OPINION

Multifunctional deadenylase complexes diversify mRNA control

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Abstract | Dynamic changes of the lengths of mRNA poly(A) tails are catalysed by diverse deadenylase enzymes. Modulating the length of the poly(A) tail of an mRNA is a widespread means of controlling protein production and mRNA stability. Recent insights illuminate the specialized activities, biological functions and regulation of deadenylases. We propose that the recruitment of multifunctional deadenylase complexes provides unique opportunities to control mRNAs and that the heterogeneity of the deadenylase complexes is exploited to control translation and mRNA stability.

DNA is transcribed into RNA, and RNA is translated into protein. The RNA link in that chain is a crucial node of control. The lifetimes of eukaryotic mRNAs vary by four orders of magnitude, from minutes to months. The regulation of mRNA stability, translation and localization determines the timing, location and how much protein is produced during mRNA translation[1]. These levels of control permeate biology, from embryonic development to learning and memory[1].

Changes in poly(A) tail length occur throughout the lifetime of an mRNA. In the nucleus, processing of newly synthesized mRNAs is accompanied by the addition of a long poly(A) tail[2] (FIG. 1). Upon export to the cytoplasm, poly(A)-binding protein (PABP) binds to the tail, stabilizes the mRNA and facilitates translation[3]. PABP binds to the translation-initiation factor eIF4G, which in turn binds to the cap-binding protein eIF4E[3] (FIG. 1). The PABP–eIF4G–eIF4E interaction enhances mRNA translation. The role of poly(A) in translation is antagonized by specialized pathways of translational repression and mRNA destruction[4] (FIG. 1). MicroRNAs (miRNAs) and regulatory proteins bind to 3′ untranslated regions (UTRs) of target mRNAs to control their translation and stability[4]. In many cases, their action is linked to changes in the poly(A) tail: repressors shorten the tail, and activators lengthen it[5]. The deadenylase enzymes, which shorten mRNA poly(A) tails, are key players in the process of translational repression.

Deadenylases constantly erode poly(A) tails. In the nucleus, the deadenylation process restricts newly added mRNA poly(A) tails to their proper lengths[4] (FIG. 1). In the cytoplasm, extensive deadenylation of an mRNA initiates its degradation or repression[4] (FIG. 1). Deadenylation is often a rate-limiting step for mRNA decay and translational silencing, making it an ideal control point for both processes. Regulation of deadenylation is widespread; deadenylation rates vary widely among mRNAs. Moreover, the length of the poly(A) tail of a single mRNA can vary under different conditions or depending on the cell-cycle phase[6,7]. Globally, as inferred from microarray measurements, mRNAs with longer poly(A) tails are more efficiently translated[6,7]. Regulated deadenylation can act as a rheostat, quantitatively modulating translation in a graduated manner. It can also function as an on–off switch, causing rapid repression and mRNA degradation. Deadenylation diminishes translation initiation by reducing or eliminating mRNA-bound PABP. Reciprocally, the termination of translation can influence deadenylation through interactions between PABP and the termination factor eRF3 (REF. 8). Deadenylation is controlled by sequence-specific factors that bind to mRNAs.

Here, we highlight the diversity, control and biological functions of deadenylases in mRNA regulation. We do not discuss translational control, mRNA stability or regulatory proteins *per se,* as they have been well considered elsewhere[1,4]. We also do not consider the removal of poly(A) from non-coding RNAs, which is associated with nuclear decay of many transcripts[9].

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We discuss new findings that indicate that, in addition to functioning in mRNA decay, deadenylases control specific biological processes. We propose that mRNA deadenylation, decay and repression by many 3′-UTR-borne regulators are all manifestations of the recruitment of multifunctional deadenylase complexes.

Diversity and complexity of deadenylases

Deadenylases are Mg^{2+}-dependent exoribonucleases that hydrolyse RNA in the 3′→5′ direction, which results in release of 5′-AMP. Poly(A) tails are the main substrate of these enzymes; however, some deadenylases can degrade other ribopolymers *in vitro*[10,11]. Once the poly(A) tail is removed from an mRNA, other degradative enzymes initiate its degradation[4] (FIG. 1).

Diversity and biochemical activities. The number of known deadenylases has recently expanded, mainly through biochemical and genetics studies; putative deadenylases have been identified through bioinformatics, but their functions have not yet been established (TABLE 1 and Supplementary information S1 (table)). Based on comparisons of nuclease domains, all known deadenylases belong to one of two groups, the DEDD or the exonuclease–endonuclease–phosphatase (EEP) superfamilies (TABLE 1). DEDD-type nucleases are named after the conserved catalytic Asp and Glu residues that are dispersed between three exonuclease motifs, which coordinate Mg^{2+} ions[12]. Members of this group include the POP2 (also known as CAF1), CAF1Z, poly(A)-specific ribonuclease (PARN) and PAN2 families (TABLE 1 and Supplementary information S1 (table)). The EEP-type enzymes have conserved catalytic Asp and His residues in their nuclease domains. EEP-type enzymes include CCR4, Nocturinin, ANGEL and 2′ phosphodiesterase (2′PDE) families[13] (TABLE 1). Other deadenylases, unrelated to DEDD or EEP enzymes, may exist, but have not yet been described.

Poly(A) tails that are added to many nuclear, non-coding RNAs are also removed through deadenylation[9]. The nuclear exosome complex is strongly implicated in this deadenylation, with the RRP6 subunit, a DEDD-type exonuclease, being the probable deadenylating enzyme[9,14].

The range of deadenylases varies among species. Members of the POP2, CCR4, PAN2 and ANGEL families are present in all eukaryotes (TABLE 1), whereas other deadenylases are less conserved. For example, *Drosophila melanogaster* lacks both PARN and CAF1Z (TABLE 1). *Arabidopsis thaliana*

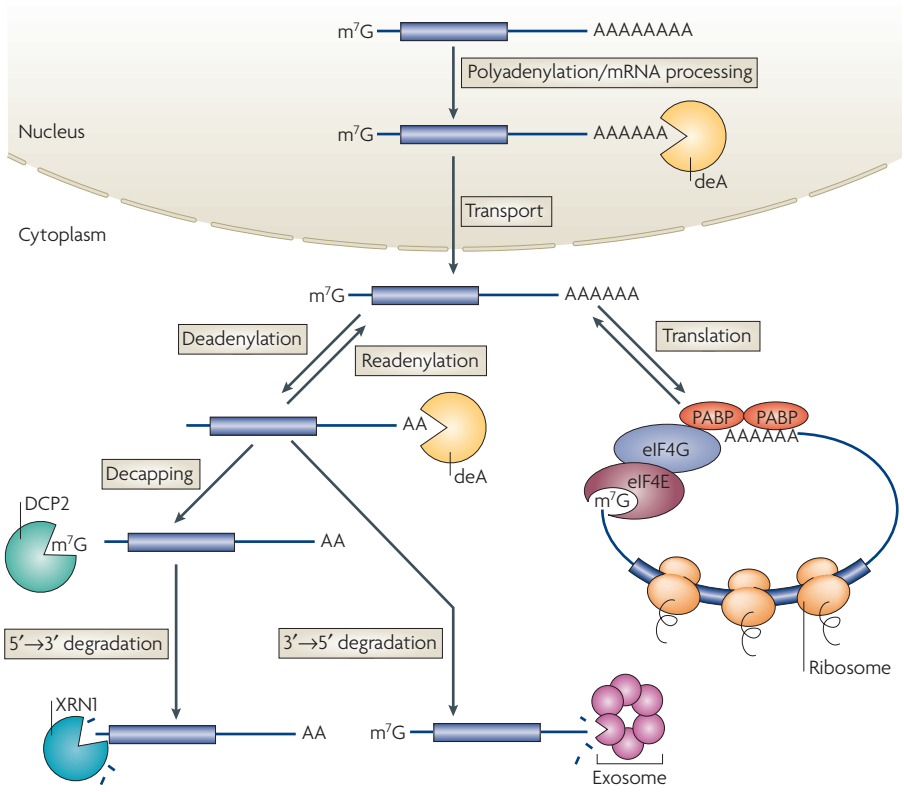

Figure 1 | **Pathways of translation and degradation.** In the nucleus, 3′-end formation and poly(A) addition are necessary for mRNA export. In yeast, the Pan2 deadenylase (deA) trims nuclear tails to their proper length. Once exported to the cytoplasm, the mRNA poly(A) tail enhances translation and stabilizes the mRNA. Poly(A)-bound poly(A)-binding protein (PABP) interacts with the 5′-cap-bound translation initiation factors eIF4G and eIF4E to synergistically enhance translation initiation. By removing the poly(A) tail from mRNA, deadenylases elicit mRNA decay or translational repression, whereas readenylation can activate some mRNAs. More commonly, deadenylated mRNAs are degraded via one of two pathways: a 5′→3′ pathway that proceeds by mRNA decapping (catalysed by the decapping enzyme DCP2) followed by degradation by the exoribonuclease XRN1; or 3′→5′ degradation is catalysed by the exosome complex.

may have as many as 26 deadenylases, probably because of genome-duplication events (Supplementary information S1 (table)). In vertebrates, the POP2, CCR4, PARN and ANGEL families have expanded, due to gene duplications (TABLE 1). Furthermore, informatics predicts several new deadenylases, such as mammalian PARNL and ANGEL1 (REFS 15,16) (TABLE 1).

At least one orthologue from each family exhibits nuclease activity, and the nuclease motifs are conserved (TABLE 1). In some instances, deadenylase activities were demonstrated using both *in vitro* and *in vivo* tests. The identification of ANGEL and 2′PDE as deadenylases was circumstantial: human ANGEL proteins associate with deadenylase complexes[16] and have conserved active-site residues. Moreover, Ngl2 — an orthologue of ANGEL in budding yeast — is an exonuclease[17]. Human 2′PDE can degrade poly(A) tails when the 5′ phosphates of each adenosine are covalently linked between either the 2′ or 3′ hydroxyl positions. Currently, however, the only demonstrated biological role of 2′PDE is in the degradation of 2′–5′-linked oligo(A)[18] (TABLE 1). Further research is needed to identify all active deadenylases and determine their enzymatic properties.

So, what is the advantage of having such a range of deadenylases? It is possible that specific deadenylases target unique subsets of mRNAs, enabling mRNA control to be dictated through regulation of the activity of one enzyme. On the other hand, multiple deadenylases can act on the same mRNA, with discrete but overlapping functions. In yeast, Pan2 deadenylates nascent mRNAs in the nucleus[4] (FIG. 1); however, following the translocation of the mRNA into the cytoplasm, Ccr4 is the predominant deadenylase[19]. In mammalian cells, PAN2 removes about half of the tail, then members of the POP2 and CCR4 families degrade the rest of the tail[20]. How these transitions are coordinated is an important open question.

Deadenylase complexes. Deadenylases are often associated with multisubunit complexes. Other proteins in these complexes can influence the activity of the deadenylase. For example, the deadenylase PAN2 binds to PAN3, which in turn interacts with PABP[4] (Supplementary information S2 (figure)). PABP regulates the activity of the PAN2–PAN3 complex by recruiting it to poly(A) tails (see below).

Certain deadenylases form homo- or heterodimers (Supplementary information S2 (figure)). For example, homodimerization of PARN is necessary for its activity[21]. Yeast Ccr4 and Pop2 form heterodimers[22]. Indeed, heterodimerization greatly expands the repertoire of deadenylase complexes in mammals; seven unique pairings between deadenylases have been documented[16,23] (Supplementary information S2 (figure)). Different heterodimers can have different enzymatic or regulatory properties. For example, CNOT8 (a member of the POP2 family in humans) binds to a PUF regulatory protein more avidly than does another member of the POP2 family in humans, CNOT7 (REF. 24) (see below).

Higher-order deadenylase complexes add further complexity and regulatory potential. For example, specific heterodimers made up of a CCR4 protein and a POP2 protein in various species associate with NOT proteins to form large multisubunit complexes[16,22] (Supplementary information S2 (figure)). The NOT proteins are implicated in both synthesis and deadenylation of mRNAs[22]. Multiple forms of CCR4–POP2–NOT complexes exist[22,25], with molecular weights ranging from 0.65 to 2.0 MDa[25]. NOT proteins might influence the activities of POP2 and CCR4 family members. NOT proteins can also act as adaptors; at least one NOT protein, *D. melanogaster* NOT4, is known to link the deadenylase complex to a 3′-UTR-bound regulatory protein[26]. Between heterodimers and multiple higher-order complexes to which they differentially bind, the repertoire of deadenylase complexes is enormous.

Biological functions of deadenylases

Deadenylases have diverse biological functions, as inferred from genetic and RNA interference experiments (TABLE 1). Some of these enzymes are essential for the viability of organisms, whereas mutants of other deadenylases exhibit specific phenotypes. In metazoans, plants and yeast, the disruption of deadenylases has yielded a range of interesting phenotypes (TABLE 1). None of the deadenylases are essential for yeast viability;

Table 1 | **Diversity of deadenylases**

Family	*Sc*	*Ce*	*Dm*	*Xl*	*Mm*	*Hs*	Active	Notes	Protein domains	Local-ization	Biological functions	Ref
DEDD nucleases												
POP2	Pop2	CCF-1	POP2	CNOT7	CNOT7	CNOT7	*Sc*, *Dm*, *Mm*, *Hs*	Inhibited by PABP	DEDD	C, PB, Sh	DNA damage, cell cycle (*Sc*)	4, 10, 20, 22, 28, 32, 33, 34, 36, 66, 68, 69
											Development, fertility (*Ce*)	
											Cell size, miRNA control (*Dm*)	
											Spermato-genesis, bone mass (*Mm*)	
				CNOT8	CNOT8	CNOT8	*Hs*		DEDD		ND	
CAF1Z		CAF-1z		CAF1Z	CAF1Z	CAF1Z	*Hs*		DEDD, C3H ZnF, NLS	N, CB, Sh	ND	16
PARN		PARN		PARN	PARN	PARN	*Xl*, *Hs*	Binds and stimulated by cap; inhibited by PABP and CBP80	DEDD, R3H, NBD, NLS	N, C, Sh	Oocyte maturation (*Xl*)	4, 20, 27, 38, 61
											NMD (*Hs*)	
					PARNL	PARNL	ND		DEDD			
PAN2	Pan2	PAN-2	PAN2	PAN2	PAN2	PAN2	*Sc*, *Mm*, *Hs*	Stimulated by PABP	DEDD, WD40, UCH	N, C, Sh	Nuclear poly(A) shortening, replication stress (*Sc*)	4, 20, 70
EEP nucleases												
CCR4	Ccr4	CCR-4	CCR4	CNOT6	CCR4	CNOT6	*Sc*, *Dm*, *Mm*, *Hs*	Inhibited by PABP	EEP, LRR	C, PB, Sh	DNA damage, cell cycle, replication stress (*Sc*)	4, 20, 23, 29, 69
											Oogenesis (*Dm*)	
											NMD (*Mm*)	
				CNOT6L	CCR4L	CNOT6L	*Mm*, *Hs*		EEP, LRR	C, Sh	Cell proliferation (*Mm*)	
Nocturnin			NOC	Noc	NOC	NOC	*Xl*, *Mm*, *Hs*		EEP	C	Circadian (*Xl*, *Mm*)	35, 40, 71
											Homeostasis (*Mm*)	
ANGEL	Ngl1	Angel	Angel	Angel1	ANGEL1	ANGEL1	ND		EEP	C, MA (*Sc*)		15–17
	Ngl2			Angel2	ANGEL2	ANGEL2	*Sc*		EEP	C (*Sc*); N, CB, Sh (*Hs*)	5.8S rRNA processing, carbon source (*Sc*)	
	Ngl3						ND		EEP	ND		
2′PDE		2′PDE	2′PDE	2′PDE	2′PDE	2′PDE	*Hs*	Degrades 2′–5′A and 3′–5′A	EEP	ND	Interferon response (*Hs*)	18

Deadenylases from each species were classified into either (Asp-Glu-Asp-Asp) DEDD or exonuclease–endonuclease–phosphatase (EEP) superfamilies on the basis of amino-acid sequences of exonuclease domains. The deadenylases were subclassified into orthologous families on the basis of amino-acid-sequence relationships. Those species in which enzymatic activity has been demonstrated are indicated under the 'Active' column. Orthologous putative deadenylases were identified in the remaining species on the basis of sequence homology. Protein domains were identified by bioinformatics analysis using InterproScan, cDART, Prosite, PredictNLS and NCBI conserved domain searches. The known biological functions and representative references are shown. C3H ZnF, Cys-His zinc finger; C, cytoplasm; CB, cajal body; *Ce*, *Caenorhabditis elegans*; *Dm*, *Drosophila melanogaster*; *Hs*, *Homo sapiens*; LRR, Leu-rich repeat; MA, mitochondrial associated; *Mm*, *Mus musculus*; N, nucleus; ND, not determined; NBD, nucleotide-binding domain, related to the RNA-recognition motif; NLS, nuclear localization signal; PB, processing body; R3H, Arg–His motif; *Sc*, *Saccharomyces cerevisiae*; Sh, nucleo-cytoplasmic shuttling; UCH, ubiquitin C-terminal hydrolase; *Xl*, *Xenopus laevis*.

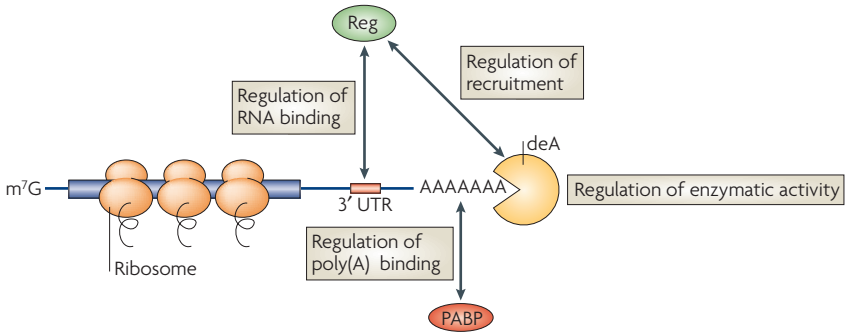

Figure 2 | **Regulation of mRNA deadenylation.** Sequence specific, RNA-binding regulatory factors (Reg) enhance deadenylation by binding to sequence elements in the 3′ untranslated region (UTR) of an mRNA and by recruiting deadenylases (deA) via physical association. The recruited deadenylase then degrades the poly(A) tail, initiating mRNA degradation and repression of translation. Specific examples are shown in TABLE 2. The regulation of deadenylation can be accomplished by affecting protein–RNA binding between the regulator and the target mRNA or poly(A)-binding protein (PABP) and the mRNA poly(A) tail. Likewise, protein–protein interactions, crucial for deadenylase recruitment, are nodes of regulation. Deadenylation can also be controlled by direct modulation of deadenylase enzyme activity. Two-way arrows indicate protein–protein or protein–RNA interactions.

however, mutants exhibit a number of different phenotypes (TABLE 1). Functional redundancy complicates the picture. Although deadenylation is compromised in either *pan2* or *ccr4* single mutants in yeast, it is completely blocked in *pan2 ccr4* double mutants[19]. The double-mutant strain, although slow growing, is viable, which indicates that compensation by other mRNA-decay pathways allows decay to continue, albeit at a reduced rate[19].

Specific deadenylases are necessary for specific biological processes, which indicates that regulating mRNAs is crucial for those processes. Several deadenylases, including *A. thaliana* and *Xenopus laevis* PARN, *D. melanogaster* CCR4 and *C. elegans* CCF-1, are essential during early development[27–31]. Other deadenylases are required for fertility. *Cnot7*-knockout mice, though viable and without conspicuous somatic defects, are defective in spermatogenesis and, therefore, the males are sterile[32,33]. Both *D. melanogaster* CCR4 and *C. elegans* CCF-1 have key roles in the germline, such that reductions in their activity causes sterility[28,29].

Deadenylases also have important roles in physiology. For instance, *Cnot7*-knockout mice have bone-mass increases that are due to enhanced bone formation[34]. Disruption of the mouse Nocturnin gene causes defects in the control of metabolic homeostasis, rendering animals resistant to fat-induced obesity[35]. Indeed, mice that lack Nocturnin gain less weight and fat, despite eating and behaving normally[35].

At the cellular level, deadenylases also control growth. *D. melanogaster Ccr4*-mutants exhibit cell-cycle defects, as do yeast *pop2* and *ccr4* mutants[22,29]. Knockdown of *pop2* expression perturbs control of cell size[36]. In mammals, overexpression of *Cnot7* or *Caf1Z*, or reduction of *Cnot6L* expression, reduces cell growth[23,37].

These intriguing findings provide a first glimpse into the physiological roles of deadenylases. Currently, it is difficult to dissect direct and indirect effects. For example, the phenotype caused by disrupting the Nocturnin gene could be due to mismanagement of many mRNAs or to a specific defect in an obesity-related mRNA. The identification of target mRNAs whose misregulation is responsible for the phenotypes described above is essential.

Regulation of mRNA deadenylation

The regulation of deadenylase activity is essential, as unbridled deadenylation would wreak havoc. Stable, translationally active mRNAs must be protected against poly(A) tail deadenylation, whereas unstable or aberrant mRNAs are deadenylated and degraded[38,39]. Rapid deadenylation of specific targets is superimposed on the basal, slow level of deadenylation of virtually all mRNAs. Therefore, deadenylases are regulated both on global and mRNA-specific bases (FIG. 2).

Expression of deadenylases and their regulators dictate when and where regulation can occur. For example, the expression of Nocturnin is rhythmic, possibly through circadian control[40]. Other deadenylases, such as the members of the POP2 and CCR4 family, are widely and constitutively expressed[23,41]. Inhibition of enzymatic activity provides a second mode of global deadenylation control (FIG. 2). Specific stresses, such as ultraviolet damage, oxidative, osmotic or heat stresses, as well as glucose deprivation, rapidly inhibit deadenylation[42–44]; the molecular mechanism (or mechanisms) that regulate this inhibition are still not known.

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Deadenylation is influenced by two forms of spatial control: nucleocytoplasmic partitioning and localization to granules. Several deadenylases shuttle between the nucleus and the cytoplasm[16,20] (TABLE 1). Changes in partitioning can have dramatic effects: for example, nuclear breakdown during amphibian meiosis releases PARN to the cytoplasm, where it then deadenylates maternal mRNAs[27].

Certain deadenylases are detected in intracellular granules (TABLE 1). These granules — such as germline, stress and neuronal granules, as well as processing (P) bodies — contain repressed mRNAs that can become active[45]. Colocalization of enzymes and substrates may enhance deadenylation kinetics, but this theory has not yet been tested. Regardless, deadenylation is unlikely to be restricted to granules because the same deadenylases are also found throughout the cytoplasm[20,46].

Features of target mRNAs can also influence deadenylation. The mRNA 5′ cap can influence mRNA deadenylation by stimulating the activity and processivity of PARN[4], whereas it has no effect on the activity of other deadenylases (TABLE 1). This unique property is mediated by the direct interaction of PARN with the 5′ cap of mRNA[4]. For some mRNAs, the poly(A) tail can be protected from deadenylases by base-pairing. For example, a uridine-rich (U-rich), *cis*-acting, RNA element of the nuclear PAN RNA from Kaposi's Sarcoma associated herpesvirus prevents its own deadenylation by base-pairing with the poly(A) tail[47]. The U-rich region of the *Saccharomyces cerevisiae Edc1* mRNA may function similarly[48].

Recruitment by regulators. The regulation of specific mRNAs hinges on sequence elements, often located in the 3′ UTRs, that enhance deadenylation. These elements are bound by factors that recruit individual deadenylases and enhance mRNA deadenylation (FIG. 2). Several RNA-binding proteins (CUG-BP, PUF and CPEB),

recruit deadenylases by directly binding to them[24,49,50] (TABLE 2). Even PABP can activate deadenylation by recruiting the PAN2–PAN3 complex to the mRNA[4] (TABLE 2). In mammals, PABP can also recruit complexes of POP2–CCR4 family members through protein interactions that are mediated by the TOB protein[8,51]. In other instances, the association of regulators and deadenylases is known, but the molecular details are not (TABLE 2).

Combinatorial action of regulators expands the versatility of the deadenylation process. Multiple repressors — whether proteins or miRNAs — often bind to the same mRNA 3′ UTR. For example, the yeast repressor proteins Puf4 and Puf5 bind to the 3′ UTR of *HO* mRNA; each protein recruits Ccr4–Pop2 complexes to accelerate deadenylation[52]. Moreover, 3′-UTR-bound complexes can recruit deadenylases through multiple subunits. In *D. melanogaster,* the RNA-binding proteins Pumilio and Nanos bind to one target mRNA *(*hunchback*)* and interact with each other. The Pumilio–Nanos complex then recruits the CCR4–POP2–NOT complex through contacts with different components; Pumilio binds POP2, whereas Nanos binds NOT4 (REF. 26) (TABLE 2).

Deadenylation can also be controlled by modulating the binding of the regulator to the mRNA or the deadenylase (FIG. 2). Phosphorylation of the RNA-binding protein KSRP by the p38 mitogen-activated protein kinase (MAPK) pathway inactivates its RNA-binding activity and disrupts the regulation of the target mRNA deadenylation during myogenic differentiation of cultured mouse cells[53]. Similarly, the *D. melanogaster* protein Oskar has been proposed to inhibit binding of the translational repressor Smaug to its mRNA targets through protein interactions, which leads to derepression of the mRNA of the posterior determinant *nanos* gene during embryogenesis[54] (FIG. 2). The regulator–deadenylase interaction is yet another point of control (FIG. 2); yeast Pbp1 binds to PABP and inhibits Pan2–Pan3 activity, probably by blocking recruitment[55].

Deadenylation antagonists and enhancers. The substrate of deadenylation — the mRNA poly(A) tail — is coated with PABP molecules, which block the access of deadenylases to the poly(A) tail[3] (FIG. 2). Yeast PABP inhibits the activity of purified Ccr4 (REF. 56). In mammals, PABP inhibits PARN deadenylase[57]. *X. laevis* ePAB, a PABP homologue, also inhibits mRNA deadenylation[58]. Regulated PABP dissociation from the mRNA poly(A) tail or PABP–deadenylase interactions can enhance deadenylation[8,51,59,60]. In humans, at least six forms of PABP exist, in addition to other proteins that can bind poly(A) tails (such as NAB2); how they influence deadenylation is unknown[3]. Regulators can also block deadenylation by stabilizing PABP–mRNA complexes. For instance, the α-CP complex of RNA-binding proteins stabilizes α-globin mRNA by binding to PABP[4]. Protein partners can also influence deadenylases activity (FIG. 2). CBP80, a subunit of the nuclear 5′-cap-binding complex, binds to and antagonizes PARN, perhaps by limiting its activity on nascent pre-mRNAs[61].

Regulatory proteins may also act through PABP to enhance mRNA deadenylation (FIG. 2). Helicase-related proteins such as RHAU can enhance deadenylation, perhaps by displacing PABP from the poly(A) tail[62]. Mammalian TOB enhances deadenylation by CCR4–POP2 family member complexes through interaction with the C-terminal (non-RNA-binding) region of PABP; TOB acts as a bridge, joining PABP with the deadenylase complex[8,51].

Table 2 | **Regulators recruit multifunctional deadenylase complexes**

Regulator	Deadenylase	RNA-binding cofactors	mRNA-decay cofactors	Translation cofactors	Refs
CUGBP ↔	PARN				50
PABP ↔	PAN2–PAN3 or POP2–CCR4–TOB				4, 8, 20, 51, 70
PUF ↔	POP2–CCR4–NOT	↔ Nanos	DCP1	Dhh1, 4EHP, Brat	24, 26, 52
CPEB	POP2–CCR4–NOT	CPSF, Symplekin		Maskin, GLD2	49
KSRP	PARN		DCP2, XRN1, exosome		72, 73
Smaug	POP2–CCR4–NOT			CUP	74
TTP	POP2–CCR4–NOT		DCP1, DCP2, HEDLS, XRN1, exosome		75
UNR	POP2–CCR4–NOT	PABP, hnRNP D		PAIP1, NSAP1	76
UPF1, UPF2, UPF3	PARN or POP2–CCR4–NOT and PAN2		DCP1, DCP2, XRN1, exosome,		20, 38
MicroRNA	POP2–CCR4–NOT	Argonaute, GW182	DCP1, DCP2, XRN1	Argonaute, RCK/p54	65, 66

Regulators control mRNA deadenylation, decay and translation by recruiting proteins and enzymes that are involved in these processes to specific mRNAs. RNA-binding cofactors can contribute to combinatorial control and to the recruitment of deadenylases (for example, Nanos). Direct protein interactions between regulators and deadenylases are indicated by two-sided arrows. In the other cases, functional associations between regulators and deadenylases have been demonstrated, although direct contacts have not. 4EHP, eIF4E-homologous protein; CPEB, cytoplasmic polyadenylation element binding protein; CPSF, cleavage polyadenylation specificity factor; DCP, decapping protein; hnRNP D, heterogeneous nuclear ribonucleoprotein D; PABP, poly(A)-binding protein; PAIP1, PABP-interacting protein-1; PARN, poly(A)-specific ribonuclease; XRN1, exoribonuclease-1.

Compensatory polyadenylation. Readenylation can offset deadenylation[5] (FIG. 1). Cytoplasmic poly(A) polymerase enzymes of the GLD2 family activate repressed mRNAs[5,49]. In *X. laevis* oocytes, the RNA-binding protein CPEB binds to cyclin B1 mRNA and simultaneously associates with the polymerase GLD2 and the deadenylase PARN[49] (TABLE 2). The activities of both enzymes are deadlocked until meiosis. During meiosis, phosphorylation of CPEB causes dissociation of PARN, allowing polyadenylation to proceed and activate the mRNA[49]. This mode of control may be common when repressed mRNAs need to be stored and mobilized at a different stage, as occurs at synapses[1].

Multifunctional control

Deadenylase complexes that are recruited by specific regulators can be multifunctional; they contain components that repress mRNA translation and enhance mRNA decay, along with the deadenylase enzymes (FIG. 3). Multifunctionality has two important consequences. First, deadenylation is not sufficient to cause rapid mRNA decay if,

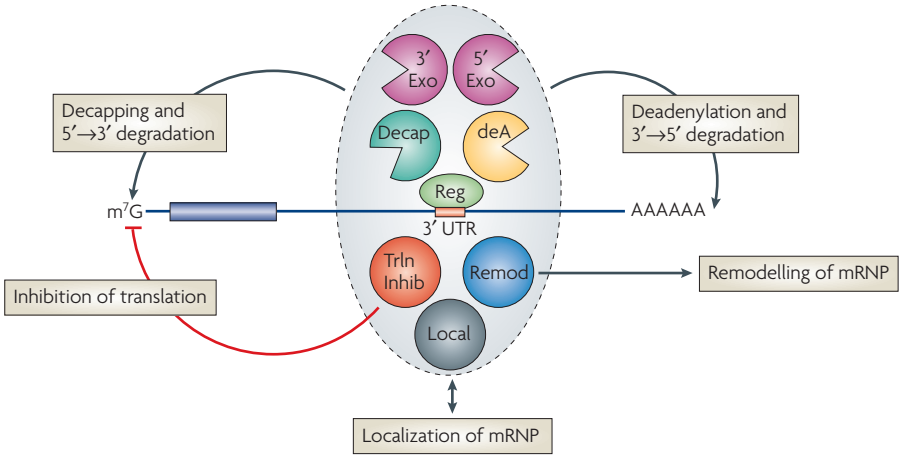

Figure 3 | **Multifunctional control of mRNA decay and translation.** Regulators (Reg) can control mRNA deadenylation, decay and translation by recruiting multifunctional complexes to mRNAs. The composition, assembly and enzymatic activities of the complex dictate the regulatory outcome. These complexes (shown in the grey oval) can include enzymes that deadenylate (deadenylases; deA), decap and degrade mRNAs (for example, 5′ exonucleases such as XRN1 or 3′ exonucleases such as the exosome). Complexes can also consist of proteins that inhibit translation (5′-cap- or eIF4E-binding proteins, or helicases such as Dhh1 orthologues; shown as Trln Inhib), remodel protein–mRNA complexes (for example, helicases; shown as Remod) or control mRNA localization (shown as Local). The remodelling of the mRNA–protein complex (mRNP) could affect one or multiple regulatory steps. The recruitment of a multifunctional regulatory complex can occur through a single molecular contact with a single subunit of that complex. Likewise, the regulator can nucleate the assembly of a multifunctional complex on the mRNA through separate contacts with multiple regulatory factors. In other cases, the recruitment can occur through combinatorial interactions between regulators that are bound to the 3′ untranslated region and several subunits of the complexes.

for that mRNA, other rate-determining steps, such as decapping, lie downstream[63]. Therefore, other factors in the deadenylase complex ensure a more complete mRNA shut-down. Second, multifunctionality provides opportunities to control deadenylation, translation and turnover independently or together, depending on the nature of the complex (FIG. 3; TABLE 2).

Many regulators (such as PUF, CPEB, KSRP, Smaug, TTP and miRNAs) recruit specific deadenylases and cofactors that either inhibit translation (such as Dhh1, Maskin, CUP and 4EHP), promote decapping (such as decapping protein-1 (DCP1) and DCP2) or rapidly degrade mRNA (such as the exosome and exoribonuclease-1 (XRN1)) (FIG. 3; TABLE 2). Recruitment of helicase-related proteins, such as Dhh1 (and its orthologues)[24] and RHAU[62], may facilitate repression and decay by remodelling mRNA–protein complexes[5] (FIG. 3; TABLE 2). Remodelling may displace factors from the mRNA, including translation and regulatory factors. At the same time, remodelling may permit other factors access to the mRNA, such as degradative enzymes.

Even a single deadenylase protein can be multifunctional. PARN binds to and is stimulated by the 5′ cap[4]. When recruited to an mRNA, the PARN–5′-cap interaction might prevent binding of the cap-binding translation initiation factor eIF4E. Consequently, PARN may simultaneously catalyse deadenylation and repress translation, which is consistent with the negative correlation between PARN and eIF4E binding to mRNAs[64]. Similarly, the PARN–5′-cap interaction may ensure that only non-translating mRNAs, no longer associated with eIF4E, are targeted to PARN. CCR4–POP2–NOT complexes also appear to be multifunctional; they possess protein subunits that are involved in the control of cell cycle, mRNA transcription and signal transduction[22], and perhaps they integrate mRNA decay with these processes.

By recruiting a single multiprotein complex, some regulators command many activities at once (FIG. 3; TABLE 2). In yeast, the Ccr4–Pop2–Not complex associates with the mRNA-decapping enzyme and Dhh1 (REF. 22). This higher-order complex is multifunctional, possessing deadenylase, decapping, remodelling and translational repressive activities. Regulators such as PUF proteins recruit this multifunctional complex by making a single interaction with the Pop2 subunit, thereby repressing translation and enhancing mRNA degradation[24,52] (TABLE 2). In other cases, an RNA-binding regulator may recruit each activity through several separate protein interactions. In these cases, the regulator nucleates the assembly of a specific multifunctional complex on the mRNA (FIG. 3). Last, the combinatorial action of several regulators may assemble a multifunctional regulatory complex, unique to a specific mRNA, that is dictated by the 3′-UTR sequence of the mRNA and the specific regulators that recognize it (TABLE 2).

miRNAs can also elicit multifunctional control; they repress mRNA translation, enhance mRNA decay and cause deadenylation[65]. miRNAs interact with Argonaute proteins to form RNA-induced silencing complexes (RISCs)[65]. These complexes enhance mRNA deadenylation through the CCR4–POP2–NOT complex, repress translation through an Argonaute–5′-cap interaction mechanism and promote mRNA decapping through the DCP1–DCP2 decapping complex[65,66] (TABLE 2). Furthermore, RISC components also associate with the RCK (also known as p54) helicase protein, a Dhh1 orthologue that is required for miRNA-mediated repression[67] (TABLE 2).

The heterogeneity of multifunctional deadenylase complexes could explain why a single regulator, or regulatory family, can sometimes promote mRNA translational repression and at other times promote mRNA decay. In yeast, both Puf4 and Puf5 recruit the Ccr4–Pop2–Not complex to their target mRNAs, but Puf5 represses mRNA translation in a deadenylation-independent manner, whereas Puf4 repression is dependent on deadenylation[52]. The difference probably lies in the composition or modification of the deadenylase complexes that they recruit. The mRNA 3′ UTR dictates which deadenylase subcomplex will be attracted. We propose that this sort of versatility is widespread and is used to differentially regulate mRNAs.

Perspectives

The presence of hundreds of RNA-binding proteins and miRNAs in the human genome suggests that mRNA-based regulation is more common than was previously thought. The diversity of mRNA deadenylases and the complexes they form provides robust and flexible opportunities for controlling mRNA. Recruitment of deadenylase complexes to specific mRNAs by RNA-binding regulators is a crucial step in regulation.

Several key issues now need to be addressed. The identity, activity and structure of the full repertoire of deadenylases must be identified. Functional tests will continue to

establish their biological roles, but specific mRNA targets must be defined. The precise composition of deadenylase complexes, and the repressor–mRNA complexes that recruit them, is needed, and their heterogeneity presents a challenge. Complexes may exist that promote mRNA decay but not translational repression, or vice versa. So, two mRNAs may each be associated with deadenylase complexes, yet have different fates — one destroyed, the other repressed.

Ultimately, the type of regulatory complex that is recruited must be dictated by the 3′ UTR of the target mRNAs. To understand how mRNAs are regulated, the RNA-binding factors that recognize it must be identified. It will be crucial to determine which deadenylase complex an RNA-bound regulator recruits and how it discriminates that particular complex through protein interactions. To reveal the functions of deadenylase complexes and their regulators, it will be essential to measure directly the degradation and repression of specific mRNAs and pin those to biological outcomes and phenotypes.

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DATABASES

UniProtKB: http://ca.expasy.org/sprot
CCF-1 | Ccr4 | CNOT7 | CNOT8 | DCP2 | Ngl2 | NOT4 | Pan2 | PAN3 | PARN | Pop2 | XRN1

FURTHER INFORMATION

Marvin Wickens's homepage:
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