Annu. Rev. Biochem. 2004. 73:861–90
doi: 10.1146/annurev.biochem.73.011303.074032

First published online as a Review in Advance on March 18, 2004

EUKARYOTIC mRNA DECAPPING

Jeff Coller and Roy Parker
Howard Hughes Medical Institute, Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721; e-mail: jmcoller@u.arizona.edu, rrparker@u.arizona.edu

Key Words mRNA turnover, mRNA stability, mRNA decay deadenylation

■ **Abstract** Eukaryotic mRNAs are primarily degraded by removal of the 3′ poly(A) tail, followed either by cleavage of the 5′ cap structure (decapping) and 5′->3′ exonucleolytic digestion, or by 3′ to 5′ degradation. mRNA decapping represents a critical step in turnover because this permits the degradation of the mRNA and is a site of numerous control inputs. Recent analyses suggest decapping of an mRNA consists of four central and related events. These include removal, or inactivation, of the poly(A) tail as an inhibitor of decapping, exit from active translation, assembly of a decapping complex on the mRNA, and sequestration of the mRNA into discrete cytoplasmic foci where decapping can occur. Each of these steps is a demonstrated, or potential, site for the regulation of mRNA decay. We discuss the decapping process in the light of these central properties, which also suggest fundamental aspects of cytoplasmic mRNA physiology that connect decapping, translation, and storage of mRNA.

CONTENTS

THE BIOLOGICAL ROLE OF mRNA TURNOVER

The process of mRNA turnover is important in numerous aspects of eukaryotic mRNA physiology. For example, mRNA turnover plays a key role in the control of gene expression both by setting the basal level of gene expression and as a site of regulatory responses. More recently, it has become clear that mRNA decay mechanisms play an important role in antiviral defenses. This antiviral role can include functions of the basic mRNA decay machinery as well as specialized systems, such as the response to dsRNA (RNA interference) [for reviews, see (113, 118)]. Finally, specialized mRNA turnover systems exist that recognize and degrade aberrant mRNAs, thereby increasing the quality control of mRNA biogenesis (72). Given these functions, it is important to understand how decay rates of different mRNAs are controlled, how aberrant mRNAs are targeted for destruction, and how viral mRNAs are distinguished from normal cellular mRNAs.

PATHWAYS OF mRNA DECAY

In every eukaryote, the regulated destruction of mRNAs can occur via four general mechanisms: deadenylation dependent 3′ to 5′ decay, endonucleolytic digestion, or by specialized quality control pathways. In most species, multiple mechanisms exist. The major pathway for degradation may vary between species, but all organisms seem to have multiple mechanisms for turnover in place. In the following section, we introduce each pathway and briefly discuss key enzymes required for each (Table 1).

Deadenylation Dependent Exonucleolytic Digestion

There are two general pathways by which polyadenylated mRNAs can be degraded in eukaryotic cells (Figure 1). In both cases, the degradation of the transcript begins with the shortening of the poly(A) tail at the 3′ end of the mRNA (77, 99). Three mRNA deadenylases have been identified. In yeast, the predominant deadenylase complex contains two nucleases, Ccr4p and Pop2p,

TABLE 1 Enzymes involved in mRNA decay

Protein	Function	Features
Deadenylation		
Ccr4p	Catalytic subunit of the deadenylase	Homology to Mg2+ dependent endonucleases
Pop2p	Regulator of deadenylation, may also have deadenylase activity	Homology to RNaseD
Pan2p/Pan3p	Minor deadenylases, required for poly(A) length control	Pan2p has homology to RNaseD
PARN	Mammalian deadenylase	Homology to RNaseD. Can bind to 5′ cap, which stimulates deadenylase activity
Decapping		
Dcp1p	Major component of decapping holoenzyme	EVH1/WH1 domain
Dcp2p	Catalytic subunit of decapping holoenzyme	NUDIX motif, conserved Box A and Box B motif
Exonucleases		
Xrn1p	Major cytoplasmic 5′→3′ exonuclease	
Rat1p	Nuclear 5′→3′ exonuclease	
Rrp4, Rrp40p, Rrp41p, Rrp42p, Rrp43p, Rrp44p, Rrp45p, Rrp46p, Mtr3p, Cs14p	A complex of 3′→5′ exonucleases termed the exosome	Domain organization similar to that of bacterial PNPase

and several accessory proteins, Not1-Not5p, Caf4p, Caf16p, Caf40p, and Caf130p (25, 31, 108, 109). Additional deadenylases include the Pan2p/Pan3p complex (13, 18, 109) and the RNase D homolog PARN, which is found in some but not all eukaryotes (61, 62). An unresolved issue is the relative importance of these individual deadenylases in different organisms and for different mRNAs [see (86) for more discussion].

In yeast, shortening of the poly(A) tail primarily leads to removal of the 5′ cap structure (decapping), thereby exposing the transcript to digestion by a 5′ to 3′ exonuclease (34, 51, 80). Several observations suggest that mRNA decapping is a decay mechanism in other eukaryotic cells. First, the decapping enzymes, Dcp1p and Dcp2p, are conserved among eukaryotes (36, 71, 110, 116). Second, analysis of the 5′ and 3′ ends of mammalian mRNAs demonstrated that decapped mRNAs are specifically detected on transcripts with short poly(A) tails (28).

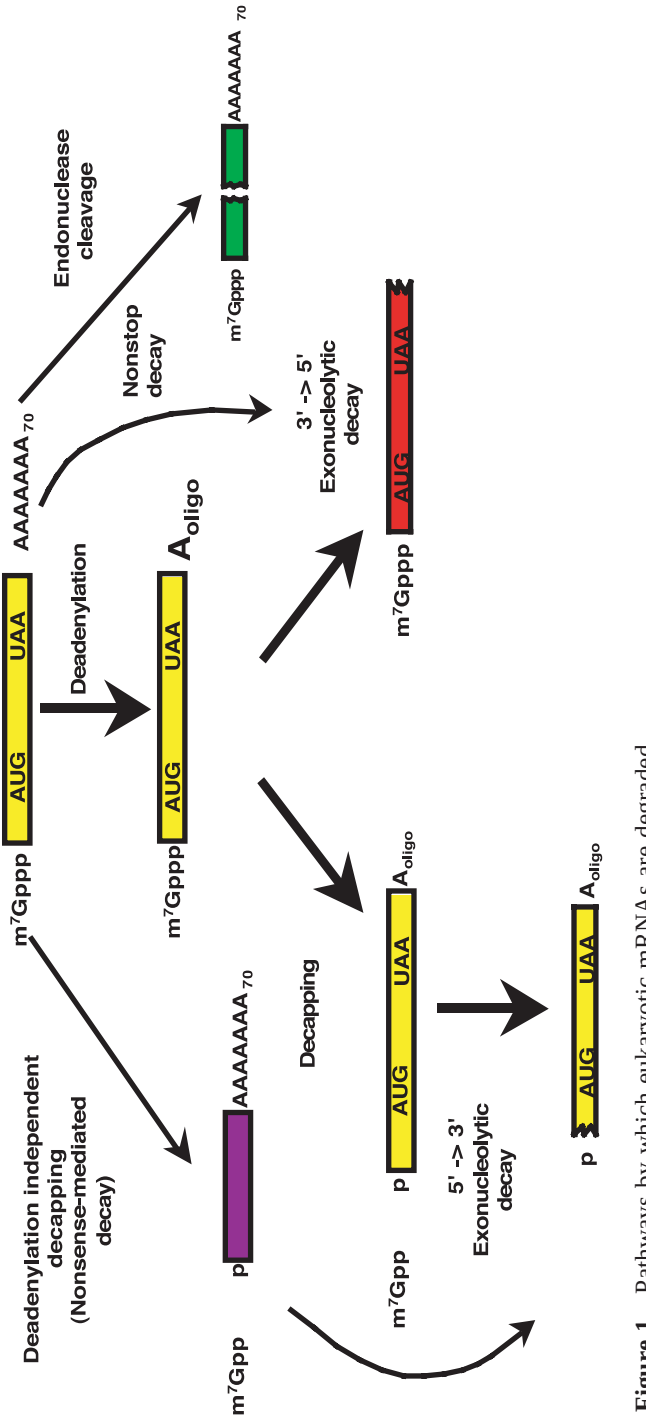

Figure 1 Pathways by which eukaryotic mRNAs are degraded.

Lastly, the aggregation of decapping factors into discrete subcellular structures is also conserved (5, 53, 71, 98, 110).

mRNAs can also be degraded in a 3′ to 5′ direction following deadenylation (Figure 1) (79). 3′ to 5′ degradation of mRNAs is catalyzed by the exosome (54, 81, 92), which is a large complex of 3′ to 5′ exonucleases functioning in several RNA degradative and processing events [for reviews, see (19, 74, 114)]. For the yeast mRNAs that have been studied, the process of 3′ to 5′ decay is slower than the decapping dependent 5′ to 3′ decay pathway (23). However, it is likely that for some yeast mRNAs, or in other eukaryotic cells, 3′ to 5′ degradation will be the primary mechanism of mRNA degradation following shortening of the poly(A) tail (46). Consistent with that view, recent results suggest that degradation of some mammalian mRNAs containing AU-rich destabilizing elements (AREs) may be predominantly in a 3′ to 5′ direction (25, 81, 117).

Decay of Eukaryotic mRNA via Endonucleolytic Cleavage

Eukaryotic mRNAs can also be degraded via endonucleolytic cleavage prior to deadenylation (Figure 1). Evidence for this mechanism comes from the analysis of transcripts, such as mammalian 9E3, transferrin receptor, *c-myc*, insulin-like growth factor II, serum albumin, vitellogenin mRNA, and Xenopus β-globin mRNA. In these cases, mRNA fragments that correspond to the 5′ and/or 3′ portions of the transcript are detected in vivo and are consistent with internal cleavage within the mRNA (11, 17, 29, 43, 103, 112). Because there does not appear to be any similarity between the cleavage sites in these mRNAs, a wide variety of endonucleases may exist with different cleavage specificities. For example, the endonuclease suggested to be responsible for β-globin decay appears to cleave at UG and UC dinucleotides (17), whereas decay of the eNOS pre-mRNA appears to occur by cleavage at CA repeats (52).

Endonucleolytic cleavage is also paramount in RNA-mediated gene silencing (RNAi) [for review, see (107)]. The process of RNAi appears to defend the genome against viruses and transposons as well as control gene expression of some endogenous mRNAs. An RNAi response is initiated by the recognition of a dsRNA sequence by the ATP-dependent endonuclease *Dicer*. *Dicer* cleaves the dsRNA to generate 21–23 nucleotide RNA species that are used to target the RNAi-induced silencing complex (RISC) to the complementary mRNA. In some cases, the RISC complex facilitates a second endonucleolytic event, which triggers the destruction of the mRNA. A simple hypothesis is that this initial cleavage leads to the 5′ and 3′ portions of the mRNA being degraded by Xrn1p and/or the exosome.

Specialized Decay Pathways for mRNA Quality Control

Eukaryotic cells have evolved quality control mechanisms to monitor mRNA biogenesis (Figure 2). These mechanisms take the form of specialized pathways for the rapid degradation of aberrant mRNAs. For example, aberrant mRNAs

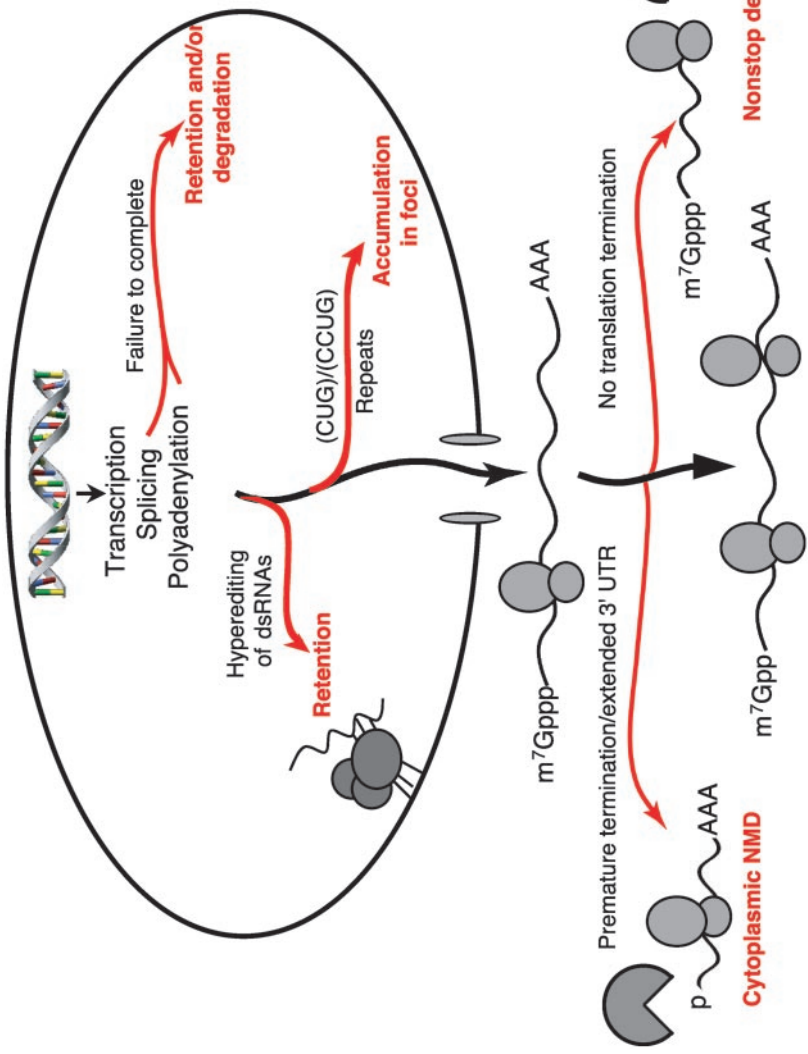

Figure 2 Mechanism of mRNA quality control.

containing a premature translational stop codon are decapped without prior poly(A) shortening (22, 78). This rapid degradation of aberrant transcripts, referred to as nonsense-mediated decay (NMD), is a specialized mRNA turnover process the cell uses to reduce the production of truncated proteins, which can have deleterious consequences to the cell (20). Another aberrancy monitored by specialized decay machinery is the absence of a stop codon, referred to as nonstop decay. In these cases, the mRNA is targeted to the cytoplasmic exosome by a specific adaptor protein, Ski7p, which is proposed to interact with the stalled ribosome (39, 123).

Quality control systems also exist within the nucleus to degrade inappropriately spliced mRNAs and other abnormal transcripts [for review, see (75)]. Nuclear decay appears to predominately occur via the exosome, although there may be some contributions by the nuclear Xrn1p homolog, Rat1p (15, 41). Other aberrancies monitored within the nucleus include hyperediting of dsRNA (122), the presence of CUG expansions (32), and incomplete transcriptional processes, such as polyadenylation and splicing (Figure 2) (30, 35, 47). A subset of these events results in exosome-mediated retention of the mRNA at the site of transcription [for review, see (56)].

Two observations suggest that the frequency with which aberrant mRNAs are produced is quite high. First, analysis of alternatively spliced mRNAs in mammalian cells suggests that one third of all splicing events produce mRNAs with premature translation termination codons, which would then be recognized and degraded by NMD (69). This indicates that splicing produces large amounts of errors, which are then rapidly degraded. Similarly, expressed sequence tags in which premature polyadenylation has occurred within the open reading frame make up ~1% of yeast libraries. Such mRNAs are degraded at least 10× faster than normal mRNAs because of nonstop decay, thus suggesting that errors during 3′ end processing may be as high as 10% for all transcripts even in a simple organism like yeast (A. van Hoof and R. Parker, unpublished observations).

The infidelity of mRNA processing may serve as an evolutionary capacitor, allowing rapid responses to changing environmental conditions. However, the accumulation of aberrant and potentially toxic mRNA products is a consequence of this plasticity. Therefore, it appears that one major function of mRNA decay in the cell is quality control, which ensures that only mRNA having an appropriate structure, i.e., cap, start codon, stop codon, and poly(A) tail, survive. Thus the cell has evolved "proofreading" mechanisms to distinguish between normal and abnormal mRNAs. Understanding how these distinctions are made will be important in improving our understanding of how turnover is regulated.

THE mRNA DECAPPING MACHINERY

The bulk of this review focuses on the regulation and execution of the decapping reaction. In this section, we introduce the various factors shown to be required for decapping and provide a description of their characteristics and demonstrated, or

TABLE 2 Regulators of yeast mRNA decapping

Protein	Properties	Function	Significant interactions
Pab1p	Contains four N-terminal RRM domains and a proline-rich C terminus	Major protein associated with poly(A) tail. Blocks mRNA decapping and stimulates translation. Primary coupler of deadenylation and decapping.	eIF-4G, eRF3, Pan2/3p
eIF-4E	Cap-binding protein	Component of the eukaryotic translational initiation complex, eIF-4F. Blocks mRNA decapping by competing with Dcp1/2p for access to the cap.	Lsm7p, eIF-4G, eIF-4A, eIF-4B, Pab1p
Lsm1–7p	Sm-like proteins	Required for the efficiency of decapping in vivo. Forms a heteroheptameric ring complex and interacts with the mRNA after deadenylation. May facilitate the assembly of the decapping complex.	Dcp1p, Dcp2p, Dhh1p, Pat1p, Xrn1p, Upf1p
Pat1p	88kDa protein with no recognizable sequence motifs	Interacts with both polyadenylated and deadenylated transcripts. Required for efficiency of both decapping and formation of P bodies in vivo. May "seed" the decapping complex on the mRNA.	Dcp1p, Dcp2p, Lsm1–7p, Dhh1p, Xrn1p, Crm1p
Dhh1p	Member of the ATP-dependent DExD/H box helicase family	Required for the efficiency of decapping in vivo. Homologs across species are required for translational repression during mRNA storage events.	Dcp1p, Dcp2p, Lsm1–7p, Ccr4p, Pop2p, Caf17p, Pbp1p, Edc3p
Edc1p, Edc2p	Small, basic proteins with weak homology to each other	Required for efficient decapping in vitro. Directly binds to the mRNA substrate.	Dcp1p, Dcp2p
Edc3p	Contains five conserved domains	A general and mRNA-specific regulator of decapping. Regulates the decapping of the RPS28a mRNA.	Dcp1p, Dcp2p, Dhh1p, Crm1p, Rps28ap, Nup157p, Lsm8p
Puf3p	Pumillo-like protein, contains eight PUF repeats	Messages specific activator of mRNA deadenylation and decapping. Homologs facilitate translational repression. Regulates the decapping of the COX17 mRNA.	
Upf1p, Upf2p, Upf3p	Upf1p is an ATP-dependent RNA helicase	Required for non-sense-mediated decapping.	eRF1, eRF3, Dcp2p, Upf2p, Lsm1p
eIF-5a, Vps16p, Mrt4p, Sla2p, Gcr5p, Ths1p		Additional proteins suggested to be involved in mRNA turnover but functions remain unclear.	

hypothesized, biochemical functions (summarized in Table 2). An emerging theme is that several different types of protein complexes can be seen interacting with mRNAs and with the decapping enzyme, thereby providing a possible

mechanism by which such proteins can affect decapping. A second theme emerging is that several of these factors appear to have additional roles in the cell and may function to integrate mRNA decapping with translation, transport, and transcription.

The mRNA Decapping Enzyme

Several observations suggest that two proteins, Dcp1p and Dcp2p, function together as a decapping holoenzyme with Dcp2p as the catalytic subunit. First, in both yeast and mammals, these proteins copurify (36, 37, 71). Second, copurification of recombinant yeast Dcp1p and Dcp2p from *Escherichia coli* yields active decapping enzyme under a variety of conditions (100). Third, yeast Dcp2p alone can have decapping activity in the presence of manganese (100) or in high magnesium (110). In contrast, recombinant mammalian Dcp2p is a robust decapping enzyme by itself (110, 116). Fourth, Dcp2p contains a NUDIX motif, which is found in a class of pyrophosphatases (10, 60), and mutations in the Dcp2p NUDIX motif inactivate decapping activity in vivo and in vitro in both the yeast and mammalian enzymes (36, 71, 100, 116). The simplest interpretation of these observations is that Dcp2p is the catalytic subunit of the decapping complex, and Dcp1p primarily functions to enhance Dcp2p activity by a currently unresolved mechanism. It is unclear whether previous results suggesting gel purified or recombinant Dcp1p could have catalytic activity were in error or whether Dcp1p can also have decapping activity (67, 115).

THE CHEMISTRY OF DECAPPING Biochemical analysis of the Dcp1/Dcp2p decapping activity demonstrated that the m^7GpppX of mRNAs is cleaved to yield the products m^7GDP and a 5′-monophosphate mRNA (Figure 1) (67, 102). Production of a transcript with a 5′-monophosphate is functionally significant in order to ensure rapid degradation by Xrn1p, the 5′ to 3′ exonuclease. Xrn1p is blocked by a cap structure (51) and preferentially degrades substrates containing a 5′-monophosphate end compared to those with a 5′-triphosphate end (101). The decapping enzyme presumably functions in vivo as it does in vitro, yielding an mRNA possessing a monophosphate at its 5′ end, which is then rapidly degraded by Xrn1p.

INTERACTION OF Dcp1/2p AND THE mRNA SUBSTRATE The *DCP1/DCP2* gene products demonstrate specificity in vitro consistent with an activity that primarily decaps mRNAs. For example, the 7-methyl group of the cap structure contributes to substrate specificity of the enzyme (67, 116). Interestingly, addition of the cap analog m^7GpppG_{OH} fails to inhibit the decapping reaction to an appreciable extent (67, 116). This suggested that the decapping enzyme might also interact with the body of the transcript or that RNA binding may allosterically regulate decapping. This interpretation has been supported by three additional observations. First, Dcp1/2p was effectively inhibited in vitro by uncapped mRNAs (67). Second, Dcp1/Dcp2p or Dcp2p alone prefers substrates that are $\geq$ 25 nucleotides

in length, with a preference for longer mRNA substrates (67, 88, 100, 116). Third, mammalian and yeast Dcp2p binds to RNA (88; C.J. Decker and R. Parker, unpublished observation). These properties suggest that Dcp2p recognizes the mRNA substrate by interactions with both the cap and the RNA moiety. This preference for longer substrates may have biological significance because the 5′ cap structure is often complexed with a set of proteins involved in translation initiation, referred to as the cap-binding complex or eIF-4F. A requirement for a significant length of RNA in addition to the cap structure for substrate recognition by Dcp1/2p may prevent decapping of mRNAs on which translation initiation complexes are assembled (see below).

FEATURES OF Dcp2p In addition to the NUDIX domain, which appears to be the active site for decapping, Dcp2p also has two additional regions that are highly conserved, termed Box A and Box B. These two regions have no homology to other functional motifs, but it has recently been shown that Box B is required for both RNA binding and decapping activity in vitro (88). The importance of Box A is unknown, but it may serve to facilitate the interaction between Dcp1p and Dcp2p. In yeast, Dcp2p has an extensively long C terminus, which is not seen in other homologs.

FEATURES OF Dcp1p The Dcp1p protein is also conserved in eukaryotes, and two human homologs, hDcp1a and hDcp1b, have been identified (71). The human homologs appear functional because hDcp1a copurifies with decapping activity (71). Experimental and modeling approaches show that the N-terminal of hDcp1a belongs to a new class of functional EVH1/WH1 domains (Ena/VASP homology 1/Wiskott-Aldrich syndrome protein homology 1). This structural feature was first suggested by modeling of one of the human Dcp1p homologs, referred to as SMIF (21). More recently, a high-resolution X-ray structure for the yeast Dcp1p has confirmed and extended this analysis (97). EVH1/WH1 domains are protein-protein interaction modules that interact with their proline-rich containing ligands, thereby providing essential links for their host proteins to various signal transduction pathways, such as actin filament assembly, synaptic transmission, and Ras signaling (4, 9, 89). Comparison of the proline-rich sequence (PRS) binding sites in this family of proteins with Dcp1p indicates that Dcp1p belongs to a novel class of EVH1 domains. Mapping the sequence conservation on the molecular surface of Dcp1p reveals two prominent sites, one of which is required for function of the Dcp1p/Dcp2p complex, and a second, corresponding to the PRS binding site, that is likely to be a binding site for decapping regulatory proteins. Moreover, a conserved hydrophobic patch is revealed to be critical for decapping.

Dcp1p may have additional functions within the cell. Indeed, hDcp1a may play a role in SMAD-mediated TGFβ signaling, having been shown to interact with Smad4 and to partially translocate to the nucleus in response to transformation growth factor β stimulation (3). The significance of this relocalization is

not known but could affect both cytoplasmic degradation and nuclear functions of Dcp1p.

The Scavenger Decapping Enzyme

Eukaryotic cells also contain a second type of decapping enzyme referred to as the scavenger decapping enzyme. These enzymes were first described over thirty years ago and decap short substrates, such as the dinucleotide cap structure or a capped oligonucleotide (64, 83, 84). Recent experiments indicate that the scavenger decapping enzyme acts to decap the capped oligonucleotides produced by exosome mediated 3′ to 5′ degradation of mRNA (117). Consistent with this role, DcpS coimmunoprecipitates with the exosome from mammalian cells (117). DcpS also has a second function in hydrolyzing the m7GDP produced by mRNA decapping to m7GMP and phosphate (111).

DcpS proteins are members of the HIT family of pyrophosphatases and use a histidine triad to perform catalysis (70). Interestingly, DcpS is unable to decap long substrates (70). This suggests that either long mRNAs cannot fit into the active site of DcpS or that RNA is a negative allosteric regulator of this enzyme. The inability to cleave long mRNAs may be biologically important to prevent DcpS from prematurely decapping mRNAs not targeted for degradation.

Regulators of Decapping

Several proteins have been identified that function to accelerate or decelerate the decapping process (see Table 2). One class of proteins includes those that inhibit the decapping process. In particular, the poly(A)-binding protein (Pab1p), is an important member of this group because it is required to couple decapping to prior deadenylation (24, 76). In addition, components of the translation initiation complex also impede decapping (94, 95). Most notably, the cap-binding protein, eIF-4E, inhibits decapping both in vitro and in vivo.

Several proteins are required for the efficient decapping of most normal mRNAs in vivo, but they are not absolutely required for decapping per se. These proteins include the *LSM1–7* complex, which is a presumed RNA-binding complex, Pat1p, which is of unknown biochemical properties, and Dhh1p, which is a member of the DEAD box ATPase family (12, 16, 27, 38, 44, 107). A related class of proteins that affects decapping, referred to as enhancers of decapping, includes Edc1p, Edc2p, and Edc3p (37, 63, 94). Lesions in these genes do not affect general decapping rates unless decapping is already compromised. However, Edc1–3p may be required for the decapping of specific mRNAs. For example, Edc3p specifically affects the decapping rate of the *RPS28a* mRNA (A. Jacquier, personal communication).

Two other classes of proteins are specifically required for the decapping of subsets of mRNAs. For example, specific mRNA-binding proteins, such as the PUF protein family members, can bind individual mRNAs and control their rate of decapping [(85), reviewed in (119)]. Another group of mRNA-specific

regulators are Upf1p, Upf2p, and Upf3p, which are primarily required for the recognition and rapid decapping induced by NMD (72).

Important issues for understanding the process of decapping are (*a*) determining how proteins, such as Pab1p, inhibit decapping and (*b*) defining the roles of proteins in activating decapping in specific substeps in the decapping pathway.

OVERVIEW OF DECAPPING

In the following sections, we discuss the process of mRNA decapping and the role of the proteins involved. To introduce this section, we describe a working model for the process of mRNA decapping with three general points. First, a key step in decapping is the removal or functional inactivation of the poly(A). Second, for decapping to be efficient, mRNAs need to exit translation and assemble into specific mRNP structures, referred to as P bodies, where mRNA decapping occurs. Third, specific mRNA-protein complexes form and may function, in part, to recruit the decapping enzyme to the mRNA.

An overall, and currently speculative, model of mRNA decapping based on these three steps is diagramed in Figure 3 and is discussed in more detail below. First, poly(A) shortening can occur while the mRNA is polysome associated and actively translating, although the process of deadenylation may also be connected to P-body formation (see below). Second, once the mRNA is deadenylated, defective translation initiation events, or stochastic variations in translation initiation, lead to the mRNA becoming ribosome free because of continued elongation in the absence of new initiation events. Third, the combination of being ribosome free and deadenylated leads to a series of mRNP transitions wherein the translation initiation complex disassembles and the decapping complex begins assembly, perhaps nucleated by Pat1p previously bound to the mRNA. Fourth, the assembly of the decapping complex leads to an mRNP structure that aggregates into P bodies wherein decapping complex assembly is completed, decapping occurs, and mRNP proteins are released. Key steps addressed in the following sections are the control of decapping by poly(A) tails, the relationship between translation and decapping, the function and role of P bodies in decapping, and the assembly of mRNA decapping complexes on mRNA.

POLY(A) TAILS AS AN INHIBITOR OF DECAPPING

Several observations indicate that the poly(A) tail can serve as an inhibitor of decapping. First, for those mRNAs examined, the time required before decapping takes place correlates to the time that is required for the mRNAs to deadenylate (34). Second, deadenylation still precedes decapping even when the rate of deadenylation has been either increased or decreased for an individual transcript

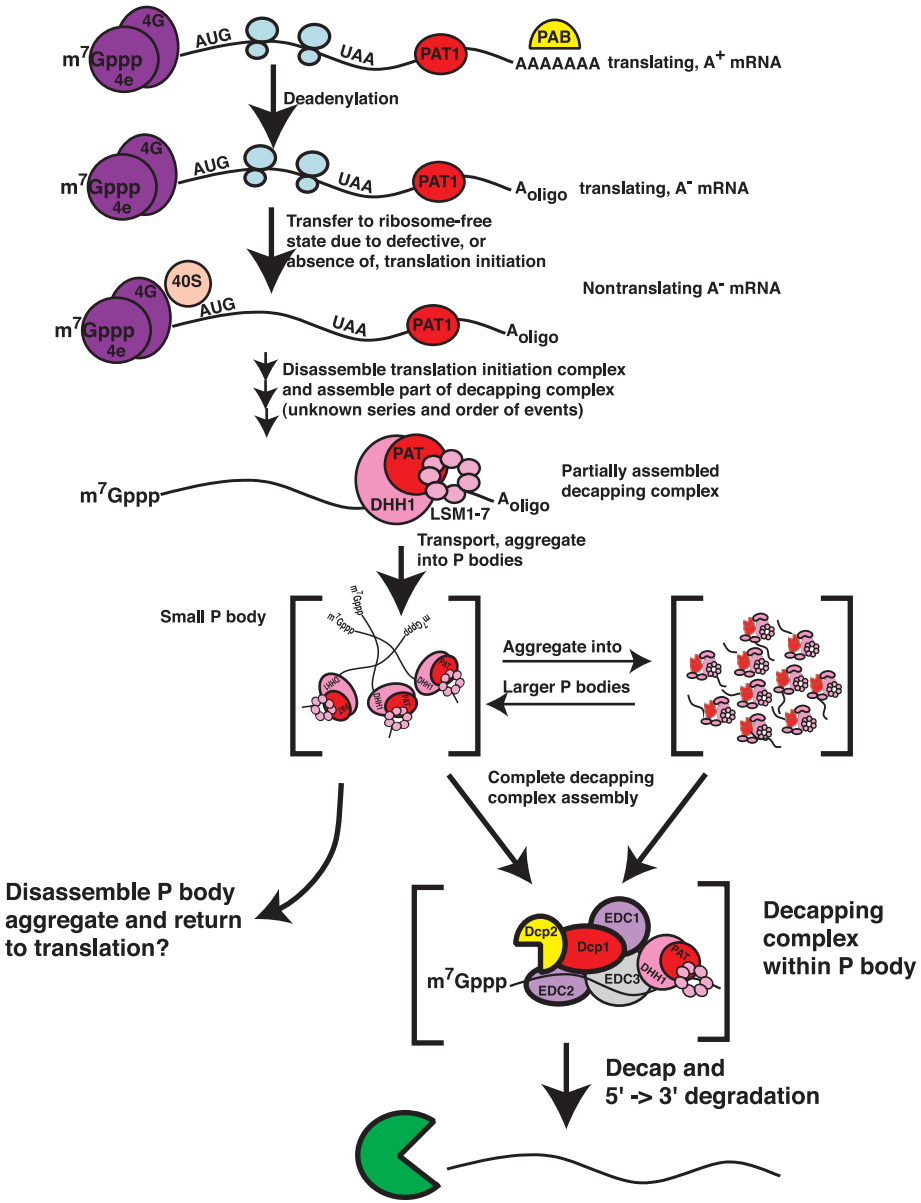

Figure 3 A working hypothesis for the decapping pathway.

by changes in the 3′ UTR sequence (34, 66). Third, decay intermediates produced by the 5′ decapping reaction only appear after at least some of the population of mRNA has undergone deadenylation (34, 79, 80). In addition, these decay intermediates possess only oligo (A)-length tails, suggesting that only deadenylated mRNAs are substrate for decapping (34, 79, 80). Finally, mRNAs with poly(A) tails are resistant to decapping in cell-free extracts in a Pab1p-dependent manner (121). The ability of the poly(A) tail to inhibit decapping adds to the function of poly(A) tails in promoting translation initiation [for review, see (55)] and suggests possible mechanisms by which decapping and translation are connected (see below).

The Poly(A)-Binding Protein Acts as a Block to Decapping

Several observations argue that the ability of the poly(A) tail to inhibit decapping is primarily mediated through Pab1p. First, it has been demonstrated in yeast that decapping occurs when the poly(A) tail has been shortened to an oligo (A) length of ~12 residues (34). This is approximately the minimum length required for Pab1p binding (93). Second, in *pab1* mutant strains, decapping is uncoupled from deadenylation (24, 76). In this case, intermediates in mRNA decay trapped by inhibiting 5′ to 3′ degradation in *cis* with strong secondary structures are produced as decapped mRNA fragments with long poly(A) species (24). This indicates that in the absence of Pab1p the requirement for prior deadenylation before decapping is relieved. It should be noted that poly(A) tails may also inhibit decapping independent of Pab1p. Cell extracts depleted of Pab1p were observed to still show some poly(A) tail inhibition of decapping (121), although the mechanism and in vivo significance of this observation remains to be established.

The block to decapping by Pab1p does not require the presence of the poly(A) tail. If Pab1p is tethered to an mRNA by the RNA-binding domain of the MS2 coat protein, its ability to block decapping becomes independent of its binding to poly(A) (26). This allows Pab1p to bind mRNAs via a MS2-binding sequence in the 3′ UTR, independent of poly(A). These mRNAs accumulate as full-length, capped, and deadenylated messages. This argues that the role of the poly(A) tail is to recruit Pab1p to the transcript and that mRNA-associated Pab1p mediates access to the cap by the decapping machinery. Given this, the process of deadenylation can be considered a process where the binding sites for Pab1p are removed from the transcript, relieving Pab1p-dependent inhibition of decapping.

How Does PAB Block mRNA Decapping?

One unresolved issue is the manner in which Pab1p inhibits decapping and leads to coupling of deadenylation and decapping. Given our current knowledge of mRNA turnover, there are three general, and overlapping, mechanisms by which Pab1p might inhibit decapping (cartooned in Figure 4). Given that no single mechanism is sufficient to explain the effect of Pab1p, it suggests that Pab1p

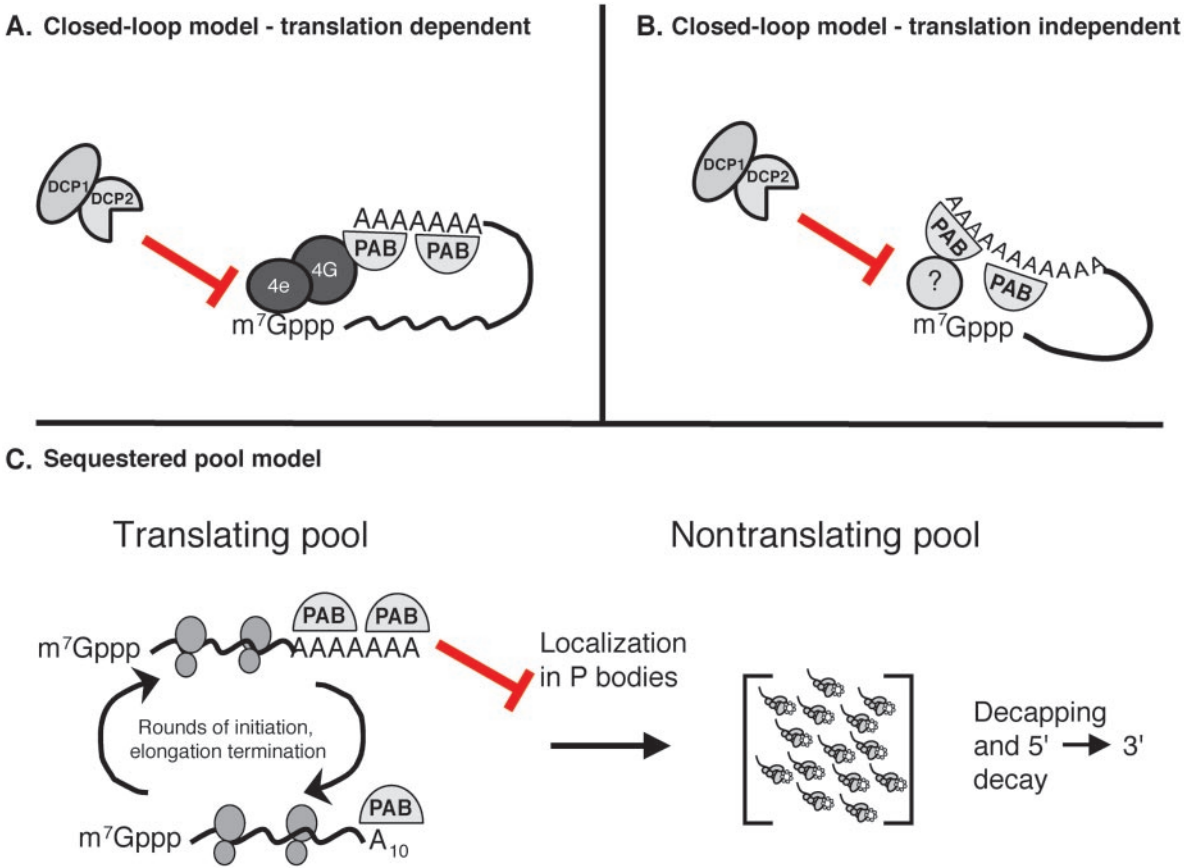

Figure 4 Possible mechanisms for the inhibition of decapping by Pab1p.

inhibits decapping by several mechanisms possibly connecting multiple processes, such as translation, localization, and turnover.

One likely mechanism for Pab1p inhibition of decapping is through interactions with the cap-binding complex (Figure 4*A*). The closed-loop model is the result of observations that in vitro Pab1p directly interacts with the cap-binding complex via protein-protein interactions with eIF-4G (104). This suggests a simple model in which Pab1p binds the poly(A) tail, then binds eIF-4G, which stabilizes eIF-4E on the mRNA. Such an interaction could inhibit decapping both by keeping the cap protected from Dcp1p/Dcp2p by eIF-4E and by increasing translation of the mRNA, which would have an indirect effect on blocking decapping (see below).

However, several observations suggest that the interaction of Pab1p with eIF-4G, though it may contribute, cannot solely explain the Pab1p inhibition of decapping. First, deletion of the eIF-4G interacting domain in Pab1p has no effect on the ability of tethered Pab1p to block decapping (26). This indicates that the region within the Pab1p that stabilizes mRNAs is distinct from the region that binds eIF-4G. Second, strains carrying mutations in translation initiation factors still exhibit dedeadenylation-dependent mRNA decapping even though translational initiation is severely compromised (95). Third, expression of a PAB1 gene from *Arabidopsis* in a *pab1*Δ yeast strain can complement the translation defect but not the mRNA turnover defects (8). This would suggest that the decay and translation functions of the Pab1p are distinct.

A second manner by which Pab1p could inhibit decapping is through interactions that are independent of translation initiation complex (Figure 4*B*). This is suggested by the observation that mutations in critical components of the translation initiation complex, including temperature-sensitive eIF-4E, eIF-4A, and deletion of eIF-4G, increase the rate of decapping. However, this decapping process is still dependent on prior deadenylation (95). One possible mechanism in this case would include protein-protein interaction through which Pab1p could directly inhibit the decapping enzyme. Alternatively, on the basis of in vitro studies, Pab1p may directly bind the cap and inhibit decapping through steric exclusion of the decapping enzyme (58).

A third manner by which Pab1p could inhibit decapping is by affecting the spatial location of transcripts within the cell (Figure 4*C*). For example, the observation that decapping can occur in P bodies suggests translating and degrading pools of mRNAs are spatially distinct (98). Thus, Pab1p might inhibit decapping by preventing mRNAs from entering P bodies or by promoting their rapid exit from P bodies back into the translating pool when Pab1p is bound. This is also consistent with the observation that mRNAs trapped in polysomes (i.e., by treating cells with cyclohexamide) are resistant to decapping and not found in P bodies (6, 98).

The possibility that Pab1p affects the spatial localization of mRNAs and thereby their fate resonates with other observations in the literature. For example, poly(A) has been shown to be preferentially colocalized with microfilaments,

suggesting translating mRNAs might be tethered to the cytoskeleton [for review, see (33)]. In addition, a possible role for Pab1p in recruiting mRNAs out of P bodies is analogous to the recruitment of stored mRNAs into a translating pool by cytoplasmic adenylation. Such processes occur in a variety of contexts, which include regulation of the cell cycle, activation of maternal mRNAs, and localized translation in neurons [for reviews, see (90, 91)].

Decapping of Nonsense mRNAs: Does a Proper Translation Termination Event Establish the Pab1p-Dependent Block to Decapping?

One interesting possibility is that the ability of Pab1p to inhibit decapping is dependent on proper translation termination. This idea results from the observation that nonsense-containing mRNAs, which terminate translation prematurely, undergo rapid decapping independently of deadenylation (22, 78). Moreover, such nonsense mRNAs also show enhanced rates of deadenylation by Ccr4p (22), which is inhibited by Pab1p (108). These two consequences of premature translation termination can be explained by inactivation of Pab1p function.

Additional evidence that proper translation termination may affect Pab1p function comes from a variety of observations. For example, the ability of tethered Pab1p to block decapping requires translation of the mRNA (26). Moreover, recent results using tethered Pab1p indicate that a proper termination event requires only that Pab1p be in close proximity to the stop codon. The critical observations are first that tethering Pab1p to the 3′UTR of a nonsense *PGK1* mRNA at a substantial distance from the termination codon has no effect on the deadenylation-independent decay of this transcript (26). However, moving the binding site for tethered Pab1p close to the early stop codon restores the deadenylation dependence for decapping on this message (A. Jacobson, unpublished data). The implication of these results indicates that Pab1p, proximal to a terminating ribosome, is sufficient to initiate the 5′ block to decapping; however, it does not address the requirements for maintenance of this inhibition. Interestingly, the yeast and mammalian polypeptide chain release factor (eRF3/GSPT) that functions in translation termination has been shown to physically interact with Pab1p (48, 49). This suggests that possibility that proper translation termination leads to enhancement of Pab1p function in inhibiting decapping, perhaps by affecting the dynamics of Pab1p-mRNA interactions such that Pab1p stays bound for prolonged periods.

DECAPPING REQUIRES A TRANSITION TO A NONTRANSLATION COMPETENT mRNP

Several observations argue that translation and decapping of the mRNA are in competition (Figure 5). For example, mutations in translation initiation factors that decrease translation rates increase the rate of decapping (95). Similarly,

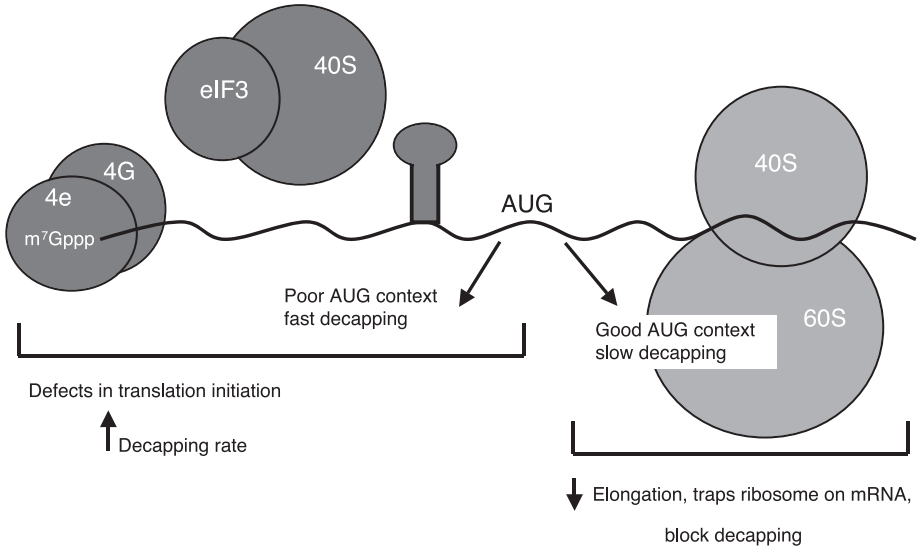

Figure 5 Relationship between mRNA translation and decapping rate.

decreasing translation initiation by placing strong secondary structures within 5′UTRs or with a poor AUG context leads to faster decapping (66, 79). Conversely, inhibition of translation elongation by mutations or by elongation inhibitors (e.g., cyclohexamide) significantly decreased the rates of decapping (6, 87). In addition, increased AUG recognition and an increased length of open reading frame (at least in some cases) led to slower rates of decapping (22, 66). These observations demonstrate a correlation wherein factors that increase an mRNA's association with ribosomes inhibit decapping rates, and factors that decrease an mRNA's association with ribosomes increase decapping rates (Figure 5).

A Critical mRNP Transition From Translation to Decay

An unresolved issue is how active translation of a transcript decreases its rate of decapping. One possibility is that protection from decapping may be due in part to a direct competition between translation and decay machineries for access to the 5′ cap structure. Translation is initiated via recognition of the 5′ m7GpppG cap by eIF-4E, part of the eIF-4F initiation complex. In vitro addition of purified eIF-4E inhibits decapping (96, 121). Consistent with this observation, addition of cap analog enhances decapping rate in mammalian extracts (40). In vivo loss of 4E function stimulates decapping, and lesions in eIF-4E suppress the decapping defect of a *dcp1–1* allele (96). Thus, a translating mRNP is protected from decapping in part by the tight association of the initiation complex with the cap. This conclusion has two clear implications. First, an a priori step in decay must

be dissolution of the translating mRNP and deposition of the decapping machinery, although the specific steps of this exchange are unclear. Second, poorly translated mRNAs must have a faster rate of loss of the cap-binding complex because of some difference in their mRNP dynamics.

Additional evidence for a specific transition in mRNP organization leading to decapping has come from using coimmunoprecipitation to examine the interactions between the mRNA decay factors, the mRNA, the cytoplasmic cap-binding complex (eIF-4E and eIF-4G), and Pab1p. After deadenylation, Pat1p, the LSM complex, and the decapping enzyme (Dcp1/2p) can coimmunoprecipitate with the mRNA. In addition, this mRNP complex does not contain eIF-4E, eIF-4G, and Pab1p (106). Although it has not been directly tested, it is likely this decay mRNP also contains other decapping factors, which include Dhh1p, Edc1p, Edc2, Edc3p, and possibly Pop2p (27). This identifies a critical transition in mRNP organization following deadenylation that leads to decapping and degradation of yeast mRNAs.

The mechanics that cause this mRNP transition are unknown. However, at least some components of the decapping machinery may assemble on the mRNP prior to completion of translation, perhaps to initiate this transition. For example, Pat1p coimmunoprecipitates with Pab1p and eIF-4E in an RNA-dependent manner (106). This suggests that Pat1p can interact with the transcript while it is still translationally competent. Consistent with that view, Pat1p can be detected in polysome fractions (14). Given the association of Pat1p with mRNAs early in the decay process, Pat1p may function to facilitate the removal of the cap-binding complex and subsequent assembly of the decapping complex following deadenylation.

Do Elongating Ribosomes Protect mRNAs From Decapping?

Another possible overlapping manner in which translation and decapping could be related is through mRNAs having different properties when associated with elongating ribosomes. This view is suggested by the several observations. First, all treatments that decrease ribosome loading increase decapping (66, 79, 96). Second, all manipulations that increase ribosome loading decrease decapping rates (6, 22, 66, 87). Third, decapping can occur in P bodies (see below), and polysome-bound mRNAs are protected from decapping and excluded from P bodies (6, 98). On the basis of these observations, we propose a hypothesis wherein elongating ribosomes protect mRNAs from decapping, and decreases in translation initiation increase decapping either by direct recognition of a defective translation initiation event or by simply reducing the probability of having an elongating ribosome present on the mRNA. The relationship between ribosome dynamics and mRNP transitions leading to decapping is unclear but may be related to the cytoplasmic localization of the mRNA (see below).

DECAPPING CAN OCCUR IN SPECIALIZED CYTOPLASMIC STRUCTURES TERMED P BODIES

The proteins involved in mRNA decapping and 5′ to 3′ exonucleolytic decay are found in specific cytoplasmic foci, referred to as P bodies. In yeast, GFP-tagged Dcp1p, Dcp2p, Lsm1p, Pat1p, Dhh1p, and Xrn1p have been localized to P bodies (98). In contrast, Ccr4p, Ski7p (a cytoplasmic component of the exosome), Puf3p (an mRNA-binding protein), and translation factors are not concentrated or show small concentrations in P bodies (98; M. Brengues and R. Parker, unpublished observations). In mammalian cells, the Lsm1–7 complex, Xrn1p, Dcp1p, and Dcp2p have been localized to analogous structures (5, 53, 71, 110).

Two experimental observations in yeast cells argue that P bodies are specific sites wherein mRNAs can be decapped and degraded 5′ to 3′ (98). First, the size and number of P bodies varies in a manner correlating with the flux of mRNA molecules through the decapping step. For example, inhibiting mRNA decay at the deadenylation step in a *ccr4Δ* strain leads to a reduction in P-body size and number. Similarly, inhibiting decapping by deleting the *PAT1* gene or by adding cyclohexamide (6) leads to a reduction or loss of P bodies. In contrast, inhibiting the enzymatic steps of decapping or 5′ to 3′ exonuclease digestion leads to an increase in the size and number of P bodies (98). The second key observation is that mRNA decay intermediates, trapped either by the insertion of strong secondary structures or by deletion of the gene for the 5′ to 3′ exonuclease Xrn1p, can be specifically localized to P bodies. The simplest interpretation of these observations is that P bodies are sites of mRNA decapping and 5′ to 3′ exonucleolytic decay. However, because the mRNA decapping factors are also found distributed throughout the cytoplasm, decapping and degradation may also occur outside of P bodies. A reasonable working hypothesis is that mRNAs undergo a transition to an mRNP state (as discussed above) that is both an mRNP precursor to the decapping reaction and has the ability to aggregate into larger structures (Figure 3). Given this, mRNA decapping and decay might take place outside of large P bodies, possibly in smaller-scale aggregates of the same biochemical nature, but too small to be easily observed in the light microscope. It should be noted that the conservation of these structures from yeast to mammals suggests they have functional significance and that decapping is occurring in mammalian cells.

P bodies are dynamic structures and can vary in size and number under different conditions. One striking example of this phenomenon is that P bodies are affected by changes in the translation status of the cell. Specifically, when cells are treated with translation elongation inhibitors, which trap mRNAs in polysomes, P bodies disappear within 5 min (98). In contrast, when mRNAs are driven off polysomes by conditions reducing translation initiation, such as glucose deprivation (1), P bodies rapidly increase in number and size (D. Teixeira and R. Parker, unpublished results). These results are consistent with the polysome and P-body pool of mRNAs being spatially distinct. Moreover, a

requirement for entry into P bodies for optimal rates of decapping could explain why elongating ribosomes protect mRNAs from decapping.

P bodies show some remarkable similarities to another form of mRNA containing cytoplasmic particles, referred to as a stress granule. Stress granules form in response to decreased translation initiation and contain poly(A)+ mRNA, translation initiation factors, specific RNA-binding proteins TIA and TIA-R, and 40S subunits [for review, see (57)]. Stress granules and P bodies are similar in their dynamics because both are increased by decreasing translation initiation and both decline when mRNAs are driven into polysomes. At this time, however, stress granules and P bodies appear to have distinct protein compositions and to physically differ. Intriguing issues for future work are (*a*) the relationship between stress granule formation and P bodies, (*b*) finding whether these represent different possible fates of nontranslating mRNAs, and (*c*) finding if there are different steps along a pathway of mRNA sequestration and degradation.

The Relationship Between Deadenylation/Decapping Control and P Bodies

An unresolved issue is the relationship between the processes that control deadenylation and decapping. Several observations argue that there are commonalities in the control of these two mechanisms and that their regulation may be intertwined. First, inhibition of translation initiation by mutations in translation initiation factors, poor AUG context, or insertion of strong secondary structures leads to increases in the rates of both deadenylation and decapping (66, 79, 95). Second, many sequence elements that accelerate mRNA degradation in yeast increase the rates of both deadenylation and decapping. These include the *MFA2* 3′ UTR, the MIE in the *MATα1* mRNA, and the binding of Puf3p to the *COX17* mRNA (24, 77, 85). Similarly, recognition of mRNAs as nonsense-containing by the Upf proteins triggers both accelerated deadenylation and decapping (22). Finally, Dhh1p physically binds to both the decapping enzyme and the deadenylase, possibly mediating the communication between these two events (27). One interpretation of these observations is that transcripts that are poorly translated have increased mRNP dynamics and cycle rapidly through states, possibly nontranslating pools of mRNAs, where they are subject to either mRNA deadenylation or decapping.

Given the similarity in factors affecting both deadenylation and decapping, one question is whether deadenylation can or needs to occur within P bodies. Multiple lines of evidence suggest that, though deadenylation probably occurs in P bodies to some extent, deadenylation can also occur outside of these structures. Evidence that P bodies are not required for deadenylation is twofold. First, treatment of yeast cells with cyclohexamide causes rapid loss of P bodies, but deadenylation of *MFA2* and *PGK1* transcripts proceeds normally (6; D. Muhlrad and R. Parker, unpublished observations). Second, Ccr4p, a component of the major yeast deadenylase, is not primarily found in P bodies when cells are grown in glucose media under standard conditions (98).

Despite these results, several other observations argue that deadenylation may be related to P bodies. First, under some conditions, Ccr4p, and additional components of the deadenylase complex, can be concentrated in P bodies (D. Teixiera and R. Parker, unpublished observations). Similarly, when the flux of mRNAs through P bodies is altered by inhibition of decapping, Ccr4p can be detected in P bodies. Interestingly, cyclohexamide treatment of mammalian cells inhibits the deadenylation of *c-myc* mRNA and raises the possibility that this specific type of deadenylation may be sensitive to P-body structure (68). These results suggest that an understanding of the mRNP dynamics and their spatial location should yield insight into both the control of deadenylation and decapping.

A Conserved Model for mRNA Decapping and Other mRNA Storage Events

The hypothesis that mRNAs enter a nontranslating state following deadenylation and prior to decapping is analogous to the storage of mRNA in numerous biological contexts where deadenylated mRNAs are translationally repressed prior to their later activation (120). Such storage occurs during early development, in neurons, and under conditions of stress in which the regulated repression and derepression of mRNA is paramount for controlling gene expression (50, 57, 59). In essence, the storage that occurs under these situations and the formation of a decapping complex may be manifestations of the same event (Figure 6).

There are three striking similarities between the formation of an untranslated mRNP prior to decapping and the storage of maternal mRNAs. First, in both cases, the mRNA is deadenylated and then enters a translational repressed state. Second, in both cases, similar proteins are involved. For example, the *Drosophila* homolog of Dhh1p, *Me31b*, is required for the masking of *bicoid* and *oskar* mRNA, and the Xenopus homolog, Xp54, is a major component of maternal mRNA storage particles and appears to directly repress mRNA translation (65, 73, 82). Third, both processes occur within distinct cytoplasmic granules (50, 98). For example, maternal mRNA storage is hallmarked by the formation of granular-like structures commonly referred to as polar granules, P granules, or germinal granules (120). In neurons, cytoplasmic structures, termed transport granules, contain stored mRNA that are transported to synaptic junctions (91).

This suggests the hypothesis that cells utilize a conserved mechanism by which mRNAs exit translation and enter a quiescent state with different fates of that quiescent mRNA utilized in different biological contexts. The primordial role of the decapping machinery may be that of mRNA storage following translational repression. In organisms such as yeast in which the decapping rate is high, translational repression following deadenylation would lead to destruction of the mRNA. In oocytes and neurons, deadenylation would lead to the storage of mRNAs as metabolically inert species until activated by cytoplasmic polyadenylation (42, 91). Given this, an understanding of the events that facilitate the assembly of the decapping complex may in fact provide insights into other areas in which mRNAs are posttranscriptionally regulated.

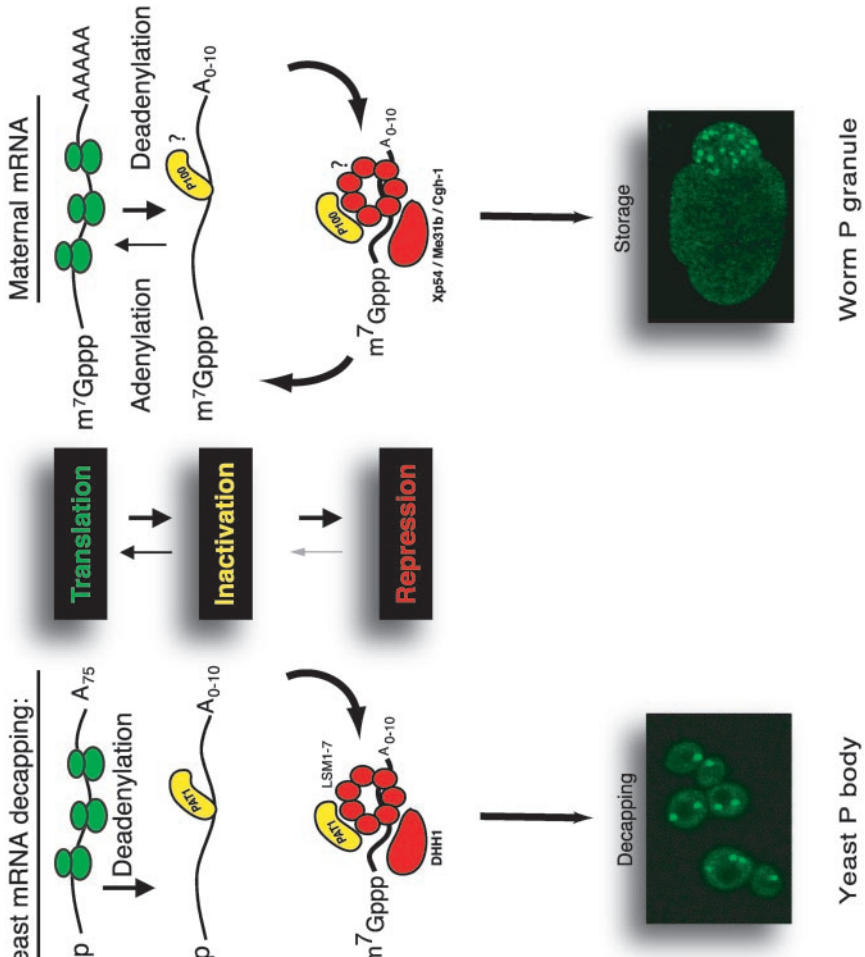

Figure 6 Possible relationship between mRNA decay and maternal mRNA storage.

Advantages of Sequestration of Decapping Factors

The observation that mRNA decapping at least primarily occurs within P bodies raises an obvious question: Why sequester mRNAs for decapping into discrete cytoplasmic foci? There are three potential reasons why sequestration of mRNAs and the decapping machinery would be beneficial to the cell. First, by sequestering the decapping machinery, or dictating it is only active when assembled into P bodies, the cell effectively partitions the degradation machinery away from the functional mRNAs. This partitioning could prevent the premature decapping of mRNAs not yet targeted for decay. In addition, by regulating delivery to the complex, the cell adds an additional layer of control, distinct from the enzymatic steps of degradation, which can be regulated. In this light, it is worth noting that most degradative processes are compartmentalized either within membrane organelles (e.g., the lysosome) or larger structures (e.g., the proteosome). This common compartmentalization must reflect functional advantages.

Another possible rationale for compartmentalization of these mRNAs in both stress granules and P bodies is that it reflects a fundamental buffering system within cells for maintaining a proper ratio of translation capacity to the pool of mRNAs that are translating (Figure 7). An excessive amount of mRNAs within the translating pool may compete for limiting translation factors. Thus, no mRNA would receive its full complement of proteins required for appropriate initiation, and overall translation rates of all mRNAs would decline. By sequestering nontranslating mRNAs in a manner that is not in competition with translation, the cell may be able to successfully translate the mRNAs remaining in the translation pool (Figure 7). In this view, the role of mRNA sequestration into stress granules and/or P bodies may be an ancient system for buffering translation capacity, which has then been co-opted by evolution for various other uses, such as the control of mRNA degradation and storage of mRNAs.

A third hypothesis for these particles is that translational repression and/or targeting a mRNA for decay may require two distinct phases (Figure 6). The first phase would simply be a slowing of translational initiation rate. This could occur quite passively by the loss of the poly(A) tail, or it may be an active consequence of binding a repressor protein. In the latter case, the ability to stay repressed is a function of the dissociation rate of the repressor with its target message. Because mRNP complexes can be highly dynamic, this particular state has the potential to reenter the translational pool, even if at a fairly low level. In order to prevent promiscuous expression of certain messages, a second state may exist in which repression is achieved and maintained. This may be a function of assembling mRNAs into large aggregated complexes. By assembling mRNA into large complexes, the dissociation rate of the binding no longer is the driving force influencing cycles of repression and derepression. Rather, it is the ability to deliver the mRNA to the repressive center (i.e., P bodies) that becomes the rate-limiting step.

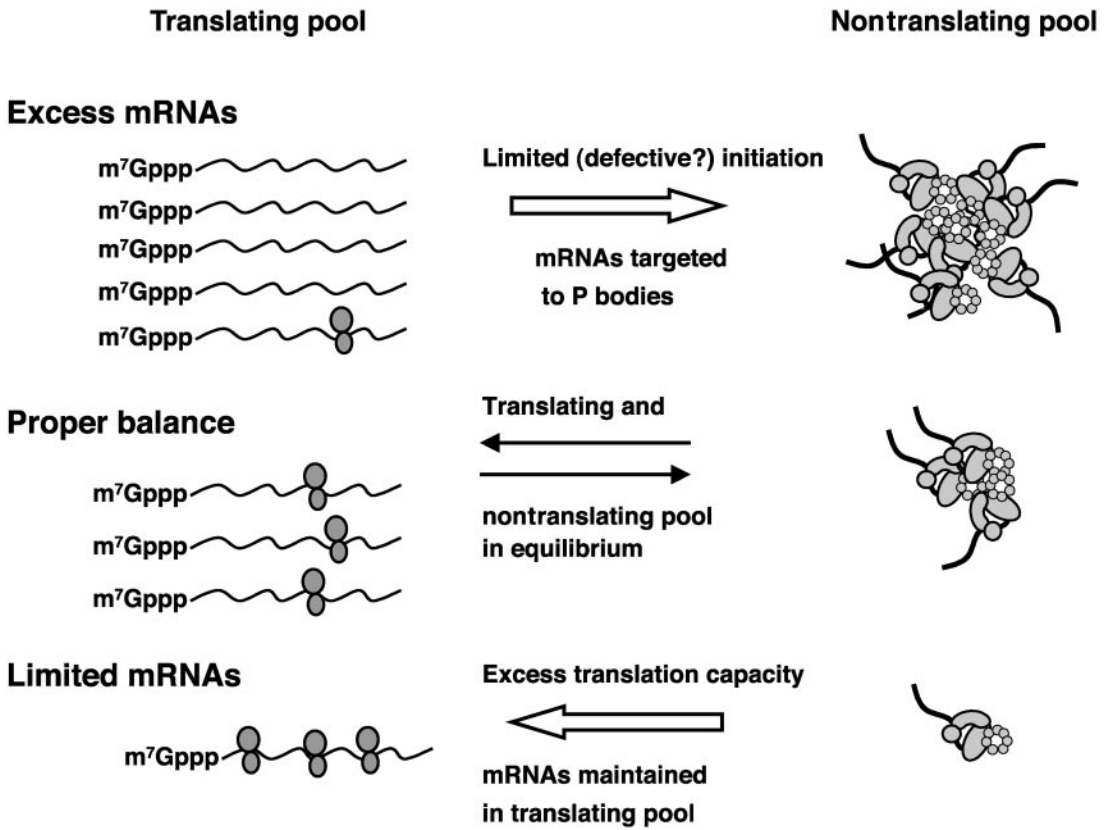

Figure 7 Model showing how compartmentalization can provide buffering to translational capacity.

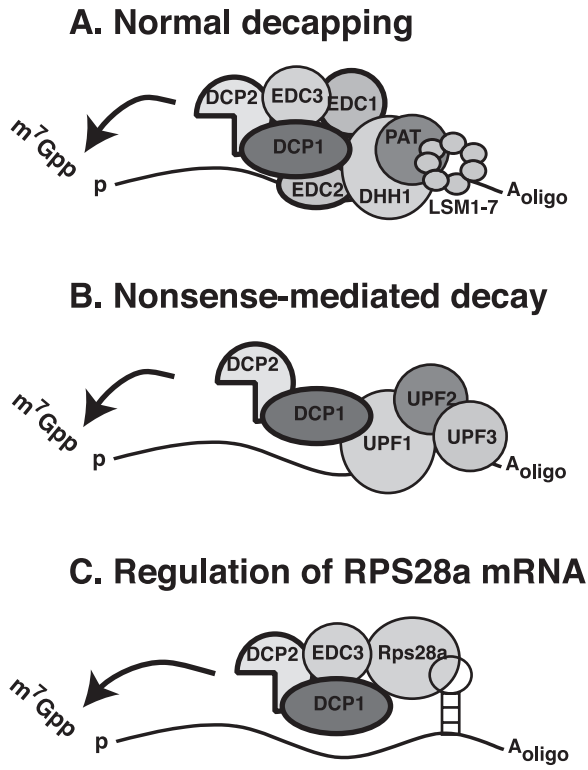

Figure 8 Illustration of three distinct decapping complexes.

ASSEMBLY OF DECAPPING COMPLEXES

Decapping of mRNAs also involves the assembly of different complexes of proteins on the mRNA that may function in vivo to enhance the interaction of the decapping enzyme with mRNA. In yeast, where several proteins affecting decapping have been identified, the majority of mRNAs appear to assemble a decapping complex consisting of the Lsm1 to Lsm7 proteins, Pat1p, Dhh1p, and Dcp1p/Dcp2p (Figure 8*A*) (7, 12, 16, 27, 38, 44, 105). In contrast, physical interactions between Upf1p and Dcp2p suggest that the process of NMD involves the assembly of a decapping complex wherein the decapping enzyme is recruited to the mRNA through interactions with Upf1p (Figure 8*B*) (45, 71). Another specialized decapping complex appears to occur on the *RPS28A* mRNA, where in an autoregulatory process, the Rps28a protein binds a stem-loop in the 3′ UTR and then interacts with Edc3p, which interacts with Dcp1p/Dcp2p and enhances their functions (Figure 8*C*) (A. Jacquier, personal communication). Thus, this set of physical interactions could recruit the decapping enzyme to the Rps28a. One

should expect to see more such complexes identified as work in this area progresses. For example, the two small RNA-binding proteins, Edc1p and Edc2p, have been shown to bind RNA and recruit Dcp1/Dcp2p for decapping in vitro. This suggests that Edc1p and Edc2p may also nucleate mRNA-specific decapping complexes on specific mRNAs yet to be identified.

FUTURE DIRECTIONS

In the past few years, decapping has emerged as an important step in the process of mRNA degradation. Moreover, the enzymes and numerous proteins that modulate decapping have been identified. However, little is known about how each factor specifically functions. A deeper understanding into the biochemical properties of the decapping enzyme and its regulators should yield increased insight into the actual process of decapping and its control.

This basic understanding should also set the stage for a mechanistic understanding of how the decay rates for individual mRNAs are initially specified and then regulated in response to environmental cues. Progress in this area should come from understanding mRNA-specific binding proteins and their mechanisms of function. In addition, this area will be aided by a basic understanding of the various mRNP states and the dynamics that exist between a translating mRNA and an mRNA associated with the decapping complex. By understanding how a translating mRNP protects an mRNA from decapping, we shall also gain insight into the process of translational control, the role of Pab1p, and how Pab1p couples deadenylation and decapping.

A new challenge for the field is understanding the role of compartmentalization of mRNAs into discrete cytoplasmic structures, such as P bodies and stress granules. Despite the conservation of these structures, their biological significance is still largely unclear. This area is only in its infancy, and we should expect to see additional biological functions connected to these structures in the future. Insight into the function of these structures will require a biochemical and genetic analysis of their assembly/disassembly and relationships with each other and other cellular structures.

ACKNOWLEDGMENTS

We thank Drs. Allan Jacobson and Alain Jacquier for sharing results prior to publication. In addition, we thank the members of the Parker lab for sharing their time, talent, friendship, and advice over the course of this work. Support was received by grants from both HHMI and NIH.

The *Annual Review of Biochemistry* is online at http://biochem.annualreviews.org

LITERATURE CITED

1. Ashe MP, De Long SK, Sachs AB. 2000. *Mol. Biol. Cell* 11:833–48
2. Deleted in proof
3. Bai RY, Koester C, Ouyang T, Hahn SA, Hammerschmidt M, et al. 2002. *Nat. Cell Biol.* 4(3):181–90
4. Ball LJ, Jarchau T, Oschkinat H, Walter U. 2002. *FEBS Lett.* 513(1):45–52
5. Bashkirov VI, Scherthan H, Solinger JA, Buerstedde JM, Heyer WD. 1997. *J. Cell Biol.* 136(4):761–73
6. Beelman CA, Parker R. 1994. *J. Biol. Chem.* 269(13):9687–92
7. Beelman CA, Stevens A, Caponigro G, Lagrandeur TE, Hatfield L, et al. 1996. *Nature* 382:642–46
8. Belostotsky DA, Meagher RB. 1996. *Plant Cell* 8(8):1261–75
9. Beneken J, Tu JC, Xiao B, Nuriya M, Yuan JP, et al. 2000. *Neuron* 26(1):143–54
10. Bessman M, Frick D, O'Handley S. 1996. *J. Biol. Chem.* 271:25059–62
11. Binder R, Horowitz JA, Basilion JP, Koeller DM, Klausner RD, Harford JB. 1994. *EMBO J.* 13(8):1969–80
12. Boeck R, Lapeyre B, Brown CE, Sachs AB. 1998. *Mol. Cell. Biol.* 18(9):5062–72
13. Boeck R, Tarun S Jr, Rieger M, Deardorff JA, Muller-Auer S, Sachs AB. 1996. *J. Biol. Chem.* 271(1):432–38
14. Bonnerot C, Boeck R, Lapeyre B. 2000. *Mol. Cell. Biol.* (16):5939–46
15. Bousquet-Antonelli C, Presutti C, Tollervey D. 2000. *Cell* 102(6):765–75
16. Bouveret E, Rigaut G, Shevchenko A, Wilm M, Séraphin B. 2000. *EMBO J.* 19: 1661–71
17. Bremer KA, Stevens A, Schoenberg DR. 2003. *RNA* 9(9):1157–67
18. Brown CE, Tarun SZ Jr, Boeck R, Sachs AB. 1996. *Mol. Cell. Biol.* 16(10):5744–53
19. Butler JS. 2002. *Trends Cell Biol.* 12(2): 90–96
20. Cali BM, Anderson P. 1998. *Mol. Gen. Genet.* 260(2–3):176–84
21. Callebaut I. 2002. *FEBS Lett.* 519(1–3): 178–80
22. Cao D, Parker R. 2003. *Cell* 113(4): 533–45
23. Cao D, Parker R. 2001. *RNA* 7:1192–212
24. Caponigro G, Parker R. 1995. *Genes Dev.* 9(19):2421–32
25. Chen CY, Gherzi R, Ong SE, Chan EL, Raijmakers R, et al. 2001. *Cell* 107(4): 451–64
26. Coller JM, Gray NK, Wickens MP. 1998. *Genes Dev.* 12(20):3226–35
27. Coller JM, Tucker M, Sheth U, Valencia-Sanchez MA, Parker R. 2001. *RNA* 7(12):1717–27
28. Couttet P, Fromont-Racine M, Steel D, Pictet R, Grange T. 1997. *Proc. Natl. Acad. Sci. USA* 94(11):5628–33
29. Cunningham KS, Dodson RE, Nagel MA, Shapiro DJ, Schoenberg DR. 2000. *Proc. Natl. Acad. Sci. USA* 97(23): 12498–502
30. Custodio N, Carmo-Fonseca M, Geraghty F, Pereira HS, Grosveld F, Antoniou M. 1999. *EMBO J.* 18(10):2855–66
31. Daugeron MC, Mauxion F, Seraphin B. 2001. *Nucleic Acids Res.* 29(12): 2448–55
32. Davis BM, McCurrach ME, Taneja KL, Singer RH, Housman DE. 1997. *Proc. Natl. Acad. Sci. USA* 94(14):7388–93
33. Decker CJ, Parker R. 1995. *Curr. Opin. Cell Biol.* 7(3):386–92
34. Decker CJ, Parker R. 1993. *Genes Dev.* 7:1632–43
35. Dower K, Rosbash M. 2002. *RNA* 8(5): 686–97
36. Dunckley T, Parker R. 1999. *EMBO J.* 18:5411–22
37. Dunckley T, Tucker M, Parker R. 2001. *Genetics* 157:27–37
38. Fischer N, Weis K. 2002. *EMBO J.* 21(11):2788–97
39. Frischmeyer PA, van Hoof A, O'Donnell K, Guerrerio AL, Parker R, Dietz HC. 2002. *Science* 295:2258–61

40. Gao M, Wilusz CJ, Peltz SW, Wilusz J. 2001. *EMBO J.* 20(5):1134–43
41. Geerlings TH, Vos JC, Raue HA. 2000. *RNA* 6(12):1698–703
42. Gray NK, Wickens M. 1998. *Annu. Rev. Cell Dev. Biol.* 14:399–458
43. Hanson MN, Schoenberg DR. 2001. *J. Biol. Chem.* 276(15):12331–37
44. Hatfield L, Beelman CA, Stevens A, Parker R. 1996. *Mol. Cell. Biol.* 16: 5830–38
45. He F, Jacobson A. 1995. *Genes Dev.* 9(4):437–54
46. Higgs DC, Colbert JT. 1994. *Plant Cell* 6(7):1007–19
47. Hilleren P, McCarthy T, Rosbash M, Parker R, Jensen TH. 2001. *Nature* 413(6855):538–42
48. Hoshino S, Imai M, Kobayashi T, Uchida N, Katada T. 1999. *J. Biol. Chem.* 274(24):16677–80
49. Hosoda N, Kobayashi T, Uchida N, Funakoshi Y, Kikuchi Y, et al. 2003. *J. Biol. Chem.* 278(40):38287–91
50. Houston DW, King ML. 2000. *Curr. Top. Dev. Biol.* 50:155–81
51. Hsu CL, Stevens A. 1993. *Mol. Cell. Biol.* 13:4826–35
52. Hui J, Reither G, Bindereif A. 2003. *RNA* 9(8):931–36
53. Ingelfinger D, Arndt-Jovin DJ, Luhrmann R, Achsel T. 2002. *RNA* 8(12): 1489–501
54. Jacobs JS, Anderson AR, Parker RP. 1998. *EMBO J.* 17(5):1497–506
55. Jacobson A, Peltz SW. 1996. *Annu. Rev. Biochem.* 65:693–739
56. Jensen TH, Dower K, Libri D, Rosbash M. 2003. *Mol. Cell* 11(5):1129–38
57. Kedersha N, Anderson P. 2002. *Biochem. Soc. Trans.* 30:963–69
58. Khanna R, Kiledjian M. 2004. *Genes Dev.* In press
59. Kiebler MA, DesGroseillers L. 2000. *Neuron* 1:19–28
60. Koonin EV. 1993. *Nucleic Acids Res.* 21(20):4847
61. Korner CG, Wahle E. 1997. *J. Biol. Chem.* 272(16):10448–56
62. Korner CG, Wormington M, Muckenthaler M, Schneider S, Dehlin E, Wahle E. 1998. *EMBO J.* 17(18): 5427–37
63. Kshirsagar M, Parker R. 2004. *Genetics* In press
64. Kumagai H, Kon R, Hoshino T, Aramaki T, Nishikawa M, et al. 1992. *Biochim. Biophys. Acta* 1119(1):45–51
65. Ladomery M, Wade E, Sommerville J. 1997. *Nucleic Acids Res.* 25:965–73
66. LaGrandeur T, Parker R. 1999. *RNA* 5: 420–33
67. LaGrandeur TE, Parker R. 1998. *EMBO J.* 17(5):1487–96
68. Laird-Offringa IA, de Wit CL, Elfferich P, van der Eb AJ. 1990. *Mol. Cell. Biol.* 10(12):6132–40
69. Lewis BP, Green RE, Brenner SE. 2003. *Proc. Natl. Acad. Sci. USA* 100(1): 189–92
70. Liu HD, Rodgers ND, Jiao X, Kiledjian M. 2002. *EMBO J.* 21(17):4699–708
71. Lykke-Andersen J. 2002. *Mol. Cell. Biol.* 22(23):8114–21
72. Maquat LE, Carmichael GG. 2001. *Cell* 104(2):173–76
73. Minshall N, Thom G, Standart N. 2001. *RNA* (12):1728–42
74. Mitchell P, Tollervey D. 2000. *Curr. Opin. Genet. Dev.* 10(2):193–98
75. Moore MJ. 2002. *Science* 298(5592): 370–71
76. Morrissey JP, Deardorff JA, Hebron C, Sachs AB. 1999. *Yeast* 15(8):687–702
77. Muhlrad D, Parker R. 1992. *Genes Dev.* 6:2100–11
78. Muhlrad D, Parker R. 1994. *Nature* 370(6490):578–81
79. Muhlrad D, Decker CJ, Parker R. 1995. *Mol. Cell. Biol.* 15:2145–56
80. Muhlrad D, Decker CJ, Parker R. 1994. *Genes Dev.* 8:855–66
81. Mukherjee D, Gao M, O'Connor JP, Raijmakers R, Pruijn G, et al. 2002. *EMBO J.* 21(1–2):165–74

82. Nakamura A, Amikura R, Hanyu K, Kobayashi S. 2001. *Development* 128(17):3233–42
83. Nuss DL, Furuichi Y. 1977. *J. Biol. Chem.* 252(9):2815–21
84. Nuss DL, Furuichi Y, Koch G, Shatkin AJ. 1975. *Cell* 6(1):21–27
85. Olivas W, Parker R. 2000. *EMBO J.* 19(23):6602–11
86. Parker R, Song H. 2004. *Nat. Struct. Biol.* 11(2):121–27
87. Peltz SW, Donahue JL, Jacobson A. 1992. *Mol. Cell. Biol.* 12(12):5778–84
88. Piccirillo C, Khanna R, Kiledjian M. 2003. *RNA* 9(9):1138–47
89. Renfranz PJ, Beckerle MC. 2002. *Curr. Opin. Cell Biol.* 14(1):88–103
90. Richter JD. 1999. *Microbiol. Mol. Biol. Rev.* 63(2):446–56
91. Richter JD. 2001. *Proc. Natl. Acad. Sci. USA* 98(13):7069–71
92. Rodgers ND, Wang Z, Kiledjian M. 2002. *RNA* 8(12):1526–37
93. Sachs AB, Davis RW, Kornberg RD. 1987. *Mol. Cell. Biol.* 7(9):3268–76
94. Schwartz DC, Decker CJ, Parker R. 2003. *RNA* 9(2):239–51
95. Schwartz DC, Parker R. 1999. *Mol. Cell. Biol.* 19(8):5247–56
96. Schwartz DC, Parker R. 2000. *Mol. Cell. Biol.* 20(21):7933–42
97. She M, Decker C, Liu Y, Sundramurthy K, Parker R, Song H. 2004. *Nat. Struct. Mol. Biol.* In press
98. Sheth U, Parker R. 2003. *Science* 300(5620):805–8
99. Shyu AB, Belasco JG, Greenberg ME. 1991. *Genes Dev.* 5(2):221–31
100. Steiger M, Carr-Schmid A, Schwartz DC, Kiledjian M, Parker R. 2003. *RNA* 9(2):231–38
101. Stevens A, Maupin MK. 1987. *Arch. Biochem. Biophys.* 252(2):339–47
102. Stevens A. 1980. *J. Biol. Chem.* 255(7): 3080–85
103. Stoeckle MY, Hanafusa H. 1989. *Mol. Cell. Biol.* 9(11):4738–45
104. Tarun S, Sachs A. 1996. *EMBO J.* 15: 7168–77
105. Tharun S, He W, Mayes AE, Lennertz P, Beggs JD, Parker R. 2000. *Nature* 404(6777):515–18
106. Tharun S, Parker R. 2001. *Mol. Cell* 8(5):1075–83
107. Tijsterman M, Ketting RF, Plasterk RH. 2002. *Annu. Rev. Genet.* 36:489–519
108. Tucker M, Staples RR, Valencia-Sanchez MA, Muhlrad D, Parker R. 2002. *EMBO J.* 21(6):1427–36
109. Tucker M, Valencia-Sanchez MA, Staples RR, Chen J, Denis CL, Parker R. 2001. *Cell* 104:377–86
110. van Dijk E, Cougot N, Meyer S, Babajko S, Wahle E, Seraphin B. 2002. *EMBO J.* 21(24):6915–24
111. van Dijk E, Le Hir H, Seraphin B. 2003. *Proc. Natl. Acad. Sci. USA* 100(21): 12081–86
112. van Dijk EL, Sussenbach JS, Holthuizen PE. 2001. *Nucleic Acids Res.* 29(17): 3477–86
113. van Hoof A, Parker R. 2002. *Curr. Biol.* 12(8):285–87
114. van Hoof A, Parker R. 1999. *Cell.* 99(4): 347–50
115. Vilela C, Velasco C, Ptushkina M, McCarthy JE. 2000. *EMBO J.* 19(16):4372–82
116. Wang ZR, Jiao XF, Carr-Schmid A, Kiledjian M. 2002. *Proc. Natl. Acad. Sci. USA* 99(20):12663–68
117. Wang ZR, Kiledjian M. 2001. *Cell* 107(6):751–62
118. Waterhouse PM, Wang MB, Lough T. 2001. *Nature* 411(6839):834–42
119. Wickens M, Bernstein DS, Kimble J, Parker R. 2002. *Trends Genet.* 18(3):150–57
120. Wickens M, Goldstrohm A. 2003. *Science* 300(5620):753–55
121. Wilusz CJ, Gao M, Jones CL, Wilusz J, Peltz SW. 2001. *RNA* 7(10):1416–24
122. Zhang Z, Carmichael GG. 2001. *Cell* 106(4):465–75
123. van Hoof A, Frischmeyer PA, Dietz HC, Parker R. 2002. *Science* 295(5563): 2262–64