DIPLOID SPORE FORMATION AND OTHER MEIOTIC EFFECTS OF TWO CELL-DIVISION-CYCLE MUTATIONS OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

The meiotic effects of two cell-division-cycle mutations of *Saccharomyces cerevisiae* (*cdc5* and *cdc14*) have been examined. These mutations were isolated by L. H. HARTWELL and his colleagues and characterized as defective in mitosis, causing a temperature-sensitive arrest in late nuclear division. When subjected to the restrictive temperature in meiosis, diploid cells homozygous for either of these mutations generally proceeded through premeiotic DNA synthesis and commitment to meiotic levels of recombination, but then arrested at a stage following spindle pole body (SPB) duplication and separation. The two SPBs lacked the interconnection by spindle microtubules typical of the complete meiosis I spindle. Challenge of these homozygotes by a semi-restrictive temperature often caused the production of asci containing two diploid spores. Genetic analysis of the viable pairs of spores revealed that each spore had become homozygous for centromere-linked markers significantly more frequently than for distal markers, indicating that the two spores each contained pairs of sister centromeres that had co-segregated in the reductional division of meiosis I. Ultrastructural analysis of the *cdc5* homozygote demonstrated that these cells had completed meiosis I and formed two meiosis II spindles, but that the latter remained unusually short. This resulted in the encapsulation of both poles of each spindle within a single spore wall. These mutations therefore are defective in both meiotic divisions, as well as in the mitotic division described originally.

NUCLEAR division of eukaryotes typically occurs in more than one manner during the life cycle. Mitotic division provides for the equational distribution of the replicated chromosomes, whereas meiotic divisions consist of both a reductional division of the centromeres and a subsequent equational division. The mode of centromeric orientation on the spindle appears to account for these differences (NICKLAS 1974), but the underlying mechanisms remain obscure. One means of approaching this problem is in the study of mutants that are defective in various aspects of nuclear division. Among mutations of this sort, many appear to have their primary effects on DNA replication or DNA metabolism associated with recombination or repair (BAKER *et al.* 1976). In order to focus more directly on the chromosomal segregation mechanisms, it may prove useful to examine mutants that do not appear to alter DNA metabolism.

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In the budding yeast *Saccharomyces cerevisiae* a set of mutations that affect mitotic nuclear division has been characterized by HARTWELL *et al.* (1973). These mutants, designated *cdc* (cell-division cycle) mutants, are temperature sensitive for cell division, the cells arresting at specific stages of the mitotic cell cycle. Several of the *cdc* mutants undergo a normal round of DNA replication and then are arrested by a specific defect in nuclear division (HARTWELL 1976). Cytological analysis reveals that these arrests occur at specific stages in the formation or elongation of the intranuclear mitotic spindle (BYERS and GOETSCH 1974). Furthermore, most of the *cdc* mutants were shown by SIMCHEN (1974) to be temperature sensitive for sporulation, which normally follows the meiotic divisions in yeast. In order to gain a better understanding of the roles played by these genes in meiosis, we have undertaken an analysis of their cytology in comparison with that of normal strains, as described previously (MOENS and RAPPORT 1971a,b; BYERS and GOETSCH 1975; ZICKLER and OLSON 1975). We report here the meiotic terminal phenotypes of strains mutant in two genes (*cdc5* and *cdc14*) that are required for late nuclear division in mitosis (CULOTTI and HARTWELL 1971; HARTWELL *et al.* 1973).

MATERIALS AND METHODS

Strains: Our standard diploid strain DA364A (*MAT***a**/*α ade1*/+ *ade2-1*/*ade2-R8 can1*/+ *leu1*/+ *ura3*/+ *gal1*/+ *his7*/+ *lys2*/+ *tyr1*/+ *ura1*/+ was wild type with regard to the *cdc* mutations and was heteroallelic at *ade2* (SCHILD and BYERS 1978). The *cdc* heterozygotes D473 (*cdc5-1*/+) and D7041 (*cdc14-1*/+ have the same nutritional markers as DA364A. The *cdc* homozygotes, D473H (*cdc5-1*/*cdc5-1*) and D7041H (*cdc14-1*/*cdc14-1*), were isolated after X-ray-induced mitotic recombination of the *cdc* heterozygotes; they were tested by dissection and complementation to insure homozygosity of the *cdc* mutation, as previously described (SCHILD and BYERS 1978).

Media and procedures for growth, sporulation, electron microscopy, measurement of DNA synthesis and assaying of commitment to intragenic recombination have been described (SCHILD and BYERS 1978).

Nuclear stain: Cells were stained with DAPI (4′, 6-diamidino-2-phenylindole) after ethanol fixation (WILLIAMSON and FENNELL 1975) and viewed in a Leitz fluorescence microscope.

RESULTS

We first assayed the ability of strains homozygous for *cdc5* and *cdc14* to sporulate at both the permissive temperature (21°) and the restrictive temperature (33.5°). The results (Table 1) show that both homozygotes are temperature sensitive for sporulation, as previously reported by SIMCHEN (1974). We also noted that sporulation of the *cdc* homozygotes at 21° differs from that of the wild-type and *cdc* heterozygous strains. At 21°, the *cdc* homozygotes produce not only four-spored asci, but also many two-spored asci containing spores that are larger than normal. Data presented below demonstrate that these large spores are diploid and that they arise from an abnormal second meiotic division at semi-restrictive temperatures (below 33.5°). At the restrictive temperature (33.5°), most cells homozygous for either *cdc5* or *cdc14* fail to form spores (Table 1). In order to determine the stage of meiosis at which these cells fail, we examined

TABLE 1

Sporulation of the late nuclear division cdc *mutations and wild-type strain*

	Strain	*cdc* Genotype	Percentage sporulation (48 hr)* 21° 2-Spored asci	21° Total asci	33.5° 2-Spored asci	33.5° Total asci
Heterozygotes	D473	*cdc5-1/+*	4	77	7	71
	D7041	*cdc14-1/+*	4	73	12	63
Homozygotes	D473H	*cdc5-1/cdc5-1*	27†	81	3†	7§
	D7041H	*cdc14-1/cdc14-1*	7†	75	0	0
Wild type	DA364A	+/+	3	76	15	65

* Results for *cdc* homozygotes and for DA364A are averages of three separate experiments.
† Larger spores than normal; see text.
§ Includes 4% 1-spored asci, but no 3- or 4-spored asci.

DNA synthesis, commitment to meiotic recombination and ultrastructure of *cdc* homozygotes at the restrictive temperature.

DNA synthesis and commitment to meiotic recombination: The *cdc* homozygotes were tested at the permissive and restrictive temperatures for premeiotic DNA synthesis by the diamino-benzoic acid (DABA) fluorometric assay and for commitment to meiotic levels of intragenic recombination (henceforth termed "commitment to recombination") by the appearance of adenine prototrophs resulting from intragenic recombination between the heteroalleles at *ade2*. Yeast cultures transferred from sporulation medium back to vegetative medium before the cells have become committed to meiotic division and sporulation may be found to have undergone commitment to meiotic levels of recombination (SHERMAN and ROMAN 1963). Therefore, mutants unable to complete meiosis, such as the present *cdc* homozygotes at the restrictive temperature, may be examined for their commitment to recombination (ESPOSITO and ESPOSITO 1974; SIMCHEN 1974). Cells arrested in meiosis by the restrictive temperature were plated on medium lacking adenine to assay for adenine prototrophs, as well as on rich (YEPD) plates to permit normalization for the number of viable cells recovering from the arrest. Unarrested cultures may already have expressed their commitment to recombination before plating; for ease of comparison with the arrested cultures, in this report we consider extant levels of recombinants in unarrested cultures to be a measure of commitment to recombination.

The *cdc5* homozygotes undergo premeiotic DNA synthesis and commitment to recombination at both 21° and 33.5° (Table 2). At 33.5°, viability is significantly reduced by 24 hr (down to 34% of that in the 21° culture), but viability is normal at 12 hr, when commitment to recombination at 33.5° is similar to both the 12- and 24-hr levels in the 21° culture. Therefore, most of the *cdc5* homozygous cells undergo commitment to recombination prior to arresting in meiosis at 33.5°. The higher level of apparent commitment to recombination after 24 hr at 33.5° than at 21° is probably due to the heterozygosity at *ade1;* the formation of spores, which is limited to the lower temperature, would render half of the *ADE2* recombinants still auxotrophic for adenine because of segrega-

TABLE 2

Premeiotic DNA synthesis and commitment to recombination in late nuclear division cdc *homozygotes and wild-type control*

Strain	DNA content (24 hr)*		Commitment to recombination†			
	21°	33.5°	21°		33.5°	
			12 hr	24 hr	12 hr	24 hr
D473H (*cdc5-1*)	2.2	2.0	187	249	215	335‡
D7041H (*cdc14-1*)	2.1	1.8	386	270	161	84‡
DA364A (*CDC+*)	2.1	2.0	338	417	120	207

* Relative to content of 1.0 at 0 hr in SPM; average of three separate experiments.
† Adenine prototrophs per 10^6 viable cells; average of two separate experiments at 12 hr and of three experiments at 24 hr. These data include *bona fide* meiotic recombinants in some cultures; see text.
‡ Viability was reduced at 33.5°; see text.

tion with *ade1*. Although segregation of *ade1* partially obscures detection of recombinants in the *ADE2* locus, we retained the *ade1* marker present in the original *cdc* isolates in order to avoid variation in the genetic backgrounds of our diploid strains.

The *cdc14* homozygote exhibited an 80% increase in DNA content prior to arresting in meiosis at 33.5° (Table 2). Although this increment might be attributable to a partial round of premeiotic DNA synthesis occurring in all cells, ultrastructural analysis (described more fully below) suggested a different explanation. In addition to many cells arrested in meiosis, a substantial fraction of the cells failed to begin meiosis, so that the observed DNA increase probably represents a full round of synthesis in only those cells that entered meiosis. Commitment to recombination in the 33.5° culture achieved 49% of the 21° control value at 12 hr, but then declined to 31% by 24 hr (Table 2). Viability declined to 26% and 13% of the control values of the respective time points, so that recombination possibly occurred in a subset of cells that had a greater probability of becoming inviable at later time points.

Ultrastructural analysis of terminal phenotypes: Cells subjected to 33.5° arrest of sporulation were fixed and embedded for serial-section electron microscopy. By 24 hr, the wild-type strain was found to have reached near-maximal levels of sporulation at 33.5°; thus, we assumed that *cdc* homozygotes had accumulated at their respective terminal phenotypes. Gradual loss of viability in cells arrested longer than 24 hr prevented effective observation of ultrastructure at later times. Among the cells of the *cdc5* homozygote and homozygotes for other *cdc* mutations observed in an arrested culture (SCHILD and BYERS 1978), 5 to 15% remain in a nonsporulating state, bearing a single spindle pole body and no modification of intranuclear anatomy. Because a similar proportion of cells remain in this state in cultures sporulating under permissive conditions, this failure does not appear to result from the *cdc5* mutation. A relatively larger proportion of cells in *cdc14* homozygote culture are arrested at this stage.

Most cells of the *cdc5* homozygote are arrested in meiosis at 33.5° after both duplication and separation of the spindle pole bodies (SPBs) (Table 3; Figure 1).

TABLE 3

Ultrastructural terminal phenotypes of late nuclear division cdc *homozygotes in meiosis, with comparison of meiotic and mitotic phenotypes*

Strain	Ultrastructure of cells at terminal phenotype*					Summary of phenotypes*				
						Meiotic			Mitotic	
	D-SPB	D-SPB and-SC	Separated D-SPB	MI-S	MII-S	Spindle	DNA‡	Recombination‡	Spindle§	DNA‖
D473H (*cdc5-1/cdc5-1*)	3	6	20†	1	0	Separated D-SPB	DS+	CR+	CS	DS+
D7041H (*cdc14-1/cdc14-1*)¶	7	1	15†	2	0	Separated D-SPB	DS+	CR+	CS	DS+

* Abbreviations : D-SPB, duplicated spindle pole body; SC, synaptonemal complex; MI-S, meiosis I spindle; MII-S, meiosis II spindle; CR, commitment to recombination; DS, DNA synthesis; CS, complete spindle (vegetative).

† Some contained polycomplex and filament aggregates; see text.

‡ Summary of Table 2.

§ Determined by BYERS and GOETSCH (1974).

‖ Determined by CULOTTI and HARTWELL (1971) and HARTWELL (1976).

¶ Also observed higher than normal level of dense cells with unduplicated spindle pole body; see text.

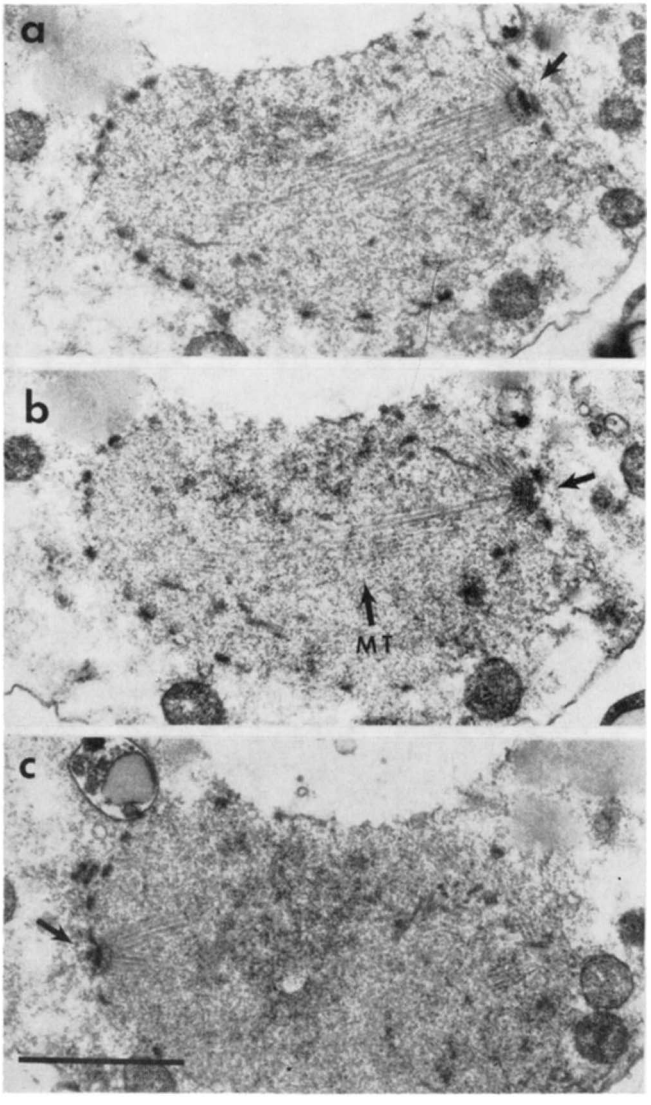

FIGURE 1.—Arrest of meiosis I in *cdc5-1* (2 hr at 21° followed by 22 hr at 33.5° in SPM): Typical appearance of separated spindle pole bodies. (a), (b) and (c) are consecutive serial sections. Note microtubules (MT) that enter nucleus from each SPB but which do not form a meiosis I spindle. Bar equals 1 μm.

Although most of these SPBs are found on opposite sides of the nucleus, the microtubules extending into the nucleus from each are not interconnected in the manner typical of ongoing meiosis I. We were unable to ascertain whether this occurrence of separate spindle poles represents a stage before meiosis I spindle formation or the aberrant separation of a previously formed spindle. In three of the cells with separated SPBs, each SPB was located in a distinct (but still connected) lobe of the single nucleus. The nuclei in five of the 20 cells with sepa-

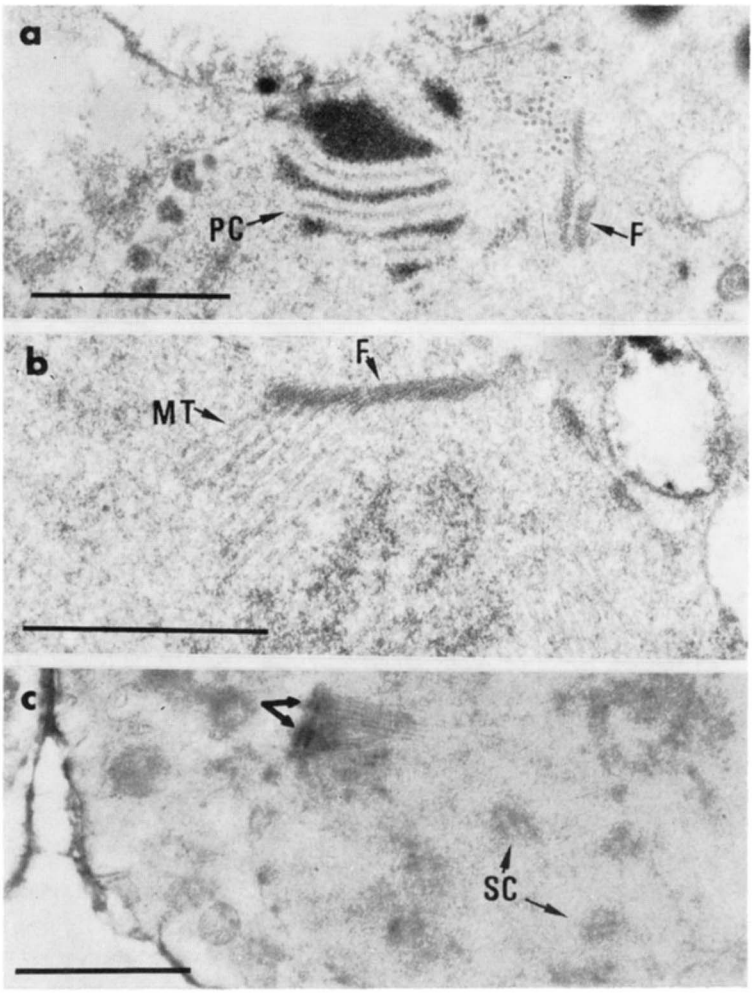

FIGURE 2.—Arrest of meiosis I by *cdc5-1* (2 hr at 21° followed by 22 hr at 33.5° in SPM): (a) Polycomplex (PC), frequently seen. Also note filamentous aggregates (F). (b) Filamentous aggregates (F), seen in some cells in association with microtubules (MT). (c) Duplicated spindle pole body (double arrow) and synaptonemal complex (SC), present in about 20% of cells. Bars equal 1 μm.

rated SPBs contained polycomplex (Figure 2a), which appears to be a polymer of the components of the synaptonemal complex (MOENS and RAPPORT 1971b) and has been interpreted by ZICKLER and OLSON (1975) to arise during diplotene. Most of the nuclei with separated SPBs also contained bundles of a filamentous component that frequently appeared to be associated with the spindle microtubules (Figure 2b). This component has previously been seen in other yeast strains in meiosis (K. SHRIVER and L. GOETSCH, personal communication).

In the *cdc5* homozygote, we observed nine cells that contained duplicated (but unseparated) SPBs; six of these also contained synaptonemal complex (Table 3;

Figure 2c). A possible explanation for the loss of viability among arrested cells is that the separation of SPBs without spindle formation renders the cell incapable of either reverting to mitosis or completing meiosis at the permissive temperature. Pachytene arrest alone does not cause inviability, as has been demonstrated by the good survival of such arrests in *cdc4* homozygotes (BYERS and GOETSCH 1975; SIMCHEN and HIRSCHBERG 1977) and wild-type cells arrested by high temperature (DAVIDOW, GOETSCH and BYERS 1980).

Cells homozygous for *cdc14* arrest predominantly with separated SPBs, such as those in *cdc5*. Of 37 cells examined, 12 had arrested with unduplicated SPBs, seven with duplicated SPBs and 15 with separated SPBs (Table 3). The separated SPBs usually did not face each other from opposite sides of the nucleus in the manner typical of the *cdc5* arrest, possibly indicating an even greater disruption of the normal functions than in the *cdc5* mutant. As with the *cdc5* arrest, the majority of 15 cells containing separated SPBs had single, spherical nuclei, while four cells bore the SPBs in separate halves of distinctly bilobed nuclei (Figure 3). Several of the cells with separated SPBs also contained polycomplex and/or filamentous aggregates similar to those seen in the *cdc5* arrested cells.

Second terminal phenotypes: Since meiosis, unlike vegetative growth, is not a cyclical process, a single transfer to 33.5° does not test for all possible stages of arrest. To test the *cdc5* and *cdc14* homozygotes for additional stages of meiotic arrest, we shifted cells from 21° to 33.5° at successively later times after initiation of sporulation. The cultures shifted sufficiently late that half-maximal sporulation was achieved were examined by electron microscopy. The rationale for this procedure was that when half of the cells had passed the last temperature-sensitive stage for that particular *cdc* mutant, most of the remaining unsporulated cells would be arrested at the latest stage(s) of arrest.

After 13 hr at the permissive temperature, approximately half as many cells in the *cdc5* homozygote culture were capable of sporulating at the restrictive temperature as in cultures left at the permissive temperature. Of the nonsporulated cells arrested in meiosis, nine were examined in serial sections: three contained duplicated SPBs (two with synaptonemal complex), five had separated SPBs and one had a meiosis I spindle. The frequencies of these different classes closely resemble those seen after the two hr shift to the restrictive temperature (Table 3); therefore, the *cdc5* gene product does not appear to have a second time of essential function among the cells unable to complete sporulation. There was, however, a change in the population of cells that succeeded in forming spores. The proportion of large two-spored asci increased from 5% to 12% in cells shifted from 21° to 33.5° after eight and 10 hr, respectively. As will be discussed later, it seems likely that the *cdc5* product is also required during meiosis II, but arrested cells are capable of effecting the encapsulation of two-spored asci.

When the *cdc14* homozygote was shifted to the restrictive temperature after 14 hr at 21°, the cells were capable of sporulating to about half of the 21° control level. Of seven unsporulated cells observed, two had unduplicated SPBs, one had a duplicated SPB, one had a duplicated SPB with synaptonemal complex, two

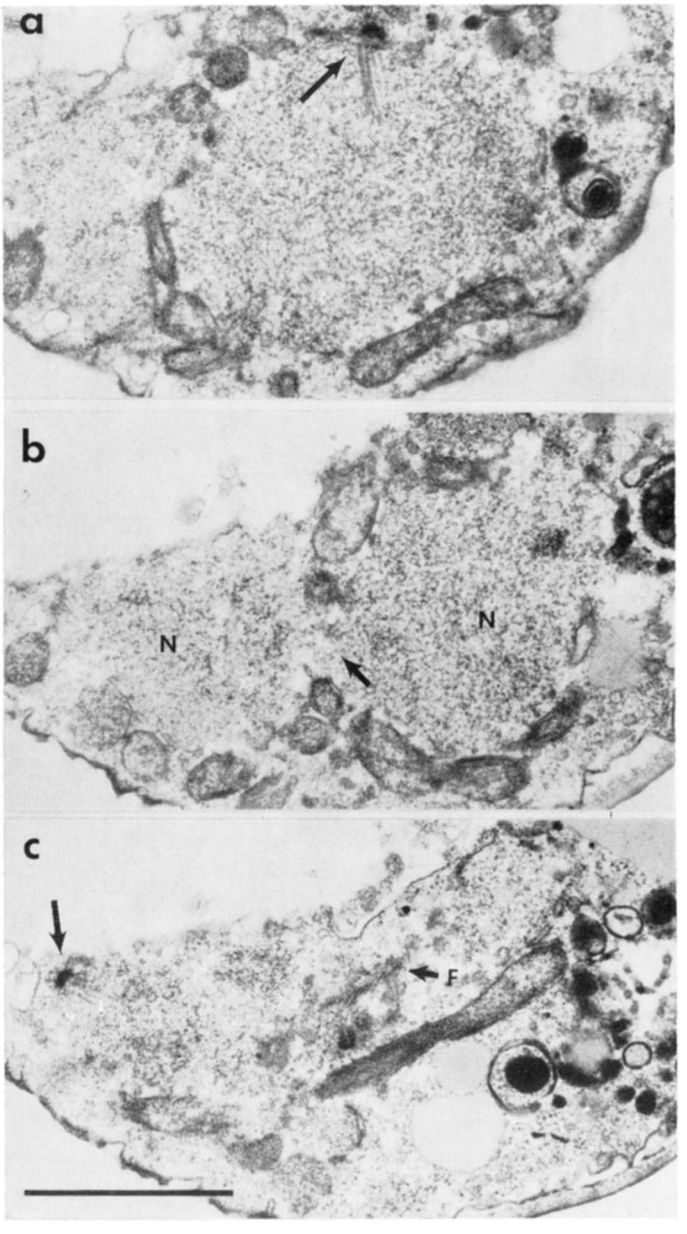

FIGURE 3.—(a-c) Sections 1, 3 and 4 of serial sections through separated spindle pole bodies [arrows in (a) and (c)] seen in the arrested *cdc14-1* homozygote (2 hr at 21° followed by 22 hr at 33.5°). Note the partial separation of this nucleus into two lobes connected by a narrow neck [arrow in (b)]. Filamentous aggregates (F) are present. Bar equals 1 μm.

had separated SPBs and one had a meiosis II spindle. Since the level of sporulation seen at 48 hr was about 4% higher than the level seen after 32 hr (when these cells were examined), the single case in meiosis II might represent an unarrested cell proceeding through meiosis. Therefore, late shifts of the *cdc14* homozygote to the restrictive temperature result in a varied pattern of arrest similar to that seen in cells shifted early in meiosis.

Diploid spores in cdc5 *and* cdc14 *homozygotes:* Several independently isolated *cdc5* homozygotes exhibited an unusually high frequency of two-spored asci (ranging from 10% to 35% in separate experiments) at 21° (Table 1). An unusual feature of these two-spored asci was that their spores were larger than those from either four-spored asci from this strain or two-spored asci from most other diploid strains. Our wild-type strain also gave many (about 15%) two-spored asci when subjected to 33.5°, but these spores were of normal size and dissection demonstrated that all were capable of mating and therefore probably haploid. We observed large two-spored asci in the *cdc14* homozygotes, but at a lower frequency (Table 1), and therefore concentrated on the analysis of the *cdc5* two-spored asci.

Because the *cdc5* heterozygote did not show high levels of two-spored asci (Table 1), the high level seen in the *cdc5* homozygote could be attributed to a recessive effect of the *cdc5* mutation on sporulation at 21°. Since this effect was not manifested in all asci, we may tentatively conclude that the *cdc5* gene product was only partially defective at this temperature. We might expect allele specificity for this phenotype; unfortunately, no other alleles of *cdc5* have been isolated (HARTWELL *et al.* 1973). As an alternative means of examining this question, we investigated the variation of the frequency of two-spored asci from the *cdc5* homozygote with change of temperature (Table 4). The frequency decreased from 25% at 21° to 11% at 17°. Although the absolute frequency remained nearly unchanged at 21°, 27.5° and 30°, the relative frequency of two-spored asci among all asci increased to 45% at the latter two temperatures, indicating that the phenotype does indeed result from partial temperature sensitivity. At 33.5°, very few asci with any spores were seen by phase-contrast microscopy, but electron microscopy revealed that many cells contained one or

TABLE 4

Temperature effect on number of spores per ascus in cdc5–1 *homozygote (D473H)*

Temperature	Percentage of cells with:* 1-spore	2-spore	3-, 4-spore	Total percentage of cells with spores	Relative† frequency of 2-spored asci
17°	1	11	71	83	13
21°	5	25	54	84	30
27.5°	7	28	27	62	45
30°	12	26	20	58	45
33.5°	4	3†	0	7	43

* 72-hr time-point for 17° culture, 48-hr time-point for all other cultures.
† Percentage of total asci with only two spores.

two spores. Eighteen (36%) of 50 cells examined by electron microscopy had sporulated and approximately ¾ of these were two-spored asci, with the remaining ¼ each containing a single large spore. Our inability to detect spores in these asci by phase contrast microscopy appears due to some defect in the structure, and hence the density, of the ascospore wall.

The two-spored asci from the *cdc5* homozygote at 21° were dissected in order to test their viability and genotype. Of 183 two-spored asci dissected, 52% contained no viable spores, 26% contained one and 22% had both spores viable. In total, only 35% of the spores were viable. If the viability of each spore were independent of the other in the ascus, one would expect to see a binomial distribution of the three classes: 42% with neither spore viable, 46% with one viable and 12% with both viable. A chi-square analysis demonstrated that the observed frequencies differ significantly (at the 99% confidence level, since $\chi^2 = 36.9$ with 2 degrees of freedom) from the results expected if viabilities of two spores of a pair were independent. Therefore, these results are skewed towards coincidence of inviability of the two spores, such as might result from coincident aneuploidy caused by multiple nondisjunction.

Genetic analysis of the asci with two viable spores revealed the classes shown in Table 5. The predominant classes (1 and 2) were tested further to determine their ploidy. Class 2 spore clones were mated to haploids of opposite mating type and the mated cells were sporulated. Dissection revealed extremely low viability, as would be typical of triploids, so that we tentatively concluded that the original spores were diploid (**a**/**a** or α/α) or near-diploid aneuploids. Class 1 spore clones were transferred directly to sporulation conditions at 21°: 12 of 16 pairs from *cdc5* and all six pairs from *cdc14* yielded predominantly four-spored asci, in addition to some of the two-spored asci with large spores. Dissection of the four-spored asci from both sets yielded good (60 to 80%) spore viability, indicating that the original spores were largely normal diploids. The genotypes of these spores are listed in Table 6.

A priori, we could entertain several models for the production of asci with two diploid spores: (1) the encapsulation of the products of the meiosis I reductional division in the absence of any equational (meiosis II) division, (2) the occurrence of meiosis II without a prior reductional division, (3) the occurrence of both divisions followed by the formation of binucleate spores, and (4) the diploidization of haploid products after germination, as occurs in homothallic strains. The genotypes of the **a**α spores (Table 6) permit us to test some of these

TABLE 5

Mating-ability of spore colonies from two-spored asci of cdc5–1 *and* cdc14–1 *homozygotes*

Class	Mating types	*cdc5–1*	*cdc14–1*
1	2 nonmaters	16	6
2	2 maters of opposite type	22	12
3	2 maters of same type	2	2
4	1 mater:1 nonmater	1	1

TABLE 6

Genotype of aα spores from class 1 two-spored asci of cdc5–1 *and* cdc14–1 *homozygotes*

Strain	Spore pair	Genotype of aα spores* *leu1*	*ade1*	*ura3*	*gal1*	*lys2*	*tyr1*	*his7*
D473H	1A	—/—	—/—	+/+	—/—	+/—	+/—	+/+
(*cdc5/cdc5*)	B	+/+	+/+	—/—	+/+	+/—	+/—	—/—
	2A	—/—	+/+	—/—	—/—	+/—	+/—	+/—
	B	+/+	—/—	+/—†	+/+	+/—	+/—	+/—
	3A	—/—	+/+	+/—	+/+	+/—	+/+	+/—
	B	+/+	—/—	+/—	—/—	+/—	—/—	+/—
	4A	—/—	—/—	+/—	+/+	+/—	+/—	+/—
	B	+/+	+/+	+/—	—/—	+/—	+/—	+/—
	5A	+/+	+/—	—/—	—/—	+/+	+/—	+/—
	B	—/—	+/—	+/+	+/+	—/—	+/—	+/—
	6A	+/+	+/—	—/—	+/+	+/+	+/+	+/+
	B	—/—	+/—	+/+	—/—	—/—	—/—	—/—
	7A	+/+	+/—	+/+	+/+	+/—	+/—	+/+
	B	—/—	+/—	—/—	—/—	+/—	+/—	—/—
	8A	+/+	—/—	+/—	+/+	—/—	+/—	+/—
	B	—/—	+/+	+/—	—/—	+/+	+/—	+/—
	9A	+/+	+/+	+/+	—/—	+/—	+/—	—/—
	B	—/—	—/—	—/—	+/+	+/—	+/—	+/+
	10A	+/+	—/—	+/+	+/+	+/—	+/—	+/+
	B	—/—	+/+	—/—	—/—	+/—	+/—	—/—
	11A	+/—	+/+	—/—	—/—	—/—	—/—	+/—
	B	+/—	—/—	+/+	+/+	+/+	+/+	+/—
	12A	+/+	—/—	+/+	+/+	+/—	+/+	+/—
	B	—/—	+/+	—/—	—/—	+/—	—/—	+/—
Frequency of homozygosis		0.92	0.75	0.73	1.0	0.33	0.33	0.42
D7041H	1A	+/+	—/—	+/+	+/+	+/—	+/+	+/—
(*cdc14/cdc14*)	B	—/—	+/+	—/—	—/—	+/—	—/—	+/—
	2A	—/—	+/+	+/+	+/+	+/—	+/+	+/—
	B	+/+	—/—	—/—	—/—	+/—	—/—	+/—
	3A	+/+	—/—	+/—	—/—	+/—	+/+	+/—
	B	—/—	+/+	+/—	+/+	+/—	—/—	+/—
	4A	—/—	—/—	—/—	+/+	+/—	+/—	+/+
	B	+/+	+/+	+/+	—/—	+/—	+/—	—/—
	5A	—/—	—/—	—/—	+/+	+/—	+/—	+/—
	B	+/+	+/+	+/+	—/—	+/—	+/—	+/—
	6A	—/—	—/—	+/—	+/+	+/—	+/—	+/—
	B	+/+	+/+	+/—	—/—	+/—	+/—	+/—
Frequency of homozygosis		1.0	1.0	0.67	1.0	0	0.50	0.17
Frequency of first division segregation‡		0.95	0.90	0.90	0.87	0.32	0.35	0.29

* aα spores are themselves sporulated, four-spored asci dissected and tested for genetic markers. *leu1*, *ade1*, *ura3* and *gal1* are centromere-linked on different chromosomes; *lys2*, *tyr1* and *his7* are not centromere-linked and are all on chromosome *II*.

† A gene conversion; left out of analysis.

‡ Data from MORTIMER and HAWTHORNE (1966).

models. Model 4 (homothallism) can easily be ruled out because all the markers in these strains would be homozygous, which is clearly not the case. With the kind assistance of J. FELSENSTEIN, we used a single-tailed *t* test to compute the probability that the data for centromere-linked markers had resulted from one of these models. This test took into account the frequency of recombination between the markers and the centromeres. The greater extent of homozygosis of the centromere-linked markers (*leu1*, *gal1*, *ade1* and *ura3*) than of distal markers (*lys2*, *tyr1* and *his7*) in most spores permits us to distinguish between the other three models. With greater than 99% confidence, we can conclude that our data do not fit model (2); nor do they fit model (3) if we assume that the incorporation of two meiotic products into each spore is random. The data are consistent with model 1, which predicts that the products of the reductional division (meiosis I) would co-segregate. Centromere-linked markers would preferentially become homozygous, whereas gene-centromere recombination would make the segregation of more distal markers random. The production of diploid spores heterozygous for mating type does not contradict this interpretation, since mating type is not tightly linked to the centromere and exhibits approximately 41% second-division segregation (MORTIMER and HAWTHORNE 1966). The data are statistically not inconsistent with the production of all **a**α spores by the reductional division of centromeres in meiosis I.

We also examined the formation of two-spored asci in *cdc5* and *cdc14* homozygotes by fluorescence microscopy after nuclear staining with DAPI. Of 50 two-spored asci from the *cdc5* culture observed, 34 had a single large nucleus per spore (Figure 4), and five had one uninucleate spore and one spore with either a bilobed nucleus or two nuclei. The remaining 11 contained two small uni-

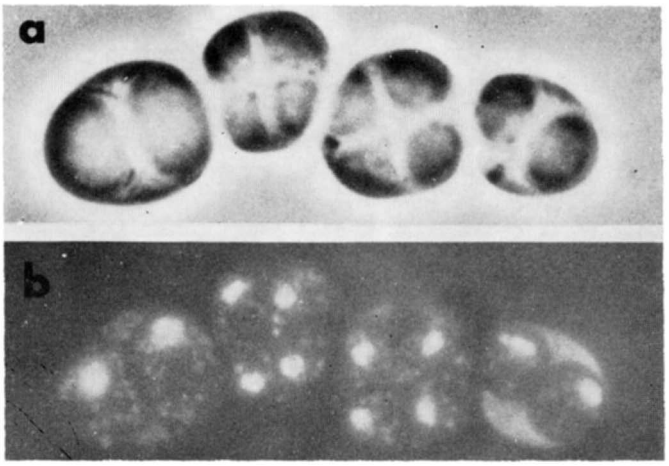

FIGURE 4.—Nuclear (DAPI) stained asci from *cdc5-1* homozygote sporulated at 21°. (a) Phase-contrast illumination. (b) Fluorescence illumination (365 nm). Most two-spored asci were similar to that at the left, containing one large nucleus in each large spore. Some two-spored asci were similar to the one on the right, which appears to contain two haploid spores; the additional staining of the ascal space probably represents the two unencapsulated haploid nuclei. The middle two asci contain the normal complement of four spores and display the discrete areas of staining representative of the mitochondria (WILLIAMSON and FENNELL 1975).

nucleate spores with DAPI-staining material external to the spores, as is normally seen in two-spored asci containing haploid spores. Among 50 *cdc14* two-spored asci, 42 contained two large uninucleate spores, and the other eight had two small uninucleate spores with DAPI-staining material outside the spores. The predominant appearance of large uninucleate spores suggests that the diploid spores arise by failure of meiosis II, but it could not be excluded on this basis that the final division succeeds and is then followed by nuclear fusion.

Ultrastructural analysis was done on *cdc5* homozygotes at 21° in order to establish the cytological basis for the formation of diploid spores. Because the culture also produced many four-spored asci, only those cells in the process of encapsulating two products were examined. A typical case (Figure 5) shows that complete meiosis II spindles were formed, but failed to elongate; thus, both poles of the short spindle were in the process of being encapsulated within a single spore at the time of fixation. Similar images from several other cells suggested that all diploid spores of the *cdc5* homozygotes arise in the same manner: insufficient elongation of the meiosis II spindle leads to a failure of each spindle pole to gain an individual prospore wall.

DISCUSSION

These observations demonstrate that the genes represented by these two cell-division cycle mutations, *cdc5* and *cdc14*, play essential roles, not only in late nuclear division of vegetative growth (HARTWELL *et al.* 1973), but also in both meiotic divisions. Challenge of the homozygous mutants with the restrictive conditions early in meiosis leads to failure of meiosis I. Cytological observation of both *cdc5* and *cdc14* homozygotes demonstrated that the majority of cells arrested in meiosis I had undergone spindle pole body separation, but failed to form a complete spindle. This stage is clearly subsequent to pachytene, in which the spindle pole bodies remain paired and the chromosomal profiles are defined by the synaptonemal complex (ZICKLER and OLSON 1975; BYERS and GOETSCH 1975). HORESH, SIMCHEN and FRIEDMANN (1979) have recently demonstrated by electron microscopy of random thin sections that the chromosomes of a *cdc5* homozygote may retain a considerable degree of condensation during arrest in meiosis I. The present analysis by serial sectioning demonstrates (as in Figure 1) that these condensed structures, which lack the clearly defined tripartite morphology of the synaptonemal complex, reside in nuclei that have already undergone spindle pole body separation. Our observations thereby confirm the suggestion by HORESH, SIMCHEN and FRIEDMANN (1979) that such densities arise after completion of pachytene.

Delayed application of the restrictive temperature to these homozygotes (or use of semi-restrictive temperatures) leads to failure of meiosis II. The latter defect is frequently not lethal because encapsulation of the diploid products of meiosis I proceeds to completion. Mutations affecting meiosis II have been infrequently found either in yeast or in other species (reviewed by BAKER *et al.* 1976). In *Drosophila melanogaster*, both *meiS332* (DAVIS 1971) and *ord* (MASON 1976)

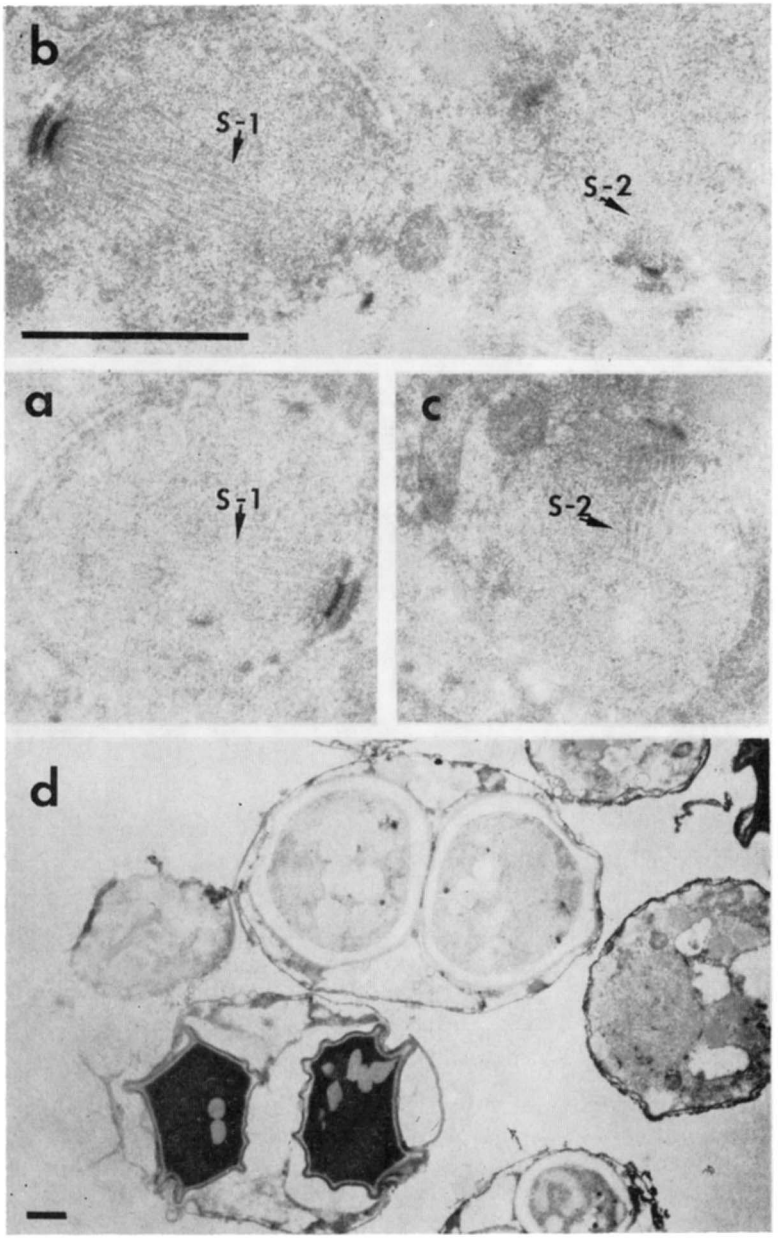

FIGURE 5.—Formation of two-spored asci in the *cdc5–1* homozygote. (a-c) Sections 1, 2 and 4 of serial sections through a cell encapsulating a complete meiosis II spindle (S1) after 15 hr at 21° in SPM. The other meiosis II spindle (S-2) has not advanced as far. (d) Two two-spored asci of *cdc5–1* homozygote, including an immature ascus with spherical spores (above) and a mature ascus with collapsed spores (below). Bars equal 1 μm for (a-c) and (d).

cause high levels of nondisjunction during both meiosis I and meiosis II. BEADLE (1932) found that maize bearing the mutation *va* (variable sterile) is defective in cytokinesis during both meiotic divisions, frequently producing *2n* and *4n* products. Another mutation (*el*, elongate) in maize causes production of diploid meiotic products, as well as near-diploid aneuploid ones. Although the latter mutation was first thought to suppress only the second (equational) meiotic division (RHOADES and DEMPSEY 1966), more recent evidence indicates that the first meiotic division is sometimes affected (NEL 1975). A similar mixture of some cells suppressed for the first and second meiotic divisions could not be ruled out in our study of two-spored asci of *cdc5* and *cdc14* if the latter failures were predominant. Among other mutations of *S. cerevisiae*, *spo2* has been found to affect meiosis II, while *spo3* is defective in both meiotic divisions. Both mutants appear unable to maintain proper coordination between nuclear segregation and ascospore formation, as indicated by the formation of anucleate spores (MOENS, ESPOSITO and ESPOSITO 1974).

In the case of the present mutants, *cdc5* and *cdc14*, the aberration of meiosis II is made particularly accessible to analysis by the formation of viable spores. Genetic analysis readily revealed that these are diploid and that most result from failure to complete meiosis II. Similar defects in the second meiotic division have been found in other cases to lead to viable products. Besides the maize mutants discussed above, mammalian teratomas may arise from failure of meiosis II (LINDER, KAISER-MCCAW and HECHT 1975) although alternative pathways of this formation cannot be excluded (MINTZ, CRONMILLER and CUSTER 1978). GREWAL and MILLER (1972) previously described yeast strains that regularly produce two diploid spores. Genetic analysis revealed the presence in these strains of alleles at two loci that suppress meiosis I and thereby lead to a single equational division (KLAPHOLZ and ESPOSITO, 1980). Examination of meiosis in such strains by electron microscopy revealed that only one spindle is formed; the poles of this single spindle are then modified to the type characteristic of meiosis II, leading to the induction of a prospore wall about the diploid genome at either pole (MOENS *et al.* 1977). The production of diploid spores in *cdc5* and *cdc14* homozygotes not only differs genetically, but is also distinct in its cytological mechanism. The spindles for meiosis II clearly form, but fail to elongate. Although we cannot tell whether there is any physical separation of the genomes, the resulting genotypes of the spores are determined by the fact that both genomes come to reside in the same diploid nucleus. The formation of modified spindle pole bodies ensues normally after the formation of the meiosis II spindles. Prospore wall formation is then initiated adjacent to the modified spindle pole bodies, as suggested from cytological observations (MOENS and RAPPORT 1971a) and demonstrated more definitively by regulation of spore number at these sites (DAVIDOW, GOETSCH and BYERS 1980). In the present case, it appears that the close proximity of the two spindle poles on the unelongated meiosis II spindle is responsible for the eventual formation of one spore wall, rather than two distinct ones, about each meiosis I product.

The failure of meiosis II in these mutants differs strikingly from that in mitosis or meiosis I. In mitosis, the spindle achieves the greatly elongated form characteristic of late nuclear division (BYERS and GOETSCH 1974); whereas, the meiosis I failure described in this study leads to the presence of two disconnected spindle pole bodies. These disparate consequences of the genetic defects suggest substantial differences in the control of these three types of nuclear division. Although all share the requirement for these gene products, they clearly differ in the cytological consequences of the primary genetic defect. The essential identity of the failures in nuclear division by mutants in two independent loci, *cdc5* and *cdc14*, suggests that the products of both loci participate in an event that is required for all three types of nuclear division, but differs in its relation to other events in each type. Future analysis of the cytological defects in other mutants affecting nuclear division may lead to a better understanding of these differences.

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