The Inner Workings of RNA Polymerase: How Genetic Information is Transcribed

The research group of Professor Roger Kornberg of Stanford University has studied RNA polymerase II for more than 20 years. In 2000, his group solved the atomic-level structure of RNA polymerase, a macromolecular machine that transcribes genetic information, using crystallography diffraction data collected at SSRL [1]. The structure was featured on the cover of *Science* Magazine in the April 28, 2000 issue (Fig. 1). One year later, his group published the structure of polymerase in the act of transcription [2].

Fig. 1. The structure of RNA polymerase II (left) and RNA polymerase II in the act of transcription (right) featured on covers of *Science* Magazine. During transcription, a template strand of DNA (shown in blue on the right) is unwinding just before the active center. Newly formed RNA is shown in red.

A key step in gene expression is the "transcription" of the DNA sequences comprising the genes into a message that can be read by the ribosome to produce protein. Transcription is the first step and a key control point in gene expression. Transcriptional regulation underlies all aspects of cellular metabolism including oncogenesis (cancer) and morphogenesis (development). RNA polymerase II is a large complex of 12 subunits that is at the heart of the transcription mechanism. Gene expression, and therefore RNA polymerase, is regulated by a number of helper molecules termed initiation and transcription factors.

In two landmark studies, the Kornberg group recently published back-to-back papers in the February 13, 2004 issue of *Science* that help to explain how polymerase carries out its tasks [3, 4]:

http://www.sciencemag.org/cgi/reprint/303/5660/983.pdf

http://www.sciencemag.org/cgi/reprint/303/5660/1014.pdf

The first paper [3] describes the structure of polymerase and a helper molecule (initiation factor) that together form a transcription initiation complex. The helper molecule inserts a 'finger' into the polymerase as shown in Fig. 2. A docking site was also revealed that may function as the starting point of transcription, a spot where the RNA polymerase aligns on a gene.

The second paper [4] describes how the team caught a snapshot of the polymerase in action in which the newly made RNA could be visualized separating from the DNA (Fig. 3). The polymerase inserts itself as a wedge between the two, with the RNA trailing out an opening in the polymerase. This is the same opening that the helper molecule extends into, allowing for direct regulation of the transcription process.

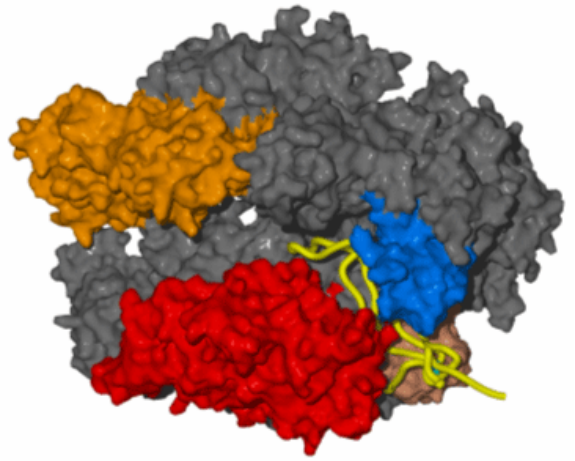

Fig.2 A 'finger' (shown in yellow) of one of the helper molecules, required for initiation of transcription, is shown inserted into the active center of polymerase.

To find the highest quality diffracting crystals among the hundreds of possible choices for the structures reported in the two recent *Science* publications, the Kornberg group used the new automatic robot screening system that has been developed at SSRL with grants from the National Institutes of Health (and implemented on all the SSRL macromolecular crystallography beam lines). This automated system operates in an integrated software environment within the Blu-Ice experiment control software. The robotic system is able to store about 300 frozen crystals in the beam line hutch (divided among 3 cassettes) and automatically mounts each crystal on the x-ray camera and tests for diffraction quality [5]. In contrast to the earlier studies published in 2000 and 2001, where about 1000 crystals were screened over many months to obtain suitable samples [1,2], the robot enabled very rapid crystal screening (a day or two). Hence the new automation system significantly decreased the required labor and accelerated these ground-breaking studies.

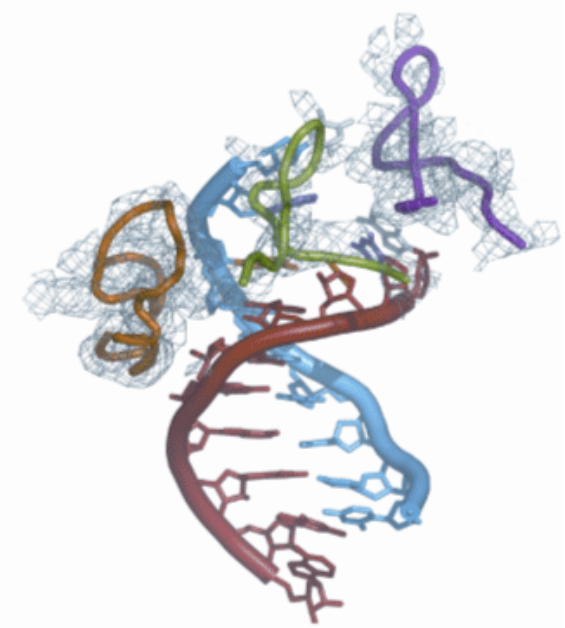

Fig 3. Separation of the RNA transcript (red) from the DNA template (blue) of the RNA polymerase transcribing complex. Electron density is shown for parts of the polymerase structure involved with strand separation (brown, green and purple).

Additional information can be found on the homepages of the Kornberg group at (http://kornberg.stanford.edu/) and in a press release by Stanford University

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2. P. Cramer, D. A. Bushnell and R. D. Kornberg, "Structural Basis of Transcription: RNA Polymerase II at 2.8 Ångstrom Resolution", *Science* **292**, 1863 (2001).
3. D. A. Bushnell, K. D. Westover, R. E. Davis and R. D. Kornberg, "Structural Basis of Transcription: An RNA Polymerase II-TFIIB Cocrystal at 4.5 Angstroms", *Science* **303**, 983 (2004).

4. K. D. Westover, D. A. Bushnell and R. D. Kornberg, "Structural Basis of Transcription: Separation of RNA from DNA by DNA Polymerase II", *Science* **303**, 1014 (2004).
5. A. E. Cohen, P. J. Ellis, M. D. Miller, A. M. Deacon and R. P. Phizackerley, “An Automated System to Mount Cryo-Cooled Protein Crystals on a Synchrotron Beamline, Using Compact Sample Cassettes and a Small-Scale Robot”, *J. Appl. Crystallogr.* **35**, 720 (2002).

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