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BIOPHYSICS

Proof that the Replication of DNA involves Separation of the Strands

IN their description of the specific pairing of the nucleotides of deoxyribonucleic acid (DNA), Watson and Crick[1] proposed that this pairing was the basis for a mechanism of duplication: if unwinding occurred at the time of replication, each strand of the double helix could act as a template for the creation of a new molecule so that each daughter molecule would be a 'hybrid' of a parental strand and a new strand. Since then there have been several demonstrations that DNA replication does indeed result in an equal division of the parent molecule between the two daughters[2,3]. This, however, has not put the hypothesis beyond the realm of cavil. The DNA which divides between the two daughters might, the argument goes[4,5], consist of a pair of double helices: if so, no unwinding of the strands would be necessary to produce the observed result, and the mechanism of replication could be radically different from that proposed by Watson and Crick.

Decision could be reached if some DNA were found which (*a*) becomes hybrid on replication and (*b*) can be isolated intact in its entirety and, in this state, shown to be two-stranded. Bacterial DNA is unsuitable; it becomes hybrid[2], but it has not yet been isolated intact and so there is no unambiguous estimate of its native length-to-mass ratio (that is, strandedness). Conversely, *T*2 bacteriophage DNA is known to be largely or totally two-stranded[6], but the transfer of parental *T*2 DNA to the progeny is accompanied by so much fragmentation that only small sections of the recipient molecules are known to be hybrid[7]; these small hybrid sections could conceivably be four-stranded without markedly disturbing the ratio of total length to total mass. However, the case of λ-bacteriophage DNA appears to be straightforward. Parental λ-DNA is transferred to progeny particles which, though typical in other respects[8], contain DNA which is half parental and half new (that is, is manifestly hybrid)[9]. Further, the entire DNA of λ resides in a single molecule which can be extracted intact[10,11]. If this molecule can be shown to have the ratio of total length to total mass of two-stranded DNA, then strand separation must occur.

To prepare labelled λ DNA for autoradiography, lysogenic *E. coli CR*34 (λ) thy$^-$ was induced by irradiation with ultra-violet light and then incubated in *A* medium[9] supplemented with 2 μgm./ml. tritiated-thymine (11·2 c./m*M*). After 100 min., the bacteria were lysed with chloroform. The resulting phage was sedimented with 10,000-fold excess of unlabelled λ. resuspended and extracted with phenol[12]. This preparation of λ-DNA was diluted to a total concentration of 5 μgm./ml. in $M/100$ phosphate buffer *p*H 7, heated at 75° C. for 20 min.[11], and collected on glass microscope slides for autoradiography. Though most labelled molecules were seen to be folded or tangled, some could be measured. These ranged up to 23μ in length (Fig. 1). This estimate of length, when combined with the reported value of 46×10^6 for the molecular weight[11], gives a ratio of about 2×10^6 mol. wt./μ. This is the expected ratio for a DNA double helix in the *B* configuration[13]. Further support for the conclusion that λ-DNA is two-stranded came from the finding that the density of grains per unit length along these molecules was approximately the same as that along *T*2 DNA, labelled with the same tritiated-thymine and exposed under film for the same time; if *T*2 DNA is largely two-stranded so must be λ-DNA.

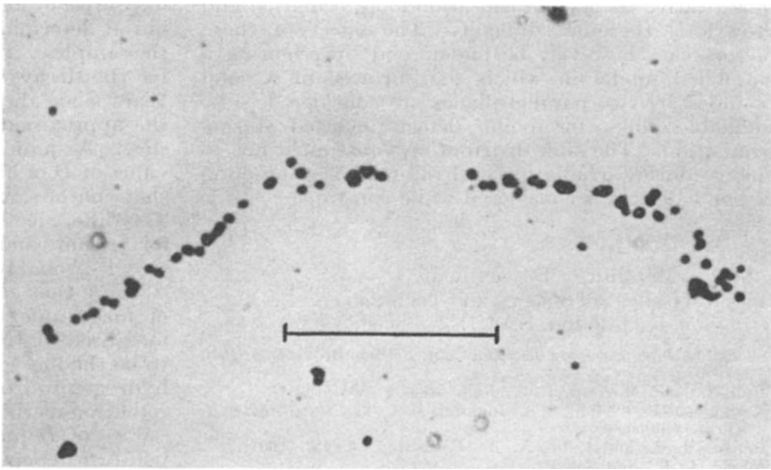

Fig. 1. Autoradiograph showing two molecules of λ-bacteriophage DNA, labelled with tritiated-thymine and exposed under Kodak '*AR* 10' stripping film for 56 days. The scale represents 10μ

In short, λ-DNA is known to form hybrids on replication. It is shown here to have the ratio of total length to total mass of two-stranded DNA. Therefore the replication of DNA must involve separation of the polynucleotide strands and the Watson–Crick model for DNA replication seems to be correct.

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