THE HUMAN CHROMOSOME

Electron Microscopic Observations on Chromatin Fiber Organization

J. G. ABUELO and DOROTHY E. MOORE

From the United States Department of Health, Education, and Welfare, Public Health Service, Bureau of Disease Prevention and Environmental Control, National Center for Radiological Health, Radiation Bio-Effects Program, Rockville, Maryland 20852

ABSTRACT

Human lymphocytes were grown in short-term tissue culture and were arrested in metaphase with Colcemid. Their chromosomes were prepared by the Langmuir trough–critical point drying technique and were examined under the electron microscope. In addition, some chromosomes were digested with trypsin, Pronase, or DNase. The chromosomes consist entirely of tightly packed, 240 ± 50-A chromatin fibers. Trypsin and Pronase treatments induce relaxation of fiber packing and reveal certain underlying fiber arrangements. Furthermore, trypsin treatment demonstrates that the chromatin fiber has a 25–50 A trypsin-resistant core surrounded by a trypsin-sensitive sheath. DNase digestion suggests that this core contains DNA.

INTRODUCTION

Chromosomal fine structure has been the subject of extensive electron microscopy research for more than a decade. Chromosomes from higher plants, insects, and various vertebrates have been observed to consist mainly of chromatin fibers 30–500 A in diameter (1–10). Radioautography (3) and enzyme digestion (2, 4, 5, 8, 11, 12) have demonstrated the presence of DNA and protein in these fibers. In addition the ultrastructure of the nucleolar organizer region (13), the kinetochore (12, 14–16), and the synaptinemal complex (17, 18) has been described.

Attempts to detect an ordered arrangement of the chromatin fibers within mitotic chromosomes have been less successful. With thin sectioning techniques, a fiber can be followed for only a very short distance in a single section. This considerably hampers the examination of the three-dimensional organization of fibers. With whole mount techniques chromatin fibers from interphase nuclei may be followed for several microns (4, 5, 7, 8, 11). However, metaphase chromosomes prepared by whole-mount techniques are characterized by closely packed, irregularly folded chromatin fibers (4, 6, 8). It may be that this tight packing precludes the observation of some regular fiber arrangement in the metaphase chromosome.

It has been shown that the packing of the chromatin fibers can be induced to loosen by treatment with trypsin (4, 19–21) or hypotonic solution (9, 11, 22, 23). In the present study, these treatments are applied to human lymphocyte chromosomes, which are then prepared by the whole-mount technique of Gall (7) and examined with the electron microscope. In addition the actions of deoxyribonuclease and Pronase[1] on chromosomes are examined.

[1] The use of commercial products does not constitute endorsement by the United States Public Health Service.

MATERIAL AND METHODS

Human Leukocyte Cultures

Blood was obtained from normal female volunteers. One portion was heparinized for plasma, and the other was allowed to clot for serum. A modification of the Moorhead method (24) of culturing leukocytes was used. The heparinized blood was allowed to sediment and the resultant plasma-cell suspension was withdrawn. The suspension was centrifuged for 15 min at 500 rpm, and the cell button was washed once with Eagle's Minimum Essential Medium (MEM) supplemented with 3 mmoles/ml glutamine, 75 units/ml penicillin, and 75 μg/ml streptomycin. The cells were resuspended in a volume of autologous serum equal to the original amount of plasma. Cultures were prepared in 30-ml plastic screwcap bottles (Falcon Plastics, Los Angeles) with 2.5 ml of the cell-serum suspension, 7.5 ml of supplemented Eagle's MEM, and 0.15 ml of phytohemagglutinin (Burroughs-Wellcome Co., Tuckahoe, N.Y.). The cultures were incubated for 48–72 hr at 37°C, with 0.2 μg/ml of Colcemid (Ciba, Fairlawn, N.J.) present for the last 4 hr.

Specimen Preparation

Cultures were centrifuged for 5 min at 500 rpm; 70% of the medium was discarded and replaced with distilled water. The cells were dispersed, recentrifuged, and the supernatant was discarded. Portions of the pellet were picked up on the tip of a stainless steel spatula which was then dipped into the cleaned surface of a Langmuir trough containing 0.03 M urea. The area was compressed with a waxed barrier, and cells were taken up by touching formvar-carbon–coated grids to the surface. The grids were placed in a plastic carrier in 50% ethyl alcohol and dehydrated by the following procedure: 70% ethyl alcohol, 1 min; 95% ethyl alcohol, 1 min; 100% ethyl alcohol, two 5-min changes followed by one 10-min change; and 100% amyl acetate, two 5-min changes followed by one 10-min change. Grids were then dried with the use of the critical-point method (25) and examined under a Siemens Elmiskop IA operated at 80 kv.

Enzymes

After the cells were picked up on grids from the surface of the Langmuir trough, grids to be treated were floated cell side downward for 20 min at 37°C in the following concentrations of enzymes dissolved in 0.1 M phosphate buffer, pH 7.3: 6.25–200 μg per ml trypsin (Worthington Biochemical Co., Freehold, N.J., 2× recrystallized); 5–100 μg per ml trypsin (Fisher Scientific Co., Pittsburgh); or 100–800 μg per ml Pronase[2] (Calbiochem, Los Angeles). Pronase was also used at concentrations of 10–400 μg per ml 0.1 M Tris-HCl, pH 7.3. Pronase was pretreated to inactivate DNase according to the method of Hotta and Bassel (26). Other grids were treated with 1–50 μg deoxyribonuclease (Calbiochem) per ml 0.003 M $MgCl_2$ in 0.1 M phosphate buffer, pH 7.3. Suitable controls were prepared by treating grids with buffer alone.

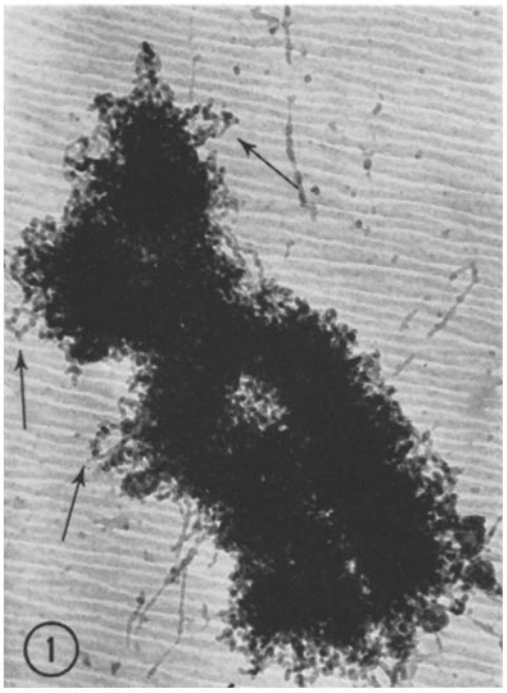

FIGURE 1 Well preserved human lymphocyte chromosome. Chromatin fibers loop out of the chromosome (arrows). × 33,000.

RESULTS

Untreated Chromosomes

Grids usually contain from four to ten metaphase spreads. However, we have not observed the full complement of 46 chromosomes in a single metaphase group.

A chromosome is considered well preserved if its general morphology resembles that found in light microscope studies and if few chromatin fibers are seen stretched out and broken. Fig. 1 shows an example of a well preserved chromosome. The length, arm ratio, and the submetacentric

[2] A protease from *Streptomyces griseus*.

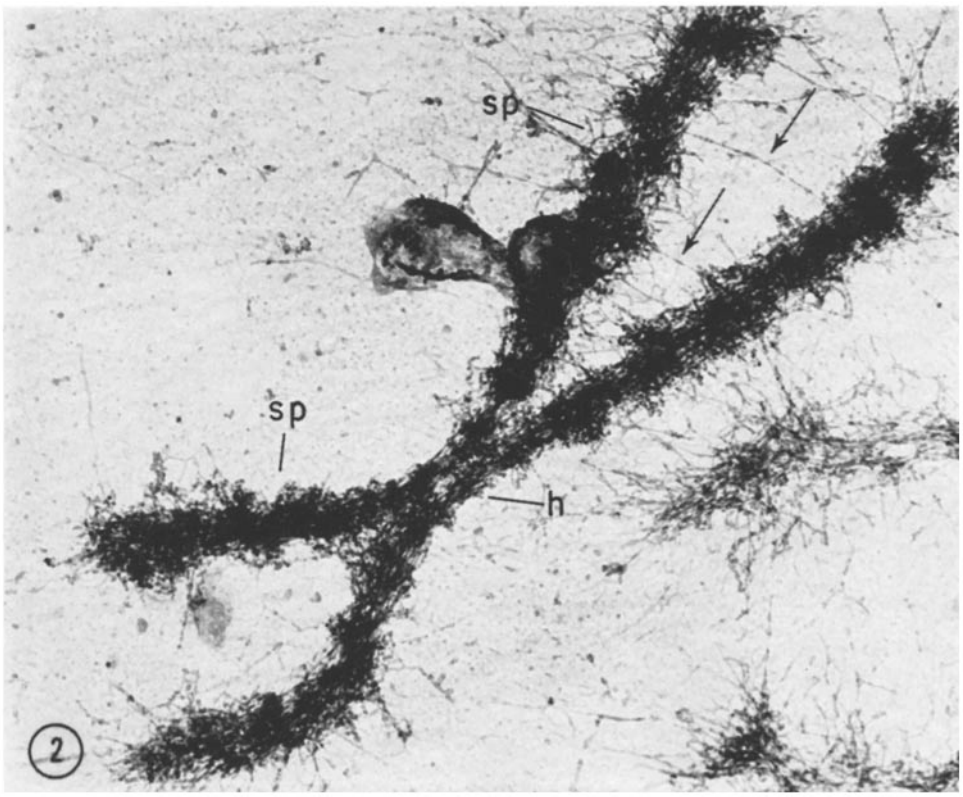

FIGURE 2 Less condensed chromosome. Longitudinally aligned chromatin fibers appear along the chromosome arms. Clumps of chromatin fibers assume a spiral configuration (*sp*). Chromatin fibers pass between sister chromatid arms (arrows). A "hole" or area of less dense chromatin packing is seen in the centromere (*h*). × 23,300.

placement of the centromere constriction suggest that this chromosome is a member of the C group of the normal human karyotype as defined by the Denver (27) and London (28) conferences. The chromosome appears to consist of fibers mostly in the range of 240 ± 50 A in diameter. The fibers are bumpy in appearance. Occasionally, bumps reach 350 A in diameter, while rare narrowed areas approach 140–150 A. Some fibers run out of the main body of the chromosome and usually loop back in. Loose ends are seldom observed. No ordered arrangement of the fibers is seen here.

Often chromosomes, parts of chromosomes, or individual fibers are found to be stretched out or broken. Occasionally, such a stretched chromosome appears to have an underlying arrangement of chromatin fibers not observed when the fibers are tightly packed. Such chromosomes are included in Figs. 2, 4–6. Fig. 2 shows a large submetacentric chromosome, probably a No. 2, in a less condensed state that that shown in Fig. 1. The less contracted condition of the chromosome may be an indication that it is in early metaphase, or may be an artifact due to increased stretching forces as evidenced by the number of individual fibers splayed out of the chromosome arms. This chromosome demonstrates four features of chromosome organization observed in the examination of some 1,500 metaphases. First, a longitudinal arrangement of bumpy 240 ± 50 A fibers is seen throughout the length of the chromosome (see also Figs. 8–10, 13, 14, 16, 18). Estimates of the number of longitudinal fibers at selected points along the chromosome vary between eight and 16. Secondly, superimposed on the longitudinal fibers are areas of similar, but tightly packed fibers (see also Figs. 8, 10, 16). These areas often assume a spiral configuration, continuous for two to four gyres. Oc-

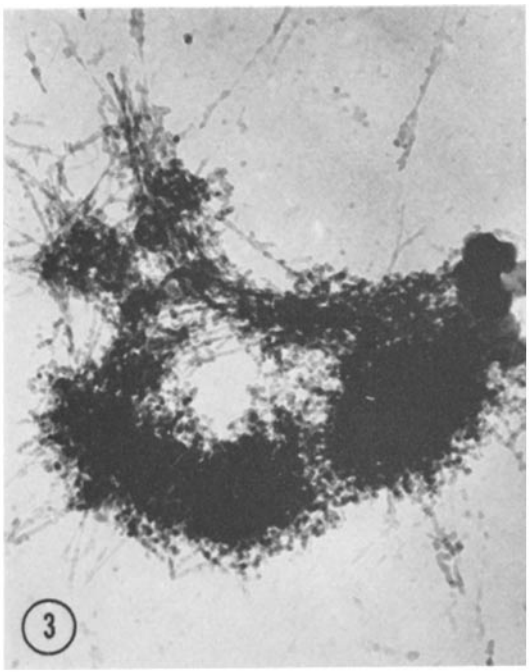

FIGURE 3 Acrocentric chromosome. The ends of the long arms are closely associated. × 36,000.

casionally, the more compact areas appear as separate units along the arms (see also Figs. 9–11, 17, 18). Third, fibers appear to pass between the sister chromatid arms well outside the centromere region (see arrows, Fig. 2) (see also Figs. 12, 16–18). These fibers resemble the other fibers of the chromosome. They tend to be more numerous near the ends of the arms and appear to bind together partially separated chromatid arms (Fig. 3). Fourth, a less dense area of fiber packing is observed in the centromere region. In severely stretched chromosomes, it can be observed that this appearance is caused by a specific configuration of the longitudinal chromatin fibers (Figs. 4–6). As one follows the longitudinal fibers toward the centromere, it can be seen that about half the fibers cross over to the sister chromatid where they appear to reverse direction and run again distally in the sister chromatid. However, some crossing fibers continue in the same direction (Fig. 6). With fibers crossing at both sides of the centromere, two decussation points are created with an empty area between them. Fig. 7 is an idealized schematic of the fiber organization in the centromere region.

Trypsin Treatment

The observations were originally made with Fisher purified trypsin (FT) which has a trypsin activity of 50 National Formulary (NF) units/mg and a chymotrypsin activity of 40 proposed NF units/mg. Subsequently, Worthington 2× recrystallized, lyophilized trypsin (WT) was used to confirm and extend our findings. WT has an activity of 3,300 NF units/mg and has less than 0.1% chymotrypsin activity.

The action of dilute trypsin (10–50 μg/ml FT; 6.25 μg/ml WT) is to induce the relaxation of tight fiber packing (Figs. 8–11). This effect varies from a slight loosening in some preparations to extensive relaxation in others, such that the chromosome becomes an almost formless meshwork of chromatin fibers.

With the loosening of fiber packing caused by trypsin, the longitudinally aligned fibers are more evident and the apparent spiral arrangement of the chromatin fibers is more clearly seen. Several gyres of the more tightly packed fibers may be observed in a chromosome arm (Figs. 8, 10). In chromosome arms that are somewhat stretched, the gyres appear to be separated into clumps of condensed chromatin fibers along the arms (Figs. 9–11). The relation between the longitudinal fibers and the chromatin fibers of the more compact "gyres-clumps" is obscure. It is unclear whether some of the longitudinal fibers pass uninterrupted through the more compact areas; Fig. 11 suggests that some fibers may.

The action of more concentrated trypsin (100 μg/ml FT; 12.5–200 μg/ml WT) is to cause thinning of the chromatin fibers along varying portions of their lengths. Thinning is observed in the fibers looping out of the chromosome arms (Fig. 12), the longitudinal fibers (Figs. 13, 14), the fibers passing between sister centromeres (Figs. 14, 15), and the fibers passing between sister arms (Fig. 12). The thin areas are 25–50 A in diameter. Beadlike structures about 200 A in diameter are observed irregularly spaced along the thinner areas.

Pronase Treatment

In concentrations of 200–400 μg/ml in phosphate buffer and 20–100 μg/ml in Tris buffer, Pronase also exhibits a relaxing action on fiber packing (Figs. 16–18). Concentrations of Pronase higher than 400 μg/ml in phosphate buffer form a precipitate and produce no increase in enzyme action. In Tris buffer at concentrations of Pronase

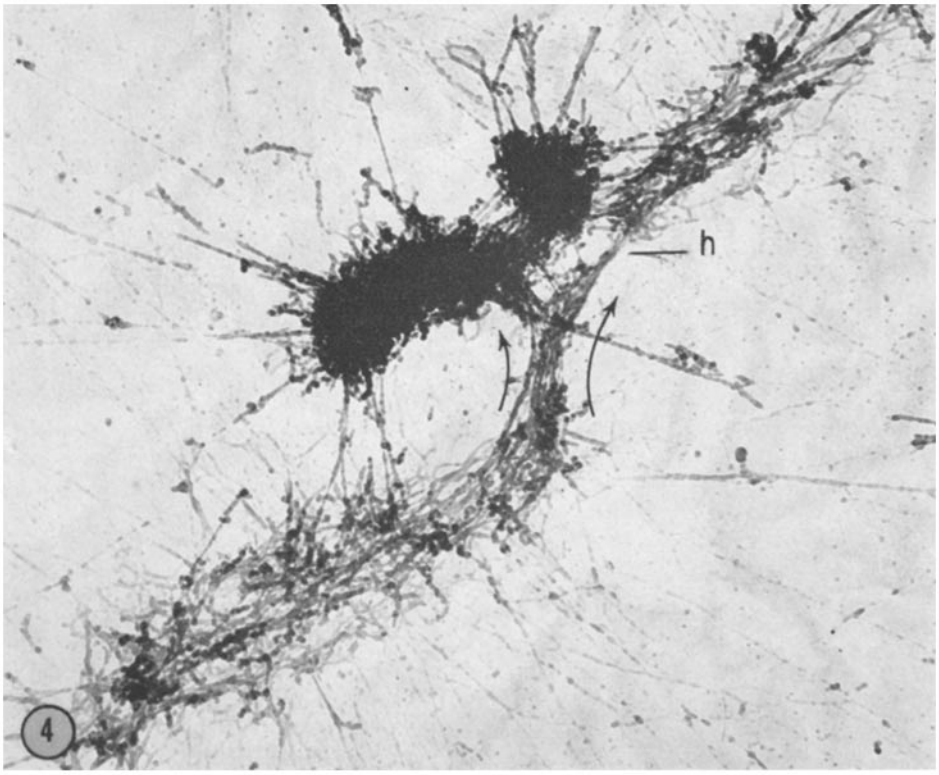

FIGURE 4 Chromosome with one of the sister chromatids severely distorted. At the centromere, the longitudinal chromatin fibers bifurcate with some crossing to the other chromatid and some continuing in the same direction (arrows). A "hole" is formed at the centromere (*h*). × 24,000.

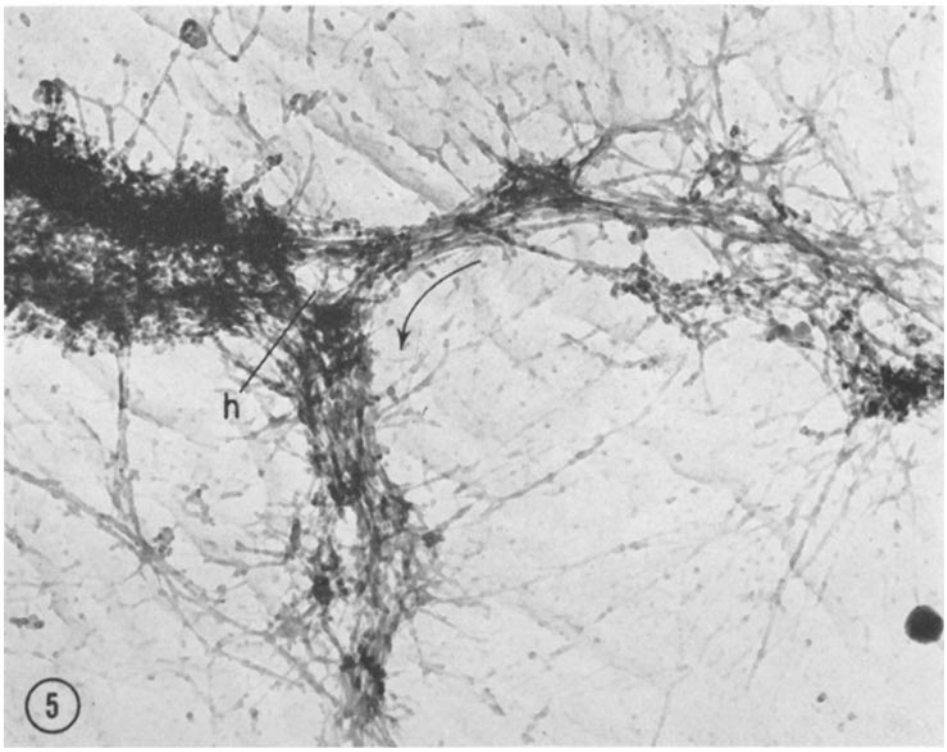

FIGURE 5 Chromosome with sister short arms pulled out. Longitudinal fibers bifurcate at the centromere. Some fibers change direction and run back along the sister arm (arrow). Other fibers continue without changing direction. A "hole" appears in the centromere (*h*). × 30,400.

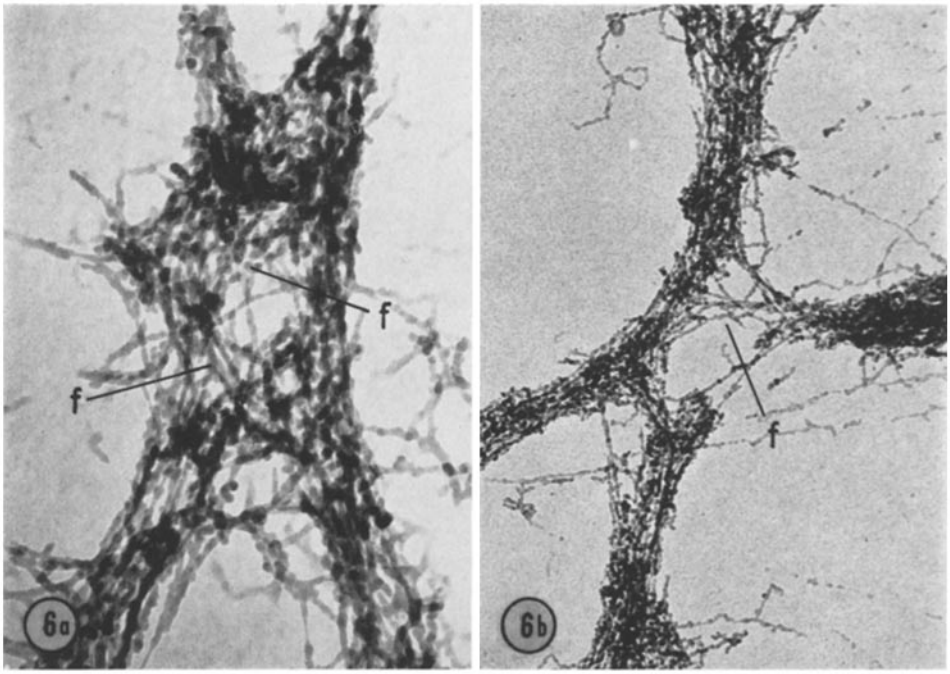

FIGURE 6 Some chromatin fibers seem to cross from one chromatid to the other and continue in the same direction (*f*). *a*, Centromere from chromosome treated with 10 μg/ml trypsin (Fisher). × 45,500. *b*, Centromere from chromosome shown in Fig. 8 *a*. Treatment is with 6.25 μg/ml trypsin (Worthington). × 25,400.

higher than 100 μg/ml, chromatin fibers are not observed on the grid.

Figs. 16–18 illustrate longitudinal fiber arrangement observed in Pronase-treated chromosomes. As with trypsin, clumps of more tightly folded fibers are present along the chromosome arms (Figs. 17, 18). Fiber thinning, although reported for interphase fibers (34), was not observed in Pronase-treated chromosomes. The fibers between the sister chromatid arms are particularly well seen in Figs. 17 and 18. Fiber thinning and relaxation are not observed when chromosomes are treated with phosphate and Tris buffers alone.

DNase Treatment

DNase activity is first detected at a concentration of 5 μg/ml (Figs. 19, 20). Here the linear integrity of the chromatin fibers is interrupted, and only short fiber segments are observed. Fibers passing between sister chromatid arms are also affected. With increasing DNase concentration, digestion is more pronounced, and at 20 μg/ml the chromatin fibers are no longer observed. Chromosomes treated with phosphate buffer with 0.003 M $MgCl_2$ do not exhibit digestion.

DISCUSSION

Chromatin Fibers

In the present study, human lymphocyte chromosomes prepared by the Langmuir-trough and critical-point drying techniques appear to be comprised entirely of 240 ± 50 A fibers. Dupraw (29) obtained similar results with human chromosomes prepared in the same manner. This technique has also yielded fibers of similar diameter in meiotic chromosomes (31), in mitotic chromosomes (4, 8, 32) and in interphase chromatin (4, 8, 10).

Wolfe and Grim (33) recently presented evidence that the 200–250 A fibers of interphase chromatin probably exist in the living cell as fibers with diameters of about 100 A. They suggest that the transition to a larger diameter occurs as the 100 A fibers of the intact cell combine with surrounding matrix material of the nucleus. On the

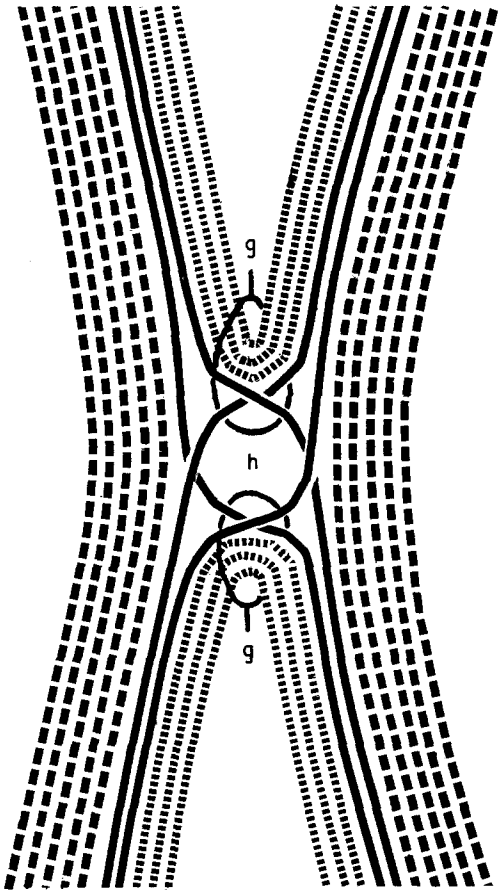

FIGURE 7 Schematic diagram of chromatin fibers at the centromere. ..... represents those fibers that cross from one sister chromatid to the other, reverse direction, and run back along the sister arm. ---------- represents those fibers which continue uninterrupted in the same chromatid. ——— represents the occasional fiber which seems to cross between chromatids at the centromere but does not change direction. Because of this pattern, the fibers cross at the centromere in two groups (*g*), forming an empty space in between (*h*).

other hand, Ris (34) observes the 250 A fiber to divide into two 100 A fibers under the influence of chelating agents. We have only rarely seen multiple fiber cores branching from a single 240 ± 50 A fiber. On the basis of our observations, then, no real information can be added to the question about possible subunit organization of 200–250 A fibers.

Ris shows that the 100 A fiber can be reduced to one, two, or more thinner fibrils and that these thinner fibrils approach the theoretical diameter of the DNA double helix (5). Thinning of fibers can be induced by various treatments which remove histone or protein: 0.2 N HCl (10), 500 μg/ml trypsin (5), Pronase (34), and 2 M NaCl (11). The thinner fibrils obtained are susceptible to digestion by DNase (4, 34). Digestion by DNase also causes a disappearance of chromatin fibers, even without prior treatment to remove protein in interphase nuclei (8, 11) and in metaphase chromosomes (12, 35, 36).

Most enzyme digestion studies have been confined to the chromatin fibers of interphase nuclei. The fibers of metaphase chromosomes are thought to result from a condensation or aggregation of interphase fibers (8, 30) and would be expected to react to enzyme treatments in a similar manner. The present studies support this reasoning. As with interphase chromatin, the 240 ± 50 A fibers of metaphase chromosomes are digested completely by DNase and are reduced to 25–50 A fibers by trypsin. In addition, the possibility that DNase merely strips intact fibers from the grid, rather than actually digesting them, is ruled out by the observation of partial digestion at low DNase concentrations. It seems likely that the chromatin fibers consist of a protein coating around a 25–50 A trypsin-resistant core. The susceptibility of the whole fiber to DNase digestion suggests that DNA is responsible for the linear integrity of the fiber and that the DNA is in the core.

Loosening of Fibers

In our studies, as in previous ones (4, 13, 20, 21), trypsin is observed to cause loosening of the chromatin fiber mass. That such loosening also occurs with Pronase suggests that this loosening is a property of any substance with proteolytic activity. It may be that proteolytic enzymes cause relaxation of chromatin fiber packing by two actions: that of breaking internal fiber bonds which hold each fiber in a rigidly folded configuration, and/or that of removing a proteinaceous interfiber glue.

Longitudinal Arrangement

Fibers longitudinally arranged along the chromosome arm have been observed with whole-mount techniques in meiotic prophase chromosomes (30, 37) and in various mitotic chromosomes (6, 31, 36, 38, 39) as well as in this report. They have also been demonstrated in one study of thin-sectioned mammalian chromosomes (35). How-

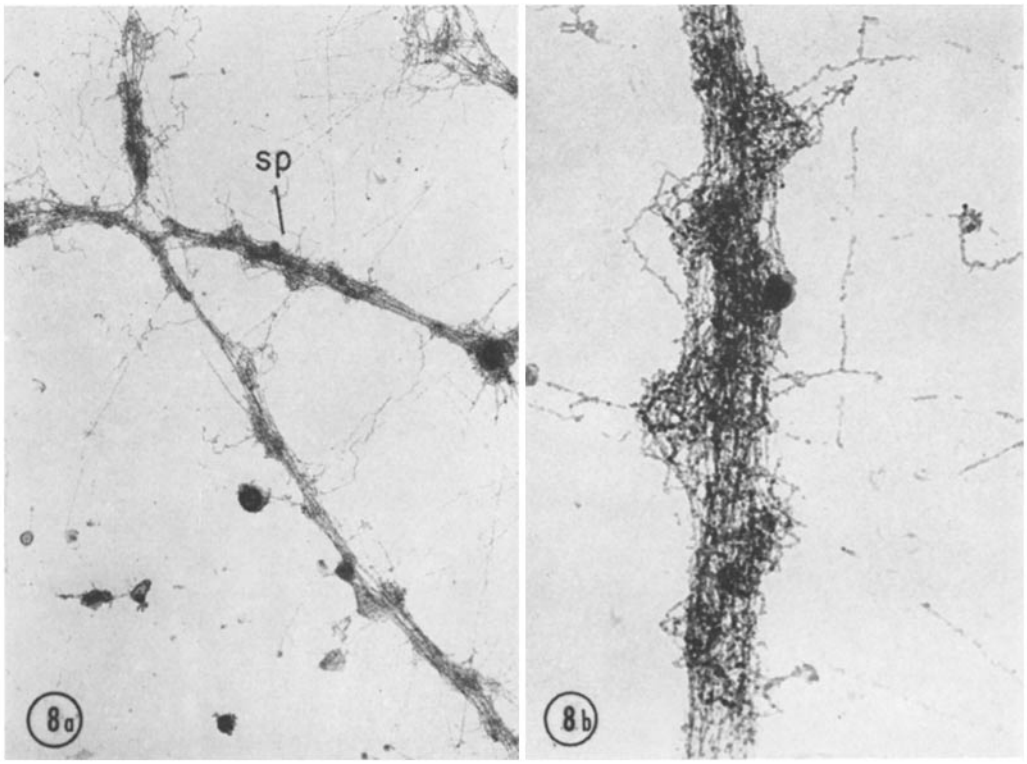

FIGURE 8 *a*, Chromosome treated with 6.25 μg/ml trypsin (Worthington). Chromatin fibers are somewhat loosened. In one arm, three gyres of a spiral appear (*sp*). × 6,600. *b*, Higher magnification of region with coiling. Relation of longitudinal fibers to those of the gyres is obscure. × 28,000.

ever, longitudinal alignment of fibers was not seen in other studies in which whole mount techniques were used (4, 8, 32). It may be that compact folding of unaligned fibers obscured the longitudinal fibers. This explanation is supported by our observation that gentle loosening of the condensed metaphase chromosomes by protease treatment seems to uncover underlying parallel fibers. Wolfe and Hewitt (40) have also reported that *Oncopeltus* meiotic chromosomes exhibit parallel fibers while extended in prophase I, but not when they are more condensed in metaphase I.

Coiling

The tendency of the arms of mitotic and meiotic chromosomes to take the form of helical gyres is well known from light microscopic observations (41, 43, 44). Although chromosome arm spiraling may be factitiously induced or exaggerated by chemical and physical agents (42, 44, 45), observations of coiling in untreated living cells indicate that this spiraling phenomenon is not artifactual (42, 46). Coiling has also been found with electron microscopy in sectioned (35) and whole-mount mammalian (8) mitotic chromosome preparations. The present observations of major gyres in human lymphocyte chromosomes are similar to those of Dupraw (29). Our use of enzymes to loosen chromatin fibers allows us to examine the fiber organization in the gyre. Evidence for minor coils (47) or a hierarchy of helical systems (37) is not found. Instead, the gyres appear to be composed of irregularly folded, closely packed chromatin fibers. How these gyres relate to the longitudinally aligned fibers is still unclear.

Longitudinal Differentiation Along the Arms

Also unclear is the appearance of gyres as separated clumps of chromatin fibers in stretched areas of the chromosome arm. While it may be that this separated state results as an artifact of stretching,

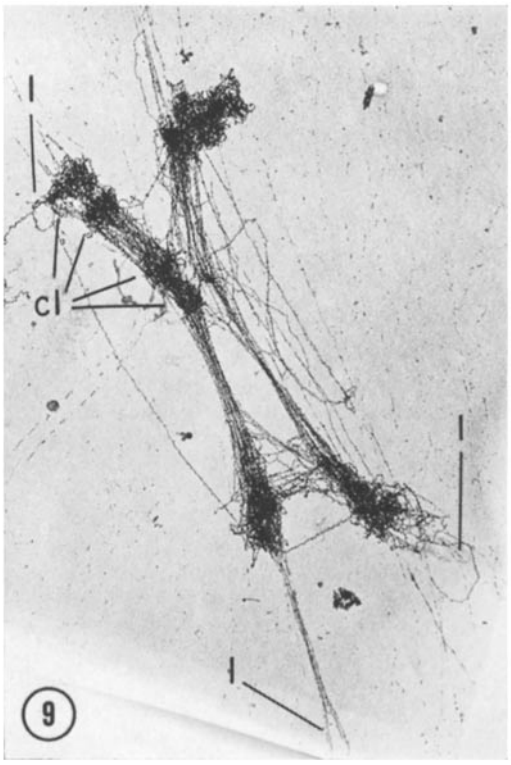

FIGURE 9 Chromosome treated with 6.25 μg/ml trypsin (Worthington). There is relaxation of the chromatin fibers. Several loops appear (*l*). There are few loose ends. Separated clumps of chromatin are seen (*cl*). × 9,600.

another possibility exists. These clumps may reflect a true longitudinal heterogeneity along the chromosome arm. This concept of linear differentiation is not new. It goes back 90 years to the first reports of beadlike structures observed along the arms of chromosomes in meiotic prophase (48). These structures, called chromomeres, are usually constant in size, number, and position. They have since been described in the zygotene and pachytene stages in a variety of organisms (48–51). Possibly related are morphologically distinct regions (also called chromomeres) which appear along the axes of certain mitotic chromosomes (52–54), lampbrush chromosomes (55), and dipteran giant salivary chromosomes (56). Electron microscopic studies have demonstrated that the chromomeres of meiotic prophase (40, 47), lampbrush (57), and giant salivary chromosomes (21, 31) are actually areas of tightly coiled chromatin fibers. The morphological variations along the chromosome may be related to certain functional variations. In giant salivary chromosomes and in maize pachytene chromosomes the chromomere patterns have a constant relationship to the respective genetic maps (58–62). Furthermore, Pelling (63) has recently speculated that the "chromomere" may represent a unit of RNA synthesis and DNA replication. He points out that DNA synthesis as determined in labeling experiments occurs independently in the bands of *Chironomus* salivary chromosomes. Similarly, DNA labeling is observed at specific loci in chromosomes of certain plants and animals (64–67). Identification of labeling loci with chromomeres has not been attempted. However, studies with Chinese hamster cells indicate that late labeling areas of the chromosome may condense later than other areas (83, 84). This suggests that condensation of chromatin is also carried out in a pattern along the chromosome arm. Further evidence that units of the chromosome arm may act independently is found in the behavior of radiation-induced acentric fragments. These fragments can replicate themselves, may condense normally in prophase and metaphase, and separate from their sister fragments in early anaphase (46). Is there evidence for similar linear differentiation in human chromosomes? Chromomeres have been observed in human pachytene chromosomes (68–70), and certain chromosomes have a constant pattern of chromomeres (68, 70). Genetic mapping in man is in a preliminary state (71–74), but the X chromosome has been shown to have several genes in linear arrangement (75). DNA labeling in a number of human chromosomes has been observed to occur in small discrete segments (76–81). There are also linear variations in chromosome condensation. Sasaki and Makino showed that certain late labeling chromosome regions condensed later than others (82). This late condensation can be prevented if the cells are incubated in calcium-free medium during the last hours of culture.

Thus, it appears that each region of the human chromosome arm may be identified with its characteristic genetic loci, its sequence of DNA replication and its sequence of condensation. The separated clumps of chromatin fibers along the chromosome arm observed in the present studies may reflect this pattern of differential condensation. Before such a hypothesis is seriously entertained,

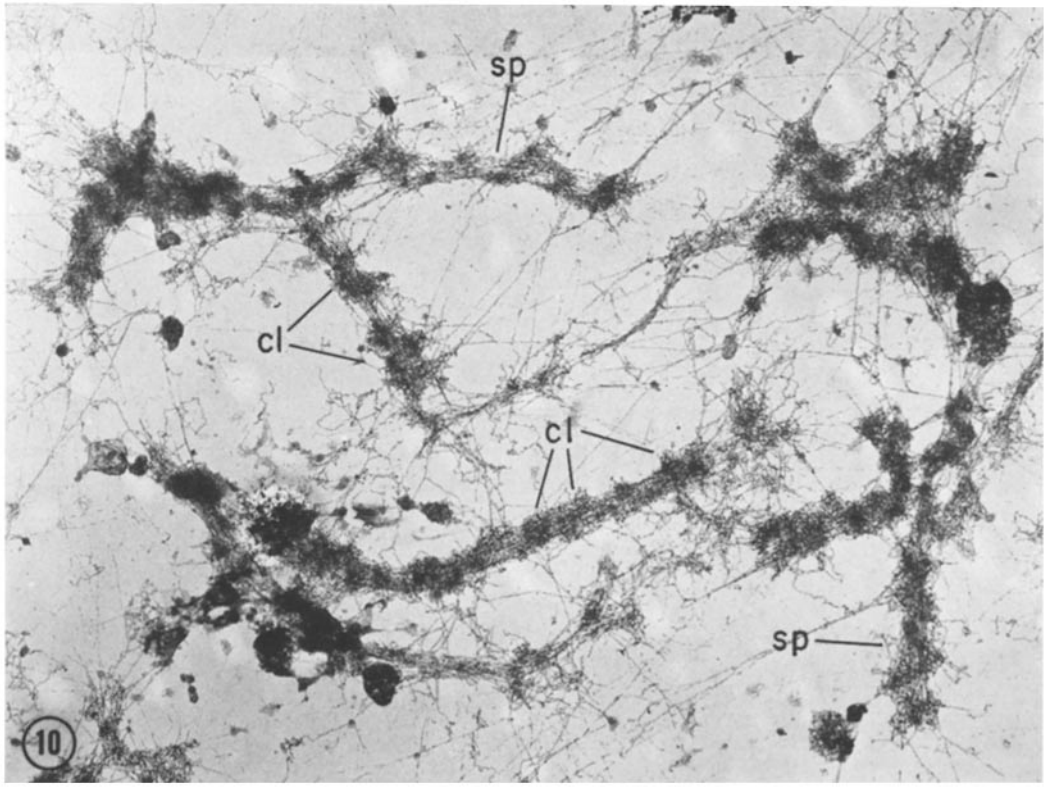

FIGURE 10 Chromosomes treated with 10 μg/ml trypsin (Fisher). Loosening of the chromatin fibers reveals spiraling (*sp*) and separated chromatin clumps (*cl*) along the arms. × 11,000.

it must be shown that a constant "clumping" pattern exists in the mitotic chromosomes and that this pattern is related to DNA labeling sequences.

Centromere

The centromere region of the mitotic chromosome has been variously described. With light microscopy, two or four densely staining centromeric spherules or chromomeres have been observed (85, 86). Similar techniques reveal an association (87) or even a band of stained material (88) between the proximal regions of sister chromatid arms. The cinemicrography of living endosperm has also demonstrated a proximal arm association (45) as well as the formation of a centromere hole (89). Dupraw (29) in his study of whole-mount human chromosomes observed that, "in the centromere proper, multiple lengths of fiber (about fifteen) pass from one chromatid to the other." The present studies corroborate Dupraw's finding of several chromatin fibers crossing at the centromere. Moreover, our observations that the fibers cross at two points and leave an empty area in between is supported by the configuration of the centromere in Dupraw's Fig. 5 (29). This would explain the appearance of a centromere hole and the association of proximal arm regions previously reported (see above). The centromere hole in human lymphocyte chromosomes is also seen in the absence of mitotic-arresting agents (90, see Figs. 3, 4, and 9), when whole-mount preparations are examined with the electron microscope.

The pathways of chromatin fibers in the centromere region as observed in the present study are represented schematically in Fig. 7. These findings must be considered tentative at present because of the small number of observations on which they are based. In addition, it should be noted that kinetochore structures as described in thin-sectioned chromosomes (12, 14–16) are not observed with the Langmuir trough–critical point

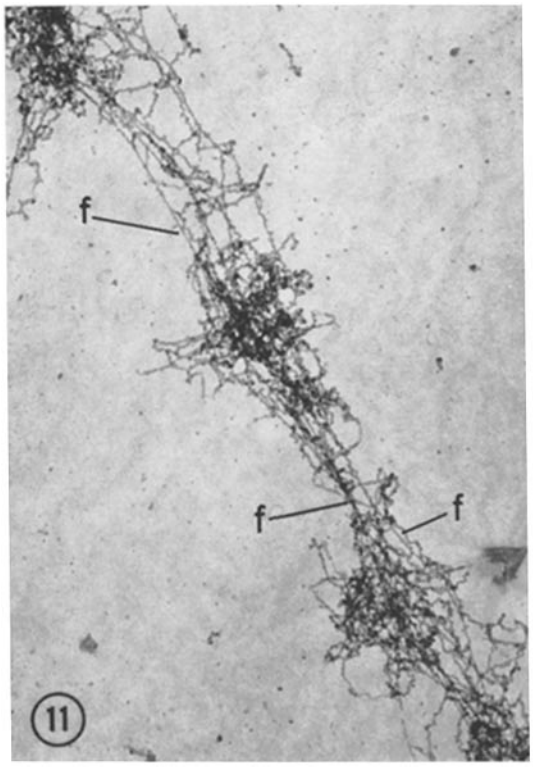

FIGURE 11 Portion of a chromosome arm treated with 25 μg/ml trypsin (Fisher). Separated clumps of chromatin fibers are connected by longitudinal fibers. Some fibers may pass uninterrupted through or around the clumps (*f*). × 17,200.

drying technique. It may be that these structures are removed during chromosome preparation.

Interarm Fibers

In whole mount chromosomes, chromatin fibers pass between sister chromatid arms outside the centromere region. Such fibers can also be seen in micrographs in previously published reports (8, 29). The fibers are particularly evident in chromosomes loosened with trypsin or Pronase. It may be that these fibers hold the metaphase chromosome arms rather tightly together. With relaxation of the chromatin meshwork, the chromosome arms may drift apart enough so that the connecting fibers are observed intact.

This hypothetical role of the fibers holding chromosome arms together finds some support in previous studies. In cinemicrography of mitotic cells, the splitting apart of sister chromatids occurs as a synchronized event before the start of anaphase (45, 46). This seems to occur independently of spindle activity and has been observed in Colcemid-mitosis (91), in radiation-produced acentric fragments (46), and in mitotic nuclei not associated with a spindle (92). If the separation of sister chromatids is mediated by a release of connecting fibers, our findings of fibers crossing more frequently at the centromere and at the ends of the arms would suggest that the telomere and centromere are the last parts of the arms to separate. This would explain the observation of human chromosomes with partially separated arms held together at their centromere and at their ends, both with Colcemid (29, see Fig. 5) and without Colcemid (90, see Figs. 8 and 9). Levan (93) observed the same configuration in colchicine-arrested *Allium* root chromosomes. He concluded that a releasing action moves down the chromosome arms from the centromere towards the ends and that when this action passes through the ends a complete separation of the arms takes place. Molé-Bajer described this phenomenon in living endosperm: "A longitudinal split appears which moves towards the ends of the arms and at the same time increases in breadth. In consequence the two daughter chromosome arms may often look as if they are connected at the kinetochore and at the ends of the arms with a split between them" (91).

The question arises whether the crossing fibers are the same type of fiber as those of the chromosome proper. Both Wolfe (8) and we have observed that these fibers are sensitive to DNase digestion. Also, high concentrations of trypsin cause thinning of these fibers. Therefore, the crossing fibers are affected by enzymes in the same way as those of the chromosome arms, and it may be that these fibers differ only in their position.

Strandedness

The recondite problem of how many strands comprise each chromatid has been debated for over 30 years (19, 43, 94–96). Even the use of radioautography (97, 98) and electron microscopy has not settled the question (37, 40, 99). Our observations of several parallel chromatin fibers aligned longitudinally along the chromosome arm would appear to support the concept of multistrandedness. However, this appearance may be accounted for by parallel folding of a single strand. Dupraw noted that the ends of the chromatid have

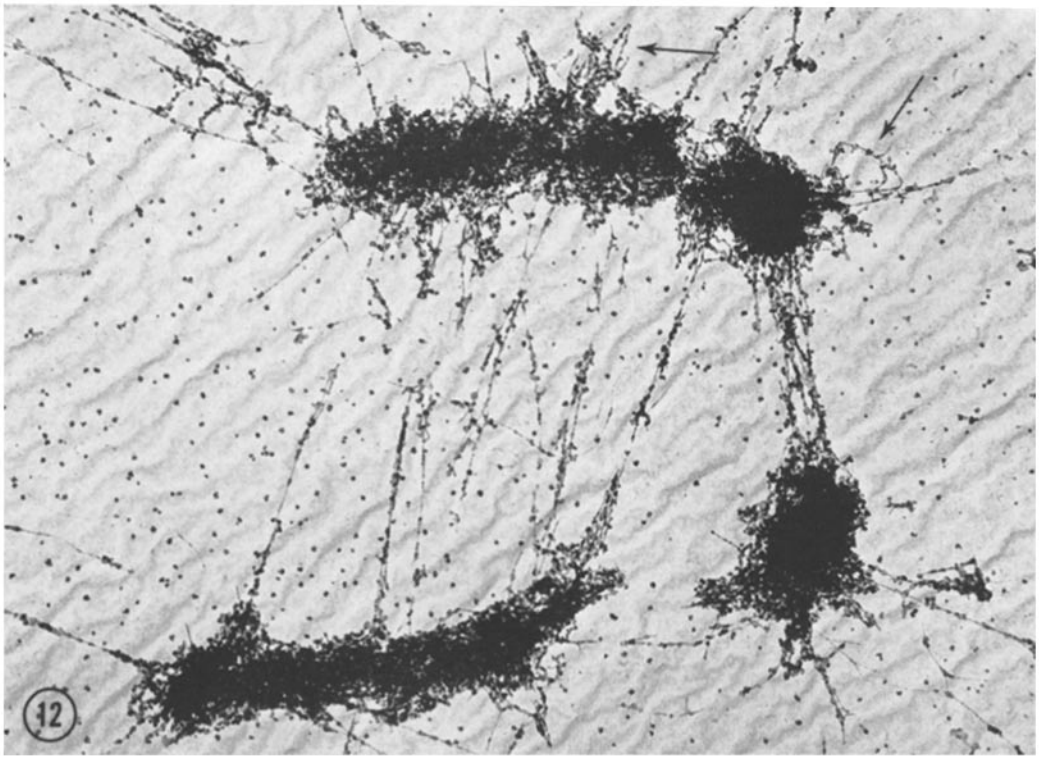

FIGURE 12 Chromosome treated with 25 μg/ml trypsin (Worthington). Thinning is observed in certain fibers looping out of the chromosome (arrows) and in fibers between the arms. × 18,800.

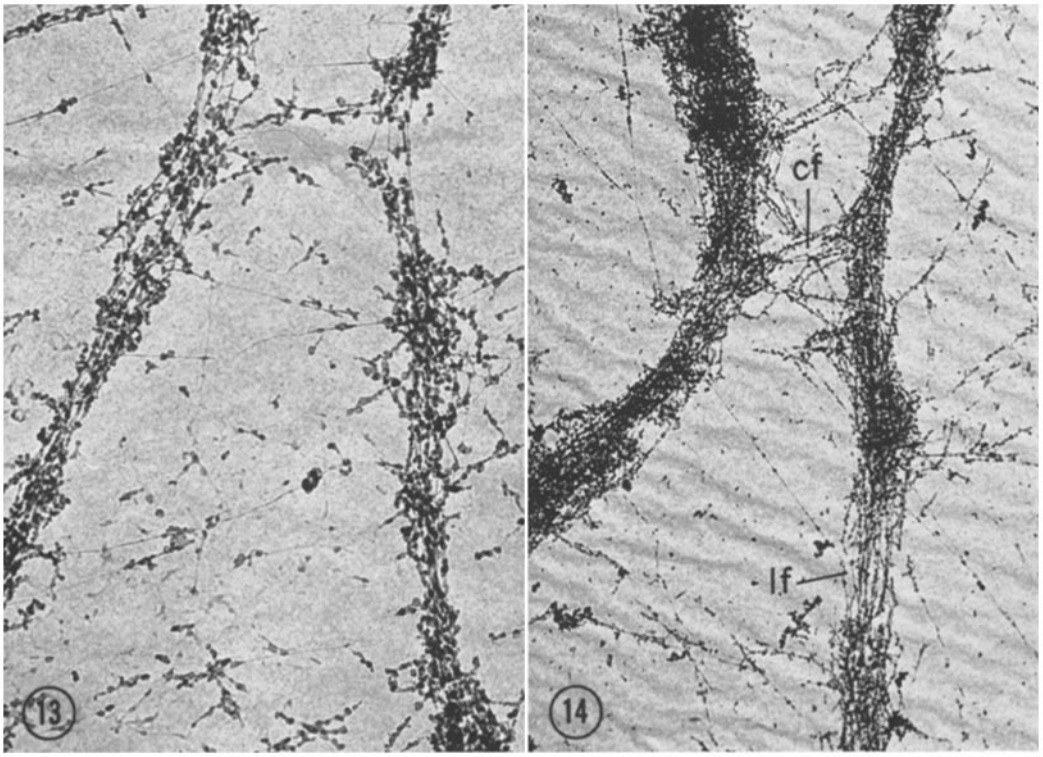

FIGURE 13 Portion of a chromosome treated with 12.5 μg/ml trypsin (Worthington). Note thinning in the longitudinal fibers. Thin regions of some fibers have diameters of 35–50 A. × 32,000.

FIGURE 14 Portion of a chromosome treated with 25 μg/ml trypsin (Worthington). Thinning is evident in the longitudinal fibers (*lf*) and the fibers passing between the sister centromeres (*cf*). × 17,100.

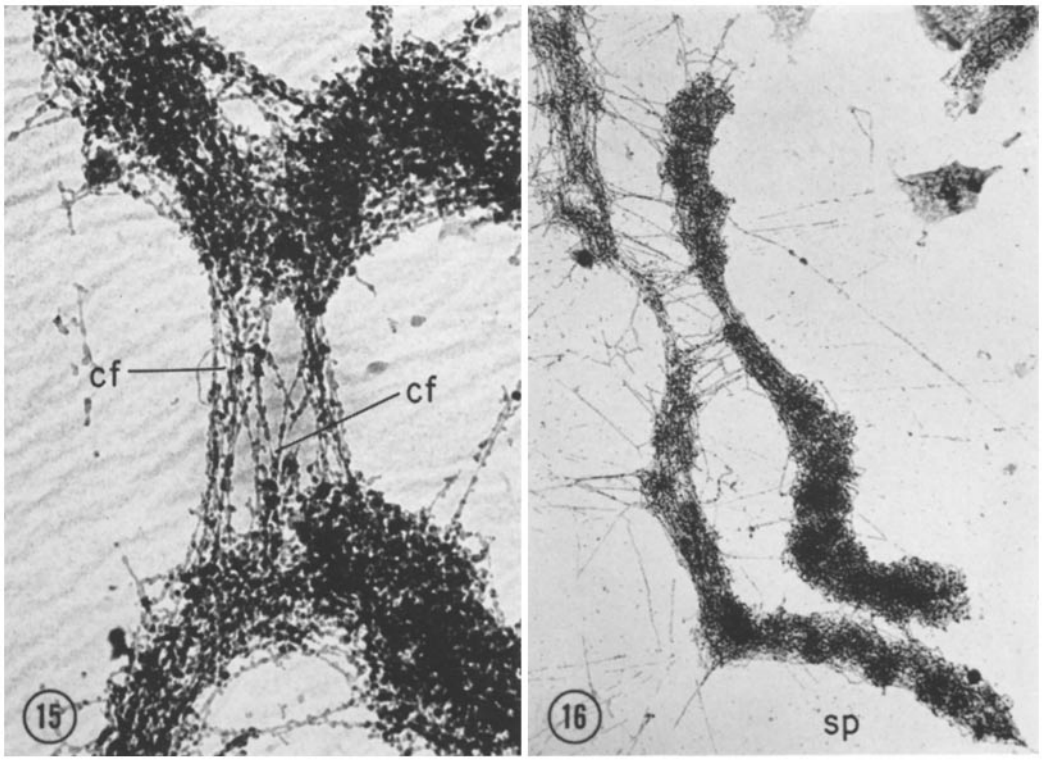

FIGURE 15 Centromere region of a chromosome treated with 12.5 μg/ml trypsin (Worthington). Thinning appears in the fibers between the sister centromeres (*cf*). × 37,300.

FIGURE 16 Chromatin fiber relaxation in chromosome treated with 400 μg/ml Pronase in phosphate buffer. Fibers pass between the arms of sister chromatids. Spiraling appears at the distal portion of one arm (*sp*). × 12,000.

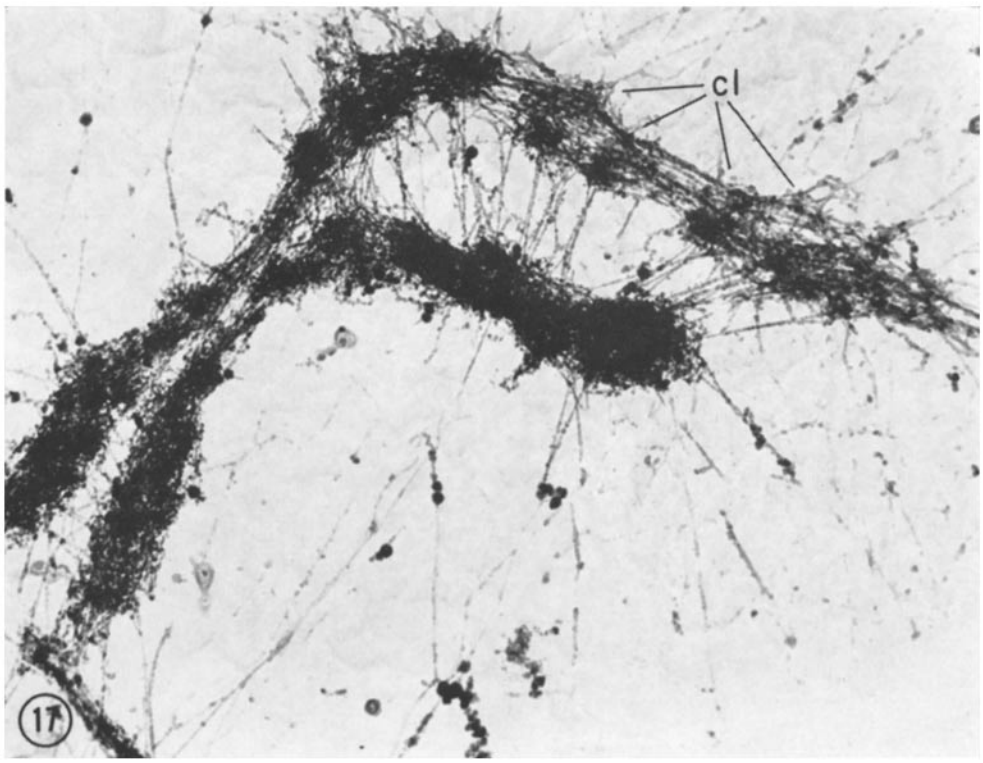

FIGURE 17 Chromosome treated with 800 μg/ml Pronase in phosphate buffer shows fibers between the arms and separated clumps of chromatin fibers (*cl*). × 17,000.

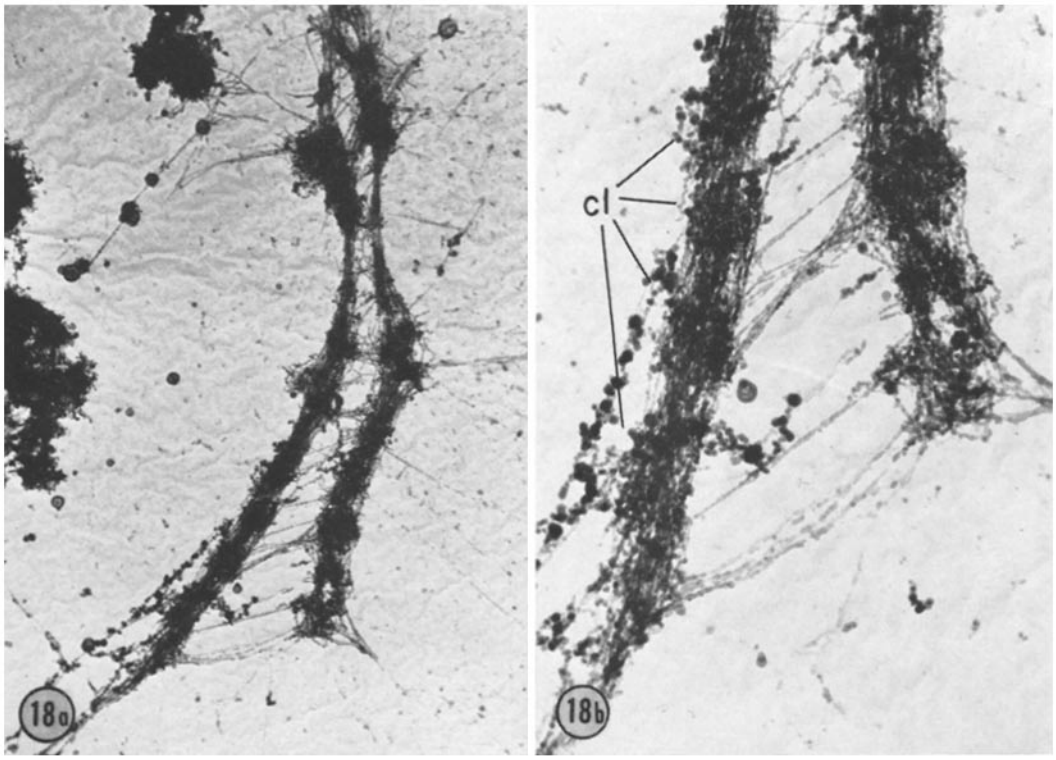

FIGURE 18 *a*, Chromosome treated with 800 μg/ml Pronase in phosphate buffer. × 9,200. *b*, Higher magnification shows fiber relaxation, fibers between the arms, and separated clumps of chromatin fibers (*cl*). × 29,000.

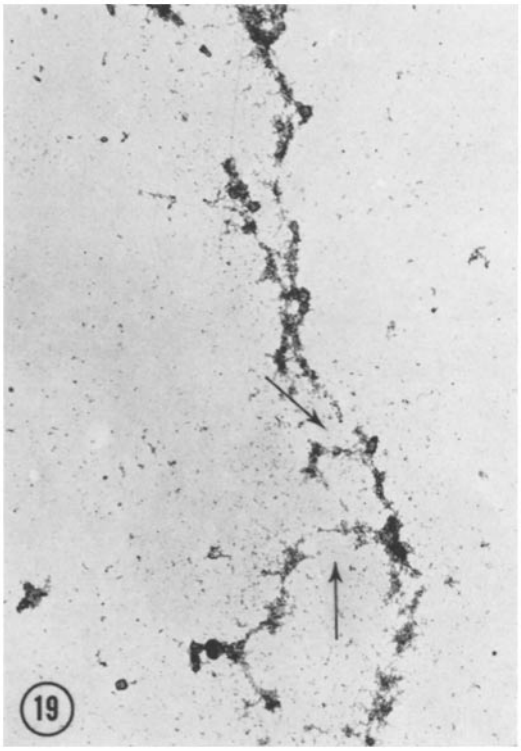

FIGURE 19 Chromosome demonstrating digested appearance following treatment with 10 μg/ml DNase. Fibers between the arms are also affected (arrows). × 9,000.

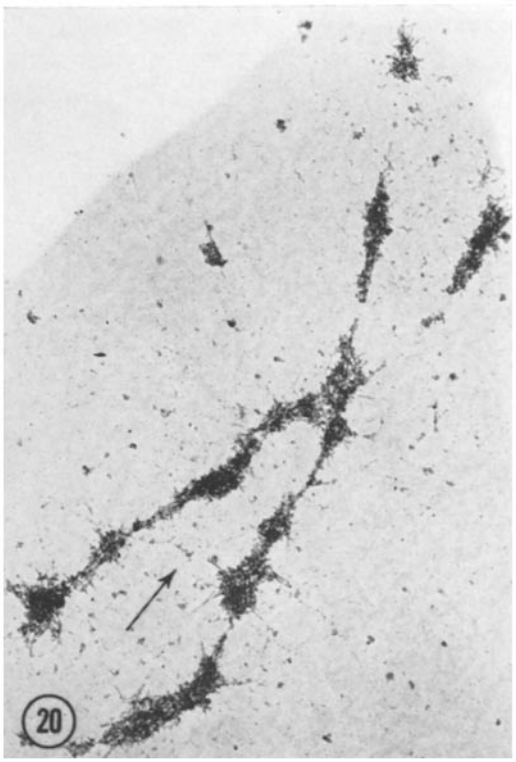

FIGURE 20 Chromosome demonstrating digestion following treatment with 5 μg/ml DNase. A digested interarm fiber appears (arrow). × 10,800.

many fibers coming out and looping back (100). He suggested that these are sites in which a fiber, which has reached the end of the arm, turns around to go back, i.e. parallel folding (38). Wolfe reported that the number of longitudinal fibers in the meiotic chromosomes of the milkweed bug doubles between zygotene and diakinesis and suggested that parallel folding may take place (40). It is difficult, however, to imagine a pattern of folding of a single fiber which would account for all the fiber arrangements we observe in the present studies.

We are very grateful to Mr. Don W. Coble, Dr. Stephen L. Wolfe, and Dr. Howard Nankin for their technical advice. We also thank Dr. William M. Leach for his comments during the preparation of this manuscript.

Received for publication 1 July 1968, and in revised form 6 November 1968.

REFERENCES

1. Ris, H. 1961. Ultrastructure and molecular organization of genetic systems. *Can. J. Genet. Cytol.* **3**:95.
2. Kaufman, B. P., H. Gay, and M. R. McDonald. 1960. Organizational patterns within chromosomes. *Int. Rev. Cytol.* **9**:77.
3. Hay, E. D., and J. P. Revel. 1963. The fine structure of the DNP component of the nucleus. *J. Cell Biol.* **16**:29.
4. Dupraw, E. J. 1965. The organization of nuclei and chromosomes in honeybee embryonic cells. *Proc. Nat. Acad. Sci.* **53**:161.
5. Ris, H. 1966. Fine structure of chromosomes. *Proc. Roy. Soc. London, Ser. B.* **164**:246.
6. Barnicot, N. A. 1967. A study of newt mitotic chromosomes by negative staining. *J. Cell Biol.* **32**:585.
7. Gall, J. 1963. Chromosome fibers from an interphase nucleus. *Science.* **139**:120.
8. Wolfe, S. L. 1965. The fine structure of isolated chromosomes. *J. Ultrastruct. Res.* **12**:104.
9. Barnicot, N. A., and H. E. Huxley. 1965. Electron microscopic observations on mitotic chromosomes. *Quart. J. Microsc. Sci.* **106**:197.
10. Ris, H., and B. L. Chandler. 1963. The ultrastructure of genetic systems in prokaryotes and eukaryotes. *Cold Spring Harbor Symp. Quant. Biol.* **28**:1.
11. Solari, A. J. 1965. Structure of the chromatin in sea urchin sperm. *Proc. Nat. Acad. Sci.* **53**:503.
12. Nebel, B. R., and E. M. Coulon. 1962. Enzyme effects on pachytene chromosomes of the pigeon evaluated with the electron microscope. *Chromosoma.* **13**:292.
13. Hsu, T. C., B. R. Brinkley, and F. E. Arrighi. 1967. The structure and behavior of the nucleolus organizers in mammalian cells. *Chromosoma.* **23**:137.
14. Jokelainen, P. T. 1967. The ultrastructure and spatial organization of the metaphase kinetochore in mitotic rat cells. *J. Ultrastruct. Res.* **19**:19.
15. Luykx, P. 1965. The structure of the kinetochore in meiosis and mitosis in *Urechis* eggs. *Exp. Cell Res.* **39**:643.
16. Brinkley, B. R., and E. Stubblefield. 1966. The fine structure of the kinetochore of a mammalian cell in vitro. *Chromosoma.* **19**:28.
17. Franchi, L. L., and A. M. Mandl. 1963. The ultrastructure of oogonia and oocytes in the foetal and neonatal rat. *Proc. Roy. Soc. London, Ser. B.* **157**:99.
18. Moses, M. J., and J. R. Coleman. 1964. Structural patterns and the functional organization of chromosomes. *In* The Role of Chromosomes in Development. Growth Symposium No. 23. M. Locke, editor. Academic Press Inc., New York and London. 11.
19. Trosko, J. E., and S. Wolff. 1965. Strandedness of *Vicia Faba* chromosomes as revealed by enzyme digestion studies. *J. Cell Biol.* **26**:125.
20. Bastia, D., and M. S. Swaminathan. 1967. Ultrastructure of interphase chromosomes. *Exp. Cell Res.* **48**:18.
21. Kaufman, B. P., and M. R. McDonald. 1956. Organization of the chromosome. *Cold Spring Harbor Symp. Quant. Biol.* **21**:233.
22. Brinkley, B. R. 1967. Effects of hypotonic culture media on the ultrastructure of mammalian cells in vitro. *J. Cell Biol.* **35**:17A. (Abstr.)
23. Schwarzacher, H. G., and W. Schnedl. 1967. Elektronmikroskopische Untersuchungen menschlicher Metaphasen-Chromosomen. *Humangenetik.* **4**:153.
24. Moorhead, P. S., P. C. Nowell, W. J. Mellman, D. M. Battips, and D. A. Hungerford. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res.* **20**:613.
25. Anderson, T. F. 1951. Techniques of preservation of three-dimensional structure in pre-

paring specimens for the electron microscope. *Trans. N. Y. Acad. Sci.* **13**:130.

26. HOTTA, Y., and A. BASSEL. 1965. Molecular size and circularity of DNA in cells of mammals and higher plants. *Proc. Nat. Acad. Sci.* **53**:356.
27. 1960. A proposed standard system of nomenclature of human mitotic chromosomes. *Lancet.* **1**:1063.
28. 1963. The London Conference on the normal human karyotype. *Cytogenetics.* **2**:264.
29. DUPRAW, E. J. 1966. Evidence for "folded fibre" organization in human chromosomes. *Nature.* **209**:577.
30. WOLFE, S. L., and B. JOHN. 1965. The organization and ultrastructure of male meiotic chromosomes in *Oncopeltus faciatus*. *Chromosoma.* **17**:85.
31. RAE, P. M. M. 1966. Whole mount electron microscopy of *Drosophila* salivary chromosomes. *Nature.* **212**:139.
32. WOLFE, S. L., and P. G. MARTIN. 1967. Strandedness of chromosomes from two species of *Vicia*. Proceedings Twenty-fifth Anniversary Meeting Electron Microscopy Society of America. 84.
33. WOLFE, S. L., and J. N. GRIM. 1967. The relationship of isolated chromosome fibers to the fibers of the embedded nucleus. *J. Ultrastruct. Res.* **19**:382.
34. RIS, H. 1966. Electron microscope studies on nucleohistone fibers from interphase nuclei. *J. Cell Biol.* **31**:134A. (Abstr.)
35. DALES, S. 1960. A study of the fine structure of mammalian somatic chromosomes. *Exp. Cell Res.* **19**:577.
36. GOVARTS. A., and D. DEKEGEL. 1966. Electron micrography of human chromosomes. *Nature.* **209**:831.
37. RIS, H. 1957. Chromosome structure. *In* The Chemical Basis of Heredity. W. D. McElroy and B. Glass, editors. The Johns Hopkins Press, Baltimore. 55.
38. DUPRAW, E. J., and P. M. M. RAE. 1966. Polytene chromosome structure in relation to the "folded fibre" concept. *Nature.* **212**: 598.
39. OSGOOD, E. E., D. P. JENKINS, R. BROOKS, and R. K. LAWSON. 1964. Electron micrographic studies of the expanded and uncoiled chromosomes from human leukocytes. *Ann. N. Y. Acad. Sci.* **113**:717.
40. WOLFE, S. L., and G. M. HEWITT. 1966. The strandedness of meiotic chromosomes from *Oncopeltus*. *J. Cell Biol.* **31**:31.
41. SWANSON, C. P. 1957. Cytology and Cytogenetics. Prentice-Hall, Inc., Englewood Cliffs, N.J. 200.
42. MANTON, I. 1950. The spiral structure of chromosomes. *Biol. Rev.* **25**:486.
43. KAUFMAN, B. P. 1948. Chromosome structure in relation to the chromosome cycle. II. *Bot. Rev.* **14**:57.
44. CLEVELAND, L. R. 1949. The whole life cycle of chromosomes and their coiling systems. *Trans. Amer. Phil. Soc.* **39**:1.
45. OHNUKI, Y. 1965. Demonstration of the spiral structure of human chromosomes. *Nature.* **208**:916.
46. BAJER, A., and J. MOLÉ-BAJER. 1963. Cine analysis of some aspects of mitosis in endosperm. *In* Cinemicrography in Cell Biology. G. G. Rose, editor. Academic Press Inc., New York and London. 363.
47. MAZIA, D. 1961. Mitosis and the physiology of cell division. *In* The Cell. Meiosis and Mitosis. J. Brachet and A. E. Mirsky, editors. Academic Press Inc., New York and London. **3**:167.
48. SWANSON, C. P. 1957. Cytology and Cytogenetics. Prentice-Hall, Inc., Englewood Cliffs, N.J. 123.
49. LIMA-DE-FARIA, A. 1952. Chromomere analysis of the chromosome complement of rye. *Chromosoma.* **5**:1.
50. NEBEL, B. R. 1939. Chromosome structure. *Bot. Rev.* **5**:563.
51. KAUFMAN, B. W. 1948. Chromosome structure in relation to the chromosome cycle. II. *Bot. Rev.* **14**:57.
52. KAKHIDZE, N. T. 1940. Chromomere structure of mitotic chromosomes in wheats. *Compt. Rend. (Doklady) Acad. Sci. URSS.* **26**:468.
53. SHMARGON, E. N. 1938. Analysis of the chromomere structure of mitotic chromosomes in rye. *Compt. Rend. Acad. Sci.* **21**:259.
54. KAUFMAN, B. P. 1934. Somatic mitoses of *Drosophila melanogaster*. *J. Morphol.* **56**:125.
55. GALL, J. G. 1955. On the submicroscopic structure of chromosomes. *Brookhaven Symp. Biol.* **8**:17.
56. SWANSON, C. P. 1957. Cytology and Cytogenetics. Prentice-Hall, Inc., Englewood Cliffs, N.J. 142.
57. MILLER, O. L. 1965. The fine structure of lampbrush chromosomes. *Nat. Cancer Inst. Monogr.* **18**:79.
58. SWANSON, C. P. 1957. Cytology and Cytogenetics. Prentice-Hall, Inc., Englewood Cliffs, N.J. 154.
59. MCCLINTOCK, B. 1951. Chromosome organization and genic expression. *Cold Spring Harbor Symp. Quant. Biol.* **16**:13.
60. CREIGHTON, H. B., and B. MCCLINTOCK. 1931. A correlation of cytological and genetical

crossing over in *Zea mays*. *Proc. Nat. Acad. Sci.* **17**:492.

61. PAINTER, T. S. 1933. A new method for the study of chromosome rearrangements and plotting of chromosome maps. *Science.* **78**:585.
62. BRIDGES, C. B. 1936. The bar "gene" a duplication. *Science.* **83**:210.
63. PELLING, C. 1966. A replicative and synthetic chromosomal unit—the modern concept of the chromomere. *Proc. Roy. Soc. London, Ser. B.* **164**:279.
64. EVANS, H. J. 1964. Uptake of H^3-thymidine and patterns of DNA replication in nuclei and chromosomes of *Vicia faba*. *Exp. Cell Res.* **35**:381.
65. HSU, T. C. 1964. Mammalian chromosomes in vitro. XVII. DNA replication in the Chinese hamster. *J. Cell Biol.* **23**:53.
66. LIMA-DE-FARIA, A. 1961. The initiation of DNA synthesis at specific segments in the meiotic chromosomes of *Melanoplus*. *Hereditas.* **47**:674.
67. HOWARD, E. F., and W. PLAUT. 1967. Ordered chromosomal DNA synthesis in *Drosophila melanogaster*. *J. Cell Biol.* **35**:59A (Abstr.)
68. VALENCIA, J. I., N. CACHEIRO, and C. SONNENSCHEIN. 1964. Human pachytene chromosomes. *In* Mammalian Cytogentics and Related Problems in Radiobiology. C. Pavan, C. Chagas, O. Frota-Pessoa, and L. R. Caldas, editors. The Macmillan Company, New York. 186.
69. YERGANIAN, G. 1957. Cytological maps of some isolated human pachytene chromosomes. *Amer. J. Human Genet.* **9**:42.
70. SCHULTZ, J., and P. ST. LAWRENCE. 1949. A cytological basis for a map of the nucleolar-chromosome in man. *J. Hered.* **40a**:30.
71. GERALD, P. S., S. WARNER, J. D. SINGER, P. A. CORCORAN, and I. UMANSKY. 1967. A ring D chromosome and anomalous inheritance of haptoglobin type. *J. Pediat.* **70**: 172.
72. SUTTON, H. E. 1967. Human genetics. *Ann. Rev. Genet.* **1**:1.
73. NANCE, W. E., and E. ENGEL. 1967. Autosomal deletion mapping in man. *Science.* **155**:692.
74. RENWICK, J. H. 1961. Elucidation of gene order. *In* Recent Advances in Human Genetics. L. S. Penrose, editor. Little, Brown, and Company, Boston. 120.
75. SANGER, R. 1965. Genes on the X chromosome. *Can. J. Genet. Cytol.* **7**:179.
76. GERMAN, J. 1692. DNA synthesis in human chromosomes. *Trans. N. Y. Acad. Sci.* **24**:395.
77. LIMA-DE-FARIA, A. 1964. DNA Replication in human chromosomes—A review. Mammalian Cytogenetics and Related Problems in Radiobiology. C. Pavan, C. Chagas, O. Frota-Pessoa, and L. R. Caldas, editors. The Macmillan Company, New York. 31.
78. MORISHIMA, A., M. M. GRUMBACH, and J. H. TAYLOR. 1962. A synchronous duplication of human chromosomes and the origin of sex chromatin. *Proc. Nat. Acad. Sci.* **48**:756.
79. SCHMID, W. 1963. DNA replication patterns of human chromosomes. *Cytogenetics.* **2**:175.
80. GAVOSTO, F., L. PEGORARO, G. BOVERA, and P. MASERA. 1967. Time sequence DNA replication in heteropycnotic X. *Nature.* **215**: 535.
81. LIMA-DE-FARIA, A., J. REITALU, and S. BERMAN. 1961. The pattern of DNA synthesis in the chromosomes of man. *Hereditas.* **47**: 695.
82. SASAKI, M. S., and S. MAKINO. 1963. The demonstration of secondary constrictions in human chromosomes. *Amer. J. Human Genet.* **15**:24.
83. ZAKHAROV, A. F., and N. A. EGOLINA. 1968. A synchrony of DNA replication and mitotic spiralization along heterochromatic portions of Chinese hamster chromosomes. *Chromosoma.* **23**:365.
84. STUBBLEFIELD, E. 1964. DNA synthesis and chromosomal morphology of Chinese hamster cells cultured in media containing Colcemid. *Symp. Int. Soc. Cell Biol.* **3**:223.
85. TJIO, H. J., and A. LEVAN. 1950. Quadruple structure of the centromere. *Nature.* **165**:368.
86. GIMENEZ-MARTIN, G., J. F. LOPEZ-SAEZ, and A. MARCOS-MORENO. 1965. Structure of the centromere in telocentric chromosomes. *Experimentia.* **21**:391.
87. LIMA-DE-FARIA, A. 1956. The role of the kinetochore in chromosome organization. *Hereditas.* **42**:85.
88. GIMENEZ-MARTIN, G., J. F. LOPEZ-SAEZ, and A. GONZALES-FERNANDEZ. 1963. Somatic chromosome structure. *Cytologia.* **28**:381.
89. BAJER, A. 1958. Cine-micrographic studies on mitosis in endosperm. IV. The mitotic contraction stage. *Exp. Cell Res.* **14**:245.
90. BARNICOT, N. A., and H. E. HUXLEY. 1961. The electron microscopy of unsectioned human chromosomes. *Ann. Hum. Genet.* **25**: 253.
91. MOLÉ-BAJER, J. 1958. Cine-micrography of C-mitosis in endosperm. *Chromosoma.* **9**:332.
92. CLEVELAND, L. R. 1955. Hormone-induced sexual cycles of flagellates. XIII. Unusual behavior of gametes and centrioles of *Barbulanympha*. *J. Morphol.* **97**:511.
93. LEVAN, A. 1938. The effect of colchicine on root mitoses in *Allium*. *Hereditas.* **24**:471.
94. KAUFMAN, B. W. 1936. Chromosome structure

in relation to the chromosome cycle. *Bot. Rev.* **2**:529.

95. SWANSON, C. P. 1957. Cytology and Cytogenetics. Prentice-Hall, Inc., Englewood Cliffs, N.J. 118.
96. SWIFT, H. 1965. Molecular morphology of the chromosome. *In* The Chromosome: Structural and Functional Aspects. C. J. Dawe, editor. (*In Vitro* 1:26). Tissue Culture Association, Inc. Boston.
97. TAYLOR, J. H. 1963. The replication and organization of DNA in chromosomes. *In* Molecular Genetics. J. H. Taylor, editor. Academic Press, Inc. New York.
98. PEACOCK, W. J. 1965. Chromosome replication. *Nat. Cancer Inst. Monogr.* **18**:101.
99. DUPRAW, E. J. 1965. Macromolecular organization of nuclei and chromosomes: a folded fiber model based on whole mount electron microscopy. *Nature.* **208**:338.
100. DUPRAW, E. J. 1966. Telomere ultrastructure in relation to chromosome strandedness. *J. Cell Biol.* **31**:30A. (Abstr.)