It would also be of interest to know the response of the calcareous organisms to high ambient partial pressures of CO_2 sufficient to produce undersaturation with respect to $CaCO_3$. Two questions require answers: can these organisms build their shells at all under unsaturated conditions; if so, what is the kinetics of the heterogeneous redissolution reaction—can the shells remain undissolved during the lifetime of the organisms? It would be reassuring if the answers to these questions were affirmative.

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Hypothesis on Differentiation and the Inheritance of Gene Superstructure

P. R. COOK

Sir William Dunn School of Pathology, South Parks Road, Oxford

It is suggested that cellular differentiation is established during development by the development of higher order structural polymorphism of the chromosomes. The differentiated superstructure, once adopted, would be maintained during replication.

It is now widely assumed that nearly all the cells of one organism contain similar genetic information; their DNA contains identical base sequences[1]. During differentiation, identical genes are therefore expressed differently in the cells of one organism. In diploid cells the two copies of each gene are probably coordinately controlled and so differences in the expression of the same gene must be studied in different cells. A more critical approach is to consider those situations where homologous DNA molecules, within one cell, behave differently. For example, genes on only one of the two X chromosomes in female cells of eutherian mammals are expressed[2–5].

Because of the success of the ideas of Jacob and Monod[6,7] in explaining the regulation of bacterial gene expression, differences in the behaviour of identical genes in differentiated cells are usually explained in terms of an association of genes with repressor or activator molecules[8]. I propose an alternative mechanism for maintaining the inactivity of the X chromosome. Differences in the expression of identical base sequences might arise from differences in gene superstructure. The term superstructure will be used to include the secondary and higher order structures that might be superimposed upon the primary base sequence of a nucleic acid. If gene superstructure is an important factor determining gene expression, perhaps superstructures of DNA, like primary base sequences, may be replicated and inherited. DNA might therefore contain two kinds of heritable information: one kind which is stored in the primary base sequence of a gene, and a second kind which is acquired during development and is contained in its superstructure. The idea is elaborated in a manuscript to be published elsewhere.

Mammalian X Chromosomes

Normal female cells from adult eutherian mammals possess two X chromosomes, for which there is convincing evidence that only one specifies the synthesis of proteins[2–5,9]. The mechanism that creates the difference in activity acts randomly and so cannot be influenced by particular maternal or paternal base sequences. Differentiation of the two X chromosomes occurs early in development and is heritable: the progeny of a cell in which the maternal X chromosome is inactive have inactive maternal X chromosomes[3]. When cell hybrids are made by fusion the X chromosomes of the hybrid cell retain the properties they had before fusion[10,11]. An X chromosome, once inactivated, is not influenced by the presence of additional X chromosomes in the cell and so an acquired characteristic is inherited. Any complete explanation of the inactivation of the X chromosome should account for the stable inheritance of the inactivity of a particular X chromosome. (The creation, *ab initio*, of differences in behaviour of the two X chromosomes will only be of peripheral concern in this discussion (see ref. 5).

"Differential"

The term "differential" is defined as that which constitutes the specific difference between nucleic acids with identical coding properties and which causes their differing behaviour. The differential is recognized by its property of permitting a gene to be expressed. The creator of the differential is

defined as the differentiator. There are two kinds of differential, simple and complex. Identical base sequences may behave differently either because their superstructures are intrinsically different or because they are associated with regulatory molecules in the environment. A difference in gene superstructure would constitute a simple differential; a complex differential depends upon the association of a differentiator with a gene.

The repressor and activator molecules discussed by Jacob and Monod[6] are examples of differentiators involved in a complex differential. Complex differentials, which depend upon the continued association of differentiators with genes, can be inherited only if the differentiator as well as the target gene is replicated. If the differentiator is a protein then a self-maintaining loop involving the protein, the gene that codes for it, and its target gene must be involved. This is because proteins, unlike nucleic acids, cannot be templates for their own synthesis. In organisms which show complex patterns of differentiation, each pattern might be associated with a specific self-maintaining loop, but it has been argued that this is unlikely[12]. Furthermore, small differentiator molecules, being diffusible, should affect all target genes in a cell so that special mechanisms must be invoked to explain the activity of only one of the two X chromosomes in a cell.

The Superstructural Differential

In one cell, two homologous DNA duplexes may be expressed differently because they differ in superstructure. Very little is known about the organization of DNA in chromosomes so that ideas about superstructures are necessarily vague, but these differences may be in base stacking, in sense or degree of supercoiling[13] or in other forms of higher order structures[14,15]. Perhaps when DNA is in the B conformation, transcription cannot occur because of the inability of a nascent RNA molecule to adopt this conformation. A gene might be expressed because its DNA exists in the A or C conformation whilst its homologous partner is not, because it is in the B conformation.

A simple form of superstructure is a coiled coil in which tertiary turns are imposed upon the helical turns of the DNA duplex. (In the following discussion two assumptions are made unless stated otherwise. The first is that no covalent bonds are broken. The second is that there is no net rotation of bases about sugar-phosphate bonds in either of the backbone polynucleotide chains of the duplex. This is a plausible assumption because the forces maintaining the double helical structure restrict such free rotation.) Extra supercoils can be maintained in DNA if net rotation about the axis of the helix is restricted, perhaps by the action of histones or by attaching the ends of the duplex to a larger structure, for example, the nuclear membrane. Extrinsic factors restrict rotation in these cases; rotation would be intrinsically restricted in a circle formed by joining the ends of the duplex as is the case in the circular DNA of viruses. (Circles are formally equivalent to linear duplexes in which free rotation of the ends is forbidden.) Additional supercoils in a linear molecule could be maintained intrinsically if the DNA were organized into loops, for example, of the type seen in the lampbrush chromosomes of amphibian oocytes[16].

How might differences in superstructure be created in similar DNA molecules? Superstructures might be introduced after synthesis by coiling the duplex or by cutting one strand of the duplex, introducing superhelical turns into it and then mending the cut. Enzymes that change the superhelical properties of DNA have recently been isolated from both animal and bacterial cells infected with viruses[17–19]. Alternatively, superstructures might be introduced during the replication of DNA. For example, if DNA synthesis on one strand occurs in the A configuration and on the other in the C configuration, on subsequent relaxation to the stable B form, progeny supercoils of opposite sense would be formed.

How might superstructures of nucleic acids be replicated and inherited? Perhaps defined superstructures are reintroduced into the DNA during each cell cycle by the action of diffusible differentiator molecules. This mechanism involves a complex differential; the superstructure, although it controls gene expression, is maintained by a continuing association with differentiator molecules. Alternatively, the replicating machinery of cells might duplicate not only the primary base sequence but also the superstructure of DNA. For example, consider the semi-conservative replication of a circular duplex of DNA proceeding by strand separation and progressive synthesis of daughter strands around the circle. If no covalent bonds are broken, two interlocking circles result, each topologically identical to the parent. (The number of times one circle interlocks with the other is a function of both duplex and superhelical turns.) It is not surprising that superstructure is conserved on replication in this way because, from the point of view of one strand of the parental double helix, all that has been done is to remove a complementary strand and replace it with a newly synthesized strand. The progeny circles can only be freed from one another by cutting covalent bonds to open the duplex. The cut ends must then be mended after separation of the progeny to reform two isolated circles. (Of course, circle opening and closing probably occurs continuously during synthesis.) If net rotation about the axis of the helix is forbidden during opening of the circle, the progeny duplexes remain topologically identical to the parent. It seems likely that during circle opening the cut ends of the duplex will be held in some way, so that they can be rejoined after separation of the progeny. They might be held by the enzymes responsible for making the cut or by some supramolecular organization of the duplex. This holding of the cut ends implies a restriction of rotation about the axis of the helix. If the circle of DNA contained superhelical turns these plausible restrictions ensure that its superstructure is automatically replicated together with its primary base sequence. If supercoils can be maintained in linear DNA molecules they, too, will be conserved on replication in this way.

It has been argued that a simple differential can be automatically replicated and inherited. Once established, a superstructural differential, unlike other differentials, does not require the continued operation of the mechanisms that created it. The idea that development proceeds by a process of directed gene mutation appealed to the early embryologists for the same reason. Specific mutations can be inherited without the operation of specific self-generating loops; so, too, might specific superstructures of DNA. The crux of the problem posed by differentiation concerns the way differentials can be inherited. Superstructural differentials, but not those of other kinds, can be inherited without invoking self-generating loops.

The superstructural hypothesis requires that DNA molecules which have identical base sequences may be polymorphic. We know that the small circular DNA molecules of viruses can adopt a variety of configurations. These molecules contain superhelical turns in addition to the helical turns of the DNA duplex. Intact and closed circular DNA of SV40 can be purified from isolated virions or from cells infected with virus. One strand of the double stranded DNA from these viruses can be cut *in vitro*, and this is followed by a dissipation of superhelical turns; the cut can be subsequently mended by polynucleotide ligase. Intact and circular DNA molecules from SV40 may therefore be obtained from the virions, from cells infected with virus and by making the "relaxed" form *in vitro*. Although these DNA molecules have identical base sequences they differ in superhelical properties[20]. Parental and progeny SV40 DNA molecules have the same superhelical density, which differs

from that of "relaxed" molecules; a defined configuration is faithfully replicated and inherited during the normal growth of the virus.

Cis and *Trans* Effects

The genes on X chromosomes are coordinately activated or inactivated. Presumably controlling elements on the X chromosome determine the activity of neighbouring genes on the same chromosome (*cis*) but not the activity of genes on the other X chromosome in the same cell (*trans*). What permits these controlling elements to transmit information to genes located *cis* but not *trans*? It is generally believed that information is transmitted to genes located *cis* through polycistronic RNA molecules or through a molecular bridge built from diffusible molecules by cooperative effects. Alternatively, information might be transmitted along a chromosome through the superstructure of DNA. Indeed, a superstructural differential might be expected to lead directly to *cis* effects. The *cis* inactivity of the genes on the X chromosome is correlated with gross condensation of the chromatin to form a Barr body[21].

How might one distinguish between the effects of the two types of differential? First, if the superstructure of a gene is determined by that of neighbouring DNA, translocation of genes might affect their function; on the other hand, translocated genes should still be subject to regulation by diffusible differentiators. Second, cell fusion mediated by Sendai virus can be used to construct binucleate cells from parental cells with genomes that probably contain identical base sequences but which have different phenotypes. Such studies should provide insight into whether the differentiated phenotype results from *cis* or *trans* phenomena. Third, if superstructures of DNA can be changed, for example by the intercalation of drugs, gene expression governed by a simple differential should be modified. Perhaps the remarkable effects of the thymidine analogue, 5-bromodeoxyuridine, on the expression of differentiated traits result from effects on gene superstructure.

Implications of Heritable Superstructural Differentials

It has been argued that superstructural differentials can be automatically replicated and inherited. Once established, a superstructural differential, unlike other differentials, does not require the continued operation of those mechanisms that created it. It also provides a simple explanation of *cis* phenomena. Only genes on a chromosome that has adopted a particular superstructure might become inactive; those on other genetic elements might be unaffected. Perhaps heritable differentials have a superstructural basis; other types of differential might have other bases. If so, the superstructure of a gene would be one factor in the hierarchy of mechanisms which govern the expression of the gene. Diffusible differentiators might regulate gene expression within the limits imposed by that superstructure and they would be involved at the creation of the superstructural differential. But the superstructure, once created, would be inherited as an acquired characteristic. The development of an organism would then proceed by an orderly acquisition of gene superstructure.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Rotation of the Earth's Magnetic Field

HALLEY[1] first noticed that the magnetic declination at a number of sites changed with time in a manner that was consistent with a steady westward drift of the magnetic field relative to the surface of the Earth. After long neglect, interest in westward drift was revived by the work of Bullard *et al.*[2], who examined the westward drift of the non-dipole part of the field, which they considered to originate less deep within the core than the slower moving dipole field. Since then, there have been numerous other studies (for example refs 3-5) introducing various refinements, such as separation of the field into drifting and standing parts, variation of drift rate with latitude and separate rates of drift for each harmonic component. A common feature of all such studies is that they consider the drift of the field to be a rotation about the geographical axis. Here we remove this constraint and examine the possibility that the secular changes in the magnetic field might be more closely represented by rotation about an axis other than the geographical axis. This should not be confused with the "northward drift"[6,7] which is merely the change in the latitude coordinate of the eccentric dipole position.

To avoid spurious effects due to different distribution of data at different epochs, we used the main field models of Malin[7] which were all based on 5-yr means of observatory data from the same set of eighty observatories for each epoch (1942.5, 1947.5, 1952.5, 1957.5 and 1962.5). These models are sets of spherical harmonic coefficients to degree and order 6 referred to a geographical polar coordinate system (r, θ, λ), where r denotes radial distance from the centre of the Earth, θ denotes colatitude and λ denotes east longitude.