Supercomputers Simulate the Molecular Machines That Replicate and Repair DNA

Imagine you are an astronaut. A piece of space junk has cut a gash into the side of the space station, and you have been tasked with repairing the damage. Your spacesuit is equipped with a clamp, which you open, slide onto a tether connecting you to the space station, and close. Then you move to the far end of the gash and begin applying composite material to fill the holes. You glide along the gash making repairs until you are done.

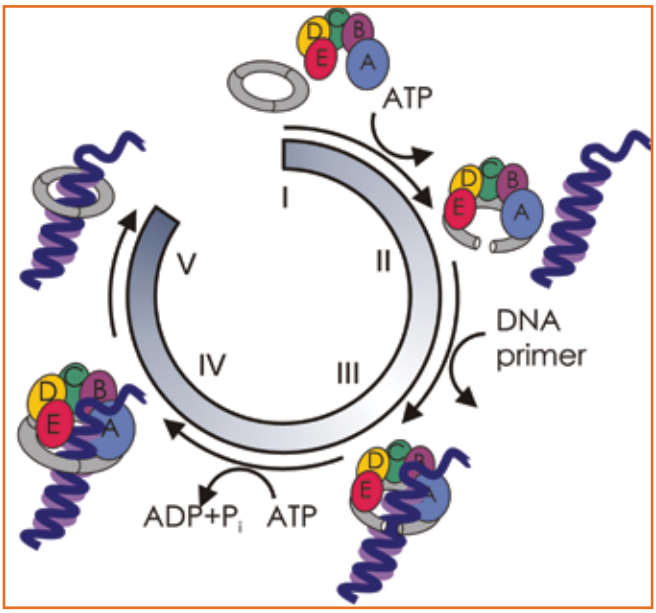
DNA replication, modification, and repair happen in a similar way. That's what groundbreaking biochemical simulations run on one of the world's fastest supercomputers have revealed. Ivaylo Ivanov of Georgia State University, John Tainer of the Scripps Research Institute, and J. Andrew McCammon of the University of California–San Diego used Jaguar to elucidate the mechanism by which accessory proteins, called sliding clamps, are loaded onto DNA strands and coordinate enzymes that enable gene repair or replication. Their findings, published in the May 10, 2010, issue of the *Journal of the American Chemical Society*, inspire a new approach for attacking diverse diseases.

"This research has direct bearing on understanding the molecular basis of genetic integrity and the loss of this integrity in cancer and degenerative diseases," said Ivanov, whose investigation was supported by the Howard Hughes Medical Institute and the National Science Foundation's Center for Theoretical Biological Physics. The project focused on the clamp-loading cycle in eukaryotes—or plants, animals, and other organisms whose genetic material is enclosed in a nuclear membrane. Prokaryotes, such as bacteria, whose genes are not compartmentalized, also have a molecular machine to load clamps, but it works a little differently. Viruses, on the other hand, do not have their own clamp loaders but instead co-opt the replication machinery of their hosts.

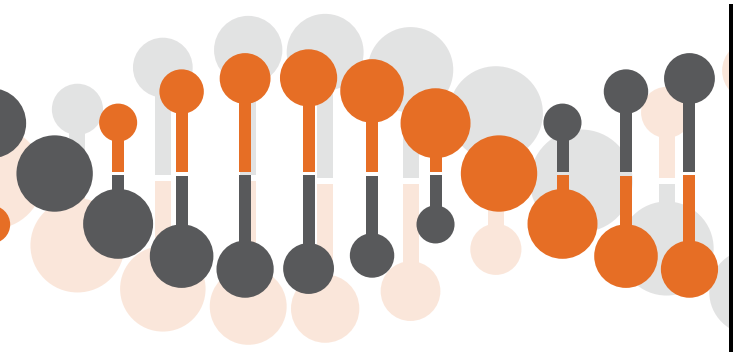
So how does the molecular machine work in a eukaryote? The researchers revealed that a clamp loader (replication factor C) places a doughnut-shaped sliding clamp (proliferating cell nuclear antigen, or PCNA) onto DNA. The clamp loader first binds to the clamp to activate its opening with energy from adenosine triphosphate (ATP). Protein secondary structures, or beta sheets, at the junctures of the clamp's three subunits, separate at one juncture. A complex made up of the open clamp and the clamp loader then encircles primer-template DNA, which is double-stranded in one region and single-stranded in another. Again activated by ATP, the clamp closes and is now free to slide along the DNA strand and coordinate enzymes needed for replication and repair.

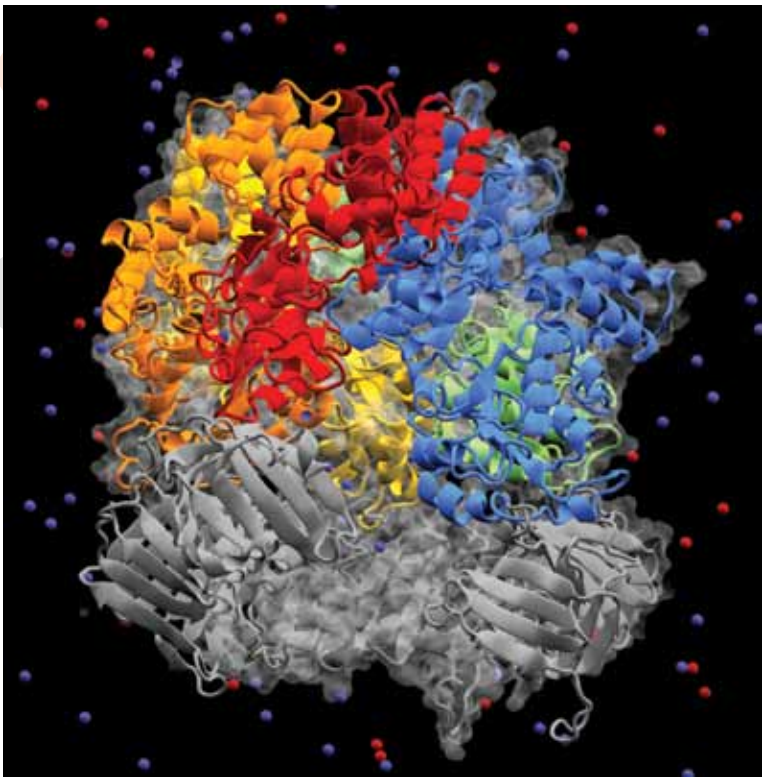
These sliding clamps and clamp loaders are part of the replisome—the molecular machinery responsible for the faithful duplication of the genetic material during cell division. "The replisome is very complex and dynamic, with interchanging parts. It's an incredibly challenging system to understand," explained Ivanov. "Simulating just a few of its constituent parts—the clamp/clamp loader assembly—required a system of more than 300,000 atoms. To make progress simulating the system in a reasonable amount of time, we needed access to large-scale computing."

An allocation of supercomputing time through the INCITE program allowed the researchers to run NAMD, a molecular dynamics code.



The clamp-loading cycle. Image courtesy Ivaylo Ivanov, Georgia State University.





OLCF supercomputers illuminate the workings of the molecular machinery that opens and loads sliding clamps onto DNA. Sliding clamps play vital roles in both DNA replication and repair. Here the clamp loader (with its subunits shown in blue, green, yellow, orange, and red) is depicted in complex with a ring-open sliding clamp (shown in gray) and counterions (spheres). Image courtesy Ivaylo Ivanov, Georgia State University, and Mike Matheson, ORNL.

The work consumed more than 2 million processor hours on the Jaguar XT4 and XT5 components in 2009 and 2010, taking a few months of total computing time. Using the kind of machine on which NAMD is usually run, a single simulation continuously running would have taken years.

Master coordinator

In DNA replication the clamp slides along a strand of genetic material made of building blocks called nucleotides. Nucleotides differ only in the type of base they carry, so bases are what determine the genetic message. Enzymes called polymerases catalyze the formation of a new DNA strand from an existing DNA template. The association of the sliding clamps with polymerases significantly increases the efficiency of strand replication, as it prevents polymerases from falling off the DNA and makes sure replication continues uninterrupted. Polymerases iteratively add one of four bases to DNA strands until they have strung together thousands of them.

In DNA repair, the sliding clamp serves as the master coordinator of the cellular response to genetic damage. A number of proteins, such as cell cycle checkpoint inhibitors or DNA repair enzymes, attach themselves to the clamp to perform their functions. In this capacity the role of the clamp is to orchestrate a variety of DNA modification processes by recruiting crucial players to the replication fork, a structure in which double-stranded DNA gives rise to single-stranded prongs that act as templates for making new DNA.

Given the dual function of PCNA in replication and repair, it is not surprising that this clamp has been implicated in diseases accompanied by excessive replication and unchecked cell growth, such as cancer. PCNA modifications are key in determining the fate of the replication fork and, ultimately, both tumor progression and treatment outcome. Therefore, PCNA has been used as a diagnostic and prognostic tool (biomarker) for cancer.

Most studies of DNA replication have focused on polymerases. Gaining a better understanding of the replisome, however, may shift the spotlight. "Instead of just focusing on polymerase, we can interfere with many different components within this complex machinery," Ivanov said. "That may allow new drug targets to be developed for hyperproliferative diseases such as cancer."

Improved understanding of the replisome may make it possible to exploit differences among organisms as diverse as viruses, bacteria, plants, and animals. Although clamp loaders from different kingdoms of life share many architectural features, significant mechanistic differences exist, specifically in the ways ATP is used. Drugs targeted to the clamp loader could selectively inhibit replication of viral DNA in diseases such as chickenpox, herpes, and AIDS without interfering with DNA replication in normal human cells. Similarly, in processes with increased DNA replication, such as cancer, inhibiting clamp loading might produce therapeutic effects without unwanted side effects.

In the future Ivanov and his colleagues will study mechanisms of alternative clamps such as a PCNA-related protein complex that signals the cell to arrest division upon detection of DNA damage. Ultimately, the researchers, fueled by enthusiasm at the therapeutic prospects, want to demystify the entire clamp-loading cycle.—*by Dawn Levy*