Review in Advance first posted online on October 5, 2015. (Changes may still occur before final publication online and in print.)

Translating the Genome in Time and Space: Specialized Ribosomes, RNA Regulons, and RNA-Binding Proteins

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Annu. Rev. Cell Dev. Biol. 2015. 31:26.1–26.24

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

This article's doi:
10.1146/annurev-cellbio-100814-125346

Keywords

ribosome-centered regulation, specialized translation machinery, ribosomal proteins, RNA regulons, RNA-binding proteins

Abstract

A central question in cell and developmental biology is how the information encoded in the genome is differentially interpreted to generate a diverse array of cell types. A growing body of research on posttranscriptional gene regulation is revealing that both global protein synthesis rates and the translation of specific mRNAs are highly specialized in different cell types. How this exquisite translational regulation is achieved is the focus of this review. Two levels of regulation are discussed: the translation machinery and *cis*-acting elements within mRNAs. Recent evidence shows that the ribosome itself directs how the genome is translated in time and space and reveals surprising functional specificity in individual components of the core translation machinery. We are also just beginning to appreciate the rich regulatory information embedded in the untranslated regions of mRNAs, which direct the selective translation of transcripts. These hidden RNA regulons may interface with a myriad of RNA-binding proteins and specialized translation machinery to provide an additional layer of regulation to how transcripts are spatiotemporally expressed. Understanding this largely unexplored world of translational codes hardwired in the core translation machinery is an exciting new research frontier fundamental to our understanding of gene regulation, organismal development, and evolution.

Contents

INTRODUCTION

For decades, the central dogma of molecular biology has explained the flow of genetic information from DNA → mRNA → protein. It has also motivated key questions as to how the same information encoded in the linear, one-dimensional genome is interpreted and translated to form myriad cell types and the complex three-dimensional (3D) morphological topology underlying organismal development. Undoubtedly, exquisite regulatory mechanisms governing when and where genes are expressed in time and space endow regulatory control to cell-fate specification, differentiation, and organismal development. In particular, countless layers of upstream regulation that ultimately lead to the production of transcripts are important drivers of key cellular and developmental processes. However, although mRNA translation is considered a complex and highly coordinated process, neither the importance nor the mechanisms of translational regulation are realized to the same extent as for transcriptional control. Importantly, an exciting body of emerging research has revealed that, in reality, both the global protein synthesis rates and translational efficiencies of specific mRNAs are highly unique to different cell types. Although the ribosome has historically been perceived as a remarkably precise but constitutive molecular machine for reading and translating the genetic code of mRNAs (Alberts 1998, Frank 2000), recent studies have discovered significant functional specificity of many core ribosomal proteins (RPs) and unveiled greater gene regulatory potential by the ribosome. In this respect, heterogeneity in the composition and modification of the ribosome provides a platform for tremendous diversity in ribosome activity and/or function.

RP: ribosomal protein

We discuss the evidence for specialized ribosomes that harbor unique compositions or activities as well as key experiments that would address whether such differential composition and modification exerts unique gene regulatory functions. Recent advances in the field have also identified diverse arrays of *cis*-regulatory elements that orchestrate when, where, and how much protein is made. These studies suggest that structured *cis*-acting elements within 5′ untranslated regions (5′UTRs) act as critical regulatory platforms or landing pads for specialized ribosomes in decoding how the genome is functionally expressed. In addition, although conveying genetic information from DNA to protein is an obvious function of transcripts, the information embedded in mRNA goes beyond specifying the amino acid sequence of proteins. In particular, UTRs, codon choice, and RNA modifications encode information important for determining the stability, localization, efficiency, and even the mode of translation initiation of the mRNA molecule; it is of outstanding importance to understand this noncoding language. In the picture emerging, translational control is a paramount mode of gene regulation rather than an additional, fine-tuning layer. In this review, we focus on the remarkable cell- and tissue-specific regulation of mRNA translation and highlight emerging paradigms for how the core translational machinery, in concert with RNA-binding proteins and *cis*-acting RNA regulons, specify unique translational programs vital to the posttranscriptional circuitry of gene regulation underlying cell and organismal development.

UTR: untranslated region

rRNA: ribosomal RNA

Rpl: ribosomal protein, large subunit

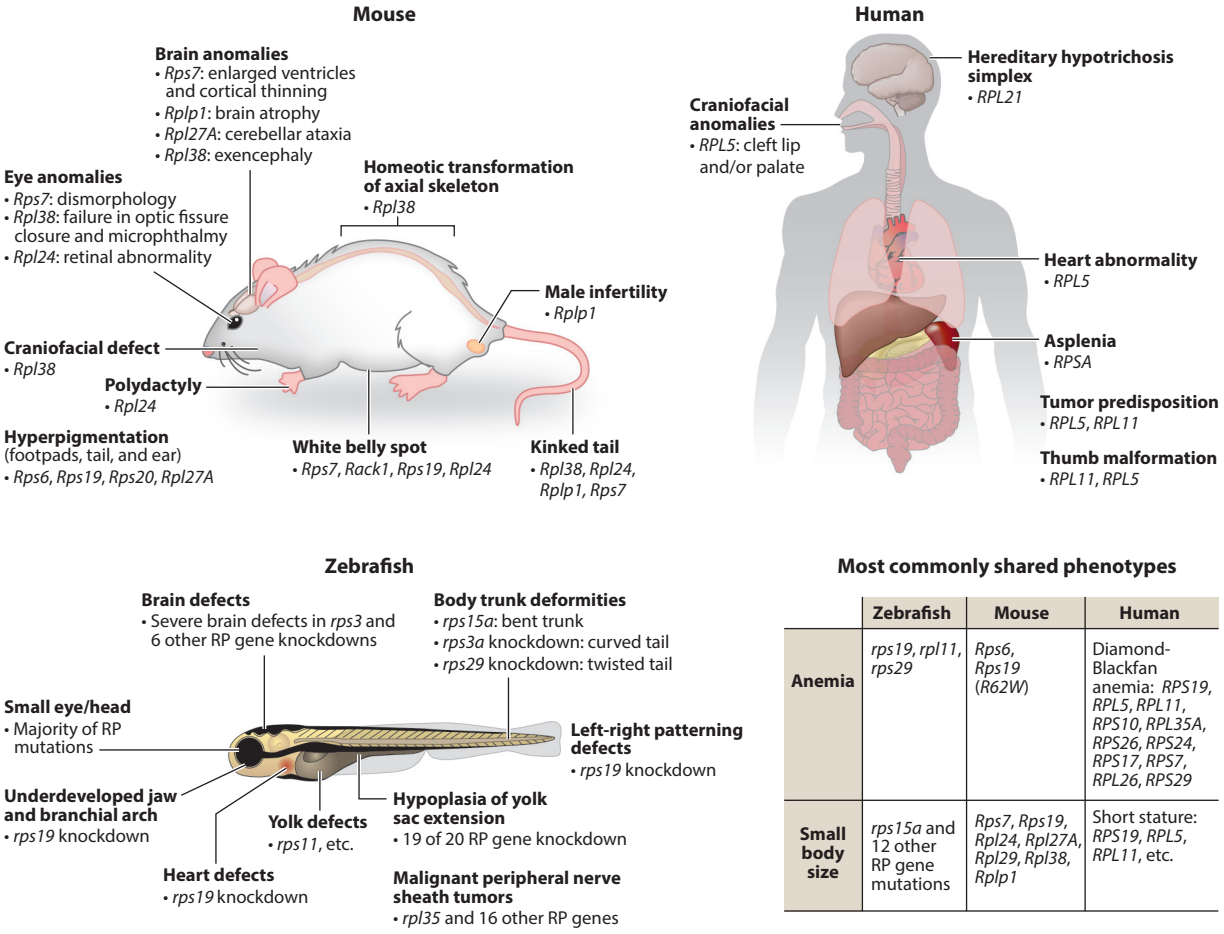
THE GENETICS OF THE TRANSLATION MACHINERY: CORE YET SPECIFIC

Surprising Functional Specificity of Core Ribosomal Proteins

The eukaryotic ribosome is composed of four ribosomal RNAs (rRNAs) and ∼80 core RPs. The coordinated functions of RPs are best illustrated by classical *Drosophila* genetics: Almost all of the ∼50 *Drosophila Minute* mutants bear mutations in RP genes and have similar phenotypes, including developmental delay, reduced fertility, short thin bristles, and recessive lethality (Lambertsson 1998, Marygold et al. 2007). Why mutations in different loci produced this shared phenotype became obvious after it was discovered that these gene products all function within the same complex: the ribosome. Curiously, additional specific phenotypes were observed in several RP mutants, hinting at the potential functional specificity of individual RPs. For example, *Rpl38* mutant flies have large, rough eyes; wing patterning defects; and abnormally large wings (Marygold et al. 2005).

Despite the manifestation of certain *Minute*-like phenotypes in vertebrates, including small body size as well as delayed development and recessive lethality, individual RP loss-of-function mutations often lead to highly specific phenotypes (**Figure 1**). Recent studies in vertebrates serve as a major point of discussion here, and we refer readers to other recent reviews (Byrgazov et al. 2013, Horiguchi et al. 2012, Xue & Barna 2012) focusing on RP functional specialization in other species.

In zebrafish (*Danio rerio*), an initial unbiased forward genetic screen identified 12 tumor-prone lines, most of which developed a very specific malignant peripheral nerve sheath tumor phenotype. Interestingly, all but one line carried heterozygous mutations in genes encoding different RPs (Amsterdam et al. 2004). Even more intriguingly, of 28 lines with heterozygous mutations in RP genes, only 17 were prone to developing the tumor, clearly suggesting the functional specifications of RPs (Lai et al. 2009). In addition, a reverse genetic screen of 20 RPs using morpholino antisense oligos revealed highly specific developmental defects, ranging from curved tails to enlarged eye lenses to telencephalon hypoplasia, owing to loss of function of *rps3a*, *rps29*, or *rps4*, respectively (Uechi et al. 2006). Although only 12 RP mutants have been characterized in mouse to date, they display a wide spectrum of tissue-specific phenotypes, from dramatic axial skeletal



Most commonly shared phenotypes

	Zebrafish	Mouse	Human
Anemia	*rps19*, *rpl11*, *rps29*	*Rps6*, *Rps19* (*R62W*)	Diamond-Blackfan anemia: *RPS19*, *RPL5*, *RPL11*, *RPS10*, *RPL35A*, *RPS26*, *RPS24*, *RPS17*, *RPS7*, *RPL26*, *RPS29*
Small body size	*rps15a* and 12 other RP gene mutations	*Rps7*, *Rps19*, *Rpl24*, *Rpl27A*, *Rpl29*, *Rpl38*, *Rplp1*	Short stature: *RPS19*, *RPL5*, *RPL11*, etc.

Figure 1

Highly specific phenotypes of ribosomal protein (RP) loss-of-function mutations/knockdown in zebrafish, mouse, and human. Brief descriptions of the phenotypes and underlying RP gene mutations are shown, and the specific organs/tissues that display the phenotype are highlighted. For a comprehensive list of references, see **Supplemental Text 1**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**.

patterning defects, including homeotic transformations in $Rpl38^{Ts/+}$ mice (Kondrashov et al. 2011) and cerebellar ataxia in $Rpl27a^{Sfa/+}$ mice (Terzian et al. 2011), to male infertility in $Rplp1^{+/-}$ mice (Perucho et al. 2014), among others. These studies highlight an emerging paradigm: A single RP or small set of RPs exerts distinct or unique functions on organismal development. Finally, in humans, a growing number of mutations in RP genes or genes required for ribosome biogenesis have been linked to tissue-specific clinical phenotypes. This includes Diamond-Blackfan anemia (DBA), in which mutations in several RPs lead to anemia as well as a distinct spectrum of congenital limb, heart, and urogenital birth defects as well as craniofacial malformations including cleft palate, growth failure, and a predisposition to cancer (Boria et al. 2010). The most prevalent cause of early mortality in DBA patients is bone marrow failure. Mutations in *Rpl21* have also been identified in hereditary hypotrichosis simplex, a disorder characterized by progressive hair loss early in childhood (Zhou et al. 2011). Mutations in *RPSA* underlie isolated congenital asplenia

DBA: Diamond-Blackfan anemia

(ICA), in which children are born without a spleen (Bolze et al. 2013). Although these patients are susceptible to bacterial infections, they do not develop additional abnormalities. Other complex human disorders associated with mutations in ribosome components include X-linked dyskeratosis congenita, Shwachman-Diamond syndrome, 5q-syndrome, Treacher Collins syndrome, and Hoyeraal-Hreidarsson syndrome (Boria et al. 2010).

Rps: ribosomal protein, small subunit

Despite the conservation of *Minute*-like defects and anemia phenotypes across species, many new phenotypes are manifest in RP mutations in mammals, suggesting increasing functional specialization of RPs during evolution in higher organisms. Multiple layers of specificity have emerged from the genetic studies discussed above: First, several tissues or cell types, including the skeleton, blood cells, neurons, and keratinocytes, are uniquely affected by specific RP loss-of-function mutations. Second, additional layers of selective RP functions during cell differentiation exist. For example, mutations in different RP genes result in defects in distinct cell lineages, producing diverse phenotypes ranging from macrocytic anemia in $Rps14^{+/-}$ mice (Barlow et al. 2010), pancytopenia in $Rpl27a^{Sfa/+}$ mice (Terzian et al. 2011), and T cell–specific defects in $Rpl22^{-/-}$ mice (Anderson et al. 2007) to no noticeable phenotypes in several other mouse RP gene mutants. Finally, a single RP mutation can lead to distinct phenotypic consequences even in the same tissue or organ. For example, in the context of a conditional *Rps6* knockout mouse, the adult liver can grow in response to nutrients similar to that of a wild-type control; however, proliferation of liver cells after partial hepatectomy is selectively blocked (Volarevic et al. 2000).

What Do Ribosomal Protein Genetics Tell Us About Ribosome Function/Activity at the Cellular and Organismal Level?

Interestingly, most, if not all, RP loss-of-function mutations are dominant owing to haploinsufficiency. At first, it was hypothesized that protein synthesis could be rate limiting to cell growth. Therefore, it seemed intuitive that these phenotypes could be explained by a cell-autonomous defect in protein synthesis, particularly within cells in which the demand for ribosome biogenesis or protein synthesis is the greatest. Several new observations challenge this simple explanation (**Figure 2**). To begin with, the overall low association between cell division rates and sensitivity to RP mutations argues against the idea that the most affected cells divide faster and therefore have higher demand for the protein synthesis machinery. For example, hematopoietic stem cells (HSCs) have a markedly lower protein production rate compared with most other hematopoietic progenitors but are particularly sensitive to either increasing or further reducing protein production, such as in the background of *Rpl24* haploinsufficiency (Signer et al. 2014). Therefore, HSCs are an important example of a cell type in which a simple correlation between cell division rates and a high capacity for protein synthesis cannot account for specific phenotypes associated with RP loss of function (**Figure 2*a***).

Although RP haploinsufficiency has been linked to slower growth rates, several studies have shown that reduced levels of specific RPs can cause hyperplasia and overgrowth in certain tissues. In *Drosophila*, heterozygous mutations in *Rps6* (Stewart & Denell 1993, Watson et al. 1992) and *Rps21* (Török et al. 1999) lead to overgrowth of hematopoietic organs as a result of cell overproliferation. *Rpl5* and *Rpl38* heterozygous flies have wings that are larger than wild type as a result of increased cell size (Marygold et al. 2005). In addition, a growing number of RP gene mutations have been associated with predisposition to cancer across many organisms (Amsterdam et al. 2004, De Keersmaecker et al. 2013). These observations indicate that specific RP gene mutations affect tissue/organs in more selective ways than can simply be ascribed to slower growth rates.

In cell lines and mouse tissues, marked differences exist in the degree to which different RP gene mutations reduce global protein synthesis rates. Surprisingly, using a [^{35}S] methionine

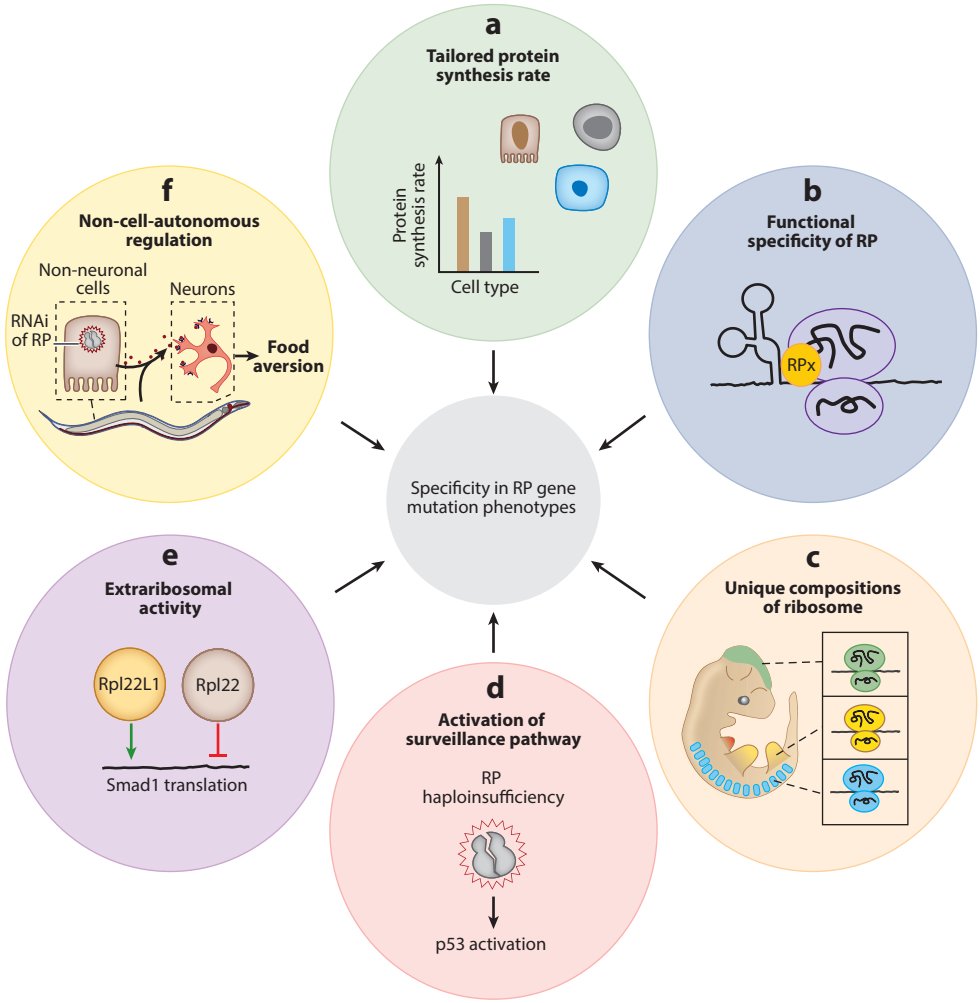


Figure 2

Interpretation of why and how RP gene mutation phenotypes produce specific phenotypes. Illustrated are several possible mechanisms that can account for the differential effects of select RPs on organismal development. (*a*) The tailored protein synthesis rate in each cell/tissue type may determine the specific sensitivity to RP mutations. (*b*,*c*) The specific RP gene mutation phenotypes can also be attributed to the functional specificity of RP in translational control as well as unique compositions of ribosomes in different cell and tissue types. (*d*,*f*) Furthermore, the phenotypes of RP gene mutations may also be due to the activation of p53 surveillance pathway and/or non-cell-autonomous regulation. (*e*) Finally, a few RPs may have extraribosomal activity and therefore cause specific phenotypes upon loss of function. Abbreviations: RNAi, RNA interference; RP, ribosomal protein.

incorporation assay to monitor the neural tube and somites of mouse embryos, $Rpl38^{Ts/+}$, $Rps19^{DSK3/+}$, and $Rps20^{DSK4/+}$ mutants showed no change in de novo protein synthesis, whereas $Rpl29^{+/-}$, $Rpl24^{BST/+}$, and $Rpl29^{-/-}$ mice exhibited 15%, 35%, and up to ~45% decreases, respectively (Kondrashov et al. 2011). Interestingly, the $Rpl29^{-/-}$ mouse, which shows the greatest reduction in global protein synthesis among these RP mutants, does not possess any major phenotypes other than small body size (Kirn-Safran et al. 2007). By contrast, $Rpl38^{Ts/+}$ mutants with no change

in global protein synthesis rates exhibit profound homeotic transformation in the axial skeleton (Kondrashov et al. 2011). The overall low association between global protein synthesis rates and the severity of RP gene mutation phenotypes indicates that these phenotypes may be attributed to more specialized functions in the translation of specific mRNAs rather than effects on global protein synthesis. In addition, RPL40 is largely dispensable for global cap-dependent translation but specifically required for the translation of a subset (~200) of cellular mRNAs, including several stress response transcripts (Lee et al. 2013). These examples have led to a growing realization that individual RPs allow the ribosome to exert more specialized control over the translation of particular subsets of mRNAs. Moreover, as discussed below (see Are All Ribosomes the Same?), the unique compositions of ribosome can further endow ribosomes with greater regulation activity in control of gene expression, thereby exerting highly specific effects on organismal development (**Figure 2*b,c***).

Interestingly, mutations in several RPs activate p53. This has been proposed to serve as a surveillance mechanism to ensure the integrity and fidelity of ribosomes (**Figure 2*d***). Curiously, in some mutants, including *Rps19*$^{Dsk3/+}$, *Rps20*$^{Dsk4/+}$, *Rpl27a*$^{Sfa/+}$, and *Rps7*$^{Zma/+}$ mice, loss of *p53* can suppress major phenotypes, in particular those associated with increased cell death (Barkic et al. 2009, McGowan et al. 2008, Terzian et al. 2011, Watkins-Chow et al. 2013). However, the midgestation or early embryonic lethality of *Rps6*$^{+/-}$ and *Rack1* homozygous mutant embryos, respectively, cannot be suppressed by *p53* loss of function. Similarly, the striking axial patterning phenotypes of *Rpl38*$^{Ts/+}$ mice (Kondrashov et al. 2011, Panic et al. 2006, Volta et al. 2013) are not affected by the removal of one copy of p53, further indicating specific functions of individual RPs. In the instances in which p53 depletion ameliorates RP phenotypes, it is not clear whether this effect is a direct or indirect consequence of p53 activation. For example, p53 itself regulates ribosome biogenesis (Budde & Grummt 1999, Golomb et al. 2012) and may also suppress RP phenotypes through a mechanism not directly tied to the proposed activation of p53 as a checkpoint for ribosome fidelity. Therefore, the link between p53 activation and ribosome function needs to be better understood mechanistically but at present cannot account for the unique phenotypes observed in many RP loss-of-function studies. It is also important to consider that as RNA-binding proteins, RPs can potentially bind to RNAs or proteins outside of the ribosome (**Figure 2*e***). A handful of RPs has been found to regulate transcription, splicing, and translation of mRNAs (Warner & McIntosh 2009). One interesting example of the extraribosomal function of RPs in translational control is RPL13A. In response to interferon-γ, RPL13A is phosphorylated and released from the ribosome, which then binds to the 3′UTR of ceruloplasmin mRNA and inhibits its translation (Mazumder et al. 2003). Another example is the RP paralogs Rpl22 and Rpl22-like1 (Rpl22l1), both of which bind *smad1* mRNA. They exert opposite effects on Smad1 translation and therefore function antagonistically in hematopoietic development (Zhang et al. 2013). This raises the possibility that additional extraribosomal functions of RPs exist, and how many of these extraribosomal functions contribute to the tissue-specific phenotypes of RP mutants also remains an outstanding question.

Finally, intriguing recent studies reveal that alterations in ribosome synthesis and/or activity could also exert nonautonomous effects on body growth and organismal behavior (**Figure 2*f***). In *Drosophila*, tissue-specific knockdown of transcription initiation factor-IA (TIF-IA), a key factor in rRNA transcription by RNA polymerase I, reduces ribosome biogenesis in muscle, causing significant developmental delays and reduced body size. By contrast, reducing ribosome biogenesis in the lymph gland or fat body only slightly delays development, if any effect is noted at all. Mechanistically, inhibiting muscle ribosome biogenesis reduces systematic insulin-like growth factor signaling via a decrease in the production of insulin-like peptides as well as an increase in inhibitor of insulin signaling (Ghosh et al. 2014). In the nematode *Caenorhabditis elegans*, RNA interference (RNAi) knockdown of RPs causes growth arrest and, interestingly, avoidance of normally

attractive nutritious bacteria. RNAi inactivation of RPs in non-neuronal tissues is sufficient to stimulate aversion behavior, revealing a neuroendocrine axis of control that additionally requires serotonergic and Jnk kinase signaling pathways (Melo & Ruvkun 2012). These studies highlight unexpected non-cell-autonomous functions of RPs.

Hox: homeobox

It has been nearly 100 years since the discovery of the first *Minute* mutation by Bridges in 1919 (Bridges & Morgan 1923), which occurred a few decades before the first description of the ribosome (Palade 1955). Remarkably, we still do not fully understand the mechanisms by which RP mutations lead to specific phenotypes. Moreover, new interpretations of these phenotypes, as discussed above, are provoking many interesting questions, motivating the field to rethink the role of RPs in regulating cell growth, differentiation, and coordinated organismal development.

Why and How Do Ribosomal Proteins Defy Genetic Analysis?

The phenotypes evident in heterozygous RP mutants reveal potentially unique or rate-limiting functions for these proteins in either translational control or additional extraribosomal functions. However, we lack a complete systematic view of RP function. It is clear that RPs fulfill distinct functions during the biogenesis and assembly of ribosomes in the nucleolus as well as during translation initiation and elongation in the cytoplasm. For example, a single RP may be critical for ribosome biogenesis but then exerts more specialized functions as part of a translating ribosome in the cytoplasm. Teasing out the former function may prove particularly challenging with conventional genetic approaches. For example, the complete loss of an RP within a cell may not be compatible with cell viability, owing to early perturbations in ribosome biogenesis. Therefore, future studies will require more selective means of inactivating RP function. Perhaps RPs could be inactivated only when translationally competent ribosomes have already formed in the cytoplasm. Without a more intelligent design for assessing RP functions at discrete steps in translational control, conventional genetic loss-of-function approaches may fail to reveal the complete picture of more specialized RP functions either at a cell or organismal level.

PARADIGMS FOR SPECIALIZED RIBOSOME ACTIVITY AND PROTEIN SYNTHESIS RATES IN CELL AND ORGANISMAL DEVELOPMENT

A Single Core Ribosomal Protein, RPL38, Controls Formation of the Mammalian Body Plan

An exciting paradigm for understanding the specialized functions of individual RPs has come from the in-depth genetic, molecular, and biochemical analysis of a single RP, RPL38, belonging to the large ribosome subunit (Kondrashov et al. 2011, Xue et al. 2015). An unbiased forward genetic screen revealed RPL38, a core RP, is required to establish the mammalian body plan. The vertebrate skeleton is built on an intricate pattern of stereotyped anatomical elements, the vertebrae, which on the basis of their relative position along the anterior-posterior (A-P) axis in the developing embryo have distinct anatomical features. The relative number and arrangement of these vertebrae types collectively give rise to the mammalian body plan. In *Rbt/+*, *Tss/+*, and *Ts/+* mice, which possess a deletion or mutation of the *Rpl38* gene, the individual morphologies of these skeletal elements are altered in a profound way; phenotypes include numerous patterning defects and compound homeotic transformations. These findings linked a core component of the ribosome to the tissue patterning required to establish the mammalian body plan for the first time. At a mechanistic level, global protein synthesis in *Rpl38* mutant embryos is surprisingly unaffected; however, transcript-specific translational control of subsets of *Hox* (homeobox) mRNAs required for A-P tissue patterning is perturbed. Unexpectedly, *Rpl38* transcripts are localized in a highly

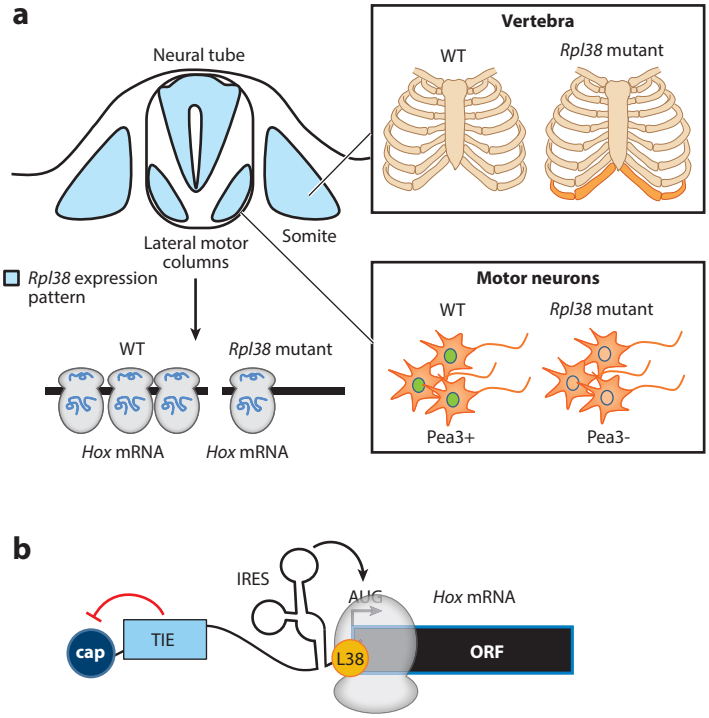


Figure 3

A paradigm for specialized ribosome function: Rpl38-mediated, transcript-specific translational control in body plan patterning. (*a*) RPL38 is highly enriched in somites and the neural tube of developing mouse embryos and is required for translating specific *Hox* mRNAs necessary for axial skeletal and motor neuron patterning (illustrated by the expression of the PEA3 marker). Adapted from Xue & Barna (2012). (*b*) A model of combinatorial TIE + IRES RNA regulons within the 5′UTRs of *Hox* mRNAs providing specificity in translational control. This specificity is key in establishing the mammalian body plan. The TIE inhibits general, cap-dependent translation. As a result, *Hox* mRNAs exploit IRES-mediated translation that specifically relies on RPL38. Adapted from Xue et al. (2015). Abbreviations: AUG, start codon; IRES, internal ribosome entry site; L38, RPL38; ORF, open reading frame; TIE, translation inhibitory element; UTR, untranslated region; WT, wild type.

tissue-specific manner. *Rpl38* expression is markedly enriched within developing somites, the vertebrae precursors, and the lateral motor column (LMC) of the neural tube, where specific *Rpl38* loss-of-function phenotypes are observed (Kondrashov et al. 2011). Collectively, these studies suggest that specialized ribosomes, harboring a unique regulatory activity by virtue of individual RP components, such as RPL38, carry out more specialized functions in the translational control of specific mRNAs within different regions of the embryo (Xue & Barna 2012) (**Figure 3*a***).

Cis-Regulatory Elements and Individual Ribosomal Proteins in Transcript-Specific Gene Regulation

An outstanding question raised by these initial studies is the nature of the regulatory elements in target mRNAs that interface with specialized ribosomes that may contain a unique RP composition and/or have unique activity. Might ribosome-mediated control of gene regulation be encoded within the genomic template, akin to a transcription factor binding site or microRNA

IRES: internal ribosome entry site

TIE: translation inhibitory element

seed sequence? Recent studies have addressed this outstanding question by employing *RPL38* and *Hox* mRNAs as a model system (**Figure 3*b***). A striking topology has been identified in mammalian 5′UTRs that enables specialized, ribosome-mediated translational regulation (Xue et al. 2015). In all eukaryotes, cellular mRNAs are capped (Sonenberg & Hinnebusch 2009). However, in many RNA viruses that do not cap their RNAs, more directed mRNA-ribosome interactions occur through an internal ribosome entry site (IRES) element, which allows an alternative mechanism of translation initiation (Kieft 2008). Although a small subset of stress-response mRNAs in the mammalian genome are known to harbor IRES elements, this mode of gene regulation has largely been considered a fail-safe mechanism to drive expression under select stress conditions when cap-dependent translation is diminished (Holcik & Sonenberg 2005, Komar & Hatzoglou 2011). The cap-dependent mechanism is considered highly efficient and thereby the predominant means for translation of most eukaryotic transcripts (Sonenberg & Hinnebusch 2009). Unexpectedly, using the *HoxA* locus as a model system has led to the identification of many conserved, IRES-like elements positioned in *Hox* genes. In fact, of the 11 *HoxA* genes, 5 (*a3*, *a4*, *a5*, *a9*, and *a11*) contain newly identified IRES-like elements. Notably, deletion of these elements in 5′UTR reporter constructs completely blocks translation initiation, and these IRES elements require RPL38 for translational activation. Moreover, the structure of Hox IRES elements was determined in vitro by carrying out selective 2′-hydroxyl acylation followed by primer extension (SHAPE) analysis (Wilkinson et al. 2006, Xue et al. 2015). Strikingly, mutate-and-map analysis (Kladwang et al. 2011) has confirmed structural features of the *Hoxa9* IRES element at base-pair resolution and revealed that an intricate 3D RNA structure is required for cellular *Hox* IRES activity within cells. Moreover, one of the first targeted knockouts of a cellular IRES element in the mouse genome demonstrated a complete block in HOXA9 protein expression within the neural tube and somites of developing $Hoxa9^{\Delta IRES/\Delta IRES}$ embryos, leading to striking axial skeletal patterning defects.

An outstanding question raised by these studies is why *Hox* mRNAs rely on IRES elements for translation under normal physiological conditions, when cap-dependent translation is not diminished. This phenomenon can be explained by an additional 5′UTR regulon present in *Hox* IRES-containing mRNAs that is sufficiently strong to inhibit cap-dependent translation under physiological conditions. This regulon is termed the translation inhibitory element (TIE) and is located in close proximity to the 5′ cap. The TIE ensures that translation of *Hox* IRES-containing mRNAs is selectively under the control of IRES elements, which require more direct interactions with the ribosome and may rely on specialized ribosome activity as a means of carefully refining where these transcripts are translated in space and time. Thus, similar to transcriptional silencers and activators, the TIE potently inhibits general cap-dependent translation and converts a generic mode of mRNA translation into a highly specialized one that relies on a unique activator of translation, the IRES. It is interesting to hypothesize that both the IRES and TIE may be differentially regulated, providing a versatile toolbox for controlling the ultimate expression of transcripts. Intriguingly, although Hox IRES elements are highly conserved and may be present in *Drosophila* Hox orthologs, the TIE lacks conservation and is absent even in vertebrate species such as fish and amphibians. These species often lay their eggs in harsh environments and may have originally used IRES elements as a fail-safe mechanism to ensure proper expression of key transcripts under stress conditions. Therefore, the acquisition of the TIE later in evolution may have enabled an additional, independent level of regulatory control of IRES-dependent translation and gene expression during normal development. These studies also hint at the possibility that greater ribosomal regulation of gene expression may be a hallmark of higher vertebrates or mammals, a consequence of parallel evolution in the *cis*-regulatory landscape of 5′UTRs.

Together, these studies have provided unique insight into how ribosome-mediated control of gene expression is embedded in the mRNA sequence. It will be interesting to determine if

RPs promote specialized translation through control of unique subsets of IRES-containing mRNAs, either directly or through RNA-binding proteins. This mechanism could explain, at least in part, the cell- and tissue-specific phenotypes evident upon RP loss of function in many organisms (see above). It will also be interesting to determine whether this paradigm of translational control is a harbinger of more widespread control of gene expression critical for organismal development.

Emerging Genetic Links Between Tailored Protein Synthesis and Cell Fate Determination

As early as 1950, it was noted that protein production rates vary significantly among different mammalian cell types, with the exocrine pancreas having the highest protein synthesis rate (Allfrey et al. 1953, Garlick et al. 1980). New techniques allow accurate quantification of protein synthesis in vivo at a single-cell level (Liu et al. 2012) and have revealed marked differences in protein production rates, even among stem cells and progenitors directly derived from them (Signer et al. 2014). HSCs and multipotent progenitors (MPPs) exhibit significantly less protein synthesis than restricted hematopoietic progenitors (Signer et al. 2014). This echoes several recent studies showing, for example, lower global mRNA translation efficiency in mouse embryonic cells (mESCs) compared with embryoid bodies (Buszczak et al. 2014, Ingolia et al. 2011, Sampath et al. 2008). A key question that then arises is whether the differences in protein production rates are consequences or active regulators of cell fate determination. Recent genetic evidence reveals a strong link between translational regulation and cell fate choice (Signer et al. 2014). In *Drosophila*, decreasing or increasing rRNA synthesis in ovarian germline stem cells (GSCs) leads to reduction of proliferation or delayed differentiation, respectively. Strikingly, modulation of rRNA transcription affects the expression of a select subset of proteins including those of the bone morphogenetic protein (BMP) pathway, which regulates cell proliferation and specification (Zhang et al. 2014). Together, these studies suggest that the delicate balance between cell renewal and differentiation is tightly linked to the control of protein synthesis. Whether changes in cell fate are modulated through global or transcript-specific translation and how this occurs are the next outstanding questions.

ARE ALL RIBOSOMES THE SAME?

The realization that the ribosome is an intricate ribonucleoprotein complex was followed by an initial wave of interest in whether or not ribosomes are uniformly homogenous. A homogeneity hypothesis was postulated almost 50 years ago (Moore et al. 1968) but was immediately challenged by a series of studies showing that bacterial ribosomes vary in protein composition under different growth conditions (Deusser 1972, Deusser & Wittmann 1972, Milne et al. 1975) and depending on functional state (free subunits, monosomes, or polysomes) (Bickle et al. 1973). Stoichiometry measurements also indicate that, although most RPs are present in one copy per ribosome, approximately a quarter of RPs are present in less than molar amounts and a few are multicopy (Kurland et al. 1969, Traut et al. 1969, Weber 1972). However, owing to a lack of standard purification procedures and clear nomenclature and classification of RPs in these early studies, it remains unclear whether ribosomes are normally heterogeneous in vivo. The impact of these studies is further weakened because no clear functional specificity has been associated with the observed physical heterogeneity. On the contrary, experimental evidence clearly shows that ribosomes can be depleted of several RPs and still retain the ability to translate mRNA. A striking example comes from bacteria treated with the antibiotic kasugamycin, in which ribosomes lack more than six RPs. These ribosomes nevertheless retain the ability to translate leaderless mRNAs (Kaberdina et al. 2009). Moreover, early attempts to delineate the minimal components necessary for ribosome activity demonstrated that peptidyl transferase, responsible for the catalysis of peptide-bond

PTM: posttranslational modification

formation, may only require rRNAs in the absence of most RPs (Noller et al. 1992). These studies reveal the potential for ribosomes with a diminished RP composition, synthesized under selective conditions, to retain functional activity. However, they do not address whether such ribosomes exist under normal physiological conditions. Therefore, heterogeneity in ribosome composition remains a tantalizing hypothesis; in multicellular organisms, greater ribosomal regulation activity could provide an invaluable new layer of spatiotemporal control over gene expression. This is shown by RPL38's remarkable functional role in controlling the formation of the mammalian body plan. We have previously proposed that the definition of specialized ribosomes (Xue & Barna 2012) encompass (*a*) differences in the activity of a specific component of the ribosome, (*b*) changes in the composition of the ribosome with respect to either core RPs or auxiliary factors, and (*c*) differences in posttranslational modifications (PTMs) (**Figure 4**). Here, we discuss the current state of our understanding of specialized ribosomes and the key experimental evidence required to substantiate the role and impact of ribosome heterogeneity in cell and organismal biology.

Core Ribosomal Proteins

Genome-wide transcriptional analysis in mammals has revealed surprisingly distinct expression profiles of RP transcripts in different tissue and cell types. In particular, one of the first comprehensive gene expression studies to directly measure and quantify RP expression during embryonic development led to the hypothesis that ribosomes in different regions of the embryo are highly

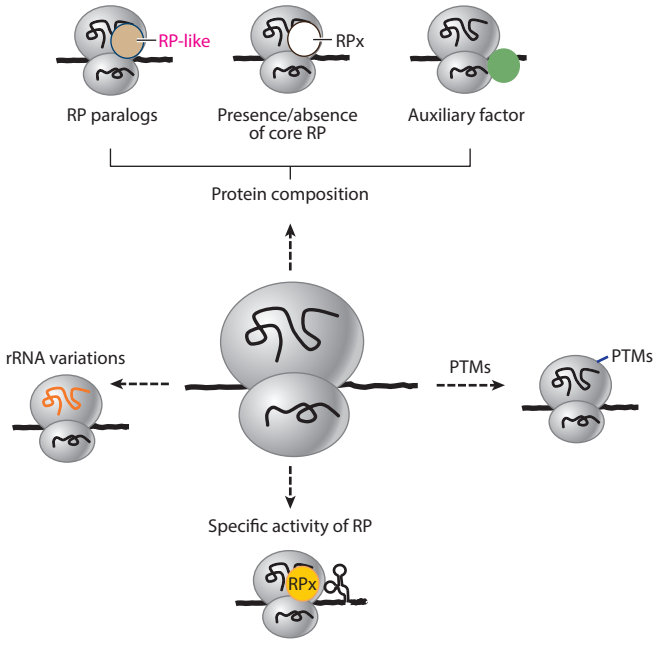


Figure 4

Different levels of regulation could produce specialized ribosomes. Specialized ribosome composition may be conferred by unique ribosomal proteins (RPs) and/or auxiliary proteins, ribosomal RNA (rRNA) sequence variation, and posttranslational modifications (PTMs) of RPs. In addition, differences in rRNA sequences or modifications may produce different types of ribosomes.

regulated and specialized (Kondrashov et al. 2011). In this study, RPs were hierarchically clustered based on expression in 14 tissue and cell types within the vertebrate embryo over a 250-fold range, expressed in $\log_2$ space, revealing dynamic regulation. In addition, more general patterns emerged, including enriched expression of most RPs in embryonic stems cells compared with more differentiated cell types, such as murine embryonic fibroblasts. A clear example of RP expression profiles being functionally significant in organismal development is the *Rpl38* loss-of-function phenotypes, including the pronounced homeotic transformations of the axial skeleton, which precisely coincide with where *Rpl38* expression is most enriched, such as in somites and the neural tube (Kondrashov et al. 2011). These findings suggest that the tissue-specific functional roles of RPs are coupled to the regulation of RP expression. It is tempting to speculate that ribosomes bearing different sets of RPs may also exist within a single cell. In neurons, direct comparison of transcripts and proteins in neuronal processes and the cell body has revealed that select sets of RPs, quantified at the level of total protein abundance (Pertz et al. 2008) or the level of transcript abundance (Moroz et al. 2006), distinguish neuronal processes. Although direct quantitative mass spectrometry data on ribosome composition is lacking at present, it is tempting to speculate that local, selective pools of ribosomes are primed to translate mRNAs bearing unique features. As a clear, functional link between protein synthesis and long-term memory exists, including the finding that translational remodeling of gene expression underlies behavioral plasticity (Sutton & Schuman 2006), a local translational program directed by specialized ribosomes could be required for synaptic plasticity and memory formation.

TOP: terminal oligopyrimidine tract

Additional examples of functional differences in ribosome composition come from the multiple paralogs encoding RPs in many organisms, most notably yeast and plants. Despite high sequence homology, many RP paralogs have drastically different expression patterns (Parenteau et al. 2011, Whittle & Krochko 2009), regulatory mechanisms (Parenteau et al. 2011), and loss-of-function phenotypes (Degenhardt & Bonham-Smith 2008, Falcone Ferreyra et al. 2010). In mammals, however, RPs are encoded by single genes, with a few exceptions. Interestingly, variation in the expression patterns of RP paralogs (e.g., *Rps4*, *Rpl10*, *Rpl22*, and *Rpl39*) is particularly evident in germ cells (Lopes et al. 2010, Sugihara et al. 2010), suggesting the potential importance of ribosome heterogeneity in germ cell biology.

In addition, certain RPs bear PTMs, such as acetylation, methylation, ubiquitination, phosphorylation, and O-GlcNAcylation (Odintsova et al. 2003, Yu et al. 2005, Zeidan et al. 2010). Ribosome modifications appear to be regulated during the cell cycle (Spence et al. 2000), during specific life stages (Ramagopal 1991), or in response to environmental conditions (Krieg et al. 1988). However, we lack a mechanistic understanding of the functional roles that ribosome PTMs play in the life of the organism. It is tempting to speculate that RP modifications modulate the activity or specificity of ribosomes, and some important examples of this are emerging. RPS6 phosphorylation has been most extensively characterized in culture cell lines. However, recently its biological significance was elucidated using a knock-in mouse in which all five conserved phosphorylated residues of RPS6 had been mutated ($Rps6^{P-/-}$) (Ruvinsky et al. 2005). It was originally believed that RPS6 phosphorylation mediates translational control of a specific class of mRNAs harboring a 5′ terminal oligopyrimidine tract (5′TOP) sequence in response to mechanistic target of rapamycin (mTOR) signaling. Surprisingly, however, the $Rps6^{P-/-}$ mice show no defect in global or 5′TOP mRNA translation but do display decreased cell size as well as impaired glucose homeostasis, revealing the importance of RPS6 phosphorylation to mammalian biology (Ruvinsky et al. 2005). Furthermore, mutations in leucine-rich repeat kinase 2 (LRRK2) are a common cause of Parkinson's disease, but the molecular mechanism underlying the LRRK2 hyperactivation that causes neurodegeneration is unknown. A recent study identified *Rps15* as a key LRRK2 substrate in *Drosophila* and human neuron Parkinson's disease models. Increased RPS15 phosphorylation

conferred by hyperactive LRRK2 mutations stimulates global mRNA translation and is a major contributor to the toxicity observed in LRRK2 mutants, as inhibition of global protein synthesis ameliorates LRRK2 mutation-induced neurodegeneration (Martin et al. 2014).

Ribosome-Associated Factors

In addition to the core RPs, hundreds of auxiliary factors ensure robustness, fidelity, and efficiency at every step of protein synthesis. The multiple layers of regulation conferred by ribosome-associated factors are best exemplified by the receptor for activated C-kinase 1 (RACK1), first identified in association with ribosomes by mass spectrometry (Link et al. 1999). Ribosome-bound RACK1 is required for IRES-mediated translation and is a key determinant of infection by IRES-containing viruses (Majzoub et al. 2014). As a scaffold protein, RACK1 displays incredibly multifaceted activity by recruiting a cohort of interacting proteins. For example, through RACK1, activated protein kinase C (PKCβII) binds to the ribosome and may stimulate translation by phosphorylating the translation initiation factor eIF6, releasing it from the 60S subunit and allowing subunit joining (Ceci et al. 2003). RACK1 also recruits the microRNA-induced silencing complex (miRISC) to ribosomes, which is critical for microRNA function (Jannot et al. 2011). Furthermore, RACK1 associates with the cell adhesion receptor integrin-β (Liliental & Chang 1998), which may recruit ribosomes to sites of cell attachment for local translation and may also control the translation of specific subsets of mRNAs through its regulation of RNA-binding proteins (Baum et al. 2004, Coyle et al. 2009).

Several additional proteins have been identified that associate with the ribosome, each exerting a specific type of regulation of translation. In embryonic stem cells, the RNA-binding protein LIN28A interacts with ribosomes at the endoplasmic reticulum (ER) and inhibits the translation of a specific set of mRNAs destined for the ER and harboring a sequence motif (Cho et al. 2012). By contrast, unlike most RNA-binding proteins, which interact with ribosomes through mRNAs, the Fragile-X mental retardation protein (FMRP) binds directly to the ribosome itself (Chen et al. 2014). Cryo-electron microscopy (cryo-EM) studies have shown that FMRP binds within the intersubunit space of the ribosome and inhibits translation elongation, likely by blocking the recruitment of tRNAs and translation elongation factors (Chen et al. 2014, Darnell et al. 2011). An additional RNA-binding motif of FMRP can interface with specific RNA structures and/or sequences, which further confers selectivity to its inhibition of translation (Brown et al. 2001, Darnell et al. 2001, Schaeffer et al. 2001). Lastly, even proteins with no obvious RNA-binding specificity may exert specific regulatory roles in functionally specialized ribosomes. For example, the transmembrane receptor DCC associates with ribosomes within neuronal axons and dendrites and may regulate protein synthesis locally in response to the extracellular ligand netrin, which is important for synaptic plasticity (Tcherkezian et al. 2010). Together, these and other examples suggest that ribosome activity is highly regulated and that ribosomes may each be armed with distinct cohorts of auxiliary factors, allowing them to be specially tuned for the tailored translation of certain mRNAs. In support of this hypothesis, proteomics analysis has identified myriad additional, poorly understood ribosome-associated factors (Fleischer et al. 2006, Link et al. 1999, Reschke et al. 2013).

In addition to diversity in the composition and modification of core RPs and associated factors, the sequence and modification of rRNAs varies (Bellodi et al. 2013, Gunderson et al. 1987). Importantly, although the distinct composition of ribosomes in different tissues could be fundamental in cell fate specification, there is a lack of experimental data in either unicellular or multicellular organisms that precisely quantify ribosome composition at the level of the protein. This will be a

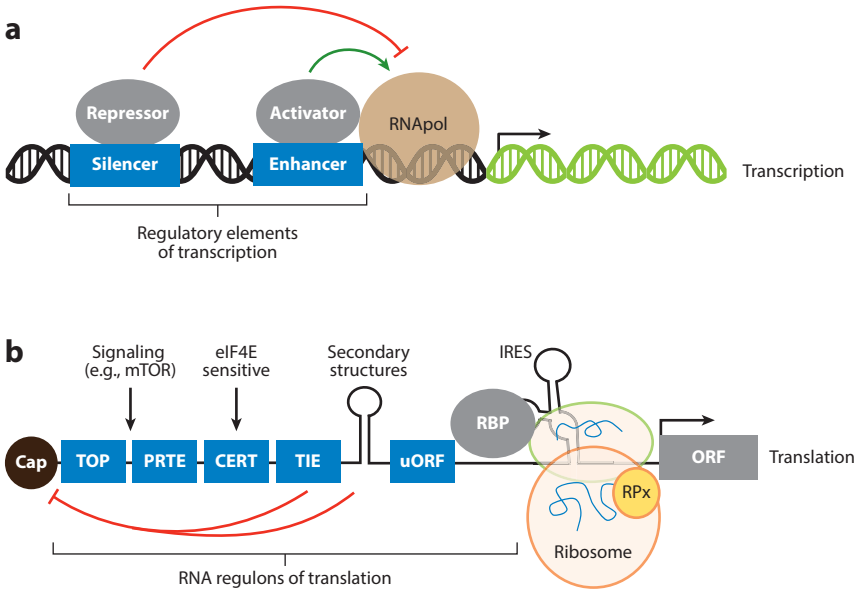


Figure 5

Emerging parallels between the regulation of transcription and translation conferred by *cis*-regulatory elements. (*a*) At the transcriptional level, silencers and enhancers repress and activate transcription by binding to repressors and activators, respectively. Here, RNApol is RNA polymerase. (*b*) Terminal oligopyrimidine tracts (TOPs), pyrimidine-rich translational elements (PRTEs), CERT (cytosine-enriched regulator of translation), RNA secondary structure translation inhibitory elements (TIEs), upstream open reading frames (uORFs), and internal ribosome entry sites (IRES) are examples of the myriad RNA regulons that mediate transcript-specific translational control by interacting with specific RNA-binding proteins (RBPs) and specialized translation machinery. RPx represents any specific ribosomal protein.

vital future direction in the field. Moreover, our understanding of more cell- and tissue-specialized roles of RPs will greatly benefit from such in-depth ribosome-omics.

RNA REGULONS: CODES BEYOND THE GENETIC CODE

5′ Untranslated Region RNA Regulons Interface with Specialized Translational Machinery

Regulatory elements embedded in mRNAs (often within the 5′ and 3′UTRs) are the focus of many studies aimed at understanding translational regulation. However, the field is just beginning to appreciate the rich regulatory information that directs selective translation of transcripts. As revealed by studies of RPL38-mediated translation of *Hox* mRNAs, RNA regulatory elements such as the TIE and IRES-like elements are just beginning to be characterized. Moreover, 5′UTRs encode a variety of *cis*-regulatory elements, including upstream AUGs (uAUGs), upstream open reading frames (uORFs), TOPs, and IRESs, among many others (**Figure 5**). These sequence motifs or structures can drive regulation through processes as diverse as cap-dependent recognition and scanning, cap-independent translation, start codon usage, translation elongation rates, localization, and transcript stability. Here, we discuss examples of the growing molecular toolbox of regulatory elements within UTRs that provide greater translational control during organismal development.

ORF: open reading frame

Cis-Acting RNA Sequence Motifs

PRTE: pyrimidine-rich translational element

CERT: cytosine-enriched regulator of translation

Occurring in almost half of human and mouse transcripts (Calvo et al. 2009), the uORF is one of the most prevalent regulons embedded in the 5′UTR. The conservation of uORFs (Calvo et al. 2009, Iacono et al. 2005) and their high prevalence in the transcripts of key regulatory genes involved in growth, proliferation, and differentiation (Kozak 1987) imply that uORF-mediated translational control has an important function in organismal development and physiology. As ribosomes need to reinitiate translation after translating a uORF, and reinitiation is generally inefficient, many uORFs impede translation of the downstream main ORF. However, numerous examples show that instead of acting as a universal brake to mRNA translation, the regulatory activity of uORFs is highly context specific and tunable.

In yeast, a genome-wide translational profiling study has revealed pervasive translational control in meiosis: uORFs with near-cognate start codons are present in many mRNAs functioning in sporulation. In contrast to many AUG uORFs, these near-cognate uORFs seem to favor rather than inhibit translation of the main ORF (Brar et al. 2012). Although uORF regulation has been extensively studied using reporter assays, providing many mechanistic insights (Morris & Geballe 2000), the physiological relevance of uORF regulation lags behind. However, a few in vivo examples reinforce the fundamental importance of uORF-mediated translational control and highlight highly dynamic and condition-dependent regulation. In one study, a recombinant mouse line was generated by introducing an ATG-to-TTG point mutation in the transcription factor CCAAT/enhancer-binding protein β (C/EBPβ) uORF start site. C/EBPβ is a key regulator involved in many processes, including cell proliferation and differentiation, metabolism, innate immunity, liver development, and regeneration (Ramji & Foka 2002). C/EBPβ encodes three different N-terminal isoforms [termed LAP* (liver-activating protein*), LAP, and LIP (liver inhibitory protein)] with antagonistic functions, initiated from three different consecutive start codons. Because only one isoform can be translated at a time, the choice of start codon determines the relative abundance of antagonistic isoforms. Mutational analysis and tissue culture assays suggest an evolutionarily conserved uORF modulates the choice of start codon (Calkhoven et al. 2000, Lincoln et al. 1998). Remarkably, C/EBPβ$^{\Delta uORF}$ mice fail to initiate translation of the inhibitory LIP isoform and exhibit multiple phenotypes, including impaired liver regeneration after partial hepatectomy and defects in osteoclast differentiation (Wethmar et al. 2010), revealing that uORF-mediated control is vital to animal physiology. Underscoring the potential importance of uORF regulation, uORF-altering polymorphisms or mutations affect protein production (Calvo et al. 2009) and are associated with many human diseases (Barbosa et al. 2013).

In addition to uORFs, several sequence motifs have been described that control translation in a specific and coordinated manner. For example, the TOP in RP 5′UTRs and several translation elongation factors are suggested to confer mTOR-mediated control in response to nutrients and growth factors (Levy et al. 1991). In addition, the newly identified pyrimidine-rich translational element (PRTE) confers translational activation of specific mRNAs downstream of mTOR in an eIF4E-dependent manner (Hsieh et al. 2012). Intriguingly, although eIF4E, the major cap-binding protein, has been considered rate limiting in global protein synthesis, a recent study revealed that eIF4E haploinsufficiency is surprisingly compatible with normal mammalian development yet selectively impedes cellular transformation. Specifically, eIF4E dosage is crucial for translating key mRNA networks underlying the oncogenic translation program, including redox balance, signaling, and proteasome control, demarcated by a unique cytosine-rich 15-nucleotide motif in the 5′UTR called the cytosine-enriched regulator of translation (CERT) domain. Most importantly, mutations of the CERT domain in several eIF4E targets are sufficient to restore translational differences awarded by eIF4E dosage, underscoring the functional significance of

the CERT domain in conferring eIF4E sensitivity. The CERT domain may therefore function as a unique 5′UTR signature to delimit a specific subset of mRNAs and sensitize them to a specific dose of eIF4E required for the cellular oncogenic program as well as stress response (Truitt et al. 2015). These findings again underscore the presence of unique *cis*-acting regulatory elements that confer transcript-specific regulation to translational control. The recent explosion in genome-wide translational analysis will undoubtedly lead to the identification of many more common sequence motifs that confer translational control specificity.

Cis-Acting RNA Structure Elements

Although cap-dependent scanning is the predominant mode of translation control in eukaryotes, a subset of mRNAs exploits an alternative mode of translation via IRES elements. This mode involves the direct recruitment of translational machinery. First observed in picornavirus RNAs (Jang et al. 1988, Pelletier & Sonenberg 1988), this mechanism allows the translation of uncapped viral mRNAs at the expense of cap-dependent translation of cellular mRNAs (Gingras et al. 1996, Gradi et al. 1998).

Following the discovery of viral IRES elements, it became apparent that certain cellular transcripts also bear IRES elements. Examples include the tumor suppressor genes *p27* (*Kip1*) and *p53* and the gene encoding the antiapoptotic factor XIAP (X-linked inhibitor of apoptosis protein) (Bellodi et al. 2010, Holcik et al. 1999, Kullmann et al. 2002, Miskimins et al. 2001, Ray et al. 2006, Yang et al. 2006, Yoon et al. 2006). IRES-mediated translation of these mRNAs is stimulated when cap-dependent translation is compromised (Bellodi et al. 2010, Holcik et al. 1999, Miskimins et al. 2001, Ray et al. 2006, Yang et al. 2006). This may serve as a fail-safe mechanism for their translational activation, especially during times of stress. Furthermore, although most transcripts in eukaryotes are capped and encode a single protein, exceptions to this general principle exist. One outstanding example is the PITSLRE protein kinase, related to the family of cyclin-dependent kinases that regulate cell cycle progression. The *PITSLRE* mRNA produces two protein isoforms: a longer isoform, $p110^{PITSLRE}$, and a shorter isoform, $p58^{PITSLRE}$. The latter is produced from the C-terminal end of the transcript, initiated from an internal in-frame AUG. Whereas $p110^{PITSLRE}$ is synthesized throughout the cell cycle by cap-dependent translation, $p58^{PITSLRE}$ translation is mediated by an IRES element and occurs in the G2/M phase of the cell cycle (Cornelis et al. 2000).

As cap-dependent translation is extremely efficient, the relevance of IRES-dependent translational control of gene expression in normal cell physiology, tissue patterning, and organismal development is unclear. As discussed above, the identification of the TIE has revealed a new mechanism for ensuring that the translation of key developmental regulators, such as the *Hox* genes, depends on IRES elements. A potent repressor of cap-dependent translation, the TIE creates a requirement for more specialized mechanisms of translation initiation, thereby converting a more generic mode of translation control into a highly regulated, 5′UTR context–dependent mechanism of gene regulation (Xue et al. 2015).

Little is known about the molecular mechanism of IRES activation and, in particular, whether a sequence or structure is important for recruiting the ribosome to these mRNAs in a cap-independent manner. Interestingly, the *Hox* IRES elements represent the first cellular IRES elements for which careful structure-function analysis has been achieved. Mutate-and-map analysis (Kladwang et al. 2011) has confirmed in vitro structural features of the *Hoxa9* IRES element at base-pair resolution via SHAPE analysis (Wilkinson et al. 2006, Xue et al. 2015) and revealed that an intricate 3D RNA structure is required for activity within cells. Given the special requirement for RPL38 in selectively promoting *Hox* IRES-dependent activation, future studies must determine how *Hox* IRES elements interface with individual RPs to confer translational specificity.

As exemplified by these IRES studies, determining RNA secondary and tertiary structures will be key in advancing our understanding of potentially numerous 5′UTR RNA regulons. Structural studies have largely been performed on RNAs extracted from cells, as probing RNA structures in living cells is extremely challenging. New approaches that chemically probe RNA are providing the first global view of RNA secondary structures in living cells. Notably, mRNAs appear to be significantly less structured in cells than in in vitro conditions, suggesting active unfolding of RNA in more physiological states (Ding et al. 2014, Rouskin et al. 2014). Unfolding of RNA structures appears to be energy-dependent, as ATP depletion in yeast cells leads to marked increases in RNA structure formation (Rouskin et al. 2014). Meanwhile, differences in RNA structures in living cells and in vitro have also been observed in *Arabidopsis*. Interestingly, the mRNA structures of genes associated with stress responses tend to adopt secondary and tertiary structures with higher free energy in living cells, displaying the most discrepancies with in silico models (Ding et al. 2014). These findings support the idea that mRNAs undergo conformational changes under different conditions, reflecting tremendous flexibility in response to context. The similar structural features of transcripts functioning in the same process (e.g., the stress response) also suggest the importance of specific shared 3D RNA structure elements in coordinated regulation of gene expression. Finally, a recent click-selective 2′-hydroxyl acylation and profiling experiment (icSHAPE) in mouse embryonic stem cells revealed structural rearrangements overlapping with RNA-modification sites and sites interacting with RNA-binding proteins (Spitale et al. 2015). These findings suggest that RNA regulons may interface with myriad RNA-binding proteins and hint at new, exciting ways of RNA-based regulation of translational control, potentially involving 5′UTRs. Indeed, growing evidence suggests that hundreds of RNA-binding proteins promote specific recognition of regulatory elements in mRNAs, including those within 5′UTRs (Castello et al. 2012). However, our understanding of their functional roles, beyond the suspicion that they may modulate the posttranscriptional fate of mRNAs within the genome, often remains incomplete. A deeper understanding of 5′UTR structure-based RNA regulons as well as the RNA-binding proteins that interface with such elements will significantly advance our understanding of the specialized mechanisms that control when, where, and how much of a protein is expressed from an mRNA template.

FUTURE ISSUES

1. Given the vital importance of ribosomal proteins (RPs) in ribosome biogenesis and organismal viability, RP haploinsufficiency mutation genetics may just represent the tip of the iceberg for RP functional specialization. Tissue/cell type–specific and conditional manipulation of RP levels combined with transcript-specific and genome-wide quantitative measurement of protein synthesis will greatly deepen our understanding of the specialized ribosome functions endowed by individual RPs.
2. What is the degree of ribosome heterogeneity? To date, we lack a systematic mass spectrometry approach to quantify ribosome heterogeneity in terms of the presence/absence of RPs, RP paralogs, posttranslational modifications of RPs, and auxiliary factors. Variation in rRNA sequence and modifications hints at possible points of further control. How many different types of ribosomes exist, and how are they structurally different? What is the biological meaning and function of these specialized ribosomes?

3. What are the upstream mechanisms that generate heterogeneous populations of ribosomes? Is heterogeneity rooted at the initial step of ribosome biogenesis? How does a ribosome missing certain core RPs evade quality control mechanisms, and, alternatively, how can an RP be removed from a fully assembled ribosome in the cytoplasm?
4. The striking example of RACK1 being identified as an auxiliary factor prior to the realization that its core function is as a bona fide RP suggests that perhaps more RPs remain to be discovered. How should we define a protein as being an auxiliary factor or an RP?
5. What is the nature of *cis*-regulatory elements that interface with RNA-binding proteins and the specialized translation machinery to provide exquisitely sensitive translational control in space and time? How specific or promiscuous is this recognition mechanism? How do we systematically identify more RNA regulatory elements?
6. The field of cell and developmental biology has focused on transcriptional control as the paramount mode of gene regulation for decades. The next exciting frontier may be to develop a similarly deep and complete roadmap for how translational control, both global and transcript specific, guides organismal development.
7. How do conservation and change within 5′UTR sequence, especially with respect to *cis*-regulatory elements, shape mRNA translational control and the evolution of the organismal body plan? Are they a mechanism for expanding the protein expression potential of a fixed number of transcripts within the genome to evolve greater diversity in life?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank the Barna lab for constant inspiration in conceptualizing specialized ribosome functions and translational regulation. This work was supported by the NIH Director's New Innovator Award (7DP2OD00850902; M.B.), an Alfred P. Sloan Research Fellowship (M.B), a Mallinckrodt Foundation Award (M.B.), a Pew Scholars Award (M.B.), and a Life Science Research Foundation Postdoctoral Fellowship (Z.S.).

LITERATURE CITED

Alberts B. 1998. The cell as a collection of protein machines: preparing the next generation of molecular biologists. *Cell* 92(3):291–94

Allfrey V, Daly MM, Mirsky AE. 1953. Synthesis of protein in the pancreas. II. The role of ribonucleoprotein in protein synthesis. *J. Gen. Physiol.* 37(2):157–75

Amsterdam A, Sadler KC, Lai K, Farrington S, Bronson RT, et al. 2004. Many ribosomal protein genes are cancer genes in zebrafish. *PLOS Biol.* 2(5):E139

Anderson SJ, Lauritsen JP, Hartman MG, Foushee AM, Lefebvre JM, et al. 2007. Ablation of ribosomal protein L22 selectively impairs αβ T cell development by activation of a p53-dependent checkpoint. *Immunity* 26(6):759–72

Barbosa C, Peixeiro I, Romao L. 2013. Gene expression regulation by upstream open reading frames and human disease. *PLOS Genet.* 9(8):e1003529

Barkic M, Crnomarkovic S, Grabusic K, Bogetic I, Panic L, et al. 2009. The p53 tumor suppressor causes congenital malformations in *Rpl24*-deficient mice and promotes their survival. *Mol. Cell. Biol.* 29(10):2489–504

Barlow JL, Drynan LF, Hewett DR, Holmes LR, Lorenzo-Abalde S, et al. 2010. A p53-dependent mechanism underlies macrocytic anemia in a mouse model of human 5q- syndrome. *Nat. Med.* 16(1):59–66

Baum S, Bittins M, Frey S, Seedorf M. 2004. Asc1p, a WD40-domain containing adaptor protein, is required for the interaction of the RNA-binding protein Scp160p with polysomes. *Biochem. J.* 380(Pt. 3):823–30

Bellodi C, Kopmar N, Ruggero D. 2010. Deregulation of oncogene-induced senescence and p53 translational control in X-linked dyskeratosis congenita. *EMBO J.* 29(11):1865–76

Bellodi C, McMahon M, Contreras A, Juliano D, Kopmar N, et al. 2013. H/ACA small RNA dysfunctions in disease reveal key roles for noncoding RNA modifications in hematopoietic stem cell differentiation. *Cell Rep.* 3(5):1493–502

Bickle TA, Howard GA, Traut RR. 1973. Ribosome heterogeneity: the nonuniform distribution of specific ribosomal proteins among different functional classes of ribosomes. *J. Biol. Chem.* 248(13):4862–64

Bolze A, Mahlaoui N, Byun M, Turner B, Trede N, et al. 2013. Ribosomal protein SA haploinsufficiency in humans with isolated congenital asplenia. *Science* 340(6135):976–78

Boria I, Garelli E, Gazda HT, Aspesi A, Quarello P, et al. 2010. The ribosomal basis of Diamond-Blackfan anemia: mutation and database update. *Hum. Mutat.* 31:1269–79

Brar GA, Yassour M, Friedman N, Regev A, Ingolia NT, Weissman JS. 2012. High-resolution view of the yeast meiotic program revealed by ribosome profiling. *Science* 335(6068):552–57

Bridges CB, Morgan TH. 1923. *The Third-Chromosome Group of Mutant Characters of* Drosophila Melanogaster. Baltimore, MD: Carnegie Inst.

Brown V, Jin P, Ceman S, Darnell JC, O'Donnell WT, et al. 2001. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in Fragile X syndrome. *Cell* 107(4):477–87

Budde A, Grummt I. 1999. p53 represses ribosomal gene transcription. *Oncogene* 18(4):1119–24

Buszczak M, Signer RAJ, Morrison SJ. 2014. Cellular differences in protein synthesis regulate tissue homeostasis. *Cell* 159(2):242–51

Byrgazov K, Vesper O, Moll I. 2013. Ribosome heterogeneity: another level of complexity in bacterial translation regulation. *Curr. Opin. Microbiol.* 16(2):133–39

Calkhoven CF, Muller C, Leutz A. 2000. Translational control of C/EBPα and C/EBPβ isoform expression. *Genes Dev.* 14:1920–32

Calvo SE, Pagliarini DJ, Mootha VK. 2009. Upstream open reading frames cause widespread reduction of protein expression and are polymorphic among humans. *PNAS* 106(18):7507–12

Castello A, Fischer B, Eichelbaum K, Horos R, Beckmann BM, et al. 2012. Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. *Cell* 149:1393–406

Ceci M, Gaviraghi C, Gorrini C, Sala LA, Offenhauser N, et al. 2003. Release of eIF6 (p27BBP) from the 60S subunit allows 80S ribosome assembly. *Nature* 426(6966):579–84

Chen E, Sharma MR, Shi X, Agrawal RK, Joseph S. 2014. Fragile X mental retardation protein regulates translation by binding directly to the ribosome. *Mol. Cell* 54(3):407–17

Cho J, Chang H, Kwon SC, Kim B, Kim Y, et al. 2012. LIN28A is a suppressor of ER-associated translation in embryonic stem cells. *Cell* 151(4):765–77

Cornelis S, Bruynooghe Y, Denecker G, Van Huffel S, Tinton S, Beyaert R. 2000. Identification and characterization of a novel cell cycle–regulated internal ribosome entry site. *Mol. Cell* 5(4):597–605

Coyle SM, Gilbert W V, Doudna JA. 2009. Direct link between RACK1 function and localization at the ribosome in vivo. *Mol. Cell. Biol.* 29(6):1626–34

Darnell JC, Jensen KB, Jin P, Brown V, Warren ST, Darnell RB. 2001. Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* 107(4):489–99

Darnell JC, Van Driesche SJ, Zhang C, Hung KY, Mele A, et al. 2011. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell* 146(2):247–61

De Keersmaecker K, Atak ZK, Li N, Vicente C, Patchett S, et al. 2013. Exome sequencing identifies mutation in *CNOT3* and ribosomal genes *RPL5* and *RPL10* in T-cell acute lymphoblastic leukemia. *Nat. Genet.* 45(2):186–90

Degenhardt RF, Bonham-Smith PC. 2008. *Arabidopsis* ribosomal proteins RPL23aA and RPL23aB are differentially targeted to the nucleolus and are disparately required for normal development. *Plant Physiol.* 147(1):128–42

Deusser E. 1972. Heterogeneity of ribosomal populations in *Escherichia coli* cells grown in different media. *Mol. Gen. Genet.* 119(3):249–58

Deusser E, Wittmann HG. 1972. Ribosomal proteins: variation of the protein composition in *Escherichia coli* ribosomes as function of growth rate. *Nature* 238(5362):269–70

Ding Y, Tang Y, Kwok CK, Zhang Y, Bevilacqua PC, Assmann SM. 2014. In vivo genome-wide profiling of RNA secondary structure reveals novel regulatory features. *Nature* 505(7485):696–700

Falcone Ferreyra ML, Pezza A, Biarc J, Burlingame AL, Casati P. 2010. Plant L10 ribosomal proteins have different roles during development and translation under ultraviolet-B stress. *Plant Physiol.* 153(4):1878–94

Fleischer TC, Weaver CM, McAfee KJ, Jennings JL, Link AJ. 2006. Systematic identification and functional screens of uncharacterized proteins associated with eukaryotic ribosomal complexes. *Genes Dev.* 20(10):1294–307

Frank J. 2000. The ribosome—a macromolecular machine par excellence. *Chem. Biol.* 7(6):R133–41

Garlick PJ, McNurlan MA, Preedy VR. 1980. A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [^{3}H]phenylalanine. *Biochem. J.* 192(2):719–23

Ghosh A, Rideout EJ, Grewal SS. 2014. TIF-IA–dependent regulation of ribosome synthesis in *Drosophila* muscle is required to maintain systemic insulin signaling and larval growth. *PLOS Genet.* 10(10):e1004750

Gingras AC, Svitkin Y, Belsham GJ, Pause A, Sonenberg N. 1996. Activation of the translational suppressor 4E-BP1 following infection with encephalomyocarditis virus and poliovirus. *PNAS* 93(11):5578–83

Golomb L, Bublik DR, Wilder S, Nevo R, Kiss V, et al. 2012. Importin 7 and exportin 1 link c-Myc and p53 to regulation of ribosomal biogenesis. *Mol. Cell* 45:222–32

Gradi A, Svitkin Y V, Imataka H, Sonenberg N. 1998. Proteolysis of human eukaryotic translation initiation factor eIF4GII, but not eIF4GI, coincides with the shutoff of host protein synthesis after poliovirus infection. *PNAS* 95(19):11089–94

Gunderson JH, Sogin ML, Wollett G, Hollingdale M, de la Cruz VF, et al. 1987. Structurally distinct, stage-specific ribosomes occur in *Plasmodium*. *Science* 238(4829):933–37

Holcik M, Lefebvre C, Yeh C, Chow T, Korneluk RG. 1999. A new internal-ribosome-entry-site motif potentiates XIAP-mediated cytoprotection. *Nat. Cell Biol.* 1(3):190–92

Holcik M, Sonenberg N. 2005. Translational control in stress and apoptosis. *Nat. Rev. Mol. Cell Biol.* 6(4):318–27

Horiguchi G, Van Lijsebettens M, Candela H, Micol JL, Tsukaya H. 2012. Ribosomes and translation in plant developmental control. *Plant Sci.* 191–192:24–34

Hsieh AC, Liu Y, Edlind MP, Ingolia NT, Janes MR, et al. 2012. The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature* 485(7396):55–61

Iacono M, Mignone F, Pesole G. 2005. uAUG and uORFs in human and rodent 5′ untranslated mRNAs. *Gene* 349:97–105

Ingolia NT, Lareau LF, Weissman JS. 2011. Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. *Cell* 147(4):789–802

Jang SK, Krausslich HG, Nicklin MJ, Duke GM, Palmenberg AC, Wimmer E. 1988. A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. *J. Virol.* 62(8):2636–43

Jannot G, Bajan S, Giguere NJ, Bouasker S, Banville IH, et al. 2011. The ribosomal protein RACK1 is required for microRNA function in both *C. elegans* and humans. *EMBO Rep.* 12(6):581–86

Kaberdina AC, Szaflarski W, Nierhaus KH, Moll I. 2009. An unexpected type of ribosomes induced by kasugamycin: a look into ancestral times of protein synthesis? *Mol. Cell* 33(2):227–36

Kieft JS. 2008. Viral IRES RNA structures and ribosome interactions. *Trends Biochem. Sci.* 33(6):274–83

Kirn-Safran CB, Oristian DS, Focht RJ, Parker SG, Vivian JL, Carson DD. 2007. Global growth deficiencies in mice lacking the ribosomal protein HIP/RPL29. *Dev. Dyn.* 236(2):447–60

Kladwang W, VanLang CC, Cordero P, Das R. 2011. A two-dimensional mutate-and-map strategy for non-coding RNA structure. *Nat. Chem.* 3(12):954–62

Komar AA, Hatzoglou M. 2011. Cellular IRES-mediated translation: the war of ITAFs in pathophysiological states. *Cell Cycle* 10(2):229–40

Kondrashov N, Pusic A, Stumpf CR, Shimizu K, Hsieh AC, et al. 2011. Ribosome-mediated specificity in *Hox* mRNA translation and vertebrate tissue patterning. *Cell* 145(3):383–97

Kozak M. 1987. An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* 15:8125–48

Krieg J, Hofsteenge J, Thomas G. 1988. Identification of the 40 S ribosomal protein S6 phosphorylation sites induced by cycloheximide. *J. Biol. Chem.* 263(23):11473–77

Kullmann M, Gopfert U, Siewe B, Hengst L. 2002. ELAV/Hu proteins inhibit p27 translation via an IRES element in the p27 5′UTR. *Genes Dev.* 16(23):3087–99

Kurland CG, Voynow P, Hardy SJ, Randall L, Lutter L. 1969. Physical and functional heterogeneity of *E. coli* ribosomes. *Cold Spring Harb. Symp. Quant. Biol.* 34:17–24

Lai K, Amsterdam A, Farrington S, Bronson RT, Hopkins N, Lees JA. 2009. Many ribosomal protein mutations are associated with growth impairment and tumor predisposition in zebrafish. *Dev. Dyn.* 238:76–85

Lambertsson A. 1998. The *Minute* genes in *Drosophila* and their molecular functions. *Adv. Genet.* 38:69–134

Lee AS, Burdeinick-Kerr R, Whelan SP. 2013. A ribosome-specialized translation initiation pathway is required for cap-dependent translation of vesicular stomatitis virus mRNAs. *PNAS* 110(1):324–29

Levy S, Avni D, Hariharan N, Perry RP, Meyuhas O. 1991. Oligopyrimidine tract at the 5′ end of mammalian ribosomal protein mRNAs is required for their translational control. *PNAS* 88:3319–23

Liliental J, Chang DD. 1998. Rack1, a receptor for activated protein kinase C, interacts with integrin β subunit. *J. Biol. Chem.* 273(4):2379–83

Lincoln AJ, Monczak Y, Williams SC, Johnson PF. 1998. Inhibition of CCAAT/enhancer-binding protein α and β translation by upstream open reading frames. *J. Biol. Chem.* 273:9552–60

Link AJ, Eng J, Schieltz DM, Carmack E, Mize GJ, et al. 1999. Direct analysis of protein complexes using mass spectrometry. *Nat. Biotechnol.* 17(7):676–82

Liu J, Xu Y, Stoleru D, Salic A. 2012. Imaging protein synthesis in cells and tissues with an alkyne analog of puromycin. *PNAS* 109(2):413–18

Lopes AM, Miguel RN, Sargent CA, Ellis PJ, Amorim A, Affara NA. 2010. The human RPS4 paralogue on Yq11.223 encodes a structurally conserved ribosomal protein and is preferentially expressed during spermatogenesis. *BMC Mol. Biol.* 11:33

Majzoub K, Hafirassou ML, Meignin C, Goto A, Marzi S, et al. 2014. RACK1 controls IRES-mediated translation of viruses. *Cell* 159(5):1086–95

Martin I, Kim JW, Lee BD, Kang HC, Xu JC, et al. 2014. Ribosomal protein s15 phosphorylation mediates LRRK2 neurodegeneration in Parkinson's disease. *Cell* 157(2):472–85

Marygold SJ, Coelho CM, Leevers SJ. 2005. Genetic analysis of *RpL38* and *RpL5*, two *Minute* genes located in the centric heterochromatin of chromosome 2 of *Drosophila melanogaster*. *Genetics* 169(2):683–95

Marygold SJ, Roote J, Reuter G, Lambertsson A, Ashburner M, et al. 2007. The ribosomal protein genes and *Minute* loci of *Drosophila melanogaster*. *Genome Biol.* 8:R216

Mazumder B, Sampath P, Seshadri V, Maitra RK, DiCorleto PE, Fox PL. 2003. Regulated release of L13a from the 60S ribosomal subunit as a mechanism of transcript-specific translational control. *Cell* 115(2):187–98

McGowan KA, Li JZ, Park CY, Beaudry V, Tabor HK, et al. 2008. Ribosomal mutations cause p53-mediated dark skin and pleiotropic effects. *Nat. Genet.* 40(8):963–70

Melo JA, Ruvkun G. 2012. Inactivation of conserved *C. elegans* genes engages pathogen- and xenobiotic-associated defenses. *Cell* 149(2):452–66

Milne AN, Mak WW, Wong JT. 1975. Variation of ribosomal proteins with bacterial growth rate. *J. Bacteriol.* 122(1):89–92

Miskimins WK, Wang G, Hawkinson M, Miskimins R. 2001. Control of cyclin-dependent kinase inhibitor p27 expression by cap-independent translation. *Mol. Cell. Biol.* 21(15):4960–67

Moore PB, Traut RR, Noller H, Pearson P, Delius H. 1968. Ribosomal proteins of *Escherichia coli*. II. Proteins from the 30 s subunit. *J. Mol. Biol.* 31(3):441–61

Moroz LL, Edwards JR, Puthanveettil S V, Kohn AB, Ha T, et al. 2006. Neuronal transcriptome of *Aplysia*: neuronal compartments and circuitry. *Cell* 127(7):1453–67

Morris DR, Geballe AP. 2000. Upstream open reading frames as regulators of mRNA translation. *Mol. Cell. Biol.* 20(23):8635–42

Noller HF, Hoffarth V, Zimniak L. 1992. Unusual resistance of peptidyl transferase to protein extraction procedures. *Science* 256:1416–19

Odintsova TI, Muller EC, Ivanov AV, Egorov TA, Bienert R, et al. 2003. Characterization and analysis of posttranslational modifications of the human large cytoplasmic ribosomal subunit proteins by mass spectrometry and Edman sequencing. *J. Protein Chem.* 22(3):249–58

Palade GE. 1955. A small particulate component of the cytoplasm. *J. Biophys. Biochem. Cytol.* 1(1):59–68

Panic L, Tamarut S, Sticker-Jantscheff M, Barkic M, Solter D, et al. 2006. Ribosomal protein *S6* gene haploinsufficiency is associated with activation of a p53-dependent checkpoint during gastrulation. *Mol. Cell. Biol.* 26(23):8880–91

Parenteau J, Durand M, Morin G, Gagnon J, Lucier JF, et al. 2011. Introns within ribosomal protein genes regulate the production and function of yeast ribosomes. *Cell* 147(2):320–31

Pelletier J, Sonenberg N. 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* 334:320–25

Pertz OC, Wang Y, Yang F, Wang W, Gay LJ, et al. 2008. Spatial mapping of the neurite and soma proteomes reveals a functional Cdc42/Rac regulatory network. *PNAS* 105(6):1931–36

Perucho L, Artero-Castro A, Guerrero S, Ramón Y Cajal S, LLeonart ME, Wang ZQ. 2014. RPLP1, a crucial ribosomal protein for embryonic development of the nervous system. *PLOS ONE* 9(6):e99956

Ramagopal S. 1991. Covalent modifications of ribosomal proteins in growing and aggregation-competent *Dictyostelium discoideum*: phosphorylation and methylation. *Biochem. Cell Biol.* 69(4):263–68

Ramji DP, Foka P. 2002. CCAAT/enhancer-binding proteins: structure, function and regulation. *Biochem. J.* 365:561–75

Ray PS, Grover R, Das S. 2006. Two internal ribosome entry sites mediate the translation of p53 isoforms. *EMBO Rep.* 7(4):404–10

Reschke M, Clohessy JG, Seitzer N, Goldstein DP, Breitkopf SB, et al. 2013. Characterization and analysis of the composition and dynamics of the mammalian riboproteome. *Cell Rep.* 4(6):1276–87

Rouskin S, Zubradt M, Washietl S, Kellis M, Weissman JS. 2014. Genome-wide probing of RNA structure reveals active unfolding of mRNA structures in vivo. *Nature* 505(7485):701–5

Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, et al. 2005. Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes Dev.* 19(18):2199–211

Sampath P, Pritchard DK, Pabon L, Reinecke H, Schwartz SM, et al. 2008. A hierarchical network controls protein translation during murine embryonic stem cell self-renewal and differentiation. *Cell Stem Cell* 2(5):448–60

Schaeffer C, Bardoni B, Mandel JL, Ehresmann B, Ehresmann C, Moine H. 2001. The Fragile X mental retardation protein binds specifically to its mRNA via a purine quartet motif. *EMBO J.* 20(17):4803–13

Signer RA, Magee JA, Salic A, Morrison SJ. 2014. Haematopoietic stem cells require a highly regulated protein synthesis rate. *Nature* 509(7498):49–54

Sonenberg N, Hinnebusch AG. 2009. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* 136(4):731–45

Spence J, Gali RR, Dittmar G, Sherman F, Karin M, Finley D. 2000. Cell cycle–regulated modification of the ribosome by a variant multiubiquitin chain. *Cell* 102(1):67–76

Spitale RC, Flynn RA, Zhang QC, Crisalli P, Lee B, et al. 2015. Structural imprints in vivo decode RNA regulatory mechanisms. *Nature* 519(7544):486–90

Stewart MJ, Denell R. 1993. Mutations in the *Drosophila* gene encoding ribosomal protein S6 cause tissue overgrowth. *Mol. Cell. Biol.* 13:2524–35

Sugihara Y, Honda H, Iida T, Morinaga T, Hino S, et al. 2010. Proteomic analysis of rodent ribosomes revealed heterogeneity including ribosomal proteins L10-like, L22-like 1, and L39-like. *J. Proteome Res.* 9(3):1351–66

Sutton MA, Schuman EM. 2006. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell* 127(1):49–58

Tcherkezian J, Brittis PA, Thomas F, Roux PP, Flanagan JG. 2010. Transmembrane receptor DCC associates with protein synthesis machinery and regulates translation. *Cell* 141(4):632–44

Terzian T, Dumble M, Arbab F, Thaller C, Donehower LA, et al. 2011. *Rpl27a* mutation in the sooty foot ataxia mouse phenocopies high p53 mouse models. *J. Pathol.* 224(4):540–52

Török I, Herrmann-Horle D, Kiss I, Tick G, Speer G, et al. 1999. Down-regulation of RpS21, a putative translation initiation factor interacting with P40, produces viable *Minute* imagos and larval lethality with overgrown hematopoietic organs and imaginal discs. *Mol. Cell. Biol.* 19:2308–21

Traut RR, Delius H, Ahmad-Zadeh C, Bickle TA, Pearson P, Tissieres A. 1969. Ribosomal proteins of *E. coli*: stoichiometry and implications for ribosome structure. *Cold Spring Harb. Symp. Quant. Biol.* 34:25–38

Truitt ML, Conn CS, Shi Z, Pang X, Tokuyasu T, et al. 2015. Differential requirements for eIF4E dose in normal development and cancer. *Cell* 162(1):59–71

Uechi T, Nakajima Y, Nakao A, Torihara H, Chakraborty A, et al. 2006. Ribosomal protein gene knockdown causes developmental defects in zebrafish. *PLOS ONE* 1:e37

Volarevic S, Stewart MJ, Ledermann B, Zilberman F, Terracciano L, et al. 2000. Proliferation, but not growth, blocked by conditional deletion of 40S ribosomal protein S6. *Science* 288(5473):2045–47

Volta V, Beugnet A, Gallo S, Magri L, Brina D, et al. 2013. RACK1 depletion in a mouse model causes lethality, pigmentation deficits and reduction in protein synthesis efficiency. *Cell. Mol. Life Sci.* 70:1439–50

Warner JR, McIntosh KB. 2009. How common are extraribosomal functions of ribosomal proteins? *Mol. Cell* 34(1):3–11

Watkins-Chow DE, Cooke J, Pidsley R, Edwards A, Slotkin R, et al. 2013. Mutation of the Diamond-Blackfan anemia gene *Rps7* in mouse results in morphological and neuroanatomical phenotypes. *PLOS Genet.* 9(1):e1003094

Watson KL, Konrad KD, Woods DF, Bryant PJ. 1992. *Drosophila* homolog of the human S6 ribosomal protein is required for tumor suppression in the hematopoietic system. *PNAS* 89:11302–6

Weber HJ. 1972. Stoichiometric measurements of 30S and 50S ribosomal proteins from *Escherichia coli*. *Mol. Gen. Genet.* 119(3):233–48

Wethmar K, Bégay V, Smink JJ, Zaragoza K, Wiesenthal V, et al. 2010. C/EBP$\beta^{\Delta uORF}$ mice—a genetic model for uORF-mediated translational control in mammals. *Genes Dev.* 24:15–20

Whittle CA, Krochko JE. 2009. Transcript profiling provides evidence of functional divergence and expression networks among ribosomal protein gene paralogs in *Brassica napus*. *Plant Cell* 21(8):2203–19

Wilkinson KA, Merino EJ, Weeks KM. 2006. Selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE): quantitative RNA structure analysis at single nucleotide resolution. *Nat. Protoc.* 1(3):1610–16

Xue S, Barna M. 2012. Specialized ribosomes: a new frontier in gene regulation and organismal biology. *Nat. Rev. Mol. Cell Biol.* 13(6):355–69

Xue S, Tian S, Fujii K, Kladwang W, Das R, Barna M. 2015. RNA regulons in *Hox* 5′ UTRs confer ribosome specificity to gene regulation. *Nature* 517(7532):33–38

Yang DQ, Halaby MJ, Zhang Y. 2006. The identification of an internal ribosomal entry site in the 5′-untranslated region of *p53* mRNA provides a novel mechanism for the regulation of its translation following DNA damage. *Oncogene* 25(33):4613–19

Yoon A, Peng G, Brandenburger Y, Zollo O, Xu W, et al. 2006. Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita. *Science* 312(5775):902–6

Yu Y, Ji H, Doudna JA, Leary JA. 2005. Mass spectrometric analysis of the human 40S ribosomal subunit: native and HCV IRES-bound complexes. *Protein Sci.* 14(6):1438–46

Zeidan Q, Wang Z, De Maio A, Hart GW. 2010. O-GlcNAc cycling enzymes associate with the translational machinery and modify core ribosomal proteins. *Mol. Biol. Cell* 21(12):1922–36

Zhang Q, Shalaby NA, Buszczak M. 2014. Changes in rRNA transcription influence proliferation and cell fate within a stem cell lineage. *Science* 343(6168):298–301

Zhang Y, Duc ACE, Rao S, Sun XL, Bilbee AN, et al. 2013. Control of hematopoietic stem cell emergence by antagonistic functions of ribosomal protein paralogs. *Dev. Cell* 24(4):411–25

Zhou C, Zang D, Jin Y, Wu H, Liu Z, et al. 2011. Mutation in ribosomal protein L21 underlies hereditary hypotrichosis simplex. *Hum. Mutat.* 32:710–14