MS06-O5

The Structural Organization of Substrate Loading in Iterative Polyketide Synthases

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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.

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MS07 Nucleic acids and interactions with proteins

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MS07-O1

Structure and function of the spliceosome

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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 3.6Å 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' ss 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' ss recognition, branching, and intron release, but lacked information on 3' ss 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 3.3Å resolution, revealing that the 3' ss recognition is driven by the interaction between 3' and 5' ss, likely facilitated by a stem-like structure formed in the intronic regions between the branch site and 3' ss. 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' ss proofreading and exon release, respectively. By providing insights into the 3' ss 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.

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MS07-O2

Post-translational regulation of gene expression in the immune system by Roquin/RC3H1 and ribonucleases of the Regnase/ZC3H12/MCPIP family

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Roquin/RC3H1 and Regnase/ZC3H12/MCPIP family members control the lifetime and degradation of their target mRNAs by binding to characteristic stem-loop structures in their 3'-untranslated regions (3'UTRs). In earlier work [1] we have determined the crystal structure of the ROQ domain of human Roquin-1 and characterized its binding to constitutive decay elements (CDEs) present in the 3'UTRs of tumor necrosis factor-α (TNF-α) and the inducible T-cell co-stimulator (ICOS) mRNA. Roquin-1 was shown to post-transcriptionally regulate the A20 mRNA, thereby modulating the activity of the IKK/NF-κB pathway [2]. Roquin-1 mediates mRNA degradation by recruiting the CCR4-CAF1-NOT deadenylase complex to mRNAs after CDE binding. Closely similar stem-loop structures are recognized by the Regnase enzymes that mediate mRNA breakdown through their intrinsic ribonuclease activity. These enzymes share a PIN-type ribonuclease domain followed by a unique CCCH zinc-finger (ZnF) domain. In recent work, we have determined crystal structures of the Regnase-3 PIN domain and of a heptaribonucleotide bound to a Regnase-3 fragment containing both the PIN and the ZnF domain. These structures reveal binding modes of the RNA to both the PIN domain and the zinc finger of Regnase-3 and suggest a basis for RNA loop recognition by the unique zinc finger. In addition, we discovered - by serendipity - a novel form of double-stranded RNA incorporating a half-turn of A-like RNA based entirely on wobble-base pairing, which we termed W-RNA [3]. The W-RNA formed under acidic crystallization conditions from oligonucleotides expected to form CDE-like RNA stem-loops and is stabilized by C-A+ wobble-base pairing. We propose that RNA duplexes based on pH-switchable wobble-base pairing may find biotechnological applications in the future.

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