

MS06-O5

The Structural Organization of Substrate Loading in Iterative Polyketide Synthases

Roman Jakob¹, Dominik A. Herbst¹, Callie R. Huitt-Roehl², Jacob M. Kravetz², Philip A. Storm², Jamie R. Alley², Craig A. Townsend², Timm Maier¹

1. Department of Biozentrum, University of Basel, Basel, Switzerland
2. Department of Chemistry, Johns Hopkins University, Baltimore, United States of America

email: roman.jakob@unibas.ch

Polyketide synthases (PKSs) are microbial multienzymes for the biosynthesis of biologically potent secondary metabolites. Polyketide production is initiated by the loading of a starter unit onto an integral acyl carrier protein (ACP) and its subsequent transfer to the ketosynthase (KS). Initial substrate loading is achieved either by multidomain loading modules or by the integration of designated loading domains, such as starter unit acyltransferases (SAT), whose structural integration into PKS remains unresolved. A crystal structure of the loading/condensing region of the nonreducing PKS CTB1 demonstrates the ordered insertion of a pseudodimeric SAT into the condensing region, which is aided by the SAT-KS linker. Cryo-electron microscopy of the post-loading state trapped by mechanism-based crosslinking of ACP to KS reveals asymmetry across the CTB1 loading/-condensing region, in accord with preferential 1:2 binding stoichiometry. These results are critical for re-engineering the loading step in polyketide biosynthesis and support functional relevance of asymmetric conformations of PKSs.

References:

Herbst, D. A., et al. (2018). *Nat Chem Biol* 14, 474-479. Keywords: mechanism-based crosslinking, multidomain loading modules, natural products

Keywords: mechanism-based crosslinking, multidomain loading modules, natural products

MS07 Nucleic acids and interactions with proteins

Chairs: Prof. Ralf Ficner, Prof. Markus Wahl

MS07-O1

Structure and function of the spliceosome

Rui Zhao¹, Xueni Li¹, Shiheng Liu², Lingdi Zhang¹, Jiansen Jiang², Sara Espinosa¹, Ryan Hill¹, Kirk Hansen¹, Hong Zhou²

1. Biochemistry and Molecular Genetics, Aurora, United States of America
2. Electron Imaging Center for Nanomachines, Los Angeles, United States of America

email: rui.zhao@ucdenver.edu

Pre-mRNA splicing is catalyzed by the spliceosome, a huge protein-RNA complex composed of the U1, U2, U4, U5, U6 snRNPs and many non-snRNP related proteins. U1 snRNP plays a critical role in 5' splice site recognition and is a frequent target of alternative splicing factors. These factors transiently associate with human U1 snRNP and are not amenable for structural studies, while their *Saccharomyces cerevisiae* (yeast) homologs are stable components of U1 snRNP. We have recently determined the structure of yeast U1 snRNP at 3.6 Å resolution using cryoEM. The structure reveals common features as well as important differences from the human U1 snRNP. It provides atomic models of nearly all essential domains of U1 snRNA, all core proteins, and five auxiliary proteins. The structure offers a framework to integrate a wealth of existing genetic and biochemical data regarding the structure and function of yeast U1 snRNP and the mechanism of 5' splice site recognition. In addition, the yeast U1 snRNP structure and biochemical analyses based on the structure provided intriguing insight into the structure and function of these auxiliary human U1 snRNP proteins in alternative splicing in higher eukaryotes.

The spliceosome undergoes dramatic changes signified by the E, A, Pre-B, B, B^{act}, B*, C, C*, P, and ILS complexes in a splicing cycle. CryoEM structures of B, B^{act}, C, C*, and ILS revealed mechanisms of 5' splice site recognition, branching, and intron release, but lacked information on 3' splice site recognition, exon ligation and release, all related to the post-catalytic P complex. We have recently determined the cryoEM structure of the yeast P complex at 3.3 Å resolution, revealing that the 3' splice site recognition is driven by the interaction between 3' and 5' splice sites, likely facilitated by a stem-like structure formed in the intronic regions between the branch site and 3' splice site. The structure shows that a new protein(s) becomes stably associated with the core components of the P complex around Prp8 and Prp22, securing the 3' exon and potentially regulating the activity of Prp22. The structure demonstrates that Prp22 binds 15-21 nucleotides downstream of the exon-exon junction, enabling it to pull the 3' intron-exon and ligated exon in a 3' to 5' direction to achieve 3' splice site proofreading and exon release, respectively. By providing insights into the 3' splice site recognition, exon ligation, and the action of RNA helicase Prp22, the P complex structure fills a major gap in our understanding of the splicing cycle.