Reversal of cell determination in yeast meiosis: Postcommitment arrest allows return to mitotic growth

(Saccharomyces cerevisiae/commitment/sporulation)

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ABSTRACT When yeast from the early stages of meiosis are transferred from sporulation to growth medium, they can reenter the mitotic cell cycle directly. In contrast, cells from later stages of meiosis (after the initiation of the first nuclear division) will complete meiosis and sporulation despite the shift to growth medium, a phenomenon known as “commitment to meiosis.” This study reports the surprising finding that when the normal progression of meiosis is arrested, cells from later stages of meiosis can return to growth. Cells were arrested after the first or second meiotic division by three independent means: the spo14 mutation, the spo3-1 mutation, and a high-temperature arrest of wild-type cells. In every case, the arrested cells were able to form buds after transfer to growth medium. These cells, however, experienced a delay upon return to growth relative to uncommitted cells. We propose that the commitment phenomenon results from a transient delay of mitotic growth, which occurs specifically during meiosis, and that commitment does not involve an irreversible inhibition of mitosis as previously thought.

In many developing systems while cell fate is initially specified by extracellular signals, cells remain determined once these signals are removed. In the last several years, dramatic progress has been made in identifying the ligands and receptors that initiate cell determination. These functions, notably tyrosine kinase receptors, are conserved in a wide range of organisms (1). In contrast, comparatively little is known of the transition, generally termed commitment, that renders cell fate independent of environment. Evidence for commitment has been established in a number of experimental systems by removing partially differentiated cells from their natural environment. These cells remain stably determined and can complete normal differentiation under appropriate conditions, indicating that the maintenance of the determined state is independent of extracellular signals (2, 3).

Meiosis and sporulation in the yeast Saccharomyces cerevisiae provide an excellent model system for examining cell determination and commitment, since both the environment of the cell and individual genes can be easily manipulated. Diploid yeast can follow two alternative cell division pathways: mitotic division and growth or meiosis and sporulation. The meiotie fate is triggered by deprivation of glucose and nitrogen. As is true in many types of cell differentiation, cells in meiosis remain uncommitted through the early stages of the process—i.e., they will abort meiosis and return to mitotic growth if the cells are transferred from sporulation to growth medium (4). Significantly, genetic and cytological analyses have revealed that meiotic DNA replication, symmetrical complex formation, chromosome pairing, and DNA recombination all occur during this uncommitted period (5–7). In contrast, cells become fully committed to meiotic development at the initiation of chromosome segregation in meiosis I—i.e., after this point they will complete the two meiotic divisions and form spores despite the addition of glucose and nitrogen. This study presents the unexpected result that when meiotic cells are arrested after either the first or second division, cells are able to return to mitotic growth. Further experiments suggest that commitment results from a transient delay in mitotic growth rather than an irreversible inhibition of the cell cycle during sporulation.

MATERIALS AND METHODS

Strains. The following diploid strains were used in this study. H65 contains MATa/MATa ade2/ade2-1 ade5/ade5 can1/can1 cyh2/CYH2 his1/HIS1 his7/HIS7 hom3-10 leu2-3/leu2-1 lys1/LYS1 lys2/LYS2 meti4/MET14 met8/MET8 pet8/PET8 TRP1/trpl ura3/URA3. H91 is isogenic to H65 except for the presence of a homozygous disruption/duplication allele of SPO14 designated spo14:URA3 spo14 (8). RREE1525 contains MATa/MATa arg4-1/arg4-1 cyh1-1/cyh1-1 HO/HO met4/met4. SH495 is congenic to RREE1525 and is spo3-1/spo3-1.

Media. Growth (YPD) and acetate (YPA) media have been described (8). The sporulation media (SPII-30 and SPII-31) contain 20 g of potassium acetate per liter supplemented with 75 μg (each) of either adenine sulfate, L-leucine, and uracil per ml (for strains H65 and H91) or L-arginine and L-methionine (for strains RREE1525 and SH493).

Assaying Landmark Events in Meiosis. Fifty-milliliter cultures were grown for 24 hr at 30°C in YPA to 5.0 × 10^6 Cells were washed three times, resuspended in 25 ml of sporulation medium (pH 6.5), and aerated by shaking at constant temperature in a New Brunswick Scientific gyratory water bath shaker at 250 rpm. At intervals, 200-μl samples were fixed by addition of 400 μl of 95% ethanol. An aliquot (6 μl) of this cell suspension was spotted on a microscope slide and allowed to dry. Nuclei were stained by placing 6 μl of a solution of 1 μg of 4,6-diamidino-2-phenylindole dihydrochloride per ml (DAPI, Boehringer Mannheim), 0.1 mg of 1,4-phenylene diamine per ml (Sigma), 50 mM sodium phosphate buffer, and 50% glycerol on the dried cells. Cells were visualized by Nomarski/fluorescence microscopy on a Jenulinar microscope. Two hundred to 300 cells were counted for each sample. DNA content was determined by DNA staining with propidium iodide (Sigma) followed by flow cytometry (9) using a Becton Dickinson FACScan 4 flow cytometer and LYSYS II software. Meiotic intragenic recombination was assayed at the leu2 locus by plating cells on synthetic complete medium lacking leucine.

Return-to-Growth Experiments. At intervals during sporulation, 0.5-ml samples were transferred to 9.5 ml of pre-

Abbreviation: DAPI, 4,6-diamidino-2-phenylindole dihydrochloride.
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warm YPDA and aerated by shaking. At various times, aliquots (1 ml) were removed from the YPDA cultures, washed once with distilled H$_2$O, resuspended in 100 µl of distilled H$_2$O, and fixed by the addition of 200 µl of 95% ethanol. Nuclei were stained as described above. Immunofluorescent staining of spindles was performed as described (10), except that Zymolyase 20T (ICN) was substituted for 100T.

RESULTS

Wild-Type Meiosis I Binucleate Cells Complete Meiosis II and Spore Formation After Transfer to Growth Medium. In wild-type cells, chromosome segregation at meiosis I results in "binucleate" cells, as visualized by DAPI nuclear staining and fluorescence microscopy. These convert to "tetranucleate" cells at the completion of the meiosis II division and then form spores to yield tetrad ascii. Electron microscopy reveals that the nuclear envelope in yeast remains intact around the separated chromatids throughout meiosis.

In a control experiment, a wild-type culture was sporulated until 10–15% of the cells had completed meiosis I and become binucleate. The culture was then shifted to growth medium and continued progression of these binucleate cells through meiosis measured by the appearance of tetranucleate cells and tetrads ascii (Fig. 1A, circles) and the disappearance of binucleate cells (Fig. 1B, left panel). The ability of cells that have completed meiosis I to return to growth was assayed by the appearance of buds associated with binucleate cells (Fig. 1B, left panel). Both criteria indicate that wild-type binucleate cells do not form buds or return to growth; instead, they proceed through meiosis and sporulation, in agreement with prior studies (see the Introduction). Equivalent observations were made for wild-type tetranucleate cells in this and another strain background (cf. Fig. 1 C and D, left panel).

Temperature-Arrested Wild-Type Binucleate Cells Can Return to Growth Directly Without Completing Meiosis. Our interest in commitment arose during the characterization of spo14, a new mutant defective in sporulation (8). In spo14 mutants, the second meiotic division and spore formation are defective (Fig. 1A, triangles), while earlier meiotic events are largely unaffected (Table 1). This results in the accumulation of binucleate cells during meiosis. Surprisingly, these binucleate cells form buds when transferred to growth medium (Fig. 1B, compare left to middle panel); furthermore, these cells continue to divide and yield viable products (8). Since

![Completion of meiosis vs. return to mitotic growth in wild-type and arrested meiotic cells. (A) Meiosis II in wild-type and spo14 cells. The % cells completing meiosis II (tetranucleate cells plus tetrad ascii/total cells) was monitored in a wild-type strain (circles) and in an isogenic spo14 disruption (triangles) at 30°C, and in the wild-type after shifting to 38°C at 9 hr of sporulation (squares). At 10 hr, cells from sporulation culture were transferred to growth medium, and their progress through meiosis II (open symbols, transfer indicated by arrow) was compared to those remaining in sporulation medium (closed symbols). In the wild type, 64% of the total cells form tetrad ascii after 17 hr of sporulation, compared to 46% tetrad ascii after 10 hr of sporulation followed by 7 hr in growth medium; in the spo14 mutant, <0.1% of the cells form ascii under either condition. (B) Return to growth of binucleate cells arrested in meiosis. The fraction of binucleate cells without buds (solid bars) or with buds (stippled bars) was measured after 10 hr of sporulation or 10 hr of sporulation followed by 4 hr in growth medium: wild-type (left panel), spo14 disruption at 30°C (middle panel), and wild-type transferred to 38°C at 9 hr (right panel). The % binucleate cells in the wild-type decreases after transfer to growth medium as these cells complete meiosis and form ascii. (C) Spore formation in wild-type and spo3-I cells. Asci were monitored in a wild-type (circles) and a spo3-I strain (triangles) in sporulation medium (closed symbols), or following transfer to growth medium after 13 hr of sporulation (open symbols, transfer indicated by arrow). (D) Return to growth of tetranucleate cells arrested in meiosis. The fraction of tetranucleate cells without buds (solid bars) or with buds (stippled bars) was measured after 10–13 hr in sporulation medium or after 10–13 hr in sporulation medium followed by 4–5 hr in growth medium: wild-type (left panel), spo3-I (middle panel), and spo14 disruption (right panel).]
tetranucleate cell that has undergone several divisions. This cell was sporulated for 13 hr and then exposed to growth medium for 4 hr. (C) A spo3-1 tetranculate cell that has undergone several divisions. This cell was sporulated for 13 hr and then exposed to growth medium for 7 hr. These cells are rare; more commonly, spo3-1 tetranculate cells undergo complete cell separation after each division. (∗×160.)
explanation for these results is that interruption of meiosis is sufficient to allow the arrested cells to return to growth.

**Mitotic Growth from Multinucleate Cells.** How do binucleate and tetranucleate cells return to growth? Although it is not always feasible to follow the nuclei of an individual cell as it buds and returns to growth (fluorescence visualization generally prevents further growth of the cell), a combination of microscopy and genetic analysis allows reconstruction of the principle events of this process. While the general mechanism of return to growth is not yet known, the following observations suggest that one nucleus of a multinucleate cell migrates into the new bud and then reenters the mitotic cycle at the G1 stage.

Immediately after transfer to growth medium, unbudded binucleate cells decrease in the population as *budded* binucleate cells appear (Fig. 1B). Later, the absolute number of binucleates declines rapidly (Fig. 3, triangles and circles) without any increase in the total number of tri- and tetranucleate cells (Fig. 1A, open squares and triangles; Fig. 1D). These data favor a mechanism in which the binucleate cells, instead of undergoing a new round of nuclear division of one or both nuclei upon return to growth, undergo a transfer of one of the two meiosis I nuclei to the bud to give rise to mononucleate mother and daughter cells. Similar results were also obtained for *spo3*-arrested tetranucleate cells; they first form buds (Fig. 1D) and then their absolute number decreases rapidly in the population (Fig. 3), and cells with more than four nuclei are not seen.

Other evidence comes from genetic characterization of colonies formed after *spo14*-arrested meiotic cells are returned to growth (8). For each chromosome tested, these colonies were shown to be homozygous diploids, containing two copies of one parental chromosome and no copies of its homolog. Their phenotypes are consistent with transfer and propagation of a single diploid nucleus of a meiosis I binucleate cell into the daughter. Their origin is incompatible with one diploid nucleus undergoing mitosis into the bud in the absence of an S phase (as that would generate haploid daughters) and one nucleus undergoing an S phase then mitosis into the bud (as the absolute number of binucleates should remain constant).

A final line of evidence is based on the finding that although the majority of *spo14* cells arrest as binucleates, a minority of the cells (5%) reach the tetranucleate stage. Transfer of these cells to growth medium is particularly informative because of the two meiosis II spindles present in tetranucleate cells (tubulin staining of these cells is also much clearer than in *spo3* cells). Eighty-four nucleate cells in which at least one nucleus is present in the bud were examined; these often reveal a spindle that extends from a nucleus in the mother to one in the bud (Fig. 4 A–D), suggesting that the preexisting meiotic spindle is reoriented during the initial cell division to promote migration of one of the nuclei into the new bud. Consistent with this view is the observation that the nucleus still in the mother cell is usually near the bud neck (Fig. 4 A–D) and that in some cells it appears that one spindle connecting two nuclei has entirely migrated into the daughter cell (Fig. 4 E and F).

**Arrested Binucleate Cells Return to Mitotic Growth More Slowly than Mononucleate Cells.** What causes a cell to become committed to meiosis (14–16)? One explanation is that in the course of meiosis, cells lose the machinery necessary to return to the mitotic cycle, for example, by losing receptors for glucose or nitrogen. However, the demonstration that arrested binucleate and tetranucleate cells can return to growth rules out models that involve irreversible changes in the cell. Rather, it suggests that commitment is caused by transiently repressing mitosis and that this regulatory state can be reversed with time. By this view, when cells are blocked in meiosis, they eventually become derepressed for mitotic functions and can return to mitotic growth. Support

![Fig. 4. Visualization of spindles in multinucleate cells after return to growth. A *spo14* strain (H91) was exposed to sporulation medium for 16 hr at 30°C and then transferred to growth medium for an additional 6 hr. Each pair of photographs shows the same cell stained with DAPI (left) or by anti-tubulin (right). (A and B) Cell indicated by arrow. A long spindle connects one of two nuclei present in the (putative) mother cell with a nucleus present in the daughter cell. (C and D) Two of three nuclei in the (putative) mother cell are connected by a spindle, while the third nucleus is connected by a second spindle to a nucleus in the daughter cell. (E and F) Cell indicated by arrow. One pair of nuclei are present in each cell, each pair connected by a spindle. Both spindles are aligned and perhaps connected. Ninety-four percent (283/300) of the total cells in the culture stain with both DAPI and anti-tubulin. Thirty-seven percent of multinucleate cells (30/80) contain a long spindle like those shown here; the remaining cells contain spindle pole bodies (38/80) or short spindles (9/80) associated with each nucleus. (×1750.)](image1)

![Fig. 5. Return to growth of *spo14* binucleate cells is delayed relative to completion of sporulation in wild-type cells. The ordinate indicates the fraction of cells that have either initiated budding or completed sporulation as a percent of the maximum levels of these processes; the abscissa indicates the time after transfer of a 10-hr sporulation culture to growth medium. Samples were removed periodically, fixed, and examined by Nomarski/fluorescence microscopy for bud emergence in asci (triangles) and mononucleate (squares) cells of the mutant and spore formation in the wild-type strain (circles). At maximum, 56% of the total binucleate cells and 52% of the total mononucleate cells contained buds; at maximum, 46% of the total cells completed sporulation.](image2)
for this idea comes from comparing the rate of return to growth in a spo14 culture to the rate of completion of meiosis in a wild-type culture (Fig. 5). Although more than half of spo14-arrested binucleate cells initiate budding upon transfer to growth medium, bud emergence occurs only after a lag of several hours (Fig. 5, triangles). In contrast, mononucleate (i.e., uncommitted) cells from the same spo14 culture return to mitotic growth much more rapidly (Fig. 5, squares), consistent with the idea that mitotic functions are transiently repressed in committed cells. Although mitotic repression in committed cells is not permanent, it is sufficiently long to allow the completion of sporulation in growth medium when meiosis is not arrested (Fig. 5, circles)—i.e., it is sufficient to cause commitment. This time delay in returning to growth may reflect the time required to reprogram the regulatory state of the cell.

**DISCUSSION**

After the initiation of chromosome segregation in meiosis I, yeast cells are able to complete meiosis and sporulation even if transferred to growth medium, a phenomenon termed "commitment to meiosis." The primary result described in this paper is that commitment is a reversible process: if meiosis is arrested, cells from the postcommitment stages of the meiotic pathway can reenter the mitotic cell cycle; they are not "stuck in the meiotic program." What does it mean for a cell to be "committed to meiosis," given that this commitment is reversed when cells are arrested? One explanation is that commitment occurs when the time period required for reinitiating mitotic growth exceeds the time required for completing meiosis. This idea, called the "kinetic choice" model for commitment, was suggested to explain how spo14-arrested binucleate cells are able to return to growth (8). By this argument, commitment is reversible when growth is arrested, and this is because now the cells have sufficient time to return to the mitotic cycle. The results presented in this paper strongly support this idea since arresting meiosis by any of three different means can reverse commitment, indicating that blocking differentiation allows this reversal regardless of the cause of the arrest.

The second major finding described above is that the resumption of mitotic growth from arrested postcommitment cells is delayed relative to uncommitted cells. While the original version of the kinetic choice model proposed that the commitment process need not "specifically inhibit mitotic functions," the results shown in Fig. 5 suggest that mitotic functions are, in fact, inhibited. We found that cells arrested after the time of commitment return to mitotic growth after a long delay (4 hr) relative to uncommitted cells from the same culture (1 hr). This last result indicates that the commitment process involves functions that (transiently) inhibit the response to growth signals. In summary, commitment is understood as a kinetic difference between the completion of meiosis (fast) and the response to nutrients (slow). This mechanism for delaying the response to nutrients in committed cells may represent a novel means of controlling differentiation.

Meiotic cells arrested at the binucleate and tetranucleate stage and allowed to return to mitosis may prove useful for examining the mechanics of mitotic cell division. Our results are most consistent with multinucleate cells returning to growth via a pathway in which a nucleus migrates into a new bud and resumes the mitotic cell cycle at the G1 stage. Nuclear migration to the bud neck occurs prior to mitosis in yeast and also occurs before nuclear fusion during mating. Although migration of a nucleus into a new cell body without nuclear division is not normally seen in wild-type yeast, it is common in some other fungi and in algae (17). Furthermore, examination of several S. cerevisiae mutants (e.g., num1 and dcl1) that transiently generate binucleate cells during mitosis has led to the suggestion that the daughter nucleus migrates into the bud after mitosis in these mutants (18, 19). Time-lapse photography of a binucleate cell that is undergoing such a nuclear migration supports this view (Ayumu Yamamoto and Doug Koshykald, personal communication). In line with the behavior of these mitotic mutants, one way to explain our results is to say that migration of preexisting meiotic spindles in multinucleate cells results in the segregation of one nucleus into the daughter cell. In many cases it appears that two sister nuclei still connected by a meiosis II spindle move together into the new bud, suggesting an interaction of either the spindle or the nuclei with an intracellular matrix.

In a variety of developmental systems, determined or even terminally differentiated cells can revert to other developmental fates under special circumstances, a phenomenon known as transdifferentiation. Two examples of this reversal in cell fate are the regeneration of cells of the retina from surrounding cells of different type and the conversion of determined imaginal disk cells in Drosophila to alternate fates after prolonged growth in culture (20, 21). The findings reported here suggest that in the case of yeast meiosis, simply blocking the preferred developmental pathway can be sufficient to allow previously committed cells to adopt a mitotic rather than a meiotic fate. Acquisition of an alternate fate as a result of a defect in normal differentiation, and particularly regaining the capacity for mitotic growth and division as a result of blocking the progression of development, could be involved in other examples of transdifferentiation, in oncogenesis, and in the normal adaptation of developing cells to environmental variations.

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