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Subtractional Heterogeneity: A Crucial Step toward Defining Specialized Ribosomes

Joseph W. Briggs[1] and **Jonathan D. Dinman**[1,*]

[1]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA

Abstract

In this issue of *Molecular Cell*, Shi et al. (2017) identify translating ribosomes which lack specific proteins and associate with specific classes of mRNAs. This challenges the popular conception of "the ribosome" as a homogeneous, monolithic molecular machine.

We refer to "the ribosome" as if all ribosomes in every cell in the body are identical. It is not clear how or why this idea became so ingrained. Indeed, from the first demonstration of somatic and oocyte versions of 5S ribosomal RNA (rRNA) in *Xenopus* (Korn and Brown, 1978) to a recent paper describing different maternal and somatic rRNAs in zebrafish (Locati et al., 2017), evidence of rRNA heterogeneity abounds. Signs of rRNA functional heterogeneity also run deep (Kiparisov et al., 2005), and the description of substoichiometric rRNA modification in normal cells (Popova and Williamson, 2014) lends further credence to this idea. A class of diseases called "ribosomopathies" (reviewed in Farley and Baserga, 2016) and the finding that specific ribosomal proteins (RPs) are required for translation of certain homeobox mRNAs (Kondrashov et al., 2011) also points to the RPs in generating ribosome heterogeneity. These and other studies have led to the proposal that cells contain specialized ribosomes (Xue and Barna, 2012), along with the complementary concept of a ribosome population biology (Dinman, 2016). In this issue of *Molecular Cell*, Shi and co-workers (2017) identify populations of ribosomes in murine embryonic stem cells (mESCs) lacking particular ribosomal proteins tasked for translation of specific mRNA subsets. These results support the idea that ribosome heterogeneity is employed to control the translation of classes of mRNA that are involved in distinct biological pathways.

Actively translating ribosomes harvested from mESCs were analyzed by a state-of-the-art mass spectroscopic protocol called selected reaction monitoring (SRM)-based proteomics. The method works by spiking experimental samples with known quantities of heavy isotope labeled standards to generate highly quantitative data on absolute protein abundance. This application identified four ribosomal proteins, two each from the small (40S) and large (60S) subunits present in substoichiometric quantities. In parallel, siRNA knockdown was used to validate the sensitivity of the SRM-based proteomics approach. Alternative approaches, including tandem mass tagging and formaldehyde crosslinking, were used to ensure that RPs were not being stripped off during purification. These results matched the SRM data and

*Correspondence: dinman@umd.edu.

provided strong orthogonal supporting evidence for the existence of naturally occurring ribosomes lacking these proteins.

Given the existence of actively translating heterogeneous populations of ribosomes, do they have special functions? CRISPR/Cas-9-mediated genome editing was employed to epitope tag one small subunit (RPS25/eS25) and one large subunit (RPL10/euL1) RP, and the mRNAs translated by these ribosomes were identified and quantified by a clever combination of affinity purification and ribosome profiling. Comparison of these datasets with data obtained from polysome profiling of total mESC mRNAs identified sets of mRNAs that were enriched or depleted in an RP-specific manner. The very small degree of overlap between the two sets provides additional support for the idea of specialization. Thus, like Monty Python's Black Knight, ribosomes lacking specific body parts can keep on fighting (Figure 1); indeed, they appear to be used by cells to control gene expression.

RPL10A/uL1 was of particular interest because of its location in the mRNA exit tunnel and its demonstrated interactions with a viral internal ribosomal entry site (IRES) element. siRNA studies demonstrated reduced translation of two well-characterized viral IRES elements, and analysis of theRPL10A/uL1-enriched transcript datasets identified a number of known IRES-containing mouse mRNAs. These findings suggest that translation of at least a subset of specific mRNAs may be regulated by controlling the amount of RPL10A/uL1 containing ribosomes. Other *cis*-acting mRNA elements requiring specific RPs are known; e.g., selenocysteine insertion sequence elements require eL30 (Donovan and Copeland, 2010). Moving forward, a true challenge will lie in identifying and characterizing novel classes of mRNAs specialized to interact with specific types of ribosomes. Further, an alternate viewpoint may be that the specialization lies with the mRNAs, i.e., that they are specialized to be translated by ribosomes containing or missing some component. In the end, this is merely a semantic distinction. The important takeaways are (1) the demonstration that cells naturally harbor heterogeneous ribosome populations and (2) their differential translation of specific classes of mRNAs.

This study raises a plethora of important questions that will have strong impact across a wide range of fields. For example, how can a ribosome missing a RP be construed as "specialized," as opposed to "defective"? We tend to think of specialization as addition to the default state. For example, all people are, by default, people, but adding a uniform and badge marks one as specialized for law enforcement. In contrast, molecular genetic systems are counterintuitive insofar as they tend to favor negative regulation, e.g., anti-termination, repression, and de-repression. Indeed, an abnormal state such as cancer is characterized by the loss of negative control. Viewed from this perspective, it is not unthinkable that ribosome specialization could be achieved via subtraction.

The fact that the four RPs map to solvent-exposed surfaces of the ribosome raises an additional question: might they be inserted into and removed from preexisting ribosomes, i.e., are ribosomes adaptive? This line of inquiry poses an entirely new set of questions: What, if any, *trans*-acting factors are required? What is their composition? Where are they stored, assembled, and dissembled? Does this kind of ribosome modification occur in any specific subcellular compartment? What are the extra- and intracellular signaling pathways

controlling this phenomenon? Can defects in any of these be linked to specific pathologies? Additionally, one wonders if the cryo-EM community has ignored such ribosomes on the presumption that they are defective; it may be worthwhile to reexamine the original, unfiltered data, for the presence of classes of ribosomes lacking specific RPs. Finally, a particularly salient feature of the current study is that the authors generated affinity-tagged ribosomes in mESCs. These (and presumably additional lines to follow) constitute the foundations for generating transgenic mice. Such tools will drive the exponential expansion of our understanding of the role of ribosome specialization in mammalian development and disease.

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Figure 1. The Ribosome Represented as Monty Python's Black Knight

Despite the loss of specific ribosomal proteins, ribosomes can translate certain classes of mRNAs. Hence, specialization may be accomplished through a subtractive process.