Poster Presentation

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The role of Ndc10 in budding yeast kinetochore establishment

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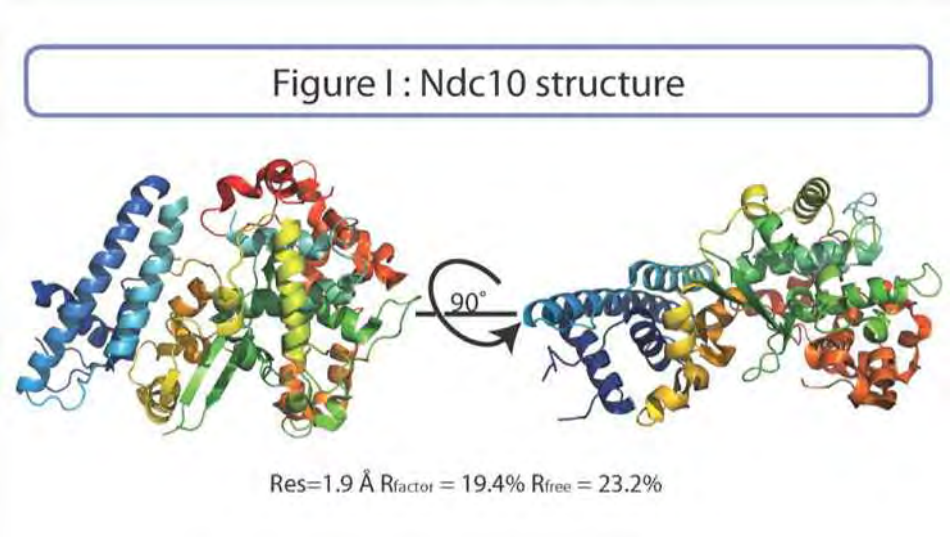
The kinetochore is a large assembly of protein that connects sister chromatids to the mitotic spindle during mitosis. It both transmits microtubule (MT) tension and controls the mitotic checkpoint. The kinetochore forms onto the centromere (CEN), a chromosomal locus classified in two groups varying dramatically in composition. In the vast majority of eukaryotes, regional CENs are long arrays of repetitive non-conserved DNA hosting multiple MTs nucleation. On the contrary, in a small group of yeast related to S.cerevisae, point CENs are characterised by a conserved short sequence hosting a single MT. Despite this difference, all CENs share a similar establishment mechanism relying on the loading of the CENP-A histone-like protein by the chaperone Scm3. This mechanism is ambiguous in regional CENs, whereas in point CENs it solely relies on the CBF3 complex binding to the centromeric DNA elements III (CDEIII). CDEIII is point CEN most conserved sequence and point mutants show totally impaired kinetochore formation. CBF3 is composed by the essential Ndc10, Cep3, Ctf13, and Skp1. Ctf13 and Skp1 link the CBF3 formation to the cell cycle, whereas Cep3 and Ndc10 bind to the CEN. Before our study, Ndc10's role was speculative but it was known that its primary function is to recruit CENP-A after S-phase through interaction with the histone chaperone Scm3. In this study, we have solved the X-ray structure of Ndc10 N-terminal domain (NTD) at 1.9 Å resolution. Unexpectedly, Ndc10 NTD fold belongs to the tyrosine recombinase/α-integrase family, which alters DNA structure through a conserved catalytic tyrosine. Interestingly, Ndc10 has lost the catalytic tyrosine but, as we suspect, might alter the CEN DNA winding, something possibly critical for CENP-A loading. By analysing the recombinase fold and by mutating key residues, we described Ndc10 NTD DNA-binding motifs. This finding offers new insights into kinetochore evolution and the adaptation of a well-studied protein fold to a novel role.

[1] *Perriches, T., & Singleton, M. R. (2012) "The structure of the yeast kinetochore Ndc10 DNA-binding domain reveals an unexpected evolutionary relationship to tyrosine recombinases." Journal of Biological Chemistry*



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