THE REPLICATION OF DNA

III. Changes in the Number of Strands in *E. coli* DNA during its Replication Cycle

LIEBE F. CAVALIERI *and* BARBARA HATCH ROSENBERG

From the Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College, New York

ABSTRACT DNA has been isolated from synchronized cultures of *E. coli* 15_{T^-} at various times. At first the DNA was four-stranded (and indistinguishable in all respects from log phase *E. coli* DNA), but at the start of DNA synthesis the DNA was found to have halved its molecular weight and to have become two-stranded. This sample had all the properties of undenatured, double-helical DNA, and behaved in all respects like DNA from non-proliferating sources. The replication cycle of the DNA molecule has thus been shown to consist of an alternation between the four- and two-stranded forms, the latter being the conserved unit. The evidence provided by the three papers of this series with respect to DNA and chromosomal structure and replication is discussed and summarized.

E. coli DNA molecules, isolated from a randomly dividing culture, are composed of two units which are independently conserved during replication (1, 2). The chromosome (or nucleoid) of *E. coli* as a whole also appears to replicate semiconservatively (3), as do the DNA molecules (4) and chromosomes (5) of human (HeLa) cells and other higher organisms (6-9). Each half of the "anaphase" chromosome is therefore composed of one unit from each DNA molecule composing the chromosome. The behavior of DNA molecules during the cell replication cycle may thus be interpreted in terms of structural changes in chromosomes.

The number of strands in the DNA molecule is indicative of the number of units it contains, since it has been shown (10) that the single conserved DNA unit consists of two polynucleotide strands whereas the double-unit (hereafter called biunial) DNA molecule from *E. coli* consists of four strands. Since the two units of this DNA molecule (and of the chromosome) eventually appear, in conjunction with newly synthesized partners, in different cells, they must separate from one another at some stage in the replication cycle. If the units separate completely before DNA synthesis, rather than concurrently with it, then DNA prepared from random cul-

tures of *E. coli* should contain a certain proportion of these single-unit, half-sized molecules. However, if the period of time between separation of units and synthesis of new partners were brief relative to the generation time, the proportion of single-unit (hereafter called unitary) molecules would be negligible. We know that the proportion is indeed negligible, since separation of the units of random culture DNA *in vitro* decreases the average molecular weight by about half (2); a significant proportion of molecules that could not split would lead to an average molecular weight change of *less* than 50 per cent. It seems probable, nevertheless, that the units do exist separately *in vivo* at some stage, however brief, for unitary DNA has been isolated from other sources (2, 10). We have therefore examined DNA from *E. coli* 15_{T-} cultures synchronized with respect to DNA synthesis and cell division, with the aim of isolating the single, conserved unit in its native state and thereby establishing the nature and sequence of some of the events in the replication cycle.

MATERIALS AND METHODS

E. coli 15_{T-}, a thymine-requiring auxotroph obtained from Dr. Seymour Cohen, was grown with constant aeration in the glucose-limiting synthetic medium described by Cohen and Arbogast (11, 11a). The thymine concentration was 0.001 mg/ml. Synchrony was achieved by rapidly cooling, centrifuging, and twice washing a log-phase culture to remove thymine, then resuspending the bacteria in thymineless medium, and continuing incubation and aeration until just before the onset of thymineless death. At this point thymine (0.002 mg/ml) was added and incubation continued, at 36°, until after the first burst of cell division. The procedure is that described by Barner and Cohen (12).

The number of viable cells was followed routinely by plating suitably diluted culture aliquots on nutrient agar containing 5 mg/ml sodium chloride. The bacterial concentration was adjusted to give about 50 colonies per plate, and plates were made in duplicate. In a preliminary experiment the total count was found to be the same as the viable count (except during thymineless death).

DNA was prepared at various times after resuspension of the bacteria in thymineless medium. Sometimes several DNA samples were obtained in one experiment by removing aliquots of the culture at different times. Part of the culture was always retained for periodic viable counts until after the first burst of cell division. At the desired preparation time the culture was poured over ice to lower the temperature instantly below 15°. It was then cooled further, centrifuged, and lysed, and the DNA isolated by the duponol method, as previously described (13). After digestion of RNA with RNase, the DNA was deproteinized by shaking with chloroform-octanol (13). Considerable amounts of protein were removed thereby. The number of treatments necessary could be reduced by using 2 M sodium chloride as the aqueous phase. When most of the protein had been removed, the solution was dialyzed to lower the salt concentration to 0.2 M. The solution was then made 0.1 M in EDTA, and adjusted to pH 8.5. (However, the presence of EDTA was not mandatory.) Chloroform-octanol treatments were continued (usually three times) until the molecular weight remained constant. During this process the molecular weight dropped to a minimum and the length-to-mass ratio increased to a maximum.

The DNA samples were studied by the methods described in the preceding papers (2,

10). Samples J and K (but not Wθ) were denatured before the kinetic experiments by heating for 10 minutes at 100° in 0.2 M sodium acetate, pH 8, followed by immediate cooling to room temperature. This treatment had no effect on molecular weight (2, 10), but produced a sharp drop in the radius of gyration. Molecular weights and radii of gyration were determined by light scattering (13).

RESULTS

The growth of *E. coli* 15_{T-} during and after the establishment of synchrony is shown in Fig. 1. DNA samples J and K were obtained in the experiment depicted. The timing of thymineless death and the division burst was the same in a number of repetitions. Sample G was isolated, in a similar experiment, just before the onset of thymineless death. Sample J was isolated 5 minutes after the addition of thymine, when preparation for DNA synthesis and cell division may have been underway. Sample K was isolated approximately at the start of DNA synthesis. The occurrence of DNA synthesis in this synchronized system has been investigated by Barner

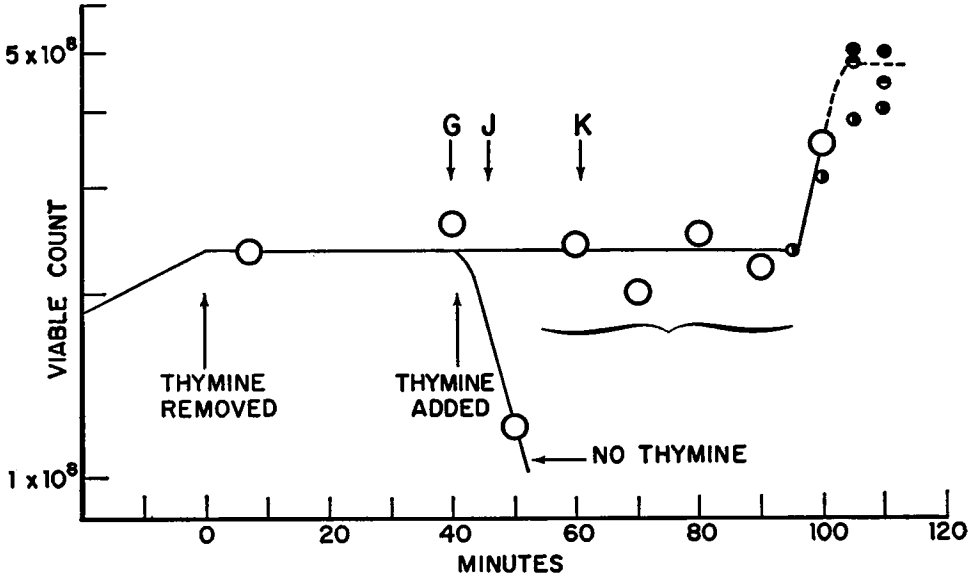


FIGURE 1 Growth curve for synchronized *E. coli* 15_{T-}. The open circles represent the experiment in which DNA samples J and K were obtained. The small, filled or partially filled circles represent data taken from three similar experiments. Thymine was removed from a log-phase culture, by washing and resuspending in thymineless medium, at time 0. The onset of thymineless death occurred about 42 minutes later (lower curve). Thymine was added to the main part of the culture at 41 minutes. Aliquots were removed for preparation of DNA samples J and K at 46 and 61 minutes, respectively; in a similar experiment, sample G was prepared at 40 minutes. The first burst of cell division took place between 95 and 105 minutes. DNA synthesis occurred within the period indicated by the brace (12).

and Cohen (12); although their time scale is somewhat more compressed than ours, it is clear that DNA is synthesized just before cell division, some time during the period within the brace in Fig. 1.

Properties of the three DNA samples are listed in Table I. Both G and J have the same molecular weight (1.2 million) as DNA from unsynchronized *E. coli* 15_{T-}; but K has half this molecular weight. When G and J were heated to 100° in 7.7 molal cesium chloride at pH 9, they decreased in molecular weight to 0.65—0.75 million, or very nearly the molecular weight of K. This treatment has been found

TABLE I

DNA sample	Time*	M_W‡	$\overline{(\rho^2)}^{\frac{1}{2}}$§	M_W‡ after heating in CsCl	Original No. of strands‖
Wθ	Unsynchronized	1.2×10^6	1120 Å		4.2
G	40 min.	1.2×10^6	1100	0.65×10^6	
J	46 min.	1.2×10^6	1140	0.75×10^6	3.7
K	61 min.	0.62×10^6	1100	0.72×10^6	1.7

* Time, after removal of thymine, at which sample was prepared.
‡ Weight-average molecular weight; accuracy, $\pm 0.1 \times 10^6$.
§ Z-average radius of gyration.
‖ Original number of strands per molecule; accuracy, ±0.3.

to separate the two conserved units in random culture *E. coli* DNA molecules (1, 2). While G and J behaved like unsynchronized DNA, the cesium chloride treatment had no effect on K. The number of polynucleotide strands per molecule was calculated from the kinetics of degradation by deoxyribonuclease II, as described in the preceding paper (10). Light-scattering and titration data were used to evaluate the number of molecular cleavages ($[1 - R]/R$) and the fraction of internucleotide bonds cleaved (T_t/m_o) as the reaction progressed. The slopes of the curves in Fig. 2 give the number of strands directly. Within the experimental error the molecules of J are four-stranded, like unsynchronized *E. coli* DNA, whereas K is two-stranded.

All these results indicate that the DNA of samples G and J is biunial and the DNA of sample K is unitary. This is possible only if, sometime during the 15 minutes' incubation between the removal of samples J and K (which were obtained from the same culture), each of the DNA molecules of the *E. coli* chromosome split laterally into two separate parts. In the portion of the culture remaining after removal of K, DNA synthesis followed immediately (12), converting the unitary molecules back to biunial molecules before cell division. Thus, unitary molecules constitute an intermediate form between parental and daughter DNA molecules.

Except with regard to molecular weight, the synchronized unitary sample K is indistinguishable from the DNA of non-proliferating cells (2). Thus it appears that

such DNA, originally biunial, soon reaches the unitary state but is prevented from replicating itself at that point. However, the fact that unsynchronized DNA is identical with the synchronized biunial samples G and J provides further evidence that the time normally spent in the unitary state by the DNA of proliferating cells is relatively brief.

All the DNA samples studied were undenatured, as shown by their hyperchromicity as well as by their large radii of gyration. Thus, biunial and unitary molecules from the same source both possess the properties of double helices, the former being composed of two such units, the latter of one. It is notable that the radii of gyration of G, J, and K are the same, in spite of the twofold difference in molecular weight. This observation, as well as the number of strands, indicates that in biunial molecules the conserved units are associated laterally. This would appear to be inevitable, of course, if one unit has served as template for the synthesis of the other.

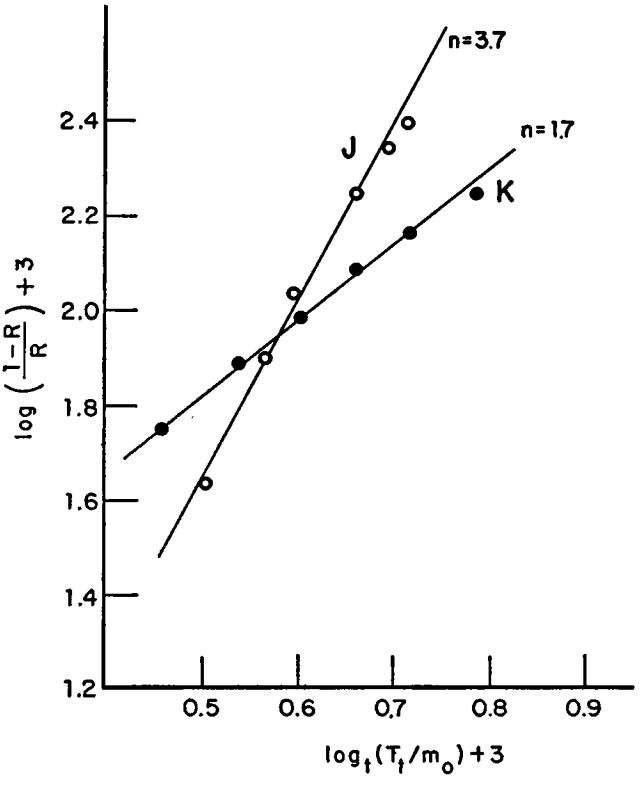


FIGURE 2 The kinetics of degradation of samples J and K by deoxyribonuclease II, plotted so that the slopes of the curves give the number of strands per molecule (10). R is the ratio of the molecular weight at time t to that at time 0; T_t is the titer (the total number of internucleotide bonds broken) at time t; m_0 is the initial number of internucleotide bonds present in the entire titrated sample.

DISCUSSION

It is not unlikely that isolation of an essentially pure sample of unitary molecules has been possible in this system only because synchrony was artificially induced. In untreated single cells the DNA molecules do not necessarily replicate simultaneously, as Taylor (14) has demonstrated. In *E. coli* cells in particular, the fact that DNA synthesis is continuous (15-17) strongly implies that they do not simultaneously replicate. In view of the brevity of the period spent in the unitary state by the DNA molecules of randomly dividing *E. coli* cells, it is probable that each molecule does not divide into its two constituent units until just before its own replication. Therefore the withholding and adding of thymine must have synchronized, intracellularly, the splitting of biunial DNA molecules and their subsequent replication, in addition to synchronizing the division cycles of the cells. However, although the relative timing of the various steps involved in replication differs in synchronized and unsynchronized cells, the steps themselves cannot differ without implying the existence of two entirely different mechanisms of replication. We feel safe, therefore, in concluding that the following sequence is characteristic of the replication cycle of normal *E. coli*: (1) cell division; (2) separation of units to produce two unitary molecules per original molecule; (3) DNA synthesis to produce two hybrid (parental plus new) biunial molecules per original molecule; (4) segregation of the daughter molecules as parts of different daughter chromosomes; (1) cell division. Different molecules apparently undergo steps 2 and 3 at staggered intervals throughout most of the life of the cell; for each molecule, these steps must be of short duration.

It has been pointed out that, because of the continuous synthesis of DNA in log-phase *E. coli,* the DNA complement of a large proportion of cells will have partially but not completely replicated at the moment of thymine withdrawal. These cells are incapable of division without further DNA synthesis. The fact that only unitary molecules were present in sample K, when DNA synthesis was about to commence once more, indicates that *all* DNA molecules, whether they had previously replicated or not, must have divided into their constituent units. During the synthetic period all these units replicate, doubling the total amount of DNA and leaving many of the cells with an aneuploid amount of DNA. However, Barner and Cohen (12) found that the average amount of DNA more than doubles before the first cell division. The factor of increase is close to that expected (2.7) if, after synchronous doubling, all molecules which had not replicated before thymine withdrawal were to replicate again (18), giving the cells a complete, quadruplicate complement of DNA. If we recall that replicating molecules are organized into a replicating chromosome, it is clear that the necessity for completing an "aneuploid" chromosome could provide the stimulus for replication of the remaining molecules. Once completed, the daughter chromatids could segregate and cell division could follow. It is interesting that in the experiments of Barner and Cohen (12) the lag between

the first and second bursts of division was very brief or non-existent, and, unlike the cell count, the amount of DNA did not double. Thus, at least some of the cells would seem to have been prepared for two divisions at the completion of the first synthetic period (95 minutes, in Fig. 1); the others apparently remain "diploid" beyond the second division. In this connection, Quastler (19) has noted (p. 583) that cells blocked after DNA synthesis and before mitosis often go through another synthetic cycle after removal of the block, thus increasing in ploidy.

CONCLUSIONS

DNA has been isolated from *E. coli* 15_{T-} synchronized with respect to DNA synthesis and cell division. The DNA was biunial until the start of DNA synthesis, when it was found to be unitary. The latter DNA had half the molecular weight of the former, but both had the same length. Both were undenatured and stiff, indicating that both must be composed of two or more strands in an ordered configuration such as a double helix. The kinetics of enzymatic degradation showed that the biunial DNA was composed of four strands, the unitary DNA, of two strands. The biunial but not the unitary molecules split into two units when heated in cesium chloride. The relationship between replication in synchronized and randomly dividing cultures has been discussed.

The evidence presented here, combined with other findings (1, 2), indicates that in *E. coli,* and probably other organisms as well, the conserved unit of DNA is a stiff, relatively low molecular weight double helix such as the unitary DNA isolated here. The unit is at first laterally attached to a similar parental unit, then separates, replicates without loss of integrity, and eventually passes (as part of a biunial molecule containing one old and one new double-helical unit) to one of the daughter cells.

SUMMARY: INTERRELATIONS AND IMPLICATIONS

The three papers of this series have shown that the unit of DNA which is conserved during replication is composed of two strands, held together by hydrogen bonds and chain entanglements to form a double helix (20-21*b*). It is, in other words, the entire DNA molecule as isolated from calf thymus, salmon sperm, or other non-proliferating sources. It has a molecular weight in the range of 0.6 to 2 million, the exact value perhaps being a species characteristic.

At the beginning of its replication cycle the *E. coli* DNA molecule is biunial; that is, it consists of two double-helical units laterally attached to one another. This molecule, inherited from the parent cell, eventually splits into two parts, each of which then synthesizes a new partner. Thus, just before cell division, there are two biunial molecules identical with the original one. At cell division, each goes to a

different daughter cell. Replication of the parental molecule is therefore semiconservative, but replication of the double helix is conservative.

The DNA molecule is unitary (consists of a single unit) just before DNA synthesis. This stage is brief, relative to the generation time, in proliferating cells, but in cells not destined to divide again (or to become polytene or polyploid) the DNA is arrested permanently in the unitary state. These generalizations are based on a study of DNA from seven sources, ranging from bacteria to mammals. However, just as the order and timing of the replication of chromosomal segments is a species characteristic, so may be the order and timing of unit separation. That is, in some cells all DNA molecules may become unitary at once, whereas in other cells, such as *E. coli,* the molecules split in sequence. It is also possible that there are species in which the unitary stage is more extended, or in which the non-dividing cell is arrested *after* DNA synthesis, making the molecules biunial; but no such species have yet been encountered.

Since in proliferating cells the DNA has been found to be biunial most of the time, DNA isolated from such cells is four-stranded. Each of these molecules can be separated into two two-stranded units by heating in concentrated cesium chloride; this treatment breaks the biunial bonds, formed at the time of DNA synthesis, which hold the two units together side by side. The nature of the biunial bonding is as yet unknown, but it is much weaker than the hydrogen bonding in the double helix and it is different from the protein or protein–metal ion linkage which is responsible for the aggregation of DNA molecules.

The replication scheme outlined above and in Fig. 3 differs in a major respect from that suggested by Watson and Crick (22). According to their hypothesis, the two strands of the double helix untwist while, simultaneously, new complementary strands form along the free parts of the parental strands. The mechanism by which the parental strands act as templates is the specific pairing of complementary bases. When the original strands have completely separated there are two identical double-helical molecules, each composed of one new and one old strand. This hypothesis, based solely on the proposed complementary double-helical structure of DNA and the fact that the hereditary material must replicate itself somehow, is appealing because of its directness and simplicity. However, apparent simplicity is not a sufficient guarantee of truth, especially in view of our extensive ignorance of the structure and function of genetic material as a whole.

Most of the evidence that has been adduced in support of the Watson-Crick replication hypothesis has shown that the replication of DNA (1) and chromosomes (7) is semiconservative. This is, of course, indirect evidence and applies equally well to the scheme presented here. Other evidence, also indirect, is related to specific base pairing; for example, in Kornberg's synthetic system, base analogs replace only those natural bases that have the same hydrogen-bonding properties (23). While there might be other ways of interpreting these data, it is not necessary to invoke

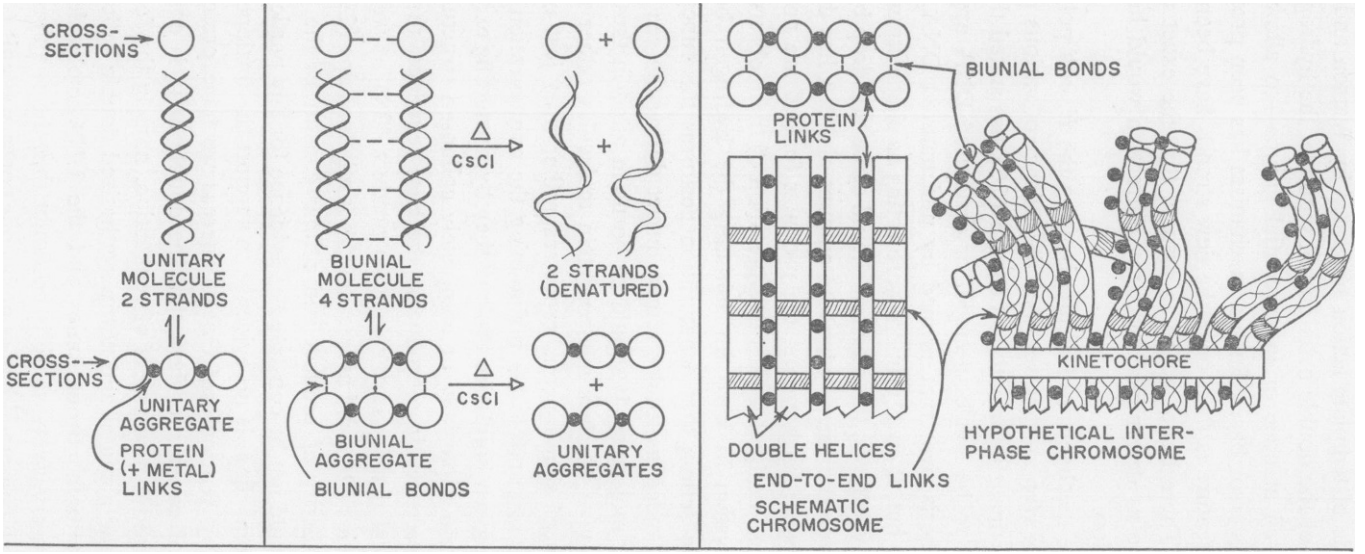
them here since there is at this time no compelling evidence (24) contradictory to the mode of template action suggested by Watson and Crick. Even though the two strands of the double helix do not separate, the hydrogen bonds between them may break—all at once or in sequential groups—to allow the bases to make contact with precursor bases. Such a mechanism has been proposed by Bloch (25). Its essential feature is that, once the new strands have been synthesized, the old strands rejoin. There is no reason to assume that the exact dimensions of the molecular structure as determined by x-ray diffraction would have to be maintained during replication.

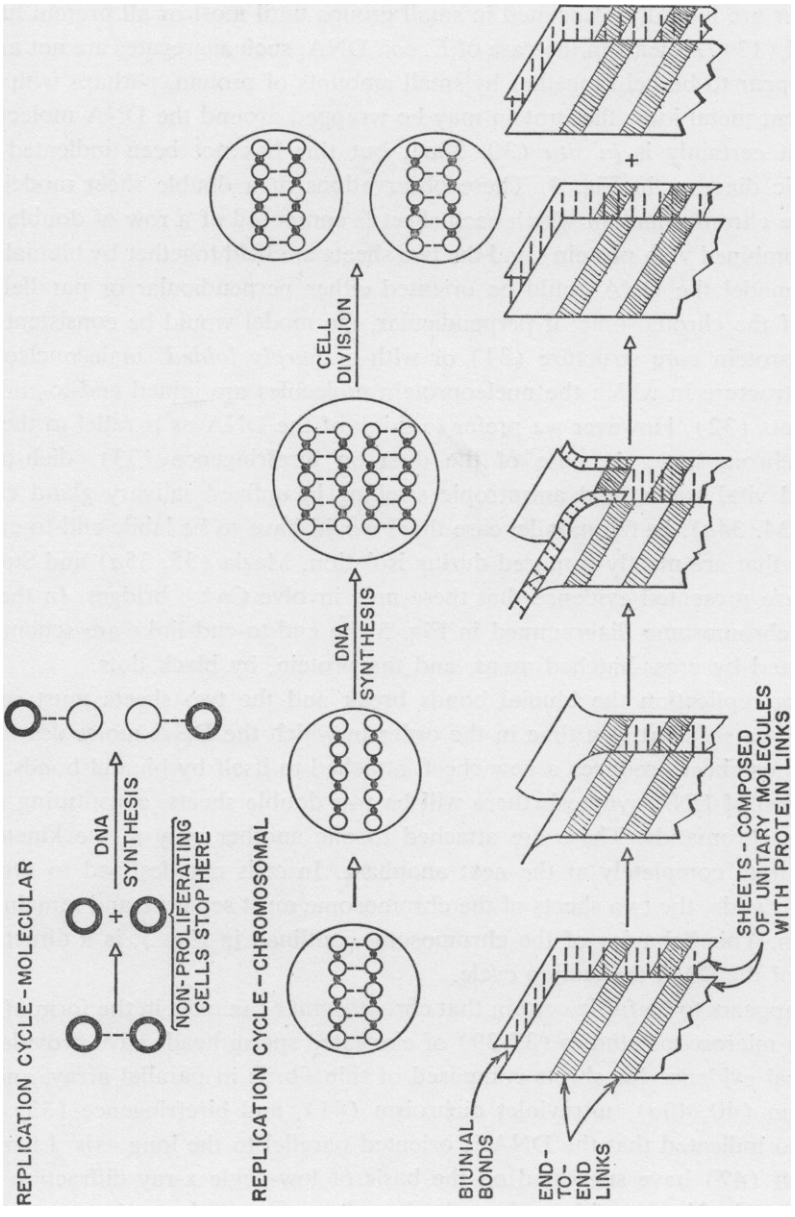
If the mechanism of synthesis using the *E. coli* polymerase *in vitro* is the same as the *in vivo* mechanism, then the efficacy of various primers in that system should give pertinent information. Lehman (26) has stated that the use of ΦX-174 DNA, thought to be single-stranded, or denatured DNA approximately doubles the rate of synthesis over that primed by undenatured DNA. However, as Kit (27) has pointed out, if such primers were acting as single strands, according to the Watson-Crick hypothesis, the reaction should cease (or drop back to the rate obtained with undenatured primer) when the amount of DNA has doubled. This apparently does not happen; furthermore, the products formed are not themselves single-stranded. The most straightforward interpretation of these observations is that there is no efficient system *in vitro* for initiating the hydrogen bond breakage necessary for template action; that denaturation performs this function; and that the denatured, multistranded molecules remain intact during the reaction, producing entirely new product molecules. It should be borne in mind in these considerations that replication *in vivo* involves nucleoprotein molecules rather than DNA alone, and that a number of simultaneous processes rather than just one must occur. The protein may be responsible for maintaining the configuration of the DNA molecule while the hydrogen bonds are broken, thereby permitting exact replication. In the absence of protein a partially or completely collapsed structure might result in only short range accuracy in reproducing the base sequence, thus accounting for the lack of biological activity in the *in vitro* product (28) as well as the reproduction of the base ratios (26) and nearest-neighbor relationships (29) of the primer. Incidentally, the similarity of product and primer constitutes the problem under investigation, and should not be construed as evidence for any particular mechanism.

In sum, there is no evidence to support the unzippering of the double helix proposed by Watson and Crick, whereas we have presented direct evidence, in this series of papers, to show that the double helix is conserved intact. We have also discovered a new aspect of replication, namely the formation of biunial bonds between the parental and newly synthesized double helices.

Since both chromosomes and the DNA molecules composing them replicate semiconservatively, there is no doubt that the anaphase chromosome is double and that each conserved half contains one conserved unit (or double helix) of each DNA

FIGURE 3 Outline of the results of papers I to III of this series. Top, unitary and biunial molecules and aggregates, showing the effects of disaggregation and of heating in cesium chloride. Protein is indicated schematically by black dots, biunial bonds by dashed lines; the cross-section of a double helix is represented by a circle. The cross-section of the schematic chromosome is supported by the data of these papers, while the end-to-end arrangement of the sheets of molecules is one of several possible arrangements (see text Summary). The hypothetical interphase chromosome, shown before separation of the units, illustrates a possible configuration in which the "sheet" structure would not be morphologically apparent. Bottom, the molecular (DNA) and chromosomal replication cycles are diagrammed in cross-section, and the replication behavior of the schematic chromosome above is suggested.




REPLICATION CYCLE - MOLECULAR
DNA
SYNTHESIS
NON-PROLIFERATING
CELLS STOP HERE
REPLICATION CYCLE - CHROMOSOMAL
DNA
SYNTHESIS
CELL
DIVISION
BIUNIAL
BONDS
END-
TO-
END
LINKS
SHEETS - COMPOSED
OF UNITARY MOLECULES
WITH PROTEIN LINKS

molecule. The doubleness of the chromosome, therefore, should not normally be resolvable under the microscope since the units of the DNA molecules are closely joined at this stage by the biunial bonds. Furthermore, we have shown that DNA molecules are laterally associated in small groups until most or all protein has been removed (13). At least in the case of *E. coli* DNA, such aggregates are not artifacts. They appear to be held together by small amounts of protein, perhaps with the aid of divalent metal ions; the protein may be wrapped around the DNA molecules, as it almost certainly is *in situ* (30, 30*a*), but this has not been indicated in the schematic diagram in Fig. 3. These observations fit a double sheet model of the anaphase chromosome, in which each sheet is composed of a row of double helices (each combined with protein) and the two sheets are held together by biunial bonds. In this model the DNA could be oriented either perpendicular or parallel to the length of the chromosome; if perpendicular, the model would be consistent with a central protein core structure (31) or with a *sharply folded* single nucleoprotein strand structure in which the nucleoprotein molecules are joined end-to-end at the fold-points (32). However we prefer to think of the DNA as parallel to the length of the chromosome, because of the data on birefringence (33), dichroism of adsorbed vital stains, and anisotropic swelling in unfixed salivary gland chromosomes (34, 34*a*). In the parallel case there would have to be labile end-to-end connections that are mostly ruptured during isolation. Mazia (35, 35*a*) and Steffensen (36) have presented evidence that these may involve Ca^{++} bridges. In the hypothetical chromosome diagrammed in Fig. 3 the end-to-end links are schematically represented by cross-hatched areas, and the protein, by black dots.

During replication the biunial bonds break and the two sheets must separate, perhaps one segment at a time in the order in which the DNA molecules replicate (14). Each sheet produces a new sheet, attached to itself by biunial bonds. At the completion of DNA synthesis there will be two double sheets, constituting the two daughter chromatids. These are attached to one another only at the kinetochore, and separate completely at the next anaphase. In cells not destined to synthesize DNA or divide, the two sheets of the chromosome must separate and remain in that condition. This behavior of the chromosome, outlined in Fig. 3, is a direct consequence of the DNA replication cycle.

It appears to be fairly certain that chromosomes can exist in the form of sheets. Electron microscope studies (37-39) of elongated sperm heads have provided morphological evidence for sheets composed of thin fibrils in parallel array, and x-ray diffraction (40, 40*a*), ultraviolet dichroism (41), and birefringence (33) studies have also indicated that the DNA is oriented parallel to the long axis. Luzzati and Nicolaieff (42) have suggested on the basis of low-angle x-ray diffraction studies that the nucleohistone of interphase fowl erythrocyte nuclei may be organized in a lamellar structure. For most interphase chromosomes, however, the sheet form may be only a potentiality. That is, small groups of molecules may split apart laterally

from one another (while remaining attached through their end-to-end neighbors to the kinetochore) and take up variously bent and coiled configurations, thus giving the appearance of randomly oriented fibrils (see Fig. 3). In prophase each double sheet would be expected to crumple and twist into a tightly coiled bundle, whose cross-section would not reveal the underlying sheet form.

Since the two halves of the anaphase chromosome eventually segregate from one another to different cells, they must contain equivalent information. However, Taylor (8) has found certain limitations on sister-strand crossover which necessitate differences between the conserved halves of the chromosome. It seems most likely that these differences lie not in the DNA but in the protein or other links that connect the ends of the molecules, in the vicinity of which crossover probably occurs.

Mazia and Bibring (43, 43*a*) have recently made an observation which is very revealing of the basic structure of chromosomes and DNA. They found that if dividing sand-dollar eggs were blocked at metaphase with β-mercaptoethanol, and the block removed after a certain interval (during which no DNA synthesis occurred), the eggs divided four ways. Since the quadruplet daughter cells remained diploid and continued to proliferate, each must have received a complete set of genetic information, and therefore each metaphase chromosome must have contained four such sets. If β-mercaptoethanol had not been added, these chromosomes would have divided two ways at anaphase. This, then, is direct cytological proof that the normal anaphase chromosome consists of two sets of information *which can separate and still remain functional and complete* in terms of information. These parts must, of course, be the two conserved halves of the anaphase chromosome, which apparently can be caused to separate independently of replication under unusual conditions. If each chromosome half had been composed of single strands from double helices, as necessitated by the Watson-Crick hypothesis, it would, first, have been a formidable problem to untwist each DNA molecule and its protein in the condensed chromosome; and second, it is doubtful whether a complex chromosome made up of single polynucleotide strands would behave normally. However, Mazia and Bibring's observations are fully consistent with the chromosome structure presented here, in which the anaphase chromosome has two parts, each composed of one double helix from each biunial DNA molecule. Premature rupture of the biunial bonds (*i.e.* at anaphase rather than the following interphase) would produce two easily separable (44) and completely stable chromatids, identical in information content, from each anaphase chromosome—or four in all from each metaphase chromosome. These chromatids could act as chromosomes in the quadripartitioning egg, without requiring any change in the normal mechanisms of function and replication.

This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant CY-3190), from the Atomic Energy Commission (Contract No. AT(30-1)-910), and from the American Cancer Society.
Received for publication, December 19, 1960.

REFERENCES

1. MESELSON, M., and STAHL, F. W., *Proc. Nat. Acad. Sc.*, 1958, **44**, 671.
2. CAVALIERI, L. F., and ROSENBERG, B. H., *Biophysic. J.*, 1961, **1**, 317.
3. FORRO, F., and WERTHEIMER, S. A., *Biochem. et Biophysica Acta*, 1960, **40**, 9.
4. SIMON, E., *J. Molecular Biol.*, in press.
5. TAYLOR, J. H., *Am. Scientist*, 1960, **48**, 365.
6. SUEOKA, N., *Proc. Nat. Acad. Sc.*, 1960, **46**, 83.
7. TAYLOR, J. H., WOODS, P., and HUGHES, W., *Proc. Nat. Acad. Sc.*, 1957, **43**, 122.
8. TAYLOR, J. H., *Genetics*, 1958, **43**, 515.
9. TAYLOR, J. H., *in* A Symposium on Molecular Biology, (R. E. Zirkle, editor), Chicago, University of Chicago Press, 1959, 304.
10. CAVALIERI, L. F., and ROSENBERG, B. H., *Biophysic. J.*, 1961, **1**, 323.
11. COHEN, S. S., and ARBOGAST, R., *J. Exp. Med.*, 1950, **91**, 619.

11*a*. BARNER, H. D., and COHEN, S. S., *J. Bact.*, 1954, **68**, 80.

12. BARNER, H. D., and COHEN, S. S., *J. Bact.*, 1956, **72**, 115.
13. CAVALIERI, L. F., DEUTSCH, J. F., and ROSENBERG, B. H., *Biophysic. J.*, 1961, **1**, 301.
14. TAYLOR, J. H., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 455.
15. SCHAECTER, M., BENTZON, M. W., and MAALØE, O., *Nature*, 1959, **183**, 1207.
16. MCFALL, E., and STENT, G., *Biochim. et Biophysica Acta*, 1959, **34**, 580.
17. ABBO, F. E., and PARDEE, A. B., *Biochim. et Biophysica Acta*, 1960, **39**, 478.
18. If *E. coli* DNA molecules are considered to replicate in a regular sequence, each replicating instantaneously, then there are at any instant in a random, log-phase culture equal numbers of cells containing N, $(N + 1)$, $(N + 2)$, . . . $(N + N)$ DNA molecules, in which N is the "haploid" number of DNA molecules in the chromosome. Since the DNA content ranges from N to $2N$, the average amount of DNA per cell will be $1.5 \times$ the haploid amount. When these cells are caused to undergo synchronous DNA synthesis, the average amount of DNA doubles to become $3 \times$ the haploid amount, distributed in the classes $2N$, $2(N + 1)$, $2(N + 2)$, . . . $4N$. If the cells containing uneven multiples of the haploid amount of DNA then continue to synthesize DNA until all reach an even multiple of the haploid amount, the DNA content per cell will become $4N$ before cell division. Thus the total amount of DNA will have increased by a factor of $4N/1.5N$, or 2.7-fold.
19. QUASTLER, H. *Ann. New York Acad. Sc.*, 1960, **90**, 580.
20. WATSON, J. D., and CRICK, F. H. C., *Nature*, 1953, **171**, 737.
21. WILKINS, M. H. F., STOKES, A. R., and WILSON, H. R., *Nature*, 1953, **171**, 740.

21*a*. LANGRIDGE, R., WILSON, H. R., HOOPER, C. W., WILKINS, M. H. F., and HAMILTON, L. D., *J. Molecular Biol.*, 1960, **2**, 19.

21*b*. LANGRIDGE, R., MARVIN, D. A., SEEDS, W. E., WILSON, H. R., HOOPER, C. W., WILKINS, M. H. F., and HAMILTON, L. D., *J. Molecular Biol.*, 1960, **2**, 38.

22. WATSON, J. D., and CRICK, F. H. C., *Cold Spring Harbor Symp. Quant. Biol.*, 1953, **18**, 123.
23. BESSMAN, M. J., LEHMAN, I. R., ADLER, J., ZIMMERMAN, S. B., SIMMS, E. S., and KORNBERG, A., *Proc. Nat. Acad. Sc.*, 1958, **44**, 633.
24. SINSHEIMER, R. L. (*Science*, 1957, **125**, 1123) has suggested that other factors may also be involved.
25. BLOCH, D. P., *Proc. Nat. Acad. Sc.*, 1955, **41**, 1058.
26. LEHMAN, I. R., *Ann. New York Acad. Sc.*, 1959, **81**, 745.
27. KIT, S., *Cancer Research*, 1960, **20**, 1121.
28. BESSMAN, M. J., LEHMAN, I. R., SIMMS, E. S., and KORNBERG, A., *J. Biol. Chem.*, 1958, **233**, 171.
29. JOSSE, J., and KORNBERG, A., *Fed. Proc.*, 1960, **19**, 305.
30. WILKINS, M. H. F., *Cold Spring Harbor Symp. Quant. Biol.*, 1956, **21**, 75.

30*a*. WILKINS, M. H. F., ZUBAY, G., and WILSON, H. R., *Tr. Faraday Soc.*, 1959, **55**, 497.
31. TAYLOR, J. H., *Am. Naturalist*, 1957, **91**, 209.
32. FREESE, E., *Cold Spring Harbor Symp. Quant. Biol.*, 1958, **23**, 13.
33. SCHMIDT, W. J., *Chromosoma*, 1941, **2**, 86.
34. AMBROSE, E. J., and GOPAL-AYENGAR, A. R., *Nature*, 1952, **169**, 652.
34*a*. AMBROSE, E. J., *Progr. Biophysics*, 1956, **6**, 26.
35. MAZIA, D., *Proc. Nat. Acad. Sc.*, 1954, **40**, 521.
35*a*. BERNSTEIN, M. H., and MAZIA, D., *Biochim. et Biophysica Acta*, 1953, **11**, 59.
36. STEFFENSEN, D., *Genetics*, 1957, **42**, 239.
37. GALL, J. G., and BJORK, L. B., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 479.
38. DASS, C. M. S., and RIS, H., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 129.
39. SOTELO, J. R., and TRUJILLO-CENÓZ, O., *Z. Zellforsch. und mikroskop. Anat.*, 1960, **51**, 243.
40. WILKINS, M. H. F., and RANDALL, J. T., *Biochim. et Biophysica Acta*, 1953, **10**, 192.
40*a*. WILKINS, M. H. F., and BATTAGLIA, B., *Biochim. et Biophysica Acta*, 1953, **11**, 412.
41. CASPERSSON, T., *Chromosoma*, 1940, **1**, 605.
42. LUZZATI, V., and NICOLAIEFF, A., *J. Molecular Biol.*, 1959, **1**, 127.
43. MAZIA, D., HARRIS, P. J., and BIBRING, T., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 1.
43*a*. MAZIA, D., *Ann. New York Acad. Sc.*, 1960, **90**, 455.
44. The two double helices of the biunial molecule appear not to be intertwined (HALL, C. E., and CAVALIERI, L. F., *J. Biophysic. and Biochem. Cytol.*, in press).