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SPOTLIGHT

A SUMOylation wave to anchor the genome

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Chromatin tethers to the nuclear envelope are lost during mitosis to facilitate chromosome segregation. How these connections are reestablished to ensure functional genome organization in interphase is unclear. Ptak et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202103036) identify a phosphorylation and SUMOylation-dependent cascade that links chromatin to the nuclear membrane during late mitosis.

Eukaryotic genomes are surrounded by a nuclear membrane that influences chromatin folding by providing an interaction surface (1). Transcriptionally silent heterochromatin preferentially associates with the inner nuclear membrane, while nuclear pore complexes tend to interact with transcriptionally active chromatin. Chromatin tethering to the nuclear envelope (NE) is linked to telomere length regulation, gene silencing, and DNA damage repair (2). However, in mitosis, chromatin-nuclear envelope contacts need to be broken to prevent chromosome segregation errors (3). How chromatin contacts are reestablished after mitosis remains elusive. In this issue, Ptak et al. (4) took advantage of budding yeast, where unlike in mammals, the nuclear membrane remains largely intact during mitosis (5), to address this question.

Small ubiquitin-related modifier (SUMO) is an ∼10-kD polypeptide that is post-translationally and reversibly attached to proteins (6). SUMO has been implicated in tethering chromatin to the NE (7). Using immunofluorescence and an antibody against yeast SUMO protein (Smt3), the authors found that SUMO conjugates are concentrated at the NE in mitosis but not interphase. Mitosis-specific SUMO conjugates were predominant in the 40–55 kD range on immunoblots, leading the authors to screen a panel of 141 mutants deleted for genes encoding known SUMOylated proteins of this molecular weight. Remarkably, the major mitotic SUMO band was missing in cells lacking Scs2 (scs2Δ) or expressing a version of Scs2 that cannot be SUMOylated ($scs2^{K180R}$). Scs2 is the yeast homologue of the highly conserved VAP (vesicle-associated membrane protein [VAMP]–associated protein) family of integral membrane proteins, which act as receptors for cytoplasmic proteins on the endoplasmic reticulum (8). Ptak et al. found that Scs2 also localizes to the inner nuclear membrane, where it functions as a protein receptor in the nucleus. At least three other proteins also were SUMOylated in mitosis, which was largely abolished in cells lacking Scs2 (*scs2Δ*) or expressing a version of Scs2 that cannot be SUMOylated ($Scs2^{K180R}$). Together, these observations suggested that SUMO conjugation to Scs2 triggers a wave of mitosis-specific SUMOylation of yet-unidentified targets.

Using yeast genetics, the authors identified that Siz2 was the SUMO E3 ligase responsible for the enrichment of Scs2-SUMO and mitotic SUMOylated species at the NE. Imaging of cells revealed that Siz2 itself relocates abruptly from the nucleoplasm to the NE in late mitosis as a consequence of its phosphorylation on serine 522. Thus, preventing Siz2 phosphorylation abolishes the wave of SUMOylation at the NE that is normally observed in mitosis. Therefore, they concluded that Siz2 phosphorylation triggers its relocalization to the NE, where it targets its substrates, including Scs2. However, the identity of the kinase targeting Siz2 and how Siz2 phosphorylation links to cell cycle regulation to induce the chromatin tethering to the NE in late mitosis remain open questions.

Ptak et al. found that Scs2 was not only a substrate of Siz2, but also its receptor in the NE. Scs2, in common with other VAP proteins, harbors a major sperm protein (MSP) motif that is known to bind FFAT (two phenylalanines in an acidic tract) motifs. The authors identified a FFAT-like motif in Siz2 and showed that it, and the reciprocal MSP motif on Scs2, were both required for Siz2–Scs2 interaction, nuclear membrane localization of Siz2, and the wave of mitotic SUMOylation at the NE. Therefore, Siz2 phosphorylation allows it to bind Scs2 via the FFAT–MSP interaction, resulting in Scs2 SUMOylation. Scs2-SUMO in turn, reinforces Siz2 binding through one of its two SUMO-interaction motifs (SIM domains).

Why is a SUMO E3 ligase recruited to the nuclear envelope? The researchers provided convincing evidence that one important role of Scs2- and Siz2-dependent SUMOylation is chromatin tethering. Telomere association with the nuclear periphery in late mitosis and G1 phase was reduced by preventing Siz2 phosphorylation, disrupting the FFAT–MSP interaction, or by abolishing the SUMO acceptor site on Scs2. Interestingly, chromatin tethering persisted into G1 while Scs2 SUMOylation and Siz2 phosphorylation did not. This suggested that while phosphorylation and SUMOylation control the establishment of chromatin tethering,

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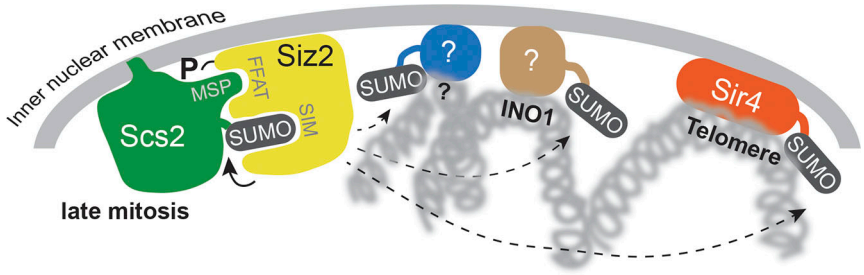

Figure 1. **Re-establishment of chromatin tethering to the nuclear envelope in late mitosis.** SUMO E3 ligase Siz2 is phosphorylated in mitosis, allowing its binding to VAP protein Scs2. The Scs2–Siz2 interaction involves the MSP motif on Scs2 and the FFAT motif on Siz2. Siz2 also SUMOylates Scs2, which stabilizes their interaction through SUMO–SIM binding. The resultant recruitment of Siz2 to the nuclear envelope leads to SUMOylation of multiple targets. This includes Sir4, SUMOylation of which promotes its association with telomeric chromatin. SUMOylation of an unknown target also leads to recruitment of the *INO1* gene to nuclear pores.

maintenance of the NE interactions is under distinct regulation. Telomere association with the NE in mitosis is known to require Sir4, which is itself SUMOylated (7). The authors found that although Siz2 association with the NE enhanced Sir4 SUMOylation, this was not important for its NE localization. Instead, Sir4 SUMOylation by NE-associated Siz2 promoted Sir4 association with telomeric chromatin. Consistently, Sir4 SUMOylation was important for NE tethering in G1 and mitosis but not in S phase.

Telomeres were not the only chromatin loci whose tethering depended on Scs2- and Siz2-mediated NE SUMOylation. The *INO1* gene relocates to the nuclear pore complex when activated in response to inositol starvation and this regulation had previously been linked to Scs2 SUMOylation (9, 10). *INO1* relocalization occurred in G1 and late mitosis, but not S phase, and required both Siz2 phosphorylation and Scs2 SUMOylation. Unlike telomeres, however, *INO1* relocalization did not require Sir4 SUMOylation. Hence, NE-directed SUMOylation uses distinct mechanisms to promote the association of telomeres with the NE and *INO1* with the nuclear pores. Which is the relevant SUMO target that directs *INO1* relocalization from the nucleoplasm to the nuclear pore complex is an intriguing question to be addressed in the future. Some of the other SUMO conjugates that the authors observed in mitosis might hold the solution to this part of the puzzle.

Overall, Ptak et al. showed that mitotic phosphorylation triggers a wave of SUMOylation at the NE to re-attach chromatin to the nuclear periphery in late mitosis (Fig. 1). Their work revealed a multilayered control centered on Scs2-Siz1 and involving phosphorylation, SUMOylation, FFAT-MSP, and SUMO–SIM interactions governing the reestablishment of NE-chromatin tethers during the cell cycle. The combinatorial mechanism of complex formation between Scs2-Siz2 is thought provoking for several reasons. First, FFAT–MSP interactions are used by VAP proteins for recruitment of cytoplasmic proteins to the ER, while here it is employed by nuclear proteins for recruitment to the inner nuclear membrane. Second, reinforcement by SUMO-SIM is a new concept in FFAT–MSP interactions. Third, cell cycle–specific phosphorylation licenses these interactions. Alongside the specific questions relating to the control of chromatin–NE tethering, this work raises the broader question of whether FFAT–MSP interactions have a more diverse role in protein targeting to membrane compartments and whether they are subject to similar regulatory cascades.

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