MUTANTS OF THE KILLER PLASMID OF *SACCHAROMYCES CEREVISIAE* DEPENDENT ON CHROMOSOMAL DIPLOIDY FOR EXPRESSION AND MAINTENANCE

REED B. WICKNER

Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

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ABSTRACT

Mutants of the killer plasmid of *Saccharomyecs cerevisiae* have been isolated that depend upon chromosomal diploidy for the expression of plasmid functions and for replication or maintenance of the plasmid itself. These mutants are not defective in any chromosomal gene needed for expression or replication of the killer plasmid.——Haploids carrying these mutant plasmids (called *d* for diploid-dependent) are either unable to kill or unable to resist being killed or both and show frequent loss of the plasmid. The wild-type phenotype (K+R+) is restored by mating the *d* plasmid-carrying strain with either (a) a wild-type sensitive strain which apparently has no killer plasmid; (b) a strain which has been cured of the killer plasmid by growth at elevated temperature; (c) a strain which has been cured of the plasmid by growth in the presence of cycloheximide; (d) a strain which has lost the plasmid because it carries a mutation in a chromosomal *mak* gene; or (e) a strain of the opposite mating type which carries the same *d* plasmid and has the same defective phenotype, indicating that the restoration of the normal phenotype is not due to recombination between plasmid genomes or complementation of plasmid or chromosomal genes.——Sporulation of the phenotypically K+R+ diploids formed in matings between *d* and wild-type nonkiller strains yields tetrads, all four of whose haploid spores are defective for killing or resistance or maintenance of the plasmid or a combination of these. Every defective phenotype may be found among the segregants of a single diploid clone carrying a *d* plasmid. These defective segregants resume the normal killer phenotype in the diploids formed when a second round of mating is performed, and the segregants from a second round of meiosis and sporulation are again defective.

SOME strains of the yeast *S. cerevisiae* (killers) secrete a protein-containing substance which kills other strains (sensitives) (BEVAN and MAKOWER 1963; WOODS and BEVAN 1968; BUSSEY 1972; BUSSEY, SHERMAN and SOMERS 1973). The ability to secrete this killer substance and the ability to resist its effects show non-Mendelian inheritance (BEVAN and SOMERS 1969). The plasmid responsible for these phenomena appears to be a double-stranded RNA of molecular weight 1.4×10^6 encapsulated in a virus-like particle. A second encapsulated double-stranded RNA species of molecular weight 2.5×10^6 is present in all killer strains

but has not been shown to be involved in the killer phenomena (BERRY and BEVAN 1972; VODKIN and FINK 1973; BEVAN, HERRING and MITCHELL 1973; VODKIN, KATTERMAN and FINK 1974). Cells may be "cured" of the plasmid by growth in low concentrations of cycloheximide (FINK and STYLES 1972) or by growth at elevated temperatures (WICKNER 1974b).

Replication of the plasmid depends on at least five chromosomal genes (SOMERS and BEVAN 1969; FINK and STYLES 1972; WICKNER 1974a) and expression of its genetic information depends on at least three different chromosomal genes (WICKNER 1974a).

Several types of plasmid mutants have also been isolated. One type, neutral or "*n*", shows a simple defect of killing, with no defect in resistance; this K^-R^+ phenotype is transmitted to all diploids and all spores in a cross with a strain carrying no plasmid (BEVAN and SOMERS 1969). A second type of plasmid mutant, called "suppressive" or "s," is deficient in both killing and resistance but excludes the normal killer plasmid (SOMERS 1973); it might be called a "defective interfering plasmid."

This report describes a third type of plasmid mutant, called "diploid dependent" or "*d*." Strains carrying a *d* plasmid have a defective phenotype if they are haploid and a normal phenotype if they are diploid.

MATERIALS AND METHODS

S. cerevisiae strain A364A (*a ade1 ade2 lys2 his7 gal1 ura1 tyr1*) HARTWELL, CULOTTI and REID 1970) is the parent of the mutants described here. Genetic techniques (see MORTIMER and HAWTHORNE 1973 for references), tests for killing and resistance (SOMERS and BEVAN 1969; FINK and STYLES 1972), and media were as previously described (WICKNER 1974a). Routinely, zygote clones were obtained by mixing the haploid parent strains in water and filtering onto a Millipore filter in a Swinnex apparatus. The filter was placed on YPAD agar (WICKNER 1974a) at 25° and after 3–4 hours the cells were diluted and plated on media selective for diploids.

A strain's *phenotype* is designed K^+, K^w, or K^- for normal killing ability, weak killing ability, or no killing ability, respectively; its resistance to killing may be, similarly, R^+, R^w, or R^-.

The killer *genotype* is denoted here as follows: (i) chromosomal mutations are indicated in the usual way, e.g., *a mak2-1* denotes a strain of *a* mating type carrying a recessive mutation in the *mak2* gene. Note that *mak* genes are needed for *ma*intenance or replication of the *k*iller plasmid, while the *kex* and *rex* chromosomal genes are needed for *ex*pression of *k*illing and *r*esistance, respectively (WICKNER 1974a); and (ii) the killer plasmid residing in a strain is indicated in square brackets, [ ]. The letters KIL indicate that one is referring to the killer genome. This is followed by a small letter corresponding to the nomenclature devised by BEVAN and SOMERS (1969) and SOMERS (1973). Finally, a number denotes the mutant or isolate number. Examples: [KIL-0], strain carries no killer plasmid; [KIL-k], normal killer plasmid; [KIL-n26], neutral plasmid (BEVAN and SOMERS 1969) which gives an otherwise normal strain, the K^-R^+ phenotype; [KIL-s3], suppressive, or defective interfering plasmid (SOMERS 1973); [KIL-h] and [KIL-c], heat-cured (WICKNER 1974b) and cycloheximide-cured (FINK and STYLES 1972), respectively; and [KIL-d30], a diploid-dependent plasmid, discussed here. This nomenclature should be replaced by the usual gene designations as soon as a method is obtained for doing complementation tests and mapping with mutants of the plasmid.

Strains used in these experiments are described in Table 1.

TABLE 1

Yeast strains

Strain designation	Killer phenotype	Markers	Source or reference
A364A	K+R+	*a ade1 ade2 tyr1 lys2 ura1 his7 gal1* [KIL-k]	HARTWELL, CULOTTI and REID (1970)
20 (= D587-4B)	K+R+	*α his1* [KIL-k]	F. SHERMAN
S37	K+R+	*α leu2 met5* [KIL-k]	J. BASSEL
AN33	K-R-	*α thr1 arg1* [KIL-0]	S. HENRY
8	K-R-	*a his4-290 trp1* [KIL-0]	G. FINK
7	K-R-	*a his7-1229 trp5* [KIL-0]	G. FINK
200	K-R-	*a lys10* [KIL-0]	J. BASSEL
201	K-R-	*α lys10* [KIL-0]	J. BASSEL
18	K+R+	*a lys1* [KIL-k]	F. SHERMAN
18c	K-R-	*a lys1* [KIL-c]	This work
18h	K-R-	*a lys1* [KIL-h]	This work
K30	K-R+	*a ade1 ade2 tyr1 lys2 ura1 his7 gal1* [KIL-d30]	R. WICKNER (1974a)
K30c	K-R-	*a ade1 ade2 tyr1 lys2 ura1 his7 gal1* [KIL-c]	This work
273-1C	K-R+	*α ade1 ade2 thr1* [KIL-d30]	Segregant of AN33 × K30
269-2A	K-R+	*α ade1 ade2 thr1 tyr1 lys2 his7* [KIL-d30]	Segregant of AN33 × K30
187-1C	K-R-	*α ade1 ade2 lys1 mak1* [KIL-0]	R. WICKNER (1974a)
226-5A	K-R-	*α his7 trp1 leu2 mak2* [KIL-0]	R. WICKNER (1974a)
297	K-R-	*a his1 mak3* [KIL-0]	R. WICKNER, unpublished
A5969A-c	K-R-	*α his4-331 leu2-3 trp1 thr4 MAL2* [KIL-c]	B. SHAFFER *et al.* (1971) this work

RESULTS

In a series of 40 mutants isolated from the killer strain A364A, ten were defective as haploids and showed no defect in chromosomal genes, but gave rise to phenotypically normal killer diploids when mated with strain AN33 [KIL-0], a wild-type strain lacking the killer plasmid. While many strains of this type have been isolated, one strain, called K30 (Wickner 1974a), has been analyzed in detail.

As K30 has the K^-R^+ phenotype, some plasmid-borne information is retained in this mutant. In fact, growth at 37° or in the presence of cycloheximide, procedures which result in loss of the killer plasmid, convert K30 to the K^-R^- phenotype with high efficiency.

Further evidence that the killed plasmid is still present comes from examination of the double-stranded RNA species present in these mutants. As will be described elsewhere, mutant K30 contains normal amounts of both the large 2.5×10^6 molecular weight and small 1.5×10^6 molecular weight species of double-stranded RNA whose presence in killer strains has been described by BEVAN, HERRING and MITCHELL (1973) and VODKIN, KATTERMAN and FINK (1974). In contrast, a mitotic derivative of K30 which has become R^- specifically

lacks the 1.5×10^6 molecular weight double-stranded RNA species whose presence has been correlated with the presence of the killer genome (BEVAN, HERRING and MITCHELL (1973) and VODKIN, KATTERMAN and FINK (1974).

Occasionally, spontaneous K+R+ haploid revertants have been found which behave in all crosses like normal killers. This revertibility suggests that K30 may have only one point mutation, although it is still possible that the revertants are not true revertants.

The mutation in K30 is nonchromosomal: Chromosomal and nonchromosomal mutants of killer yeast are easily distinguished by introducing a normal killer plasmid by mating the mutant with a normal killer strain. Chromosomal mutations resulting in defective maintenance (SOMERS and BEVAN 1969; FINK and STYLES 1972; WICKNER 1974a) or expression (WICKNER 1974a) of the plasmid genome segregate 2 : 2 in such a cross. None of the K30 × killer segregants were defective. All segregants showed the normal K+R+ phenotype (Table 2). Moreover, all the diploids were normal killers (Table 2). Thus, the mutation in K30 is "recessive", nonchromosomal, and is not of the defective interfering plasmid ("suppressive") type described by SOMERS (1973). In accordance with the nomenclature proposed above in MATERIALS AND METHODS, the K30 plasmid will be referred to as [KIL-d30] or as the *d* plasmid.

Mating a strain carrying [KIL-d] with any haploid yields phenotypically normal diploids: As previously reported, diploids formed on mating K30 [KIL-d30] (K-R+) with AN33 [KIL-0] are often K+R+ (WICKNER 1974a; Fig. 1 and Table 3). Since this could be due to recombination of the K30 plasmid and an inapparent plasmid present in AN33, K30 [KIL-d30] was mated with heat-cured (WICKNER 1974b) or cycloheximide-cured (FINK and STYLES 1972) strains or with strains carrying the chromosomal *mak1*, *mak2*, or *mak3* muta-

TABLE 2

*Mating of strains carrying [KIL-d30] with normal killers**

Parents [KIL-d]†		× Wild-type killer		Diploid progeny		
Strain designation	Phenotype	Strain designation	Phenotype	Phenotype	Segregation‡	No. of tetrads
K30 [KIL-d30]	K-R+	20	K+R+	K+R+ (497 of 498 zygote clones tested)	4 K+R+ : 0	(6)
273-1C [KIL-d30]	K-R+	18	K+R+	K+R+ (all of > 500)	4 K+R+ : 0	(18)
269-2A [KIL-d30]	K-R+	18	K+R+	K+R+ (all of > 500)	4 K+R+ : 0	(20)

* Each strain carrying [KIL-d] was mated on rich medium with a wild-type killer and zygote clones were isolated by dilution and plating on selective medium after 3–4 hours. Zygote clones were tested for killing and resistance and a single clone was sporulated. Tetrads were dissected and the phenotypes of spore clones determined. All markers other than killing and resistance in each cross showed the expected 2 : 2 segregation.

† The *d* plasmid in each of these strains was derived from strain K30.

‡ Spore survival was > 90% in each cross. Only complete tetrads were scored.

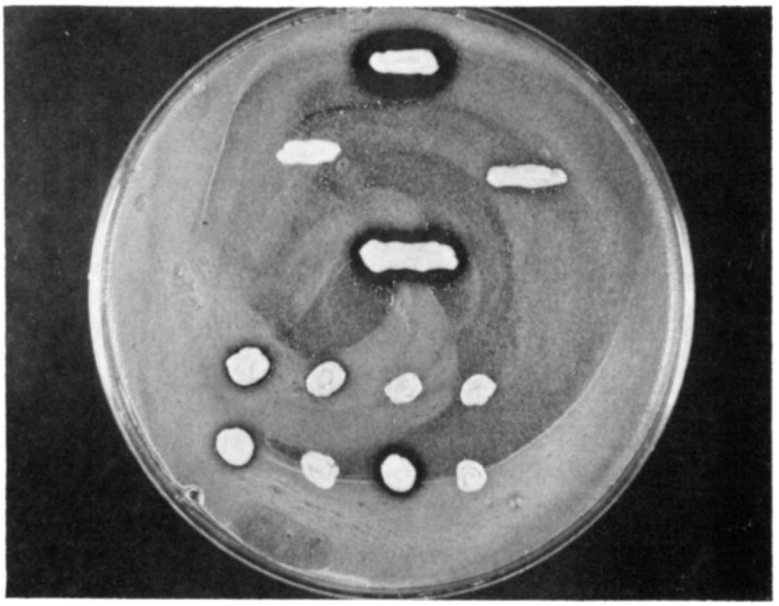

FIGURE 1.—Toxin production by a wild-type killer (top center); a wild-type nonkiller, [KIL-0] (second row, left); a strain carrying the *d* plasmid [KIL-d30] (second row, right); a diploid formed by [KIL-0] × [KIL-d30] (third row, center); and two tetrads derived from this diploid (third and fourth rows). These spore clones would be scored as follows: third row, K^w, K^w, K−, K−; fourth row, K^w, K−, K+, K−.

TABLE 3

Frequency of K+R+ diploids in [KIL-d] matings

		Strain designation:	AN33	20	α Parent 269-2A	273-1C	187-1C	226-5A	A4969Ac	247-5D	200-4A
a Parent		Killer genotype:	[KIL-0]	[KIL-k]	[KIL-d30]	[KIL-d30]	*mak1* [KIL-0]	*mak2* [KIL-0]	[KIL-c]	*kex2-1*	*kex1-1*
Strain designation	Killer genotype	% K+R+ haploids:*	0	100	0	0	0	0	0	0	0
					% *of diploids formed having K+R+ phenotype**						
8	[KIL-0]	0	0	100	17	100	0	0	0	100	100
7	[KIL-0]	0	0	100	—	100	0	0	0	100	100
18	[KIL-k]	100	100	100	100	100	100	100	100	100	100
K30	[KIL-d30]	0	65	100	84	100	90	60	80	100	100
A364A	[KIL-k]	100	100	100	100	100	100	100	100	100	100
A364Ac	[KIL-c]	0	0	100	—	—	—	—	—	—	—
K30c	[KIL-c]	0	0	100	—	—	—	—	—	—	—
18h	[KIL-h]	0	0	100	74	100	—	—	—	—	—
18c	[KIL-c]	0	0	100	67	100	—	—	—	—	—
297	*mak3* [KIL-0]	0	0	100	50	100	—	—	—	—	—

* Fifty or more single colonies of each haploid parent or zygote clones of each diploid were tested for killing ability and resistance. Results are shown as percent K+R+ clones. The balance was invariably K−R−, except for *kex*− haploids, which were K−R+.

tions, which are known to be unable to maintain the plasmid (WICKNER 1974a, and unpublished results). In each case, a large proportion of the diploids formed were K^+R^+, although each of the cured or *mak*$^-$ strains behaved as if it lacked the killer plasmid. A number of wild-type nonkillers were crossed with K30 [KIL-d30] in an attempt to find one which would not yield killer diploids. None has been found. The K^+R^+ diploids formed by mating K30 [KIL-d30] and AN33 [KIL-0] are not due to the mating of K^+ revertants of K30 with AN33 because, while about 20% of the total K30 cells form zygotes in a routine mating experiment such as that shown in Figure 1, most yielding K^+R^+ diploids, only a very rare mitotic segregant of K30 (less than 1 in 10^4) has become K^+R^+. K30 [KIL-d30] was also mated with *kex1* [KIL-k] and *kex2* [KIL-k] strains since the *kex* genes might actually be genes for maintenance of a hypothetical second plasmid required for the killer phenotype to be expressed. This second plasmid might be present in all [KIL-0] strains and mutant in [KIL-d30]. However, K30 [KIL-d30] × *kex1* (or *kex2*) [KIL-k] diploids are all K^+R^+. The findings suggest that K30 carries a plasmid with all the genetic information necessary to allow killing and resistance, but for some reason, this information is not expressed in K30.

The K30 plasmid [KIL-d30] is mitotically unstable: Those diploids in the K30 [KIL-d30] by AN33 [KIL-0] cross which were not K^+R^+ were K^-R^- (Table 3). During the course of this study, hundreds of K^- diploids from this type of cross have been tested for resistance and none have been found to be R^+. We will return to this point below.

The following experiments indicate that the K^-R^- diploids come from K30 haploids which have lost their plasmid. A mating mixture of K30 [KIL-d30] × AN33 [KIL-0] was plated for diploid clones at various times, and the diploid clones were tested for killing and resistance (Figure 2). The proportion of diploid clones which were K^+R^+ was independent of time after mating began. This indicates that, since the earliest diploid clones observed are zygote clones, some zygote clones were K^+R^+ while the remainder were K^-R^-. Indeed, replating the K^+R^+ zygote clones produced only K^+R^+ secondary clones, and replating K^-R^- zygote clones produced only K^-R^- secondary clones (Table 4), as is true for wild-type killer and sensitive strains. In order to further determine the source of the K^-R^- diploids, single colonies of K30 [KIL-d30] and AN33 [KIL-0] were mated in pairs and the proportion of K^+R^+ and K^-R^- zygote clones determined in each cross (Table 5). The proportion of K^+R^+ diploids varied only with the K30 single colony and not with the AN33 [KIL-0] single colony. Those K30 single colonies (K30–3 and K30–10 in Table 5) which give rise to only K^-R^- diploids had lost resistance and had, in fact, lost the 1.5×10^6 molecular weight double-stranded RNA (unpublished results). Many K30 single colonies gave intermediate percentages of K^+R^+ diploids suggesting frequent loss of the plasmid during growth. This instability in the haploid state contrasts with the high mitotic stability of the normal killer plasmid (BEVAN and SOMERS 1969; FINK and STYLES 1972) and of the K^+R^+ diploids formed in the K30 × AN33 crosses described above.

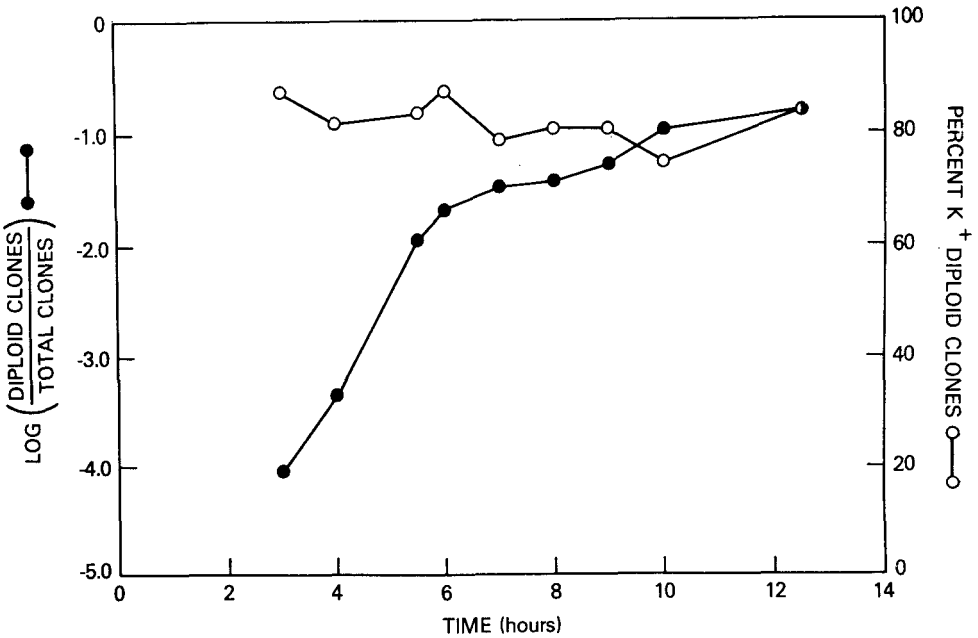

FIGURE 2.—Strains K30 (A364A[KIL-d30]) and AN33 [KIL-0] were mated as described in MATERIALS AND METHODS. At the times indicated the mating mixture was plated on YPAD (total clones) and SD (diploid clones). Diploid clones (50–200 for each point) were then tested for killing ability.

TABLE 4

Stability of zygote clones of K30 [KIL-d30] × AN33 [KIL-0]

Zygote clone		Replated clones		Zygote clone		Replated clones
K30 [KIL-d30]						
× AN33 [KIL-0]	: K-1	0 K+ :	1070 K-	K30 × AN33	: K+7	1570 K+ : 0 K-
	: K-2	0 K+ :	910 K-		: K+8	> 1000 K+ : 0 K-
	: K-3	0 K+ :	> 1000 K-		: K+9	900 K+ : 0 K-
	: K-4	0 K+ :	524 K-		: K+10	1340 K+ : 0 K-
					: K+11	1090 K+ : 0 K-
K30 × AN33	: K+1	203 K+ :	0 K-		: K+12	1870 K+ : 0 K-
	: K+2	1170 K+ :	1 K-		: K+13	890 K+ : 3 K-
	: K+3	810 K+ :	0 K-		: K+14	304 K+ : 0 K-
	: K+4	1205 K+ :	1 K-		: K+15	835 K+ : 0 K-
	: K+5	1080 K+ :	5 K-		: K+16	915 K+ : 0 K-
	: K+6	> 1000 K+ :	2 K-		: K+17	578 K+ : 1 K-

Single zygote clones (denoted K+1, K+2, . . . , or K-1, K-2, . . .) from the 3- and 4-hour time points in Figure 2 were replated for single colonies and these were tested for killing ability.

TABLE 5

Mating of K30 clones with AN33 clones

Parents		Zygotes	Parents		Zygotes
K30 clone	AN33 clone		K30 clone	AN33 clone	
1 K-R+	1 K-R-	345 K+ : 1 K-	1 K-R+	1 K-R-	345 K+ : 1 K-
2 K-R+	1 K-R-	770 K+ : 1 K-	1 K-R+	2 K-R-	490 K+ : 2 K-
3 K-R-	1 K-R-	0 K+ : 212 K-	1 K-R+	3 K-R-	355 K+ : 0 K-
4 K-R+	1 K-R-	179 K+ : 0 K-	1 K-R+	4 K-R-	>200 K+ : 1 K-
5 K-R+	1 K-R-	131 K+ : 3 K-	1 K-R+	5 K-R-	475 K+ : 1 K-
6 K-R+	1 K-R-	56 K+ : 89 K-	1 K-R+	6 K-R-	540 K+ : 0 K-
7 K-R+	1 K-R-	43 K+ : 24 K-	1 K-R+	7 K-R-	410 K+ : 2 K-
8 K-R+	1 K-R-	222 K+ : 2 K-	1 K-R+	8 K-R-	615 K+ : 3 K-
9 K-R+	1 K-R-	181 K+ : 52 K-	1 K-R+	9 K-R-	195 K+ : 0 K-
10 K-R-	1 K-R-	0 K+ : 268 K-	1 K-R+	10 K-R-	265 K+ : 0 K-
11 K-R+	1 K-R-	251 K+ : 4 K-	1 K-R+	11 K-R-	250 K+ : 0 K-
12 K-R+	1 K-R-	208 K+ : 34 K-	1 K-R+	12 K-R-	645 K+ : 0 K-

Strains K30 and AN33 were plated for single clones. Twelve of each (numbered 1–12 above) were picked, their phenotypes tested, and each was mated with clones of the other as indicated. Zygote clones were isolated and tested for killing ability.

Sporulation of a [KIL-d30] K+R+ diploid yields defective haploid segregants: Sporulation of K-R- diploids from K30 [KIL-d30] × AN33 [KIL-0] yields consistently 4 K-R- : 0 segregation. When the K+R+ diploids formed by mating K30 [KIL-d30] with AN33 [KIL-0] are sporulated, almost all the haploid segregants showed defective killing, resistance, or stability, or some combination of these (Table 6, cross I, and Figure 1). Even the few K+R+ segregants observed were not normal since when these were again crossed with a wild-type nonkiller such as 200[KIL-0] or 201[KIL-0], they again segregated out defective haploids (Table 6, crosses II and IV). Some of the segregants of cross I (Table 6) had the same K-R+ phenotype as the parent, K30, but many were K-R-, K+R-, or showed weak killing and/or resistance. All of the nutritional markers in these crosses showed normal $2^+ : 2^-$ segregation, and no correlation has been found between killing or resistance and any other marker segregating in these crosses. A number of these defective haploid segregants were again crossed with wild-type nonkiller strains and they each showed the same behavior as the original mutant. In each case, the diploids were K+R+ or K-R- (Table 7), and segregants showed a mixture of defective phenotypes (Table 6, crosses II–IV). Thus, among the meiotic haploid segregants of a single diploid K+R+ clone of K30 × AN33 are found all possible phenotypes. The factors affecting this phenotype are obscure at this time. The segregation pattern described above can best be described as 4 defective : 0 and provides further evidence that the mutation described here is cytoplasmic rather than chromosomal. The alteration between defective haploid to nondefective killer diploid to defective haploid could be continued for at least three cycles of mating and sporulation. Mutants of A364A with K+R- or K-R- phenotypes and showing "diploid-dependent" behavior, similar to that described here for K30, have also been isolated.

TABLE 6

Sample meiotic products involving the [KIL-d30] plasmid

Cross I: K30[KIL-d30] × AN33[KIL-0] : K+R+ single colony sporulated							
Tetrad no.		1	2	3	4	5	6
Spore	A	K- R+	K- R-	K- R-	K- R^w	K- R+	K- R-
phenotypes	B	K- R^w	K+R+	K^w R-	K- R+	K^w R^w	K^w R+
	C	K^w R+	K^w R-	K- R-	K- R-	K- R^w	K^w R+
	D	K^w R+	K- R+	K^w R^w	K+R-	K- R-	K- R-
Tetrad no.		7	8	9	10	11	
Spore	A	K- R+	K+R+	K- R+	K^w R+	K+R+	
phenotypes	B	K+R^w	K+R^w	K^w R-	K+R-	K+R-	
	C	K^w R-	K^w R+	K+R+	K+R+	K- R+	
	D	K+R^w	K^w R-	K+R+	K+R+	K+R^w	
Cross II: I-9C[KIL-d30] × 201[KIL-0] : K+R+ single colony sporulated							
Tetrad no.		1	2	3	4	5	6
Spore	A	K+R+	K- R+	K- R+	K^w R+	K- R-	K^w R-
phenotypes	B	K^w R+	K^w R+	K^w R+	K- R+	K^w R^w	K- R+
	C	K- R+	K^w R-	K^w R+	K^w R-	K^w R^w	K^w R+
	D	K^w R+	K^w R+	K^w R^w	K^w R+	K^w R+	K^w R+
Cross III: I-8C[KIL-d30] × [KIL-0] : K+R+ single colony							
Tetrad no.		1	2	3	4	5	6
Spore	A	K^w R^w	K^w R+	K^w R^w	K- R-	K+R+	K+R+
phenotypes	B	K- R^w	K^w R+	K^w R+	K- R+	K- R+	K^w R+
	C	K^w R+	K- R^w	K^w R^w	K+R+	K- R+	K^w R^w
	D	K+R+	K- R-	K^w R^w	K+R+	K^w R^w	K- R-
Cross IV: I-10C[KIL-d30] × [KIL-0] : K+R+ single colony							
Tetrad no.		1	2	3	4	5	6
Spore	A	K^w R+	K^w R^w	K^w R+	K- R+	K^w R+	K- R -
phenotypes	B	K^w R^w	K^w R+	K^w R+	K^w R^w	K^w R+	K^w R+
	C	K- R+	K^w R+	K^w R+	K^w R^w	K^w R+	K- R-
	D	K^w R+	K^w R+	K^w R+	K^w R-	K^w R+	K^w R^w

TABLE 7

All meiotic segregants carrying the [KIL-d30] plasmid yield K+R+ diploids with a [KIL-0] or α [KIL-0]

Segregant carrying [KIL-d30]	Diploid phenotypes	Segregant carrying [KIL-d30]	Diploid phenotypes
I-7A	100 K+ : 2 K-	I-10A	100 K+ : 0 K-
B	100 K+ : 0 K-	B	100 K+ : 0 K-
C	90 K+ : 12 K-	C	100 K+ : 0 K-
D	100 K+ : 3 K-	D	100 K+ : 0 K-
I-8A	100 K+ : 2 K-	I-11A	100 K+ : 4 K-
B	90 K+ : 10 K-	B	100 K+ : 4 K-
C	100 K+ : 24 K-	C	100 K+ : 0 K-
D	100 K+ : 2 K-	D	50 K+ : 1 K-
I-9A	100 K+ : 0 K-		
B	100 K+ : 0 K-		
C	100 K+ : 0 K-		
D	100 K+ : 3 K-		

Each spore clone from tetrads 7–11 in cross I of Table 6 was mated with strain 200 (*a* [KIL-0]) or strain 201 (α [KIL-0]) and, after 4 hours, plated on minimal medium for zygote clones. These were then tested for killing (usually 100 killers were counted for each cross).

"Selfing" [KIL-d30] × [KIL-d30] yields K⁺R⁺ diploids: Each of 2 α K⁻R⁺ segregants carrying the K30 plasmid was mated with the original mutant, K30, and with wild-type killers or nonkillers (Table 3). The K30 [KIL-d30] (K⁻R⁺) × 273–1c [KIL-d30] (K⁻R⁺) diploids, for example, were again either K⁺R⁺ or K⁻R⁻. This "selfing" experiment shows again that one is not dealing with either a chromosomal mutant or a simple plasmid recombination or complementation. In this cross, both parents have killer plasmids derived from the same mutant, K30. More extensive "selfing" crosses have been performed with similar results. These include crossing each member of a tetrad with each other member. All of the diploids formed (including rare *a/a* and *α/α* diploids) included stable K⁺R⁺ clones.

DISCUSSION

The K⁻R⁺ mutant of A364A, designated K30 [KIL-d30], has been studied in some detail. The mutation is clearly recessive and nonchromosomal since introduction of a normal plasmid yields all killer diploids which yield only killer segregants on meiosis. The surprising finding was that these diploid-dependent strains become phenotypically normal killers on mating with *any* strain, even one lacking the plasmid as a result of heat or cycloheximide curing or because it had a mutant chromosomal *mak* gene, or one carrying the *same mutant plasmid*. That these phenotypically normal killer diploids were not genotypically normal is seen immediately when one undergoes meiosis and sporulation. Instead of yielding all haploid killers, these K⁺R⁺ diploids kielded all defective segregants having a mixture of all possible defective phenotypes in no discernible pattern (Figure 3).

A number of hypotheses have been considered in an attempt to explain these results:

1) Heterozygosity at the mating-type locus is necessary to allow proper expression and replication of the mutant K30 plasmid. This notion was ruled out when it was found that *a/a* and *α/α* diploids (identified as *a/a* or *α/α* by their parentage and by their inability to sporulate) carrying the K30 plasmid were again either K⁺R⁺ or K⁻R⁻ as for *a/α* diploids.

2) K30 carries some sort of defective interfering or "suppressive" plasmid (SOMERS 1973) which interferes only with the A364A plasmid. Diploid formation with a wild type might somehow relieve the interference, which was then re-imposed on sporulation. This hypothesis was ruled out by crossing an *α* [KIL-d30] strain with A364A. The diploids were all K⁺R⁺ and segregation on meiosis was uniformly 4 K⁺R⁺ : 0. Thus, the K30 plasmid does not interfere with the normal A364A killer plasmid (its parent) in either haploids or diploids.

3) The conditions of mating (such as amino acid or purine starvation employed in diploid selection) or the mating hormones themselves convert the K⁻R⁺ K30 to the K⁺R⁺ or K⁻R⁻ phenotype independent of the actual process of mating. Similarly, normal killers might be converted to defectives by incubation on sporulation medium.

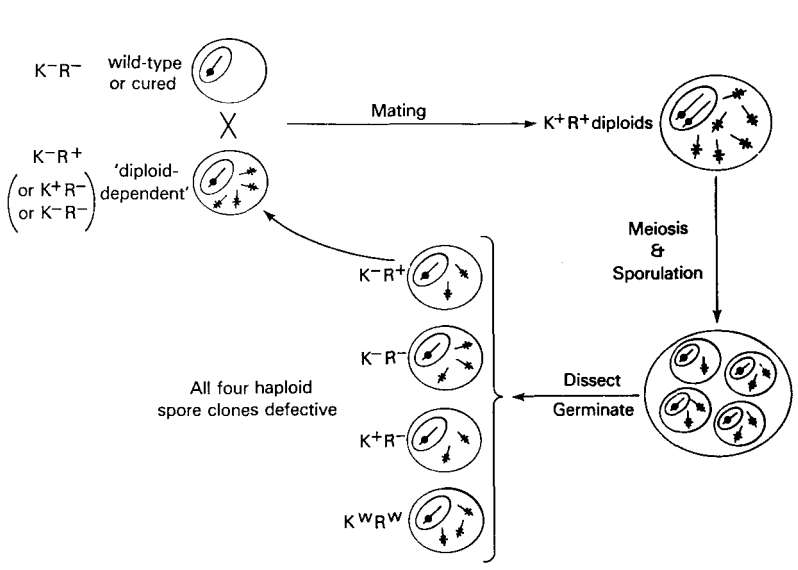

FIGURE 3.—Summary of the pattern of inheritance of killing and resistance for cytoplasmic "diploid-dependent" mutants.

To investigate these possibilities, *a* or *α* cells carrying [KIL-k] or [KIL-d30] were incubated on complete agar either (a) without adenine; (b) without histidine; (c) without dextrose; (d) without ammonium sulfate; (e) with acetate or glycerol replacing dextrose; (f) without ammonium sulfate and with acetate or glycerol replacing dextrose; (g) on sporulation medium; or (h) on pH 4.7 MB medium. None of these treatments changed the phenotypes of either the killers or the [KIL-d30] nonkillers.

When mixtures of *a* [KIL-0] and *a* [KIL-d30] (or mixtures of *α* [KIL-0] and *α* [KIL-d30]) cells were incubated on YPAD (mating conditions), no killers could be detected among the parents although, as mentioned above, the rare *a/a* or *α/α* diploids formed were K+R+. Similarly, when *a* [KIL-0] and *α* [KIL-d30] were cultivated on opposite sides of a Millipore filter, none of the parents became K+R+. Thus, neither the conditions of mating or sporulation, nor the mating hormones, nor other soluble diffusible factors, are responsible for converting [KIL-d30] strains from a defective phenotype to the K+R+ phenotype.

4) K30 is a complex multiple mutant with a plasmid altered so that it now needs the functions of many different chromosomal genes which have now become mutant or which were defective in the original A364A parent. This hypothesis would explain the defective phenotype of meiotic segregants of the cross K30 [KIL-d30] × AN33 [KIL-0] by their carrying one or more of the many chromosomal defects in K30 along with the defective *d* plasmid. When such segregants were then mated with the original mutant, K30, all diploids formed should have been defective. In fact, all the diploids formed were normal killers. While this is not a true selfing experiment, it does rule out this hypothesis.

The frequency with which *d* mutants are found suggests further that multiple changes in A364A are not needed to produce them. In a series of 40 killer mutants of A364A (WICKNER 1974a), 6 were single chromosomal mutants, 5 appeared to have lost the killer plasmid completely, 4 were of the neutral (K^-R^+) type described by BEVAN and SOMERS (1969), none were of the defective interfering plasmid type ("suppressive") described by SOMERS (1973), and at least 10 were of the diploid-dependent type. The remaining 15 were incompletely characterized. Thus *d* mutants represent a very frequent class of mutants.

5) Another hypothesis is that the cell fusion process activates, and the process of meiosis deactivates, the *d* plasmid, independent of their effects on ploidy *per se*.

6) A final hypothesis is that the diploid state is in some way able to compensate the defect in strain K30. This is certainly sufficient to explain all the data presented here, but it would be more satisfying if one knew what part or parts of the genome were needed in duplicate. The data do not distinguish, as yet, between (5) and (6).

While *d* mutants are frequent, and defective interfering plasmids ("suppressive") (SOMERS 1973) are as yet unknown among mutants derived from strain A364A, others have observed (SOMERS 1973; VODKIN KATTERMAN and FINK 1974), and we have confirmed, that other parent strains give rise to suppressive mutants with great frequency. The explanation for this finding is not yet clear.

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Corresponding editor: F. SHERMAN