKGHAVGDIPGV
MusS23             AKQPNSAIRKCVRVQLIKNGKKITAFVPNDGCLNFIEENDEVLVAGFGRKGHAVGDIPGV
DrosophilaS23      AKQPNSAIRKCVRVQLIKNGKKITAFVPRDGSLNYIEENDEVLVAGFGRKGHAVGDIPGV
CelegansS23        AKQPNSAIRKCVRVQLIKNGKKITAFVPNDGCLNFVEENDEVLVSGFGRSGHAVGDIPGV
ArabiS23           AKQPNSAIRKCARVQLIKNGKKIAAFVPNDGCLNYIEENDEVLIAGFGRKGHAVGDIPGV
YeastS23           SKQPNSAIRKCVRVQLIKNGKKVTAFVPNDGCLNFVDENDEVLLAGFGRKGKAKGDIPGV
BacillusS12        PKKPNSALRKYARVRLT-NGIEVTAYIPGIG--HNLQEHSVVLIRGGRVKNLPRVRYHIV
EcoliS12           PKKPNSALRKVCRVRLT-NGFEVTSYIGGEG--HNLQEHSVILIRGGRVKDLPGVRYHTV
                   .*:****:**  **:*  ** ::::::   *  : ::*:. :*: *   .. .      *

HumanS23           RFKVVKVANVSLLALYKGK-KERPRS-
MusS23             RFKVVKVANVSLLALYKGK-KERPRS-
DrosophilaS23      RFKVVKVANVSLLALYKEK-KERPRS-
CelegansS23        RFKIVKVANTSLIALFKGK-KERPRS-
ArabiS23           RFKVVKVSGVSLLALFKEK-KEKPRS-
YeastS23           RFKVVKVSGVSLLALWKEK-KEKPRS-
BacillusS12        RGALDTAGVENRAQGRPKYGTKKPKAK
EcoliS12           RGALDCSGVKDRKQARSKYGVKRPKA-
                   *  :   .  .          ::*::
```

S24-A YER074W

```
S24_a_Homo                 MN-DTVTIRTRKFMTNRLLQRKQMVIDVLHPGKATVPKTEIREKLAKMYK 49
S24_3_Mus                  MN-DTVTIRTRKFMTNRLLQRKQMVIDVLHPGKATVPKTEIREKLAKMYK 49
S24_Drosophila             MSGTTATIRTRKFMTNRLLARKQMVCDVLHPGLSSVNKTEIREKLAAMYK 50
RPS-24_Caenorhabditis      MG-DVVTIRTRKVLTNKLLYRKQMVVEVIHPGRPTVPKADIREKIAKLYK 49
S24-1_Arabidopsis          MAEKAVTIRTRKFMTNRLLSRKQFVIDVLHPGRANVSKAELKEKLARMYE 50
S24B_Saccharomyces         MS-DAVTIRTRKVISNPLLARKQFVVDVLHPNRANVSKDELREKLAEVYK 49
                           *   ..******.::* ** ***:* :*:**. ..* * :::**:* :*:

S24_a_Homo                 TT-PDVIFVFGFRTHFGGGKTTGFGMIYDSLDYAKKNEPKHRLARHGLYE 98
S24_3_Mus                  TT-PDVIFVFGFRTHFGGGKTTGFGMIYDSLDYAKKNEPKHRLARHGLYE 98
S24_Drosophila             VT-PDVVFAFGFRTNFGGGRSTGFALIYDTLDFAKKFEPKYRLARHGLFE 99
RPS-24_Caenorhabditis      TT-PDTVIPFGFESKIGGGKSKGFALVYDTIDFAKKFEPKYRLVRMGLAT 98
S24-1_Arabidopsis          VKDPNAIFVFKFRTHFGGGKSSGFGLIYDTVESAKKFEPKYRLIRNGLDT 100
S24B_Saccharomyces         AE-KDAVSVFGFRTQFGGGKSVGFGLVYNSVAEAKKFEPTYRLVRYGLAE 98
                           .   :.:  * *.:::***:: **.::*:::  *** **.:** * **

S24_a_Homo                 KK-KTSRKQRKERKNRMKKVRGTAK------------ 122
S24_3_Mus                  KK-KTSRKQRKERKNRMKKVRGTAK------------ 122
S24_Drosophila             QK-KQTRKQRKERRNRMKKVRGTAKAK---------- 125
RPS-24_Caenorhabditis      KVEKPGRKQRKERKNRQKKVRGTAKAK---------- 125
S24-1_Arabidopsis          KI-EKSRKQIKERKNRAKKI----------------- 119
```

```
S24B_Saccharomyces         KVEKASRQQRKQKKNRDKKIFGTGKRLAKKVARRNAD 135
                           :  :  *:* *:::** **:
```

S25-A YGR027C

```
S25_A_Drosophila           MPPKKDAKS-SAKQPQKTQKKKEGSGGGKAKKKKWSKGKVRDKLNNQVLF 49
RPS-25_Caenorhabditis      MPPKKDPKG-GKAPPS---KKKEGSGGGKAKKKKWSKGKVRDKLNNMVLF 46
S25_Homo                   MPPKDDKKKKDAGKSAKKDKDPVNKSGGKAKKKKWSKGKVRDKLNNLVLF 50
S25-3_Arabidopsis          -------------------SKPAKSGGGKQKKKKWSKGKQKEKVNNMVLF 31
S25A_Saccharomyces         MPPK--------QQLSKAAKAAAALAGGKKSKKKWSKKSMKDRAQHAVIL 42
                                                .     .*** .****** . ::: :: *::

S25_A_Drosophila           DKATYEKLYKEVPAYKLITPSVVSERLKIRGSLAKRALIELREKGLIKQV 99
RPS-25_Caenorhabditis      DQATYDKLYKEVITYKLITPSVVSERLKVRASLAKAGLKELQAKGLVKCV 96
S25_Homo                   DKATYDKLCKEVPNYKLITPAVVSERLKIRGSLARAALQELLSKGLIKLV 100
S25-3_Arabidopsis          DQATYDKLLSEAPKFKLITPSILSDRLRINGSLARRAIRELMAKGTIRMV 81
S25A_Saccharomyces         DQEKYDRILKEVPTYRYVSVSVLVDRLKIGGSLARIALRHLEKEGIIKPI 92
                           *: .*::: .*.  :: :: ::: :**:: .***: .: .*  :* :: :

S25_A_Drosophila           VQHHSQVIYTRAT--- 112
RPS-25_Caenorhabditis      VHHHGQVVYTRAT--- 109
S25_Homo                   SKHRAQVIYTRNT--- 113
S25-3_Arabidopsis          SAHSSQQIYTRAT--- 94
S25A_Saccharomyces         SKHSKQAIYTRATASE 108
                             *  * :*** *
```

S26-A YGL189C

```
S26_Homo                MTKKRRNNGRAKKGRGHVQPIRCTNCARCVPKDKAIKKFVIRNIVEAAAVRDISEASVFD 60
S26_Drosophila          MTKKRRNGGRNKHNRGHVKPVRCTNCARCVPKDKAIKKFVIRNIVEAAAVRDITEASIWD 60
S26-3_Arabidopsis       ---KRRNGGRNKHNRGHVKPIRCSNCGKCCPKDKAIKRFIVRNIVEQAAIRDVQEASVYE 57
S26A_Saccharomyces      MPKKRASNGRNKKGRGHVKPVRCVNCSKSIPKDKAIKRMAIRNIVEAAAVRDLSEASVYP 60
                           ** ..** *:.****:*:** **.:. *******:: :***** **:**: ***::

S26_Homo                AYVLPKLYVKLHYCVSCAIHSKVVRNRSREARKDRTPP--PRFRPAGAA---------- 107
S26_Drosophila          SYVLPKLYAKLHYCVSCAIHSKVVRNRSREARRIRTPP--LR---------------- 100
S26-3_Arabidopsis       GYTLPKLYAKTQYCVSCAIHSHVVRVRSRTNRRVRTPP--PRFAR-------------- 100
S26A_Saccharomyces      EYALPKTYNKLHYCVSCAIHARIVRVRSREDRKNRAPPQRPRFNRENKVSPADAAKKAL 119
                         *.*** * * :********:::** ***  *: *:**   *
```

S27-A YKL156W

```
S27_Homo                   MPLA--KDLLHPSPEEEKRKHKKKRLVQSPNSYFMDVKCPGCYKITTVFS 48
RPS-27_Caenorhabditis      MPLA--VDLLHPEPQREIRCHKLKRLVQHPNSYFMDVKCSGCFKISTVFS 48
S27_Drosophila             MPLA--KDLLHPLPAEEKRKHKLKRLVQHPNSYFMDVKCPGCYRITTVFS 48
S27-2_Arabidopsis          MVLQNDIDLLNPPAELEKRKHKLKRLVQSPNSFFMDVKCQGCFNITTVFS 50
S27A_Saccharomyces         MVLV--QDLLHPTAASEARKHKLKTLVQGPRSYFLDVKCPGCLNITTVFS 48
                           * *    ***:* .  * * ** * *** *.*:*:**** ** .*:****

S27_Homo                   HAQTVVLCVGCSTVLCQPTGGKARLTEGCSFRRK 82
RPS-27_Caenorhabditis      HATTVVVCVGCNTVLCQPTRGKAKLTEGCSFRKK 82
S27_Drosophila             HAQGVVVCAGCATILCQPTGGRAKLTEGCSFRRK 82
S27-2_Arabidopsis          HSQTVVVCGNCQTILCQPTGGKAKLTEGCSFRRK 84
S27A_Saccharomyces         HAQTAVTCESCSTILCTPTGGKAKLSEGTSFRRK 82
                           *:  .* * .* *:** ** *:*:*:** ***:*
```

S28-A YOR167C

```
S28_Homo                   MDTSRVQPIKLARVTKVLGRTGSQGQCTQVRVEFMDDTS-RSIIRNVKGP 49
RPS-28_Caenorhabditis      MDK-----LTLARVTKVIGRTGSQGQCTQVRVEFINDQNNRSIIRNVKGP 45
S28b_Drosophila            MDK----PVVWARVMKVLGRTGSQGQCTQVKVEFLGEQN-RQIIRNVKGP 45
S28-1_Arabidopsis          -------------VVKVMGRTGSRGQVTQVRVKFTDSDR--YIMRNVKGP 35
S28A_Saccharomyces         MDN--KTPVTLAKVIKVLGRTGSRGGVTQVRVEFLEDTS-RTIVRNVKGP 47
                                     * **:*****:*  ***:*:*  .     *:******
```

```
S28_Homo                  VREGDVLTLLESEREARRLR 69
RPS-28_Caenorhabditis     VREGDILTLLESEREARRLR 65
S28b_Drosophila           VREGDILTLLESEREARRLR 65
S28-1_Arabidopsis         VREGDILTLLESEREARRLR 55
S28A_Saccharomyces        VRENDILVLMESEREARRLR 67
                          ***.*:*.*:**********
```

S29

```
HumanS29          MGHQQ----------------------------------------LYWSHPRKFGQGSR
MusS29            MGHQQ----------------------------------------LYWSHPRKFGQGSR
DrosophilaS29     MGFAT----------------------------------------LWYSHPRKYGQGSR
CelegansS29       MGFQN----------------------------------------LWFSHPRKFGPGSR
YeastS29          MAHEN----------------------------------------VWFSHPRRYGKGSR
ArabiS29          MGHSN----------------------------------------VWNSHPKKYGPGSR
BacillusS29       LAKKSKVAKELKRQQLVEQYAGIRRELKEKG------------DYEALSKLPRDSAPGRL
EcoliS14          MAKQSMKAREVKRVALADKYFAKRAELKAIISDVNASDEDRWNAVLKLQTLPRDSSPSRQ
                  :.                                                . *:  . .

HumanS29          --SCRVCSNRHGLIRKYGLN-MCRQCFRQYAKDIGFIKLD-
MusS29            --SCRVCSNRHGLIRKYGLN-MCRQCFRQYAKDIGFIKLD-
DrosophilaS29     --CCRACSNRHGLIRKYGLN-ICRQCFREYANDIGFKKLD-
CelegansS29       --SCRVCAGHHGLIRKYGLD-LCRRCFREQARDIGFKKLD-
YeastS29          --QCRVCSSHTGLIRKYGLN-ICRQCFREKANDIGFNKFR-
ArabiS29          --LCRVCGNSHGLIRKYGLN-CCRQCFRSNAKEIGFIKYR-
BacillusS29       HNRCMVTGRPRAYMRKFKMSRIAFRELAHKGQIPGVKKASW
EcoliS14          RNRCRQTGRPHGFLRKFGLSRIKVREAAMRGEIPGLKKASW
                     *   .   . :**: :.    :     ..  *. *
```

S30-A YLR287BC

```
S30_A_Drosophila          --KVHGSLARAGKVKGQTPKVEKQEKKKKKTGRAKRRIQYNRRFVNFVQGFGRRR---GP 55
S30_Mus                   --KVHGSLARAGKVRGQTPKVAKQEKKKKKTGRAKRRMQYNRRFVNV------------- 45
S30_Arabidopsis           MGKVHGSLARAGKVRGQTPKVAKQDKKKKPRGRAHKRLQHNRRFVTAVVGFGKKR---GP 57
S-30_Caenorhabditis       --KVHGSLARAGKVRAQTPKVDKQDKKKKKRGRAFRRVQYTRRYVNVASGPGKKR---GP 55
S30A_Saccharomyces        MAKVHGSLARAGKVKSQTPKVEKTEKPKKPKGRAYKRLLYTRRFVNVTLVNGKRRMNPGP 60
                            ************:.***** * :* **  *** :*: :.**:*.

S30_A_Drosophila          ---
S30_Mus                   ---
S30_Arabidopsis           ---
S-30_Caenorhabditis       ---
S30A_Saccharomyces        SVQ 63
```

S31 YLR167W

```
S27a_Homo                 MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYN 60
S27a_Mus                  MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYN 60
S27A_Drosophila           MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYN 60
S27a-2_Arabidopsis        MQIFVKTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYN 60
S31_Saccharomyces         MQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYN 60
                          ******************.****:***:****************************:***

S27a_Homo                 IQKESTLHLVLRLRGGAKKRKKKSYTTPKKNKHKRKKVKLAVLKYYKVDENGKISRLRRE 120
S27a_Mus                  IQKESTLHLVLRLRGGAKKRKKKSYTTPKKNKHKRKKVKLAVLKYYKVDENGKISRLRRE 120
S27A_Drosophila           IQKESTLHLVLRLRGGAKKRKKKNYSTPKKIKHKRKKVKLAVLKYYKVDENGKIHRLRRE 120
S27a-2_Arabidopsis        IQKESTLHLVLRLRGGAKKRKKKTYTKPKKIKHKHKKVKLAVLQFYKVDGSGKVQRLRKE 120
S31_Saccharomyces         IQKESTLHLVLRLRGGGKKRKKKVYTTPKKIKHKHKKVKLAVLSYYKVDAEGKVTKLRRE 120
                          ****************.****** *:.*** ***:********.:**** .**: :**:*

S27a_Homo                 CPSDECGAGVFMASHFDRHYCGKC-------- 144
S27a_Mus                  CPSDECGAGVFMGSHFDRHYCGKC-------- 144
S27A_Drosophila           CPGENCGAGVFMAAHEDRHYCGKC-------- 144
```

```
S27a-2_Arabidopsis      CPNATCGAGTFMASHFDRHYCGKC-------- 144
S31_Saccharomyces       CSNPTCGAGVFLANHKDRLYCGKCHSVYKVNA 152
                        *..  ****.*:. * ** *****
```

L2A

```
HumanL8          MGRVIRGQRKGAGSVFRAHVKHRKGAARLRAVDFAERHGYIKGIVKDIIHDPGRGAPLAK 60
MusL8            MGRVIRGQRKGAGSVFRAHVKHRKGAARLRAVDFAERHGYIKGIVKDIIHDPGRGAPLAK 60
DrosophilaL8     MGRVIRAQRKGAGSVFKAHVKKRKGAAKLRSLDFAERSGYIRGVVKDIIHDPGRGAPLAV 60
CelegansL2       MGRRIRIQRKGAGGIFKSHNKHRKGASKLRPLDYAERHGYIKGLVKDIIHDPGRGAPLAI 60
YeastL2          MGRVIRNQRKGAGSIFTSHTRLRQGAAKLRTLDYAERHGYIRGIVKQIVHDSGRGAPLAK 60
                 *** ** ******.:* :* : *:**::**.:*:*** ***:*:**:*:**.*******
                                          Conserved, buried

HumanL8          VVFRDPYRFKKRTELFIAAEGIHTGQFVYCGKKAQLNIGNVLPVGTMPEGTIVCCLEEKP 120
MusL8            VVFRDPYRFKKRTELFIAAEGIHTGQFVYCGKKAQLNIGNVLPVGTMPEGTIVCCLEEKP 120
DrosophilaL8     VHFRDPYRYKIRKELFIAPEGMHTGQFVYCGRKATLQIGNVMPLSQMPEGTIICNLEEKT 120
CelegansL2       IAFRDPYKYKTVKTTVVAAEGMHTGQFIHCGAKAQIQIGNIVPVGTLPEGTTICNVENKS 120
YeastL2          VVFRDPYKYRLREEIFIANEGVHTGQFIYAGKKASLNVGNVLPLGSVPEGTIVSNVEEKP 120
                 : *****:::     .:* **:*****::.* ** :::**::*:. :**** :. :*:*.

HumanL8          GDRGKLARASGNYATVISHNPETKKTRVKLPSGSKKVISSANRAVVGVVAGGGRIDKPIL 180
MusL8            GDRGKLARASGNYATVISHNPETKKTRVKLPSGSKKVISSANRAVVGVVAGGGRIDKPIL 180
DrosophilaL8     GDRGRLARTSGNYATVIAHNQDTKKTRVKLPSGAKKVVPSANRAMVGIVAGGGRIDKPIL 180
CelegansL2       GDRGVIARASGNYATVIAHNPDTKKTRIRLPSGAKKVVQSYNRAMIGLVAGGGRTDKPLL 180
YeastL2          GDRGALARASGNYVIIIGHNPDENKTRVRLPSGAKKVISSQARGVIGVIAGGGRVDKPLL 180
                 **** :**:****. :*.** : :***::****:***: *  *.::*::***** ***:*
                                                Conserved, buried

HumanL8          KAGRAYHKYKAKRNCWPRVRGVAMNPVEHPFGGGNHQHIGKPSTIRRDAPAGRKVGLIAA 240
MusL8            KAGRAYHKYKAKRNCWPRVRGVAMNPVEHPFGGGNHQHIGKPSTIRRDAPAGRKVGLIAA 240
DrosophilaL8     KAGRAYHKYKVKRNSWPKVRGVAMNPVEHPHGGGNHQHIGKASTVKRGTSAGRKVGLIAA 240
CelegansL2       KAGRSYHKYKAKRNSWPRVRGVAMNPVEHPHGGGNHQHIGHPSTVRRDASAGKKVGLIAA 240
YeastL2          KAGRAFHKYRLKRNSWPKTRGVAMNPVDHPHGGGNHQHIGKASTISRGAVSGQKAGLIAA 240
                 ****::***: ***.**:.********:**.*********:.**: *.: :*:*.*****

HumanL8          RRTGRLRGTKTVQEKEN--- 257         methylation
MusL8            RRTGRLRGTKTVQEKEN--- 257
DrosophilaL8     RRTGRIRGGK--GDSKDK-- 256
CelegansL2       RRTGRIRGGKPVKFTKEENV 260
YeastL2          RRTGLLRGSQKTQD------ 254
```

L3

```
HumanL3          MSHRKFSAPRHGSLGFLPRKRSSRHRGKVKSFPKDDPSKPVHLTAFLGYKAGMTHIVREV 60
MusL3            MSHRKFSAPRHGSLGFLPRKRSSRHRGKVKSFPKDDASKPVHLTAFLGYKAGMTHIVREV 60
DrosoL3          MSHRKFSAPRHGSMAFYPKKRSARHRGKVKAFPKDDASKPVHLTCFIGYKAGMTHIVREA 60
CelegansL3       MSHRKFSAPRHGHMGFTPKKRSRTYRGRIKAFPKDDKSKPIHLTAFLGYKAGMTHIVRDV 60
YeastL3          MSHRKYEAPRHGHLGFLPRKRAASIRARVKAFPKDDRSKPVALTSFLGYKAGMTTIVRDL 60
Arabi            MSHRKFEHPRHGSLGFLPRKRANRHRGKVKAFPKDDQTKPCKFTAFMGYKAGMTHIVREV 60
Bacillus         ------------------------------------------MTKGILGRKIGMTQVFAEN 19
EcoliL3          -------------------------------------------MIGLVGKKVGMTRIFTED 18
                                                                ::* * *** :. :

HumanL3          DRPGSKVNKKEVVEAVTIVETPPMVVVGIVGYVETPRGLRTFKTVFAEHISDECKRRFYK 120
MusL3            DRPGSKVNKKEVVEAVTIVETPPMVVVGIVGYVETPRGLRTFKTVFAEHISDECKRRFYK 120
DrosoL3          DRPGSKINKKEVVEAVTVLETPPMIVVGAVGYIETPFGLRALVNVWAQHLSEECRRRFYK 120
CelegansL3       DKPGSKVNKKEVVEAVTIVETPPMVIAGVTGYVDTPQGPRALTTIWAEHLSEEARRRFYS 120
YeastL3          DRPGSKFHKREVVEAVTVVDTPPVVVVGVVGYVETPRGLRSLTTVWAEHLSDEVKRRFYK 120
Arabi            EKPGSKLHKKETCEAVTIIETPAMVVVGVVAYVKTPRGLRSLNTVWAQHLSEEVRRRFYK 120
Bacillus         G----------DLIPVTVIEAAPNVVLQKKTAEND--GYEAIQLGFDDKREKLSNKPEKG 67
EcoliL3          G----------VSIPVTVIEVEANRVTQVKDLAND--GYRAIQVTTGAKKANRVTKPEAG 66
                              .**:::. .  :       .   * .::      :  .   :
```

```
HumanL3         NWHKSKKKAFTKYCKKWQDEDGKKQLEKDFSSMKKYCQVIRVIAHTQMRLLP-LRQKKAH 179
MusL3           NWHKSKKKAFTKYCKKWQDDTGKKQLEKDFNSMKKYCQVIRIIAHTQMRLLP-LRQKKAH 179
DrosoL3         NWYKSKKKAFTKASKKWTDDLGKKSIENDFRKMLRYCKVIRVIAHSQIRLIK-QRQKKAH 179
CelegansL3      NWAKSKKKAFTKYAKKWQDEDGKKLIEADFAKLKKYCSSIRVIAHTQMKILR-RRQKKAH 179
YeastL3         NWYKSKKKAFTKYSAKY-AQDG-AGIERELARIKKYASVVRVLVHTQIRKTP-LAQKKAH 177
Arabi           NWAKSKKKAFTGYAKQYDSEDGKKGIQAQLEKMKKYATVIRVLAHTQIRKMKGLKQKKAH 180
Bacillus        HVAKAE-----------------------------------------------TAPKRF 79
EcoliL3         HFAKAG-----------------------------------------------VEAGRG 78
                :  *:

HumanL3         LMEIQVNGGTVAEKLDWARERLEQQVPVNQVFGQDEMIDVIGVTKGKGYKGVTSRWHTKK 239
MusL3           LMEIQVNGGTVAEKLDWARERLEQQVPVNQVFGQDEMIDVIGVTKGKGYKGVTSRWHTKK 239
DrosoL3         VMEIQLNGGSIEDKVKWAREHLEKPIQVSNVFGQDEMIDCVGVTKGKGFKGVTSRWHTKK 239
CelegansL3      LVEIQVNGGTIEQKVDWAREHLEKQVQVDTVFAQDEMIDTIGVTRGHGFKGVTSRWHTKK 239
YeastL3         LAEIQLNGGSISEKVDWAREHFEKTVAVDSVFEQNEMIDAIAVTKGHGFEGVTHRWGTKK 237
Arabi           MMEIQINGGTIAQKVDFAYSFFEKQIPIEAVFQKDEMIDIIGVTKGKGYEGVVTRWGVTR 240
Bacillus        VKELRGVEM---------DAYEVGQEVKVEIFSAGEIVDVTGVSKGKGFQGAIKRHGQSR 130
EcoliL3         LWEFRLAEG---------EEFTVGQSISVELFADVKKVDVTGTSKGKGFAGTVKRWNFRT 129
                : *::                          :*   : :*  ..::*:*: *.  *

HumanL3         --LPRKTHRGLRKVACIGA-WHPARVAFSVARAGQKGYHHRTEINKKIYKIGQGYLIKDG 296
MusL3           --LPRKTHRGLRKVACIGA-WHPARVAFSVARAGQKGYHHRTEINKKIYKIGQGYLIKDG 296
DrosoL3         --LPRKTHKGLRKVACIGA-WHPSRVSTTVARAGQKGYHHRTEINKKIYRIGAGIHTKDG 296
CelegansL3      --LPRKTHKGLRKVACIGA-WHPSRVAFTVARAGQKGFHHRTIINNKIYRIGKSALTEEG 296
YeastL3         --LPRKTHRGLRKVACIGA-WHPAHVMWSVARAGQRGYHSRTSINHKIYRVGK------G 288
Arabi           --LPRKTHRGLRKVACIGA-WHPARVSYTVARAGQNGYHHRTELNKKIYRLGK------V 291
Bacillus        GPMSHG-SRYHRRPGSMGP-VDPNRVFKGKLLPGRMGGEQITVQNLEIVKVDA------- 181
EcoliL3         QDATHGNSLSHRVPGSIGQNQTPGKVFKGKKMAGQMGNERVTVQSLDVVRVDA------- 182
                   .:      *  ..:*    * :*      .*: * .  *  . .: ::.

HumanL3         KLIKNNASTDYDLSDKSINPLGGFVHYGEVTNDFVMLKGCVVGTKKRVLTLRKSLLVQTK 356
MusL3           KLIKNNASTDYDLSDKSINPLGGFVHYGEVTNDFIMLKGCVVGTKKRVLTLRKSLLVQTK 356
DrosoL3         KVIKNNASTEYDLTDKSITPMGGFPHYGEVNNDFVMIKGCCIGSKKRIITLRKSLLKHTK 356
CelegansL3      ---KNNGSTEFDLTQKTITPMGGFPRYGIVNQDYIMLRGAVLGPKKRLITLRKSLITQTK 353
YeastL3         DDEAN-GATSFDRTKKTITPMGGFVHYGEIKNDFIMVKGCIPGNRKRIVTLRKSLYTNTS 347
Arabi           GTEAHTAMTEYDRTEKDVTPMGGFPHYGIVKDDYLMIKGCCVGPKKRVVTLRQSLLTQTS 351
Bacillus        ------------------------------ERNLLLIKGNVPGAKKSLITVKSAVKSK-- 209
EcoliL3         ------------------------------ERNLLLVKGAVPGATGSDLIVKPAVKA--- 209
                                                : ::::*   *     : :: ::

HumanL3         RRALEKIDLKFIDTTSKFGHGRFQTMEEKKAFMGPLKKDRIAKEEGA------------- 403
MusL3           RRALEKIDLKFIDTTSKFGHGRFQTMEEKKAFMGPLKKDRIAKEEGA------------- 403
DrosoL3         RSALEQIKLKFIDTSSKMGHGRFQTPADKLAFMGPLKKDRLKEEAAATTAAAAAATTTSA 416
CelegansL3      RVAHEKINLKWIDTSSKTGHGRFQTTAEKRAFMGKLKRDFLAEAEAKA------------ 401
YeastL3         RKALEEVSLKWIDTASKFGKGRFQTPAEKHAFMGTLKKDL-------------------- 387
Arabi           RLALEEIKLKFIDTASIFGHGRFQTSLEKMRFYNRVTK---------------------- 389
Bacillus        ------------------------------------------------------------
EcoliL3         ------------------------------------------------------------
```

L4A

```
humanL4           --MACARPLISVYSEKGESSGK-NVTLPAVFKAPIRPDIVNFVHTNLRKNNRQPYAVSEL 57
Mus               --MACARPLISVYSEKGESSGK-NVTLPAVFKAPIRPDIVNFVHTNLRKNNRQPYAVSEL 57
DrosophilaL4      MSLGNARPLVSVYTEKNEPAKDKNICLPAVFKAPIRPDVVNEVHQLLRRNNRQAYAVSEL 60
CelegansL4        ---MAARPLVTVYDEKYEATQS-QIRLPAVFRTPIRPDLVSFIADQVRRNRRQAHAVNTK 56
yeastL4           ----MSRPQVTVHSLTGEATAN-ALPLPAVFSAPIRPDIVHTVFTSVNKNKRQAYAVSEK 55
                       :** ::*:  . *.: .  : ***** :*****:*  :   :.:*.**.:**.

humanL4           AGHQTSAESWGTGRAVARIPRVRGGGTHRSGQGAFGNMCRGGRMFAPTKTWRRWHRRVNT 117
Mus               AGHQTSAESWGTGRAVARIPRVRGGGTHRSGQGAFGNMCRGGRMFAPTKTWRRWHRRVNT 117
DrosophilaL4      AGHQTSAESWGTGRAVARIPRVRGGGTHRSGQGAFGNMCRGGRMFAPTKTFRRWHRKVNV 120
CelegansL4        AGKQHSAESWGTGRAVARIPRVRGGGTHRSGQGAFGNMCRGGHMFAPLKVFRRWHRNVNI 116
yeastL4           AGHQTSAESWGTGRAVARIPRVGGGGTGRSGQGAFGNMCRGGRMFAPTKTWRKWNVKVNH 115
```

```
                    **:* ***************** **** **************:**** *.:*:*: .**
R Highly conserved methylated

humanL4             TQKRYAICSALAASALPALVMSKGHRIEEVPELPLVVEDKVEGYKKTKEAVLLLKKLKAW 177
Mus                 TQKRYAICSALAASALPALVMSKGHRIEEVPELPLVVEDKVEGYKKTKEAVQLLKKLKAW 177
DrosophilaL4        NQRRYALVSAIAASGVPALVQSKGHVIDGVSEFPLVVSDEVQKVQKTKQAVIFLRRLKIW 180
CelegansL4          AQKRYAVSSAIAASGIPALLQARGHVIDQVAEVPLVVSDKVESFRKTKEAVVFLRRSHLW 176
yeastL4             NEKRYATASAIAATAVASLVLARGHRVEKIPEIPLVVSTDLESIQKTKEAVAALKAVGAH 175
                     ::***  **:**:.:.:*: ::** :: :.*.****. .::  :***:**  *:


Serine and threonine phosphorylation conserved, buried, need to check close proteins and RNAs


humanL4             NDIKKVYASQRMRAGKGKMRNRRRIQRRGPCIIYNEDNGIIKAFRNIPGITLLNVSKLNI 237
Mus                 NDIKKVYASQRMRAGKGKMRNRRRIQRRGPCIIYNEDNGIIKAFRNIPGITLLNVSKLNI 237
DrosophilaL4        ADIQKVYKSQRFRAGRGTMRDRRRIARRGPLVVYDKDEGLRKAFRNIPGIETINVDKLNL 240
CelegansL4          ADIEKVYNSKRNRAGKGKLRNRQHKQKLGPVVIYGQDAECARAFRNIPGVDVMNVERLNL 236
yeastL4             SDLLKVLKSKKLRAGKGKYRNRRWTQRRGPLVVYAEDNGIVKALRNVPGVETANVASLNL 235
                     *: **  *:: ***:*. *:*:   : ** ::* :*    :*:**:**:   **  **:
                    Same region


humanL4             LKLAPGGHVGRFCIWTESAFRKLDELYG-TWRKAASLKSNYNLPMHKMINTDLSRILKSP 296
Mus                 LKLAPGGHVGRFCIWTESAFRKLDELYG-TWRKAASLKSNYNLPMHKMMNTDLSRILKSP 296
DrosophilaL4        LKLAPGGHVGRFVIWTESAFARLNDLFG-TWKKPSTLKKGYNLPQPKMANTDLSRLLKSE 299
CelegansL4          LKLAPGGHLGRLIIWTESAFKKLDTIYGTTVANSSQLKKGWSVPLPIMANSDFSRIIRSE 296
yeastL4             LQLAPGAHLGRFVIWTEAAFTKLDQVWG--SETVASSKVGYTLPSHIISTSDVTRIINSS 293
                    *:****.*:**: ****:** :*: ::*    . :  * .:.:*   : .:*.:*::.*
                                                         This region


humanL4             EIQRALRAPRKKIHRR--VLKKNPLKNLRIMLKLNPYAKTMRRNTILRQARNHKLRVDKA 354
Mus                 EIQRALRAPRKKIHRR--VLKKNPLKNLRIMLKLNPYAKTMRRNTILRQARNHKLRVKKL 354
DrosophilaL4        EIRKVLRDPRKRVFRS--VRRLNPLTNVRQLIKLNPYAEVLKRRAALAAEKRTVAKVLAK 357
CelegansL4          EVVKAIRAPKKNPVLP--KVHRNPLKKRTLLYKLNPYASILR-----------------K 337
yeastL4             EIQSAIRPAGQATQKRTHVLKKNPLKNKQVLLRLNPYAKVFAAEKLGS----------KK 343
                    *:  .:* . :         : ***.:   : :*****. :

humanL4             AAAAAALQAKSD---EKAAVAGKKPVVGKKGKKAAVGVKKQKKPLVGKKAAATKKPAPEK 411
Mus                 EAAATALATKSEKVVPEKGTADKKPAVGKKGKK--VDAKKQKP--AGKKVVA-------K 403
DrosophilaL4        AKKQNVELAKSHFANVATKAAANRAKLLAARKK---------------------------- 390
CelegansL4          ASKANVKK------------------------------------------------------ 345
yeastL4             AEKTGTKPAAVFTETLKHD------------------------------------------ 362
                          .

humanL4             KPAEKKPTTEEKKPAA 427
Mus                 KPAEKKPTTEEKKPAA 419
DrosophilaL4        KVAAKKPAAKK----- 401
CelegansL4          ----------------
yeastL4             ----------------
```

L5

```
HumanL5         MGFVKVVKNKAYFKRYQVKFRRRREGKTDYYARKRLVIQDKNKYNTPKYRMIVRVTNRDI 60
MusL5           MGFVKVVKNKAYFKRYQVRFRRRREGKTDYYARKRLVIQDKNKYNTPKYRMIVRVTNRDI 60
Drosophila      MGFVKVVKNKQYFKRYQVKFRRRREGKTDYYARKRLIFQDKNKYNTPKYRLIVRLSNKDI 60
CelegansL5      MGLVKVIKNKAYFKRYQVKLRRRREGKTDYYARKRLTVQDKNKYNTPKYRLIVRITNKDV 60
ArabiL5         MVFVKSSKSNAYFKRYQVKFRRRRDGKTDYRARIRLINQDKNKYNTPKYRFVVRFTNKDI 60
YeastL5         MAFQKDAKSSAYSSRFQTPFRRRREGKTDYYQRKRLVTQHKAKYNTPKYRLVVRFTNKDI 60
BacillusL5      -MITKTSKNAARLKR-HARVRAKLSGTAE------------------RPRLNVFRSYKHI 40
EcoliL5         -----MDKKSARIRR-ATRARRKLQELG-------------------ATRLVVHRTPRHI 35
                       *.     *  .  * : .                       *: *  : :.:
```

```
HumanL5         ICQIAYARIEGDMIVCAAYAHELPKYGVKVGLTNYAAAYCTGLLLARRLLNRFGMDKIYE 120
MusL5           ICQIAYARIEGDMIVCAAYAHELPKYGVKVGLTNYAAAYCTGLLLARRLLNRFGMDKIYE 120
Drosophila      TVQIAYARIEGDRVVCAAYSHELPKYGIQVGLTNYAAAYCTGLLVARRVLNKLGLDSLYA 120
CelegansL5      VAQLAYSKIEGDVVVASAYSHELPRYGLKVGLTNYAAAYATGLLLARRHLKTIGLDSTYK 120
ArabiL5         VAQIVSASIAGDIVKASAYAHELPQYGLTVGLTNYAAAYCTGLLLARRVLKMLEMDDEYE 120
YeastL5         ICQIISSTITGDVVLAAAYSHELPRYGITHGLTNWAAAYATGLLIARRTLQKLGLDETYK 120
BacillusL5      YAQIIDDVNGVTLASASTLDKDLN--VESTGDT--SAATKVGELVAKRAAEKGISDVVFD 96
EcoliL5         YAQVIAPNGSEVLVAASTVEKAIAEQLKYTGNK--DAAAAVGKAVAERALEKGIKDVSFD 93
                  *:           .::  : :       * .   **  .*  :*.*  :    *  :

HumanL5         GQVEVTGDEYNVESIDGQPGAFTCYLDAGLARTTTGNKVFGALKGAVDGGLSIPHSTKRF 180
MusL5           GQVEVNGGEYNVESIDGQPGAFTCYLDAGLARTTTGNKVFGALKGAVDGGLSIPHSTKRF 180
Drosophila      GCTEVTGEEFNVEPVDDGPGAFRCFLDVGLARTTTGARVFGAMKGAVDGGLNIPHSVKRF 180
CelegansL5      GHEELTGEDYNVE-EEGDRAPFKAVLDIGLARTTTGSKIFAVMKGVADGGINVPHSESRF 179
ArabiL5         GNVEATGEDFSVEPTDSRR-PFRALLDVGLIRTTTGNRVFGALKGALDGGLDIPHSDKRF 179
YeastL5         GVEEVEGEYELTEAVEDGPRPFKVFLDIGLQRTTTGARVFGALKGASDGGLYVPHSENRF 180
BacillusL5      ------------------------------RGGYLYHGRVKALADAAREAGLKF------- 120
EcoliL5         ------------------------------RSGFQYHGRVQALADAAREAGLQF------- 117
                                                   :: .  ... :.*: .

HumanL5         PGYDSESKEFNAEVHRKHIMGQNVADYMRYLMEEDEDAYKKQFSQYIKNSVTPDMMEEMY 240
MusL5           PGYDSESKEFNAEVHRKHIMGQNVADYMRYLMEEDEDAYKKQFSQYIKNNVTPDMMEEMY 240
Drosophila      PGYSAETKSFNADVHRAHIFGQHVADYMRSLEEEDEESFKRQFSRYIKLGIRADDLEDIY 240
CelegansL5      FGFDQESKEYNAEAHRDRILGKHVADYMTYLKEEDEDRYKRQFSKFLAAGLNADNLVATY 239
ArabiL5         AGFHKENKQLDAEIHRNYIYGGHVSNYMKLLGEDEPEKLQTHFSAYIKKGVEAESIEEMY 239
YeastL5         PGWDFETEEIDPELLRSYIFGGHVSQYMEELADDDEERFSELFKGYLADDIDADSLEDIY 240
BacillusL5      ------------------------------------------------------------
EcoliL5         ------------------------------------------------------------

HumanL5         KKAHAAIRENPVYEKKPKK------EVKKKRWNRPKMSLAQKKDRVAQKKASFLRAQERA 294
MusL5           KKAHAAIRENPVYEKKPKR------EVKKKRWNRPKMSLAQKKDRVAQKKASFLRAQERA 294
Drosophila      KKAHQAIRNDPTHKVTAKKS----SAVTKKRWNAKKLTNEQRKTKIAAHKAAYVAKLQSE 296
CelegansL5      QKVHSAIRADASP--AAKK-----AAKPSKRHTAKRLTYDERKQRVADKKALLLQLKEQQ 292
ArabiL5         KKVHAAIRAEPNHKKTEKS-----APKEHKRYNLKKLTYEERKNKLIERVKALNGAGGDD 294
YeastL5         TSAHEAIRADPAFKPTEKKFTKEQYAAESKKYRQTKLSKEERAARVAAKIAALAGQQ--- 297
BacillusL5      ------------------------------------------------------------
EcoliL5         ------------------------------------------------------------

HumanL5         AES---- 297
MusL5           AES---- 297
Drosophila      TEA---- 299
CelegansL5      E------ 293
ArabiL5         DDEDDEE 301
YeastL5         -------
BacillusL5      -------
EcoliL5         -------
```

L6A

```
MusL6               MAGEKA--PDTKEKKPAAKKAGSDAAASRPRAAKVAKKVHPKGKKPKKAKPHCSRNPVLV 58
HomoL6              MAGEKVEKPDTKEKKPEAKKVD---------AGGKVKKGNLKAKKPKKGKPHCSRNPVLV 51
DrosophilaL6A       MAPIEK--------------------------AKKVAKSAKKGKK-------HPVNSYLK 27
CaenorhabditisL6    MVGKRN--------------------------LPVISR-----------------NFDLS 17
ArabidopsisL6-3     MPAKQR--------------------------TPKVNR-----------------NPDLI 17
SaccharomycesL6A    ------------------------------------------------------------
```

```
Mus                 RGIGRYSRSAMYSRKALYKRKYSAAKTK--VEKKKKKEKVLATVTKTVGGDKNGGTRVVK 116
Homo                RGIGRYSRSAMYSRKAMYKRKYSAAKSK--VEKKKK-EKVLATVTKPVGGDKNGGTRVVK 108
Drosophila          GGILRYSKAQMYKRRALYRLKDKKSPVV--EKAKVPIKKSKASYPTKTFVKKRPS----- 80
Caenorhabditis      PGVLRFSAS---------RLRLKKG-----EKKPKFTKDTSAKLPK---LQRNGT----- 55
Arabidopsis         RGVGKYSRSQMYHKRGLWAIKAKNGGVFPRHDAKSKVDAPVEKPPKFYPAEDVKKP---- 73
Saccharomyces       --------------------------------------MSAQKAPKWYPSEDVAAL---- 18
                                                            . ..    .

Mus                 LRKMPRYYPTEDVPRKLLSHGKKPFSQHVRRLRSSITPGTVLIILTGRHRGKRVVFLKQL 176
Homo                LRKMPRYYPTEDVPRKLLSHGKKPFSQHVRKLRASITPGTILIILTGRHRGKRVVFLKQL 168
Drosophila          --------------KANFSEHKR------NTRRN-LTPGTVLILLAGRHQGKRVVLLKVL 119
Caenorhabditis      --------------KFALGHSKT------VTLRKTLTPGTVLIVLAGRHKGKRVVFLKQL 95
Arabidopsis         --------------LPNRRTAKP------TKLRASITPGTVLIILAGRFKGKRVVFLKQL 113
Saccharomyces       --------------KKTRKAARP------QKLRASLVPGTVLILLAGRFRGKRVVYLKHL 58
                                        :          *  :.***:**:*:**.:***** ** *

Mus                 D-SGLLLVTGPLVINRVPLRRTHQKFVIATSTKVDISDVKIPKHLTDAYFKKKQLR-KPR 234
Homo                A-SGLLLVTGPLVLNRVPLRRTHQKFVIATSTKIDISNVKIPKHLTDAYFKKKKLR-KPR 226
Drosophila          A-SGLLLVTGPFALNSCPLRRVSQRYVIGTSSKVDLGAFKVPEHLNDAYFRRLKAKKDKK 178
Caenorhabditis      PQSGLLLVTGPHKINGFPLRRIGQAFVIATSLKVNVSGVKIPEHINDEYFKRKST--AQK 153
Arabidopsis         A-SGLLLVTGPFKINGVPLRRVNQAYVIGTSTKVDISGVTL-DKFDDKYFGKVAEK-KKK 170
Saccharomyces       E-DNTLLISGPFKVNGVPLRRVNARYVIATSTKVSVEGVNV-EKFNVEYFAKEKLT-KKE 115
                      .. **::**  :*  ****    :**.** *:.:  ..: .::   ** :       .

Mus                 HQEGEIFDTE-KEKYEITEQRKADQKAVDLQILPKIKAVPQ---LQGYLRSQFSLTNGMY 290
Homo                HQEGEIFDTE-KEKYEITEQRKIDQKAVDSQILPKIKAIPQ---LQGYLRSVFALTNGIY 282
Drosophila          TGEADIFAAK-KERFVPNEQRKKDQKEVDAALLKVIKAHPEGKFFAKYLQNMFALHSSQY 237
Caenorhabditis      TGKN-IFASG-KTEYTVSEQRKKDIKTVDAPILAAIKKHPEHKFLFGYLGTRFSLGKNQY 211
Arabidopsis         KTEGEFFEAEKEEKKEIPQVKKDDQKAVDAALIKAIEAVPE---LKTYLGARFSLKQGMK 227
Saccharomyces       KKEANLFPE--QQNKEIKAERVEDQKVVDKALIAEIKKTPL---LKQYLSASFSLKNGDK 170
                      :  :*    : .      :  * * **  ::  *:  *    :  **   *:* ..

Mus                 PHKLVF 296
Homo                PHKLVF 288
Drosophila          PHRMRF 243
Caenorhabditis      PHKMQF 217
Arabidopsis         PHELVF 233
Saccharomyces       PHMLKF 176
                    ** : *
```

L7A

```
HumanL7             MEGVEEKKK----------------------EVPAVPPTLKKKRRNFAELKIKRLRKKFA 38
MusL7               MEAVPEKKKKVATVPGTLKKKVPAGPKTLKKKVPAVPPTLKKKRRNFAELKVKRLRKKFA 60
DrosophilaL7        MPAPVVKKPA-------------------AKKLPAVPPSKLKFSKKQISKRVAESKRRLK 41
CelegansL7          -------MAP-------------------TKKVPQVPPTVLKRRKQRADARTKAAQHKVT 34
YeastL7             -------MAA-------------------EKIL--TPPSQLKKSKAQQKTAEQVAAERAA 32
ArabidopsisL7       ----------------------------MVESKVVVPPSVLKKRKREEEWALEKKQNVEA 32
BacillusL7          ------------------------------------------------------------
EcoliL7             ------------------------------------------------------------

HumanL7             QKMLRKARRKLIYEKAKHYHKEYRQMYRTEIRMARMARKAGNFYVPAEPKLAFVIRIRGI 98
MusL7               LKTLRKARRKLIYEKAKHYHKEYRQMYRTEIRMARMARKAGNFYVPAEPKLAFVIRIRGI 120
DrosophilaL7        KAAVIALRKKENLVRAEKYQNEYIKAEQREIKLRRLAKKRNQFYVPAEAKLAFVVRIRGI 101
CelegansL7          VAAKNKEKKTQYFKRAEKYYQEYRNAQKEGLRLKREAEAKGDFYVPAEHKVAFVVRIRGI 94
YeastL7             RKAANKEKRAIILERNAAYQKEYETAERNIIQAKRDAKAAGSYYVEAQHKLVFVVRIKGI 92
ArabidopsisL7       AKKKNAENRKLIFKRAEQYSKEYAEKEKELISLKREAKLKGGFYVDPEAKLLFIIRIRGI 92
BacillusL7          ------------------------------------------------------------
EcoliL7             ------------------------------------------------------------

HumanL7             NGVSPKVRKVLQLLRLRQIFNGTFVKLNKASINMLRIVEPYIAWGYPNLKSVNELIYKRG 158
```

```
MusL7              NGVSPKVRKVLQLLRLRQIFNGTFVKLNKASINMLRIVEPYIAWGYPNLKSVNELIYKRG 180
DrosophilaL7       NKVAPKVRKVLQLFRLRQINNGVFIKLNKATINMLRIAEPYITWGYPNLKSVRELIYKRG 161
CelegansL7         NQLHPKPRKALQILRLRQINNGVFVKLNKATLPLLRIIEPYVAWGYPNNKTIHDLLYKRG 154
YeastL7            NKIPPKPRKVLQLLRLTRINSGTFVKVTKATLELLKLIEPYVAYGYPSYSTIRQLVYKRG 152
ArabidopsisL7      NAIDPKTKKILQLLRLRQIFNGVFLKVNKATMNMLRRVEPYVTYGFPNLKSVKELIYKRG 152
BacillusL7         --------------------------MAK-----LEITLKRSVIGRP--EDQRVTVRTLG 27
EcoliL7            --------------------------MAKT----IKITQTRSAIGRL--PKHKATLLGLG 28
                                            : *     :.      . *       .  :   *

HumanL7            YGKINKKRIALTDNALIARSLGKYG-IICMEDLIHEIYTVGKRFKEANNFLWPFKLSSPR 217
MusL7              YGKINKKRIALTDNSLIARSLGKFG-IICMEDLIHEIYTVGKRFKEANNFLWPFKLSSPR 239
DrosophilaL7       FVKHNRQRVPITDNFVIERKLRQAHQIQCVEDLVHEIFTVGPNFKYASNFLWPFKLNTPT 221
CelegansL7         YAKVDGNRVPITDNTIVEQSLGKFN-IICLEDLAHEIATVGPHFKEATNFLWPFKLNNPT 213
YeastL7            FGKINKQRVPLSDNAIIEANLGKYG-ILSIDDLIHEIITVGPHFKQANNFLWPFKLSNPS 211
ArabidopsisL7      YGKLNHQRIALTDNSIVEQALGKHG-IICTEDLIHEILTVGPHFKEANNFLWPFQLKAPL 211
BacillusL7         LKKTN-QTVVHEDNAAIRGMINKVSHLVSVKEQ---------------------------- 59
EcoliL7            LRRIG-HTVEREDTPAIRGMINAVSFMVKVEE----------------------------- 59
                     : . : :   *.  :   :     :   .:

HumanL7            GGM--KKKTTHFVEGGDAGNREDQINRLIRRMN 248
MusL7              GGM--KKKTTHFVEGGDAGNREDQINRLIRRMN 270
DrosophilaL7       GGW--RKKANHYVNGGDFGNREDQINRLLRKMV 252
CelegansL7         GGW--TKKTNHFVEGGDFGNREDQINNLLRKMV 244
YeastL7            GGWGVPRKFKHFIQGGSFGNREEFINKLVKSMN 244
ArabidopsisL7      GGL--KKKRNHYVEGGDAGNRENFINELIRRMN 242
BacillusL7         ----------------------------------
EcoliL7            ----------------------------------
```

L8A

```
Homo_L7a                   MPKGKKAKGKKVAPAPAVVKKQ--EAKKVVNPLFEKRPKNFGIGQDIQPK 48
Mus_L7a                    MPKGKKAKGKKVAPAPAVVKKQ--EAKKVVNPLFEKRPKNFGIGQDIQPK 48
Caenorhabditis_RPL-7A      MP-SKKVIKKKVAAVPAHIRAQTQVQKEVKNPLFEKRARNFNIGQDIQPK 49
Saccharomyces_L8A          -----MAPGKKVAPAPFGAKSTK--SNKTRNPLTHSTPKNFGIGQAVQPK 43
Arabidopsis_L7a-1          -----MAPKKGVKVA---AKKKT--AEKVSNPLFERRPKQFGIGGALPPK 40
                                 .  * *  .    :      ::. *** .  .::*.**  : **

Homo_L7a                   RDLTRFVKWPRYIRLQRQRAILYKRLKVPPAINQFTQALDRQTATQLLKL 98
Mus_L7a                    RDLTRFVKWPRYIRLQRQRAILYKRLKVPPAINQFTQALDRQTATQLLKL 98
Caenorhabditis_RPL-7A      KDVTRFVKWPKYIRLQRQSAILQKRLKVPPTINQFRTALDSQSARQAFKL 99
Saccharomyces_L8A          RNLSRYVKWPEYVRVQRQKKILSIRLKVPPTIAQFQYTLDRNTAAETFKL 93
Arabidopsis_L7a-1          KDLSRYIKWPKSIRLQRQKRILKQRLKVPPALNQFTKTLDKNLATSLFKV 90
                           ::::*::***. :*:***  **  ******:: **  :** : * . :*:

Homo_L7a                   AHKYRPETKQEKKQRLLARAEKKAAGKGDV-PTKRPPVLRAGVNTVTTLV 147
Mus_L7a                    AHKYRPETKQEKKQRLLARAEKKAAGKGDV-PTKRPPVLRAGVNTVTTLV 147
Caenorhabditis_RPL-7A      LDKYRPESTEAKKNRLRARAEARAAGKKEE-VTKRPNTVRHGVNTITRLV 148
Saccharomyces_L8A          FNKYRPETAAEKKERLTKEAAAVAEGKSKQDASPKPYAVKYGLNHVVALI 143
Arabidopsis_L7a-1          LLKYRPEDKAAKKERLVKKAQAEAEGKPSE--SKKPIVVKYGLNHVTYLI 138
                             *****    **:**  .*   * ** .   : :* .:: *:* :. *:

Homo_L7a                   ENKKAQLVVIAHDVDPIELVVFLPALCRKMGVPYCIIKGKARLGRLVHRK 197
Mus_L7a                    ENKKAQLVVIAHDVDPIELVVFLPALCRKMGVPYCIIKGKARLGHLVHRK 197
Caenorhabditis_RPL-7A      ETRRAQLVLIAHDVNPLEIVLHLPALCRKYNVPYAIIKGKASLGTVVRRK 198
Saccharomyces_L8A          ENKKAKLVLIANDVDPIELVVFLPALCKKMGVPYAIVKGKARLGTLVNQK 193
Arabidopsis_L7a-1          EQNKAQLVVIAHDVDPIELVVWLPALCRKMEVPYCIVKGKSRLGAVVHQK 188
                           * .:*:**:**:**:*:*:*: *****:*  ***.*:***: ** :*.:*

Homo_L7a                   TCTTVAFTQVNSEDKGALAKLVEAIRTNYNDRYDEIRRHWGGNVLGPKSV 247
Mus_L7a                    TCTTVAFTQVNSEDKGALAKLVEAIRTNYNDRYDEIRRHWGGNVLGPKSV 247
Caenorhabditis_RPL-7A      TTAAVALVDVNPEDKSALNKLVETVNNNFSERHEEIRKHWGGGVMSAKSD 248
Saccharomyces_L8A          TSAVAALTEVRAEDEAALAKLVSTIDANFADKYDEVKKHWGGGILGNKAQ 243
Arabidopsis_L7a-1          TASCLCLTTVKNEDKLEFSKILEAIKANFNDKYEEYRKKWGGGIMGSKSQ 238
                           * :  .:. *. **:  : *::.::  *: ::::* :::***.::. *:
```

```
Homo_L7a                  ARIAKLEKAKAKELATKLG 266
Mus_L7a                   ARIAKLEKAKAKELATKLG 266
Caenorhabditis_RPL-7A     AKKLKIERARARDLG-KL- 265
Saccharomyces_L8A         AK--MDKRAKNSDSA---- 256
Arabidopsis_L7a-1         AKTKAKERVIAKEAAQRMN 257
                          *:    ::.   : .
```

L9A

```
Mus_L9                    MKTILSNQTVDIPENVEITLKGRTVIVKGPRGTLRRDFNHINVELSLLGK---KKKRLRV 57
Homo_L9                   MKTILSNQTVDIPENVDITLKGRTVIVKGPRGTLRRDFNHINVELSLLGK---KKKRLRV 57
Drosophila_L9             MRTINSNQCVKIPKDIKASVKARVVTITGTRGTLKRSFKHLALDMYMP-----DKRTLKV 55
Caenorhabditis_RPL-9      MKLIESNDTVVFPEGVTFTVKNRIVHVTGPRGTIRKDFRHLHMEMERIG-----KSTLRV 55
Arabidopsis_L9-2          MKTILSSETMDIPDGVAIKVNAKVIEVEGPRGKLTRDFKHLNLDFQLIKDQVTGKRQLKI 60
YEAST_L9-A                MKYIQTEQQIEVPEGVTVSIKSRIVKVVGPRGTLTKNLKHIDVTFTKVN-----NQLIKV 55
                          *: * :.: : .*..:  .:: : : : *.**.: :.:.*: : :         :  :::

Mus_L9                    DKWWGNRKELATVRTICSHVQNMIKGVTLGFRYKMRSVYAHFPINVVIQENG--SLVEIR 115
Homo_L9                   DKWWGNRKELATVRTICSHVQNMIKGVTLGFRYKMRSVYAHFPINVVIQENG--SLVEIR 115
Drosophila_L9             EKWFGTKKELAAVRTVCSHIENMIKGVTFGFQYKMRAVYAHFPINCVTSENN--TVIEIR 113
Caenorhabditis_RPL-9      RKWFGVRKELAAIRTVCSHIKNMIKGVTVGFRYKMRSVYAHFPINVTLQDGN--RTVEIR 113
Arabidopsis_L9-2          DSWFGSRKTSASIRTALSHVDNLIAGVTQGFLYRMRFVYAHFPINASIDGNN--KSIEIR 118
YEAST_L9-A                AVHNGGRKHVAALRTVKSLVDNMITGVTKGYKYKMRYVYAHFPINVNIVEKDGAKFIEVR 115
                              * :*  *::**  * :.*:* *** *: *:** ********      .    :*:*

Mus_L9                    NFLGEKYIRRVRMRTGVACSVSQAQKDELILEGNDIELVSNSAALIQQATTVKNKDIRKF 175
Homo_L9                   NFLGEKYIRRVRMRPGVACSVSQAQKDELILEGNDIELVSNSAALIQQATTVKNKDIRKF 175
Drosophila_L9             NFLGEKYIRRVEMAPGVTVVNSTAQKDELIVEGNDIESVSGSAALIQQSTTVKNKDIRKF 173
Caenorhabditis_RPL-9      NFLGEKIVRRVPLPEGVIATISTAQKDEIVVEGNDVQFVSQAAARIQQSTAVKEKDIRKF 173
Arabidopsis_L9-2          NFLGEKKVRKVEMLDGVKIVRSEKVKDEIILEGNDIELVSRSCALINQKCHVKKKDIRKF 178
YEAST_L9-A                NFLGDKKIRNVPVRDGVTIEFSTNVKDEIVLSGNSVEDVSQNAADLQQICRVRNKDIRKF 175
                          ****:* :*.* :  **    *   ***:::.**.:: **  .* ::*   *::******

Mus_L9                    LDGIYVSCKGTVQQADE 192
Homo_L9                   LDGIYVSCKGTVQQADE 192
Drosophila_L9             LDGLYVSCKTTVVKLES 190
Caenorhabditis_RPL-9      LDGIYVSCKTTIVPTD- 189
Arabidopsis_L9-2          LDGIYVSCKGKIAVEE- 194
YEAST_L9-A                LDGIYVSHKGFITEDL- 191
                          ***:***.*  :
```

L10 Chain l

```
HumanL10          MGRRPARCYRYCKNKPYPKSRFCRGVPDAKIRIFDLGRKKAKVDEFPLCGHMVSDEYEQL 60
MusL10            MGRRPARCYRYCKNKPYPKSRFCRGVPDAKIRIFDLGRKKAKVDEFPLCGHMVSDEYEQL 60
DrosophilaL10     MGRRPARCYRYCKNKPYPKSRFCRGVPDPKIRIFDLGRKKATVEDFPLCVHLVSDEYEQL 60
CelegansL10       MGRRPARCYRYIKNKPYPKSRFCRGVPDAKIRIFDLGNKRANVDTFPACVHMMSNEREHL 60
YeastL10          MARRPARCYRYQKNKPYPKSRYNRAVPDSKIRIYDLGKKKATVDEFPLCVHLVSNELEQL 60
                  *.********* *********: *.***.****:***.*:*.*: ** * *::*:* *:*

HumanL10          SSEALEAARICANKYMVKSCGKDGFHIRVRLHPFHVIRINKMLSCAGADRLQTGMRGAFG 120
MusL10            SSEALEAARICANKYMVKSCGKDGFHIRVRLHPFHVIRINKMLSCAGADRLQTGMRGAFG 120
DrosophilaL10     SSEALEAGRICCNKYLVKYCGKDQFHIRMRLHPFHVIRINKMLSCAGADRLQTGMRGAFG 120
CelegansL10       SSEALEAARICANKYMVKNCGKDGFHLRVRKHPFHVTRINKMLSCAGADRLQTGMRGAYG 120
YeastL10          SSEALEAARICANKYMTTVSGRDAFHLRVRVHPFHVLRINKMLSCAGADRLQQGMRGAWG 120
                  *******.***.***:.. .*:* **:*:* ***** *************** *****:*
```

```
HumanL10          KPQGTVARVHIGQVIMSIRTKLQNKEHVIEALRRAKFKFPGRQKIHISKKWGFTKFNADE 180
MusL10            KPQGTVARVHIGQVIMSIRTKLQNKEHVIEALRRAKFKFPGRQKIHISKKWGFTKFNADE 180
DrosophilaL10     KPQGTVARVRIGQPIMSVRSSDRYKAQVIEALRRAKFKFPGRQKIYVSKKWGFTKYERER 180
CelegansL10       KPQGLVARVDIGDILFSMRIKEGNVKHAIEAFRRAKFKFPGRQIIVSSRKWGFTKWDRED 180
YeastL10          KPHGLAARVDIGQIIFSVRTKDSNKDVVVEGLRRARYKFPGQQKIILSKKWGFTNLDRPE 180
                  **:* .*** **: ::*:* .      .:*.:***::****:* *  *:*****: :

HumanL10          FEDMVAEKRLIPDGCGVKYIPNRGPLDKWRALHS------- 214
MusL10            FEDMVAEKRLIPDGCGVKYIPNRGPLDKWRALHS------- 214
DrosophilaL10     YEELRDDNRLEPDGCNVKYRPEHGPIAAWEKAQRDVYA--- 218
CelegansL10       YERMRAEGRLRSDGVGVQLQREHGPLTKWIENPI------- 214
YeastL10          YLKKREAGEVKDDGAFVKFLSKKGSLENNIREFPEYFAAQA 221
```

L11A Chain J

```
HumanL11          MA------DQGEKE--NPMRELRIRKLCLNICVGESGDRLTRAAKVLEQITGQTPVFSKA 52
MusL11            MAQ-----DQGEKE--NPMRELRIRKLCLNICVGESGDRLTRAAKVLEQITGQTPVFSKA 53
DrosophilaL11     MAAVTKKIKRDPAK--NPMRDLHIRKLCLNICVGESGDRLTRAAKVLEQITGQQPVFSKA 58
CelegansL11       MTDVEKQTEIREKKGRNVMRELKIQKLCLNICVGESGDRLTRAAKVLEQITGQTPVFSKA 60
YeastL11          MS----------TKAQNPMRDLKIEKLVLNISVGESGDRLTRASKVLEQISGQTPVQSKA 50
                  *:           :  * **:*:*.** ***.*****:******:******:** ** ***

HumanL11          RYTVRSFGIRRNEKIAVHCTVRGAKAEEILEKGLKVREYELRKNNFSDTGNFGFGIQEHI 112
MusL11            RYTVRSFGIRRNEKIAVHCTVRGAKAEEILEKGLKVREYELRKNNFSDTGNFGFGIQEHI 113
DrosophilaL11     RYTVRSFGIRRNEKIAVHCTVRGAKAEEILERGLKVREYELRRENFSSTGNFGFGIQEHI 118
CelegansL11       RYTVRTFGIRRNEKIAVHCTVRGPKAEEILEKGLKVKEYELFKENFSDTGNFGFGVQEHI 120
YeastL11          RYTVRTFGIRRNEKIAVHVTVRGPKAEEILERGLKVKEYQLRDRNFSATGNFGFGIDEHI 110
                  *****:************ ****.*******:****:**:*  .*** *******::***

HumanL11          DLGIKYDPSIGIYGLDFYVVLGRPGFSIADKKRRTGCIGAKHRISKEEAMRWFQQKYDGI 172
MusL11            DLGIKYDPSIGIYGLDFYVVLGRPGFSIADKKRRTGCIGAKHRISKEEAMRWFQQKYDGI 173
DrosophilaL11     DLGIKYDPSIGIYGLDFYVVLGRPGYNVNHRKRKSGTVGFQHRLTKEDAMKWFQQKYDGI 178
CelegansL11       DLGIKYDPGIGIYGMDFYVVLNRNGVRVSKRRRAPGRIGPSHRVDKEETIKWFQQKYDGI 180
YeastL11          DLGIKYDPSIGIFGMDFYVVMNRPGARVTRRKRCKGTVGNSHKTTKEDTVSWFKQKYDAD 170
                  ********.***:*:*****:.* *  :  ::*  * :* .*:  **::: **:****.

HumanL11          ILPGK----------- 177
MusL11            ILPGK----------- 178
DrosophilaL11     ILNTKK---------- 184
CelegansL11       ILPPKPKVKKNFFRRR 196
YeastL11          VLDK------------ 174
                  :*
```

L12 Not in ribosome structure

L13A

```
HumanL13          MAPSRNGMVLKPHFHKDWQRRVATWFNQPARKIRRRKARQAKARRIAPRPASGPIRPIVR 60
MusL13            MAPSRNGMILKPHFHKDWQQRVDTWFNQPARKIRRRKARQAKARRIAPRPASGPIRPIVR 60
DrosophilaL13     MGKG-NNMIPNQHYHKWWQRHVKTWFNQPARKVRRHANRVKKAKAVFPRPASGALRPVVR 59
CelegansL13       MAPRGNQMLGNAHFRKHWHKRIKTWFDQPARKLRRRQNRQAKAVEIAPRPVAGLLRSVVR 60
YeastL13          MAISKNLPILKNHFRKHWQERVKVHFDQAGKKVSRRNARAARAAKIAPRPLD-LLRPVVR 59
                  *.   *  : : *::* *:.:: . *:*..:*: *:  *  :*  : ***    :*.:**
```

```
HumanL13          CPTVRYHTKVRAGRGFSLEELRVAGIHKKVARTIGISVDPRRRNKSTESLQANVQRLKEY 120
MusL13            CPTVRYHTKVRAGRGFSLEELRVAGIHKKVARTIGISVDPRRRNKSTESLQANVQRLKEY 120
DrosophilaL13     CPTIRYHTKLRAGRGFTLEELKGAGIGANFAKTIGIAVDRRRKNKSLESRQRNIQRLKEY 119
CelegansL13       CPQKRYNTKTRLGRGFSLQELKAAGISQAQARTIGIAVDVRRTNKTAEGLKANADRLKEY 120
YeastL13          APTVKYNRKVRAGRGFTLAEVKAAGLTAAWARTIGIAVDHRRQNRNQEIFDANVQRLKEY 119
                  .*  :*: * * ****:* *:: **:    *:****:** ** *:. *  . * :*****

HumanL13          RSKLILFPRKPSAPKKGDSSAEELKLATQLTGPVMPVRN-VYKKEKARVITEEEKNFKAF 179
MusL13            RSKLILFPRKPSAPKKGDSSAEELKLATQLTGPVMPIRN-VYKKEKARVITEEEKNFKAF 179
DrosophilaL13     RSKLILFPINEKKIRAGESSLEECKLATQLKGPVLPIKNEQPAVVEFREVTKDEKKFKAF 179
CelegansL13       KAKLILFPKKASAPKKGDSSAEELKVAAQLRGDVLPLSH-TITFDEPRQVTDAERKVEIF 179
YeastL13          QSKIIVFP------RDGKAPEAEQVLSAAAT---FPIAQ-PATDVEARAVQDNGE--SAF 167
                  ::*:*:**      : *.:.  *  :::      :*: :      : * : .  .  . *
                Conservered serine phosphorylation, changed to proline

HumanL13          ASLRMARANARLFGIRAKRAKEAAE-QDVEK---KK--- 211
MusL13            ASLRMARANARLFGIRAKRAKEAAE-QDVEK---KK--- 211
DrosophilaL13     ATLRKARTDARLVGIRAKRAKEAAESEDAAKGDPKKAKK 218
CelegansL13       RLLRKERADKKYRGKREKRAREAAE-ENK---------- 207
YeastL13          RTLRLARSEKKFRGIREKRAREKAEAEAEKKK------- 199
                    **  *:: :  * * ***:* ** :
```

RPL14A

```
HumanL14          MVFR--------RFVEVGRVAYVSFGPHAGKLVAIVDVIDQNRALVDGPCTQVRRQAMPF 52
MusL14            MVFR--------RYVEVGRVAYISFGPHAGKLVAIVDVIDQNRALVDGPCTRVRRQAMPF 52
CelegansL14       MVFN--------RVVQIGRVVFIASGKDQGKLAAIVNVIDGNRVQIDGPSSDVTRTVRNL 52
YeastL14          MSTDSIVKASNWRLVEVGRVVLIKKGQSAGKLAAIVEIIDQKKVLIDGPKAGVPRQAINL 60
DrosophilaL14     MPFE--------RFVQTGRIAKASAGPLKGRLVAIVDVIDQNRVLVDGPLTGVPRQEYRL 52
                  *           * *: **:.    *   *:*.***::** ::. :*** : * *    :

HumanL14          KCMQLTDFILKFPHSAHQKYVRQAWQKADINTKWAATRWAKKIEARERKAKMTDFDRFKV 112
MusL14            KCMQLTDFILKFPHSARQKYVRKAWEKADINTKWAATRWAKKIDARERKAKMTDFDRFKV 112
CelegansL14       KDLQLTKFVLKLRVGQRTKGVKAAFDAAKVTENFQKTQWAKKIAQRAIRAKLTDFERYKL 112
YeastL14          GQVVLTPLTFALPRGARTATVSKKWAAAGVCEKWAASSWAKKIAQRERRAALTDFERFQV 120
DrosophilaL14     NNLHLTKYRIKFPYTAPTRIVRKAWTESDLKAQWKVSPWSVKAQNICKRSSLNDFDRFKL 112
                    : **   : :        *   :  : :  ::  : *: *      :: :.**:*:::

HumanL14          MKAKKMRNRIIKNEVKKLQKAALLKASPKKAPGTKG--TAAAAAAAAAAKVPAKKITAAS 170
MusL14            MKAKKMRNRIIKTEVKKLQRAAILKASPKKAAVAKAAIAAAAAAAAAAKAKVPAKKATGPG 172
CelegansL14       MKAKQMRNRIVRVELAKLKKAQK------------------------------------- 135
YeastL14          MVLRKQKRYTVKKALAKA-------------------------------------------- 138
DrosophilaL14     RYAKRQRNKLLTIAFNTLKKRTKADGTPR-----------------------------VL 143
                     :: :.  :   . .

HumanL14          KKAPAQKVPAQKATGQKAAPAPKAQKGQKAPAQKAPAPKASGKKA 215
MusL14            KKAAGQKAPAQKAAGQKAAPPAKGQKGQKTPAQKAPAPKAAGKKA 217
CelegansL14       ----------------------------------------------
YeastL14          ----------------------------------------------
DrosophilaL14     KKDRRERLRAEKAKGGKKAAAKK---------------------- 166
```

L15A

```
HumanL15          MGAYKYIQELWRKKQSDVMRFLLRVRCWQYRQLSALHRAPRPTRPDKARRLGYKAKQGYV 60
MusL15            MGAYKYIQELWRKKQSDVMRFLLRVRCWQYRQLSALHRAPRPTRPDKARRLGYKAKQGYV 60
YeastL15          MGAYKYLEELQRKKQSDVLRFLQRVRVWEYRQKNVIHRAARPTRPDKARRLGYKAKQGFV 60
DrosophilaL15     MGAYRYMQELYRKKQSDVMRYLLRIRVWQYRQLTKLHRSPRPTRPDKARRLGYRAKQGFV 60
CelegansL15       MGAYKYMQEIWRKKQSDALRYLLRIRTWHYRQLSAVHRVPRPTRPEKARRLGYRAKQGFV 60
                  ****:*::*: ******.:*:* *:* *.*** . :** .*****:*******:****:*

HumanL15          IYRIRVRRGGRKRPVPKGATYGKPVHHGVNQLKFARSLQSVAEERAGRHCGALRVLNSYW 120
MusL15            IYRIRVRRGGRKRPVPKGATYGKPVHHGVNQLKFARSLQSVAEERAGRHCGALRVLNSYW 120
YeastL15          IYRVRVRRGNRKRPVPKGATYGKPTNQGVNELKYQRSLRATAEERVGRRAANLRVLNSYW 120
DrosophilaL15     IYRIRVRRGGRKRPVPKGCTYGKPKSHGVNQLKPYRGLQSIAEERVGRRLGGLRVLNSYW 120
CelegansL15       VYRVRVRRGNRKRPVCKGQTYGKPKTHGVNELKNAKSKQAVAEGRAGRRLGSLRVLNSYW 120
                  :**:*****.***** ** ******  :***:**  :. :: ** *.**: . ********

HumanL15          VGEDSTYKFFEVILIDPFHKAIRRNPDTQWITKPVHKHREMRGLTSAGRKSRGLGKGHKS 180
MusL15            VGEDSTYKFFEVILIDPFHKAIRRNPDTQWITKPVHKHREMRGLTSAGRKSRGLGKGHKF 180
CelegansL15       VAEDSTYKFYEVVLIDPFHKAIRRNPDTQWITKPVHKHREQRGLTSAGRKSRGLGKGWRF 180
DrosophilaL15     IAQDASYKYFEVILIDTHHSAIRRDPKINWICKHVHKHRELRGLTSAGKSSRGIGKGYRY 180
YeastL15          VNQDSTYKYFEVILVDPQHKAIRRDARYNWICDPVHKHREARGLTATGKKSRGINKGHKF 180
                  : :*::**::**:*:*. *.****:.  :** . ****** ****::*:.***:.** :

HumanL15          HHTIGGSRRAAWRRRNTLQLHRYR- 204
MusL15            HHTIGGSRRAAWRRRNTLQLHRYR- 204
CelegansL15       SATRGGSQAKNWKRKNTKVFHRKR- 204
DrosophilaL15     SQTIGGSRRAAWKRKNREHMHRKR- 204
YeastL15          NNTKAG-RRKTWKRQNTLSLWRYRK 204
                    * .* :   *:*:*   : * *
```

L16A

```
HumanL13          -----MAEV-----QVLVLDGRGHLLGRLAAIVAKQVLL------------GRKVVVVRC 38
MusL13            -----MAEG-----QVLVLDGRGHLLGRLAAIVAKQVLL------------GRKVVVVRC 38
ArabiL13          -----MVSGSGICSKRVVVDARHHMCGRLASIIAKELLN------------GQSVVVVRC 43
YeastL16          -----MSVEP-----VVVIDGKGHLVGRLASVVAKQLLN------------GQKIVVVRA 38
DrosophilaL13     -----MTGLTN---RTVVIDGRGHLLGRLASVVAKYLLQ------------GGKVAVVRC 40
CelegansL13       -----MGLSN----RAIIIDGKNHLLGRLASIVAKKLLQ------------GDKVVVLRA 39
BacillusL13       MRTTPMANASTIERKCLVVDAAGKTLGRLSSEVAAILRGKHKPTYTPHVDTGDHVIIINA 60
EcoliL13          MKTF-TAKPETVKRDWYVVDATGKTLGRLATELARRLRGKHKAEYTPHVDTGDYIIVLNA 59
                                  ::*.  :  ***:: :*  :              *  : ::..

HumanL13          EGINISGNFYRNKLKYLAFLRKRMNTNPSRGPYHFRAPSRIFWRTVRGMLPHKTKRGQAA 98
MusL13            EGINISGNFYRNKLKYLAFLRKRMNTNPSRGPYHFRAPSRIFWRTVRGMLPHKTKRGQAA 98
ArabiL13          EEICLSGGLVRQKMKYMRFLRKRMNTKPSHGPIHFRAPSKIFWRTVRGMIPHKTKRGAAA 103
YeastL16          EELNISGEFFRNKLKYHDFLRKATAFNKTRGPFHFRAPSRIFYKALRGMVSHKTARGKAA 98
DrosophilaL13     EELNLSGHFYRNKIKFLAYLRKRCNVNPARGPFHFRAPSRIFYKAVRGMIPHKTKRGQAA 100
CelegansL13       EEIVISGNFHRSKLKYMSFLRKRCNINPARGAFHYRAPGKIFWRTVRGMLPHKTNRGNEA 99
BacillusL13       EKIELTGKKLTDKIYYRHTQHPGGLKSRTALEMRTNYPEKMLELAIKGMLPKGS-LGRQM 119
EcoliL13          DKVAVTGNKRTDKVYYHHTGHIGGIKQATFEEMIARRPERVIEIAVKGMLPKGP-LGRAM 118
                  : : ::*    .*: :    :     . :      . * :::  :::**:.: .  *

HumanL13          LDRLKVFDGIPPPYDKKKRMVVPAALKVVRLKPTRKFAYLGRLAHEVGWKYQAVTATLEE 158
MusL13            LERLKVLDGIPPPYDKKKRMVVPAALKVVRLKPTRKFAYLGRLAHEVGWKYQAVTATLEE 158
ArabiL13          LARLKVFEGVPPPYDKVKRMVIPDALKVLRLQAGHKYCLLGRLSSEVGWNHYDTIKELEV 163
YeastL16          LERLKVFEGIPPPYDKKKRVVVPQALRVLRLKPGRKYTTLGKLSTSVGWKYEDVVAKLEA 158
DrosophilaL13     LARLRVFDGIPSPYDKRRRVVVPIAMRVLTLRSDRKYCQVGRLSHEVGWHYQDVIKSLER 160
CelegansL13       LKNLRAYEGVPAKYQKTKSLHAPSASR-FRLQPRRKFCVVGRLSHEVGWQFQDVVAKLEA 158
BacillusL13       FKKLNVYRGSEHPHEAQK----PEVYELRG------------------------------ 145
EcoliL13          FRKLKVYAGNEHNHAAQQ----PQVLDI-------------------------------- 142
                  : .*..  *    :   :    * .
```

```
HumanL13          KRKEKAKIHYRKKKQLMRLRKQAEKNVEKKIDKYTEVLKTHGLLV 203
MusL13            KRKEKAKMHYRKKKQILRLRKQAEKNVEKKICKFTEVLKTNGLLV 203
ArabiL13          KRKERSQALYERKKQLTKLRAKAEKVAEEKLGSQLDVLASIKY-- 206
YeastL16          KRKVSSAEYYAKKRAFTKKVASANATAAES--DVAKQLAALGY-- 199
DrosophilaL13     KRKAKLRVTLKHNRELKKLTVKARENIAKAAEPFNKIIKSYGYEV 205
CelegansL13       KRKVKGAAYFEQKKKMDKLAVQAKKNAAPKIAQYQKIIEALGYN- 202
BacillusL13       ---------------------------------------------
EcoliL13          ---------------------------------------------
```

RPL17A

```
humanL17        --MVRYSLDPENPTKSCKSRGSNLRVHFKNTRETAQAIKGMHIRKATKYLKDVTLQKQCV 58
musL17          --MVRYSLDPENPTKSCKSRGSNLRVHFKNTRETAQAIKGMHIRKATKYLKDVTLKKQCV 58
C.elegans       MTKVHYSRAPENSTKSCKARGSDLRVHFKNTHEAAMALRGMPLRRAQAFLNHVKEHKEIV 60
Drosophila      MG--RYSRESDNVAKSCKARGPNLRVHFKNTHETAQAIKRMPLRRAQRYLKAVIDQKECV 58
Yeast           --MARYGATSTNPAKSASARGSYLRVSFKNTRETAQAINGWELTKAQKYLEQVLDHQRAI 58
                    :*.  . * :**..:**. *** ****:*:* *:.   : :*  :*: *  ::. :

humanL17        PFRRYNGGVGRCAQAKQWGWTQGRWPKKSAEFLLHMLKNAESNAELKGLDVDSLVIEHIQ 118
musL17          PFRRYNGGVGRCAQAKQWGWTQGRWPKKSAEFLLHMLKNAESNAELKGLDVDSLVIEHIQ 118
C.elegans       PFRRFHGGIGRAAQTKQWNTTQGRWPVKSADFLLDLLKNAESNAEYKGLDVDHLVIEHIN 120
drosophila      PFRRFNGGVGRCAQAKQWKTTQGRWPKKSAEFLLQLLRNAEANADCKGLDADRLVVHHIQ 118
Yeast           PFRRFNSSIGRTAQGKEFGVTKARWPAKSVKFVQGLLQNAAANAEAKGLDATKLYVSHIQ 118
                ****::..:** ** *::  *:.*** **..*:  :*:** :**: ****.  * : **:

humanL17        VNKAPKMRRRTYRAHGRINPYNSSPCHIEMILTEKEQIVPKPEEEVAQKKKISQKKLKKQ 178
musL17          VNKAPKMRRRTYRAHGRINPYNSSPCHIEMILTEKEQIVPKPEEEVAQKKKISQKKLKKQ 178
C.elegans       VQRAAKLRRRTYRAHGRINPYNSSPCHIEVILAEKEDVVSKPTDDAAPKVKKESKRKQRR 180
drosophila      VNRAQCLRRRTYRAHGRINPYNSSPCHVEVILTEKEELVSKATDDEPAKKKLSKKKLQRQ 178
Yeast           VNQAPKQRRRTYRAHGRINKYESSPSHIELVVTEKEEAVAKAAEKKVVRLTSRQRGRIAA 178
                *::*   ************ * ***.*:*::::***: *.*. :.   : .  .:

humanL17        K--LMARE- 184
musL17          K--LMARE- 184
C.elegans       Q--LARGEF 187
drosophila      KEKMLRSE- 186
Yeast           QKRIAA--- 184
                :  :
```

L18A Chain Q

```
HumanL18        MGVDIR-HNKDRKVRRKEPKSQDIYLRLLVKLYRFLARRTNSTFNQVVLKRLFMSRTNRP 59
MusL18          MGVDIR-HNKDRKVRRKEPKSQDIYLRLLVKLYRFLARRTNSTFNQVVLKRLFMSRTNRP 59
DrosophilaL18   MGIDIN-HKYDRKVRRTEPKSQDVYLRLLVKLYRFLQRRTNKKFNRIILKRLFMSKINRP 59
CelegansL18     MGIDIN-HKHDRVARRTAPKSENPYLRLLSKLYAFLARRTGEKFNAIVLKRLRMSRRNRQ 59
YeastL18A       MGIDHTSKQHKRSGHRTAPKSDNVYLKLLVKLYTFLARRTDAPFNKVVLKALFLSKINRP 60
                **:*   :: .*  :*. ***:: **:** *** ** ***.  ** ::** * :*: **

HumanL18        PLGLSRMIRKMKLPGRENKTAVVVGTITDDVRVQEVPKLKVCALRVTSRARSRILRAGGK 119
MusL18          PLSLSRMIRKMKLPGRENKTAVVVGTVTDDVRILEVPKLKVCALRVSSRARSRILKAGGK 119
DrosophilaL18   PLSLQRIARFFKAANQPESTIVVVGTVTDDARLLVVPKLTVCALHVTQTARERILKAGGE 119
CelegansL18     PLSLAKLARAVQKAGNENKTVVTLSTVTDDARLYTVPKISVAALHVTEGARARILAAGGE 119
YeastL18A       PVSVSRIARALKQEGAANKTVVVVGTVTDDARIFEFPKTTVAALRFTAGARAKIVKAGGE 120
                *:*: :: * .:  .  :.* *.:.*:***.*:  .** .*.**:.:  ** :*: ***:
```

```
HumanL18          ILTFDQLALDSPKGCGTVLLSGPRKGREVYRHFGKAPGTPHSHTKPYVRSKGRKFERARG 179
MusL18            ILTFDQLALESPKGRGTVLLSGPRKGREVYRHFGKAPGTPHSHTKPYVRSKGRKFERARG 179
DrosophilaL18     VLTFDQLALRSPTGKNTLLLQGRRTARTACKHFGKAPGVPHSHTRPYVRSKGRKFERARG 179
CelegansL18       IITLDQLALKSPKGENTVFLQGPRSAREAEKHFGPAPGVPHSHTKPYVRSKGRKFERARG 179
YeastL18A         CITLDQLAVRAPKGQNTLILRGPRNSREAVRHFGMG---PHKGKAPRILSTGRKFERARG 177
                   :*:****: :*.* .*::* * *..* . :*** .   **. . * : *.*********

HumanL18          RRASRGYKN 188
MusL18            RRASRGYKN 188
DrosophilaL18     RRSSCGYKK 188
CelegansL18       RRASRAYKN 188
YeastL18A         RRRSKGFKV 186
                  ** * .:*
```

L19A

```
HumanL19          MSMLRLQKRLASSVLRCGKKKVWLDPNETNEIANANSRQQIRKLIKDGLIIRKPVTVHSR 60
MusL19            MSMLRLQKRLASSVLRCGKKKVWLDPNETNEIANANSRQQIRKLIKDGLIIRKPVTVHSR 60
DrosophilaL19     MSSLKLQKRLAASVLRCGKKKVWLDPNEINEIANTNSRQNIRKLIKDGLIIKKPVVVHSR 60
CelegansL19       MSNLRLQKRLASAVLKCGKHRVWLDPNEVSEISGANSRQSIRRLVNDGLIIRKPVTVHSR 60
YeastL19          MANLRTQKRLAASVVGVGKRKVWLDPNETSEIAQANSRNAIRKLVKNGTIVKKAVTVHSK 60
                  *: *: *****:*:*:  **::******* .**: :***: **:*:::* *::*.*.***:
                         Conserved phosphor, buried site

HumanL19          ARCRKNTLARRKGRHMGIGKRKGTANARMPEKVTWMRRMRILRRLLRRYRESKKIDRHMY 120
MusL19            ARCRKNTLARRKGRHMGIGKRKGTANARMPEKVTWMRRMRILRRLLRRYRESKKIDRHMY 120
DrosophilaL19     YRVRKNTEARRKGRHCGFGKRKGTANARMPTKLLWMQRQRVLRRLLKKYRDSKKIDRHLY 120
CelegansL19       FRAREYEEARRKGRHTGYGKRRGTANARMPEKTLWIRRMRVLRNLLRRYRDAKKLDKHLY 120
YeastL19          SRTRAHAQSKREGRHSGYGKRKGTREARLPSQVVWIRRLRVLRRLLAKYRDAGKIDKHLY 120
                   * *    ::*:*** * ***:** :**:* :  *::* *:**.** :**:: *:*:*:*

HumanL19          HSLYLKVKGNVFKNKRILMEHIHKLKADKARKKLLADQAEARRSKTKEARKRREERLQAK 180
MusL19            HSLYLKVKGNVFKNKRILMEHIHKLKADKARKKLLADQAEARRSKTKEARKRREERLQAK 180
DrosophilaL19     HDLYMKCKGNVFKNKRVLMEYIHKKKAEKQRSKMLADQAEARRQKVREARKRREERIATK 180
CelegansL19       HELYLRAKGNNFKNKKNLIEYIFKKKTENKRAKQLADQAQARRDKNKESRKRREERQVVK 180
YeastL19          HVLYKESKGNAFKHKRALVEHIIQAKADAQREKALNEEAEARRLKNRAARDRRAQRVAEK 180
                  * ** . *** **:*: *:*:* : *::  * * * ::*:*** * : :*.** :*   *

HumanL19          KEEIIKTLSKEEETKK------- 196
MusL19            KEEIIKTLSKEEETKK------- 196
DrosophilaL19     KQELIALHAKEDEIAAKAATAGH 203
CelegansL19       RAELLRKISQSEKVIAGK----- 198
YeastL19          RDALLKEDA-------------- 189
                  :  ::   :
```

L20A

```
L18a_Homo               MKASGTLREYKVVGRCLPTPKCHTPPLYRMRIFAPNHVVAKSRFWYFVSQLKKMKKSSGE 60
L18a_Mus                MKASGTLREYKVVGRCLPTPKCHTPPLYRMRIFAPNHVVAKSRFWYFVSQLKKMKKSSGE 60
L18A_Drosophila         MRAKGLLKEYEVVGRKLPSEKEPQTPLYKMRIFAPDNIVAKSRFWYFLRQLKKFKKTTGE 60
L20A_Saccharomyces      ---MAHFKEYQVIGRRLPTESVPEPKLFRMRIFASNEVIAKSRYWYFLQKLHKVKKASGE 57
L18a-2_Arabidopsis      -MGAFRFHQYQVVGRALPTEKDVQPKIYRMKLWATNEVRAKSKFWYFLRKLKKVKKSNGQ 59
                              :::*:*:** **: .   . :::*:::*.:.: ***::***: :*:*.**:.*:

L18a_Homo               IVYCGQVFEKSPLRVKNFGIWLRYDSRSGTHNMYREYRDLTTAGAVTQCYRDMGARHRAR 120
L18a_Mus                IVYCGQVFEKSPLRVKNFGIWLRYDSRSGTHNMYREYRDLTTAGAVTQCYRDMGARHRAR 120
L18A_Drosophila         IVSIKQVYETSPVKIKNFGIWLRYDSRSGTHNMYREYRDLTVGGAVTQCYRDMGARHRAR 120
L20A_Saccharomyces      IVSINQINEAHPTKVKNFGVWVRYDSRSGTHNMYKEIRDVSRVAAVETLYQDMAARHRAR 117
L18a-2_Arabidopsis      MLAINEIYEKNPTTIKNFGIWLRYQSRTGYHNMYKEYRDTTLNGAVEQMYTEMASRHRVR 119
                        ::   :: *  *  :****:*:**:**:* ****:* ** :  .**   * :*.:***.*
```

```
L18a_Homo               AHSIQIMKVEEIA-ASKCRRPAVKQFHDSKIKFPLPHRVLRRQH---KPRFTTKRPNTFF 176
L18a_Mus                AHSIQIMKVEEIA-AGKCRRPAVKQFHDSKIKFPLPHRVLRRQH---KPRFTTKRPNTFF 176
L18A_Drosophila         AHSIQIIKVDSIP-AAKTRRVHVKQFHDSKIKFPLVQRVHHKGN---RKLFSFRKPRTYF 176
L20A_Saccharomyces      FRSIHILKVAEIEKTADVKRQYVKQFLTKDLKFPLPHRVQKS-----TKTFSYKRPSTFY 172
L18a-2_Arabidopsis      FPCIQIIKTATVP-AKLCKRESTKQFHNSKIKFPLVFRKVRPPSRKLKTTYKANKPNLFM 178
                          .*:*:*.  :  :   :*  .***  ..:****  *  :         :. .:*  :

L18a_Homo               -
L18a_Mus                -
L18A_Drosophila         Q 177
L20A_Saccharomyces      -
L18a-2_Arabidopsis      -
```

L21A

```
L21_Mus                    MTNTKGKRRGTRYMFSRPFRKHGVVPLATYMRIYKKGDIVDIKGMGTVQK 50
L21_Homo                   MTNTKGKRRGTRYMFSRPFRKHGVVPLATYMRIYKKGDIVDIKGMGTVQK 50
L21_Drosophila             MTNSKGYRRGTRDMFSRPFRKHGVIPLSTYMRVFKIGDIVDIKGHGAVQK 50
RPL-21_Caenorhabditis      MTNSKGLRRGTRYMFARDFRKHGVEHLSTYYTQYKRGDLVDIKTNGAFQK 50
L21A_Saccharomyces         MGKSHGYRSRTRYMFQRDFRKHGAVHLSTYLKVYKVGDIVDIKANGSIQK 50
                           * :::* *  ** ** * *****.  *:**   :* **:****  *:.**

L21_Mus                    GMPHKCYHGKTGRVYNVTQHAVGIIVNKQVKGKILAKRINVRIEHIKHSK 100
L21_Homo                   GMPHKCYHGKTGRVYNVTQHAVGIVVNKQVKGKILAKRINVRIEHIKHSK 100
L21_Drosophila             GLPYKAYHGKTGRIFNVTQHAVGVIVNKRVRGKILAKRVNVRIEHIHHSK 100
RPL-21_Caenorhabditis      GMPFKAYHGRTGRIFNVTRGAVGIIVNKRVRGNILPKRINIRIEHIKPSK 100
L21A_Saccharomyces         GMPHKFYQGKTGVVYNVTKSSVGVIINKMVGNRYLEKRLNLRVEHIKHSK 100
                           *:*.* *:*:** ::***: :**:::** * .. * **:*:*:***: **

L21_Mus                    SRDSFLKRVKENDQKKKEAKEKGTWV-QLKRQPAPPREAHFVRTNGKEPE 149
L21_Homo                   SRDSFLKRVKENDQKKKEAKEKGTWV-QLKRQPAPPREAHFVRTNGKEPE 149
L21_Drosophila             CREDFLRRVKENERLLKEAKEKGQWV-SLKRQPEQPKKAHFVKK-LEEPI 148
RPL-21_Caenorhabditis      CRTDFLNRVKSNDEKRKAAKSAGQPVPALKRLPVAPRGAHTVTTQNNEPE 150
L21A_Saccharomyces         CRQEFLERVKANAAKRAEAKAQGVAV-QLKRQPAQPRESRIVSTEGNVPQ 149
                           .* .**.*** *      **  *  *  *** *  *: :: * .  : *

L21_Mus                    LLEPIPYEFMA 160
L21_Homo                   LLEPIPYEFMA 160
L21_Drosophila             ALAPIPYEFIA 159
RPL-21_Caenorhabditis      LLAPLRFEIVA 161
L21A_Saccharomyces         TLAPVPYETFI 160
                            * *: :* .
```

L22A

```
L22-like_Homo              --------------------------------------------------
L22-like_1_Mus             --------------------------------------------------
RPL-22_a_Caenorhabditis    --------------------------------------------------
L22_Drosophila             MAPTAKTNKGDTKTAAAKPAEKKAAPAAAAAKGKVEKPKAEAAKPAAAAA 50
L22A_Saccharomyces         --------------------------------------------------

L22-like_Homo              --------------------------------------------------
L22-like_1_Mus             --------------------------------------------------
RPL-22_a_Caenorhabditis    --------------------------------------------------
L22_Drosophila             KNVKKASEAAKDVKAAAAAAKPAAAKPAAAKPAAASKDAGKKAPAAAAPK 100
L22A_Saccharomyces         --------------------------------------------------

L22-like_Homo              --------------------------------------------------
L22-like_1_Mus             --------------------------------------------------
RPL-22_a_Caenorhabditis    --------------------------------------------------
L22_Drosophila             KDAKAAAAPAPAKAAPAKKAASTPAAAPPAKKAAPAKAAAPAAAAPAPAA 150
L22A_Saccharomyces         --------------------------------------------------
```

```
L22-like_Homo                -------MAPQ--------------------KDRKPKRSTWRFNLDLTHP 23
L22-like_1_Mus               -------MAPQ--------------------KDKKPKKSTWRFHLDLTHP 23
RPL-22_a_Caenorhabditis      -------MVPKPHAKSA-------------KKALRKKKVHLKFNVECKNP 30
L22_Drosophila               AAPAVAKPAPKPKAKAAPAPSKVVKKNVLRGKGQKKKKVSLRFTIDCTNI 200
L22A_Saccharomyces           -------MAPN--------------------TSRKQKIAKTFTVDVSSP 22
                                     .*:                       : ::    * :: .

L22-like_Homo                VEDGIFDSGNFEQFLREKVKVNGKTGNLG-NVVHIERFKNKITVVSEKQF 72
L22-like_1_Mus               VEDGIFDSGNFEQFLREKVKVNGKTGNLG-NVVHIERLKNKITVVSEKQF 72
RPL-22_a_Caenorhabditis      VEDGILRIEDLEAFLNEKIKVNGKTGHLAANNVKVEVAKSKVSVVSEVPF 80
L22_Drosophila               AEDSIMDVADFEKYIKARLKVNGKVNNLG-NNVTFERSKLKLIVSSDVHF 249
L22A_Saccharomyces           TENGVFDPASYAKYLIDHIKVEGAVGNLG-NAVTVTEDGTVVTVVSTAKF 71
                             .*:.::   .   ::  ::**:* ..:*. * * .      : * *   *

L22-like_Homo                SKRYLKYLTKKYLKKNNLRDWLRVVASDKETYELRYFQISQDEDEGGEGGED 122
L22-like_1_Mus               SKRYLKYLTKKYLKKNNLRDWLRVVASDKETYELRYFQISQDEDGGGEGGED 122
RPL-22_a_Caenorhabditis      SKRYLKYLTKKYLKRNSLRDWLRVVAVNKNTYEVRYFHINDGEDAGGGDHE 130
L22_Drosophila               SKAYLKYLTKKYLKKNSLRDWIRVVANEKDSYELRYFRISSNDDEDDDAE 299
L22A_Saccharomyces           SGKYLKYLTKKYLKKNQLRDWIRFVSTKTNEYRLAFYQVTPEEDEEEDEE 121
                             *  ***********:*.****:*.*: ..: *.: ::::.  :*  .. :
```

L23

```
HumanL23A            -------------------------------------------------------MAPK 4
MusL23a              -------------------------------------------------------MAPK 4
ArabidopsisL23       ---------------------------------------------------------MSP- 3
DrosophilaL23a       MPPKKPTEKSAKPGDKKPEQKKTAAAPAAGKKEAAPSAAKPAAAAPKKAAAPAAKKAAPA 60
CelegansL25.1        -------------------------------------------------------MAPS 4
YeastL25             -------------------------------------------------------MAP- 3
BacillusL23          ------------------------------------------------------------
EcoliL23             ------------------------------------------------------------

HumanL23             AKKEAPA----------PPKAEAKAKALKAKKAVLKG------------------------ 31
MusL23               AKKEAPA----------PPKAEAKAKALKAKKAVLKG------------------------ 31
ArabidopsisL23       AKVDT------------TKKADPKAKALKAAKAVKSGQ----------------------- 29
DrosophilaL23        AKKPATAGAAAKKPAAVKTTAAAKAKSKDAKKKVLAGKKPQSVLAKLSAKARAAAKAKKG 120
CelegansL23          APAKT-------------------AKALDAKKKVVKGK----------------------- 23
YeastL23             ----------------------SAKATAAKKAVVKG------------------------ 17
BacillusL23          ------------------------------------------------------------
EcoliL23             ------------------------------------------------------------

HumanL23             --------------------------------VHSHKKKKIRISPTFRRPKTLRLRRQPK 59
MusL23               --------------------------------VHSHKKKKIRISPTFRRPKTLRLRRQPK 59
ArabidopsisL23       --------------------------------AFKKKDKKIRTKVTFHRPKTLTKPRTGK 57
DrosophilaL23        VKPVTKPAKGTAKAKAVALLNAKKVQKKIIKGAFGTRARKIRTNVHFRRPTTLKLPRSPK 180
CelegansL23          ---------------------------------RTTHRRQVRTSVHFRRPVTLKTARQAR 50
YeastL23             --------------------------------TNGKKALKVRTSATFRLPKTLKLARAPK 45
BacillusL23          ----------------------------------------------------MKDPRD-- 6
EcoliL23             ---------------------------------------------MIREERLLKVLRAPH 15
                                                                        :   *

HumanL23             YPRKSAPRRNKLDHYAIIKFPLTTESAMKKIEDNNTLVFIVDVKANKHQIKQAVKKLYDI 119
MusL23               YPRKSAPRRNKLDHYAIIKFPLTTESAMKKIEDNNTLVFIVDVKANKHQIKQAVKKLYDI 119
ArabidopsisL23       YPKISATPRNKLDHYQILKYPLTTESAMKKIEDNNTLVFIVDIRADKKKIKDAVKKMYDI 117
DrosophilaL23        YPRKSVPTRNRMDAYNIIKYPLTTEAAMKKIEDNNTLVFLTHLRANKNHVRAAVRKLYDI 240
CelegansL23          FPRKSAPKTSKMDHFRIIQHPLTTESAMKKIEEHNTLVFIVSNDANKYQIKDAVHKLYNV 110
YeastL23             YASKAVPHYNRLDSYKVIEQPITSETAMKKVEDGNILVFQVSMKANKYQIKKAVKELYEV 105
BacillusL23          VLKRPVITERSADLMTEEKYTFEVDVRANKTEAKDAVESIFGVKVDKVNIMN-----YKG 61
EcoliL23             VSEKAS---TAMEKSNTIVLKVAKDATKAEIKAAVQKLFEVEVEVVNTLVVKG------- 65
                         .       :        .  :    : :            . :  :
```

```
HumanL23            DVAKVNTLIRPDGEK-KAYVRLAPDYDALDVANKIGII 156
MusL23              DVAKVNTLIRPDGEK-KAYVRLAPDYDALDVANKIGII 156
ArabidopsisL23      QTKKVNTLIRPDGTK-KAYVRLTPDYDALDVANKIGII 154
DrosophilaL23       KVAKVNVLIRPDGQK-KAYVRLARDYDALDIANKIGII 277
CelegansL23         QALKVNTLITPLQQK-KAYVRLTADYDALDVANKIGVI 147
YeastL23            DVLKVNTLVRPNGTK-KAYVRLTADYDALDIANRIGYI 142
BacillusL23         KSKRVGRYTGMTSRRRKAIVKLTADSKEIEIFEA---- 95
EcoliL23            KVKRHGQRIGRRSDWKKAYVTLKEG-QNLDFVGGAE-- 100
                    .  : .          ** * *  . . ::.
```

L24A

```
L24_Homo                    --MKVELCSFSGYKIYPGHGRRYARTDGKVFQFLNAKCESAFLSKRNPRQ 48
L24_Drosophila              --MKIGLCAFSGYKIYPGHGKTMVKIDGKSFTFLDKKCERSYLMKRNPRK 48
RPL-24.1_Caenorhabditis     --MKVETCVYSGYKIHPGHGKRLVRTDGKVQIFLSGKALKGAKLRRNPRD 48
L24-2_Arabidopsis           MVLKTELCRFSGQKIYPGRGIRFIRSDSQVFLFLNSKCKRYFHNKLKPSK 50
L24A_Saccharomyces          --MKVEIDSFSGAKIYPGRGTLFVRGDSKIFRFQNSKSASLFKQRKNPRR 48
RLP24_Mus                   --MRIEKCYFCSGPIYPGHGMMFVRNDCKVFRFCKSKCHKNFKKKRNPRK 48
                              ::     :..  *:**:*    : * :   * . *.      : :*

L24_Homo                    INWTVLYRRKHKKGQS--EEIQKKRTRRAVKFQ-RAITGASLADIMAKRN 95
L24_Drosophila              VTWTVLYRRKHRKGIE--EEASKKRTRRTQKFQ-RAIVGASLAEILAKRN 95
RPL-24.1_Caenorhabditis     IRWTVLYRIKNKKGTHGQEQVTRKKTKKSVQVVNRAVAGLSLDAILAKRN 98
L24-2_Arabidopsis           LAWTAMYRKQHKKDAA--QEAVKRRRRATKKPYSRSIVGATLEVIQKKRA 98
L24A_Saccharomyces          IAWTVLFRKHHKKGIT--EEVAKKRSRKTVK-AQRPITGASLDLIKERRS 95
RLP24_Mus                   VRWTKAFRKAAGKELTVDNSFEFEKRRNEPVKYQRELWNKTIDAMKRVEE 98
                            : **  :*    *     :.   .: :       * : . ::  :   .

L24_Homo                    QKPEVRKAQREQAIRAAKEAKKAKQASKKTAMAAAKAPTKAAPKQKIVKP 145
L24_Drosophila              MKPEVRKAQRDQAIKVAKEQKRAVKAAKKAAAPAP--AKKSAPKQKAAKV 143
RPL-24.1_Caenorhabditis     QTEDFRRQQREQAAKIAKDANKAVRAAKAAANKEKK-ASQPKTQQKTAKN 147
L24-2_Arabidopsis           EKPEVRDAAREAALREIKERIKKTKDEKKAKKVEFASKQQKVKANFPKAA 148
L24A_Saccharomyces          LKPEVRKANREEKLKANKEKKKAEKAARKAEKAKSAGTQS---SKFSKQQ 142
RLP24_Mus                   IKQKRQAKFIMNRLKKNKELQKVQDIKEVKQNIHLIRAPLAGKGKQLEEK 148
                             . . :        :  *:  :     .                :

L24_Homo                    VKVSAPRVGGKR--- 157
L24_Drosophila              TQKAAPRVGGKR--- 155
RPL-24.1_Caenorhabditis     VKTAAPRVGGKR--- 159
L24-2_Arabidopsis           AASKGPKVGGGGGKR 163
L24A_Saccharomyces          AKGAFQKVAATSR-- 155
RLP24_Mus                   MVQQLQEDVDMEEAS 163
```

L25

```
L23A_Drosophila             MPPKKPTEKSAKPGDKKPEQKKTAAAPAAGKKEAAPSAAKPAAAAPKKAA 50
L23a-2_Arabidopsis          --------------------------------------------------
L23a_Homo                   --------------------------------------------------
RPL-25.2_Caenorhabditis     --------------------------------------------------
L25_Saccharomyces           --------------------------------------------------

L23A_Drosophila             APAAKKAAPAAKKPATAGAAAKKPAAVKTTAAAKAKSKDAKKKVLAGKKP 100
L23a-2_Arabidopsis          ----------------------MSPAKVDVTKKADPK--------------- 15
L23a_Homo                   ------------------MAPKAKKEAPAPPKAEAK--------------- 18
RPL-25.2_Caenorhabditis     ----------------------MAPSSNKVG-------------------- 9
L25_Saccharomyces           ----------------------MAPS------------------------- 4

L23A_Drosophila             QSVLAKLSAKARAAAKAKKGVKPVTKPAKGTAKAKAVALLNAKKVQKKII 150
L23a-2_Arabidopsis          --------AKALKAAKAVKSGQIVKKPAK---------------------- 36
L23a_Homo                   --------AKALKAKKAVLKGVHSHKKKK---------------------- 39
RPL-25.2_Caenorhabditis     ---------KAIQAKKAVVKGSKTNVRKN---------------------- 29
```

```
L25_Saccharomyces            --------AKATAAKKAVVKGTNGKKALK-------------------- 25
                                     **  * **           :

L23A_Drosophila              KGAFGTRARKIRTNVHFRRPTTLKLPRSPKYPRKSVPTRNRMDAYNIIKY 200
L23a-2_Arabidopsis           ---------KIRTKVTFHRPKTLTVPRKPKYPKISATPRNKLDHYQILKY 77
L23a_Homo                    ----------IRTSPTFRRPKTLRLRRQPKYPRKSAPRRNKLDHYAIIKF 79
RPL-25.2_Caenorhabditis      ----------VRTSVHFRRPKTLVTARAPRYARKSAPARDKLDSFAVIKA 69
L25_Saccharomyces            ----------VRTSATFRLPKTLKLARAPKYASKAVPHYNRLDSYKVIEQ 65
                                      :**.  *: *.**   * *:*.  :..  :::* : :::

L23A_Drosophila              PLTTEAAMKKIEDNNTLVFLTHLRANKNHVRAAVRKLYDIKVAKVNVLIR 250
L23a-2_Arabidopsis           PLTTESAMKKIEDNNTLVFIVDIRADKKKIKDAVKKMYDIQTKKVNTLIR 127
L23a_Homo                    PLTTESAMKKIEDNNTLVFIVDVKANKHQIKQAVKKLYDIDVAKVNTLIR 129
RPL-25.2_Caenorhabditis      PHTTESSMKKIEDHNTLVFIVDEKANKHHIKRAVHALYNVKAVKVNTLIT 119
L25_Saccharomyces            PITSETAMKKVEDGNILVFQVSMKANKYQIKKAVKELYEVDVLKVNTLVR 115
                             * *:*::***:** * *** .  :*:* ::: **: :*::.. ***.*:

L23A_Drosophila              PDGQKKAYVRLARDYDALDIANKIGII 277
L23a-2_Arabidopsis           PDGTKKAYVRLTPDYDALDVANKIGII 154
L23a_Homo                    PDGEKKAYVRLAPDYDALDVANKIGII 156
RPL-25.2_Caenorhabditis      PLQQKKAYVRLASDYDALDVANKIGFI 146
L25_Saccharomyces            PNGTKKAYVRLTADYDALDIANRIGYI 142
                             *   *******: ******:**:** *
```

L26A

```
HumanL26          -MKFNPFVTSDRSKNRKRHFNAPSHIRRKIMSSPLSKELRQKYNVRSMPIRKDDEVQVVR 59
MusL26            -MKFNPFVTSDRSKNRKRHFNAPSHIRRKIMSSPLSKELRQKYNVRSMPIRKDDEVQVVR 59
DrosophilaL26     -MKQNPFVSSSRRKNRKRHFQAPSHIRRRLMSAPLSKELRQKYNVRSMPIRRDDEVQVIR 59
CelegansL26       -MKVNPFVSSDSGKSRKAHFNAPSHERRRIMSAPLTKELRTKHGIRAIPIRTDDEVVVMR 59
YeastL26          MAKQSLDVSSDRRKARKAYFTAPSSQRRVLLSAPLSKELRAQYGIKALPIRRDDEVLVVR 60
                    * .  *:*.  * ** :* ***  ** ::*:**:**** ::.::::*** **** *:*
                                 Serine conserved, buried

HumanL26          GHYKGQQIGKVVQVYRKKYVIYIERVQREKANGTTVHVGIHPSKVVITRLKLDKDRKKIL 119
MusL26            GHYKGQQIGKVVQVYRKKYVIYIERVQREKANGTTVHVGIHPSKVVITRLKLDKDRKKIL 119
DrosophilaL26     GHFKGNQVGKVVQAYRKKFVVYVEKIQRENANGTNVYVGIHPSKVLIVKLKLDKDRKAIL 119
CelegansL26       GRHKGN-TGRVLRCYRKKFVIHIDKITREKANGSTVHIGIHPSKVAITKLKLDKDRRALV 118
YeastL26          GKKGQ-EGKISSVYRLKFAVQVDKVTKEKVNGASVPINLHPSKLVITKLHLDKDRKALI 119
                  *  **:  *::   ** *:.: :::: :*:.**:.* :.:****: *.:*:*****: ::

HumanL26          ERKAKSR--QVGKEKGKYKEETIEKMQE-- 145
MusL26            ERKAKSR--QVGKEKGKYKEETIEKMQE-- 145
DrosophilaL26     ERRGKGRLAALGKDKGKYTEETAAQPMETA 149
CelegansL26       ERKAAGRSRVTGILKGKHTDETVN------ 142
YeastL26          QR------------KGGKLE---------- 127
```

L27

```
L27_Homo                  MGKFMKPGKVVLVLAGRYSGRKAVIVKNIDDGTSDRPYSHALVAGIDRYP 50
L27_Mus                   MGKFMKPGKVVLVLAGRYSGRKAVIVKNIDDGTSDRPYSHALVAGIDRYP 50
RPL-27_Caenorhabditis     MGKIMKPGKVVLVLRGKYAGRKAVVVKQQDEGVSDRTYPHAIIAGIDRYP 50
L27_A_Drosophila          MRKIMKQGKIVIVLSGRYAGRKAIIVKTHDDGTPEKPFGHALVAGIDRYP 50
L27A_Saccharomyces        MAKFLKAGKVAVVVRGRYAGKKVVIVKPHDEGSKSHPFGHALVAGIERYP 50
L27-3_Arabidopsis         MVKFLKQNKAVILLQGRYAGKKAVIIKSFDDGNRDRPYGHCLVAGLKKYP 50
```

```
                          * *::* .* .::: *:*:*:*.:::*  *:*  .:.: *.::**:.:**


L27_Homo                  RKVTAAMGKKKIAKRSKIKSFVKVYNYNHLMPTRYSV-DIPLDKTVVNKD 99
L27_Mus                   RKVTAAMGKKKIAKRSKIKSFVKVYNYNHLMPTRYSV-DIPLDKTVVNKD 99
RPL-27_Caenorhabditis     LKVTKDMGKKKIEKRNKLKPFLKVVSYTHLLPTRYSV-DVAFDKTNINKE 99
L27_A_Drosophila          RKVTKKMGKNKLKKKSKVKPFLKSLNYNHLMPTRYTAHDISFEK--LSPK 98
L27A_Saccharomyces        LKVTKKHGAKKVAKRTKIKPFIKVVNYNHLLPTRYTL-DVEAFKSVVSTE 99
L27-3_Arabidopsis         SKVIRKDSAKKTAKKSRVKCFIKLVNYQHLMPTRYTL-DVDLKE-VATLD 98
                           **    . :*  *:.::* *:*  .* **:****:  *:   :   . .

L27_Homo                  VFRDPALKRKARREAKVKFEERYKTGKNKWFFQKLRF 136
L27_Mus                   VFRDPALKRKARREAKVKFEERYKTGKNKWFFQKLRF 136
RPL-27_Caenorhabditis     ALKAPSKKRKALVEVKSKFEERYKTGKNKWFFTKLRF 136
L27_A_Drosophila          DLKDPVKRKTHRFQTRVKFESVYKEGKNKWFFQKLRF 135
L27A_Saccharomyces        TFEQPSQREEAKKVVKKAFEERHQAGKNQWFFSKLRF 136
L27-3_Arabidopsis         ALQSKDKKVAALKEAKAKLEERFKTGKNRWFFTKLRF 135
                           :.    :      .:  :*. .: ***:*** ****
```

L28

```
L27a_Homo                   MPSRLRKTRKLRGHVSHGHGRIGKHRKHPGGRGNAGGLHHHRINFDKYHP 50
L27a_Mus                    MPSRLRKTRKLRGHVSHGHGRIGKHRKHPGGRGNAGGMHHHRINFDKYHP 50
Y37E3.8_a_Caenorhabditis    MAHALRKTRKLRGHVSHGHGRIGKHRKHPGGRGNAGGQHHHRINRDKYHP 50
L27a-3_Arabidopsis          MTTRFKKNRKKRGHVSAGHGRIGKHRKHPGGRGNAGGMHHHRILFDKYHP 50
L28_Saccharomyces           MPSRFTKTRKHRGHVSAGKGRIGKHRKHPGGRGMAGGQHHHRINMDKYHP 50
                            *.  : *.** ***** *:************** *** *****  *****

L27a_Homo                   GYFGKVGMKHYHLKRNQSFCPTVNLDKLWTLVSEQTRVNAAKNKTGA-AP 99
L27a_Mus                    GYFGKVGMRHYHLKRNQSFCPTVNLDKLWTLVSEQTRVNAAKNKTGV-AP 99
Y37E3.8_a_Caenorhabditis    GYFGKVGMRVFHLNKNQHYCPTVNVERLWSLVPQEVRDKATGGKS----P 96
L27a-3_Arabidopsis          GYFGKVGMRYFHKLRNKFFCPIVNLDKLWSLVPEDVKAKSTKDNV----P 96
L28_Saccharomyces           GYFGKVGMRYFHKQQAHFWKPVLNLDKLWTLIPEDKRDQYLKSASKETAP 100
                            ********: :*  : : : * :*:::**:*:.:: : :   .      *

L27a_Homo                   IIDVVRSGYYKVLGKGKLPK-QPVIVKAKFFSRRAEEKIKSVGGACVLVA 148
L27a_Mus                    IIDVVRSGYYKVLGKGKLPK-QPVIVKAKFFSRRAEEKIKGVGGACVLVA 148
Y37E3.8_a_Caenorhabditis    VIDCTKLGYFKVLGKGLLPE-TPLIVKARFFSHEAEQKIKKAGGACVLVA 145
L27a-3_Arabidopsis          LIDVTQHGFFKVLGKGHLPENKPFVVKAKLISKTAEKKIKEAGGAVVLTA 146
L28_Saccharomyces           VIDTLAAGYGKILGKGRIPN-VPVIVKARFVSKLAEEKIRAAGGVVELIA 149
                            :**    *: *:**** :*:  *.:***::.*: **:**: .**.  * *
```

L29

```
L29_Homo                  MAKSKNHTTHNQSRKWHRNGIKKPRSQRYESLKGVDPKFLRN-------- 42
L29_Mus                   MAKSKNHTTHNQSRKWHRNGIKKPRSQRYESLKGVDPKFLRN-------- 42
L29_Saccharomyces         MAKSKNHTAHNQTRKAHRNGIKKPKTYKYPSLKGVDPKFRRNHKHALHGT 50
RPL-29_Caenorhabditis     MAKSKNHTNHNQNKKAHRNGITKPKKHIFLSMKGVDAKFIKNLRFSRKNN 50
L29_A_Drosophila          MAKSKNHTNHNQNKKAHRNGIKRP-------------------------- 24
L29-2_Arabidopsis         MAKSKNHTAHNQSAKAHKNGIKKPRRHRHTPTRGMDPKFLRNQRYARKH- 49
                          ******** ***. * *:***.:*

L29_Homo                  ---------
L29_Mus                   ---------
L29_Saccharomyces         AKALAAAKK 59
RPL-29_Caenorhabditis     K-------- 51
L29_A_Drosophila          ---------
L29-2_Arabidopsis         ---------
```

L30

```
RPL-30_Caenorhabditis      MAPAAKPQVKKNAENINSRLSMVMKTGQYVLGYKQTLKSLLNGKAKLVII 50
L30_Homo                   ----------KSLESINSRLQLVMKSGKYVLGYKQTLKMIRQGKAKLVIL 40
L30_Drosophila             MVAVKK--QKKALESTNARLALVMKSGKYCLGYKQTLKTLRQGKAKLVLI 48
L30-2_Arabidopsis          ----------KSHEGINSRLALVMKSGKYTLGYKSVLKSLRGSKGKLILI 40
L30_Saccharomyces          ------MAPVKSQESINQKLALVIKSGKYTLGYKSTVKSLRQGKSKLIII 44
                                    *  *. * :* :*:*:*:* ****..:* :  .*.**:::

RPL-30_Caenorhabditis      ANNTPPLRKSEIEYYAMLAKTGVHHYNGNNIELGTACGRLFRVCTLAVTD 100
L30_Homo                   ANNCPALRKSEIEYYAMLAKTGVHHYSGNNIELGTACGKYYRVCTLAIID 90
L30_Drosophila             ASNTPALRKSEIEYYAMLAKTEVQHYSGTNIELGTACGKYFRVCTLSITD 98
L30-2_Arabidopsis          STNCPPLRRSEIEYYAMLAKVGVHHYNGNNVDLGTACGKYFRVSCLSIVD 90
L30_Saccharomyces          AANTPVLRKSELEYYAMLSKTKVYYFQGGNNELGTAVGKLFRVGVVSILE 94
                           : * * **:**:******:*. * ::.* * :**** *: :**  ::: :

RPL-30_Caenorhabditis      AGDSDI----- 106
L30_Homo                   PGDSDI----- 96
L30_Drosophila             PGDSDI----- 104
L30-2_Arabidopsis          PGDSDI----- 96
L30_Saccharomyces          AGDSDILTTLA 105
                           .*****
```

L31

```
HumanL31            MAPAKKGGEKKKGRSAINEVVTREYTINIHKRIHGVGFKKRAPRALKEIRKFAMKEMGTP 60
MusL31              MAPAKKGGEKKKGRSAINEVVTREYTINIHKRIHGVGFKKRAPRALKEIRKFAMKEMGTP 60
DrosophilaL31       MTKTK--GEKIN-KSAINEVVTRECTIHLAKRVHNIGFKKRAPRAIKEIRKFAEREMGTT 57
CelegansL31         -MAPK--NEKKS-RGTINEVVTREYTIHIHARIRGIGSKKRAPRAIDEIKKFAKIQMKTN 56
Yeast               -------------MAGLKDVVTREYTINLHKRLHGVSFKKRAPRAVKEIKKFAKLHMGTD 47
                                 : :::***** **::  *::.:. *******:.**:***  .* *

HumanL31            DVRIDTRLNKAVWAKGIRNVPYRIRVRLSRKRNEDEDSPNKLYTLVTYVPVTTFKNLQTV 120
MusL31              DVRIDTRLNKAVWAKGIRNVPYRIRVRLSRKRNEDEDSPNKLYTLVTYVPVTTFKNLQTV 120
DrosophilaL31       DVRIDTRLNKHIWSKGIRSTPFRIRVRLARRRNDDEDSPNKLYTTVTYVPVSTFKNLQTE 117
CelegansL31         DVRVDTKLNKFIWSKGIKNVPYRVRVRLSRRRNEDEDSAQKLYTLCTYVPCTNFHGLTNV 116
Yeast               DVRLAPELNQAIWKRGVKGVEYRLRLRISRKRNEEEDAKNPLFSYVEPVLVASAKGLQTV 107
                    ***: ..**: :* :*::.. :*:*:*::*:**::**: : *::    *  :. :.* .

This region looks important
HumanL31            NVDEN-- 125
MusL31              NVDEN-- 125
DrosophilaL31       NVESSDD 124
CelegansL31         NVDSEE- 122
Yeast               VVEEDA- 113
                     *:..
```

L32

```
L32_Homo                   MAALRPLVKPKIVKKRTKKFIRHQSDRYVKIKRNWRKPRGIDNRVRRRFK 50
L32-like_Mus               MAALRPLVKPKIVKKRTKKFIRHQSDRYVKIKRNWRKPRGIDHRVRR--R 48
L32_A_Drosophila           ---------PKIVKKRTKHFIRHQSDRYAKLSHKWRKPKGIDNRVRRRFK 41
L32-1_Arabidopsis          ----------KVVKKRSAKFIRPQSDRRITVKESWRRPKGIDSRVRRKFK 40
RPL-32_Caenorhabditis      ----------KVVKKKLTKFKRHESDRYRRVAPSWRKPKGIDNRVRRRFR 40
L32_Saccharomyces          ---MASLPHPKIVKKHTKKFKRHHSDRYHRVAENWRKQKGIDSVVRRRFR 47
                                    *:***:  :* * .***   :  .**: :***  ***  :
```

```
L32_Homo                  GQILMPNIGYGGNKKTKHMLPSGFRKFLVHNVKELEVLLMCNKSYCAEIA 100
L32-like_Mus              GQILMPNIGYGGNKKTKHTLPSSFRKFLVHNVKELEVLLMCNRSYCAEIA 98
L32_A_Drosophila          GQYLMPNIGYGSNKRTRHMLPTGFKKFLVHNVRELEVLLMQNRVYCGEIA 91
L32-1_Arabidopsis         GVTLMPNVGYGSDKKTRHYLPNGFKKFVVHNTSELELLMMHNRTYCAEIA 90
RPL-32_Caenorhabditis     GMRAMPTIGHGSDRRTRFVLPNGYKKVLVQNVKDLDMLLMQSYKYIGEIG 90
L32_Saccharomyces         GNISQPKIGYGSNKKTKFLSPSGHKTFLVANVKDLETLTMHTKTYAAEIA 97
                          *    *.:*:**:::*:.  *...:..:* *. :*: * * .  * .**.

L32_Homo                  HNVSSKNRKAIVERAAQLAIRVTNPNARL---- 129
L32-like_Mus              HKVTSKNRKAIVERAAQLAIRVTNPNARL---- 127
L32_A_Drosophila          HGVSSKKRKEIVERAKQLSVRLTNPNGRL---- 120
L32-1_Arabidopsis         HNVSTKKRKAIVERASQLDVVVTN---RLA--- 117
RPL-32_Caenorhabditis     HGVSAKSRKGIVERAAQLNIKLTNGNARL---- 119
L32_Saccharomyces         HNISAKNRVVILARAKALGIKVTNPKGRLALEA 130
                          * :::*.*  *: **  * : :**   **
```

L33-A

```
RPL-33_Caenorhabditis     -----RLYVKAIFTGFKRGLRTQSEHTSLLKLEGVFNKEDAGFYAGKRVV 45
L35A_Drosophila           ------------------------------KIEGARRKEHGSFYVGKRCV 20
L35a_Mus                  ----------------------------LLKIEGVYARDETEFYLGKRCA 22
L35a_Homo                 ----------------------------LLKIEGVYARDETEFYLGKRCA 22
L33A_Saccharomyces        MAESHRLYVKGKHLSYQRSKRVNNPNVSLIKIEGVATPQDAQFYLGKRIA 50
L35a-2_Arabidopsis        -----RLYVRGTVLGYKRSKSNQYPNTSLIQIEGVNTQEEVNWYKGKRLA 45
                                                      ::**.   :.  :* *** .

RPL-33_Caenorhabditis     YLYKAHNKTLKTGHT-VATRTRAIWGKITRPHGNAGAVRAKFHHNIPPSA 94
L35A_Drosophila           YVYKAETKKCVPQHPERKTRVRAVWGKVTRIHGNTGAVRARFNRNLPGHA 70
L35a_Mus                  YVYKAKNNTVTPGGK--PNKTRVIWGKVTRAHGNSGMVRAKFRSNLPAKA 70
L35a_Homo                 YVYKAKNNTVTPGGK--PNKTRVIWGKVTRAHGNSGMVRAKFRSNLPAKA 70
L33A_Saccharomyces        YVYRASKEVR-------GSKIRVMWGKVTRTHGNSGVVRATFRNNLPAKT 93
L35a-2_Arabidopsis        YIYKAKTKKN-------GSHYRCIWGKVTRPHGNSGVVRSKFTSNLPPKS 88
                          *:*:* .:          .: * :***:** ***:* **: *  *:*  :

RPL-33_Caenorhabditis     LGKRIRVLLYPSNI 108
L35A_Drosophila           MGHRIRIMLYPSRI 84
L35a_Mus                  IGHRIRVMLYPSRI 84
L35a_Homo                 IGHRIRVMLYPSRI 84
L33A_Saccharomyces        FGASVRIFLYPSNI 107
L35a-2_Arabidopsis        MGARVRVFMYPSNI 102
                          :*  :*:::***.*
```

L34A

```
RPL-34_Caenorhabditis     MSLRVTYRRRLSYNTTSNKKRLVKTPGGRLVVQYIKKRGQIPK--CRDTG 48
L34-1_Arabidopsis         MVQRLVYRSRHSYATKSNQHRIVKTPGGKLVYQTTKKRASGPK--CPVTG 48
L34A_Saccharomyces        MAQRVTFRRRNPYNTRSNKIKVVKTPGGILRAQHVKKLATRPK--CGDCG 48
L34_2_Mus                 MVQRLTYRRRLSYNTASNKTRLSRTPGNRIVYLYTKKVGKAPKSACGVCP 50
L34_Homo                  MVQRLTYRRRLSYNTASNKTRLSRTPGNRIVYLYTKKVGKAPKSACGVCP 50
L34a_Drosophila           MVQRLTLRRRLSYNTRSNKRRIVRTPGGRLVYQYVKKNPTVPR--CGQCK 48
                          *  *:. * * .* * **: :: :***. :     **    *:  *

RPL-34_Caenorhabditis     VKLHGITPARPIALR--LLKRNERTVTRAYGGCLSPNAVKERITRAFLVE 96
L34-1_Arabidopsis         KRIQGIPHLRPSEYKRSRLSRNRRTVNRAYGGVLSGSAVRERIIRAFLVE 98
L34A_Saccharomyces        SALQGISTLRPRQYA--TVSKTHKTVSRAYGGSRCANCVKERIIRAFLIE 96
L34_2_Mus                 GRLRGVRAVRPKVLM--RLSKTQKHVSRAYGGSMCAKCVRDRIKRAFLIE 98
L34_Homo                  GRLRGVRAVRPKVLM--RLSKTKKHVSRAYGGSMCAKCVRDRIKRAFLIE 98
L34a_Drosophila           EKLHGITPSRPSERP--RMSKRLKTVSRTYGGVLCHSCLRERIVRAFLIE 96
                            ::*:   **       :.:  : *.*:***  . ..:::** ****:*

RPL-34_Caenorhabditis     EQKIVN----------KVIKHQ------------- 108
L34-1_Arabidopsis         EQKIVK----------KVLKLQKAKEKVAPKA--- 120
L34A_Saccharomyces        EQKIVK----------KVVKEQTEAAKKSEKKAKK 121
```

```
L34_2_Mus                  EQKIV----------VKVLKAQAQSQKAK------ 117
L34_Homo                   EQKIV----------VKVLKAQAQSQKAK------ 117
L34a_Drosophila            EQKIVKALKSQREALVKPVKKVVEKKPVKAAAKK- 130
                           *****           * :*
```

L35

```
HumanL35           MAKIKARDLRGKKKEELLKQLDDLKVELSQLRVAKVTGGAASKLSKIRVVRKSIARVLTV 60
MusL35             MAKIKARDLRGKKKEELLKQLDDLKVELSQLRVAKVTGGAASKLSKIRVVRKSIARVLTV 60
CelegansL35        MTKLKCKSLRGEKKDALQKKLDEQKTELATLRVSKVTGGAASKLSKIRVVRKNIARLLTV 60
DrosophilaL35      MVKVKCSELRIKDKKELTKQLDELKNELLSLRVAKVTGGAPSKLSKIRVVRKAIARVYIV 60
YeastL35           MAGVKAYELRTKSKEQLASQLVDLKKELAELKVQKLS---RPSLPKIKTVRKSIACVLTV 57
                   *. :*. .** :.*. * .:* : * **  *:* *::    ..*.**:.*** ** :  *

HumanL35           INQTQKENLRKFYKGKKYKPLDLRPKKTRAMRRRLNKHEENLKTKKQQRKERLYPLRKYA 120
MusL35             INQTQKENLRKFYKGKKYKPLDLRPKKTRAMRRRLTKHEEKLKTKKQQRKERLYPLRKYA 120
CelegansL35        INQTQKQELRKFYADHKYKPIDLRLKKTRAIRRRLTAHELSLRSAKQQAKSRNQAVRKFA 120
DrosophilaL35      MHQKQKENLRKVFKNKKYKPLDLRKKKTRAIRKALSPRDANRKTLKEIRKRSVFPQRKFA 120
YeastL35           INEQQREAVRQLYKGKKYQPKDLRAKKTRALRRALTKFEASQVTEKQRKKQIAFPQRKYA 117
                   ::: *:: :*:.: .:**:* *** *****:*: *.  : .  : *:  *    . **:*

HumanL35           VKA 123
MusL35             VKA 123
CelegansL35        VKA 123
DrosophilaL35      VKA 123
YeastL35           IKA 120
```

L36

```
L36_Homo                   ----------AVGLNKGHKVT----------KNVSKPRHSRRRGRLTKHT 30
L36_A_Drosophila           ---MAVRYELAIGLNKGHKTSKIRNVKYTGDKKVKGLRGSRLKNIQTRHT 47
RPL-36_Caenorhabditis      --------GLAVGLNKGHAAT----------QLPVKQRQNRHKGVASKKT 32
L36-1_Arabidopsis          MTTPAVKTGLFVGLNKGHVVT----------RRELAPRPNSRKGKTSKRT 40
L36A_Saccharomyces         ---MTVKTGIAIGLNKGKKVT----------SMTPAPKISYKKGAASNRT 37
                                     :*****: .:                : .  :.  :.:*

L36_Homo                   KFVRDMIREVCGFAPYERRAMELLKVSKDKRALKFIKKRVGTHIRAKRKR 80
L36_A_Drosophila           KFMRDLVREVVGHAPYEKRTMELLKVSKDKRALKFLKRRLGTHIRAKRKR 97
RPL-36_Caenorhabditis      KIVRELVREITGFAPYERRVLEMLRISKDKRALKFLKRRIGTHRRAKGKR 82
L36-1_Arabidopsis          IFIRKLIREVAGMAPYEKRITELLKVGKDKRALKVAKRKLGTHKRAKRKR 90
L36A_Saccharomyces         KFVRSLVREIAGLSPYERRLIDLIRNSGEKRARKVAKKRLGSFTRAKAKV 87
                            ::*.::**: * :***:*  :::: . :*** *. *:::*:. *** *

L36_Homo                   EELSNVLAAMR-- 91
L36_A_Drosophila           EELSNI------- 103
RPL-36_Caenorhabditis      EELQNVIIAQR-- 93
L36-1_Arabidopsis          EEMSSV------- 96
L36A_Saccharomyces         EEMNNIIAASRRH 100
                           **:..:
```

L37

```
HumanL37           MTKGTSSFGKRRNKTHTLCRRCGSKAYHLQKSTCGKCGYPAKRKRKYNWSAKAKRRNTTG 60
MusL37             MTKGTSSFGKRRNKTHTLCRRCGSKAYHLQKSTCGKCGYPAKRKRKYNWSAKAKRRNTTG 60
DrosophilaL37      MTKGTSSFGKRHNKTHTLCRRCGRSSYHIQKSTCAQCGYPAAKLRSYNWSVKAKRRKTTG 60
YeastL37           MGKGTPSFGKRHNKSHTLCNRCGRRSFHVQKKTCSSCGYPAAKTRSYNWGAKAKRRHTTG 60
CelegansL37        MTKGTQAFGKKHVKSHTLCKRCGKSSFHIQKKRCASCGYQDAKKRTYNWGAKSIRRRTTG 60
                   * *** :***:: *:****.***  ::*:**. *..***   : *.***..*: **.***
```

```
HumanL37          TGRMRHLKIVYRRFRHGFREGTIPKPKRAAVAASSSS 97
MusL37            TGRMRHLKIVYRRFRHGFREGTTPKPKRAAVAASSSS 97
DrosophilaL37     TGRMQHLKVVRRRFRNGFREGTQAKPKKAVASK---- 93
YeastL37          TGRMRYLKHVSRRFKNGFQTGSASKASA--------- 88
CelegansL37       TGRTRHLRDVNARFRNGFR-GTTPKPRAQPTN----- 91
                  *** ::*: *  **::**: *: .*.
```

Buried, conserved

L38

```
RPL-38_Caenorhabditis     MPKEIKEIKDFLVKARRKDAKSVKIKKNSNN---------TKFKVRCASY 41
L38_A_Drosophila          MPREIKEVKDFLNKARRSDARAVKIKKNPTN---------TKFKIRCSRF 41
L38_Mus                   MPRKIEEIKDFLLTARRKDAKSVKIKKNKDN---------VKFKVRCSRY 41
L38_Homo                  MPRKIEEIKDFLLTARRKDAKSVKIKKNKDN---------VKFKVRCSRY 41
L38_Arabidopsis           MPKQIHEIKDFLLTARRKDARSVKIKRSKDI---------VKFKVRCSRY 41
L38_Saccharomyces         MAREITDIKQFLELTRRADVKTATVKINKKLNKAGKPFRQTKFKVRGSSS 50
                          *.::* ::*:**  :** *.::..:* .             .***:* :

RPL-38_Caenorhabditis     LYTLVVADKDKAEKLKQSLPP------- 62
L38_A_Drosophila          LYTLVVQDKEKADKIKQSLPPGLQV--- 66
L38_Mus                   LYTLVITDKEKAEKLKQSLPPGL----- 64
L38_Homo                  LYTLVITDKEKAEKLKQSLPPGL----- 64
L38_Arabidopsis           LYTLCVFDQEKADKLKQSLPPGLSV--- 66
L38_Saccharomyces         LYTLVINDAGKAKKLIQSLPPTLKVNRL 78
                          **** : *  **.*: *****
```

L39

```
Drosophila39      MAAHKSFRIKQKLAKKLKQNRSVPQWVRLRTGNTIRYNAKRRHWRRTKLKL 51
Yeast39           MAAQKSFRIKQKMAKAKKQNRPLPQWIRLRTNNTIRYNAKRRNWRRTKMNI 51
HumanL39          MSSHKTFRIKRFLAKKQKQNRPIPQWIRMKTGNKIRYNSKRRHWRRTKLGL 51
MusL39            MSSHKTFRIKRFLAKKQKQNRPIPQWIRMKTGNKIRYNSKRRHWRRTKLGL 51
Celegans39        MSALKKSFIKRKLAKKQKQNRPMPQWVRMKTGNTMKYNAKRRHWRRTKLKL 51
                  *:: *.  **: :**  ****.:***:*::*.*.::**:***:*****: :
```

No modification site

L41

```
L41_Mus                 MRAKWRKKRMRRLKRKRRKMRQRSK 25
L41_Arabidopsis         MRAKWKKKRMRRLKRKRRKMRQRSK 25
L41_Drosophila          MRAKWRKKRMRRLKRKRRKMRARSK 25
L41A_Saccharomyces      MRAKWRKKRTRRLKRKRRKVRARSK 25
                        *****:*** *********:* ***
```

PHOSPHORYLATION FROM MY MASS SPEC

```
HumanL5         MGFVKVVKNKAYFKRYQVKFRRRREGKTDYYARKRLVIQDKNKYNTPKYRMIVRVTNRDI 60
MusL5           MGFVKVVKNKAYFKRYQVRFRRRREGKTDYYARKRLVIQDKNKYNTPKYRMIVRVTNRDI 60
Drosophila      MGFVKVVKNKQYFKRYQVKFRRRREGKTDYYARKRLIFQDKNKYNTPKYRLIVRLSNKDI 60
CelegansL5      MGLVKVIKNKAYFKRYQVKLRRRREGKTDYYARKRLTVQDKNKYNTPKYRLIVRITNKDV 60
ArabiL5         MVFVKSSKSNAYFKRYQVKFRRRRDGKTDYRARIRLINQDKNKYNTPKYRFVVRFTNKDI 60
YeastL5         MAFQKDAKSSAYSSRFQTPFRRRREGKTDYYQRKRLVTQHKAKYNTPKYRLVVRFTNKDI 60
BacillusL5      -MITKTSKNAARLKR-HARVRAKLSGTAE------------------RPRLNVFRSYKHI 40
EcoliL5         -----MDKKSARIRR-ATRARRKLQELG-------------------ATRLVVHRTPRHI 35
                       *.     *  .  * : .                       *: *  : :.:

HumanL5         ICQIAYARIEGDMIVCAAYAHELPKYGVKVGLTNYAAAYCTGLLLARRLLNRFGMDKIYE 120
MusL5           ICQIAYARIEGDMIVCAAYAHELPKYGVKVGLTNYAAAYCTGLLLARRLLNRFGMDKIYE 120
Drosophila      TVQIAYARIEGDRVVCAAYSHELPKYGIQVGLTNYAAAYCTGLLVARRVLNKLGLDSLYA 120
CelegansL5      VAQLAYSKIEGDVVVASAYSHELPRYGLKVGLTNYAAAYATGLLLARRHLKTIGLDSTYK 120
ArabiL5         VAQIVSASIAGDIVKASAYAHELPQYGLTVGLTNYAAAYCTGLLLARRVLKMLEMDDEYE 120
YeastL5         ICQIISSTITGDVVLAAAYSHELPRYGITHGLTNWAAAYATGLLIARRTLQKLGLDETYK 120
BacillusL5      YAQIIDDVNGVTLASASTLDKDLN--VESTGDT--SAATKVGELVAKRAAEKGISDVVFD 96
EcoliL5         YAQVIAPNGSEVLVAASTVEKAIAEQLKYTGNK--DAAAAVGKAVAERALEKGIKDVSFD 93
                  *:           .::  : :       * .   **  .*  :*.*  :    *  :

HumanL5         GQVEVTGDEYNVESIDGQPGAFTCYLDAGLARTTTGNKVFGALKGAVDGGLSIPHSTKRF 180
MusL5           GQVEVNGGEYNVESIDGQPGAFTCYLDAGLARTTTGNKVFGALKGAVDGGLSIPHSTKRF 180
Drosophila      GCTEVTGEEFNVEPVDDGPGAFRCFLDVGLARTTTGARVFGAMKGAVDGGLNIPHSVKRF 180
CelegansL5      GHEELTGEDYNVE-EEGDRAPFKAVLDIGLARTTTGSKIFAVMKGVADGGINVPHSESRF 179
ArabiL5         GNVEATGEDFSVEPTDSRR-PFRALLDVGLIRTTTGNRVFGALKGALDGGLDIPHSDKRF 179
YeastL5         GVEEVEGEYELTEAVEDGPRPFKVFLDIGLQRTTTGARVFGALKGASDGGLYVPHSENRF 180
BacillusL5      ------------------------------RGGYLYHGRVKALADAAREAGLKF------- 120
EcoliL5         ------------------------------RSGFQYHGRVQALADAAREAGLQF------- 117
                                                :: .  ... :.*: .

HumanL5         PGYDDEGKEFNAEVHRKHIMGQNVADYMRYLMEEDEDAYKKQFSQYIKNSVTPDMMEEMY 240
MusL5           PGYDSESKEFNAEVHRKHIMGQNVADYMRYLMEEDEDAYKKQFSQYIKNNVTPDMMEEMY 240
Drosophila      PGYSAETKSFNADVHRAHIFGQHVADYMRSLEEEDEESFKRQFSRYIKLGIRADDLEDIY 240
CelegansL5      FGFDQESKEYNAEAHRDRILGKHVADYMTYLKEEDEDRYKRQFSKFLAAGLNADNLVATY 239
ArabiL5         AGFHKENKQLDAEIHRNYIYGGHVSNYMKLLGEDEPEKLQTHFSAYIKKGVEAESIEEMY 239
YeastL5         PGWDFETEEIDPELLRSYIFGGHVSQYMEELADDDEERFSELFKGYLADDIDADSLEDIY 240
BacillusL5      ------------------------------------------------------------
EcoliL5         ------------------------------------------------------------

HumanL5         KKAHAAIRENPVYEKKPKK------EVKKKRWNRPKMSLAQKKDRVAQKKASFLRAQERA 294
MusL5           KKAHAAIRENPVYEKKPKR------EVKKKRWNRPKMSLAQKKDRVAQKKASFLRAQERA 294
Drosophila      KKAHQAIRNDPTHKVTAKKS----SAVTKKRWNAKKLTNEQRKTKIAAHKAAYVAKLQSE 296
CelegansL5      QKVHSAIRADASP--AAKK-----AAKPSKRHTAKRLTYDERKQRVADKKALLLQLKEQQ 292
ArabiL5         KKVHAAIRAEPNHKKTEKS-----APKEHKRYNLKKLTYEERKNKLIERVKALNGAGGDD 294
YeastL5         TSAHEAIRADPAFKPTEKKFTKEQYAAESKKYRQTKLSKEERAARVAAKIAALAGQQ--- 297
BacillusL5      ------------------------------------------------------------
EcoliL5         ------------------------------------------------------------

HumanL5         AES---- 297
MusL5           AES---- 297
Drosophila      TEA---- 299
CelegansL5      E------ 293
ArabiL5         DDEDDEE 301
```

```
YeastL5         -------
BacillusL5      -------
EcoliL5         -------
```

Bibliography

Albert, B., Knight, B., Merwin, J., Martin, V., Ottoz, D., Gloor, Y., Bruzzone, M.J., Rudner, A., and Shore, D. (2016). A Molecular Titration System Coordinates Ribosomal Protein Gene Transcription with Ribosomal RNA Synthesis. Mol Cell *64*, 720-733.

Andersson, D.I., Bohman, K., Isaksson, L.A., and Kurland, C.G. (1982). Translation rates and misreading characteristics of rpsD mutants in Escherichia coli. Mol Gen Genet *187*, 467-472.

Bachand, F., and Silver, P.A. (2004). PRMT3 is a ribosomal protein methyltransferase that affects the cellular levels of ribosomal subunits. EMBO J *23*, 2641-2650.

Ban, N., Beckmann, R., Cate, J.H., Dinman, J.D., Dragon, F., Ellis, S.R., Lafontaine, D.L., Lindahl, L., Liljas, A., Lipton, J.M.*, et al.* (2014). A new system for naming ribosomal proteins. Curr Opin Struct Biol *24*, 165-169.

Biever, A., Valjent, E., and Puighermanal, E. (2015). Ribosomal Protein S6 Phosphorylation in the Nervous System: From Regulation to Function. Front Mol Neurosci *8*, 75.

Briones, E., Briones, C., Remacha, M., and Ballesta, J.P. (1998). The GTPase center protein L12 is required for correct ribosomal stalk assembly but not for Saccharomyces cerevisiae viability. J Biol Chem *273*, 31956-31961.

Calidas, D., Lyon, H., and Culver, G.M. (2014). The N-terminal extension of S12 influences small ribosomal subunit assembly in Escherichia coli. RNA *20*, 321-330.

Chen, W., Xie, Z., Yang, F., and Ye, K. (2017). Stepwise assembly of the earliest precursors of large ribosomal subunits in yeast. Nucleic Acids Res.

Christiano, R., Nagaraj, N., Fröhlich, F., and Walther, T.C. (2014). Global proteome turnover analyses of the Yeasts S. cerevisiae and S. pombe. Cell Rep *9*, 1959-1965.

Dai, M.S., and Lu, H. (2004). Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. J Biol Chem *279*, 44475-44482.

Davis, J.H., Tan, Y.Z., Carragher, B., Potter, C.S., Lyumkis, D., and Williamson, J.R. (2016). Modular Assembly of the Bacterial Large Ribosomal Subunit. Cell *167*, 1610-1622.e1615.

DeLabre, M.L., Kessl, J., Karamanou, S., and Trumpower, B.L. (2002). RPL29 codes for a non-essential protein of the 60S ribosomal subunit in Saccharomyces cerevisiae and exhibits synthetic lethality with mutations in genes for proteins required for subunit coupling. Biochim Biophys Acta *1574*, 255-261.

Deshmukh, M., Tsay, Y.F., Paulovich, A.G., and Woolford, J.L. (1993). Yeast ribosomal protein L1 is required for the stability of newly synthesized 5S rRNA and the assembly of 60S ribosomal subunits. Mol Cell Biol *13*, 2835-2845.

Donati, G., Peddigari, S., Mercer, C.A., and Thomas, G. (2013). 5S ribosomal RNA is an essential component of a nascent ribosomal precursor complex that regulates the Hdm2-p53 checkpoint. Cell Rep *4*, 87-98.

Dresios, J., Derkatch, I.L., Liebman, S.W., and Synetos, D. (2000). Yeast ribosomal protein L24 affects the kinetics of protein synthesis and ribosomal protein L39 improves translational accuracy, while mutants lacking both remain viable. Biochemistry *39*, 7236-7244.

Fernández-Pevida, A., Martín-Villanueva, S., Murat, G., Lacombe, T., Kressler, D., and de la Cruz, J. (2016). The eukaryote-specific N-terminal extension of ribosomal protein S31 contributes to the assembly and function of 40S ribosomal subunits. Nucleic Acids Res *44*, 7777-7791.
Fernández-Pevida, A., Rodríguez-Galán, O., Díaz-Quintana, A., Kressler, D., and de la Cruz, J. (2012). Yeast ribosomal protein L40 assembles late into precursor 60 S ribosomes and is required for their cytoplasmic maturation. J Biol Chem *287*, 38390-38407.
Gamalinda, M., and Woolford, J.L. (2014). Deletion of L4 domains reveals insights into the importance of ribosomal protein extensions in eukaryotic ribosome assembly. RNA *20*, 1725-1731.
Gasch, A.P. The environmental stress response: a common
yeast response to diverse environmental
stresses In Yeast Stress Responses, W.H.M. Stefan Hohmann, ed. (Topics in Current Genetics).
Gazda, H.T., Zhong, R., Long, L., Niewiadomska, E., Lipton, J.M., Ploszynska, A., Zaucha, J.M., Vlachos, A., Atsidaftos, E., Viskochil, D.H.*, et al.* (2004). RNA and protein evidence for haplo-insufficiency in Diamond-Blackfan anaemia patients with RPS19 mutations. Br J Haematol *127*, 105-113.
Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S. (1983). The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell *35*, 849-857.
Guillier, M., Allemand, F., Graffe, M., Raibaud, S., Dardel, F., Springer, M., and Chiaruttini, C. (2005). The N-terminal extension of Escherichia coli ribosomal protein L20 is important for ribosome assembly, but dispensable for translational feedback control. RNA *11*, 728-738.
Gómez-Herreros, F., Rodríguez-Galán, O., Morillo-Huesca, M., Maya, D., Arista-Romero, M., de la Cruz, J., Chávez, S., and Muñoz-Centeno, M.C. (2013). Balanced production of ribosome components is required for proper G1/S transition in Saccharomyces cerevisiae. J Biol Chem *288*, 31689-31700.
He, X., Li, Y., Dai, M.S., and Sun, X.X. (2016). Ribosomal protein L4 is a novel regulator of the MDM2-p53 loop. Oncotarget *7*, 16217-16226.
Herbst, K.L., Nichols, L.M., Gesteland, R.F., and Weiss, R.B. (1994). A mutation in ribosomal protein L9 affects ribosomal hopping during translation of gene 60 from bacteriophage T4. Proc Natl Acad Sci U S A *91*, 12525-12529.
Johnson, S.P., and Warner, J.R. (1987). Phosphorylation of the Saccharomyces cerevisiae equivalent of ribosomal protein S6 has no detectable effect on growth. Mol Cell Biol *7*, 1338-1345.
Kamath, R.S., and Bungay, H.R. (1988). Growth of yeast colonies on solid media. J Gen Microbiol *134*, 3061-3069.
Kruger, K., Grabowski, P.J., Zaug, A.J., Sands, J., Gottschling, D.E., and Cech, T.R. (1982). Self-splicing RNA: autoexcision and autocyclization of the ribosomal RNA intervening sequence of Tetrahymena. Cell *31*, 147-157.
Lam, Y.W., Lamond, A.I., Mann, M., and Andersen, J.S. (2007). Analysis of nucleolar protein dynamics reveals the nuclear degradation of ribosomal proteins. Curr Biol *17*, 749-760.
Lawrence, M.G., Shamsuzzaman, M., Kondopaka, M., Pascual, C., Zengel, J.M., and Lindahl, L. (2016). The extended loops of ribosomal proteins uL4 and uL22 of Escherichia coli contribute to ribosome assembly and protein translation. Nucleic Acids Res *44*, 5798-5810.

Mager, W.H., Planta, R.J., Ballesta, J.G., Lee, J.C., Mizuta, K., Suzuki, K., Warner, J.R., and Woolford, J. (1997). A new nomenclature for the cytoplasmic ribosomal proteins of Saccharomyces cerevisiae. Nucleic Acids Res *25*, 4872-4875.
Mailliot, J., Garreau de Loubresse, N., Yusupova, G., Meskauskas, A., Dinman, J.D., and Yusupov, M. (2016). Crystal Structures of the uL3 Mutant Ribosome: Illustration of the Importance of Ribosomal Proteins for Translation Efficiency. J Mol Biol *428*, 2195-2202.
Melnikov, S., Ben-Shem, A., Garreau de Loubresse, N., Jenner, L., Yusupova, G., and Yusupov, M. (2012). One core, two shells: bacterial and eukaryotic ribosomes. Nat Struct Mol Biol *19*, 560-567.
Melnikov, S., Ben-Shem, A., Yusupova, G., and Yusupov, M. (2015). Insights into the origin of the nuclear localization signals in conserved ribosomal proteins. Nat Commun *6*, 7382.
Moore, P.B., and Steitz, T.A. (2002). The involvement of RNA in ribosome function. Nature *418*, 229-235.
Müller, U.F. (2009). Evolution of ribozymes in an RNA world. Chem Biol *16*, 797-798.
Nicolas, E., Parisot, P., Pinto-Monteiro, C., de Walque, R., De Vleeschouwer, C., and Lafontaine, D.L. (2016). Involvement of human ribosomal proteins in nucleolar structure and p53-dependent nucleolar stress. Nat Commun *7*, 11390.
Noller, H.F. (2017). The parable of the caveman and the Ferrari: protein synthesis and the RNA world. Philos Trans R Soc Lond B Biol Sci *372*.
Olivier, M., Hollstein, M., and Hainaut, P. (2010). TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol *2*, a001008.
Pagliara, V., Saide, A., Mitidieri, E., d'Emmanuele di Villa Bianca, R., Sorrentino, R., Russo, G., and Russo, A. (2016). 5-FU targets rpL3 to induce mitochondrial apoptosis via cystathionine-β-synthase in colon cancer cells lacking p53. Oncotarget.
Peltz, S.W., Hammell, A.B., Cui, Y., Yasenchak, J., Puljanowski, L., and Dinman, J.D. (1999). Ribosomal protein L3 mutants alter translational fidelity and promote rapid loss of the yeast killer virus. Mol Cell Biol *19*, 384-391.
Pestov, D.G., Strezoska, Z., and Lau, L.F. (2001). Evidence of p53-dependent cross-talk between ribosome biogenesis and the cell cycle: effects of nucleolar protein Bop1 on G(1)/S transition. Mol Cell Biol *21*, 4246-4255.
Petrov, A.S., Bernier, C.R., Hsiao, C., Norris, A.M., Kovacs, N.A., Waterbury, C.C., Stepanov, V.G., Harvey, S.C., Fox, G.E., Wartell, R.M.*, et al.* (2014). Evolution of the ribosome at atomic resolution. Proc Natl Acad Sci U S A *111*, 10251-10256.
Piepersberg, W., Böck, A., and Wittmann, H.G. (1975). Effect of different mutations in ribosomal protein S5 of Escherichia coli on translational fidelity. Mol Gen Genet *140*, 91-100.
Randall-Hazelbauer, L.L., and Kuland, C.G. (1972). Identification of three 30S proteins contributing to the ribosomal A site. Mol Gen Genet *115*, 234-242.
Raué, H.A., Mager, W.H., and Planta, R.J. (1991). Structural and functional analysis of yeast ribosomal proteins. Methods Enzymol *194*, 453-477.
Root-Bernstein, M., and Root-Bernstein, R. (2015). The ribosome as a missing link in the evolution of life. J Theor Biol *367*, 130-158.

Schlünzen, F., Zarivach, R., Harms, J., Bashan, A., Tocilj, A., Albrecht, R., Yonath, A., and Franceschi, F. (2001). Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature *413*, 814-821.
Schmeing, T.M., Voorhees, R.M., Kelley, A.C., Gao, Y.G., Murphy, F.V., Weir, J.R., and Ramakrishnan, V. (2009). The crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA. Science *326*, 688-694.
Sharma, S., and Lafontaine, D.L. (2015). 'View From A Bridge': A New Perspective on Eukaryotic rRNA Base Modification. Trends Biochem Sci *40*, 560-575.
Sloan, K.E., Warda, A.S., Sharma, S., Entian, K.D., Lafontaine, D.L., and Bohnsack, M.T. (2016). Tuning the ribosome: The influence of rRNA modification on eukaryotic ribosome biogenesis and function. RNA Biol, 1-16.
Soifer, I., and Barkai, N. (2014). Systematic identification of cell size regulators in budding yeast. Mol Syst Biol *10*, 761.
Steitz, T.A., and Moore, P.B. (2017). Perspectives on the ribosome. Philos Trans R Soc Lond B Biol Sci *372*.
Stelzl, U., and Nierhaus, K.H. (2001). A short fragment of 23S rRNA containing the binding sites for two ribosomal proteins, L24 and L4, is a key element for rRNA folding during early assembly. RNA *7*, 598-609.
Sun, X.X., Wang, Y.G., Xirodimas, D.P., and Dai, M.S. (2010). Perturbation of 60 S ribosomal biogenesis results in ribosomal protein L5- and L11-dependent p53 activation. J Biol Chem *285*, 25812-25821.
Sung, M.K., Reitsma, J.M., Sweredoski, M.J., Hess, S., and Deshaies, R.J. (2016). Ribosomal proteins produced in excess are degraded by the ubiquitin-proteasome system. Mol Biol Cell *27*, 2642-2652.
Tait-Kamradt, A., Davies, T., Cronan, M., Jacobs, M.R., Appelbaum, P.C., and Sutcliffe, J. (2000). Mutations in 23S rRNA and ribosomal protein L4 account for resistance in pneumococcal strains selected in vitro by macrolide passage. Antimicrob Agents Chemother *44*, 2118-2125.
Thapa, M., Bommakanti, A., Shamsuzzaman, M., Gregory, B., Samsel, L., Zengel, J.M., and Lindahl, L. (2013). Repressed synthesis of ribosomal proteins generates protein-specific cell cycle and morphological phenotypes. Mol Biol Cell *24*, 3620-3633.
Tsay, Y.F., Thompson, J.R., Rotenberg, M.O., Larkin, J.C., and Woolford, J.L. (1988). Ribosomal protein synthesis is not regulated at the translational level in Saccharomyces cerevisiae: balanced accumulation of ribosomal proteins L16 and rp59 is mediated by turnover of excess protein. Genes Dev *2*, 664-676.
Warner, J.R. (1999). The economics of ribosome biosynthesis in yeast. Trends Biochem Sci *24*, 437-440.
Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H.*, et al.* (1999). Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science *285*, 901-906.
Wittmann-Liebold, B., Kopke, A., Arndt, E., Kromer, W., Hatakeyama, T., and Wittmann, H. (1990). Sequence comparison and evolution of ribosomal proteins and their genes. In The Ribosome Structure, Function, & Genetics, W. Hill, A. Dahlberg, R. Garrett, P. Moore, D. Schlessigner, and J. Warner, eds. (American Society for Microbiology), pp. 598-616.
Wolfe, K.H., and Shields, D.C. (1997). Molecular evidence for an ancient duplication of the entire yeast genome. Nature *387*, 708-713.

Wool, I.G., Chan, Y.L., and Glück, A. (1995). Structure and evolution of mammalian ribosomal proteins. Biochem Cell Biol *73*, 933-947.
Xirodimas, D.P., Sundqvist, A., Nakamura, A., Shen, L., Botting, C., and Hay, R.T. (2008). Ribosomal proteins are targets for the NEDD8 pathway. EMBO Rep *9*, 280-286.
Yu, Y., Maggi, L.B., Brady, S.N., Apicelli, A.J., Dai, M.S., Lu, H., and Weber, J.D. (2006). Nucleophosmin is essential for ribosomal protein L5 nuclear export. Mol Cell Biol *26*, 3798-3809.
Zhang, J., Harnpicharnchai, P., Jakovljevic, J., Tang, L., Guo, Y., Oeffinger, M., Rout, M.P., Hiley, S.L., Hughes, T., and Woolford, J.L. (2007). Assembly factors Rpf2 and Rrs1 recruit 5S rRNA and ribosomal proteins rpL5 and rpL11 into nascent ribosomes. Genes Dev *21*, 2580-2592.
Zhang, L., Wu, C., Cai, G., Chen, S., and Ye, K. (2016). Stepwise and dynamic assembly of the earliest precursors of small ribosomal subunits in yeast. Genes Dev *30*, 718-732.
Zhang, Y., Wang, J., Yuan, Y., Zhang, W., Guan, W., Wu, Z., Jin, C., Chen, H., Zhang, L., Yang, X.*, et al.* (2010). Negative regulation of HDM2 to attenuate p53 degradation by ribosomal protein L26. Nucleic Acids Res *38*, 6544-6554.
Zhou, F., Roy, B., and von Arnim, A.G. (2010). Translation reinitiation and development are compromised in similar ways by mutations in translation initiation factor eIF3h and the ribosomal protein RPL24. BMC Plant Biol *10*, 193.