Title

Fix it, dont trash it: Ribosome maintenance by chaperone-mediated repair of damaged subunits.

Journal

Molecular Cell, 83(9)

Authors

Spiri, Silvan
Brar, Gloria

Publication Date

2023-05-04

DOI

10.1016/j.molcel.2023.04.006

Peer reviewed

HHS Public Access

Author manuscript

Mol Cell. Author manuscript; available in PMC 2023 September 06.

Published in final edited form as:

Mol Cell. 2023 May 04; 83(9): 1374–1376. doi:10.1016/j.molcel.2023.04.006.

Fix it, don't trash it: Ribosome maintenance by chaperone-mediated repair of damaged subunits

Silvan Spiri[1,2], **Gloria Ann Brar**[1,2,*]

[1]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, 94720, USA

[2]California Institute for Quantitative Biosciences (QB3), University of California, Berkeley, CA, 94720, USA

Summary:

Acute stressors or normal cellular function may result in ribosomal protein damage, which threatens the functional ribosome pool and translation. In this issue, Yang et al.[1] show that chaperones can extract damaged ribosomal proteins and replace them with newly synthesized versions to repair mature ribosomes.

Cellular components are exposed to many detrimental exogenous and endogenous conditions, leading to inevitable damage over time. Efficient strategies must counteract the deterioration of key components, including protein complexes and nucleic acids, to maintain cellular fitness. While DNA repair mechanisms are known and necessary, whether protein complexes can be repaired - as opposed to being degraded and resynthesized - has been unclear. In a recent study, Yang et al.[1] elucidate how the ribosome can be repaired to regain functionality after partial oxidative damage through replacement of impaired protein components.

Ribosomes, the conserved cellular machines that control protein synthesis, are large protein-RNA complexes, containing ~80 different ribosomal proteins (RP).[2] Ribosome assembly is a highly regulated process that is enabled by ribosome-associated chaperones, which bind the aggregation-prone free RPs until they are integrated into the maturing ribosome.[4,5] A subset of RPs have dedicated chaperones, including Tsr2 for Rps26, a 40S subunit protein.[4] In yeast cells exposed to high salt or low pH conditions, Tsr2 reversibly releases Rps26 from ribosomes, generating Rps26-lacking ribosomes with transcript preferences distinct from Rps26-containing ribosomes to counteract these adverse conditions.[6,7] Intriguingly, RPs in general, and the cysteines in Rps26 specifically, are preferentially oxidized in yeast and *Drosophila* cells in response to conditions that increase oxidative damage[1,9]. The fate of these damaged ribosomes is the focus of Yang et al.[1].

*Correspondence: gabrar@berkeley.edu.

Declaration of interests:

The authors declare no competing interests.

Cells must tightly control the ribosomal lifecycle, as even modest changes in number of ribosomes can lead to cellular growth defects and mRNA-specific translational changes.[8] Roughly half of all transcriptional and translational capacity is dedicated to ribosome synthesis and assembly, and once made, ribosomes are highly stable.[2] So how do cells deal with oxidatively damaged ribosomes? At least three non-mutually exclusive possibilities exist. One strategy could be to cleanse the ribosome pool of damage through replacement of entire complexes. However, this would be energy-intensive, and since oxidative stress causes downregulation of overall translation,[9] may also be slow and inefficient. Alternatively, cells could retain damaged ribosomes and rectify downstream consequences through quality control pathways that sense stalling or collisions during translation. A third possibility is that damaged ribosomes are repaired by extracting and replacing the specific damaged components (Figure 1). From a cellular efficiency and integrity perspective, this third model is most attractive. However, it has not been directly observed until now.

Using a cell-permeable probe to detect cysteinyl oxidation, followed by purification of oxidized ribosomes and mass spectrometry, Yang et al.[1] showed that Rps26 is prone to oxidation *in vivo*, even in the absence of the oxidative stressor H_2O_2. Furthermore, purified Tsr2 can specifically release oxidized Rps26 from ribosomes *in vitro*. This role depends on Tsr2's ability to bind both Rps26 and the 40S subunit, demonstrating that Tsr2 actively extracts $Rps26_{ox}$ rather than just "catching" released Rps26. *In vivo* experiments, in which oxidative stress was coordinated with a switch to tagged Rps26, showed that $Rps26_{ox}$ release is followed by Tsr2-dependent incorporation of newly synthesized Rps26 into pre-existing ribosomes. Notably, since this process also occurs in unstressed cells, although to a much lesser extent, Tsr2-mediated replacement of damaged Rps26 acts in ribosome maintenance even in the absence of acute stress.

How does Tsr2 remove $Rps26_{ox}$? Yang et al.[1] showed that oxidative damage led to release of a Zn^{2+} ion that is important for Rps26 structure. This Zn^{2+} is normally chelated by four cysteines in Rps26, two of which were identified as sites of oxidation. Cys23 and Cys74, are essential for viability and *in vitro* incorporation into the ribosome. Purified Rps26 mutated for either residue was Zn^{2+}-free, suggesting that binding of Zn^{2+} to Rps26 is essential for its function. Because Tsr2 selectively releases Rps26 from ribosomes when its binding is weakened by Mg^{2+} ion loss at an rRNA interface[7], the authors hypothesize that $Rps26_{ox}$ is recognized and released in a similar way. Lack of Rps26 exchange, due to specific mutations in Tsr2, caused poor resistance to oxidative stress even in the presence of excess Rps26, and reduced growth even in the absence of H_2O_2 treatment, arguing that this repair mechanism is important for cellular fitness.

This type of repair is not unique to Rps26. Rpl10 is a preferentially oxidized 60S component that Yang et al.[1] showed was similarly replaced in response to oxidative damage. When Rpl10 is oxidized, its dedicated chaperone Sqt1 is recruited to ribosomes where it removes $Rpl10_{ox}$ from 60s subunits. Sqt1 mutants deficient in Rpl10 release are sensitive to oxidative stress. Since both Tsr2 and Sqt1 are highly conserved and Rps26 and Rpl10 undergo oxidation in other organisms, this mechanism may be general to eukaryotes. While the possibility of ribosomal repair has been suggested previously in studies investigating remodeling of ribosomes in vertebrate neurons, these studies could not

rule out late association of subunits with immature ribosomes rather than their removal and replacement.[10,11] Here, Yang et al.[1] provide molecular evidence that ribosome repair occurs, and put forth a potential general mechanism to protect the critical integrity of the ribosome pool by replacing damaged parts of the ribosome with dedicated chaperones.

Tsr2 and Sqt1, like all dedicated RP chaperones described to date, function in ribosome biogenesis, often within the nucleus (as in the case of Tsr2). Some of the others may also serve to repair ribosomes. Moreover, ribosome repair would be expected to occur in the cytoplasm, and as is not necessary for chaperones involved in ribosome repair to also contribute to ribosome biogenesis, there may be RP chaperones that repair ribosomes but have not yet been identified for an RP-related role. More widespread use of ribosome repair, beyond Rps26 and Rpl10, and beyond oxidative damage, warrants further study. It will also be interesting to investigate the fate of damaged proteins downstream of extraction from the ribosome. This might reveal an active pathway with additional regulatory factors or a passive model whereby damaged ribosomal protein interactions with their chaperone are unstable or outcompeted by newly synthesized protein.

Compared to the complete ribosome degradation upon damage, the repair mechanism demonstrated here presents a cost-efficient, fast, and flexible way for cells to respond to a variety of challenging endogenous and exogenous conditions (Figure 1). The modularity of the ribosome, which is composed of many very small proteins, seems particularly well-suited to such a strategy, and indeed could represent an advantage of this evolutionarily conserved structure.

Acknowledgments:

S.S is supported by the Swiss National Science Foundation mobility fellowship P500PB_214421, G.B is supported by the National Institutes of Health (1R01AG071869 and 1R35GM134886) and the Astera Fund.

References:

1. Yang Y, Jung Y, Abegg D, Adibekian A, Caroll A, and Karbstein K (2023). Chaperon-Directed Ribosome Repair after Oxidative Damage. Mol Cell.
2. Warner JR (1999). The economics of ribosome biosynthesis in yeast. Trends in Biochemical Sciences 24, 437–440. [PubMed: 10542411]
3. Parker MD, and Karbstein K (2023). Quality control ensures fidelity in ribosome assembly and cellular health. J Cell Biol 222 (4), e202209115. [PubMed: 36790396]
4. Schütz S, Fischer U, Altvater M, Nerurkar P, Peña C, Gerber M, Chang Y, Caesar S, Schubert OT, Schlenstedt G, et al. (2014). A RanGTP-independent mechanism allows ribosomal protein nuclear import for ribosome assembly. eLife 3, e03473. [PubMed: 25144938]
5. Rössler I, Embacher J, Pillet B, Murat G, Liesinger L, Hafner J, Unterluggauer JJ, Birner-Gruenberger R, Kressler D, and Pertschy B (2019). Tsr4 and Nap1, two novel members of the ribosomal protein chaperOME. Nucleic Acids Res 47, 6984–7002. [PubMed: 31062022]
6. Ferretti MB, Ghalei H, Ward EA, Potts EL, and Karbstein K (2017). Rps26 directs mRNA-specific translation by recognition of Kozak sequence elements. Nat Struct Mol Biol 24, 700–707. [PubMed: 28759050]
7. Yang Y, and 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. [PubMed: 35213229]

8. Mills EW, and Green R (2017). Ribosomopathies: There's strength in numbers. Science 358 (6363), eaan2755. [PubMed: 29097519]

9. Topf U, Suppanz I, Samluk L, Wrobel L, Böser A, Sakowska P, Knapp B, Pietrzyk MK, Chacinska A, and Warscheid B (2018). Quantitative proteomics identifies redox switches for global translation modulation by mitochondrially produced reactive oxygen species. Nat Commun 9, 324. [PubMed: 29358734]

10. Shigeoka T, Koppers M, Wong HH-W, Lin JQ, Cagnetta R, Dwivedy A, de Freitas Nascimento J, van Tartwijk FW, Ströhl F, Cioni J-M, et al. (2019). On-Site Ribosome Remodeling by Locally Synthesized Ribosomal Proteins in Axons. Cell Rep 29, 3605–3619.e10. [PubMed: 31825839]

11. Fusco CM, Desch K, Dörrbaum AR, Wang M, Staab A, Chan ICW, Vail E, Villeri V, Langer JD, and Schuman EM (2021). Neuronal ribosomes exhibit dynamic and context-dependent exchange of ribosomal proteins. Nat Commun 12, 6127. [PubMed: 34675203]

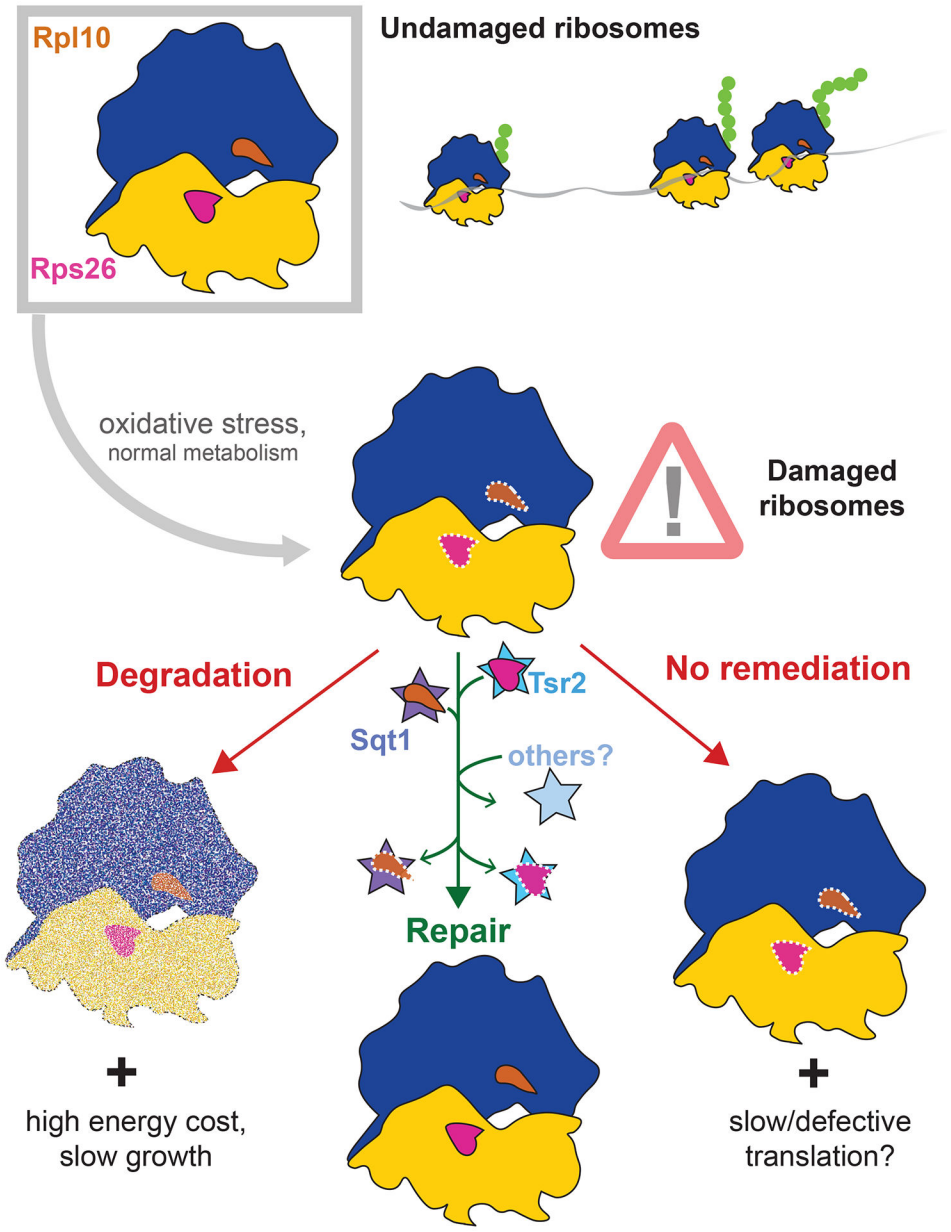

Figure 1. Possible strategies to counteract damage to the ribosome complex.

1. degradation of the complete complex; 2. oxidized Rps26 and Rpl10 are extracted from ribosomes and replaced, by chaperones Tsr2 and Sqt1 respectively, to restore ribosome integrity; 3. damage remains and other quality control pathways handle defective translation. These strategies are not mutually exclusive; the first and third occur in some instances. Yang et al.[1] show for the first time that the second strategy happens and is important.