

# 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 licens-

ing, 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  $\alpha$  (Pol $\alpha$ ). 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-Pol $\alpha$  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 Pol $\delta$  or Pol $\epsilon$ , which take over from Pol $\alpha$ . 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 Pol $\delta$  than for Pol $\epsilon$  (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 ( $\beta$  clamps), which are structurally and functionally conserved. Although there is barely any sequence similarity between the  $\beta$  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  $\alpha$  helices, which associates with DNA, and an outer surface composed of  $\beta$  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 Qxx $\Psi$  ( $\Psi$  being the hydrophobic residues L, M, or I), which in most cases is C-terminally flanked by the sequence xx99 (9 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  $\alpha$  helix and a  $\beta$  sheet) that acts as a hydropho-

Activities	Proteins
DNA polymerases	Pol $\delta$ , Pol $\epsilon$ , Pol $\eta$ , Pol $\iota$ , Pol $\kappa$ , Pol $\zeta$ , Pol $\lambda$ , Pol $\beta$ , Rev1
Clamp loader	Rfc1, Rfc3, Rfc4
Flap-endonuclease	FEN-1
DNA ligase	DNA Ligase I
Topoisomerase	Topo II $\alpha$
Replication licensing factor	Cdt1
E3 ubiquitin ligases	Rad18, Rad5
E2 SUMO-conjugating enzyme	Ubc9
Helicases, ATPases	Srs2, Rrm3, Mgs1, WRN, RECQ5
Mismatch repair enzymes	Msh3, Msh6, Mlh1, EXO1
Base excision repair enzymes	UNG2, MPG, NTH1, hMYH, APE1, APE2, XRCC1
Nucleotide excision repair enzyme	XPG
Poly (ADP-ribose) polymerase	PARP-1
Histone chaperone	CAF-1
Chromatin remodeling factor	WSTF
Histone acetyltransferase	p300
Histone deacetyltransferase	HDAC1
DNA methyltransferase	DNMT1
Sister-chromatid cohesion factors	Eco1, Chl1, Ctf18
Protein kinases	CDK2, EGF Receptor
Cell-cycle regulators	p21, p57, Cyclin D1
Apoptotic factors	Gadd45, ING1b, p53

**Figure 2. PCNA-Binding Proteins**

PCNA interacts directly with a host of proteins involved in many different cellular processes. Listed are a selection of key PCNA-dependent activities and the corresponding PCNA-interacting proteins. Proteins in orange are known to contain PIP-box sequences, which bind to a groove in PCNA (orange) buried underneath the interdomain connecting loop. (See Table S1 for a full list of currently known PCNA-binding proteins and the sequences of their PIP boxes).

bic plug, docking into a hydrophobic pocket of PCNA buried under the interdomain connecting loop (Bruning and Shamoo, 2004; Gulbis et al., 1996). Some proteins that contain PIP boxes may use additional regions to interact with PCNA. Importantly, because proteins that contain PIP boxes bind to the same region in PCNA (the pocket, the interdomain connecting loop, and neighboring regions), binding of these factors to PCNA is often mutually exclusive and competitive. However, because PCNA is a homotrimer, it is theoretically possible that PCNA can bind to more than one PIP box-containing protein at the same time.

### Orchestrating Replication Events

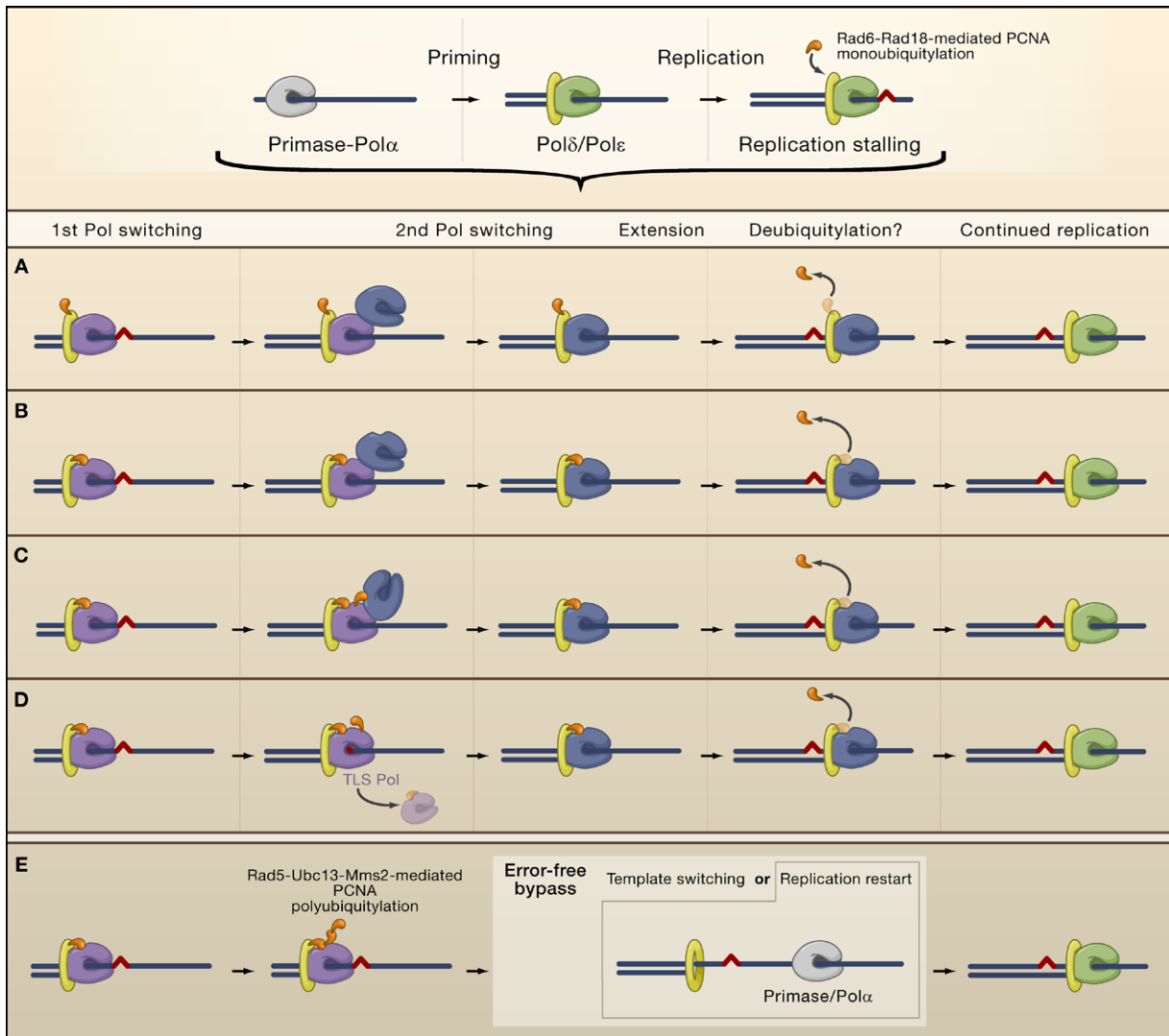
Binding of PCNA to the catalytic subunit of Pol $\delta$  takes place only when the complex is loaded onto DNA and might not involve PIP boxes (Garg and Burgers, 2005a). However, PIP boxes are found in the small (perhaps regulatory) subunits of Pol $\delta$  (p12 and p66 in humans and Pol32 in *S. cerevisiae*) and in the human catalytic p125 subunit and in two of the four subunits of Pol $\epsilon$ , including its catalytic subunit Pol2 (Figure 2 and Table S1).

The switch from the primase-Pol $\alpha$  complex to a replicative polymerase during replication initiation, both on the leading strand and at every Okazaki fragment on the lagging strand, requires the RFC-dependent loading of PCNA onto the primer terminus of the RNA-DNA hybrid (Garg and Burgers, 2005a). Both FEN-1 and DNA ligase I—which are part of the replication complex on the lagging strand—have PIP boxes required for their recruitment to PCNA, and although PCNA binding was shown

to stimulate FEN-1 activity, its effect on DNA Ligase I is unclear (Jonsson et al., 1998; Montecucco et al., 1998; Tom et al., 2001). Structural data, however, suggests that the catalytic reactions of both FEN-1 and DNA ligase I might be promoted by conformational changes induced upon DNA binding of the PCNA-bound enzymes (Chapados et al., 2004; Pascal et al., 2004). Therefore, it appears that during replication PCNA not only stimulates DNA polymerases but also crucially coordinates Okazaki fragment processing and joining, apparently in a stepwise reaction.

### Prevention of Rereplication

At each replication origin, replication must initiate only once in the cell cycle to prevent aneuploidy and genomic instability. To prevent rereplication (“origin refiring”), licensing and replication initiation are temporally separated during the cell cycle (Diffley, 2004). In metazoans, licensing is downregulated in S phase by several measures, including ORC inactivation, degradation of Cdc6, and Cdt1 inhibition by binding to the protein geminin. Notably, another key protective mechanism against origin refiring is the S-phase-specific ubiquitin-dependent degradation of Cdt1 on chromatin. Recent studies in *Xenopus* egg extracts have shown that the coupling of Cdt1 destruction to DNA replication is achieved by a direct interaction between Cdt1 and PCNA (Arias and Walter, 2006). Cdt1 contains a PIP box, and defects in PCNA-Cdt1 binding result in a loss of Cdt1 ubiquitylation, Cdt1 stabilization, and rereplication. As PCNA appears to remain on DNA after DNA synthesis is com-



### Figure 3. Polymerase Switching and Bypass Replication

The primase-Pol $\alpha$  complex (gray) catalyzes a short RNA-DNA primer to which PCNA (yellow circle) is loaded by RFC. RFC recruits the replicative polymerases Pol $\delta$  and Pol $\epsilon$  (green), which catalyze the bulk of DNA synthesis in S phase. DNA lesions that lead to replication stalling (red) induce Rad6-Rad18-mediated ubiquitylation of PCNA at lysine-164 (orange). Several scenarios—both error-prone (A–D) or error-free (E)—are possible for bypass replication.

(A) Monoubiquitylation of PCNA displaces the blocked Pol $\delta$  and Pol $\epsilon$  and allows translesion synthesis (TLS) polymerases (blue and purple) to bind (1st Pol switching). The optimal TLS polymerase to replicate a specific lesion is selected by a trial-and-error mechanism. TLS sometimes takes place in two steps, in which one TLS polymerase (purple) replicates across the lesion, and a secondary TLS polymerase (blue) continues for a short while (2nd Pol switching; extension) until the replicative polymerases Pol $\delta$  and Pol $\epsilon$  are able to take over. Whether or when deubiquitylation takes place is not known.

(B) Alternatively, monoubiquitylated PCNA specifically recruits TLS polymerases via ubiquitin-binding sites in these enzymes.

(C) In a variation of (B), binding of the first TLS polymerase triggers its monoubiquitylation, which in turn recruits secondary TLS polymerases.

(D) Alternatively, monoubiquitylation of the first TLS polymerase only occurs if the polymerase is unable to replicate across the lesion. This results in intramolecular binding of its ubiquitin modification to its ubiquitin binding domain, thereby releasing the unsuccessful polymerase from PCNA.

(E) The error-free mode might occur after recruitment of a TLS polymerase that is unable to replicate across the lesion. Prolonged stalling might trigger Rad5-Ubc13-Mms2-dependent PCNA (K-63-linked) polyubiquitylation. How the error-free path operates is unknown, but it might involve template switching, and/or the polyubiquitin modification might displace PCNA from the polymerase, and the detached PCNA molecule might label the site for later repair. Replication will continue beyond the lesion by a fresh restart involving the primase-Pol $\alpha$  complex.

pleted, it has been proposed that accumulated PCNA on DNA might recruit free Cdt1, promoting its ubiquitylation by the DDB1-Cul4 ubiquitin ligase (Arias and Walter, 2006). In this model, the bulk of nuclear Cdt1 is ubiquitylated by this mechanism, thereby clearing the

nucleus from Cdt1-mediated licensing activity. How PCNA promotes Cdt1 ubiquitylation is unknown, but it is conceivable that PCNA might stimulate the ubiquitin ligase, or it might turn Cdt1 into a substrate for ubiquitylation upon binding.



### Bypass Replication

DNA lesions that are not repaired prior to S phase are dangerous obstacles for the replication machinery, as most lesions cannot be accommodated into the active sites of replicative DNA polymerases, thereby blocking progression of the replication fork. Prolonged stalling of replication forks can lead to a collapse of the replication machinery, which might result in double-strand breaks and gross chromosomal rearrangements, or even to a permanent cell-cycle arrest and cell death. To steer clear of this potential crisis, cells have evolved bypass mechanisms that handle stalled replication forks (Barbour and Xiao, 2003). One important means is the switch from accurate replicative polymerases (i.e., Pol $\delta$  or Pol $\epsilon$ ) to so-called translesion polymerases that can read through lesions as they can accommodate even bulky lesions at their active sites. These polymerases incorporate either a correct or an incorrect nucleotide opposite to the lesion, but a downside of this “sloppiness” is that translesion synthesis (TLS) is intrinsically error prone. Yet, also an error-free bypass mechanism exists, which uses the undamaged information of the sister duplex perhaps by template switching. Unfortunately, the mechanism of this bypass is largely unknown, but it might share similarities with homologous recombination.

How bypass replication is activated had long been an enigma. However, recent studies in yeast have revealed that both modes of bypass replication are directly controlled through covalent modifications of PCNA by ubiquitin (Hoege et al., 2002). Notably, ubiquitylation of PCNA is carried out by enzymes encoded by the *RAD6* group of genes that function in “postreplicative DNA repair” (Barbour and Xiao, 2003). This often-used term, however, is a misnomer, as the *RAD6* pathway allows stable replication without removing the damage (thus formally not a DNA-repair function). Remarkably, half of the known genes of the *RAD6* group are enzymes of the ubiquitin system, whereas the remaining others are mainly TLS polymerases (Hoege et al., 2002; Jentsch et al., 1987; Ulrich and Jentsch, 2000).

In response to DNA damage (or stalled replication forks), PCNA is ubiquitylated at the conserved lysine (K) residue 164. It can either be modified by a single ubiquitin moiety (monoubiquitylation) or by a polyubiquitin chain, in which the ubiquitin moieties are linked via K63 of ubiquitin (Figure 1). This noncanonical polyubiquitin chain does not normally promote proteasomal degradation but often affects cell signaling. PCNA monoubiquitylation requires the ubiquitin-activating enzyme E1; the Rad6 protein, which is an E2 ubiquitin-conjugating enzyme; and Rad18, a RING-finger-containing E3 ubiquitin ligase of the *RAD6* group (Hoege et al., 2002). Rad18 not only binds Rad6 and PCNA but also DNA, thereby possibly recruiting the ubiquitylation machinery to the chromatin-bound target. Polyubiquitylation of PCNA at K164, in addition to Rad6 and Rad18, requires another RING-finger ubiquitin ligase, Rad5, which in turn recruits the proteins Ubc13 and Mms2 (Ulrich and

Jentsch, 2000). Rad5 is also a DNA-binding protein and additionally a member of the SWI/SNF family, whereas Ubc13 and Mms2 form a heterodimeric E2 enzyme that catalyzes specifically K63-linked polyubiquitin chains. Whether PCNA monoubiquitylation always comes before PCNA polyubiquitylation is currently not clear. Importantly, however, monoubiquitylation of PCNA triggers the error-prone pathway through TLS, whereas PCNA polyubiquitylation is needed for the error-free mode of the bypass (Figure 3). Therefore, this “ubiquitin-PCNA switch” (Hoege et al., 2002) not only allows the replication fork to pass lesions but also determines whether bypass replication operates in the error-prone or error-free mode. Yeast genetic studies indicated that monoubiquitylated PCNA is linked to the TLS polymerases Pol $\eta$  (Rad30) and Pol $\zeta$  (Rev3/Rev7) (Stelter and Ulrich, 2003), and indeed human Pol $\eta$  was found to interact specifically with monoubiquitylated PCNA (Kannouche et al., 2004; Watanabe et al., 2004) (Figure 3B). Ubiquitin-binding domains were found in Y-family TLS polymerases (Pol $\eta$ , Pol $\iota$ , Rev1) (Bienko et al., 2005), and these domains in addition to their PCNA-binding motifs (PIP boxes of Pol $\eta$  and Pol $\iota$  but a BRCT domain in Rev1; Table S1) are required for recruiting them specifically to monoubiquitylated PCNA at stalled replication forks. Moreover, in vitro assays of DNA replication across abasic sites showed that monoubiquitylated PCNA is able to activate Pol $\eta$  (Rad30 in yeast) and Rev1 (Garg and Burgers, 2005b). Alternatively, monoubiquitylation may prevent binding of replication factors to PCNA (Haracska et al., 2006), whereas TLS polymerases might tolerate the modification (Figure 3A). Intriguingly, the TLS polymerases Pol $\eta$ , Pol $\iota$ , and Rev1 are themselves substrates for monoubiquitylation (Bienko et al., 2005; Guo et al., 2006). It has been proposed that these modifications might facilitate a sequential action of different TLS polymerases, which is often characteristic for TLS. In this model (Figure 3C), monoubiquitylated PCNA recruits the first TLS polymerase, which inserts the nucleotide across the lesion, whereas subsequent monoubiquitylation of this recruited enzyme will attract specific TLS polymerases that extend a few additional nucleotides past the lesion (Friedberg et al., 2005; Guo et al., 2006). Alternatively, as attachment of ubiquitin to Pol $\eta$  and Pol $\iota$  appears to block their ubiquitin-interaction domain (Bienko et al., 2005), PCNA-triggered monoubiquitylation of the TLS polymerase might also prevent the enzyme from rebinding monoubiquitylated PCNA if it failed to replicate across the lesion (Figure 3D). Such a mechanism would facilitate the productive pairing of PCNA with specifically those TLS polymerases that are optimal for a particular lesion.

Intriguingly, the induction of TLS by PCNA monoubiquitylation is not only used for bypass replication but also for immunoglobulin gene hypermutation (Arakawa et al., 2006). This indicates that this error-prone replication safeguard mechanism has been borrowed in evolution for setting desired mutations in a locus-specific manner.

How polyubiquitylated PCNA might activate the error-free pathway remains speculative (Figure 3E). One possibility is that PCNA polyubiquitylation induces template switching, perhaps by promoting a controlled reversion of the replication fork (creating a “chicken foot” structure), which would expose the newly synthesized undamaged DNA duplex for recombination-like processes. Alternatively, it might detach PCNA from the polymerase and hence the moving replication fork and label the site of lesion for later repair (Figure 3E). Perhaps in line with the latter idea, it has been shown that certain DNA lesions can uncouple leading and lagging strand synthesis with the result that single-stranded gaps are formed behind the replication fork, which are filled by TLS or recombination (Lopes et al., 2006). An interesting feature of this model is that the ubiquitin modification will not affect normal replication as new PCNA molecules will be loaded 3' of the lesion.

Not mutually exclusive to these models is the possibility that the polyubiquitin chain on PCNA might recruit specific factors, or it might inhibit TLS. Indeed, studies suggest that mammalian Pol $\delta$  and Pol $\eta$  are able to bind not only monoubiquitin but also polyubiquitin chains, which led to the idea that perhaps TLS polymerases are removed from PCNA (thereby preventing TLS) if they recognize a polyubiquitin modification on PCNA (Plosky et al., 2006). Importantly, one way to keep TLS in check is apparently mediated by the human deubiquitylation enzyme USP1, which is able to remove monoubiquitin from PCNA (Huang et al., 2006). However, whether USP1 also removes the polyubiquitin chain from PCNA, and whether this enzyme is crucial for the error-free bypass, has not been addressed. Whatever the mechanism, it is of note that both mono- and polyubiquitylation of PCNA and the responsible ubiquitylation enzymes are conserved from yeast to mammals, suggesting that the functional consequences of the two modifications might be conserved as well (Unk et al., 2006).

### Prevention of Sister-Chromatid Recombination

Even in the absence of exogenously applied DNA damage, a fraction of PCNA at steady state in S phase (at least in *S. cerevisiae*, *Xenopus*, and chicken) is also modified by the ubiquitin-related modifier SUMO (Arakawa et al., 2006; Hoege et al., 2002; Leach and Michael, 2005). Notably, SUMOylation targets the same residue K164 of PCNA, indicating that ubiquitin and SUMO are alternative modifications of this site (Figure 1). PCNA of *S. cerevisiae* is additionally SUMOylated at residue K127, which is located within the interdomain connecting loop (see later). It has been proposed that SUMOylation of PCNA is equivalent to PCNA monoubiquitylation for promoting TLS, albeit for specific damaged sites (Stelter and Ulrich, 2003). However, genetic studies indicated that PCNA SUMOylation is inhibitory for DNA repair if the RAD6 bypass pathway is not functional (Hoege et al., 2002; Pfander et al., 2005). Indeed, SUMOylation of PCNA in yeast (at either site) recruits the helicase Srs2, which is

an antirecombinogenic enzyme, as it disrupts Rad51 nucleoprotein filaments that are crucial for recombination (Papouli et al., 2005; Pfander et al., 2005). Apparently, RAD52-dependent recombination between sister chromatids represents a salvage pathway for stalled replication forks, which is, however, normally kept in check by PCNA SUMOylation and Srs2 recruitment (Pfander et al., 2005). In fact, employment of recombination for reviving replication forks seems disadvantageous for cells, as it can lead to deleterious gross chromosomal rearrangements (Lambert et al., 2005). The PCNA-SUMO-Srs2 check thus appears to function as a guardian, preventing unwanted sister-chromatid recombination during replication and thereby facilitating the use of the RAD6 ubiquitin-dependent bypasses. Although Srs2 is not present in vertebrate cells, PCNA SUMOylation is apparently conserved across species (Arakawa et al., 2006), suggesting that perhaps different antirecombinogenic activities are recruited to SUMOylated PCNA in higher eukaryotes.

### Mismatch Repair

Mismatch repair (MMR) (Jiricny, 2006) corrects base-base mismatches and small insertion/deletion loops generated during faulty replication. It acts by removing the error-containing portion of the newly synthesized DNA strand and the targeting of the DNA-synthesis machinery to the freshly formed single-stranded gap. The major MMR sensor complex, MutS $\alpha$  (the heterodimeric MSH2-MSH6 complex), recognizes base-base mismatches and small insertion/deletion loops, whereas the less frequently used MutS $\beta$  complex (MSH2-MSH3) recognizes insertion/deletion loops of larger sizes. Other MMR components are the transducers MutL $\alpha$  (MLH1-PMS2) and related complexes, exonucleases like EXO1, which remove the flawed strand, single-strand binding-protein RPA, the nonhistone chromatin protein HMGB1 (in higher eukaryotes), and finally the DNA-synthesis machinery, which fills the gap.

An unexpected finding was that PCNA is not only required for repair DNA synthesis but also for early events of MMR (Umar et al., 1996). Indeed, PCNA interacts directly with MSH6, MSH3, MLH1, and EXO1, and at least MSH6, MSH3, and MLH1 possess PIP boxes (Table S1). Most importantly, MMR is strand specific; i.e., it selectively repairs the newly synthesized strand, which contains the error produced by faulty replication. It seems that this strand is identified by the MMR machinery by the presence of a nick or gap (e.g., the end of an Okazaki fragment), and probably also PCNA loaded with its C side oriented toward the growing end of the new DNA molecule. Starting from this nick or gap, and perhaps also from other nicks actively introduced by the MMR machinery, excision of the defective strand occurs (Modrich, 2006). The default direction of excision is apparently in 5'-to-3' direction, and EXO1 seems to be the major enzyme that carries out this reaction. MMR is more efficient on the lagging strand, probably because

nicks and gaps 5' from the detected mismatch (required for 5'-to-3' excision) are inherent to this discontinuous strand. How nicks 5' from the error are introduced into the leading strand had long been an enigma. However, recent studies indicate that PCNA, RFC, and EXO1 are required to activate a cryptic endonucleolytic activity of the mammalian mediator PMS2, which creates a nick 5' of the mispair, allowing EXO1 to act in its usual 5'-to-3' polarity (Kadyrov et al., 2006).

Still enigmatic is how the two landmarks of MMR, the mismatch and the 5'-positioned nick or gap, which can be several hundred nucleotides apart, communicate with each other. One possibility is that the MMR complex (MutS $\alpha$  perhaps in association with MutL $\alpha$ ) migrates (perhaps actively) from the mismatch in 3'-to-5' direction along the defective strand toward the 5'-located nick, where it productively encounters PCNA (Jiricny, 2006). This might lead to a recruitment of EXO1 to PCNA, which will then conduct strand excision, followed by repair DNA synthesis that requires PCNA. Alternatively (resembling events of transcription initiation), proteins at the mismatch and the nick might directly communicate through protein-protein interactions, thereby inducing a DNA loop. At any rate, the apparently mutually exclusive PCNA binding of sensors, transducers, and effectors (EXO1 and polymerases) suggests a stepwise recruitment of these factors to PCNA to allow MMR to function in an ordered pathway (Lee and Alani, 2006).

### Base Excision Repair

Base excision repair (BER) (Sancar et al., 2004) repairs chemical alterations of the nucleotide bases in DNA, including alkylated, oxidized, reduced, or deaminated bases, as well as misincorporated uracils. BER is initiated by the recognition of such damaged bases by specific DNA glycosylases, which remove the bases from the nucleotides, forming abasic sites. The phosphodiester bond 5' of the abasic site is then cleaved by apurinic/apyrimidinic (AP) endonucleases, and BER continues in two different ways. In "short-patch repair," Pol $\beta$  can fill the single nucleotide gap, and the nick is sealed by the XRCC1-DNA ligase 3 complex. In "long-patch repair," the RFC-PCNA-Pol $\delta$ /Pol $\epsilon$  complex is recruited to perform repair DNA synthesis, and the process is completed analogous to lagging strand synthesis, involving FEN-1 and DNA ligase I (and sometimes the PCNA-related 9-1-1 complex; see later).

In a manner reminiscent of MMR, PCNA appears to be involved in BER well before the step of repair DNA synthesis takes place. Also here factors that function early (glycosylases) or later (AP endonuclease) interact with PCNA, suggesting a PCNA-guided ordered reaction. Uracil-DNA glycosylase 2 (UNG2) contains a PIP box, whereas DNA glycosylase (MPG) contains an "inverted" PIP box (YFCMNISSQ) (Ko and Bennett, 2005; Otterlei et al., 1999; Xia et al., 2005). NTH1 (endonuclease III homolog), another DNA glycosylase, also binds PCNA, but not through a PIP box (Oyama et al., 2004). Importantly,

PCNA not only colocalizes with these enzymes in foci but also stimulates their enzymatic activities. Likewise, PCNA also binds and stimulates AP endonucleases (e.g., human APE2, *S. cerevisiae* Apn2) involving PIP boxes of these enzymes and colocalizes in foci (Tsuchimoto et al., 2001; Unk et al., 2002). Lastly, PCNA also recruits the repair cofactor XRCC1 to replication foci. Although no PIP box had been found in XRCC1, it binds PCNA and thereby possibly couples repair to replication (Fan et al., 2004).

### Nucleotide Excision Repair

Nucleotide excision repair (NER) (Sancar et al., 2004) involves PCNA as well. NER removes bulky DNA lesions generated by exposure to radiation or chemicals. Lesion detection is conducted by the XP (Xeroderma pigmentosum) protein XPC (Rad4 in *S. cerevisiae*). Subsequently, XPD (also known as ERCC2 in human and Rad3 in *S. cerevisiae*) and the XPB (ERCC3) helicases of the TFIIH transcription DNA-repair factor complex open up the DNA around the lesion, and the endonucleases XPG (ERCC5; Rad2 in *S. cerevisiae*) and XPF (ERCC1) cut the strand approximately 6 nucleotides 3' and 20 nucleotides 5' to the damage, respectively. The single-stranded gap is finally closed by DNA synthesis and ligation. Notably, a PIP box was identified in the C terminus of the nuclease XPG, and this domain is required for PCNA binding and NER activity in cells (Gary et al., 1997). How the PCNA-XPG interaction promotes NER remains unclear, but analogous to the cases discussed before, it seems likely that it coordinates DNA excision and synthesis.

### Chromatin Assembly

DNA is typically packaged in chromatin fibers (Polo and Almouzni, 2006). The basic unit of chromatin is the nucleosome, consisting of 146 base pairs of DNA wrapped around an octameric protein core, containing two molecules of each of the histones H2A, H2B, H3, and H4. Higher-order chromatin structures are formed by further deposition of structural (e.g., histone H1) and regulatory proteins to nucleosome arrays. During replication, the approaching replication fork apparently triggers a local destabilization of the nucleosomes in front of the replication fork and a disassembly of higher-order chromatin structure. However, once the replisome has passed, chromatin is quickly reassembled on the two daughter strands. Although the precise mechanism is currently unknown, the histones of the parental nucleosomes are apparently transferred directly (at least H3-H4 dimers or tetramers) on the two daughter strands during replication. To arrive at the original number of nucleosomes per DNA, and to preserve the chromatin state, half of the nucleosomes of the daughter strands must be formed de novo from histones synthesized during S phase. Histone chaperones are targeted to sites of chromatin assembly, where they discharge their histone load on DNA in a highly regulated process, starting by dep-

osition of histones H3 and H4 as a tetramer (or two dimers), followed by addition of two H2A-H2B dimers (Polo and Almouzni, 2006).

Nucleosome assembly and disassembly is conducted by partially redundant pathways, involving the conserved histone chaperones CAF-1 or the HIR complex (Hir1-3, Hpc2) and their common cofactor Asf1 (Mousson et al., 2006). The heterotrimeric complex CAF-1 appears to deliver histones H3 and H4 to replicating DNA during S phase but also in chromosomal replication-independent chromatin assembly (Smith and Stillman, 1989). Targeting of CAF-1 to sites of DNA synthesis requires its direct interaction with PCNA via a PIP box of its largest subunit Cac1, thereby physically linking histone deposition activity to the replication fork (Krawitz et al., 2002; Zhang et al., 2000). Indeed, in yeast PCNA mutants defective in this interaction, CAF-1 is not located properly on chromatin, and these cells exhibit gene-silencing defects (Zhang et al., 2000). PCNA-dependent recruitment of CAF-1 was also observed at sites of NER, where it might re-establish chromatin after the repair process is finished (Gaillard et al., 1996). Moreover, as indicated by genetic interactions, CAF-1-dependent chromatin assembly seems crucial for bypass replication by the *RAD6* pathway as well (Game and Kaufman, 1999), indicating that PCNA-CAF-1-dependent chromatin assembly is perhaps generally important for events that require DNA synthesis.

Asf1 binds to the histones H3 and H4 and cooperates with CAF-1 to deposit histones on newly replicated DNA (Tyler et al., 1999) and with the HIR complex for chromosomal replication-independent chromatin assembly (Green et al., 2005). Although the HIR complex does not appear to bind PCNA directly, mutations in yeast PCNA impaired the contribution of Asf1/Hir to silencing (Sharp et al., 2001), pointing to a possible role of PCNA in the HIR-dependent pathway as well. Another histone chaperone from *S. cerevisiae*, Rtt106, also binds Cac1 and shows genetic links to PCNA, emphasizing again the importance of PCNA for nucleosome assembly pathways (Huang et al., 2005).

### Epigenetic Inheritance and Chromatin Remodeling

During cell division, not only the DNA sequence but also the gene-expression pattern must be transmitted to the progeny. This epigenetic information is encoded by covalent modifications of DNA and histones ("histone code"), which during replication must be copied with high fidelity from the parental chromosome to the sister chromatids. This is achieved by coupling the activities required for the maintenance of epigenetic patterns directly to the replication machinery.

One epigenetic mechanism controlled by PCNA is the methylation at the C5 position of cytosine residues, predominantly found in CpG sequences (Bird, 2002). Cytosine methylation at promoter regions is associated with gene silencing, acting either through masking the binding sites for transcription factors, or by recruiting methyl-

CpG binding proteins like MeCP2 or MBD1, which function as transcription corepressors. Responsible for the preservation of the methylation pattern during DNA replication is the enzyme DNA cytosine methyltransferase 1 (DNMT1, also known as MCMT) (Hermann et al., 2004), which can methylate cytosines at hemimethylated CpG sequences resulting from semiconservative DNA replication. Owing to a PIP box within its N-terminal region, DNMT1 is targeted to replication foci via PCNA, which stimulates its enzymatic activity (Chuang et al., 1997; Iida et al., 2002). Notably, DNMT1 is also recruited by PCNA to sites of DNA repair, presumably to restore the DNA methylation status after repair DNA synthesis took place (Mortusewicz et al., 2005).

A key epigenetic marker is methylation of the histone H3 at the K9 residue of its N-terminal tail (abbreviated H3K9), which in turn recruits the heterochromatin-associated protein HP1 to set up a repressed (heterochromatic) state (Hediger and Gasser, 2006). Notably, the transmission of the H3K9 methylation pattern to daughter cells is directly coupled to replication through the recruitment of DNA methyltransferases to PCNA. Remarkably, in the case of the mammalian histone methyltransferase G9a, this coupling is achieved through complex formation with the DNA methyltransferase DNMT1, which binds PCNA via its PIP box (Esteve et al., 2006). In a variation of this theme, another H3K9 methyltransferase, SETDB1, binds to methyl-CpG binding protein MBD1 at methylated (repressed) promoters. During replication of these loci, the MBD1-SETDB1 complex from the parental strand is recruited by PCNA-bound CAF-1 to the replication fork, where SETDB1 methylates histone H3 deposited by CAF-1 (Sarraf and Stancheva, 2004). This intriguing mechanism therefore provides the means to copy the repressed state of chromatin directly from the sites before the replication fork onto the two newly formed sister strands involving PCNA of the passing replisome.

Main regulators of the chromatin state are histone acetyltransferases (HATs) and histone deacetylation enzymes (HDACs), and some of these enzymes also are linked to PCNA. HDAC1, which plays an important role in gene silencing, was shown to interact with PCNA in vitro and colocalizes in foci in cells (Milutinovic et al., 2002). Furthermore, the mammalian transcriptional coactivator p300, a protein with HAT activity, forms a complex with PCNA. PCNA-p300 association does not depend on S phase but occurs preferentially at sites of DNA damage (Hasan et al., 2001). Therefore, it has been proposed that PCNA recruits p300 to sites of damage, where p300's HAT activity induces chromatin structures suitable for repair.

As a matter of fact, coupling of regulators of chromatin organization with PCNA, either directly or indirectly through PCNA binding factors, appears to be a more general principle. Additional examples are the human WSTF-SNF2H chromatin-remodeling complex, which binds PCNA through four PIP boxes (Poot et al., 2004),



and human heterochromatin protein HP1, which is recruited to the replication fork possibly through PCNA-bound CAF-1 (Murzina et al., 1999).

### Sister-Chromatid Cohesion

Faithful inheritance not only requires flawless replication but also the accurate distribution of the genetic information to the two daughter cells. Crucial for correct segregation of the chromosomes is that the sister chromatids, the products of replication, are kept together throughout the G2 phase of the cell cycle until anaphase, when their correct alignment is a prerequisite for segregating homologous chromosomes into the daughter cells. This physical coupling, known as cohesion, is mediated by a proteinaceous ring, termed cohesin, which is composed of the four subunits Smc1, Smc3, Scc1, and Scc3 (Nasmyth and Haering, 2005). It has been suggested that the cohesin ring encircles the two sister chromatids, but other ideas have been entertained as well. Before anaphase, the cohesion ring is enzymatically dissolved, thereby allowing the forces of the spindle to pull the sister chromosomes to the two daughter cells.

Sister-chromatid cohesion is already established in S phase, apparently in order to tie up the sister chromatids instantly after they have been formed by replication (Uhlmann and Nasmyth, 1998). An essential factor for cohesion establishment in yeast is Eco1 (also known by Ctf7), a protein with acetyltransferase activity, for which its crucial substrate is not known however. Notably, Eco1 and apparently all members of this family (including the human proteins ESCO1 and ESCO2) possess a PIP box, but only its core element (QxxY), lacking the hydrophobic flank characteristic for most other PIP boxes (Moldovan et al., 2006). Yeast Eco1 binds directly to PCNA via its PIP box, and this interaction is essential for normal loading of Eco1 on chromatin, establishment of cohesion in S phase, and thus viability. Because also human PCNA binds ESCO2 (Moldovan et al., 2006) (which is deficient in an inherited disease known as Roberts syndrome), it is highly likely that the PCNA-Eco1 pathway for coupling establishment of cohesion with replication is conserved across species.

### Cell-Cycle Control and Survival

In vertebrate cells, PCNA is crucially regulated by the tumor suppressor protein p21 (WAF1) (Dotto, 2000), which was initially identified as a potent inhibitor of cell-division cycle kinases (CDKs). PCNA binding is mediated by a PIP box located in p21's C-terminal tail (Waga et al., 1994; Warbrick et al., 1995). Binding of p21 to PCNA inhibits replication *in vitro* and *in vivo* by blocking activity of PCNA to stimulate polymerases (Rousseau et al., 1999; Waga et al., 1994). Structural data indicated that p21 directly blocks the surface required for polymerase binding (Gulbis et al., 1996), and biochemical studies broadened this idea by showing that p21 is an effective competitor of many PIP-box proteins. Proteins that are barred *in vitro* from PCNA

binding by p21 include the p66 subunit of Pol $\delta$ , FEN-1 (Ducoux et al., 2001; Warbrick et al., 1997), the licensing cofactor Cdt1 (Arias and Walter, 2006), the chromatin-remodeling factor WSTF (Poot et al., 2004), the DNA methyltransferase DNMT1 (MCMT) (Chuang et al., 1997), and the repair protein XPG (Gary et al., 1997). Moreover, *in vitro* assays showed that p21 also functionally inhibits MMR (Umar et al., 1996), and the ATPase activity of RFC (Oku et al., 1998), whereas the effect on BER is unclear.

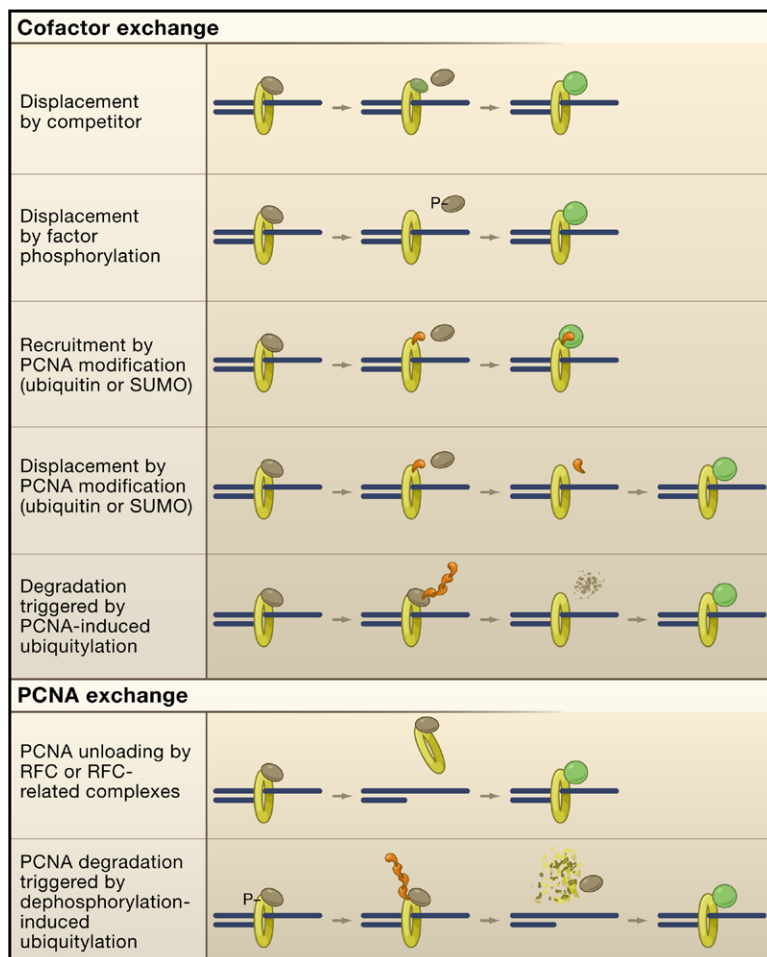
On the other hand, PCNA is also found associated with CDK-cyclin complexes (Zhang et al., 1993). Therefore, CDK activity might locally be coupled to the replisome. In fact, CDK2-cyclin A in complex with PCNA phosphorylates RFC and DNA ligase I (Koundrioukoff et al., 2000), and CDK2-catalyzed phosphorylation of FEN-1 dissociates this enzyme from PCNA (Henneke et al., 2003).

PCNA is also crucial for the balance of survival and cell death. The p33(ING1b) isoform of the ING1 tumor suppressor contains in its N-terminal domain a PIP box required for PCNA interaction (Scott et al., 2001). In response to UV irradiation, PCNA binding of ING1b is increased tenfold, thereby inducing apoptosis. On the other hand, PCNA displays also an antiapoptotic activity through interaction with proteins of the Gadd45 family (Gadd45, MyD118, and CR6), which are implicated in growth control, apoptosis, and DNA repair. All three members of this protein class have PCNA-interaction domains in their C-terminal regions; however, in these cases, PCNA interaction inhibits their activities (Azam et al., 2001; Vairapandi et al., 1996). Finally, it was recently reported that the tumor suppressor p53 and its negative regulator Mdm2 contain PIP boxes and interact with PCNA (Banks et al., 2006). Although the significance of PCNA binding is not clear in these cases, depletion of PCNA results in an accumulation of p53, suggesting that PCNA might indeed contribute directly to p53 stability.

### Regulation of PCNA Functions

There is hardly any protein known to date that rivals PCNA in its capacity to associate with so many different and alternative cofactors. Indeed, the already stunning number of confirmed PCNA binding proteins (Figure 2 and Table S1) is surely not final, as more PCNA-binding proteins will undoubtedly be discovered in the future. PCNA also stands out because it functions not merely as a scaffold or binding platform for a plethora of proteins, but apparently also as a stimulator and conductor, orchestrating complicated mechanisms to perform in an ordered pathway. Superior to the proverbial Jack-of-All-Trades, PCNA masters them all. But why do these multiple choices of protein binding not lead to confusion?

As detailed above, most PCNA interactions take place on the C side of PCNA, where a hydrophobic pocket accommodates the PIP-box motif present in the majority of PCNA's binding partners. Given that PCNA is a homotrimer, the clamp could bind theoretically up to three (possibly different) PIP-box proteins



**Figure 4. Alternative PCNA-Cofactor Interactions**

(Upper panel) Cofactor exchange can be triggered by affinity-driven displacement (e.g., by p21), by cofactor phosphorylation (P; e.g., FEN-1), by PCNA monoubiquitylation (perhaps also K63-linked polyubiquitylation), or by SUMO modification (see Figure 1). SUMOylation can lead to either recruitment (e.g., ubiquitin-TLS polymerase or SUMO-Srs2) or inhibition of cofactor binding (e.g., SUMO: Eco1). Cofactor polyubiquitylation (perhaps the K48-linked polyubiquitin chain) promotes its proteasomal degradation (e.g., Cdt1). New binding partners (green) can then bind to unmodified or modified PCNA.

(Lower panel) PCNA exchange can proceed either by unloading mediated by RFC or RFC-related enzymes, or by PCNA polyubiquitylation (perhaps the K48-linked polyubiquitin chain) and proteasomal degradation, e.g., after phosphorylated (P) PCNA becomes dephosphorylated. Newly bound PCNA molecules can engage in interactions with new cofactors (green).

at a time. In fact, the arc-shaped RFC complex binds with three of its five different subunits to two subunits of the PCNA clamp (Bowman et al., 2004). By contrast, in case of FEN-1, three copies can bind to the three PCNA subunits simultaneously, but interestingly in a way that shifts the bulk of FEN-1 away from the PCNA trimer, perhaps admitting other proteins (e.g., DNA ligase) access to PCNA (Sakurai et al., 2005). Notably, if indeed a PCNA trimer could be furnished with three different activities, it might function as a revolver (e.g., of a microscope), perhaps shifting a pathway forward step by step. Also attractive is the idea that the partners will associate with PCNA in a sequential reaction, by which for example binding of partner-1 to subunit-1 of PCNA will allow and facilitate binding of partner-2 to subunit-2. Such a hypothetical mechanism would be optimal for complex pathways (e.g., lagging strand synthesis, MMR, BER), in which multiple PIP-box proteins are known to function one after the other. Intriguing insights indicating that this might indeed be the case for lagging strand synthesis came from studies of PCNA from the archaeon *Sulfolobus solfataricus*. Interestingly, in this organism PCNA is a heterotrimer, and the three different

subunits, PCNA2, PCNA1, PCNA3, distinctively bind polymerase, FEN-1, and DNA ligase, respectively (Dionne et al., 2003). Remarkably, DNA ligase appears to associate initially with PCNA in an extended “open” conformation (Pascal et al., 2006). Yet upon DNA binding (in cells, perhaps when the flap structure has been removed by FEN-1), it apparently forms a ring (also in humans), clamping on DNA and thereby shielding PCNA from other potential binding partners (Pascal et al., 2004, 2006). Corresponding to its role as the last enzyme in the DNA-replication pathway, this locking mechanism will effectively close the reaction.

In many cases, however, binding of different PIP-box proteins to PCNA is competitive for sterical reasons (see models in Figure 4; upper panel). One key parameter that decides which partner will win is the respective affinity of the protein for PCNA. Mutagenesis studies showed that binding of PIP-box proteins to the hydrophobic pocket of PCNA is dramatically altered by even modest changes in the canonical PIP-box sequence, as well as in flanking sequences (Bruning and Shamoo, 2004). Although it has not been systematically addressed, it seems likely that PIP-box proteins can be hierarchically ordered according to their PCNA affinities. The protein with the highest affinity for PCNA currently known is p21, which practically jams the binding pocket, explaining its dominant role in inhibiting replication and other PCNA-linked functions. In fact, the affinity of p21 for PCNA can be by nearly three orders of magnitude higher than those of other PIP-box proteins (Bruning and Shamoo, 2004).

An effective means to reorder the hierarchy of PCNA interactors and to control PCNA interaction is through factor phosphorylation. In keeping with its role as a key

regulator of S phase, CDK2 appears to be crucial for PCNA associations as well. Interestingly, CDK2 lacks a PIP box, which allows the kinase to function in triad with PCNA and PIP-box proteins (Riva et al., 2004). CDK2-PCNA complexes are known to phosphorylate RFC, FEN-1, and DNA ligase I (Koundrioukoff et al., 2000), and in case of FEN-1, this leads to the dissociation of the nuclease from PCNA (Henneke et al., 2003). Indeed, this mechanism might be broadly used to remove specific binding factors from PCNA and might control Okazaki fragment processing during lagging strand synthesis. Moreover, as already discussed in the context of TLS, it is also conceivable that perhaps PCNA-coupled ubiquitylation of binding proteins could prevent their re-association.

In addition to modification of binding partners, another way to control PCNA interactions is through reversible modification of PCNA (Hoegge et al., 2002). As accentuated in this review, modification of PCNA by ubiquitin and SUMO significantly expands the repertoire of binding partners and influences their PCNA-binding properties. Remarkably, this can happen in both positive and negative ways (Figure 4; upper panel). Modification of PCNA with monoubiquitin and SUMO attracts specific binding partners (TLS polymerases and Srs2, respectively) (Bienko et al., 2005; Kannouche et al., 2004; Papouli et al., 2005; Pfander et al., 2005), due to the presence of specific binding motifs in these proteins. Although it is not yet settled whether ubiquitylation of PCNA reduces its affinity toward the replicative polymerases (Pol $\delta$  and Pol $\epsilon$ ), PCNA SUMOylation (in addition to its positive function in recruiting Srs2) clearly exhibits repressive effects on the association with PIP-box proteins. In *S. cerevisiae*, SUMOylation of PCNA, in particular at residue K127, interferes with Eco1-dependent sister-chromatid cohesion (Moldovan et al., 2006). Because K127 is positioned right within the interdomain connecting loop, it is likely that the bulky SUMO modification at this site will mask the binding pocket, thereby preventing Eco1 to collaborate with PCNA in cohesion. However, it is plausible that this repressive effect by SUMO is not restricted to Eco1 and that binding of other PIP-box proteins is affected as well. Notably, only a small fraction of PCNA is modified by SUMO at steady state. This suggests that perhaps only certain PCNA molecules are selected for SUMOylation (perhaps at specific chromosomal sites). But it is equally possible that all PCNA molecules engaged in replication are modified at some point, but the modification might be a very transient event. It has been suggested that PCNA SUMOylation at K127 might function as a “reset button” by kicking off PCNA interactors (Moldovan et al., 2006). In this model, fresh rebinding can occur only after SUMO has been removed from PCNA by a de-SUMOylation enzyme. Notably, K127 of PCNA resides within a consensus site for SUMOylation ( $\Psi$ KxD/E, where  $\Psi$  is a hydrophobic residue), which is known to bind the SUMO-conjugation enzyme Ubc9 directly. In fact, in vitro studies showed that Ubc9 can

effectively displace Eco1 from PCNA (Moldovan et al., 2006). Therefore, it is attractive to speculate that the reset button might function with two settings: in the first step, Ubc9 will displace PIP-box proteins from PCNA (perhaps analogous to p21, which does not exist in yeast), whereas in the second step, inhibition is made longer lasting by attaching SUMO covalently to PCNA. However, where Ubc9 is positioned in the affinity hierarchy of PCNA-binding proteins, and which factors are displaced by Ubc9 in vivo is currently not known.

An irreversible intervention into PCNA associations proceeds via protein degradation involving the ubiquitin-proteasome pathway (Figure 4; upper panel). In addition to PCNA-triggered Cdt1 ubiquitylation and degradation in order to prevent rereplication (Arias and Walter, 2006), also degradation of Xic1, a *Xenopus leavis* homolog of the human CDK inhibitor p27, appears to be directly coupled to PCNA (Chuang and Yew, 2005). Because of the effectiveness of this mechanism, it is tempting to speculate that PCNA-triggered degradation might represent a more general tactic to clear PCNA from superseded binding factors.

Lastly, there are two ways that affect PCNA and its binding partners in a more indiscriminate sense: PCNA unloading and PCNA degradation (Figure 4; lower panel). Obviously, both mechanisms will disrupt functional interactions with chromatin-bound partners, but concomitantly will allow fresh engagements with a newly loaded PCNA molecule.

PCNA unloading can be mediated by RFC (Rfc1-5), the same complex that also mediates loading. Interestingly, however, eukaryotes possess three additional Rfc1-related proteins, Rad17 (Rad24 in *S. cerevisiae*), Ctf18, and Elg1 of *S. cerevisiae*, which can replace Rfc1 in the heteropentameric complex (Majka and Burgers, 2004). Whereas Rad17 (Rad24)-containing RFC acts primarily on the PCNA-related heterotrimeric checkpoint protein complex 9-1-1 (Rad9-Rad1-Hus1 in mammals and Ddc1-Rad17-Mec3 in *S. cerevisiae*), the other two Rfc1-like proteins seem to act on PCNA. Indeed, Ctf18 and Rad24-containing RFC-like complexes are able to remove PCNA from DNA as well (Bylund and Burgers, 2005; Yao et al., 2006). Due to its involvement in cohesion, it is possible that Ctf18 unloads PCNA bound to cohesion proteins. By contrast, yeast genetic data link Elg1 to replication, suggesting that perhaps an alternative Elg1-containing complex might load or unload PCNA molecules involved in replication or repair. The interesting possibility that loading/unloading and the use of distinct Rfc1-like proteins might be influenced by PCNA modifications has not been addressed. Equally, whether RFC and RFC-like complexes not only chaperone PCNA but also guide PCNA partners remains unexplored.

PCNA was originally called “cyclin” as its protein level in higher eukaryotes fluctuates during the cell cycle (apparently not much in *S. cerevisiae*) with a peak in S phase. In particular, as indicated by its current name, PCNA levels are particularly high in proliferating cells.

Recent results show that human PCNA degradation is triggered by PCNA dephosphorylation. Interestingly, the crucial kinase is the nuclear form of epidermal growth factor receptor (EGFR), which, triggered by its ligand EGF, phosphorylates PCNA apparently in the nucleus (Wang et al., 2006). Phosphorylated PCNA is stably associated with chromatin, whereas a phosphorylation-defective PCNA variant is subject to ubiquitylation and degradation. Notably, ubiquitylation of PCNA that leads to PCNA degradation does not involve enzymes of the *RAD6* pathway, indicating that other ubiquitylation enzymes can act on PCNA as well. Interestingly, PCNA degradation causes reduced DNA synthesis and defects in mismatch repair, suggesting that the proliferation-promoting activity of EGF is in part linked to its ability to trigger PCNA stabilization.

### Epilogue

Although the above examples explain how PCNA interactions might be coordinated on chromatin and in time, they also add another layer of complexity. In addition to the basic replication machinery and the orchestra of PCNA cofactors, one now has to add a pile of different regulators and ask how these proteins are activated. These regulators seem to comprise protein kinases and phosphatases, ubiquitylation and deubiquitylation enzymes, SUMO-conjugating and deconjugating enzymes, and dominantly acting inhibitors like p21. Replication in higher eukaryotes takes place in so-called "replication factories," which seem to encompass a dozen or more replication forks. It is tempting to speculate that these subnuclear assemblies also concentrate some of the regulators, perhaps thereby stimulating their activities. Moreover, this arrangement will also facilitate a simultaneous global regulation of many replication forks by cellular cues.

Although most PCNA interactions seem to occur directly at the replication fork of replicating chromosomes, PCNA also acts independently of chromosomal replication, e.g., for gap-filling repair DNA synthesis and other functions that are often linked to DNA repair. Despite the fact that PCNA is coupled to bountiful replication-linked functions, it surprisingly does not seem to bind directly to proteins of the checkpoint cascade that signal cell-cycle arrest upon DNA damage. In eukaryotes, this function is associated with the PCNA-related protein complex 9-1-1. Although this clamp performs far fewer functions than PCNA (as it seems to be dedicated primarily to activation of the damage checkpoint), it contains three different subunits. As in the heterotrimeric PCNA clamp of archaea, this arrangement might allow a defined association of individual 9-1-1 subunits with checkpoint proteins (e.g., checkpoint kinases) and other partners. Indeed, the 9-1-1 protein complex of *S. cerevisiae* appears to interact via two specific subunits also with the TLS polymerase Pol $\zeta$  (Sabbioneda et al., 2005), but it is not yet clear how it might contribute to TLS and how this differs from TLS in union with PCNA.

The 9-1-1 clamp also binds to Pol $\beta$ , FEN-1, and perhaps DNA ligase I (Helt et al., 2005), however apparently only in connection with BER and not for lagging strand synthesis. Therefore, it seems reasonable to assume that PCNA and 9-1-1 have evolved from a common  $\beta$  clamp ancestor perhaps because a spatial separation of the replisome and checkpoint components turned out to be beneficial for cells. This spatial segregation of activities might perhaps facilitate a molecular distinction between the discontinuities that normally take place during lagging strand synthesis and other gaps that should trigger cell-cycle arrest. On the other hand, this division of labor might also facilitate ongoing replication without disturbance by checkpoint signaling proteins.

DNA replication has once more surfaced as an extremely interesting field of research. As the field has progressed from a classical topic of genetics to a subject for modern protein biochemistry and cell biology, the replication orchestra with its maestro PCNA are now in the minds of a larger audience. In particular, the links to other hot topics, like cell-cycle control, genome stability, chromatin function, and cancer, guarantee that the challenging open questions concerning the regulation and the nuclear organization of these activities will be profitably addressed in the future.

### Supplemental Data

Supplemental Data include one table and Supplemental References and can be found with this article online at <http://www.cell.com/cgi/content/full/129/4/665/DC1/>.

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### REFERENCES

- Arakawa, H., Moldovan, G.L., Saribasak, H., Saribasak, N.N., Jentsch, S., and Buerstedde, J.M. (2006). A role for PCNA ubiquitination in immunoglobulin hypermutation. *PLoS Biol.* 4, e366.
- Arias, E.E., and Walter, J.C. (2006). PCNA functions as a molecular platform to trigger Cdt1 destruction and prevent re-replication. *Nat. Cell Biol.* 8, 84–90.
- Azam, N., Vairapandi, M., Zhang, W., Hoffman, B., and Liebermann, D.A. (2001). Interaction of CR6 (GADD45gamma) with proliferating cell nuclear antigen impedes negative growth control. *J. Biol. Chem.* 276, 2766–2774.
- Banks, D., Wu, M., Higa, L.A., Gavrilova, N., Quan, J., Ye, T., Kobayashi, R., Sun, H., and Zhang, H. (2006). L2DTL/CDT2 and PCNA interact with p53 and regulate p53 polyubiquitination and protein stability through MDM2 and CUL4A/DDB1 complexes. *Cell Cycle* 5, 1719–1729.
- Barbour, L., and Xiao, W. (2003). Regulation of alternative replication bypass pathways at stalled replication forks and its effects on genome stability: a yeast model. *Mutat. Res.* 532, 137–155.
- Bell, S.P., and Dutta, A. (2002). DNA replication in eukaryotic cells. *Annu. Rev. Biochem.* 71, 333–374.



- Bienko, M., Green, C.M., Crosetto, N., Rudolf, F., Zapart, G., Coull, B., Kannouche, P., Wider, G., Peter, M., Lehmann, A.R., et al. (2005). Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. *Science* 310, 1821–1824.
- Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes Dev.* 16, 6–21.
- Bowman, G.D., O'Donnell, M., and Kuriyan, J. (2004). Structural analysis of a eukaryotic sliding DNA clamp-clamp loader complex. *Nature* 429, 724–730.
- Bruning, J.B., and Shamoo, Y. (2004). Structural and thermodynamic analysis of human PCNA with peptides derived from DNA polymerase-delta p66 subunit and flap endonuclease-1. *Structure* 12, 2209–2219.
- Bylund, G.O., and Burgers, P.M. (2005). Replication protein A-directed unloading of PCNA by the Ctf18 cohesion establishment complex. *Mol. Cell. Biol.* 25, 5445–5455.
- Chapados, B.R., Hosfield, D.J., Han, S., Qiu, J., Yelent, B., Shen, B., and Tainer, J.A. (2004). Structural basis for FEN-1 substrate specificity and PCNA-mediated activation in DNA replication and repair. *Cell* 116, 39–50.
- Chuang, L.C., and Yew, P.R. (2005). Proliferating cell nuclear antigen recruits cyclin-dependent kinase inhibitor Xic1 to DNA and couples its proteolysis to DNA polymerase switching. *J. Biol. Chem.* 280, 35299–35309.
- Chuang, L.S., Ian, H.I., Koh, T.W., Ng, H.H., Xu, G., and Li, B.F. (1997). Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. *Science* 277, 1996–2000.
- Diffley, J.F. (2004). Regulation of early events in chromosome replication. *Curr. Biol.* 14, R778–R786.
- Dionne, I., Nookala, R.K., Jackson, S.P., Doherty, A.J., and Bell, S.D. (2003). A heterotrimeric PCNA in the hyperthermophilic archaeon *Sulfolobus solfataricus*. *Mol. Cell* 11, 275–282.
- Dotto, G.P. (2000). p21(WAF1/Cip1): more than a break to the cell cycle? *Biochim. Biophys. Acta* 1471, M43–M56.
- Ducoux, M., Urbach, S., Baldacci, G., Hubscher, U., Koundrioukoff, S., Christensen, J., and Hughes, P. (2001). Mediation of proliferating cell nuclear antigen (PCNA)-dependent DNA replication through a conserved p21(Cip1)-like PCNA-binding motif present in the third subunit of human DNA polymerase delta. *J. Biol. Chem.* 276, 49258–49266.
- Esteve, P.O., Chin, H.G., Smallwood, A., Feehery, G.R., Gangisetty, O., Karpf, A.R., Carey, M.F., and Pradhan, S. (2006). Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. *Genes Dev.* 20, 3089–3103.
- Fan, J., Otterlei, M., Wong, H.K., Tomkinson, A.E., and Wilson, D.M., 3rd. (2004). XRCC1 co-localizes and physically interacts with PCNA. *Nucleic Acids Res.* 32, 2193–2201.
- Friedberg, E.C., Lehmann, A.R., and Fuchs, R.P. (2005). Trading places: how do DNA polymerases switch during translesion DNA synthesis? *Mol. Cell* 18, 499–505.
- Gaillard, P.H., Martini, E.M., Kaufman, P.D., Stillman, B., Moustacchi, E., and Almouzni, G. (1996). Chromatin assembly coupled to DNA repair: a new role for chromatin assembly factor I. *Cell* 86, 887–896.
- Game, J.C., and Kaufman, P.D. (1999). Role of *Saccharomyces cerevisiae* chromatin assembly factor-I in repair of ultraviolet radiation damage in vivo. *Genetics* 151, 485–497.
- Garg, P., and Burgers, P.M. (2005a). DNA polymerases that propagate the eukaryotic DNA replication fork. *Crit. Rev. Biochem. Mol. Biol.* 40, 115–128.
- Garg, P., and Burgers, P.M. (2005b). Ubiquitinated proliferating cell nuclear antigen activates translesion DNA polymerases eta and REV1. *Proc. Natl. Acad. Sci. USA* 102, 18361–18366.
- Garg, P., Stith, C.M., Sabouri, N., Johansson, E., and Burgers, P.M. (2004). Idling by DNA polymerase delta maintains a ligatable nick during lagging-strand DNA replication. *Genes Dev.* 18, 2764–2773.
- Gary, R., Ludwig, D.L., Cornelius, H.L., MacInnes, M.A., and Park, M.S. (1997). The DNA repair endonuclease XPG binds to proliferating cell nuclear antigen (PCNA) and shares sequence elements with the PCNA-binding regions of FEN-1 and cyclin-dependent kinase inhibitor p21. *J. Biol. Chem.* 272, 24522–24529.
- Gomes, X.V., and Burgers, P.M. (2001). ATP utilization by yeast replication factor C. I. ATP-mediated interaction with DNA and with proliferating cell nuclear antigen. *J. Biol. Chem.* 276, 34768–34775.
- Green, E.M., Antczak, A.J., Bailey, A.O., Franco, A.A., Wu, K.J., Yates, J.R., 3rd, and Kaufman, P.D. (2005). Replication-independent histone deposition by the HIR complex and Asf1. *Curr. Biol.* 15, 2044–2049.
- Gulbis, J.M., Kelman, Z., Hurwitz, J., O'Donnell, M., and Kuriyan, J. (1996). Structure of the C-terminal region of p21(WAF1/CIP1) complexed with human PCNA. *Cell* 87, 297–306.
- Guo, C., Tang, T.S., Bienko, M., Parker, J.L., Bielen, A.B., Sonoda, E., Takeda, S., Ulrich, H.D., Dikic, I., and Friedberg, E.C. (2006). Ubiquitin-binding motifs in REV1 protein are required for its role in the tolerance of DNA damage. *Mol. Cell. Biol.* 26, 8892–8900.
- Haracska, L., Unk, I., Prakash, L., and Prakash, S. (2006). Ubiquitylation of yeast proliferating cell nuclear antigen and its implications for translesion DNA synthesis. *Proc. Natl. Acad. Sci. USA* 103, 6477–6482.
- Hasan, S., Hassa, P.O., Imhof, R., and Hottiger, M.O. (2001). Transcription coactivator p300 binds PCNA and may have a role in DNA repair synthesis. *Nature* 410, 387–391.
- Hediger, F., and Gasser, S.M. (2006). Heterochromatin protein 1: don't judge the book by its cover! *Curr. Opin. Genet. Dev.* 16, 143–150.
- Helt, C.E., Wang, W., Keng, P.C., and Bambara, R.A. (2005). Evidence that DNA damage detection machinery participates in DNA repair. *Cell Cycle* 4, 529–532.
- Henneke, G., Koundrioukoff, S., and Hubscher, U. (2003). Phosphorylation of human Fen1 by cyclin-dependent kinase modulates its role in replication fork regulation. *Oncogene* 22, 4301–4313.
- Hermann, A., Gowher, H., and Jeltsch, A. (2004). Biochemistry and biology of mammalian DNA methyltransferases. *Cell. Mol. Life Sci.* 61, 2571–2587.
- Hoege, C., Pfander, B., Moldovan, G.L., Pyrowolakis, G., and Jentsch, S. (2002). RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* 419, 135–141.
- Huang, S., Zhou, H., Katzmman, D., Hochstrasser, M., Atanasova, E., and Zhang, Z. (2005). Rtt106p is a histone chaperone involved in heterochromatin-mediated silencing. *Proc. Natl. Acad. Sci. USA* 102, 13410–13415.
- Huang, T.T., Nijman, S.M., Mirchandani, K.D., Galardy, P.J., Cohn, M.A., Haas, W., Gygi, S.P., Ploegh, H.L., Bernards, R., and D'Andrea, A.D. (2006). Regulation of monoubiquitinated PCNA by DUB autocleavage. *Nat. Cell Biol.* 8, 339–347.
- Iida, T., Suetake, I., Tajima, S., Morioka, H., Ohta, S., Obuse, C., and Tsurimoto, T. (2002). PCNA clamp facilitates action of DNA cytosine methyltransferase 1 on hemimethylated DNA. *Genes Cells* 7, 997–1007.
- Jentsch, S., McGrath, J.P., and Varshavsky, A. (1987). The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. *Nature* 329, 131–134.
- Jiricny, J. (2006). The multifaceted mismatch-repair system. *Nat. Rev. Mol. Cell Biol.* 7, 335–346.
- Johnson, A., and O'Donnell, M. (2005). Cellular DNA replicases: components and dynamics at the replication fork. *Annu. Rev. Biochem.*

74, 283–315.

Jonsson, Z.O., Hindges, R., and Hubscher, U. (1998). Regulation of DNA replication and repair proteins through interaction with the front side of proliferating cell nuclear antigen. *EMBO J.* 17, 2412–2425.

Kadyrov, F.A., Dzantiev, L., Constantin, N., and Modrich, P. (2006). Endonucleolytic function of MutLalpha in human mismatch repair. *Cell* 126, 297–308.

Kannouche, P.L., Wing, J., and Lehmann, A.R. (2004). Interaction of human DNA polymerase eta with monoubiquitinated PCNA: a possible mechanism for the polymerase switch in response to DNA damage. *Mol. Cell* 14, 491–500.

Ko, R., and Bennett, S.E. (2005). Physical and functional interaction of human nuclear uracil-DNA glycosylase with proliferating cell nuclear antigen. *DNA Repair (Amst.)* 4, 1421–1431.

Koundrioukoff, S., Jonsson, Z.O., Hasan, S., de Jong, R.N., van der Vliet, P.C., Hottiger, M.O., and Hubscher, U. (2000). A direct interaction between proliferating cell nuclear antigen (PCNA) and Cdk2 targets PCNA-interacting proteins for phosphorylation. *J. Biol. Chem.* 275, 22882–22887.

Krawitz, D.C., Kama, T., and Kaufman, P.D. (2002). Chromatin assembly factor I mutants defective for PCNA binding require Asf1/Hir proteins for silencing. *Mol. Cell. Biol.* 22, 614–625.

Krishna, T.S., Fenyo, D., Kong, X.P., Gary, S., Chait, B.T., Burgers, P., and Kuriyan, J. (1994). Crystallization of proliferating cell nuclear antigen (PCNA) from *Saccharomyces cerevisiae*. *J. Mol. Biol.* 241, 265–268.

Lambert, S., Watson, A., Sheedy, D.M., Martin, B., and Carr, A.M. (2005). Gross chromosomal rearrangements and elevated recombination at an inducible site-specific replication fork barrier. *Cell* 121, 689–702.

Leach, C.A., and Michael, W.M. (2005). Ubiquitin/SUMO modification of PCNA promotes replication fork progression in *Xenopus laevis* egg extracts. *J. Cell Biol.* 171, 947–954.

Lee, S.D., and Alani, E. (2006). Analysis of interactions between mismatch repair initiation factors and the replication processivity factor PCNA. *J. Mol. Biol.* 355, 175–184.

Lopes, M., Foiani, M., and Sogo, J.M. (2006). Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable UV lesions. *Mol. Cell* 21, 15–27.

Majka, J., and Burgers, P.M. (2004). The PCNA-RFC families of DNA clamps and clamp loaders. *Prog. Nucleic Acid Res. Mol. Biol.* 78, 227–260.

Milutinovic, S., Zhuang, Q., and Szyf, M. (2002). Proliferating cell nuclear antigen associates with histone deacetylase activity, integrating DNA replication and chromatin modification. *J. Biol. Chem.* 277, 20974–20978.

Modrich, P. (2006). Mechanisms in eukaryotic mismatch repair. *J. Biol. Chem.* 281, 30305–30309.

Moldovan, G.L., Pfander, B., and Jentsch, S. (2006). PCNA Controls Establishment of Sister Chromatid Cohesion during S Phase. *Mol. Cell* 23, 723–732.

Montecucco, A., Rossi, R., Levin, D.S., Gary, R., Park, M.S., Motycka, T.A., Ciarrocchi, G., Villa, A., Biamonti, G., and Tomkinson, A.E. (1998). DNA ligase I is recruited to sites of DNA replication by an interaction with proliferating cell nuclear antigen: identification of a common targeting mechanism for the assembly of replication factories. *EMBO J.* 17, 3786–3795.

Mortusewicz, O., Schermelleh, L., Walter, J., Cardoso, M.C., and Leonhardt, H. (2005). Recruitment of DNA methyltransferase I to DNA repair sites. *Proc. Natl. Acad. Sci. USA* 102, 8905–8909.

Mousson, F., Ochsenbein, F., and Mann, C. (2006). The histone chap-

erone Asf1 at the crossroads of chromatin and DNA checkpoint pathways. *Chromosoma* 116, 79–93.

Murzina, N., Verreault, A., Laue, E., and Stillman, B. (1999). Heterochromatin dynamics in mouse cells: interaction between chromatin assembly factor 1 and HP1 proteins. *Mol. Cell* 4, 529–540.

Nasmyth, K., and Haering, C.H. (2005). The structure and function of SMC and kleisin complexes. *Annu. Rev. Biochem.* 74, 595–648.

Oku, T., Ikeda, S., Sasaki, H., Fukuda, K., Morioka, H., Ohtsuka, E., Yoshikawa, H., and Tsurimoto, T. (1998). Functional sites of human PCNA which interact with p21 (Cip1/Waf1), DNA polymerase delta and replication factor C. *Genes Cells* 3, 357–369.

Otterlei, M., Warbrick, E., Nagelhus, T.A., Haug, T., Slupphaug, G., Akbari, M., Aas, P.A., Steinsbekk, K., Bakke, O., and Krokan, H.E. (1999). Post-replicative base excision repair in replication foci. *EMBO J.* 18, 3834–3844.

Oyama, M., Wakasugi, M., Hama, T., Hashidume, H., Iwakami, Y., Imai, R., Hoshino, S., Morioka, H., Ishigaki, Y., Nikaido, O., and Matsunaga, T. (2004). Human NTH1 physically interacts with p53 and proliferating cell nuclear antigen. *Biochem. Biophys. Res. Commun.* 321, 183–191.

Papouli, E., Chen, S., Davies, A.A., Huttner, D., Krejci, L., Sung, P., and Ulrich, H.D. (2005). Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. *Mol. Cell* 19, 123–133.

Pascal, J.M., O'Brien, P.J., Tomkinson, A.E., and Ellenberger, T. (2004). Human DNA ligase I completely encircles and partially unwinds nicked DNA. *Nature* 432, 473–478.

Pascal, J.M., Tsodikov, O.V., Hura, G.L., Song, W., Cotner, E.A., Clasen, S., Tomkinson, A.E., Tainer, J.A., and Ellenberger, T. (2006). A flexible interface between DNA ligase and PCNA supports conformational switching and efficient ligation of DNA. *Mol. Cell* 24, 279–291.

Pfander, B., Moldovan, G.L., Sacher, M., Hoege, C., and Jentsch, S. (2005). SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. *Nature* 436, 428–433.

Plosky, B.S., Vidal, A.E., Fernandez de Henestrosa, A.R., McLenigan, M.P., McDonald, J.P., Mead, S., and Woodgate, R. (2006). Controlling the subcellular localization of DNA polymerases iota and eta via interactions with ubiquitin. *EMBO J.* 25, 2847–2855.

Polo, S.E., and Almouzni, G. (2006). Chromatin assembly: a basic recipe with various flavours. *Curr. Opin. Genet. Dev.* 16, 104–111.

Poot, R.A., Bozhenok, L., van den Berg, D.L., Steffensen, S., Ferreira, F., Grimaldi, M., Gilbert, N., Ferreira, J., and Varga-Weisz, P.D. (2004). The Williams syndrome transcription factor interacts with PCNA to target chromatin remodelling by ISWI to replication foci. *Nat. Cell Biol.* 6, 1236–1244.

Riva, F., Savio, M., Cazzalini, O., Stivala, L.A., Scovassi, I.A., Cox, L.S., Ducommun, B., and Prosperi, E. (2004). Distinct pools of proliferating cell nuclear antigen associated to DNA replication sites interact with the p125 subunit of DNA polymerase delta or DNA ligase I. *Exp. Cell Res.* 293, 357–367.

Rousseau, D., Cannella, D., Boulaire, J., Fitzgerald, P., Fotedar, A., and Fotedar, R. (1999). Growth inhibition by CDK-cyclin and PCNA binding domains of p21 occurs by distinct mechanisms and is regulated by ubiquitin-proteasome pathway. *Oncogene* 18, 4313–4325.

Sabbioneda, S., Minesinger, B.K., Giannattasio, M., Plevani, P., Muzi-Falconi, M., and Jinks-Robertson, S. (2005). The 9-1-1 checkpoint clamp physically interacts with polzeta and is partially required for spontaneous polzeta-dependent mutagenesis in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 280, 38657–38665.

Sakurai, S., Kitano, K., Yamaguchi, H., Hamada, K., Okada, K., Fukuda, K., Uchida, M., Ohtsuka, E., Morioka, H., and Hakoshima, T. (2005). Structural basis for recruitment of human flap endonuclease 1

to PCNA. *EMBO J.* 24, 683–693.

Sancar, A., Lindsey-Boltz, L.A., Unsal-Kacmaz, K., and Linn, S. (2004). Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu. Rev. Biochem.* 73, 39–85.

Sarraf, S.A., and Stancheva, I. (2004). Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. *Mol. Cell* 15, 595–605.

Scott, M., Bonnefin, P., Vieyra, D., Boisvert, F.M., Young, D., Bazett-Jones, D.P., and Riabowol, K. (2001). UV-induced binding of ING1 to PCNA regulates the induction of apoptosis. *J. Cell Sci.* 114, 3455–3462.

Sharp, J.A., Fouts, E.T., Krawitz, D.C., and Kaufman, P.D. (2001). Yeast histone deposition protein Asf1p requires Hir proteins and PCNA for heterochromatic silencing. *Curr. Biol.* 11, 463–473.

Sheng, W., and Liao, X. (2002). Solution structure of a yeast ubiquitin-like protein Smt3: the role of structurally less defined sequences in protein-protein recognitions. *Protein Sci.* 11, 1482–1491.

Smith, S., and Stillman, B. (1989). Purification and characterization of CAF-I, a human cell factor required for chromatin assembly during DNA replication in vitro. *Cell* 58, 15–25.

Stelter, P., and Ulrich, H.D. (2003). Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. *Nature* 425, 188–191.

Tom, S., Henriksen, L.A., Park, M.S., and Bambara, R.A. (2001). DNA ligase I and proliferating cell nuclear antigen form a functional complex. *J. Biol. Chem.* 276, 24817–24825.

Tsushimoto, D., Sakai, Y., Sakumi, K., Nishioka, K., Sasaki, M., Fujiwara, T., and Nakabeppu, Y. (2001). Human APE2 protein is mostly localized in the nuclei and to some extent in the mitochondria, while nuclear APE2 is partly associated with proliferating cell nuclear antigen. *Nucleic Acids Res.* 29, 2349–2360.

Tyler, J.K., Adams, C.R., Chen, S.R., Kobayashi, R., Kamakaka, R.T., and Kadonaga, J.T. (1999). The RCAF complex mediates chromatin assembly during DNA replication and repair. *Nature* 402, 555–560.

Uhlmann, F., and Nasmyth, K. (1998). Cohesion between sister chromatids must be established during DNA replication. *Curr. Biol.* 8, 1095–1101.

Ulrich, H.D., and Jentsch, S. (2000). Two RING finger proteins mediate cooperation between ubiquitin-conjugating enzymes in DNA repair. *EMBO J.* 19, 3388–3397.

Umar, A., Buermeyer, A.B., Simon, J.A., Thomas, D.C., Clark, A.B., Liskay, R.M., and Kunkel, T.A. (1996). Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. *Cell* 87, 65–73.

Unk, I., Haracska, L., Gomes, X.V., Burgers, P.M., Prakash, L., and Prakash, S. (2002). Stimulation of 3'→5' exonuclease and 3'-phosphodiesterase activities of yeast apn2 by proliferating cell nuclear an-

tigen. *Mol. Cell. Biol.* 22, 6480–6486.

Unk, I., Hajdu, I., Fattyol, K., Szakal, B., Blastyak, A., Bermudez, V., Hurwitz, J., Prakash, L., Prakash, S., and Haracska, L. (2006). Human SHPRH is a ubiquitin ligase for Mms2-Ubc13-dependent polyubiquitination of proliferating cell nuclear antigen. *Proc. Natl. Acad. Sci. USA* 103, 18107–18112.

Vairapandi, M., Balliet, A.G., Fornace, A.J., Jr., Hoffman, B., and Liebermann, D.A. (1996). The differentiation primary response gene MyD118, related to GADD45, encodes for a nuclear protein which interacts with PCNA and p21WAF1/CIP1. *Oncogene* 12, 2579–2594.

Vijay-Kumar, S., Bugg, C.E., Wilkinson, K.D., Vierstra, R.D., Hatfield, P.M., and Cook, W.J. (1987). Comparison of the three-dimensional structures of human, yeast, and oat ubiquitin. *J. Biol. Chem.* 262, 6396–6399.

Waga, S., Hannon, G.J., Beach, D., and Stillman, B. (1994). The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. *Nature* 369, 574–578.

Wang, S.C., Nakajima, Y., Yu, Y.L., Xia, W., Chen, C.T., Yang, C.C., McIntush, E.W., Li, L.Y., Hawke, D.H., Kobayashi, R., and Hung, M.C. (2006). Tyrosine phosphorylation controls PCNA function through protein stability. *Nat. Cell Biol.* 8, 1359–1368.

Warbrick, E., Lane, D.P., Glover, D.M., and Cox, L.S. (1995). A small peptide inhibitor of DNA replication defines the site of interaction between the cyclin-dependent kinase inhibitor p21WAF1 and proliferating cell nuclear antigen. *Curr. Biol.* 5, 275–282.

Warbrick, E., Lane, D.P., Glover, D.M., and Cox, L.S. (1997). Homologous regions of Fen1 and p21Cip1 compete for binding to the same site on PCNA: a potential mechanism to co-ordinate DNA replication and repair. *Oncogene* 14, 2313–2321.

Watanabe, K., Tateishi, S., Kawasuji, M., Tsurimoto, T., Inoue, H., and Yamaizumi, M. (2004). Rad18 guides poleta to replication stalling sites through physical interaction and PCNA monoubiquitination. *EMBO J.* 23, 3886–3896.

Xia, L., Zheng, L., Lee, H.W., Bates, S.E., Federico, L., Shen, B., and O'Connor, T.R. (2005). Human 3-methyladenine-DNA glycosylase: effect of sequence context on excision, association with PCNA, and stimulation by AP endonuclease. *J. Mol. Biol.* 346, 1259–1274.

Xu, H., Zhang, P., Liu, L., and Lee, M.Y. (2001). A novel PCNA-binding motif identified by the panning of a random peptide display library. *Biochemistry* 40, 4512–4520.

Yao, N.Y., Johnson, A., Bowman, G.D., Kuriyan, J., and O'Donnell, M. (2006). Mechanism of proliferating cell nuclear antigen clamp opening by replication factor C. *J. Biol. Chem.* 281, 17528–17539.

Zhang, H., Xiong, Y., and Beach, D. (1993). Proliferating cell nuclear antigen and p21 are components of multiple cell cycle kinase complexes. *Mol. Cell. Biol.* 13, 897–906.

Zhang, Z., Shibahara, K., and Stillman, B. (2000). PCNA connects DNA replication to epigenetic inheritance in yeast. *Nature* 408, 221–225.