Mechanistic basis for the role of acidic tail of Rad6 (ubiquitin-conjugating enzyme) in Bre1 (E3 ligase) mediated recognition of histones in Saccharomyces cerevisiae

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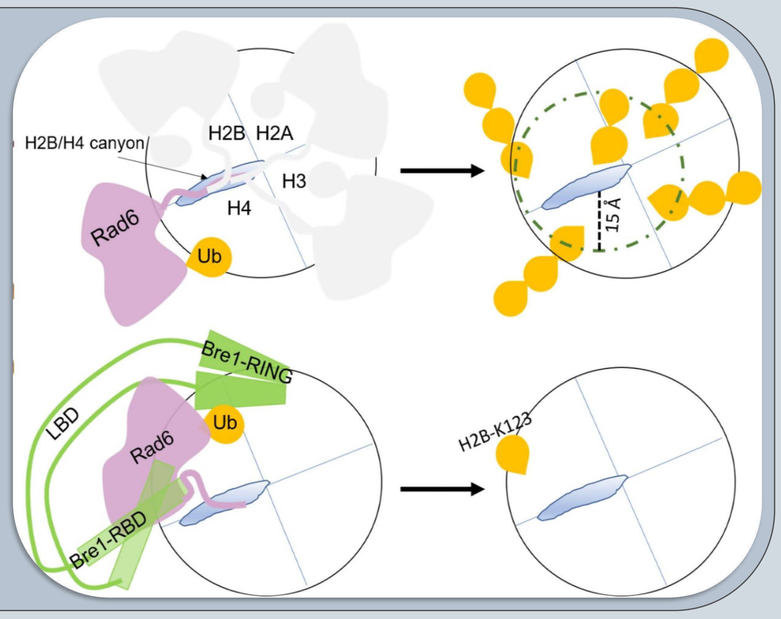
Abstract

Mono-ubiquitation at histone $H2B_{K123}$ in yeast ($H2B_{K120}$ in humans) plays an important role in gene activation and regulation. It is a highly conserved process which involves E2 conjugating enzyme (Rad6) and E3 Ligase (Bre1). Previous studies have highlighted the role of Rad6 and Bre1 in mono-ubiquitination at histone $H2B_{K123}$ site in yeast, however the mechanistic basis of Rad6/Bre1 dependent histone H2B mono-ubiquitination was unknown. In our present study, we aim to characterize the role of a C-terminal acidic tail of Rad6 and its interaction with Bre1 for site-specific H2B mono-ubiquitination. Through solution-based structural biology, *in-vitro* assays and binding studies we have shown that Rad6 acidic tail binds to positively charged surface on H2B restricting the access of Rad6 to 15 A˚ radii on NCP surface. This allows only promiscuous ubiquitination of various lysine residues present in the vicinity in absence of Bre1.Using different structural and biophysical approaches, this study for the first time uncovers the direct role of Rad6-acidic tail in interaction with the Bre1 Rad6-Binding Domain (RBD) and recognition of histones surface to facilitate histone H2B mono-ubiquitination. On the other hand, RBD (Rad6 Binding Domain) along with RING domain in Bre1 inhibits non-specific transfer of ubiquitin by Rad6 on histones by modulating the dynamics of acidic tail and restricting only H2BK123 mono-ubiquitination. Taken together, we aim to reveal the mechanistic basis of constrains in E2:E3 and E2:NCP recognition that ensure the fidelity of histone H2B monoubiquitination reaction.



Results

Acidic tail in Rad6 is critical in mediating ubiquitin transfer to histone lysine

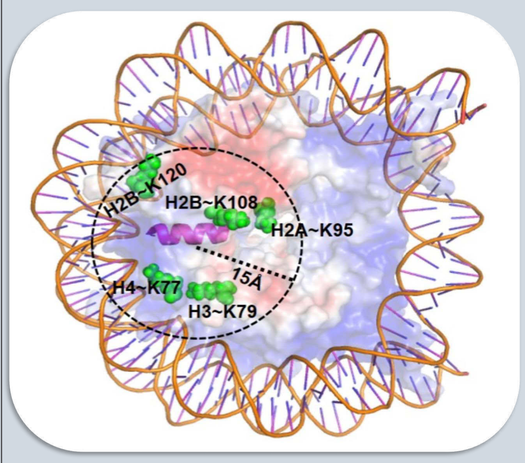


Figure 1-Model of recombinant *Xenopus laevis* nucleosome with positively charged region of H2B

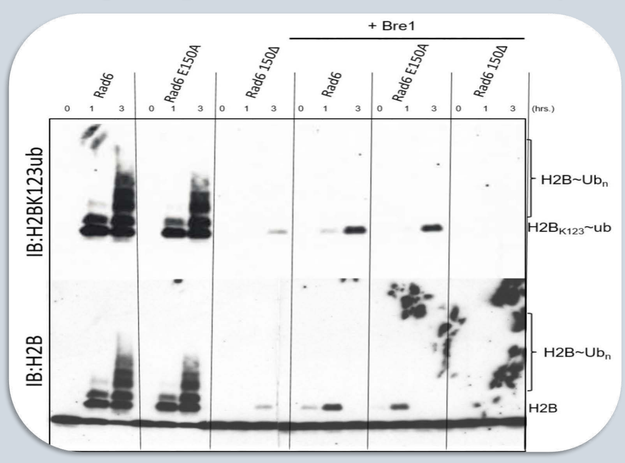


Figure 2-Ubiquitination of natural nucleosome by Rad6 and mutants with and without Bre1 enzyme

Rad6 acidic tail and a positively charged histone H2B region are involved in E2:NCP recognition

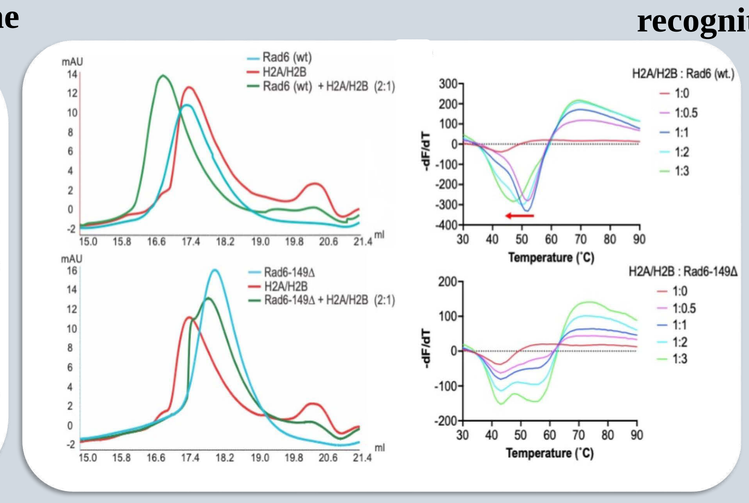


Figure 3-Size-Exclusion chromatography and DSF of Rad6 and Rad6-149Δ with H2A/H2B heterodimer

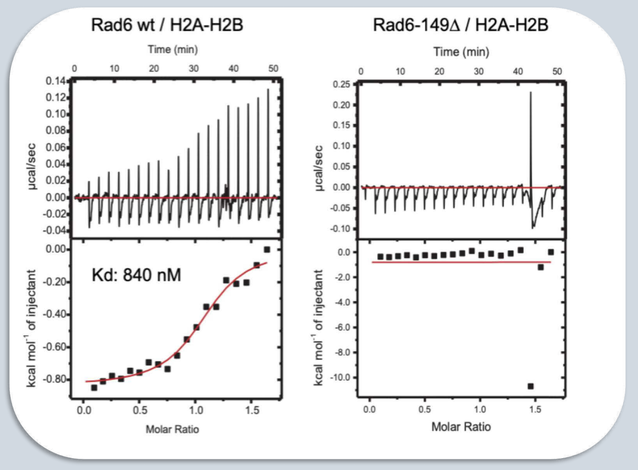


Figure 4- Interaction study of H2A/H2B heterodimer with full-length Rad6 and Rad6-149Δ using ITC

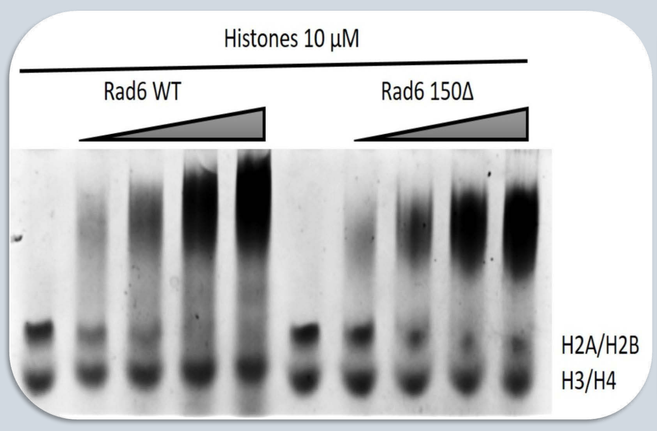


Figure 9- Rad6(wt) or Rad6-149Δ with histones complex (H2A/H2B + H3/H4)

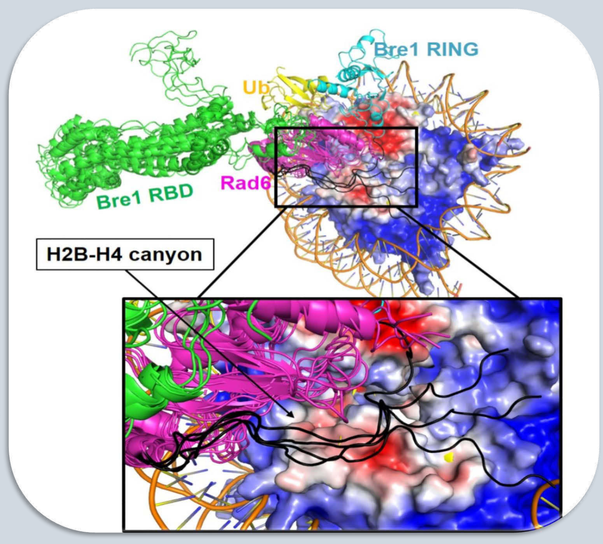


Figure 12- Docking of Rad6 acidic tail over the yeast nucleosome

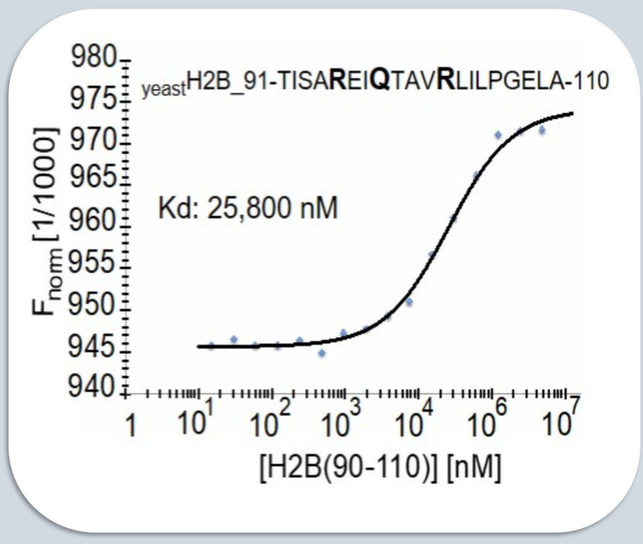


Figure 5- Interaction study between Rad6 and peptide from yeast H2B using Microscale Thermophoresis

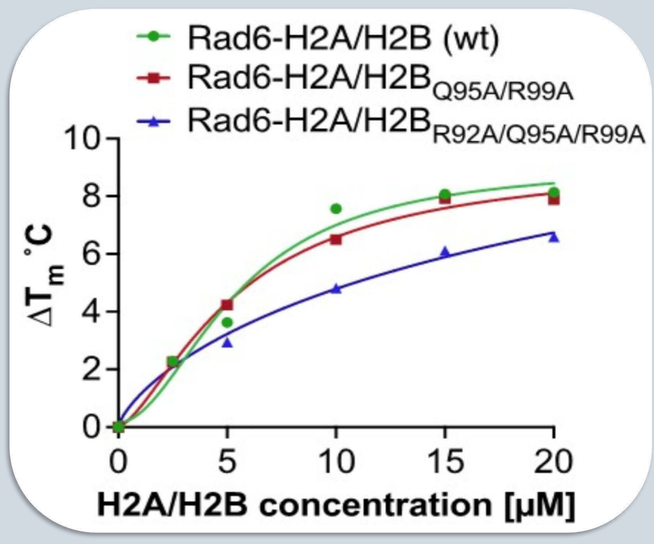


Figure 6-Interaction between Rad6 and H2A/H2B heterodimers using ThermoFluor assay

Bre1 RBD domain prevents non-specific ubiquitin transfer to histone lysine residues

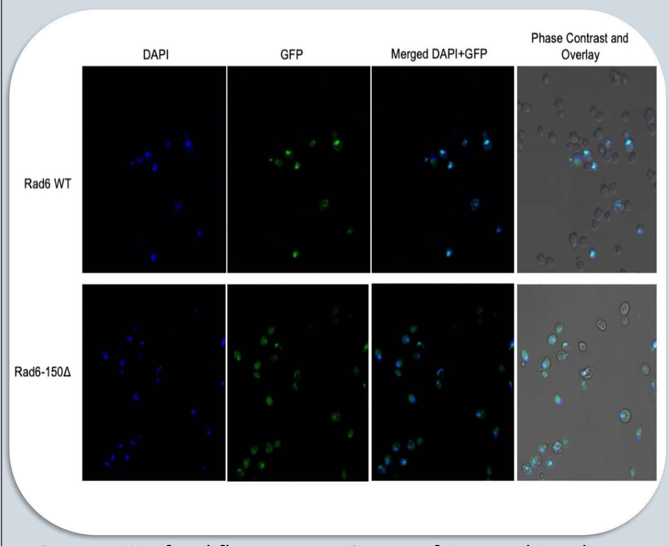


Figure 7- Confocal fluorescence image of GFP-Rad6 and GFP-Rad6-150Δ expressed in *Saccharomyces cerevisiae*

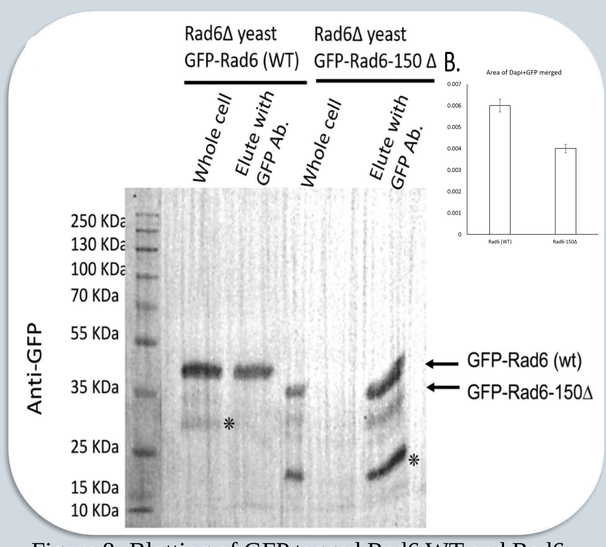


Figure 8- Blotting of GFP tagged Rad6 WT and Rad6 150Δ and intensity graph of confocal images

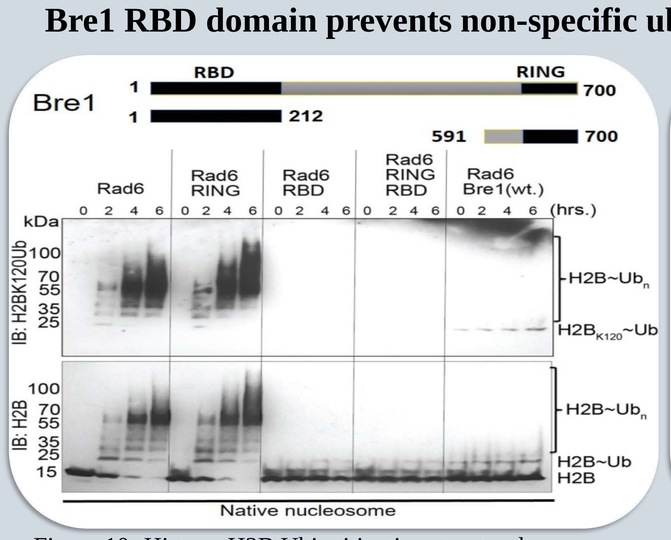


Figure 10- Histone H2B Ubiquitination at natural nucleosome by Rad6 alone and in the presence of Bre1 full-length and its domains RBD and RING

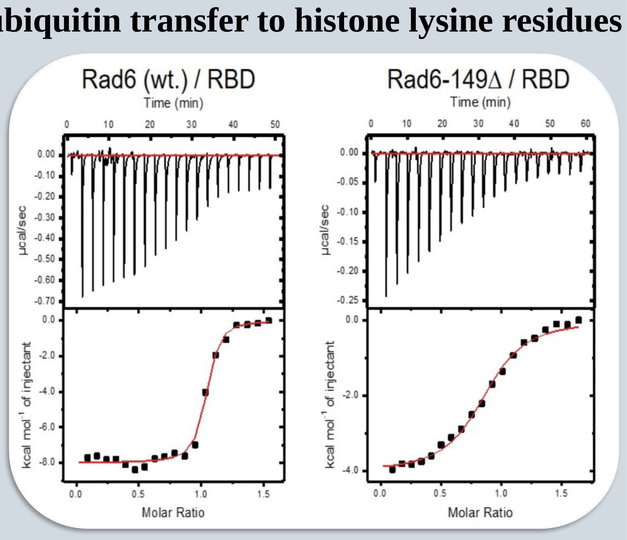


Figure 11- Interaction studies of RBD domain with Rad6 (wt.) and Rad6-149Δ by Isothermal Titration Calorimetry

Conclusion

E2 Rad6 and E3 Bre1 are indispensable for $H2B_{K123}$ monoubiquitination in budding yeast however, the mechanistic basis for the fidelity of the ubiquitination reaction was not clear. The present study reveals two important findings: (i)) the involvement of Rad6 acidic tail and a positive charged surface of histone H2B in E2-NCP recognition prior to ubiquitin transfer, and (ii) direct interaction of Bre1 RBD with Rad6 prevents promiscuous ubiquitin transfer to histone lysine residues, allowing site-specific monoubiquitination in conjunction with the RING domain

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Refferences

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Publication

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