Roles of mRNA poly(A) tails in regulation of eukaryotic gene expression

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Abstract | In eukaryotes, poly(A) tails are present on almost every mRNA. Early experiments led to the hypothesis that poly(A) tails and the cytoplasmic polyadenylate-binding protein (PABPC) promote translation and prevent mRNA degradation, but the details remained unclear. More recent data suggest that the role of poly(A) tails is much more complex: poly(A)-binding protein can stimulate poly(A) tail removal (deadenylation) and the poly(A) tails of stable, highly translated mRNAs at steady state are much shorter than expected. Furthermore, the rate of translation elongation affects deadenylation. Consequently, the interplay between poly(A) tails, PABPC, translation and mRNA decay has a major role in gene regulation. In this Review, we discuss recent work that is revolutionizing our understanding of the roles of poly(A) tails in the cytoplasm. Specifically, we discuss the roles of poly(A) tails in translation and control of mRNA stability and how poly(A) tails are removed by exonucleases (deadenylases), including CCR4–NOT and PAN2–PAN3. We also discuss how deadenylation rate is determined, the integration of deadenylation with other cellular processes and the function of PABPC. We conclude with an outlook for the future of research in this field.

mRNA decay
The process of removing mRNA from the cytosolic pool of transcripts. Decay of mRNA occurs in a defined pathway with each mRNA having an intrinsic half-life.

Translation efficiency
The amount of protein output relative to the amount of transcribed mRNA.

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Early studies of mRNA in eukaryotes were quick to note that these emissaries of the genetic code are polyadenylated on their 3′ end[1–6]. Poly(A) tails are present on almost every eukaryotic mRNA, with the only known exception being some mammalian histone transcripts. Poly(A) tails are added co-transcriptionally and are required for the export of mature mRNAs to the cytoplasm (FIG. 1). Eukaryotic transcripts receive a poly(A) tail with an average length of ~200 nt in mammals[7] and ~70 nt in yeast[8].

The poly(A) tail contributes to both the translational status and the stability of mRNAs, thereby functioning as a master regulator of gene expression in the cytoplasm (FIG. 1). Specifically, the poly(A) tail can function synergistically with the 7-methylguanosine (m^7G) cap on the 5′-end of the mRNA to stimulate translation[9]. Accordingly, a transcript without a poly(A) tail exhibits low levels of translation and is also a substrate for removal of the 5′ cap (decapping). Thus, poly(A) shortening (deadenylation) ultimately triggers translation repression and subsequently mRNA decay. The rate of deadenylation can be regulated in response to cellular cues in a transcript-specific manner[10]. Moreover, in specific cases, poly(A) tails can be extended in the cytoplasm to reactivate translationally repressed transcripts or to maintain their stability. The dynamic nature of poly(A) tails is therefore vital in regulating gene expression and is important in almost every aspect of eukaryotic biology, including in early development, the inflammatory response and synaptic plasticity[10]. Consistent with a conserved and crucial role in gene regulation, many viral transcripts either have a poly(A) tail or use alternate mechanisms that functionally substitute for a poly(A) tail[11,12]. Likewise, in metazoans, mRNAs of replication-dependent histones substitute the poly(A) tail with a unique 3′ ribonucleoprotein (RNP) complex[13].

Post-transcriptional regulation of mRNAs, including changes in the length of the poly(A) tail, is a quintessential aspect of gene expression that determines the composition of the proteome. Transcripts are differentially regulated, resulting in large variations in translation efficiency and mRNA stability. For instance, translation efficiency can vary more than 1,000-fold between transcripts, and mRNA half-lives vary by a similar order of magnitude[14–18]. Recent insights from new sequencing techniques, biochemical reconstitution and structural biology are now providing new molecular insights into the biology of poly(A) tails. We now know that poly(A) tail lengths are not as uniform as once hypothesized, that they can contain non-A nucleotides and that the connection between tail length, translation efficiency and mRNA stability is not straightforward.

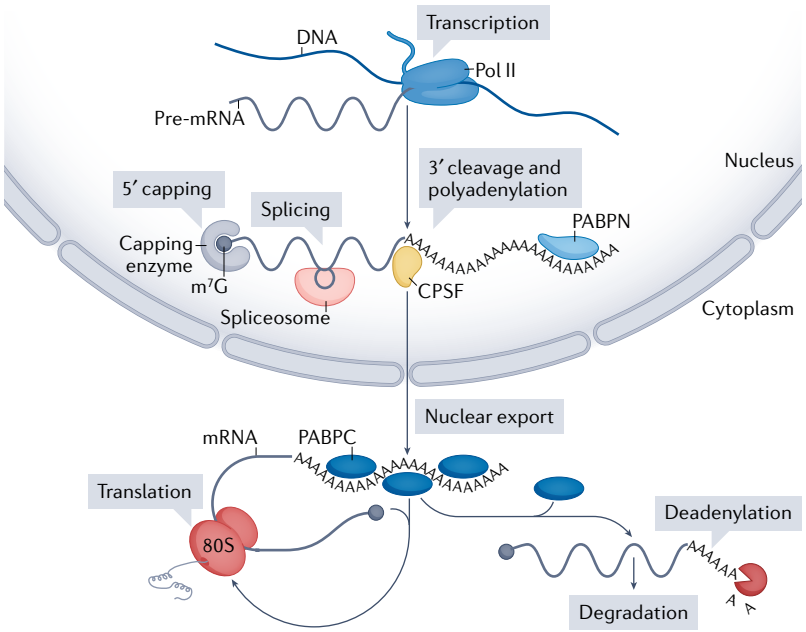


Fig. 1 | **Overview of the function of mRNA poly(A) tails as master regulators of gene expression in the cytoplasm.** In the nucleus, pre-mRNAs are transcribed by RNA polymerase II (Pol II) and processed, which includes 5′ capping, splicing, and 3′ cleavage and polyadenylation. Nuclear poly(A)-binding protein (PABPN) controls poly(A) tail addition. Mature, polyadenylated mRNAs are exported to the cytoplasm. Cytoplasmic poly(A)-binding protein (PABPC) binds poly(A) tails and promotes translation by the 80S ribosome. Poly(A) tails and PABPC also influence mRNA stability: removal or shortening of the poly(A) tail (deadenylation) releases PABPC and leads to mRNA degradation. CPSF, cleavage and polyadenylation specificity factor; m^7G, 7-methylguanosine.

In this Review, we discuss the roles of poly(A) tails in the cytoplasm — specifically in translation and control of mRNA stability — and the mechanisms of poly(A) tail removal by the CCR4–NOT and PAN2–PAN3 deadenylation complexes. We discuss how the rate of deadenylation is controlled, how deadenylation interfaces with other cellular processes and the role of the major cytoplasmic polyadenylate-binding protein (PABPC; Pab1 in yeast and PABPC1 in humans). We begin with describing key historical findings and then discuss recent discoveries that extend our understanding of the roles of poly(A) tails in the cytoplasm, which are now leading to a resurgent interest in the field. Finally, we provide a perspective of the focus of future research on cytoplasmic mRNA translation and decay.

Poly(A) tails support mRNA translation

Pioneering work in the 1970s demonstrated that poly(A) tails are important for efficient translation. For example, sea urchin eggs were shown to have a large burst of cytoplasmic transcript polyadenylation shortly after fertilization[19,20] and maternal mRNAs were found to be translationally quiescent, often with very short poly(A) tails[21–24] that are extended during oocyte maturation, coincident with expression of their cognate protein products[25,26]. Perhaps most dramatically, cytoplasmic polyadenylation was shown to be both necessary and sufficient for the activation of the proto-oncogene *c-mos*, driving meiotic maturation of frog oocytes and suggesting that it was the newly formed poly(A) tail that turned on translation[27,28]. At about the same time, it was shown that, in synaptic junctions, some transcripts are translationally silent and have short poly(A) tails much like maternal mRNAs in oocytes[29]. Synaptic stimulation results in both polyadenylation and concomitant translational activation of these dendritic mRNAs. Collectively, these data suggested that mRNA translation is broadly influenced by the control of poly(A) tail length.

The storage and activation of maternal mRNAs in oocytes and of neuronal mRNAs is analogous in almost every molecular detail, including the protein factors that mediate these processes. In both of these specialized cases (but not in all situations), the length of the poly(A) tail correlates with translation efficiency — the longer the poly(A) tail, the more efficiently the mRNA is translated[30–33]. Thus, poly(A) tail metabolism is dynamic and a crucial node of gene regulation that is leveraged in multiple biological contexts. The discovery of cytoplasmic polyadenylation opened up a new research field and the details have been reviewed extensively elsewhere[34,35].

During translation initiation, factors assemble at the 5′ end of the mRNA to recruit the small, 40S ribosomal subunit and promote scanning of the mRNA 5′ untranslated region (UTR) to identify a start codon. Following its assembly on the start codon, the initiation complex is competent to join with the large, 60S ribosomal subunit, thereby forming a complete 80S ribosome with initiator tRNA in the ribosomal P site. It is now clear that the poly(A) tail at the 3′ end of the mRNA can influence translation initiation at the 5′ end[9,36]. However, despite a litany of information spanning 30 years demonstrating that poly(A) enhances translation, a clear mechanistic understanding of this connection is lacking. This is partly because methods to study poly(A) tail lengths were slow to develop and in vitro translation systems do not always recapitulate poly(A) effects. Additionally, homeostatic mechanisms in cells balance out disruptions in gene expression levels, further complicating the interpretation of in vivo mechanistic studies: disruption of one pathway may be compensated for by modulation of another. In addition, the role of poly(A) tails likely differs between embryonic cells and post-embryonic cells[18], as we discuss below. However, perhaps most importantly, a major factor known to mediate the effects of poly(A) tails — PABPC — has been difficult to study.

The role of PABPC

In the cytoplasm, poly(A) tails are bound by PABPC[37]; this was discovered at about the same time as mRNAs were found to bear a poly(A) tail[38,39]. There is one PABPC in yeast (Pab1) but multiple isoforms in mammals: PABPC1 is the best-studied mammalian isoform and likely the most abundant in most cell types[40]. Other mammalian isoforms include PABPC4, which may act in a transcript-specific manner, the embryonic ePABP and the testes-specific tPABP[40]. PABPC is highly conserved in eukaryotes and has four N-terminal RNA recognition motif (RRM) domains, which bind poly(A) RNA with a nanomolar affinity[41,42] (FIG. 2a). RRM1 and RRM2 have a higher affinity and specificity for poly(A) than RRM3 and RRM4 (REFS[41,42]). A crystal structure of RRM1

Bypass suppressors
A mutation at a distinct locus, which restores viability following mutation of an essential gene. Bypass suppressors often provide insight into the function of essential genes.

and RRM2 showed that RRM1 is located 3′ of RRM2 on the bound poly(A)[43] (FIG. 2a). The RRMs are followed by a proline-rich linker and a C-terminal mademoiselle (MLLE) domain (FIG. 2a). The MLLE domain recognizes a peptide motif called poly(A)-interacting motif 2 (PAM2), which is found in a number of PABPC partner proteins that regulate poly(A) tail dynamics[44]. Nuclear PABPs — PABPN1 (also known as PABP2) in humans and Nab2 in yeast — have a different domain architecture to PABPCs and influence the process of polyadenylation itself. PABPN function will not be covered here[45].

PABPC requires about 12 adenosines for high-affinity binding (through RRM1 and RRM2) but physically covers about 30 nucleotides[37] (FIG. 2b). Longer tails can bind more PABPC and a poly(A) tail of 90 nt can bind three molecules[46]. Interaction between adjacent PABPC molecules promotes cooperative binding to poly(A) RNA, facilitating PABPC multimerization[47]. However, recent data[18,48] suggest that PABPC concentrations in cells may be limiting and that steady-state poly(A) tail length in cells does not necessarily correlate with the amount of associated PABPC, as discussed in more detail below.

Most of our understanding of PABPC function comes from early studies in yeast. In *Saccharomyces cerevisiae*, bypass suppressors of *PAB1* mutants have been identified and are divided into two classes. First are mutations in genes encoding the large (60S) ribosomal subunits and 60S biogenesis factors[49]. The other class of *PAB1* mutation suppressors are found in genes that encode factors involved in mRNA degradation, including decapping regulators (*PAT1*, *LSM1* and *DCP1*) and the exonucleases 5′–3′ exoribonuclease 1 (*XRN1*) and the exosome 3′–5′ exoribonuclease *RRP6* (REFS[50,51]). Although these genetic findings are consistent with a role for PABPC in translation, they also point to a vital role for PABPC (and poly(A) tails) in controlling mRNA stability. PABPC is both necessary and sufficient for the roles of poly(A) in mediating transcript translation and stability[52–54] and this is discussed in more detail below.

The closed-loop model

The m^{7}G cap at the 5′ end of the mRNA also has an important role in regulating translation. The cap binds directly to translation factors to promote translation initiation[55]. Synergy between the 5′ cap and the 3′ poly(A) tail further stimulates this process as demonstrated by monitoring the translation of reporter mRNAs in plant, animal and yeast cells[9]. In these experiments, the combination of both cap and tail on the same mRNA strongly enhanced translation efficiency compared with mRNAs with either modification (FIG. 2c).

How does the 3′ end of the mRNA stimulate translation initiation at the 5′ end? Eukaryotic translation initiation factor 4E (eIF4E) recognizes the 5′ cap[55] and also interacts with another translation initiation factor, eIF4G, which in turn binds PABPC[56]. Thus, mRNAs can form a ‘closed loop’ that enables direct physical communication between the 5′ cap and the 3′ poly(A)

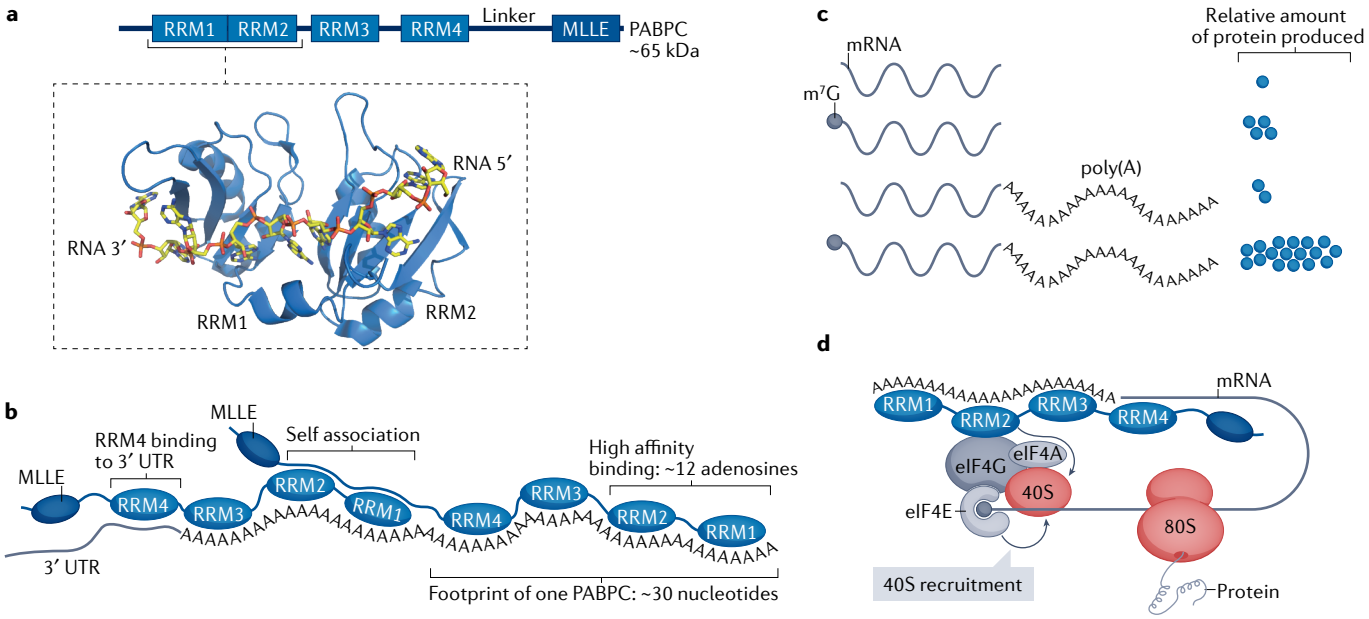


Fig. 2 | **mRNA poly(A) tails stimulate translation. a** | Domain diagram of the conserved eukaryotic cytoplasmic polyadenylate-binding protein (PABPC) is shown, indicating the relative positions of the four RNA-recognition motif (RRM) domains and the mademoiselle (MLLE) domain[40,44] (top). A crystal structure of the RRM1–RRM2 region of human PABPC1 bound to poly(A) RNA (PDB 1CVJ), showing that RRM1 is located 3′ of RRM2 on the bound RNA[43] (bottom). **b** | Arrangement of PABPC on RNA. Two PABPC molecules can bind a 60 nt poly(A) tail. RRM1 and RRM2 have the highest affinity and specificity for poly(A) and require about ~12 adenosines for high affinity binding[41,42]. Full-length PABPC has a footprint of about 30 nt, and adjacent PABPC molecules interact with each other[37,46,47]. RRM4 may bind to the 3′untranslated region (3′UTR)[130]. **c** | The mRNA 5′ cap (7-methylguanosine (m^{7}G)) and 3′ poly(A) tail act synergistically to stimulate gene expression in eukaryotes. The relative amounts of protein produced from reporter mRNAs with and without 5′ cap and poly(A) tail in plant, animal and yeast cells are depicted[9]. **d** | The closed-loop translation initiation model. Eukaryotic translation initiation factor 4E (eIF4E) binds the 5′ cap; eIF4G binds both eIF4E and PABPC as well as the RNA helicase eIF4A, and these interactions are thought to stimulate recruitment of the small (40S) ribosomal subunit. 40S assembles with the large (60S) ribosomal subunit on a start codon to form a translation-competent 80S ribosome.

tail[57] (FIG. 2d). The interaction of eIF4G with PABPC stabilizes the eIF4E–cap interaction[58] and, similarly, the interaction of PABPC with poly(A) RNA stabilizes its interaction with eIF4G[58]. PABPC also stimulates eIF4A, another translation initiation factor, by enhancing its ATPase and helicase activity[59]. Together, the cap–eIF4E–eIF4G–PABPC–poly(A) complex is thought to stimulate translation, at least in part, by recruiting the small (40S) ribosomal subunit[60–62] (FIG. 2d).

It is tantalizing to compare the mRNA closed loop in translation to that of a rolling circle in DNA replication. In DNA replication, circular molecules of DNA can undergo a unidirectional process of replication that greatly enhances production, with DNA polymerase going round and round the circle ad infinitum. For mRNA translation, the situation is different: whereas DNA replication can be continuous, translation is discontinuous with distinct steps of initiation, elongation and termination for each polypeptide produced. Nevertheless, an mRNA closed loop would result in translation termination occurring in close physical proximity to the 5′ end of the mRNA, potentially allowing for rapid recycling of ribosomes back to the initiation codon of the same transcript. Circularization of the transcript could also act as a quality control mechanism to ensure that translation does not initiate on a partially degraded mRNA.

Direct biochemical data demonstrate that PABPC and eIF4G can mediate circularization of an mRNA[63,64] but this 'closed loop' may not be as simple as was originally thought. For example, single-molecule imaging of RNA in cells supports a model in which, although the mRNAs are not fully extended, the 5′ and 3′ ends of transcripts are often not in close enough proximity to be physically connected by eIF4E, eIF4G and PABPC[65]. Some of these inconsistencies between in vitro and in vivo studies might be explained by differences in experimental setup. Nevertheless, although the 'closed loop' model may apply to some mRNAs, circularization may be dynamic and may not occur in all transcripts and/or in all biological contexts[66]. There is ample evidence for communication between the 5′ and 3′ ends of mRNAs but whether it is mediated by a closed loop and the nature of the relationship between the cap and tail are still somewhat ambiguous. Some of the key discrepancies in this model are discussed below but we refer the reader to a recent Review that extensively discusses this topic[36].

Poly(A) tails and mRNA stability

Early experiments showed that poly(A) tails are shortened exonucleolytically in a time-dependent manner[67–69]. mRNAs injected into *Xenopus laevis* oocytes with a poly(A) tail of at least 32 nt were translated as efficiently as mRNAs with long (150 nt) poly(A) tails[70]. By contrast, poly(A) tails of 16 nt or less were not translated[70] and tails shorter than 30 adenosines are not often observed in cells[71]. Together, these data suggested that poly(A) tails stabilize mRNAs (allowing their translation) and that a minimum tail length of about 30 nt is generally required to confer stability. Agreeably, this minimum length corresponds with the footprint of PABPC. However, some mRNAs are stable and efficiently translated despite bearing a very short (fewer than 20 nt) or no poly(A) tail, including histone mRNAs, some viral transcripts and mRNAs with a conserved sequence that limits poly(A) tails to lengths shorter than 20 nt (REFS[11–13,72]). Efficiently translated mRNAs without poly(A) tails are most likely exceptions.

How do poly(A) tails confer stability? One hypothesis was that PABPC protects the 3′ end of the mRNA by preventing access to exonucleases. In agreement with the hypothesis, a series of experiments including transcriptional pulse-chase and in vitro reconstitution, showed that there is an ordered progression of mRNA degradation where poly(A) tail shortening, or deadenylation, is required to release PABPC before mRNA decay can proceed[73–75]. The addition of excess poly(A) RNA into an in vitro degradation system sequesters PABPC, thereby exposing the poly(A) tail on reporter RNAs and resulting in their destabilization[76]. Conversely, the addition of excess PABPC to in vitro assays inhibits deadenylation[77–79].

In a general model of canonical mRNA decay, the poly(A) tail is first shortened to 10–12 nt, the 5′ cap is then removed (decapping) and the transcript is degraded in a 5′–3′ direction by XRN1 or in a 3′–5′ direction by the cytoplasmic exosome[80–84] (FIG. 3). Deadenylation is hypothesized to be the rate limiting step of canonical mRNA decay[80]. The deadenylation rate varies across different transcripts in a sequence-dependent manner, which can result in mRNAs with vastly different half-lives[73,80,85–88].

A physical interaction between the 5′ and 3′ ends of mRNAs through eIF4E–eIF4G–PABPC in a 'closed loop' provides a possible explanation for how the poly(A) tail influences decapping. These interactions stabilize eIF4E on the cap[58] and could prevent the association of decapping enzymes. In agreement with this possibility, eIF4E and the decapping complex compete for cap access, at least in vitro[89,90]. Moreover, it has also been suggested that PABPC1 in humans can interact directly with the 5′ cap[91]. Therefore, stabilization of mRNA by PABPC could be due to simple steric inhibition of the association between the cap and decapping enzymes. Consistent with this idea, in yeast, deadenylated mRNAs with short oligo(A) tails do not efficiently bind PABPC but are capable of binding the heptameric Lsm1–7 complex of proteins, which in turn physically binds the decapping complex, thereby connecting the 5′ and 3′ ends[92–94].

Taken together, these data indicate that the 5′ end of the mRNA contributes to poly(A)-mediated control of mRNA stability and translation. However, some data do not agree with this model. For example, deletion of *PAB1* in yeast results in an increase in the steady state length of poly(A) tails[49,51] and a reduced rate of poly(A) tail shortening[51]. The opposite effect would be expected if Pab1 simply acts to block 3′ exonucleases from accessing the poly(A) tail. Similarly, PABPC1 is required for efficient deadenylation in a mouse extract system[95]. These in vivo experiments are complicated by the pleotropic effects of PABPC and need to be integrated with data addressing how poly(A) tails are removed. This process is discussed next.

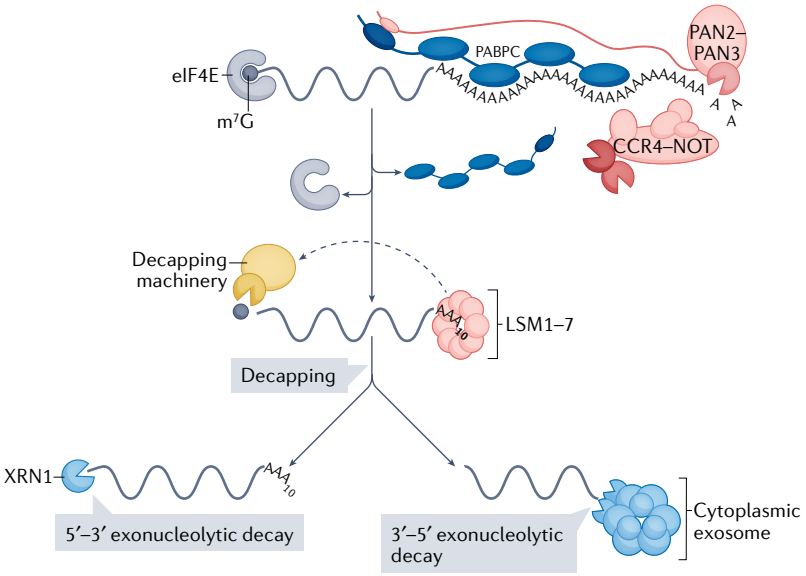


Fig. 3 | **Eukaryotic mRNA deadenylation and decay.** Before an mRNA can be degraded, its poly(A) tail is removed by the PAN2–PAN3 and/or CCR4–NOT deadenylation complexes. This releases cytoplasmic polyadenylate-binding protein (PABPC) and may weaken the association of eukaryotic translation initiation factor 4E (eIF4E) with the 5′ cap. The decapping machinery can then access and remove the 5′ cap. The LSM1–7 complex can associate with oligo(A) tails or with 3′ uridyl tails (not shown) to help recruit the decapping machinery. Decapping is followed by degradation of the mRNA in the 5′–3′ direction by 5′–3′ exoribonuclease 1 (XRN1) or in the 3′–5′ direction by the cytoplasmic exosome complex.

Modulation of poly(A) tail length

Poly(A) tails are shortened or removed by dedicated exonucleases that act specifically on adenosines (deadenylases). Two protein complexes account for the major cytoplasmic deadenylation activities in eukaryotes: PAN2–PAN3 and CCR4–NOT (TABLE 1).

The deadenylation enzymes

Pan2–Pan3 was first identified through fractionation of yeast extracts and contains a DEDD/RNaseD-type exonuclease in its Pan2 subunit[96–98]. Within the complex, two copies of the Pan3 protein form an asymmetric homodimer, which functions as a scaffold for one Pan2 molecule[99–101]. The genes encoding Pan2 and Pan3 are not essential in yeast: deletion of either gene results in longer poly(A) tails at steady state but does not eliminate deadenylation because Pan2–Pan3 is partially redundant with Ccr4–Not[97,98].

PAN2–PAN3 is recruited to poly(A) RNA through at least three interactions: a PAN3 N-terminal zinc finger specifically binds adenosines[99], a PAM2 motif in PAN3 binds PABPC[102], and additional regions of the complex interact with the PABPC–poly(A) RNP[46]. Thus, by recruiting PAN2–PAN3 to poly(A) RNA, PABPC stimulates deadenylation. This has been observed both in vivo and using fully purified components in vitro[99,103]. Additionally, the exonuclease active site of Pan2 is selective for poly(A) RNA because it recognizes an intrinsic, single-stranded helical conformation of RNA that is uniquely formed by poly(A)[104] (BOX 1). PAN2–PAN3 can be recruited to specific transcripts via an interaction with the GW182 protein within the microRNA-induced silencing complex[105,106] but it remains unclear how or whether PAN2–PAN3 is specifically recruited to other transcripts.

The second major deadenylase, CCR4–NOT, is a 0.5 MDa complex of seven core subunits, including two exonucleases: CCR4-associated factor 1 (Caf1; also known as CNOT7 or CNOT8 in mammals and as Pop2 in fission yeast) (TABLE 1), which is a DEDD-type exonuclease; and Ccr4 (also known as CNOT6 or CNOT6L in mammals), which is an EEP-type exonuclease[78,107–110]. Lack of Caf1 or Ccr4 in yeast results in slowed and incomplete deadenylation[107,111]. The Not1 subunit functions as a scaffold to assemble Ccr4–Not and is the only subunit essential for viability in yeast, possibly because Not1 integrates several non-redundant functions of Ccr4–Not[112,113]. The subunits CNOT2 (Not2 in yeast) (TABLE 1) and CNOT3 (Not3 and Not5 in yeast) are linked with decapping[114,115] and CNOT9 (Caf40 and Rcd1 in budding and fission yeast, respectively) is required for interaction with RNA and with several RNA-binding proteins (RBPs)[116–118]. Finally, CNOT4 (Not4 in budding yeast) is an E3 ubiquitin ligase that monoubiquitylates ribosomal proteins (as discussed below) and may also promote protein degradation[119–122]. CCR4–NOT is specifically recruited to transcripts by RBPs as discussed below.

A third deadenylase, poly(A)-specific exoribonuclease (PARN), has also been identified[123–125]. PARN is not found in all eukaryotes — orthologues have been identified in vertebrates only. It may have more specialized roles, for example, in the maturation and stability of small nucleolar RNAs, PIWI-interacting RNAs, or microRNAs[126–129] and will not be discussed further.

The biphasic model of deadenylation. A major question is why there are multiple deadenylation enzymes in the cell. Do they target different sets of mRNAs or do they respond to different stimuli? One hypothesis is that the major deadenylation complexes function in a biphasic (or sequential) manner, where PAN2–PAN3 removes the distal part of the poly(A) tail and CCR4–NOT removes adenosines that are more proximal to the 3′ UTR (FIG. 4a).

Deletion of *PAN2* in yeast results in longer poly(A) tails at steady state (up to 90 nt, compared with about 70 nt in the wild type)[97], which is consistent with a defect in removing the distal part of the poly(A) tail. By contrast, deletion of *CCR4* results in the accumulation of poly(A) tails with a length of 20–40 nt and changes the endpoint of deadenylation from about 10 nt to about 20 nt (REFS[107,130]), suggesting that it has a major role in removing poly(A) sequences proximal to the 3′ UTR. Deletion of both *PAN2* and *CCR4* exacerbates the defects in deadenylation[107]; similar conclusions were obtained from in vitro studies[46]. Experiments in mammalian cells[126,131] also showed that PAN2 is important for a slower, initial phase of deadenylation involving removal of the distal part of the longer mammalian poly(A) tail (200–110 nt), whereas CCR4 functions in a second, fast phase to remove the final 110 nt of the poly(A) tail. The activity of the CCR4–NOT complex may be more processive than that of PAN2–PAN3 in many cases, which may account for its more rapid

Table 1 | **Major deadenylases in eukaryotes**

Complex	Subunit name			Function
	Homo sapiens	*Saccharomyces cerevisiae*	*Schizosaccharomyces pombe*	
PAN2–PAN3	PAN2	Pan2	–	DEDD exonuclease
	PAN3	Pan3	–	RNA binding, scaffold
CCR4–NOT	CNOT1	Not1	Not1	Scaffold
	CNOT2	Not2	Not2	NOT box scaffold
	CNOT3	Not3, Not5	Not3	NOT box scaffold
	(CNOT4)	Not4	Mot2	RING E3 ligase
	CNOT6, CNOT6L	Ccr4	Ccr4	EEP exonuclease
	CNOT7, CNOT8	Pop2	Caf1	DEDD exonuclease
	CNOT9	Caf40	Rcd1	Protein–protein interaction
	CNOT10	–	–	–
	CNOT11	–	–	RNA binding
	–	Caf130	–	–

deadenylation. Thus, PAN2–PAN3 and CCR4–NOT have partially overlapping functions but they appear to act predominantly at different points in poly(A) tail removal. PAN2–PAN3 does not appear to substantially affect mRNA half-life and may be more important in specific cellular circumstances[126].

A recent cryoEM structure of the yeast Pan2–Pan3 complex provides an explanation for why it preferentially acts on the distal part of the poly(A) tail[46]. In the structure, a 90 nt poly(A) RNA is bound by three Pab1 molecules. The poly(A)–Pab1 RNP winds across the surface of Pan2–Pan3 in a zigzag configuration (FIG. 4b). Adjacent Pab1 molecules interact with each other and with the deadenylase complex. Since Pan2–Pan3 recognizes the Pab1 oligomerization interface, it preferentially acts on poly(A) RNA that is long enough to accommodate multiple Pab1 molecules. This model therefore suggests that, as the poly(A) tail is shortened, its affinity for Pan2–Pan3 progressively decreases. It will be interesting to determine how mammalian PAN2–PAN3 achieves a similar function on longer poly(A) tails.

Both PAN2–PAN3 and CCR4–NOT function specifically on poly(A) sequences. Therefore, they remove the poly(A) tail and do not act on the 3′ UTR; mRNA decay occurs only when the poly(A) tail becomes very short (fewer than 10 nt). Thus, in the sequential model, CCR4–NOT has a more important role than PAN2–PAN3 in triggering mRNA decay because PAN2–PAN3 initiates deadenylation and CCR4–NOT completes it. In agreement with this model, depletion of CCR4–NOT components in mammalian cells results in an overall increase in mRNA half-lives but depletion of PAN2–PAN3 does not have a major effect on mRNA half-lives[126].

The separate roles of CCR4 and CAF1. Another question is what are the roles of the two different nucleases within CCR4–NOT? In budding yeast, deletion of *CCR4* has a greater effect on deadenylation than deletion of *CAF1* (REFS[107,108,130]). However, as mentioned above, homeostatic mechanisms can compensate for the disruption of gene expression in vivo, complicating the interpretation of these experiments.

Using purified, recombinant *Schizosaccharomyces pombe* proteins, it is possible to make point mutations in active-site residues to dissect the roles of individual nucleases[130,132]. In these in vitro experiments, both Ccr4 and Caf1 shorten poly(A) tails. Point mutations in either nuclease did not strongly influence deadenylation activity on naked poly(A) RNA. However, Caf1 did not deadenylate Pab1-bound RNA. By contrast, Ccr4 binds Pab1 and can release it from poly(A) tails; similar conclusions were obtained for human CCR4–NOT[126]. Together, these data suggest that the two nucleases in CCR4–NOT have different functions: CAF1 degrades naked poly(A) RNA and is blocked by PABPC, whereas CCR4 is able to release PABPC to deadenylate PABPC-bound poly(A) RNA (FIG. 4c). Moreover, CAF1 functions in a translation-dependent manner, whereas CCR4 is not dependent on translation per se[130]. Moreover, this work showed that PABPC does not simply block the 3′ end of the RNA but stimulates both PAN2 and CCR4, thereby explaining why deletion of *PAB1* results in a reduced rate of poly(A) tail shortening in yeast. These data also suggest that there may be regulatory mechanisms that control which nuclease is used.

What controls deadenylation rate?

The deadenylation rates and half-lives of different transcripts can vary by more than a 1,000-fold[17]. This raises the question of how poly(A) tails are differentiated by deadenylases to allow transcript-specific mRNA decay. The answer to this lies, unsurprisingly, not in the poly(A) tail itself but in other parts of the transcript. First, specific sequences (often in the 3′ UTR) are recognized by RBPs that recruit deadenylases to specific transcripts. Second, RNA sequence affects the translation elongation rate, which is also a major determinant of mRNA half-life. A recent model suggests that mRNA sequence accounts for almost 60% of the variation in mRNA half-lives[133]: 3′ UTR motifs explain 5.5% of the variation while codon usage explains 55%. These two factors are discussed in detail below.

Most known deadenylation regulation functions through the CCR4–NOT complex. It remains unclear whether PAN2–PAN3 activity is regulated (for example, by translation) and whether PAN2–PAN3 is targeted to specific transcripts by different RNA adapter proteins or whether it acts as a general factor that 'trims' long poly(A) tails. Given that PAN2–PAN3 is conserved across evolution, we expect it to have a major, as-yet-undiscovered role in mRNA decay — perhaps on a subset of transcripts or only in response to specific situations.

Transcript-targeted deadenylation. It is well-established that CCR4–NOT is recruited to specific mRNAs through association with RBPs, which function as adapters between specific sequences in the 3′ UTR and the deadenylases (FIG. 5a). Widely studied examples

Intrinsically disordered regions
(IDRs). Polypeptide segments enriched in polar or charged amino acids and lacking hydrophobic amino acids that would mediate cooperative folding. IDRs generally lack a secondary structure.

include the tristetraprolin RBPs, which recognize AU-rich elements[134,135], and the Pumilio/FBF RBPs, which use a modular system to recognize Pumilio-response elements[136–139]. The microRNA-induced silencing complex also mediates targeted deadenylation as its GW182 subunit interacts directly with the PAN2–PAN3 and CCR4–NOT complexes[140]. Many other RNA–deadenylase adapters have been identified in recent years, including nanos, roquin and YTHDF2 (REFS[116,117,132,141]).

The RNA adapter proteins that mediate targeted deadenylation often contain extended intrinsically disordered regions (IDRs) in addition to their RNA-binding domains. Short motifs within these IDRs interact with CCR4–NOT and often multiple motifs from the same RNA adapter interact with one deadenylase complex, suggesting the existence of complex multipartite interfaces[142–146]. The interacting sequences within IDRs are difficult to identify because they are not highly conserved at the sequence level and because binding mechanisms of orthologous proteins sometimes vary between species[147].

Artificially tethering RNA adapters to reporter transcripts in cells results in increased deadenylation and RNA decay[117]. Similarly, in fully reconstituted in vitro systems, RNA adapters, including tristetraprolin and Pumilio/FBF proteins, substantially accelerate deadenylation of target RNAs[139,142]. RNA adapter proteins therefore act as molecular tethers between mRNAs and deadenylation complexes. By physically tethering enzyme and substrate together, the deadenylation rate is increased in a transcript-specific manner and deadenylation activity is more processive. This process is highly tunable since regulatory mechanisms can modulate affinities between the RNA adapter and mRNA and between the RNA adapter and the deadenylation complex. For example, the phosphorylation of tristetraprolin in cells disrupts its binding to CCR4–NOT and stabilizes mRNAs of the inflammatory response[148]. Other mechanisms for regulating how RNA adapters affect deadenylation exist, for example, through collaboration or competition between RNA adapters for RNA binding[149–151].

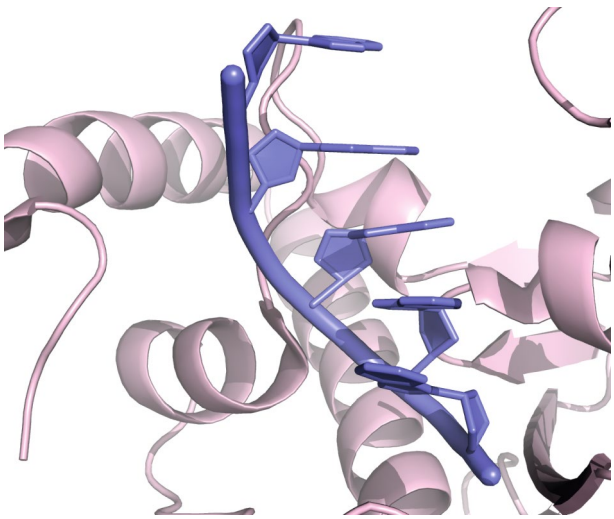
The relationship between translation and deadenylation rate. A growing body of data suggests that translation affects poly(A) tail dynamics. For instance, the inhibition of translation initiation in either *cis* or *trans* enhances the deadenylation rate of long-lived transcripts[90,152–154]. Moreover, the rate of translation elongation can modulate deadenylation rates from yeast to humans and this is mediated by codon optimality[130,155–165]. Codon optimality is the concept that each of the 61 codons is not read by the ribosome at the same rate. Subtle distinctions in functional tRNA concentration and the identity of flanking codons can alter decoding kinetics and these effects are additive across a transcript, setting the overall elongation rate for the mRNA[166]. Surprisingly, the elongation rate is detected by the mRNA deadenylation and decapping complexes: mRNAs hosting slow-moving ribosomes are targeted for rapid deadenylation and decapping in a CAF1-dependent manner, whereas mRNAs with relatively fast ribosome movement evade poly(A) tail shortening and decapping more effectively[130,156]. It is important to note that the classic definition of codon optimality, which is variability in tRNA concentration, is not the only feature that can slow elongation in such a way that elicits faster poly(A) shortening and decapping. Indeed, other features, such as tRNA charging, tRNA and mRNA modifications, amino acid identity and concentration, mRNA sequence and structure, codon context, polypeptide composition within the ribosome exit channel, and folding of the nascent polypeptide, may also slow translation elongation in a manner that is read by the deadenylase and decapping complexes[166].

The emerging picture is that the deadenylase and decapping complexes sense a particular ribosome conformation, which is the result of slowed elongation. There are now several documented interactions of CCR4–NOT with the translation apparatus (FIG. 5b). The yeast Ccr4–Not complex binds and ubiquitylates (through Not4) the nascent-associated polypeptide complex on the ribosome[167]. Not4 also binds and ubiquitylates the ribosomal protein eS7 (also known as 40S ribosomal protein S7) in response to stress[168,169]. Lastly, Not5 was recently shown to bind to translating ribosomes with empty A and E sites, a condition that occurs on transcripts with non-optimal codon content[122]. Importantly, deletion of the Not5 domain that binds the ribosomal E site or mutation of the ubiquitylation sites

Box 1 | The structure of poly(A) RNA

What makes poly(A) RNA unique? Biophysicists have long known that poly(A) has unique properties among polyribonucleotides. Poly(A) RNA forms a single-stranded A-form-like helix, whose stacked arrangement was originally proposed from a crystal structure of two adenosines[192]. More recent crystal structures with longer segments of RNA directly show that single-stranded poly(A) RNA adopts this A-form-like helix configuration, in which the bases are stacked on top of each other[104,193]. The figure shows poly(A) RNA (purple) bound to the exonuclease domain of Pan2 (pink) (PDB 6R9J)[104]. Although Pan2 has nucleotide specificity, no base-specific contacts were observed in the co-crystal structure. Instead, Pan2 (and likely also the deadenylase Caf1) contacts the ribophosphate backbone and specifically recognizes poly(A) RNA through this unique A-form helical structure.

The structure of poly(A) is not only recognized by nucleases but also by ribosomes that have translated through the 3′ untranslated region into the poly(A) tail, for example, on mRNAs lacking a translation termination codon[194,195]. This recognition contributes to activation of a quality control pathway that eliminates those mRNAs and their associated polypeptide chains.



in eS7 stabilizes transcripts that contain non-optimal codons and prevents association of the decapping activator Dhh1 (REF.[122]). Thus, Not5 binding to the ribosome and Not4-mediated ubiquitylation of eS7 likely directly detect and/or signal slow elongation to the deadenylation and decapping machinery.

Together, these data suggest that a key feature in regulating transcript-specific deadenylation is the monitoring of distinct ribosome activities or states by the deadenylases. A slowly elongating ribosome may, in fact, be another type of RNA adapter that connects mRNAs with CCR4–NOT. Thus, it is possible that almost all cellular deadenylation occurs through a conceptually similar recruitment step by an RNA adapter — either an RBP, a ribosome or another unknown factor — and that deadenylation is too slow to have a major role in mRNA decay in the absence of an RNA adapter.

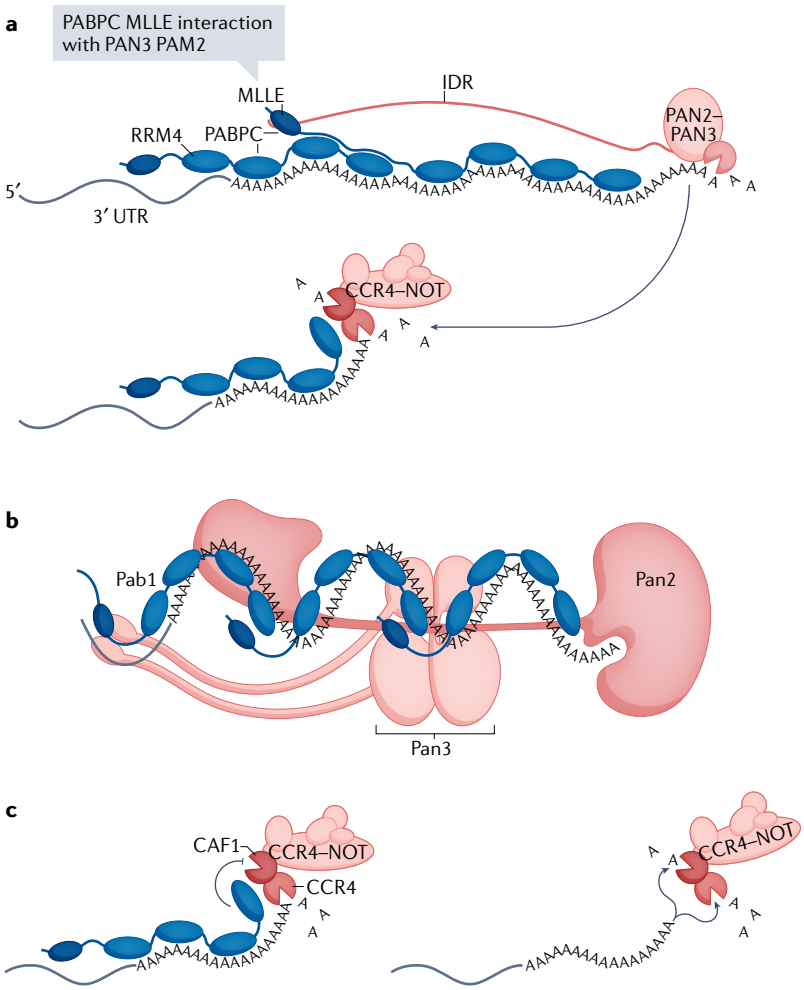


Fig. 4 | **Deadenylation by PAN2–PAN3 and CCR4–NOT. a** | Sequential (biphasic) model of deadenylation. In this model, PAN2–PAN3 preferentially removes the distal part of the poly(A) tail. A cytoplasmic poly(A)-binding protein (PABPC)-interacting motif 2 (PAM2) within an intrinsically disordered region (IDR) of PAN3 interacts with the mademoiselle (MLLE) domain of PABPC. Following PAN2–PAN3 function, CCR4–NOT removes the part of the poly(A) tail that is more proximal to the 3′ untranslated region (3′UTR) of the mRNA. The PABPC protein at the 5′-most position on the mRNA may be positioned on the poly(A) tail such that its RNA recognition motif 4 (RRM4) is located on the 3′UTR. **b** | Model of the budding yeast Pan2–Pan3–Pab1–poly(A) tail complex. A cryoEM structure (PDB 6R5K)[46] shows that Pan2–Pan3 contacts the interface between adjacent Pab1 molecules, thereby providing an explanation for why PAN2–PAN3 preferentially functions on longer poly(A) tails (which bind multiple Pab1 proteins). In the structure, three Pab1 molecules are bound to a 90 nt poly(A) tail. **c** | CCR4 is a general deadenylase, which can degrade poly(A) RNA when it is bound by PABPC. CCR4-associated factor 1 (CAF1) is a specialized deadenylase that degrades naked poly(A) RNA and is blocked by PABPC[130].

Other contributing factors. A large-scale study of mRNA half-life in yeast as well as studies in human cells implicated RNA sequence and structure as major determinants of mRNA stability[16,170]. For example, the inclusion of poly(U) within the 3′ UTR, which can base pair with poly(A) and prevent Pab1 binding, or of other stem-loop structures within the 3′UTR results in an increased mRNA half-life (FIG. 5c). Stem-loops may block deadenylase recruitment to single-stranded RNA[132] or may alter the binding of PABPC RRM4 to the 3′UTR[130]. Additionally, small variations in the final 3 nt of a transcript due to alternative cleavage and polyadenylation can result in isoforms that have a twofold or greater change in half-lives[16] (FIG. 5c). In agreement with these findings, RNA sequence can have a direct effect on the deadenylation rate in vitro[132]. Thus, the direct recruitment of deadenylase complexes to specific sequences may also have an influence on the deadenylation rate[118].

Other factors regulate the interplay between deadenylases and PABPC to control deadenylation. Many of these regulators have PAM2 motifs that allow direct interaction with the MLLE domain of PABPC[44]. For example, TOB proteins interact with CCR4–NOT and also contain a PAM2 motif that binds PABPC to promote deadenylation[171]. The interaction between TOB2 and PABPC is regulated by phosphorylation[172]. LARP1 and LARP4 bind directly to poly(A) RNA and to PABPC1 through a PAM2 motif[173]. In doing so, they protect mRNAs from deadenylation, possibly by stabilizing PABPC binding[174]. The translation termination factor eRF3 interacts with PABPC through a PAM2 motif, thereby linking the poly(A) tail with translation[175]. Other poly(A) regulatory proteins also contain PAM2 motifs, including GW182 and PABPC-interacting protein 1 (PAIP1) and PAIP2 (REF.[44]). Together, PAM2-containing proteins contribute to poly(A) tail dynamics.

Finally, the insertion of ribonucleotides other than adenosine may slow deadenylation in a transcript-specific manner at least in part because guanosine disrupts the helical conformation of poly(A) RNA (BOX 1; FIG. 5c). Sequencing methods have shown that non-adenosine residues are sometimes found in poly(A) tails in cells and the presence of guanosine correlates with mRNA stability[176–178]. Guanosine is least efficiently removed by the deadenylation machinery in vitro[104]. Cytosines and uracils are removed by PAN2–PAN3 slightly less efficiently than adenosine but CCR4–NOT is more selective for adenosine than PAN2–PAN3 (REF.[104]). Interestingly, viruses can promote the addition of non-adenosines into poly(A) tails of viral transcripts, thereby protecting them from the host deadenylation machinery[179]. By contrast, 3′ oligo-uridyl tails are often found on transcripts downstream of short (fewer than 25 nt) poly(A) tails, which is consistent with uridylation acting as a decay-promoting signal[176,180].

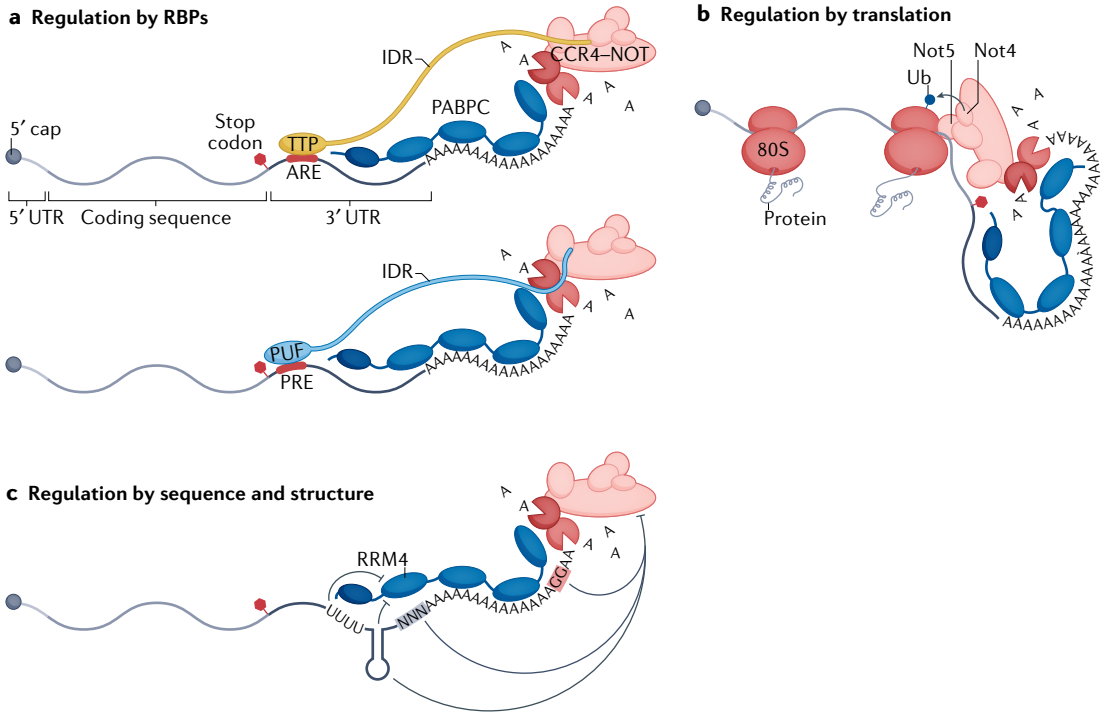


Fig. 5 | **Factors that influence deadenylation rate.** The deadenylation rate of CCR4–NOT is modulated by several factors. **a** | RNA-binding proteins (RBPs), such as tristetraprolin (TTP) proteins and Pumilio/FBF (PUF) proteins act as RNA adapters to tether CCR4–NOT to specific transcripts, thereby accelerating their deadenylation[139,142]. TTP binds to AU-rich elements (AREs) in the mRNA 3′ untranslated region (3′UTR)[134,135] and PUF proteins bind to Pumilio-recognition elements (PREs)[136]. **b** | A slow translation elongation rate results in more rapid deadenylation, likely through direct interactions between *Saccharomyces cerevisiae* Ccr4–Not and the ribosome: Not5 associates with ribosomes having vacant A and E sites and Not4 ubiquitylates the ribosomal protein eS7 (REF.[122]). These interactions likely function to sense the translation elongation rate. **c** | The inclusion of poly(U) within the 3′UTR (which can base pair with poly(A); not shown) and of other RNA structures and changes in the final nucleotides of the 3′UTR (NNN) can all attenuate deadenylation, possibly by altering the affinity of CCR4–NOT or cytoplasmic poly(A)-binding protein (PABPC) for the 3′UTR[16,118,132,170]. The insertion of nucleotides other than adenosine (for example, guanosines) into the poly(A) tail can also stall deadenylation by CCR4–NOT and PAN2–PAN3 (REFS[104,176]).

Reconsidering mRNA metabolism

There has been a recent resurgence in interest in poly(A) tail biology. Developments in sequencing methods, protein expression technology and cryoEM have enabled new investigations into the length and composition of poly(A) tails and the mechanisms of function of deadenylase complexes. These have changed our view on how poly(A) tail length, translation and RBPs, including PABPC, influence gene expression.

The significance of poly(A) tail length

Until recently, an understanding of how poly(A) tail length regulates mRNA metabolism remained limited in part because analysis of poly(A) tail length was difficult to perform on a transcriptome-wide level. New developments in sequencing methodologies (including TAILseq, mTAILseq, PALseq, FLAMseq, PATseq and Nanopore sequencing) now enable the analysis of both the length and sequence of poly(A) tails[32,33,174,176,178,181,182]. Using these methods, the range of poly(A) tail lengths was found to be large and poly(A) tails that were much longer than expected (longer than 250 nt in humans) were found[178,182]. However, very long tails are relatively rare. Surprisingly, multiple studies found that the length of poly(A) tails of highly translated, stable RNAs is relatively short (about 30 nt) at steady state[33]. By contrast, poorly translated mRNAs have comparatively long poly(A) tails. Overall, the modal poly(A) tail length is 30 nt in yeast and 50–100 nt in other eukaryotes, including human, mouse, *Drosophila melanogaster* and *Caenorhabditis elegans*. These results are consistent with earlier data showing that stable transcripts in the amoebae *Dictyostelium discoideum* can have relatively short poly(A) tails of 40–60 nt (REF.[183]). Poly(A) tail lengths in vivo and in vitro are phased in ~30 nt increments, which is consistent with the binding of multiple PABPC proteins to the poly(A) tail[33,46,126,130].

These findings have a number of important implications. First, highly translated transcripts have short poly(A) tails of about 30 adenosines, a length that would accommodate only a single PABPC, suggesting that one PABPC is sufficient to promote efficient translation. Second, it was previously assumed that a longer poly(A) tail correlates with increased mRNA stability. However, these new data showed that transcripts with poly(A) tails that have been shortened to about 30 nt can also be stable. In fact, steady-state poly(A) tail length and mRNA half-life were found to be poorly or inversely correlated

and PABPC occupancy on mRNAs does not correlate with steady-state poly(A) tail length[48,184]. However, a study examining the kinetics of deadenylation instead of steady-state tail length does show a correlation between mRNA half-life and deadenylation rate[17]. Third, these findings provided a strong link between translation efficiency and deadenylation, supporting the idea that translation rate is directly related to mRNA stability (FIG. 6).

Finally, the global phasing of poly(A) tail length in 30 nt increments suggested that PABPC does not bind randomly across the poly(A) tail. Instead, the first PABPC binds at the junction of the 3′ UTR and the poly(A) tail. Subsequent PABPC molecules bind downstream of this PABPC and any adenosines not bound by PABPC are likely removed rapidly. In vitro reconstitution of deadenylation agrees with this model: one Pab1 molecule binds at the junction of the 3′ UTR and poly(A) tail and Ccr4–Not releases one Pab1 molecule at a time during deadenylation[126,130]. RRM4 of the Pab1 molecule most proximal to the transcript body may bind the 3′ UTR[130], particularly when the poly(A) tail length is insufficient to accommodate RRM4 binding (FIG. 4a). In agreement with these findings, RRM4 is highly conserved but is not selective for poly(A) binding[41]. Although CCR4–NOT activity removes the final part of the poly(A) tail and it is often thought of as being 'faster' than PAN2–PAN3, it is clear that CCR4–NOT does not function equally on all short poly(A) tails because stable, highly translated transcripts are more resistant to deadenylation. The mechanisms of such resistance remain unclear. We speculate that fast deadenylation by CCR4–NOT may only occur when it is tethered to a transcript by an RNA adapter protein.

In summary, it is now clear that poly(A) tail length is not only regulated in a transcript-specific manner: poly(A) tail metabolism also controls gene expression, in a more complex, transcript-specific way that likely depends on translation. Whether other factors, such as mRNA localization, also determine poly(A) tail function remains to be tested.

Mediating poly(A) tail effects by PABPC

Despite its omnipresence in translation and mRNA decay, the roles of PABPC remain largely enigmatic. How does PABPC stimulate translation? Do all mRNAs form closed loops? If not, how do the 5′ and 3′ ends of the mRNA communicate? How many PABPC molecules are loaded on an average poly(A) tail and how are they arranged on the poly(A) tail? Is PABPC binding different on different RNAs? What is the effect of PABPC stoichiometry on deadenylation?

A complete molecular understanding of PABPC loading onto poly(A) would facilitate an understanding of its function. The recent structure of Pan2–Pan3 bound to a Pab1–poly(A) RNP combined with the earlier structure of Pab1 RRM1 and RRM2 provides important new insight into the overall arrangement of Pab1 on poly(A) RNA[43,46]. However, due to the limited resolution of the cryoEM structure, the precise molecular details of which amino acids of Pab1 contact RNA and adjacent Pab1 molecules remain unclear. It will be important to obtain this information to allow perturbation experiments and the evaluation of the importance of Pab1 oligomerization in gene expression.

The closed-loop model for how PABPC controls mRNA metabolism is enticing and has made its way into numerous textbooks as canon. However, the described interaction between PABPC and eIF4G does not seem to fully capture the role of PABPC in either controlling mRNA decapping or translation. In yeast, the loss of Pab1 results in premature decapping while the mRNA still has a long poly(A) tail. Nevertheless, a similar phenotype is not observed when eIF4G function is impaired in a wild-type Pab1 background or when Pab1 is tethered to mRNAs; deadenylation is still a prerequisite to mRNA decapping[90,185]. Thus, Pab1 is required to render decapping dependent on deadenylation. Blocking translation initiation in either *cis* or *trans* also does not change the coupling between deadenylation and decapping[89,152,153]. Depletion of PABPC in mammalian cells destabilizes mRNAs and this phenotype can be rescued by the addition of PABPC mutants that cannot bind eIF4G[18].

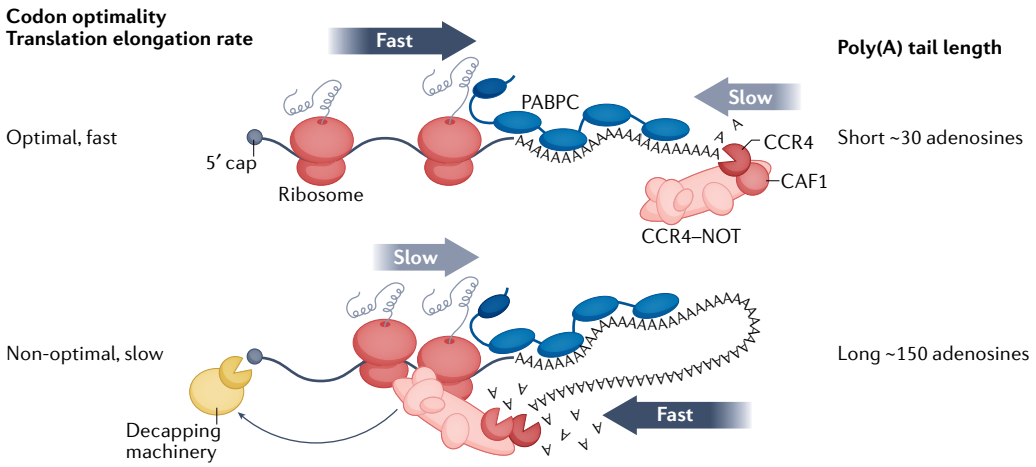


Fig. 6 | **Summary of recent insights into gene regulation by poly(A) tails.** Poly(A) tails can be short (about 30 adenosines) on stable, highly translated mRNAs (top). CCR4–NOT may sense the rate of translation elongation by detecting ribosomes containing empty A and E sites (not shown) and consequently recruit the decapping machinery (bottom). The concentration of cytoplasmic poly(A)-binding protein (PABPC) may also affect the role of poly(A) tails in gene expression.

Thus, simply breaking the PABPC–eIF4G interaction does not abrogate the ability of PABPC to stabilize mRNA.

Perhaps a simple model of PABPC in contact with eIF4G does not completely capture what is truly occurring on mRNA. In one study, at least two distinct closed-loop states were found to exist on mRNAs[64]: the 'classic' closed loop is mediated by PABPC and eIF4G (with other initiation factors) and a second closed loop is mediated by PABPC interacting with the 80S ribosome and the translation termination factors eRF3 and eRF1. PABPC serves an important role in translation termination by facilitating the distinction between normal termination events and aberrant termination events[186]. Thus, it is emerging that communication between the 5′ and 3′ ends may occur through mechanisms that do not solely rely on the PABPC–eIF4G-mediated closed loop and instead may involve other factors, including the ribosome[36]. The architecture of the translating RNP is complex and the true role of PABPC in protecting mRNA from degradation and promoting translation remains unknown. To gain further insight into the importance and prevalence of the closed loop model, we will need to monitor how translation and mRNA stability are modulated by mutations that disrupt the closed loop without disrupting PABPC or eIF4E association, ideally in a cellular environment.

Recent work suggests that PABPC concentration in cells might also be crucial for mediating poly(A) tail effects on translation and stability[18]. In early oocytes, PABPC concentrations in the cell are limited, which results in longer-tailed mRNAs outcompeting shorter-tailed mRNAs for PABPC binding and thus the transcripts with longer tails are more robustly expressed. By contrast, in many somatic cells, PABPC concentrations are not limiting and it is therefore presumed that most poly(A) RNA is bound by PABPC. Unlike in oocytes, in many somatic cells, deadenylated mRNAs are unstable and PABPC does not strongly influence translation efficiency. Together, these data help to rationalize why poly(A) tail length has different effects in different cell types, that is, why poly(A) tail length correlates with translation efficiency only in specific cell types.

Consistent with these findings, PABPC levels are known to be tightly regulated within the cell. The PABPC mRNA includes an extensive A-rich sequence in its 5′UTR, which binds to the PABPC protein and represses its own translation through steric inhibition of ribosome joining[187]. Thus, an autoregulatory feedback determines the ratio of PABPC to poly(A) tails within the cell. This mechanism may be important for determining how poly(A) tail length mediates post-transcriptional regulation. Future work should further address how PABPC levels are controlled.

The influence of PABPC on deadenylation also remains unclear. The CCR4 enzyme can release PABPC from RNA but CAF1 cannot[126,130] — is this regulated? What is the effect of PABPC stoichiometry on CCR4–NOT activity and how does this influence mRNA stability? PABPC has very high affinity for poly(A), so one could imagine that there are active mechanisms to remove PABPC from RNA and remodel its distribution over the transcriptome. If PABPC straddles the 3′ UTR and poly(A) tail[130], some 3′ UTR sequences might be more favourable for PABPC binding, especially in situations where PABPC concentration is limiting. There is also some evidence that active translation affects the stability of PABPC binding[130]. Elucidating the details of these processes will be important.

Coupling of decapping and deadenylation

RNA maturation processes (pre-mRNA splicing, ribosome maturation, microRNA biogenesis, etc.) are characterized by a series of RNP transitions in which one complex is serving as the foundation upon which the next complex is formed. In mRNA splicing, for example, the order of RNP transitions ensures precise intron excision and exon ligation[188]. By contrast, however, there is little information on how over 30 polypeptides interact with each other and with the mRNA in the case of mRNA deadenylation, decapping and degradation[83]. It can be assumed that higher-order RNP assembly is, at least in part, responsible for how the rates of both deadenylation and decapping are achieved. Moreover, it is likely that ordered complex assembly also helps ensure that decapping does not occur prior to deadenylation.

Deadenylation and decapping appear to directly communicate with each other through the ribosome. First, the deadenylase components Not1, Not2, Not4 and Not5 are all required for efficient mRNA decapping[114,122]. Second, the decapping helicase Dhh1 (DDX6 in humans) is also known to communicate with Ccr4–Not through Not1 (REFS[189,190]). Third, Dhh1 and the deadenylase proteins Not4 and Not5 physically bind the ribosome[122,191]. Lastly, Not5 binding to the ribosome is required for Dhh1 binding to the ribosome[122]. This latter observation provides the first evidence that the deadenylase directly influences the decapping complex. Of interest, Not5 can only associate with ribosomes having vacant A and E sites, which may explain how CCR4–NOT senses the translation elongation rate (FIG. 6).

It is tantalizing to speculate that both deadenylase and decapping proteins not only bind to unique ribosome states but also stabilize them, thereby facilitating downstream factor assembly and committing the bound mRNA for decay, which is perhaps exemplified by the observation that Dhh1 binding to the ribosome depends on Not5–ribosome interaction. We anticipate that understanding how the decapping and deadenylase complexes interface and interact with the translating ribosome will be crucial for solving the mystery of how the mRNA 3′ end controls the 5′ end.

Conclusions and future perspectives

It has been over 50 years since mRNAs were shown to be polyadenylated at their 3′ end. In this time, the poly(A) tail has emerged as a central regulator of mRNA translation and stability. Thanks to numerous advances, dogmatic notions are being challenged by new experimental tools, structural analysis and biochemical reconstitution experiments. The relationship between poly(A) tail length and translation efficiency is being reconsidered, the pervasive 'closed loop' model is being challenged and major advances in understanding deadenylase function have emerged that are providing insight into poly(A) tail biology.

In our view, the next few years promise to bring a renaissance into the study of post-transcriptional mRNA regulation, especially as mediated by the poly(A) tail. Moving forwards, we see several major goals as being important.

- To document and understand the regulation of deadenylation rates.
- To understand how RNP context (RNA sequence and structure, codon content, RNA modifications, etc.) modulates deadenylase activity.
- To understand the specificity and roles of the various deadenylase enzymes.
- To gain a clearer picture of the translating mRNP and the events involved in stimulating translation and mRNA stability.
- To understand the differential roles of PABPC isoforms and determine whether they provide regulatory flexibility.
- To fully understand how PABPC functions in mediating 5′-end function and fate.
- To revisit how poly(A) length controls mRNA metabolism; moreover, we must gain a more detailed view of how poly(A) metabolism functions in specific biological contexts and cell types.
- Lastly, we must understand how the deadenylase and its various components interface with the ribosome.

The emergence of mRNA-based therapeutics and vaccines will undoubtedly advance research in this area as fundamental biology is leveraged to advance human health. These applications are dependent on understanding the cytoplasmic life of an mRNA and manipulating it accordingly for therapeutic efficacy. We therefore anticipate that this tail is far from over.

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