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# Observation of an E2 (Ubc9)-homodimer by crystallography

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ABSTRACT

Post-translational modifications by the small ubiquitin-like modifiers (SUMO), in particular the formation of poly-SUMO-2 and -3 chains, regulates essential cellular functions and its aberration leads to life-threatening diseases (Geoffroy and Hay, 2009) [1]. It was shown previously that the non-covalent interaction between SUMO and the conjugating enzyme (E2) for SUMO, known as Ubc9, is required for poly-SUMO-2/3 chain formation (Knipscheer et al., 2007) [2]. However, the structure of SUMO-Ubc9 non-covalent complex, by itself, could not explain how the poly-SUMO-2/3 chain forms and consequently a Ubc9 homodimer, although never been observed, was proposed for poly-SUMO-2/3 chain formation (Knipscheer et al., 2007) [2]. Here, we solved the crystal structure of a heterotrimer containing a homodimer of Ubc9 and the RWD domain from RWDD3. The asymmetric Ubc9 homodimer is mediated by the N-terminal region of one Ubc9 molecule and a surface near the catalytic Cys of the second Ubc9 molecule (Fig. 1A). This N-terminal surface of Ubc9 that is involved in the homodimer formation also interacts with the RWD domain, the ubiquitin-fold domain of the SUMO activating enzyme (E1), SUMO, and the E3 ligase, RanBP2 (Knipscheer et al., 2007; Tong et al.. 1997; Tatham et al., 2005; Reverter and Lima, 2005; Capili and Lima, 2007; Wang et al., 2009, 2010; Wang and Chen, 2010; Alontaga et al., 2015) [2–10]. The existence of the Ubc9 homodimer in solution is supported by previously published solution NMR studies of rotational correlation time and chemical shift perturbation

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(Alontaga et al., 2015; Yuan et al., 1999) [10,11]. Site-directed mutagenesis and biochemical analysis suggests that this dimeric arrangement of Ubc9 is likely important for poly-SUMO chain formation (Fig. 1B and C). The asymmetric Ubc9 homodimer described for the first time in this work could provide the critical missing link in the poly-SUMO chain formation mechanism. The data presented here are related to the research article entitled, "RWD domain as an E2 (Ubc9) interaction module" (Alontaga et al., 2015) [10]. The data of the crystal structure has been deposited to RCSB protein data bank with identifier: 4Y1L.



## Specifications Table

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| Subject area | *Biological Chemistry/Structure Biology* |
| More specific subject area | *Ubiquitin-like modifications, SUMO, RWD* |
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| Data accessibility | *The X-ray crystal structure data is available at RCSB protein data bank with PDB identifier 4Y1L* (http://www.rcsb.org/pdb/explore/explore.do?structureId=4Y1L) |

**Fig. 1.** Proposed mechanism of poly-SUMO chain formation. (A) A model of how Ubc9 homodimer could stimulate SUMO-chain formation. The Ubc9 homodimer observed in the crystal structure of the heterotrimer contains two Ubc9 molecules (green) and one RWD (not shown). The residues at the binding interfaces are indicated in cyan (the top Ubc9 molecule) and yellow (the bottom Ubc9 molecule) and with their sidechains shown. The catalytic Cys residue of Ubc9 is shown with spheres. Y134 at the interface is indicated. The structure of non-covalent Ubc9-SUMO complex is superimposed onto the top Ubc9 molecule of the Ubc9 homodimer, resulting in the position of the SUMO molecule, shown in red, on the upper left side. The dashed red line represents the flexible N-terminal segment of SUMO that contains the SUMOylation site but did not have electron density in X-ray diffraction. A hypothetical SUMO molecule that forms a thioester conjugate with the bottom Ubc9 is shown on the lower right side. (B) To test whether the Ubc9 homodimer observed here is important for poly-SUMO chain formation, the Y134A mutant at the Ubc9 homodimer interface was used to test the ability of Ubc9 in stimulating the formation of poly-SUMO chains. Residues at the N-terminal surface of Ubc9 were not mutated, because this surface is directly involved in a higher affinity interaction with E1 for the transfer of SUMO from E1 to E2 than that of surface containing Y134 near the catalytic Cys [7,12]. SDS-PAGE analysis of poly-SUMO chain formation in the presence of wild-type and Ubc9 Y134A mutant is shown to the left. Quantification of gel band intensity using the ImageJ software is shown to the right. Y134A showed severe defects in catalyzing poly-SUMO-2 chain formation. (C) Because Y134A plays a role both in the transfer of SUMO from E1 to E2, and from E2 to target proteins [13,14], we examined whether the defects in poly-SUMO-2 chain formation is only due to the effect of the mutation on SUMO transfer from E1-E2 and E2-substrate using mono-SUMO-1 modification of the substrate Sp100. SDS-PAGE analysis of mono-SUMO-1 modification of Sp100 in the presence of wild-type and Ubc9 Y134A mutant is shown to the left. Quantification of gel band intensity using the ImageJ software is shown to the right. The Y134A mutant showed more severe defects in catalyzing poly-SUMO-2 chains than mono-SUMO-1 modification of Sp100, indicating that its defect in catalyzing poly-SUMO-2 chain formation was not only due to its effect on general SUMOylation. These data suggest that the Ubc9 dimer observed in this crystal structure is likely important to the formation of poly-SUMO chains.