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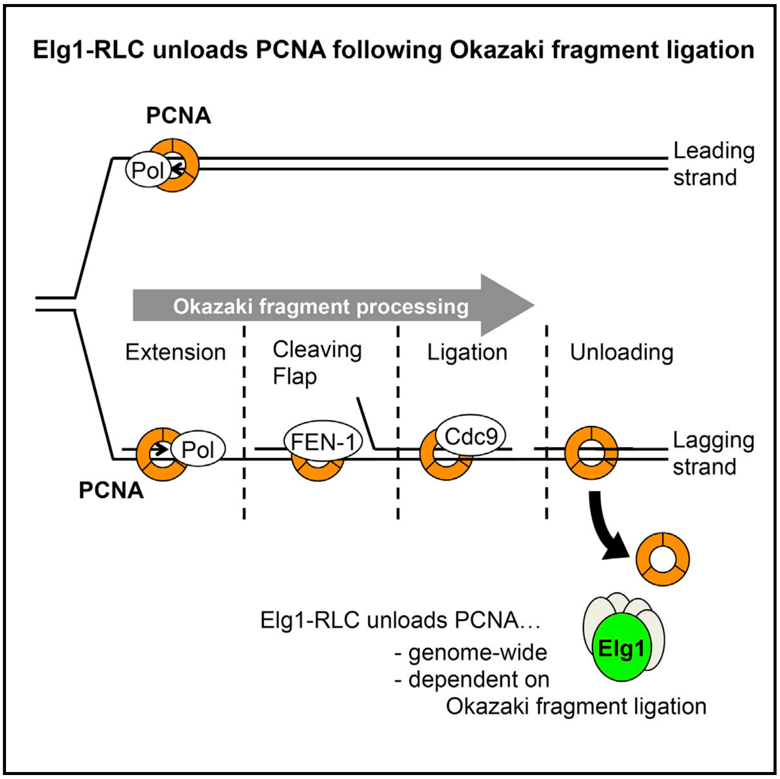
Replication-Coupled PCNA Unloading by the Elg1 Complex Occurs Genome-wide and Requires Okazaki Fragment Ligation

Graphical Abstract



Highlights

- PCNA is retained behind all replication forks that lack Elg1
- Elg1-RLC cannot unload PCNA from unligated lagging-strand DNA
- An exogenous ligase can join Okazaki fragments to promote PCNA unloading
- Elg1-RLC acts as a general PCNA unloader, dependent upon Okazaki fragment ligation

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In Brief

The sliding clamp PCNA is crucial to DNA replication fidelity, and loading and unloading of PCNA must be strictly regulated. Kubota et al. find that the Elg1 complex unloads PCNA behind all replication forks and is dependent upon successful DNA ligation of Okazaki fragments on the lagging strand.

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Replication-Coupled PCNA Unloading by the Elg1 Complex Occurs Genome-wide and Requires Okazaki Fragment Ligation

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SUMMARY

The sliding clamp PCNA is a crucial component of the DNA replication machinery. Timely PCNA loading and unloading are central for genome integrity and must be strictly coordinated with other DNA processing steps during replication. Here, we show that the *S. cerevisiae* Elg1 replication factor C-like complex (Elg1-RLC) unloads PCNA genome-wide following Okazaki fragment ligation. In the absence of Elg1, PCNA is retained on chromosomes in the wake of replication forks, rather than at specific sites. Degradation of the Okazaki fragment ligase Cdc9 leads to PCNA accumulation on chromatin, similar to the accumulation caused by lack of Elg1. We demonstrate that Okazaki fragment ligation is the critical prerequisite for PCNA unloading, since *Chlorella* virus DNA ligase can substitute for Cdc9 in yeast and simultaneously promotes PCNA unloading. Our results suggest that Elg1-RLC acts as a general PCNA unloader and is dependent upon DNA ligation during chromosome replication.

INTRODUCTION

Integrity of the DNA replication machinery is crucial to ensure accurate duplication of the genetic information and subsequent transfer to daughter cells. The ring-shaped homotrimeric protein PCNA (proliferating cell nuclear antigen) has a central role in DNA replication, coordinating the action of many replisome-associated proteins (Krishna et al., 1994). PCNA encircles DNA to act as a sliding clamp, ensuring processivity of DNA polymerases, and a platform for recruitment of numerous other replication proteins (Moldovan et al., 2007). Two important components whose recruitment is assisted by direct interaction with PCNA are the flap endonuclease FEN-1 and DNA ligase I (Beattie and Bell, 2011). Both these proteins are involved in the processing of Okazaki fragments, the series of short fragment precursors first synthesized and then ligated to assemble the nascent lagging strand. On the lagging strand, PCNA must be loaded on the DNA repeatedly, at the initiation of each Okazaki fragment. PCNA is loaded onto primer-template junctions by replication factor C (RFC), a hetero-pentameric complex consisting of one large subunit, Rfc1, and four smaller ones, Rfc2–5 (Bowman et al., 2004; Gomes and Burgers, 2001; Kelch et al., 2011). After completion of each Okazaki fragment, PCNA is believed to be unloaded from DNA and recycled to promote fidelity of synthesis of subsequent Okazaki fragments.

The Elg1 RFC-like complex (Elg1-RLC), in which Elg1 replaces Rfc1 to associate with Rfc2–5, acts in DNA replication (Kanellis et al., 2003). Previous results indicate one probable molecular function of *S. cerevisiae* Elg1-RLC is unloading of PCNA during DNA replication (Kubota et al., 2013a, 2013b). The function of the Elg1-RLC in PCNA unloading appears to be conserved in humans, since ATAD5 (the human Elg1 homolog) is required for proper removal of PCNA from chromatin in human cell lines (Lee et al., 2013; Shiomi and Nishitani, 2013).

When DNA synthesis is blocked, PCNA becomes mono-ubiquitinated at K164 to promote polymerase exchange, which enables DNA repair (Bienko et al., 2005; Hoege et al., 2002). In contrast, SUMOylation of PCNA (at K164 and K127) is stimulated simply by association with DNA and occurs during S phase even in the absence of exogenous damage (Hoege et al., 2002; Parker et al., 2008). One role for PCNA SUMOylation appears to be recruitment of the antirecombinogenic helicase Srs2 to prevent inappropriate recombination (Armstrong et al., 2012; Papouli et al., 2005; Pfander et al., 2005). Elg1-RLC preferentially binds SUMOylated PCNA, although SUMOylation of PCNA is not necessary for its unloading by Elg1-RLC (Kubota et al., 2013b; Parnas et al., 2010).

Loss of yeast Elg1 causes genome instability including gross chromosomal rearrangements, increased spontaneous sister chromatid recombination, defective sister chromatid cohesion, and derailed telomere length maintenance (Bellaoui et al., 2003; Ben-Aroya et al., 2003; Kanellis et al., 2003; Maradeo and Skibbens, 2009; Parnas et al., 2009; Smolikov et al., 2004). This requirement for Elg1 for genome maintenance seems to be conserved in higher eukaryotes, since mice with reduced expression of ATAD5 (the mammalian Elg1 ortholog) show genome instability and develop tumors (Bell et al., 2011). Elg1 is therefore crucial for genome maintenance.

Where and how the Elg1-RLC ensures timely unloading of PCNA from chromatin has until now remained obscure. In particular, the defective sister chromatid cohesion and derailed