PCNA on the crossroad of cancer

Ivaylo Stoimenov* and Thomas Hellday*†

*Department of Genetics, Microbiology and Toxicology, Stockholm University, S-106 91 Stockholm, Sweden, and †Gray Institute for Radiation Oncology and Biology, Old Road Campus Research Building, Roosevelt Drive, University of Oxford, Oxford OX3 7DQ, U.K.

Abstract
Cancer is caused by genetic changes that often arise following failure to accurately replicate the DNA. PCNA (proliferating-cell nuclear antigen) forms a ring around the DNA to facilitate and control DNA replication. Emerging evidence suggests that PCNA is at the very heart of many essential cellular processes, such as DNA replication, repair of DNA damage, chromatin structure maintenance, chromosome segregation and cell-cycle progression. Progression of the DNA replication forks can be blocked by DNA lesions, formed either by endogenous damage or by exogenous agents, for instance anticancer drugs. Cellular response often results in change of PCNA function triggered either by specific post-translational modification of PCNA (i.e. ubiquitylation) or by exchange of its interaction partners. This puts PCNA in a central position in determining the fate of the replication fork. In the present article, we review PCNA modifications and interaction partners, and how those influence the course of events at replication forks, which ultimately determines both tumour progression as well as the outcome of anticancer treatment.

Introduction
The accurate transmission of genetic material to daughter cells is critical for the control of cell proliferation and survival. Loss of accurate replication results in mutations that, in certain unfavourable circumstances, allow cells to bypass normal growth control and genome stability checkpoints. Such changes are sufficient to transform a normal cell into a cancer cell.

Understanding the regulation of DNA replication is not only essential for gaining insights into tumour development, but is also critical in cancer treatments. The majority of drugs used for cancer treatments result in DNA damage, which in turn causes more toxic lesions during DNA replication. Replicating cells will obtain more toxic lesions that trigger cell death which results in tumour shrinkage, while non-dividing healthy cells are spared.

It is clear that there are several choices for the damaged replication forks: the replication fork may either pause in front of the damage to allow repair [1] or it may bypass the damage using specific polymerases [2], or it may convert the damage into a second lesion that in turn will either trigger a different DNA-repair pathway or will activate programmed cell death [3]. Emerging evidence suggests that modifications of a key replication protein, PCNA (proliferating-cell nuclear antigen) play an important role in deciding which pathway is to be used.

PCNA is an evolutionarily well conserved protein found in all eukaryotic species from yeast to humans, as well as in archaea. PCNA functions are associated not only with DNA replication, but also with other vital cellular processes such as chromatin remodelling, DNA repair, sister-chromatid cohesion and cell-cycle control. PCNA was originally described as an antigen for autoimmune disease in systemic lupus erythematosus patients, detected only in the proliferating-cell populations [4]. Later it was shown that expression levels of PCNA during cell cycle are differential and associated with proliferation or transformation [5,6]. In the following years, much has been done to uncover the role of PCNA in DNA replication, and one of the first functions assigned was a sliding clamp for DNA polymerase δ [7,8]. However, the progress in the field not only strengthened the importance of PCNA, but also even placed PCNA at the crossroad of many essential pathways. The crucial involvement of PCNA in cellular proliferation and its tight association with cancer transformation resulted in the frequent use of PCNA as a diagnostic and prognostic cell-cycle marker [9]. As a protein with significant clinical importance, extensive research has been dedicated to determine PCNA structure, function and therapeutic applications.

Structural considerations for PCNA functions
Alignment of amino acid sequences of PCNA from different species shows considerable homology and evolutionary conservation (Figure 1A). Even more striking is the similarity in molecular structure: yeast and human PCNA share 35% amino acid sequence identity, but their three-dimensional structure is highly superimposable [10].

The eukaryotic PCNA consists of three identical monomers, interacting head-to-tail and forming a homotrimer with
Figure 1 | Sequence and structural features of PCNA

(A) Amino acid sequence alignment of PCNA from human (*Homo sapiens*), mouse (*Mus musculus*), chicken (*Gallus gallus*), *Drosophila melanogaster* and *Saccharomyces cerevisiae*. (B) Front view of the three-dimensional structure of human PCNA, different subunits are coloured red, green and blue, interdomain connecting loop is coloured yellow, and important amino acid residues are designated with yellow balls (small balls indicate Tyr211, and big balls indicate Lys164). (C) Side view of human PCNA showing a smooth front side and visible protrusions in the back side.

---

an overall toroidal shape (Figures 1B and 1C). Each monomer has two very similar domains linked by an interdomain connecting loop (Figure 1B). Interaction between those domains clearly resembles the interaction between individual monomers, which gives the molecule a pseudo-hexagonal symmetry. The homotrimer has a distinguishable front and back side (Figure 1C). The front side is believed to be the interface for protein interactions, whereas the back side might have a different function (see below).

The overall surface of the PCNA molecule has a negative charge; however, many amino acid residues facing the hole form a positive potential field. The positively charged interior side interacts with the negative charges of the sugar-phosphate backbone of DNA and that interaction is believed to facilitate sliding of PCNA along DNA [11].

**Post-translational modifications of PCNA**

PCNA is the subject of several post-translational modifications that affect its function. Although the vast majority of PCNA molecules in the cell are not post-translationally modified, it is believed that PCNA is ubiquitylated, phosphorylated, acetylated, methylated and even SUMOylated.
One of the well-documented post-translational modifications of PCNA is ubiquitylation. First described in yeast and later found in mammalian cells [12–14], ubiquitylation is targeted on a highly conserved lysine residue at position 164. There are two distinct ubiquitylation events: attachment of a single ubiquitin moiety (mono-ubiquitylation) and building of a polyubiquitin chain via an ‘unusual’ Lys63 interubiquitin linkage (Lys63 polyubiquitylation) [12]. Each of these requires a separate set of modification enzymes and each is believed to result in different protein interactions. Mono-ubiquitylation is achieved by consequent action of an ubiquitin-activating enzyme E1, specific ubiquitin-conjugation enzyme E2 (which in humans might be either hRad6A or hRad6B) and RING (really interesting new gene)-finger-containing E3 ubiquitin ligase (Rad18). Building a polyubiquitin chain requires the heterodimeric ubiquitin-conjugation enzyme Ubc13–Mms2 and a specific RING-finger-containing E3 ubiquitin ligase. In yeast, this ligase is believed to be Rad5, whereas, in humans, three different enzymes are shown to polyubiquitylate PCNA on position 164 by Lys63 linkages, SHPRH (SNF2 histone linker PHD RING helicase), HLTF (helicase-like transcription factor) and RNF8 (RING finger protein 8), at least in vitro [15–18]. However, it is currently unknown whether mono-ubiquitylation always precedes PCNA poly-ubiquitylation. Attachment of a single ubiquitin molecule results in a new surface property of PCNA and induces a change in the interaction partners. This is a key concept in the proposed polymerase-switch during one of the DNA-damage-avoidance pathways: TLS (translesion synthesis).

In yeast, another PCNA modification, targeted on the same conservative lysine residue at position 164, has been described [12]. This modification involves attachment of a protein, designated as SUMO (small ubiquitin-related modifier). SUMOylation of PCNA in yeast might also occur at another position, Lys127, which is not present in higher eukaryotes (Figure 1A). Attachment of a SUMO on yeast PCNA is a signal for recruitment of a specific helicase (ySrs2), which blocks recombinational events during replication [19]. However, SUMOylation of PCNA has not yet been characterized in mammals, and the precise function of this modification remains to be uncovered. In yeast, even a poly-SUMOylation on PCNA has been described recently [20]. Poly-SUMOylation is controlled by DNA and is proposed to be a consequence of PCNA loading on DNA [21].

The data on modification of PCNA by phosphorylation are contradictory. The first report on PCNA phosphorylation [22] was investigated further by Naryzhny and Lee [23]. The conclusion in the latter paper was that a highly phosphorylated protein was co-purified with PCNA, and Naryzhny and Lee [23] argued that phosphorylation on PCNA does not happen in vivo. It should be noted that both research groups used cells derived from different mammals. However, more recent data clearly show phosphorylation on Tyr211 [24]. Using electrospray ionization MS/MS (tandem MS), phosphorylation on Tyr211 in both human and mouse cells was demonstrated, strengthened by the use of phospho-specific antibodies. It is estimated that approx. 6% of chromatin-bound PCNA is subjected to phosphorylation on Tyr211. This modification has been suggested to stabilize chromatin-bound PCNA as opposed to polyubiquitylation [24].

Acetylation is another modification detected on PCNA [23]. Analysis of high-resolution two-dimensional protein electrophoresis shows the presence of three forms of PCNA, two of which correspond to acetylation and hyperacetylation. The presence of the acetylated and hyperacetylated PCNA on the chromatin and localization of the non-acetylated form in the nucleosol clearly suggests a dynamic PCNA translocation dependent on acetylation status [23]. The only detailed report speculates about the involvement of PCNA acetylation in the initiation of DNA replication [23].

A new modification on PCNA has also been described: methyl esterification on many glutamic acid and aspartic acid residues [25]. Interestingly, PCNA methylation is associated with breast cancer and is believed to be cancer-specific [26]. This fact points to a possible new cancer marker; however, it is currently not known what aspect of PCNA metabolism could be altered. Methylated PCNA is commonly referred as csPCNA (cancer-specific PCNA).

PCNA-interacting partners and corresponding PCNA-interaction motifs

PCNA interacts with a plethora of proteins that are involved in many vital cellular processes (summarized in Figure 2). These include DNA replication proteins, proteins of DNA-repair machineries, cell-cycle regulators, chromatin remodelling and assembly factors, sister-chromatid cohesion proteins, transcription and translation factors, metabolic enzymes and even membrane and cytoskeletal proteins. Many of these interactions are well characterized, but it should be pointed out that some of them are only detected in vitro and their functional significance in cells remains to be discovered.

From the two PCNA-specific binding motifs described, the better studied and widely represented in proteins is the PIP-box (PCNA-interaction protein box). The consensus sequence of the PIP-box is QXX(M/L/I)XX(F/Y)(F/Y), although it sometimes might be N-terminally flanked by a KAX sequence [27]. The PIP-box has an unusual secondary structure: a 310-helix that fits quite well in the hydrophobic pocket of PCNA, which is situated in the interdomain connecting loop [28]. Interactions involving the PIP-box are hydrophobic and as such they are highly favourable in water solvents. Another PCNA-binding motif which is related to the canonical PIP-box is termed the KA-box [KA(A/L/I)(A/L/Q)XX(L/V)]. Although theoretically each PCNA trimer could interact with three different proteins at the same time and even through different PCNA-binding motifs, sometimes the size of the interacting proteins or complexes makes this scenario impossible. However, in archaea, PCNA is a heterotrimer, and each subunit shows binding preferences for either DNA polymerase, FEN1 (flap
PCNA-interacting proteins

Surface model of PCNA is shown with individual monomers coloured red, green and blue. Characteristic interfaces on PCNA are emphasized: interdomain connecting loop (purple), C-terminal tail (orange) and inner α-helices (yellow). For clarity, only one interface per subunit is shown. Different interacting proteins are grouped by function, their respective PCNA-interactive domains are denoted by coloured boxes, and the respective PCNA interface (if known) is presented as a coloured circle.

BLM, Bloom’s syndrome protein; CDC25C, cell-division cycle 25C; HLTF, helicase-like transcription factor; hMYH, human MutY homologue; ING1b, inhibitor of growth 1b; MCL1, myeloid cell leukaemia sequence 1; MDM2, murine double minute 2; MPG, methylpurine-DNA glycosylase; NTHL1, nth endonuclease III-like 1; PAF, PCNA-associated factor; PARP-1, poly(ADP-ribose) polymerase 1; Pol., polymerase; RNF8, RING finger protein 8; SHPRH, SNF2 histone linker PHD RING helicase; UNG2, uracil-DNA glycosylase 2; WRN, Werner’s syndrome protein; WSTF, Williams–Beuren syndrome transcription factor.

dNA endonuclease-1) or DNA ligase I [29], suggesting a model of co-operative action. Eukaryotic PCNA, on the other hand, is a homotrimer and binding preferences are not observed, although similar co-operation between DNA polymerase, FEN1 and DNA ligase I cannot be excluded.

PCNA interactions play a key role in DNA replication

Interestingly, existing evidence suggests interaction with PCNA occurs only at the front side of the homotrimer. This makes interactions mutually exclusive when the PCNA partner is a huge protein complex. A binary mode of those interactions places PCNA at the cross-point of the several switching events that are associated with the progression of the replication forks. The very first of these events concerns exchange of the priming replicative enzyme, DNA polymerase α-primase, with a processive DNA polymerase. The general model of DNA replication involves DNA polymerase α-primase as a complex, which initiates DNA synthesis. This is achieved by synthesis of a short RNA primer (∼10 nucleotides), later extended with a short DNA sequence of an additional ∼20 nucleotides. The polymerase α-primase complex, however, has a high error rate owing to a lack of proofreading and possesses limited processivity. In contrast with many other DNA polymerases, DNA polymerase α-primase does not need PCNA to function, and furthermore, PCNA might even repulse it from the replication fork. When priming of DNA is complete, processive DNA synthesis is needed in

©The Authors Journal compilation ©2009 Biochemical Society
In eukaryotes, the PCNA clamp is loaded around DNA by an RFC (replication factor C) protein complex. This is achieved by temporarily breaking interactions between subunits of PCNA and reassembling the homotrimer structure around DNA. The subunits hRFC140, hRFC36 and hRFC40 interact with the front side of PCNA in an ATP-dependent manner. The whole complex is recruited to DNA, where the RFC–PCNA complex is able to bind the template–primer junction generated by polymerase α-primase [31]. The binding of RFC and the loading of PCNA on to the DNA displaces polymerase α-primase. However, the front side of PCNA, which is the side for interaction with polymerase δ or ε, is blocked by the bulky RFC complex. A conformational change in RFC facilitated by ATP hydrolysis disrupts the RFC–PCNA complex and clears the way for PCNA and polymerase δ/ε interaction. In this manner, PCNA, with the help of RFC, conveys the DNA synthesis from one polymerase (α) to another one (δ or ε).

Another event orchestrated by PCNA during DNA replication is the maturation of Okazaki fragments. These fragments are remnants of discontinuous synthesis of the lagging strand. When the polymerization of a newly primed fragment reaches the primer of the previous fragment, several enzymatic activities are needed to complete the replication, all stimulated by PCNA. Polymerization continues with a strand displacement of the ribonucleotides of the old primer by a process called nick translation. This is carried out by the replicative polymerase in conjunction with a helicase/nuclease yDNA2. Displaced nucleotides form a flap structure, which hinders the polymerase processivity and eventually triggers dissociation of the DNA polymerase from PCNA. Another PCNA-interacting protein is recruited, FEN1, which cleaves the flap overhang. The enzymatic activity of FEN1 is stimulated by PCNA interaction, and the result of the reaction is a nick in the double-stranded DNA. That nick is sealed by yet another PCNA-interacting enzyme: DNA ligase I. The analysis of three-dimensional structures suggests the binding of DNA ligase I to PCNA via a PIP-box. This interaction is believed to trigger a conformational change that allows DNA ligase I to adopt a more organized structure around DNA and will eventually lead to the nick being sealed. It appears that co-ordination of Okazaki fragment maturation occurs in a stepwise fashion and each step might involve a different PCNA interaction.

PCNA at the crossroad of the DNA-damage-avoidance switch

Conveying replication initiation to elongation and later to the final ligation step (once in the leading strand synthesis and multiple times in the lagging strand) is not the only process for PCNA to coordinate. However, this is believed to happen under normal circumstances of undisturbed DNA replication. If DNA contains damage once replication has begun, the replication fork may encounter that damage, and it may stall and eventually might collapse. Stalled replication forks are the signal for activation of special pathways involved in DNA-damage avoidance. Again, the control is believed to be carried out through interactions with PCNA; however, the key step is a post-translational modification of the PCNA molecule. The current model suggests that, at the stalled replication forks, PCNA becomes mono-ubiquitylated in a Rad6A (or Rad6B)–Rad18 dependent manner on an evolutionarily conserved position: a lysine residue at position 164. This is a signal for recruitment of a special polymerase, which is able to continue DNA replication even on a damaged template. Humans, together with other eukaryotes, have several such polymerases commonly referred as TLS polymerases [32]. Mono-ubiquitylation of PCNA is a key event in the exchange of the conventional replicative polymerase (δ or ε) with a TLS polymerase, since this modification increases the affinity of the TLS polymerase for PCNA [33]. Indeed, UBMs (ubiquitin-binding motifs) are found in many TLS polymerases involved in DNA damage bypass mechanisms, for example in Y-family TLS polymerases (η, ι and κ and Rev1), together with PIP-boxes in some polymerases (η and ι) or another domain in other polymerases [BRCT (BRCA1 C-terminal) domain in Rev1]. However, the initial hypothesis that the PCNA-interaction motif and UBM co-operate in the recruitment of TLS polymerases at sites of damage has recently been challenged. The latest results suggest the more important role of the PCNA-interaction motif and assign a secondary function to UBM [34]. However, mono-ubiquitylation of PCNA might be the mechanism of repulsion of the replicative polymerase and a platform for recruitment of accessory proteins in order to bypass a DNA lesion. Although TLS polymerases can bypass damaged bases, their processivity and fidelity are very low, which imposes the idea that these polymerases act on a short sequence around the damage. Little is known of whether there is a back switch to a replicative polymerase (δ or ε) or whether TLS polymerases are simply filling gaps simultaneously and/or behind the replication forks; however, it has been proved that mono-ubiquitylation of PCNA is directly reversible at least after UV damage [35]. The presence of a deubiquitylating enzyme in humans, which can act on PCNA (ubiquitin-specific protease 1) implies a mechanism for recycling of intact PCNA [35].

TLS is one of the two main pathways of DNA-damage avoidance during replication. The second pathway is poorly characterized, but is believed to involve a recombination event around the forks, which is independent of Rad52 [36]. However, from genetic studies and models in yeast, it is
proposed that this pathway requires a modification of PCNA, but this time that is polyubiquitylation on the same residue: Lys164. Building a polyubiquitin chain on PCNA is achieved by the consecutive action of specific enzymes and results in a chain where the ubiquitins are linked via the ‘unusual’ Lys63 position. This is not a signal for proteasome degradation, but rather for recruitment of recombinational factors. However, the levels of PCNA polyubiquitylation in mammals are extremely low, and usually only mono-ubiquitylation is easily detectable, which might be a direct result of different utilization of the DNA-damage-avoidance pathways or the existence of another model in higher eukaryotes.

The model built from yeast studies suggests an elegant mechanism for PCNA in conveying the replication to one or another DNA-damage-avoidance pathway [37]. If the fork encounters damage, which obstructs the replication progression, the cellular machinery utilizes the quicker but error-prone pathway (TLS) by mono-ubiquitylation of PCNA. If that is enough, the damage is bypassed and replication continues. If none of the TLS polymerases is able to cope with the problem, a second more massive protein modification event takes place: polyubiquitylation of PCNA. This is believed to recruit factors for recombinational avoidance of the damage and is generally considered to be error-free. PCNA SUMOylation is suggested to be a mechanism for suppression of unwanted recombination during replication, since it is normally detected in S-phase or it might be an event which places the PCNA ubiquitylation in another phase of the cell cycle.

More recently, new insights were added to the model, suggesting coupling between DNA-damage-avoidance pathways and DNA replication [38]. Accumulation of ssDNA (single-stranded DNA) regions after replication fork stalling might be the primary signal for recruitment of the PCNA ubiquitylation machinery. Those ssDNA regions are quickly covered by RPA (replication protein A). RPA may function as a mediator for recruitment of the PCNA ubiquitin ligase Rad18 at the replication forks [38].

**PCNA is an indispensable factor for DNA repair**

The role of PCNA in replication seems to be important in many aspects; however, that is not the only function of the protein. PCNA is an indispensable part of several repair pathways such as MMR (mismatch repair), NER (nucleotide excision repair) and BER (base excision repair).

MMR is a mechanism to correct misincorporated bases (mismatches) or insertion/deletion loops generated after imprecise replication. In MMR, PCNA is required not only for the actual repair synthesis, but also in the initial step of damage recognition [39]. MMR needs to discriminate between the original and newly synthesized strand in order to function properly. PCNA provides this opportunity simply because of the interactions being directional, occurring on the face of the molecule. Since PCNA is loaded on the DNA in the only possible orientation, facing the 3’-end of the daughter strand, discrimination is possible and indeed exonuclease excisions of incorrectly incorporated nucleotides in the growing strand are carried out in the 5’-3’ direction. PCNA interacts with MSH3 (MutS homologue 3), MSH6 (MutS homologue 6), MLH1 (MutL homologue 1) and EXO1 (exonuclease 1), components of MMR, from which at least MSH3, MSH6 and MLH1 have a PIP-box. Later in the pathway, repair synthesis takes place, which also depends on PCNA. However, all of these interactions are believed to be mutually exclusive, suggesting that PCNA is conveying the function from the sensor proteins (MSH3, MSH6 and MLH1) to the actual excision effectors (EXO1) and later to the polymerases [40].

NER is a pathway which deals with bulky DNA lesions, generated after interaction of DNA with certain chemicals and UV-irradiation. PCNA is proven to interact with a PIP-box of at least one of the NER proteins, the endonuclease XPG (xeroderma pigmentosum complementation group G) [41]. The main role of PCNA in NER is associated with the repair synthesis, which occurs after the reaction catalysed by XPG. However, the interaction between PCNA and XPG suggests a cross-talk between the proteins involved in different stages of NER. Mutations in XPG may cause a disorder referred to as xeroderma pigmentosum, which is tightly associated with cancer. Indeed, some of those mutations in XPG are in close proximity to the PCNA-binding site or cause a truncated protein unable to bind PCNA. There exists a patent application claiming a method for purifying cPCNA, on the basis of its specific interaction with XPG [42].

The pathway for the repair of small chemical alternation of nucleotides after exposure to oxidizing, reducing or alkylating agents as well as of detected misincorporated uracils is denoted as BER. There are two modes of BER: short-patch and long-patch. The role of PCNA is associated with the repair of DNA synthesis in the long-patch, carried by DNA polymerase δ or ε. However, PCNA is recruited to the BER machinery in the steps of damage recognition before the actual repair synthesis takes place. As can be seen in Figure 2, PCNA interacts with proteins involved in all steps of BER: glycosylases [UNG2 (uracil-DNA glycosylase 2), MPG (methylpurine-DNA glycosylases), NTHL1 (nth endonuclease III-like 1) and hMYH (human MutY homologue)], AP-endonucleases (APE1 and APE2), polymerases (DNA polymerase β, δ and ε) and even a repair cofactor XRCC1 (X-ray repair complementing defective repair in Chinese-hamster cells 1). It is possible that PCNA functions as a bridge between different BER proteins, stimulates their activities and co-ordinates the whole process.

**PCNA in chromatin assembly and maintenance**

More enigmatic is the role of PCNA in chromatin assembly and maintenance. The organization of genetic material in eukaryotes is a real barrier for many aspects of DNA metabolism, which imposes dynamic changes in the chromatin
structure, especially while the cell is dividing. During DNA replication, chromatin is completely disrupted in front of the replication fork, but is quickly restored when the fork passes. At present, it is not clear how big a region around the fork is remodelled; however, it is known that at least the nucleosomal organization is restored once the replication machinery leaves behind a couple of hundred nucleotides [43]. This suggests a very tight regulation between chromatin remodelling and DNA replication. Indeed, among the many chromatin-remodelling factors, several are believed to function around the forks. One of them is the histone chaperone complex known as CAF1 (chromatin assembly factor 1). Interestingly enough, a protein of the complex (CAF1 subunit A) possesses a PIP-box and is found to interact with the front side of PCNA. There have been studies exploring that interaction and also suggesting a mechanism for coupling of chromatin remodelling with DNA replication. The working model is based on the observation that PCNA might function as a double trimer in vivo [44,45]. The double-trimer formation is possible if two PCNA molecules are interacting with their back sides. Site-directed mutagenesis experiments mapped this interaction between the conserved Arg5 and Lys110 [44].

Double-trimer formation is highly speculative, it might possibly different molecules of the double trimer. Although two processes involve front-side PCNA interactions, but at nucleosomal assembly happening behind the fork. These processes are building the sister-chromatid cohesion and also suggesting a mechanism for coupling of chromatin remodelling with DNA replication. The working model is based on the observation that PCNA might function as a double trimer in vivo [44,45]. The double-trimer formation is possible if two PCNA molecules are interacting with their back sides. Site-directed mutagenesis experiments mapped this interaction between the conserved Arg5 and Lys110 [44]. Speculation of double-trimer formation places PCNA in the crossroad of genetic and epigenetic inheritance, with DNA replication proceeding in a chosen direction and nucleosomal assembly happening behind the fork. These two processes involve front-side PCNA interactions, but at possibly different molecules of the double trimer. Although double-trimer formation is highly speculative, it might describe some specific situation. One of the challenges of the model is to explain simultaneous loading of two PCNA molecules around DNA in opposite direction.

There are several other PCNA-interacting proteins, whose function is dedicated to chromatin remodelling and maintenance (Figure 2), although the role of the corresponding interactions has not been clearly defined. For example, DNMT1 (DNA methyltransferase 1) is an enzyme which has a PIP-box and which function is related to replication. DNMT1 methylates semi-methylated CpG sequences, resulting from semi-conservative DNA replication, which is a function dedicated to preserve the epigenetic methylation pattern. This pattern is directly connected to the chromatin structure. Another example is HDAC1 (histone deacetylase 1). HDAC1 interacts directly with PCNA in vitro, and this interaction is a proposed mechanism for recruitment of HDAC1 into distinct foci in vitro [46]. HDAC1 is involved in gene silencing by deacetylation of the core histone components and, as such, altering epigenetic information and chromatin structure.

PCNA is involved in establishment of sister-chromatid cohesion

Successful replication of the genome is one of the important steps required in cell division, while the next step is a segregation of the genetic material into progeny cells. A special structure is involved in keeping the homologous chromosomes together, termed the sister-chromatid cohesion complex (or simply cohesin). Cohesin is already established in S-phase of the cell cycle, coupling the DNA replication to sister-chromatid cohesion. An essential protein for cohesin assembly in yeast is yEco1 [in humans, the family contains ESCO (establishment of cohesion) 1 and 2] and that protein was shown to bind PCNA via a defined PIP-box [47]. Since the human protein ESCO2 was also shown to interact with PCNA, there is a clear intersection at PCNA between the two processes, building the sister-chromatid cohesion and progression of the replication forks.

Cell-cycle regulators interact with PCNA

In higher eukaryotes, PCNA functions are regulated by an interaction with one of the cell-cycle inhibitors, p21CIF1/WAF1. Binding of p21 to PCNA is mediated by a PIP-box (Figure 2), and it is one of the strongest PCNA interactions. The experimental results show that, in vitro, p21 abrogates DNA replication and MMR, but does not interfere with PCNA-dependent NER. A proposed explanation is a competitive binding on the same PCNA interface:interdomain connection loop and stronger affinity for p21 in comparison with the competitors such as DNA polymerase δ66 subunit, FEN1, DNA ligase I and the replication factor Cdt1. Stable quaternary complexes between p21, PCNA, CDK (cyclin-dependent kinase) and cyclins were reported, which suggests a more complex model of regulation. In fact, PCNA can bind CDK–cyclin couples separately from p21. For example, CDK2–cyclin A, stimulated by PCNA, is able to phosphorylate RFC, DNA ligase I and FEN1. Interestingly enough, PCNA forms complexes with cyclins alone, and, for D-type cyclins, the interactions have been mapped in a very distinct PCNA region: interior α-helices (Figure 2, yellow dot).

Cell-cycle regulators such as CDK–cyclins as well as the inhibitors of the cell cycle such as p21 and p27 are crucial components of the checkpoint system, a barrier very often overcome in the progression of cancer. It seems that extensive cross-talk, mediated by PCNA, exists between cell-cycle regulation, DNA replication and repair.

Another process related to cell-cycle-checkpoint control is apoptosis. It has been shown that PCNA participates actively in the regulation of damage-induced apoptosis, by inducing programmed cell death, either by stimulating proteins such as ING1b (inhibitor of growth 1b) or suppressing anti-apoptotic proteins such as Gadd45 (growth-arrest and DNA-damage-inducible protein 45), MyD118 (myeloid differentiation factor 118) and CR6. An interesting fact is that the interaction between Gadd45 and PCNA involves a different interface, a C-terminal tail of PCNA (Figure 2, orange dot), which is in close proximity to the interdomain
connecting loop, but is a separate region. Thus integration of all processes in cell-cycle progression might involve distinct parts of the PCNA molecule: interdomain connecting loop, C-terminal tail or interior α-helices (Figure 2). There is a correlation between the PCNA interface and the motifs present in PCNA-interacting proteins; however, the current knowledge is insufficient for drawing a strong conclusion.

Conclusion and future perspectives

PCNA is at the very heart of many essential cellular processes such as DNA replication, repair of DNA damage, chromatin structure maintenance, chromosome segregation and cell-cycle progression and can be regarded as one of their common integrators. In order to achieve this goal, PCNA must interact with many proteins, either simultaneously or separately in a mutually exclusive manner. In the process of the replication of DNA, many lesions, formed accidentally by environmental factors or intentionally by anticancer drugs, might block the progression of the replication forks. The cellular response to this often results in a change of PCNA function triggered either by a specific post-translational modification of PCNA (i.e. ubiquitylation) or by a change of its interaction partners, making PCNA determine the fate of the replication fork. Successful repair of the lesion and restart of the replication, or alternatively failure of the DNA repair machinery or even cell death, might ultimately be translated into a simple question of whether a patient will die from cancer or respond to anticancer therapy.

The role of PCNA is established through its interaction partners; however, little is known about the compartmentalization of the respective interactions. It is very likely that we have many subpopulations of PCNA, dedicated to a particular process. The logic implies that there are at least three main fractions of PCNA: a cytosolic fraction produced after translation, a nucleosolic pool and, most importantly, a chromatin-bound PCNA pool. From published work, it is known that even chromatin-bound PCNA is not a homogeneous fraction, as, depending on the methods of fixation or extraction procedures, at least two populations could be described [48]. The mechanical model of a replication fork suggests that PCNA on the leading and lagging strands might be different: one should stay with the polymerase until termination occurs, the other should be loaded and unloaded constantly at each Okazaki fragment. Despite this, PCNA is engaged in processes other than mere DNA replication.

All of these predicates allow us to speculate that every set of PCNA molecules is unique with respect to its properties. One possible explanation for such a variety is the change of the post-translational status of PCNA by different modifications (phosphorylation, ubiquitylation, SUMOylation, acetylation and methylation), which might result in different interaction affinities and different intracellular localizations. An example for importance of post-translational modifications of PCNA is the existence of csPCNA, which is methylated [25]. However, the post-translational modifications occur as a consequence of protein interactions with the respective modifying enzymes. It is tempting to believe that we have a chain of events (localization, interaction, modification, change of interacting partners or localization) and a new sequence of events. Since the chain is highly branched, the result will be a different subset of PCNA molecules dedicated to a particular cellular process.

A critical issue that needs to be addressed in future experiments is revealing how different anticancer drugs influence PCNA modifications or interactions. Since the choice of error-prone or error-free DNA-damage avoidance is believed to be dependent on the simple switch between mono- and poly-ubiquitylation of PCNA, guidance of anticancer therapy in one or another direction might be achieved in the near future. However, it is currently unknown how different cancers will respond to chemicals knocking down a particular PCNA-related function. The wide variety of anticancer drugs exhibit highly different activities in different tumours, despite many of them being alkylating agents producing DNA adducts. Thus it is likely that specific DNA lesions will signal for different PCNA modifications which may be of importance for the outcome of the treatment. Furthermore, it is possible that genetic and epigenetic difference among tumours influences the modifications of PCNA, which is an area that needs further attention.

Acknowledgements

We apologize to those scientists whose work we were unable to cite in the present article. We thank Dr Anne Lagerqvist for critically reading the manuscript before submission.

Funding

We thank the Swedish Cancer Society, the Swedish Children’s Cancer Foundation, the Swedish Research Council, the Swedish Pain Relief Foundation and the Medical Research Council for financial support.

References
