27 July 1956, Volume 124, Number 3213 SCIENCE

Molecular Organization of Genetic Material

H. Kacser

From a physicochemical point of view, the cytological and genetical behavior of chromosomes raises two important problems. One is the duplication of a physical entity, the other the replication of specific functional properties. In what follows, the view will be taken that the functional aspect of the genetic material is the more fundamental of the two problems and that duplication should arise as a special case operating under specified conditions. In spite of considerable cytological information about their later behavior, there is little relevant information concerning the chromosomes at the critical duplicating stage. Similarly, although it is certain that functional properties are associated with well-defined portions of the structure, we have no direct evidence how these functions are carried out. By taking biochemical and physicochemical evidence into account, it is possible, however, to arrive at what might be called the minimum requirements for the functioning of the genetic material.

It is convenient to start by discussing the suggestion (*1*) that a proposed structure of crystalline desoxyribose nucleic acid (DNA) is relevant to the genetic problem. The complementary pairing of the bases in the double helix makes it conceivable how two identical double helices could result if a mechanism for their "untwisting" could be imagined. However, "the DNA replicating scheme does not provide a mechanism for separating the two intertwined chains" (*2*). The suggestion (*3*) that systematic breaks take place along the length of the structure avoids the difficulties of "untwisting" but raises problems of equal magnitude in the "systematic breaking."

Although further investigations may prove the proposed structure to be substantially correct (see, for example, Feughelman *et al.*, *4*), it does not, so far, provide a satisfactory mechanism for one of the minimum requirements—namely, the duplication of a physical entity. In any case, the cytological evidence of relic spirals suggests that what "survives" from one division to the next is of an order of magnitude very much greater than the double helix.

It is, however, in the functional aspect that the greatest difficulties of this model are encountered. In the first place, the model fails to account for the protein in the chromosome ("We really have no comparable model for the protein part of nucleoprotein," *5;* see also Watson and Crick, *6*). References to some "nonspecific function" of the protein (*7*) (actually the polypeptide) are unaccompanied by any suggestion how the peptide would be attached to the desoxyribose nucleic acid. Some gallant attempts to close this gap also give no indication how such attachment would be possible (*7–10*). A more rigorous attempt (*4*) envisages combination of the phosphate with the basic amino acid residues only. Even if successful, all such models are concerned solely with polypeptides, while the biological problem is concerned with proteins—that is, polypeptides organized in a specific manner. In fact, the evidence from x-ray scattering (*11*) indicates that protein and nucleic acid, separately organized in large aggregates, constitute the components of native nucleoproteins. The absence of a mechanism for attaching protein to the desoxyribose nucleic acid reflects a more general shortcoming of the model, namely the absence of a mechanism for transmitting to other molecules the specificity that is assumed to reside in the sequence of the bases. Mere duplication of desoxyribose nucleic acid is biologically rather sterile, and consequently the interest of this interpretation of x-ray diffraction is somewhat reduced.

The model that we would like to propose does not concern itself with detailed arguments of particular crystalline structures, which are the proper domain of x-ray crystallography. It is probably not incompatible with the structure of desoxyribose nucleic acid currently held or any other that might emerge. It proposes a mechanism for the chromosome's functional properties which result in the duplication of the structure under specified conditions. The main assumptions are that the chromosome is a "solid phase" (that is, that the system of the nucleus is heterogeneous in the kinetic sense) and that the functional properties are essentially catalytic in nature. It envisages the structure as consisting of an adsorption complex between protein and nucleic acid, the interface of which constitutes the functional part of the assembly.

Surface Specificity

Perhaps one of the most discussed hypotheses arising from the body of biochemical genetics is that the main (if not the only) function of the genetic material is the determination of the specificity of macromolecules, such as various antigens and general proteins and possibly enzymes. The evidence points strongly to a close connection between the genes on the one hand and enzymes and antigens on the other. Whatever the merits of the "one gene-one enzyme" hypothesis, it is clear that the carrier of the genetic material, the chromosome, must be responsible, directly or indirectly, for the production of the enzymes and the antigens or that it must participate in such production in a critical way. We further know that the enzyme, with regard to its specificity and probably its catalytic ac-

The author is a member of the staff of the department of animal genetics, University of Edinburgh, Edinburgh, Scotland.

tivity, acts through its surface and that it is the surface structure which gives the antigen its specific property. In consequence, it is with these surface specificities that the chromosome must be concerned. This function of the chromosome will itself involve a surface since surface specificity cannot be transmitted by anything less than a surface.

The argument that the determination of sequence of the units constituting the macromolecules is sufficient to account for their specificity can be shown to be inadequate. In the case of the proteins and enzymes, for instance, we know that the specificity depends on the particular way in which they are folded or assembled. That this is not fully determined by the sequence of the amino acids is shown by the fact that these structures can be denatured, with a consequent change (or loss) in specificity. This change is due, not to a change in the sequence within the polypeptide, but to a change in organization of the polypeptide within the more compact protein. Denaturations are spontaneous processes, resulting in structures of lower free energy. If nothing but sequence were genetically determined, spontaneous assembly or folding would result in these lowest free energy structures—which is contrary to the evidence.

Some additional mechanism producing assemblies of low entropy must therefore be involved. This is possible if a surface is provided which can determine sequence as well as intersequence organization. This is the familiar template concept, but it should be pointed out that this argument does not necessarily involve a commitment to a particular theory of protein synthesis. All that is implied is that the chromosome should contain specific surfaces, no matter how indirect or by what mechanism the surface specificity is eventually transmitted to its metabolic descendants. The interface of the nucleoprotein complex would fulfill this requirement by providing such surfaces.

There is, of course, another conceivable mechanism—namely, the stepwise assembly of units in a manner similar to the reverse process in, for example, the stepwise degradation of proteins. There is, however, no convincing evidence that such intermediate compounds are formed. In any case, it would involve the presence of specific enzymes for each step—an argument resulting in an infinite regress. (It should be noted that this does not imply a denial of stepwise assembling as a mechanism of processes subsequent to the initial transfer of specificity.) A stepwise folding could equally yield low-entropy structures. If this process is not surface controlled, it implies essentially a rate control. This would be subject to the usual variations of concentration, temperature, pH, and so forth; hence it is difficult to see how an invariable product could be produced even if such mechanisms were known. In contrast, surface mechanisms are in fact known to operate in biological systems, and therefore preference will be given to this type of process in the ensuing sections.

Variability

Specificity associated with variability (in the allelic sense) is another important property which must be accounted for. The constituents of the genetic material must be capable of being organized in a very large number of ways. The specificity from point to point on the surface of an assembly of elementary units (amino acids or nucleotides) depends on both the nature and the relative position of the surface groups present, but it will be relatively unaffected by the nature of the underlying "internal" structure. The large number of possible combinations and relative positions of the constituents of proteins and nucleic acid makes it evident how a very great number of highly specific surface configurations can arise. If it is granted that the area of a specific "patch" extends beyond the width of a peptide or a double helix, the influence of neighboring chains permits extremely subtle variations to be imposed on the "shape" of the surface forces, for instance, by simply shifting the relative positions of neighboring units.

While these surface configurations are in our view functionally important, the underlying organization, constituting the bulk of the assembly, may conform to any regular pattern appropriate to the crystal properties of the molecular species in question. There is good evidence that native protein and nucleic acid are paracrystalline (see Feughelman *et al.*, *4*)—that is, that they contain a large fraction of imperfectly crystalline material. Such assemblies may show a sufficiently orderly arangement of the crystalline portions to allow x-ray determination of their structure to be carried out. Such methods, however, can give no information about the amount of cross-linkage or branching, let alone the nature of a surface a few angstroms in depth.

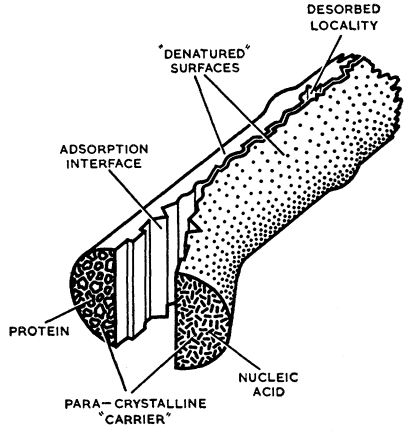

Fig. 1. Diagrammatic view of genetic material.

We take the view that both the protein and the nucleic acid contain specific surfaces and that in fact they form an adsorption complex. Since most of the hydrogen-bonding portions will be internally occupied, the main forces operating on the surface will be van der Waals and coulombic forces. These are short range in nature and therefore demand a very close approach for any potential adsorbate. Unlike hydrogen bonds, these surface forces depend strongly on the nature of the substituents and hence present a highly specific force field. Since the heat of adsorption, a measure of the "tightness of binding," depends on the "area of interaction," it is clear that, if conditions for good adsorption are to be fulfilled, the adsorbate must be "complementary" to the surface. If the adsorbate is itself a surface, it means that, if two such surfaces are adsorbed, their surfaces represent mirror images of force distribution.

Stability

The adsorption complex of protein and nucleic acid is therefore envisaged as containing an interface generated by the complementary surfaces of the two moieties each of which consists of paracrystalline assemblies of the appropriate molecules (Fig. 1). The external surface of the whole complex is not regarded as specific or functional but rather as a denatured surface—that is, a surface having lowest free surface energy and random structural organization. The functional pattern, the genetic "information," is therefore considered to lie only "inside" the genetic material. Like all structures of high free energy content, this pattern will be subject to "decay" in the same manner as proteins and enzymes are denatured and inactivated. Apart from the free energy of activation for this decay, there is another mechanism which tends to maintain surface structure intact. This, in the case of enzymes, is the well-known phenomenon of stabilization by substrates or analogs. The sorption equilibrium between enzyme and substrate "protects" the surface for part of the time so that the rate of inactivation is reduced. With these small molecules as adsorbates, the rate constants of the sorption process are high

and the thermodynamic constants are such that the enzyme is protected only part of the time. An increase in molecular weight would reduce the rate constants, and an increase in the area of interaction would increase the heat of adsorption; both factors tend to increase the stabilization. With two complementary surfaces belonging to high-molecular-weight molecules, the stabilization of one by the other would tend to be exceedingly high. This high stability is, of course, another requirement of a genetic material. That the stability is not absolute is inevitable and may have evolutionary significance. The protective effect may be one of the biological reasons for the existence of two substances in the chromosome (*12*). Another will be evident from the following consideration of the duplication process.

Autosynthesis

When one is discussing the synthesis of new genetic material, it is not helpful to explain it in terms of the hypothetical property of a substance or body called a "self-duplicating ability." No property of this type is known for any molecule except the trivial one of autocatalysis, where already preexisting material is released. The relevant phenomenon is autosynthesis which is the property of a *system*. The general kinetic conditions have been carefully analyzed (*13, 14*) for homogeneous systems. The existence of chromosomes, however, raises problems of macromolecular mechanics not immediately evident from a purely kinetic treatment.

For this purpose, we shall treat the genetic material as a solid phase formed from soluble precursors, the nature of which need not be specified at this stage. Consider the system

$$n\,A + m\,B \leftrightharpoons C$$

where the component C is a solid. The equilibrium constant for this system is defined by the product of the soluble reactants only, the active mass of a solid phase being constant.

How quickly the equilibrium is approached, however, will, apart from concentration considerations, depend on conditions favoring crystallization, since C is a solid. If the structure of C is complex, there will, in the absence of nuclei, be a long delay before the onset of crystallization. Systems with high negative entropies of crystallization may remain supersaturated for very long times. Conversely, the provision of suitable surfaces, particularly the system's "own" lattice, will provide ready centers for rapid crystallization. In such a case, the rate of production of C may depend only on the supply of A and B.

When there are several ways in which A and B can combine to form a number of solids, say, C_1, C_2, C_3 . . . C_n, and if the free energy of the various C's does not differ greatly, the presence of a particular component, C_i, will result in the nearly total conversion of A and B into C_i only. This is, of course, not an equilibrium state, but may have any desired degree of metastability if the free energies of activation for transitions are sufficiently high. We therefore see that, if two (or more) components can associate in more than one way, the actual solid produced will be strongly influenced by the presence of any preexisting surface structure and in the case of a complex compound may be entirely determined by it.

The idea that chromosome duplication is akin to crystallization has been expressed by various authors. There are a number of objections to the unqualified application of this simple view. If a "template" is said to be responsible for the "guiding" of the new material to produce a new crystal, then (unless the template has a high degree of symmetry) it will produce a crystal with a mirror image template. It has been suggested that this objection may be overcome by allowing this second complementary structure in turn to produce a third, its complement, which would be identical with the original. There is, however, no evidence that three structures are involved in the process, as this idea suggests.

There is one type of structure, with any desired degree of asymmetry, which could result in an identical unit by a single act. This structure consists of two parts joined by an interface. The face of each part is the complement of the other. Which of these would be designated "the template" is a verbal matter only.

Duplication

The processes that take place in organisms are rarely simple. As a rule, they are the result of a large number of coordinated reaction sequences, and this is certainly true of cell division and chromosomal duplication. For the present purpose, however, we need only consider the interaction of the solid-phase adsorption complex with soluble precursors no matter how the supply of these may be controlled. Since the "external" surface of the complex is regarded as denatured, it follows that the only surface which can readily initiate new solid-phase formation is the one that carries the specific genetic pattern. The availability of this interface will depend on the frequency of desorption of its parts. Any portion of the structure that has accumulated sufficient thermal energy may separate by desorbing but, since adjacent portions are still adsorbed, it cannot "leave," and the tendency of the distorted structure to straighten out will bring the two portions together again. All along the length of the structure these desorption-adsorption openings may occur, but the probability of the whole structure separating at the same time is very small. While the probability of separation at any point is roughly the same, there are two points, the ends, where they may be more frequent. If an end portion separates, the tendency to close again operates only from one side and therefore this portion may stay open longer, or separate more frequently. These end portions may therefore be positions where the laying down of new material is more likely to begin. Sorption equilibrium is of course sensitive to the conditions of the medium such as ionic strength and *p*H, and a change in these conditions may be instrumental in beginning the process (Fig. 2).

The nature of the sorption forces involved will ensure that the first layer has a close "fit"—that is, will result in a precise complementary surface. Only certain molecules and sequences will be stereochemically suitable for particular portions of the surface. Particular amino acids will best fit the relevant nucleic acid surface and similarly for nucleotides on the protein surface. The situation is not altered if it is assumed that more

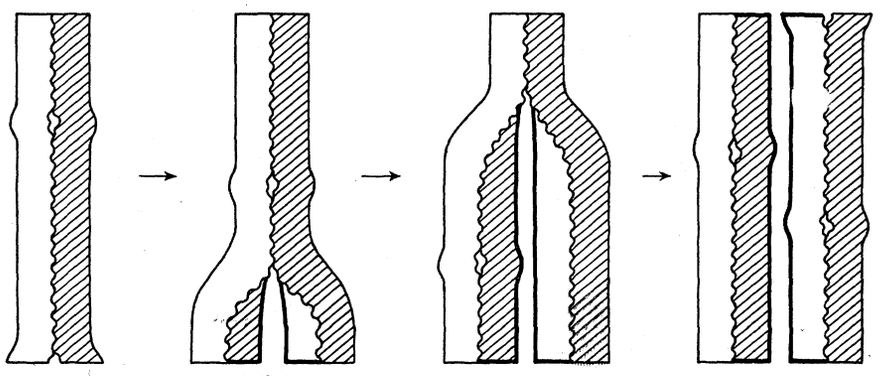

Fig. 2. Diagrammatic view of duplication of absorption complex.

complex condensation products of the simple precursors are supplied. While the first layer will be formed in this manner, further building up will take place by covalent and hydrogen bond formation. This can proceed rather easily since both these bonds are relatively unspecific and consequently may have greater freedom with respect to the nature and sequence of the units. This is because the entropy of adsorption onto a specific surface is very large (and negative), whereas the entropy of hydrogen and covalent bonding is much smaller.

While adsorption is a spontaneous process, the building up of the main bulk of the structure requires the supply of free energy mainly for covalent bonding. If we assume that this is effected by coupling with free energy-yielding reactions, duplication may be triggered by conditions determining these reactions which may also be rate determining.

Once the process has started at one point, wherever that point may be, it may continue along the interface in a zipper-like fashion since the building up of the material will always result in an "open" portion just in front. The supply of the complementary precursors need not be strictly synchronous. It is seen that eventually two structures are obtained, one containing "old" protein and "new" nucleic acid, and other "new" protein and "old" nucleic acid, provided that no sister strand crossing over takes place. However, the two structures are identical as far as internal surface structure is concerned. The building up of the paracrystalline "carrier" will terminate when, for example, the surface volume ratio reaches a critical value, possibly determined by such factors as surface energy or charge density or when the supply of some critical material is exhausted. This final size, however, will be influenced by factors of the environment such as temperature, concentration, hydration, *p*H, and so forth. It is also possible that the composition of the bulk material could vary within limits since strict specificity requirements are operating only in the surface layer. The supply of precursors, controlled by the system as a whole, may, however, be considerably buffered, tending to produce identical structures.

We thus have a mechanism whereby a solid structure is duplicated and at the same time a replication and preservation of a specific interface takes place. We may identify this structure with the chromosome, but the diagrammatic view of Fig. 1 is not meant to imply that the interface necessarily runs along the chromosome axis, nor that it is dimensionally correct, nor, indeed, that this is the only way adsorption complexes can be organized into one structure. Variations of the model could, for example, account for "discreteness" of the genetic material in the chromosome. (Chromomeres? see also, Mazia, *15*).

The special conditions described here in some detail do not necessarily apply to all structures containing genetic material nor to all variations of a changing system of which it forms a part. Thus, for instance, the evidence that in certain systems (phage, transforming principle) the nucleic acid only is transferred is not incompatible with the idea that the participation of this material in the host's cell economy results in the same *type* of structure as discussed here. Neither is the principle of the duplicating mechanism restricted to desoxyribose nucleic acid as one of the participants. The evidence that the plant viruses contain ribose nucleic acid only (where one definitely does not find base pairs) should serve as a reminder that genetic mechanisms should not be looked for in the properties of particular substances but in the way the whole system is organized (*16, 17*).

References and Notes

1. J. D. Watson and F. H. C. Crick, *Nature* 171, 964 (1953).
2. J. D. Watson, *J. Cellular Comp. Physiol.* 45, Suppl. 2, 109 (1955).
3. M. Delbrück *Proc. Natl. Acad. Sci. U.S.* 40, 783 (1954).
4. M. Feughelman *et al., Nature* 175, 834 (1955).
5. F. H. C. Crick, *J. Cellular Comp. Physiol.* 45, Suppl. 2, 183 (1955).
6. J. D. Watson and F. H. C. Crick, *Cold Spring Harbor Symposia Quant. Biol.* 18, 123 (1953).
7. G. Gamov, *Nature* 173, 318 (1954).
8. N. Arley, *ibid.* 176, 465 (1955).
9. D. Schwartz, *J. Cellular Comp. Physiol.* 45, Suppl. 2, 171 (1955).
10. L. S. Lockingen and A. G. DeBusk, *Proc. Natl. Acad. Sci. U.S.* 41, 925 (1955).
11. D. P. Riley and U. W. Arndt *Nature* 172, 294 (1953).
12. R. D. Hotchkiss, *J. Cellular Comp. Physiol.* 45, Suppl. 2, concluding remarks (1955).
13. C. N. Hinshelwood, *J. Chem. Soc.* 1952, 745 (1952).
14. ———, *ibid.* 1953, 1947 (1953).
15. D. Mazia, *Proc. Natl. Acad. Sci. U.S.* 40, 521 (1954).
16. A discussion of these aspects and the problems connected with meiosis, mutations, and differentiation is in preparation.
17. My thanks are due to my colleagues for helpful criticism. The basic ideas underlying the present approach were developed in discussions with Per Oftedal during his stay in this department in 1952.

Lamont Natural Radiocarbon Measurements III

W. S. Broecker, J. L. Kulp, C. S. Tucek

The previously published radiocarbon measurements from this laboratory (*1, 2*) were made in 1950–1952 with the black-carbon method (*3, 4*). Subsequent to this period, the increasing frequency of atomic tests and, later, the larger shots which produced continuous long-term fallout, caused sufficient air contamination to render the black-carbon method unreliable unless elaborate precautions were taken and multiple runs were employed. By the end of 1953, the pioneer work of de Vries and Barendsen (*5, 6*) with carbon dioxide and Suess (*7*) with acetylene had shown that proportional gas-counting methods have some distinct advantages. A gas proportional-counting system was then designed and constructed at Lamont Observatory (*8*). This paper (*9*) reports the results obtained by the black-carbon method during 1953 which are considered reliable and those obtained by the new carbon dioxide proportional-counting method.

Techniques, Assumptions, and Errors

The carbon dioxide proportional-counting system is superior to the black-carbon method for several reasons: (i) it has virtually 100-percent counting efficiency compared to 6-percent; (ii) it is free from air-borne fission product contamination; and (iii) it can be readily adapted to samples containing as little as 0.1 gram of carbon. The use of carbon dioxide is preferred over acetylene because of the absence of explosion hazards, the possibility of going to higher pressures with a consequent increase in sample/

The authors are on the staff of the Lamont Geological Observatory, Columbia University.