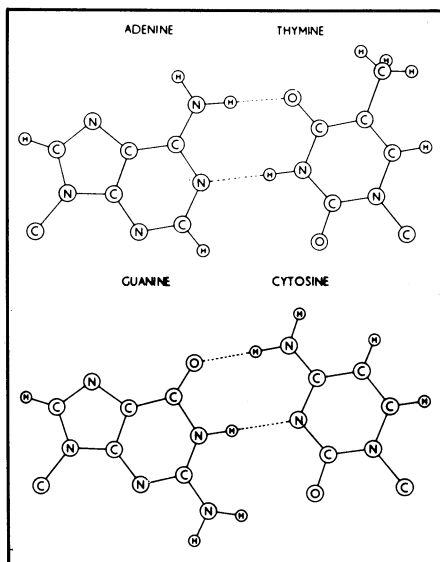
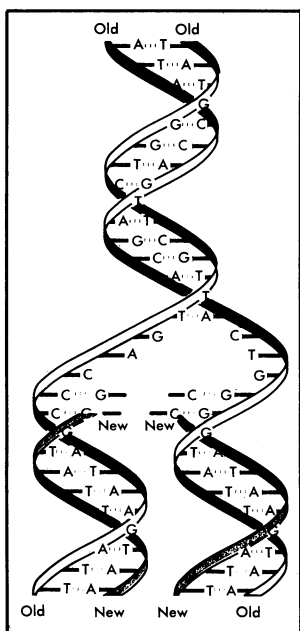


# DNA: ON TO THE LOOSE ENDS

*"We should like to propose that each of our complementary DNA chains serves as a template for the formation onto itself of a new companion chain."* James D. Watson and Francis Crick, 1953.



The double helix model of DNA suggested the mechanism by which it reproduces accurately. Hydrogen bonds (dotted lines) dictate that the nucleotides pair most stably in the combinations shown.

The DNA double-helix model wound up many biological problems, but after twenty-five years there are still unsolved strands

BY JULIE ANN MILLER

It's the silver jubilee for the DNA double helix, at least as a scientific model. Four hundred biologists flocked to the Cold Spring Harbor Laboratory on Long Island, N.Y., to celebrate by participating in this year's version of the symposium where the model was first announced. James D. Watson is now director of that laboratory, so Franklin W. Stahl of the University of Oregon was prompted to comment, "This 25th anniversary celebration is a well-deserved tribute, even if Jim [Watson] did have to throw the party himself."

The "party" was not typical congratulatory fluff. To absorb grueling sessions of detailed talks, scientists jammed the main lecture room (usually arriving an hour early) or stationed themselves before closed-circuit televisions in a huge tent. The talks focused on several areas including: new visions of the genetic material that are emerging mostly from DNA sequencing studies, the nitty-gritty of DNA reproduction in systems ranging from virus to human and high hopes for dissecting the steps involved in recombinations of genetic material.

To these scientists, DNA no longer appears to be a stiff, reticent structure. With recently developed techniques, they can examine its dynamics and delve into its contents.

## DNA dynamics and contents

The DNA double helix stretches, bends, shears and unwinds, says Henry M. Sobell of the University of Rochester in New York. He suggests that waves of distortion travel along the structure. Because their nucleotide compositions make some areas of the molecule more flexible than others, regions differ in ability to trap energy and convert it to structural change. Sobell suggests that "localized premelting" or partial unstacking of DNA components characterizes the regions where the DNA strands must be forced apart, for example to initiate DNA reproduction.

The most pervasive new approach is the simple determination of a DNA molecule's exact nucleotide sequences (SN: 3/5/77, p. 148; 4/2/77, p. 216). The DNA code for speci-

fying proteins is well known — a chart of 3-nucleotide words, each specifying an amino acid of the protein or a start or stop signal. But much of the chromosome is involved in regulatory activity: when DNA will replicate, how pieces of DNA move about and which of the genes will be active at any time. It is unclear what characteristics of the DNA sequence specify areas that interact with proteins to perform those controlling functions.

Again and again speakers at the symposium showed slides of DNA sequences, often blocks of hundreds of As, Cs, Ts and Gs, far too small for the audience to read. So little is known about the important signals in the sequence that the researchers



Watson and Dressler chat during a break.

seemed to hate to put aside any of their data.

One approach to the puzzle is to look for similarities in specific regions of different organisms. Perhaps such a series of Rosetta stones will provide the answer, although scientists now are not fluent in any of the languages.

The origin of DNA reproduction, the spot where a DNA molecule begins to reproduce, may be one Rosetta stone. At the meeting researchers reported and compared sequences of that region for viral, bacterial and mitochondrial DNA. Similarities across species might point out what DNA sequences have special meaning to proteins within a cell.

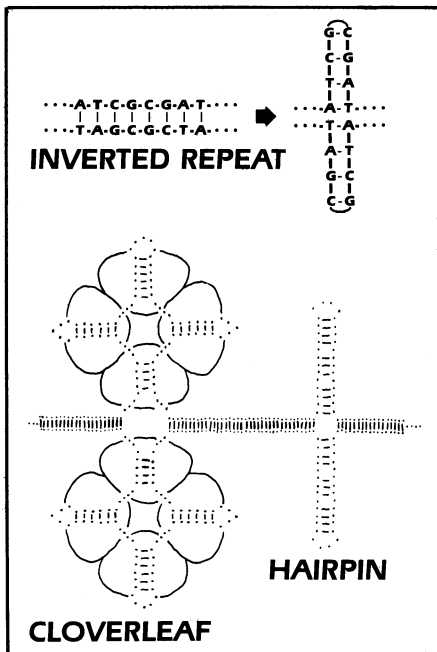
The origin of replication falls between genes on the chromosome in some cases,

in others it is within the DNA region coding for a specific protein. In the bacterial virus lambda, Mark Furth, William Dove and Frederick Blattner and co-workers at the University of Wisconsin find the origin sitting within the gene for a protein (O) required for lambda replication. Gerd Hobom of the University of Freiburg in West Germany argues that two DNA regions are necessary for originating replication in lambda. One is the O gene and the other a section of the neighboring gene cII, which produces a protein that blocks expression of the O gene.

"Meltability" is one feature researchers have examined in a variety of origins of replication. The double helix can most easily separate into single strands in areas where there are clusters of the nucleotides containing bases adenine and thymine and also where all the adenine- and guanine-containing nucleotides are on one strand and all the thymine and cytosine nucleotides are on the other. In a melted area, enzymes may be best able to approach a DNA strand and read the nucleotide sequence or the folding. At the origins of replication of lambda and of the plasmid colE1, the sequences suggest easy melting.

Inverted repeats, or palindromes, are another characteristic that researchers notice. A palindrome is a stretch of DNA where the nucleotides on one strand are the mirror image of nucleotides adjacent or nearby on the opposite strand. The reason for an interest in inverted repeats is that such a region may explain replication that goes in both directions, as seen in lambda, animal tumor virus SV40 and bacterium *Escherichia coli*.

In addition, such regions may offer a



Inverted repeats (palindromes) of DNA have an alternative to double-helical structure. Hobom proposes a cloverleaf where eight protein molecules can bind.



Gellert and Mizuuchi show how, with twisting, loops spring out of a double helix.

stable alternative to double-helical structure. The base sections of the nucleotides, instead of pairing across the helix with those of the other strand, might pair with bases on the same strand to form "hairpin" loops. In single-stranded DNA viruses, loops would be the only possibility for double-strand stability.

There is clear evidence for hairpin loops in the chromosomes of single-stranded DNA viruses, such as phiX174 and G4. Nigel Godson of Yale Medical School reports that G4 has two origins of replication. He finds three loops in the origin region where the virus begins forming double-stranded DNA from its own single strand. SV40 also has a clear inverted repeat at the origin.

A forward (non-inverted) repeat is another structural feature under consideration. Copies of a short sequence could allow proteins to bind sequentially and, perhaps, cooperatively. A shape more elaborate than a simple hairpin was suggested for the origin region of the lambda virus DNA. Hobom proposes a cruciform (or cloverleaf) shape, novel for DNA but similar to the structure of transfer RNA molecules, the go-betweens of nucleic acid instructions and a growing protein chain. Four sections of the DNA cross have similar sequences; thus they may be binding sites for a protein.

But do strands of DNA really leave their ladder-like configuration to pop out into hairpin loops or crosses? Martin Gellert and Kiyoshi Mizuuchi of the National Institutes of Health are using special DNA circles to examine the problem. By joining two identical strips of double-helix DNA head-to-head and tail-to-tail, they con-



Godson describes G4 virus replication.

structed a ring that is itself entirely a palindrome. If large areas of the molecule make hairpin loops, it would appear as a smaller circle with "rabbit ears" sticking out on opposite sides.

Using a plastic-tubing model of a circular double helix, Gellert and Mizuuchi demonstrate that they must put extra twists into the molecule to pull out hairpin loops. In the test tube they add gyrase, an enzyme that normally supercoils DNA helices (SN: 6/10/78, p. 372), to the suspension of circular palindromes. With an electron microscope Gellert and Mizuuchi observe "rabbit ears" sprouting from the circles. "It can work *in vitro*," Gellert says. The next question is whether such changes occur in living cells.

While no unifying structural feature has so far been discovered among the origins of replication, people are beginning to think about synergism, Dove explains. First, high meltability could allow regions to become single stranded, and then enzymes would have access to repeated stretches, even if the loops are less stable than the original double helix.

## DNA replication

Protein-nucleic acid interactions, whether or not they involve DNA hairpins and cloverleaves, dominate the complex process by which two identical DNA molecules are produced from one. Scientists at the meeting fluctuated at being impressed with the simplicity of the basic mechanism and with the complexity of some of its manifestations. In his summary of the meeting, Stahl reverently read the Watson-Crick text from the 1953 symposium (quoted above) and remarked, "Not only did they say it right, they damn near said it all."

Yet given that the basic idea dates from 1953, working out the mechanisms has taken longer than most biologists expected. Watson reminded the scientists of a "murky" meeting 10 years ago on DNA replication. "Now we almost know what's up," he says. Stahl added after the meeting, "Never before have I seen such an awesome display of virtuoso biochemistry."

Among the virtuosos at the meeting were Arthur Kornberg of Stanford University Medical School and Jerard Hurwitz of Albert Einstein College of Medicine in New York. Much of the biochemical dissection of DNA replication has been performed in their laboratories and in those of their former students.

Part of the delay in working out the mechanics was that the simple bacterium *E. coli* in this case, as in others, turned out not to be so simple. Kornberg now estimates that the products of 20 genes are involved in *E. coli*'s DNA replication. And although the Watson-Crick model made valuable predictions, it left some aspects of DNA replication unexplained. For instance, how replication begins and how new DNA strands are simultaneously formed on both of the parent strands. Harvard geneticist Matt Meselson once drew a modest diagram of a DNA molecule replicating, but with a fig leaf covering the junction where the two new helices fork from the old. Now, Kornberg says, the fig leaf has become, at least, translucent.

Although in the past heated debates have arisen between the laboratories, "more basic similarities are emerging," Kornberg says. The first step to understanding an operation, he says, is to know all its parts. His strategy is "divide and conquer." Kornberg's penchant for isolating enzymes from their surrounding contaminants is widely recognized. Generations of students have heard, "Don't waste clean thinking on dirty enzymes." In fact, one of Kornberg's own graduate students drives a car with California license plate "PURIFY." Kornberg himself claims that Watson said in a lecture that he couldn't think of anything clever to do with a new protein, so he purified it "like Arthur Kornberg."

But the purification strategy is not the only approach. Other researchers work with less pure enzymes in an environment

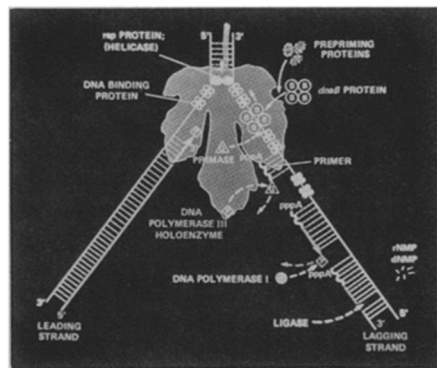
more like a cell's interior. Although that attack can become hindered by the action of irrelevant ingredients, it decreases the likelihood of washing away key components.

Results of both approaches now support a basic outline of how DNA replicates accurately and efficiently. Replication involves an array of enzymes. In analogy to the ribosome, the complicated structure that produces proteins, the DNA replication apparatus is called a "replisome."

What gets the protein show on the DNA road? About six proteins prepare the DNA helix for the start of replication. One pries the strands apart, another stabilizes the separated strands. Others guide the stabilizing protein to its site.

Most researchers now agree that replication involves a primer stretch, stitched together by an enzyme called primase. Many groups report that small strips of RNA are laid down first at the replication fork, then links of DNA start being added. Some groups instead find primers made of DNA or even, at least under test-tube conditions, of a mixture of RNA and DNA. Bruce Alberts of the University of California at San Francisco suggests that the initial segment of a nucleotide chain will contain more errors than later portions. Thus he sees an "erasable" primer as essential for accuracy.

The main action in replication is linkage of the nucleotides that line up along each single-strand template. Because DNA strands are not symmetric and are held together in opposite orientations in the



The fig leaf is translucent. A complex of processes make up DNA replication.

double helix, scientists originally expected to find two enzymes joining the nucleotides, one that could travel from a 5' to a 3' end and another that could work in the reverse direction. But nature apparently did not take that option.

Over the years three enzymes have been identified that link DNA nucleotides, but they all do it with the same directionality. Therefore one strand (called the leading strand) may be completed in an uninterrupted zip, but the other (lagging) strand must be filled in with short stretches of complementary DNA that are later joined by a patching enzyme called ligase.

The researchers at the meeting gen-

erally agreed that the lagging strand is synthesized in chunks, although they did not agree about the size of the pieces. However, Hurwitz and some others believe that even the leading strand is originally made in pieces that are later connected.

The most important DNA nucleotide-linking enzyme, polymerase III, is comprised of about seven different subunits and so is called a "holoenzyme." In future experiments the researchers expect to learn the function of the different subunits.

A different enzyme, now called polymerase I, was first thought to do the DNA nucleotide linking. Now researchers agree that its most important function is the reverse reaction. Traveling in the opposite direction from a polymerase, it snips out nucleotides that are not correctly matched to the opposite strand.

The successful dissection of the replication system, like the elaboration of metabolic pathways, has relied on mutant bacteria, especially those that can replicate at one temperature but not at another. Recently, simple bacterial viruses have also served as important probes. There are profound similarities, Kornberg says, among bacteria and the viruses that live in and emerge from them. Although each virus provides its own models of some replication enzymes to reproduce their small chromosomes, they all rely, to varying degrees, on the infected bacterium's machinery. For example, phiX174 requires the prepriming proteins, while G4 bypasses them. Primase recognizes a promoter region of the G4 chromosome.

Among higher organisms, the details of DNA replication still must be uncovered. Kornberg says there are many converts among biologists to the *in vitro* camp, where simple experiments are done with isolated elements. Then the researchers can return to the intact cell and challenge it with their findings. Kornberg predicts further work on higher organisms will provide more embellishments on the basic outline.

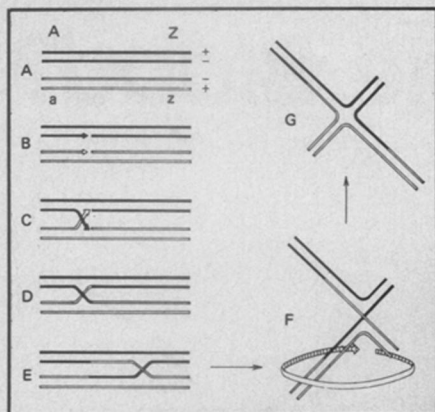
Stahl jokes that the details, in addition to being a tribute to virtuoso biochemistry, are also a tribute to the ingenuity of nature. "Not only has nature solved the problems posed by the Watson-Crick model and its proposed mechanism of replication, but she has done so in more ways than you can shake a stick at."

## Chromosomal recombination

The progress in replication reported at the symposium contrasted with continuing confusion over the mechanism of another DNA process. Recombination, also called crossing-over, is one means by which groups of genes on a chromosome can change their associations, and it is also the method by which plasmids and viral chromosomes slip into cellular DNA. In recombination, the double helices line

Continued on page 30

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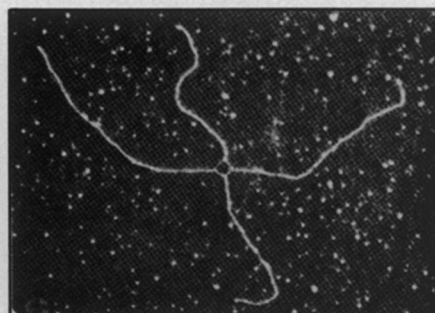
Potter and Dressler/PNAS

Break and exchange of DNA strands produces recombination in Holliday model.

up, homologous areas side-by-side. Each helix then breaks, they switch pieces and reseat. The Watson-Crick model does not apply as facily to recombination as it does to replication. Watson marvels, "I never thought anything as intelligent as DNA would want to be broken, let alone untwisted."

Stahl laments that replication is already "serious biochemistry" with clean substrates and clean enzymes, while recombination is still propelled by "colored chalk on smudgy blackboards." But he believes the processes are not unrelated. Replication can stimulate recombination and vice-versa. He suggests they may both require the same open conformation of DNA, as at the replication fork.

With their colored chalk and experiments on fungi, as well as on viruses and bacteria, the geneticists have made progress. By looking at distributions of traits after recombination is finished, they describe what recombination must do. Researchers at the meeting presented genetic studies whose complicated results are a challenge for any molecular model.



Potter and Dressler/PNAS

Electron microscope reveals Chi-shaped crossover intermediate, produced by snipping recombining DNA rings. The double helix is opened at the center to show the linkage.

One set of genetic studies asks the question: What cues the recombination machinery to the chromosome? Stahl and colleagues, including Dhruva K. Chatteraj and Daryl Faulds, have identified a genetic change that stimulates recombination. They call the recombination-promoting factor "Chi" in honor of the X-shaped crossover intermediate, first proposed by Robin Holliday of the National Institute for Medical Research in England.

Stahl and co-workers find hundreds of sites for Chi mutations along the long *E. coli* chromosome (approximately one every ten thousand base pairs). Similar mutations (called *cog*) have been detected by biologists working on fungi. The Chi mutations Stahl finds are required on only one chromosome to stimulate recombination of adjacent regions; the partner chromosome may even be missing the entire Chi site area. The site has been sequenced, but the researchers don't yet know what characteristic of the site attracts proteins involved in recombination. The recombination apparatus may be like gyrase or certain DNA cutting enzymes, Faulds explains, a protein that recognizes a specific site but acts a distance away from it.

Biochemical, as well as further genetic, analyses appear to be close at hand. Huntington Potter and David Dressler of Harvard University report a useful test-tube system for studying genetic recombination. They use extracts of bacteria as the source of enzymes; the substrates are rings of DNA with regions of homologous nucleotide sequences. With electron microscopy, Potter and Dressler observe conformations identical to crossover intermediates (such as the Chi shape) that have been isolated from intact cells. The researchers find that replication is not required for making recombination intermediates. "The ability to fuse plasmid chromosomes *in vitro* to give molecules joined by crossover-type connections should allow enzymological progress."

"Enzymology doesn't spring *de novo*," Dressler points out. The recent biochemical data on replication rest on years of molecular biological work characterizing the viral and bacterial chromosomes. Studies on recombination now seem to be at a similar jumping-off point.

"I suspect that this symposium will someday be recognized as the one at which essential *in vitro* analyses mark the end of recombination as the geneticists' playground," geneticist Stahl concludes. "I can already hear the biochemists circling in the night." □

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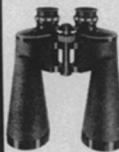


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