

A STUDY OF CHROMOSOMES WITH THE ELECTRON MICROSCOPE*

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PLATES 130 AND 131

“The central problem of biology is the physical nature of living substance. It is this that gives drive and zest to the study of the gene, for the investigation of the behavior of genic substance seems at present our most direct approach to this problem” (Stadler (14)). This genic substance is localized in the chromosomes. The morphological, chemical, and physiological investigation of chromosomes is therefore one of the main approaches to the nature of the genic substance. Lately the chemical analysis of chromosomes has provided interesting information (1). Most significant is perhaps the behavior of the deoxyribonucleic acid (DNA) and the non-histone protein fraction. The DNA is present in constant amounts per chromosome set of a species, it is remarkably stable biochemically, and has a very interesting molecular structure which has suggested a useful model for speculations on the process of replication of genes and chromosomes (15). The non-histone protein and ribonucleic acid (RNA) on the other hand vary greatly in amount and this variation is closely related to the physiological activity of the nucleus. Ultimately we want to describe the behavior of chromosomes and genes in terms of these molecules. However, there is still a large gap between the level of the DNA molecule and that of the chromosome as we know it, a fact too often neglected by those who have tried to explain chromosome behavior with the Watson-Crick model of DNA. To help fill this gap we need information on chromosome organization that may be obtained with the electron microscope.

During mitosis, when they can be clearly recognized, chromosomes are generally too thick for electron microscopy. Suspensions of interphase chromosomes mechanically isolated from vertebrate tissues are not suitable, since it is difficult in the electron microscope to distinguish chromosome material from contaminations and it is not known how the isolation procedures affect the structure of chromosomes. Thin sections on the other hand are very difficult to interpret by themselves. We found that lampbrush chromosomes dissected from oocyte nuclei of amphibians and pachytene chromosomes of insects and certain plants are favorable material. They can be followed from the light

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microscope level into the electron microscope and pictures of whole chromosomes or at least short regions of them can be obtained (8-10). These prophase chromosomes consist of bundles of microfibrils. The microfibrils are helically coiled (in lampbrush chromosomes) or twisted less regularly, and the bundle in turn forms a helix which corresponds to the chromonema visible in the light microscope. The microfibrils are from 500 to 600 A thick in all prophase chromosomes studied and are split into two finer fibrils which are about 200 A thick. Fragments of interphase nuclei were also obtained in Feulgen-squash preparations and were seen to contain twisted fibrils about 200 A thick. For a detailed study and higher resolutions such preparations are not suitable however, and one has to resort to thin sections. In the following I should like to give a brief report on work now in progress on the organization of chromosomes in interphase nuclei and at various stages of mitosis and meiosis.

Material and Methods

Anthers of *Lilium longiflorum* were chosen for a study of meiotic chromosomes. The development of sporogenous cells from premeiotic stages to mature microspores is synchronous in all anthers of a bud (4). One anther was analyzed in the light microscope and the others fixed for 1 hour in 1 per cent OsO₄ buffered with acetate-veronal to pH 7.5, embedded in methacrylate, and sectioned with a Porter-Blum microtome. Anthers of *Tradescantia paludosa*, onion root tips, oocytes of *Triturus viridescens*, and testis of the rat were fixed and sectioned in the same way. Small pieces of onion root were fixed also in 10 per cent neutral formalin and treated with deoxyribonuclease (1 mg./cc. in 0.003 M MgSO₄ at 40°C. for 2½ hours) (7). A sample was stained with the Feulgen reagent and the nuclei found to give a negative reaction. The material was then fixed in buffered OsO₄, embedded in methacrylate, and sectioned.—In some of the sections the methacrylate was removed with amyloacetate and the preparation shadowed with uranium.

The micrographs were taken with an RCA electron microscope type EMU-2 on Ilford process plates N.40 and developed in D-19.¹

OBSERVATIONS

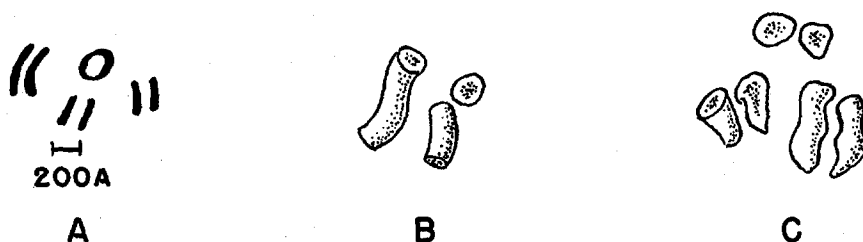
Intact Leptotene Chromosome.—Part of a leptotene chromosome from a formalin-fixed Feulgen-squash is shown in Fig. 1. The chromosome is made of fibrils, about 500 A thick. In a few places the double nature of these fibrils is visible (arrow). The thicker regions labelled *ch* correspond to the chromomeres of the light microscopist. They apparently contain the same kind of fibrils as the interchromomeric regions, but seem more closely coiled and packed together. The exact number of fibrils per chromosome is not known, but appears to be around 8 in some interchromomeric regions. While it cannot be proven that these fibrils are continuous for the length of the chromosome, no other continuous structures have been found. Since genetic data and the longitudinal

¹ The shadow casting and electron microscopy were done in the Electron Microscope Laboratory of the University of Wisconsin. I wish to thank Dr. P. Kaesberg for his advice and practical help.

division of chromosomes require some continuous unit through the length of the chromosome it is assumed at present that these fibrils are the continuous subunits of chromosomes and that their number is a multiple of two.

Sections of Meiotic Prophase Chromosomes.—Knowing what the intact free chromosome looks like in the electron microscope, it becomes possible to interpret thin sections through entire nuclei. We expect to find random sections through bundles of coiled microfibrils. They should be about 200 A thick and in prophase chromosomes two of them should be closely associated into 500 A fibrils.

Fig. 6 shows a section through a leptotene nucleus of lily. Instead of the expected 200 A fibers we see much thinner lines, about 60 A thick, but they occur generally in pairs of parallel lines. The width of the double unit is 200 A. In addition to the double lines we find circles with a diameter of 200 A (Text-fig. 1 A). Similar structures are visible also in sections through metaphase chromo-



TEXT-FIG. 1. Diagrammatic representation of the chromosomal microfibrils in sections. A, methacrylate not removed. B, methacrylate removed, shadowed with uranium, somatic interphase. C, methacrylate removed, shadowed, prophase.

somes (Fig. 7). In order to decide whether we are dealing with double fibrils or cylinders with the osmium deposited on the outside only, the methacrylate was removed and the sections shadowed with uranium. In Fig. 4 we see that after shadowing the 200 A fibrils have a solid contour and there is no evidence of any split. Where the fibrils are cut across, the sections look like doughnuts, the center being less electron-dense than the outer part of the fibrils. Since Fig. 4 represents a section through a late prophase chromosome we find always two of the elementary fibrils running parallel and closely associated (Fig. 4, brackets, Text-fig. 1 C). These pairs correspond to the 500 A fibrils seen in the intact leptotene chromosome (Fig. 1). While this association in pairs is clearly visible after shadowing, it is less obvious in micrographs of unshadowed preparations with their confusing array of black lines.

Somatic Interphase Nuclei.—Sections through somatic interphase nuclei from lily anthers show similar double lines and circles (Text-fig. 1 A). After shadowing they appear as random sections through coiled fibrils 200 A in thickness. These fibrils again are less electron-dense in the center with a denser outer region (Figs. 2 and 3, arrows, Text-fig. 1 B). In the interphase nucleus the micro-

fibrils are spaced farther apart than in mitotic chromosomes where they appear to be as closely packed as possible (compare Figs. 2 and 4). The spacing between the microfibrils is affected by fixation. Fixatives which produce a coarse nuclear structure cause the microfibrils to stick together in clumps. After fixation with osmium, which preserves the life-like optically homogeneous appearance of nuclei, the microfibrils are separated by a space about equal to their width or larger. The shrinking and swelling of chromosomes (12) appear to be due largely to the closer or wider spacing of the microfibrils. Between the chromosomal areas there are irregular spaces which contain scattered dense granules and rodlets. Their significance is not known.

Interphase Nuclei of Other Tissues.—The pattern described for nuclei of the lily is found also in other tissues. Interphase nuclei from onion roots, from somatic cells in anthers of *Tradescantia* (Fig. 5), from thecal cells in the ovary of *Triturus*, lampbrush chromosomes in oocyte nuclei of *Triturus* (Fig. 8), and spermatid nuclei of the rat (Fig. 9) show the double lines and circles in sections with the methacrylate left in and segments of microfibrils 200 Å thick after shadowing. These microfibrils seem to be the universal and basic component of chromosomes in both animal and plant cells.

Appearance of Microfibrils after Treatment with Deoxyribonuclease.—Sections through onion root nuclei treated with deoxyribonuclease show pieces of microfibrils that are not different from those of control sections. The removal of DNA from the microfibrils therefore does not visibly alter their structure.

DISCUSSION

In previous publications (8–10) we have shown that amphibian lampbrush chromosomes and pachytene chromosomes of various insects and plants are made of microfibrils of similar thickness in all species. There was some evidence that these units were double. Interphase nuclei were found to contain microfibrils half as thick. It was suggested that the double units in prophase chromosomes were the result of duplication of the 200 Å fibrils. This duplication appeared to occur before visible prophase.

Thin sections through prophase and interphase nuclei have confirmed these findings. Interphase nuclei contain masses of microfibrils 200 Å thick. Similar fibrils are present in chromosomes during mitosis, but they occur here as pairs which correspond to the 500 Å fibrils described earlier. In mitotic chromosomes the microfibrils are tightly packed together while during interphase they are more widely spaced.

It is now generally agreed that chromosomes are multiple structures. Even the light microscope has revealed two or even four units (chromonemata) in anaphase chromosomes. With the electron microscope these chromonemata are found to be still further subdivided. In the 200 Å fibrils we seem to have reached a definite structural unit, since it is not further split into thinner fibers,

but appears to have a more complex organization. While the exact number of these units per chromosome has not been determined yet, there is evidence that it may not be the same in all species (9, 10).

For some time now the nature of the longitudinal differentiation of chromosomes into chromomeres, interchromomeric fibers, hetero-, and euchromatin has been debated. Some ten years ago I suggested that these chromosomal regions did not differ in kind, but in the spatial arrangement or degree of coiling of the same unit, the chromonema (11). The electron microscopic study of chromosomes has confirmed this point of view. The morphological unit of chromosomes is a coiled fibril. Chromomeres and interchromomeric regions contain the same fibrils, but they are differently arranged in space. This does, of course, not exclude chemical differences along their length. No doubt such differences are very important, one striking example being the loops and chromomeres in lampbrush chromosomes (8). Both structures consist of similar fibrils, but in chromomeres they contain DNA, while loops are Feulgen-negative and are made essentially of protein and RNA.

The organization of the elementary microfibril in terms of its chemical constituents is of special interest. Speculation on the nature of the gene, gene action, and gene duplication, or chromosome physiology and reproduction must take into account the organization of these chromosomal units. What is the arrangement of DNA, histone, and non-histone protein in the fibril? While little factual evidence is yet available, the appearance of the fibrils as described in this paper suggests an interesting model. Superficially at least there is a striking resemblance to the structure of the tobacco mosaic virus as described recently (5, 13). Apparently the virus particle contains a core of RNA surrounded by a sheath of protein. Perhaps the chromosomal fibril contains a core of DNA surrounded by protein. The data so far are in agreement with the model: The DNA can be removed without changing the outer appearance of the microfibril; osmium is present in the outer layer, but not in the core of the fibril, which is expected if OsO_4 combines with protein but not with nucleic acid (2); x-ray diffraction studies suggest that the protein is arranged around the DNA double helix (6). Such an organization of the chromosomal unit could bridge the gap between the duplication of the DNA macromolecule and that of the chromosomal unit. The DNA double helix could be duplicated in the core of the fibrils according to one or the other of the schemes proposed (see for instance reference 3), followed by a reorganization of the protein shell resulting in a doubling of the microfibril. It should be noticed that since most chromosomes contain more than one elementary fibril, separation of daughter chromosomes at anaphase does not separate the "old" and "new" microfibril, but the two halves (chromatids) of the bundle of fibrils which were already separate units in previous anaphase. Daughter chromosomes therefore must have equal numbers of "old" and "new" microfibrils.

SUMMARY

Amphibian lampbrush chromosomes and meiotic prophase chromosomes of various insects and plants consist of a bundle of microfibrils about 500 A thick. These fibrils are double, being made of two closely associated fibrils 200 A thick. Fragments of interphase nuclei contain a mass of fibrils 200 A thick. Ultrathin sections through nuclei in prophase or interphase show sections of these double or single fibrils cut at various angles. A comparison of sections with the methacrylate left in and sections that were shadowed after removing the methacrylate suggests that the OsO_4 reacts only with the outer part of the fibrils either because it does not penetrate, or as a result of a chemical difference of the inner core and the outside of the fibril. It is suggested that in analogy to the structure of the tobacco mosaic virus the chromosomal microfibril may have an inner core of DNA surrounded by a shell of protein.

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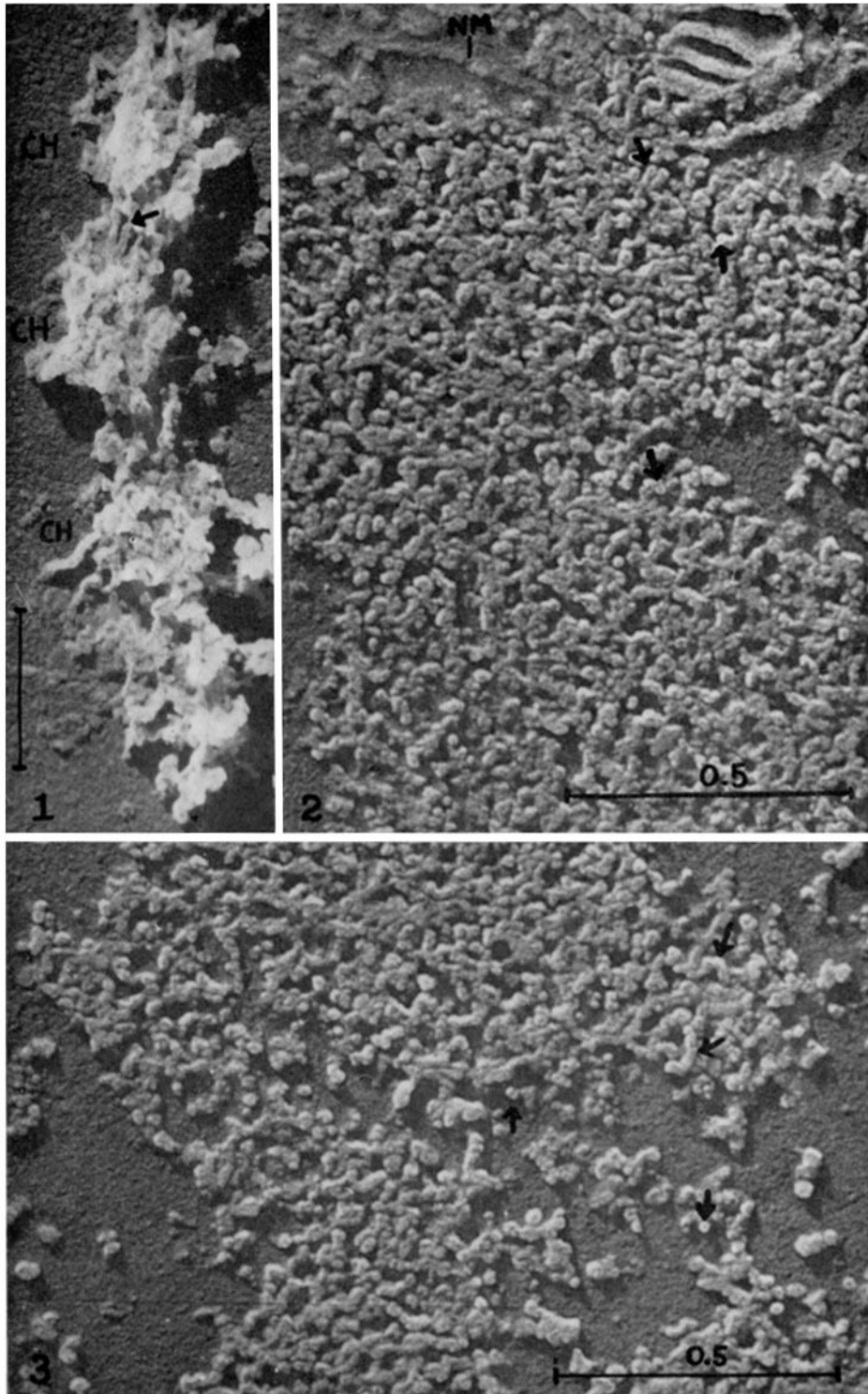
PLATES

EXPLANATION OF PLATES

PLATE 130

FIG. 1. Part of a leptotene chromosome of *Lilium*. Feulgen-squash. The chromosome consists of microfibrils, 500 A thick. In some regions they are visibly double (arrow). Regions in which the bundle of fibrils is doubled up and more closely packed correspond to the chromomeres of the light microscopist (*CH*). $\times 22,000$.

FIGS. 2 and 3. Sections through somatic interphase nuclei in the anther of *Lilium*. Methacrylate removed, uranium-shadowed. Sections through large numbers of twisted microfibrils are visible. End views of the cut fibrils show a dark center (arrows), indicating that the fibrils have a less electron-dense core. $\times 80,000$.



(Ris: Chromosomes studied with electron microscope)

PLATE 131

FIG. 4. Section through a late prophase chromosome of a microspore mother cell (*Lilium*). Methacrylate removed, uranium-shadowed. The 200 A fibrils occur in pairs (brackets) corresponding to the 500 A units seen in Fig. 1. In end views a darker core is visible in each fibril (circle). $\times 49,000$.

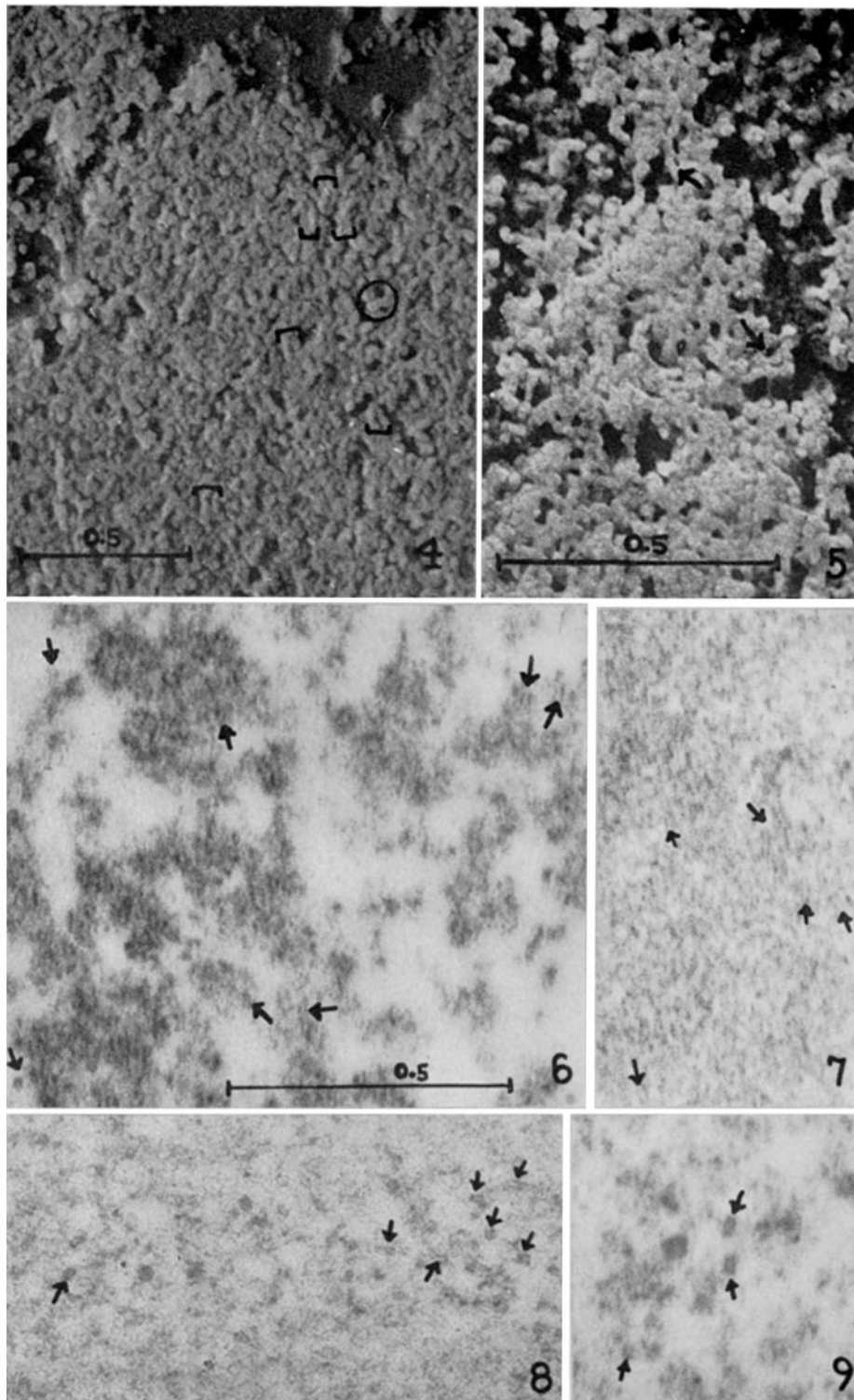
FIG. 5. Section through a somatic interphase nucleus of a *Tradescantia* anther. Methacrylate removed, uranium-shadowed. Randomly cut segments of twisted microfibrils are visible (arrows). $\times 80,000$.

FIG. 6. Section through a leptotene chromosome in a microspore mother cell of *Lilium*. Note the double lines and circles representing the more electron-dense outer region of the microfibrils (arrows). Compare with Text-fig. 1 A. $\times 80,000$.

FIG. 7. Section through a chromosome in first meiotic metaphase of a microspore mother cell (*Lilium*). Sections through microfibrils appear as double lines and circles 200 A in diameter (arrows). $\times 80,000$.

FIG. 8. Section through an oocyte nucleus of *Triturus*. The microfibrils of the lampbrush chromosomes appear again as double lines and circles (arrows). $\times 80,000$.

FIG. 9. Section through a rat spermatid. Arrows indicate sections through chromosomal microfibrils. $\times 80,000$.



(Ris: Chromosomes studied with electron microscope)