THE STRUCTURE OF THE CENTRAL REGION IN THE SYNAPTONEMAL COMPLEXES OF HAMSTER AND CRICKET SPERMATOCYTES

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ABSTRACT

The fine structure of bivalents from golden hamster and house cricket spermatocytes has been studied with a whole mount surface-spreading method combined with negative staining. The elements of the synaptonemal complex show detail of structure which is absent in other preparative procedures. The transverse filaments found in the central region of the synaptonemal complex from both species are straight and have a similar width, 1 6–1 8 nm These filaments occur mainly in bundles The central element differs in architecture in the two species In hamster bivalents it is formed of longitudinal stretches of filaments 1.6–1 8 nm wide and a small amount of an amorphous material similar to that of the lateral elements In the cricket, the central element contains transverse fibrils which are continuous with the transverse filaments of the central region, and an amorphous material lying mainly along the sides of the central element All of the components of the central region of the synaptonemal complex are resistant to pancreatic DNase. The overlapping ends of the transverse filaments, together with additional protein material, make up the central element The widespread occurrence and close morphological and histochemical interspecies similarities of the transverse filaments indicate that they serve an essential role, probably one concerned with holding synapsed bivalents together via the lateral elements. Restrictions placed by the observations reported here on current models of the synaptonemal complex are discussed.

INTRODUCTION

The synaptonemal complex (SC) (Moses, 1969) is a structure composed of two longitudinal lateral elements (LE) and an enclosed central region, which contains a longitudinal central element (CE) that is joined to the LE's by thin transverse filaments (TF) (see reviews by Moses, 1968, 1969; and by Sotelo, 1969). The bivalents of mammals, exemplified by the hamster, and those of certain insects as typified by the house cricket, *Acheta domesticus*, respectively represent two extremes of organization of the central region Both contain fine transverse filaments bridging the space between lateral and central elements. However, the CE in the cricket is conspicuous in having a complex, highly ordered structure (Guenin, 1965; Schin, 1965, Sotelo and Wettstein, 1965), while that of the mammal is neither prominent nor so highly organized Underlying these differences presumably are certain common structural features which a rigorous comparison between the two types would be expected to reveal. It follows that any model of

the structure or function of the SC that is to be generally applicable must accord with such general features, rather than with special ones. This note presents more detailed information regarding the size, shape, and number of transverse filaments in relation to the CE, as seen in negatively stained, surface-spread bivalents of the two species. Evidence that in both cases the filaments are composed of a protein that is distinguishable from the bulk protein of the lateral and central elements is presented and discussed.

MATERIALS AND METHODS

The species used were the golden hamster, *Mesocricetus auratus*, and the house cricket, *A. domesticus*. The spreading technique used in this work has been previously described (Solari, 1972). Seminiferous tubules of hamster testes are freed from the tunica albuginea and a small piece of tissue is chopped on a wax plate with a razor blade. A needle is dipped into the chopped tissue and immediately touched to the clean surface of a 0.45% NaCl solution in water. Carbon-coated, Formvar-covered grids are touched to this surface and then floated for 30 s on a 0.5% solution of uranyl acetate in water (deionized). The edge of the grid is then touched to filter paper to drain the uranyl acetate and to leave a thin film that is air-dried, resulting in staining of the material by negative contrast. Cricket testes are dissected and soaked in insect Ringer solution. Groups of follicles are cut near the ductal end, the ducts are removed, and the follicles chopped in a small drop of insect Ringer. A small portion of this drop of suspended tissue is placed on the tip of a triangle-shaped piece of slide, and the adhering material is then rubbed with another glass piece, the glass piece is then touched to a clean surface of a 0.9% NaCl solution in deionized water. The material spread on that surface is recovered on grids and stained as described above.

Pancreatic DNase (Sigma Chemical Co., St. Louis, Mo., electrophoretically purified) 50 μg/ml is dissolved in 0.9% NaCl plus 2.5 mM $MgCl_2$ in deionized water. Incubations are carried out at 37°C for 2 and 3 h.

Single micrographs and stereo pairs (tilted $\pm 6°$ from the horizontal plane) are taken using a Philips 200 electron microscope (Philips Electronic Instruments, Mount Vernon, N. Y.). Measurements are made on prints with the aid of a calibrated magnifier.

RESULTS

The Central Region of the SC in Hamster Spermatocytes

The central region of the SC measures 130 nm in width, and shows transverse filaments, axial (longitudinal) filaments, and patches of amorphous material similar to that found in the LE's (Fig. 1). These structures are better shown after DNase digestion, which clears the central region from the overlapping chromatin that otherwise obscures the structures proper to this region.

TABLE I

Widths in Ångstroms of the Transverse Filaments and Related Fibers in Cricket and Hamster Spermatocytes

Source	Average Width	SD	*N*
	Å	Å	
Hamster, DNase-treated transverse filaments	16	3	20
Cricket, DNase-treated transverse filaments	16	4	20
Cricket, untreated transverse filaments	18	5	17
Free filaments from cricket spermatocytes	18	4	20

The transverse filaments have an average width of 1.6 nm (see Table I). These filaments are generally straight and cross the space between the LE and CE at different angles with respect to the axis of the bivalent. The filaments cannot be followed after they reach the CE because, at that point, a fuzzy material is attached to the end of the filaments (Fig. 1). However, it is possible to observe that most of the filaments do not go beyond the CE (Fig. 6). The number of TF's per unit length of the LE's is variable. In many regions, 50–80 TF's/μm of SC length can be counted.

Longitudinal filaments (or filamentous pieces) are axial in the CE. Short stretches, 50–100 nm in length, can be followed (Fig. 1), but it is not possible to show that they are continuous throughout the CE. The average width of these axial filaments is the same as that of the transverse filaments (Fig. 1). In some regions of the CE, two or three axial filaments are seen parallel to each other.

The transverse and longitudinal filaments, as well as the bulk of the LE's and the patches of amorphous material in the central region, are resistant to DNase (Fig. 6). However, it has been found that the transverse and longitudinal filaments are removed by digestion with chymotrypsin and pronase (Solari, 1972).

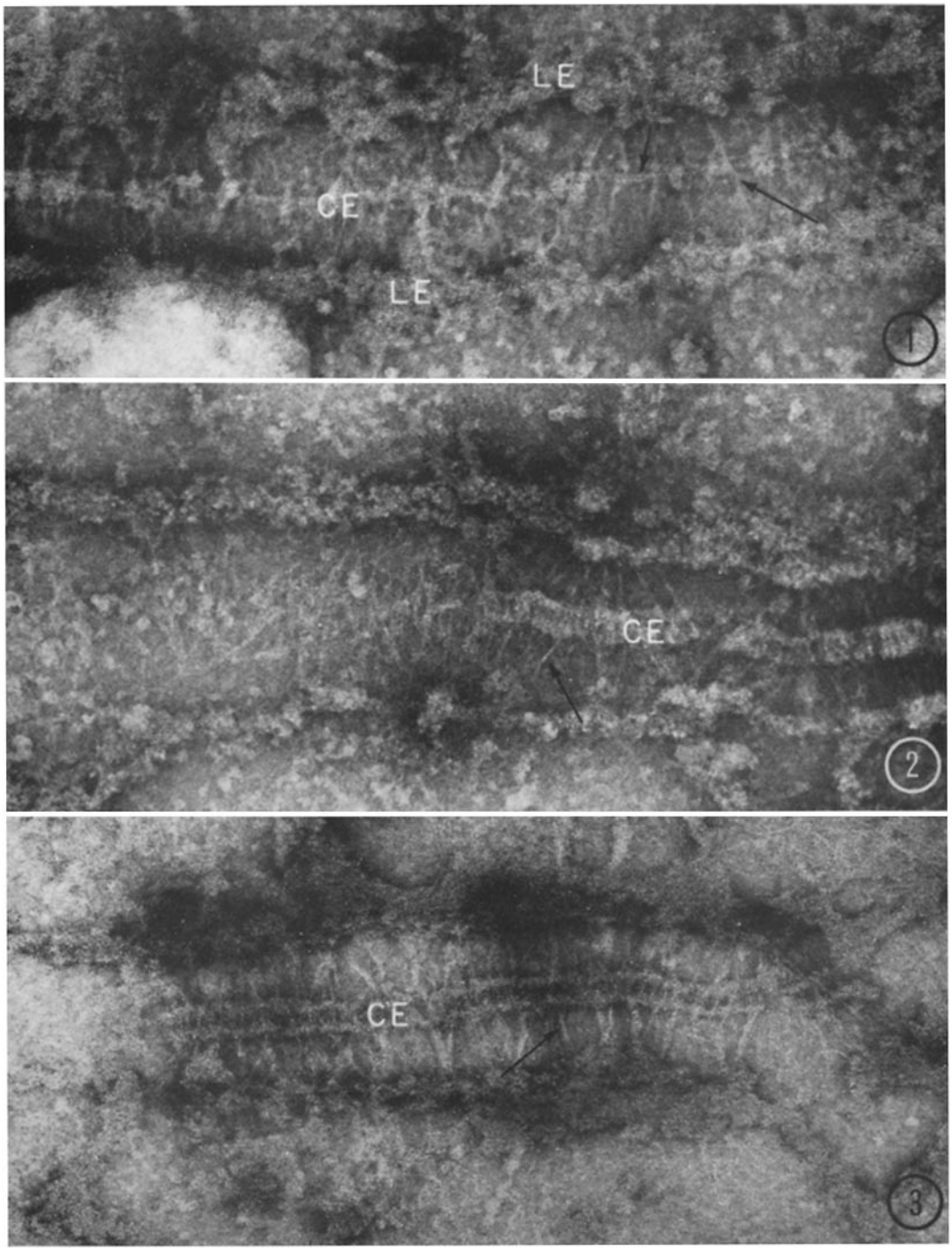


FIGURE 1 Bivalent of golden hamster spread on 0.45% NaCl, digested with DNase for 2 h, and negatively stained with uranyl acetate. *LE*, lateral element; and *CE*, central element of the synaptonemal complex. Transverse filaments (long arrow) join the LE's to the CE Stretches of longitudinal filaments (short arrow) run along the CE. × 150,000.

FIGURE 2 Bivalent of cricket, untreated. The central element (*CE*) is more prominent and becomes disorganized at the left side. The transverse filaments (arrow) are present both in the places where the CE is intact and in regions where it is disorganized. Uranyl acetate, negative stain. × 150,000.

FIGURE 3 Bivalent of cricket digested with DNase for 2 h. The central element (*CE*) and the transverse filaments (arrow) remain undigested. The CE is bounded at the sides by denser regions and contains transverse fibrils. × 150,000.

The Central Region of the SC in Cricket Spermatocytes

The central region of the SC from cricket spermatocytes measures 110 nm in average width; it is clearly seen in untreated bivalents because the sparsely distributed and condensed chromatin that surrounds the central region does not overlap it at many places. The CE is more prominent than that of hamster spermatocytes (Fig. 2) and measures 28 nm in average width.

The CE is bounded at each side by a linear density which is especially prominent in DNase-digested preparations (Fig. 3), but no evidence has been obtained that these densities contain axial filaments. The CE contains transverse filaments that are continuous with the TF's of the central space (Fig. 5) and that bridge the gap between the two lateral densities of the CE. However, these bridging filaments are not strictly parallel to each other, and thus the ladderlike aspect is distorted as compared with that seen in sections.

The TF's of the central region have an average width of 1.6 nm in the preparations digested with DNase (Fig. 5), which is almost the same (1.8 nm) as in the untreated preparations (Fig. 4). Single TF's can be seen in many places along the SC, but they are generally grouped in bundles containing two to five filaments (Figs. 4 and 5). Stereoscopic pairs of micrographs reveal that patches of material traversing the central region are often bundles of filaments. When the bundles are resolved into individual filaments, each filament has the 1 6-nm average width. The bundles themselves show some imperfect periodicity in usually being spaced 10–20 nm apart.

The number of TF's per unit length of the SC is variable. Regions in which the CE is well-preserved show 120–250 filaments/μm of SC length In some places where the CE is disrupted, the TF's run at higher tilting angles (Figs. 2 and 7), and occasionally they seem to cross the whole central region. TF's may exist in undiminished quantities, even in the absence of a complete CE (Fig. 2), and, conversely, an occasional piece of CE may not show any TF attaching to one of its sides (Fig. 6). It is evident that TF's do not alone make up the CE, and that some additional material takes part in its formation.

In some places where the SC is extensively disrupted, thin fibers similar to the TF's appear in the background surrounding the SC (Fig. 8). The average width of these fibers is 1.8 nm, similar to the filaments, but they may be longer (150 nm) than the diameter of the central region The TF's, the CE, and the free filaments in the periphery of the cricket bivalents are resistant to DNase digestion.

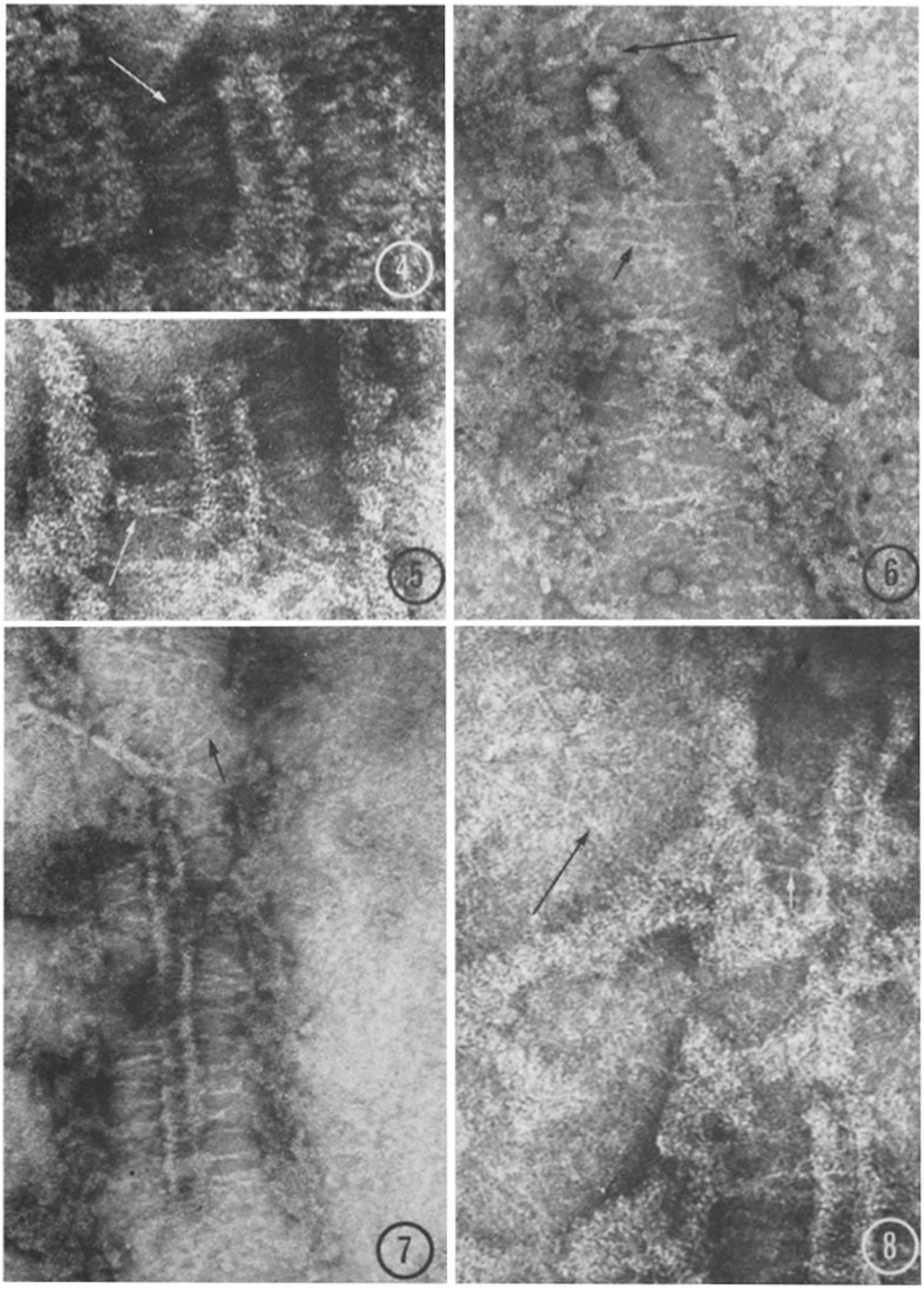
DISCUSSION

The Structure of the Central Region

Previous cytochemical studies on thin sections have shown that the CE does not contain DNA as a major constituent (Coleman and Moses, 1964, Westergaard and von Wettstein, 1970). In agreement with these previous data, Comings and Okada (1970, 1971) showed that the central region of the SC from quail and mouse spermatocytes spread on water and dried with the critical point method contained DNase-resistant, fibrillar material On the other hand, the observations of Comings and Okada on the fine structure of the CE differ markedly from those obtained with negative staining of spread preparations as shown in the present results and in those of Solari (1972).

In the present study, DNase-resistant, straight filaments have been shown to cross at least part of the central region in the bivalents of both cricket and hamster spermatocytes A remarkable similarity in the width (1.6–1.8 nm) of these filaments in both species and their similar properties (resistance to DNase, insolubility in high concentrations of salt, and sensitivity to protease [Solari, 1972]) suggest that such filaments are formed by a similar class of protein in these two and possibly other different species Although the width of these filaments is extremely small, there are other instances of protein filaments similar in size. Thus, tail fibers from T_4 bacteriophage are 2.0 $\times$ 130 nm in size, and are formed of protein units with a minimal molecular weight of 100,000 (Brenner et al , 1959). Furthermore, tropomyosin B aggregates show pairs of filaments, 1.0–1.5 nm each in width, and it has been suggested that they represent single alpha helices or pairs of coiled alpha helices (Huxley, 1963).

The width of the TF's reported here agrees with that reported by Wettstein and Sotelo (1971) for the TF's in sectioned cricket bivalents These widths, however, as well as the morphology of the filaments, do not agree with the 4 5 nm pairs of so-called "LC fibers" forming loops at the medial plane described by Comings and Okada (1970,



FIGURES 4 and 5 Untreated (Fig 4) and DNase-digested (Fig 5) bivalents of a house cricket Bundles of transverse filaments (arrows) can be observed both in the untreated and in the digested bivalents. Single filaments have approximately the same width (16–18 Å) in both preparations. Uranyl acetate, negative stain. × 300,000.

FIGURE 6 DNase-digested hamster bivalent. Some transverse filaments (short arrow) do not go beyond the CE. A fuzzy material (long arrow) is found in the places where the transverse filaments attach to the CE. Uranyl acetate, negative stain. × 150,000.

FIGURE 7 DNase-digested cricket bivalent. The transverse filaments (arrow) are found in places where the CE is disrupted, as well as where it is intact Uranyl acetate, negative stain. × 150,000.

FIGURE 8 DNase-digested cricket bivalent partially disrupted during the spreading before DNase digestion. Transverse filaments (short arrow) are seen in the central region of the synaptonemal complex and free, similar filaments (long arrow) are found in the outside regions. Uranyl acetate, negative stain. × 300,000.

1971) in the mouse and Japanese quail. On the basis of previous observations on the effect of ethanol dehydration (Solari, 1971, 1972) and the present evidence, it is reasonable to assume that these transverse "LC fibers" represent bundles of clumped 1.6–1.8-nm filaments.

The structural pattern of the central region of the SC cannot be reconstructed on the exclusive basis of spread preparation, as the structures are flattened and stretched initially during the surface spreading. Sectioned material has shown a high degree of order in the CE in some species (Sotelo, 1969; Moses, 1968; King and Akai, 1971; and Roth, 1966). Especially pertinent to the present results is the pattern found in the cricket (Sotelo and Wettstein, 1965, Sotelo, 1969, Wettstein and Sotelo, 1971). Sections through the CE in a median or sagittal plane show an ordered array with two periodicities: one 10-nm axial spacing and a lesser, but distinct, antero-posterior periodicity The spread preparations, because of their intrinsic distortion, do not, at the present time, permit any firm conclusions about this pattern Nevertheless, periodic stacking of the filamentous bundles described here is most probably responsible for the axial, 10-nm repeat pattern. While the three longitudinal parallel lines seen in thin sections of the CE from cricket have no exact counterpart in our spread preparations, the dense material flanking the CE in the latter could represent the outermost two, but the middle one is missing in both undigested and DNase-treated specimens, having evidently been either lost or dispersed in the spreading process.

The Structure of the Central Region and Models of the SC

Evidence to date points to the synaptonemal complex as participating intimately in the close pairing of meiotic chromosomes, and in the events surrounding crossing-over. Although various studies have suggested ways in which the SC might function, neither the causal role, if any, nor the detail of how the SC actually participates has been established. A number of extensive schemata have been presented, however, to account for participation of the SC in effective pairing and crossing-over, based on observations restricted to one or a few biological forms or technical procedures (Moens, 1968, on *Lilium*, King, 1970, and King and Akai, 1971, on *Drosophila* and *Bombyx*; von Wettstein, 1971; Westergaard and von Wettstein, 1970, on the fungus *Neottiella*; and Comings and Okada, 1970, 1971, on surface-spread whole mounts of bivalents from various genera) Probably as a consequence of such limitations, the models are not in agreement, though some have significant points in common. In all, the CE and associated TF's play a key role, though there is no agreement as to whether it concerns holding homologues together, providing the locus for molecular recombination, or both In fact, there is even inconsistency over the detailed structure of the central region among the models mentioned and also with the architectural scheme proposed by Sotelo and Wettstein (1965). Thus, the selection or devising of a model that is broadly applicable requires resolution of discrepancies by improving techniques and applying them to a range of contrasting SC variations While the present limited observations cannot serve to test any of the models that have been proposed, they do at least put constraints on the extent to which these models may be generalized to other organisms.

Comings and Okada (1971) proposed that the transverse filaments are projections from the LE's that loop to the CE and return to the LE, the CE is thus said to be formed by the alignment of the loops. Solari (1972) has shown that the TF's in negatively stained mammalian bivalents spread in saline do not, in fact, form loops, and that the CE contains additional material of protein nature These observations are borne out by the present evidence on *Acheta*. Furthermore, a simple, planar (or double-planar) model of the central region, such as the one proposed by Comings and Okada (1971), does not fit with the finding of bundles of TF's lying in different planes, as reported in this paper, and with the total number of filaments per unit length of the bivalent.

The model proposed by von Wettstein (1971) and based on observations on the fungus *Neottiella* has pairing specificity residing in a tandem series of DNA segments located in the LE. Recombination is said to occur in loops of chromatin that enter the central region and make contact with homologous loops from the opposite side. No specific reference is made to the transverse filaments in this model The postulated DNA-containing loops can hardly correspond to the TF's in mammals and in *Acheta* because they are not recognizable as loops and their DNase resistance is evidence against their being chromatin.

King (1970) and King and Akai (1971) also

propose hypothetical pairing segments in the chromatin of the LE, though, in contrast to von Wettstein's model, each segment is nonspecific in King's model. The transverse filaments play a precise role: they are the nonspecific binding units (zygosomes) that attach to the pairing segments (synaptomeres) through their uncoiled proximal ends, and overlap in the axis of the central region by their coiled distal ends to form the CE and to keep the homologues closely spaced Our observations agree with theirs on *Bombyx*, on which the model is based, with respect to the diameter of the TF's (2 nm compares with our values of 1.6–1 8 nm) and the number of TF's per micrometer of SC (420 per bivalent agrees with 120–250 per homologue in the cricket). However, the model is not supported in that neither the transverse filaments of the hamster nor those of the cricket show any evidence of coiling at their distal ends, and the filaments are straight, even in the disorganized regions of the CE Furthermore, while the ends of the TF's may, in fact, overlap in the region of the CE, they are not the only structures that comprise it. In both the hamster and the cricket, additional protein material is present, evidently variable in amount and nature in different species.

The above models appear in various ways to be derivatives of the one proposed earlier by Moens (1968) on the basis of his observations on *Lily*. Here, the transverse filaments are envisioned to be non-DNA strands that project laterally from the LE's and interdigitate across the central space to form the CE, and thus to establish and maintain the correct pairing distance between homologues. As in King's model, there is no provision for additional material, which, according to our observations, makes up the bulk of the CE, and most probably accounts for its scalariform appearance in *Acheta*. Our evidence in both hamster and cricket is, however, consistent with the interlocking of TF's as a spacing and holding device. This is most evident where the nonfilamentous bulk material of the CE is lost, leaving the interdigitating TF's intact (e.g., Figs. 2, 6, and 7). The fine linear filaments sometimes seen by Moens to be axial to the CE in *Lily* have counterparts in the hamster, but apparently not in *Acheta*, where either they have been lost, or they are obscured by the CE material. In any case, quite apart from the question of whether they are of general occurrence, when they are present, they are DNase resistant. Our evidence is thus inconsistent with Moens' hypothesis that they represent strands of DNA from opposite homologues, capable of genetic exchange.

The less speculative suggestions of Sotelo and Wettstein (1965), Sotelo (1969), and Roth (1966) are in general agreement with the present evidence. However, as pointed out above, certain features of the CE, such as the medial axial density, are not conspicuous in the spreads, and the elaborate path of the transverse filaments when they reach the CE, pictured by Sotelo (1969), has not been confirmed.

In summation, our observations give weight to what is becoming the prevailing view that the transverse filaments are nongenetic subunits that serve to align and join homologous chromosomes via the lateral elements of the SC, and to stabilize them at a fixed separation distance The results further show that the TF's are fine (1.5–2.0 nm), straight, nonlooping protein fibers that originate in the LE's, have a remarkably constant length in the SC (though threads of the same diameter found outside the SC seem to be considerably longer), and interdigitate in a complex fashion in the central region, where, together with additional protein material, they form the CE Our observations suggest that the CE stabilizes the interlocking of TF's from opposite homologues, and thus the CE and TF's together maintain the critical spacing All major components of the central region are resistant to DNase, but the possibility cannot presently be excluded of a DNase-resistant DNA-protein complex, or of an occasional enzyme-susceptible DNA strand in the region.

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