

# Scientists at NSLS Discover how Papillomavirus ‘unzips’ DNA

Finding may lead to drugs to prevent sexually transmitted disease and cervical cancer

Infection with the human papillomavirus (HPV) is the most common sexually transmitted disease in the United States. According to the Centers for Disease Control and Prevention in Atlanta, Georgia, an estimated 20 million Americans are currently infected – but the vast majority does not know it.

Though HPV sometimes causes genital warts, in most cases, it infects people without causing visible symptoms. Women with persistent infections from certain types of HPV are at risk for cervical cancer, as 99 percent of cervical cancers around the world are associated with HPV infection.

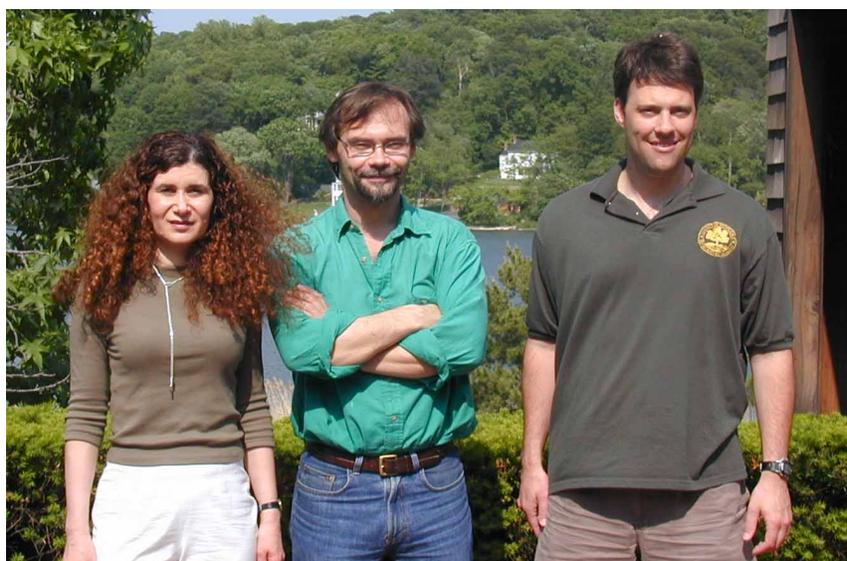
Preventing papillomavirus from

have gained new insight into how papillomavirus – in this case, cow, or bovine papillomavirus, commonly used as a model system – starts to multiply, causing infection. This new understanding could be used to design drugs to stop HPV infection, which is of particular significance since no cure or vaccine are currently available, although vaccine development is underway.

“We know very little about how papillomavirus replicates,” says biologist Leemor Joshua-Tor, the Cold Spring Harbor team leader. “So we decided to look at the molecular details of how the replication mechanism is initiated, with the aim of helping to design drugs that act like monkey wrenches in the replication process.”

The infection process starts as follows: The papillomavirus first inserts its DNA – a double-stranded helix containing the virus’s genetic information – into the host cell. The virus hijacks the protein production machinery of the host cell to produce a viral protein called E1. By attaching to the viral DNA, E1 proteins can initiate the DNA replication process, so that more viruses can be formed, and later multiply further.

“The DNA double helix can be replicated only if it is ‘unzipped,’ which allows proteins called DNA polymerases to make copies of each strand,” Joshua-Tor explains. “The E1 protein is known to initiate the ‘unzipping’ process, but how it does it is not very well understood.”



*Authors of the study (from left to right): Leemor Joshua-Tor, Arne Stenlund and Eric Enemark.*

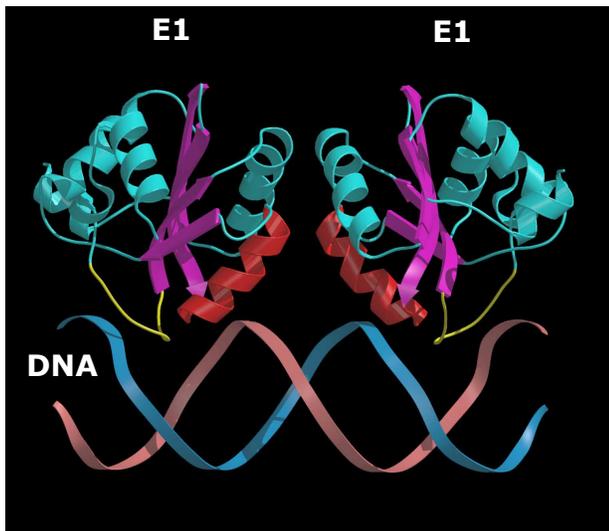
multiplying is one way of stopping the infection. Toward that goal, a team of scientists from Cold Spring Harbor Laboratory (CSHL) in New York, working at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory,

Like all viruses, a papillomavirus is an infectious agent that uses the cells it infects to reproduce itself. Replication of HPV does not kill the host cells, but can make them cancerous.

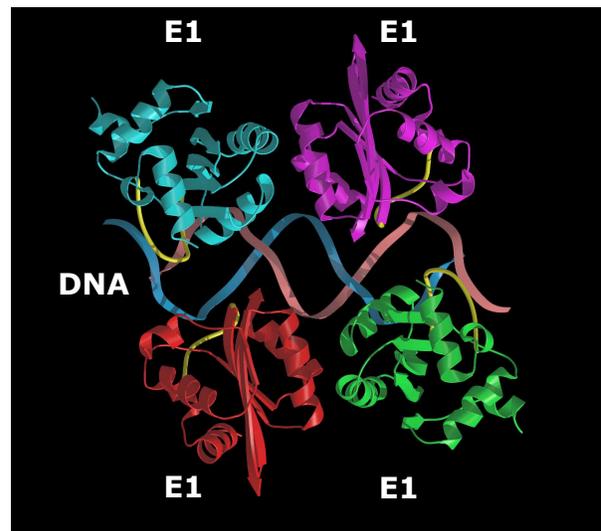
To look carefully at how E1 proteins attach to viral DNA, Joshua-Tor and her postdoctoral associate, Eric Enemark, in collaboration with Arne Stenlund, a renowned papillomavirus expert at CSHL, grew crystals of E1 and papillomavirus DNA at two different stages of the attachment process, in which either two or four E1 proteins bind to DNA.

The researchers then used a technique called x-ray crystallography to determine the positions of the atoms making up the E1 proteins and DNA. X-rays produced by the NSLS were projected toward the crystals, and the positions of the atoms were determined by looking at how the x-rays scattered off the crystal.

To their surprise, Joshua-Tor and her colleagues observed that E1 uses two separate modules, one shaped like a loop and the other



**Figure 1.** Crystal structure of two E1 proteins binding to papillomavirus DNA. Each E1 protein exhibits two modules: a loop (yellow) and a helix (red), each binding to a separate DNA strand, the loop binding more tightly than the helix to the DNA strands.



**Figure 2.** Crystal structure of four E1 proteins binding to papillomavirus DNA. The loops (yellow) of the two proteins on the right (purple and green) bind one DNA strand (pink), while the loops of the two proteins on the left (red and blue) bind the other DNA strand (blue).

like a helix, to bind DNA, each one binding to a different DNA strand (**Figure 1**). “This is very unusual,” Joshua-Tor says. “We expected that both modules would bind the two strands simultaneously.” The scientists also noticed that the loop bind more tightly than the helix, giving loops a larger role in E1-DNA binding than helices.

When two E1 proteins attach the DNA, Joshua-Tor and her collaborators observed that the loops bind different strands (**Figure 1**). When four proteins bind to DNA, they form two pairs facing each other, with proteins in each pair binding the same DNA strand as the ones on the opposite side (**Figure 2**).

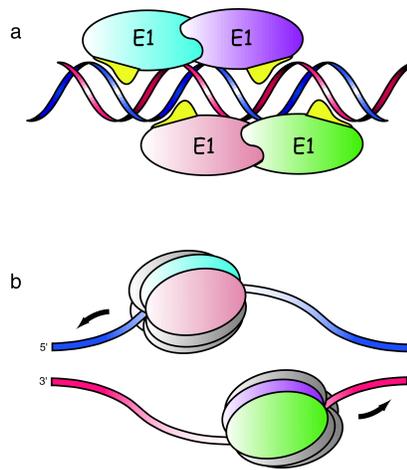
These results suggested a mechanism by which the double-stranded DNA might ‘unzip’ (**Figure 3**). “We already know that, ultimately, the unzipping process involves two bundles of six E1 proteins each, called hexamers, each ‘unzipping’ the DNA in opposite directions,” Joshua-Tor says. “So, we think that the initial assembly of the two

hexamers from the four proteins shown in our structure is what causes the strands to separate by forming around the single strands.”

Joshua-Tor and her colleagues suggest that the four proteins separate into two pairs, each recruiting four additional E1 proteins, thus creating two hexamers that would

move in opposite directions (**Figure 3**). Each hexamer would encircle either strand, and act like a little propeller that rotates around the strand, thus ‘unzipping’ it from its partner DNA strand along the way.

“If this is the way these proteins operate, it is pretty clever,” Joshua-



**Figure 3.** Schematic representation of how hexamers are formed and DNA ‘unzipping’ process is initiated. (a) When four E1 proteins bind to the double stranded DNA (red and blue), they link DNA strands with a separate loop (yellow) that acts like a clip and allows E1 to hold tight onto the strand. Note that two proteins facing each other hold onto the same strands: The blue and red E1 proteins bind to the blue DNA strand, while the purple and green E1 proteins bind to the red DNA strand. (b) The E1 proteins that face each other (blue and red on one side, purple and green on the other side) attach to each other, and recruit four more E1 proteins to form a hexamer on each side of the DNA. The hexamers act like little propellers moving in opposite directions and ‘unzipping’ each DNA strand from its partner.

Tor says. "This is the first time that it has been found that the two individual DNA strands bind to two separate protein modules prior to the DNA 'unzipping' process."

While Joshua-Tor and her colleagues are still investigating the papillomavirus-induced DNA replication, they are also starting to test compounds that interfere with papillomavirus DNA replication. For example, Anitra Auster, a graduate student, is developing compounds that could interfere with the

DNA replication induced by a high-risk type of human papillomavirus that can lead to cervical cancer.

"Understanding these binding mechanisms could significantly improve the treatment of this sexually transmitted disease," Joshua-Tor says. "We can now design and test drugs aiming to prevent E1 proteins from attaching to the viral DNA, which is one of the first steps to making much-needed antivirals against HPV infections and HPV-induced cervical cancer."

## **BEAMLINE**

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## **PUBLICATION**

E. Enemark, A. Stenlund, and L. Joshua-Tor, "Crystal Structures of Two Intermediates in the Assembly of the Papillomavirus Replication Initiation Complex," *The EMBO Journal*, **21**, 6, 1487-1496 (2002).

-Patrice Pages

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