

Minireview

# Integration of splicing, transport and translation to achieve mRNA quality control by the nonsense-mediated decay pathway

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## Abstract

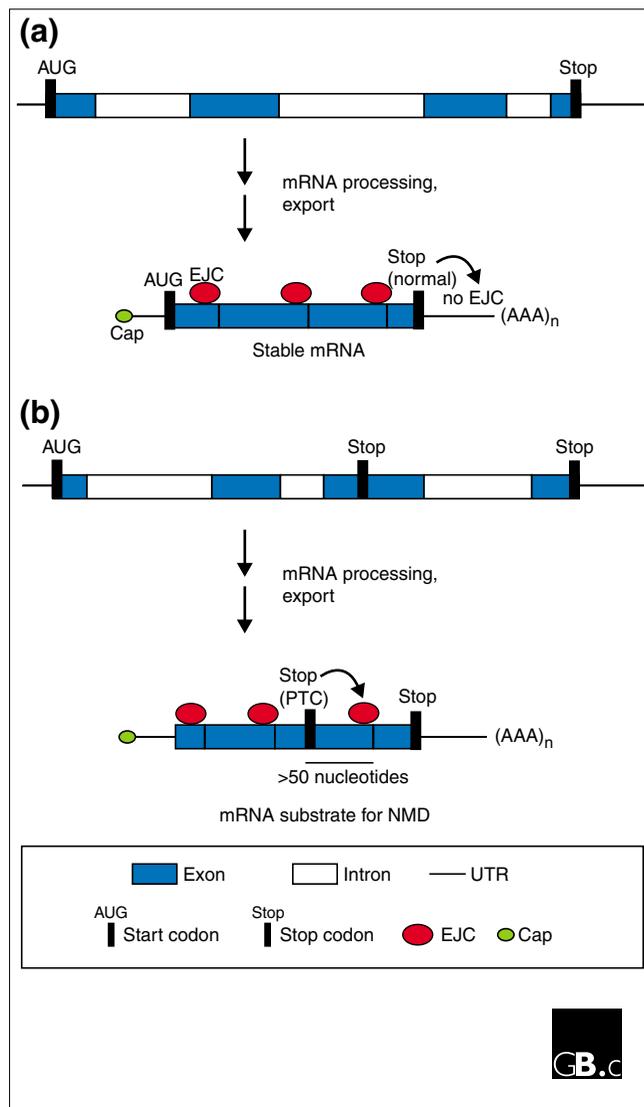
When pre-mRNAs are spliced, a multi-component complex is deposited onto them, close to the sites of intron removal. New findings suggest that these exon-exon junction complexes and the complexes that bind mRNA caps are key effectors of the fate of spliced mRNAs and may regulate whether mRNAs containing premature stop codons are degraded.

Nonsense-mediated mRNA decay (NMD) is a specific RNA-degradation process by which eukaryotic cells can eliminate mRNAs that contain premature translation-termination codons (PTCs). Such mRNAs encode truncated polypeptides that could, when translated, exert dominant-negative effects; it is therefore advantageous to cells to minimize translation of these mRNAs. NMD has been found in yeast, plants, nematodes, flies and vertebrates [1].

There are several examples that illustrate the medical importance of NMD. Most of the mutations that introduce a PTC into the human  $\beta$ -globin mRNA cause a form of  $\beta$ -thalassemia that is recessive; heterozygous carriers of these mutations are thought to be protected by degradation of the mutant mRNAs via NMD. A mutation that escapes NMD is associated with dominant  $\beta$ -thalassemia, however [2]. Similarly, certain PTCs in the human *ROR2* mRNA, which encodes an orphan receptor tyrosine kinase, fail to trigger NMD and cause a dominantly inherited form of brachydactyly type B [3]. Finally, in B and T lymphocytes, the somatic mutation and recombination events of the immune system frequently lead to the production of PTC-containing mRNAs. The NMD pathway is thought to prevent the expression of truncated immunoglobulin and T-cell-receptor polypeptides that would arise from such aberrant mRNAs [4].

Three proteins have been shown to be directly and specifically involved in NMD in *Saccharomyces cerevisiae*: Upf1p/Nam7p, Upf2p/Nmd2p and Upf3p (reviewed in [1,5]). Functional homologs of Upf1p, Upf2p and Upf3p have been described in humans and in other organisms, and the three Upf proteins can interact with each other [6-10]. Each of the mammalian Upf proteins has a characteristic subcellular localization. Human Upf1p (hUpf1p) is cytoplasmic and associated with polysomes [11]; most hUpf2p localizes to the nuclear periphery; and the two human Upf3p homologs (encoded by two genes, *hUpf3a* and *hUpf3b*) are shuttling proteins that are found predominantly in the nucleus [8-10]. The interaction of Upf1p with the translation-termination factors eRF1 and eRF3 [12] links NMD biochemically to the termination of translation.

A central question about the mechanism of NMD is how the cell distinguishes a premature from a normal stop codon (Figure 1). Remarkably, the position of introns seems to play a critical role in metazoans: a PTC is functionally defined as an in-frame stop codon that is located at least 50 nucleotides upstream of the last exon-exon junction in a spliced mRNA (see Figure 1; reviewed in [13,14]). Consequently, naturally intronless PTC-containing mRNAs are not usually destroyed by NMD [15,16], and transfection of PTC-containing cDNAs



**Figure 1**  
Specification of premature (versus normal) stop codons in mammalian cells. **(a)** In the nucleus, a normal intron-containing transcript is subjected to multiple modifications, such as splicing, addition of a <sup>7</sup>mGpppN cap and polyadenylation, to yield a mature mRNA. This mRNA can be translated after export to the cytoplasm. The exon-exon junction complex (EJC) is associated with the mRNA just upstream of each splice site. **(b)** If an mRNA carries a premature termination codon (PTC), however, it can be degraded by the NMD system. Note that the physiological stop codon has no EJC positioned downstream of it, whereas NMD-sensitive PTCs are usually followed by at least one EJC (curved arrows in (a,b)). The EJC is therefore proposed to play an important role in mRNA surveillance.

of human  $\beta$  globin yields ‘NMD immune’ mRNAs [17]. Furthermore, insertion of an intron more than 50 nucleotides downstream of a normal stop codon triggers NMD of an mRNA that in fact has an intact open reading frame [18,19]. As might be expected, it is unusual to find an intron in the 3’ untranslated region of an mRNA. When such introns do occur, they are almost always positioned less than 50

nucleotides downstream of the stop codon [20]. These observations collectively support a model in which splicing deposits a ‘mark’ at or near exon-exon junctions and thus tags the position of excised introns for later ‘inspection’ by the translation machinery. In yeast, in which only few genes have introns, a downstream sequence element seems to serve as such a positional mark [5,13,21]. When the mRNA first passes through the ribosome, NMD is thought to be triggered when the mark is encountered downstream from the position of translation termination [13,14,21] (Figure 1).

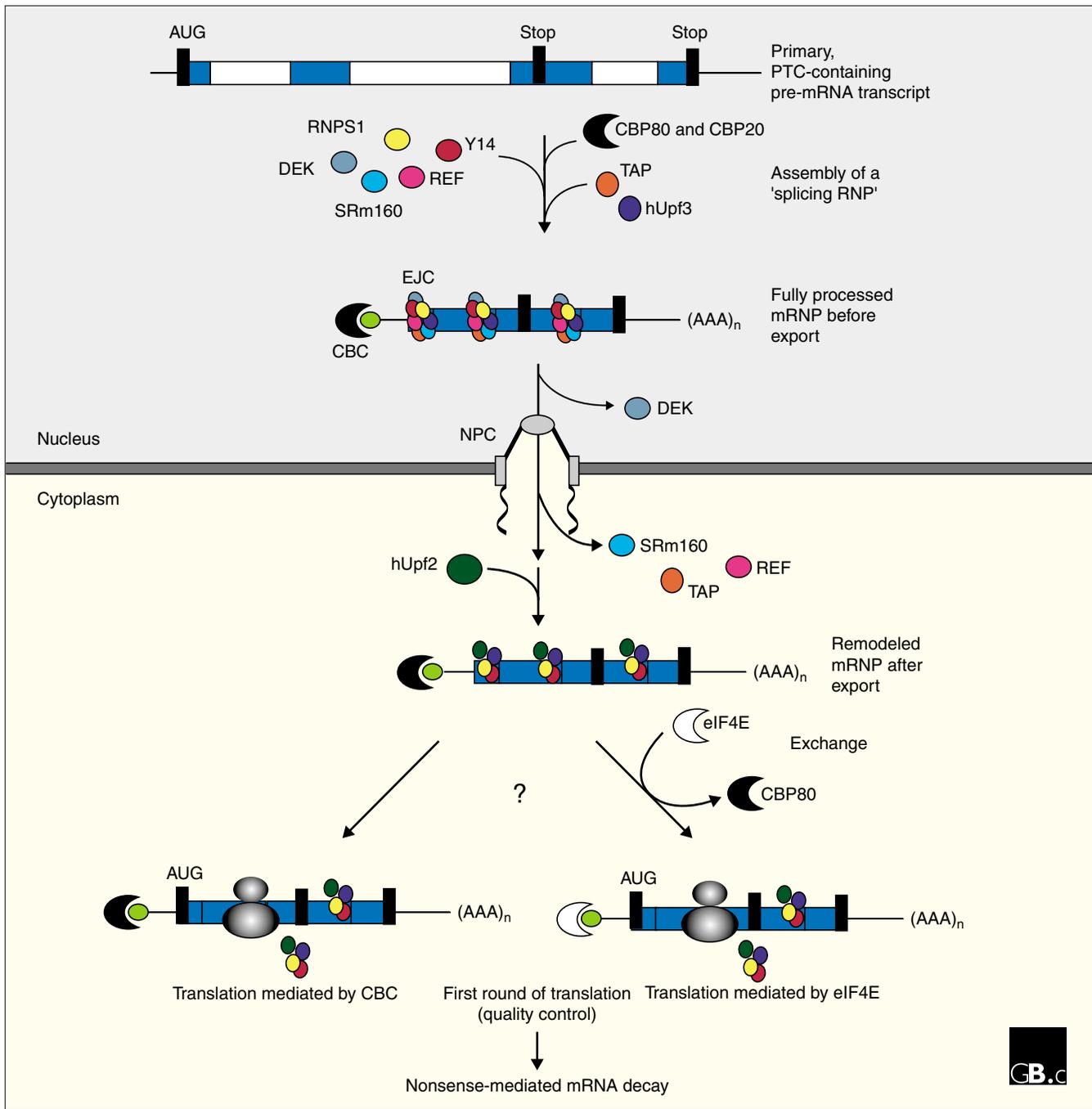
### Beyond a model: a multi-component exon-exon junction complex

Recent work has identified a multi-subunit complex that could serve as a mark of intron removal (Figure 2). This exon-junction complex (EJC) consists of at least five proteins: the splicing-associated factors SRm160, DEK and RNPS1, the mRNA export factor REF/Aly and the mRNA-shuttling protein Y14. It is deposited approximately 20 nucleotides upstream of exon-exon junctions [22] and at least part of it is exported together with the spliced RNA to the cytoplasm (see below). Using a shortened  $\beta$ -globin mRNA with a first exon of either 38 or 17 nucleotides ( $\beta/38$  or  $\beta/17$ , respectively) as a reporter, Le Hir *et al.* [23] showed that the  $\beta/38$  mRNA but not the  $\beta/17$  bound the EJC after splicing. Deposition of the EJC onto the mRNA was associated with a substantial increase in RNA export from the nucleus. This increase can be explained by the ability of the EJC to interact with the export factors REF/Aly and TAP/p15, which in turn interact with the nuclear pore complex (reviewed in [24]). As the EJC travels from the nucleus to the cytoplasm, its composition changes (Figure 2): only antibodies against SRm160 (weakly) and Y14 (strongly) co-immunoprecipitated spliced  $\beta/38$  mRNA from the cytoplasm, suggesting that DEK, RNPS1 and REF/Aly dissociate from the EJC before or soon after export [23].

### Does the exon-junction complex play a role in NMD?

Although it cannot yet be determined conclusively whether the EJC has a role in NMD, there are some strong clues that it might. As mentioned above, the Y14 and SRm160 proteins escort the spliced mRNA into the cytoplasm. Furthermore, recent reports implicate the EJC component RNPS1 in NMD [25] and show an association between the EJC and the NMD factors hUpf3 and hUpf2 [23,26].

Lykke-Andersen *et al.* [25] used a tethering approach to examine the role of the different components of the EJC in NMD. Using as a reporter gene a human  $\beta$ -globin mRNA with binding sites for the bacteriophage MS2 coat protein in its 3’ untranslated region (UTR), they examined whether EJC proteins fused to the MS2 coat protein could trigger NMD when the MS2 binding sites were located at an appropriate distance



**Figure 2**

A model of the changes in PTC-containing mRNA-protein complexes (mRNPs) during nuclear export. A PTC-containing transcript, depicted as in Figure 1, is capped, spliced and polyadenylated. Concomitantly, the cap-binding complex (CBC, consisting of CBP80 and CBP20), the exon junction complex (EJC, which consists of the DEK, REF/Aly, RNPS1, SRm160 and Y14 proteins), the NMD protein hUpf3 and the export factor TAP/p15 are all added, to give a fully processed mRNP. Before or soon after export from the nucleus through the nuclear pore complex (NPC), DEK, RNPS1 and REF/Aly dissociate from the EJC and hUpf2 binds. It is not clear whether the first round of translation can be mediated by CBC (left) or whether CBC is first exchanged for eIF4E. After this first round, the PTC-containing mRNA undergoes nonsense-mediated decay. See text for further details. Figure modified from [23,26].

(over 50 nucleotides) downstream of the stop codon. Like any of the hUpf proteins [9], when tethered in this manner RNPS1, and to a lesser extent also Y14, induced NMD of the

reporter  $\beta$ -globin mRNA in transfected HeLa cells (with RNPS1, 22-34% of the RNA remained compared with control RNA without binding sites; with Y14, 56-72% remained).

Importantly, this effect could be partially inhibited by a dominant-negative mutant of hUpf1. None of the other components of the EJC exhibited NMD activity in this assay. RNPS1 is known to shuttle between the nucleus and the cytoplasm of HEK293 human embryonic kidney cells, and thus could contribute to NMD in both subcellular locations.

When the EJC components were tagged individually, only RNPS1 could co-immunoprecipitate hUpf1, hUpf2 and hUpf3 from lysates of transfected HEK293 cells; this result suggests that RNPS1 is the component of the EJC that interacts with the NMD factors [25]. On the other hand, Kim *et al.* [26] found using a tag-specific antibody that tagged hUpf3a or hUpf3b could co-immunoprecipitate Y14. Furthermore, recombinant Y14 could bind hUpf3a or hUpf3b translated *in vitro*. Although these experiments suggest a direct interaction between Y14 and hUpf3a/b, the possibility that proteins (such as RNPS1) present in the *in vitro* translation reactions could mediate the interaction cannot be excluded. In support of the hypothesis that hUpf3 is a *bona fide* component of the EJC at post-splicing stages of mRNA maturation [9,23], Kim *et al.* [26] showed that FLAG-tagged hUpf3b stably associated with spliced mRNA at a position similar to that of the EJC. Taken together, the experiments of Lykke-Andersen *et al.* [25] and Kim *et al.* [26] provide biochemical support for the idea that the EJC subunits RNPS1 and/or Y14 are involved in NMD by providing a bridge between the splicing 'mark' and the Upf proteins (Figure 2), which in turn are linked to translation termination.

### Translation and NMD

For NMD to protect the cell from aberrant proteins, it is crucial to destroy PTC-containing mRNAs as soon as possible and before multiple truncated polypeptides have been translated. Could this destruction happen as early as when the mRNA is still in the nucleus? Although the possibility of 'nuclear translation' has recently received renewed attention [27,28], cytoplasmic translation appears to be required for NMD [13,14,18,21]; the first round of translation, that is, the first time that the mRNA passes through the ribosome, is considered to be particularly important [29]. What distinguishes the initial round from subsequent rounds of translation? Conceivably, NMD could take place while the mRNA is being exported from the nucleus. During this phase, nuclear factors bound to the mRNA could be removed and potentially replaced by cytosolic proteins. Is this initial round of translation initiated by the canonical translation initiation factors or does it involve any special translation initiation factors?

In the nucleus, the 7mGpppN cap structure of mRNAs is bound by a heterodimeric cap-binding complex (CBC, consisting of CBP20 and CBP80), which can affect splicing and 3' end processing [30]. It appears that mRNAs are exported with their 5' ends first, exposing the CBC to the cytoplasm early in the transport process [31]. The initiation factor

eIF4E binds the cap structure in the cytoplasm, displacing the CBC, and mediates translation by recruiting eIF4G, other translation factors and ultimately the ribosome. This raises the question of whether the CBC and the cytoplasmic eIF4E exchange before, during or after the initial round of translation (Figure 2). Notably, both yeast and mammalian CBCs can, like eIF4E, interact with eIF4G (via their CBP80 subunits) [32,33], and the yeast CBC has been shown to be able to support cell-free translation, albeit quite inefficiently [32].

Recently, Ishigaki *et al.* [34] proposed that a "pioneer round of translation" may be initiated while the mRNA is still associated with the CBC, and that this could act to survey the mRNA for the presence of PTCs. Two versions of a human  $\beta$ -globin mRNA served as a reporter system for the experiments of Ishigaki *et al.*: a  $\beta$ -globin mRNA with a PTC (' $\beta$ -globin PTC') and one without (' $\beta$ -globin normal') [34]. Following immunoprecipitation with antibodies against CBP80, the level of co-immunoprecipitated  $\beta$ -globin PTC RNA was reduced to 20% of the level of  $\beta$ -globin normal RNA. This reduction quantitatively reflects NMD of the  $\beta$ -globin PTC RNA in the total cellular RNA fraction, and corresponds to the level of reduction in the  $\beta$ -globin PTC RNA (15%) that was precipitable with antibodies to eIF4E. Thus, the authors suggest that surveillance and NMD of  $\beta$ -globin PTC take place while the mRNA is associated with CBP80 and before the remaining intact RNA is handed over to eIF4E [34].

Ishigaki *et al.* [34] also showed that hUpf2, hUpf3 (but not hUpf1) and the nuclear poly(A)-binding protein (PABP2) co-immunoprecipitated with anti-CBP80 antibodies in an RNA-dependent manner, whereas complexes precipitated with the anti-eIF4E antibody did not contain any of these proteins. Given that hUpf2 and hUpf3 are involved in NMD, these results can be interpreted to suggest that NMD occurs while the mRNA is still bound by CBP80, but not detectably when the RNA is bound to eIF4E [34].

Do the majority of mRNAs undergo the initial round of translation without the support of eIF4E, which was thought to be a very important translation initiation factor? This idea offers a very interesting and unexpected twist to our perception of the translation and NMD pathway, and will surely stimulate many interesting future experiments. Before accepting the notion of a 'pioneer round' of mRNA translation under the guidance of CBP80 as a general aspect of mRNA export, however, some additional considerations may be warranted. At least *in vitro*, the CBC is an ineffective translation-initiation factor compared with eIF4E [32]. Moreover, a yeast strain with the *CBP80* gene deleted does not display any overt translational defects nor any lack of NMD [35]; CBP80 is thus not essential either for NMD or for translation in yeast. The function of the CBC in mammalian cells may differ from its functions in yeast, however, and/or it may act as a more efficient translation factor *in vivo* than

*in vitro*. The finding that the amount of  $\beta$ -globin PTC mRNA associated with CBP80 was reduced to 20% of the level of  $\beta$ -globin normal mRNA [34] certainly suggests that translation and NMD can occur before CBC has been exchanged for eIF4E. Only about 15%-30% of the CBC and eIF4E proteins and their associated RNAs from the extract was accessible to immunoprecipitation [34], however, and we therefore do not know about the mRNAs associated with the other 70-85% of these proteins. Comparisons of mRNA levels were made between PTC-containing and wild-type RNAs each co-immunoprecipitated with either CBP80 or eIF4E, but we do not know the percentage of the  $\beta$ -globin PTC and  $\beta$ -globin normal mRNAs present in the cells that was analyzed [34]. Thus, we do not yet know to what extent the analyzed mRNA-protein complexes (mRNPs) are representative of the majority of mRNAs being exported from the nucleus and being surveyed for NMD. Undoubtedly, future experiments will address this question as well as some of the intriguing implications of this work.

From a broader perspective, the recent progress highlights the dynamic nature of mRNP remodeling during mRNA processing, export, translation and degradation. We are on the way to a better understanding of how the different levels of gene expression are integrated and coordinated, how they affect and influence each other, and what the players and mechanisms are at the biochemical level.

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