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An emerging role for the ribosome as a nexus for post-translational modifications

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The ribosome is one of life's most ancient molecular machines that has historically been viewed as a backstage participant in gene regulation, translating the genetic code across all kingdoms of life in a rote-like fashion. However, recent studies suggest that intrinsic components of the ribosome can be regulated and diversified as a means to intricately control the expression of the cellular proteome. In this review, we discuss advances in the characterization of ribosome post-translational modifications (PTMs) from past to present. We specifically focus on emerging examples of ribosome phosphorylation and ubiquitylation, which are beginning to showcase that PTMs of the ribosome are versatile, may have functional consequences for translational control, and are intimately linked to human disease. We further highlight the key questions that remain to be addressed to gain a more complete picture of the array of ribosome PTMs and the upstream enzymes that control them, which may endow ribosomes with greater regulatory potential in gene regulation and control of cellular homeostasis.

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Within the central dogma of molecular biology, that describes the linear flow of information from DNA to RNA to protein, translational control is considered the final step. The ribosome, a multi-component assembly of RNA and protein, is responsible for accurately decoding messenger RNAs (mRNAs) and converting their sequences into proteins. Since the first electron microscopy observation of the ribosome as "a particular component of small dimensions and high density" in 1955 [1], decades of research have led to the discovery of multiple mechanisms that regulate translation. However, most of these investigations have mainly focused on additional translation factors (e.g. translation initiation and elongation factors) rather than the ribosome itself [2,3]. In all organisms, Ribosomal Proteins (RPs) and ribosomal RNAs (rRNAs) make up the small and large ribosomal subunits. The space between the two subunits harbors the catalytic activity of the ribosome. Although the majority of the RNA and protein components, including the peptidyl transferase center that catalyzes peptide bond formation, are well-conserved throughout all kingdoms of life from bacteria to humans, ribosomes have undergone structural and compositional changes throughout evolution that reflect differences in the translational mechanisms utilized and the complexity of the proteomes synthesized [4,5].

Observations that ribosomes within a single species may differ in composition have been reported since the 1970s, primarily from the bacterial response to antibiotics or changing growth conditions [6,7]. Despite these intriguing observations, ribosomes have traditionally been considered housekeeping structures, invariable in composition within the same species. Only recently has this perception been challenged by studies demonstrating that the absence or presence of specific RPs or rRNA segments actually has functional significance and can tune the ribosome to preferentially translate specific mRNAs. Under stress, bacteria express a site-specific endonuclease that cleaves both mRNAs, which renders them leaderless, as well as rRNAs, to form specialized ribosomes that can only translate these cleaved mRNA species [8]. In mammalian cells, a key example of ribosome heterogeneity is Rpl38/eL38, a core RP, which is differentially expressed in specific regions of the developing mouse embryo, such as the somites that give rise to the mammalian body axis. Reduction of Rpl38/eL38 in a mouse model revealed that it does not affect global translational regulation but is selectively required for the translation of specific *Hox* genes that control patterning of the mammalian body plan through *cis*-regulatory elements residing in their 5′ untranslated regions (UTRs) [9,10•]. Emerging studies that highlight the functional significance of different RPs serving specific roles are reviewed elsewhere [11,12] and they suggest that the ribosome is not merely a static machine, but rather a dynamic one whose intrinsic components can be regulated and diversified to promote intricate regulation of translation.

It is tempting to hypothesize that an additional layer of dynamic regulation to ribosome activity may be achieved

by RP PTMs such as phosphorylation and ubiquitylation. PTMs are covalent modifications that can change the stability, subcellular localization, and/or interaction partners of the modified substrate and can regulate such properties within developmental and physiological timescales. Initial proteomic studies of ribosomes crudely isolated through centrifugation methods attempted to identify PTMs via database searches of either intact RPs or digested RP-peptides [13–15]. However, PTM assignment based on mass difference, such as acetylation or methylation, is not comprehensive and is prone to misinterpretation as specific masses could result from a combination of multiple PTMs or could be due to artifacts arising from sample preparation methods. Recent advances in PTM enrichment methods from digested peptides, combined with high-resolution quantitative mass spectrometry (MS), permit systems-level PTM mapping for phosphorylation and ubiquitylation, whereby the conjugation sites of the modification can also be identified with higher confidence [16,17]. Interestingly, many of these near-comprehensive static PTM maps or dynamic PTM snapshots in response to different perturbations contain RPs as candidates [18–22,23••]. Although the presence of diverse PTMs increases the combinatorial diversity of ribosomes, analogously to the histone PTMs that make up the histone code, our understanding of the mechanisms that underlie ribosomal PTMs and their potential functional roles in regulating translational output is in its infancy. In this review, we will summarize recent findings, concentrating on examples of phosphorylation and ubiquitylation of RPs identified by both focused, case-by-case studies and by unbiased, large-scale proteomic studies that enrich for a specific PTM.

Phosphorylated ribosomes in sickness and in health

The first inducible PTM identified at the ribosome was the phosphorylation of mammalian Rps6/eS6, which was discovered almost 40 years ago by analyzing ribosomes from regenerating livers, utilizing radioactive phosphate incorporation and two dimensional (2D) gel electrophoresis [24]. Phosphorylation events on Rps6/eS6 are mapped to five sites that can all be catalyzed by S6K1/S6K2 kinases. RSK, PKA, and CK1 kinases can also phosphorylate distinct sites of Rps6/eS6, whereas all the phosphorylation can be reversed by phosphatase PP1 (Figure 1a) [25]. Numerous studies have shown that Rps6/eS6 phosphorylation occurs downstream of multiple external stimuli (e.g. growth factors), which are transduced via the PI3K and mTOR pathways. As a result, Rps6/eS6 phosphorylation is frequently used as a readout for mTORC1 activation [25]. Moreover, in mice physiological or pharmacological stimuli that activate neurons, also lead to an increase in Rps6/eS6 phosphorylation [26,27]. This observation led to the development of phosphorylated Rps6/eS6 as a marker of activated neurons and antibodies that specifically recognize this modification enabled the enrichment and isolation of ribosomes and associated mRNAs translated in response to neuronal activation [27]. A knock-in mouse model in which all five Rps6/eS6 phosphorylation sites were mutated, along with mouse models deficient in S6K1/S6K2 kinases, paved the way for a better understanding of the role of Rps6/eS6 phosphorylation. Surprisingly, Rps6/eS6 phosphorylation-deficient mice are viable and only display subtle, tissue-specific phenotypes, for example, smaller pancreatic beta cells accompanied by impaired glucose homeostasis [28], and smaller myoblasts with decreased muscle mass [29]. Moreover, studies using Rps6/eS6 phosphorylation-deficient mice in different cancer mouse models suggested a role of Rps6/eS6 phosphorylation in the initiation of pancreatic cancer, but found it was dispensable for AKT-mediated thymic lymphomas [30–32]. Therefore, although the regulatory inputs leading to Rps6/eS6 phosphorylation have been extensively studied and Rps6/eS6 phosphorylation has been successfully used as a readout of these inputs, the physiological roles of this modification Rps6/eS6 are not yet entirely known.

A second prominent example of RP phosphorylation comes from human monocytic cells and the interferon (IFN)-γ-mediated innate immune response (Figure 1a). Upon IFN-γ incubation, the DAPK1-ZIPK kinase-signaling cascade results in the phosphorylation of Rpl13a/uL13 at stoichiometric levels at a single serine. This process results in the release of Rpl13a/uL13 from the assembled large ribosomal subunit [33]. Released phosphorylated Rpl13a/uL13 has an extra-ribosomal function as an essential component of the interferon-γ-activated inhibitor of translation (GAIT) complex, which binds to a defined element in the 3′ UTRs of a select group of inflammation-related mRNAs to inhibit their translation [34]. Phosphorylation of Rpl13a/uL13 is the rate-limiting step in GAIT complex-mediated translational repression. After the complex is recruited to target mRNAs, phosphorylated Rpl13a/uL13 interacts specifically with the initiation factor eIF4G and suppresses translation by blocking the recruitment of the small ribosomal subunit [33,35]. Interestingly, DAPK1-ZIPK kinases themselves are translational targets of phosphorylated Rpl13a/uL13, thereby forming a negative feedback loop [33].

A more recent study showed how phosphorylation of an RP can be critical for the etiology of the neurodegenerative Parkinson's disease (PD) (Figure 1a) [36]. Rps11/uS17, Rps15/uS19, and Rps27/eS27 were found to be the major interactors of LRRK2, a kinase frequently mutated in familial and sporadic PD. Intriguingly, 19 of 67 RPs tested can be phosphorylated directly by LRRK2 [37••]. For Rps15/uS19, the mutation of a single phosphorylation site rescues the neurotoxicity caused by the LRRK2 mutation in Drosophila, and phospho-mimetic Rps15/