Genetic Block of Outer Plaque Morphogenesis at the Second Meiotic Division in an hfdl-1 Mutant of Saccharomyces cerevisiae

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An hfdl-1 mutant of Saccharomyces cerevisiae, SOS4, characterized by predominant production of two-spored asci at 29 °C, undergoes normal meiotic nuclear divisions and produces four haploid nuclei, but only two non-sister nuclei among them are incorporated into mature ascospores. Spindle pole bodies and prospore wall membranes at the second meiotic division at 29 °C were observed in this mutant by electron microscopy. The spindle pole body at one pole of each spindle had a normal outer plaque which was larger than the inner plaque. At the other pole, the outer plaque was entirely absent and a normal prospore wall membrane was not detected. It was concluded that at 29 °C the hfdl-1 mutation blocks the morphogenesis of outer plaques and prospore wall membranes at the two non-sister poles in the second meiotic division, and consequently only non-sister nuclei in the resulting meiotic cell are incorporated into ascospores.

INTRODUCTION

Meiosis and ascospore formation in Saccharomyces cerevisiae provides an excellent model system for the investigation of developmental pathways (Hartwell, 1974; Esposito & Esposito, 1975). When diploid cells of S. cerevisiae heterozygous for mating-type locus are transferred into nitrogen-poor medium containing acetate as a carbon source they complete meiosis and ascospore formation to form four-spored asci. However, asci containing less than four spores can be produced physiologically and genetically (Takahashi, 1962; Haber & Halvorson, 1972; Tsuboi & Yanagishima, 1974; Davidow et al., 1980; Grewal & Miller, 1972; Moens et al., 1974; Srivastava et al., 1981). Analyses of abnormal asci are useful for elucidating the cause–effect sequences in sporulation (Moens et al., 1977; Davidow et al., 1980; Okamoto & Iino, 1981).

A mutant, SOS4, which predominantly produces two-spored asci, was isolated from a homothallic diploid, MT13 of S. cerevisiae, and its mutant phenotype (Hfd; High frequency of dyad production) was shown to be caused by a single nuclear mutation, hfdl-1 (Okamoto & Iino, 1981). The hfdl-1 mutant was distinct from other mutants in that it was deficient in the incorporation of two non-sister nuclei in a post-meiotic cell into mature spores (Okamoto & Iino, 1981).

Electron microscopic studies on the incorporation of nuclei into ascospores in wild-type diploids of S. cerevisiae have revealed a series of changes in intracellular structures (Moens, 1971; Moens & Rapport, 1971; Zickler & Olson, 1975; Horesh et al., 1979). Before the second meiotic division, the outer plaques of spindle pole bodies are modified and become larger than mitotic and first meiotic ones. At the second meiotic division, two spindles form in

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a nucleus and the nucleus buds to form a lobe at one side of each spindle pole. Then, each lobe is included by a prospore wall membrane which originates at the cytoplasmic side of the outer plaque. Eventually, each nuclear lobe 'pinches off' and the prospore wall membrane closes to form an immature ascospore. These observations suggest that the outer plaque plays an essential role in the formation of a prospore wall membrane (Moens, 1971; Moens & Rapport, 1971; Zickler & Olson, 1975; Horesh et al., 1979), and indicate that two sister nuclei, one of which is incorporated into an ascospore in the hfdl-1 mutant at 29 °C, share the same spindle at the second meiotic division.

This led us to expect that structural abnormalities might be detected in the mutant cell at, or near, one of the two poles of a second meiotic spindle. Therefore, we examined in serial thin sections the structures near the poles of the second meiotic spindles in the hfdl-1 mutant and its parental strain. We report here results which indicate that the outer plaque at one of the two poles of the second meiotic spindle is entirely absent in the mutant.

METHODS

Media. The composition of the presporulation medium (YPA) and the liquid sporulation medium (SPOL) were described previously (Fast, 1973; Okamoto & Iino, 1981).

Strains. For electron microscopy, an hfdl-1 mutant, SOS4 (hfdl-1/hfdl-1), and its parent, MT13 (HFD1+/HFD1+), were used. The former produces about 87% two-spored asci and the latter produces about 72% four-spored asci when sporulation is induced in SPOL at 29 °C (Okamoto & Iino, 1981). The genetic backgrounds of these two homothallic diploids differ only in a single nuclear gene, hfdl (Okamoto & Iino, 1981).

Electron microscopy of meiotic cells. SOS4 or MT13 cells were inoculated into 100 ml YPA at a concentration of 1–2 \times 10^5 cells ml^{-1}. After 15 h incubation at 29 °C, the cells were washed with 100 ml distilled water and resuspended in 100 ml SPOL in a 1 l flask. The flask was aerated on a Taiyo incubator (model M-100N) at 29 °C and at 100–120 strokes min^{-1}. At 7 h after transfer of the cells into SPOL, 33 ml of the SPOL culture was removed and 1 ml 70% (w/v) glutaraldehyde (Ladd Res.) was added. Cells were immediately pelleted and fixed with 5 ml 4% glutaraldehyde at room temperature overnight. The cell wall was subsequently digested by glusulase (Endo Laboratory Inc.) according to the method of Zickler & Olson (1975) except that 0.5 m-dithiothreitol was used instead of 0.1 m-β-mercaptoethanol. The cells were post-fixed with 4% (w/v) osmium tetroxide for 2 h, contrasted in 0.5% (w/v) aqueous uranyl acetate solution for 2 h, dehydrated in a graded ethanol series and embedded in Spurr's resin (Spurr, 1969). Serial sections were cut on a Reichert OMU2 ultramicrotome with a glass knife and picked up with Formvar-coated single-hole grids. Sections were stained in saturated uranyl acetate solution for 2 h and then with lead citrate for 5 min (Reynolds, 1963). Samples were observed in a JEM 100C electron microscope operating at an accelerating voltage of 100 kV.

RESULTS

The structures observed around five pairs of spindle poles in five, second meiotic cells of strain MT13 and seven pairs of spindle poles in six, second meiotic cells of mutant SOS4 are summarized in Table 1.

In strain MT13, two stages during the second meiotic division were discriminated based on the presence or absence of prospore wall membranes as previously reported (Moens & Rapport, 1971). For convenience, we call these stage I and stage II, respectively. Cells at stage I had no prospore wall membranes, but these developed in cells at stage II. All spindle pole bodies detected in MT13 cells, irrespective of the stage, had inner plaques and large outer plaques characteristic of the second meiotic division (Fig. 1) and normal prospore wall membranes developed at both poles in stage II cells. These observations are consistent with the results reported on other wild-type strains (Moens & Rapport, 1971).

In mutant SOS4 cells, three types of pairs of spindle pole bodies were detected (Table 1). In five of the seven pairs examined, the outer plaque was entirely absent at one of the poles, while the other pole had a normal outer plaque (Fig. 2). In one of seven pairs, the outer plaque was entirely absent at one of the poles, while the other pole had an outer plaque smaller than the normal outer plaque. In the remaining pair, the normal outer plaque was detected at one


**Meiosis in S. cerevisiae**

Table 1. *Structures observed in the spindle pole area during the second meiotic division*

Structures were identified with serial sections by electron microscopy. The two pole areas of each spindle were designated as X and Y. The structures observed were a prospore wall membrane (PSW), outer plaque (OP) and inner plaque (IP). Cells at stage I had no prospore wall membranes, but these developed in cells at stage II. The structures corresponding to 2a and 2b were observed in the same cell.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Stage</th>
<th>Observed cell no.</th>
<th>X</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PSW</td>
<td>OP</td>
<td>IP</td>
</tr>
<tr>
<td>MT13 (+/+)</td>
<td>I</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOS4 (hfd1-1/hfd1-1)</td>
<td>I</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>2a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>6</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

| +  | Presence of normal structures; ±, presence of smaller structures than those indicated as +; −, absence of structures; A, presence of abnormal structures.

of the poles and the other pole had an outer plaque which was smaller and thinner than normal. Four of the seven pairs described above corresponded to stage II. In these pairs, an abnormal prospore wall membrane, which appeared to be less dense and less rigid than the normal prospore wall membrane, developed near the pole where the outer plaque was entirely absent (Figs 3 and 4). In addition to these seven pairs of spindle poles, one spindle pole which had a normal inner plaque, an outer plaque and a prospore wall membrane was detected in both cell no. 3 and cell no. 4 of Table 1. One which had a much smaller and thinner outer plaque than that detected in pole area X of cell no. 6 was detected in the same cell.

**Discussion**

In each of the four pairs of spindle poles observed in the hfd1-1 mutant cells at stage II, there was a normal prospore wall membrane at one pole but not at the other. In two of three pairs observed at stage I, one spindle pole had a normal outer plaque and the other did not. Only the former is expected to develop a normal prospore wall membrane at the subsequent stage. These results are consistent with the previous conclusion that the two non-sister nuclei were incorporated into ascospores (Okamoto & Iino, 1981). The exceptional one in which outer plaques were detected at both poles at stage I, may correspond to a minor fraction producing three-spored ascii. Therefore, we conclude that the genetic block caused by the hfd1-1 mutation at 29 °C affects the morphogenesis of outer plaques and prospore wall membranes at the non-sister poles of the second meiotic spindle.

At a late phase of the first meiotic division, each spindle pole duplicates and side-by-side structures are formed (Moens & Rapport, 1971; Zickler & Olson, 1975). Then, the parental and daughter spindle pole bodies separate and the second meiotic spindle extends between the two (Moens & Rapport, 1971; Zickler & Olson, 1975). Considering these processes, we infer that in mutant SOS4 the pre-first meiotic spindle pole body duplication is normal but the pre-second meiotic duplications are so defective at 29 °C that newly synthesized spindle pole bodies lack the outer plaques (Fig. 5). This means that duplication of outer plaques at the pre-first meiotic division was not affected at 29 °C by the hfd1-1 mutation, but those at the pre-second meiotic division were affected.
Fig. 1. Electron micrographs of a second meiotic spindle of strain MT13 (HFD1+/HFD1+) in two serial sections. (a) A spindle pole body having normal inner (IP) and outer (OP) plaques, with microtubules (MT) extending from it. (b) A spindle pole body at the opposite pole, again having normal inner and outer plaques. The bar markers represent 0.2 μm.
Davidow et al. (1980) reported that under certain physiological conditions, presumably of nutrient deprivation, strain AP-1 of *S. cerevisiae* manifested an Hfd phenotype and formed non-sister spindle pole bodies entirely lacking outer plaque at the second meiotic division (Davidow et al., 1980). These phenotypes are similar to those of the *hfdl-1* mutant. However, no prospore wall membrane was detected in AP-1 near the pole lacking the outer plaque (Davidow et al., 1980), while an abnormal one was detected at this pole in the *hfdl-1* mutant.

There are three possible sequences of events which might cause the phenotype of the *hfdl-1* mutation. The first is that the *hfdl-1* mutation blocks the morphogenesis of outer plaques at the pre-second meiotic division and the absence of outer plaques results in the development of abnormal prospore wall membranes. The second is that the development of prospore wall membranes is blocked by the *hfdl-1* mutation and the resulting abnormalities lead to the absence of outer plaques. The third is that the *hfdl-1* mutation causes both abnormalities in parallel. Since morphogenesis of outer plaques at the pre-second meiotic division always precedes the development of prospore wall membranes in wild-type strains (Moens & Rapport, 1971; Horesh et al., 1979), the second possibility is unlikely. The results reported here do not discriminate between the remaining two possibilities.

The observed structures of 17 stage II spindle poles of MT13 or SOS4 cells can be divided into four classes as follows (numbers in parentheses represent the observed numbers of poles belonging to that class): 1 – a pole which has full length outer plaque and a normal prospore...
Fig. 3. Electron micrographs of the spindle pole bodies in Fig. 2(b) at higher magnification. (a, b) Two serial sections of the spindle pole body having the outer plaque (OP), with a normal prospore wall membrane (PSW) near the pole. (c, d) Two serial sections of the opposite spindle pole body lacking the outer plaque, with an abnormal prospore wall membrane (APSW) near the pole. The bar markers represent 0.2 μm.

wall membrane (11); 2 – a pole which has no detectable outer plaque and an abnormal prospore wall membrane (4); 3 – a pole which has a smaller and thinner outer plaque than normal and a normal prospore wall membrane (1); 4 – a pole which has a much smaller and thinner outer plaque than that described in class 3 and has an abnormal prospore wall membrane (1).
Fig. 4. Electron micrograph of a second meiotic cell in mutant SOS4. A spindle pole body (arrow) which lacks the outer plaque has an abnormal prospore wall membrane (APSW) near the pole. Double arrows indicate the location of the opposite spindle pole body (which appeared in subsequent sections), with a normal prospore wall membrane (PSW) developing near the pole. The bar markers represent 0.1 μm.
Fig. 5. Schematic representation of meiosis and ascospore formation in mutant SOS4 (hfdl-1/hfdl-1) at 29 °C. The intermediate figure for spindle formation shown in parenthesis has not been detected in meiosis but has been detected in mitosis (Peterson & Ris, 1976). Cell walls are omitted. 1, Nuclear envelope; 2, inner plaque; 3, outer plaque; 4, prospore wall membrane; 5, abnormal prospore wall membrane.

Poles belonging to class 1 have been observed by many workers and a role for the outer plaque in the formation of the prospore wall membrane has been suggested (Moens & Rapport, 1971; Zickler & Olson, 1975; Horesh et al., 1979; Davidow et al., 1980). Poles belonging to classes 2, 3 and 4 have not been reported previously. The presence of these classes suggests that the full length of the outer plaque is not a prerequisite for the formation of a prospore wall membrane. Moreover, it indicates that in the absence of the outer plaque, formation of the prospore wall membrane can proceed partially.

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REFERENCES


Meiosis in *S. cerevisiae*


