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CHROMOSOME STRUCTURE IN RELATION TO THE CHROMOSOME CYCLE. II[1]

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CONTENTS

INTRODUCTION

The discovery that chromosomes divide longitudinally, and that the halves so produced separate into two nuclei in the cycle of mitosis, represented a fundamental step in the analysis of the processes by which living cells effect their reduplication. A more precise definition in terms of chromosomal materials followed the disclosure that the chromonema is not a transitory component of the chromosome but that it maintains the linear order of the genes through successive mitosis. In the cycle of coiling and uncoiling attendant on its multiplication, the chromonema reflects on a microscopically de-

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tectable level the adjustments requisite to duplication of the constituent genes and their distribution to daughter nuclei. Submicroscopic alterations that occur in the form and substance of the chromosomes during the mitotic processes represent a further level of analysis, toward which an approach has been made by application of physical and chemical methods developed for studies of cellular constituents. The contributions that have been made in recent years toward an understanding of these phenomena form the basis of the present report. This serves as a supplement to a previous review (185), which outlined the more general features of the chromonema cycle and appraised the divergent interpretations of such particulars as number, linear organization, mechanism of coiling, and relation of the chromonemata to other materials of the chromosome. Since the 1936 review was written, a series of other summaries, critical reviews and expressions of opinion on these controversial matters has been presented (87, 92, 132, 133, 140, 143, 169–171, 209, 273, 283, 294, 295, 320, 356, 358, 360, 383, 403, 404, 427, 430, 431). Because of this extensive series the review offered here makes no pretense at completeness in surveying the literature that has accumulated during the past ten years but aims rather to indicate the present level of our knowledge of chromosome structure and prospective approaches to further studies.

A considerable portion of the information now available concerning the organization of the chromosome during somatic and meiotic mitoses has been obtained from studies of such plants as *Tradescantia* and *Trillium,* although in recent years a number of supplementary observations of the behavior of chromonemata have been made on several species of animals belonging to various phyla (*e.g.,* 72, 78, 80, 135, 193, 199, 235, 265, 287, 302, 315, 332, 414, 428, 432). Studies on giant chromosomes, such as those of the salivary glands of Diptera and the oocytes of Amphibia, have been of paramount importance in resolving conflicting viewpoints. Whereas the larger plant chromosomes provided favorable material for basic studies of the pattern of coiling of the chromonemata, the giant chromosomes have been indispensable in the more recent histochemical approaches to problems of fundamental organization.

Newer methods of analysis developed in these studies have involved the use of monochromatic ultraviolet radiation for identification and determination of the distribution of cellular components

during the cycle of mitosis. The technique permits measurements to be made of amounts of substances that could not be detected by other chemical or physico-chemical methods (51). Different parts of the spectrum and different ranges of absorption require different procedures. A photoelectric method with electrometers as recording instruments, which is applicable to objects of a diameter of 1 μ or larger, has been designed for use with ultraviolet radiation beginning at 2,000 Å, as well as with the visible portion of the spectrum. For analysis of finer detail, the method of measuring density differences on photographic plates with a recording microphotometer is applicable within the limits of the resolving power of the microscope lenses. (Descriptions of these methods of analysis, and illustrations of the apparatus used, are to be found in papers 47, 51 and 74.)

Other advances in methods of analysis include the development of the phase-contrast and electron microscopes. In the phase-contrast system, slight invisible phase changes in the light passing through the specimen are converted into intensity differences that can be seen (the physical principles involved are discussed in 18, 38, 197, 329 and 441). As a result, a wealth of detail may be disclosed in cells that appear relatively homogeneous under the ordinary microscope (see 17, 18, 229, 264, 327–329 for a discussion of the general adaptability for cytological studies). Since chromosomes may be identified in the living or unstained cell, phase-difference microscopy gives promise of serving as a useful supplementary method in experimental approaches to problems of chromosome structure.

The electron microscope, on the other hand, provides a manifold extension of the power of resolution beyond that available with optical systems using ordinary light, and should ultimately prove serviceable in the analysis of the finer details of chromosome organization. Sharp definition, combined with great depth of field, is attainable at magnifications as high as 20,000 diameters (442). However, a vacuum is required throughout the instrument to permit passage of the electrons, which have low power of penetration; and for the same reason only extremely thin layers of thoroughly dehydrated material can be analyzed. These requirements constitute severe handicaps in the study of biological materials. Some progress has been made in analyzing the structure of dispersed particles of small dimensions, such as viruses, bacteriophages (see review in

443) and isolated mitochondria (69), and in determining the organization of the cytoplasmic ground substance and inclusions in smears (326) and outgrowths of explants of embryonic cells (309). Such methods have but limited application for the study of chromosome structure (*cf.* 68), and sectioning must be employed if normal spatial relationships are to be maintained. Several types of microtome, each of which has certain limitations, have accordingly been designated to permit preparation of sections as thin as 0.1 μ (4, 5, 129, 145, 289, 324, 325). In addition to the difficulties of sectioning, special problems arise in efforts to insure faithful preservation of structural details during the processes of fixing, embedding and dehydrating (70, 115, 116, 145, 440). It is thus apparent that the technology of electron microscopy is at present in the preliminary stages of development with respect to its utilization for cytological studies; but if new methods are developed that will permit examination of thin slices of embedded cellular material, the resolution of finer details of chromosome structure may be anticipated. The need for such information will be apparent as the conflicting viewpoints arising from different interpretations of the same type of material are presented in the following pages.

ORGANIZATION OF THE CHROMOSOME

The compact, cylindrical chromosome, such as is seen at anaphase of mitosis, contains within its limiting membrane (sheath or pellicle) the helically coiled chromonemata and the associated matrix. A primary constriction marks the position of the centromere (spindle attachment region or kinetochore). Secondary constrictions are often detectable, such as those that separate terminal satellites and reveal the positions at which the nucleoli are organized during the telophase stages. Other secondary constrictions may exist at various positions along the chromosome, and are usually seen most clearly during late prophase stages. Utilizing the constrictions and length of arms as topographical markers, the karyotypes of many species of plants have been described in recent years. These studies need not be reviewed here, especially since many of them have been concerned primarily with phylogenetic relationships rather than with problems of chromosome structure. Moreover, a comprehensive index to the literature dealing with chromosome numbers in plants has recently been published (94).

Sheath

The existence of a membrane which delimits the condensed chromosome has been inferred from various aspects of chromosomal behavior in normal and experimental material (*e.g.*, 261, 262, 356). In microsporocytes that have been partially flattened by spreading beneath a scalpel on a glass slide prior to fixation, the coiled chromonemata are surrounded by a material differing in its microscopic appearance and staining capacity from the cytoplasm, and presumably separated from it by a delicate interfacial membrane. The aspect in a general way resembles that of the karyomeres that are formed at telophase in some grasshoppers, fishes and other animals. Prefixation treatment—as with KCN—of the condensed chromosome of metaphase or anaphase will produce a similar appearance (176). Demonstration of a chromosomal membrane in the living cell is extremely difficult, but has been obtained by application of micrurgical methods to the salivary-gland chromosomes of the larva of *Chironomus* (82), confirming the observations made by other workers on fixed and stained preparations of these giant chromosomes (*e.g.*, 196, 358).

In some species of animals more conspicuous perichromosomal coatings have been observed. The sheath of the metaphase chromosomes in some Hemiptera appears to be modified to form interzonal connections between the separating daughter chromosomes at anaphase (352, 353). Treatment with osmic acid will blacken a broad zone of material surrounding the chromosome in spermatocytes of the beetle *Palomena* (164). Confluence of perichromosomal sheaths under special conditions may lead to formation of chromosome aggregates (*262, cf.* 357).

From this limited description it is evident that the sheathing materials vary greatly in their morphological aspects in different cells. Further information concerning the chemical nature of the materials of the pellicle, and their relation to other chromosomal and cytoplasmic materials, is to be sought in the application of histochemical methods such as are described subsequently.

Matrix

Although it is generally agreed that the condensed chromosome is bounded by a sheath of Feulgen-negative material, there is no unanimity of opinion concerning either the nature of the matrix

material or its distribution within the chromosome. The term "matrix" has been used in a variety of ways, some authors employing it as a synonym for sheath or pellicle, others applying it to that portion of the chromosome, seen in fixed preparations, that envelops the chromonemata. On the other hand, some workers (especially 84 and 96), although admitting the existence of a chromosome pellicle, have maintained that the matrix of fixed material has no relevance to the living chromosome. This point of view could be supported more readily if it were assumed that the material of the so-called matrix seen in stained preparations represents the Feulgen-positive coating of the individual chromonema, and if there were no additional material lying between the chromonemata and the chromosomal membrane. With respect to the first of these qualifications, available evidence suggests that the desoxyribose nucleic acid is not a superficial deposit on the genonema, in the sense of a loosely-associated or enveloping matrix substance, but that it is bound up with the proteins of the thread itself. The second qualification has been critized repeatedly (*e.g.*, 242). Although in some chromosomes the chromonematic coils are packed so tightly that they seem to constitute a closed cylinder (101, 404, 428), it has been demonstrated, both in living material (66) and in fixed preparations, that the end view of a chromosome often presents the aspect of a hollow cylinder, with the more refractive or densely staining material peripherally disposed (*e.g.*, 415, 391, 144). Additional and more striking evidence is provided by those preparations in which, as a result of prefixation treatment, both pellicle and chromonemata have been separated to reveal an intervening zone—the matrix—as has been shown in photographs of *Trillium* (176), of *Tradescantia* (408, 412), and of the grasshopper *Podisma* (235). Since a different interpretation of this type of fixation image has recently been proposed (332), it is necessary to consider its validity insofar as it bears on the problem of interpretation of interchromonematic materials. Ris suggests that Makino's photographs of *Podisma* indicate that four chromonemata are present in each of the homologues, and that two of the strands are peripherally disposed and stain faintly, the other two remaining appressed in the medial region and staining intensely. This interpretation leaves unanswered a series of questions concerning the disposition of sister chromatids and half-chromatids in the axial and peripheral regions

of the chromosome, and their capacity for differential staining. Moreover, the appraisal need not be based exclusively on orthopteran material, since the same general aspect of a bivalent that is shown in Makino's photographs was also presented by those preparations of *Tradescantia* in which the four chromatids of first meiotic metaphase were first demonstrated to be helically coiled threads (182, figs. 62 and 63). There seems little question, in the light of the considerable number of studies on the chromosomes of this plant in the intervening years (see especially 208), that the two chromatids constituting the dyad are intimately associated, and that, although each may be further subdivided (to provide an octopartite bivalent, as will be described subsequently), the half-chromatids are closely appressed and not loosely associated in the two-by-two relationship assumed by Ris to exist in the grasshopper chromosome.

The material constituting the matrix in these *Tradescantia* preparations does not stain with gentian violet-iodine, aceto orcein or the leucobasic fuchsin used in the Feulgen technique for detection of desoxyribonucleic acid; accordingly, it has often been designated as achromatic. From the evidence obtained by studies of salivary-gland chromosomes it seems that the interchromonematic material contains a pepsin-digestible protein, since chromosomes exposed to the action of this enzyme undergo a marked reduction in diameter (127, 128, 257, 258, 365). A similar but less pronounced effect of pepsin on the root-tip chromosomes of the onion has been reported (reference in 257). Removal of the interchromonematic protein does not disrupt the "skeletal" proteins to cause structural disorganization of these chromosomes, nor is the Feulgen-staining capacity of the desoxyribose nucleic acid impaired by pepsin digestion. It has also been reported (358) that the interbands of the salivary-gland chromosomes of *Drosophila,* which are Feulgen-negative in contrast with the Feulgen-positive bands, can be counter-stained with fast green, using the method employed for the differential staining of nucleoli (24–26, 367–369). Similarity in the staining quality of matrix and nucleolus is not in itself unequivocal evidence in support of the contention—previously suggested on morphological and cytogenetic grounds—that they contain similar materials. However, the enzyme ribonuclease will remove the fast-green staining component from the chromosome (358). It appears, therefore, that ribonucleoproteins, which are known to exist in the nucleolus

(363), also represent a major component of the matrix (*cf.* 32). However, the conclusions that may be derived from these tests are limited by questions concerning the purity of the enzyme and its specificity of action. Some samples of crystalline ribonuclease tested have indeed been found to possess proteolytic activity (73, 349).

Both the ribose and the desoxyribose nucleic acids show maximum absorption in ultraviolet radiation of about wave length 2,600 Å. When chromosomes of the pollen tubes of *Tradescantia* were exposed to radiation of wave length 2,537 Å, they were markedly shortened and disclosed a hyaline matrical zone. It was suggested that this effect might be attributable to absorption of the ultraviolet radiation by ribonucleic acid of the matrix (408). Chromosomes may be broken as a result of exposure to ultraviolet rays as well as X-rays, although there are marked qualitative differences in the effects of the two kinds of radiation. The possible rôle of the matrix in the process of chromosome breakage was outlined briefly in the original (1936) review. More recently it has been suggested that the difference in the types of alteration produced by X-rays and by ultraviolet rays may be due to the fact that the latter can not disrupt the matrix to produce a thoroughgoing break, whereas the X-ray break may involve both chromonema and matrix (272). The inhibition of X-ray-induced breaks in *Tradescantia* by ultraviolet radiation of wave length 2,537 Å has also been attributed to the effect of the supplementary treatment on the substance of the matrix (413).

In contrast with the point of view that the matrix material is essentially achromatic, a number of cytologists in recent years have accepted the interpretation that the matrix represents the Feulgen-positive coating of the gene string or genonema (*e.g.*, 140, 160, 356, 371). Designation of this portion of the chromosome as the matrix may be referable to a series of factors—among them the gradual accretion of thymonucleoproteins by the genonemata as the chromosome increases in diameter during the prophases; the aspect of uniformly colored, Feulgen-positive metaphase and anaphase chromosomes when fixation does not disclose the details of internal organization; the presence of chromatic interchromosomal connections, even in living cells of some species; and the assumption that the material of the matrix is indispensable in the processes of gene

multiplication and metabolism and must therefore contain thymonucleic acid as an essential component. Thus the connotation of the term "matrix" has undergone considerable revision in the course of recent years (see 283 for various interpretations). A more rigid definition seems required if confusion is to be avoided in describing the findings of the increasing number of cytochemical studies of chromosomes (*e.g.*, 121). Much remains to be determined about the synthesis and distribution of materials within the chromosome; and little precise information can be offered at present concerning the relationship of the matrix to the chromonemata during the various phases of mitosis, although an elaborate scheme has been proposed relating the rôle of the matrix in the spiralization cycle to changes in its colloidal state (209). Despite these limitations, the results available from physical and chemical methods of analysis, together with the older morphological considerations, lead to the suggestion that the term "matrix" should be reserved for the portion of the chromosome surrounding the chromonemata, which can be dissociated from them by various techniques. The term "chromonema" may continue to serve as a morphological description of the genonema and its associated desoxyribose nucleoproteins. With an increasing knowledge of the chemical nature of chromosomes, a terminology based on morphological considerations may undergo drastic revision; until such precise information is available there seems little advantage in applying new terms to describe the Feulgen-positive material around the genonema.

Centromere

Before turning to a consideration of the number of chromonemata and the patterns of their coiling, attention will be given to various aspects of the problem of linear organization of the chromosome. The centric region is a primary topographical feature. It occupies a definite position with respect to the ends of the chromosome that may be altered by such processes as pericentric inversion, duplication, unequal reciprocal translocation or misdivision of the centromere. Compound chromosomes, as occur in the germ line of *Ascaris megalocephala,* presumably contain the centromeres of the several component units (300, 353, 426). A type of centromere that is not localized but spread over the chromosome occurs in

the Hemiptera (166–168, 331, 352, 353, 418). When chromosomes of *Steatococcus* and *Tamalia,* having such diffuse centromeres, were fragmented by X-rays, each of the segments maintained the normal centric functions; it continued to divide through successive mitoses and did not behave as an acentric body.

It is generally assumed that the centromere represents a compound structure, although the distribution of its components has been the subject of much speculation. Various considerations indicate that the chromonemata, perhaps in a modfied form, traverse the body of the centromere (*e.g.,* 253). Such structural organization would best explain the free separation in successive stages of the numerous chromonemata that constitute diplo- and polychromosomes (10, 151, 152). Following differential staining, small spherical bodies (spindle spherules or attachment chromomeres) have been observed within the centromere (*e.g.,* 355), presumably at the point of union of the chromonemata extending in from the two arms of the chromosome. At the time of initiation of the anaphasic movements, the spherules may be drawn out but maintain their connection with the body of the chromosome by attenuation of the chromonematic threads. Counts of the number of detectable attachment chromomeres suggest that one is present for each functional chromatid (*e.g.,* 177, 354). Thus in *Trillium* and in *Lilium* at first meiotic metaphase there are six granules detectable in a trivalent, four in a bivalent, and two in a univalent (177). The observation that two granules are present in the metaphase chromosome of the second meiotic division is regarded as evidence consistent with the interpretation that the chromosomes during the first meiotic division are octopartite. In some plants the fine threads and the attachment chromomeres were found to be Feulgen-positive (177, 312); in others the material of the centromere gave a negative Feulgen reaction (343, 344). Such dissimilarities may possibly be attributable to difficulties in interpreting the Feulgen reaction when applied to bodies of minute size, and not to fundamental chemical differences between the materials tested (*cf.* 356).

Modification of chromosome form in the course of mitosis—for example, the production of an isochromosome with a median centromere and two structurally similar arms from a chromosome with arms of unequal length—has been observed in a number of plant genera, *e.g., Tulipa* (422), *Pisum* (198), *Fritillaria* (88, 90), *Zea*

(322), the B-type chromosomes of *Sorghum* (100), *Nicandra* (93), *Secale* (219, 275–277), *Nothoscordum* (130) and *Godetia* (155, 156). The origin of such modified types has been attributed to misdivision of the centromere (90). A pattern of organization of the centromere which would permit such misdivision has accordingly been visualized (88, 283). The essential organ of division in this pattern is a centric fluid, normally "exploding", according to Darlington, so as to divide the centromere parallel to the long axis of the chromosome in accordance with the distribution of the fibrous determinants or centrogenes, but capable of division in other planes under special conditions. On the other hand, the possibility of origin of isochromosomes from abnormal attachment of chromatid segments to the halves of a normally divided centromere has been advanced (277). Metacentric chromosomes, morphologically indistinguishable from isochromosomes, apparently originate from J-shaped chromosomes during meiosis in *Gasteria* as a result of crossing-over in a heterozygous pericentric inversion (148).

When a chromosome with a truly terminal centromere (a telocentric or telomitic chromosome) originates, it apparently either is eliminated in natural selection or gives rise to an isochromosome. The behavior of a telocentric chromosome in maize indicates that it regularly undergoes structural changes in somatic cells (322). Such instability may apply to all such chromosomes and account for the fact that they are rarely, if ever, found in nature. Even such small chromosomes as the dot-like fourth of *Drosophila melanogaster* are two-armed, as cytological and genetical evidence has shown. The confirmation in recent years by several workers (78, 81, 86, 265) of White's earlier observation that the chromosomes of grasshoppers possess minute short as well as long arms appears to invalidate the descriptions of truly telomitic chromosomes in these insects (e.g., 161). However, the pronounced short arm shown in Coleman's photographs of chromosomes of *Chortophaga viridifasciata* (78) is not indicated in Carlson's illustrations of this material (43, 44).

Division of the body of the centromere occurs with the onset of anaphase. As the daughter chromosomes move apart the centromere takes precedence, the attachment chromomere marking the position of the spindle fiber that extends from the chromosomes to the pole. Exceptional cases have been reported of chromosomes

having secondary centric regions that lead the way to the poles in meiotic divisions, sometimes even before the primary constriction has divided (323, in maize; 181 and 310, in some inbred strains of rye). A further analysis of these actively mobile, so-called T-ends of the rye chromosome in meiosis indicates that they are furnished with chromosomal fibers similar to those attached to the centromere (292). The possibility of a shift in position of a functioning centromere within a chromosome had been suggested previously by Carothers (45), although her theory that the centromere is merely a temporarily modified chromomere is not supported by the evidence presented in the preceding paragraphs, nor by the discovery in certain viviparid molluscs of an intimate centromere-centrosome relationship (306).

Nucleolus-Organizing Region

The nucleolus is ordinarily formed at the telophase of mitosis by the nucleolus-organizer, which occupies a specific position in one of the chromosomes of the set. As the nucleolus enlarges it may displace parts of the chromosome—such as the satellite and the main body—which maintain continuity, nevertheless, by extension of the connecting chromonematic threads. During the succeeding prophases, as the nucleolus diminishes in volume, the separated parts are again brought into contact, although the position formerly occupied by the nucleolus is often revealed at late prophase or at metaphase by a marked constriction (a so-called secondary constriction). Its width, or the length of the satellite thread, at these stages, however, does not necessarily bear a direct relation to the size of the nucleolus at telophase (317, 319, 320).

Modifications of this standard pattern have frequently been reported. Duplication of the region containing the organizer may permit the development of nucleoli in additional chromosomes of the set. A striking example is provided by the male flies of *Drosophila ananassae* (186), in which the fourth chromosomes and the Y-chromosome are concerned with nucleolus formation. In the females only the fourth chromosomes carry the organizers. Since in females of related species of *Drosophila,* nucleoli are organized in the X-chromosomes, it seems probable that interchromosomal rearrangement in the course of phylogeny has resulted in the transfer of the organizer from the X to the fourth chromosomes. Duplication

within the chromosome set of the segment carrying the organizer has likewise been suggested as an explanation of the extra nucleolar chromosomes of *Rhoeo* (27).

In several species of *Trillium,* in some lines of *Pisum,* in *Allium amplectans* and in *Paris polyphylla* the nucleoli, sometimes several in number, are produced at the ends of the chromosomes (250, 96, 218, 158). However, in the related species *Paris hexaphylla* and *Medeola virginiana* the nucleoli are organized at definite positions in specific chromosomes (250, 400). Such differences between closely related species could be attributed either to the absence or presence of the organizer or to relative degrees of its activity. It has been suggested that in *Trillium* the organizer is probably not absent, but of small size (316, 400). The presence of nucleoli on several chromosomes of the set, as in the somatic and pollen mitoses of *Allium amplectans,* may seem to indicate that a definitive organizer is lacking (218), although the association in this species of a single nucleolus with one pair of homologues during pachytene and diplotene stages of meiosis suggests that an organizer exists whose capacity to function varies in different tissues.

Another modification affects the timing of the cycle of organization and dissolution of the nucleoli, so that they may appear precociously at anaphase, or persist until metaphase (123, 158, 220, 260; other references in 87, p. 306). Both the precocious and the persistent types have been observed in somatic cells of *Bellevalia hackeli* (220). Satellited chromosomes, which presumably carry the organizers, have been observed in this species and also in *Pisum* (158). The nucleoli, however, were not localized in the region of the satellite, but either were budded off from the ends of the chromosomes or appeared in regions close to the centromeres. Disturbances in the normal genotypic control may thus be assumed to upset the functional capacity of the organizer, which loses its competitive advantages to other portions of the chromosomes in the organization and timing of the process of nucleolus formation (*cf.* 250). Studies of the nucleolus of several species of *Salix* indicate that persisting nucleoli are more frequent at lower temperatures than at higher ones (114). These observations have led to an elaboration of the concept of the nucleolus as a coacervate—a separated phase out of a saturated solution. Differences in the activity of the region that includes the organizer—perhaps as a result of

alterations in cellular environment during phylogeny—are also shown by observations that at corresponding positions on the long chromosome in three species of *Chironomus* (*C. tentans, C. lobiferus,* and *C. plumosus*) there may occur either a nucleolus, a nucleolus-like "puff", or a dark band rich in nucleic acid (259).

It has been demonstrated in several species of animals and in some plants that the organizers occur in parts of the chromosome that are heterochromatic. During pachytene in *Zea* (232) and in *Medeola* (400), for example, the nucleolus-organizing regions are detectable as heteropyknotic knobs. In some plants, such as *Narcissus* (118), *Uvularia* (140) and members of the Aloinae (316), the amount of detectable heterochromatin is either negligible or confined to a small satellite, although the process of nucleolus formation is not impaired thereby. The relation of size of the heterochromatic region to capacity for nucleolus formation is not always a direct one; although a quantitative correspondence was found in *Solanum* (217), in *Zea mays* the smaller portion of a fragmented organizer produced the larger nucleolus (232). It has been reported that in *Narcissus* both euchromatic and heterochromatic supernumerary chromosomes effect an increase in volume of nucleus and nucleolus as compared with plants lacking such chromosomes, but that the essentially euchromatic chromosome exerts its effect primarily on the nucleus, whereas the heterochromatic supernumerary exerts a much stronger effect on the nucleolus (121).

These heterochromatic regions, which have the capacity to form large amounts of thymonucleoproteins within the chromosomes of which they are a part, appear therefore to be concerned also with the ribonucleoprotein metabolism of the nucleolus (60, 362, 363). Caspersson (54) has suggested that the essential function of the heterochromatic regions is the production of histones, which accumulate in the nucleolus during the telophase and interphase and then diffuse through the nuclear membrane to form in the cytoplasm the ribose nucleic acids that are concerned with the synthesis of cytoplasmic proteins. The method used in reaching an estimate of high histone content, on which these conclusions are based, involves interpretation of curves of absorption spectra; its validity has been questioned (268). More recently it has been reported in a brief abstract that, following application of the Millon test for protein,

the ultraviolet absorption peak of tyrosine-containing proteins such as the histones is shifted away from the region (*ca.* 2,800 Å) which is largely dominated by strong absorption of the purine and pyrimidine bases characteristic of nucleic acids, into a region (*ca.* 3,500 Å) where the absorption due to nucleic acid is negligible. Using this criterion for detecting the tyrosine content before and after removal of histone, it has been found that in the nucleolus of maize histone does not constitute more than 10 per cent of the total protein (307). More data will be required before such results can be appraised adequately with respect to Caspersson's hypothesis, especially since earlier work had shown that different types of nucleoli may vary with respect to the proportions of protein and nucleic acid (363). Caspersson's conclusion has been questioned, however, on the basis of observations that supernumerary euchromatic as well as heterochromatic chromosomes exert an effect on the volume of the nucleolus in *Narcissus* (121, see also 320). These observations suggest that the nucleolus is derived from materials provided by all the chromosomes at telophase, the heterochromatic sections being more efficient than the euchromatic in nucleolus formation. This represents essentially a reaffirmation—in terms of chromosomal constituents—of the transportation hypothesis visualized by some earlier workers from morphological considerations of the chromosome-nucleolus relationship.

Other Secondary Constrictions

Secondary constrictions other than those associated with nucleolus formation have been observed in the chromosomes of many plants and animals. They are generally most conspicuous during the prophases, and may be lost to view in the condensed chromosome (*e.g.,* 405). A striking illustration of a secondary constriction is provided in the left limb of the second chromosome of *Drosophila melanogaster,* the adjacent parts of the chromosome sometimes being so widely separated during the early prophases as to appear as separate chromosomes, although critical observation reveals that an attenuated chromonematic thread bridges the intervening gap (184).

It has been suggested that secondary constrictions, other than those attributable to the activity of nucleolus organizers, represent intercalary heterochromatic regions. This generalization rests on

the discovery that exposure to low temperature will produce in the chromosomes of *Trillium* short terminal or intercalary segments, having so little thymonucleic acid that they present the appearance of a series of secondary constrictions (95–97). A similar annulated appearance has been produced in the chromosomes of a series of plants (142, 179, 180, 219, 321, 433) and in the salamander, *Triton* (39, 432), by exposure to cold. Factors other than cold treatment may be effective in disclosing these "differential segments" in chromosomes that do not reveal them under normal conditions. They may be induced by starvation of animals (432); and they have appeared in hybrids of *Mecosthetus* (194). In haploids of rye "nucleic acid starvation" seems to occur under normal conditions (219, see also 320). Intercalary segments with a staining reaction different from that of adjoining regions have been revealed in some root-tip chromosomes merely by the application of special methods of fixing and staining (203, 222).

It has been reported that the differential segments are constant in number, although they may vary in length (96, 433, 434), and that they occupy specific positions along the chromosomes. Differences in homologous chromosomes of *Trillium* have been attributed to the hybrid nature of the material (96), although other workers have found that in plants of this genus a chromosome may be regionally differentiated in some cells of a single plant and not in others. Considerable variability in the expression of "negative heterochromacy" has also been reported to occur in different cysts of a single testis of the grasshoppers, *Mecosthetus* and *Chorthippus* (194). Similar variations have been reported among the chromosomes of different nuclei of an individual in the Salmonidae (405). The possibility of utilizing patterns of distribution of differential segments for detecting variations in chromosomal organization may accordingly prove useful in some species but not in others.

Darlington and LaCour have suggested that the differential segments represent regions in which the accumulation of nucleic acid that occurs under normal conditions has been inhibited. In support of this interpretation they have reported that there is a positive correlation between the number of differential segments and the number of "chromocenters" detectable in the resting stage. These deeply staining bodies have long been recognized as heterochromatic portions that differ from other parts of the chromosome in the tim-

ing of the nucleic acid attachment in relation to the cycle of reproduction ("Heterochromatin is allocyclic."). It is also known that the chromocenters exhibit a constancy in form, number and position that is characteristic for the individual or species (*e.g.*, 109, 260, 270, 313, 316, 320). Darlington and LaCour conclude, therefore, that the over-nucleated chromocenters of the resting stage represent the under-nucleated differential segments of metaphase. In haploid rye the differential segments and the heteropyknotic knobs seen at pachytene occupy corresponding positions in the chromosomes (219). The possibility has not been excluded, however, that the regions which presumably show "nucleic acid starvation" represent exaggerated secondary constrictions, such as occur in *Drosophila* between the proximal heterochromatic and the more distal essentially euchromatic regions (the pronounced constriction in the left limb of the second chromosome of *D. melanogaster* mentioned previously provides a good illustration). This type of organization would also provide a positive correlation between chromocenters and "differential segments". As has been pointed out (359), the "starvation" interpretation is difficult to reconcile with the opinion expressed by Darlington (92) that heterochromatic regions are centers of nucleic acid synthesis. It must also be kept in mind that differential staining may be referable primarily to a difference in contraction of various parts of the chromosome, which is a manifestation of differential coiling (434; however, *cf.* 319, 321).

A comparative study of several species of *Paris* suggested that the same genes that control allocycly in one species may not do so in another. This control has been related to the activity of the nucleolus organizer, and the suggestion offered that the "absence of allocyclic behavior goes with the presence of nucleolus organizers" (91). The validity of the cytological observations on which this suggestion was based has been questioned (320); and allocycly and organizers have been reported to exist together in several species of plants and animals (39, 143, 419, 432).

Confirmation or refutation of the generalization that all secondary constrictions reveal the location of heterochromatic regions must await the accumulation of information concerning the organization of specific chromosomes. Present knowledge of the distribution of euchromatic and heterochromatic segments, even in such a carefully studied chromosome as the X of *D. melanogaster,* is far from

complete. From the data now available (187, 192, 311) it is evident that some of the intercalary heterochromatic regions of the X-chromosome are not revealed as constrictions in the ordinary fixed and stained preparations of mitotic prophases. An additional indication that secondary constrictions may exist apart from heterochromatic regions is provided by the observation that in neuroblast chromosomes of stocks carrying translocations a constriction frequently marks the point of attachment between the non-homologous chromosomes (*e.g.*, 105). Since the induced breaks involved in rearrangement have been found to be distributed at random along the chromosomes, and not restricted to heterochromatin, it appears unlikely that the sample of translocations in which the constrictions were observed would include only exchanges involving heterochromatin. Chromosomal rearrangement occurs frequently in nature, and some of the constrictions that now characterize specific chromosomes, as in *D. melanogaster,* may have arisen in this process.

Chromomeres

A consideration of the linear differentiation of the chromosome must inevitably face the problem of the nature of the chromomere-like regions seen along the extended chromonemata during the prophases of somatic and meiotic mitoses. These regions frequently have been shown to be so constant in number and form that their pattern of distribution serves to identify specific chromosomes (*e.g.*, the studies of the chromomeres during meiotic prophases in liliaceous plants, 15, 16, 159, 172; those on the chromosomes of *Zea mays* and related genera, 223–227, 230; on *Crepis* and *Trillium,* 203, 204; on grasshoppers, 42; on the pachytene pattern in human spermatocytes, 361; and many others). The term "chromomere" has thus received a connotation that in modern cytological usage implies the existence of constantly reproducible regions that are set off from the adjoining portions of the chromonema by differences in diameter and staining capacity. Alexander and Bridges (3) suggested several years ago that the chromomeres seen at prophase are local accumulations of freshly synthesized materials, and serve as an index of the relative activity of the synthetic and accumulative processes carried out during these stages at different loci. In more modern terminology the chromomeres are chemically identifiable by specificity in the cycle of attachment and detachment of nucleic

acid (96, 99, 204); or, as stated by Pontecorvo (308), "each chromomere shows a distinct reactivity of its own in nucleic acid synthesis; it is 'allocyclic' in relation to others, either because its cycle is shifted in time, relative to that of other chromomeres, or because different chromomeres synthesize at different rates or reach different final charges".

Interpretations that involve regional structural differences in the chromonema due to differences in the time of attachment of nucleic acid have been questioned from time to time, most recently by Ris (332), who attributes the observed linear differentiation to modifications in the degree and pattern of coiling of a thread of essentially uniform diameter. Certainly some of the chromomere-like bodies can be resolved into differentially coiled regions under proper conditions of fixation and staining. The condensed regions lying adjacent to the centromeres have been shown in some plants to represent precociously coiled threads (*e.g.*, in *Velthemia* and *Rhoeo*, 76, 77); it has also been demonstrated that the chromocenters of a series of plants and animals can be transformed experimentally into a loose mass of uncoiled chromonemata (215, 381). The chromonemata of the heteropyknotic regions that lie adjacent to the centromeres in the chromosomes of *D. melanogaster* are coiled during the prophases of somatic mitoses (184), but it has not been determined by cytological observation whether the chromomeres that appear along these chromosomes—which presumably represent intercalary heterochromatic regions—likewise represent coiled portions of the thread. On the basis of present knowledge of the organization of somatic and meiotic chromosomes, it seems certain that helically disposed chromonemata must constitute the chromomere-like segments that can be produced in some condensed chromosomes by pretreatment or by fixation which accentuates the intervening secondary constrictions (*e.g.*, 136, 179, 203, 382, 406). Even in well-extended chromosomes, such as those of the leptotene and zygotene stages of meiosis, the small chromomeric bodies have been identified as tight coils (28, 183, 201, 202, 279, 412).

The conflict between the interpretation advocated by Ris and the more widely accepted view of the reality of ultimate chromomeres, as visualized by Belling, is not to be resolved, however, by showing that some chromomere-like bodies are coiled structures, but rather by the demonstration that all chromomeres represent differentially

coiled parts of the thread. Any analysis directed toward this end must consider the organization of giant chromosomes such as are seen in the salivary glands of the larvae of the Diptera. The appearance of discs or bands that traverse these so-called polytene chromosomes depends, according to the most widely accepted interpretation, on the close approximation or confluence of homologous chromomeres of a series of parallel-lying chromonemata. Adjacent discs vary sufficiently in thickness and intensity of staining to form a constant and precise pattern of linear organization that has been utilized by cytogeneticists in the analysis of naturally occurring and induced chromosomal changes. It is generally believed that the extraordinary length of the giant chromosomes is attained primarily by extension of the visible and submicroscopic coils of the chromosomes of the ancestral mitotic cells. According to this view, the Feulgen-positive nodal points along the chromonemata are ultimate chromomeres, and not differentially coiled portions of the thread. However, this interpretation has been contested by Ris and Crouse (333) who suggest that the apparent banding represents only the surface aspect of helically-coiled chromonemata that weave back and forth across the width of the chromosome. These authors state that "the chromonema itself is uniformly Feulgen-positive", but observational evidence lends little support to this interpretation. Since the bands vary greatly in their staining capacity, it follows that the nucleic-acid content of the threads varies from region to region, even if it is assumed that the bands represent adjacent turns of a system of coiled threads. The additional pertinent observation has been made that various protein-staining dyes gives the same differential pattern of banding as orcein and the Feulgen reaction (365). These are indications of regional differentiation, regardless of the interpretation accepted concerning the disposition of the chromonemata. Moreover, since the pattern of banding of a specific region is constant, even when extensive realignment of parts occurs as a result of spontaneous or induced chromosomal rearrangement, it is necessary on the theory proposed by Ris and Crouse to assume that the course of the chromonemata as they traverse the chromosome, and the pattern of association of sister strands at any one level, must be under the precise control of the individual loci in the regions involved. The constancy of the pattern of banding, even when larvae are raised under a wide range of experimental condi-

tions, also indicates that any assumed coiling mechanism which permits such stability is not so sensitive to environmental modification as the mechanism that controls coiling of the chromonemata in plants (*e.g.*, see 409 for a report of the effect of heat on modification of the pattern of coiling in *Tradescantia.*)

The theory that the bands of the salivary-gland chromosome merely represent turns of the chromonematic coils disregards much of the experimental evidence that has been accumulated in recent years. Attention may be called to observations that stretching of the salivary-gland chromosomes of *Chironomus* by micromanipulation involves primarily the interband regions and is accompanied by a decrease in the diameter of the chromosome (36, *cf.* 12, 304, 305). Objections that might be raised to interpretations of chromosome structure based on such fixed preparations have been met by micrurgical studies of living chromosomes of *Chironomus* (82). It was found that chromosomes may regain their initial length after a tenfold elongation, that in the process of stretching the interband regions undergo the greatest elongation, and that the thick bands are more resistant to stretching than the thinner ones but can be separated in some cases into a series of component bands. Fibrils detached from the chromosome in other experiments revealed a pattern of differentiation corresponding to that of the discs of the region from which the strand was removed. Stretching of the chromosome laterally produced a distinctly beaded appearance in bands that were more or less uniformly homogeneous prior to the micromanipulation. These observations and the cytogenetic studies that have revealed the loss or gain of single delicate bands, such as has occurred at the roughest (*rst*) locus in *D. melanogaster* (190), support the theory that the giant chromosome is polytene and that each component strand consists of alternating chromomeric and non-chromomeric segments. The evidence now available is more difficult to reconcile with the theory that these large chromosomes consist of a system of helically arranged chromonemata.

This briefing of the argument must suffice, since a full consideration of any one theory lies beyond the scope of the present article and since another critique has recently been presented (163). In passing it seems desirable, however, to point out that Ris's effort to fit the lampbrush type of chromosome into his general interpretation, by assuming that the side-chains represent loops of a system of

coiled threads, likewise disregards much of the experimental evidence. These chromosomes of the amphibian oocyte may be stretched micrurgically without disturbing the specific chromomeric pattern seen in the living condition, or without opening the side loops (110–113). Moreover, the loops, which normally disappear in later prophase stages, may be separated precociously from the main strands that represent the chromatids as a consequence of alteration in pH, changes in salt concentration, the action of proteolytic enzymes, or treatment with X-rays. Evidence of this type led Duryee to the conclusion that the lateral loops grow out from the chromomeres that are distributed along the chromatids. Little support can be derived from such experiments for the contention that "the side branches are the gyres of major coils of the individual chromonemata, which have laterally separated from each other" (332).

Efforts directed toward the denial of chromomeres as definite structural units inevitably emphasize the differences inherent in the more restricted interpretations of the chromomere and the chromonema hypotheses. The assumption that they represent alternative, or mutually exclusive, theories of chromosome organization was a natural consequence of the belief that the longitudinal division of a coiled thread would disrupt the linear order of the genes contained therein. Since that conflict was settled many years ago in a manner that eliminates such objections, it seems desirable to continue to utilize the term "chromomere" to describe the constant, morphologically differentiated segments of the chromonema seen in the living cell as well as in fixed and stained preparations. The further resolution of such regions into tightly coiled threads may be revealed at the microscopic level in some cases; in others the evidence now available suggests that chromomeric organization exists in the fully extended chromonema. As judged by their appearance and staining capacity, the chromomeres can be explained best, at our present level of knowledge, on the basis of the variable capacities of different loci with respect to the timing or amplitude of the nucleic-acid cycle.

Euchromatin and Heterochromatin

Linear differentiation of the extended chromonema is thus revealed in both euchromatic and heterochromatic regions. Accord-

ingly, euchromomeres and heterochromomeres have been described (13), but morphological differentiation is not always possible. In the salivary-gland chromosomes the heterochromatic regions lying adjacent to the centromeres frequently show a vesiculate type of chromomere in contrast with the compact type characteristic of euchromatic regions. As indicated previously, this has not been a useful criterion for revealing the location of intercalary heterochromatic segments in *D. melanogaster;* but, as Schultz noted (271) and as the author has observed in induced rearrangements, the nucleic-acid content of a disc and the appearance of its chromomeres will depend not only on the general environment provided within the nucleus but also on its position within the chromosome. Such position effect might account for the absence of heterochromomeres in regions of the salivary-gland chromosome that, on the basis of their non-specific pairing and frequency of breakage with respect to length, appear to contain heterochromatin. Alterations in more general conditions within the cell may account for the type of flexible heterochromatization reported for *D. palidipennis* (106). In the salivary-gland chromosomes the boundary between heterochromatin and euchromatin is variable; many discs that appear euchromatic in some cells have in other preparations the diffuse appearance characteristic of heterochromatin. Moreover, in mitotic cells the rate of nucleination of the euchromatic portions of the distal section of one arm of the X-chromosome appears to depend on proximity to heterochromatin.

The suggestion has been advanced that the essential difference between euchromatin and heterochromatin may depend on the proximity of chromomeres with the same nucleic-acid cycle, a heterochromatic segment being one with a high proportion of similar or identical chromomeres (308). However, in some plants the heterochromatin in different parts of the same nucleus or in different cells at the same stage may present different cytological appearances (119, 139, review by 320). Cytogenetic studies on *Drosophila* have revealed that certain position effects depend on the proximity of a locus to a specific portion of the heterochromatic material (*e.g.,* 190). In this genus also, marked differences may exist in the salivary-gland chromosomes in the degree of development of regions which appear in mitotic cells to be uniformly heterochromatic. It is known that long segments of chromosomes, or

even entire chromosomes, may be represented in the salivary-gland nucleus by very few discs. For example, the heterochromatic segment which constitutes about one-fifth of the left limb of the second chromosome of *D. melanogaster* during mitosis has been reported to be reduced to a single band in the salivary-gland nucleus, whereas the bulk of the heterochromatin seen in the salivary-gland chromosome is derived from another segment of the mitotic chromosome (162). A striking case has been described in *D. nebulosa* (303); one type of heterochromatin shows relatively little "reduction" in the salivary-gland cells in proportion to euchromatin, but another type—not distinguishable from the first by its staining properties in the mitotic chromosome—is represented by a proportionally much smaller number of discs. Following prefixation treatment of root-tips of *Allium* with 0.005 M mercuric nitrate, and other reagents, pronounced differential staining was obtained of heterochromatic regions in the contracted metaphase and anaphase chromosomes. The heterochromatin of the centric regions was found to retain the stain longer in destaining than that in other parts of the chromosome, and the conclusion was accordingly reached that the proximal heterochromatin is of a special kind, essential for the functioning of the centromere (222). (For a fuller consideration of the various types of heterochromatin that have been described, see 320.)

The knowledge of the distribution of heterochromatic regions is slowly accumulating (see 191, 205, 387 for a survey of the various criteria being used in these studies). Further information is also being gained concerning the location and properties of the included genes. The original concept of genetic inertness applied to heterochromatin has been replaced by that of specialized function. Some workers have suggested that heterochromatin may contain a series of replicated units that serve essentially as modifiers of characters determined by other loci (246, 308). Other workers have suggested that heterochromatic genes function primarily in the control of nucleic-acid metabolism.

Chromosomes such as have already been described consist of sections of euchromatin and heterochromatin. Some chromosomes are entirely heterochromatic or nearly so. A classical example, the Y-chromosome of *D. melanogaster,* is essentially "inert" in the sense that it does not carry genes requisite to normal growth and development, but is indispensable because its presence is necessary to insure

fertility to the male. The supernumeraries (supernumerary fragments) found in natural populations of several species of plants and animals provide other examples of essentially "inert" chromosomes (87 and 118 present lists of species in which such fragments have been observed). The accessory B-type chromosomes of maize have been reported to show a staining reaction during the resting stage of mitosis that is characteristic of heterochromatin (102), although regional differences in staining capacity are evident at pachytene (231). As many as 34 B-type chromosomes have been observed in the cells of some plants of maize (314); in other plants they are wholly lacking. They are often distributed irregularly at mitosis and undergo alterations in size (for a consideration of the possible causes of such changes see 120); but nevertheless they are maintained in the population from generation to generation. It has therefore been suggested that their presence confers some advantage to gamete or zygotes that favors their selection (100, 102, 246, 274–276). Under such conditions the supernumeraries can hardly be designated as "inert" in the sense that they are entirely devoid of genic material concerned with nuclear and cellular activity. The opinion has been advanced that the additional heterochromatin they provide permits a more elastic regulation of the nucleic-acid metabolism of the nucleus as a whole than is possible in their absence, or perhaps favors the production of proteins of the histone type (92, 102, see also 50, 54). This explanation is in harmony with the evidence from studies of the effects of extra Y-chromosomes in *Drosophila;* the nucleic-acid content of oocytes in XXY-females is consistently higher than in XX-females (58, 358). In maize, however, it has been found that high numbers of the B-chromosomes cause reduction in vigor and fertility of the plant, and that they can rarely be accumulated in excess of 30 (314). Differential behavior of the supernumeraries may occur in different tissues. In *Sorghum purpureo-sericeum,* the B-chromosomes are lost by lagging in the cells of the radicle before seed ripening and in the shoot tissues as they attain maturity. Chromosomes that reach the cells of the anthers and ovaries, however, are maintained regularly. At the second division in the pollen grain, the B-chromosomes pass to the generative pole undivided. Following this the vegetative nucleus may undergo a series of additional divisions, presumably as a result of the effect of heterochromatin on the cytoplasm (100,

178). In a strain of rye it has been found that the chromatids of the "standard fragments" fail to disjoin at the first pollen mitosis, and pass to the generative pole. A similar process occurs at a corresponding stage in the ovule (277).

Håkansson (157) reports that the supernumeraries of *Godetia nutans,* which he prefers to call "accessories", are not heterochromatic, and cannot for that reason have a significance in the nucleic-acid relationships of the cell. It has been suggested that the accessory chromosomes in this species may have a kind of "parasitic" existence, accumulating as a result of non-disjunction and being maintained because of their relatively harmless effects (291). According to this view, the fragment chromosomes have persisted in the population despite selection that favors plants without fragments.

Supernumerary fragments of varying number and size are also represented in the sex-chromosome complement of the bedbug *Cimex* (89, 386, 388, 389). Probably only two of the three to 15 fragments observed in different individuals have a sex-determining function, the remainder representing supernumeraries. The various possible methods of origin that have been suggested include misdivision of the centromere (89), loss of parts of the X containing the active genes (429), and fragmentation of the X or Y (418, on the basis of a study of the fragments in the reduviid, *Gelastocoris,* which appear to represent nonhomologous sections of an original single X with a diffuse centromere).

In this general discussion heteropyknosis has frequently been mentioned as an index to the location of heterochromatic regions. However, chromosomes that are heteropyknotic in some divisions may condense at a slower rate or at the same rate as the autosomes in other mitoses. The terms "positive" and "negative" heteropyknosis have been applied to these aspects of alteration in the timing cycle. If heteropyknosis is accepted as the sole criterion for identifying heterochromatin, the X-chromosome in certain grasshoppers must be regarded as heterochromatic in spermatocytes, in which it condenses precociously, and euchromatic in oocytes, in which it condenses at the same rate as the autosomes (78). In closely related species of *Gerris,* Geitler (141) observed that the X-chromosome could be either somatically euchromatic or heterochromatic, and concluded on that basis that such heterochromatin could not have an essential physiological function. Among the males of certain

scale insects one entire haploid set of chromosomes is heteropyknotic in somatic tissues and during spermatogenesis (165, 350, 351). Probably the entire set is paternal in origin (see 431), having been altered in passing through the gamete so as to produce heteropyknosis in the male but not in the female. Obviously, heteropyknosis in this situation does not provide an index to genic inertness of the chromosomes involved.

SUBMICROSCOPIC ORGANIZATION

An approach to analysis of the finer structure of the chromosome and the distribution of its constituent materials has been made in recent years by various chemical, histochemical and physicochemical methods. A brief summary of some of the contributions will be presented here. Other methods of potential value in the study of chromosome structure have been considered by Loofbourow (228) in his review of borderline problems in biology and physics.

Chemical and Histochemical Analyses

In 1924 Feulgen and Rossenbeck (122) described a technique, now known as the Feulgen or nucleal reaction, for demonstrating the presence of desoxyribose nucleic acid in tissues. Application of this test has shown that desoxyribose nucleic acid represents a major component of the nucleus but not of the cytoplasm; it is detectable in limited quantities in some types of nucleolus, *e.g.*, the salivary-gland cells of *Drosophila* (184). During the growth stages in oogenesis the chromosomes may not give the characteristic reaction, and it has been assumed that they were Feulgen-negative (200), but the work of Brachet (30) suggests that they are never entirely free of desoxyribose nucleic acid, although the degree of dispersion in the large chromosomes may provide only a feeble staining reaction (*cf.* 407). The "in situ" specificity of the Feulgen reaction has recently been questioned (46, 67, 83, 396–399) and defended (11, 32, 33, 40, 56, 107, 370, 401, 402). As the latter group has indicated, the point of view presented by the former workers appears untenable, and more convincing evidence is required if it is to be sustained.

Both desoxyribose and ribose nucleic acids, because of their purine and pyrimidine bases, show maximum ultraviolet absorption at about wave length 2,600 Å. The position in the spectrum of

the region of maximum absorption, and its intensity, as indicated by an extraordinarily high extinction coefficient, provide a type of absorption curve that permits detection of nucleic acid in biological systems, even when it is present together with proteins and other substances (47). Thus by comparing the cytochemical evidence obtained by analysis of absorption spectra with that provided by the Feulgen reaction it has been possible to determine the distribution of desoxyribose nucleic acid during the cycle of mitosis. The detection of ribose nucleic acid presents greater difficulties; the criterion of maximum absorption at about 2,600 Å and failure to give the Feulgen reaction is not infallible, inasmuch as purines and pyrimidines may exist in forms other than nucleic acid; *e.g.*, in striated muscle the concentration of adenyl nucleotides is sufficient to permit their localization (61). An additional method of analysis involves use of the enzyme ribonuclease. Structures assumed to contain ribose nucleic acid are tested before and after application of the enzyme, either by measurements of absorption spectra or by staining in Unna's pyronin-methyl green combination (see especially 32). It has previously been pointed out that the conclusions which may be derived from these tests are limited by questions concerning the purity of the enzyme and its specificity of action.

Despite such limitations—and Danielli (83) has pointed out many of the complications inherent in the techniques now used—modern cytochemical methods permit an approach to a quantitative expression of differences in nucleic-acid concentration. They have shown that the quantity of desoxyribose nucleic acid increases during the prophases (48, 49), accumulating locally at each chromomere, with the heterochromatic regions serving as especially potent centers of synthesis (362). During the telophases the quantity of desoxyribose nucleic acid decreases. Evidence has also been obtained that indicates a direct connection between the nucleic-acid metabolism of the nucleus and that of the cytoplasm. A supernumerary Y-chromosome in a female of *Drosophila* increases markedly the ribose-nucleic-acid content of her oocytes as compared with those of the ordinary XX-female (362). Cells of growing tissues exhibit in their cytoplasms an absorption peak around 2,600 Å, characteristic of the cyclic nitrogenous bases present in the nucleic acids, whereas the cells of mature tissues exhibit an absorption spectrum similar to that of the proteins. This has been shown by

comparisons of meristematic and differentiated cells in the root tip of *Allium,* and of rapidly growing and inactive yeast cells; by studies of the embryo of rye; and by analysis of embryonic development in the chick (59, 62, 57, 34; see also Brachet's studies on marine eggs, 29). By measuring cytoplasmic basophily following digestion with ribonuclease, it was demonstrated that young pollen-mother and tapetal cells of *Rhoeo discolor* are rich in ribonucleic acid, but that it is used up during the progress of meiosis (296). Accumulation of ribonucleic acid occurs subsequently in the cytoplasm of the pollen grain, presumably as a result of the cytolysis of tapetal tissue. Painter suggests that this accumulation serves to facilitate the rapid synthesis of material during the formation of the pollen tube. In the seeds of *Echinocystis macrocarpa* the giant cells of the nucellus and the endosperm presumably function as the immediate source of the materials necessary for the innumerable mitoses which produce the dense small-celled tissue of the rapidly developing cotyledons of the embryo (366). The phenomenon of endomitosis provides a method for increasing the nucleoprotein content of the nucleus, and the presence of multiple chromosomes or chromosome complexes in a variety of cells with nutritional or secretory functions has elicited various suggestions concerning their rôle. In *D. melanogaster* cleavage mitoses may follow each other within intervals as short as ten minutes, and it has been suggested that the ability of the chromosomes to synthesize the material needed for their rapid duplication is made possible by the reassembling of products, such as nucleotides, that are made available following the disintegration of the nurse cells (299). The question has been raised whether the chromosomes produced endomitotically during oogenesis themselves serve to produce the cytoplasmic precursors (60), since it has been found that in the sea urchin, and in *Drosophila,* the cytoplasmic synthesis takes place before the breakdown of the nuclei. More recently Painter (297) studied the secretory processes in the lateral pharyngeal glands of the worker honey bee, as well as in the salivary glands of the larva of *Drosophila,* and concluded that "endomitotic growth is the cytological mechanism by which heterochromatic centers and nucleolar organizers are increased in gland cells thus making it possible for a single cell to secrete large amounts of protein or other complex substances".

It has been possible to demonstrate in some rapidly synthesizing

cells that concentrations of ribose nucleic acid occur around the nuclear membrane and in the nucleolus. In the sea urchin the cytoplasm close to the germinal vesicle shows a much higher nucleic-acid maximum than that in the more peripheral regions (60); and in the nurse cells of *Drosophila* the concentration around the membrane is equally marked (358). Oocytes of the toad likewise show a perinuclear concentration of ribonucleic acid when tested by Unna's stain in combination with ribonuclease (301). In cells of root tips of *Allium* and *Spinacia,* local accumulations of ribose nucleic acid have not been detected in the cytoplasm, but will be determinable only if synthesis exceeds the rate of transport (60). However, the arginine test for protein indicates that during the prophases in root-tip mitoses of *Allium* and *Vicia* there is a synthesis (or differentiation) of basic proteins taking place at the same time that the nucleolus is disappearing (376). The possible rôle of the nucleolus in building up the cytoplasmic nucleic acids has been mentioned previously.

Within the chromosomes themselves quantitative changes in the concentration of desoxyribose nucleic acid have been measured in studies of spermatogenesis in the grasshopper, *Gomphocerus* (48, 49). The amount of nucleic acid was found to be much smaller in the spermatogonia than in cells at early leptotene. Since neither the cytoplasm nor the fluids of the testes showed any appreciable absorption at 2,600 Å during the period of rapid cell division, it was concluded that an active synthesis takes place in the chromosomes. From mid-leptotene to diplotene the quantity of nucleic acid in the nucleus is approximately constant; it appears, therefore, that there is no direct correlation between nucleic-acid synthesis and the chromatid coiling which occurs during these stages. Since the increase in concentration occurs at the time that the splitting of the chromosomes becomes apparent, Caspersson has suggested a fundamental relation between this increase and gene reproduction—in line with a similar suggestion made by Caspersson and Schultz (58), which was based primarily on a study of nucleic-acid metabolism of the salivary-gland chromosome and of the egg cytoplasm in certain variegated races of *D. melanogaster* (see also 92). However, the multiplication of genes and chromonemata apparently occurs one or more mitoses in advance of the time of separation of the chromatids, so that Caspersson's data may merely indicate that

the concentration of nucleic acid permits recognition of a previously established line of demarcation between chromonemata (see 171). It has been reported that chromatic materials, when injected intravenously into rats, increase the rate of mitosis in liver following partial hepatectomy (244). On the other hand, the finding that the percentage of histones is low in carcinoma nuclei and chick embryos, and high in resting nuclei, has led to the suggestion that these proteins regulate the growth and division of cells (398).

The relation of nucleic acid to the other components of the chromosome has been studied in a variety of ways. Analysis of absorption spectra indicates that the dark bands of the salivary-gland chromosomes contain high concentrations of nucleic acid, the pale bands much less, but that both contain proteins (47). A trypsin solution to which lanthanum had been added was used to digest the proteins and leave the nucleic acid as an insoluble lanthanum thymonucleate. Other enzyme studies (126–128, 257, 258, 358, 365) have extended these observations. Pepsin did not remove any constituent of the chromosome concerned in maintaining its integrity, although a marked decrease in volume occurred, perhaps owing to removal of the more complex proteins of the matrix. Following treatment with trypsin in the absence of lanthanum ions, the nucleus appeared empty. By the use of nucleases, both the sugar and the nitrogenous components of the nucleic acid presumably were removed; but the continuity of the chromosome was not destroyed, the protein component still giving a characteristic blue color with ninhydrin. It seems, therefore, that the chromosome has a continuous protein structure which is not disrupted when the nucleic acid is split off (258). These experiments indicate that chromosomes contain nucleic acid and proteins in intimate association as nucleoproteins.

An additional series of histochemical tests for localization of cellular components has been developed (31, 32, 366, 372–379). In addition to the ninhydrin reaction, the biuret, xanthoproteic and Millon tests have been utilized, together with special methods for the detection of arginine and tryptophane in sections and entire cells. Nucleic acids have been localized by testing for phosphorus. As an illustration of the method of application: A high concentration of arginine is interpreted as indicating the presence of basic proteins resembling the histones, since they alone—except for the protamines

of fish sperms and the edestins found in plant seeds—possess a great amount of this amino acid. Furthermore, histones contain only traces of tryptophane, the more complex proteins a much larger proportion, and this provides a chemical basis for their differentiation. Quantitative determinations proceed on the assumptions that the color intensity is approximately proportional to the amount of the compound present, that the density does not vary greatly in the regions compared, and that secondary color impregnation and adsorption do not occur. The first two involve factors of judgment; the third can be determined experimentally. The arginine test, as used by Serra and Queiroz Lopes, confirms essentially the findings of Caspersson (52), obtained from an analysis of absorption spectra, that the discs in the salivary-gland chromosome contain proteins of the histone type, and probably also proteins of a "higher type", whereas the interbands consist wholly of proteins of the "higher type," perhaps similar to globulin. Tests made on the mitotic chromosomes of the root tips of *Vicia* and *Allium* suggest that the proteins are similar to those found in the discs of the salivary-gland chromosome. The action of nuclease does not diminish the strength of the reaction. The phosphorus test is reported to give a strong reaction in the chromosomes of dividing cells and in spermatozoa, which are rich in nucleoproteins. The detailed procedures utilized in these and other tests for proteins have been summarized by Serra (375); and additional discussion concerning the application of the methods is to be found in other reviews (32, 150, 359).

Considerations such as these lead inevitably to problems of chromosome chemistry and gene constitution (for discussion see 54, 92, 99, 153, 154, 266, 273, 359, 360, 371). The isolation and analysis of parts of the cell by physical and chemical methods (*e.g.*, 71, 108, 256, 267–269, 396) have provided quantitative estimations of amounts of nucleic acids and proteins of various types in chromosomes obtained from normal and malignant tissues. The chemical evidence suggests that the chromosome contains three groups of components—desoxyribose nucleic acids, histones, and tryptophane-containing proteins—the quantities varying greatly according to the type of tissue and its physiological activity (269, 396)*.

* Since the foregoing was written in the summer of 1946, studies by Mirsky and Ris, and histochemical analyses carried out by the author have confirmed the earlier intimations that the chromosome also contains ribose nucleic acid.

Histochemical tests have also included microincineration and ash analysis. The ash deposits of incinerated chromosomes represent primarily the phosphate residues of the nucleic acid. In the salivary glands the bands differ considerably in their ash content. Details of the methods and their applicability to problems of chromosome structure have been presented in other reviews (116, 420).

Physicochemical Analyses

Birefringence in polarized light, and X-ray and electron diffraction patterns, have been utilized in efforts to determine the submicroscopic molecular architecture of the chromosome.

The use of double refraction of nucleic-acid molecules as a guide to patterns of coiling of the chromonemata (206–208, 210–212, 280, 345, 346) has not provided critical information beyond that available from studies with the ordinary microscope (see 14, 124, 212, 283). Orientation of the thymonucleic-acid molecules, as determined by their birefringence in polarized light, has led to the suggestion that the nucleic-acid chains as well as the polypeptide chains of the giant chromosomes probably lie parallel to the long axis (124, 304, 305, 346, 347; for similar evidence from other methods of analysis see 7, 53, 258, 385). By X-ray diffraction it has been found that the distance between the nucleotides corresponds almost exactly with the backbone spacing (3.34 Å) of the fully extended polypeptide chain of a protein reported by Astbury and Bell (7). The observation that the nucleic-acid chains lie parallel to the long axis of the chromosome contrasts with an earlier interpretation (438) that they are interwoven with the protein chains at right angles to their axis of alignment. A further suggestion that only the protein which is combined with the nucleic acid in the bands is in the fully extended state, that of the interband regions being in the folded state (305), has been questioned (53). No marked orientation of the molecules of thymonucleic acid was detected by means of a sensitive test involving dichroism in the ultraviolet. It thus becomes necessary, in interpreting birefringence of fixed preparations in polarized light, to consider the possibility of artifact resulting from the tendency of the molecules of nucleic acid to become

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The histochemical studies in this laboratory have involved the use of a ribonuclease preparation that had been freed of measurable traces of proteolytic activity through methods developed by Dr. Margaret McDonald.

aligned artificially. (For further discussion of these problems see 348 and 55.)

Since, "in the usual heterogeneous biological material, birefringence is likely to be due simply to orientation of micelles . . .", an attempt was made to employ diffraction methods in the analysis of chromosome structure (37). By painstaking and laborious methods, packets of thousands of salivary glands were obtained from various species of *Chironomus* for X-ray and electron diffraction photography. The results did not warrant formulation of any definite conclusions, but Buck and Melland suggest that the technique has not been tested adequately.

It is by a combination of methods as have been described in the foregoing paragraphs that a more precise understanding will be reached of the sequence of events that leads to alterations in chromosome structure in the cycle of mitosis. Determination of the proportions of the different proteins, their relation to the nucleic acids, and the various degrees of polymerization and depolymerization occurring during the different mitotic phases provides one of the more promising approaches to problems of chromosome structure and gene activity.

CHROMONEMATA

Determination of the number of chromonemata and their patterns of coiling represents the first approximation to an understanding of the method of chromosome duplication. Efforts have been made, therefore, to secure unequivocal fixation images and to discriminate between different observational interpretations by utilizing accessory methods of analysis, such as ionizing radiations. Despite these efforts, conflicting conclusions have often been drawn from different studies on the same organism. Possible differences among species, individuals and types of cells are also complicating factors which prevent formulation of a uniform and general interpretation. Although the studies of recent years have not answered fully all the questions advanced in the 1936 review, several of the observations have provided the information necessary for a critical appraisal of theories of chromosome mechanics based on assumptions of simple numerical relations.

Numerical Relations

Microscopic observations. At the time that the 1936 review was prepared, the evidence derived from cytological observations favored

the following interpretation. Intertwined chromonemata seen in early prophase nuclei of somatic mitoses are residual from the chromosomes of the preceding telophase. As the prophases advance, these coils loosen, and each discloses another pair of intertwined chromonemata. Metaphase chromosomes are accordingly quadripartite with respect to chromonemata, and following the separation of the chromatids at anaphase each chromosome reveals two intertwined strands. Since premeiotic anaphases and telophases showed the same type of organization, it was assumed that the leptotene threads of meiosis were longitudinally double, although appearing unsplit under the microscope. Pairing of these threads and subsequent appearance of a tertiary split accounted for the octopartite chromosome observed at metaphase of the first meiotic division. Anaphase chromosomes of this division would accordingly have four chromonemata, those of the second meiotic division two.

More recent observations, although differing widely with respect to such details as the time of splitting of the chromosome, have confirmed the existence of multiple chromonemata at the various stages of somatic and meiotic mitoses (*e.g.*, 1, 2, 8, 23, 42, 75, 80, 104, 131, 132, 134, 140, 169, 202, 207–209, 212, 221, 236–239, 241–243, 278, 279, 281, 285, 318, 319, 334, 335, 391, 424, 425, 436, 437, 439).

Among the clearest illustrations of coiled chromonemata are those obtained by treatment of smears of microsporocytes with weak solutions of acids, alkalies and other agents preceding their fixation (*e.g.*, 79, 95, 214, 251, 293, 380). Application of such treatment to the growing pollen tube, whose mitotic chromosomes are readily reached by the reagents, has provided fixation images that indicate the presence in each metaphase chromatid or anaphase chromosome of a tightly coiled thread rather than loosely intertwined chromonemata. Some workers have maintained that the coil is composed of an undivided strand, an interpretation that stems from the concept that the chromatid, which behaves as a unit in mitosis and in crossing over, is likewise the fundamental structural unit (see 87 for exposition of this viewpoint). The thread, even when extended, may appear single, but this can hardly be accepted as conclusive evidence that it is undivided or that well defined cleavage planes for succeeding mitoses are not determinable. In somatic chromosomes, split ends or bipartite satellites, which are occasionally observed during anaphase, furnish evidence that the thread is divided (*e.g.*,

75, 137, 185). Since such observations were made on chromosomes that had not received prefixation treatment as well as on those that had, there seems no valid basis for the interpretation (432) that the appearance of duality following certain types of prefixation treatment is attributable to the action of the agent and does not reveal the normal pattern of organization of the chromosome. In the living germinal vesicle of the frog each of the homologues appears as a single thread under the conditions of microdissection, although the tetrad nature of the bivalent is implied by the presence of chiasmata, and four chromonemata may be revealed if the cells are fixed, or treated with X-rays (113). It has been suggested that dehydration of the nucleus may cause the close approximation and optical obliteration of the individual strands of a complex spiral, and that "reversible gelation-peptisation changes", especially in the matrix of the chromosome, would account for the variable expression of a prophasic or telophasic split (209, 214).

The compounding of several strands into an optically single one is strikingly represented in the chromosomes that presumably originate by repeated duplication of the chromonemata without intervening mitosis. In the epithelium of the hind gut of the larva of the mosquito, *Culex pipiens,* each chromosome may be composed of from two to 32 strands united in a common centric region and frequently appearing under the microscope as a single thread rather than as an aggregate (19–22, 151, 152). Similarly, the origin of the giant chromosomes of the salivary glands of the Diptera has been traced to the fusion of a pair of homologues, each of which is longitudinally double (35, 298). Nevertheless, in the smaller cells of the salivary gland the chromosomes appear single (263); in the larger cells the homologues are easily distinguishable in unsynapsed regions, but only rarely are indications of the fundamental quadripartite structure detectable (125, 195, 259, 298, 417).

When cells constitute masses of tissue to which the reagents do not have immediate access, the problem of securing adequate fixation becomes more difficult. Following prefixation treatment with a dilute solution of sodium cyanide (75), a tightly coiled spiral was observed in chromosomes of the premeiotic and root-tip metaphases of the cultivated tulip, and also in metaphase and anaphase chromosomes of the second meiotic division in *Gasteria.* Because of such observations (see also 95, 137, 139, 140, 342, 421) some workers

regard the interlocking double-coil type of structure so frequently described by plant cytologists as a fixation artifact. Others, proceeding on the assumption that the appearance of the single-coil type of structure does not imply that the chromonema is undivided, have suggested that the transition from one type to the other might conceivably follow a change in the form of the chromosome, a redistribution of its materials or an alteration in the expression of the standard and subsidiary coils (see 209, 213, 260, 283, 319). Observational evidence verifying the existence of the interlocking-double-coil type of structure continues to accumulate. By treatment of root tips of *Allium* with salts of heavy metals prior to fixation it was possible to detect intertwined chromonemata in each metaphase chromatid (221). "The half-chromatids often form a relational spiral of great clearness", and the space between them is sometimes so considerable that "the phenomenon cannot be due to an optical error". Moreover, metaphase and anaphase chromosomes of microspores of *Trillium* show closely coiled somatic-type spirals, each of which is composed of two chromonemata that in certain regions "can be seen to be wound in the form of a plectonemic spiral" (391).

Some evidence has been presented in favor of the view that the somatic telophase chromosome contains at least four microscopically detectable chromonemata (2, 209, 214, 239, 282, 283, 285–287). Determination of the number of strands by cytological methods involves interpretation of diffraction patterns that are made more confusing by uncertainties concerning the helical relationships of sister chromonemata and the possible existence of a minor gyre along each of the more conspicuous coils. Use of the ultraviolet microscope may possibly afford some advantages in resolving these details. Photographs made with this instrument suggest that late-anaphase chromosomes in germinating spores of the fern, *Todea,* have four differentiable chromonemata (239).

Four coiled chromatids can readily be demonstrated in first meiotic metaphase chromosomes of microsporocytes of various species of plants. Further subdivision into half-chromatids, which will separate at the first pollen grain division, has been detected in some species with large chromosomes (for summaries of earlier work see 185, 209, 283). Physically complete separation of meiotic half-chromatids occurred in plants of *Trillium kamtschaticum* that

had been subjected to heat treatment prior to meiosis (255). Among animals, half-chromatids were observed in the grasshoppers *Podisma* (235) and *Romalea* (265); but the most striking demonstration is provided by coccids of the genus *Llaveiella* (166). In these insects primary spermatocytes were observed in which the chromosomes were subdivided along a tertiary split, each pair of half-chromatids possessing an independent spindle. Occasionally one or more chromosomes of the complex revealed a fourth or quaternary split. From such evidence it may be inferred that in this material the leptotene chromosomes and those of the preceding anaphase and telophase are multiple stranded.

Studies utilizing radiations. Treatment of chromosomes with X-rays has been utilized in an effort to discriminate between the divergent interpretations of the descriptive cytologists. The first experiments proceeded on the assumption that irradiation of the divided, or double, chromosome would produce breaks independently in the two chromatids (chromatid breaks), whereas irradiation of the undivided, or single, chromosome would produce breaks that would be transmitted with the subsequent division equally to the daughter chromatids (chromosome breaks). It was assumed that the energy required to induce breakage is derived from a single ion pair, and that, following the longitudinal division of the chromosome, the sister strands would soon become separated so widely that they could not be included within the sphere of action of a single ionization—a sphere whose radius could hardly extend over microscopically detectable distances (103). In fact, the experimental results indicated in a general way that prior to a certain stage in the course of mitosis—either in interphase (245, see also 63) or early prophase (330, see also 43, 44, 337)—the chromosome reacts as a unit in response to the ionizing radiation, and that subsequently each of the two chromatids is affected independently. It remained a question, however, as Huskins and Hunter pointed out in 1935, whether this reaction represented in all cases a valid guide to structural singleness and doubleness, and whether two or more chromonemata might not act as a unit in response to the radiation. In addition to the cytological observations that lent support to this possibility, types of induced aberrations had been observed whose production appeared to depend on the simultaneous breakage of two adjacent chromatids (281).

Additional evidence was subsequently obtained from studies on *Tradescantia,* in which the processes of breakage and recombination occur in comparatively rapid succession (216, 339) and in which the splitting of the chromosome is presumably effected in a comparatively short time (341). Irradiation of the prophase nucleus of the first pollen-grain mitosis has yielded shortened, dicentric chromosomes and accompanying U-shaped acentric fragments whose production is attributed to the fusion of ends of sister chromatids broken by a single "hit" (341). Additional evidence that microscopically differentiable sister chromatids may be severed conjointly is provided by X-ray-induced double deletions having corresponding parts detached from both chromatids (338, 411). Aberrations of this type are comparatively infrequent in cells rayed at late prophase, but more abundant following irradiation at early prophase when the chromatids are closer together. Moreover, the frequency with which they are produced is proportional to the dosage, as might be expected if the breaks involved were produced by a single ionizing particle. If these same stages are treated with very soft X-rays (64), or ultraviolet rays (408, 411; also see 395), single chromatid deletions rather than isochromatid breaks are produced almost exclusively. Such results suggest that there is a marked qualitative difference between the effects of different kinds of radiation, the sphere of action of the very soft X-rays and the ultraviolet quanta rarely including more than one strand of a divided chromosome, that of the harder X-rays often encompassing both.

The path to an understanding of such results was indicated by studies of neutron-induced rearrangements (146, 147, 416). It was found that neutrons are effective in inducing two-break chromosomal rearrangements with a frequency proportional to the dosage. This indicated that the densely ionizing track of a single recoil proton causes breakage of two separate chromosomes, which it traverses successively. By analogy, Lea and Catcheside (216) reached the conclusion—which is fairly well substantiated (*e.g.,* 117)—that X-rays may produce isochromatid breaks by the action of a single secondary electron, which crosses successively the two chromatids even when they are widely separated. Thus the progress of splitting of the chromosome appears to reduce the chance of simultaneous breakage of the two strands, not by removing them from a single possible sphere of action, but by reducing the chance

that both will be hit by a single electron in rapid succession. It was also necessary for Lea and Catcheside to modify earlier conceptions of the breakage process by postulating that it is not dependent on a single ionization, even when a single strand is involved, but on a dense cluster of perhaps 20 ionizations within the thickness of the strand. Such clustering takes place not only at the tail end of the electron track, or at places where the main track branches out into other short tracks, but along the entire length of the track of a recoil proton.

Studies of the effects of different types of radiation on chromosomes in comparable stages of division have lent support to some of the earlier observations that suggested the occurrence of aberrations involving half-chromatids (*e.g.*, 43, 44, 243, 282, 283, 287). In his study of the differential sensitivity of prophase pollen-tube chromosomes of *Tradescantia* to X-rays and ultraviolet radiation, Swanson (411) found ultraviolet-induced breaks extending only part way across the diameter of the chromatid, suggesting that only one of two half-chromatids had been broken. He also observed an unequivocal case of a half-chromatid translocation induced by X-rays: "the half-chromatids at the point of breakage could be traced with ease and clarity".

The evidence provided by the studies of *Tradescantia* leads to the question whether the chromosome and chromatid breaks obtained following irradiation of the spermatozoa of *Drosophila* may likewise be derived from chromosomes that are longitudinally divided. Since the individual chromosomes are not identifiable in the spermatozoon, and since the induced breaks do not recombine until after the sperm has entered the egg in the process of fertilization (189, 272), the actual time of division of the chromosome into chromatids remains unknown, and the probability must also be considered that the presumptive cases of chromatid breakage actually result from differential recombination of sister chromatids that were effectively separated after the time of irradiation. The significance of the various chromosomal mosaics and duplications in evaluating these alternative interpretations has been discussed at length (*e.g.*, 188, 272; see also 65 for a consideration of the ring-X chromosome). Fractionals or mosaics for changes that presumably represent point mutations may also be produced by irradiation of the spermatozoa of *Drosophila,* and it is especially difficult to reconcile the production

of some of the observed types with the theory that subdivision of the chromonema has not occurred prior to the time of treatment.

From the various considerations presented in the foregoing paragraphs it is apparent that the methods of radiation biology tend to support rather than deny the interpretations of chromosome structure derived from the major portion of the cytological observations. Analytical procedures have in general involved treatment of cells assumed to be in a certain stage of mitosis and observation of the induced changes in the cells at a considerably later stage. It is to be hoped that some of the ambiguities and uncertainties inherent in this indirect method of approach may be avoided by direct observations of the effects of ionizing radiation on chromosomes, utilizing such techniques as Carlson (44) has developed for the study of living neuroblasts of grasshoppers. There is the further possibility that a comparative study of X-ray-induced and chemically induced gene and chromosomal mosaics—such as Auerbach (9) proposes—may cast additional light on the structure of the chromosomes at the time of treatment.

Mechanism and Patterns of Coiling

Patterns of coiling. Interpretations of methods of coiling have varied according to the premises accepted with respect to the number, disposition and organization of the spiral threads at the various stages of mitosis. Earlier efforts to envisage a simple mechanism whereby a relatively straight thread might assume a helical form to facilitate distribution of the chromosomes on the spindle, soon met with such difficulties as changes in direction of coiling within a chromosome arm, persistence of some degree of coiling throughout the cycle of mitosis, and occurrence of closed or ring chromosomes in which multiplication of chromonemata and separation of chromatids could occur without their entanglement. As a result, a series of critical studies of patterns of coiling have been carried out in recent years, using the favorable material provided by large plant chromosomes. Many of the observations have been evaluated in other publications (*e.g.*, 170, 394) and need not be presented in detail at this time.

During the early prophase of somatic mitosis in plants with large chromosomes, the chromonemata are seen in loose spirals with wide gyres. These are the relic coils derived from the standard coils of

the preceding mitosis. As the prophase advances it can be observed that the chromatids, which are contorted into these wide loops, are closely twisted around each other, or relationally coiled. Their constituent chromonemata are helically disposed, forming the so-called standard coils (283). The existence of a subsidiary coil along the standard coil has been inferred from observational and experimental evidence (*e.g.*, 95, 98, 260).

The chromonematic coils are especially conspicuous during meiosis, and have been studied extensively in the chromosomes of *Tradescantia* and *Trillium*. Each of the chromatids of the bivalent of *Tradescantia* at first meiotic metaphase is coiled into five to eight major gyres. The degree of coiling is under genetic control and therefore varies in different species and their hybrids; it is modifiable by environmental variables such as temperature (see 98, 409 for summary of earlier studies). Along the major coil there is a small-gyred minor coil (79, 170, 209, 293). Swanson (409) even reports the presence of a subminor coil, but since he regards chromomere-like aspects as evidence of minute coils, his observations must await confirmation. In *Trillium* the minor coils are apparently less conspicuous. Photographs suggesting their existence have been published (79, 176, 384), although in some preparations only a waviness rather than a tight coiling is apparent along the wide-gyred spiral (170). The observed divergency in form is probably not of fundamental importance but attributable to variable conditions existing during the spiralization cycle (208).

The two chromatids of each dyad are intimately paired in *Tradescantia,* whereas in *Trillium* each is a relatively independent coil. These chromatid major coils in *Trillium* are clearly paranemic (this type of coil, also called an "anorthospiral", permits separation without interlocking). When first-meiotic-metaphase chromosomes of *Tradescantia* were artificially uncoiled by ammonia (reported in 208), the chromatid major spirals were interlocked, as if derived from a plectonemic, or orthospiral. However, if the two chromatid major coils were normally paranemic, and the treatment causes uncoiling by rotation, that would account for the intertwining (170). It is certainly clear in smears of microsporocytes of *Tradescantia* (unpublished data of the author) that the chromatids constituting the dyad at first meiotic anaphase are free to separate—and can be forced apart when the pressure used in smearing is sufficient to

crush the cell—without any apparent loosening or uncoiling of the major gyres. This is evidence of a paranemic arrangement; and there is no convincing observational evidence that a plectonemic arrangement existed during earlier stages and became transformed to the paranemic type by breakage and reunion of chromatids (crossing over, as postulated in 252).

Indications of separation into half-chromatids are sometimes apparent in condensed chromosomes at metaphase, but are seen most clearly following artificial uncoiling of the major spiral (208) or in temperature-treated material (170, 173, 394, 409, 412). When clearly defined, the half-chromatids of *Trillium erectum* appear to be arranged in a plectonemic coil (170). On the contrary, in heat-treated material of *Trillium* and *Tradescantia* lateral separation of sister half-chromatids occurs without entanglement (255, 409). The free separation indicates a paranemic relationship of the sister strands, although it may possibly have been derived secondarily as a result of rotation induced by the treatment. It is particularly important to note that in *Tradescantia* (410), as in *Trillium,* the microspore chromosomes show plectonemic relational coils. In the grasshopper, *Romalea,* at first meiotic anaphase each of the chromatids of the X-chromosome reveals two half-chromatids which are loosely coiled around each other and presumably have been derived from a plectonemic coil (265).

During interkinesis in *Tradescantia* the major coils of the chromatids relax considerably, the spirals of the second division resembling those of somatic mitosis. In *Trillium* there is practically no interkinesis, and the chromosomes pass relatively unchanged from the anaphase of the first division to the metaphase of the second. The major coil of second anaphase is almost as large-gyred as that of the first (172, 249, 435). That its chromatids are plectonemically arranged was determined by direct observation and by analysis of relic and relational coiling of the first microspore division (391, 394).

The sequence of changes by which the conspicuous meiotic coils of metaphase and anaphase are derived from the attenuated leptotene chromosomes can not readily be detected cytologically. Inability to follow the coils from the time of their inception constitutes a severe handicap in the analysis. As indicated previously, interpretations have not agreed that the chromomere-like bodies of leptotene

represent minute coils along the chromonemata. It has generally been assumed that new coils are not formed until full extension has eliminated the spirals of the premeiotic anaphase chromosomes and the homologues have been associated throughout a part or all of their length (*e.g.*, 175, 236). Studies of normal and heat-treated material of *Tradescantia* suggest that the minute gyres that appear in early zygotene, or perhaps even in leptotene, are destined to become the major coils of metaphase by a process of despiralization which involves reduction in chromosome length and gyre number and increase in gyre diameter. During the later prophases the minor coil makes its first visible appearance, becoming increasingly evident as anaphase approaches (409, 410, 412; *cf.* 206–209 on *Tradescantia,* 212 on *Trillium*). Other observations have been interpreted as indicating that the minor coils precede the major ones in their formation (*e.g.*, 336 on *Tradescantia*). In support of this interpretation it has been reported that during mid and late diplotene in *Trillium* there is present only a small-gyred spiral with some intimations at the ends of the chromatids of the beginning of the larger-gyred coil (79).

Direction of coiling. An extensive series of observations on the direction of coiling serves as a further basis for evaluating the various hypotheses that have been advanced to explain the mechanisms involved. In somatic chromosomes it is only rarely possible to map accurately the course of the chromonemata through successive gyres. When the gyres are few in number, as in the flagellate, *Spirotrichonympha,* or in spermatogonial chromosomes of grasshoppers, occasional reversals in direction along the chromosome have been observed (72, 428). The chromonematic spirals in somatic chromosomes of plants are generally more compact and greater in number (20–25 in *Tradescantia, ca.* 80 in *Fritillaria*). Occasional preparations of metaphase and anaphase chromosomes reveal clearly the direction of coiling (336), but studies have more generally been made of the system of relational coiling during the late prophase stages. These observations suggest that the direction is essentially at random for the two arms of a chromosome and for corresponding arms of homologous chromosomes. There is a tendency for the coiling to be in the same direction from the centromere to the distal end of the chromosome, although reversals do occur (336; *cf.* 85). Sparrow (391) suggests that reversals observed in the somatic-type

coils of the microspore chromosomes of *Trillium* may play a part in maintaining uniformity of coiling along the chromonema and in facilitating the untwisting of prophase relational coiling.

Explanations of the origin of the relational coiling observed in somatic prophases vary according to the premises accepted with respect to the number and relation of the chromonemata during anaphase and telophase (see 209 and 213 for an extended discussion of the alternative possibilities; also see 391 for a listing of the proponents of the various interpretations). Darlington (85, 87) contends that the "relational coiling" of the chromatids seen in early prophase results from the division of the chromosomes at the end of the resting stage along a cleavage plane that will enable the chromatids to lie parallel at metaphase and separate freely at anaphase. This coiling, which compensates for the relic coiling and is therefore necessarily in the opposite direction to it, presumably disappears along with the relic coiling during prophase. The relational coiling seen at late prophase and metaphase is attributed to the chromatids' being dragged round one another as a consequence of forces determined by the assumption of an internal spiral in each chromatid. Consistency in direction of coiling within a chromosome arm, arising from an internal molecular torsion, is a basic tenet of this hypothesis (however, see preceding paragraph for evidence that the direction is not always consistent).

Twisting of chromatids, presumably in connection with the origin of new spirals, must also be considered as a mechanism involved in the production of prophase relational coiling, if it is assumed that two chromatids arranged in the form of a paranemic coil are present in the anaphase chromosome; for, under such conditions, extension of these coils will provide strands that are not interlocked, and intertwining must occur subsequently. The sequence of events would thus parallel in a general way that reported for diploid somatic mitoses of Diptera, in which somatically paired but independent telophase homologous chromosomes become intertwined during the resting stage, presumably in relation to chromosome reduplication (151, 152, 390). If, however, we accept the evidence, previously outlined, that the anaphase chromatids form either a loose or a compact plectonemic coil, then the intertwining observed during the prophases may be regarded as residual from the preceding mitosis (*e.g.*, 182; for discussion see 209, 394).

A further question arises concerning the pattern of association of the half-chromatids in those anaphase chromosomes reported to have four closely associated chromonemata. Some observers suggest that the arrangement is paranemic (239, 285). As visualized by Kuwada (209, 213), the two chromatids of the anaphase chromosome run parallel or twist around each other to some degree, each being composed of plectonemically arranged half-chromatids. In the untwisting of half-chromatids in the prophase, the two chromatids become interwined, since it is assumed that the half-chromatid spirals are to a large extent coiled in the same direction. Studies of the multiple-complex cells of *Culex pipiens* indicate that several chromonemata may exist in a complex plectonemic relationship and subsequently dissociate freely. The relational coiling between sister chromonemata is presumably established at the time of their formation, and at the same time there occurs a loosening of the relational coiling between the strands that originated at an earlier division. Some descriptions of the behavior of chromonemata in an ordinary somatic mitosis conform to such a general pattern; the anaphase chromatids form a plectonemic spiral; and their relational coiling is reduced gradually during the prophases when the plectonemic coils of the half-chromatids first become clearly defined. Whether the parallel thus drawn represents more than a superficial analogy depends on the validity of the underlying assumptions concerning the number of chromonemata and the origin of relational coiling in association with chromonema duplication.

The relational coiling derived from the somatic coils of the last premeiotic anaphase is presumably eliminated during the early meiotic prophases by the straightening out of the chromosomes (410). Kuwada (209) has suggested that synapsis takes place between homologues while they are unwinding the relational coils derived from the paired half-chromatids of the last premeiotic anaphase, and that when such untwisting occurs in parts of the homologues that happen to be twisted in the same direction, intertwining of the homologues will result, with possible chiasma formation at the points where the threads cross each other. Other evidence indicates that the threads are more or less fully extended and lie essentially parallel at the time of synapsis (*e.g.*, 254). The relational coiling between homologous chromosomes at pachytene has been attributed by Darlington (84, 87) to an internal molecular

torsion, and the suggestion advanced that chiasmata may replace relational coiling at the time that the chromatids are assumed to divide at the end of pachytene. However, as indicated previously, the basic assumptions on which this theory rests are open to serious criticism. If relational coiling at meiotic prophase is responsible for crossing over, and is the same in origin and nature as the relational coiling of mitotic prophase, a correlation might be expected to exist between the amount of relational coiling per unit of chromosome length and the number of chiasmata. Analyses carried out on the chromosomes of *Tradescantia, Vicia* and *Allium* (175, 336), and on a series of species of grasshoppers (81), suggest that such a correlation may possibly exist, although the data do not permit any definite conclusions.

The direction of coiling of the major spirals of the first meiotic division has been determined in a number of species. Changes in direction occur more commonly at the centromere, but also at chiasmata and other intercalary regions. The direction is essentially at random for homologous chromosomes. In *Vicia faba* and in *Tradescantia,* reversals were observed at chiasmata and occasionally at other loci (336). An extensive series of observations on synaptic, desynaptic and asynaptic chromosomes of *Trillium erectum* (170, 173, 394) led to formulation of the following hypotheses: *a*) The direction of coiling is random on either side of the centromere; this will cause half as many changes in direction as there are chromatid attachments. (Occasional cases of non-random change across the centromere were attributed subsequently—392—to a disproportionate or non-random untwisting in chromosomes with interbrachial reversals, and not to coiling that was non-random at its inception.) *b*) Chiasmata may cause changes in direction at a rate equal to the chiasma frequency—the basic assumptions here being that coiling begins after chiasma formation and that direction of coiling is random with changes optional rather than obligatory at the chiasmata (however, *cf.* 247, 248). *c*) Other reversals of direction ("adventitious reversals") occur with a frequency that is proportional to chromonema length and gyre number.

The frequency of changes in direction in a limited number of second-anaphase and microspore-relic coils in *Trillium* was found to correspond closely with that of first anaphase (394). Other analyses of relational coiling in gametophytic divisions have been

made on *Tradescantia paludosa, Allium cepa, Vicia faba* and *Hyacinthus orientalis* (174, 175, 423).

Mechanism of coiling. In the preceding paragraphs it has been shown that reversals of direction of coiling within a chromosome limb may occur in root-tip, meiotic and postmeiotic mitoses. Earlier studies (summarized in 185) have indicated that the direction of coiling of the minor spiral may be the same as or different from that of the major one. These observations run counter to any simple explanation of the mechanism of coiling which assumes a dependence on internal torsion directed from the centromere (*e.g.,* the hypotheses of Darlington, 84, and MacKnight, 234). Svärdson (405), on the basis of observations of changes in the relative lengths of the two arms of the chromosomes of salmon in the course of a mitosis and in consecutive mitoses—the effect being more marked in the longer arms than in the shorter arm—suggested a "migration" of spiralization over the chromosome with successive impulses originating from the centromere or from the telomere (terminal centromere). Such a mechanism would permit occasional changes in direction but does not account for a distribution that is proportional to chromonema length. A further suggestion that the distal portion of the chromosome may initiate the coiling process has been made by Coleman and Hillary (79) on the basis of their observation that the major coil of *Trillium erectum* is first detectable in this part of the chromosome. "Forces developing in the chromonema itself . . . independent of any forces exerted by the matrix" are assumed to cause spiralization. Nebel (284) has supplied a hypothesis which retains the molecular spiral envisaged by Darlington but endows it with properties that permit the direction of coiling to be facultatively determined anew at each period of gene multiplication. It is suggested that the gene strand is made up of "nemameres", intermediate in size between the gene and the chromomere, regionally differentiated in such a fashion that torsional repulsion permits a randomness of direction of the molecular spiral. Random changes in direction of coiling within a chromosome arm can be explained on the basis of this hypothesis, but the concept that reversals can take place only concomitantly with nemameric reproduction is not in accordance with the fact that there is a correlation between chiasmata and changes of direction.

Other theories have been formulated on the general assumption

that an elastic chromonema is thrown into a helical form as a result of being confined within some encasing material. Kuwada (207) proposed the theory that anisotropic swelling causes an intensification of the internally directed tendency of the chromonema to twist, and that when imbibition water is distributed evenly the threads are forced to untwist. The untwisting involves transformation of the twist into a spiral which assumes a regular and compact form as a consequence of the contracting force of the matrix. According to this theory the direction of the internal twist is the same as that of the spirals themselves, the twist and spiral being "different morphological expressions of the same twisted condition" (*cf.* Darlington's molecular-spiral theory which assumes an internal twist that is compensatory for and in the opposite direction to that of the larger spirals). Matsuura (254) argues that the properties of the matrix alone can not account for a uniformly regular spiral as is seen in *Trillium*. He concedes that the matrix imposes space limitations but believes that the elasticity of the thread, and its electrical charges, influence the pattern of coiling. Sax and Humphrey (340) had suggested earlier that contraction or compression of the two flexible chromatids without rotation within the chromosome pellicle would account for the primary coils of meiosis. This interpretation has been contested (435) on the grounds that the required shortening in the over-all chromosome length does not occur between late diplotene—when the major coil is first laid down—and anaphase (see also 79). However, this criticism is based on a study of *Trillium* which may differ markedly from *Tradescantia* in this respect. Observations in general suggest that a reduction of the over-all chromosome length occurs between pachytene and metaphase—to about one-ninth in *Tradescantia* (342) and to about one-fourth in *Osmunda* (240). The theory of Sax and Humphrey has also been contested on the grounds that the chromatids do not necessarily coil together but may coil independently (410). Wilson and Huskins have in turn advanced the theory that coiling results from elongation of the chromonema within a confined space bounded by the pellicle. Measurements on *Trillium erectum* indicate that elongation of the chromonema occurs between early diakinesis and first anaphase, and between second anaphase and microspore prophase (391, 394). Contraction occurs between zygotene and early diakinesis, between first and second

anaphase, and during microspore prophase. It is suggested that the chromonemata increase in length within a matrix that is neither expanding nor contracting longitudinally, although capable of increasing in diameter, and that they are forced into a spiral thereby. Reversals of direction may be due either to the effect of "bends in the pellicle" or to the fact that coiling begins at various places along the chromosome. Objections to this theory have been made on the grounds that the inception of the major spiral is at the ends of the chromatid, not at random along its length (79), and that assumed changes in length may be referable to alterations in the amplitude of the minor spirals rather than to pronounced modification of the real length of the chromonema. Measurements made by some investigators have indeed suggested that the length of the chromonema remains relatively constant during the various phases of mitosis (236, 279, 302). Decisions here depend on the ability to follow precisely the turns of spirals, which lie close to the threshold of microscopic visibility.

Clarification of these details should lead to a more rigid definition of the coiling process in terms of molecular patterns, although conceivably the physico-chemical approach may provide the clue for integration of the microscopic observations. Some efforts in this direction have already been recorded. Elvers (116) has advanced a hypothesis based on the existence of nucleic-acid-charged chromomeres in the form of dipoles oriented across the chromosome, the attraction between poles of adjacent chromomeres causing spiralization. Sparrow (393) has observed that reduced spiralization follows X-ray treatment of meiotic-prophase chromosomes, and has suggested that the effect may be due to an induced deficiency of desoxyribose nucleic acid. Its normal increase in prophase would, in this case, play a rôle in the mechanism of spiralization. It has been demonstrated by Astbury (6) that changes in length of protein fibers may follow folding and unfolding of the polypeptid chains. Östergren (290) has recently suggested that folding of this type, rather than increased spiralization, may be responsible for the excessive contraction of chromosomes induced by cold, colchicine and other agents. In this way fibrous protein chains are brought to a more or less corpuscular form, the contraction being manifested directly in the contraction of the chromosome. The reverse process of unfolding of submicroscopic convolutions has been visualized as

a possible cause of chromosome elongation in leptotene (236) and as a basis for the great increase in length attained by the giant chromosomes of the Diptera. However, Wilson and Huskins have pointed out that reversible changes in length can take place in fibrous proteins, such as keratin and myosin, without any evidence of intramolecular reorientation appearing in the X-ray diagrams.

From all these considerations it is apparent that the existence of a universal coiling mechanism has not been established. The evidence in favor of a torsional mechanism seems most convincing, especially in the light of the observations that once the chromonematic coils have been established, in the mitotic chromosomes or in the endomitotically produced complexes, there is a progressive reduction in their number by a process of continuous despiralization. Reversals in the direction of coiling are difficult to harmonize with a torsion mechanism unless control in localized regions permits orientation of blocks of smaller or greater size. The basis for such differentiation is in turn not apparent. There also remains the problem of the ring chromosome whose chromatids are frequently able to separate at anaphase and therefore must have been lying parallel without any interlocking. If it is assumed that a plectonemic coil exists from the time of the inception of the ring, there remains the problem of reducing the consequent relational coiling so as to permit free separation of chromatids. Rotation of chromatids at the centromere, or breakage with subsequent attachment, would provide possible methods of relieving the strain set up in such a relationally coiled system at the time that new coils were being enlarged. Of 65 X-ray-induced rings observed at metaphase and anaphase in microspores of *Tradescantia,* only 10 were "disjunctional", permitting free separation of the chromatids; the remainder had interlocked chromatids or they had opened out to form a dicentric ring (174). Since the X-ray treatment was given during the resting stages or early prophases, these data suggest that the chromatids do not rotate at the centromere or break during the course of the prophase stages. The dicentric rings are similar to those of *Zea* studied extensively by McClintock (summary in 233), which presumably arise as a result of a single exchange or crossover between the two sister chromatids. The continuous, double-sized, dicentric ring breaks in anaphase as a result of the strain imposed by the movement of the centromeres toward

opposite poles of the spindle. There are ring chromosomes, however, such as those of *Drosophila melanogaster,* which, although capable of forming dicentric rings, are nevertheless perpetuated in unaltered form through successive generations (65, 364). Little is known of their pattern of coiling aside from the casual statement of Schultz and Catcheside that they have seen no difference between the spirals in these closed X-chromosomes and those of the rod X. It has been suggested that the ability of ring chromosomes to separate indicates that relational coiling may be facultative rather than obligatory (85). A study of patterns of coiling in chiasma loops of *Trillium* led to the conclusion that when strands are "fixed" at their ends they are unable, if below a certain length, to assume regular cylindrical spirals, but merely assume corrugated configurations (254). Whatever the "compensatory" mechanism for relational coiling may be, it does not lead to interference with the orderly progress of mitosis or crossing over in ring chromosomes. Certainly a more precise description of the cycle of coiling in these forms is desirable. Perhaps the newer methods of microscopic analysis outlined in the introductory section of this review will provide a further step in securing the basic data required.

SUMMARY

In this review an effort is made to indicate the present level of our knowledge of chromosome structure, and prospective approaches to further studies. Morphological considerations based on observational evidence have been supplemented in recent years by various chemical, histochemical and physico-chemical methods of analysis, using large plant chromosomes and the giant polytene chromosomes of the salivary glands of the Diptera. The findings are appraised in so far as they bear on such particulars as linear organization, mechanism of coiling and relation of the chromonemata to other materials of the chromosome.

The compact cylindrical chromosome contains within its limiting membrane the helically coiled chromonemata and the associated matrix. Consideration of the evidence available from physical and chemical methods of analysis, together with the older morphological considerations, leads to the suggestion that the term "matrix" should be reserved for the Feulgen-negative portion of the chromosome which surrounds the chromonemata and which can be dis-

sociated from them by various techniques. The linear differentiation of the chromosome is discussed with respect to constrictions, chromomeres, and euchromatic and heterochromatic regions. The term "chromomere" is used to describe the constant, morphologically differentiated segments of the chromonema seen in the living cell as well as in fixed and stained preparations. The further differentiation of such regions into tightly coiled threads has been revealed microscopically in some cases; in others, the evidence now available suggests that the chromomeric organization exists in the fully extended chromonema. Evidence concerning the distribution of heterochromatin among the members of the chromosome complex is considered, and the possible rôle of this material in the mitotic cycle is outlined.

Analysis of the finer structure of the chromosome and the distribltion of its materials has involved the use of monochromatic ultraviolet radiation and various chemical tests for identification and determination of the distribution of cellular components. These cytochemical methods have permitted an approach to an expression of differences in concentration of nucleic acids and proteins, and have suggested possible relations between the nucleic-acid metabolism of the nucleus and that of the cytoplasm.

Determination of the number of chromonemata and their patterns of coiling has involved efforts to secure unequivocal fixation images and to discriminate between different observational interpretations by accessory methods of analysis, such as ionizing radiations. The observations, although differing widely with respect to such details as the time of splitting of the chromosome, have confirmed the existence of multiple chromonemata at the various stages of somatic and meiotic mitoses. On the basis of these findings and of extensive observations of patterns, direction of coiling and changes in the length of the chromonemata, a series of hypotheses concerning the coiling mechanism have been proposed.

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