Name: SAMBIT DALUI

Index No. 35/17/Life Sc./25

Thesis title:

Structural and Functional characterization of Testis-specific Y encoded-like protein 5 : a novel member of NAP histone chaperone superfamily

Abstract

In eukaryotic organisms, genetic information is packaged into a chromatin and all DNA dependent cellular events require chromatin assembly and disassembly. This chromatin dynamicity is governed by a specific group of proteins, histone chaperones. Nucleosome assembly protein (NAP) superfamily is a growing set of histone chaperone proteins that shows sequence similarity with NAP1 protein but the function and expressions varies between cell/tissue types. The efficacy of Testis-specific protein Y-encoded Like 5 (TSPYL5), a member of NAP superfamily, in chromatin context and its function as a histone chaperone during DNA mediated processes still remains unclear. In this report, we identified and characterized human TSPYL5 that harbours the signature NAP Like fold at the C-terminal region. In vitro and ex vivo immuno pull-down assay revealed histone H3/H4 specificity of TSPYL5 over H2A/H2B. We were able to map the H3/H4 interacting regions of TSPYL5 and observed that TSPYL5-NLD (NAP like domain) specifically interacts with H3/H4. We showed that H3 with its C-terminal tail region (105-136 amino acids) interacts with TSPYL5. We further characterized the amino acid residues of TSPYL5 responsible for histone association. This helped us express and purify the TSPYL5-H3/H4 complex. Using DSS crosslinking assay and SEC-MALS, we established a stable complex of TSPYL5with H3/H4 at a stoichiometric ratio of 2:2:2, where one TSPYL5 dimer interacts with two histone H3/H4 dimers or one tetramer. H3-H3' interaction surface at the C-terminal region is responsible for H3/H4 tetramerization. Mutating the tetramer forming residues of H3 and using them in IP assays, we illustrated that both dimeric and tetrameric conformation could bind TSPYL5. Biochemical and biophysical analyses demonstrated that the bottom of the earmuff domain i.e. TSPYL5-NLD interacts with H3/H4 and is important for nucleosome assembly in vitro. The complex facilitated nucleosome assembly by depositing H3/H4 to naked DNA template confirming activity of TSPYL5 as a histone chaperone. In conclusion, we described TSPYL5 as a novel member of NAP histone chaperone family that specifically binds H3/H4. Histone deposition activity validated its role in chromatin context and this work thus provides useful information to resolve the enigma of histone re-cycling associated with DNA mediated processes.

Siddhartha Roy 28/06/22
(Signature of the Supervisor with date & official seal)

Sambit Dalui 29/06/2022
(Signature of the Candidate)

SIDDHARTHA ROY
Principal Scientist
Structural Biology & Bioinformatics
CSIR-Indian Institute of Chemical Biology
4, Raja S C Mullick Road
Jadavpur, Kolkata-700 032