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Genetics

A chromosomal gene required for killer plasmid expression, mating, and spore maturation in *Saccharomyces cerevisiae*

(pheromones/sterile mutants/double-stranded RNA/meiosis)

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ABSTRACT **"Killer" strains of *Saccharomyces cerevisiae* are those that harbor a double-stranded RNA plasmid and secrete a toxin that kills only strains not carrying this plasmid (sensitives). Two chromosomal genes (*kex1* and *kex2*) are required for the secretion of toxin by plasmid-carrying strains. The *kex2* gene, which maps at a site distinct from the mating-type locus, is also required for normal mating by α strains and meiotic sporulation in all strains. Strains that are α mating-type and *kex2* fail to secrete the pheromone α-factor or to respond to the *a*-factor II pheromone which causes a morphological change, but they do respond to *a*-factor I which causes G_1 arrest in α cells. Strains that are *a* mating-type and *kex2* show no defect in mating, pheromone secretion, or response to α-factor. Diploids that are homozygous for the *kex2* mutation, unlike wild-type or heterozygous diploids, fail to undergo sporulation, with the defect occurring in the final spore maturation stage. These same defects in the sexual cycle are present in all *kex2* mutants independent of the presence of the "killer" plasmid.**

"Killer" strains of *Saccharomyces cerevisiae* secrete a toxin into the medium that kills other yeast strains, called sensitives (1–3). The ability to secrete the toxin and to resist killing is inherited in a non-Mendelian fashion (4, 5). The presence of the cytoplasmic killer genome is associated with the presence of a 1.4 to 1.7×10^6 molecular weight double-stranded RNA molecule (6, 7). This RNA plasmid is associated with virus-like particles in extracts of killer strains (8). The killer plasmid is further distinguished from chromosomal genes by its curability by growth in cycloheximide (5) or at elevated temperatures (9), treatments that result in the simultaneous loss of killing ability, resistance, and the killer plasmid.

The function of the killer genome requires the presence of at least 13 chromosomal genes. Replication or *ma*intenance of the *k*iller plasmid requires at least 10 chromosomal genes (refs. 4, 5, and 10 and R. B. Wickner and M. J. Leibowitz, unpublished). In strains carrying the killer plasmid, production of toxin requires the action of two chromosomal genes, *kex1* (chromosome VII) and *kex2* (chromosome XIV), for *k*iller *ex*pression (10, 11). At least one other gene is required for plasmid-carrying strains to resist killing (10).

In the course of genetic analysis of *kex2* mutants (11), it was found that all such mutants showed defects in the sexual cycle, indicating the function of this gene in both "host" and plasmid functions.

MATERIALS AND METHODS

Strains. *S. cerevisiae* strains used are listed in Table 1. Isolation of *kex* mutants and their genetic analysis have been described (10, 11).

Media. YPAD (rich medium), medium 4.7 MB, and SD (synthetic dextrose) were as described (11), with specific nutrients added to SD where indicated. Presporulation agar contained 0.8% yeast extract, 0.3% peptone, 10% dextrose, and 2% agar; sporulation agar was 1% potassium acetate, 0.1% yeast extract, 0.05% dextrose, and 2% agar. Liquid acetate presporulation and sporulation media (13) contained adenine sulfate (40 mg/liter) and L-leucine (20 mg/liter).

Purification of α-Factor. α-Factor (14), which induces G_1 arrest and elongation in *a* cells, was purified from liquid SD + uracil medium in which strain 47 had grown to stationary growth (15).

Sporulation Ability Assay. Diploids were tested for sporulation ability by growing on presporulation agar and then on sporulation agar. After 5 days at 26° the cells were suspended in 0.6 M NaCl, and extent of sporulation was measured as percent of cells that were 3- or 4-spored asci (1000 cells observed).

Abbreviation: SD, synthetic dextrose medium.

RESULTS

Mating reaction of yeast

S. cerevisiae haploid strains exist in two mating types, *a* and α, which are determined by allelic genes at the mating-type locus (reviewed in ref 16). Cells of the two types can fuse to form an *a*/α diploid, which can undergo meiotic sporulation to produce four haploid progeny (spores).

Prior to mating in liquid cultures, haploid cells of both mating types show arrest of growth in the G_1 phase of the cell cycle (unbudded) and morphological elongation, effects due to the actions of pheromones (17–19). The origins and effects of these substances, and their detection, are summarized in Fig. 1. Haploid α cells produce a single polypeptide pheromone α-factor, which causes both changes in responding *a* cells. Haploid *a* cells produce *a*-factor I (which causes G_1 arrest of α cells), *a*-factor II (which causes elongation of α cells), and barrier function (which inactivates α-factor).

α-Specific mating defect of *kex2* strains

All α *kex2* strains (18 independent *kex2* isolates) were defective in mating, whereas all 10 isolates of mutants in *kex1* showed normal mating. In 21 crosses (212 tetrads) involving various *kex2* mutants, all 169 α *kex2* segregants showed decreased mating ability. In the same crosses only one *a kex2* and one α *kex2*$^+$ segregant showed defective mating (rare nonmating segregants are seen in *all* crosses). The cosegregation of α-sterility and *kex2* indicates that the *kex2* mutation results in α-specific sterility. Quantitation of this mating defect (Table 2) indicates that α *kex2* strains mate less than 10^{-4} as efficiently as do wild-type α cells (crosses 1 and 4). However, *a kex2* cells mate as frequently with α wild-type (cross 7) or α *kex2* cells (cross 1) as do wild-type *a* yeast (crosses 4 and 10). Cross 13

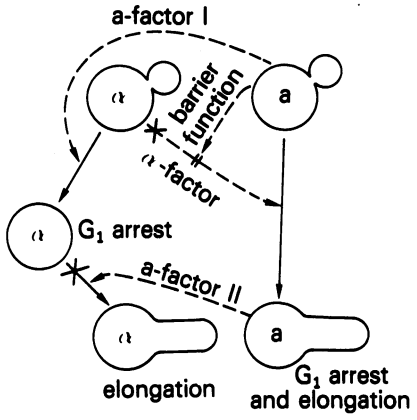

FIG. 1. Sexual pheromones of yeast and their detection. Here the broken arrows indicate substances secreted into the medium; solid arrows indicate response of cells to pheromones. Haploid α cells constitutively produce the α-factor polypeptide (14), which causes ***a*** cells to undergo G_1 arrest and elongation similar to that observed in mating mixtures. The factor(s) causing G_1 arrest and elongation of α cells in mating mixtures have been difficult to measure (12, 17). Haploid ***a*** cells constitutively produce a diffusible substance, ***a***-factor I, which acts transiently to block DNA synthesis (18) and causes G_1 arrest in α cells (20). A second pheromone, ***a***-factor II, is secreted by ***a*** cells and causes α cell elongation. This factor is produced by ***a*** cells only after induction by α-factor, except in one strain (X2180-G2C), which produces it constitutively (V. MacKay, personal communication). Furthermore, only cells of the ***a***-mating type can inactivate α-factor (barrier function), and this inactivation may be due to a diffusible substance (21). Normal ***a***/α diploids fail to secrete or respond to any of these substances. The X's indicate the defects in pheromone production and response observed in *kex2* strains. The assay of α-factor was as described (12). Detection of ***a***-factor I is described in Table 3. In order to obtain ***a***-factor II, strain X2180-G2C was grown to stationary phase in liquid SD, and the medium was concentrated 10-fold by evaporation and adjusted to pH 5.0 with 2 M NaOH (crude ***a***-factor II). Cells to be tested for response to ***a***-factor II were first grown in YPAD and then resuspended in SD at 4×10^7 cells per ml for 2 hr at 30°. Assays were then performed in 25-ml flasks at 30° with vigorous aeration by mixing 0.3 ml of 5 times concentrated SD, 0.2 ml of cells, and 1.0 ml of crude ***a***-factor II. After 8 hr a positive response to ***a***-factor II was indicated by the elongation of the cells (V. MacKay, personal communication). Barrier function was assayed as described (21). Strain 47 (α-factor source) was applied with a toothpick to YPAD agar and grown for 3.5 hr at 30°. Then a similar streak of the strain tested was applied immediately adjacent, and incubation was continued for 1 hr. Then a thin suspension of strain 200 (***a*** tester) was streaked immediately beside the strain tested, and after 8 hr the tester strain was examined. The barrier-plus phenotype was indicated by microcolonies of round cells, as were seen in the absence of α-factor. Formation of single elongated ***a*** tester cells indicated the barrier-minus phenotype and was the same response seen if there were no cells between the α source and ***a*** tester cells.

demonstrates that the presence of α *kex2* cells does not inhibit the mating of wild-type ***a*** and α strains.

α *kex2* strains do not secrete α-factor

The failure of α *kex2* strains to mate is accompanied by their inability to produce α-factor, assayed as described in the legend of Fig. 1. α *kex2* cells carrying 11 independently isolated *kex2* mutations all failed to produce α-factor. On the other hand, α *kex1* strains, which mated normally, produced α-factor. In a cross of an ***a*** *kex2*-3 strain with a wild-type nonkiller (α +), all 15 α + segregants found in 15 tetrads produced α-factor, whereas all 15 α *kex2* segregants did not. Thus, *kex2*, α-sterility, and the α-factor secretion defect segregate together. In similar tests ***a*** *kex2* cells, which mate normally, showed the normal response to α-factor secreted by α + cells.

"Helper" α + cells secreting α-factor did not help α *kex2* cells to mate (Table 2, crosses 2 and 5) nor did ***a*** + "helpers" (Table 2, crosses 3 and 6). Thus the α *kex2* strains must be deficient in additional α-specific functions.

α *kex2* strains respond to ***a***-factor I, but not to ***a***-factor II

Although none of the ***a*** strains we have tested were induced by α-factor to produce ***a***-factor II, we have confirmed that strain X2180-G2C does constitutively produce this pheromone, which causes α + cells to elongate. No effect of ***a***-factor II was observed on an α *kex2-2* strain (M78), indicating that the α-sterility in *kex2* mutants includes failure to respond to this factor. However, some G_1 arrest-effect of ***a***-factor I on α *kex2* cells was observed (Table 3).

The response of α *kex2* cells to ***a***-factor I is consistent with the observation that mutations in *kex2* do not abolish all α functions. Strains that are α *kex2* did not mate with other α cells, nor did ***a*** *kex2* strains mate with ***a*** cells. Like normal ***a***/α diploids, but unlike ***a***/***a*** diploids, diploids that were ***a***/α and heterozygous or homozygous for *kex2-1*, *kex2-2*, or *kex2*-3 failed to mate with haploid ***a*** or α strains, indicating that some α-specific functions still occurred in the *kex2* mutant background. Further supporting this hypothesis is the failure of ***a***/α *kex2* homozygotes (M349) to show "barrier function" for α-factor, an ***a***-specific reaction. The *kex2* mutation does not block barrier activity of ***a*** cells, and ***a*** *kex2-1*, ***a*** *kex2-2*, and ***a*** *kex2*-3 strains are barrier-plus. The corresponding α *kex2* strains are negative, both for α-factor secretion and barrier activity, as are wild-type ***a***/α diploids. Table 3 indicates that the *kex2* mutation does not block all pheromone secretion, since ***a*** *kex2* cells secrete ***a***-factor I, which causes transient mitotic arrest of α cells.

Mutations in *kex2* are distinct from other sterile mutations

Since the *kex2* locus is a site needed for killer expression, mutations in which confer α-specific sterility, sterile mutants (ref. 23; all K^-R^-) were crossed with wild-type killers to determine if they also carried *kex* type defects. Some could not be tested because they failed to mate. However, the two parental strains (XT1172-S245c and XT1177-S47c) and all of the mutants in which low frequency matings occurred showed no K^- haploid progeny, implying the absence of any *kex* or *mak* mutations. Strains tested [phenotypic mutant classes and proposed *ste* (sterile) loci indicated] included VC2 (class 1, *ste1*), VN33 (class 2, *ste1-5*), VAB2 (class 3, *ste2*), VQ3 (class 4, *ste3*), VZ4 (class 4, *ste4*), VAC1 (class 5, *ste5*), VI14 (class 6), VW9 (class 7), VC8 (class 8), VC73 (class 10), VT1 (class 11), VB5 (class 12), and VY3 (class 13). Furthermore, none of the 15 phenotypic classes of sterile mutants described (23) exhibited the *kex2* sterility phenotype of α-specific sterility with a defect in α-factor production.

Diploid *kex2* homozygotes arrest late in sporulation

When an ***a***/α diploid is starved for nitrogen with acetate as a carbon source, it undergoes meiosis to produce an ascus containing a tetrad of haploid spores. This sporulation process involves sequential steps which include an early doubling of DNA content accompanied by meiotic recombination, followed by meiotic division of the diploid nucleus into four haploid nuclei, each of which is incorporated into a mature spore (24–26).

Table 2 (cross 1) shows that ***a*** *kex2* × α *kex2* matings resulted in the formation of rare ***a***/α *kex2*/*kex2* diploids. All *kex2* homozygotes (11 *kex2* alleles tested) showed markedly decreased sporulation despite normal respiratory competence,

Table 1. Strains of *Saccharomyces cerevisiae*

Designation	Killer phenotype[a]	Genotype[b]	Source
80	K^-R^+	*a ade2 ura1 kex2-1* [KIL-k]	(10)
M78	K^-R^+	*α leu2-1 kex2-2* [KIL-k]	This work
660-3D	K^-R^+	*a leu2-1 thr1 arg1 kex2-3* [KIL-k]	This work
X2180-G2C	K^-R^-	*a gal2* [KIL-o] (*a*-factor II constitutive)	V. MacKay
XT1172-S245c	K^-R^-	*α ade6 his6 leu1 met1 trp5-1 gal2 can1* [KIL-o]	(12)
47	K^-R^-	*α ura3* [KIL-o]	J. Marmur
200	K^-R^-	*a lys10* [KIL-o]	R. K. Mortimer
M348	K^+R^+	*a/α ade2-1/ade2-1 leu2-27/leu2-1 thr1/+ arg1/+ +/his5-35 +/tyr1* [KIL-k]	This work
M349	K^-R^+	*a/α ade2-1/ade2-1 leu2-27/leu2-1 thr1/+ arg1/+ +/his5-35 +/ura3 kex2-2/kex2-2* [KIL-k]	This work

[a] Killing ability (K) and resistance (R) to killing are phenotypes of the strains (4, 10). Killing ability was tested by growth on a lawn of a sensitive strain at pH 4.7. Resistance was assayed by cross-streaking a cell suspension with a killer strain. In both cases, killer strains produced a clear halo that was bordered by growth of sensitive cells, whereas no effect was produced on resistant cells.

[b] The killer plasmid genotypes are: [KIL-k], wild-type killers; [KIL-o], sensitives lacking the plasmid.

whereas *kex1* homozygous or heterozygous diploids and *kex2* heterozygotes could undergo normal sporulation. Among fifteen tetrads of haploid segregants from the cross *α kex*$^+$ K^-R^- × *a kex2-2* K^-R^+, all 30 *kex2* haploid segregants, upon crossing with other *kex2* strains, formed diploids that were defective in sporulation (no spores seen in 22 crosses, maximum sporulation in one was 1.1%). Although occasional *kex2*$^+$ strains showed defective sporulation (2 of 30 < 1.1% sporulation), the *kex2*$^+$ progeny had an average of 21% sporulation. No *kex2-2* haploids sporulated normally upon formation of *kex2-2* homozygotes, indicating that a recessive sporulation defect cosegregated with *kex2*. In crosses among different *kex* mutants, diploids hetero-allelic for *kex2* showed deficient sporulation, whereas *kex1/kex2* diploids formed spores at normal frequencies.

The kinetics of sporulation in *kex2* homozygotes and wild-type diploids indicate that the block in sporulation in *kex2* strains occurs late in sporulation, after meiotic recombination and DNA synthesis have occurred (Fig. 2). Meiotic nuclear divisions could be seen in the mutant strain, although at reduced frequency, and the formation of mature spores was markedly reduced. The rare tetrads seen after the double selection of mating and sporulation contained no *kex2*$^+$ spores, indicating

Table 2. Mating efficiencies of *kex2* strains[a]

Cross	*a* Parent	α Parent	Helper	Diploids[b] α parent
1	*a kex2*	*α kex2*	—	2.5×10^{-4}
2	*a kex2*	*α kex2*	*α +*	2.6×10^{-5}
3	*a kex2*	*α kex2*	*a +*	1.4×10^{-4}
4	*a +*	*α kex2*	—	1.0×10^{-4}
5	*a +*	*α kex2*	*α +*	2.8×10^{-5}
6	*a +*	*α kex2*	*a +*	9.2×10^{-5}
7	*a kex2*	*α +*	—	2.0
8	*a kex2*	*α +*	*α +*	1.8
9	*a kex2*	*α +*	*a +*	2.6
10	*a +*	*α +*	—	1.4
11	*a +*	*α +*	*α +*	1.9
12	*a +*	*α +*	*a +*	1.6
13	*a +*	*α +*	*α kex2*	2.4

[a] The *a* and *α* parents, and "helper" strains where present, were mixed in 5:1:1 ratios (approximately 10^7 cells of the *α* parent) in water, collected on a Millipore filter (0.45 μm), and allowed to mate for 5.5 hr at 23° on YPAD agar. The filters were then removed from the plates, the mixtures were suspended in water, and serial dilutions were plated on SD agar with appropriate supplements to measure diploid formation. In each cross only the diploids formed by fusion of the *a* and *α* "parent" strains could be protrophic, whereas any matings occurring between either parent and the "helper" would result in auxotrophic diploids that would not grow on the selective medium used. All *kex2* strains used carried *kex2-3*.

[b] Numbers are expressed as diploids recovered per *α* parent at the start of the mating; presumably, numbers greater than 1.0 represent mitotic division of parents or diploids during the mating incubation.

Table 3. G_1 arrest caused by *a*-factor I: Secretion by *a kex2*, and response by *α kex2* cells

Supernatant[a]	Tester cells (% budded)[b] *a +*	*α +*	$\frac{a+}{\alpha+}$	*α kex2-2*
a +	40	6	67	19
α +	10	59	66	53
$\frac{a+}{\alpha+}$	31	62	NT[c]	NT
$\frac{a\ kex2\text{-}2}{\alpha\ kex2\text{-}2}$	30	48	NT	NT
a kex2-1	NT	11	NT	NT
a kex2-2	NT	13	NT	NT

[a] Cells were grown in 50 ml of liquid SD medium (with required nutrients added) in 250-ml flasks with vigorous aeration at 30° for 32 hr (final cell density, about 10^8 cells per ml). The flasks were then chilled, and after centrifugation, the supernatants were concentrated 10-fold by evaporation.

[b] Reactions were run by a modification of the method described (20). Each reaction included 1.5 ml of 10-fold concentrated supernatant, 1 ml of twice concentrated YPAD medium, and 3.5 ml of an exponentially growing culture of tester cells (5×10^6 cells per ml in YPAD medium at 23°) in a 50-ml flask. After 2 hr at 23°, 0.5-ml aliquots were fixed in 3.7% formaldehyde containing 0.15 M NaCl and were washed and digested with glusulase (22) to separate cells that had completed cytokinosis. Budded and total cells were then counted with a light microscope. At least 200 cells were counted from each sample. All zero-time samples showed 40–54% budded cells. All 5-hr samples contained 43–56% budded cells except for that in which supernatant from *α +* acted on *a +* cells, where only 8% budded cells were seen at 5 hr; this was also the only sample that contained elongated cells (57% of cells at 5 hr).

[c] NT indicates not tested.

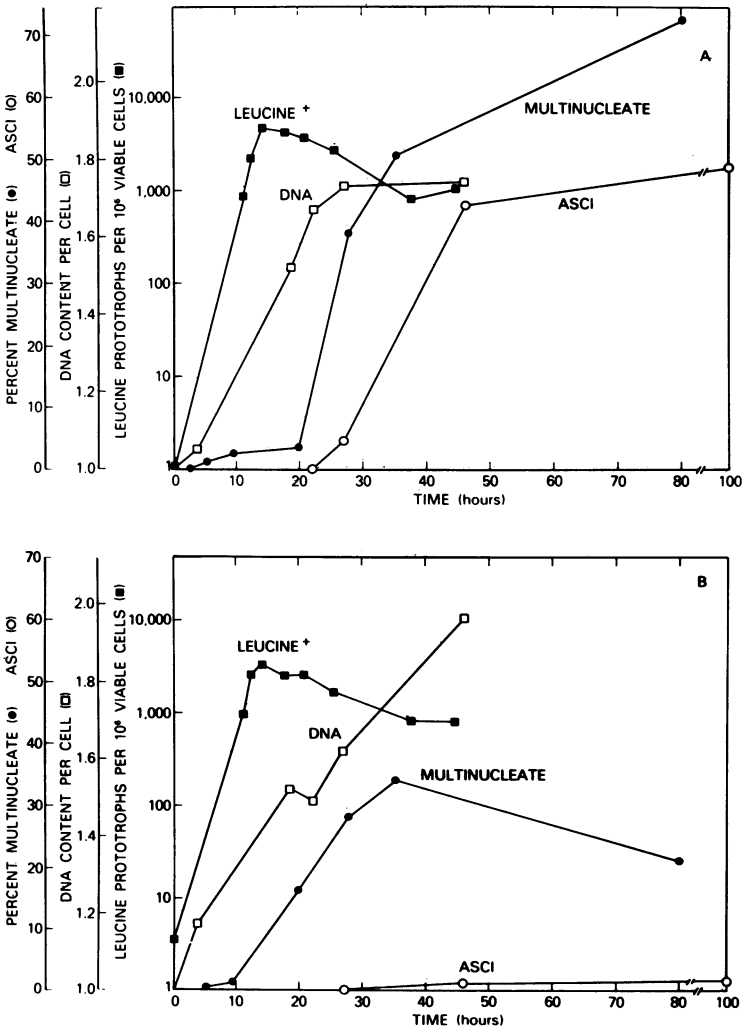

FIG. 2. Kinetics of sporulation of *kex2* homozygotes. Strains (A) M348 (wild-type diploid) and (B) M349 (*kex2* diploid) were constructed to be homozygous for *ade2-1* and hetero-allelic for *leu2-1* and *leu2-27*, noncomplementary alleles at *leu2* which undergo meiotic recombination by the gene conversion mechanism (27). Both strains were grown logarithmically (5×10^6 cells per ml) at 30° in liquid presporulation medium containing [^{14}C]adenine, centrifuged, washed twice in water, and then resuspended in an equal volume of sporulation medium containing [8-^{14}C]adenine (time 0). The culture was again aerated, and aliquots were periodically withdrawn. Leucine prototrophs, recombinants at *leu2*, were determined by plating dilutions on SD + adenine agar; growth on YPAD agar was used to estimate viable cell count. DNA synthesis was measured as described (28), except that [^{14}C]adenine was used as the radioactive DNA precursor. Cell number was determined in a hemocytometer chamber; each cell or spore tetrad was counted as a single cell. DNA synthesis is expressed here as cpm per cell number at each time relative to the value at 0 time, arbitrarily set at 1. All samples (1 ml of culture) contained at least 2000 cpm of alkali-stable trichloroacetic acid-precipitable radioactivity. Meiotic nuclear division (percent of multinucleate cells) was measured by staining cell nuclei with Giemsa stain (29), using RNase treatment in the place of acid (30), and then examining them in a light microscope (200 counted from each sample) as described (24). Extent of sporulation was scored as described in *Materials and Methods*. During these experiments both viable and visible cell counts remained approximately constant.

that the low levels of α-mating and homozygote sporulation observed were due to these processes occurring at reduced frequencies rather than reversion at *kex2*.

There is no evidence for involvement of the killer plasmid in the mating or sporulation defects of *kex2* mutants. Four heat-cured (K^-R^-) isolates (9) of strain M348 (wild-type diploid) showed 19.6–33.1% sporulation, whereas four cured isolates of M349 (*kex2*-2 homozygous diploid) showed 0–0.4% asci under standard conditions (only strains retaining respiratory competence were tested). Similarly, α strains mutant in *kex2*, which were cured of their plasmid, showed the same mating defect as their uncured parents.

DISCUSSION

The *kex2* gene product is required both for α-mating functions and meiotic sporulation, and for toxin production in yeast carrying the killer plasmid. The α-specific mating defect correlates with the absence of α-factor activity and the inability to respond to *a*-factor II. However, α *kex2* strains retain some α-functions, as is indicated by the ability of such strains to respond to *a*-factor I and the absence in *a*/α *kex2*/*kex2* diploids of *a*-specific mating functions, including ability to mate with α strains, *a*-factor I secretion, and barrier effect for α-factor, all of which are present in *a kex2* strains. Furthermore, such *kex2* homozygotes undergo normal meiotic recombination and DNA synthesis, functions depending upon the presence of both the *a* and α genetic loci (25, 27). However, sporulation does not continue to completion in *kex2* diploids; it is unknown if this spore maturation defect is another aspect of α-specific sterility or if it is an independently arising phenotype of the *kex2* mutation.

The *kex2* locus is a genetic site distant from mating-type

which is involved in the mating process. Other mapped sites influencing the mating reaction include *nul3* on chromosome IV (ref. 31 and D. C. Hawthorne, personal communication) and *tup1*, a gene controlling deoxythymidine-5′-monophosphate utilization, on chromosome III and linked to a/α (32). Many other mutations resulting in sterility map at unknown sites distant from the mating-type locus, and at least four such loci were identified, *ste2*, *ste3*, *ste4*, and *ste5* (23). Since these mutations are unlinked to mating-type and mutants at these four sites are kex^+, it is doubtful that these loci are *kex2* or *tup1*.

The sporulation of diploids depends upon multiple genes, and mutations have been isolated in many of these (26–28). Some sterile mutants also have sporulation defects (12, 23). In addition to *kex2*, a number of other loci required for sporulation have been mapped, including *tup1* (32), *SUP3*, an ochre nonsense suppressor (33), some sites controlling x-ray sensitivity (34), many of the cell division cycle (*cdc*) genes (35), and the loci (*pet*) required for respiratory competence (36).

The biochemical nature of the *kex2* gene product, which is required for the expression of a double-stranded RNA plasmid and for α-mating and diploid sporulation, remains unknown. The observation that the mating and sporulation abilities of *kex2* and wild-type strains are the same in plasmid-bearing and cured cells indicates that the sexual cycle defects are not mediated by the *kex2* gene effects on the killer plasmid. This gene's sexual functions may involve the other double-stranded RNA species that are present in nearly all yeast (7, 8) or might reflect cell wall or enzyme functions which are required in common by the specific processes that are defective in *kex2* mutants.

We thank Dr. E. Cabib for instruction in staining techniques and Dr. J. B. Hicks for allowing us to examine his manuscript (21) prior to its publication. Dr. R. Roth generously provided strains carrying the *leu2-1* and *leu2-27* markers. Drs. V. MacKay and T. R. Manney provided many strains used in mating experiments. Preliminary accounts of parts of this research have been presented (37, 38). M.J.L. is a Research Associate in Pharmacology, National Institute of General Medical Sciences, National Institutes of Health.

1. Makower, M. & Bevan, E. A. (1963) *Proc. Int. Congr. Genet. 11th* **1**, 202.
2. Woods, D. R. & Bevan, E. A. (1968) *J. Gen. Microbiol.* **51**, 115–126.
3. Bussey, H. (1972) *Nature New Biol.* **235**, 73–75.
4. Somers, J. M. & Bevan, E. A. (1969) *Genet. Res.* **13**, 71–83.
5. Fink, G. R. & Styles, C. A. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2846–2849.
6. Bevan, E. A., Herring, A. J. & Mitchell, D. J. (1973) *Nature* **245**, 81–86.
7. Vodkin, M., Katterman, F. & Fink, G. R. (1974) *J. Bacteriol.* **117**, 681–686.
8. Herring, A. J. & Bevan, E. A. (1974) *J. Gen. Virol.* **22**, 387–394.
9. Wickner, R. B. (1974) *J. Bacteriol.* **117**, 1356–1357.
10. Wickner, R. B. (1974) *Genetics* **76**, 423–432.
11. Wickner, R. B. & Leibowitz, M. J. (1976) *Genetics* **82**, 429–442.
12. MacKay, V. & Manney, T. R. (1974) *Genetics* **76**, 255–271.
13. Roth, R. & Halvorson, H. O. (1969) *J. Bacteriol.* **98**, 831–832.
14. Duntze, W., Stötzler, D., Bücking-Throm, E. & Kalbitzer, S. (1973) *Eur. J. Biochem.* **35**, 357–365.
15. Bücking-Throm, E., Duntze, W., Hartwell, L. H. & Manney, T. R. (1973) *Exp. Cell Res.* **76**, 99–110.
16. Mortimer, R. K. & Hawthorne, D. C. (1969) in *The Yeasts*, eds. Rose, A. H. & Harrison, J. S. (Academic Press, New York), Vol. 1, pp. 385–460.
17. Levi, J. D. (1956) *Nature* **177**, 753–754.
18. Shimoda, C. & Yanagishima, N. (1973) *Physiol. Plant.* **29**, 54–59.
19. Hartwell, L. H. (1973) *Exp. Cell Res.* **76**, 111–117.
20. Wilkinson, L. E. & Pringle, J. R. (1974) *Exp. Cell Res.* **89**, 175–187.
21. Hicks, J. B. & Herskowitz, I. (1976) *Nature* **260**, 246–248.
22. Hartwell, L. H. (1971) *Exp. Cell Res.* **69**, 265–276.
23. MacKay, V. & Manney, T. R. (1974) *Genetics* **76**, 273–288.
24. Croes, A. F. (1967) *Planta* **76**, 209–226.
25. Roth, R. & Lusnak, K. (1970) *Science* **168**, 493–494.
26. Roth, R. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3087–3091.
27. Fogel, S. & Roth, R. (1974) *Mol. Gen. Genet.* **130**, 189–201.
28. Esposito, M. S. & Esposito, R. E. (1974) *Genetics* **78**, 215–225.
29. Rabinow, C. F. (1961) *J. Biophys. Biochem. Cytol.* **9**, 879–892.
30. Hartwell, L. H. (1970) *J. Bacteriol.* **104**, 1280–1285.
31. Mortimer, R. K. & Hawthorne, D. C. (1973) *Genetics* **74**, 33–54.
32. Wickner, R. B. (1974) *J. Bacteriol.* **117**, 252–260.
33. Rothstein, R. J., Esposito, R. E. & Esposito, M. S. (1975) *Genetics* **80**, s70–s71.
34. Game, J. C. & Mortimer, R. K. (1974) *Mutat. Res.* **24**, 281–292.
35. Ephrussi, B. (1953) in *Nucleo-Cytoplasmic Relations in Micro-Organisms* (Clarendon Press, Oxford), pp. 29–44.
36. Simchen, G. (1974) *Genetics* **76**, 745–753.
37. Leibowitz, M. J. & Wickner, R. B. (1975) *Fed. Proc.* **34**, 503.
38. Leibowitz, M. J. & Wickner, R. B. (1975) in *Abstracts of the Annual Meeting of the American Society for Microbiol.*, p. 115.