Ubiquitin—a beacon for all during quality control on the ribosome

Liewei L Yan & Hani S Zaher

Ribosome stalling triggers no-go decay (NGD) and ribosome-associated quality control (RQC) pathways to rapidly degrade the aberrant mRNA and the incomplete nascent peptide, respectively. Two recent studies in yeast and mammalian systems reveal the importance of stalling-induced ribosomal protein ubiquitination by Hel2/ZNF598 for both NGD and RQC. The studies also structurally explain how collided ribosomes generate a unique interface not present in monosomes, which can be recognized by Hel2/ZNF598 ubiquitin ligases.

The EMBO Journal (2019) 38: e101633

See also: K Ikeuchi *et al* (March 2019)

The elongation phase of translation is an imperfect process that often results in stalling events, during which the ribosome halts movement along the mRNA template. If left unresolved, stalling leads to sequestration of ribosomes and the accumulation of potentially toxic protein products, which is detrimental for the cell (Simms *et al*, 2017a). Indeed, the inability of cells to recognize and recycle arrested ribosomes has been associated with neurodegenerative disease (Ishimura *et al*, 2014). In eukaryotes, the mRNA surveillance pathway of “no-go decay” (NGD) has evolved to rapidly degrade the aberrant mRNA by initiating endonucleolytic cleavage of the transcript upstream of the stalled ribosome. In addition to mRNA degradation, this process involves ribosome rescue by Dom34, Hbs1 and Rli1, and ubiquitin-mediated degradation of the nascent peptide *via* the ribosome-associated quality control (RQC) pathway. Following recycling of the 80S ribosome, the RQC E3 ubiquitin ligase Ltn1, in cooperation

with Rqc1, recognizes the peptidyl-tRNA-bound 60S subunit and then conjugates K48-linked polyubiquitin chains to the nascent peptide, thus marking it for degradation by the proteasome. The processes of ribosome rescue and RQC also depend on Hel2, another E3 ligase that adds K48-linked or K63-linked ubiquitin chains to a number of ribosomal proteins upon stalling (Saito *et al*, 2015; Matsuo *et al*, 2017). In the absence of Hel2, stalling is alleviated and Ltn1 fails to ubiquitinate the nascent peptide, presumably due to the inability of Dom34/Hbs1/Rli1 to rescue the stalled ribosomes. The main challenge faced by Hel2, and many of the quality control factors for that matter, is how to selectively recognize and distinguish stalled ribosomes from slowed ones.

While initial models suggested that stalled ribosomes might exhibit a distinct conformation that is recognized by the E3 ligase, two recent studies have suggested that yeast Hel2 and its mammalian counterpart ZNF598 distinguish stalled ribosomes from normal ones by recognizing collided ribosomes (Simms *et al*, 2017b; Juszkiwicz *et al*, 2018). In particular, structural analysis by Juszkiwicz *et al* revealed that a collided di-ribosome (disome) provides an extensive interface between the leading and trailing 40S subunit for ZNF598 to recognize its target ribosomal proteins. In addition, Simms and colleagues provided data that argued for a role for collisions in NGD, suggesting that both RQC and NGD are initiated by ribosomes running into each other. How these two processes are triggered by the same molecular signal is not obvious. The new results from the Inada and Beckmann groups in this issue (Ikeuchi *et al*, 2019) now provide compelling evidence

suggesting that ribosomal protein ubiquitination by Hel2 is required for both processes. It is thus likely that ubiquitination of ribosomal proteins is the first reaction to proceed after stalling has occurred, and is utilized by all downstream events to manifest the different facets of NGD and RQC.

Using a reporter mRNA that contains twelve inhibitory consecutive CGN codons (R(CGN)₁₂), which trigger robust NGD in yeast, Ikeuchi *et al* (2019) showed that Hel2 is required for the endonucleolytic cleavage of mRNA. In aiming to identify the mechanistic details of how Hel2 coordinates endonucleolytic cleavage during NGD and nascent-peptide ubiquitination during RQC, the authors revealed that there are two branches of NGD pathways. These rely on different domains of Hel2 protein and require different ubiquitination patterns on the 40S subunit (Fig 1). Hel2 is composed of an N-terminal RING domain, followed by three C₂H₂-type zinc finger (ZnF) domains and a C-terminal proline-rich domain. The RING and C₂H₂-ZnF domains are likely to be important for ubiquitination, and as expected their deletions phenocopy Hel2 deletion during NGD and RQC. On the other hand, the C-terminal proline-rich domain appears to be dispensable for NGD. This domain, however, is still important for Ltn1-dependent ubiquitination and hence RQC. Therefore, Hel2 appears to be responsible for independently activating both RQC and NGD. Interestingly, even though Northern blotting showed similar-sized mRNA decay intermediates in the presence of Hel2 C-terminal truncation as in the presence of the full-length factor, careful mapping of these products revealed that such Hel2 mutations result in a different cleavage pattern. In

Figure 1. 5'-capped and 3'-polyadenylated mRNA is translated by rapidly translocating poly-ribosomes (polysomes) until one of them stalls (top); such stalling causes a collision and formation of a "disome" by the first two colliding ribosomes (bottom).

Within this disome, the ubiquitin ligase Hel2 is recruited and ubiquitinates ribosomal protein uS10 with K63-linked ubiquitin chains, a prerequisite for upstream mRNA cleavage (NGD) and ribosomal nascent peptide degradation (RQC). In the absence of Hel2-mediated uS10 ubiquitination, mRNA cleavage ensues further upstream *via* a non-canonical RQC⁻ NGD mechanism involving monoubiquitination of eS7 by the E3 ligase Not4, followed by Hel2-mediated K63-chain elongation.

particular, in cells expressing full-length Hel2, cleavage products could be mapped to the P site of the ribosome and 26–36 nucleotides upstream; while in cells expressing Hel2(1–315), the cleavage products were mapped 45–51 nucleotides upstream. The authors referred to these two types of cleavage reactions as RQC⁺ and RQC⁻, respectively, with both being dependent on the ability of Hel2 to introduce K63-linked ubiquitin chains. This first set of experiments highlights the intricate role of Hel2, and likely its ribosomal protein target specificity, in bifurcating the processes of NGD and RQC downstream of the initial stalling event.

The observation that different Hel2 deletions activate different branches of NGD suggests that the process is critically dependent on the identity of the target ribosomal protein. Previous proteomic analysis by the same group showed ribosomal protein uS10 to be a substrate for Hel2. After confirming these results, Ikeuchi *et al* (2019) went on to demonstrate that K63-linked polyubiquitination of uS10 is critical for the RQC⁺ branch of NGD. More importantly, the authors used *in vitro* ubiquitination assays to show that

Hel2 prefers higher-order polysomes over monosomes for its activity on uS10, suggesting that this branch of NGD requires ribosome collisions. These findings are in complete agreement with two previous studies that showed a dependence on ribosome collision for Hel2 and ZNF598 activation (Simms *et al*, 2017b; Juszkievicz *et al*, 2018). Moreover, similar to the analysis of the mammalian ribosome, cryo-EM structural analysis of the yeast disome unit revealed that collisions provide an interface between the small subunits serving as a specific-molecular-recognition site for Hel2 (Ikeuchi *et al*, 2019).

What about the RQC⁻ branch of NGD? It is independent of uS10 ubiquitination—in fact, it is only robustly active when uS10 can no longer be ubiquitinated. However, it is still dependent on the RING domain of Hel2, suggesting that it requires ubiquitination of some other ribosomal protein(s). Screening multiple ribosomal components, the authors identified ribosomal protein eS7 as the key Hel2 target in the RQC⁻ branch of NGD. Ikeuchi *et al* further showed that eS7 is initially mono-ubiquitinated by the Not4 ubiquitin ligase within the Ccr4-NOT complex

(Panasenko & Collart, 2012), with subsequent K63-linked-chain elongation mediated by Hel2. The authors provided a number of experiments that add support for this model, but most compelling is the observation that Not4 deletion results in inhibition of the RQC⁻ branch of NGD. Consequently, deletion of Not4 in a yeast strain in which uS10 can no longer be ubiquitinated by Hel2 completely abolishes NGD. How Not4 is activated to add monoubiquitin to eS7 outside the disome unit is still unclear, as is the downstream recruitment of Hel2 in this context.

An important question related to the mechanism of mRNA quality control has been how stalling-induced ribosome collisions activate endonucleolytic cleavage of the mRNA and ubiquitination of the incomplete nascent peptide for downstream degradation. Here, Ikeuchi *et al* (2019) demonstrated that Hel2-mediated ubiquitination of collided ribosomes act as a master regulator for both processes of NGD and RQC. Interestingly, K63-linked ubiquitination also serves as a signaling platform for DNA double-strand break (DSB) repair (Lee *et al*, 2017). This newly discovered role of

K63-linked ubiquitination in initiating endonucleolytic cleavage on mRNA expands the diverse function of K63-linked ubiquitination in signaling and showcases the effectiveness of this signaling mechanism in responding to damage to nucleic acids. How ubiquitination activates both NGD and RQC processes is currently unknown. A simple model that could explain these observations is that K63-ubiquitination of ribosomal proteins somehow activates/recruits the endonuclease(s). The resulting cleavage reaction predisposes ribosomes, which have little to no mRNA downstream, for dissociation by Dom34/Hbs1/Rli1. This in turn presents the peptidyl-tRNA-bound 60S subunit to Ltn1 and the RQC machinery for nascent-peptide ubiquitination. The appealing feature of this model is that it ensures that the two processes of NGD and RQC are coupled and activated by the same originating signal. However, K63-linked ubiquitination of ribosomal proteins activates two branches of NGD, suggesting that there are multiple signals that are activated upon stalling. Whether these signals activate the same endonuclease or not is unknown. At a minimum, the potential existence of multiple endonucleases could explain why it has been difficult to identify

the factor/s that are directly involved in this reaction. Furthermore, this requirement for two types of endonucleolytic reactions during NGD is not especially obvious, as the reactions do not appear to be redundant. Whether they are utilized for different types of stalling events or distinct ribosome conformations remains to be addressed. These are exciting questions for the field to explore in the future. The answers to these questions are likely to have far-reaching consequences for our understanding of the mechanistic details of quality control processes and their utility in responding to defective molecules as well as potentially regulating gene expression.

References

- Ikeuchi K, Tesina P, Matsuo Y, Sugiyama T, Cheng J, Saeki Y, Tanaka K, Becker T, Beckmann R, Inada T (2019) Collided ribosomes form a unique structural interface to induce Hel2-driven quality control pathways. *EMBO J* 38: e100276
- Ishimura R, Nagy G, Dotu I, Zhou H, Yang X-L, Schimmel P, Senju S, Nishimura Y, Chuang JH, Ackerman SL (2014) Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration. *Science* 345: 455–459
- Juszkiewicz S, Chandrasekaran V, Lin Z, Kraatz S, Ramakrishnan V, Hegde RS (2018) ZNF598 is a quality control sensor of collided ribosomes. *Mol Cell* 72: 469–481.e7
- Lee BL, Singh A, Mark Glover JN, Hendzel MJ, Spyropoulos L (2017) Molecular basis for K63-linked ubiquitination processes in double-strand DNA break repair: a focus on kinetics and dynamics. *J Mol Biol* 429: 3409–3429
- Matsuo Y, Ikeuchi K, Saeki Y, Iwasaki S, Schmidt C, Udagawa T, Sato F, Tsuchiya H, Becker T, Tanaka K, Ingolia NT, Beckmann R, Inada T (2017) Ubiquitination of stalled ribosome triggers ribosome-associated quality control. *Nat Commun* 8: 159
- Panasenko OO, Collart MA (2012) Presence of Not5 and ubiquitinated Rps7A in polysome fractions depends upon the Not4 E3 ligase. *Mol Microbiol* 83: 640–653
- Saito K, Horikawa W, Ito K (2015) Inhibiting K63 polyubiquitination abolishes no-go type stalled translation surveillance in *Saccharomyces cerevisiae*. *PLoS Genet* 11: e1005197
- Simms CL, Thomas EN, Zaher HS (2017a) Ribosome-based quality control of mRNA and nascent peptides. *Wiley Interdiscip Rev RNA* 8: e1366
- Simms CL, Yan LL, Zaher HS (2017b) Ribosome collision is critical for quality control during no-go decay. *Mol Cell* 68: 361–373.e5