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Citation: Gilbert, Wendy V. "Functional Specialization of Ribosomes?" Trends in Biochemical Sciences 36, no. 3 (March 2011): 127–132.

As Published: <http://dx.doi.org/10.1016/j.tibs.2010.12.002>

Publisher: Elsevier

Persistent URL: <http://hdl.handle.net/1721.1/99128>

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

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Published in final edited form as:

Trends Biochem Sci. 2011 March ; 36(3): 127–132. doi:10.1016/j.tibs.2010.12.002.

Functional Specialization of Ribosomes?

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Abstract

Ribosomes are highly conserved macromolecular machines responsible for protein synthesis in all living organisms. Work published in the past year shows that changes to the ribosome core can affect the mechanism of translation initiation that is favored in the cell, potentially leading to specific changes in the relative efficiencies with which different proteins are made. Here I examine recent data from expression and proteomic studies suggesting that cells make slightly different ribosomes under different growth conditions and discuss genetic evidence that such differences are functional. In particular, I will argue that eukaryotic cells likely produce ribosomes that lack one or more ‘core’ ribosomal proteins (RPs) under some conditions, and that ‘core’ RPs contribute differentially to translation of distinct subpopulations of mRNAs.

There are many potential sources of heterogeneity in eukaryotic ribosomes

The last ten years have witnessed spectacular progress in structure-function determination for bacterial and archaeal ribosomes (reviewed in [1–4]), yet the elucidation of high-resolution ribosome crystal structures has produced a tendency to regard ribosomes as unchanging homogeneous entities. Perhaps as a result, the dominant paradigms for translational control of eukaryotic gene expression emphasize the functional significance of heterogeneity among mRNA substrates and their associated RNA-binding proteins, and treat recruitment of “the ribosome” as a uniform endpoint of regulation. This view contradicts provocative evidence of potentially regulated ribosomal heterogeneity in eukaryotes that raises the possibility of functional specialization of the core translation machinery.

Eukaryotic ribosomes consist of small (40S) and large (60S) subunits comprising four ribosomal RNAs (18S, 25S, 5.8S, and 5S) and 79 core proteins that are conserved from yeast to humans [5]. In addition to this conserved core, ribosomes can vary in protein composition and/or modification state in a number of ways. A recent proteomic study of yeast ribosomes identified sub-stoichiometric translation machinery associated (TMA) proteins that could potentially modulate ribosome function under certain conditions. TMA proteins stably bound only a subset of ribosomes and were not required for normal global translation rates under standard lab conditions [6]. Nevertheless, by biochemical criteria some of the TMA proteins are indistinguishable from canonical ribosomal proteins; the conditions required to dissociate them from ribosomes are equivalently harsh. Ten of these TMA proteins are conserved from yeast to humans. In addition to this source of ribosome heterogeneity, many of the core RPs are encoded by duplicated genes in fungi and plants. In many cases, these paralogous genes encode subtly different proteins. (A useful compendium of ribosomal protein genes from a variety of organisms can be found at

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<http://ribosome.med.miyazaki-u.ac.jp/>) Ribosomal proteins are also subject to numerous post-translational modifications including phosphorylation, methylation, acetylation, and ubiquitylation [7–11]. Finally, the ribosomal RNAs are themselves extensively modified, the most frequent post-transcriptional modifications being 2'-O-methylation of ribose moieties (54 sites in yeast, directed by 42 non-coding guide small nucleolar RNAs (snoRNAs)) and conversion of uridine to pseudouridine (44 sites in yeast, targeted by 28 guide snoRNAs) at sites that are largely conserved from yeast to humans [12]. Thus, multiple opportunities for ribosome specialization exist.

Biochemical and proteomic evidence for production of different ribosomes in different circumstances

“Functional specialization of ribosomes” requires that two conditions be satisfied. First, that cells produce mature ribosomes that are biochemically distinct under different growth conditions; and second, that the production of different ribosome variants affects cell physiology by affecting translation. To illustrate the concept, consider two examples from prokaryotes. The first example comes from the halophilic archaeon *Haloarcula marismortui*, whose genome includes three rDNA operons. One of the three, *rrnB*, is highly divergent, having more than 100 nucleotide changes in the mature ribosomal RNA sequence compared to ribosomes produced from the *rrnA* and *rrnC* operons. The *rrnB* operon is specifically induced at high temperatures and repressed at low temperatures, and deletion of *rrnB* causes a temperature-sensitive growth phenotype [13]. Thus, *H. marismortui* cells make ribosomes with different rRNA sequences at high temperature, and failure to do so causes a growth defect. This study did not identify any specific differences in translational activity of ribosomes containing the *rrnB* rRNA variant, but noted that many of the rRNA sequence changes in *rrnB* replace A-U base pairs with more stable G-C pairs, suggesting that the ‘specialization’ in this case might be a simple matter of increasing structural stability at high temperature.

A second example from prokaryotes provides compelling evidence for mRNA-specific effects on translation caused by ribosome specialization. The antibiotic kasugamycin binds to ribosomes and inhibits translation of typical prokaryotic mRNAs that rely on specific features of their 5'-untranslated regions (UTRs) (such as Shine-Delgarno sequences) to recruit ribosomes [14,15]. Certain mRNAs are resistant to translational inhibition by kasugamycin. The resistant mRNAs are naturally leaderless (beginning with a 5' AUG initiation codon) [16,17]. Investigations into the mechanism responsible for the kasugamycin resistance of leaderless mRNA translation made the surprising discovery that *Escherichia coli* cells cultured with kasugamycin produced 61S ribosomes with small subunits that lacked six ‘core’ RPs (S1, S2, S6, S12, S18, and S21) and contained substoichiometric amounts of several other RPs. These novel protein-deficient ribosomes preferentially translated leaderless messages [18]. Although the conditions that led to production of protein-deficient 61S ribosomes were somewhat artificial, the study is nevertheless a striking demonstration that not all ‘core’ RPs are equally required for translation of all mRNAs. These protein-deficient ribosomes are clearly competent for the essential business of ribosomes: decoding and peptide bond formation. Moreover, they illustrate the potential for functional specialization of ribosomes by modulation of ‘core’ RP protein content.

Developmentally regulated synthesis of cell-type specific eukaryotic ribosomes was proposed almost thirty years ago, based on observations, by 2-D gel analysis, of differences between ribosomes purified from vegetative amoebae and differentiated spores of *Dictyostelium discoideum* [19]. More recent studies in maize and *Arabidopsis thaliana* similarly provide proteomic evidence for tissue type- and developmental state-specific

ribosome variants for which the authors propose active roles in translational control in the service of cellular differentiation [11], [20,21]. What is currently missing from these intriguing stories is any evidence that the biochemically distinct pools of ribosomes found in different tissues have different activities [Box 1].

Box 1

Potential Differences in the Activities of Specialized Ribosomes

Alternative ribosomes could differ in activities required for initiation, the process of recruiting 40S subunits to mRNAs and locating the start of the open reading frame, or elongation, which includes decoding, peptide bond formation and translocation. Such differences might be global in impact or could preferentially affect translation of specific messages. Here I mention a few specific mechanisms to illustrate the possibilities.

The most direct way for changes in ribosomes to affect the relative efficiencies with which different mRNAs initiate translation would be through altered affinity of 40S subunits for specific 5' untranslated regions (5'UTRs). Many viral 5'UTRs contain internal ribosome entry sites (IRESs) that interact directly with ribosomes [53]. Some cellular 5'UTRs may too, although this is not the canonical view of cellular translation. Consistent with this hypothesis, mutations in dyskerin, a protein component of H/ACA snoRNPs required for pseudouridylation of rRNAs, reduce both viral IRES-dependent initiation and some cellular translation in mammalian cells [36]. Likewise, loss of the non-essential RP Rps25 abolishes binding of 40S to some viral IRESs and slightly reduces global cellular translation through effects on as yet unidentified mRNAs [32]. Effects of altered 40S subunits on recruitment of specific mRNAs might also be mediated by bridging interactions between RPs and mRNA-binding proteins, (e.g. Asc1 and Sep160 [45]).

In addition to affecting the efficiency of mRNA recruitment, alternative ribosomes could affect the site of initiation. 40S ribosome subunits are recruited to capped eukaryotic mRNAs through the cooperative action of initiation factors (eIFs) that also regulate the ribosome's recognition of AUG initiation codons during scanning. Changes to the 40S subunit that altered the binding of eIF1, eIF1A or eIF2 (which delivers the initiator tRNA) could affect its scanning properties. Such effects would preferentially influence translation of mRNAs containing upstream open reading frames (uORFs) in their 5'UTRs.

Altered ribosomes might also differ in global or mRNA-specific elongation activity. For example, slowing the global rate of elongation could be an adaptive response to stress situations in which initiation rates are greatly reduced, ensuring that mRNAs remain ribosome associated and stable. (The most useful mechanisms for stress responses would involve reversible modification of pre-existing ribosomes.) Global or codon-specific changes in elongation rates could also enhance or alter patterns of co-translational protein folding. Importantly, not all regulated ribosome specializations would necessarily be beneficial to cells. In particular, viruses that rely on 'aberrant' behavior by translocating ribosomes (e.g. frame-shifting, slipping, reinitiation) in order to translate their genomes might target host ribosomes for alteration. These are just a handful of speculative models. In principle, the efficiency, selectivity, fidelity or rate of any ribosome-dependent reaction could be affected by ribosome specialization.

Production of alternative ribosome variants might be a frequent response to altered growth conditions

There is currently little published proteomic evidence for regulated production of alternative ribosomes in eukaryotes other than plants and slime molds, but if we consider data from mRNA expression studies, a rich picture of potential alternative ribosomes emerges. Genome-wide expression data reveal coordinated changes in expression of individual ribosome components (RPs and TMA proteins) and modification guide snoRNAs in response to changing cellular environments and tissue differentiation states [22–26]. Budding yeast reduce expression of most canonical ribosomal protein gene (RPG) mRNAs precipitously in response to a variety of environmental perturbations [22], likely because the cells are transitioning from a phase of rapid division, in which ~200,000 new ribosomes are synthesized every 90 minutes [23,24], to a period of much slower mass doubling and, in some cases, cellular differentiation. (Examples of environmentally regulated cellular differentiation programs in yeast include invasive growth, pseudohyphal growth, and sporulation.) Against this backdrop of overall decreased new ribosome synthesis, expression of some TMA genes increases [22]. This finding suggests that the ribosome occupancy of some TMA proteins, which are present at levels sufficient to bind only ~1% of ribosomes in rapidly dividing cells [6,25], might increase under certain stress conditions. Both *tma10Δ* and *tma17Δ* grow poorly on minimal media but normally on rich media, suggesting that their function is important only under the low-nutrient conditions in which they are more highly expressed [26] [22].

Gene expression profiling data from multicellular eukaryotes suggest a need to reconsider our thinking about the function of ‘core’ RPs. Although there is a strong assumption of equal RP stoichiometry in the literature (i.e., every ribosome is presumed to contain one molecule of each of the core proteins), evidence that this is always the case is lacking. Quantitative determination of relative RP abundance in purified ribosomes is very rarely performed outside of structural studies, which by necessity attempt to obtain a homogeneous pool of ribosomes from cells in a single defined growth state. Taken at face value, a number of gene expression studies suggest that the RP composition of ribosomes differs among tissues and developmental states [31–33]. Although this is an unorthodox notion, several ‘core’ RPs are dispensable for life in yeast, showing that ‘core’ RPs could play specific, rather than general, roles in translation, affecting only subpopulations of mRNAs. The example of kasugamycin-specialized ribosomes from *E. coli* demonstrates the potential for regulating translation of specific subpopulations of mRNAs (e.g. leaderless mRNAs) through changes in the ‘core’ RPs.

Putting the ‘function’ in functional specialization

Although there is currently little proteomic evidence for regulated production of alternative ribosomes in budding yeast, a wealth of genetic evidence shows that mutating individual non-essential ribosome components and modifiers leads to distinct cellular phenotypes that are not likely to be explained by a view of “the” ribosome as a monolithic entity with uniform effects on translation of all mRNAs. A few examples, discussed below, will illustrate this point: *rps25Δ* effects on initiation mechanism, distinctive phenotypes of individual *snoRNAΔ* mutants, and differential effects of RPG paralogs on a variety of cellular processes. In considering the possibility that even ‘core’ components of ribosomes (RP proteins and rRNA nucleotides) might play specialized roles in translation, it is important to remember that eukaryotic ribosomes contain many more proteins and rRNA modifications than bacterial ribosomes, despite the fact that ribosomes from all organisms perform the same basic task of protein synthesis by a highly conserved molecular

mechanism. For a more in-depth discussion of the probable roles of the ‘extra’ ribosomal proteins found in eukaryotes, see [34].

Rps25 is a non-essential RP that might play a selective role in translation of mRNAs that rely on alternative initiation mechanisms. Recent work showed that mutant ribosomes lacking Rps25 are defective for translation of certain viral mRNAs [35]. The cricket paralysis virus (CrPV) and the hepatitis C virus (HCV) initiate translation by direct binding of host 40S subunits to internal ribosome entry sites (IRESs) in their 5'-UTRs. 40S subunits lacking RPS25 do not bind the CrPV IRES, and mammalian cells depleted of RPS25 by siRNA knockdown show reduced CrPV and HCV IRES activity. However, RPS25 knockdown did not affect translation efficiency of a typical m⁷G-capped mRNA in mammals, and *rps25Δ* yeast showed nearly normal global translational activity (81% of wild type levels) under standard conditions [35]. No cellular mRNAs have yet been identified that initiate translation via a direct ribosome-binding mechanism. The mRNAs responsible for the slight reduction in global translation in *rps25Δ* cells could hold the clues to understanding the specialized function of Rps25 in translation initiation. It would be interesting to know whether cells regulate the expression of Rps25 to favor IRES-dependent translation under some conditions. Viruses might also up-regulate RPS25 expression to enhance viral protein expression. Hepatitis B or adenovirus infection leads to up-regulation of another 40S protein, RPS15A [36,37]; however, the consequences of increasing RPS15A levels for viral or cellular translation have not been determined. If viral mRNAs exploit specific RPs to enhance their translation, it seems likely that some cellular mRNAs do too.

Expression profiling data show that yeast modification guide snoRNAs are divergently regulated under some conditions [27], suggesting that at least a subset of snoRNAs might regulate changes in ribosome function, via alternative rRNA modification states, that are relevant to cellular stress responses. These data contradict the prevailing idea that snoRNAs play constitutive roles in ribosome biogenesis, and that all rRNA target sites are fully modified under all conditions. Characterization of individual snoRNA deletion mutants provides genetic evidence in support of the hypothesis that regulated changes in rRNA modifications could play a role in cellular adaptive responses [28]. Loss of single snoRNAs makes cells sensitive to environmental perturbations that require the cell to alter its program of gene expression to survive. It is tempting to speculate that altered rRNA modifications adapt ribosomes for enhanced translation of substantially altered pools of mRNA substrates. The failure of snoRNA deletion mutants to show identical or even similar phenotypes refutes the hypothesis that the net result of deleting any individual snoRNA is a generic and weak reduction in overall translation activity through a reduction in the production of functional ribosomes. Recent work in mammals supports the hypothesis of mRNA-specific requirements for rRNA modifications: a modest reduction in pseudouridine synthesis led to strong defects in translation of a handful of cellular and viral mRNAs [38]. The ease of genetic manipulation in yeast, coupled with recent advances in genome-wide translation state profiling methods [29,30], should make it possible to determine if and how specific changes in ribosome composition and/or rRNA modification state affect translation of individual mRNAs [Box 2].

Box 2

Future Directions

Moving the field of ribosome specialization beyond the current stage of description and speculation will require direct tests of the functional significance of ribosome alterations in vivo, and quantitative mechanistic comparisons of ribosome variants' activities in vitro. A crucial step towards understanding the mechanistic differences between specialized ribosome populations is the identification of sensitive mRNA substrates. Two

recently described methods give quantitative genome-wide measurements of mRNA-specific translational activity and are sensitive enough to detect even small effects of putative ribosome specializations: polysome profiling with gradient encoding (GE) and ribosome footprint profiling (FP) [37,38]. GE is cost-effective and involves relatively simple sample preparation, making it the method of choice for investigating large numbers of mutants or performing fine-grained developmental time courses of single mutants. FP has the advantage of revealing the positions of ribosomes along mRNAs, which would be essential for discovering specific effects of ribosome specializations on initiation site selection or elongation. Given expression data that suggest tissue-specific differences in ribosome composition, it might be fruitful to examine genome-wide translation activity in mouse embryonic stem (ES) cells subjected to various *in vitro* differentiation regimes, with or without RNAi knockdown of putative ribosome specialization factors (ribosome accessory proteins and specific RPs). Similarly, morpholinos (modified antisense oligonucleotides) targeting snoRNAs could be used to prevent modification of individual rRNA targets in order to investigate their impact on translation of specific mRNAs.

Once mRNA substrates that respond to ribosome specializations *in vivo* have been identified, the next step will be to quantitatively investigate the step(s) in their translation affected by differences in ribosomes purified from genetically or developmentally distinct cell types [Box 1]. Fully reconstituted translation initiation systems suitable for such experiments are available for yeast, mammals and *E. coli*. Although the current mammalian system uses rabbit ribosomes, it should be possible to develop alternative protocols using ribosomes purified from cell types more amenable to genetic manipulation. The development of a reconstituted translation system from the genetic model plant *Arabidopsis thaliana* would aid functional studies of developmentally regulated ribosome variants previously identified by proteomic approaches [20,21].

Both genome-wide and targeted genetic studies have shown that many of the yeast RP paralogs give distinct phenotypes when deleted [26,39–43] [28]. Based on these observations, Komili *et al.* [41] proposed the existence of a ‘ribosome code’ for translational regulation of gene expression in analogy with the ‘histone code’ model [44], whereby distinct constellations of histone protein variants and post-translational modification states contribute to cell-type appropriate patterns of transcription. The problem with this analogy is that many of the RP gene paralogs, including those that show different phenotypes when deleted, encode 100% identical proteins. Thus, in at least some cases, the phenotypic differences between RP gene paralogs must be due to differences in the regulation of their expression. If expression differences are responsible for growth phenotypes, it follows logically that either each of these RPs must have extra-ribosomal functions in the cell (reviewed in [48]), or that under some circumstances (minimally when one RPG paralog is missing) the cell makes some ribosomes that lack a core RP. Given the evidence that ribosomes lacking one or more core RPs are functional and affect translation of specific mRNAs, it seems likely that some of the yeast RPG paralog-specific phenotypic differences arise from differences in the amounts or circumstances of production of specific RP-deficient ribosomes.

One ribosome ‘flavor’ at a time vs. many ribosome types simultaneously

So far, we have mainly considered a mode of ribosome specialization in which global changes in the cellular environment lead to a concerted change in the type of ribosome produced, in order to enhance translation of an altered pool of mRNA substrates. An alternative view of ribosome specialization posits the co-existence of diverse ribosome variants within a cell, each subtly optimized for translation of a distinct subpopulation of

messages. One problem with this idea is how to achieve one-to-one pairing. How could specific recruitment be achieved? Our current understanding of the mechanism of ribosome recruitment during translation initiation in eukaryotes does not readily accommodate such a notion. According to the canonical model of cap- and scanning-dependent translation initiation, constitutive, presumably unspecialized, translation factors bind all mRNAs through recognition of their m⁷GpppN caps. A small ribosomal subunit (40S) is then recruited to the mRNA through a network of protein–protein interactions between these factors. Following recognition of an AUG initiation codon, a large (60S) subunit is recruited, again through the action of general translation factors not known to have differential activity towards distinct mRNAs, much less towards distinct ribosome variants. Of course, the absence of evidence should not be taken as evidence of absence, but many new players would need to be discovered to accommodate a model for widespread specificity in pairing between mRNAs and alternative ribosome variants.

mRNA-specific RNA-binding proteins are obvious candidates to facilitate such pairings, but little is currently known about direct interactions between mRNPs and ribosomes. The conserved multi-KH domain RNA-binding protein Scp160 (vigilin in mammals) is one potential example. Scp160 requires the ribosome-associated protein Asc1 (RACK1) to be bound to 40S subunits in order to associate with polysomes *in vivo* [45]. Scp160 is thought to interact with a specific pool of mRNAs [46], implying that these mRNAs will only be translated by Asc1-containing ribosomes. Although Asc1 (RACK1) is thought to be near-stoichiometric with core ribosomal proteins, hence providing little or no opportunity for ribosome specialization, there are reports of selective depletion of Asc1 from 40S subunits in starved or quiescent yeast cells, which have a distinctive pattern of protein expression [45] [47]. The ribosomal proteins themselves are RNA-binding proteins, known in a few cases to bind and regulate expression of specific mRNAs [44]. It is not currently known whether any eukaryotic RPs can bind simultaneously to mRNA and rRNA targets, but this would be an attractive mechanism to explain the mRNA-specific effects of individual non-essential RPs (*e.g.* Rps25) on translation. An alternative mechanism for direct interaction between specific mRNAs and ribosomes involves base pairing between rRNA and complementary mRNA sequences [48,49]. Such a mechanism could also be regulated by changes in RP composition or rRNA modification state that affect the accessibility or conformation of the rRNA.

At its extreme case (one message being translated by one kind of ribosome), the idea of specific pairings between mRNAs and ribosome variants within a cell seems excessively complicated. A more palatable idea involves subcellular colocalization of specific mRNA subpopulations with particular ribosome variants. For example, ribosomes associated with the endoplasmic reticulum (ER) membrane might be specialized for translation of membrane and secreted proteins. Specialization could enhance interactions between the ribosome and the translocon, or render the ribosome more amenable to transient translational arrest by the signal recognition particle (SRP). The proteomic approaches used successfully to identify tissue- and developmental phase-specific ‘flavors’ of ribosomes could be applied to fractionated cell extracts to investigate this possibility [11,19–21].

Concluding remarks

A wealth of gene expression data suggest that eukaryotic ribosomes might vary in protein composition and/or modification state in a number of ways. Although biochemical studies demonstrating the production of alternative ribosomes lag behind, a handful of successes illustrate the potential of quantitative proteomic methods to reveal instances of ribosome specialization. Currently, the idea that specialized ribosomes are adapted for translation of the different pools of mRNA substrates expressed in different tissues and growth conditions

is largely speculation. Nevertheless, recent work reviewed here establishes a paradigm for specific effects of ‘core’ RP proteins on translation of particular messages [18,35].

On a final note, reconsidering the assumption that ‘core’ ribosomal components contribute equally to translation of all mRNAs could also lead to new perspectives on human diseases. For example, Diamond-Blackfan anemia is associated with mutations in specific RPGs [50], and dyskeratosis congenita occurs in patients with mutations that decrease rRNA modifications [38]. Moreover, several, but not all, RP genes behave as haploinsufficient tumor suppressors in zebrafish [51]. In addition to these genetic links between individual ribosome components and disease states, new evidence suggests roles for certain RPs in viral infection: Adenovirus and HBV manipulate the expression of specific host RPs [36,37], and HCV translation can be inhibited by knocking down a single RP without substantially inhibiting host cell translation [35]. Given the current level of knowledge in the field, mechanistic characterization of the basis for altered translation of even one mRNA through ribosome specialization will be an exciting development.

Acknowledgments

I would like to thank my lab for many stimulating discussions on the subject. I apologize to colleagues whose work was not discussed due to space constraints.

References

1. Korostelev A, Noller HF. The ribosome in focus: new structures bring new insights. *Trends Biochem Sci* 2007;32:434–441. [PubMed: 17764954]
2. Moore PB, Steitz TA. The structural basis of large ribosomal subunit function. *Annu Rev Biochem* 2003;72:813–850. [PubMed: 14527328]
3. Ramakrishnan V. Ribosome structure and the mechanism of translation. *Cell* 2002;108:557–572. [PubMed: 11909526]
4. Steitz TA. A structural understanding of the dynamic ribosome machine. *Nat Rev Mol Cell Biol* 2008;9:242–253. [PubMed: 18292779]
5. Venema J, Tollervey D. Ribosome synthesis in *Saccharomyces cerevisiae*. *Annu Rev Genet* 1999;33:261–311. [PubMed: 10690410]
6. Fleischer TC, Weaver CM, McAfee KJ, Jennings JL, Link AJ. Systematic identification and functional screens of uncharacterized proteins associated with eukaryotic ribosomal complexes. *Genes Dev* 2006;20:1294–1307. [PubMed: 16702403]
7. Kruiswijk T, de Hey JT, Planta RJ. Modification of yeast ribosomal proteins. Phosphorylation. *Biochem J* 1978;175:213–219. [PubMed: 367365]
8. Lee SW, et al. Direct mass spectrometric analysis of intact proteins of the yeast large ribosomal subunit using capillary LC/FTICR. *Proc Natl Acad Sci USA* 2002;99:5942–5947. [PubMed: 11983894]
9. Louie DF, Resing KA, Lewis TS, Ahn NG. Mass spectrometric analysis of 40 S ribosomal proteins from Rat-1 fibroblasts. *J Biol Chem* 1996;271:28189–28198. [PubMed: 8910435]
10. Spence J, et al. Cell cycle-regulated modification of the ribosome by a variant multiubiquitin chain. *Cell* 2000;102:67–76. [PubMed: 10929714]
11. Szick-Miranda K, Bailey-Serres J. Regulated heterogeneity in 12-kDa P-protein phosphorylation and composition of ribosomes in maize (*Zea mays* L.). *J Biol Chem* 2001;276:10921–10928. [PubMed: 11278810]
12. Samarsky DA, Fournier MJ. A comprehensive database for the small nucleolar RNAs from *Saccharomyces cerevisiae*. *Nucleic Acids Res* 1999;27:161–164. [PubMed: 9847166]
13. López-López A, Benlloch S, Bonfá M, Rodríguez-Valera F, Mira A. Intragenomic 16S rDNA divergence in *Haloarcula marismortui* is an adaptation to different temperatures. *J Mol Evol* 2007;65:687–696. [PubMed: 18026684]

14. Schlutzen F, et al. The antibiotic kasugamycin mimics mRNA nucleotides to destabilize tRNA binding and inhibit canonical translation initiation. *Nat Struct Mol Biol* 2006;13:871–878. [PubMed: 16998488]
15. Schuwirth BS, et al. Structural analysis of kasugamycin inhibition of translation. *Nat Struct Mol Biol* 2006;13:879–886. [PubMed: 16998486]
16. Chin K, Shean CS, Gottesman ME. Resistance of lambda cI translation to antibiotics that inhibit translation initiation. *J Bacteriol* 1993;175:7471–7473. [PubMed: 8226693]
17. Moll I, Bläsi U. Differential inhibition of 30S and 70S translation initiation complexes on leaderless mRNA by kasugamycin. *Biochem Biophys Res Commun* 2002;297:1021–1026. [PubMed: 12359258]
18. Kaberdina AC, Szaflarski W, Nierhaus KH, Moll I. An unexpected type of ribosomes induced by kasugamycin: a look into ancestral times of protein synthesis? *Mol Cell* 2009;33:227–236. [PubMed: 19187763]
19. Ramagopal S, Ennis HL. Regulation of synthesis of cell-specific ribosomal proteins during differentiation of *Dictyostelium discoideum*. *Proc Natl Acad Sci USA* 1981;78:3083–3087. [PubMed: 16593020]
20. Chang IF, Szick-Miranda K, Pan S, Bailey-Serres J. Proteomic characterization of evolutionarily conserved and variable proteins of *Arabidopsis* cytosolic ribosomes. *Plant Physiol* 2005;137:848–862. [PubMed: 15734919]
21. Giavalisco P, et al. High heterogeneity within the ribosomal proteins of the *Arabidopsis thaliana* 80S ribosome. *Plant Mol Biol* 2005;57:577–591. [PubMed: 15821981]
22. Bévort M, Leffers H. Down regulation of ribosomal protein mRNAs during neuronal differentiation of human NTERA2 cells. *Differentiation* 2000;66:81–92. [PubMed: 11100899]
23. Gasch AP, et al. Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell* 2000;11:4241–4257. [PubMed: 11102521]
24. Halbeisen RE, Gerber AP. Stress-Dependent Coordination of Transcriptome and Translatome in Yeast. *PLoS Biol* 2009;7:e105. [PubMed: 19419242]
25. Tang YP, Wade J. Sexually dimorphic expression of the genes encoding ribosomal proteins L17 and L37 in the song control nuclei of juvenile zebra finches. *Brain Res* 2006;1126:102–108. [PubMed: 16938280]
26. Whittle CA, Krochko JE. Transcript Profiling Provides Evidence of Functional Divergence and Expression Networks among Ribosomal Protein Gene Paralogs in *Brassica napus*. *Plant Cell*. 2009
27. von der Haar T. A quantitative estimation of the global translational activity in logarithmically growing yeast cells. *BMC systems biology* 2008;2:87. [PubMed: 18925958]
28. Warner JR. The economics of ribosome biosynthesis in yeast. *Trends Biochem Sci* 1999;24:437–440. [PubMed: 10542411]
29. Ghaemmaghami S, et al. Global analysis of protein expression in yeast. *Nature* 2003;425:737–741. [PubMed: 14562106]
30. Hillenmeyer ME, et al. The chemical genomic portrait of yeast: uncovering a phenotype for all genes. *Science* 2008;320:362–365. [PubMed: 18420932]
31. Dresios J, Panopoulos P, Synetos D. Eukaryotic ribosomal proteins lacking a eubacterial counterpart: important players in ribosomal function. *Mol Microbiol* 2006;59:1651–1663. [PubMed: 16553873]
32. Landry DM, Hertz MI, Thompson SR. RPS25 is essential for translation initiation by the Dicistroviridae and hepatitis C viral IRESs. *Genes Dev* 2009;23:2753–2764. [PubMed: 19952110]
33. Lam YW, Evans VC, Heesom KJ, Lamond AI, Matthews DA. Proteomics analysis of the nucleolus in adenovirus-infected cells. *Mol Cell Proteomics* 2010;9:117–130. [PubMed: 19812395]
34. Lian Z, et al. Human S15a expression is upregulated by hepatitis B virus X protein. *Mol Carcinog* 2004;40:34–46. [PubMed: 15108328]
35. Esguerra J, Warringer J, Blomberg A. Functional importance of individual rRNA 2'-O-ribose methylations revealed by high-resolution phenotyping. *RNA* 2008;14:649–656. [PubMed: 18256246]

36. Yoon A, et al. Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita. *Science* 2006;312:902–906. [PubMed: 16690864]
37. Hendrickson DG, et al. Concordant regulation of translation and mRNA abundance for hundreds of targets of a human microRNA. *PLoS Biol* 2009;7:e1000238. [PubMed: 19901979]
38. Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science* 2009;324:218–223. [PubMed: 19213877]
39. Enyenihi AH, Saunders WS. Large-scale functional genomic analysis of sporulation and meiosis in *Saccharomyces cerevisiae*. *Genetics* 2003;163:47–54. [PubMed: 12586695]
40. Haarer B, Viggiano S, Hibbs MA, Troyanskaya OG, Amberg DC. Modeling complex genetic interactions in a simple eukaryotic genome: actin displays a rich spectrum of complex haploinsufficiencies. *Genes Dev* 2007;21:148–159. [PubMed: 17167106]
41. Komili S, Farny NG, Roth FP, Silver PA. Functional specificity among ribosomal proteins regulates gene expression. *Cell* 2007;131:557–571. [PubMed: 17981122]
42. Ni L, Snyder M. A genomic study of the bipolar bud site selection pattern in *Saccharomyces cerevisiae*. *Mol Biol Cell* 2001;12:2147–2170. [PubMed: 11452010]
43. Parsons AB, et al. Exploring the mode-of-action of bioactive compounds by chemical-genetic profiling in yeast. *Cell* 2006;126:611–625. [PubMed: 16901791]
44. Jenuwein T, Allis CD. Translating the histone code. *Science* 2001;293:1074–1080. [PubMed: 11498575]
45. Baum S, Bittins M, Frey S, Seedorf M. Asc1p, a WD40-domain containing adaptor protein, is required for the interaction of the RNA-binding protein Scp160p with polysomes. *Biochem J* 2004;380:823–830. [PubMed: 15012629]
46. Hogan DJ, Riordan DP, Gerber AP, Herschlag D, Brown PO. Diverse RNA-binding proteins interact with functionally related sets of RNAs, suggesting an extensive regulatory system. *PLoS Biol* 2008;6:e255. [PubMed: 18959479]
47. Fuge EK, Braun EL, Werner-Washburne M. Protein synthesis in long-term stationary-phase cultures of *Saccharomyces cerevisiae*. *J Bacteriol* 1994;176:5802–5813. [PubMed: 8083172]
48. Warner JR, McIntosh KB. How common are extraribosomal functions of ribosomal proteins? *Mol Cell* 2009;34:3–11. [PubMed: 19362532]
49. Mauro VP, Edelman GM. The ribosome filter hypothesis. *Proc Natl Acad Sci USA* 2002;99 : 12031–12036. [PubMed: 12221294]
50. Mauro VP, Edelman GM. The ribosome filter redux. *Cell Cycle* 2007;6:2246–2251. [PubMed: 17890902]
51. Narla A, Ebert BL. Ribosomopathies: human disorders of ribosome dysfunction. *Blood* 2010;115:3196–3205. [PubMed: 20194897]
52. Lai K, et al. Many ribosomal protein mutations are associated with growth impairment and tumor predisposition in zebrafish. *Dev Dyn* 2009;238:76–85. [PubMed: 19097187]
53. Filbin ME, Kieft JS. Toward a structural understanding of IRES RNA function. *Curr Opin Struct Biol* 2009;19:267–276. [PubMed: 19362464]