The Enzymology of Transcriptional Regulation: the Work of E. Peter Geiduschek

Construction and Properties of a Cell-free System for Bacteriophage T4 Late RNA Synthesis

Transcription at Bacteriophage T4 Variant Late Promoters. An Application of a Newly Devised Promoter-mapping Method Involving RNA Chain Retraction

E. Peter Geiduschek was born in Vienna, Austria in 1928. He attended Columbia University and graduated with a degree in chemistry in 1945, after which he enrolled at Harvard University, intending to study physical chemistry. However, instead he joined Paul Doty's biophysical chemistry laboratory where he studied the interactions between macromolecules. Geiduschek earned his doctoral degree in 1952 and then left Cambridge for New Haven, where he had been offered an instructorship in chemistry at Yale University.

At the end of his first year of teaching, Geiduschek began two years of military service, during which he was posted to the biochemistry department at the Walter Reed Hospital in Washington, D.C. He returned to Yale and later did a brief stint at the University of Michigan. In 1959, he moved again, this time to Chicago to accept a position in the Committee on Biophysics at the University of Chicago, where he was first introduced to enzymology and phage. In 1970, Geiduschek joined the biology department at the University of California, San Diego, where he has remained since.

Geiduschek's research has dealt primarily with the enzymology of transcriptional regulation in phage-infected bacteria, eukaryotes, and archaea. One topic he has researched extensively is the regulation of the late genes of bacteriophage T4. The story of how Geiduschek came to that subject can be found in his Journal of Biological Chemistry (JBC) Reflections (1).

By 1978, when Geiduschek's first JBC Classic was published, it was known that after infecting Escherichia coli, the T4 bacteriophage made different proteins at different times of the viral infection cycle. The genes for these proteins were referred to as early, middle, and late genes. It also was known that the late genes required T4 DNA replication for their transcription and that the RNA polymerase that functioned in T4-infected cells during the late period of infection was an extensively modified host enzyme, containing ADP-ribosylation and T4-specific subunits gp33 and gp55.

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Geiduschek had begun attempting to develop an in vitro system to mimic T4 late transcription in 1965, but it wasn’t until Dietmar Rabussay arrived in his lab that they were able to create a working system. Rabussay and Geiduschek developed a cell-free system based on the “cellophane disc” technique devised by Schaller et al. (2), who lysed bacteria on permeable cellophane discs in a way that minimally disrupted the mechanically fragile DNA. Schaller was able to retain high concentrations of cellular components and still allow diffusion of small molecule components, including the nucleotide substrates of DNA replication and transcription, into and out of the lysate.

Rabussay used Schaller’s technique but inhibited the lysate’s endogenous transcription using the antibiotic streptolydigin and then added streptolydigin-resistant RNA polymerase (RNAP). Using cell disc lysates from E. coli infected with gene 33 and gene 55 phage, he analyzed the in vitro synthesized RNA by hybridization-competition. Rabussay was able to show that RNAP purified from wild type T4-infected cells generated a high proportion of T4 late RNA whereas RNAP from uninfected bacteria generated essentially none (3). In 1978, Rabussay and Geiduschek submitted their Classic paper to the JBC, describing the properties of this in vitro system in detail, specifying its requirements, operating parameters, optimization, and pitfalls.

The second Classic reprinted here is the result of Geiduschek’s search for a single T4 late promoter. In the mid-1980s, it was known that T4 late promoters contained a conserved sequence that extended over about 18 base pairs and that the central 8-base pair sequence (TATAAATA in the non-transcribed strand) was absolutely conserved. The Geiduschek lab’s work with the phage SPO1 and the chromatin-binding protein TFI (a diverged and virus-specific member of the HU/IHF family of bacterial chromatin proteins) had led them to a DNA segment that encompassed 6 variant T4 late promoters with variations in the downstream start site sequence. Geiduschek and his colleagues took advantage of this DNA to recognition of variant T4 late promoters: They used DNA sequence-dependent RNA chain initiation with dinucleotides as well as the DNA sequence-dependent characteristics of RNA chain-terminating nucleotide analogs (3’-O-methylribonucleoside triphosphates) and pyrophosphate-mediated RNA chain retraction to map transcriptional initiation to the precise base pair. They then used gel exclusion chromatography to separate the large transcript-elongating RNAP-DNA complexes from their small molecule substrates. As reported in the Classic, they discovered that not every base pair of the absolutely conserved 10 sequence of T4 late promoters is essential and that other sequences are significant for late promoter function in vitro.

Geiduschek has received numerous awards and honors for his research, including the Harvard University Paul Doty Lecture (1993), the Order of Merit of the Italian Republic (1997), the University of Geneva’s Jean Weigle Lecture (2001), and the Gregor J. Mendel Medal from the Academy of Sciences of the Czech Republic (2004). He also was elected to the National Academy of Sciences and the American Academy of Arts & Sciences.1,2

Nicole Kresge, Robert D. Simoni, and Robert L. Hill

REFERENCES


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2 Biographical information on E. Peter Geiduschek was taken from Refs. 1 and 4.
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