Applying the brakes on gene expression

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Eukaryotic messenger RNAs are degraded through a pathway that starts with the removal of the poly(A) tail. A recent study shows that cytoplasmic mRNA deadenylation in mammals occurs by the consecutive action of two distinct deadenylases and that degradation of nonsense mRNA involves the same biphasic deadenylation pathway.

For driving a car, control over the brakes is just as important as control of the accelerator pedal. Likewise, for regulating gene expression, regulation of mRNA degradation is as important as regulation of its synthesis. In fact, expression of many genes is controlled mainly by regulating their mRNA-turnover rate[1]. Yet, our understanding of the mRNA degradation pathways in mammalian cells is still sparse compared to the tremendous amount of information on mRNA synthesis. From the available data on mammalian systems combined with the more detailed understanding of mRNA-degradation pathways in the yeast *Saccharomyces cerevisiae*, the following general picture emerges. Most mRNAs seem to decay by a deadenylation-dependent pathway that initiates with a 3′→5′ exonucleolytic removal of the poly(A) tail. After deadenylation, the mRNA body is degraded by one of two pathways. In the first, removal of the 7-methylguanosine cap at the 5′ end by the decapping enzyme Dcp1–Dcp2 leads to a rapid 5′→ 3′ exonucleolytic degradation by Xrn1, whereas the second pathway relies on 3′→ 5′ exonucleolytic degradation by the exosome (reviewed in refs. 1,2).

The recent findings reported by Yamashita *et al.*[3] on page 1054 of this issue shed light on several central aspects of mammalian mRNA decay. First, the authors show that deadenylation of β-globin mRNA in the cytoplasm of mouse cells involves the consecutive action of two different deadenylases (**Fig. 1**). The first deadenylase, which consists of the catalytically active subunit PAN2 and its binding partner PAN3, initiates mRNA deadenylation on fully polyadenylated mRNAs. After removal of about half the ~200 initial A nucleotides, the second deadenylase, the multiprotein complex CCR4–CAF1 (ref. 2), takes over and removes the remaining poly(A) tail. The deadenylation mediated by PAN2–PAN3 is relatively slow and occurs synchronously in an mRNA population produced by a transcriptional pulse. After PAN2–PAN3 has been exchanged with the CCR4–CAF1 complex, deadenylation proceeds less synchronously and decay of the mRNA body is observed. On the basis of the cytoplasmic localization of PAN2 and PAN3 in mouse NIH3T3 fibroblasts, Yamashita *et al.*[3] concluded that PAN2–PAN3 represents the first step of cytoplasmic mRNA deadenylation. This seems to be different in *S. cerevisiae*, where there is evidence that PAN2–PAN3 functions in the nucleus to trim the poly(A) tails of freshly polyadenylated mRNAs to the correct length and thereby has an important role in making these messenger ribonucleoprotein particles export competent[4]. For cytoplasmic mRNA deadenylation, however, PAN2 and PAN3 are dispensable in the presence of active CCR4 in *S. cerevisiae*[5]. Residual deadenylation only depends on PAN2 in a *ccr4*Δ yeast strain[5].

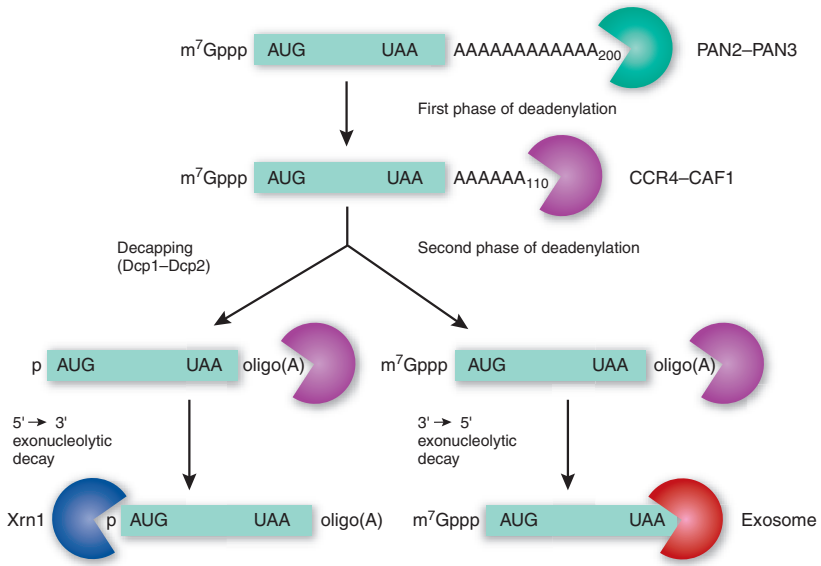

Figure 1 Incorporation of the recent results from Yamashita *et al.*[3] into the current model of mammalian mRNA degradation pathways leads to the following scheme. Cytoplasmic mRNA degradation starts with deadenylation, a 3′→5′ exonucleolytic shortening of the poly(A) tail mediated by the consecutive activity of two different deadenylases. During the first phase of deadenylation, PAN2–PAN3 shortens the poly(A) tails to ~110 A nucleotides. PAN2–PAN3 is then replaced by CCR4–CAF1 for the second phase of deadenylation. During the CCR4–CAF1–mediated phase of deadenylation, the decapping enzyme (consisting of Dcp2, Dcp1 and additional factors) can remove the 7-methylguanosine cap from the 5′ end of the mRNA, which renders the mRNA susceptible to rapid 5′→3′ exonucleolytic degradation by Xrn1. Alternatively or in parallel, CCR4–CAF1 is replaced by the exosome when only a few As are left at the 3′ end. The exosome then degrades the mRNA body from the 3′ end.

The second important aspect of the paper concerns nonsense-mediated mRNA decay (NMD), a degradation pathway that specifically recognizes and degrades mRNAs with premature translation-termination codons (PTCs), which if translated would encode potentially deleterious C-terminally truncated proteins[6,7]. Initial studies in *S. cerevisiae* indicated that NMD is initiated by direct decapping of fully polyadenylated nonsense mRNA[8], in contrast to the deadenylation-dependent decapping observed in 'normal' turnover of PTC-free mRNA[2]. More recently, however, PTCs in yeast mRNAs have been shown to induce accelerated deadenylation followed by exosome-mediated 3′→5′ exonucleolytic degradation of the mRNA body[9]. There is also evidence from mammalian cell cultures

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for the involvement of deadenylation in NMD[10]. Likewise, kinetic studies with PTC-containing β-globin transcripts in mammalian cells have shown that decay of the mRNA body is preceded by rapid deadenylation[11]. Yamashita *et al.*[3] report that this accelerated deadenylation of PTC-containing β-globin transcripts has biphasic kinetics similar to those for deadenylation of normal, PTC-free β-globin mRNA. The effects on deadenylation and decay of PTC-containing β-globin mRNA by overexpression of the wild-type or catalytically inactive mutant PAN2 and CCR4 suggest that, as for wild-type β-globin mRNA, deadenylation by PAN2–PAN3 precedes CCR4-mediated deadenylation and decay of the mRNA body. The main difference between degradation of wild-type and PTC-containing β-globin mRNAs seems to be that the PAN2–PAN3-mediated first phase is much shorter for the nonsense transcripts; by contrast, there is no substantial difference in the decay kinetics of the CCR4-mediated second phase for the two transcript types. Collectively, these results suggest that upon PTC recognition, mammalian NMD shunts its target mRNAs to the basic mRNA-turnover machinery, where they get degraded in a deadenylation-dependent manner that involves the subsequent action of PAN2–PAN3 and CCR4–CAF1. It is currently not known exactly how and where the NMD pathway connects to the basic mRNA-turnover pathway.

The third important finding reported by Yamashita *et al.*[3] involves the coupling between deadenylation and decapping of the mRNA. According to our current understanding of mRNA turnover, removal of the 5′ cap by the DCP1–DCP2 decapping enzyme is followed by Xrn1-mediated 5′→3′ degradation of the mRNA body[2]. Consistent with this view, the authors found that depletion of DCP2 by RNA-mediated interference resulted in the accumulation of mRNAs with a poly(A) tail length of ~110 nucleotides, indicating that decapping takes place after the PAN2–PAN3–mediated first phase of deadenylation, during the CCR4–CAF1–mediated second phase. When the catalytically inactive PAN2 mutant was overexpressed in combination with a DCP2 knockdown, mRNAs with full-length poly(A) tails accumulated. Intriguingly, overexpression of the same PAN2 mutant alone affected mRNA deadenylation and decay only slightly. This suggests that DCP2-directed decapping can also function as a fail-safe mechanism to trigger mRNA decay if deadenylation is impeded. This finding supports other recent evidence for a functional link between PAN2, CCR4 and DCP2 in deadenylation and decapping[12–14].

Notably, Yamashita *et al.*[3] also investigated a third enzyme reported to function in mRNA deadenylation, PARN. Surprisingly, given that PARN provides the major deadenylation activity in *in vitro* mammalian decay systems[15,16], neither depletion nor overexpression of PARN substantially affected the turnover of wild-type or PTC-containing β-globin mRNA *in vivo*. The reason for the discrepancy between *in vitro* and *in vivo* data is currently not clear and will certainly be a subject for future studies.

One of the most obvious questions raised by the results of Yamashita *et al.*[3] concerns how and according to which parameters the handover from PAN2–PAN3 to CCR4–CAF1 takes place on the poly(A) tail of an mRNA. This was not experimentally addressed in the paper, but the authors refer to studies from other laboratories for their working model. PAN2–PAN3 activity has been shown to be stimulated by poly(A)-binding protein (PABP) both in *S. cerevisiae*[17] and in mammals[18]. In contrast, CCR4 deadenylation activity and DCP2 decapping activity are both inhibited by PABP[19,20]. Combining these observations with their own results, Yamashita *et al.*[3] hypothesize that the number of PABP molecules bound to a full-length poly(A) tail is sufficient to interact with PAN2–PAN3 and to stimulate it to start shortening the poly(A) tail. By contrast, they propose that interactions of PABP with the CCR4–CAF1 complex and DCP2 inhibit their activities at this stage. During poly(A)-tail shortening mediated by PAN2–PAN3, PABPs would gradually be lost from the mRNA, resulting in the diminished stimulation of PAN2–PAN3 activity and increased activity of CCR4–CAF1 and DCP2. Their model assumes that when the poly(A) tail reached a length of around 110 nucleotides, the balance would tilt toward CCR4-mediated deadenylation and onset of mRNA decapping, which represents the beginning of the second phase of mRNA degradation. Although this model is simple and elegant, one drawback is that it cannot explain the observed decapping of fully polyadenylated mRNAs under conditions where PAN2–PAN3 is inactivated. According to the model, DCP2 should be kept inactive by the presence of PABP, independent of PAN activity. It therefore seems that in reality, the activities of the three enzyme complexes are more intimately coupled to each other than the model suggests, or that there exists an additional dimension of regulation. This additional dimension could be, for example, regulation through spatial separation of certain steps in the degradation pathway. Evidence for an important spatial component in regulation of mRNA turnover comes from the recent discovery of P-bodies (also called processing bodies or Dcp-bodies): data indicate that mRNAs destined for degradation are transported from the sites of translation to these small cytoplasmic foci, which are enriched with mRNA-decay factors and function as mRNA-degradation centers[12,21].

Although significant progress has been made during the last few years, we are still far away from a complete understanding of the 'brakes' of gene expression. Luckily, it is not we scientists who have to control these brakes, but rather the cells that are responsible. And most of the time, they manage to reach their destination safely.

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