REPLICATION OF DOUBLE-STRAND NUCLEIC ACIDS

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Communicated by Linus Pauling, April 20, 1965

The conventional explanation of replication of double-strand nucleic acid suggested that the parental double-strand Watson-Crick helix, forming the stem of a Y-like structure, opens up at the Y juncture and that it is there that the two semiconservative replica arms of the Y originate. The Watson-Crick H bond base-pair specificity was supposed to operate here so discriminately that only nucleotides whose bases are complementary to the bases of the just recently separated parental strands are admitted to the formation of complementary filial strands, the parental strands acting as templates.

The hydrogen bonds, particularly at the time and place where the parental strands separate, are, however, quite unreliable in achieving correct complementary base choice without fail.[1] Besides, the two nucleic acid single strands lack structural definition exactly where, as a help for correct nucleotide incorporation, it would be needed most, i.e., at the Y juncture. One has thus to look for a way which would guarantee accurate selection of filial nucleotides in such a replication process. When an enzyme is proposed to perform that task, one is faced with the question of what the structural conditions are which make such an enzyme work.

In 1963 we proposed a scheme which may help toward understanding accurate replication.[2] It involved the hypothesis of a reinforcement of the Watson-Crick structure by molecules (presumably polymerases[3] and some cations) laid snugly into its two grooves in a manner as suggested in another connection by Wilkins[4] when he brought evidence indicating that histones or protamines associate with nucleic acids in their grooves. Such a Watson-Crick-Wilkins helix (WCW helix) would form a very compact and tightly wound structure. The stabilizing molecules might be laid or folded in the grooves in a highly specific manner, such as to be charge-complementary and structurally complementary to the nucleic acid sections to which they are attached. (The attachment might be temporary for the purpose of replication of a section, and the groove-populating molecules might later even shift to the next nucleic acid section.)

A local change in the ionic condition of the surrounding medium, in particular a lowering of the hydrogen ion concentration, would at some point pry the WCW helix slightly open, weakening the hydrogen bonds and evicting a base from its position in the WCW helix; that base would remain attached to its original phosphate-pentose backbone chain. There remains then a cavity in the otherwise approximately intact WCW helix. A free nucleotide triphosphate, carried by Brownian motion into the neighborhood of that cavity, may enter it, provided it has the same base as the one which just before moved out of the cavity. The replacement of the evicted parental nucleotide by the filial nucleotide triphosphate might be understood on the basis that at this time the filial, unlike the parental base, is not constrained by attachment to a phosphate-pentose backbone chain.

The extraordinary accuracy of the DNA replication process is here seen as due to exact structural and charge complementarity.[5] This is a mold and cast situation,

provided by complementary H bonds, and still more so provided by the complementarity of molecules filling both grooves of the Watson-Crick helix. The H-bond complementarity becomes really effective only after normal ionic conditions have been re-established. It should be looked upon as an important, necessary (by itself not sufficient) condition for correct selection of nucleotides.

It has also been pointed out[2] that London-force specificity[6] helps essentially in providing for a preferential choice of the correct filial nucleotide triphosphate, to be brought in proper position, near the cavity which is to be refilled by it.

The outcome of this process is a parental single-strand nucleic acid being peeled off from the WCW helix which, from this replication region onward, becomes a semiconservative double-strand helix, after the polymerization of the newly incorporated filial nucleotide triphosphates.

Formation of Two Semiconservative Double-Strand Helices.—The experiment of Meselson and Stahl proved the semiconservative nature of replication of double-strand DNA of *E. coli*.[7] Pulse-labeling techniques also suggested that the two new filial strands (when formed in a complementary fashion against each of the parental

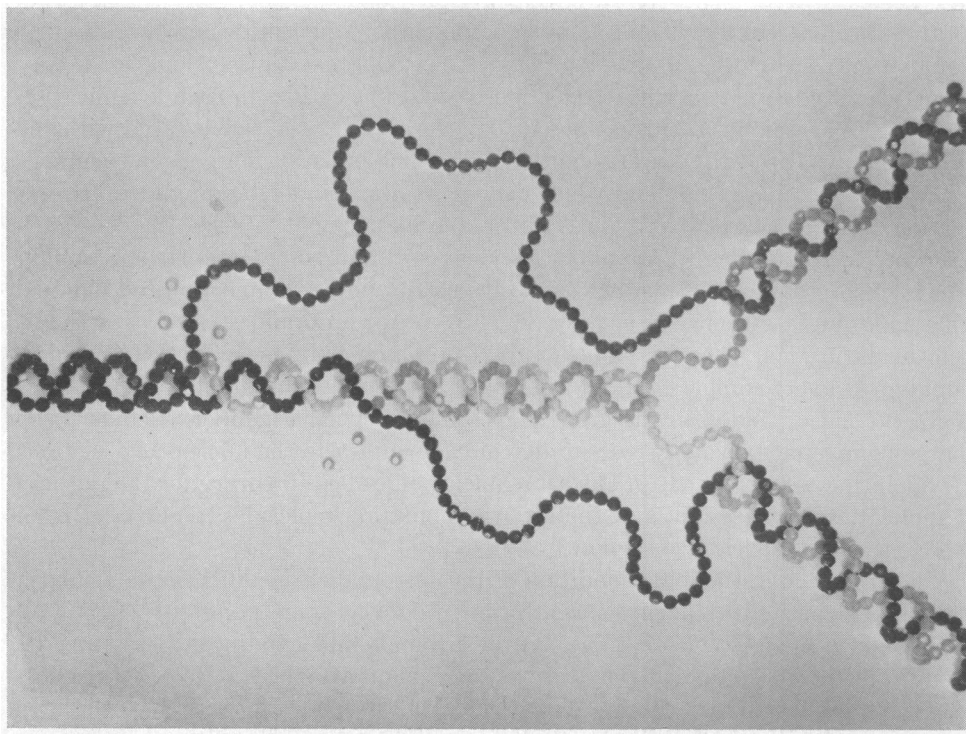

FIG. 1.—The strand synthesis process described in the introduction[2] occurs twice over, one time after the other, on both nucleic acid strands of a "Watson-Crick-Wilkins helix." Both parental strands (dark beads) peel off from the stem region of the Y structure while filial nucleotide triphosphates (lighter beads) get incorporated, replacing the parental nucleotides, and polymerization occurs. All along the stem region the WCW helix remains structurally intact because of the groove-filling molecules (not shown in picture) which reinforce the structure. The peel-off process implies a torque on the stem which therefore performs a screwlike motion and which thus gets eventually opened up at the stem's filial-filial end, i.e., the Y juncture. The peeled-off parental strands base-pair with the complementary filial strands. This proposal differs from the conventional one in that the synthesis region is relegated to the stem of the Y (rather than to the Y juncture) where a well-defined Watson-Crick helix may assure correct replica formation.

strands) are synthesized "in parallel," i.e., in similar direction, thus implying synthesis in a direction which in one of the synthesized strands is in the direction of its polarity, in the other strand opposite to its polarity.[8]

This brings up the question of how two semiconservative helices may arrive in such a replication process. These experiments suggest that the replicating peel-off process occurs on both strands of the parental double-strand nucleic acid helix. Once the process is initiated on an end or on a break of one strand, the other strand's replication is also initiated about at the same time and place; the two replication processes may follow each other, presumably at some distance along the WCW helix. The net result would be a totally filial double-strand nucleic acid with proteins in both grooves, and the two parental single strands peeling off as two random coils.

As the process proceeds some distance along the helix, the frictional drag on the two off-peeling coils increases so that these random coils, instead of swinging around the WCW helix, oblige the helix to spin in a right-hand helical forward motion, sliding along the directions of its backbone helices, while both energy and entropy considerations oblige the two parental single-strand nucleic acids to get peeled off from the helix. They are being replaced in the helix by filial nucleotide triphosphates which thereupon will form nucleic acid chains in a polymerization process.

Subsequently, the upper forward (filial-filial) section of the helix may suffer kinkage or formation of temporary knots or some attachment hindering its helical motion. The off-peeling torques (exerted by the parental single strands), which always act toward a tightening of the parental section and loosening of the filial section of the double-strand nucleic acid, will then cause some untwining of the filial section; whereupon the H bonds between the bases may be broken, exposing the bases of the two filial single-strand nucleic acids while the two protein strands might remain attached to them, one to each of the filial nucleic acid strands, thus forming two nucleoprotein strands.

The peeled-off parental single-strand random coils might then form complementary base pairs with these filial nucleoprotein strands leading to the formation of two semiconservative helices, in the form of two arms of a Y-shaped structure. The underlying hypothesis is that these two nucleoprotein strands (one nucleic acid with one specific protein each) have a better tendency toward the formation of complementary base pairing with single-strand nucleic acids than two naked single-strand random coil nucleic acids have; similarly, two nucleic acid strands with *two* specific protein strands (i.e., a complete WCW helix) may have a still stronger tendency of staying together. That will give preference to the double filial stem region (a complete WCW helix) over the arms of the Y which have less protein strands (incomplete WCW helixes); this preference might prevent untwining of the stem backwards from the Y juncture. In Figure 1 the development would be, in the conventional language, a screwlike speedometer cable motion, the material flowing from the stem to the two arms which grow longer and longer.

With this proposal the conventional interpretation of Meselson-Stahl's experiments, and of Cairn's radioautographs, is almost retained. It would be good to understand the occurrence and the repair of eventual breaks of strands.[9]

The process of reading out single-strand RNA from a double-strand DNA WCW

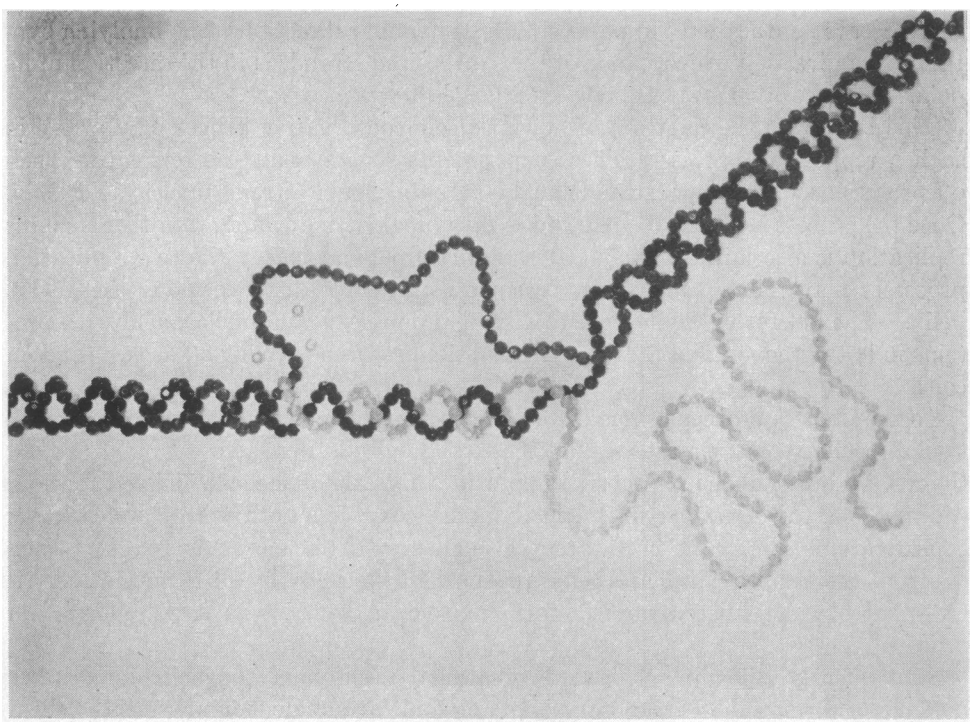

FIG. 2.—Reading out an RNA, from a double-strand DNA functioning as a template. This process might differ from the DNA replication process of Fig. 1. in that here only one of the parental nucleic acid strands gets peeled off and replaced, in this case by filial ribonucleic acid monomers. Along the stem a hybrid double-strand helix is being formed. The stem is again presumably reinforced by tightly fitting polymerases laid into the grooves. The off-peeling parental DNA strand exerts a torque at the synthesis site which eventually causes untwining of the stem at the hybrid side, thus giving also the newly formed RNA a chance to peel off and perhaps be replaced on the helix by the formerly peeled-off parental DNA strand. The motion is, in both Figs. 1 and 2, a helical flow channeled along the backbone helix directions; the conformation of the replicating region would remain essentially unchanged as time goes on.

helix—which eventually stays conserved after the process—follows the same pattern (Fig. 2). There is, in this case, only one of the two DNA strands temporarily peeled off from the double-strand DNA helix; the RNA forms against the other DNA strand,[10] while the polymerases which specifically mark the sections to be read out give that nucleic acid strand its firm structure. The torque caused by the peel-off eventually untwines the hybrid section of the helix and liberates the newly formed single-strand RNA, leaving the complementary DNA strands free to be rejoined.[11, 12]

Hypotheses about details of biosynthesis processes are of necessity conjectural at this time. These nucleic acid synthesis proposals are, in their general outlines, however, a direct consequence of the experimental data and with due regard to structural requirements.

The author is deeply indebted to many colleagues for suggestions and discussions, and in particular to Mr. M. L. Ingerman at George Washington University. He would like to acknowledge generous support from the George Washington University Committee on Research, and from the National Cancer Institute (NIH research grant CA 04989 BBC).

[1] The difficulty of understanding accurate base pairing on the basis of complementary H bonds alone has been emphasized by R. D. Hotchkiss, Dyer lecture (1962); by Rosenberg, B. H., and L. Cavalieri, *Progr. Nucl. Acid Res.*, **2**, 2 (1963) and these PROCEEDINGS, **51**, 826 (1964); and by many authors. Among the literature references to such H bonding, one may point to Pimentel, G. C., and A. L. McClellan, *The Hydrogen Bond* (New York: Reinhold, 1960); Lipsett, M. N., L. A. Heppel, and D. F. Bradley, *J. Biol. Chem.*, **193**, 265 (1961).

[2] Jehle, H., M. L. Ingerman, R. M. Shirven, W. C. Parke, and A. A. Salyers, these PROCEEDINGS, **50**, 738 (1963), Figs. 1 and 2.

[3] Reich, E., *Science*, **143**, 684 (1964).

[4] Wilkins, M. H. F., in *Genetic Mechanisms: Structure and Function*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 21 (1956), p. 75.

[5] Pauling, L., *Festschrift Arthur Stoll* (Basel: Birkhauser, 1957) p. 597; *Nature of the Chemical Bond* (Cornell University Press, 1960); *Aspects of the Origin of Life*, ed. M. Florkin (Pergamon Press, 1960), p. 132; *Aspects of Synthesis and Order in Growth* (Princeton Univ. Press, 1955), p. 3; Pauling, L., and R. B. Corey, *Arch. Biochem. Biophys.*, **65**, 164 (1956); Pauling, L., and R. Hayward, *The Architecture of Molecules* (Freeman, 1964); Pauling, L., and H. A. Itano, *Molecular Structure and Biological Specificity* (Am. Inst. Biol. Sci., 1957).

[6] Yos, J. M., W. L. Bade, and H. Jehle, *Phys. Rev.*, **110**, 793 and 800 (1958); Jehle, H., W. C. Parke, R. M. Shirven, and D. K. Aein, in *Biopolymer Symposium on Quantum Aspects of Polypeptides and Polynucleotides*, ed. M. Weissbluth (New York: Interscience, 1964), p. 209; Jehle, H., these PROCEEDINGS, **50**, 516 (1963), *Biophysika*, **9**, 401 (1964), *Advan. Quantum Chem.*, **2** (1965); Jehle, H., W. C. Parke, and A. Salyers, in *Electronic Aspects of Biochemistry*, ed. B. Pullman (New York: Academic Press, 1964), p. 313; Yos, J. M., W. L. Bade, and H. Jehle, these PROCEEDINGS, **43**, 341 (1957).

[7] Meselson, M., F. W. Stahl, and J. Vinograd, these PROCEEDINGS, **43**, 581 (1957); Meselson, M., and F. Stahl, these PROCEEDINGS, **44**, 671 (1958); Meselson, M., and J. J. Weigle, these PROCEEDINGS, **47**, 857 (1961).

[8] Cairns, J., *J. Mol. Biol.*, **6**, 208 (1963); in *Synthesis and Structure of Macromolecules*, Cold Spring Harbor Symposia on Quantitative Biology, v9l. 28 (1963);, p. 43; Hanawalt, P. C., and D. S. Ray, these PROCEEDINGS, **52**, 125 (1964).

[9] Meselson, M., *J. Mol. Biol.*, **9**, 734 (1964); Thomas, C. A., Jr., and L. A. MacHattie, these PROCEEDINGS, **52**, 1297 (1964); Fong, P., these PROCEEDINGS, **52**, 239 and 641 (1964).

[10] As to the formation of hybrid nucleic acids, apart from replication processes, cf. Schildkraut, C. L., J. Marmur, J. R. Fresco, and P. Doty, *J. Biol. Chem.*, **236**, PC 2 (1961).

[11] The peel-off and rejoining of parental strands, though in this instance initiated by the incorporation of a "filial" RNA strand, has some resemblance to reversible denaturation. Steiner, R. F., and R. F. Beers, Jr., *Polynucleotides* (New York: Elsevier, 1961); Doty, P., J. Marmur, J. Eigner, and C. Schildkraut, these PROCEEDINGS, **46**, 461 (1960); Freese, E. B., and E. Freese, *Biochemistry*, **2**, 707 (1963); Kuhn, W., *J. Mol. Biol.*, **3**, 473 (1961), and *Experientia*, **13**, 301 (1957); Longuet-Higgins, H. C., and B. H. Zimm, *J. Mol. Biol.*, **2**, 1 (1960); Geiduschek, E. P., these PROCEEDINGS, **47**, 950 (1961); Beer, M., and C. A. Thomas, Jr., *J. Mol. Biol.*, **3**, 699 (1961); Fixman, M., *J. Mol. Biol.*, **6**, 39 (1963).

[12] The relationship of single-strand primer processes to double-strand processes, in particular through a "replicative form," is outside the scope of this paper; cf. Sinsheimer, R. L., B. Starman, C. Nagler, and S. Guthrie, *J. Mol. Biol.*, **4**, 142 (1962); Chamberlin, M., and P. Berg, *J. Mol. Biol.*, **8**, 297 (1964); Dounce, A. L., *J. Theoret. Biol.*, **2**, 152 (1962).