CHROMOSOMAL AND NONCHROMOSOMAL MUTATIONS AFFECTING THE "KILLER CHARACTER" OF *SACCHAROMYCES CEREVISIAE*¹

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ABSTRACT

The "killer character" of *Saccharomyces cerevisiae* is a nonchromosomal genetic element which imparts to cells carrying it (a) the ability to kill cells which lack it, by secreting the soluble macromolecular killer substance and (b) the ability to resist the killing effects of the killer substance.—Mutants have been isolated from *S. cerevisiae* carrying the "killer character". Mutants were found in two nuclear genes (*mak1* and *mak2*) involved in maintenance of the genetic element of the killer character. Mutants in three other nuclear genes could maintain the cytoplasmic genetic element normally; two of these were able to express resistance to killing, but not the ability to kill (*kex1* and *kex2*), while the third could express the killing function, but was sensitive to killing and is thus a "suicide" strain (*rex1*). These five nuclear genes were all distinct.—Several mutants were detected which showed non-mendelian segregation indicating mutation of the "cytoplasmic" genetic element. Some such mutants had normal resistance to killing but were either unable to kill at all or showed marked reduction in the ability to kill. Others had lost both killing ability and resistance. None were suppressive.—Two nonkilling mutants, both of which showed cytoplasmic inheritance, were mated. A large, but variable, proportion of diploid colonies, plated after several generations of growth, showed normal killing which has then stably inherited on further subcloning.

THE "killer character" of *S. cerevisiae* is a non-mendelian genetic element which gives strains carrying it (1) the ability to kill sensitive strains by secreting a killer substance and (2) immunity to the action of the killer substance, a protein-containing macromolecule whose structure and mode of action are as yet unclear (BEVAN and MAKOWER 1963; SOMERS and BEVAN 1969; BEVAN and SOMERS 1969; WOODS and BEVAN 1968; FINK and STYLES 1972; and BUSSEY 1972). The killer character is widespread in the usual laboratory strains and can be "cured" by growth on low concentrations of cycloheximide (FINK and STYLES 1972) or by growth at elevated temperatures (WICKNER 1974). A double-

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stranded RNA in a particulate fraction has been found specifically in killer strains, but not in nonkillers (BERRY and BEVAN 1972; VODKIN and FINK 1973). It is not yet clear whether this represents the cytoplasmic genetic element of the killer character. The ability to kill requires the presence of at least two chromosomal genes, *M* and *pets* (SOMERS and BEVAN 1969; FINK and STYLES 1972), and a non-mendelian genetic element, (*k*). Some strains are immune to the action of the killer substance, but do not produce it. These have been called "neutral" (SOMERS and BEVAN 1969; BEVAN and SOMERS 1969). The neutral phenotype is also governed by the presence of a cytoplasmic element (*n*) and the chromosomal gene *M*. Somatic segregation of (*n*) and (*k*) in a diploid of the type *M*(*k*) $\times$ *M*(*n*) has been demonstrated (BEVAN and SOMERS 1969).

The isolation of mutants of a killer strain reported here was undertaken with the aim of understanding killer as a genetic system and of studying the maintenance, replication, segregation, and expression of the cytoplasmic genetic element. Although the study of naturally occurring variants referred to above has been valuable in outlining many of this system's essential features, a detailed understanding requires the isolation of a series of mutants derived from a single strain. Both nuclear and non-nuclear mutants have been found. The nuclear mutants include those in three new genes needed for expression of killing or of resistance to killing and two genes, at least one of which is new, needed for maintenance of the killer genome. The cytoplasmic mutants include weak killers, "neutral" strains, and "sensitive" strains. Zygotes formed by mating two of these haploid cytoplasmic mutants, each unable to kill by itself, were found to give rise to mitotically stable strains with normal killing ability. This new finding opens the possibility of genetic studies of the nonchromosomal killer genome.

MATERIALS AND METHODS

Media: YPAD contained 1% yeast extract, 2% peptone, 2% dextrose, 2% agar, and 0.04% adenine sulfate. 4.7 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.

Notation: There are at least four possible *phenotypes* of strains with regard to their killing ability and resistance to killing. These are denoted *K*+*R*+, *K*-*R*+, *K*+*R*-, and *K*-*R*-. These can arise as a result of many different genotypes, as will be discussed below. *K*^w means weak killing and is seen as a narrower zone of killing in the usual assay (see below).

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 4.7 MB medium which had been previously spread with a lawn of the sensitive strain 5X47. Resistance to killing was checked by streaking a suspension of the strain to be tested on 4.7 MB medium and cross-streaking with the killer strain 18X19. 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.

Mutagenesis: A364A (*K*+*R*+) was grown overnight at 30° on YPAD slants, suspended at about 10⁸ cells/ml in 0.1 M potassium phosphate, pH 7.5, and made 5% with ethylmethane sulfonate. After 40 minutes of incubation with shaking at 30°, the cell suspension was diluted 1 : 10 with 5% sodium thiosulfate. Ten minutes later, the cells were plated for single colonies at 30° on YPAD plates, and the single colonies were replica plated to a lawn of a sensitive strain on 4.7

TABLE 1

Strain designation	Killer phenotype	Other markers	Source or reference
A364A	K ⁺ R ⁺	<i>a ade1 ade2 tyr1 lys2 ura1 his7 gal1</i>	HARTWELL, CULOTTI and REID 1970
A5969A	K ⁺ R ⁺	α <i>his4 thr4 MAL2 leu2 trp1</i>	SHAFFER <i>et al.</i> 1971
20 (=D587-4B)	K ⁺ R ⁺	α <i>his1</i>	F. SHERMAN
AN33	K ⁻ R ⁻	<i>a thr1 arg1</i>	S. HENRY
5X47	K ⁻ R ⁻	<i>a his1 trp1 ura3</i> α $\frac{-}{+}$ $\frac{-}{+}$ $\frac{-}{+}$	
18X19	K ⁺ R ⁺	<i>a lys1 his1</i> α $\frac{-}{+}$ $\frac{-}{+}$	

MB medium. Colonies showing no killing or blue staining were picked from the original plates and purified twice by picking single colonies. Finally, these colonies were tested for their killing ability and resistance to killing.

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).

RESULTS

Isolation of Mutant "Killer" Strains

Following mutagenesis with ethylmethane sulfonate, about 100 mutants with abnormal killing characteristics were isolated as described in MATERIALS AND METHODS. The purified mutant strains were tested for their killing ability and their resistance to killing by a known killer strain. Four mutant phenotypes were observed, namely, K⁻R⁻, K⁻R⁺, K⁺R⁻, and K⁺R⁺. The first two correspond to the "sensitive" and "neutral" phenotypes of SOMERS and BEVAN (1969).

The third phenotype, K⁺R⁻, or "suicide," has not been previously reported. This strain (K44) grew well on unbuffered YPAD (pH ~ 6), probably because the killer substance is not active at this pH (WOODS and BEVAN 1968; FINK and STYLES 1972). At pH 4.7 in the presence of methylene blue this strain shows definite killing of a sensitive strain, the colony grows poorly, and it is dark blue, due to methylene blue staining of the dead cells killed by the killer substance they themselves produced. The killing observed is reduced, probably due to the poor cell growth at this pH. A lawn of the K⁺R⁻ strain K44 on 4.7 MB medium shows no resistance to killing by a standard killer strain.

The fourth phenotype, K⁺R⁺, or weak killer, was frequently found. This strain was not a mixture of normal killer and nonkiller cells. Every colony showed the same degree of weak killing.

All mutants described here were able to grow on a nonfermentable carbon source (grande), as judged by their ability to grow on YPG plates.

Meiotic Segregation Analysis of Killer Mutants

Each strain was mated with the killer (K⁺R⁺) strain 20, and a sensitive (K⁻R⁻) strain AN33. The phenotype of the diploids was determined. Each diploid

TABLE 2

Nuclear mutants

Mutant	Designation	Mutant phenotype	Diploid with strain 20 (K ⁺ R ⁺) Diploid phenotype	Segregation of K and R*	Diploid with strain AN33 (K ⁻ R ⁻) Diploid phenotype	Segregation of K and R*
K26	<i>kex</i> 1-1	K-R ⁺	K ⁺ R ⁺	(6) 2K-R ⁺ :2K ⁺ R ⁺	K ⁺ R ⁺	(22) 2K-R ⁺ :2K ⁺ R ⁺ (1) 3K-R ⁺ :1K ⁺ R ⁺ (1) 1K-R ⁺ :3K ⁺ R ⁺
K34	<i>kex</i> 2-1	K-R ⁺	K ⁺ R ⁺	(25) 2K-R ⁺ :2K ⁺ R ⁺ (1) 3K-R ⁺ :1K ⁺ R ⁺	K ⁺ R ⁺	(28) 2K-R ⁺ :2K ⁺ R ⁺ (1) 3K-R ⁺ :1K ⁺ R ⁺
K56	<i>mak</i> 1-1	K-R ⁻	K ⁺ R ⁺	(39) 2K-R ⁻ :2K ⁺ R ⁺	K-R ⁻	(10) 4K-R ⁻ :0
K58	<i>mak</i> 2-1	K-R ⁻	K ⁺ R ⁺	(14) 2K-R ⁻ :2K ⁺ R ⁺ (1) 1K-R ⁻ :3K ⁺ R ⁺ (1) 0:4K ⁺ R ⁺	K-R ⁻	(10) 4K-R ⁻ :0
K44	<i>rex</i> 1-1	K ⁺ R ⁻	K ⁺ R ⁺	(6) 2K ⁺ R ⁻ :2K ⁺ R ⁺	K ⁺ R ⁺	(24) 2K ⁺ R ⁻ :2K ⁺ R ⁺

* Numbers in parentheses indicate number of tetrads examined.

was sporulated, tetrads were dissected, and the phenotypes of the spore colonies were checked. For purposes of analysis of nuclear mutants, it has been assumed that AN33 lacks all cytoplasmic components of the killer genome. Although evidence is presented below suggesting that this may not be true, the conclusions drawn in the studies on nuclear mutants appear justified in any case. When the parent of the mutants described here, A364A (K⁺R⁺), is crossed with AN33 (K⁻R⁻), the diploids are K⁺R⁺ and sporulation yields uniformly 4 K⁺R⁺ : 0 tetrads. Thus, strain AN33 is wild type with respect to the nuclear genes needed to harbor and express the killer cytoplasmic genome.

Nuclear mutants: Five mutants (K26, K34, K56, K58, and K44) showed consistent 2 : 2 segregation, indicative of a single chromosomal mutation (Table 2). These mutants fell into three phenotypic classes (Table 2) and five distinct genes (see below).

Both K26 and K34 were K-R⁺ but apparently contained the complete cytoplasmic killer genome since diploids formed from these mutants and a sensitive (K-R⁻) strain were normal killers. Since both these strains and those formed by mating the mutants with a normal killer (K⁺R⁺) showed 2 : 2 segregation of killing and 4R⁺ : OR⁻ segregation of resistance, it seems likely that K26 and K34 are mutants defective in recessive chromosomal functions necessary for expression of killing, but not needed for either expression of resistance or maintenance of the cytoplasmic genetic element. These mutants are thus called *kex* (killer expression).

Another chromosomal mutant, K44, was found which, though able to kill, was itself sensitive to killing. This "suicide" strain gave rise to normal K⁺R⁺ diploids when mated with either a K-R⁻ (AN33) or a K⁺R⁺ (20) haploid (Table 2). The "suicide" phenotype segregated out when either of these diploids was sporulated (Table 2). Thus, the "suicide" character is recessive and reflects an inability to express the resistance (*rex*) and not a loss of some of the nonchromosomal genetic information. Further studies of this mutant will be needed to conclusively prove that it is not in the same gene as *kex1* or *kex2*.

K56 and K58 showed a K^-R^- phenotype. When mated with a normal K^+R^+ strain (Table 2), each gave rise to K^+R^+ diploids. On sporulation, each tetrad yielded 2 K^-R^- and 2 K^+R^+ spore colonies. Thus, these represent single recessive, chromosomal genes. To determine whether or not K56 and K58 still contain the cytoplasmic genome, each was mated with AN33 (K^-R^-), a strain known to be wild type with regard to all genes needed to express and maintain the cytoplasmic genome. If K56 or K58 still contained the cytoplasmic genome, diploids with AN33 would be K^+R^+ , since the mutations in K56 and K58 are recessive. Since the diploids were K^-R^- , K56 and K58 must lack the cytoplasmic genome. Thus, it may be concluded that K56 and K58 are defective in maintenance of the non-chromosomal killer genetic element. I propose that K56 and K58 be called *mak1* and *mak2*, respectively (maintenance of killer).

It was possible that K56 and K58 had lost their cytoplasmic killer genome during mutagenesis by an event independent of the chromosomal mutation. If K56 or K58 had been unable to *express* killing and resistance due to a nuclear mutation and lacked the killer genome due to a second unrelated mutational event, then the K^-R^- meiotic segregants of $K56 \times 20$ or $K58 \times 20$ would have the cytoplasmic killer genome and would be expected to yield K^+R^+ diploids with AN33 and segregate 2 K^-R^- : 2 K^+R^+ . In fact, however, the diploids were K^-R^- and segregation was 4 K^-R^- : 0 (Table 3, lines 5 and 7). Similar crosses with derivatives of K26 and K34 gave the expected results. Crosses of K^+R^+ segregants from Table 2 gave segregation of killing and resistance typical of wild-type killer strains. This is especially important in the case of the K^+R^+ segregants of the crosses K26 (*kex1*) $\times$ AN33 and K34 (*kex2*) $\times$ AN33. One might hypothesize that these K^+R^+ segregants arise because AN33 has an abnormal killer genome expressible only when present with one of the abnormal *kex* genes. This hypothesis is ruled out by the crosses in lines 2 and 4 of Table 3 with AN33.

Another hypothesis which might be considered is that *kex1* and *kex2* strains are each unable to *maintain* segments of the cytoplasmic genome which are (a) necessary for killing but not necessary for resistance; (b) present in AN33 and thus not sufficient for either killing or resistance; and (c) supplied by AN33 in the diploids with *kex1* or *kex2* strains to make a complete killer genome. This notion implies that: (a) there must be two such segments in AN33, one which it supplied to *kex1* strains and one which it supplied to *kex2* strains because *kex1* complements *kex2* (Table 3); and (b) both *mak1* and *mak2* strains must be able to maintain both of these segments since these strains complement either *kex1* or *kex2* strains (Table 4). Further data will be needed to rule out this hypothesis.

Complementation tests were performed with the five chromosomal mutants described above (Table 4). Complementation was observed between each non-identical pair except between *mak1* and *mak2*. However, since *mak1* and *mak2* strains have lost their cytoplasmic killer genome, the heterozygote formed between them would not be expected to kill. *Mak1* and *mak2* are distinct since *mak1* is tightly centromere-linked, while *mak2* shows no centromere linkage (Table 5).

The complementation of *kex1*, *kex2*, and *rex1* strains by *mak1* and *mak2* is further evidence that the former carry the normal killer genome.

TABLE 3

Segregation of meiotic derivatives of kex-1, kex-2, mak-1, and mak-2

Strain	Mutant phenotype	Diploid with strain 20 (K ⁺ R ⁺) Diploid phenotype	Segregation of K and R [*]	Diploid with strain AN33 (K ⁻ R ⁻) Diploid phenotype	Segregation of K and R [*]
1. <i>a kex 1-1</i> (K ⁻ R ⁺ segregant from K26 × AN33)	K ⁻ R ⁺	K ⁺ R ⁺	(6) 2K ⁻ R ⁺ :2K ⁺ R ⁺	K ⁺ R ⁺	(11) 2K ⁻ R ⁺ :2K ⁺ R ⁺
2. <i>a kex 1+</i> (K ⁺ R ⁺ segregant from K26 × AN33)	K ⁺ R ⁺	K ⁺ R ⁺	(5) 4K ⁺ R ⁺ :0	K ⁺ R ⁺	(5) 4K ⁺ R ⁺ :0
3. <i>a kex 2-1</i> (K ⁻ R ⁺ segregant from K34 × AN33)	K ⁻ R ⁺	K ⁺ R ⁺	(22) 2K ⁻ R ⁺ :2K ⁺ R ⁺ (1) 4K ⁺ R ⁺ :0	K ⁺ R ⁺	(18) 2K ⁻ R ⁺ :2K ⁺ R ⁺ (1) 3K ⁻ R ⁺ :1K ⁺ R ⁺ (1) 4K ⁻ R ⁺ :0
4. <i>a kex 2+</i> (K ⁺ R ⁺ segregant from K34 × AN33)	K ⁺ R ⁺	K ⁺ R ⁺	(11) 4K ⁺ R ⁺ :0	K ⁺ R ⁺	(6) 4K ⁺ R ⁺ :0
5. <i>a mak 1-1</i> (K ⁻ R ⁻ segregant from K56 × 20)	K ⁻ R ⁻	K ⁺ R ⁺	(35) 2K ⁻ R ⁻ :2K ⁺ R ⁺	K ⁻ R ⁻	(12) 4K ⁻ R ⁻ :0
6. <i>a mak 1+</i> (K ⁺ R ⁺ segregant from K56 × 20)	K ⁺ R ⁺	K ⁺ R ⁺	(6) 4K ⁺ R ⁺ :0	K ⁺ R ⁺	(6) 4K ⁺ R ⁺ :0
7. <i>a mak 2-1</i> (K ⁻ R ⁻ segregant from K58 × 20)	K ⁻ R ⁻	K ⁺ R ⁺	(10) 2K ⁻ R ⁻ :2K ⁺ R ⁺	K ⁻ R ⁻	(12) 4K ⁻ R ⁻ :0
8. <i>a mak 2+</i> (K ⁺ R ⁺ segregant from K58 × 20)	K ⁺ R ⁺	K ⁺ R ⁺	(6) 4K ⁺ R ⁺ :0	K ⁺ R ⁺	(5) 4K ⁺ R ⁺ :0

* Numbers in parentheses indicate number of tetrads examined.

TABLE 4

Complementation tests among chromosomal killer mutants

<i>a</i> Parent	Phenotype	<i>kex 1</i> K ⁻ R ⁺	<i>kex 2</i> K ⁻ R ⁺	<i>α</i> Parent <i>mak 1</i> K ⁻ R ⁻	<i>mak 2</i> K ⁻ R ⁻	<i>rex 1</i> K ⁺ R ⁻
<i>kex 1</i>	K ⁻ R ⁺	K ⁻ R ⁺	K ⁺ R ⁺	K ⁺ R ⁺	K ⁺ R ⁺	K ⁺ R ⁺
<i>kex 2</i>	K ⁻ R ⁺	K ⁺ R ⁺	K ⁻ R ⁺	K ⁺ R ⁺	K ⁺ R ⁺	K ⁺ R ⁺
<i>mak 1</i>	K ⁻ R ⁻	K ⁺ R ⁺	K ⁺ R ⁺	K ⁻ R ⁻	K ⁻ R ⁻	K ⁺ R ⁺
<i>mak 2</i>	K ⁻ R ⁻	K ⁺ R ⁺	K ⁺ R ⁺	K ⁻ R ⁻	K ⁻ R ⁻	K ⁺ R ⁺
<i>rex 1</i>	K ⁺ R ⁻	K ⁺ R ⁺	K ⁺ R ⁺	K ⁺ R ⁺	K ⁺ R ⁺	K ⁺ R ⁻

Single colony isolates of the indicated parental strains having the phenotypes indicated were mated on YPAD plates and diploids selected by their complementary nutritional requirements. Single diploid colonies were reisolated and tested for killing and resistance as described in "MATERIALS AND METHODS."

TABLE 5

Preliminary linkage data of chromosomal killer mutants

Mutant	Designation		ade 1	a/a
K26	<i>kex 1</i>	PD	4	8
		NPD	7	5
		T	16	32
K34	<i>kex 2</i>	PD	15	11
		NPD	3	13
		T	26	45
K56	<i>mak 1</i>	PD	26	13
		NPD	20	14
		T	3	7
K58	<i>mak 2</i>	PD	7	4
		NPD	5	1
		T	18	12

"PD" = parental ditype; "NPD" = nonparental ditype; and "T" = tetratype.

TABLE 6

Allele tests of chromosomal killer mutants

	K ⁻ : K ⁺	<i>kex 1</i>	<i>kex 2</i>	<i>mak 1</i>	<i>mak 2</i>
<i>kex 1</i>	2 : 2	—	3	4	2
	3 : 1	—	5	13	5
	4 : 0	12	3	1	2
<i>kex 2</i>	2 : 2	..	—	5	12
	3 : 1	..	—	14	20
	4 : 0	..	6	4	3
<i>mak 1</i>	2 : 2	..	..	—	—
	3 : 1	..	..	—	—
	4 : 0	..	..	9	24
<i>mak 2</i>	2 : 2	..	..	..	—
	3 : 1	..	..	..	—
	4 : 0	..	..	..	17

Two chromosomal genes, *M* and *pets* (SOMERS and BEVAN 1969; FINK and STYLES 1972), have previously been shown to be involved in maintenance of the killer genome. Neither K56 nor K58 is *pets* since neither is linked to the mating type locus (Table 5) and both are grande, while *pets* is tightly linked to the mating type locus and has the petite phenotype. One of these might be identical to *M*, the maintenance gene described by SOMERS and BEVAN (1969).

Allele tests have been done to confirm the complementation tests (Table 6). These indicate that *kex1* and *kex2* are non-allelic and that each of these are non-allelic with *mak1* and *mak2*.

TABLE 7
Cytoplasmic killer mutants

6/18/73 mutant	Phenotype	× 20 (K ⁺ R ⁺)		× AN33 (K ⁻ R ⁻)	
		Diploid phenotype	Segregation	Diploid phenotype	Segregation
K23	K ⁻ R ⁻	K ⁺ R ⁺ → (537 of 540 zygote clones tested)	4K ⁺ R ⁺ :0 (5)	K ⁻ R ⁻ →	4K ⁻ R ⁻ :0 (6)
K30	K ⁻ R ⁺	K ⁺ R ⁺ → (497 of 498 zygote clones tested)	4K ⁺ R ⁺ :0 (6)	K ⁺ R ⁺ * →	4K ^w R ⁺ :0* (20)
K10	K ⁻ R ⁺	K ⁺ R ⁺ → (all of 252 zygote clones tested)	4K ⁺ R ⁺ :0 (4)	K ⁺ R ⁺ →	4K ^w R ⁺ :0 (6)
K15	K ⁻ R ⁻	K ⁺ R ⁺ → (all of 470)	4K ⁺ R ⁺ :0 (6)	K ⁻ R ⁻ →	4K ⁻ R ⁻ :0 (6)
K21	K ^w R ⁺	K ⁺ R ⁺ → (110 of 111)	4K ⁺ R ⁺ :0 (2)	K ^w R ⁺ →	4K ^w R ⁺ :0 (6)
K22	K ^w R ⁺	K ⁺ R ⁺ → (all of 450)	4K ⁺ R ⁺ :0 (6)	K ^w R ⁺ →	4K ^w R ⁺ :0 (6)
K28	K ⁻ R ⁻	K ⁺ R ⁺ → (687 of 690)	4K ⁺ R ⁺ :0 (6)	K ⁻ R ⁻ →	4K ⁻ R ⁻ :0 (6)
K29	K ⁻ R ⁺	K ⁺ R ⁺ → (245 of 245)	4K ⁺ R ⁺ :0 (6)	K ⁺ R ⁺ →	4K ⁺ R ⁺ :0 (6)

Over 100 single colonies of each haploid mutant were tested for killing and resistance. Each mutant was crossed with the K⁺R⁺ strain 20 and zygote clones isolated on minimal medium were tested for killing and resistance. This constitutes a test for suppressiveness. Each mutant was also mated with the K⁻R⁻ strain AN33. Otherwise the phenotype indicated is that of diploids isolated *en masse* from a mating mixture by complementary nutritional requirements. Diploids were sporulated and dissected and spore clones were scored *en masse* for killing and resistance.

* As noted in the text, not all diploids of K30 × AN33 were K⁺R⁺. Moreover, some K⁺R⁺ diploids of this type gave more complicated segregation patterns to be described in detail elsewhere.

Cytoplasmic mutants. Mutants in this group fell into three classes: (a) K⁻R⁺; (b) K^wR⁺, *i.e.*, those displaying reduced killing; and (c) K⁻R⁻. Those in class (b) were not a mixture of killer and nonkiller cells, since every clone derived from class (b) mutants showed the same low level of killing. Similarly, K⁻R⁺ and K⁻R⁻ strains do not show mitotic segregation of K⁺ colonies. (None were observed in > 2,000 colonies of each strain examined.)

In all three classes, diploids formed with a normal killer strain were killers, and segregated 4 K⁺R⁺ : 0 (Table 7). This indicates that their deficient killing was not due to a defect in a nuclear gene needed for the expression or maintenance of the cytoplasmic element, nor were any of these mutants "suppressive." Indeed, the mutant killer genome is thus recessive and, at most, rarely segregates out from the normal killer genome. In crosses of these "cytoplasmic" mutants with the K⁻R⁻ strain (AN33) used to test nuclear mutants (above), 4 : 0 meiotic segregation was usually observed, all of the spores having the same phenotype. This is again consistent with the notion that these mutants are defective in the cytoplasmic genetic element.

Unexpectedly, however, diploids of the type $K-R^+ \times K-R^-$ often were K^+R^+ or K^+R^- . The mitotic stability of this phenotype was investigated in a particular cross of this type: K30 ($K-R^+$) $\times$ 128-4D ($K-R^-$). Strain 128-4D is a segregant of K23 $\times$ AN33. Each parent showed no K^+ colonies in over 500 colonies tested. Diploids were isolated by their complementary nutritional requirements and, after 2 days of growth, plated for single diploid colonies. Of 801 colonies tested, 713 were killers and 88 were nonkillers. Five colonies of each type were picked and replated for single colonies, and over 2,000 were tested for killing. The killers gave rise only to killers, and nonkillers gave rise only to nonkillers. Similar experiments were done in which zygote clones were isolated on minimal medium after 5 hours of mating on rich medium. Thirty-two percent of 457 such zygote clones showed normal killing, 38% showed reduced but significant killing, while the remaining 30% of such clones showed no significant killing. Thus, by an as yet unknown mechanism, two nonkiller strains can give rise to a killer strain. Further studies are now underway to elucidate the mechanism of this phenomenon.

DISCUSSION

Mutants of killer strains of *S. cerevisiae* have been isolated by mutagenesis and replica plating. Those analyzed here include both chromosomal and nonchromosomal mutants. Among the chromosomal mutants are those in genes necessary for (1) maintenance of the nonchromosomal element (*mak1* and *mak2*); (2) for expression of killing (*kex1* and *kex2*); or (3) expression of resistance to killing (*rex1*). Either *mak1* or *mak2* may be identical to the maintenance gene *M* described by SOMERS and BEVAN (1969), but neither can be *pets* since both are grande and neither is linked to the mating type locus while *pets* is tightly linked to the mating type (BEVAN and SOMERS, cited in FINK and STYLES 1972).

The *kex* and *rex* genes have not been previously described. All previously described $K-R^+$ strains were, like mutants K29 and K30 described here, defective in the cytoplasmic genetic element. Mutants in the *kex* and *rex* genes will be useful both in studying the mechanism of expression of the killer genome and as a means to dissociate possession of the nonchromosomal genetic element from ability to kill (or to resist killing). It will be of interest, for example, to determine whether or not the double-stranded RNA shown by VODKIN and FINK (1973) and BERRY and BEVAN (1972) to be associated with the possession of the killer phenotype is present in *kex*⁻ or *rex*⁻ mutants.

Among the nonchromosomal mutants are (1) nonkillers which remain fully resistant to killing; (2) weak killers which are resistant to killing; and (3) nonkillers which are sensitive to killing.

On mating two different nonkilling, nonchromosomal mutants, some killer diploids often arise. This was not the case in the studies of SOMERS and BEVAN (1969) where crosses of neutral ($K-R^+$) and sensitive ($K-R^-$) strains invariably gave rise to neutral diploids. After several generations, these killer diploids appear to be stable and do not often throw off nonkiller segregants. The mechanism of this phenomenon is not yet clear.

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