

PCNA: The Sliding Clamp that Tethers Essential Binding Partners to the DNA Template During Replication and Repair

Proliferating Cell Nuclear Antigen, (PCNA) was discovered in 1978 by Miyachi et al. as an antigen for the autoimmune disease systemic lupus erythematosus (1). PCNA's function eluded researchers at the time. Subsequent research noted its appearance during the S-phase of the cell cycle implicating involvement in replication. PCNA was later identified as an essential factor for replication *in vitro* for the SV40 (simian virus 40) (2). The 36kDa PCNA is now understood to be the processivity factor during replication and repair. It tethers the DNA polymerase to the template allowing the synthesis of tens of thousands of base pairs versus approximately ten to fifteen base pairs in its absence, and is essential for chromosomal DNA replication (3). Functioning as a processivity factor in replication appears to be the main role of PCNA. However, ongoing research is showing it plays supporting roles in a multitude of cellular functions pertaining to replication and repair.

PCNA's structure is intimately involved in multiple functions, sliding along DNA tethering binding partners to the template. It was hypothesized that a ring structure would be plausible. In 1995 the crystal structure of PCNA was solved and submitted to the protein data bank (1PLQ, 1PLR) by Krishna et al., (4).

With the mystery of structure solved the details of exactly how this trimeric protein functioned could be explored in detail. The trimer is composed of three identical subunits. Each subunit is composed of 256 residues forming three β -sheets and five α -helices. The helices pack into the center of the ring and the β -sheets form the exterior of the protein (*Fig 1*). Two of the five helices are 3-10 helices, which display fewer residues per turn and cause a noticeable kink. It is not uncommon to find proline residues within these helices, as is the case with the larger of the 3-10 helices of PCNA. The smaller helix is comprised of only three residues S, E, G. The exterior of the protein is primarily hydrophilic and negatively charged, the interior positively charged and hydrophobic. Since DNA is a negatively charged molecule, the interior constitution of PCNA is an ideal place for DNA to enter. There appear to be two major sites of activity in PCNA, the center of the ring where DNA is fed through and the inter-domain linker loops (*fig 2*). The inter-domain linker loops connect the N and C domains of each subunit and provide a docking platform for many of the proteins associated with replication and repair. The dynamics of interaction between DNA and PCNA are not yet fully understood.

The formation of the trimer also lacks many details. The head to tail hydrophobic interactions at the interface of the

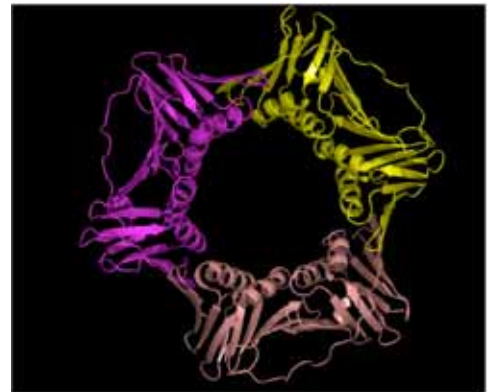


fig. 1 Trimer of PCNA

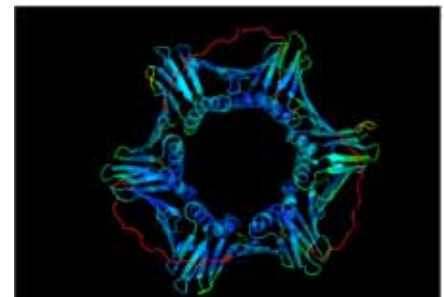


fig. 2 Inter-domain linkers in red

monomers are the main stabilizing feature of the molecule (*fig 3*). The research of Piard et al., has shown that a single point mutation at residue 69 involving the change of glycine to aspartate is enough to halt trimerization in *S. pombe* PCNA (5). The comparison of *S. pombe* and human PCNA is relevant and applicable as the sequence identity amongst the two proteins is greater than 95%. Sequence identity amongst eukaryotic PCNA averages greater than 90% with only a few exceptions, namely the fly at only 70%. Sequence identity between prokaryotic and eukaryotic PCNA is virtually non-existent, however structural homology is strikingly similar and the core functions of both are that of processivity factor for their respective polymerases(*fig. 4*). The ancillary actions of the sliding clamp in eukaryotes and prokaryotes are continually being discovered.

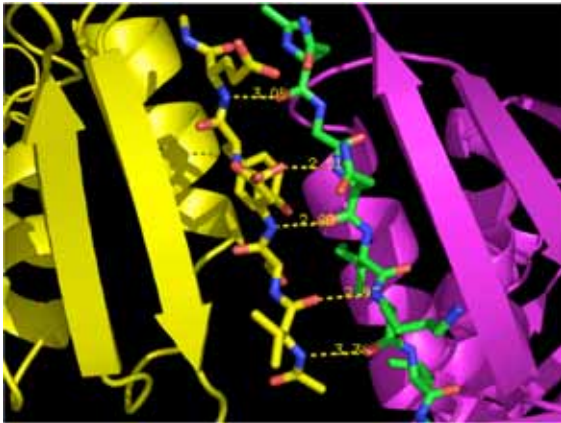


fig. 3 Interactions for trimerization

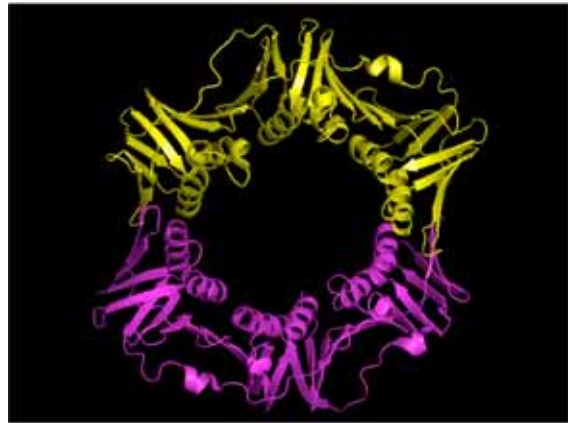


fig. 4 Prokaryotic beta clamp

The ancillary processes of PCNA include binding interactions with the following:

- DNA (interior interaction)
- Polymerase δ and ϵ (exterior interaction)
- p21, a CDK inhibitor (exterior interaction)
- Replication factor C (RFC), the PCNA clamp loader and unloader (exterior interaction)
- FEN1, an endonuclease active in mismatch repair (exterior interaction)
- DNA ligase (exterior interaction)
- XPG, an endonuclease active in nucleotide excision repair (exterior interaction)
- UNG2, MSH2, MLH1 – MMR and NER repair systems (exterior interaction)

There exists a common feature among the majority of docking partners whose interactions lie within the inter-domain linker on the C-face of PCNA. They contain the conserved sequence, -Q--I/L--FF- that interacts with the loop. One of PCNA's docking partners, p21 is the superstar of cyclin dependant kinase inhibitors and can shut down the cell cycle at three of the four CDK/cyclin checkpoints. To completely insure that synthesis of corrupted DNA does not occur it also interacts with PCNA causing a slight conformational shift that renders the protein inactive. It is not just this conformational shift that destroys function but mainly that p21 binds to the docking site required by the polymerase in a competitive manner. As the corrupted DNA

is sensed, p53 transcriptionally activates p21, the amount of p21 surpasses that of the polymerase and blocks its docking site (*fig 5,6*).

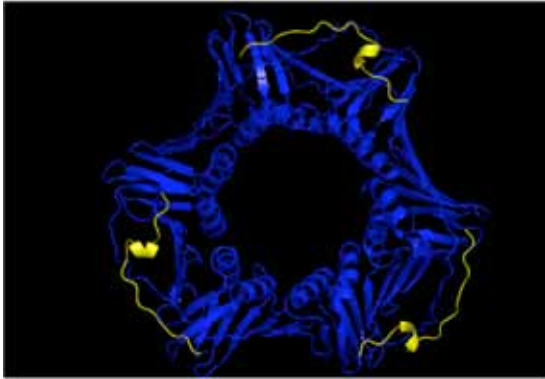


fig. 5 Bound p21

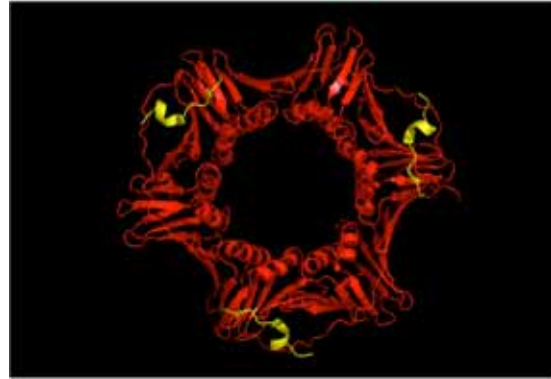


fig. 6 Bound Pol Delta

The following table lists the specific residues in PCNA that interact with each protein respectively:

Docking Partner	Asp41-His44 of Center loop	Leu121-Glu32 of Interdomain linker	Lys254-Glu256 of C-term tail
Pol delta	Yes	Yes	No
Pol epsilon	No	No	Yes
RFC	Yes	No	Yes
FEN1	Yes	Yes	Yes
P21	Yes	Yes	Yes

However, all of these interactions are moot unless Replication Factor C (RFC) loads PCNA on to DNA. RFC complexes with PCNA at the residues indicated in the above table. RFC actually opens one interface of the trimer in an ATP dependant manner to load it on DNA. Once hydrolysis of ATP occurs the clamp is loaded, its affinity for PCNA becomes weak and it dissociates to load another PCNA onto the template (6). The RFC-PCNA interface is also located on the C-side of the protein. Analysis of mutant PCNA has shown that Lys254-Glu256 is essential for the ATPase activity of RFC (*fig. 7,8*). Residues 41-44 are essential for docking of RFC. A single mutation in this region will either block docking of RFC or hydrolysis of ATP.

In figure 7, the critical interactions of the above-mentioned residues occur at the interface of the orange PCNA monomer and the gamma (yellow) subunit of RFC.

Figure 8 demonstrates a view through the top of PCNA, the C-side is facing down. DNA is loaded through the RFC towards the PCNA, which is then oriented towards the 3' end of the template.

PCNA appears to represent divergent evolution amongst eukaryotes, as it displays high sequence identity from yeast to humans. When comparing with prokaryotes it would seem to be divergent evolution as virtually no sequence identity exists between the two. Also, the prokaryotic beta clamp only contains two subunits compared with three subunits in eukaryotic PCNA.

PCNA represents one of the many circles of life. The application of a single point mutation in a critical region renders this protein inutile. Without a means of replicating chromosomal DNA in a processive fashion or being able to repair it, an organism cannot sustain life. This is why I chose PCNA as MFP.



fig. 7 Side view RCF/PCNA

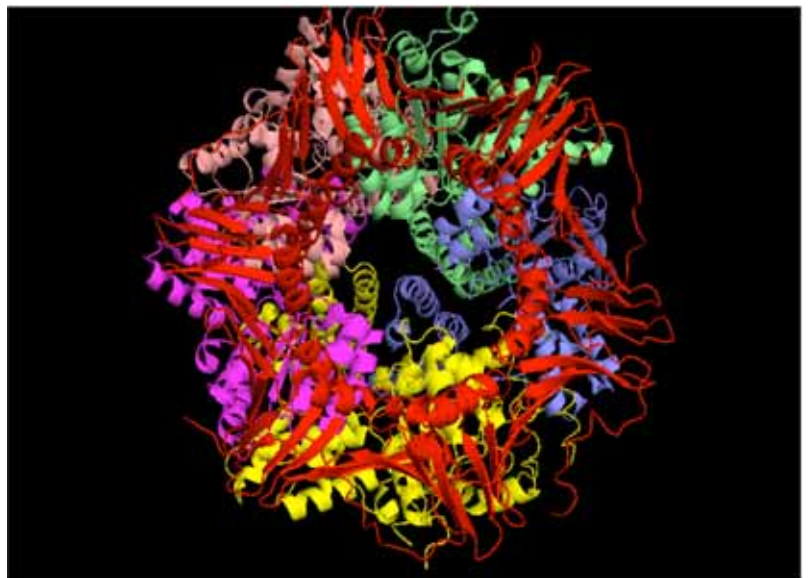


fig. 8 Top view PCNA/RFC

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