ANNUAL REVIEWS

Annual Review of Cell and Developmental Biology

Ribosome Assembly and Repair

Yoon-Mo Yang[1,2] and Katrin Karbstein[1,3]

[1]Department of Integrative Structural and Computational Biology, The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology, Jupiter, Florida, USA

[2]Current affiliation: Graduate School of Biomedical Science and Engineering and Hanyang Institute of Bioscience and Biotechnology, Hanyang University, Seoul, Republic of Korea; email: ymyang@hanyang.ac.kr

[3]Current affiliation: Department of Biochemistry, Vanderbilt School of Medicine, Vanderbilt University, Nashville, Tennessee, USA; email: katrin.karbstein@vanderbilt.edu

Annu. Rev. Cell Dev. Biol. 2024. 40:241–64

First published as a Review in Advance on May 9, 2024

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

https://doi.org/10.1146/annurev-cellbio-111822-113326

Keywords

ribosome assembly, ribosome repair, ribosomal protein turnover, ribosome turnover, ribosomal protein chaperone, ribosome damage

Abstract

Ribosomes synthesize protein in all cells. Maintaining both the correct number and composition of ribosomes is critical for protein homeostasis. To address this challenge, cells have evolved intricate quality control mechanisms during assembly to ensure that only correctly matured ribosomes are released into the translating pool. However, these assembly-associated quality control mechanisms do not deal with damage that arises during the ribosomes' exceptionally long lifetimes and might equally compromise their function or lead to reduced ribosome numbers. Recent research has revealed that ribosomes with damaged ribosomal proteins can be repaired by the release of the damaged protein, thereby ensuring ribosome integrity at a fraction of the energetic cost of producing new ribosomes, appropriate for stress conditions. In this article, we cover the types of ribosome damage known so far, and then we review the known repair mechanisms before surveying the literature for possible additional instances of repair.

Contents

OVERVIEW OF RIBOSOME ASSEMBLY 242
RIBOSOMES ARE EXTREMELY STABLE 245
Ribosome Half-Lives 245
Cells with Long-Lived Ribosomes 246
CHEMICAL DAMAGE TO RIBOSOMES 246
Ribosomal Protein Damage by Oxidative Stress 246
rRNA Damage by Oxidative Stress 249
rRNA Damage by Cisplatin 250
Ultraviolet Damage 250
Other Types of Ribosome Damage 250
CONSEQUENCES OF RIBOSOME DAMAGE 251
Ribosome Degradation 251
Ribosomes Can Be Repaired 251
Other Examples of Chaperone-Mediated Ribosomal Protein Repair? 252
The Repair Machinery Can Be Used for Ribosome Remodeling 253
How Are Extraction and Incorporation Regulated and Balanced? 255
Additional Evidence of Ribosomal Protein Exchange 256
SUMMARY AND FUTURE OUTLOOK 257

OVERVIEW OF RIBOSOME ASSEMBLY

Ribosomes are universally conserved RNA-protein complexes that enable protein synthesis in all cells. Ribosomes comprise two subunits, a small subunit (40S or 30S in eukaryotes or bacteria, respectively) and a large subunit (60S or 50S in eukaryotes or bacteria, respectively). These are made up of four ribosomal RNAs (rRNAs) (three for bacteria) onto which 79 ribosomal proteins (RPs) assemble (54 in bacteria and 79 in eukaryotes). While both rRNAs and RPs have universally conserved elements (which for RPs are denoted with the prefix u), there is also an elaboration of the structure in the transition from bacteria to eukaryotes, with eukaryote-specific expansion segments in the rRNA and additional RPs.

Ribosomes are highly abundant in all cells, and their assembly requires significant cellular resources, including approximately 60% of all transcription events, 90% of all splicing events, and about half of all translation events in yeast (Warner 1999). Thus, it is not surprising that under all characterized cellular stress conditions ribosome assembly is rapidly downregulated (Gasch et al. 2000), presumably to conserve energy. Importantly, conserving ribosome numbers is critically linked to protein homeostasis, as different messenger RNAs (mRNAs) respond differently to changes in ribosome numbers (Ivanov et al. 2022, Mills & Green 2017). Thus, given that stress conditions temporarily block both new ribosome assembly and cell division, ribosome numbers remain constant if there is no significant ribosome degradation. Such degradation could occur either to provide nutrients (such as autophagy) or because ribosomes are damaged. Conversely, extensive ribosome damage and decay during stress would alter protein homeostasis.

Ribosome assembly begins with transcription of the rRNA and the cotranscriptional modification, folding, and processing of the rRNA from longer precursor transcripts (reviewed in Henras et al. 2015). These processes are integrated with the hierarchical binding of a subset of early binding RPs (de la Cruz et al. 2015). Assembly is mediated by a large machinery (reviewed in Klinge &

Woolford 2019), which is essential in eukaryotes and required under stress conditions in bacteria. This machinery promotes the processing and modification of the rRNA, chaperones the folding of the rRNA (Blomqvist et al. 2023, Huang & Karbstein 2021, Liu et al. 2021), enables hierarchy in RP binding (Blomqvist et al. 2023), and allows for regulation and quality control (Garcia-Gomez et al. 2011; Ghalei et al. 2015, 2017; Huang et al. 2020; Lo et al. 2010; Mitterer et al. 2023; Parker et al. 2019; Schafer et al. 2006; Strunk et al. 2012). While quality control mechanisms have been recently reviewed (Parker & Karbstein 2023) and therefore are not a focus here, the elaborate system of multiple quality control systems—as well as the finding that they are frequently bypassed in disease states, which leads to defective ribosomes in the translating pool (Parker & Karbstein 2023)—suggests the importance of quality control during ribosome assembly and thus the integrity of ribosomes for cellular and organismal health.

Most RPs are small, highly basic proteins that tend to aggregate outside of ribosomes (Gorenstein & Warner 1977, Warner 1977). Thus, they are stabilized by binding to the ribosome-associated chaperones ribosome-associated complex (RAC) and nascent polypeptide-associated complex (NAC) (Koplin et al. 2010), preventing their aggregation and enabling the production of ribosomes at the levels necessary to maintain cell growth and protein homeostasis. Interestingly, 13 RPs have specialized chaperones that solely bind and stabilize these RPs outside the ribosome (Black et al. 2019, Pillet et al. 2017, Rossler et al. 2019, Ting et al. 2017, Yang et al. 2016) (**Table 1**). In addition, yeast Fap7, yeast Sef1, and human NAP1L1 are chaperone candidates for uS11 (Rps14), uS19 (Rps15), and uL2 (Rpl2), respectively (Hellmich et al. 2013, Yip et al. 2022).

Table 1 Ribosomal proteins with personalized chaperones

Ribosomal protein	Yeast	*Caenorhabditis elegans*	Mice	*Drosophila*	Humans	Zebrafish
40S proteins						
uS3/Rps3	Yar1	ND	ND	ND	ND	ND
uS5/Rps2	Tsr4	ND	Pdcd2	Zfrp8	PDCD2	pdcd2
eS6/Rps6	Nap1[b]	nap-1	Nap1l1	Nap1	NAP1L1	nap1l4a
uS11/Rps14[a]	Fap7[a]	E02H1.6	Ak6	Ak6	AK6	zgc:86811
uS19/Rps15[a]	Sef1[a]	ND	ND	ND	ND	ND
eS26/Rps26	Tsr2	Y51H4A.15	Tsr2	CG14543	TSR2	Tsr2
60S proteins						
uL2/Rpl2[a]	Nap1	nap-1	Nap1l1	Nap1	NAP1L1[a]	nap1l4a
uL3/Rpl3	Rrb1	Y54H5A.1	Grwd1	l(2)09851	GRWD1	grwd1
uL4/Rpl4	Acl4	ND	ND	ND	ND	si:dkey-12j5.1
uL5/Rpl11	Syo1[b]	ND	Heatr3	CG10286	HEATR3	heatr3
uL14/Rpl23	Bcp1	ZK1127.4	Bccip	CG9286	BCCIP	bccip
uL16/Rpl10	Sqt1	Y111B2A.12	Aamp	CG5114	AAMP	aamp
uL18/Rpl5	Syo1[b]	ND	Heatr3	CG10286	HEATR3	heatr3
eL39/Rpl39	Nap1[b]	nap-1	Nap1l1	Nap1	NAP1L1	nap1l4a
eL42/Rpl42	Nap1[b]	nap-1	Nap1l1	Nap1	NAP1L1	nap1l4a
eL43/Rpl43	Puf6/Loc1	puf-12/ND	Pum3/ND	Peng/ND	PUM3/ND	ND/ND

Abbreviation: ND, not determined.

[a]Possible personalized chaperones.

[b]Chaperones interacting with multiple targets.

Typically, although not universally, these chaperones are conserved from yeast to humans (**Table 1**), and their deletion or depletion produces cell growth phenotypes that can be rescued by overexpression of the client RP (Black et al. 2019, Koch et al. 2012, Pausch et al. 2015, Pillet et al. 2015, Rossler et al. 2019, Schutz et al. 2018, Ting et al. 2017, Yang & Karbstein 2022, Yang et al. 2016). These chaperones have been suggested to deliver their client RPs to nascent ribosomes, which has been demonstrated for Tsr2 and eS26 (Rps26) and for Yar1 and uS3 (Rps3) (Ghalei et al. 2015, Yang & Karbstein 2022). In addition, chaperones might regulate nuclear import of some RPs (Iouk et al. 2001, Koch et al. 2012, Kressler et al. 2012, Mitterer et al. 2016, Pillet et al. 2015, Schutz et al. 2014, Stelter et al. 2015, Ting et al. 2017) and ensure the stoichiometric assembly of RPs into ribosomes (Kressler et al. 2012). Interestingly, while many RPs with chaperones are universally conserved, chaperones are not known to exist in bacteria. Moreover, many RPs with personalized chaperones [except uL4 (Rpl4) and eL42 (Rpl42)] are located on the periphery of the ribosome (**Figure 1*a***). Together, these features suggest that a critical role of the chaperones is not the stabilization of their client proteins. Indeed, we have recently shown that the eS26 (Rps26) chaperone Tsr2 and the uL16 (Rpl10) chaperone Sqt1 can release their client proteins from ribosomes under specific conditions, allowing for ribosome remodeling or repair (Yang & Karbstein 2022, Yang et al. 2023).

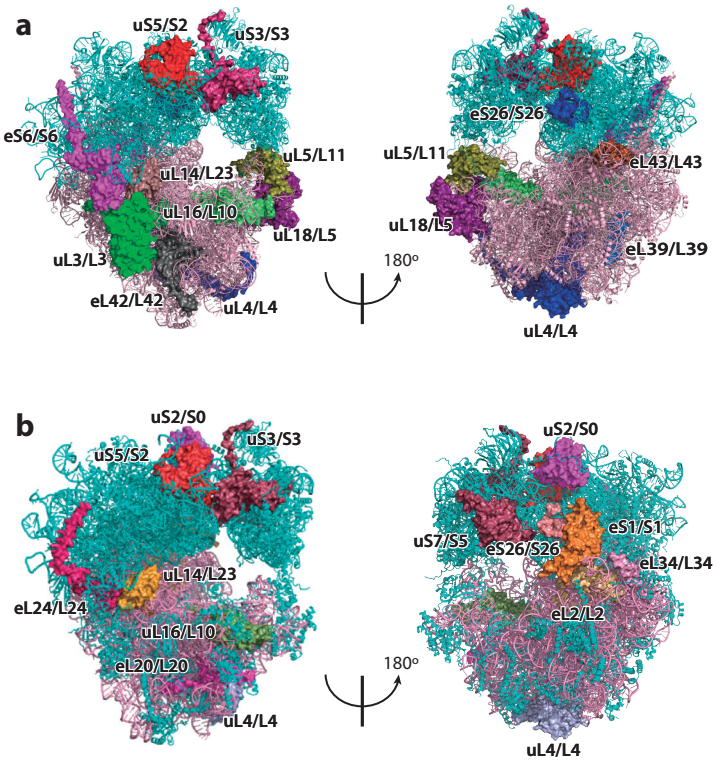

Figure 1

The location of RPs with chaperones or evidence of damage. (*a*) RPs with known personalized chaperones are highlighted in multiple colors and in space-filling models of yeast 80S ribosomes (PDB ID 4V88). The small ribosomal subunit is shown in cyan and the large subunit in pink. (*b*) Frequently oxidized RPs are highlighted in multiple colors and depicted as space-filling models, as in panel *a*. Abbreviations: PDB, Protein Data Bank; RPs, ribosomal proteins.

RIBOSOMES ARE EXTREMELY STABLE

Ribosome Half-Lives

The stability of ribosomes can be measured by the stability of their constituent RPs or rRNAs. While both contribute to the stability of ribosomes, the half-lives of different RPs can vary, suggesting that a subset of RPs are exchangeable (see the section titled Ribosomes Can Be Repaired). In addition, unassembled RPs are rapidly degraded (Ju et al. 2023, Shigeoka et al. 2019, Sung et al. 2016). Therefore, the stability of ribosomes is likely better represented by long-lived RPs rather than by an average half-life of all RPs, which includes RPs outside of ribosomes. On the other hand, the half-lives of rRNAs, which form the ribosome core and cannot be exchanged without entirely disassembling (and thus likely degrading) the ribosome, may directly represent ribosome stability.

As detailed with several examples below, the ribosome half-life generally exceeds 100 h, with RPs presenting longer half-lives than rRNAs. These differences in the apparent stability of RPs and rRNAs might be experimental artifacts, or they might indicate that some RPs are recycled. Importantly, these half-lives are much longer than the doubling times of the cells that they are found in, indicating that ribosome populations are turned over by cell division and not by the decay of ribosomes. In addition, the average half-lives for RPs from the small subunit are generally longer than those for the large subunit, indicating that small subunits are more stable, perhaps simply reflecting fewer opportunities to incur damage due to their smaller size.

A proteomic analysis using *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* has shown that a number of RPs (including uS2, eS27, uL24, and eL31 in *S. cerevisiae*) have a half-life exceeding 100 h, providing a lower limit for the half-life of ribosomes in yeast (Christiano et al. 2014). Similarly, no degradation was observed for 18S (~3.5 h) and 25S (~2.5 h) rRNA (Cole et al. 2009), although the timescale of the experiment was very short.

Compared with higher eukaryotic organisms, it is difficult to measure the half-life of ribosomes in bacteria due to their short doubling time (~20 min in *Escherichia coli*) relative to the stability of ribosomes. Yet, studies again suggest that their stability far exceeds the doubling time of bacteria (Meselson et al. 1964). Moreover, in *E. coli*, ribosomes are degraded under certain stress conditions, including entrance into the stationary phase (reviewed in Deutscher 2003).

In *Caenorhabditis elegans*, the half-lives of RPs in young worms varied around ~100 h [with the longest-lived from each subunit being uS14 (~118 h) and eL28 (~140 h)]. However, in aged worms, the numbers drastically changed, now varying between different RPs and ranging from 7 h to 660 h (eS8, ~660 h; uL15, ~420h) (Dhondt et al. 2017).

In *Drosophila melanogaster*, average RP half-lives were initially measured as ~200 h [with the longest-lived from each subunit being uS10 (~270 h) and uL3 (~220 h), respectively (Vincow et al. 2013)]. A subsequent study showed that, just as in *C. elegans*, the half-life of RPs in young flies (eS6, ~265 h; eL15, ~315 h) is shorter than in older flies (eS6, ~310 h; uL1, ~345 h), with average half-lives of ~189 h and ~210 h in young and older flies, respectively (Vincow et al. 2021).

Finally, a correlation between ribosome half-life and age was observed in ribosomes from rat brains. Total ribosomes (RNA and protein) turned over with a half-life of ~18 days (~15.7 days for rRNA and ~21.4 days for RPs) for 24-month-old animals, while for younger (12-month-old) animals the ribosome half-life averaged ~7 days [~8.2 days for rRNA and ~6.7 days for RPs (Menzies & Gold 1972)]. In contrast, no significant age-dependent difference was observed in other rat tissues, including liver (5.9 or 6.1 days), kidney (6.5 or 6.4 days), lung (9.0 or 10.2 days), spleen (8.6 or 9.1 days), and intestinal mucosa (8.1 or 8.3 days) (Menzies et al. 1972). Moreover, the half-life of rRNA in male brains (~12 days) was significantly longer than in females (~7 days) (Novakovic et al. 1979). Whether this finding represents premature aging in male rats, or

intrinsic differences in the stability of ribosomes, remains unknown. Together, these observations from three distinct organisms suggest that ribosomes might be stabilized in aging animals, which downregulate the production of ribosomes (D'Aquila et al. 2017, Jung et al. 2015). The prolonged stability of ribosomes may also require the repair of damaged subunits, potentially explaining why the half-lives of different RPs start to vary in aged animals. Thus, the stability of ribosomes appears to be regulated to respond to the cellular demand for ribosomes and the ability to assemble new ribosomes. In addition, high levels of ribosome damage in different tissues or growth conditions might lead to increased degradation of damaged ribosomes (Cole et al. 2009, LaRiviere et al. 2006). Therefore, a fundamental question is how ribosomes are selectively degraded or stabilized under different cellular conditions.

Cells with Long-Lived Ribosomes

As indicated above, there is evidence for tissue-specific differences in ribosome half-lives. Studies carried out with both rat livers and brains show faster turnover of ribosomes in livers than in brains (Dawson 1967, Retz & Steele 1980, Stoykova et al. 1983). Moreover, the half-lives of RPs in rat brains vary within a wider range (3–10 days), suggesting the exchange of a subset of RPs and perhaps the repair of long-lived ribosomes that become damaged (Dorrbaum et al. 2018).

Moreover, Mathieson et al. (2018) have analyzed the RP half-life in four nondividing human cell types [B cells, monocytes, natural killer (NK) cells, and hepatocytes]. Interestingly, while on average the RP half-life was ~100 h, in NK cells the half-life was ~520 h. Similarly, the longest-lived RPs varied, with NK cells having the slowest turnover (eS12, ~1,020 h; P0, ~830 h), followed by hepatocytes (uS14, ~410; eL42, ~200 h), monocytes (uS8, ~155 h; eL34, ~510 h), and B cells (eS27, ~130 h; eL22, ~130 h). rRNA stability similarly varied between 72 and 700 h, depending on the cell type (Liebhaber et al. 1978).

An extreme example might be presented by oocytes, where ribosomes (and mRNAs) made during the fetal development of the animal are stored in a translational resting state (Abelson et al. 1974, Bachvarova & De Leon 1977). Depending on the animal, these dormant ribosomes can be stored for years or decades, becoming activated and transitioning to actively translating polysomes after fertilization (Leesch et al. 2023). Dormant ribosomes in zebrafish are associated with Habp4-eEF2 and Dap1b/Dap-eIF5a, which stabilize 80S ribosomes and repress translation. Habp4 (SERBP1 or Stm1 in humans or yeast, respectively) knockout animals, but not Dap1 knockout animals, have fewer ribosomes, suggesting the importance of Habp4 for ribosome stability in oocytes.

As in oocytes, other studies have similarly shown that ribosomes are typically stored in 70S or 80S complexes rather than individual subunits. In bacteria, RaiA, HPF, RMF, and LHPF are used to preserve ribosomes in 70S or 100S (two 70S) complexes (Prossliner et al. 2018). In eukaryotes, Stm1, IFRD2, and Lso2 are used to store ribosomes in 80S complexes to prevent translation and stabilize ribosomes (Ben-Shem et al. 2011, Brown et al. 2018, Wells et al. 2020). One advantage of this arrangement might be that if these complexes are damaged and then decayed, the ratio of small and large subunits will be maintained. In addition, and likely even more importantly, subunit joining reduces the surface area of the ribosome, likely protecting the subunits from damage. Indeed, eS26 (Rps26) in 80S complexes is less prone to oxidation and decay relative to eS26 (Rps26) in 40S subunits, consistent with its location at the subunit interface (Yang et al. 2023).

CHEMICAL DAMAGE TO RIBOSOMES

Ribosomal Protein Damage by Oxidative Stress

Reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), are ubiquitous in cells. They cause oxidative stress by damaging cellular

components, including protein and RNA (Apel & Hirt 2004, Cross et al. 1987, Imlay 2003). In proteins, cysteine, histidine, methionine, tryptophan, and tyrosine are all sensitive to oxidative stress (Sies 1986). Nonetheless, both because cysteine is most readily oxidized (Shacter 2000) and because its oxidation can serve signaling functions (Paulsen & Carroll 2013), its oxidation has been the most extensively characterized (Reddie & Carroll 2008), using a variety of probes that take advantage of cysteine's reactivity and covalently attach themselves to cysteine or its oxidized forms (Shi & Carroll 2020).

RPs, and specifically their cysteine residues, have been identified as targets of oxidation in various species (Fu et al. 2017, Leichert et al. 2008, Meng et al. 2021, Petrova et al. 2018, Topf et al. 2018, van der Reest et al. 2018), which is perhaps not surprising given the abundance of ribosomes. Moreover, by comparing previous studies using various eukaryotic model organisms, we noticed that specific RPs were frequently oxidized in many species, conserving even oxidation sites in some cases (Yang et al. 2023). Interestingly, the most frequently oxidized RPs [uS2 (Rps0), eS1 (Rps1), uS5 (Rps2), uS3 (Rps3), uS7 (Rps5), and eS26 (Rps26) from the 40S subunit and uL4 (Rpl4), uL16 (Rpl10), eL20 (Rpl20), uL14 (Rpl23), eL24 (Rpl24), and eL34 (Rpl34) from the 60S subunit] are all located in functionally important regions (**Figure 1*b***) (**Table 2**). Four of the six oxidized small subunit proteins [uS5 (Rps2), uS3 (Rps3), uS7 (Rps5), and eS26 (Rps26)] line the mRNA binding channel; from the large subunit, one [uL16 (Rpl10)] is located in the peptidyl transferase center; one [uL4 (Rpl4)] lines the peptide exit tunnel; and three proteins, from both subunits [eS1 (Rps1), uL14 (Rpl23), and eL24 (Rpl24)], are located at the subunit interface. These locations might simply reflect their accessibility to solvent and thereby ROS, but they also suggest that the oxidation of these RPs might adversely affect ribosome function. Interestingly, half of these RPs are known to have a personalized chaperone [uS5 (Rps2), uS3 (Rps3), eS26 (Rps26), uL4 (Rpl4), uL16 (Rpl10), and uL14 (Rpl23)]. In addition, uS11 (Rps14) and uL2 (Rpl2), RPs with chaperone candidates, are oxidized, and Fap7, the chaperone candidate for uS11 (Rps14), is important for the oxidative stress response in yeast (Juhnke et al. 2000).

Other RPs that appear to be selectively prone to oxidation are those with zinc-finger motifs. Six out of nine RPs with bound zinc [eS26, eS27, uS14 (Rps29), eS31, eL34, eL37, el40, eL42, and eL43] are found to be oxidized, likely reflecting the high susceptibility of cysteines in zinc fingers to oxidation (Topf et al. 2018).

Similarly, in the bacterial model system *E. coli*, specific RPs are prone to oxidation (Leichert et al. 2008, Xie et al. 2019). Leichert et al. (2008) observed the cysteine oxidation of uS4, uS17, uL14, and bL31 in *E. coli* treated with either H_2O_2 or hypochlorite. Interestingly, three of these RPs (uS17, uL14, and bL31) were also identified as being oxidized during phagocytosis, along with six additional RPs (bS1, uS2, uS12, uS13, bS21, and bL35) (Xie et al. 2019). Out of these nine RPs, six are universally conserved, and four of them (uS2, uS13, uS17, and uL14) are also oxidized in eukaryotic systems (Yang et al. 2023). Notably, while bS21 and eS26 (Rps26) share no discernable homology, they bind at the same location on the ribosome and both RPs are oxidized, again suggesting that accessibility to ROS might be a main driver of susceptibility to oxidation.

The oxidation of the other sulfur-containing amino acid, methionine, affects L7/L12 (for bL12, note that L7 and L12 are both encoded by the same gene but differ by the presence and absence of an N-terminal acetylation) binding to ribosomes in *E. coli* (Caldwell et al. 1978, Gudkov & Behlke 1978). While two L7/L12 dimers bind per ribosome in *E. coli* (Diaconu et al. 2005), H_2O_2 treatment converts the dimerized L7/L12 to a monomer by oxidizing methionine and weakening the binding of one monomer to ribosomes (Caldwell et al. 1978, Gudkov & Behlke 1978), which decreases EF-G binding (Diaconu et al. 2005, Koteliansky et al. 1978). Similarly, a study in *Arabidopsis thaliana* showed that methionine oxidation in RPs increased in

Table 2 Oxidized ribosomal proteins with evidence of exchange

Ribosomal protein	Cysteine oxidation[a]	Exchange	Chaperone[a]	Zinc finger
40S proteins				
eS1 (Rps1)	**Eukaryotes**	Eukaryotes		
uS2 (Rps0)	**Bacteria, eukaryotes**	Bacteria		
uS3 (Rps3)	**Eukaryotes**	Eukaryotes	Eukaryotes	
uS5 (Rps2)	**Eukaryotes**	Bacteria	Eukaryotes	
uS8 (Rps22)	Eukaryotes	Eukaryotes		
uS11 (Rps14)	Eukaryotes	Eukaryotes	(Eukaryotes)	
eS19 (Rps19)	Eukaryotes	Eukaryotes		
bS21	Bacteria	Bacteria		
eS26 (Rps26)	**Eukaryotes**	Eukaryotes	Eukaryotes	Eukaryotes
60S proteins				
uL1 (Rpl1)	Eukaryotes	Bacteria, eukaryotes		
uL2 (Rpl2)	Eukaryotes	Eukaryotes	(Eukaryotes)	
uL3 (Rpl3)	Eukaryotes	Eukaryotes	Eukaryotes	
uL4 (Rpl4)	**Eukaryotes**	ND	Eukaryotes	
uL5 (Rpl11)	Eukaryotes	Bacteria	Eukaryotes	
uL10 (Rpp0)	Eukaryotes	Bacteria, eukaryotes		
uL11 (Rpl12)	Eukaryotes	Eukaryotes		
eL13 (Rpl13)	Eukaryotes	Eukaryotes		
uL14 (Rpl23)	**Bacteria, eukaryotes**	Eukaryotes[b]	Eukaryotes	
uL16 (Rpl10)	**Eukaryotes**	Eukaryotes	Eukaryotes	
uL18 (Rpl5)	Eukaryotes	ND	Eukaryotes	
eL21 (Rpl21)	Eukaryotes	Eukaryotes		
eL24(Rpl24)	**Eukaryotes**	Eukaryotes		
bL31	Bacteria	Bacteria		Bacteria
eL34 (Rpl34)	**Eukaryotes**	Eukaryotes		Eukaryotes
eL36 (Rpl36)	Eukaryotes	Eukaryotes		
eL38 (Rpl38)	Eukaryotes	Eukaryotes		
eL42 (Rpl42)	#[c]	Eukaryotes	Eukaryotes	Eukaryotes
eL43 (Rpl43)	Eukaryotes	ND	Eukaryotes	Eukaryotes
Rpp1	Eukaryotes	Eukaryotes		
Rpp2	(Eukaryotes)	Eukaryotes		

Abbreviation: ND, not determined.

[a]Parentheses indicate that the ribosomal protein has a suggested chaperone.

[b]Data from Y.-M. Yang & K. Karbstein (unpublished observations).

[c]The number sign (#) indicates cysteine-containing peptides that are too small to be detected by mass spectrometry, precluding the detection of oxidized eL42/Rpl42. The most frequently oxidized ribosomal proteins appear in bold.

the catalase knockout plant (Jacques et al. 2015). Yet, whether and how ribosome function is altered from the methionine oxidation of RPs, and whether these RPs are repaired by methionine sulfoxide reductases (Moskovitz 2005), remain to be addressed.

Together, these observations suggest that certain RPs are more prone to oxidation than others, likely due to their accessibility to ribosome ligands, which also exposes them to solvent. Because of the functional importance of these residues, it is likely that their oxidation might result in compromised ribosome activity, explaining why a repair function is needed.

rRNA Damage by Oxidative Stress

While cysteine oxidation in proteins is caused by the relatively stable H_2O_2 (Gough & Cotter 2011, Kiley & Storz 2004), RNA is most likely targeted by the highly reactive •OH, which is generated by Fe^{2+} and H_2O_2 in the so-called Fenton reaction. Of the various types of base damage caused by •OH (Barciszewski et al. 1999), 8-oxo-7,8-dihydroguanosine (8-oxoG) is the most frequently observed chemical modification, presumably due to the relatively low redox potential of guanine (Steenken & Jovanovic 1997). While the presence of RPs protects guanosine residues in rRNA from oxidation to 8-oxoG (Estevez et al. 2021), 8-oxoG in rRNA has been observed in several studies (Shcherbik & Pestov 2019). Together, these observations suggest that regions without bound RPs should be more prone to oxidation. However, such recurring oxidation sites have not (yet) been identified.

While the alkylation of both DNA and RNA is repairable in an enzymatic manner (Aas et al. 2003), whether oxidized guanosines in rRNA (or other RNAs) can be repaired or reduced remains unknown. mRNAs containing 8-oxoG are recognized by the ribosomal collisions they incur and then degrade (Simms et al. 2014, Yan et al. 2019), perhaps suggesting that 8-oxoG in RNA cannot be repaired or reduced, even though (*a*) RNA is more susceptible to this damage than DNA both in vitro (Hofer et al. 2006) and in vivo (Hofer et al. 2005) and (*b*) a repair system for oxidized guanine in DNA has been identified (Poetsch 2020).

While •OH can cause oxidative damage by chemically modifying rRNA, it can also cleave the rRNA backbone. Interestingly, the Shcherbik and Pestov groups have shown that increased levels of endogenous oxidative stress cause cleavage at a specific site in 25S rRNA (Shedlovskiy et al. 2017). In a follow-up study, they provided evidence that this site-specific cleavage arises in an Fe^{2+}-dependent manner (Zinskie et al. 2018). The Fe^{2+}-dependent cleavage was blocked by the addition of Mn^{2+}, suggesting that there is a binding site for Fe^{2+} that can be blocked by Mn^{2+}, which causes cleavage by locally generated •OH (Smethurst et al. 2020). Finally, the authors observed similar patterns of degradation in vivo and in vitro for other rRNAs (18S, 5.8S, and 5S). Together, these observations suggest that site-specific Fe^{2+}-dependent oxidation–mediated cleavage occurs within rRNA.

RNA damaged by backbone cleavage can be repaired via ligation. Bacterial RtcB and its homologs in eukaryotes (Trl1 in yeast and HSPC117 in humans) catalyze the ligation of RNAs with a 2′,3′-cyclic phosphate and 5′-OH. Its function is important for the maturation of transfer RNAs and the XBP1 mRNA in mammalian cells (Jurkin et al. 2014, Lu et al. 2014, Popow et al. 2011). RtcB is conserved in prokaryotes but not in all eukaryotes, where plants lack this enzyme (Englert et al. 2011, Popow et al. 2011, Tanaka & Shuman 2011). Interestingly, RtcB is also activated by multiple stress-causing agents, especially those that cause oxidative stress (Engl et al. 2016). Capillary electrophoresis experiments with *E. coli* ribosomes from RtcB knockout cells show the accumulation of site-specifically cleaved rRNA, suggesting that there are cleavage-prone sites in *E. coli* rRNA and that the Rtc system is important for its repair and, by extension, rRNA stability (Engl et al. 2016). Another study showed that RtcB accelerated the repair of rRNA (Manwar et al. 2020). Interestingly, rRNA cleavage and religation were observed during and after antibiotic stress, respectively (Manwar et al. 2020), suggesting that rRNA cleavage may be caused by ROS generated during antibiotic treatment (Dwyer et al. 2014, Kohanski et al. 2007). Additionally, RtcB was suggested to function in ribosome repair during recovery from stress (Temmel et al. 2017). In *E. coli*, the endoribonuclease MazF cleaves 16S rRNA, thereby removing its 3′ end, which contains the anti-Shine-Dalgarno sequence (Vesper et al. 2011), thereby inactivating ribosomes against mRNAs whose translation is driven by a Shine-Dalgarno sequence. RtcB reverses this inactivation by the religation of the 16S rRNA 3′ end (Temmel et al. 2017). Since rRNA repair by

RtcB has been demonstrated only in *E. coli*, it needs to be elucidated whether this rRNA repair system is conserved in eukaryotes, which should be greatly aided by a recent direct sequencing methodology that detects the products of repair (White et al. 2023).

rRNA Damage by Cisplatin

Cisplatin is one of the most widely used chemotherapy drugs, and it functions by intercalation into DNA (Wang & Lippard 2005). However, cisplatin can target other cellular molecules, including RNA, to which it covalently attaches (Jamieson & Lippard 1999). Indeed, cisplatin accumulates in rRNA from both prokaryotic and eukaryotic ribosomes (Dedduwa-Mudalige & Chow 2015, Hostetter et al. 2012, Melnikov et al. 2016, Osborn et al. 2014, Plakos & DeRose 2017, Rijal & Chow 2009). Specifically, experiments in *S. cerevisiae* show that cisplatin accumulates ~4–20-fold more in RNA than DNA as well as ~4–6-fold more in rRNA than in mRNA (Hostetter et al. 2012), which is likely at least partially a reflection of the high abundance of rRNA. Moreover, specific platinated sites in rRNA have been identified, including functionally important sites (Dedduwa-Mudalige & Chow 2015, Hostetter et al. 2012, Melnikov et al. 2016, Osborn et al. 2014, Plakos & DeRose 2017, Rijal & Chow 2009). These include the peptidyl transferase center and the intersubunit region of 25S rRNA (Osborn et al. 2014, Plakos & DeRose 2017), as well as the mRNA channel of 18S rRNA (Hostetter et al. 2012). Similarly, in prokaryotic ribosomes, cisplatin targets the mRNA channel, subunit interface, and GTPase center of 23S rRNA (Melnikov et al. 2016, Rijal & Chow 2009). Finally, cisplatin accumulates at helix 69 of 23S rRNA in bacteria (Dedduwa-Mudalige & Chow 2015) and functions by inhibiting ribosome recycling (Borovinskaya et al. 2007). What happens to these platinated ribosomes remains unknown.

Ultraviolet Damage

Like oxidative stress, UV light can cause chemical modifications of RNA (Wurtmann & Wolin 2009). Interestingly, UV-induced lesions of mouse 28S rRNA were observed in a site-specific and dose-dependent manner (Iordanov et al. 1998). Because the ribosome active site was particularly affected, its translation activity was decreased (Iordanov et al. 1998).

UV damage also leads to ribosome collisions, but whether these arise solely from damage to the mRNA and/or the ribosome itself remains to be determined (Stoneley et al. 2022, Wu et al. 2020). Notably, it appears that collided complexes both sensitive and resistant to resolution by Asc-1/Rqt are formed (Stoneley et al. 2022), consistent with a model in which some collisions arise due to mRNA damage, which should allow them to be resolved by Asc-1/Rqt, while others may represent damaged ribosomes, whose collided complexes do not respond to Rqt (Parker et al. 2024).

Finally, UV stress can lead to RNA-RNA or RNA-protein cross-linking (Wurtmann & Wolin 2009). uS11, uL23, eL29, and eL32 cross-linked to RNA in UV-exposed plants (Casati & Walbot 2004), and a subset of RPs (eS1, eS6, uL18, and eL6) were cross-linked to mRNA after UV treatment in rat liver polysomes (Takahashi & Ogata 1981). These results suggest that RP-mRNA cross-linking could arise during translation under UV stress, which may trigger ribosome stalling and the subsequent degradation of these ribosomes. Importantly, this does not seem to account for the Asc-1/Rqt-resistant collided disomes (Stoneley et al. 2022).

Other Types of Ribosome Damage

While ribosomes are targets of various posttranslational modifications (including ubiquitylation, phosphorylation, acetylation, and methylation), the functional effects of these modifications remain mostly unclear (Lee et al. 2002, Nesterchuk et al. 2011, Odintsova et al. 2003, Yu et al. 2005).

While many efforts are focused on identifying these modifications as causes of functional ribosome heterogeneity (Gay et al. 2022), it appears likely that in many, perhaps most, cases, these modifications are nonspecific and simply reflect the extreme cellular abundance of ribosomes. In addition, modifications that disrupt ribosome function and consequentially cause disease (Simsek & Barna 2017) should be considered ribosome damage.

While whether and which posttranslational modifications cause ribosome damage require clarification, Young et al. (2012) observed the methylation of one of the zinc-binding cysteine residues in eS27, a zinc-finger protein. Because cysteine methylases have not been discovered, the authors suggested that this modification occurs in a nonenzymatic but zinc-dependent manner, as in the case of the *E. coli* Ada protein (He et al. 2005). Cysteine methylation in eS27 may lead to the release of its bound zinc, as we have shown for the oxidation of analogous cysteines in eS26 (Yang et al. 2023). While the structural disorder caused by zinc release recruits Tsr2 for the release of eS26 from ribosomes (see below), a personalized chaperone for eS27 has not (yet) been identified. Nevertheless, cysteine demethylase activity has not been described, suggesting that this lesion must be managed by degradation.

CONSEQUENCES OF RIBOSOME DAMAGE

Ribosome Degradation

Above we describe observations demonstrating that ribosomes are exceptionally long-lived. This long life should allow for ample opportunity to incur damage to both the rRNA and RPs, and, as we also describe above, there is substantial evidence for such damage. These observations lead to the question of how cells deal with damaged ribosomes. While no response might be required for a subset of the damaged sites, because they do not alter the structure of the function of the molecule, consideration of their location or data in the literature support the functional impairment of the ribosome arising from many of the documented damages, as discussed above.

Data in the literature indicate that nonfunctional 18S and 25S rRNAs are rapidly degraded (Cole et al. 2009, LaRiviere et al. 2006), providing for a paradigm where nonfunctional ribosomes are selectively removed from cells. It is believed that these genetically encoded, nonfunctional ribosomes are stalled at the start codon (Garshott et al. 2021, Sugiyama et al. 2019), because they either cannot load an incoming amino acid or catalyze the addition of that second amino acid to the starting methionine. However, if damage is incurred during an ongoing round of translation, then these ribosomes might slow down or even stall inside the open reading frame of the mRNA. This would lead to collisions with the subsequent ribosomes (Simms et al. 2017). Indeed, we have shown that genetically encoded, partially functional ribosomes (which can translate but move more slowly) invite collisions with fully functional ribosomes and are then degraded (Parker et al. 2024). This depends on the presence of functional ribosomes. Thus, one way in which damage might be recognized is via collisions, mirroring the way that ribosomes detect damage in mRNAs (D'Orazio & Green 2021, Yan & Zaher 2019, Yan et al. 2019). A potential downside of this mechanism would be that extensive damage could thereby significantly lower ribosome numbers, with effects on protein homeostasis and cell growth (Ivanov et al. 2022, Mills & Green 2017).

Ribosomes Can Be Repaired

Given the impressive stability of ribosomes, the large amount of cellular resources that are required to assemble ribosomes, and the extensive oxidative damage to RPs, the repair of damaged ribosomes might be an alternative to their degradation that enables the maintenance of ribosome numbers and preserves the energetic investment that went into ribosome assembly, while safeguarding ribosome integrity.

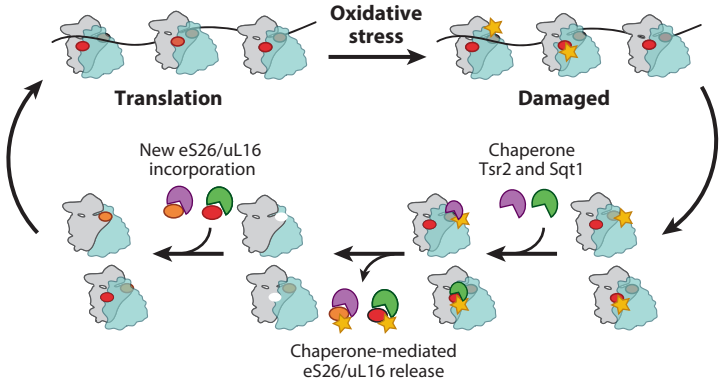

Figure 2

Model for ribosome repair. Oxidative stress preferentially damages eS26/Rps26 and uL16/Rpl10 in yeast. Ribosomes containing the damaged proteins are removed from the polysomes and converted into idle 80S complexes, from which Tsr2 and Sqt1 release eS26/Rps26 and uL16/Rpl10, respectively. Newly made eS26/Rps26 and uL16/Rpl10 are then incorporated into these ribosomes to repair the lesion and allow for the resumption of translation by these subunits. Figure adapted with permission from Yang et al. (2023).

Utilizing a probe that enables the labeling of oxidized cysteines in live cells, we showed that eS26 (Rps26) and uL16 (Rpl10) are preferentially oxidized and that this impairs their function. To mitigate the harm from dysfunctional ribosomes, the damaged proteins are then selectively released from ribosomes via their chaperones Tsr2 and Sqt1, respectively, to allow for their relatively rapid turnover. The eS26- or uL16-deficient ribosomes are then repaired with newly made RPs. Our data indicate that these processes occur within idle 80S ribosomes (**Figure 2**) (Yang et al. 2023). Together, these data reveal for the first time that ribosomes are extensively and site-specifically damaged via cysteine oxidation and that this damage can be repaired, conserving ribosome numbers and cellular resources. Thus, the novel repair mechanism is akin to the repair of damaged photosystem complexes by the replacement of individual subunits (Tikkanen et al. 2014).

We speculate that the evolution of this repair machinery was necessary because oxidative stress also damages mRNA, which is detected in a translation-dependent manner (Simms et al. 2014, Yan et al. 2019). If damaged ribosomes were degraded, it would reduce ribosome numbers enough to potentially impair the clearance of damaged mRNAs, which is concentration dependent (Simms et al. 2017).

Other Examples of Chaperone-Mediated Ribosomal Protein Repair?

While we have observed the chaperone-dependent release of eS26, uL16 (Rpl10) (Yang et al. 2023), and ul14 (Rpl23) (Y.-M. Yang & K. Karbstein, unpublished observations) under oxidative stress, no release by their personalized chaperones was observed for uS5 (Rps2), uS3 (Rps3), or eL43 (Rpl43) (Y.-M. Yang & K. Karbstein, unpublished observations). Interestingly, while this finding aligns with the resistance of these RPs to oxidation in yeast (Topf et al. 2018, Yang et al. 2023), the fact that these RPs were sensitive to oxidation in other species suggests the possibility that the chaperone-dependent repair of these RPs may exist in other eukaryotic systems, or may occur after distinct physiological forms of damage.

While these are the only confirmed cases of ribosome repair, data in the literature suggest several other instances of repair. First, two independent mass spectrometry–based studies observed some locally translated RPs to be incorporated into ribosomes in the dendrites of neuronal cells

(Fusco et al. 2021, Shigeoka et al. 2019). Moreover, using mass spectrometry, Fusco et al. (2021) showed that some newly made RPs appear in assembled ribosomes faster than the rest, indicating incorporation into premade, assembled ribosomes under oxidative stress [eS30 (Rps30), RACK1, uL10 (P0), Rplp2, and uL16] (**Table 3**). While it is difficult to entirely rule out artifacts from pools of free RPs and differences in turnover rates in such studies, the subset of exchangeable RPs includes eS26 (Rps26) and uL16 (Rpl10), which are repaired in yeast (Yang et al. 2023), as well as other RPs with (suggested) personalized chaperones, including uL3 (Rpl3), uS11 (Rps14), uS19 (Rps15), and uL2 (Rpl2). While this finding suggests that ribosome repair may occur in neuronal cells, whether such repair requires chaperones and whether chaperones are also locally translated or axonally transported (Maday et al. 2014) need to be confirmed.

In addition, Leesch et al. (2023) observed the chaperone Nap1l4a (possible chaperone for eS6, uL2, eL39, and eL42) (**Table 1**) bound to ribosomes, and this binding decreased during fertilization. This observation is consistent with a model whereby these chaperones detect damage in their client RPs and repair the damaged ribosomes after fertilization, when new RPs are translated. More interestingly, a subset of RPs showed significantly altered levels during fertilization. These RPs include eS17, uS19, eS26, eS27, eS30, eS31, uL11, eL28, eL29, eL37, eL40, P1, and P2. While many of these overlap with oxidized RPs, they also include five zinc-containing RPs (**Table 2**), again indicating repair of these proteins after fertilization.

The Repair Machinery Can Be Used for Ribosome Remodeling

As described above, we hypothesize that the requirement for ribosome repair might have driven the evolution of at least a subset of RP chaperones, which can both extract and deliver RPs. However, there is also evidence that these abilities might be utilized under different cellular stress conditions to rapidly and reversibly remodel ribosomes (Yang & Karbstein 2022).

It has been suggested that different ribosome populations might exist within cells to preferentially translate different subsets of mRNAs (reviewed in Ferretti & Karbstein 2019,

Table 3 Ribosomal proteins with evidence for exchange

Ribosomal protein	Exchange	Cysteine oxidation[a]	Virus homolog	Chaperone
40S proteins				
eS1 (Rps1)	Eukaryotes	Eukaryotes		
uS2 (Rps0)	Bacteria	Bacteria, eukaryotes		
uS3 (Rps3)	Eukaryotes	Eukaryotes		Eukaryotes
eS4 (Rps4)	Eukaryotes	ND		
uS5 (Rps2)	Bacteria	Eukaryotes		Eukaryotes
eS7 (Rps7)	Eukaryotes	ND		
uS8 (Rps22)	Eukaryotes	Eukaryotes		
eS10 (Rps10)	Eukaryotes	ND		
uS11 (Rps14)	Eukaryotes	Eukaryotes		Eukaryotes[b]
eS19 (Rps19)	Eukaryotes	Eukaryotes		
bS20	Bacteria	ND	Bacteria	
bS21	Bacteria	Bacteria	Bacteria	
eS26 (Rps26)	Eukaryotes	Eukaryotes		Eukaryotes
eS30 (Rps30)	Eukaryotes	(Eukaryotes)		
RACK1 (Asc1)	Eukaryotes	(Eukaryotes)		

(*Continued*)

Table 3 (*Continued*)

Ribosomal protein	Exchange	Cysteine oxidation[a]	Virus homolog	Chaperone
60S proteins				
uL1 (Rpl1)	Bacteria, eukaryotes	Eukaryotes		
uL2 (Rpl2)	Eukaryotes	Eukaryotes	Bacteria	Eukaryotes[b]
uL3 (Rpl3)	Eukaryotes	Eukaryotes		Eukaryotes
uL5 (Rpl11)	Bacteria	Eukaryotes		Eukaryotes
bL9	Bacteria	ND		
uL10 (Rpp0)	Bacteria, eukaryotes	Eukaryotes	Bacteria	
uL11 (Rpl12)	Eukaryotes	Eukaryotes	Bacteria	
eL13 (Rpl13)	Eukaryotes	Eukaryotes		
uL14 (Rpl23)	Eukaryotes[c]	Bacteria, eukaryotes		Eukaryotes
uL16 (Rpl10)	Eukaryotes	Eukaryotes		Eukaryotes
eL21 (Rpl21)	Eukaryotes	Eukaryotes		
eL22 (Rpl22)	Eukaryotes	ND		
eL24 (Rpl24)	Eukaryotes	Eukaryotes		
eL27(Rpl27)	Eukaryotes	ND		
uL29 (Rpl35)	Eukaryotes	ND		
uL30 (Rpl7)	Bacteria, eukaryotes	ND		
bL31	Bacteria	Bacteria	Bacteria	
eL31 (Rpl31)	Eukaryotes	ND		
bL33	Bacteria	ND	Bacteria	
eL34 (Rpl34)	Eukaryotes	Eukaryotes		
bL36	Bacteria	ND	Bacteria	
eL36 (Rpl36)	Eukaryotes	Eukaryotes		
eL38 (Rpl38)	Eukaryotes	Eukaryotes		
eL42 (Rpl42)	Eukaryotes	ND		Eukaryotes
Rpp1	Eukaryotes	Eukaryotes		
Rpp2	Eukaryotes	(Eukaryotes)		

Abbreviation: ND, not determined.

[a]Parentheses indicate that increased exchange under conditions of oxidative stress has been observed but cysteine oxidation has not been confirmed.

[b]Possible personalized chaperones.

[c]Data from Y.-M. Yang & K. Karbstein (unpublished observations).

Gay et al. 2022, Genuth & Barna 2018, Xue & Barna 2012). While this is an intriguing proposal, there remain limited data for such distinct subsets of ribosomes with distinct functionalities (Ferretti et al. 2017, Shi et al. 2017, Thompson et al. 2016, Werner et al. 2015), and several major conceptual obstacles have been raised (Barna et al. 2022, Ferretti & Karbstein 2019). These include the stability of ribosomes, which slows the turnover of ribosome populations and is exacerbated under stress conditions. Additionally, stress conditions impair ribosome turnover, as they generally block ribosome assembly to make altered ribosomes, as well as cell division, which dilutes ribosomes. Moreover, the observation that ribosome assembly is carefully quality controlled to ensure that only fully assembled ribosomes enter the translating pool (Blomqvist et al. 2023, Ghalei et al. 2017, Huang et al. 2020, Parker et al. 2019), as well as the finding that ribosome misassembly is generally pathogenic (reviewed in Parker & Karbstein 2023), argues

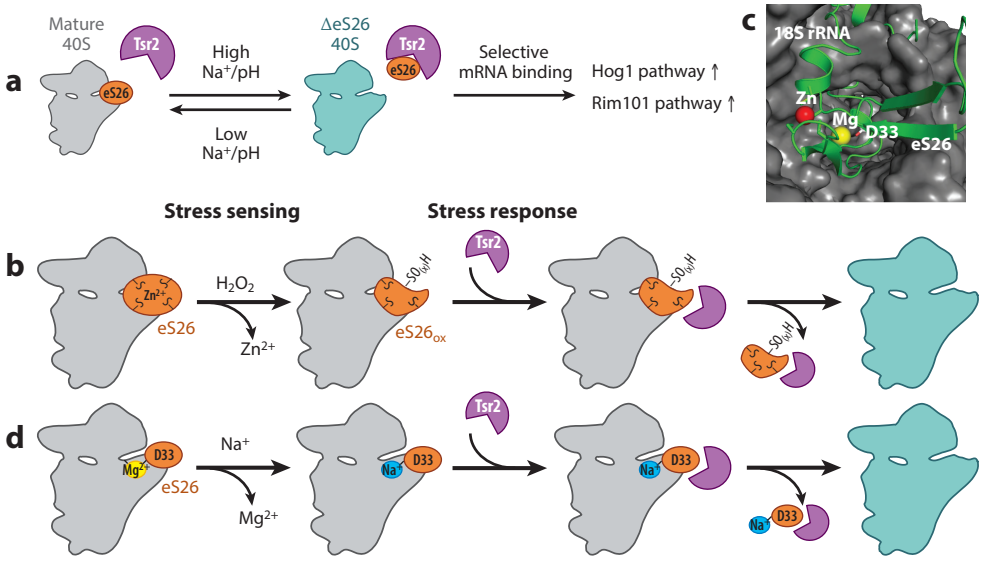

Figure 3

Chaperone-dependent ribosome remodeling. (*a*) Tsr2-dependent release of eS26/Rps26 under high-salt conditions or pH stress produces eS26-deficient ribosomes (Yang & Karbstein 2022), which have altered mRNA selectivity (Ferretti et al. 2017) and support the translation of mRNAs encoding for proteins involved in the response to high salt/pH stress. (*b*) Oxidation of the zinc-finger cysteines in eS26/Rps26 leads to zinc release and partial protein unfolding, thus weakening its binding to ribosomes and enabling its release by Tsr2 (Yang et al. 2023). (*c*) Portion of the structure of eS26/Rps26 within ribosomes (PDB ID 4V88), showing the Zn^{2+} and Mg^{2+} ions that support the folding and binding of eS26/Rps26, respectively. (*d*) Model for the Na^+-dependent release of eS26/Rps26. Mg^{2+} binding to Asp33 is required for eS26 binding (Schutz et al. 2014). Na^+ competes for this Mg^{2+} ion (Yang & Karbstein 2022), weakening eS26/Rps26 binding to ribosomes while strengthening binding to Tsr2 (Schutz et al. 2018) and thus enabling the Tsr2-mediated release of eS26/Rps26 under high-salt conditions. Abbreviations: PDB, Protein Data Bank; rRNA, ribosomal RNA.

against widespread ribosome heterogeneity. Finally, the finding that ribosomes with different elongation speeds are removed via collisions (Parker et al. 2024) suggests limits to heterogeneity. Remodeling of preexisting ribosomes would address these conceptual flaws, as it could be potentially rapid, conservative in terms of ribosome numbers, and regulated, thereby avoiding pathogenic effects. Indeed, we have shown that under conditions of high salt or high pH stress, Tsr2, the chaperone for eS26 (Rps26), which is required for the repair of damaged ribosomes, also extracts eS26/Rps26 from mature, undamaged ribosomes to generate eS26/Rps26-deficient ribosomes (Yang & Karbstein 2022) (**Figure 3*a***). Because eS26 (Rps26) directly interacts with the mRNA upstream of the start codon, in a region that forms part of the Kozak sequence (Hussain et al. 2014), the eS26-deficient ribosomes are blinded from some of the information contained in the Kozak sequence and enrich and translate a different subset of mRNAs relative to the eS26-containing ribosomes (Ferretti et al. 2017). Importantly, mRNAs enriched in eS26-deficient ribosomes encode proteins required for the cellular response to high salt and high pH, and their translation by eS26-deficient ribosomes promotes resistance to these stresses.

How Are Extraction and Incorporation Regulated and Balanced?

If chaperones can both extract and incorporate RPs into ribosomes, then how is this reversible reaction sometimes driven toward incorporation and sometimes toward extraction? Our data

strongly suggest that the relative affinity of ribosomes and chaperones for the RP dictates the directionality of this process (**Figure 3*b–d***). On the one hand, the data indicate that eS26 (Rps26) oxidation leads to the release of a Zn^{2+} ion, which is bound to the four cysteines in eS26 (Yang et al. 2023) (**Figure 3*b***). This is expected to lead to unfolding of the zinc-finger domain stabilized by this Zn^{2+} ion and would render the protein dysfunctional. Because the zinc-finger domain is at the interface between eS26 (Rps26) and rRNA (**Figure 3*c***), the expectation would be that the zincless protein would bind ribosomes weakly. Indeed, we have shown that zincless eS26 (Rps26) cannot be incorporated into ribosomes (Yang et al. 2023). Moreover, while both Na^+ and K^+ can promote eS26 release from ribosomes in vitro, much lower concentrations of Na^+ than K^+ are required, indicating that this is not a general electrostatic effect but rather reflects the specific binding of a monovalent cation. In addition, increasing the Mg^{2+} concentration protects against the Na^+- or K^+-dependent eS26 (Rps26) release (Yang & Karbstein 2022), indicating that the monovalent cations compete with an Mg^{2+} ion that is crucial for eS26 binding (**Figure 3*d***). Indeed, the crystal structure of 40S ribosomes shows an Mg^{2+} ion bridging the interaction between eS26 and rRNA (Ben-Shem et al. 2011) (**Figure 3*c***). Mutating the eS26-derived ligand for this Mg^{2+} ion, Asp33 essentially abolishes eS26 binding to ribosomes (Schutz et al. 2014, Yang & Karbstein 2022), demonstrating the importance of the Mg^{2+} ion for eS26 (Rps26) binding. Finally, biophysical experiments also indicate that high Na^+ concentrations strengthen the Tsr2-eS26 interaction (Schutz et al. 2018), which might further enable eS26 (Rps26) binding to Tsr2 rather than ribosomes. Taken together, these data support a model where high concentrations of Na^+ (or even higher concentrations of K^+) evict a structural Mg^{2+} ion, which is required for eS26 (Rps26) binding, thus weakening eS26 (Rps26) binding to ribosomes while strengthening its interaction with Tsr2 and ultimately allowing for its extraction by Tsr2.

Interestingly, in bacterial ribosomes, differences in rRNA folding at the binding sites of uL1 and uL11 have been observed, which are caused by the availability of Mg^{2+} or Fe^{2+} (Bray et al. 2018). We speculate that these RPs, which are known to exchange both in and out of ribosomes (see the next section), may be extracted in a metal ion–dependent mechanism.

Additional Evidence of Ribosomal Protein Exchange

The exchange of individual RPs out of ribosomes has been observed in several species. For instance, ribosomes from bacteria in the stationary phase contain bL31B and bL36B instead of bL31A and bL36A, respectively (Lilleorg et al. 2019) (**Table 3**). While it has not been shown that the difference involved the exchange of the bL31 and bL36 proteins rather than the replacement of bL31A/bL36A ribosomes, bL31A can be exchanged for bL31B at low pH in vitro. Similarly, pulse-chase experiments with radioactively labeled proteins show the binding of labeled bL31, uL1 (Rpl1), uL5 (Rpl11), uL10 (Rpp0), uL11 (Rpl12), uL30 (Rpl7), uS2 (Rps0), and uS5 (Rps2) as well as bL9, bL33, and bS21 into ribosomes under conditions where ribosome assembly is not detectable (Pulk et al. 2010, Robertson et al. 1977, Subramanian & van Duin 1977). Consistently, in pulse-chase mass spectrometry experiments, N^{15}-labeled bS20, bS21, and bL33 are also detected in ribosomes faster than expected if these ribosomes were produced by de novo ribosome assembly, suggesting that they exchange into preexisting ribosomes and not newly made ribosomes (Chen et al. 2012). While it may be unlikely that all of these proteins are exchangeable, we note that for three of them, bS21, bL31, and bL33, multiple independent studies provide evidence for exchange as described above, bolstering this evidence (**Table 3**). Nonetheless, evidence for the extraction of these RPs, as opposed to spontaneous dissociation and equilibration, has not yet been provided.

Interestingly, some viruses encode their own RP paralogs (e.g., bS6, uS9, uS15, bS20, bS21, uL2, uL10, uL11, bL12, bL19, bL31, bL33) in their limited genomes, suggesting another subset of exchangeable RPs (Mizuno et al. 2019). Six of these putative viral RP paralogs (bS20, bS21, uL10,

uL11, bL31, and bL33) are likely exchangeable. Notably, bS21 and bL31 are observed oxidized and exchangeable and have virus-encoded paralogs, making them strong candidates for ribosome remodeling or repair in prokaryotes.

Finally, a study in yeast that combined cryo-electron microscopy and mass spectrometry to investigate ribosome composition after the switch of the carbon source from glucose to glycerol found increased levels of ribosomes lacking uL16, eS1, uS11, and eS26 (Sun et al. 2021) (**Table 3**). uL16 and eS26 are released by their chaperones for repair (Yang et al. 2023) or remodeling (Yang & Karbstein 2022), and eS1 and uS11 bind directly to eS26. Whether these ribosomes are being repaired due to increased oxidative stress after a carbon shift or remodeled to produce alternate ribosomes, or even degradation intermediates, remains unknown.

In mammalian cells, it has been demonstrated that uL29 (Rpl35), Rpp2, Rps7, and Rps10 as well as uL16 (Rpl10), Rpl24, eL34 (Rpl34), Rpl36A (Rpl42), and Rpl38 turn over faster than the other RPs (An et al. 2020, Mathis et al. 2017), indicating that they could be exchanged and then degraded from a free pool. Additionally, several studies have shown that mRNAs in localized dendrites, whose local translation is important for neuronal function, include those encoding RPs (reviewed in Holt et al. 2019). Moreover, locally translated RPs were detected with preexisting ribosomes (Fusco et al. 2021, Shigeoka et al. 2019). Altogether, the data from bacteria and eukaryotes suggest that the exchange of RPs between a free and a ribosome-bound pool appears possible for a sizable subset of these proteins (**Table 3**).

SUMMARY AND FUTURE OUTLOOK

The importance of ribosomes for protein homeostasis necessitates mechanisms to ensure the structural and functional integrity of ribosomes. These include quality control mechanisms during assembly to ensure that only correctly assembled and fully functional ribosomes enter the translating pool (Parker & Karbstein 2023), as well as quality control mechanisms after assembly and during their functional cycle that detect defective or damaged ribosomes and either degrade them (Parker et al. 2024) or repair their damage (Yang et al. 2023). While these mechanisms have so far been documented only in yeast and in limited cases, the data reviewed here suggest that these mechanisms may be conserved and that different forms of physiological ribosome damage might be repaired in other instances. Future research will be required to better characterize the sites of damage and identify repair machineries, as well as mechanisms. Moreover, while the long lifetime of ribosomes might necessitate repair mechanisms, the finding that in one case the repair machinery can also be utilized to remodel ribosomes raises the tantalizing possibility that this might occur for other RPs as well.

DISCLOSURE STATEMENT

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

ACKNOWLEDGMENTS

We thank members of the Karbstein lab for comments on the manuscript. The writing of this review was supported by National Institutes of Health grant R35-GM136323 to K.K. and by the research fund of Hanyang University (HY-202300000003039) to Y.-M.Y.

LITERATURE CITED

Aas PA, Otterlei M, Falnes PO, Vagbo CB, Skorpen F, et al. 2003. Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. *Nature* 421:859–63

Abelson HT, Johnson LF, Penman S, Green H. 1974. Changes in RNA in relation to growth of the fibroblast. II. The lifetime of mRNA, rRNA, and tRNA in resting and growing cells. *Cell* 1:161–65

An H, Ordureau A, Korner M, Paulo JA, Harper JW. 2020. Systematic quantitative analysis of ribosome inventory during nutrient stress. *Nature* 583:303–9

Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55:373–99

Bachvarova R, De Leon V. 1977. Stored and polysomal ribosomes of mouse ova. *Dev. Biol.* 58:248–54

Barciszewski J, Barciszewska MZ, Siboska G, Rattan SI, Clark BF. 1999. Some unusual nucleic acid bases are products of hydroxyl radical oxidation of DNA and RNA. *Mol. Biol. Rep.* 26:231–38

Barna M, Karbstein K, Tollervey D, Ruggero D, Brar G, et al. 2022. The promises and pitfalls of specialized ribosomes. *Mol. Cell* 82:2179–84

Ben-Shem A, Garreau de Loubresse N, Melnikov S, Jenner L, Yusupova G, Yusupov M. 2011. The structure of the eukaryotic ribosome at 3.0 Å resolution. *Science* 334:1524–29

Black JJ, Musalgaonkar S, Johnson AW. 2019. Tsr4 is a cytoplasmic chaperone for the ribosomal protein Rps2 in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 39:e00094-19

Blomqvist EK, Huang H, Karbstein K. 2023. A disease associated mutant reveals how Ltv1 orchestrates RP assembly and rRNA folding of the small ribosomal subunit head. *PLOS Genet*. 19:e1010862

Borovinskaya MA, Pai RD, Zhang W, Schuwirth BS, Holton JM, et al. 2007. Structural basis for aminoglycoside inhibition of bacterial ribosome recycling. *Nat. Struct. Mol. Biol.* 14:727–32

Bray MS, Lenz TK, Haynes JW, Bowman JC, Petrov AS, et al. 2018. Multiple prebiotic metals mediate translation. *PNAS* 115:12164–69

Brown A, Baird MR, Yip MC, Murray J, Shao S. 2018. Structures of translationally inactive mammalian ribosomes. *eLife* 7:e40486

Caldwell P, Luk DC, Weissbach H, Brot N. 1978. Oxidation of the methionine residues of *Escherichia coli* ribosomal protein L12 decreases the protein's biological activity. *PNAS* 75:5349–52

Casati P, Walbot V. 2004. Crosslinking of ribosomal proteins to RNA in maize ribosomes by UV-B and its effects on translation. *Plant Physiol.* 136:3319–32

Chen SS, Sperling E, Silverman JM, Davis JH, Williamson JR. 2012. Measuring the dynamics of *E. coli* ribosome biogenesis using pulse-labeling and quantitative mass spectrometry. *Mol. Biosyst.* 8:3325–34

Christiano R, Nagaraj N, Frohlich F, Walther TC. 2014. Global proteome turnover analyses of the yeasts *S. cerevisiae* and *S. pombe*. *Cell Rep*. 9:1959–65

Cole SE, LaRiviere FJ, Merrikh CN, Moore MJ. 2009. A convergence of rRNA and mRNA quality control pathways revealed by mechanistic analysis of nonfunctional rRNA decay. *Mol. Cell* 34:440–50

Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, et al. 1987. Oxygen radicals and human disease. *Ann. Intern. Med.* 107:526–45

D'Aquila P, Montesanto A, Mandalà M, Garasto S, Mari V, et al. 2017. Methylation of the ribosomal RNA gene promoter is associated with aging and age-related decline. *Aging Cell* 16:966–75

Dawson DM. 1967. Turnover of ribosomal RNA in the rat brain. *J. Neurochem.* 14:939–46

de la Cruz J, Karbstein K, Woolford JL Jr. 2015. Functions of ribosomal proteins in assembly of eukaryotic ribosomes in vivo. *Annu. Rev. Biochem.* 84:93–129

Dedduwa-Mudalige GNP, Chow CS. 2015. Cisplatin targeting of bacterial ribosomal RNA hairpins. *Int. J. Mol. Sci.* 16:21392–409

Deutscher MP. 2003. Degradation of stable RNA in bacteria. *J. Biol. Chem.* 278:45041–44

Dhondt I, Petyuk VA, Bauer S, Brewer HM, Smith RD, et al. 2017. Changes of protein turnover in aging *Caenorhabditis elegans*. *Mol. Cell. Proteom.* 16:1621–33

Diaconu M, Kothe U, Schlünzen F, Fischer N, Harms JM, et al. 2005. Structural basis for the function of the ribosomal L7/12 stalk in factor binding and GTPase activation. *Cell* 121:991–1004

D'Orazio KN, Green R. 2021. Ribosome states signal RNA quality control. *Mol. Cell* 81:1372–83

Dorrbaum AR, Kochen L, Langer JD, Schuman EM. 2018. Local and global influences on protein turnover in neurons and glia. *eLife* 7:e34202

Dwyer DJ, Belenky PA, Yang JH, MacDonald IC, Martell JD, et al. 2014. Antibiotics induce redox-related physiological alterations as part of their lethality. *PNAS* 111:E2100–9

Engl C, Schaefer J, Kotta-Loizou I, Buck M. 2016. Cellular and molecular phenotypes depending upon the RNA repair system RtcAB of *Escherichia coli*. *Nucleic Acids Res.* 44:9933–41

Englert M, Sheppard K, Aslanian A, Yates JR 3rd, Soll D. 2011. Archaeal 3′-phosphate RNA splicing ligase characterization identifies the missing component in tRNA maturation. *PNAS* 108:1290–95

Estevez M, Valesyan S, Jora M, Limbach PA, Addepalli B. 2021. Oxidative damage to RNA is altered by the presence of interacting proteins or modified nucleosides. *Front. Mol. Biosci.* 8:697149

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.* 24:700–7

Ferretti MB, Karbstein K. 2019. Does functional specialization of ribosomes really exist? *RNA* 25:521–38

Fu L, Liu K, Sun M, Tian C, Sun R, et al. 2017. Systematic and quantitative assessment of hydrogen peroxide reactivity with cysteines across human proteomes. *Mol. Cell. Proteom.* 16:1815–28

Fusco CM, Desch K, Dorrbaum AR, Wang M, Staab A, et al. 2021. Neuronal ribosomes exhibit dynamic and context-dependent exchange of ribosomal proteins. *Nat. Commun.* 12:6127

Garcia-Gomez JJ, Lebaron S, Froment C, Monsarrat B, Henry Y, de la Cruz J. 2011. Dynamics of the putative RNA helicase Spb4 during ribosome assembly in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 31:4156–64

Garshott DM, An H, Sundaramoorthy E, Leonard M, Vicary A, et al. 2021. iRQC, a surveillance pathway for 40S ribosomal quality control during mRNA translation initiation. *Cell Rep.* 36:109642

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, et al. 2000. Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* 11:4241–57

Gay DM, Lund AH, Jansson MD. 2022. Translational control through ribosome heterogeneity and functional specialization. *Trends Biochem. Sci.* 47:66–81

Genuth NR, Barna M. 2018. The discovery of ribosome heterogeneity and its implications for gene regulation and organismal life. *Mol. Cell* 71:364–74

Ghalei H, Schaub FX, Doherty JR, Noguchi Y, Roush WR, et al. 2015. Hrr25/CK1δ-directed release of Ltv1 from pre-40S ribosomes is necessary for ribosome assembly and cell growth. *J. Cell Biol.* 208:745–59

Ghalei H, Trepreau J, Collins JC, Bhaskaran H, Strunk BS, Karbstein K. 2017. The ATPase Fap7 tests the ability to carry out translocation-like conformational changes and releases Dim1 during 40S ribosome maturation. *Mol. Cell* 67:990–1000.e3

Gorenstein C, Warner JR. 1977. Synthesis and turnover of ribosomal proteins in the absence of 60S subunit assembly in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 157:327–32

Gough DR, Cotter TG. 2011. Hydrogen peroxide: a Jekyll and Hyde signalling molecule. *Cell Death Dis.* 2:e213

Gudkov AT, Behlke J. 1978. N-terminal sequence protein of L7-L12 is responsible for its dimerization. *Eur. J. Biochem.* 90:309–12

He C, Hus JC, Sun LJ, Zhou P, Norman DPG, et al. 2005. A methylation-dependent electrostatic switch controls DNA repair and transcriptional activation by *E. coli* Ada. *Mol. Cell* 20:117–29

Hellmich UA, Weis BL, Lioutikov A, Wurm JP, Kaiser M, et al. 2013. Essential ribosome assembly factor Fap7 regulates a hierarchy of RNA-protein interactions during small ribosomal subunit biogenesis. *PNAS* 110:15253–58

Henras AK, Plisson-Chastang C, O'Donohue MF, Chakraborty A, Gleizes PE. 2015. An overview of pre-ribosomal RNA processing in eukaryotes. *Wiley Interdiscip. Rev. RNA* 6:225–42

Hofer T, Badouard C, Bajak E, Ravanat JL, Mattsson A, Cotgreave IA. 2005. Hydrogen peroxide causes greater oxidation in cellular RNA than in DNA. *Biol. Chem.* 386:333–37

Hofer T, Seo AY, Prudencio M, Leeuwenburgh C. 2006. A method to determine RNA and DNA oxidation simultaneously by HPLC-ECD: greater RNA than DNA oxidation in rat liver after doxorubicin administration. *Biol. Chem.* 387:103–11

Holt CE, Martin KC, Schuman EM. 2019. Local translation in neurons: visualization and function. *Nat. Struct. Mol. Biol.* 26:557–66

Hostetter AA, Osborn MF, DeRose VJ. 2012. RNA-Pt adducts following cisplatin treatment of *Saccharomyces cerevisiae*. *ACS Chem. Biol.* 7:218–25

Huang H, Ghalei H, Karbstein K. 2020. Quality control of 40S ribosome head assembly ensures scanning competence. *J. Cell Biol.* 219:e202004161

Huang H, Karbstein K. 2021. Assembly factors chaperone ribosomal RNA folding by isolating helical junctions that are prone to misfolding. *PNAS* 118:e2101164118

Hussain T, Llacer JL, Fernandez IS, Munoz A, Martin-Marcos P, et al. 2014. Structural changes enable start codon recognition by the eukaryotic translation initiation complex. *Cell* 159:597–607

Imlay JA. 2003. Pathways of oxidative damage. *Annu. Rev. Microbiol.* 57:395–418

Iordanov MS, Pribnow D, Magun JL, Dinh TH, Pearson JA, Magun BE. 1998. Ultraviolet radiation triggers the ribotoxic stress response in mammalian cells. *J. Biol. Chem.* 273:15794–803

Iouk TL, Aitchison JD, Maguire S, Wozniak RW. 2001. Rrb1p, a yeast nuclear WD-repeat protein involved in the regulation of ribosome biosynthesis. *Mol. Cell. Biol.* 21:1260–71

Ivanov IP, Saba JA, Fan CM, Wang J, Firth AE, et al. 2022. Evolutionarily conserved inhibitory uORFs sensitize *Hox* mRNA translation to start codon selection stringency. *PNAS* 119:e2117226119

Jacques S, Ghesquiere B, De Bock PJ, Demol H, Wahni K, et al. 2015. Protein methionine sulfoxide dynamics in *Arabidopsis thaliana* under oxidative stress. *Mol. Cell. Proteom.* 14:1217–29

Jamieson ER, Lippard SJ. 1999. Structure, recognition, and processing of cisplatin-DNA adducts. *Chem. Rev.* 99:2467–98

Ju D, Li L, Xie Y. 2023. Homeostatic regulation of ribosomal proteins by ubiquitin-independent cotranslational degradation. *PNAS* 120:e2306152120

Juhnke H, Charizanis C, Latifi F, Krems B, Entian KD. 2000. The essential protein Fap7 is involved in the oxidative stress response of *Saccharomyces cerevisiae*. *Mol. Microbiol.* 35:936–48

Jung M, Jin SG, Zhang XY, Xiong WY, Gogoshin G, et al. 2015. Longitudinal epigenetic and gene expression profiles analyzed by three-component analysis reveal down-regulation of genes involved in protein translation in human aging. *Nucleic Acids Res.* 43:E100

Jurkin J, Henkel T, Nielsen AF, Minnich M, Popow J, et al. 2014. The mammalian tRNA ligase complex mediates splicing of XBP1 mRNA and controls antibody secretion in plasma cells. *EMBO J.* 33:2922–36

Kiley PJ, Storz G. 2004. Exploiting thiol modifications. *PLOS Biol.* 2:e400

Klinge S, Woolford JL. 2019. Ribosome assembly coming into focus. *Nat. Rev. Mol. Cell Biol.* 20:116–31

Koch B, Mitterer V, Niederhauser J, Stanborough T, Murat G, et al. 2012. Yar1 protects the ribosomal protein Rps3 from aggregation. *J. Biol. Chem.* 287:21806–15

Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* 130:797–810

Koplin A, Preissler S, Ilina Y, Koch M, Scior A, et al. 2010. A dual function for chaperones SSB-RAC and the NAC nascent polypeptide-associated complex on ribosomes. *J. Cell Biol.* 189:57–68

Koteliansky VE, Domogatsky SP, Gudkov AT. 1978. Dimer state of protein L7/L12 and EF-G-dependent reactions of ribosomes. *Eur. J. Biochem.* 90:319–23

Kressler D, Bange G, Ogawa Y, Stjepanovic G, Bradatsch B, et al. 2012. Synchronizing nuclear import of ribosomal proteins with ribosome assembly. *Science* 338:666–71

LaRiviere FJ, Cole SE, Ferullo DJ, Moore MJ. 2006. A late-acting quality control process for mature eukaryotic rRNAs. *Mol. Cell* 24:619–26

Lee SW, Berger SJ, Martinovic S, Pasa-Tolic L, Anderson GA, et al. 2002. Direct mass spectrometric analysis of intact proteins of the yeast large ribosomal subunit using capillary LC/FTICR. *PNAS* 99:5942–47

Leesch F, Lorenzo-Orts L, Pribitzer C, Grishkovskaya I, Roehsner J, et al. 2023. A molecular network of conserved factors keeps ribosomes dormant in the egg. *Nature* 613:712–20

Leichert LI, Gehrke F, Gudiseva HV, Blackwell T, Ilbert M, et al. 2008. Quantifying changes in the thiol redox proteome upon oxidative stress in vivo. *PNAS* 105:8197–202

Liebhaber SA, Wolf S, Schlessinger D. 1978. Differences in rRNA metabolism of primary and SV40-transformed human fibroblasts. *Cell* 13:121–27

Lilleorg S, Reier K, Pulk A, Liiv A, Tammsalu T, et al. 2019. Bacterial ribosome heterogeneity: changes in ribosomal protein composition during transition into stationary growth phase. *Biochimie* 156:169–80

Liu X, Huang H, Karbstein K. 2021. Blocking a dead-end assembly pathway creates a point of regulation for the DEAD-box ATPase Has1 and prevents platform misassembly. bioRxiv 2021.09.06.459192. **https://doi.org/10.1101/2021.09.06.459192**

Lo KY, Li Z, Bussiere C, Bresson S, Marcotte EM, Johnson AW. 2010. Defining the pathway of cytoplasmic maturation of the 60S ribosomal subunit. *Mol. Cell* 39:196–208

Lu Y, Liang FX, Wang X. 2014. A synthetic biology approach identifies the mammalian UPR RNA ligase RtcB. *Mol. Cell* 55:758–70
Maday S, Twelvetrees AE, Moughamian AJ, Holzbaur EL. 2014. Axonal transport: cargo-specific mechanisms of motility and regulation. *Neuron* 84:292–309
Manwar MR, Shao C, Shi X, Wang J, Lin Q, et al. 2020. The bacterial RNA ligase RtcB accelerates the repair process of fragmented rRNA upon releasing the antibiotic stress. *Sci. China Life Sci.* 63:251–58
Mathieson T, Franken H, Kosinski J, Kurzawa N, Zinn N, et al. 2018. Systematic analysis of protein turnover in primary cells. *Nat. Commun.* 9:689
Mathis AD, Naylor BC, Carson RH, Evans E, Harwell J, et al. 2017. Mechanisms of *in vivo* ribosome maintenance change in response to nutrient signals. *Mol. Cell. Proteom.* 16:243–54
Melnikov SV, Söll D, Steitz TA, Polikanov YS. 2016. Insights into RNA binding by the anticancer drug cisplatin from the crystal structure of cisplatin-modified ribosome. *Nucleic Acids Res.* 44:4978–87
Meng J, Fu L, Liu K, Tian C, Wu Z, et al. 2021. Global profiling of distinct cysteine redox forms reveals wide-ranging redox regulation in *C. elegans*. *Nat. Commun.* 12:1415
Menzies RA, Gold PH. 1972. The apparent turnover of mitochondria, ribosomes and sRNA of the brain in young adult and aged rats. *J. Neurochem.* 19:1671–83
Menzies RA, Mishra RK, Gold PH. 1972. The turnover of ribosomes and soluble RNA in a variety of tissues of young adult and aged rats. *Mech. Ageing Dev.* 1:117–32
Meselson M, Nomura M, Brenner S, Davern C, Schlessinger D. 1964. Conservation of ribosomes during bacterial growth. *J. Mol. Biol.* 9:696–711
Mills EW, Green R. 2017. Ribosomopathies: There's strength in numbers. *Science* 358:eaan2755
Mitterer V, Gantenbein N, Birner-Gruenberger R, Murat G, Bergler H, et al. 2016. Nuclear import of dimerized ribosomal protein Rps3 in complex with its chaperone Yar1. *Sci. Rep.* 6:36714
Mitterer V, Thoms M, Buschauer R, Berninghausen O, Hurt E, Beckmann R. 2023. Concurrent emodelling of nucleolar 60S subunit precursors by the Rea1 ATPase and Spb4 RNA helicase. *eLife* 12:e84877
Mizuno CM, Guyomar C, Roux S, Lavigne R, Rodriguez-Valera F, et al. 2019. Numerous cultivated and uncultivated viruses encode ribosomal proteins. *Nat. Commun.* 10:752
Moskovitz J. 2005. Methionine sulfoxide reductases: ubiquitous enzymes involved in antioxidant defense, protein regulation, and prevention of aging-associated diseases. *Biochim. Biophys. Acta Proteins Proteom.* 1703:213–19
Nesterchuk MV, Sergiev PV, Dontsova OA. 2011. Posttranslational modifications of ribosomal proteins in *Escherichia coli*. *Acta Nat.* 3:22–33
Novakovic MB, Petrovic SL, Rakic LM, Ivanus JJ. 1979. Different turnover rates of brain ribosomal ribonucleic acids in male and female rats. *J. Neurochem.* 33:661–67
Odintsova TI, Müller EC, Ivanov AV, Egorov TA, Bienert R, et al. 2003. Characterization and analysis of posttranslational modifications of the human large cytoplasmic ribosomal subunit proteins by mass spectrometry and Edman sequencing. *J. Protein Chem.* 22:249–58
Osborn MF, White JD, Haley MM, DeRose VJ. 2014. Platinum-RNA modifications following drug treatment in *S. cerevisiae* identified by click chemistry and enzymatic mapping. *ACS Chem. Biol.* 9:2404–11
Parker MD, Collins JC, Korona B, Ghalei H, Karbstein K. 2019. A kinase-dependent checkpoint prevents escape of immature ribosomes into the translating pool. *PLOS Biol.* 17:e3000329
Parker MD, Brunk ES, Getzler AJ, Karbstein K. 2024. The kinase Rio1 and a ribosome collision-dependent decay pathway survey the integrity of 18S rRNA cleavage. *PLOS Biol.* 22:e3001767
Parker MD, Karbstein K. 2023. Quality control ensures fidelity in ribosome assembly and cellular health. *J. Cell Biol.* 222:e202209115
Paulsen CE, Carroll KS. 2013. Cysteine-mediated redox signaling: chemistry, biology, and tools for discovery. *Chem. Rev.* 113:4633–79
Pausch P, Singh U, Ahmed YL, Pillet B, Murat G, et al. 2015. Co-translational capturing of nascent ribosomal proteins by their dedicated chaperones. *Nat. Commun.* 6:7494
Petrova B, Liu K, Tian C, Kitaoka M, Freinkman E, et al. 2018. Dynamic redox balance directs the oocyte-to-embryo transition via developmentally controlled reactive cysteine changes. *PNAS* 115:E7978–86
Pillet B, Garcia-Gomez JJ, Pausch P, Falquet L, Bange G, et al. 2015. The dedicated chaperone Acl4 escorts ribosomal protein Rpl4 to its Nuclear pre-60S assembly site. *PLOS Genet.* 11:e1005565

Pillet B, Mitterer V, Kressler D, Pertschy B. 2017. Hold on to your friends: dedicated chaperones of ribosomal proteins. *BioEssays* 39:1–12

Plakos K, DeRose VJ. 2017. Mapping platinum adducts on yeast ribosomal RNA using high-throughput sequencing. *Chem. Commun.* 53:12746–49

Poetsch AR. 2020. The genomics of oxidative DNA damage, repair, and resulting mutagenesis. *Comput. Struct. Biotechnol. J.* 18:207–19

Popow J, Englert M, Weitzer S, Schleiffer A, Mierzwa B, et al. 2011. HSPC117 is the essential subunit of a human tRNA splicing ligase complex. *Science* 331:760–64

Prossliner T, Skovbo Winther K, Sorensen MA, Gerdes K. 2018. Ribosome hibernation. *Annu. Rev. Genet.* 52:321–48

Pulk A, Liiv A, Peil L, Maivali U, Nierhaus K, Remme J. 2010. Ribosome reactivation by replacement of damaged proteins. *Mol. Microbiol.* 75:801–14

Reddie KG, Carroll KS. 2008. Expanding the functional diversity of proteins through cysteine oxidation. *Curr. Opin. Chem. Biol.* 12:746–54

Retz KC, Steele WJ. 1980. Ribosome turnover in rat brain and liver. *Life Sci.* 27:2601–4

Rijal K, Chow CS. 2009. A new role for cisplatin: probing ribosomal RNA structure. *Chem. Commun.* 2008:107–9

Robertson WR, Dowsett SJ, Hardy SJ. 1977. Exchange of ribosomal proteins among the ribosomes of *Escherichia coli*. *Mol. Gen. Genet.* 157:205–14

Rossler I, Embacher J, Pillet B, Murat G, Liesinger L, et al. 2019. Tsr4 and Nap1, two novel members of the ribosomal protein chaperOME. *Nucleic Acids Res.* 47:6984–7002

Schafer T, Maco B, Petfalski E, Tollervey D, Bottcher B, et al. 2006. Hrr25-dependent phosphorylation state regulates organization of the pre-40S subunit. *Nature* 441:651–55

Schutz S, Fischer U, Altvater M, Nerurkar P, Pena C, et al. 2014. A RanGTP-independent mechanism allows ribosomal protein nuclear import for ribosome assembly. *eLife* 3:e03473

Schutz S, Michel E, Damberger FF, Oplova M, Pena C, et al. 2018. Molecular basis for disassembly of an importin:ribosomal protein complex by the escortin Tsr2. *Nat. Commun.* 9:3669

Shacter E. 2000. Quantification and significance of protein oxidation in biological samples. *Drug Metab. Rev.* 32:307–26

Shcherbik N, Pestov DG. 2019. The impact of oxidative stress on ribosomes: from injury to regulation. *Cells* 8:1379

Shedlovskiy D, Zinskie JA, Gardner E, Pestov DG, Shcherbik N. 2017. Endonucleolytic cleavage in the expansion segment 7 of 25S rRNA is an early marker of low-level oxidative stress in yeast. *J. Biol. Chem.* 292:18469–85

Shi Y, Carroll KS. 2020. Activity-based sensing for site-specific proteomic analysis of cysteine oxidation. *ACC Chem. Res.* 53:20–31

Shi Z, Fujii K, Kovary KM, Genuth NR, Rost HL, et al. 2017. Heterogeneous ribosomes preferentially translate distinct subpools of mRNAs genome-wide. *Mol. Cell* 67:71–83.e7

Shigeoka T, Koppers M, Wong HH, Lin JQ, Cagnetta R, et al. 2019. On-site ribosome remodeling by locally synthesized ribosomal proteins in axons. *Cell Rep.* 29:3605–19.e10

Sies H. 1986. Biochemistry of oxidative stress. *Angew. Chem. Int. Ed.* 25:1058–71

Simms CL, Hudson BH, Mosior JW, Rangwala AS, Zaher HS. 2014. An active role for the ribosome in determining the fate of oxidized mRNA. *Cell Rep.* 9:1256–64

Simms CL, Yan LWL, Zaher HS. 2017. Ribosome collision is critical for quality control during no-go decay. *Mol. Cell* 68:361–73.e5

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

Smethurst DGJ, Kovalev N, McKenzie ER, Pestov DG, Shcherbik N. 2020. Iron-mediated degradation of ribosomes under oxidative stress is attenuated by manganese. *J. Biol. Chem.* 295:17200–14

Steenken S, Jovanovic SV. 1997. How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution. *J. Am. Chem. Soc.* 119:617–18

Stelter P, Huber FM, Kunze R, Flemming D, Hoelz A, Hurt E. 2015. Coordinated ribosomal L4 protein assembly into the pre-ribosome is regulated by its eukaryote-specific extension. *Mol. Cell* 58:854–62

Stoneley M, Harvey RF, Mulroney TE, Mordue R, Jukes-Jones R, et al. 2022. Unresolved stalled ribosome complexes restrict cell-cycle progression after genotoxic stress. *Mol. Cell* 82:1557–72.e7

Stoykova AS, Dudov KP, Dabeva MD, Hadjiolov AA. 1983. Different rates of synthesis and turnover of ribosomal RNA in rat brain and liver. *J. Neurochem.* 41:942–49

Strunk BS, Novak MN, Young CL, Karbstein K. 2012. A translation-like cycle is a quality control checkpoint for maturing 40S ribosome subunits. *Cell* 150:111–21

Subramanian AR, van Duin J. 1977. Exchange of individual ribosomal proteins between ribosomes as studied by heavy isotope-transfer experiments. *Mol. Gen. Genet.* 158:1–9

Sugiyama T, Li S, Kato M, Ikeuchi K, Ichimura A, et al. 2019. Sequential ubiquitination of ribosomal protein uS3 triggers the degradation of non-functional 18S rRNA. *Cell Rep.* 26:3400–15.e7

Sun M, Shen B, Li W, Samir P, Browne CM, et al. 2021. A time-resolved cryo-EM study of *Saccharomyces cerevisiae* 80S ribosome protein composition in response to a change in carbon source. *Proteomics* 21:e2000125

Sung MK, Porras-Yakushi TR, Reitsma JM, Huber FM, Sweredoski MJ, et al. 2016. A conserved quality-control pathway that mediates degradation of unassembled ribosomal proteins. *eLife* 5:e19105

Takahashi Y, Ogata K. 1981. Ribosomal proteins cross-linked to natural mRNA by UV irradiation of rat liver polysomes. *J. Biochem.* 90:1549–52

Tanaka N, Shuman S. 2011. RtcB is the RNA ligase component of an *Escherichia coli* RNA repair operon. *J. Biol. Chem.* 286:7727–31

Temmel H, Muller C, Sauert M, Vesper O, Reiss A, et al. 2017. The RNA ligase RtcB reverses MazF-induced ribosome heterogeneity in *Escherichia coli*. *Nucleic Acids Res.* 45:4708–21

Thompson MK, Rojas-Duran MF, Gangaramani P, Gilbert WV. 2016. The ribosomal protein Asc1/RACK1 is required for efficient translation of short mRNAs. *eLife* 5:e11154

Tikkanen M, Mekala NR, Aro EM. 2014. Photosystem II photoinhibition-repair cycle protects photosystem I from irreversible damage. *Biochim. Biophys. Acta Bioenerg.* 1837:210–15

Ting YH, Lu TJ, Johnson AW, Shie JT, Chen BR, et al. 2017. Bcp1 is the nuclear chaperone of Rpl23 in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 292:585–96

Topf U, Suppanz I, Samluk L, Wrobel L, Boser A, et al. 2018. Quantitative proteomics identifies redox switches for global translation modulation by mitochondrially produced reactive oxygen species. *Nat. Commun.* 9:324

van der Reest J, Lilla S, Zheng L, Zanivan S, Gottlieb E. 2018. Proteome-wide analysis of cysteine oxidation reveals metabolic sensitivity to redox stress. *Nat. Commun.* 9:1581

Vesper O, Amitai S, Belitsky M, Byrgazov K, Kaberdina AC, et al. 2011. Selective translation of leaderless mRNAs by specialized ribosomes generated by MazF in *Escherichia coli*. *Cell* 147:147–57

Vincow ES, Merrihew G, Thomas RE, Shulman NJ, Beyer RP, et al. 2013. The PINK1-Parkin pathway promotes both mitophagy and selective respiratory chain turnover in vivo. *PNAS* 110:6400–5

Vincow ES, Thomas RE, Merrihew GE, MacCoss MJ, Pallanck LJ. 2021. Slowed protein turnover in aging *Drosophila* reflects a shift in cellular priorities. *J. Gerontol. A Biol. Sci. Med. Sci.* 76:1734–39

Wang D, Lippard SJ. 2005. Cellular processing of platinum anticancer drugs. *Nat. Rev. Drug Discov.* 4:307–20

Warner JR. 1977. In the absence of ribosomal RNA synthesis, the ribosomal proteins of HeLa cells are synthesized normally and degraded rapidly. *J. Mol. Biol.* 115:315–33

Warner JR. 1999. The economics of ribosome biosynthesis in yeast. *Trends Biochem. Sci.* 24:437–40

Wells JN, Buschauer R, Mackens-Kiani T, Best K, Kratzat H, et al. 2020. Structure and function of yeast Lso2 and human CCDC124 bound to hibernating ribosomes. *PLOS Biol.* 18:e3000780

Werner A, Iwasaki S, McGourty CA, Medina-Ruiz S, Teerikorpi N, et al. 2015. Cell-fate determination by ubiquitin-dependent regulation of translation. *Nature* 525:523–27

White LK, Strugar SM, MacFadden A, Hesselberth JR. 2023. Nanopore sequencing of internal 2′-PO_4 modifications installed by RNA repair. *RNA* 29:847–61

Wu CC, Peterson A, Zinshteyn B, Regot S, Green R. 2020. Ribosome collisions trigger general stress responses to regulate cell fate. *Cell* 182:404–16.e14

Wurtmann EJ, Wolin SL. 2009. RNA under attack: cellular handling of RNA damage. *Crit. Rev. Biochem. Mol. Biol.* 44:34–49

Xie K, Bunse C, Marcus K, Leichert LI. 2019. Quantifying changes in the bacterial thiol redox proteome during host-pathogen interaction. *Redox Biol*. 21:101087

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

Yan LL, Simms CL, McLoughlin F, Vierstra RD, Zaher HS. 2019. Oxidation and alkylation stresses activate ribosome-quality control. *Nat. Commun.* 10:5611

Yan LWL, Zaher HS. 2019. How do cells cope with RNA damage and its consequences? *J. Biol. Chem.* 294:15158–71

Yang YM, Jung Y, Abegg D, Adibekian A, Carroll KS, Karbstein K. 2023. Chaperone-directed ribosome repair after oxidative damage. *Mol. Cell* 83:1527–37.e5

Yang YM, Karbstein K. 2022. The chaperone Tsr2 regulates Rps26 release and reincorporation from mature ribosomes to enable a reversible, ribosome-mediated response to stress. *Sci. Adv.* 8:eabl4386

Yang YT, Ting YH, Liang KJ, Lo KY. 2016. The roles of Puf6 and Loc1 in 60S biogenesis are interdependent, and both are required for efficient accommodation of Rpl43. *J. Biol. Chem.* 291:19312–23

Yip MCJ, Sedor SF, Shao S. 2022. Mechanism of client selection by the protein quality-control factor UBE2O. *Nat. Struct. Mol. Biol.* 29:774–80

Young BD, Weiss DI, Zurita-Lopez CI, Webb KJ, Clarke SG, McBride AE. 2012. Identification of methylated proteins in the yeast small ribosomal subunit: a role for SPOUT methyltransferases in protein arginine methylation. *Biochemistry* 51:5091–104

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

Zinskie JA, Ghosh A, Trainor BM, Shedlovskiy D, Pestov DG, Shcherbik N. 2018. Iron-dependent cleavage of ribosomal RNA during oxidative stress in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* 293:14237–48