ABSTRACT :

Efficient chromosome segregation and the maintenance of genome stability during cell division require precise coordination between multiple molecular processes. In this study, we investigated the intricate relationships between the cohesin complex, PCNA regulation, and genome stability maintenance in *Saccharomyces cerevisiae*, with a particular focus on the essential roles of the cohesin subunit Pds5 and Elg1 proteins in these processes.

PCNA (proliferating cell nuclear antigen) is a highly conserved eukaryotic protein that forms a homotrimeric sliding clamp. PCNA encircles DNA and tethers DNA polymerases δ and ε to the replication fork, dramatically increasing their processivity during DNA synthesis. Besides replication, PCNA coordinates numerous DNA metabolic processes including Okazaki fragment maturation, DNA repair, and chromatin remodeling through its interactions with various cofactors and enzymes. PCNA undergoes post-translational modifications such as ubiquitination and SUMOylation that regulate DNA damage response and PCNA protein-interaction network in yeast cells. PCNA is loaded onto the chromatin by the RFC complex while it's unloading strictly depends upon the Elg1-RFC-like complex (RLC). The Elg1-RLC is crucial for timely unloading and recycling of PCNA during DNA replication. Besides its core function in DNA replication, Elg1 ensures efficient nucleosome assembly and sister chromatid cohesion during DNA replication.

Cohesin is a ring-shaped multi-subunit complex that physically tethers sister chromatids from the time of their synthesis until anaphase, a process known as sister chromatid cohesion (SCC). SCC is essential for proper chromosome segregation and chromosome alignment on the metaphase plate. In budding yeast, the cohesin complex consists of four core subunits: Smc1, Smc3, Mcd1 (also called Scc1), and Scc3, which together form a large proteinaceous ring that topologically entraps sister chromatids. The Mcd1 subunit additionally serves as a binding platform for accessory proteins Wpl1/Rad61, Scc2/4 and Pds5. While the HEAT-repeat containing protein Pds5 is required for proper sister chromatid cohesion, its exact molecular role remains unclear and continues to be investigated.

Elg1 shows complex genetic interactions with the different components of the sister chromatid cohesion (SCC) machinery. The deletion of *ELG1* can partially suppress the

temperature sensitivity and the cohesion defects of mutants of auxiliary cohesin factors such as *PDS5* and *ECO1* by unknown mechanisms.

In this study, we explored the genetic interactions between *ELG1* and *PDS5*. We observed that *pds5Δ elg1Δ* double mutants are inviable, and using a high copy number genetic screen, we found that the overexpression of Mcd1 can completely restore this mutant's viability. In a second genetic screen, we discovered that spontaneous mutations that inactivate the gene encoding the G1 cyclin Cln2 or mutations in the cohesin subunit Smc3 localized to the Mcd1-Smc3 interaction interface can also restore the viability of a *pds5Δ elg1Δ* double mutant. We find that the deletion of *CLN2* in *pds5Δ elg1Δ* restores Mcd1 protein levels by increasing the expression of the *MCD1* gene. The simultaneous deletion of *ELG1* and *CLN2* significantly alleviates the sister chromatid cohesion defects associated with the loss of Pds5, allowing the triple deletion to grow normally. We observed that the *ELG1* deletion suppresses defects in *PDS5* by accumulating SUMOylated-PCNA on the chromatin, and this, in turn, results in increased recruitment of the Srs2 DNA helicase. We propose that higher levels of Srs2 evict Rad51 from replication fork, resulting in the availability of ssDNA that might promote cohesin loading. Thus, a combination of *ELG1* and *CLN2* deletions promotes cohesin loading through independent mechanisms that eventually compensate for the loss of cohesion in the absence of Pds5.

In a follow-up study, we explored the mechanism by which the *SMC3* point mutations at the Smc3-Mcd1 interphase (SMI) restore the viability of *pds5Δ elg1Δ* strain. We confirmed that the suppressor points mutations in *SMC3* cohesin subunits can rescue the sister chromatid cohesion defects, cell inviability and reduced Mcd1 levels associated with the lack of Pds5. Interestingly, the suppressor mutations also rescue the lethality and cohesion defects associated with *smc3-RR* or with the lack of Eco1. We find that Pds5 is epistatic to the Eco1-dependent Smc3 acetylation pathway, promoting acetylation events that inhibit cohesin ATPase activity and ensure proper cohesion establishment. The identification of functionally relevant mutations within the conserved ABC-signature motif of *SMC3* provides new insights into the relationship between cohesin ATPase regulation and sister chromatid cohesion maintenance via Pds5 cohesin subunit. Thus, our comprehensive genetic screens and mechanistic analyses demonstrates that Pds5, a critical component of the cohesin complex, functions as a key regulator of sister chromatid cohesion through multiple

interconnected pathways. The research reveals that Pds5 serves a dual role: protecting the structural integrity of the cohesin ring while also facilitating cohesion establishment by promoting the replication-coupled Eco1 dependent Smc3-acetylation pathway.

In an additional study, we explored the role of Elg1 PCNA unloader in maintaining genome stability. Elg1 loss results in a variety of genome instability phenotypes such as sensitivity to DNA damage, defective heterochromatin silencing and elevated mutation rates. In this work we demonstrated that the genome stability functions attributed to Elg1 are directly correlated with its role in PCNA chromatin dynamics rather than with any additional protein function. Through systematic analysis of the *elg1* mutants we establish that proper PCNA unloading is essential for maintaining chromatin structure and preventing genomic instability, including DNA damage sensitivity, silencing defects, and elevated mutation rates. Collectively, this work advances our understanding of the molecular mechanisms involved in genome stability by revealing how cohesin regulation, PCNA dynamics, and replication fork progression are coordinated to ensure faithful chromosome segregation and maintain genomic integrity.