More than one way to make a tail

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The EMBO Journal (2010) 29, 4066–4067. doi:10.1038/emboj.2010.306

The vast majority of eukaryotic messenger mRNAs undergo 3' end cleavage and polyadenylation. The noncanonical poly(A) polymerase (PAP), STAR-PAP, adds a poly(A) tail to specific transcripts under oxidative stress conditions. These transcripts have the AAUAAA polyadenylation signal and use the same factors for cleavage, but are not polyadenylated by the canonical polyA polymerase. In this issue of *The EMBO Journal*, Leishram and Anderson ask what determinants are required for the modification of specific mRNAs by STAR-PAP. They report a surprising role for STAR-PAP in binding to the pre-mRNA and recruiting the processing factors required for cleavage, which suggests that there is still much to be discovered regarding the regulation of 3' end processing.

Eukaryotic messenger RNAs, with the exception of animal histone mRNAs, end in a poly(A) tail. The basic set of factors required for polyadenylation in mammalian cells was established by the work of the laboratories of Jim Manley and Walter Keller during the early 1990s (reviewed in Mandel *et al.*, 2007). The polyadenylation signal AAUAAA located 5' of the site of cleavage is recognized by the cleavage polyadenylation specificity factor (CPSF) and the downstream sequence element is recognized by cleavage stimulatory factor (CstF), with cleavage occurring between these two elements catalyzed by CPSF73, a component of CPSF (Mandel *et al.*, 2006). Coincident with cleavage, PAP adds the poly(A) tail to the 3' end. PAP is required *in vitro* for cleavage of many substrates, guaranteeing coupling of cleavage and polyadenylation. Two years ago, Richard

Anderson and coworkers published the first example of a second PAP, termed STAR-PAP, that adds a polyA tail to the 3' end of a specific pre-mRNA, heme oxygenase (HO-1) mRNA (Mellman *et al.*, 2008), when cells are subjected to oxidative stress. STAR-PAP is a non-canonical PAP (Speckle targeted PIPK1 α regulated poly A polymerase) that requires PI4,5P₂ for activity, linking phosphatidylinositol signalling pathways to RNA processing. Surprisingly, the canonical AAUAAA polyadenylation signal is present in HO-1 mRNA, and the same CPSF complex and other polyadenylation factors are involved in polyadenylation by STAR-PAP. The question then is what determines that an mRNA will be polyadenylated by STAR-PAP and what prevents polyadenylation by the canonical PAP. In this issue, Leishram and Anderson (2010) provide a partial answer to this question by showing that STAR-PAP is required not only for polyadenylation but also for cleavage of HO-1 mRNA.

Using *in vitro* studies, they show that STAR-PAP specifically binds to the 3'UTR of HO-1 mRNA 5' of the polyadenylation signal. STAR-PAP also binds to CPSF160 and helps recruit it stably to the HO-1 mRNA polyadenylation signal (Figure 1A). Using recombinant proteins, they then show that three polypeptides, STAR-PAP, CPSF-160 and CPSF-73, are sufficient to cleave HO-1 pre-mRNA *in vitro*, in a reaction that is dependent on the AAUAAA polyadenylation signal and CPSF-73, the endonuclease that cleaves pre-mRNAs (Mandel *et al.*, 2006). Surprisingly, PI4,5P₂ is not required for cleavage although it is required for polyadenylation.

Figure 1 Two mechanisms of polyadenylation. (A) The proposed mechanism of assembly of a cleavage-polyadenylation complex using Star-PAP to recruit polyadenylation factors and add the poly(A) tail (adapted from Leishram and Anderson, 2010). The precise interactions among different components are not known. (B) The canonical cleavage-polyadenylation mechanism, which involves binding of CPSF, CstF and canonical PAP to the pre-mRNA and then recruitment of the accessory factors required to activate cleavage (adapted from Mandel *et al.*, 2007).

Polyadenylation of HO-1 pre-mRNA is activated in response to oxidative stress, resulting in rapid production of mRNAs from pre-mRNAs that are already being synthesized, followed by rapid production of proteins to deal with the stress. An attractive hypothesis is that the group of RNAs upregulated in stress (Mellman *et al*, 2008) will share conserved binding sites for STAR-PAP. What also remains unknown is how STAR-PAP is activated and recruited for cleavage. Extracts from normally growing cells are active in cleavage of HO-1 mRNA, although the activity is threefold higher in extracts from cells in which the oxidative stress pathway is activated. The activation is unlikely to be due to a modification of STAR-PAP, since recombinant protein can restore activity to a depleted extract. Thus, in the cell, STAR-PAP may be sequestered away from its target RNAs until mobilized by oxidative stress. There may well be other such pathways where the ability to express a class of mRNAs is determined by the presence of novel factors required to form the poly(A) tail.

STAR-PAP is a member of the non-canonical PAPs, which include the cytoplasmic polyA polymerase, Gld2 (Rouhana *et al*, 2005). This family of seven enzymes in animals also includes terminal uridylyl transferases, which can add U's to the 3' end of RNA (Kwak and Wickens, 2007). Indeed STAR-PAP was previously identified as the enzyme that adds U's to U6 snRNA (Trippe *et al*, 2006), and other family members can

add U's to miRNAs (Heo *et al*, 2009). It is likely that some other enzymes in this family may add either A's or U's to the 3' end of RNAs.

Why isn't HO-1 pre-mRNA processed by the canonical polyadenylation pathway? The HO-1 mRNA has what looks like a canonical polyadenylation site, including the consensus AAUAAA motif and GU elements. The processing complex contains all the polyadenylation factors they tested for, including at least one subunit of CstF, although it lacks canonical PAP. Note that both PAP and STAR-PAP interact with CPSF-160 (Figure 1), although whether they bind to the same site is not known. These results point out how much there is still to learn about regulation of polyadenylation. Recently there has been increased interest in alternative polyadenylation as an important regulatory event in gene expression, with the finding that there are large changes in polyadenylation site use in protooncogene mRNAs between normal and tumour cells (Mayr and Bartel, 2009). This suggests that there is differential regulation of translation and stability of different polyadenylated forms of the same mRNA by miRNAs and RNA-binding proteins, as a result of having different 3' UTRs on the same open reading frame.

Conflict of interest

The author declares that he has no conflict of interest.

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