FINE STRUCTURE OF CHROMOSOMES*

By BERWIND P. KAUFMANN, Ph.D., and DEEPESH N. DE

(From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, and the Brookhaven National Laboratory, Upton, Long Island, New York)

PLATE 142

The essential feature of somatic mitosis is reflected on a gross scale in the longitudinal splitting of the chromosome and the separation of its halves into daughter nuclei. More specific information about the time at which each of the component strands originates as a replicated unit has been sought by cytologists in determination of the number of subsidiary chromonemata at the various phases of mitosis. Some thirty years ago evidence was presented from studies with the ordinary light microscope that the somatic anaphase chromosome contains at least two identifiable chromonemata and the metaphase chromosome four (1). Subsequent observations indicated that under appropriate conditions of treatment four chromonemata could be detected in telophase as well as in metaphase chromosomes (2). Since the structures under consideration approached in size the resolving powers of the best optical systems then available, it is not surprising that the validity of the multiple-strand as compared with the single-strand pattern of organization of these chromosomes was subjected to extensive debate. The observational and experimental evidence mustered in support of these alternative views has been summarized in various reviews (*e.g.*, 3–5), which afford a frame of reference for the information that is now becoming available from studies with the electron microscope.

As papers presented in this conference by Gall, Gay, Ris, and others have demonstrated, analysis of the structure of plant and animal chromosomes is now proceeding in many laboratories at the level of resolution afforded by the electron microscope (6–10). In our approach to the problems involved, we have focused attention on the plant, *Tradescantia*, since it figured so prominently in the earlier light microscope analyses of chromonematic patterns (1, 2), although supplementary studies have been made on cells of other plants and of insects (grasshoppers and *Drosophila*). The results of these investigations have sustained the interpretation that the somatic chromosome is composed of helically disposed chromonemata (1), but have also indicated that the number of com-

* This investigation was supported in part by a research grant (RG-149) from the National Institutes of Health, United States Public Health Service.

A portion of this work was carried out at the Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.

Downloaded from http://rupress.org/jcb/article-pdf/2/4/419/436237/419.pdf by guest on 29 September 2020

ponent strands is far greater than was originally inferred from the light microscope observations.

Materials and Methods

Staminate hairs of *Tradescantia reflexa* Raf. were used in this study. The hairs are composed of cylindrical cells arranged end to end in single file. It was assumed that this arrangement would minimize problems of fixation and dehydration as compared with those encountered in preservation of cells aggregated into compact tissues. The hairs were detached from the filaments while immersed in a buffered solution of osmium tetroxide. Preliminary tests involved a series of concentrations of OsO_4 in acetate, phosphate, and veronal buffers at different pH's. The differences afforded by these systems of reagents appeared in general to have less effect on the quality of fixation than did changes in the osmotic properties of the solutions, possibly because of the high water content of the cells and the presence of large vacuoles. Solutions that were isotonic—as determined by their effects in the absence of OsO_4 with respect to plasmolysis and modification of cyclosis—proved most satisfactory. Fixation was reduced to a brief period (15 to 30 minutes) and the hairs were then gradually dehydrated through increasing concentrations of ethanol. The materials were embedded in a mixture of butyl and methyl methacrylate (9:1) and the blocks were sectioned with a Porter-Blum microtome.

The cylindrical form of the staminate hair permits ready identification of component cells during the course of preparation of the material for electron microscopy. In the younger hairs, dividing cells are not uncommon, so that specific phases of the mitotic process can be selected for study. To facilitate this procedure, we adapted the method suggested by Gay (11) of placing the materials to be embedded on a flat glass plate, covering with a capsule containing methacrylate, and peeling from the glass surface after polymerization. The cells to be examined then lie near the face of the block, and can readily be identified with the compound microscope for orientation in the microtome.

OBSERVATIONS

The observations presented here will be restricted to the pattern of organization of the chromosomes during early prophase stages of somatic mitosis. Changes occurring in this pattern during the course of the mitotic cycle will be discussed in another publication.

Micrographs of thin sections of very early prophase cells reveal highly electron-scattering chromonematic strands aggregated into chromosomes separated by narrow zones of less dense karyolymph (Figs. 1 and 2). The two primary chromatids constituting a chromosome are relationally coiled, as is indicated by the caduceus-like pattern shown in Figs. 1 *a* and 1 *b*. Each chromatid is subdivided into two secondary or half-chromatids (Figs. 1 *b* and 2), and each of these in turn is subdivided successively into lower orders of chromonematic pairs until the smaller detectable units present the appearance shown at high magnification in Fig. 1 *c*. If our interpretation is correct—and it should be noted that it rests on a comparison of successive serial sections as well as the isolated patterns presented in Figs. 1–3—the somatic chromosome of *Tradescantia* contains at early prophase an aggregate of 64 identifiable chromonemata. They are presumably arranged as intertwined pairs of pairs, although the definitive

Downloaded from http://rupress.org/jcb/article-pdf/2/4/419/436237/419.pdf by guest on 29 September 2020

observational evidence in support of this aspect of the interpretation has not been obtained for all levels of organization.

Several orders of coiling of the finer detectable strands are shown in Fig. 1 *c*. Each of the loops of the ring-like configuration lying to the left of the black arrow in this micrograph has a diameter of about 250 A, but it is in turn composed of two subsidiary 125 A units. In cross-sectional view under high magnification such a subunit appears as a ring of electron-scattering material surrounding a less dense "core" (as is to be seen at the tip of the white arrow in Fig. 1 *c*). The wall of this ring has a thickness of about 40 A, and apparently represents another pair of coiled threads surrounding the 40 A central axis. Other fine strands of approximately 125 A diameter appear elsewhere in this micrograph.

A similar pattern of chromosomal organization is represented in Fig. 2. Each of the two chromatids indicated by the vertical arrows placed on this figure shows a number of paired, coiled threads. The more prominent units are approximately 1000 A in diameter (see horizontal arrow) and therefore constitute a higher order of coiling than that described in the preceding paragraph.

As prophase advances and the chromosomes condense the gross coiling pattern becomes more clearly defined and the interchromosomal spaces more conspicuous (Fig. 3), although the finer strands may be less readily identifiable than at very early prophase.

Some additional observations, which are not concerned with patterns of chromonematic organization, are recorded here because of their possible interest to plant cytologists. The first concerns the existence of a nuclear membrane, which has been identified in the better preparations as double layered and "fenestrated," although its structural details have not been characterized with accuracy. Some students of the staminate hairs of *Tradescantia* (*e.g.*, Wada (12)) have suggested that nucleus and cytosome are separated by only a phase boundary rather than a morphologically differentiated membrane, but our findings do not support that interpretation. The second of these supplementary observations concerns the existence of a membrane, apparently also double layered, between the cytosome and the vacuolar contents (the tonoplast of de Vries). Part of a vacuole with its limiting membrane is shown at the upper edge of Fig. 1 *a*; more detailed illustrations will be furnished in another publication.

DISCUSSION

The identification of a multiplicity of chromonemata in somatic chromosomes of *Tradescantia* sheds new light on some problems of structural organization with which cytologists have long been occupied. Although the number of discrete chromonematic units now disclosed in the electron micrographs far exceeds that previously detected in cytological analysis, it should not be over-

Downloaded from http://rupress.org/jcb/article-pdf/2/4/419/436237/419.pdf by guest on 29 September 2020

looked that radioisotope experiments of Friedrich-Freksa and Kaudewitz (13) in which individual amebas were traced through successive generations, suggest that the chromosomes of this protozoan contain at least 16 strands (see also the study of Henke and Pohley (14) on radiation-induced mutations in *Ephestia*).

Our interpretation that the subsidiary strands of *Tradescantia* are aggregated into a hierarchical assembly of pairs of pairs agrees with the observations of Gay (8) concerning the structure of the salivary-gland chromosomes of *Drosophila*, and those of Beermann (15) on the Balbiani ring of *Chironomus*. At the limits of resolution afforded by the electron micrographs the distinction between chromomeres and coils becomes difficult, a type of problem that was encountered in the earlier light microscope studies with respect to the higher orders of coiling. It was shown then that chromomere-like swellings often represent nodal points caused by turns of the chromonematic coils (16), and it now seems possible that the coiled pattern of organization extends down to the level of simpler nucleoprotein complexes (*cf.* reference 10), a pattern of organization proposed many years ago by Seifriz (17) on a speculative basis and codified more recently in terms of specific models (*e.g.*, reference 18).

The presence of a "matrix" material between the intertwined strands at the lowest orders of pairing raises the question whether the pair rather than the single strand represents a functional unit (in the sense suggested by Ris in this conference) in which protein is associated with—or surrounds—the double helix of the nucleic acid. Ris's studies have involved the shadowing technique, and until this method is applied to our material and we have in addition extended our cytochemical analysis to determine the nature of the electron-scattering materials, no definitive answer can be given to the question of the arrangement of nucleic acids and proteins in the ultimate chromonemata.

Finally, it may be noted that detection of a multiplicity of chromonemata clarifies some problems of chromosome structure but that it also presents new complexities in analysis of such mechanisms as are involved in induced chromosome breakage and recombination and in crossing-over. (It may be remarked in this connection that we have also detected numerous chromonemata in the chromosomes of *Tradescantia* during the prophases of the first meiotic division.) It now seems apparent that satisfactory answers to these problems are not to be found in models that are formulated on the assumption that the chromatid is a homogeneous unit, but are rather to be sought in an understanding of the nature and patterns of association of its constituent materials. Studies of this type are being extended in this laboratory to encompass the finer structures revealed by electron microscopy.

SUMMARY

Electron micrographs of staminate hair cells of *Tradescantia reflexa* indicate that early prophase chromosomes are composed of a number of helically ar-

Downloaded from http://rupress.org/jcb/article-pdf/2/4/419/436237/419.pdf by guest on 29 September 2020

ranged chromonemata. Favorable preparations reveal as many as 64 identifiable subsidiary strands, assumedly arranged as intertwined pairs to form a hierarchy of pairs of pairs. The helices of the smallest discernible units have a diameter of about 125 A, with highly electron-scattering material disposed peripherally around a less dense "core." The wall of this peripheral ring has a thickness of about 40 A, and apparently represents another pair of coiled threads surrounding a 40 A central axis. The implications of the findings are discussed briefly.

The authors desire to express their sincere thanks to Dr. Helen Gay for counsel during the course of the investigation. They also wish to thank Mr. Henry H. Jones for preparation of the accompanying plate of illustrations.

BIBLIOGRAPHY

1. Kaufmann, B. P., *Am. J. Bot.*, 1926, **13,** 59.
2. Nebel, B. R., *Z. Zellforsch. u. mikr. Anat.*, 1932, **16,** 251.
3. Kaufmann, B. P., *Bot. Rev.*, 1936, **2,** 529.
4. Kaufmann, B. P., *Bot. Rev.*, 1948, **14,** 57.
5. Manton, I., *Biol. Rev.*, 1950, **25,** 486.
6. Gall, J., *J. Morphol.*, 1954, **94,** 283.
7. Gall, J., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 393.
8. Gay, H., *Dissertation Abstr.*, 1955, **15,** 899.
9. Gay, H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 407.
10. Ris, H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 385.
11. Gay, H., *Stain Technol.*, 1955, **30,** 239.
12. Wada, B., *Cytologia*, 1950, **16,** 1.
13. Friedrich-Freksa, H., and Kaudewitz, F., *Z. Naturforsch.*, 1953, **8b,** 343.
14. Henke, K., and Pohley, H.-J., *Z. Naturforsch.*, 1952, **7b,** 65.
15. Beermann, W., *Chromosoma*, 1952, **5,** 139.
16. Kaufmann, B. P., *Am. Naturalist*, 1931, **65,** 280.
17. Seifriz, W., *Science*, 1933, **78,** 361.
18. Watson, J. D., and Crick, F. H. C., *Cold Spring Harbor Symp. Quant. Biol.*, 1953, **18,** 123.

Downloaded from http://rupress.org/jcb/article-pdf/2/4/419/436237/419.pdf by guest on 29 September 2020

EXPLANATION OF PLATE 142

Micrographs of ultrathin sections of chromosomes of staminate hair cells of *Tradescantia reflexa*. From material fixed in buffered OsO_4: Fig. 1, at pH 6.5; Fig. 2, at pH 7.5; Fig. 3, at pH 8.5. Markers on all figures represent 1 μ except on Fig. 1 *c* where the marker represents 0.1 μ.

FIG. 1 *a*. Over-all view of very early prophase nucleus showing chromosomes and at top a segment of a vacuole with its double, limiting membrane. The white rectangle frames the caduceus-like pattern formed by intertwined chromatids. Compare Fig. 1 *b*. × *ca.* 14,000.

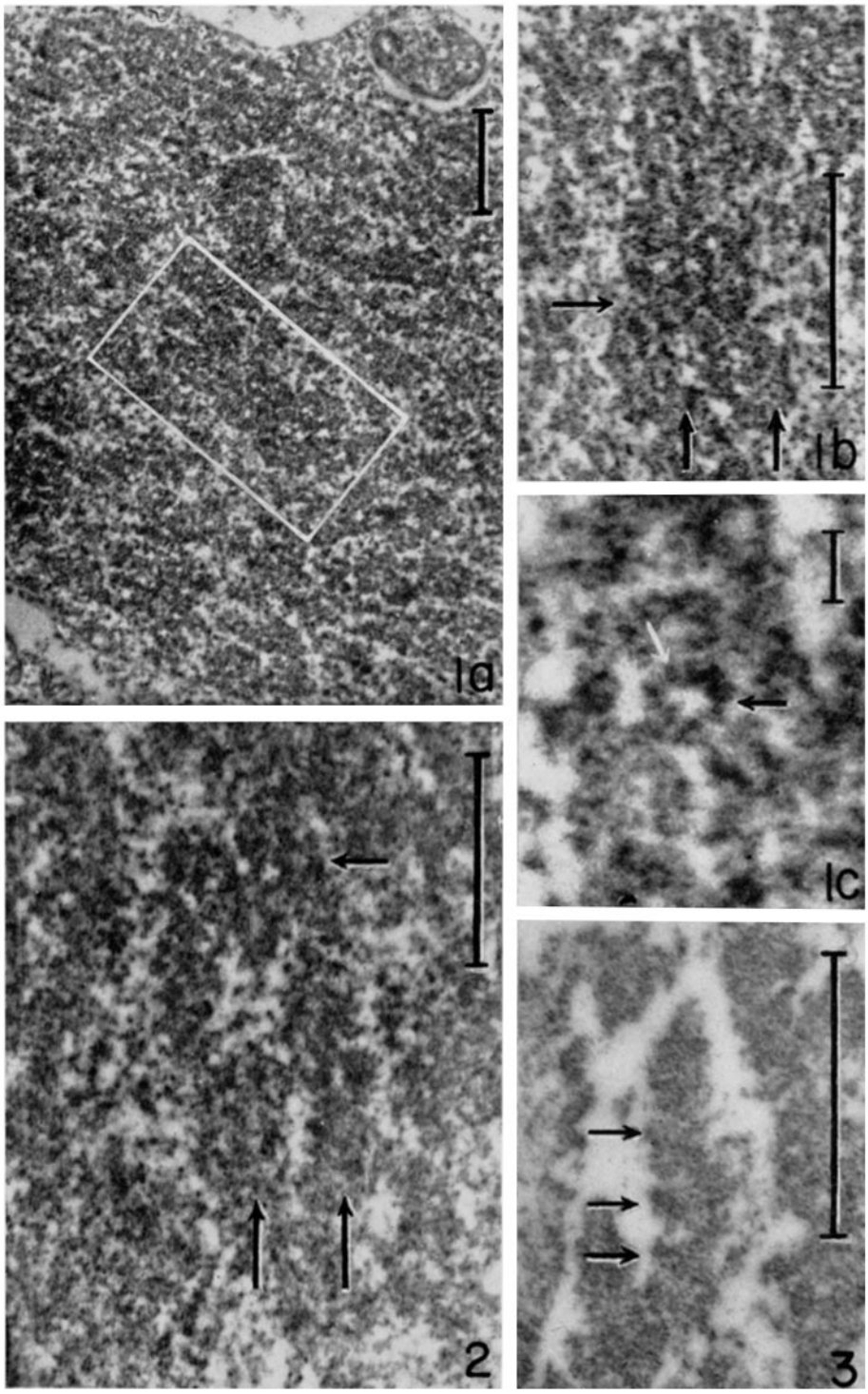
FIG. 1 *b*. Higher magnification of section framed in Fig. 1 *a*. A region of overlap of the two chromatids lies to the right of the horizontal arrow. Below this level the two primary chromatids (vertical arrows) lie side by side separated by a median zone of less dense material. Each chromatid is seen to be composed of subsidiary units of highly electron-scattering coiled strands separated by less dense material. × *ca.* 29,000.

FIG. 1 *c*. A higher magnification of a portion of one of the chromatids (the part lying slightly to the left of center near the top of Fig. 1 *b*). Several orders of coiling of the finer strands are represented here. Tip of white arrow indicates a cross-sectional view of a subsidiary unit of *ca.* 125 A diameter, having a highly electron-scattering border around a less dense central axis (fuller description in text). × *ca.* 97,000.

FIG. 2. Another early prophase nucleus showing chromatids with subsidiary chromonemata. Each of the sister chromatids is denoted by a vertically placed arrow at bottom. The paired parallel threads lying immediately to the left of the horizontally placed arrow constitute a unit of approximately 1000 A diameter. Similar units are visible elsewhere in these chromatids. × *ca.* 29,000.

FIG. 3. A section of a more advanced prophase nucleus with pronounced patterns of gross coiling. Note chromatid in center with its large gyres (arrows) and subsidiary coiled strands. A segment of the nucleolus lies to the left in this photograph. × *ca.* 39,000.

Downloaded from http://rupress.org/jcb/article-pdf/2/4/419/436237/419.pdf by guest on 29 September 2020



(Kaufmann and De: Fine structure of chromosomes)

Downloaded from http://rupress.org/jcb/article-pdf/2/4/419/436237/419.pdf by guest on 29 September 2020