The Paf1 transcription elongation complex interacts directly with the N-terminal helix of Rad6 to facilitate H2B ubiquitylation

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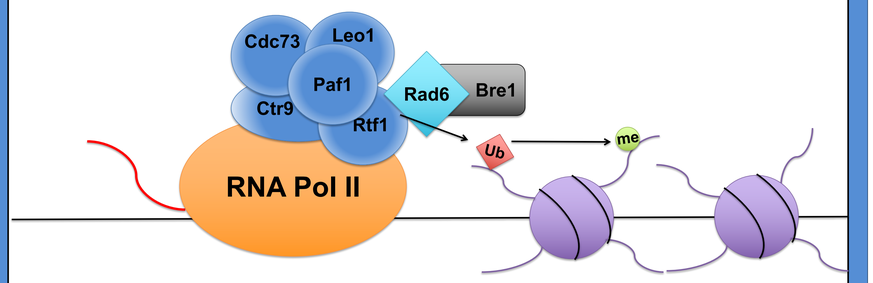
- The Polymerase Associated Factor 1 Complex (Paf1C) subunit Rtf1 is necessary for post-translational mono-ubiquitylation of histone H2B K123 (H2Bub)[1].
- H2Bub is required for the H3 K4 and H3 K79 di- and trimethylation, and dysregulation of H2Bub or these downstream marks is associated with neurological defects and cancer.
- A direct interaction between the Histone Modification Domain (HMD) of Rtf1 and the E2 ubiquitin conjugase Rad6 is necessary for H2Bub, but the Rad6 interface was unknown.



Key Questions: What is the nature of the HMD interaction surface of Rad6? Is this surface specific to Rad6's role in H2Bub?

BPA crosslinking: site-specific protein crosslinking to identify direct interactions using mass spectrometry

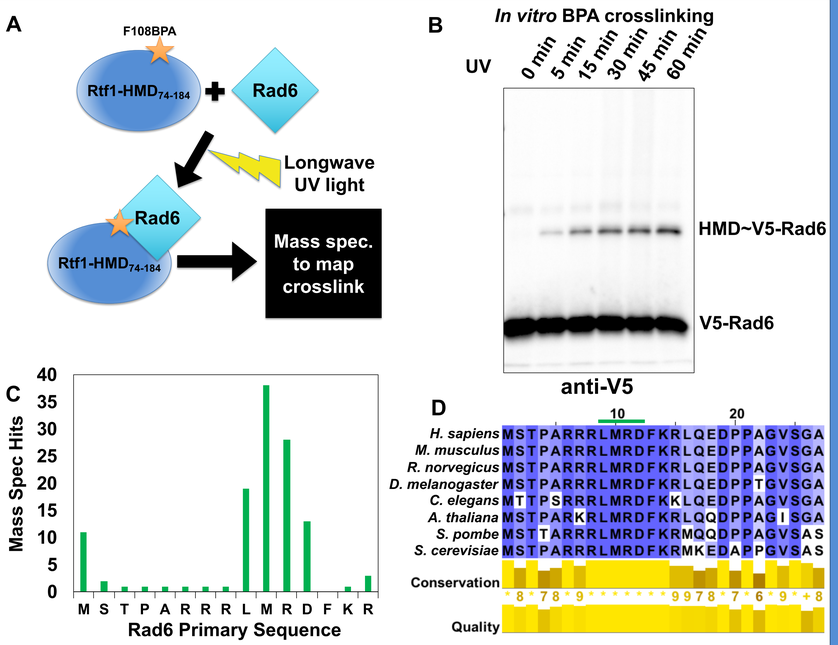


Figure 1. A. Cartoon depiction of *in vitro* BPA crosslinking between Rtf1-HMD$_{74-184}$ and Rad6. Star represents BPA, a photoreactive phenylalanine analog. B. Western blot showing UV-dependent crosslink between Rtf1-HMD$_{74-184}$ and V5 -Rad6. C. Crosslink locations were mapped to Rad6's N-terminal helix by mass spectrometry. D. Jalview alignment of Rad6 homologs across eukaryotes shows conservation of the putative HMD-interacting region. Green line highlights crosslinked residues.

Acknowledgements

I thank members of the Arndt, Hainer, Kaplan, Lee, and VanDemark labs for helpful discussion and shared resources. I thank Jaehoon Kim and Song Tan for generously sharing recombinant proteins for *in vitro* assays. Funding for this project was provided by NIH grant GM052593 to K.M.A.

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Functional consequences of *rad6* mutations *in vivo* assessed by western blots and telomeric silencing

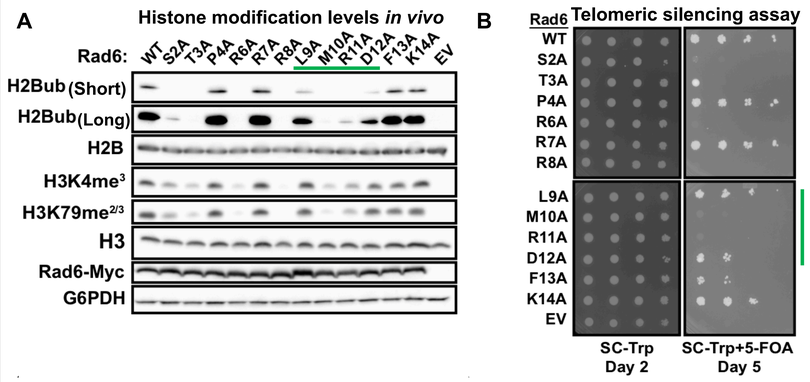

Figure 2. A. *rad6* mutants show global defects in H2Bub and downstream H3 methylation marks (K4me, K79me) independent of H2B, H3, or Rad6 protein expression levels. G6PDH serves as loading control. B. Using an orthogonal telomeric silencing reporter[2], the same mutants with defects in H2Bub by western analysis have defects in chromatin architecture.

Most *rad6* mutants showing H2Bub defects appear normal for DNA damage repair and N-degron proteolysis

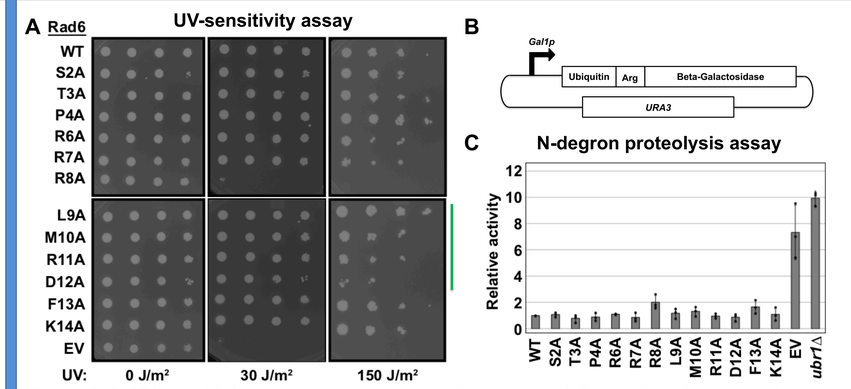

Figure 4. A. UV sensitivity spot assays were used to assess defects of *rad6* mutants in post-replication DNA damage repair (PRR)[3]. Of mutants assayed, only *rad6-R8A* had a defect comparable to *rad6*Δ (EV). B. Schematic of N-degron reporter plasmids[4]. An unstable Arg-β-Galactosidase reporter was used to assess defects in N-degron proteolysis. C. Quantification of activity of each *rad6* mutant shows that no mutant has defects comparable to *rad6*Δ or *ubr1*Δ, the E3 ubiquitin ligase required for N-degron proteolysis.

Substitutions in the Rad6 N-terminal helix disrupt HMD-mediated stimulation of H2Bub *in vitro*

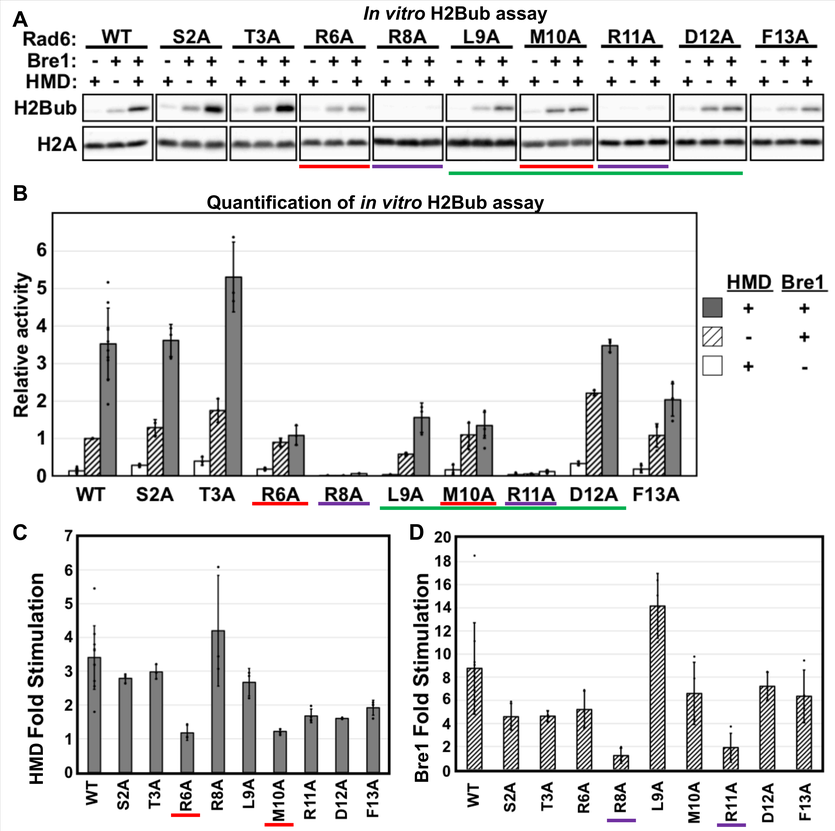

Figure 3. A. H2Bub activity of alanine-substituted Rad6 proteins in an *in vitro* H2Bub assay[1]. B. Quantification of blots in A. Note that Rad6-R6A and Rad6-M10A (red lines) have no increased activity upon addition of the HMD but perform comparably to wild type Rad6 in the absence of HMD. C. Data shown in B. transformed to show the stimulation factor upon addition of HMD to the reaction. D. Data shown in B. transformed to show Bre1 stimulation factor. Note that Rad6-R8A and Rad6-R11A (purple lines) are unable to be stimulated by Bre1, the E3 ubiquitin ligase required for H2Bub.

Current model of HMD-Rad6 interaction

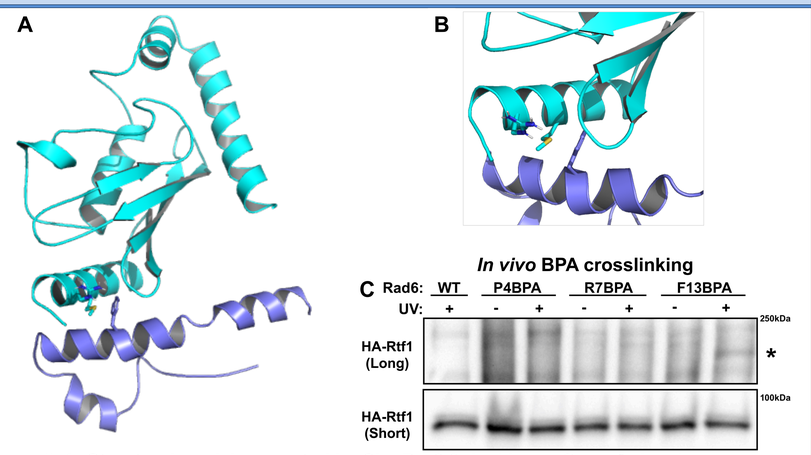

Figure 5. A. Structural model generated by ClusPro2.0 docking software[5] showing interaction of Rad6 (cyan, PDB:1AYZ) with the HMD of Rtf1 (Blue, PDB:5E8B). B. Zoom of A. showing interaction interface. Rad6-R6, Rad6-M10, and Rtf1-F108 side chains are shown. C. Validation of model using *in vivo* BPA crosslinking. Appearance of a UV-specific band (*) when Rad6-F13 is substituted with BPA indicates that the residue is within ~5Å of Rtf1.

Conclusions

- **The N-terminal helix of Rad6, specifically residues R6 and M10, are necessary residues for productive HMD-Rad6 interactions.**
- **This interaction surface is specific to the H2Bub process as no defects in post-replication DNA damage repair or N-degron proteolysis were observed for *rad6-R6A* or *rad6-M10A* mutants.**
- **The N-terminal helix of Rad6 also makes critical contacts with other proteins involved in H2Bub, including Bre1.**
- **The high conservation of these residues implies this interaction is present in higher eukaryotes, but further inquiry is needed.**
- **This region could be a promising drug target for diseases associated with aberrant H2B ubiquitylation.**