Connections Underlying Translation and mRNA Stability

Aditya Radhakrishnan [1,2] and Rachel Green [2]

*1 - **Program in Molecular Biophysics,** Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA*
*2 - **Howard Hughes Medical Institute,** Johns Hopkins School of Medicine, Department of Molecular Biology and Genetics, Baltimore, MD 21205, USA*

Correspondence to Rachel Green: *ragreen@jhmi.edu*
http://dx.doi.org/10.1016/j.jmb.2016.05.025
Edited by Daniel N. Wilson

Abstract

Gene expression and regulation in organisms minimally depends on transcription by RNA polymerase and on the stability of the RNA product (for both coding and non-coding RNAs). For coding RNAs, gene expression is further influenced by the amount of translation by the ribosome and by the stability of the protein product. The stabilities of these two classes of RNA, non-coding and coding, vary considerably: tRNAs and rRNAs tend to be long lived while mRNAs tend to be more short lived. Even among mRNAs, however, there is a considerable range in stability (ranging from seconds to hours in bacteria and up to days in metazoans), suggesting a significant role for stability in the regulation of gene expression. Here, we review recent experiments from bacteria, yeast and metazoans indicating that the stability of most mRNAs is broadly impacted by the actions of ribosomes that translate them. Ribosomal recognition of defective mRNAs triggers "mRNA surveillance" pathways that target the mRNA for degradation [Shoemaker and Green (2012)]. More generally, even the stability of perfectly functional mRNAs appears to be dictated by overall rates of translation by the ribosome [Herrick et al. (1990), Presnyak et al. (2015)]. Given that mRNAs are synthesized for the purpose of being translated into proteins, it is reassuring that such intimate connections between mRNA and the ribosome can drive biological regulation. In closing, we consider the likelihood that these connections between protein synthesis and mRNA stability are widespread or whether other modes of regulation dominate the mRNA stability landscape in higher organisms.

Introduction

The life of a coding RNA can be divided into three phases: birth by transcription, production of protein through translation, and death by mRNA decay. The steady-state level of coding mRNAs achieved by transcription (which, for ease of discussion, we take to include all subsequent processing required to ensure proper translation by ribosomes) and decay largely determines the level of gene expression, given that protein synthesis depends on the availability of mRNA [4–6]. However, the basis of heterogeneity in mRNA stability, often spanning multiple orders of magnitude in a given organism, remains poorly understood [7,8]. Despite substantial knowledge of the major pathways and enzymatic complexes responsible for mRNA degradation in both bacteria [9,10] and eukaryotes [11–15], we still do not understand what dictates the heterogeneity in stability of mRNAs. Perhaps, this is not surprising, as considerable diversity exists in the mRNA decay pathways; endonuclease-initiated mRNA decay serves as the committed step for decay in bacteria while exonucleolytic decay predominates in eukaryotes. While some of the heterogeneity in stability can likely be attributed to *trans*-acting factors [i.e., 3′-untranslated region (3′-UTR) binding proteins, microRNAs, etc.], the effects of these 3′-UTR binding factors on mRNA decay have, in many cases, not been separated from their effects on translation, and these interconnections may be central to their action [16–19]. For example, microRNAs are recruited to mRNA targets through Argonaute proteins that bind to machineries implicated in mRNA decay [20,21] and in translational repression [22]. Similarly, the CUP

protein, initially implicated in translational repression [23–25], also recruits the deadenylase complex to synergistically contribute to the regulation of gene expression [26].

A simple starting point for such a discussion is that mRNA abundance is regulated by the balance between the processes of transcription and decay. While it is immediately apparent how the process of transcription affects mRNA abundance, the extent to which the process of translation can directly impact mRNA abundance is less clear (Fig. 1). Recent studies, however, have established causal links between translation and mRNA stability, suggesting that these processes are intricately coupled, sometimes through nothing more than the rate of ribosome movement along the mRNA. These interconnections are reminiscent of those identified in the earlier steps in the mRNA life cycle, where the action of molecular machines at one step determines the fate of an mRNA in a later step. It is well known, for example, that mRNA processing and maturation are coupled to RNA transcription through RNA polymerase–CTD interactions [27–29], and that nuclear export, translation, and nonsense-mediated decay (NMD) are directly impacted by the presence of exon-junction complexes deposited during splicing [30–33]. In this perspective, we will discuss the role that translating ribosomes play in coupling translation to decay in mRNA surveillance, general decay of mRNAs, and microRNA-mediated gene regulation.

Generalizing mRNA Quality Control

Ribosomes that are arrested in nonproductive coding events (typically, on aberrant mRNAs) trigger mRNA quality control (or surveillance) pathways that ultimately lead to mRNA decay [34]. In all bacteria, ribosomes stalled on truncated mRNAs are "rescued" by tmRNA, a factor which couples ribosome release and recycling with decay of mRNA and the protein product [35,36]. In eukaryotes, examples of aberrant translation events include premature stop codons in the middle of an open reading frame (ORF; triggering NMD), poly(A) tails at the end of mRNAs due to the mRNA lacking a stop codon (non-stop decay), and dramatic kinetic traps (e.g., hairpins or truncated mRNAs) that prevent further translation (no-go decay) [1,37]. In each case, the ribosome (and associated factors) is thought to "sense" the defect in the mRNA and recruit additional machinery to implement downstream events including mRNA decay, proteolysis of the nascent peptide, and recycling of the stalled ribosome [38–42]. For example, in NMD, the UPF proteins are thought to be involved in identifying premature termination codons, while for non-stop decay, iterated lysines in the active site and exit channel are thought to contribute to critical ribosome pausing. We see that mechanisms linking aberrant translation to RNA decay are ubiquitous from bacteria to man.

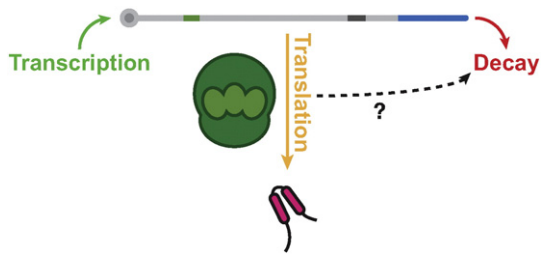

Fig. 1. The half-life of an mRNA is governed by the processes (colored arrows) of transcription and decay. However, recent findings suggest the act of translation itself governs mRNA decay.

While mRNA quality control couples decay of a nonfunctional mRNA to an aberrant translational event, there is a great deal of literature showing that decay is directly coupled with translation in more general contexts on functional mRNAs. In bacteria, decay is primarily governed by endonucleolytic activity, and barren mRNA appears to be an ideal substrate for the decay machinery. These conclusions derived from studies showing that when mRNAs are depleted of ribosomes—either by inhibiting efficient translation initiation through perturbation of the Shine–Dalgarno sequence [43,44] or by using exogenous RNA polymerases to outrun the translational machinery [45]—they are more susceptible to decay. These findings in bacteria provide precedent for direct coupling between the translational state of the mRNA and its decay.

In eukaryotes, there is also strong evidence for widespread coupling of decay with translation. For example, while mRNA surveillance is primarily thought to act on aberrant mRNA transcripts, there are numerous instances where mRNA surveillance pathways are co-opted to link translation to decay on functional mRNA transcripts. Specifically, genes with actively translated upstream ORFs in the transcript leader have been shown to be subject to decay by NMD machinery [46–48]. More generally, Hu *et al.* showed that mRNAs that sediment deep in a polysome profile with associated ribosomes are already substantially decapped and partially degraded by the exonuclease Xrn1 [49]. These initial observations were extended by experiments aimed at determining how widespread such co-translational decay might be across the genome. Employing high-throughput 5′ P-sequencing (trapping the natural product of Xrn1-mediated exonucleolytic decay), studies found that approximately a tenth of all cellular mRNAs in yeast were in the process of being degraded. Moreover, the position of the 5′ ends of these decay intermediates exhibited 3-nucleotide periodicity, suggesting that exonucleases are running into actively translating ribosomes [49,50].