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REVIEW

mRNA surveillance in eukaryotes: Kinetic proofreading of proper translation termination as assessed by mRNP domain organization?

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ABSTRACT

In the last few years it has become clear that a conserved mRNA degradation system, referred to as mRNA surveillance, exists in eukaryotic cells to degrade aberrant mRNAs. This process plays an important role in checking that mRNAs have been properly synthesized and functions, at least in part, to increase the fidelity of gene expression by degrading aberrant mRNAs that, if translated, would produce truncated proteins. A critical issue is how normal and aberrant mRNAs are distinguished and how that distinction leads to differences in mRNA stability. Recent results suggest a model with three main points. First, mRNPs have a domain organization that is, in part, a reflection of the completion of nuclear pre-mRNA processing events. Second, the critical aspect of distinguishing a normal from an aberrant mRNA is the environment of the translation termination codon as determined by the organization of the mRNP domains. Third, the cell distinguishes proper from improper termination through an internal clock that is the rate of ATP hydrolysis by Upf1p. If termination is completed before ATP hydrolysis, the mRNA is protected from mRNA degradation. Conversely, if termination is slow, then ATP hydrolysis and a structural rearrangement occurs before termination is completed, which affects the fate of the terminating ribosome in a manner that fails to stabilize the mRNA. This proposed system of distinguishing normal from aberrant transcripts is similar to, but distinct from other systems of kinetic proofreading that affect the accuracy of other biogenic processes such as translation accuracy and spliceosome assembly.

Keywords: 3′ UTR; mRNA turnover; NMD; Upf1

WHAT IS mRNA SURVEILLANCE?

The process of mRNA surveillance refers to a mechanism by which aberrant mRNAs are distinguished from normal mRNAs and rapidly degraded by the cell. Aberrant transcripts can be generated by two different methods. First, transcripts can be aberrant due to mutations in the DNA, such as premature nonsense codons. Alternatively, errors in pre-mRNA processing can yield aberrant transcripts that are degraded by mRNA surveillance. For example, the yeast CYH2 pre-mRNA is inefficiently spliced, which leads to intron-containing precursors being exported to the cytoplasm. These unspliced CYH2 transcripts contain an intron-encoded premature stop codon, and are degraded by the mRNA surveillance mechanism (He et al., 1993).

mRNA surveillance is commonly referred to as nonsense-mediated decay (NMD) because the majority of transcripts known to be degraded by this mechanism contain premature translation termination codons (e.g., Losson & Lacroute, 1979; Maquat et al., 1981). However, the mRNA surveillance mechanism also works on other types of aberrant transcripts. For example, transcripts with retained introns (which include premature termination codons; He et al., 1993), extended 5′ UTRs (which include upstream ORFs including a termination codon; Oliveira & McCarthy, 1995), or extended 3′ UTRs (which change the relationship of the termination co-

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don to the 3′ UTR; Zaret & Sherman, 1984; Pulak & Anderson, 1988, 1993; Muhlrad & Parker, 1999b) are all degraded by the mRNA surveillance mechanism. A striking feature common to all these types of mRNA surveillance substrates is an alteration of the normal spatial relationship between the termination codon and the 3′ UTR.

DECAPPING AND RIBOSOMES: THE HOW OF mRNA SURVEILLANCE

During mRNA surveillance, aberrant substrates are rapidly shunted into the normal mRNA turnover pathway, bypassing the normal control events that allow a transcript a prolonged life for translation. The normal mRNA turnover pathway occurs in a process where deadenylation leads to a decapping event, which exposes the body of the mRNA to 5′-to-3′exonucleolytic degradation (reviewed in Beelman & Parker, 1995). In contrast, the degradation of a transcript in response to mRNA surveillance occurs by rapid decapping while the transcript is still fully adenylated (Shyu et al., 1991; Lim & Maquat, 1992; Muhlrad & Parker, 1994).

Several observations suggest that decapping triggered by the process of mRNA surveillance requires the translation of the mRNA by ribosomes. First is the simple fact that it is nonsense codons that are stimulating mRNA decay (Losson & Lacroute, 1979; Maquat et al., 1981). Second, the presence of suppressor tRNAs can reduce or eliminate the effect of nonsense codons on mRNA degradation (Losson & Lacroute, 1979; Belgrader, 1993). Third, blocking translation *in cis* either by insertion of a stem-loop structure in the 5′ UTR or by mutation of the AUG start codon stabilizes nonsense-containing transcripts (Naeger et al., 1992; Belgrader et al., 1993; Simpson & Stoltzfus, 1994; Ruiz-Echevarria et al., 1998). Fourth, nonsense containing transcripts cofractionate with polysomes (Leeds et al., 1991; He et al., 1993; Stephenson & Maquat, 1996; Zhang & Maquat, 1997; Thermann et al., 1998). Fifth, critical components of the mRNA surveillance system (i.e., the Upf 1, Upf2, and Upf3 proteins) likewise cofractionate with polysomes (Atkin et al., 1995, 1997) and are themselves, translation termination factors (see below). These observations argue that a critical step in distinguishing normal from aberrant transcripts is the nature of the translation termination event. This raises the questions of (1) how a terminating ribosome determines whether a termination event is proper or improper, and (2) how that distinction leads to different stabilities of the transcripts. As discussed below, several lines of evidence now suggest that the sum and substance of mRNA surveillance is that differences in the environment of the termination codon influence events at that termination codon, and as a result, alter the manner in which termination occurs, with consequences for mRNA degradation.

CIS-ACTING SEQUENCES AND THE NATURE OF TRANSLATION TERMINATION

Changes in the sequence downstream of a termination codon can affect the process of mRNA surveillance. For example, in the yeast *Saccharomyces cerevisiae*, the rapid decay of mRNA induced by a premature termination codon is prevented if the 3′ UTR is positioned in close proximity to the premature termination codon by deletion of the intervening, untranslated coding region (Peltz et al., 1993). Moreover, rapid mRNA decay can be restored in this case by the insertion of a variety of different sequences (termed Downstream Elements or DSEs) between the premature termination codon and the 3′ UTR (Peltz et al., 1993; Hagan et al., 1995, Zhang et al., 1995; Ruiz-Echevarria & Peltz, 1996; Ruiz-Echevarria et al., 1998). Conversely, a 68-nt region [referred to as the STE (Stabilizer Element)] normally found downstream of a small upstream open reading frame (uORF4) in the GCN4 mRNA protects mRNAs from surveillance-mediated decay when positioned downstream of a premature termination codon (Ruiz-Echevarria et al., 1998). These results indicate that RNA sequences can act in both a positive and negative manner to influence whether or not a transcript is perceived as aberrant or normal.

In mammalian transcripts, one critical determinant in distinguishing normal from aberrant transcripts is the spatial relationship between the termination codon and the 3′ most exon–exon junction (reviewed in Nagy & Maquat, 1998). Translation termination codons positioned greater than 50–55 nt upstream from the 3′ most exon–exon junction are generally perceived as improper and lead to mRNA surveillance decay (Cheng et al., 1994; Thermann et al., 1998; Zhang et al., 1998a, 1998b). This explains why the vast majority of natural (proper) termination codons are positioned downstream of the 3′ most exon–exon junction (Hawkins, 1988).

An important issue is how these downstream sequence elements function to distinguish proper and improper termination events. One popular model is that the downstream sequences are specifically recognized after improper termination by a “surveillance complex” that then triggers mRNA decay (Peltz et al., 1993; Cheng et al., 1994; Zhang et al., 1995; Zhang et al., 1998a, 1998b; Thermann et al., 1998). An alternate possibility is that the downstream sequences affect the nature of the termination event. This concept is based on the fact that a ribosome positioned at a termination codon can undergo several different fates, depending on the sequence context surrounding the termination codon. For example, local sequence context can determine whether ribosomes read through a termination codon (Skuzeski et al., 1991; Wills et al., 1991), or following an actual termination event, continue to scan for reinitiation (Kozak, 1987; Miller & Hinnebusch, 1989; Grant & Hinnebusch, 1994). Moreover, distant sequences can also

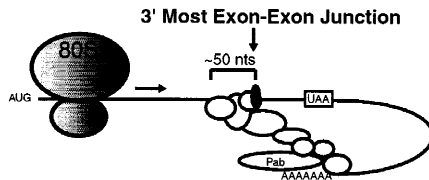
dramatically influence the activity of a terminating ribosome. The most striking example of this phenomenon is the specification of selenocysteine incorporation at UGA codons in some mammalian mRNAs (Berry et al., 1991, 1993). In this case, a structural element (SECIS) within the 3′ UTR of Se-GPx1 dictates ribosome read-through by stimulating the incorporation of the nonstandard selenocysteine amino acid at the prescribed UGA codon located some 500 nt upstream. Thus, both the local and distal context of a termination codon can affect the activity of a ribosome positioned at a termination codon.

THE mRNP ENVIRONMENT OF A TERMINATION EVENT

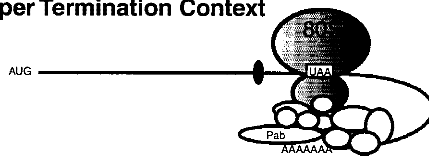
Relationship of introns and translation termination in mammalian cells

Several observations suggest that a critical determinant of whether an mRNA is targeted to mRNA surveillance is the environment of the termination event as defined by functional domains within the mRNP (Fig. 1). In mammalian transcripts, the critical relationship between the termination codon and the 3′ most

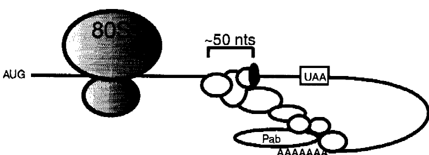
A) Mammalian 3' Terminal mRNP Domain



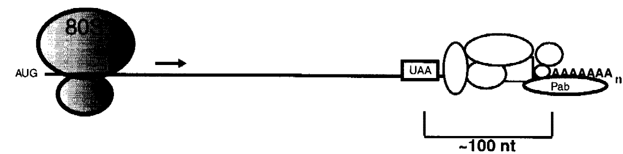
B) Proper Termination Context



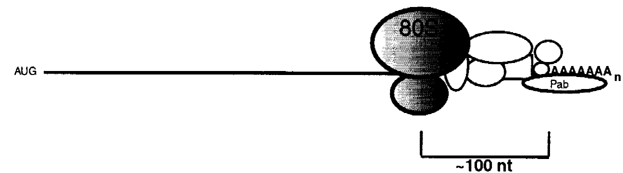
C) Improper Termination Context



D) Yeast 3' Terminal mRNP Domain



E) Proper Termination Context



F) Improper Termination Context

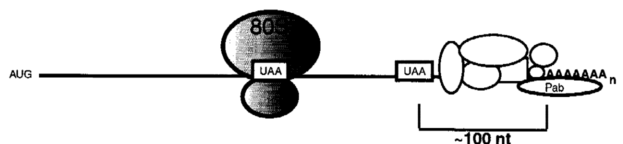


FIGURE 1. A model of how 3′ terminal mRNP domains in mammalian and yeast transcripts may provide a proper context for translation termination. **A**: The mammalian 3′ terminal mRNP domain is formed during 3′-end formation and terminal-exon splicing of pre-mRNA transcripts. This mRNP domain is retained in the mature mRNA and serves as the mark of the 3′ most exon–exon junction (filled oval). **B**: A translating ribosome that approaches (within 50 nt) or traverses the boundary of the 3′ terminal mRNP domain is now in the proper environment for termination. Translation termination leads to a reorganization of the mRNP, and subsequent stabilization of the transcript. **C**: A translating ribosome that terminates before encountering the boundary of the 3′ terminal mRNP domain is in an improper context. This termination event leads to a failure to reorganize the mRNP, and subsequent destabilization of the transcript. **D**: The 3′ terminal mRNP domain in yeast may be defined by 3′UTR-bound factors that originally functioned in 3′ end formation. The ~100-nt length of most yeast 3′UTRs may represent a spatial limitation to physical interactions between the 3′ UTR-bound factors and a terminating ribosome that helps to define the 3′ end of the transcript. **E**: A terminating ribosome that can engage in interactions with the 3′ UTR-bound factors is in the proper termination context and leads to a stabilization of the transcript. **F**: A terminating ribosome that is unable to engage the 3′ UTR-bound factors in interactions is in an improper context, and leads to a destabilization of the transcript.

exon–exon junction has led to the suggestion that the process of splicing deposits a mark on the mRNA, at the 3′ most exon–exon junction, that is subsequently interpreted by the translating ribosome (for review, see Li & Wilkinson, 1998; Nagy & Maquat, 1998; Hentze & Kulozik, 1999).

An important point is that the 3′ most exon–exon junction will be different from every other exon–exon junction in a mammalian mRNA. This is because during mammalian pre-mRNA processing, discreet domains are believed to be formed as a result of the processes of exon and intron definition (reviewed in Berget, 1995). In most mammalian pre-mRNAs, exons, rather than introns, are believed to be the unit of definition whereby an assembly of splicing factors physically spans or bridges across the exon in response to splice-site signals. Internal exons are defined by an upstream 3′ splice site and a downstream 5′ splice site. Terminal exons however, are defined differently. Most relevant to this discussion, the 3′ terminal exon is defined by an upstream 3′ splice site region, and the polyadenylation site within the 3′ UTR of the transcript. Thus, the mark that defines the 3′ most exon–exon junction in mammalian transcripts for distinguishing proper termination, might very well be a remnant of the physical bridge that once served to define the terminal exon. We refer to this mark as the boundary of a 3′ terminal mRNP domain.

An unresolved issue is whether or not the 3′ exon–exon mark serves to stimulate mRNA degradation when positioned too far downstream of the stop codon, or serves to stabilize the mRNA when positioned upstream of, or near, the termination codon. In this latter view, a ribosome would need to encounter the boundary of the 3′ terminal mRNP domain before or during termination for the termination event to be considered proper (Fig. 1B). In contrast, translation-termination events that occur without prior interaction with the 3′ terminal mRNP boundary would be considered improper and would lead to degradation of the transcript (Fig. 1C). Interestingly, essentially all natural termination codons are either beyond or are within 50 nt upstream of the 3′ terminal mRNP boundary (reviewed in Nagy & Maquat, 1998). This would ensure that ribosomes would either translate through the mRNP boundary, or that they would terminate in close enough proximity to engage an interaction with components of the 3′ mRNP boundary.

An interesting variant on this spacing rule is seen with the lymphoid specific, T-cell receptor (TCR-B) transcript, wherein a termination codon as close as 8–10 nt upstream from the 3′ most exon–exon junction still leads to destabilization of the transcript (Carter et al., 1996). This variation can be interpreted as a more stringent requirement for interactions between the terminating ribosome and constituents of the 3′ terminal mRNP to define the transcript as aberrant. In support of a more stringent mechanism of discrimination, the nonsense-containing TCR-B transcript undergoes a 10–100-fold down regulation whereas the down regulation of other nonsense-containing mRNAs tends to be more modest, within the two- to fourfold range (reviewed in Li & Wilkinson, 1998).

The requirement for translating ribosomes to encounter the 3′ terminal mRNP domain prior to termination to stabilize the mRNA might also help to explain the observation that transfection constructs bearing at least one intron tend to yield higher expression levels (e.g., Zhang et al., 1998a). Intronless mammalian mRNAs may fail to form a proper terminal mRNP domain, and therefore would be subject to mRNA surveillance. Consistent with this view, the insertion of an intron can, at least in some cases, increase not only the stability, but also the translation rate of some mRNA transcripts in *Xenopus oocytes* (Matsumoto et al., 1998). It should be noted, however, that intronless transcripts may also be defective in proper 3′ end formation, and subsequently degraded by a nuclear decay mechanism (Nesic & Maquat, 1994). Future experiments should easily test if intronless mRNAs are indeed, subject to mRNA surveillance. For example, this view predicts that the levels of intronless mRNAs would increase if mRNA surveillance was inhibited either *in cis*, by the insertion of a stem-loop structure that precludes 80S formation, or *in trans* by mutations in the mRNA surveillance machinery.

mRNP structure in yeast

Akin to the proposed model for 3′ terminal mRNP domain function in mammalian transcripts, the effects of sequence information downstream of termination codons in yeast mRNAs can also be understood in terms of mRNP structure (Fig. 1). However, because very few yeast transcripts undergo pre-mRNA splicing and exon definition is not believed to be operative in yeast, the primary derivation of mRNP domain organization in yeast may rely more strictly upon other pre-mRNA processing events, such as 5′ and 3′ end formation. It follows then that discreet mRNP domain organization in yeast may be limited to the terminal regions of the transcript. In this light, it is interesting to note that yeast 3′ UTRs are strikingly homogeneous in length, with an average length of approximately 100 nt (Graber et al., 1999). This strongly preferred short 3′ UTR length in yeast may reflect the need for interactions between the terminating ribosome and the assembly of proteins on the 3′ UTR that initially played a role in 3′ end formation. Interestingly, the one yeast mRNA that has been described to have a long 3′ UTR (~2 kb) is the CTF13 mRNA. Increased levels of the CTF13 transcript are observed in strains deficient in mRNA surveillance, although the mechanisms leading to the higher mRNA levels have yet to be determined (Dahlseid et al., 1998).

The effects of yeast sequences that affect mRNA surveillance can also be interpreted in consideration of the mRNA and its associated proteins. In the extreme view, yeast DSEs could be envisioned as sequence elements that disrupt the proper spatial interactions between the normal 3′ UTR and the translation termination machinery, perhaps due to the failure to associate with the "normal" 3′ UTR binding proteins. In contrast, the STE sequence element of the GCN4 mRNA could be envisioned as a 3′ UTR-like module that positions a functional "3′ UTR" environment downstream of the GCN4 uORF termination codons, thereby preventing these uORFs from triggering mRNA surveillance. This view suggests that other uORF-containing mRNA that are resistant to mRNA surveillance will contain similar STE-like elements and that these elements will bind proteins that also associate with 3′ UTRs.

Extended 3′ UTRs as aberrant terminal mRNP domains

As previously discussed, a second class of aberrant transcripts subject to mRNA surveillance are those mRNAs bearing abnormally extended 3′ UTRs due to mutations that eliminate the normal site of 3′ end cleavage and polyadenylation (Zaret & Sherman, 1984; Pulak & Anderson, 1988, 1993; Muhlrad & Parker, 1999b). These transcripts differ from mRNAs with early nonsense codons in that what has changed is not the location of the termination codon, per se, but the inclusion of novel 3′ untranslated sequence, the length of which depends upon the choice of cryptic 3′ end cleavage and polyadenylation sites. This class of aberrant mRNAs would be predicted to have a significantly altered 3′ terminal mRNP domain that would no longer have the proper relationship with the termination codon. The fact that these types of mutations result in mRNA surveillance-dependent decay in yeast and nematodes supports a conserved role for mRNP domain function in the discrimination of proper from improper translation termination events.

DISTINGUISHING PROPER FROM IMPROPER TERMINATION: IS THE Upf1 ATPase AN INTERNAL CLOCK?

The above results suggest the model that mRNP domains influence the nature of the termination event. Given this, an important question is how the cell is able to sense the differences in the nature of the termination event caused by proper or improper mRNP environments. Insight into this issue has come from the analysis and characterization of the proteins required for mRNA surveillance. A number of genetic loci have been identified that are required to trigger mRNA surveillance. These loci include the yeast Upf1, Upf2, and Upf3 proteins (Leeds et al., 1991, 1992; Cui et al., 1995; He & Jacobson, 1995; Lee & Culbertson, 1995) and a set of genetic loci in nematodes, termed *smg1* to *smg7* (Pulak & Anderson, 1993). The Upf1 protein is a nucleic-acid-dependent ATPase whose function is required for mRNA surveillance in yeast, nematodes, and humans (Leeds et al., 1991; Czaplinski et al., 1995; Sun et al., 1998; P. Anderson, pers. comm.).

How might the Upf1p function to distinguish proper from improper termination? Several observations discussed below lead to a hypothesis that Upf1p serves as a kinetic clock, providing a time frame within which proper termination events can be completed. In the simplest scenario, a proper termination event would be defined by productive interactions between the terminating ribosome and the 3′ terminal mRNP domain leading to successful completion of termination, perhaps including a specific fate for the terminating ribosome. Conversely, termination events that lag because of an inappropriate termination environment would not be completed before Upf1p ATP hydrolysis. ATP hydrolysis by Upf1p would then lead to rapid decay of the transcript. It is important to note that it is the helicase function of the Upf1p protein, presumably driven by the ATPase clock, that affects the fate of the ribosome, perhaps because of a structural rearrangement within the termination complex, thereby generating a signal to decap the transcript. Similar to other proofreading mechanisms, in this model a fixed rate of ATP hydrolysis by Upf1p is used to create a window of opportunity for termination to be completed. However, the proposed mode of proofreading here is unusual in that NTP hydrolysis is not required for the process to proceed, but instead serves to direct the mRNA to a discard pathway.

Critical to the hypothesis presented above, several genetic and biochemical observations support an intimate association between Upf1p and the terminating ribosome, affecting both the assembly of the termination complex and the fate of the terminating ribosome. For example, physical interactions between the Upf1 protein and the translation release factors Erf1 and Erf3 have been described based on coaffinity purification and coimmunoprecipitations (Czaplinski et al., 1998). In addition, deletion of any of the three yeast UPF genes results in an increase in the observed nonsense suppression or ribosomal readthrough of various reporter genes (Cui et al., 1995; Lee & Culbertson, 1995). Based on these observations, one function of the Upf1p appears to be to enhance the assembly of a termination complex (Fig. 2). In the absence of this function, termination complex assembly is less efficient and increased levels of nonsense codon readthrough are observed (Weng et al., 1996a).

The second function of Upf1p appears to be uniquely required for the process of mRNA surveillance. The key observation is that there are specific upf1 alleles (DE572AA, TR800AA, RR793AA) that show efficient translation termination, but fail to activate the mRNA

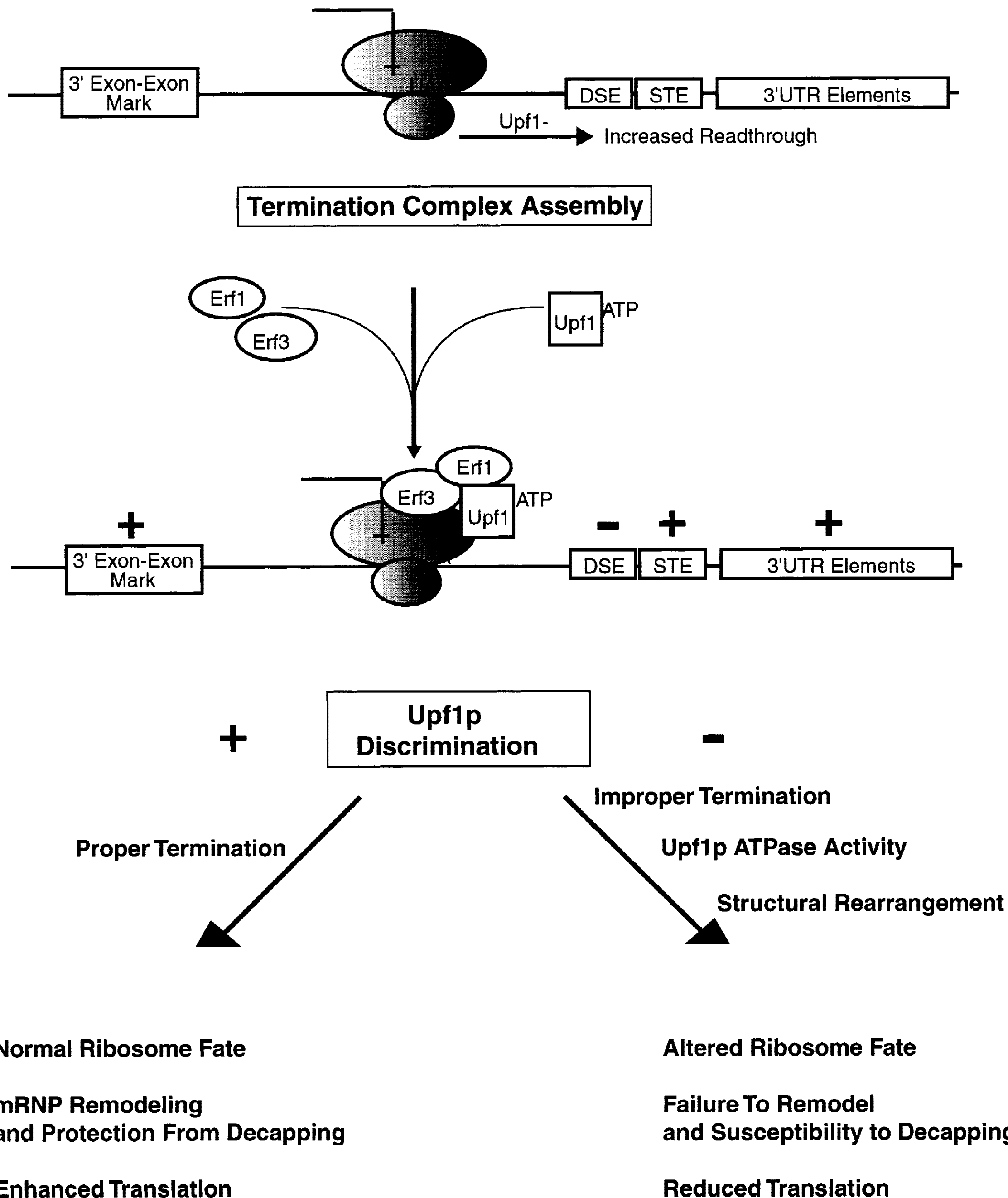


FIGURE 2. A model of how Upf1p may function as a kinetic clock in the discrimination of proper from improper termination events. Upf1p associates with the translation termination release factors Erf1 and Erf3 in promoting efficient translation termination complex assembly. Local and distal sequence elements and associated factors influence the termination event, and either promote (+) or inhibit (−) key events in the translation-termination process. If the termination process is improper or too slow, Upf1p hydrolyzes its bound ATP, which leads to a structural rearrangement within the terminating ribosome, and subsequently alters the fate of that ribosome.

surveillance pathway, resulting in high levels of nonsense-containing transcripts (Weng et al., 1996a).

Insight into the mechanism of action of the Upf1 protein in mRNA surveillance and how that relates to its function in termination complex assembly has come from a detailed analysis of the biochemical properties of the Upf1 protein. The ∼109-kDa Upf1 protein is a member of the superfamily group I helicases based on the presence and organization of ATP binding, ATPase, RNA binding, and helicase domains (Koonin, 1991, 1993; Leeds et al., 1992). The purified Upf1 protein demonstrates nucleic-acid-dependent ATPase activity, as well as RNA binding and RNA helicase activities in vitro (Czaplinski et al., 1995, Weng et al., 1998). A thorough analysis of Upf1p variants and their activities has revealed key features about the function of the protein.

First, the ability of Upf1p to bind ATP is central to any and all functions of the protein. A single point mutation within the highly conserved ATP binding domain (K436Q) results in a complete loss of function allele (Weng et al., 1996a). This is consistent with the observed requirement for ATP to be bound to the Upf1p to allow it to assemble with the termination factors (Czaplinski et al., 1998). Second, ATPase and helicase activities are required for the Upf1 mRNA turnover function (Weng et al., 1996b). These results are consistent with a model wherein ATP hydrolysis by Upf1p leads to an RNA–Upf1p interaction that causes rapid degradation of the transcript.

Based on the biochemical properties of Upf1p, a simple hypothesis is that the cell takes advantage of a subtle difference in the efficiencies of proper versus improper termination to funnel the ribosome into different fates (Fig. 2). In addition to the observations that *cis*-acting sequences can affect the fate of the terminating ribosome (see above), this model builds on the fact that mutations in prokaryotic ribosome release factors can alter the fate (e.g., continued downstream scanning) of the terminating ribosome in prokaryotes (Hirashima & Kaji, 1973; Ogawa & Kaji, 1975; Janosi et al., 1998). In eukaryotes, the normal fate of a terminating ribosome may include ribosome recycling to the 5′ end as a consequence of mRNP reorganization, or alternatively, complete dissociation from the mRNA (see below). Continued ribosome scanning and reinitiation downstream of a premature termination event could be considered an alternate ribosome fate. In this light, it is noteworthy that reinitiation by a scanning ribosome downstream of a premature termination codon can block surveillance-mediated decay of the transcript (Zhang & Maquat, 1997; Ruiz-Echevarria & Peltz, 1996; Ruiz-Echevarria et al., 1998).

A critical distinction that governs the fate of a terminating ribosome is whether or not ATP hydrolysis by Upf1p occurs before termination is completed. ATP hydrolysis by Upf1p is proposed to lead to a conformational change in the termination complex such that the fate of the ribosome is altered. In this view, Upf1p serves as internal clock to monitor the rate of translation termination and thereby to assess whether or not termination is occurring in the proper context.

BACK TO THE BEGINNING: DIFFERENT RIBOSOME FATES AND CONTROLLING DECAPPING

There are two simple mechanisms by which differences in ribosome fate could dictate whether the mRNA is decapped. In one view, a Upf1p-altered ribosome traversing downstream of the termination codon would itself trigger decay. This seems unlikely for two reasons. First, the insertion of stem-loop structures downstream of the termination codon, which should be sufficient to block downstream ribosome scanning, does not prevent decay of the mRNA in response to a nonsense codon (Zhang et al., 1998a). Second, when ribosomes are able to reinitiate translation downstream of an improper termination codon, the mRNA is not subject to mRNA surveillance, provided that the second termination codon is in the proper context (Zhang & Maquat, 1997; Ruiz-Echevarria & Peltz, 1996; Ruiz-Echevarria et al., 1998). This suggests that it is not the act of improper termination, but rather the lack of a proper termination event that triggers mRNA surveillance-mediated decay.

An alternate hypothesis is that during proper termination the ribosome adopts a fate that leads to mRNA stabilization. One appealing possibility is that during proper termination the ribosome is efficiently recycled to the 5′ end of the mRNA in a manner that boosts translation initiation. Interestingly, this hypothesis is supported by the observation that aberrant mRNAs are poorly translated in wild-type cells but their translation is increased in upf1Δ strains, even in the absence of accelerated decay (Muhlrad & Parker, 1999a). This is also a simplifying model in that it mechanistically links the rapid decay promoted by the process of mRNA surveillance with the decapping event that normally requires prior deadenylation. Recent results from our lab suggest that the decapping rate of an mRNA is dictated by its translation rate (LaGrandeur & Parker, 1999; Schwartz & Parker, 1999). Thus, the cell takes advantage of mechanistic interplays between translation initiation and decapping, as well as translation termination and translation initiation to sense the integrity of the transcript.

mRNP REMODELING: ESTABLISHMENT VERSUS MAINTENANCE AND ITS ROLE IN mRNA STABILITY

An unresolved issue is whether the differences in proper and improper termination affect the establishment and/or maintenance of the proper mRNP organization. One popular model is that successful completion of the first round of translation triggers a conformational rearrangement in the architecture of the individual mRNP domains, such that the 3′ and 5′ terminal domains of the mRNP become linked. Evidence that the distinction between normal and aberrant mRNAs may occur during the first round of translation has come from the observation that nonsense codons in some mammalian transcripts cause a reduction in the levels of mature mRNAs that biochemically cofractionate with nuclei (e.g., mammalian triosephosphate isomerase (TPI) transcript) and are therefore assumed to be in the process of nuclear export (Cheng & Maquat, 1993; Belgrader & Maquat, 1994). Moreover, at least for TPI, the few nonsense-containing transcripts that reach the cytoplasmic pool then show a normal decay rate in that subcellular com-

partment (Stephenson & Maquat, 1996). A simple interpretation of these observations is that the first round of translation is occurring on the nuclear-associated transcripts and that mRNAs that successfully complete this round of translation establish a proper mRNP organization and show normal decay thereafter. However, other mammalian and viral mRNAs show increased rates of cytoplasmic mRNA degradation in response to nonsense codons (Maquat et al., 1981; Barker & Beemon, 1991), suggesting that the discrimination of proper versus improper termination may not be limited to first-round translation events. In this view, proper versus improper termination could be interpreted to affect the maintenance of the mRNP organization.

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