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mak Mutants of Yeast: Mapping and Characterization

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Killer strains of *Saccharomyces cerevisiae* are those carrying a 1.5×10^6-dalton double-stranded (ds) ribonucleic acid (RNA) (*M*) in virus-like particles and secreting a protein toxin. Most yeast (killer or not) also carry a 3×10^6-dalton dsRNA (*L*). We have mapped mutations in eight of the chromosomal genes needed for maintaining *M* (*mak* genes). The *mak* genes are widely distributed on the yeast map, with no multigene complexes. We show that mutants defective in these and other *mak* genes lose *M* dsRNA, but not *L* dsRNA. The *mak3-1* mutation results in markedly decreased cellular levels of *L* dsRNA, but *mak3-1* strains do not lose *L* dsRNA completely. Mutation of *mak16* results in temperature-sensitive growth, whereas mutations in *mak13*, *mak15*, *mak17*, *mak20*, *mak22*, and *mak27* result in slow growth at any temperature. No effect of *mak* mutations on mating, meiosis, sporulation, germination, homothallism, or ultraviolet sensitivity has been found. The specificity of *mak* mutations is discussed.

Killer strains of *Saccharomyces cerevisiae* carry two linear double-stranded (ds) RNA species called *L* (3×10^6 daltons) and *M* (1.5×10^6 daltons), both separately encapsulated in intracellular virus-like particles (1, 2, 4, 9, 11, 12, 16, 20, 22, 26). *L* codes for the major coat protein of the particles containing *L* (13), whereas *M* codes (20; K. A. Bostian, J. E. Hopper, D. T. Rogers, and D. J. Tipper, 9th Int. Conf. Yeast Genet. Mol. Biol. Abstr., 1978, p. 103) for the protein toxin secreted by such cells (5, 20, 35). *M* also makes cells carrying it immune to the effects of the toxin (2, 3, 9, 26). Wild-type sensitive strains generally have *L* dsRNA, but lack *M* (2, 26), although a few strains lacking both have been described (2, 17, 25). The virus-like particles are not known to be naturally infectious and are not found outside the cell. Transmission occurs only by cytoplasmic mixing during mating (reviewed in 31).

Mutations in any of 27 chromosomal genes (*mak* [25 genes], *spe2*, *pet18*) result in loss of the killer plasmid as defined genetically (2, 7, 9, 15, 21, 27, 29). Some of these genes have been mapped (30, 32), and some of the mutant strains have been shown to lose only *M* dsRNA and not *L* dsRNA (2, 26, 32). We report here mapping of an additional group of such mutations, dsRNA analysis of strains carrying these mutations, and the growth defects displayed by several. We have found that one, the *mak3-1* mutation, also results in a decrease of cellular *L* dsRNA.

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MATERIALS AND METHODS

Notation: Phenotypes. K^+ or K^- means ability or inability to secrete an active killer toxin. R^+ or R refers to resistance or sensitivity to the killer toxin. Chromosomal genes needed to *ma*intain the *k*iller plasmid are called *mak* genes. *mak* mutations are scored in meiotic crosses as K^- R^- segregants. Two additional chromosomal genes required for maintenance of the killer plasmid are *pet18*, needed also to maintain mitochondrial DNA (15), and *spe2*, the gene coding for adenosylmethionine decarboxylase, an enzyme in spermidine and spermine biosynthesis (6, 7). Mutation of one of the four chromosomal *ski* genes results in increased production of toxin activity (superkiller) (23), suppression of various *mak* mutations (24; Toh-e and Wickner, unpublished data) and, in the case of *ski2*, *ski3*, and *ski4*, increased cellular *M* dsRNA (23). The wild-type killer plasmid is denoted [KIL-k]. The absence of the killer plasmid is denoted [KIL-o].

Strains. Some of the yeast strains used are listed in Table 1.

Media. Media were as previously described (29, 33).

Isolation of dsRNA. dsRNA was isolated as previously described (33), or as described by Fried and Fink (10), and analyzed by electrophoresis in 1% agarose slabs as previously described (23).

RESULTS

Genetic mapping. We present the genetic localization of three new genes (*mak9*, *mak14*, *and mak27*) and five genes previously described (29) as complementation groups (*mak11*, *mak12*, *mak15*, *mak16*, and *mak21*). The initial locali-

TABLE 1. *Strains of S. cerevisiae*

Designation	Killer phenotype	Genotype	Source or reference
T-1269-38C	$K^- R^-$	**a** *HO HMα* **HMa** / **a** *HO HMα* **HMa** *lys2 met4 suc* / *lys2 met4 suc*	Takano
1024	$K^- R^-$	**a** *trp3 ade1 mak9-1* [KIL-o], isolate MK 34	This work
560	$K^- R^-$	**a** *leu2 mak11-1* [KIL-o]	29
1532-15B	$K^- R^-$	**a** *his6 ura1 mak12-1* [KIL-o]	29
1043	$K^- R^-$	**a** *ade2 ura1 mak14-1* [KIL-o], isolate MK 20	This work
943	$K^- R^-$	α *leu2 mak15-1* [KIL-o]	29
1091	$K^- R^-$	**a** *ade1 ura1 mak16-1* [KIL-o]	29
716	$K^- R^-$	α *ade2 mak21-1* [KIL-o]	29
1303	$K^- R^-$	**a** *lys1 mak27-1* [KIL-o], isolate K335	This work
1117	$K^+ R^+$	α *his1 ura1 ade2 lys1 rna1-1* [KIL-k]	30

zation of these genes was by centromere linkage, chance observation of linkage to a known marker, or by noting trisomic segregation in a cross with a strain known to be disomic for chromosome XI. For a review of the yeast map, see references 18 and 19.

Mutations in *mak9*, *mak11*, and *mak15* were located on chromosome XI (Table 2). The gene order was found to be *mak9-fas1-trp3-ura1* for the fragment, based on the data in Table 2 as well as on analysis of individual tetrads, assuming that double crossovers are infrequent in this interval (data not shown). This is consistent with previous results (8). No linkage of *mak9*, *trp3*, *fas1*, or *ura1* to other markers on XI was detected. Linkage of a *cly7* mutation to *fas1* was not detected (parental ditype = 4, nonparental ditype = 8, tetratype = 13). The *mak11-1* mutation was tightly linked to *cdc16-1*, but *cdc16-1* did not show a *mak* phenotype, nor was *mak11-1* temperature sensitive for growth or a slow grower. The *mak15-1* mutation was located between *met1* and *MAL4* (Table 2).

The data in Table 3 place *mak12* on the left arm of chromosome XII; it is the only known marker to the left of the centromere (18, 19). In addition to the data shown, no linkage of *mak12* to *gal12* could be detected (parental ditype = 5, nonparental ditype = 3, tetratype = 7).

The *mak14* gene is on chromosome III close to *thr4* (Table 4). Its order relative to *tsm5*, *SUP61*, and *rad18* is not certain.

mak16 is located on chromosome I linked to *cys1* and *ade1* (Table 5). The order *mak16-cys1-ade1* is based on both the linkage data in Table 5 and analysis of individual tetrads (data not shown).

Table 6 shows mapping data for *mak21* with various chromosome IV markers. The linkage of *mak21* to *trp1* and *aro1D*, two markers which are unlinked to each other (18), places *mak21*

TABLE 2. *Mapping of mak9, mak11, and mak15 on chromosome XI*[a]

		fas1	*trp3*	*ura1*	*cdc16*	*met14*	*met1*	*MAL4*
	PD	37	57	59	1	15	5	5
mak9-1	NPD	0	6	11	5	12	2	2
	T	5	101	135	12	36	11	15
	cM	6	42	49				
	PD		27	47	6	17	12	1
fas1	NPD		1	3	5	17	12	1
	T		51	72	19	50	36	4
	cM		36	37				
	PD			258	8	17	27	16
trp3	NPD			1	11	19	24	12
	T			42	41	60	98	41
	cM			8				
	PD			11	52	45		
mak11-1	NPD			7	0	1		
	T			27	0	24		
	cM				<1	21		
	PD					0	55	12
mak15-1	NPD					3	1	0
	T					7	22	7
	cM						18	18

[a] Segregation data are given as the number of parental ditype (PD), nonparental ditype (NPD), and tetratype (T) asci observed. In a cross of the type ab × AB, a PD ascus would be ab, ab, AB, AB. An NPD ascus would be aB, aB, Ab, Ab, and a T ascus would be ab, Ab, aB, AB. Genetic distances are calculated in percent recombination units (map units or centimorgans [cM]) by the formula: 1 map unit = [(T + 6NPD)100]/[2(PD + NPD + T)].

TABLE 3. *Mapping of mak12 on chromosome XII*[a]

		trp1	*asp5*
	PD	25	45
mak12-1	NPD	21	9
	T	56	88
	cM	27	47
	PD	57	
asp5	NPD	70	
	T	56	
	cM	15	

[a] Centromere linkage is detected by a decrease in second meiotic division segregation frequency. Second division segregation frequency is measured as tetratype (T) ascus frequency for the unmapped marker and a standard marker known to be linked to its centromere (such as *trp1* which is <1 centimorgan [cM] from the centromere of IV). This frequency is corrected for the known second division segregation frequency of the standard marker and converted to map units by dividing by 2. PD, Parental ditype; NPD, nonparental ditype.

TABLE 4. *mak14 located on chromosome III*[a]

		thr4	a/α
	PD	38	67
mak14-1	NPD	0	15
	T	8	74
	cM	9	36
	PD	39	
a/α	NPD	1	
	T	16	
	cM	20	

[a] Analysis of individual tetrads in which *thr4* and *mak14* were tetratype (T) suggested the gene order, **a/α-*thr4-mak14***. PD, Parental ditype; NPD, nonparental ditype; cM, centimorgans.

TABLE 5. *mak16 located on chromosome I*[a]

		cys1	*ade1*
	PD	42	46
mak16	NPD	1	1
	T	8	21
	cM	14	20
	PD	44	
ade1	NPD	0	
	T	7	
	cM	7	

[a] The location of *cys1* is due to S. Fogel (personal communication). PD, Parental ditype; NPD, nonparental ditype; T, tetratype; cM, centimorgans.

TABLE 6. *Mapping of mak21 on chromosome IV*[a]

		rna11	*cdc2*	*trp1*	*aro1D*	*pet14*	*ade8*
	PD	30	18	71	66	44	12
mak21	NPD	12	8	10	9	12	13
	T	110	60	218	121	118	35
	cM	(60)	(54)	46	45	55	
	PD	15		33		87	
aro1D	NPD	13		27		2	
	T	65		108		91	
	cM					29	
	PD	84	19			22	
trp1	NPD	0	6			28	
	T	45	32			98	
	cM	17					

[a] PD, Parental ditype; NPD, nonparental ditype; T, tetratype; cM, centimorgans.

between these genes. Attempts to confirm this location using *rna11* showed that *mak21* was further from *rna11* than either *trp1* or *aro1D*, suggesting that the order is *rna11*-(*trp1*-centromere)-*mak21-aro1D-pet19-ade8*. This order was confirmed by Toh-e (personal communication) in the course of his locating *pho2* (=*phoB*) on the left arm of chromosome IV. Finally, the original data of Mortimer and Hawthorne (18) seem to us most consistent with a location of *rna11* to the left of the centromere of IV. It is also possible that *rad55* is to the left of the centromere of IV.

The *mak27* gene is tightly linked to *rna1* (parental ditype = 165, nonparental ditype = 0, tetratype = 7, 2.1 centimorgans). The genetic map of yeast emphasizing killer-related genes is shown in Fig. 1.

dsRNA analysis. From mutants defective in each of *mak9*, *mak11* through *mak24*, *mak26*, and *mak27*, dsRNA was isolated by CF11 column chromatography. Electrophoresis of these samples (Fig. 2) showed that in each case, *L* dsRNA (about 3×10^6 daltons) was present, but *M* dsRNA (about 1.5×10^6 daltons) was absent.

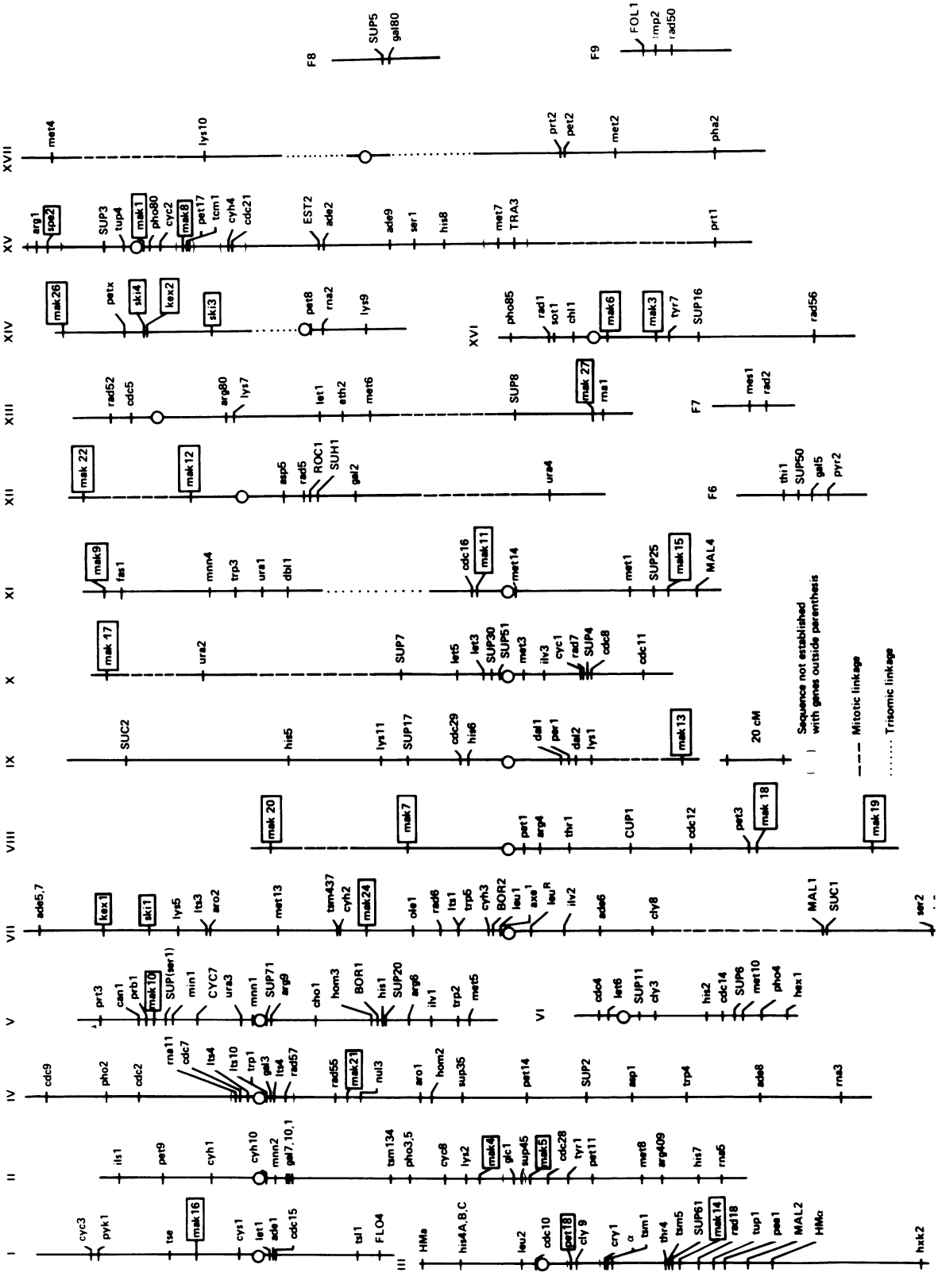
We previously showed that a single *mak3-1* strain lacked *M* dsRNA but carried *L* in apparently reduced amounts (32). We now have found that this reduction in *L* dsRNA cosegregates in 10 tetrads with the *mak3-1* mutation (Fig. 3). This is the first case of a *mak* gene affecting both *L* and *M* dsRNA maintenance.

The small amount of dsRNA in the *mak3-1* segregants was isolated by CF11 chromatography and compared with *L* dsRNA from a *mak3*$^+$ segregant. They were identical in migration on agarose gel electrophoresis.

Growth defects of *mak* mutants. We have previously reported that *mak1-3* and *pet18* mutants are temperature sensitive for growth (15, 32) and that *kex2* (*k*iller *ex*pression) mutants have defects in mating and meiosis (14). We have now found that *mak16-1* results in temperature-sensitive growth, with slow growth at 20 or 25°C and no growth at 30°C or above. Temperature sensitivity for growth and *mak* cosegregated in each of 132 tetrads. *mak16-1* reverts only very rarely. One true revertant showed coreversion of the *mak* and temperature-sensitive phenotypes, whereas another revertant was due to a dominant suppressor mutation unlinked to *mak16* and suppressing the temperature-sensitive phenotype, but not the *mak* phenotype.

Small colony size at 25°C was found to cosegregate with the *mak* phenotype for *mak13-1* (22 tetrads), *mak15-1* (88 tetrads), *mak17-1* (68 tetrads), *mak20-1* (12 tetrads), *mak22-1* (46 tet-

FIG. 1. *Genetic map of S. cerevisiae. Markers in boxes are those related to the killer character (see text). KRB1 (34) is not shown here but is tightly centromere linked and is not on chromosomes I through XVI. Most of the markers not related to the killer character are referenced in Mortimer and Hawthorne (18). Unpublished markers include cyc2, cyc3, and cyc8 (Rothstein and Sherman); cys1 (Fogel); pho2, pho4, and pho85 (Toh-e); arg409 and arg80 (Hilger and Mortimer); pep16 and prb1 (Mitchell and Jones); SUP17 (Ono, Stewart, and Sherman); sot1 (Haber and Remer); and tse (McCusker and Haber); arg1 (Hilger and Mortimer).*


I
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Sequence not established with genes outside parenthesis
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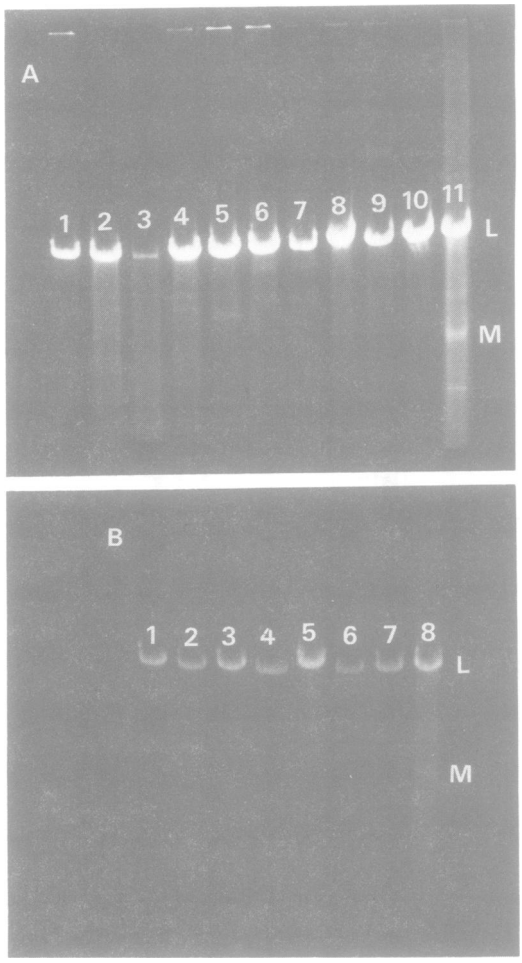

FIG. 2. *Agarose slab gel electrophoresis of dsRNA from mak strains. (A) 1, mak9-1 (strain 1025); 2, mak11-1 (strain 559); 3, mak12-1 (strain 707); 4, mak13-1 (strain 1433-13B); 5, mak14-1 (strain 1043); 6, mak15-1 (strain 943); 7, mak16-1 (strain 710); 8, mak17-1 (strain 701); 9, mak18-1 (strain 721); 10, mak19-1 (strain 719); 11, wild-type killer (strain A364A). (B) 1, mak20-1 (strain 722); 2, mak21-1 (strain 716); 3, mak22-1 (strain 552); 4, mak23-1 (strain 557); 5, mak24-1 (strain 531); 6, mak26-1 (strain 1533-9B); 7, mak27-1 (strain 704); 8, wild-type killer (strain A364A).*

rads), and *mak27-1* (131 tetrads). Colony size was clearly not altered by *mak11-1*, *mak12-1*, *mak18-1*, *mak18-2*, *mak19-1*, *mak23-1*, *mak24-1*, or *mak25-1*.

Since *mak27-1* showed slow growth at 25, 30, and 37°C and mapped very close to *rna1-1* which is temperature sensitive for growth at 37°C, it was of interest to determine whether these might be mutations in the same gene. The complementation of the slow-growth phenotype of *mak27-1* by *rna1-1* was equally efficient at 25, 30, and 37°C, suggesting that they are probably defective in different genes. Growth of K^+ *rna1-1* strains at semipermissive temperatures did not yield K⁻ mitotic segregants, and none of 12 spontaneous temperature-resistant revertants had become K⁻.

Other defects. Diploids homozygous for a number of *mak* mutations were prepared to test for an effect of the *mak* genes on meiosis, sporulation, or spore germination. No effect was found in any of the cases tested (*mak-1*, *mak2-1*, *mak3-1*, *mak4-1* [30°C], *mak5-1*, *mak6-1*, *mak7-1*, *mak7-2*, *mak8-1*, *mak9-1*, *mak10-1*, *mak11-1*, *mak12-1*, *mak13-1*, *mak14-1*, *mak15-1*, *mak17-1*, *mak18-1*, *mak18-2*, *mak19-1*, *mak20-1*, *mak21-1*, *mak22-1*, *mak25-1*, and *mak26-1*). No *mak* mutations prevented mating by **a** or α strains.

To test for an effect of *mak* mutations on the homothallic interconversion of mating types, diploids of the type $\frac{\mathbf{a}\ HO}{\alpha\ HO}\ \frac{HM\mathbf{a}}{HM\mathbf{a}}\ \frac{HM\alpha}{HM\alpha}\ \frac{makx}{makx}$ were constructed. In each case tested, these diploids sporulated normally and the spore clones failed to mate with either **a** or α tester strains, except at the very low frequency observed for a normal **a**/α diploid. This gross test indicates that mating type interconversion was operative in the *mak* spores. This test was carried out for *mak1-1*, *mak3-1*, *mak9-1*, *mak10-1*, *mak13-1*, *mak16-1*, *mak18-1*, *mak19-1*, *mak22-1*, and *mak24-1*.

Patch tests for UV sensitivity were negative for all *mak* gene mutants.

DISCUSSION

We have now located a total of 26 *mak* genes on 15 of the 17 chromosomes comprising the genetic map of *S. cerevisiae*. These mapping studies confirm the assignments of mutations to genes by complementation tests reported earlier (29). The frequent occurrence of suppressors of the *mak2*, *mak23*, and *mak25* mutations in our mapping strains has, to date, prevented our mapping these genes. Like most groups of genes of known functional relation, the *mak* genes are apparently scattered at random on the map.

A visiting genome, such as the killer plasmid, insinuates itself into the host's molecular machinery, using host proteins with host-specific functions, to act on behalf of the visitor. It is our goal to define these host genes, their host-specific functions, and their role in the maintenance and expression of the killer plasmid genome. Mutants defective in *pet18*, *mak1*, and *mak16* are temperature sensitive for growth, and several

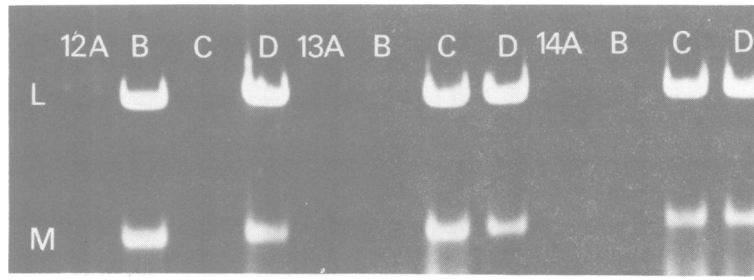

FIG. 3. *Cosegregation of low L dsRNA with mak3-1. Strain 297 (a his1 mak3-1 [KIL-o]) was crossed with strain 1101 (α his4 [KIL-k]). Meiotic segregants (10 complete tetrads) were grown and dsRNA was prepared by the rapid method of Fried and Fink (10). The RNA was electrophoresed on 1% agarose gels, stained with ethidium bromide, and photographed under UV light. In each tetrad, the two K⁻ spore clones had no M dsRNA and very low amounts of L dsRNA. Tetrads 12, 13, and 14 are shown. A, B, C, and D are the four spore clones of a tetrad.*

other *mak* mutations result in small colony size at any temperature. Mutants defective in the *pet18* gene lose both the killer plasmid (*M* dsRNA) and the mitochondrial genome, becoming nonsuppressive ρ° petites with no detectable mitochondrial DNA. *pet18* is not needed for maintenance of other yeast plasmids (15).

The only chromosomal gene for killer plasmid maintenance whose product is known is the *spe2* gene. *spe2* codes for adenosylmethionine decarboxylase (6), an enzyme in the pathway for biosynthesis of the polyamines, spermidine, and spermine. *spe2* mutants completely deficient in spermidine and spermine grow with a sixfold-increased doubling time, cannot undergo meiotic sporulation (6), and lose the *M* dsRNA when starved of polyamines (7). All of these defects are prevented by supplying either spermidine or spermine.

It is evident that more *mak* genes remain to be found. Excluding *spe2*, for which there is a specific screening method (6), there are two genes (*mak1* and *pet18*) for which four mutant representatives have been isolated. There are two mutants defective in each of the *mak7* and *mak18* genes, and every other *mak* gene has but a single mutant representative. Assuming a Poisson distribution of mutations in genes, there must be over 100 *mak* genes. This indicates that the maintenance and replication of the killer plasmid (*M* dsRNA) are a complex process with complex regulation. Also, a group of several *mak* genes may, for example, code for enzymes in a pathway leading to a single product which is involved in killer plasmid replication.

While several *mak* mutations also result in decreased cellular growth rate, the decreased growth rate alone is not sufficient to explain the killer plasmid loss. (i) Most clones of a mutagenized stock grow slowly, but almost all retain the killer plasmid. In particular, the *ski1-1* mutation results in slow growth, increased toxin production (23), and suppression of many *mak* mutations (Toh-e and Wickner, unpublished data). (ii) Starvation of auxotrophs for adenine or histidine does not result in killer plasmid loss. This includes the slow growth of *ade2-1* (ochre) SUQ5 [PSI⁺] strains where, in effect, adenine-limited cell growth is not accompanied by killer plasmid loss (15). (iii) Cells growing slowly on glycerol or glycerol-minimal medium do not lose the killer plasmid, nor do *cdc* temperature-sensitive mutants or *rna1* strains grown at temperatures where growth is slowed but not stopped. (iv) Suppression of *pet18* by *KRB1* (34) or *ski1* (Toh-e and Wickner, unpublished data) corrects the *mak* defect without affecting the temperature-sensitive growth defect; similarly, suppression of *spe2* by *ski1* through *ski4* reverses the *mak* defect without affecting the growth rate defect (7). The suppression of *mak10-1* by deletion of mitochondrial DNA is also accompanied by a decreased (28) growth rate. Although the role of some *mak* gene products in killer plasmid replication may be indirect, each *mak* mutation is specific in that the effect on plasmid replication is greater than any effect on cell growth. If cell growth rate and plasmid replication rate were each halved by a particular mutation, the plasmid would not be lost from the cell and the mutation would not be scored as a *mak* mutation.

Mutants defective in each of the 28 *mak* genes lose *M* dsRNA but retain *L* dsRNA. Thus, *L* dsRNA can do without any one of the *mak* gene products. Except for *mak3-1*, the chromosomal genes responsible for the maintenance and replication of *L* have not yet been defined.

Whereas *mak3-1* strains have not completely lost *L* dsRNA, they have a markedly reduced copy number. Either the *mak3* gene product is independently involved in both *L* and *M* main-

tenance or the effect of *mak3* on *L* results in the loss of *M*. It would be useful to isolate other *mak3* mutant alleles. Mitchell et al. (17) reported an apparently mutant *L* which had become dependent on the *mak10* gene product, unlike the *L* found in most strains.

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