

**Experiment title:**

Structural studies on the Lsm1-7 complex involved in mRNA decapping

Experiment**number:**

MX-976

Beamline:

ID23 1

Date of experiment:

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Shifts:

3

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Report:

mRNA turnover and its regulation is an effective mechanism to modulate gene expression. In eukaryotes, the process of mRNA decay usually initiates with removal of the poly(A) tail (Decker et al. 1993). Two major pathways of mRNA decay after deadenylation has been described (Parker and Song, 2004). Decapping is a key and rate-limiting step in 5'-3' mRNA decay pathway and mainly occurs in cytoplasmic foci, also referred as P-bodies (Sheth and Parker, 2003). In yeast, it has been shown that the Lsm1-7 complex, which is composed of seven Sm-like proteins (Bouveret et al. 2000) can associate with the 3' end of deadenylated mRNA with high affinity (Chowdhury et al. 2007). This association seems to inhibit 3'-5' mRNA decay while simultaneously promoting decapping at the 5' end of the mRNA (Tharun et al. 2001) by interacting with decapping enzyme Dcp1p-Dcp2p and decapping activator Pat1p. Moreover, the Lsm1-7 complex has been shown to bind and stabilize mRNAs containing 5' poly(A) tracts (Bergman et al. 2007), thereby blocking both decapping and the 3'-5' decay. This finding suggests that the Lsm1-7 complex may play an important role in viral infection by stabilizing the viral mRNA containing 5' poly(A) tracts.

As a first step towards elucidating the structure of Lsm1-7 complex in free form and in complex with a short oligo(A), we have solved the crystal structure of the subcomplex Lsm5/6/7 using the data collected on ID23-1 (Table 1). The structure provide insight into the assembly of the Lsm1-7 complex and will serve as a model for ultimately determining the structure of the Lsm1-7 complex.

Table 1 Data collection and refinement statistics

Data collection	Se-Lsm5/6/7
Wavelength (Å)	0.9793
Resolution limit (Å)	2.3
Unique reflections (N)	18881
I/σ	10.9 (2.5)
Completeness (%)	96.3(80.5)
R _{merge} ^a	0.097(0.542)
Refinement Statistics	
Data range (Å)	20-2.3
Used Reflections (N)	17467
Nonhydrogen atoms	1643
R _{work} ^b (%)	24.4
R _{free} ^c (%)	26.3
R.m.s deviation	
Bond length (Å)	0.012
Bond angles (°)	1.50

Values in parentheses indicate the specific values in the highest resolution shell.

^aR_{merge} = $\sum |I_j - \langle I \rangle| / \sum I_j$, where I_j is the intensity of an individual reflection, and $\langle I \rangle$ is the average intensity of that reflection.

^bR_{work} = $\sum ||F_o| - |F_c|| / \sum |F_c|$, where F_o denotes the observed structure factor amplitude, and F_c denotes the structure factor amplitude calculated from the model.

^cR_{free} is as for R_{work} but calculated with 5.0% of randomly chosen reflections omitted from the refinement.

References:

Bergman N, Moraes KC, Anderson JR, Zaric B, Kambach C, Schneider RJ, Wilusz CJ, Wilusz J. (2007). Lsm proteins bind and stabilize RNAs containing 5' poly(A) tracts. *Nat Struct Mol Biol.* 14:824-31.

Bouveret E, Rigaut G, Shevchenko A, Wilm M, Séraphin B. (2000) A Sm-like protein complex that participates in mRNA degradation. *EMBO J* 19:1661–1671.

Chowdhury A, Mukhopadhyay J, Tharun S. (2007) The decapping activator Lsm1p-7p-Pat1p complex has the intrinsic ability to distinguish between oligoadenylated and polyadenylated RNAs. *RNA.* 13:998-1016.

Decker CJ and Parker R. (1993). A turnover pathway for both stable and unstable mRNAs in yeast: evidence for a requirement for deadenylation. *Genes Dev.* 7:1632-43.

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Sheth U, Parker R. (2003) Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science.* 300:805-8.

Tharun S, Parker R. (2001) Targeting an mRNA for decapping: Displacement of translation factors and association of the Lsm1p-7p complex on deadenylated yeast mRNAs. *Mol Cell* 8:1075–1083.

