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Seminar series

Interactions between the nucleosome histone core and Arp8 in the INO80 Chromatin Remodeling Complex

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Wednesday, 7 November, 2012
11:00 AM
Seminar Room

Biochemical analyses of chromatin remodeling by various complexes suggest that these machineries have diverse strategies to disrupt histone-DNA interactions. Ino80 complex is an ATP-dependent chromatin-remodeling complex that plays important roles in transcription, DNA repair and recombination. However, the biochemical mechanism of chromatin remodeling and its association with these events are unclear. Hence it is important to understand the mechanism of the Ino80 complex and resolve the roles of its individual subunits in chromatin remodeling. The complex contains 15 subunits, amongst these actin, actin related proteins (ARPs) Arp4, Arp5 and Arp8, bacterial RuvB like helicases Rvb1 and Rvb2, Ino eighty subunits (IES) 2, and 6 are conserved between yeast and human. Recent studies from different groups showed that ARPs are essential components of the chromatin-remodeling complex, however their exact role in chromatin remodeling is unclear. To understand the role of ARPs, we have over expressed and purified yArp8, an essential subunit of the complex, and performed structural and biochemical analyses. Yeast Arp8 (yArp8) comprises two domains; a 25KDa N-terminal, only found in yeast, and a 75KDa C-terminal domain (yArp8CTD) that contains the actin fold and is conserved across other species. The crystal structure reveals yArp8CTD contains
three insertions within the actin core. Using a combination of biochemistry and electron microscopy, we show that Arp8 forms a complex with nucleosomes and that the principal interactions are via the H3 and H4 histones, mediated through one of the yArp8 insertions. We show that recombinant yArp8 exists in monomeric and dimeric states but the dimer is the biologically relevant form required for stable interactions with histones that exploits the two-fold symmetry of the nucleosome core. yArp8 CTD, stoichiometrically binds both ATP and ADP with micromolar affinity. Together these data provide unique insight into the stoichiometry, architecture and molecular interactions between components of the INO80 remodelling complex and nucleosomes, providing a first step towards building up the structure of the complex.