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Review

Proliferating cell nuclear antigen: a proteomics view

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Abstract. Proliferating cell nuclear antigen (PCNA), a cell cycle marker protein, is well known as a DNA sliding clamp for DNA polymerase delta and as an essential component for eukaryotic chromosomal DNA replication and repair. Due to its mobility inside nuclei, PCNA is dynamically presented in a soluble or chromatin-associated form. The heterogeneity and specific modifications of PCNA may reflect its multiple functions and the presence of many binding partners in the cell. The recent proteomics approaches applied to characterizing PCNA interactions revealed multiple PCNA partners with a wide spectrum of activity and unveiled the possible existence of new PCNA functions. Since more than 100 PCNA-interacting proteins and several PCNA modifications have already been reported, a proteomics point of view seems exactly suitable to better understand the role of PCNA in cellular functions.

Keywords. Proliferating cell nuclear antigen, proteomics, interaction, functions.

Introduction

Proliferating cell nuclear antigen (PCNA) is well known as a cell cycle marker. However, this is just one aspect of its very complicated and thrilling nature. The complexity of PCNA function is reflected in the history of its discovery and subsequent investigation. Studies in different areas such as immunology, proteomics and biochemistry all intersected to identify exactly the same protein. This protein was initially identified by Miyachi 30 years ago as an antigen for autoimmune disease in systemic lupus erythematosis patients [1]. A few years later, Bravo and Celis, using two-dimensional gel electrophoresis (2-DE) found an 'acidic protein' that was differentially expressed during the cell cycle [2]. Accordingly, they gave it the name 'cyclin' [3]. The periodic appearance of PCNA in S phase nuclei, co-localized with incorporated bromodeoxyuridine [2–4], suggested an involvement in DNA replication. Later, it was revealed that PCNA and cyclin were the same protein [5]. An essential factor for SV40 (simian virus 40) DNA replication *in vitro* was also identified as PCNA [6, 7]. As the name 'cyclin' was given to another class of proteins, PCNA/cyclin is now usually called PCNA. Subsequent biochemical and genetic studies with budding yeast demonstrated that PCNA is essential for chromosomal DNA replication as a DNA sliding clamp for DNA polymerase delta [8–10]. A structural analysis of PCNA had a tremendous impact on studies of its function(s), revealing that PCNA is organized as a ring that can encircle DNA and work as a sliding clamp for DNA polymerase delta and epsilon, and a docking station, or scaffold, for other proteins [11–16]. Many of these proteins are involved in DNA replication, DNA repair and cell cycle control. Additionally, there are many proteins involved in chromatin assembly and remodeling, chromatid cohesion, transcription and other functions. PCNA is known as a docking station that encircles DNA and coordinates multiple genetic functions during DNA replication and repair [17–20]. But there is a substantial pool of

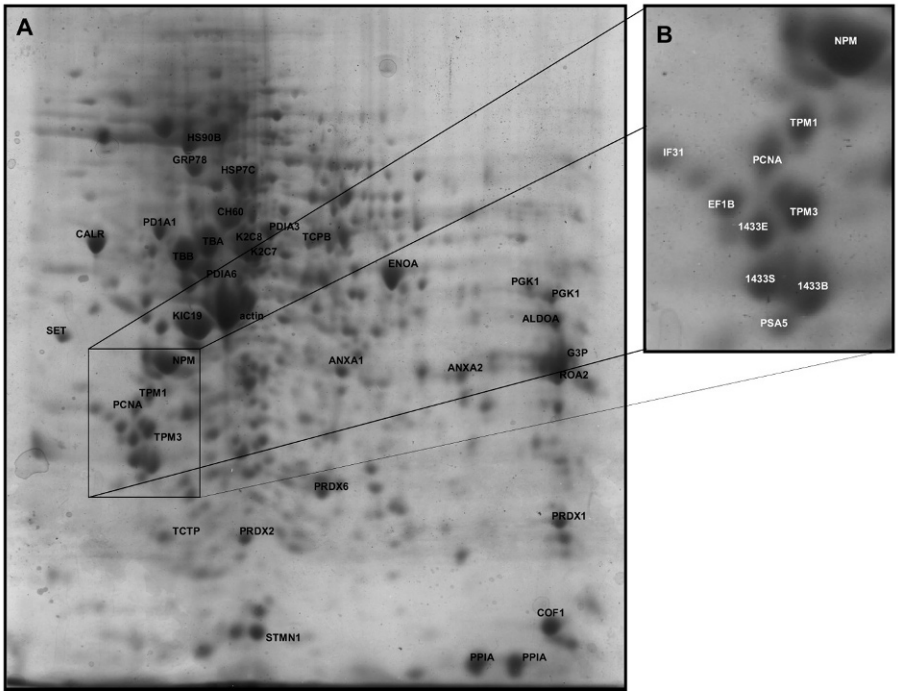

Figure 1. A two-dimensional (2-DE) map of proteins from MB468 cells and PCNA position. (*A*) Proteins (0.4 mg) were separated by 2-DE (pH3–10, 13 cm – first dimension, 10% SDS-PAGE, 13 cm – second dimension) and stained with Coomassie R350. Protein identification was done according to protein fingerprints (spectra) produced by trypsin digestion of material from gel plugs and detected by MALDI-TOF instrument (Waters) [35]. The marked proteins are: actin (ACTB_HUMAN), aldolase A (ALDOA_HUMAN), heterogeneous nuclear ribonucleoproteins A2/B1 (ROA2_HUMAN), annexin A2 (ANXA2_HUMAN), annexin A1 (ANXA1_HUMAN), stathmin (STMN1_HUMAN), alpha-enolase (ENOA_HUMAN), keratin, type II cytoskeletal 7 (K2C7_HUMAN), keratin, type II cytoskeletal 8 (K2C8_HUMAN), keratin, type I cytoskeletal 17 (K1C17_HUMAN), glyceraldehyde-3-phosphate dehydrogenase (G3P_HUMAN), peroxiredoxin-1 (PRDX1_HUMAN), peroxiredoxin-2 (PRDX2_HUMAN), peroxiredoxin-6 (PRDX6_HUMAN), calreticulin precursor (CALR_HUMAN), heat shock cognate 71 kDa protein (HSP7C_HUMAN), heat shock protein HSP 90-beta (HS90B_HUMAN), 78 kDa glucose-regulated protein precursor (GRP78_HUMAN), 60 kDa heat shock protein, mitochondrial precursor (CH60_HUMAN), tubulin alpha-1B chain (TBA1B_HUMAN), tubulin beta chain (TBB5_HUMAN), protein disulfide-isomerase precursor (PDIA1_HUMAN), peptidyl-prolyl cis-trans isomerase A (PPIA_HUMAN), cofilin-1 (COF1_HUMAN), translationally-controlled tumor protein 9 (TCTP_HUMAN), stathmin (STMN1_HUMAN), phosphoglycerate kinase 1 (PGK1_HUMAN), protein SET (SET_HUMAN). (*B*) Zoomed area around PCNA from the same 2-DE map is shown. Tropomyosin alpha-1 (TPMI_HUMAN, 4.69/32700), tropomyosin alpha-3 (TPM3_HUMAN, 4.68/32800), 14-3-3 protein epsilon (1433E_HUMAN, 4.63/29300) 14-3-3 protein sigma (1433S_HUMAN, 4.69/27900), 14-3-3 protein beta/alpha (1433B_HUMAN, 4.76/28082), elongation factor 1-beta (EF1B_HUMAN, 4.5/24900), nucleophosmin (numatrin, B23) (NPM_HUMAN, 4.64/32700) are detected around PCNA.

nucleoplasmic PCNA that may work even without interacting with DNA [4, 21, 22]. The growth-related properties of PCNA, as well as its physicochemical properties (p*I*, mass, antigenicity) have been well conserved during evolution [23], suggesting that it plays a vital role in cellular metabolism.

2-DE map (proteome) and PCNA

In 1980, Bravo and Celis published their 2-DE analysis of protein synthesis during the cell cycle of HeLa cells [2]. Using [35S] methionine labeling, they found that all detected polypeptides are synthesized throughout the cell cycle. When differences appeared, they were clearly in relative intensity (rate of synthesis) rather than the appearance of new polypeptides [2]. As they pointed out, the most interesting protein was a '36,000 mol wt acidic cytoarchitectural protein that increases in S-phase' [2]. Lately, several different 2-DE databases have been established [24] and the position of PCNA has been determined and analyzed in more detail [5, 25–30]. PCNA is usually seen as a single spot. Protein spots close to PCNA in the 2-DE map are housekeeping proteins that can be present in different ratios. In particular, these include tropomyosins and different forms of 14-3-3 proteins (Fig. 1). Since isoelectric point (p*I*) and molecular mass are fundamental physicochemical parameters for any protein, their correct estimation is very important. Initial estimates of PCNA mass gave a value of 36 kDa [2, 31], although the theoretical mass is 30 kDa. This shift from theoretical parameters could be the result of post-translational modification. Unfortunately, some proteins, like histones, migrate abnormally in SDS-PAGE based on abnormal SDS binding or basic

character. Separation on gels with different acrylamide concentrations (pore sizes) can sometimes solve the problem and give more accurate mass values. Accordingly, PCNA migrates as a single band around 36 kDa [5, 32] on 12–20% polyacrylamide gel, but around 30 kDa, very close to the theoretical value, in 8–9% gel [33]. On 2-DE (Fig. 1), PCNA is usually detected as a single spot, as initially described [31]. However, higher resolution and better sensitivity allow the detection of additional acidic and basic satellite spots (Fig. 2) [5, 25, 28, 34]. The main (M) PCNA spot corresponds to a polypeptide of p*I* 4.57 and mass 30 kDa. These numbers match almost exactly the PCNA theoretical parameters, showing that the majority of PCNA is not post-translationally modified [35]. The minor spots are produced because of mass and charge shift and represent PCNA modifications [30, 34]. These modifications can be biological (functional) or technical (produced during sample analysis). Multiple lower-mass PCNA fragments (Fig. 2) are produced by proteolysis during 2-DE and can be prevented by using the proteosome inhibitor MG132 or thiourea [34–36]. This proteolysis also takes place *in vivo* and possibly has a biological function, although its detection needs to be more sensitive [34, 37]. PCNA ubiquitination is another well-documented modification [34, 38–40] that produces spots of higher mass (~40 kDa) and p*I* (~5.0) on the 2-DE map (Fig. 2). The nature of other modifications that produce a shift in PCNA p*I* is less clear. Although several different modifications have been reported, some of these data may not be correct since the conclusions are not always consistent [30, 41–44]. One example is the issue of PCNA phosphorylation, which was initially described as a potential mechanism for regulatory PCNA function. However, this issue was addressed using several different experimental approaches, including *in vivo* labeling, immunoprecipitation, Western blot analysis, high-resolution 2-DE and mass spectrometry [30]. The data obtained strongly suggest that PCNA is not phosphorylated [30]. Acetylation and deacetylation might explain the changes in PCNA charge shift and the production of additional spots [30]. The transcription factor p300 and HDAC1 are responsible, at least in part, for PCNA acetylation and deacetylation, respectively. PCNA treated with HDAC1 showed much lower binding affinity to the DNA polymerases beta and delta than did the sample treated with TSA [30]. The decrease in affinity was particularly notable between deacetylated PCNA and DNA polymerase delta. Consistent with this result, deacetylated PCNA showed lower activity in DNA polymerization than did highly acetylated PCNA [30]. However, the possibility that these isoforms (A-, B-spots, Fig. 2) contain other modifications such as oxidation [unpublished observation] or methylation cannot presently be ruled out. In particular, methylation is a modification that can produce a p*I* shift in the basic direction (B-spot), and its presence in PCNA has been reported [42]. As the p*I* of the main (M) form exactly matches the theoretical value for PCNA (p*I* 4.57), the corresponding spot likely represents the unmodified form. However, there is still a chance that a portion of the protein in the M-spot contains a peptide having two modifications simultaneously. One modification that leads to the A-form (acetylation or oxidation, p*I* shift is –0.05) and another that leads to the B-form (methylation, p*I* shift is +0.05) could result in no apparent change in charge, and the peptide would have the same p*I* 4.57.

PCNA quantity in a cell

PCNA was initially found to be a protein that was differentially expressed during the cell cycle [2, 31], and has lately became a marker of cell proliferation [45]. Therefore, the amount of PCNA in the cell was the subject of special attention, especially in the context of cell transformation [28, 46, 47]. Although there may be an increase in PCNA synthesis in cells traversing from G0 to S phase [48, 49], variations in the PCNA level are only 2–3 times greater in cycling cells, which reflects comparable changes in the level of PCNA mRNA [26, 29, 50]. As the cell ages, the PCNA level doesn't change significantly, although the cell doubling time dramatically increases at the late phase of cell aging [51]. Cancer often correlates with an enhanced level of PCNA, and PCNA levels can be used as a prognostic marker in some cases [52, 53]. Analysis of cell cultures showed that cancer cells have a significantly higher level of PCNA than normal cells even in cases where they have the same doubling time [35]. Cells maintain a large pool of free PCNA throughout the cell cycle. Even in S phase, only a small portion of PCNA (20–30%) is directly involved in DNA replication, co-localizing with DNA replication factories (foci) [4]. There is no change in the stability of PCNA in relation to total cellular proteins as a function of the cell cycle. The half-life for PCNA, determined in cycling HeLa cells, is 8 h, or similar to the half-life of total cellular protein [26]. In 3T3 cells, the half-life of PCNA is even larger, 20 h [4]. The absolute amount of PCNA in a cellular proteins is about 0.06–0.08% [51, 54]. That gives a concentration as high as ~500000 molecules (monomers)/cell for non-transformed, or normal cell lines where the molar actin/PCNA ratio is ~200/1. In transformed cell lines, the concentration

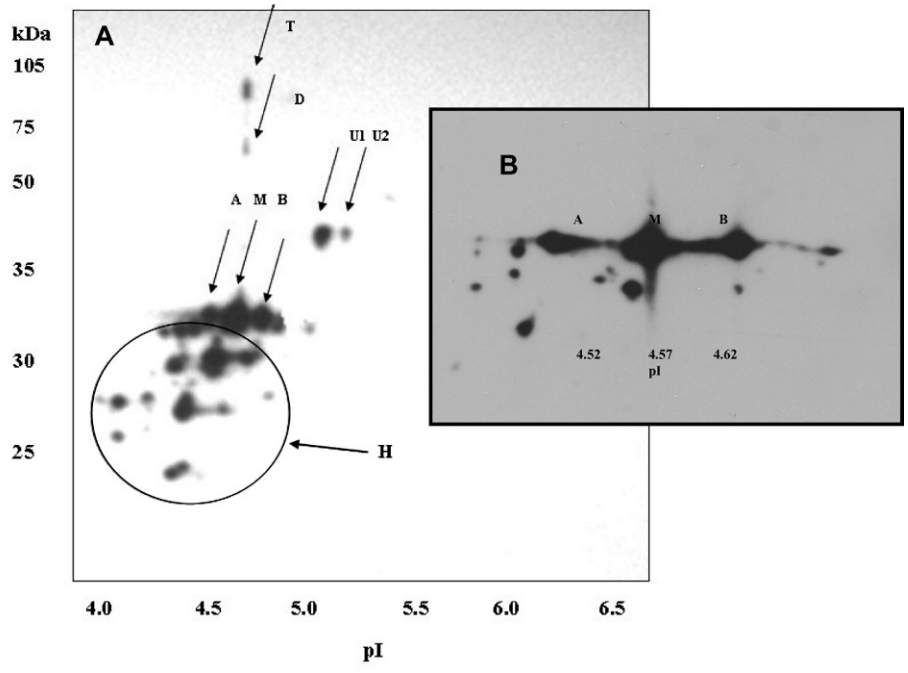

Figure 2. Multiple spots of PCNA (isoforms) can be detected by 2-DE with increased resolution and better sensitivity. Immunostaining using PCNA-specific antibody PC10 after 2-DE separation and transfer to Immobilon-P membrane. A, M, B, T and D denote acidic, main, basic, probable trimer and probable dimer forms of PCNA, respectively. U1 and U2 are ubiquitinated forms of PCNA monomer. H is a cluster of PCNA hydrolysis products, produced by proteosome action [34]. (*A*) 2-DE (pH 4–7, 13 cm – first dimension, 10% SDS-PAGE, 13 cm – second dimension). (*B*) 2-DE (pH 4–5, 18 cm – first dimension, 10% SDS-PAGE, 13 cm – second dimension).

is about 4000000 monomers/cell, and the actin/PCNA ratio goes down to ~20/1 [28, 35].

PCNA structure

PCNA structure has been highly conserved during evolution; its amino acid sequences in rats and humans differ by only 4 of 261 amino acids. As was shown by crystallography and small-angle neutron scattering, PCNA is organized into a toroid structure (Fig. 3) [13, 14, 16, 55]. This ring-shaped organization is characteristic and very similar to the 'sliding clamps' needed for processivity of DNA synthesis in bacteriophages, archaeons and eukaryotes [56]. For example, the protein structures of *Saccharomyces cerevisiae* and human PCNA are closely superimposable over the core secondary structural elements, even though the similarity at the amino acid sequence level between the two is only 35%. Each PCNA monomer comprises two domains that are joined firmly together by forming an extended ß sheet across the interdomain boundary [13, 14]. Three monomers are joined in an anti-parallel interaction between strand ßD2 in one molecule and ßI1 in the next, which results in a total of eight main chain amide-to-carboxyl hydrogen bonds across the intermolecular surface of the trimer ring (Fig. 3) [13, 14]. In addition, a hydrophobic core is formed by packing together the αA2 helix in one molecule and the αB1 in the adjacent PCNA monomer [14]. The overall structure of the PCNA trimer ring is estimated to be ~34 Å for the internal diameter ~80 Å for the external diameter and ~30 Å in thickness. Even though PCNA has an overall negative charge, the inner surface is positively charged due to the presence of many lysine and arginine residues (10 per subunit). This suggests that the negatively charged DNA can pass through the ring without electrostatic repulsions [13, 14]. The PCNA ring has distinct 'front' and 'back' sides: the front side includes the interdomain connecting loop (IDCL, 118LMDLDVEQLGIPEQEYSC135), linking the N- and C-terminal domains of each monomer, and the back contains several pronounced loops connecting anti-parallel ß strands that protrude into the solvent (e.g., ßD2ßE2 and ßH1-ßI1) [14]. PCNA interacts with protein partners only on the front side. No interaction with other proteins was found with the back side of the trimer. Henderson et al., working on PCNA in a *Drosophila melanogaster* model system, speculated that the results of their genetic studies can be explained if two PCNA trimers form a back-to-back complex [57]. A detailed analysis, based on cross-linking and mutagenesis, strongly confirmed that PCNA presents in mammalian cells as a double homotrimer and that Arg5 and Lys110 are crucial for its formation and detection [17, 33]. The available crystal structure of the PCNA trimer allows for a model where a double homotrimer is organized through back-to-back interaction [17]. As the $\beta D_2 \beta E_2$ loop (back loop, BL, 184QTSNVDKEEEAV195) protrudes from the back of the ring, a direct interaction between Arg5 of one trimer and Lys110 in the

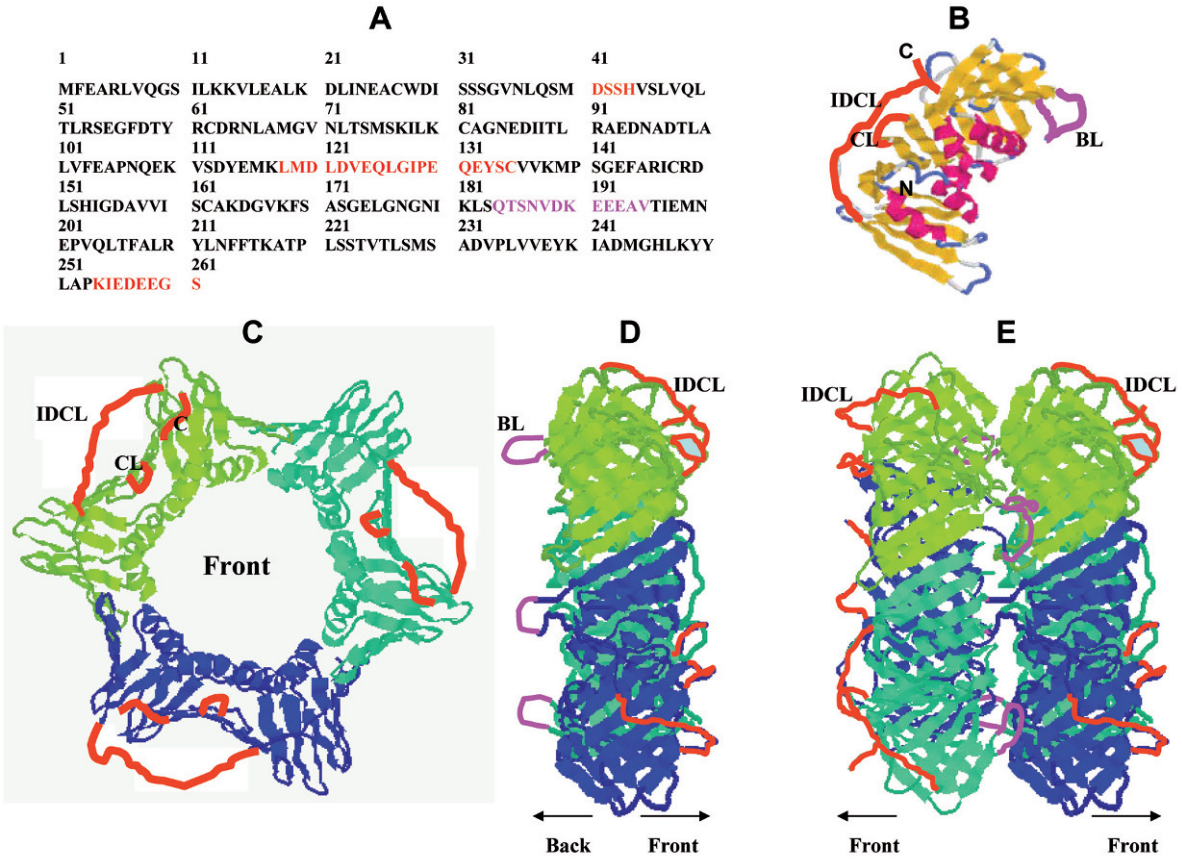

Figure 3. Different levels of PCNA organization. (*A*) An amino acid sequence of human PCNA. Interdomain connecting loop, IDCL (118LMDLDVEQLGIPEQEYSC135), center loop, CL (41DSSH44), C-terminus, C (254KIEDEEGS261) are shown in red; back side loop, BL (184QTSNVDKEEEAV195) is shown in pink. (*B*) A three-dimensional structural model of PCNA monomer (chain E from 1AXC file is shown, http://www.rcsb.org/pdb/home/home.do). IDCL, CL, C are shown in red; BL in pink. Helix areas (αA1, αB1, αA2, αB2) are pink and β sheets (βA1, βB1, βC1, βD1, βE1, βF1, βG1, βH1, βI1, βA2, βB2, βC2, βD2, βE2, βF2, βG2, βH2, βI2) are yellow. (*C*) A trimer PCNA organization (1XC) is shown from front. (*D*) A side view. (*E*) A model of PCNA double trimer [17]. IDCL, CL, C are shown in red; BL in pink.

another trimer might only be possible if the rings are twisted against each other to make the loops fit into groves on the opposite trimer (Fig. 3). However, some scientists are still sceptical about the presence of PCNA double trimer in the replication fork. The main objection is a question about how PCNA is loaded by RFC on DNA at its 3′ recessed end [56]. Since the crystal structure of RFC bound to PCNA trimer (not double trimer) was determined [11], it means that all loaded trimers should be in the same, stereospecific orientation [55, 56]. Although controversy exists on this aspect, practically all mammalian cellular PCNA molecules can be cross-linked by formaldehyde as a double trimer [17, 33].

It is interesting that cross-linking using formaldehyde treatment doesn't produce a double trimer in yeast [unpublished observation], even though yeast PCNA has the same pair of Arg and Lys in the 5 and 110 positions (only in reversed combination, Lys5 and Arg110) and the overall PCNA structures from yeast and human are superimposable. The reason may be technical. For example, the distance between these residues in yeast may be slightly longer than formaldehyde can cross-link. Another reason may be that the PCNA double-trimer organization is a property only of higher eukaryotes where a more complicated network of interactions with PCNA is required. It should be mentioned that the double-ring structure is not unique to PCNA. Many prokaryotic and eukaryotic proteins have a similar organization, among them glutamine synthetase [58], ATP-sulfurolase [59], the MCM complex [60], SW40 large antigen helicase [61], a meiosis-specific DNA recombinase (Dmc1, a RecA/Rad51 homolog) [62] and chaperonin GroEL [63]. Interestingly, some of these proteins do not have any functions that involve interactions with DNA.

Table 1. PCNA-binding proteins involved in DNA replication and repair.

#	Swiss Prot	Protein name / synonyms	Theoretical pI/ Mw	Reference
1	PCNA_HUMAN	**Proliferating cell nuclear antigen /** PCNA / Cyclin	4.57 / 28768	[12, 17]
2	DNL1_HUMAN	**DNA ligase 1** / Polydeoxyribonucleotide synthase [ATP] 1	5.49 / 101736	[99, 149]
3	DPOD1_HUMAN	**DNA polymerase delta catalytic subunit** / DNA polymerase delta subunit p125	6.64 / 123631	[87]
4	DPOD3_HUMAN	**DNA polymerase subunit delta-3** / DNA polymerase subunit delta p66	9.38 / 51400	[124]
5	DPOD4_HUMAN	**DNA polymerase subunit delta-4 /** DNA polymerase subunit delta p12	6.29 / 12433	[150]
6	Q6GQH3_XENLA CDT1_HUMAN	**DNA replication factor Cdt1 /** Double-parked homolog / DUP	9.83 / 69530 9.91 / 60413	[121]
7	RFA1_HUMAN	**Replication protein A 70 kDa DNA-binding subunit** / RP-A / RF-A / Replication factor-A protein 1 / Single-stranded DNA-binding protein / p70	6.92 / 68138	[151]
8	DPOE_YEAST RFC3_HUMAN	**DNA polymerase epsilon, catalytic subunit A /** DNA polymerase II subunit A	5.98 / 261518	[152]
9	RFC1_HUMAN	**Replication factor C subunit 1** / Replication factor C large subunit / RF-C 140 kDa subunit / Activator 1 140 kDa subunit / Activator 1 large subunit / A1 140 kDa subunit / DNA-binding protein PO-GA	9.38 / 128255	[99]
10	RFC2_HUMAN	**Replication factor C subunit 2** / Replication factor C 40 kDa subunit / RF-C 40 kDa subunit / RFC40 / Activator 1 40 kDa subunit / A1 40 kDa subunit	6.04 / 39157	[11]
11	RFC3_YEAST RFC3_HUMAN	**Replication factor C subunit 3** / Replication factor C 38 kDa subunit / RFC38 / Activator 1 38 kDa subunit / A1 38 kDa subunit / RF-C 38 kDa subunit	6.07 / 38204	[11]
12	RFC4_YEAST RFC3_HUMAN	**Replication factor C subunit 4** / Replication factor C 37 kDa subunit / RF-C 37 kDa subunit / RFC37 / Activator 1 37 kDa subunit / A1 37 kDa subunit	9.16 / 36149	[11]
13	RFC5_HUMAN	**Replication factor C subunit 5 /** Replication factor C 36 kDa subunit / RF-C 36 kDa subunit / RFC36 / Activator 1 36 kDa subunit / A1 36 kDa subunit	6.72 / 38497	[151]
14	FEN1_HUMAN	**Flap endonuclease 1** / Flap structure-specific endonuclease 1 / FEN-1 / Maturation factor 1 / MF1 / hFEN-1 / DNase IV	8.80 / 42593	[123]
15	KCD13_HUMAN	**BTB/POZ domain-containing protein KCTD13 /** Polymerase delta-interacting protein 1 / TNFAIP1-like protein	6.77 / 36357	[153]
16	PDIP2_HUMAN	**Polymerase delta-interacting protein 2** / 38 kDa DNA polymerase delta interaction protein / p38	8.80 / 42033	[154]
17	TOP1_HUMAN	**DNA topoisomerase 1**	9.33 / 90726	[101]
18	TOP2A_CHICK TOP2A_HUMAN	**DNA topoisomerase 2**	8.90 / 175000 8.82 / 174385	[102]
19	MCM10_YEAST MCM10_HUMAN	**Minichromosome maintenance protein 10 /** Protein DNA43 **Protein MCM10 homolog /** HsMCM10	9.67 / 65815 8.96 / 98183	[155]
20	Q5TCJ4_HUMAN	**Lamin A/C /**	6.13 / 53250	[107]
21	Q5TCJ2_HUMAN	**Lamin A/C /**	6.57 / 74139	[107]
22	LMNB1_HUMAN	**Lamin-B1**	5.11 / 66408	[107]

PCNA interactions

Interaction with different protein partners is an important regulatory mechanism for the diverse cellular functions of PCNA. One could speculate that mammalian cells utilize PCNA for more diverse cellular functions than monocellular organisms because of their greater potential for different fates. Consistent with this speculation, a literature and protein database search identified a long list of PCNA-binding proteins. This list includes proteins involved in DNA replication (Table 1), DNA repair (Table 2), cell cycle control (Table 3), chromatin remodeling/epigenetic inheritance, chromatid cohesion (Table 4), transcription (Table 5) and other miscellaneous functions (Table 6) [20, 39, 64–85]. PCNA can interact with protein partners through the hydrophobic groove on the front side organized by its center loop (CL, 41DSSH44), the C-terminal tail (CT, 254KIE257) and the interdomain-connecting loop (IDCL, 118LMDLDVEQLGIPEQEYSC135). It is thought that the binding of a given protein to the

PCNA trimer ring occurs in a competitive manner at the expense of other PCNA-binding proteins, although a PCNA trimer contains three identical surfaces [17]. Most of the PCNA-binding proteins contain the consensus PCNA-binding motif termed the PIP (PCNA-interacting protein) box QXX(L/M/I)XX(F/Y)(F/Y), suggesting that these proteins bind to the same sites on PCNA [19, 20]. This PIP-box peptide is folded into a 3_{10} helix (different from an α helix or β sheet) and acts as a hydrophobic plug, docking into the hydrophobic pocket of PCNA produced by IDCL, CL and CT (Fig. 3) [13]. Not all PCNA-interacting proteins have a canonical PIP box. Binding to PCNA may occur through the PIP-related sequence QLXLF presented in chromatin assembly factor (CAF-1), DNA polymerase beta or the p50 subunit of DNA polymerase delta [86]. A shorter amino acid sequence, referred to as the KA motif, has also been suggested to mediate PCNA interactions with various proteins [87]. However, some PCNA-binding proteins can interact with PCNA independently of any of these motifs [77, 88, 89]. There is a chance that some PCNA-binding proteins can utilize another binding site on PCNA. So far, this situation has been reported only once where the N-terminal region comprising the inner α helices of PCNA was shown to form the binding site for cyclin D [21, 90]. The cyclin-dependent kinase inhibitor 1 or p21 (CDN1A_HUMAN) interacts more strongly with PCNA than other binding proteins in accordance with its role as a 'brake' that blocks access of DNA polymerase delta to PCNA [21, 91]. Having the highest affinity to PCNA, p21 can also compete with replication factor C (RFC) and inhibit PCNA loading onto DNA *in vitro*. Surprisingly, at a low-ratio p21/PCNA (around 1:1), p21 can actually stimulate loading of PCNA onto DNA, and inhibition starts only at a very high p21/PCNA ratio (more than 10:1) [92]. It is possible that p21-PCNA interaction partially destabilizes the PCNA structure that initially helps RFC promote PCNA opening and DNA loading, but that at a higher ratio, p21 blocks all binding sites on PCNA and prevents any RFC-PCNA interaction. A database search for potential PCNA partners that contain the PIP box or a related sequence identified a number of new potential candidates [93]. Many PCNA-binding proteins belong to pathways that are unrelated to DNA replication, DNA repair or chromatin organization, raising the important question of whether these interactions really take place *in vivo*. Confirmation of these interactions may reveal new areas of PCNA functionality.

DNA replication (Table 1)

Mammalian DNA replication is a multi-stage and multi-protein process. Involvement of PCNA in this process has been thoroughly investigated, documented and reviewed [21, 56, 94, 95]. So far, the best-characterized function of PCNA is its involvement in DNA synthesis as a DNA sliding clamp. Comparison of the DNA elongation processes in *Escherichia coli*, phage and eukaryotic systems have revealed that the DNA replication apparatus is highly conserved in terms of structure and function [96]. DNA sliding clamps exist in all of these systems and increase efficiency of individual polymerases to synthesize long DNA strands. For example, the *E. coli* β subunit and PCNA are representative of prokaryotic and eukaryotic DNA sliding clamps and are able to specifically stimulate their respective partners, DNA polymerase III and DNA polymerase delta. Clamps have a common ring shape that allows them to wrap around DNA and slide along the double helical DNA freely in both directions [13, 14] so that their interactions with DNA polymerases can stabilize the enzymes on the template. Repetitive loading/unloading of the PCNA ring onto the lagging DNA strand, as well as leading strand, is performed in an ATP-dependent manner by chaperonine-like replication factor C (RFC), a heteropentameric AAA+ protein complex of RFC1, RFC2, RFC3, RFC4 and RFC5 subunits [56]. This arc-shaped complex interacts with PCNA in a screw-cap-like arrangement [11], opening the PCNA ring and loading it on the DNA. Stabilized by PCNA, DNA polymerase delta or epsilon replicate a leading strand continuously through the 5′-to-3′ direction in an error-free manner due to the presence of a 3'-to-5' editor exonuclease [97]. The lagging strand, however, is replicated in a discontinuous fashion, producing short (Okazaki) fragments through the consecutive action of DNA polymerase alpha, flap structure-specific endonuclease-1 (FEN-1) and DNA polymerase delta. DNA ligase I seals the nicks between the fragments. FEN-1 and DNA ligase interact with PCNA through a PIP box. The interaction of FEN-1 with PCNA even stimulates its activity [98–100]. Topological problems that accumulate in DNA (catenation and supercoiling) because of the moving replicative forks are counteracted by topoisomerases I and II that are also able to bind PCNA [101, 102]. PCNA works as a moving platform or binding station for factors involved in or concomitant with DNA replication [17, 21]. The double ring organization could ideally suit to coordinating these functions, since two symmetrical protein interaction areas facing opposite directions on the replicating DNA allow PCNA to be

Table 2. PCNA-binding proteins involved in DNA repair.

#	Swiss Prot	Protein name / synonyms	Theoretical pI/ Mw	Reference
1	APEX2_HUMAN	**DNA-(apurinic or apyrimidinic site) lyase 2 /** Apurinic-apyrimidinic endonuclease 2 / AP endonuclease 2 / APEX nuclease 2 / APEX nuclease-like 2 / AP endonuclease XTH2	8.65 / 57400	[156]
2	APEX1_HUMAN	**DNA-(apurinic or apyrimidinic site) lyase /** AP endonuclease 1 / APEX nuclease / APEN / Protein REF-1	8.33 / 35554	[157]
3	3MG_HUMAN	**DNA-3-methyladenine glycosylase /** 3-methyladenine DNA glycosidase / ADPG / 3-alkyladenine DNA glycosylase / N-methylpurine-DNA glycosylase	9.65 / 32863	[111]
4	ERCC5_HUMAN	**DNA-repair protein complementing XP-G cells /** Xeroderma pigmentosum group G-complementing protein / DNA excision repair protein ERCC-5	5.19 / 133329	[70]
5	DPOLB_HUMAN	**DNA polymerase beta**	9.01 / 38177	[158]
6	CCNO_HUMAN	**Cyclin-O** / Uracil-DNA glycosylase 2 / UDG2 / Cyclin-like uracil-DNA glycosylase	9.43 / 35352	[110, 159]
7	PRKDC_HUMAN	**DNA-dependent protein kinase catalytic subunit /** DNA-PK catalytic subunit / DNA-PKcs / DNPK1 / p460	6.75 / 469089	[151]
8	EXO1_HUMAN	**Exonuclease 1** / hExo1 / Exonuclease I / hExoI	8.59 / 94103	[160]
9	GA45A_HUMAN	**Growth arrest and DNA-damage-inducible protein GADD45 alpha** / DNA-damage-inducible transcript 1 / DIT-1	4.36 / 18336	[161]
10	GA45B_HUMAN	**Growth arrest and DNA-damage-inducible protein GADD45 beta /** Negative growth regulatory protein MyD118 / Myeloid differentiation primary response protein MyD118	4.33 / 17818	[161]
11	GA45G_HUMAN	**Growth arrest and DNA-damage-inducible protein GADD45 gamma** / Cytokine-responsive protein CR6 / DNA-damage-inducible transcript 2 / DDIT-2	4.26 / 17121	[64]
12	KU70_HUMAN	**ATP-dependent DNA helicase 2 subunit 1** / ATP-dependent DNA helicase II 70 kDa subunit / Lupus Ku autoantigen protein p70 / Ku70 / 70 kDa subunit of Ku antigen / Thyroid-lupus autoantigen / TLAA / CTC box-binding factor 75 kDa subunit / CTCBF / CTC75 / DNA-repair protein XRCC6	6.23 / 69712	[162]
13	KU86_HUMAN	**ATP-dependent DNA helicase 2 subunit 2** /ATP-dependent DNA helicase II 80 kDa subunit / Lupus Ku autoantigen protein p86 / Ku86 / Ku80 / 86 kDa subunit of Ku antigen / Thyroid-lupus autoantigen / TLAA / CTC box-binding factor 85 kDa subunit / CTCBF / CTC85 / Nuclear factor IV /DNA-repair protein XRCC5	5.55 / 82573	[151]
14	PAF_HUMAN	**PCNA-associated factor** / p15PAF / Overexpressed in anaplastic thyroid carcinoma 1 / OEATC-1 / Hepatitis C virus NS5A-transactivated protein 9 / HCV NS5A-transactivated protein 9	9.85 / 11986	[163, 164]
15	MSH2_HUMAN	**DNA mismatch repair protein Msh2** / MutS protein homolog 2	5.58 / 104743	[66]
16	MSH3_HUMAN	**DNA mismatch repair protein Msh3 /** Divergent upstream protein / DUP / Mismatch repair protein 1 / MRP1	8.30 / 127455	[165]
17	MSH6_HUMAN	**DNA mismatch repair protein MSH6** / MutS-alpha 160 kDa subunit / G/T mismatch-binding protein / GTBP / GTMBP / p160	6.50 / 152786	[66, 165]
18	WRN_HUMAN	**Werner syndrome ATP-dependent helicase**	5.96 / 162495	[166]
19	TDT_HUMAN	**DNA nucleotidylexotransferase** / Terminal addition enzyme / Terminal deoxynucleotidyltransferase / Terminal transferase	8.55 / 58437	[167]
20	MUTYH_HUMAN	**A/G-specific adenine DNA glycosylase /** MutY homolog / hMYH	8.99 / 60069	[168]
21	SRS2_YEAST	**ATP-dependent DNA helicase SRS2**	8.86 / 134306	[169]
22	PRKDC_HUMAN	**DNA-dependent protein kinase catalytic subunit /** DNA-PK catalytic subunit / DNA-PKcs / DNPK1 / p460	6.75 / 469088	[151]
23	PARP1_HUMAN	**Poly [ADP-ribose] polymerase 1 /** PARP-1 / ADPRT / NAD(+) ADP-ribosyltransferase 1 / Poly[ADP-ribose] synthetase 1	8.99 / 113083	[170]
24	RAD18_YEAST RAD18_HUMAN	**E3 ubiquitin-protein ligase RAD18 /** Postreplication repair E3 ubiquitin-protein ligase RAD18 / Radiation sensitivity protein 18 / hRAD18 / hHR18 / RING finger protein 73	7.53 / 55230 7.17 / 56194	[38]
25	RAD5_YEAST	**DNA repair protein RAD5 /** Radiation sensitivity protein 5 / Revertibility protein 2	5.98 / 134002	[38]
26	POLI_HUMAN	**DNA polymerase iota**	6.37 / 80346	[171]

Table 2 *(Continued)*

#	Swiss Prot	Protein name / synonyms	Theoretical pI/ Mw	Reference
27	POLK_HUMAN	**DNA polymerase kappa**	8.42 / 98809	[172]
28	DPOLL_HUMAN	**DNA polymerase lambda**	7.96 / 63482	[173]
29	DPOLQ_HUMAN	**DNA polymerase theta**	6.57 / 197597	[174]
30	POLH_HUMAN	**DNA polymerase eta**	8.74 / 78413	[39]
31	NTHL1_HUMAN	**Endonuclease III-like protein 1**	9.72 / 34389	[78]
32	XRCC1_HUMAN	**DNA-repair protein XRCC1 /** X-ray repair cross-complementing protein 1	6.02 / 69525	[67]

involved simultaneously in DNA synthesis and additional processes such as chromatin remodeling or DNA methylation [17, 103]. Many of the details regarding the mode of PCNA interaction are missing, since the process of mammalian DNA replication is extremely dynamic and complex. However, DNA replication machineries are organized in replication factories (foci), and these organizations are spatially distributed inside nuclei which require interactions with special structural proteins (nuclear matrix) [104, 105]. These nuclear matrix proteins include lamins, heterogeneous nuclear ribonucleoproteins, actin, vimentin and numatrin (B23) [106]. Interestingly, PCNA is also present in some nuclear matrix preparations [106]. This may be due to the ability of PCNA to interact with lamins through highly conserved Ig-fold motifs located in their C-terminus [107].

DNA repair (Table 2)

DNA repair is a process where many details of PCNA involvement have been described. More information can be obtained from several recent reviews [95, 108]. Multiple specialized pathways required to repair DNA damage involve a DNA synthesis step which is performed by DNA polymerase delta or epsilon together with PCNA. It means that the DNA replication machinery has to be flexible enough to work in both normal and stressed situations. PCNA is able to coordinate different reactions and can be involved not only in DNA synthesis regulation. In the case of nucleotide excision repair (NER), PCNA binds to the endonuclease XP-G through a PIP box located in the C-terminus, possibly allowing PCNA to coordinate DNA excision and DNA synthesis [70]. A similar situation takes place during base excision repair (BER), where practically all of the factors involved [uracil-DNA glycosylase 2, DNA-3-methyladenine glycosylase (MPG), endonuclease III-like protein 1 (NTH1)] interact with and are stimulated by PCNA, suggesting a PCNA ordered reaction. Uracil-DNA glycosylase 2 (Cyclin-O), DNA-lyase 2 (APEX2) and DNA polymerase beta all contain a PIP box, while DNA glycosylase (MPG) has an 'inverted' PIP box [109–111]. Other factors, like NTH1 or XRCC1, also bind PCNA but not through a PIP box [67, 78]. Bypass replication and mismatch repair also involve aspects of PCNA functions that have recently been studied, and an attractive model of 'a ubiquitination-PCNA switch' was proposed [38, 95, 108]. In this model, the response to DNA damage (or stalled replication forks) results in PCNA that is mono- or poly-ubiquitylated at the conserved lys164. Importantly, PCNA monoubiquitylation triggers the error-prone DNA repair pathway through translesion synthesis (TLS), while polyubiquitylation is required for the error-free mode of bypass repair [95]. The whole family of TLS polymerases has been described and have been shown to specifically interact with PCNA through PIP boxes and ubiquitin-binding domains [40, 112, 113]. In addition, the TLS polymerases can themselves be monoubiquitylated [112, 114], and this may provide a negative switch preventing their interaction with PCNA [95, 112]. Intriguingly, PCNA can interact with E3 ubiquitin ligase and trigger its activity [115]. Additionally, TLS induced by PCNA monoubiquitylation is not only involved in bypass replication but in immunoglobulin gene hypermutation as well [116].

Cell cycle control, survival (Table 3)

The level of PCNA oscillates during the cell cycle, and this is may be connected to the participation of PCNA in cell cycle control. These functions could be mediated via interaction with cyclins and cdks. These interactions could be independent from the DNA replication machinery, producing a quaternary complex PCNA-CDK-CyclinD-p21 [83, 117, 118], or could be locally coupled to the replisome as is the case for Cdk2-cyclin A, which forms in a complex with

Table 3. PCNA-binding proteins involved in cell cycle control and survival.

#	Swiss Prot	Protein name/synonyms	Theoretical pI/ Mw	Reference
1	RAD9A_HUMAN	**Cell cycle checkpoint control protein RAD9A** / DNA repair exonuclease rad9 homolog A / hRAD9	5.41 / 42547	[175]
2	CDC2_SCHPO CDC2_HUMAN	**Cell division control protein 2 homolog**/ p34 protein kinase / Cyclin-dependent kinase 1 / CDK1	7.13 / 34359 8.38 / 34095	[72]
3	MPIP3_HUMAN	**M-phase inducer phosphatase 3** / Dual specificity phosphatase Cdc25C	6.34 / 53365	[176]
4	CDC6_HUMAN	**Cell division control protein 6 homolog** / CDC6-related protein / p62(cdc6) / HsCDC6 / HsCDC18	9.64 / 62734	[177, 178]
5	CDK6_HUMAN	**Cell division protein kinase 6** / Serine/threonine-protein kinase PLSTIRE	6.02 / 36938	[177-179]
6	CCNA1_HUMAN	**Cyclin-A1**	4.99 / 52358	[72]
7	CCNA2_HUMAN	**Cyclin-A2**	6.10 / 48537	[72]
8	CCNB1_HUMAN	**G2/mitotic-specific cyclin-B1**	7.09 / 48337	[180]
9	CCNA_HUMAN	**G1/S-specific cyclin-A**		[72]
10	CCND1_HUMAN	**G1/S-specific cyclin-D1**	4.97 / 33729	[83, 90, 118]
11	CCND2_HUMAN	**G1/S-specific cyclin-D2**	5.06 / 33067	[83, 90, 118]
12	CCND3_HUMAN	**G1/S-specific cyclin-D3**	6.66 / 32520	[83, 90, 118]
13	PARP1_HUMAN	**Poly [ADP-ribose] polymerase 1 /** PARP-1 / ADPRT / NAD(+) ADP-ribosyltransferase 1 / Poly[ADP-ribose] synthetase 1	8.99 /112953	[181]
14	CDK2_HUMAN	**Cell division protein kinase 2**/ p33 protein kinase	8.80 / 33929	[72]
15	CDN1A_HUMAN	**Cyclin-dependent kinase inhibitor 1** / p21 / CDK-interacting protein 1 / Melanoma differentiation-associated protein 6 / MDA-6	8.69 / 18119	[118, 182]
16	CHK1_DROME CHK1_HUMAN	**Serine/threonine-protein kinase Chk1**	8.37 / 57833 8.50 / 54419	[147, 183]
17	CDK5_HUMAN	**Cyclin dependent kinase 5** / Cyclin-dependent kinase 5 / Tau protein kinase II catalytic subunit / TPKII catalytic subunit / Serine/threonine-protein kinase PSSALRE	7.57 / 33304	[184]
18	RAD1_HUMAN	**Cell cycle checkpoint protein RAD1 /** DNA repair exonuclease rad1 homolog / Rad1-like DNA damage checkpoint protein / hRAD1	4.73 / 31827	[185]
19	RAD17_HUMAN	**Cell cycle checkpoint protein RAD17** / hRad17 / RF-C/activator 1 homolog	6.63 / 77055	[175]
20	HUS1_HUMAN	**Checkpoint protein HUS1** / hHUS1	6.37 / 31691	[175]
21	ING1_HUMAN	**Inhibitor of growth protein 1**	9.33 / 46781	[80]
22	CDN1C_HUMAN	**Cyclin-dependent kinase inhibitor 1C** / Cyclin-dependent kinase inhibitor p57 / p57KIP2	5.39 / 32177	[178]
23	MCL1_HUMAN	**Induced myeloid leukemia cell differentiation protein Mcl-1** / Bcl-2-related protein EAT/mcl1 / mcl1/EAT	5.51 / 37337	[69]
24	MD2L2_HUMAN	**Mitotic spindle assembly checkpoint protein MAD2B /** MAD2-like 2 / hREV7	6.05 / 24334	[147]
25	PP1G_HUMAN	**Serine/threonine-protein phosphatase PP1-gamma catalytic subunit /** PP-1G / Protein phosphatase 1C catalytic subunit	6.12 / 36983	[90, 186]
26	P53_HUMAN	**Cellular tumor antigen p53 /** Tumor suppressor p53 / Phosphoprotein p53 / Antigen NY-CO-13	6.33 / 43653	[130]
27	MDM2_HUMAN	**E3 ubiquitin-protein ligase Mdm2 /** p53-binding protein Mdm2 / Oncoprotein Mdm2 / Double minute 2 protein / Hdm2	4.60 / 55232	[130]

PCNA and phosphorylates RFC and DNA ligase [72] or involves Cdk2-catalyzed phosphorylation and dissociation of FEN-1 [119]. Among the proteins involved in cell cycle regulation, p21 (CDN1A_HUMAN) is known as a crucial regulator of PCNA function [120]. The N-terminus (AA17–24) of p21 is responsible for its binding and inhibition of Cdk, and the C-terminus contains the PIP sequence that allows p21 to efficiently bind PCNA and competitively block interaction with other PCNA-binding proteins [65, 70, 121–124]. Accordingly, p21 can efficiently stop DNA replication and can promote the switch or brake PCNA from DNA replication to DNA repair. However, it is not known how p21 blocks DNA replicative

Table 4. PCNA-binding proteins involved in chromatin assembly, remodeling and chromatid cohesion.

#	Swiss Prot	Protein name / synonyms	Theoretical pI/ Mw	Reference
1	HDAC1_HUMAN	**Histone deacetylase 1** / HDAC1 / HD1	5.31 / 55103	[77]
2	ECO1_YEAST ESCO1_HUMAN	**N-acetyltransferase ECO1 /** Establishment of cohesion protein 1 / Chromosome transmission fidelity protein 7	9.94 / 31845 9.27 / 94982	[138, 187]
3	CAF1A_HUMAN	**Chromatin assembly factor 1 subunit A** / CAF-1 subunit A / Chromatin assembly factor I p150 subunit / CAF-I 150 kDa subunit / CAF-Ip150	5.77/105223	[188]
	CAC1_YEAST	**Chromatin assembly factor 1 subunit p90 /** CAF-1 90 kDa subunit / RAP1 localization factor 2	6.38 / 70210	[189]
4	EP300_HUMAN	**Histone acetyltransferase p300** / E1A-associated protein p300	8.78 / 264143	[71]
5	DCC1_YEAST DCC1_HUMAN	**Sister chromatid cohesion protein DCC1** / Defective in sister chromatid cohesion protein 1	6.04 / 44074 5.05 / 44825	[138, 190, 191]
6	BAZ1B_HUMAN	**Bromodomain adjacent to zinc finger domain protein 1B** / Williams-Beuren syndrome chromosomal region 9 protein / Williams syndrome transcription factor / hWALP2	8.70 / 170902	[141]
7	DNMT1_HUMAN	**DNA (cytosine-5)-methyltransferase 1** / Dnmt1 / DNA methyltransferase HsaI / DNA MTase HsaI / MCMT / M.HsaI / CXXC-type zinc finger protein 9	7.99 / 183165	[65]
8	CTF8_YEAST Q8WV66_HUMAN	**Chromosome transmission fidelity protein 8** / Clamp loading **Chromosome transmission fidelity factor 8 homolog**	7.95 / 15169 7.90 / 13214	[191]
9	CTF18_YEAST Q8WVB6_HUMAN	**Chromosome transmission fidelity protein 18 /** CTF18/CHL12 protein / Chromosome transmission fidelity factor 18 homologue / Clamp loading	8.54 / 84373 6.78 / 107383	[191]
10	PTMA_HUMAN	**Prothymosin alpha**	3.69 / 12072	[192]
11	SETD8_HUMAN	**Histone-lysine N-methyltransferase SETD8** / H4-K20-HMTase SETD8 / SET domain-containing protein 8 / PR/SET domain-containing protein 07 / PR/SET07 / PR-Set7 / Lysine N-methyltransferase 5A	9.69 / 42889	[193]
12	A6ZST7_YEAS7 PIF1_HUMAN	**DNA helicase /** RRM3 **ATP-dependent DNA helicase PIF1 /** PIF1/RRM3 DNA helicase-like protein	9.71 / 81592 9.81 / 69798	[194]
13	UBC9_HUMAN	**SUMO-conjugating enzyme UBC9 /** SUMO-protein ligase / Ubiquitin-conjugating enzyme E2 I / Ubiquitin-protein ligase I / Ubiquitin carrier protein I / Ubiquitin carrier protein 9 / p18	8.87 / 18006	[147]

synthesis but still allows it to perform repair synthesis. It is possible that this is regulated through specific degradation of p21 [125, 126] or through phosphorylation [127]. Cyclin D1 is another interesting candidate for a negative cell cycle regulator of PCNA. Baldin et al. showed that cyclin D1 is normally localized to the nucleus but disappears from the nucleus in S phase [128]. They proposed that the interaction of cyclin D1 with PCNA might prevent PCNA from binding to the replication complex [128]. Interestingly, it has been shown that the cyclin D family interacts with PCNA in a unique manner from all the other interacting proteins [21, 90]. Another mechanism to explain the cell cycle regulation of PCNA exists at the transcriptional level. PCNA is transcribed under the control of p53 [129]. However, PCNA itself can also contribute directly to p53 stability, since p53 and its negative regulator, Mdm2, have PIP boxes and can interact with PCNA [130].

Chromatin assembly, chromatid cohesion (Table 4)

The process of duplication in eukaryotes is not just about DNA replication but is also about the replication of chromatin, which bears genetic and epigenetic information [131]. Chromatin is disassembled during progression of the replication fork and immediately reassembled on the two daughter strands. A special histone chaperone, the CAF-1 complex, delivers histones to replicating DNA using a direct interaction with PCNA via the PIP box located on its largest subunit (human CAF-1 subunit A or Cac1 in yeast). Similarly, PCNA-dependent recruitment of CAF-1 was observed at sites of NER or during bypass replication [132, 133], indicating that this process is generally important for DNA synthesis in chromatin. An interesting model of DNA synthesis and chromatin assembly coordination due to the double-trimer organization that allows PCNA to simultaneously bind DNA polymerase delta and CAF-1 on opposite faces was proposed [17, 103]. Since epigenetic information is encoded by covalent modifications of DNA and histones ('histone code'), the required enzyme

Table 5. PCNA-binding proteins involved in transcription.

#	Swiss Prot	Protein name / synonyms	Theoretical pI/ Mw	Reference
1	EP300_HUMAN	**Histone acetyltransferase p300** / E1A-associated protein p300	8.78 / 264143	[71]
2	BAZ1B_HUMAN	**Bromodomain adjacent to zinc finger domain protein 1B** / Williams-Beuren syndrome chromosomal region 9 protein / Williams syndrome transcription factor / hWALP2	8.70 / 170902	[141]
3	MBB1A_HUMAN	**Myb-binding protein 1A**	9.34 / 148855	[151]
4	RPC1_YEAST RPC1_HUMAN	**DNA-directed RNA polymerase III subunit RPC1**	8.50 / 162301 8.76 / 155748	[195]
5	TF65_HUMAN	**Transcription factor p65 /** Nuclear factor NF-kappa-B p65 subunit	5.46 / 60219	[196]
6	YBOX1_HUMAN	**Nuclease sensitive element-binding protein 1** / Y-box-binding protein 1/ Y-box transcription factor / YB-1 / CCAAT-binding transcription factor I subunit A / CBF-A / Enhancer factor I subunit A / EFI-A / DNA-binding protein B / DBPB	9.87 / 35793	[197]
7	RUVB2_HUMAN	**RuvB-like 2** / 48 kDa TATA box-binding protein-interacting protein / 48 kDa TBP-interacting protein / TIP49b / Repressing pontin 52 / Reptin 52 / 51 kDa erythrocyte cytosolic protein / ECP-51 / TIP60-associated protein 54-beta / TAP54-beta	5.49 / 51025	[151]
8	DHX9_HUMAN	**ATP-dependent RNA helicase A** / Nuclear DNA helicase II / NDH II / DEAH box protein 9	6.41 / 140958	[101, 151]
9	T22D1_HUMAN	**TSC22 domain family protein 1 /** Transforming growth factor beta-1-induced transcript 4 protein / Regulatory protein TSC-22 / TGFB-stimulated clone 22 homolog / Cerebral protein 2	5.12 / 15679	[144]
10	NONO_HUMAN	**Non-POU domain-containing octamer-binding protein /** NonO protein / 54 kDa nuclear RNA- and DNA-binding protein / p54(nrb) / p54nrb / 55 kDa nuclear protein/ NMT55 / DNA-binding p52/p100 complex, 52 kDa subunit	9.01 / 54231	[151]
11	DDX17_HUMAN	**Probable ATP-dependent RNA helicase DDX17 /** DEAD box protein 17 / RNA-dependent helicase p72 / DEAD box protein p72	8.82 / 72371	[151]
12	TCP4_HUMAN	**Activated RNA polymerase II transcriptional coactivator p15 /** SUB1 homolog / Positive cofactor 4 / PC4 / p14	9.60 / 14395	[198]
13	P53_HUMAN	**Cellular tumor antigen p53 /** Tumor suppressor p53 / Phosphoprotein p53 / Antigen NY-CO-13	6.33 / 43653	[130]

activity must also be directly coupled to the replication machinery. The diverse covalent modifications (acetylation, methylation, phosphorylation, ubiquitination, sumoylation) form a 'histone code' that regulates chromatin function by defining the accessibility of the genome to transcriptional and other machineries [134, 135]. DNA cytosine methyltransferase 1 (DNMT1) is responsible for cytosine methylation in CpG sequences which are involved in gene silencing. DNMT1 is activated and then targeted to replication foci by interacton with PCNA through a PIP box [65, 136]. Histone modification enzymes (histone deacetylase HDAC, histone acetyltransferase p300) are also recruited to the replication fork through direct interaction with PCNA [71, 77, 88]. A complete picture of PCNA involvement in faithful inheritance includes chromosome segregation as well. For accurate distribution of the genetic material between daughter cells, the sister chromatids are kept together by a cohesion ring throughout the G2 phase until anaphase. This ring is enzymatically dissolved before anaphase, allowing the tubulin spindle to pull the sister chromosomes to the daughter cells. Sister-chromatid cohesion is established in S phase, instantly after replication [137], with the help of an essential factor N-acetyltransferase called ECO1. ECO1 directly binds to PCNA via a PIP box, and this interaction is essential for its loading on chromatin, establishment of cohesion and daughter cell viability [95, 138].

Transcription (Table 5)

The scaffold proteins that form sliding clamps in viruses and bacteriophages can interact with the transcription machinery and regulate gene expression [139, 140]. Not very much information about PCNA involvement in transcription in eukaryotes has been published. PCNA may be involved in transcription via interaction and regulation of remodeling factors such as Williams syndrome transcription factor (BAZ1B_-

Table 6. PCNA-binding proteins involved in other miscellaneous functions.

#	Swiss Prot	Protein name / synonyms	Theoretical pI/ Mw	Reference	Function
1	ANXA2_HUMAN	**Annexin A2 /** Annexin-2 / Annexin II / Lipocortin II / Calpactin I heavy chain / Chromobindin-8 / p36 / Protein I / Placental anticoagulant protein IV / PAP-IV	7.57 / 38604	[198]	membrane
2	41_HUMAN	**Protein 4.1 /** Band 4.1 / P4.1 / EPB4.1 / 4.1R	5.42 / 97016	[144]	membrane
3	TC1D1_HUMAN	**Tctex1 domain-containing protein 1**	9.04 / 20729	[199]	cytoskeleton
4	EF1A1_HUMAN	**Elongation factor 1-alpha 1** / EF-1-alpha-1 / Elongation factor 1 A-1 / eEF1A-1 / Elongation factor Tu / EF-Tu / Leukocyte receptor / cluster member 7	9.10 / 50140	[146, 146, 200]	translation / cytoskeleton
5	RRMJ1_HUMAN	**Putative ribosomal RNA methyltransferase 1 /** rRNA (uridine-2'-O-)-methyltransferase	5.42 / 36079	[144]	translation
6	EIF1B_HUMAN	**Eukaryotic translation initiation factor 1b /** eIF1b / Protein translation factor SUI1 homolog GC20	6.82 / 12823	[144]	translation
7	CBS_YEAST CBS_HUMAN	**Cystathionine beta-synthase /** Serine sulfhydrase / Beta-thionase	6.25 / 56021 6.20 / 60586	[195]	cysteine metabolism
8	KIN20_CAEEL KC1D_HUMAN	**Casein kinase I isoform delta**	8.09 / 56808 9.77 / 47330	[185]	circadian clock
9	CSK22_YEAST CSK22_HUMAN	**Casein kinase II subunit alpha /** CK II	8.68 / 39403 8.65 / 41213	[201]	Wnt signaling pathway.
10	TCOF_HUMAN	**Treacle protein** / Treacher Collins syndrome protein	9.06 / 144312	[151]	transport
11	KCNA2_HUMAN	**Potassium voltage-gated channel subfamily A member 2 /** Voltage-gated potassium channel subunit / Kv1.2 / HBK5 / NGK1 / HUKIV	4.78 / 56716	[199]	ion transport
12	GLYC_HUMAN	**Serine hydroxymethyltransferase, cytosolic /** Serine methylase / Glycine hydroxymethyltransferase / SHMT	7.61 / 53082	[185]	interconversion of S and G
13	G3P_HUMAN	**Glyceraldehyde-3-phosphate dehydrogenase /** GAPDH	8.57 / 36053	[202]	glycolysis
14	Q9V9R2_DROME CUL2_HUMAN	**CG1512-PA, isoform A** **Cullin-2 /** CUL-2 drosophila	6.46 / 87391 6.46 / 86983	[147]	proteolysis
15	TRAF6_HUMAN	**TNF receptor-associated factor 6 /** Interleukin-1 signal transducer / RING finger protein 85	6.00 / 59573	[144]	proteolysis
16	CRCM_HUMAN	**Colorectal mutant cancer protein /** Protein MCC	5.40 / 93055	[144]	cancer
17	Q13537_HUMAN	**Putative transposase /** Tigger / Pogo	8.89 / 51666	[203]	transposition
18	1C04_HUMAN	**HLA class I histocompatibility antigen, Cw-4 alpha chain [Precursor] /** MHC class I antigen Cw*4	6.04 / 40994	[144]	immune response
19	IKKE_HUMAN	**Inhibitor of nuclear factor kappa-B kinase subunit epsilon** / I kappa-B kinase epsilon / IkBKE / IKK-epsilon / IKK-E / Inducible I kappa-B kinase / IKK-i	7.91 / 80462	[144]	immune response
20	1B08_HUMAN	**HLA class I histocompatibility antigen, B-8 alpha chain [Precursor] /** MHC class I antigen B*8	5.46 / 40330	[144]	immune response
21		**MHC Class II proteins** **AA65-79 NIAVLKHNLNIVIKR**		[143]	immune response

HUMAN) or p300 (EP300_HUMAN) [141, 142]. In addition, a direct interaction between PCNA and RNA polymerase III (RPC1_NUMAN), and some other transcriptional factors, like p53 (P53_HUMAN), has been detected (Table 5).

Other miscellaneous functions (Table 6)

Surprisingly, there is an additional list of PCNA-interacting proteins (Table 6) which are involved in cellular functions where the role of PCNA is difficult to explain. PCNA-dependent processes are thought to occur in the nuclei, on DNA or chromatin, and PCNA is thought to function as a loading platform for coordination of multiple activities. However, the functions of the majority of PCNA-binding proteins presented in Table 6 are cytoplasmic or membranous. This indicates additional PCNA functional aspects that are still waiting to be disclosed. One of these functions is immunological. Interestingly, although PCNA was initially discovered 30 years ago as a lupus

antigen [1], the mechanism(s) connecting PCNA and the immune response remain unknown. It was hypothesized that PCNA presents itself as a specific target or 'sensor' for the immune system [143]. This could explain the interaction of PCNA with HLA class I histocompatibility antigens and MHC class II proteins [143, 144]. It is tempting to speculate that interaction of PCNA with membrane and cytoskeletal proteins could be involved in the process of PCNA transport, a practically unknown aspect of PCNA metabolism. PCNA doesn't have any known nuclear localization signal (NLS) sequence and is a very acidic protein, which is uncharacteristic for nuclear proteins, but is normally localized almost exclusively inside nuclei. Other mechanisms, not regulated by importin alpha and beta, must be responsible for PCNA import into nuclei. The interaction of PCNA with translation factors (EF1A, E1F1B) or metabolic enzymes (GAPDH, beta-thionase, serine methylase) indicates other possible functions of these proteins. For example, besides being a major translation factor, EF1A could be a component of the ubiquitin-dependent proteolytic system [145] and could be involved in the maintenance of the balance between the mono- and polyubiquitinated forms of PCNA [146]. Since PCNA can be regulated through ubiquitination and sumoylation, it may also act to direct ubiquitination and proteolysis of additional proteins, like Cdt1 [121]. The ability of PCNA to interact with proteolytic enzymes like cullin-2 (CUL2_HUMAN), TNF receptor-associated factor 6 (TRAF6_HUMAN) and E3 ubiquitin-protein ligase Mdm2 (MDM2_HUMAN) [130, 144, 147], provides some support for its role in proteolysis regulation.

Summary

PCNA has no enzyme activity, and its role in a cell depends on its ability to mediate interactions between proteins and DNA. The function of PCNA in chromatin is directly related to the coupling of DNA replication/repair processes with other cellular functions such as chromatin remodeling and epigenetic modifications. PCNA may achieve this function by providing a moving 'scaffolding' platform for DNA polymerases and other proteins such as p300, CAF-1 and HDAC1 [17, 33, 77, 84, 95, 103, 148]. An interesting property of PCNA is that it can act as a scaffold for different proteins even without interaction with DNA [83, 117]. In this case, a potential question is whether PCNA is located on special structures like the nuclear matrix or freely distributed in nuclei, or even cytoplasm. Another important question involves determine the regulation of the binding of a specific protein to the PCNA platform at a specific time and space. Keeping in mind that the list of potential PCNA-binding partners is getting longer and longer, this should be a crucial regulatory mechanism. Post-translational modifications of PCNA (and other proteins) may be part of this regulation. Therefore, examination of the signal networks leading to the post-translational modification and association/dissociation of protein complexes with PCNA is necessary to provide a more complete understanding of PCNA-involved control mechanisms. However, studying these mechanisms is very challenging, since the association and dissociation of PCNA with other proteins appears to be very dynamic. Nevertheless, this kind of dynamic interaction between the same and different protein partners in a given time and space may be extremely important for the regulation of diverse and complex cellular functions. As proteomics approaches such as 2DE or large-scale mass spectrometry have already been successful in PCNA studies, we can expect that further proteomics-based studies will increase our understanding of mechanisms of PCNA interactions and its role in the network that regulates cell behavior. Completely new methods or new combinations of known proteomics methods will be the crucial factors in this breakthrough. A wide spectrum of activity of PCNA-binding proteins indicates a possible involvement of PCNA in some unexpected functions. Possibly, these functions may be related to processes where proteins listed in the Table 6 are involved or could be absolutely different.

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