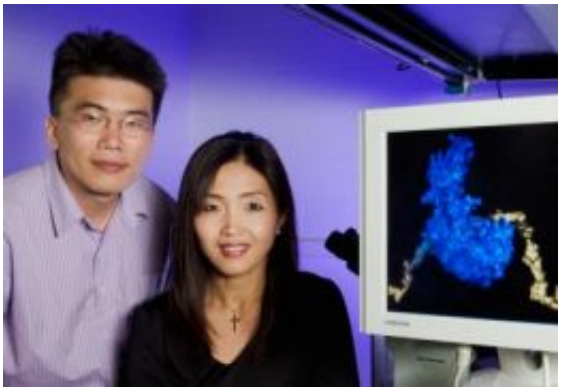
Hepatitis C helicase unwinds DNA in a spring-loaded, 3-step process

July 26 2007



Using single-molecule fluorescence analysis, Institute for Genomic Biology professor Sua Myong and physics professor Taekjip Ha led a team that discovered the mechanism by which the hepatitis C helicase unwinds DNA and RNA for replication. Ha is also affiliated with the Institute for Genomic Biology and the Howard Hughes Medical Center. Credit: Photo by L. Brian Stauffer, U. of I. News Bureau

The process by which genes are duplicated is mysterious and complex, involving a cast of characters with diverse talents and the ability to play well with others in extremely close quarters. A key player on this stage is an enzyme called a helicase. Its job is to unwind the tightly coiled chain of nucleic acids – the DNA or RNA molecule that spells out the organism's genetic code – so that another enzyme, a polymerase, can faithfully copy each nucleotide in the code.

Researchers at the University of Illinois, Yale University and the Howard Hughes Medical Institute have shed new light on how the Hepatitis C helicase plays this role, using a technique developed at Illinois that can track how a single molecule of RNA or DNA unwinds. Their research findings appear tomorrow in the journal, *Science*.

Getting at the underlying mechanisms of replication is no easy task. Structural studies involve crystallizing the DNA-protein complexes to see how they interact. Biochemists look at the agents of a reaction, the energy used and how much time lapses between steps. Such studies measure the behavior of hundreds of thousands of molecules at a time, and the results describe a whole population of reactions.

Using single-molecule fluorescence analysis, the research team tracked how the hepatitis C helicase, NS3, unwound a duplexed DNA molecule tagged with a fluorescent label on each strand of its double-stranded region. (The NS3 helicase is primarily involved in unwinding the single-stranded RNA of the hepatitis virus, but it can also act on DNA. This suggests that the helicase plays a role in unwinding double-stranded host DNA during infection. The duplex created for the experiment included both single- and double-stranded DNA; fluorescent labels were located in the double-stranded region.)

By tracking the gradually increasing distance between the two marked nucleotides as the strands separated in an unwinding event, the researchers were able to measure the rate at which the unwinding occurred. What they found was that the DNA unwound in discrete jumps: Three nucleotide pairs (base pairs) had to be unhitched from one another before an unwinding event occurred.

“It’s like you’re adding tension to a spring,” said U. of I. physics professor Taekjip Ha, a researcher on the study and an affiliate of the Institute for Genomic Biology and the Howard Hughes Medical Institute.

“You are loading the spring with small mechanical movements until finally you have accumulated enough tension on the DNA-protein complex to cause the rapid unwinding of three base pairs.”

Such reactions are energetically intensive, requiring the input of adenosine triphosphate (ATP) a cellular fuel source. The researchers observed that three ATP molecules were consumed in each unwinding reaction, indicating that three “hidden steps,” each involving the unhitching of one base pair, occurred for each unwinding event.

Although one molecule of ATP contains enough energy to unwind as many as 10 base pairs, the researchers said they were not surprised by the high-energy costs of the reaction.

“Helicases work hand in hand with polymerases in replication, so it makes sense that the helicase would work on one base pair at a time,” said Institute for Genomic Biology professor Sua Myong, lead author on the study. “It’s a very systematic, one-base-pair translocation that may help the polymerase accurately copy genes one base at a time.”

The helicase must also navigate around a lot of obstacles: proteins and other co-factors that are involved in replication. This requires extra energy. Ha compared the energy needs of the NS3 helicase to those of a sport utility vehicle.

“It’s not fuel efficient but in principle it could also go off-road, carry some luggage or maneuver around barriers,” he said. “So it may actually make sense to develop a low-efficiency motor because then you have extra energy to do extra work when needed.”

Myong noted that NS3 is the only helicase in the viral genome, and that it is already being targeted in pharmaceutical studies to combat Hepatitis C infection. It also belongs to the largest of four helicase superfamilies,

so the new findings could have relevance across many organisms.

Source: University of Illinois at Urbana-Champaign