Researchers unveil new, detailed images of DNA transcription

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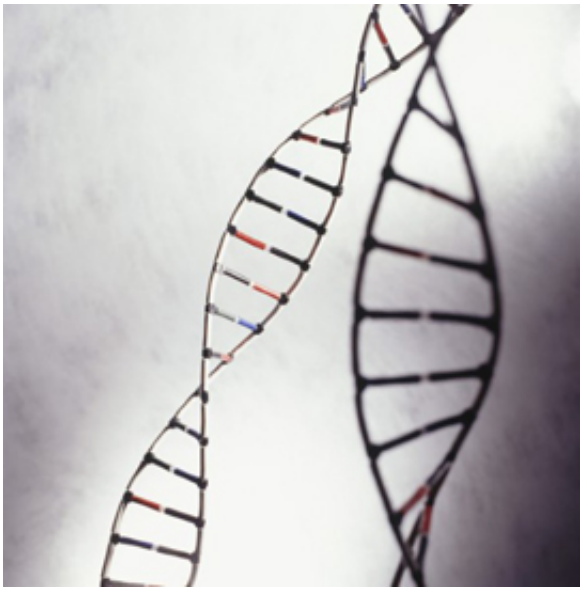

Credit: NIH

An unprecedented molecular view of the critical early events in gene expression, a process essential for all life, has been provided by researchers at Georgia State University, the University of California at Berkeley and Northwestern University.

Cryo-electron microscopy (cryo-EM), a technique that studies samples at cryogenic temperatures, combined with state-of-the-art computational modeling, allowed researchers to visualize large transcription pre-initiation complexes (PIC) at near-atomic resolution. The PIC is a

protein assembly that positions the enzyme RNA polymerase so it can start transcription.

The new structures shed light on the sequential conformational changes in the PIC throughout the transcription initiation process, including recognizing the promoter region of DNA where transcription of a gene starts, opening this promoter region and initiating transcription. The study was published in the journal *Nature*.

Genes are made up of DNA, which serves as a repository of all our genetic information. To use the information encoded in a gene, RNA polymerase must make a copy in the form of messenger RNA. The copying process, called transcription, is one of the central activities necessary for life.

At the beginning of this tightly controlled process, RNA polymerase and general transcription factor proteins assemble at a specific site along the DNA to form a PIC. The PIC assembly is required for opening the double-stranded DNA helix of the promoter, positioning the DNA in the active site of RNA polymerase and starting the transcription process. The messenger RNA transcripts are then used to produce proteins, the building blocks of human bodies.

"This paper provides detailed structural information on the complexes that participate in the early stages of the transcription process," said Ivaylo Ivanov, associate professor of chemistry at Georgia State. "We explore the steps that RNA polymerase and general transcription factors take in order to open the transcription bubble and begin the process of transcription. This is a very important system that wasn't accessible by either crystallography or any other structural method before. This is the very first near-atomic Cryo-EM reconstruction of the human PIC assembly."

Chemical cross-linking and crystallography had provided glimpses of partial RNA polymerase complexes from eukaryotic organisms such as yeast, but these techniques could not solve the structure of the entire PIC complex. The events and processes leading to DNA unwinding by the PIC and the formation of a transcription bubble, a molecular structure that occurs during transcription when a portion of the DNA double strand is unwound, were insufficiently understood.

To build detailed atomic models of the PIC complex, Ivanov and his team applied integrative molecular modeling techniques. The calculations relied on modern supercomputing technology available though the National Science Foundation Extreme Science and Engineering Discovery Environment program and the National Energy Research Scientific Computing Center. The researchers showed that judicious combination of complementary techniques—molecular dynamics flexible fitting and refinement of atomic coordinates with the Phenix crystallography software package—led to models comparable in quality to crystal structures in the same resolution range.

The researchers captured the human PIC in three different functional states: 1) a closed state engaged with the DNA double helix of the promotor region, 2) an open state engaged with the transcription bubble and 3) an initial transcribing complex poised to carry out the chemistry of messenger RNA synthesis. They were also able to visualize numerous previously undetermined components of the human PIC assembly. The findings revealed the complete subunit organization of a transcription factor called TFIIH, which has a critical role in opening the promoter region. TFIIH proved one of the most difficult pieces of the PIC assembly to resolve.

"We have a lot of newly visualized structural elements that were never established for the human complex before," Ivanov said.

Comparisons between the closed, open and initial transcribing states of the PIC provide new mechanistic insights into the processes of DNA engagement, promoter melting and transcription bubble stabilization.

"None of this would have been possible without advances in electron microscopy (EM) and without recent advances in integrative computational modeling," Ivanov said. "The ability to get near-atomic resolution EM structures has only happened recently through combination of direct electron detector technology and new powerful computing algorithms to analyze the images.

"In the last few years Cryo-EM has undergone a revolution making it possible for the first time to achieve resolution comparable to crystallography. This opens up tremendous opportunities for the field of structural biology to study large macromolecular complexes in atomic detail without the need to produce protein crystals."

More information: Yuan He et al, Near-atomic resolution visualization of human transcription promoter opening, *Nature* (2016). DOI: 10.1038/nature17970

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