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Messenger RNA Turnover in Eukaryotes: Pathways and Enzymes

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The control of mRNA degradation is an important component of the regulation of gene expression since the steady-state concentration of mRNA is determined both by the rates of synthesis and of decay. Two general pathways of mRNA decay have been described in eukaryotes. Both pathways share the exonucleolytic removal of the poly(A) tail (deadenylation) as the first step. In one pathway, deadenylation is followed by the hydrolysis of the cap and processive degradation of the mRNA body by a 5′ exonuclease. In the second pathway, the mRNA body is degraded by a complex of 3′ exonucleases before the remaining cap structure is hydrolyzed. This review discusses the proteins involved in the catalysis and control of both decay pathways.

Keywords mRNA decay, cap, poly(A) tail, regulation of gene expression

INTRODUCTION

A high rate of turnover is one of the distinguishing properties of messenger RNA. Rapid synthesis and equally rapid destruction were postulated early on as key features of a "structural messenger" carrying information from genes to ribosomes, based on the observation that both gene induction and repression happened within minutes. These kinetic considerations were a principal argument to suggest that an experimentally observed small fraction of cellular RNA showing rapid turnover was in fact "messenger RNA" (Jacob & Monod, 1961). The argument leading to the discovery of mRNA also highlights the biological significance of the molecule's instability: It is this feature of mRNA that permits a cell to adapt its pattern of protein synthesis continuously to changing physiological needs. The approach to a new steady state upon both up- and down-regulation of the transcription of any gene is faster if the corresponding mRNA is unstable (Ross, 1995). If mRNA were a stable molecule, it could only be diluted out by cell growth once the gene has been turned off; a doubling of cell mass would be required to reduce the relative rate of synthesis of the encoded protein to one half.

In any given cell, half-lives of mRNAs differ: In *E. coli*, typical mRNAs have half-lives of 1–2 min, whereas the most stable RNAs have half-lives of about 15 min (Coburn & Mackie, 1999). In yeast, the most unstable RNAs have half-lives of approximately 2–3 min, and stable RNAs have half-lives of 90 min or more (Herrick *et al.*, 1990; Wang *et al.*, 2002a). In mammalian cells, the unstable *c-fos* message decays with a half-life of about 15 min; the half-life of the stable β-globin mRNA exceeds 24 h (Shyu *et al.*, 1989). In other words, the half-lives of stable RNAs approach or even exceed the doubling times of the cells in which they are made; if the production of such an RNA ceases, dilution by cell growth can contribute as much to the decreasing mRNA concentration as mRNA decay. In contrast, unstable mRNAs have twentyfold shorter half-lives, and cell growth contributes little to changes in their concentration. (Note that half-life is not really an appropriate parameter to describe mRNA stability, as its decay is not a stochastic process. Consequently, half-life measurements by decay from steady state underestimate the true average life span of an RNA (Cao & Parker, 2001).) Not surprisingly, short-lived mRNAs usually encode proteins of regulatory significance or, for example, enzymes necessary or dispensable, depending on the carbon source of the cell or similar parameters that may change rapidly. The stabilities of individual mRNAs can be regulated by specific signals; stabilization contributes to gene induction and destabilization to gene repression.

This review will focus on the two general pathways of mRNA decay known and the proteins that act in them. We will treat mRNA-specific regulatory proteins only briefly, and we will not discuss additional regulatory aspects. Related reviews have been published recently (Parker & Song, 2004; Wilusz *et al.*, 2001).

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PATHWAYS OF mRNA DECAY

RNA is usually considered an unstable molecule: RNases are ubiquitous and notoriously difficult to inactivate, and RNA is also chemically more labile than DNA. Thus, it is perhaps surprising that the "default state" of an mRNA in a eukaryotic cell is one of relative stability; specific signals are required to accelerate the decay of individual mRNAs. The main reason appears to be that mRNA decay is catalyzed almost exclusively by exonucleases, and the ends of eukaryotic mRNAs are protected against these enzymes by specific terminal structures and their associated proteins: a m^7GpppN cap at the 5′ end and a poly(A) tail at the 3′ end. Removal of these two terminal modifications is thus considered rate limiting for mRNA decay.

mRNA half-lives and degradation pathways are often investigated by an inhibition of transcription and subsequent analysis of the decaying RNA population by Northern blotting or other methods. In mammalian cells, transcription can be inhibited by actinomycin. In yeast, thiolutin or a temperature-sensitive mutation in the largest subunit of RNA polymerase II (*rpb1-1*), which permits mRNA production to be shut off by a shift to the nonpermissive temperature, can be used. In addition to concerns about nonspecific effects of inhibitors, one disadvantage of this type of experiment is the steady-state population of RNA present at the beginning of the time course of degradation, a heterogeneous mixture of "old" and "young" molecules. More clear-cut results are obtained with transcriptional pulse–chase experiments, in which a promoter is activated briefly and then turned off so that the decay of a relatively homogeneous population of mRNA molecules can be observed. In yeast, this is usually done with the help of the GAL1 promoter, which is induced by the addition of galactose and repressed by the addition of glucose. A combination of glucose repression with a temperature shift of a *rpb1-1* mutant allows particularly tight control of transcription (Decker & Parker, 1993). In mammalian cells, the *c-fos* promoter, which is activated by the addition of serum to serum-starved cells and turned off after a short while, or an artificial tetracyclin-regulated system have been used for pulse–chase protocols (Shyu *et al.*, 1989; Xu *et al.*, 1998). In *Drosophila* cells, the same can be achieved through the use of the heat-regulated HSP70 promoter (Dellavalle *et al.*, 1994; Temme *et al.*, 2004). Such experiments have almost invariably shown that mRNAs first lose their poly(A) tails by a process of more or less continuous shortening from the 3′ end; only after the poly(A) tail has been degraded below a certain limit does the total amount of mRNA begin to decrease (Decker & Parker, 1993; Shyu *et al.*, 1991; Wilson & Treisman, 1988). In yeast, the poly(A) tail has to be shortened to about 10 nucleotides (Decker & Parker, 1993), and in mammalian cells to 30–60 nucleotides (Chen *et al.*, 1994), before the RNA begins to disappear. Although RNAs with poly(A) tails of this length sometimes accumulate, suggesting that the second step of decay can also be slow, the product of this second and any subsequent steps is not detectable under normal conditions, so that the pathway of decay following deadenylation cannot be deduced. In yeast, further analysis has been possible by two methods: First, mutant strains blocked at certain steps in the decay pathway accumulate the corresponding intermediates, which can then be examined (Hsu & Stevens, 1993; Muhlrad *et al.*, 1994). Second, the incorporation of short oligo(G) tracts at internal positions of an mRNA blocks the activity of exonucleases; the structure of the accumulating intermediates demonstrates that exonuclease digestion is responsible for mRNA decay and reveals the enzymes' directionality (Decker & Parker, 1993; Vreken & Raué, 1992).

These experiments have shown that, in the major mRNA decay pathway of *S. cerevisiae*, the step following deadenylation is hydrolysis of the 5′ cap to m^7GDP and an mRNA with a 5′ monophospate. Cap hydrolysis depends on almost complete deadenylation, and thus this pathway is called the *deadenylation-dependent decapping pathway*. The RNA product of the decapping step is then degraded by a 5′ exonuclease (Hsu & Stevens, 1993; Muhlrad *et al.*, 1994). Mutational inactivation of this pathway revealed a second pathway in which, after deadenylation, the RNA is degraded from the 3′ end (Jacobs Anderson & Parker, 1998). The remaining cap structure is then hydrolyzed to m^7GMP (Liu *et al.*, 2002; Wang & Kiledjian, 2001). Significant quantities of oligo(G)-stabilized intermediates reflecting a 3′ exonucleolytic decay of mRNA are seen only when 5′ decay is blocked (Jacobs Anderson & Parker, 1998; Muhlrad *et al.*, 1995). Also, mutations affecting 3′ decay do not increase the half-lives of most mRNAs when the strain is wildtype with respect to 5′ decay. Both observations suggest that 3′ decay is of secondary importance for the turnover of the mRNAs examined (Jacobs Anderson & Parker, 1998). However, a genome-wide survey of mRNA abundance showed that mutations affecting the 5′ pathway changed the abundance of less than 20% of all yeast mRNAs, indicating that the 3′ pathway might actually be more important (He *et al.*, 2003). The reciprocal experiment of examining mRNA abundance in mutants defective in the 3′ decay pathway has not been reported, though. In mammalian cells, 3′ decay has been argued to be the predominant pathway, based mostly on *in vitro* evidence (Chen *et al.*, 2001a; Mukherjee *et al.*, 2002; Wang & Kiledjian, 2001).

Simultaneous inactivation of both decay pathways in *S. cerevisiae* is lethal and, in conditional mutants, leads to almost complete stability of mRNAs under nonpermissive conditions, indicating that no other pathway of general importance exists (Jacobs Anderson & Parker, 1998). A mathematical model for cytoplasmic mRNA turnover

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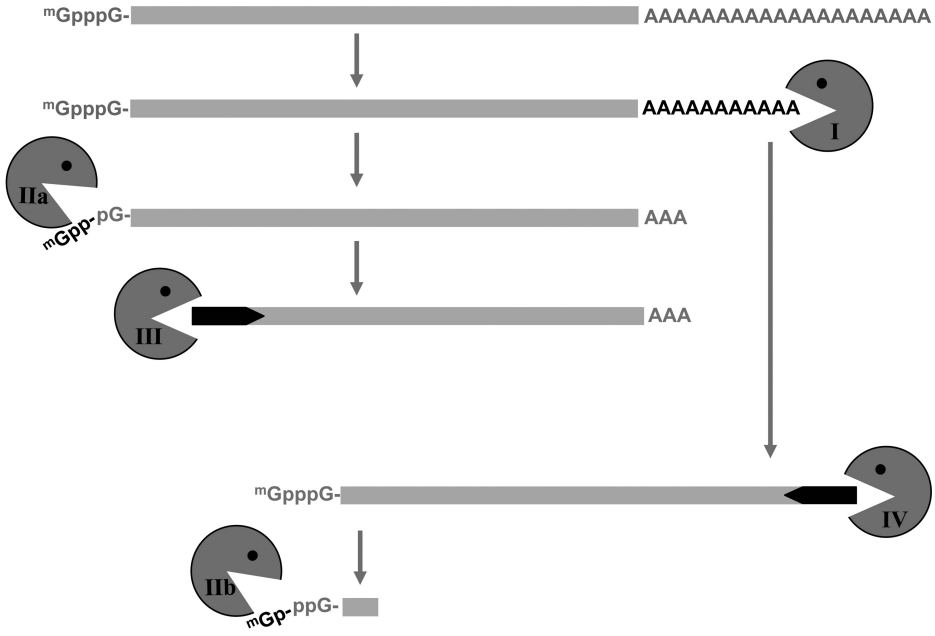
has been developed, based on the two pathways described above and experimentally determined rate constants (Cao & Parker, 2001). This model reproduces experimental observations. It shows, for example, that the rate of deadenylation has the greatest influence on the overall rate of mRNA decay, in agreement with the experimental observation that this is the main regulated step (see below).

In mammalian cells, inactivation of decay enzymes has barely been used so far for the analysis of decay pathways, and accumulation of stable intermediates due to oligo(G) tracts inserted into mRNAs has been reported only *in vitro* (Wang & Kiledjian, 2001). The conviction that the two pathways described above also exist in mammalian cells rests on four types of data. First, deadenylation can be directly observed as the first step in mRNA decay *in vivo* (Chen & Shyu, 1995; Shyu *et al.*, 1991; Wilson & Treisman, 1988). Second, homologs for all enzymes catalyzing the two yeast pathways exist in higher eukaryotes, and their catalytic activities have been confirmed (see below). Third, the 5′-monophosphorylated RNA product of the decapping reaction has been detected by an indirect method relying on mRNA circularization and reverse transcription polymerase chain reaction (RT-PCR) analysis (Couttet *et al.*, 1997). These experiments also provided evidence for a dependence of decapping on prior deadenylation. Finally, deadenylation and 3′ decay followed by cap hydrolysis has been observed in cell-free extracts from mammalian cells (Chen *et al.*, 2001a; Mukherjee *et al.*, 2002; Rodgers *et al.*, 2002b; Wang & Kiledjian, 2001).

The two general pathways of mRNA decay and the proteins that act in them are summarized in Figure 1.



Reaction		Enzymes		Effectors
		Yeast	Mammals	
I	deadenylation	• Pan2p/Pan3p • CCR4-NOT complex	• Pan2/Pan3 • CCR4-NOT complex • PARN	PABPC PABPC cap
IIa	decapping	• Dcp1p/Dcp2p	• Dcp1/Dcp2	Edc1p; Edc2p; Edc3p; Pat1p; Lsm1-7; Dhh1p; PABPC
IIb	cap hydrolysis	• Dcs1p	• DcpS	
III	5′-3′exonucleolytic decay	• Xrn1p	• Xrn1	
IV	3′-5′exonucleolytic decay	• exosome	• exosome	Ski2p; Ski3p; Ski7p; Ski8p

FIG. 1. A summary of the two general pathways of eukaryotic mRNA decay and the participating proteins. Both inhibitory and stimulatory molecules are listed under the heading "effectors." See text for details.

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Whereas the majority of mRNAs are degraded by one or both of the two general pathways described above, the decay of some mRNAs is initiated by endonucleases (Binder *et al.*, 1994; Chernokalskaya *et al.*, 1998; Gallouzi *et al.*, 1998; Ioannidis *et al.*, 1996; Rodgers *et al.*, 2002a; van Dijk *et al.*, 2001). The 5′ cleavage fragment produced by endonucleolytic scission is likely to be equivalent to a deadenylated RNA and may thus be degraded by the 3′ decay pathway. Similarly, the 3′ cleavage fragment, which lacks a cap, is probably a substrate for the 5′ decay pathway. As both cap and poly(A) tail are required for the initiation of translation (Sachs, 2000), none of the intermediates of any of the pathways of mRNA decay is likely to be translated at a significant rate (see, however, Thoma *et al.*, 2001). This is important to prevent the synthesis of truncated proteins, which might be harmful for the cell. Removal of decay intermediates from the translatable RNA pool should be most efficient for the deadenylation-dependent decapping pathway since the mRNA is deprived of both cap and poly(A) tail before the coding sequence is attacked.

All cells have quality control pathways for the rapid and specific elimination of aberrant mRNAs. These pathways include nuclear or cytoplasmic degradation of mRNA precursors that failed to be properly processed (Hilleren & Parker, 2003; Moore, 2002); nonsense-mediated decay (NMD), which eliminates mRNAs carrying a premature stop codon (Maquat, 2004); and nonstop decay, a degradation pathway for mRNAs lacking a stop codon (Frischmeyer *et al.*, 2002; van Hoof *et al.*, 2002). These pathways use the same enzymes responsible for general mRNA decay but, at least in the cases of nonsense-mediated decay and nonstop decay, involve mechanisms for specific targeting of these enzymes to aberrant mRNAs. These specialized pathways will not be discussed here.

ENZYMES AND PROTEINS OF THE DEADENYLATION-DEPENDENT DECAPPING PATHWAY

Deadenylating Enzymes

The first poly(A) degrading 3′ exoribonuclease to be described was the poly(A) nuclease (PAN) of *S. cerevisiae*, which consists of the Pan2p and Pan3p subunits (Boeck *et al.*, 1996; Brown *et al.*, 1996). PAN has also been characterized in human cells and appears to be conserved in all metazoans (Uchida *et al.*, 2004). Pan2 is the catalytic subunit of the heterodimer; it is a Mg^{2+}-dependent enzyme liberating 5′-AMP from the 3′ end (Lowell *et al.*, 1992; Uchida *et al.*, 2004). Pan2 is a member of a family of 3′ exoribonucleases, which was originally described as the RNase D family (Moser *et al.*, 1997) and later renamed DEDD superfamily, with RNase D-like enzymes forming a subfamily (Zuo & Deutscher, 2001). These enzymes, which are related to the 3′ exonuclease domain of *E. coli* DNase I, contain four conserved acidic residues in three exonuclease motifs, which bind the two divalent metal ions involved in catalysis. A characteristic feature of PAN is the dependence of its activity on coating of the poly(A) substrate by the cytoplasmic poly(A) binding protein (PABPC; Pab1p in yeast) (Sachs & Deardorff, 1992; Uchida *et al.*, 2004). The PAN3 subunit binds directly to PABPC (Uchida *et al.*, 2004). In the absence of PABPC, PAN can also be activated by rather high salt concentrations or by spermidine. The enzyme still prefers poly(A) as a substrate under these conditions, i.e., its substrate specificity is not determined solely by PABPC (Lowell *et al.*, 1992; Uchida *et al.*, 2004).

Deletions of PAN2 or PAN3, singly or in combination, do not affect the viability of yeast. Steady-state bulk poly(A) is approximately 20 nucleotides longer in such strains, confirming a role of the enzyme in poly(A) tail metabolism *in vivo* (Boeck *et al.*, 1996; Brown *et al.*, 1996). However, PAN is clearly not the main deadenylating enzyme in yeast; its main role is thought to be an initial shortening of poly(A) tails (Brown & Sachs, 1998). Even though this reaction occurs very rapidly, very soon after poly(A) tail synthesis, PAN is localized in the cytoplasm both in yeast (Huh *et al.*, 2003) and in human cells (Uchida *et al.*, 2004). Thus, PAN-catalyzed shortening may occur after nuclear export of the RNA. The fact that residual deadenylation upon knockout of the main deadenylase is abolished if PAN2 is also deleted (Tucker *et al.*, 2001) argues for a cytoplasmic role of PAN as well.

The major deadenylase of yeast is the CCR4-NOT complex (Daugeron *et al.*, 2001; Tucker *et al.*, 2001), and this complex is conserved in mammals (Albert *et al.*, 2000) and in *Drosophila* (Temme *et al.*, 2004). The experiments through which the yeast complex was initially identified as a potential regulator of transcription have been reviewed recently (Collart, 2003; Denis & Chen, 2003). The Ccr4p subunit of the complex has sequence similarity to a family of Mg^{2+}-dependent nucleases related to *E. coli* exonuclease III. Deletion of the CCR4 gene causes a decrease in the rate of deadenylation and a stabilization of mRNAs (Tucker *et al.*, 2001). Point mutations affecting the predicted active site of Ccr4p cause a similar deadenylation defect, demonstrating that the catalytic activity of the protein is required for deadenylation (Chen *et al.*, 2002; Tucker *et al.*, 2002). *Drosophila ccr4* mutants also have a deadenylation defect (Temme *et al.*, 2004). Both yeast Ccr4p and its mammalian homolog are active as poly(A)-specific 3′ exoribonucleases *in vitro* in the absence of the other subunits of the CCR4-NOT complex (Chen *et al.*, 2002; Viswanathan *et al.*, 2003). The activity of an immunoaffinity-purified CCR4-NOT complex

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is inhibited by Pab1p (Tucker *et al.*, 2002). The reaction product of this complex is 5′ AMP (Tucker *et al.*, 2001). In addition to its C-terminal nuclease domain, Ccr4p contains a conserved leucine-rich repeat that is essential for binding the Pop2p/Caf1p subunit of the complex and also affects exonuclease activity (Clark *et al.*, 2004).

The Pop2p/Caf1p subunit of the CCR4-NOT complex is a member of the RNase D (or DEDD) family of exonucleases (Daugeron *et al.*, 2001; Moser *et al.*, 1997). While the predicted catalytic site of yeast Pop2p is not very well conserved, the isolated polypeptide is active as a 3′ exonuclease with some preference for poly(A) *in vitro*, but the activity is very much weaker than that of Ccr4p (Daugeron *et al.*, 2001; Thore *et al.*, 2003; Viswanathan *et al.*, 2004). The active sites of Pop2/Caf1 orthologs in other organisms are better conserved, and therefore these proteins might have a more robust activity. Crystallographic analysis revealed a structure of yeast Pop2p/Caf1p closely resembling that of the DnaQ proofreading exonuclease, thus confirming the protein's membership in the DEDD family (Thore *et al.*, 2003). Deletion of yeast POP2/CAF1 leads to a similar deadenylation defect as a *ccr4*Δ mutation (Daugeron *et al.*, 2001; Tucker *et al.*, 2001). Similarly, inactivation of *Drosophila* CAF1 causes an increased steady-state length of bulk poly(A) and a decrease in the rate of HSP70 mRNA deadenylation (Temme *et al.*, 2004). However, in yeast the catalytic activity of Pop2p/Caf1p appears to be irrelevant under normal conditions, as point mutations in the active site do not generate a deadenylation phenotype and do not affect the catalytic activity of the CCR4-NOT complex *in vitro* (Chen *et al.*, 2002; Tucker *et al.*, 2002; Viswanathan *et al.*, 2004; C. Temme and E. Wahle, unpublished data). The phenotype of the *pop2*Δ*/caf1*Δ mutant may be explained by the fact that Pop2p/Caf1p links Ccr4p to the remainder of the CCR4-NOT complex (Chen *et al.*, 2001b).

The yeast Ccr4 and Pop2/Caf1 proteins have been purified as part of a complex that also contained the Not1–5 proteins, Caf40p and Caf130p, and all these polypeptides were also associated in immunoprecipitations (Chen *et al.*, 2001b). An even larger complex containing additional proteins has also been described (Denis & Chen, 2003). In contrast, TAP-tag purifications using different "entry points" consistently resulted in copurification of Ccr4p, Pop2p/Caf1p, Caf40p, Caf130p, and Not1p, but not of Notp2p–5p (Gavin, 2002). In agreement with earlier data (Bai *et al.*, 1999; Chen *et al.*, 2001b) this suggests that the CCR4-NOT complex may be composed of two physically and functionally distinct units, one containing the two catalytic polypeptides as well as Caf40p and Caf130p, the other comprised of Not2p–5p. Both appear to be linked primarily *via* Not1p (Chen *et al.*, 2001b). Nevertheless, *not2*Δ, *not5*Δ, and possibly *not3*Δ and *not4*Δ mutants have a deadenylation phenotype (Tucker *et al.*, 2002), and the *Drosophila* Not proteins are also important for deadenylation (Temme *et al.*, 2004). These data argue that the entire CCR4-NOT complex functions in deadenylation. In agreement with this, all components of the complex that have been examined are localized mainly in the cytoplasm (Cougot *et al.*, 2004a; Huh *et al.*, 2003; Temme *et al.*, 2004; Tucker & Parker, 2000; Tucker *et al.*, 2002). In yeast, all subunits examined are also present in roughly similar amounts with the exception of Caf40p, which is more abundant (Huh *et al.*, 2003). Beyond their apparent involvement together with Ccr4p and Pop2p/Caf1p in mRNA deadenylation, not much is known about the functions of the additional subunits of the complex except that the human Not4 protein functions as a ubiquitin–protein ligase (Albert *et al.*, 2002).

The Pop2p/Caf1p subunit of the yeast CCR4-NOT complex is phosphorylated by the Yak1p kinase upon glucose depletion. A *pop2/caf1* mutant that cannot be phosphorylated causes a defect in cell cycle control upon glucose depletion (Moriya *et al.*, 2001). The CCR4-NOT complex is also associated with the cell-cycle-regulated protein kinase Dbf2p, and *dbf2* mutants share several phenotypes with mutations in subunits of the CCR4-NOT complex (Liu *et al.*, 1997). Modification and subsequent degradation of the Not3p and Not5p subunits upon stress has also been reported (Lenssen *et al.*, 2002).

Many eukaryotes contain not only orthologs of yeast CCR4, identified by the presence of both the nuclease domain and the leucine-rich repeat, but also paralogs that lack the latter. Most of these paralogs can be grouped into three families (Dupressoir *et al.*, 2001). *Xenopus* nocturnin, a representative of one of these families, which has been implicated in the generation of a circadian rhythm and is itself expressed in a 24 h rhythm, has poly(A)-specific 3′ exonuclease activity *in vitro* (Baggs & Green, 2003). The two other families of Ccr4-related proteins have not been examined for catalytic activity. The human genome also contains two different Pop2/Caf1 homologs (Albert *et al.*, 2000). These data suggest that there may be different versions of the CCR4-NOT complex that catalyze mRNA deadenylation.

Although the CCR4-NOT complex is inhibited by Pab1p, *pab1* mutants have a defect in deadenylation (Caponigro & Parker, 1995; Sachs & Davis, 1989). One aspect of this defect is an extended lag phase before the onset of deadenylation. This might reflect a defect in the initial poly(A) tail shortening catalyzed by PAN—recall that this enzyme is Pab1p-dependent (Chekanova *et al.*, 2001; Kühn & Wahle, 2004). Unexpectedly, *pab1* strains display not only the lag phase but also lower rates of deadenylation. This is currently unexplained but might be caused by other proteins taking the place normally occupied by Pab1p and acting as a stronger barrier to the deadenylase (Caponigro & Parker, 1995).

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Most eukaryotes contain, in addition to the CCR4-NOT complex and PAN, a third poly(A)-degrading enzyme, the poly(A)-specific ribonuclease (PARN; initially called DAN; Körner & Wahle, 1997; Körner *et al.*, 1998). PARN, a member of the DEDD nuclease family (Zuo & Deutscher, 2001), is a poly(A)-specific 3′ exoribonuclease, releases 5′-AMP in a Mg^{2+}-dependent manner and is inhibited by PABPC (Körner & Wahle, 1997; Ren *et al.*, 2002). The enzyme consists of a single polypeptide of 74 kDa but functions as a homooligomer (Martinez *et al.*, 2000). Unexpectedly, a 5′ cap stimulates PARN activity by increasing the processivity of degradation (Dehlin *et al.*, 2000; Gao *et al.*, 2000; Martinez *et al.*, 2001). An analysis of the *in vivo* function of PARN has been hampered by the fact that neither *S. cerevisiae* nor *Drosophila* contain such an enzyme. The so-called default deadenylation during *Xenopus* oocyte maturation, a general, sequence-independent poly(A) degradation reaction that serves in translational regulation and does not lead to mRNA degradation, is catalyzed by PARN (Körner *et al.*, 1998). So far, there is little direct evidence that PARN participates in regular mRNA turnover in somatic cells. The enzyme has been reported to be associated with RHAU, a protein thought to promote the decay of urokinase plasminogen activator mRNA, but the role of PARN in its deadenylation was not examined (Tran *et al.*, 2004). *In vitro* evidence for ARE-dependent deadenylation catalyzed by PARN and regulated by the RNA-binding protein tristetraprolin has been published (Lai *et al.*, 2003; see below). Reduction of PARN abundance by RNA interference has been found to increase the abundance of mRNAs that are subject to NMD; however, the rate of deadenylation was not measured (Lejeune *et al.*, 2003). *Arabidopsis* contains a single PARN-like gene that is ubiquitously expressed and essential for plant development (Chiba *et al.*, 2004). A role in mRNA decay has not yet been tested.

The Decapping Enzyme and Associated Proteins

Deadenylation prepares the mRNA for cap hydrolysis. This process has been reviewed very recently (Cougot *et al.*, 2004b). Cap cleavage is catalyzed by the yeast Dcp2 protein or its mammalian homolog (Lykke-Andersen, 2002; van Dijk *et al.*, 2002; Wang *et al.*, 2002b). Earlier reports attributing the activity to Dcp1p are no longer considered correct (Parker & Song, 2004; van Dijk *et al.*, 2002). Dcp2p is a member of the Nudix family of pyrophosphatases, enzymes that hydrolyse nucleoside diphosphates linked to some other moiety, **X** (Bessman *et al.*, 1996). The recombinant Dcp2 protein is active *in vitro* and releases m^7GDP from a capped RNA, leaving a 5′ monophosphate on the RNA. The enzyme requires a divalent cation for catalysis and is specific for cap methylated on N7. It also prefers capped RNA as a substrate; the rate of hydrolysis of free m^7GpppN is very low. The activity increases significantly when the length of the RNA is increased from around 20–50 to ∼100 nucleotides (Steiger *et al.*, 2003; Stevens, 1988; van Dijk *et al.*, 2002), suggesting the existence of an RNA-binding site on Dcp2p that is distinct from the active site. Deletion analysis has identified such a site on the C-terminal side of the Nudix domain (Piccirillo *et al.*, 2003). *dcp2*Δ mutants have a defect in mRNA decay, accumulating oligoadenylated, capped mRNA degradation intermediates (Dunckley & Parker, 1999). The Dcp2 protein is associated with the Dcp1 protein (Dunckley & Parker, 1999; Gavin, 2002; Lykke-Andersen, 2002; Steiger *et al.*, 2003). Dcp1p is required for decapping *in vivo* (Beelman *et al.*, 1996; Hatfield *et al.*, 1996) and stimulates the activity of Dcp2p *in vitro* (Steiger *et al.*, 2003). The structure of yeast Dcp1p has been solved by X-ray crystallography, and point mutations have been identified that affect cap hydrolysis without interfering with the binding of Dcp1p to the catalytic subunit, Dcp2p (She *et al.*, 2004). The mechanism by which Dcp1p stimulates cap hydrolysis remains to be investigated. Human cells contain two different Dcp1 homologs, Dcp1a and Dcp1b (Lykke-Andersen, 2002). A stimulatory effect of these proteins on the activity of Dcp2 has not been found so far.

Genetic evidence first indicated that decapping in yeast was stimulated by the Edc1 and Edc2 proteins, two small basic proteins that have sequence similarity to each other. Edc1p associates with the Dcp1/Dcp2 complex (Dunckley *et al.*, 2001). Both Edc1p and Edc2p bind RNA and are able to stimulate the *in vitro* decapping reaction individually and quite dramatically (Schwartz *et al.*, 2003; Steiger *et al.*, 2003). Nevertheless, *edc1*Δ and *edc2*Δ mutations do not affect decapping under normal conditions *in vivo* (Dunckley *et al.*, 2001), indicating that the catalytic activity is normally not rate-limiting for cap hydrolysis *in vivo* (Schwartz *et al.*, 2003). Interestingly, Edc1p is involved in changes in gene expression upon a shift from glucose to glycerol as a carbon source (Schwartz *et al.*, 2003). The product of the *S. cerevisiae* EDC3 gene also enhances mRNA decapping *in vivo*, presumably by directly affecting the Dcp1p/Dcp2p decapping enzyme (Kshirsagar & Parker, 2004). Homologs of the Edc proteins in higher eukaryotes have not been characterized.

Mutations in the PAT1 (=MRT1) gene reduce the rate of decapping *in vivo* but have no effect on cap hydrolysis in extracts; thus, they probably do not affect the catalytic step (Bonnerot *et al.*, 2000; Bouveret *et al.*, 2000; Hatfield *et al.*, 1996). Pat1p also appears to be involved in the initiation of translation (Wyers *et al.*, 2000). It is unknown if and how this function is connected to the protein's role in mRNA decay. Pat1p, which has been described only in yeast so far, is associated with the cytoplasmic Lsm complex (Bouveret *et al.*, 2000; Tharun *et al.*, 2000). The Lsm proteins, which are conserved in

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evolution, are related to the Sm proteins, which form the core of the spliceosomal snRNPs: Both types of proteins contain the so-called Sm domain, and both form seven-membered, doughnut-shaped rings (Achsel *et al.*, 1999; Salgado-Garrido *et al.*, 1999). Whereas a nuclear Lsm complex, containing Lsm2p–8p, associates with the U6 spliceosomal snRNA and thus participates in splicing, the cytoplasmic version, containing the Lsm1–7 proteins, is involved in mRNA decapping (Boeck *et al.*, 1998; Bouveret *et al.*, 2000; He & Parker, 2000; Tharun *et al.*, 2000). Surprisingly for a factor promoting decapping, the Lsm complex probably binds the 3′ end of the mRNA (He & Parker, 2001). It has been proposed that the Lsm–Pat1p complex facilitates cap hydrolysis either by promoting rearrangements in the mRNP complex that favor access to the cap (see below) or by directly recruiting the decapping enzyme (He & Parker, 2000). Another protein associated with the cytoplasmic Lsm complex is the DEAD box protein Dhh1p, predicted to be a RNA-dependent NTPase and possibly a RNA helicase. The homolog of Dhh1p in higher eukaryotes is the rck/p54 protein, which has been demonstrated to have ATP- or GTP-dependent RNA helicase activity (Cougot *et al.*, 2004a; Ladomery *et al.*, 1997; Minshall *et al.*, 2001). *dhh1*Δ mutants display increases in mRNA half-life due to a defect in cap hydrolysis (Coller *et al.*, 2001; Fischer & Weis, 2002). Why cap hydrolysis should depend on a helicase is unclear. Like Pat1p, Dhh1p appears to have a second function, possibly in translation (Coller *et al.*, 2001). Surprisingly, Dhh1p is also associated with the CCR4-NOT complex (Coller *et al.*, 2001; Hata *et al.*, 1998). However, the rate of deadenylation is not affected by a *dhh1* mutation (Coller *et al.*, 2001).

In NMD, decapping is also catalyzed by the Dcp1p/Dcp2p complex but is independent of deadenylation (Beelman *et al.*, 1996; Dunckley & Parker, 1999). In contrast to the decapping enzyme itself, Pat1p (Hatfield *et al.*, 1996), Dhh1p (Coller *et al.*, 2001; Fischer & Weis, 2002), and the Lsm complex (Boeck *et al.*, 1998) are dispensable for NMD and required only for deadenylation-dependent decapping in the decay of regular mRNAs. This implies that they are not involved in the catalytic step itself but in the control of decapping, presumably recognition of the deadenylated substrate RNA. The dependence of decapping upon prior deadenylation is mediated by the cytoplasmic poly(A)-binding protein, Pab1p, acting as an inhibitor of decapping; shortening of the poly(A) tail to a length insufficient for binding of Pab1p prevents the inhibition, and artificial tethering of Pab1p to the RNA makes the protein a poly(A)-independent inhibitor of cap hydrolysis (Caponigro & Parker, 1995; Coller *et al.*, 1998).

Deadenylation may favor decapping in three ways. First, deadenylation may lead to the dissociation of translation initiation factors and thus to exposure of the cap: Pab1p interacts with eIF4G and thus, indirectly, with the cap-binding protein eIF4E, stabilizing their interaction with polyadenylated RNA (Tarun & Sachs, 1996; reviewed by Kühn & Wahle, 2004; Sachs, 2000). The dissociation of Pab1p should therefore deprive the cap of initiation factors, baring it for hydrolysis. In fact, it has been shown experimentally that the loss of Pab1p from the RNA is accompanied by the loss of translation initiation factors and the association of the Lsm complex and the decapping enzyme (Tharun & Parker, 2001). A competition between cap-binding translation factors and decapping has been confirmed by biochemical and genetic experiments (Schwartz & Parker, 1999, 2000a, 2000b). However, there are reasons to believe that stabilization of the cap-eIF4E interaction is not the only way for PABPC to prevent decapping (Kühn & Wahle, 2004). Second, it has been reported that human PABPC can associate directly with the cap, protecting it from hydrolysis independently of eIF4E. Since cap binding was favored by a poly(A) tail on the same RNA molecule, loss of the poly(A) tail would again be expected to favor cap cleavage (Khanna & Kiledjian, 2004). However, as direct binding to cap was not found for yeast Pab1p, this cannot be a general mechanism to explain the deadenylation-dependence of decapping. As a third way for deadenylation to promote decapping, the loss of Pab1p may make the RNA's 3′ end available for association with the Lsm complex. This, in turn, is thought to recruit the Dcp1p/Dcp2p complex.

The 5′ Exonuclease XRN1

After cap hydrolysis, the mRNA is rapidly degraded by Xrn1p, a divalent cation-dependent processive 5′ exonuclease. Capped RNA is resistant to Xrn1p, and RNA carrying a 5′ monophosphate is strongly preferred over RNA with a 5′ hydroxyl end. The products are 5′-NMPs (Stevens, 1980). The enzyme was first characterized in yeast, but homologous proteins exist in mammals (Bashkirov *et al.*, 1997), flies (Till *et al.*, 1998) and worms (Newbury & Woollard, 2004). Yeast *xrn1* mutants accumulate full-length mRNAs lacking a cap structure (Hsu & Stevens, 1993; Muhlrad *et al.*, 1994). Xrn1p activity is inhibited by the accumulation of adenosine 3′–5′ bisphosphate upon amino acid starvation in yeast so that the relative importance of the alternative 3′ decay pathway increases under these conditions (Benard, 2004).

Localization of the Deadenylation-Dependent Decapping Pathway

The mammalian Xrn1 nuclease was initially found to be concentrated in a number of cytoplasmic foci (Bashkirov *et al.*, 1997), and similar observations were made for the Xp54 helicase, the likely *Xenopus* orthologue of the Dhh1 protein (Ladomery *et al.*, 1997). Later, additional

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components of the 5′–3′ decay pathway were also detected in cytoplasmic foci in mammalian cells: The human Lsm1–7 proteins colocalize with each other as well as with Xrn1, rck/p54, and the Dcp1-Dcp2 decapping enzyme (Cougot *et al.*, 2004a; Ingelfinger *et al.*, 2002; Lykke-Andersen, 2002; van Dijk *et al.*, 2002). An additional component of these foci is GW182, a 182 kD protein that contains an RNA recognition motif (RRM) (Eystathioy *et al.*, 2003). Fluorescence energy transfer measurements and other experiments indicate that polypeptides in these foci associate with each other in the anticipated combinations (Cougot *et al.*, 2004a; Ingelfinger *et al.*, 2002). Similar foci (P bodies) containing the Lsm proteins Dcp1p, Dcp2p, Pat1p, Xrn1p, and Dhh1p have been found in yeast (Sheth & Parker, 2003). Manipulations that change the flow of RNA through the 5′ decay pathway cause changes in the number or size of both yeast and mammalian foci. For example, inhibiting 5′ decay by mutation leads to an accumulation of the corresponding decay intermediates and increases the abundance and size of cytoplasmic foci in yeast (Sheth & Parker, 2003). Similar experiments using RNA interference in mammalian cells cause an accumulation of RNA in the foci (Cougot *et al.*, 2004a). Most importantly, a specific trapped mRNA decay intermediate has been localized to the foci in yeast (Sheth & Parker, 2003). These data strongly suggest that the foci are sites where cytoplasmic mRNA decay takes place. However, even in those cases where the localization of endogenous mRNA decay factors was examined, as opposed to products of transfected genes with their unknown and uncontrolled expression levels, widespread cytoplasmic staining was usually seen in addition to the foci, suggesting that mRNA decay may not be limited to the foci.

The Ccr4 subunit of the mammalian CCR4-NOT deadenylase has been found in the same cytoplasmic foci that contain the polypeptides contributing to decapping and 5′ decay (Cougot *et al.*, 2004a). The *Drosophila* Ccr4 and Caf1 proteins also accumulate in cytoplasmic dots, although in this case no colocalization experiments have been performed (Temme *et al.*, 2004). The intracellular distribution of yeast Ccr4p was more uniform, and a colocalization with cytoplasmic decay foci was uncertain (Sheth & Parker, 2003). However, the Puf proteins, which are likely to induce Ccr4p-catalyzed mRNA deadenylation *via* binding to the mRNA 3′ UTR (see below) are present in cytoplasmic foci in yeast (Gerber *et al.*, 2004). A colocalization of Puf proteins with other components of the mRNA decay machinery has not been tested.

Decapping and 5′ decay are usually rapid once deadenylation is complete. Also, the completion of deadenylation apparently leads to a stop of translation initiation and association of the mRNA with a new set of proteins promoting decay (Tharun & Parker, 2001). Thus, it would not seem unreasonable that decapping and 5′ decay should be restricted to specific locations in the cell. Localization of the degradation enzymes may contribute to the protection of active mRNAs from accidental destruction. The localization of the deadenylase in specific structures is more surprising: The shortening of the poly(A) tail is a slow and continuous process proceeding throughout the lifetime of the mRNA, presumably while the RNA is being translated. Thus, localized deadenylation would imply localized translation. Since the Puf proteins each bind a distinct set of mRNAs, encoding proteins with related functions (see below), it has been hypothesized that their concentration in cytoplasmic foci may reflect a colocalization of such sets of mRNAs serving to coordinate not only their decay but also their regulation and the fates of the encoded proteins (Gerber *et al.*, 2004). However, it has not been examined whether the different Puf proteins are present in distinct foci.

ENZYMES OF THE 3′ DECAY PATHWAY

The Exosome

The exosome is a complex of 3′ exonucleases and associated polypeptides that was discovered through its role in 5.8S rRNA processing in the cell nucleus (Mitchell *et al.*, 1997). The exosome exists both in the nucleus and in the cytoplasm; the complexes located in the two different compartments are distinguished by specific subunits and associated proteins. The nuclear exosome is involved not only in 5.8S rRNA synthesis but also in additional steps in rRNA processing, in snRNA and snoRNA processing, and in turnover of incompletely processed mRNAs or mRNA precursors (Butler, 2002). The cytoplasmic version plays a role in nonstop decay (van Hoof *et al.*, 2002) and in cytoplasmic disposal of trapped splicing intermediates (Hilleren & Parker, 2003). We will confine ourselves to summarizing the role of the exosome in cytoplasmic decay of "regular" mRNAs.

The cytoplasmic exosome can degrade the mRNA body, following deadenylation, in a 3′–5′ direction (Jacobs Anderson & Parker, 1998). Since deadenylation is the first and rate-limiting step in both 5′ and 3′ decay, and exosome mutations do not affect mRNA half-life in an otherwise wild-type yeast background, the exosome does not contribute significantly to deadenylation (Jacobs Anderson & Parker, 1998). This is confirmed by the fact that an exosome mutation does not cause any additional deadenylation defect in a *ccr4*Δ mutant, whereas a *pan2*Δ mutation does abolish residual deadenylation activity in a *ccr4*Δbackground (Tucker *et al.*, 2001). Immunodepletion experiments in mammalian cell extract also showed that the exosome does not contribute to deadenylation (Chen *et al.*, 2001a). Nevertheless, there are indications that under some circumstances or in some organisms the cytoplasmic exosome

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can contribute to deadenylation: In yeast, an exosome mutation prevents nonstop decay and leads to the stabilization of a fully polyadenylated RNA (van Hoof *et al.*, 2002). In contrast, a *ccr4* mutation has no effect (Frischmeyer *et al.*, 2002). Thus, in this case the exosome degrades not only the RNA body but also the poly(A) tail. A poly(A) tail makes RNA susceptible to *in vitro* digestion by a processive subunit of the *Arabidopsis* exosome (Chekanova *et al.*, 2000). This is reminiscent of poly(A) tails in bacteria serving as unstructured entry points for processive 3′ exonucleases (Coburn & Mackie, 1999; Dreyfus & Régnier, 2002; Steege, 2000). Knockdown of exosome subunits by RNA interference in trypanosomes retards the onset of mRNA decay, suggesting the possibility that deadenylation is impaired (Haile *et al.*, 2003).

The nuclear and the cytoplasmic exosome from yeast share a set of ten subunits. Nine of these form a very stable core, whereas Rrp44p is somewhat more loosely attached (Allmang *et al.*, 1999; Mitchell & Tollervey, 2000). Rrp44p homologs, although they exist, are also not part of highly purified exosomes from human cells (Chen *et al.*, 2001a) and from trypanosomes (Estévez *et al.*, 2001, 2003). Both types of exosomes have a composition very similar to the yeast complex. Six subunits, Rrp41p/Ski6p, Rrp42p, Rrp43p, Rrp45p, Rrp46p, and Mtr3p, have sequence similarity to *E. coli* polynucleotide phosphorylase (PNPase) and RNase PH, suggesting that like these two enzymes they catalyze the 3′–5′ degradation of RNA by phosphorolytic attack, releasing nucleoside 5′ diphosphates (Allmang *et al.*, 1999; Mitchell *et al.*, 1997). Indeed, recombinant yeast Rrp41p has been shown to have such an activity (Mitchell *et al.*, 1997), and so has the homologous protein from *Arabidopsis thaliana* (Chekanova *et al.*, 2000). Two additional subunits of the yeast exosome, Rrp4p and Rrp44p, have hydrolytic 3′ exonuclease activities (Mitchell *et al.*, 1997). This type of activity has been confirmed for the Rrp4p homologs from *Trypanosoma brucei* (Estévez *et al.*, 2001) and *Arabidopsis* (Chekanova *et al.*, 2002). The Rrp40p subunit may also be a hydrolytic enzyme, based on its sequence similarity to Rrp4p. No catalytic activity has been predicted for the Csl4p/Ski4p subunit. While the exosome contains one proven and five potential phosphorolytic subunits, the purified complexes from yeast, HeLa cells, and trypanosomes exhibit only hydrolytic activity. Therefore, the activities of some or many subunits are repressed in the complex and may need to be activated by a cofactor (Brouwer *et al.*, 2001; Estévez *et al.*, 2001; Mitchell *et al.*, 1997). Possible models for this type of regulation have been discussed (Mitchell & Tollervey, 2000; van Hoof & Parker, 1999). It is also interesting that, in spite of the multiplicity of catalytic subunits, the mutation of any single subunit leads to essentially the same defects in 5.8S rRNA processing and cytoplasmic mRNA turnover (Allmang *et al.*, 1999; Jacobs Anderson & Parker, 1998; Mitchell *et al.*, 1997). Presumably individual subunits do not function on their own but only as parts of an intact complex, and the absence of any single subunit prevents complex assembly.

Prokaryotic PNPase has been crystallized as a homotrimeric complex with a central channel. As each monomer is composed of two domains of almost identical fold, the structure can be considered a hexamer. Only one of the two domains in each monomer is thought to be catalytically active (Symmons *et al.*, 2000). The fact that the exosome contains six different subunits, which each resemble one domain of PNPase, has led to the suggestion that a PNPase-like hexamer formed from these six subunits lies at the core of the exosome (Symmons *et al.*, 2002). High resolution electron microscopy and protein interaction data support this hypothesis, although various proposals differ in the exact arrangement of the six phosphorolytic subunits. The location of the remaining four subunits is also uncertain (Aloy *et al.*, 2002; Estévez *et al.*, 2003; Rajmakers *et al.*, 2002). As further support of a hexameric structure, RNase PH, another bacterial phosphorolytic 3′ exonuclease, which, in its monomeric form, resembles a single domain of PNPase, forms a trimer of dimers closely corresponding to the structure of PNPase (Harlow *et al.*, 2004; Ishii *et al.*, 2003). The active sites of PNPase and RNase PH have been located in the central channel, close to its "lower" end (Harlow *et al.*, 2004; Ishii *et al.*, 2003; Symmons *et al.*, 2002), and entrapment of substrate RNA in this channel may contribute to the processive mode of action of PNPase (Symmons *et al.*, 2002). PNPase also carries accessory RNA-binding domains of the KH- and S1-type at its "upper" end (Symmons *et al.*, 2002), and these probably also contribute to processivity. In the exosome, the central channel appears conserved (Aloy *et al.*, 2002), and the nonphosphorolytic subunits Rrp4p, Rrp40p, and Csl4p are predicted to possess S1-type RNA-binding domains (Allmang *et al.*, 1999). Therefore, the exosome is likely to degrade RNA in a processive manner. Surprisingly, even several isolated exosome subunits have been reported to be processive (Chekanova *et al.*, 2000; Mitchell *et al.*, 1997).

Four additional polypeptides are not stably associated with the exosome but contribute to exosomal mRNA degradation in the cytoplasm: Ski2p, Ski3p, Ski7p, and Ski8p. These proteins are not involved in nuclear functions of the exosome (Jacobs Anderson & Parker, 1998; van Hoof *et al.*, 2000). Ski2p, Ski3p, and Ski8p form a complex (Ski complex; Brown *et al.*, 2000). Ski2p is a predicted RNA helicase. Association with a RNA helicase is a recurring theme in 3′ exonuclease complexes. The nuclear exosome is associated with a different helicase, Dob1p/Mtr4p (Butler, 2002). A mitochondrial 3′ exoribonuclease complex also contains a RNA helicase, and nuclease activity depends on this enzyme and a ribonucleoside triphosphate

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(Margossian *et al.*, 1996). In the *E. coli* "degradosome" a RNA helicase is associated with the endonuclease RNaseE and PNPase. The enzyme helps PNPase digest structured RNAs in an ATP hydrolysis-dependent manner (Py *et al.*, 1996). Ski2p may likewise facilitate exonuclease activity by removing RNA secondary structure. In addition, protein complexes tightly bound to RNA, which might also obstruct the exonuclease, can be removed by RNA helicases (Fairman *et al.*, 2004; Jankowsky *et al.*, 2001). The Ski complex might also be involved in targeting the exosome to specific substrates. Ski7p is not a stable component of the Ski complex, but it can associate both with this complex and with the exosome (Araki *et al.*, 2001; van Hoof *et al.*, 2002). Ski7p has a C-terminal domain that resembles the GTPase domains of translation elongation factor EF1α and termination factor eRF3. While Ski7p is required for cytoplasmic mRNA decay, the GTPase domain is dispensable (Araki *et al.*, 2001). However, this domain is required for nonstop decay and thus may mediate the recognition of mRNAs lacking a stop codon, possibly by binding to an empty A site on the ribosome (van Hoof *et al.*, 2002).

Not unexpectedly, all ribonucleases involved in mRNA turnover are Mg^{2+}-dependent enzymes that liberate nucleoside 5′ monophosphates or diphosphates, which can be converted into new precursors for RNA synthesis by one or two kinase reactions. In contrast, simpler ribonucleases of the RNase A type, whose robust nature makes an RNA biochemist's life difficult, catalyze a Me^{2+}-independent intramolecular attack of the 2′ hydroxyl on the phosphodiester bond, generating a 2′–3′ cyclic phosphodiester as an intermediate and a nucleoside 3′ monophosphate as a product (Saenger, 1991). Conversion of such products into RNA precursors would require two additional enzymatic steps.

The Scavenger Decapping Enzyme

Complete degradation of the mRNA from the 3′ end generates the free cap dinucleotide, m^7GpppN, as a product. This is then attacked by a m^7G-specific pyrophosphatase distinct from the Dcp1/Dcp2 decapping enzyme described above. This "scavenger" decapping enzyme, DcpS in mammalian cells or Dcs1p in yeast (Kumagai *et al.*, 1992; Liu *et al.*, 2002; Nuss & Furuichi, 1977; Nuss *et al.*, 1975; Wang & Kiledjian, 2001) differs in two functional aspects from the Dcp2 enzyme: DcpS prefers the free cap dinucleotide or capped oligonucleotides as substrates as opposed to capped long RNA molecules, and the cleavage products are m^7GMP and the corresponding nucleoside diphosphate or oligonucleotide with a 5′ diphosphate. With respect to catalytic mechanism, DcpS, unlike Dcp2, is not a member of the Nudix family but of the HIT family of pyrophosphatases (Liu *et al.*, 2002). These enzymes are characterized by a histidine triad (HXHXH) and catalyze the metal-independent cleavage of pyrophosphate bonds through transient formation of a covalent intermediate, in which the product (m^7GMP in the case of DcpS) is covalently attached to a histidine side chain before it is released by hydrolysis (Lima *et al.*, 1997). A DcpS variant inactivated by a point mutation substituting the active site histidine with alanine has been crystallized in a complex with substrate (Gu *et al.*, 2004). The crystal shows a homodimer with a two-domain structure in each monomer. The homodimer is distinctly asymmetric: The two domains of one subunit are in close contact (closed conformation), while those of the other subunit are separated by a wide gap (open conformation). In each subunit, the substrate is bound to the C-terminal domain containing the HIT motif such that it is sandwiched between the two domains in the closed conformation, which is considered to be the catalytically active one. It has been speculated that the conformational change required to generate this structure may be involved in preventing precocious cap hydrolysis on complete mRNAs.

The *S. cerevisiae* genome contains another gene, DCS2, which is closely related to DCS1. No catalytic activity has yet been found for Dcs2p, and its biological function is unknown (Liu *et al.*, 2002; van Dijk *et al.*, 2002). A DcpS-like protein, Nhm1, has also been characterized in *S. pombe*. In contrast to the proteins from mammals and *S. cerevisiae*, this enzyme has been reported to hydrolyze the cap attached to a long RNA (Salehi *et al.*, 2002).

What becomes of the m^7G nucleotides produced by mRNA turnover? At least two considerations suggest they may have to be disposed of. First, they might compete with capped RNA for cap-binding proteins. Second, they would likely find their way into nucleic acids: The product of Dcp2-catalyzed cap cleavage, m^7GDP, would be expected to be converted to deoxy-m^7GDP by ribonucleotide reductase, as this enzyme has no base specificity, acting on all ribonucleoside diphosphates. Moreover, m^7GDP is converted to m^7GTP by nucleoside diphosphate kinase (van Dijk *et al.*, 2002; Wang & Kiledjian, 2001), and deoxy-m^7GDP would also be expected to be a substrate for this enzyme: NDP kinase has neither base specificity nor does it distinguish between ribose and deoxyribose (Kornberg & Baker, 1992). m^7GTP and deoxy-m^7GTP should then be used as precursors for RNA and DNA synthesis. The extent to which the methyl group, after incorporation of m^7G, would interfere with the function of RNA is hard to predict, but N7 can take part in non-Watson-Crick hydrogen bonding, for example in the guanosine base quadruple (Burkard *et al.*, 1999; Saenger, 1984). Thus, N7 methylation can be expected to interfere with RNA interactions. While the N7 guanosine methylation in DNA is not considered a particularly harmful modification, the methylated purine ring may open spontaneously, and cells possess enzymatic activities able to remove either the open purine ring or N7-methyl

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guanosine itself from DNA (Asaeda *et al.*, 2000; Begley *et al.*, 1999; Boiteux *et al.*, 1990; Chetsanga & Lindahl, 1979). Thus it seems logical that there should be a pathway to discard the products of the decapping reaction before their incorporation into nucleic acids. Indeed, the DcpS enzyme acts not only on m^7GpppN but also accepts the Dcp2 reaction product m^7GDP as a substrate, hydrolysing it to m^7GMP and thereby preventing its conversion into either the triphosphate or the deoxyribonucleotide. In this sense, DcpS acts both in the 3′ and the 5′ decay pathway. In extracts from various organisms, m^7GMP can be further converted into an unidentified phosphorylated compound or lose its phosphate through the activities of unknown enzymes (van Dijk *et al.*, 2003).

While decay of active mRNA produces the two identified substrates for DcpS, m^7GpppN and m^7GDP, in the cytoplasm, the mammalian enzyme is localized primarily in the nucleus (Cougot *et al.*, 2004a), and this has also been reported for the *S. pombe* Nhm1 protein (Salehi *et al.*, 2002). Thus, the possibility should be considered that m^7GTP and deoxy-m^7GTP are additional *in vivo* substrates for DcpS. In other words, DcpS might act as a "sanitizing agent" not only by preventing the conversion of m^7GDP into ribo- and deoxyribonucleoside triphosphates but also by degrading the triphosphates themselves in the location in which they would otherwise be used as precursors for nucleic acid synthesis. In a similar manner, members of the Nudix family of enzymes have been shown to degrade mutagenic derivatives of deoxyribonucleoside triphosphates in *E. coli* (Bessman *et al.*, 1996). It should be informative to compare the various known or suspected substrates of DcpS in a quantitative manner.

DCS1 is not an essential gene in yeast, but *dcs1* mutants have a slow growth phenotype (Liu *et al.*, 2002). Levels of m^7G in RNA or DNA in a *dcs1* mutant strain and phenotypic consequences have not been tested to our knowledge. In a growing yeast culture, continuous de novo synthesis of guanosine nucleotides would be expected to limit the proportion of m^7G to a relatively low steady-state level even in the absence of DcpS activity. In contrast, in terminally differentiated nondividing cells in multicellular organisms, ongoing synthesis and turnover of mRNA in combination with a constant nucleotide pool might lead to a gradual accumulation of m^7G nucleotides if these are not removed.

REGULATION OF mRNA DECAY BY RNA-BINDING PROTEINS

Differences in the rates of mRNA decay are determined by sequence elements in the RNAs themselves. Many of these, including the well-studied AU-rich elements (AREs) (Chen & Shyu, 1995), are located in the 3′ UTR, but others are found in the coding sequence or the 5′ UTR (Guhaniyogi & Brewer, 2001). Although a stabilizing element has been characterized in the 3′ UTR of the α-globin mRNA (Waggoner & Liebhaber, 2003; Weis & Liebhaber, 1995), RNA sequences affecting turnover of eukaryotic mRNAs typically act to promote decay, and they usually do so by accelerating deadenylation (Chen & Shyu, 1995). It is generally believed that destabilizing sequences function through specific binding proteins, although alternative mechanisms can be envisioned. We will discuss a few proteins that bind destabilizing elements and have been shown to affect mRNA turnover.

The yeast Puf3p belongs to the family of Puf proteins, members of which function as posttranscriptional regulators in metazoan development (Wickens *et al.*, 2002). Deadenylation and degradation of COX17 mRNA are retarded in *puf3*Δ mutants. Puf3p binds the COX17 3′ UTR (Olivas & Parker, 2000). Rapid deadenylation of the COX17 mRNA depends on the CCR4-NOT complex (Tucker *et al.*, 2002). Thus, this deadenylase is probably regulated by Puf3p. There is some reason to believe that Puf3p may also accelerate decapping (Olivas & Parker, 2000). Another yeast Puf protein, Mpt5p (= Puf5p; Olivas & Parker, 2000), inhibits the expression of the HO gene by promoting the decay of the HO message through binding its 3′ UTR (Tadauchi *et al.*, 2001). Whether Mpt5p induces deadenylation has not been investigated, but the fact that the MPT5 gene was discovered as a multicopy suppressor of a *pop2* mutation (Hata *et al.*, 1998) suggests that this may be the case. A systematic identification of mRNAs associated with each of the five yeast Puf proteins led to the conclusion that every member of this protein family binds a relatively large and distinct set of mRNAs. A fraction of these mRNAs (around 700 total) is bound by more than one Puf protein. Comparison of the target mRNAs suggested specific sequences recognized by Puf3p, Puf4p, and Puf5p (Gerber *et al.*, 2004). As mentioned above, the mRNAs bound by a single type of Puf protein encode proteins that share functional characteristics. This is in agreement with the previous observation that mRNAs encoding proteins with related functions have similar half-lives (Wang *et al.*, 2002a). Unexpectedly, the mRNAs bound by a specific Puf protein very often encode proteins that share a common cellular localization. For example, the Puf3p-associated mRNAs encode proteins with mitochondrial functions (at least 162 out of 220 total; Gerber *et al.*, 2004).

One of the founding members of the Puf family, the *Drosophila* Pumilio protein, acts together with a second protein, Nanos, to promote the deadenylation of *hunchback* mRNA at the posterior end of the *Drosophila* embryo, as shown by RNA injection experiments (Wreden *et al.*, 1997). Pumilio binds Nanos response elements (NREs) in the *hunchback* 3′ UTR and recruits Nanos protein as well as another protein, Brain Tumor (Sonoda & Wharton, 1999, 2001; Wickens *et al.*, 2002). The *bicoid* message also

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contains a NRE and is deadenylated in a Nanos-dependent manner if exposed to Nanos protein *in vivo* (Wharton & Struhl, 1991; Wreden *et al.*, 1997). In normal embryos, however, Nanos protein at the posterior pole of the embryo and *bicoid* mRNA at the anterior pole do not overlap. Therefore, Pumilio is thought to cooperate with a different partner for bicoid mRNA deadenylation (Gamberi *et al.*, 2002).

The *nanos* mRNA itself is translationally regulated with the help of the protein Smaug, which binds to Smaug response elements (SREs) in the *nanos* 3′ UTR (Dahanukar *et al.*, 1999; Smibert *et al.*, 1996, 1999). Studies of the SRE and the RNA binding domain of Smaug have led to the identification of a Smaug homolog in *S. cerevisiae*, Vts1p. This protein also bound to SRE RNAs *in vitro*. Expression of a reporter RNA carrying three SREs in its 3′ UTR was inhibited by Vts1p *in vivo*. As Vts1p destabilized this reporter RNA in a Ccr4p-dependent manner, the protein may facilitate deadenylation catalyzed by the CCR4-NOT complex (Aviv *et al.*, 2003). The biological function of Vts1p and natural target RNAs are not known. It is also unknown whether the regulation of *Drosophila nanos* mRNA by Smaug involves deadenylation.

Mammalian AUF1 (= hnRNP D) was first identified by its ability both to bind specifically to AREs and to destabilize the c-myc mRNA in an *in vitro* system (Brewer, 1991). However, the purified protein retained only its specific binding capacity, not the ability to induce mRNA degradation (Zhang *et al.*, 1993). There is a fairly large body of correlative evidence for an involvement of AUF1 in mRNA decay (De Maria & Brewer, 1996; Wilson *et al.*, 2003, and references cited therein). AUF1 overexpression in various cell lines has been found to accelerate ARE-dependent mRNA decay in two studies (Loflin *et al.*, 1999; Sarkar *et al.*, 2003), but a stabilizing effect was reported in a different study (Xu *et al.*, 2001). Results of RNA interference experiments were also equivocal: While a uniform knockdown of all four isoforms of AUF1 had no effect on the decay of an unstable mRNA, a selective knockdown of the p40 and p45 isoforms stabilized the same reporter mRNA (Raineri *et al.*, 2004).

Another protein binding specifically to AREs is Hu-R, a member of the ELAV family of RNA-binding proteins (Myer *et al.*, 1997). However, overexpression of HuR inhibited ARE-dependent rapid mRNA decay (Fan & Steitz, 1998; Peng *et al.*, 1998), and a stabilizing role of HuR has been confirmed by RNA interference experiments (Raineri *et al.*, 2004). The stabilizing effect of HuR is probably due to competition with destabilizing proteins for binding to the same RNA sequences.

Mice deficient in tristetraprolin (TTP), a zinc finger protein, suffer from a complex inflammatory syndrome caused by overexpression of tumor necrosis factor alpha (TNF-α). Overexpression of TNF-α is due to a stabilization of its mRNA in the TTP-deficient mice (Carballo *et al.*, 1998). The role of TTP in ARE-dependent decay has been confirmed by other studies and extended to GM-CSF and interleukin-2, -3, and -6 mRNAs (Carballo *et al.*, 2000; Stoecklin *et al.*, 2000, 2001, 2003). In contrast, the stability of the *c-fos* mRNA was not affected by TTP (Carballo *et al.*, 2000), suggesting that the protein is specific for the so-called class II AREs (Chen & Shyu, 1995). TTP binds to AREs in the 3′ UTR of the TNF-α message (Carballo *et al.*, 1998; Lai *et al.*, 1999), and promotes deadenylation *in vivo* (Carballo *et al.*, 2000) and also in a cell-free system (Lai *et al.*, 2003). In the *in vitro* system, PARN appears to be the enzyme responsible for TTP-dependent deadenylation. Transfection studies showed that TTP can also destabilize mRNAs that do not carry a poly(A) tail (Lai & Blackshear, 2001). BRF1, a protein related to TTP, has been identified as a mRNA destabilizing, ARE-binding protein by a genetic selection scheme (Stoecklin *et al.*, 2002). Which step of mRNA decay is promoted by BRF1 has not been reported.

Analysis of an *in vitro* system that recapitulated ARE-dependent mRNA decay led to the conclusion that the AREs promote decay of the mRNA body catalyzed by the exosome (Chen *et al.*, 2001a; Mukherjee *et al.*, 2002). A highly purified exosome preparation was active in ARE-dependent decay (Chen *et al.*, 2001a). Three ARE-binding proteins, KSRP, TTP, and AUF1, were found to be associated with the exosome in substoichiometric amounts. The addition of either KSRP or TTP, but not AUF1, to depleted extracts or purified exosomes stimulated ARE-dependent decay. In the same *in vitro* system, and in contrast to mRNA body decay, the rate of deadenylation was not affected by the presence or absence of an ARE in the substrate RNA. Acceleration of mRNA body decay by AREs is consistent with the observation that TTP can destabilize nonpolyadenylated mRNAs (see above), with *in vivo* results showing that AREs can facilitate both deadenylation and subsequent decay (Chen *et al.*, 1994; Shyu *et al.*, 1991) and also with ARE-dependent destabilization of snRNAs that do not carry a poly(A) tail (Fan *et al.*, 1997). More generally, deadenylated RNAs sometimes accumulate transiently (Carballo *et al.*, 2000; Dellavalle *et al.*, 1994; Stoeckle & Guan, 1993), indicating that the step after deadenylation can also be slow and is, therefore, a potential target for regulation. How does an ARE accelerate two distinct phases of mRNA decay, deadenylation and decay of the RNA body, which are catalyzed by two different enzymes? One possibility is that proteins bound to the ARE might influence a third process (e.g., translation) that then affects both deadenylation and subsequent decay. However, as the ARE effect on exosome-catalyzed decay of the RNA body has been seen in a reconstituted system (Chen *et al.*, 2001a), the involvement of additional factors, in particular of translation, is unlikely. Similar

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arguments apply to deadenylation (Lai *et al.*, 2003). A common regulation of deadenylation and mRNA body decay *via* translation is also unlikely because *in vivo* AREs destabilize snRNAs that are not translated (Fan *et al.*, 1997). A second possibility is that a single protein or complex of proteins bound to the ARE might directly affect two different enzymes sequentially, first the deadenylase and then the exosome. As a third possibility, two different proteins or protein complexes or two different components of a protein complex bound to an ARE might each affect a distinct decay enzyme. The fact that some ARE mutations can separate the ARE function in deadenylation from its function in RNA body decay (Chen *et al.*, 1994; Shyu *et al.*, 1991) favors the latter model. However, as explained above, TTP appears to be able to stimulate both deadenylation and mRNA body decay.

It has also been reported that AREs stimulate cap hydrolysis to m^7GDP *in vitro* (Gao *et al.*, 2001). However, as the TLC system used did not reliably separate all primary and secondary products of the Dcp2 and the DcpS reactions (Bergman *et al.*, 2002; van Dijk *et al.*, 2003), and the products of both reactions are further metabolized in extract (see above), it is possible that the primary ARE effect in this system was really on exosomal decay and a higher rate of DcpS-catalyzed cap hydrolysis was only the secondary consequence.

In contrast to exosomal degradation of the mRNA body, sequence-dependent deadenylation has been difficult to recapitulate *in vitro* (but see Lai *et al.*, 2003). Although ARE-dependent deadenylation in a mammalian cell extract has been reported (Ford *et al.*, 1999), such a reaction has not been observed by others (Chen *et al.*, 2001a), and it appears that the main effect of AREs in this system is on exosome activity as discussed above. A very convincing ARE-dependent deadenylation reaction has been seen in *Xenopus* egg extracts (Voeltz *et al.*, 2001). However, this system has not been used so far to identify the enzyme involved and the mechanism of its stimulation. *Xenopus* egg and embryo extracts also perform a rapid deadenylation reaction dependent on a 3′ UTR sequence (EDEN) that directs deadenylation during early development (Legagneux *et al.*, 1995). Like the PARN-catalyzed default deadenylation mentioned above, the EDEN-dependent reaction does not lead to mRNA decay. A protein binding to EDEN, EDEN-BP, has been identified, and the *in vitro* system has been used to provide evidence for an involvement of this protein in the deadenylation reaction (Paillard *et al.*, 1998, 2003). Again, the enzyme responsible and the mechanism of its regulation are both unknown.

mRNA DECAY AND TRANSLATION

The poly(A) tail is the first target of attack in both pathways of mRNA decay, and the cap is the second in one major pathway. As both structures are bound by proteins involved in translation initiation, translation is predicted to interfere with mRNA decay. As outlined above, the expectation that binding of the translation initiation complex to the cap competes with cap hydrolysis has been experimentally confirmed, and this competition can, to a large extent, explain the deadenylation dependence of cap hydrolysis. However, similar reasoning also applies to deadenylation: Formation of the cap-eIF4E-eIF4G-PABPC-poly(A) complex should strengthen the association of PABPC with the poly(A) tail, and this has been verified (Le *et al.*, 1997). To the extent that PABPC is an inhibitor of deadenylation, formation of the translation initiation complex should thus interfere with poly(A) tail removal. If PARN is involved, competition of the translation initiation complex for cap binding would also be inhibitory. There are several reasons to believe that the effect of translation on deadenylation may be more interesting than on decapping: First, deadenylation is essential in both pathways of mRNA decay, not just one, and it appears to be the main rate-limiting and regulated step. Second, mRNAs are efficiently translated while they are being deadenylated, but current views on translation initiation predict that translation should effectively cease once deadenylation is complete. Thus, it may not matter much for the mRNA's functional half-life whether cap hydrolysis occurs more or less rapidly. Third, whereas cap hydrolysis is obviously an all-or-none decision for an individual RNA molecule, deadenylation, consisting of the hydrolysis of many phosphodiester bonds, can vary in rate for an individual mRNA molecule. Therefore, the rate of translation initiation might regulate the rate of deadenylation.

Several experiments in yeast support a competition between translation initiation and deadenylation: inhibition of the translation of the PGK1 mRNA by insertion of secondary structures in the 5′ UTR accelerated deadenylation and decay (Muhlrad *et al.*, 1995). Similarly, changes in the sequences surrounding the PGK1 initiation codon showed a correlation between poor translation and rapid deadenylation (LaGrandeur & Parker, 1999). Deadenylation was also favored by mutations in translation initiation factors (Schwartz & Parker, 1999). In mammalian cells, inhibition of translation by a protein bound to the 5′ UTR induced poly(A) shortening (Muckenthaler *et al.*, 1997). However, the situation is far from clear, as many contradictory results have been reported. For example, in one study the insertion of secondary structure into the 5′ UTR of yeast mRNAs had stabilizing, destabilizing, or no effects, depending on the gene used (Linz *et al.*, 1997). A mammalian mRNA destabilized by the GM-CSF ARE was stabilized by the introduction of a stem-loop into the 5′ UTR, and instability was restored by introduction of an internal ribosome entry site downstream of the stem loop (Aharon & Schneider, 1993). While this appears to prove that rapid degradation

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in fact depends on translation—quite the opposite of the expectation formulated above—secondary structures 3′ to the coding region could also stabilize the RNA as long as they were upstream of the ARE (Curatola *et al.*, 1995), and so their mechanism of action remains unclear. Blocks to translation elongation also stabilize mRNAs (Schwartz & Parker, 2000a). In most studies of the relationship between mRNA decay and translation, only overall stability of the RNA was examined, not individual steps. Most of these studies are also hard to interpret because the processes of deadenylation and decapping should not be inhibited by translation per se but by the binding of translation factors to the cap, and it is unknown how cap occupancy is affected by secondary structures in the 5′ UTR.

If translation indeed competes with mRNA decay, destabilizing elements might function indirectly by inhibiting translation. In fact, AREs have been reported to have such an activity (Kruys & Huez, 1994; Kruys *et al.*, 1989). However, inhibition of translation and destabilization of mRNA appear to be two separate functions of the ARE mediated by different proteins (Zhang *et al.*, 2002).

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REFERENCES

Achsel, T., Brahms, H., Kastner, B., Bachi, A., Wilm, M., and Lührmann, R. 1999. A doughnut-shaped heteromer of human Sm-like proteins binds to the 3′-end of U6 snRNA, thereby facilitating U4/U6 duplex formation in vitro. *EMBO J* **18:**5789–5802.

Aharon, T. and Schneider, R.J. 1993. Selective destabilization of short-lived mRNAs with granulocyte-macrophage colony-stimulated factor AU-rich 3′ noncoding region is mediated by a cotranslational mechanism. *Mol Cell Biol* **13:**1971–1980.

Albert, T.K., Lemaire, M., van Berkum, N.L., Gentz, R., Collart, M., and Timmers, H.T.M. 2000. Isolation and characterization of human orthologs of yeast CCR4-NOT complex subunits. *Nucleic Acids Res* **28:**809–817.

Albert, T.K., Hanzawy, H., Legtenberg, Y.I.A., de Ruwe, M.J., van den Heuwel, F.A.J., Collart, M.A., Boelens, R., and Timmers, H.T.M. 2002. Identification of a ubiquitin-protein ligase subunit within the CCR4-NOT transcription repressor complex. *EMBO J* **21:**355–364.

Allmang, C., Petfalski, E., Podtelejnikov, A., Mann, M., Tollervey, D., and Mitchell, P. 1999. The yeast exosome and human PM-Scl are related complexes of 3′-5′ exonucleases. *Genes Dev* **13:**2148–2158.

Aloy, P., Ciccarelli, F.D., Leutwein, C., Gavin, A.-C., Superti-Furga, G., Bork, P., Böttcher, B., and Russell, R.B. 2002. A complex prediction: three-dimensional model of the yeast exosome. *EMBO Rep* **3:**628–635.

Araki, Y., Takahashi, S., Kobayashi, T., Kajiho, H., Hoshino, S., and Katada, T. 2001. Ski7p G protein interacts with the exosome and the Ski complex for 3′-to-5′ mRNA decay in yeast. *EMBO J* **20:**4684–4693.

Asaeda, A., Ide, H., Asagoshi, K., Matsuyama, S., Tano, K., Murakami, A., Takamori, Y., and Kubo, K. 2000 Substrate specificity of human methylpurine DNA N-glycosylase. *Biochem* **39:**1959–1965.

Aviv, T., Lin, Z., Lau, S., Rendl, L.M., Sicheri, F., and Smibert, C.A. 2003. The RNA binding SAM domain of Smaug defines a new family of post-transcriptional regulators. *Nature Struct Biol* **10:**614–621.

Baggs, J.E. and Green, C.B. 2003. Nocturnin, a deadenylase in Xenopus laevis retina: a mechanism for posttranscriptional control of circadian-related mRNA. *Curr Biol* **13:**189–198.

Bai, Y., Salvadore, C., Chiang, Y.-C., Collart, M., Liu, H.-Y. and Denis, C.L. 1999. The CCR4 and CAF1 proteins of the CCR4-NOT complex are physically and functionally separated from NOT2, NOT4 and NOT5. *Mol Cell Biol* **19:**6642–6651.

Bashkirov, V.I., Scherthan, H., Solinger, J.A., Buerstedde, J.-M., and Heyer, W.-D. 1997. A mouse cytoplasmic exoribonuclease (mXRN1p) with preference for G4 tetraplex substrates. *J Cell Biol* **136:**761–773.

Beelman, C.A., Stevens, A., Caponigro, G., LaGrandeur, T.E., Hatfield, L., Fortner, D.M., and Parker, R. 1996. An essential component of the decapping enzyme required for normal rates of mRNA turnover. *Nature* **382:**642–646.

Begley, T.J., Haas, B.J., Noel, J., Shekhtman, Y., Williams, W.A., and Cunningham, R.P. 1999. A new member of the endonuclease III family of DNA repair enzymes that removes methylated purines from DNA. *Curr Biol* **9:**653–656.

Benard, L. 2004. Inhibition of 5′ to 3′ mRNA degradation under stress conditions in Saccharomyces cerevisiae: from GCN4 to MET16. *RNA* **10:**458–468.

Bergman, N., Opychral, M., Bates, E.J., and Wilusz, J. 2002. Analysis of the products of mRNA decapping and 3′-to-5′ decay by denaturing gel electrophoresis. *RNA* **8:**959–965.

Bessman, M.J., Frick, D.N., and O'Handley, S.F. 1996. The MutT proteins or "nudix" hydrolases, a family of versatile, widely distributed, "housecleaning" enzymes. *J Biol Chem* **271:**25259–25062.

Binder, R., Horowitz, J.A., Basilion, J.P., Koeller, D.M., Klausner, R.D., and Harford, J.B. 1994. Evidence that the pathway of transferrin receptor mRNA degradation involves an endonucleolytic cleavage within the 3′-UTR and does not involve poly(A) tail shortening. *EMBO J* **13:**1969–1980.

Boeck, R., Lapeyre, B., Brown, C.E., and Sachs, A.B. 1998. Capped mRNA degradation intermediates accumulate in the yeast spb8-2 mutant. *Mol Cell Biol* **18:**5062–5072.

Boeck, R., Tarun, S., Rieger, M., Deardorff, J.A., Müller-Auer, S., and Sachs, A.B. 1996. The yeast Pan2 protein is required for poly(A)-binding protein-stimulated poly(A)-nuclease activity. *J Biol Chem* **271:**432–438.

Boiteux, S., O'Connor, T.R., Lederer, F., Gouyette, A., and Laval, J. 1990. Homogeneous Escherichia coli FPG protein. *J Biol Chem* **265:**3916–3922.

Bonnerot, C., Boeck, R., and Lapeyre, B. 2000. The two proteins Pat1p (Mrt1p) and Spb8p interact in vivo, are required for mRNA decay, and are functionally linked to Pab1p. *Mol Cell Biol* **20:**5939–5946.

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Bouveret, E., Rigaut, G., Shevchenko, A., Wilm, M., and Seraphin, B. 2000. A Sm-like protein complex that participates in mRNA degradation. *EMBO J* **19:**1661–1671.

Brewer, G. 1991. An A+U-rich element RNA-binding factor regulates c-myc mRNA stability in vitro. *Mol Cell Biol* **11:**2460–2466.

Brouwer, R., Allmang, C., Raijmakers, R., van Aarsen, Y., Vree Egberts, W., Petfalski, E., van Venrooij, W.J., Tollervey, D., and Pruijn, G.J.M. 2001. Three novel components of the human exosome. *J Biol Chem* **276:**6177–6184.

Brown, C.E. and Sachs, A.B. 1998. Poly(A) tail length control in Saccharomaces cerevisiae occurs by message-specific deadenylation. *Mol Cell Biol* **18:**6548–6559.

Brown, C.E., Tarun, S.Z., Boeck, R., and Sachs, A.B. 1996. PAN3 encodes a subunit of the Pab1p-dependent poly(A) nuclease in Saccharomyces cerevisiae. *Mol Cell Biol* **16:**5744–5753.

Brown, J.T., Bai, X., and Johnson, A.W. 2000. The yeast antiviral proteins Ski2p, Ski3p, and Ski8p exist as a complex in vivo. *RNA* **6:**449–457.

Burkard, M.E., Turner, D.H., and Tinoco, I. 1999. The interactions that shape RNA structure. In *The RNA World*, pp. 233–264. Gesteland, R.F., Cech, T.R., and Atkins, J.F., Eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Butler, J.S. 2002. The yin and yang of the exosome. *Trends Cell Biol* **12:**90–96.

Cao, D., and Parker, R. 2001. Computational modeling of eukaryotic mRNA turnover. *RNA* **7:**1192–1212.

Caponigro, G. and Parker, R. 1995. Multiple functions for the poly(A)-binding protein in mRNA decapping and deadenylation in yeast. *Genes Dev* **9:**2421–2432.

Carballo, E., Lai, W.S., and Blackshear, P.J. 1998. Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. *Science* **281:**1001–1005.

Carballo, E., Lai, W.S., and Blackshear, P.J. 2000. Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. *Blood* **95:**1891–1899.

Chekanova, J.A., Dutko, J.A., Mian, I.S., and Belostotsky, D.A. 2002. Arabidopsis thaliana exosome subunit AtRrp4p is a hydrolytic 3′-5′ exonuclease containing S1 and KH RNA binding domains. *Nucleic Acids Res* **30:**695–700.

Chekanova, J.A., Shaw, R.J., and Belostotsky, D.A. 2001. Analysis of an essential requirement for the poly(A) binding protein function using cross-species complementation. *Curr Biol* **11:**1207–1214.

Chekanova, J.A., Shaw, R.J., Wills, M.A., and Belostotsky, D.A. 2000. Poly(A) tail-dependent exonuclease AtRrp41p from Arabidopsis thaliana rescues 5.8S rRNA processing and mRNA decay defects of the yeast ski6 mutant and is found in an exosome-sized complex in plant and yeast cells. *J Biol Chem* **275:**33158–33166.

Chen, C.-Y., Gherzi, R., Ong, S.-E., Chan, E.L., Raijmakers, R., Pruijn, G.J.M., Stoecklin, G., Moroni, C., Mann, M., and Karin, M. 2001a. AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell* **107:**451–464.

Chen, C.-Y. and Shyu, A.-B. 1995. AU-rich elements: characterization and importance in mRNA degradation. *Trends Biochem Sci* **20:**465–470.

Chen, C.-Y.A., Chen, T.-M., and Shyu, A.-B. 1994. Interplay of two functionally and structurally distinct domains of the c-fos AU-rich element specifies its mRNA-destabilizing function. *Mol Cell Biol* **14:**416–426.

Chen, J., Chiang, Y., and Denis, C.L. 2002. CCR4, a 3′-5′ poly(A) RNA and ssDNA exonuclease, is the catalytic component of the cytoplasmic deadenylase. *EMBO J* **21:**1414–1426.

Chen, J., Rappsilber, J., Chiang, Y.-C., Russell, P., Mann, M., and Denis, C.L. 2001b. Purification and characterization of the 1.0 MDa CCR4-NOT complex identifies two novel components of the complex. *J Mol Biol* **314:**683–694.

Chernokalskaya, E., Dubell, A.N., Cunningham, K.S., Hanson, M.N., Dompenciel, R.E., and Schoenberg, D.R. 1998. A polysomal ribonuclease involved in the destabilization of albumin mRNA is a novel member of the peroxidase gene family. *RNA* **4:**1537–1548.

Chetsanga, C.J. and Lindahl, T. 1979. Release of 7-methylguanine residues whose imidazole rings have been opened from damaged DNA by a DNA glycosylase from Escherichia coli. *Nucleic Acids Res* **6:**3673–3684.

Chiba, Y., Johnson, M.A., Lidder, P., Vogel, J.T., van Erp, H., and Green, P.J. 2004. AtPARN is an essential poly(A) ribonuclease in Arabidopsis. *Gene* **328:**95–102.

Clark, L.B., Viswanathan, P., Quigley, G., Chiang, Y.-C., McMahon, J.S., Yao, G., Chen, J., Nelsbach, A., and Denis, C.L. 2004. Systematic mutagenesis of the leucine-rich repeat (LRR) domain of CCR4 reveals specific sites for binding to CAF1 and a separate critical role for the LRR in CCR4 deadenylase activity. *J Biol Chem* **279:**13516–13623.

Coburn, G.A. and Mackie, G.A. 1999. Degradation of mRNA in Escherichia coli: an old problem with some new twists. *Prog Nucleic Acids Res Mol Biol* **62:**55–108.

Collart, M. 2003. Global control of gene expression in yeast by the Ccr4-Not complex. *Gene* **313:**1–16.

Coller, J.M., Gray, N.K., and Wickens, M.P. 1998. mRNA stabilization by poly(A) binding protein is independent of poly(A) and requires translation. *Genes Dev* **12:**3226–3235.

Coller, J.M., Tucker, M., Sheth, U., Valencia-Sanchez, M.A., and Parker, R. 2001. The DEAD box helicase, Dhh1p, functions in mRNA decapping and intercts with both the decapping and deadenylase complexes. *RNA* **7:**1717–1727.

Cougot, N., Babajko, S., and Seraphin, B. 2004a. Cytoplasmic foci are sites of mRNA decay in human cells. *J Cell Biol* **165:**31–40.

Cougot, N., van Dijk, E., Babajko, S., and Séraphin, B. 2004b. Captabolism. *Trends Biochem Sci* in press.

Couttet, P., Fromont-Racine, M., Steel, D., Pictet, R., and Grange, T. 1997. Messenger RNA deadenylylation precedes decapping in mammalian cells. *Proc Nat Acad Sci USA* **94:**5628–5633.

Curatola, A.M., Nadal, M.S., and Schneider, R.J. 1995. Rapid degradation of AU-rich element (ARE) mRNAs is activated by ribosome transit and blocked by secondary structure at any position 5′ to the ARE. *Mol Cell Biol* **15:**6331–6340.

Dahanukar, A., Walker, J.A., and Wharton, R.P. 1999. Smaug, a novel RNA-binding protein that operates a translational switch in Drosophila. *Mol Cell* **4:**209–218.

Daugeron, M.-C., Mauxion, F., and Seraphin, B. 2001. The yeast POP2 gene encodes a nuclease involved in mRNA deadenylation. *Nucleic Acids Res* **29:**2448–2455.

De Maria, C.T. and Brewer, G. 1996. AUF1 binding affinity to A+U-rich elements correlates with rapid mRNA degradation. *J Biol Chem* **271:**12179–12184.

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Decker, C.J. and Parker, R. 1993. A turnover pathway for both stable and unstable mRNAs in yeast: evidence for a requirement for deadenylation. *Genes Dev* **7:**1632–1643.

Dehlin, E., Wormington, M., Körner, C.G., and Wahle, E. 2000. Cap-dependent deadenylation of mRNA. *EMBO J* **19:**1079–1086.

Dellavalle, R.P., Petersen, R., and Lindquist, S. 1994. Preferential deadenylation of Hsp70 mRNA plays a key role in regulating Hsp70 Expression in Drosophila melanogaster. *Mol Cell Biol* **14:**3646–3659.

Denis, C.L. and Chen, J. 2003. The CCR4-NOT complex plays diverse roles in mRNA metabolism. *Prog Nucleic Acids Res Mol Biol* **73:**221–250.

Dreyfus, M. and Régnier, P. 2002. The poly(A) tail of mRNAs: bodyguard in eukaryotes, scavenger in bacteria. *Cell* **111:**611–613.

Dunckley, T. and Parker, R. 1999. The DCP2 protein is required for mRNA decapping in Saccharomyces cerevisiae and contains a functional MutT motif. *EMBO J* **18:**5411–5422.

Dunckley, T., Tucker, M., and Parker, R. 2001. Two related proteins, Edc1p and Edc2p, stimulate mRNA decapping in Saccharomyces cerevisiae. *Genetics* **157:**27–37.

Dupressoir, A., Morel, A.-P., Barbot, W., Loireau, M.-P., Corbo, L., and Heidmann, T. 2001. Identification of four families of yCCR4- and Mg-dependent endonuclease-related proteins in higher eukaryotes, and characterization of orthologs of yCCR4 with a conserved leucine-rich repeat essential for hCAF1/hPOP2 binding. *BMC Genomics* **2:**9–22.

Estévez, A.M., Kempf, T., and Clayton, C. 2001. The exosome of Trypanosoma brucei. *EMBO J* **20:**3831–3839.

Estévez, A.M., Lehner, B., Sanderson, C.M., Ruppert, T., and Clayton, C. 2003. The roles of intersubunit interactions in exosome stability. *J Biol Chem* **278:**34943–34951.

Eystathioy, T., Jakymiw, A., Chan, E.K.L., Seraphin, B., Cougot, N., and Fritzler, M.J. 2003. The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSm4 in cytoplasmic GW bodies. *RNA* **9:**1171–1173.

Fairman, M.E., Maroney, P.A., Wang, W., Bowers, H.A., Gollnick, P., Nilsen, T.W., and Jankowsky, E. 2004. Protein displacement by DExH/D "RNA helicases" without duplex unwinding. *Science* **304:**730–734.

Fan, X.C., Myer, V.E., and Steitz, J.A. 1997. AU-rich elements target small nuclear RNAs as well as mRNAs for rapid degradation. *Genes Dev* **11:**2557–2568.

Fan, X.C. and Steitz, J.A. 1998. Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. *EMBO J* **17:**3448–3460.

Fischer, N. and Weis, K. 2002. The DEAD box protein Dhh1 stimulates the decapping enzyme Dcp1. *EMBO J* **21:**2788–2797.

Ford, L.P., Watson, J., Keene, J.D., and Wilusz, J. 1999. ELAV proteins stabilize deadenylated intermediates in a novel in vitro mRNA deadenylation/degradation system. *Genes Dev* **13:**188–201.

Frischmeyer, P.A., van Hoof, A., O'Donnell, K., Guerrerio, A.L., Parker, R., and Dietz, H.C. 2002. An mRNA surveillance mechanism that eliminates transcripts lacking termination codons. *Science* **295:**2258–2261.

Gallouzi, I.-E., Parker, F., Chebli, K., Maurier, F., Labourier, E., Barlat, I., Capony, J.-P., Tocque, B., and Tazi, J. 1998. A novel phosphorylation-dependent RNase activity of GAP-SH3 binding protein: a potential link between signal transduction and RNA stability. *Mol Cell Biol* **18:**3956–3965.

Gamberi, C., Peterson, D.S., He, L., and Gottlieb, E. 2002. An anterior function for the Drosophila posterior determinant Pumilio. *Development* **129:**2699–2710.

Gao, M., Fritz, D.T., Ford, L.P., and Wilusz, J. 2000. Interaction between a poly(A)-specific ribonuclease and the 5′ cap influences mRNA deadenylation rates in vitro. *Mol Cell* **5:**479–488.

Gao, M., Wilusz, C.J., Peltz, S.W., and Wilusz, J. 2001. A novel mRNA-decapping activity in HeLa cytoplasmic extracts is regulated by AU-rich elements. *EMBO J* **20:**1134–1143.

Gavin, A.C.e.a. 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415:**141–147.

Gerber, A.P., Herschlag, D., and Brown, P.O. 2004. Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast. *PLOS Biol* **2:**342–354.

Gu, M., Fabrega, C., Liu, S.-W., Liu, H., Kiledjian, M., and Lima, C.D. 2004. Insight into the structure, mechanism and regulation of scavenger mRNA decapping activity. *Mol Cell* **14:**67–80.

Guhaniyogi, J. and Brewer, G. 2001. Regulation of mRNA stability in mammalian cells. *Gene* **265:**11–23.

Haile, S., Estévez, A.M., and Clayton, C. 2003. A role for the exosome in the in vivo degradaton of unstable mRNAs. *RNA* **9:**1491–1501.

Harlow, L.S., Kadziola, A., Jensen, K.F., and Larsen, S. 2004. Crystal structure of the phosphorolytic exoribonuclease RNase PH from Bacillus subtilis and implications for its quaternary structure and tRNA binding. *Prot Sci* **13:**668–677.

Hata, H., Mitsui, H., Liu, H., Bai, Y., Denis, C.L., Shimizu, Y., and Sakai, A. 1998. Dhh1p, a putative RNA helicase, associates with the general transcription factors Pop2p and Ccr4p from Saccharomyces cerevisiae. *Genetics* **148:**571–579.

Hatfield, L., Beelman, C.A., Stevens, A., and Parker, R. 1996. Mutations in trans-acting factors affecting mRNA decapping in Saccharomyces cerevisiae. *Mol Cell Biol* **16:**5830–5838.

He, F., Li, X., Spatrick, P., Casillo, R., Dong, S., and Jacobson, A. 2003. Genome-wide analysis of mRNAs regulated by the nonsense-mediated and 5′ to 3′ mRNA decay pathways in yeast. *Mol Cell* **12:**1439–1452.

He, W. and Parker, R. 2000. Functions of Lsm proteins in mRNA degradation and splicing. *Curr Op Cell Biol* **12:**346–350.

He, W. and Parker, R. 2001. The yeast cytoplasmic LsmI/Pat1p complex protects mRNA 3′ termini from partial degradation. *Genetics* **158:**1445–1455.

Herrick, D., Parker, R., and Jacobson, A. 1990. Identification and comparison of stable and unstable mRNAs in Saccharomyces cerevisiae. *Mol Cell Biol* **10:**2269–2284.

Hilleren, P.J. and Parker, R. 2003. Cytoplasmic degradation of splice-defective pre-mRNAs and intermediates. *Mol Cell* **12:**1453–1465.

Hsu, C.L. and Stevens, A. 1993. Yeast cells lacking 5′-3′ exoribonuclease 1 contain mRNA species that are poly(A) deficient and partially lack the 5′ cap structure. *Mol Cell Biol* **13:**4826–4835.

Huh, W.-K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S., and O'Shea, E.K. 2003. Global analysis of protein localization in budding yeast. *Nature* **425:**686–691. http://yeastgfp.ucsf.edu.

Ingelfinger, D., Arndt-Jovin, D.J., Lührmann, R., and Achsel, T. 2002. The human LSm1-7 proteins colocalize with the mRNA-degrading enzymes Dcp1/2 and Xrn1 in distinct cytoplasmic foci. *RNA* **8:**1489–1501.

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Ioannidis, P., Havredaki, M., Courtis, N., and Trangas, T. 1996. In vivo generation of 3′ and 5′ truncated species in the process of c-myc mRNA decay. *Nucleic Acids Res* **24:**4969–4977.

Ishii, R., Nureki, O., and Yokoyama, S. 2003. Crystal structure of the tRNA processing enzyme RNase PH from Aquifex aeolicus. *J Biol Chem* **278:**32397–32404.

Jacob, F. and Monod, J. 1961. Genetic regulatory mechanisms in the synthesis of proteins. *J Mol Biol* **3:**318–356.

Jacobs Anderson, J.S. and Parker, R. 1998. The 3′ to 5′ degradation of yeast mRNAs is a general mechanism for mRNA turnover that requires the SKI2 DEVH box protein and 3′ to 5′ exonucleases of the exosome complex. *EMBO J* **17:**1497–1506.

Jankowsky, E., Gross, C.H., Shuman, S., and Pyle, A.M. 2001. Active disruption of an RNA-protein interaction by a DExH/D RNA helicase. *Science* **291:**121–125.

Khanna, R. and Kiledjian, M. 2004. Poly(A)-binding-protein-mediated regulation of hDcp2 decapping in vitro. *EMBO J* **23:**1968–1976.

Kornberg, A. and Baker, T.A. 1992. *DNA Replication*, W. H. Freeman, New York.

Körner, C. and Wahle, E. 1997. Poly(A) tail shortening by a mammalian poly(A)-specific 3′-exoribonuclease. *J Biol Chem* **272:**10448–10456.

Körner, C.G., Wormington, M., Muckenthaler, M., Schneider, S., Dehlin, E., and Wahle, E. 1998. The deadenylating nuclease (DAN) is involved in poly(A) tail removal during the meiotic maturation of Xenopus oocytes. *EMBO J* **17:**5427–5437.

Kruys, V. and Huez, G. 1994. Translational control of cytokine expression by 3′ UA-rich sequences. *Biochimie* **76:**862–866.

Kruys, V., Marinx, O., Shaw, G., Deschamps, J., and Huez, G. 1989. Translational blockade imposed by cytokine-derived UA-rich sequences. *Science* **245:**852–855.

Kshirsagar, M. and Parker, R. 2004. Identification of Edc3p as an enhancer of mRNA decapping in Saccharomyces cerevisiae. *Genetics* **166:**729–739.

Kühn, U. and Wahle, E. 2004. Structure and function of poly(A) binding proteins. *Biochim Biophys Acta* **1678:**67–84.

Kumagai, H., Kon, R., Hoshino, T.T.A., Nishikawa, M., Hirose, S., and Igarashi, K. 1992. Purification and properties of a decapping enzyme from rat liver cytosol. *Biochim Biophys Acta* **1119:**45–51.

Ladomery, M., Wade, E., and Sommerville, J. 1997. Xp54, the Xenopus homologue of human RNA helicase p54, is an integral component of stored mRNP particles in oocytes. *Nucleic Acids Res* **25:**965–973.

LaGrandeur, T. and Parker, R. 1999. The cis acting sequences responsible for the differential decay of the unstable MFA2 and stable PGK1 transcripts in yeast include the context of the translational start codon. *RNA* **5:**420–433.

Lai, W.S. and Blackshear, P.J. 2001. Interactions of CCCH zinc finger proteins with mRNA. Tristetraprolin-mediated AU-rich element-dependent mRNA degradation can occur in the absence of a poly(A) tail. *J Biol Chem* **276:**23144–23154.

Lai, W.S., Carballo, E., Strum, J.R., Kennington, E.A., Philipps, R.S., and Blackshear, P.J. 1999. Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. *Mol Cell Biol* **19:**4311–4323.

Lai, W.S., Kennington, E.A., and Blackshear, P.J. 2003. Tristetraprolin and its family members can promote the cell-free deadenylation of AU-rich element-containing mRNAs by poly(A) ribonuclease. *Mol Cell Biol* **23:**3798–3812.

Le, H., Tanguay, R.L., Balasta, M.L., Wei, C.-C., Browning, K.S., Metz, A.M., Goss, D.J., and Gallie, D.R. 1997. Translation initiation factors eIF-iso4G and eIF-4B interact with the poly(A)-binding protein and increase its RNA binding activity. *J Biol Chem* **272:**16247–16255.

Legagneux, V., Omilli, F., and Osborne, H.B. 1995. Substrate-specific regulation of RNA deadenylation in Xenopus embryo and activated egg extracts. *RNA* **1:**1001–1008.

Lejeune, F., Li, X., and Maquat, L.E. 2003. Nonsense-mediated mRNA decay in mammalian cells involves decapping, deadenylating and exonucleolytic activities. *Mol Cell* **12:**675–687.

Lenssen, E., Oberholzer, U., Labarre, J., De Virgilio, C., and Collart, M. 2002. Saccharomyces cerevisiae Ccr4-Not complex contributes to the control of Msn2p-dependent transcription by the Ras/cAMP pathway. *Mol Microbiol* **43:**1023–1037.

Lima, C.D., Klein, M.G., and Hendrickson, W.A. 1997. Structure-based analysis of catalysis and substrate definition in the HIT protein family. *Science* **278:**286–290.

Linz, B., Koloteva, N., Vasilescu, S., and McCarthy, J.E.G. 1997. Disruption of ribosomal scanning on the 5′-untranslated region, and not restriction of translational initiation per se, modulates the stability of nonaberrant mRNAs in the yeast Saccharomyces cerevisiae. *J Biol Chem* **272:**9131–9140.

Liu, H., Rodgers, N.D., Jiao, X., and Kiledjian, M. 2002. The scavenger mRNA decapping enzyme DcpS is a member of the HIT family of pyrophosphatases. *EMBO J* **21:**4699–4708.

Liu, H.-Y., Toyn, J.H., Chiang, Y.-C., Draper, M.P., Johnston, L.H., and Denis, C.L. 1997. DBF2, a cell cycle-regulated protein kinase, is physically and functionally associated with the CCR4 transcriptional regulatory complex. *EMBO J* **16:**5289–5298.

Loflin, P., Chen, C.-Y.A., and Shyu, A.-B. 1999. Unraveling a cytoplasmic role for hnRNP D in the in vivo mRNA destabilization directed by the AU-rich element. *Genes Dev* **13:**1884–1897.

Lowell, J.E., Rudner, D.Z., and Sachs, A.B. 1992. 3′-UTR-dependent deadenylation by the yeast poly(A) nuclease. *Genes Dev* **6:**2088–2099.

Lykke-Andersen, J. 2002. Identification of a human decapping complex associated with hUpf proteins in nonsense-mediated decay. *Mol Cell Biol* **22:**8114–8121.

Maquat, L.E. 2004. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. *Nature Rev Mol Cell Biol* **5:**89–99.

Margossian, S.P., Li, H., Zassenhaus, H.P., and Butow, R.A. 1996. The DExH box protein Suv3p is a component of a yeast mitochondrial 3′-to-5′ exoribonuclease that suppresses group I intron toxicity. *Cell* **84:**199–209.

Martinez, J., Ren, Y.-G., Nilsson, P., Ehrenberg, M., and Virtanen, A. 2001. The mRNA cap structure stimulates rate of poly(A) removal and amplifies processivity of degradation. *J Biol Chem* **276:**27923–27929.

Martinez, J., Ren, Y.-G., Thuresson, A.-C., Hellman, U., Astrom, J., and Virtanen, A. 2000. A 54-kDa fragment of the poly(A)-specific ribonuclease is an oligomeric, processive and cap-interacting poly(A)-specific 3′ exonuclease. *J Biol Chem* **275:**24222–24230.

Minshall, N., Thom, G., and Standart, N. 2001. A conserved role of a DEAD box helicase in mRNA masking. *RNA* **7:**1728–1742.

Mitchell, P., Petfalski, E., Shevchenko, A., Mann, M., and Tollervey, D. 1997. The exosome: a conserved eukaryotic RNA processing complex containing multiple 3′-5′ exoribonuclease activities. *Cell* **91:**457–466.

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Mitchell, P. and Tollervey, D. 2000. Musing on the structural organization of the exosome complex. *Nature Struct Biol* **7:**843–846.

Moore, M.J. 2002. Nuclear RNA turnover. *Cell* **108:**431–434.

Moriya, H., Shimizu-Yoshida, Y., Omori, A., Iwashita, S., Katoh, M., and Sakai, A. 2001. Yak1p, a DYRK family kinase, translocates to the nucleus and phosphorylates yeast Pop2p in response to a glucose signal. *Genes Dev* **15:**1217–1228.

Moser, M.J., Holley, W.R., Chatterjee, A., and Mian, I.S. 1997. The proofreading domain of Escherichia coli DNA polymerase I and other DNA and/or RNA exonuclease domains. *Nucleic Acids Res* **25:**5110–5118.

Muckenthaler, M., Gunkel, N., Stripecke, R., and Hentze, M.W. 1997. Regulated poly(A) tail shortening in somatic cells mediated by cap-proximal translational repressor proteins and ribosome association. *RNA* **3:**983–995.

Muhlrad, D., Decker, C.J., and Parker, R. 1994. Deadenylation of the unstable mRNA encoded by the yeast MFA2 gene leads to decapping followed by 5′-3′ digestion of the transcript. *Genes Dev* **8:**855–866.

Muhlrad, D., Decker, C.J., and Parker, R. 1995. Turnover mechanism of the stable yeast PGK1 mRNA. *Mol Cell Biol* **15:**2145–2156.

Mukherjee, D., Gao, M., O'Connor, J.P., Raijmakers, R., Pruijn, G., Lutz, C.S., and Wilusz, J. 2002. The mammalian exosome mediates the efficient degradation of mRNAs that contain AU-rich elements. *EMBO J* **21:**165–174.

Myer, V.E., Fan, X.C., and Steitz, J.A. 1997. Identification of HuR as a protein implicated in AUUUA-mediated mRNA decay. *EMBO J* **16:**2130–2139.

Newbury, S. and Woollard, A. 2004. The 5′-3′ exoribonuclease xrn-1 is essential for ventral epithelial enclosure during C. elegans embryogenesis. *RNA* **10:**59–65.

Nuss, D.L. and Furuichi, Y. 1977. Characterization of the m7G(5′)pppN-pyrophosphatase activity from HeLa cells. *J Biol Chem* **1977:**2815–2821.

Nuss, D.L., Furuichi, Y., Koch, G., and Shatkin, A.J. 1975. Detection in HeLa cell extracts of a 7-methyl guanosine specific enzyme activity that cleaves m7GpppNm. *Cell* **6:**21–27.

Olivas, W. and Parker, R. 2000. The Puf3 protein is a transcript-specific regulator of mRNA degradation in yeast. *EMBO J* **19:**6602–6611.

Paillard, L., Legagneux, V., and Osborne, H.B. 2003. A functional deadenylation assay identifies human CUG-BP as a deadenylation factor. *Biol Cell* **95:**107–113.

Paillard, L., Omilli, F., Legagneux, V., Bassez, T., Maniey, D., and Osborne, H.B. 1998. EDEN and EDEN-BP, a cis element and an associated factor that mediate sequence-specific mRNA deadenylation in Xenopus oocytes. *EMBO J* **17:**278–287.

Parker, R. and Song, H. 2004. The enzymes and control of eukaryotic mRNA turnover. *Nature Struct Mol Biol* **11:**121–127.

Peng, S.S.-Y., Chen, C.-Y.A., Xu, N., and Shyu, A.-B. 1998. RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. *EMBO J* **17:**3461–3470.

Piccirillo, C., Khanna, R., and Kiledjian, M. 2003. Functional characterization of the mammalian RNA decapping enzyme hDcp2. *RNA* **9:**1138–1147.

Py, B., Higgins, C.F., Krisch, H.M., and Carpousis, A.J. 1996. A DEAD box RNA helicase in the Escherichia coli RNA degradosome. *Nature* **381:**169–172.

Raineri, I., Wegmueller, D., Gross, B., Certa, U., and Moroni, C. 2004. Roles of AUF1 isoforms, HuR and BRF1 in ARE-dependent mRNA turnover studied by RNA interference. *Nucleic Acids Res* **32:**1279–1288.

Rajmakers, R., Egberts, W.V., van Venrooij, W.J., and Pruijn, G.J.M. 2002. Protein-protein interactions between human exosome components support the assembly of RNase PH-type subunits into a six-membered PNPase-like ring. *J Mol Biol* **323:**653–663.

Ren, Y.-G., Martinez, J., and Virtanen, A. 2002. Identification of the active site of poly(A)-specific ribonuclease by site-directed mutagenesis and Fe^{2+}-mediated cleavage. *J Biol Chem* **277:**5982–5987.

Rodgers, N.D., Wang, Z., and Kiledjian, M. 2002a. Characterization and purification of a mammalian endoribonuclease specific for the alpha-globin mRNA. *J Biol Chem* **277:**2597–2604.

Rodgers, N.D., Wang, Z., and Kiledjian, M. 2002b. Regulated alpha-globin mRNA decay is a cytoplasmic event proceeding through 3′-to-5′ exosome-dependent decapping. *RNA* **8:**1526–1537.

Ross, J. 1995. mRNA stability in mammalian cells. *Microbiol Rev* **59:**423–450.

Sachs, A. 2000. Physical and functional interactions between the mRNA cap structure and the poly(A) tail. In *Translational control of gene expression*, Sonenberg, N., Hershey, J.W.B., and Mathews, M.B. Eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Sachs, A.B. and Davis, R.W. 1989. The poly(A) binding protein is required for poly(A) shortening and 60S ribosomal subunit-dependent translation initiation. *Cell* **58:**857–867.

Sachs, A.B. and Deardorff, J.A. 1992. Translation initiation requires the PAB-dependent poly(A) ribonuclease in yeast. *Cell* **70:**961–973.

Saenger, W. 1984. *Principles of Nucleic Acid Structure*, Springer, New York.

Saenger, W. 1991. Structure and catalytic function of nucleases. *Curr. Op. Struct. Biol.* **1:**130–138.

Salehi, Z., Geffers, L., Vilela, C., Birkenhäger, R., Ptushkina, M., Berthelot, K., Ferro, M., Gaskell, S., Hagan, I., Stapley, B., and McCarthy, J.E.G. 2002. A nuclear protein in Schizosaccharomyces pombe with homology to the human tumour suppressor Fhit has decapping activity. *Mol Microbiol* **46:**49–62.

Salgado-Garrido, J., Bragado-Nilsson, E., Kandels-Lewis, S., and Seraphin, B. 1999. Sm and Sm-like proteins assemble in two related complexes of deep evolutionary origin. *EMBO J* **18:**3451–3462.

Sarkar, B., Xi, Q., He, C., and Schneider, R.J. 2003. Selective degradation of AU-rich mRNAs promoted by the p37 AUF1 protein isoform. *Mol Cell Biol* **23:**6685–6693.

Schwartz, D., Decker, C.J., and Parker, R. 2003. The enhancer of decapping proteins, Edc1p and Edc2p, bind RNA and stimulate the activity of the decapping enzyme. *RNA* **9:**239–251.

Schwartz, D. and Parker, R. 2000a. Interaction of mRNA translation and mRNA degradation in Saccharomyces cerevisiae. In *Translational control of gene expression*, pp. 807–825. Sonenberg, N., Hershey, J.W.B., and Mathews, M.B. Eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Schwartz, D.C. and Parker, R. 1999. Mutations in translation initiation factors lead to increased rates of deadenylation and decapping of mRNAs in Saccharomyces cerevisiae. *Mol Cell Biol* **19:**5247–5256.

Schwartz, D.C. and Parker, R. 2000b. mRNA decapping in yeast requires dissociation of the cap binding protein, eukaryotic translation initiation factor 4E. *Mol Cell Biol* **20:**7033–7942.

She, M., Decker, C.J., Sundramurthy, K., Liu, Y., Chen, N., Parker, R., and Song, H. 2004. Crystal structure of Dcp1p and its functional

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implications in mRNA decapping. *Nature Struct Mol Biol* **11:**249–256.

Sheth, U. and Parker, R. 2003. Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science* **300:**805–808.

Shyu, A.-B., Belasco, J.G., and Greenberg, M.E. 1991. Two distinct destabilizing elements in the c-fos message trigger deadenylation as a first step in rapid mRNA decay. *Genes Dev* **5:**221–231.

Shyu, A.-B., Greenberg, M.E., and Belasco, J.G. 1989. The c-fos transcript is targeted for rapid decay by two distinct mRNA degradation pathways. *Genes Dev* **3:**60–72.

Smibert, C.A., Lie, Y.S., Shillinglaw, W., Henzel, W.J., and Macdonald, P.M. 1999. Smaug, a novel and conserved protein, contributes to repression of nanos mRNA translation in vitro. *RNA* **5:**1535–1547.

Smibert, C.A., Wilson, J.E., Kerr, K., and Macdonald, P.M. 1996. Smaug protein represses translation of unlocalized nanos mRNA in the Drosophila embryo. *Genes Dev* **10:**2600–2609.

Sonoda, J. and Wharton, R.P. 1999. Recruitment of Nanos to hunchback mRNA by Pumilio. *Genes Dev* **13:**2704–2712.

Sonoda, J. and Wharton, R.P. 2001. Drosophila Brain Tumor is a translational repressor. *Genes Dev* **15:**762–773.

Steege, D.A. 2000. Emerging featurs of mRNA decay in bacteria. *RNA* **6:**1079–1090.

Steiger, M., Carr-Schmid, A., Schwartz, D.C., Kiledjian, M., and Parker, R. 2003. Analysis of recombinant yeast decapping enzyme. *RNA* **9:**231–238.

Stevens, A. 1980. Purification and characterization of a Saccharomyces cerevisiae exoribonuclease which yields 5′-mononucleotides by a 5′-3′ mode of hydrolysis. *J Biol Chem* **255:**3080–3085.

Stevens, A. 1988. mRNA-decapping enzyme from Saccharomyces cerevisiae: Purification and unique specificity for long RNA chains. *Mol Cell Biol* **8:**2005–2010.

Stoeckle, M.Y. and Guan, L. 1993. High-resolution analysis of groalpha mRNA poly(A) shortening: regulation by interleukin-1ß. *Nucleic Acids Res* **21:**1613–1617.

Stoecklin, G., Colombi, M., Raineri, I., Leuenberger, S., Mallaun, M., Schmidlin, M., Gross, B., Lu, M., Kitamura, T., and Moroni, C. 2002. Functional cloning of BRF1, a regulator of ARE-dependent mRNA turnover. *EMBO J* **21:**4709–4718.

Stoecklin, G., Gross, B., Ming, X.-F., and Moroni, C. 2003. A novel mechanism of tumor suppression by destabilizing AU-rich growth factor mRNA. *Oncogene* **22:**3554–3561.

Stoecklin, G., Ming, X.-F., Looser, R., and Moroni, C. 2000. Somatic mRNA turnover mutants implicate tristetraprolin in the interleukin-3 mRNA degradation pathway. *Mol Cell Biol* **20:**3753–3763.

Stoecklin, G., Stoeckle, P., Lu, M., Muehlemann, O., and Moroni, C. 2001. Cellular mutants define a common mRNA degradation pathway targeting cytokine AU-rich elements. *RNA* **7:**1578–1588.

Symmons, M.F., Jones, G.H., and Luisi, B.F. 2000. A duplicated fold is the structural basis for polynucleotide phosphorylase catalytic activity, processivity and regulation. *Structure* **8:**1215–1226.

Symmons, M.F., Williams, M.G., Luisi, B.F., Jones, G.H., and Carpousis, A.J. 2002. Running rings around RNA: a superfamily of phosphate-dependent RNases. *Trends Biochem Sci* **27:**11–18.

Tadauchi, T., Matsumoto, K., Herskowitz, I., and Irie, K. 2001. Post-transcriptional regulation through the HO 3′-UTR by Mpt5, a yeast homolog of Pumilio and FBF. *EMBO J* **20:**552–561.

Tarun, S.Z. and Sachs, A.B. 1996. Association of the yeast poly(A) tail binding protein with translation initiation factor eIF-4G. *EMBO J* **15:**7168–7177.

Temme, C., Zaessinger, S., Simonelig, M., and Wahle, E. 2004. A complex containing the CCR4 and CAF1 proteins is involved in mRNA deadenylation in Drosophila. *EMBO J* **23:**2862–2871.

Tharun, S., He, W., Mayes, A.E., Lennertz, P., Beggs, J.D., and Parker, R. 2000. Yeast Sm-like proteins function in mRNA decapping and decay. *Nature* **404:**515–518.

Tharun, S. and Parker, R. 2001. Targeting an mRNA for decapping: displacement of translation factors and association of the Lsm1p-7p complex on deadenylated yeast mRNAs. *Mol Cell* **8:**1075–1083.

Thoma, C., Hasselblatt, P., Köck, J., Chang, S.-F., Hockenjos, B., Will, H., Hentze, M.W., Blum, H.E., von Weizsäcker, F., and Offensperger, W.-B. 2001. Generation of stable mRNA fragments and translation of N-truncated proteins induced by antisense oligodeoxynucleotides. *Mol Cell* **8:**865–872.

Thore, S., Mauxion, F., Seraphin, B., and Suck, D. 2003. X-ray structure and activity of the yeast Pop2 protein: a nuclease subunit of the mRNA deadenylase complex. *EMBO Rep* **4:**1150–1155.

Till, D.D., Linz, B., Seago, J.E., Elgar, S.J., Marujo, P.E., Elias, M.L., Arraiano, C.M., McClellan, J.A., McCarthy, J.E., and Newbury, S.F. 1998. Identification and developmental expression of a 5′-3′ exoribonuclease from Drosophila melanogaster. *Mech Dev* **79:**51–55.

Tran, H., Schilling, M., Wirbelauer, C., Hess, D., and Nagamine, Y. 2004. Facilitation of mRNA deadenylation and decay by the exosome-bound DEXH protein RHAU. *Mol Cell* **13:**101–111.

Tucker, M. and Parker, R. 2000. Mechanisms and control of mRNA decapping in Saccharomyces cerevisiae. *Annu Rev Biochem* **69:**571–595.

Tucker, M., Staples, R.R., Valencia-Sanchez, M.A., Muhlrad, D., and Parker, R. 2002. Ccr4p is the catalytic subunit of a Ccr4p/Pop2/Notp mRNA deadenylase complex in Saccharomyces cerevisiae. *EMBO J* **21:**1427–1436.

Tucker, M., Valencia-Sanchez, M.A., Staples, R.R., Chen, J., Denis, C.L., and Parker, R. 2001. The transcription factor associated Ccr4 and Caf1 proteins are components of the major cytoplasmic mRNA deadenylase in Saccharomyces cerevisiae. *Cell* **104:**377–386.

Uchida, N., Hoshino, S., and Katada, T. 2004. Identification of a human cytoplasmic poly(A) nuclease complex stimulated by poly(A)-binding protein. *J Biol Chem* **279:**1383–1391.

van Dijk, E., Cougot, N., Meyer, S., Babajko, S., Wahle, E., and Séraphin, B. 2002. Human Dcp2: a catalytically active mRNA decapping enzyme located in specific cytoplasmic structures. *EMBO J* **21:**6915–6924.

van Dijk, E., Le Hir, H., and Séraphin, B. 2003. DcpS can act in the 5′-3′ mRNA decay pathway in addition to the 3′-5′ pathway. *Proc Natl Acad Sci USA* **100:**12081–12086.

van Dijk, E., Sussenbach, J.S., and Holthuizen, P.E. 2001. Kinetics and regulation of site-specific endonucleolytic cleavage of human IGF-II mRNAs. *Nucleic Acids Res* **29:**3477–3486.

van Hoof, A., Frischmeyer, P.A., Dietz, H.C., and Parker, R. 2002. Exosome-mediated recognition and degradation of mRNAs lacking a termination codon. *Science* **295:**2262–2264.

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van Hoof, A. and Parker, R. 1999. The exosome: a proteasome for RNA? *Cell* **99:**347–350.

van Hoof, A., Staples, R.R., Baker, R.E., and Parker, R. 2000. Function of the Ski4p (Csl4p) and Ski7p proteins in 3′-to-5′ degradation of mRNA. *Mol Cell Biol* **20:**8230–8243.

Viswanathan, P., Chen, J., Chiang, Y.-C., and Denis, C.L. 2003. Identification of multiple RNA features that influence CCR4 deadenylation activity. *J Biol Chem* **278:**14949–14955.

Viswanathan, P., Ohn, T., Chiang, Y.-C., Chen, J., and Denis, C.L. 2004. Mouse CAF1 can function as a processive deadenylase/3′-5′ exonuclease in vitro but in yeast the deadenylase function of CAF1 is not required for mRNA poly(A) removal. *J Biol Chem* **279:**23988–23955.

Voeltz, G.K., Ongkasuwan, J., Standart, N., and Steitz, J.A. 2001. A novel embryonic poly(A) binding protein, ePAB, regulates mRNA deadenylation in Xenopus egg extracts. *Genes Dev* **15:**774–788.

Vreken, P., and Raué, H.A. 1992. The rate-limiting step in yeast PGK1 mRNA degradation is an endonucleolytic cleavage in the 3′-terminal part of the coding region. *Mol Cell Biol* **12:**2986–2996.

Waggoner, S.A. and Liebhaber, S.A. 2003. Regulation of alpha-globin mRNA stability. *Exp Biol Med* **228:**387–395.

Wang, Y., Liu, C.L., Storey, J.D., Tibshirani, R.J., Herschlag, D., and Brown, P.O. 2002a. Precision and functional specificity in mRNA decay. *Proc Natl Acad Sci USA* **99:**5860–5865.

Wang, Z., Jiao, X., Carr-Schmid, A., and Kiledjian, M. 2002b. The hDcp2 protein is a mammalian mRNA decapping enzyme. *Proc Natl Acad Sci USA* **99:**12663–12668.

Wang, Z. and Kiledjian, M. 2001. Functional link between the mammalian exosome and mRNA decapping. *Cell* **107:**751–752.

Weis, I.M. and Liebhaber, S.A. 1995. Erythroid cell-specific mRNA stability elements in the alpha2-globin 3′ nontranslated region. *Mol Cell Biol* **15:**2457–2465.

Wharton, R.P. and Struhl, G. 1991. RNA regulatory elements mediate control of Drosophila body pattern by the posterior morphogen nanos. *Cell* **67:**955–967.

Wickens, M., Bernstein, D.S., Kimble, J., and Parker, R. 2002. A PUF family portrait: 3′ UTR regulation as a way of life. *Trends Genet* **18:**150–157.

Wilson, G.M., Lu, J., Sutphen, K., Sun, Y., Huynh, Y., and Brewer, G. 2003. Regulation of A+U-rich element-directed mRNA turnover involving reversible phosphorylation of AUF1. *J Biol Chem* **278:**33029–33038.

Wilson, T. and Treisman, R. 1988. Removal of poly(A) and consequent degradation of c-fos mRNA facilitated by 3′ AU-rich sequences. *Nature* **336:**396–399.

Wilusz, C.J., Wormington, M., and Peltz, S.W. 2001. The cap-to-tail guide to mRNA turnover. *Nat Rev Mol Cell Biol* **2:**237–246.

Wreden, C., Verrotti, A.C., Schisa, J.A., Lieberfarb, M.E., and Strickland, S. 1997. Nanos and pumilio establish embryonic polarity in Drosophila by promoting posterior deadenylation of hunchback mRNA. *Development* **124:**3015–3023.

Wyers, F., Minet, M., Dufour, M.E., Vo, L.T.A., and Lacroute, F. 2000. Deletion of the PAT1 gene affects translation initiation and suppresses a PAB1 gene deletion in yeast. *Mol Cell Biol* **20:**3538–3549.

Xu, N., Chen, C.-Y.A., and Shyu, A.-B. 2001. Versatile role for hnRNP D isoforms in the differential regulation of cytoplasmic mRNA turnover. *Mol Cell Biol* **21:**6960–6971.

Xu, N., Loflin, P., Chen, C.-Y.A., and Shyu, A.-B. 1998. A broader role for AU-rich element-mediated mRNA turnover revealed by a new transcriptional pulse strategy. *Nucleic Acids Res* **26:**558–565.

Zhang, T., Kruys, V., Huez, G., and Gueydan, C. 2002. AU-rich element-mediated translational control: complexity and multiple activities of trans-activating factors. *Biochem Soc Transact* **30:**952–958.

Zhang, W., Wagner, B.J., Ehrenman, K., Schaefer, A.W., DeMaria, C.T., Crater, D., DeHaven, K., Long, L., and Brewer, G. 1993. Purification, characterization, and cDNA cloning of an AU-rich element RNA-binding protein, AUF1. *Mol Cell Biol* **13:**7652–7665.

Zuo, Y. and Deutscher, M.P. 2001. Exoribonuclease superfamilies: structural analysis and phylogenetic distribution. *Nucleic Acids Res* **29:**1017–1026.

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