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New Insights into Decapping Enzymes and Selective mRNA Decay

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Abstract

Removal of the 5' end cap is a critical determinant controlling mRNA stability and efficient gene expression. Removal of the cap is exquisitely controlled by multiple direct and indirect regulators that influence association with the cap and the catalytic step. A subset of these factors directly stimulate activity of the decapping enzyme, while others influence remodeling of factors bound to mRNA and indirectly stimulate decapping. Furthermore, the components of the general decapping machinery can also be recruited by mRNA-specific regulatory proteins to activate decapping. The Nudix hydrolase, Dcp2, identified as a first decapping enzyme, cleaves capped mRNA and initiates 5' to 3' degradation. Extensive studies on Dcp2 led to broad understanding of its activity and the regulation of transcript specific decapping and decay. Interestingly, seven additional Nudix proteins possess intrinsic decapping activity *in vitro* and at least two, Nudt16 and Nudt3, are decapping enzymes that regulate mRNA stability in cells. Furthermore, a new class of decapping proteins within the DXO family preferentially function on incompletely capped mRNAs. Importantly, it is now evident that each of the characterized decapping enzymes predominantly modulates only a subset of mRNAs, suggesting the existence of multiple decapping enzymes functioning in distinct cellular pathways.

Graphical Abstract

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Keywords

decapping; mRNA decay; Nudix proteins; Dcp2; Nudt3; Nudt16; DXO family

Eukaryotic transcripts are capped at their 5' ends by a 5'-5' triphosphate bridged 7-methylguanosine to the first transcribed nucleotide of the mRNA chain¹. The cap fulfills a variety of functions in all aspects of mRNA metabolism - synthesis, nucleo-cytoplasmic transport, translation, silencing and turnover². The presence of the 5' cap structure increases both the accuracy and efficiency of pre-mRNA splicing^{3,4} and participates in mRNA processing and exports⁵. In the cytosol, the cap is specifically recognized by the translation initiation factor eIF4E and required for efficient mRNA translation⁶. The cap is also an important determinant of mRNA stability where capped mRNAs are more stable than uncapped transcripts⁷. The cap protects the RNA from 5'-3' exonuclease decay^{8,9} and its removal is a critical regulatory event that influences transcript expression¹⁰ and affects overall protein synthesis within the cell.

Dcp2 was initially discovered in yeast through a genetic screen¹¹ and subsequently identified as the first eukaryotic decapping enzyme¹²⁻¹⁴. For almost a decade since its discovery, Dcp2 has been assumed to be the only enzyme responsible for the majority of eukaryotic mRNA decapping. Interestingly, the findings in recent years have demonstrated that at least three additional enzymes are also capable of removing the cap from an mRNA in cells¹⁵⁻¹⁷. Moreover, it is now evident that each decapping enzyme regulates only a small fraction of cellular transcripts suggesting that a number of distinct decapping complexes exist, each with their own substrate specificities and modulatory pathways. In this review, we will initially summarize the properties that confer transcript specificity to the extensively characterized Dcp2 protein and expand to our current understanding of additional decapping enzymes, Nud16 and Nudt3 as well as the DXO family of proteins that hydrolyze incompletely capped RNAs.

Regulators of Dcp2 decapping

Dcp2 (Nudt20) is a member of the evolutionary conserved Nudix hydrolase superfamily of proteins that are known to catalyze hydrolysis of a wide range of small nucleotide substrates composed of a nucleoside diphosphate linked to another moiety X (Nudix)¹⁸. They contain a conserved Nudix motif with the consensus sequence GX₅EX₇REUXEEXGU (where U represents a hydrophobic residue, and X represents any amino acid), which forms part of the versatile catalytic site for diphosphate hydrolysis¹⁸ (Figure 1). The catalytic site within the Nudix motif of Dcp2 contains three conserved glutamate residues that coordinate a divalent cation essential for cap hydrolysis^{19,20}. Dcp2 also possesses a conserved region known as Box A and Box B (Figure 1). Box A is important for catalytic fidelity of decapping²⁰ and at least in yeast, for an interaction with the decapping activator Dcp1p²¹ (Figure 2). Box B is required for the RNA binding property of Dcp2 and its removal decreases both RNA-binding and decapping activity *in vitro*²⁰. Dcp2 cleaves between the α- and β-phosphate moieties of the cap generating m⁷GDP and 5'-end monophosphate RNA^{12,14} (Figure 3). Importantly, the RNA body contributes to the Dcp2 substrate specificity²⁰ and both the cap and an

oligonucleotide chain of approximately 25 nucleotides are required for the enzyme activity¹⁹. For more extensive reviews on Dcp2 structure and its interaction with the cap see^{22–24}.

In yeast, decapping is carried out by a single enzyme composed of a catalytic subunit (Dcp2p) and a regulatory subunit (Dcp1p) and facilitated by decapping activators²⁵ (Figure 2). Based on the mechanistic models that rely on *in vitro* enzymatic assays, protein-protein interaction studies and structural analyses, the activators mainly function to promote decapping by inducing conformational changes in Dcp2p required for the decapping reaction^{26, 27}. Structural analyses of a ternary complex consisting of Dcp2p-Dcp1p with the decapping enhancer Edc1p reveals the requirement of a rotation of the Dcp2p catalytic domain in the presence of Dcp1p and Edc1p to position Dcp2p and Dcp1p residues to bind the capped mRNA substrate explaining the decapping enhancement by Dcp1p and Edc1p²⁸. Conserved from yeast to humans, Pat1p, Dhh1p, Edc3p and the Lsm1–7 complex activate general decapping whereas Upf1p, Upf2p and Upf3p are NMD-specific regulators. Edc1p and Edc2p decapping stimulators are present exclusively in yeast¹⁹. In metazoans, the Hedls protein (also known as Edc4 or Ge-1) enhances Dcp2 decapping *in vitro* by promoting a stable interaction of Dcp1 with Dcp2^{29, 30}. Pat1p, initially identified in yeast as a decapping activator³¹, interacts directly with Dcp1p³² to stimulate decapping³³. Pat1p associates with the Lsm1p–7p protein complex and binds selectively to deadenylated mRNAs to promote decapping. The Dhh1p decapping activator (known as DDX6 or Rck/p54 in human and Me31B in *Drosophila*) was also initially identified in yeast where its depletion results in the accumulation of capped mRNAs^{34–36}. Mammalian Dhh1 interacts with Dcp2, Edc3 and Pat1 decapping components to stimulate decapping^{32, 37, 38}. Importantly, a point mutation in the human Edc3 that compromises its ability to activate Dcp2 decapping *in vitro* was identified in a family with cognitive disability³⁹, indicating that Edc3 and Dcp2 contribute to neural functions.

There are at least two categories of decapping stimulatory proteins, general activators and transcript specific activators. In *Drosophila* S2 cells, Dcp1 and Edc4 regulate overlapping mRNAs indicating a general decapping stimulatory function, whereas Edc3 targets a group of transcripts distinct from Dcp1 and Edc4⁴⁰. Moreover, positive regulation of decapping can be mediated through interaction of the Dcp2 C-terminal domain with specific decapping activators that control substrate specificity and trigger Dcp2 enzyme activation (Figure 2). In *S. cerevisiae*, Edc3p specifically promotes decapping of the Rps28 mRNA⁴¹. Rps28p protein recognizes a conserved hairpin structure in the 3' UTR of Rps28 mRNA and recruits Edc3p. The decapping is stimulated by an interaction of Edc3p with Dcp1p and Dcp2p^{34, 42, 43}. Pat1p and Upf1p can also bind both their respective substrate mRNAs and distinct sites in Dcp2p^{44–47} (Figure 2), implying that these decapping activators directly control the targeting or substrate specificity of the decapping enzyme.

In addition to positive mediators of Dcp2p, Dcp2p is also subjected to a surprising negative regulatory circuit by a *cis*-inhibitory element in its C-terminus⁴⁷ (Figure 2). Removal of the inhibitory element results in constitutive activation of Dcp2p decapping and loss of substrate mRNA specificity. In the presence of the inhibitory element, Edc3p is an obligate cofactor for Dcp2p-mediated YRA1 pre-mRNA decay. However, when the inhibitory element is

removed, YRA1 pre-mRNA degradation could bypass its requirement for Edc3p. The negative element appears to inhibit the enzymatic or substrate-binding activity of Dcp2p whereas the positive elements promote the binding of specific decapping activators such as Edc3p, Pat1p, and Upf1p to Dcp2p to alleviate the inhibitory activity and promote formation of distinct decapping complexes.

mRNA *cis*-elements and RNA binding protein-mediated recruitment of Dcp2

Dcp2 is an RNA-binding protein that selectively binds a subset of mRNAs containing a stem-loop structure within 10 bases of the 5' cap⁴⁸. The structure, termed the Dcp2 binding and decapping element, was initially identified in the mRNAs including the mRNA encoding human exosome component, Rrp41 and promotes preferential Dcp2-directed decapping on a heterologous mRNA⁴⁸. Importantly, the secondary structure, but not primary sequence, is critical for Dcp2 recognition and decapping⁴⁹. A genome-wide analysis predicts several hundred human mRNAs could harbor a similar stem loop structure at their 5' end.

In addition to direct RNA recruitment, *cis* elements can also serve as a platform to recruit RNA binding proteins and activate decapping. The AU-rich element (ARE) located in the 3' UTRs of many mRNAs encoding transcription factors, cytokines and proto-oncogenes can recruit ARE-binding proteins and subject the mRNA to rapid decapping and degradation⁵⁰. One well-characterized ARE-binding protein is tristetraprolin (TTP), which recruits Edc3 and Dcp2⁵¹ and promotes ARE-dependent Dcp2 decapping activity *in vitro*²⁹. Knockdown of Dcp2 reduces 5'-decay of c-fos ARE-containing mRNAs⁵² while overexpression of Dcp2 results in accelerated degradation of an mRNA containing the GM-CSF ARE²⁹.

The Lsm1p-7p-Pat1p complex is important for the recruitment of the decapping complex to deadenylated mRNA and activation of decapping⁵³. The purified Lsm1-7-Pat1p complex from yeast has an inherent ability to distinguish between oligoadenylated and polyadenylated mRNAs and binds preferentially to deadenylated transcripts containing a U-tract at their 3'-end⁴⁴. In mammalian cells, a U-tract at the 3'-end of a generic RNA triggers Dcp2-mediated decapping in an Lsm1-7 complex-dependent manner⁵⁴. Stimulation of decapping by oligouridylation is also observed for an endogenous mRNA. Mammalian histone mRNAs contain a stem-loop structure instead of a poly(A) tail at their 3'-end and undergo tailing by unconventional terminal ribonucleotidyltransferases⁵⁵. Uridylation induces rapid decay of histone mRNAs through both a Dcp2 dependent 5'-3' degradation and exosome mediated 3'-5' degradation⁵⁵⁻⁵⁷. Interestingly, uridylation, which was initially detected at the 3' ends of miRNA-directed cleavage products in *Arabidopsis* and mammalian cells⁵⁸, also occurs on polyadenylated mRNAs and is a conserved feature of mRNA metabolism in eukaryotes⁵⁹⁻⁶³.

Dcp2 protein modification and controlled levels as a means to regulate decapping

In addition to the regulation of mRNA decapping by direct or protein-mediated indirect Dcp2 recruitment, modifications of the Dcp2 protein and its expression can also determine the fate of a subset of mRNAs. In particular, the C-terminus of human Dcp2 can act as a regulatory domain to modulate Dcp2 activity (Figure 1) by either promoting decapping complex assembly through Hedls or restricting Dcp2 levels by targeting uncomplexed Dcp2

for ubiquitin-mediated proteasome degradation⁶⁴. Moreover, Dcp2 levels are limiting relative to Dcp2 decapping stimulatory proteins⁶⁴ and ~10-fold less abundant than other decapping factors⁶⁵, indicative of the importance of maintaining its expression in check within a cell.

A role for Dcp2 in autophagy provides another example of regulated Dcp2 decapping by protein modification. A conserved pathway in both yeast and humans negatively regulates autophagy through modulation of the autophagy-related (ATG) mRNA stability in an mTOR-dependent manner⁶⁶. Under normal growth conditions RCK promotes Dcp2-dependent decapping of the ATG mRNA and subjects it to 5'-3' degradation. mTOR-associated phosphorylation of Dcp2 is required for transcript decapping and subsequent degradation. Exposure to stress by nutrient deprivation inactivates mTOR and prevents Dcp2 phosphorylation leading to dissociation of RCK from the ATG mRNA. This, in turn, facilitates accumulation of ATG transcripts and induction of autophagy.

Levels of Dcp2 mRNA and protein are responsive to viral challenge in mouse embryonic fibroblast (MEF) cells where Dcp2 levels are elevated⁶⁷. Importantly, MEF cells deficient in Dcp2 upregulate a group of innate immune response genes involved in type I IFN response⁶⁷. In particular, the stability of a key transcription factor in the antiviral response, IFN regulatory factor 7 (IRF-7) mRNA is regulated by Dcp2 decapping. MEF cells defective in Dcp2 expression are more resistant to viral challenge than wild type MEF cells indicating Dcp2 may be involved in curtailing the innate immune response following activation⁶⁷. Induction of Dcp2 expression is also important in the modulation of inflammatory response genes through the celiac disease-associated lnc13 RNA. In celiac patient macrophages, or lipopolysaccharide induced macrophages, Dcp2 levels are transcriptionally induced by nuclear factor kappa B (NF- κ B) which initiates decay of lnc13 RNA⁶⁸. lnc13 represses the expression of a subset of inflammatory genes and reduction of lnc13 levels relieves the repression thus promoting the disease associated inflammatory response⁶⁸. Whether regulation of Dcp2 decapping can be exploited for the amelioration of celiac disease remains to be determined.

Mammalian cells contain multiple mRNA decapping enzymes

Since its discovery, Dcp2 has been postulated as the major decapping enzyme in eukaryotic cells. However, recent studies have demonstrated that Dcp2 is not the default all encompassing decapping enzyme in multicellular organisms, but is one member of a growing list of decapping enzymes. Several lines of evidence were critical for the postulate that multiple decapping enzymes likely exist in mammals. First, Dcp2 is an RNA binding protein that must bind to the 5' end of an RNA to recognize and hydrolyze the cap^{14, 19, 20}. As an RNA-binding protein, it would be expected to bind different RNA sequences/structures with different affinities and thus preferentially hydrolyze different mRNAs. This hypothesis was substantiated by the demonstration that Dcp2 can selectively regulate a subset of mRNAs possessing a Dcp2 element at their 5' end^{48, 49}. Second, Dcp2 is not ubiquitously expressed in all mouse tissues. Of the eight mouse tissues tested, Dcp2 protein is highly expressed in brain and testes, detected in lung and spleen but devoid in liver, muscle, heart and kidney¹⁵. Moreover, Dcp2 is developmentally regulated. In two adult

tissues that do not express detectable levels of Dcp2 protein, heart and liver, robust Dcp2 expression is detected in mouse embryos and the levels decrease to undetectable upon birth¹⁵. In contrast, Dcp2 can be detected in the brain throughout development. Most significantly, MEF cell lines either containing or lacking measurable Dcp2 expression are equally competent to decap exogenous RNA substrates introduced into these cells¹⁵ indicating that decapping proteins other than Dcp2 are also functional in mammalian cells.

Nudt16 is an mRNA decapping enzyme—The first direct demonstration that mammalian mRNA 5'-end degradation is more complex and extends beyond just Dcp2-directed decapping came with the finding that Nudt16 can function as a decapping enzyme in cells. Nudt16 is a nucleolar U8 snoRNA binding protein⁶⁹ that possesses decapping activity and was initially proposed to be a U8 snoRNA specific nucleolar RNA decapping enzymes^{69, 70}. More recently analysis of mouse Nudt16 uncovered it is ubiquitously expressed across mouse tissues, predominantly expressed in the cytoplasm and is involved in mRNA decapping^{15, 71}.

Consistent with the premise of multiple mRNA decapping enzymes that each function on a subset of mRNAs, Dcp2 and Nudt16 uniquely influence the stability of only a subset of mRNA transcripts. Global profiling of mRNA in Nudt16 knockdown cells following one and four-hour transcriptional arrest identified 40 and 134 mRNAs, respectively whose stabilities increase¹⁵. Importantly mRNAs responsive to Nudt16 are minimally responsive to Dcp2 and vice versa. Furthermore, it appears Dcp2 preferentially functions on more labile mRNAs since a greater number of mRNAs are altered at the one-hour transcriptional arrest while Nudt16 may function on more long lived mRNAs that are apparent after four hours of transcriptional arrest¹⁵. The two decapping enzymes also appear to differentially function in different decay mechanisms, where nonsense-mediated mRNA decay predominantly utilizes Dcp2, while degradation of ARE containing mRNAs and miRNA-mediated silencing can use either Dcp2 or Nudt16⁷². Decapping, at least in mammals, appears to involve multiple enzymes that catalyze hydrolysis of the mRNA cap and each preferentially function on distinct subsets of transcripts.

Multiple Nudix proteins can decap RNA *in vitro*—There are 22 Nudix proteins in the human genome, with each protein reported to hydrolyze distinct and overlapping substrates including dNTPs, diadenosine polyphosphates, nucleotide sugars⁷³ and in the case of Dcp2 (Nudt20) and Nudt16, capped mRNAs⁷⁴. Six additional Nudix proteins (Nudt2, Nudt3, Nudt12, Nudt15, Nudt16, Nudt17 and Nudt19 shown schematically in Figure 1) have the capacity to decap RNA *in vitro*⁷⁴. Similar to Dcp2 and Nudt16, Nudt17 and Nudt19 primarily cleave the pyrophosphate linkage between the α - and β -phosphates to generate $m^7GDP + pRNA$ (Figure 3A). Nudt12 and Nudt15 hydrolyze the β - γ -phosphate bond to generate $m^7GMP + ppRNA$ products (Figure 3B). Nudt2 and Nudt3 cleave between the α - β or the β - γ -phosphates with comparable activity (Figure 3C)⁷⁴. The function of ppRNA in cells is currently unknown. Since all known 5' to 3' exoribonucleases utilize a 5' end monophosphorylated RNA substrate, ppRNAs are unlikely to be direct targets for 5' end decay. Alternatively, they may be substrates for cytoplasmic recapping that utilizes ppRNA⁷⁵. The reported accumulation of uncapped and untranslated mRNA⁷⁶ may be

constituted by decapping generated ppRNA maintained in a silenced state prior to restoration into the translating pool by cytoplasmic recapping.

With the exception of Nudt12 and Nudt16, all the decapping competent Nudix proteins only hydrolyze a cap that is at the 5' end of an RNA and not free cap structure⁷⁴. The requirement of an RNA for the majority of the Nudix decapping enzymes suggests that like Dcp2, they are also RNA-binding proteins and likely selectively function on specific mRNAs as is also the case for Nudt16¹⁵ and Nudt3 (¹⁷ and below). In addition to RNA decapping, Nudt12 and Nudt16 can hydrolyze m⁷GpppG cap structure *in vitro*⁷⁴ and Nudt16 can also hydrolyze inosine diphosphate (IDP) and its cognate deoxyribose (dIDP) into IMP and Pi^{77, 78}. Cap structure decapping is a well-characterized function of the scavenger decapping protein, DcpS^{79–81}. DcpS is a member of the histidine triad family of proteins⁸⁰ that cleaves m⁷GpppG to m⁷Gp + ppG. The Fragile Histidine Triad (FHIT) protein can also hydrolyze cap structure *in vitro*⁸². However, the significance of Nudt12, Nudt16 and FHIT in hydrolyzing cap structure in cells remains an open question considering all three enzymes have several orders of magnitude lower cap structure decapping activity than does DcpS (⁷⁴ and unpublished).

Surprisingly, decapping is not restricted to eukaryotic proteins. The bacterial Nudix protein RppH, which accelerates decay of *E. coli* mRNA decay by converting their triphosphorylated 5' ends to monophosphorylated form⁸³, can hydrolyze m⁷G capped RNA exclusively between the α - and β -phosphates to release m⁷GDP + pRNA⁷⁴. Similar to the eukaryotic decapping enzymes above, it requires a cap substrate linked to an RNA moiety and does not function on cap structure alone. The significance of this observation is currently unclear since prokaryotes lack an m⁷G cap. However, the recent demonstration that bacterial mRNAs can possess a nicotinamide adenine dinucleotide (NAD) on their 5' end and the ability of a bacterial Nudix protein, NudC, to hydrolyze NAD at the 5' end of an RNA⁸⁴ suggests there might yet exist additional 5' end modifications in prokaryotes that are targeted by RppH. Moreover, the RppH decapping activity also indicates the ability of Nudix proteins to hydrolyze a triphosphorylated nucleoside at the 5' end evolved prior to eukaryotic decapping.

Nudt3 is an mRNA decapping enzyme—Nudt3 is a cytoplasmic protein that, along with Nudt4, Nudt10, and Nudt11, belongs to the diphosphoinositol polyphosphate phosphohydrolase (DIPP) family of proteins and can hydrolyze diphosphoinositol polyphosphates (DIPs) and Ap_nA dinucleotides⁷³. However, only Nudt3 contains decapping activity *in vitro*⁷⁴ and in cells¹⁷. Moreover, the *S. cerevisiae* ortholog, Ddp1p, contains robust decapping activity *in vitro*⁷⁴ suggesting that like Nudt3, Ddp1p may be a decapping enzyme in yeast.

A genome-wide RNA-seq analysis performed in MCF-7 cells with reduced levels of Nudt3 reveals that, similarly to Dcp2 and Nudt16, steady state levels of a small subset of RNAs are altered as a function of Nudt3 with a disproportional percentage of the responsive mRNAs involved in cell motility¹⁷. Intriguingly, many of the mRNAs regulated by Nudt3 are either cell surface receptors or secreted extracellular proteins involved in cell migration, suggesting an important role for Nudt3 in controlling cell motility. Indeed, reduction of Nudt3 levels in

MCF7 cells promotes enhanced lamellipodia/filopodia formation and corresponding increase in cell migration¹⁷.

Of the four characterized Nudt3 responsive mRNAs involved in cell motility, Nudt3 decapping is directly involved in the stability of integrin $\beta 6$ and lipocalin-2 mRNAs. A similar alteration in mRNA levels are not observed in cells knocked down for the related Nudt4 protein which demonstrates the changes in integrin $\beta 6$ and lipocalin-2 mRNAs are not due to DIPP or Ap_nA dinucleotide hydrolysis activities shared by Nudt3 and Nudt4⁷³, but rather due to the unique decapping property of Nudt3. Interestingly, the steady state increase in the remaining two mRNAs, fibronectin and S100A8, following Nudt3 knock down are independent of mRNA stability and likely a consequence of indirect modulation by Nudt3 decapping.

DXO Family of Decapping Enzymes

Historically, the cotranscriptional addition of the N7-methyl guanosine cap to a nascent pre-mRNA was thought to be a default process that always proceeded to completion. This perception was upended by the identification of a novel class of enzymes that specifically recognize and remove incomplete caps at the 5' end of an mRNA in a capping quality control mechanism (reviewed in^{85, 86}). In *S. cerevisiae*, Rai1p which exists in a heterodimeric complex with the nuclear 5'-3' exonuclease Rat1⁸⁷ possesses RNA 5' pyrophosphohydrolase (PPH) activity, releasing pyrophosphate (PP_i) from 5' triphosphorylated RNA⁸⁸ (Table 1). Interestingly, Rai1p homologs from *Ashbya gossypii*, *Candida glabrata* and *Scheffersomyces stipites*, remove the entire first nucleotide of an RNA 5' triphosphate with a 5' triphosphonucleotide hydrolase (TPH) activity, releasing pppN + RNA from 5' pppN-RNA⁸⁹. All the fungal Rai1 proteins tested thus far also cleave incompletely capped GpppN-RNA releasing unmethylated GpppN¹⁶ (Table 1), in contrast to the canonical decapping enzymes, which cleave within the pyrophosphate linkage (see above). All the Rai1p activities generate an RNA with a 5' monophosphate that is subsequently degraded by Rat1p. Furthermore, both Rai1p and Rat1p mutually stimulate each others activity.

Yeast also contain a second protein with extensive structural identity to Rai1 termed Decapping exonuclease 1(Dxo1p)⁹⁰. *S. cerevisiae* Dxo1p is predominantly a cytoplasmic protein⁹¹ and similar to Rai1, the *K. lactis* Dxo1 can preferentially hydrolyze an unmethylated cap from an mRNA, but lacks PPH activity (Table 1). Surprisingly, *K. lactis* Dxo1p also has intrinsic 5'-3' exonuclease activity and can single handedly both decap and degrade an incompletely capped RNA⁹⁰ an outcome analogous to the Rai1p-Rat1p heterodimer within the cytoplasm. Most significantly, aberrantly capped mRNAs accumulate in *rai1* *S. cerevisiae* under stress conditions¹⁶, or *rai1 dxo1* double disrupted *S. cerevisiae*, suggesting capping is a regulated process and Rai1p and Dxo1p function in a capping quality control mechanism to ensure 5' end fidelity.

Mammalian cells appear to have one homolog of Rai1p and Dxo1p, termed DXO (formerly DOM3Z) and it possesses the cumulative actives of both proteins. It contains PPH activity to cleave between the α - β -phosphates of the triphosphate 5' end, it hydrolyzes unmethylated incompletely capped RNA, and possesses 5'-3' exonuclease activity⁹² (Table 1). The

decapping activity of DXO does not discriminate between methylated and unmethylated caps *in vitro*. However, cap binding proteins inhibit DXO activity⁹² and likely protect the mature, methylated cap from DXO-mediated degradation. Surprisingly, DXO preferentially degrades incompletely capped pre-mRNAs in cells suggesting a role in pre-mRNA quality surveillance. Of particular note, incompletely capped pre-mRNAs are impaired in their efficiency to splice internal introns which extends earlier observations that demonstrated a role for the 5' cap in splicing of the first intron^{3, 4, 93} and provides a link between 5' capping quality and subsequent splicing.

Conclusion

Regulation of mRNA 5' end decapping occurs at multiple levels. First is the availability of the decapping enzyme, whether it is constitutively or temporally expressed and its presence in a particular cell type. Second is its recognition of the mRNA substrate which involves both direct and indirect association with the 5' end cap and modulatory proteins that influence the access and activity of the enzyme. Studies focused on Dcp2 have led to an extensive understanding of its activity and function including the surprising preferential targeting of only a small fraction of cellular mRNAs, in particular, mRNAs involved in the innate immune response. More recently, it has become apparent that mammalian cells possess a multitude of decapping enzymes in addition to Dcp2. Of the seven additional Nudix proteins with intrinsic decapping activity, two thus far have been shown to function as decapping enzymes that modulate mRNA stability in cells, Nudt3 and Nudt16. Similar to Dcp2, both do not indiscriminately function on all mRNAs and preferentially target a select subset. Furthermore, a new class of decapping enzymes within the DXO family preferentially function on incompletely capped mRNAs. It is anticipated that the remaining Nudix decapping-competent enzymes are also cellular decapping enzymes that function on select mRNAs with each decapping protein modulating a subset of distinct cellular pathways.

The identification of multiple decapping proteins raises intriguing future areas of inquiries. Considering the number of regulatory proteins involved in Dcp2 decapping (Figure 2), it is tempting to speculate additional confirmed decapping proteins will have both stimulatory and inhibitory proteins that control their activity. Whether each will have unique or overlapping associated proteins remains to be determined. Are the enzymes polysome associated and are they active when polysome bound? Does decay occur in a specific subcellular location or throughout the cytoplasm? These and many other questions can now be addressed with Nudt3 and Nudt16 and others as they are also confirmed as decapping enzymes. The presence of numerous modified nucleotides, such as pseudouridine, 5-methylcytidine, 5-hydrozylmethylcytidine, and N⁶-methyladenosine⁹⁴⁻⁹⁷ in RNA provides further potential avenues of transcript specific degradation by decapping enzymes. In particular, modifications of the first encoded nucleotide following the cap would be predicted to have a disproportional influence. The last one and a half decades since the demonstration of Dcp2 as a decapping enzyme has provided a tremendous wealth of unexpected findings. Nevertheless, it is anticipated that we are only at the beginning phase of understanding all the decapping components that function in cells and the physiological roles that they fulfill.

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Figure 1. Schematic representation of the Nudix proteins with decapping activity

Nudix proteins with decapping activity are aligned relative to their Nudix motif. The human Dcp2 protein is schematically depicted at the top with the evolutionarily conserved domains, with Nudix Motif (*red*), Box A (*green*), Box B (*blue*) and regulatory domains encompassing the binding sites for Hedls (*orange*) and ubiquitin ligases (*orange with stripes*).

Figure 2. Schematic representation of the yeast Dcp2p decapping protein and its interacting partners

S. cerevisiae Dcp2p is schematically depicted with Nudix Motif (*red*), Box A (*green*), Box B (*blue*), *cis*-inhibitory element (*grey*) and binding sites for Dcp1p, Edc3p, Pat1p and Upf1p interacting proteins denoted along with their corresponding amino acid regions numbered.

Figure 3. A summary of the predominant cleavage site(s) within the cap triphosphate bridge for Nudix proteins with decapping activity

The position of the α , β and γ phosphates are shown with the cleave sites indicated by the arrow

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Table 1

Summary of DXO family member biochemical activities

Activity	Decapping				5'-3' Exo
	PPH	TPH	m ⁷ GpppRNA	GpppRNA	
Substrate	pppRNA	pppRNA	m ⁷ GpppRNA	GpppRNA	pRNA
Cleavage site	↑	↑	↑	↑	↑
Products	PP _i + pRNA	pppN + pRNA	m ⁷ GpppN + pRNA	GpppN + pRNA	pN + pRNA
<i>A.gossypii</i> Rai1	-	++	++	++	++
<i>C.glabrata</i> Rai1	-	+	-	+	-
<i>S.pombe</i> Rai1	+	-	-	+	-
<i>K.lactis</i> Dxo1	-	-	++	++	++
<i>M.musculus</i> DXO	++	-	++	++	++

+ 50% activity; ++ 50% activity

Data derived from ⁸⁹