Crystal structure of the 5′→3′ exoribonuclease Xrn1
Jeong Ho Chang, Liang Tong
Department of Biological Sciences, Columbia University, New York, NY10027. E-mail: jhc2158@columbia.edu

The 5′→3′ exoribonucleases (XRNs) have important functions in transcription, RNA metabolism, and RNA interference. The recent structure of Rat1 (Xrn2) showed that the two highly conserved regions of XRNs form a single, large domain, defining the active site of the enzyme. Xrn1 has a 510-residue segment following the conserved regions that is required for activity but is absent in Rat1. We report here the crystal structures at 2.9 Å resolution of Klyuyveromyces lactis Xrn1 (residues 1-1245, E178Q mutant), alone and in complex with a Mn2+ ion in the active site. Mn2+ is directly coordinated by three acidic residues, Asp206, Asp208 and Asp291. The 510-residue segment contains four domains (D1-D4), which are located far from the active site. Our mutagenesis and biochemical studies demonstrate that their functional importance is due to their stabilization of the conformation of the N-terminal segment of Xrn1. These domains may also constitute a platform for interacting with protein partners of Xrn1. Three of the domains (D1, D2 and D4) share the same backbone fold as chromo and tudor domains, but are unlikely to bind methylated lysine.

Keywords: exoribonuclease, chromo domain, tudor domain

Structure analysis of eukaryotic translation initiation factor complex 5B-1A
Min Yao,a Aiping Zheng,a Isao Tanaka,b *Graduate School of Life Sciences, Hokkaido University, cFaculty of Advanced Life Sciences, Hokkaido University, Sapporo 060-0810, (Japan). E-mail: yaoa@castor.sci.hokudai.ac.jp

The translation initiation of protein synthesis on the ribosome is one of the crucial processes in the cell for all organisms, and requires three initiation factors (IF1, IF2, and IF3) in bacteria and at least eight initiation factors (eIFs) in eukaryotes. In this process, after the recognition of start codon, eIF5B binds to eIF1A on the 48S initiation complex and facilitates ribosomal subunits joining.

The interaction between eIF5B and eIF1A is important for the conversion of 48S complex to a functional 80S ribosome. Unlike their bacterial homology IF1 and IF2, eIF1A and eIF5B have direct interaction even off the ribosome. The details of this interaction are not yet understood although previous studies have suggested that C-terminus of eIF1A interacts with domain IV of eIF5B. To elucidate the molecular bases for this interaction, we have determined structure of eIF5B-eIF1A complex from Saccharomyces cerevisiae by X-ray crystallography.

The crystal of eIF5BAN-eIF1AAN complex diffracted to 3.3 Å resolution and belonged to space group P2_12_1, with unit-cell parameters a = 101.9, b = 120.9, c = 132.8 Å. The complex structure was solved by MR method. The structure shows different conformations of two complex molecules in the asymmetry unit. The relative positions of domain IV to domain I-III of two eIF5B in the complex are different. Their structures are also different from that of isolated eIF5B structure. Such high flexibility of domain IV may reflect the conformation change requirement of eIF5B between pre- and post-state of subunit joining.

Keywords: eukaryotic translation initiation, ribosomal subunits joining, eIF5B-eIF1A interaction

Structure of hetR, a master regulator of heterocyst differentiation
Y. Kim, a G. Joachimiak, a,b Z. Ye, a,b,c T. A. Binkowski, a R.G. Zhang, a P. Gornicki, a S.M. Callahan, a W.R. Hess,a R. Haselkorn, a A. Joachimiak, a,f *Midwest Center for Structural Genomics and Structural Biology Center, Biosciences, Argonne National Laboratory, 9700 South Cass Avenue, Building 202, Argonne, Illinois 60439, (USA). 1The University of Chicago, Department of Molecular Genetics & Cell Biology, 920 E. 58th St., Chicago, IL 60637, (USA). 2Peking University, College of Life Sciences, Beijing, 1000871, (China). 3The University of Hawaii, Department of Microbiology, Honolulu, Hawaii 96822, (USA). 4The University of Freiburg, Faculty of Biology, Schänzlestr. 1, D-79104 Freiburg, (Germany). 5The University of Chicago, Department of Biochemistry and Molecular Biology, 929 East 57 Street, Chicago IL 60637, (USA). E-mail: ykim@anl.gov

HetR was discovered as an essential regulator required for differentiation of nitrogen-fixing heterocysts in the cyanobacterium Anabaena. A mutation in the hetR gene prevented the earliest steps in heterocyst differentiation, yielding a culture incapable of nitrogen fixation. Complementation of such a mutant with a copy of the wild-type hetR gene on a plasmid produced a strain that could fix nitrogen and differentiate the same number of heterocysts with the same spacing pattern as the wild type.

The purified HetR protein is a dimer of 299-amino acid monomers. The dimer binds to several sites in Anabaena DNA, one of which is a palindrome upstream of the start site for transcription of the hetP gene. The crystal structure of HetR from Fischerella was determined at 3.0 Å. The protein is a dimer comprised of three structural units: a central DNA-binding unit containing the N-terminal regions of the two subunits organized with two HTH motifs; two globular flaps protruding away from a 2-fold dyad; and two C-terminal domains joined to form a hood over the central core. The flaps and hood have no precedent in the protein structure database, therefore representing new folds in this transcription factor. The structural assignments are supported by site-directed mutagenesis and DNA-binding studies. We suggest that HetR serves as a scaffold for assembly of components critical for heterocyst development.

To understand the details of protein-DNA interactions and the functional implication, the structure of the HetR complexed with cognate DNA is in progress.

This work was supported by National Institutes of Health grantGM094585 (AJ), NSF grant IOS-0919878 (SC), BMBF grant 0313921 (WRH) and by the U. S. Department of Energy, Office of Biological and Environmental Research, under contract DE-AC02-06CH11357 (AJ).

Keywords: HetR, transcription, heterocyst