The Decade-long Pursuit of a Reconstituted Yeast Transcription System: the Work of Roger D. Kornberg

Reconstitution of Transcription with Five Purified Initiation Factors and RNA Polymerase II from *Saccharomyces cerevisiae*


Purification and Properties of *Saccharomyces cerevisiae* RNA Polymerase II General Initiation Factor a


Roger David Kornberg was born in St. Louis, Missouri in 1947. He was the first of three children born to Nobel laureate Arthur Kornberg and his wife Sylvy, both of whom were biochemists. “Both my parents had fine scientific minds and taught by example how to approach questions and problems in a logical, dispassionate way,” Kornberg once said. “Science was a part of dinner conversation and an activity in the afternoons and on weekends. Scientific reasoning became second nature. Above all, the joy of science became evident to my brothers and me.”

Kornberg was able to engage his scientific interests at an early age when, as a high school student, he worked in the laboratory of Nobel laureate Paul Berg, a colleague of his father’s at Stanford University. Kornberg published his first research paper in 1965 (1). His coauthors were, among others, his father, Berg, and H. Gobind Khorana, all three of whom have authored previous *Journal of Biological Chemistry* (JBC) Classics (2–4).

Kornberg earned his bachelor’s degree in chemistry from Harvard University in 1967 and began graduate study in chemical physics with Harden McConnell at Stanford. For his thesis research he looked at the rotation of lipid molecules in the membrane, which led to his discovery, by nuclear and paramagnetic resonance methods, of phospholipid flip-flop and lateral diffusion (5, 6). After graduating in 1972, he decided he wanted to learn about x-ray diffraction and chose to do a postdoctoral fellowship at the Laboratory of Molecular Biology in Cambridge, England, where protein crystallography was developed. Working with Nobel laureate Aaron Klug, Kornberg studied chromatin structure and proposed that the basal unit of chromatin, the nucleosome, is made of 200 base pairs of DNA and 8 histones (7).

Kornberg returned to the United States in 1976 to become an assistant professor of biological chemistry at Harvard Medical School. Two years later, he joined the Department of Structural Biology at Stanford Medical School, where he has remained for his entire scientific
career. He served as department chair from 1984 to 1992 and is currently the Mrs. George A. Winzer Professor in Medicine at Stanford University Medical School.

Kornberg began his independent research career by continuing his studies on nucleosomes. He found that RNA polymerases are capable of reading right through a nucleosome, which eventually led to the discovery that nucleosomes play a regulatory role in transcription. This led to Kornberg’s studies on transcriptional regulation in yeast. His first priority in this project was reconstituting the system, which was problematic, since the starting transcription signal was a thousand-fold less than that in mammalian systems. Kornberg and his colleagues were able to fractionate a yeast nuclear extract and resolve five yeast RNA polymerase II initiation factors that he designated a, b, c, d, and e (8). Factor d was identified as yeast TFIID, and factor b was found to be a multisubunit protein kinase that phosphorylates the repetitive carboxyl-terminal domain in the largest subunit of RNA polymerase II (9). However, isolation of the remaining factors was hampered by the loss of essential components and an accumulation of inhibitors. Fortunately, Kornberg’s postdoctoral fellow, Michael H. Sayre, devised a new fractionation procedure that yielded, from whole cell extract, a fraction enriched for RNA polymerase II and all factors except TFIID. The scheme was refined and extended and published in three back-to-back papers in the JBC, two of which are reprinted here as JBC Classics.

In the first paper, Kornberg, Sayre, and Herbert Tschochner present the fractionation scheme and show that it can be used to isolate initiation factors a, b, and e, as well as a fifth essential factor, designated g, which co-purifies with factor b through several chromatographic steps. These factors were found to be sufficient, when combined with bacterially expressed yeast transcription factor TFIID, to enable RNA polymerase II to initiate transcription. In the second Classic paper, the researchers describe factor a and discuss its possible relationship to mammalian initiation factors. The purification and polymerase-binding activity of factor g was described in the final JBC paper (10).

These papers represent the culmination of Kornberg’s decade-long pursuit of a reconstituted RNA polymerase II transcription system from yeast. They show that the yeast system is virtually identical to that from mammalian cells, dispelling a long held notion to the contrary and setting the stage for combined genetic and structural studies possible only with proteins from yeast. Using this system, Kornberg was able to discover a multiprotein complex called Mediator, composed of about 200 different proteins, that transfers signals from transcription factors to RNA polymerase II and other transcription factors (11).

In parallel with his biochemical studies of transcription, Kornberg pursued the structure of the transcription machinery. He began with pol II, the core of the transcription complex that consists of a dozen proteins. To crystallize this massive complex, he took advantage of the expertise with lipid membranes he gained from his graduate studies to devise a technique for the formation of two-dimensional protein crystals on lipid bilayers. He used the two-dimensional crystals to seed the formation of three-dimensional crystals for x-ray analysis. Nine years after growing these first two-dimensional crystals, Kornberg published a 3 Å resolution backbone model of a 10-subunit yeast RNA polymerase II (12). He has recently extended these studies to obtain structural images of RNA polymerase associated with accessory proteins (13).

As a result of this work, Kornberg was awarded the 2006 Nobel Prize in Chemistry “for his studies of the molecular basis of eukaryotic transcription.” He has also received many additional awards, including the 1981 Eli Lilly Award, the 1982 Passano Award, the 1990 Ciba-Cardio Award, the 2000 Gairdner Foundation International Award, the 2001 Welch Award in Chemistry, the 2002 ASBMB-Merck Award, the 2002 Pasarow Award in Cancer Research, the 2002 Le Grand Prix Charles-Leopold Mayer, the 2003 Massey Prize, and the 2005 General Motors Cancer Research Foundation’s Alfred P. Sloan Jr. Prize. He is also a member of the National Academy of Sciences (1993) and a fellow of the American Academy of Arts and Sciences (1999).1

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REFERENCES


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1 Biographical information for this article was taken from Ref. 14.


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