Synaptonemal Complex Complement of Man in Spreads of Spermatocytes, with Details of the Sex Chromosome Pair

Abstract. *Human pachytene chromosome pairs have been characterized electron microscopically in spread preparations on the basis of synaptonemal complex length, kinetochore position and attached nucleoli when present. The X and Y chromosomes can be followed by their filamentous axial cores from partial synapsis, through precocious disjunction and end-to-end attachment, to differentiation of a network in the sex chromosome pair.*

A new approach to the analysis of meiotic chromosomes with the electron microscope has been made possible by observations on the synaptonemal complex (SC). This filamentous, proteinaceous structure, which regularly appears as the axis of pachytene bivalents in organisms in which crossing-over occurs (*1*), has a remarkably constant morphology over a wide range of animal and plant species (*2*). The behavior of the SC so closely parallels that of the bivalent in synapsis and disjunction that characteristics of the bivalent, such as length, orientation, and stage of pairing, may be taken directly from it.

Karyotype analysis using SC's is possible when SC lengths and kinetochore (centromere) positions can be determined for the entire complement. *Locusta migratoria* and *Zea mays* are the only species so far in which kinetochores have been identified at pachytene (*3*). In both cases, the entire bivalent complement has been characterized from kinetochore positions and lengths of the SC's in reconstructed serial sections.

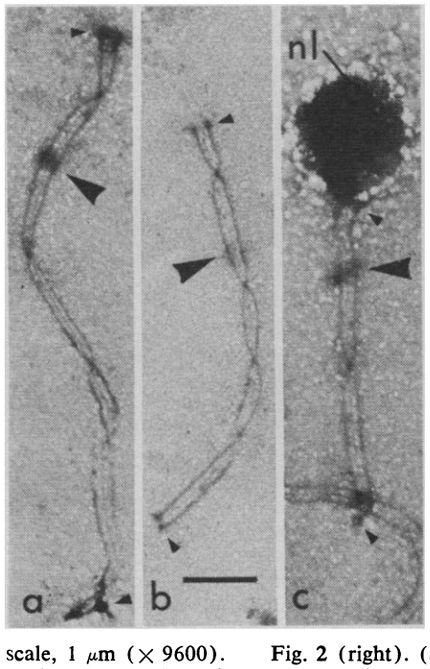
In *Locusta* spermatocytes (*4*) spread on the surface of a saline solution, fixed with formaldehyde, and treated with alcoholic phosphotungstic acid, the SC is selectively stained because of its protein nature and is rendered visible for its entire length, distinct from the surrounding unstained chromatin. The kinetochore is also strongly stained: moreover, it is seen as a differentiation of parts of the SC. We have adapted this method and applied it to mammalian spermatocytes; it makes possible the karyotypic analysis of mammalian meiosis through the SC in unsectioned spread nuclei.

Our observations on meiotic cells of the hamster (*5*) and of man (as shown here) extend the first results in *Locusta* to other species (*4*). Figure 1 shows three SC's from human pachytene spermatocytes (*6*). The parallel lateral elements are strongly stained and, together with the central element, can be followed from the dense points of attachment to the nuclear envelope at one end to those at the other. The kinetochore is represented as a positively stained region of the SC; in Fig. 1b it can be seen as a local differentiation of the lateral elements.

Kinetochores of mammalian pachytene chromosomes have not hitherto been observed with the electron microscope in either sections (*7, 8*), or spreads (*9*); the Counce and Meyer procedure (*4*) demonstrates these structures for the first time. Each bivalent can now be classified by the length of its SC and the position of its kinetochore.

Spermatocyte nuclei are often intact and flattened after drying with this technique. Intact SC's remain within such nuclei, probably because of their attachments to the nuclear envelope (Fig. 2). The entire complement of SC's can be recognized in such nuclei; 22 SC's, representing the 22 autosomal bivalents, are seen in Fig. 2, together with the XY pair.

The use of SC's for meiotic karyotyping depends on a proportionality be-



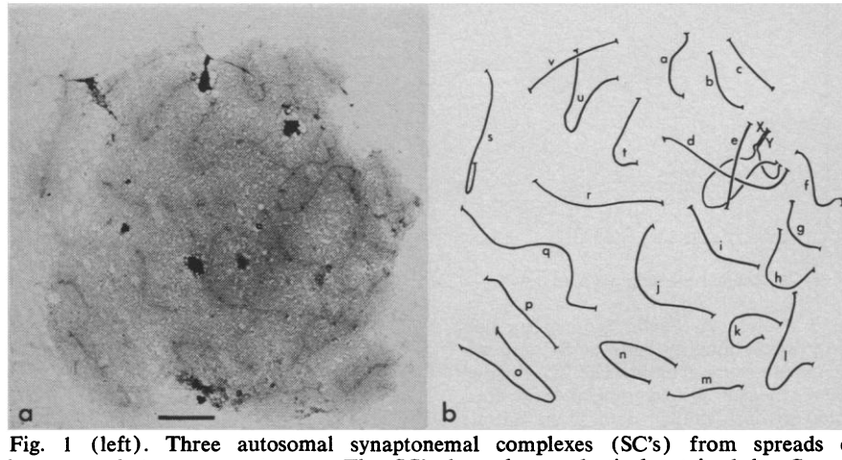


Fig. 1 (left). Three autosomal synaptonemal complexes (SC's) from spreads of human pachytene spermatocytes. The SC's have been selectively stained by Counce and Meyer's method (*4*). The twisting, filamentous SC, seen for its entire length, consists of two parallel lateral elements, each flanking and equidistant from a finer central element to which they are connected by transverse filaments. The lateral elements terminate in dense points of attachment to the nuclear envelope (small arrows). The kinetochore regions (large arrows), one per chromosome, are seen as pronounced thickenings of the lateral elements. In (c), a nucleolus (*nl*) is attached to the SC close to the termination of its short arm. Another autosomal SC crosses the long arm attachment region; scale, 1 μm ($\times$ 9600). Fig. 2 (right). (a) A human pachytene nucleus spread and stained by Counce and Meyer's method. Distortion due to flattening and spreading is minimal, but the SC's are not always distinct, particularly at this magnification. Also, many kinetochores are indistinguishable in this thick preparation. (b) Tracing of SC's from the nucleus in (a). Positive identification of termini and of the paths of the SC's was made at a higher magnification than that shown here. There is a full complement of 22 autosomal SC's (bivalents) and an XY pair; the short Y and the long X are indicated; scale, 5 μm ($\times$ 2350).

tween SC and chromosome length. This is demonstrable in Fig. 2, where the SC's have not been stretched by spreading. Here the SC's were measured and ranked according to length, expressed as percentage of total length of autosomal SC's. When these values are plotted against a corresponding array of published values of measured mitotic karyotypes (*10*), the regression curve is approximately linear (Fig. 3).

A full SC karyotype cannot be derived from this example because many kinetochores are obscured. But studies on the hamster (*5*) have shown positive correspondence between the SC karyotype and the somatic metaphase karyotype. An analysis of the human SC karyotype will be presented elsewhere.

Individual bivalents can be characterized by three SC criteria. The normalized length of any autosomal bivalent can be designated for size grouping by dividing the measured length of its SC by the length ratio (*11*) calculated from a particular reference mitotic karyotype. The position of the kinetochore is then taken as the second criterion for grouping the bivalent. Thus, according to current designations of human chromosome groups (*12*), the bivalent in Fig. 1a appears to be a member of the E group (which includes chromosome Nos. 16 to 18); that in Fig. 1b, the F group (Nos. 19 and 20); and that in Fig. 1c, the G group (Nos. 21 and 22). The presence of nucleoli provides a third characteristic for classification. Depending on the stage, nucleoli are seen attached near the terminal ends of certain SC's (Fig. 1c). From cytological (*13*) and in situ hybridization evidence (*14*), these sites represent the nucleolar organizer regions of chromosomes 13, 14, 15, 21, and 22. The attachment of a nucleolus close to the termination of the short arm of the SC in Fig. 1c confirms that this is either chromosome 21 or 22.

The XY pair is clearly identified in our spreads (Fig. 4). It is morphologically distinct from the 22 autosomal bivalents, and its structure and behavior conform to those established for the human X and Y chromosomes from other studies [for instance, (*8*)]. We have observed various configurations of the sex chromosome pair. It is possible in our preparations to distinguish the prophase stages (*15*), and, therefore, the configurations can be arranged in sequence. They thus appear to represent stages in XY pairing.

A paired region (Fig. 4a) is often evident at late zygotene and early pachytene (*15*) in spread preparations, the lateral elements of the SC extending as filamentous "cores" (*8*) into the unpaired parts. The shorter core is axial to the Y; and the longer, to the X (*8*). Granules that accumulate along the cores may represent differentiations of the filamentous material. The observed features agree with three-dimensional reconstructions of serial sections of the partially synapsed human XY pair made by Solari and Tres (*8*).

So far, electron microscope studies have not indicated which arms of the X and Y are paired (*8*). However, observations after quinacrine staining (*16*) have shown that the short, nonfluorescent arm of the Y is associated with the X. Indirect evidence (*17*) suggests that the short arm of the X may be involved in synapsis. Verification should be possible from the kinetochore positions in our preparations. Although the kinetochores cannot be distinguished from granular accumulations along the cores, their positions may be estimated by calculating short arm lengths from ratios of long to short arms of mitotic X and Y chromosomes (*18*). Only when the SC region is assumed to involve the short arms of both chromosomes do visible

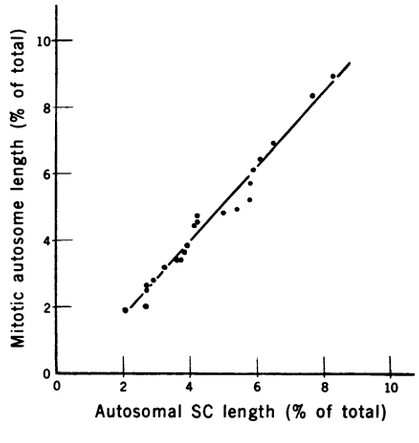


Fig. 3 (left). A linear regression indicating proportionality between SC and mitotic chromosome lengths. The SC's in Fig. 2 were measured and ranked, and then plotted against similar data from mitotic karyotypes (*10*). Lengths are expressed as percentages of the summed lengths of the autosomes in each case.

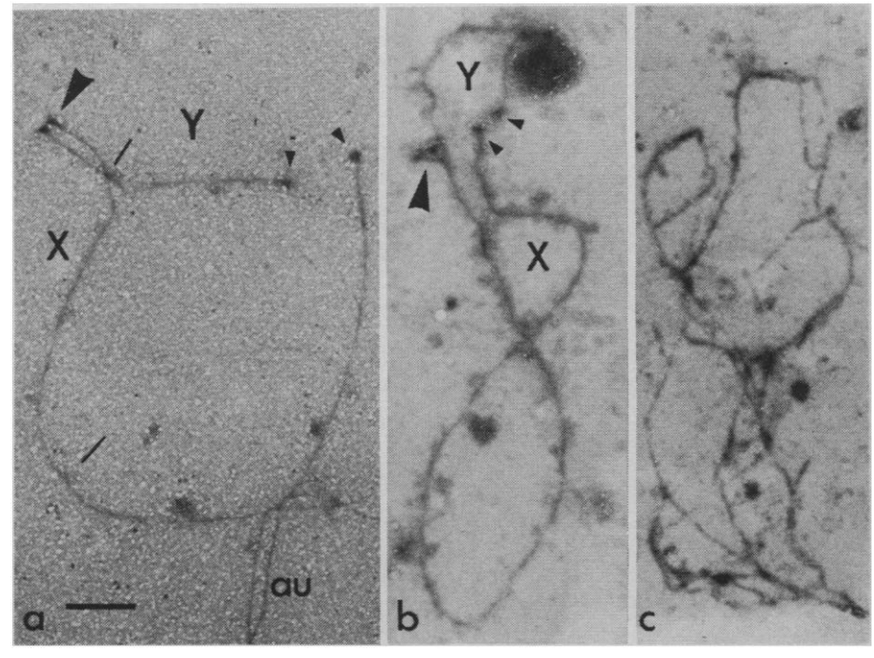


Fig. 4 (right). The XY complex at three different stages in human pachytene nuclei. The complex is represented by filamentous cores thought to be axial to the sex chromosomes. In (a), found at early pachytene, a short core represents the Y chromosome; and a long core, the X. The long and short cores pair to form a short (1.5 μm) length of SC, ending in attachment points (large arrow). The unpaired ends are also marked by attachment points (small arrows). Dashes mark the positions of the X and Y kinetochores that were predicted (*18*) by assuming the SC region to involve the short arms of both chromosomes. An autosomal SC (*au*) crosses the long core, but is not part of it. In (b), found at early or mid pachytene, disjunction has evidently occurred and the pairing of the cores has become terminal, the X and Y remaining associated at their attachment points (arrows). The long arm of the Y is characterized by an attached dense body. The attachment points of the short arms (large arrow) appear connected, while those of the long arms are more separate (small arrows). In (c), found at late pachytene, the cores have elaborated a network of filamentous material in the sex vesicle. Neither X and Y cores nor their attachment points can be distinguished; scale 1 μm ($\times$ 9400).

differentiations of both X and Y cores coincide with the predicted kinetochore positions (Fig. 4a, dashes). It follows that, insofar as the SC reflects chromosome homology, the distal portion of the X short arm is homologous with the short arm of the Y.

At diplotene and diakinesis in light microscopic preparations, the X and Y chromosomes are seen attached end-to-end. The existence of a SC in the XY pair implies synapsis and is thus consistent with the possibility that the tandem arrangement results from chiasma terminalization (*8*). Our observations do not bear directly on this point, but they do contribute to an understanding of the nature of the end-to-end associations.

Figure 4b shows the X and Y cores in a later stage of pairing. The SC is no longer present, but four attachment points are visible; two of these are closely spaced (large arrowhead), as in the terminus of the SC, and represent ends of the X and Y cores. The other ends of these cores terminate in neighboring but more separate attachment points (small arrowheads). The X and Y short arms cannot be verified by kinetochore position because of the many dense accumulations along the cores. However, a large dense body, resembling that shown (*8*) to be associated with the distal portion of the Y core, is seen near the distal attachment end of what is then presumed to be the long arm of the Y (Fig. 4b). Thus, it seems likely that the configuration in Fig. 4b results from disjunction of the synapsed region (with or without chiasma terminalization) and loss of the SC, with the common ends remaining joined at their attachment points on the nuclear envelope (large arrowheads). This conclusion is supported by the observation of progressively shorter SC lengths from late zygotene to early pachytene (*15*), as would be expected if disjunction with loss of SC were occurring.

Because all of the autosomes in Fig. 4b are still in pachytene, the XY disjunction is regarded as precocious. It is reasonable to suppose that the persistent nuclear envelope attachments of the SC terminus constitute the structural device by which end-to-end association is realized.

A third configuration of the sex pair is found at late pachytene (*15*) (Fig. 4c). X and Y cores are no longer distinguishable in the filamentous network, nor can attachment points be identified. It appears that, concomitant with precocious disjunction, a proteinaceous component is elaborated in or on the cores; this leads to an anastomosing framework whose area is roughly equivalent to that delineated by the cores at earlier stages. This structure probably represents the filamentous components of the XY chromosome pair which become more elaborate as prophase progresses (*8*). Consistent with earlier observations (*8*), formed nucleoli are not attached to the sex chromosome pair.

The simple and rapid method of spreading and staining human pachytene spermatocytes offers a new and unique approach to such problems as the identification and fate of chromosomal rearrangements in meiosis and the manner and extent of XY pairing. It could conceivably be the source of routine diagnostic information on autosomal meiotic behavior as well as on that of the sex chromosomes, whose morphology is known to be highly variable in both clinically normal and abnormal males [for example, see (*19*)].

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References and Notes

1. M. J. Moses, *Annu. Rev. Genet.* **2**, 363 (1968); *Genetics* **61** (Suppl.), 41 (1969); M. Westergaard and D. von Wettstein, *Annu. Rev. Genet.* **6**, 71 (1972). The presence of the SC at meiosis suggests that it may play a key role in meiotic processes. Whether and how it functions in chromosome pairing and genetic recombination, and what its significance is to the organization of the chromosome, are questions that were raised when the structure was first observed and remain without clear answers.
2. Reconstruction of entire SC complements from serial sections [R. Wettstein and J. R. Sotelo, *J. Microsc. (Paris)* **6**, 557 (1967); P. B. Moens, *Chromosoma* **28**, 1 (1969); C. B. Gillies, *ibid.* **36**, 119 (1972); P. B. Moens and F. O. Perkins, *Science* **166**, 1289 (1969); A. T. C. Carpenter, in *Symposium on Mechanisms in Recombination*, R. Grell, Ed. (Plenum, New York, 1974)] and study of whole mount spread preparations [for example, D. E. Comings and T. A. Okada, *Chromosoma* **30**, 269 (1970); A. J. Solari, *ibid.* **39**, 237 (1972); A. J. Solari and M. J. Moses, *J. Cell Biol.* **56**, 145 (1973)] have proved that the SC is axial to paired chromosomes, joins homologues along a line of synapsis, and extends from one end of every chromosomal bivalent to the other, with its termini usually applied to the nuclear envelope. Unpaired homologous chromosomes contain single corelike axial elements, which become lateral elements of the SC upon synapsis or are derived from them when homologues disjoin. Components of the SC are not usually found in late prophase or later.
3. P. B. Moens, *Cold Spring Harbor Symp. Quant. Biol.* **38**, 99 (1974); C. B. Gillies, *Chromosoma* **43**, 145 (1973).
4. S. J. Counce and G. F. Meyer, *Chromosoma* **44**, 231 (1973).
5. M. J. Moses and S. J. Counce, in *Symposium on Mechanisms in Recombination*, R. Grell, Ed. (Plenum, New York, 1974), pp. 385–390.
6. Small portions of testicular biopsies, taken from two adult males during the course of surgical exploration for obstruction of the vas deferens, were promptly placed in Eagle's minimal essential medium at 37°C. A 2- to 3-mm² piece of tissue, covered with medium, was chopped a few times with a razor and then macerated with fine blunt forceps to express spermatogenic cells from the tubules. After large pieces of tissue were discarded, the cell suspension was taken up in a 1-ml syringe without needle to a volume of 0.6 ml with additional medium. The contents were expressed and taken up in the syringe two or three times to break up clumps of cells. The suspension was then centrifuged for 5 minutes at about 150*g*. The supernatant was reduced to a volume about two to three times that of the pellet, and the pellet was resuspended. Phase microscopic examination of the suspension showed all stages of apparently normal spermatogenesis. A small drop of the suspension was applied by micropipette to the clean surface of a spreading solution consisting of 0.5 percent NaCl. After the spread had stabilized for at least 2 minutes, carbon- and Formvar-coated, glow-discharged grids were touched to the surface and then floated on 4 percent formaldehyde, *p*H 8, for 5 minutes. The subsequent detergent rinse, drying, staining with phosphotungstic acid in ethanol, and drying after an ethanol rinse were performed as described by Counce and Meyer (*4*). Grids were examined in a Philips EM-200 at 80 kv.
7. D. H. M. Woollam, E. H. R. Ford, J. W. Millen, *Exp. Cell Res.* **42**, 657 (1966).
8. A. J. Solari and L. L. Tres, *J. Cell Biol.* **45**, 43 (1970); in *The Human Testis*, E. Rosenberg and A. Paulsen, Eds. (Plenum, New York, 1970), pp. 127–138.
9. D. E. Comings and T. A. Okada, *Exp. Cell Res.* **65**, 99 (1971).
10. J. Reitalu, *Hereditas* **59**, 1 (1968).
11. The length ratio for a given SC complement $= 2\Sigma l_{sc}/\Sigma l_a$, where Σl_{sc} is the sum of the measured lengths of the 22 autosomal SC's and Σl_a is the sum of the autosomal lengths in the reference mitotic karyotype.
12. Denver Study Group, *Suppl. Cerebr. Palsy Bull.* **2**, 1 (1960).
13. M. A. Ferguson-Smith, *Cytogenetics* **3**, 124 (1964).
14. A. S. Henderson, D. Warburton, K. C. Atwood, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3394 (1972).
15. In our spread preparations, we have defined zygotene as the condition (with respect to the autosomes) in which unpaired axial elements are found continuous with lateral elements of the SC, a condition indicating that pairing is incomplete. The designation early or late depends on the predominance of single axial elements or complete SC's, respectively. Pachytene is defined as the condition in which only SC's are found. At early pachytene (as at zygotene) the chromatin is evenly dispersed in the spreads, with little or no stain, while at late pachytene, the chromatin is more condensed and stainable, with individual chromosomes becoming distinguishable.
16. P. L. Pearson and M. Bobrow, *Nature (Lond.)* **226**, 959 (1970).
17. M. Hulten, J. Lindsten, P. M. Ming, M. Fraccaro, *Ann. Hum. Genet.* **30**, 119 (1966).
18. The length of the short arms of the X or Y core is $l_c/(R+1)$, where l_c is the measured length of the core and R is the ratio of long to short arm lengths taken from the X and Y chromosome of the reference mitotic karyotype.
19. V. McKusick and G. Chase, *Annu. Rev. Genet.* **7**, 435 (1973); J. A. Robinson and K. E. Buckton, *Chromosoma* **35**, 342 (1971); M. Bobrow, P. L. Pearson, M. C. Pike, O. S. El-Alfi, *Cytogenetics* **10**, 190 (1971); J. Reitalu, S. Bergman, B. Ekwall, *Hereditas* **65**, 97 (1970).
20. We gratefully acknowledge the assistance of Ms. Katherine Moses. This research was supported in part by NSF grant GB-40562 and PHS grant CA-1-4236. The research of D.F.P. was supported by grant 5R262 CA-1541 from the National Cancer Institute.

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