RNA turnover: **The helicase story unwinds**

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Recent results show that RNA helicases play important roles in RNA decay, both as exoribonuclease accessory factors and in communicating signals to RNA decay machinery.

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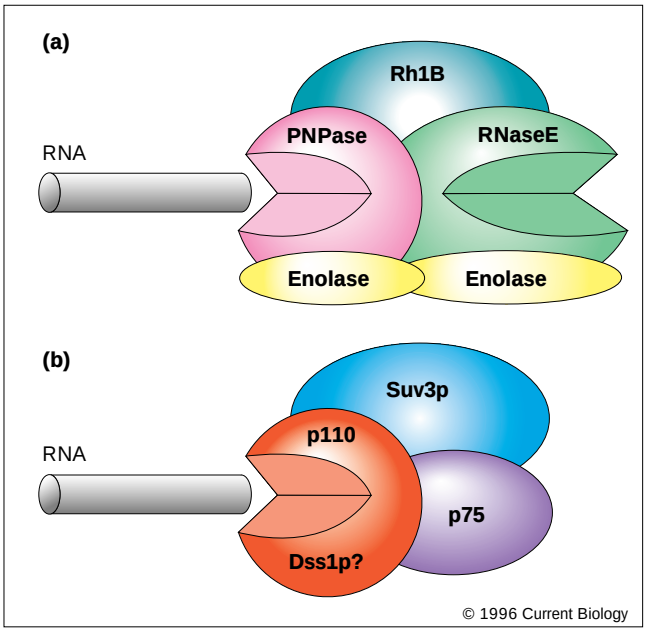
The process of RNA turnover is important both for the control of mRNA levels and for the degradation of excised introns. In several cases, the degradation of transcripts is now known to involve exonucleolytic digestion of the RNA, either directly, or following an initiating nucleolytic event, such as decapping (the removal of the structure that characteristically 'caps' the 5′ end of eukaryotic mRNAs) [1,2]. We shall discuss recent observations indicating that several members of the two RNA helicase protein families play important roles in RNA turnover, both in aiding exoribonuclease digestion and in recognizing RNA features that specify the decay rate. Helicases catalyze the nucleoside triphosphate (NTP)-dependent unwinding of nucleic acid duplexes. These proteins have been organized into two superfamilies based on conserved sequence motifs [3]. Although only a few of these proteins have been demonstrated to have helicase activity *in vitro*, the conserved regions that define the families are required for this helicase activity, suggesting that many of these proteins are likely to have helicase activity. Members of the two helicase superfamilies have been demonstrated to have important roles in translation, pre-mRNA splicing and ribosomal biogenesis [3]. Several recent reports have shown that RNA helicases also act in RNA decay pathways [4–6].

During RNA turnover, an RNA helicase could act to unwind strong secondary structures that might otherwise stall or block an exoribonuclease. Two new reports provide an example of such an RNA helicase in *Escherichia coli* [4,5]. In *E. coli*, a major mechanism of RNA decay involves both endonucleolytic cleavage by RNase E and 3′-to-5′ exoribonucleolytic degradation of the transcript body by polynucleotide phosphorylase (PNPase). These proteins are found in a multicomponent complex, termed the degradosome [7,8]. Recently, Miczak *et al.* [4] and Py *et al.* [5] have demonstrated by several criteria that the enolase and RhlB proteins are also present in this complex. (Fig. 1a) Enolase is a glycolytic enzyme whose role in RNA turnover is unclear. RhlB is a protein with DEAD box sequence motifs, characteristic of superfamily II RNA helicases.

Figure 1



RNA helicases as exoribonuclease accessory proteins. (a) Rh1B in the *E. coli* degradosome. Only components identified in both [4] and [5] are shown. The stoichiometry is based on quantification of protein gels and may be inaccurate. (b) Components of the yeast mitochondrial enzyme mtEXO.

Clues as to how this putative RhlB helicase functions in RNA turnover have come from the analysis of degradosome activity *in vitro*. Py *et al.* [5] have observed that the purified degradosome can, in the presence of ATP, completely degrade an RNA containing a stem–loop element of secondary structure. In contrast, digestion with purified PNPase, or reactions lacking ATP, led to the accumulation of RNAs degraded to a stem–loop structure. Evidence that the RhlB protein is required for the degradosome to be able to proceed beyond the stem–loop came from the observation that addition of anti-RhlB antibodies also led to the accumulation of RNAs only degraded at the 3′ end. These results imply that the role of RhlB is to aid PNPase in proceeding through strong RNA secondary structures.

As RhlB deletions are lethal, the protein has an essential function in the cell. There are at least two essential roles RhlB could play. PNPase deletions are viable, because RNase II, another 3′-to-5′ exoribonuclease, can substitute for PNPase [9]. RhlB could assist both of these enzymes in degrading structured RNA. Losing RhlB activity would then be equivalent to losing both 3′-to-5′ exoribonucleases,

which is lethal [9]. An alternative explanation is offered by Miczak *et al.* [4], who suggest that RhlB may be involved in generating appropriate RNase E cleavage sites. This could occur by RhlB either influencing the RNA substrate directly, or affecting the activity of RNase E through protein–protein interactions in the degradosome. Consistent with this model, RNase E deletions are lethal.

Stem–loops located at the 3′ end of *E. coli* RNAs have been shown to have stabilizing effects [2]. These results suggest that the RhlB-containing degradosome provides one mechanism for removing these structures. In addition, recent work has indicated that the addition of a 3′ poly(A) tail can also promote the degradation of a structured RNA by PNPase [10]. These observations imply that the ability of a stem–loop to stabilize a transcript will depend upon as yet unknown features of the RNA that influence polyadenylation and/or RhlB function. This provides the potential for apparently simple 3′ stem–loop structures to influence the RNA decay rate in several different ways. It should be noted, however, that 3′ stem–loops are not the only determinants of stability, as cleavage in the 5′ end by RNase E can be rate determining for many RNAs. Future work is likely to determine the relationships between these decay pathways and may well reveal additional, as yet unidentified, nucleases with important roles in RNA turnover (see [2] for discussion).

A second example of an exoribonuclease-associated helicase has come from the characterization of a 3′-to-5′ exoribonuclease complex found in yeast mitochondria [11,12]. (Fig. 1b). This complex consists of three polypeptides and possesses NTP-dependent 3′-to-5′ exoribonuclease activity [11]. Several biochemical observations indicate that a member of the DExH box subfamily of helicase superfamily II, Suv3p, is a component of this mitochondrial exoribonuclease [12]. In addition, the phenotypes of *suv3* mutants *in vivo* are consistent with a role for Suv3p in RNA turnover. The mutants accumulate several excised group I introns, in some cases to very high levels [13]. These results clearly show that this exoribonuclease complex is involved in degrading excised introns.

It is likely that Suv3p has no intrinsic exoribonucleolytic activity, and that its function is to assist the actual nuclease subunit. A good candidate for the nuclease subunit is the protein encoded by the *DSS1* gene, identified as a high-copy suppressor of *suv3* mutants [14]. Strikingly, Dss1p shows some sequence similarity to *E. coli* RNase II, and the predicted molecular weight of Dss1p (111 kDa) is quite similar to that of the largest component of the mitochondrial exoribonuclease complex (110 kDa). These observations suggest that, at least in some cases, overexpressing the corresponding exoribonuclease may suppress defects in associated RNA helicases. One interesting question is whether this same exoribonuclease complex also plays a role in the degradation of mRNAs transcribed in mitochondria.

The presence of putative helicases in both the *E. coli* degradosome and the mitochondrial exoribonuclease complex suggest other exoribonucleases may also have associated helicases. In yeast, both 5′-to-3′ and 3′-to-5′ exoribonucleases have been proposed to function in cytoplasmic mRNA decay [1]. Although there is no strong evidence to date for associated helicases in either of these cases, mutations in the 5′-to-3′ exoribonuclease Xrn1p are synthetically lethal with mutations in the DExH box protein Ski2p, another putative RNA helicase [15]. One simple explanation is that this helicase is important for the function of the 3′-to-5′ exoribonuclease, and that loss of the major 5′-to-3′ and 3′-to-5′ exoribonuclease activities leads to the death of the cell.

Proteins in the general class of RNA helicases may also play a role in the recognition of RNA features that modulate decay rates. This function would be analogous to the role in pre-mRNA splicing of several putative RNA helicases, which are thought to act in a sequence-specific manner to promote RNA rearrangements [16]. Such an RNA helicase appears to function in the decay of mRNAs containing an early nonsense codon, which are rapidly decapped, and then degraded exoribonucleolytically in a 5′-to-3′ direction. Upf1p, which is a member of the helicase superfamily I, is required for this degradation in yeast [1]. Importantly, purification of Upf1p has shown that this protein has 5′-to-3′ RNA and DNA helicase activities, as well as a nucleic-acid-dependent ATPase activity. The helicase activity is likely to be important for the function of this protein in RNA decay, as mutations that inactivate the helicase activity also prevent rapid decay of mRNAs with early nonsense codons [6].

What might be the role of an RNA helicase in this pathway of decay? One possible function for Upf1p would be to assist the 5′-to-3′ exoribonuclease, encoded by the *XRN1* gene, that degrades nonsense-codon-containing transcripts following decapping. This is unlikely for several reasons. First, Xrn1p also functions in the decay of normal yeast mRNAs, and Upf1p is known not to be required in this case [1]. In addition, some mutations in the *UPF1* gene have also been shown to affect translational termination, suggesting that Upf1p may be involved both in the process of normal termination and in signaling when termination is premature [6]. Although the exact mechanism is unknown, Upf1p may function to trigger some specific RNA structural transition. For example, Upf1p could promote the dissociation of the codon:anticodon duplex between the mRNA and the final peptidyl-tRNA, a process which could be required both for termination and for triggering decay.

It is now evident that RNA helicases play essential roles in multiple RNA decay pathways, both in signal transmission

and in facilitating the decay of structured RNA. The involvement of these proteins adds a new layer of complexity to the body of knowledge concerning RNA decay pathways and suggests that as our understanding of RNA turnover progresses we should look forward to the involvement of additional helicases around the next turn.

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