MS06-O2

A hub for 3'-end processing: structural insights into mrna polyadenylation

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Almost all eukaryotic pre-mRNAs must undergo 5′ capping, splicing and 3′-end processing before they can be transported to the cytoplasm for their translation into proteins. 3′-end processing involves over 20 different protein factors that also co-ordinate transcription termination. The cleavage and polyadenylation factor (CPF) is an essential component of the 3′-end machinery that cleaves pre-mRNA transcripts and adds the 3' polyA tails. Despite its fundamental importance, we are still far from understanding the molecular mechanisms of CPF. Here, we identify a sub-complex of the yeast CPF, the polyadenylation module (pAm), which acts as a hub for protein-protein interactions. Using cryo-EM we determine a 3.5 Å structure of the Cft1-Pfs2-Yth1 subunits of pAm. This consists of 4 beta propellers in Cft1 and Pfs2 that are strikingly similar to other interaction hubs involved in DNA and RNA processing. The zinc finger Yth1 protein extends from the side, providing an RNA binding surface. Biochemical studies confirm the structural observations and indicate the important role of pAm as the scaffold element of CPF to assemble other CPF subunits, including the poly(A) polymerase, and accessory factors of the 3'end processing machinery on RNA. We now aim to understand how the enzymatic activities are regulated. Our most recent results will be presented.

References:

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MS06-O3

Structural insights into the recognition between tri-ubiquitin and ubiquitin binding protein

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Poly-ubiquitin chains are important signaling molecules that were assembled by the ubiquitination process in order to trigger different linkage-specific signaling pathways through subsequent protein-protein interaction with downstream signaling molecules. The K63 and linear polyubiquitins are involved in non-degradative signaling pathways that mostly regulate the NF-kB activation and tumor necrosis factor receptor (TNFR)-mediated cell death, as well as necroptosis, in which deubiquitinases play a significant role in deciphering ubiquitin codes for regulating signaling. However, the molecular mechanism of how a polyubiquitin chain recognizing signaling molecules remains unclear. Emerging evidence in recent years shows that A20, a deubiquitinase, down-regulates the NF-kB activation signaling by interacting and degrading the polyubiquitin chains. The interaction is mediated by A20-binding inhibitors of NF-kB, ABINs, which contain both A20 and linear polyubiquitin interacting domains. Here we report our recent works on the ABIN2:polyubiquitin complex. Structural analyses together with the mutagenesis, pull-down, and isothermal titration calorimetry assays show that ABIN2 has a primary and a secondary linear ubiquitin-binding site. Surprisingly, a tri-ubiquitin molecule could simultaneously interact with two ABIN2 dimers, in which the ubiquitins form a helical trimer when bridging two hABIN2 dimers. Our studies suggest the formation of a higher-order complex between a linear polyubiquitin chain, ABIN2, and A20.

References:

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