PERSPECTIVES: MOLECULAR BIOLOGY

Skiing Toward Nonstop mRNA Decay

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Prokaryotic and eukaryotic cells have evolved remarkable quality assurance mechanisms at virtually every step of gene expression. One well-studied example in eukaryotes is nonsense-mediated messenger RNA (mRNA) decay (NMD). NMD prevents the synthesis of potentially deleterious truncated proteins when translation terminates prematurely (*1*). On pages 2258 and 2262 of this issue, the Dietz and Parker labs join forces to describe a new quality assurance mechanism that allows eukaryotes to identify and degrade mRNAs that lack a termination codon (*2, 3*).

Decay of nonstop and nonsense mRNA occurs by different mechanisms, but both require translation. In NMD, a termination codon is identified as premature by its position relative to downstream destabilizing elements. In *Saccharomyces cerevisiae*, these are loosely defined mRNA sequences. In mammalian cells, they are protein complexes that are deposited at exon-exon junctions during pre-mRNA splicing, regardless of whether termination is premature. The destabilizing elements recruit a subset of Upf proteins. If the recruited Upf proteins are a sufficient distance downstream of the termination site, they are activated to function in NMD as a consequence of translation termination. Otherwise they are removed by translating ribosomes.

Translation of mRNAs that lack a termination codon will result in a stalled ribosome at the 3′ end of the mRNA. In eubacteria, stalled ribosomes are released for recycling by a transfer-messenger RNA (tmRNA) (*4*). A tRNA-like region of tmRNA donates alanine to the stalled peptide chain using the A site of the stalled 30*S* ribosomal subunit. A messenger RNA-like region of tmRNA then displaces the mRNA and directs the synthesis of the last 10 amino acids (*5*). The resulting carboxyl-terminal tag targets the protein for proteolysis (*6*). Although the fate of the stalled mRNA is less certain, the finding that tmRNA copurifies with ribonuclease (RNase) R, a 3′-to-5′ exonuclease, suggests that the mRNA is also degraded (*7*).

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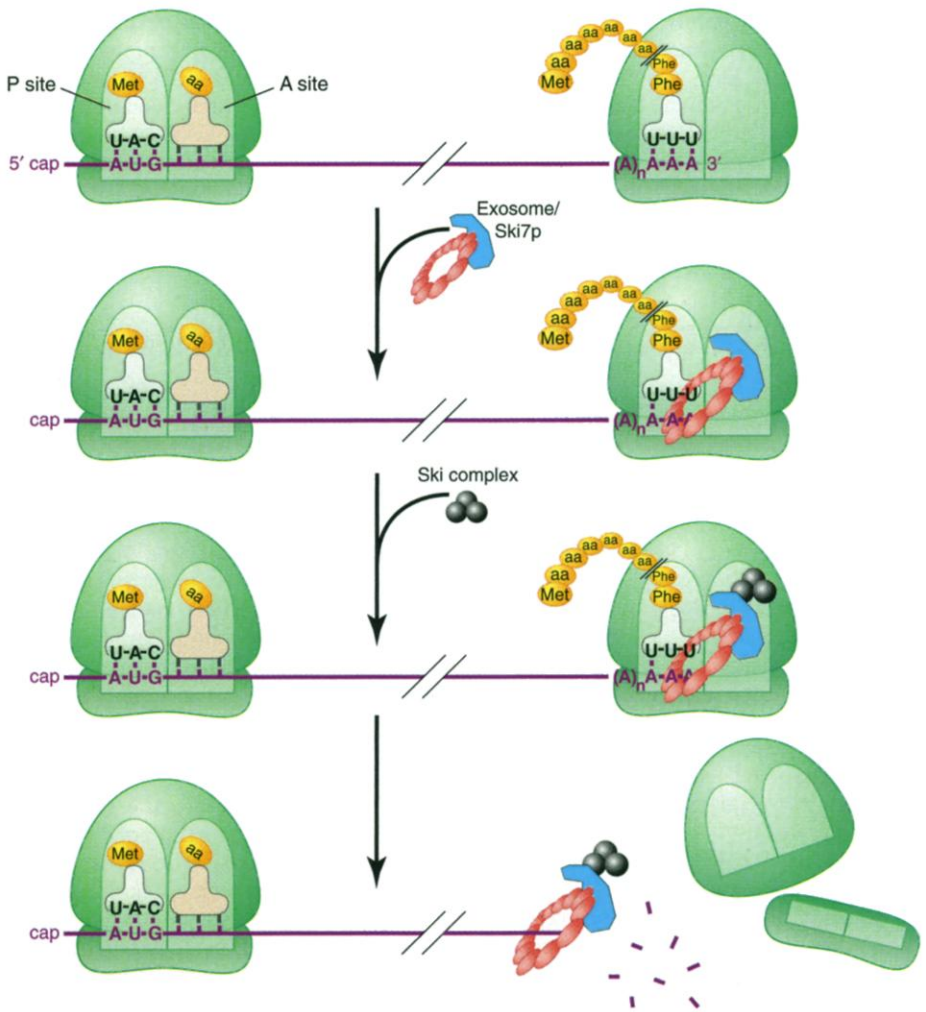
Data from the Dietz and Parker labs indicate that, in eukaryotes, the mechanism for assuring that protein products do not build-up from mRNA lacking a termination codon is quite different from NMD and from the tmRNA mechanism in eubacteria. Normally, mRNAs in *S. cerevisiae* are degraded through a major 5′-to-3′ pathway or a minor 3′-to-5′ pathway (*8–10*). Both pathways begin with shortening of the 3′-polyadenylate [poly(A)] tail by the deadenylase Ccr4p/Caf1p. The major pathway then involves removal of the 5′ cap by the decapping enzyme Dcp1p, followed by rapid degradation of the mRNA by the 5′-to-3′ exonuclease Xrn1p. The minor pathway involves the cytoplasmic exosome (a protein complex that is structurally related to the nuclear exosome, which processes or degrades diverse nuclear RNAs). NMD in *S. cerevisiae* can be viewed as a derivative of the major pathway, initiating with decapping followed by 5′-to-3′ decay but without the need for poly(A) tail shortening. In contrast to *S. cerevisiae*, mRNAs in mammalian cells may be degraded primarily in the 3′-to-5′ direction by the cytoplasmic exosome (*11*); the mechanism of NMD remains unclear.



A model for nonstop mRNA decay in eukaryotes. The stalling of an 80*S* ribosome at the 3′ end of a nonstop mRNA in the cytoplasm results in recognition of the ribosomal A site [associated with zero to three nucleotides of the poly(A) tail] by Ski7p. Recognition is mediated by the Ski7p carboxyl-terminal domain, which structurally mimics the GTPase domains of EF1A and eRF3. The amino-terminal domain of Ski7p recruits the exosome as well as the Ski complex. Because Ski7p is not detectable free of the exosome (*3*), Ski7p and the exosome are thought to bind the nonstop complex together. Finally, nonstop mRNA is degraded from the 3′ end. A_n, poly(A) tail; P, peptidyl-tRNA binding site on the ribosome; A, aminoacyl-tRNA binding site on the ribosome; aa, amino acid.

Frischmeyer *et al.* (*2*) and van Hoof *et al.* (*3*) demonstrate that nonstop mRNA decay in both *S. cerevisiae* and mammalian cells requires neither the major deadenylase nor players in either the 5′-to-3′ or NMD pathways. Instead it involves cytoplasmic exosome-mediated decay that begins at the 3′-poly(A) tail. The protein Ski7p associates with the cytoplasmic form of the exosome and with an auxiliary complex of Ski proteins (Ski2p, Ski3p, and Ski8p) through its amino-terminal domain (*12*). The completely or partially codon-free A site of the leading ribosome that is stalled at the 3′ end of the poly(A) tail is recognized by the carboxyl-terminal domain of Ski7p. This domain closely resembles the GTPase domains of the translation factors EF1A and eRF3 (*13*), which interact with the ribosomal A site occupied by a sense codon and a nonsense codon, respectively. An exosome mutation that inhibits Ski7p from interacting with the exosome also inhibits nonstop mRNA decay. Thus, van Hoof and co-workers (*3*) propose that the amino-terminal domain of Ski7p recruits both the exosome and the Ski complex to the 3′ end of nonstop mRNAs (see the figure).

Frischmeyer and co-workers (*2*) justify the need for nonstop mRNA decay by citing evidence that 40 of 3266 expressed sequence tags in *S. cerevisiae* (1.2%) derive from mRNAs having a 3′ end located upstream of the termination codon. They also show that 52 of 6357 translational reading frames in *S. cerevisiae* (0.8%) and 239 of 32,755 translational reading frames in humans (0.7%) harbor a putative 3′-end processing signal. In theory, nonstop mRNAs can also be generated when 3′-end formation takes place upstream of the normal termination codon as a result of RNA polymerase pausing (which is a known contributor to 3′-end formation), when ribosomes pause at rare codons or normal termination codons (as they do to trigger tmRNA function in bacteria), or when 3′-to-5′ decay is initiated on ribosome-bound mRNA.

One of a number of issues that remain to be resolved is whether eukaryotic nonstop mRNA decay involves a protein that mimics tRNA, similar to eRF1 [which functions with eRF3 in nonsense codon recognition (*14*)] or a tRNA-like RNA. Furthermore, the fate of the protein products of nonstop mRNAs remains a mystery. Molecular tags for proteolysis in eukaryotes usually consist of multiubiquitin chains that target the protein for degradation by the 26*S* proteasome (*15*). Because proteases responsible for the degradation of the protein products of nonstop mRNAs in bacteria are related to components of the proteasome (*15*), it is conceivable that the protein products of nonstop mRNAs in eukaryotes are ubiquitinated and then degraded by the proteasome. How the degradation of nonstop—and, for that matter, nonsense—mRNAs and the proteins they produce are coordinated is an area for future study.

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PERSPECTIVES: CHEMICAL REACTIONS

Steric and Solvent Effects in Ionic Reactions

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Noncovalent interactions play a central role in chemical structure and reactivity. Chemical reaction rates are influenced strongly by nonbonding interactions, particularly the solvation processes that evolve as reactants approach, and the short-range repulsions that constrain motion through the transition state. But separating the effects of solvation from the repulsive interactions (the steric effect) has challenged chemists for over a century (*1–3*).

On page 2245 of this issue, Regan *et al.* (*4*) take an important step toward resolving this problem. They report gas phase rate measurements and computer simulations on a classic nucleophilic substitution (S_N2) reaction. The results provide quantitative estimates of the distinct contributions of the solvation and steric effects to the free energy of activation in this important organic reaction.

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In an S_N2 reaction, an incoming reactant X^- (the "nucleophile") approaches a saturated carbon atom in a "backside attack" (see the figure). As the nucleophile approaches the central carbon atom and the incipient C-X bond forms, the umbrella associated with the tetrahedral geometry about the carbon atom begins to invert. The noncovalent interactions that influence motion through the transition state are very sensitive to the size of substituents R_1, R_2, and R_3 bound to the reaction center (*5*, *6*). The rates of nucleophilic substitution in alkyl halides decrease by several orders of magnitude as the alkyl substituents increase in size.

The study of chemical reactions such as the S_N2 reaction in the gas phase is a valuable approach for probing their dynamics without the complexities of solvent interactions (*5*, *6*). According to the double minimum potential model for the gas phase S_N2 reaction (see the figure) (*7*), the reaction proceeds through an electrostatically bound reactant-like complex $X^-•RY$, which rearranges to a product-like complex $RX•Y^-$. The latter intermediate then decays to reaction products.

Experimental studies that isolate the precursor complexes for both the forward and reverse processes and probe their reactions (*8–10*) demonstrate that these complexes are true reactive intermediates on the S_N2 reaction coordinate. The barrier that separates these complexes provides a direct measure of the steric effect in the reaction, after correction for differences in the bond strengths of the forming and breaking bonds.

Even though the steric barrier in the gas phase lies below the energies of the approaching reactants and therefore does not manifest itself directly as a reaction threshold, it serves as an entropic bottleneck to product formation. Once the reactant-like complex forms, it can proceed through the sterically hindered transition state and cross the barrier to form products, or it can revert to reactants. The calculated branching ratio, evaluated with Rice-Ramsperger-Kassel-Marcus (RRKM) statistical rate theory (*11*), is a parametric function of the steric barrier height. The best estimate of the barrier height is the value that reproduces the experimentally observed rate. A significant body of experimental and theoretical research (*12*) confirms the validity of the statistical hypothesis in the present system.

Regan *et al.*'s careful experiments provide a direct measure of the steric barrier.