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

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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 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).

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.

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 RPB 6 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 machinery 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