

the bacterial transcription elongation factors, which modulate the enzymatic activity of RNAP. GreA is a transcript cleavage factor, which binds to a stalled elongation complex caused by an RNAP back tracking. It rescues the RNAP by invoking the inherent RNAP activity to cleave the extruded RNA 3' end. On the other hand, Gfh1, a close paralog of GreA, inhibits the enzymatic activity. To elucidate details of how these factors modulate the RNAP activity, we crystallized *Thermus thermophilus* RNAP complexed with them. Crystallization, data collection and preliminary crystallographic analysis will be presented.

Keywords: RNA polymerase, transcription regulation, macromolecular X-ray crystallography

P04.16.389

Acta Cryst. (2008). A64, C352

Crystal structure of the full-length Hsp110 molecular chaperone in the nucleotide-free state

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The 70kDa heat shock proteins (Hsp70) play essential roles in diverse cellular processes as molecular chaperones by preventing the substrate proteins from unfavorable aggregation. The 110 kDa heat shock protein (Hsp110) is a distal member of Hsp70 superfamily found in eukaryotes. In spite of the existence of two domains both characteristic of Hsp70, Hsp110 shows some significant different properties than canonical members of Hsp70; most importantly, Hsp110 lacks ATPase activity that is necessary for the Hsp70 function. Recently, the function of Hsp110 as a nucleotide exchange factor for canonical Hsp70s has become clear. The crystal structure of the *Saccharomyces cerevisiae* ortholog of Hsp110 in the ATP-bound state has been more recently reported, although the detailed mechanism is still poorly understood. We report the crystallization and X-ray crystallographic analysis of Hsp110 from *Schizosaccharomyces pombe*. The protein was crystallized in the absence of nucleotides and includes the C-terminal portion that was truncated in the previous study. The biochemical data suggests that the C-terminal helix bundle is critical for the nucleotide exchange activity. By comparing the two structures, possible roles of the ATP binding and the C-terminal helix bundle will be discussed.

Keywords: X-ray structure determination, protein crystallography, protein chaperone

P04.16.390

Acta Cryst. (2008). A64, C352

Structural insights into asymmetric cell division in *Drosophila*

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Miranda is a multidomain adaptor protein involved in neuroblast asymmetric cell division in *Drosophila*. The central domain of miranda is necessary for cargo binding of the neural transcription factor prospero. We solved the first solution structure of miranda central cargo-binding domain using small angle x-ray scattering (Yousef et al, 2008). Additionally, the complex structure of prospero homeodomain bound to its dna target has been previously solved by x-ray crystallography (Yousef and Matthews, 2005). We have also expressed different segments of prospero and miranda in soluble forms for further structural analyses. The structural information available thus far has shed light on important mechanisms involved in asymmetric cell division.

Yousef MS, Kamikubo H, Kataoka M, Kato R and Wakatsuki S. *Protein Science* (2008). 17:908-917
Yousef MS and Matthews BW. *Structure* (2005). 13:601-607

Keywords: asymmetric cell division, small angle x-ray scattering, X-ray crystallography

P04.17.391

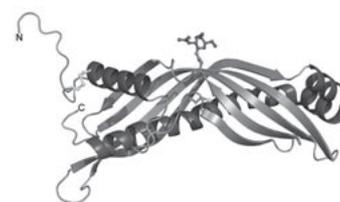
Acta Cryst. (2008). A64, C352

Ancestral lipid-binding fold of insect juvenile hormone binding protein

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As specific carriers of Juvenile Hormone (JH), Juvenile Hormone Binding Proteins (JHBPs) have a profound effect on insect development. The crystal structure of *G. mellonella* JHBP has a remarkable fold, consisting of a long helix wrapped almost completely in a highly curved beta-sheet. JHBP resembles the folding pattern of some human lipid binding proteins, with similar organization of one cavity and an S-S bond between the helix and the beta wrap. The human molecules, however, have a tandem of JHBP-like domains, probably as a result of gene duplication. Thus, JHBP reveals an ancestral fold used for hydrophobic ligand binding. Unexpectedly, the JHBP molecule possesses two hydrophobic pockets, one at each pole. Although it was not possible to crystallize a JHBP-JH complex, several experiments indicate that JHBP binds JH in only one cavity. The fact that JHBP crystals disintegrate on contact with JH confirms a report of a conformational change on hormone binding. The nature of the second ligand is unknown but it is intriguing that with its two binding pockets the JHBP molecule has a similar binding potential as the human molecules, in which each domain has only one hydrophobic pocket.



Keywords: insect development, juvenile hormone, hemolymph