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Visions & Reflections

What's new in translation initiation? The first translation determines the fate of mRNA

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Abstract. The two terms 'translation' and 'protein synthesis' are interchangeable in describing the process whereby the genetic code in the form of messenger RNA (mRNA) is deciphered such that amino acids cognate with the triplet code are joined end to end to form a peptide chain. However, new data suggest that the initial act of translation on newly synthesised mRNA also functions to proofread mRNA for errors. Aberrant mRNAs detected in this way are rapidly degraded before their encoded proteins impede normal cell function. Initiation of surveillance translation appears to differ from that of regular protein synthesis in three ways: (i) composition of the substrate; (ii) temporal and spatial restrictions; (iii) factors used to recruit the ribosome. This review discusses translational aspects of mRNA surveillance, primarily in the context of the mammalian system, although much information has come from studies in yeast and other organisms.

Key words. eIF4E; CBC; translation; nonstop decay; NMD; NAS; PTC; nucleus.

What errors can ribosomal scanning detect?

While errors in the DNA sequence of the genome can be easily detected due to an inability of altered bases to correctly pair with the adjacent strand, the ability to detect errors in a single-stranded messenger RNA (mRNA) appears a daunting, and perhaps impossible task. In the nucleus many potential errors in RNA processing are eliminated by the tight coordination of the processing and export procedures, ensuring that mRNA export is not permitted until the mRNA is correctly processed [1–4]. Two additional errors are detected during ribosomal scanning and result in targeted degradation of the aberrant mRNA; the absence of a termination codon [5, 6], and the presence of a premature termination codon (PTC) [7, 8]. These processes are known as nonstop decay and nonsense-mediated decay (NMD), respectively.

Nonstop decay

mRNAs lacking a stop codon are most likely to be produced by either inappropriate cleavage and polyadenylation during nuclear processing or by incomplete degradation of an mRNA undergoing translation [5]. The degradation of mRNA lacking a stop codon requires the cytoplasmic exosome with two associated factors: the heterotrimeric complex of Ski proteins (namely Ski2p, Ski3p and Ski8p) and Ski7p [6]. The N terminus of Ski7p links the exosome to the other Ski proteins [9] and is required for cytoplasmic exosome activity. The C terminus is dispensable for exosome function, and is instead required for nonstop decay [6]. The C terminus of Ski7p has homology to the GTPase domains of eukaryotic elongation factor 1 A (eEF1 A) and release factor 3 (eRF3) [10] that interact with the A site of the ribosome during elongation and termination, respectively. Nonstop decay has been localised to the cytoplasm [5], which is consistent with the cytoplasmic localisation of Ski2p and Ski7p, as well as the

translational requirement. The current model for nonstop decay is as follows: in the absence of a termination codon, the ribosome translates through the poly(A) tail and becomes stalled at the 3′ end of the mRNA with an empty A site. Ski7- binds to this site via its C terminus (in an analogous manner to the binding of eEF1 A or eRF3) and recruits the Ski complex and cytoplasmic exosome to degrade the aberrant mRNA [5, 6].

Although initially discovered in yeast, nonstop decay has also been shown to occur in mammalian cells, and since the Ski proteins are conserved, it is likely to proceed by a mechanism similar to that described for yeast [5, 6].

NMD

In contrast to the recent discovery of nonstop decay, NMD was discovered in 1979, when the absence of β-globin expression [7] and reduced Ura 3 mRNA [8] were both found to be due to the presence of nonsense codons within the coding sequence.

Genetic mutations that are the cause of genetic diseases and inherited cancer frequently result in NMD. For example, 77% of mutations in BRCA1 that are associated with breast cancer [11] and 89% of mutations in the ATM gene that cause ataxia telangiectasia [12] lead to premature chain termination. A notable example of the importance of mRNA surveillance in inherited genetic disease is evident in β-thalassaemia, characterised by defects in the β-globin gene. The majority of PTC-generating mutations in the β-globin gene are recessive due to a functional NMD pathway. However, if a PTC occurs in the final exon, it is immune to detection by the surveillance pathway and a truncated protein is synthesised, resulting in a dominantly inherited, severe dyserythropoiesis [13]. In addition to eliminating mRNAs containing PTCs generated either as a result of DNA mutations, or by errors that occur during gene expression or DNA metabolism, NMD also regulates the levels of specific 'normal' mRNAs. Inactivation of the NMD pathway has been shown to cause global changes in hundreds of transcripts from the normal repertoire of gene expression [14].

How is a PTC detected?

The arguments for nuclear vs. cytoplasmic NMD are discussed in detail later, and this section relates to the model of cytoplasmic NMD. Some confusion has been generated by the fact that mRNA can theoretically undergo cytoplasmic NMD while copurifying with nuclei. This is due to the fact that the 5′ end of some mRNAs can initiate translation in the cytoplasm, while the 3′ end is still in transit through the nuclear pore [15] (fig. 1), a phenomenon sometimes described as nucleus-associated NMD [16, 17]. It is interesting to note that 60% of mRNA degraded by the unrelated process of RNA interference (RNAi), a process that is totally restricted to the cytoplasm, also co-purifies with nuclei [18].

The current model proposes that following termination of the initial round of translation on newly synthesised mRNA, a posttermination scanning complex searches mRNAs for specific 'marker' proteins (discussed in detail below), and checks that there are no such marker proteins downstream of the termination codon. If, as in normal circumstances, this is the case, the mRNA is released into the translatable pool. Alternatively, if a specific marker protein is detected, the termination codon is deemed to be premature and the mRNA is degraded [19, 20] (fig. 1).

In mammalian cells, the 'marker' that distinguishes a PTC from an authentic stop codon is produced as a result of splicing [21–23]. Specifically, during splicing, a complex of proteins is deposited 20–24 nucleotides upstream of each exon-exon junction [24, 25]. Known as the exon junction complex (EJC), this complex includes proteins required for pre-mRNA splicing and mRNA export. The initial complex is sequentially modified to allow nuclear proteins to dissociate before mRNP export and proteins required for NMD to be recruited. Four proteins (RNPS1, Upf1, Upf2 and Upf3) have been shown experimentally to be capable of defining a termination codon as being premature when they are artificially tethered to the mRNA downstream of it, and are thus candidate 'marker' proteins [26, 27]. RNPS1 is a splicing factor that is part of the EJC [25] and serves as an anchorage point for the recruitment of Upf2 and Upf3 [28]. The protein Upf1 appears to link the termination of translation to the downstream protein complex, and is described in detail below. Essentially, following splicing, a protein complex including RNPS1 remains bound 20–24 nucleotides upstream of exon-exon junctions, and recruits other 'marker' proteins (Upf2 and Upf3) that serve to define any termination codon upstream of this as being premature. The modified EJC (i.e. the marker protein complex) is thought to be displaced during ribosomal translocation, such that if a PTC is not detected during the initial round of translation, the mRNA joins the cellular pool of mRNA (fig. 1).

What happens following detection of a PTC?

Termination of translation occurs following recognition of any of three stop codons by interaction of eukaryotic release factor 1 (eRF1) with the ribosome [29–32]. eRF1 catalyses peptidyl transfer RNA (tRNA) hydrolysis [33], and this activity is enhanced by the eRF1- and ribosome-dependent GTPase activity of eRF3 [34–36], which interacts with eRF1 to form a heterodimeric complex [35–39]. The presence of EJC proteins is probably

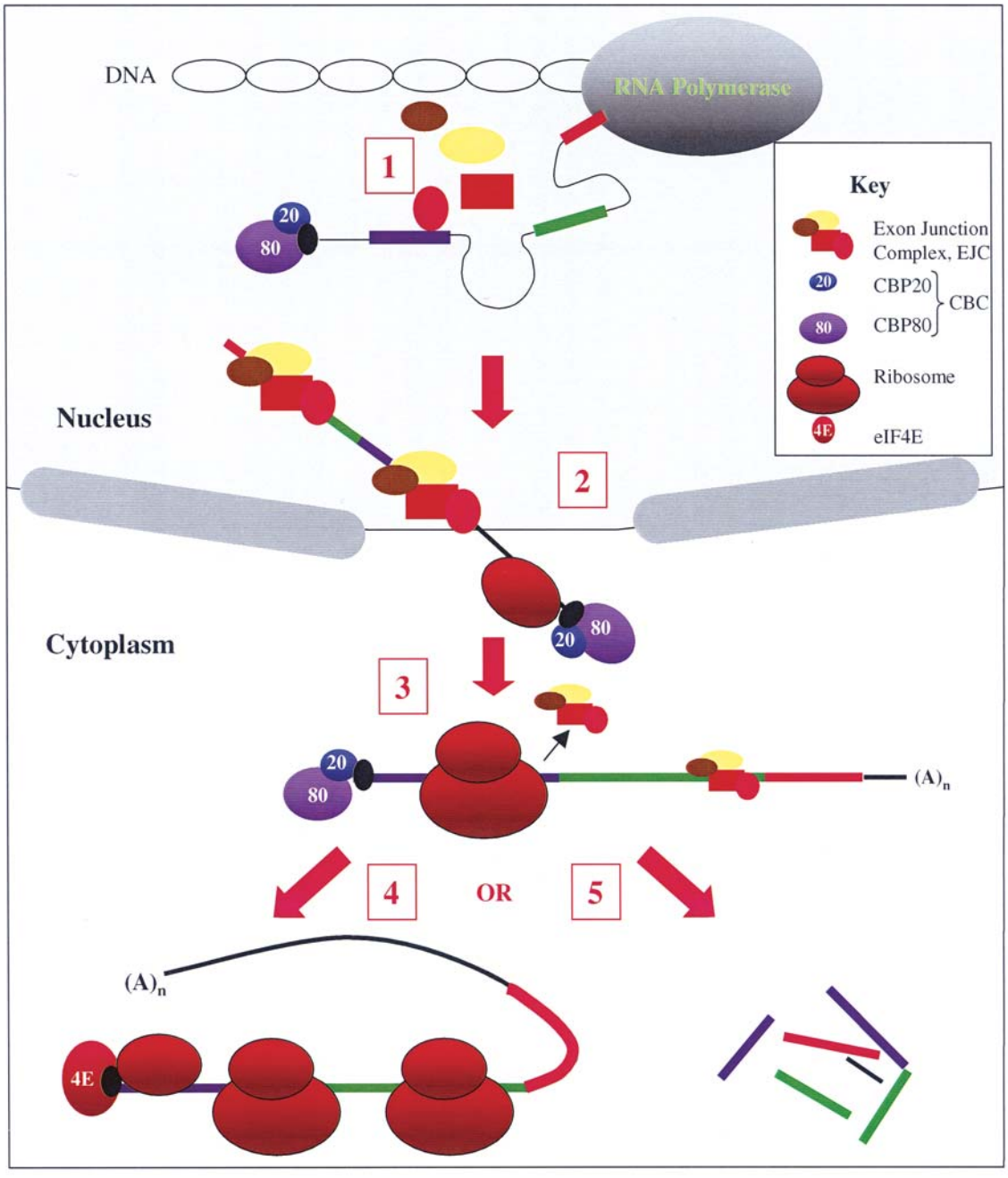

Figure 1. Illustration of the current model for cytoplasmic NMD in mammalian cells.
Step 1: Cotranscriptional processing of pre-mRNA, including splicing and association of EJC components. Step 2: Export of mRNA bound by CBC in a 5′–3′ direction, at which point CBC could initiate translation by recruiting the small ribosomal subunit. Step 3: Surveillance translation on mRNA still associated with CBC. During this round of translation, EJC components are displaced by the ribosome. Step 4: Following termination at an authentic stop codon, mRNA joins the translatable pool of cytoplasmic mRNA, and is bound at the 5′ cap by eIF4E. Step 5: Following termination at a PTC, mRNA is degraded.

relayed to the termination complex by the protein Upf1. Upf1 is a multidomain protein demonstrating RNA binding, RNA-dependent ATPase and RNA helicase activities [40–44]. It is associated with translating ribosomes in yeast [45–47] and mammalian cells [48], and interacts with release factors eRF1 and eRF3 [49] as well as Upf2 [50, 51] (fig. 2A).

The mechanism by which the aberrant mRNA is degraded is not yet known for mammalian cells. In yeast it occurs by deadenylation-independent decapping followed by 5′-3′ exonuclease activity. Recently, human Upf1 was shown to interact with human decapping enzymes, suggesting that degradation may occur by a similar mechanism in mammalian cells [52].

How does (and why should) initiation of surveillance translation differ from initiation of regular translation?

The mechanism of regular translation initiation

The majority of eukaryotic translation occurs in the cytoplasm and is thought to be initiated by recruitment of the 40S ribosomal subunit to the inverted and methylated guanosine cap structure at the 5′ end of the mRNA by eukaryotic translation initiation factors (eIFs), followed by scanning of the 5′-untranslated region (5′UTR) until the initiation codon, AUG, is recognised by Met-tRNA. At this point the large ribosomal subunit is recruited, and elongation occurs. Prior to mRNA recruitment, the 40S ribosomal subunit is primed to initiate translation by interaction with the 'ternary complex' (consisting of eIF2 bound to GTP and Met-tRNA) and with eIF3, resulting in the formation of the 43S preinitiation complex.

This complex is then recruited to the mRNA by two more initiation factors: eIF4G, a large, multidomain protein, often described as a scaffold protein, due to its interaction with many different initiation factors, and eIF4E, a protein that specifically recognises the modified 5′ 'cap' structure of mRNAs (fig. 2 B; reviewed in [53]).

Therefore, two features of eIF4E enable it to direct the 43S preinitiation complex to the 5′ end of mRNA: its ability to specifically recognise the cap and its interaction with eIF4G, which in turn binds eIF3. However, recent evidence suggests that eIF4E might not be unique in possessing these qualities [54–56] (fig. 2 C).

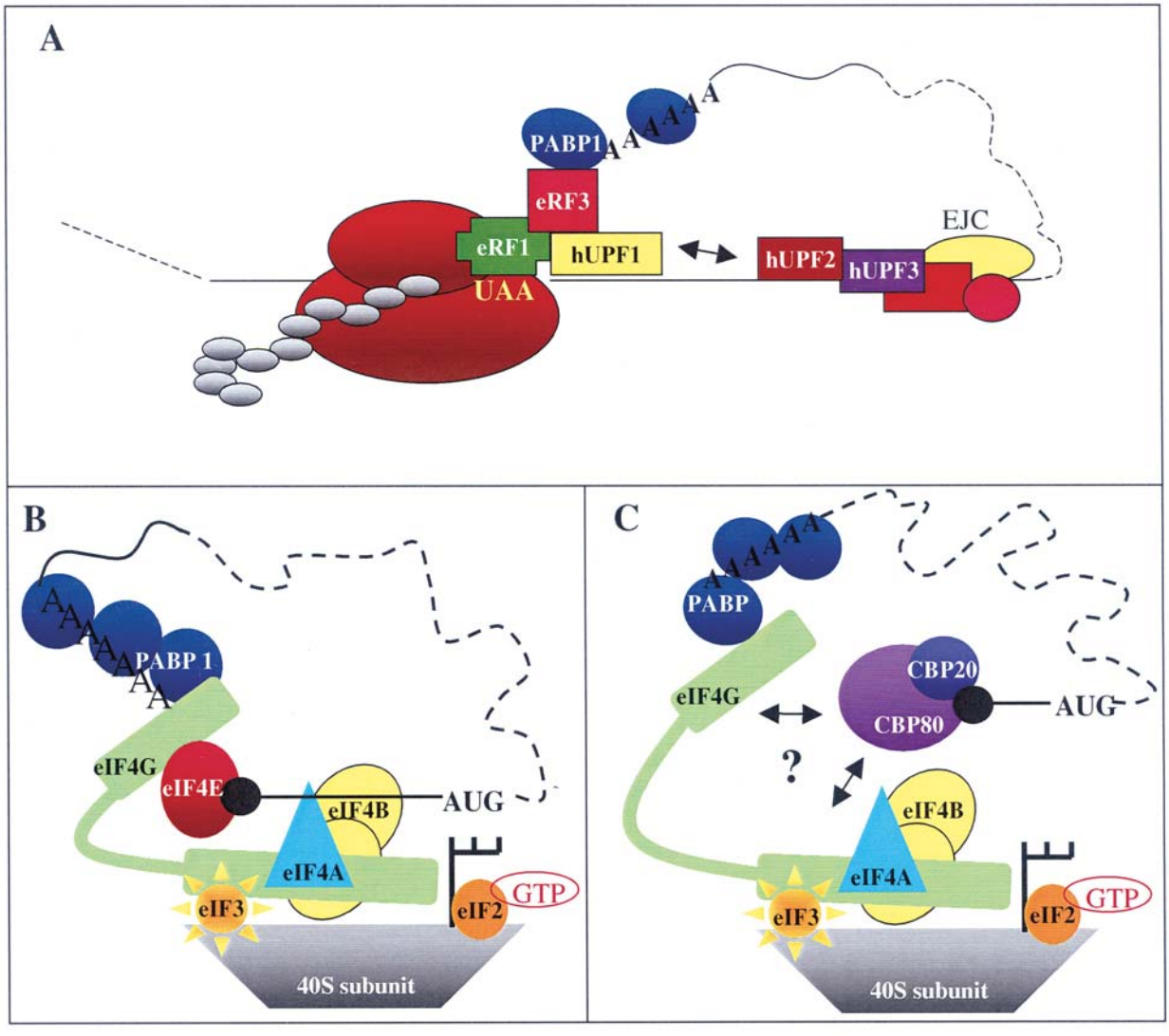

Figure 2. (*A*) Diagram of possible interactions during and post termination at a PTC. See main text for descriptions of proteins involved. (*B*) Diagrammatic representation of the 43S preinitiation complex promoting 'normal' translation of cytoplasmic mRNA. The 5′ cap structure is bound by eIF4E (red), and the poly (A) tail is bound by PABP1 (purple), and both proteins interact with eIF4G (green). (*C*) Possible interactions during initiation of surveillance translation.

Protein composition at the cap during the initial round of cytoplasmic translation

In the nucleus, the cap is initally bound by the predominantly nuclear cap binding complex, CBC, a heterodimer of 80- and 20-kDa subunits [57, 58] (CBP80 and CBP20; fig. 1), and is required for efficient splicing, polyadenylation and nuclear export of the RNA, as well as protection against exonucleolytic digestion [57–59]. Electron microscopy data indicate that for the Balbiani ring particle in salivary gland cells of *Chironomous tentans*, the CBC-mRNA complex is not only intact during export from the nucleus, but also maintained on ribosomes associated with the endoplasmic reticulum [15].

Further evidence that CBC may recruit the ribosome in an analogous manner to eIF4E has come from interaction studies in both yeast [54] and mammalian [56] cells, where CBC was found in complex with eIF4G (the translation initiation factor that links cap-binding protein eIF4E with the 43S preinitiation complex [53]). Yeast eIF4G and CBP80 were shown to directly interact, and when competition from eIF4E was removed by mutation of the eIF4E binding site on eIF4G, yeast CBC could support a limited amount of translation [54]. The most compelling evidence for the ability of CBC to support the initial round of cytoplasmic translation in mammalian cells comes from the demonstration that CBC-bound cytoplasmic mRNA was reduced when the mRNA has a premature termination codon [55]. Furthermore, this reduction in CBC-bound, PTC-containing mRNA was, like NMD itself, dependent on ongoing translation. In these experiments, mRNA associated with eIF4E was also reduced by the presence of a PTC [55]. This is consistent with two models: in model 1, eIF4E receives mRNA after it has undergone an initial round of translation in complex with CBC and thus receives a reduced amount of mRNA with a PTC; in model 2, the exchange of CBC for eIF4E does not require an initial round of translation. Instead, NMD occurs in parallel on mRNAs bound by either eIF4E or CBC, and with a similar efficiency of PTC detection and mRNA degradation in each case. A role for eIF4E in NMD cannot yet be excluded, but evidence that EJC components coimmunoprecipitate with CBC and not eIF4E [55, 60], favours the first model. Relevant to this discussion is the hypothesis that eIF4E may associate with and promote the export of specific mRNAs [61]. This appears to be the case for cyclin D1 [62, 63], but other pre-mRNAs do not detectably copurify with eIF4E, under conditions that allow copurification with CBC [60].

Why should the initiation of translation make a difference to termination?

A good question to consider at this stage is why should the factor bound to the mRNA cap during translation initiation have any influence on the events that follow termination? The true answer is not yet known, and it is also not known whether the same cap-binding protein that initiated translation still remains bound to the cap by the time the ribosome reaches the termination codon. It is known that the poly(A)-binding protein PABP1 interacts with initiation factors eIF4G [64] and eIF4B [65, 66], and by doing so is thought to promote the synergistic effect of having both a cap and a poly (A) tail on translational efficiency [53, 65–67]. The translation termination factor eRF3 also interacts with PABP1 [68] (fig. 2A), and so could relay information from the termination complex to the both ends of the mRNA and regulate the initiation of subsequent rounds of translation. This linkage becomes particularly important when the hypothesis is considered that decapping constitutes the initial step of mammalian PTC-containing mRNA degradation [52]. While decapping enzymes will remove the cap of mRNA, and expose the 5′ end to rapid degradation by exonucleases, their activity is blocked by interaction of eIF4E with the cap [69]. This suggests that prior to decapping, there needs to be a modification of the cap-binding proteins, such that the cap is exposed. This remodelling could be initiated by the protein-protein interactions mentioned above that link eRF3 to eIF4G. Therefore, it is possible that different cap-binding activities of CBC and eIF4E in response to signals from the termination complex may determine which protein allows an aberrant mRNA to be decapped and degraded.

Where in the cell does NMD occur?

The data presented above are all compatible with a model of NMD that occurs during the initial round of cytoplasmic translation, even though this might occur while mRNA is in transit through the nuclear pore. The argument that NMD is restricted to the cytoplasm is strengthened by experiments where translation and therefore NMD of a specific mRNA was able to be controlled by the regulated binding of iron regulatory protein (IRP) to a hairpin-forming element in the 5′UTR of the mRNA, called the IRE [70]. IRP was shown to be localised to the cytoplasm only and was able to stabilise reporter mRNA with a PTC suggesting that NMD was also localised to the cytoplasm [70]. One caveat to the method is that although IRP appeared to be restricted to the cytoplasm, it is technically difficult to disprove that there was not a small amount present in the nucleus also.

Also supporting cytoplasmic NMD is the localisation of Upf proteins that are essential for NMD [45, 71–73]. Although Upf3 is predominantly nuclear, export to the cytoplasm is required for NMD to occur [72, 73]. The cytoplasmic localisation of Upf1 and Upf2 is also compatible with NMD occurring during or shortly after nuclear ex-

port [45, 71, 72]. However, more recent evidence indicates that while the majority of Upf1 is cytoplasmic, it shuttles between cytoplasm and nucleus, such that it accumulates in the nucleus of cells treated with leptomycin B, an inhibitor of the nuclear export factor CRM1 (chromosomal region maintenance) [74]. This nuclear fraction of Upf1 appears to function in additional nuclear surveillance events (described below), although it may also function in intranuclear NMD [74].

One of the main arguments used to defend exclusively cytoplasmic NMD is the requirement for translation – an event considered to be exclusively cytoplasmic. Challenges to the exclusively cytoplasmic nature of translation (where isolated nuclei were demonstrated to incorporate radiolabelled amino acids into high molecular weight material) were not able to convince the sceptical scientific community that nuclear translation occurred (reviewed in [75]). However, a more recent publication by Iborra et al. has reopened the long-standing debate [76]. These researchers demonstrated translation within the nucleus of permeabilised cells and purified nuclei using suboptimal conditions that allowed only a few amino acids from biotinylated aminoacyl tRNA precursors to become incorporated into the ongoing elongation of nascent peptide chains [76]. The nuclear translation was found to be tightly coupled with transcription, and is supported by data showing the presence of ribosomes at the sites of transcription, the partial nuclear localisation of various translation factors as well as aminoacyl tRNA and the presence of polysomes in the nuclear fraction of *Dictyostelium* [61, 76–79].

Presumably the substrate for nuclear translation is fully spliced mRNA, although it might be possible for a ribosome to recognise and bind to sequences present in introns such that it could initiate translation downstream. However, this fanciful hypothesis would also require non-AUG initiation and for the ribosome to know which reading frame to choose, for which a likely mechanism cannot easily be invoked.

There are many questions that follow the demonstration of nuclear translation, not least whether it represents a nuclear mRNA proofreading step with a mechanism analogous to that described above. Either of the two models for NMD (intranuclear or cytoplasmic, but on nucleus-associated mRNA) could explain the apparent stability of cytoplasmic mRNA bearing a PTC, since both models predict that surveillance occurs during the initial round of translation, and that mRNA that escapes this proofreading-linked degradation is immune to detection thereafter. However, the demonstration that inhibition of mRNA export did not affect the extent of NMD in the nuclear fraction suggests that NMD can occur in the nucleus proper [80]. Perhaps the initial round of translation could occur in either location, giving the cell double protection against the production of truncated proteins.

Other nuclear phenomena resulting from PTCs

Due to the large amount of recombination and sequence alterations required to obtain immunoglobulin and T-cell-receptor diversity, these transcripts appear to respond particularly dramatically to the presence of PTCs (reviewed in [81]). A number of nuclear phenomena have been demonstrated in response to the presence of a PTC within the coding sequence of these genes, including accumulation of pre-mRNA at the sites of transcription and nonsense-mediated altered splicing (NAS; also observed for other RNAs) [82, 83]. The question considered here is whether these responses are due to translation or detection by other means, for example by disruption of sequences required for correct splicing. One approach to differentiating between these two explanations is by demonstrating that missense mutations do not give the same phenotype. However, certain missense mutations have been shown to be less efficient at disrupting exonic splice enhancers than PTCs [84], and this data has led to reevaluation of the requirement of nuclear translation for some examples of NAS (reviewed in [85]). An elegant way to demonstrate that it is the PTC itself and not the change in sequence that elicits the response is to create a PTC by causing a frameshift. This is achieved by inserting extra sequence (for example 10 bases) some distance upstream of the induced PTC and controlling for the effects of the insertion by repeating the experiment with an insertion that would not cause a frameshift (e.g. 9 bases). This approach has been successfully used for nonsense-mediated accumulation of pre-mRNA [82] and certain examples of NAS [85], and suggests that translation is probably responsible for the proofreading in these cases.

It is perhaps hard to envisage how the steric problem of translation and splicing occurring in cis are overcome, particularly when splicing is altered 5′ to the PTC. Perhaps the surveillance translation occurs in trans. There is little evidence regarding the precise mechanisms by which these processes occur, although the factor requirement appears to differ from those required for NMD [74].

Conclusion

In summary, there are a number of nuclear responses to the presence of a PTC, which appear to involve a nuclear translation event. In addition, aberrant mRNAs not detected in the nucleus have a further chance to be proofread during the initial round of cytoplasmic translation that may occur during mRNA export. The mechanism of ribosomal recruitment to the 5′ cap during these surveillance translation events may involve the predominantly nuclear cap-binding complex, CBC, although a role for the usual cap-binding translation factor, eIF4E, has not been ruled out. The precise mechanism by which

mammalian CBC recruits the ribosome, the efficiency of CBC-initiated translation and the mechanism for exchange of CBC for eIF4E remain exciting questions to be answered.

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