

Cytoplasmic deadenylation: regulation of mRNA fate

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Abstract

The poly(A) tail of mRNA has an important influence on the dynamics of gene expression. On one hand, it promotes enhanced mRNA stability to allow production of the protein, even after inactivation of transcription. On the other hand, shortening of the poly(A) tail (deadenylation) slows down translation of the mRNA, or prevents it entirely, by inducing mRNA decay. Thus deadenylation plays a crucial role in the post-transcriptional regulation of gene expression, deciding the fate of individual mRNAs. It acts both in basal mRNA turnover, as well as in temporally and spatially regulated translation and decay of specific mRNAs. In the present paper, we discuss mRNA deadenylation in eukaryotes, focusing on the main deadenylase, the Ccr4–Not complex, including its composition, regulation and functional roles.

Introduction

Regulated transcription is often the focus of studies on gene expression where polymerases, transcription factors and chromatin structure play important roles. However, post-transcriptional regulation is also a key factor in modulating gene expression [1], and much of this is controlled through the mRNA poly(A) tail. With the exception of some histone mRNAs, all protein coding mRNAs in eukaryotes contain a 3' poly(A) tail. mRNAs exit the nucleus with a uniform poly(A) tail length (~70 nucleotides in yeast, ~250 nucleotides in mammals), but deadenylases act in the cytoplasm to shorten or remove it. Deadenylation occurs in both gene- and context-specific manners to allow differential control of poly(A) tail lengths [2]. Importantly, poly(A) tail length affects gene expression in two major ways: it controls mRNA stability and translational efficiency (Figure 1). This is essential for many processes including periodic expression of cell-cycle-related genes, microRNA-mediated gene silencing, and expression of maternal, masked mRNAs during oogenesis and early development [3–7]. By regulating poly(A) tail length, eukaryotes possess a highly sophisticated mechanism to allow exquisite control of gene expression.

In the present review, we aim to give an overview of deadenylation, with a focus on the Ccr4–Not complex, the major deadenylase in eukaryotes. We emphasize proteins from the budding yeast *Saccharomyces cerevisiae*, but also cover mechanisms in other organisms.

Key words: Ccr4–Not, deadenylation, gene expression, mRNA, poly(A) tail.

Abbreviations used: Ago, Argonaute; EEP, exonuclease/endonuclease/phosphatase; EGD, enhancer of Gal4p DNA binding; eIF4F, eukaryotic translation initiation factor 4F; miRNA, microRNA; miRISC, miRNA-induced silencing complex; NAC, nascent polypeptide-associated complex; PABP, poly(A)-binding protein; PARN, poly(A)-specific ribonuclease; Puf, Pumilio/FBF (fem-3-binding factor); UTR, untranslated region.

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Poly(A) tails and mRNA stability

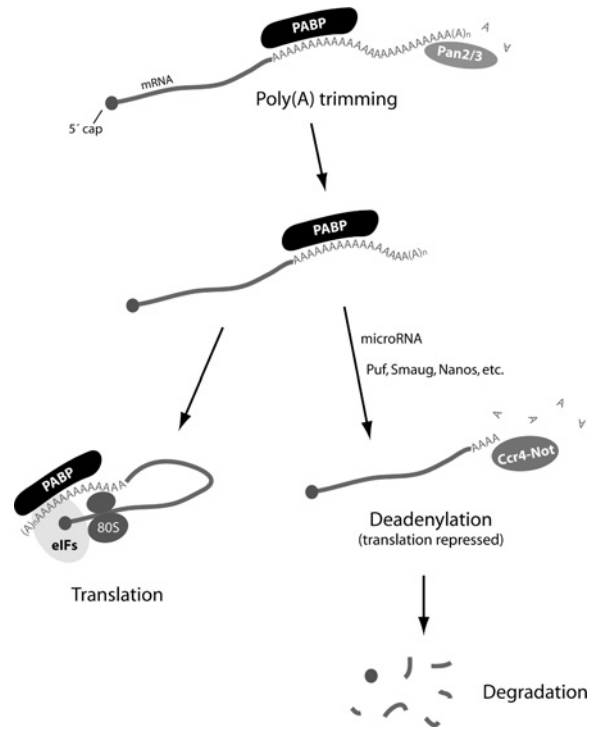
PABPs [poly(A)-binding proteins] (Pab1p in yeast) protect the 3'-ends of mRNAs from exonucleases [8]. Indeed, the first step of mRNA degradation is normally the shortening or removal of the poly(A) tail [9,10]. Computational studies have shown that the most significant factor determining mRNA degradation is the rate of deadenylation [9]. Deadenylation is followed by one of two alternative degradation pathways: 3'→5' degradation by the cytoplasmic exosome, or removal of the 5'-cap (decapping) by Dcp1p/Dcp2p and 5'→3' degradation by the Xrn1p exonuclease [8]. Recently, it was shown that this latter type of degradation can occur co-translationally, allowing the ribosome to complete the current round of translation before the mRNA is degraded completely [11].

Poly(A) tails and translation

Poly(A) tails also function during initiation of translation. PABPs interact with eIF4F (eukaryotic translation initiation factor 4F) that is bound to the 5'-cap [12]. These interactions effectively circularize the mRNA, acting as a control measure to ensure that only intact mRNAs are translated (Figure 1). Importantly, PABPs and eIF4F enhance recruitment of the small ribosomal subunit to promote translation initiation. Thus a longer poly(A) tail can result in stimulation of translation, whereas deadenylation decreases the efficiency of translation initiation [3]. Experiments showing co-regulation of poly(A) tail lengths of functionally related genes supports this [3,4]. For example, in yeast, the lengths of mRNA poly(A) tails of cell-cycle-regulated genes are generally short, suggesting that the strict temporal regulation of their expression depends not only on transcription, but also on deadenylation [3]. In higher eukaryotes, cycles of deadenylation and cytoplasmic polyadenylation act in

Figure 1 | Deadenylation of mRNAs in eukaryotes

PABPs protect the 3'-end of mRNA from exonucleases and stimulate translation. The Pan2/3 complex trims poly(A) tails, whereas the Ccr4-Not complex removes them. Regulatory signals (e.g. miRNAs and Puf proteins) accelerate deadenylation by recruiting Ccr4-Not to target mRNAs.



a general mechanism to control mitosis by regulating translation of cell cycle mRNAs [4].

The eukaryotic deadenylases**Ccr4-Not complex**

A major deadenylase activity involved in both basal mRNA decay and regulated deadenylation of specific transcripts is found within the evolutionarily conserved Ccr4-Not complex [10,13]. Ccr4-Not is composed of nine different protein subunits (including the deadenylase subunits Ccr4p and Pop2p/Caf1p) and is discussed in detail below.

Pan complex

In *ccr4* mutant yeast, a complex containing the Pan2p and Pan3p proteins is responsible for mRNA deadenylation, suggesting functional redundancy with Ccr4-Not [14]. Pan2p is the deadenylase subunit, containing a nuclease domain belonging to the DEDD (Asp-Glu-Asp-Asp) superfamily. Pan3p acts as a regulator, binding to both Pan2p and PABPs. A *ccr4/pan2* double-mutant yeast strain grows very slowly and demonstrates no deadenylation, indicating that Ccr4p and Pan2p are responsible for most deadenylation [14]. The precise role of the Pan complex is unclear, but it is thought

to 'trim' poly(A) tails to mature lengths, dependent on PABPs and on mRNA-specific signals [13,15] (Figure 1). In agreement with this, deletion of either *pan2* or *pan3* results in longer poly(A) tails, but only causes a minor deadenylation defect [15]. There may be a complex interplay between the Ccr4-Not and Pan complexes, such that each contributes to cytoplasmic deadenylation. For example, Pan2/3 may shorten the poly(A) tail to a certain length, allowing Ccr4-Not to complete the deadenylation [13]. Further research is needed to clarify this.

PARN [poly(A)-specific ribonuclease]

PARN [previously known as DAN (deadenylating nuclease)] is found only in vertebrates. It forms a homodimer and differs from the other deadenylases because it binds to and is stimulated by the mRNA 5'-cap [13,16]. Deadenylase activity is inhibited by PABPs and cap-binding proteins. PARN is responsible for deadenylation of a number of mRNAs, and often these are subject to intricate regulation. For example, PARN acts on specific mRNAs to repress their translation [until cytoplasmic poly(A) polymerases once again lengthen the poly(A) tail]. This occurs during mitosis, with maternal mRNA in oocytes and in plant embryogenesis [4,17,18].

Nocturnin and Ngl

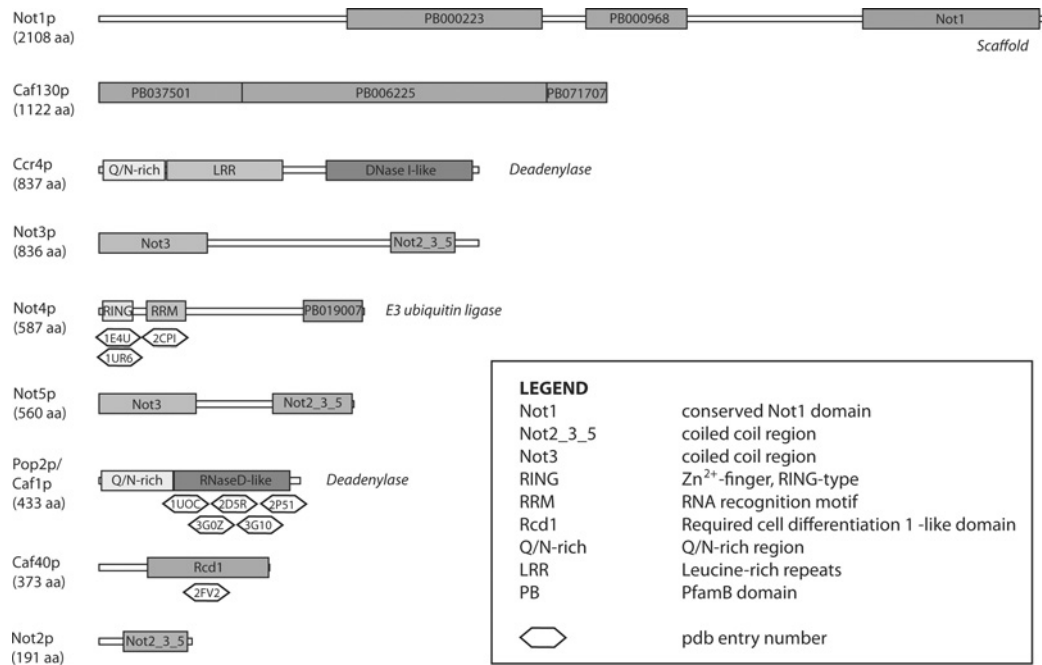
Two further deadenylases, Nocturnin and Ngl/ANGEL, share nuclease domain similarity with Ccr4 [i.e. they are members of the EEP (exonuclease/endonuclease/phosphatase) superfamily]. Nocturnin has been identified as a rhythmically expressed deadenylase in the cytoplasm of the retinal photoreceptor cells of *Xenopus laevis* and is thought to play a role in post-transcriptional regulation of circadian-related mRNAs [19]. Ngl is a deadenylase involved in 5.8S rRNA processing [20].

Regulated removal of the poly(A) tail by the Ccr4-Not complex

The Ccr4-Not complex is a 3'→5' exoribonuclease with a preference for poly(A). Many of its subunits were first identified as transcriptional regulators. The Ccr4-Not complex localizes to promoter regions, interacts with TFIID (transcription factor IID) and SAGA (Spt/Ada/Gcn5/acetyltransferase), and facilitates chromatin modifications such as trimethylation of Lys⁴ of histone H3 (H3K4me3) [21–23]. Thus the Ccr4-Not complex may regulate gene expression not only through post-transcriptional deadenylation, but also at a transcriptional level. Both functions respond to extra- or intra-cellular stimuli. For example, the complex plays key roles in the cellular response to DNA replication stress and DNA damage [24], cell stress [25] and cell cycle control [26]. A recent microarray-based study suggested that the Ccr4-Not complex is important for the expression of most (>85%) of the yeast genome [27].

Figure 2 | Schematic representation of yeast Ccr4–Not complex subunits

Domains are depicted according to known structures and predictions using the Pfam database. PfamB domains are computationally generated and unannotated, but can give insights into functional conserved regions. Functional roles are given on the right of each subunit, in italics. PDB codes are given underneath each protein for any available structures. It should be noted that in higher eukaryotes, the composition of the Ccr4–Not complex varies slightly. Not3p and Not5p are very similar proteins and in humans they have only one orthologue, CNOT3. In contrast, the deadenylase subunits Ccr4p and Pop2p have two human orthologues (CNOT6/hCcr4a and CNOT6L/hCcr4b; CNOT7/hCaf1 and CNOT8/hPop2/CALIF). aa, amino acids.



The Ccr4–Not complex is conserved across eukaryotes. In yeast, it is composed of nine subunits that can be purified as a 1 MDa particle [21,22,28]. The core complex consists of two deadenylases (Ccr4p and Pop2p/Caf1p), five Not proteins (Not1p–Not5p), Caf40p and Caf130p (Figure 2) [29]. At least two ‘modules’ appear to exist within the Ccr4–Not complex; genetic and biochemical experiments have suggested that the Not proteins associate in one complex, whereas Ccr4p, Pop2p, Caf40p and Caf130p exist in a second subcomplex. The Not and deadenylase modules are joined through the 240 kDa Not1p protein which acts as a scaffold and is necessary for yeast viability [30].

The two deadenylase subunits are unrelated: Ccr4p contains a DNaseI-like domain of the EEP superfamily, whereas Pop2p contains a RNaseD-like domain belonging to the DEDD family. It is unclear why both are present. Deletion of either *ccr4* or *pop2* results in defects in the rate and extent of deadenylation [14]. Experiments so far have indicated that Ccr4p activity is predominant [10,31]. For example, overexpression of Ccr4p overcomes the deadenylation defects in a yeast strain with a *pop2* deletion, and point mutations in catalytic residues of Ccr4p, but not Pop2p, inhibits deadenylation *in vivo* and *in vitro* [31,32]. Correspondingly, overexpression of Ccr4p, but not Pop2p,

in a wild-type yeast strain increases mRNA deadenylation [31].

Although it appears to have lower activity, Pop2p is also a functional deadenylase and may be responsible for regulated deadenylation of specific transcripts, with Ccr4p being responsible for basal mRNA degradation [32,33]. In addition, Pop2p probably plays a role in stabilizing the complex and/or activating Ccr4p. Indeed, Ccr4p associates with the Ccr4–Not complex primarily through Pop2, and catalytically inactive Pop2p can complement a yeast strain containing a *pop2* deletion [34].

In human cells, there are two isoforms of both Ccr4 (CNOT6/hCcr4a and CNOT6L/hCcr4b) and Pop2 (CNOT7/hCaf1 and CNOT8/hPop2). Since each isoform may have different substrate specificities, this allows formation of multiple types of Ccr4–Not deadenylase complexes.

In addition to its deadenylase activity, the Ccr4–Not complex possesses E3 ubiquitin ligase activity in the RING finger of its Not4p subunit. The substrates of this activity remain poorly defined, but include the heterodimeric NAC (nascent polypeptide-associated complex) or EGD (enhancer of Gal4p DNA binding) complex [35]. NAC/EGD binds to the ribosome, near the polypeptide exit tunnel to protect

nascent chains as they emerge, participating in protein folding and ribosome biogenesis [36]. Its ubiquitination promotes ribosome association, but does not affect protein stability [37]. Not4p also ubiquitinates translational arrest products that occur after translation of poly(A) [38]. Poly(A) sequences are not normally translated and therefore, by recognizing arrested ribosomes, Not4p may promote degradation of aberrant proteins. Finally, Not4p ubiquitinates the histone H3 Lys⁴ (H3K4) demethylase Jhd2p/JARID1C (Jumonji AT-rich interactive domain 1C), targeting it for degradation by the proteasome to regulate H3K4 trimethylation and gene expression [23].

It was shown recently that the Ccr4–Not complex (and Not3 in particular) is a conserved regulator of heart function [39]. Neely et al. [39] performed an RNAi (RNA interference) screen in *Drosophila* and identified a number of Ccr4–Not components as being important for cardiac function. These include Not1, Not2, Not3, Not4, the *Drosophila* homologue of NAC and the E2 Ubc4. Importantly, *not3*^{-/-} mice are embryonic lethal, whereas *not3*^{+/-} mice have heart defects (spontaneous impairment of cardiac contractility and increased susceptibility to heart failure). Moreover, the Ccr4–Not complex is likely to be involved in heart function in humans since SNPs (single nucleotide polymorphisms) in Ccr4–Not genes are correlated with altered cardiac QT intervals [39].

Together, the Ccr4–Not complex probably plays an integrated role in transcription, mRNA stability and translational regulation. Its two catalytic activities (deadenylation and E3 ubiquitin ligase) could mediate most of the functions ascribed to the Ccr4–Not complex. However, the extent and relevance of the E3 ubiquitin ligase activity, and how this is linked to deadenylation, is still unclear. Further research will be needed to clarify this.

miRNA (microRNA)-targeted deadenylation

miRNAs are short RNAs that bind to sequences in the 3'-UTRs (untranslated regions) of their target mRNAs with imperfect complementarity. This recruits Ago (Argonaute) proteins and GW182/TNRC6 to form a miRISC (miRNA-loaded RNA-induced silencing complex) that silences expression of the target mRNA (reviewed in [40,41]). At least part of the silencing mechanism involves mRNA deadenylation. Specifically, miRISC recruits the Ccr4–Not complex, triggering deadenylation of target mRNAs and leading to mRNA decay and/or translational repression [5,42,43].

miRNA-induced deadenylation requires the deadenylase activity of Pop2/Caf1 and an interaction between Ago and GW182 [5,42,44]. GW182 is thought to recruit the Ccr4–Not complex, although no direct interactions have been shown. Tethering of GW182 to mRNA results in deadenylation, independently of miRNA or Ago [7]. The details of this

process remain unclear, including whether Ccr4 activity is also required and how the recruitment is mediated.

Interestingly, PABP is also necessary for miRNA-induced deadenylation [5], even though *in vitro* assays have demonstrated that PABP inhibits deadenylation by Ccr4–Not complexes [31]. A possible explanation is given by recent studies showing that the C-terminal domain of GW182 interacts with PABP (reviewed in [7]). Thus GW182 might antagonize PABP function and thereby destabilize the PABP–poly(A) interaction. This, in turn, could increase the accessibility of poly(A)-tails for deadenylases.

The temporal order of miRNA-mediated repression is heavily debated. On one hand, it has been suggested that repression of translation initiation occurs first, followed by mRNA deadenylation and degradation [5]. On the other hand, deadenylation may take place first, and, consequently, mRNAs with shorter poly(A) tails are destabilized and translation initiation becomes less efficient [45].

Protein-targeted deadenylation

The Ccr4–Not complex can be recruited to mRNA by conserved regulatory proteins {e.g. Puf [Pumilio/FBF (fem-3-binding factor)] proteins} bound to specific sequences in the 3'-UTR [46]. Indeed, tethering of either Pop2 or specific 3'-UTR-binding proteins to a model mRNA is sufficient to trigger rapid deadenylation and mRNA decay [47–49].

Puf proteins are found in all eukaryotes and they control mRNA stability and translation, for example in development, memory and stem cell maintenance. Puf proteins increase the efficiency of Ccr4–Not-mediated deadenylation of specific mRNAs by interacting with both binding elements in the 3'-UTR and Pop2p, thereby recruiting the complex directly to target mRNAs [50]. They bind to large groups of specific mRNAs (e.g. they bind 10–15% of yeast transcripts) and each of the six yeast Puf proteins seems to regulate a different set of functionally related genes [51].

Similarly, other evolutionarily conserved proteins bind sequences in the 3'-UTR and recruit the Ccr4–Not complex: Nanos binds to Not4 [49,52], Bicaudal-C/Bic-C binds Not3/5 [53], and Smaug/Vts1p recruits the Ccr4–Not complex, although its direct interaction partner has not been characterized [48]. These conserved recruitment factors all play crucial roles in early development and have been characterized in the context of *Drosophila* embryonic development. However, this fine-tuning of translational control plays a general role wherever spatial or temporal regulation of protein synthesis occurs, for example in mitosis and in synapses [4,54]. Future work should address exactly how these proteins control gene expression.

Perspectives

Many questions remain unanswered about the mechanisms of regulated deadenylation of mRNAs in eukaryotes. For example, what is the relationship between different deadenylases, specifically the Ccr4–Not and Pan complexes?

How is deadenylation coupled to other mechanisms of gene regulation? What are the precise mechanisms of recruitment factors? It has been suggested that deadenylation requires remodelling of PABPs on the poly(A) tail to make it more accessible [55,56]. However, it is unclear what promotes this reorganization; perhaps additional regulatory proteins assist in releasing or altering the structure of PABPs. Structural models of intact deadenylation complexes as well as detailed biochemical characterizations will begin to answer these questions and explain the cellular determinants of poly(A) tail length.

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