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.

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 maintain the killer 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; Tobc 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-
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-fasl-trp3-ural 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 ural 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 mak1 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 gall12 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

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**Table 1. Strains of S. cerevisiae**

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<th>Killer pheno-type</th>
<th>Genotype</th>
<th>Source or reference</th>
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<td></td>
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**Table 2. Mapping of mak9, mak11, and mak15 on chromosome XI**

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**Table 3. Mapping of mak12 on chromosome XII**

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* Segregation data are given as the number of parental ditype (PD), nonparental ditype (NPD), and tetratype (T) ascii observed. In a cross of the type ab × AB, a PD ascii would be ab, ab, AB, AB. An NPD ascii would be aB, aB, AB, AB, and a T ascii 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 = (1T + 6NPD + 100PD) × (PD + NPD + T).

* Centromere linkage is detected by a decrease in second meiotic division segregation frequency. Second division segregation frequency is measured as tetratype (T) ascii frequency for the unmapped marker and a standard marker known to be linked to its centromere (such as trpl 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.

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 trpl and aro1D, two markers which are unresolved to each other (18), places mak21...
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. Electrolysis of these samples (Fig. 2) showed that in each case, L dsRNA (about 3 x 10^6 daltons) was present, but M dsRNA (about 1.5 x 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 htx2 (killer expression) 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 cosegregates 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-

Table 4. mak14 located on chromosome III^a

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Table 5. mak16 located on chromosome I^a

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<td>8</td>
<td>21</td>
</tr>
<tr>
<td>cM</td>
<td>14</td>
<td>20</td>
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Table 6. Mapping of mak21 on chromosome IV^a

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<td>(54)</td>
<td>46</td>
<td>45</td>
<td>15</td>
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</table>

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

The location of cysl is due to S. Fogel (personal communication). 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 trpl or aro1D, suggesting that the order is rna11-(trpl-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.
tation 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 a HO HMa HMa makx α HO HMa HMa 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 petl8, mak1, and mak16 are temperature sensitive for growth, and several...
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 ρ⁰ petetics 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 skil-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 skil (Toh-e and Wickner, unpublished data) corrects the mak defect without affecting the temperature-sensitive growth defect; similarly, suppression of spe2 by skil4 through skil4 reverses the mak defect without affecting the growth rate defect (7). The suppression of makI0-1 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-

**Fig. 3. Cosegregation of low L dsRNA with mak3-I.** Strain 297 (a his4 mak3-I [KIL-ol]) was crossed with strain 1101 (a 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.
tenance or the effect of \textit{makh} on \textit{L} results in the loss of \textit{M}. It would be useful to isolate other \textit{makh} mutant alleles. Mitchell et al. (17) reported an apparently mutant \textit{L} which had become dependent on the \textit{makh10} gene product, unlike the \textit{L} found in most strains.

ACKNOWLEDGMENTS

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LITERATURE CITED