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The Nature and Extent of Synaptonemal Complex Formation in Haploid Barley

C. B. Gillies

From the Department of Physiology, Carlsberg Laboratory, Copenhagen

Abstract. Normal synaptonemal complexes have been found in haploid barley meiotic prophase at stages equivalent to pachytene in diploids. Reconstructions of serially sectioned nuclei have shown that up to 60% of the haploid chromosomes may pair in either intra- or interchromosomal associations. The extent and nature of the synaptonemal complex formation suggest that the chromosome pairing is non-homologous. From the virtual absence of chiasmata in metaphase I stages of the haploids it is inferred that crossing over requires a more precise DNA alignment than is provided by synaptonemal complex formation alone.

Introduction

The synaptonemal complex (SC) is usually associated with the occurrence of homologously paired chromosomes in early meiotic prophase, and appears to be necessary for the subsequent high-efficiency recombination and chiasma formation in late prophase (Westergaard and von Wettstein, 1972; Stern and Hotta, 1973). However, a number of cases have been reported where synaptonemal complexes are present but crossing over and/or chiasmata formation is reduced or absent (Moens, 1969; Nilan *et al.*, 1969; Gassner, 1969). Apparently normal SCs have been reported from the microsporocytes of haploid plants in maize (Ting, 1971), tomato (Menzel and Price, 1966), *Petunia* and *Antirrhinum* (Sen, 1970). In such haploids it is expected that chromosome pairing is non-homologous, with the exception of possible duplicated regions, which in most cases are not extensive (Weber and Alexander, 1972). The question therefore arises as to whether the segments of SC reported in haploids involve small homologous duplicated regions in otherwise non-homologous chromosomes, or whether the SC formation is between non-homologous regions.

The development by Kasha and Kao (1970) of a technique whereby large numbers of haploid barley plants could be obtained, has allowed some extensive light microscopic studies of meiosis in this haploid (Sadasivaiah and Kasha, 1971). Chromosome pairing is extensive in a stage comparable to pachytene, but few if any chiasmata are found at

metaphase I. The availability of plentiful supplies of haploid microsporocytes has enabled the present study of the occurrence and extent of SC formation in haploid barley.

Materials and Methods

The haploid plants were kindly made available by Dr. C. J. Jensen of the Danish Atomic Energy Establishment at Risø (Jensen, 1973). They were derived from crosses of *Hordeum vulgare* × *H. bulbosum* (both 2n = 14) by the method of Kasha and Kao (1970). Subrahmanyam and Kasha (1973) have shown that the *H. bulbosum* chromosomes are selectively eliminated in early mitoses of the developing zygote, and haploid *H. vulgare* embryos can be cultured about two weeks after pollination. Chromosome numbers of the plants were checked by root tip squashes. The haploids used in the present studies were derived from Sultan, a Dutch cultivar, and from an F_1 of Sultan × Carlsberg II mutant 29 (a high lysine line produced by ethyl methane sulfonate treatment).

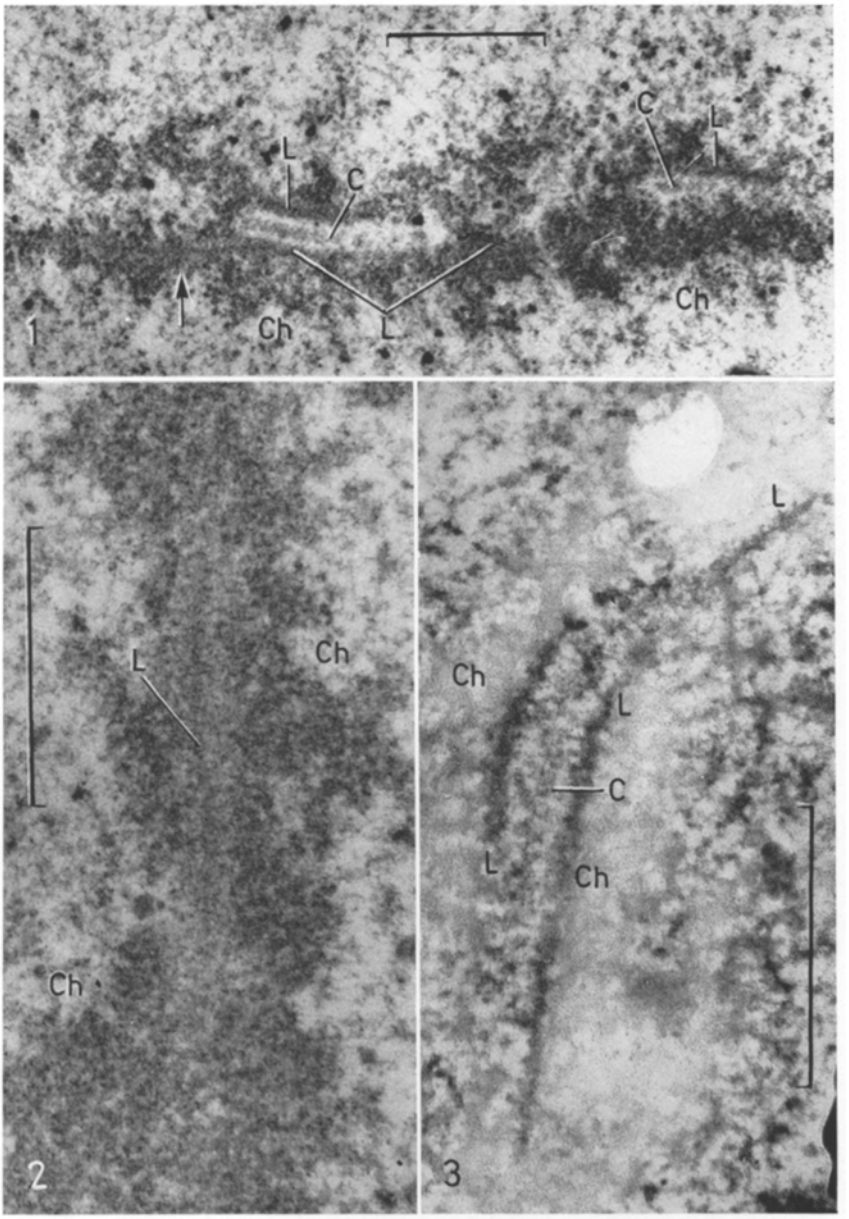
Meiotic stages of anthers were determined by acetocarmine squashes, and adjacent anthers fixed for electron microscopy. Initial fixation was for $2^1/_2$ hours in 4% formaldehyde/4% glutaraldehyde in 0.1 M phosphate buffer, with or without added sucrose (Gillies, 1973a). After several buffer washes, anthers were either fixed for $1^1/_2$ hours in 2% OsO_4, or processed with phosphotungstic acid (PTA) staining (Sheridan and Barrnett, 1969) or by Bernhard's (1969) method. Anthers were dehydrated, and embedded in Spurr's (1969) low viscosity resin.

Anthers were sectioned and picked up on single hole grids as described by Gillies (1972). Staining in uranyl acetate and lead citrate, or by Bernhard's method was carried out where appropriate. Serial micrographs of PTA stained PMCs were used to reconstruct the components of the SC (Gillies, 1973b).

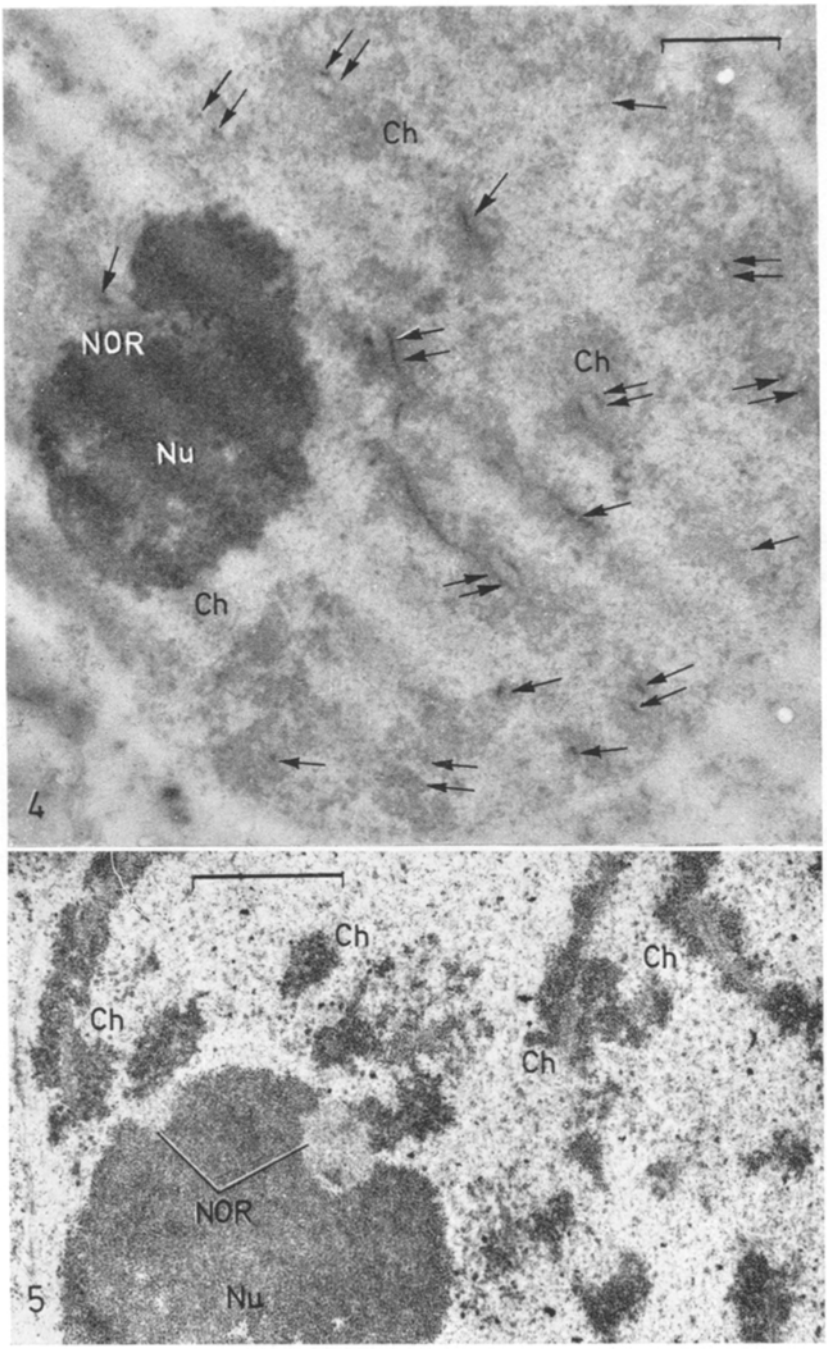
Results

All three staining methods readily visualize synaptonemal complexes in haploid barley at stages equivalent to pachytene (Figs. 1, 3 and 4). The dimensions of the complex components are the same as commonly reported for SCs [central region 1000–1100 Å; lateral components (LC) 350–450 Å; central component (CC) 400–500 Å]. With the osmium fixation and uranium acetate and lead citrate staining (U/Pb) shown in Fig. 1, the LCs and CC of a fully formed SC are amorphous, the LCs being denser than the CC and of about the same electron density as the surrounding chromatin. No substructure is evident in either component.

With Bernhard's staining reaction for ribonucleoprotein, the SC lateral components are strongly stained and the CC less strongly contrasted (Fig. 3). Chromatin is largely unstained. Fifteen hours staining of dehydrated anthers in alcoholic 1% PTA resulted in distinct staining of the SC, and very faint staining of the CC in the unstained central region (Figs. 4, 6 and 7). The chromatin was lightly stained. No centromeres could be recognized by any of the staining methods, but as serial sections were made only of PTA stained material it is possible that centromeres were missed in the U/Pb and Bernhard's stained material.



Figs. 1–3. Fig. 1. U/Pb stained synaptonemal complex in haploid barley. *L* lateral component, *Ch* chromatin. Arrow indicates unpaired lateral component. Bar 0.5 μ. × 40000. Fig. 2. Unpaired lateral component (*L*) and associated chromatin (*Ch*). U/Pb stained. Bar 0.5 μ. × 75000. Fig. 3. Synaptonemal complex stained by Bernhard's reaction. *L* lateral component, *C* central component, *Ch* chromatin (unstained). Bar 0.5 μ. × 77400


Ch
NOR
Nu
Ch
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4
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5

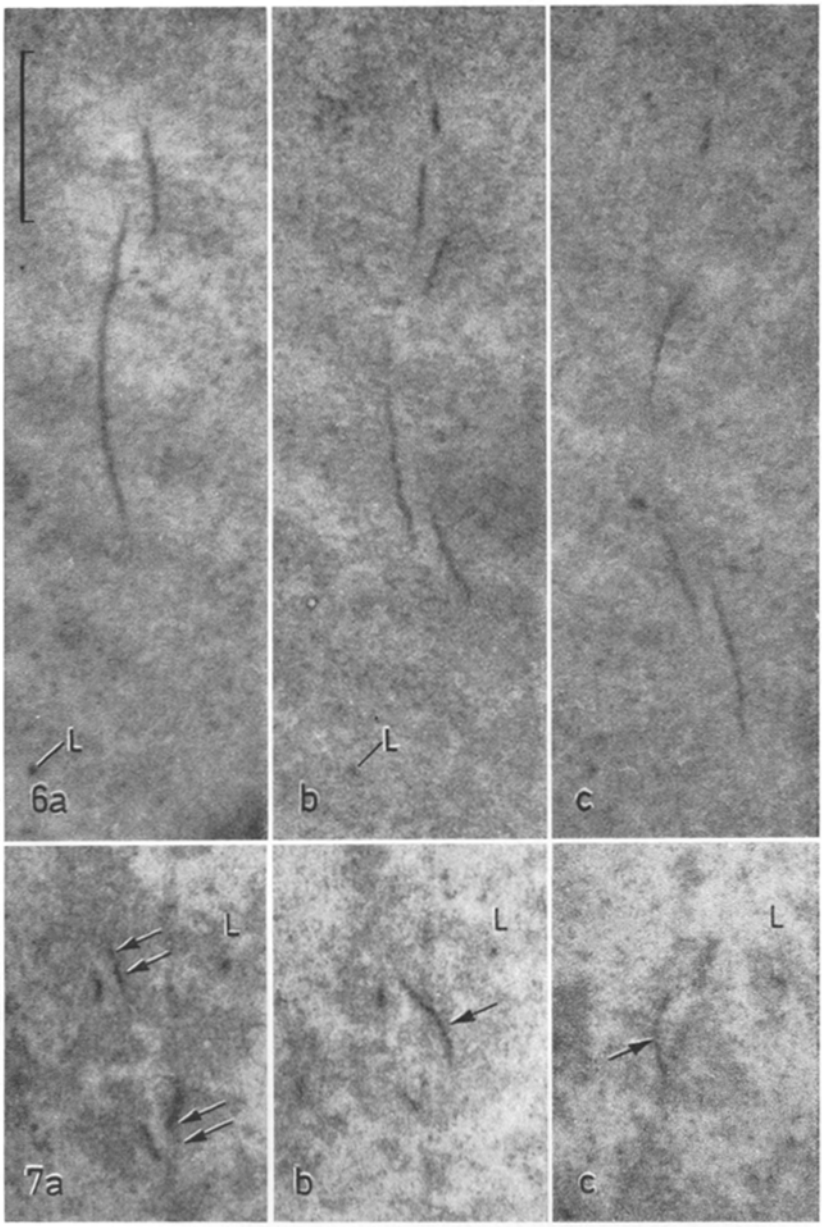
The lateral components of unpaired chromosomes, when stained with U/Pb (Figs. 2 and 5) appear as dark rods about 120–180 Å wide, centrally located in a lighter contrasted cylinder about 800–1000 Å wide. The dark rod shows some evidence of cross striations (Fig. 2) but these are irregular in occurrence. With Bernhard stained material there is some suggestion that the darkly stained LCs of unpaired regions are narrower than those of paired SCs. In some unpaired LCs so stained there appears to be a lightly stained region flanking the darkly stained central part of the LC. With PTA staining it is not possible to see a difference in width between LCs in paired and unpaired regions (Figs. 4 and 7). With U/Pb stain, the two components of the LCs are difficult to recognize in fully paired SCs but are immediately obvious in any parts where pairing is interrupted or incomplete (Fig. 1, arrow).

In regions of chromosome pairing the LCs are adjacent to the unstained central region, while most unpaired LCs appear to be completely encased in chromatin (Figs. 2, 5 and 7).

Two lightly stained fine grained nucleolus organizer regions (NOR) are found embedded in the periphery of the nucleolus (Fig. 5). The two chromosomes carrying the NORs are usually univalent for some distance adjacent to the NOR (Figs. 4 and 5), and with PTA staining the LCs of these chromosomes can be seen to penetrate into the interior of the nucleolus (Fig. 4). The satellite usually appears to terminate inside the nucleolus, the LC either folding back on itself or thickening to form a kind of knob, readily visible with PTA and Bernhard's staining (Fig. 8b). Reconstructions of three serially sectioned nuclei showed that the two NORs were in two cases widely separated on the single nucleolus, and in the third case the two NORs had coalesced but the LCs of their two chromosomes were unassociated. In all three nuclei the remainder of the NOR chromosomes showed no propensity to pair in SCs with each other (Fig. 9).

Using PTA stained material, three nuclei from two anthers have been serially sectioned and the LCs reconstructed. Figs. 4, 6 and 7 show a section of the whole nucleus, and paired and unpaired LCs. In two of the nuclei from different anthers the LCs have been completely reconstructed, while in the third nucleus all paired LCs and some unpaired

Figs. 4 and 5. Fig. 4. PTA stained nucleus, median section nucleus I. *Nu* nucleolus, *NOR* nucleolus organizer region, *Ch* chromatin, Double arrows — synaptonemal complex, Single arrow — unpaired lateral component. Bar 1.0 μ. × 12900. Fig. 5. Portion of nucleus, U/Pb stained. Nucleolus (*Nu*) with two lightly stained nucleolus organizer regions (*NOR*). Unpaired chromosomes with lateral components (*Ch*). Bar 1.0 μ. × 20000



Figs. 6 and 7. Fig. 6a–c. Three consecutive sections of chromosomes showing lateral components of the synaptonemal complex stained with PTA. Unpaired lateral component in cross section (*L*). Fig. 7a–c. Three consecutive sections showing diverging unpaired lateral components (single arrows) between normal synaptonemal complexes (double arrows). Other unpaired lateral components (*L*). PTA stain. Bar 1.0 μ. Both × 23300

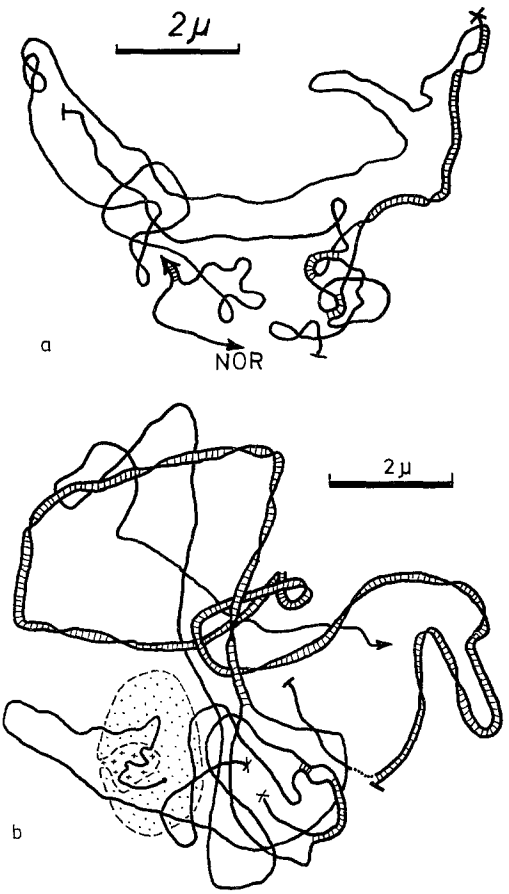


Fig. 8a and b. Reconstructions of portions of chromosomes from two nuclei; (a) from nucleus II showing long stretches of unpaired lateral components and short stretches of synaptonemal complexes (cross hatched); (b) from nucleus I showing more extensive synaptonemal complex formation. Nucleolus (stippled) and nucleolus organizer (crosses) are shown. Arrow heads indicate continuation of synaptonemal complexes or lateral components. X indicates lateral components whose ending is uncertain. Perpendicular lines indicate chromosomes ending at the nuclear envelope

LCs were reconstructed. Figs. 8a and 8b show portions of the reconstructions from the two completed nuclei. Extensive regions of fully paired SCs were found in all three nuclei, but nucleus I from anther 103 had considerably more SCs than the nuclei II and III from anther 116. Nucleus I had a total corrected SC length of 117μ, while nuclei II and III had 69μ and 51.6μ respectively. Nucleus I had 159μ of unpaired LCs, while nucleus II had 298.5μ of unpaired LCs. These give total haploid

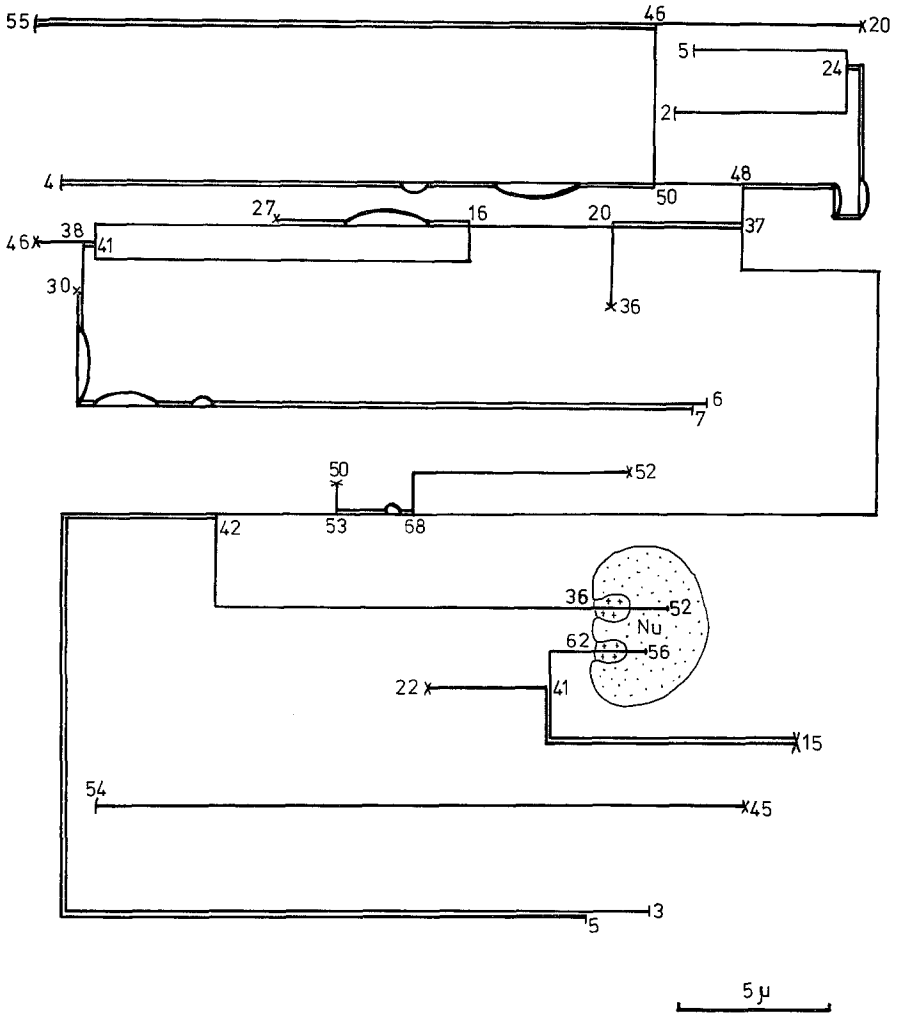


Fig. 9. Diagrammatic representation of synaptonemal complexes (parallel double lines) and unpaired lateral components (single lines) of nucleus I. Numbers are section numbers. Remainder of legend as for Fig. 8

chromosome length of 393μ and 436.5μ respectively. The amount of pairing in nuclei I and II is respectively 60% and 31% of total chromosome lengths. Nucleus III has approximately 25% pairing, based on the mean total chromosome length of nuclei I and II.

The difference in total amount of SCs in the nuclei of the two anthers was also reflected in the number and length of discrete pieces of SC.

Nucleus I had 19 pieces of SC, the largest being 35μ, while nuclei II and III had 22 and 15 pieces respectively, and the longest SC pieces were 14.9μ and 11μ. Nucleus I had five pieces 10μ or longer, while nucleus II had 2 pieces and nucleus III had one piece of this length. In all three nuclei the smallest recognizable length of SC was 0.3μ. The unpaired lateral components were naturally greater in length and number than the SC. The longest unpaired LC in nucleus I was 29μ, and in nucleus II was 38μ.

SCs are formed at many places on most chromosomes. Because of twisting in paired regions it was not always possible to continuously distinguish between the two LCs, but several cases were noted in which foldback loops appeared to form from one LC pairing with itself (Fig. 8a). An attempt was made to display the pairing configurations of the complete haploid chromosome complement in nuclei I and II, but in both cases the incompleteness of the reconstructions (due to ends being lost for technical reasons) meant that chromosomes could not be identified. Fig. 9 shows the results for nucleus I in which 13 chromosome ends were identified, two SCs appearing to pair chromosome ends. In nucleus II only a few ends were identified, two being paired in an SC. The reconstructions of several SCs with connecting LCs can only be interpreted if pairing involves two different chromosomes.

Discussion

The occurence of SCs in prophase I of haploid barley is not unexpected in the light of similar findings in other haploids. The extensive pachytene-type pairing visible at the light microscope level (Sadasivaiah and Kasha, 1971) is in agreement with the extensive presence of SCs. The complexes appear to be normal in most respects, and are very similar morphologically to those in diploid barley (Westergaard and von Wettstein, 1972).

The morphology of the unpaired LCs consisting of the dark rod in a lighter cylinder (Figs. 2 and 5), is also seen in the LCs of diploid zygotene nuclei (von Wettstein, pers. comm.), and is similar to the structure shown by Sen (1970) for leptotene in haploid petunia. Cross sections of unpaired LCs show this same image (Fig. 5). In contrast the LCs of paired SCs seem to be homogeneous. The sharp difference between the LCs of paired and unpaired regions (Fig. 1) suggests that some modification of the LC occurs, either during synapsis, or postsynaptically to stabilize the newly formed SC. This is in some ways similar to the situation in *Drosophila* (Rasmussen, 1974) and *Locusta* (Moens, 1973) where LCs only become fully elaborated after synapsis has occured. The change in arrangement of chromatin from roughly symmetrical around the LCs in unpaired regions, to asymmetrical in SCs, also suggests a rearrange-

ment of the chromosome as part of the pairing process. It is reminiscent of the model of von Wettstein (1971) which postulated the relocation of the LC at zygotene from between the two sister chromatids, to the outside of the chromosome, which would facilitate pairing with its homologue.

The total haploid chromosome lengths measured from the reconstructed LCs of nuclei I and II (393 μ and 436 μ) are approximately 83% and 92% respectively of the pachytene complement length of diploid barley (ca. 474 μ) reported in the light microscopic study of Sarvella *et al.* (1958). Such shorter electron microscopically determined lengths have been reported previously by Gillies (1972, 1973b) in *Neurospora* and maize, and probably reflect differences between preparative techniques for light and electron microscopy. Sarvella *et al.* (1958) found no morphological evidence for suspecting gross duplication or aneuploidy in the evolution of the barley karyotype.

Sadasivaiah and Kasha (1971) found that in haploid barley the pachytene pairing was as intimate as in diploids, and involved both foldback and interchromosomal pairing. The high proportion of SCs found here in nucleus I (60% of chromosome length paired) and the data from the reconstructions lead to similar conclusions. The difference in amount of pairing between nuclei II, III, and nucleus I, may represent progressive stages in the pairing process (equivalent to early and late zygotene) or it may represent variability in the amount of pairing in different nuclei or anthers. Both anthers were from the same spike, anther 103 (nucleus I) coming from the bottom 1/3 and anther 116 (nuclei II and III) coming from the top 1/3. The pairing and SC formation is so extensive that it must involve largely non-homologous associations. The alternative is to postulate duplications or partial polyploidy, which has been discounted by the studies of both Sarvella *et al.* (1958) and Sadasivaiah and Kasha (1971). The very low chiasmata frequencies at metaphase I (0.03–0.05 per cell) found by the latter authors supports this lack of duplication. Even the NORs, which would be expected to have essentially identical ribosomal RNA coding sequences, do not show any tendency for their LCs to pair, and in two cases the NORs were widely separated (cf. Fig. 5).

Non-homologous pairing of chromosomes at pachytene was extensively described in maize by McClintock (1933). She found a tendency for the ends of non-homologously paired chromosomes to be matched, even though non-pairing was found in proximal parts. This has been recently extended by Burnham *et al.* (1972) using double translocation heterozygotes in maize. Both Levan (1942) studying haploid rye, and Rieger (1957) studying haploid *Antirrhinum* also found extensive pairing at "pachytene" and synapsis of chromosome ends. In the present study,

matching of the ends completely or almost so, occurred in five cases in nucleus I (Fig. 9). Sadasivaiah and Kasha (1971) likewise noted a frequent matching of chromosome ends in their light microscope study of haploid barley "pachytene". Several authors have suggested that the nuclear envelope has a role in initiation of synaptonemal complex formation (Stern and Hotta, 1973), and the observed matching of ends seems to support this. However, the apparent lack of pattern in pairing, with both intra- and interchromosomal pairing, suggests that in haploids, the pairing itself is at random. Thus the absence of homologues seems to remove the constraint of matching homologous sites (perhaps a pre-alignment condition), and allows SC formation by chance collisions of LCs. The small differences in the lengths of the seven barley chromosomes (58μ at pachytene — Sarvella *et al.* 1958) may mean that this process can be almost completely random in barley haploids. The extensive nature of the SC formation probably means that synapsis is not initiated by matching of unique sites in the DNA (Stern and Hotta, 1973). The extended time which barley haploids spend in meiotic prophase up to "pachytene" allows a long period during which pairing can occur.

The extremely low chiasmata frequency reported in haploid barley (Sadasivaiah and Kasha, 1971) would indicate that essentially no crossing over occurred, in spite of the extensive SC formation. This may be a result of the synaptic and recombinational processes involving different sites or structures in the chromosomes. The synaptic process of SC formation may be non-selective in the absence of homologous regions. The recombinational process, in contrast, would require close matching of homologous sites, a condition absent in the non-homologously paired chromosome segments. This could be manifested as slight abnormalities in the SCs which are not detectable by the electron microscopic techniques used. The essential conclusion however, is that extensive regions of SC can be formed by non-homologous pairing, but that these do not lead to crossing over.

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Dr. C. B. Gillies,
Botany Building A12.
School of Biological Sciences
University of Sydney
New South Wales 2006
Australia