Multi-faceted regulation of the sumoylation of the Sgs1 DNA helicase in genome maintenance

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Abstract

To minimize DNA damage-induced genome instability and cancer formation, the DNA repair system requires proper regulation to adjust its efficiency and actions. Sumoylation is emerging as an important regulatory means for many forms DNA repair pathways, including homologous recombination (HR) repair. However, how HR proteins are dynamically sumoylated to modulate their functions remains poorly understood. The Sgs1-Top3-Rmi1 (STR) complex in budding yeast and its human counterpart BLM-Topo IIIa-RMI1-RMI2 (BTRR) play pivotal roles in genome maintenance. They affect multiple steps during HR. We recently reported that all three STR subunits are sumoylated and this requires the SUMO E3 ligase, Nse2 (aka Mms21), a subunit of the Smc5/6 complex. Further, STR sumoylation positively influence HJ removal as sumoylation promotes subunit interaction and recruitment to DNA repair foci.

Our recent effort addresses the factors that directly promote STR sumoylation using a combination of *in vitro* sumoylation systems and cellular assays. We demonstrated that DNA binding per se enhances Sgs1 sumoylation *in vitro*, providing one mechanism for the observed HJ requirement in STR sumoylation in cells. In addition, we show that a scaffold protein Esc2 stimulates Mms21-mediated STR sumoylation *in vivo* and *in vitro*. Esc2's action requires two distinct domains. Esc2 stimulates STR sumoylation through its C-terminal SLD2 domain binding to the backside of SUMO E2. A separate effect is mediated by the Esc2 mid-region (MR). Interestingly, though Esc2-MR binds HJ DNA, its stimulation of Sgs1 sumoylation is separable from this DNA binding activity, suggesting a dual role of the Esc2-MR domain. Consistent with the *in vitro* data, cellular results provided evidence that Sgs1 function and sumoylation are positively affected by the two Esc2 domains. In summary, our finding defined multiple stimulatory elements that render efficient Sgs1 sumoylation in promoting its functions, thus advancing our understanding of how sumoylation regulates DNA repair and genome maintenance.

Results

Figure 1. Sumoylation of Sgs1-Top3-Rmi1 is mediated by Mms21 and functions in HJ removal

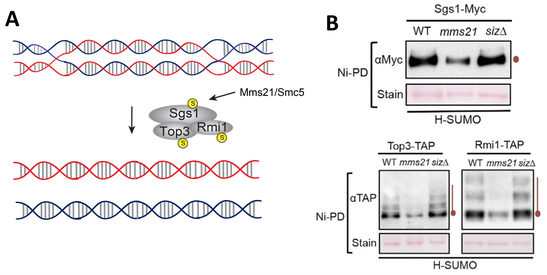


Figure 2. Genome stability factor Esc2 promotes STR sumoylation in cells

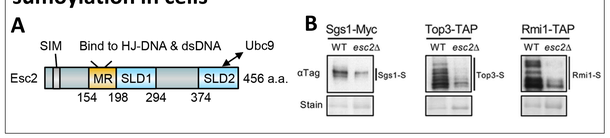


Figure 3. DNA binding per se enhances Sgs1 sumoylation

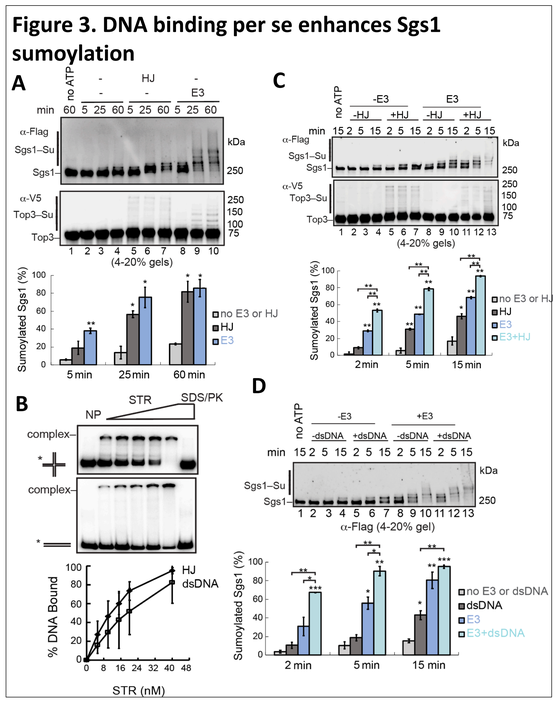


Figure 4. DNA and Esc2 additively increase Sgs1 sumoylation

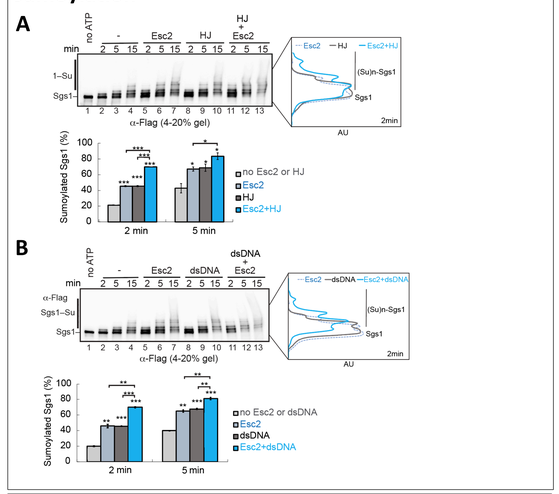


Figure 5. Esc2 interacts with E2 (Ubc9), but not E3 or SUMO substrates

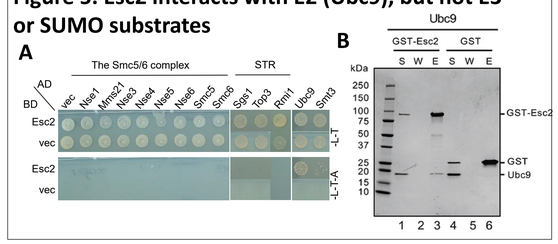


Figure 6. Esc2 stimulates STR sumoylation through its C-terminal SLD2 domain binding to the backside of SUMO E2

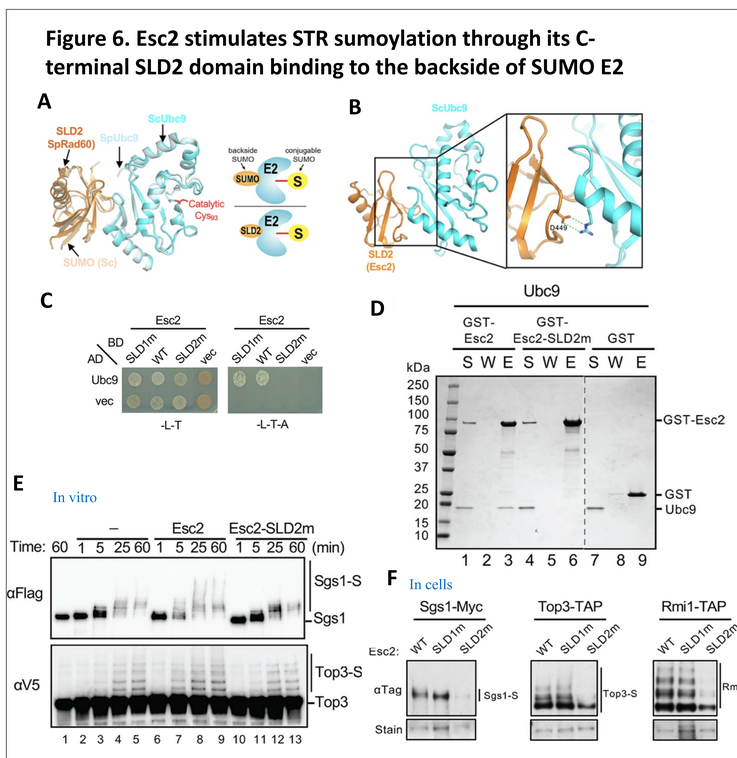


Figure 7. Esc2-MR promotes Sgs1 sumoylation independent of its DNA-binding ability

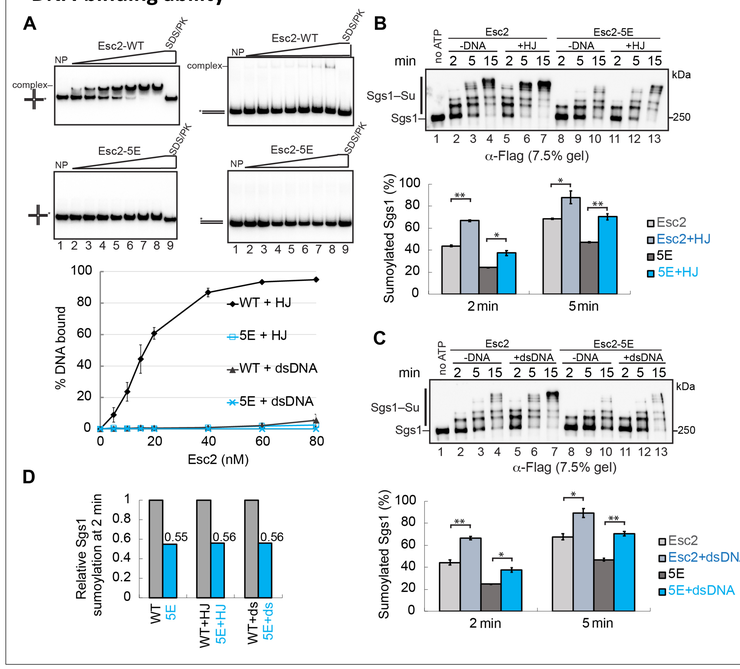


Figure 8. Esc2 mutants worsen genotoxic sensitivity of cells

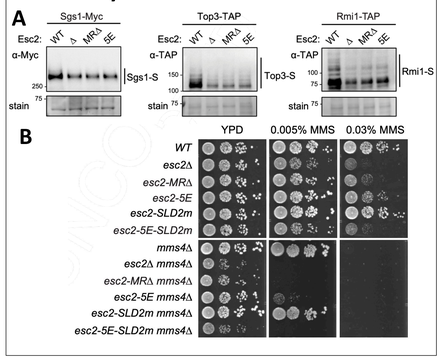
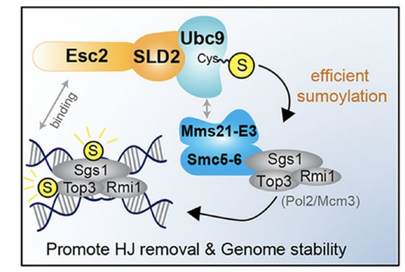


Figure 9. Working model showing Esc2 and DNA stimulates STR sumoylation in HJ removal and genome maintenance



Conclusions:

1. Sgs1 binding to DNA per se promotes its sumoylation.
2. The observed DNA-based stimulation of Sgs1 sumoylation is enhanced by the Esc2 protein, which requires its two distinct domains.
3. Esc2 stimulates STR sumoylation through its C-terminal SLD2 domain binding to the backside of SUMO E2.
4. Esc2-MR contributes to Sgs1 sumoylation but through a DNA-independent manner, thus suggesting a dual role for this domain.
5. In the future, whether other components of the Smc5/6 complex will enhance Mms21's E3 efficiency, whether DNA binding of the Smc5/6 complex will affect E3 activity, will be addressed.

Publications:

This work has resulted in two publications:

(1) Li, S.[#], Mutchler, A.[#], Zhu, X., So, S., Epps, J., Guan, D., Zhao, X.*, and Xue, X*. ***J Biol Chem***. 2022, 298(7): 102092.

(2) Li, S., Bonner, J. N., Wan, B., So, S., Mutchler, A., Gonzalez, L., Xue, X.*, and Zhao, X*. ***Genes Dev.*** 2021, 35(3-4): 261-272.