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PCNA, the Maestro of the Replication Fork

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Inheritance requires genome duplication, reproduction of chromatin and its epigenetic information, mechanisms to ensure genome integrity, and faithful transmission of the information to progeny. Proliferating cell nuclear antigen (PCNA)—a cofactor of DNA polymerases that encircles DNA—orchestrates several of these functions by recruiting crucial players to the replication fork. Remarkably, many factors that are involved in replication-linked processes interact with a particular face of PCNA and through the same interaction domain, indicating that these interactions do not occur simultaneously during replication. Switching of PCNA partners may be triggered by affinity-driven competition, phosphorylation, proteolysis, and modification of PCNA by ubiquitin and SUMO.

Duplication of the genome occurs during the synthesis (S) phase of the eukaryotic cell cycle. Accompanying this crucial event are several other important processes, such as replication of chromatin modifications to maintain epigenetic information and maintenance of centromere and telomere structure. Moreover, sister chromatids, the products of replication, must be tethered together instantly after replication, as their alignment is required for faithful chromosome segregation. Although replication is normally highly accurate and proceeds in eukaryotes at about 2900 bases per minute, obstacles such as DNA lesions can lead to replication failure or even broken chromosomes, which endanger genome integrity and viability. Therefore, several safeguards are directly coupled to replication, which permit replication through problematic regions, repair DNA damage on site, or signal cell-cycle arrest through a checkpoint pathway. Here, we focus on how crucial S-phase functions are coupled to DNA replication. In particular, we highlight the role of PCNA as a conductor of replication-linked processes and discuss models for how these functions are orchestrated in harmony.

DNA Replication

The duplication of the genome is mediated by a dynamic protein complex called the replisome (Bell and Dutta, 2002; Johnson and O'Donnell, 2005). DNA replication starts at DNA elements termed origins of replication. In the budding yeast *Saccharomyces cerevisiae* these origins are short sequence-specific DNA elements, whereas in metazoa these origins of replication are much less defined at the sequence level. Replication initiation proceeds in two temporally distinct steps during the cell cycle. During G1 phase, in a process called origin licensing, a prereplicative complex (pre-RC) binds to DNA at origins of replication (Bell and Dutta, 2002; Diffley, 2004). This complex contains a six subunit ATPase called the origin recognition complex (ORC; Orc1-6 [we use the terminology for human proteins but refer to others if it is informative]). In addition, the hexameric Mcm2-7 complex is recruited to this site in a reaction that requires the licensing cofactor Cdt1 and the ATPase Cdc6.

The next step is activation of the origin through formation of a replication fork. This step is promoted by subsequent recruitment of additional factors (Mcm10, Cdc45, Dpb11, Sld2, Sld3, and the GINS complex in *S. cerevisiae*), and activation of S-phase cyclin-dependent kinases (CDKs) and the Cdc7-Dbf4 kinase (DDK), which both phosphorylate proteins of the replisome (e.g., Mcm proteins, Sld2, Sld3) and other targets. This reaction serves to assemble the replicative helicase—which may comprise the Mcm2-7 complex together with associated factors—and to recruit the DNA polymerases and other factors required for DNA synthesis. The helicase unwinds the DNA duplex, and the resulting single-stranded DNA is stabilized through binding of multiple copies of the heterotrimeric single-strand binding protein RPA, and a bidirectional replication fork is formed.

The two DNA strands are synthesized by different mechanisms. The leading strand can be replicated continuously through the 5′-to-3′-polymerase activity of DNA polymerases. The lagging strand, however, is replicated in a discontinuous fashion, each (Okazaki) fragment being smaller than the stretch unwound in the replication fork structure. The initial RNA primer for DNA synthesis is made by the primase enzyme, followed by a short stretch of DNA synthesized by polymerase α (Polα). Both enzymatic activities reside within a sin-

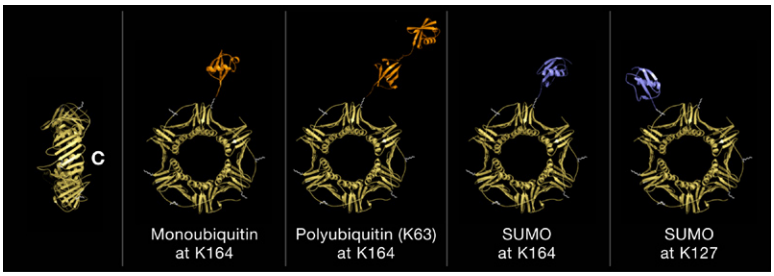

Figure 1. PCNA Modifications
Structure of yeast PCNA (yellow) (Krishna et al., 1994), shown in side and front view of the C-side (C). PCNA can be modified in several ways including monoubiquitylation, K63-linked polyubiquitylation, or SUMOylation at K164. In *S. cerevisiae*, SUMO also can be attached to K127, which is positioned in the interdomain connecting loop that connects two similar lobes of a PCNA monomer. The models are assembled from published structures of PCNA (Krishna et al., 1994), ubiquitin (Vijay-Kumar et al., 1987), and SUMO (*S. cerevisiae* Smt3) (Sheng and Liao, 2002). The orientation of the modifications is randomly chosen and might be different in vivo. The modification sites K164 and K127 are shown on all three subunits. Ubiquitin, red; SUMO, blue.

gle primase-Polα protein complex. Replication factor C (RFC) binds to the primer template junction and catalyzes the loading of the ring-shaped replication factor PCNA (Pol30 in *S. cerevisiae*) that encircles DNA. This leads to association with the replicative polymerases Polδ or Polε, which take over from Polα. PCNA enhances the processivity of these enzymes, which carry out the bulk of DNA synthesis. These polymerases contain 3′-to-5′-exonuclease (proofreading) activity, which strongly reduces stable misincorporation of nucleotides. In lagging strand synthesis, when the replicative polymerase reaches an end from a previous Okazaki fragment, it partially displaces this fragment by ongoing DNA synthesis, and a flap structure is generated. Through the activity of flap structure-specific endonuclease-1 (FEN-1, Rad27 in *S. cerevisiae*), this structure is cut out, and the resulting nick is sealed by DNA ligase I (Cdc9 in *S. cerevisiae*). As the coordination between the polymerase and FEN-1 is more efficient for Polδ than for Polε (and with additional arguments), the former might act on the lagging strand, whereas the latter might act on the leading strand (Garg et al., 2004). Topological problems caused by the replication forks (catenation and positive super-coiling ahead of the replication fork) are finally counteracted by the action of topoisomerases I and II.

PCNA and Its Mode of Action

PCNA belongs to the family of DNA sliding clamps (β clamps), which are structurally and functionally conserved. Although there is barely any sequence similarity between the β clamps in all branches of life, crystallographic studies have shown that they have almost superimposable three-dimensional structures (Krishna et al., 1994). They form ring-shaped complexes (homodimers in eubacteria, homotrimers in eukaryotes and T4 bacteriophage, heterotrimers in archaea) with a pseudohexameric symmetry, which encircle the DNA and are able to slide freely in both directions. PCNA monomers have two similar globular domains, linked by a long, possibly flexible loop, called interdomain connecting loop. Head-to-tail arrangement of three monomers form the ring (Figure 1), which has an inner positively charged surface formed by α helices, which associates with DNA, and an outer surface composed of β sheets.

PCNA is loaded around DNA by the conserved chaperone-like complex RFC (Majka and Burgers, 2004). RFC is an arc-shaped complex of five similarly structured essential proteins (AAA+ type ATPases) and associates with PCNA like a screw cap with a bottle. RFC specifically recognizes template-primer 3′ ends and loads PCNA to these sites. ATP binding is required for the formation of a stable PCNA-RFC complex and for its loading to primed DNA. DNA binding in turn activates the ATP hydrolysis activity of RFC, apparently leading to its dissociation from the loaded clamp (Gomes and Burgers, 2001). RFC binds to the so-called C side of PCNA (termed so because the C termini of the PCNA monomers protrude from this face; Figure 1) and loads it with this side positioned toward the 3′ end of the elongating DNA. This ensures that polymerases, which also bind to the C side of PCNA, are oriented toward the growing end. Orientation-dependent loading of PCNA by RFC additionally serves as a discriminator between the parental and the newly synthesized strand, which is important for example in mismatch repair (see later).

The PCNA ring, which encircles DNA, tethers polymerases firmly to DNA, making the sliding clamp an essential cofactor for DNA synthesis. Early in vitro studies have shown that the presence of PCNA increases the processivity of DNA polymerases from tens to thousands of nucleotides. Moreover, being devoid of enzymatic activity (in a strict sense), PCNA is ideally suited to function additionally as a moving platform for factors that act concomitantly with replication (Figure 2, Table S1, and references therein). This "matchmaker" activity is mediated largely by the C side of PCNA, where most interactors bind. In fact, a conserved motif termed PIP (PCNA-interacting protein) box, was found in Rfc1 (also known as RFC-A), Rfc3 (RFC-C), and most other PCNA-binding partners. The core element of the PIP box is a peptide with the sequence QxxΨ (Ψ being the hydrophobic residues L, M, or I), which in most cases is C-terminally flanked by the sequence xxϑϑ (ϑ being the aromatic residues F or Y) and in some cases N-terminally flanked by the sequence KAx (Xu et al., 2001). Structural studies have shown that the PIP-box peptide sequence is folded into a 3_{10} helix (a structure different from an α helix and a β sheet) that acts as a hydropho-