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## Degradation of mRNAs that lack a stop codon: A decade of nonstop progress

A. Alejandra Klauer and Ambro van Hoof

Ambro van Hoof: ambro.van.hoof@uth.tmc.edu

### Abstract

Nonstop decay is the mechanism of identifying and disposing aberrant transcripts that lack in-frame stop codons. It is hypothesized that these transcripts are identified during translation when the ribosome arrives at the 3' end of the mRNA and stalls. Presumably the ribosome stalling recruits additional cofactors, Ski7 and the exosome complex. The exosome degrades the transcript using either one of its ribonucleolytic activities and the ribosome and the peptide are both released. Additional precautionary measures by the nonstop decay pathway may include translational repression of the nonstop transcript after translation, and proteolysis of the released peptide by the proteasome. This surveillance mechanism protects the cells from potentially harmful truncated proteins, but it may also be involved in mediating critical cellular functions of transcripts that are prone to stop codon read-through. Important advances have been made in the past decade as we learn that nonstop decay may have implications in human disease.

### Keywords

nonstop decay; exosome; ski complex; review; surveillance; mRNA degradation; Ski7

### Introduction

In all three kingdoms of life, gene expression is a precisely regulated process. As a way to control the gene expression in the cell, eukaryotes have evolved two pathways to rid the cell of normal mRNAs. Normal mRNA decay has been characterized in yeast and it is conserved throughout eukaryotes<sup>1</sup>. The rate limiting step in both pathways includes the deadenylation, or poly(A) shortening, of the target mRNA<sup>2, 3</sup>. Once the mRNA is deadenylated, it can be degraded in either 5' to 3' or 3' to 5' directions. Deadenylated mRNAs quickly become prone to decapping by the Dcp2 enzyme and are subsequently degraded by the 5' to 3' exonuclease Xrn1<sup>2, 4–11</sup>. The 3' to 5' degradation pathway involves the 3' exonucleolytic digestion of the deadenylated mRNA by the exosome<sup>12</sup>. The exosome is a complex of ten essential proteins that performs RNA maturation and degradation functions in the nucleus and cytoplasm of eukaryotic cells.

In addition to the exosome, the 3' to 5' decay pathway requires a complex of three proteins, Ski2, Ski3, and Ski8, as well as the cofactor Ski7<sup>8, 12–14</sup>. Yeast cells remain viable when either the decapping or exosome pathway is disrupted, however simultaneous inactivation of these mRNA degradation pathways is lethal<sup>12, 15</sup>. The observations that mutants of the 3' to 5' degradation pathway show little increase in the half-lives of most mRNAs indicated that this pathway may be secondary to the 5' to 3' pathway<sup>12</sup>. However, for two mRNAs whose decay kinetics have been carefully characterized, the decapping pathway is only 2–5 fold faster than the exosome pathway and genome-wide analysis of the mutants in the 5' to 3' decay pathway indicated that 20% of mRNAs depend on this pathway for degradation, arguing that the 3' to 5' decay pathway may be the main degradation pathway for some mRNAs<sup>16, 17</sup>.

In addition to controlling the amount of normal mRNAs, the cell needs to maintain fidelity in the production of such mRNAs. Since mistakes can arise from transcription to translation and anywhere in between, the cell has evolved ways to rapidly identify, target, and eliminate these errors. These aberrancies can occur at the gene, mRNA or protein levels. Through mRNA surveillance mechanisms, the cell distinguishes between transcripts suitable for translation and those that are unsuitable. Examples of aberrant mRNAs include those with premature stop codons, rare codons, or those that lack a poly(A) tail<sup>18–22</sup>. Each of these different types of aberrant mRNAs triggers a different surveillance response. Transcripts with premature stop codons activate nonsense-mediated decay<sup>23</sup> and mRNAs that contain rare codons or secondary structures that promote long translations pauses trigger no-go decay<sup>24</sup>. Transcripts that lack a poly(A) tail are degraded through an exosome-mediated decay pathway<sup>22</sup>. Moreover, transcripts that promote translation beyond the normal stop codon are degraded through ribosome-extension mediated decay<sup>25, 26</sup>. As more of these pathways become unveiled, we expect that we will discover more aberrant substrates, which may be degraded through a variety of decay pathways. Finally, this review focuses on the degradation pathway that targets transcripts that lack in-frame stop codons, the nonstop decay pathway. Interestingly, some of these mRNA surveillance pathways are related and use similar trans-acting factors. We refer the interested reviewer to thorough and recent reviews on this relatedness<sup>27, 28</sup>.

## NONSTOP mRNAs ARE COMMON PRODUCTS OF MISTAKES IN GENE EXPRESSION

Nonstop transcripts can arise in instances when transcription aborts, when polyadenylation occurs prematurely or through point mutations that disrupt the stop codon. It is also possible that certain transcripts are prone to nonstop decay in order to maintain low levels of gene expression under certain/normal physiological conditions.

*In silico* analysis provided evidence that premature polyadenylation occurs in about 1% of random yeast and human cDNA clones<sup>20, 29</sup>. Furthermore, species like yeast and plants have poorly conserved poly(A) signals, indicating that polyadenylation may be occurring at alternative sites more often than thought<sup>29</sup>. Through premature polyadenylation, nonstop mRNAs may be generated perhaps 5 to 10% of the time<sup>20, 30</sup>. Cryptic poly(A) sites have been found in about 1% of *S. cerevisiae* cDNAs upstream of the termination codon<sup>20</sup>.

Given that cryptic polyadenylation occurs, one should expect that organisms use this to regulate genes under certain physiological conditions. Indeed, the premature polyadenylation of four yeast genes has been shown to be regulated by nutrient source<sup>31</sup>. Transcripts for *CBP1 AEP2/ATP13* are known to be involved in respiration, so it is no surprise that these would be regulated with different nutrient sources. However *RNAI4* and *SIRI* have no apparent involvement in respiration and, interestingly, respond to different types of carbon sources. It is still unclear mechanistically how gene expression is regulated through premature/alternative polyadenylation, and furthermore how nonstop decay may be coupled.

Premature polyadenylation is not restricted to yeast, but has also been described in *Arabidopsis* using a transcriptome sequencing<sup>32</sup>. Most transcriptome sequencing studies focus on functional mRNAs and therefore filter out mRNAs that result from polyadenylation within the coding region. However, the study from Hunt and coworkers indicates that such premature polyadenylation sites reflect widespread premature polyadenylation. In *Arabidopsis* about 10% of all the mapped poly(A) sites mapped within coding regions of more than 4000 genes. We anticipate that what is true for yeast and *Arabidopsis* is also true

for other eukaryotes, but most of the available transcriptome data have not been analyzed in way that would discover these.

In addition to premature polyadenylation of endogenous genes, this phenomenon has also been shown to be a problem in expressing exogenous genes. For example, transgenic crops expressing a toxin from *Bacillus thuringiensis* are widespread in agriculture. These crops all contain codon optimized genes because the original gene failed to be expressed. Premature polyadenylation, and subsequent rapid degradation of the mRNA severely limits the expression of the Bt toxin<sup>33, 34</sup>.

## THE MECHANISM OF NONSTOP DEGRADATION IN YEAST

### Nonstop mRNAs are degraded in the cytoplasm

An initial hint that nonstop mRNAs are rapidly degraded came in 1989 when Herrick and Jacobson described two chimeric mRNAs between the yeast ACT1 and HIS3 genes<sup>35</sup>. One of these mRNAs contained the HIS3 stop codon and was stable (half-life of 79 minutes), while the other lacked an in-frame stop codon due to a 4-nucleotide deletion and was unstable (half-life of 9 minutes). Independently, this was also shown for yeast PGK1 mRNA, and this reporter was used for a more mechanistic analysis of nonstop mRNA decay<sup>20, 21</sup>. The PGK1-nonstop reporter was shown to degrade with a half-life of less than 2 minutes in wild-type yeast cells, while it is stabilized to a half-life of 15 minutes in a yeast strain with a point mutation in the exosome. The exosome, a complex of ten essential proteins, contains only one catalytic subunit, Rrp44. Recently, it was shown that the exosome has two catalytic sites in Rrp44, an exonucleolytic and an endonucleolytic domain<sup>36–38</sup>. Surprisingly, either the endo- or the exonuclease activity can promote the degradation of the nonstop transcript<sup>39</sup>.

In addition to requiring the exosome, nonstop mRNA decay requires the Ski7 protein and the Ski complex. The Ski complex is composed of the DEVH-box RNA helicase Ski2, the tetratricopeptide repeat protein Ski3 and two copies of the WD repeat protein Ski8<sup>13, 40–43</sup>. The *SKI* genes were initially identified as part of an antiviral mechanism of the yeast *S. cerevisiae* and later identified as cofactors required for the cytoplasmic functions of the exosome<sup>7, 12, 14, 44–46</sup>. Specifically, Ski2, Ski3, Ski8, and Ski7 physically and functionally interact with the exosome and promote the exosome-mediated degradation of normal and aberrant mRNAs in the cytoplasm<sup>21, 22, 46</sup>. It is not completely clear what the specific function of each of these Ski proteins in nonstop decay, however all four are required for nonstop mRNA degradation.

The nonstop decay pathway is different from the general mRNA decay mechanism and the nonsense-mediated degradation (NMD) pathway. Dcp2, Xrn1, and Upf1, which are proteins involved in general cytoplasmic mRNA decay and NMD, are not required for nonstop decay (see fig 1)<sup>20</sup>. The mechanism for nonstop decay is also distinct from the exosome-mediated decay of normal mRNAs in three aspects (see figs 1 and 2 for comparison). First, nonstop decay occurs without prior deadenylation of the mRNA<sup>21</sup>. Second, nonstop mRNA decay requires the C-terminal domain of Ski7, which is dispensable for exosome-mediated decay of normal mRNAs<sup>21</sup> (see below). Third, as mentioned above, nonstop mRNAs can be efficiently degraded by either the endo- or the exonuclease activities of the exosome, while exosome-mediated decay of normal mRNAs requires only the exonuclease activity<sup>39</sup>.

### Nonstop mRNA degradation may be initiated when Ski7 recognizes a stalled ribosome

Translation of the nonstop transcripts is required for initiation of the nonstop decay pathway<sup>20</sup>. This conclusion is based on the observation that treatment with cycloheximide which prevents translation elongation, stabilizes nonstop transcripts<sup>20</sup>. Furthermore,

addition of a stop codon just upstream of the poly(A) tail also blocked nonstop decay<sup>20</sup>. During *in vitro* translation, nonstop transcripts form stable complex with ribosomes<sup>47, 48</sup> indicating that unlike RNA or DNA polymerases, the ribosome does not dissociate from its template when it reaches the end. Furthermore *in vivo* sucrose gradient experiments confirm that nonstop mRNAs remain associated with polysomes<sup>26</sup>. These observations suggest a model where nonstop decay requires at least one round of translation, whereby the ribosome travels to the 3' end of the transcript and stalls.

Genetic evidence and sequence similarity suggests that Ski7 recognizes ribosomes stalled on nonstop mRNA through its C-terminal domain, and promotes the exosome-mediated degradation of the transcript<sup>21</sup>. The C-terminus of Ski7 is a member of a family of ribosome-associated GTPases, which also include the eukaryotic elongation factor 1 $\alpha$  (eEF1 $\alpha$ ), the eukaryotic release factor 3 (eRF3), and the no-go decay protein Hbs1<sup>49</sup>. All three of these factors recognize the ribosome, depending on what codon is in the ribosomal A-site. During translation elongation the A-site contains a sense codon, which is recognized by an aminoacyl-tRNA/eEF1 $\alpha$  complex. During translation termination the A-site contains a stop codon, which is recognized by the eRF1/eRF3 complex. Finally, when the ribosome stalls during translation elongation, it is recognized by the Dom34/Hbs1 complex<sup>19, 50-53</sup>. (Please see<sup>24</sup> for more details on no-go decay)

Based on the similarity between Ski7 and these translation factors, it was proposed that the C-terminal domain of Ski7 recognizes a stalled ribosome on a nonstop mRNA<sup>21</sup>. As predicted, C-terminal truncations of Ski7 affect nonstop decay without disrupting the general cytoplasmic function of the exosome<sup>21</sup>. Alternatively, mutations in *SKI7* that disrupt Ski7 binding to the exosome and the Ski complex (N-terminal truncations) affect both normal and nonstop mRNA degradation<sup>21</sup>. Thus, genetic evidence suggests that the C-terminal part of Ski7 may be responsible for recognizing ribosomes stalled on nonstop mRNAs.

Although Ski7 shares characteristics with the translation factors eEF1 $\alpha$ , eRF3 and the no-go decay factor Hbs1, two differences are striking. First, eEF1 $\alpha$ , eRF3, and Hbs1 each act as a heterodimer, with an interacting partner responsible for recognizing the state of the ribosome (tRNA, eRF1 and Dom34, respectively). No such interacting partner has been identified for Ski7, and thus it is not clear why Ski7 is specific for ribosome stalled on nonstop mRNAs. Second, GTPase activity is critical for the specificity of eEF1 $\alpha$ , eRF3, and Hbs1, and requires a conserved active site His residue<sup>54-56</sup>. This residue is not conserved in Ski7, and thus Ski7 may accomplish this function without the use of energy provided by GTP. Biochemical characterization of Ski7 and its interaction with the ribosome will be needed to further understand the recognition of nonstop mRNAs during translation.

### **Additional mechanisms may down-regulate nonstop gene expression**

As described above, one consequence of detecting the lack of a stop codon in an mRNA is the rapid degradation of the mRNA. This aspect is fairly well understood, but may not be the only manner in which the cell limits the consequences of these aberrant mRNAs. For example, translation of the nonstop transcript may be repressed, possibly due to the translation of the poly(A) tract<sup>26, 57</sup> and several studies have argued that the protein translated from nonstop mRNA is also very rapidly degraded (see below). One caveat of this statement is that it is difficult to decipher whether these consequences are due to the absence of a stop codon in the mRNA or unrelated to the absence of the stop codon. For example, nonstop reporter mRNAs are typically generated by mutating the normal stop codon in the mRNA, causing the 3' UTR and poly(A) to become part of the open reading frame. The protein encoded by such transcripts has a C-terminal extension with a poly-Lysine tail. The encoded protein is generally unstable, which in several cases has been attributed to the

absence of a stop codon, but contribution of the C-terminal extension or poly(Lys) tail have not been ruled out in all cases. For example, it has been shown that a poly(Lys) tail can contribute to rapid protein decay, but a poly(Arg) tail, and several other Komopolymers were also effective<sup>57, 58</sup>.

The abundance of the protein translated from nonstop mRNAs is much lower than can be explained simply from the lower mRNA levels<sup>57</sup>. Protein degradation significantly contributes to the low levels of reporter expression. It has been shown that the proteasome inhibitor MG132 increases expression of the protein encoded by the nonstop mRNA<sup>58</sup>. Wilson and coworkers showed that mutations of a proteasome subunit (*pre9Δ*), two proteasome assembly factors (*umplΔ* and *irc25Δ*, a.k.a. *poc3Δ*, *pba3Δ* and *dmp2Δ*) and a RING ubiquitin E3 ligase (*Int1Δ*, a.k.a. *rkr1Δ*) cause the stabilization of proteins translated from two different nonstop reporter mRNAs<sup>59</sup>. The function of the Lnt1 was confirmed by a subsequent study that also showed that Lnt1 specifically promotes the ubiquitination and proteasome-dependent degradation of the nonstop product<sup>60</sup>. Additional results indicate that Lnt1 mediates cotranslational recognition of the poly(Lys) tail on the nascent peptide. Notably, this recognition occurred both when poly(Lys) was encoded by a poly(A) tail, or by 12 AAA or AAG codons followed by a stop codon<sup>58</sup>. Thus, recognition of the nascent peptide triggers Lnt1-mediated protein decay, independent of the lack of a stop codon. In addition, the effect of *ski7Δ* is additive with the *pre9Δ* or *Int1Δ* effect, indicating that the exosome-mediated decay of nonstop mRNAs and the proteasome-mediated decay of the encoded peptide are independent of each other<sup>59, 60</sup>. Interestingly, the poly(Lys) tail would be inside the protein exit tunnel of the ribosome, and thus unavailable for direct co-translational recognition. A reasonable model appears that some peptide sequences mediate ribosome stalling, and the stalled ribosome is recognized by Lnt1, which mediates ubiquitination and protein degradation. This phenomenon might also depend on protein context, since a different set of experiments using different reporters suggest that (Lys)<sub>12</sub>-triggered ribosome stalling led to degradation of the nascent peptide mediated by E3 enzyme Not4<sup>58</sup>. Ribosome stalling Triggered by features in the RNA is known to activate no-go decay, so it will be interesting to see whether this generates additional substrates for Lnt1 or Not4.

## NONSTOP DECAY IN HUMAN CELLS

### Nonstop decay factors are conserved throughout eukaryotes and important for human health

The exosome and its cytoplasmic cofactors are conserved in all eukaryotes<sup>61–64</sup>. Most of the ten exosome subunits and four cytoplasmic cofactors each have one corresponding human ortholog, suggesting that cytoplasmic exosome functions are also conserved between yeast and humans. In addition the capacity of Ski2, Ski3, and Ski8 to form a Ski complex is also conserved in humans<sup>65</sup>. The molecular function of human Ski2, 3, and 8 has not been studied in detail, but the function of the drosophila homologs is similar to the yeast genes. Specifically, both the yeast and fly proteins have shown to be responsible for the degradation of 5' mRNA fragments generated by various endonucleases<sup>22, 66–69</sup>. Thus the function of the Ski complex and presumably the exosome in cytoplasmic mRNA decay was already established in the common ancestor of fungi and animals.

The physiological function of the human Ski complex has been revealed by the observation that loss of function mutations in the human *SKI3* (TTC37) or *SKI2* gene causes a rare congenital disorder (Tricho-Hepato-Enteric Syndrome)<sup>70–72</sup>. The major defect in Tricho-Hepato-Enteric syndrome occurs in the small intestine, and therefore the initial presentation of the syndrome is diarrhea starting shortly after birth, which can be treated by long-term parenteral nutrition. These findings, suggest that some function of the human ski complex is



especially important in the small intestine, although other symptoms can not be explained defects in the small intestine. Whether Tricho-Hepato-Enteric syndrome is caused by a defect in nonstop decay, or a more general cytoplasmic exosome disruption, remains to be determined.

The two exceptions of a one-to-one correspondence between human and yeast cytoplasmic exosome subunits and cofactors are the two proteins most central in the yeast nonstop decay, namely the Ski7 nonstop mRNA recognition protein, and the Rrp44 RNase responsible for degrading nonstop mRNAs. The human genome includes three homologs of Rrp44, named hDis3, Dis3L1, and Dis3L2<sup>73, 74</sup>. The functions of hDis3 and hDis3L1 have been studied to some extent, and are more specialized than the single yeast homolog. While hDis3 functions in the nuclear exosome, hDis3L1 localizes to the cytoplasm<sup>73, 74</sup>. The reverse is true for Ski7, which is a more specialized protein in a subset of the saccharomycete yeasts, but whose function is carried out by a more general protein in other eukaryotes<sup>75, 76</sup>.

### Whole genome duplication (WGD) in *Saccharomyces cerevisiae* led to a specialized nonstop decay factor-Ski7

Approximately 100 million years ago a genome duplication occurred in an ancestor of *Saccharomyces cerevisiae*<sup>77, 78</sup>. Initially the whole genome of *S. cerevisiae* was duplicated, but subsequently most duplicated genes were lost, and a minority retained. Genomic and molecular analyses that compared ancestors of *S. cerevisiae* pre-duplication with *S. cerevisiae* have shed light to the gene specialization that occurred after this WGD<sup>77, 79–81</sup>. Prior to genome duplication, the genome of ancestors of the *Saccharomyces* complex contained an ancestral gene, which we will refer to as *SKI7/HBS1*. WGD gave rise to separate *HBS1* and *SKI7* genes, with *HBS1* functioning in no-go decay and *SKI7* in nonstop decay. *Lachancea kluyveri* (previously named *Saccharomyces kluyveri*) diverged from *S. cerevisiae* just before genome duplication and thus has only a single *SKI7/HBS1* gene. The *L. kluyveri* *SKI7/HBS1* gene can complement both a *ski7Δ* and an *hbs1Δ*, strongly suggesting that this gene functions in both no-go and nonstop decay. Furthermore, other eukaryotes including humans, *Drosophila*, *Arabidopsis*, and *Trypanosoma* each have a single *SKI7/HBS1* gene like *L. kluyveri*, which presumably functions in both nonstop and no-go decay. It remains to be elucidated whether and how this gene specialization of *SKI7* and *HBS1* conferred an increased fitness for the *Saccharomyces* species.

### Nonstop mRNAs are rapidly degraded in mammalian cells without prior deadenylation

Nonstop reporter mRNA are also preferentially degraded in human cells, but whether all aspects of the nonstop decay mechanism are conserved, remains to be elucidated. An initial analysis of the stability of a nonstop mRNA reporter in HeLa cells showed that its cytoplasmic steady state levels were reduced about 3-fold<sup>20</sup>. A second paper used a nonstop  $\alpha$ -globin-nonstop reporter mRNA as a control in a study of a different mRNA decay pathway<sup>25</sup>.  $\alpha$ -globin-nonstop mRNA was very rapidly degraded in human HeLa cells, as well as mouse MEL and C127 cells (half-life of 2h versus 11.5h). Similarly to yeast nonstop mRNA decay, the rapid degradation of the nonstop transcript was not preceded by deadenylation and dominant negative versions of the deadenylase enzyme had no effect on the stability of  $\alpha$ -globin-nonstop mRNA<sup>25</sup>. This study however did not analyze whether rapid decay of these nonstop reporter mRNAs requires ongoing translation. A different study using a nonstop version of the human RPS19 mRNA, showed that the rapid degradation of the nonstop transcript is blocked by cycloheximide<sup>82</sup>. Thus, translation-dependent, but deadenylation-independent rapid decay of nonstop mRNA seems to be conserved in yeast and mammals. Whether the mammalian decay also required the exosome and its cofactors has not been tested, but it was shown to not require the nonsense-mediated decay factor Upf1<sup>25</sup>.

## Rapid degradation of nonstop mRNAs contributes to several human diseases

With the understanding that the steady state level of RNAs contributes greatly to gene expression, researchers are paying more attention to RNA stability. Gene therapy that attempts to disrupt the normal stability of mRNAs through miRNAs continues to be a hot topic in the cancer research field. Likewise, mutated genes that produce mRNAs with altered stability have been shown to contribute to human diseases.

As explained above, mutations in the human ski complex cause Tricho-Hepato-Enteric Syndrome. A second connection between nonstop decay and disease results from a growing number of reports that link nonstop mutations in specific genes to disease. By far the most prevalent nonstop mutation, perhaps affecting 100's of millions of people, was reported for the DEF126 gene<sup>83</sup>. The encoded  $\beta$ -defensin protein is important for human sperm function<sup>83–86</sup>, but 20% of males are homozygous for a nonstop mutation in DEF126<sup>83</sup>. A population study of Chinese couples with and without this polymorphism showed that the nonstop mutation in the DEF126 gene causes lower fertility which manifests as a longer time to achieve pregnancy<sup>83</sup>. More rare instances of nonstop mutations are listed in table 1<sup>82, 83, 87–95</sup>. In most of these cases the nonstop mutation correlates with reduced mRNA levels. Importantly, the nonstop mutation leads to reduced levels of a C-terminally extended protein and in these cases the lack of protein is the main cause for the symptoms. In some cases the mutant protein is functional, so that increased stability of the nonstop proteins and increased production of the protein could provide therapeutic benefit.

Two different genome-wide analyses have been performed on either mutations that cause disease or on SNPs<sup>96, 97</sup>. Each of them identified over 100 mutations where a stop codon is changed to a sense codon. In a subset of these, no other in frame stop codon is found in the 3'UTR, and thus these mutations generate a nonstop mRNA, which may cause disease<sup>96</sup>. In contrast, mutating stop codon that was followed by an additional next in frame stop codon within 50nt appears less likely to cause deleterious/clinically relevant phenotype<sup>97</sup>. More careful characterization of these cases will be required to fully understand the impact of nonstop mRNA decay on human genetic diseases.

## CONCLUSIONS AND FUTURE DIRECTIONS

Great advances have been made in the past ten years, unveiling and deciphering the nonstop degradation pathway. We have only begun to scratch the surface in understanding the mechanisms of nonstop decay. Other surveillance pathways such as no-go and the prokaryotic trans-translation pathway have detailed structural and functional mechanisms elucidated, the lack of structural and biochemical data on Ski7, the SKI complex and their interactions with the stalled ribosome and the exosome limit our understanding of nonstop decay at the molecular level.

Learning how to suppress the nonstop decay pathway will have important implications in treating the aforementioned diseases. Recently, genetic therapies that suppress NMD, are being analyzed in clinical trials to prevent/treat Cystic fibrosis and Duchenne's muscular dystrophy among other genetic diseases<sup>98</sup>. Likewise, nonstop decay could be targeted to prevent mRNA and/or protein degradation. There is still a long and exciting road ahead to fully understand and learn how to control the mechanism of nonstop mRNA and peptide degradation.

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**Sidebar****Trans-translation versus nonstop decay**

Similarly to the eukaryotic nonstop decay, bacteria have a surveillance mechanism to relieve the cell of stalled ribosomes. Trans-translation is the process whereby mRNAs that lack a termination codon are released from the ribosome and targeted for degradation. The recycling of ribosomes is a necessary feature for the cells to continue efficient translation of other transcripts. The details of the trans-translation have been reviewed by Barends et al.<sup>99</sup>. Briefly, trans-translation uses a tmRNA molecule, which functions as both a tRNA and a mRNA. The tmRNA is required for recognition of the stalled ribosome with the peptidyl-tRNA bound to the P-site. The “tRNA” side of the tmRNA binds the A-site of the ribosome and donates its charged alanine to the still bound polypeptide, which triggers the release of the tRNA from the P-site. While tmRNA is now bound to the polypeptide, the ribosome continues to translate the “mRNA” side of the tmRNA, which encodes a protein degradation tag and a stop codon. Translation termination occurs normally with the stop codon from the tmRNA and the tagged polypeptide is targeted for degradation by specific proteases.

**Recognition and degradation of bacterial and eukaryotic nonstop mRNAs requires homologous factors**

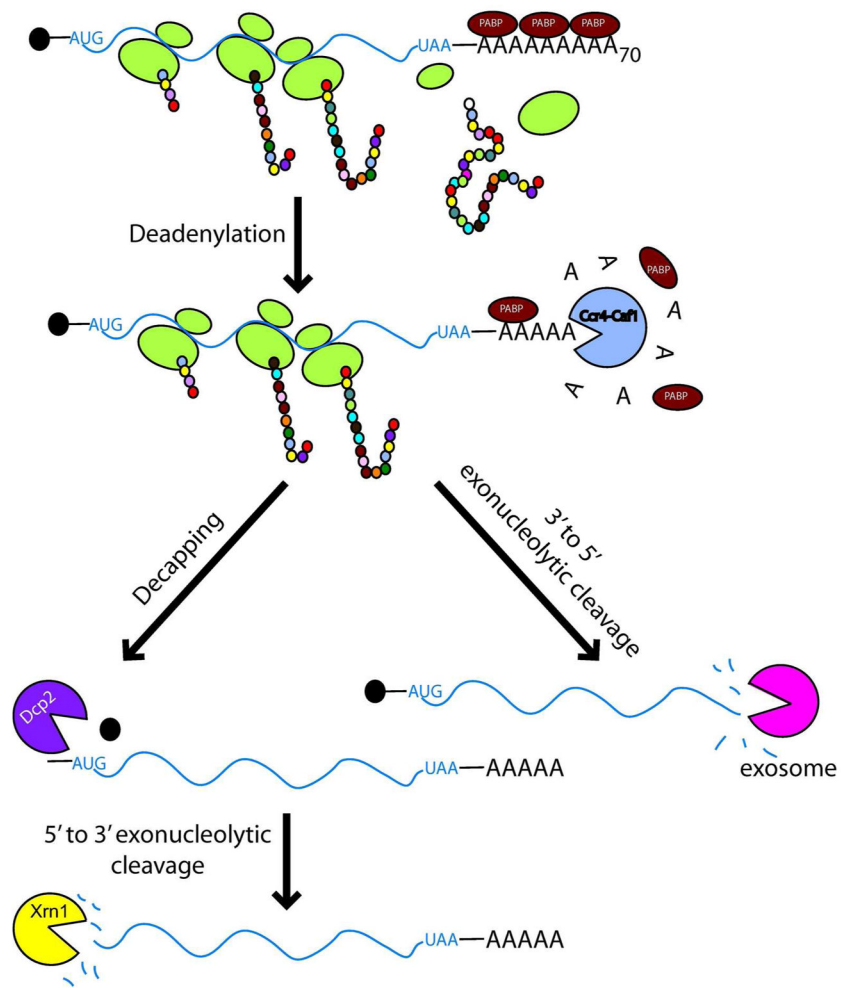
Both trans-translation and nonstop decay are triggered by stalled ribosomes caused by the lack of stop codons. tmRNA is delivered to the ribosome by EF-tu, the same GTPase that delivers tRNAs during translation elongation. EF-tu is a distant relative of Ski7. Whether this involvement of a GTPase is a conserved feature of bacterial trans-translation and eukaryotic nonstop mRNA decay or whether bacteria and eukaryotes have independently recruited a GTPase to these processes is not clear. Additionally, there is no evidence of Ski7 associating with a homologous eukaryotic tmRNA molecule. Furthermore, there is no evidence of a transpeptidation reaction occurring in eukaryotic nonstop decay like it does in trans-translation. After transpeptidation the tmRNA is positioned in a way that allows the release of the aberrant mRNA and proper placement of the “mRNA” side of the tmRNA in decoding center of the ribosome. The released mRNA is then targeted for degradation by RNase R, a homolog of Rrp44, the catalytic subunit of the exosome<sup>100</sup>. SimNar to EF-tu and Ski7 involvement RNase R and Rrp44 involvement may reflect conservation from an ancient ancestor, or a case where bacteria and eukaryotes have recruited similar proteins for similar functions.

**Release and degradation of nonstop peptide in trans-translation and eukaryotic nonstop decay**

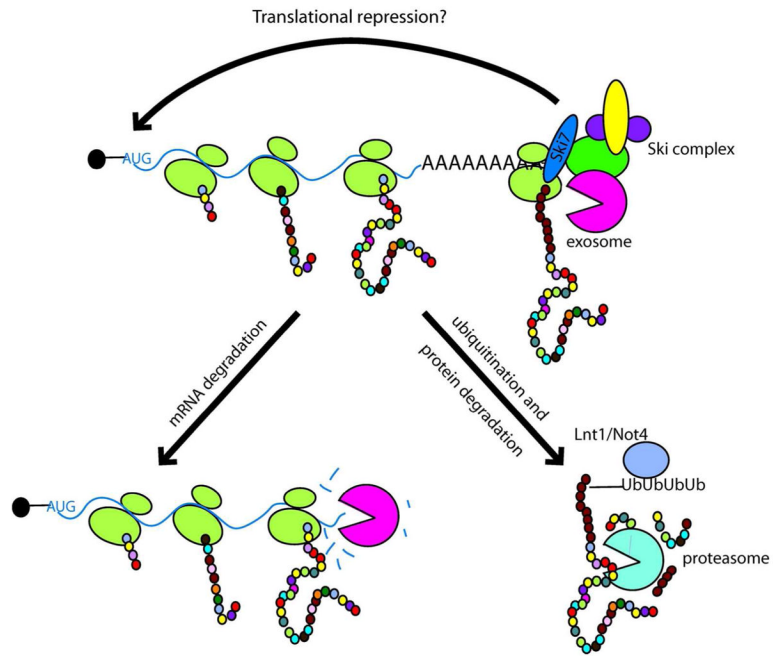
One clear difference between the trans-translation and nonstop decay mechanism is that fact that in the bacterial system addition of a proteolysis tag is intimately linked recognition of the stalled ribosome. In contrast to bacterial mRNAs eukaryotic mRNAs carry a long poly(A) tail. This distinction may explain why the eukaryotic mechanism does not require a tmRNA, since this C-terminal poly(lysine) tail may provide the tag for protein.

Finally, it is unclear how the mRNA, ribosomal subunits and the peptide are released in the eukaryotic nonstop decay pathway. In the trans-translation translation termination occurs normally with the help of the stop codon encoded by the tmRNA molecule.





**Figure 1.** Pathways for the degradation of normal yeast mRNAs. See text for details.



**Figure 2.** nonstop mRNAs are rapidly degraded, and may also trigger translational repression and proteolysis. See text for details.

Table 1

Human diseases caused by nonstop mutations.

Gene	Disease/symptoms	Reduction in mRNA level	reference
<i>DEFB126</i>	Reduced fertility	Y	[81]
Dysterlin	Muscular dystrophy		[92]
<i>RPS19</i>	Diamond Blackfan anemia	Y	[80,90,91]
Factor X	Haemophilia	Y	[89]
<i>GPR54</i>	idiopathic hypogonadotropic hypogonadism, infertility	Y	[88]
<i>TYMP</i>	Mitochondrial neurogastrintestinal encephalomyopathy	N	[93]
<i>APRT</i>	2,8-dihydroxyadenine urolithiasis, kidney stones, kidney failure	Y	[87]
<i>FOXE3</i>	Anterior segment dysgenesis, Peters anomaly	Y	[86]
<i>HSD3B2</i>	Adrenal hyperplasia	N	[85]