Published in final edited form as:

DNA Repair (Amst). 2009 May 1; 8(5): 570–578. doi:10.1016/j.dnarep.2008.12.014.

Loading Clamps for DNA Replication and Repair

Linda B. Bloom

Department of Biochemistry & Molecular Biology, University of Florida, Gainesville, FL 32610-0245

Abstract

Sliding clamps and clamp loaders were initially identified as DNA polymerase processivity factors. Sliding clamps are ring-shaped protein complexes that encircle and slide along duplex DNA, and clamp loaders are enzymes that load these clamps onto DNA. When bound to a sliding clamp, DNA polymerases remain tightly associated with the template being copied, but are able to translocate along DNA at rates limited by rates of nucleotide incorporation. Many different enzymes required for DNA replication and repair use sliding clamps. Clamps not only increase the processivity of these enzymes, but may also serve as an attachment point to coordinate the activities of enzymes required for a given process. Clamp loaders are members of the AAA+ family of ATPases and use energy from ATP binding and hydrolysis to catalyze the mechanical reaction of loading clamps onto DNA. Many structural and functional features of clamps and clamp loaders are conserved across all domains of life. Here, the mechanism of clamp loading is reviewed by comparing features of prokaryotic and eukaryotic clamps and clamp loaders.

1. Introduction

Sliding clamps and clamp loaders are essential components of DNA replication and DNA repair machinery. Sliding clamps are ring-shaped complexes of protein subunits that encircle and slide along duplex DNA [1,2]. By virtue of these properties, proteins that bind sliding clamps remain tightly associated with DNA yet able to translocate along duplexes. Sliding clamps and clamp loaders were first identified as elongation factors required for DNA replication [3]. When bound to a sliding clamp, the processivity of a replicative DNA polymerase increases from tens to thousands of nucleotides. Other DNA polymerases, including those required for translesion synthesis and DNA repair, also use sliding clamps. There is evidence to support the “toolbelt” model [4] that sliding clamps coordinate the activities of replicative and translesion polymerases by binding both at the same time so that when the replicative polymerase stalls at a lesion, the translesion polymerase is present to synthesize DNA past the lesion [5]. Since their initial discovery as polymerase processivity factors, sliding clamps have been found in all domains of life, and are now known to be involved in many other aspects of DNA metabolism (reviewed in [6,7]). Some examples of other proteins that bind sliding clamps include flap endonuclease I [8–10] and DNA ligase I [11–13] that participate in Okazaki fragment maturation and base excision repair; MutS and MutL homologs [14–19] required for mismatch repair; DNA glycosylases [20–23] that initiate base excision repair; and DNA methyltransferase 1 [24] and chromatin assembly factor 1 [25] that modify and reassemble DNA into chromatin following replication. Clamps may increase the efficiency of this diverse group of enzymes by tethering them to DNA, or alternatively, clamps may coordinate enzyme

Contact info: Email: E-mail: lbloom@ufl.edu, Phone: (352) 392 – 8708, Fax: (352) 392-6511.

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activities for a given process by helping to recruit a series of enzymes to a specific site on DNA, or clamps could do both.

Sliding clamps do not spontaneously assemble onto genomic DNA, but instead must be loaded. Multi-subunit clamp loaders catalyze the assembly of clamps on DNA in an ATP-dependent reaction. As with clamps, there is a great deal of structural and functional similarity in clamp loaders from different organisms. Clamp loaders from bacteria, eukaryotes, and even bacteriophage T4, contain five core subunits which are members of the AAA+ family of ATPases and contain conserved sequence motifs required for ATP binding and hydrolysis. Although the details of the mechanisms may differ somewhat, clamp loaders use energy associated with ATP binding and hydrolysis to promote conformational changes within the clamp loader complex. These conformational changes modulate binding to the clamp and DNA, and allow the clamp loaders to catalyze the mechanical clamp loading reaction. The main focus of this review will be on the mechanism of clamp loading as revealed by structural and biochemical studies of clamps and clamp loaders with the main focus on comparing and contrasting the eukaryotic and *E. coli* clamps and clamp loaders.

2. 1. Structural features of sliding clamps

Many structural features of sliding clamps are conserved. The majority of clamps are composed of three subunits arranged in a ring. The eukaryotic clamp, proliferating cell nuclear antigen (PCNA) [26,27], the gp45 clamps from bacteriophage T4 [28] and the closely related bacteriophage RB69 [29], and most archaeal PCNA clamps [30] are homotrimers. However, at least one species of archaeobacteria, *Sulfolobus solfataricus*, [31] uses a heterotrimeric clamp. Bacterial clamps, such as the *E. coli* β sliding clamp are ring-shaped homodimers [1,32]. Even though the *E. coli* sliding clamp (β) has only two subunits and shares little sequence homology with the eukaryotic sliding clamp, the overall structures of the β -clamp and PCNA are quite similar. Each clamp is composed of six globular domains organized into a ring largely through noncovalent interactions between neighboring domains (Fig. 1). Peptide loops on the outer edge of the rings covalently link globular domains to form individual subunits. For trimeric clamps such as PCNA, a single subunit is formed by covalently linking two domains, and for dimeric clamps such as β , three domains are linked to form a subunit. Thus, PCNA contains three interdomain interfaces, and β contains two, that interact only through noncovalent bonds, and that can be opened to allow DNA to pass into the center of the ring.

Individual subunits within a clamp are arranged in a head-to-tail fashion giving the clamps a rotational axis of symmetry through the center, and importantly, different front and back faces. In principle, sliding clamps could bind proteins on both the front and back faces to coordinate enzyme activities. However, all proteins shown to bind sliding clamps interact with the same face via a conserved peptide sequence motif. A key feature of clamp binding motifs in both prokaryotes and eukaryotes is the presence of hydrophobic amino acid residues including Phe that bind to a hydrophobic pocket on one face of the clamps [26,33,34]. Based on sequence alignments and binding studies, consensus binding sequences of QL(S/D)LF [35] and QxxL(x)F [36] have been proposed for the *E. coli* β -clamp. A similar consensus sequence, Qxx(I/L/M)xxF(F/Y), has been proposed for proteins that interact with PCNA [37,38]. Clamps contain one binding pocket per monomer, PCNA contains three and the β -clamp contains two. Even though, there are multiple binding pockets, the clamp loader and replicative polymerase cannot bind the clamp at the same time due to steric effects. The clamp loader must disengage from the clamp before the DNA polymerase can bind.

2. 2. Structural features of clamp loaders

Clamp loaders are molecular machines that catalyze the physical reaction of assembling clamps on DNA. These enzymes are part of the AAA+ family of ATPases that use energy derived

from ATP binding and hydrolysis to promote the mechanical reactions that they catalyze (reviewed in [39–43]). The functional core of clamp loaders is composed of five structurally homologous subunits [33,44,45], each of which contains three domains joined by flexible linkers (Fig. 2A). The N-terminal domains (I & II) share homology with members of the AAA + family of ATPases, and the C-terminal domain (III) is unique to clamp loaders. These five subunits are arranged in a ring via tight interactions between the C-terminal domains (Fig. 2B). The N-terminal domains I and II are loosely packed as if they were suspended from a “collar” formed by the C-terminal domains, and a large opening exists on the N-terminal face. The structure of clamp loaders is somewhat analogous to a hand where the C-terminal domains are assembled into the palm and the N-terminal domains are represented by the fingers. The flexible linkers in the individual subunits provide conformational flexibility and allow the domains to move relative to one another just as fingers can bend at joints. To simplify nomenclature in comparing clamp loaders from different species, the five core clamp loader subunits will be referred to as subunits A – E (Fig. 2C) based on their positions in the ring (e.g. RFC-A).

The five-subunit core of the *E. coli* clamp loader is composed of three different proteins, three copies of the DnaX protein and a single δ and δ' subunit arranged such that δ occupies position A, δ' position E, and the DnaX proteins positions B, C, and D (Fig. 2B and C). Beyond the conserved five-subunit core, the *E. coli* clamp loader contains two additional subunits, the ψ and χ subunits, and these additional subunits are also found in other bacterial clamp loaders [46,47]. The ψ and χ subunits form a 1:1 complex that binds the clamp loader via interactions between ψ and DnaX [48,49] and stabilizes the clamp loader complex [50]. The χ subunit also interacts with single-stranded DNA binding protein (SSB). This χ -SSB interaction facilitates the displacement of primase to hand-off the RNA primer to the DNA polymerase [51], increases the efficiency of clamp loading [52], and stabilizes the interaction of the holoenzyme with the SSB-coated template [52,53]. Although the five-subunit complex is capable of loading clamps, the clamp loading efficiency of this minimal clamp loader (DnaX₃ $\delta\delta'$) is not as high as that for the complete seven-subunit complex (DnaX₃ $\delta\delta'\psi\chi$) [46,49,53,54]. The ψ subunit may increase the activity of the clamp loader either by facilitating ATP-induced conformational changes or stabilizing an ATP-bound conformational state that give the clamp loader a high affinity for DNA [54].

In *E. coli*, two different forms of the DnaX protein are expressed, a long form, τ , and a short form, γ . This is true for some but not all bacteria (reviewed in [47]). The τ subunit is the full-length product of the *dnaX* gene and the γ subunit results from a translational frameshift that truncates the protein prematurely so that γ is about 2/3 of the length of τ and of identical sequence except for the last amino acid. The γ subunit contains three domains and can be assembled into a fully active clamp loader complex [55] referred to as the γ complex ($\gamma_3\delta\delta'\psi\chi$), however, bacteria that express only γ are not viable whereas those that express τ grow normally [56]. The C-terminal extension on τ is required for mediating protein•protein interactions and coordinating activities at the replication fork. The τ subunit binds to DNA polymerase III via domain V [57] to physically tether the polymerase to the clamp loader forming the DNA polymerase holoenzyme. The form of the holoenzyme isolated from cells contains two copies of DNA polymerase III and a clamp loader of the composition, $\tau_2\gamma\delta\delta'\psi\chi$ [58]. This dimeric polymerase holoenzyme is capable of coupled leading and lagging strand DNA replication [59]. Recently, a trimeric DNA polymerase III holoenzyme composed of three copies of DNA polymerase III and a clamp loader containing τ only ($\tau_3\delta\delta'\psi\chi$), was shown to be fully active in reconstituted replication assays [60]. This raises intriguing possibility that a trimeric DNA polymerase holoenzyme may function in DNA replication so that the third DNA polymerase can take over when the leading or lagging strand polymerase encounters a block to DNA synthesis. Besides coupling the polymerase to the clamp loader, the τ subunit mediates other key protein•protein interactions at the replication fork. The τ subunit interacts with the DnaB helicase to couple duplex unwinding at the replication fork to DNA synthesis [61]. The

τ subunit prevents the β clamp from being removed while the DNA polymerase is actively synthesizing DNA [62], but then mediates DNA polymerase dissociation and recycling on completing synthesis of an Okazaki fragment [63].

The eukaryotic clamp loader, RFC, is a heteropentamer containing four small subunits (B – E), 36 – 40 kDa in size, and one large subunit (A), 95 kDa in *S. cerevisiae* and 140 kDa in humans. The large subunit, RFC-A, contains a large N-terminal extension and a smaller C-terminal extension that the other subunits lack. The cellular functions of these two extensions are not yet known. The N-terminal region has homology to bacterial DNA ligases and to poly (ADP-ribose) polymerase and is conserved in eukaryotes [64–66]. The N-terminal region binds DNA [65,67], but is not required directly for clamp loading or viability in yeast [68–70]. Deletion of the N-terminal region actually increases clamp loading activity *in vitro* [70]. There is no evidence that RFC binds directly to DNA polymerases ϵ or δ to form a DNA polymerase-clamp loader holoenzyme complex as found in bacteria.

3. The A-subunit of RFC and Alternative Clamp Loaders

Three alternative RFC complexes exist in which RFC-A is replaced by another protein [71–83]. In one such complex, Rad24 in *S. cerevisiae* [76], or Rad17 in humans and *S. pombe* [77], is substituted for RFC-A to form a clamp loader required for a checkpoint response during S-phase (reviewed in [84,85]). The biochemistry and cellular functions of this alternative clamp loader are perhaps the best understood of the three. Substituting Rad24/Rad17 for RFC-A alters the substrate specificity of the clamp loader. This checkpoint clamp loader interacts with and loads an alternative clamp called the 9-1-1 complex (composed of Rad9, Hus1, and Rad1 in humans and *S. pombe*, and Ddc1, Mec3, and Rad17, respectively, in *S. cerevisiae*) onto DNA [77,86,87]. Although the Rad24/Rad17-RFC complex can bind PCNA, it is unable to productively load PCNA onto DNA [86,88]. Rad24-RFC can, however, unload PCNA from DNA [88]. Substitution of RFC-A with Rad24/Rad17 also alters the DNA substrate specificity such that the checkpoint clamp loader no longer has the specificity for ss/ds DNA junctions with 3' recessed ends that RFC has, and binds any ss/ds DNA junction [87,89,90]. Coating ss DNA with RPA gives the checkpoint clamp loader a preference for ss/ds DNA junctions with 5' recessed ends [90,91]. These biochemical properties are consistent with the cellular function of the checkpoint clamp loader (recently reviewed in [92–95]). The Rad24/Rad17-RFC complex loads the 9-1-1 complex at sites of DNA damage and stalled replication forks to mediate a checkpoint response by activating the ATR kinase [96–98].

Replacement of RFC-A with Ctf18 forms an alternative RFC complex (Ctf18-RFC) that is required for sister chromatid cohesion [78–80]. Ctf18 binds Ddc1 and Ctf8 and recruits these proteins to the complex to form seven-subunit RFC complex. Both the five- and seven-subunit Ctf18-RFC can load PCNA onto DNA and unload PCNA from DNA [99–101].

The third alternative RFC complex contains Elg1, contributes to chromosome stability, and suppresses chromosomal rearrangements [81–83] (reviewed in [102,103]). An interaction between Elg1 and PCNA has been demonstrated by co-immunoprecipitation of Elg1 and PCNA from yeast cell extracts [81], but a productive Elg1-RFC•PCNA interaction has not been demonstrated in biochemical assays *in vitro*. It is not yet clear whether the cellular target of Elg1-RFC is PCNA or whether an alternative clamp has yet to be identified.

4. Clamp and DNA binding

Structural [33,34,104,105] and biochemical studies [88,106–109] support a model in which the clamp loader binds the clamp via contacts made between the N-terminal domain (I) of each clamp loader subunit and one face of the clamp (Fig. 3). Hydrophobic residues in a conserved sequence motif in the A-subunit bind a hydrophobic pocket on the clamp [33,34]. The

hydrophobic pocket is located near the interface of neighboring domains within a clamp monomer. There are three such pockets in PCNA and a RFC•PCNA structure (Fig. 3A) shows that the C-subunit makes similar contacts, but to a lesser extent, with the adjacent PCNA monomer [33]. The B-subunit in the RFC•PCNA structure contacts PCNA near the interface of two domains between adjacent monomers. In this structure the clamp is not opened, and it is likely that productive formation of an open clamp loader•clamp complex requires each of the five clamp loader subunits to make similar contacts with the clamp. Given the six-domain structure of the clamp, clamp loader subunits could bind the clamp at five of the six interdomain regions and the sixth would be free to open (Fig. 3B and C).

Subunits within the clamp loader adopt a spiral conformation relative to an axis through the center of the ring [33,105], and this likely opens the clamp in an out-of-plane conformation [105,110]. It is believed that the duplex portion of a primed template enters the clamp loader•clamp complex via a large opening present on the N-terminal face of the clamp loader and extends up towards the collar (see Fig. 3C). Modeling primed template DNA into the RFC•PCNA structure shows that the clamp loader can bind DNA in a manner similar to a “screw cap” with the RFC subunits and clamp spiraling around the duplex with the same pitch as the helix [33,105]. This model also explains how clamp loaders specifically recognize ss/ds DNA junctions of primed templates. About one helical turn of the duplex portion of the primed template fits into RFC such that the 3′-primer end extends to the collar formed by the C-terminal domains of the RFC subunits [33,111]. This would then place the 5′ single-stranded overhang in position to exit through the gap between the A- and E- subunits. Given this model, it is interesting to speculate how substituting Rad24/Rad17 for RFC-A alters the DNA substrate specificity of the checkpoint clamp loader.

5. ATP Binding and hydrolysis

Clamp loaders require ATP to load clamps. Ultimately, ATP binding and hydrolysis promote conformational changes in the clamp loader that modulate its affinity for the clamp and DNA. A common feature of AAA+ ATPase family members, including clamp loaders, is the arrangement of multiple ATP binding subunits within a ring such that ATP binding sites are located at the interface of adjacent subunits (reviewed in [39–43]). Although the five core clamp loader subunits share homology with AAA+ proteins, only a subset, four (A – D) in RFC [66], four in the bacteriophage T4 gp44/62 clamp loader, and three (B – D) in the *E. coli* clamp loader [112,113], are functional ATPases. The ATP binding sites are located at the interface of domains I and II within a subunit (Fig. 2A) and each contains conserved Walker A and Walker B sequence motifs [33,66]. Conserved Arg fingers extend from one subunit to interact with ATP bound to the neighboring subunit [33,44,45]. This location of ATP sites enables dynamic coupling of ATP binding and hydrolysis to promote conformational changes in the complex that regulate clamp and DNA binding.

Although clamp loaders show a great deal of similarity in the architecture and arrangement of ATP sites, there appears to be some variation in the mechanisms by which clamp loaders use ATP binding and hydrolysis to promote clamp loading. In general, ATP binding promotes conformational changes that give the clamp loader a high affinity for the clamp and DNA [106,114–117], and ATP hydrolysis has the opposite effect to reduce the affinity of the clamp loader for the clamp and DNA (Fig. 3C). But, there are variations on this theme with differences in how the ATP sites fill, and at what point in the clamp loading reaction ATP is hydrolyzed.

The weakly hydrolyzable ATP analog, ATPγS, has been used to identify steps in clamp loading reactions that require ATP binding but not hydrolysis. Binding studies with RFC show that ATP sites fill sequentially such that binding two molecules of ATPγS promotes binding of either PCNA or DNA, and binding of PCNA or DNA promotes binding of a third molecule of

ATP γ S, and formation of a ternary RFC•PCNA•DNA complex promotes binding a fourth ATP γ S molecule [118]. In contrast, all three sites in the *E. coli* γ complex ($\gamma_3\delta\delta'$ 'ψχ) bind ATP in the absence of the clamp or DNA [119]. Similarly, the bacteriophage T4 clamp loader binds four molecules of ATP prior to binding the clamp or DNA. Binding of ATP promotes binding and opening of clamps by both the *E. coli* γ complex and RFC [88,116,117,120,121], as well as formation of ternary clamp loader•clamp•DNA complexes [122,123]. The situation may be different for the bacteriophage T4 gp44/64 clamp loader in that binding four molecules of ATP is required to bind the gp45 clamp, but clamp opening may require hydrolysis of two of the four molecules of ATP [124,125]. However, other studies indicate that gp44/62 does not need to hydrolyze ATP to form an open clamp loader•clamp complex [126,127].

Hydrolysis of ATP is required for isolated clamp loaders to release clamps on DNA [122–124,128,129]. DNA binding to clamp loader•clamp complexes triggers the hydrolysis of ATP, and this in turn causes the clamp loader to release the clamp on DNA. The mechanism by which clamp loaders recognize the appropriate sites to load clamps, that is ss/ds DNA junctions with 3' recessed ends, is dynamic in that these sites preferentially trigger ATP hydrolysis and clamp release [130,131]. An intriguing study using the *E. coli* DNA polymerase III holoenzyme ((DNA polymerase III) $_{2\tau_2\gamma\delta\delta'}$ 'ψχ) showed that the clamp loader could productively load a clamp on the leading strand in the absence of ATP hydrolysis using ATP γ S, but required ATP hydrolysis to load a second clamp on the lagging strand [132,133]. It is possible that protein•protein interactions within the holoenzyme impart this asymmetry in the requirement for ATP hydrolysis by the clamp loader.

Clamp loaders are exquisitely fine tuned in their response to ATP, and the γ -phosphoryl group of ATP is likely to be a key feature by which the clamp loader senses and responds to changes in the nucleotide to differentiate between ATP and hydrolyzed ADP + P_i. Conserved Arg fingers in clamp loaders extend from one subunit towards the γ -phosphoryl group of ATP bound to the adjacent subunit. One function these Arg fingers may serve is to aid in catalyzing the hydrolysis reaction by stabilizing the charge that forms on the γ -phosphate in the transition state as seen for GTPase-activating proteins [134]. Although Arg fingers may play a role in ATP hydrolysis by clamp loaders, this is difficult to dissect because Arg fingers also play a role in clamp and DNA binding which comes before hydrolysis. Mutation of Arg fingers to Ala in both the *E. coli* γ complex ($\gamma_3\delta\delta'$ 'ψχ) and RFC does not affect ATP binding to the clamp loaders [119,120]. However, in the *E. coli* γ complex, the Arg to Ala mutations reduce both the DNA binding and clamp binding activities [135]. Interestingly, mutation of Arg fingers in RFC reduces DNA binding activity but does not affect PCNA binding as assessed by a clamp opening assay [120]. In both systems, mutation of Arg fingers in some subunits has a greater effect on DNA binding than mutations at other sites which suggests that ATP binding at some sites may be more important for regulating DNA binding. These studies suggest that the conserved Arg fingers may have a role in sensing bound ATP or in responding to ATP binding by conformational changes that move the Arg fingers close enough to interact with the γ -phosphoryl group of ATP. As a technical note, given the potential importance of the γ -phosphoryl group in promoting conformational changes, mechanistic studies that use ATP γ S should be interpreted cautiously. Even though clamp loaders may bind ATP and ATP γ S with similar affinities [45,115,116,118], ATP γ S may not have the efficacy of ATP in promoting conformational changes. The affinity of the *E. coli* γ complex ($\gamma_3\delta\delta'$ 'ψχ) for the β -clamp is at least an order of magnitude weaker in assays with ATP γ S than ATP, and binding of the minimal five-subunit complex ($\gamma_3\delta\delta'$ ') to the β -clamp is not stimulated much, if at all, by the addition of ATP γ S [54]. DNA binding is also affected by substituting ATP γ S for ATP, such that the rate of γ complex binding to DNA is slower in assays with ATP γ S than with ATP [130].

6. Concluding remarks

Thus far, a fairly static view of clamp loading has been presented, but to successfully load clamps many changes in interactions between the clamp loader and clamp, and between the clamp loader and DNA must occur. It is likely that the interactions the clamp loader makes with its binding partners are dynamic in that each binding (or hydrolysis) event promotes conformational changes in the clamp loader that facilitate the next step in the reaction. On a very basic level, the clamp loader must initially have a high affinity for the clamp and DNA to bring these macromolecules together, but then must have a low affinity to release the clamp on DNA for use by a DNA polymerase. This affinity modulation is accomplished in part by ATP binding and hydrolysis. In an ATP-bound conformational state, the clamp loader has a high affinity for the clamp and DNA, and on hydrolysis of ATP, the affinity is decreased and the clamp loader releases the clamp on DNA (Fig. 3C). However, ATP binding and hydrolysis *alone* cannot provide a sufficient mechanism for ordered affinity modulation that supports an efficient clamp loading reaction. And, this reaction must be efficient to support clamp loading on the lagging strand, particularly in *E. coli* where a clamp must be loaded for each 1 – 2 kb Okazaki fragment synthesized and these fragments are synthesized every 1 – 2 s. An additional level of regulation of ATP binding and hydrolysis is likely to exist. For example, consider a mechanism in which the clamp loader were simply to bind and hydrolyze ATP on its own. In this case, the clamp and DNA may or may not be bound prior to ATP hydrolysis, and futile cycles of ATP binding and hydrolysis would occur. This would decrease the efficiency of the clamp loading reaction. Ideally, the system would be set so that ATP hydrolysis would only occur *after* the clamp loader bound both the clamp and DNA. Similarly, a defined temporal order for binding and releasing the clamp and DNA would increase the efficiency of the clamp loading reaction. For example, if the clamp loader were to release its grip on DNA prior to clamp closing, the DNA may slip out of the open clamp. We hypothesize that additional levels of temporal regulation in the clamp loading reaction could come from three sources: 1) individual clamp loader subunits are largely responsible for regulating different interactions, 2) ATP binding and hydrolysis occurs sequentially at individual sites, and 3) interactions with the clamp and DNA promote changes in the clamp loader. This regulation could favor a defined temporal order of events that leads to efficient clamp loading. That is not to say that alternative pathways do not exist, but that the system is biased to favor one pathway. Similarly, ordered binding/hydrolysis of ATP and binding/release of the clamp and DNA are not necessarily all-or-none processes (e.g. the clamp loader must bind one before the other), but there may be a kinetic preference for one event to occur before the other. Further mechanistic studies are needed to address these questions and to uncover the detailed mechanism and temporal order of events in the clamp loading reaction.

Acknowledgments

I thank Ankita Chiraniya and Jennifer A. Thompson for critical reading and thoughtful comments on this manuscript. Research on clamp loading mechanisms in the Bloom laboratory is supported by the National Institutes of Health grants GM055996 and GM082849.

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Fig. 1. Structural features of sliding clamps

Ribbon diagrams of the *E. coli* β -clamp (left panel), human PCNA (center panel), and bacteriophage RB69 gp45 (right panel) are shown with each subunit in a different color. Beneath the ribbon diagrams are cartoons that illustrate key structural features of the clamps. Each clamp is composed of six globular domains that are arranged in a ring and interact mainly through noncovalent interactions. Peptide linkers on the outside of the rings covalently join globular domains into monomeric subunits. In β , three globular domains are covalently joined by peptide linkers to form a monomer, and in PCNA and gp45, two domains are covalently linked to form a monomer. Structures were generated from PDB files 2POL [1], 1AXC [26], and 1B77 [29], for β , PCNA, and gp45, respectively, using PyMol from DeLano Scientific LLC.

Fig. 2. Structural features of clamp loaders

A) Ribbon diagrams of the D-subunits from *S. cerevisiae* RFC (upper panel) and *E. coli* γ complex (lower panel) with bound ATP γ S (yellow spheres) are shown. The subunits are oriented such that the C-terminal domain III is at the top. B) Ribbon diagrams of the five-subunit core clamp loaders, *S. cerevisiae* RFC (upper panel) and *E. coli* $\gamma_3\delta\delta'$ (lower panel) are shown. The view of RFC is looking down on the “collar” formed by the C-terminal domains (III). The view of the *E. coli* clamp loader is rotated 90° relative to that of RFC such that the collar formed by the C-terminal domains (III) is at the top of the structure. In this view, the tight packing of the C-terminal domains relative to the N-terminal domains and the gap between the A- and E-subunits can be seen. Bound ATP γ S is shown as gray spheres. The A-subunit in the RFC structure was truncated for crystallography to residues 295 – 785 (of 861) and the other subunits are full-length. The γ subunits are truncated to residues 1 – 373 (of 431). C) Cartoon diagrams illustrating the arrangement of the five core subunits in eukaryotic and *E. coli* clamp loaders. The subunits are referred to as A – E to make comparisons between clamp loaders from different species. The subunit nomenclature is also given, and for RFC both the yeast (Rfc1 – 5) and human (e.g. p140) designations are given. All ribbon diagrams were generated from PDB files 1SXJ [33] and 1XXH [44] for the yeast and *E. coli* proteins, respectively, using PyMol from DeLano Scientific LLC.

Fig. 3. Clamp and DNA binding

A) A ribbon diagram of a *S. cerevisiae* RFC•PCNA complex is shown. The N-terminal domains (I) of subunits A, B, and C contact one face of the clamp. Contacts with the clamp are the greatest for subunit A and decrease around the ring to the point at which subunits D and E do not contact the clamp at all. The clamp is closed in this structure. In the open clamp loader•clamp complex, the cyan and magenta PCNA subunits are anticipated to swing up so that the N-terminal domain (I) of each subunit contacts the surface of the ring as illustrated in the cartoon in panel C. Arginine fingers in the conserved Ser-Arg-Cys motif were mutated to glutamine and ATP γ S was substituted for ATP to prevent hydrolysis of ATP. The large A subunit was truncated to residues 295 – 785 (of 861), and is closer in size to the small RFC subunits in this structure. This diagram was generated from PDB file 1SXJ [33] using PyMol from DeLano Scientific LLC. B) A hypothetical model is shown that illustrates the footprint the clamp loader makes when binding the clamp to form an open clamp loader•clamp complex. The black circles represent contacts with the face of PCNA made by each RFC subunit (A – E). C) In the cartoon diagrams of RFC and PCNA, each subunit is colored as in the ribbon diagrams. Individual domains of each subunit are represented by spheres or ovals. The cartoon diagrams are based on structural and biochemical data discussed in the text. In general, the clamp loading reaction can be divided into two phases based on ATP requirements. In the first phase, formation of a ternary clamp loader•clamp•DNA complex is promoted by ATP binding. And in the second phase, DNA binding triggers hydrolysis of ATP and the release of the clamp on DNA.