THE REPLICATION OF DNA

I. Two Molecular Classes of DNA

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ABSTRACT Two classes of DNA have been distinguished on the basis of their reaction to heating in cesium chloride. DNA from proliferating sources is cleaved in half by such treatment, whereas DNA from non-proliferating sources is unaffected in molecular weight. This has been shown by two independent techniques. The relationship between the molecular units produced by cesium chloride treatment and the units conserved during DNA replication is discussed.

The DNA of *E. coli* (1) and of certain higher organisms (2, 3) contains two units, each of which replicates without loss of integrity, producing a new partner once during each generation. The same is true of the chromosomes of *E. coli* (4) and of higher organisms (5). What is the nature of these conserved units? Are they composed of single polynucleotide strands, double-helical molecules, or still more complex entities? These questions must be answered before we can achieve an understanding of the replication process.

We propose to answer these questions in three steps. The first step, presented in this paper, shows that there are two types of DNA molecule, one of which can be separated into two constituent units corresponding to the independently conserved units of *E. coli* DNA, while the other type cannot. The second step is presented in the following paper, which is concerned with the number of polynucleotide strands in the two types of DNA molecule before and after separation of the units. Thirdly, in the final paper a replication hypothesis is set forth and confirmed experimentally, and the native structure of the conserved unit is demonstrated.

To return to the first step, one would like to generalize the observations of Meselson and Stahl (1) concerning the two units of *E. coli* DNA to include DNA from all sources. However, a disturbing discrepancy was found (1) in the behavior of DNA from salmon sperm, as compared to that from *E. coli*. When heated in a concentrated solution of cesium chloride, *E. coli* DNA molecules separated into their constituent units, thus halving their molecular weight and increasing the width of the band formed by centrifugation in a cesium chloride density gradient. The

band formed by salmon sperm DNA, however, did not broaden after heating in cesium chloride (1). Either an actual decrease in molecular weight was obscured by density heterogeneity (6), or salmon sperm DNA is composed of only one unit, or its units are held together differently from those of *E. coli* DNA. In this paper we present evidence which eliminates the first and favors the second of these three alternatives, and which supports a hypothesis (7) concerning the existence of the two classes of DNA molecule: those possessing two separable units (hereafter called biunial molecules), and those apparently possessing only one unit (hereafter called unitary molecules).

MATERIALS AND METHODS

The sources, preparation, and properties of all the DNA samples used here are described in the preceding paper (8). All samples except one, *E. coli* B I, were disaggregated, and all were undenatured, as indicated by hyperchromicity with acid, alkali, and/or heat. Mouse sarcoma-180 DNA contained about 10 per cent of material which sedimented more slowly than the main boundary in the ultracentrifuge. All other samples sedimented as single boundaries, which became sharper as protein was removed during preparation.

The procedure for heating DNA in the absence of cesium chloride is also presented in the preceding paper (8). Heating of DNA in the presence of cesium chloride was carried out in 7.7 molal cesium chloride (9), 0.01 M tris, and 0.0 to 0.1 M EDTA, at pH 8.5-9.7, for 20 to 30 minutes at 100°. The sample was then either thoroughly dialyzed against 2 M sodium chloride followed by 0.01 M EDTA—0.2 M sodium chloride, or passed through a sephadex (10) column to remove salts and then adusted to 0.2 M sodium chloride. The latter procedure proved more successful. Lastly, the sample was shaken with chloroform-octanol (8) before light-scattering measurements were made.

Molecular weights were determined by light scattering in a Brice-Phoenix photometer (8). Equilibrium centrifugation in a density gradient was performed in 7.7 molal cesium chloride—0.01 M tris, pH 8.5-9.0, at 25° and 44,770 R.P.M., in a Spinco model E ultracentrifuge with ultraviolet optics.

RESULTS AND DISCUSSION

Molecular weights were determined by the method of light scattering, which was shown in the preceding paper (8) to give the weight-average molecular weights of the DNA samples studied here. It was also shown that these samples are essentially unaggregated.

Using the light-scattering technique, we have confirmed that DNA from *E. coli* B, when heated in cesium chloride, does indeed halve its molecular weight (Table I). However, no change in molecular weight takes place when the DNA is heated without cesium chloride. The DNA used in these experiments had been prepared by the duponol procedure (8) from bacteria grown and lysed in the same manner employed by Meselson and Stahl (1), in the experiment in which they demonstrated that *E. coli* B DNA separates into two conserved units upon heating in cesium

TABLE I

Source	Sample code	M_W*	$\overline{(\rho^2)^{\frac{1}{2}}}$‡	M_W* after heating§	M_W* after heating in CsCl	σ^2/σ_0^2 after heating in CsCl‖	Hybrid¶	Approximate generation time
E. coli B	I (aggregate)	11×10^6	1600 A	10×10^6	5.6×10^6	3.6 2.9 (1)	+(1)	50 min.
E. coli B	Ib (disaggregated)	2.4×10^6	1620	2.3×10^6	1.3×10^6			
E. coli 15_{T-}	G	1.2×10^6	1100	1.1×10^6	0.65×10^6			50 min.
Pneumococcus R6	LC–I	1.5×10^6	1300	1.5×10^6	0.85×10^6	2.3		45 min.
Mouse sarcoma-180	LC–SI	1.3×10^6	1160	1.3×10^6	0.61×10^6			22 hrs.
HeLa							+(3)	20 hrs.
Chlamydomonas reinhardi							+(2)	3 hrs.
Salmon sperm	KS	2.0×10^6	1480		1.9×10^6	1 (1)		∞**
Calf thymus	OS	2.2×10^6	1700		2.1×10^6	1.1 1 (6)		Long‡‡
Sea urchin sperm	TS	2.2×10^6	1490		2.3×10^6			∞**
Phage T4 (mature particles)						1 (13)		∞**

* Weight-average molecular weight, derived from light scattering.

‡ Z-average radius of gyration, derived from light scattering.

§ No contact with cesium chloride at any time.

‖ Ratio of (band width)^2 after, to that before heating in cesium chloride. Numbers in parentheses are references.

¶ + Indicates that hybrid (half-labeled) DNA has been found in one-generation labeling experiments. Numbers in parentheses are references.

** Incapable, *per se*, of DNA synthesis and division.

‡‡ In young Wistar rats, the generation time of thymus lymphocytes is greater than 5 days. In cattle, thymus nuclei contain an average of two times the amount of DNA in sperm, indicating that few cells are in preparation for division. (14)

chloride. Their DNA preparation and ours (sample I, Table I) formed bands of the same shape and width when centrifuged under the same conditions in a cesium chloride density gradient. Therefore the two must have the same molecular weight since, in the absence of density heterogeneity (11), (band width)^2 is inversely proportional to molecular weight (12). When heated in cesium chloride, both show a similar increase in band width. We conclude that our DNA sample was identical with that of Meselson and Stahl. This DNA could be disaggregated, by treatment with chymotrypsin or by shaking with chloroform-octanol (8), to a molecular weight about four times lower than the original one. The molecular weight of this disaggregated DNA (sample Ib), like that of the original aggregate, dropped to half upon heating in cesium chloride. This shows that disaggregation does not affect the association (hereafter referred to as the biunial bond) between the two conserved units of the molecule. The biunial bond apparently differs in nature from the electrostatic protein link in the aggregate, for the two types of bond can be broken independently.

Although our results indicate that the DNA of Meselson and Stahl was aggregated, their conclusions remain unaffected. Furthermore, the facts that a hybrid aggregate could be dissociated into two differently labeled components, and that these components, also aggregates, are discretely conserved in the cell, demonstrate that the aggregates dealt with by Meselson and Stahl and by us are chromosomal remnants rather than artifacts. Conclusion regarding the organization of such aggregates may thus be extended to the chromosome itself.

Having established that disaggregated DNA behaves with respect to its conserved units in the same way as the originally studied aggregates, we then investigated disaggregated DNA from a number of different sources. In accord with its unchanged band width (1), salmon sperm DNA was found by light scattering to maintain its original molecular weight when heated in cesium chloride. Light scattering thus confirms the centrifugal results that show the existence of two classes of DNA, based on separability of units when heated in cesium chloride. Several of the samples studied fell into each class (Table I, above and below the horizontal line). DNA of both classes was at first laterally aggregated; then, after deproteinization, the molecular weights dropped to low and reproducible values (8). These molecular weights did not change when the samples were heated without cesium chloride (8). (It has been shown (8) that there was no question of aggregation or renaturation in these experiments.)

Table I presents data obtained in this and other laboratories. In every case for which both measurements are available, the halving of the light-scattering molecular weight correlates with the widening of the band formed during density-gradient centrifugation, after heating the DNA in cesium chloride. These two observations appear also to be correlated with the existence of hybrid DNA molecules. Such molecules, composed of half old, half new material, have been detected by virtue

of their intermediate density in lysates of initially "heavy" labeled cells grown for one or more generations in "light" medium (1-3).

Observe that the light-scattering data for salmon sperm DNA and calf thymus DNA support the gradient sedimentation data indicating no change in molecular weight on heating in cessium chloride. Thus, the constancy of the band width for these materials cannot be attributed to a masking effect of density heterogeneity. For bacteriophage T4, the question of heterogeneity does not arise; for bacteria, it has been shown to be negligible (11). In general, therefore, changes in band width should and do correspond fairly well with changes in molecular weight observed by light scattering, in spite of the fact that, in our hands, the error in the former experiments was considerably greater than in the latter. Only the aggregated *E. coli* B DNA shows a notable discrepancy, possibly because of disaggregation phenomena in the presence of cesium chloride; however, the fact that the hybrid aggregate split into equal amounts of heavy and light material, in the gradient sedimentation experiment of Meselson and Stahl (1), indicates that a halving of the molecules does occur and that it is independent of aggregation.

CONCLUSIONS

It has been shown by two independent methods—light scattering and equilibrium sedimentation in a density gradient—that there are two classes of DNA molecule: those molecules possessing two separable units (biunial molecules) and those apparently possessing only one unit (unitary molecules). The units correspond to those which are discretely conserved during replication, in *E. coli*. DNA does not change in molecular weight, when heated, unless cesium chloride is present. Such treatment separates the units of biunial molecules but has no effect on unitary molecules. The agreement of the light-scattering and gradient sedimentation experiments rules out the possibility that density heterogeneity of the unitary DNA masked an actual band broadening in the latter type of experiment.

Since separation of the units of biunial DNA can be accomplished regardless of whether or not the sample is aggregated, while disaggregation can be brought about without affecting these biunial links, these links must be different from those causing aggregation (see Fig. 3 in Paper III of this series (15)). The lateral aggregation observed between molecules of incompletely deproteinized DNA is, in some cases at least, a remnant of the chromosomal organization.

For all the DNA samples so far studied, the biunial-unitary classification corresponds to the characterization of their sources as either proliferating or non-proliferating. Table I is divided into two parts on this basis. This correlation suggests that the two DNA classes represent different stages in the metabolism of DNA. Hybrid molecules, containing half parental and half newly synthesized material, have so far been found only in, and may be limited to, proliferating sources. Such

sources yield biunial DNA. However, unitary DNA has been obtained only from sources which are not destined for immediate proliferation. Perhaps the latter DNA has been arrested at a stage which lasts only a relatively short time in proliferating cells—a stage in which the units have separated, as indeed they must do in the course of replication. This interpretation is supported by the occurrence in mammals of both biunial DNA (from proliferating mouse sarcoma-180) and unitary DNA (from non-proliferating calf thymus).

The distinctions in separability and source between biunial and unitary DNA make it plausible, if not probable, that the latter is in fact a single conserved unit. If this is so, then the replicating unit must be double-helical (or still more complex), since unitary DNA is undenatured; that is, it possesses properties which are incompatible with a single-stranded structure. Furthermore, biunial DNA, which is composed of two units, must then consist of two linked double helices, and the hybrid molecules that have been observed must then contain one old and one new double helix.

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