CHROMOCENTRES AND THE SYNAPTINEMAL COMPLEX

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SUMMARY

The 1-4 chromocentres seen in nuclei of Fritillaria lanceolata, which derive from fusion of heterochromatic segments situated proximal to the centromere in all but two of the 24 chromosomes, were studied with the electron microscope in thin sections of pollen mother cells at zygotene and pachytene, in respect of the synaptinemal complex. Prophase stages of meiosis in two plants were also surveyed briefly with the light microscope.

The latter observations revealed that the timing of the separation of heterochromatic segments from chromocentres is genetically controlled. In one plant the segments were still contained in chromocentres at pachytene, whereas in the other they were free at zygotene. At this time they could be identified by a near-surface position in the nucleus and an even condensation concomitant with an absence of chromomeres.

In thin section, the fine structure of the chromocentres in zygotene nuclei was distinctive in that the chromatin fibrils were less condensed and more widely dispersed than those in euchromatic regions. The fibrillar network was also interspersed with 'clear areas' or channels. After further chromosome condensation, the condensation of fibrils in the chromocentres became equivalent at pachytene to those in euchromatic regions.

Synaptinemal complexes were seen at zygotene and pachytene both in euchromatic regions and chromocentres. Their presence in the chromocentres signifies that homologous chromosomes must have been closely paired in regions extending from the centromeres to the distal ends of the heterochromatic segments already at telophase of the last pre-meiotic mitosis.

Configurations involving entangled pairs of axial cores, peculiar to zygotene and chromocentres and parts of euchromatic regions proximal to them, are interpreted as resulting from restricted movement.

INTRODUCTION

The involvement of the synaptinemal complex (Moses, 1958) in pairing of homologous chromosomes at meiotic prophase and in genetic exchange is now firmly established (see Moses, 1968). A great deal of the supporting evidence has come from electron-microscopical studies of the relevant stages of meiosis in animal cells. The function of the complex is seemingly to ensure close register of homologous parts of the paired chromosomes, which is required for the process of genetic exchange, though such exchange does not always follow.

A variant in chromosome behaviour which has yet to receive attention in relation to the complex is the formation of large chromocentres that derive from the fusion of two or more heterochromatic segments. In certain plants, e.g. some New World species of Fritillaria, this may include most if not all members of the chromosome complement. In the present investigation, involving both the light and electron microscope, the early stages of meiosis have been studied in diploid Fritillaria lanceolata.
As shown previously in root tips, all but two of its 24 chromosomes contain heterochromatic segments proximal to the centromere (La Cour, 1951). The close proximity of these regions at telophase favours their fusion, so that 1-4 chromocentres containing all these parts in a condensed condition are found at interphase.

**MATERIALS AND METHODS**

For studies with the light microscope, strings of pollen mother cells, expelled from anthers cut at one end, were fixed in ice-cold acetic-ethanol (1:3) for 4-6 h, and stored overnight or longer in 70% ethanol at 5°C. Squash preparations were made by an aceto-orcein method used previously for the study of leptotene (L. F. La Cour & B. Wells, unpublished).

For studies with the electron microscope, the strings of cells were fixed in ice-cold 10% (v/v) formalin in phosphate buffer (pH 7.6) plus 0.1% (w/v) calcium chloride and 0.3% (w/v) sucrose for 18-24 h. After fixation, the specimens were rinsed a few times in water and then transferred to Caulfield's osmium-sucrose (plus calcium chloride as above) for 2 h, in order to enhance contrast of the image in the electron microscope. After fixation the specimens were rinsed in water and then dehydrated through an ethanol series. To obtain further contrast they were stained for 1 h in 2% (w/v) uranyl acetate in absolute ethanol as the last step in dehydration. Cross-linked methacrylate (Kushida, 1961) was used for embedding. Sections were cut with a Cambridge-Huxley microtome, mounted supported on 'Parlodion' and then stained in lead citrate. All the electron micrographs were obtained with a Siemens Elmiskop 1 a.

**RESULTS**

**Light-microscope observations**

Anthers of two plants of *F. lanceolata* from different sources were studied. The plants were grown together in an unheated glasshouse and flower buds at similar stages of development were obtained from both plants on the same day. In respect of formation of chromocentres, the two plants were identical; 1-4 of these bodies were seen in nuclei of anther walls, tapetum and in those at pre-meiotic interphase. Unexpectedly, however, the timing of the separation of the heterochromatic (hetero-) segments from the chromocentres in prophase of meiosis differed in the two plants. In one, the segments were still fused at pachytene (Fig. 1), whereas in the other they had already separated at zygotene (Fig. 2). In Fig. 2 it can be seen that, as in mitotic interphase nuclei, the segments lie near the surface of the nucleus and, unlike the euchromatic (eu-) parts since they remain condensed, show no chromomeres.

**Electron-microscope observations**

The present investigation is concerned only with meiotic prophase in which chromocentres persist at pachytene.

The chromocentral chromatin could readily be distinguished from euchromatin in most thin sections of nuclei at zygotene. Apart from the larger area usually covered, the fibrillar structure was more loosely knit and the fibrils less condensed. This created an impression, perhaps real, that the fibrils were less intensely stained by uranyl/lead ions. The network of fibrils was also interspersed with 'clear areas', possibly due to channels. All these distinctive features are apparent in Figs. 3 and 4. From our experience with other plants, the character of the chromocentral chromatin
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more closely resembled that of chromatin flanking the axial cores of leptotene chromosomes.

Stretches of synaptinemal complexes involving the eu-parts of homologous chromosomes were fairly common in sections of nuclei at zygotene. In most of them the central component, in the pairing space between axial cores, was well defined (Fig. 5). In a few sections long stretches were seen which involved both the eu- and hetero-regions of homologous pairs (Figs. 3, 4). No central component is evident in these two particular sections, probably because of the plane in which they were cut. Although this may be so, it may be more than a coincidence that we saw only one complex with a conspicuous central component (Fig. 6) in all the numerous sections of chromocentres in zygotene nuclei which were examined. Usually the configurations appeared as paired axial cores, many of which were associated with the clear areas in the chromocentres, as in Fig. 4.

It is obvious that chromocentre formation must restrict the movement of the imprisoned hetero-regions and parts of eu-segments proximal to the chromocentres. In the chromosomes of *F. lanceolata* therefore it is only the distal parts of eu-segments which will have unrestricted freedom. The configuration seen in Figs. 4, 7 and 8, which appears to indicate a stress placed on paired chromosomes presumably entangled with other pairs, may be assumed to have arisen as a consequence of this restriction. As would be expected on this view, the anomalous behaviour appeared to be confined to chromocentres and eu-segments proximal to them. Further, it was evidently capable of correction, for the aberrations were not seen at pachytene, when the chromosomes are shorter.

In root tips it has been shown that minute eu-segments are intercalated singly in most of the hetero-regions of the chromosomes of *F. lanceolata* (La Cour, 1951). We were not able to recognize these segments in thin sections of the chromocentres. At pachytene, the chromatin fibrils in the chromocentres were as much condensed as those in the eu-parts, and there was an impression that they were now more closely packed. The channels seen at zygotene were still evident. Stretches of synaptinemal complexes showing the central component were seen more frequently in sections of the chromocentres at this time. An example is shown in Fig. 9.

**DISCUSSION**

So far as we are aware, evidence of the genetic control of timing of the separation of hetero-segments from chromocentres has not been noted previously. The size of the chromocentres found in many American species of *Fritillaria* (La Cour, 1951) probably makes this material more suitable than any other for its detection. In *Fritillaria* the persistence of chromocentres at pachytene may be the general rule, since they were also seen at this time by Darlington & La Cour (1941) in *F. pudica*, another Californian species.

It is evident that, as shown by the present study, observations with the light microscope are not always a reliable guide to the degree of condensation of chromatin. This is shown by the fact that, at zygotene, the chromatin fibrils in the chromo-
centres were more dispersed and more loosely coiled than those in euchromatin. The difference in degree of condensation between the eu- and hetero-regions, which is almost a reverse of that found in interphase nuclei, must be due largely to the coiling initiated at leptotene in the chromomeres of eu-segments (Ris, 1957; L. F. La Cour & B. Wells, unpublished). Another possible contributing factor is the difference in time at which these two parts of the chromosome replicate their DNA.

In root tips of this species, Pelc & La Cour (1960) found that the hetero-segments complete the synthesis of their DNA last, and this now appears to be a characteristic feature of these segments and hetero-chromosomes in both plant and animal cells (Lima-de-Faria & Jaworska, 1968). The bulk of the DNA of meiotic chromosomes is synthesized during pre-meiotic interphase (Taylor & McMaster, 1954; compare Hotta, Ito & Stern, 1966). Recently Callan & Taylor (1968) have found that during pre-meiotic synthesis of DNA in the newt *Triturus vulgaris*, the hetero-regions finish replicating their DNA about 1 day later than other parts of the chromosome. The possibility cannot be dismissed, then, that the hetero-regions lag behind the eu-parts at early prophase of meiosis. It is because of this possibility that we are inclined to believe that some of the configurations seen in the chromocentres during zygotene do not represent typical synaptinemal complexes. In other words, at this time they may consist only of paired axial cores.

The body of evidence accumulated elsewhere (see Moses, 1968) suggests that formation of the central component is a secondary event dependent on the coming together of the axial cores. In the pollen mother cells of a lily, Moens (1968) has shown that transverse filaments in the central element which extend from the inner (pairing) face of the lateral components (axial cores) correspond to fine fibrils coming from one side of homologous axial cores just before pairing. The transverse filaments were thought to interdigitate during synapsis. The body of evidence indicates that these filaments consist of protein. A protein fraction essential for pairing and formation of chiasmata has been shown to be synthesized at zygotene/pachytene by Hotta, Parchman & Stern (1968). It is tempting to consider that the hetero-segments may be out of phase with the eu-parts also in this respect.

There are few other studies of the synaptinemal complex which involve heterochromatin. In mammals the hetero-X and Y chromosomes associate in spermatocytes to form a sex vesicle. The fine structure of the sex vesicle in man and the mouse has been studied. In both, the interpretation of pairing is complicated by the fact that only a single minute segment in the X and Y is thought to be strictly homologous. A study of the fine structure of the sex vesicle in the mouse (Solari, 1964) indicates that here also the heterochromatin is out of phase with euchromatin during zygotene. This is suggested by the fact that synaptinemal complexes entailing the largely euchromatic autosomes were already in evidence at zygotene, whereas those involving the small homologous segments in the X and Y did not appear until pachytene. A study of the sex vesicle in man (Solari & Tres, 1967) did not reveal typical synaptinemal complexes at pachytene; the homologous segment in the X and Y was considered as perhaps being too short for their formation.

It is apparent that, as shown by the presence of synaptinemal complexes in the
chromocentres at zygotene/pachytene, homologous chromosomes must have been closely paired in regions extending from the centromeres to the distal ends of the hetero-segments prior to leptotene. As shown by Darlington (1935), initial pairing in *Fritillaria* begins close to the centromere, irrespective of the number of chiasmata formed, and evidently irrespective of whether the chromosomes have hetero-segments or not (compare La Cour, 1951). In *F. lanceolata* the bivalents at metaphase have 4–5 chiasmata freely distributed along the chromosomes (Darlington, 1936).

The possibility that there is some degree of alignment of homologous chromosomes at pre-meiotic interphase or telophase has long been considered. Slight evidence favouring this possibility was obtained in maize by Maguire (1967): Wagenaar (1969) has found evidence of end-to-end attachments of possibly homologous chromosomes in nuclei at interphase, early prophase and telophase in somatic cells of several plants, and has reviewed previous evidence for end-associations and homologous alignment of other chromosomal regions at interphase.

Studies on chiasma formation in a variety of organisms (Darlington, 1935, 1937; Frankel, Darlington & La Cour, 1940) indicate that in organisms in which at meiosis the cells have fewest chiasmata, pairing begins either near the centromere or near an end, and possibly sometimes at both these points when chiasmata are numerous. This does not, of course, prove that homologous chromosomes are closely paired at these sites prior to leptotene, though in respect of the centromere, it is obvious that, as a natural consequence of polarization at telophase, the centromeres of all the chromosomes are brought close together. Where they exist, this must also apply to relatively short arms of chromosomes. Proximal hetero-segments, as we have seen in *Fritillaria*, can effectively extend the distance of close alignment.

A previous study with the electron microscope of cores in metaphase chromosomes of other plants, where hetero-segments are absent or inconspicuous, did not reveal any suggestion of pre-leptotene alignment of homologous chromosomes along their length (L. F. La Cour & B. Wells, unpublished). It is unfortunate that we were unable to obtain material at leptotene in the present study.

The formation of chromocentres in *F. lanceolata* is closely similar to that found in *Drosophila melanogaster*, where at interphase all the chromosomes are held together at a single chromocentre, as shown by Prokofyeva-Belgovskaya (1935) in the polytene nuclei of salivary glands. Crossing over does not occur in the male and meiosis is achiasmate. Cytogenetic studies have shown that genetic exchanges involving the heterochromatin occur between heterologous chromosomes in gonial nuclei (see Cooper, 1949). The regular chiasma formation found in *F. lanceolata* excludes the possibility that such exchanges occur there.

Both large and relatively small fibrillar-free areas were seen in thin sections of the chromocentres. It seems most likely that the former represent occasional channels or spaces between individual hetero-segments. The paired axial cores seen at zygotene were frequently associated with the latter, but the reason why chromatin fibrils should be minimal in these instances is obscure.
REFERENCES


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Fig. 1. Photomicrograph of a pachytene nucleus in a pollen mother cell of *F. lanceolata*, showing 2 large chromocentres. Note bunching of eu-regions of the chromosomes proximal to the chromocentres. × 1200.

Fig. 2. Photomicrograph of a zygotene nucleus in a PMC from another plant of *F. lanceolata* grown under identical conditions to the plant from which the pachytene nucleus (Fig. 1) was obtained. The hetero-segments, which have no chromomeres, lie free near the surface of the nucleus; some are seen at extreme right and show evidence of pairing. × 1400.
Fig. 3. Electron micrograph of a thin section of a PMC of *F. lanceolata* at zygotene, showing part of a synaptinemal complex (sc) which includes eu-regions (e) and hetero-regions (h) of the homologous pair. Owing to the angle of the section part of one of the axial cores (ac) is not seen. Note less intense coiling of chromatin fibrils in hetero-segments contained in the chromocentre (c). The crystal at the left of the complex may be a virus contaminant. × 40000.
Fig. 4. Electron micrograph of a thin section of a PMC of *F. lanceolata* at zygotene, showing part of a synaptinemal complex which includes eu- and hetero-segments; the short arrows indicate paired axial cores, one of them distorted (see Figs. 7, 8). (e, eu-region; h, hetero-region; sc, synaptinemal complex.) × 28000.

Fig. 5. Electron micrograph of a thin section of a PMC of *F. lanceolata* at late zygotene, showing a synaptinemal complex involving homologous eu-segments. × 50000.

Fig. 6. Electron micrograph of a thin section of a PMC of *F. lanceolata* at zygotene, showing (arrow) a synaptinemal complex involving homologous hetero-segments. × 50000.
Fig. 7. Electron micrograph of a thin section of a PMC of *F. lanceolata* at zygotene, showing (arrow) paired axial cores involving eu- and hetero-segments which in the latter appear entangled with other paired cores. ×45,000.

Fig. 8. Electron micrograph of a thin section of a PMC of *F. lanceolata* at zygotene, showing (arrow) entangled pairs of axial cores in eu-regions proximal to a chromo-centre. ×60,000.

Fig. 9. Electron micrograph of a thin section of a PMC of *F. lanceolata* at pachytene, showing a synaptinemal complex involving hetero-segments. (*e*, eu-region; *h*, hetero-region.) ×60,000.