



RESEARCH PROFILE

Bruce Stillman

Understanding the chromosome cycle

Bruce Stillman still speaks passionately about the subject that has been at the center of his working life for more than three decades. Ever since he began his doctoral studies, at age 22, he has been trying to learn more about how multiplying cells make precise copies of their genetic material.

"Just imagine," he says. "In almost every one of our cells — the approximately 100 trillion cells that make up the organs and tissues of the human body — we've got 23 pairs of chromosomes, one set from each parent.

Postdoc Anthony Mazurek and graduate student Marlies Rossmann in the lab with their mentor.

So each time a cell divides, six billion base-pairs of genetic information within the six feet of DNA in our chromosomes have to be copied, precisely. Try to imagine tiny machines copying this information, different parts of which accurately read and copy each bit of the double helix, and at the same time edit the copies for errors. It's an absolutely extraordinary process."

Stillman's career in research, which has continued without interruption throughout the years he has been Director and President of Cold Spring Harbor Laboratory, has been full of discoveries about a portion of the replication machinery. He is interested in the chromosome cycle, the series of exquisitely timed processes and mechanisms by which chromosomes are duplicated and then segregate themselves in cells that are preparing to divide.

The study of how chromosomes are copied is an excellent example of basic science — it is one of the predicates upon which advances in the treatment of serious disease is based. Cancer, for instance, is a disease of uncontrolled cellular proliferation; surely we must

understand the exact mechanisms used by cells to proliferate if we are to achieve better results in our efforts to fight cancer. Replication of the genetic material, and errors and defects that occasionally crop up in the process — some of which can set cells on a malignant course — are therefore of acute interest.

For 18 years Stillman has headed CSHL's National Cancer Institute-designated Cancer Center. Yet to regard him strictly as a cancer researcher would be to miss something important about both basic science and his career. For he did not set out at age 22 to understand cancer, but rather, the rudiments of the replication machinery that are active in every eukaryotic cell — the nucleated cells of higher organisms. "As I have counseled my students, it is important to work on problems that are fundamental in biology. But as I also like to say, there are lots of unintended consequences in science — you never know where the work is going to take you. And therein lies much of its value."

Captivated by tumor viruses

Stillman, who grew up in Melbourne, Australia, was an undergraduate at the University of Sydney in the period when recombinant DNA was developed. This was also when work on viruses that cause cancer was advancing. "One of my Sydney professors took a sabbatical year at Stanford and I received letters from him saying, 'You wouldn't believe what's going on here.'" Stillman was hooked. He applied and was accepted at the John Curtin School of Medical Research at Australian National University, where he devoted himself to the study of human adenovirus, one of a number of very simple viruses that causes cancer in animals and helped teach scientists some of the secrets of DNA replication.

Stillman's work fit right in at Cold Spring Harbor Laboratory, where he arrived in 1979 to do postdoctoral research. Two important figures in molecular biology, both British and both associated with Stillman's Australian graduate school, were affiliated with the Laboratory: former Director John Cairns, famous for his work on replication of the bacterial genome; and former CSHL Assistant Director Joseph Sambrook, a pioneer in the study of DNA tumor viruses. In the early '80s Stillman, by this time running his own lab, began studying the biochemistry of another very simple virus, a tumor virus called SV40. The SV40 genome was a wonderful target for research: a small, double-stranded circle of DNA, which became exposed once the virus penetrated the membrane of cells it attacked.

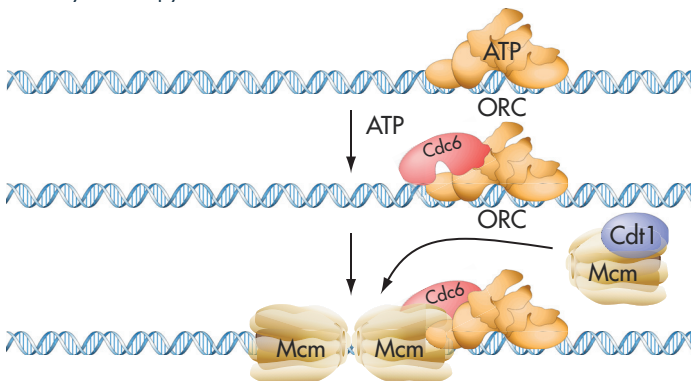
"An experiment done in 1978 here at CSHL by Bob Tijian [now president of the Howard Hughes Medical Institute] was very interesting to me," Stillman remembers. "He had purified a protein called T antigen that was encoded by the SV40 genome." When T antigen was injected into cultured human cells, the cells started duplicating their DNA. What particularly intrigued Stillman was the fact that T antigen was known to be an oncoprotein — a protein encoded by an oncogene, a cancer-promoting gene. The notion of a protein somehow initiating DNA replication would be something Stillman would study in great detail in the years to come.

Over the next decade, his lab and a few others in the U.S. used a technique called fractionation to pick apart the elements that enabled SV40 to replicate its tiny genome in host cells. This painstaking process involved isolating and then purifying a number of individual proteins. "Eventually we were able to reconstitute the



Replication's exquisite choreography

The attachment of ORC (origin recognition complex) at the replication start site is the first step in the assembly of a complex called the pre-RC (pre-replication complex). Stillman's team has been in on all of the major discoveries: the recruitment by ORC of two proteins, Cdc6 and Cdt1, which in turn load a third and structurally more elaborate protein, the Mcm complex, also known as helicase. Arrayed adjacent to ORC, these barrel-shaped enzymes are poised to unwind a segment of the double helix, thus creating a replication fork — the opening step of the DNA copying process. In every iteration of the cell cycle, pre-RCs assemble at every origin site on the genome. (This is called licensing.) Although many thousands "fire" during any given replication, not every pre-RC fires every time. Those that do fire must collectively make one and only one copy of chromosomal DNA.



replication of the entire SV40 genome in a test tube, using the proteins we had purified.”

The next logical step was to apply the same approach to replication of the genome in eukaryotic cells, cells that contain a nucleus full of DNA, such as our own cells. Stillman turned to yeast, a single-celled eukaryote, and succeeded in identifying many of the same proteins he had found in the SV40 replication system. All of the proteins except one turned out to be present in human

cells. The exception was the virus-encoded T antigen — the protein that had the very specific role of attaching itself to the double helix and starting the replication process in SV40-infected cells. Not only did it recognize the “origin of replication” in virus DNA; T antigen was also shown by Stillman’s group and several others to be central in the unwinding of the double helix — a necessary prelude to the bi-directional copying of each DNA “template” strand — and in the recruitment of

proteins to the replication start site. These proteins were the building blocks of the molecular machinery that actually synthesized new genetic material.

Stillman was convinced that there was something like T antigen in eukaryotic cells. This was controversial. “There was evidence that there were *no* specific start sites for copying DNA in eukaryotic cells, implying that they lacked a specific start protein. I never liked the idea. The problem was, if the process was random, how could you insure that the all the DNA in chromosomes was copied *once and only once* per cell cycle?”

This reduced to the question of how chromosomal replication was regulated in nucleated cells, which, unlike simple bacteria, had *multiple* replication start sites. (The yeast genome, it turned out, has about 300 origins — specific sequences in its DNA from which replication proceeds; in the human genome, we now know, there are more than 30,000 origins.) Stillman strongly believed that in the evolution of complex cells and organisms, very fine control mechanisms had to have emerged, in order to protect the integrity of the chromo-

somes as they were duplicated and segregated into “daughter” cells during the process of cell division.

ORC — a landmark discovery

Stillman was vindicated after years of hard work. In May 1992, he and then-postdoc Stephen Bell published a landmark paper in *Nature* in which they described their discovery of a multi-protein complex that recognized and bound to specific DNA sequences in yeast that were the start sites for copying DNA. They called this molecular machine ORC, for origin recognition complex. As subsequent work would show, just as ORC was the “initiation protein” for DNA replication in yeast, so its analogs would perform this crucial role in all other organisms, including humans [see box, p. 3].

ORC in humans, and indeed the entire process of DNA replication in human cells, is “much more complex” than in yeast, Stillman notes. Implicit in that statement are the gleanings of nearly 20 additional years of basic research, work that continues in Stillman’s lab to this day — and, much to his pride, in the labs of several of his former students and postdocs, notably Steve Bell, John Diffley and others. In 1992, they scarcely knew how pervasive a role ORC and related proteins would prove to have in the chromosome cycle, a fact with interesting evolutionary implications.

Take for example the questions of redundancy and timing. There are thousands of replication start sites along each linear human chromosome. Why so many? What determines when and how they fire? The great number of sites can be attributed to the need of replicating a large genome rapidly — within a defined period in the cell division cycle. As for the number of sites, “They tend to fire in little clusters, some earlier and some later during the DNA synthesis ‘S’-phase,” Stillman says. “That has got to do, in part, with the way the genetic material is packaged in the nucleus.” One unexpected finding was that ORC could participate in the organization of the structure of chromosomes and hence influence the expression of genes as well as when during S phase chromosomes are copied.

Much more recently, components of the ORC complex have been found by Stillman and colleagues to be involved in duplication of material that insures proper chromosome segregation during the part of the cell cycle called mitosis, when chromosomes segregate before a cell divides into two daughter cells, each

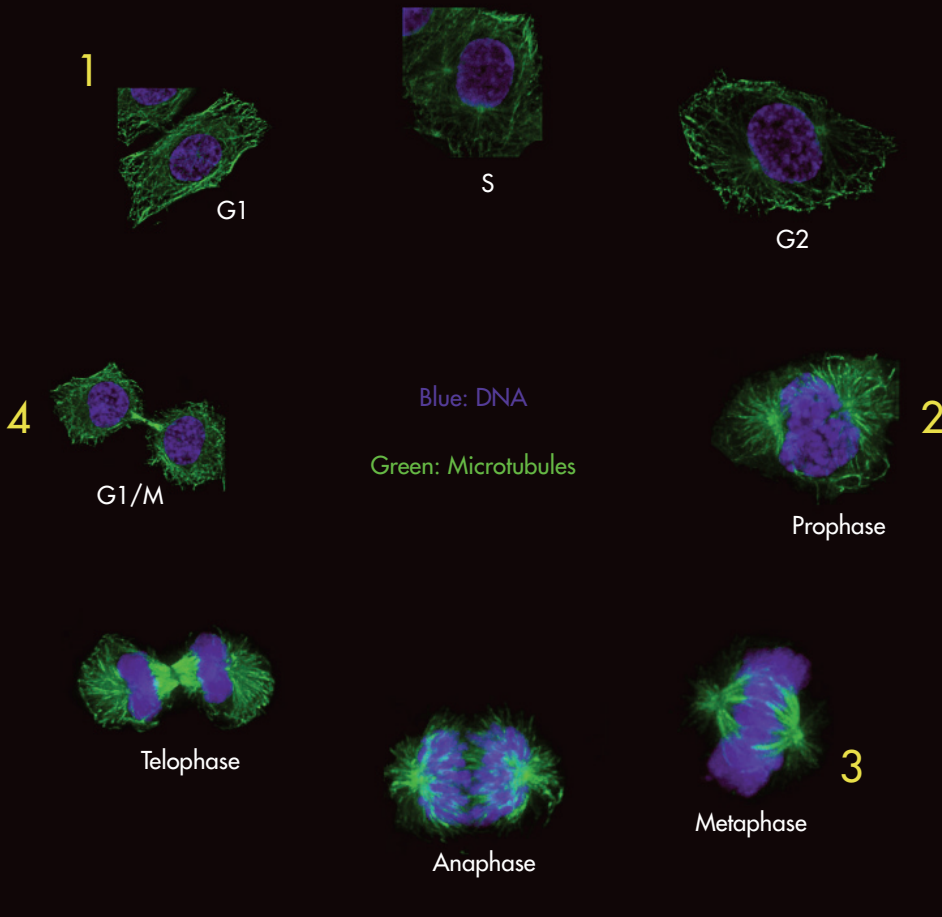
“It is in the very nature of science that the more we find out, the more we realize we don’t know. Therefore we continue to explore.”

Bruce Stillman, Ph.D.



ORC and the chromosome cycle

In cells of higher organisms ORC is a complex of six proteins whose diverse functions were not anticipated when ORC was discovered in yeast in 1992. In this series of images made by Stillman's team, we see cells at 4 distinct moments in one full cell cycle at which ORC plays roles related to chromosome replication and separation (the "chromosome cycle"). In the G1 (Gap1) phase of the cell cycle (1), ORC binds to sequence-specific origin sites across the genome and recruits other proteins to form the pre-replication complex [see box, p. 3]. After a given origin fires in S (synthesis) phase, various ORC subunits help regulate chromosome assembly and separation. In the M (mitosis) phase of the cell cycle: (2) ORC subunits localize to centrosomes, and the Orc1 subunit is involved in control of duplication of centrioles; (3) Orc2 and Orc3 subunits are required for stable attachment of the spindle, the structure that separates "sister" sets of chromosomes during cell division; (4) Orc6 is required for cytokinesis, the division of the cytoplasm into two daughter cells; it binds proteins essential to the process called septins.



containing one full set of chromosomes. "We published a paper in *Science* last year that shows how ORC controls duplication of the centrosome" — an organelle in cells that organizes the spindles that pull the duplicated chromosomes into two equal sets just prior to cell division; "and we have just submitted a paper showing that ORC is required for proper function of the centromere" — the region in each of the duplicated chromosomes that is tethered to the spindles so that they segregate correctly to the daughter cells.

Why would ORC be involved? "In evolutionary terms, my guess is that ORC is a chromosome-organization protein," Stillman says. "When the first chromosomes acquired the ability to control when they duplicate and how they segregate, the origins — the replication start sites — would logically be linked to sites on the genome where the chromosomes would be pulled apart."

In trying to sum up a long and productive career, Stillman is philosophical. "We certainly have a good sense, now, of how the genome is copied. When I first started out we didn't have a clue. But it is in the very nature of science, of course, that the more you find out, the more you realize you don't know. So the process continues, and we continue to explore." For example, many of the proteins his lab discovered are also involved in processes that repair DNA when it is damaged by chemicals or UV light, and some are involved in the signal processes that arrest cell proliferation if the genome is damaged, biochemical steps that are lost in all cancer cells.

Stillman's body of work is a superb example of the reason our society invests great sums each year in fundamental science. For as he points out, no one can know where the process leads — "and therein lies its unique value." **Peter Tarr**