On the Replication of Desoxyribonucleic Acid (DNA)

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also be masked in hemoglobins with more of them blocked in the abnormal hemoglobins. Other more complicated masking possibilities may be imagined to account for the differences in charge, but the evidence provided by the experiments reported above makes it very probable that the three hemoglobins differ in their content of free carboxyl groups.

The method of differential titration described in this paper should be applicable to the study of other sets of closely related proteins.

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ON THE REPLICATION OF DESOXYRIBONUCLEIC ACID (DNA)

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The discoveries of Hershey and Chase[1] concerning the role of DNA in transmitting genetic information in phage and of Watson and Crick[2] concerning the structure of DNA have brought the problem of the replication of DNA into focus. The structure proposed by Watson and Crick consists of two polynucleotide chains wound helically around a common axis, tied together by hydrogen bonds between the

purine and pyrimidine side chains. These side chains of the two chains are arranged so that adenine is always matched with thymine and guanine with cytosine. The sequence of bases along either chain is not subject to any restrictions, but once the sequence along one chain is given, the sequence along the other chain is completely determined. This sequence, then, constitutes the genetic information, a linear message written in a four-symbol code. The duplex of the two chains contains the information in a twofold redundance. Each chain has a directional polarity because of the nonequivalence of the 3- and 5-positions through which each pentose is linked to the preceding and the following phosphate group in the chain. This polarity runs in opposite directions in the two chains of the duplex.

Watson and Crick[3] have proposed that this structure is replicated by a process in which the chains of a duplex are separated and each catalyzes the synthesis of a complementary chain. This is schematically represented in Figure 1. One imagines that the synthesis of the complementary chains occurs in a zipper-like

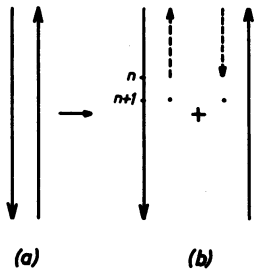

FIG. 1.—Replication of a duplex of complementary chains through complement formation. Each chain is represented by an arrow. Parental chains are represented by solid lines; new chains by dashed lines; last link by a dot. *a*, Parental duplex. *b*, Daughter-duplex in the process of adding link $n + 1$.

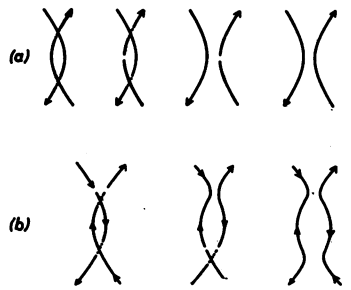

FIG. 2.—Two methods of resolving an interlock between two chains: *a*, By breaking one chain and slipping the other through the gap. *b*, By breaking both chains at each overlap and rejoining them crisscross. *Two pairs of breaks* are needed to resolve one interlock.

fashion along both the parental chains, so that, when synthesis has progressed to point n, the niche formed by link n of the new chain and link $n + 1$ of the old chain provides a suitable enzymatic surface for the insertion of the correct nucleotide as link $n + 1$ of the new chain and for the rejection of the three incorrect nucleotides.

The principal difficulty of this mechanism lies in the fact that the two chains are wound around each other in a large number of turns and that, therefore, the daughter-duplexes generated by the process just outlined are wound around each other with an equally large number of turns. There are three ways of separating the daughter-duplexes: (*a*) by slipping them past each other longitudinally; (*b*) by unwinding the two duplexes from each other; (*c*) by breaks and reunions.

We reject the first two possibilities as too inelegant to be efficient and propose to analyze the third possibility.

If one tries to separate the two chains of a duplex by moving the two chains laterally in opposite directions, an interlock occurs for each turn of the helix, i.e., at

each tenth link. Such an interlock can be resolved in two ways: (*a*) by breaking one of the chains, slipping the other chain through the gap, and rejoining the broken ends (Fig. 2, *a*); (*b*) by breaking both chains at each half-turn and rejoining them crisscross (Fig. 2, *b*).

We reject both these mechanisms—the first one because it introduces an asymmetry between the two chains (only one of them being broken) which is contrary to the symmetry of the structure and the second one because it rejoins chains with opposite polarity, which is chemically not permissible. We conclude that it is not feasible to separate by breaks and rejoins the two chains of a single duplex. The situation is quite different, however, when one considers a duplex during the process of replication. Let us consider a duplex in which replication has proceeded synchronously along the two chains up to link n. We will call this point the "growth point." If we now break both the old chains between links n and $n + 1$, we may join the lower terminals of the breaks in a crisscross fashion, not to the upper terminals of the breaks but to the open ends of the *new* chains of equal polarity. The upper terminals of the breaks now become the open ends for the continuation of the replication process. This process is illustrated in Figures 3 and 4. In Figure 3 we illustrate the breaks and rejoins in lateral view. Figure 4 illustrates a series of successive cross-sections through the replicating duplex, viewed from above, one gross-section for each successive link, in the vicinity of the growth point. The lower cross-sections are below the growth point and illustrate the rotation of the two chains around the common axis by an angle of 36° for each link, in a counterclockwise direction as we proceed upward (*right-handed helix*). The upper cross-sections are above the growth point and illustrate the two daughter-duplexes, and in each of them they indicate again the rotation of the chains of the daughter-duplexes around the axis of each duplex. As the growth point moves down one notch, the two members of the next pair of complementary links of the parental duplex move laterally, to the left and right, respectively, *without change in orientation*, and to each of them a new complementary link is added as the next link of the new chains of the daughter-duplexes. As this process continues from link to link, obstructions arise at every half-turn of the helix, i.e., at every fifth link. These obstructions can be resolved by making at every half-turn breaks and rejoins indicated in Figure 3 in lateral view and in Figure 5 in cross-sectional view.

Chemically these breaks and rejoins amount to exchanges of one nucleotide bond for another, i.e., to transnucleotidations. No energy is consumed or liberated in the process. The activation energy needed is probably similar to the activation energy involved in the addition of a new link, since the pool of DNA precursors probably exists not as free nucleotides but in such a form that the functional groups to be linked are tied up by chemical groups which are exchanged for the new partner.

The daughter-duplexes generated by this process are correctly coiled from the start; in fact, there does not occur at any time any twisting of the parental chains.

We consider it an attractive feature of this proposed mechanism that the complement formation proceeds *synchronously* down the two chains. This means that at any one time only one short section of a duplex is the "working section." Only in this section will the structure be different from that of a stable duplex. This is attractive for two reasons: (1) the energy required at any one time for the separation of the parental duplex is a minimum; (2) since the total length of a duplex,

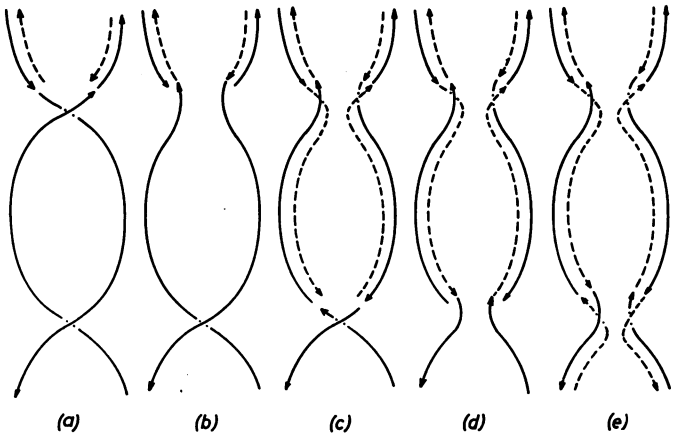

FIG. 3.—Resolution of an interlock in a replicating duplex by breaking both old chains at each half-turn of the helix and rejoining the lower terminals of the breaks to the open ends of equal polarity of the new chains. Lateral view. *a*, Location of first pair of breaks. *b*, Rejoining of lower terminals of breaks. *c*, Location of second pair of breaks. *d*, Rejoining of lower terminals.

Parental chains are represented by solid lines; new chains, by dashed lines. At the overlaps the lower chains are dotted.

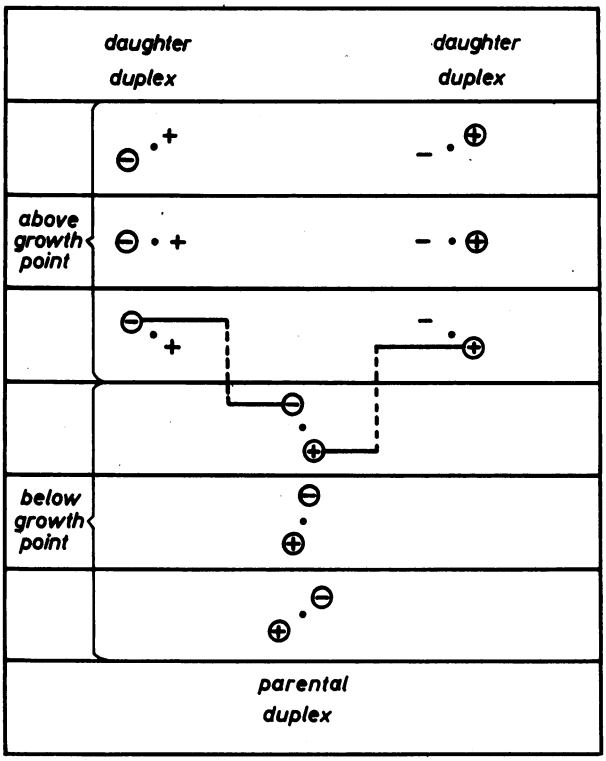

FIG. 4.—Successive cross-sectional views from above of a replicating duplex standing vertically. The sections above and below the growth point are shown. Distance between cross-sections equals one link. Polarities of the chains are indicated by + and −. Parental chains are ringed; new chains are not ringed. Growth proceeds from top downward.

when fully extended, is very large compared to the intracellular space available, the actual length of the duplex must be enormously reduced by some process of supercoiling (or, less likely, folding). The working section, and this section only, has to be in direct contact with the pool of precursors and must therefore be unencumbered by obstructions due to neighboring coils or folds. If there is only one short working section, we may assume that only this section is straightened out, as in a sewing machine only that section of a large piece of material which is under the needle needs to be flat.

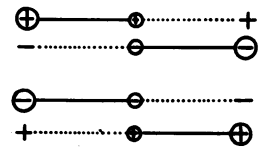

FIG. 5.—The breaks and rejoins shown in Fig. 3 in lateral view are here shown in a superposition of two cross-sectional views. Polarity is indicated by + and −. Old chains are ringed; new chains are not ringed. Old connections are represented by full lines; new connections, by dotted lines. Upper cross-section is large; lower cross-section is small.

It is an important implication of the proposed mechanism that the chains of the daughter-duplexes consist of alternating sections of parental and assimilated nucleotides, each section with an average length of five nucleotides. If a labeled duplex replicates repeatedly at the expense of an unlabeled pool, then, according to this model, the label will be statistically equally distributed to the daughter-duplexes *at each successive replication* (Fig. 6, *a*). Without the breaks

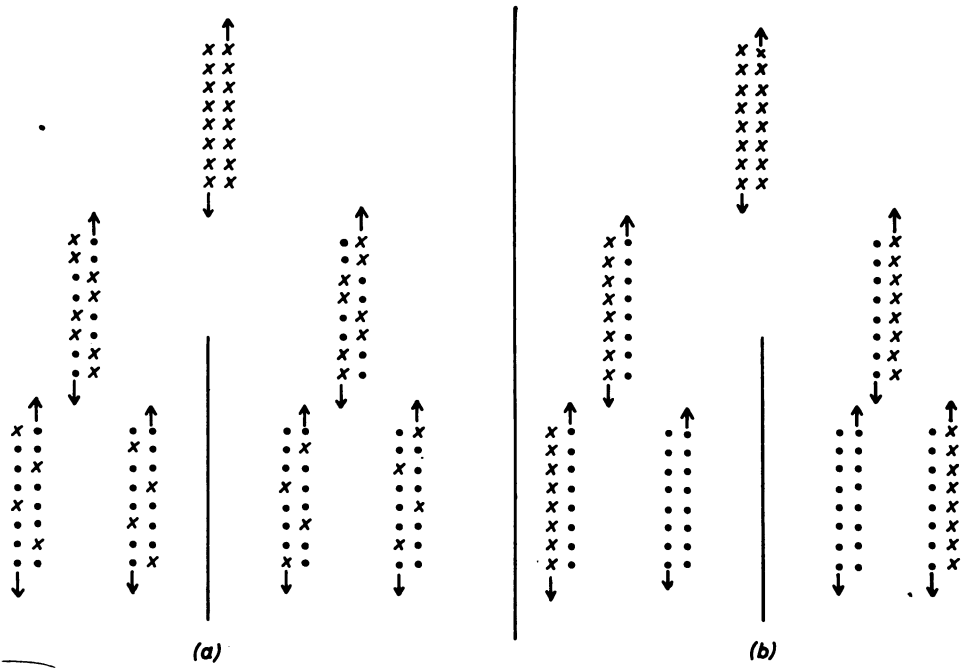

FIG. 6.—Distribution of labeled parental chains to daughter-duplexes in two successive cycles of replication. × = labeled parental chains; ● = unlabeled chain material assimilated from pool of precursors during replication. *a*, With breaks and rejoins as postulated in the theory here presented. *b*, Without breaks.

For simplicity of representation it is assumed that the breaks in *a* occur at exactly every second link and that the break points during the second replication are intermediate between those of the first replication.

and reunions the distribution of label would occur only at the first replication. At each subsequent replication one daughter-duplex would receive all of the label, the other none (Fig. 6, *b*). At present it does not seem possible to discuss the bearing of this implication on the experiments of Stent[4] concerning the mortality, due to the decay of incorporated P^{32} of phage infective centers at various stages of the reproductive cycle. These mortality experiments are complicated by the phenomenon of multiplicity reactivation, i.e., an interaction between different duplexes, the nature of which is still uncertain.

Another implication of the proposed mechanism is the fact that after each odd-numbered exchange the open ends of the chains are those of *parental* material. If the addition of new links is a reversible process, then these open ends of parental nucleotides offer the possibility of exchange of parental labeled nucleotides with unlabeled nucleotides from the pool. One may estimate that such exchange reactions could lead to a maximum loss of label of 10 per cent per replication cycle from the aggregate of vegetative phage particles. Since there are 5–10 replication cycles during one intrabacterial growth cycle, such losses could easily account for the observed low values (30–50 per cent) of transmission of parental P^{32} to the progeny.[5]

Another implication concerns hypothetical ring duplexes, analogous to ring chromosomes. In the first place, such a ring duplex contains necessarily a whole number of turns, since a half-integer number of turns would necessitate joining chains of unequal polarity. In the second place, the daughter-duplexes are separate rings if there are two pairs of breaks and rejoins for exactly every turn. How likely such an exact coincidence would be for large rings depends on details of strain relationships, about which we know too little at present. In any event, exact equality should be more probable than any specified inequality, and its absolute probability should be greater the smaller the ring.

Summary.—Watson and Crick have proposed a mechanism for the replication of DNA. This mechanism involves the synthesis of complementary polynucleotide chains on each of the complementary chains of the parental structure. In this paper a mechanism is proposed for resolving the interlocks which prevent the separation of the daughter-duplexes.

It is assumed that (1) the complementary synthesis proceeds synchronously along the two chains, and (2), as synthesis proceeds, the chains break at the growth point at every half-turn of the helix (every fifth link); the lower terminals of the breaks are immediately rejoined to the open ends of equal polarity of the new chains. Some implications of this mechanism are pointed out.

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