SYNAPTIC STRUCTURES IN THE NUCLEI OF
SPORULATING YEAST, SACCHAROMYCES
CEREVISIAE (HANSEN)

P. B. MOENS AND E. RAPPORT
York University, Downsview, Ontario and Simon Fraser University, Burnaby, B.C.,
Canada

SUMMARY

After 4 h on sporulation medium the larger cells have formed, within the nucleolus, a
spherical body of amorphous substance which stains less densely than the nucleolus. At 8 h most
of these bodies contain synaptonemal complex-like structures. There is usually only one such
body per nucleus. Only rarely are normal synaptonemal complexes detectable in the nucleus.
At the first meiotic division these bodies are still present but they no longer have polycytophases
associated with them. At this time they become sequestered in a nuclear evagination and they
are no longer detectable after the second meiotic division. It is argued that the polycomplex
body may elaborate synaptic elements which function in meiotic chromosome pairing but that
the resulting complexes are difficult to detect because of the lack of chromosome condensation,
a characteristic of several fungi.

INTRODUCTION

The report by Engels & Croes (1968) on the occurrence of synaptonemal complexes
in the yeast Saccharomyces cerevisiae suggests that the cytology of meiosis, which has
been difficult to resolve with the light microscope, may be clarified at the fine-structure
level. Such information may be particularly useful in this organism where the morpho-
logy, general cytology, physiology, biochemistry, and genetics are already well docu-
mented (Rose & Harrison, 1969).

The function of synaptonemal complexes in chromosome pairing and chiasma
formation is supported by observational evidence (Moses, 1968) and by experimental
evidence. Inhibitors of DNA synthesis and of protein synthesis have been found to
affect the formation of synaptonemal complexes, the presence of chiasmata and the
maintenance of chromosome integrity in Lilium pollen mother cells (Ito, Hotta &
Stern, 1967; Roth & Ito, 1967; Parchman & Stern, 1969). Temperature treatments of
the fungus Coprinus lagopus affect genetic recombination only while the synaptonemal
complexes are present (Lu, 1970). Such cytogenetic correlations have not been studied
in yeast, but may be done to advantage because meiosis can be synchronized (Roth &
Halvorson, 1969; Croes, 1967), because treatments with inhibitors are easily accom-
plished in this single-celled organism, and because genetic analysis can differentiate
the treatment effects on recombination between as well as within genes. This report
describes the development of synaptic structures and details of their fine structure
during normal meiotic prophase.
MATERIALS AND METHODS

Saccharomyces cerevisiae, of Hansen strain CBS 5525, were given to us by Dr. A. F. Croes. We used his clone no. 10 which Dr. Engels and Dr. Croes had found to have a high frequency of synaptonemal complexes (Engels & Croes, 1968). Cells were grown on an acetate presporulation medium (Roth & Halvorson, 1969). After 4, 7, 8, 9, 10 and 12 h at 30 °C on sporulation medium samples were taken and fixed in phosphate-buffered 3% glutaraldehyde for about 24 h and post-fixed in a phosphate-buffered 2% osmium tetroxide solution for 1 h. The samples were dehydrated in a graded series of ethanols and propylene oxide. Cells were infiltrated with Epon (Luft, 1961) at 60 °C for 2 days in the absence of accelerator. After reinfiltation with Epon and accelerator embedding was continued for 2 days at 60 °C. This method preserves the synaptic structures well and it gives well embedded cells, which is necessary for serial sections. The method does not preserve the membranes. On the contrary, the membranes are often negatively stained. If the membranes need to be preserved, fixation in KMnO₄ or pretreatment with gluulsulase may be more suitable.

Sections were cut about 0.09 μm thick with a Porter-Blum MT-2 microtome with a Dupont diamond knife. Serial sections were collected and mounted on single hole, Formvar-covered grids according to a modified Galey & Nilsson technique (Galey & Nilsson, 1966; Moens, 1970) and stained with a saturated aqueous uranyl acetate stain (Reynolds, 1963). Routine photography was done with 35-mm Kodak fine-grain positive film and details were recorded on Kodak electron image plates with a Philips EM 200.

RESULTS

After cells have been on sporulation medium for 8 h the larger cells have completed early sporulation and contain 4 immature ascospores while the smaller cells and buds have not as yet undergone meiotic divisions. All intermediate developmental stages can be found in such a sample. A large percentage of cells, possibly 50%, contain the synaptonemal complex-like structures (Figs. 2B, 3). Since there are frequently several such structures together the term polycomplex is used here. These complexes do not have detectable lateral elements. They consist of an electron-transparent central region of specific width, 0.1 μm, transected by an electron-opaque central element and bounded on both sides by a dense amorphous substance (Fig. 3A). The complexes are continuous from one section to the next in the plane of section and perpendicular to it. They therefore have a 3-dimensional structure rather than the essentially 2-dimensional structure of conventional synaptonemal complexes.

Serial sections of complete nuclei showed that, with few exceptions, the nucleus contains only one polycomplex. The polycomplex is usually located at the periphery of the nucleus, where it may or may not be associated with the nucleolus (Figs. 2B, 3A). (Identification of the nucleolus and ribosomes is based on the biochemical and electron-microscope study of isolated yeast nuclei by Molenaar, Smitt, Rozijn & Tonino, 1970). The polycomplexes were observed at the time that the spindle plaque on the nuclear membrane and the intranuclear spindle tubules became apparent in electron-microscope preparations, and at the time that the plaque divides into 2 plaques, a preliminary to the formation of the intranuclear spindle at the first meiotic division. (The development of spindles, spindle plaques and ascospores has been reported in 2 separate papers, Moens & Rapport, 1971; Moens, 1971).

While scanning large numbers of sections it became apparent that single synapto-
nemal complexes do also occur, but that they are difficult to detect. The lack of lateral elements renders the structure nearly unrecognizable. The occasional accumulation of chromatin frames the central region and the central element so that the characteristic structure and dimension of the synaptonemal complex can be recognized (Fig. 2C, arrow). All the same the continuity of these complexes is poor and their identification is equivocal so that no quantitative study can be made. Where there is insufficient chromatin flanking the complex the clear central region is not expressed and only the dense central element remains. By itself the central element is difficult to identify.

Table 1. Numbers of cells with synaptic structures at different stages of meiosis. Entire nuclei of cells on sporulation medium for 8 and 4 h were examined in serial sections

<table>
<thead>
<tr>
<th></th>
<th>No plaque observed</th>
<th>2 plaques side by side</th>
<th>2 plaques opposite</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) After 8 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No synaptic structures</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Polycrplex body</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Polycrplexes</td>
<td>5</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>(b) After 4 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No synaptic structures</td>
<td>2</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>Polycrplex body</td>
<td>3</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Polycrplexes</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The polycomplexes are surrounded by a fine, even, amorphous substance which stains more densely than the nuclear material but less than the nucleolus (Figs. 2, 3). The nucleolus, moreover, seems to be coarser textured and unevenly structured. The fine substance around the polycomplexes is in turn surrounded by a layer of dense granules resembling ribosomes. Whenever the fine amorphous substance was encountered the adjacent sections were checked for the presence of polycomplexes. It appeared that some did have complexes (Figs. 2A, 2B), but others did not (Figs. 1, 4). This spherical body which frequently contains the polycomplexes is here referred to as the polycomplex body, or pc body. It occurs only in cells undergoing meiosis and not in vegetatively growing cells.

The temporal relationship between the polycomplex body and the polycomplexes was studied in cells that had been on sporulation medium for 8 h. Because the formation of the spindle follows an obligatory sequence of developmental stages it can be used as a means of staging the progression of sporulation (Moens & Rapport, 1971). In early meiotic cells neither the spindle plaque nor the spindle tubules are well defined and such cells are scored as having 'no plaques.' As meiotic prophase progresses the single plaque and associated tubules become better differentiated. Where the disk-shaped plaque lies in the plane of section, it is difficult to identify, but the cross-sectioned tubules in adjacent sections signal the presence of the plaque. Sub-
sequently the plaque divides into 2 side-by-side plaques. Later these separate and rotate so as to bracket the intranuclear spindle at meiosis I. A total of 51 complete nuclei, of the 8-h sample, were scanned. The results, shown in Table 1, (a), show that the polycomplex and the pc body can be found at all stages. The impression is gained that the pc body forms early in meiotic prophase and then usually, but not necessarily, differentiates to include the polycomplexes. At the time of spindle elongation (anaphase I) the pc body does not contain complexes any longer. It becomes sequestered in an evagination of the nucleus (Fig. 4) and at later stages it can no longer be detected. (The nucleolus also forms one or more spherical structures which become similarly sequestered but they are, as before, darker staining, coarser textured, less well delineated and they remain visible until the early stages of ascospore development, located between the 4 immature ascospores.)

The occurrence of the pc body prior to polycomplex differentiation was checked in a sample of cells grown for 4 h on sporulating medium. The results from 11 complete nuclei are shown in Table 1, (b). In addition, no polycomplexes were found in a large number of single sections. The pc body does appear to precede the formation of polycomplexes. The pc body itself appears to be preceded by one or more light-staining, amorphous patches inside the nucleolus. In early cells the pc body is small and usually located inside the nucleolus (4 out of 5 in Table 1, (b)). At later stages it may remain associated with the nucleolus (Figs. 1, 2) or become separated from it (Figs. 3, 4). In nuclei undergoing the first meiotic division the pc body is separate from the nucleolus.

**DISCUSSION**

*Synaptonemal complexes*

The most striking feature of meiotic prophase nuclei in the yeast *Saccharomyces cerevisiae* is the virtual absence of conventional synaptonemal complexes. With the exception of some achiasmatic dipteran males and mutant *Drosophila melanogaster* females (Meyer, 1964) the synaptonemal complex occurs in all sexually reproducing organisms so far examined (Moses, 1968), including protists, fungi, plants and animals. It would seem unlikely that yeast which undergoes sexual reproduction, as well as meiotic genetic exchange, should be an exception. The presence of polycomplexes in many nuclei and of true synaptonemal complexes in some nuclei suggests that the machinery for the production of these synaptic structures exists but that the product may be unusually difficult to discern. A possible explanation for the scarcity of synaptonemal complex may be that the chromosomes of sporulation yeast remain so diffuse as to be indistinguishable in electron micrographs. (Also true of mitotic chromosomes, Robinow & Marak, 1966.) In most organisms the condensed chromatin delineates and accentuates the clear central region of the complex. Where the chromatin is extremely dispersed the dense lateral elements protect the complex from obscurity as is the case in the Basidiomycete *Coprinus lagopus* (Lu, 1967) and *Schizophyllum commune* (Volz, Heintz, Jersild & Niederpruem, 1968). Where the chromatin and lateral elements are not well defined only the central element remains and it is not sufficiently unique or continuous to be recognized as such, with certainty, in electron
Synaptic structures in yeast nuclei

Micrographs. Alternative, but less appealing, explanations are that the complexes occur over very short distances, or that they are present for very short times only, or that they are usually not present at all.

Polycomplexes

Synaptonemal complex-like structures, not associated with paired homologous chromosomes have been observed in a number of insects (reviewed by Moses, 1968). In the fungus Neottiella (Westergaard & Wettstein, 1970) and in the protist Pyrsonymphpha (Holland & Carruette-Valentin, 1970) and in the slime mould Stemonitis herbatica (Aldrich & Mims, 1970) such structures have been reported in association with the nucleolus. In the case of Neottiella the authors propose that 'The central region of the synaptinemal complex is assembled in the nucleolus and then laid down between the lateral components of homologous chromosomes.' Because the lateral elements of the Neottiella complexes are striking it can readily be seen that they do not occur in the nucleolus but only in the chromosomes. Jaworska & Lima-de-Faria (1969), on the other hand, propose that the multiple synaptonemal complexes of Acheta oocytes are located between the multiple DNA copies of the ribosomal cistrons. In either case the complexes express a pairing function and they may therefore serve as a useful parameter of the synopsis of genetic material during meiosis in this yeast.

We thank Dr A. F. Croes of the Department of Botany, University of Nijmegen, The Netherlands, for supplying the yeast. Financial assistance was given by the National Research Council of Canada.

References

(Received 23 March 1971)

Fig. 1. A, Yeast cells on sporulation medium for 4 h or more develop a spherical body of amorphous substance which is less densely staining and finer textured than the nucleolus (no) in which it is usually embedded. Because of its later association with the polycomplexes the structure is referred to as the polycomplex body (pcb). The pcb body is surrounded by granules which resemble the ribosomes of the cytoplasm (cy) (n, nucleus.) x 50000. B, A higher magnification to show the details of the pcb body. x 100000.
Synaptic structures in yeast nuclei
Fig. 2. Sections 17, 14 and 13 of one nucleus containing 2 side-by-side spindle plaques (in sections 25, 26 and 27, not shown here), a polycomplex (sections 13–16) and a single synaptonemal complex (section 17). × 50000.

A, The light-staining area within the nucleolus is marked by an arrowhead. It is similar in substance and position to the pc body of Fig. 1 but it now contains the polycomplex shown in Fig. 2B.

B, One section of a polycomplex (arrow) within the nucleolus (n). The electron-transparent central region is flanked by electron-opaque substance and it is transected by a darker-staining central element. (n, nucleus.)

C, The synaptonemal complex is marked by an arrow. The central element is distinct but the central region is revealed only because of the condensed chromatin framing it. It is suspected that there are more such complexes in the nucleus but that usually the chromatin is not condensed and the complex thereby loses an important identification characteristic.
Fig. 3. A–G, serial sections of a polycomplex and a summary sketch. There are side-by-side spindle plaques elsewhere in this nucleus (n). The polycomplex (arrow) is separate from the nucleolus (no). The dark-staining area in the middle of the nucleus is an invagination of the cytoplasm (cy). × 50000.
Fig. 4. A, At late stages of meiotic prophase and during the meiotic divisions the pc body (pcb) no longer has polycomplexes and it becomes sequestered from the nucleus (n). (cy, cytoplasm.) × 150000. B, A higher magnification of the pc body shows its amorphous, finely textured, evenly structured substance, surrounded by granules, similar to the ribosomes of the cytoplasm. × 100000.
Synaptic structures in yeast nuclei