Revisiting The Non-go Decay Endonucleolytic Pathway

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Abstract

The process of translation in eukaryotes is subject to rigorous quality controls to mitigate proteotoxicity, which has the potential to cause deleterious effects on cellular function. During the process of elongation, ribosomes may experience stalling events, which can result in collisions between ribosomes. The phenomenon of ribosome rescue and mRNA decay taking place in the context of ribosome collisions is commonly known as Non-go Decay (NGD). Recent progresses have revealed the machinery of NGD in yeast and with multiple pathways being proposed. Among these pathways, the endonucleolytic pathway has been proposed. However, a recent *in vivo* study conducted in *c. elegans* showed a contradiction with commonly accepted NGD endonuclease mechanism. This review will explore the possibility of accepting this proposed idea by considering the findings of previous studies.

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Introduction

The process of mRNA translation is a complex phenomenon that includes the participation of numerous proteins and RNA molecules, which aid in the assembly of ribosomal subunits and the binding of ribosomes onto the mRNA. Subsequently, the ribosome undergoes translocation along the coding region and disassembles upon reaching the stop codon, thereby releasing the recently produced protein. Translation process is subject to quality control to prevent proteotoxicity that could be harmful for the cells[1,2]. During the elongation process, ribosomes may experience stalling due to the mRNA sequence itself or being subjected to environmental condition. The prior could be caused by the premature termination codon (PTC), stem loop secondary structure of the mRNA, CGA repeats, or lacking stop codon[3-6].

In order to address the diverse factors that contribute to challenges in the translation process, eukaryotes have developed three known distinct quality control mechanisms, namely Nonsense-Mediated mRNA Decay (NMD), Non-stop Decay (NSD), and No-go Decay (NGD). NMD is a surveillance pathway where the mRNAs contain PTC that might be resulted from genetic mutations or errors in the splicing process. This mRNA decay pathways involve UPF proteins and additional SMG proteins for metazoans to disassociate ribosome and further promoting mRNA degradation[5]. Moreover, non-stop decay (NSD) takes place when ribosomes encounter a stall at the 3' end of mRNA. The SKI complex identifies this event and facilitates mRNA degradation through the exosome[6].

In this review, we will deep dive more into NGD, which resulted from the collision of stalled ribosome and the trailing ribosome(s). We will start with the collision sensing mechanism, and their downstream processes, including mRNA degradation and ribosome rescue. In addition, we will discuss an interesting *in vivo* NGD study in *c. elegans* where it suggested that Hbs1, aside forming Pelota:Hbs1 complex (Dom34:Hbs-1 in Yeast) for rescuing ribosome, enables endonuclease process in NGD[7]. Furthermore, the authors suggested rescue first, cleavage second NGD model which contradicts with previous studies[8,9].

Ribosome Collision Sensing

It has been reported that ubiquitination by E3 ubiquitin ligase ZNF598 (Hel2 in yeast) is required for recognizing the ribosome collisions in NGD[7,10,11] and cells lacking ZNF598/Hel2 showed increase in the NGD reporter products[7,11,12]. ZNF598/Hel2 selectively ubiquitinate the small ribosomal subunit of the trailing stalled ribosome. It was reported in an in vitro study that in yeast, Hel2 ubiquitinates uS10[12] on the small ribosomal subunit 40s which then activates the downstream process for rescuing ribosome. In addition, it was also reported that ZNF598 targets uS10 and eS10, which are both components of ribosomal subunit 40s in the in vitro experiment of mammalian cells[11]. Additionally, it is also showed that the knocked out of ubiquitination increased the level of the protein reporter level, suggesting that ribosome ubiquitination is essential for sensing collision in NGD[7,11]. Nevertheless, it was also reported that in yeast, Syh1 provides an additional distinct pathway from ubiquitination for collision sensing[13,14]. However, its homolog in mammalian cells GIGYF1/2 thought to be dependent to ubiquitination and together with 4EHP, which binds into the mRNA 5' cap, repress the translation process in NGD[14].

mRNA Degradation

It is known that canonical mRNA degradation in eukaryotes occurs through the exonucleolytic pathways. In yeast, the degradation of mRNA from the 5' to 3' end occurs in a co-translational manner and is widely regarded as the primary pathway involved in canonical mRNA degradation[15]. Furthermore, the process of 3' to 5' exonuclease activity is carried out by the exosome, which is recruited by the SKI complex. This complex is responsible for the degradation of mRNA following deadenylation[16].

In the context of NGD in yeast, the Xrn1 plays major role for the degradation of aberrant mRNAs[17]. This degradation process, which occurs in 5' to 3' direction, is thought to be triggered by Syh1[13]. When Syh1 and RQC-trigger (RQT) complex are not available, the degradation of the mRNAs occurs through the endonucleolytic pathway, resulting in cleavage by Cue2 within the stalled ribosomes. This cleavage event gives rise to two intermediate NGD products. In addition, the ribosome of 5'-NGD intermediate will be rescued[18] and the mRNA will be degraded 3' to 5' by the SKI complex while the 3'-NGD mRNA product will be degraded 5' to 3' by Xrn1.

It is important to acknowledge that in a separate study, NONU-1, the Cue2 homolog in *c. elegans*, was reported to function as an endonuclease[19]. Furthermore, it has been reported that small MutS related (SMR) domain, which is conserved through eukaryotes, on both NONU-1 and Cue2 is crucial for the endonuclease during NGD[17,19].

Ribosome Rescuing Mechanism

While the disassociation of ribosomes is not required for the degradation of aberrant mRNA in the canonical mRNA degradation process in yeast[15], in case of NGD, it is coupled with two distinct ribosomal rescue pathways. First, the ribosomal rescue is primary catalysed by the RQT complex (ASC-1 complex in mammals). In yeast, RQT complex consists of RNA helicase Slh1/Rqt2, ubiquitin binding protein Cue3/Rqt3, and zinc-finger type Rqt4 (In mammals their orthologs are ASCC3, ASCC2, and TRIP4)[20,21]. It was reported that the ATPase dependent helicase by Slh1/ASCC3 is

important for the ribosome dissociation into 40s subunit and 60s-peptidyl-tRNA[20,21]. The latter then undergoes Ribosome-bound Quality Control (RQC) to degrade nascent peptide and 60s subunit recycle[22]. In addition, an *in vitro* study suggested that the CUE domain of Cue3/Rqt3 was showed to be important for the binding of RQT into the ubiquitinated uS10[12]. However, the importance of CUE domain in ASCC3 has not been consistently reported in the two different *in vitro* studies[21,23].

Furthermore, in the absence or inaccessibility of RQT complex, the cleavage in vicinity of collided ribosomes is preferred[17]. Then, the ribosome on 5'-NGD intermediate product will be rescued by Dom34:Hbs1, which is paralog of eRF1:eRF3[8,9]. In mammals, the ribosomal dissociation requires ABCE1 and Pelota:Hbs1 complex[8]. Furthermore, due to lack of GGQ motif as in eRF1, both Dom34:Hbs1 and Pelota:Hbs1 do not release nascent peptide[8,24]. In addition, despite the suggested role of the Hbs1 in sensing stalled ribosomes from a cryo-EM structural study, it has been commonly accepted that in case of the NGD endonuclease pathway in yeast, the cleavage comes first before ribosomal rescue[8,9,17]. However, a study in *c. elegans* proposed contrasting perspective[7].

Rescue First, Cleavage Second

An in vivo study was aimed to know the order of the known NGD factors through endonucleolytic pathway in *c. elegans* [7]. In the study, the authors used mutated unc-54 gene that inserted with rare arginin codons (CGG, AGG) to induce ribosome collision and coupled it with GFP. Subsequently, the fluorescence was assessed in order to quantify the final products of translation. Furthermore, to check whether the Pelota and Hbs1 are important for the NGD in *c. elegans in vivo*, they measured the GFP fluorescent of Pelota(Δ), Hbs1(Δ) and Pelota(Δ)Hbs1(Δ). The findings indicated the two might function together and each of the mutants could affect the GFP level. Furthermore, due the poorly understood role of Hbs1, they checked whether the Hbs1 could influence GFP level when it mutated together with NONU-1. The result showed that the Hbs1 might function in the same pathway as NONU-1 in the cleavage process. Moreover, in order to determine the necessity of Pelota:Hbs1 prior to cleavage, they used the inverse relationship of overexpression factor (mCherry) to NGD (GFP). The study also demonstrated that the overexpression of NONU-1 in the Pelota(Δ) Hbs1(Δ) condition resulted in a negative overlap value indicating a potential rescue first, cleavage later mechanism. In the subsequent section, we will examine the potential acceptance of this idea considering prior studies. At the end, we will also discuss the future outlook of it.

Examining the Rescue-Cleavage Model

Early *in vitro* biochemical study in yeast implicated that Dom34:Hbs1 (Pelota:Hbs1 in mammals) was suggested to be required for the endonuclelytic cleavage in NGD[4]. Although the endonuclease responsible for cleavage in NGD had not been identified at that particular time, the results obtained from northern blotting indicated a complete absence of 5'-NGD mRNA fragments on the Xrn1ΔDom34Δ strain. The absence of 3'-NGD mRNA fragments was also observed in the Ski7ΔDom34Δ strain suggesting the Dom34 role in NGD[4]. However, in the other study, the earlier results were not replicable[25] suggesting neither Dom34 nor Hbs1 were required for the NGD cleavage *in vitro*. In addition, it was also suggested that Dom34 did not have nuclease

activity *in vivo* and it was proposed Dom34 would interact with empty A site of ribosome due to its similarity with eRF1[25]. Further *in vitro* study showed that Dom34:Hbs1 dissociates ribosomes codon-independently which suggested due to Dom34 lacks the GGQ motif as eRF1 has to recognise the stop codons[24,25]. Furthermore, an *in vitro* study conducted on mammals demonstrated that Pelota:Hbs1 (Dom34:Hbs1 in yeast) was capable of dissociating ribosomes that possess an empty A site in a codon-independent manner. However, this process required the presence of ABCE1. Additionally, it was proposed that the Pelota:Hbs1:ABCE1 complex does not have the capability of dissociating pre-cleavage NGD[8].

Additional structural analyses provided further evidence in support of the idea that Dom34 binds into empty A-site. A study revealed that Dom34:Hbs1 complex adopts EF-Tu-tRNA complex. Notably, the structure of Dom34 N-terminal and central domain matched with the anticodon and aminoacyl acceptor of tRNA[26]. Moreover, another structural study also supported the importance of the empty A site of ribosome for the binding of Dom34 which leads to the pre-cleavage NGD requirement for the ribosome dissociation by Dom34:Hbs1 complex[9]. Furthermore, a Cryo-EM study on the structure of collided disomes revealed that the trailing ribosome was in the rotated state (hybrid A/P and P/E tRNA positions) supporting the cleavage requirement[10]. In addition, a ribosome profiling study found that the 16nt fragments were enriched in Dom34(Δ), providing additional evidence supporting the notion of cleavage occurring prior to the ribosome dissociation[27].

Based on the prior biochemical and structural studies, it is tempting to suggest that the idea of rescue first, cleavage second migt not be true. However, it is still possible that the Pelota, Hbs1 or Pelota:Hbs1 complex might recruit inderectly the endonuclease in *c.elegans in vivo.* However, if it is true, it should have direct or indirect sensing to the stalled ribosome to activate the endonuclease. A study suggested N domain of Hbs1 might recognise the stalled ribosome[9]. However, the mutation on the N domain of Hbs1 was proved *in vivo* to not affecting the NGD in *c. elegans*[7] suggesting the indirect sensing of stalled ribosome.

Future Outlook

Despite the recent studies, the NGD mechanism in metazoans, especially *c.elegans*, is still poorly understood. Moreover, from the prior study which proposed the NGD endonuclease in *c. elegans*, the ribosome profiling results were questionable[19]. There were no dramatic accumulations upstream the rareArginine seqeunce for 28-30nt fragments when Skih-2, Pelo, and NONU-1 were mutated indicating no ribosom collisions. In the same mutant background, the 15-18nt fragments were also not significantly accumulated suggesting either low level or inconcistent NONU-1 endunuclase activity. Finding either more robust endonuclease or replicating the study of NONU-1 is important to validate and reveal the mechanism of ribosomal rescue and mRNA decay for the NGD in *c. elegans*. Moreover, it is also tempting to find the other role of either Hbs1 or Pelota in NGD aside of forming Pelota:Hbs1 complex to rescue the ribosomes. Furthermore, it would be valuable to investigate the potential presence of the NGD RQT mechanism in metazoans, given its status as the predominant NGD pathway in yeast.

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