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CONTROL OF GENE EXPRESSION BY TRANSLATIONAL RECODING

By JONATHAN D. DINMAN

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland, USA

Abstract

Like all rules, even the genetic code has exceptions: these are generically classified as ''translational recoding.'' Almost every conceivable mode of recoding has been documented, including signals that redefine translational reading frame and codon assignation. While first described in viruses, it is becoming clear that sequences that program elongating ribosomes to shift translational reading frame are widely used by organisms in all domains of life, thus expanding both the coding capacity of genomes and the modes through which gene expression can be regulated at the posttranscriptional level. Instances of programmed ribosomal

ADVANCES IN PROTEIN CHEMISTRY AND STRUCTURAL BIOLOGY, Vol. 86
DOI: 10.1016/B978-0-12-386497-0.00004-9

frameshifting and stop codon reassignment are opening up new avenues for treatment of numerous inborn errors of metabolism. The implications of these findings on human health are only beginning to emerge.

I. Introduction

Many years ago, I took a Japanese colleague on a walking tour of Manhattan, starting in SoHo and ending up at Rockefeller Center. Having come from Tokyo, my guest was not so much overwhelmed by the crowds as he was perplexed by the fact that New Yorkers tended to cross the street against the light. By the time we had reached Times Square, he turned to me and said ''I understand: don't walk means run.'' The point is that sometimes rules can be safely and advantageously broken. Indeed, exceptions to the rules provide the contrasting points of reference that enable definition of the rules themselves. Every hero needs a foil: there could be no Hamlet without Laertes.

Consider the ''Central Dogma'' of molecular biology: information flows from DNA to RNA to protein. The discovery of reverse transcriptase shattered this rule and opened up broad new vistas in both our understanding and ability to manipulate biological systems. Not insignificantly, this discovery garnered Nobel Prizes for Drs. Temin and Varmus, demonstrating that challenging the status quo can (sometimes) be very rewarding. The genetic code is another set of rules defining how amino acids are encoded in nucleic acid sequences. Given that four nucleotide bases must encode 20 amino acids, Nirenberg surmised that a minimum of three bases had to be used to encode one amino acid. Based on this and using defined templates in well-defined *in vitro* translation assay systems, he deciphered the genetic code and demonstrated that it is the same in *Escherichia coli*, *Xenopus laevis,* and guinea pig tissues: a universally conserved genetic code composed of nucleoside triplets. Given the organization of genetic information into triplet codons, it became apparent that ribosomes, the universally conserved protein synthetic machinery, have to accurately recognize and decode bases three at a time in order to accurately translate the genetic information contained in mRNAs into proteins. These findings laid the foundation for investigations designed to answer the fundamental questions of translational accuracy and reading frame maintenance. As described elsewhere, the discoveries of exceptions to the genetic code, generically termed translational ''recoding,'' have helped to

address these questions and have opened new vistas in our understanding of many other biological questions (reviewed in Atkins and Gesteland, 2010). This chapter focuses on recent efforts to identify translational recoding signals in cellular genomes, and their known and hypothetical roles as *cis*-acting elements in controlling gene expression.

II. Programmed −1 Ribosomal Frameshifting

A. *Introduction and History*

Programmed ribosomal frameshifting (PRF) is a translational recoding phenomenon historically associated with viruses and retrotransposons. PRF signals are *cis*-acting elements embedded in mRNAs that stochastically redirect translating ribosomes into a new reading frame (i.e., by +1 or −1 nucleotide). In the typical viral context, a PRF signal allows ribosomes to bypass the 0-frame encoded in-frame stop codon and continue synthesis of a C-terminally extended fusion protein. The most well-defined −1 PRF phenomena are directed by an mRNA sequence motif composed of three important elements: a ''slippery site'' composed of seven nucleotides where the translational shift in reading frame actually takes place; a short spacer sequence of usually <12 nucleotides; and a downstream stimulatory structure, typically an mRNA pseudoknot. In eukaryotic viruses, the slippery site has the heptameric motif N NNW WWH, where the incoming reading frame is indicated, and *N*=any three identical nucleotides, *W*=AAA or UUU, and *N*≠*G* (Harger et al., 2002). Current models posit that aminoacyl- (aa-) and peptidyl-tRNAs are positioned on this sequence while the ribosome pauses at the downstream secondary structure (Lopinski et al., 2000; Kontos et al., 2001; Plant et al., 2003; Plant and Dinman, 2005). The nature of the slippery sequence enables re-pairing of the non-wobble bases of both the aa- and peptidyl-tRNAs with the −1 frame codons. While it is generally accepted that mRNA pseudoknots are the most common type of downstream stimulatory structures, other mRNA structures are capable of filling this role as well (Kollmus et al., 1996; Baril et al., 2003). Generally, it is thought that the essential function of the stimulatory structure is to provide an energetic barrier to a translating ribosome and to position it over the slippery site. A number of models have been presented to predict at which point during the course of the

translation elongation cycle −1 PRF occurs (reviewed in Brierley et al., 2010). However, recent kinetic analyses revealed that −1 PRF merely represents an endpoint accessible by at least three different kinetic pathways that can yield two possible frameshift products (Liao et al., 2010). Again, this finding serves as a cautionary tale against dogmatic thinking and illustrates a fundamental truth of biological systems: rather than searching for the right answer, evolution merely selects for solutions to problems.

B. PRF in Viruses: The "Golden Mean" and Possible Therapeutic Applications

First discovered in retroviruses, and now documented in many other RNA viruses, PRF is used to synthesize C-terminally extended Gag-pol fusion peptides. It has been proposed that 1 PRF solves a number of problems posed to RNA viruses. For example, PRF allows RNA viruses to synthesize multiple proteins from a single, unaltered RNA template, thus solving the problem of how to maximize genomic coding space while maintaining genome integrity. Additionally, PRF enables nesting of coding sequences, enabling viruses to maximize genomic coding space into the smallest possible genome size, a very important issue considering the limited volumes inside of viral capsids available to viral genomes.

PRF is also used by viruses to ensure production of viral gene products in their correct stoichiometric ratios. For example, the L-A virus of yeast has a simple icosahedral structure of T=1. In Euclidian solid geometry, the simplest sphere (T=1) is composed of 60 identical subunits: this resembles a traditional soccer ball. Translating this structure to viruses, these subunits are the capsid proteins (also called Gag for "*G*roup specific *a*ntig*e*n"). These typically self-assemble to form the viral capsid, which protects the genome from environmental assault. The only other component required for L-A virus propagation is a single molecule of an enzyme capable of replicating the genome, typically called the viral replicase, or Pol (for *Pol*ymerase). Thus, the ideal ratio of capsid to replicase is 60:1. The L-A viral −1 PRF signal has evolved to shift ribosomes from the Gag open reading frame to the Pol open reading frame at a rate of 1.8%, thus producing the desired 60:1 ratio of capsid to replicase proteins (Dinman and Wickner, 1992). Further, changing the rate of −1 PRF, either by mutations of the frameshift signal, as a consequence of mutations in the

host cellular translational apparatus, or with small molecule inhibitors of the protein synthesis, alters the stoichiometric ratio of capsid to replicase, consequently interfering with the viral particle self-assembly program (reviewed in Dinman, 1995; Dinman et al., 1998). Beyond the simple L-A virus of yeast, this general concept has been shown to apply to the Ty*1* retrotransposable element of yeast (Kawakami et al., 1993), HIV-1 (Biswas et al., 2004), flaviviruses (Melian et al., 2010), and the SARS-associated coronavirus (Plant et al., 2010). These findings have led to the suggestion that each virus has evolved a unique PRF signal designed to deliver the optimum stoichiometries of viral gene products for viral particle assembly, that is, a ''Golden Mean.'' In theory, small molecule agents that can alter PRF efficiency would upset this Golden Mean, and thus have antiviral activities.

C. *Computational Identification of −1 PRF Signals: Genomic Frameshifting*

With few exceptions, almost every basic molecular mechanism was first discovered in viruses. −1 PRF is no exception, and indeed there are a small number of well-documented bacterial and archaeal examples (reviewed in Baranov et al., 2002; Cobucci-Ponzano et al., 2005). Until recently, few such examples were documented in eukaryotes, and their discovery tended to be serendipitous. For example, −1 PRF in the mRNA encoding the edr protein in mice was only discovered because DNA sequencing revealed that the open reading frame was noncontiguous, and identification of the −1 PRF signal was facilitated by its similarity with retroviral −1 PRF signals (Shigemoto et al., 2001; Manktelow et al., 2005). The human edr homolog, PEG10, also uses −1 PRF, and is highly expressed in placenta and embryonic tissues (Lux et al., 2005, 2010). Edr and PEG10 are members of a large family of functional neogenes called Mart (Mammalian retrotransposon-derived), that are widely distributed among mammals, and appear to be related to the *gag* gene of the Sushi-like long terminal repeat retrotransposons (Brandt et al., 2005).

Beginning approximately a decade ago, computational approaches have been applied to identify −1 PRF signals in the large genome databases. In the beginning, these approaches were particularly challenging, primarily because of computer memory limitations which have been solved by increased CPU speeds and cloud computing. In general, four different

strategies have been employed to identify −1 PRF signals: searches for overlapping reading frames (Moon et al., 2004a,b; Bekaert et al., 2005), searches for known slippery sites (Shah et al., 2002; Wills et al., 2006), neural networks approaches (Bekaert et al., 2003), and programs designed to identify sequence and structure motifs resembling viral −1 PRF signals (Hammell et al., 1999; Jacobs et al., 2007; Belew et al., 2008; Theis et al., 2008). The first method rests on the assumption that −1 PRF events always result in the production of C-terminally extended fusion products. While this can identify interrupted open reading frames, it is incapable of identifying new classes of frameshifted genes. The second, although computationally rapid, only represents a first approximation of potential frameshift sites and does not query for the presence of 3′ stimulatory elements. While the third approach is neutral with regard to what may or may not constitute a −1 PRF signal, in practice, its computational complexity has hindered its development as a widespread tool. The reliance of the fourth on known stimulatory elements precludes its ability to identify new ones, although as discussed below, it has led to a new paradigm of posttranscriptional regulation of gene expression.

A few studies have translated these approaches to the bench. A search for overlapping ORFs combined with the application of hidden Markov models was used to identify 189 candidate genes in the *Saccharomyces cerevisiae* genome (Bekaert et al., 2003), and a later investigation showed that 28 of 58 candidates expressed full-length mRNAs encompassing both ORFs, 11 of which promoted highly efficient −1 PRF (Bekaert et al., 2005). An important feature of this latter study was the demonstration that most candidates do not contain typical virus-like −1 PRF signals, thus revealing new classes of −1 PRF promoting *cis*-acting elements. The approach designed to identify sequence and structure motifs resembling viral −1 PRF signals has also yielded surprises. The first study identified (a) large numbers of putative −1 PRF signals in many genomes, (b) apparently evolutionarily conserved −1 PRF signals in homologous genes from different species, (c) known disease alleles that were predicted to abolish frameshifting, and (d) empirically demonstrated efficient −1 PRF promoted by two computationally identified signals (Hammell et al., 1999). A later investigation of the yeast genome suggested that ∼10% of genes contain at least one strong candidate −1 PRF signal and demonstrated that eight out of eight such elements assayed at the bench were able to promote efficient −1 PRF (Jacobs et al., 2007). A later study

employing more stringent cutoff parameters for prediction of mRNA pseudoknots suggested that the number of −1 PRF signals may be significantly lower, that is, 257 genes or ∼4% of genes in the yeast genome (Theis et al., 2008). While the current issue remains to determine the actual number of functional −1 PRF signals per genome, the argument about whether or not −1 PRF is employed by a significant number of cellular mRNAs has been effectively settled.

D. −1 PRF in Cellular mRNAs: mRNA Destabilization Elements and Regulation of Gene Expression

A surprising observation from the bioinformatics studies was that, in contrast to viruses, >95% of predicted ''cellular'' −1 PRF events were predicted to direct elongating ribosomes into premature termination events. This engendered the hypothesis that −1 PRF may be employed to target mRNAs for degradation via the nonsense-mediated mRNA decay pathway (NMD; reviewed in Conti and Izaurralde, 2005). Proof-of-principle experiments using a viral −1 PRF signal inserted in the ''genomic orientation'' into a reporter mRNA validated this idea (Plant et al., 2004). Additional experiments demonstrating an inverse relationship between −1 PRF efficiency and mRNA half-lives suggested that regulation of −1 PRF could be employed to posttranscriptionally regulate gene expression. Subsequently, studies using endogenous −1 PRF signals from yeast demonstrated that these can also function as mRNA destabilizing elements, not only through NMD but also through the No-Go decay pathway; (Belew et al., 2010). This study also suggested that (a) −1 PRF may be centrally involved in yeast telomere maintenance and (b) −1 PRF signals appear to evolve rapidly, and thus may play a significant role in speciation. In unpublished studies from our laboratory, we have shown that endogenous −1 PRF signals from human genes can also function as mRNA destabilizing elements in mammalian cells through NMD, and that −1 PRF may play an important role in regulation of the immune response.

If −1 PRF is used to control expression of cellular genes, then it should be regulated. However, the fact that viruses which require fixed levels of − 1 PRF are nonetheless able to be successfully propagated suggests that, if regulation does occur, it has to happen with some degree of frameshift-signal specificity. Indeed, attempts to identify nonspecific regulators of −1 PRF using gel-shift and competition assays have been unsuccessful

(see Brierley et al., 2010). How might specificity be achieved? The characterization of numerous mutants in yeast, and the demonstration that siRNA knockdown of eRF1 stimulated −1 PRF in human cells (Kobayashi et al., 2010), suggests one possible avenue, consistent with the production of ''specialized ribosomes'' by cell (reviewed in Dinman, 2009). Alternatively, regulation of specific −1 PRF signals could be effected by small noncoding RNAs. For example, the demonstration that oligonucleotides capable of disrupting −1 PRF mRNA pseudoknot formation can inhibit −1 PRF *in vitro* (Plant and Dinman, 2005) provides proof-of-principle for this concept. Similarly, antisense RNAs have been shown to stimulate −1 PRF (Olsthoorn et al., 2004; Henderson et al., 2006; Yu et al., 2010). This strategy would enable individual cells to rapidly regulate −1 PRF on specific mRNAs by synthesizing small RNA species capable of interacting with −1 PRF signals in a sequence-specific manner, and would bypass having to pay the energetic costs of producing new or modifying preexisting ribosomes. In addition, single nucleotide polymorphisms (SNPs) that alter the slippery site or change the thermodynamic stability of the mRNA pseudoknot could affect −1 PRF efficiency, and thus mRNA stability, thus affecting gene expression and potentially causing disease phenotypes in humans. Current studies in our laboratory are investigating these intriguing possibilities.

III. Programmed +1 Ribosomal Frameshifting

A. *History of +1 PRF: Retrotransposable Elements*

Programmed +1 ribosomal frameshifting (+1 PRF) is the result of a net shift of the translational reading frame by one base in the 3′ direction (reviewed in Farabaugh, 1996). In eukaryotes, +1 PRF was discovered in the Ty*1* retrotransposable element of yeast. The first sequence analysis of Ty*1* revealed a 38bp overlap between the TYA (gag) and TYB (pol) genes with the latter shifted into the +1 frame, and immunoblot analyses demonstrated the presence of a TYA–TYB fusion protein (Clare and Farabaugh, 1985). Deletion analysis studies eventually reduced the frameshift signal to a 7-nt sequence in the TYA–TYB overlap region (Clare et al., 1988). The Ty*1* ''slippery site'' was determined to be CUU AGG C (the incoming TYA reading frame is indicated by spaces). The +1 PRF event

happens when the ribosome harboring a Leu-tRNA^{UAG} base-paired at the CUU codon in the P-site pauses at a rare AGG codon in its A-site. Slippage of the Leu-tRNA by one base in the 3′ direction enables decoding of the +1 frame UUA Leu codon in the P-site and positions the GGC codon, corresponding to a highly abundant Gly-tRNA, in the A-site (Belcourt and Farabaugh, 1990). Overexpression of the rare Arg-tRNA^{CCU} caused a 50-fold decrease in +1 PRF, while deleting it caused +1 PRF efficiency to approach 100% (Kawakami et al., 1993). The +1 frameshifts of Ty*2* and Ty*4*, and many other members of the *copia* family of retrotransposable elements, are thought to utilize this mechanism of tRNA slippage (reviewed in Farabaugh, 1996).

The Ty*3* *gypsy*-like yeast retrotransposon has a similar genome organization (Hansen et al., 1988). In Ty*3*, +1 PRF occurs at the sequence GCG AGU U. Importantly, the inability of the 0-frame tRNA in the P-site to base pair with the +1 frame codon suggested a completely different mechanism of establishing the frameshift (Farabaugh et al., 1993). Instead, +1 PRF in Ty*3* requires skipping the first A of the 0-frame A-site codon and recognizing the +1 frame GUU codon by a Val-tRNA. The Ty*3* +1 PRF also requires a downstream stimulatory element that has been suggested to enable base-pairing with sequence in the decoding center of 18S rRNA, optimizing the positioning of the slippery site (Li et al., 2001).

B. Cellular +1 PRF

1. Ornithine Decarboxylase Antizyme

Ornithine decarboxylase antizyme (AZ) is a negative regulator of polyamine biosynthesis, and AZ and its substrate (ornithine decarboxylase) coregulate one another *in vivo* (Heller and Canellakis, 1981). +1 PRF was first identified in the rat AZ gene (Miyazaki et al., 1992). It was subsequently found that the frequency of this event is dependent on polyamine levels and that increased levels of polyamines increase +1 frameshifting. This increases AZ production, which in turn decreases the abundance of ornithine decarboxylase, resulting in lower polyamine levels, thus completing the autoregulatory feedback loop between AZ and polyamine levels (Rom and Kahana, 1994). The AZ +1 slippage occurs at the heptameric sequence UCC UGA U in all metazoa (reviewed in Ivanov et al., 2000), although there is some degeneracy in this sequence in fungi and

arthropods (Palanimurugan et al., 2004). AZ +1 PRF is stimulated by sequences both 5′ and 3′ of the slippery site, although the nature of these stimulatory elements remains ill-defined.

As noted above, AZ gene-directed +1 PRF efficiency is increased in the presence of polyamines, as is synthesis of the key polyamine biosynthetic enzyme AdoMetDC (Hanfrey et al., 2005). In *S. cerevisiae*, increased levels of putrescine consequent to depletion of spermidine synthase promoted increased +1 PRF directed by the Ty*1* +1 PRF signal, but not in −1 PRF (Balasundaram et al., 1994), suggesting that polyamines play a general role in +1 PRF.

2. *Other Examples of Cellular +1 Frameshifting*

One of the first cellular +1 PRF signals was identified in the *E. coli prfB* gene, encoding release factor 2 (RF2; Craigen and Caskey, 1986), which promotes translation termination at the UGA and UAA codons. Decoding of the first 15% of this gene is initiated at the start codon, while the three-prime 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 distinct parameters help to promote high efficiency +1 PRF on this mRNA. The presence of an upstream Shine–Dalgarno-like sequence positions the ribosome at the +1 PRF site, directing the UGA termination codon to the A-site. It is not known whether the SD-like sequence serves to ''pull'' on the mRNA, thus promoting frameshifting, or merely serves to enhance translational pausing at the frameshift signal. Termination is efficient in the presence of high levels of RF2, thus downregulating synthesis of the full-length gene product. In contrast, low RF2 levels result in inefficient UGA codon recognition, thus stimulating frameshifting. Like AZ, this also constitutes an autoregulatory feedback circuit in which RF2 levels control production of RF2 through regulation of +1 PRF. There are only two known instances of +1 PRF in eukaryotic cellular mRNAs: in the EST3 and APB140 mRNAs of *S. cerevisiae* (Lundblad and Morris, 1997; Asakura et al., 1998). The +1 PRF signals in these two mRNAs are identical to the Ty*1* +1 signal, suggesting that a low-abundance amino acyl-tRNA directs ribosome pausing at the shift site. However, the physiological roles of +1 PRF in these cases remain unknown. Interestingly, while all known mitochondrial *nad3* and *cytb* genes contain single nucleotide insertions, the high degree of sequence conservation suggests that they are nonetheless functional,

suggesting that they are expressed through a +1 PRF mechanism (Mindell et al., 1998; Beckenbach et al., 2005). The +1 PRF mechanism appears to involve ribosome pausing at an AGY codon in the A-site. In *Euplotes* spp., mass spectrometric methods detected a frameshifted telomerase protein (Aigner et al., 2000). The presence of insertions and deletions resulting in the requirement for +1 frameshifting has also been described for a number of genes in this genus (Mollenbeck et al., 2004).

IV. Selenocysteine and Pyrrolysine: The 21st and 22nd Amino Acids

A. *Selenocysteine*

Selenium is an essential micronutrient, and selenium deficiency has been directly linked to a fatal cardiomyopathy termed Keshan disease, an osteoarthropathy known as Kashin–Beck disease, and myxedematous endemic cretinism. Selenium is incorporated into proteins in the form of selenocysteine, a version of cysteine in which sulfur is replaced by selenium. Numerous studies have revealed roles for the involvement of specific selenoproteins in antioxidant defenses, thyroid hormone metabolism, spermatogenesis, neuronal development and function, and many other processes. Disruption of the entire repertoire of selenoproteins results in early embryonic lethality, underscoring the essential nature of selenium (Bosl et al., 1997).

Incorporation of selenium into proteins requires specific signals in selenoprotein-encoding mRNAs, and specific *trans*-acting factors. Common features required for selenium incorporation in all three kingdoms are: (a) the recoding of the UGA codon to specify the position of selenocysteine in the coding region; (b) a unique tRNA species, $tRNA^{(Ser)Sec}$, which serves as both the site of selenocysteine biosynthesis and the means to deliver selenocysteine to the ribosome; and (c) specialized structures in selenoprotein-encoding mRNAs, which recruit and deliver the factors that promote recoding of the UGA codon (reviewed in Hoffmann and Berry, 2005). These latter *cis*-acting mRNA signals are termed selenocysteine incorporation signals or SECIS elements. In bacteria, SECIS elements are located immediately 3′ of the UGA codons, while in eukaryotes, they are typically located in 3′ untranslated regions (UTRs). Interestingly, they are located in either the 5 or 3′ UTRs *archaea*. In all cases, they function to deliver

sec-tRNA$^{(Ser)Sec}$ to the ribosome, albeit via different mechanisms. In prokaryotes, sec-tRNA$^{(Ser)Sec}$ is bound by and delivered to the ribosome by a specialized elongation factor called SelB. In eukaryotes, the analogous eEFsec contains a C-terminal region analogous to bacterial SelB, but this extension does not bind the SECIS element. Rather, a second protein called SBP2 interacts with eEFsec, thus ensuring that the tRNA$^{(Ser)Sec}$ is specifically delivered to the UGA codon in the presence of a SECIS element (Copeland et al., 2000). Interestingly, ribosomal proteins L30 and SBP2 have overlapping specificity for SECIS elements, and the two proteins compete for SECIS binding, generating the hypothesis that the SECIS element acts as a molecular switch, alternating between SBP2 and L30 binding during the recoding process, and suggesting a mechanism in which L30 and SBP2 may bind and act sequentially during UGA recoding to recruit eEFsec and deliver Sec-tRNA$^{(Ser)Sec}$ to the ribosomal A-site (Chavatte et al., 2005).

B. Pyrrolysine

Pyrrolysine is lysine in which a pyrroline ring is linked to the end of the lysine side chain. Its discovery in *archaea* and bacteria, and the observation that it is universally encoded by UAG, resulted in its designation as the 22nd amino acid (Atkins and Gesteland, 2002; Hao et al., 2002; Srinivasan et al., 2002). Currently, studies on the mechanism of pyrrolysine incorporation are in their infancy, but there are clear differences with selenocysteine: pyrrolysine has only been identified in a small number of methanogenic *archaea* and a few other microbes; pyrrolysine appears to be ligated directly to its cognate tRNA; and this tRNA is recognized by the standard elongation factor EF-Tu. While the mechanism by which UAG is recoded for pyrrolysine remains unknown, three possibilities have been proposed: (a) redefinition of a subset of UAG codons by *cis*-acting mRNA signals; (b) reassignment of all UAG codons within an organism to pyrrolysine; and/or (c) competition between readthrough and termination. Empirical evidence exists for the first two possibilities (Theobald-Dietrich et al., 2005; Zhang et al., 2005). Interestingly, *archaea* species that incorporate pyrrolysine use UAG codons very infrequently, which would minimize the potentially deleterious effects of reassignment of this codon on the whole organism. Whether a single or multiple strategies exist for incorporation of this unusual amino acid in different organisms is the focus of current efforts in the field.

V. Termination Codon Readthrough

A. *History: In Viruses*

Programmed suppression of termination codons represents another common strategy that viruses have evolved to solve the problem of regulating the ratios of structural to enzymatic proteins. The translational readthrough signal of Murine Leukemia virus (MuLV) remains the paradigm in the field, having enabled identification of critical sequence and structural features, including the requirement for an mRNA pseudoknot (Wills et al., 1991, 1994; Alam et al., 1999). The ability of the MuLV reverse transcriptase to interact with eRF1 suggests that depletion of this termination factor may be used to enhance the frequency of translational readthrough (Goff, 2004). Analyses of other examples of programmed translational readthrough suggest that the general rules pertaining to the makeup and context of termination codons that govern termination efficiency have been adopted by many other RNA viruses. (reviewed in Maia et al., 1996).

B. *Termination Suppression in Cellular Genes*

Examples of translational readthrough in cellular mRNAs continue to accumulate. A computational analysis of the yeast genome identified eight genes containing ''poor context'' termination signals (Namy et al., 2003), and a follow-up study demonstrated that termination suppression in the PDE2 mRNA is used to modulate cAMP levels (Namy et al., 2002). A few examples of translational readthrough have also been documented in the *Drosophila*: *oaf*, *hdc*, and *keltch* mRNAs (Xue and Cooley, 1993; Bergstrom et al., 1995; Stenberg et al., 1998), and regulation of translational readthrough is thought to be important for *Drosophila* development. Approximately 4% of predicted mRNAs in the mouse genome contain premature termination codons, suggesting that this mechanism may be widely used to posttranscriptionally regulate vertebrate gene expression (Xing and Lee, 2004). The emerging view is that that termination suppression may represent a significant mode of the posttranscriptional regulation of cellular gene expression.

C. *Therapeutic Applications*

It is estimated that as many as one-third of inherited genetic disorders and many forms of cancer are caused by frameshift or nonsense mutations, resulting in destabilization of the mutant mRNAs through the NMD pathway. The list of such diseases includes Duchenne muscular dystrophy (reviewed in Nelson et al., 2009), recessive von Willebrand disease types 1 and 2 (reviewed in Castaman et al., 2009), cystic fibrosis (reviewed in Proesmans et al., 2008), lysosomal storage disorders (reviewed in Brooks et al., 2006), aortic valve disease (reviewed in Garg, 2006), inherited afibrinogenaemia (reviewed in Neerman-Arbez, 2001), and epidermolysis buillsa (reviewed in Bauer et al., 2002). The observation that expression of as little of 10–15% of normal levels of the cystic fibrosis transmembrane conductance regulator (CFTR) can rescue patients from clinical disease (Chu et al., 1992), and the ability of aminoglycoside antibiotics such as gentamycin to promote readthrough of stop codons, suggests that this class of drugs may be of clinical utility in treating this class of diseases. However, the nephro- and ototoxicity of this drug precludes its broad use, although numerous groups are investigating the usefulness of chemically derivatized variants of this class of drugs (Zingman et al., 2007). Alternatively, NMD may be an attractive target for therapeutic manipulation. One drug, PTC124 [{3-[5-(2-fluorophenyl)-1,2,4-oxadiazol-3-yl]benzoic acid}, or ataluren], has been specifically developed to treat genetic disorders caused by nonsense mutations (Welch et al., 2007). Although there is some controversy regarding whether it actually inhibits PTC or merely inhibits the activity of the firefly luciferase reporter protein used in screens to identify PTC124 (Auld et al., 2009, 2010), this drug is currently being used in clinical trials of cystic fibrosis and other diseases (Nelson et al., 2009; Kohli et al., 2010; Dranchak et al., 2010; Goldmann et al., 2011; Tan et al., 2011; Wilschanski et al., 2011).

VI. Summary

Rules are made to be broken. Indeed, the exceptions to rules aid in the definition of rules themselves, which in turn expand our understanding of how diversity is generated in living organisms. Both viruses and free living cells have evolved numerous molecular mechanisms to subvert the genetic code, allowing expansion of genomic coding space and providing novel

modes by which to regulate gene expression. Current studies are focused on investigating the molecular mechanisms underlying translational recoding, the roles played by these events in gene expression, and possible therapeutic strategies based on these findings. The coming years will surely witness a broad expansion in this field, particularly as it relates to the role of translational recoding in medicine and biotechnology.

Acknowledgments

I would like to thank past and present members of my laboratory, particularly Arturas Meskauskas, Ashton Trey Belew, and Jonathan Jacobs, without whom the genomic frameshifting project would not have been possible. Thanks also to Ron Taylor, who wrote the original code to identify −1 PRF signals in the yeast genome. This work was supported by a grant to J. D. D. by the National Institutes of Health (R01 GM058859).

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