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The Synaptinemal Complex and Four-Strand Crossing Over

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ABSTRACT The synaptinemal complex provides a structural basis for four-strand crossing over: prior to chromosome pairing, both sister chromatids of each homologous chromosome participate in the genesis of one lateral component. During precise pairing, the two lateral components are combined into one synaptinemal complex per bivalent.

THE SYNAPTINEMAL COMPLEX

At pachytene of meiosis the synaptinemal complex (1) joins the paired homologous chromosomes along their entire length at a uniform distance. Cytochemical techniques reveal the synaptinemal complex to consist of protein (1–3) and ribonucleic acids (2). Tests for the presence of DNA, by means of deoxyribonuclease treatments, have been negative (1–3).

Especially well-defined images of the components in the synaptinemal complex, and its association with the chromatin of the two homologous chromosomes, are obtained from the ascomycete genus *Neottiella* (Fig. 1) (2). Embedded in the chromatin surface of each homologue is one lateral component constructed of alternating thick (10-nm) and thin (5-nm) bands. These have a diameter of 50–60 nm and are spaced at a center-to-center distance of 19.3 nm. The two, banded, lateral components are held in register, about 100 nm apart, by the ribonucleoprotein of the central region, which includes the 18- to 20-nm thick dense rod that is called the central component.

By treatment of formalin-fixed asci with ammoniacal silver ions at pH 8–10, the bands of the lateral components in the synaptinemal complex of *Neottiella* can be specifically labeled with metallic silver grains (Fig. 2) (2). This differential-labeling procedure was used to follow the biogenesis of the components of the synaptinemal complex. Before chromosome pairing, central-region material accumulates in large layers in the nucleolus (Fig. 3), which suggests that the nucleolus is the primary assembly site of the central regions for the synaptinemal complexes. A single lateral component is formed in the split between the two sister chromatids of each homologous chromosome prior to their pairing (Fig. 4).

Chromosome pairing is terminated by removal of the synaptinemal complex from the bivalent (2, 4–6). In *Neottiella*, amorphous remnants of the synaptinemal complex remain associated with the bivalents (Fig. 5) until late diplotene, whereafter the remnants disappear from the nucleus. Chiasmata at diplotene are short, structurally modified, regions of the synaptinemal complex that have been retained and hold the repulsing homologous chromosomes together (Fig. 5) (2).

Functions of the synaptinemal complex

The proposed functions are discussed with the aid of Fig. 6, which is based on the meiosis of an ascomycete. The nuclear events that lead to the onset of meiotic prophase are summarized in stages 1–6. DNA replication takes place in the haploid nuclei before karyogamy (7). The zygote nucleus in stage 6 contains the 4 N amount of DNA; each of its chromosomes consists of two chromatids. In the diagram, a chromatid is drawn as a single continuous nucleohistone fibril consisting of one continuous double helix of DNA

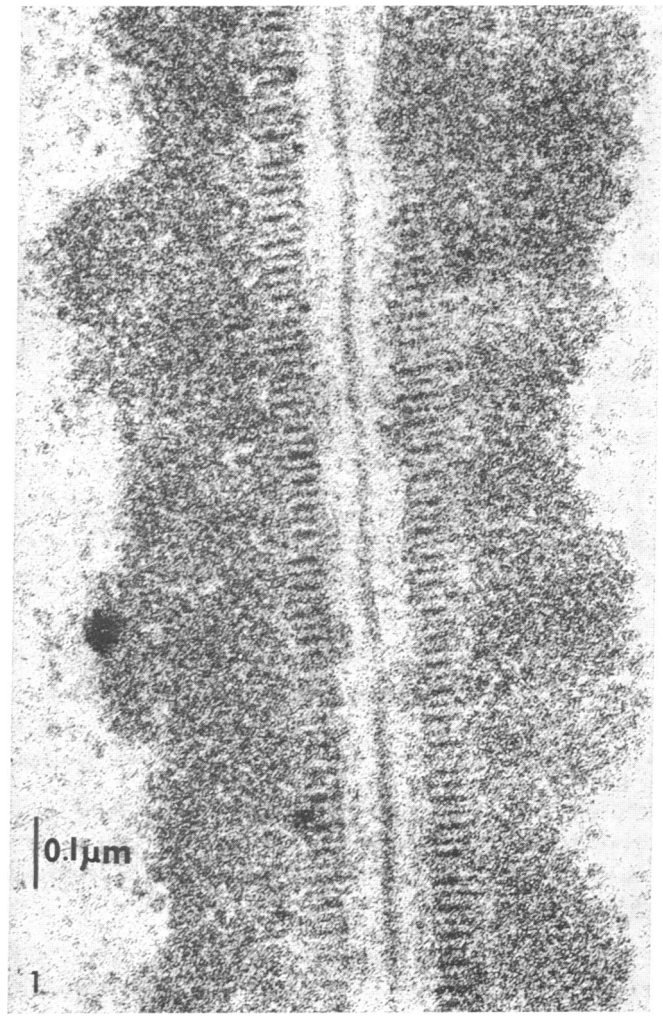

FIG. 1. Longitudinal section through bivalent with synaptinemal complex at pachytene of meiosis in *Neottiella*. ×91,000.

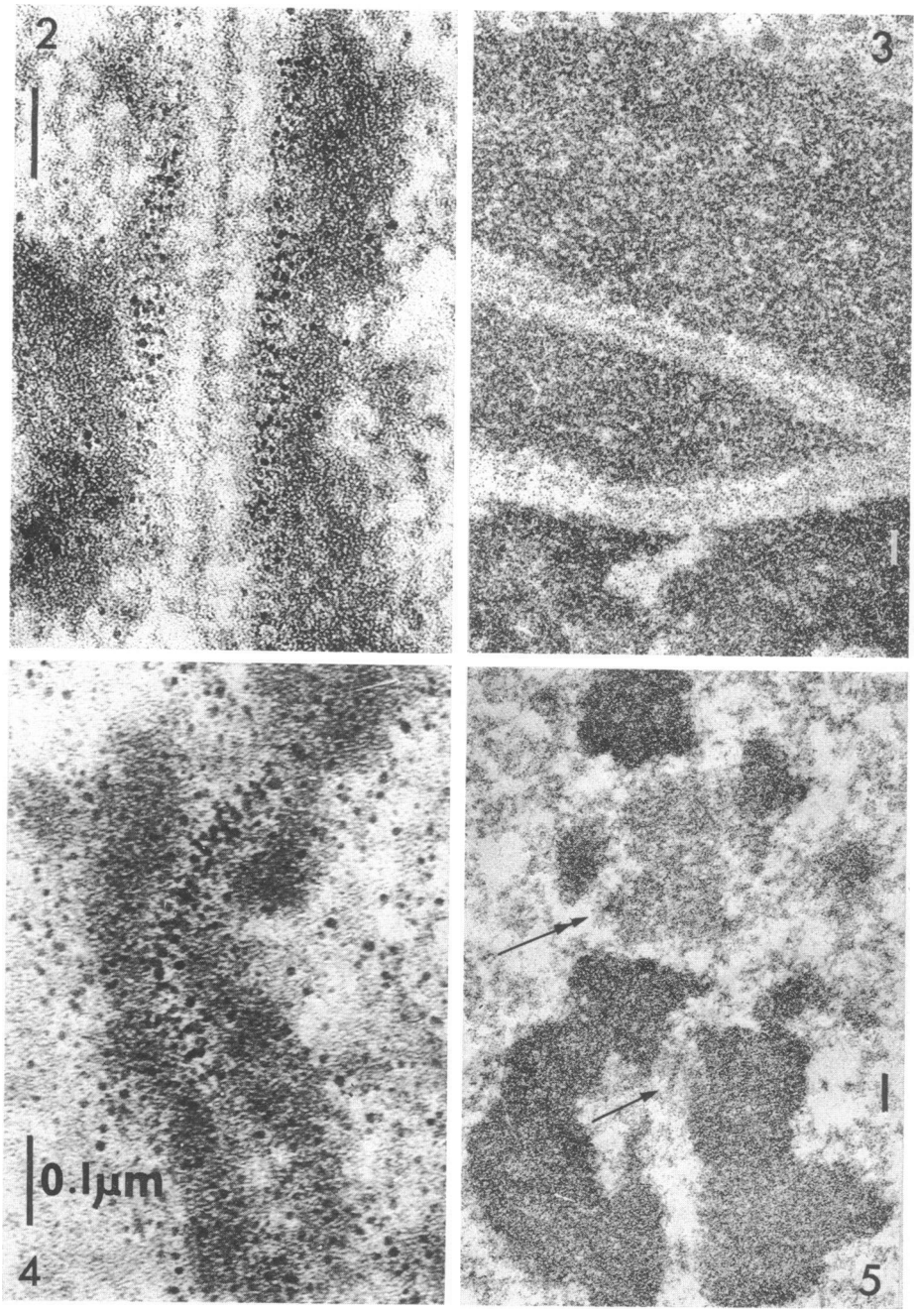

FIG. 2. Silver labeling of the lateral components in a synaptinemal complex. ×117,000.

FIG. 3. Central regions of the synaptinemal complex in nucleolus prior to chromosome pairing. ×48,000.

FIG. 4. Silver-labeled lateral component in split between sister chromatids of unpaired chromosome. ×117,000.

FIG. 5. Bivalent at diplotene with two chiasmata (*arrow*) and shed synaptinemal complex (*double arrow*). ×48,000.

complexed with histones and nonhistone proteins (8). One sister chromatid is shown as a continuous line, the other as a broken line.

The lateral components prior to chromosome pairing (leptotene; Fig. 6, stage 7)

A single lateral component is formed *de novo* in the split between the sister chromatids of each chromosome (*a*, *b*): Its strong reaction with ammoniacal silver ions is probably due to basic residues in proteins (2). Central regions assemble in the particulate part of the nucleolus (*a*, *top*).

I propose that short regions of the nucleohistone fibril are associated with the banded segments of the lateral component (*a*, *b*). The loops of the fibrils that connect these short regions are thought to be very long. The lateral component conceivably serves two functions: (*i*) it is endowed with the information for the specific site-to-site recognition of the lateral component of the homologous chromosome, information used in precise pairing. (*ii*) It incorporates only one sister chromatid in a given segment, but in adjacent segments different sister chromatids can be represented. The sister chromatid attached to the lateral component at a given point is the one to participate in a potential crossing over.

These two interpretations are implicit in the right-hand drawing (*c*) of stage 7. The DNA in both sister chromatids in the short regions of the nucleohistone fibrils that border the split and connect the extending loops codes for specific recognition sites in the lateral components (e.g., segments comprising a thick and a thin band). The recognition sites could consist of messenger-like RNA (9) and/or protein translated from it. Each segment of the lateral component

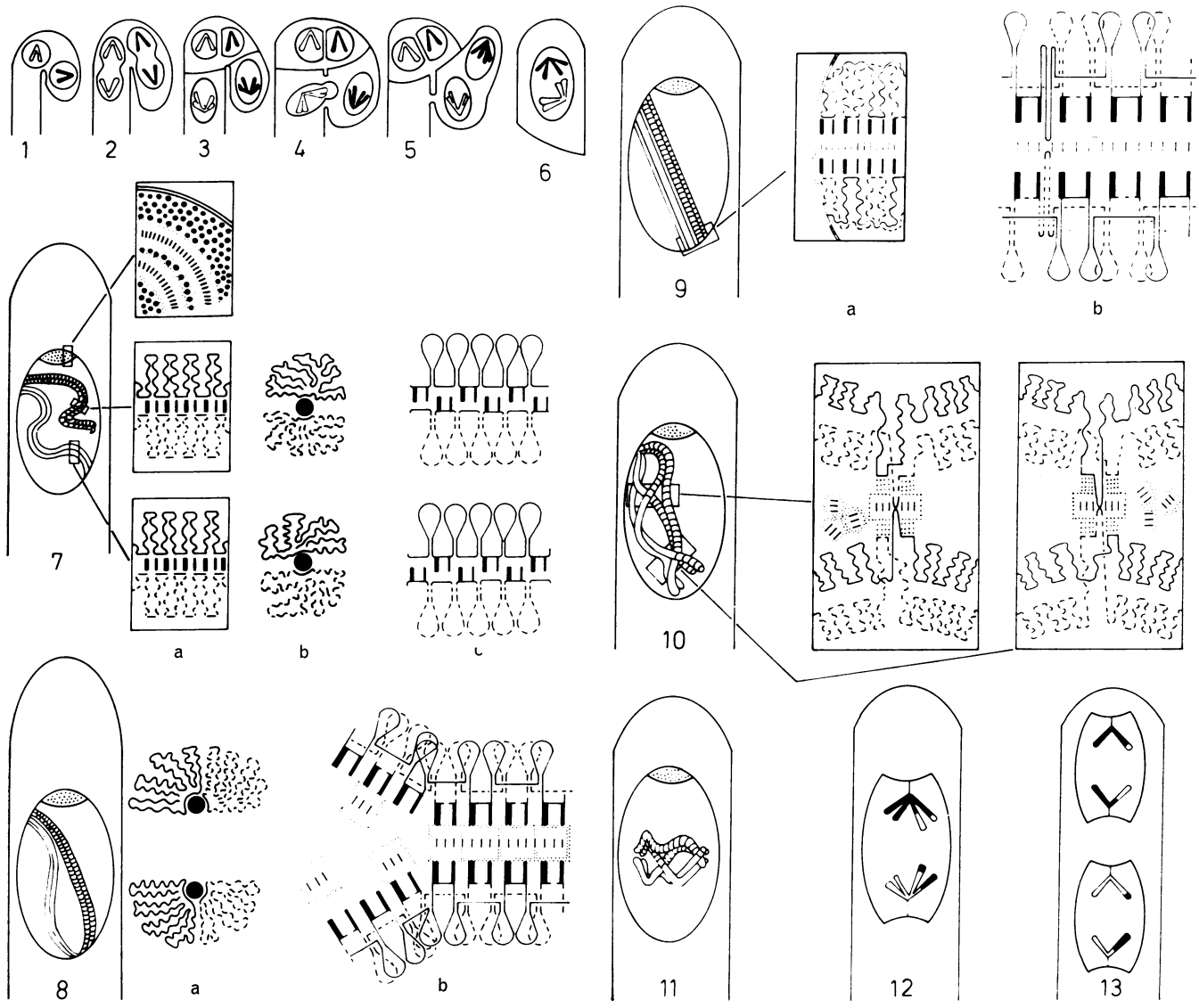

FIG. 6. Diagram of the synaptinemal complex at the different stages of meiosis in an ascomycete (*Neottiella*).

would be the product of a recognition gene. The segment attaches to its recognition gene in one chromatid only, but adjacent segments would attach to different sister chromatids. In this way, sister chromatids became represented alternatingly in the lateral component, as is required by the genetic results of half-tetrad and tetrad analyses which reveal that only two nonsister chromatids are involved in any one crossing over and that different chromatids can be involved when more than one exchange takes place in a chromosome arm (10).

At synapsis (zygotene; Fig. 6, stage 8)

Pairing of homologous chromosomes proceeds in three steps (11): (*i*) Approximate alignment to within 300 nm. (*ii*) Precise point-to-point pairing, at a distance of 100 nm, by the synaptinemal complex. (*iii*) Base-by-base pairing of two nonsister chromatids.

(*i*) Parallel alignment of the homologous is initiated at the telomeres, centromeres, or other regions of the chromosome (12). In some organisms, at least, the telomeres of the unpaired homologous chromosomes are attached at distant points on the nuclear envelope and move together during alignment until they are combined to one end of the synaptinemal complex (11). Both ends of all synapsed bivalents can be attached to the nuclear envelope (11, 13, 14). A drastic increase in nuclear volume during early meiotic prophase (2) may facilitate approximate homologue alignment.

(*ii*) Homologous chromosomes may pair precisely, with the aid of the synaptinemal complex, in the following way: the lateral component of the unpaired chromosome is relocated into a position lateral to both sister chromatids. This is accomplished by the rotation of the sister chromatids relative to the lateral component, whereby the space between the sister chromatids becomes eliminated (Fig. 6, stage 8*a*).

The two lateral components of the homologous chromosomes are then arranged in register directly opposite each other, at a distance of 100 nm, with the aid of the central region material, as indicated in the diagram (*b*): the ribonucleoprotein blocks of central-region material coming

from the nucleolus are nonspecific. When a given segment of the lateral component associates with the surface of such a block, its opposite surface is patterned by conformational change into a structure fitting only to the segment's counterpart in the homologue.

The precise pairing of two large chromosomes in this manner requires the production of a large number of similar variants of specific protein and RNA molecules that are coded for by recognition genes spaced along the chromosomes. A specific molecule for precise pairing may be analogous to the gamma-globulin molecule (15), which by permutations of a restricted number of amino acids in the variable portion of its peptide chains provides a large number of specific antibodies against antigens.

In *Neottiella*, the lateral component contains 53 electron-opaque bands per μm of chromosome (Fig. 1). Since the largest chromosomes are approximately 15 μm long at meiotic prophase, they have 781 bands in their lateral components. A very rough estimate of 250 μm for the total length of the lateral components in a haploid genome would result in about 13,000 bands. If a recognition gene extends between a pair of thick and thin bands, there would have to be 6500 recognition genes per genome.

In various groups of eukaryotes, the lateral and central components differ in fine structure (1, 2, 4); this probably reflects differences in composition rather than function. Banded patterns may arise from the clustering of certain amino acids in the repetitive polypeptide chains of the lateral or central component.

At pachytene (Fig. 6, stage 9)

The two homologous chromosomes in a bivalent are paired throughout their length by a continuous synaptinemal complex, spanning from one site to another on the nuclear envelope (*a*).

(*iii*) In the diagram (*b*) a possible, but so far strictly hypothetical, mechanism for the effective pairing of short regions of two homologous nonsister chromatids is indicated. The long loops between the recognition genes, which are firmly fastened to segments of the lateral component, could grow into the central component. After removal of histones and other proteins from the fibrils, physical contact and base-by-base pairing of the two DNA double helices is established, then followed by breakage and reciprocal reunion, as is required for crossing over and chiasma formation (16). As evidenced by gene conversion (10), the molecular events of recombination may involve heteroduplex formation with cutting and repairing of DNA strands in the effectively paired regions (17). A small amount of DNA synthesis during pachytene has been demonstrated (18). Good evidence places crossing over at pachytene (16, 19), and the recombination frequency in a chromosome region is directly correlated with the intimacy of pairing at pachytene, as observed by light microscopy (20–22).

At diplotene (Fig. 6, stage 10)

When pairing of the homologous chromosomes is replaced by their repulsion, the synaptinemal complex is shed from the bivalents, except at places where the homologous chromosomes are held together by chiasmata. These consist of retained regions of the synaptinemal complex with an altered fine structure (2). The banded lateral component is no longer discernible (Fig. 5), and dense material fills the space between the chromatin and the central component. Most probably the short pieces of the synaptinemal complex are retained because of the breakage and reunion of two nonsister chromatids in connection with a crossing over. As the lateral components disappear, the sister chromatids rotate back into the position they occupied prior to pairing and are again visible as separate strands.

Late stages of meiosis (Fig. 6, stages 11–13)

At diakinesis and *metaphase I*, chiasmata are no longer characterized by modified short stretches of the synaptinemal complex but reveal a continuous chromatin structure between the rejoined chromatids (2).

CONCLUDING REMARKS

Crossing over is a four-strand event in the sense that all four chromatids may be involved in two crossing-overs of a chromosome arm. Since there is only one synaptinemal complex per bivalent, the difficulty has been to decide what role it plays in the mechanism of four-strand crossing over. Participation of both sister chromatids in the genesis of one lateral component per homologous chromosome eliminates this difficulty.

Several facts favor a system of specific recognition between the lateral components of homologous chromosomes in synapsis. Firstly, precise synapsis is initiated at several independent sites. Five partner-switches in one chromosome arm of a quadrivalent at pachytene, for instance, require six independent points of initiation in the two-by-two pairing of four chromosomes (23). At zygotene, short stretches of two homologous chromosomes can be joined by a complete piece of synaptinemal complex before their centromeres and telomeres are (11). Secondly, in inversion heterozygotes precise synapsis takes place with the aid of a loop in one chromosome and a reverse loop in its homologue. Since anaphase I bridges and fragments resulting from one or two crossing-overs in the loop can occur in 49% of the meiocytes (24), exact pairing in the loop configuration must be the rule.

A simple, unspecific zipper mechanism from telomere-to-telomere mediated by the synaptinemal complex, as suggested by King (25), cannot account for independent initiation points. An unspecific zipper mechanism with multiple initiation points, as suggested by Comings and Okada (26), still requires specific recognition between the initiation points as well as a mechanism by which, at a given moment, only homologous segments of the lateral components are available for precise synapsis in inversion heterozygotes.

Recognition specificity between all segments of the lateral components would allow precise synapsis to start at any point along the chromosomes and can be tested for in two ways: (*i*) Synaptinemal complex formation should be permitted to begin at several independent sites in the loop of an inversion heterozygote. (*ii*) Isolated pieces of the lateral component should contain heterogeneous constituent molecules.

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