ARE MITOTIC FUNCTIONS REQUIRED IN MEIOSIS?

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ABSTRACT

Sporulation of diploid yeasts (*Saccharomyces cerevisiae*), homozygous or heterozygous for temperature-sensitive mitotic cell-cycle mutations, was examined at the restrictive and permissive temperatures. Twenty genes, represented by 32 heterozygotes and 60 homozygotes, were divided into three groups, showing (i) normal sporulation, (ii) no sporulation at the restrictive temperature but normal sporulation at the permissive temperature, (iii) no sporulation at both temperatures. Group (i) as well as several other strains were tested for their meiotic behavior with regard to intragenic recombination and haploidization. The conclusion reached was that all the mitotic nuclear-division and DNA-synthesis functions were required in meiosis. The only cell-division mutations not to affect meiosis were in three cytokinesis loci and in one bud-emergence locus.

APPROXIMATELY ten percent of the temperature-sensitive mutants of *Saccharomyces cerevisiae* were characterized by HARTWELL, CULOTTI and REID (1970) and by HARTWELL *et al.* (1973) as cell-cycle mutants (*cdc*, for cell division cycle). Upon transfer to the restrictive temperature, cell-cycle mutants arrest uniformly at a typical stage of the cycle and consequently may display unusual cellular and nuclear morphologies. It may be assumed from this behavior of the mutants that the products of the *cdc* genes are required at distinct times in the cell cycle. Since most of the mutants arrest within 1–2 cell generations, products from previous cycles are probably not available. Mutants belonging to 20 of the 32 complementation groups (HARTWELL *et al.* 1973) arrest at various stages of nuclear division, mutants in four genes are defective in cytokinesis (but the nuclei continue to divide) and the remaining *cdc* mutants arrest as unbudded cells. Five genes are involved in DNA synthesis (HARTWELL 1971a, 1973) and mutants in four of these arrest in nuclear division.

The cell-cycle temperature-sensitive mutants represent functions that are indispensable for the mitotic vegetative cycle. It is of interest to inquire whether these functions are also required in the meiotic process. The latter differs from the vegetative mitotic cycle in yeast in a number of important features, notably those related to nuclear division, cell separation, the formation of spores following meiosis and recombination. Meiotic and sporulation-specific mutants in yeast (ESPOSITO and ESPOSITO 1969; ROTH and FOGEL 1971; SIMCHEN, PIÑON and SALTS 1972 and unpublished), which do not affect vegetative growth, demonstrate that certain meiotic functions are not required during the vegetative cycle. We may ask, however, whether in meiosis these meiotic-specific functions are

superimposed on the requirements of a vegetative cycle, thus implying that meiosis evolved as a specialized mitotic cycle. The alternative would be that mitosis and meiosis have diverged to such a degree that mitotic functions would not be required for meiosis.

MATERIALS AND METHODS

The mutants: Most of the temperature-sensitive cell-cycle mutants (*cdc*) were obtained as survivors of mutagenic treatments given to the haploid A364A, genotype *a ade1 ade2 ura1 tyr1 his7 lys2 gal1* (HARTWELL *et al.* 1973). The mutagenic agent was mostly N-methyl-N′-nitro-N-nitrosoguanidine, but some mutants were obtained from a treatment with ethylmethane sulfonate. A few of the mutants contained more than one temperature-sensitive mutation and were therefore crossed by HARTWELL *et al.* to a related α haploid to give *a* progeny with only one temperature-sensitive mutation. All the *cdc* mutants used in the present study were supplied by DR. L. H. HARTWELL, University of Washington, as haploids, mostly of the original genotype (same as A364A) or as derivatives of mating type *a*.

Heterozygous and homozygous diploids: The diploid 419 was obtained from a mating of the parental haploid A364A to a standard α strain of genotype *ade2-R8 met8 can1-11*. The latter was derived from the α parent of diploid 131 (SIMCHEN, PIÑON and SALTS 1972), as a canavanine-resistant colony. The *can1-11* mutation is allelic to *can1* on chromosome V (MORTIMER and HAWTHORNE 1973); diploids carrying both mutations are canavanine-resistant (both mutations are recessive), and progeny of such diploids do not contain canavanine-sensitive colonies in a frequency higher than 1 in 1000. The mutations *ade2* and *ade2-R8* do not complement each other. Diploids containing both *ade2* mutations are heteroallelic and yield *ADE2* prototrophic recombinants.

Diploids comparable to 419 but heterozygous for the various *cdc* mutations were obtained by mating the appropriate haploid mutants with the standard α strain. Thus each of the heterozygous diploids was expected to differ from 419 by a single *cdc* mutation.

Diploids homozygous for the *cdc* mutations were derived from the heterozygous diploids by the following procedure. A vegetative logarithmic culture in liquid YEP medium was washed, resuspended in water and diluted to 5000 plating units/ml. Approximately 5 ml of this suspension were exposed to ultraviolet irradiation dose of 1000 erg/mm^2 while being shaken in a small petri dish, followed by 1:10 dilution in liquid YEP medium. This culture was incubated in a shaking water bath at 25° for 3–4 hours and was then spread on YEP plates, 0.1 ml/plate, to give approximately 100 plating units/plate. The irradiated cultures were kept away from photoreactivating light throughout this part of the procedure. The plates were incubated for 2–3 days at the permissive temperature of 25° and were thereafter each replicated onto two other YEP plates, one to be incubated at 25° and the other, usually prewarmed, to be incubated at the restrictive temperature of 35° (the restrictive temperature for *cdc28* was 38°). The plates were examined the following day and independent temperature-sensitive colonies were isolated and retested for temperature sensitivity. The morphology of the temperature-arrested cells was compared to the morphology of the parental haploid mutant at the restrictive temperature (HARTWELL *et al.* 1973) and found to be as expected in each case. These homozygous diploids resulted most probably from mitotic crossing over events between the *cdc* mutation sites and their respective centromers. The diploids did not mate with either *a* or α haploids and were therefore assumed to have remained a/α.

Sporulation: Diploids were grown in liquid PSP2 medium, supplemented with 40 μg/ml adenine, to a titer of 10^7 cells/ml. The cells were washed in sterile distilled water and resuspended in liquid SPM medium (see SIMCHEN, PIÑON and SALTS 1972 for details). To test sporulation at the restrictive and permissive temperatures, a 10-ml culture in SPM was split into 5-ml portions in 50-ml flasks. Both flasks started sporulation in a 25° shaking water bath, but one was transferred to a 33.5° shaker after 1½–2 hours. This allowed most of the cells to complete their vegetative cycle at the permissive temperature. The restrictive temperature for sporulation was chosen as 33.5° ± 0.5° since a notable reduction in sporulation was observed at temperatures higher than 34° in the non-mutant parental diploid 419 (and in many other diploids).

Sporulation was evaluated microscopically in formaldehyde-fixed samples with phase-contrast optics. Counts were made on 200–300 cells relating to mother-cells and buds as separate cells.

Media: YEP and PSP2 are the glucose and acetate vegetative media respectively, and SPM is the sporulation medium (for details see SIMCHEN, PIÑON and SALTS 1972). —AD medium serves to detect adenine prototrophs and contains Yeast Nitrogen Base (without amino acids) 6.7 g, glucose 20.0 g, lysine, methionine and tyrosine 40 mg each, histidine and uracil 10 mg each, Difco Bacto-agar 15 g, water to 1 liter. CAN medium supports the growth of canavanine-resistant colonies only, and is similar to —AD medium but contains also adenine 10 mg and canavanine sulfate 40 mg.

RESULTS

Criteria for sporulation and meiosis

Sporulation and meiotic events of the parental, non-mutant, diploid 419 at 30° are similar in timing and synchrony to those obtained with the related diploid 131 (SIMCHEN, PIÑON and SALTS 1972; SIMCHEN, SALTS and PIÑON 1973). The temperatures used for sporulation, 25° and 33.5°, reduce the rate and synchrony of the sporulation events in comparison to the optimal temperature of 30°. The following observations are based on five experiments with 419.

i) Premeiotic DNA synthesis, as measured by fluorospectrophotometry (SIMCHEN, PIÑON and SALTS 1972), occurs between 7 and 10 hrs in SPM at both 25° and 33.5°.

ii) The number of *ADE2* prototrophs upon plating of 419 cells in sporulation medium is increased 20–50-fold between 5 and 8 hrs at 33.5° and 5 and 9 hrs at 25°. These are produced by events of intragenic recombination.

iii) The number of canavanine-resistant colonies increases from 5–10% at 8 hrs to 50% and greater at 10 hrs, at both 25° and 33.5°. Such colonies signal mostly meiotic haploidization (SHERMAN and ROMAN 1963).

iv) Ascus frequency at 33.5° increases from 5% to 55% between 12 and 15 hrs and at 25° from 9% to 60% between 12 and 17 hrs. Further frequency increases are observed within the next 24 hrs.

The relation among the four events is comparable to previous descriptions (SHERMAN and ROMAN 1963; SIMCHEN, SALTS and PIÑON 1973).

Sporulation of mutant diploids

To determine ascus frequencies, samples from the cultures at the restrictive and the permissive temperatures were taken at two times, 24 hrs and 48 hrs, in SPM. Table 1 summarizes the sporulation data of 32 diploids heterozygous for mutant alleles of 20 *cdc* genes, and of 60 homozygotes obtained from them.

Based on these data, the following categories of mutants can be recognized with regard to their effects on sporulation (stages of arrest given in brackets—see note to Table 1).

1) No effect on ascospore formation: *cdc1* (BE), *cdc3* (CK), *cdc6* (mND), *cdc10* (CK), *cdc11* (CK), *cdc15* (lND), *cdc24* (BE).

2) Ascospore formation in homozygotes poor or absent at the restrictive temperature while apparently normal at the permissive temperature: *cdc2* (mND), *cdc4* (iDS), *cdc13* (mND), *cdc14* (lND) and probably also *cdc5* (lND) and

TABLE 1

Sporulation of diploids homozygous and heterozygous for cdc *mutations*

		Sporulation of heterozygotes			Sporulation of homozygotes		
*cdcx-a**	Role†	Designation	25°	33.5°	Number‡	25°	33.5°
cdc1–1	BE	445	++	++	1	++	++
cdc2–1	mND	435	++	++	2	++	—
cdc3–1	CK	422	++	++	4	++	++
cdc3–3	CK	423	++	++	1	++	++
cdc4–1	iDS	152	++	++	1	++	—
		165	++	++	1	++	—
		403	++	++	1	++	—
cdc4–3	iDS	212	++	++	2	++	—
cdc5–1	lND	425	++	++	1	+	—
cdc6–1	mND	436	++	++	2	++	++,+
cdc7–1	iDS	447	++	++	2	+—	+—,—
cdc7–2	iDS	446	++	++	1	+	+—
cdc7–3	iDS	448	++	++	1	+	+—
cdc7–4	iDS	204	+—	—	3	+—	—
cdc8–1	DS	405	++	++	1	+—	—
cdc8–2	DS	414	++	++	2	+—	—
cdc8–3	DS	438	++	++	3	+—	+—,—
cdc8–4	DS	409	++	++	3	+—	—
cdc9–2	mND	429	++	++	2	+	+—
cdc10–1	CK	424	++	++	4	++	++
cdc11–1	CK	439	++	++	1	++	++
cdc13–1	mND	210	++	++	1	++	—
cdc14–1	lND	426	++	++	1	++	—
cdc14–2	lND	431	++	++	3	++	+—,—
cdc15–1	lND	206	++	++	1	++	++
cdc15–2	lND	421	++	++	3	++	++,+
cdc16–1	mND	208	++	++	2	+—	—
cdc17–1	mND	432	++	++	3	+,+—	+—
cdc20–1	mND	434	+	+	3	+—,—	—
cdc23–1	mND	433	++	++	1	—	—
cdc24–1	BE	437	++	++	1	++	+
cdc28–1	iDS	443	++	++	2	+—	+—

* x-complementation group, a-allele.

† Stage of arrest at the restrictive temperature, and probable role in the cell cycle: BE—bud emergence; CK—cytokinesis; DS—DNA chain elongation; iDS—initiation of DNA synthesis; lND and mND—late and middle nuclear division, respectively. See HARTWELL *et al.* (1973); HARTWELL, CULOTTI and REID (1970); HARTWELL (1971a, b, 1973); CULOTTI and HARTWELL (1971).

‡ Number of independent homozygous diploids tested.

++ Full sporulation (50–70% at 24 hours); + slightly reduced sporulation (20–50% at 24 hours), +— drastically reduced sporulation (none or very little at 24 hours and up to 20% at 48 hours), — no sporulation.

cdc9 (mND) (although the latter two homozygous diploids show somewhat reduced sporulation at 25°).

3) Ascospore formation in homozygotes poor at both permissive and restrictive temperatures: *cdc7* (iDS), *cdc8* (DS), *cdc16* (mND), *cdc17* (mND), *cdc20* (mND), *cdc23* (mND), *cdc28* (iDS).

4) Ascospore formation in homozygotes and heterozygotes impaired: *cdc7–4* (iDS).

The test for sporulation at the two temperatures is a very general one and a detailed analysis of the effects on meiosis is obviously required for each of the mutants. With reference to the main question that was asked in the introduction of this paper, however, we felt it necessary to verify that the insensitivity to the restrictive temperature of mutants of category (1) was genuine. The fact that they were selected as cell-cycle mutants at 36° (HARTWELL *et al.* 1973) could mean that 33.5° was in fact not a restrictive temperature. This was tested by growing the various homozygotes and heterozygotes belonging to category (1) in liquid vegetative medium (PSP2 + adenine) at 25° and transferring the cultures at a titer around 2.5×10^6/ml to a shaking water bath at 33.5°. In such experiments, the cytokinesis mutants (CK) *cdc3*, *cdc10* and *cdc11* developed the characteristic "star" morphology (HARTWELL 1971b). The diploids homozygous for *cdc6*, *cdc15* and *cdc24* arrested within one cell-cycle (Table 2), as expected from mutants that arrest at a distinct stage in the cell cycle. It should be noted, however, that the increase in titer of *cdc6* in Table 2 is exactly twofold, and other experiments with this mutant gave similar increases, sometimes even slightly more than a twofold increase. With *cdc1* the homozygote arrested within two cycles. These results do not ensure the complete sensitivity of *cdc1* and *cdc6* to

TABLE 2

Titers (in millions/ml) of liquid cultures at the permissive and restrictive temperatures of heterozygotes and homozygotes at four cdc *genes*

Genotype	Stage of arrest	Strain no.	25°			33.5°		11 hrs
			0 hrs	4 hrs	11 hrs	4 hrs	11 hrs	0 hrs*
CDC1/cdc1		445	2.5	6.8	32.4	7.3	24.9	10.0
cdc1/cdc1	BE	445–2	2.4	5.3	25.4	4.4	8.5	3.5
CDC6/cdc6		436	2.2	5.8	24.1	7.4	29.2	13.3
cdc6/cdc6	mND	436–1	2.3	7.1	19.5	4.1	4.6	2.0
cdc6/cdc6	mND	436–10	3.2	6.4	14.5	5.4	6.5	2.0
CDC15/cdc15–1		206	3.8	9.5	26.0	7.9	28.5	7.5
cdc15–1/cdc15–1	lND	206–4	2.0	4.4	14.1	2.2	2.6	1.3
CDC15/cdc15–2		421	1.4	3.4	13.0	3.7	12.2	8.7
cdc15–2/cdc15–2	lND	421–5	1.5	3.7	14.0	2.3	2.6	1.7
CDC24/cdc24		437	2.0	5.5	18.4	6.3	22.1	11.1
cdc24/cdc24	BE	437–6	2.3	4.6	11.7	2.5	3.3	1.4

* The 0-hr titer is the same at 25° and 33.5°.

the restrictive temperature of 33.5°. For the other five genes (*cdc3*, *cdc10*, *cdc11*, *cdc15* and *cdc24*), however, we may conclude that ascospore development is not affected at a temperature that is restrictive for vegetative growth.

Recombination and haploidization of the mutant diploids in SPM

The mutants of category (1) that have no effect on ascus formation at the restrictive temperature were tested to determine whether they had any effect on intragenic recombination and haploidization during meiosis. The mutant diploids were sampled from SPM cultures at 0 hrs from the permissive temperature, and 10 hrs and 24 hrs from both the permissive and restrictive temperatures, and spread on −AD and CAN plates. The plates were incubated at 25°. The results for five mutants are shown in Table 3.

The control parental strain 419 exhibits a 20–50-fold increase in intragenic recombination at the *ade2* locus and 81% to 97% haploidization as indicated by *can1–11* segregation. It should be emphasized that the canavanine-resistant colonies reflect the growth of canavanine-resistant haploid ascospores within whole asci plated at the later times.

The cytokinesis mutants (CK), like 439–4 (*cdc11*), do not show a marked change in recombination or haploidization. One bud-emergence mutant, 445–2 (*cdc1*), has no effect on recombination and haploidization while the other bud-emergence mutant, 437 (*cdc24*), has an effect on haploidization. This is seen in the heterozygote 437 as well as in the homozygote 437–6. The two nuclear division genes, *cdc6* and *cdc15*, affect both recombination and haploidization. *Cdc6* (436–1) has a less drastic effect, which might be related to the possible incomplete sensitivity to 33.5° noted above. *Cdc15* (206–4) shows an interesting phenomenon of reduced haploidization and no recombination at the *ade2* locus. This result is likely to be an artifact, however, because selection of the *cdc15* homozygote (206–4) probably also resulted in homozygosis for *ade1* due to the close linkage between *cdc15* and *ade1* (MORTIMER and HAWTHORNE 1973). Thus in this diploid *ADE2* prototrophs would remain undetected.

In summary (Table 3 and other unpublished data), among the *cdc* genes that do not affect ascus formation, only the cytokinesis genes *cdc3*, *cdc10* and *cdc11*, and one bud emergence gene, *cdc1*, do not affect *ade2* intragenic recombination and meiotic haploidization.

Among the genes which do effect ascus production it may be asked whether recombination and haploidization proceed normally or whether spore formation alone is affected while meiosis proceeds undisturbed. Since the mitotic behavior of these mutants at the restrictive temperature shows effects on nuclear division or DNA synthesis, which do not seem to be related to spore formation *per se*, but rather to events occurring prior to or during meiosis, the latter possibility seems unlikely. Hence, at least meiotic haploidization is expected to be interfered with in those cases where ascus formation is absent. This expectation is born out by preliminary data with four mutants. Haploidization is absent at 33.5° in *cdc4–3*, *cdc7–4*, *cdc8–3* and *cdc9–2*, while *ade2* recombination is not affected in *cdc9–2*, only partly affected in *cdc4–3* and very low in the other two mutants. It is recognized that this approach has to be extended in further studies. Until addi-

TABLE 3

Frequencies of adenine prototrophs ($\times 10^{-6}$) and canavanine-resistant colonies of heterozygotes and homozygotes at five cdc *genes*

Genotype	Stage of arrest	Strain no.	25°						33.5°			
			0 hrs		10 hrs		24 hrs		10 hrs		24 hrs	
			ADE	*can1*	*ADE*	*can1*	*ADE*	*can1*	*ADE*	*can1*	*ADE*	*can1*
Parental		419	4.5	.02%	230	46%	252	97%	83	60%	81	81%
CDC1/cdc1-1		445	<0.2	.01%	98	69%	106	73%	83	55%	80	63%
cdc1-1/cdc1-1	BE	445-2	41	.08%	150	68%	151	61%	94	52%	89	70%
CDC6/cdc6-1		436	<0.2	.06%	78	43%	80	79%	49	40%	42	70%
cdc6-1/cdc6-1	mND	436-1	<0.2	.02%	22	22%	21	32%	28	17%	11	33%
CDC11/cdc11-1		439	6.5	.02%	202	52%	211	100%	106	70%	95	78%
cdc11-1/cdc11-1	CK	439-4	8.7	.01%	334	49%	379	86%	132	46%	175	76%
CDC15/cdc15-1		206	0.2	.02%	153	11%	161	40%	102	32%	89	36%
cdc15-1/cdc15-1	lND	206-7	<0.2	.02%	<0.2	8%	<0.2	13%	<0.2	11%	<0.2	17%
CDC24/cdc24-1		437	2.2	.04%	126	3%	121	26%	108	16%	73	18%
cdc24-1/cdc24-1	BE	437-6	22.2	.02%	127	11%	134	12%	94	7%	69	17%

At each point, five plates each of YEP, —AD and CAN media were spread with the appropriate dilutions. Adenine prototrophs are given as prototrophs per 10^6 cells plated.

tional data are available, we assume that in the *cdc* mutants, interference with sporulation also implies interference with meiosis.

DISCUSSION

From the data presented here we may conclude that mitotic functions are indeed required in meiosis. The only *cdc* mutations that seem not to affect meiosis are in the three cytokinesis genes (*cdc3*, *cdc10* and *cdc11*) and in one bud-emergence gene (*cdc1*). That those functions are not required for meiosis is not surprising because even in the vegetative cell cycle they are not directly related to mitosis. In fact, several rounds of nuclear division are observed in CK mutants at the restrictive temperature while cytokinesis is arrested (HARTWELL 1971b). The inclusion in the study of *cdc* mutants that do not affect meiosis adds strength to our conclusion about the essential role of mitotic functions in meiosis, because the cytokinesis mutants can serve as internal controls on the methodology and experimental approach taken. Thus not every temperature-sensitive or *cdc* mutation interferes with meiosis, but only those that relate to mitosis in the sense of nuclear division and/or DNA synthesis.

From seventeen of the 32 heterozygous diploids we obtained two or more independent *cdc* homozygotes, and in all cases these homozygotes corresponded well with each other. Furthermore, for six of the twenty *cdc* genes reported in Table 1, we obtained homozygotes from heterozygotes constructed from independently isolated alleles of the same gene. The correspondence between the meiotic behavior of the different alleles and of the different homozygotes of the same allele adds support to our conclusions about the meiotic behavior of the mutants.

The only gross inconsistency observed between alleles of the same *cdc* gene is the behavior of *cdc7–4* compared with alleles *cdc7–1*, *cdc7–2* and *cdc7–3*. The former shows a unique behavior in sporulation in that it acts as a dominant effect, reducing sporulation (Table 1), recombination and haploidization drastically in the heterozygote. We propose that the haploid parent carrying *cdc7–4* (HARTWELL's 4008) also carries a dominant sporulation-specific mutation, similar to the one reported by SIMCHEN, PIÑON and SALTS (1972) in diploid 132. This is a likely possibility in view of the fact that several of the *cdc* mutants were reported by HARTWELL *et al.* (1973) to harbor more than one mutation, resulting from the potent mutagenic treatment to which they were exposed (in this study we refrained, as far as possible, from using these multiple mutants).

A more detailed analysis of the effects of the various mutants on meiosis is in progress. In addition to tests for haploidization and recombination in the permissive and restrictive conditions, we are employing temperature shifts for mutants of category (2) to determine the time in sporulation after which they are not sensitive to the restrictive conditions. This point is comparable to the execution point (HARTWELL, CULOTTI and REID 1970) in the vegetative cycle and to the *te* point of temperature-sensitive sporulation-deficient mutants of yeast (ESPOSITO *et al.* 1970). For mutants of category (3), the approach is more limited, but the stage they reach in sporulation can be studied in relation to

meiotic events and stages of commitment (SIMCHEN, PIÑON and SALTS 1972; SIMCHEN, SALTS and PIÑON 1973).

The effects on sporulation of mutants of our category (3) deserve additional comments. With respect to vegetative growth these mutants are temperature-sensitive, whereas in sporulation their deleterious effects are not conditional upon temperature. This difference in behavior might stem from the delicacy of balance between various cellular processes in meiosis that could be more easily disturbed, at any temperature, than in mitosis. It could also be suggested that for such temperature-sensitive mutants there is in mitosis an alternative functional pathway which is available only at the permissive temperature, but not during meiosis.

From our preliminary data on recombination and haploidization (including Table 3), we observe that certain heterozygous diploids exhibit changes in meiotic behavior, though sporulation is apparently normal. It is premature to draw any conclusions at this stage but from the complexity of meiosis such effects could be expected.

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