



HAL
open science

Coupling mRNA and peptide degradation pathways into the ribosome-associated quality control

Ferrand Gabin, Claire Torchet, Benard Lionel

► **To cite this version:**

Ferrand Gabin, Claire Torchet, Benard Lionel. Coupling mRNA and peptide degradation pathways into the ribosome-associated quality control. RNA meeting 2024, FASEB, May 2024, Edingburgh, United Kingdom. hal-04802392

HAL Id: hal-04802392

<https://hal.science/hal-04802392v1>

Submitted on 25 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

Coupling mRNA and peptide degradation pathways into the ribosome-associated quality control

Ferrand Gabin (1), Torchet Claire (1), Benard Lionel (1)

1 - Institut de biologie physico-chimique; CNRS UMR 8226; Sorbonne Université (France)

Accumulation of impaired proteins poses a critical issue for all cells, and elaborate quality control systems have evolved to minimize their effects. In particular, cells have developed failsafe mechanisms to cope with deleterious ribosomal stalls that result from damaged mRNA. Though temporary ribosome stalls can have biological functions, like co-translational protein folding or targeting, persistent stalls are generally indicative of a stress, leading to ribosomal collisions, promoting degradation of the nascent peptide (NP) and triggering mRNA decay.

Recognition of ribosomal collisions involves factors and mechanisms that are highly conserved in eukaryotes, as exemplified in the *S. cerevisiae* model. Thus, ribosome collisions form a particular conformation recognized by a conserved ubiquitin ligase, Hel2. Specific ribosomal proteins are then ubiquitinated and associate with the Ribosome Quality Triggering (RQT) complex, which disassembles the colliding ribosomes and generates a 60S subunit still attached to a peptidyl RNA. Then, the Ribosome-mediated Quality Control (RQC) pathway can interact with this particular 60S subunit, and triggers the degradation of the arrested product by the proteasome system. It is proposed that this quality control is coupled to mRNA decay processes that target problematic mRNAs and prevent ribosomes from continuously producing aberrant peptides, potentially toxic to the cell. It is now admitted that problematic mRNAs (stalling within coding sequence) are mostly degraded through 5'-decapping and 5'-3' exonucleolytic processing by the canonical degradation machinery, with the 5'-3' exoribonuclease Xrn1 playing a major role. How all these processes are coordinated has not been clarified. Nor has the interconnection of these processes been elucidated, if it exists at all.

To investigate whether mRNA and peptide degradation are coupled, we wondered whether mRNA decay factors are able to affect RQT and/or RQC processes. We thus designed different mRNA reporters containing rare codons, and analyzed the impact of a set of mRNA decay mutants on aberrant peptide production. The tricky part of this approach is that a greater stabilization of faulty mRNAs may simply result, in a passive mode, in the accumulation of aberrant peptides. We have therefore designed our mRNA reporters to limit this issue and we propose promising candidates interconnecting all these quality control processes via mechanisms and interactions to be deciphered.

Title (required)

Beyond tRNA splicing and No Go mRNA Decay, the Importance of the RNA Kinase Trl1

Abstract (required)

The No-Go Decay (NGD) mRNA surveillance pathway degrades mRNAs containing stalled ribosomes during the elongation step of translation. NGD occurs when ribosomes are blocked by the presence of stable intra- or intermolecular RNA structures, enzymatic cleavage,

chemically damaged sequences, or rare codons. Other mRNA surveillance pathways can also ultimately lead to NGD. For instance, transcripts synthesized without a stop codon due to premature polyadenylation have stalled ribosomes that are initially detected by the Non-Stop Decay (NSD) pathway. NSD targets mRNAs are cleaved and become targets of NGD when ribosomes reach the new 3'-end and stall. NGD thus plays a key role in resolving translational issues potentially detrimental to cellular homeostasis.

These problematic mRNAs can be degraded through 5'-decapping and 5'-3' exonucleolytic processing by the canonical degradation machinery, with the 5'-3' exoribonuclease Xrn1 playing a major role. When ribosomal collisions are too robust, Cue2-mediated endonucleolytic cleavage activity can serve as a fail-safe system. Cue2 displays an SMR (small MutS-related) hydrolase Cter-domain and two conserved CUE ubiquitin-binding domains, allowing its recruitment to collided ribosomes after the ubiquitination of specific ribosomal proteins by the ubiquitin ligase Hel2. Cue2 cleavage proximal to the stalling site results in the production of 5' NGD and 3' NGD- RNA intermediates. 5' NGD RNA intermediates are degraded by the 3'-5' exonucleolytic pathway, which involves the Ski complex (Ski2, Ski3 & Ski8), Ski7, and the cytoplasmic exosome, while the 3' NGD- RNA intermediates are degraded by the 5'-3' Xrn1 exoribonuclease.

Several years ago, we focused on 3' NGD-RNA intermediates and demonstrated that these RNAs were 5'-hydroxylated. Surprisingly, these intermediates, now known to be produced by Cue2, are degraded by the 5'-3' Xrn1 exoribonuclease, which exclusively requires a 5'-monophosphorylated extremity for degradation. Our investigation revealed that the RNA kinase activity of Trl1 is responsible for the phosphorylation of these 5'-hydroxylated RNAs generated by NGD.

Trl1 is a well-characterized factor with RNA kinase, cyclic phosphodiesterase activity, and ligase activities essential for tRNA processing. Indeed, splicing of tRNAs is known to generate 5'OH-intronic RNAs, which require Trl1 kinase activity to permit their degradation by Xrn1. Here we show that its RNA kinase activity is not limited to tRNA splicing and NGD-targeted mRNAs but also degrades the majority of 5'-hydroxylated RNA species present in the cell. The importance and cellular advantage of such RNA kinase activity are discussed.

Authors and affiliations (required)

Rénette SAINT-FORT (Presenting) renette.saint-fort@ibpc.fr

CNRS SORBONNE UNIVERSITE, PARIS, France

Presenting author position: Not student or post-doc

Presenting author gender: Female

Sébastien CHAMOIS

CNRS SORBONNE UNIVERSITE, PARIS, France

Cristina PANOZZO cristina.panozzo@ibpc.fr

CNRS SORBONNE UNIVERSITE, PARIS, France

Lionel BENARD lionel.benard@ibpc.fr
CNRS SORBONNE UNIVERSITE, PARIS, France
Topic category (required)
Surveillance and degradation

Contribution of Ski Factors to Nascent Peptide Degradation During Ribosome Quality Control

Cristina Panozzo (1), Claire Torchet (1), Sébastien Chamois(1), Benoit Palancade(2), Lionel Benard (1)

1 - Institut de Biologie Physico-Chimique; CNRS UMR 8226; Sorbonne Université, 13 rue Pierre et Marie Curie, Paris (France)

2-Institut Jacques Monod ; Université Paris Cité - Bât. Buffon- 15 Rue Hélène Brion, Paris (France)

Aberrant or damaged mRNAs can stall ribosomes during translation and adversely affect cellular functions. Eukaryotic cells exhibit multiple quality control mechanisms to manage problematic protein synthesis. For instance, Ribosome Quality Control (RQC) monitors incomplete nascent peptides associated with such stalled ribosomes. Any failure to complete RQC can result in the production of incomplete and dysfunctional proteins, as well as the formation of potentially toxic cellular aggregates.

In these defective mRNAs, collisions of ribosomes are considered the primary trigger for quality control mechanisms, leading to the degradation of nascent peptides (NPs) along with mRNA decay. Although significant progress has been made in understanding the mechanisms leading to NP degradation, the coordination between NP decay and RNA decay pathways remains unclear. It is proposed that NP degradation coincides with mRNA degradation, offering the advantage of curtailing translation cycles of aberrant messengers, thereby preventing excessive burden on the NP quality control system.

Upon ribosome collisions, problematic mRNAs are primarily degraded through 5'-decapping and 5'-3' exonucleolytic processing by the canonical degradation machinery, with the 5'-3' exoribonuclease Xrn1 playing a major role. When ribosomal collisions are too robust, or in the absence of RQC, Cue2-mediated endonucleolytic cleavage activity may serve as a fail-safe system. Cue2 cleavage, proximal to the stalling site, results in the production of 5' NGD mRNA intermediates, which are further degraded by the 3'-5' exonucleolytic pathway involving the Ski complex (Ski2, Ski3 & Ski8), Ski7 and the cytoplasmic exosome and in the production of 3' NGD-mRNA intermediates degraded by the 5'-3' exoribonuclease Xrn1.

Based on preliminary genetics and proteomic studies using mRNA reporters known to produce ribosome collisions, we have focused on the importance of 3'-5' mRNA degradation in RQC and obtained evidence for the importance of the interaction of 3'-5' mRNA decay activators for proper NP degradation.

mRNA Decay
Methods and Protocols Book © 2025
Editor Maxime Wery

RNA Blotting by Electrotransfer and RNA Detection
Gabin Ferrand, Rénette Saint-Fort, Cristina Panozzo, Claire Torchet, Lionel Benard
Pages 29-41
<https://doi.org/10.1007/978-1-0716-4176-7>

Book Title mRNA Decay

Book Subtitle Methods and Protocols

Editors Maxime Wery

Series Title Methods in Molecular Biology

DOI <https://doi.org/10.1007/978-1-0716-4176-7>

Publisher Humana New York, NY

eBook Packages Springer Protocols

Copyright Information The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Science+Business Media, LLC, part of Springer Nature 2025

Hardcover ISBN978-1-0716-4175-0Published: 14 November 2024

Softcover ISBN978-1-0716-4178-1Due: 28 November 2025

eBook ISBN978-1-0716-4176-7Published: 13 November 2024

Series ISSN 1064-3745

Series E-ISSN 1940-6029

Edition Number 1

Number of Pages XIV, 423

Number of Illustrations 15 b/w illustrations, 64 illustrations in colour

Topics Biochemistry, general, Cell Biology