

Classics

A PAPER IN A SERIES REPRINTED TO CELEBRATE THE CENTENARY OF THE JBC IN 2005

JBC Centennial 1905–2005

100 Years of Biochemistry and Molecular Biology

Discovery and Characterization of DNA Excision Repair Pathways: the Work of Philip Courtland Hanawalt

Expression of Wild-type p53 Is Required for Efficient Global Genomic Nucleotide Excision Repair in UV-irradiated Human Fibroblasts

(Ford, J. M., and Hanawalt, P. C. (1997) *J. Biol. Chem.* 272, 28073–28080)

Structural Characterization of RNA Polymerase II Complexes Arrested by a Cyclobutane Pyrimidine Dimer in the Transcribed Strand of Template DNA

(Tornaletti, S., Reines, D., and Hanawalt, P. C. (1999) *J. Biol. Chem.* 274, 24124–24130)

G4-forming Sequences in the Non-transcribed DNA Strand Pose Blocks to T7 RNA Polymerase and Mammalian RNA Polymerase II

(Tornaletti, S., Park-Snyder, S., and Hanawalt, P. C. (2008) *J. Biol. Chem.* 283, 12756–12762)



Philip C. Hanawalt

Philip Courtland Hanawalt was born in Akron, Ohio, in 1931 and grew up in Midland, Michigan. An early interest in electronics earned him an honorable mention in the 1949 Westinghouse Science Talent Search which, in turn, led to a scholarship to Deep Springs College. He transferred to Oberlin College and earned his bachelor's degree in 1954.

Hanawalt then entered the biophysics graduate program at Yale University. There he became interested in using short wavelength ultraviolet light to probe the workings of bacterial cells and joined the research group of Richard B. Setlow, an expert in UV spectroscopy and photochemistry. With Setlow, Hanawalt began to study how UV affects macromolecular synthesis in *Escherichia coli*, and he confirmed that low UV doses temporarily inhibit DNA synthesis in a dose-dependent manner (1). He also found that exposing UV-irradiated cells to visible light shortened the lag before replication resumed, suggesting a photoreactivating activity that removed blocks to replication (2). (At that time, the principal DNA photoproducts induced by UV exposure and their ability to be repaired were not known.)

In the course of his studies, Hanawalt became interested in thymineless death (TLD), a phenomenon discovered by *Journal of Biological Chemistry* (JBC) Classic author Seymour Cohen (3), who had isolated and characterized a thymine-requiring *E. coli* mutant. This led to Hanawalt's comparison of the effects of UV *versus* thymine deprivation on macromolecular syntheses. He earned his doctorate degree and went on to the University of Copenhagen in

1958 to do a postdoctoral fellowship with Ole Maaløe. Hanawalt brought some thymidylate synthetase mutants with him and continued his TLD research in Maaløe's laboratory. He found that the cells, which were additionally auxotrophic for amino acids and uracil, could complete their replication cycles in the absence of protein and RNA synthesis, making them resistant to TLD until the cycle was reinitiated (4).

Hanawalt returned to the U. S. in 1960 to do a second postdoctoral fellowship with Robert Sinsheimer at Caltech. There he enrolled in a course on UV photobiology taught by Max Delbruck that reignited his interest in how UV affects DNA replication. In 1961, Hanawalt joined the faculty of the Biophysics Laboratory at Stanford University and focused his research on how UV-induced pyrimidine dimers (CPDs) in parental DNA affect the behavior of replication forks.

In 1963, Hanawalt and his first graduate student, David Pettijohn, observed an unusual density distribution of newly synthesized DNA during labeling with 5-bromouracil in UV-irradiated *E. coli* (5). These studies, along with the discovery of CPD excision by the Setlow and Paul Howard-Flanders groups, represented the co-discovery of nucleotide excision repair (6, 7).

It is now known that there are two subpathways of nucleotide excision repair: global genomic repair (GGR), which deals with damage throughout the genome, and transcription-coupled repair (TCR), which targets DNA lesions that arrest the translocating RNA polymerase. Hanawalt and his colleagues discovered TCR in the mid-1980s (8).

James Ford, a postdoc in Hanawalt's group, discovered that Li-Fraumeni syndrome fibroblasts, homozygous for mutations in the p53 tumor suppressor gene, are deficient in GGR but proficient in TCR (9). In the first JBC Classic paper reprinted here, Ford and Hanawalt use monoclonal antibodies, specific for the respective UV-photoproducts, CPDs and the more distorting 6-4 pyrimidine-pyrimidone photoproducts, to show that CPD repair is primarily affected by p53 deficiency. Using human cells in which p53 expression could be tetracycline-regulated, they established that the wild-type p53 gene product is an important determinant of GGR but not TCR. In subsequent work in several laboratories it was shown that p53 regulates the DDB2 component of a DNA damage-binding protein complex required for efficient recognition of some types of lesions, such as CPDs, in chromatin.

The second JBC Classic paper details the characterization of RNA polymerase II (RNAPII) transcription complexes arrested at the site of a CPD (in the transcribed DNA strand) in a reconstituted mammalian RNAPII transcription system with initiation factors. Hanawalt and his colleagues mapped the polymerase footprint and showed that it extended about 10 nucleotides beyond the CPD and 25 nucleotides behind it, explaining why the lesion is inaccessible to GGR. They also found that the footprint at a natural pause site was similar to that at a CPD, raising the question of how the system can distinguish the two. A proof-of-principle experiment, relevant to the mechanism of TCR, showed that the polymerase (with its partially degraded RNA product) could regress from the lesion far enough to facilitate access and repair by the small DNA repair enzyme photolyase, permitting subsequent read-through at the site when transcription resumed.

Hanawalt continued to focus on the question of how the translocating RNA polymerase and its associated factors can distinguish between a *bona fide* lesion and a naturally occurring DNA sequence forming an unusual secondary structure that arrests transcription. In the third JBC Classic paper, he and his colleagues consider the behavior of RNAP in G4 quadruplex-forming sequences, which are found in telomeres and in the immunoglobulin switch regions. In previous analyses, the lesions that arrested transcription were always in the template DNA strand, but Hanawalt found that in this case, the arrest sites involved short runs of guanine nucleotides in the non-template strand. The structures are stabilized by hydrogen bonding within the quadruplex and stacking interactions between the planar G tetrads. Hanawalt speculated that the arrest of transcription in non-canonical DNA structures might initiate futile cycles of "gratuitous" TCR, which could be deleterious.

In 1965, Hanawalt joined the department of biological sciences at Stanford as an associate professor. He was promoted to professor in 1970 and now holds the Morris Hertzstein professorship in biology. He also completed two 3-year stints as department chair after which he "passed the baton" to JBC Deputy Editor Robert Simoni.

Hanawalt has received many honors and awards for his contributions to science including the Excellence in Teaching Award from Northern California Phi Beta Kappa (1991), the Environmental Mutagen Society's Annual Research Award (1992), the Peter and Helen Bing

Award for Distinguished Teaching (1992), the American Society for Photobiology Research Award (1996), the International Mutation Research Award (1997), and the John B. Little Award in Radiation Sciences from the Harvard School of Public Health (2002). He also is a member of the National Academy of Sciences and a Fellow of the American Academy of Arts and Sciences.¹

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

REFERENCES

1. Hanawalt, P. C., and Setlow, R. B. (1960) Effect of monochromatic UV on macromolecular synthesis in *E. coli*. *Biochim. Biophys. Acta* **41**, 283–294
2. Hanawalt, P. C., and Buehler, J. (1960) Photoreactivation of macromolecular synthesis in *E. coli*. *Biochim. Biophys. Acta* **37**, 141–143
3. JBC Classics: Flaks, J. G., and Cohen, S. S. (1959) *J. Biol. Chem.* **234**, 1501–1506; Barner, H. D., and Cohen, S. S. (1959) *J. Biol. Chem.* **234**, 2987–2991 (<http://www.jbc.org/cgi/content/full/282/39/e31>)
4. Maaløe, O., and Hanawalt, P. C. (1961) Thymine deficiency and the normal DNA replication cycle I. *J. Mol. Biol.* **3**, 144–155
5. Pettijohn, D., and Hanawalt, P. C. (1963) Deoxyribonucleic acid replication in bacteria following ultraviolet irradiation. *Biochim. Biophys. Acta* **72**, 127–129
6. Pettijohn, D., and Hanawalt, P. C. (1964) Evidence for repair-replication of ultraviolet damaged DNA in bacteria. *J. Mol. Biol.* **9**, 395–410
7. Hanawalt, P. C. (2004) Density matters: the semiconservative replication of DNA. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 17889–17894
8. Hanawalt, P. C., and Spivak, G. (2008) Transcription-coupled DNA repair: two decades of progress and surprises. *Nat. Rev. Mol. Cell Biol.* **9**, 958–970
9. Ford, J. M., and Hanawalt, P. C. (1995) Li-Fraumeni syndrome fibroblasts homozygous for p53 mutations are deficient in global DNA repair but exhibit normal transcription-coupled repair and enhanced UV resistance. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 8876–8880

DOI 10.1074/jbc.O110.000232

¹ We thank Philip C. Hanawalt for providing background information for this article.