Charles University in Prague
Faculty of Science

Study programme: Special Chemical and Biological Programmes

Field of study: Molecular Biology and Biochemistry of Organisms

Filip Nemčko

Gene expression regulation of ribosomal protein genes

Regulácia expresie génov pre ribozomálne proteíny

Bachelor's thesis

Supervisor: Mgr. Kateřina Abrhámová, Ph.D.
Prague, 2016

Acknowledgements

I would like to express my sincere gratitude to my supervisor, Katka Abrhámová, for her support and valuable advices. Her guidance helped me in writing of this thesis.

Prehlásenie

Prehlasujem, že som záverečnú prácu spracoval samostatne a že som uviedol všetky použité informačné zdroje a literatúru. Táto práca ani jej podstatná časť nebola predložená k získaniu iného alebo rovnakého akademického titulu.

V Prahe, 09.05.2016

Filip Nemčko

ABSTRACT

Saccharomyces cerevisiae cells produce 2000 ribosomes per minute under normal conditions. The expression of ribosomal proteins is massive – it takes 50% of RNA polymerase II transcription and 90% of pre-mRNA splicing in rapidly growing cells. Since cells need an equimolar amount of individual ribosomal proteins, the tight coregulation of gene expression is required. The transcription is a main target of regulation, however, it is inherently unable to set a stoichiometric balance of ribosomal proteins. Various types of post-transcriptional regulation deal with fluctuations of individual ribosomal proteins and fine-tune their expression. Intron-dependent regulation appears to by predominant among ribosomal protein genes. Besides balancing their expression, presence of introns provides a rapid global regulation (repression) of ribosomal protein genes in response to environmental stress.

KEY WORDS

ribosomal protein genes, RPG, ribosomal protein, gene expression regulation, coregulation, *Saccharomyces cerevisiae*

ABSTRAKT

Kvasinka *Saccharomyces cerevisiae* produkuje za normálnych podmienok približne 2000 ribozómov za minútu. Produkcia ribozomálnych proteínov je rozsiahla – odhadom predstavuje až 50% celkovej transkripcie prevádzanej RNA polymerázou II a 90% celkového zostrihu pre-mRNA v bunkách. Expresia génov ribozomálnych proteínov je koordinovaná, čím zabezpečuje ekvimolárne množstvo jednotlivých ribozomálnych proteínov. Regulácia prebieha prevažne na úrovni transkripcie, avšak rôznorodá post-transkripčná regulácia je potrebná pre odstránenie fluktuácie koncentrácií jednotlivých ribozomálnych proteínov, a teda pre ich stoichiometrickú vyváženosť. Prevládajúcou je regulácia závislá na intrónoch, ktorá okrem vyvažovania jednotlivých ribozomálnych proteínov zabezpečuje ich rýchlu reguláciu (represiu) v odpovedi na enviromentálny stres.

KĽÚČOVÉ SLOVÁ

gény pre ribozomálne proteíny, RPG, ribozomálne proteíny, regulácia génovej expresie, koregulácia, *Saccharomyces cerevisiae*

LIST OF ABBREVIATIONS

AA	amino acid
bp	base pair
Δ	gene deletion
Δi	intron deletion
GFP	green-fluorescent protein
ChIP-seq	chromatin immunoprecipitation followed by a high-throughput DNA sequencing
K-turn	kink-turn
mRNA	messenger RNA
NMD	nonsense-mediated decay
NMR	nuclear magnetic resonance
pre-mRNA	precursor mRNA
RNAPII	RNA polymerase II
RPG	ribosomal protein gene
r-protein	ribosomal protein
rRNA	ribosomal RNA
snoRNA	small nucleolar RNA
snRNP	small nuclear ribonucleoprotein
TOR pathway	target of rapamycin signaling pathway
UTR	untranslated region
wt	wild-type
YFP	yellow-fluorescent protein

TABLE OF CONTENTS

DNA neither cares nor knows. DNA just is. And we dance to its music.

-Richard Dawkins-

1 INTRODUCTION

The ribosome is an essential translational machinery of all organisms, which decodes genetic information encoded by messenger RNAs (mRNAs) by the formation of peptide bonds between particular amino acids – ribosome catalyzes the synthesis of proteins. It is a ribonucleoprotein particle of 70S in prokaryotes and 80S in eukaryotes, composed of two subunits.

The large 60S subunit of the *Saccharomyces cerevisiae* 80S ribosome consists of three ribosomal RNA (rRNA) molecules (25S, 5.8S, and 5S) and 46 different ribosomal proteins (r-proteins), whereas the small 40S subunit includes an 18S rRNA molecule and 33 r-proteins (Planta and Mager, 1998). All 79 r-proteins are encoded by 137 ribosomal protein genes – 59 of them are duplicated, most likely as a consequence of the whole genome duplication, which occurred roughly 100 million years ago in *S. cerevisiae* ancestor (Planta and Mager, 1998; Wolfe and Shields, 1997).

Unlike many bacterial ribosomal protein genes (RPGs), which are clustered in a few operons and hence simply coregulated (Fujita et al., 1998), eukaryotic RPGs are widely spread across genome (Planta and Mager, 1998; Uechi et al., 2001). Since the expression of ribosomal proteins is massive – it takes 50% of RNA polymerase II transcription and 90% of pre-mRNA splicing in rapidly growing yeast cells – it has to be tightly coordinated to maintain a stoichiometric balance in ribosome assembly (Li et al., 1999; *Warner, 1999).

The aim of my bachelor thesis is to review what is known about the tight coregulation of ribosomal proteins in budding yeast, especially at the post-transcriptional level. Furthermore, it ought to outline the recent view of ribosome as an active machine capable to optimize translation of specific mRNAs or to adapt to specific conditions.

2 TRANSCRIPTIONAL REGULATION OF RIBOSOMAL PROTEIN GENES

Ribosomal protein genes count among the most highly expressed and coordinated genes (Li et al., 1999). The tight coregulation mainly occurs at the transcriptional level. The understanding of RPGs transcriptional regulation is still fragmentary, however, several transcriptional regulators of ribosomal protein genes have been identified.

Roughly 98% of RPG promoter regions contain predominantly two binding sites for a transcriptional regulator Rap1 (repressor activator protein 1), known as RPG boxes (Leer et al., 1985; Shore and Nasmyth, 1987; Lascaris et al., 1999; Brown et al., 2001). Besides RP genes, Rap1 is also involved in a transcriptional regulation of genes encoding glycolytic enzymes (Chambers et al., 1989) and in other cellular functions, such as the regulation of telomere length (Buchman et al., 1988). Rap1 binds to the RPG promoter constantly (Reid et al., 2000; Martin et al., 2004), thus its function seems to be in a recruitment of specific (co)factors, which determine the transcriptional activity of respective ribosomal protein genes. Rap1 is required for the binding of all other transcriptional factors (Yu and Morse, 1999; Zhao et al., 2006; Knight et al., 2014).

One of the known factors recruited by Rap1 is Fhl1, also constitutively bound to (~90%) RPG promoter regions (Lee et al., 2002; Martin et al., 2004; Kasahara et al., 2007). It was shown that Fhl1 could interact with coactivator Ifh1 or corepressor Crf1 through a conserved Forkhead-associated domain. Ifh1 and Crf1 compete with each other for the binding site on Fhl1. A nutrient-sensitive target of rapamycin (TOR) signaling pathway controls association of these cofactors with Fhl1 and therefore, it regulates the transcriptional activity of RPGs. Inactive Crf1 is localized in cytoplasm under normal condition, when TOR signaling pathway is active. The inactivation of TOR pathway due to unfavorable conditions leads to the Crf1 phosphorylation and its localization to nucleus, where it represses the RPGs transcription (Martin et al., 2004; Schawalder et al., 2004; Wade et al., 2004). Another known mechanism of RPGs transcriptional repression lies on a sequestration of Ifh1 by CURI, a complex of rRNA processing factors CK2, Utp22 and Rrp7. This process might coordinate the amount of rRNAs and r-proteins, since decreased rRNAs production presumably 'releases' processing factors, which could sequestrate Ifh1 and thus adjust the RPGs transcription to reduced rRNA expression (Rudra et al., 2007).

Hmo1 transcriptional activator requires Rap1 for its association with ~70% of the RPG promoters. Hmo1 and Fhl1 bind cooperatively to RPG promoters, which is demonstrated by their proposed physical interactions (Ho et al., 2002) and by a radical reduction of Fhl1 (and consequently Ifh1) association with RPG promoters due to the Hmo1 loss. Interestingly, the lack of Hmo1 does not lead to change in mRNA levels of studied ribosomal protein genes (Hall et al., 2006). Another study showed that Fhl1 is able to bind to some RPG promoters in a Hmo1-independent manner (Kasahara et al., 2007).

Sfp1 is a different transcriptional activator of RPGs. It appears that Sfp1 interacts with RPG promoters indirectly (Gordân et al., 2009). A broad range of stresses (e.g. nutrient deprivation, oxidative stress, osmotic stress, TOR inactivation) lead to the relocalization of Sfp1 to cytoplasm and therefore to the RPG transcription downregulation. Sfp1 deletion leads to the partial downregulation of RPG expression and to improper response to environmental conditions (Fingerman et al., 2003; Jorgensen et al., 2004; Marion et al., 2004).

Recent investigation has brought an overall view of RPG promoters. RP genes have two prevalent promoter architectures as seen in the Figure 1. The first category (~50%) of promoters strongly associates with Hmo1, whereas the second one (~44%) does not. The region of depleted nucleosomes seems to be larger for the first category. There are typically two closely spaced Rap1-binding sites (RPG boxes) in both types of promoters. Ifh1 appears to interact also with Rap1 and therefore serves as a bridge between Fhl1 and Rap1 (Mallick and Whiteway, 2013; Knight et al., 2014). Furthermore, Fhl1 probably associates by the protein-protein interaction with Hmo1 rather than by its DNA-binding domain to promoters of the first category (Knight et al., 2014).

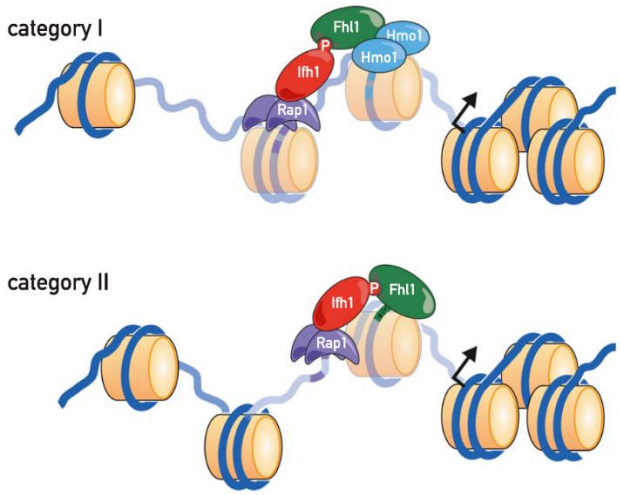

Figure 1: Two predominant promoter architectures of RP genes. Depicted nucleosomes and the position of Rap1, Hmo1, Fhl1 and Ifh1 on RPG promoter regions with respect to the transcription start site (arrow). (Knight et al., 2014)

There is no significant difference in an average transcriptional activity between these two promoter structures. Furthermore, the response to various stresses is similar for both classes, except the slight difference in response to a heat shock (Knight et al., 2014).

One of the principal questions is how r-proteins encoded by different number of genes are able to reach equimolar amounts in the cell. The analysis of 6 independent public RNA-seq and DNA microarray data indicated that the expression of single-copy RPGs is significantly higher than of duplicated genes. Using yellow-fluorescent protein (YFP) reporters fused downstream of 110 out of 137 RPG promoters has revealed that the difference in mRNA levels of single-copy and duplicated RP genes is encoded within their promoters. Promoter activities of RPG pairs (treated as a single promoter) are reduced sufficiently to reach the proper stoichiometry of ribosomal protein transcripts in the cell (Zeevi et al., 2011). Albeit the transcription creates different RPG transcripts in roughly equimolar amounts, a precise balancing occurs at the post-transcriptional level.

3 POST-TRANSCRIPTIONAL REGULATION OF RIBOSOMAL PROTEIN GENES

Despite transcriptional regulation as a primary level of regulation which controls r-proteins globally, secondary regulation on post-transcriptional level deals with fluctuations of individual ribosomal proteins and keeps their expression balanced.

Pioneering experiments done by Nancy Pearson with colleagues showed post-transcriptional regulation of RP genes in *S. cerevisia*e for the first time. Increase of *RPL3* transcription does not lead to the change in Rpl3 protein level, which remains equal to other r-proteins. They supposed that cells control the translational rate of *RPL3* mRNA (Pearson et al., 1982). Afterward, six additional RPGs have been investigated and potential mechanisms of regulation have been proposed (Warner et al., 1985). Similar feedback inhibition of translation was suggested for *RPL28* – fivefold excess of its mRNA level does not lead to the elevated Rpl28p amount. Additionally, *RPL28* as well as *RPL30* increased transcription leads to the accumulation of their precursor mRNA (pre-mRNA) as a consequence of mRNA processing regulation. In contrast, overproduction of *RPL24A/B, RPS7, RPS10A* or *RPS10B* transcripts lead to an excess of corresponding proteins, which are immediately degraded (Warner et al., 1985). These primary experiments have shown a robustness in the ribosomal protein genes regulation.

3.1 Intron-dependent regulation

Unlike higher eukaryotes, where genes are often interrupted by multiple long introns – 95% of human genes are interrupted by an intron with a mean size of more than 3300 base pairs (bp) and sometimes exceeding 10 kb (Frazier et al., 2001); only 4% of yeast genes are interrupted by an intron of a mean size about 260 bp (Spingola et al., 1999).

Ribosomal protein genes represent merely 2% of genes in *S. cerevisiae*. However, they are over-represented among yeast intron-containing genes as summarized in Table I. Up to 74% of ribosomal protein genes contain introns compared to 5% of non-ribosomal protein genes (Spingola et al., 1999; Parenteau et al., 2008).

The fact, that all 101 intron-containing RP genes produce ~24% of total cellular mRNA (Ares et al., 1999), indicates the evolutionary reason for their retention in otherwise intron- poor *S. cerevisiae*. Presence of introns in ribosomal protein genes may serve for an additional level of their regulation. I will try to propose a few predictions of their evolutionary retention and function.

	RP genes	All genes	Source
Total	137	5820	http://www.yeastgenome.org/genomesnapshot (as of 4/17/2016)
Num. of introns	104	296	Spingola et al., 1999; Parenteau et al., 2011
Intron-containing genes	101	283	Spingola et al., 1999; Parenteau et al., 2008
	74%	5%	
Ohnologs*	118	1102	Byrne and Wolfe, 2005; Parenteau et al., 2011
	86%	19%	
Intron-containing ohnologs	94	121	Plocik and Guthrie, 2012
	80%	11%	

Table I: The characteristics of ribosomal protein genes compared to all defined (verified and uncharacterized) yeast open reading frames (ORF). *Ohnologs, a proposed name for paralogs arisen from a whole genome duplication in honor of Susumu Ohno (Wolfe, 2000).

3.1.1 Ribosomal protein genes pre-mRNA's splicing seems to be distinctive

Kinetic profiling of splicing in diverse mutants for core components of the spliceosome revealed that transcripts are not equally affected by these mutations. Interestingly, the splicing of RPG pre-mRNAs seems to be different from other, non-RPG transcripts. It suggests that the spliceosome is capable to operate in a transcript-specific manner (Pleiss et al., 2007a).

Nonetheless, not all RPGs show similar behavior. Some r-protein paralogous genes respond differently to particular spliceosomal mutations. While the *RPS30B* pre-mRNA splicing is affected by most of the spliceosome mutants used in the study of Pleiss et al., the *RPS30A* pre-mRNA is not. Similar bias has been shown for *RPL19* and *RPL14* pairs (Pleiss et al., 2007a).

The nature of a distinct RPG pre-mRNAs splicing has not been revealed yet. The time between transcription and splicing seems to be shorter for RP genes, since the pre-mRNA level of RP genes is remarkably low relative to non-RP genes (Pleiss et al., 2007a). It suggests that the RPG pre-mRNAs splicing might occur co-transcriptionally, which is consistent with the higher density of the U1 snRNP (small nuclear ribonucleoprotein) on RPG transcripts compared to non-RP genes measured by the whole-genome chromatin immunoprecipitation followed by a DNA microarray analysis (Tardiff et al., 2006).

3.1.2 Introns of ribosomal protein genes are dispensable under normal conditions

Generally, intron-containing genes produce 3.9-fold more RNA on average and 3.3-fold more protein on average than intronless genes in *S. cerevisiae*. The same result appears for a subset of ribosomal genes annotated as being part of the 'ribosome cellular component' (e.g. RPGs, translation initiation factors, elongation factors) – 3.7-fold excess of RNA and 4.1-fold more proteins for intron-containing ribosomal genes on average (Juneau et al., 2006). However, the trend in RNA and protein levels between intron-containing and intronless r-protein genes has not been significant – probably as a consequence of a needful balance of ribosomal proteins in the cell (Juneau et al., 2006; Plocik and Guthrie, 2012).

Evaluation of the impact of RPGs introns on the expression and cell growth has been investigated by a precise intron deletion in each of 101 intron-containing RP genes. None of the deletion changed cell growth in rich media, suggesting that RPGs introns are not essential under normal conditions (Parenteau et al., 2011). The intron deletion changed expression of 84% intron-containing RP genes. An intron-dependent negative regulation (presence of intron decreases expression) occurs in 43% of RPGs, whereas intron-dependent positive regulation (presence of intron increases expression) affects 41% of RPGs. The expression of 17 intron-containing RP genes does not depend on the presence of their introns (Parenteau et al., 2011).

3.1.3 Introns provide rapid regulation of RP genes in response to environmental stress

An amino acid starvation leads to the rapid splicing inhibition of the majority of RP genes and hence to their pre-mRNAs accumulation. The starvation does not affect splicing globally, since there is a little change in the splicing of non-RP genes compared to ribosomal protein genes. Response to amino acid starvation is often regulated by the protein kinase Gcn2, but the downregulation of RPG splicing is Gcn2-independent (Pleiss et al., 2007b).

Another example is a specific decrease in the RP pre-mRNA levels as a response to osmotic stress. This global regulation of RP expression is mediated by cytoplasmic nonsense-mediated decay, a degradation pathway that eliminates RNAs with premature termination codons (Bergkessel et al., 2011; Garre et al., 2013). Furthermore, the osmotic stress leads to the destabilization of RPG mRNAs (Romero-Santacreu et al., 2009). As a result, the translation might be focused on osmo-protective proteins rather than abundant ribosomal proteins (Soufi et al., 2009).

Ribosomal protein genes are one of the most highly expressed genes and therefore consume noticeable cellular energy resources. Thus, a rapid regulation of RPGs expression is crucial under conditions that

endanger survival. The rapid response to environmental stress could explain the prevalence of introns in ribosomal protein genes.

3.1.4 Introns allow auto-regulated expression of ribosomal protein genes

The size distribution of yeast introns is bimodal. A specific characteristics of RPG introns is their relative larger size compared to non-RPGs in budding yeast (Figure 2). The length of just *RPL27B, RPL7A* and *RPS22B* introns has been elucidated, since their introns harbor sequences coding small nucleolar RNA (snoRNA) (Spingola et al., 1999; Vincenti et al., 2007).

Genes with longer introns are on average more expressed than genes with shorter introns. The correlation relates to an RNA as well as to a protein abundance (Juneau et al., 2006). Whole genome experiments showed that RPG transcripts are spliced more effectively compared to non-RPG transcripts (Pleiss et al., 2007a).

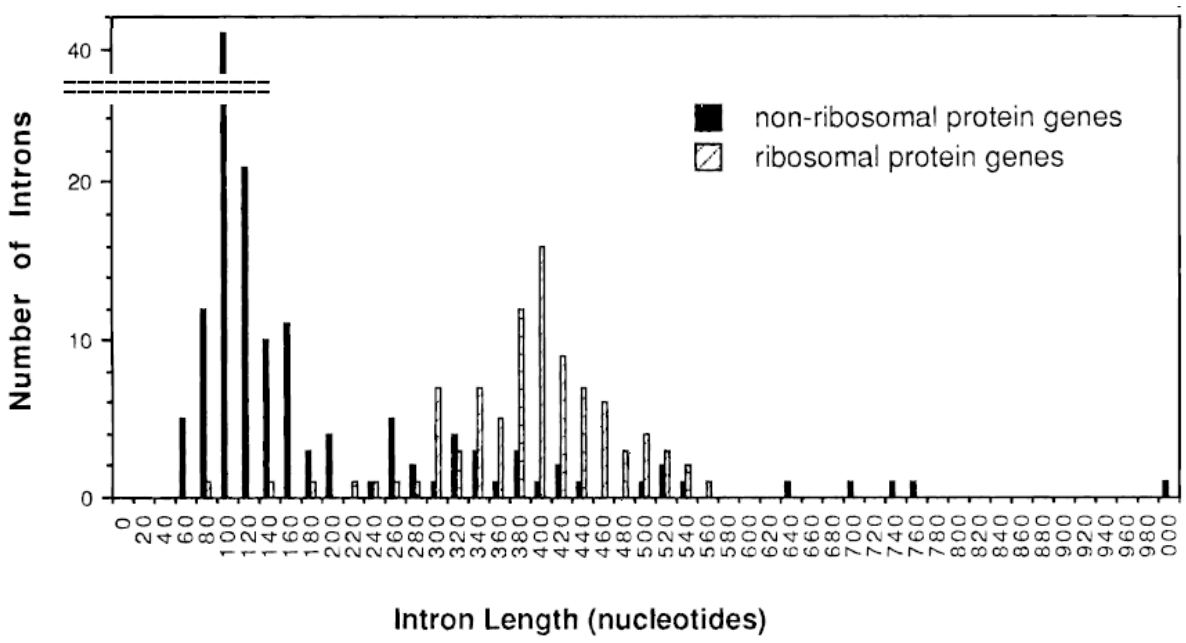

Figure 2: The length distribution of yeast introns is bimodal. RPG introns tend to be larger than introns of non-ribosomal protein genes. (modified from Spingola et al., 1999)

Many longer yeast introns contain intramolecular secondary structures, which have a role in a pre-mRNA splicing (Charpentier and Rosbash, 1996; Howe and Ares, 1997). These basepairing interactions can bring distant and hence ineffective splicing signals to proximity and resemble short introns, thus support an optimal spliceosome assembly (Howe and Ares, 1997; Gahura et al., 2011; Meyer et al.,

2011). Furthermore, secondary structures within premature RNA can act as epitopes for regulatory proteins. One of the examples is *RPL30* gene, which is described below with a historical context.

3.1.4.1 *RPL30* regulation

Intron-containing gene *RPL30* (*YGL030W*), formerly known as *RPL32*, encodes an essential 105 amino acids long protein Rpl30 of the 60S ribosomal subunit (Dabeva and Warner, 1987). Pioneering experiments showed that increased transcription of *RPL30* leads to the accumulation of *RPL30* pre-mRNA (Warner et al., 1985). Hence, one could hypothesize that the Rpl30 protein negatively regulates the splicing of its own transcript – like that time already known examples of *E.coli* ribosomal proteins, which negatively regulate translation of their own transcripts (e.g. Nomura et al., 1980).

The hypothesis that Rpl30p itself is needed for inhibition of the *RPL30* pre-mRNA splicing was confirmed shortly after (Dabeva et al., 1986). Secondary structure on *RPL30* pre-mRNA serving as the binding site has been postulated between the first exon and initial intron sequence encompassing the 5‘ splice site (Eng and Warner, 1991). Surprisingly, similar RNA structure is conserved within the spliced mRNA – expression of *RPL30* is also regulated on the translational level by blocking access of ribosome to the mRNA (Dabeva and Warner, 1993). Rpl30p can prevent assembly of spliceosome on its pre-mRNA as well as reduces the formation of ribosome on its mRNA.

The explanation for this dual regulation can be the fact that each of created *RPL30* mRNA is translated to approximately 50 proteins. Thus, an imbalance of only 2% between the synthesis of rRNA and Rpl30p would provide enough unbound Rpl30p to inhibit its own production (Kim and Warner, 1983; Dabeva and Warner, 1993).

It was proved that the *RPL30* pre-mRNA splicing is regulated by a direct binding of Rpl30p to the RNA structure on *RPL30* pre-mRNA (Vilardell and Warner, 1994). The RNA structure is defined as a helix-internal loop-helix element, the kink-turn (K-turn), showed schematically in the Figure 3B (Li et al., 1995a; Klein et al., 2001). The high-resolution structure of complex Rpl30p: *RPL30* pre-mRNA was solved using NMR (nuclear magnetic resonance) spectroscopy (Mao et al., 1999) and X-ray crystallography (Chao and Williamson, 2004). A joint structure refinement using both X-ray and NMR data resulted in improved image of the complex (Figure 3A). K-turn fold on the pre-mRNA complementarily interacts with Rpl30p binding surface (Chao and Williamson, 2004). The internal loop enriched with purines is crucial for the binding (H. Li et al., 1995).

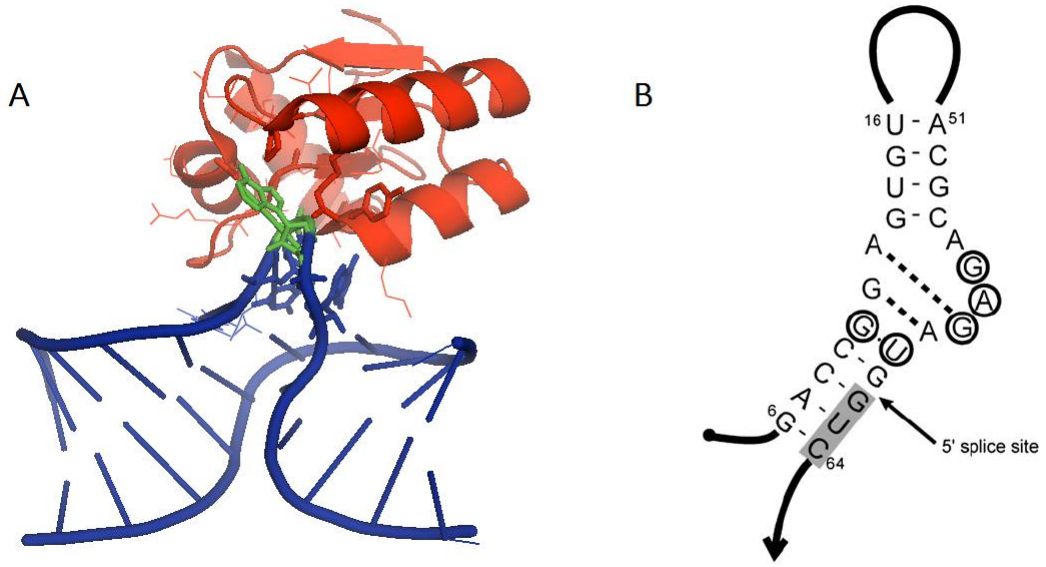

Figure 3: *RPL30* pre-mRNA splicing is regulated by a direct binding of Rpl30p to the RNA structure on *RPL30* pre-mRNA
A. The structure of complex Rpl30p protein – *RPL30* pre-mRNA created as a joint refinement of X-ray and NMR data. Rpl30p (red) interacts with RNA internal loop (blue). Adenine 57 (green) is inserted into a pocket of the protein (Chao and Williamson, 2004). Figure was generated with PyMOL, PDB ID: 1T0K.
B. Schematic representation of the *RPL30* pre-mRNA kink-turn, made up of the first exon and initial intron sequence (gray) encompassing the 5‘ splice site. Nucleotides responsible for binding with Rpl30p are marked with circles. Numbers represent order from the transcription start site. (Macías et al., 2008)

Regulation of Rpl30p production is required for an efficient cell growth – strain with disrupted *RPL30* pre-mRNA splicing regulation and translational regulation co-cultivated with wild-type cells has been lost after 3 days of continual cultivation. However, translational regulation alone as well as pre-mRNA splicing regulation alone is sufficient to maintain optimal level of Rpl30p – cells with disrupted one of the two regulations compete effectively with wild-type cells under normal conditions (Li et al., 1996).

Because 5‘splice site is a component of established RNA structure, the mechanism of regulation was suggested as a blocking access of the U1 snRNP (small nuclear ribonucleoprotein) to 5‘splice site (Eng and Warner, 1991). Subsequent *in vitro* experiments showed that the *RPL30* pre-mRNA associates with U1 snRNP, but the association of U2 snRNP is blocked (Vilardell and Warner, 1994). A detailed *in vitro* and *in vivo* analysis has revealed that the presence of 5’ splice site inside the Rpl30p binding site is not required for the *RPL30* pre-mRNA splicing repression. Additionally, a next step in the spliceosome assembly – the branch point recognition by BBP (branch point binding protein) and Mud2 factors, is also maintained during the repression. The Rpl30p somehow prevents the following branch point recognition by U2 snRNP (Macías et al., 2008). A genetic screen for mutations capable to reestablish the *RPL30* pre-mRNA splicing regulation, using mutants with disrupted Rpl30p binding site on the pre-mRNA

and hence resistant to the Rpl30p repression, revealed a role of Cbp80 protein, a component of the cap-binding complex. Besides the role in 5' splice site recognition and U1 snRNP recruitment, Cbp80 assists in the U2 snRNP recruitment – a function, which is possibly target of the splicing regulator Rpl30p (Bragulat et al., 2010).

Interestingly, a distant ortholog of *RPL30* from a thermoacidophilic archaeon *Sulfolobus acidocaldarius* binds to the yeast *RPL30* pre-mRNA kink-turn indistinguishably from the yeast Rpl30p and inhibits *RPL30* pre-mRNA splicing both *in vitro* and *in vivo*. These two orthologous Rpl30 proteins have just a 33% sequence identity, but highly conserved structural elements. Since there is no binding site on the *Sulfolobus RPL30* mRNA transcript, it predicates that this evolutionary conservation has preserved because the binding site resembles the 25S rRNA site for Rpl30p. The specific stem-loop on the 25S rRNA has been identified to cross-link with the yeast, archaeal and human Rpl30p. Moreover, the primary sequence as well as the secondary structure of this stem-loop is one of the most highly conserved in the whole rRNA (Vilardell et al., 2000).

To sum up findings above, a ribosomal protein Rpl30 competes between newly synthetized rRNA and the *RPL30* pre-mRNA. When in excess, Rpl30p inhibits its own synthesis by blocking the splicing and the translation. This is an example of autoregulatory feedback loop.

3.1.5 Introns regulate expression of paralogous RP genes

There are 78 distinct r-proteins in yeast. Fifty-nine of them are encoded by a pair of paralogous genes, which are functionally redundant (Planta and Mager, 1998; Dean et al., 2008). Despite the functional redundancy of paralogs, the loss of one of them has often distinct effect on phenotype. The reason is their unequal contribution to the pool of their product, a ribosomal protein. Nearly 70% of all duplicated r-protein genes are expressed asymmetrically (Parenteau et al., 2011).

Interestingly, 5 paralogs (*RPL15A, RPL18A, RPL42A, RPS28A, RPS30B*) are essential, whereas their counterparts are not (Steffen et al., 2012). A transcriptionally silent *RPL15B* gene explains the essentiality of the *RPL15A* (Simoff et al., 2009). The reason for essentiality of the other paralogs has not been elucidated yet.

Twenty-one out of 59 RPG pairs encode identical proteins, 14 pairs produce proteins that vary in only one amino acid. Fifty-one pairs of r-proteins differ no more than in 5% of their amino acid sequence. The highest difference accounts for ribosomal proteins P1, P2 and L22 with 54, 61 and 82% amino acid sequential identity, respectively (Steffen et al., 2012). It points out a high sequential identity of paralog's coding sequences and their functional redundancy (Dean et al., 2008). The conservation of RP paralogs

points to the evolutionary benefit of having both copies. Presumably, duplicated RP genes have a buffering capacity – they are capable to compensate the desired r-protein level, which is important for maintaining the stoichiometrically precise balance of r-proteins in cells (Dean et al., 2008; Deutschbauer et al., 2005; Gu, 2003; Kafri et al., 2006; Kellis et al., 2004; Papp et al., 2003)

Contrariwise the coding sequences, noncoding sequences including introns have diverged completely (Parenteau et al., 2011). The presence of introns is preferentially conserved in duplicated RPGs, as it is depicted in the Figure 4. Only 24 of 118 duplicated ribosomal protein genes are intronless (Parenteau et al., 2011). From the other site of view, 55% of intron-containing RPGs are duplicated whereas only 17% of intronless RPGs are duplicated (Juneau et al., 2006). This indirectly indicates the importance of introns in paralogous RP genes.

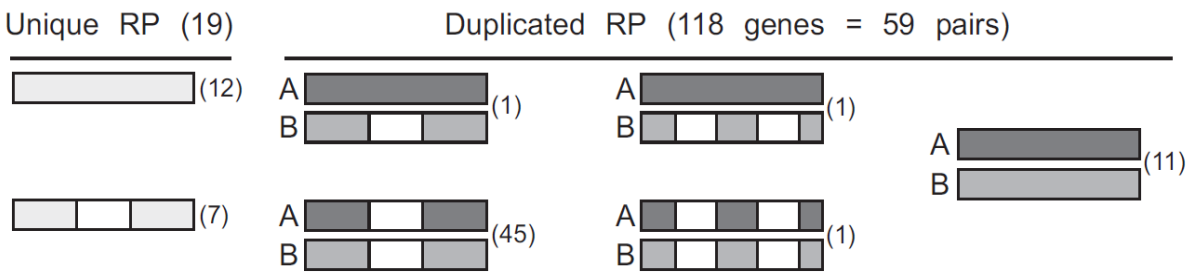

Figure 4: A huge prevalence of intron-containing genes among paralogous RP genes. 101 of 137 ribosomal protein genes contain totally 104 introns – 98 genes carry single intron and 3 genes carry 2 introns. Ribosomal protein genes are depicted with the corresponding numbers in parentheses. From 118 duplicated RPGs, 94 genes carry at least one intron. Introns (white), Exons (gray). (modified, Parenteau et al., 2011)

Study of RPG introns and their impact on the expression done by Julie Parenteau with colleagues showed that for the majority of RPG pairs, deletion of intron in one of the paralogs affects the expression of not just the own gene (intragenic regulation discussed above), but also the another paralog in the pair (Parenteau et al., 2011). One would expect that changes in the expression are compensatory to maintain balanced level of individual r-proteins. Nonetheless, the intron loss has the opposite effect on both paralogs – increase the expression of one copy and decrease of the other – just for 18 out of 94 intron-containing duplicated RPGs. Only 15 paralogous RP-genes are able to compensate wild-type (wt) level of mRNA encoded by the RPG pair. Simultaneous positive or negative change in expression has been observed for 41 r-protein genes (Parenteau et al., 2011). Introns appear to have the role in maintaining the paralog specific ratio of expression.

To illuminate a potential mechanism of this extensively widespread intergenic regulation among duplicated r-protein genes, the necessity of intron presence in the opposite paralog has been examined. Merely 33% of duplicated RPGs responded to intron deletion in one paralog by changing the expression of the other paralog only in the presence of its own intron – an intron-dependent intergenic regulation. Thirty percent of duplicated RPGs run intron-independent intergenic regulation, accordingly the intron loss in one paralog led to change in expression of the responsive paralog independently on the presence of its own intron (Parenteau et al., 2011).

3.1.5.1 *RPS9A/B* regulation

Ribosomal protein Rps9 is encoded by 2 paralogous genes, *RPS9A* (YPL081W) and *RPS9B* (YBR189W), both containing intron. While their coding sequence is almost identical and final protein differs only in 5 amino acids, introns differ noticeably with just 43% sequential identity. There is unequal contribution to the *RPS9* mRNA level – *RPS9A* encodes 6%, while *RPS9B* encodes 94% of the *RPS9* mRNA under normal conditions (Plocik and Guthrie, 2012). The contribution of *RPS9A* and *RPS9B* paralogs to the total Rps9p level was quantified as 2% and 98%, respectively (Petibon et al., 2016). Chromatin immunoprecipitation followed by a high-throughput DNA sequencing (ChIP-seq) experiments with RNA polymerase II (RNAPII) and nucleosomes revealed that unexpectedly, *RPS9A* is equally (and perhaps even more) transcribed than *RPS9B* (Petibon et al., 2016 by Kaplan et al., 2009; Bonnet et al., 2014). This suggests very fascinating post-transcriptional regulation.

In 2012, the splicing cross-regulation, a negative feedback circuit between *RPS9A* and *RPS9B* has been proposed. The deletion of intron in any one of the paralogs causes the increase of its own mRNA level (intron-dependent negative intragenic regulation) and simultaneously, decrease in the expression of the counterpart. Moreover, the accumulation of pre-mRNA of the paralog with kept intron has been observed. As predicted, no regulation occurs in the absence of introns (Plocik and Guthrie, 2012).

This splicing cross-regulation leads to the compensation of the *RPS9* mRNA quantity as depicted in the Figure 5A. Since *RPS9B* encodes the majority of *RPS9* mRNA, increase of its expression due to intron deletion results in the total *RPS9* mRNA level higher than for WT cells, however no *RPS9A* mRNA is produced as a result of the maximal possible compensation (Plocik and Guthrie, 2012).

Unexpectedly, recent findings partially contradict this results. While mutant with deleted *RPS9A* intron behaves as described above, *RPS9B* intron deletion (*Δi*) decreases the expression of its own gene – an intron-dependent positive intragenic regulation for the *RPS9B* gene. The expression of *RPS9B* remains unaffected in a double-intron deleted (*AΔiBΔi*) mutant, whereas the expression of *RPS9A* is as in the

single *RPS9AΔi* mutant. Deletion of the whole *RPS9B* gene leads to the *RPS9A* overexpression. The expression has been measured on mRNA as well as on protein level (Petibon et al., 2016). Relative mRNA levels of *RPS9A* and *RPS9B* in all discussed mutants are summarized for a comparison with the previous data (Plocik and Guthrie, 2012) in the Figure 5B (Petibon et al., 2016).

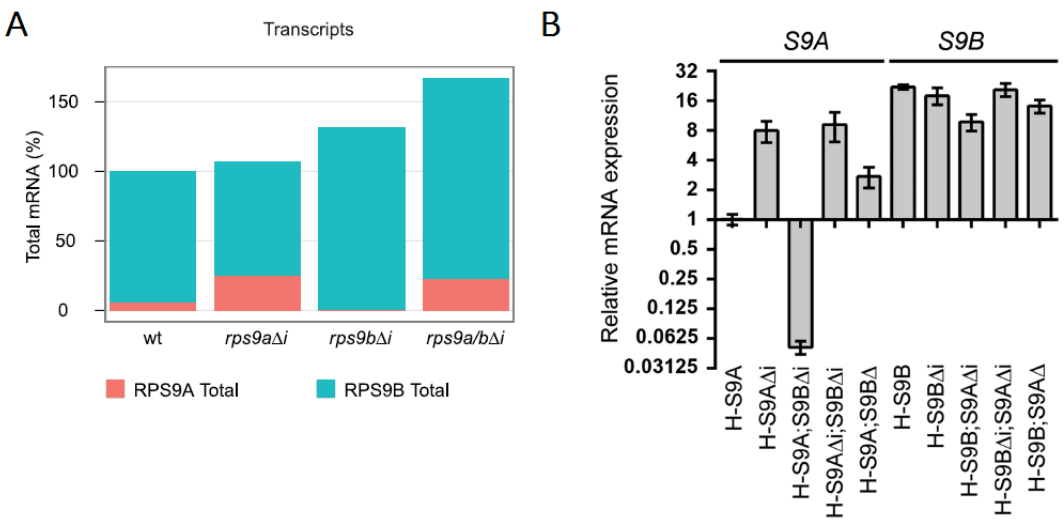

Figure 5: Introns asymmetrically regulate the expression of *RPS9* mRNA. Relative mRNA level of strains with wild type genes *RPS9A* (*S9A*) and *RPS9B* (*S9B*), as well as strains with deleted introns (*Δi*) and whole gene (*Δ*) has been determined. Changes in the mRNA levels accounts for the presence of intron-dependent intergenic regulation of the Rps9p expression.
A. Data published by Plocik and Guthrie indicates the presence of cross-regulation between *RPS9A* and *RPS9B*, which balances Rps9p level in the cell. (Plocik and Guthrie, 2012)
B. Recent findings by Petibon et al. show that the synthesis of RPS9 genes is regulated by asymmetric splicing of duplicated genes. Relative mRNA level of *RPS9A* or *RPS9B* was always measured just in the strain with His-tagged version of the investigated gene (marked as H-*S9*). (Petibon et al., 2016)

The intron sequence is required for different expression of *RPS9A* and *RPS9B* definitely, since the replacement of introns between paralogs switched expression levels of both genes. The mRNA level of *RPS9A* gene with *RPS9B* intron increased significantly, whereas its pre-mRNA level remained the same as for the wild type *RPS9A* gene. Reversely, *RPS9B* gene with *RPS9A* intron is extensively downregulated. Additionally, it was shown that the splicing of *RPS9A* pre-mRNA is less effective than of *RPS9B*. The RPS9A expression enhancement in *RPS9ΔB* mutant is a result of the improvement of *RPS9A* pre-mRNA splicing efficiency. Hence, Rps9p (Rps9B, since it represents about 98% of total Rps9p in the cell) somehow represses the splicing of *RPS9A* pre-mRNA (Petibon et al., 2016).

A two-way helical structure inside the intron of *RPS9A* pre-mRNA – very similar to the binding site of Rps9p on 18S rRNA (Petibon et al., 2016 by Ben-Shem et al., 2011) – is responsible for the regulation. Its deletion leads to the increase in the *RPS9A* mRNA and decrease in the *RPS9A* pre-mRNA level and finally to the overexpression of Rps9Ap. The increase in the Rps9Ap level is followed by the Rps9B

expression downregulation without any change in the *RPS9B* mRNA level – an indication of a potential *RPS9B* translational regulation. Additionally, authors declare that Rps9 protein binds to the intronic two-way helical structure on the *RPS9A* pre-mRNA. This observation accounts for the splicing repression of the *RPS9A* pre-mRNA as a result of bound Rps9 protein (Petibon et al., 2016).

Besides intron-dependent regulation, 3' untranslated region (UTR) modulates the expression of *RPS9* paralogs – the mRNA level of *RPS9B* gene with *RPS9A* 3' UTR is partially reduced, while the 3' UTR of *RPS9B* is responsible for the increase in the *RPS9A* mRNA level when replaced. Moreover, the *RPS9B* 3' UTR is responsible for reduction of the amount of the *RPS9A* pre-mRNA when substituted. Both, the intron and the 3' UTR uniqueness can independently regulate the mRNA level and hence the amount of Rps9 created by *RPS9A* and *RPS9B*. The Rps9p regulatory circuit proposed by authors is depicted in the Figure 6 (Petibon et al., 2016).

Personally, I see the recent work as a very questionable. When the model works and Rps9p represses splicing of the RPS9A pre-mRNA, how the RPS9A splicing can be totally inhibited in the case of RPS9BΔi, where the expression of Rps9B is also downregulated? In addition to that, the previous work of the same authors! (Parenteau et al., 2011) *as well as the work of others* (Plocik and Guthrie, 2012) *contradict the recent findings. Nevertheless, my role is to summarize, not to judge. The work has been already judged by the peer review in Nucleic Acids Research journal.*

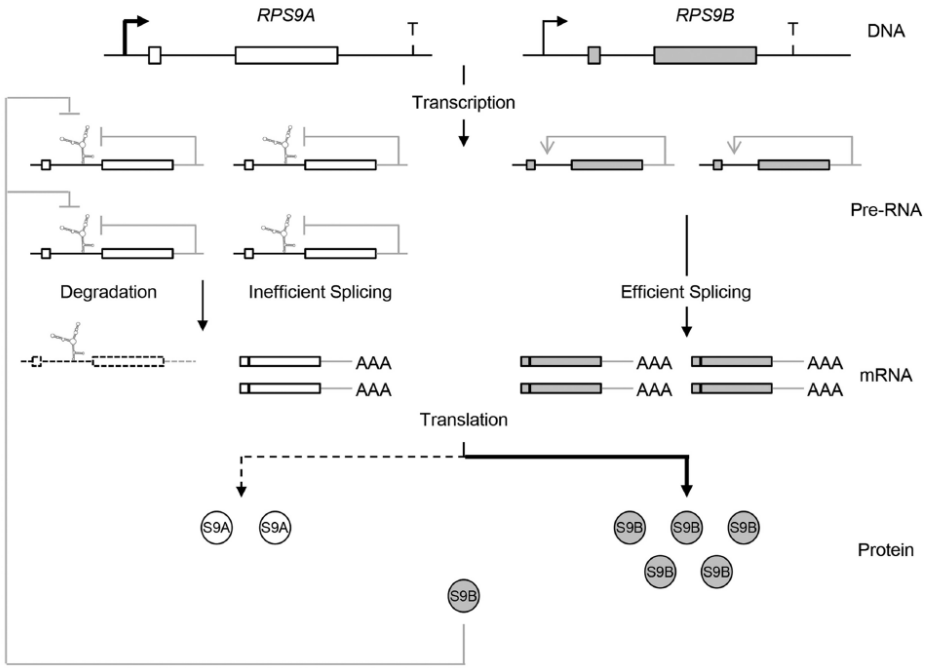

Figure 6: The *RPS9* regulatory circuit. *RPS9A* is transcribed more effectively than *RPS9B*. However, its intronic secondary structure serves as a binding site for Rps9, which repress splicing of *RPS9A* pre-mRNA and hence predetermines it to degradation. Moreover, 3' UTR sequence of *RPS9A* independently decreases efficiency of *RPS9A* pre-mRNA splicing. Thus, *RPS9B* contributes to 98% of Rps9p in the cell under normal conditions. (Petibon et al., 2016)

Independently on the model of regulation, the fact is that *RPS9B* creates 98% and *RPS9A* 2% of total Rps9p under normal conditions. The question is, why both RPS9A and RPS9B genes are preserved in the genome and why the regulation (against *RPS9A*) occurs. The nature of distinctive role of Rps9Ap and Rps9Bp has not been elucidated yet. Nevertheless, Rps9 protein encoded by both *RPS9* paralogs differs only in 5 amino acids. Four of them are located at the acidic C-terminal end, which has been shown to have role in proper translation (Pnueli and Arava, 2007).

Surprisingly, stress exposure alters the ratio of *RPS9A* and *RPS9B* mRNA level in the intron-dependent manner. While the *RPS9B* expression is preferred under normal conditions, exposing cells to higher concentration of NaCl (and hence osmotic stress) or apoptosis-inducing drug straurosporine favors the expression of *RPS9A*. Furthermore, deletion of *RPS9A* gene leads to the sensitivity of mutant cells to straurosporine (Parenteau et al., 2011). This is an example of non-redundant function of duplicated ribosomal protein genes. More arguments against redundancy of duplicated RPGs and concept of 'specialized ribosomes' are presented further in this work.

3.1.5.2 *RPS14A/B* regulation

The essential Rps14 protein (originally rp59) is encoded by duplicated intron-containing genes, *RPS14A* (YCR031C) and *RPS14B* (YJL191W). Under normal conditions, *RPS14A* produces 8- to 10-times more mRNA than *RPS14B*. The deletion of dominant *RPS14A* gene leads to a 10-fold upregulation of *RPS14B* and presence of roughly 80% of wild-type *RPS14* mRNA – thus cells compensate level of Rps14p by intergenic regulation (Paulovich et al., 1993).

The expression of *RPS14B* but not *RPS14A* is normally repressed by Rps14 protein. The regulation occurs post-transcriptionally without *RPS14B* pre-mRNA accumulation under repressive conditions. However, the *RPS14B* pre-mRNA significantly accumulates in the mutant with blocked nuclear RNA export. Analogous increase of the *RPS14B* pre-mRNA level has been noticed in the mutant with abolished nonsense-mediated decay (NMD), a degradation pathway that eliminates RNAs with premature termination codons in cytoplasm (Z. Li et al., 1995).

It was shown that the *RPS14B* pre-mRNA contains a RNA secondary structure formed from 5' exon and first 62 nucleotides of the intron, which is important for the regulation (Z. Li et al., 1995). A three-hybrid system and *in vitro* assays showed that Rps14p directly interacts with the *RPS14B* pre-mRNA. Similarly, Rps14p binds to a conserved helix in 18S rRNA with a 5-fold higher affinity. The C-terminus of Rps14p is essential for the interaction with both RNAs (Fewell and Woolford, 1999; Antúnez De Mayolo and Woolford, 2003).

The model of *RPS14B* autogenous regulation has been proposed. The Rps14p is synthetized in cytoplasm from both – the major *RPS14A* and minor *RPS14B* paralogs; then imported to nucleus, where it is assembled into emerging ribosomes. When in excess, Rps14p binds to the *RPS14B* pre-mRNA and blocks its expression. Presumably, the pre-mRNA with bound Rps14p is exported to cytoplasm and degraded by the nonsense-mediated decay pathway (Fewell and Woolford, 1999).

3.2 Intron-independent regulation

Parenteau et al. studied all 11 pairs of intronless ribosomal protein genes. While a coding sequence of these paralogs is conserved with 93% sequence identity, surrounding 5' and 3' sequences (including e.g. promoters, terminators and UTR) share only 42% of primary structure on average (Parenteau et al., 2015). The expression of RP genes in ten out of 11 RPG pairs is asymmetrical. Differential mRNA level of paralogs is controlled by a selective RNA degradation rather than by a distinctive transcriptional rate of responsible genes (Bonnet et al., 2014; Parenteau et al., 2015).

Interestingly, the underexpressed paralogs often generate alternative mRNA forms with different length of 3' UTR, as a result of an alternative transcription termination. For instance, a major *RPL8B* paralog contains canonical 3' end, whereas a minor *RPL8A* contains at least two transcription termination sites. The first 3' end of *RPL8A* is generated by a canonical polyadenylation-dependent termination, while the second one is less effective Rnt1p-dependent site, which usage leads to the RNA degradation rather than to polyadenylation. Subsequent experiments showed that transcription termination sites are responsible for the low expression level of *RPL8A*. Thus, the *RPL8A* terminator sequence seems to serve as a negative regulator of *RPL8A* expression, while the *RPL8B* terminator sequence positively affects the *RPL8B* expression (Nagalakshmi et al., 2008; Ghazal et al., 2009; Parenteau et al., 2015).

The loss of one copy is compensated by an equivalent increase in the expression of the other RP paralog for only 4 intronless RPG pairs. Indeed, the deletion of ribosomal paralog led to non-correlated or even opposite effect on the expression of the counterpart. Additionally, the presence of directional negative feedback loops has been proposed for some RPG pairs – the loss of only one paralog in the pair increases expression of the other one – e.g. the deletion of a major *RPL8B* or a minor *RPL11A* increases the expression of their paralogs, but not in reverse (Parenteau et al., 2015).

The measurement of drugs impact on mutants with deleted RP paralogs has shown the paralog-specific stress response. Certain r-protein paralogs seem to be favorable under different conditions and thus are not functionally redundant, as also discussed in the chapter 4 (Cracking the idea of functionally redundant duplicated r-proteins). For example, deletion of *RPL8A* increases sensitivity to hygromycin B,

whereas the deletion of major *RPL8B* does not. Subsequent experiments have shown that while the minor *RPL8A* is repressed under normal conditions (relying on transcription termination sites, see above), the exposure to stress presumably induces its expression through promoter induction (Parenteau et al., 2015).

From this study of intronless RPG pairs (Parenteau et al., 2015) as well as the study of intron-containing RPG pairs (Parenteau et al., 2011), authors proposed a model in which duplicated RP genes subfunctionalized in the ability to support growth under normal conditions (predominantly one paralog) as well as under the stress (another one).

3.2.1 *RPL4A/B* regulation

Rpl4 protein (originally Rpl2p) of large ribosomal subunit is encoded by 2 paralogous genes – *RPL4A* (YBR031W) and *RPL4B* (YDR012W) – both lacking intron (Presutti et al., 1988). They do not contribute equally to the pool of Rpl4p. *RPL4A* produces 72%, whereas the *RPL4B* gene produces 28% of total *RPL4* mRNA. The difference in contribution of both genes to final protein level is reflected by the distinct phenotype of strain lacking one of the paralog. The disruption of *RPL4A* gene results in a reduction of the growth rate caused by the limited amount of Rpl4p, while strain lacking *RPL4B* has enough Rpl4p for the 60S subunit synthesis and the wild-type phenotype (Lucioli et al., 1988).

The presence of extra copies of *RPL4A* gene down-regulates level of both *RPL4A* and *RPL4B* transcripts and leads to the accumulation of truncated *RPL4* mRNA molecules. Rpl4 protein is required for the feedback regulation (Presutti et al., 1991). Subsequent experiments pointed out that surplus Rpl4p regulates abundance of *RPL4A/B* mRNA by an endonucleolytic cleavage on a 3' region of mRNA followed by an exonucleolytic degradation. The long region on the 5' region of *RPL4* mRNAs (in the -21 to +339 position, with respect to the translation start site) named as L4 responsive element has been identified as a sufficient regulatory target for destabilization of any transcript when in excess of Rpl4p (Presutti et al., 1995).

3.2.2 *RPS28A/B* regulation

Rps28p protein of small ribosomal subunit is encoded by 2 intronless paralogous genes, *RPS28A* (YOR167C) and *RPS28B* (YLR264W). The Rps28Ap and Rps28Bp differs in only one amino acid (Planta and Mager, 1998).

It was shown that the *RPS28B* expression and not *RPS28A* is autoregulated at the level of mRNA decay. When in excess, the Rps28p (produced by both, *RPS28A* and *RPS28B*) might bind to the RNA stem-loop present in the 3' UTR of the *RPS28B* mRNA (Badis et al., 2004). Rps28p directly interacts with Edc3, an enhancer of mRNA decapping. The result is a formation of decapping machinery complex with *RPS28B* mRNA and mRNA decapping followed by the 5' to 3' degradation. The degradation does not involve deadenylation. Presence of Edc3 is required for the regulation, since *edc3*Δ mutant does not exploit regulation of *RPS28B* mRNA level (Badis et al., 2004; Kolesnikova et al., 2013).

Recent findings have unpredictably shown that Edc3, but not Rps28p, directly binds to the 3' UTR of *RPS28B* mRNA. Edc3 mediates an interaction of Rps28p with Dcp1 and Dcp2, a decapping enzyme complex. Authors suggest that Rps28p allows Edc3 to dimerize as well as it regulates the ability of Edc3 to bind to the 3' UTR regulatory element (He et al., 2014).

To sum up, *RPS28A* and *RPS28B* produce Rps28 protein, which is assembled into emerging ribosomes. When in excess, Rps28p binds to an mRNA decapping activator Edc3, a component of Dcp1/Dcp2 decapping enzyme. The interaction Edc3-Rps28p results in the Edc3 dimerization and binding of the decapping complex to 3' UTR of *RPS28B* mRNA. Finally, surplus *RPS28B* mRNA is degraded. The feedback regulation is depicted in the Figure 7.

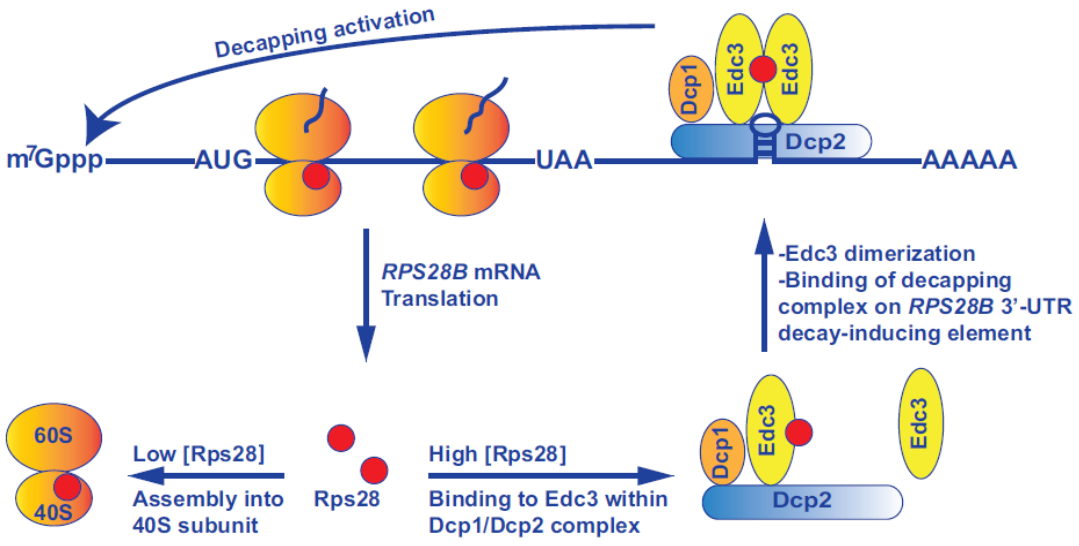

Figure 7: Edc3-mediated RPS28B mRNA degradation requires the Rps28 protein. Rps28 protein encoded either by *RPS28A* or *RPS28B* is assembled into the 40S subunit of ribosome. When in excess, Rps28p interacts with an mRNA decapping activator Edc3. The interaction directs Edc3 to dimerization. As a result, a decapping complex binds to the 3' UTR of *RPS28B* mRNA and degrades surplus *RPS28B* mRNA. (He et al., 2014, modified)

4 CRACKING THE IDEA OF FUNCTIONALLY REDUNDANT DUPLICATED R-PROTEINS

A first indication of RP paralogous genes specialization arose from the study of *RRP7* (ribosomal RNA processing 7), an essential gene required for a pre-rRNA processing and consequently a proper 40S ribosomal subunit production. It has been shown that *RPS27A* or *RPS27B* gene on multi-copy plasmid suppresses the lethality of *RRP7* deletion and restore the synthesis of 40S subunits of the ribosome. The profile of pre-rRNA intermediates appears different with elevated level of 35S and 23S rRNA in the case of *RPS27B*, while accumulation of the 32S and 21S rRNA has been shown for *RPS27A*. *RPS27A* better supports rRNA processing than *RPS27B* when overexpressed in strain lacking *RRP7*. Surprisingly, Rps27 protein encoded by *RPS27A* and *RPS27B* paralogs differs only in one amino acid. Nonetheless, this phenomenon has not been further examined and even authors have been skeptical about their observation (Baudin-Baillieu et al., 1997).

Paralogous genes *RPL6A*, *RPL13B*, *RPL14A*, *RPL19B*, *RPL23A*, *RPL31A* and *RPP1A*, but not their counterparts, are coupled with actin cytoskeleton processes, since diploid strains hemizygous for *ACT1* (gene encoding actin) and simultaneously RP genes named above, have a growth deficit and severe cell and morphology defects. It has been shown that Rpl13 protein expressed either from *RPL13A* or *RPL13B* paralogs with total length of 199 amino acids (AA) differs only in 2 AA. Surprisingly, two-thirds of total Rpl13p are encoded by *RPL13A*, a gene not contributing to the function of the actin cytoskeleton. Thus, another example of a ribosomal paralog with specialized function has been shown (Haarer et al., 2007).

A large-scale genomic analysis of genes essential for meiosis and sporulation revealed that 8 ribosomal protein paralogous genes (*RPL7A*, *RPL13A*, *RPL19A*, *RPL27A*, *RPL31A*, *RPL34B*, *RPL35A* and *RPL40A*), but not their counterparts, are required for proper meiosis and sporulation (Enyenihi and Saunders, 2003). Another study of polarized cell division has found genes required for a bipolar bud site selection. The loss of *RPL12B*, *RPL17A*, *RPL22A*, *RPS0B*, *RPS1B*, *RPS7A*, *RPS27B*, *RPS28A* or *RPS30B* paralog leads to altered budding pattern (random budding) and for some gene deletions also to reduced growth rate (Ni and Snyder, 2001). The reason of these functional specificities between duplicated RP genes has not been elucidated and thus might be (simply) caused by differential expression of paralogs.

Finally, a study of *ASH1* gene has brought a conclusive evidence. *ASH1* mRNA is targeted and anchored to the bud tip at the end of anaphase. Thus, Ash1 protein is localized exclusively in the daughter cell, where it suppresses mating-type switching upon cell division (Sil and Herskowitz, 1996). The localization of *ASH1* mRNA is translation-dependent, since inhibition of *ASH1* translation as well as a total inhibition of translation by cycloheximide disrupts bud-tip anchoring of *ASH1* mRNA (Gonzalez et al., 1999; Kruse et al., 2002). Paralogous genes *RPL7A*, *RPL12B*, *RPL22A* and *RPS18B* are required for proper *ASH1* mRNA localization, whereas their counterparts have little if any effect on the localization, even when

overexpressed (Komili et al., 2007). Same paralogs have been identified as required for the bud-site selection (discussed above; Ni and Snyder, 2001). Interestingly, the paralog specificity for *ASH1* mRNA localization is not a result of the differential expression or any general effect on the ribosomal assembly (Komili et al., 2007). It is believed that Loc1p, a protein which operates in the *ASH1* mRNA localization as well as in the ribosome assembly (Harnpicharnchai et al., 2001; Long et al., 2001), is required for the assembly of specific ribosomal paralogous proteins during ribosome biogenesis – a creation of 'specialized' ribosomes, which selectively translate and mediate a proper *ASH1* mRNA localization (Komili et al., 2007).

Transcriptional profiling of cells lacking various RP paralogs revealed that duplicated RP genes affect mRNA levels of genes with different functions. For example, the absence of *RPL12A* leads to the induction of genes involved in amino acid biosynthesis, whereas loss of *RPL12B* represses genes involved in cell wall and RNA modification. Loss of paralogs also results in a distinct sensitivity to inhibitors – e.g. deletion of *RPL41A* (but not *RPL41B*) results in a phenantroline sensitivity, while cells lacking *RPL41B* are sensitive to benomyl. Detected differences in phenotype between paralogs seem not to be a consequence of their expression level. Additionally, clustering analysis showed that no two paralogous ribosomal proteins display identical phenotype effects. Despite the high sequence similarity between duplicated r-proteins, their cellular roles are different. It indicates a model in which right combinations of RP paralogs are required for different cellular processes. Even though these differences have not been linked to translation as in the case of *ASH1* localization, authors hypothesize about the role of 'specialized' ribosomes with different combinations of paralogous ribosomal proteins. Translation might be extensively regulated by different combinations of ribosomal proteins – a 'ribosome code', analogous to 'histone code', which regulates transcription state of adjacent DNA by combining of histones and their modifications (Komili et al., 2007). Similar idea has been proposed as a ribosome filter hypothesis 14 years ago. Authors speculated there about sequences within some mRNAs, which might arrange specific mRNA-rRNA and/or mRNA-ribosomal protein interactions and thus affect their translation. These competitive interactions between ribosomal subunits and various mRNAs are possibly modulated by a ribosome heterogeneity (Mauro and Edelman, 2002).

RPL7A and *RPL7B* genes, both containing two introns, encode ribosomal protein Rpl7, which share 239 out of 244 amino acids (Mizuta et al., 1992). Despite the high sequence homology, the Rpl7B-GFP (green-fluorescent protein) fusion protein localizes in both the cytoplasm and nucleolus, whereas Rpl7A-GFP is present exclusively in the cytoplasm. Interestingly, the level of Rpl7A is about 14-times higher that of Rpl7B. *RPL7A* deletion leads to the cytoplasmic localization of Rpl7B-GFP. Therefore, Rpl7A seems to be preferentially incorporated into the ribosome during its assembly and so it is detected only in cytoplasmic ribosomes (Ghaemmaghami et al., 2003; Kim et al., 2009). The loss of Loc1,

a factor required for ribosome assembly and perhaps for folding of specific paralogous r-proteins to ribosome as proposed by Komili et al. (2007), leads to the sole localization of Rpl7B-GFP into the nucleolus – accordingly supports a role of Loc1 in a creation of 'specialized' ribosomes (Kim et al., 2009).[1]

In 2013, the use of diploid yeast strains hemizygous for one of the duplicated RP genes has allowed investigation of altered ribosomes. The deletion of one copy of RP paralog causes that at least a sub-population of ribosomes is enriched with a paralogous r-protein encoded by 2 copies in genome. Strains with altered ribosomes are able to specifically modulate the translation of reporter mRNAs compared to WT strain. One of the examples is *RPL35A* deletion, which does not change the translation of any of reporter mRNAs, whereas the *RPL35B* deletion does. It leads to the overexpression of LAMB3+PTC reporter (a human extracellular matrix protein laminin β3 gene fused to firefly luciferase gene, containing a premature termination codon) – without change in the expression of LAMB3-PTC reporter. The *RPL35B* deletion has no effect on the expression of firefly luciferase mRNA alone with or without PTC. At least, the artificial modification of ribosome paralogous RP composition is responsible for a distinctive modification of translational efficiency of specific mRNAs (Bauer et al., 2013).

Recently, a proteomics analysis has shown that cells contain ribosomes with different stoichiometry of ribosomal proteins. The different stoichiometry depends on the number of ribosomes per mRNA and the carbon source. Ribosomal proteins whose stoichiometry differ the most are often located on the surface of ribosome. Moreover, the differential ratio of r-proteins in monosomes and polysomes seems to be evolutionary conserved, since results with yeast ribosomes resemble that of mouse embryonic stem cells ribosomes. As an example, Rpl37A protein is enriched in mono-ribosomal fraction, whereas Rpl37B protein predominates in polysomes, when grown on glucose carbon source (Slavov et al., 2015).

Historically, the ribosome has been seen as a molecular machine with fixed composition and rather as a passive participant in translation (Frank, 2000; Ben-Shem et al., 2011). Recent findings suggest an idea that cells can modulate the ribosome composition and function. Similar, and sometimes even more attractive findings have been shown for species across the tree of life, as reviewed in (*Shi and Barna, 2015).

[1] Besides being part of ribosomes, many r-proteins have auxiliary, moonlighting roles known as extraribosomal function. One of the example is an ability of r-proteins to regulate their own expression, which was discussed above. It was shown that r-proteins influence also DNA repair, replicative lifespan or RNA polymerase III transcription in budding yeast, as reviewed in (*Lu et al., 2015). Therefore, it is a difficult task to separate the ribosomal and extraribosomal function of r-proteins in phenotypic outcomes described above.

5 CONCLUSION

The purpose of a previous text was to show that the expression of ribosomal protein genes is fine-tuned to maintain equimolar amounts of r-proteins in cells. RP genes are regulated mainly at the transcriptional level. Despite the fact that we know many transcriptional factors regulating RP genes, there are more questions than answers at this point. For instance, 10 RP genes are not regulated by any of known RPG transcriptional regulators (Reja et al., 2015).

The transcription appears to be precise and displays low level of noise in the expression of RP genes. However, cells have a second 'line of defense', covered by various types of post-transcriptional regulation, which deals with fluctuations of individual ribosomal proteins. RP genes are enriched with introns in otherwise intron-poor yeast genome. Numerous examples of post-transcriptional regulation are intron-dependent. Thus, introns seem to be required for the fine-tuning of ribosomal proteins expression.

Lastly, the idea that paralogous ribosomal proteins might have altered functions and therefore affect specific aspects of cell physiology has been proposed. Under various stresses, the ratio of paralogs is changed. The expression of specific paralogs in cells might lead to ribosomes with a specific combination of these r-proteins (a ribosome code) and therefore to specific ribosome properties. This suggests the presence of specialized ribosomes capable to optimize translation of specific mRNA or to adapt to specific environmental conditions.

6 REFERENCES

Antúnez De Mayolo, P., Woolford, J.L., 2003. Interactions of yeast ribosomal protein rpS14 with RNA. Journal of Molecular Biology 333, 697–709.

Ares, M., Grate, L., Pauling, M.H., 1999. A handful of intron-containing genes produces the lion's share of yeast mRNA. Rna 5, 1138–1139.

Badis, G., Saveanu, C., Fromont-Racine, M., Jacquier, A., 2004. Targeted mRNA degradation by deadenylation-independent decapping. Molecular Cell 15, 5–15.

Baudin-Baillieu, A., Tollervey, D., Cullin, C., Lacroute, F., 1997. Functional analysis of Rrp7p, an essential yeast protein involved in pre-rRNA processing and ribosome assembly. Molecular and cellular biology 17, 5023–32.

Bauer, J.W., Brandl, C., Haubenreisser, O., Wimmer, B., Weber, M., Karl, T., Klausegger, A., Breitenbach, M., Hintner, H., von der Haar, T., Tuite, M.F., Breitenbach-Koller, L., 2013. Specialized Yeast Ribosomes: A Customized Tool for Selective mRNA Translation. PLoS ONE 8.

Ben-Shem, A., Garreau de Loubresse, N., Melnikov, S., Jenner, L., Yusupova, G., Yusupov, M., 2011. The structure of the eukaryotic ribosome at 3.0 Å resolution. Science (New York, N.Y.) 334, 1524–9.

Bergkessel, M., Whitworth, G.B., Guthrie, C., 2011. Diverse environmental stresses elicit distinct responses at the level of pre-mRNA processing in yeast. RNA (New York, N.Y.) 17, 1461–78.

Bonnet, J., Wang, C.Y., Baptista, T., Vincent, S.D., Hsiao, W.C., Stierle, M., Kao, C.F., Tora, L., Devys, D., 2014. The SAGA coactivator complex acts on the whole transcribed genome and is required for RNA polymerase II transcription. Genes and Development 28, 1999–2012.

Bragulat, M., Meyer, M., Macías, S., Camats, M., Labrador, M., Vilardell, J., 2010. RPL30 regulation of splicing reveals distinct roles for Cbp80 in U1 and U2 snRNP cotranscriptional recruitment. RNA (New York, N.Y.) 16, 2033–41.

Brown, P.O., Lieb, J.D., Botstein, D., Liu, X., Botstein, D., Brown, P.O., 2001. Promoter-specific binding of Rap1 revealed by genome-wide maps of protein-DNA association. Nature Genetics 28, 327–334.

Buchman, a R., Kimmerly, W.J., Rine, J., Kornberg, R.D., 1988. Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences, and telomeres in Saccharomyces cerevisiae. Molecular and cellular biology 8, 210–225.

Byrne, K.P., Wolfe, K.H., 2005. The Yeast Gene Order Browser: Combining curated homology and syntenic context reveals gene fate in polyploid species. Genome Research 15, 1456–1461.

Dabeva, M.D., Post-Beittenmiller, M.A., Warner, J.R., 1986. Autogenous regulation of splicing of the transcript of a yeast ribosomal protein gene. Proc Natl Acad Sci U S A 83, 5854–7.

Dabeva, M.D., Warner, J.R., 1987. The yeast ribosomal protein L32 and its gene. Journal of Biological Chemistry 262, 16055–16059.

Dabeva, M.D., Warner, J.R., 1993. Ribosomal protein L32 of Saccharomyces cerevisiae regulates both splicing and translation of its own transcript. Journal of Biological Chemistry 268, 19669–19674.

Dean, E.J., Davis, J.C., Davis, R.W., Petrov, D.A., 2008. Pervasive and persistent redundancy among duplicated genes in yeast. PLoS Genetics 4.

Deutschbauer, A.M., Jaramillo, D.F., Proctor, M., Kumm, J., Hillenmeyer, M.E., Davis, R.W., Nislow, C.,

Giaever, G., 2005. Mechanisms of haploinsufficiency revealed by genome-wide profiling in yeast. Genetics 169, 1915–1925.

Eng, F.J., Warner, J.R., 1991. Structural basis for the regulation of splicing of a yeast messenger RNA. Cell 65, 797–804.

Enyenihi, A.H., Saunders, W.S., 2003. Large-scale functional genomic analysis of sporulation and meiosis in Saccharomyces cerevisiae. Genetics 163, 47–54.

Fewell, S.W., Woolford, J.L., 1999. Ribosomal protein S14 of Saccharomyces cerevisiae regulates its expression by binding to RPS14B pre-mRNA and to 18S rRNA. Molecular and cellular biology 19, 826–34.

Fingerman, I., Nagaraj, V., Norris, D., Vershon, A.K., 2003. Sfp1 Plays a Key Role in Yeast Ribosome Biogenesis Sfp1 Plays a Key Role in Yeast Ribosome Biogenesis. Eukaryotic Cell, 2, 1061–1068.

Frank, J., 2000. The ribosome - A macromolecular machine par excellence. Chemistry and Biology 7, 133–141.

Frazier, M., Gibbs, R.A., Muzny, D.M., Scherer, S.E., Bouck, J.B., Sodergren, E.J., Worley, K.C., Rives, C.M., Gorrell, J.H., Metzker, M.L., FitzHugh, W., Naylor, S.L., Kucherlapati, R.S., Nelson, D.L., Weinstock, G.M., Sakaki, Y., Fujiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Funke, R., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Weissenbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Gage, D., Pelletier, E., Robert, C., Wincker, P., Smith, D.R., Doucette-Stamm, L., Rubenfield, M., Weinstock, K., Lee, H.M., Dubois, J., Rosenthal, A., Harris, K., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Hood, L., Heaford, A., Rowen, L., Madan, A., Qin, S., Davis, R.W., Federspiel, N.A., Abola, A.P., Proctor, M.J., Myers, R.M., Schmutz, J., Dickson, M., Howland, J., Grimwood, J., Cox, D.R., Olson, M. V, Kaul, R., Raymond, C., Shimizu, N., Kawasaki, K., Minoshima, S., Evans, G.A., Athanasiou, M., Kann, L., Schultz, R., Roe, B.A., Chen, F., Pan, H., Ramser, J., Lehrach, H., Reinhardt, R., McCombie, W.R., de la Bastide, M., Dedhia, N., Lehoczky, J., Blocker, H., Hornischer, K., Nordsiek, G., Agarwala, R., Aravind, L., Bailey, J.A., Bateman, A., Batzoglou, S., Birney, E., Bork, P., LeVine, R., Brown, D.G., Burge, C.B., Cerutti, L., Chen, H.C., Church, D., Clamp, M., Copley, R.R., Doerks, T., Eddy, S.R., Eichler, E.E., McEwan, P., Lander, E.S., Furey, T.S., Galagan, J., Gilbert, J.G., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L.S., McKernan, K., Jones, T.A., Kasif, S., Kaspryzk, A., Kennedy, S., Kent, W.J., Kitts, P., Koonin, E. V, Korf, I., Kulp, D., Lancet, D., Meldrim, J., Lowe, T.M., McLysaght, A., Mikkelsen, T., Moran, J. V, Mulder, N., Pollara, V.J., Ponting, C.P., Schuler, G., Schultz, J., Slater, G., Mesirov, J.P., Smit, A.F., Stupka, E., Szustakowski, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y.I., Miranda, C., Wolfe, K.H., Yang, S.P., Yeh, R.F., Collins, F., Guyer, M.S., Peterson, J., Felsenfeld, A., Wetterstrand, K.A., Patrinos, A., Morgan, M.J., Morris, W., de Jong, P., Catanese, J.J., Osoegawa, K., Shizuya, H., Choi, S., Chen, Y.J., Szustakowki, J., Naylor, J., Rosetti, M., Santos, R., Sheridan, A., Linton, L.M., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Birren, B., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Nusbaum, C., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Zody, M.C., Milne, S., Mullikin, J.C., Mungall, A., Plumb, R., Ross, M., Shownkeen, R., Sims, S., Waterston, R.H., Wilson, R.K., Hillier, L.W., Baldwin, J., McPherson, J.D., Marra, M.A., Mardis, E.R., Fulton, L.A., Chinwalla, A.T., Pepin, K.H., Gish, W.R., Chissoe, S.L., Wendl, M.C., Delehaunty, K.D., Devon, K., Miner, T.L., Delehaunty, A., Kramer, J.B., Cook, L.L., Fulton, R.S., Johnson, D.L., Minx, P.J., Clifton, S.W., Hawkins, T., Branscomb, E., Dewar, K., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J.F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Doyle, M., 2001. Initial sequencing and analysis of the human genome. Nature 409, 860–921.

Fujita, K., Baba, T., Isono, K., 1998. Genomic Analysis of the Genes Encoding Ribosomal Proteins in Eight Eubacterial Species and Saccharomyces cerevisiae. Genome informatics. Workshop on Genome Informatics 9, 3–12.

Gahura, O., Hammann, C., Valentová, A., Puta, F., Folk, P., 2011. Secondary structure is required for 3′ splice site recognition in yeast. Nucleic Acids Research 39, 9759–9767.

Garre, E., Romero-Santacreu, L., Barneo-Muñoz, M., Miguel, A., Pérez-Ortín, J.E., Alepuz, P., 2013. Nonsense-Mediated mRNA Decay Controls the Changes in Yeast Ribosomal Protein Pre-mRNAs Levels upon Osmotic Stress. PLoS ONE 8.

Ghaemmaghami, S., Ghaemmaghami, S., Huh, W.-K., Huh, W.-K., Bower, K., Howson, R., Bower, K., Belle, a, Howson, R.W., Belle, A., Dephoure, N., O'Shea, E., Dephoure, N., Weissman, J., O'Shea, E.K., Weissman, J.S., 2003. Global analysis of protein expression in yeast. Nature 425, 737–41.

Ghazal, G., Gagnon, J., Jacques, P. Étienne, Landry, J.R., Robert, F., Abou Elela, S., 2009. Yeast RNase III Triggers Polyadenylation-Independent Transcription Termination. Molecular Cell 36, 99–109.

Gonzalez, I., Buonomo, S.B.C., Nasmyth, K., Von Ahsen, U., 1999. ASH1 mRNA localization in yeast involves multiple secondary structural elements and ASH1 protein translation. Current Biology 9, 337–340.

Gordan, R., Hartemink, A.J., Bulyk, M.L., 2009. Distinguishing direct versus indirect transcription factor-DNA interactions. Genome Research 19, 2090–2100.

Gu, X., 2003. Evolution of duplicate genes versus genetic robustness against null mutations. Trends in Genetics 19, 354–356.

Haarer, B., Viggiano, S., Hibbs, M.A., Troyanskaya, O.G., Amberg, D.C., 2007. Modeling complex genetic interactions in a simple eukaryotic genome: Actin displays a rich spectrum of complex haploinsufficiencies. Genes and Development 21, 148–159.

Hall, D.B., Wade, J.T., Struhl, K., 2006. An HMG Protein , Hmo1 , Associates with Promoters of Many Ribosomal Protein Genes and throughout the rRNA Gene Locus in Saccharomyces cerevisiae An HMG Protein , Hmo1 , Associates with Promoters of Many Ribosomal Protein Genes and throughout the rRNA Gene 26, 3672–3679.

Harnpicharnchai, P., Jakovljevic, J., Horsey, E., Miles, T., Roman, J., Rout, M., Meagher, D., Imai, B., Guo, Y., Brame, C.J., Shabanowitz, J., Hunt, D.F., Woolford, J.L., 2001. Composition and functional characterization of yeast 66S ribosome assembly intermediates. Molecular Cell 8, 505–515.

He, F., Li, C., Roy, B., Jacobson, A., 2014. Yeast Edc3 targets RPS28B mRNA for Decapping by Binding to a 3′ Untranslated Region Decay-Inducing Regulatory Element. Molecular and Cellular Biology 34, 1438–51.

Ho, Y., Gruhler, A., Heilbut, A., Bader, G.D., Moore, L., Adams, S.-L., Millar, A., Taylor, P., Bennett, K., Boutilier, K., Yang, L., Wolting, C., Donaldson, I., Schandorff, S., Shewnarane, J., Vo, M., Taggart, J., Goudreault, M., Muskat, B., Alfarano, C., Dewar, D., Lin, Z., Michalickova, K., Willems, A.R., Sassi, H., Nielsen, P. a, Rasmussen, K.J., Andersen, J.R., Johansen, L.E., Hansen, L.H., Jespersen, H., Podtelejnikov, A., Nielsen, E., Crawford, J., Poulsen, V., Sørensen, B.D., Matthiesen, J., Hendrickson, R.C., Gleeson, F., Pawson, T., Moran, M.F., Durocher, D., Mann, M., Hogue, C.W. V, Figeys, D., Tyers, M., 2002. Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry. Nature 415, 180–183.

Howe, K.J., Ares, M., 1997. Intron self-complementarity enforces exon inclusion in a yeast pre-mRNA. Proceedings of the National Academy of Sciences of the United States of America 94, 12467–72.

Chambers, A., Tsang, J.S., Stanway, C., Kingsman, A.J., Kingsman, S.M., 1989. Transcriptional control of the Saccharomyces cerevisiae PGK gene by RAP1. Molecular and cellular biology 9, 5516–24.

Chao, J.A., Williamson, J.R., 2004. Joint X-ray and NMR refinement of the yeast L30e-mRNA complex. Structure 12, 1165–1176.

Charpentier, B., Rosbash, M., 1996. Intramolecular structure in yeast introns aids the early steps of in vitro spliceosome assembly. RNA 2, 509–522.

Jorgensen, P., Rupeš, I., Sharom, J.R., Schneper, L., Broach, J.R., Tyers, M., 2004. A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. Genes and Development 18, 2491–2505.

Juneau, K., Miranda, M., Hillenmeyer, M.E., Nislow, C., Davis, R.W., 2006. Introns regulate RNA and protein abundance in yeast. Genetics 174, 511–518.

Kafri, R., Levy, M., Pilpel, Y., 2006. The regulatory utilization of genetic redundancy through responsive backup circuits. Proceedings of the National Academy of Sciences 103, 11653–11658.

Kaplan, N., Moore, I.K., Fondufe-Mittendorf, Y., Gossett, A.J., Tillo, D., Field, Y., Leproust, E.M., Hughes, T.R., Lieb, J.D., Widom, J., Segal, E., 2009. The DNA-encoded nucleosome organization of a eukaryotic genome. Nature 458, 362–366.

Kasahara, K., Ohtsuki, K., Ki, S., Aoyama, K., Takahashi, H., Kobayashi, T., Shirahige, K., Kokubo, T., 2007. Assembly of regulatory factors on rRNA and ribosomal protein genes in Saccharomyces cerevisiae. Molecular and cellular biology 27, 6686–6705.

Kellis, M., Birren, B.W., Lander, E.S., 2004. Proof and evolutionary analysis of ancient genome duplication in the yeast Saccharomyces cerevisiae. TL - 428. Nature 428 VN - , 617–624.

Kim, C.H., Warner, J.R., 1983. Messenger RNA for ribosomal proteins in yeast. Journal of Molecular Biology 165, 79–89.

Kim, T.Y., Ha, C.W., Huh, W.K., 2009. Differential subcellular localization of ribosomal protein L7 paralogs in Saccharomyces cerevisiae. Molecules and Cells 27, 539–546.

Klein, D.J., Schmeing, T.M., Moore, P.B., Steitz, T.A., 2001. The kink-turn: A new RNA secondary structure motif. EMBO Journal 20, 4214–4221.

Knight, B., Kubik, S., Ghosh, B., Bruzzone, M.J., Geertz, M., Martin, V., Dénervaud, N., Jacquet, P., Ozkan, B., Rougemont, J., Maerkl, S.J., Naef, F., Shore, D., 2014. Two distinct promoter architectures centered on dynamic nucleosomes control ribosomal protein gene transcription. Genes and Development 28, 1695–1709.

Kolesnikova, O., Back, R., Graille, M., Séraphin, B., 2013. Identification of the Rps28 binding motif from yeast Edc3 involved in the autoregulatory feedback loop controlling RPS28B mRNA decay. Nucleic Acids Research 41, 9514–9523.

Komili, S., Farny, N.G., Roth, F.P., Silver, P.A., 2007. Functional Specificity among Ribosomal Proteins Regulates Gene Expression. Cell 131, 557–571.

Kruse, C., Jaedicke, A., Beaudouin, J., Bohl, F., Ferring, D., Guttler, T., Ellenberg, J., Jansen, R.P., 2002. Ribonucleoprotein-dependent localization of the yeast class V myosin Myo4p. Journal of Cell Biology 159, 971–982.

Lascaris, R.F., Mager, W.H., Planta, R.J., 1999. DNA-binding requirements of the yeast protein Rap1p as selected in silico from ribosomal protein gene promoter sequences. Bioinformatics 15, 267–277.

Lee, T.I., Rinaldi, N.J., Robert, F., Odom, D.T., Bar-Joseph, Z., Gerber, G.K., Hannett, N.M., Harbison,

C.T., Thompson, C.M., Simon, I., Zeitlinger, J., Jennings, E.G., Murray, H.L., Gordon, D.B., Ren, B., Wyrick, J.J., Tagne, J.-B., Volkert, T.L., Fraenkel, E., Gifford, D.K., Young, R.A., 2002. Transcriptional Regulatory Networks in Saccharomyces cerevisiae. Science 298, 799–804.

Leer, R.J., Van Raamsdonk-Duin, M.M.C., Mager, W.H., Planta, R.J., 1985. Conserved sequences upstream of yeast ribosomal protein genes. Current Genetics 9, 273–277.

Li, B., Nierras, C.R., Warner, J.R., 1999. Transcriptional elements involved in the repression of ribosomal protein synthesis. Molecular and cellular biology 19, 5393–5404.

Li, B., Vilardell, J., Warner, J.R., 1996. An RNA structure involved in feedback regulation of splicing and of translation is critical for biological fitness. Proceedings of the National Academy of Sciences of the United States of America 93, 1596–1600.

Li, H., Dalal, S., Kohler, J., Vilardell, J., White, S. a, 1995. Characterization of the pre-mRNA binding site for yeast ribosomal protein L32: the importance of a purine-rich internal loop. Journal of molecular biology 250, 447–59.

Li, Z., Paulovich, A.G., Woolford, J.L., 1995. Feedback inhibition of the yeast ribosomal protein gene CRY2 is mediated by the nucleotide sequence and secondary structure of CRY2 pre-mRNA. Molecular and cellular biology 15, 6454–64.

Long, R.M., Gu, W., Meng, X., Gonsalvez, G., Singer, R.H., Chartrand, P., 2001. An exclusively nuclear RNA-binding protein affects asymmetric localization of ASH1 mRNA and Ash1p in yeast. Journal of Cell Biology 153, 307–318.

Lucioli, A., Presutti, C., Ciafrè, S., Caffarelli, E., Fragapane, P., Bozzoni, I., 1988. Gene dosage alteration of L2 ribosomal protein genes in Saccharomyces cerevisiae: effects on ribosome synthesis. Molecular and cellular biology 8, 4792–8.

Macías, S., Bragulat, M., Tardiff, D.F., Vilardell, J., 2008. L30 Binds the Nascent RPL30 Transcript to Repress U2 snRNP Recruitment. Molecular Cell 30, 732–742.

Mallick, J., Whiteway, M., 2013. The evolutionary rewiring of the ribosomal protein transcription pathway modifies the interaction of transcription factor heteromer Ifh1-Fhl1 (interacts with forkhead 1-forkhead-like 1) with the DNA-binding specificity element. Journal of Biological Chemistry 288, 17508–17519.

Mao, H., White, S. a, Williamson, J.R., 1999. A novel loop-loop recognition motif in the yeast ribosomal protein L30 autoregulatory RNA complex. Nature structural biology 6, 1139–47.

Marion, R.M., Regev, A., Segal, E., Barash, Y., Koller, D., Friedman, N., O'Shea, E.K., 2004. Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression. Proceedings of the National Academy of Sciences of the United States of America 101, 14315–22.

Martin, D.E., Soulard, A., Hall, M.N., 2004. TOR regulates ribosomal protein gene expression via PKA and the Forkhead Transcription Factor FHL1. Cell 119, 969–979.

Mauro, V.P., Edelman, G.M., 2002. The ribosome filter hypothesis. Proc Natl Acad Sci U S A 99, 12031–12036.

Meyer, M., Plass, M., Pérez-Valle, J., Eyras, E., Vilardell, J., 2011. Deciphering 3'ss Selection in the Yeast Genome Reveals an RNA Thermosensor that Mediates Alternative Splicing. Molecular Cell 43, 1033–1039.

Mizuta, K., Hashimoto, T., Otaka, E., 1992. Yeast ribosomal proteins: XIII. Saccharomyces cerevisiae YL8A gene, interrupted with two introns, encodes a homolog of mammalian L7. Nucleic acids research 20, 1011–6.

Nagalakshmi, U., Wang, Z., Waern, K., Shou, C., Raha, D., Gerstein, M., Snyder, M., 2008. The transcriptional landscape of the yeast genome defined by RNA sequencing. Science (New York, N.Y.) 320, 1344–9.

Ni, L., Snyder, M., 2001. A genomic study of the bipolar bud site selection pattern in Saccharomyces cerevisiae. Molecular biology of the cell 12, 2147–2170.

Nomura, M., Yates, J.L., Dean, D., Post, L.E., 1980. Feedback regulation of ribosomal protein gene expression in Escherichia coli: structural homology of ribosomal RNA and ribosomal protein MRNA. Proceedings of the National Academy of Sciences 77, 7084–7088.

Papp, B., Pál, C., Hurst, L.D., 2003. Dosage sensitivity and the evolution of gene families in yeast. Nature 424, 194–197.

Parenteau, J., Durand, M., Morin, G., Gagnon, J., Lucier, J.F., Wellinger, R.J., Chabot, B., Elela, S.A., 2011. Introns within ribosomal protein genes regulate the production and function of yeast ribosomes. Cell 147, 320–331.

Parenteau, J., Durand, M., Veronneau, S., Lacombe, A.-A., Morin, G., Guerin, V., Cecez, B., Gervais-Bird, J., Koh, C.-S., Brunelle, D., Wellinger, R.J., Chabot, B., Abou Elela, S., 2008. Deletion of many yeast introns reveals a minority of genes that require splicing for function. Molecular biology of the cell 19, 1932–1941.

Parenteau, J., Lavoie, M., Catala, M., Malik-Ghulam, M., Gagnon, J., Abou Elela, S., 2015. Preservation of Gene Duplication Increases the Regulatory Spectrum of Ribosomal Protein Genes and Enhances Growth under Stress. Cell Reports 13, 2516–2526.

Paulovich, A.G., Thompson, J.R., Larkin, J.C., Li, Z., Woolford, J.L., 1993. Molecular Genetics of Cryptopleurine Resistance in. DNA Sequence 730.

Pearson, N.J., Fried, H.M., Warner, J.R., 1982. Yeast use translational control to compensate for extra copies of a ribosomal protein gene. Cell 29, 347–355.

Petibon, C., Parenteau, J., Catala, M., Elela, S.A., 2016. Introns regulate the production of ribosomal proteins by modulating splicing of duplicated ribosomal protein genes. Nucleic Acids Research 8000, gkw140.

Planta, R.J., Mager, W.H., 1998. The list of cytoplasmic ribosomal proteins of Saccharomyces cerevisiae. Yeast 14, 471–477.

Pleiss, J.A., Whitworth, G.B., Bergkessel, M., Guthrie, C., 2007a. Transcript specificity in yeast pre-mRNA splicing revealed by mutations in core spliceosomal components. PLoS Biology 5, 745–757.

Pleiss, J.A., Whitworth, G.B., Bergkessel, M., Guthrie, C., 2007b. Rapid, Transcript-Specific Changes in Splicing in Response to Environmental Stress. Molecular Cell 27, 928–937.

Plocik, A.M., Guthrie, C., 2012. Diverse forms of RPS9 splicing are part of an evolving autoregulatory circuit. PLoS Genetics 8, e1002620.

Pnueli, L., Arava, Y., 2007. Genome-wide polysomal analysis of a yeast strain with mutated ribosomal protein S9. BMC genomics 8, 285.

Presutti, C., Ciafré, S. a, Bozzoni, I., 1991. The ribosomal protein L2 in S. cerevisiae controls the level of accumulation of its own mRNA. The EMBO journal 10, 2215–21.

Presutti, C., Lucioli, A., Bozzoni, I., 1988. Ribosomal protein L2 in Saccharomyces cerevisiae is homologous to ribosomal protein L1 in Xenopus laevis. Isolation and characterization of the

genes. Journal of Biological Chemistry 263, 6188–6192.

Presutti, C., Villa, T., Hall, D., Pertica, C., Bozzoni, I., 1995. Identification of the cis-elements mediating the autogenous control of ribosomal protein L2 mRNA stability in yeast. The EMBO journal 14, 4022–30.

Reid, J.L., Iyer, V.R., Brown, P.O., Struhl, K., 2000. Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. Molecular Cell 6, 1297–1307.

Reja, R., Vinayachandran, V., Ghosh, S., Pugh, B.F., 2015. Molecular mechanisms of ribosomal protein gene coregulation 1942–1954.

Romero-santacreu, L., Moreno, J., Perez-Ortin, J.E., Alepuz, P., 2009. Specific and global regulation of mRNA stability during osmotic stress in Saccharomyces cerevisiae. Rna 1110–1120.

Rudra, D., Mallick, J., Zhao, Y., Warner, J.R., 2007. Potential interface between ribosomal protein production and pre-rRNA processing. Molecular and cellular biology 27, 4815–24.

Shore, D., Nasmyth, K., 1987. Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. Cell 51, 721–732.

Schawalder, S.B., Kabani, M., Howald, I., Choudhury, U., Werner, M., Shore, D., 2004. Growth-regulated recruitment of the essential yeast ribosomal protein gene activator Ifh1. Nature 432, 1058–61.

Sil, A., Herskowitz, I., 1996. Identification of an asymmetrically localized determinant, Ash1p, required for lineage-specific transcription of the yeast HO gene. Cell 84, 711–722.

Simoff, I., Moradi, H., Nygärd, O., 2009. Functional characterization of ribosomal protein L15 from Saccharomyces cerevisiae. Current Genetics 55, 111–125.

Slavov, N., Semrau, S., Airoldi, E., Budnik, B., van Oudenaarden, A., 2015. Differential Stoichiometry among Core Ribosomal Proteins. Cell Reports 13, 865–873.

Soufi, B., Kelstrup, C.D., Stoehr, G., Fröhlich, F., Walther, T.C., Olsen, J. V, 2009. Global analysis of the yeast osmotic stress response by quantitative proteomics. Molecular bioSystems 5, 1337–1346.

Spingola, M., Grate, L., Haussler, D., Ares, M., 1999. Genome-wide bioinformatic and molecular analysis of introns in Saccharomyces cerevisiae. RNA (New York, N.Y.) 5, 221–234.

Steffen, K.K., McCormick, M.A., Pham, K.M., Mackay, V.L., Delaney, J.R., Murakami, C.J., Kaeberlein, M., Kennedy, B.K., 2012. Ribosome deficiency protects against ER stress in Saccharomyces cerevisiae. Genetics 191, 107–118.

Tardiff, D.F., Lacadie, S.A., Rosbash, M., 2006. A Genome-Wide Analysis Indicates that Yeast Pre-mRNA Splicing Is Predominantly Posttranscriptional. Molecular Cell 24, 917–929.

Uechi, T., Tanaka, T., Kenmochi, N., 2001. A complete map of the human ribosomal protein genes: assignment of 80 genes to the cytogenetic map and implications for human disorders. Genomics 72, 223–30.

Vilardell, J., Warner, J.R., 1994. Regulation of splicing at an intermediate step in the formation of the spliceosome. Genes and Development 8, 211–220.

Vilardell, J., Yu, S.J., Warner, J.R., 2000. Multiple functions of an evolutionarily conserved RNA binding domain. Mol Cell 5, 761–766.

Vincenti, S., De Chiara, V., Bozzoni, I., Presutti, C., 2007. The position of yeast snoRNA-coding regions

within host introns is essential for their biosynthesis and for efficient splicing of the host pre-mRNA. RNA (New York, N.Y.) 13, 138–50.

Wade, J.T., Hall, D.B., Struhl, K., 2004. The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes. Nature 432, 1054–1058.

Warner, J.R., Mitra, G., Schwindinger, W.F., Studeny, M., Fried, H.M., 1985. Saccharomyces cerevisiae coordinates accumulation of yeast ribosomal proteins by modulating mRNA splicing, translational initiation, and protein turnover. Molecular and cellular biology 5, 1512–1521.

Wolfe, K., 2000. Robustness - it's not where you think it is. Nature Genetics 25, 3–4.

Wolfe, K.H., Shields, D.C., 1997. Molecular evidence for an ancient duplication of the entire yeast genome. Nature 387, 708–713.

Yu, L., Morse, R.H., 1999. Chromatin opening and transactivator potentiation by RAP1 in Saccharomyces cerevisiae. Molecular and cellular biology 19, 5279–5288.

Zeevi, D., Sharon, E., Lotan-pompan, M., Lubling, Y., Shipony, Z., Raveh-sadka, T., Keren, L., Levo, M., Weinberger, A., Segal, E., 2011. Compensation for differences in gene copy number among yeast ribosomal proteins is encoded within their promoters 2114–2128.

Zhao, Y., McIntosh, K.B., Rudra, D., Schawalder, S., Shore, D., Warner, J.R., 2006. Fine-structure analysis of ribosomal protein gene transcription. Mol Cell Biol 26, 4853–4862.

*Lu, H., Zhu, Y.F., Xiong, J., Wang, R., Jia, Z., 2015. Potential extra-ribosomal functions of ribosomal proteins in Saccharomyces cerevisiae. Microbiological Research 177, 28–33.

*Shi, Z., Barna, M., 2015. Translating the Genome in Time and Space: Specialized Ribosomes, RNA Regulons, and RNA-Binding Proteins. Annual Review of Cell and Developmental Biology 31, annurev–cellbio–100814–125346.

*Warner, J.R., 1999. The economics of ribosome biosynthesis in yeast. Trends in Biochemical Sciences 24, 437–440.

http://www.yeastgenome.org/genomesnapshot (4/17/2016)

*review