[9] For example, Jordan, D. O., *The Chemistry of Nucleic Acids* (London: Butterworths, 1960), p. 171.

[10] Bissot, T. C., R. W. Parry, and D. H. Campbell, *J. Am. Chem. Soc.*, **79**, 796 (1957).

[11] Bendich, A., E. Borenfreund, G. C. Korngold, and M. Krim, *Federation Proc.*, **22**, 582 (1963).

[12] Hsu, T. C., and C. F. Somers, these PROCEEDINGS, **47**, 396 (1961); Somers, C. F., and T. C. Hsu, these PROCEEDINGS, **48**, 937 (1962).

[13] Oehlkers, F., *Z. Vererbungslehre*, **81**, 313 (1943); Oehlkers, F., *Heredity*, **6**, suppl., 95 (1952).

---

## *CIRCULAR T2 DNA MOLECULES*

BY C. A. THOMAS, JR., AND L. A. MACHATTIE

DEPARTMENT OF BIOPHYSICS, JOHNS HOPKINS UNIVERSITY

*Communicated by A. D. Hershey, September 30, 1964*

Each T2 bacteriophage particle yields a single *linear* duplex DNA molecule, an example of which may be seen in the electron micrograph (Fig. 2*B*). Although each molecule contains the same genetic message, the order of the nucleotides appears to be different from molecule to molecule. Renaturation experiments indicate that those sequences that are found near the ends of some molecules are found near the middles of others.[1] This is precisely what would be expected if each linear molecule had a nucleotide sequence which was a different circular permutation of a common basic sequence. (A collection of linear molecules with circularly permuted sequences can be generated by making a single random break in each of a collection of identical circular molecules.) Other bacteriophage DNA molecules such as T5 (ref. 1) and λ (ref. 2) are known to be "unique" in that most of them have the same nucleotide sequence. The unusual situation in regard to T2 or the related T4 is probably the physical basis for the circular genetic map observed in this phage.[3]

If these molecules are circular permutations of each other, and if they consist mainly of two continuous polynucleotide chains as shown previously,[4] then denaturation followed by reannealing as depicted in Figure 1 should lead to the formation of artificial circular molecules.

*Experiments.*—DNA molecules from T2 bacteriophage were extracted with phenol and purified by chromatography[5] as described previously.[4] These molecules were diluted tenfold into 0.20 *M* NaOH at a final concentration of 1.25 γ/ml. After one min, $^1/_{10}$ vol of 3.0 *M* NaCl, 0.30 *M* Na citrate, was added and the solution dialyzed for 10 hr against 0.30 *M* NaCl, 0.03 *M* Na citrate. This solution was then heated at 65°C for 40 min and cooled to 4°C. Visualization in the electron microscope was accomplished by the method of Kleinschmidt[6] as previously described.[7] The present procedure renders duplex DNA clearly visible, but single polynucleotide chains are not seen. An aliquot of the same solution receiving all treatments except that of denaturation by NaOH served as a control. In addition to the experiments done on T2 whole molecules, T2 half molecules (41% relative length or 54 million molecular weight), and T5 whole molecules were given the same experimental and control treatments.

*Results.*—Grids prepared from the solutions of unbroken T2 molecules that had been treated with NaOH followed by reannealing at 65° showed many closed circles,

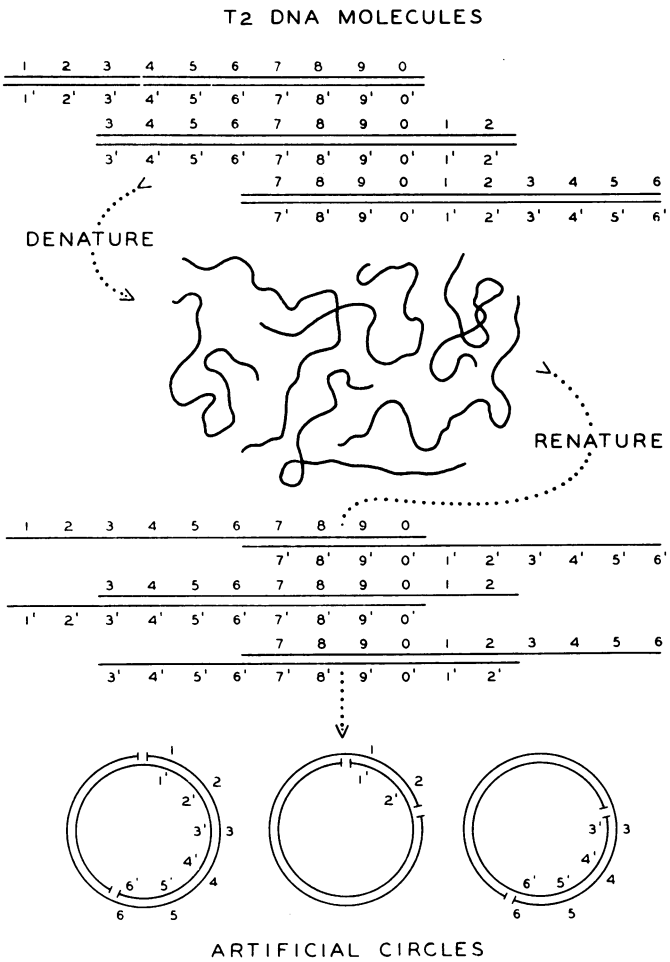



FIG. 1.—The diagram shows how a collection of linear duplex DNA molecules with sequences that are circular permutations of each other can produce circular molecules by chain separation followed by random association and reformation of the duplex structure. In principle nearly every final molecule could be circular.

while the grids made from the control solutions showed no circles, only linear molecules. Examples of the two types are shown in Figure 2. The measured contour lengths of many circular and linear molecules are compiled in Figure 3. All circles had nearly the same contour length (55 ± 3 $\mu$) as the unbroken linear control molecules (56 ± 2 $\mu$).[8] The linear molecules found in the circularized preparations had a variety of lengths as might be expected if these were overlapped structures (Fig. 1) which had not yet circularized.

The circles were abundant. In order to estimate their frequency, the entire areas of two (200 mesh) grid squares were photographed and inventoried. Of the 168 molecules seen, 25 ran into grid wires or were otherwise obscured. Of the remaining 143, 29 were circles, 4 of which must be classified as doubtful since it was impossible to verify strand continuity in these cases. The remaining 114 molecules were of various lengths with about half of them longer than 35 $\mu$.

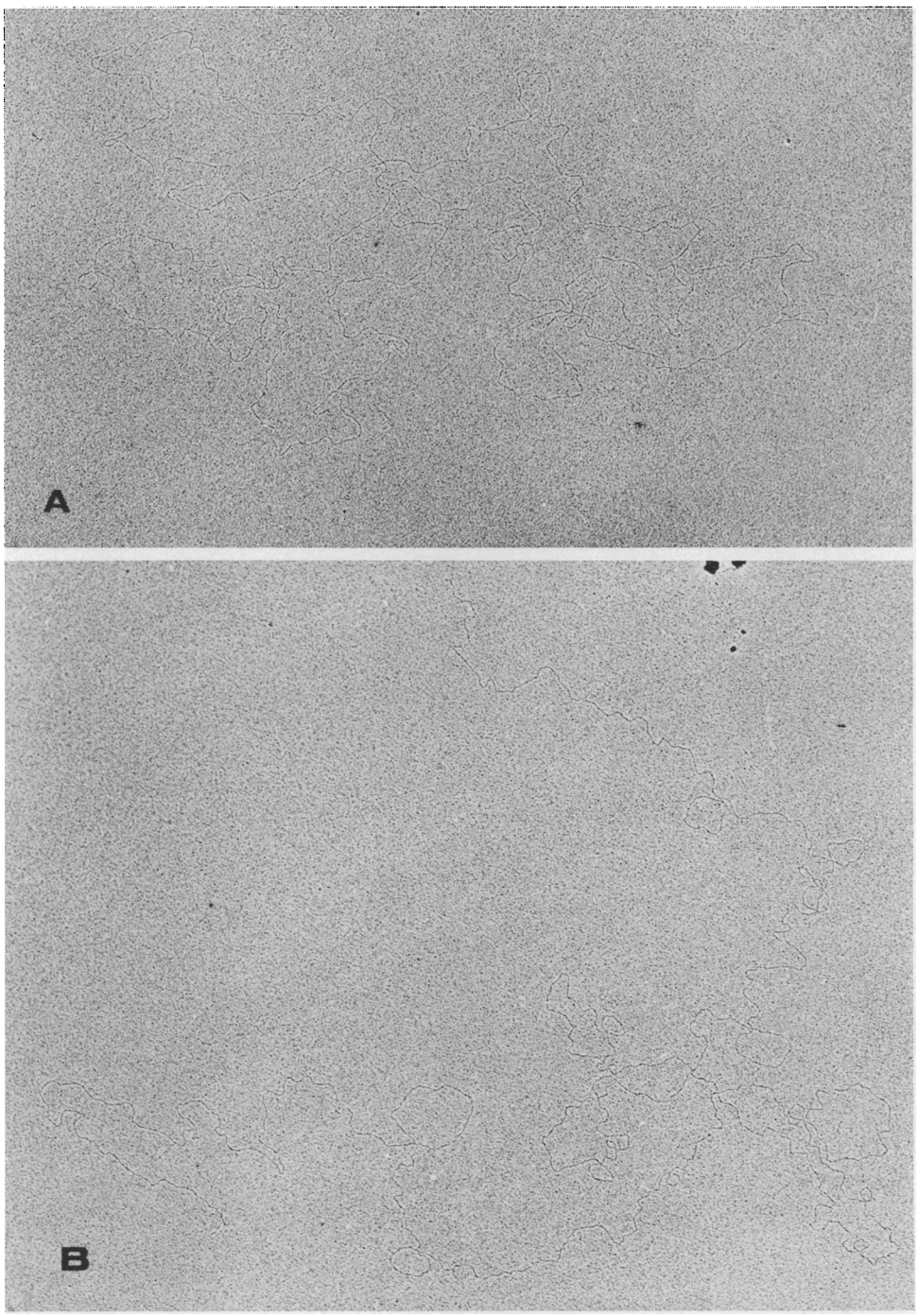



FIG. 2.—(*A*) A circular T2 DNA molecule from a solution that had been exposed to denaturation and renaturation conditions. (*B*) T2 DNA molecule from a solution that had been exposed to renaturation conditions only.

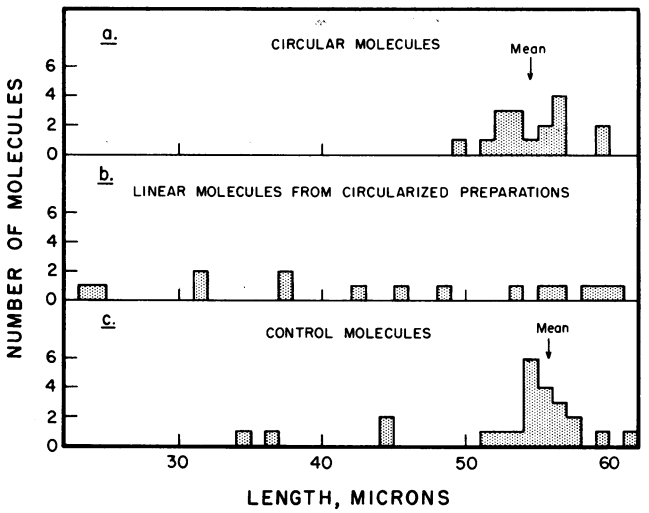



FIG. 3.—Histograms of measured contour lengths of duplex T2 DNA: (*a*) and (*b*), after denaturation and renaturation; (*c*) after renaturation treatment only.

We were interested to learn whether polynucleotide chains which were interrupted could form circles under these conditions. It is theoretically possible for four polynucleotide chains of less than full length to cooperate to form a circle of full contour length, albeit possibly with single-chain regions. The basic experiment with undenatured controls was performed with column-fractionated fragments of 41 per cent relative length, repeated again with a fourfold increased concentration of these fragments, and unbroken T2 molecules as a control. As expected, circles were found in the solutions which contained whole molecules, but no circles were found in the solutions containing 41 per cent fragments. We take this to mean that the polynucleotide chains must be continuous over the entire polynucleotide map in order for circle formation to be likely under these conditions.

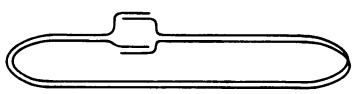
When T5 whole molecules are examined in the same way, no circles are found in spite of an exhaustive search. None are expected because T5 DNA molecules are thought to have identical sequences.[1]

*Discussion.*—The finding that T2 DNA molecules can be caused to assume the form of a closed loop or circle by exposure to conditions which cause the separation and subsequent reunion of polynucleotide chains is in exact accord with expectation, provided that the original linear T2 DNA molecules have nucleotide sequences which are different circular permutations of each other. The fact that the contour length of the circular molecules is almost the same as that of the undenatured linear molecules means that the cycle of permutation extends over the majority of the molecule. This point is further supported by the inability of fragments of half-size to form circles. This question was left unresolved by earlier studies.[1]

On the basis of genetic experiments[3] Streisinger has proposed that each T4 molecule, in addition to being a different circular permutation of a common sequence, posesses a terminal reduplication of part of the genetic message, and that this terminal redundancy is responsible for a certain group of heterozygous phage particles. Thus, the nucleotide sequences found at the left end of each molecule would be repeated at the right end of the same molecule. These duplicated se-

quences would be different in different molecules depending on the permutation they represent. If circle formation comes about by the scheme shown in Figure 1, these repeated sequences would not be formed into the circle, but be left as unpaired single polynucleotide chains attached to the circle at two points. Since the present procedure does not reveal single polynucleotide chains, one would not expect to see them. The contour length of the circle would correspond to only one complete genetic map; thus, the fact that the observed mean contour length of the circles (55 $\mu$) is shorter than that of the linear control molecules (56 $\mu$) may be significant.

It might be supposed that the circle formation could be the result of complementary pairing of the end redundancies to produce a molecule of the following form:



This appears unlikely to us for the following reasons: (1) it presumes that the original chains never completely separate before renaturation; (2) it supposes that these terminal polynucleotide chains find their complement at the opposite end of the molecule rather than reuniting with their original partners which are close by and already in register; and (3) this scheme predicts the formation of loops as shown above about 1 $\mu$ in length. No convincing example of such a loop exists in our collection of circular molecules.

*Summary.*—T2 DNA molecules can be caused to assume a circular form by denaturation with alkali followed by renaturation at neutral pH. This result confirms the hypothesis that native linear T2 DNA molecules have nucleotide sequences which are various circular permutations of a common sequence. When broken T2 DNA molecules or whole T5 DNA molecules are treated in the same way, no circles are found.

We are grateful to Messrs. John Abelson and Marcus Rhoades for chromatographed DNA samples. During this period one of us (L. A. M.) held a Damon Runyon Fellowship (#327). This work was supported by the National Science Foundation (G-10726) and the Atomic Energy Commission (AT(30-1)2119).

[1] Thomas, C. A., and I. Rubenstein, *Biophys. J.*, **4**, 93 (1964).

[2] Kaiser, A. D., and D. S. Hogness, *J. Mol. Biol.*, **2**, 392 (1960); Kaiser, A. D., *J. Mol. Biol.*, **4**, 275 (1962); Hogness, D. S., and J. R. Simmons, *J. Mol. Biol.*, in press.

[3] Streisinger, G., R. S. Edgar, and G. H. Denhardt, these PROCEEDINGS, **51**, 775 (1964).

[4] Thomas, C. A., T. C. Pinkerton, and I. Rubenstein, *Informational Macromolecules* (Academic Press, Inc., 1963), p. 89; Thomas, C. A., and T. C. Pinkerton, *J. Mol. Biol.*, **5**, 356 (1962); Berns, K. I., and C. A. Thomas, *J. Mol. Biol.*, **3**, 289 (1961).

[5] Mandell, J., and A. D. Hershey, *Anal. Biochem.*, **1**, 66 (1960).

[6] Kleinschmidt, A. K., and R. K. Zahn, *Z. Naturforsch.*, **14b**, 770 (1959); Kleinschmidt, A. K., D. Lang, D. Jacherts, and R. K. Zahn, *Biochim. Biophys. Acta*, **61**, 857 (1962).

[7] MacHattie, L. A., and C. A. Thomas, *Science*, **144**, 1142 (1964).

[8] While this is longer than the previously published value of 47 $\mu$, [6] it is still somewhat shorter than 63 $\mu$, the length predicted for a duplex in the B-form with a molecular weight of 133 million.[9]

[9] Rubenstein, I., C. A. Thomas, and A. D. Hershey, these PROCEEDINGS, **47**, 113 (1961).