New insights into the formation of active nonsense-mediated decay complexes

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In the nonsense-mediated mRNA decay (NMD) pathway, an exon-junction protein complex (EJC) and hUpf proteins mediate rapid downregulation of aberrant mRNAs that terminate translation upstream of the last splice junction. Two EJC subunits, Y14 and RNPS1, have been proposed to act as a link between splicing and NMD by recruiting hUpf3 and the other hUpf proteins. New studies now present evidence that Y14 is directly involved in NMD, and that Y14 is required for hUpf3 activity. These findings suggest unforeseen intricacies in the formation of active NMD complexes.

In eukaryotes, an mRNA undergoes several processing steps before it can be translated into the corresponding protein. Numerous mechanisms mediated by the eukaryotic gene expression machinery ensure that only properly processed mRNAs that are capable of encoding functional polypeptides are translated. One such mechanism, the nonsense-mediated mRNA decay (NMD) pathway, is a post-transcriptional process that rapidly degrades mRNAs with premature translation termination codons. This limits the expression of truncated polypeptides. The NMD pathway is initiated during translation when the responsible proteins discriminate premature from normal translation termination and mark the transcript for rapid decay.

In human NMD, human Upf (hUpf) proteins cooperate with an exon-junction complex (EJC) to identify mRNAs with premature stop codons [1–3]. The EJC consists of multiple proteins that are deposited 20–24 nucleotides upstream of exon–exon junctions after splicing [4]. Two components of the EJC – RNPS1 and Y14 – have been proposed to recruit hUpf proteins to trigger NMD [1–3]. New studies now provide evidence that Y14 is required for NMD, and that Y14 might have a role beyond simply recruiting hUpf proteins to the EJC [5]. This indicates an unexpected complexity in the assembly of active NMD complexes.

Normal or premature translation termination: the basis of discrimination

Even before the identification of molecular players involved in NMD it was known that, in mammals, the distinction between a normal and a premature translation stop codon is made on the basis of its location with respect to the last exon–exon junction: if the termination codon is positioned >50–55 nucleotides upstream it is considered premature, and the mRNA is targeted for rapid decay [6–9]. How does the position of an intron influence translation termination and susceptibility of an mRNA to undergo NMD? It was hypothesized that splicing would leave a mark at exon–exon junctions, which would help differentiate between normal and premature termination events [7,9,10]. The discovery of the multi-protein EJC deposited upstream of exon–exon junctions provided a rationale for this enigmatic premise [4] (Fig. 1). Y14 and

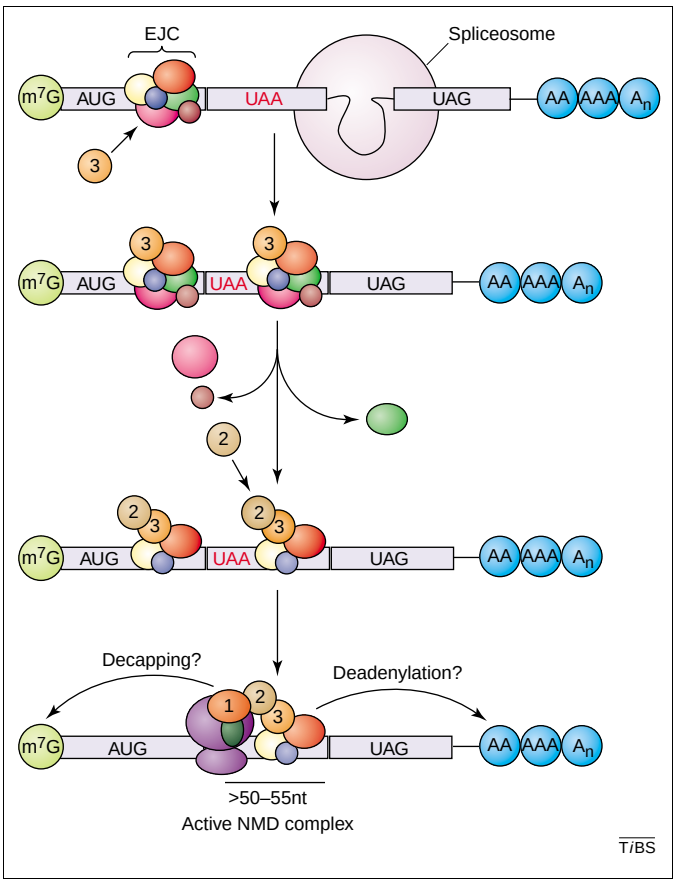

Fig. 1. A model for the nonsense-mediated mRNA decay (NMD) pathway based on the current evidence. A multi-protein exon-junction complex (EJC) is deposited 20–24 nucleotides (nt) upstream of each exon–exon junction after splicing. Y14 and RNPS1 recruit hUpf3 to the EJC followed by hUpf2. Some proteins are believed to leave the EJC before translation. The first translation event removes the EJCs from the mRNA. If translation terminates >50–55 nt upstream of the last exon–exon junction, one or more EJCs remain associated downstream of the termination complex. hUpf1, which could be recruited to the mRNA either by translation release factors or hUpf2, might bridge the terminated ribosome and the downstream EJC to form an active NMD complex that triggers rapid decay of the mRNA. The Y14–Magoh heterodimer and RNPS1 might form a part of this active NMD complex, whereas the role of the other EJC proteins is currently unknown. The components of the EJC are colored as follows: SRm160, pink; Y14–Magoh, yellow and dark blue; Aly/REF, green; RNPS1, red; UAP56, dark brown. The hUpf proteins hUpf1, hUpf2, and hUpf3a and 3b are labeled 1, 2 and 3, respectively. The translation apparatus consists of the cap-binding complex (light green), the ribosome (purple), poly(A)-binding protein (PABP; light blue) and release factors (dark green).

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RNPS1 were subsequently demonstrated to associate with the Upf proteins, which are the effector proteins of NMD [1–3,11]. This evidence provided a link between splicing and NMD.

The Upf proteins and a current model of the NMD pathway

First identified in genetic screens in *Saccharomyces cerevisiae*, the Upf proteins directly mediate the process of NMD [12,13]. In humans, four Upf proteins exist: hUpf1, hUpf2, hUpf3a and hUpf3b [14–16]. The proteins hUpf3a and hUpf3b are highly similar and probably functionally redundant. Several studies have implicated the hUpf proteins in NMD in cultured human cells: the expression of a dominant negative mutant hUpf1 protein, depletion of hUpf1 and hUpf2 by RNA interference or expression of hUpf2 antisense RNA all inhibit NMD [17–19]. The hUpf proteins exist in a complex in human cell extracts but, interestingly, localize to distinct subcellular regions [14]. It is intriguing how these proteins come together to form a complex on an mRNA targeted for NMD, which, in itself, is an indication of the dynamic nature of the formation of active NMD complexes.

According to a current view of the NMD pathway (Fig. 1), the formation of active NMD complexes on a human mRNA is initiated by the deposition of the EJC upstream of exon–exon junctions after splicing in the nucleus. Next hUpf3a or hUpf3b and then hUpf2 is recruited to form an NMD-competent EJC. This complex is believed to form on all mRNAs that undergo splicing [1,3,4,11]. In case of an mRNA with a natural stop codon, which is most often present in the last exon [9], the first translating ribosome will displace the complexes assembled at the exon–exon junction [20]. When translation terminates prematurely, >50–55 nucleotides upstream of the last exon–exon junction, at least one EJC remains bound to the mRNA. This is believed to lead to the recruitment and activation of hUpf1 via its interactions with hUpf2 and translation release factors eRF1 and eRF3 [14,16,21]. The activation of hUpf1, which involves phosphorylation by the kinase hSmg-1 [22–24], triggers NMD. At present, it is unclear how the activation of hUpf1 subsequently leads to stimulation of the mRNA decay machinery.

The formation of active NMD complexes

Much of the data supporting the current model of the NMD pathway has been obtained by tethering assays, where specific NMD components have been fused to an RNA binding MS2 coat protein and tethered to MS2 coat protein-binding sites in an mRNA downstream of the normal termination codon (Fig. 2). This is thought to directly nucleate the formation of active NMD complexes on the mRNA and, thereby, bypass the requirement for splicing. Thus, each of the four hUpf proteins induce NMD when tethered to the 3′ untranslated region (UTR) of β-globin mRNA [14]. When individual EJC components, RNPS1, Y14, SRm160 and Aly/REF were tethered to the 3′ UTR of β-globin mRNA using MS2 coat protein fusions, only RNPS1 triggered robust NMD [1]. By contrast, Y14 triggered only modest NMD in these assays [1], even though its interaction with hUpf3a or hUpf3b *in vitro* suggests that Y14 plays a role in NMD [2].

Now, Gehring *et al.* have used a different RNA-binding protein, the λN protein, and its corresponding RNA binding site to show that tethered Y14 can also trigger robust NMD [5]. Importantly, NMD triggered by tethered Y14 depends on the hUpf1 and hUpf2 proteins. In addition, depletion of Y14 by RNA interference inhibits NMD of β-globin mRNA with a premature stop codon, which directly implicates Y14 in NMD [5]. Gehring *et al.* also discovered a conserved domain in the C terminus of hUpf3b, which is required for the association with Y14. Deletion or a single amino-acid substitution within this domain results in disruption of the hUpf3b–Y14 complex and disables the ability of hUpf3b to induce NMD when tethered to an mRNA [5]. This domain might be an interaction

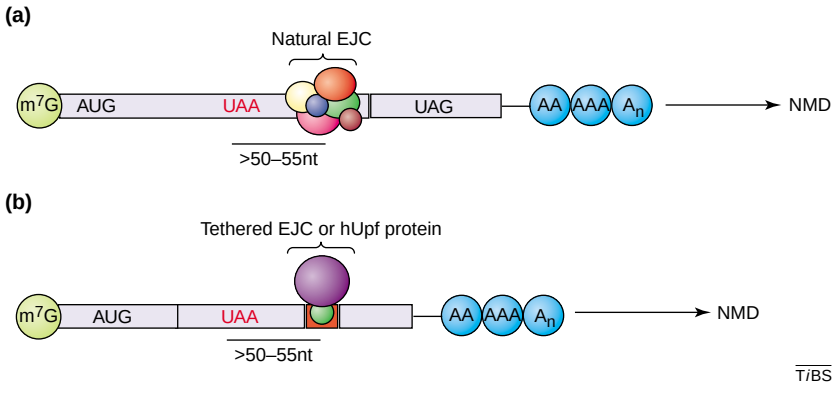

Fig. 2. The tethering approach to recapitulate nonsense-mediated mRNA decay (NMD). (a) The presence of an exon-junction complex >50–55 nucleotides (nt) downstream of the translation stop codon acts as a trigger for natural NMD. (b) When Y14 is fused to a λN RNA-binding protein (green) and tethered to the corresponding λN RNA-binding sites (red) on an mRNA downstream of the natural stop codon, the tethered protein nucleates the formation of active NMD complexes and triggers decay [5]. A similar approach using MS2 coat protein and its corresponding RNA-binding sites has previously implicated RNPS1 and the human Upf (hUpf) proteins in NMD [1,14]. Although multiple RNA-binding sites are used in these assays, only one site is shown in this simplified illustration.

interface between hUpf3b and Y14, although it cannot be ruled out that it mediates the interaction with Y14 indirectly via other EJC proteins. Does the C-terminal domain of hUpf3b play a similar important role in natural NMD, which is not initiated from tethered proteins? This question can now be tested by replacing endogenous hUpf3b with mutant hUpf3b.

The present data collectively suggest that the NMD complexes are built around the RNPS1–Y14 core. But to what extent are these EJC proteins required for the formation of active NMD complexes? Their role might be limited to nucleating the formation of the hUpf complex. Alternatively, as part of the final active NMD complexes, they may play a more direct role in destabilizing the transcript. We do not yet know the answers to these questions; however, the studies by Gehring *et al.* show that Y14 is required for NMD triggered by tethered hUpf3b [5]. This observation suggests that the role of Y14 is not restricted to just recruiting hUpf3a or hUpf3b, in which case, NMD triggered by tethered hUpf3b should not depend on Y14. Therefore, Y14 might, in addition, be required at a later step in the NMD pathway.

Concluding remarks

Although the findings by Gehring *et al.* contribute significantly to our understanding of the NMD pathway in human cells, several important questions about the formation of active NMD complexes remain to be addressed. For example, what are the roles of the EJC subunits other than Y14 in NMD? This can now be tested in depletion studies similar to those done for Y14 by Gehring *et al.* Such experiments should help test the basis of the curious, albeit negative, results that other EJC subunits such as Aly/REF and SRm160 do not trigger tethered NMD [1]. If RNPS1 and other EJC proteins are required for NMD, do they have a role beyond the nucleation of the hUpf complex? In addition, the order of assembly of different subunits into active NMD complexes remains largely unknown. Also, once assembled, how does the active NMD complex stimulate the RNA degradation machinery? Future studies should answer these unexplored questions and lead to a better understanding of the molecular mechanism behind the enigmatic NMD machinery.

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