

# A sliding clamp monkey wrench

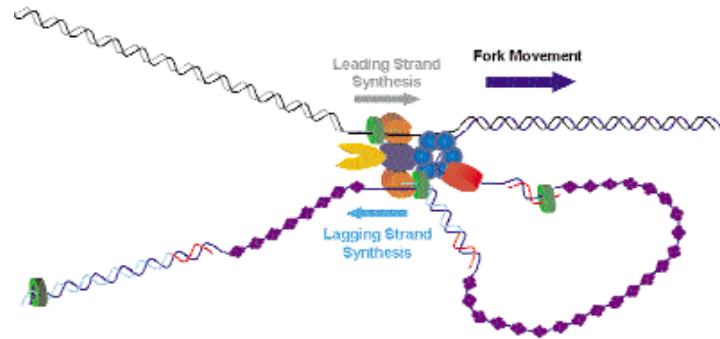
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Crystal structures of the *Escherichia coli* DNA replication  $\gamma$  clamp loading complex and of a subunit of the clamp loader bound to a  $\beta$  clamp monomer provide a physical framework in which to view ATP-dependent modulation of  $\gamma$  complex- $\beta$  interactions. The structural data suggest how the  $\beta$  ring is opened and loaded onto DNA in the absence of a direct interaction between the  $\gamma$  complex and the  $\beta$  dimer interface.

Efficiency and accuracy is the name of the game when duplicating genomic DNA. Accuracy is ensured by high fidelity replicative polymerases, but these enzymes are notoriously inefficient on two counts — they copy DNA with grossly insufficient processivity, and they cannot copy leading and lagging strand templates in a temporally coordinated manner. To alleviate these deficiencies, the polymerases bind a sizable group of accessory proteins, forming 'replisomes' able to catalyze coupled processive leading and lagging strand replication. Replisome components are highly conserved from simple unicellular organisms to humans and are composed of leading and lagging strand DNA polymerases, sliding processivity clamps and a clamp-loading protein complex (Fig. 1). Kuriyan, O'Donnell and colleagues have now solved the structures of a subassembly of the *Escherichia coli* clamp loader and of a subunit of the clamp loader bound to a  $\beta$  monomer, as published recently in *Cell*<sup>1,2</sup>. These structures provide a foundation to model the clamp loading reaction cycle and highlight many challenging questions that remain to be answered.

## Opening the clamp

The effect of the sliding clamp on polymerase processivity is most impressive in *E. coli*. The number of nucleotides incorporated by the *E. coli* DNA polymerase III core (pol III core) in a single DNA binding event is ~10–20, increasing to many thousands once the  $\beta$  clamp and  $\gamma$  clamp-loading complex come into play. The crystal structure of  $\beta$ , a ring-shaped dimer large enough to encircle DNA (Fig. 2a), provides a literal representation of the mechanism used to enhance pol III core processivity<sup>3</sup>. When bound to the pol III core and encircling DNA,  $\beta$  acts as a sliding clamp tethering the polymerase to DNA, thereby preventing premature dissociation until synthesis is complete. This seemingly simple mechanism is complicated by the inability of the clamp to load itself onto DNA, requiring the action of the multisubunit clamp loading motor to cycle  $\beta$  on and



**Fig. 1** Schematic diagram of the *E. coli* replisome containing a dimeric polymerase capable of coordinated leading and lagging strand synthesis. The leading strand polymerase synthesizes DNA in one continuous strand moving in the same direction as the advancing replication fork. The opposite polarity of the DNA template on the lagging strand requires the lagging strand polymerase to synthesize DNA in the opposite direction. This is accomplished by synthesis of DNA in shorter Okazaki fragments. The clamp loading complex, illustrated by the blue ovals and the yellow C-shaped structure, must assemble and disassemble a clamp for every Okazaki fragment synthesized. The clamp loader contains two  $\tau$  and one  $\gamma$  subunit, both of which are encoded by the *DnaX* gene.  $\tau$  is the full length gene product and  $\gamma$ , produced by a translational frameshift, is about two thirds the length of  $\tau$ . The two  $\tau$  subunits (blue ovals) provide the 'glue' that joins the dimeric polymerase with the clamp loader and with the DNA helicase.

off the DNA during lagging strand synthesis (Fig. 1). In *E. coli*, the clamp loader also plays a second essential role by providing a protein scaffold that binds both leading and lagging strand pol III core molecules via the dimeric  $\tau$  subunits (Fig. 1).

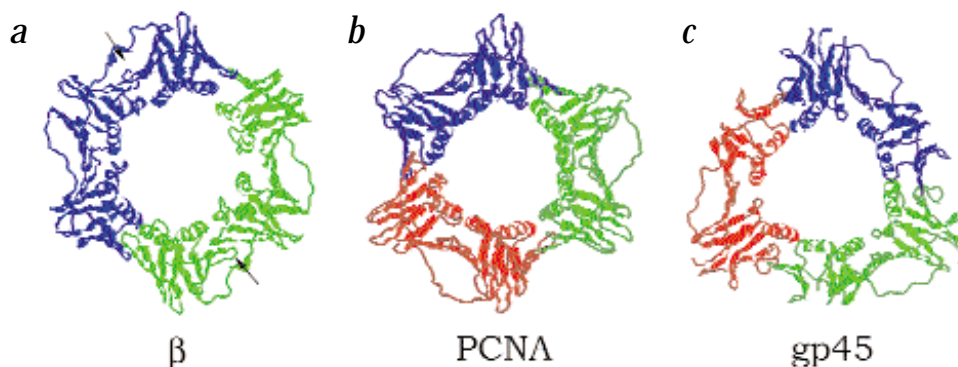
The key feature of the clamp loading model proposed by D. Jeruzalami *et al.*<sup>1</sup> is that the  $\delta$  subunit of the clamp loader acts as a molecular wrench to induce or trap  $\beta$  in a conformation where one of the two dimer interfaces is open (Fig. 3). This model is based primarily on the structure of the  $\delta$  subunit bound to a  $\beta$  monomer containing two mutations that prevent dimerization.

The  $\delta$  subunit does not interact directly with either of the dimer interfaces, but instead inserts amino acid residues into a hydrophobic pocket between two neighboring domains within the  $\beta$  monomer (indicated by arrowheads in Fig. 2a). The  $\beta$  monomer structure in the  $\delta$ - $\beta$  complex differs from that in the  $\beta$  dimer in two ways: there is a conformational change at one dimer interface, and there is an overall reduction in the curvature of the crescent-shaped  $\beta$  monomer. Both of these changes are attributed to  $\delta$  binding interactions.

However, the region of the dimer interface undergoing the conformational change in this  $\beta$  monomer contains two mutations that inhibit dimerization in the absence of  $\delta$  and may contribute to the conformational change at the interface. The  $\delta$  subunit could either trap a  $\beta$  monomer in this relaxed conformation or induce this state by cranking open the dimer interface. Once  $\delta$  binds one monomer of a  $\beta$  dimer, steric hindrance would prevent a second  $\delta$  binding to the other monomer.

Dynamic simulations suggest that monomeric  $\beta$  subunits could exist in a relaxed conformation similar to that seen in the structure of the  $\delta$ - $\beta$  complex<sup>1</sup>. Interactions occurring at the dimer interface might stabilize the monomers within the  $\beta$  clamp into a strained and curved conformation. Weaker interactions between  $\beta$  monomers are likely to increase the propensity for monomers to adopt a relaxed conformation with reduced curvature and favor binding of the  $\delta$  subunit. In agreement, the  $\delta$  subunit binds 50-fold more tightly to the monomeric mutant  $\beta$  than the wild type dimer<sup>1</sup>. The clamp opening model is further supported by kinetic data showing that  $\beta$  dimer mutants with

## news and views



**Fig. 2** Ribbon diagrams based on crystal structures of sliding clamps from **a**, *E. coli* ( $\beta$ )<sup>3</sup>, **b**, humans (PCNA)<sup>5</sup>, and **c**, bacteriophage T4 (gp45)<sup>6</sup>. Arrows indicate the general regions of the  $\beta$  monomers found to interact with the  $\delta$  subunit of the clamp loader.

weaker interactions at the dimer interface severely inhibit dissociation of the clamp loading motor from the  $\beta$ -DNA complex.

Ring-shaped clamp structures are common to *E. coli*, bacteriophage T4 and eukaryotes. In contrast to the  $\beta$  dimer, the T4 and eukaryotic (PCNA) clamps are composed of three identical monomers (Fig. 2)<sup>5,6</sup>. Are the trimeric clamps assembled on DNA in a manner similar to the dimeric  $\beta$  clamp? Similar assembly mechanisms are possible because the domain organization of the subunits in the three clamps are generally analogous. Each clamp loader could insert amino acid residues between two neighboring domains of a clamp monomer to 'pop' open an interface. Alternatively, relaxed rings having one open interface could pre-exist in solution in equilibrium with closed rings. The latter model may account for T4 clamp assembly based on fluorescence data showing that one of three gp45 clamp monomer interfaces is present in an open conformation in aqueous solution<sup>7</sup>. The T4 clamp loading complex may trap the clamp in open conformations, possibly facilitating further opening for assembly around the DNA.

### Structure of the clamp loader

Within the *E. coli* clamp loader, the  $\beta$  binding surface on the  $\delta$  subunit is not accessible<sup>8</sup>. Addition of ATP alters the conformation of subunits within the clamp loader to expose the  $\beta$  binding site of  $\delta$ . The nature of this conformational change is

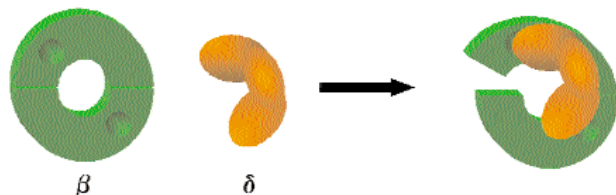
suggested by the crystal structure of a subassembly of the *E. coli* clamp loader containing N-terminal fragments of three  $\gamma$  subunits in addition to the  $\delta$  and  $\delta'$  subunits<sup>2</sup>. The stoichiometry of the components in the crystal structure supports the recent findings of McHenry and colleagues<sup>9</sup> showing that the clamp loader contains three copies of the *DnaX* gene product ( $\gamma$  and  $\tau$  subunits; see legend of Fig. 1), and the  $\gamma_3\delta\delta'$  complex is the minimal complex that supports clamp loading *in vitro*. The  $\gamma$ ,  $\delta$  and  $\delta'$  subunits have the same overall fold and are composed of three domains that form a 'C-shape'. In the complex, the C-terminal domains of the five subunits form a pentameric ring, whereas the middle and the N-terminal domains extend outward from the ring. Although the five subunits have the same fold, the relative orientations between domains within each subunit differ substantially, particularly between the middle and C-terminal domains. Consequently, the C-terminal domains of the five subunits form a quasi-symmetric arrangement without equivalent symmetry for the N-terminal domains; the entire complex is a ' $\sigma$ -shape' structure.

Although the crystal contains no ATP, the N-terminal domain of the  $\delta$  subunit protrudes from the complex partially exposing the  $\beta$  binding site. Kuriyan and coworkers<sup>2</sup> suggest that crystal packing forces may enable the clamp loading complex to adopt an asymmetric open conformation similar to the conformation

induced by ATP binding. They propose that in solution in the absence of ATP, the clamp loader adopts a more symmetric closed conformation where there is less of a difference in interdomain orientations in the individual subunits (Fig. 4).

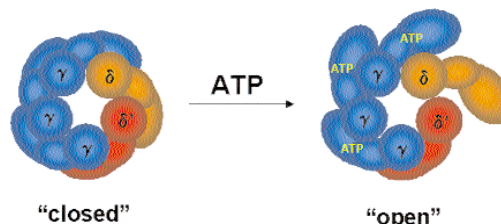
Binding and hydrolysis of ATP are required to accomplish the mechanical task of assembling clamps onto DNA. Although it might seem that ATP hydrolysis would be necessary to pry open the stable  $\beta$  ring, it is not. The ATP-independent interaction of the  $\delta$  subunit alone with  $\beta$  is sufficient to open the  $\beta$  ring<sup>10</sup>, and based on the recent structure<sup>1</sup>, it is likely that removal of the  $\beta$ - $\delta$  interaction allows the ring to 'snap' shut. Instead, the functions of ATP binding and hydrolysis may be to modulate clamp loader- $\beta$  and clamp loader-DNA interactions. A high affinity between the clamp and the loader is required prior to loading, but a subsequent reduction in this affinity is required to avoid interfering with the DNA polymerase binding to the clamp. In *E. coli*, oscillation between high and low affinity clamp binding must occur rapidly enough on the lagging strand to assemble and disassemble a clamp for each 1–2 kb Okazaki fragment synthesized every few seconds.

The structure of the *E. coli*  $\gamma_3\delta\delta'$  complex suggests a mechanism by which ATP binding and hydrolysis could alter the conformations of subunits within the clamp loader to expose or mask binding sites for the clamp and DNA. Although it is not yet known which subunit(s) binds DNA, ATP binding converts the clamp loader into a complex with high affinity for both  $\beta$  and DNA. But what then induces a decreased affinity of the clamp loader for the clamp, thereby allowing the clamp to close and the clamp loading complex to move away from the polymerase- $\beta$  binding surface? The mechanism for clamp release has yet to be established, but it is known to require ATP hydrolysis<sup>4,10,11</sup> and may also involve inter-



**Fig. 3** Schematic diagram of the  $\delta$ - $\beta$  interaction that results in clamp opening. Residues of the N-terminal domain of the  $\delta$  subunit bind to a hydrophobic binding pocket in one  $\beta$  monomer<sup>1</sup>. This interaction either induces or traps a relaxed conformation of the  $\beta$  monomer with reduced curvature, causing the opening of one dimer interface.

**Fig. 4** Schematic diagram of the ATP-induced conformational change in the *E. coli* clamp loader<sup>2</sup>. In the absence of ATP, the clamp loader exists in a conformation where the  $\beta$  binding site of the  $\delta$  subunit is blocked from interactions with  $\beta$ . On addition of ATP, a conformational change in the complex exposes the  $\beta$  binding site of the  $\delta$  subunit.



actions between the clamp loader and DNA that trigger a decrease in the affinity of the clamp loader for DNA<sup>12</sup> and the clamp. Perhaps the ATP-bound form of the clamp loader is cocked and ready to spring, and the interaction with the primed template provides the trigger to release the clamp and DNA. A DNA-induced switch would not only provide a mechanism for modulating binding activities of the clamp loader but also a dynamic mechanism for the clamp loader to recognize primed template sites where DNA synthesis is slated to begin.

The crystal structure of the  $\gamma_3\delta\delta'$  complex lays the groundwork for modeling structural changes that modulate the clamp loader's affinity for the clamp and DNA. At least three different conformational states may be required for a loading cycle — ATP-free, ATP-bound, and ADP-bound. In addition, each of the  $\gamma$  (or  $\tau$ ) subunits in the *E. coli* clamp loader contains an ATP binding site so that as many as three molecules of ATP may be hydrolyzed to load a single clamp onto DNA. These ATP molecules may be bound and hydrolyzed simultaneously or perhaps sequentially during the loading cycle, creating additional intermediate conformational states. Identification of these individual conformational states awaits future biochemical and structural investigations.

The structure of  $\gamma_3\delta\delta'$  offers the first view of a clamp loading complex and hints at what the complete clamp loading motor might look like. *In vivo*, the *E. coli* clamp

loader most likely contains two  $\tau$  subunits and one  $\gamma$  subunit in addition to  $\chi$  and  $\psi$  subunits, having a stoichiometry of  $\tau_2\gamma\delta\delta'\chi\psi$ . These additional subunits expand the functions of the clamp loader at the replication fork. The  $\chi$  subunit binds to single-stranded binding protein and helps orchestrate the switch from primase to DNA polymerase once a primer is made<sup>13</sup>. The full length  $\tau$  subunits contain additional binding domains for the polymerase and helicase not present in  $\gamma$ . These subunits are responsible for forming a dimeric polymerase<sup>14</sup> and coupling the DNA polymerase III holoenzyme to DnaB helicase<sup>15</sup> at the replication fork. Although both *DnaX* gene products are capable of binding  $\delta'$  and  $\psi$ , the  $\gamma$  subunit, rather than  $\tau$ , binds both.<sup>16</sup> The  $\chi$  subunit joins the complex through interactions with  $\psi$ <sup>17</sup>, whose function is not yet known. Perhaps the asymmetry introduced by the presence of a single  $\gamma$  subunit allows the clamp loader to function differently on the lagging strand where it must continuously assemble and disassemble clamps than on the leading strand where only a single clamp is needed. How these additional subunits are arranged in the complete clamp loader complex remains to be seen.

The structures reported by Kuriyan and colleagues<sup>1,2</sup> offer a physical basis to explain how the prototypical *E. coli* clamp loading complex pries open a  $\beta$  processivity clamp. Would the kinetics, energetics and clamp loading dynamics based on the *E. coli*

structures also apply to the functionally equivalent human clamp loading RFC motor and PCNA clamp? These structures have now pried open the door to the answer of this more general question.

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## history

### The X-ray files

Alone in the laboratory in a late autumn evening in 1895, the German physicist Wilhelm Röntgen began his experiment on a modified cathode ray tube. He was planning to study a phenomenon that had been reported earlier by Philipp Lenard — that is, cathode rays penetrated through a thin layer of aluminum coating the end of the tube. Röntgen put his cathode ray tube

in a 'light-proof' black cardboard box and turned on the device. In the darkened laboratory, he noticed that a piece of paper coated with a fluorescent material a few feet away began to glow.

What could produce this mysterious fluorescence? From the results of past experiments, Röntgen knew that it could not be the cathode rays because they could

not penetrate the cardboard box. Not knowing the origin of this new form of energy, he called it the 'X-rays'.

Over the next few weeks, the careful scientist studied the properties of the X-rays. He quickly found that X-rays penetrated most of the materials placed in their path, such as a book or a block of wood; the most opaque material to the X-rays was