Spermidine or Spermine Requirement for Killer Double-stranded RNA Plasmid Replication in Yeast*

(Received for publication, March 20, 1978, and in revised form, May 29, 1978)

Murray S. Cohn, Celia W. Tabor, Herbert Tabor, and Reed B. Wickner

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

SUMMARY

The spe2 gene of Saccharomyces cerevisiae codes for S-adenosylmethionine decarboxylase, an enzyme in the biosynthetic pathway for the polyamines spermidine and spermine. We previously found that spe2 mutants completely lacking enzymic activity have no spermidine or spermine when grown in polyamine-free medium and require spermidine or spermine for meiotic sporulation and optimal growth. We now show that the killer plasmid, a 1.5 x 10^8 molecular weight double-stranded RNA (M) in intracellular virus-like particles, is lost from spe2 killer strains grown without spermidine or spermine. In contrast, the 3.0 x 10^6 molecular weight double-stranded RNA (L), the mitochondrial genome (p), and the cytoplasmic element that potentiates weak ochre suppressors (4) are not lost under the same conditions. Once the killer plasmid is lost from a polyamine-deficient strain, it cannot be restored by polyamine addition, and such a strain is sensitive to the killer toxin excreted by strains containing the plasmid. Recessive mutations in any of four chromosomal genes (skl1, skl2, skl3, and skl4) that result in overproduction of killer toxin activity (A. Toh-e and R. B. Wickner, personal communication) bypass the polyamine requirement for killer plasmid maintenance, but not the spe2-related growth defect. Thus, spe2 skl strains grown on polyamine-free medium are killers. The polyamine requirement for killer plasmid maintenance is not bypassed by the absence of the mitochondrial genome or the chromosomal KRBI mutation, which do bypass other chromosomal genes needed for killer plasmid maintenance.

The in vivo function(s) of the ubiquitous polyamines, spermidine and spermine, remain uncertain in spite of many studies of the regulation of their biosynthesis and of their effects. (For references, see Ref. 1.) Recently, yeast mutants deficient in adenosylmethionine decarboxylase have been isolated (2, 3). Two of these mutants (2) totally lack S-adenosylmethionine decarboxylase and contain no spermidine or spermine when grown in their absence. These mutants, in the absence of spermidine or spermine, grow at about one-sixth the rate of the wild type and are defective in meiotic sporulation; both growth and sporulation are normal if either polyamine is added (2).

Yeast strains containing a double-stranded RNA plasmid (called the killer plasmid or M, 1.5 x 10^8 molecular weight) secrete a protein toxin which is lethal to strains not carrying this plasmid (reviewed in Ref. 4). Strains that are deficient in adenosylmethionine decarboxylase, when grown in medium containing polyamines, can maintain the killer plasmid and secrete the killer toxin. We now report that if these strains are made deficient in polyamines by growth in polyamine-free medium, they lose the killer double-stranded (ds) RNA plasmid, while retaining other plasmids.

MATERIALS AND METHODS

Yeast Strains—Strains used in this study are listed in Table I. Media—YPAD contained 1% yeast extract, 2% peptone, 2% dextrose, 2% agar; YPD medium contained 0.67% yeast nitrogen base without amino acids (Difco), 2% agar, and 2% dextrose. Complete minimal medium was SD supplemented with adenine sulfate, 400 mg/liter; uracil, 24 mg/liter; tryptophan, 24 mg/liter; histidine, 24 mg/liter; arginine, 24 mg/liter; methionine, 24 mg/liter; tyrosine, 36 mg/liter; leucine, 36 mg/liter; lysine, 36 mg/liter; aspartic acid, 100 mg/liter; threonine, 200 mg/liter; and phenylalanine, 60 mg/liter.

Mortality—Phenotypes of strains with regard to their killing ability (K) and resistance to killing (R) are denoted K'R', K' R', K'R, and K' R. The genotype of the wild type killer plasmid is denoted by [KIL-k] and its absence by [KIL-o]. Chromosomal skili, skl2, skl3, and skl4 mutants result in increased killing ability, a phenotype denoted by K'" and called superkiller. Chromosomal mak genes are those needed to maintain the normal killer plasmid, p', indicates the presence and p" the absence of normal mitochondrial DNA. Growth on YPD medium requires p'. The mitochondrial genome, p, can be completely eliminated by growth of cells to single colonies on YPAD plates containing 30 mg/ml of ethidium bromide (5). K91 is a dominant chromosomal mutation resulting in bypass of the requirement for the mak7 and pet18 genes for killer plasmid maintenance (6).

Polyamine Depletion—Depletion was accomplished by two successive single colony isolations on complete minimal medium.

Assay of Killing and Resistance—The ability of a strain to kill was assayed at 20°C or 30°C essentially as described by Somers and Bevan (7). Colonies grown on YPAD were transferred to MB medium which had been previously spread with a lawn of the sensitive strain 5X47 [KIL-o]. Resistance to killing was checked by making a lawn of the strain to be tested on MB medium and streaking with a killer strain. In each case, killing is indicated by a clear zone surrounding the killing strain and surrounded in turn by growth of the lawn. Superkiller strains have a larger zone of killing than wild type strains, especially at 30°C where wild type killers produce almost no clear zone.

Genetic Analysis—Matings were carried out on YPAD. Diploids were isolated utilizing the complementary nutritional requirements of their parents. Sporulation and dissection were by the usual methods (see Mortimer and Hawthorne (8) for references).

RESULTS

Requirement of Spermidine or Spermine to Maintain the Killer Plasmid—All haploid strains containing both the killer...

* A preliminary report was given at the American Society of Biological Chemists/American Association of Immunologists Meeting, Atlanta, Georgia, June, 1978. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

A. Toh-e and R. B. Wickner, unpublished observations.
Polyamine Requirement for Killer Plasmid Replication

plasmid and the spe2 mutation are killers when grown on rich (YPAD) medium. However, these strains become nonkillers and sensitive to the killer toxin when exhausted of their polyamine content by extended growth on polyamine-free complete minimal medium (Table I). To obtain loss of the killer phenotype, spe2 strains must be cloned twice on polyamine-free medium. After a single cloning, some cells are killers while some have lost the killer phenotype. After the second cloning, all cells have become K^-R^- This requirement for extensive depletion is similar to that previously described for demonstration of the slow growth rate of such strains (2). Supplementation of complete minimal medium with 10^{-6} M spermidine or spermine prevents the loss of the killer phenotype. However, once lost, the killer phenotype cannot be restored to polyamine-depleted strains by subsequent polyamine addition. This suggests that the polyamine-depleted strains have lost the killer plasmid. A spe2 strain which had been depleted and had become K^- was crossed with a spe^+ [KIL-o] strain. The diploids were nonkillers. This is further evidence that the polyamine-depleted spe2 strain had become a nonkiller due to killer plasmid loss.

Loss of the killer plasmid is directly attributed to the spe2 defect and not to some other inadvertently introduced mutation. The loss of the killer phenotype upon polyamine depletion co-segregated with spe2 in all of the 12 tetrads tested from a cross of a spe2 K^- strain with a wild type killer (41-8B x 14-22B). Also, the killer plasmid loss was observed with two independently isolated spe2 alleles (strains 52-6D and 53-6B). Furthermore, these two strains did not complement one another (in the 52-6D x 53-6B diploid) with respect to the spe2 defect or the loss of the killer phenotype upon polyamine depletion. The prevention of killer plasmid loss by inclusion of spermidine or spermine in the medium is, of course, the strongest evidence on this point.

An analysis of the double-stranded RNA content of a polyamine-depleted spe2 strain which has lost the killer plasmid reveals a lack of the M (1.5 X 10^6 molecular weight) species but not L (3.0 X 10^6 molecular weight) (Fig. 1). Therefore, the effect of polyamine deficiency resembles that of other chromosomal mak (maintenance of killer) genes previously described (10). Spe2 strains depleted of polyamines remain able to use glycerol as a carbon source indicating that the mitochondrial genome, p, is still present. ψ is a non-Mendelian genetic element which potentiates weak ochre suppressors, such as SUQ5 (11). Spe2 ade2-1 SUQ5 ψ strains, which are able to grow without adenine supplementation because of the

FIG. 1. Agarose slab gel electrophoresis of ds RNA prepared from strain 47-10B [spe2-2] grown under various conditions: A, grown on YPAD; B, depleted of polyamines and then grown on YPAD; C, grown on complete minimal medium in the presence of spermidine; and D, depleted of polyamines and then grown on complete minimal medium. Preparation of ds RNA and electrophoresis were carried out as described (9).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>27-1B</td>
<td>a adel arg1 spe2-2 [KIL-o]</td>
<td>(2)</td>
</tr>
<tr>
<td>28-8D</td>
<td>a adel thr1 spe2-3 [KIL-o]</td>
<td>(2)</td>
</tr>
<tr>
<td>52-6D</td>
<td>a adel his4 leu2 spe2-3 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>53-6B</td>
<td>a adel arg1 leu2 spe2-3 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>47-10B</td>
<td>a adel arg1 leu2 ura1 rna1 spe2-2 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>47-10B</td>
<td>a adel arg1 leu2 ura1 rna1 spe2-2 [KIL-o]</td>
<td>This work</td>
</tr>
<tr>
<td>48-13B</td>
<td>a adel arg1 leu2 ura1 rna1 spe2-2 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>AT95</td>
<td>a his7 skil-1 [KIL-k]</td>
<td>A. Toh-e</td>
</tr>
<tr>
<td>W87-5D</td>
<td>a thr1 skil-2 [KIL-k]</td>
<td>A. Toh-e</td>
</tr>
<tr>
<td>W88-8B</td>
<td>a adel thr1 skil-3 [KIL-k]</td>
<td>A. Toh-e</td>
</tr>
<tr>
<td>W7-5A</td>
<td>a adel thr1 skil-4 [KIL-k]</td>
<td>A. Toh-e</td>
</tr>
<tr>
<td>41-8B</td>
<td>a adel ura1 adel spe2-2 [KIL-o]</td>
<td>This work</td>
</tr>
<tr>
<td>14-22B</td>
<td>a ura1 rna1 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>M394</td>
<td>a adel2-1 his3-35 SUQ5 [PSI] [KIL-k]</td>
<td>M. Leibowitz</td>
</tr>
<tr>
<td>58-5C</td>
<td>a met14 adel spe2-2 KRB-1 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>64-1C</td>
<td>a adel1 his7 spe2-2 skil-1 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>61-6D</td>
<td>a adel2 adel spe2-2 spe2-3 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>60-2D</td>
<td>a adel2 adel thr1 spe2-2 skil-1 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>63-6C</td>
<td>a adel2 adel thr1 spe2-2 skil-1 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>63-6C</td>
<td>a adel2 adel thr1 spe2-2 skil-1 [KIL-k]</td>
<td>This work</td>
</tr>
</tbody>
</table>

**TABLE I**

Yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>27-1B</td>
<td>a adel arg1 spe2-2 [KIL-o]</td>
<td>(2)</td>
</tr>
<tr>
<td>28-8D</td>
<td>a adel thr1 spe2-3 [KIL-o]</td>
<td>(2)</td>
</tr>
<tr>
<td>52-6D</td>
<td>a adel his4 leu2 spe2-3 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>53-6B</td>
<td>a adel arg1 leu2 spe2-3 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>47-10B</td>
<td>a adel arg1 leu2 ura1 rna1 spe2-2 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>47-10B</td>
<td>a adel arg1 leu2 ura1 rna1 spe2-2 [KIL-o]</td>
<td>This work</td>
</tr>
<tr>
<td>48-13B</td>
<td>a adel arg1 leu2 ura1 rna1 spe2-2 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>AT95</td>
<td>a his7 skil-1 [KIL-k]</td>
<td>A. Toh-e</td>
</tr>
<tr>
<td>W87-5D</td>
<td>a thr1 skil-2 [KIL-k]</td>
<td>A. Toh-e</td>
</tr>
<tr>
<td>W88-8B</td>
<td>a adel thr1 skil-3 [KIL-k]</td>
<td>A. Toh-e</td>
</tr>
<tr>
<td>W7-5A</td>
<td>a adel thr1 skil-4 [KIL-k]</td>
<td>A. Toh-e</td>
</tr>
<tr>
<td>41-8B</td>
<td>a adel ura1 adel spe2-2 [KIL-o]</td>
<td>This work</td>
</tr>
<tr>
<td>14-22B</td>
<td>a ura1 rna1 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>M394</td>
<td>a adel2-1 his3-35 SUQ5 [PSI] [KIL-k]</td>
<td>M. Leibowitz</td>
</tr>
<tr>
<td>58-5C</td>
<td>a met14 adel spe2-2 KRB-1 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>64-1C</td>
<td>a adel1 his7 spe2-2 skil-1 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>61-6D</td>
<td>a adel2 adel spe2-2 spe2-3 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>60-2D</td>
<td>a adel2 adel thr1 spe2-2 skil-1 [KIL-k]</td>
<td>This work</td>
</tr>
<tr>
<td>63-6C</td>
<td>a adel2 adel thr1 spe2-2 skil-1 [KIL-k]</td>
<td>This work</td>
</tr>
</tbody>
</table>

**TABLE II**

Polyamine dependence of killer plasmid maintenance: suppression by superkiller mutations

<table>
<thead>
<tr>
<th>Designation</th>
<th>Partial genotype</th>
<th>Before polyamine depletion</th>
<th>After polyamine depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-22B</td>
<td>+ [KIL-k]</td>
<td>K^+</td>
<td>K^-</td>
</tr>
<tr>
<td>53-6B</td>
<td>spe2-2 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
<tr>
<td>52-6D</td>
<td>spe2-3 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
<tr>
<td>53-6B x 52-6D</td>
<td>spe2-2/spe2-3 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
<tr>
<td>47-10B-EB4</td>
<td>spe2-2 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
<tr>
<td>58-5C</td>
<td>spe2-2 KRB-1 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
<tr>
<td>AT95</td>
<td>shi1-1 [KIL-k]</td>
<td>K^+</td>
<td>K^-</td>
</tr>
<tr>
<td>64-1C</td>
<td>shi1-1 spe2-2 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
<tr>
<td>W87-5D</td>
<td>shi2-3 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
<tr>
<td>W88-8B</td>
<td>shi3-3 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
<tr>
<td>63-6C</td>
<td>shi4-1 spe2-2 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
<tr>
<td>60-2D</td>
<td>spe2-2 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
<tr>
<td>53-6B</td>
<td>spe2-2 [KIL-k]</td>
<td>K^-</td>
<td>K^-</td>
</tr>
</tbody>
</table>

**"At least three different strains with each indicated genotype were examined for their killer phenotype before and after polyamine depletion was carried out as described under "Materials and Methods." Only one strain designation is shown in each case."**
suppression by SUQ5 and ψ of the octobr ade2-1 mutation (11), retain this ability upon polyamine depletion. Therefore, ψ is not lost from polyamine-depleted strains even though the killer plasmid is lost.

Bypass of Polyamine Requirement for Killer Plasmid Replication by Superkiller Mutations—Most, if not all, mak mutations can be bypassed (suppressed) by second site mutations; thus, the killer plasmid and phenotype are retained in spite of the presence of the mak mutation. Three classes of mutations are known which bypass mak genes: (i) loss of the mitochondrial genome, ρ, bypasses mak10 (12); (ii) the dominant chromosomal KRBl (killer replication bypass) mutation makes the mak7 and pet18 genes dispensable for plasmid maintenance (6); and (iii) the recessive chromosomal ski1, ski2, ski3, and ski4 mutations result in both increased killing ability (super killer) and bypass of various mak mutations.2

Loss of the mitochondrial genome or presence of the KRBl mutation has no effect on the polyamine requirement for killer plasmid maintenance. However, each of the ski1, ski2, ski3, and ski4 mutations bypasses the polyamine requirement, so that a ski spe2 strain depleted of polyamines remains a superkiller (Table II). Superkiller strains are recessive for their phenotype. As expected, they are also recessive for bypassing the polyamine requirement, so that a spe2/spe2 ski*/ski diploid depleted of polyamines becomes a nonkiller (spe2/spe2 ski*/ski diploids remain superkillers). Therefore, the loss of any of at least four gene products allows maintenance of the killer plasmid under conditions of polyamine depletion where the plasmid would normally be lost. This bypass is not due to the biosynthesis of spermidine or spermine by an alternate pathway, nor to the presence of a new polyamine, since an analysis of a polyamine-depleted ski2 spe2 strains grow as slowly as ski+ spe2 strains, but retain the killer plasmid.

The polyamine requirement for killer plasmid maintenance is bypassed by mutations in any of the four chromosomal ski (superkiller) genes, but not by either the KRBl mutation or by deletion of the mitochondrial genome. The mak1-1, mak4-1, and mak6-1 mutations are also bypassed by mutations in any of the four ski genes, but not by either KRBl or ρ0 (Toh-e and Wickner1). This suggests that these mak genes and spe2 are functionally related in killer plasmid maintenance.

DISCUSSION

Our findings show that the wild type killer plasmid requires either spermidine or spermine for its maintenance. As in the case of the 26 known chromosomal genes designated mak that are required for killer plasmid maintenance (4, 10, 19), the spe2 effect is specific in that only M ds RNA and not L ds RNA is lost. Furthermore, neither the mitochondrial genome, ρ, nor the ψ plasmid is lost from spe2 mutants. Of the various mak mutants, the spe2 mutant is of particular interest since it is the only gene of the mak type whose biochemical function is known. Polyamine deficiency does not explain the effects of any of the previously described mak mutants, since all of these were isolated on polyamine-containing medium. As with the other 26 mak genes, the role of spe2 in killer plasmid maintenance may be direct or indirect.

The defects in sporulation (2) and in killer plasmid maintenance of spe2 mutants are the first examples of an absolute requirement for spermidine or spermine in vivo. The diamine, putrescine, which is present in excess in all spe2 strains (2), does not function in place of spermidine or spermine. Although polyamine starvation of spe2 mutants results in slow cell growth, the decreased growth rate alone is not sufficient to explain the killer plasmid loss: (i) starvation of auxotrophs for adenine or histidine does not result in killer plasmid elimination; (ii) most clones grown from a mutagenized stock grow slowly, but almost all retain the killer plasmid; and (iii) polyamine-depleted ski spe2 strains grow as slowly as ski+ spe2 strains, but retain the killer plasmid.

REFERENCES


H. B. Wickner, unpublished observations.
Spermidine or spermine requirement for killer double-stranded RNA plasmid replication in yeast.
M S Cohn, C W Tabor, H Tabor and R B Wickner