A POSSIBLE MECHANISM FOR THE REPLICATION OF THE HELICAL STRUCTURE OF DESOXYRIBONUCLEIC ACID

By David P. Bloch†

HISTOCHEMICAL RESEARCH LABORATORY, COLUMBIA UNIVERSITY
COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK

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Desoxyribonucleoprotein, the genetically active complex of the cell, carries on two important functions: that of self-replication and that of maintaining a cellular environment compatible with its own existence. An adjunct to the latter function among the differentiated cells of the multicellular organism is the accommodation of these processes within individual cells to the needs of the total organism. At present very little is known, at the molecular level, of the manner in which these processes take place. Recent advances in the knowledge of the chemical structure of this complex, however, make it possible to formulate a scheme for the replication of DNA which accords with the present-day conceptions of the complex and its cytological behavior during duplication of the chromosomal material prior to cell division.

On the basis of X-ray diffraction studies and the chemical composition of the complex, the desoxyribonucleoprotamine molecule has been pictured by Feughelman et al.1 as a triple coaxial, relationally coiled helix. Two relationally coiled polynucleotide strands (o and o' in Fig. 1, a and b) are connected across the center of the helix by hydrogen bonding through the bases, guanine being linked to cytosine and adenine to thymine as proposed by Watson and Crick.2 A third chain, a polypeptide, is relationally coiled about the two polynucleotide strands in such a manner that the 2 basic groups of adjacent amino acids, projecting in opposite directions but parallel to the axis of the helix, can form ionic linkages alternately with the phosphate groups of both polynucleotide helices. The two polynucleotide helices are therefore connected one with the other, both by hydrogen bonding through their complementary bases and by ionic linkage through the associated protamine (Fig. 1, a and b). The desoxyribonucleohistone complex is assumed here to be similar to the protamine counterpart. The differences between the protamines and histones are probably of degree rather than of kind. Histones are of an extremely variegated nature; their properties—molecular weight,3 amino acid composition,4 even their occurrence in tissues5—appear to overlap somewhat with the less complex protamines. Both of these basic proteins form salt-like complexes with DNA. The protamine might be regarded as an extreme form of histone. The apparently globular nature of histone6 can be compared with the more extended protamine configuration by assuming the globularity of the former to be due to an abundance of nonbasic loops such as were postulated by Feughelman et al.1 to occur to a limited extent even in the otherwise extended protamine.

Replication of the polynucleotide in the desoxyribonucleoprotein complex can be visualized by first postulating a slight rotation or vertical displacement of one of the helices relative to the other, the structure more closely approximating that originally proposed by Watson and Crick,2 in order to be able to accommodate additional strands of polynucleotide. Such a configuration would not contradict the results
of X-ray diffraction studies on the structure of this molecule,\textsuperscript{1,7} since, to date, these have been carried out on material obtained from nonproliferating tissues or from cells in which DNA doubling probably does not occur to any great extent. Figure 1, \textit{a} and \textit{b}, schematically illustrates the desoxyribonucleoprotein model as it might appear at the onset of DNA duplication. Figure 1, \textit{a}, shows the helix in cross-section. Throughout the following description this complex, consisting of polynucleotide helices \( o \) and \( o' \) and the attached histone, will be referred to as the "original." The newly synthesized polynucleotides \( r \) and \( r' \), and histone, will be called the "replicate."
If, prior to replication, the hydrogen bonds are broken and a temporary distortion of the molecule enables the bases to rotate approximately 180° about the single nonresonating glycoside bonds, as indicated by the arrows in Figure 2, a, the bases would now be in position to combine by hydrogen bonding to complementary unpolymerized nucleotides, such as were demonstrated by Schmitz et al. to be present in proliferating tissues. The original polynucleotides might meanwhile be held rigidly in place by means of their attachment through the histone (Fig. 2). In this manner the small molecules would be oriented for synthesis of new polynucleotide. The unpolymerized nucleotides, once aligned, would be expected to have a rotational configuration of the base about the glycoside bond similar to that of their
homologues in the original molecule, so that, after alignment, synthesis of the nucleotide bond would result in two new replicate polynucleotide helices. The $o$ strand of the original would now be associated with the $r'$ strand of the replicate, and $o'$ of the original with $r$ of the replicate. What had been the “left-hand” helix in the original association is now the “right-hand” helix in the new association between the original and the replicate, and vice versa.

Bloch and Godman\(^6\) and Alfert\(^9\) have shown that during the interphase period, while duplication of chromosomal material takes place, there is a simultaneous and parallel synthesis of both DNA and histone. This may reflect a simultaneous or rapid succession of the syntheses of both substances at the same site on the molecule. The complex, after duplication of chromosomal material but before separation, might then consist of four relationally coiled polynucleotide strands and two relationally coiled polypeptide strands. Each of the original polynucleotide strands would be associated with its complementary replicate strand by hydrogen bonding through the bases. The original strands remain in association with each other by means of their attachment to the original basic polypeptide; the replicate polynucleotides become similarly associated by means of attachment to the newly formed polypeptide (Fig. 2).

At a later stage, the hydrogen bonds between the original and replicate DNA might again be broken, and, either prior to or following separation of the complexes, the bases could rotate about the glycoside bonds, assuming the proper orientation for hydrogen-bond formation within the respective complexes (Fig. 3, a and b).

Separation of the original from the replicate complex might be visualized by assuming the histone and polynucleotides to form a permanent association during the whole process of replication and separation, the hydrogen bonds maintaining the only temporary linkage. As suggested by Watson and Crick\(^11\) and Gamow,\(^12\) separation of the relationally coiled strands (in the present instance, relationally coiled complexes) may be made possible by the chromosome coiling which takes place during prophase prior to cell division.\(^13\) Winding of a double relationally coiled helix about an axis perpendicular to the axis of the helix, once for every turn in the helix, allows separation\(^12\) (Fig. 4). Of course, any such process leading to separation of originals and replicates would be greatly complicated where chromosomes consist of great numbers of such paired complexes, as may be the case among the higher organisms.

The scheme for replication of the desoxyribonucleoprotein complex as presented here can claim no direct evidence in its support. Partial evidence might be provided by X-ray diffraction studies on material from cell populations containing doubled amounts of DNA. A number of phenomena occur in the duplicating cell which can be explained in terms of such a scheme. Furthermore, this scheme can overcome a few of the objectional features of previously suggested mechanisms, based upon replication of separated single units\(^11\) rather than of associated double units. If DNA replication follows separation of the original double polynucleotide helices, the single polypeptide strand would have a “choice” to make as to which polynucleotide strand to follow, unless the cell uneconomically discards the old histone fraction and synthesizes anew a double complement. Either of these alternatives is more complicated than the presently proposed mechanism. More important, a permanent association between the histone and polynucleotides might serve
to stabilize the helical structure after disruption of the hydrogen bonds during DNA replication.

The present scheme also accords with recent work as reported in preliminary form which suggests that during separation of daughter-chromosomes the newly synthesized DNA goes to one chromosome and the original to the other. If, on the other hand, replication follows separation, the daughter-chromosomes would be expected to contain one strand of original for every strand of replicate material. The complete report of Plaut on the partition of old and new DNA during division will be of great interest.

If, as suggested above, chromosome coiling and contraction during mitosis are visible expressions of a separation occurring at a molecular level, the fact that DNA synthesis occurs during the interphase period prior to visible signs of chromosome
coiling, rather than in the reverse order, would appear to support the present scheme of replication of DNA before separation of the helices.

Finally, histone has been proposed as a regulator of genetic expression among the differentiated cells of an organism\textsuperscript{16,17} the desoxyribonucleohistone complex being characteristic of a cell type much as DNA is characteristic of a species. This hypothesis is supported by evidence of a relative degree of histone constancy within cells of the same type\textsuperscript{9,18} and, at the same time, histone variation between tissues.\textsuperscript{16,18}
and low histone turnover rates,\textsuperscript{19} \textit{inter alia}. A permanent association of DNA with the histone during replication of DNA might provide the basis for replication of the histone also. If the nature of the histone attached to DNA at any one point could impress changes in the configuration of the DNA which could then be passed on to the replicated DNA, and hence to the newly synthesized histone, the scheme might conceivably provide for the transmission of a particular structural pattern of the desoxyribonucleohistone complex governing cellular activity. An accurate transmission of such an "operational" genetic complement through division of differentiated cells may be as important to the proper functioning of the multicellular organism as the accurate transmission of the total genetic complement through the germ cells is to the maintenance of a species.

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\footnote{† Fellow of the Damon Runyon Fund for Cancer Research.}

\textsuperscript{12} G. Gamow, these \textit{Proceedings}, 41, 7–9, 1955.
\textsuperscript{13} C. L. Huskins, \textit{Cold Spring Harbor Symposium Quant. Biol.}, 9, 13–17, 1941.