Molecular structure and biological function of proliferating cell nuclear antigen

Song Nanmeng[1], Sang Jianli[1] and Xu Heng[2 *]

(1. Institute of Cell Biology, College of Life Science, Beijing Normal University, Beijing 100875, China; 2. Emory University School of Medicine, GA 30332, USA)

Accepted on June 8, 2007

Abstract Proliferating cell nuclear antigen (PCNA) is the core component of replication complex in eukaryote. As a processive factor of DNA polymerase delta, PCNA coordinates the replication process by interacting with various replication proteins. PCNA appears to play an essential role in many cell events, such as DNA damage repair, cell cycle regulation, and apoptosis, through the coordination or organization of different partners. PCNA is an essential factor in cell proliferation, and has clinical significance in tumor research. In this article we review the functional structure of PCNA, which acts as a function switch in different cell events.

Keywords: PCNA, Function Switch, PCNA Binding Motif, Cell Cycle, DNA Replication, DNA Repair.

Proliferating cell nuclear antigen (PCNA) was originally discovered in the systemic lupus erythematosus patients as an antigen found only in the nucleus of dividing cells (including normal dividing cells and cancer cells). It was originally characterized as a DNA sliding clamp for replicative DNA polymerases and as an essential component of the eukaryotic chromosomal DNA replisome. However, subsequent studies revealed its striking ability of interacting with multiple partners, which are involved in several metabolic pathways, including Okazaki fragment processing, DNA repair, cell cycle regulation, chromatin remodeling, DNA methylation and apoptosis. It arouse great interests to study PCNA which appears to play a key role in controlling several reactions as a function switch through the coordination and organization of different partners.

1 Functional structure of PCNA

The protein function is dependent on its structure, and the structure adapts well to the function. The molecular structure feature of PCNA is the basis of its interactions with other proteins.

1.1 Molecular structure of PCNA

Three identical PCNA monomers, each comprising two similar structural domains, are joined in a head-to-tail arrangement to form a homotrimer. And these form the six repeating domains and exhibit in the shape of symmetry hexagon[1,2] (Fig. 1). A PCNA trimer is composed of 81 asparagine, 54 glutamic acid, 24 lysine and 6 histidine residues, so its net charge is negative. Whereas, the charge of the inner loop surface which contains 9 lysine and histidine residues in each monomer is positive, so it can be attracted by the phosphate backbone of DNA. The internal diameter of the hole in the center is 35 Å, so duplex DNA which has a similar cross-sectional width of 20 Å can readily pass through the centers of the rings[3].

The ring has two nonequivalent surfaces. The inner side is composed of α-helices rich in basic residues, 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 is still able to freely slide along the DNA lattice by virtue of the α-helices lining the inner channel. There are nine anti-parallel β-sheets flanking each domain, which expose the hydrophobic structure and subsequently bind other proteins by hydrophobic force. The major interaction site is the interdomain connecting loop, a coiled structure on the side of PCNA, spanning residues from L121 to E132 (Fig. 1(a)). This loop is recognized by several proteins, such as DNA polymerase delta (polδ), p21, flap endonuclease 1 (Fen1), DNA methyltransferase (MeCTr), and DNA ligase 1 (Lig1). Other important sequences are the C terminal tail, which is im-

* To whom correspondence should be addressed. E-mail: heng6888@yahoo.com

portant for the interaction with DNA polymerase ε (Polε), replication factor C (RF-C), CDK2 and GADD45. And the N-terminal region comprising the inner α-helices forms part of the binding site for cyclin D[1].

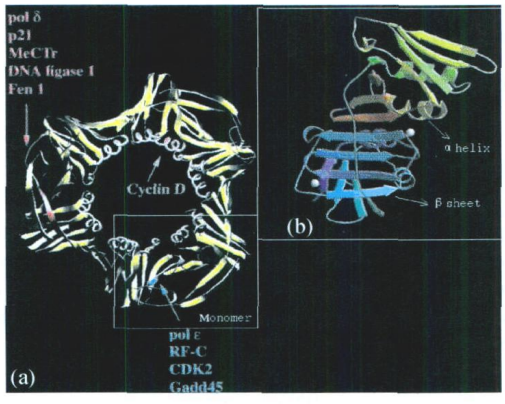

Fig. 1. Tertiary structure of PCNA monomer and trimer. (a) PCNA trimer: the interdomain loop (L121 to E132, red), the inner side α-helices at the N-terminal (pink), and the C-terminal tail (blue) (From Ref. [1]). (b) PCNA monomer (From SWISSPROT database).

1.2 PCNA structure and its regulation mechanism

The possible regulation mechanism of PCNA and its parteners might lie in the homotrimeric structure of PCNA which, in principle, can allow it to bind different partners simultaneously. There is some evidence for this speculation. For instance, p21 has been shown to bind to PCNA with a 3∶1 stoichiometry, so that it prevents other interactions by occupying all the possible binding sites on the trimer[4]. Another interacting partner, Gadd45, binds to PCNA with a 2∶1 stoichiometry, thus leaving a binding site free for other proteins[5]. The switch between alternative binding sites might be one way in which PCNA can regulate its interaction with different partners.

Binding kinetics studies suggested that Fen1 and Lig1 bind with somewhat lower affinity to PCNA than p21, so p21 could be an effective competitor of Fen1 and Lig1 binding, which is consistent with biochemical studies[1,6]. That different partners bind with different affinity to PCNA is one possible mechanism to facilitate function switch.

It is known that the enzyme activity of the protein can be enhanced by interacting with PCNA. Most DNA relative proteins have specific DNA recognizing sequences. However, proteins that have no DNA recognizing sequences could utilize PCNA as an adapter in order to interact with their DNA substrates. PCNA also seems to be one of the ways in which the cell recruits particular proteins to a particular place at a particular moment, though the mechanism is not so clear so far.

1.3 PCNA structure is constant in evolution

There are similar polymerase processors in virus and proeukaryotic cells (Table 1). A structure-based sequence alignment shows that the sequence identity between gp45, β-clamp and PCNA, which are auxiliary replication factors, is only 10%. Despite the lack of similarity in their sequences, the structures of the three proteins are strikingly similar and it seems probable that they interact with DNA in a similar manner, and they all need ATP-dependent proteins (such as RF-C in eukaryote) to provide energy by hydrolyzing[3]. These similarities strongly suggest that the mechanism of achieving processivity by hooking the polymerase to a ring-shaped sliding clamp was arrived at early evolution and has been elaborated on ever since.

Table 1. DNA polymerase and auxiliary factor in different organisms

Origin protein	DNA polymerase	Auxiliary factor	ATP-dependent
T4 phage	?	gp45	gp44/62
E. coli	DNA polymerase III	β-clamp	γ-complex
Eukaryote	DNA polymerase δ	PCNA	RF-C

2 Interaction of PCNA with DNA and proteins

2.1 Interaction of PCNA with DNA

It is the essential step for PCNA to encircle DNA strand. The reason that binding behavior of PCNA to DNA could not be observed by the conventional gel shift assay or surface plasmon resonance (SRP) probably due to the weak binding and slipping behavior.

Furusawa et al. first reported the kinetics of slipping binding behavior of PCNA to DNA strands by a QCM method[7]. They employed DNA-immobilized QCM to analyze the kinetics of the sequence-specific binding of PCNA peptide to DNA strands in an aqueous buffer solution, and demonstrated that PCNA could bind 1∶1 to the dsDNA. It was found that PCNA only binds to a large extent to a simple dsDNA with a blunt end. This suggests that a toroidal PCNA binds to dsDNA by penetrating from

the blunt end, not by slide binding[7]. These kinetic parameters for PCNA were compared with other side-binding proteins, and the toroidal PCNA showed slipping binding with a low k_1 value, while the k_{-1} values were almost the same among these proteins. It indicates that the slipping binding of PCNA from the terminal end decreases the binding rate compared with side-binding behavior but not the dissociation rate constants[8].

2.2 Interaction of PCNA with proteins

Recent studies have indicated that PCNA specifically interacts with more than one hundred factors involved in cell cycle control, DNA replication, DNA repair, and apoptosis. To understand its broad significance, the regions on PCNA interacting with other proteins have been sought with various methods, including peptide scanning, deletion mutants, random site mutagenesis, site directed mutagenesis, yeast two-hybrid assays and random peptide library. One of the main conclusions from mutagenic studies of PCNA is that the molecule can sustain a surprising amount of mutation without losing activity or showing obvious effects either *in vivo* or *in vitro*, and that the most clear-cut effects are seen when residues in exposed loops on the PCNA surface are mutated, such as SHV43AAA, QLGI125AAAA, VDK188AAA, and LAPK251AAAA[9].

Post-translational modifications of both PCNA and its binding proteins, such as acetylation, SUMOylation or phosphorylation, can also positively or negatively regulate the interaction. PCNA is mono-ubiquitinated through RAD6 and RAD18, modified by multi-ubiquitination, which additionally requires MMS2, UBC13 and RAD5, and is conjugated to SUMO by UBC9[10]. All the three modifications affect the same lysine residue of PCNA, suggesting that they label PCNA for alternative functions.

Many PCNA-binding proteins contain a common PCNA binding motif: the PCNA interaction protein (PIP)-box, which has the consensus sequence Q-xx-(h)-x-x-(a)-(a), where h represents residues with moderately hydrophobic side chains, a represents residues with highly hydrophobic, aromatic side chains and x is any residue[6,9]. Table 2 is a summary of proteins containing PIP-box. 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[11]. Searching for related sequences in the SWISSPROT database obtained nearly 1500 hits, and among which were a number of proteins related to cell cycle control, DNA replication, DNA repair, and apoptosis. The identification of a second motif that may be involved in PCNA binding is of particular significance, as it may be of utility in identifying PCNA-binding domains in candidate proteins that bind to PCNA, and it would provide insights to the mechanism of PCNA as a function switch.

Table 2. PCNA interacting proteins containing PIP box

Protein	Function
*polδ(Polymeraseδ)	DNA replication and repair
*polε(Polymeraseε)	DNA replication and repair
polβ(Polymeraseβ)	Base excision repair
polη(Polymeraseη)	Translesion synthesis
polι(Polymeraseι)	Translesion synthesis
polκ(Polymeraseκ)	Translesion synthesis
polλ(Polymeraseλ)	Translesion synthesis
AP-Endonucleases APN1, APN2	Base excision repair
CAF-1(Chromatin assembly factor)	Chromatin assemblage
CDK2(Cyclin dependent kinase)	Cell cycle control
CHL12	Sister chromatin cohesion
CoLim15[12]	Early meiosis
Ctf7p (Chromosomal transmission fidelity)	Sister chromatin cohesion
*Cyclin D[3]	Cell cycle control
DNA ligase I	DNA replication and repair
FEN 1(Flap endonuclease 1)	DNA replication and repair
Gadd45(Growth arrest- and DNA damage-induced gene)	Apoptosis inhibitor
Ing1p33[ING1][13]	Protection from UV-induced apoptosis
*MCMT (DNA (Cytosine-5) methyltransferase) MeCTr/Dnmt[3]	Maintenance of methylation pattern
MCL1 (Myeloid cell leukemia 1)[14]	Apoptosis inhibitor
*MLH1, MSH2/3/6	Mismatch repair
MyD118(Myeloid differentiation primary response)	Apoptosis inhibitor
p21	Cell cycle control
p57[3]	Cell cycle control
P300 (Transcriptional coactivator)	Facilitation of PCNA function in DNA repair
*RF-C(Replicative factor-C)	DNA replication and repair

(To be continued)

Continued

Protein	Function
Topo I	DNA replication and repair
Topo IIα	DNA replication and repair
UBC9[10]	SUMO modification
* UNG2 (Uracil-DNA glycosylase)	Base Excision Repair
WRN helicase	DNA double-strand break repair
*XP-G denonuclease	Nucleotide excision repair
XRCC1(X-ray repair cross complementing 1)[15]	Nucleotide excision repair / DNA double-strand break repair

* represents proteins containing novel KA box.

3 Different functions of PCNA in cell metabolic pathways

Based on the molecular structure, PCNA can utilize many proteins to realize the functional switch, which regulates different cell events (Fig. 2).

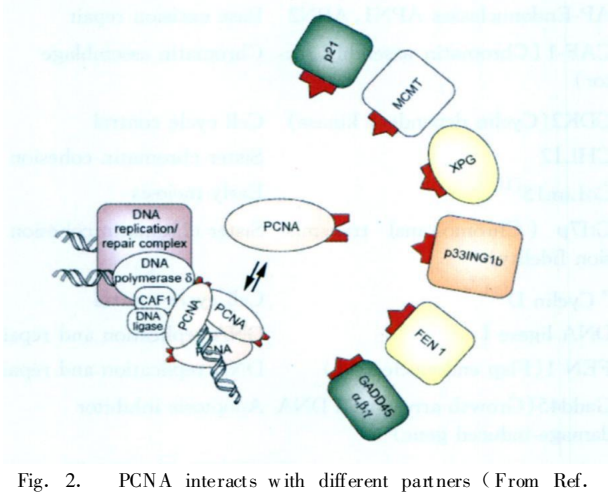

Fig. 2. PCNA interacts with different partners (From Ref. [14]). Function swith of PCNA with several partners, such as cell cycle inhibitor p21, apoptosis inhibitor Gadd45, p33, DNA replication complex Fen1, Lig1, and DNA repair complex XPG.

3.1 PCNA in DNA replication

Our current view of DNA replication in eukaryotes is that pol-α/primase synthesizes the first RNA/DNA primer on the leading strand. Then the PCNA trimer opens and then closes at the 3' OH end of the nascent DNA strands by the enzymatic activity of the clamp loader RF-C, which triggers the displacement of polα and subsequent recruitment of polδ for processive synthesis. Pol-α/primase is also involved in discontinuous DNA synthesis of the lagging strand. Completion of Okazaki fragment synthesis, however, requires the processive polδ (or polε) holoenzyme (polδ, RF-C and PCNA). PCNA/Fen1 complex then efficiently removes the flap structure generated by strand displacement synthesis. This is followed by binding of Lig1 to PCNA, which performs the final ligation step, thus sealing the nick[1, 2, 16, 17].

In vitro experiments show that the competition for PCNA binding among polδ, Fen1 and Lig1 coordinates the ordered action of these enzymes[17]. The PCNA binding domain in Lig1 N terminal is important for its localization in the replication complex, which is regulated by the cell-cycle dependent phosphonation[18]. It seems to be a significant principle of proteins recruiting to the replication site. In summary, an ordered sequence of association and dissociation events that involve different proteins ensures the coordinated action of the various components of the DNA synthesome. PCNA is a key mediator in these processes[16].

3.2 PCNA and polymerase δ

DNA polymerase δ is a central enzyme essential for eukaryotic DNA replication. PCNA acts as a processive factor to increase the binding of polδ to template-primer DNA by at least 2000-fold. Processivity is defined as the number of nucleotides incorporated by the polymerase per binding event[19]. This resembling fix to DNA polymerase δ of PCNA confirms that the polymerase cannot fall from DNA strand during replication[20, 21].

It has been reported that an inhibitory monoclonal hPCNA antibody (74B1) inhibits the ability of PCNA to stimulate DNA synthesis catalyzed by polδ *in vitro*. The epitope of this antibody has been mapped to residues 121—135 of PCNA, which are located in the loop that connects the two conformationally conserved domains. Mutations in these residues affected the interactions between PCNA and polδ as determined by enzyme-linked immunosorbent assays, and also affected their abilities to form a ternary complex with a DNA template-primer, as determined by electrophoretic mobility gel shift assays[22].

It is believed that the catalytic subunit of polδ p125 interacts directly with PCNA. N2 is a conserved region in p125 from numerous materials, which is located in the N terminal and contains PIP box[23]. p125 and PCNA can co-immunoprecipitate in crude

calf thymus and Hela extracts, and when co-expressed in Sf9 cells they can form a physical complex that can be detected on gel filtration. The direct interaction between PCNA and p125 could also be demonstrated in the yeast two hybrid system and overlay experiments[22].

Whereas Hughes showed that the third subunit of human DNA polymerase δ, p66, interacts with PCNA through a canonical PCNA-binding sequence by pull-down assays, immunoprecipitation, and Far-Western[24]. Recent study in Pol32 subunit of *Saccharomyces cerevisiae*, a homologue of mammalian p66 subunit, shows that during DNA synthesis the C terminus interacts with the carboxyl-terminal region of PCNA, instead of the hydrophobic pocket at the interdomain connector loop region of PCNA[25].

It is not clear whether the binding of PCNA to different subunits of polδ means some kind of functional switch. Are there any choice of binding to different subunits, and any alternation between different subunits and different binding sites for single subunit? All these questions need to be investigated further.

3.3 PCNA in DNA damage repair

There are many aggressive factors in the environment that may cause abnormal gene expression. To guarantee the stability of the genomes, cells activate complex signaling networks in response to DNA damage and replication stress. They detect the damage and then transduce the signal to downstream effectors that block cell cycle progression. Three proteins, Rad9, Hus1, and Rad1, which resemble a ring-shaped sliding clamp, interact in a heterotrimeric complex (dubbed the 9-1-1 complex) with PCNA. It is suggested that this multi-protein complex may be loaded onto DNA at sites of damage, and coordinates the transition of cell-cycle progression and DNA repair[26, 27]. A new checkpoint protein called Claspin that is believed to transduce the checkpoint DNA damage signals to Chk1 kinase has been found in PCNA complex[28].

3.3.1 PCNA in nucleotide excision repair (NER)

The DNA-binding protein XPA is involved in damage recognition before repair, in concert with RP-A, which binds single stranded DNA. This XPA protein-related recognition step is tightly linked to the recruitment of PCNA to the damaged sites[29]. The XPF-ERCC1 and XP-G complex cleaves the damaged strand about 27—29 nucleotides from the lesion. Indeed, PCNA is loaded specifically at the site of the XP-G incision, and 3′ of the lesion to be repaired. Thus, PCNA participates in damage recognition and repair via XP-A and XP-G. In addition, XP-G and polymerase δ bind at the same site of PCNA, so it is presumed that competitive binding of PCNA promotes the function switch from lesion excision to DNA resynthesis[30]. After the incision has been made, PCNA can promote the transition to the next steps by binding polδ or polε for re-synthesis of the complementary strand and subsequently recruiting Lig1 for the final ligation step.

3.3.2 PCNA in mismatch repair (MMR)

Early studies showed that PCNA interacts with mispair binding proteins MSH2, MSH3 and MSH6 and enhances their mispair binding specificity[31]. Recently it was reported 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[32].

3.3.3 PCNA in base excision repair (BER)

There are two pathways in BER, polβ-dependent and PCNA-dependent mechanism. The latter one also needs AP endonuclease (AP1), Fen1, RF-C, uracil DNA glycosylase (UNG), and polδ. The observation that PCNA physically interacts with Apn1, and colocalizes in replication foci with UNG supports the notion that PCNA participates both in the incision and in the resynthesis steps of BER[33—35].

A PCNA binding protein XRCC1 interacts with several proteins such as DNA polymerase β (polβ), Ap1, polynucleotide kinase/phosphatase (PNKP), tyrosyl DNA phosphodiesterase (TDP1), poly (ADP-ribose) polymerases 1 and 2 (PARP1/2), 8-oxoguanine DNA glycosylase (OGG1), which participate in related pathways. But XRCC1 has no known enzymatic activity or recognition of the gap/nick/damaged DNA[15]. XRCC1 functions as a scaffolding protein in BER through binding to PCNA, resembling the function of PCNA in DNA replication.

3.3.4 PCNA in translesion synthesis (TLS)

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. 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 specialized TLS polymerases.

TLS enzymes are polη, polκ, polι and polλ and they all have PCNA binding motifs. Pull-down assay using Ni-NTA beads showed the formation of a complex between polκ and PCNA. When replication factor C (RFC), and replication protein A (RPA) act cooperatively, Polκ can synthesize DNA *in vitro*. Although the enzyme activity of polκ is not influenced by PCNA, the DNA binding capability is enhanced[36]. The similar results have been showed in polη study[37]. Recent studies have found hPCNA mutator E85K can form a more stable polδ-PCNA-template/primer complex. So the polymerase substitution induced by DNA damage is inhibited, and polδ completes the DNA synthesis using mutated strand as the template[17]. It is speculated that PCNA appears to be an ideal candidate for coordinating the functions of TLS polymerases, as well as for recruiting them at the replication fork. It was reported recently that only mono-ubiquited PCNA can bind to polη, but unmodified PCNA cannot. Conclusively ubiquition-modification of PCNA is possibly a way to alter polymerases[38].

3.4 PCNA in cell cycle control

Cyclin, CDK, and CDK inhibitor (CKI) can form special complexs in cell cycle to modulate the transition of cell cycle phases[39, 40]. PCNA interacts with several eukaryotic cell cycle proteins (Fig. 3).

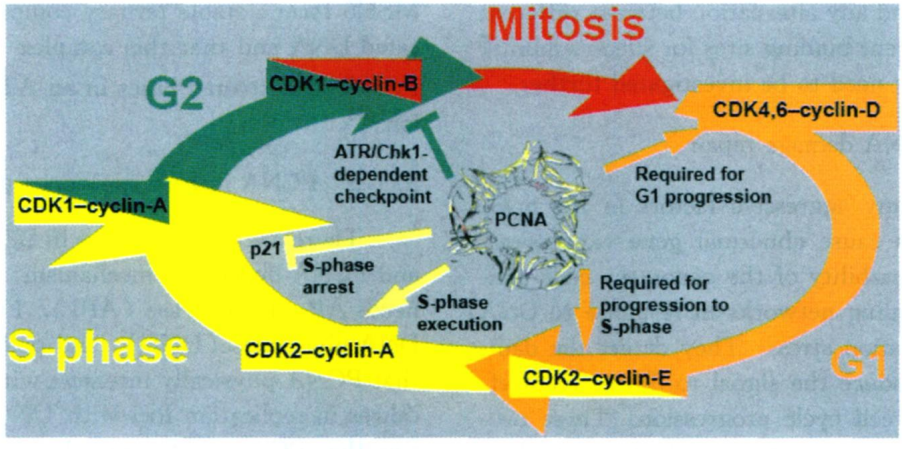

Fig. 3. The interactions between PCNA and cell cycle regulatory networks. PCNA forms complexes with all these CDK-cyclin complexes as well as with critical checkpoint proteins, transducing both positive (indicated as arrows) and negative signals (indicated as T-bars). (From Ref. [1])

3.4.1 PCNA and CDK/cyclin

Zhang et al. reported the existence of PCNA-p21/CDK-cyclin quaternary complexes. PCNA interacts with cyclin A, cyclin B, cyclin D, and cyclin-dependent kinases CDK1, CDK2, CDK4, and CDK5[41].

Biochemical studies showing that PCNA interacts with the S-phase-specific CDK2-cyclin-A complex, suggesting a functional role for binding of PCNA to cyclin-CDK complexes. 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, stimulating their phosphorylation[42].

3.4.2 PCNA and p21

p21 is a universal CDK inhibitor, and it can repress all CDK/cyclin activity. 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 CDK/cyclin through its N-terminal region and to PCNA through its C-terminal region. PCNA-p21 com-

plex inhibits CDK activity to arrest the cell cycle in G1 phase by influencing RB phosphorylation and transcription factors releasing[40]. Also, p21 competes with pol δ for PCNA interaction, which directly blocks the replication process[43].

As a product of tumor suppress gene, p21 negatively regulates cell cycle through PCNA. In many tumor cells, p21 low expression or missing causes cell cycle regulated and/or abnormal proliferation. In a recent study in ovarian cancer cells, p21 was found functional, and overexpression of p21 was associated with increased expression of its partners PCNA, so was in vascular smooth muscle cells of atherosclerosis[44]. Accordingly, the inhibitory effect of p21 may be titrated by overexpression of PCNA.

3.5 PCNA and its partners in other cellular events

3.5.1 PCNA and chromatin metabolism

During replication of the eukaryotic cell genome, not only is the DNA replicated, but the newly synthesized DNA must also be assembled into chromatin. DNA replication-coupled chromatin assembly is essential for the inheritance of chromatin structures. PCNA binds directly to P150, the largest subunit of chromatin assembly factor-1 (CAF-1). CAF-1 is a molecular chaperone that deposits nucleosome onto newly replicated DNA[45]. Through the interaction with CAF-1, PCNA is indispensable for chromosome structure stability maintenance.

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[46]. A proteomic approach to identify PCNA-binding proteins in human cell lysates identified another cohesion factor, CHL12[47,48]. The fact that CHL12 can bind to RFC suggests that it might act as an alternative clamp loader for PCNA and further indicates a connection between chromatid cohesion and DNA replication[45].

3.5.2 PCNA and apoptosis

Apoptosis plays an important role in cell development and illness. Considerably, now there are three signal pathways to regulate it, death signal receptor pathway, mitochondrial pathway, and p53-dependent pathway. p53 is a tumor suppress protein, and it induces cell apoptosis by regulating specific genes expression[49]. A tumor suppressor ING1 contains a PIP motif and physically interacts with PCNA. Cells expressing ING1 mutants that cannot bind to PCNA are protected from UV-induced apoptosis[5,50].

Tumor suppressor Gadd45, MyD118, and MCL1 all contain PCNA interaction motifs. When mutants of Gadd45 and MyD188 that lack the PCNA-interaction domain are ectopically expressed, they induce apoptosis more efficiently and inhibit the colony fomation[50,51].

3.5.3 PCNA and methylation

DNA methylation is involved in damage repair and genome stability. DNA-cytosine-5 methyltransferase (MCMT) exists to ensure the maintenance of precise methylation pattern. Various diseases, such as fragile X syndrome, are associated with abnormal DNA methylation. Research with both cellular and recombinant PCNA indicated that MCMT binds to PCNA directly[52]. The quaternary PCNA-p21-CDK4/cyclinD1 complex, which regulates G1-S transition of the cell cycle, is disturbed by MCMT. The released CDK4/cyclinD1 complex would phosphorylate and inactivate Rb, so the cell cycle would enter the S phase. 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 depends on PCNA.

4 Conclusions and perspectives

To conclude, the analysis of PCNA and its so many protein partners will provide us with a fascinating field for future study. It will be possible to analyze why and how PCNA acquires the functions in coordinating different proteins, and how PCNA acts as a function switch, and also to study the mechanisms that spatially and temporally regulate the ability of PCNA to bind with the right partner at the right time.

Besides the theoretical significance, PCNA relates to clinical research closely. Abnormally expressed PCNA appears in stomach cacer, lung cacer, and liver cacer[53]. Synoviocytes from patients with

rheumatoid arthritis, lens epithelial cells of age-related cataract, and vascular smooth muscle cells of atherosclerosis all proliferate abnormally which associate with PCNA[54]. PCNA is also used as a biomarker of breast cancer[55]. The major research on PCNA now is focused on the discovering of its clinical significance in pathogenesis, but there is very few to concern the mechanism of its action. There will be much extensive prospect and in-service-use significance if we can put the mechanism of sickness occurrence and the fundamental research of PCNA together into consideration and take PCNA as the breakthrough to conquer the disease.

References

1 Maga G and Hubscher U. Proliferating cell nuclear antigen (PCNA): a dancer with many partners. Journal of Cell Science, 2003, 116(15): 3051—3060

2 Krishna TS, Kong XP, Gary S, et al. Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. Cell, 1994, 79: 1233—1243

3 Moarefi I, Jeruzalmi D, Turner J, et al. Crystal structure of the DNA polymerase processivity factor of T4 bacteriophage. Journal of Molecular Biology, 2000, 296: 1215—1223

4 Gulbis JM, Kelman Z, Hurwitz J, et al. Structure of the C-terminal region of of $p21^{WAF1/CIP1}$ complexed with human PCNA. Cell, 1996, 87: 297—306

5 Vairapandi M, Azam N, Balliet AG, et al. Characterization of MyD118, Gadd45 and proliferating cell nuclear antigen (PCNA) interacting domains. The Journal of Biological Chemistry, 2000, 275(22): 16810—16819

6 Warbrick E. The puzzle of PCNA's many partners. BioEssays, 2000, 22: 997—1006

7 Furusawa H, Kitamura Y, Hagiwara N, et al. Binding kinetics of the toroid-shaped PCNA to DNA strands on a 27 MHz quartz crystal microbalance. Chemphyschem, 2002, 5: 446—448

8 Okahata Y, Niikura K, Sugiura Y, et al. Kinetic studies of sequence-specific binding of GCN4-bZIP peptides to DNA strands immobilized on a 27-MHz quartz-crystal microbalance. Biochemistry, 1998, 37: 5666—5672

9 Jonsson ZO, Hindges R, Hubscher U. Regulation of DNA replication and repair proteins through interaction with the front side of proliferating cell nuclear antigen. The EMBO Journal, 1998, 17(8): 2412—2425

10 Hoege C, Pfander B, Moldovan GL, et al. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature, 2002, 419: 135—141

11 Xu H, Zhang P, Liu L, et al. A novel PCNA-binding motif identified by the panning of a random peptide display library. Biochemistry, 2001, 40(14): 4512—4520

12 Hamada FN, Koshivama A, Namekawa SH, et al. Proliferating cell nuclear antigen (PCNA) interacts with a meiosis-specific RecA homologues, Lim15/Dmc1, but does not stimulate its strand transfer activity. Biochem Biophys Res Commun, 2007, 352(4): 836—842

13 Feng X, Hara Y, Riabowol K. Different HATS of the ING1 gene family. Trends in Cell Biology, 2002, 12: 532

14 Loor G, Zhang SJ, Zhang P, et al. Identification of DNA replication and cell cycle proteins that interact with PCNA. Nucleic Acids Research, 1997, 25(24): 5041—5046

15 Fan J, Otterlei M, Wong HK, et al. XRCC1 co-localizes and physically interacts with PCNA. Nucleic Acids Research, 2004, 32(7): 2193—2201

16 Frouin I, Montecucco A, Spadari S, et al. DNA replication: a complex matter. EMBO reports. 2003, 7(4): 666—670

17 Tom S, Henricksen LA, Bambara RA. Mechanism whereby proliferating cell nuclear antigen stimulates flap endonuclease 1. Journal of Biological Chemistry, 2000, 275(14): 10498—10505

18 Levin DS, McKenna AE, Motycka TA, et al. Interaction between PCNA and DNA ligase I is critical for joining of Okazaki fragments and long-patch base-excision repair. Current Biology, 2000, 10: 919—922

19 Fisher PA, Moutsiakis DL, McConnell M, et al. A single amino acid change (E85K) in human PCNA that leads, relative to wild type, to enhanced DNA synthesis by DNA polymerase δ past nucleotide base lesions (TLS) as well as on unmodified templates. Biochemistry, 2004, 43: 15915—15921

20 McConnell M, Miller H, Mozzherin DJ, et al. The mammalian DNA polymerase delta—proliferating cell nuclear antigen—template-primer complex: molecular characterization by direct binding. Biochemistry, 1996, 35: 8268—8274

21 Mozzherin DJ, Shibutani S, Tan CK, et al. Proliferating cell nuclear antigen promotes DNA synthesis past template lesions by mammalian DNA polymerase delta. Proc Natl Acad Sci USA, 1997, 94: 6126—6131

22 Zhang P, Sun Y, Hsu H, et al. The interdomain connector loop of human PCNA is involved in a direct interaction with human polymerase δ. The Journal of Biological Chemistry, 1997, 273(2): 713—719

23 Zhang SJ, Zeng XR, Zhang P, et al. A conserved region in the amino terminus of DNA polymerase δ is involved in proliferating cell nuclear antigen binding. The Journal of Biological Chemistry, 1995, 270(14): 7988—7992

24 Ducoux M, Urbach S, Baldacci G, et al. 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 δ. The Journal of Biological Chemistry, 2001, 276(52): 49258—49266

25 Johansson E, Garg P, Burgers PM. The Pol32 subunit of DNA polymerase contains separable domains for processive replication and proliferating cell nuclear antigen (PCNA) binding. The Journal of Biological Chemistry, 2004, 279(3): 1907—1915

26 Parrilla-Castellar ER, Arlander SJ, Karnitz L. Dial 9-1-1 for DNA damage: the Rad9-Hus1-Rad1(9-1-1) clamp complex. DNA Repair, 2004, 3: 1009

27 Friedrich-Heineken E, Toueille M, Tannler B, et al. The two DNA clamps Rad9/Rad1/Hus1 complex and proliferating cell nuclear antigen differentially regulate flap endonuclease 1 activity. The Journal of Biological Chemistry, 2005, 353(5): 980—989

28 Brondello JM, Duocommun B, Fernandez A, et al. Linking PCNA-dependent replication and ATR by human Claspin. Biochem Biophys Res Commun, 2007, 354(4): 1028—1033

29 Miura M and Sasaki T. Effect of XPA gene mutations on UV-induced immunostaining of PCNA in fibroblasts from xeroderma pigmentosum group A patients. Mutation Research, 1996, 364: 51—56

30 Gary R, Ludwig DL, Cornelius HL, et al. 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. The Journal of Biological Chemistry, 1997, 272(39): 24522—24529

31 Kleczkowska HE, Marra G, Lettieri T, et al. hMSH3 and hMSH6 interact with PCNA and colocalize with it to replication foci. Genes & Development, 2001, 15: 724—736

32 Lau PJ and Kolodner RD. Transfer of the MSH2-MSH6 complex from proliferating cell nuclear antigen to mispaired bases in DNA. The Journal of Biological Chemistry, 2003, 278(1): 14—17

33 Matsumoto Y, Kim K, Hurwitz J, et al. Reconstitution of proliferating cell nuclear antigen-dependent repair of apurinic/apyrimidinic sites with purified human proteins. The Journal of Biological Chemistry, 1999, 274(47): 33703—33708

34 Krokan HE, Otterlei M, Nilsen H, et al. Properties and functions of human uracil-DNA glycosylase from the UNG gene. Prog Nucleic Acid Res Mol Biol, 2001, 68: 365—386

35 Dianova II, Bohr VA, Dianov GL. Interaction of human AP endonuclease 1 with flap endonuclease 1 and proliferating cell nuclear antigen involved in long-patch base excision repair. Biochemistry, 2001, 40: 12639—12644

36 Haracska L, Unk I, Johnson RE, et al. Stimulation of DNA synthesis activity of human DNA polymerase κ by PCNA. Molecular and Cellular Biology, 2002, 22(3): 784—791

37 Haracska L, Johnson RE, Unk I, et al. Physical and functional interactions of human DNA polymerase η with PCNA. Molecular and Cellular Biology, 2001, 21(21): 7199—7206

38 Kannouche PL, Wing J, Lehmann AR. Interaction of human DNA polymerase eta with monoubiquitinated PCNA: a possible mechanism for the polymerase switch in response to DNA damage. Mol Cell, May 21, 2004, 14(4): 491—500

39 Israels ED, Israels LG. The cell cycle. Fundamentals of Cancer Medicine, 2001, 19: 88—91

40 Zhang H, Xiong Y, Beach D. Proliferating cell nuclear antigen and p21 are components of multiple cell cycle kinase complexes. Molecular and Cellular Biology, 1993, 4: 897—906

41 Wang KR, Xue SB, Liu HT. Cell Biology. 2ed Edition. Beijing: Publishing Company of Beijing Normal University, 1998, 448

42 Koundrioukoff S, Jonsson ZO, Hasan S, et al. A Direct interaction between proliferating cell nuclear antigen (PCNA) and Cdk2 targets PCNA-interacting proteins for phosphorylation. The Journal of Biological Chemistry, 2000, 275(30): 22882—22887

43 Cazzalini O, Perucca P, Riva F, et al. $p21^{CDKN1A}$ does not interfere with loading of PCNA at DNA replication sites, but inhibits subsequent binding of DNA polymerase delta at the G1/S phase transition. Cell Cycle, 2003, 2(6): 596—603

44 Barboule N, Baldin V, JOzan S, et al. Increased level of p21 in human ovarian tumors is associated with increased expression of CDK2, Cyclin A and PCNA. International Journal of Cancer, 1998, 76: 891—896

45 Shibahara K and Stillman B. Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. Cell, 1999, 96: 575—585

46 Skibbens RV, Corson LB, Koshland D, et al. Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes & Development, 1999, 13: 307—319

47 Shiomi Y, Shinozaki A, Sugimoto K, et al. The reconstituted human Chl12-RFC complex functions as a second PCNA loader. Genes to Cells, 2004, 9: 279—290

48 Ohta S, Shiomi Y, Sugimoto K, et al. A proteomics approach to identify proliferating cell nuclear antigen (PCNA)-binding proteins in human cell lysates. The Journal of Biological Chemistry, 2002, 277(43): 40362—40367

49 Haupt S, Berger M, Goldberg Z, et al. Apoptosis-P53 network. Journal of Cell Science, 2003, 116(20): 4077—4085

50 Scott1 M, Bonnefin P, Vieyra D, et al. UV-induced binding of ING1 to PCNA regulates the induction of apoptosis. Journal of Cell Science, 2001, 114: 3455—3462

51 Fujise K, Zhang D, Liu J, et al. Regulation of apoptosis and cell cycle progression by MCL1 differential role of proliferating cell nuclear antigen. The Journal of Biological Chemistry, 2000, 275(50): 39458—39465

52 Chuang Ls, Ian HI, Koh TW, et al. Human DNA-(Cytosine-5) methyltransferase-PCNA complex as a target for $p21^{WAF1}$. Science, 1997, 277(26): 1996—2000

53 Gumurdulu D, Erdogan S, Kayaselcuk F, et al. Expression of COX-2, PCNA, Ki-67 and p53 in gastrointestinal stromal tumors and its relationship with histopathological parameters. World Journal of Gastroenterology, 2007, 03: 014

54 Heng X, Wei H, Qian L. Proposal concerning the mid and long term strategic development for the national biomedical science and construction of the biomedical strategic platform. Bulletin of National Science Foundation of China. 2004, 04: 011

55 Malkas LH, Herbert BS, Abdel-Aziz W, et al. A cancer-associated PCNA expressed in breast cancer has implications as a potential biomarker. Proc Natl Acad Sci USA. 2006, 103(51): 19472—19477