Chromosome Structure Under the Electron Microscope

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OUR PRESENT KNOWLEDGE OF chromosome structure, based entirely upon light microscopy, has revealed that the basic structure of the chromosome is a long, slender thread of granules or chromomeres. These chromonemal threads are wound or coiled compactly in forming the chromosome of midmitosis. It is in the prophase of mitosis, before they have become closely coiled, that the individual chromonemal threads may be observed to best advantage. In a critical review with a bibliography of more than 700 titles, Geitler (*5*) has brought together extensive literature bearing on chromosome structure.

Since it has long been recognized that the structure of chromatin is most clearly observable in the early prophase of mitosis, anyone would plan to attack this problem in prophase. The researches of McClintock (*7*) on meiotic prophase chromosomes have, in fact, demonstrated directly that in the hereditary mechanism we are concerned with a linear order of visible particles or chromomeres united in some way into a thread. She has shown that definite sections of these pachytene chromonemas may, by their manner of synaptic approximation, be related to peculiarities in the genetic constitution of the plant. This was probably the closest demonstration by direct observation of the essential correctness of Morgan's theory of the linear arrangement of genes on chromosomes that had appeared up to the discovery and interpretation of giant salivary chromosomes, independently by Painter (*8*) in *Drosophila* and by Heitz and Bauer (*6*) in *Bibio*. Salivary chromosomes have also confirmed the linear arrangement of genes and led to a better understanding of the nature of the genic elements.

The limit of resolution in light microscopy was approached before the turn of the century with the perfection of modern lens systems. Improvement since Abbe computed the lens formulas that are still in use may be expected to result only from greater precision or perfection of workmanship in manufacture. Theoretically, one should be able to distinguish two objects when separated by about 1/5 μ or more—an optical perfection usually not realized. The employment of light of short wave length and ultraviolet improves resolution above this, but the use of ultraviolet depends upon photographic images rather than direct visual methods. The electron microscope gives, theoretically, 100 times better resolution than microscopes dependent upon light; it would separate points that are 1/100 of these distances apart and should therefore give much clearer definition.

Some of the conditions necessary for the examination of objects with an electron microscope are very discouraging to biologists. The objects to be examined must be placed in a very high vacuum and cannot be observed while immersed in fluid media. They are unavoidably subjected to extreme desiccation. Wyckoff (*12*) has recently given an account of the special requirements in preparations examined with the electron microscope. These conditions would not arouse much enthusiasm on the part of cytologists.

Images of dried objects, when examined under light microscopy, appear very unfamiliar and are frequently not recognized as identical when compared with the image of the same object while immersed in water, oil, or other fluid media. Bacteriologists dry the suspensions of bacteria on slides as a routine procedure before and after staining them, but during examination these are usually immersed in oil or other fluid medium of high refractive index. However, biologists must become accustomed to a study of the dried remains of organic matter and learn to employ techniques that differ radically from routine methods.

In using the electron microscope, preparations of objects may be carried out under light microscopy. Hence, one must learn to recognize selected objects in both conditions (immersed in fluid as well as dry), even when the dry object shows much less detail and appears very different from the customary mount.

Since the installation of an electron microscope at the University of Illinois in 1942 it has been the writer's ambition to render service in adapting this equipment to the study of chromosomes. Sporadic efforts made to mount and observe prophase chromosomes failed completely, but such efforts serve to familiarize one with the general technique and requirements in selection of objects suitable for study. It was soon realized that very special techniques must first be developed. For long periods all efforts other than reflection and speculation were abandoned. The present period of intensive experimentation began in June 1946.

Schmitt's claim (*11*) that sections of organic tissue must be cut as thin as 0.1 μ or less in order to be sufficiently penetrated by electrons to form images may be an overstatement, but he did not specify degree of magnification and definition. Claude and Fullam (*4*) have actually obtained electron micrographs from sections 0.3–0.6 μ in thickness but under magnifications not greatly exceeding those attainable with light microscopy. Their photographs, made at initial magnifications of 1,650, demonstrate that electronically recorded images actually show

much greater resolution than those of photomicrography and that they are subject to considerable enlargement. However, sections of more highly magnified organic objects should be made much thinner than has been attainable thus far.

Porter, Claude, and Fullam (*9*) have given electron micrographs of parts of entire cells obtained from tissue cultures—cells derived from chick embryos. Due to their thickness, these were photographed at magnifications of 900–1,600 and enlarged to ×15,000. In the photographs the superior resolving power of the electron microscope is evident by the sharp contrast and definition of the images. Only resting interphase nuclei were included. In regions of nuclei these isolated cells were too thick to show structure. As far as the writer has been able to find, chromosomal structure has not been certainly demonstrated with the electron microscope.

Some preliminary results on "lampbrush chromosomes" had been reported from our laboratories by Clark, Barnes, and Baylor (*2*) and illustrated in Clark, Quaife, and Baylor (*3*). Interpretations of these results have been complicated through subsequent investigations by Quaife (*10*) on protein salt systems, and some of these investigations remain incomplete and unpublished.

It occurred to the writer that the difficulty of obtaining small, thin objects might be overcome by teasing parts from cells. This is essentially the general method employed by Schmitt (*11*) in the study of muscle and collagen fibers. However, this technique involves new difficulties when used in a study of cells. Cytologists will realize at once that a thread stained with hematoxylin and located within the nuclear membrane is usually interpreted as part of a chromosome; similar objects encountered in cytoplasm outside of the nuclear membrane might be interpreted as mitochondria, chromidia, etc. When a cell is torn open and dismembered, how can one be certain as to the origin of the residues? Even when the cell has been stained, the chromosomes are not the only objects that may possess color.

Some of these difficulties have been overcome. It was found possible to recognize parts of chromosomes in prophase after they were desiccated on a slide. Through repeated rehearsal of dissection methods it was possible to isolate parts of chromosomes and separate them completely from the surrounding cytoplasm. Parts of prophase chromosomes from the pollen mother cells of maize could be isolated by operating on individual cells that had been transferred to clean slides. Furthermore, a technique has been developed by which an object such as an isolated bit of chromosome, definitely recognized as coming from within the nucleus, may be dried, mounted in a film, and transferred to the object holder of an electron microscope. The selected object is carefully centered when mounted or so related to peculiarities of the mount that its position may be recognized in the finder of the microscope. There is still some lack of perfection in this technique, which may be responsible for the considerable loss in contrast and definition thus far obtained from these objects at the magnifications used. An account of the technique will be given in detail in another publication. It is from such isolated bits of prophase chromosomes that the accompanying electron micrographs were obtained.

While the writer has dissected and prepared all objects and selected the images to be photographed, he does not operate the electron microscope by himself.[1] Another microscope, a new Universal model, was used in one session on January 2, 1947, at the Battelle Memorial Institute, Columbus, Ohio.[2] This new model gave excellent results and seems ideally adapted for biological investigations such as these. With more patience, equally good and some superior electron micrographs were obtained with the older RCA model (Serial No. 1) installed in G. L. Clark's laboratory at the University of Illinois. Even though this microscope is capable of higher magnifications, nearly all of this work was done at initial enlargements of 10,000–12,000. There are now more than 50 negatives showing chromosome structure in maize.

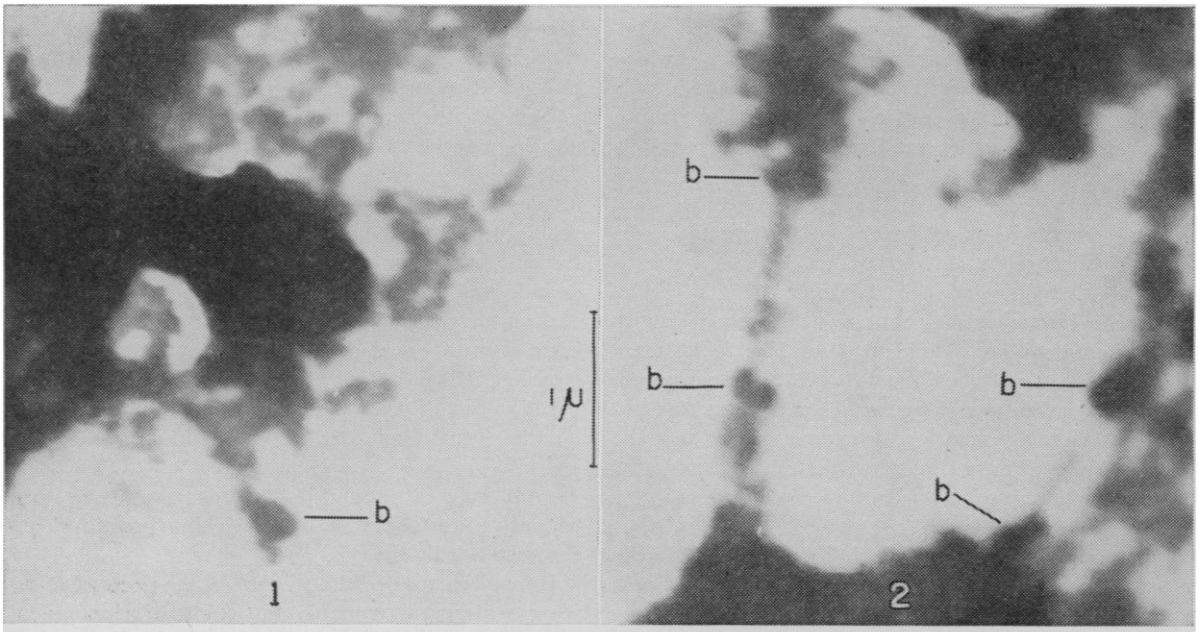
The picture of chromosome structure obtained has not greatly altered the general concept derived from light microscopy. There is close agreement with previously known features. Some details only partially known may be ascertained, and the method gives promise of supplying information concerning many other unknown or little known facts.

There is a thread that connects the granules or chromosomes or to which a great variety of chromomeres are attached. In places this thread appears to be flattened, and granules appear to be attached laterally. Both its width and thickness may not be constant. Some regions would indicate a width of 100 mμ or more in places that may border heterochromatic regions. The smallest values estimated for width were 50, and the smallest for thickness were about 30 mμ. Granules were usually found attached in pairs at the rate of 4–5/μ. In regions in which they were most crowded there were as many as 8/μ along the length of the thread. In some regions there were relatively few granules, but one cannot be certain that some of these may not have become detached and washed away during dissection and mounting. Data such as these, when given with greater precision, may be useful in estimating the actual size of the smallest visible elements of the gene.

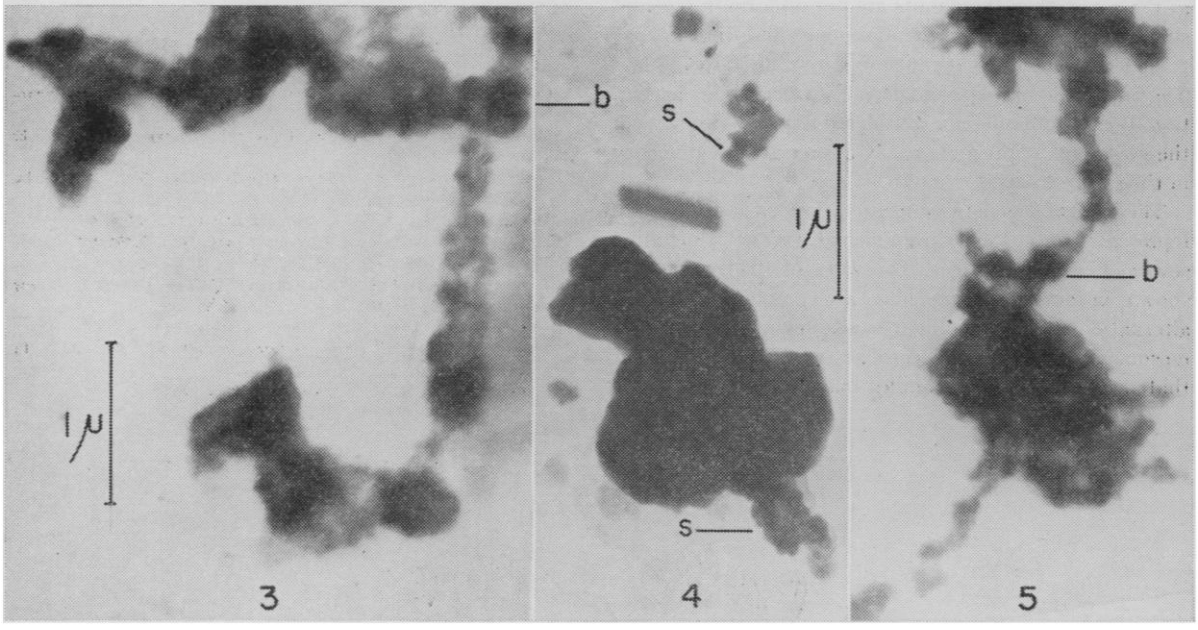
The chromomeres or granules that are found attached to the supporting thread vary through a wide range of sizes, shapes, and "density." Some are spherical and relatively translucent to electrons and range in size from 75 to 165 mμ. The spherical translucent granules are observ-

[1] The writer is greatly indebted to A. Eisenstark, who is employed by the Graduate School of the University of Illinois to operate this equipment for biological departments; to Martha B. Baylor, who was in charge of the electron microscope prior to September 1, 1946; and to H. B. Gillin who substituted for Mrs. Baylor on several occasions.

[2] This was made available through the courtesy of the Institute and Charles M. Schwartz, who operated the equipment.



FIGS. 1–5. Electron micrographs of fragments of chromosomes at or near pachytene, dissected from pollen mother cells of maize. All figures are × 20,000, enlarged from original recording made at × 11,000, except Fig. 4, which was recorded at × 12,000, and reproduced here at × 26,000. The granules are shown attached to a "linin" thread and vary greatly in size, shape, and "transparency" to electrons. Those shown at b scatter the electrons and appear as if "opaque". Those shown at s are spool shaped. Fig. 5 shows a long thread in snarls which appear as chromomeres under light microscopy.



able in all except Fig. 4. The spherical and spheroidal granules that appear as if opaque to electrons (Figs. 1–3, b) range in size from 85 to 660 mμ. Spheroidal "dark" granules were seen that may be estimated at 150 x 180, 132 x 220, 210 x 330, and 330 x 430 mμ.

Very many other granules seem to be lobed bodies—possibly spool shaped. Some of these may be seen in Fig. 4 near the s markers. When symmetrically lobed, these suggest the possibility that they are granules in stages of division, which could also apply if they are actually more nearly spool shaped. In some regions these measure approximately 150 x 210 mμ; others were estimated at 130 x 165 mμ.

Synapsis may be observed in some of the chromonemal

threads where they appear paired. Fig. 3–4 shows a section of two synapsed chromonemal threads, each of which may be double. However, Figs. 1, 3, and 5 suggest single threads, the double nature of which may be due to doubling of the chromonema itself, giving sister strands as in ordinary mitosis.

Nothing decisive and not previously recognized concerning synapsis is shown, nor would interpretation be considered critical in material that has been subjected to considerable manipulation in dissection.

Not all objects that may appear as chromomeres under light microscopy are individual units. Fig. 5 illustrates this, for here the bodies about 1 x 2 μ in size that are visible under light and would be interpreted as chromomeres are actually made up of snarls of beaded threads. Similar snarls in Figs. 1 and 2 would appear as rounded beads when observed under light microscopy so near the limits of resolution.

The granules that appear smooth and spherical in the electron micrographs may represent homogeneous substance, made up of uniform molecules. This might suggest that we are now actually dealing with the genic elements, with only the individual molecules of these units remaining unrevealed.

But where is the gene? Unfortunately, the answer may not be given with assurance. If the granules are considered as the genes, then these units are not uniform in size and shape. Granules may be found among those that appear to be homogeneous that range in size from 50 to 750 mμ. They vary also in shape and density to electronic penetration. If these granules are considered as initial products of the gene, one might more easily appreciate the diversity in their appearance.

The gene of genetic interpretations may, however, represent sections of chromonemal threads, including several kinds of granules. The work of Bridges (*1*) has shown, in fact, that some genes are composed of several alternations of dark and light bands in the salivary chromosomes of *Drosophila*. A corollary to this would suggest that numerous unlike granules may be included in the gene referred to by geneticists. It would appear logical to consider the granules shown here as parts of the elements of genes. This suggests that the gene is composite.

However, the chromonemal threads to which the granules are attached cannot be excluded from this concept. This thread is a vital part of the living substance that divides during the mitotic cycle, and if the granules are visible products of molecules, incorporated within the thread to which they appear attached, then we should not exclude, while they remain attached, these granules as parts of the genic elements. In a somewhat different sense the genic element would therefore be composite, even if one considers the position of a granule as its locus. This unit would consist of at least one molecule within the supporting thread that duplicates itself during each mitotic cycle and also produces the substance given off to form granules. It may be presumed that the granules or their derivative substance would be given off to produce effects elsewhere within the cell.

It may be expected that the use of the electron microscope will reveal much in the study of chromosome structure. In any event, it would seem that a concept of gene elements should emerge from a continued study of chromosomal structure by this means.

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