Review

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Changed in translation: mRNA recoding by −1 programmed ribosomal frameshifting

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Programmed −1 ribosomal frameshifting (−1PRF) is an mRNA recoding event commonly utilized by viruses and bacteria to increase the information content of their genomes. Recent results have implicated −1PRF in quality control of mRNA and DNA stability in eukaryotes. Biophysical experiments demonstrated that the ribosome changes the reading frame while attempting to move over a slippery sequence of the mRNA – when a roadblock formed by a folded downstream segment in the mRNA stalls the ribosome in a metastable conformational state. The efficiency of −1PRF is modulated not only by *cis*-regulatory elements in the mRNA but also by *trans*-acting factors such as proteins, miRNAs, and antibiotics. These recent results suggest a molecular mechanism and new important cellular roles for −1PRF.

Accurate decoding versus programmed recoding

Ribosomes (see Glossary) are cellular factories that produce proteins in all cells using the nucleotide sequence of mRNAs as a blueprint. Nucleotide triplets of an mRNA – the codons – are translated into an amino acid sequence of a protein. The selection of the translation start and the reading frame on an mRNA is tightly controlled during the initiation phase of protein synthesis. The subsequent elongation phase entails repeated cycles of codon decoding by aminoacyl-tRNA, peptide bond formation, and tRNA–mRNA translocation. Elongation cycles continue until the ribosome reaches a stop codon, on which translation is terminated. It is intuitively clear that translation of an mRNA sequence into a protein must be colinear and highly accurate. Errors can lead to the formation of toxic or misfolded proteins, increase the energetic cost of translation, and cause additional load on the cellular clean-up and quality-control machineries [1]. To avoid this burden, cells have evolved sophisticated control mechanisms that ensure the fidelity of decoding and reading frame maintenance. However, in special cases, the ribosomes, guided by signals encoded in the mRNA, abandon the principle of mRNA–protein colinearity and read the message in an alternative way, which results in mRNA recoding (Box 1) [2–4]. Programmed frameshifting is a recoding event that can occur in the + or − direction relative to the normal 0-frame mRNA translation by shifting the ribosome by one or two nucleotides, thereby producing two (or even three) different proteins from one mRNA. In contrast to spontaneous frameshifting, which is infrequent, the efficiency of programmed frameshifting may be as high as 80%, although in many cases it is only a few percent [5]. Programmed frameshifting increases the coding potential of the genome and is often used to expand the variability of cellular proteomes, adapt to changing environments, or ensure a defined stoichiometry of protein products. The mechanisms of +1 and −1 frameshifting appear to be different, and particularly the mechanism of −1 programmed ribosome frameshifting (−1PRF), its abundance, and physiological significance have remained unclear for a long time. The advances of biophysical techniques and reconstitution of highly-purified translation systems has recently provided new insight into how and when ribosomes slip into the −1 reading frame. New examples of −1PRF in eukaryotes and the identification of previously unknown *trans*-acting elements show how cells can regulate gene expression through frameshifting. This review is intended to summarize these recent breakthroughs in understanding the mechanism and biological importance of −1PRF.

Prevalence of ribosomal frameshifting

Although the large majority of reported frameshifting sequences have so far been found in viral genomes, programmed frameshifting exists in all branches of life from bacteria to higher eukaryotes. The important role of −1PRF in viruses and bacteria is well documented [6]. In bacteria, −1PRF is required to produce the γ subunit of DNA polymerase III and is particularly abundant in bacterial transposable elements, which use −1PRF to generate their transposase [2,7]. −1PRF is also found in several families of eukaryotic viruses, where it is often used for the expression of viral replicases [8]. In retroviruses such as HIV, −1PRF is necessary to produce the DNA polymerase Pol and defines the ratio of Gag to Pol proteins. Because this ratio should not exceed a particular threshold value to maintain efficient virus assembly, genome packaging and maturation [9], changing −1PRF efficiency can be detrimental to the virus [10,11]. In comparison to viruses and bacterial mobile elements, only a few examples of −1PRF have been described in higher eukaryotes, although computational

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Glossary

Avian infectious bronchitis virus (IBV): a coronavirus. The IBV 1a/1b sequence encodes two proteins: a shorter protein 1a and a longer 1a/1b polypeptide which is synthesized due to −1PRF. IBV is one of the best-studied examples of −1PRF. The efficiency of −1PRF is very high: 30–70%. The IBV frameshifting sequence is also operational in mammalian, plant, yeast and bacterial cells, suggesting that the principles of −1PRF are universal.
***Cis*-acting elements:** the elements in the mRNA that modulate −1PRF.
Colinearity: denotes the linear correspondence between the mature mRNA and its protein product. Colinearity is ensured by the ribosomes, because they usually translate codons one after another in a very accurate manner.
***dnaX*:** an example of dual coding in a chromosomal gene in bacteria. The *dnaX* gene encodes two products, the τ and γ subunits of DNA polymerase III. τ is the longer protein; the shorter γ polypeptide is synthesized as a result of −1PRF.
Elongation factor G (EF-G): the translocase in bacteria that promotes the movement of the ribosome over the mRNA at the cost of GTP hydrolysis. The homologous eukaryotic translocase is eEF2.
Fluorescence resonance energy transfer (FRET): energy transfer can occur between two fluorescent reporter groups where the donor in the excited state transfers energy to an acceptor, provided that the two are close to one another (<10 nm). FRET efficiency is inversely proportional to the sixth power of the distance between donor and acceptor, making FRET suitable for measuring small changes in distance. FRET can be utilized to measure the thermodynamics, kinetics, and dynamics of interactions between interacting molecules or within macromolecules. smFRET, single-molecule FRET, is a technique to monitor FRET changes of individual molecules or molecular assemblies. smFRET has been extensively used to study tRNA–mRNA translocation on the ribosome and, recently, −1PRF.
Frameshift (FS) efficiency: a measure for the preference for translation in the −1-frame compared to overall translation, calculated as FS% = −1-frame product/(−1-frame + 0-frame products) × 100%.
Optical tweezers: a single-molecule technique for measuring the force required to unwind an mRNA secondary structure (or any other process that generates force). An experiment is performed by focusing a laser beam on a bead attached to the macromolecule under study such that small movements from the center of the laser beam generate a counteracting force. In the experimental setup to measure unwinding forces with optical tweezers, the ends of the RNA molecules are flanked by DNA handles that are attached to beads. While one bead is trapped by the laser beam, the second is pulled by a micropipette or another laser beam to generate the tension force. The force is proportional to the displacement of the object from the center of the optical trap.
Pseudoknot: a complex RNA structure that is formed by base-pairing between the loop of a stem-loop structure in an RNA molecule and a single-stranded region located upstream or downstream of the stem-loop structure. Pseudoknots have been implicated in gene expression and viral genome replication.
Quench flow: a rapid-kinetics technique which allows monitoring the kinetics of reactions in the ms to s time-range. After rapid mixing of two ligands, the reaction is allowed to proceed for a desired time and then stopped by the addition of a quencher. In the present context the technique was used for performing a codon walk on the mRNA.
Rapid kinetics: an approach to monitor reactions in the μs to s time-range and to determine the rates of rapid reactions. The term is usually applied to measurements on ensembles of molecules.
Ribosomes: large cellular organelles (about 2.5 MDa in prokaryotes) that synthesize proteins. They are ribonucleoprotein complexes composed of two subunits, the large (50S) and the small (30S).
30S subunit: the small subunit of bacterial ribosomes that is responsible for mRNA recruitment and decoding of mRNA codons. The number indicates the sedimentation coefficient of the particle (in Svedberg units).
50S subunit: large subunit of bacterial ribosomes; responsible for peptide bond formation and provides the exit tunnel for the emerging peptide.
Stopped flow: a rapid kinetics technique in which, following rapid mixing of the reactants, the reaction progress is monitored by changes of an optical parameter, such as fluorescence, FRET, light scattering, or absorption. The technique has been useful in dissecting different steps of the translation cycle and gaining kinetic information about individual steps. Stopped flow has been used to solve the mechanism of −1PRF by measuring changes in fluorescence of tRNA, FRET between tRNA and the 30S ribosome head, as well as EF-G binding and dissociation.
***Trans*-acting elements:** modulators of −1PRF that do not reside in the mRNA itself.

analysis have predicted that ~10% of cellular mRNAs may be controlled by −1PRF [12]. Recent evidence suggests that −1PRF may have unexpected roles in regulating not only the production of particular proteins but also in regulating mRNA and DNA stability (Table 1). Dinman and colleagues demonstrated operational −1PRF signals in four mRNAs encoding proteins crucial for yeast telomerase maintenance [13], and reported an example of eukaryotic −1 PRF in *ccr5* and six other genes for cytokine receptors [14]. Overexpression of the telomerase gene *Est2* through inhibition of frameshifting leads to formation of shorter telomeres, suggesting the importance of −1PRF for the maintenance of eukaryotic genomes [13]. Translation in the −1 frame of *ccr5* leads to a premature stop, which targets the mRNA for degradation by nonsense-mediated decay (NMD) [14]. This appears to be a general phenomenon because, for the large majority of predicted genes, −1PRF leads to premature termination less than 30 codons beyond the frameshifting site [15]. Thus, in eukaryotic genomes −1PRF can be used to regulate gene expression. Another notable example is the embryonal carcinoma differentiation regulated gene (*edr*), where −1PRF regulates the synthesis of two distinct polypeptides [16]. *Edr* is a single-copy gene in mice and humans. Its expression is regulated in a spatiotemporal manner during embryogenesis, suggesting a role of −1PRF in development [17]. Global analysis of dual decoding potential in human cells revealed several new examples of alternative frame reading in ribosome profiling data [18]. By analogy, one can expect more examples of −1PRF emerging with advances in high-throughput analysis of cellular proteomes. Together, these findings indicate that −1PRF may be more abundant and more important in eukaryotic cells than previously thought.

When do ribosomes slip?

−1PRF is promoted by several stimulatory elements embedded in the mRNA sequence (Figure 1) [5,19]. The crucial regulatory element is the so-called slippery sequence, which is usually a heptameric sequence with the pattern X XXY YYZ (the underlined sequence denotes the 0-frame codons). The slippery sequence ensures correct base-pairing between the mRNA codon and the tRNA anticodon before and after the slippage, provided that the 0-frame and −1-frame codons can be decoded by the same tRNA through wobble interactions at the third codon position [XXY (0-frame) vs XXX (−1-frame), or YYZ (0-frame) vs YYY (−1-frame)]. Another stimulatory element is an mRNA secondary structure element, usually a pseudoknot or a stem-loop located 5–8 nt downstream of the slippery sequence. Interestingly, antisense oligonucleotides or guanine-rich sequences that can fold into four-stranded structures of stacked guanine-tetrads, so-called G-quadruplexes, can also stimulate −1PRF, reaching efficiencies up to 40% [20,21]. In addition, in some cases a Shine–Dalgarno-like element 11–14 nt upstream of the first slippery codon [22] or even long-distance base-pairing appear to stimulate frameshifting [23].

To understand the mechanism of −1PRF, it is first essential to identify the precise timing of slippage and the position of the frameshifting ribosome on the slippery sequence. One straightforward way to determine the timing of slippage is to analyze the kinetics of translation at each codon before, over, and after the slippery sequence (through a 'codon walk') to find the step which is different for ribosomes that undergo −1PRF compared to those continuing unperturbed translation in the 0-frame. Previous reports suggested that a ribosome, when it