COMMENTARY

Nonsense-mediated decay breaks the circle?

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This Commentary discusses new data from the laboratory of Matthias Hentze (Schell et al., in this issue) that begins to dissect the RNA–protein and protein–protein interactions important for the process of nonsense-mediated decay (NMD). Schell and co-workers have developed a novel tool for analysis of such interactions *in vivo*. Using a proteomics approach, they have identified two different protein–RNA complexes that contain

human Upf (up-frameshift) proteins central to the NMD process. Intriguingly, they identified a poly[A]-binding protein associated with these complexes.

Key words: nonsense-mediated decay, RNA–protein interactions, up-frameshift (Upf) protein, poly[A]-binding protein.

In all cells, accuracy of gene expression is vital for proper growth. Usually, RNAs transcribed from mutant alleles or resulting from defective processing events in the nucleus are rapidly degraded to ensure that this is so. This is particularly important for mutations that give rise to a termination codon upstream of the normal stop codon, as translation would yield a C-terminal truncated polypeptide. Such polypeptides can often act in a dominant-negative manner, leading to deleterious effects on the cell or organism. Fortunately, the eukaryotic cell has in place a surveillance mechanism, the nonsense-mediated decay (NMD) pathway, to ensure that only error-free mRNAs are accurately translated [1].

How does NMD work? In the nucleus, most pre-mRNAs are processed by capping, splicing and polyadenylation in large multiprotein complexes (messenger ribonucleoprotein particles). Once introns have been removed from pre-mRNAs during splicing, some components of these complexes remain in association with the mature transcript, 20–24 nucleotides upstream of exon–exon junctions, and accompany it during export from the nucleus and loading on to the polysomes for translation [2]. The complex involved in this post-splicing tagging of mRNAs is termed the exon junction complex (EJC). The 3' ends of transcripts should not be associated with EJCs, as 3' terminal exons are always downstream of exon–exon junctions. This is important for distinguishing between transcript-internal premature termination codons (PTC) and the normal translation stop codon that resides within the 3' terminal exon. The rule appears to be that any stop codon followed by a downstream intron comprising 50 or more nucleotides is tagged as a PTC through cross-talk between components of the EJC and the NMD process [3]. In support of this model, experiments where an intron was inserted downstream of a normal stop codon in a 3' terminal exon resulted in the mRNA being targeted for NMD. Most mammalian mRNAs are probably targeted for NMD while still associated with the nucleus. For an mRNA to be marked for NMD, one initial round of translation is required. If the transcript is recognized as full-length and error-free, then the messenger ribonucleoprotein particle is redesigned so that those factors that could tag the RNA for NMD are lost and further translation can proceed. It seems likely that this initial round of translation occurs in the nucleus or at least is associated with the nucleus, for example, coupled to transcript export [4].

Three yeast proteins Upf1, Upf2 and Upf3 (up-frameshift mutants) form a complex that is important for efficient translation

termination and interact with the termination factors eRF1 and eRF3 [5]. However, Upf proteins also recruit an mRNA surveillance complex in concert with engagement of the translation termination machinery on the ribosome. For the human Upf orthologues, hUpf3 is predominantly nuclear, but shuttles continuously between the nucleus and cytoplasm, whereas hUpf2 is perinuclear and hUpf1 is a cytoplasmic protein. It has been proposed that hUpf3 interacts first with the EJC in the nucleus and recruits hUpf2 and hUpf1 sequentially upon nuclear export. In yeast and man, Upf1 is a key player in NMD, as it can bind RNA, carry out RNA-dependent ATP binding and hydrolysis and act as a 5'-to-3' ATP-dependent RNA helicase. In yeast, Upf2 and Upf3 can regulate the activities of Upf1, and Upf3 may interact directly with components of the EJC. Upf2 can bind to Upf1 along with the termination factors eRF1 and eRF3, and terminate translations, leading to decapping and mRNA decay from the 5' end. Linking any one of these proteins to the 3' UTR of β -globin mRNA leads to recognition of the true termination codon as a PTC and degradation by the NMD pathway [6].

As reported in this issue of the *Biochemical Journal*, Schell et al. [7] have made a significant technical advance in this complex field by generating a transgenic HeLa cell line stably expressing an affinity-tagged form of hUpf1. They carefully demonstrate that the tagged protein has very similar functional and biochemical properties to the untagged protein, and that there is no detectable difference in the subcellular localization of the tagged and untagged version. As expected, hUpf1 interacts with hUpf2 and the two hUpf3 proteins (hUpf3a and hUpf3b) that are present in HeLa cells. Next, they use the tagged protein to isolate protein complexes to which hUpf1 contributes in the cell, and components of the complexes are identified by an elegant proteomics approach. The data indicate that hUpf1 exists largely as a monomer and is present in a very large complex of approx. 1–1.3 MDa, and a smaller complex of approx. 400–600 kDa. The larger complex contains, among other proteins, hUpf2 and hUpf3aL (a long isoform of hUpf3a), whereas the smaller complex contains hUpf3aS (a short isoform of hUpf3a) but no hUpf2. Technical problems ruled out the possibility of using co-immunoprecipitation to confirm any direct interaction between hUpf1 and the other hUpf proteins in the complexes. However, the approach *in vivo* makes it likely that these proteins co-exist in some sort of complex. The discovery of two distinct

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complexes containing either the short or long version of hUpf3a could indicate that the protein variants perform different functions in NMD. One of the most intriguing findings is that the smaller complex did not contain hUpf2, but still managed to recruit hUpf1. Either it is possible for hUpf3 to recruit hUpf1 directly or hUpf2 can dissociate from the complex once it has recruited hUpf1. The next step will be to identify what targets the proteins to these different complexes. One possibility is the differential phosphorylation states of the proteins that are mediated by human homologues of the SMG (suppressor with morphogenetic effect on genitalia) proteins first isolated from *Caenorhabditis elegans* [5].

A key issue for NMD is its proposed functional association with the EJC. It would make a lot of sense for these complexes to interact with one another. However, this elegant approach *in vivo* has failed to identify key components of the EJC in the hUpf1-containing complexes. As found in another study [8], hUpf1 does not co-immunoprecipitate EJC-complexed mRNAs, and this protein may not be directly involved in the association between the EJC and NMD.

Finally, it is surprising that poly[A]-binding protein (PABP) is one of the main proteins pulled out by hUpf1. Addition of up to approx. 200 A residues to the 3' end of a transcript is an important step in its maturation, and serves to protect the mature mRNA from degradation, facilitate its export from the nucleus and ensure its efficient translation. PABP binds the nascent poly[A] tail and aids progressive polyadenylation. Recently, PABP has been shown to interact with eIF4G (eukaryotic initiation factor 4G), one component of the cap-binding complex that serves to optimize loading of the ribosome on to the 5' end of the transcript. This interaction between proteins at the 5' and 3' ends of the message circularizes the template, and is proposed to protect the cap to

preclude 5'-to-3' exonuclease activity and increase efficiency of successive rounds of translation. Does the interaction of PABP and hUpf1 have a functional role in NMD? One interesting possibility is that any sequestration of PABP in the hUpf1 NMD complex could abrogate its role in mediating mRNA stability, leading to degradation of aberrant transcripts. Clearly this hypothesis requires functional testing in future.

REFERENCES

- 1 Henzle, M. and Kulozik, A. E. (1999) A perfect message: RNA surveillance and nonsense-mediated decay. *Cell* (Cambridge, Mass.) **96**, 307–310
- 2 Le Hir, H., Izaurralde, E., Maquat, L. E. and Moore, M. J. (2001) The spliceosome deposits multiple proteins 20–24 nucleotides upstream of mRNA exon–exon junctions. *EMBO J.* **19**, 6860–6869
- 3 Thermann, R., Neu-Yilik, G., Deters, A., Frede, U., Wehr, K., Hagemeier, C., Hentze, M. and Kulozik, A. E. (1998) Binary specification of nonsense codons by splicing and cytoplasmic translation. *EMBO J.* **17**, 3848–3494
- 4 Muhlemann, O., Mock-Casagrande, C. S., Wang, J., Li, S., Custodio, N., Carmo-Fonseca, M., Wilkinson, M. G. and Moore, M. J. (2001) Precursor RNAs harbouring nonsense codons accumulate near the site of transcription. *Mol. Cell* **8**, 33–43
- 5 Culbertson, M. R. and Leeds, P. F. (2003) Looking at mRNA decay pathways through the window of molecular evolution. *Curr. Opin. Genet. Dev.* **13**, 207–214
- 6 Lyyke-Andersen, J., Shu, M. D. and Steitz, J. A. (2000) Human Upf proteins target an mRNA for nonsense-mediated decay when bound downstream of a termination codon. *Cell* (Cambridge, Mass.) **103**, 209–223
- 7 Schell, T., Kocher, T., Wilm, M., Seraphin, B., Kulozik, A. E. and Hentze, M. W. (2003) Complexes between the nonsense-mediated decay (NMD) pathway factor Hupf1 and essential NMD factors in HeLa cells. *Biochem. J.* **373**, 775–783
- 8 Le Hir, H., Gattfield, D., Izaurralde, E. and Moore, M. J. (2001) The exon–exon junction complex provides a binding platform for factors involved in mRNA export and nonsense-mediated decay. *EMBO J.* **20**, 4987–4997

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