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Genetics

Specificity of polyamine requirements for the replication and maintenance of different double-stranded RNA plasmids in *Saccharomyces cerevisiae*

(killer plasmids/double-stranded RNA replication/putrescine/spermidine)

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ABSTRACT **We have shown previously that the M_1 double-stranded (ds) RNA (i.e., the killer plasmid [KIL-k_1]) that codes for a protein toxin requires spermidine or spermine for its replication. We now report that replication of two other ds RNA plasmids of yeast also requires polyamines: (*i*) M_2 ds RNA ([KIL-k_2]) and (*ii*) L-A-E, a ds RNA plasmid carrying the non-Mendelian genetic element [EXL]. Putrescine alone is sufficient to maintain L-A-E but is not sufficient to maintain either M_1 ds RNA or M_2 ds RNA, which require either spermidine or spermine. Once M_1 or M_2 or L-A-E is lost, it cannot be restored by the addition of polyamines. In contrast, L-A-HN, a ds RNA molecule that carries the cytoplasmic genes [HOK] and [NEX], is not lost during polyamine deprivation. It is striking that polyamine deprivation differentially affects L-A-E and L-A-HN, even though these two ds RNA molecules have more than 99% homology. L-C, which is the same size as L-A but very different in sequence, is also not lost on polyamine starvation.**

It is difficult to study the role of polyamines in virus or plasmid replication in most eukaryote cells largely because, in most such systems, genetic studies of the host cell are difficult. Consequently, we have been studying the role of polyamines in the maintenance of some double-stranded (ds) RNA plasmids of *Saccharomyces cerevisiae*.

We have previously reported that spermidine or spermine is required for the maintenance of [KIL-k_1], a plasmid of *S. cerevisiae* identified as M_1 ds RNA (1). M_1 ds RNA codes for a "killer" toxin that is excreted and kills sensitive strains of *S. cerevisiae* (2–5). Mutants of *S. cerevisiae* that cannot synthesize polyamines permanently lose the [KIL-k_1] plasmid (M_1 ds RNA) if grown without added spermidine or spermine (1). Putrescine cannot substitute for spermidine and spermine in strains unable to convert putrescine to these amines. We now report a similar polyamine requirement for a closely related killer plasmid, [KIL-k_2] (M_2 ds RNA).

These experiments demonstrated polyamine requirements for the maintenance or replication of the M forms of ds RNA. We thought it of interest to see if the other types of ds RNA plasmids of *S. cerevisiae* also require polyamines. Several ds RNAs (L ds RNAs), which are larger than M ds RNAs, carry non-Mendelian genes that influence the maintenance or replication of the killer plasmids (2–6). Three such genetic elements, [HOK], [NEX], and [EXL], are located on certain variants of the L-A class of L ds RNA plasmids (4). If [EXL] is present (i.e., on a L-A-E ds RNA plasmid), [KIL-k_2] is excluded from the cell and no M_2 ds RNA is found (5); however, if [NEX] is also present, then the action of [EXL] is neutralized (3–5). The presence of [HOK] is necessary for the maintenance or replication of a mutant form of [KIL-k_1] in a *SKI*$^+$ host (see Table 1) (3, 6). The cytoplasmic genes [HOK] and [NEX] are carried on a single ds RNA molecule called L-A-HN (4). L-B and L-C are different classes of the L ds RNA plasmid, showing no cross-hybridization with L-A. Their role in the killer system, if any, is unknown.

In the present work we show that the maintenance of the L-A-E ds RNA (carrying [EXL]) also requires polyamines, but, in contrast to the results with [KIL-k_1] and [KIL-k_2], putrescine can substitute for spermidine or spermine. No requirement for polyamines was found for the maintenance of L-A-HN ds RNA (carrying [HOK] and [NEX]) or L-C ds RNA.

MATERIALS AND METHODS

Strains. The nomenclature for the killer system is given in Table 1. The yeast strains used are listed in Table 2.

Media. YPAD medium contained 2% dextrose, 2% peptone, 1% yeast extract, 2% agar, and 0.04% adenine sulfate. MB medium was YPAD with 0.1 M sodium citrate buffered to pH 4.7 and contained 0.003% methylene blue. SD medium contained 2% dextrose, 2% agar, and 0.67% yeast nitrogen base without amino acids (Difco). Complete minimal medium was SD supplemented with adenine, uracil, and amino acids (1). Noble agar (Difco) was used, and the medium was not autoclaved but was sterilized by filtration.

Polyamine Depletion. Polyamine depletion was accomplished by 3–5 successive colony isolations on complete minimal medium at 30°C. As described (7), *spe2-4* mutants grow indefinitely in minimal medium; the doubling time after amine deprivation is about 12 hr. *spe10-3* mutants, on the other hand, stop growing after several colony isolations on complete minimal medium (8).

Assay of Killing. Colonies to be tested for killing ability were replicated to MB medium, which previously had been spread with a lawn of a strain sensitive to the killer function being tested; strain 5X47 was used, unless otherwise specified. MB plates were incubated at 20°C for 2–3 days. The killing was indicated by a clear zone surrounding the killing strain carrying the killer plasmid, which was surrounded in turn by growth of sensitive cells.

Genetic Analysis. Unbuffered YPAD medium (pH ≈ 6.0) was used for mating experiments because the toxin does not act at that pH (11, 12). Diploids were purified on the basis of the complementary nutritional requirements of their parents. Sporulation and dissection were as described (13).

ds RNA Extraction and Electrophoresis. ds RNAs were purified by a rapid method described by Fried and Fink (9).

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Abbreviation: ds, double-stranded.

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Table 1. Nomenclature

Phenotypes	
K_1^+ or K_1^-	Ability (K_1^+) or inability (K_1^-) to kill a lawn of a $K_2^+R_2^+$ or a K^-R^- strain; K_1^+ is unable to kill a lawn of another $K_1^+R_1^+$ strain.
R_1^+ or R_1^-	Resistance (R_1^+) or sensitivity (R_1^-) to K_1 killer toxin.
K_1^{++}	Superkiller phenotype; increased toxin production.
K_2^+ or K_2^-	Ability (K_2^+) or inability (K_2^-) to kill a lawn of a $K_1^+R_1^+$ or a K^-R^- strain; K_2^+ is unable to kill a lawn of another $K_2^+R_2^+$ strain.
R_2^+ or R_2^-	Resistance (R_2^+) or sensitivity (R_2^-) to K_2 killer toxin.
Genotypes	
[KIL-o]	Absence of killer plasmid.
[KIL-k_1]	Normal killer plasmid (M_1 ds RNA).
[KIL-k_2]	Normal killer plasmid (M_2 ds RNA).
[KIL-sd_1]	Mutant killer plasmid derived from [KIL-k_1]; depends for its replication on a host *ski*$^-$ mutation (6) or on the presence of the plasmid [HOK]. Cells that carry [KIL-sd_1] are $K_1^+R_1^+$.
[HOK]	Plasmid that allows [KIL-sd_1] to replicate in a *SKI*$^+$ host (*h*elper *o*f *k*iller).
[HOK-o]	Absence of [HOK] plasmid.
SKI	Chromosomal gene, mutation of which produces the superkiller (K^{++}) phenotype. There are four *SKI* genes. The *ski*$^-$ mutants are recessive to wild type.
[EXL]	Plasmid that prevents the replication of [KIL-k_2].
[NEX]	Neutralizer of [EXL]. This non-Mendelian trait prevents the action of [EXL].
ds RNA	
M_1	M_r 1.25×10^6 linear ds RNA identified as [KIL-k_1]; it codes for K_1 toxin (not present in K^- or K_2 strains).
M_2	M_r 1.0×10^6 linear ds RNA identified as [KIL-k_2]; it codes for K_2 toxin (not present in K^- or K_1 strains).
L-A	M_r 3.0×10^6 linear ds RNA present in most *S. cerevisiae*; it codes for major coat protein of particles containing L-A and M ds RNAs.
L-A-E	A variety of L-A ds RNA that carries the cytoplasmic gene [EXL].
L-A-HN	A variety of L-A ds RNA that carries the cytoplasmic genes [HOK] and [NEX].
L-B and L-C	M_r 3×10^6 ds RNAs unrelated to L-A and present in virus-like particles with different major proteins. L-B and L-A or L-C and L-A are compatible. L-B and L-C show some sequence homology.

Plasmids are given in brackets, and chromosomal genes are presented in italics. Dominant alleles of chromosomal genes are in uppercase letters, and the recessive alleles are in lowercase.

Briefly, the cells, treated with 2-mercaptoethanol, were extracted with a sodium dodecyl sulfate/phenol mixture, and the extract was precipitated with ethanol. Electrophoresis was carried out on 1.5% agarose gels by the method of Toh-e *et al.* (10).

RESULTS

Polyamine Requirement for Replication of [KIL-k_2]. The strains containing the [KIL-k_2] plasmid and *spe10* or *spe2* mutations are killers when they are grown on rich (YPAD) medium. Both the *spe2* and *spe10* strains, however, become nonkillers and sensitive to killer toxin when exhausted of their polyamine content by growth in the absence of polyamines as described in *Materials and Methods* (Table 3). Putrescine is not sufficient to maintain [KIL-k_2] because *spe2* mutants continue to make large amounts of putrescine and lack only spermidine and spermine. When 100 μM spermidine was included in the polyamine-free complete minimal medium during the growth of these strains, neither *spe10* nor *spe2* strains lost the [KIL-k_2] plasmid. However, supplementation with polyamines did not restore the [KIL-k_2] plasmid once it had been lost from either *spe10* or *spe2* strains as a result of polyamine deprivation.

When total cellular ds RNA of the *spe2* and *spe10* strains that had lost [KIL-k_2] after polyamine deprivation was examined by electrophoresis on agarose gels, no M_2 ds RNA (M_r 1.0×10^6) was found (Fig. 1, lanes D, E, and F). However, the L ds RNA (M_r 3.0×10^6) was not lost (Fig. 1).

Polyamine Requirement for the Replication of [EXL] (L-A-E ds RNA Plasmid). The presence or absence of [EXL] was measured by mating the strain to be tested with a [KIL-k_2] [NEX-o] strain and determining whether the diploids exhibit the killer phenotype. Because both the *spe10* strain M4 and the *spe2* strain 177 carry the [EXL] plasmid, when either strain was mated with strain 1387, which carries [KIL-k_2], the diploids did not show any killing. However, when the *spe10* strain, depleted of its polyamine content by extended growth on polyamine-free complete minimal medium, was mated with strain 1387, the diploids clearly showed killing. This indicates that the *spe10* strain lost the [EXL] plasmid and that polyamines are required for the replication of [EXL] (Table 4, experiment A). Once [EXL] was lost, it could not be restored to polyamine-depleted strains by subsequent growth in putrescine-supplemented medium.

When the *spe2* strain was depleted of spermidine and spermine by extended growth on polyamine-free complete minimal medium, [EXL] was not lost; that is, diploids formed by mating the *spe2* strain (after polyamine depletion) with strain 1387 did not show killing of sensitive strains. This *spe2* strain, in contrast with the *spe10* strain, continued to make putrescine in greater than wild-type amounts when depleted of spermidine and spermine. Thus, putrescine alone, in the absence of spermidine and spermine, is sufficient to maintain the [EXL] plasmid (Table 4, experiment A). We also showed that addition of 100 μM putrescine to minimal medium during the growth period of the *spe10* strain prevented the loss of [EXL].

Further evidence for the loss of [EXL] was shown by gel electrophoresis of the RNA of the *spe10* strain before and after polyamine deprivation (Fig. 2). The amount of L ds RNA was decreased by about 50% once the strain was deprived of polyamines (Fig. 2*A*, lane 2). This is consistent with the observations that strains carrying [EXL] have twice as much L ds RNA as the same strain cured of [EXL] (4). The *spe10-3* mutant, which is derived from wild-type AN33 strain, contains two types of L ds RNAs, L-A and L-C, in equal proportions. The [EXL] genetic element is located on L-A (4). During polyamine depletion, although [EXL] (L-A-E)

Table 2. Yeast strains

Strain	Genotype*†	Ref.
AN33	*α thr1 arg1* [EXL] [KIL-o] [HOK-o] [NEX-o]	5
177	*α thr1 arg1 spe2-4* [EXL] [KIL-o]	9
M4	*α arg1 thr1 spe10-3* [EXL] [KIL-o]	10
Y331‡	*α arg1 thr1 spe10-3* [KIL-k_1] [HOK] [NEX]	§
1387	*a ura1* [KIL-k_2] [NEX-o] [HOK-o]	5
1479	*a lys1 can*^{r} [KIL-k_2] [NEX] [HOK]	5
1466	*a leu1 kar1-1* [HOK-o] [NEX-o] [EXL] [KIL-o]	5
T1	*α his4 kar1-1* [HOK-o] [NEX-o] [EXL] [KIL-o]	§
1405	*a ade1 ski2-2* [KIL-sd_1-3]	6
1406	*α ade1 ski2-2* [KIL-sd_1-3]	6
72-3C	*α arg1 thr1 can*^{r} *spe2-4* [KIL-k_2] [HOK] [NEX]	§
72-5D	*α arg1 thr1 can*^{r} *spe2-4* [KIL-k_2] [HOK] [NEX]	§
Y330	*a lys1 can*^{r} *spe10-3* [KIL-k_2] [HOK] [NEX]	§
5X47	*a/α his1/+ trp1/+ ura3/+* [KIL-o]	5
S121	*a/α mkt1/mkt1 lys1/+ met13/+ can1/+* [KIL-k_2]	3

*Killer genotypes in all cases refer to strains not depleted of polyamines. Strains 72-3C and 72-5D were constructed by mating strain 177 with 1479 and selecting appropriate segregants. Strain Y330 was constructed by mating strain M4 with strain 1479 and selecting an appropriate segregant. The presence of [NEX] in strains 72-3C, 72-5D, and Y330 was demonstrated by mating them with the [EXL]-carrying strains 1466 and T1 and showing that the diploids formed were all K_2^+.

†*spe2-4* is a mutation in the gene for adenosylmethionine decarboxylase. *spe2* mutants make putrescine but not spermidine or spermine (7). *spe10* is a mutation in the gene controlling ornithine decarboxylase. *spe10* mutants make no putrescine, spermidine, or spermine (8).

‡Strain Y331 was constructed by "cytoducing" [KIL-k_1] [HOK] [NEX] from a wild-type K_1 killer strain into strain M4. Strain Y331 thus has the same nuclear genotype as strain M4 but a different cytoplasmic genotype. Cytoduction (transfer of cytoplasmic markers without transfer of nuclear markers) was carried out as described (5).

§This work.

Table 3. Requirement of polyamines for the replication of [KIL-k_2] (M_2 ds RNA)

Strain	Genotype	Killer phenotype: Before polyamine depletion	Killer phenotype: After polyamine depletion
72-3C	*α spe2-4* [KIL-k_2]	K^+	K^-
72-5D	*α spe2-4* [KIL-k_2]	K^+	K^-
Y330	*a spe10-3* [KIL-k_2]	K^+	K^-
Y331	*α spe10-3* [KIL-k_1]	K^+	K^-

Strains 72-3C, 72-5D, and Y330 were isolated by mating *spe* strains with strain 1479, which carries [KIL-k_2], and selecting *spe*$^-$ [KIL-k_2] segregants. Strain Y331 was constructed as described in Table 2, footnote ‡, and selected as a [KIL-k_1] cytoductant. Polyamine depletion was carried out as described. All of these strains also carry [NEX] and [HOK].

was lost, only a 50% reduction in the amount of L ds RNA was seen. Thus, L-C remained, and so we conclude that L-C can replicate in the absence of polyamines. In addition, the analysis of RNA from the diploids formed by mating the *spe10* strain and the 1387 strain, which carries [KIL-k_2], showed that the M_2 ds RNA band was not present if the diploids were generated before the *spe10* strain was deprived of polyamines; however, if the *spe10* strain was first deprived of polyamines and then the diploids were generated by crossing the *spe10* strain with the same 1387 strain, then the M_2 ds RNA band was present in the RNA of these diploids (Fig. 2).

Polyamines Are Not Required for the Replication of [HOK] and [NEX] (i.e., L-A-HN ds RNA Plasmid). L-A-HN is a ds RNA molecule that carries the cytoplasmic genes [HOK] (*h*elper *o*f *k*iller) and [NEX] (a gene that prevents [EXL] from excluding M_2 ds RNA) (4). The presence of the L-A-HN plasmid was demonstrated in certain *spe2* and *spe10* strains by the following procedure. *Spe2* strains (72-3C and 72-5D) were mated with strain 1405, and the *spe10* strain (Y330) was mated with strain 1406. Both strains 1405 and 1406 carry [KIL-sd_1] and the *ski2-2* (recessive) mutation.

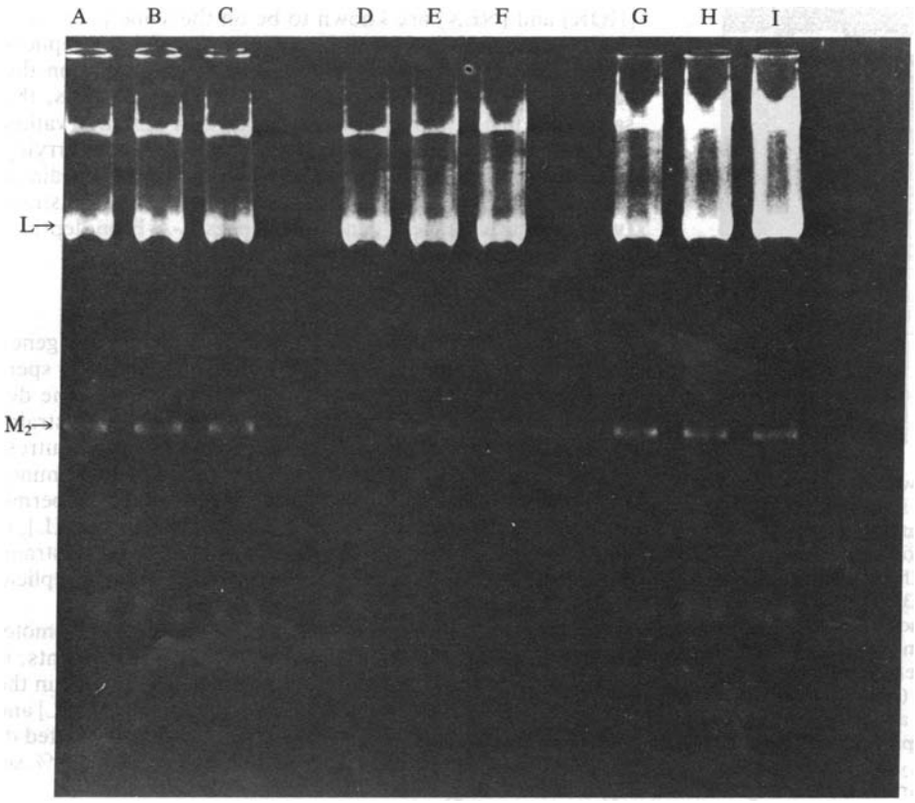

FIG. 1. Loss of M_2 ds RNA after growth of *spe2* and *spe10* strains in the absence of polyamines. Lanes: A, *α arg1 thr1 can*^{r} *spe2-4* [KIL-k_2] (strain 72-3C) before polyamine deprivation; B, *α arg1 thr1 can*^{r} *spe2-4* [KIL-k_2] (strain 72-5D) before polyamine deprivation; C, *α lys1 can*^{r} *spe10-3* [KIL-k_2] (strain Y330) before polyamine deprivation; D, E, and F, strains 72-3C, 72-5D, and Y330, respectively, after they have been depleted of their polyamine contents and then grown on YPAD medium; and G, H, and I, strains 72-3C, 72-5D, and Y330, respectively, after they have been grown on complete minimal medium in the presence of spermidine.

Table 4. Polyamines are required for replication of [EXL] (L-A-E ds RNA) but not for [HOK] (L-A-HN ds RNA)

Exp.	*spe*⁻ haploid		Polyamine starvation	Haploid used for mating	Diploid phenotype
A	M4	α *spe10-3* [EXL] [KIL-o]	Unstarved	1387 *a* [KIL-k_2] [NEX-o]	K^-
			Starved	1387 *a* [KIL-k_2] [NEX-o]	K^+
	177	α *spe2-4* [EXL] [KIL-o]	Unstarved	1387 *a* [KIL-k_2] [NEX-o]	K^-
			Starved	1387 *a* [KIL-k_2] [NEX-o]	K^-
B	72-3C	α *spe2-4 SKI* [KIL-k_2] [HOK] [NEX]	Unstarved	1405 *a ski2-2* [KIL-sd_1]	K_1^+
			Starved	1405 *a ski2-2* [KIL-sd_1]	K_1^+
	72-5D	α *spe2-4 SKI* [KIL-k_2] [HOK] [NEX]	Unstarved	1405 *a ski2-2* [KIL-sd_1]	K_1^+
			Starved	1405 *a ski2-2* [KIL-sd_1]	K_1^+
	Y330	*a spe10-3 SKI* [KIL-k_2] [HOK] [NEX]	Unstarved	1406 α *ski2-2* [KIL-sd_1]	K_1^+
			Starved	1406 α *ski2-2* [KIL-sd_1]	K_1^+
	Y331	α *spe10-3 SKI* [KIL-k_1] [HOK] [NEX]	Unstarved	Test cannot be done*	
			Starved	1405 *a ski2-2* [KIL-sd_1]	K_1^+

Depletion of polyamines and testing for killer phenotype were carried out as described. Only the *spe10-3* mutant and *spe2-4* mutants were depleted of polyamines. Testing of diploids for K_1 in experiment B was done by using a $K_2^+R_2^+$ lawn (strain S121), which is resistant to the K_2 toxin but sensitive to the K_1 toxin. In all cases 100 or more diploid single colonies were examined and all had the indicated phenotype.
*Because strain Y331 carries [KIL-k_1], it cannot be tested for [HOK] in the same way as the [KIL-k_2] strains were tested. But the starved colonies of Y331 were [KIL-o] (see Table 3) and so could be tested and had [HOK], showing that the unstarved Y331 cells also had [HOK].

The diploids formed were tested for [KIL-k_1] on a $K_2^+R_2^+$ lawn, which is resistant to [KIL-k_2] but is killed by [KIL-k_1]. Because the diploids formed were all K^+ and because [HOK] is required for the replication of [KIL-sd_1] in a *SKI*⁺ strain (2), these data indicate the presence of [HOK]. Similarly, the presence of [NEX] in these *spe2* and *spe10* strains was demonstrated by mating *spe2* strains with strain 1466 and the *spe10-3* strains with strain T1. (Both strains 1466 and T1 carry [EXL] but no [NEX] or [HOK].) When tested on a lawn sensitive to K_2, these diploids were all K^+, indicating that these *spe* strains carry [NEX].

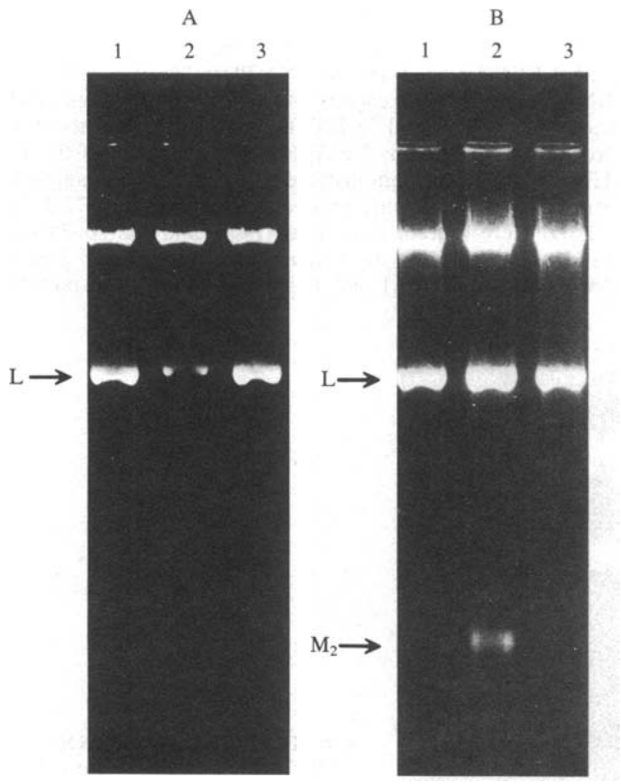

FIG. 2. Loss of [EXL] plasmid after growth of *spe10-3* strain in the absence of polyamines. (*A*) α *arg1 thr1 spe10-3* [EXL] [KIL-o] (strain M4). Lanes show electrophoretic patterns of ds RNA after growth on YPAD (lane 1), after depletion of polyamines and then growth on YPAD (lane 2), and after growth on complete minimal medium in the presence of putrescine (lane 3). The 50% decrease in L in lane 2 confirms the loss of L-A-E and shows that L-C is not lost. (*B*) α *arg1 thr1 spe10-3* [EXL] [KIL-o] (strain M4) × *a ura1* [KIL-k_2] [NEX-o] [HOK-o] (strain 1387). Diploids were generated before depleting the polyamine content of strain M4 (lane 1), after depleting the polyamine content of strain M4 (lane 2), and after growing strain M4 on complete minimal medium in the presence of putrescine. Electrophoretic patterns of ds RNA show M_2 ds RNA only in lane 2, indicating the absence of [EXL] by its failure to exclude M_2.

When these *spe* strains were depleted of their polyamine contents by successive cloning on polyamine-free complete minimal medium and then were mated with strain 1405 or 1406, the diploids still remained K^+. This result indicates that there was no loss of the plasmid that carries [HOK] (L-A-HN ds RNA) (Table 4, experiment B). This shows that replication of L-A-HN in yeast is not dependent upon polyamines. (The presence of [NEX] was not measured after polyamine deficiency, but its presence was assumed because [HOK] and [NEX] are known to be on the same plasmid.)

The testing of the polyamine requirement for the replication of L-A-HN (Table 4, experiment B) was done on the same polyamine-deprived cells used in Table 3. Thus, the same cells lost M_2 ds RNA but retained L-A-HN. Starvation of strain Y331 (isogenic to the *spe10* strain M4, but carrying [KIL-k_1] [HOK] [NEX] instead of [EXL]) resulted again in loss of M_1 ds RNA but not L-A-HN. Thus, the same strain (with regard to nuclear genes) that loses L-A-E on deprivation of polyamines retains L-A-HN.

DISCUSSION

Our results show that M_2 ds RNA, which carries the genes for the K_2 toxin and resistance, requires spermidine or spermine for maintenance and replication. After polyamine deprivation, M_2 ds RNA is lost both from the *spe2* strain, which lacks spermidine and spermine but contains putrescine (7), and the *spe10* strain, which lacks all three amines (8), indicating that putrescine does not substitute for spermidine or spermine. However, L-A-E, which carries [EXL], is lost from the *spe10* strain but is not lost from the *spe2* strain, indicating that putrescine alone is sufficient for the replication of L-A-E plasmid.

In contrast to the above results, L-A-HN, a ds RNA molecule that carries the [NEX] and [HOK] genetic elements, is not lost during polyamine deprivation. This difference in the effect of polyamine depletion on the replication of [EXL] and of [HOK] and [NEX] is surprising because the associated ds RNA molecules (i.e., L-A-E and L-A-HN) show >99% sequence homology (4).

Table 5. Polyamine requirements for maintenance or replication of ds RNA plasmids of *S. cerevisiae*

ds RNA	Plasmid	Polyamine requirement
M_1	[KIL-k_1]	Spermidine or spermine (not putrescine)
M_2	[KIL-k_2]	Spermidine or spermine (not putrescine)
L-A-E	[EXL]	Putrescine, spermidine, or spermine
L-A-HN	[HOK] [NEX]	None
L-C	—	None

The amine requirements for the maintenance or replication of the different ds RNAs are summarized in Table 5. We have shown (7, 8) that no spermidine or spermine is detectable in *spe2-4* strains, and no putrescine, spermidine, or spermine in *spe10-3* strains grown on a minimal medium; the analytical methods used indicated a reduction of putrescine to <1/400th, of spermidine to <1/2,000th, and of spermine to <1/400th of the concentrations found in wild-type strains. Although it is conceivable that some undetectable amount of polyamines is required for the maintenance of L-A-HN, the amounts needed would be small compared to the requirements for L-A-E ds RNA, M_1 ds RNA, or M_2 ds RNA.

SPE10 and *SPE2* are two of the 29 chromosomal (*MAK*) genes that are required for the maintenance or replication of the killer plasmid (2). In only one other case is the gene product known—namely, *MAK8*, which codes for ribosomal protein L3 (14).‡ The biochemical defects in *spe2* and *spe10* mutants are well-defined, even though we do not know the specific steps in which the polyamines exert their effect.

These data indicate that polyamines are important in the replication of [KIL-k_1], [KIL-k_2], and [EXL] for specific (but still undefined) steps, which are probably not involved in the replication or maintenance of [HOK] and [NEX]. Similarly, the finding that putrescine satisfies the amine requirement for [EXL] but not for [KIL-k_1] and [KIL-k_2] demonstrates a specificity of polyamine requirements for the replication of these ds RNA plasmids.

‡*MAK1* has been identified recently as the gene coding for DNA topoisomerase I activity (15).

1. Cohn, M. S., Tabor, C. W., Tabor, H. & Wickner, R. B. (1978) *J. Biol. Chem.* **253,** 5225–5227.
2. Wickner, R. B. (1981) in *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 415–444.
3. Sommer, S. S. & Wickner, R. B. (1982) *J. Bacteriol.* **150,** 545–551.
4. Sommer, S. S. & Wickner, R. B. (1982) *Cell* **31,** 429–441.
5. Wickner, R. B. (1980) *Cell* **21,** 217–226.
6. Wickner, R. B. & Toh-e, A. (1982) *Genetics* **100,** 159–174.
7. Cohn, M. S., Tabor, C. W. & Tabor, H. (1978) *J. Bacteriol.* **134,** 208–213.
8. Cohn, M. S., Tabor, C. W. & Tabor, H. (1980) *J. Bacteriol.* **142,** 791–799.
9. Fried, H. M. & Fink, G. R. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4224–4228.
10. Toh-e, A., Guerry, P. & Wickner, R. B. (1978) *J. Bacteriol.* **136,** 1002–1007.
11. Palfree, R. & Bussey, H. (1979) *Eur. J. Biochem.* **93,** 487–493.
12. Young, T. W. & Yagiu, M. (1978) *J. Microbiol. Serol.* **44,** 59–77.
13. Mortimer, R. K. & Hawthorne, D. C. (1975) in *Methods in Cell Biology*, ed. Prescott, D. M. (Academic, New York), Vol. 11, pp. 221–233.
14. Wickner, R. B., Ridley, S. P., Fried, H. M. & Ball, S. G. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4706–4708.
15. Thrash, C., Voelkel, K., DiNardo, S. & Sternglanz, R. (1984) *J. Biol. Chem.* **259,** 1375–1379.