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Names and affiliations of applicants (* indicates experimentalists):

Zhihong Cheng* and Haiwei Song

Laboratory of Macromolecular Structure, Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore 138673.

Report:

Nonsense-mediated mRNA decay (NMD) is a cellular quality control mechanism that promotes rapid decay of nonsense-containing mRNAs. The NMD pathway is conserved in all eukaryotic organisms that have been studied[1,2]. The key players in the NMD pathway were initially identified by genetic screens in *S. Cerevisiae* (Upf1,-2, -3) and *C.elegans* (SMG2, -3, -4). Human homologs of these proteins have also been characterized (named as hUpf1, -2, 3). These proteins are highly conserved from yeast to humans[3]. These three proteins form a surveillance complex that is essential for NMD[4]. Among them, Upf1 is a cytoplasmic protein with ATP-dependent RNA helicase activity and belongs to superfamily group I helicases[5,6]. In addition to working in concert with Upf2/3 proteins, Upf1 protein acts with many cellular molecules in NMD pathway, such as translation release factors eRF1/3[7], a GTP-binding protein Ski7p[8] and human decapping complex[9]. It was found that NMD can also regulate the expression of broad classes of physiologic transcripts[10]. Most recently, it has been shown that hUpf1 can interact with Staufen proteins without other Upf proteins for regulating natural targets such as Arf1 mRNA, in so called SMD (staufen-mediated decay) pathway[11]. hUpf1 is also indispensable for replication-dependent histone mRNAs degradation[12]. To understand the molecular mechanism of NMD, and the structural basis of how Upf1 interacts with other cellular molecules in mRNA decay, we perform structural studies on hUpf1 protein.

The truncated hUpf1 (hUpf1c) protein has been crystallized in three states (Apo, AMPPNP-bound and ADP-bound). The structure of the AMPPNP-bound hUpf1c has been solved by SAD phasing at 2.6Å resolution. The structures of the Apo and ADP-bound hUpf1c were solved by molecular replacement based on the AMPPNP-bound structure. Further structural and functional analysis is in progress. The data collection and structure determination statistics is summarized in Table 1.

Data collection	hUpf1c:AMPPNP	hUpf1c	hUpf1c:ADP
Derivative	Se	-	-
Wavelength(Å)	0.9793	0.9793	0.9793
resolution(Å)	2.6	2.8	2.4
Observations(unique)	357045(27959)	393150(24896)	82629(43423)
I/σ	6.0	4.5	8.8
Completeness(last shell, %)	97.2(98.9)	100(100)	90.8(90.8)
Rsym	0.094(0.379)	0.102(0.395)	0.059(0.24)
Refinement			
Data range(Å)	20-2.6	20-2.8	20-2.4
Reflections(crossvalidation)	24065(1288)	21121(1138)	41163(2207)
Nonhydrogen atoms(water)	4872(110)	4715(63)	9545(243)
Rwork(Rfree)	24.5(27.1)	25.8(29.8)	23.5(27.2)
RMS bond length(Å)(bond angles)	0.006(1.048)	0.012(1.294)	0.008(1.011)

hUpf1c:AMPPNP(P43212)(Å): a=b=188.979, c=44.872; $\alpha=\beta=\gamma=90°$ and one molecule per asymmetric unit.
hUpf1c:Apo(P43212)(Å): a=b=195.189, c=45.458; $\alpha=\beta=\gamma=90°$ and one molecule per asymmetric unit.
hUpf1c:ADP(P1)(Å): a=63.284, b=67.836, c=87.25, $\alpha=114.41°$, $\beta=90.13°$, $\gamma=110.22°$ and two molecules per asymmetric unit.

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