Review of: "Frameshifting at collided ribosomes is modulated by elongation factor eEF3 and by Integrated Stress Response regulators Gcn1 and Gcn20"

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Review for Manuscript 2021.08.26.457827v1 by Grayhack lab entitled "Frameshifting at collided ribosomes is modulated by elongation factor eEF3 and by Integrated Stress Response regulators Gcn1 and Gcn20"

The authors have been investigating how the ribosome maintains the reading frame during stalling. They previously reported the roles of Asc1 and uS3, and Mbf1 in the +1 ribosomal frameshifting (+1RFS) by rare codon repeat. In this study, they identified two factors GCN1/20 and eFF3 that are involved in the +1RFS by rare codon repeat. GCN1 and GCN0 on the collided ribosome reduce the +1RFS distinct from their role in the Integrated Stress Response (ISR) pathway. The authors provide evidence that eEF3 suppresses frameshifting at CGA codon repeats, but not at TRM140. These indicate that the repression of frameshifting by eEF3 is CGA codon repeats specific. The finding in this study is the mutually exclusive involvement of quality control factor Hel2 and the ISR factor GCN1/20 in the suppression of +1RFS, probably competition in the association with the rare codon-disome. They also demonstrated the novel function of eEF3 in the facilitation of the +1RFS by the rare codon repeats. It leads to the important question of how eEF3 acts on the GCN1-disome and Hel2-disome to facilitates the +1RFS. It will be of general interest to the fields of translation regulation and quality controls. These are important discoveries that will be of broad interest and are appropriate for publication. However, more experiments before publication will clarify the molecular mechanism of how the reading frame maintains during ribosome stalling.

Major comments:
The results in the study and previous studies demonstrated that there three factors, Mbf1, GCN1, and Hel2 are involved in the suppression of +1RFS by CGA4. Recent structural analysis revealed that GCN1 and Hel2 bind to the disome mutually exclusive manner but Mbf1 could bind to GCN1-disome and Hel2-disome. The genetic analysis mentioned is crucial to clarify the roles of the three modes and their relations in +1RFS induced by rare codons-mediated ribosome stalling.

1. The role of Mbf1 in the suppression of +1RFS in the GCN1-disome and the Hel2-disome: The

*gcn1*D or *hel2*D single mutation did not increase +1RFS but synergistically increased in the gcn1D *hel2*D double mutation in the presence of Mbf1 (Figure 6E). In the mbf1-R89K mutant background, the *gcn1*D or *hel2*D single mutations increased +1RFS and the *gcn1*D*hel2*D double mutation additively increased the +1RFS. Importantly +1RFS increased drastically in the g*cn1*D*mbf1*D mutant cells (Figure 6E), probably due to that +1RFS is more efficient in the *mbf1*D mutant cells than that in the mbf1-R89K mutant cells (Figure 3). To clarify the role of Mbf1 in +1RFS in the GCN1-disome and the Hel2-disome, +1RFS by CGA4 in *gcn1*D, *hel2*D, *gcn1*D*hel2*D mutant cells should be investigated in the *mbf1*D background.

2. The relation between eEF3-mediated facilitation and two-suppression modes of the +1 RFS: In the *mbf1-R89K* background, eEF3 increased +1 RFS by Hel2-disome and GCN1-disome (Figure 6C, YEF3 wt lanes). In the presence of Mbf1, the +1 RFS increased only in the *hel2*D*gcn1*D double mutant cells, and the +1 RFS in *hel2*D*gcn1*D deletion mutant cells are the almost same as that in the *mbf1*D deletion, suggesting that eEF3 increased +1 RFS in the Hel2-disome and GCN1-disome. To clarify who eEF3 facilitates +1RFS in the GCN1-disome and the Hel2-disome in the presence of Mbf1, +1RFS by CGA4 in *gcn1*D, *hel2*D, *gcn1*D*hel2*D mutant cells should be investigated in the YEF3-m background.

3. The role of eEF3 in the absence of three suppression factors: eEF3 facilitates +1RFS in the *gcn1*D*hel2*D mutant cells in the presence of Mbf1 (Figure 6C) and the mbf1-R89K background (Figure 6F). To demonstrate that eEF3 in the absence of three suppression factors, it is important to demonstrate how eEF3 facilitates the +1RFS by CGA4 in the *gcn1*D*hel2*D*mbf1*D triple mutant cells.

4. The role of Hel2-mediated uS10 ubiquitination in the suppression of +1RFS: Gcn1 and Gcn20 on the collided ribosome reduce the +1RFS distinct from their known role in the ISR pathway. It is also evident that Hel2 reduces the +1RFS by CGA4 independent of the GCN1-mediated reduction. Given that the role of Hel2 in RQC and NGD is dependent on the ubiquitination of ribosome proteins, and the CGACGG-disome is the substrate for Hel2-mediated ubiquitination of lysine 4 and lysine 8 on ribosome protein uS10, it is informative to investigate the effect of the *uS10-K6R/K8R* mutation in the +1RFS in combination with the *gcn1*D or *mbf1*D as well as the eEF3 mutation.

5. The effect of the RPS3-S104Y mutation in the suppression of +1 RFS: The deletion of GCN1 and GCN20 synergistically increased the +1 RFS by CGA4 with the *RPS3-S104Y* mutant cells (Figure 5). Since the involvement of uS3 mutation in the quality control induced by the ribosome stalling, and the deletion of quality control factor Hel2 synergistically increased the +1RFS with *gcn1*D or *gcn20*D mutation, and it should be examined the increase of +1RFS in RPS3-S104Y*hel2*D double mutation in the combination with *mbf1*D and Mbf1-R89K mutation.