

This Week's Citation Classic®

Riggs A D. X inactivation, differentiation, and DNA methylation.
Cytogenet. Cell Genet. 14:9-25, 1975.
[City of Hope National Medical Center, Duarte, CA]

This paper proposed the existence of a "maintenance" methylase that would postreplicationally form 5'-methylcytosine at symmetrical DNA sites. It was further proposed that this enzyme would prefer hemimethylated sites. With such an enzyme, methylation patterns would be somatically heritable and could be important for X-chromosome inactivation and cellular differentiation. [The *SCI*® indicates that this paper has been cited in over 225 publications, making it the most-cited paper published in this journal.]

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In the summer of 1969 I left the Salk Institute, where I had done postdoctoral work on the *Escherichia coli lac* repressor, and moved to a staff position at the City of Hope Medical Center. I intended to start work on X-chromosome inactivation, a gene-regulation phenomenon that occurs only in mammals. The City of Hope Medical Center was an appropriate place to begin such work because my department chairman was Susumu Ohno, who was well known as a discoverer of X-chromosome inactivation.

This phenomenon results in the coordinate genetic silencing of most of the thousands of genes located on one of the two X chromosomes in female cells. I thought it presented an important molecular puzzle, the solution of which would advance our understanding of gene regulation in higher organisms. I believe that I was correct in this thought, but I did not realize that it would be years before I could think of any molecular mechanism to even partly explain X-chromosome inactivation. Without a model to stimulate research on X inactivation I chose not to start such work, and instead I continued studying the interaction of the *lac* repressor with DNA. These studies resulted in learning that the 5'

position of a pyridine ring, which is exposed in the major groove of DNA, is very important for protein-DNA interactions. I also became familiar with bacterial restriction systems, some of which modify DNA by forming 5'-methylcytosine.

In 1973 I realized that one of the properties of the *EcoK* restriction enzyme, its preference for hemimethylated DNA, could provide a new type of cellular heredity and a mechanism helpful for explaining X-chromosome inactivation. I gave some "in-house" talks on the idea and was much encouraged by the enthusiastic responses of Ohno and Ernest Beutler, two experts on X inactivation. In early 1974 I began writing about the idea, mostly on Saturday afternoons in the California Institute of Technology library after playing basketball in the morning. There was no rush because I did not think that anyone else interested in X-chromosome inactivation would be thinking along these lines. When I finally did send the manuscript to one of the journals commonly read by molecular biologists, it was promptly rejected. Discouraged, I asked Ohno what I should do, and he suggested that I send it to *Cytogenetics and Cell Genetics*. I followed his advice, but not enthusiastically, because as a biochemist I never read this journal. I remember Ohno commenting that because of *Current Contents*® it didn't matter much which journal published the article; those interested would see the title and read the paper. As it turned out, he was right, but I certainly did not believe it when I was "scooped" by a paper by R. Holliday and J.E. Pugh,¹ who independently had the idea that methylation patterns could be somatically heritable and who suggested, among other things, that this was relevant to X inactivation.

With the passage of time it has become clear that both of our papers, which emphasized different aspects of DNA modification, have had considerable impact. My paper is cited for two reasons: first, because I suggested that DNA modification by enzymatic methylation was important for the X-inactivation process, and second, because I suggested a new, somatically heritable, information-coding system based on methylation patterns. By the time the field was reviewed in 1984,² enough evidence had accumulated for it to seem virtually certain that a DNA methylation system maintains X-chromosome inactivation and is one of the mechanisms used by mammalian cells for the somatically heritable silencing of many genes. I think my paper would have been cited even more often except that it was supplanted by a review I wrote with Aharon Razin,³ which has been cited nearly 700 times since 1980.

1. Holliday R & Pugh J E. DNA modification mechanisms and gene activity during development. *Science* 187:226-32, 1975. (Cited 355 times.)
2. Razin A, Cedar H & Riggs A D, eds. *DNA methylation: biochemistry and biological significance*. New York: Springer-Verlag, 1984. 392 p.
3. Razin A & Riggs A D. DNA methylation and gene function. *Science* 210:604-10, 1980. (Cited 685 times.)

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