How RNA polymerase II gets the go-ahead for gene transcription

October 9 2009

All cells perform certain basic functions. Each must selectively transcribe parts of the DNA that makes up its genome into RNAs that specify the structure of proteins. The set of proteins synthesized by a cell in turn determines its structure and behaviour, and enables it to survive and reproduce. So it is crucial that the appropriate stretches of DNA are transcribed in each cell type.

In today's issue of the journal *Nature*, a team of researchers at the Gene Center of Ludwig-Maximilians-Universität (LMU) in Munich, led by Professor Patrick Cramer, provides the first detailed description of how the RNA polymerase II initiates gene transcription. "The findings led us to propose a model of the whole complicated process of transcription initiation," says Cramer. "This operation is of crucial importance in all organisms, because it determines which genes are expressed, and when. Our work thus represents a milestone in the quest to understand gene regulation."

Cell types such as liver cells and nerve cells differ from one another because they make distinct sets of proteins. Therefore, gene transcription and protein synthesis must be carried out with great precision. This requires the use of complicated assemblies made up of many different proteins, often referred to as molecular machines. The basic structure of RNA polymerase II, the protein complex that transcribes genes encoding proteins in multicellular organisms, was worked out some years ago, but this structure could not explain how the initial steps in transcription take place.

Signals encoded in the DNA sequence tell RNA polymerase II where to start and stop transcription. The regions in which transcription begins are called promoters. In many genes, the promoter region is marked by a short DNA sequence called the TATA box. The actual transcription start site (TSS) is located 30-40 nucleotides downstream. It was already known that the protein TBP recognizes and binds to the TATA box, producing a sharp kink in the DNA. TBP in turn binds TFIIB, to which the polymerase enzyme (comprising 12 different proteins) then attaches. So it is TFIIB that actually gives the start signal for transcription initiation.

What the LMU researchers in Cramer's group have now done is to determine the three-dimensional structure of the complex formed between RNA polymerase II and TFIIB from brewer's yeast. Analysis of this complex using X-ray diffraction gave them a map that could be compared with one obtained for the polymerase alone. The differences between the two enabled the scientists to localize the TFIIB with respect to both the polymerase and the DNA. On the basis of this structure they were able to deduce how the initiation of transcription occurs, how the TSS is selected and the first segment of RNA is synthesized and, finally, how the polymerase "shifts gear" from the initiation to the elongation mode, as it leaves the region of the promoter and proceeds on through the gene. In a fruitful collaboration with Professor Michael Thomm's lab at the University of Regensburg the researchers also confirmed important aspects of their model experimentally.

It turns out that TFIIB acts as a bridge between TBP and polymerase, so that the polymerase faces the DNA, in the so-called closed complex. This is converted into an open complex when part of the TFIIB (called the B-linker) inserts between the two DNA strands. One of the strands (the template strand) is displaced into a tunnel formed by TFIIB and the polymerase. The complex then searches the sequence in the tunnel for an initiator sequence that defines the TSS, "using a second element (the B-

reader) in TFIIB, which functions rather like the reading head in a tape recorder", explains Cramer. When the TSS is located, the first two nucleotides of the new RNA transcript pair with their complementary partners on the DNA and are linked together by the polymerase. This marks the real initiation of transcription. After the addition of additional nucleotides, TFIIB is released from the complex.

The resulting elongation complex continues to synthesize an RNA sequence complementary to that of the template DNA strand, which later determines the structure of a specific protein. As Cramer points out, "The findings led us to propose a model of the whole complicated process of transcription initiation, an operation that is of crucial importance in all organisms, because it determines which genes are expressed, and when." The work of the LMU group thus represents a milestone in the quest to understand how genes are regulated. The results also provide the framework for investigating the mechanisms underlying the regulation of transcription initiation, which governs cellular gene expression. (PH)

Source: Ludwig-Maximilians-Universität München