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THE CHROMOSOME

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During the past two decades, an ever-increasing body of scientific workers, drawn not only from genetics and cytology, but from chemistry, physics and mathematics as well, have been engaged in the fascinating game of attempting to unravel the intricacies of the genetic material. As a consequence of their efforts, the most remarkable chapter in the history of biology is presently being written. Part of the interest surrounding the problem arises from the knowledge that its solution will bring man much closer to understanding the origin and nature of life itself; and while the origin of life may "only represent a definite milepost along the general historic road of the evolution of matter," as Oparin so aptly put it, yet to man this constitutes a particularly intriguing milepost.

One phase of this work necessarily involves a study of the chromosome, for it is in these bodies that the genetic material of most organisms is found. The story of the chromosome begins in the last century with the discovery of deeply staining material within the nucleus which displayed characteristically complex behavior when the cell divided and to which Waldeyer in 1888 gave the name "chromosomes." Actually, the christening came a little late, for Flemming had observed and described the mitotic process six years earlier. Two other events, the formulation of the laws of inheritance by Mendel in 1865 and the discovery of nucleic acid in the nuclei of pus cells by Miescher (1869) were to provide function and substance to these bodies, but the implications of these discoveries were not immediately apparent. Mendel's findings, which in point of time preceded the discovery of the chromosome, were ignored until 1900. Yet it was only two years after their rediscovery that Sutton suggested that the physical basis of the laws of heredity might be found in the behavior of chromosomes during meiosis. The vast body of knowledge that has accumulated since that time concerning the inheritance of chromosomes and their component parts, the genes, constitutes the science of genetics. The information derived from genetic sources must play an essential part in concepts of chromosome structure.

Recognition of nucleic acid as the primary genetic material was much slower in coming. Caspersson (1936) presented evidence for the nucleoprotein nature of the gene based on UV-absorption studies with the salivary gland chromosomes of *Drosophila melanogaster*. Shortly afterward, Hollaender (1939) and Emmons and Hollaender (1939) demonstrated that the UV action spectrum for mutation was similar to the UV absorption spectrum for nucleic acid, a finding that strongly implicated nucleic acid as the genetic material. In 1944, Avery, MacLeod and McCarty isolated the factor causing genetic transformation of characters in bacteria (the transforming principle) in nearly pure form and identified it as deoxyribonucleic acid. Probably this discovery, above all else, served to focus attention on the chemical nature of the gene and to usher in the modern phase of chromosome research.

There is currently an almost bewildering fund of information concerning various aspects of chromosome structure, chemistry and function. The task of organizing this material into a reasonably coherent picture is complicated by wide gaps in knowledge on the one hand and frequent conflicts of opinion on the other. In the following discussion, the chromosome is considered first at the gross, visual level, as revealed by the light microscope; next, at the molecular level concerning which there exists a wealth of biochemical, biophysical and chemical-genetic studies; and briefly, at the electron microscopy level, the meeting ground for chemical and visual information. Finally, an attempt is made to examine the various proposals for chromosome models in the light of the most cogent information and evaluate their suitability as working hypotheses.

GROSS LEVEL MORPHOLOGY

Description of chromosome morphology, except in rare instances, is limited to the chromosomes of cells in the process of division because interphase chromosomes are diffuse and unresolvable. At the beginning of prophase, the mitotic chromosome is maximally extended and consists of two chromatids that are loosely wound about one another and that can only separate freely by a rotation of their distal ends (plectonemic coiling). At this stage one sees in the chromosome a loose spiral (relic coil) that is the remnant of a tighter coil that traces back to the prophase of the previous division. The relic coil is soon eliminated and the chromosome undergoes a new coiling cycle (somatic or standard coil). As prophase progresses, the gyres of the somatic

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coil decrease in number but increase in diameter so as to produce a shortening of the chromosome. While this contraction is occurring, the two chromatids disengage themselves, presumably through a rotation of their ends, and come to lie side by side, held together only by the undivided centromere or proximal region. At metaphase and anaphase, the two coiled chromatids that comprise each chromosome reach their maximum contraction and have a characteristic appearance, determined to a large extent by the position of the centromere, which is constant from cell to cell.

As soon as one attempts to describe the more intimate details of chromosome structure, the subject matter becomes highly controversial. The fundamental unit of the chromosome is considered to be the chromonema, the deeply staining, Feulgen-positive material within which the genes are located. According to one hypothesis, this fundamental unit is a coiled thread without microscopic differentiation whose linearly differentiated regions are all attributable to differential coiling (Ris, 1945). The chromomere hypothesis, on the other hand, maintains that the chromonema consists of chromomeres (bead-like structures) of different but constant size, rich in nucleic acid and connected together by protein fibrils (Kaufmann, 1948). The chromomeres bear the genes, they reproduce as specific units and, in the case of the salivary gland chromosome, they may represent the visual expression of the linear order of the genes. It may well be that light microscope observations, upon which these two extreme viewpoints are based, are incapable of resolving chromosome structure and that the truth may lie somewhere in between.

There is no agreement as to the number of chromonemata making up a chromosome or chromatid; neither is there agreement as to the presence or absence of a matrix around the chromonema or a pellicle around the chromosome. Ris (1957), whose views have colored much of the thinking concerning chromosome structure, holds that the chromomere is only an expression of differential coiling of the chromonema; that the matrix is a shrinkage space; that the pellicle is nonexistent; and that the chromosome is multistranded but that the number of chromonemata may vary widely from species to species and, of course, among the higher taxonomic groups. Other workers, such as Gall (1956) and Callan (1956) believe the chromosome consists of only a single chromonema.

In their attempts to elucidate details of chromosome structure, workers have turned to the giant polytene chromosomes found in the salivary glands of *Drosophila*, *Chironomus*, *Sciara*, and *Rhynchosciara* larvae as well as to the giant diplotene chromosome of amphibia, descriptively termed "lampbrush." The dipteran salivary gland chromosomes in the third larval instar are about 100 times the length of the somatic metaphase chromosomes. The increase in length cannot be attributed to straightening out of the somatic coils alone but may result from an unfolding of the molecular structure of the chromosomes. The increase in width is generally interpreted as a consequence of polyteny, the total chromonemata numbering 1,000 or more. Caspersson's very early work with cytochemical and UV analysis of the salivary chromosomes of *D. melanogaster* (1941) indicated that the banded areas consisted of DNA probably combined with a histone, a globulin and a protamine-type protein; that the interband areas lacked the nucleic acid and histone but contained the two other proteins; and that the nucleolus contained histone and RNA. (Some question has been raised as to whether the spectrophotometric methods used could reliably distinguish between the various proteins.) D'Angelo (1950) dissected out the chromonema and upon stretching the swollen regions has observed that the bands, generally believed to correspond to chromomeres, do not disappear as a consequence of the treatment, as might be expected if they were merely tightly coiled portions. She has concluded from her micromanipulation studies that the chromomeres are not more tightly coiled portion. and that the chromosome possesses a delicate, elastic membrane and a jelly-like matrix. Kaufmann (1956-57) fed tritiated thymidine to Drosophila larvae of various ages and then observed the pattern of incorporation of the radioactive material into the salivary gland nuclei. The label appeared to be restricted to the banded regions since the radioautographs revealed no distintegration of tritium in intervals between bands. The most revealing radioautographs were obtained from larvae fed labelled food throughout development for the bands were then clearly de fined as rows of dots traversing the width of the chromosome.

Although this is admittedly a highly specialized and deviant type of chromosome, nonetheless it is reasonable to suppose that the banded structure observed in the salivary chromosome represents in exaggerated form a basic pattern of organization common to, but invisible in most chromosomes. The correlation between the absence of a particular band and the appearance of a mutant phenotype, as has been demonstrated in *D. melanogaster* (Slizynska, 1938), suggests that the genetic material controlling the ultimate phenotype is located in the banded region. Beerman (1952) using *Chironomus* larvae and Breuer and Pavan (1955) using *Rhynchosciara* have described remarkable transformations that occur in certain bands. These changes take place at definite stages in development and since polyteny is not peculiar to the salivary gland chromosomes, they can be shown to differ from tissue to tissue. Specific bands enlarge into structures known as puffs, bulbs or, in the case of *Chironomus*, Balbiani rings. According to Breuer and Pavan the enlargement is associated with accumulations of DNA, RNA and protein, portions of which are subsequently eliminated and other portions of which are retained, resulting in a temporary and permanent differentiation of the chromosome.

The puffs of polytene chromosomes resemble to some extent the side loops of the lampbrush chromosomes found in the developing oocytes of certain vertebrates that possess a yolky egg. During the diplotene stage of such vertebrates, their chromosomes undergo a tremendous growth and assume a characteristic brush-like appearance due to the formation of radiating hairs or side loops arising from the chromomeres. According to Gall (1956), the loops are part of the continuous chromonema rather than lateral secretions of the chromomeres. An indication of the number of chromonemata present in a chromosome is afforded by the lampbrush chromosomes for although they have grown enormously in length, they are not polytene. Significantly, only a single chromonema is observed in the interchromomeric regions, although Gall (1956) finds in his electron micrographs a basic duality of the structure. Callan and MacGregor (1958) treated the lampbrush chromosomes of *Triturus* with proteolytic enzymes, RNase and DNase, and found that only DNase will disrupt the continuity of the chromosome by causing the submicroscopic fibrils connecting adjacent chromomeres to snap. Their studies also revealed the presence of DNA in the loops. Although, quantitatively, the predominant material appears to be protein and RNA, these fail to keep the loops intact once the axis is destroyed by DNase. The normal disappearance of the loops in post-diplotene stages might be attributed to the shedding of the ribosomal and protein material, which eventually finds its way into the cytoplasm, and the withdrawal of the DNA portion of the loop into the chromomere.

A constant feature of most chromosomes is the presence within them of two types of chromatin, euchromatin, and heterochromatin. As originally defined by Heitz (1929), heterochromatic regions are those that remain condensed and darkly staining during interphase. White (1936) added the concept of negative heteropycnosis for those regions lightly staining and less contracted during metaphase. An extension of these definitions might consider heterochromatin as that chromatin that is out of phase with euchromatin. The discovery that the X chromosomes of some species of grasshoppers are heteropycnotic in the primary spermatocyte but not in the primary oocyte indicates that the expression of heteropycnosis may be variable and dependent upon the internal environment. A number of observations have contributed to the impression that heterochromatin is inert genetic material that may be easily omitted without great detriment to the organism. These include the facts that few genes have been located in heterochromatic regions; that heterochromatic chromosomes, such as the Y chromosome of *Drosophila*, may be added or subtracted from the genome (as in XXY, XXYY, XO, XYY, XYYY aneuploids in Drosophila) without inviability ensuing; that the evolution of the sex-determining mechanism usually proceeds by heterochromatization and subsequent loss of the Y; that supernumerary chromosomes are largely, if not completely heterochromatic; and that chromosome diminution and elimination almost invariably involves heterochromatic material. It has been proposed that heterochromatin is formed from euchromatin by a process of genic deterioration resulting from isolation. Isolation of a chromosome from its homologue can be accomplished by any mechanism that effectively prevents crossing-over between them. A chromosome so isolated would tend to undergo functional degeneration of most of its genes through mutation if natural selection were prevented by diploidy from acting upon it. The evidence, taken in its entirety, indicates that heterochromatin plays, at best, a minor role in heredity.

On the other hand, it appears that heterochromatin is involved in a subtle way in the process of differentiation. For instance, extra heterochromatin added to the genome is effective in suppressing variegation associated with a certain class of position effects in Drosophila. Also, the remarkable studies of McClintock (1951) with the Ac-Ds system in maize indicate that particular "loci" can control events within the nucleus leading to differentiation and that these "loci" may consist of blocks of heterochromatin. Recently Brink (1960) has advanced an admittedly speculative but most interesting model for chromosome organization in which he suggests that two components, orthochromatin and parachromatin, make up the genome. Brink implies that these substances may prove to coincide with euchromatin and heterochromatin. Orthochromatin, which contains the DNA template, is the genetic material proper that is self-replicating, capable of permanent change through mutation, and whose linear arrangement is revealed by the conventional methods of genetic analyses. Parachromatin, although coextensive in distribution with orthochromatin, and self-replicating as well, differs from orthochromatin functionally in that it serves as a kind of messenger between orthochromatin and the metabolically active parts of the nucleus. Thus, its role is a regulatory rather than a genetic one. Whereas orthochromatin is visualized as remaining qualitatively unaltered from one nucleus to another, parachromatin undergoes a succession of different states during ontogeny correlated with the kind of cellular environment in which it finds itself. These qualitatively altered states are mitotically transmissible but they may be reversed. Brink's theory stems from his work with paramutation at the R locus in maize and from the data on mutable alleles. When paramutation occurs, the parachromatin is thought to be irreversibly altered in that generation and these changes are carried over into the gamete nuclei simulating true mutation. Paramutation, unlike true mutation, is not a permanent modification of the genetic material, for there is a reversion to the previous condition after several generations.

Chemistry of the Genetic Material

If we turn now from the morphology of the chromosome to its chemical nature, we begin to deal with material that is better defined, less speculative and hence less controversial. The study

of chromosome chemistry may be approached in a variety of ways. One method is to perform a direct chemical analysis following mass isolation of nuclei and chromosomes. This type of procedure was employed by Mirsky and Ris (1947) using calf thymus lymphocytes. The isolated chromosomes were separated into two fractions, each of which contained nucleoprotein. The fraction soluble in 1 M NaCl consists largely of deoxyribonucleohistone and constitutes 90-92% of the chromosome mass, DNA and histone being present in the ratio of 45:55, respectively. The insoluble residue, called the residual chromosome, accounts for 8-10% of the chromosome and consists of 12-14% RNA, 2-3% DNA and 83-86% non-histone protein. Mirsky and Ris considered that the form of the chromosome was due primarily to the protein thread of the residual chromosome and that either the genes formed part of its substance or alternately that they were organized around it.

A second approach that the cytochemist may take is to determine the effect of specific enzymes on chromosomes. The use of purefied enzymes in combination with various staining procedures affords a reliable method for identifying the chromosomal components and determining their pattern of association. Kaufmann, McDonald and Gay (1951) found that treatment of cells with ribonuclease did not affect stainability with Feulgen but did destroy stainability with methyl green (an RNA stain) demonstrating that the chromosome contains both RNA and DNA. Treatment with deoxyribonuclease showed that this enzyme effaces stainability with Feulgen, thereby serving to locate chromosomal DNA. The use of ribonuclease and acidic dyes, specific for protein, demonstrated that RNA is combined with protein. Treatment with ribonuclease and dilute HCl (the acid impairs stainability of the histone component by acidic dyes) preceding the use of acidic dyes indicates that the protein moiety of the chromosomal ribonucleoprotein is probably a histone. Treatment with DNase and dilute HCl before staining with acid dyes showed that the DNase releases from the chromosomal deoxyribonucleoprotein a non-histone protein in addition to histone. Kaufmann studied the effects of proteolytic enzymes on salivary gland chromosomes. Structural deformation of the chromosome did not occur in aqueous solution of trypsin but was induced by subsequent treatment with phosphate buffer and was attributed to the swelling of partially degraded nucleoproteins in the presence of electrolytes, since removal of DNA inhibited the swelling. This study did not support the view that trypsin causes degradation of the chromosome by digesting those bonds essential to the maintenance of structural integrity. Mazia *et al.* (1947) found, by contrast, that pepsin caused a distinct shrinkage of the chromosome, possibly by the removal of proteins with acidic properties.

The fact that nucleic acids are major cell components suggested that they were involved in some important biological role but with a single exception, there had been no proof of a specific biological function performed by a polynucleotide. The highly significant exception was the case in which DNA was demonstrated to be the bacterial transforming principle. Boivin *et al.* (1948), among others, inferred from this phenomenon that DNA must be very close to the actual genic material. This idea led them to the test the following hypothesis: if DNA does in fact constitute genic material, then its distribution should run parallel to that of the chromosome and gene cycle. They isolated masses of nuclei from various beef organs, determined first the total amont of DNA in each mass and then, by counting an aliquot, the number of nuclei in each organ sample. In this way they showed that the amount of DNA per nucleus was very nearly the same for the different organs, and furthermore, that approximately one-half this amount was found in the haploid nuclei of the bull sperm.

Pollister *et al.* (1951) approached the question of the DNA content of nuclei in a different way. Quantitative analysis of the amount of DNA within single cells may be obtained by spectrophotometric means. This procedure depends upon the Feulgen reaction. The amount of light absorbed by a stained nucleus is translated into the amount of DNA present within the nucleus. The values obtained are not absolute but are multiples of a unit amount corresponding to the amount of DNA present in the haploid chromosome set. The studies of Pollister and coworkers have demonstrated that a close correlation exists between the number of chromosome sets and the number of units of DNA present in any nucleus.

The identification of nucleic acid as the most probable carrier of genetic material radically altered the beliefs held up to that time. Before 1940 most cytologists and geneticists, if queried as to the chemical nature of the gene, would have selected protein as its prime constituent since it alone appeared to possess the specificity necessary for this role. Nucleic acid, which had proved highly refractory to detailed analysis, was in a conveniently simplified manner conceived to consist of four different bases present in equimolar proportions and structurally arranged as regular repeating units of tetranucleotides. A new approach to the study of nucleic acids was made possible through the development of chromatographic techniques for quantitative estimation of the purines and pyrimidines. In 1948, Visher and Chargaff and Hotchkiss, through the introduction of these methods, were able to show that the four bases do not occur in equimolar proportions as postulated by the tetranucleotide hypothesis (Levine and Bass, 1931) and it became possible to consider the nucleic acids as substances comparable to protein in specificity. These studies, and those of Avery, have led to a complete reappraisal of the structure and function of DNA and RNA.

The evidence that supports the hypothesis of DNA as genetic material comes from a variety of sources. One line of evidence is based on the correlation between the properties of DNA and those of genetic material. Thus, if DNA is the chief constituent of genes, it should be located exclusively within the chromosomes and its quantity should be proportional to the number of chromosome sets. The chemical studies noted above have demonstrated that (1) DNA is found almost entirely within the nucleus, (2) its amount is quantitatively correlated with the number of chromosome sets. Experiments indicated that DNA, once formed, is metabolically inert. This property is consistent with its role as a repository of genetic information. A correlation was shown to exist between the UV absorption spectrum for nucleic acids and the UV action spectrum for mutation. This relation has been interpreted to mean that nucleic acid is the target molecule (Hollaender, 1939; Emmons and Hollaender, 1939). Also, experiments with 5-bromouracil have shown that where this analogue of thymine is incorporated into the DNA of a cell, its rate of mutation is greatly increased (Litman and Pardee, 1956).

The best evidence comes, however, from genetic experiments with microorganisms. In 1928 Griffith first observed transformation in pneumococcus. Pneumococcus exists in two forms: a smooth one, which possesses a type-specific polysaccharide capsule, and which is virulent; and a rough one, which lacks virulence and the polysaccharide coat. Griffith showed that when living rough forms and killed smooth forms are mixed and injected into mice, living smooth forms could be removed and these continued to produce smooth progeny. In 1944 Avery *et al.* identified the substance responsible for the transformation as DNA. Recently Hotchkiss and Marmur (1954) showed that when wild-type pneumococci are placed in a medium which contains pure DNA from pneumococci that carried two genetic markers, three types of transformed pneumococci could be recovered, those transformed for each marker and those transformed for both. The frequency of double transformation was much greater than the product of the frequencies of both single transformations, indicating that these were not independent events but that there was a linked transfer of two units in a single event.

Further evidence that genetic continuity resides in the nucleic acids comes from studies with bacteriophage. Phage particles consist of a head made up of a protein membrane surrounding a packet of DNA and a tail exclusively protein. Hershey and Chase (1952) labelled the protein of the phage with S^{35} and the DNA with P^{32}. The P^{32} was transferred into the bacterium and subsequently 30-50% of it was recovered in the phage progeny. Only 3% of the protein was found to enter the bacterium and none of the S^{35} label appeared in the progeny.

Finally, experiments with plant viruses demonstrate, perhaps most convincingly, that it is the nucleic acid portion that carries genetic specificity. Plant viruses differ from most bacterial viruses and from cellular organisms in that they contain no DNA. Fraenkel-Conrat and Williams (1955) were able to separate tobacco mosaic virus (TMV) into two components, protein and RNA and to reconstitute infective particles from them. In 1956 Fraenkel-Conrat and Gierer and Schramm succeeded in showing that only the RNA is infective. Since different strains of TMV are known that possess different and identifiable proteins, it was possible to reconstitute particles carrying protein from one strain and RNA from another. The particles so reconstituted always had the genetic properties of the strain from which the RNA came (Fraenkel-Conrat and Singer, 1957).

The realization of the key role of nucleic acids in heredity led to a concerted effort to establish a structural model for the polynucleotides since an understanding of their function is predicated to a large extent upon the way the nucleotides are joined together. The study of DNA structure, both the molecular composition and the arrangement of the component parts in three dimensions, constitutes one of the most absorbing chapters in biochemical history. Initially, sedimentation and light-scattering studies had shown that the molecular weight of DNA was very large, somewhere between 5 and 10 million or greater, while viscosity and electron microscopy studies indicated the molecule was long, thin, and fairly straight. Such studies, however, told nothing of the detailed arrangement in space of the atoms within the molecules. For this it was necessary to use X-ray diffraction, and elegant studies along these lines were carried on in England by Franklin and Gosling (1953), Wilkins and Randall (1953) and others. Their work showed that the X-ray patterns of DNA from different sources were remarkably similar. They suggested that a uniform molecular pattern for all DNA existed. They also showed that DNA structure could take two forms depending on the water content of the molecule. In relatively high humidity, the DNA molecules gave a paracrystalline pattern showing the molecules were packed in a rather irregular manner; when the humidity was lowered and the fibers contained less water, they decreased in length by about 30%, and the protein became crystalline, indicating that the molecules were aligned regularly in all three dimensions. Finally, the X-ray pictures disclosed that the repeats in the crystallographic pattern came at much longer intervals than the chemical repeat units in the molecule, i.e., the distance from one nucleotide to the next was about 7 A, whereas the crystallographic repeat came at intervals of 28 A in the crystalline form and 34 A in the paracrystalline form.

Titration studies indicated the polynucleotide chains in the DNA molecule were joined together through bonding between the base residues. Furthermore, analytical studies by Chargaff (1951) of DNA samples from a host of biological sources established the following regularities in the ratios of the bases: (1) a stoichiometric equality between the sum of the purines and the sum of the pyrimidines, (2) an equivalence in the content of

adenine and thymine and in the content of guanine and cytosine (or the sum of cytosine and its substitution products), and (3) an equality in the content of 6-amino groups and 6-keto groups.

All of these experimental data were utilized by Watson and Crick (1953) to formulate their model for DNA. This consists of two polynucleotide chains that run in opposite direction and are wound round a common axis forming a double helix. Each chain consists of a sugar-phosphate backbone to which the purine and pyrimidine bases are attached. The two chains are held together by hydrogen bonds between the bases. One of the critical assumptions of their model is that there is a specific type of base pairing, adenine with thymine and guanine with cytosine, which makes each pair symmetrical and equivalent with respect to the cross-linking of the two sugar-phosphate backbones. The second critical assumption is that the molecule consists of two parts, each of which is the complement of the other so that either chain can act as a template upon which a complementary chain can be synthesized. This complementarity has very great genetic significance. Watson and Crick succeeded in constructing a model based on these assumptions, in which the distances between atoms coincided with those calculated from the X-ray diffraction patterns.

Support for a double helix structure of DNA has subsequently come from the studies of Thomas (1956). Thomas followed the enzymatic degradation of DNA by the cleavage of phosphorus diester bonds as evidenced by the production of hydrogen ions and simultaneously by the fall in molecular weight determined by light-scattering and viscosity measurements. In this way he could directly relate molecular weight decay to the number of ester bonds broken. During the initial stages of degradation, the molecular weight remained approximately constant. The breakage of about 200 hydrogen bonds was required before any decrease in molecular weight occurred, and these results were in good agreement with the concept of a long, double-chain molecule.

Impressive evidence for the mode of DNA duplication envisioned by Watson and Crick has been provided by labeling experiments. If each strand of the double helix acts as a template upon which a new strand is fashioned, each "new" double helix should consist of one old strand and one new strand. This kind of replication has been termed semiconservative, as opposed to the conservative type, in which the two old strands remain together and the two new strands remain together, or the dispersive type, in which the old and new DNA is distributed randomly in all strands. Using a method developed by Vinograd in which strong solutions of cesium chloride are forced into a density gradient when exposed to high centrifugal fields, Meselson and Stahl (1958) took advantage of the fact that a macromolecular substance like DNA, dissolved in the salt solution, will seek its own density in the gradient. By obtaining a population of *Escherichia coli* fully labeled with N^{15} isotope and then transferring the cells during the logarithmic phase to a medium containing N^{14} precursors, they were able to compare DNA before replication, with DNA after one and two replications had occurred. That prepared from the fully labeled bacteria gave a single band corresponding to the density expected for N^{15} material. After one generation in unlabeled precursors this band was entirely replaced by a new band having the density to be expected for equal amounts of N^{14} and N^{15} DNA and after two generations, two bands were observed, one corresponding to 100% N^{14} DNA and the other to one-half N^{14} and one-half N^{15}. These results appear interpretable only on the semiconservative model proposed by Watson and Crick.

Convincing as the above facts may be, additional evidence of the two-stranded nature of DNA has recently been provided by the simple, yet elegant experiments of Doty *et al.* (1960) and of Marmur and Lane (1960). Doty found that when solutions of bacterial DNA were denatured by heating and then cooled, two different molecular states could be recovered in essentially pure form, depending principally on the rate of cooling. One state, obtained by fast cooling, had about one-half the molecular weight of the original DNA and consisted of single strands. The other state, obtained by slow cooling, consisted of recombined strands united by a complementary base-pairing over most of their length. The latter form, which has as much as 50% of its original transforming activity, is called renatured whereas the quickly cooled, single stranded form is practically inactive and is called denatured. Doty was able to demonstrate, by means of density gradient experiments using N^{14} and N^{15} *E. coli* DNA, the existence of hybrids in the DNA renatured from the mixture. Marmur and Lane (1960) employed genetically marked DNA to form hybrids. DNA from *Diplococcus pneumoniae* bearing streptomycin-resistant and streptomycin-sensitive (wild-type) markers were heated together and then cooled slowly. The increase in biological activity of the hybrid DNA as evidenced by the increase in its transforming ability (streptomycin-sensitive to streptomycin-resistant) over renatured, nonhybrid, mutant DNA was dependent upon the amount of excess homologous wild-type DNA present and appeared to result from the formation of hybrids between strands of marked and wild-type DNA. In the process of hybridization the duplicate set of genetic information in the native DNA molecule of the mutant strain is distributed among a greater number of renatured molecules in which in many cases only one strand now carried the mutant information. These results strongly suggest that genetic information can be carried by a single strand.

The implication that genetic information can be carried in a single strand is consistent with the recent discovery of two viruses with a single-stranded DNA structure. These are ΦX 174 and S-13, originally suspected by Tessman (1959) to be single on the basis of their susceptibility in inactivation by P^{32} decay. Both viruses display an efficiency of one inactivation per disintegration which is about 10 times greater than the efficiency of inactivation of the double-stranded phage DNA. Sinsheimer (1959) has confirmed the single-stranded nature of their DNA by (1) formaldehyde studies, which indicate their hydrogen bonding is similar to RNA and denatured DNA, but not to native DNA; (2) by their UV-absorption properties; (3) by their curve of molecular weight decay with DNase and (4) in the case of ΦX 174, by the demonstration of the absence of stoichiometric equality between adenine and thymine and between guanine and cytosine. Instead, the four bases, as determined by enzymatic digestion to nucleotides, are found in the ratio of 1.00:1.33:0.98:0.75, respectively.

Elucidation of the pathway of DNA synthesis is, of course, of great interest. It was not known whether a free base (adenine, guanine, thymine, cytosine), supplied to an organism, could be incorporated into its DNA or if instead, it was necessary to supply the base combined with a sugar moiety, i.e., the nucleoside (adenosine, guanosine, thymidine, cytidine) or finally, whether the phosphorylated nucleoside, i.e., the nucleotide (adenylic acid, guanylic acid, thymidylic acid, cytidylic acid) was required. Plentl and Schoenheimer (1944) showed that the free N^{15}-labeled bases, guanine and thymine, are not utilized in the synthesis of DNA. Bendich *et al.* (1949) obtained the same negative results using cytosine as the isotopic precursor. Hammarsten *et al.* (1950) found that cytidine was incorporated to some extent and later Reichard and Estborn (1951) observed that deoxycytidine was incorporated into cytosine and thymine, that thymidine was incorporated into thymine, but that deoxyhypoxanthosine was not incorporated into any DNA base. McQuade *et al.* (1955) compared the induction of aberations in onion root tips treated with C^{14}-labeled thymine and C^{14} thymidine and corroborated the finding that thymidine but not thymine is a precursor of DNA. Apparently free adenine can be incorporated into polynucleotides, for Brown *et al.* (1948) recovered both labeled guanine and labeled adenine from the nucleic acid of rats fed isotopically labeled adenine. The most remarkable work concerning DNA synthesis was the discovery by Kornberg (1957) of an enzyme in *E. coli*, DNA polymerase, which catalyzes the condensation of deoxyribonucleotides, in the presence of DNA primer, to form large polydeoxynucleotide chains with the liberation of pyrophosphate. The amazing property of the system is that the composition of the newly synthesized polymer displays approximately the same purine and pyrimidine base composition as that of the primer in spite of the initial equality in concentration of the four triphosphates. Absence of any one of the four nucleotides reduces the reaction rate to about 1% of its maximum. The activity of the primer is increased by heating, indicating that it is a single strand of DNA which serves as a model.

The Meselson-Stahl experiment followed the distribution of DNA for several generations at the molecular level; an equivalent experiment which followed DNA distribution at the chromosome level had been performed earlier by Taylor *et al.* (1957). Use of tritiated thymidine in their radioautographic studies provided excellent resolution since tritium emits β particles of rather low energy resulting in localized film blackening that permits accurate identification of the labeled portions. The experiments were done with *Vicia faba* root tips grown on tritiated thymidine for one generation and then removed to a medium containing colchicine but lacking thymidine. The distribution of label within the chromosome was determined at intervals by autoradiography. Since colchicine blocks cell division but presumably does not affect chromosome replication, the number of chromosome sets within a cell indicates the number of post-transfer mitotic divisions that have occurred. The results were fully consistent with those expected on the Watson-Crick model if the chromosome carries only one double helix of DNA. After one generation in label all chromatids were labeled; after one generation in the absence of label, only one chromatid of each chromosome was labeled; finally, after two generations in the absence of label, as far as analysis permitted, it appeared that approximately one-half of the chromosomes were unlabeled and the other half had one labeled chromatid. There is not unanimous agreement concerning the mode of DNA replication envisioned by Watson and Crick. Cavalieri and Rosenberg (1961) believe that the DNA molecule, as it exists in proliferating cells, is biunial. This means it consists of two double helical units that are laterally attached to one another. The nature of the bonds linking the two double helices is unknown but it is considered to be much weaker than the hydrogen bonding linking the two strands of the double helix. At the beginning of replication, the biunial molecule is thought to split and each double helix to synthesize a new partner so that just before cell division two biunial molecules are present in the cell, one of which goes to each daughter cell. Since the two strands remain together at cell division, the replication of the double helix is conservative, whereas the replication of the biunial molecule is semiconservative.

Autoradiographic studies of chromosome duplication have been of value in respect to another aspect of chromosome behavior. For many years cytologists had observed that condensed chromosomes had a high concentration of Feulgen-staining material, whereas early prophase chromosomes did not. As a result of this observation it was generally believed that DNA was increasing during prophase. Studies during the past 10 years, utilizing two new techniques, the cytophotometric measurements of dye content of individual nuclei and autoradiography, have revealed that previous concepts concerning time of DNA and chromosome replication

were entirely incorrect. Swift (1950) employing cytophotometric measurement of Feulgen to observe quantitative changes in DNA content accompanying cell division in mouse embryos and *Ambystoma* larvae, obtained clear cut evidence that DNA replication in the mitotic chromosome occurred during interphase. Howard and Pelc (1951a), using autoradiographic techniques to measure the time required between incorporation of P^{32} label into DNA and its presence in metaphase chromosomes, also placed DNA synthesis at interphase and showed that prophase and metaphase chromosomes did not incorporate P^{32}. Taylor (1953) obtained similar evidence concerning the incorporation of P^{32} in *Lilium* and *Tradescantia* microspore nuclei. Since the time of chromosome duplication need not necessarily coincide with the time of DNA synthesis, Howard and Pelc (1951b) attempted to determine the time of protein synthesis by measuring the uptake of S^{35} in *Vicia* root cells. They found it was incorporated at the same time DNA synthesis occurred. The time of histone synthesis is a better criterion for chromosome duplication since this protein has about the same constancy as DNA. Both Alfert (1956) and Bloch and Godman (1955) showed cytophotometrically, using fast green at an elevated pH, a reaction which is specific for histone, that synthesis of basic protein and DNA occurred simultaneously during mitotic interphase.

Evidence concerning the time of duplication of the meiotic chromosome, has shown that here again the earlier concepts were incorrect. Swift (1950) determined cytophotometrically that in the mouse spermatocyte DNA synthesis was practically complete by the beginning of leptotene. Incorporation of P^{32} into DNA during meiosis of *Lilium* was shown by Taylor (1953) to cease before leptotene; in the case of *Tradescantia* (Taylor, 1953), it extended into early leptotene but ceased well before zygotene pairing. To test the assumption that incorporation of P^{32} was correlated with a net increase of DNA, Taylor and McMaster (1954), combined the cytophotometric and autoradiographic techniques. The two events were shown to occur at the same time during the premeiotic interphase in *Lilium* microsporocytes, while in *Tradescantia*, Moses and Taylor (1955) found the events coincided during leptotene. These results were seriously questioned by some geneticists on the grounds that both the Belling and Darlington hypotheses for crossing over require chromosome duplication to occur after zytogene pairing. Taylor and Taylor (1953) have since shown, by determination of the time of S^{35} incorporation in the meiotic chromosomes of *Lilium*, that the bulk of protein synthesis occurs at the same time as DNA synthesis, i.e., during the premeiotic interphase. Taylor concluded that since chromosome duplication occurs before zygotene pairing, intergenic crossing over in higher organisms is probably not a part of the replication process. As the time of crossing over can, at present, only be inferred, in much the same way that the time of DNA synthesis was inferred and subsequently shown to be incorrect, several alternatives are possible that would invalidate Taylor's conclusion.

Autoradiographic and cytophotometric techniques have provided a means for measuring the synchrony as well as the time of DNA and protein replication. The first evidence of an oriented asynchrony in the duplication of chromosomes was found in *Crepis capillaris* by Taylor (1958). A gradient in the labeling of these chromosomes with tritiated thymidine indicated DNA synthesis proceeded from the tip of the chromosome to the centromere region. Gall (1959) studied this phenomenon in the ciliate, *Euplotes eurystomus*. This protozoa possesses a ribbon-like macronucleus which pinches in half amitotically at each cell division. Several hours before division, two lightly staining reorganization bands appear at the ends of the nucleus and approach each other slowly, finally meeting near the middle. Autoradiographic and photometric observations showed that the tritiated thymidine is incorporated only in a limited region immediately distal to the bands and that the average amount of Feulgen dye bound by the nucleus rise concomittantly reaching two times the presynthesis value by the time the bands meet. A similar rise in alkaline fast green dye is observed in the duplicating nuclei. These data support the hypothesis that DNA histone synthesis takes place in a sequential fashion starting at the tips of the nucleus and proceedings toward the middle.

In male grasshoppers, of the genus *Melanoplus*, the early prophase meiotic chromosomes are visibly differentiated into the euchromatic autosomes and heterochromatic sex chromosomes. Lima-de-Faria (1959) injected tritiated thymidine into males during the last nymphal stage and studied the labeling of the chromosomes during early meiotic prophase. He found the sex chromosome was synthesizing DNA at a different period of time than the autosomes. Since there is a developmental sequence of spermatocyte cysts along the testicular tube, it was possible to show that the heterochromatin synthesizes DNA later than euchromatin. In order to determine if asynchrony was characteristic of heterochromatin in general, Lima-de-Faria studied uptake of tritiated thymidine in *Secale*. The chromosomes of rye have a large proximal block of heterochromatin while the median and distal portions are euchromatic. Here asynchrony was found to occur within each chromosome, corresponding to the euchromatic and heterochromatic blocks, although it was not possible to order the sequence. Taylor (1960) found in the case of cultured cells of the Chinese hamster, studied by following the uptake of tritiated thymidine, that five or six chromosomes of the complement have segments that duplicate late, including the heterochromatic Y and long arm of the X. Taylor believes that asynchrony in duplication is genetically controlled and that it may have a functional significance.

Chromosome Models

If we pause now to reassess the information that has been described above, several facts stand out. First, and most important, has been the identification of nucleic acid rather than protein as the genetic material. Although histones, judging from their position and their constancy, may play a fairly important role in heredity, the possibility of protein alone acting as the primary genetic material seems to be conclusively ruled out. Second, duplication of the mitotic chromosome occurs during interphase and of the meiotic chromosome before zygotene pairing. This necessitates a revamping of earlier concepts regarding crossing over, but the picture is far from clear at the present time. Third, there is now available an excellent model of DNA in molecular terms that satisfies its requirement as a genetic template. The great gap that remains lies between the visible chromosome at the level of the light microscope and the chromosome at the molecular or macromolecular level of DNA. How is the nucleoprotein joined together to produce the integrated structure we call a chromosome? Electron microscopy, which was expected to be of great value in clarifying this problem, has proved so far to be fairly unrewarding. Chromosomes are recognizable as dense accumulations of granular material without membranes. In the case of the spermatocyte, Moses (1956) has observed what he terms "synaptinemal complexes" which may be involved in the pairing of leptotene chromosomes. These consist of thread-like elements with delicate side chains extending laterally, and while the dimensions of the side chain are consistent with a DNA double helix, their association with chromosome structure remains rather tenuous.

In order to provide some sort of working hypothesis in terms of structure, several chromosome models have recently been proposed. At this stage, the best we can hope to do is to postulate the simplest working model not inconsistent with what is known genetically, biochemically, and cytologically. What, then, is known for certain? The greatest body of information comes from genetic data. These data tell us in unequivocal terms that the genes are arranged in a linear sequence along the chromosome. Equally important, they tell us that each gene acts in a unitary fashion. We know from chemical and genetic evidence that information resides in the DNA. Ultraviolet and chemical studies tell us that the structural integrity of the chromosome probably resides in the nucleic acid. We know that the DNA is arranged according to the Watson-Crick model of a double helix and that, at least for microorganisms, a single strand of the DNA helix can carry information. Beyond this, we know very little.

Of the four models that will be considered, only one assumes that more than two duplicate strands of DNA are present in a chromosome. The evidence for a multistranded chromosome comes largely from electron micrographs in which many fibrillae are observed. A fibril was originally estimated to have a diameter of 500 A. Ris (1961) has now resolved the fibril into two double helices of 40 A each, and found that upon removal of their protein, they fit precisely the 20 A width of DNA. That the fibrillae observed in electron micrographs of nuclei are DNA and that they are separate strands rather than part of a continuous, closely packed single strand is only inferred. The difficulty of packing a meter or more of length of DNA into a chromosome is readily apparent, but whether more information is present in a unitary amount or less information is present multiply seems immaterial to the solution. On the other hand, a multiple-stranded chromosome presents almost insurmountable difficulties functionally, because it is inconsistent with genetic data. Crossing over, which is inferred to occur by breakage and reunion of two strands at identical points, would require breakage and reunion of perhaps hundreds of strands, all precisely at the same nucleotide. In the case of mutation, each strand would have to be simultaneously altered in the same way in order for the mutation to show so that the probability of obtaining a point mutation would decrease with the number of strands involved. If one assumes that instead of remaining together, the strands segregated so that each one, ultimately in some generation gave rise to all identical DNA strands, delayed mutation should then be the rule. Irradiation of Drosophila would, on this assumption, producepredominantly mosaic flies, yet the frequency of mosaics is about 7% of the total mutants recovered (Altenburg and Browning, 1961). With respect to duplication, the distribution of label to the chromatids, observed by Taylor *et al.* (1957), points to the bipartite nature of the chromosome in which each half could correspond to a single strand of the DNA helix. Finally, Marmur and Lane (1960) have shown that in bacteria, the genetic material is present as a DNA double helix and that the information is carried independently in each strand. Unless some compelling reason for a multistranded chromosome appears, models based on this premise must be considered genetically unsatisfactory.

Taylor *et al.* (1957) had considered a model in which many Watson-Crick helices, each representing a gene, are attached at right angles to a central protein core. This arrangement would not be expected to give, for intragenic crossing over, the strictly one-dimensional recombination that is genetically observed. Moreover, Stadler and Uber (1942) have demonstrated that the UV spectrum for chromosome breakage is closely correlated with the UV absorption spectrum for nucleic acids while Callan and Macgregor (1958) have shown the chromosome's structural integrity depends on its core of DNA, so that a chromosome built on a protein backbone seems to be inconsistent with the facts. Taylor's model is predicated on a single-stranded chromosome and is in accord with his chromosome labeling results if the chromosome is, in fact, single stranded.

The earliest of the four chromosome models under consideration is that proposed by Schwartz (1958). He suggests that the molecules of DNA project as side chains from a discontinuous protein axis. To satisfy the need for chromosomal continuity, the DNA double helix is tied together at the free ends. Crossing over could occur intragenically or intergenically, but in the former case the chromosome would behave as a branched structure. Schwartz (1958) assumes, on the basis of half-chromatid splits seen by many cytologists at anaphase and half-chromatid aberrations observed after irradiation, that the basic condition of the chromosome is a two-stranded one. Replication of each DNA molecule, on this scheme is necessarily conservative since the two ends of the double helix are joined. Duplication, as a consequence of the bipartite nature of the chromosome, should give the results observed in the incorporation experiments of Taylor *et al.* (1957).

Finally, Freese's model (1958) consists of a single strand made up of alternate DNA molecules and connecting units. Each end of a DNA duplex is attached at one end and free at the other. In this way, two parts of a double helix are attached to different connecting units, or, conversely, a single connecting unit holds two different DNA molecules. Chromosome replication, in this case, would be required to occur sequentially, molecule by molecule, since the structural integrity of the whole depends on the binding of the constituent molecules via the double helix.

The last three models discussed are all based on the assumption that the DNA is divided into many molecules connected by a matrix material of protein. Another possibility is that the DNA is present as a single, continuous thread. The resolution of these alternatives is tied in with the molecular weight of DNA. Measurement of many kinds of DNA gave a weight of about 10×10^6 and seemed to indicate that it was homogeneous as to size. Recent studies of Davison (1959) have made these results questionable for he has shown that the method of loading DNA into the cell of the analytical centrifuge could damage it. Davison found that when the material was loaded very carefully, the DNA studied had a molecular weight of about 80×10^6 or eight times that originally calculated. It is difficult to imagine that all the DNA of a chromosome exists as a continuous double strand but it is also difficult to refute the idea. In conclusion, a note of caution should be injected as to the validity of extrapolating from knowledge obtained from microorganisms to construct chromosome models of higher forms. The possibility must not be overlooked that genetic material itself has undergone considerable evolution. The genetic material of a bacterium or a phage may be organized in a rather simple way, whereas that of higher organisms has probably acquired a complexity of organization corresponding to the increased complexity of their structure and function.

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