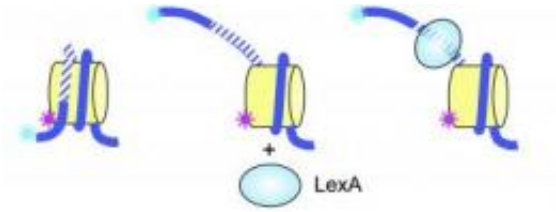
All-access genome: New study explores packaging of DNA

September 23 2011, by Richard Harth



Fluorescence resonance energy transfer (FRET): experimental design. Nucleosomes are constructed having a fluorescent donor (cyan) attached to one end of the DNA, and a fluorescent acceptor (magenta) attached nearby on the histone protein core. In the middle diagram, spontaneous partial unwrapping of the DNA thread exposes a hidden DNA target site (hatched area), which is site-specific for the DNA binding protein LexA. When LexA is added in sufficient concentration, nucleosomes are temporarily trapped in their unwrapped state. The distance between the two fluorescent molecules changes as the DNA unwraps and rewraps, allowing the process to be precisely measured. Credit: Reprinted from: *Journal of Molecular Biology*, volume 411(2), Tims HS, Gurunathan K, Levitus M, Widom J, Dynamics of nucleosome invasion by DNA binding proteins, pgs 430-48, with permission from Elsevier.

While efforts to unlock the subtleties of DNA have produced remarkable insights into the code of life, researchers still grapple with fundamental questions. For example, the underlying mechanisms by which human genes are turned on and off -- generating essential proteins, determining our physical traits, and sometimes causing disease -- remain poorly understood.

Biophysicists Marcia Levitus and Kaushik Gurunathan at the Biodesign Institute at Arizona State University along with their colleagues Hannah S. Tims, and Jonathan Widom of Northwestern University in Evanston, Illinois have been preoccupied with tiny, spool-like entities known as nucleosomes. Their latest insights into how these structures wrap and unwrap, permitting regulatory proteins to access, bind with and act on regions of DNA, recently appeared in the Journal of Molecular Biology.

Nucleosomes, Levitus explains, are essential components of the genome, acting to regulate access to DNA and protect it from harm. Nucleosome structure permits the entire strand of human DNA, roughly 6 feet in length, to be densely packed into the nucleus of every cell—an area just 10 microns in diameter. This occurs after nucleosomes assemble and fold into higher order structures, culminating in the formation of chromosomes.

Each nucleosome (there are roughly 30 million per cell) consists of a 147 base pair segment of DNA. This length of DNA thread is wound 1.67 times around the spool-like protein units, known as histones. The histone complex, together with its windings of DNA, forms the nucleosome core particle.

A multitude of proteins must act on regions of the DNA strand, by binding with appropriate target sites. Essential functions rely on these operations, including gene expression, replication and repair of damaged regions of the DNA molecule. But in eukaryotic cells like those of humans, some 75-80 percent of the DNA strand is curled up and hidden in the nucleosomes—inaccessible to protein binding interactions.

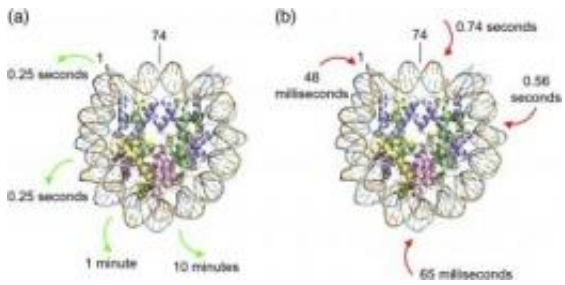
In earlier work, the group was able to show that nucleosomes are dynamic structures, quite different from the static pictures produced by X-ray crystallography. Lengths of DNA make themselves available for protein interaction by unwrapping and rewrapping around the histone

core. When nucleosomes unwrap, proteins present in sufficient concentration can find their DNA targets and bind with them.

In order to observe and characterize the dynamic behavior of nucleosomes, the team relied on a versatile imaging method known as Fluorescence Resonance Energy Transfer or FRET. The technique allows researchers to look at a pair of fluorescent molecules or fluorophores, one of which is attached to the end of the exposed DNA strand, the other, to one of the histones around which the DNA is coiled, (see figure 1).

As Levitus explains, spontaneous unwrapping and rewrapping of DNA changes the distance between fluorophores, signaling that the process has occurred and allowing the group to quantify the frequency and rate of DNA exposure and concealment.

"Although FRET has been used for decades to measure molecular distances in biological systems, dynamic biomolecules such as nucleosomes present particular challenges," notes Levitus. Traditionally, FRET experiments are performed with protein solutions containing many billions of particles. In the case of nucleosomes however, the dynamic behavior of each particle is crucial and bulk measurements using FRET are not effective.



This graphic shows the time elapsed during DNA unwrapping (a) and re-wrapping (b) as measured by FRET analysis. FRET works by measuring the

distance between a pair of fluorescent molecules of fluorophores -- one attached to the end of the DNA and the other attached to the histone protein spool around which the DNA "thread" winds and unwinds. Credit: Reprinted from: *Journal of Molecular Biology*, volume 411(2), Tims HS, Gurunathan K, Levitus M, Widom J, Dynamics of nucleosome invasion by DNA binding proteins, pgs 430-48, with permission from Elsevier.

"In simple terms, if one wanted to understand how humans clap, it would be useless to listen to the whole planet clapping at once. Instead, one would listen to a few individuals, and that is exactly what we did with nucleosomes," Levitus says.

The results of initial studies were revealing. For base pair sequences along the nucleosomes' outer rind, spontaneous DNA unwrapping occurs at a rapid rate— about 4 times per second. This corresponds to a period of only 250 milliseconds during which this region of DNA remains fully wrapped and occluded by the histone complex. Once unwrapped, the DNA remains exposed for 10-50 milliseconds.

These findings present a plausible mechanism to allow protein binding with unwrapped DNA in vivo, so long as the binding sites occur near the ends of wrapped nucleosomal DNA.

The new study also examines, for the first time, the condition of DNA sequences occurring further along the wound length of nucleosomal DNA, that is, closer to the nucleosome's center. Here, rates of DNA unwrapping decreased by orders of magnitude, (see figure 2).

To examine this phenomenon, the group used a site-specific binding protein of Escherichia coli (known as LexA) to identify binding site exposure caused by nucleosome unwrapping. The nucleosomes were

labeled with a FRET dye, which allowed the binding process of LexA and its target to be visualized. In successive experiments, the team shifted the binding sites in 10 base pair increments from the end of the nucleosome toward the middle.

The changes in unwrapping rate observed as the binding site was successively moved further inside the nucleosome were dramatic. In one case, a change in position of just 10 base pairs could produce a 250-fold decrease in unwrapping rate of the binding region.

These results prompt the question of how DNA binding sites more deeply wound within the nucleosome are able to successfully interact with their respective protein binders in vivo. The team proposes several possible mechanisms that would permit rapid access to hidden DNA binding regions, even where intrinsic rates of nucleosome unwrapping are low.

One hypothesis is that two or more proteins with target sites on the same nucleosome can act cooperatively, with one protein holding the momentarily unwrapped DNA open as the other enters the nucleosome and invades more inward regions of the DNA sequence, in what the authors describe as a ratcheting process.

Jonathan Widom, Dr. Levitus' collaborator and a co-author of the new study was responsible for much of the pathbreaking research into nucleosome complexity. Dr. Widom died unexpectedly this past month. He was honored for his generosity, prolific research and outstanding contributions to biology in the August 25th issue of the journal *Nature*.

"I consider myself tremendously fortunate to have had the chance to collaborate with Jon Widom," Levitus says. "Jon has been, and will continue to be, an incredible role model. His generosity, humility, and scientific genius has touched my life in many ways, and his death will

leave a void that will be felt for many years to come."

Ongoing research into the subtleties of nucleosome behavior promises to yield rich dividends for genomic science in general and provide a deeper appreciation for foundational issues of health and disease.

Provided by Arizona State University

Citation: All-access genome: New study explores packaging of DNA (2011, September 23) retrieved 9 April 2024 from https://phys.org/news/2011-09-all-access-genome-explores-packaging-dna.html

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