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Mms2/Ubc13 with Covalently Bound Ubiquitin: Structural Basis of Linkage-Specific Ubiquitin Chain Formation. M.J. Eddins¹, C.M. Carlile³, K.M. Gomez⁴, C.M. Pickart³, C. Wolberger^{1,2}, ¹Dept. of Biophysics, Johns Hopkins School of Medicine, Baltimore, MD, ²Howard Hughes Medical Inst., ³Dept. of Biochemistry, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, ⁴Clafin Univ., Orangeburg, SC.

The E2 ubiquitin conjugating enzyme Ubc13 in complex with the UEV protein Mms2 is involved in forming K63-linked polyubiquitin chains which are implicated in nonproteolytic signaling pathways. The Mms2/Ubc13 complex plays vital roles in DNA repair and the NF- κ B pathway. We have determined the structure of the Mms2/Ubc13/ubiquitin complex by trapping the covalently linked donor ubiquitin to the active site residue of Ubc13. The complex shows the details of the active site of Ubc13 including the covalent link and specific interactions of the donor ubiquitin with the UEV/E2 complex. Crystal packing places an ubiquitin from one complex in the acceptor site of an adjacent complex placing K63 of the acceptor ubiquitin into the active site. Mutations that disrupt this acceptor site and confer UV sensitivity provide evidence that the ubiquitin in the crystal is in the acceptor site. Along with K63 of this acceptor ubiquitin making specific interactions with residues in the active site, this suggests a model for ubiquitin chain formation and substrate specificity.