TWO CHROMOSOMAL GENES REQUIRED FOR KILLING EXPRESSION IN KILLER STRAINS OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

The killer character of yeast is determined by a 1.4×10^6 molecular weight double-stranded RNA plasmid and at least 12 chromosomal genes. Wild-type strains of yeast that carry this plasmid (killers) secrete a toxin which is lethal only to strains not carrying this plasmid (sensitives). ——— We have isolated 28 independent recessive chromosomal mutants of a killer strain that have lost the ability to secrete an active toxin but remain resistant to the effects of the toxin and continue to carry the complete cytoplasmic killer genome. These mutants define two complementation groups, *kex1* and *kex2*. *Kex1* is located on chromosome *VII* between *ade5* and *lys5*. *Kex2* is located on chromosome *XIV*, but it does not show meiotic linkage to any gene previously located on this chromosome. ——— When the killer plasmid of *kex1* or *kex2* strains is eliminated by curing with heat or cycloheximide, the strains become sensitive to killing. The mutant phenotype reappears among the meiotic segregants in a cross with a normal killer. Thus, the *kex* phenotype does not require an alteration of the killer plasmid. ——— *Kex1* and *kex2* strains each contain near-normal levels of the 1.4×10^6 molecular weight double-stranded RNA, whose presence is correlated with the presence of the killer genome.

CERTAIN strains of *Saccharomyces cerevisiae* (called killers) secrete a protein which kills other strains (called sensitives) (MAKOWER and BEVAN 1963; WOODS and BEVAN 1968; BUSSEY 1972). Ability to secrete this toxin shows non-Mendelian inheritance (SOMERS and BEVAN 1968; BEVAN and SOMERS 1969; FINK and STYLES 1972), and a 1.4×10^6 molecular weight double-stranded RNA has been associated with the presence of the cytoplasmic killer genome (BERRY and BEVAN 1972; VODKIN and FINK 1973; BEVAN, HERRING and MITCHELL 1973; VODKIN, KATTERMAN and FINK 1974). This genome is reported to be encapsulated in virus-like particles (HERRING and BEVAN 1974). Cells may be "cured" of the cytoplasmic killer genome by growth in cycloheximide (FINK and STYLES 1972) or at elevated temperatures (WICKNER 1974a).

At least ten chromosomal genes are essential for maintenance or replication of the killer plasmid. These are *M* (SOMERS and BEVAN 1969), *pets* (FINK and STYLES 1972), *mak1* and *mak2* (WICKNER 1974b), and *mak3*, *mak4*, *mak5*, *mak6*, *mak7*, and *mak8* (WICKNER and LEIBOWITZ, unpublished). Three other

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genes, distinct from these, are necessary for expression of plasmid functions (WICKNER 1974b). Two of these, *kex1* and *kex2* (for *k*illing *ex*pression), are necessary for a strain to secrete an active toxin but are not needed for resistance to the toxin or for maintenance of the plasmid. The third, called *rex1* (for *r*esistance *ex*pression) is needed only for ability to resist being killed (WICKNER 1974b). In this communication we report the analysis of 28 mutants of the *kex* type.

MATERIALS AND METHODS

Strains: Strains of *S. cerevisiae* used are shown in Table 1.

Mutant isolation: All *kex* mutants were derived from A364A, S37, or 18 by the procedures previously described (WICKNER 1974b) except that mutagenized cells were plated at 20° instead of 30° to reduce the frequency of heat curing.

TABLE 1

Strains of Saccharomyces cerevisiae

Designation	Killer phenotype	Genotype	Source or reference
A364A	K+R+	**a** *ade1 ade2 tyr1 lys2 ura1 his7 gal1* [KIL-k]	HARTWELL, CULOTTI and REID, 1970
AN33	K-R-	*α thr1 arg1* [KIL-o]	S. HENRY
5X47	K-R-	**a**/*α his1*/+ *trp1*/+ *ura3*/+ [KIL-o]	R. WICKNER 1974b
20 (= D587-4B)	K+R+	*α his1* [KIL-k]	F. SHERMAN
M49 (= XS144-519)	K+R+	**a** *cyh2 met13 leu1 trp5 aro2 lys5 ade5* [KIL-k]	R. K. MORTIMER
95	K-R+	*α his1 kex1-1* [KIL-k]	R. WICKNER 1974b
54	K-R-	*α ade1* [KIL-o]	S. HENRY
S37	K+R+	*α leu2-1 met5* [KIL-k]	R. K. MORTIMER
81	K-R+	**a** *thr1 arg1 lys2 kex2-1* [KIL-k]	R. WICKNER 1974b
18 (= D585-11C)	K+R+	**a** *lys1* [KIL-k]	F. SHERMAN
1086-28D	K-R+	**a** *kex1-1 his1 ade5 lys5 aro2 met13 cyh2 trp5* [KIL-k]	This work
1086-15A	K-R+	**a** *kex1-1 his1 ade5 lys5 aro2 met13 cyh2 trp5* [KIL-k]	This work
5	K-R-	**a** *his1-315 trp1* [KIL-o]	G. FINK
ts 179	K+R+	A364A *rna2-2* [KIL-k]	J. WARNER (HARTWELL *et al.* 1970)
ts 368	K+R+	A364A *rna2-1* [KIL-k]	J. WARNER (HARTWELL *et al.* 1970)
H146-2-3	K+R+	**a** *ade1 ade2 ura1 lys2 gal1 cdc21-1* [KIL-k]	L. HARTWELL
23	K-R-	**a** *ade2 lys9* [KIL-o]	F. SHERMAN
230	K+R+	**a** *thr4 pet8 ilv3 met14* [KIL-o]	This work
82	K-R-	*α ade2 mak1-1* [KIL-o]	R. WICKNER 1974b
79	K-R+	*α arg1 ura1 his7 kex2-1* [KIL-k]	R. WICKNER 1974b

Media: YPAD contained 1% yeast extract, 2% peptone, 2% dextrose, 2% agar, and 0.04% adenine sulfate. MB medium was YPAD buffered at pH 4.7 with 0.05 M sodium citrate and containing 0.003% methylene blue (SOMERS and BEVAN 1969). YPG medium contained 1% yeast extract, 2% peptone, 2% agar, 0.04% adenine, and 3% (v/v) glycerol. SD medium contained 0.67% yeast nitrogen base (Difco), 2% agar, and 2% dextrose. Complete minimal medium was SD supplemented with: adenine sulfate, 400 mg/l; uracil, 24 mg/l; tryptophan, 24 mg/l; histidine, 24 mg/l; arginine, 24 mg/l; methionine, 24 mg/l; tyrosine, 36 mg/l; leucine, 36 mg/l; isoleucine, 36 mg/l; lysine, 36 mg/l; aspartic acid 100 mg/l; valine, 150 mg/l; threonine, 200 mg/l; and phenylalanine, 60 mg/l. Sensitivity to cycloheximide was determined on YPAD in the presence of 1 μg/ml of cycloheximide.

Notation: Phenotypes of strains with regard to their killing ability (K) and resistance (R) to killing are denoted K+R+, K-R+, K+R-, and K-R-. These can arise as a result of many different genotypes. The genotype of the killer plasmid is denoted by: [KIL-k] wild-type killers, [KIL-o] wild-type sensitives (carrying no plasmid), [KIL-n6] "neutral" mutants (BEVAN and SOMERS 1969), [KIL-s4] "suppressive" mutants (SOMERS 1973), or [KIL-d30] "diploid-dependent" mutants (WICKNER 1976). The lower case letter indicates the type of plasmid present, and the number indicates the allele.

Assay of killing and resistance: The ability of a strain to kill was assayed essentially as described by SOMERS and BEVAN (1969). Colonies grown on YPAD were replica plated to MB medium which had been previously spread with a lawn of the sensitive strain 5X47 [KIL-o]. Resistance to killing was checked by streaking a suspension of the strain to be tested on MB medium and cross-streaking with the killer strain 20. In each case, killing is indicated by a clear zone surrounding the killing strain and surrounded in turn by growth of the lawn or streak of sensitive cells.

Genetic analysis: Matings were carried out on unbuffered YPAD which, because its pH is about 6, does not permit the killer substance to function (WOODS and BEVAN 1968). Diploids were isolated utilizing the complementary nutritional requirements of their parents. Sporulation and dissection were by the usual methods (see MORTIMER and HAWTHORNE 1973 for references).

Purification and analysis of double-stranded RNA: All solutions were sterilized prior to use. Strains were grown to stationary phase at 28° with aeration in 3 liters of YPAD broth. Cells (30–60 g wet weight) were harvested at 4°, washed once with H_2O, and resuspended in 100 ml of 0.5 M sodium thioglycolate containing 0.1 M Tris-chloride, pH 8.8. After 30 minutes at 23°, the cells were centrifuged, washed once with H_2O, and suspended in 125 ml of 1 M sorbitol, to which was added 3 ml of Glusulase (Endo Laboratories). Alternatively, spheroplasts were often prepared by treatment of the harvested H_2O-washed cells with 0.4 mg of Zymolase (Kirin Brewery Co., Takasaki, Japan) per gram of cells in 1 M sorbitol containing 0.1 M potassium phosphate, pH 7.5 and 10 mM mercaptoethanol (KANEKO, KITAMURA and YAMAMOTO 1973). After 2 or 3 hours at 30°, the spheroplasted cells were centrifuged and washed twice with 60 ml of 1 M sorbitol. The pellet was suspended in 10 ml of 1% bentonite [prepared as described by FRAENKEL-CONRAT, SINGER and TSUGITA (1961)], 10 ml of "nuclease-free" pronase [10 mg/ml (Calbiochem), self-digested by the method of YOUNG and SINSHEIMER (1967)], and 100 ml of 0.15 M NaCl-0.1 M EDTA-2% sodium dodecylsulfate. This suspension was frozen and thawed once, incubated for 4 hours at 50°, and then centrifuged at 12,000 × g for 10 minutes. When cells were labeled with $^{33}PO_4$, the trichloroacetic acid-precipitable material in this supernatant contained 83% of the total trichloroacetic acid-precipitable material in the washed cells. The supernatant was extracted twice with neutralized water-saturated crystalline phenol containing 0.5 g 8-hydroxyquinoline per 500 g phenol. Losses of total nucleic acids at this step were negligible if the pronase digestion was complete. Nucleic acids in the aqueous phase were precipitated with 2 volumes of ethanol at —20° overnight, pelleted at 12,000 × g for 10 minutes, and dissolved in 200 ml of 0.05 M Tris-chloride, pH 7.3, 0.1 M NaCl, and 1 mM EDTA containing 15% ethanol. Double-stranded RNA was isolated from this material (up to 18,000 A_{260} units) by chromatography on a 2.5 × 30 cm column of CF11 cellulose by the method of FRANKLIN (1966). The ds RNA fraction generally constituted about 0.1% of the total nucleic acids applied to the column (or about 35 nmoles per g wet weight of cells) as measured by either ^{33}P or A_{260}.

This amount was not increased by increasing the size of the column 4-fold relative to the amount of nucleic acid applied and agrees well with the results of VODKIN, KATTERMAN and FINK (1974). The ds RNA fractions prepared from various strains were precipitated with 2 volumes of ethanol, dissolved in 0.02 M potassium phosphate, pH 6.8, 1 mM EDTA, and subjected to electrophoresis on 5% acrylamide slab gels using the system described by VODKIN, KATTERMAN and FINK (1974). The gels were stained by immersion in electrophoresis buffer containing 0.4 μg/ml of ethidium bromide. Photographs were taken under short-wave UV light illumination.

RESULTS

Among mutants of a killer strain which had lost the ability to kill, two were found, K26 and K34, which retained the killer plasmid and the ability to resist the effects of the killer toxin (K^-R^+ phenotype) (WICKNER 1974b). These two mutants were each recessive to wild type and yielded K^+R^+ diploids when mated with either a wild-type killer [KIL-k] or a wild-type K^-R^- [KIL-o] (sensitive) strain. Each showed Mendelian segregation on meiosis in both of these crosses, yielding two K^-R^+ and two K^+R^+ segregants in each tetrad. K26 and K34 also complemented one another and were non-allelic. They were thus denoted *kex1–1* and *kex2–1*, respectively.

We have since isolated 28 mutants that show this pattern (data not shown). Ten of these are certainly independent since they arose from different clones of strains A364A, S37, or 18. All are probably independent because no period of growth was allowed between mutagenesis and plating cells for single colonies to test for loss of killing ability.

Several other types of mutants with the K^-R^+ phenotype have been observed. Strains carrying a mutant plasmid of the "neutral" type, [KIL-n] (BEVAN and SOMERS 1969), are K^-R^+. Strains carrying a mutant plasmid of the "diploid-dependent" type, [KIL-d] (WICKNER 1974b and 1976), may be K^-R^+ as a haploid, but are K^+R^+ as diploids. A third type of K^-R^+ mutant can arise from a wild-type K^-R^- strain, [KIL-o], by mutation in any one of at least three chromosomal genes which we call *sek* for *sen*sitivity to *k*iller (BUSSEY, SHERMAN and SOMERS 1973; LEIBOWITZ and WICKNER, unpublished).

Complementation tests (Table 2): Complementation analysis showed that all *kex* mutants isolated fell into two complementation groups. This was confirmed in several cases by allelism tests (not shown). In the course of the allelism tests it was also noted that *kex1 kex2* double mutants have the same K^-R^+ phenotype as the single mutants. A few cases of intracistronic complementation were noted. If there are more than two genes of the *kex* type, those not found here are probably essential for growth.

Curing of kex *strains*: To show that the *kex* phenotype did not involve defects in the killer plasmid, *kex1–1* and *kex2–1* strains were treated with either heat (WICKNER 1974a) or cycloheximide (FINK and STYLES 1972) in order to "cure" them of their killer plasmid. *Kex1–1* or *kex2–1* colonies which had become R^- as a result of one of these treatments yielded only K^-R^- diploids in crosses with wild-type K^-R^- [KIL-o] strains and all of the meiotic segregants of these crosses were likewise K^-R^-. When cured *kex1–1* or *kex2–1* strains (presumably [KIL-o]) were

TABLE 2

Complementation testing of kex *mutants*

kex mutation in a parent		*kex* mutation in α parent																	
		K26 *kex1-1*	MK37 *1-2*	J16 *1-3*	K214 *1-4*	K242 *1-5*	K243 *1-6*	K34 *kex2-1*	MK1 *2-2*	J32 *2-3*	K215 *2-4*	K220 *2-5*	K221 *2-6*	K230 *2-7*	K237 *2-8*	K245 *2-9*	K250 *2-10*	K603 *2-11*	K606 *2-12*
K26	*kex1-1*	—	+	±	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
MK37	*1-2*	±	—	±	—	—	—	+	+	+	+	+	+		+	+	+	+	+
J16	*1-3*	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
K214	*1-4*	—	—	—	—			+	+	+									
K242	*1-5*	—	—	—		—		+	+	+									
K243	*1-6*	—	—	—			—	+	+	+									
K34	*kex2-1*	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
MK1	*2-2*	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
J32	*2-3*	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
K215	*2-4*	+	+	+				—	—	—	—								
K220	*2-5*	+	+	+				—	—	—		—							
K221	*2-6*	+	+	+				—	—	—			—						
K230	*2-7*	+	+	+				—	—	—				—					
K237	*2-8*	+	+	+				—	—						—				
K245	*2-9*	+	+	+				—	—	—						—			
K250	*2-10*	+	+	+				—	—								—		
K603	*2-11*	+	+	+				—	—	—								—	
K606	*2-12*	+	+	+				—	—	—									—

All diploids had R+ phenotype. Entries in the table indicate the killer phenotypes (K+ or K-) of the indicated diploids; ± indicates weak killing. Additional complementation tests (not shown) indicated that K226, K602, K251, and K721 were mutant in the *kex1* gene, while K234, K247, K605, K255, K730, and K731 had defects in the *kex2* gene.

crossed with wild-type K^+R^+ strains, the diploids were all K^+R^+ and the mutant K^-R^+ and wild-type K^+R^+ phenotypes segregated 2 : 2 on meiosis. Thus, a normal killer plasmid requires both *kex* genes for expression of the K^+ phenotype.

Kex *strains carry the same species of double-stranded RNA as does a wild-type killer*: Total cellular double-stranded RNA was prepared as described in MATERIALS AND METHODS and samples were subjected to electrophoresis on polyacrylamide slabs (Figure 1). As previously shown by BEVAN, HERRING and MITCHELL (1973) and VODKIN, KATTERMAN and FINK (1974), there are two major species detected in this way, one of which (arrow in Figure 1) is correlated with the presence of the killer plasmid. This species is present in normal killer strains (e.g., A364A) and is absent in wild-type nonkillers (e.g., AN33) and in strains carrying a mutation in one of the chromosomal genes needed to replicate the killer plasmid [e.g., 82, which is *mak1* (WICKNER 1974b)]. As shown in Figure 1, we find that both *kex1* and *kex2* mutants carry the killer ds RNA species, as expected from their genetic properties (WICKNER 1974b). All strains examined to date show various additional minor ds RNA species.

Mapping of kex1: Mapping by the multiple disome method of MORTIMER and HAWTHORNE (1973) narrowed the possible location of *kex1* to chromosomes *VII*, *XII*, and *XIII* (data not shown). The location of *kex1* between *ade5* and *lys5* on chromosome *VII* was then demonstrated by linkage in the crosses shown in

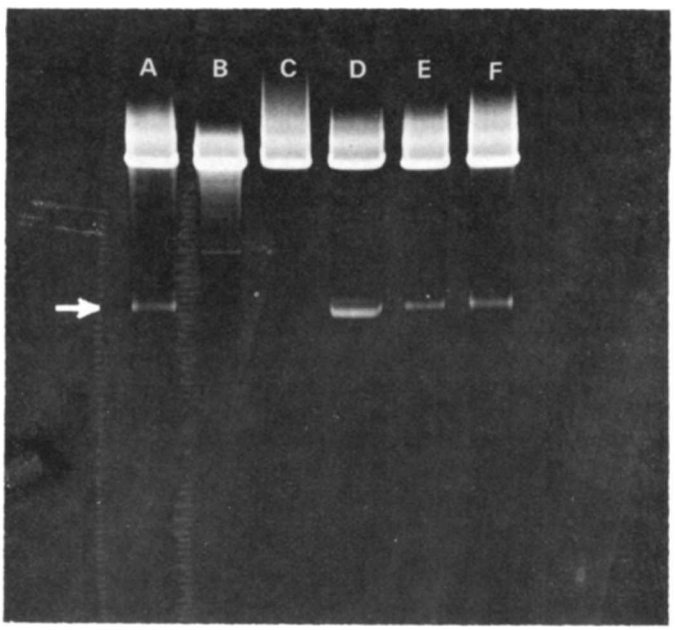

FIGURE 1.—Acrylamide gel electrophoresis of ds RNA preparations stained with ethidium bromide (see MATERIALS AND METHODS). (A) A364A ds RNA (wild-type killer); (B) AN33 ds RNA (wild-type nonkiller); (C) 82 ds RNA (*mak1-1* mutant); (D) 95 ds RNA (*kex1-1* mutant); (E) 79 ds RNA (*kex2-1* mutant); (F) A364A ds RNA (wild-type killer). Electrophoresis was from top to bottom. The arrow indicates the position of the 1.4×10^6 molecular weight ds RNA species correlated with the killer plasmid.

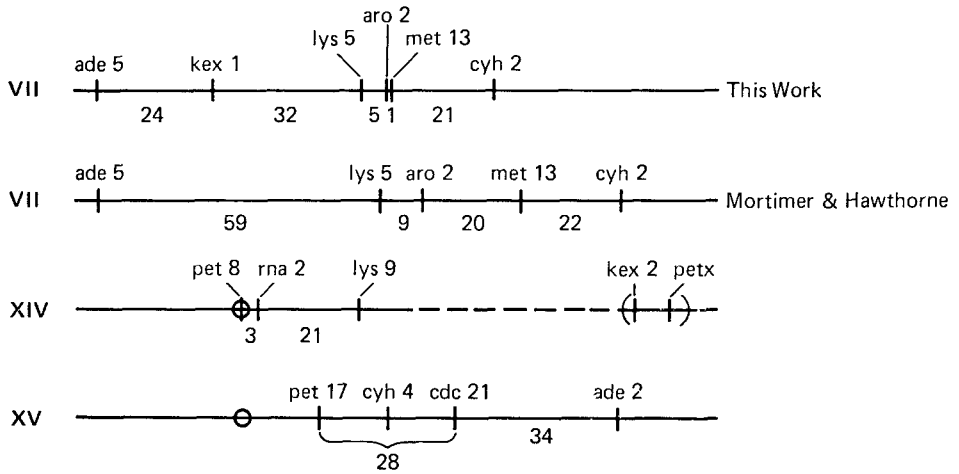

FIGURE 2.—Genetic maps of parts of chromosomes *VII* (Tables 3 and 4 and MORTIMER and HAWTHORNE 1966), *XIV* (Table 7), and *XV* (Table 8). Distances in centimorgans are indicated. The parentheses around *kex2* and *petx* indicate that their order relative to the rest of the chromosome is not known.

TABLE 3

Linkage of kex1 *to* ade5 *and* lys5 *on chromosome* VII*

		ade5	*lys5*	*aro2*	*met13*	*cyh2*	*trp5*
	PD	38	30	22	22	10	6
kex1	NPD	—	1	2	2	5	7
	T	35	38	46	45	53	32
	cM	*24*	*32*	*41*	*41*		
	PD		21	19	17	15	10
ade5	NPD		5	7	6	11	8
	T		57	56	59	55	30
	cM		*52*				
	PD			76	74	44	16
lys5	NPD			—	—	1	4
	T			9	10	40	29
	cM			*5*	*6*	*27*	
	PD				85	56	15
aro2	NPD				—	1	4
	T				2	31	20
	cM				*1*	*21*	
	PD					56	
met13	NPD					1	
	T					30	
	cM					*21*	

* For each pair of genes, the number of parental ditype (PD), nonparental ditype (NPD), and tetratype (T) asci observed is given. The data shown represent the pooled data from three crosses: M49 × 95, AN33 × 1086-15A, and AN33 × 1086-28D. In each cross, spore survival was greater than 90%. Distances are calculated in centimorgans (cM) by the method of PERKINS (1949).

TABLE 4

Gene order of ade5, kex1, *and* lys5 *based on tetrad data**

Type of tetrad	Crossovers needed for indicated gene order			Number observed
	ade5–kex1–lys5	*kex1–ade5–lys5*	*ade5–lys5–kex1*	
ade5 and *kex1* PD; *lys5* T with *ade5* and *kex1*	1	1	2	23
lys5 and *kex1* PD; *ade5* T with *lys5* and *kex1*	1	2	1	15
ade5 and *lys5* PD; *kex1* T with *ade5* and *lys5*	2	1	1	3

* The "number observed" was obtained from an analysis of the same 68 tetrads used in Table 3 to demonstrate linkage.
Conclusion: The results observed are explained with the fewest crossovers if one assumes the gene order of *ade5–kex1–lys5*.

Table 3. The gene order *ade5–kex1–lys5* was confirmed by analysis of specific tetrads in these crosses (Table 4). The map of chromosome *VII* (Figure 2) constructed from the data of Table 3 is consistent with that of MORTIMER and HAWTHORNE (1966) except for the distance between *aro2* and *met13*. Our ratio of PD : NPD : T was 85 : 0 : 2, while MORTIMER and HAWTHORNE obtained 53 : 1 : 26 for the same interval and ZIMMERMAN and VIG (1975) found 103 : 0 : 33. Conceivably, a deletion in this interval has occurred in the strain M49 (obtained from the Berkeley collection). It is also possible that these results represent the effects of site-specific recombination genes (reviewed by CATCHESIDE 1974; LAM *et al.* 1974).

Mapping of kex2: When strain 54 (*α ade1* [KIL-o]) was crossed with 81 (*α kex2–1 thr1 arg1 lys2* [KIL-k]), there was an excess of wild-type over mutant phenotypes in most tetrads (Table 5, cross 319), instead of the expected 2 K^-R^+ : 2 K^+R^+ segregation. The same phenomenon was observed with two other independent *kex2* alleles (Table 5, crosses L161 and 849). Mating *kex2* strains with any other strain gave consistent 2 : 2 segregation in meiosis. To determine if the *kex2–1* mutation was present, but masked in the tetrads which did not show 2 K^-R^+ : 2 K^+R^+ segregation, two tetrads from cross 319 showing 4 K^+R^+ : 0 segregation were examined by further crosses. This was indeed the case in two of each four spore clones (Table 6).

These results indicate the presence in strain 54 of a suppressor of *kex2* (functional or translational) or an extra copy of the *kex2*$^+$ gene, such as a disome. A translation suppressor was unlikely since all three independently isolated alleles tested were bypassed; furthermore, strain 54 did *not* carry a suppressor of *trp1–1* (amber), *lys1–1* (ochre), or *ade2–1* (ochre).

The presence of a disome for chromosome *XIV* was confirmed when segregants from cross 319, carrying the *kex2–1* mutation but having the K^+R^+ phenotype, were crossed with various marked strains. Markers on all chromosomes segregated $2^+ : 2^-$ except for those on chromosome *XIV* (Table 5), while in each cross, *kex2* showed an excess of wild-type spores, indicating the continued presence of the extra chromosome. *Pet8*, *lys9*, and *rna2* are the only markers located

TABLE 5

Mapping of kex2 *using a disomic strain**

Chromosome	Marker	Cross no.	Disomic strain	Segregation				
				$4^+ : 0^-$	$3^+ : 1^-$	$2^+ : 2^-$	$1^+ : 3^-$	$0^+ : 4^-$
	kex2-1	319	54	26	35	7	1	—
		333, 411, 519	319-62B	5	25	—	—	—
		334, 357, 397, 419, 421	319-62D	9	12	3	—	—
		338	319-63A	—	6	—	—	—
		337, 348, 383, 396, 398, 413	319-63B	38	59	12	—	—
		350	319-20A	4	8	3	1	—
	kex2-2	L161	54	2	14	4	—	—
	kex2-9	849	54	1	3	2	—	—
I†	*ade1*	357, 419, 421	319-62D	—	—	14	—	—
II	*lys2*	319	54	—	1	68	—	—
III	**a**/α	319	54	—	1(3**a**:1α)	68	—	—
IV	*trp1*	338	319-63A	—	—	6	—	—
		333	319-62B	—	—	6	—	—
V	*his1*	338	319-63A	—	—	6	—	—
		333	319-62B	—	—	6	—	—
VI	*his2*	350	319-20A	3	1	12	—	—
VII	*trp5*	348	319-63B	—	—	11	1	—
VIII	*thr1*	319	54	—	2	66	1	—
IX	*lys1*	334	319-62D	—	1	5	—	—
		337	319-63B	—	—	9	—	—
X	*ilv3*	397	319-62D	—	—	6	—	—
		411	319-62B	—	—	12	—	—

TABLE 5—Continued

Chromosome	Marker	Cross no.	Disomic strain	Segregation 4+ : 0-	3+ : 1-	2+ : 2-	1+ : 3-	0+ : 4-
XI	*ura1*	357	319-62D	—	—	6	—	—
	met14	413	319-63B	—	—	11	—	—
XII	*asp5*	350	319-20A	—	1	15	—	—
XIII	*lys7*	383	319-63B	—	—	29	—	—
XIV	*lys9*	396	319-63B	4	16	3	—	—
		519	319-62B	5	7	—	—	—
	pet8	413	319-62B	10	—	7	1	—
	rna2	419, 421	319-62D	3	2	3	—	—
XV	*ade2*	350	319-20A	—	—	16	—	—
		348	319-63B	—	—	12	—	—
XVI	*tyr7*	1156	54	—	—	18	—	—
XVII	*lys10*	398	319-63B	—	—	18	—	—

* In each cross, the marker(s) indicated were in a haploid strain which was crossed with a strain carrying an extra copy of the chromosome on which *kex2* is located. Consistent 2 : 2 segregation of a marker indicates that its chromosome does not carry *kex2*. In each cross, *kex2* showed an excess of wild-type spores, indicating the continued presence of the extra chromosome.

† Crosses 357, 419, and 421 were crosses of A364A, A364A *rna2–1*, and A364A *rna2–2* with 319–62D. While 319–62D has 2 copies of the *kex2* chromosome, neither copy carries *ade1* (see legend to Table 6). Thus, the 2 : 2 segregation of *ade1* in these crosses indicates *kex2* is not located on chromosome *I*.

TABLE 6

Some K+R+ segregants of cross 319 carry kex2-1*

Parents		Segregation		
K+R+ segregant of cross 319	Tester strain	4 K+R+ : 0	3 K+R+ : 1 K-R+	2 K+R+ : 2 K-R+
319-62A	20	12	—	—
319-62B	5	1	5	—
319-62C	20	12	—	—
319-62D†	18	1	5	—
319-63A	5	—	6	—
319-63B	18	5	4	—
319-63C	20	6	—	—
319-63D	20	9	—	—

* The 4 spore clones of each of two 4 K+R+ : 0 tetrads from cross 319 were crossed with tester strains known to be *kex+*. The appearance of K-R+ segregants derived from 2 members of each tetrad indicates that the *kex2-1* mutation is segregating 2 : 2 in each tetrad of cross 319, but is not observed because of either a suppressor or a disome (see text).

† In the cross of 319-62D with strain 18, none of the segregants were ade-, indicating that neither copy of the *kex2* chromosome in strain 319-62D carried *ade1*.

on chromosome *XIV* (MORTIMER and HAWTHORNE 1973). Each of these showed segregation consistent with the presence of an extra chromosome *XIV* in the strains disomic for the *kex2* chromosome (Table 5). We conclude that *kex2* is on chromosome *XIV*. After prolonged storage, single colonies of 319-62B, 319-62D, 319-63A, and 319-63B were crossed with a *pet8 lys9 rna2* strain. Killing segregated consistently 4 K+ : 0, indicating loss of the extra chromosome carrying the *kex2* mutation, while *pet8*, *lys9*, and *rna2* all segregated 2+ : 2-, indicating concomitant loss of the extra chromosome *XIV*. This result confirms the location of

TABLE 7

Kex2 *is unlinked to chromosome* XIV *markers*

		lys9	*rna2*	*pet8*	*petx**
kex2	PD	11	9	6	44
	NPD	12	17	11	—
	T	50	54	33	7
	cM				*7*
lys9	PD		23	24	
	NPD		1	—	
	T		15	22	
	cM		*28*	*24*	
rna2	PD			35	
	NPD			—	
	T			2	
	cM			*3*	

* *Petx* is a nuclear *pet* gene, in which a mutation was present in the original isolate of *kex2-6*. Symbols are as in Table 3.

TABLE 8

Cdc21 *is located between* ade2 *and* pet17*

		ade2	*pet17*	*trp1*
	PD	46	10	11
cdc21	NPD	2	0	6
	T	62	13	34
	cM	*34*	*28*	

* Symbols are as in Table 3.

kex2 on chromosome *XIV*. However, no linkage was observed between *kex2* and any of the markers on chromosome *XIV* (Table 7). In these latter crosses, *pet8*, *lys9*, and *rna2* showed consistent 2 : 2 segregation. It is also unclear on which arm of chromosome *XIV* *kex2* lies.

A nuclear petite mutation found in the original isolate of *kex2–6* was found linked to *kex2* (Table 7) and is denoted *petx*. *Petx* complements *pet4*, *pet5*, and *pet7* and is different from *pet1*, *pet2*, *pet3*, *pet8*, *pet9*, *pet11*, *pet14*, *pet17*, and *pet18* based on map position. *Pet6* is not linked to *kex2* in meiosis.

In the course of other studies, we have mapped the *cdc21* gene (Table 8), one of the "immediate-stop" DNA synthesis genes described by HARTWELL (1971, 1973). It is located between *pet17* and *ade2* on chromosome *XV*.

DISCUSSION

We have isolated 28 independent mutants defective in chromosomal genes needed for the production or secretion of an active killer toxin in the presence of a wild-type killer plasmid. These comprise two genes by complementation and allele tests—*kex1* and *kex2*. *Kex1* is on chromosome *VII* between *ade5* and *lys5*, while *kex2* is on chromosome *XIV* unlinked in meiosis to any genes previously located on this chromosome. Strains carrying either *kex1* or *kex2* mutations contain approximately normal amounts of the 1.4×10^6 molecular weight ds RNA which is thought to be the cytoplasmic killer genome.

None of the *kex* mutants described here show any tendency to lose the killer plasmid and no *chromosomal* mutants of the K^-R^- phenotype have been found that still carry the killer plasmid [but see "diploid-dependent" plasmid mutants (WICKNER 1976)]. Thus, the division of chromosomal mutants of killer strains into *kex* (*k*iller *ex*pression, K^-R^+ phenotype), *rex* (*r*esistance *ex*pression, K^+R^-), and *mak* (*ma*intenance and replication of the *k*iller plasmid, K^-R^-) mutants (WICKNER 1974b) remains valid. It remains possible that some *mak* genes may also be essential for expression of killing or resistance, but once the plasmid is lost this cannot be tested.

Because the cytoplasmic killer genome is only large enough to code for proteins with molecular weights totalling 100,000, it must utilize host enzymes for its replication (*mak* genes) and expression (*kex* and *rex* genes). These host enzymes presumably function in host processes independent of the killer genome. We

have found (LEIBOWITZ and WICKNER 1975) that the *kex2* product is necessary for mating by cells of the α mating type, but not for mating by **a** cells. The *kex2* product is also needed for meiosis and sporulation (LEIBOWITZ and WICKNER 1975). It is possible that other *kex* genes exist, but were not isolated in this study because they are essential for host cell growth.

The mapping of *kex2* illustrates the usefulness of the aneuploid mapping method of MORTIMER and HAWTHORNE (1973). Since this gene is not linked to any of the gees previously located on chromosome *XIV*, it might not have been possible to locate it without the disome method.

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LITERATURE CITED

BERRY, E. A. and E. A. BEVAN, 1972 A new species of double-stranded RNA from yeast. Nature New Biol. **239**: 279–280.

BEVAN, E. A. and J. M. SOMERS, 1969 Somatic segregation of the killer (k) and neutral (n) cytoplasmic genetic determinants in yeast. Genet. Res. **14**: 71–77.

BEVAN, E. A., A. J. HERRING and D. J. MITCHELL, 1973 Preliminary characterization of two species of *ds* RNA in yeast and their relationship to the "killer" character. Nature **245**: 81–86.

BUSSEY, H., 1972 Effects of yeast killer factor on sensitive cells. Nature New Biol. **235**: 73–75.

BUSSEY, H., D. SHERMAN and J. M. SOMERS, 1973 Action of yeast killer factor: a resistant mutant with sensitive spheroplasts. J. Bacteriol. **113**: 1193–1197.

CATCHESIDE, D. G., 1974 Fungal genetics. Ann. Rev. Genet. **8**: 279–300.

FINK, G. R. and C. A. STYLES, 1972 Curing of a killer factor in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S. **69**: 2846–2849.

FRAENKEL-CONRAT, H., B. SINGER and A. TSUGITA, 1961 Purification of viral RNA by means of bentonite. Virology **14**: 54–58.

FRANKLIN, R. M., 1966 Purification and properties of the replicative intermediate of the RNA bacteriophage R17. Proc. Natl. Acad. Sci. U.S. **55**: 1504–1511.

HARTWELL, L. H., 1971 Genetic control of the cell division cycle in yeast. II. Genes controlling DNA replication and its initiation. J. Mol. Biol. **59**: 183–194. ——, 1973 Three additional genes required for DNA synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **115**: 966–974.

HARTWELL, L. H., J. CULOTTI and B. REID, 1970 Genetic control of the cell-division cycle in yeast. I. Detection of mutants. Proc. Natl. Acad. Sci. U.S. **66**: 352–359.

HARTWELL, L. H., C. S. MCLAUGHLIN and J. R. WARNER, 1970 Identification of ten genes that control ribosome formation in yeast. Mol. Gen. Genet. **109**: 42–56.

HERRING, A. J. and E. A. BEVAN, 1974 Virus-like particles associated with the double-stranded RNA species found in killer and sensitive strains of the yeast *Saccharomyces cerevisiae*. J. Gen. Virol. **22**: 387–394.

KANEKO, T., K. KITAMURA and Y. YAMAMOTO, 1973 Susceptibilities of yeasts to yeast cell wall lytic enzyme of *Arthrobacter luteus*. Agric. Biol. Chem. **37**: 2295–2302.

LAM, S. T., M. M. STAHL, K. D. MCMILIN and F. W. STAHL, 1974 Rec-mediated recombinational hot spot activity in bacteriophage lambda. II. A mutation which causes hot spot activity. Genetics **77:** 425–433.

LEIBOWITZ, M. J. and R. B. WICKNER, 1975 Mating, sporulation, respiration, and growth defects associated with chromosomal mutations affecting a double-stranded RNA plasmid: the "killer" of *Saccharomyces cerevisiae*. Fed. Proc. **34:** 503.

MAKOWER, M. and E. A. BEVAN, 1963 The inheritance of a killer character in yeast (*Saccharomyces cerevisiae*). Proc. Int. Congr. Genet. XI **1:** 202.

MORTIMER, R. K. and D. C. HAWTHORNE, 1966 Genetic mapping in *Saccharomyces*. Genetics **53:** 165–173. ——, 1973 Genetic mapping in *Saccharomyces*. IV. Mapping of temperature-sensitive genes and use of disomic strains in localizing genes. Genetics **74:** 33–54.

PERKINS, D. D., 1949 Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34:** 607–626.

SOMERS, J. M., 1973 Isolation of suppressive mutants from killer and neutral strains of *Saccharomyces cerevisiae*. Genetics **74:** 571–579.

SOMERS, J. M. and E. A. BEVAN, 1969 The inheritance of the killer character in yeast. Genet. Res. **13:** 71–83.

VODKIN, M. H., and G. R. FINK, 1973 A nucleic acid associated with a killer strain of yeast. Proc. Natl. Acad. Sci. U.S. **70:** 1069–1072.

VODKIN, M., F. KATTERMAN and G. R. FINK, 1974 Yeast killer mutants with altered double-stranded ribonucleic acid. J. Bacteriol. **117:** 681–686.

WICKNER, R. B., 1974a "Killer character" of *Saccharomyces cerevisiae*: Curing by growth at elevated temperatures. J. Bacteriol. **117:** 1356–1357. ——, 1974b Chromosomal and nonchromosomal mutations affecting the "killer character" of *Saccharomyces cerevisiae*. Genetics **76:** 423–432. ——, 1976 Mutants of the killer plasmid of *Saccharomyces cerevisiae* dependent on chromosomal diploidy for expression and maintenance. Genetics **82:** .....–.....

WOODS, D. R. and E. A. BEVAN, 1968 Studies on the nature of the killer factor produced by *Saccharomyces cerevisiae*. J. Gen. Microbiol. **51:** 115–126.

YOUNG, E. T. and R. L. SINSHEIMER, 1967 Vegetative bacteriophage λ DNA. II. Physical characterization and replication. J. Mol. Biol. **30:** 165–200.

ZIMMERMAN, F. K. and B. K. VIG, 1975 Mutagen specificity in the induction of mitotic crossing-over in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **139:** 255–268.

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