Philadelphia, September, 1964

When someone has something profound to say about the mechanism of cancer or its cure, it will not be reserved for the last paper of a National Cancer Conference. In this instance I have less to say about cancer than any of the preceding speakers in this 3-day conference. However I will say something about nucleic acids and ~~its~~ as Dr. Stanley has so already pointed out ~~cinch that~~ the cancer story will not be written without this character.

What we are is pretty much what our DNA designs or permits us to be. Yet it is astonishing and dismaying that the chemistry of nucleic acids is never considered in a general chemistry course in a University and hardly mentioned in organic chemistry courses, introductory or advanced. In fact nucleic acid chemistry is a poverty pocket. This is not the proper forum to make a special plea for a war on this appalachia of science. But a campaign to get nucleic acids into chemistry curricula is worth waging.

Native DNA, as you know, is long fibrous molecules composed of two strands hydrogen-bonded to one another and spirally wound about each other to form a double helix. Six years ago the molecular weight of E. coli DNA was 6 million; 2 strands each contained 10,000 nucleotides. Today we're being convinced that the molecular weight is 1000 times greater and that the entire bacterial chromosome is in fact an uninterrupted chain of 10 million nucleotides. To make matters worse the bacterial chromosome appears from electron micrographs and genetic evidence to be a circle rather than a rod segment.

How is a biochemist working on DNA to cope with this fantastic molecule with limited organic reagents and physical methods? He resorts 1) to the use of enzymes to modify and manipulate these macromolecules, 2) to the electron microscope to measure their size and conformation, and 3) to methods of virology and genetics to assay their function. I'd like now to review briefly some recent studies at Stanford that illustrate these points.

The basic theme of our work during the past 8 years has been the way in which the cell replicates its DNA. An enzyme we have purified from E. coli called DNA polymerase uses each strand of the DNA as a

Slide 1

template. The enzyme follows the simple base-pairing rules originally postulated by Watson and Crick. A new chain is assembled by putting an A in place wherever the template contains a T and by matching a T for an A in the template. Similarly G-C pairing dictates the assembly of a G for a C in the template, a C for a G in the template.

Our measurements indicate that the copy has correctly reproduced a complementary image of the template with an error level below the detection of our methods. In one instance we have used as template a simple DNA containing only adenine and thymine and looked to see whether guanine residues in an incubation mixture were incorporated into the enzymatically synthesized replica. We could detect none during an interval in which 500,000 adenine and thymine nucleotides were polymerized.

What we had not clarified was the physical structure of enzymatically synthesized DNA. In the past two years we have come to recognize that the enzymatic product resembled native DNA in most physical parameters but differed in two ways: 1) the product showed an unusual capacity to

resume a helical conformation after denaturing treatments and 2) it had a branched appearance in the electron microscope (2).

Finally, I'd like to present some recent unpublished experiments of Hans Strack and Dale Kaiser in which exonuclease III and polymerase are used on a phage DNA which has infectious activity. This DNA is obtained from the lysogenic phage λ and is capable of entering E. coli and becoming the source of new bacteriophages. The chromosome of this phage is a DNA molecule 15 microns long and at least a dozen distinct genes have been mapped by genetic recombinational tests. These genes or cistrons are arranged in a linear array throughout the expanse of the molecule. The interchangeable use of the words DNA molecule and λ chromosome is correct in a strict sense as a result of the shearing experiments carried out by Kaiser and by Hogness and their colleagues. Using Hershey's techniques, they have been able to shear the molecules in half. The molecules are sheared across to produce double helices of half the length and also half the molecular weight. The right half can be separated from the left half. A recent paper by Radding & Kaiser is devoted to the left half of the molecule. The two They have different base compositions and form separate bands in cesium chloride density gradients. What's more, the right half has genetic markers distinct from the left half. The distribution of genes on the DNA is at this point in the investigation entirely consistent with the recombinational mapping of whole phage chromosomes. The DNA half-molecules can be further cleaved into quarter molecules. A remarkable finding

is that the terminal quarters are infective but the inner quarters are not. There is something crucial about the terminal parts of the DNA molecule that is required for its function. It would seem that the capacity of the two ends to find each other and cohere is an essential feature of a biologically active or infective molecule; when the two ends of an intact DNA molecule adhere, a circle results.

Slide 14

Strack and Kaiser exposed λ DNA to the graded action of Exo III. Very early in the action of the enzyme all the genetic markers were lost. When ~~only~~ as few as 100 nucleotides were removed from each end, representing far less than 1% of the length of the molecules, internal as well as terminal genes were lost. The measurements are accurate down to 5 orders of magnitude. These molecules could be restored by incubating with polymerase and the 4 triphosphates required for DNA synthesis. The graded use of polymerase could restore genetic activity up to 20% of the original λ activity and included all markers.

Now something new and, at the same time, unexpected happened. If this polymerase action was permitted to continue, activity was again destroyed. ~~Or~~ starting with native λ DNA, polymerase action promptly destroyed genetic activity. Polymerase action always required the triphosphates and could be clearly correlated with its synthetic action rather than any adventitious degradative activity. As this model indicates, the cause of this loss may be the production of a fully matched double-stranded molecule which lacks the two free complementary end portions necessary for adhesion and circle formation.

Finally DNA molecules inactivated by polymerase action could be restored by a brief exposure to Exo III; a more extensive exposure led to inactivation as expected.

I'll conclude with a brief summary. A purified enzyme, polymerase, produces a DNA molecule in the test tube which is a faithful replica of any DNA molecule supplied to it as a template. This copy is correct, nucleotide by nucleotide; errors, if ever made, are rarer than one in 10^5. However the longer range ordering of the molecule can be confused, presumably because of the proximity of the portion of the complementary strand which is not being copied. The mechanism by which the replication process in the cell avoids this confusion still escapes us.

In the course of studies designed to understand the DNA replication process, highly specific nucleases have been sought and discovered which provide refined reagents for analyzing and manipulating DNA molecules. When applied to DNA molecules with genetic activity we gain new insights into the way in which DNA molecules exert their biological functions.

Slides

1. DNA replication scheme
2. Branched DNA -- EM
3. " " EM
4. Model of pleated DNA
5. Expt. with Neurospora nuclease
6. Exo III action
7. Exo III ⟶ Polymerase
8. EM -- native T7
9. EM -- Exo III
10. EM -- Required
11. EM -- Past repair
12. EM -- Length distributions
13. B. subtilis genetic activity
14. Strack-Kaiser expt.