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Some European Contributions to the Prehistory of Molecular Biology

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Coils, spirals and helices before the days of DNA. This is an account of the first meetings of geneticists and crystallographers addressed to the nature of chromosome and gene.

THE establishment of the double helix structure of DNA by Crick and Watson is certainly the greatest discovery in biology in this century. The events that led up to it are already engaging the attention of historians of science, and will continue to do so. The subject has so far been dealt with chiefly by American authors—particularly Gunther Stent[1-3]. It therefore seems appropriate to place on record some data about relevant events which were little if at all published at the time, and of which I am probably one of the few people to have preserved records.

During the middle thirties, a number of small meetings of biologists and interested physicists and chemists were organized, with financial help from the Rockefeller Foundation, at various places in Europe. The leading people were H. J. Muller, Timoféeff-Ressovsky, Niels Bohr and Max Delbrück. The early meetings were, I think, almost entirely concerned with gene mutation, particularly as affected by X-rays. I have a typewritten "Summary of Discussions on Mutation" drawn up by Delbrück and Timoféeff-Ressovsky following a meeting held in Copenhagen on November 28 and 29, 1936, which was also attended by Muller and Bohr. (A copy has been deposited in the library of the Institute of Animal Genetics, Edinburgh.) The essence of the statement was to establish a quantum-chemical picture of the gene, and to consider how mutations might result from ionizations or excitations causing the molecular configuration to shift into another stable state. There was no discussion (at any rate recorded) of the nature of the chemical compounds involved.

More relevant to the development of general molecular biology was another similar meeting, held at Klampenborg near Copenhagen from April 2 to 5, 1938. Neither Bohr nor Muller attended—the latter was in Russia at the time. The meeting was not concerned so much with mutation, but rather with cytology and the chemical nature of the chromosome and the gene. The participants were W. T. Astbury, P. Auger, H. Bauer, J. D. Bernal, C. D. Darlington, B. Ephrussi, A. Fischer, L. Rapkine, H. Stubbe, N. W. Timoféeff-Ressovsky, C. H. Waddington and K. Zimmer. Physics was represented not only by quantum theory men such as Auger and Zimmer, but by X-ray crystallographers such as Astbury and Bernal; this was the first time that there was a real meeting of geneticists and crystallographers. I remember that the British contingent had a rough crossing from Harwich to the Hook of Holland. We were travelling second class—this was before the days of reasonable travel grants. Most of us tried to sleep on the benches in the general saloon, but Darlington and Bernal kept sea-sickness at bay by the former teaching the latter "all the genetics and cytology anyone needs to know" throughout the course of the night. Before dawn, Bernal had already decided that the mitotic spindle must be a positive tactoid, and that the separation of chromosomes probably resulted from the production by the centromeres of some fluid which formed negative tactoids within the larger positive one.

The discussions were based on reports prepared by the two cytologists Darlington and Bauer. After the meeting, I wrote summaries of these and of the discussions for circulation to the participants; it is from my copy of this document that most of the following notes are taken.

I was at that time also engaged in writing a general account of genetics, which was published the following year as *An Introduction to Modern Genetics* (Allen and Unwin, London; Macmillan, New York, 1939). Privately, I thought of the book as an introduction to future genetics, and I incorporated many far-out ideas such as those aired at Klampenborg*. This book sold fairly widely on both sides of the Atlantic, and probably provided the principal channel by which the Klampenborg ideas reached a wider public (the most important summarizing chapter was also printed as an article in the *American Naturalist*, **73**, 300 (1939)), although Darlington's pre-Klampenborg contributions were also available through his *Recent Advances in Cytology* (1937) and his *Evolution of Genetic Systems*—the preface of which is dated December 1938—was completed a few months after the Klampenborg meeting.

Much of the discussion centred around problems of chromosome mechanics—spiralization and contraction, the attractions which led to pairing in meiotic prophase, the repulsions leading to a chromosome separation in anaphase, and so on. The physical principles invoked were London–van der Waals forces (can they be effective over large enough distances?), the energy of attraction between homologous as opposed to non-homologous stretches, and so on. Much the same questions seem to be in people's minds still at the present day. The point I want to bring out, however, is the extent to which ideas which were later built by Watson and Crick into their great synthesis were already floating around. At that time these notions were not properly connected with one another, and sometimes arose at a different scale from that on which they were finally utilized—for example, in connexion with whole chromosomes rather than with DNA helices; but at least they were in the minds of molecular-minded biologists.

* The Genetics Establishment of the time was not impressed; to such an extent that a group of forward looking geneticists took the almost unprecedented step of writing to protest against the review which had appeared in *Nature*[4].

Perhaps the most obvious of these general notions is that which we then called "spirals", which are now more usually referred to as helices. Darlington's report described the observable phenomena of coiling in some detail, and goes on to deduce that these must be the results of an underlying coiling at the molecular level.

From Darlington's Report to Klampenborg

"*Internal coiling.* The chromosome threads contract to form an internal coil in the prophase of every mitosis, the two daughter chromatids of each chromosome separately side by side. They do so by increasing the amplitude and reducing the number of their coils. In the daughter nuclei the coiling is undone by continuing the same process. Owing to lack of space the uncoiling of the relic spiral is delayed and not completed until the metaphase of the next mitosis. A new coiling therefore arises underneath the old one. At meiosis the coiling is carried a stage further, a *major* spiral developing above the minor one (or vice versa). Is this due to a greater surface charge ?

"*Systems of coiling.* Two chromosome threads form a joint spiral in meiosis but not in mitosis, owing perhaps to stronger attraction. Two chromosomes under torsion develop relational coiling instead of internal coiling at the pachytene stage in meiosis, perhaps owing to the coiling being in the opposite direction to that causing internal coiling, or owing to greater attraction preventing slipping ?

"*Molecular spiral.* Direction of relational coiling of chromosomes and of internal coiling is consistent for whole arms, that is, between a centromere and an end. Torsion of two paired chromosomes must be in same direction to produce any relational coiling at all. Yet their internal coiling may be in opposite directions. Therefore torsion producing the two kinds of coiling in the same chromosome at different times need not be in the same direction. Thus although the same molecular structure must underlie both kinds of coiling, the direction of coiling must be optional and under the unitary control of the centromere."

Note that Darlington describes "relational coiling", in other words, two threads mutually coiling round each other, producing a structure which we would now call a double helix; but he describes this at the scale observable through the light-microscope. We had at that time no inkling of the great Watson–Crick breakthrough which solved so many problems by transferring this same idea down to the molecular level. We did, however, consider the problem of why chromosomes tend to have a double structure rather than consisting of three or more unit-strands, and we developed a notion of a "sticky face" which (again making allowance for a change of scale) is quite like the idea of the double helix being held together by the attractions of the base sides of the chains.

From the Klampenborg Report

"The greatest difficulty of a theory of pairing is to account for the general limitation of attraction to twos. . . . (Section on polyteny as a special case omitted.)

"Possible theories of the limitation of attraction to pairs are:

"1. The chromosome has a single attracting face. This would raise difficulties about mirror-imaging when the chromosome divides, and perhaps about the splitting of already paired chromosomes at pachytene.

"2. An explanation might be sought in the phenomena of spiralization. The relational coiling of two paired homologues may make it physically impossible for a third to approach. This suggestion was not fully worked out.

"3. The facts would be explicable if the two paired threads caused some alteration in one another, which prevented attraction of any further threads. But the nature of the alteration is quite obscure. There may be some indication of such an alteration, at least at the pachytene stage, in the fact that at this stage the chromosomes are so tightly attached that they cannot turn and have to develop relational coiling. At later stages they can turn independently and the failure of attraction in more than pairs is due to a general increase in the repulsive forces, since at mitotic metaphase even the paired chromatids are not very strongly attracted.

"The last remark draws our attention to the necessity of considering the chemical aspects of chromosome pairing. If we assume that chromomeres are comparable to molecules which, under the influence of change in the environment, become capable of homopolar reaction, we can understand that in general there is pairing by twos, the encounter by threes being very improbable. The alteration mentioned above would consist of the loss of reactivity of already polymerized molecules (in analogy with the bimolecular reactions)."

Some quotations from my *Introduction* illustrate other aspects of the ideas of that time.

" . . . the interpretation of coiling in terms of the internal structure of the chromosome. There are two general lines which such an interpretation can take. It may, on the one hand, be suggested that the individual genes have some internal spiral arrangement (perhaps to be compared with the indications of spiral structure in the molecules of proteins such as insulin (Crowfoot *et al.*, 1938)), and that this determines the formation of the visible spirals; these would then be expected to be consistent for considerable lengths of the chromosome. Or, on the other hand, one may point out that any elongated body consisting of fibres orientated parallel to its length tends to become a spiral if its surface contracts; but in this case there are usually frequent reversals of the direction of coiling. In both cases the actual assumption of the coil must be regarded as a response to changed environmental conditions. . . .

" . . . This relational coiling suggests one of two conclusions about the nature of the chromosome thread. Either the attractive forces in zygotene are much more intense than at later stages when each chromosome coils independently of its partner; or each chromosome has a single 'sticky face' along which the adhesion takes place. The latter conclusion is perhaps unlikely; it would certainly raise considerable difficulties about the mechanism of reduplication of the chromosomes. . . ."

Structure and Chemical Nature of the Gene

First of all, and rather as a parenthesis, it may be remarked that we had already realized the necessity to distinguish between the gene as a unit of heredity (in recombination), as a unit of developmental action, and as a unit of mutation (later christened the recon, the cistron and the muton). Darlington (chapter 10, "The Atom of Genetics", in *Evolution of Genetic Systems*) defined the unit of mutation in terms of visible chromosome breakages by X-rays and states that . . . "It is only possible therefore to take a unit of breakage by X-rays as having unconditional validity. . . . It is the gene of physics, biologically absolute". In my *Introduction* (page 397) I allowed more importance to gene mutation, as opposed to chromosome breakage, and to the small size of the "sensitive volumes" which had been deduced for the regions involved in a single mutational event.

Our attempts to go further in imagining a structure for the genetic material were largely frustrated by two defects in our knowledge. At that time, all the genetic material known to us contained protein as well as nucleic acid. Further, although proteins were known to have a structure complex enough to form many different genes, it was not clear that nucleic acid also had; current theory regarded it as merely regular repetitions of a sequence of four bases. As will be clear from the suggestions of Astbury, to be quoted in a moment, we did not attach much credence to this theory, but were ready to attribute to the nucleic acid a structure complementary to, and therefore as complex as, that of the protein. Gunther Stent[5] states that the virus geneticists of a few

years later, who he claims were totally sceptical about all previous work, took this particular doctrine so seriously that it prevented them accepting Avery's demonstration of the leading role of nucleic acid until it had been confirmed on their own favourite material by one of their own group—an unfortunate misplacement of belief, if true.

The chemical nature suggested for the genetic material at Klampenborg was therefore that of a compound of protein and nucleic acid. Earlier ideas, such as those of Wrinch[6] and Koltzoff[7], supposed that the chromosome is composed of a "genonema" thread in the form of a polypeptide chain, to which the genes are attached as side-chains, which might either be active compounds, such as steroid hormones, or other polypeptides, perhaps combined with nucleic acid. In the Klampenborg suggestions, the chain stretching along the length of the chromosome was considered as constituting the gene itself, rather than forming a string to which the genes are attached.

From the Klampenborg Report

Division of chromosomes and genes. "Different views were expressed on the molecular models necessary to account for the cytological and X-ray genetical evidence. Although the structure of the chromosomes has not yet been directly studied by X-rays, analogies can be drawn with the structures of known substances. The two main suggestions for its structure are:

"(*a*) A set of more or less extended peptide chains (Astbury) (repeat unit of protein chain peptide link 3·34 Å (backbone spacing); closest distance between chains 4·5 Å; side-chain spacing 10 Å). The nucleic acid has a repeat unit 3·36 Å, nearly the same as that of peptide. Astbury suggests that nucleic acid chains form links which enable peptide chains identical with the original chain to be synthesized. Nucleic acid chains have two sides, one with purine residue and one with phosphoric acid. One of these would be attached to the original protein chain and the other would build an amino-acid into a new chain, the interposition of the nucleic acid ensuring that the new chain would have the same configuration as the old and not be a mirror image of it. The general hypothesis has the advantage that the identity of any particular part of the chromosome can be preserved in growth, but only on the assumption that the effective part of the chromosome consists of one chain can the division into two equal parts be simply explained. This seems unlikely in view of the estimate of the size of the chromosome, of the order of 2000–6000 Å in diameter, or of Muller's estimate of gene size $200 \times 200 \times 1000$ Å. (*b*) The chromosome is of the virus type nucleoprotein (Bernal), a chain of sub-molecules of approximately $150 \times 150 \times 70$ Å as observed in tobacco mosaic virus. The difficulty here is to explain how such a chain can multiply laterally, but as we do not know the mechanism of any ordinary protein formation, the question is probably premature.

"The difficulty is not so much how to imagine such a chain multiplying itself, but how that multiplication can be limited to two. Any hypothesis that the original chromosome has one particular face to which new material is added gives rise to the difficulty about the mirror image formation, though this itself may be more formal than real. It is suggested (Bernal) that if the proteins did not merely multiply two-fold, but indefinitely, they might tend to form hollow or solid fibres which might beyond a certain size become unstable and divide along its length into two fibres, in the two-dimensions analogy of the splitting of a liquid drop."

Linearity of the sub-structure of the gene. In my *Introduction* I carried what may be called the Astbury line of thought a stage further. There was at that time little genetic evidence about the sub-structure of the gene. Muller had shown that deletions and inversions may occur down to the limits of visibility, and suggested they might occur below that: Dubinin had advanced a somewhat shaky theory of a linear order of "step-alleles" at the *scute* locus in *Drosophila*; students of X-ray mutation had come up with estimates of "sensitive volumes" only a few per cent of the estimated size of the gene; but that was about all. As we saw, Darlington accepted the unit of (visible) chromosome break as the ultimate unit. I pointed out that it seemed more reasonable to suppose that the genetic material is linearly organized right down to the individual peptides (or peptide–nucleotide units postulated by Astbury).

"It is clear that the old picture of the chromosome, as a linear array of individual indivisible particles, each of which is a gene, is too simple. In attempting to work out a more adequate picture, one can start from the fundamental fact that the chromosome is an elongated structure which, whenever we can analyse it, has differences arranged in a linear order along it; these differences can be detected by linkage studies, chromosome structures, etc. The units, between which differences are noted, may be of different sizes according to the different methods of investigation; there are, in roughly descending order, inert or precociously condensing regions, large chromomeres, ultimate chromomeres or salivary gland chromomeres, and the units of cross-over and X-ray breakage. One might symbolically represent the chromosome thus: abcd′e′f′g′hijklMNOPQRSTU′V′W′ where there are differences on three scales, between the capitals and lower-case letters; normal, underlined and dashed letters; and finally the letters themselves. The smallest units of this scheme, symbolized by the individual letters, are the units of crossing-over and X-ray breakage, and probably measure, as we have seen, about 100 mμ in length.

"If we view the chromosome as it were through the other end of the telescope, attempting to build it up from chemical units, we arrive at a somewhat similar scheme of a linear order of units of different orders of magnitude. The ultimate units now are the links in a polypeptide chain, with a length of only 0·334 mμ. Exactly what the larger units are is more doubtful, but we have a range of possibilities; there are the periodicities along the chains, the repeat units out of which protein crystals are built, the protein molecules such as they exist in solution, and finally virus particles, all of which may be considered as providing suggestions as to the kinds of units which may be involved. These units range in size nearly up to the 100 mμ which we took as an estimate of the smallest units to be considered when we approached the chromosome structure from the other end. It is, then, possible to conceive of the chromosome as a linear array of units, the units themselves forming a hierarchy all the way from heterochromatic and euchromatic regions, some tens of thousands of mμ long, to polypeptide links only a few tenths of a mμ long."

This point is, of course, one of the principal contributions of the American phage group to the origins of molecular biology. It is the "Benzerization" of the gene into individual small-molecular building blocks which is one of the main points of the story Gunther Stent has to tell—the other is the fact that these building blocks are nucleotides, not peptides, but in that they were really anticipated by Avery. The American work, of course, provided the solid evidence by which the point was established, but the theory which was thus confirmed had originated in Europe some years earlier*.

* In a letter, Gunther Stent writes " . . . In any case, it is my belief that though men like Darlington and Muller certainly posed the problem of the molecular basis of the gene thirty-five years ago, their ideas had, in fact, little influence on the later development of molecular genetics. Indeed, even the "one-gene-one-enzyme" theory was of less significance for the young Turks of the late 1940's than is generally supposed nowadays. For one main characteristic of the members of the American Phage Group was that they didn't believe *anything* that anyone had said or done before and insisted on working out everything for themselves. . . ." It would, of course, be a poor lookout for the advancement of science if young men started believing what their elders tell them, but perhaps it is legitimate to remark that young Turks look younger, or more Turkish or what have you, if the conclusions they eventually reach are different from what anyone had said before.

The Role of Schroedinger

The main vehicle for "Klampenborg-type" ideas, which the molecular biological "breakers-through" recognized as an influence on their outlook, is Schroedinger's short but brilliant book, *What is Life?* (Cambridge University Press, 1944). There is no doubt that, deservedly, it had very great influence, both because of its elegance and intellectual quality, and because it carried the authority of one of the leaders of theoretical physics. But although it is actually the first statement of a profound theoretical question, it is not because of this that it has been so influential.

The part of *What is Life?* that made the original impact is in fact a re-writing of the classical paper which we used to refer to as TZD (ref. 8) in which X-ray mutagenesis was interpreted in terms of a "gene molecule" the stability of which was ensured by quantum-mechanical considerations. This was the first public origin of the whole idea that the genetic material can be thought of in quantum-mechanical terms, and it was this, in Schroedinger's version, which fired the imagination of the early molecular biologists of the immediate post-war years. However, his epigram, that the gene is an aperiodic crystal, is an exceedingly paradoxical phrase, because crystals normally not only have periodicity but three dimensions, whereas the main point about the carrier of heredity is that it is linear—one dimensional. I still feel that the model presented in my *Introduction* is both less equivocal in meaning and nearer the actual facts.

What is Life ? did, however, contain a genuine new and important idea. The stability of the gene molecule is a matter of quantum laws; there is only one (or in diploids, two) such molecules per cell. Yet the gene controls the production of massive quantities of protein molecules, of such a magnitude that they enter the domain of classical, rather than quantum, physics. How is this transition made ? This is the general problem of "quantum measurement". It did not, however, form any noticeable part of what the physicists who entered molecular genetics in the late forties took away from their reading of Schroedinger; and it is only quite recently that some theoretical physicists interested in biology are taking it up again[9].

Thus by 1940 the advanced views about the genetic material contained the following points. (1) Some sort of coiled conformation is incorporated into the structure at a very basic, probably molecular level. (2) Two coiling threads may twist round one another to form a double helix. (3) These coiling threads may not be radially symmetrical around the long axis, but probably exhibit a bilaterality, evidenced by the possession of one "sticky face" by which two threads can adhere to one another. (4) The genetic material is probably linearly organized down to the level of small molecular building blocks, of the order of size of peptides, as opposed to consisting of larger genetic units attached like beads on a string. (5) There is not adequate evidence to refute the hypothesis that nucleic acid has as complicated a linear structure as proteins. (6) An attractive notion is to suppose that the genetic material is composed of a compound of two complementary linear sequences; at that time, this was thought of as complementary polypeptide–polynucleotides.

The principal new ideas involved in the Crick–Watson synthesis were the following. (*a*) That the essential genetic material is entirely nucleic acid (Avery, followed by Hershey and Chase). (*b*) That the chemical constitution of DNA is such that a complementary relation between two DNA strands is possible, marrying purine with pyrimidine (a brilliant interpretation of Chargaff). (*c*) That a complementary-duplex structure, at the level of magnitude at which synthetic processes operate (at the molecular level), solves the problem of identical duplication without mirror-image formation. (*d*) That the molecular DNA double helix can be built as a model, and fits the available X-ray data.

Of these later points, only (*c*) is a matter of pure logic, which might have been seen (but was not) in 1940. But I wonder how much sooner the factual information on which the others depend might have been discovered, if the Second World War had not disrupted the lines of thought which led in the direction of them ?

In the historical beginnings of molecular biology there were then three major influences. (1) German quantum physics: Bohr, Delbrück and Schroedinger. (2) Anglo-American genetics and cytogenetics, derived chiefly from Muller and Darlington, but with less penetrating contributions from German sources, such as Bauer and Timoféeff. (3) Principally English molecular morphology, using methods derived from the Braggs as developed by Astbury and Bernal.

[1] *Phage and the Origins of Molecular Biology* (edit. by Cairns, J., Stent, G. S., and Watson, J. D.) (Cold Spring Harbor, NY, 1966).

[2] Stent, G. S., *Science*, **160**, 390 (1968).

[3] Lamanna, C., and Stent, G. S., *Science*, **160**, 1398 (1968).

[4] Haldane, J. B. S., Huxley, J. S., and Muller, H. J., *Nature*, **144**, 78 (1939).

[5] Cf. correspondence in *Science*, **160**, 1398 (1968).

[6] Wrinch, D. M., *Nature*, **134**, 978 (1934).

[7] Koltzoff, N. K., *Les Molécules Héréditaires* (Hermann et Cie, Paris, 1939), containing earlier references to his publications in Russian.

[8] Timoféeff-Ressovsky, Zimmer and Delbrück, *Natur. Ges. Wissensch. Gottingen*, Fachgr. VI, N.F.Bd.1 (1935).

[9] See, for example, Pattee, H. H., in *Towards a Theoretical Biology*, I, *Prolegomena*, and II, *Sketches* (edit. by Waddington, C. H.) (Edinburgh Univ. Press, 1968 and 1969).