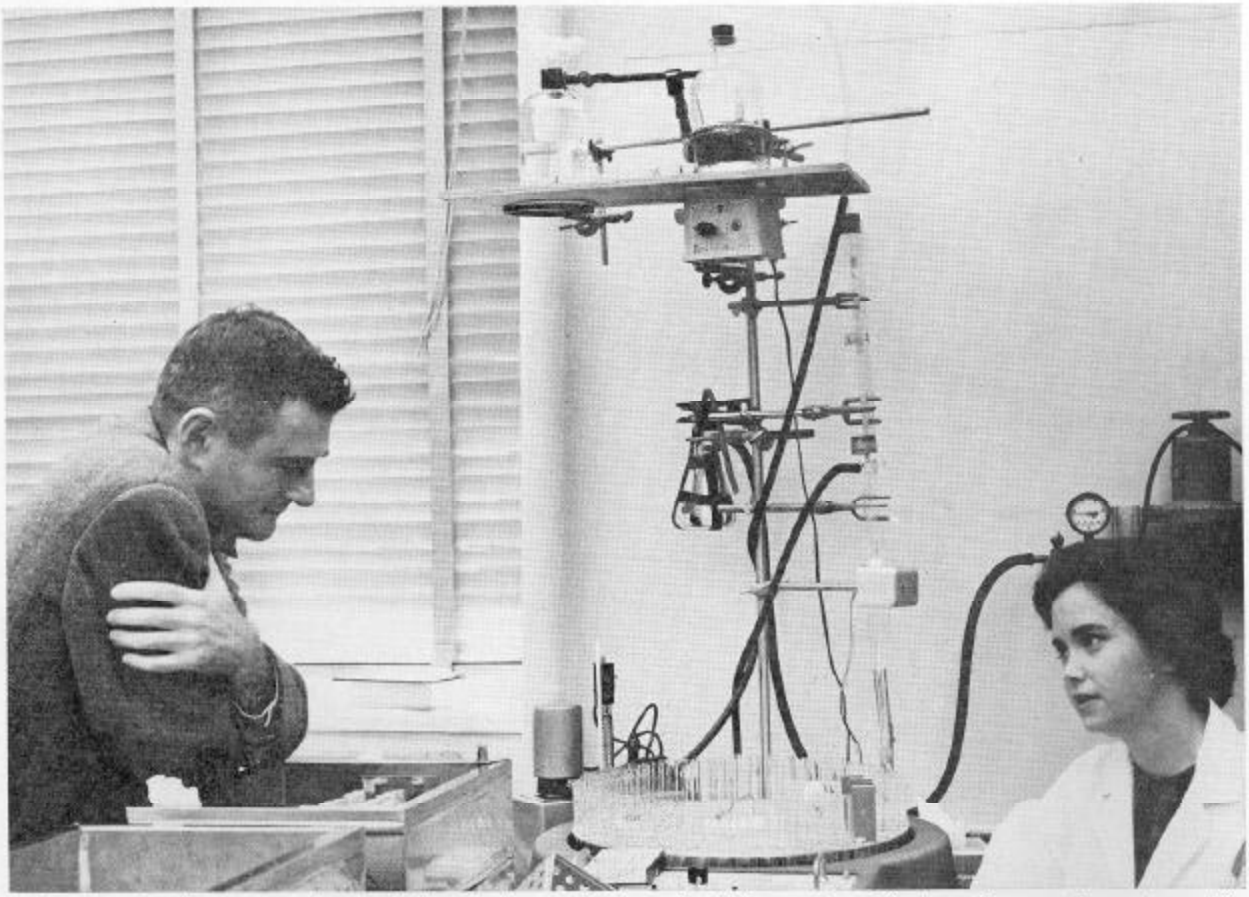


Robert L. Sinsheimer, professor of biophysics, and Research Fellow Alice Burton discuss chromatographic experiments in the laboratory where DNA is fractionated before ultracentrifugal analysis.

RINGS OF LIFE — AND DEATH

by Robert L. Sinsheimer

Recent discoveries about DNA structures could lead to further insight into reproduction of viruses.

In the past two decades biologists and biochemists have convincingly demonstrated that the genetic substance of all living creatures, from viruses to man, is nucleic acid—most usually deoxyribonucleic acid, or DNA. Molecules of DNA are long chains of thousands—or tens, or hundreds of thousands—of subunits called nucleotides. There are four principal types of nucleotides, and the sequence in which they occur in the chain is the basis of the genetic inheritance.

"Take a circle, caress it, and it will turn vicious."
—IONESCO, *The Bald Soprano*

Until very recently the attention of biochemists has been focused upon the interactions and sequences of these chains. Little attention has been given to their termini – to their beginnings and endings. While obviously of relative infrequence, these are unique points in each chain and it would seem plausible that Nature may have introduced some special features at such sites.

Within the last two years it has been shown, in considerable part at Caltech, that in several instances, and particularly in certain virus particles, DNA chains have no ends; they are rings.

The relatively short and defined lengths of the nucleic acids of virus particles afford a particular advantage to an investigator interested in the nature of nucleic acid endings. Hence it is not surprising that the first demonstration of a ring structure for a DNA was achieved with the smallest viral DNA known, that of the bacteriophage Phi X 174. This result was obtained at Caltech by Dr. Walter Fiers, a Rockefeller Fellow, and myself.

The DNA of this virus is a single chain of 5500 nucleotides with a molecular weight of 1.7×10^6. The proof that this chain is a closed ring rests upon the observation that the introduction of any single break into the chain does not change the molecular weight of the chain (while it does alter its configuration, as indicated by its properties when observed in the ultracentrifuge), whereas the introduction of any second break invariably produces a decrease in molecular weight.

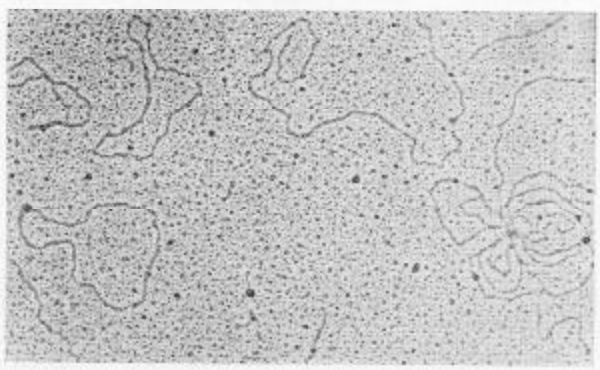
During the process of infection of bacterial cells by this virus, the single-stranded viral DNA is converted to the more familiar double-stranded DNA form. This was named a "replicative form" to indicate that this is the form the viral DNA assumes while it is replicating in a host cell.

This replicative form has very recently been isolated by Dr. Alice Burton, a research fellow in my laboratory. As a double-stranded molecule it is much stiffer and has a more extended configuration than does the viral DNA and it is much more suitable for direct observation in the electron microscope.

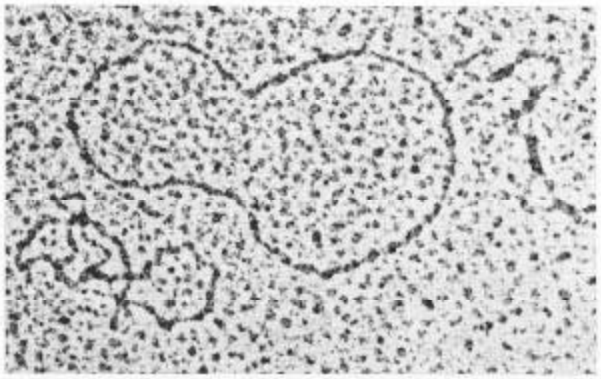
Electron micrographs of this replicative form of Phi X DNA have been taken in collaboration with Dr. A. Kleinschmidt, in the virus laboratory of the University of California at Berkeley, and one is shown below. Ring molecules, in single loops or frequently twisted into several loops, can be clearly seen, and contour lengths of these rings are readily measured. The mean contour length of 200 rings was 1.64 microns.

The detailed structure of double-stranded DNA has been deduced from X-ray diffraction analysis. In this instance, the structure leads to a calculated mass per unit length of 1.96×10^6 atomic weight units per micron. The mean molecular weight of these rings will then be 3.22×10^6, or 1.6×10^6 per strand. This conclusion is in splendid agreement with the much earlier measurement of the molecular weight of the single-strand DNA of the virus as 1.7×10^6. The visualization of these rings, of course, confirms the earlier analytical deduction of their form.

During the last year similar lines of evidence have led two groups at Caltech – Professor Renato Dulbecco and Senior Research Fellow Marguerite Vogt, in the biology division; and Research Associate Jerome Vinograd and Research Fellow Roger Weil, in chemistry – to the conclusion that the DNA of the tumorigenic virus, polyoma, is



Electron micrograph of the replicative form of Phi X DNA shows ring molecules in single loops, or frequently twisted into several loops.



Electron micrograph of the polyoma virus DNA clearly shows ring structure. The mean contour length of this ring DNA is almost identical to that of the replicative form of Phi X DNA.

also a double-stranded ring structure. An important facet of this proof was the analysis by Drs. Vinograd and Weil of the behavior of the polyoma DNA upon centrifugation in alkali. Under such conditions the hydrogen bonds linking the two strands into a stiff structure are destroyed. However, being entwined several hundred times, and lacking ends, the two strands cannot untwine and cannot separate. This situation results in a previously undescribed structure composed of two multiply-intertwined, but unlinked, polynucleotide strands with unusual centrifugal properties.

An electron micrograph of the polyoma virus DNA is shown above. By an extraordinary coincidence, the mean contour length of this ring DNA is 1.56 microns, almost identical to that of the Phi X replicative form DNA. It is remarkable that these two very distinct types of virus carry essentially identical amounts of genetic information.

It thus appears that, in these instances, the ends of the DNA chain are joined. In each case, however, there is more than a suggestion that there may be a special junction or coupling between the ends which differs from the usual internucleotide linkage along the chain. In Phi X DNA, one special enzyme-resistant linkage has been described in the ring. Polyoma DNA is found to be present to a small extent in an open-chain form.

Such a coupling may play a special role in the replication of these ring molecules. Ordinarily, during the duplication of DNA, the two chains of the double-strand molecule are untwisted and separated, one going to each daughter molecule. However, the two entwined chains of a ring DNA cannot be separated unless at least one is opened. The couplings may play an important part in such a step.

This recent work, then, has shown that DNA rings can and do exist and multiply. The detailed structure of these rings and the manner of their function and reproduction, remain to be elucidated. Such studies on the DNA from Phi X 174, and from polyoma virus, are continuing at Caltech.

Research Associate Jerome Vinograd uses the analytical ultracentrifuge to study buoyant density and sedimentation velocity of the poluoma DNA.