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Judge, Jury and Executioner: DXO functions as a decapping enzyme and exoribonuclease in pre-mRNA quality control

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Abstract

In this issue of *Molecular Cell*, Jiao et al (2013) describe the mammalian enzyme DXO, which has pyrophosphohydrolase, decapping and 5′-3′ exoribonucease activity, and functions as an important checkpoint in co-transcriptional capping of RNA polymerase II (pol II) pre-mRNA transcripts.

All eukaryotic mRNA possess a 5′ end cap structure. The N-7 methyl guanosine modification of cap and 5′-5′ triphosphate linkage with the first transcribed nucleotide creates a unique interaction site for binding proteins that promote splicing, polyadenylation, transport, translation and protects the transcript from 5′-to-3′ degradation (Moore, 2005). Decapping enzymes cleave the cap structure from the 5′ end of mRNA transcripts and often require the assistance of protein cofactors, acting as a judge and jury that ‘sentence’ a transcript to degradation (Arribas-Layton et al, 2012). Following decapping, conserved exoribonucleases act as ‘executioners’, carrying out the transcript’s death sentence by attacking the exposed 5′ monophosphate and processively degrading the transcript body in the 5′-to-3′ direction. Other executioners include the exosome, which has endonuclease and 3′-5′ exonuclease activity and functions in RNA quality control in the nucleus and mRNA decay in the cytoplasm (Doma and Parker, 2007). In this issue, Kiledjian, Tong and coworkers describe the structure and function of the mammalian enzyme DXO – formerly called Dom3Z – which acts simultaneously as judge, jury and executioner on pre-mRNA transcripts in the nucleus. DXO selectively decaps nascent transcripts with an incomplete 5′ terminal cap, and acts as a 5′-3′ exoribonuclease, in a novel pre-mRNA quality control pathway.

Nascent RNA transcripts synthesized by pol II initially have a triphosphate group at the 5′ terminal end of the growing RNA chain (Figure 1). The nuclear capping enzyme carries out sequential triphosphatase and guanylyltransferase reactions on the first transcribed nucleotide (N) to co-transcriptionally generate the GpppN 5′ cap structure. Using S-adenosylmethionine as the methyl donor, RNA (guanine-7)-methyltransferase then methylates the cap to give m7GpppN (Shuman and Lima 2004). The mature cap structure is next recognized by the cap binding complex (CBC) composed of CBP20/80, and CBC is then thought to help coordinate pre-mRNA splicing (Moore, 2005). The mRNA transcript is subsequently cleaved, the 3′ end polyadenylated, and transcription termination is carried out

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through the so-called torpedo mechanism promoted by the 5′-3′ exoribonuclease Xrn2, or Rat1 in yeast (Kuehner et al, 2011). The CBC next helps export the mature mRNA to the cytoplasm, where it is exchanged for the cap binding translation initiation factor eIF4E and its associated cofactors, and is translated by the ribosome into protein. Finally, upon translational repression and deadenylation, eIF4E can be replaced with the cytoplasmic decapping machinery, the mRNA transcript is decapped, and degraded by exonuclease Xrn1 (Franks and Lykke-Andersen, 2008).

Recently, Kiledjian, Tong and coworkers have elaborated the above eukaryotic mRNA life story through characterization of two enzymes in yeast, Rai1 and Dxo1, which function in a quality control mechanism to clear defective mRNA transcripts that have incomplete 5′ caps (Jiao et al 2013; Kuehner et al, 2011 and references therein). Rai1 functions in complex with the processive 5′-3′ exonuclease Rat1. It preferentially hydrolyzes unmethylated capped and 5′ triphosphate RNA to generate a 5′ monophosphate RNA product and GpppN or pyrophosphate, respectively. A yeast homolog of Rai1, Dxo1, also possesses decapping activity toward partially capped transcripts, but additionally has distributive 5′-3′ exonuclease activity. This work showed that in yeast, co-transcriptional capping is incomplete under nutrient deprivation and also under normal growth conditions. In this issue of *Molecular Cell*, the authors report on the enzyme DXO, a mammalian homolog of Rai1 and Dxo1, which possesses pyrophosphohydrolase, decapping and 5′-3′ exoribonuclease activities. Cap binding proteins CBP20 and eIF4E inhibit the action of DXO on RNA containing a complete cap but allow decapping on RNA containing unmethylated caps *in vitro*. Crystal structures of oligonucleotide substrate and a product mimic show that the same active site carries out all of the distinct hydrolysis reactions and that substrate positioning appears to control which phosphate groups are cleaved. Single stranded RNA threads through a short tunnel in the enzyme that ends with conserved residues coordinating two metal ions required for decapping and exonucleolytic activity. The authors show that knock down of DXO results in an increase in steady-state levels of incompletely capped pre-mRNA transcripts, which are shown to be inefficiently spliced at all introns and inefficiently polyadenylated. Likewise, the half-life for pre-mRNA transcripts was significantly increased in cells where DXO was knocked down. In contrast, steady state levels of mRNA are only modestly affected when DXO is knocked down. DXO preferentially decaps and degrades pre-mRNA lacking m7GpppN 5′ cap structures *in vivo* (Figure 1, pink box), keeping the population of incompletely capped transcripts in check.

These findings implicate DXO as a crucial arbiter of the co-transcriptional capping process in mammalian cells. The large increase in partially capped pre-mRNA population upon DXO knock down *in vivo* suggests that the enzyme identifies, decaps and degrades a significant number of incompletely capped pol II transcripts. Therefore DXO functions as an important quality control of pre-mRNA capping. However, DXO may also play a broader role in the control of gene expression, since recent studies suggest that cap methylation may be regulated in a transcript-specific manner in mammalian cells (reviewed by Topisirovic et al, 2011).

How does DXO discriminate between normal and incomplete cap structures in cells? An attractive model is that DXO activity is governed by a kinetic competition with the CBC for cap. Normal, completely capped pre-mRNA would bind CBP20/80, which blocks degradation by DXO, and then accelerates splicing and subsequent steps in pre-mRNA maturation. Incompletely capped pre-mRNA would not bind CBP20/80 efficiently, allowing DXO to decap and degrade the defective pre-mRNA in a discard pathway. This type of kinetic partitioning has been suggested in other RNA decay pathways (Doma et al, 2007). An additional question for future work is whether DXO acts alone or with other factors. *In vitro*, DXO is a distributive exonuclease and may require assistance from the processive

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Xrn2 to complete digestion of the RNA body. Consistent with this, Xrn2 was recently shown to function in pre-mRNA quality control (Davidson et al, 2012).

Identification of DXO as a player in pre-mRNA quality control adds to growing evidence that there are a multiplicity of decapping enzymes that work early and late in the mRNA life cycle to coordinate and promote quality control of gene expression. For example, Dcp2 acts during cytoplasmic 5′-3′ decay and may also function to remove the m7G cap on newly pol II transcribed mRNA in the nucleus, triggering premature transcription termination by Xrn2 (Brannan et al, 2012; Figure 1, blue box). This Dcp2-mediated nuclear decapping appears to regulate productive pol II elongation on thousands of genes. Likewise, in yeast, long non-coding RNA generated by pol II are decapped by Dcp2 and subject to 5′-3′ decay by either a nuclear (Rat1, Xrn2) or cytoplasmic (Xrn1) 5′-3′ exoribonuclease. Control of long non-coding RNA stability through removal of cap may help regulate expression of nearby protein coding genes (Geisler et al, 2012). In summary, there are multiple judges and juries that affect decapping of coding and non-coding pol II transcripts in different cellular courtrooms. A challenge for the future is to understand the role of incomplete capping and pre-mRNA decapping by DXO as a way to control gene expression in mammalian cells.

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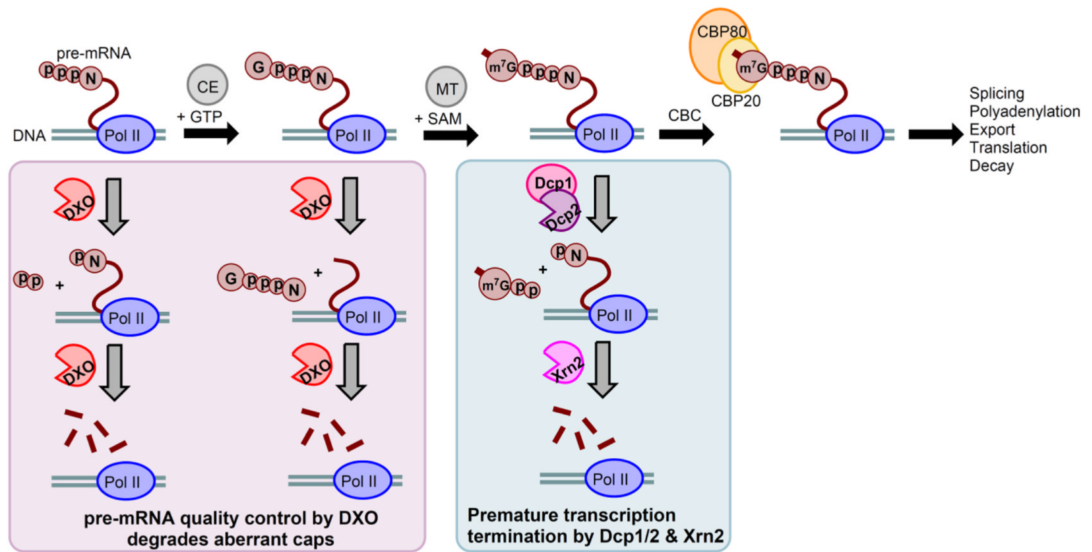

Figure 1. Overview of known nuclear decapping activity on nascent pol II transcripts

DXO selectively decaps incompletely capped pre-mRNA, and can also function as the 5′-3′ exonuclease to degrade the RNA transcript body (pink box). Dcp1/2 can remove the m7G cap and trigger transcription termination by the Xrn2-mediated torpedo mechanism (blue box; Brannan et al, 2012). Pol II is RNA polymerase II, CE is the capping enzyme, MT is the guanosine methyltransferase, SAM is S-adenosylmethionine, and CBC is the cap binding complex (CBP80/20).