Pathways to Specialized Ribosomes: The Brussels Lecture

Jonathan D. Dinman

Department of Cell Biology and Molecular Genetics, *University of Maryland, 4062 Campus Drive, College Park, MD 20742, USA*

http://dx.doi.org/10.1016/j.jmb.2015.12.021
Edited by D. N. Wilson

Abstract

"Specialized ribosomes" is a topic of intense debate and research whose provenance can be traced to the earliest days of molecular biology. Here, the history of this idea is reviewed, and critical literature in which the specialized ribosomes have come to be presently defined is discussed. An argument supporting the evolution of a variety of ribosomes with specialized functions as a consequence of selective pressures acting on a near-infinite set of possible ribosomes is presented, leading to a discussion of how this may also serve as a biological buffering mechanism. The possible relationship between specialized ribosomes and human health is explored. A set of criteria and possible approaches are also presented to help guide the definitive identification of "specialized" ribosomes, and this is followed by a discussion of how synthetic biology approaches might be used to create new types of special ribosomes.

In the Beginning

By 1959, it was known that genetic information is encoded by nucleic acids and that proteins are synthesized by RNA-rich ribosomes. This led Francis Crick to suggest that the each ribosome contains a unique RNA encoding a single gene: the "one gene–one ribosome–one protein" hypothesis [1]. A year later at the so-called "Good Friday Meeting" [2], Jacob, Brenner and Crick realized that a previously described unstable DNA-like RNA of T2 phage [3,4] possessed the properties of an intermediate required for protein synthesis. This conceptual breakthrough catalyzed the discovery of mRNA (reviewed in Ref. [5]). However, that germ of an idea, that is, the notion that heterogeneity may confer special properties upon ribosomes, has remained alluring. Indeed, the ribosomes of extremophiles such as *Haloarcula marismortui* or *Thermus thermophilus* are specialized to optimally perform protein synthesis in high-salt and high-temperature environments, properties that were critical to their characterization by X-ray crystallography [6]. Similarly, mitochondrial, chloroplastid and other organellar ribosomes have evolved to optimally translate limited genomes in specialized intracellular niches. Thus, technically, yes, special ribosomes are known to exist and the discussion could conclude here. However, the general idea that ribosomes may be fine-tuned to translate specific mRNAs and/or have special properties in specific cell types and/or in response to certain environmental cues is commonly what is understood by the term "specialized ribosome". With this in mind, this essay first explores the idea of specialized ribosomes. It is not intended to be an exhaustive review of the subject: readers interested in a deep investigation of the evidence supporting specialized ribosomes are directed to the outstanding review by Xue and Barna [7] serving to define the state of the art in this field. Rather, this essay seeks to contribute an additional perspective to this fundamental question in the field of molecular genetics. This includes an evolutionary argument in favor of ribosome heterogeneity, a discussion of the consequences of disruption of ribosomal homeostasis and a summary of the challenges that must be overcome in order to definitively demonstrate the existence of specialized ribosomes. A final set of

musings addresses the evolving relationship between classical discovery science and synthetic biology.

First Inklings: The Hunt for Special Ribosomes and Hints Regarding Where They May Be Found

One early hypothesis was that differences in between natural and laboratory environments might exert differential selective pressures upon ribosomes within a species and that this might facilitate identification of ribosomes with specialized properties. A test of this hypothesis revealed that the enhanced growth and survival phenotypes of naturally occurring *Escherichia coli* relative to laboratory strains correlated with enhanced interactions between ribosomes and ternary complex [8]. While these findings showed that the ribosomes of laboratory strains have "devolved" relative to their wild-type counterparts, it is also consistent with the notion that ribosomal diversity is selected for under natural conditions. More recent studies in bacterial systems have examined the possibility that ribosome specialization might be achieved by differential post-translational modification of ribosomal proteins. Two such studies revealed that such modifications may contribute to maintenance of stationary phase [9] and stress-adapted cell growth [10].

In contrast to bacteria, the compartmentalized subcellular structures and complex developmental pathways of eukaryotes present richer environments in which to hunt for ribosomes with special properties. The yeast *Saccharomyces cerevisiae* has historically served as a robust genetic model for identification and characterization of gene function. The whole genome of yeast was duplicated approximately 200 million years ago [11,12]. Although nearly 90% of the duplicated genes were eliminated between then and the present, ribosomal protein genes remain over-represented among the 457 remaining paralogous gene pairs. One possible explanation is that the different protein paralogs may confer differential properties upon ribosome. Perhaps the first evidence supporting this idea came from genetic screens performed in the late 1970s and in early 1980s for mutants no longer capable of supporting replication of the endogenous "Killer" virus; this approach identified a large family of genes named *MA*intenance of *K*iller (*MAK*). Gene ontology analysis reveals enrichment in two general functions among the *MAK* genes: N-terminal protein maturation and modification and the large ribosomal subunit (biogenesis, maturation and integral ribosomal proteins). The first class of *MAK* genes is explained by the requirement of the viral capsid protein for a specific type of N-terminal modification [13,14], while the latter points to the ribosome. Intriguingly, *MAK7* and *MAK18* are also known as *RPL8A* and *RPL42B*, which encode ribosomal proteins L8A and L42B (also known as eL8A and eL42B [15]). Curiously, only deletion of these genes, but not of their paralogs (*RPL8B* or *RPL42A*), promoted the Mak$^-$ phenotype [16,17], suggesting that these proteins, and not their paralogs, are specifically required to translate the mRNAs of this virus family, which lack 5′ cap structures and polyA tails. Since then, additional genetic studies in yeast revealed similar ribosomal protein paralog-specific phenotypes. For example, a large-scale screen for yeast mutants unable to support replication of the yeast Ty*1* retrotransposable element revealed 87 Ty*1* co-factor mutants, 8 of which corresponded to one, but not the other paralog of a ribosomal protein-encoding gene [18]. A more wide ranging study that screened the entire collection of yeast ribosomal protein deletion mutants identified paralog-specific differences with regard to a large number of cellular and metabolic functions including localized mRNA translation, drug sensitivity/resistance and macromolecular assembly and localization [19]. Another study applied a series of comparative protein synthesis assays to the gene deletion collection, identifying paralog-specific differences in translational accuracy [20]. In unpublished studies, my laboratory is currently investigating ribosomal protein paralog-specific differences in translational fidelity. However, as discussed below, while these studies provide evidence for the existence of ribosomes with ribosomal protein paralog-specific phenotypes, they do not provide direct evidence for specialized ribosomes.

Breakthrough: The complex developmental programs of metazoan organisms provide rich hunting grounds for specialized ribosomes

A growing number of genetic approaches support the hypothesis of ribosome specialization through differential expression of ribosomal genes or ribosomal gene-like paralogs over a broad range of functions in many organisms. These are reviewed in Ref. [7]. Perhaps the most important breakthrough came from studies of development in mice revealing tissue-specific patterns of *RPL38* expression during embryogenesis in a manner that overlaps with embryonic tissues that are affected by loss of function of the eukaryote-specific ribosomal protein eL38 [21]. This was followed by an unbiased large-scale expression profiling screen in developing mouse embryos revealing significant differences among different cell types and tissues in the expression of ribosomal protein mRNAs and revealing that groups of ribosomal protein mRNAs were co-regulated [21]. These observations, plus the prior observation that eL38 is predominantly expressed in the pancreatic ductal epithelium in adult mice [22], brought into question the common assumption that all core ribosomal proteins are stoichiometrically

expressed, suggesting the possibility of cell-type-specific and tissue-type-specific ribosome heterogeneity. These studies led to a concrete definition of what is meant by specialized ribosomes: those in which "…a unique composition or specialized activity confer(s) regulatory control in gene expression" [7].

That the lack of eL38 results in a well-defined homeotic transformation and that this correlates with eL38 requiring IRES's in specific Hox mRNAs [23] may be akin to the requirement for ribosomal protein eL30 to recruit SECIS elements for eukaryotic selenoprotein mRNA recoding [24]. In this case, ribosome-associated eL38 may act as receptor for an RNA ligand (Hox) only in the neural tube and somites, while in the prostate where eL38 is not expressed, ribosomes may possess a different receptor (another ribosomal protein? A *trans*-acting protein or RNA?) to engage a different RNA ligand. While this somewhat indirectly supports the specialized ribosome hypothesis, it raises a chicken *versus* egg conundrum. Did the IRES's in these mRNAs evolve so as to capitalize on the presence of eL38 in eukaryotic ribosomes as a means to participate in a receptor (eL38) ligand (the IRES) interaction to recruit these mRNAs to ribosomes or did eL38 evolve later, in response to the more complex vertebrate developmental program? Finally, it should be noted that the demonstration of tissue-specific differences in ribosomal protein mRNA expression does not prove the existence of specialized ribosomes. As discussed below, biochemical/biophysical approaches are needed to follow up on these exciting genetic studies.

Human health and the specialized ribosome: Inherited and acquired diseases

An apparent paradox known as Dameshek's Riddle [25] is used to describe a class of patients who initially present with too few of a specific type of blood cell but in which survivors of this phase of the disease are at higher risk of developing diseases involving over-production of the same type of cell. The first identification of the gene responsible for one of these diseases, Diamond-Blackfan anemia, was the ribosomal protein coding gene *RPS19* [26] (encoding eS19 [15]), leading this class of diseases to be termed "ribosomopathies". The list of ribosomopathies has expanded to include diseases caused by mutations in genes that encode ribosomal proteins, ribosome biogenesis factors and the machinery that modifies ribosomal RNAs (reviewed in Refs. [27–31]). Intriguingly, while all of these diseases originate with the ribosome, each has its unique clinical presentation: this is consistent with a model in which different cell/tissue types may require different types of ribosomes. More recently, associations between somatically acquired mutations in ribosomal protein genes and a variety of cancers have been established. These include the genes encoding ribosomal protein L5 (RPL5/uL18), RPL11/uL5, RPL10/uL16 and RPL22/eL22 [32–37]. Excitingly, the comprised translational efficiency of GATA1 mutants, a critical hematopoietic transcription factor with a highly structured 5′ untranslated region in Diamond-Blackfan anemia [38], and important studies on dyskerin that link rRNA modification and IRES-dependent translation in ribosomopathies [39], as well as differences in the actual pattern of rRNA modifications in patients [40], provide robust models for recapitulating ribosomopathies at the organismal level, opening windows into understanding how gene expression is affected in these syndromes. However, it should be noted that, while associations between specific ribosomal proteins and particular cancers are consistent with the idea that different tissues may require different populations of ribosomes, no studies have produced specialized ribosomes for biochemical characterization.

An evolutionary argument for specialized ribosomes

The very language that we use to refer to the ribosome implies the image of a monolithic machine. For example, a Google search for the term "the ribosome is" yielded approximately 3,130,000 results compared to 952,000 results for "ribosomes are". We persist with this conceptual construct despite the fact that there is no basis in empirical evidence for this view [29]. Indeed, it is unrealistic to assume that the evolutionary process has selected for a unique macromolecule that has been maximized for its ability to translate a near-infinite number of mRNAs in response to a near-infinite number of stimuli. Evolution is a process in which diverse populations are acted upon by selective pressures. Explicit in this is that selection for specialized function is maximized as the potential for diversity is increased. With this in mind, let us explore potential sources of diversity in ribosomes (limiting the discussion to only one species, humans, so as not to conflate interspecific differences with the definition of specialized ribosomes described above). First, consider that the diploid human genome encodes a minimum of two copies of at least 80 ribosomal protein genes [41]. Because the ribosome is so central to cellular function, it is commonly assumed that there is little to no allelic diversity among the human ribosomal protein genes. However, a cursory analysis of single nucleotide polymorphisms in the core and essential *RPL3* gene encoding ribosomal protein uL3 in the NCBI dbSNP database[†] revealed over 100 annotated missense alleles in its protein coding region alone, suggesting that there is a significant amount of allelic diversity among human ribosomal proteins. (Call to action: someone should undertake systematic analysis of

ribosomal protein allelism.) With this in mind, the genome of each individual human has the capacity to encode $\binom{80}{2}$ (i.e., 80 choose 2 = 3160) unique ribosomes based on protein content alone. Additional potential for a very large amount of diversity in ribosomes comes from the fact that at least 212 modified bases have been identified in the four human rRNAs [42]. Thus, cells are theoretically capable of generating an astounding 212! unique rRNAs (a number that is approximately 4 followed by 360 zeros). Multiply these by an unknown number of post-translational ribosomal protein modifications and transiently associated proteins and factors, and the total possible number of unique ribosomes is practically limitless. These numbers suggest a system with the capacity to generate a highly diverse set of variations of the same machine, each endowed with its own idiosyncratic properties.

With these ideas in mind, I propose that the evolutionary process has had a nearly limitless number of potential ribosomes upon which to select for specialized function (Fig. 1). "The Ribosome" can be visualized as a cloud of potential individuals capable of occupying an *n*-dimensional space. First, consider ribosomes as they occupy the standard four dimensions (*x*, *y* and *z* spatial axes plus *t* = time). At this level, ribosome heterogeneity is already beginning to be revealed at the quantum level; approximately 50 metastable free-energy states have been described for ribosomes [43]. Furthermore, a recent analysis revealing that these may follow a distinct and closed set of low-energy trajectories through the free-energy landscape [44] can be interpreted as evidence of selective

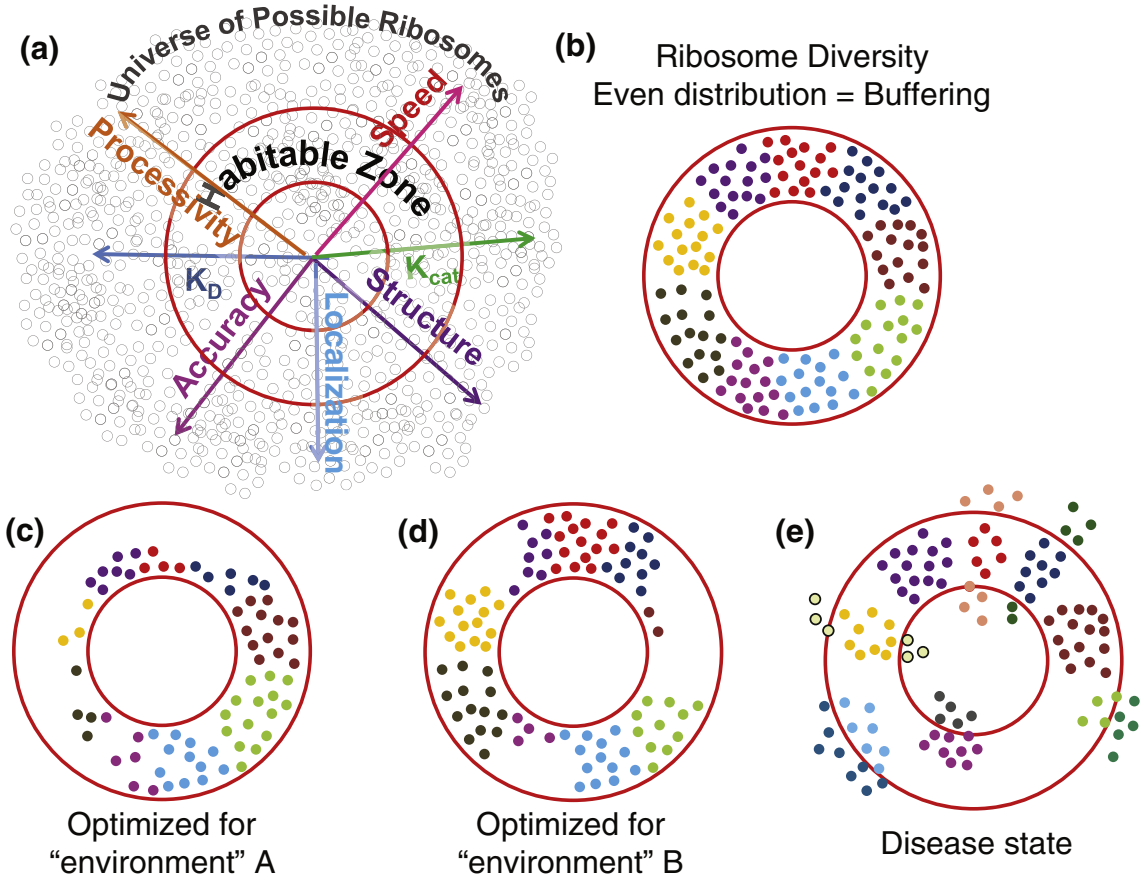

Fig. 1. "The Ribosome" as a diverse collection of non-identical individuals. (a) The nearly infinite universe of possible ribosomes is represented as grey-outlined small circles. These can be characterized in *n*-dimensional space along axes including processivity, structure, affinities for various ligands (K_D), ability to directly or indirectly catalyze enzymatic reactions (K_{cat}), translational accuracy, localization (subcellular, tissue and/or development specific) and speed. Ribosomes occupying the "Habitable Zone" are those that are capable of supporting vital cellular functions. (b) "The Ribosome" is defined as all possible ribosomes occupying the habitable zone. Evenly distributed throughout the zone provides a well-buffered system. (c and d) Different distributions of ribosomes enable optimization of the translational apparatus for different environments. (e) Expression of defective ribosomes lying outside of the habitable zone leads results in disease (ribosomopathies). Ribosomapathies may also result from depletion of a particular type of ribosome or ribosomes due to the decreased expression of one or more ribosomal protein genes.

forces at work. Dimensionality, and thus selective pressures, can be further expanded to include and operate along additional axes. Examples include affinities for different ligands, ranges of catalytic activities (both intrinsic, for example, peptidyltransferase activity, and extrinsic, for example, GTPase stimulating activity), processivity, accuracy, speed, localization and so on. The idea of selective forces operating on heterogeneous populations of ribosomes may apply at all biological levels and to all processes, maximizing fitness and maintaining homeostasis in different subcellular compartments, in different cells, in response to differentiation programs (external stimuli) and in the specialized niches presented by different tissues and organs. Importantly, the notion of "specialized ribosomes" is implicit in this model. Furthermore, different individuals within a species may harbor subtly different distributions of ribosomes, setting the ground for the speciation process in the event that groups become geographically or environmentally separated. Additionally, from this point of view, reducing the ability of the population of ribosomes to fully occupy the ribosome's functional space or altering the homeostatic distribution of ribosomes within or outside of this space could explain the genetic findings discussed above and may in part underly the tissue specificities and disease penetrance differences observed in the ribosomopathies.

This line of reasoning is not without counterarguments. For example, the existence of ribosomopathies *per se* demonstrates that variations in ribosome content can result in decreased fitness. However, preventing one or even a few rRNA modifications does not result in demonstrable phenotypes [45,46], suggesting that at least some degree of variability along this axis is tolerable. Thus, rather than confirming the monolithic ribosome view, this merely challenges us to define limits for the range of "tolerable" ribosome heterogeneity, that is, a ribosomal "habitable zone" (to borrow a term from Astronomy). Importantly, a deeper understanding of the selective pressures exerted on ribosomes in different environments may enable application of quantitative modeling approaches [47] to this field. From this point of view, ribosomopathies may be used as indicators of the boundaries of the habitable zone. The field of ribosomopathies also reveals our ignorance with regard to the importance of ribosomal protein gene dosage on cellular homeostasis. For example, ribosomal stress is associated with changes in ribosomal protein availability, both within and outside of the context of the ribosome (reviewed in Refs. [48–52]). It is possible that ribosomal protein dosage may be epistatically controlled *via* imprinting; for example, differences in local chromatin structure between maternal and paternal copies of the same chromosome may affect the expression of ribosomal protein genes. Expanding upon this, while it is well known that eukaryotic genomes harbor hundreds of copies of rDNA genes, little is known regarding their allelism, nor do we know anything about their differential expression. Germane to this, studies in yeast revealed that over-expression of different naturally occurring alleles of 5S rRNA can affect translational fidelity [53]. Indeed, it is possible that epistatic control of ribosomal protein and rRNA gene expression may provide a buffering mechanism, for example, enabling a species to respond to environmental changes more quickly than allowed by natural rates of mutation.

The Challenge of Identifying Specialized Ribosomes: Genetics Predicts, Biochemistry Confirms

As discussed above, there is a steadily growing trove of genetic evidence supporting the specialized ribosome hypothesis. The problem is that genetics is a subtractive process: identifying the specific function of a special ribosome in an assay system containing $n - 1$ ribosomes will at best lead to inferential conclusions. Indeed, we are fortunate that these systems actually yield phenotypes (although it should be cautioned that phenotypic changes could also be indicative of extra-ribosomal function for ribosomal proteins; e.g., see Ref. [54]). Thus, while the author considers himself to be a card carrying geneticist, biochemical and biophysical approaches hold the greatest promise for providing definitive evidence for the existence of specialized ribosomes.

Extraordinary claims require extraordinary evidence. In this regard, demonstration of specialized ribosomes must satisfy the "one enzyme, one (set of) substrate(s)" standard. To this end, three barriers must be overcome: (1) homogenization, (2) characterization and (3) substrate matching (see Box 1). Homogenization entails separating one type of ribosome from all others. Classically, this is performed by either additive or subtractive approaches. The additive approach requires generating homologous ribosomes by reconstituting them from purified recombinant proteins and rRNAs. While this has been achieved for some bacterial species [55] and, indeed, "specialized" (drug-resistant) ribosomes have been so assembled [56], tales of the many failed attempts to replicate this tactic with eukaryotic ribosomes have achieved near-legendary status in the ribosome community's oral history. The other approach is subtractive: purify an enzyme to homogeneity. While affinity-tagging ribosomal proteins and rRNAs methods are well developed [57,58], it is difficult to envision how this could be used to purify one specific type of naturally occurring ribosome. One alternative to purifying homogeneous ribosomes from mixed samples is to enhance resolution to the point where single molecules can be observed and characterized. Enhanced resolution will be technology driven. Emerging technologies such as atomic-resolution

Box 1
Criteria for demonstration of "specialized ribosomes": One enzyme, one substrate.

Demonstrate that one specific type of ribosome is optimized for one specific function.
Three barriers to overcome:

(1) **Homogenization**
  (a) Classic approaches:
    Additive (reconstitution)
    Subtractive (purification)
  (b) New technologies:
    Single-molecule cryo-electron microscopy
    Femtosecond X-ray crystallography
    High-throughput rRNA mod-seq
    High-resolution proteomics
(2) **Characterization**
  (a) Structural
  (b) Biochemical
  (c) Molecular
  (d) Cellular
(3) **Substrate identification and validation.**
  Substrates include:
  (a) Specific mRNAs, tRNAs and other RNAs
  (b) Translation-associated factors
  (c) Subcellular localization factors
  (d) Specific translational profiles (immunoribosomes, fidelity, etc.)

cryo-electron microscopy [59] and femtosecond X-ray crystallography [60] may enable visualization of individual ribosomes, thus allowing structural differences (and, in the latter case, different dynamic properties) to be discerned. Alternatively, emerging technologies are beginning to allow discernment of differences in the compositions of ribosomes in bulk samples isolated from different types of cells or cells subjected to different external challenges. For example, high-throughput rRNA sequencing (RiboMeth-seq) was used to reveal that 2′-O-methylation and pseudo-uridylation are sub-stoichiometric [61]; such heterogeneity may be indicative of specialized ribosomes [62]. Advances in mass spectrometry are also beginning to enable identification of differences among populations of ribosomes isolated from different cells and tissues [9,63–67].

Once homogenized, ribosomes must be characterized. As shown, in Fig. 1, this may be achieved along many different axes including (a) structural properties, (b) biochemical properties (e.g., affinities for various ligands, catalytic properties, decoding accuracy, speed and processivity) and (c) cellular properties (e.g., subcellular localization, cell type and developmental stage expression). As discussed above, the emerging cryo-electron microscopy and femtosecond X-ray crystallography appear to be emerging as the best ways to identify unique structural properties. Single-molecule translation systems [68–72] currently hold the best promise for matching individual ribosomes' specific biochemical activities. In contrast, cell-biology-based approaches may be problematic because of the uncertainty principle: methods used to identify unique ribosomes may alter their properties.

The third and last challenge is substrate identification and validation. "Substrate" can be either narrowly or broadly defined. A narrow definition may be limited to demonstrate that a specific type of ribosome is optimized to translate a specific mRNA or class of mRNAs. Slightly more broadly defined substrates may include tRNA isoacceptor species or different types of initiation, elongation, termination and recycling factors. Even more broadly, unique features that specify interactions with subcellular localization factors may be considered. Lastly, "substrate" may be intrinsic to the ribosome and its interaction with features in specific mRNAs. For example, "immunoribosomes" have been posited to generate defective ribosomal products in specialized subcellular compartments to enable efficient intracellular antigen presentation with major histocompatibility complex class I molecules [73,74]. Additionally, unpublished observations from our laboratory suggest that some ribosomes may be better than others at recognizing stop codons and shifting reading frame in response to specific *cis*-acting mRNA control elements.

Plot twist: If you can't beat 'em, join 'em

Throughout this essay, the issue of specialized ribosomes has been discussed from the point of view of a classical "discovery science". This conjures up the late 19th century romantic image of heroic explorers braving the hazards of nature in the quest to unveil its mysteries. However, as discoveries have accumulated and we have gained deeper understanding of nature's inner workings, we are increasingly adroit in manipulating nature to our own ends. This is the view of the technologist, who does not care whether or not something naturally exists as long as it can be synthesized. Genetic methods have long been used to create "designer" ribosomes in living cells. For example, pure populations of mutant ribosomes can be synthesized in cells lacking chromosomal rDNA genes [75,76], and orthogonal systems have been constructed enabling one type of synthetic ribosome to utilize one specific isoacceptor tRNA [77,78]. A fascinating recent technological breakthrough was the development of a

"monopartite" ribosome in which the subunits are tethered together [79]; this will allow the engineering of ribosomes with new functions and the development of orthogonal genetic systems. Thus, should the process of discovering and validating specialized ribosomes prove too onerous, adopting the technologist's mindset may be just the recipe for maintaining one's sanity.

Acknowledgements

I wish to thank the organizers of the 10th EMBO Conference on Ribosome Synthesis that was held in Brussels this past August for inviting me to present a lecture on the topic of specialized ribosomes; their invitation was the genesis for this piece, hence the presumptuously tongue-in-cheek title. I wish to thank also Wolfgang Losert for helping with some of the calculations and for sharing his thoughts on quantitative modeling. I also extend my sincere thanks to members of the ribosome community, my laboratory and the reviewers for their constructive feedback. This work was supported by grants from the National Institutes of Health (R01GM117177 and R01HL119439).

Received 28 September 2015;
Received in revised form 23 December 2015;
Accepted 24 December 2015
Available online 4 January 2016

Keywords:
development;
rRNA;
translation;
evolution;
single molecule

†http://www.ncbi.nlm.nih.gov/SNP/.

References

[1] F.H.C. Crick, Biochemical activities of nucleic acids. The present position of the coding problem, Brookhaven Symp. Biol. 12 (1959) 35–39.
[2] A.M. Weinberg, Messenger RNA: Origins of a discovery, Nature 414 (2001) 485.
[3] L. Astrachan, E. Volkin, Properties of ribonucleic acid turnover in T2-infected *Escherichia coli*, Biochim. Biophys. Acta 29 (1958) 536–544.
[4] E. Volkin, L. Astrachan, J.L. Countryman, Metabolism of RNA phosphorus in *Escherichia coli* infected with bacteriophage T7, Virology 6 (1958) 545–555.
[5] E.P. Geiduschek, R. Haselkorn, Messenger RNA, Annu. Rev. Biochem. 38 (1969) 647–676.
[6] V. Ramakrishnan, The ribosome emerges from a black box, Cell 159 (2014) 979–984.
[7] S. Xue, M. Barna, Specialized ribosomes: A new frontier in gene regulation and organismal biology, Nat. Rev. Mol. Cell Biol. 13 (2012) 355–369.
[8] R. Mikkola, C.G. Kurland, Is there a unique ribosome phenotype for naturally occurring *Escherichia coli*? Biochimie 73 (1991) 1061–1066.
[9] D.T. Ladror, B.L. Frey, M. Scalf, M.E. Levenstein, J.M. Artymiuk, L.M. Smith, Methylation of yeast ribosomal protein S2 is elevated during stationary phase growth conditions, Biochem. Biophys. Res. Commun. 445 (2014) 535–541.
[10] A. Shirai, M. Sadaie, K. Shinmyozu, J. Nakayama, Methylation of ribosomal protein L42 regulates ribosomal function and stress-adapted cell growth, J. Biol. Chem. 285 (2010) 22448–22460.
[11] M. Kellis, B.W. Birren, E.S. Lander, Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*, Nature 428 (2004) 617–624.
[12] K.H. Wolfe, D.C. Shields, Molecular evidence for an ancient duplication of the entire yeast genome, Nature 387 (1997) 708–713.
[13] J.C. Tercero, R.B. Wickner, MAK3 encodes an N-acetyltransferase whose modification of the L-A gag N-terminus is necessary for virus particle assembly, J. Biol. Chem. 267 (1992) 20277–20281.
[14] J.C. Tercero, J.D. Dinman, R.B. Wickner, Yeast MAK3 N-Acetyltransferase recognizes the N-terminal four amino acids of the major coat protein (gag) of the L-A double-stranded RNA virus, J. Bacteriol. 175 (1993) 3192–3194.
[15] N. Ban, R. Beckmann, J.H. Cate, J.D. Dinman, F. Dragon, S.R. Ellis, et al., A new system for naming ribosomal proteins, Curr. Opin. Struct. Biol. 24 (2014) 165–169.
[16] Y. Ohtake, R.B. Wickner, Yeast virus propagation depends critically on free 60S ribosomal subunit concentration, Mol. Cell. Biol. 15 (1995) 2772–2781.
[17] K. Carroll, R.B. Wickner, Translation and M1 dsRNA propagation: MAK18 = RPL41B and cycloheximide curing, J. Bacteriol. 177 (1995) 2887–2891.
[18] A. Dakshinamurthy, K.M. Nyswaner, P.J. Farabaugh, D.J. Garfinkel, BUD22 affects Ty1 retrotransposition and ribosome biogenesis in *Saccharomyces cerevisiae*, Genetics 185 (2010) 1193–1205.
[19] S. Komili, N.G. Farny, F.P. Roth, P.A. Silver, Functional specificity among ribosomal proteins regulates gene expression, Cell 131 (2007) 557–571.
[20] J.W. Bauer, C. Brandl, O. Haubenreisser, B. Wimmer, M. Weber, T. Karl, et al., Specialized yeast ribosomes: A customized tool for selective mRNA translation, PLoS One 8 (2013) e67609.
[21] N. Kondrashov, A. Pusic, C.R. Stumpf, K. Shimizu, A.C. Hsieh, S. Xue, et al., Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning, Cell 145 (2011) 383–397.
[22] F. Sahin, W. Qiu, R.E. Wilentz, C.A. Iacobuzio-Donahue, A. Grosmark, G.H. Su, RPL38, FOSL1, and UPP1 are predominantly expressed in the pancreatic ductal epithelium, Pancreas 30 (2005) 158–167.
[23] S. Xue, S. Tian, K. Fujii, W. Kladwang, R.B.M. Das, RNA regulons in Hox 5′UTRs confer ribosome specificity to gene regulation and body plan formation, Nature 517 (2015) 33–38.

[24] L. Chavatte, B.A. Brown, D.M. Driscoll, Ribosomal protein L30 is a component of the UGA-selenocysteine recoding machinery in eukaryotes, Nat. Struct. Mol. Biol. 12 (2005) 408–416.
[25] W. Dameshek, Riddle: What do aplastic anemia, paroxysmal nocturnal hemoglobinuria (PNH) and "hypoplastic" leukemia have in common? Blood 30 (1967) 251–254.
[26] N. Draptchinskaia, P. Gustavsson, B. Andersson, M. Pettersson, T.N. Willig, I. Dianzani, et al., The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia, Nat. Genet. 21 (1999) 169–175.
[27] D.M. Raiser, A. Narla, B.L. Ebert, The emerging importance of ribosomal dysfunction in the pathogenesis of hematologic disorders, Leuk. Lymphoma (2013)http://dx.doi.org/10.3109/10428194.2013.812786.
[28] J. Armistead, B. Triggs-Raine, Diverse diseases from a ubiquitous process: The ribosomopathy paradox, FEBS Lett. (2014)http://dx.doi.org/10.1016/j.febslet.2014.03.024.
[29] K.L. McCann, S.J. Baserga, Genetics. Mysterious ribosomopathies, Science 341 (2013) 849–850.
[30] K. De Keersmaecker, S.O. Sulima, J.D. Dinman, Ribosomopathies and the paradox of cellular hypo- to hyperproliferation, Blood 125 (2015) 1377–1382.
[31] A.P. Ross, K.S. Zarbalis, The emerging roles of ribosome biogenesis in craniofacial development, Front. Physiol. 5 (2014) 26.
[32] K. De Keersmaecker, Z.K. Atak, N. Li, C. Vicente, S. Patchett, T. Girardi, et al., Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia, Nat. Genet. 45 (2013) 186–190.
[33] G. Tzoneva, A. Perez-Garcia, Z. Carpenter, H. Khiabanian, V. Tosello, M. Allegretta, et al., Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL, Nat. Med. 19 (2013) 368–371.
[34] S. Rao, S.Y. Lee, A. Gutierrez, J. Perrigoue, R.J. Thapa, Z. Tu, et al., Inactivation of ribosomal protein L22 promotes transformation by induction of the stemness factor, Lin28B, Blood 120 (2012) 3764–3773.
[35] A.P. Novetsky, I. Zighelboim, D.M. Thompson, M.A. Powell, D.G. Mutch, P.J. Goodfellow, Frequent mutations in the RPL22 gene and its clinical and functional implications, Gynecol. Oncol. 128 (2013) 470–474.
[36] S.-C. Zhang, W. Jin, H. Liu, M.-J. Jin, Z.-X. Chen, Z.-Y. Ding, et al., RPSA gene mutants associated with risk of colorectal cancer among the Chinese population, Asian Pac. J. Cancer Prev. 14 (2013) 7127–7131.
[37] K. Wang, J. Kan, S.T. Yuen, S.T. Shi, K.M. Chu, S. Law, et al., Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer, Nat. Genet. 43 (2011) 1219–1223.
[38] L.S. Ludwig, H.T. Gazda, J.C. Eng, S.W. Eichhorn, P. Thiru, R. Ghazvinian, et al., Altered translation of GATA1 in Diamond-Blackfan anemia, Nat. Med. 20 (2014) 748–753.
[39] A. Yoon, G. Peng, Y. Brandenburger, O. Zollo, W. Xu, E. Rego, et al., Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita, Science 312 (2006) 902–906.
[40] C. Bellodi, M. Mcmahon, A. Contreras, D. Juliano, N. Kopmar, T. Nakamura, et al., H/ACA small RNA dysfunctions in disease reveal key roles for noncoding RNA modifications in hematopoietic stem cell differentiation, Cell Rep. 3 (2013) 1493–1502.
[41] L. Jenner, S. Melnikov, N.G. De Loubresse, A. Ben-Shem, M. Iskakova, A. Urzhumtsev, et al., Crystal structure of the 80S yeast ribosome, Curr. Opin. Struct. Biol. 22 (2012) 759–767.
[42] D. Piekna-Przybylska, W.A. Decatur, M.J. Fournier, The 3D rRNA modification maps database: With interactive tools for ribosome analysis, Nucleic Acids Res. 36 (2008) D178–D183.
[43] N. Fischer, A.L. Konevega, W. Wintermeyer, M.V. Rodnina, H. Stark, Ribosome dynamics and tRNA movement by time-resolved electron cryomicroscopy, Nature 466 (2010) 329–333.
[44] A. Dashti, P. Schwander, R. Langlois, R. Fung, W. Li, A. Hosseinizadeh, et al., Trajectories of the ribosome as a Brownian nanomachine, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 17492–17497.
[45] T.H. King, B. Liu, R.R. McCully, M.J. Fournier, Ribosome structure and activity are altered in cells lacking snoRNPs that form pseudouridines in the peptidyl transferase center, Mol. Cell 11 (2003) 425–435.
[46] J.L. Baxter-Roshek, A.N. Petrov, J.D. Dinman, Optimization of ribosome structure and function by rRNA base modification, PLoS One 2 (2007) e174.
[47] O. Rivoire, S. Leibler, A model for the generation and transmission of variations in evolution, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) E1940–E1949.
[48] X. Zhou, W.-J. Liao, J.-M. Liao, P. Liao, H. Lu, Ribosomal proteins: Functions beyond the ribosome, J. Mol. Cell Biol. 7 (2015) 92–104.
[49] T.-H. Kim, P. Leslie, Y. Zhang, Ribosomal proteins as unrevealed caretakers for cellular stress and genomic instability, Oncotarget 5 (2014) 860–871.
[50] D. Ruggero, Revisiting the nucleolus: From marker to dynamic integrator of cancer signaling, Sci. Signal. 5 (2012) pe38.
[51] K.A. Spriggs, M. Bushell, A.E. Willis, Translational regulation of gene expression during conditions of cell stress, Mol. Cell 40 (2010) 228–237.
[52] C. Deisenroth, Y. Zhang, Ribosome biogenesis surveillance: Probing the ribosomal protein-Mdm2-p53 pathway, Oncogene 29 (2010) 4253–4260.
[53] S. Kiparisov, A. Petrov, A. Meskauskas, P.V.V. Sergiev, O.a.A. Dontsova, J.D.D. Dinman, Structural and functional analysis of 5S rRNA, Mol. Genet. Genomics 27 (2005) 235–247.
[54] S. Chaudhuri, K. Vyas, P. Kapasi, A.a. Komar, J.D. Dinman, S. Barik, et al., Human ribosomal protein L13a is dispensable for canonical ribosome function but indispensable for efficient rRNA methylation, RNA 13 (2007) 2224–2237.
[55] K. Hosokawa, R.K. Fujimura, M. Nomura, Reconstitution of functionally active ribosomes from inactive subparticles and proteins, Proc. Natl. Acad. Sci. U.S.A. 55 (1966) 198–204.
[56] P. Nomura, M. Traub, Streptomycin resistance mutation in *Escherichia coli*: Altered ribosomal protein, Science 160 (1968) 198–199.
[57] P. Stelter, E. Hurt, A pulse-chase epitope labeling to study cellular dynamics of newly synthesized proteins: A novel strategy to characterize NPC biogenesis and ribosome maturation/export, Methods Cell Biol. 122 (2014) 147–163.
[58] A.A. Leonov, P.V. Sergiev, A.A. Bogdanov, R. Brimacombe, O.A. Dontsova, Affinity purification of ribosomes with a lethal G2655C mutation in 23S rRNA that affects the translocation, J. Biol. Chem. 278 (2003) 25664–26670.
[59] A. Bartesaghi, A. Merk, S. Banerjee, D. Matthies, X. Wu, J.L.S. Milne, et al., 2.2 Å resolution cryo-EM structure of β-galactosidase in complex with a cell-permeant inhibitor, Science 348 (2015) 1147–1151.
[60] R. Neutze, Opportunities and challenges for time-resolved studies of protein structural dynamics at X-ray free-electron

lasers, Philos. Trans. R. Soc. Lond. B Biol. Sci. 369 (2014) 20130318.
[61] U. Birkedal, M. Christensen-Dalsgaard, N. Krogh, R. Sabarinathan, J. Gorodkin, H. Nielsen, Profiling of ribose methylations in RNA by high-throughput sequencing, Angew. Chem. Int. Ed. Engl. 54 (2015) 451–455.
[62] D.L.J. Lafontaine, Noncoding RNAs in eukaryotic ribosome biogenesis and function, Nat. Struct. Mol. Biol. 22 (2015) 11–19.
[63] A.M. Popova, J.R. Williamson, Quantitative analysis of rRNA modifications using stable isotope labeling and mass spectrometry, J. Am. Chem. Soc. 136 (2014) 2058–2069.
[64] K.A. Wilson-Edell, A. Kehasse, G.K. Scott, C. Yau, D.E. Rothschild, B. Schilling, et al., RPL24: A potential therapeutic target whose depletion or acetylation inhibits polysome assembly and cancer cell growth, Oncotarget 5 (2014) 5165–5176.
[65] C. Loenarz, R. Sekirnik, A. Thalhammer, W. Ge, E. Spivakovsky, M.M. Mackeen, et al., Hydroxylation of the eukaryotic ribosomal decoding center affects translational accuracy, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 4019–4024.
[66] M.L. Falcone Ferreyra, R. Casadevall, M.D. Luciani, A. Pezza, P. Casati, New evidence for differential roles of l10 ribosomal proteins from *Arabidopsis*, Plant Physiol. 163 (2013) 378–391.
[67] N. Slavov, S. Semrau, E. Airoldi, B. Budnik, O.A. Van, Differential stoichiometry among core ribosomal proteins, Cell Rep. 13 (2015) 865–873.
[68] J. Chen, A. Petrov, A. Tsai, S.E. O'Leary, J.D. Puglisi, Coordinated conformational and compositional dynamics drive ribosome translocation, Nat. Struct. Mol. Biol. 20 (2013) 718–727.
[69] C.E. Aitken, A. Petrov, J.D. Puglisi, Single ribosome dynamics and the mechanism of translation, Annu. Rev. Biophys. 39 (2010) 491–513.
[70] M.C. Capece, G.L. Kornberg, A. Petrov, J.D. Puglisi, A simple real-time assay for *in vitro* translation, RNA 21 (2014) 296–305.
[71] S. Yan, J.-D. Wen, C. Bustamante, I. Tinoco, Ribosome excursions during mRNA translocation mediate broad branching of frameshift pathways, Cell 160 (2015) 870–881.
[72] G. Rosenblum, B.S. Cooperman, Engine out of the chassis: Cell-free protein synthesis and its uses, FEBS Lett. 588 (2014) 261–268.
[73] J.W. Yewdell, DRiPs solidify: Progress in understanding endogenous MHC class I antigen processing, Trends Immunol. 32 (2011) 548–558.
[74] L.C. Antón, J.W. Yewdell, Translating DRiPs: MHC class I immunosurveillance of pathogens and tumors, J. Leukoc. Biol. 95 (2014) 551–562.
[75] H.H. Wai, L. Vu, M. Oakes, M. Nomura, Complete deletion of yeast chromosomal rDNA repeats and integration of a new rDNA repeat: Use of rDNA deletion strains for functional analysis of rDNA promoter elements *in vivo*, Nucleic Acids Res. 28 (2000) 3524–3534.
[76] R. Rakauskaite, J.D. Dinman, Mutations of highly conserved bases in the peptidyltransferase center induce compensatory rearrangements in yeast ribosomes, RNA 17 (2011) 855–864.
[77] T.L. Hendrickson, V. De Crécy-Lagard, P. Schimmel, Incorporation of nonnatural amino acids into proteins, Annu. Rev. Biochem. 73 (2004) 147–176.
[78] B.J. Des Soye, J.R. Patel, F.J. Isaacs, M.C. Jewett, Repurposing the translation apparatus for synthetic biology, Curr. Opin. Chem. Biol. 28 (2015) 83–90.
[79] C. Orelle, E.D. Carlson, T. Szal, T. Florin, M.C. Jewett, A.S. Mankin, Protein synthesis by ribosomes with tethered subunits, Nature 524 (2015) 119–124.