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DNA Sliding Clamps: Just the Right Twist to Load onto DNA

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October 26, 2005

Current Biology

DNA SLIDING CLAMPS: JUST THE RIGHT TWIST TO LOAD ONTO DNA

Two recent papers illuminate a long sought step in DNA sliding clamp loading. One paper reveals the structure of the PCNA clamp wrapped around DNA—still open from being loaded—while a second paper discovers that the clamp may assist this process by forming a right-handed helix upon opening.

DNA sliding clamps were first characterized as DNA polymerase processivity factors. Without their presence, cell division would be inconceivably slow; replication of long stretches of DNA would be hopelessly inefficient because DNA polymerases tend to fall off the DNA after elongating a strand by only a handful of bases. By tethering the polymerase to the DNA, such processivity factors enable the polymerase to add thousands of bases in a few seconds without detaching from the DNA [1,2]. The term “sliding clamp” aptly described a protein that, while holding on to the polymerase and binding tenaciously to DNA, can still travel vast distances along the DNA. Direct observation of the remarkable ring-shaped structure of the dimeric polymerase III β subunit (or β clamp) [3] provided a beautifully simple mechanism for sliding without falling off: The protein can form a closed ring around DNA and slide along like a washer on a very long screw.

Since their discovery as processivity factors, DNA sliding clamps have been found to be involved in almost every process dealing with DNA metabolism such as replication, modification, and repair [4–7]. The major clamps associated with DNA replication are the dimeric β clamp in bacteria and the trimeric Proliferating Cell Nuclear Antigen (PCNA) clamp in eukaryotes and archaea. Seen to interact with a vide variety of proteins and protein complexes, they have been described as molecular tool-belts and moving platforms [8,9]. Yet, many mechanistic details of how these ring-shaped clamps end up wrapped around DNA are still unknown. Nature might have invented a clamp that spontaneously opens (like the letter C), binds to DNA, and then, due to the presence of DNA, simply closes into a ring

around DNA; but this does not happen.

Instead, all cellular DNA sliding clamps are stable in the closed ring form [3,10,11], and consequently they must be actively loaded onto DNA at double-stranded/single-stranded (ds/ss) DNA junctions, placing them at exactly the right place for DNA replication and repair. This process is accomplished by an ATP-fueled clamp-loader protein complex, Replication Factor C (RFC) in eukaryotes and archaea, and the γ-complex in bacteria. Once on the DNA, the clamp reseals around the DNA and the clamp loader is ejected. Implicit in this description of loading are two postulates: that the clamp actually forms a closed ring around DNA, and, that at some prior moment, the clamp ring must be somehow opened. While there has been ample evidence for this, direct observations of these two points have been lacking. For example, last year a crystal structure was determined of the PCNA clamp bound to a clamp-loader complex in the presence of non-hydrolysable ATP analogue, but the clamp remained closed [12].

Most recently, however, electron microscopy (EM) has afforded a glimpse into the process of the PCNA clamp being loaded onto DNA [13]. In the reported images, two features stand out: first, the DNA helix was clearly observed running through the middle of the PCNA clamp and into the RFC clamp loader and, secondly, the clamp is open and resembles a right-handed lock- or spring-washer (see Figure 1A). Thus, the two postulates above have now been directly confirmed. Due to limits of EM resolution no atomistic details about molecular interactions within the complex could be observed, but a modeling and simulation paper in the same journal issue [14] provides some important clues as to what it takes for PCNA to adopt such a distorted shape. Within the context of molecular dynamics (MD) simulations of dimeric PCNA (normally a homo-trimer), it was discovered that once opened the clamp tended to move toward the spring-washer conformation similar to that seen by EM (see Figure 1B). Analysis of the simulations also suggests that most of the flexibility occurs at the intermolecular interfaces. These two papers, therefore, are mutually consistent in furthering our knowledge of the mechanics of DNA sliding clamps.

In their breakthrough paper [13], Miyata et al. report averaging nearly 20,000 EM images of the archaeal RFC-PCNA-DNA complex to achieve high enough resolution (12 Å) to unambiguously identify the relative positions, orientations, and even the conformational state of the biomolecular complex. Even absent atomistic detail, this EM structure represents the first view of a double-stranded DNA helix (a 30-mer strand primed by 11 bases) running through the middle of the clamp. Using biotin/streptavidin labeling the authors were also able to infer an approximate exit path for ssDNA out of the clamp loader. The way out for DNA may lie in the RFC crack lined up, mostly it seems, by individual domains of a large RFC subunit (See the red arrow in Figure 1A).

Perhaps most interesting is that the PCNA clamp is cracked open by about a quarter the width of DNA (the authors report 5 Å) and appears flush with the right-handed surface formed by nucleotide-binding domains of the RFC clamp loader. This is in stark contrast with the features of the homologous RFC-PCNA complex from yeast previously determined [12] where the PCNA clamp remains flat and closed and only partly attached to the RFC clamp loader. In both structures the clamp loaders are in the ATP-bound state, which is known to result in clamp opening upon engagement [15,16], yet the clamp is open only in the current EM structure. Do these complexes represent different steps in the clamp loading or does the presence of the DNA make a critical difference? Or could the artificiality of either the protein crystallography or of negative-stain EM account for the difference?

There could be a number of explanations, but a simple, hitherto unaddressed issue might explain the incompatibility of the two conformations of the RFC-bound PCNA clamp. Apparently, the crystallized form of PCNA (in the yeast RFC-PCNA complex) has an extra sequence tag of 25 residues at the N-terminus [12]. Although parts of this additional tag are significantly disordered (and therefore unresolved), at least part of it can be seen binding to the neighboring subunit, effectively bridging across one of the interfaces, creating significant hydrophobic contact, and likely stabilizing the interface. It has been

thought that the mechanism of clamp opening depends on the loader destabilizing one of the subunit interfaces, a notion supported by the crystal structure of a complex of one loader subunit (δ) from *E. coli* with half the β clamp [17]. Although the extra N-terminal residues may not prevent the clamp from being actively loaded, it is certainly possible that the energy provided by these contacts is sufficient to bias the clamp toward the closed state, preventing it in the all-or-none process of crystal formation from opening fully and engaging the spiral surface of the clamp loader.

The second paper [14] illuminates the question of how the PCNA clamp might get into the open conformation observed. The authors investigate the dynamics of an open clamp by performing a series of molecular dynamics (MD) simulations on the PCNA clamps from humans, yeast, and an archaebacterium for a total of nearly 100 nanoseconds of simulation time. The simulations were begun with the removal of one subunit from the closed PCNA homo-trimer, a tactic that might seem quite drastic except that it avoids having to otherwise modify or distort the clamp. The dynamics of the dimeric form of the various PCNA structures were then analyzed for gap widening in the plane of the clamp (lateral motions) as well as out-of-plane motions. The analysis was related to the full trimeric clamp by the addition of the “shadow” of the missing subunit, in the place it would be if it moved relative to the second subunit the way that the second subunit moves relative to the first. The analysis revealed that the opened clamp opened further, analogous to previous MD simulations on a monomer of the β clamp [17].

Unlike the β clamp monomer simulations, these dimeric PCNA simulations allow an analysis of the motions between subunits. In the simulations the flexibility of PCNA appeared mainly in the subunit interfaces rather than in the domain interfaces. Perhaps more surprising was that in seven out of nine simulations, out-of-plane motions tended toward a right-handed helix. This is a remarkable result, suggesting that the clamp has evolved to cooperate in the loading process. The emerging picture is that the clamp loader in the presence of ATP binds to the clamp and interrupts one of the interfaces between the clamp

subunits, causing the clamp to “spring open” into a right-handed helix, which is then stabilized further by binding to the clamp loader. At that point, the open clamp can be loaded onto DNA, after further widening (at least beyond the 5 Å observed in the EM structure) to allow dsDNA to enter or possibly letting only single-stranded portion of DNA to squeeze in. Exactly how the negatively charged DNA enters the open clamp may also depend strongly on the details of the charge distribution of the clamp and clamp loader complexes.

It may be useful to clarify what is meant by the clamp “springing open” or being “spring-loaded”—terms first introduced to rationalize the reduced curvature of a single subunit of the β clamp, relative to the full dimeric β clamp, consistent with MD simulations of the β monomer [17]; and also used to refer to the gap widening of the PCNA dimer observed in the latest MD simulations [14]. What is not meant is that the closed clamp is like a compressed spring, higher in energy than an open clamp. In fact, since both PCNA and β remain closed in solution (as well as in the crystals so far resolved) [3,10,11], the energy of the closed ring must be the minimum energy conformation. This minimum, however, can consist of two parts: one part is due to the favorable contacts at the interfaces between subunits and the second part that is due to the rest of the latent energy of the conformation (see Figure 2). What has been observed by molecular dynamics simulations is that once the interface is removed (by removing one of the subunits), the structure relaxes and adopts a more open, less-curved, tending-towards-right-helical shape. The implication, of course, is that if the loader is able to abolish one of the interfaces between clamp subunits, the clamp will then open further without requiring an *additional* energetic contribution from the loader.

This, however, asks the question, that if the loader can, in the presence of ATP, bind to the clamp strongly enough to outcompete the favorable contacts between clamp subunits, how is it able to release the clamp? Although the details are not yet clear, this must be the role of ATP hydrolysis. It is likely that, just as the ATP binding to the clamp loader is a coordinated process [16,18], ATP hydrolysis is also a coordinated process, at least in eukaryotes and archaea. In any case,

hydrolysis of ATP and release of ADP should restore the initial orientation of N-terminal domains in the clamp loader [19], making this orientation incompatible with the high affinity for the clamp and thus allowing the loaded clamp to disengage.

As we have reported here, new data is rapidly completing the picture of how clamp loading occurs. As for the technologies involved in the work reported on here, electron microscopy and molecular dynamics simulations, these methods are surging forward as algorithms and computational power improve and—with judicious application and careful interpretation—are fast becoming primary tools in structural biology. When it was first realized that sliding clamp loaders involve a complex of five proteins that must open the clamp, load it onto DNA and release itself in an ATP-driven process, the mechanistic details were expected to be quite complicated, and they have lived up to those expectations. Somewhat surprising, however, has been that the seemingly passive clamp would also hold such fascinating features as the conformational agility observed by EM and by MD simulations. As the role of DNA sliding clamps continues to expand from processivity factor to central player in DNA metabolism, it is also being realized how finely tuned these proteins are for binding other proteins, for tenaciously grabbing and sliding on DNA, and now it seems for assisting in the loading process. It might well be that sliding clamps still have surprises left to be discovered.

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FIGURE CAPTIONS

Figure 1. A) Recent EM structure (top) of the PCNA clamp, RFC clamp loader, and ss/dsDNA [13]. Below are the separate components, all of which are right-handed helical, except for the PCNA clamp. Upon opening, however, MD simulations suggest that the PCNA clamp also adopts a right-handed helical conformation. B) Schematic illustration of the dimeric PCNA clamp MD simulations with the third "shadow" subunit shown in grey. Most MD simulations displayed a planar to right-handed helix transition [14].

Figure 2. Free energy landscape (ΔG) versus gap opening distance *d* for the PCNA clamp (shown as in Fig. 1B). When d=0 the interface stabilizes the closed trimeric PCNA ring (black, solid), while the RFC loader stabilizes the open ring (red, dotted). With no third interface, MD simulations of the PCNA dimer display a tendency toward the open ring (blue, dashed).

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ACKNOWLEDGMENTS

This work was performed under the auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

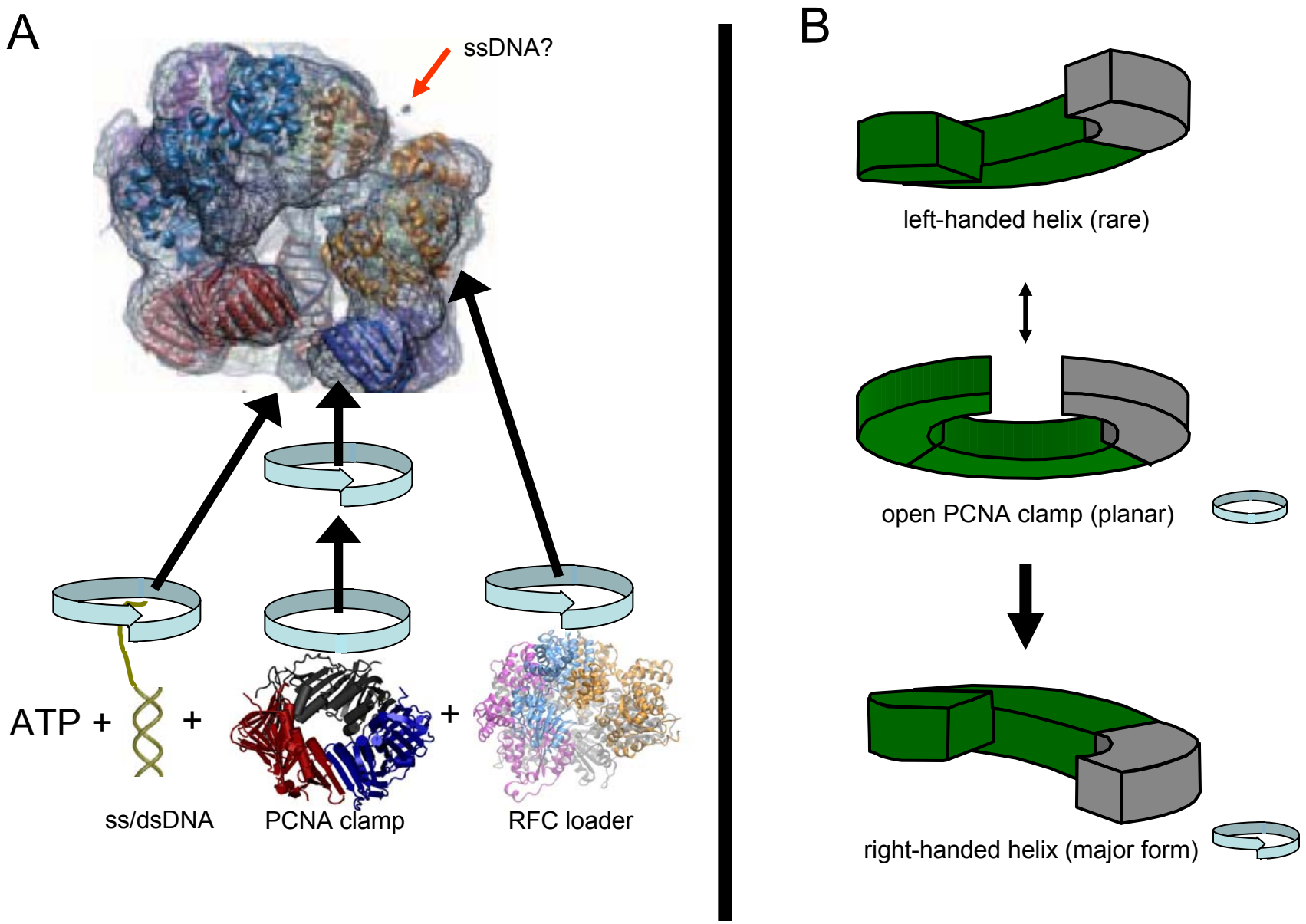
A
ssDNA?
ATP +
+
+
ss/dsDNA
PCNA clamp
RFC loader
B
left-handed helix (rare)
open PCNA clamp (planar)
right-handed helix (major form)

Figure 1

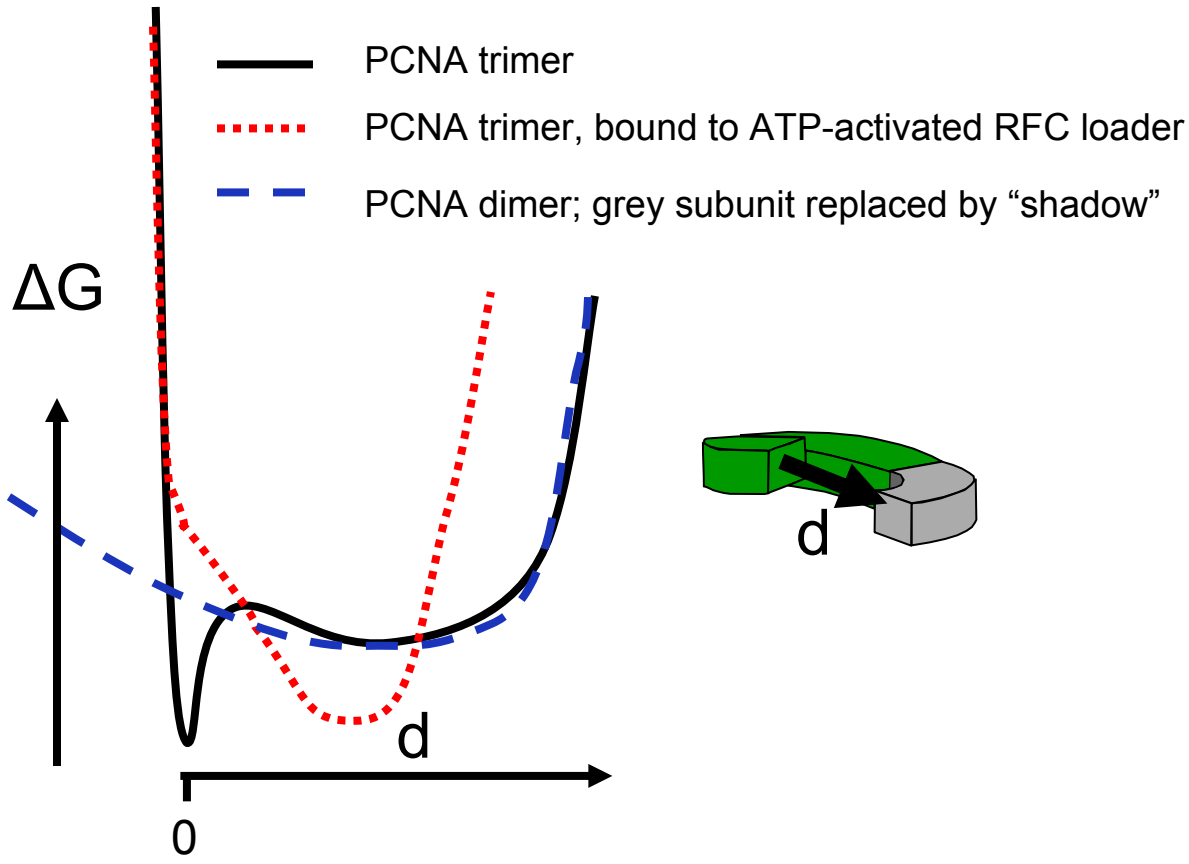
PCNA trimer
PCNA trimer, bound to ATP-activated RFC loader
PCNA dimer; grey subunit replaced by “shadow”
ΔG
d
0
d

Figure 2