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# Decapper Comes into Focus

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## Abstract

In this issue of Structure, Scarsdale et al. (2006) report structures of the Xenopus X29 Nudix decapping protein, including homodimer structures in complex with cap nucleotides. These structures reveal insights into the mechanism of cap substrate recognition and predict an RNA binding path on the protein surface.

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The small nucleolar RNAs (snoRNAs) are a group of non-coding RNAs that associate with nucleolar proteins to form small nucleolar ribonucleoprotein particles (snoRNPs) that play well-established roles in ribosomal RNA (rRNA) processing. Although over a hundred snoRNPs are involved in the various rRNA modifications, only a handful are necessary for cleavage of the 45S pre-rRNAs into the three mature rRNAs. Among these, the U8 snoRNP is essential for both 5.8S and 28S rRNA production (Peculis and Steitz, 1993). The U8 snoRNA is transcribed by RNA polymerase II and, like many small nuclear RNAs and snoRNAs, is subsequently hypermethylated at the 5′ end to a $m^{2,2,7}G$ trimethylated capped RNA.

Initial gel shift analysis of the U8 snoRNA revealed a 29 kDa polypeptide from *Xenopus* ovary extract, termed X29, that specifically bound the RNA (Tomasevic and Peculis, 1999). The novel protein was identified and found to contain an evolutionarily conserved Nudix (*nu*-cleotide diphosphatase linked to moiety *X*) motif consisting of an ∼23 amino acid consensus sequence, $GX_5EX_7REUXEEXGU$, where X denotes any residue and U represents lle, Leu, or Val (Mildvan et al., 2005). Similar to Dcp2, a Nudix protein which hydrolyzes capped messenger RNA to release $m^7GDP$ (known as the decapping reaction), X29 was also shown to possess de-capping activity and to decap U8 snoRNA preferentially, releasing the $m^{2,2,7}GDP$ trimethyl nucleoside diphosphate (Ghosh et al., 2004). In this issue, Scarsdale et al. (2006) provide structural insight into the substrate binding of the X29 Nudix decapping protein to a cap moiety.

Decapping enzymes play well-characterized roles in mRNA degradation. The crystal structures of the three known catalytically active decapping enzymes have now been solved: the scavenger decapping protein DcpS, the mRNA decapping protein Dcp2, and the nucleolar decapping protein X29 (Gu et al., 2004; Scarsdale et al., 2006; She et al., 2006). DcpS is distinct in that it harbors a histidine triad decapping motif, while X29 and Dcp2 are both Nudix-containing proteins. Scarsdale et al. (2006) report a series of crystal structures for the homodimeric X29 apo protein and the metal-and nucleotide bound X29 holo-protein. The structures confirm the presence of the characteristic α/β/α sandwich Nudix fold structure within X29. A comparison of the structural alignment of X29 with that of the recently reported amino-terminal fragment of the *Schizosaccharomyces pombe* Dcp2 monomer reveals conservation in the overall Nudix fold structure (Figure 1A). As expected, the core α helix of the Nudix motif is highly conserved, particularly in the identical positioning of the critical gluta-mates previously shown to be essential for X29 and Dcp2 decapping activities (Coller and Parker, 2004).

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The X29 Nudix protein is surprisingly unique among cap binding proteins structurally characterized thus far. Previous structural studies have broadly illustrated that cap substrates insert into a binding pocket that provides general interactions and specific contacts to the $m^7G$ nucleobase (Marcotrigiano et al., 1997). Common features of proteins that form a complex with cap analog are pi-pi stacking via two aromatic residues sandwiching the $m^7G$ base, and hydrogen bonding between the $m^7G$ base and the vicinal side chain of an acidic amino acid, as initially revealed for the eIF4E cap binding protein (Marcotrigiano et al., 1997). For example, in the crystal structure of cap bound DcpS, a plethora of van der Waals, general stacking, and hydrogen bond interactions contribute to the stabilization of the complex formation (Gu et al., 2004). However, Scarsdale et al. (2006) clearly demonstrate that the $m^7GpppAX29$ complex is unusual in that the $m^7G$ nucleobase is exposed to the solvent rather than residing within a binding pocket. Surprisingly, a stacking interaction between the nucleoside ribose and phenylalanine 49 of X29 represents the one major contact made between the $m^7G$ base and the enzyme. The observed minimal contact between the methyl cap nucleobase with X29 and its exposure to the solvent provides an important explanation for the ability of X29 to recognize and hydrolyze U8 snoRNA substrates irrespective of their cap methyl moiety. The deduced structure is consistent with previous biochemical experiments demonstrating that X29 hydrolyzes a variety of U8 snoRNA substrates that vary in degree of methylation, including unmethylated, monomethylated, and trimethylated capped RNAs (Ghosh et al., 2004). As a corollary, DcpS preferentially hydro-lyzes methylated over unmethylated cap structure, indicating that the network of contacts between the $m^7G$ base and the enzyme contributes to the specific substrate recognition (Gu et al., 2004).

An additional interesting note pertains to the carboxyl-terminal end of the X29 Nudix fold that contains a 20 amino acid stretch initially defined as Box B in Dcp2. Box B was shown to be necessary for both decapping and RNA binding activities (Piccirillo et al., 2003). Moreover, the recent Dcp2 crystal structure indicates that the α-helical Box B is not a distinct domain, but rather an integral component of the Nudix fold congruent with its requirement for both substrate binding and hydrolysis. Despite the lack of an apparent homologous Box B in X29 by sequence, structure-based alignment of the two Nudix fold domains reveals amino acids 181 to 194 of X29 form an α helix that superimposes with the Dcp2 Box B (Figure 1). Although the RNA binding site is not yet known on the X29 homodimer, Scarsdale et al. (2006) have proposed a binding path for U8 extending from the cap dinucleotide along a positive surface of the protein. Consistent with a role for Box B in RNA binding, the Box B helix seems to lie along this path. Future site-directed mutagenesis studies should clarify the precise amino acids required for general RNA binding by Nudix domains as well as for preferential U8 snoRNA binding demonstrated by X29. Based on the extent of the overall Nudix fold conservation between X29 and Dcp2, it is likely that the two proteins will share similar modes of binding to capped RNA.

The X29 crystal structures represent the first example of a Nudix decapping protein bound to a cap substrate and provide meaningful insight into the molecular mechanism behind substrate recognition and RNA binding. However, as the natural substrate for catalysis of this enzyme is a capped RNA polymer, it is likely that future cocrystal structures of X29 with capped RNA have the potential to reveal interesting conformational differences and even some surprises.

## Selected Reading

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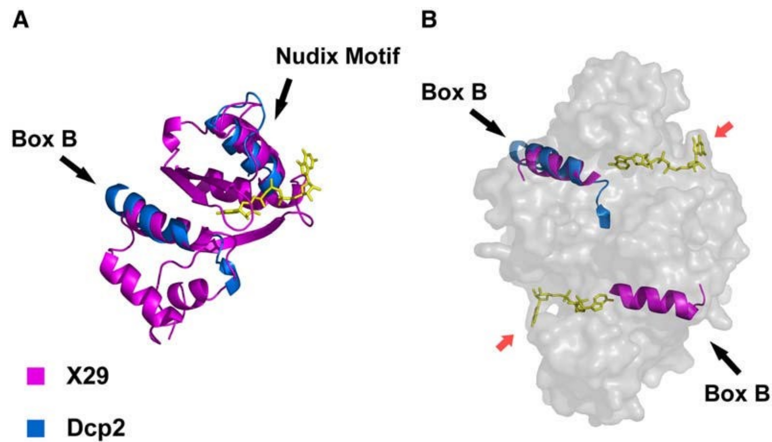


**Figure 1.**

Structure-Based Alignment of X. laevis X29 and S. pombe Dcp2

(A) Ribbon diagram for $m^7$GpppA-X29 (magenta) and Dcp2 (blue). The Nudix fold of an X29 monomer is shown. The α helices of Dcp2 Nudix motif and Box B are shown. The $m^7$GpppA (yellow) cap substrate is represented as a stick model.

(B) A transparent surface representation of the $m^7$GpppA-X29 homodimer with a ribbon diagram for the Box Bs of $m^7$GpppA-X29 (magenta) and monomer of Dcp2 (blue) is shown. The $m^7$GpppA (yellow) cap substrates are represented as stick models. The red arrows point to the respective $m^7$G nucleobase exposed to solvent.

(PDB coordinates for X29 and Dcp2 were kindly provided by J.N. Scarsdale and H. Song prior to publication, and we thank C. Lima for assistance with the structure superimposition).