The ribosome epitranscriptome: inert—or a platform for functional plasticity?

JOSEPH GEORGESON and SCHRAGA SCHWARTZ

Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 7610001, Israel

ABSTRACT

A universal property of all rRNAs explored to date is the prevalence of post-transcriptional (“epitranscriptional”) modifications, which expand the chemical and topological properties of the four standard nucleosides. Are these modifications an inert, constitutive part of the ribosome? Or could they, in part, also regulate the structure or function of the ribosome? In this review, we summarize emerging evidence that rRNA modifications are more heterogeneous than previously thought, and that they can also vary from one condition to another, such as in the context of a cellular response or a developmental trajectory. We discuss the implications of these results and key open questions on the path toward connecting such heterogeneity with function.

Keywords: epitranscriptome; modifications; rRNA; review; ribosome

Ribosomes are massive macromolecular complexes, often conceptualized as uniform entities and “factories” of protein translation. The assumption that ribosomes are an invariable homogenous entity suggests that they lack an inherent ability of *regulating* the translational output of a cell or organism. In recent years, however, it has become clear that ribosomes are considerably more heterogeneous than had previously been thought. Specifically, it was shown that ribosomes can differ in their primary ribosomal RNA (rRNA) sequence, in the composition of ribosomal proteins, as well as in the post-translational modification profiles of the ribosomal proteins (López-López et al. 2007; Slavov et al. 2015; Brown et al. 2017; Shi et al. 2017; Simsek et al. 2017; Fujii et al. 2018; Małeck et al. 2021); this has been extensively reviewed (Sergiev et al. 2011; Xue and Barna 2012; Kobayashi 2014; Sloan et al. 2016a; Bates et al. 2018; Genuth and Barna 2018a,b; Gerst 2018; Ferretti and Karbstein 2019). An emerging layer of heterogeneity between ribosomes lies in diverse post-transcriptional modifications to which the rRNA is subjected. This layer, and its potential for regulating the functional properties of the ribosome, form the topic of this mini-Review.

rRNA MODIFICATIONS: DISTRIBUTION AND BIOGENESIS

Ribosomes are composed of a highly conserved catalytic rRNA core and dozens of auxiliary proteins. A universal

property of all rRNAs explored to date is the prevalence of post-transcriptional (“epitranscriptional”) modifications, which expand the chemical and topological properties of the four standard nucleosides (Sharma and Lafontaine 2015; Sloan et al. 2016a). Dozens to hundreds of residues are modified across ribosomes from different domains of life, with *E. coli* rRNA harboring 36 modifications (Golovina et al. 2012), yeast 112 (Taoka et al. 2016), human 228 (Taoka et al. 2018), and the protist *Euglena gracilis* rRNA containing 350 modified sites (Schnare and Gray 2011). A core set of rRNA modifications is conserved across the three domains of life (Sergiev et al. 2018), and these modifications typically cluster around the functional centers of the ribosome (Ben-Shem et al. 2011; Sloan et al. 2016b). There is a remarkable diversity of modifications that adorn the ribosome, including diverse forms of methylation, acetylation, and pseudouridylation. In terms of density of modification patterns, rRNA is second only to tRNA.

rRNA modifications are catalyzed through a diverse set of RNA-modifying enzymes. The vast majority of enzymes directly recognize and modify their targets. In eukaryotes and archaea, a subset of RNA modifying enzymes are recruited to their targets via small nucleolar RNAs (snoRNAs), which harbor stretches of complementarity toward specific rRNA targets and thereby guide the modifying enzyme into place (Kiss-László et al. 1996; Ganot et al. 1997; Ni et al. 1997; Sharma et al. 2017b). The appearance of snoRNAs in evolution correlates with a dramatic

Corresponding author: schwartz@weizmann.ac.il

Article is online at <http://www.rnajournal.org/cgi/doi/10.1261/rna.078859.121>. Freely available online through the RNA Open Access option.

© 2021 Georgeson and Schwartz This article, published in *RNA*, is available under a Creative Commons License (Attribution-NonCommercial 4.0 International), as described at <http://creativecommons.org/licenses/by-nc/4.0/>.

expansion of rRNA modifications guided by them, primarily pseudouridine and ribose methylations (Lafontaine and Tollervey 1998). This may be due to the relative ease with which snoRNAs can evolve or duplicate and acquire new targets, in comparison to the greater difficulty of an entire enzyme evolving new specificity without compromising its catalytic activity.

rRNA MODIFICATIONS: FUNCTIONS

Although the first rRNA modifications were already discovered six decades ago (Lane and Allen 1961; Lane 2014), and have been subjected to considerable investigation ever since, their functions are understood only to a limited extent. We currently lack a systematic understanding of which properties are conferred via which modifications, and how. Nonetheless, it is becoming increasingly clear that there is no single function of rRNA modifications. This need not come as a surprise, given the highly heterogeneous chemical nature of rRNA modifications, the widely distinct machineries giving rise to their formation, the different regions of the ribosome at which they are catalyzed, the variability in the relative timing of their deposition with respect to ribosome biogenesis and the different cellular compartments at which they are deposited.

Given the paramount importance of the ribosome, the universality of rRNA modifications across all domains of life, their enrichment at functional regions of the ribosome, and their high conservation between very distantly related species, it is to be expected that rRNA modifications would be critical components of cells, and that their absence results in dramatic phenotypes. Indeed, such is the case for a number of modifications, where for example in yeast, absence of a single modifying enzyme installing modifications at one of several sites required for 18S biogenesis is lethal (Lafontaine et al. 1994, 1995; Liang et al. 2009; Schilling et al. 2012; Peifer et al. 2013; Ito et al. 2014; Zorbas et al. 2015). Yet, genetic dissection of roles played by rRNA modifications have consistently given rise to two surprises. First, despite the high conservation of many modifications and associated modifying enzymes, their disruption often results in subtle or even indiscernible phenotypes. For example, elimination of one or even two modifications in helix 69 of the ribosome, which interacts with both A and P site tRNAs, resulted in no discernible phenotype (Liang et al. 2007). Only when three or more modifications were eliminated from this helix, did phenotypes become apparent. In one case, a lethal phenotype caused by deletion of an rRNA modifying enzyme involved in ribosome biogenesis could even be *rescued* by deletion of additional genes involved in rRNA modifications (Buchhaupt et al. 2006, 2007; García-Gómez et al. 2011). A second—related—surprise, consistently manifesting itself in yeast studies, is that catalytically defective rRNA modifying enzymes often give rise to much milder phenotypes than deletions of the entire

genes (Lafontaine et al. 1995, 1998; Zebardjian et al. 1999; Sardana and Johnson 2012; Peifer et al. 2013; Sharma et al. 2013a; Gigova et al. 2014; Zorbas et al. 2015; Liger et al. 2016; Shen et al. 2020). These studies suggest that in many cases rRNA modifications are dispensable (or partially so), whereas the rRNA modifying enzyme is not.

These insights from genetic studies coupled with extensive follow-ups have given rise to two broad roles of ribosome modifying enzymes—modification dependent functions and modification independent ones. A first set of roles, which is often at least partially modification independent, is in facilitating the multistep rRNA maturation. rRNA is typically transcribed as a single precursor, which is subsequently subject to complex exo- and endonucleolytic cleavage events, giving rise to the large and small rRNA subunits that concomitantly need to fold and assemble into their proper structure (Demirci et al. 2010; Polikanov et al. 2015; Jiang et al. 2016; Sloan et al. 2016a; Aubert et al. 2018; Birkedal et al. 2020). Loss-of-function assays have revealed that some rRNA modifying enzymes are required for this processing (Lafontaine et al. 1995; Liang et al. 2009; Sharma et al. 2013b) and their loss results in accumulation of ribosome precursors. The fact that rRNA modifying enzymes, but often not the modifications themselves, are required for mediating this role suggests that in these contexts the enzymes serve a scaffolding or chaperoning function, assisting in the proper folding of the ribosome (Lafontaine et al. 1995; Shen et al. 2020). Indeed, a general chaperoning function was recently also proposed for snoRNAs (Huang and Karbstein 2021). Such a modification-independent function of RNA modifying enzymes is reminiscent of modification-independent chaperone-like roles for tRNA modifying enzymes. Among the best characterized examples for this is TruB, a highly conserved tRNA pseudouridine synthase, installing pseudouridine at position 55 of tRNA. Remarkably, studies in bacteria found that the catalytic activity of this enzyme is dispensable for bacterial fitness, whereas the RNA-binding domain of this enzyme is essential for proper folding and aminoacylation of the tRNA (Gutgsell et al. 2000; Keffer-Wilkes et al. 2016), strongly suggesting that the primary function of this enzyme is to chaperone the folding of the tRNA. A second set of roles, dependent on the modifications, is in facilitating RNA:RNA or RNA:protein contacts between the key components of the ribosome—rRNA, tRNA, mRNA, and proteins (Sergiev et al. 2011; Polikanov et al. 2015; Sharma and Lafontaine 2015; Sas-Chen et al. 2020). In principle, both modification-dependent and independent roles can manifest in the broad range of functional outcomes associated with disruption of diverse rRNA modifying enzymes, including aberrant assembly, aberrant structures, aberrant translational activity, reduced translational output, reduced amino acid incorporations, increased stop codon readthrough, and modulation of frameshift rate (King et al. 2003; Liang et al. 2007, 2009;

Baudin-Baillieu et al. 2009; Sloan et al. 2016a; Sergiev et al. 2018). Yet, due to the subtlety of the phenotypes and the difficulties in dissecting them, the functions of most modifications remain to a large extent elusive (Lafontaine et al. 1995; Phillips and de Crécy-Lagard 2011; Spenkuch et al. 2014; Sharma and Lafontaine 2015; Popis et al. 2016; Ayadi et al. 2019).

HETEROGENEOUS AND DYNAMIC rRNA MODIFICATIONS

In recent years, compelling evidence has accumulated for heterogeneity in ribosome composition at various levels. In zebrafish, ribosomes are expressed from two separate genomic loci, differing significantly in sequence, whereby one locus serves for transcription of maternal rRNA and the other for zygotic rRNA (Locati et al. 2017). Similarly, in *Plasmodium falciparum*, different diverging copies of rRNA are encoded, one of which is utilized during the mosquito-stage and another during the human-stage of the infection (Rogers et al. 1996; Vembar et al. 2016). At the level of protein composition, compelling evidence has emerged that ribosomes lacking specific ribosomal proteins exist within mouse embryonic stem cells (Slavov et al. 2015; Shi et al. 2017), yeast (Ferretti et al. 2017; Collins et al. 2018; Samir et al. 2018) and bacteria (Loveland et al. 2016), though questions remain as to the functionality of such ribosomes. Moreover, there are numerous amino acids within ribosomal proteins that accommodate post-translational modifications, affecting stability, structure, localization, and function (Hornbeck et al. 2015; Simsek and Barna 2017; Emmott et al. 2019; Li and Wang 2020). Many post-translational modifications are heterogeneous, and are responsive to stimuli like growth signals (Imami et al. 2018), and immune response (Mukhopadhyay et al. 2008), often leading to preferential translation of mRNAs. Protein and rRNA paralogs provide yet another source of heterogeneity, where gene duplication has resulted in very similar or identical isoforms but expressed from different genomic loci (Gerst 2018; Segev and Gerst 2018; Nurk et al. 2021).

These discoveries, combined with advances in genomic and mass-spectrometry based approaches for systematically measuring RNA modifications, have spurred explorations into the extent of heterogeneity of rRNA modifications. Such heterogeneity is of interest at two levels: First, how heterogeneous is an rRNA modification *within* a specific condition, that is, what is the stoichiometry of that modification in the ribosomes. Second, how heterogeneous is an rRNA modification *between* samples, for example, across different conditions, stimuli or pathological states. These two levels are not completely unrelated: sites that are substoichiometric within a sample also tend to change across samples (Sharma and Lafontaine 2015; Ayadi et al. 2019). To date, both dimensions have been

sampled relatively sparsely, and our knowledge is hence partial at best. Below we review some of the key themes that have been uncovered to date.

1. **Less than half of modified sites in yeast and human ribosomes are substoichiometric:** To date, most studies have focused primarily on yeast and human ribosomes. Quantitative mass-spectrometry based measurements revealed that in yeast, 12 (of 112) sites have stoichiometries ranging from 50%–80% and 28 sites from 80%–95%. In addition, one site (25S:U2345) can be either modified with a ribose methylation or with a pseudouridine or both (Taoka et al. 2016), highlighting that heterogeneity can stem from a single position harboring multiple modification types. In human the heterogeneity is even more dramatic, whereby almost half the sites are modified at substoichiometric levels: 22 (of 228) sites are modified at levels ranging from 5%–49%, 23 sites from 50%–79%, and 64 sites falling in the range of 80%–95% (Taoka et al. 2018). Similar findings, pertaining to a subset of sites exhibiting substoichiometric pseudouridines and 2'-O methylation sites were found by later studies (Sharma et al. 2017a; Marchand et al. 2020).
2. **A minority of modified sites exhibit changes in modification levels across different conditions in human and yeast:** Variability in rRNA modification levels across conditions have been observed, to date, in a minority of instances in human and yeast. In a study in *S. pombe*, modification stoichiometries were sampled via mass-spectrometry across a range of different temperatures, and of the 40 sites that could be quantified across all temperatures, six sites displayed a >20% difference in stoichiometries across conditions, all of which harbor pseudouridines (Taoka et al. 2015). An analysis of pseudouridine on rRNA during human chondrogenic differentiation revealed that a small subset of sites displayed relatively moderate changes in stoichiometry over the course of this process. The same study reported that typically subtle changes in pseudouridylation levels were present across many sites, when comparing fibroblasts, HEK293, and HeLa cells (Marchand et al. 2020). Another recent study observed a change in pseudouridylation levels of one site during stem cell differentiation, corresponding to induced levels of the snoRNA targeting it (McCann et al. 2020). Differences in ribose methylation levels were also observed in p53 knockout models, whereupon 13 (of 106) sites in human were hypomodified in comparison to WT (Sharma et al. 2017a). Furthermore, it was recently observed that many types of cancer show substoichiometric levels of the highly conserved hypermodified base m1acp3Y (Babaian et al. 2020). Finally, it was found that the universally conserved tandem adenosines at the 3' end

of 18S rRNA, thought to be constitutively di-methylated (m6,6A), can also be mono-methylated (m6A), and that the extent of monomethylation versus dimethylation is regulated by sulfur levels (Liu et al. 2021).

3. **The majority of substoichiometric and “dynamic” sites are ribose methylations and pseudouridines:** A shared finding in many of the above studies is that the majority of sites reported to be either “substoichiometric” or “dynamically regulated” in human and yeast are modified either with pseudouridine or with 2'-O-methylation. To some extent, this mirrors the relative abundance of these modifications, and that the techniques used by some studies were directed exclusively against these modifications and hence blind to all others. Nonetheless, this conclusion is also based on mass-spectrometry based approaches, which do not suffer from these limitations, and hence may suggest that these two modifications may be inherently less “constitutive” in human and yeast. Given that these two modifications are both guided by snoRNAs, it is tempting to speculate that the substoichiometric modifications associated with a subset of them may reflect this snoRNA-mediated biogenesis. By separating the catalytic from the targeting machinery, individual snoRNAs may have acquired more flexibility in evolving optimal affinities toward their targets (whereby “optimal” can, at times, also be substoichiometric) and may have attained more freedom in evolving optimal expression levels for their individual targets. With respect to the latter, snoRNAs certainly provide a *potential* platform via which rRNA modifications could be controlled, given that dramatic variations in snoRNA levels have been observed between different tissues, stimuli and disease states (Jorjani et al. 2016; Gong et al. 2017; Warner et al. 2018; McCann et al. 2020). However, only few studies have linked such heterogeneous expression with differences in modification levels (Khoshnevis et al. 2019; McCann et al. 2020). This notwithstanding, additional mechanisms for achieving substoichiometric levels of these modifications have been documented. Depletion of fibrillarin—the rRNA ribose methyltransferase—impacts methylation of different sites in varying ways, establishing that alterations of fibrillarin levels could serve as a potential mechanism for achieving heterogeneous levels of methylation across sites (Erales et al. 2017; Sharma et al. 2017a). It was also shown that methylation at a subset of sites requires the nucleolar protein Nucleophosmin (NPM1), and hence disruption of NPM1, as occurs in dyskeratosis congenita, a rare bone marrow disease, can lead to substoichiometric modification of its targets (Nachmani et al. 2019). Heterogeneity in rRNA modifications was also shown to be associated with the relative timing at which a modification is deposited, with modifications arriving late in

ribosome biogenesis being more prone to substoichiometric levels (Birkedal et al. 2015). In this context, it is important to rule out that the observed heterogeneity is present in mature ribosomes and does not merely reflect the relative composition of mature and immature ribosomes in a sample.

4. **Two examples for dramatic and systematic changes in rRNA modification levels:** As indicated above, in human and yeast, differences in modification levels of rRNA across conditions are relatively rare and often subtle. Two cases have been reported, to date, in which rRNA modifications are dramatically and systematically altered in response to an environmental cue. *Trypanosoma brucei*, the parasitic kinetoplastid responsible for widespread sleeping sickness in sub-Saharan Africa, was found to have life-cycle dependent rRNA pseudouridylation patterns. In total, 68 pseudouridine modifications were identified on rRNA in both the procyclic and bloodstream forms, and 21 of these sites were hypermodified (>1.3-fold) during the bloodstream form, during which the corresponding H/ACA snoRNAs were also induced (Chikne et al. 2016). It is thought that this is a developmentally regulated adaptation resulting from the 10°C difference between the tsetse fly vector and human host, and interestingly enough the sites cluster around functional domains of the ribosome. The second case, discovered in our laboratory, is a dramatic induction of cytidine acetylation (ac4C) in ribosomes of an archaeal hyperthermophile in response to increased growth temperatures. *T. kodakarensis* ribosomes grown under 55°C are modified at only seven sites whereas under optimal growth conditions of 85°C the ribosomes undergo acetylation at >170 sites. Consistently, loss of the single acetyltransferase enzyme, required for acetylation of all sites, led to growth defects at higher—but not at lower—temperatures. Acetylation at a minority of the target sites contributed to RNA:RNA and RNA:protein interactions, or to interactions with solvents, whereas the vast majority of sites were proposed to contribute to thermostabilization of the RNA structure at higher temperature (Sas-Chen et al. 2020). Common to both cases is that a single modification was found to be dramatically and systematically induced across the majority of harboring sites.

HORIZONS

Our understanding pertaining to rRNA modifications is by and large limited to few modifications, few species, and few conditions. Many fundamental questions thus remain wide open. Key questions include the following:

1. **Extent of heterogeneity:** To what extent are substoichiometric modifications an exception or a rule? And

to what extent are dynamically modulated rRNA modifications an exception or a rule? How abundant are these phenomena across evolution? And how abundant are they across different tissues or across physiological and pathological responses? We anticipate that exploration of these dimensions will be hugely facilitated by the ever-expanding arsenal of genomic methodologies for assaying distinct modifications (Schaefer et al. 2009; Meyer et al. 2012; Ryvkin et al. 2013; Carlile et al. 2014; Schwartz et al. 2014a; Birkedal et al. 2015; Hauenschild et al. 2015; Linder et al. 2015; Zheng et al. 2015; Marchand et al. 2016; Dai et al. 2017; Li et al. 2017; Safra et al. 2017; Marchand et al. 2018; Enroth et al. 2019; Lin et al. 2019; Pandolfini et al. 2019; Zhang et al. 2019; Sas-Chen et al. 2020).

2. **Rules of heterogeneity:** Can rules be defined pertaining to which modifications are prone to be substoichiometric or regulated, versus constitutive? Are certain modifications more prone to be so than others? Might modifications catalyzed by snoRNAs be more heterogeneous than ones catalyzed by site specific enzymes? Are modifications within certain domains of the ribosome more prone to be regulated or substoichiometric? In our studies into dynamic ac4C in archaea, we found, for example, that a small subset of sites clustered around functional regions of the ribosome were invariably acetylated across all temperatures, typically at relatively high stoichiometries, whereas the vast majority of the remaining sites were distributed randomly throughout the ribosome and only catalyzed, typically at lower stoichiometries, under higher temperatures (Sas-Chen et al. 2020). Similarly, in an analysis of substoichiometric 2'-O methylated sites it was found that nucleotides participating directly in translation at the A- and P-sites, intersubunit bridges, and peptide exit tunnel were susceptible to variation in methylation, while the peptidyl transferase center and decoding center were not affected (Erales et al. 2017; Sharma et al. 2017a). These findings suggest that there may be a structurally coherent set of constitutive modifications.
3. **Sources of heterogeneity:** What regulates heterogeneity in rRNA modifications? Why are some sites substoichiometric? What gives rise to changes in rRNA modifications across different conditions? Is this due to changes in the levels of modifying enzymes? Or in their activity? Or also due to variability in the accessibility of the rRNA between conditions? In assessing these questions it will be critical to consider the populations of ribosomes in which such heterogeneity is observed. Is heterogeneity observed in total RNA? Or in rRNA purified from polysomes? In the case of the former, such heterogeneity can potentially also reflect a mixture of mature and immature ribosomes, modified at varying levels.

4. **Consequences of heterogeneity:** Arguably the most important question is whether differentially modified ribosomes give rise to differential functions. Such functionality should ideally be established at multiple levels, among which are the structural level (how does the modification impact rRNA structure), the molecular level (how does the modification impact the catalytic properties of the ribosome) and the phenotypic level (what fitness benefit is provided by the modification), with the ultimate, highly challenging goal of drawing a causal, connecting line between these three layers. The challenges of drawing such a causal line from heterogeneity to function is by no means unique to rRNA modifications. Indeed, in the vast majority of cases in which ribosome heterogeneity has been unequivocally observed, directly linking such heterogeneity to a function has proven to be challenging (for review, see Ferretti and Karbstein 2019). Such difficulties are a consequence of the complex nature of the ribosome and of its processing and assembly pathways, rendering it highly challenging to address functions *in vitro*, but also limiting the conclusions that can be drawn from *in vivo* studies. One powerful game-changer in recent years are the major leaps in cryoEM (Kirmizialtin et al. 2015; Shalev-Benami et al. 2016; Natchiar et al. 2017; Nikolay et al. 2021), permitting the relatively rapid acquisition of ribosome structures at low Å resolutions.
5. **Evolution of heterogeneity:** Finally, a fascinating dimension to explore is how rRNA modifications in general, and heterogeneous modifications in particular, evolved over the course of evolution. How did they emerge? How did the machineries regulating them emerge? From an evolutionary perspective, are substoichiometric or dynamic modifications relatively recently acquired sets of modifications that have not yet undergone fixation? Or, in contrast, did they originate from evolutionary-fixed stoichiometric modifications, and evolve to become heterogeneous over time? A comprehensive dissection of these questions will require measurements of rRNA modifications across a wide set of species. Such endeavors, conducted via cryoEM and mass-spectrometry based approaches (Taoka et al. 2016, 2018), are likely to also give rise to discoveries of new rRNA modifications, of which our knowledge is likely still incomplete, as suggested by the continuous discovery of new forms of modifications (Boccalletto et al. 2018; Flynn et al. 2021).

From a historical perspective, it is surprising that investigations into substoichiometric and dynamically modified modifications on ribosomes are somewhat lagging behind with respect to their counterparts on mRNA (Schwartz et al. 2014b; Darnell et al. 2018), given that modifications on rRNA were discovered and explored decades before their

mRNA counterparts. To some extent, this may reflect the different disciplines, and associated philosophies, through which these modifications were studied: rRNA modifications were classically studied through the lens of structure and of post-transcriptional processing, whereas mRNA modifications—coined “RNA epigenetics” (He 2010) and the “epitranscriptome” (Meyer et al. 2012)—were intuitively connected with “epigenetics,” wherein key components are dynamics and reversibility. We anticipate that new insights into ribosome heterogeneity in recent and forthcoming years, combined with the advent of new tools for systematically interrogating rRNA modifications, will allow revisiting this exciting field, pertaining to the regulatory potential of the core translational apparatus.

ACKNOWLEDGMENTS

We acknowledge support from the Israel Science Foundation (grant no. 543165).

REFERENCES

- Aubert M, O'Donohue M-F, Lebaron S, Gleizes P-E. 2018. Pre-ribosomal RNA processing in human cells: from mechanisms to congenital diseases. *Biomolecules* **8**: 123. doi:10.3390/biom8040123
- Ayadi L, Galvanin A, Pichot F, Marchand V, Motorin Y. 2019. RNA ribose methylation (2'-O-methylation): occurrence, biosynthesis and biological functions. *Biochim Biophys Acta Gene Regul Mech* **1862**: 253–269. doi:10.1016/j.bbagrm.2018.11.009
- Babaian A, Rothe K, Girodat D, Minia I, Djondovic S, Milek M, Spencer Miko SE, Wieden HJ, Landthaler M, Morin GB, et al. 2020. Loss of m¹ acp³ Ψ ribosomal RNA modification is a major feature of cancer. *Cell Rep* **31**: 107611. doi:10.1016/j.celrep.2020.107611
- Bates C, Hubbard SJ, Ashe MP. 2018. Ribosomal flavours: an acquired taste for specific mRNAs? *Biochem Soc Trans* **46**: 1529–1539. doi:10.1042/BST20180160
- Baudin-Baillieu A, Fabret C, Liang X-H, Piekna-Przybylska D, Fournier MJ, Rousset J-P. 2009. Nucleotide modifications in three functionally important regions of the *Saccharomyces cerevisiae* ribosome affect translation accuracy. *Nucleic Acids Res* **37**: 7665–7677. doi:10.1093/nar/gkp816
- Ben-Shem A, de Loubresse NG, Melnikov S. 2011. The structure of the eukaryotic ribosome at 3.0 Å resolution. *Science* **334**: 1524–1529. doi:10.1126/science.1212642
- Birkedal U, Christensen-Dalsgaard M, Krogh N, Sabarinathan R, Gorodkin J, Nielsen H. 2015. Profiling of ribose methylations in RNA by high-throughput sequencing. *Angew Chem Int Ed Engl* **54**: 451–455. doi:10.1002/anie.201408362
- Birkedal U, Beckert B, Wilson DN, Nielsen H. 2020. The 23S ribosomal RNA from *Pyrococcus furiosus* is circularly permuted. *Front Microbiol* **11**: 582022. doi:10.3389/fmicb.2020.582022
- Boccalletto P, Machnicka MA, Purta E, Piatkowski P, Baginski B, Wirecki TK, de Crécy-Lagard V, Ross R, Limbach PA, Kotter A, et al. 2018. MODOMICS: a database of RNA modification pathways. 2017 update. *Nucleic Acids Res* **46**: D303–D307. doi:10.1093/nar/gkx1030
- Brown CW, Sridhara V, Boutz DR, Person MD, Marcotte EM, Barrick JE, Wilke CO. 2017. Large-scale analysis of post-translational modifications in *E. coli* under glucose-limiting conditions. *BMC Genomics* **18**: 301. doi:10.1186/s12864-017-3676-8
- Buchhaupt M, Meyer B, Kötter P, Entian K-D. 2006. Genetic evidence for 18S rRNA binding and an Rps19p assembly function of yeast nucleolar protein Nep1p. *Mol Genet Genomics* **276**: 273–284. doi:10.1007/s00438-006-0132-x
- Buchhaupt M, Kötter P, Entian K-D. 2007. Mutations in the nucleolar proteins Tma23 and Nop6 suppress the malfunction of the Nep1 protein. *FEMS Yeast Res* **7**: 771–781. doi:10.1111/j.1567-1364.2007.00230.x
- Carlile TM, Rojas-Duran MF, Zinshteyn B, Shin H, Bartoli KM, Gilbert WV. 2014. Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. *Nature* **515**: 143–146. doi:10.1038/nature13802
- Chikne V, Doniger T, Rajan KS, Bartok O, Eliaz D, Cohen-Chalamish S, Tschudi C, Unger R, Hashem Y, Kadener S, et al. 2016. A pseudouridylation switch in rRNA is implicated in ribosome function during the life cycle of *Trypanosoma brucei*. *Sci Rep* **6**: 25296. doi:10.1038/srep25296
- Collins JC, Ghalei H, Doherty JR, Huang H, Culver RN, Karbstein K. 2018. Ribosome biogenesis factor Ltv1 chaperones the assembly of the small subunit head. *J Cell Biol* **217**: 4141–4154. doi:10.1083/jcb.201804163
- Dai Q, Moshitch-Moshkovitz S, Han D, Kol N, Amariglio N, Rechavi G, Dominissini D, He C. 2017. Nm-seq maps 2'-O-methylation sites in human mRNA with base precision. *Nat Methods* **14**: 695–698. doi:10.1038/nmeth.4294
- Darnell RB, Ke S, Darnell JE Jr. 2018. Pre-mRNA processing includes N⁶ methylation of adenosine residues that are retained in mRNA exons and the fallacy of 'RNA epigenetics'. *RNA* **24**: 262–267. doi:10.1261/rna.065219.117
- Demirci H, Murphy F, Belardinelli R, Kelley AC, Ramakrishnan V, Gregory ST, Dahlberg AE, Jogi G. 2010. Modification of 16S ribosomal RNA by the KsgA methyltransferase restructures the 30S subunit to optimize ribosome function. *RNA* **16**: 2319–2324. doi:10.1261/rna.2357210
- Emmott E, Jovanovic M, Slavov N. 2019. Ribosome stoichiometry: from form to function. *Trends Biochem Sci* **44**: 95–109. doi:10.1016/j.tibs.2018.10.009
- Enroth C, Poulsen LD, Iversen S, Kirpekar F, Albrechtsen A, Vinther J. 2019. Detection of internal N7-methylguanosine (m⁷G) RNA modifications by mutational profiling sequencing. *Nucleic Acids Res* **47**: e126. doi:10.1093/nar/gkz736
- Erales J, Marchand V, Panthu B, Gillot S, Belin S, Ghayad SE, Garcia M, Laforêts F, Marcel V, Baudin-Baillieu A, et al. 2017. Evidence for rRNA 2'-O-methylation plasticity: control of intrinsic translational capabilities of human ribosomes. *Proc Natl Acad Sci* **114**: 12934–12939. doi:10.1073/pnas.1707674114
- Ferretti MB, Karbstein K. 2019. Does functional specialization of ribosomes really exist? *RNA* **25**: 521–538. doi:10.1261/rna.069823.118
- Ferretti MB, Ghalei H, Ward EA, Potts EL, Karbstein K. 2017. Rps26 directs mRNA-specific translation by recognition of Kozak sequence elements. *Nat Struct Mol Biol* **24**: 700–707. doi:10.1038/nsmb.3442
- Flynn RA, Pedram K, Malaker SA, Batista PJ, Smith BAH, Johnson AG, George BM, Majzoub K, Villalta PW, Carette JE, et al. 2021. Small RNAs are modified with N-glycans and displayed on the surface of living cells. *Cell* **184**: 3109–3124.e22. doi:10.1016/j.cell.2021.04.023
- Fujii K, Susanto TT, Saurabh S, Barna M. 2018. Decoding the function of expansion segments in ribosomes. *Mol Cell* **72**: 1013–20.e6. doi:10.1016/j.molcel.2018.11.023
- Ganot P, Bortolin ML, Kiss T. 1997. Site-specific pseudouridine formation in preribosomal RNA is guided by small nucleolar RNAs. *Cell* **89**: 799–809. doi:10.1016/S0092-8674(00)80263-9
- García-Gómez JJ, Babiano R, Lebaron S, Froment C, Monsarrat B, Henry Y, de la Cruz J. 2011. Nop6, a component of 90S pre-

- ribosomal particles, is required for 40s ribosomal subunit biogenesis in *Saccharomyces cerevisiae*. *RNA Biol* **8**: 112–124. doi:10.4161/rna.8.1.14143
- Genuth NR, Barna M. 2018a. Heterogeneity and specialized functions of translation machinery: from genes to organisms. *Nat Rev Genet* **19**: 431–452. doi:10.1038/s41576-018-0008-z
- Genuth NR, Barna M. 2018b. The discovery of ribosome heterogeneity and its implications for gene regulation and organismal life. *Mol Cell* **71**: 364–374. doi:10.1016/j.molcel.2018.07.018
- Gerst JE. 2018. Pimp my ribosome: ribosomal protein paralogs specify translational control. *Trends Genet* **34**: 832–845. doi:10.1016/j.tig.2018.08.004
- Gigova A, Duggimpudi S, Pollex T, Schaefer M, Koš M. 2014. A cluster of methylations in the domain IV of 25S rRNA is required for ribosome stability. *RNA* **20**: 1632–1644. doi:10.1261/rna.043398.113
- Golovina AY, Dzama MM, Osterman IA, Sergiev PV, Serebryakova MV, Bogdanov AA, Dontsova OA. 2012. The last rRNA methyltransferase of *E coli* revealed: the *yhiR* gene encodes adenine-N6 methyltransferase specific for modification of A2030 of 23S ribosomal RNA. *RNA* **18**: 1725–1734. doi:10.1261/rna.034207.112
- Gong J, Li Y, Liu CJ, Xiang Y, Li C, Ye Y, Zhang Z, Hawke DH, Park PK, Diao L, et al. 2017. A pan-cancer analysis of the expression and clinical relevance of small nucleolar RNAs in human cancer. *Cell Rep* **21**: 1968–1981. doi:10.1016/j.celrep.2017.10.070
- Gutgsell N, Englund N, Niu L, Kaya Y, Lane BG, Ofengand J. 2000. Deletion of the *Escherichia coli* pseudouridine synthase gene *truB* blocks formation of pseudouridine 55 in tRNA in vivo, does not affect exponential growth, but confers a strong selective disadvantage in competition with wild-type cells. *RNA* **6**: 1870–1881. doi:10.1017/S1355838200001588
- Hauenschild R, Tserovski L, Schmid K, Thüning K, Winz ML, Sharma S, Entian KD, Wacheul L, Lafontaine DL, Anderson J, et al. 2015. The reverse transcription signature of N¹-methyladenosine in RNA-seq is sequence dependent. *Nucleic Acids Res* **43**: 9950–9964.
- He C. 2010. Grand challenge commentary: RNA epigenetics? *Nat Chem Biol* **6**: 863–865. doi:10.1038/nchembio.482
- Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V, Skrzypek E. 2015. PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Res* **43**: D512–D520. doi:10.1093/nar/gku1267
- Huang H, Karbstein K. 2021. Assembly factors chaperone ribosomal RNA folding by isolating helical junctions that are prone to misfolding. *Proc Natl Acad Sci* **118**: e2101164118. doi:10.1073/pnas.2101164118
- Imami K, Milek M, Bogdanow B, Yasuda T, Kastelic N, Zauber H, Ishihama Y, Landthaler M, Selbach M. 2018. Phosphorylation of the ribosomal protein RPL12/uL11 affects translation during mitosis. *Mol Cell* **72**: 84–98.e9. doi:10.1016/j.molcel.2018.08.019
- Ito S, Akamatsu Y, Noma A, Kimura S, Miyauchi K, Ikeuchi Y, Suzuki T. 2014. A single acetylation of 18 S rRNA is essential for biogenesis of the small ribosomal subunit in *Saccharomyces cerevisiae*. *J Biol Chem* **289**: 26201–26212. doi:10.1074/jbc.M114.593996
- Jiang J, Seo H, Chow CS. 2016. Post-transcriptional modifications modulate rRNA structure and ligand interactions. *Acc Chem Res* **49**: 893–901. doi:10.1021/acs.accounts.6b00014
- Jorjani H, Kehr S, Jedlinski DJ, Gumienny R, Hertel J, Stadler PF, Zavolan M, Gruber AR. 2016. An updated human snoRNAome. *Nucleic Acids Res* **44**: 5068–5082. doi:10.1093/nar/gkw386
- Keffer-Wilkes LC, Veerareddygar GR, Kothe U. 2016. RNA modification enzyme TruB is a tRNA chaperone. *Proc Natl Acad Sci* **113**: 14306–14311. doi:10.1073/pnas.1607512113
- Khoshnevis S, Elizabeth Dreggors R, Hoffmann TFR, Ghalei H. 2019. A conserved Bcd1 interaction essential for box C/D snoRNP biogenesis. *J Biol Chem* **294**: 18360–18371. doi:10.1074/jbc.RA119.010222
- King TH, Liu B, McCully RR, Fournier MJ. 2003. Ribosome structure and activity are altered in cells lacking snoRNPs that form pseudouridines in the peptidyl transferase center. *Mol Cell* **11**: 425–435. doi:10.1016/S1097-2765(03)00040-6
- Kirmizialtin S, Loerke J, Behrmann E, Spahn CMT, Sanbonmatsu KY. 2015. Using molecular simulation to model high-resolution cryo-EM reconstructions. *Methods Enzymol* **558**: 497–514. doi:10.1016/bs.mie.2015.02.011
- Kiss-László Z, Henry Y, Bachellerie JP, Caizergues-Ferrer M, Kiss T. 1996. Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. *Cell* **85**: 1077–1088. doi:10.1016/S0092-8674(00)81308-2
- Kobayashi T. 2014. Ribosomal RNA gene repeats, their stability and cellular senescence. *Proc Jpn Acad Ser B Phys Biol Sci* **90**: 119–129. doi:10.2183/pjab.90.119
- Lafontaine DL, Tollervey D. 1998. Birth of the snoRNPs: the evolution of the modification-guide snoRNAs. *Trends Biochem Sci* **23**: 383–388. doi:10.1016/S0968-0004(98)01260-2
- Lafontaine D, Delcour J, Glasser A-L, Desgres J, Vandenhaute J. 1994. The *DIM1* gene responsible for the conserved m⁶₂Am⁶₂A dimethylation in the 3'-terminal loop of 18 S rRNA is essential in yeast. *J Mol Biol* **241**: 492–497. doi:10.1006/jmbi.1994.1525
- Lafontaine D, Vandenhaute J, Tollervey D. 1995. The 18S rRNA dimethylase Dim1p is required for pre-ribosomal RNA processing in yeast. *Genes Dev* **9**: 2470–2481. doi:10.1101/gad.9.20.2470
- Lafontaine DL, Preiss T, Tollervey D. 1998. Yeast 18S rRNA dimethylase Dim1p: a quality control mechanism in ribosome synthesis? *Mol Cell Biol* **18**: 2360–2370. doi:10.1128/MCB.18.4.2360
- Lane BG. 2014. Historical perspectives on RNA nucleoside modifications. In *Modification and editing of RNA* (ed. Grosjean H, Benne R), pp. 1–20. ASM Press, Washington, DC.
- Lane BG, Allen FW. 1961. The terminal residues of wheat germ ribonucleates. *Biochim Biophys Acta* **47**: 36–46. doi:10.1016/0006-3002(61)90826-5
- Li D, Wang J. 2020. Ribosome heterogeneity in stem cells and development. *J Cell Biol* **219**: e202001108. doi:10.1083/jcb.202001108
- Li X, Xiong X, Zhang M, Wang K, Chen Y, Zhou J, Mao Y, Lv J, Yi D, Chen XW, et al. 2017. Base-resolution mapping reveals distinct m¹A methylome in nuclear- and mitochondrial-encoded transcripts. *Mol Cell* **68**: 993–1005.e9. doi:10.1016/j.molcel.2017.10.019
- Liang X-H, Liu Q, Fournier MJ. 2007. rRNA modifications in an inter-subunit bridge of the ribosome strongly affect both ribosome biogenesis and activity. *Mol Cell* **28**: 965–977. doi:10.1016/j.molcel.2007.10.012
- Liang X-H, Liu Q, Fournier MJ. 2009. Loss of rRNA modifications in the decoding center of the ribosome impairs translation and strongly delays pre-rRNA processing. *RNA* **15**: 1716–1728. doi:10.1261/rna.1724409
- Liger D, Mora L, Lazar N, Figaro S, Henri J, Scrima N, Buckingham RH, van Tilbeurgh H, Heurgué-Hamard V, Graille M. 2016. Mechanism of activation of methyltransferases involved in translation by the Trm112 'hub' protein. *Nucleic Acids Res* **39**: 6249–6259. doi:10.1093/nar/gkv1172
- Lin S, Liu Q, Jiang Y-Z, Gregory RI. 2019. Nucleotide resolution profiling of m⁷G tRNA modification by TRAC-seq. *Nat Protoc* **14**: 3220–3242. doi:10.1038/s41596-019-0226-7
- Linder B, Grozhik AV, Olarerin-George AO, Meydan C, Mason CE, Jaffrey SR. 2015. Single-nucleotide-resolution mapping of m⁶A and m⁶Am throughout the transcriptome. *Nat Methods* **12**: 767–772. doi:10.1038/nmeth.3453

- Liu K, Santos DA, Hussmann JA, Wang Y, Sutter BM, Weissman JS, Tu BP. 2021. Regulation of translation by methylation multiplicity of 18S rRNA. *Cell Rep* **34**: 108825. doi:10.1016/j.celrep.2021.108825
- Locati MD, Pagano JFB, Girard G, Ensink WA, van Olst M, van Leeuwen S, Nehrlich U, Spaik HP, Rauwerda H, Jonker MJ, et al. 2017. Expression of distinct maternal and somatic 5.8S, 18S, and 28S rRNA types during zebrafish development. *RNA* **23**: 1188–1199. doi:10.1261/rna.061515.117
- López-López A, Benlloch S, Bonfá M, Rodríguez-Valera F, Mira A. 2007. Intragenomic 16S rDNA divergence in *haloarcula marismortui* is an adaptation to different temperatures. *J Mol Evol* **65**: 687–696. doi:10.1007/s00239-007-9047-3
- Loveland AB, Bah E, Madireddy R, Zhang Y, Brilot AF, Grigorieff N, Korostelev AA. 2016. Ribosome•RelA structures reveal the mechanism of stringent response activation. *Elife* **5**: e17029. doi:10.7554/eLife.17029
- Malecki JM, Odonohue MF, Kim Y, Jakobsson ME, Gessa L, Pinto R, Wu J, Davydova E, Moen A, Olsen JV, et al. 2021. Human METTL18 is a histidine-specific methyltransferase that targets RPL3 and affects ribosome biogenesis and function. *Nucleic Acids Res* **49**: 3185–3203. doi:10.1093/nar/gkab088
- Marchand V, Blanloeil-Oillo F, Helm M, Motorin Y. 2016. Illumina-based RiboMethSeq approach for mapping of 2'-O-Me residues in RNA. *Nucleic Acids Res* **44**: e135. doi:10.1093/nar/gkw547
- Marchand V, Ayadi L, Ernst FGM, Hertler J, Bourguignon-Igel V, Galvanin A, Kotter A, Helm M, Lafontaine DLJ, Motorin Y. 2018. AlkAniline-Seq: profiling of m⁷G and m³C RNA modifications at single nucleotide resolution. *Angew Chem Int Ed Engl* **57**: 16785–16790. doi:10.1002/anie.201810946
- Marchand V, Pichot F, Neybecker P, Ayadi L, Bourguignon-Igel V, Wacheul L, Lafontaine DLJ, Pinzano A, Helm M, Motorin Y. 2020. HydraPsiSeq: a method for systematic and quantitative mapping of pseudouridines in RNA. *Nucleic Acids Res* **48**: e110. doi:10.1093/nar/gkaa769
- McCann KL, Kavari SL, Burkholder AB, Phillips BT, Tanaka Hall TM. 2020. H/ACA snoRNA levels are regulated during stem cell differentiation. *Nucleic Acids Res* **48**: 8686–8703. doi:10.1093/nar/gkaa612
- Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. 2012. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell* **149**: 1635–1646. doi:10.1016/j.cell.2012.05.003
- Mukhopadhyay R, Ray PS, Arif A, Brady AK, Kinter M, Fox PL. 2008. DAPK-ZIPK-L13a axis constitutes a negative-feedback module regulating inflammatory gene expression. *Mol Cell* **32**: 371–382. doi:10.1016/j.molcel.2008.09.019
- Nachmani D, Bothmer AH, Grisendi S, Mele A, Bothmer D, Lee JD, Monteleone E, Cheng K, Zhang Y, Bester AC, et al. 2019. Germline *NPM1* mutations lead to altered rRNA 2'-O-methylation and cause dyskeratosis congenita. *Nat Genet* **51**: 1518–1529. doi:10.1038/s41588-019-0502-z
- Natchiar SK, Myasnikov AG, Kratzat H, Hazemann I, Klaholz BP. 2017. Visualization of chemical modifications in the human 80S ribosome structure. *Nature* **551**: 472–477. doi:10.1038/nature24482
- Ni J, Tien AL, Fournier MJ. 1997. Small nucleolar RNAs direct site-specific synthesis of pseudouridine in ribosomal RNA. *Cell* **89**: 565–573. doi:10.1016/S0092-8674(00)80238-X
- Nikolay R, Hilal T, Schmidt S, Qin B, Schwefel D, Vieira-Vieira CH, Mielke T, Bürger J, Loerke J, Amikura K, et al. 2021. Snapshots of native pre-50S ribosomes reveal a biogenesis factor network and evolutionary specialization. *Mol Cell* **81**: 1200–1215.e9. doi:10.1016/j.molcel.2021.02.006
- Nurk S, Koren S, Rhie A, Rautiainen M, Bizakdzé AV, Mikheenko A, Vollger MR, Altemose N, Uralsky L, Gershman A, et al. 2021. The complete sequence of a human genome. *bioRxiv* doi:10.1101/2021.05.26.445798
- Pandolfini L, Barbieri I, Bannister AJ, Hendrick A, Andrews B, Webster N, Murat P, Mach P, Brandi R, Robson SC, et al. 2019. METTL1 promotes *let-7* microRNA processing via m⁷G methylation. *Mol Cell* **74**: 1278–90.e9. doi:10.1016/j.molcel.2019.03.040
- Peifer C, Sharma S, Watzinger P, Lamberth S, Kötter P, Entian K-D. 2013. Yeast Rps8p, a novel methyltransferase responsible for m¹A 645 base modification of 25S rRNA. *Nucleic Acids Res* **41**: 1151–1163. doi:10.1093/nar/gks1102
- Phillips G, de Crécy-Lagard V. 2011. Biosynthesis and function of tRNA modifications in archaea. *Curr Opin Microbiol* **14**: 335–341. doi:10.1016/j.mib.2011.03.001
- Polikanov YS, Melnikov SV, Söll D, Steitz TA. 2015. Structural insights into the role of rRNA modifications in protein synthesis and ribosome assembly. *Nat Struct Mol Biol* **22**: 342–344. doi:10.1038/nsmb.2992
- Popis MC, Blanco S, Frye M. 2016. Posttranscriptional methylation of transfer and ribosomal RNA in stress response pathways, cell differentiation, and cancer. *Curr Opin Oncol* **28**: 65–71. doi:10.1097/CCO.0000000000000252
- Rogers MJ, Gutell RR, Damberger SH, Li J, McConkey GA, Waters AP, McCutchan TF. 1996. Structural features of the large subunit rRNA expressed in *Plasmodium falciparum* sporozoites that distinguish it from the asexually expressed subunit rRNA. *RNA* **2**: 134–145.
- Ryvkin P, Leung YY, Silverman IM, Childress M, Valladares O, Dragomir I, Gregory BD, Wang L-S. 2013. HAMR: high-throughput annotation of modified ribonucleotides. *RNA* **19**: 1684–1692. doi:10.1261/rna.036806.112
- Safra M, Sas-Chen A, Nir R, Winkler R, Nachshon A, Bar-Yaacov D, Erlacher M, Rossmanith W, Stern-Ginossar N, Schwartz S. 2017. The m¹A landscape on cytosolic and mitochondrial mRNA at single-base resolution. *Nature* **551**: 251–255. doi:10.1038/nature24456
- Samir P, Browne CM, Rahul MS, Shen B, Li W, Frank J, Link AJ. 2018. Identification of changing ribosome protein compositions using mass spectrometry. *Proteomics* **18**: e1800217. doi:10.1002/pmic.201800217
- Sardana R, Johnson AW. 2012. The methyltransferase adaptor protein Trm112 is involved in biogenesis of both ribosomal subunits. *Mol Biol Cell* **23**: 4313–4322. doi:10.1091/mbc.e12-05-0370
- Sas-Chen A, Thomas JM, Matzov D, Taoka M, Nance KD, Nir R, Bryson KM, Shachar R, Liman GLS, Burkhart BW, et al. 2020. Dynamic RNA acetylation revealed by quantitative cross-evolutionary mapping. *Nature* **583**: 638–643. doi:10.1038/s41586-020-2418-2
- Schaefer M, Pollex T, Hanna K, Lyko F. 2009. RNA cytosine methylation analysis by bisulfite sequencing. *Nucleic Acids Res* **37**: e12. doi:10.1093/nar/gkn954
- Schilling V, Peifer C, Buchhaupt M, Lamberth S, Lioutikov A, Rietschel B, Kötter P, Entian K-D. 2012. Genetic Interactions of yeast NEP1 (EMG1), encoding an essential factor in ribosome biogenesis. *Yeast* **29**: 167–183. doi:10.1002/yea.2898
- Schnare MN, Gray MW. 2011. Complete modification maps for the cytosolic small and large subunit rRNAs of *Euglena gracilis*: functional and evolutionary implications of contrasting patterns between the two rRNA components. *J Mol Biol* **413**: 66–83. doi:10.1016/j.jmb.2011.08.037
- Schwartz S, Bernstein DA, Mumbach MR, Jovanovic M, Herbst RH, León-Ricardo BX, Engreitz JM, Guttman M, Satija R, Lander ES, et al. 2014a. Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. *Cell* **159**: 148–162. doi:10.1016/j.cell.2014.08.028
- Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, Bushkin GG, Mertins P, Ter-Ovanesyan D, Habib N,

- Cacchiarelli D, et al. 2014b. Perturbation of m⁶A writers reveals two distinct classes of mRNA methylation at internal and 5' sites. *Cell Rep* **8**: 284–296. doi:10.1016/j.celrep.2014.05.048
- Segev N, Gerst JE. 2018. Specialized ribosomes and specific ribosomal protein paralogs control translation of mitochondrial proteins. *J Cell Biol* **217**: 117–126. doi:10.1083/jcb.201706059
- Sergiev PV, Golovina AY, Prokhorova IV, Sergeeva OV, Osterman IA, Nesterchuk MV, Burakovsky DE, Bogdanov AA, Dontsova OA. 2011. Modifications of ribosomal RNA: from enzymes to function. In *Ribosomes: structure, function, and dynamics* (ed. Rodnina MV, et al.), pp. 97–110. Springer Vienna, Vienna.
- Sergiev PV, Aleksashin NA, Chugunova AA, Polikanov YS, Dontsova OA. 2018. Structural and evolutionary insights into ribosomal RNA methylation. *Nat Chem Biol* **14**: 226–235. doi:10.1038/nchembio.2569
- Shalev-Benami M, Zhang Y, Matzov D, Halfon Y, Zackay A, Rozenberg H, Zimmermann E, Bashan A, Jaffe CL, Yonath A, et al. 2016. 2.8-Å Cryo-EM structure of the large ribosomal subunit from the eukaryotic parasite *Leishmania*. *Cell Rep* **16**: 288–294. doi:10.1016/j.celrep.2016.06.014
- Sharma S, Lafontaine DLJ. 2015. 'View from a bridge': a new perspective on eukaryotic rRNA base modification. *Trends Biochem Sci* **40**: 560–575. doi:10.1016/j.tibs.2015.07.008
- Sharma S, Watzinger P, Kötter P, Entian K-D. 2013a. Identification of a novel methyltransferase, Bmt2, responsible for the N¹-methyladenosine base modification of 25S rRNA in *Saccharomyces cerevisiae*. *Nucleic Acids Res* **41**: 5428–5443. doi:10.1093/nar/gkt195
- Sharma S, Yang J, Watzinger P, Kötter P, Entian K-D. 2013b. Yeast Nop2 and Rcm1 methylate C2870 and C2278 of the 25S rRNA, respectively. *Nucleic Acids Res* **41**: 9062–9076. doi:10.1093/nar/gkt679
- Sharma S, Marchand V, Motorin Y, Lafontaine DLJ. 2017a. Identification of sites of 2'-O-methylation vulnerability in human ribosomal RNAs by systematic mapping. *Sci Rep* **7**: 11490. doi:10.1038/s41598-017-09734-9
- Sharma S, Yang J, van Nues R, Watzinger P, Kötter P, Lafontaine DLJ, Granneman S, Entian K-D. 2017b. Specialized box C/D snoRNPs act as antisense guides to target RNA base acetylation. *PLoS Genet* **13**: e1006804. doi:10.1371/journal.pgen.1006804
- Shen H, Stoute J, Liu KF. 2020. Structural and catalytic roles of the human 18S rRNA methyltransferases DIMT1 in ribosome assembly and translation. *J Biol Chem* **295**: 12058–12070. doi:10.1074/jbc.RA120.014236
- Shi Z, Fujii K, Kovary KM, Genuth NR, Röst HL, Teruel MN, Barna M. 2017. Heterogeneous ribosomes preferentially translate distinct subpools of mRNAs genome-wide. *Mol Cell* **67**: 71–83.e7. doi:10.1016/j.molcel.2017.05.021
- Simsek D, Barna M. 2017. An emerging role for the ribosome as a nexus for post-translational modifications. *Curr Opin Cell Biol* **45**: 92–101. doi:10.1016/j.celb.2017.02.010
- Simsek D, Tiu GC, Flynn RA, Byeon GW, Leppek K, Xu AF, Chang HY, Barna M. 2017. The mammalian ribo-interactome reveals ribosome functional diversity and heterogeneity. *Cell* **169**: 1051–1065.e18. doi:10.1016/j.cell.2017.05.022
- Slavov N, Semrau S, Airoldi E, Budnik B, van Oudenaarden A. 2015. Differential stoichiometry among core ribosomal proteins. *Cell Rep* **13**: 865–873. doi:10.1016/j.celrep.2015.09.056
- Sloan KE, Warda AS, Sharma S, Entian K-D, Lafontaine DLJ, Bohnsack MT. 2016a. Tuning the ribosome: the influence of rRNA modification on eukaryotic ribosome biogenesis and function. *RNA Biol* **14**: 1138–1152. doi:10.1080/15476286.2016.1259781
- Sloan KE, Warda AS, Sharma S, Entian K-D, Lafontaine DLJ, Bohnsack MT. 2016b. Tuning the ribosome: the influence of rRNA modification on eukaryotic ribosome biogenesis and function. *RNA Biol* **14**: 1138–1152. doi:10.1080/15476286.2016.1259781
- Spenkuch F, Motorin Y, Helm M. 2014. Pseudouridine: still mysterious, but never a fake (uridine)! *RNA Biol* **11**: 1540–1554. doi:10.4161/15476286.2014.992278
- Taoka M, Nobe Y, Hori M, Takeuchi A, Masaki S, Yamauchi Y, Nakayama H, Takahashi N, Isobe T. 2015. A mass spectrometry-based method for comprehensive quantitative determination of post-transcriptional RNA modifications: the complete chemical structure of *Schizosaccharomyces pombe* ribosomal RNAs. *Nucleic Acids Res* **43**: e115. doi:10.1093/nar/gkv560
- Taoka M, Nobe Y, Yamaki Y, Yamauchi Y, Ishikawa H, Takahashi N, Nakayama H, Isobe T. 2016. The complete chemical structure of *Saccharomyces cerevisiae* rRNA: partial pseudouridylation of U2345 in 25S rRNA by snoRNA snR9. *Nucleic Acids Res* **44**: 8951–8961. doi:10.1093/nar/gkw564
- Taoka M, Nobe Y, Yamaki Y, Sato K, Ishikawa H, Izumikawa K, Yamauchi Y, Hirota K, Nakayama H, Takahashi N, et al. 2018. Landscape of the complete RNA chemical modifications in the human 80S ribosome. *Nucleic Acids Res* **46**: 9289–9298. doi:10.1093/nar/gky811
- Vembar SS, Droll D, Scherf A. 2016. Translational regulation in blood stages of the malaria parasite *Plasmodium* spp.: systems-wide studies pave the way. *Wiley Interdiscip Rev RNA* **7**: 772–792. doi:10.1002/wrna.1365
- Warner WA, Spencer DH, Trissal M, White BS, Helton N, Ley TJ, Link DC. 2018. Expression profiling of snoRNAs in normal hematopoiesis and AML. *Blood Adv* **2**: 151–163. doi:10.1182/bloodadvances.2017006668
- Xue S, Barna M. 2012. Specialized ribosomes: a new frontier in gene regulation and organismal biology. *Nat Rev Mol Cell Biol* **13**: 355–369. doi:10.1038/nrm3359
- Zebarjadian Y, King T, Fournier MJ, Clarke L, Carbon J. 1999. Point mutations in yeast CBF5 can abolish in vivo pseudouridylation of rRNA. *Mol Cell Biol* **19**: 7461–7472. doi:10.1128/MCB.19.11.7461
- Zhang LS, Liu C, Ma H, Dai Q, Sun HL, Luo G, Zhang Z, Zhang L, Hu L, Dong X, et al. 2019. Transcriptome-wide mapping of internal N⁷-methylguanosine methylome in mammalian mRNA. *Mol Cell* **74**: 1304–1316.e8. doi:10.1016/j.molcel.2019.03.036
- Zheng G, Qin Y, Clark WC, Dai Q, Yi C, He C, Lambowitz AM, Pan T. 2015. Efficient and quantitative high-throughput tRNA sequencing. *Nat Methods* **12**: 835–837. doi:10.1038/nmeth.3478
- Zorbas C, Nicolas E, Wacheul L, Huvelle E, Heurgué-Hamard V, Lafontaine DLJ. 2015. The human 18S rRNA base methyltransferases DIMT1L and WBSR22-TRMT112 but not rRNA modification are required for ribosome biogenesis. *Mol Biol Cell* **26**: 2080–2095. doi:10.1091/mbc.E15-02-0073

RNA

A PUBLICATION OF THE RNA SOCIETY

The ribosome epitranscriptome: inert—or a platform for functional plasticity?

Joseph Georgeson and Schraga Schwartz

RNA 2021 27: 1293-1301 originally published online July 26, 2021
Access the most recent version at doi:[10.1261/rna.078859.121](https://doi.org/10.1261/rna.078859.121)

References This article cites 118 articles, 27 of which can be accessed free at:
<http://rnajournal.cshlp.org/content/27/11/1293.full.html#ref-list-1>

Open Access Freely available online through the RNA Open Access option.

Creative Commons License This article, published in RNA, is available under a Creative Commons License (Attribution-NonCommercial 4.0 International), as described at <http://creativecommons.org/licenses/by-nc/4.0/>.

Email Alerting Service Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

To subscribe to RNA go to:
<http://rnajournal.cshlp.org/subscriptions>