UPF1 P-body localization

Saverio Brogna1, Preethi Ramanathan and Jikai Wen
School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

Abstract

NMD (nonsense-mediated mRNA decay) is a mechanism that degrades transcripts containing PTCs (premature translation termination codons). NMD is a translation-associated process that is expected to take place throughout the cytoplasm. However, recent studies have indicated that the core NMD factors UPF1 (up-frameshift-1), UPF2 and UPF3 can associate with P-bodies (processing bodies), which are large cytoplasmic granules replete with proteins involved in general mRNA decay and related processes. It has been proposed that UPF1 directs PTC-containing mRNAs to P-bodies and triggers decay. Here, we discuss the link between P-bodies and NMD in view of recent studies that suggest that P-bodies are not required for NMD in Drosophila.

NMD (nonsense-mediated mRNA decay)

NMD is a process that degrades mRNAs containing PTCs (premature translation termination codons). The mechanism has been observed in all eukaryotes studied and it is closely linked to translation [1]. It is believed that PTC-containing transcripts are distinguished by the fact that premature translation termination is intrinsically different from normal termination, because it occurs upstream of an abnormally configured RNP (ribonucleoprotein) [2]. Premature termination is believed to recruit specific NMD factors to the mRNA, which in turn promote association with general mRNA decay enzymes. The core NMD factors are the UPF (up-frameshift) proteins UPF1, UPF2 and UPF3, which were initially identified in genetic screens in Saccharomyces cerevisiae and later found to be involved in NMD in other eukaryotes [3]. In higher eukaryotes, additional factors are involved in NMD, these are the SMG-1 (suppressor with a morphogenetic effect on genitalia-1), -5, -6 and -7 proteins, which were initially identified in genetic screens as allele-specific suppressors of mRNA decay, nonsense-mediated mRNA decay (NMD), both in yeast and in mammalian cells [5,6].

The core NMD factors are the UPF (up-frameshift) proteins UPF1, UPF2 and UPF3, which were initially identified in genetic screens in Saccharomyces cerevisiae and later found to be involved in NMD in other eukaryotes [3]. In higher eukaryotes, additional factors are involved in NMD, these are the SMG-1 (suppressor with a morphogenetic effect on genitalia-1), -5, -6 and -7 proteins, which were initially identified in genetic screens as allele-specific suppressors of mutations in a variety of genes in Caenorhabditis elegans [4]. SMG-2, -3 and -4 were also identified in these screens and correspond to UPF1, UPF2 and UPF3. The best-characterized factor is UPF1, an evolutionarily well-conserved protein of over 100 kDa which contains a cysteine- and histidine-rich domain at the N-terminal and a large middle domain containing seven motifs typical of group I RNA helicases [3]. Mutations in the RNA helicase domain impair NMD, both in yeast and in mammalian cells [5,6]. S. cerevisiae and human UPF1 have been purified and show RNA binding activity, RNA-dependent ATPase activity and RNA helicase activity [5,7]. UPF2 and UPF3 stimulate UPF1 helicase activity in vitro, suggesting that the essential role of UPF2 and UPF3 in NMD is due to their requirement in UPF1 activation [8]. Activation of the helicase activity of UPF1 is believed to induce remodelling of the RNP, which somehow promotes association with general mRNA decay factors and drives rapid decay [8,9]. At least in human cells and in C. elegans, UPF1 is a phosphoprotein; phosphorylation requires SMG-1, UPF2 and UPF3, whereas SMG-5, -6 and -7 promote its dephosphorylation [3,9,10]. The phosphorylation state of UPF1 does not affect its helicase activity; the role of UPF1 phosphorylation in NMD remains indefinite.

Standard mRNA decay and P-bodies (processing bodies)

The half-life of mRNA varies greatly in the cell ranging from minutes to hours and it is increasingly appreciated that the regulation of mRNA decay is important in the control of eukaryotic gene expression. The current understanding, based on studies in yeast and mammalian cells, is that mRNA degradation initiates with shortening of the poly(A) tail, which is followed by either 5′–3′ or 3′–5′ exonucleolytic degradation [11]. In the 5′–3′ pathway, the key event is the removal of the 5′-cap by the DCP (decapping protein) 2–DCP1 holozyme complex, which is followed by 5′–3′ degradation by XRN1 (exoribonuclease 1) [11]. Following transcription inhibition, many mRNAs decay stochastically, with rate constants specific for different mRNAs [12]. Different decay rates probably result from transcript-specific differences in the rate of deadenylation [12]. It was also understood that individual decay events are independent of one another and occur in isolation in a state of free diffusion. This simplified view of mRNA decay has been challenged by the recent discovery that in many eukaryotic cells, key decay factors such as the decapping enzyme and XRN1 accumulate in large granules called P-bodies, which are readily visible by light microscopy; mRNA degradation intermediates are also found in P-bodies [13]. The P-bodies found in mammalian and Drosophila S2 cells are also known as GW bodies because, in addition to general decay factors, they contain the GW182 protein, which is involved in the miRNA (microRNA) pathway (Figure 1
shows an example in *Drosophila* [14–16]. Therefore the emerging view is either that mRNA decay factors are only active when assembled into P-bodies or that at least decay takes place preferentially in P-bodies. The emerging concept is that partition of decay enzymes to restricted regions of the cytoplasm increases the efficiency of the process and prevents accidental degradation of functional mRNA.

**NMD and P-bodies**

The idea that NMD may take place in P-bodies is based on the observation that UPF proteins localize to P-bodies in yeast strains lacking either DCP1, DCP2 or XRN1 [17]. It was also shown that PTC-containing mRNA accumulates in P-bodies and that the accumulation was reduced in UPF1-deleted strains [17]. UPF proteins do not accumulate in P-bodies in wild-type cells, but it was argued that this is due to NMD substrates transiting through P-bodies only transiently; neither NMD factors nor substrates are normally detected, but they accumulate when NMD is either impaired or slowed down by mutations in essential NMD factors or general decay enzymes required in the terminal degradation steps. In fact, it was shown that in UPF2- or UPF3-deleted strains UPF1 and NMD substrates accumulate in P-bodies [17].

Since P-bodies appear to be evolutionarily conserved entities, an important question is: can efficient mRNA decay and NMD take place in the absence of P-bodies? Given that in higher eukaryotes P-bodies contain GW182 and other factors involved in RNAi (RNA interference), the second relevant question is: are P-bodies required for RNAi? The issue was thoroughly addressed in a recent study with *Drosophila* S2 cells [18]. It was reported that S2 cells have the typical P-bodies seen in yeast and mammalian cells: they contain mRNA decay factors and proteins involved in the RNAi and miRNA pathways. In addition, their appearance is enhanced in cells accumulating mRNA intermediates or mRNA that is not being translated [18]. However, in contrast with what was reported for yeast, depletion of NMD factors by RNAi did not affect P-body formation [18]. In addition, NMD can still occur in cells in which factors required for P-body formation were depleted by RNAi. Depletion of P-bodies also did not affect RNAi or miRNA silencing; P-bodies appear to be the consequence of gene silencing rather than the cause [18].

**Conclusion**

With the discovery of P-bodies, the emerging view is that general mRNA decay, NMD and possibly other post-transcriptional processes are confined to P-bodies rather than taking place diffusely throughout the cytoplasm. Along with the idea that P-bodies may be involved in NMD, *Drosophila* UPF1, when overexpressed, co-localizes with large GW182-containing bodies in S2 cells (Figure 1). However, in contrast with this burgeoning view, the study of Eulalio et al. [18] indicates that typical P-bodies, defined as large cytoplasmic granules/foci visible by light microscopy, may not be required for NMD and RNA silencing; they may simply arise as the level of RNA degradation intermediates increases as a consequence of saturating the degradation or translation capabilities of the cell. However, it is still possible that post-transcriptional reactions are at least in part associated with submicroscopic cytoplasmic bodies/compartmenta; more work would be required to address the issue.

![P-body visualization](image)

**Figure 1** | P-body visualization

Fluorescent micrographs of a *Drosophila* S2 cell co-expressing a GFP (green fluorescent protein) fusion of UPF1 (left panel) and an RFP (red fluorescent protein) fusion of GW182 (middle panel). The right panel shows a merge of the two micrographs on the left with the DAPI (4′,6-diamidino-2-phenylindole) staining to visualize the nucleus (indicated by the arrow).

---

**References**


Received 16 March 2008
doi:10.1042/BST0360698