

A Long Time in the Making— The Nobel Prize for RNA Polymerase

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DOI 10.1016/j.cell.2006.036

The 2006 Nobel Prize in Chemistry has been awarded to Roger Kornberg for elucidating the molecular basis of eukaryotic transcription. The prize caps a decades-long quest to unlock one of the central mysteries of molecular biology—how RNA transcripts are assembled.

Roger Kornberg (Figure 1) has spent three decades studying how DNA is packaged in eukaryotic cells and how RNA polymerase II, the central enzyme of transcription, extracts genetic information from this DNA. He achieved the central breakthrough in 2001 with crystal structures of yeast RNA polymerase II alone and in a transcribing complex with its DNA template and RNA product (Cramer et al., 2001; Gnatt et al., 2001). Many factors lay behind this breakthrough, including Kornberg's keen insight, the hard work of a talented group of postdocs and graduate students, and advances in crystallographic tools that brought large biomolecules within reach. Key among these was the choice to work with a eukaryotic microbe, the budding yeast *Saccharomyces cerevisiae*, which is amenable to powerful genetics and large-scale biochemistry. Kornberg's path to the Nobel-winning discoveries beautifully illustrates how the most important fruits of scientific discovery are almost never the low-hanging ones, but instead grow from years of rigorous experimental practice and from pulling together multiple diverse threads of research.

RNA Polymerase: The Engine of Gene Expression

As the first step in the expression of genetic information, transcription is among the most highly regulated biological processes. Transcriptional regulation is a primary determinant of homeostatic, adaptive, and developmental cellular functions when it

operates normally and of disease states when it goes awry or is co-opted by pathogens.

Since formulation of the central dogma of molecular biology half a century ago, a full understanding of the molecular machinery of transcription and its regulation has been a prized goal. The key enzyme in this process, RNA polymerase, was discovered in 1959 by Samuel Weiss and Leonard Gladstone in extracts of mammalian cells (Weiss and Gladstone, 1959). Shortly thereafter, RNA polymerase was purified from different species

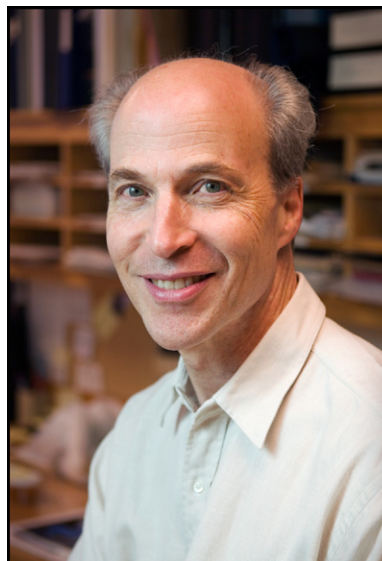


Figure 1. Twenty years of diligence pays off Roger Kornberg has been awarded this year's Nobel Prize in Chemistry for his decades-long dedication to elucidating the molecular underpinnings of eukaryotic transcription and the part played by RNA polymerase II. Photo courtesy of L.A. Cicero/Stanford University News Service.

of bacteria by Michael Chamberlin, Jerard Hurwitz, and others. It took another decade for researchers, most notably Robert Roeder, to tease out the existence of three separate forms of RNA polymerase in eukaryotes. These different forms divide responsibilities for synthesis of rRNA (RNA polymerase I), mRNA (RNA polymerase II), and tRNA (RNA polymerase III) (Roeder and Rutter, 1969).

Studies in the final three decades of the twentieth century amassed a wealth of information about the subunit composition, biochemical properties, and accessory factors of RNA polymerase. Several key insights emerged that proved remarkably concordant with the crystal structures of bacterial RNA polymerase, yeast RNA polymerase II, and its transcribing complexes, when the structures became available. First, RNA polymerases from bacteria to humans contain an essential and highly conserved core of two large subunits (called β' and β in bacteria and RPB1 and RPB2 in eukaryotic RNA polymerase II), and smaller subunits (α dimer and ω in bacteria; RPB3/11 and RPB6 in RNA polymerase II) that stabilize association of the two large subunits. Additional small subunits of varying essentiality associate with the core enzyme (RPB4, 5, 7, 8, 9, 10, and 12 in RNA polymerase II). The two large subunits form the catalytic center and make all essential contacts to the nucleic-acid scaffold. This scaffold consists of a ~ 9 base pair RNA:DNA hybrid within a ~ 14 base pair melted DNA bubble, ~ 7 nucleotides of sin-

gle-stranded nascent RNA upstream from the hybrid, and 15–20 base pairs of duplex DNA downstream of the catalytic center. Second, transcription occurs in a cycle of events that begins when initiation factors (σ in bacteria; TFIIA, B, D, E, F, and H for RNA polymerase II) program recognition and melting of promoter sequences and in so doing permit RNA polymerase to launch synthesis of the RNA transcript. Transcript elongation then commences when RNA polymerase releases the initiation factors, allowing the enzyme to escape from the promoter and to form an exceptionally stable transcribing complex. The transcription cycle ends when accessory factors, nucleic-acid sequence/structure, or both program dissociation of the transcribing complex and release of RNA polymerase for a new round of transcription. Third, a multitude of regulators modulate every step in the transcription cycle. This is best understood for initiation. In eukaryotes, an elaborate cascade of interacting regulators controls assembly and activation of transcription complexes at promoters. Some transcription regulators interact directly with RNA polymerase; others modulate the nucleosomal structure of the DNA template for transcription or mediate interactions among regulators, chromatin, and RNA polymerase. Roger Kornberg made several fundamental contributions to this transcription model, including recognition of the nucleosomal structure of DNA, characterization of some of the chromatin-modifying factors, and discovery of a bridging complex that mediates transcriptional activation (called Mediator).

The Long Road to the RNA Polymerase Structure

Roger Kornberg grew up immersed in science and developed a keen interest in physical chemistry during undergraduate studies at Harvard. Upon graduating in 1967, his interest in magnetic resonance led him to Harden McConnell's lab at Stanford. Here, he completed a thesis measuring the rates of lateral and *trans*-bilayer diffusion of membrane lipids. His key finding was that, whereas

trans-bilayer movement was slow, lateral diffusion occurred on the same timescale as diffusion in solution. Although seemingly unconnected to transcription, this knowledge of lipid dynamics would later provide Kornberg with a key foundation for structural studies of RNA polymerase.

He then started postdoctoral research with Aaron Klug at the Medical Research Council (MRC) in Cambridge, UK in 1972, an exciting time when the MRC was a nexus in the molecular biology revolution. At Cambridge, Kornberg grew fascinated with chromatin as an object in need of structural comprehension and made a fundamental contribution by recognizing that chromatin consists of nucleosomes arrayed along DNA in the form of beads on a string (Kornberg, 1974). He also came to appreciate the centrality of transcription and the importance of understanding its structural basis. Exposed in Cambridge to the emerging tool of electron crystallography, Kornberg surmised that lateral diffusion in lipid bilayers might allow molecules tethered to the bilayer to pack into two-dimensional crystals suitable for analysis by electron crystallography. Proof-of-principle was achieved by attaching antibodies to a lipid hapten and decorating them with the first component of complement (Uzgiris and Kornberg, 1983).

Upon assuming a faculty position at Stanford in 1976, Kornberg initiated his quest for the structures of RNA polymerase and its transcribing complex. Having seen the success of lipids as a platform for formation of 2D crystals amenable to electron crystallography, he began searching for ways to anchor RNA polymerase to lipid bilayers. The early work targeted the bacterial RNA polymerase from *Escherichia coli*, which by then was being studied intensely by several research groups around the world. Kornberg decided wisely to go after yeast RNA polymerase II as a source of an RNA polymerase that has evolved to negotiate the complex terrain of eukaryotic chromatin. Thus, work went forward in parallel to dissect the yeast transcription machin-

ery and to visualize the RNA polymerase structure.

The initial approach of binding RNA polymerase to lipid bilayers using DNA-conjugated lipids proved frustrating. Indeed, it wasn't until the arrival in 1987 of Seth Darst, a new postdoc trained as a chemical engineer, that the first structures were obtained. Using *E. coli* RNA polymerase provided by Mike Chamberlin, whose research group did much of the pioneering biochemistry on RNA polymerase, Darst hit upon the idea of spiking the lipid bilayers with lipids containing positively-charged head groups. These patches of positive charge proved to be good binding sites for RNA polymerase (which is now known to be negatively charged over most of its surface) and allowed the bacterial RNA polymerase to assemble into 2D crystals. By analyzing these 2D crystals using electron crystallography, Darst and Kornberg produced the first low-resolution (at ~ 25 Å) structures of *E. coli* RNA polymerase (Darst et al., 1989).

Meanwhile, the yeast transcription work began to yield results. An *in vitro* transcription assay using yeast cell extracts was made possible in 1987 when Neal Lue realized that replacement of chloride with acetate allowed better RNA polymerase-DNA interactions and robust *in vitro* transcription. Using this assay, Kornberg and coworkers then fractionated and purified the activities required for yeast RNA polymerase II to initiate efficient transcription at promoters. This work was given impetus by the pioneering purification and reconstitution of RNA polymerase II and its accessory factors from mammalian cells by Robert Roeder, with important contributions from Joan and Ron Conaway and from several other researchers.

Three key breakthroughs, each resulting from the diligence and insight of Kornberg and the outstanding group of researchers he attracted, led to the demonstration of fully functional transcription from yeast RNA polymerase II, which was then purified to the homogeneity required for crystallization. This crucial demonstration provided confidence that the

target of structural study was indeed the active form of RNA polymerase II. First, Kornberg and coworkers realized that TFIIH, a ten-subunit helicase/kinase required to melt promoter DNA and phosphorylate RNA polymerase II, separated into subcomplexes during purification and required special treatment to minimize dissociation. Second, the group discovered that activators needed another multisubunit complex they called Mediator to promote transcription initiation. Genetic dissection of the yeast transcription machinery by many labs was invaluable to Kornberg's research, but this

proved especially true for Mediator. Rick Young's work at MIT identifying suppressors of partial deletions of the C-terminal repeat domain on RNA polymerase II yielded a set of proteins termed SRBs, initially thought to be components of TFIID. By the mid 1990s, however, it became clear that Kornberg's Mediator contained the SRBs. Although initially Mediator was considered a peculiarity of yeast, its mammalian homologs are now well established. Finally, in 1990, Al Edwards in the Kornberg lab realized that the purified yeast RNA polymerase II contained substoichiometric levels of subunits RPB4/7. Using a yeast strain lacking RPB4 and an anti-RNA polymerase II monoclonal antibody developed by Dick Burgess in Wisconsin, Edwards was able to obtain yeast RNA polymerase II pure and homogeneous enough to form large, well-ordered crystals. Darst and Edwards then used the new positively charged lipid method to produce 2D crystals of yeast RNA polymerase II and a 16 Å electron crystal structure in 1991 (Darst et al., 1991).

At this point, the group realized that the 2D crystals might yield 3D crystals if used as seeds in crystallization trials. Immediately, Edwards set up the experiment, yielding 3D crystals in short order, and, by 1993, the first dif-

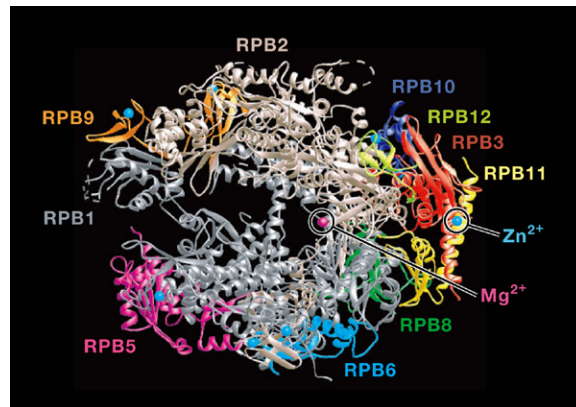


Figure 2. The 2.8 Å Resolution Crystal Structure of Yeast RNA Polymerase II Lacking Subunits RPB4 and RPB7

The bridge helix extends across the main cleft in this view, which shows the upstream face of RNA polymerase II. The mobile clamp domain is formed by the segments of RPB1 in the lower left and a small portion of RPB2. Some of the loops that extend into the cleft or the channels of the enzyme are visible. A Mg^{2+} ion (magenta sphere) marks the position of the catalytic center. Reprinted with permission from Cramer et al., 2001, copyright 2006 AAAS.

fraction patterns were in hand. Then came the tedious effort of producing isomorphous crystals, improving the diffraction, and obtaining heavy-metal derivatives for phasing of the diffraction pattern. Again, success relied both on making connections to advances by other researchers and on the group's persistence and experimental rigor. The construction of high-intensity X-ray sources from synchrotron beamlines (especially at the Stanford Linear Accelerator, where the group had ready access) as well as the development of improved computational methods for crystallography, made the work possible. The size of RNA polymerase II meant that conventional heavy metals were too small for phasing; success came from systematic screening of various heavy metal clusters (e. g., W_{10}) to find those that would bind appropriately. Jianhua Fu and David Bushnell were the unsung heroes in this story for their persistence in finding derivatives that allowed solution of the structure (Fu et al., 1999).

Just as Kornberg's work on RNA polymerase II was coming to fruition, Seth Darst, who had moved to Rockefeller in 1993, obtained a 3.4 Å resolution crystal structure of bacterial RNA polymerase from *Thermus aquaticus* (Zhang et al., 1999). Darst's

breakthrough work gave the first high-resolution view of RNA polymerase, revealing the remarkable complexity with which the two large subunits formed a nucleic-acid binding cleft. Simultaneous publication of a 5 Å resolution structure of yeast RNA polymerase II (Fu et al., 1999) showed the remarkable similarity of the two enzymes. Joined by Averell Gnatt and later by Patrick Cramer, Kornberg's team finished the high-resolution structure, obtaining not only a 2.8 Å resolution structure of the ten-subunit yeast RNA polymerase II lacking RPB4/7 (Figure 2) but also a 3.4 Å resolution structure of its transcrib-

ing complex showing the locations of RNA and DNA in the active site and the nine-base pair RNA-DNA hybrid in the main channel (Cramer et al., 2001; Gnatt et al., 2001).

Lessons from the RNA Polymerase Crystal Structure

Entry into the 21st century proved to be a watershed in the hunt for the structural basis of transcription. New insights into the molecular basis of transcription using structural analysis of RNA polymerase and its complexes prompted other research groups to join the effort, and many new structures appeared (Davis et al., 2002; Murakami et al., 2002; Vassylyev et al., 2002; Kettenberger et al., 2003; Bushnell et al., 2004; Westover et al., 2004; Tuske et al., 2005). Space and citation limits preclude a complete accounting, but these structures included views of RNA polymerase at increased resolution, with bound NTP substrates, in complex with inhibitors and in complex with basal initiation factors, including σ factor from bacteria and Mediator, TFIIB, TFIIS, and TFIIF from yeast. Among these, several key structures have been produced by the Kornberg group and its alumni, including a key snapshot of the catalytic center poised for catalysis, which appeared in the December

1 issue of *Cell* (Wang et al., 2006). Thus, the work recognized by the Nobel prize continues today. These structures have redefined the field of transcription, moving it away from “factorology” and toward a molecular description of mechanism. Coupled with structure-based biochemical experiments, these structures are revealing the unanticipated complexities of RNA polymerase dynamics and the remarkable mechanistic similarities of bacterial and eukaryotic transcription complexes.

From the pathway opened by Kornberg’s research we have learnt much, including the fact that RNA polymerase has several moving parts near the active site (the bridge helix, trigger loop, and various loops) that are crucial for NTP loading, catalysis, and translocation. However, learning how these movements are choreographed to accomplish the nucleotide-addition cycle remains an unmet challenge for investigators. RNA polymerase also contains mobile domains (the clamp, flap, Zn²⁺ binding domain, and jaw) and features (the rudder, lid, zipper, and other loops that protrude into the DNA and RNA binding channels). These domains and features must move, for instance to allow binding and release of regulators like bacterial σ factors and eukaryotic TFIIB and to transmit regulatory signals. Future research will focus on how these movements occur, how they are influenced by regulators and signals encoded in the DNA and nascent RNA, and how they influence the catalytic center of RNA polymerase. Finally, the structures illustrate a remarkable commonality among evolution’s diverse manifestations. RNA polymerase is essentially the same enzyme in all organisms. At least some regulators, like bacterial σ factors and eukaryotic TFIIB and TFIIF, turn out to have strikingly similar interactions with RNA polymerase. The key features of RNA polymerase, created by the two large subunits, are almost superimposable in the struc-

tures of bacterial RNA polymerase and yeast RNA polymerase II. The structures diverge with direct proportionality to distance from the catalytic center, beautifully reflecting the evolutionary divergence of transcriptional regulation.

In addition to honoring his central role in delivering new insights into the molecular basis of eukaryotic transcription, the awarding of this year’s Nobel prize in Chemistry to Roger Kornberg serves to punctuate a transition in scientific approach that will now focus on elucidating the molecular mechanisms of transcription in atomic detail. Perhaps equally important, it offers powerful lessons about the pursuit of scientific knowledge. Kornberg’s exceptional scientific talent certainly underpins his Nobel-winning research. However, success came not from a brief flash of brilliance or a single stroke of luck but from the deeply thoughtful, sustained, and careful practice of experimental strategy over many years. Selection of the model microbe *S. cerevisiae* as the experimental system was crucial, as it afforded invaluable connections to genetic analysis as well as the ability to build on knowledge and time-tested paradigms derived in studies of bacterial transcription. Sustaining the work toward an RNA polymerase structure over two decades, through periods of frustration as well as success, required the foresight of NIH study sections and program officers to focus on the open-ended goals of basic research rather than on the number of high-visibility papers produced over short time periods or on whether the preliminary results made the work a sure bet. Most of all, success came from scientific teamwork, both among diverse and strong-minded colleagues who cooperated while also competing, and among a group of brilliantly led young researchers whose diligence, intellect, and hard work ultimately brought home the prize.

ACKNOWLEDGMENTS

I thank Roger Kornberg for enjoyable discussion of the hunt for the structure of RNA polymerase.

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