Chromosoma (Berl.) 30, 269—286 (1970)

Whole Mount Electron Microscopy of Meiotic Chromosomes and the Synaptonemal Complex*

David E. Comings and Tadashi A. Okada
Department of Medical Genetics, City of Hope Medical Center, Duarte, California

Received October 3, 1969 / Accepted March 11, 1970

Abstract. The mechanism by which homologous chromosomes pair and crossover has been a major unsolved problem in genetics. Thin section electron microscopy of the synaptonemal complex has not provided enough details to allow any significant insight into this problem. Whole mount preparations of the testis of mice, quail, crayfish, and frogs provided a striking improvement in visualization of the morphological features of meiotic chromosomes. These studies, when combined with the use of deoxyribonuclease and trypsin allowed the following conclusions. 1. The synaptonemal complex (lateral and central elements with connecting L-C fibers) is composed of protein. 2. Contrary to common speculation the central element is not the pairing surface of homologous chromosomes. 3. The L-C fibers, averaging 75—100 Å in width, extend from the lateral elements and meet to form the central element which is usually composed of four fibers. 4. During leptotene, homologous axial elements, although unpaired for most of their length, attach next to each other at the nuclear membrane. 5. Short segments of the chromatin fibers attach to the lateral elements. These points of attachment are clustered, producing the chromomeres seen by light microscopy. 6. The chromatin fibers extend out from the lateral element as loops. Lampbrush chromosomes are thus not restricted to oogenesis but are common to all meiotic chromosomes.

Since the morphological features of the central element of the synaptonemal complex persist despite extensive deoxyribonuclease digestion, pairing is perhaps best visualized as a two-step process consisting of a) *chromosomal pairing* during which the proteinaceous synaptonemal complex pulls homologous chromosomes into approximate association with each other, and b) *molecular pairing*, which probably takes place in the area around the synaptonemal complex.

Introduction

The mechanism by which two homologous chromosomes pair and cross over remains a major unsolved problem in genetics. The discovery of the synaptonemal[1] complex (Moses, 1956) provided a structure which has served as a focus for morphological investigation of this problem. This tripartite complex is formed during zygonema in association with pairing of homologous chromosomes and consists of two lateral elements and a single central element. The extensive literature on the synaptonemal

* Supported by NIH Grants GM-15886 and C-2568, and The Charles and Henrietta Detoy Research Fellowship.

1 This spelling has been adopted at the proposal of Moses (1969).

complex has recently been reviewed (Moses, 1968). Some of the conclusions concering this complex are as follows: (a) It is a regular synaptic structure joining homologous bivalents along their lengths. The lateral elements constitute the central axis of homologous chromosomes. (b) Crossing over requires the presence of the synaptonemal complex but the synaptonemal complex does not insure that crossing over will coccur. (c) The complex may serve to provide highly specific pairing and a structural framework for recombination.

Although electron microscopy of thin sections of meiotic chromosomes have provided a great deal of information concerning the structure of the synaptonemal complex, so many important details are missing that it is virtually impossible to construct a reasonable model to explain the interrelationship between the complex and the chromosome fibers, or to explain the mechanism for homologous pairing and crossing over. Just as whole mount electron microscopy has added significantly to our knowledge of mitotic chromosome structure so may it add to our knowledge of meiotic chromosome structure and the synaptonemal complex. On previous occasions when this had been attempted (Ris and Chandler, 1963; Wolfe and John, 1965; Wolfe and Hewitt, 1966) the synaptonemal complexes were not observed. In the present study, however, they were observed and a significant improvement in the visualization of their morphological features was provided. The use of deoxyribonuclease showed that following the complete removal of chromatin fibers the synaptonemal complex persists and the lateral and central axes are composed of protein. This indicated that contrary to previous speculations the central axis probably does not represent the pairing surface of the two homologous chromosomes.

Materials and Methods

Whole Mount Preparations. The testes of the mouse (White Swiss Ha/ICR), Japanese quail (*Coturnix coturnix japonica*), frog (*Rana pipiens*), and a species of crayfish (*Cambarus*) were used. For the mouse and quail, the tunica was removed and the tubules placed in Gay's solution at room temperature. They were then cut into small pieces and dispersed into a single cell suspension by repeated pipetting. This concentrated suspension was then placed directly on a distilled water trough and after uniform spreading, the cells were picked up on EFFA bar grids coated with formvar and carbon. The grids were then either treated as below, or if untreated, they were placed in 2% uranyl acetate for 10 minutes, rinsed in distilled water and then passed through a graded series of ethanol washes ending with two 10-minute washes in amyl acetate. The grids were then either air-dried or dried in the critical point apparatus of Anderson (Anderson, 1951). Some specimens were shadowed with platinum-carbon.

Enzyme Treatments. After being picked up from the water surface some of the cells were treated by placing the grids on the following different types of solutions: (1) RNase (Sigma) 25—50 μg/ml and RNase T_1 (CalBiochem) 15—25 units per ml,

in NaCl 0.14 M, tris 0.01 M, EDTA 0.01 M, pH 7.6 (NET) at 37° C for 5—10 minutes. Both ribonucleases were first heated to 80° C for 10 minutes to destroy residual DNase. (2) DNase (Worthington) 25—50 μg/ml in 0.14 M NaCl, 0.01 M tris, 3×10^{-3} M magnesium chloride at pH 8.6 (NMT) at 37° C for 5—60 minutes. (3) 0.01% trypsin in NET at 24° C for 5—30 minutes. Following these treatments the grids were processes as described above.

Thin Sections. Testes of the mouse, quail, crayfish and frog were fixed in 2.5% glutaraldehyde at 4° C for 1 hour, rinsed for 30 minutes with phosphate buffer, pH 7.2, fixed with 2% osmic acid for 1 hour, rinsed for 30 minutes with buffer, and stained in 5% uranyl acetate for 1 hour, and then passed through a graded series of ethanol washes into propylene oxide and embedded in Epon. Sections were cut at 600 Å, stained with uranyl acetate and lead citrate and examined in an Hitachi HU-11 B electron microscope.

Results

I. Water Spread Preparations — Untreated

In our first attempt to use the water spread technique to study meiotic chromosomes, we utilized mouse testes and the results were gratifying. A low power view of a typical pachytene cell is shown in Fig. 1. Multiple bivalents can be seen with chromatin fibers spreading laterally from them. The ends of the synaptonemal complexes attach to the nuclear membrane as has been noted in thin section studies. A higher magnification of a typical pachytene bivalent in the mouse is shown in Fig. 2. Both lateral elements can be seen as well as the densities at the ends of the complex where it was associated with the nuclear membrane. The chromatin is diffusely dispersed around the synaptonemal complex. In most preparations all of the typical characteristics of the

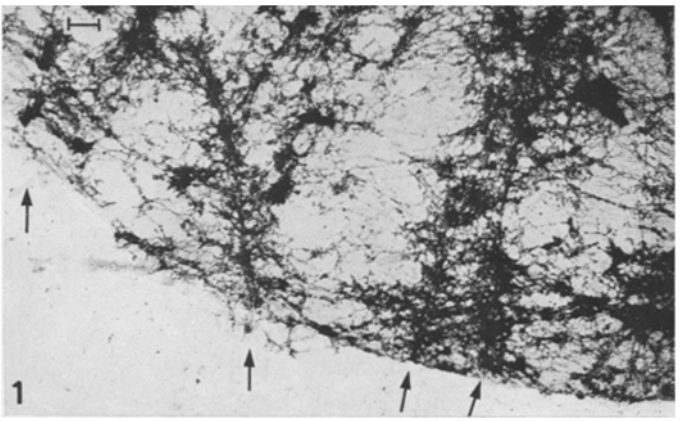

Fig. 1. A water spread preparation of a mouse pachytene cell illustrating the numerous points at which the ends of the synaptonemal complex are attached to the nuclear membrane (arrows). The marker = 1.0 μ

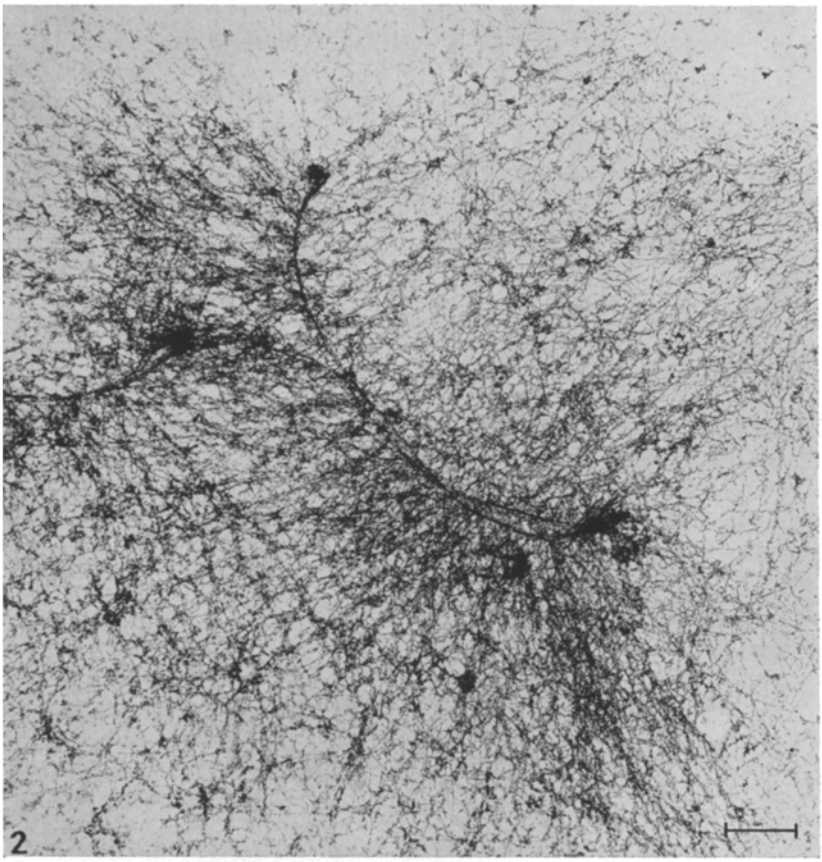

Fig. 2. A higher magnification of a bivalent from a water spread preparation of a mouse pachytene cell. The two lateral elements are readily visible. The ends of the synaptonemal complex are thickened where they were attached to the nuclear membrane. The marker = 1 μ

synaptonemal complex were readily observed (Figs. 3—4). Fig. 3 illustrates the relationship of two synaptonemal complexes to each other with chromatin fibers extending between them. To demonstrate that the technique was not unduly disruptive a thin section preparation of the complex was mounted at approximately the same magnification next to a view of the complex prepared by the water spreading technique

Fig. 3. Two prominent and two partially obscured synaptonemal complexes from a quail testes. The central element can be readily seen. The chromatin fibers form a lace-like network between the two synaptonemal complexes. The marker = 0.5 μ

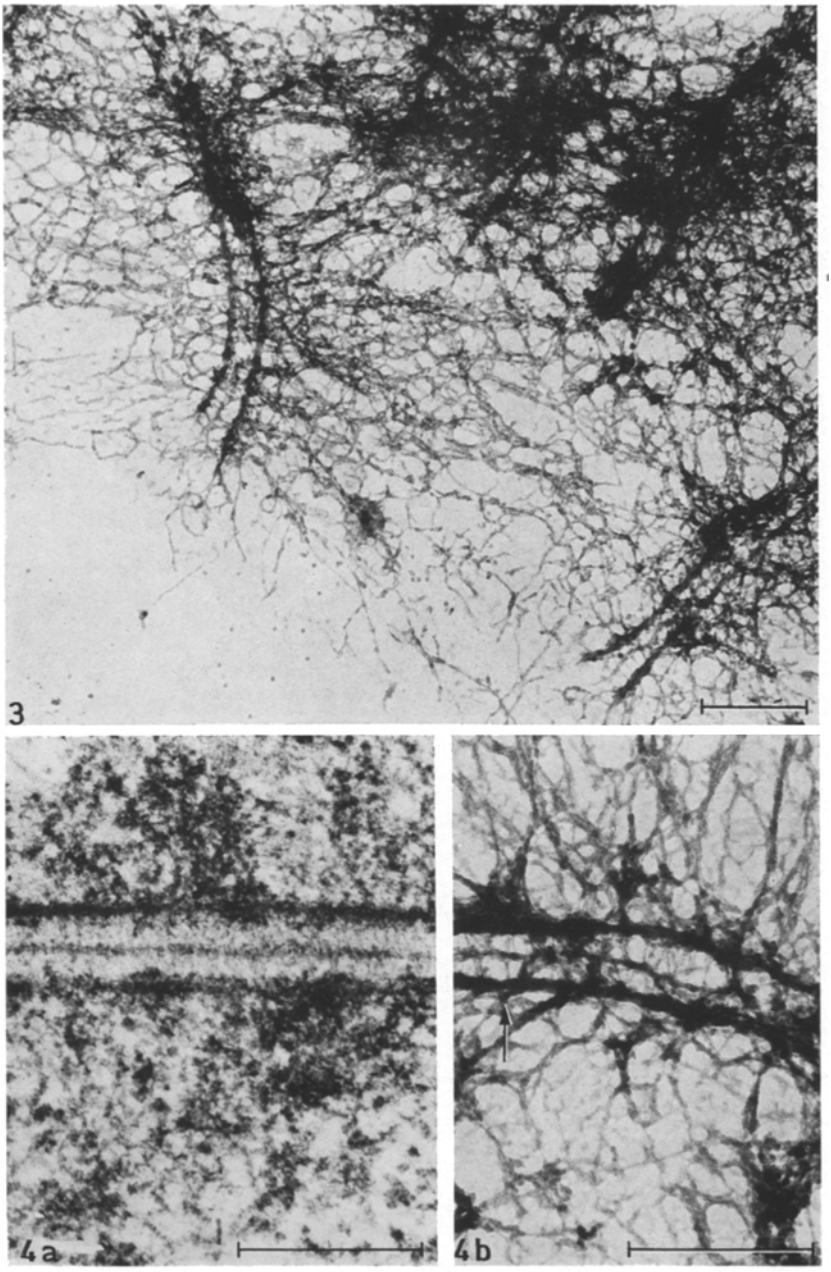

Fig. 4. a A thin section, and b water spread preparation of a synaptonemal complex from mouse testes each at approximately the same magnification. There is an increased resolution of structures and better appreciation of their relationship to each other in the water spread material. The L-C fibers passing between the lateral and central element can be seen (arrow in Fig. 4b). Approximately $\times 49{,}000$, the marker $= 0.5\mu$

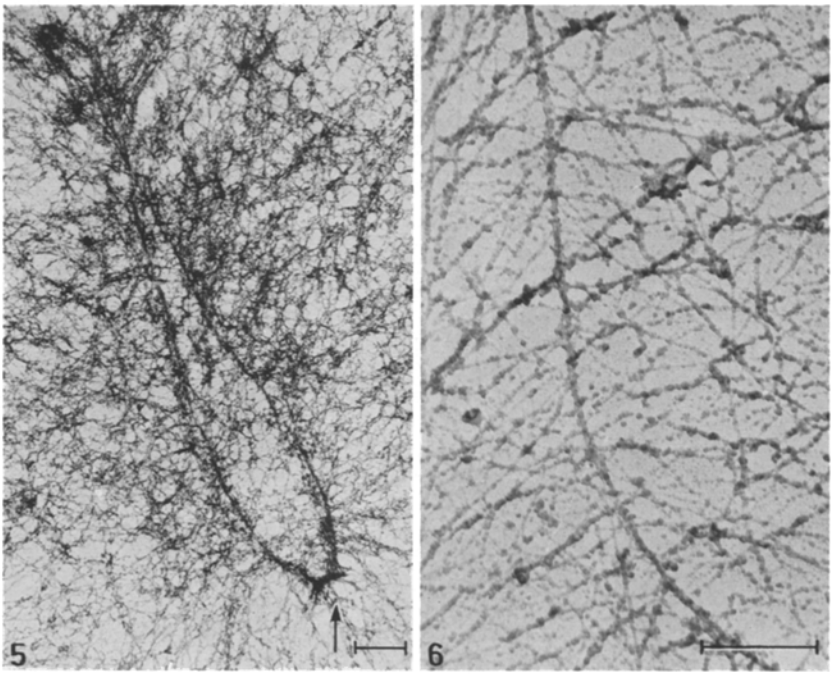

Fig. 5. A synaptonemal complex with the lateral elements separated but retaining their attachment to a common area of the nuclear membrane (arrow). The marker = 0.5 μ

Fig. 6. The axial filament (lateral element) in a quail leptotene cell passing through a field of chromatin fibers. The marker = 0.5 μ

(Fig. 4a, b). It can be appreciated that the water spreading technique gives a more detailed view of the interrelationship between the different elements involved. The fine fibers which pass from the lateral to the central element can be seen (Fig. 4b, arrow). In the rest of the paper these will be referred to as the *L-C fibers*. Despite the increased clarity of these water spread preparations it was still difficult to unambiguously ascertain the relationship between the chromatin fibers, the synaptonemal complex, and the nature of the central element. We felt that this might be resolved by using much smaller chromosomes. Quail testes were found to fall apart into a single cell suspension very readily and the cells contained a large number of micro-chromosomes. Unfortunately, despite their smaller size and a considerable reduction in the amount of chromatin around the synaptonemal complex of the micro-chromosomes, the interrelationship of the above factors was still not apparent.

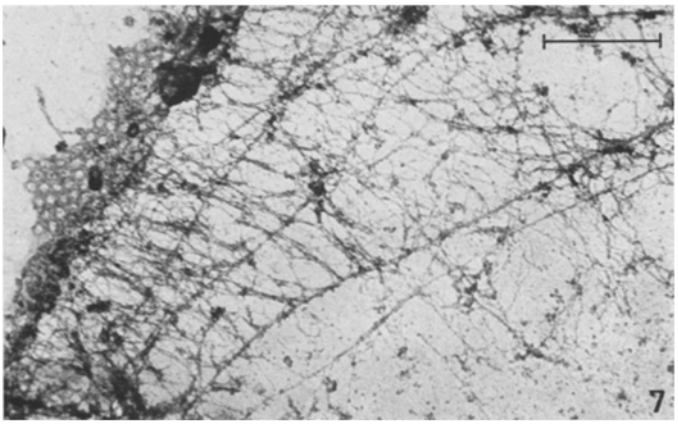

Fig. 7. A quail leptotene cell demonstrating the attachment of the chromatin fibers to both the axial element to the nuclear membrane. The marker = 1.0 μ

As seen below, the use of deoxyribonuclease resolved many of these questions.

In some cells, perhaps because they may have been at a very early stage in the formation of the synaptonemal complex, the two lateral elements were readily pulled apart (Fig. 5). The central element was not present, and the nuclear membrane plate was apparent. Fig. 6 illustrates the axial element (lateral element) in a leptotene cell. It passes through a field of chromatin fibers and the association of the chromatin fibers with it appears to be brief.

The nuclear membrane was found to be associated not only with the ends of the synaptonemal complex but also with the chromatin fibers which looped out from the axial elements. This was well shown in Fig. 7 in which the chromatin was associated on one side with the axial element, and on the other side with remnants of the nuclear membrane.

II. Deoxyribonuclease Studies

The Proteinaceous Synaptonemal Complex

Despite the use of micro-chromosomes of birds we were unable to definitively determine the structure of the synaptonemal complex, especially in relationship to the central axis. A number of investigators have attacked this problem by the use of DNase treatment (Nebel and Coulon, 1962) or by selective staining (Coleman and Moses, 1964; Brinkley and Bryan, 1964) of thin sections. It was concluded that the lateral elements were composed of protein and DNA while the central element was free of DNA. Moses and Coleman (1964), however, hedged

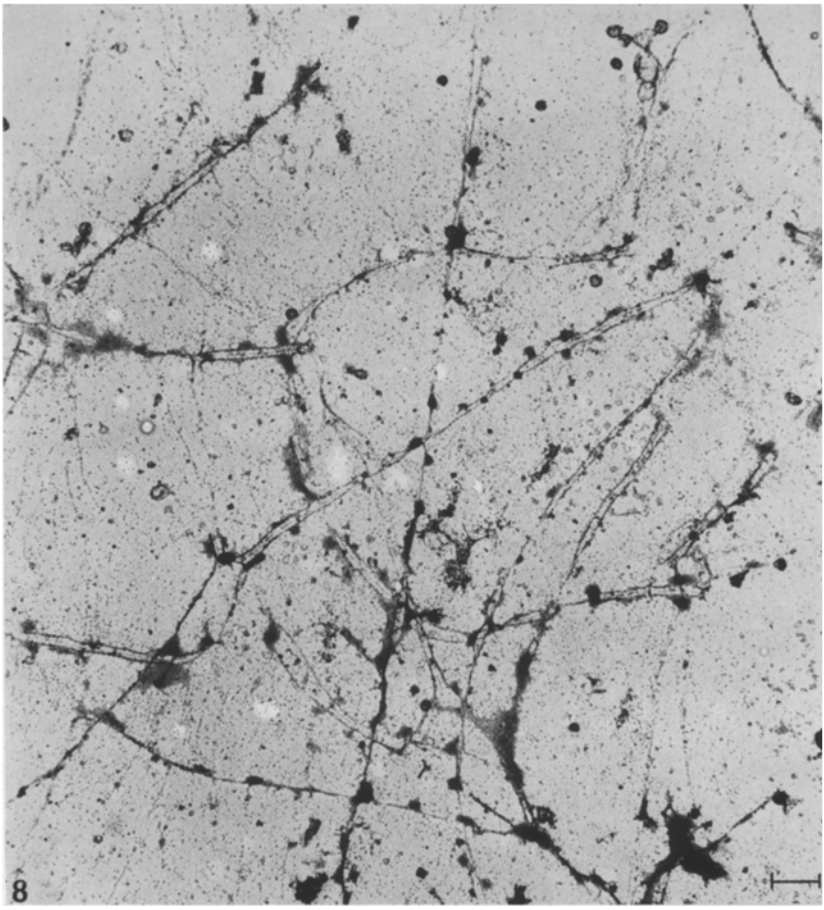

Fig. 8. A quail pachytene cell which has been treated with DNase. Only multiple criss-crossing synaptonemal complexes remain. The marker = 1.0 μ

their bets on this point by stating that because of low contrast an indium chloride stained 30—50 Å filament would be barely visible, and felt that the L-C fibers and the central element did contain DNA as well as protein. This continued to leave room for the hypothesis that the central element represents the pairing surface of homologous chromosomes (Moses, 1968). The distinct advantage of the use of DNase in conjunction with the water spread technique is that the enzyme digestion can take place prior to fixation of the cells and at a stage when the cells are ruptured and very thinly spread upon a grid, thus allowing the degradation products to be easily washed away. These studies demon-

strated that the lateral elements, the central element, and the L-C fibers of the synaptonemal complex are resistant to DNase. They were, however, digested with trypsin thus confirming their proteinaceous nature. Fig. 8 shows a low power view of a quail pachytene cell following DNase digestion. The many criss-crossing synaptonemal complexes can be readily seen after the removal of most of the chromatin fibers. Higher power views of the resulting complexes are shown in Figs. 9 through 11. The dimensions of the different components of the synaptonemal complex after DNase digestion are listed in the Table. The DNase affected neither the attachment of the synaptonemal complex to the nuclear membrane nor the structure of the nuclear membrane itself. Thin section views of embedded material have frequently shown that the lateral element appears to consist of two parts, a less dense medial portion and a more dense lateral portion. Comparison of this with a partially DNase digested synaptonemal complex suggests that the less dense medial portion viewed in thin section material probably corresponds to the protein component of the lateral element of the synaptonemal complex, while the more dense lateral material is chromatin (Fig. 10). The synaptonemal complex of mouse testicular cells following

Table. *Measurements of components of the synaptonemal complex after deoxyribonuclease digestion (in Å)*

	Quail			Mouse		
	$\bar{X}$	n	S.D.	$\bar{X}$	n	S.D.
L-C fiber width[a]	75	15	21	97	10	21
Central element width	125	14	49	195	9	64
Lateral element width	321	13	69	412	10	77
Distance between lateral and central element	670	30	136	696	16	117
Distance between inner portions of lateral elements	1,525	26	228	1,685	13	277
Distance between outer portions of lateral elements	2,308	17	370	2,565	8	291
Distance between adjacent L-C fibers	165	25	60	303	6	64
Distance between 1st and 3rd L-C fibers	640	22	106	767	6	167

[a] The difference in width of the L-C fibers between quail and mice may be the result of differences in the two preparations rather than a true difference in fiber width.

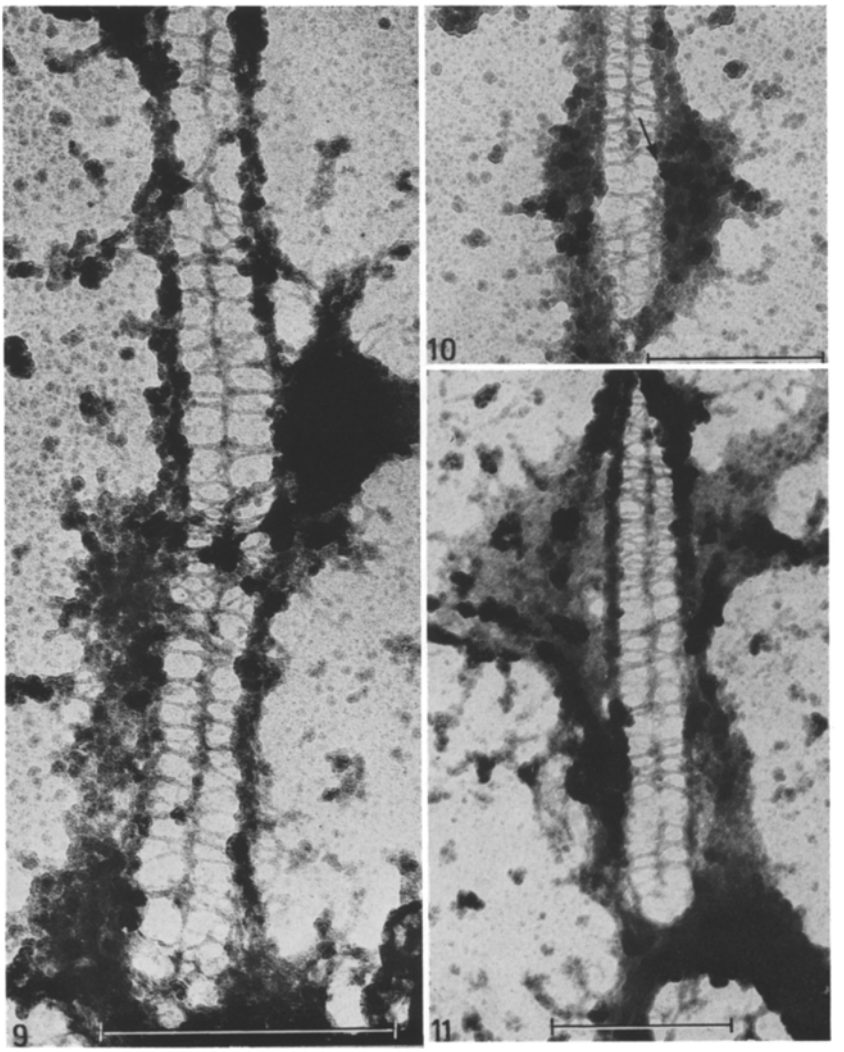

Fig. 9. The synaptonemal complex from a quail treated with DNase. Most of the chromatin has been removed and only the synaptonemal complex with its L-C fibers and the central and lateral elements remain. The marker = 0.5 μ

Fig. 10. A water spread DNase treated preparation from a quail. The less dense medial component (arrow) is the proteinaceous lateral element, and the more dense lateral portion is incompletely digested chromatin. The marker = 0.5 μ

Fig. 11. A water spread preparation of the synaptonemal complex from the mouse treated with DNase. The marker = 0.5 μ

DNase treatment was similar to that seen in the quail (Fig. 11). The L-C fibers were frequently seen to be associated in pairs. In the Table two sets of distances were measured, that between adjacent paired L-C fibers, and that between first and third fibers. These measurements of the spacing of the L-C fibers along the complex varied from preparation to preparation depending upon the degree of aggregation of the fibers and should be taken as only an estimate. The central element was continuous with the L-C fibers, and was invariably composed of at least two and usually four fibers.

The Association of Homologous Axial Elements at the Nuclear Membrane during Leptotene

The term axial element will be used to refer to the state of the lateral element before it becomes paired. Fig. 12 shows two homologous axial elements in a leptotene cell identified as such because the axial elements are present but the synaptonemal complexes have not yet formed. It is apparent that at this stage the homologous axial elements are associated with each other at their ends by attachment close together on the nuclear membrane. This type of configuration was observed many times in leptotene cells. A comparable observation can be made by light microscopy. Thus in Fig. 13, which is a light microscope view of a leptotene cell, the arrows point to places at which the axial elements appear to be associated with each other at their ends, presumably at a site of their attachment to the nuclear membrane. This type of configuration obviously has important implications in regard to the manner in which the unpaired axial elements are able to find and associate with each other during the formation of the synaptonemal complex.

Urea, Hydrochloric Acid, and Ribonuclease

The use of unfixed cells in water spread studies allowed the examination of the effect of different solutions on the structure of the synaptonemal complex. For example, three questions could be investigated: (a) Is the synaptonemal complex composed of a basic protein? (b) Do the L-C fiber loops hold the lateral elements together by hydrogen bond forces? (c) Is RNA a significant component of the synaptonemal complex? The use of specific staining procedures led Moses and Coleman (1964) and Wolstenholme and Meyer (1966) to conclude that the synaptonemal complex was composed, at least in part, of a histone-like, arginine-rich, basic protein. When cells from a quail testis were water spread, picked up on grids, treated with DNase and then placed in 0.2 M HCl for 5 minutes, the lateral elements were observed to readily dissociate from each other. In the same manner, when they were treated

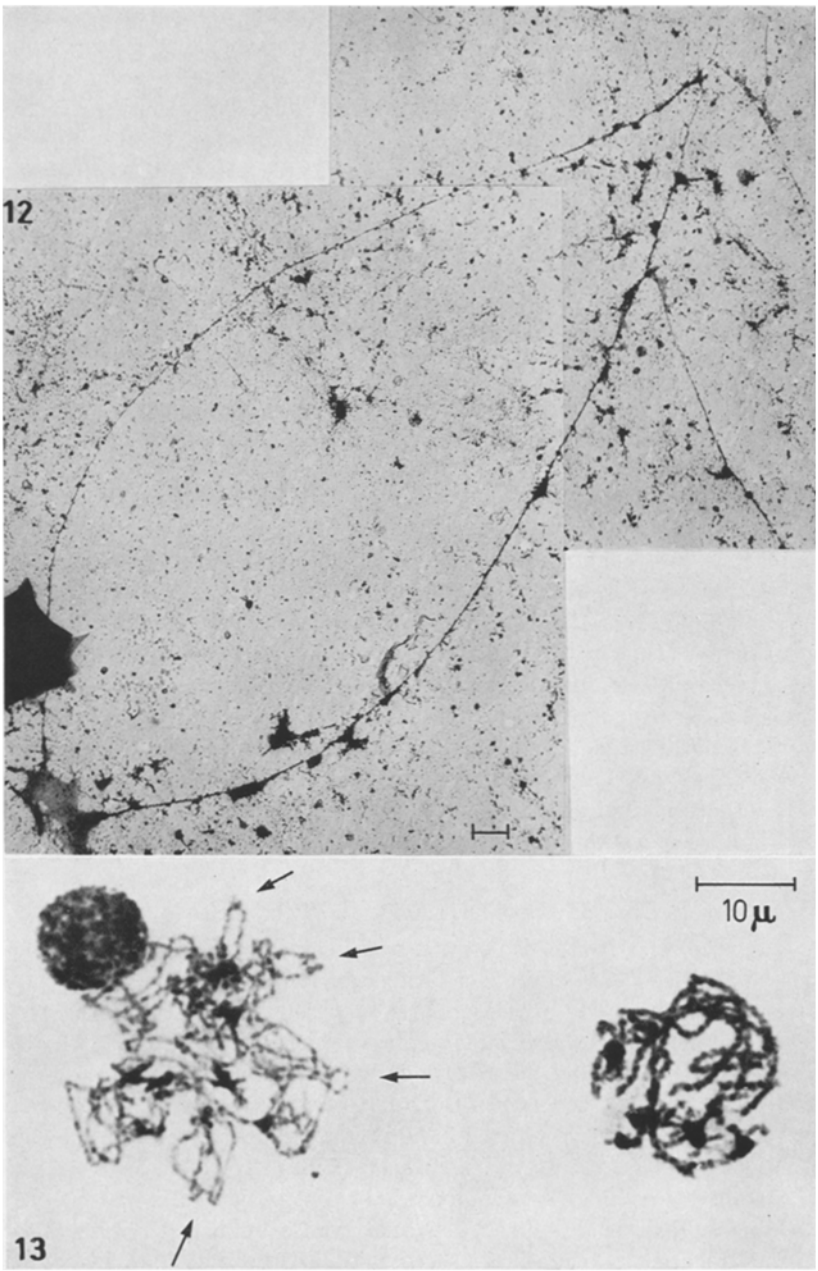

Fig. 12. A water spread preparation of a mouse leptotene cell teated with DNase. The homologous axial elements are unpaired but both ends are attached close to each other on the nuclear membrane. The marker = 1.0 μ

with DNase and then placed in 8 M urea for 5 minutes, the lateral elements dissociated from each other, and the L-C fibers as well as parts of the lateral fibers tended to disappear. Although more work involving a greater number of different solutions is necessary before definitive conclusions can be drawn, these findings suggest that the synaptonemal complex may be composed of a basic protein and that these elements may be bound together by hydrogen bond forces.

When pachytene cells of the mouse were exposed to DNase and then to RNase (see Methods), the synaptonemal complex looked no different than it did following exposure to DNase and then NET. RNA thus does not seem to be a significant component of the synaptonemal complex. This is in aggreement with studies on thin section material (Coleman and Moses, 1964).

Chromomeres

The DNase digestion studies frequently showed residual lumps of chromatin attached to the axial elements. It seemed logical to assume

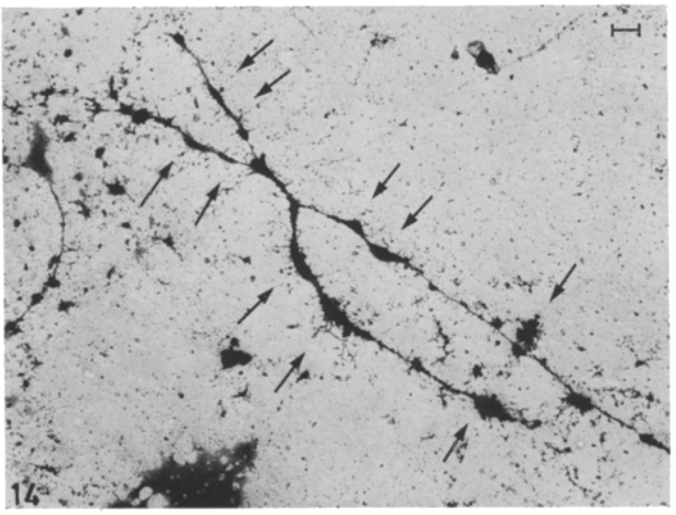

Fig. 14

Fig. 13. A quail leptotene cell comparable to that in Fig. 12 as seen by light microscopy. The arrows indicate the points of association of the ends of the axial elements at what presumably represents the nuclear membrane. On the right is a pachytene cell after pairing has been completed

Fig. 14. A mouse meiotic cell treated with DNase illustrating lumps of residual chromatin attached to the lateral elements. The suggestion that these represent chromomeres or sites of dense binding of chromatin to the lateral element is borne out by this photograph which illustrates that on these homologous lateral elements chromomeres form at homologous sites (arrows). The marker = 1.0 μ

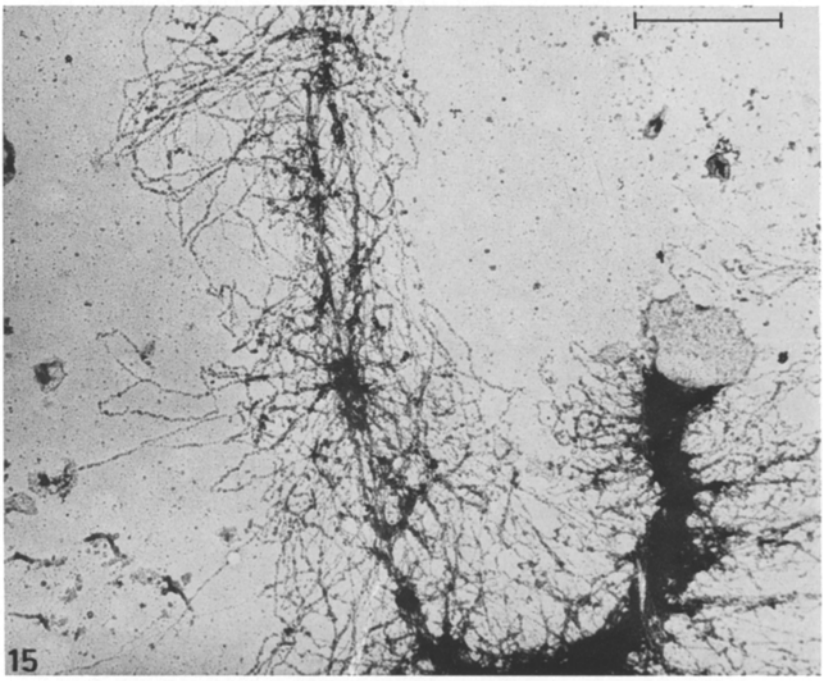

Fig. 15. A water spread preparation of a crayfish pachytene chromosome treated with RNase. The chromatin fibers branch out in loop-like arrangements similar to lampbrush chromosomes. The end of the bivalent can be seen to be associated with a remnant of the nuclear membrane. The marker = 1 μ

that these might be areas where a greater density of chromatin fibers was attached to the axial element and that as such they represented the chromomeres which are so easily seen by light microscopy. This conclusion is borne out by Fig. 14 which shows that two homologous axial elements have homologous areas of increased concentrations of residual undigested chromatin. Similar aggregations of chromatin fibers can be seen in crayfish pachytene chromosomes (Fig. 15).

III. Ribonuclease Studies

Lampbrush Chromosomes in Spermatogenesis

In an attempt to obtain still smaller chromosomes and hopefully get a better appreciation of the relationship of chromatin fibers to the synaptonemal complex, we looked at meiotic cells of the crayfish. Nuclei of crustaceans contain only a fraction of the DNA present in higher organisms (Mirsky and Ris, 1951) and at the same time crayfish

have over 200 chromosomes (Makino, 1956), thus making the DNA content of each chromosome extremely small. We found that in these studies the best visualization of the configuration of the chromatin fibers was obtained in cells which had been treated with RNase (see Methods). The lampbrush-like configuration of the chromatin fibers in these pachytene chromosomes is shown in Fig. 15. The fibers extend out and loop back upon themselves. The longest of the lateral loops measured 7 microns. They appeared to be composed of only a single chromatin fiber.

Discussion

These studies of whole mount preparations of meiotic chromosomes allow the following conclusions:

1. The synaptonemal complex (lateral and central elements with connecting L-C fibers) is composed of protein.

2. The central element, contrary to common speculation, is probably not the pairing surface of homologous chromatin. It is composed of protein and is resistant to deoxyribonuclease.

3. The L-C fibers, averaging 75—100 Å in width, extend from the lateral elements and meet to form the central element, which is usually composed of four fibers.

4. During leptotene, homologous axial elements (lateral elements) although unpaired for most of the their length, attach to each other at a common site on the nuclear membrane.

5. Short segments of the chromatin fibers attach to the lateral elements. The attachment sites may occur in clusters to produce the chromomeres visible by light microscopy.

6. The chromatin fibers extend out in a loop-like configuration from the synaptonemal complex. The lampbrush type chromosome is not restricted to oogenesis but is a configuration common to spermatogenesis as well.

The most striking implication of these conclusions is that since the central element is not a pairing surface for chromatin then molecular pairing must take place elsewhere and the synaptonemal complex serves only to bring homologous chromosomes in the greater proximity with each other. Pairing seems best conceived as a two-step process consisting of (1) *Chromosomal pairing* during which homologous chromosomes are brought in close approximation with each other by the synaptonemal complex, and (2) *Molecular pairing* or the intimate pairing of homologous DNA bases which leads to recombination and production of chiasmata.

Once relieved of the conceptually difficult task of providing a mechanism for intimate molecular pairing, the synaptonemal complex seems superbly suited to the task of providing only chromosomal pairing.

Sometime after DNA replication and before leptotene the axial elements are synthesized. The observation (Fig. 12) that homologous axial elements attach near each other at the nuclear membrane suggests that these elements may begin synthesis together at a common site on the nuclear membrane. When this process is completed the leptotene state would exist with axial elements unpaired except at the nuclear membrane. In some organisms where this is particularly marked, it would correspond to the bouquet stage. During zygotene the L-C fibers are synthesized, and in some fashion pull together the two axial filaments (lateral elements). A detailed knowledge of the structure of the synaptonemal complex will be essential to an understanding of this mechanism of chromosomal pairing. We are presently utilizing stereo-electron micrographs of the synaptonemal complex in an attempt to obtain better insight into its structure. To date they suggest that the L-C fibers extend out from the lateral elements as loops and associate to form the central element.

The relegation of the synaptonemal complex to the task of merely bringing the homologous chromosomes into a site-by-site or chromomere-by-chromomere approximation with each other leaves the question: Where and how does base specific molecular pairing take place? After the synaptonemal complex has pulled the homologous chromosomes together the concentration of homologous DNA would be significantly greater than that which is necessary to produce efficient hybridization of DNA in vitro (Hoyer *et al.*, 1964) and it seems logical to assume that recombination may take place in the area around the synaptonemal complex by the same mechanisms proposed for recombination in fungi, bacteria and bacteriophage (Thomas, 1966; Whitehouse and Hastings, 1965; Holliday, 1968).

Studies of a *Lycopersicon esculentum*(tomato) — *Solanumlycopersicoides* hybrid indicate that synaptonemal complexes occur between homologous chromosomes but that chiasmata do not form (Menzel and Price, 1966). This is consistent with the proposal that chromosomal pairing is relatively non-specific and thus succeeds while molecular pairing and recombination, which depends upon precise homology of DNA strands, fail.

Although many thousands of cells were scanned we did not find any that were obviously in diplotene. Two factors that presumably contributed to this are that pachytene is a long stage while diplotene is relatively short, and the pipetting may have ruptured the nuclear membrane of these cells and caused them to be dispersed. There was no evidence for synaptonemal complexes or lateral elements in spermatids.

The morphological findings of the structure of the synaptonemal complex following DNase digestion, and other considerations, suggest

that the primary function of the synaptonemal complex is to pull homologous chromosomes together and that it plays no role in molecular pairing of DNA strands. The following corollaries would thus be expected.

1. The synaptonemal complex should serve to increase the efficiency of recombination but some recombination should be able to occur in its absence.

2. The presence of the synaptonemal complex would not insure crossing-over since the integrity of many enzymes would be necessary for molecular pairing and recombination to occur.

The observations which have accumulated concerning the role of the synaptonemal complex in these phenomena are reviewed in the recent paper by Moses (1968). None of them contradict the above corollaries. Recombination can occur in the absence of the synaptonemal complex and the presence of the complex does not insure recombination. These whole mount studies also confirm the assumptions (Moses, 1968) that any bivalent has one and only one synaptonemal complex, and that the synaptonemal complex occurs along the entire synapsed length of the bivalent.

Note Added in Proof: Recent studies using stereo-electron microscopy of the quail synaptonemal complex following careful DNase digestion, indicate that the L-C fibers as measured here actually consist of two fibers each approximately 45 Å in width. The fibers emerge from the lateral element as a series of loops that fuse in the center to form the central element. These studies also show that the lateral element is composed of two filaments. One row of fibers loops emerges from each filament (Comings, D. E., and T. A. Okada,: Stereo-electron microscopy of deoxyribonuclease treated whole mount preparations of the synaptonemal complex, submitted). All of these observations have been synthesized into an hypothesis on the mechanism of chromosomal pairing (Comings, D. E., and T. A. Okada: A mechanism of chromosome pairing during meiosis, submitted).

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Dr. D. E. Comings
Department of Medical Genetics
City of Hope Medical Center
Duarte, Calif. 91010
U.S.A.