Name: Anirban Dasgupta

Index No. 100/18/Life Sc./25

Thesis title:

Molecular characterization of human histone H2BK120 Ubiquitin Ligase UBR7

Abstract

Ubiquitination of histones is involved in the maintenance of chromatin dynamics and genome stability through its ability to regulate gene expression and DNA damage repair. Nucleosomal histones H2A and H2B have been predominantly targeted for monoubiquitination. In mammals, Histone H2B gets mono-ubiquitinated in the conserved Lys-120 (K120), corresponding to Lys-123 (K123) in Budding Yeast. This initiates a degradation independent pathway which facilitates recruitment of other 'writer' enzymes involved in H3K4 and H3K79 methylation which subsequently helps establish an epigenetic crosstalk network with the H2BK120Ub mark. In humans, H2B K120 monoubiquitination is associated with disruption of chromatin compaction and increased transcription. Recently, our work had identified Ubiquitin Protein Ligase E3 Component N-Recognin 7 (UBR7) to be a novel H2BK120 monoubiquitin ligase which pairs with E2 conjugate UbcH6 for its E3 ligase function. In the present thesis work, to gain insight into the previously unknown mechanism of UBR7 mediated ubiquitin transfer, I have extensively mapped the E3-E2 binding interface between UBR7 and UbcH6. The results obtained suggest that atypical PHD finger of UBR7 is crucial for interaction with UbcH6 and concomitant substrate histone H2B monoubiquitination. The critical loop regions of UbcH6 involved in UBR7 interaction were identified and their role in UBR7 mediated H2B monoubiquitination was assessed. The histone H2B C-terminal tail (114-125) is necessary and was found sufficient by itself to undergo UBR7/UbcH6-mediatedmonoubiquitination. PHD finger was found to mediate dimerization of UBR7 and I used SEC-MALS to determine the molecular mass of UBR7-PHD in solution. Furthermore, the residues mapped to the dimerization interface were found implicated in cancer and were also critical in regulating E3 ubiquitin ligase function of UBR7. Upon dimer deficiency, the E2 and substrate binding of UBR7 was found to be compromised. Finally, I have also compared the mode of ubiquitin transfer of UBR7 to RNF20, a previously reported H2B K120 ubiquitin ligase, through single turnover ubiquitin discharge assays. Interestingly, unlike RNF20, the UbcH6~Ub hydrolysis mediated by UBR7 requires substrate histone H2B association. Substrate H2B binding to UBR7 brings about a change in conformation within the PHD finger which was found to be critical for efficient ubiquitin transfer. RNF20 was not subjected to any such conformational change. Thus, the mechanism of ubiquitin transfer by UBR7 was found significantly distinct from that of RNF20.

Anirban Dasgupta

(ANIRBAN DASGUPTA)

Forwarded & Recommended

Siddhartha Roy

08/06/2022

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