

Programmed Ribosomal Frameshifting Goes beyond Viruses

Organisms from all three kingdoms use frameshifting to regulate gene expression, perhaps signaling a paradigm shift

Jonathan D. Dinman

Ordinarily, ribosomes rigorously maintain the reading frame of messenger RNA (mRNA) molecules being translated into proteins. However, some mRNAs of viruses—and other, more complex organisms—sometimes carry special sequence information and structural elements in their mRNA molecules that enable ribosomes to slip, and adjust, reading frame. This programmed ribosomal frameshifting (PRF) enables viruses to pack more information into their genomes, whose sizes are limited due to the small volumes of viral capsids into which they are packaged. PRF also adjusts the ratios of structural and enzymatic proteins in viruses. Moreover, because small changes in PRF efficiency can alter these ratios and thereby disrupt viral assembly, PRF is a potential target for antiviral agents.

Ribosomes can be directed to shift either

backward or forward. In the case of a -1 PRF, ribosomes slip in the 5' direction on mRNAs by 1 nucleotide, whereas +1 PRF results in forward, or 3', slippage by one base. While researchers continue to find new mechanisms that drive PRF, a common feature involves *cis*-acting elements that lead ribosomes to pause at specific “shifty” sequences, altering the usual kinetics of translation elongation to favor side reactions such as PRF (Fig. 1).

The shifts can involve bypass or double reading of a base, or tRNA slippage on the mRNA. In viruses, PRF typically allows a single mRNA transcript to code for two proteins: a non-frameshift-encoded protein, and a longer, frameshift-encoded fusion protein. The function of such PRF signals is to bypass the usual stop codon by shifting the ribosome out of frame by a single nucleotide, thus enabling the viral genome to augment its coding potential. In viruses, the frequency of frameshifting determines the stoichiometric ratios between structural and enzymatic proteins.

Although many researchers consider PRF a virus-specific phenomenon, it provides a potentially powerful molecular regulatory mechanism. Indeed, mounting evidence indicates that organisms from all three kingdoms of life use PRF to regulate gene expression, perhaps signaling a paradigm shift in how we think about protein translation and what constitutes the “correct” reading frame.

Examples of +1 PRF Affecting Gene Expression in Microbial and Eukaryotic Cells

The *prfB* gene of *Escherichia coli* that encodes release factor 2 (RF2) was one of the first +1 PRF signals to be identified. This

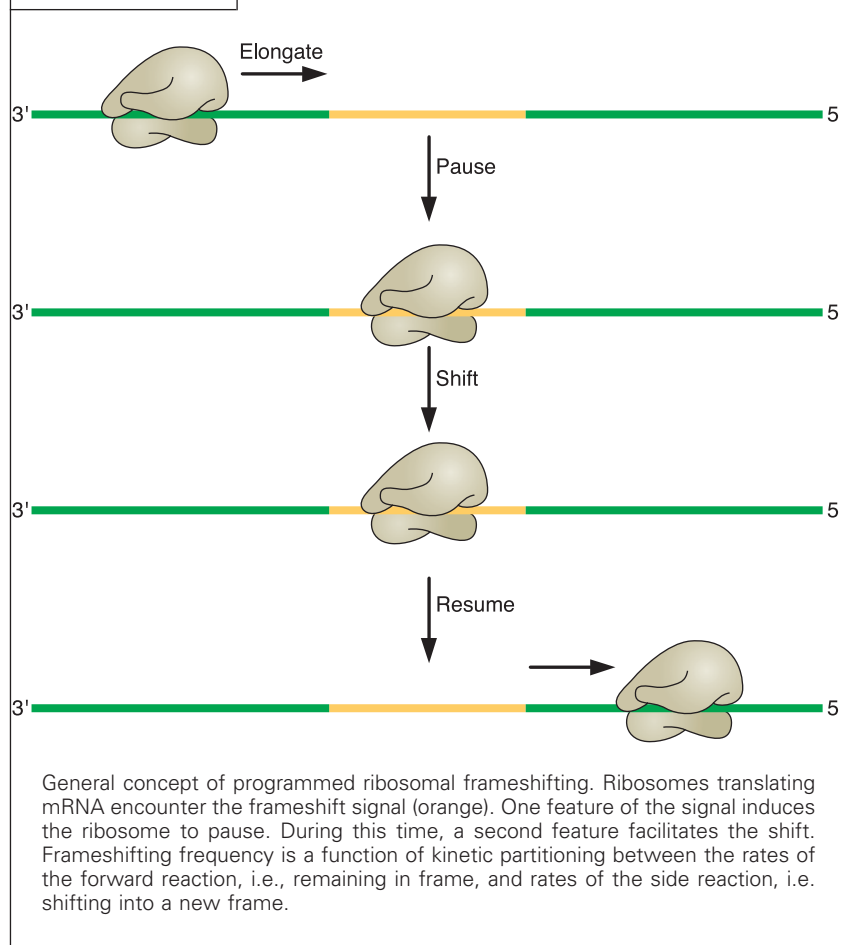
Summary

- Programmed ribosomal frameshifting (PRF) enables mainly viruses but also other organisms to adjust the reading frame of messenger RNA and thus to pack more information into their genomes.
- Organisms from all three kingdoms use PRF as a posttranscriptional mechanism to regulate gene expression, perhaps signaling a paradigm shift as to what constitutes the “correct” reading frame for mRNA molecules.
- Despite the use of several bioinformatic strategies, identifying -1 PRF signals remains daunting, even as computational power continues to increase.
- PRF might be an extremely old remnant of a prebiotic RNA world.

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FIGURE 1



protein recognizes and promotes translation termination at the UGA and UAA codons. The reading frame of the first 15% of this gene is determined by the start codon, while the remaining 85% of the mRNA is translated as a consequence of a +1 PRF event that involves bypassing UGA, a codon that ordinarily would terminate further translation.

Three separate parameters combine to promote 30–50% frameshifting efficiency (Fig. 2A). An upstream Shine-Dalgarno-like sequence positions the ribosome at the frameshift site that contains a UGA termination codon. When RF2 levels are high, termination is efficient, and synthesis of RF2 is downregulated. Conversely, low RF2 levels result in inefficient recognition of the UGA codon, stimulating frameshifting. The result is an autoregulatory feedback circuit in which RF2 levels control production of RF2 via frameshifting.

In many eukaryotes, PRF regulates expression

of the ornithine decarboxylase (ODC) anti-enzyme (AZ). AZ degrades ODC, the enzyme that catalyzes the first and rate-limiting step of polyamine biosynthesis, decreasing polyamine production. PRF occurs before the mRNA encoding this AZ can be fully translated. When levels of polyamines rise, +1 frameshifting also increases, thus producing more AZ, consequently reducing the abundance of ornithine decarboxylase, lowering polyamine levels. Thus, a +1 PRF on the ODC AZ mRNA provides an autoregulatory feedback loop between AZ and polyamine levels (Fig. 2B). The mechanism for this frameshift might also involve ribosomal pausing and positioning via mRNA:rRNA base pairing, along with neutralization of negative charge repulsion by positively charged polyamines.

Two more examples of a +1 PRF in eukaryotic cells come from *Saccharomyces cerevisiae*, in which PRF is required for making the Est3p and Abp140p proteins. The frameshift signals for these two proteins are identical to the signal of the Ty1 retrotransposable element of yeast, where a low-abundance amino acyl-tRNA directs ribosome pausing at the shift site.

Bioinformatic studies suggest that such sequences are stringently selected against in the yeast genome. All sequenced mitochondrial *nad3* and *cytb* genes contain single-nucleotide insertions, and the high degree of sequence conservation argues that they are functional. Analyses of ciliate genomes reveal that these organisms have assigned new meanings to stop codons, including translational readthrough and +1 PRF. Insertions and deletions that necessitate +1 frameshifting are found among a large number of genes in ciliates. In metazoans, evidence for +1 PRF is limited to sequence analyses, suggesting that several nuclear protein kinases and transposons are produced through PRF.

Examples of –1 PRF Affecting Gene Expression

Viruses appear to use –1 PRF more frequently than they use +1 PRF. However, there are few examples of –1 PRF chromosomally encoded mRNAs. The *dnaX* gene of *E. coli*, which encodes the τ and γ subunits of DNA polymerase

Dinman: from Philosophy to Music to Science to . . . Music?

Jonathan Dinman, who majored in philosophy as an undergraduate, found its discipline and approach helpful when he later switched to a career path in science. “Philosophy tells you what questions have been asked, and what approaches have been taken,” he says. He took plenty of science courses while studying philosophy. What he didn’t like were premedical courses “that turned science into a bunch of factlets that are memorized to take a test,” he adds. “That turned me off.”

After he finished his A.B. degree at Oberlin College in 1980, Dinman worked for three years as a recording engineer in New York, experimenting with a career in music. But his doubts grew, particularly after spending three hours in the studio babysitting Tiny Tim, the ukulele-strumming, falsetto-voiced recording artist whose version of “Tiptoe Through the Tulips” made him popular during the 1960s. “My job was to keep Tiny happy,” Dinman recalls. “He showed up holding four . . . shopping bags, and talking about what a hard life music was—and how (ex-wife) Miss Vicki had used him. He was like a bag lady.”

Such encounters likely contributed to Dinman’s return to science, as did his growing realization that the recording industry seemed to be more about business than music. “It’s not about art,” says Dinman, who plays jazz piano. “The focus on making a buck made it boring.”

Luckily, the studio was located near Bryant Park, next to the main branch of the New York Public Library. “One day I went out the back door, went into the library, picked up *Scientific American*, and looked up the originals,” Dinman says, referring to the journal articles on which some of the magazine features were based. “And I got re-interested in science.” This rekindling of interest came at a time when AIDS was emerging in New York, and music artists were “coming into the studio with mysterious purple skin lesions,” he says. “I went back to school and started [studying] immunology.” By 1988, he completed his Ph.D. at the Johns Hopkins University School of Hygiene and Public Health in Baltimore, Maryland.

Today Dinman, 48, is associate professor in the Department of Cell Biology and Molecular Genetics, and affiliate associate professor in the Department of Chemistry and Biochemistry, both at the University of Maryland, College Park. His passions are genetics, cell biology, and biochemistry. “I want to be free to just do science for the sake of doing science,” he says. “I understand that it, too, is a business, and you have to get funding. I know how to do that, but I’m not in it to be famous or gain recognition.”

Dinman is interested in ribosome structure and function, and post-transcriptional regulation of gene expression. He recently received a

grant from the National Institute of Allergy and Infectious Diseases for a project to examine ribosomal frameshifting in the SARS coronavirus.

“We have people who want to reduce a clock to its smallest bits and put it back together looking at one piece at a time,” he says. “And we have people who will close their eyes, hit the clock with a hammer, and see why the clock is no longer telling time. The former would be the biochemist, and the latter would be the geneticist. What I am trying to do is live in both worlds.”

Dinman calls himself a “faculty brat,” who grew up in Columbus, Ohio; Ann Arbor, Michigan, and Pittsburgh, Pennsylvania. His father’s work as a physician and, later, as an industrial toxicologist and college-level teacher, helps to explain those changes of scenery. His mother is a docent at the Sarah Scaife Galleries at the Carnegie Museums of Pittsburgh.

Dinman is married to a former social worker, and they have two daughters, 9 and 7. In his spare time, Dinman is working his way through the American Songbook of Jazz Standards, learning to play each song on the piano. “When I know 1,000 songs, I will retire, find a bar, roll my piano in there—and play,” he says.

Marlene Cimons

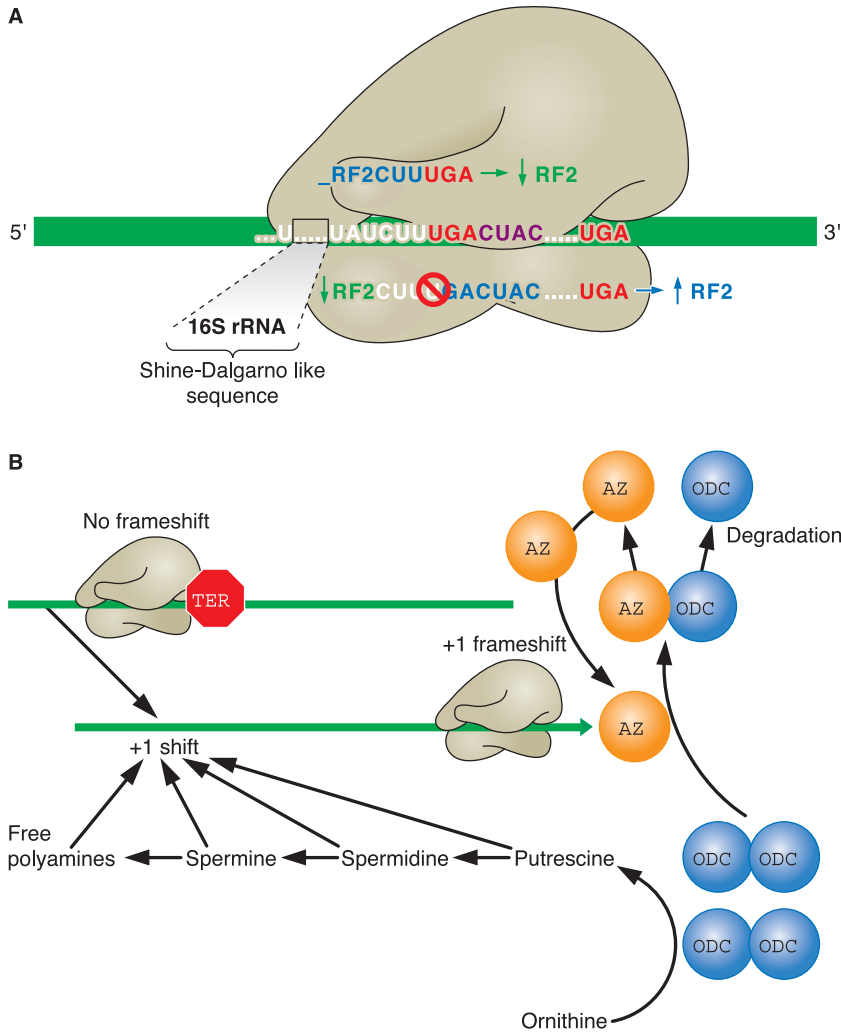
Marlene Cimons is a freelance writer in Bethesda, Md.

III, is the bacterial prototype in this category (Fig. 3). The τ subunit is encoded by the full-length gene, while synthesis of γ results from a -1 PRF that directs ribosomes to a premature stop codon, resulting in a protein that is 213 amino acids shorter. As with the RF2 protein, a

Shine-Dalgarno sequence helps to position the ribosome at the shift site. In addition, a stem-loop structure in the mRNA that is downstream of the shift site may facilitate ribosomal pausing. The additional amino acids in τ allow it to bind directly to the core polymerase and to DNA.



FIGURE 2



(A) Autoregulation of Release Factor 2 (RF2) synthesis by programmed +1 ribosomal frameshifting. The open reading frame encoding RF2, the translation termination factor that recognizes the UGA and UAA termination codons, contains an in-frame UGA codon that must be bypassed for complete translation of the protein. Low levels of RF2 promote inefficient recognition of the UGA codon. Ribosome pausing at this site enables base-pairing between an upstream Shine-Dalgarno like sequence, repositioning the ribosome to the +1 reading frame. The resulting +1 PRF event results in RF2 production. As RF2 levels rise, UGA codon recognition increases, dampening frameshifting and RF2 production. (B) Autoregulation of polyamine synthesis by programmed +1 ribosomal frameshifting. Ornithine decarboxylase (ODC) converts ornithine into putrescine, the substrate for all subsequent polyamine biosynthesis. Excess levels of polyamines stimulate +1 programmed ribosomal frameshifting on the antizyme mRNA, resulting in synthesis of antizyme (AZ). This enzyme sequesters ODC, triggering ODC degradation by the 26S proteasome. AZ is not degraded, but is recycled into the pathway.

Meanwhile, the absence of γ may make the polymerase less dedicated to the replication fork.

Conservation of the -1 PRF signal in homologous genes of closely related *Enterobacteria-*

cea, in more distantly related *Vibrio cholerae*, and in the still more distantly related genus *Neisseria* suggests that maintaining this capacity to regulate τ and γ subunits is important. The only other known -1 PRF-containing bacterial gene is in the *Bacillus subtilis* cytidine deaminase gene (*cdd*). This unusual frameshift mechanism involves a shift of only one rather than two tRNAs on the mRNA. Although the precise function of this frameshift is unknown, this -1 PRF event may be involved in regulating transcriptional initiation of the downstream *bex* gene.

Among archaea, only a single -1 PRF-containing gene has been identified, α -L-fucosidase (EC3.2.1.51) in *Sulfolobus solfataricus*, which is encoded by the *fucA1* gene. Like *dnaX*, a downstream stem-loop structure in *fucA1* may promote ribosome pausing at the slip site. However, unlike eubacterial PRF signals, an upstream Shine-Dalgarno sequence is not present in the *fucA1* -1 PRF signal. Although the function of this frameshift is unknown, the α -fucosidases help to regulate cell growth in higher plants and animals. Moreover, because this enzyme can hydrolyze fucosidated oligosaccharides, frameshifting might regulate *fucA1* expression in response specifically to these substrates and more generally to control energy metabolism.

Among mammals, -1 PRF affects expression of the mouse *Edr* gene. The *edr* -1 PRF signal is very similar to viral signals. Thus, it contains (from 5' to 3') a heptameric slippery site, a spacer, and an mRNA pseudoknot, and the frameshift is required for the 3' region portion of the mRNA to be translated. Among these signals, an mRNA pseudoknot may direct ribosomes to pause at the slippery site.

The human *edr* homolog, PEG10, also uses -1 PRF, and is highly expressed in the placenta. *Edr* and PEG10 are members of a large family of functional neogenes called *Mart* (for mammalian retrotransposon derived) that are widely distributed among mammals, and appear to be related to the *gag*

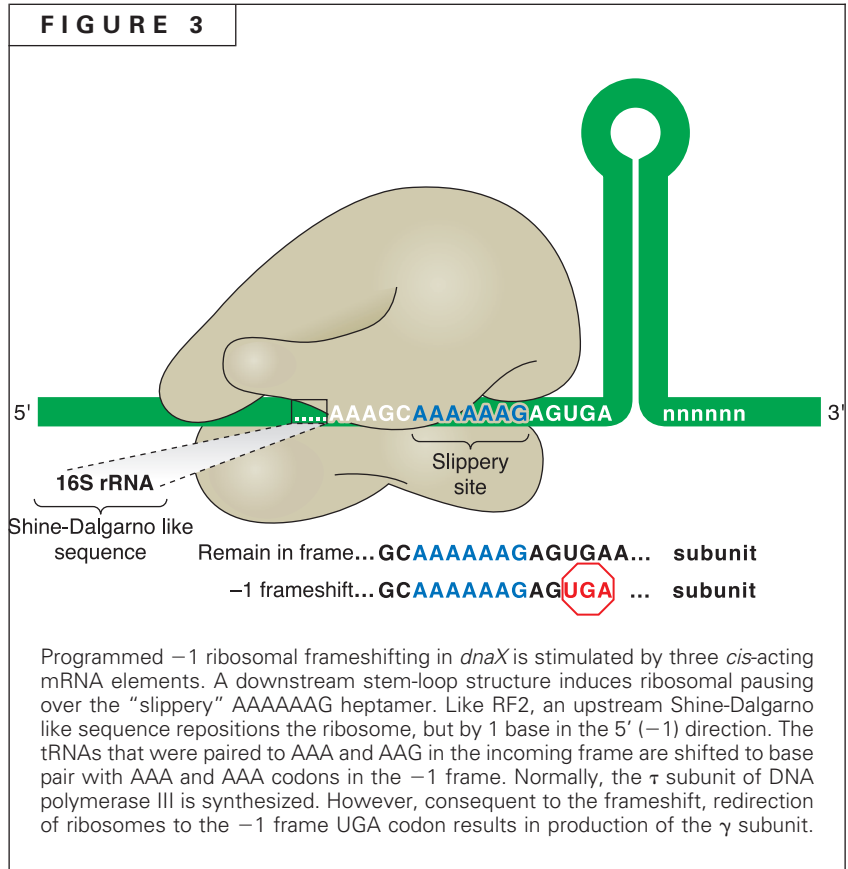
gene of repeat retrotransposons. The functions of these frameshifts are unknown.

Using Bioinformatics To Identify -1 PRF signals

Yeast cells with mutations that specifically boost -1 PRF efficiency typically are defective in regulating gene expression. Such mutants inspired investigators to use bioinformatics to find -1 PRF signals in eukaryotic genomes. The first such search found that these were represented in coding regions of eukaryotic genomes as much as sixfold more frequently than would be expected if distribution were random. Identifying -1 PRF signals remains daunting, even as computational power and sophistication continue to increase. Four main bioinformatic strategies are being employed, including (i) searches for overlapping reading frames, (ii) queries for slippery sites, (iii) neural networks approaches, and (iv) programs to identify sequence and structure motifs resembling viral -1 PRF signals.

The first method rests on the assumption that -1 PRF events always yield C-terminally extended fusion products, and thus this approach cannot identify new classes of frameshifted genes. The second, although computationally fast, is only a first approximation of potential frameshift sites and does not address the issue of 3' stimulatory elements. The strength of the third approach is it lacks preconceptions as to what may constitute a -1 PRF signal, although in practice it runs into computational hurdles that are only beginning to be overcome. The reliance of the fourth on known stimulatory elements would seem to preclude its ability to identify new ones, although it has potentially uncovered a new posttranscriptional means for regulating gene expression.

Taken together, these complementary approaches promise to expose new modalities of -1 PRF. For instance, one recent study involved a two-step approach combining a search for overlapping ORFs with hidden Markov models. It identified almost 200 candidate PRF-related genes in the *S. cerevisiae* genome. Importantly, the majority of those candidates do not contain typical virus-like -1 PRF signals, suggesting the existence of new classes of -1 PRF-promoting signals.

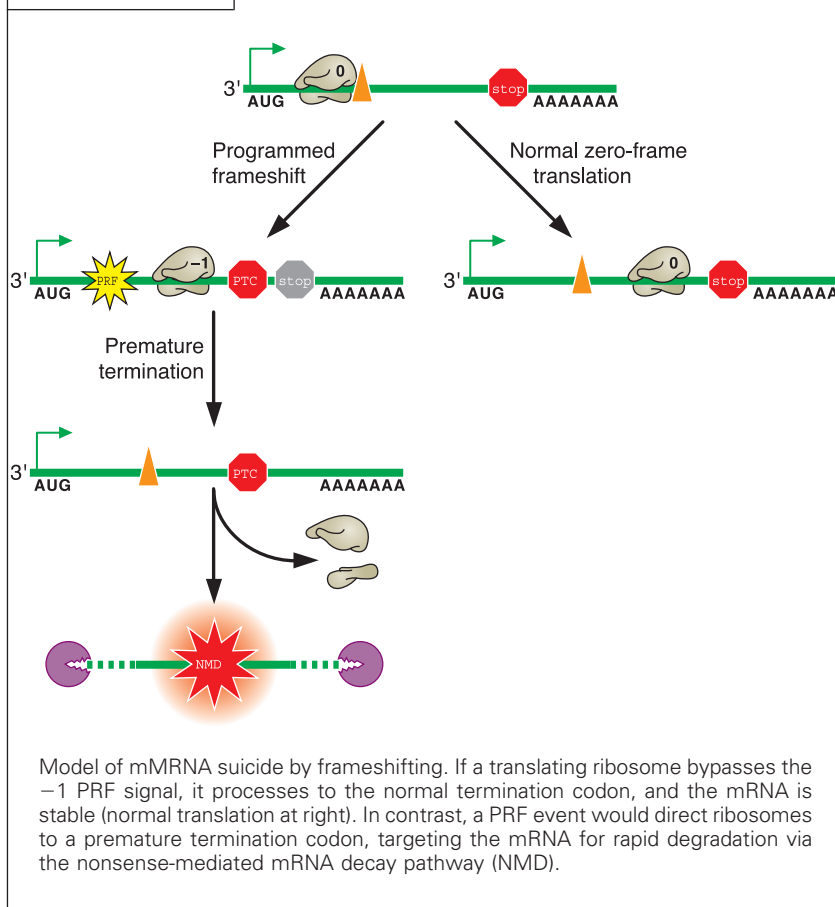


Other studies to identify sequence and structure motifs resembling viral -1 PRF signals are also yielding surprises. The first such study identified significant numbers of putative -1 PRF signals in multiple genomes, apparently conserved -1 PRF signals in homologous genes from different species, and disease alleles that might abolish frameshifting. Moreover, at least two of those motifs appear capable of promoting efficient -1 PRF. Recent, more advanced work has increased the number of reliable candidates to almost 800, and added another 8 functional -1 PRF signals to the list.

Nearly all of these -1 PRF events are predicted to direct elongating ribosomes into prematurely terminating the proteins being made. If so, maybe -1 PRF could be used to target mRNAs for rapid degradation via the nonsense-mediated mRNA decay pathway. In experiments with a viral -1 PRF signal inserted into a cellular reporter mRNA, -1 PRF signals indeed act as mRNA destabilizing elements (Fig. 4). These findings suggest that -1 PRF may be used to regulate gene expression by destabilizing mRNA.



FIGURE 4



Researchers are currently working to identify the mRNA decay pathways involved and to determine how each of them destabilizes mRNA.

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Perspective

When PRF was discovered, investigators surmised that it was an evolutionarily recent development specific to a few RNA viruses. However, the emerging picture of PRF as a widely distributed posttranscriptional regulatory mechanism suggests the opposite is true and that PRF might be a remnant of the extremely old, prebiotic RNA world. The ability of a molecule of RNA to encode more than one outcome would add to the information content of cells bearing it without altering its sequence, providing that molecule with a greater range of options. Perhaps PRF signals confer selective advantages on frameshifting RNA molecules.

Looking forward, PRF is more than a mere curiosity specific to a few exceptional viruses. Rather, the field entails a far wider variety of organisms and thus is expanding in many exciting directions. The discovery and characterization of new mechanisms of PRF will further expand our understanding of the interplay between ribosomes and *cis*-acting signals encoded in mRNAs. The finding that PRF efficiency influences mRNA abundance and, hence, protein expression suggests that PRF regulates gene expression. With that door opened, other important possibilities are manifold: maybe PRF regulates development and, the flip side of this coin, diseases such as birth defects and cancer that arise when development is dysregulated.

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