Molecular Mimicry of SUMO by Rad60 Helps Protect the Genome from Toxic Stress

DNA is subject to multiple forms of damage that can occur either spontaneously, through sources such as reactive oxygen species produced by cellular metabolism, or through exogenous sources such as UV light and X-rays. Different cellular pathways exist to detect and repair the distinct forms of damage, and thus maintain the stability of the genome. The importance of the DNA repair pathways is highlighted by inherited diseases that contain defects in pathway components, resulting in marked increase in cancer incidence, rapid-aging and/or neurological pathologies. One important family functioning in DNA repair is the Rad60 family of proteins, with Rad60 being conserved from yeast to humans. Sequence analysis of this family has revealed that members contain two regions of similarity to SUMO, which have been termed SUMO-like domains (SLDs, SLDs1 & 2). This inclusion of SLDs into a polypeptide chain is unusual because SUMO (Small Ubiquitin-like Modifier) is a small protein normally covalently attached to target proteins, thereby altering target protein function and/or cellular localization. The attachment of SUMO occurs via an enzymatic cascade that uses three steps for ligation (1). The first step uses an E1 enzyme that activates SUMO, the second step uses an E2 enzyme for conjugation, and the third use an E3 protein for substrate recognition. Multiple rounds of this SUMOylation cascade can also arise and this results in the covalent attachment of SUMO chains, which appear to have roles in signaling and in promoting the assembly of larger protein complexes (2). Interestingly however, Rad60 SLDs lack a Gly-Gly motif that is present in SUMO and is required for the activation and conjugation of SUMO to target proteins. This suggests that Rad60 has instead novel interactions with SUMO pathway components, potentially modulating the function of the SUMO pathway in DNA repair and genome stability.

Studies were conducted by a team of Structural Biologists, including Jeff Perry and Andy Arvai, in the laboratory of John Tainer at The Scripps Research Institute to define the roles of Rad60 proteins and their SLDs. Data was collected on the C-terminal SLD2 of fission yeast (*S. pombe*) Rad60 at SSRL beamline 11-1, which enabled structural determination to an ultra-high 0.97 Å resolution. This exceptional resolution allowed for a detailed structural model that included hydrogen atoms in its final refinement (Fig. 1). Interestingly, the Rad60 SLD2 backbone structure is well conserved with SUMO (Fig. 1). Yet, the surface

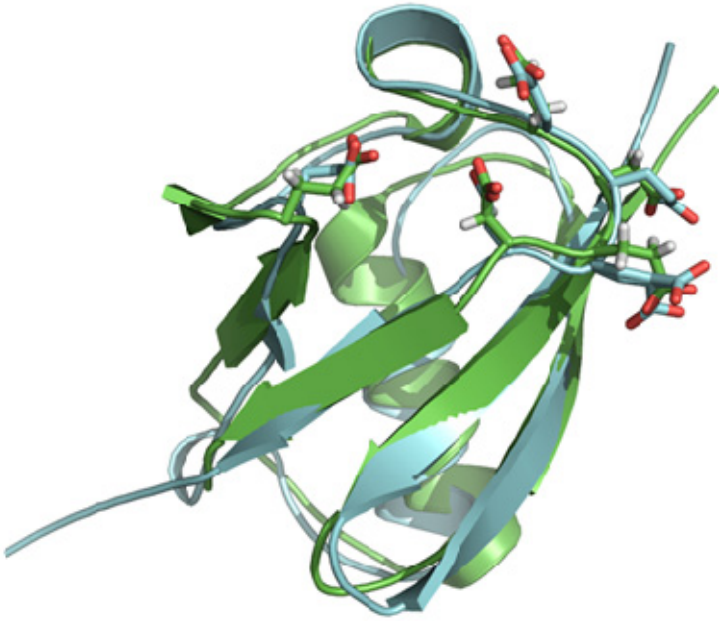

Figure 1. The 0.97 Å crystal structure of Rad60 SLD2. The Rad60 SLD crystal structure depicted as cartoon, in green, structurally superimposed onto human SUMO-1, in blue. The two structures share a common fold, though many interaction motifs differ, except the E2 non-covalent interface residues that are highlighted as sticks. Note that the ultra-high resolution allowed for the refinement with hydrogen atoms, as indicated on Rad60 SLD2 stick side chains.

features differ between these proteins, suggesting that most of the SUMO interactions with SUMO pathway components are not conserved in Rad60. However, one exception is the conserved interface on SUMO and on Rad60 SLD2 for a non-catalytic binding site on the SUMO E2 (Fig. 1); this non-catalytic site is specifically used by the E2 to promote the formation of SUMO chains (3,4,5). In collaboration with Nick Boddy's yeast genetics laboratory, also at Scripps, a structure-based mutation in Rad60 SLD2 was created that uncoupled its interaction with the E2 partner on its chain-forming interface. Notably, breaking this Rad60:E2 interaction resulted in a cellular hypersensitivity to genotoxic stress, and also an increase in spontaneous recombination associated with aberrant replication forks.

Overall, these results have provided the first detailed structural analysis of SUMO-like domains, their interaction interfaces, and a mechanistic basis for Rad60 functions in DNA-damage-responses, via interaction with the SUMO E2. Understanding SUMO pathway interactions is of great significance as this pathway is implicated in much human pathology, including cancer and neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases, and in viral infections.

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