The Molecular Basis of Eukaryotic Transcription

A complete RNA polymerase II transcription system has been derived by the fractionation of yeast and mammalian cell extracts. The required polypeptides comprise the 12-subunit RNA polymerase II, multiple "general transcription factors", and a 20-subunit "Mediator". The general transcription factors are responsible for promoter recognition and for melting the DNA template for the initiation of transcription. Mediator makes the key connection between enhancers and promoters. It transduces regulatory information from activator and repressor proteins to RNA polymerase II.

Structural studies of the RNA polymerase II transcription machinery began with electron microscope analysis of two-dimensional protein crystals formed on lipid layers. This led to the derivation of a 10-subunit form of RNA polymerase II especially conducive to crystallization, and to the use of two-dimensional crystals as seeds for the growth of large single crystals for X-ray analysis. The large size of the polymerase, over half a million Daltons, presented unusual technical difficulties, eventually overcome, and the structure was determined at 2.8 Angstroms resolution.

RNA polymerase II was also crystallized in the form of an actively transcribing complex, containing template DNA and product RNA. The structure of this complex was solved by molecular replacement, revealing the DNA entering and unwinding in the active center cleft. Nine base pairs of DNA-RNA hybrid could be seen extending from the active center at nearly right angles to the entering DNA. Protein-nucleic acid contacts help explain DNA and RNA strand separation, the specificity of RNA synthesis, and RNA and DNA translocation during transcription elongation.

RNA polymerase II crystallography has been extended to general transcription factors. The results have been assembled in a preliminary picture of a complete transcription initiation complex. From this picture, principles of both the initiation of transcription and its regulation may be derived.