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PCNA BINDING PROTEINS

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1. ABSTRACT

PCNA (proliferating cell nuclear antigen), originally characterized as a DNA polymerase accessory protein, functions as a DNA sliding clamp for DNA polymerase delta and is an essential component for eukaryotic chromosomal DNA replication. Recent studies have revealed a striking feature of PCNA in its ability to interact with multiple partners, involved, for example, in Okazaki fragment joining, DNA repair, DNA methylation and chromatin assembly. Since these reactions take place mainly on replicating DNA, PCNA has applications as a marker for DNA synthesis. It is of interest that proteins involved in cell cycle regulation may also exhibit PCNA binding activity. For example, the CDK inhibitor, p21 (Cip1/Waf1) interacts with PCNA blocking its activity necessary for DNA replication and also affecting interactions with other PCNA binding proteins. The available data indicate that DNA sliding clamps have generated additional functions with evolution of eukaryotes from simple prokaryotes. In mammalian cells, they play key roles in controlling DNA synthesis reactions and the reorganization of replicated DNA at replication forks. Several cell cycle regulation proteins target these processes by affecting PCNA actions.

2. INTRODUCTION

The well-known cell cycle marker protein, PCNA (proliferating cell nuclear antigen) was originally identified as an antigen for autoimmune disease in systemic lupus erythematosis patients (1). Its periodic appearance in S phase nuclei, co-localized with incorporated bromodeoxyuridine (2), suggested an involvement in DNA replication, and indeed, PCNA was later identified as an essential factor for SV40 (simian virus 40) DNA replication *in vitro* (3). Since this DNA replication mimics that of mammalian chromosomes, PCNA was hypothesized as generally required for eukaryotic chromosomal DNA replication. Subsequent biochemical and genetic studies with budding yeast demonstrated that PCNA is in fact essential for chromosomal DNA replication (4-6).

Comparisons of elongation processes in *E. coli*, phage and eukaryotic systems have revealed that the replication apparatus is highly conserved in terms of structure and function (7). Factors called DNA sliding clamps exist in common in all of the systems and make individual polymerases to synthesize long DNA strands efficiently. For example, the *E. coli* beta-subunit and PCNA are representative of prokaryotic and eukaryotic DNA sliding clamps, specifically stimulating their respective partners, DNA polymerase III (pol III) and DNA polymerase delta (pol delta). Clamps have a common ring shape that is able to rap around DNA and slide along double helical DNA freely in both directions (8-10) so that their interactions with DNA polymerases stabilize the enzymes on template. Regarding their closed ring structure, the topological problem of how a sliding clamp is formed on DNA requires explanation. Factors called clamp loaders are necessary to open the closed rings. Replication factor C (RFC) plays this role for PCNA through its specific binding and ATPase activity (11-14).

In addition to functioning as a DNA polymerase accessory factors, PCNA interacts with a number of proteins. Some of them are involved in Okazaki fragment joining, DNA mismatch repair and chromatin

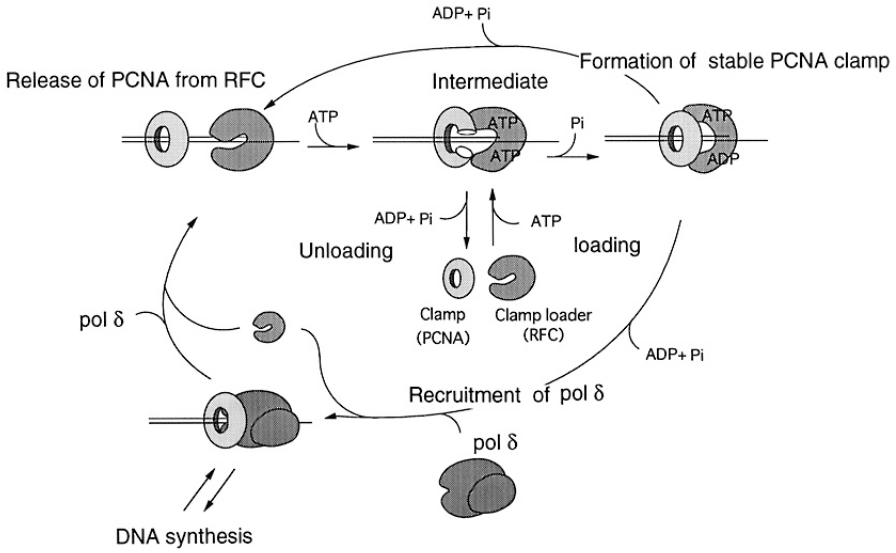

Figure 1. Loading and unloading pathway of PCNA at the 3' end of DNA by interaction with RFC. PCNA and RFC form an intermediate complex by binding of ATP to RFC. Number of bound ATP is arbitrary. Their sequential hydrolysis drives formation of stable PCNA clamp, and the completion of hydrolysis releases PCNA from RFC. If pol delta exists, its recruitment links with this step. Reverse reaction of this loading will unload PCNA from DNA in ATP hydrolysis dependent manner.

reorganization as in DNA methylation or chromatin assembly (15-17). Interestingly, since most PCNA binding proteins function on newly replicated DNA, PCNA may have a central role by functioning as a docking partner. Several cell cycle control proteins can also interact with PCNA and may modulate its functions. Thus, the eukaryotic DNA sliding clamp appeared to have more roles than a simple DNA polymerase accessory protein. This review will summarize data on PCNA binding proteins and propose a possible mechanism to explain their recruitment to replicating DNA strands through PCNA interactions.

3. DNA SLIDING CLAMP

PCNA is analogous to the pol III beta-subunit and the T4 gene45 protein functioning as DNA sliding clamps. This type of protein encircles DNA and freely slides along DNA with its closed ring structure. This characteristic well demonstrated by an experiment in which the beta-subunit stayed on circular DNA but quickly fell off upon its linearization with a restriction enzyme (18, 19), implies a role as a processivity factor for DNA polymerases by stabilizing the enzymes on a template DNA through specific protein-protein interactions. Crystallography of the beta-subunit and PCNA demonstrated well-conserved ring structures with a sufficiently large central hole to accommodate double helical DNA in both cases (8), though there is almost no similarity regarding their primary amino acid sequences. They form head to tail joined homodimer or homotrimer structures composed of six repeating domains in six-fold symmetry (8-10). Both proteins have a two layer structure composed of 12 inside alpha-helices and outside beta-sheets. The inside alpha-helices carry an array of basic residues and are positioned roughly perpendicular to the phosphate backbone of DNA and do not form a tight contact. This structure allows the clamps to slide freely on DNA.

4. CLAMP LOADING

The head to tail connection at the interface of clamp monomers is maintained by stable interactions of hydrophobic residues. Loading of clamps onto DNA requires opening of their closed ring temporally to pass through the DNA strands. Since this topological problem exists for all clamp molecules, they share the feature of specific associated chaperons called clamp loaders (20). RFC play this role for PCNA. O'Donnell's group has studied the clamp loading process in detail using *E. coli* factors (21-23). They confirmed opening of the beta-subunit ring during its loading driven by a structure change with the clamp loading protein, gamma-subunit. Binding of ATP to the gamma-subunit is an inducing factor in this process. Similarly, RFC forms a complex with PCNA and the interaction mode is changed by ATP (24) indicating an ATP dependent common mechanism to transfer DNA into the center holes of clamps. Recent studies have indicated that binding of ATP to RFC allows the PCNA ring to open (25). If its hydrolysis occurs, a stable clamp on DNA is formed and interactions between RFC and PCNA become weak and are disrupted (figure 1). Through these cycles, multiple PCNA molecules can be loaded on DNA by single RFC.

Analyses of mutant PCNAs have indicated that the major RFC interaction sites are the loop located at the center of PCNA monomer (Asp41 to His44) and the carboxyl-terminal tail (Lys254-Glu256), both of which protrude on one face, called the C-side (26-28). The distribution means that this C-side face makes contact with RFC and thus orientates to the 3' end of a synthesizing DNA strand when it is loaded on DNA. Since pol delta

subsequently substitutes for RFC and associates with PCNA on the same face, it will be precisely recruited to the elongating DNA end (figure 1). After completion of its task as a pol delta processivity factor, PCNA must be released from DNA. It has been proposed that RFC causes this unloading through hydrolysis of ATP as the reverse reaction of loading (19, 29). As described below, several PCNA binding proteins can access newly replicated DNA. In this case, PCNA remaining attached after DNA synthesis by pol delta may play a further active role.

5. DNA POLYMERASES INTERACT WITH PCNA

PCNA is required for the elongation stage of SV40 DNA replication *in vitro*, especially for simultaneous synthesis of leading and lagging DNA strands (30). Its omission from the reaction results in an accumulation of unligated short Okazaki-DNA, due to the requirement of PCNA and its partner, pol delta for complete synthesis (31). PCNA has been identified as a processivity factor for pol delta (32-34) and the polymerase alone elongates only several nucleotides from primers. This increases to more than one thousand, however, on its interaction with PCNA. A long loop structure of PCNA from Leu121 to Glu132, called the interdomain connecting loop interacts with pol delta (26, 27, 35, 36) and a direct physical interaction between the two has been detected using PCNA-fixed beads (14). PCNA alone is sufficient to allow pol delta to synthesize long DNA on a synthetic DNA template. Pol delta is composed of at least two subunits with apparent molecular weights of 125 and 50 Kdal in mammalian cells but bigger complexes with additional subunits have also been reported in yeasts (37-39). Recently, a potential third 66Kdal polypeptide subunit for mammalian pol delta has been discovered by PCNA-fixed resin affinity chromatography (40). A PCNA interaction site of pol delta was mapped to the N-terminal region of the largest subunit (41,42), but the complex of 125 and 50 Kdal subunits is necessary for PCNA dependent DNA synthesis (43). Interestingly, the novel subunit has a typical PCNA binding motif allowing provision of a further additional contact with PCNA to pol delta.

In addition, PCNA is able to stimulate the other eukaryotic DNA polymerase, pol epsilon under physiological salt conditions (44, 45). In contrast to the pol delta case, the carboxyl-terminal region of PCNA is necessary for this (35). Thus, though PCNA is a processivity factor for both pol delta and epsilon, the underlying mechanisms of action are different. Yeast genetic studies have revealed that pol delta and epsilon are both essential for progression through the S phase, and they must have distinct roles during chromosomal DNA replication (46, 47). A recent report indicated that the catalytic action of pol epsilon is not essential (48), so that it may have some regulatory function in S phase.

6. INVOLVEMENT OF PCNA IN DNA REPAIR

DNA repair reactions feature DNA re-syntheses which occurs after removal of DNA lesions. The DNA elongation apparatus, including pol delta and epsilon and their accessory proteins is essential for this purpose. Indeed, a requirement for PCNA has been reported for *in vitro* nucleotide excision repair (49-52), base excision repair (53-56), and mismatch repair (57, 58). PCNA accumulates in DNA repair active cell nuclei after UV irradiation, even though the cells are not in S phase (59, 60). DNA polymerases, RFC and PCNA recognize 3' ends of gapped DNA and fill the gaps by the same mechanism as used for joining of Okazaki fragments. One obvious phenotype due to PCNA mutation in budding and fission yeasts is an increased sensitivity to DNA damage (5, 6, 35, 61), indicating an active involvement in cellular DNA repair process.

In addition, there are specific interactions between PCNA and several DNA repair related gene products. For example, DNA fragment processing and joining proteins, FEN1, DNA ligase and XP-G bind specifically to PCNA (62-67). As described below, such interactions would be expected to increase efficiency of DNA re-synthesis reactions. MSH2 and MLH1 have also been reported to interact with PCNA (57), and detailed analyses shown that the requirement for PCNA in mismatch repair is not only in the DNA re-synthesis step but also in the earlier repair initiation for which these factors are required. A possible involvement of PCNA in a base excision repair reaction was further indicated by recent demonstration of PCNA binding to uracil-DNA glycosylase (68). The available data thus strongly suggest that PCNA is a general DNA repair factor involved in DNA damage recognition and DNA re-synthesis steps through its interaction with multiple repair factors.

7. GENERAL FEATURE OF PCNA BINDING PROTEINS

The amount of PCNA in cells is more than ten-fold that of pol delta or RFC and has therefore been surmised that PCNA might possess additional roles other than as a DNA polymerase accessory factor. Indeed, several PCNA binding proteins have been discovered by various means. Some of their PCNA binding regions have been localized within relatively narrow amino acid stretches and one common feature elucidated as a PCNA binding motif was - Q - - I/L - - FF - (28, 69). This motif exists in various PCNA binding proteins, such as p21(CIP1), FEN1, XP-G, DNA cytosine methyltransferase, DNA ligase I and pol delta 66Kdal subunit, but not in other pol delta subunits nor RFC subunits. So far, more than ten PCNA binding proteins have been reported to exist (70). However, the significance of their interactions with PCNA has not been elucidated with few exceptions. One feature common to more than half of them is a function on replicated DNA, such as in processing of primer RNA, DNA repair, DNA methylation and chromatin assembly. Thus, it is likely that in the normal cell growth phase, PCNA may remain on DNA for a certain period after progression of replication forks, and function as a docking tool (figure 2). Additional roles of prokaryotic clamp molecules remaining on replicated DNA have been reported with the *E. coli* beta subunit and the T4 gene45 protein, showing involvement in regulation of the initiator protein, DnaA reactivation (71), and in transcription

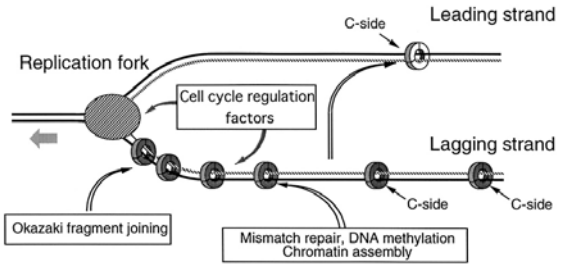

Figure 2. Multiple roles of PCNA in association with DNA replication. Partners of PCNA are exchanged after DNA synthesis, coordinating with movement of a replication fork. The newly synthesized DNA strands (broken lines) are distinguishable by the direction of C-side (darker side) if PCNA remains on DNA. Mismatch repair proteins and MeTase may use their interaction with PCNA for discrimination of a new DNA strand.

of the genes expressed in late stages of T4 phage infection (72), respectively. Systematic analyses of PCNA binding proteins from mammalian cell lysates by affinity chromatography have been reported (40, 73). Most PCNA binding proteins were reproducibly detected in the PCNA bound fractions, indicating the reliability and usefulness of this method.

8. PCNA BINDING PROTEINS FOR DNA FRAGMENT PROCESSING AND JOINING (FEN1, DNA LIGASE AND XP-G)

Before completion of DNA elongation, discontinuous lagging strands are joined to each other by the coordinated action of RNaseH, DNA ligase and a specific nuclease (maturation factor 1, MF1), in addition to the pol delta complex (31). The MF1 nuclease is a flap endonuclease, also called FEN1, which exhibits a specific interaction with PCNA from yeast to human (62, 63). The interaction results in stimulation of FEN1 nuclease is necessary to process newly synthesized Okazaki fragments prior to ligation. DNA ligase I, one component in the DNA joining process, has also been found to interact with PCNA (65), thereby affecting DNA synthesis by pol delta and epsilon (66), suggesting exchange of the PCNA partner from pol delta to ligase I during the Okazaki fragment joining step. Thus, PCNA could function as a linker between DNA polymerases and other factors to coordinate DNA synthesis and processing of lagging strands in a replication fork complex.

A similar situation may also prevail in DNA repair as introduced above. FEN1 is encoded by the *RAD27/RTH1* gene in budding yeast (74, 75) and the *Rad2* gene in fission yeast (76), shown to be involved in sensitivity to DNA damage and chromosome instability. Indeed, the substrate of FEN1, a flap DNA structure, is frequently produced at damaged or mismatched sites, or in the process of DNA repair or recombination. Thus, specific interactions of PCNA with FEN1 appear to be important for processing of repaired DNA fragments. Xeroderma pigmentosum (XP) G protein, which is a structure-specific repair endonuclease similar to FEN1 and required for nucleotide excision repair, is also reported to interact with PCNA (67). Since the PCNA binding domain in XP-G is crucial for its nucleotide excision repair activity, the interaction may represent a mechanistic linkage between excision and repair re-synthesis by recruiting various PCNA binding proteins after the reaction.

9. PCNA BINDING PROTEINS FOR DNA MISMATCH REPAIR (MSH2 AND MLH1)

Mismatch repair proteins, MSH2 and MLH1 interact with PCNA in both budding yeast and human (57). The results strongly suggest an important role for PCNA in mismatch repair processes. The requirement for PCNA in mismatch repair is not only in the DNA re-synthesis step but also in the earlier repair initiation and it might be involved in a DNA error recognition step through these interactions. Mismatch repair is one crucial mechanism to increase accuracy of DNA synthesis and proceeds following a DNA replication fork. Important step for the reaction is discrimination of a DNA strand to be repaired. Thus, meaning of the interaction could be speculated that mismatch recognition proteins will distinguish which DNA strand is newly synthesized and to be repaired through interaction with PCNA. Indeed, as mentioned above, PCNA is always loaded on DNA in a fixed orientation to face its C-side to DNA synthesis direction. Therefore, if mismatch repair proteins interact with the specific face of PCNA on DNA, they could access to a newly replicated DNA strand precisely through this protein-protein interaction (figure 2).

10. A QUATERNARY COMPLEX OF PCNA/p21/CDK/CYCLIN (LINKAGE OF PCNA WITH CELL CYCLE CONTROL)

The existence of a quaternary complex of PCNA/p21/CDK/cyclin in normal fibroblasts has been reported (77, 78). p21 (CIP1/WAF1) is induced by DNA damage, senescence or differentiation of cells through p53 dependent or independent pathways(79), inhibiting CDK/cyclin activity and blocking progression of cell cycle at G1 to S. It is known to form a tight complex with CDK through its amino-terminal half region and with PCNA through its carboxyl-terminal half region, thus acting as a bridge between the two (80, 81). The precise roles of this complex have yet to be elucidated yet, but it may contribute to coordination of cell cycle progression and DNA replication. In addition to the quaternary complex, CyclinD directly binds to PCNA (82) and it has been reported that its overproduction in fibroblasts inhibits chromosomal DNA replication, this being relieved by co-expression of PCNA (83). Therefore, induction of DNA replication at G1/S boundaries might be explained by release of PCNA from PCNA/cyclinD complexes.

In vitro binding of p21 to PCNA results in the block of SV40 DNA replication but not PCNA-dependent nucleotide excision repair *in vitro* (84-86). Further analyses showed that p21 inhibits DNA elongation by pol delta (epsilon) efficiently but not their short gap filling activity

(87, 88). *In vivo* studies support these different effects of p21 on DNA replication and repair (89). However, the controversial observation that p21 inhibits nucleotide excision repair (90) and mismatch repair (57) as well as replication should be noted. The reasons for this await explanation.

p21 also efficiently inhibits RFC ATPase stimulation by PCNA (27). This ATP hydrolysis is required not only for the formation of PCNA clamps on DNA but also for its unloading. Thus, blocking of the RFC ATPase results in inefficient turn-over of DNA polymerases during the elongation stage in SV40 DNA replication. In contrast, DNA repair requires only a single binding of a DNA polymerase to a template DNA. Therefore, it is probable that semi-saturated amounts of p21would inhibit DNA replication reactions selectively. From this aspect, production of p21 in response by DNA damage may control S phase progression by decreasing the efficiency of replication fork movement, providing time for DNA damage repair or recruitment of replication proteins. Indeed, it has been demonstrated that in UV-irradiated fibroblast cells, comparable amounts of p21 and PCNA are produced and co-localize at distinct structures in the nuclei where DNA repair occurs (89).

Relating to the p21-PCNA interaction, it has been reported that the human papillomavirus oncoprotein (HPV-16 E7 protein) binds to p21 and blocks its inhibitory function, resulting in abrogation of DNA damage-induced cell cycle arrest, even in the presence of high levels of p21 (91, 92). The E7 binding site on p21 maps to its C-terminal region, overlapping with the PCNA binding site and the second cyclin binding motif. Thus the E7 binding to p21 interferes with quaternary complex interactions. The data strongly indicate that the status of the latter in growing cells is an important determinant of cell cycle progression from G1 to the S phase. This oncogene may thus allow cells to escape from DNA damage checkpoint control by blocking p21 activity and thus result in fixation of mutations and neoplastic development.

11.GROWTH ARREST INDUCED PROTEINS AND THEIR PCNA BINDING

The growth arrest and DNA damage induced protein, GADD45 and the related product of the myeloid differentiation primary response gene, MyD118, have also been identified as PCNA binding proteins (93-95). In the GADD45 case, binding is competitive with that of p21 (96) and is presumably involved in a DNA damage response pathway, since there is an intimate relation between GADD45 expression and DNA damage. Interestingly, both MyD118 and GADD45 modestly stimulate DNA excision repair *in vitro* (95), indicating possible roles in DNA repair. However, it should be noted that there is a controversial result in which GADD45 had no positive role in excision repair (97). The two proteins being structurally and functionally similar are involved in cell growth arrest. Thus they may be members of a common mechanisms of negatively modulating cell growth, their PCNA binding possibly playing roles in this regulation.

12. PCNA BINDING PROTEINS FOR MAINTENANCE OF CHROMOSOME STRUCTURES

DNA replication is an occasion when chromatin structure is reorganized, and programs for inheritable cellular functions are reset. It has been demonstrated that a mutation of the *mus209* gene encoding PCNA in Drosophila causes pleiotropy of temperature-sensitive lethality, hypersensitivity to DNA damage and suppression of position-effect variegation. The last indicates that with the *mus209* mutant background, a marker gene located in a transcription-inactive region becomes active, with remodeling of chromatin (98). Thus, there may be a role for PCNA in maintaining the chromatin state. Indeed, direct involvement of PCNA in replication coupled chromatin assembly has been reported. In SV40 DNA replication *in vitro*, chromatin assembles preferentially on replicated DNA. A nuclear protein complex called CAF1 has been identified as an essential factor for the coupling of chromatin assembly and DNA replication (99). Further studies elucidated that CAF150, the large subunit of CAF1, interacts with PCNA specifically and that this has a role in the replication coupled chromatin assembly (100).

It is known that methylated CpG sequences in the mammalian genome are heritable, and that density of methylation in certain gene loci affects gene expression (101). Methylation patterns in parent DNA are maintained during replication by the maintenance type DNA (cytosine) methyltransferase (MeTase) which selectively methylates the hemimethylated sites on newly replicated DNA. It has been reported that MeTase interacts with PCNA (102). Maintenance of the DNA methylation pattern after DNA replication might also be dependent on PCNA, since p21 affects methylation of the genome. Thus, cancer cells lacking p21 function would be expected to have an altered pattern of DNA methylation due to unregulated PCNA/MeTase interactions. This may be linked with genomic instability and abnormal regulation of several crucial genes.

Sister chromatid cohesion is also crucial for maintenance of replicated chromosome structures, since it is necessary to separate sisters precisely into daughter cells during mitosis. Cohesion between sisters is established during DNA replication, exists along their entire length through metaphase, and is lost at the metaphase/anaphase transition. Recent progress of studies on sister chromatid cohesion in molecular basis has identified several essential factors involved in this process. *CTF7* (chromosomal transmission fidelity) gene in budding yeast encodes an essential protein for cohesion and functions in its establishment stage, since Ctf7p activity is required only during S phase (103). Interestingly, *ctf7* genetically interacts with a DNA replication mutant *pol30*, which encodes PCNA. These findings provide the first evidence that links the establishment of sister chromatid cohision to DNA replication machinery and suggest that PCNA is involved in the process through interaction with the component of cohesion complexes.

Table 1. Sorting of the PCNA binding proteins

PCNA binding protein	Center loop (Asp41-His44)	Interdomain connecting loop (Leu 121-Glu132)	C-terminal tail (Lys254-Glu256)
pol delta	+	+	-
pol epsilon	ND	-	+
RFC	+	-	+
FEN1*	+	+	+
P21 (CIP1, WAF1)	+	+	+
GADD45	ND	ND	+
MeTase	+	+	+

PCNA binding proteins are sorted according with their target sites on PCNA. Only proteins whose binding sites have been studied were chosen for demonstration of their ability to interact with three major target sites. +: required for interaction, -: dispensable for interaction, ND: not determined. *: own unpublished data

Taking all the available observations into consideration, PCNA could have a master function for maintenance of heritable information in the genomic structure when it is retained on replicated DNA after completion of its function as a pol delta processivity factor (figure 2).

13. TARGET STRUCTURE FOR PCNA BINDING PROTEINS

Functional sites on PCNA have been mapped with mutant PCNAs. Most of them are required for interaction with specific partners and are located in loop structures protruding on the C-side (28, 70). The sequence from Asp41 to His44 (center loop) is the site necessary for specific interaction with RFC. Pol delta also makes contact in the same region. Interestingly, most PCNA binding proteins, including p21, whose PCNA binding has been studied so far use this region to associate with PCNA.

A long loop structure at the side of PCNA, corresponding to residues from Leu121 to Glu132 spans from one domain to another, and is called the interdomain connection loop (9). Analyses of mutations in this region have revealed that it is necessary for pol delta DNA synthesis (26-29, 35,36). In contrast, interactions of RFC or pol epsilon were not affected. Several PCNA interacting proteins, for example p21 and FEN1, also recognize this loop (27). One more important region is the C-terminal tail located beside the center-loop on the C-side. Mutations in this region specifically affect the ability to interact with RFC, pol epsilon, FEN1, GADD45 and p21 (26-28). Table 1 summarizes data for the overlapping nature of target sites for PCNA binding proteins. This means that simultaneous association of different PCNA binding proteins with PCNA is unlikely to occur, and partners must be exchanged sequentially for their functions in processes extending from DNA synthesis to chromatin assembly. In addition to these major target sites, several other sites affecting interaction with specific partners exist, for example Asp97 for RFC (26) and Ala231-Val233 for p21 (27). This may mean that each PCNA binding protein has its specific contact site(s), and forms its first contact through this prior to partner exchange.

14. PERSPECTIVES

Alongside the intrinsic importance of PCNA as replication factor, a number of cellular functions requiring PCNA have now been discovered involving various PCNA binding proteins with diverse actions. However, they are linked by their contributions to the precise inheritance of cellular functions to new generations of cells. From this aspect, we must change our concept of PCNA from that of just a clamp to one of a master molecule for chromosome processing. It is apparent that the existence of a DNA sliding clamp is a necessity for all organisms. However, during evolution, clamp molecules have gained increasing number of functions. Indeed, PCNA binding proteins active in cell cycle regulation or replicated chromosome re-organization have only been identified in mammalians. One could thus speculate that mammalian cells utilize PCNA for more diverse cellular functions than monocellular organisms because of their greater potential for different fates. Although the number of PCNA binding proteins continues to increase, the significance of their interactions with PCNA in many cases remains unclear. Therefore, more attention should be concentrated on understanding of PCNA functions in association with such partners. Their cellular behavior is an important target for the future studies to elucidate PCNA functions in detail.

15. ACKNOWLEDGEMENTS

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