Proliferating cell nuclear antigen (PCNA): a dancer with many partners

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Summary

Proliferating cell nuclear antigen (PCNA) was originally characterised as a DNA sliding clamp for replicative DNA polymerases and as an essential component of the eukaryotic chromosomal DNA replisome. Subsequent studies, however, have revealed its striking ability to interact with multiple partners, which are involved in several metabolic pathways, including Okazaki fragment processing, DNA repair, translesion DNA synthesis, DNA methylation, chromatin remodeling and cell cycle regulation. PCNA in mammalian cells thus appears to play a key role in controlling several reactions through the coordination and organisation of different partners. Two major questions have emerged: how do these proteins access PCNA in a coordinated manner, and how does PCNA temporally and spatially organise their functions? Structural and biochemical studies are starting to provide a first glimpse of how both tasks can be achieved.

Key words: PCNA, PCNA-interacting proteins

Too many dancing partners for PCNA

PCNA is a member of the so called DNA sliding clamp family, which also includes the *E.coli* DNA polymerase (pol) III β-subunit and the phage T4 gene45 protein (Kelman and O'Donnell, 1995; Wyman and Botchan, 1995). These proteins perform the essential function of providing replicative polymerases with the high processivity required to duplicate an entire genome. Crystallographic studies of the β-subunit and PCNA revealed that these proteins adopt superimposable ring-like structures in solution, with a central hole sufficiently large to accommodate the double helix of DNA, even though they show almost no sequence similarity (Krishna et al., 1994).

Three identical PCNA monomers, each comprising two similar domains, are joined in a head-to-tail arrangement to form a homotrimer. This is thus composed of six repeating domains and exibits six-fold symmetry (Krishna et al., 1994; Schurtenberger et al., 1998). The resulting ring has two non-equivalent surfaces: an outside surface composed of β-sheets; and a layer of α-helices rich in basic residues lining the inner side of the hole, which are positioned perpendicularly to the phosphate backbone of DNA. Owing to this unique structure, PCNA is topologically linked to the double helix, encircling it, but it is still able to freely slide along the DNA lattice by virtue of the α-helices lining the inner channel. Thus, PCNA and its homologs increase the processivity of a polymerase by engaging in protein-protein interactions with its outer surface and tethering it to the DNA. This property of PCNA prevents the polymerase from dissociating while advancing along the template DNA and is the reason for the name sliding clamp (Kelman and O'Donnell, 1995).

The binding sites on PCNA for many of its partners have been mapped (Fig. 1) (reviewed by Jonsson and Hubscher, 1997; Warbrick, 2000). A major interaction site is the interdomain connecting loop, a coiled structure at the side of PCNA, spanning residues from L121 to E132. This loop is recognised by several proteins, such as pol δ, p21, flap endonuclease 1 (Fen1), DNA (cytosine) methyltransferase (MeCTr) and DNA ligase 1 (Lig1). Other important sequences are the N-terminal region comprising the inner α-helices, which forms part of the binding site for cyclin D, and the C-terminal tail, which is important for the interaction with pol ε, replication factor C (RF-C), CDK2 and GADD45.

Many PCNA-binding proteins contain a common PCNA-binding motif: the PIP-box (Jonsson et al., 1998; Warbrick, 2000), which has the consensus sequence Q-xx-(h)-x-x-(a)-(a), where h represents residues with moderately hydrophobic side chains (e.g. L, I, M), a represents residues with highly hydrophobic, aromatic side chains (e.g. F, Y) and x is any residue. Recently, by exploiting a random peptide display library, Xu et al. identified a novel PCNA binding motif, K-A-(A/L/I)-(A/L/Q)-x-x-(L/V), termed the KA-box. It is distinct from the 'classical' PIP-box, and is also present in several PCNA interacting proteins (Xu et al., 2001).

Tables 1 and 2 summarise PCNA-interacting proteins and indicate their functions in the cell. These proteins can be divided into two groups: those that have a known enzymatic activity (Table 1) and those that are involved in cell-cycle progression, checkpoint control or cellular differentiation (Table 2). The discovery of the overlapping nature of the binding sites for these PCNA binding proteins immediately suggested that the different partners must bind and dissociate sequentially to perform their functions (Jonsson et al., 1998; Warbrick, 2000). Below we provide examples of the importance of PCNA interactions in different DNA metabolic pathways.

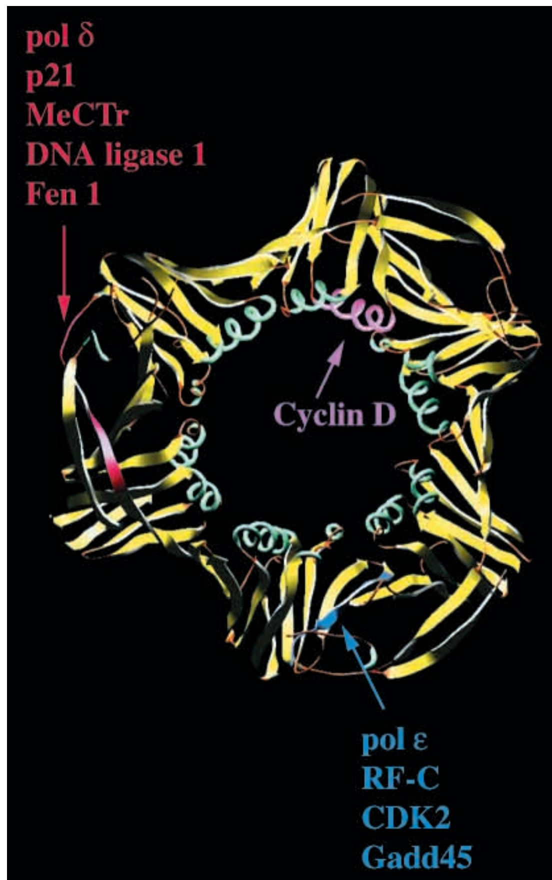

Fig. 1. Many PCNA partners share overlapping binding sites. The main regions of the PCNA ring involved in protein-protein interactions are highlighted along with the relevant partners. These are: (1) the interdomain connecting loop (aa L121 to E132; red); (2) the inner side α-helices at the N-terminus (pink); and (3) the C-terminal tail (blue). For clarity, only one region per each monomer is highlighted. The PCNA structure was generated with the program Swiss-PDBViewer from the PDB file 1AXC, rendered with QuickTime 3D and composed in Adobe PhotoShop.

PCNA in DNA replication

The identification of PCNA as a processivity factor for replicative DNA polymerases placed it at the heart of the replisome. However, other work has revealed additional roles for this protein in coordinating the complex network of interactions at the replication fork. Our current view of DNA replication in eukaryotes is that pol-α/primase synthesises the first RNA/DNA primer on the leading strand. Then pol δ, together with its processivity factor PCNA, performs continuous leading strand synthesis; pol-α/primase is involved in RNA priming and discontinuous DNA synthesis of the lagging strand (Waga et al., 1994a). Completion of Okazaki fragment synthesis, however, requires the processive pol δ (or pol ε) holoenzyme (pol δ/ε, RF-C and PCNA). Thus, both initiation of leading strand DNA replication and discontinuous lagging strand synthesis require a switch from pol α to pol δ (or pol ε). PCNA has been shown to play a central role in coordinating this process. Infact, owing to its toroidal structure, the PCNA trimer has to be opened and then closed around the nascent DNA strand by the enzymatic activity of the clamp loader RF-C. This event triggers the displacement of pol α and subsequent recruitment of pol δ (or pol ε) for processive synthesis (Yuzhakov et al., 1999; Maga et al., 2000). Re-binding of pol α is prevented by the presence of PCNA bound at the 3′-OH end, which functions as a specific recruitment signal for the more processive pol δ and pol ε.

Synthesis of an Okazaki fragment is terminated when the pol δ or pol ε holoenzyme meets the 5′ end of the RNA portion of the previously synthesised fragment and performs strand displacement synthesis. Finally, specialised proteins are recruited that remove the RNA part, fill the gap and ligate the two adjacent fragments. The two PCNA-binding proteins Fen1 and Lig1 are involved in this process (Bae and Seo, 2000; Levin et al., 2000; Bae et al., 2001). Moreover, PCNA has been shown to stimulate Fen1 activity (Jonsson et al., 1998; Tom et al., 2000). In vitro reconstitution of the Okazaki fragment maturation process showed that competition for PCNA binding among pol δ, Fen1 and Lig1 coordinates the ordered action of these enzymes (Yuzhakov et al., 1999; Maga et al., 2001).

Table 1. PCNA-interacting proteins (PIPs) in DNA replication, DNA repair and cell cycle control

PIP	Function	References
Pol δ	DNA replication and repair	Zhang et al., 1999; Zhou et al., 1997; Ducoux et al., 2001
Pol ε	DNA replicationand repair	Eissenberg et al., 1997; Dua et al., 2002
RF-C	DNA replication and repair	Fotedar et al., 1996
DNA ligase I	DNA replication and repair	Levin et al., 1997
Fen1	DNA replication and repair	Warbrick et al., 1997
Topo I	DNA replication and repair	Loor et al., 1997
Topo IIα	DNA replication and repair	Niimi et al., 2001
MLH1, MSH 2/3/6	Mismatch DNA repair	Umar et al., 1996; Clark et al., 2000
XP-G endonuclease	Nucleotide excision repair	Gary et al., 1997
WRN helicase	Double strand breaks DNA repair; linked to the Werner Syndrome disease	Lebel et al., 1999
UBC9	SUMOilation	Hoege et al., 2002
AP-endonucleases APN1, APN2	Base excision repair	Dianova et al., 2001; Unk et al., 2002
Uracil-DNA glycosylase	Base excision repair	Krokan et al., 2001
Pol β	Base excision repair	Kedar et al., 2002
Pol η	Translesion synthesis; linked to the XP-V disease	Haracska et al., 2001a
Pol ι	Translesion synthesis	Haracska et al., 2001b
Pol κ	Translesion synthesis	Haracska et al., 2002
Pol λ	Translesion synthesis	Maga et al., 2002
Cyclin/CDKs	Cell cycle control	Xiong et al., 1992; Zhang et al., 1993
p21	Cell cycle control	Waga et al., 1994c

Table 2. PCNA-interacting proteins (PIPs) in chromatin metabolism, sister chromatin cohesion and apoptosis

PIP	Function	References
CAF-1	Topological marker for CAF-1	Shibahara and Stillman, 1999
CAF-1	Epigenetic inheritance	Zhang et al., 2000
CAF-1	Recruitment to DNA damages	Moggs et al., 2000
P300	Facilitation of PCNA function in DNA repair	Hasan et al., 2001
MeCTr	Maintenance of methylation pattern	Chuang et al., 1997
Ctf7p	Connection of sister chromatin cohesion to DNA replication	Skibbens et al., 1999
CHL12	Alternative clamp loader in sister chromatin cohesion	Ohta et al., 2002
Gadd45	Negative growth control, prevention of apoptosis	Vairapandi et al., 2000
MyD118	Negative growth control, prevention of apoptosis	Vairapandi et al., 2000
Ing1p33^{ING1}	Protection from UV-induced apoptosis	Scott et al., 2001

When the pol δ holoenzyme encounters the 5′ end of the previous fragment, it performs strand displacement synthesis in conjunction with the helicase/endonuclease Dna2. The displaced strand generates a flap structure, which is bound by the ssDNA-binding protein RP-A, which, in turn, triggers dissociation of pol δ from PCNA. Following the recruitment of Fen1, the PCNA-Fen1 complex efficiently removes the flap, leaving a nick in the double-stranded DNA. This is followed by binding of Lig1 to PCNA, which performs the final ligation step, thus sealing the nick (Ayyagari et al., 2003; Jin et al., 2003).

PCNA in DNA repair

There are several specialised metabolic pathways for DNA damage repair, including nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR) and double-strand break repair (DSBR). All involve a DNA synthesis step requiring pol δ or pol ε, which thus indicates a function for PCNA. However, as we outline below, besides functioning as an auxiliary factor for the polymerase, PCNA plays other roles in some of these pathways.

In vitro reconstitution of NER showed a requirement for PCNA (Shivji et al., 1992), which is probably due to the involvement of pol δ and/or pol ε in the resynthesis step (Aboussekhra et al., 1995). The observation that PCNA also binds to the endonuclease XP-G, which acts at a stage preceding DNA synthesis, suggested an additional role for PCNA (Gary et al., 1997). Indeed, PCNA is loaded specifically at the site of the XP-G incision, 3′ of the lesion to be repaired. A protein important for the damage-recognition step in the NER pathway is XP-A. Immunofluorescence studies on wild-type or XP-A mutant cells showed that, upon UV irradiation, the nuclear pattern and localisation of PCNA at the sites of DNA damage are influenced by the presence or absence of a functional XP-A protein, suggesting a role for PCNA in the early steps of the NER process (Aboussekhra and Wood, 1995; Li et al., 1996; Miura and Sasaki, 1996). Thus, PCNA might help to recruit the essential proteins XP-G and XP-A to the site of the lesion. After the incision has been made, PCNA can promote the transition to the next steps by binding pol δ (or pol ε) for re-synthesis of the complementary strand and subsequently recruiting Lig1 for the final ligation step.

PCNA also functions in a step preceding DNA synthesis in MMR. Early studies showed that PCNA interacts with mispair-binding proteins MSH2, MSH3 and MSH6 and enhances their mispair binding specificity (Clark et al., 2000; Flores-Rozas et al., 2000; Kleczkowska et al., 2001). Recently, Lau and Kolodner have shown that PCNA and MSH2-MSH6 form a stable ternary complex on newly replicated DNA and that this complex is transferred from PCNA to mispaired bases in an ATP-dependent fashion (Lau and Kolodner, 2003). Thus, PCNA has also a role in recruiting and coordinating repair proteins in the MMR pathway.

PCNA similarly functions in long-patch BER in vitro, where the DNA synthesis step is dependent upon pol δ and/or pol ε (Klungland and Lindahl, 1997; Fortini et al., 1998; Gary et al., 1999; Pascucci et al., 1999; Levin et al., 2000; Matsumoto, 2001). Moreover, Matsumoto et al. have identified a PCNA-dependent pathway in *Xenopus laevis* for repairing abasic sites, which is an alternative to the pol β-dependent mechanism (Matsumoto et al., 1994; Matsumoto et al., 1999). This reaction depends on AP endonuclease, Fen1, RF-C and pol δ. The observation that PCNA physically interacts with the AP endonucleases Apn and Apn2, and the uracil DNA glycosylase UNG (Dianova et al., 2001; Krokan et al., 2001; Unk et al., 2002) supports the notion that PCNA participates both in the incision and in the resynthesis steps of BER.

A recent observation provided a link between PCNA and the post-replicational RAD6-dependent repair pathway (Hoege et al., 2002). Essential elements of this pathway are the ubiquitin-conjugating enzymes RAD6 and the MMS2-UBC13 heterodimer, the RING-finger proteins RAD18 and RAD5, and the small ubiquitin-related modifier (SUMO)-conjugating enzyme UBC9. PCNA is mono-ubiquitylated by RAD6 and RAD18, multi-ubiquitylated in an MMS2, UBC13 and RAD5-dependent manner, and conjugated to SUMO by UBC9 (Hoege et al., 2002). These modifications affect a specific residue (K63) of PCNA and they are essential for damage-induced DNA repair, differentially affecting resistance to DNA damage.

An alternative pathway for DNA damage tolerance in eukaryotic cells is translesion synthesis (TLS), which relies on specialised polymerases able to carry on DNA synthesis on a damaged template (Goodman, 2002; Hubscher et al., 2002). This pathway plays a major role in S phase, where replication forks can stall at damaged sites, owing to the inability of replicative pols to replicate damaged DNA. Replication fork stalling can lead to DSBs, which trigger S phase checkpoint mechanisms leading either to recombinational repair, which gives an increased incidence of deletion/duplication and chromosomal alteration, or to apoptosis. In order to avoid such dramatic consequences, cells can replace replicative polymerase at stalled forks with specialised TLS polymerases (see hypothetical model in Fig. 2) that can incorporate a nucleotide in front of the lesion, and thus create a 3′-OH primer that can be extended by replicative polymerases. Examples of

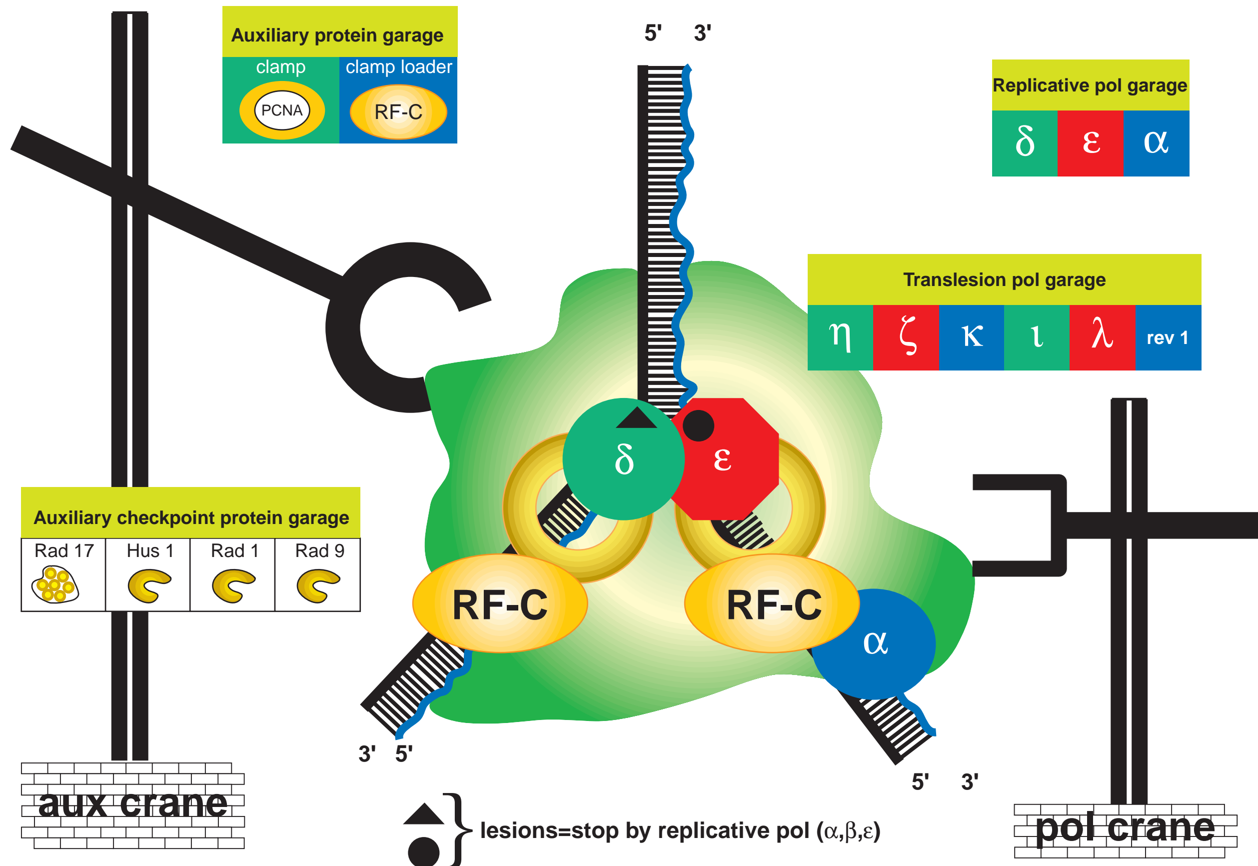

Fig. 2. A hypothetical model for the interplay between DNA polymerases involved in replication and translesion DNA synthesis. During DNA synthesis, the DNA replication machinery [schematically drawn as the pol δ/ε holoenzyme (pol δ/ε, RF-C, and PCNA)], eventually meets lesions on the DNA (represented with black symbols). Pol δ and ε are unable to traverse DNA lesions and their arrest causes the block of the replication fork. The current model predicts the existence of subnuclear compartments or foci (the 'garages' in the drawing), where replicative or translesion (TLS) polymerases and their cognate auxiliary proteins are stored. When a replication fork stalls, checkpoints are activated leading to the recruitment of specific factors at the lesion through a yet unidentified machinery (drawn as an 'auxiliary crane'). Consequently, replicative polymerases are lifted off and replaced by specialised TLS pols by a 'polymerase crane'. After damage bypass, the normal replication machinery is reconstituted through an inverse mechanism. PCNA constitutes the common element to all these pathways, providing essential interactions with both replicative and TLS polymerases, as well as acting in the checkpoint process.

these TLS enzymes are pol η, pol ι, pol κ and pol λ (Hubscher et al., 2002). Recent results have shown that the lesion bypass activity of these enzymes is increased by physical interaction of PCNA (Haracska et al., 2001a; Haracska et al., 2001b; Haracska et al., 2002; Maga et al., 2002), and immunofluorescence studies have shown that pol κ and pol ι accumulate at stalled replication forks following DNA damage (Kannouche et al., 2002). Since TLS requires interplay between these specialised polymerases and the replicative pol δ and pol ε, PCNA appears to be the ideal candidate for coordinating their functions, as well as for recruiting them at the replication fork (Fig. 2).

A mutation in the gene encoding pol η has been shown to be associated with the variant form of the genetic disease *Xeroderma Pigmentosum* (XP-V) (Masutani et al., 1999). Pol η binds to PCNA (Haracska et al., 2001b), as does the product of another disease-linked gene, the RecQ-like DNA helicase WRN. Mutations in WRN cause increased genetic instability, leading to the disease Werner Syndrome. WRN is involved in the recombinational repair of double-strand breaks and has been shown to interact with PCNA through its N-terminal domain (Lebel et al., 1999). Moreover, WRN also physically interacts with pol δ and Fen1, two other PCNA-binding proteins, stimulating their catalytic activities (Kamath-Loeb et al., 2000; Brosh et al., 2001; Kamath-Loeb et al., 2001). The role of PCNA in the recombinational repair pathway might thus depend on the WRN helicase.

Given the central role of PCNA in almost all DNA repair pathways, an intriguing scenario in which PCNA is bound to the DNA and works as general recruiting agent for specific factors can be envisaged. Depending on the kind of damage, the chromosomal context and the particular moment of the cell cycle, PCNA might help to create an optimal 'window' for the action of a particular repair pathway, allowing access to the lesioned DNA only to a specific set of proteins. Indeed, PCNA been shown to facilitate the catalytic turnover of repair-specific enzymes involved in the incision step of NER, presumably through interaction with repair-specific proteins (Nichols and Sancar, 1992). Moreover, the tight association of p21 (CIP1/WAF1) with PCNA in the nucleus following DNA

damage (Li et al., 1996) suggests that p21, together with PCNA, can link DNA replication, DNA repair and cell-cycle progression. For example, upon DNA damage, p21 may bind to PCNA and this may contribute to the XP-A and XP-G-dependent damage-recognition and incision steps. If the repair machinery fails to repair the lesions promptly, p21 and PCNA may continue to accumulate at those damaged sites, recruiting alternative factors, which, in turn, determine the cell's fate.

PCNA in cell cycle control

PCNA interacts with several eukaryotic cell cycle proteins (Fig. 3). It binds to cyclin-CDK complexes (Xiong et al., 1992), as well as to the CDK inhibitor p21 (Gulbis et al., 1996; Knibiehler et al., 1996). Moreover, Zhang et al. have reported the existence of PCNA-p21/CDK-cyclin quaternary complexes (Zhang et al., 1993).

Biochemical studies showing that PCNA interacts with the S-phase-specific CDK2–cyclin-A complex, suggest a functional role for binding of PCNA to cyclin-CDK complexes (Koundrioukoff et al., 2000). Physical binding of CDK2 to the C-terminal region of the PCNA trimer produces an active ternary PCNA–CDK2–cyclin-A complex. PCNA appears to act as a connector between CDK2 and its substrates (e.g. RF-C and Lig1), stimulating their phosphorylation. PCNA might thus target the CDK2–cyclin-A complex to PCNA-binding DNA replication proteins. This might represent an important regulatory mechanism for the recruitment of specific proteins to sites of DNA replication. For example, in mammalian cells two immunologically distinct pol-α-primase subpopulations have been identified (Dehde et al., 2001), which differ in the phosphorylation status of the p68 regulatory subunit of pol α. This post-translational modification is catalysed by the CDK2–cyclin-A complex. By using monoclonal antibodies selective for the two enzyme populations it was shown that only the phosphorylated enzyme co-immunoprecipitates with cyclin A and colocalizes with replication factories.

DNA damage, senescence or differentiation of cells through either p53-dependent or -independent pathways induces the expression of the p21 protein, which blocks progression from G1 to S phase of the cell cycle. p21 binds to CDKs through its N-terminal region and to PCNA through its C-terminal region (Chen et al., 1995; Luo et al., 1995; Chen et al., 1996; Moskowitz et al., 1996). In vitro binding of p21 to PCNA results in inhibition of DNA replication but not of PCNA-dependent NER. Biochemical analyses showed that p21 efficiently inhibits DNA elongation during processive synthesis by pol δ (or pol ε) but not during the short gap-filling activity required in NER reactions (Podust et al., 1995; Shivji et al., 1998). These results suggest that PCNA is an essential mediator of the regulatory action of p21 (Waga et al., 1994c). Recent results have shown that, in terminally differentiated cardiomyocytes, cell cycle arrest is dependent on the maintainance of high levels of p21, which, in turn, are required for the downregulation of PCNA (Engel et al., 2003). One possible explanation is that p21 prevents PCNA from binding

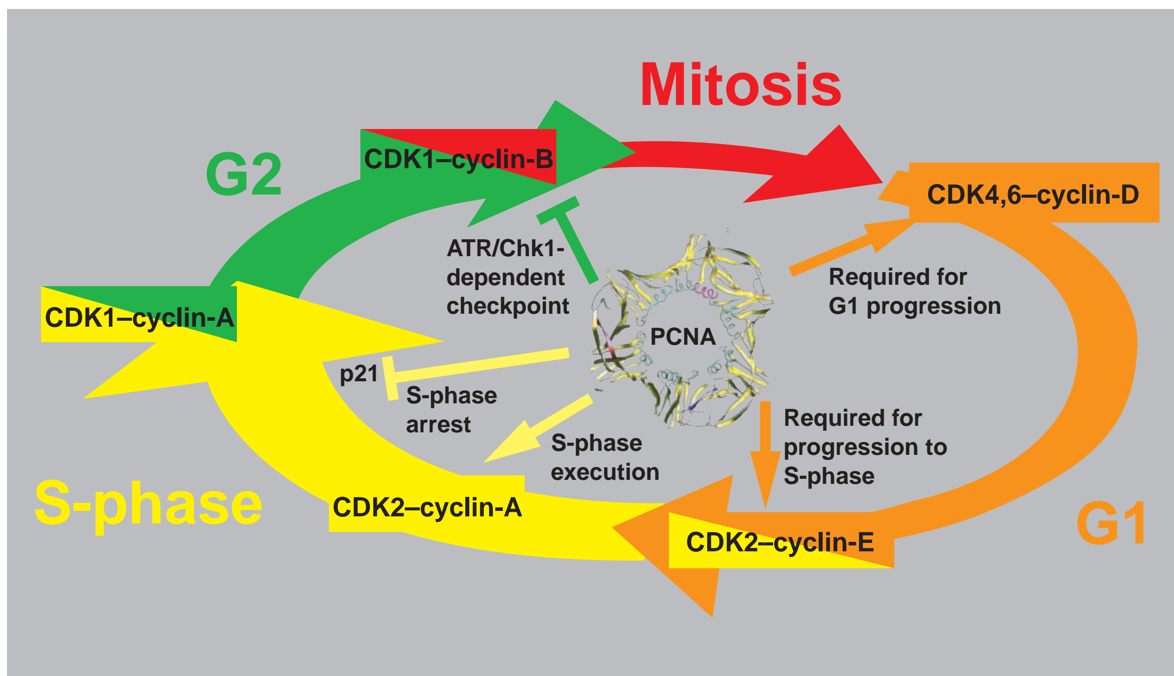

Fig. 3. The interactions between PCNA and cell cycle regulatory networks. The eukaryotic cell cycle can be divided into the four phases: G1 (green), where cells grow in size, assess their metabolic status and get ready to divide; S (yellow), where the actual genome duplication takes place; G2 (green), where cells check for completion of DNA replication and prepare to divide and M (red), where mitosis and cytodieresis take place. Each phase is under the general control of specific CDK-cyclin complexes: the CDK4,6–cyclin-D complex regulates progression through G1, CDK2–cyclin-E is involved in regulating the transition from G1 to S phase (also known as the restriction point), CDK2–cyclin-A and CDK1–cyclin-A act throughout the S phase, whereas CDK1–Cyclin-B regulates mitosis. Moreover, specific checkpoint mechanisms can be activated halting the progression through the cell cycle in case problems arise. PCNA forms complexes with all these CDK–cyclin complexes as well as with critical checkpoint proteins, transducing both positive (indicated as arrows) and negative signals (indicated as T-bars).

to the cell cycle or DNA replication machinery, thus targeting the protein for destruction. Indeed, p21 can form a stable complex with PCNA on DNA, preventing further interaction with the replication proteins RF-C and pol δ (Waga et al., 1994b).

Together, these observations allow us to envisage a possible scenario in which PCNA is both a transducer and a target of positive and negative signals. Binding of PCNA to cyclin-CDK complexes might help to bring these regulatory proteins to their targets, whereas disruption of these interactions by the competitive binding of p21 is a signal for DNA replication arrest.

DNA replication proteins appear to be organised in large macromolecular complexes or 'factories', which in S-phase colocalise with the sites of newly synthesized DNA (Hozak et al., 1993; Hozak et al., 1994; Jackson, 1995). It has been shown that the PCNA-binding domain located in the N-terminal half of Lig1 and RF-C is necessary and sufficient to target these proteins to replication foci (Montecucco et al., 1998). Moreover, association of Lig1 with PCNA as well as its recruitment to replication factories is regulated by phosphorylation in a cell-cycle-dependent fashion (Rossi et al., 1999). These results suggested that PCNA acts as a recruiting center for DNA replication proteins, helping to build the dynamic replisome at the replication fork.

PCNA and its partners in other cellular events

PCNA and chromatin metabolism

PCNA shows little turnover at established replication sites, which suggests that it remains associated with the replication machinery through multiple rounds of Okazaki fragment processing (Sporbert et al., 2002). Moreover, PCNA can diffuse to newly assembled replication sites. Activation might therefore occur through changes in local chromatin structure and accessibility. In fact, some PCNA-binding proteins function in the maintenance of chromosome structure. Chromatin assembly factor 1 (CAF-1), for example, binds to PCNA and is a molecular chaperone that deposits histones H3 and H4 onto newly replicated DNA. The role of PCNA is to topologically mark newly replicated DNA for chromatin assembly by CAF-1 (Shibahara and Stillman, 1999). Genetic experiments in yeast suggest that binding of PCNA to CAF-1 effects epigenetic inheritance of DNA and chromatin structures during S-phase of the cell cycle (Zhang et al., 2000). In addition, nucleosome assembly is triggered at nicks and gaps in the DNA and requires PCNA. PCNA, through its binding to the p150 subunit of CAF-1, is recruited to damaged DNA (Moggs et al., 2000). Furthermore, Hasan et al. have proposed that binding of PCNA to the transcriptional coactivator p300 is important for chromatin remodelling at DNA lesions to facilitate PCNA function in DNA repair (Hasan et al., 2001).

PCNA and gene expression

The methylated CpG sequences of the mammalian genome are heritable and affect gene expression. The enzyme responsible for inheritance of the methylated status is MeCTr. PCNA can bind to MeCTr, which favours the idea that maintenance of the methylation pattern in the genome is also dependent on PCNA (Chuang et al., 1997; Iida et al., 2002).

PCNA and sister chromatin cohesion

Sister chromatin cohesion is essential for the coordinated separation of replicated chromosomes into daughter cells during mitosis. A component of the cohesion complex called Ctf7p (for chromosomal transmission fidelity) links mitotic chromosome structure to the DNA replication machinery, since it is only required during S phase. This connection might involve PCNA since it genetically interacts with Ctf7p (Skibbens et al., 1999). A proteomic approach to identify PCNA-binding proteins in human cell lysates identified another cohesion factor that can bind PCNA, CHL12 (Ohta et al., 2002). The fact that CHL12 can in addition bind to the four small subunits of RF-C (see above) suggests that it might act as an alternative clamp loader for PCNA and further indicates a connection between chromatid cohesion and DNA replication.

PCNA and apoptosis

It has been known for some years that both the growth arrest and DNA damage gene product Gadd45 and the myeloid differentiation primary response gene product MyD118 can physically interact with PCNA (reviewed by Jonsson and Hubscher, 1997). More recently, Vairapandi et al. found that similar domains in Gadd45 and MyD188 mediate the interaction with PCNA (Vairapandi et al., 2000). When mutants of Gadd45 or MyD188 that lack the PCNA-interaction domain are ectopically expressed, they induce apoptosis more efficiently. This led to the conclusion that the interaction of Gadd45 and MyD188 with PCNA triggers negative growth control.

ING1 is another protein that might link PCNA to inhibition of cell growth and/or apoptosis. It is a potential tumour suppressor and the isoform $p33^{ING1}$ contains a PIP motif and physically interacts with PCNA (Scott et al., 2001). Cells expressing ING1 mutants that cannot bind to PCNA are protected from UV-induced apoptosis.

Conclusions and perspectives

PCNA plays a coordinating role for numerous proteins involved in many processes involving DNA. As a consequence of their interaction with PCNA, several enzymes show an increase in catalytic efficiency. Interestingly, most of these enzymes either recognise specific structures on DNA or have limited DNA-sequence specificity. An intriguing possibility is that proteins that have low sequence specificities use PCNA as an adapter to facilitate interaction with their DNA substrates. In an analogous way, PCNA appears to be able to facilitate the interaction between cyclin-CDK complexes and their substrates. PCNA also seems to be one of the ways the cell recruits particular proteins to a particular place at a particular moment.

How are these interactions co-ordinated? Table 3 summarises the known affinities of different partners for PCNA. All are in the nanomolar range, even if there are some differences. Pol δ, pol ε, RF-C and p21 show the strongest interactions, whereas Fen1 and Lig1 bind with somewhat lower affinity to PCNA. This suggest that p21 could be an effective competitor of Fen1 and Lig1 binding, which is consistent with biochemical studies. However, the generally similar affinities

Table 3. Binding affinities of different PCNA partners

Partner	Affinity (K_D, nM)	References
pol δ/RF-C holoenzyme*	10-20	Maga and Hübscher, 1996
RF-C	21	Gomes and Burgers, 2001
pol ε	10	Maga and Hübscher, 1995
pol λ	25-30	Maga et al., 2002
Fen1	60	Warbrick et al., 1997
Lig1	40-80*	Levin et al., 1997
p21	10 – 15	Knibiehler et al., 1996; Zheleva et al., 2000

*Value estimated from inhibition data, assuming a K_D of PCNA for pol δ of 10-20 nM.

for PCNA of its partners indicate that some additional level of regulation must exist. One possible mechanism, supported by experimental data, is regulation of the levels and the localisation of the different PCNA-interacting proteins. This is well exemplified by the dynamics of p21 and PCNA during the cell cycle. In normal quiescent fibroblast, p21 and PCNA are present in the cell at an almost equimolar ratio but show different distributions in the nucleus (Li et al., 1996). Upon entering in S phase, PCNA levels rise, whereas p21 levels remain low. This allows PCNA to bind other proteins without being challenged by p21, and indeed several replication proteins colocalise in discrete foci with PCNA during S phase. Upon DNA damage, by contrast, p21 levels increase dramatically and p21 colocalises at PCNA foci, which is consistent with the idea that it can displace other partners from PCNA by virtue of its high binding affinity. Similarly, Chuang et al. have shown that the relative levels of p21 and MeCTr, another partner competing for PCNA binding, are correlate with the proliferative state of the cells. In normal cells, p21 levels are higher than MeCTr levels whereas, in SV40-transformed cells, MeCTr is in excess over p21. Accordingly, PCNA can be co-immunoprecipitated with MeCTr only from transformed cells. Upon DNA damage, p21 colocalises with PCNA at repair sites and can effectively prevent methylation of damaged DNA by precluding MeCTr interaction with PCNA, but only in non-transformed cells (Chuang et al., 1997). Thus, it is clear that expression levels as well as cellular localisation play an important role in regulating the balance between PCNA-interacting proteins.

Another possible mechanism of regulation might lie in the homotrimeric structure of PCNA which, in principle, can allow it to bind different partners simultaneously. There is some indirect evidence suggesting that this can happen. For example, p21 has been shown to bind to PCNA with a 3:1 stoichiometry, which is consistent with the notion that it prevents other interactions by occupying all the possible binding sites on the trimer (Gulbis et al., 1996). Biochemical studies suggested that another interacting partner, Gadd45, binds to PCNA with a 2:1 stoichiometry, thus leaving a binding site free for other proteins to interact (Hall et al., 1995). Also, PCNA can have distinct binding modes. For example, in the absence of DNA, Fen1 and Apn2 interact with PCNA mainly through the interdomain-connecting loop; however, when PCNA encircles the DNA, the C-terminal domain of PCNA becomes more important for binding of the two partners (Gomes and Burgers, 2000; Unk et al., 2002). The switch between alternative binding sites might be another way in which PCNA can regulate its interaction with different partners.

Very recently, experiments with the heterotrimeric PCNA from the hyperthermophylic archeon *Sulfolobus solfataricus* suggested that one subunit of the trimer interacts with the archeal polymerase, whereas the other two subunits interact with the *S. solfataricus* homologs of Lig1 and Fen1, respectively (Dionne et al., 2003).

Post-translational modifications of both PCNA and its binding proteins, such as acetylation, SUMOylation or phosphorylation, can also positively or negatively regulate the interaction. We have now a profile of the PCNA partners. What is needed is a more mechanistic view of the processes: we must determine binding constants, the order of activation and other biochemical parameters. This will enable us to understand the mechanisms that spatially and temporally regulate the ability of PCNA to 'dance' with the right partner at the right time.

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