

LETTER

Function and Structure of the RWD Domain

Alontaga *et al.* (1) analyzed the structure of RSUME/RWDD3 and confirmed our findings (2, 3) that RWD is the only well structured domain in RSUME and participates in its binding to Ubc9 and SUMOylation action. Based on experimental data, we proposed that RSUME interacts with Ubc9 prior to the Ubc9-SUMO complex formation (2). The absence of NMR chemical shifts on Ubc9 induced by RWD after the Ubc9-SUMO complex is already formed and the higher K_D of RSUME for Ubc9 than SUMO are to be expected and are fully consistent with our model.

The authors obtained crystals of a loose heterotrimeric complex bearing two Ubc9 molecules and one RWD domain. The NMR results and analysis of the Ubc9-RWD interaction based on the HSQC (heteronuclear single quantum coherence) data do not exclude other possible Ubc9-RWD complexes that are also compatible with all NMR data. Moreover, the NMR shift histogram proves that the N-terminal helix of RSUME interacts with Ubc9, as we predicted (2). An evolutionarily conserved interaction for Ubc9-RSUME similar to established E2-UEV structures (4), as we previously proposed, remains likely.

The authors confirmed that RSUME stimulates the formation of the Ubc9-SUMO thioester conjugate and added the stimulation of the SAE2-SUMO thioester and SAE2-SUMO isopeptide. The inference

from the global SUMOylation assay performed in cells different from those reported, without Ubc9 co-transfection (optimal condition) and without confirmation of the SUMO identity of the bands, is inconclusive. *In vitro*, RSUME showed no effect on the SUMOylation of sp100, supporting the notion that RSUME acts on several, but specific, targets as HIF-1 α , I κ B, GR, and pVHL (2, 3, 5).

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