An Electron Microscope Study of Lampbrush Chromosomes*

BY JEAN G. LAFONTAINE, PH.D.,‡ AND HANS RIS, PH.D.

(From the Department of Zoology, University of Wisconsin, Madison, Wisconsin)

PLATES 43 TO 46

(Received for publication, July 25, 1957)

ABSTRACT

Lampbrush chromosomes were isolated from germinal vesicles of oocytes from *Necturus maculatus*, *Triturus viridescens*, *Pseudotriton montanus* and *Rana pipiens*.

After treatment of isolated nuclei with 10 per cent sucrose, chromosomes free of nuclear sap are obtained for examination in either the light microscope or in the electron microscope. For electron microscopy the chromosomes were prepared either by Anderson's critical-point procedure or were embedded in methacrylate and sectioned.

The evidence presented in favor of the view that the loops, axis, and the chromomeres of lampbrush chromosomes are formed by two chromonemata is based on the following observations:

1. Treatment of isolated chromosomes with 0.002 M KCN loosens the structure of the loops, and a more or less coiled organization is then observed in most of them with the light microscope. At the electron microscope level, each loop consists of a bundle of microfibrils. The latter are 500 A in diameter, and their complex arrangement within the loops is best studied in stereoscopic preparations.

2. Treatment of chromosomes with 0.002 M KCN also unravels the "chromomeric" regions of the axis. A fibrillar organization then becomes visible in the light microscope. In the electron microscope, wide strands are seen within some chromomeres; their diameter corresponds closely to that of the chromonemata forming the loops associated with the same chromomeres. In thin transverse sections of isolated chromosomes, no special structure is visible in the axial region except random profiles of fibrils similar to those seen in the loops of the same preparations.

3. Two strands sometimes connect adjacent chromomeres. Where gaps exist along the axis, after stretching of the chromosomes, a loop occasionally straddles the break and returns to a chromomere on each side.

INTRODUCTION

Lampbrush chromosomes, since their discovery by Flemming (10) and Rückert (24), have been studied in greater detail by Duryee (6–8), Koltzoff (17), Ris (19–21, 23), Dodson (5), Guyénot *et al.* (15, 16), Gall (11–14), Tomlin and Callan (25) and Callan (3, 4), and as a result of these studies, two entirely different interpretations of the structure of these giant chromosomes have been set forth. There is, on the one hand, the view proposed by Duryee, Koltzoff, Dodson, Gall, Guyénot, Tomlin, and Callan which states that the basic structure of the chromosome is a fine thread, the chromonema, along which specific granules or chromomeres are arranged in a characteristic order. During the growth of the chromosome the chromomeres are said to produce side loops which later on are shed from the chromosome, and it has been proposed that such side loops are specific products of the genes located in the chro-

*This work was supported by a grant from the Research Committee of the University of Wisconsin, from funds contributed by the Wisconsin Alumni Research Foundation. We wish to thank Dr. Paul Kaesberg for his help and interest in the course of this work.

‡Present address: Cytology Department, The Rockefeller Institute for Medical Research.

momeres. The other and different interpretation was previously suggested by Rückert (24), and was elaborated further by Ris (19) in connection with his general view on chromosome organization. The essential postulate here is that the loops are not secretion products of the chromomeres, but part of the chromonemata themselves. This implies an increase in the length of the chromonemata as the loops are formed. "Chromomers," at first considered to be points of overlap of the chromonemata (Rückert (24), Ris (19)), were later regarded as specific regions in which the chromonemata remain tightly coiled (Ris (21, 23)). In a critical review of salivary gland and lampbrush chromosomes, Alfert (1) has suggested a structure that is essentially the modified interpretation of Ris (21, 23).

It must be noted that in their most recent papers both Callan (3, 4) and Gall (13, 14) have revised their opinion and now agree that loops are part of the chromonema. While there is thus increasing agreement with regard to the nature of loops, some important details of loop structure remain controversial. According to some authors (Callan (3, 4); Gall (13, 14)), a submicroscopic axial thread continuous with the chromonema forms the axis of each loop; it is wound up in the chromomeres and spun out from them during loop growth. Surrounding this axis are granules of various sizes which are visualized as products of the axial thread, thus giving each loop its characteristic properties.

The other interpretation (Ris (20, 21, 23)), however, is that the loops consist of a bundle of fibrils. Each fibril is about 500 A thick and is made of a pair of finer filaments 200 A wide. Mitotic chromosomes of several animals and plants (Ris (22)) are also thought to consist of similar fibrils. The fine structure of loops would thus be essentially like that of chromonemata of ordinary prophase chromosomes.

The present paper reports further electron microscopic studies on the detailed structure of loops. In order to minimize distortion during preparation, Anderson's critical point method was used for drying the isolated chromosomes and stereoscopic micrographs, which greatly facilitate analysis of the spatial organization of the loops, were prepared.

Materials and Methods

Ovarian eggs of *Necturus maculatus*, *Triturus viridescens*, *Pseudotriton montanus*, and *Rana pipiens* were used as sources of lamp brush chromosomes. Nuclei were isolated in 10 per cent sucrose, which causes dissolution of the nuclear sap (Ris (20)). After the nuclear membrane is broken with fine forceps or needles, the chromosomes float out free of extraneous material.

For studies with the light microscope, chromosomes were isolated in a small container made by sealing on a glass slide a plastic disc 3 mm. thick and with a hole 7 mm. in diameter in the center. Washing with distilled water, fixation, staining, and dehydration were accomplished by using a microsyringe consisting of a ¼ c.c. syringe with a plunger controlled by a finely threaded screw. The chromosomes were fixed either in 2 per cent buffered OsO_4, or 1 per cent neutral formalin, or 70 per cent ethanol, and stained with Heidenhain's haematoxylin for 1 hour or less. By carrying out all the steps without subjecting the chromosomes to any air-liquid interface, it is possible to obtain specimens that appear to have suffered a minimum of distortion.

For electron microscopy, chromosomes were isolated, fixed, and dehydrated as described above, except that the small plastic holder was previously sealed over on one side with a thin formvar film. The chromosomes were transferred to a grid by lowering the chamber, while immersed in absolute alcohol, over a lucite peg supporting a grid. The film carrying the chromosomes thus became firmly attached to the grid while still under absolute alcohol. The grid plus chromosomes was then placed in a special holder for drying according to Anderson's critical point method. The holder consisted of a brass block with circular cavities (3 mm. x 0.07 mm.) for the grids. A thin brass sheet 0.75 mm. thick with holes 6 mm. wide fitting over those in the block was covered with a formvar film and then tightly screwed over the block. Each grid was thus immersed in ethanol in a tiny chamber covered by the formvar film. Diffusion takes place freely across this thin formvar film. The holder was then immersed in amyl acetate and finally sealed into the carbon dioxide bomb and dried as described by Anderson (2).

In making these preparations, formalin was generally used as fixative, since OsO_4 tended to make the chromosomes too brittle.

For microtomy[1], the chromosomes were first iso-

[1]Transverse sections of chromosomes (Fig. 10) were obtained in 1954 with a Spencer microtome modified by C. E. Grey (*J. Appl. Phys.*, 1953, **24**, 113) at the Sloan-Kettering Institute, where one of us (Jean G. Lafontaine) was a Damon Runyon Fellow from 1954 to 1956.

We wish to express our gratitude to Dr. J. J. Biesele and Dr. A. R. T. Denues for providing us with the facilities of the electron microscope laboratory and for their interest in this work.

lated over a formvar film, according to the above technique, and the film attached to a 100 mesh metal screen in such a way that a preselected chromosome would be located over a tiny slot (0.5 x 0.8 mm.) in this screen. This metal support was then placed inside a gelatin capsule so that the chromosome would be oriented with its axis parallel to the long axis of the capsule. Embedding was done as usual in *n*-butyl methacrylate. When the block had hardened and was ready for sectioning, a part of the metal screen was trimmed off so as not to interfere with the actual cutting. The tip of the block was thus reduced to a size of 0.5 mm. x 0.5 mm. or less.

OBSERVATIONS

A. Chromosome "Axis":

At low magnifications the lampbrush chromosome appears to consist of a beaded central axis with a large number of "loops" radiating from it (Figs. 1 and 2). The Feulgen-positive dense granules along the axis are of various sizes and shapes. These are the "chromomeres" of previous authors. They are usually so crowded together that a detailed analysis of the axial structure is not easy. The difficulty of interpreting the beaded appearance of the axis is also often increased by the presence in lampbrush chromosomes of a number of extremely short loops (Fig. 2) which, especially in unstained preparations, may easily be mistaken for particles. In spite of these, it is occasionally possible to observe interchromomeric regions consisting of two strands (Fig. 1) apparently connecting two neighboring chromomeres. Interestingly enough, the diameter of these strands corresponds closely to that of the narrow base of many of the loops. On account of the usual closeness of the chromomeres along the axis, such interconnecting elements are observed to best advantage in chromosomes that have been slightly stretched during their isolation from the nucleus (Fig. 1). In such specimens, one or more breaks may be observed in the axis, but continuity of the chromosome itself is not usually affected by these breaks, since a loop often straddles the gap and is attached to a chromomere on each side.

If a lampbrush chromosome isolated in sucrose is treated for a few minutes with 0.002 M KCN, fixed in osmium tetroxide, and then observed in the phase microscope, structural details become visible even in the dense chromomeres. Though the resolution of the light microscope is not sufficient to give a clear picture, it is evident that chromomeres have a fibrous structure similar to that of loops (Fig. 3). They react, therefore, to KCN (which is a classical reagent used to reveal the coiling in condensed chromosomes) much as do heterochromatic regions in meiotic prophase chromosomes (*cf.* Ris (19)).

In electron micrographs of entire chromosomes prepared with the critical-point method (Figs. 5 and 6) the fine structure of chromomeres is not easily analyzed on account of their highly complex organization. Stereoscopic examination of such preparations reveals indeed that chromomeres are much less simple structures than had previously been thought. The dense bodies which appear to be distributed along the axis are shown, under closer examination, to be regions where strands are twisted in a complex fashion (Figs. 5 and 6). Likewise the apparent density of these structures is seen to result from the superposition of these strands rather than from packing of material into some sort of granule. In Fig. 5, for example, a great deal of the opacity of the chromomeres disappears when examined stereoscopically.

As will be discussed in the next section, loops seem to be continuous with the strands within the chromomeres. It is, therefore, interesting to note that thin sections cut perpendicularly to the axis of isolated lampbrush chromosomes show nothing but randomly arranged profiles of microfibrils (Fig. 10). No special structure can be recognized in the axial region of such sections, and it would thus appear that the fine structure of chromomeres and consequently of the axis can be assumed to be identical to that of the loops.

B. Relation of Loops to the Axis:

Lampbrush chromosomes are generally too dense for one to ascertain the relation of loops to chromomeres and the nature of the connection running from one chromomere to another. However, chromosomes that were stretched during preparation show some interesting features. As noted above, one often finds gaps in the axis which are usually bridged by a loop passing from one chromomere to another. While some loops return to the chromomere near their origin, others connect chromomeres that are easily separated by stretching (Fig. 1). Some are, on the other hand, connected by two distinct short strands (Fig. 1, *ct*).

In electron micrographs (Figs. 5 and 6) many loops are clearly seen to go into the chromomeres, but it is unfortunately impossible to establish the continuity of any given loop with the twisted

strands observed within the chromomere to which the loop is attached.

C. Structure of the Loops:

With the light microscope, the loops of stained lampbrush chromosomes are seen to differ from one another in appearance. They may vary considerably in thickness, show thicker and thinner segments along their length, and often one basal attachment of a loop is observed to be thinner than the other (Fig. 1). In *Necturus*, for instance, some chromosomes have a number of loops approximately two or three times as wide as the others.

Besides these variations in thickness, however, all loops appear to have the same basic morphology. A more or less complex helical structure is visible in the light microscope in certain segments of loops, and the presence of more than one strand is suggested at these places (Fig. 1). This multiplicity of the units constituting the loops is especially well demonstrated by treatment with KCN, which loosens up their structure (Figs. 3 and 4). Suggestion of coiling is then observed in most, if not all of the loops.

Electron microscopic analyses of air-dried chromosomes has indicated that the loops consist of fibrils about 500 A thick. In favorable places this unit may be seen to split into two fibrils, each about 200 A thick (Ris (20, 21, 23)). Air drying causes considerable distortion and therefore Anderson's critical-point method was used in the present study. Stereoscopic photographs of such preparations allow a three-dimensional analysis of the organization of the loops.

The degree of preservation of the fine structure of the loops varies quite widely in different preparations, ranging from specimens in which many of the loops are more or less disorganized (Figs. 6 and 7) to others where loops appear to have suffered to a much lesser extent from the preparation procedure (Figs. 5, 8, and 9).

In the case of loops that have almost fallen apart (Figs. 6 and 7), examination of one picture only of the stereo pair gives the impression that the loop strand has a multitude of small whiskers radiating from its center. At places (Fig. 7), an axial filament in the strand could even be recognized. Stereoscopic observation of these same pictures suggest, on the other hand, that the partly disorganized structure and the whisker-like appearance of the loop strand result from the breaking up of many of their constituting fibrils, the free ends of which are now sticking out laterally.

Although such observations help to understand the organization of the loops, their fine structure may be decided, more convincingly perhaps, from intact preparations. In higher magnification pictures of better preserved loops (Figs. 8 and 9), individual fibrils can be seen more clearly. It is evident that due to the complex arrangements of the units and their extensive overlapping within the loops, individual fibrils within a given segment of a loop are sometimes observed only with difficulty. Nevertheless, well separated fibrils are seen at various places (Figs. 8 and 9). In formalin-fixed preparations, the smallest units seen are approximately 200 A in diameter (Fig. 8), whereas after ethanol fixation, they are about 500 A in diameter (Fig. 9). This latter fixative apparently causes the two 200 A subunits to clump together. Units which appear to be paired are also occasionally observed in formalin-fixed specimens, but they are more readily seen after osmium tetroxide fixation (Fig. 10). Examination of thin sections through isolated lampbrush chromosomes confirms the structure of the loops deduced from electron micrographs of whole chromosomes. Shorter and longer profiles of the microfibrils are visible and, in favorable places, paired units may also be observed (Fig. 10).

DISCUSSION

Structure of the "Axis":

Several different views have been put forward concerning the structure of the "axis" of lampbrush chromosomes. According to Duryee (8), it is made of a single chromonema in which chromomeres are firmly embedded. He believes that this axial filament is enveloped by a gelatinous coating. Tomlin and Callan (25) state that the axis is formed by a single fibril approximately 200 A in diameter. Guyénot and Danon (16) present a somewhat more complex picture of the axis of lampbrush chromosomes. They think that it consists of two chromonemata, 150 A wide, each of which is coated by a thick fibrous layer of acid protein acting as a matrix. The total width of the chromosome axis, according to these authors, is approximately 1600 A. Gall (11, 12) first described the axis to be a single thread less than 1000 A in diameter, but more recently (13, 14) he has concluded that it is formed of two tiny chromonemata, each a few hundred A

units in diameter. Although some of Gall's arguments for the doubleness of the axis seem to originate from Callan's stretching experiments (see Gall (14)), Callan (3, 4), in his latest paper, still believes that the axis of lampbrush chromosomes consists in general of a single chromonema. He does believe, however, that true doubleness occurs in exceptional instances as in certain chromosomes of *Triturus marmoratus*, in which two rows of chromomeres are present. Some of these chromomeres give rise to a single loop, instead of the paired loops that characterize the chromomeres of a single axis.

From the examination of stretched chromosomes in this study, it is clear that each chromosome of the bivalent consists of two chromonemata. However, these chromonemata are not submicroscopic as previously reported (Tomlin and Callan (25); Gall (13, 14)). Their diameter appears to be roughly equal to that of the narrow strand at the base of some of the loops (Fig. 1).

There is as yet no agreement on the nature of the chromomeres. Some workers (Duryee (8); Gall (12)) maintain that they are firmly attached to, or embedded in the axial filament. Others claim that these bodies correspond to deposition of discrete masses of nucleoprotein material over the straight axial chromonemata (Guyénot and Danon (16)). Both Callan (3, 4) and Gall (13, 14) now think that a single submicroscopic thread, continuing into the loops, is tightly coiled in the chromomeres. The hypothesis that chromomeres correspond to regions of the axis where the chromonemata are tightly coiled is supported by our observation that such structures are loosened up and unraveled when unfixed chromosomes are treated with a 0.002 M solution of KCN (Fig. 3). Thus they behave like heterochromatic regions in other prophase chromosomes. Also, with the electron microscope, loops are seen going into chromomeres, and the diameter of the wide strands visible within the chromomeres (Fig. 6) corresponds closely to that of the chromonema forming the loops leading to them. Moreover, in thin transverse sections of isolated chromosomes nothing but random profiles of fibrils similar to those in the loops appear in the axial region (Fig. 10). From thin sections it is impossible to tell how many of these fibrils are present. In view of the apparent continuity of the loops with the chromomeres, it is possible that the chromonemata in the chromomeres contain the same number of microfibrils as do the chromonemata forming the loops. Our observations do not actually demonstrate a linear continuity between individual fibrils in the base of a loop and those in the chromomere to which it is attached, but such a possibility is suggested as an hypothesis. It is evident that more work will be necessary to test it.

As noted above, adjacent chromomeres are occasionally connected along the axis by two strands some 0.1 to 0.2 μ in diameter. In other instances, they are united by a loop only (Fig. 1). Although such cases have not been observed, it is possible that neighboring chromomeres may be connected by only one chromonema along the axis; the second strand of the pair could then form a loop.

If a certain number of chromomeres are connected through loops only, as suggested by previous workers (Callan (3); Gall (13, 14)) and by some of our observations (Fig. 1), the question arises: what gives the axis its continuity, preserved even where the loops are dissolved (Duryee (8); Gall 12))? The answer may be found in the fact that such chromomeres connected by loops are rather easily separated by mechanical means, and perhaps it is the general "stickiness" of heterochromatin which keeps these adjacent chromomeres together.

Structure of the Loops:

Loops of lampbrush chromosomes are usually described as granular in structure (Duryee (8); Gall (11, 12)). In *Triturus pyrrhogaster*, for instance, Duryee (8) believes that they are hyaline cylinders about 1 μ in diameter, with dense particles about 1.5 μ in diameter embedded in them. The majority of the loops in *Triturus viridescens* chromosomes are said to consist of a strand of "tightly packed" granules, each somewhat less than 0.5 μ in diameter. According to Guyénot and Danon (16), the side loops consist of submicroscopic chains or rodlets arranged in a zigzag fashion.

In the different species examined for this study the loops of lampbrush chromosomes prepared by swelling isolated nuclei in 10 per cent sucrose consist of fibrils and not granules. These fibrils are either 200 A (Fig. 8) or 500 A in diameter (Figs. 9 and 10), and the latter sometimes appear to consist of two subunits, each 200 A wide (Fig. 10).

Our observations both with the electron micro-

scope on the arrangement of the bundle of fibrils within the loops and, at the light optical level, on the more or less coiled appearance of parts of loops, before and especially after treatment with KCN, do not agree with the view that loops consist of a single submicroscopic axis on which ribonucleoprotein granules (Gall (13, 14)) or secretion products (Callan (3)) are deposited. The "granules" seen previously in thin sections with the electron microscope (Gall (14)) are interpreted here as sections through the fibrils. These are better observed in whole chromosomes (Figs. 8, 9, and 10).

Recent work has shown that certain loops have a very characteristic appearance (Callan (3); Gall (12–14)). But in the course of this study, evidence was obtained that all loops have the same internal structure. The differences between individual loops and between regions of the same loop are therefore assumed to be accounted for by variations in the degree of packing of the microfibrils and the degree of coiling of the chromonema.

Cytochemical studies indicate that chromomeres and loops are different in chemical composition: chromomeres are strongly Feulgen-positive; loops are Feulgen-negative and consist mainly of protein and ribonucleic acid. In spite of these obvious chemical differences between loops and chromomeres, the electron micrographs obtained show that loops are made of several fibrils that are all similar and resemble, furthermore, the fibrils in the Feulgen-positive chromomeres. Therefore, we suggest that the chromonema, consisting of a bundle of fibrils, may be continuous through chromomeres and loops, but may vary along its length in chemical composition. The complex arrangement of the fibrils within the loops, as demonstrated in stereoscopic preparations, makes it difficult, if not impossible, to follow individual fibrils more than approximately 0.2 μ along the length of a loop. Thus, it is obvious that our observations furnish no information on the continuity of individual fibrils over extended segments of the loops or on the likelihood of their continuity from loops to chromomeres. Nevertheless, some sort of continuity exists at the fibrillar level over short segments of the loops, since the structure of the loops is unraveled by KCN treatment (Figs. 3 and 4), and since, moreover, they do not fall apart completely, even when a large proportion of their 500 A fibrils are broken (Figs. 6 and 7). Serial sectioning might be useful to evaluate the hypothesis as well as other aspects of our model of lampbrush chromosome structure.

Recently, an interesting "core" structure has been described in the prophase chromosome of primary spermatocytes of different animal species (Moses (18); Fawcett (9)), but it is unfortunately not yet clear what this axial structure looks like during the diplotene stage in the species studied. It is, therefore, too early to try to make any correlation between such a "core" and the axial structure in lampbrush chromosomes. One can anticipate that additional information on the structure of diplotene chromosomes in spermatocytes will be useful in understanding the much more complex organization of these same chromosomes in the oocytes.

Neither the structure of lampbrush chromosomes nor the relationship of these structures to the functional role they play in the growth of the oocyte is fully understood at present. We think, nevertheless, that the present work clarifies the structure of the loops, shows the similarities in organization of chromomeres and loops, and also contributes further evidence for the continuity of the chromonemata from chromomeres to loops.

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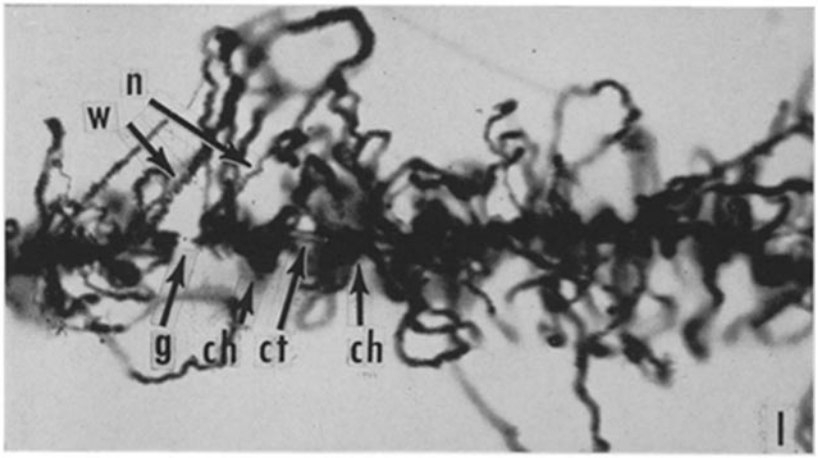
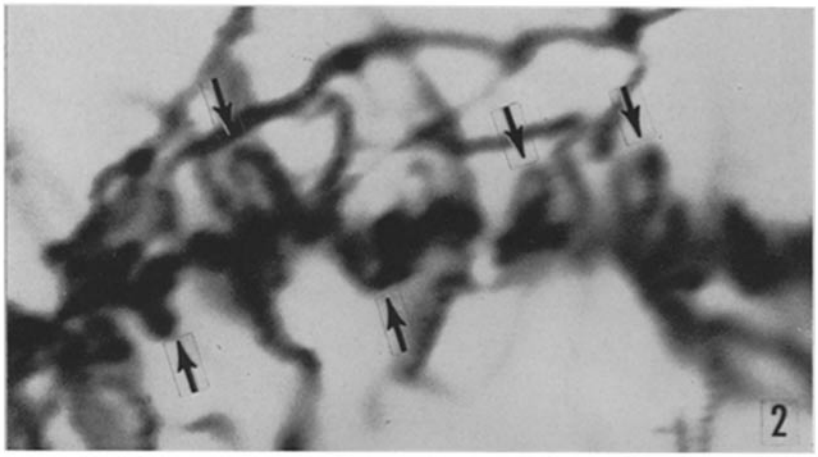
PLATE 43

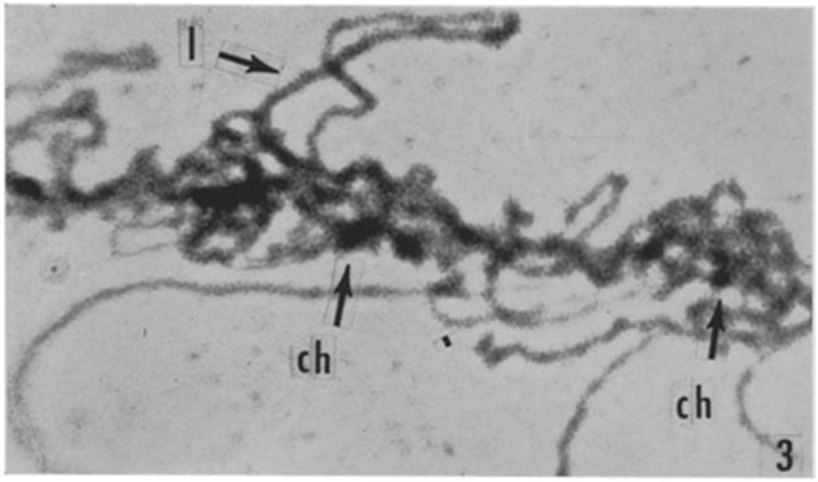
FIG. 1. Light micrograph of chromosome isolated from *Necturus maculatus* oocyte after swelling the nucleus in 10 per cent sucrose. It was fixed in buffered (pH 7.4) 1 per cent osmium tetroxide, stained with Heidenhain's hematoxylin, and mounted in balsam without being subjected to any air-liquid interface. A loop is seen straddling a gap (*g*) in the "axis" of the chromosome. One extremity of this loop is wide (*w*) and shows a coiled structure. The other end (*n*) is much narrower. Note the two chromonemata (*ct*) forming the axis between the chromomeres (*ch*). × 1200.

FIG. 2. *Necturus* chromosome prepared as above. In this specimen, there are fewer long loops, so that the axial region is seen in more detail. Note, for instance, the many extremely short loops (arrows) which can hardly be distinguished from the chromomeres. The arrow at the extreme left most probably points to one of these loops observed edge on. × 4200.

FIG. 3. *Necturus maculatus* chromosome prepared as in Fig. 2 and photographed with phase contrast. Treatment with a 0.002 M solution of KCN "unravels"the chromomeres (*ch*) and loosens the structure of the loops (*l*). Fixed with osmium tetroxide. × 2000.





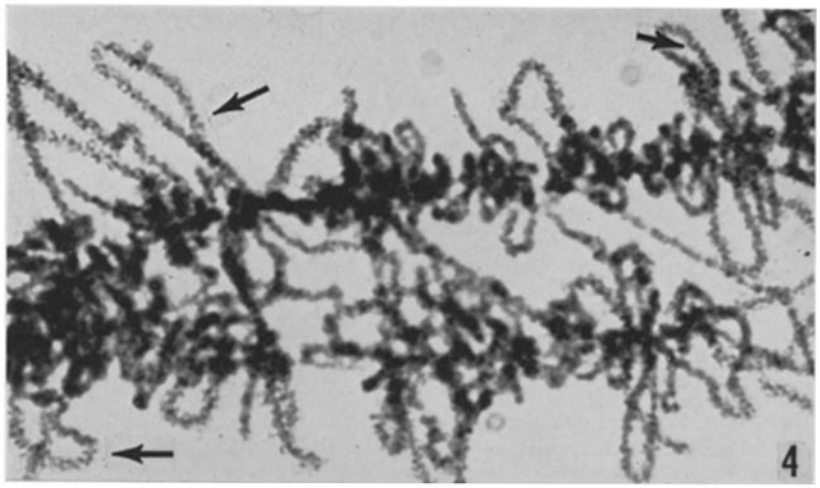


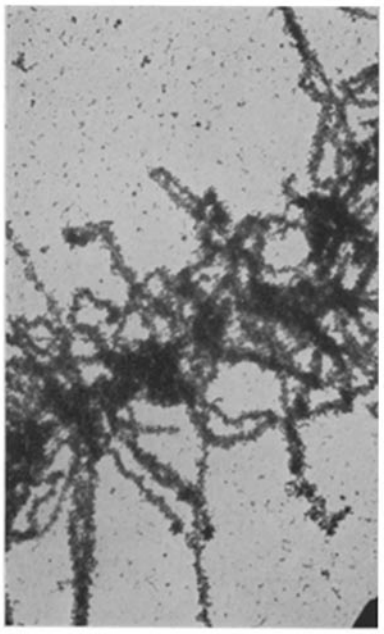
(Lafontaine and Ris: Lampbrush chromosomes)

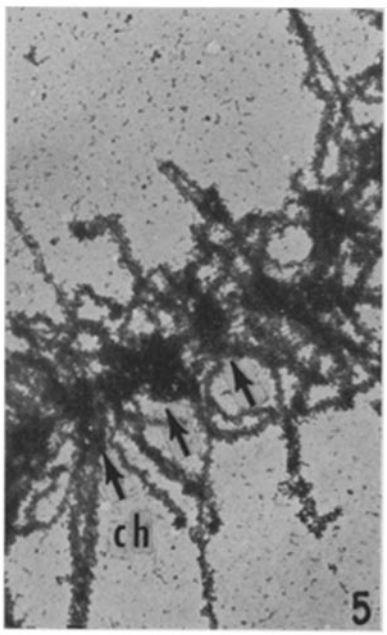
PLATE 44

FIG. 4. *Pseudotriton* chromosome isolated in 10 per cent sucrose, treated for 2 minutes in a 0.002 M solution of KCN, and then fixed in buffered osmium tetroxide (pH 7.4). Specimen stained with Heidenhain's hematoxylin and air-dried. Coiling in the loops is indicated by the arrows. × 2000.

FIG. 5. Stereo electron micrograph of *Triturus viridescens* chromosome isolated as above, but fixed in neutral formalin. For electron microscopy the specimen was prepared by means of Anderson's critical-point method. Note that the apparent density of the chromomeres (arrows) results from superposition of material in the third dimension, and is not due to a real compactness of their structure. The attachment of the loops to the axis is clearly demonstrated. × 3500.





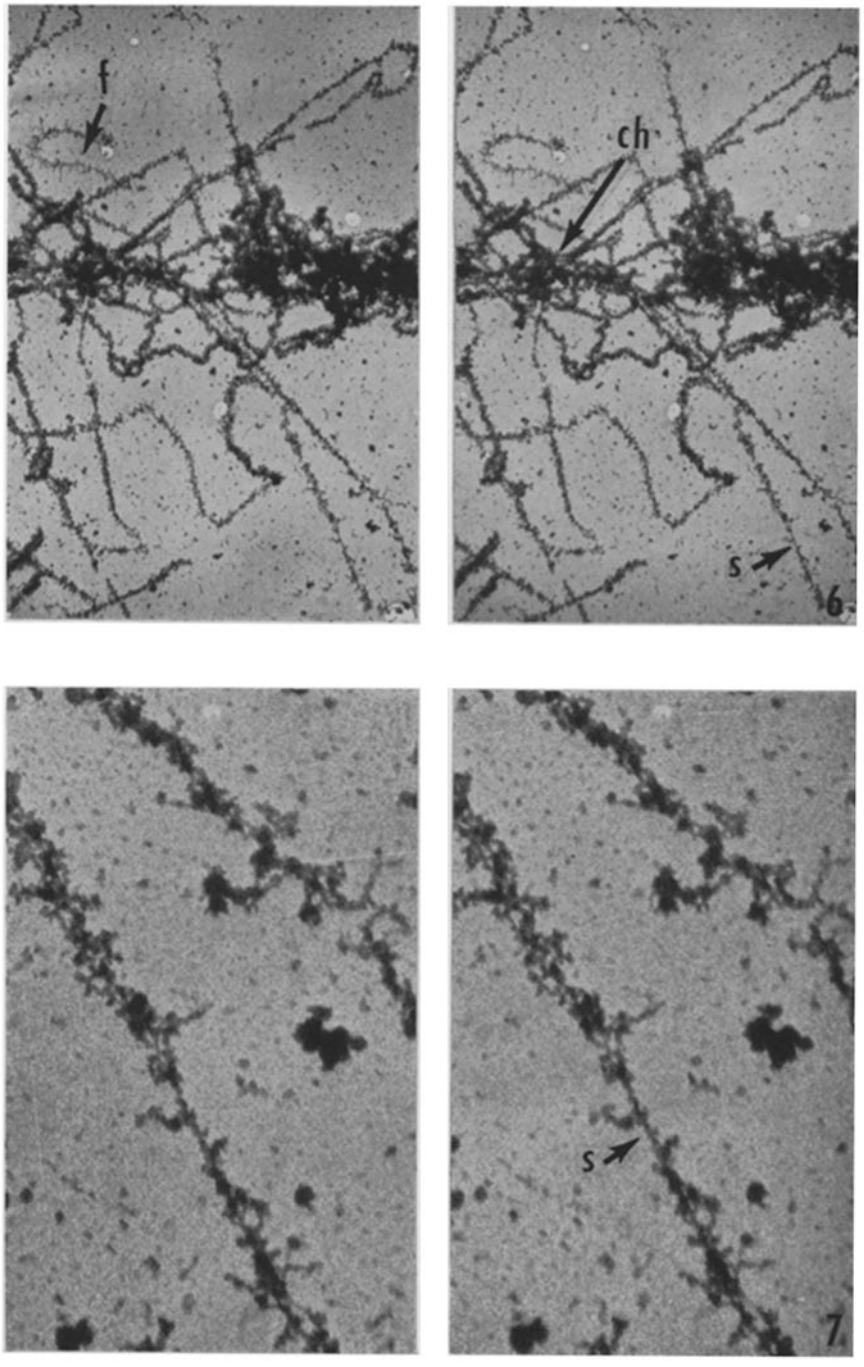


(Lafontaine and Ris: Lampbrush chromosomes)

PLATE 45

FIG. 6. *Triturus viridescens* chromosome isolated in 10 per cent sucrose and fixed in ethanol. The specimen was prepared by means of the critical-point method and photographed stereoscopically with the electron microscope. Fraying of the many fibrils (*f*) of the loops is more easily seen in such preparations. Note the single fibril (*s*) maintaining the continuity of one of the loops. × 2500.

FIG. 7. Higher magnification stereopicture of lower right hand loop in Fig. 6, showing many broken fibrils sticking out from the loops. Continuity of the loop structure seems to be maintained at one place by a single fibril (*s*) some 500 A in diameter. × 24,000.



(Lafontaine and Ris: Lampbrush chromosomes)

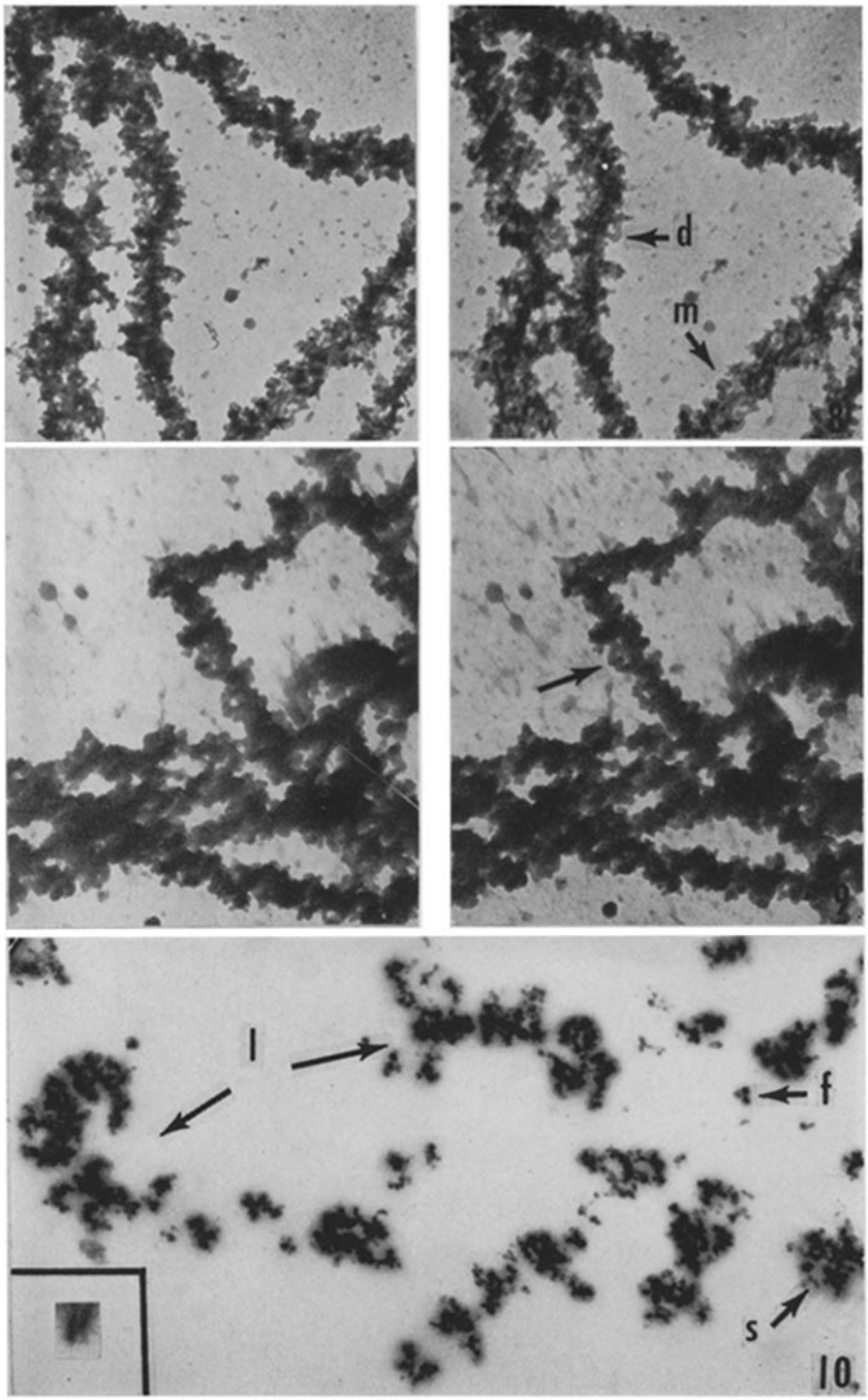
PLATE 46

FIG. 8. Stereomicrograph of *Triturus viridescens* chromosome isolated in 10 per cent sucrose, fixed in neutral formalin and prepared by means of the critical-point method. Note the multiplicity (*m*) of the fibrils in the loops. The fibrils are about 200 A wide, and some of them associate to form a double (*d*), 500 A wide fibril. × 21,000.

FIG. 9. Same as above, but fixed in ethanol. The diameter of the fibrils has doubled and is now approximately 500 A wide. Coiling (arrow) of these fibrils is sometimes suggested. × 22,000.

FIG. 10. Cross-section of *Triturus viridescens* chromosome. Specimen isolated in 10 per cent sucrose, fixed 3 minutes in 1 per cent buffered (pH 7.4) osmium tetroxide, stained 1 hour with Heidenhain's hematoxylin, and embedded in *n*-butyl methacrylate. More or less oblique sections of loops (*l*) appear at the left. Many profiles of fibrils about 500 A wide (*f*) and others approximately 200 A in diameter (*s*) are found in the cross-sections of the loops. × 20,000.

FIG. 10 (insert). Oblique section of a 500 A wide fibril, showing pairing of two subunits, each approximately 200 A in diameter. × 50,000.



(Lafontaine and Ris: Lampbrush chromosomes)