Riding the (mono)rails: the structure of catenated DNA-tracking proteins

New evidence suggests that the 'sliding clamp' processivity factors of DNA polymerases can carry a variety of protein factors along the DNA 'track'. Recent information on the structure of these factors sheds light on how they slide on DNA and why they confer processivity.

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The crystal structure of the *Saccharomyces cerevisiae* proliferating cell nuclear antigen (PCNA) has recently been determined at 2.3 Å resolution on a mercury complex of the protein, taking advantage of multi-wavelength anomalous dispersion to determine phases [1]. The structure of unmercurated PCNA has also been refined to 3 Å resolution; no significant conformational changes are generated by mercuration. PCNA is the component of the eukaryotic replication machinery that confers processivity and, *pari passu*, greater rapidity on DNA chain elongation. Its functional homologues in prokaryotic cellular and viral DNA replication are the *Escherichia coli* β subassembly of the DNA polymerase III holoenzyme and the gene 45 protein (gp45) of bacteriophage T4, respectively [2].

The attention-compelling feature of the trimeric PCNA is that it is in the form of a ring, with a central hole that is amply big enough to accommodate a DNA helix (Fig. 1). A three-fold symmetry axis passes through the center of the hole, signifying that the inter-monomer connections are head-to-tail, without rotation, and that each PCNA monomer faces the same way in the structure. A single PCNA monomer is composed of two very similar domains, so that the overall structure has the general aspect of six-fold symmetry. Since each domain can moreover be seen as comprising two elements of structure, each consisting of four β strands and one α helix, a twelve-fold substructure can also be discerned. The twelve α helices of the PCNA trimer face into the central hole (Fig. 2). The sequences of human and rice PCNA are also known and the conservation of amino-acid identity with the yeast protein offers a high probability that the mammalian and plant proteins will turn out to share all the general features of yeast PCNA.

The structure of the *E. coli* β component of DNA polymerase III holoenzyme was previously determined in the same laboratory [3]. The β protein is a dimer, but each monomer is composed of three very similar domains, each approximately the same size as its corresponding domain in PCNA. (The length of the *E. coli* β polypeptide chain is 366 amino acids; human, yeast and rice PCNA are 261, 258 and 263 amino-acid residues in length, respectively.) Thus, the structures of PCNA trimer and β dimer are strikingly similar (Fig. 1) as was already guessed to be the case when the structure of β was first presented [3]. The central cavity of β is also lined with twelve α helices. In both yeast PCNA and *E. coli* β, these helices bear a net positive charge on their inward-facing sides, whereas the overall preponderance of negative charge of these two acidic proteins is spread over their outward-facing and lateral surfaces. The central hole in PCNA is slightly smaller (by ~4 Å in the diameter) than that of β, but each hole readily admits a DNA helix. Direct protein–DNA interactions are conceivable for some of the side chains of PCNA, which extend to ~12 Å from the central axis. However, the inward-facing α helices of β and of PCNA are tilted in such a way that they cross rather than

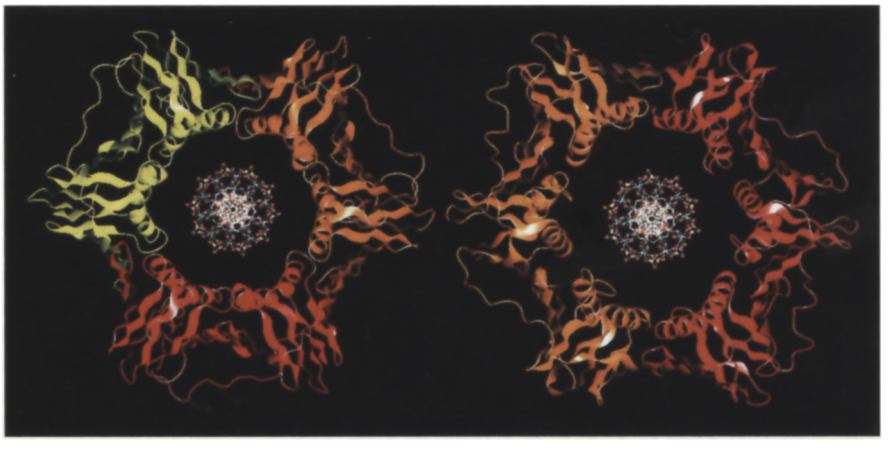

Fig. 1. Ribbon representations of the structures of trimeric yeast PCNA (left) and of the dimeric β subassembly of *E. coli* DNA polymerase III holoenzyme (right). The α helices are shown as spirals, and strands of β sheets are shown as ribbons. A B-form DNA helix is modeled on the axis of each central cavity, which is slightly smaller for the PCNA trimer than for the β dimer. (Reproduced from [1] with permission.)

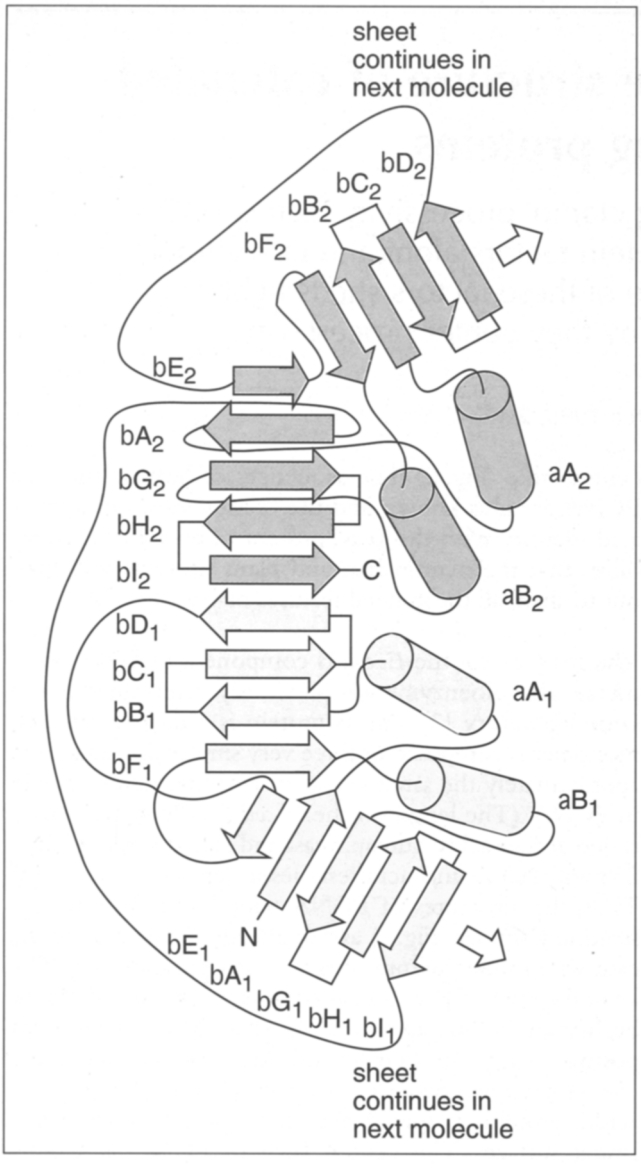

Fig. 2. Schematic diagram of the elements of secondary structure in a monomer of PCNA, with α helices shown as cylinders and β strands as arrows. The two topologically identical domains, 1 and 2, of the monomer are differently shaded; two similar subdomains, each consisting of one α helix and four strands of β sheets, can be discerned in each domain. The amino- (N) and carboxy- (C) termini are marked. (Reproduced from [1] with permission.)

penetrate the major grooves of DNA B-helices. Thus, the dominant interactions of the protein ring are electrostatic, and with the exterior of the DNA.

PCNA and β serve as the 'sliding clamps' of their respective DNA polymerases [2] and increase the rate of DNA chain elongation. This is one of those precious instances in which molecular form compellingly implies function and mechanism. When they are mounted on DNA, these two protein catenanes are constrained to move in a one-dimensional space without being confined to specific sites. Within the life-times of the corresponding complexes, PCNA and β also constrain their ligands to that one-dimensional space. Processivity can be conferred on DNA polymerization solely by virtue of that constraint, for two reasons. First, a complex between PCNA and DNA polymerase δ that releases from its primer–template junction during DNA chain elongation travels in a one-dimensional space, and will therefore reassociate with the site that it has just vacated more rapidly than it could find other primer–template junctions if obliged to explore three-dimensional space. Thus, the dimensional reduction tends to generate more continuous occupancy of the primer–template junction, even when polymerization is not rendered processive, in the strictest chemical sense. Second, processivity is commonly assessed by resistance to dilution in the presence of competing DNA templates, but this test does not distinguish between true continuity of polymerization and topological linkage.

It is easy to imagine why the sliding clamps might require accessory factors for loading onto DNA, particularly onto closed circular DNA. In fact, each of the sliding-clamp proteins has, as its essential partner in DNA replication, a multiprotein assembly that serves as its 'clamp loader' [2,4]. These 'clamp loaders', replication factor (RF)-C for PCNA, the γ complex of the DNA polymerase holoenzyme for *E. coli* β, and the complex of the protein products of gene 44 and gene 62 for the T4 gene 45 protein, are DNA-dependent ATPases. Their mechanisms of action in assisting the DNA entry of the sliding clamp are not known, but an ATP hydrolysis-driven structural change for the clamp loader that is required either at the step of opening the protein ring so that it can be slipped over a DNA helix [2], or at the step of detaching the protein catenane from its assembly factor so that it is free for tracking along DNA, can be readily imagined.

The ability to bring ligands other than DNA polymerases to track along DNA enlarges the scope of action of these proteins beyond DNA replication. Such alternative interactions have now been documented for PCNA and for the T4 gene 45 protein. The honors in regard to diversity belong to PCNA, which binds to D-type cyclins [5], to cyclin-dependent protein kinases, to the p21 protein encoded by the *CIN/CIP1/WAF1* gene [6,7] and to the Gadd45 protein [8]. The interactions with p21 and Gadd45 appear to be important for coordinating the repair of radiation damage to DNA with replication by inhibiting ongoing DNA replication and redirecting DNA polymerases [6,8]. If it were able to bring active protein kinases to track along DNA, PCNA might also be able to exert profound influence on the structure of chromatin.

A concrete example of involvement of a 'sliding clamp' protein in regulating gene activity is provided by the phage T4 gene 45 protein, whose structure has not yet been determined, although its ability to track along DNA, just as β and PCNA do [4,9], has been directly demonstrated [10]. Transcriptional initiation at T4 late promoters (there are ~40 late promoters in the T4 genome) *in vivo* requires the gene 45 protein, which is

loaded onto DNA at enhancer-like entry sites by its 'clamp loader', the gene 44/62 protein complex, then tracks along DNA, presumably interacting with RNA polymerases as it does so. It ends up as part of the activated transcription-initiation complex at a T4 late promoter through interactions with an RNA polymerase complex composed of the *E. coli* RNA polymerase's core enzyme and virus-encoded promoter-recognition and co-activator subunits [11,12]. The protein–protein interactions that cement this union remain to be worked out. The gene 45 protein of T4 is a trimer, like PCNA, and it might be able to form multiple reinforcing points of association with the transcription machinery through its constituent subunits.

Other instances in which similar mechanisms of transcriptional regulation might be at work have been suggested [13,14]. Since one is tempted to anticipate every conceivable capability for these newfound and beautiful objects, it is important to keep in mind that the catenated DNA-tracking proteins are present in cells in somewhat limited supply. The ability to mobilize them for diverse tasks by regulating the impermanence of their catenated state (that is, taking them off DNA) is therefore likely to be a significant aspect of their ability to perform these other functions.

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E Peter Geiduschek, Department of Biology and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093-0634, USA.