RNA decapping inside and outside of processing bodies
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Decapping is a central step in eukaryotic mRNA turnover. Recent studies have identified several factors involved in catalysis and regulation of decapping. These include the following: an mRNA decapping complex containing the proteins Dcp1 and Dcp2; a nucleolar decapping enzyme, X29, involved in the degradation of U8 snoRNA and perhaps of other capped nuclear RNAs; and a decapping ‘scavenger’ enzyme, DcpS, that hydrolyzes the cap structure resulting from complete 3’-to-5’ degradation of mRNAs by the exosome. Several proteins that stimulate mRNA decapping by the Dcp1:Dcp2 complex co-localize with Dcp1 and Dcp2, together with Xrn1, a 5’-to-3’ exonuclease, to structures in the cytoplasm called processing bodies. Recent evidence suggests that the processing bodies may constitute specialized cellular compartments of mRNA turnover, which suggests that mRNA and protein localization may be integral to mRNA decay.

Introduction
Messenger RNA turnover plays a key role in the regulation of gene expression. Removal of the 5’ mRNA cap by the process of decapping is an important step in both general mRNA turnover and in specific mRNA decay pathways (recently reviewed in [1,2]). Here, we discuss the proteins responsible for catalysis and regulation of decapping, and how they are integrated into mRNA decay pathways in eukaryotic cells.

Decapping is a central step in the deadenylation-mediated mRNA decay pathway, which appears to be the prominent mRNA decay pathway in the budding yeast Saccharomyces cerevisiae. In this pathway, shortening of the poly-A tail by a Ccr4:Not deadenylase complex results in recruitment of the Lsm 1–7 complex, which in turn activates the Dcp1:Dcp2 decapping enzyme complex by an unknown mechanism [2–6] (Figure 1a). The body of the mRNA is subsequently degraded from the 5’ end by the 5’-to-3’ exonuclease Xrn1 [3]. Alternatively, following deadenylation, mRNAs can be degraded from the 3’ end by a complex of 10 or more 3’-to-5’ exonucleases called the exosome. The resulting cap structure is hydrolyzed by the ‘scavenger’ decapping enzyme, DcpS [7–9] (Figure 1a).

Decapping also plays a key role in specific mRNA decay pathways. For example, in the nonsense-mediated decay (NMD) pathway, which degrades mRNAs with premature termination codons (PTCs), mRNAs are subjected primarily to deadenylation-independent decapping in S. cerevisiae [10] (Figure 1b), whereas deadenylation is activated to a minor extent [11–13,14]. Similarly, in human cells, depletion by RNA interference of the decapping enzyme Upf1, a component central to NMD, associates with the Dcp1:Dcp2 decapping complex [15,16]. Decapping also plays an important role in the AU-rich element (ARE)-mediated decay pathway, which renders mRNAs that contain AREs in their 3’UTRs unstable in mammalian cells [17,18] (Figure 1c). The human Dcp1:Dcp2 complex and other mRNA decay enzymes, including deadenylases, are thought to be recruited to ARE-containing mRNAs by ARE-binding proteins that activate mRNA decay [18*,19]. In addition, recent studies indicate that decapping also plays a key role in turnover of nuclear RNAs, including U8 snoRNA and pre-mRNAs [20**,21]. Decapping thus plays a central role in RNA turnover in eukaryotic cells. Recent research has shed light on the factors involved in the specific decapping processes.

The decapping enzymes and their distinct roles in RNA turnover
Multiple decapping enzymes have been identified that play distinct roles in the cell (Figure 2). The initial protein that was co-purified with decapping activity in S. cerevisiae was Dep1 [22]. In later studies, a screen for suppressors of Dep1 mutations in S. cerevisiae identified an additional decapping subunit, Dep2, which associates with Dep1 [23]. Homologs of Dep1 and Dep2 proteins have been identified in other eukaryotes, including humans [16,24,25].

The Dcp1:Dcp2 complex catalyzes the removal of m7GDP (7-methyl-GDP) from a capped RNA of more
than ~25 nucleotides [22,26]. The identity of the catalytic subunit of the Dcp1:Dcp2 complex has generated some controversy. Initially, Dcp1 was proposed to be the catalytic subunit on the basis of an in-gel decapping assay showing activity of Dcp1 purified from S. cerevisiae cells [22]. However, neither yeast nor human Dcp1 possesses catalytic activity when purified from bacteria [16,24,26]. By contrast, recombinant Dcp2 from both yeast and humans shows decapping activity in vitro [16,24–26]. Dcp2 contains a pyrophosphatase NUDIX domain, and mutation of critical residues in this domain inactivates decapping activity [16,23,25]. These observations favor Dcp2 as the catalytic subunit of the decapping complex. However, several point mutations have been identified in

Decapping plays a key role in different mRNA degradation pathways. (a) Deadenylation-mediated decay. mRNAs degraded by the major general decay pathway in S. cerevisiae are decapped by the Dcp1:Dcp2 complex following deadenylation and association with the Lsm 1–7 proteins and then degraded from the 5’ end by Xrn1. The cap structure generated by complete 3’-to-5’ degradation of an mRNA is hydrolyzed by another decapping enzyme, DcpS. ‘Pacmen’ symbolize exonucleases, including the Ccr4:Not deadenylase complex, the 5’-to-3’ exo-

exonuclease Xrn1 and the exosome, as indicated. (b) Decapping is involved in NMD. The Dcp1:Dcp2 complex interacts with Upf1, an essential factor in NMD, and decapping can be triggered without prior deadenylation. Deadenylation is separately activated as well. A ribosome terminating at a PTC is shown in brown. A gray ‘pacman’ symbolizes an unknown deadenylase. (c) Decapping is involved in AU-rich element (ARE)-mediated decay in mammalian cells. ARE-binding proteins, such as TTP shown here, interact with the Dcp1:Dcp2 decapping complex as well as other mRNA decay enzymes and trigger mRNA decay. A gray ‘pacman’ symbolizes a deadenylase.
yeast and human Dcp1 that do not interfere with Dcp2 association, but disrupt the decapping activity of the Dcp1:Dcp2 complex [16,27,28]. Most likely, the Dcp2 NUDIX motif plays a central role in catalysis by the Dcp1:Dcp2 complex, but the enzymatic activity of Dcp2 is critically dependent on the Dcp1 subunit in vivo. More insights into the catalytic mechanism of the Dcp1:Dcp2 complex will surely arise from structural studies, which have recently been initiated with the crystal structure of the S. cerevisiae Dcp1 protein [28].

An additional decapping activity has recently been identified in the Xenopus nucleus. The protein X29, which has a NUDIX domain similar to Dcp2 (Figure 2), localizes primarily in the nucleolus and shows specificity for U8 snoRNA [20]. These observations suggest that X29 plays a role in the regulation of U8 snoRNA turnover. However, whether X29 also plays a broader role in nuclear RNA decapping and decay is not known. Putative homologs of X29 were identified in the genomes of other vertebrates [20].

A different decapping activity, catalyzed by DcpS, hydrolyzes the m7GpppN (7-methyl-GpppN) cap that results from complete 3′-to-5′ mRNA degradation by the exosome [9,29]. DcpS exists in complex with the exosome and is believed to play an important role in ensuring that no excess unhydrolyzed cap accumulates, which could titrate the cap-binding translation initiation factor, eIF4E, away from translated mRNAs [30]. In contrast to the Dcp1:Dcp2 complex and X29, DcpS catalyzes the release of m7GMP [9]. Structural and biochemical studies have revealed that a central histidine in a histidine triade (HIT) domain in DcpS is responsible for hydrolysis of the cap structure [29,31].

Interestingly, a distinct decapping activity is found in the yeast L-A virus coat protein, which possesses a catalytic domain that functions to decap cellular mRNAs [32–34]. This mechanism is thought to help stabilize the uncapped, unadenylated mRNAs encoded by the virus by titrating away the cellular mRNA decay machinery [32,34].

Proteins that associate with the Dcp1:Dcp2 complex and stimulate mRNA decapping

Several proteins that interact with and stimulate the activity of the Dcp1:Dcp2 complex have been identified in yeast and mammalian cells, including Dhh1 (a putative RNA helicase), Lsm proteins, Pat1, and enhancer of decapping (Edc) proteins.

In S. cerevisiae, Dhh1, the Lsm 1–7 complex and Pat1 interact directly or indirectly with the Dcp1:Dcp2 complex [5,35–38]. Depletion of any of these factors in S. cerevisiae results in accumulation of capped, deadenylated mRNAs, which suggests that they are critical for deadenylation-mediated decapping [5,35–39] (Figure 1a). Dhh1 is a putative DEAD-box RNA helicase, and it has been speculated that it may play a role in rearranging mRNPs targeted for degradation, thus making the 5′ end of the mRNA available to decapping enzymes [35,39]. The Lsm 1–7 proteins form a complex in the cytoplasm that preferentially interacts with deadenylated mRNAs [5], which suggests that the Lsm 1–7 complex may function to recruit the decapping complex to deadenylated mRNAs [5,36] (Figure 1a). Interestingly, recent evidence has shown that a nuclear Lsm complex, the U6-associated Lsm 2–8 complex, may be involved in the decapping of nuclear pre-mRNAs in S. cerevisiae [21]. Homologs of Dhh1, Pat1 and Lsm 1–7 exist in other
eukaryotic cells, including human cells, where Dhh1 (called rck/p54) and Lsm 1–7 have been implicated in mRNA turnover [40,41].

Edc proteins have been identified in yeast as proteins that interact with the Dcp1:Dcp2 complex and enhance decapping activity [42,43,44]. Edc1 and Edc2 are homologous proteins that bind to mRNA and enhance decapping in vitro [42]. Although depletion of these proteins in S. cerevisiae has no apparent effect on mRNA degradation, synthetic effects are observed in decapping-compromised strains, and overexpression of Edc1 or Edc2 can suppress conditional alleles of decapping enzymes [43]. Another Edc protein, Edc3, which is not related to Edc1 and Edc2 by sequence, associates with the Dcp1:Dcp2 complex and activates decapping [44]. Although the specific role of Edc proteins in general mRNA decapping is currently unknown, Edc3 has been shown to play a key role in autoregulatory degradation of the mRNA encoding ribosomal protein Rps28b [45].

**Processing bodies – sites of decapping enzyme localization and mRNA decay**

One of the most surprising observations about the Dcp1:Dcp2 complex was its enrichment in cytoplasmic foci, termed processing bodies (also called Dcp-bodies or GW-bodies in human cells) [24,40,41,46,47] (Figure 3). In these foci, Dcp1 and Dcp2 were found to co-localize with other proteins involved in mRNA decapping and degradation, including Xrn1, Dhh1, the Lsm 1–7 complex, Edc3, and, in human cells, the deadenylase Ccr4 [40,41,44,46,47]. The discovery of these cytoplasmic foci raised the question of whether they constitute sites of mRNA degradation or sites where mRNA decay enzymes are stored or assembled.

Several lines of evidence in both S. cerevisiae and human cells suggest that processing bodies may be directly involved in mRNA degradation [41,46]. mRNA decay intermediates that are induced to accumulate by either depletion of Xrn1 in yeast and human cells [41,46] or by a secondary structure in the mRNA that impairs exonucleolytic decay in yeast are concentrated in processing bodies [46]. In addition, yeast cells that are blocked at a step downstream in the mRNA decay pathway by mutations in decapping enzymes, Lsm proteins or Xrn1 have larger and more abundant processing bodies, whereas a block upstream in the pathway by depletion of Ccr4 and Pat1 leads to disappearance of the processing bodies [46] (Figure 3). In human and yeast cells, blockage of translation elongation by the drug cycloheximide leads to mRNA stabilization and disappearance of processing bodies [41,46]. Moreover, inhibition of transcription by Actinomycin D results in loss of processing bodies in human cells [41]. Taken together, these data suggest that mRNA decay can take place in the processing bodies.

![Figure 3](image360x448to505x566)

**mRNA decay factors in the processing bodies. Human Hela cell processing bodies visualized by indirect immunofluorescence using anti-hDcp1a antibodies (green). Nuclei are visualized by DAPI staining (blue). mRNA degradation enzymes are shown above in the order in which they act on mRNAs undergoing deadenylation-mediated decay in yeast. Proteins in the green box localize to processing bodies. In addition, in human cells, the deadenylase Ccr4 and the protein GW182, which appears to have no homolog in yeast, may localize to processing bodies [41,47].**

**Conclusions and perspectives**

Decapping plays a central role in RNA turnover, a key step in the regulation of gene expression. Recent studies have unveiled several factors that catalyze or enhance decapping. Although several factors have been identified that stimulate the activity of the Dcp1:Dcp2 mRNA decapping complex, their roles in the regulation and activation of mRNA decapping in the cell is poorly understood. The identification of a nucleolar decapping enzyme, X29, that displays a preference for U8 snoRNA in vitro [20,48] raises the question of whether other specialized decapping enzymes exist that function in the turnover of specific capped RNAs, such as certain snRNAs and snoRNAs. In addition, the interesting finding that the yeast L-A virus has adopted decapping of cellular mRNA as a strategy to ensure the stability of virus-encoded transcripts raises the question of whether modulation of the cellular mRNA turnover machinery is a more general viral strategy than was previously anticipated [32–34].

The exciting discovery that mRNA decay can take place in cytoplasmic processing bodies raises several important questions. For example, what cellular processes are responsible for localizing mRNAs targeted for decay
and mRNA decay factors to the processing bodies? Do mRNA decay pathways other than deadenylation-mediated decay, such as NMD, ARE-mediated decay and RNA interference, take place in processing bodies? In addition to these questions, it remains to be established whether mRNA decay is exclusive to processing bodies, or if a fraction of cellular mRNA decay, or specific mRNA decay pathways, can take place in other regions of the cell. For instance, the exosome, which has been implicated in both NMD and ARE-mediated decay in human cells [14,18,49,50], does not appear to localize to processing bodies [46**]. Additionally, the discovery of the nucleolar X29 decapping enzyme [20**] and the finding that nuclear pre-mRNA decay may involve decapping [21*] provide two examples of decapping taking place outside of processing bodies. Future studies should address these questions and are likely to reveal unanticipated links between mRNA transport/localization and degradation. Along these lines, it is interesting to note that a protein complex, Y14:mago, has been implicated in mRNA decay pathways other than deadenylation-decapping [21*]. In addition to these questions, it remains to be established whether mRNA decay, or specific mRNA decay pathways, can take place outside of processing bodies. Future studies should address these questions and are likely to reveal unanticipated links between mRNA transport/localization and degradation. Along these lines, it is interesting to note that a protein complex, Y14:mago, has been implicated in mRNA decay pathways other than deadenylation-decapping [21*].

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
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