MOLECULAR AND CELLULAR BIOLOGY, Apr. 1984, p. 761–770
0270-7306/84/040761-10$02.00/0
Copyright © 1984, American Society for Microbiology
Vol. 4, No. 4

Superkiller Mutations in *Saccharomyces cerevisiae* Suppress Exclusion of M_2 Double-Stranded RNA by L-A-HN and Confer Cold Sensitivity in the Presence of M and L-A-HN

SUSAN PORTER RIDLEY,* STEVE S. SOMMER, AND REED B. WICKNER

Section on Genetics of Simple Eukaryotes, Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, Maryland 20205

Received 10 November 1983/Accepted 24 January 1984

In an *mktl* host, L-A-HN double-stranded RNA excludes M_2 double-stranded RNA at 30°C but not at 20°C. Recessive mutations suppressing the exclusion of M_2 by L-A-HN in an *mktl* host include six *ski* (superkiller) genes, three of which (*ski6*, *ski7* and *ski8*) are new genes. The dominant mutations in one gene (*MKS50*) and recessive mutations in at least two genes (*mksl* and *mks2*) suppress M_2 exclusion by L-A-HN but do not show other characteristics of *ski* mutations and thus define a new class of killer-related chromosomal genes. Mutations in *ski2*, *ski3*, *ski4*, *ski6*, *ski7*, and *ski8* result in increased M copy number at 30°C and prevent the cells from growing at 8°C. Elimination of M double-stranded RNA from a cold-sensitive *ski*$^-$ strain results in the loss of cold sensitivity. *ski*$^-$ [KIL-sd$_1$] strains lack L-A-HN, carry L-A-E, and have a lower M_1 copy number than do *ski*$^-$ [KIL-k$_1$] strains and are only slightly cold sensitive. The *LTS5* (=*MAK6*) product is required both for low temperature growth and for M_1 maintenance or replication. We propose that the elevated levels of M in *ski*$^-$ strains divert the host *LTS5* product away from the host and to the M replication process. We also suggest that the essential role of L-A in M replication is protection of M double-stranded RNA from the negative influence of *SKI*$^+$ products.

The killer system of *Saccharomyces cerevisiae* is unique among eucaryotic virus and plasmid systems in the detail in which interactions of viral and host components have been explored and in the large number of host components shown to be involved. This probably reflects the ease with which these aspects can be investigated in *S. cerevisiae* rather than any difference in the degree to which viral and host functions are intertwined. Numerous examples are known of host restriction of viral growth in mammalian systems, but analysis of the host genes involved is generally not practical. We show here that overproduction of a *S. cerevisiae* virus, due to a host mutation, results in host pathology, apparently due to over-utilization by the virus of a specific, essential host component.

Killer strains of *S. cerevisiae* carry M_1, M_2, or M_3 double-stranded RNA (dsRNA) specifying the secreted protein toxin (K_1^+, K_2^+, or K_3^+) and immunity to it (R_1^+, R_2^+, or R_3^+) (1, 2, 22; reviewed in references 5, 26, 28). All killer strains, and most other strains, also carry a class of larger dsRNA molecules called L (4.5 kilobase [kb] pairs) which, in most strains, consists of two or more species which do not cross-hybridize (16, 17, 27, 31). One of these, L-A, encodes the major coat protein of the virus-like particles in which both M and L-A dsRNAs are found (9, 11, 17). L-A comes in at least four highly homologous natural variants, L-A-HN, L-A-E, L-A-HE, and L-A-H. They are differentiated by which of the three genes, [HOK], [NEX], and [EXL], they carry (Table 1) (17, 25, 31). L-A-H (the L_2 of Brennan et al. [4]) carries only [HOK] (R. B. Wickner, unpublished data). Wild-type K_1 killer strains all carry L-A-HN (17, 27), whereas wild-type K_2 killers carry either L-A-HN or L-A-H (4, 17, 25; Wickner, unpublished data).

Other L dsRNA species include L-B and L-C which are related to each other in sequence and have different coat proteins than does L-A (16, 17, 31). In addition to L and M dsRNAs, cells may have dsRNAs of intermediate size, T and W dsRNAs (2.7 and 2.25 kb, respectively). T and W, which do not cross-hybridize with each other, other dsRNAs, or cellular DNAs, show cytoplasmic inheritance and heat inducibility (22).

At least 29 chromosomal genes are needed to maintain or replicate M_1 dsRNA and, in the cases tested, M_2 dsRNA (reviewed in references 26 and 28). Among these genes is *LTS5*, whose product is necessary for both cell growth at 8°C (15) and M_1 replication at 30°C (30). *lts5* was mapped near the centromere of chromosome XVI (30), and we show here that it is the same as *mak6*. Mutations in 11 other *MAK* genes (*mak3*, *4*, *7*, *8*, *10*, *11*, *12*, *16*, *18*, *19*, and *27*) do not cause cold sensitivity for growth (R. B. Wickner, unpublished data), and among the low temperature-sensitive mutants of Singh and Manney, only *lts5* (*mak6*) shows the *mak*$^-$ phenotype (30).

The *ski* mutants were first discovered based on their increased production of the K_1 toxin (the superkiller phenotype) (18). The *ski* mutations were then found to suppress many of the *mak* mutations, such that *ski mak* double mutants were able to replicate and maintain M_1 dsRNA (20). In *ski*$^-$ killer strains, mitotic segregants in which the killer plasmid had become dependent upon the *ski* mutation were frequently isolated (19). This phenotype was assumed at that time to reflect a spontaneous mutation in the M_1 dsRNA, the mutant form being denoted [KIL-sd$_1$] (sd for *ski* dependent). Subsequent work revealed the existence of a cytoplasmic gene (called [HOK] and located on L-A dsRNA) which enables [KIL-sd$_1$] to replicate in a *SKI*$^+$ host (16, 17, 31).

Since A364A, the parent of the *ski* mutants, carries L-A-HN (a form of L-A with [HOK] and [NEX] activities) (27), *ski* dependence of the killer plasmid could not have been detected unless this L-A-HN was lost or unexpressed. As we will describe in this paper, the loss of L-A-HN does, in fact, accompany the generation of [KIL-sd$_1$], and these two events may be identical.

* Corresponding author.

TABLE 1. Components of the killer systems

Component	Description
M_1	dsRNA (1.8 kb) that codes for polypeptide toxin (3) and a resistance function. Strains carrying M_1 have the genotype [KIL-k_1]. Secretion of an active K_1 toxin is the K_1^+ phenotype. Resistance to the K_1 toxin is the R_1^+ phenotype.
M_2	dsRNA (1.5 kb) that codes for a second toxin which kills M-o- and M_1-containing cells. M_2, [KIL-k_2], K_2^+, and R_2^+ are analogous to M_1, [KIL-k_1], K_1^+, and R_1^+.
[KIL-sd_1]	*ski*-dependent [KIL-k_1]: a derivative of the K_1 killer whose M_1 is lost from *SKI* (wild-type) strains lacking [HOK] but stably maintained in *ski*$^-$ strains. Although originally this *ski*-dependent phenotype was thought to reflect a mutation in M_1 (hence its designation as [KIL-sd_1]), we present evidence here that the phenotype is actually due to the presence of a wild-type M_1 together with L-A-E rather than the L-A-HN present in wild-type [KIL-k_1] strains.
[HOK]	Helper of [KIL-sd_1]. This nonMendelian trait allows [KIL-sd_1] to replicate in a *SKI*$^+$ host. It is located on certain forms of L-A dsRNA, namely, L-A-HN and L-A-HE. Based on evidence presented here, [HOK] is the function of L-A (presumably a characteristic of the coat protein gene) that enables M_1 dsRNA to be maintained in wild-type strains.
[EXL]	Excluder of M_2 dsRNA. This nonMendelian trait prevents the replication of [KIL-k_2] if [NEX] is absent but not if [NEX] is present. [EXL] is located on certain forms of L-A, namely, L-A-E and L-A-HE.
[NEX]	M_2 nonexcludable by [EXL]. This nonMendelian trait prevents the action of [EXL] but does not prevent exclusion of M_2 by strains carrying M_1. [NEX] has a second phenotype which is described under *MKT*. [NEX] is located on L-A-HN, the form of L-A found in wild-type K_1 killer strains.
MAK	Maintenance of [KIL-k_1]. *MAK* genes comprise at least 29 chromosomal genes necessary to maintain M_1 dsRNA. Mutants carrying the recessive alleles, *mak*, are K^- R^- M-o. At least some of the genes are also required for the maintenance of M_2.
SKI	Superkiller. Mutants carrying the recessive allele produce more killer toxin. *ski* mutations eliminate the need for some of the *MAK* genes.
MKT	Maintenance of [KIL-k_2] in the presence of [NEX]. Strains having the recessive allele, *mkt* (ca. 80% of laboratory strains), cannot maintain [KIL-k_2] at 30°C if [NEX] (L-A-HN) is present.
L-A	dsRNA (4.5 kb) that is noninfectious and stably maintained like a plasmid but encapsidated like a virus. It encodes its major capsid protein (81 kilodaltons). [EXL], [NEX], and [HOK] are present in various combinations on various natural variants of L-A, denoted L-A-E ([EXL] alone), L-A-HN ([HOK] and [NEX]), L-A-HE ([HOK] and [EXL]), or L-A-H ([HOK] alone; found in certain K_2 strains). L-A depends on *MAK3*, *MAK10*, and *PET18* for replication.
L-B and L-C	dsRNAs (4.5 kb) 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. L-(BC) refers to a L dsRNA like L-B or L-C but not yet characterized.
T and W	Minor dsRNAs (2.7 and 2.25 kb, respectively). They do not cross-hybridize with each other, other dsRNAs, or cell DNA. They are cytoplasmically inherited. Heat inducibility of T and W is under control of a nonMendelian gene.

Study of the K_2 killer system showed that M_2 dsRNA, unlike M_1 dsRNA, is excluded at 30°C by a cytoplasmic gene called [NEX] if the host is *mkt1* (25). [NEX] is located on L-A (16, 17). In all cases tested, all forms of L-A with [NEX] activity also have [HOK] activity (16, 17).

The work reported here explores the relationships among the different L-A dsRNAs, the *ski* mutations, M dsRNAs, and host cell growth. Based on our results, we propose explanations for the generation and properties of [KIL-sd_1] mutants and the cold sensitivity of *ski*$^-$ [KIL-k] strains.

MATERIALS AND METHODS

Strains and media. Many of the strains of *S. cerevisiae* used are listed in Table 2. Media have been described previously (24).

Assays of killing and resistance. Colonies to be tested for killing ability were replica plated onto MB medium which had just been seeded with a lawn of the appropriate sensitive strain (0.5 ml of a suspension with an optical density at 650 nm [OD_{650}] of 1 per ml spread on each plate and allowed to dry). To test K_2, a lawn of the K_1^+ R_1^+ K_2^- R_2^- strain S37 was used. To test K_1, a lawn of a K_2^+ R_2^+ K_1^- R_1^- strain or, if no ambiguity was possible, the K^- R^- strain 5 X 47 was used. Resistance to killing was checked by streaking a dilute suspension of the strain to be tested on MB medium and cross-streaking with a K_1^+ strain or a K_2^+ strain. MB plates were incubated at 20°C for 2 to 3 days. In each case, killing was indicated by a clear zone surrounding the killing strain and surrounded in turn by growth of the lawn or streak of sensitive cells.

Genetic analysis. Matings were carried out on unbuffered YPAD which, because its pH is ca. 6, does not permit the toxin to function (13, 32). Diploids were isolated by utilizing the complementary nutritional requirements of their parents.

Sporulation and dissection were by the usual methods (see Mortimer and Hawthorne [12] for references).

Cytoduction. A cytoplasmic genome can be transferred from one haploid strain to another without diploidization or other change of nuclear genotype by transient heterocaryon formation (cytoplasmic mixing, cytoduction) by using the *kar1* mutant, defective in nuclear fusion (7). Usually recipient cells were ρ^0 (mitochondrial DNA eliminated by growth on ethidium bromide), and donor cells were ρ^+. Recipient and donor cells mate on YPAD plates for 6 h, cytoplasmic mixing occurs, and the parental nuclei, failing to fuse, separate at the next cell division. Cells with donor nuclei were counterselected by plating the mating mixture for single colonies on appropriate media. Matings were not allowed to proceed too long, as the *kar1* mutation is leaky, and eventually only diploids (and no cytoductants) are found. Diploids were identified by replica plating to minimal medium and ρ^+ colonies were detected by replica plating to YPG plates. ρ^+ clones having the recipient nuclear genotypes were the cytoductants. These were then further tested for their acquisition or loss of K_1, K_2, R_1, R_2, cold sensitivity, etc.

dsRNA. dsRNA was purified by cellulose chromatography and analyzed on agarose gels as previously described (18) or by the rapid method described by Fried and Fink (8). Briefly, spheroplasts produced by zymolyase were lysed by sodium dodecyl sulfate, and the extract was digested with proteinase K, extracted with phenol, and precipitated with ethanol; dsRNA was purified by cellulose chromatography and analyzed on 1% agarose gels. In the rapid method, cells treated with 2-mercaptoethanol were extracted with a sodium dodecyl sulfate-phenol mixture, and the extract was precipitated with ethanol and analyzed on 1% agarose gels.

Cold sensitivity for growth. Cold sensitivity for growth was tested on YPAD (rich) plates at 8°C. Usually, the clearest results were obtained by making cell suspensions in water and applying a small volume of the suspension on YPAD. However, colonies or patches could also be replica plated onto YPAD.

Generation of a strain isogenic to S331 but with opposite mating type. A *ura*$^+$ revertant of S331 was selected on complete medium lacking uracil. A *lys2* mutant of that strain was selected by its ability to use aminoadipiate as a nitrogen source (6). Diploids obtained by force mating this *lys2 URA*$^+$ derivative with the original S331 were sporulated, and S442 was isolated as a meiotic segregant of the one in seven sporulating colonies that gave tetrads with good spore survival.

Isolation of mutants by the haploid screening procedure. S331 (*mkt1* [NEX] [KIL-k$_2$]) was suspended at 10^7 cells per ml in 0.1 M potassium phosphate, pH 7.5, with 5% ethyl methane sulfonate and incubated at 25°C for 1 h. Nine volumes of 5% sodium thiosulfate was added, and cells were pelleted 15 min later. Cells were resuspended, diluted, grown to single colonies at 30°C on YPAD plates, and tested for killing activity. A total of 8% of the cells survived, and 0.4% of the single colonies tested were killers; none of the 2,000 single colonies were killers in the unmutagenized control. Killer colonies were repurified and mated with strains 1480 and S140. Of 119 mutants isolated, 90 gave nonkiller diploids at 30°C with both 1480 and S140. Of the possible mutations (see below), only a recessive suppressor of *mkt1* would give nonkiller diploids with both strains.

Diploid selection procedure. Mutagenesis of strain 1478 (α *his4 mkt1* [NEX] [KIL-o]) was carried out as described above. Samples of the suspension of mutagenized cells were grown for 2 days on YPAD plates. Cells from each sample were mated en masse at 30°C with an excess of strain 1426 (α *ade5 cyh2 can1 mkt1* [KIL-k$_2$] [NEX-o]). The mating mixtures were grown on 4.7 MB plates at 30°C to kill the K^- diploids formed. The mixtures were then plated on minimal medium to select diploid clones. K_2^+ diploid clones were presumed mutants and were analyzed as described below.

RESULTS

***ski2* and *ski3* mutations suppress [NEX] exclusion of M_2 in an *mkt1*$^-$ host.** When a *ski2-2 mkt1* strain (1383) was crossed with a *SKI*$^+$ *MKT*$^+$ [NEX] [KIL-k$_2$] strain (1475), a mixture of 4 K_2^+:0, 3 K_2^+:1 K^-, and 2 K_2^+:2 K^- tetrads resulted, indicating suppression of the *mkt1*-[NEX] exclusion of M_2. All 10 *ski2 mkt1* segregants were K_2^+, and only the 13 *SKI*$^+$ *mkt1* segregants were nonkillers. This indicates that the *ski2* mutation was suppressing the *mkt1* mutation. As has been previously shown for M_1 dsRNA (18), M_2 dsRNA copy number increased in *ski2* segregants compared with that in *SKI*$^+$ segregants (Fig. 1A). The bypass of *mkt*-[NEX] exclusion by *ski2-2* is not due to loss of [NEX] from the *ski2* segregants. This was shown by mating the segregants with *mkt1 SKI*$^+$ [NEX-o] [KIL-k$_2$] strains. The *mkt1/mkt1* diploids formed were nonkillers, showing that [NEX] was present. A similar analysis demonstrated that the *ski3-1* mutation also suppresses the *mkt*-[NEX] exclusion of M_2.

Isolation of suppressors of *mkt1*-[NEX] exclusion of M_2 dsRNA. At 30°C, exclusion of M_2 dsRNA by [NEX] in an *mkt1* host is rapid and efficient, but at 20°C, *mkt1* [NEX] strains stably maintain M_2 (25). Such an *mkt1* [NEX] [KIL-k$_2$] strain was grown at 20°C, mutagenized with ethyl methanesulfonate, and plated for single colonies on YPAD medium at 30°C, and the colonies formed were tested for killing activity (haploid screening procedure). As a second procedure, an *mkt1* [NEX] [KIL-o] strain was mutagenized, the mutagenized cells were mated en masse with an *mkt1* [NEX-o] [KIL-k$_2$] strain, and the rate K_2^+ R_2^+ diploids were selected by their resistance to the K_2 toxin (diploid selection procedure). The K_2^+ meiotic segregants from these diploids were then analyzed. The phenotypes of the mutants isolated by either procedure could have been due to (i) loss of [NEX] (which would show cytoplasmic inheritance), (ii) reversion of *mkt1* (which would be dominant), (iii) mutation of M_2 to an unexcludable form, or (iv) a second-site mutation suppressing the exclusion. The mutants described here were in the fourth group, as shown by genetic analysis below. Each mutant had the genotype *mkt1* [NEX] [KIL-k$_2$] plus the suppressor mutation and was crossed with an *mkt1* [NEX] [KIL-o] strain to examine dominance and segregation of the suppressor mutations.

Dominant suppressors. The diploid selection procedure produced dominant suppressors of the *mkt1*-[NEX] exclusion of M_2, three of which were characterized. Each segregated 2+:2− in crosses with *mkt1* [NEX] [KIL-o] strains. The appearance of K^- segregants in matings with *MKT*$^+$ [KIL-k$_2$] [NEX] strains showed that in none of these mutants had *mkt1* reverted to *MKT*$^+$. Their failure to segregate relative to each other in a total of 31 tetrads indicates that all three mutants were alleles of a single gene or closely linked genes. The mutations did not suppress known amber or ochre mutations, and allelism tests showed that they were unlinked to *ski2*, *ski3*, or *ski4*. This gene is called *MKS50* (*mkt* suppressor). None of the meiotic segregants from a mating of an *MKS50* strain with a K_1 killer were superkillers at 30°C, and unlike *ski* mutations, the presence of *MKS50* in

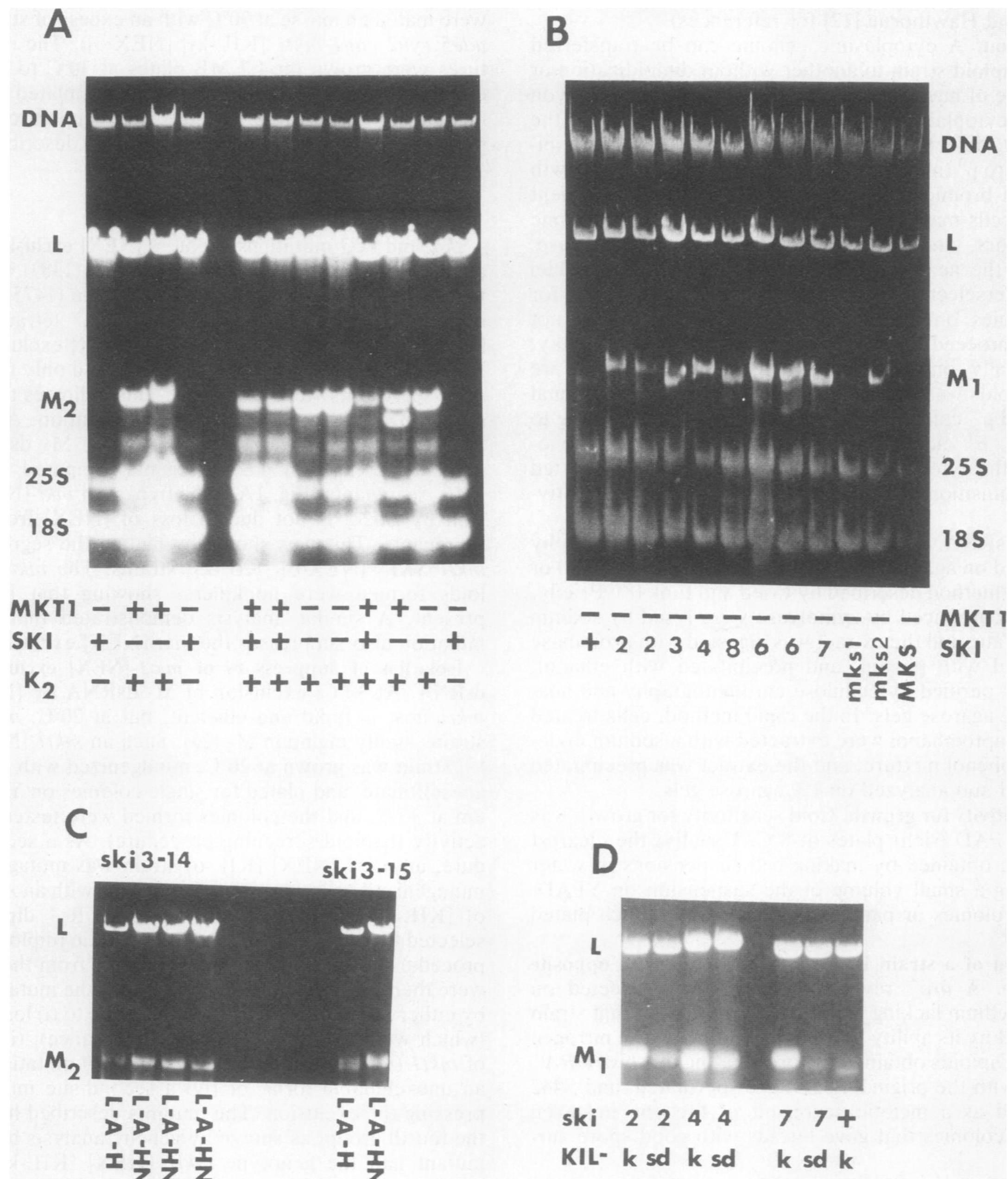


FIG. 1. Control of M copy number by *SKI*, *MKT1*, and L-A. (A) Levels of M_2 dsRNA in meiotic segregants of the cross 1383 (*ski2-2 mkt1*) × 1475 (*SKI*$^+$ *MKT*$^+$ [NEX] [KIL-k_2]). Three complete tetrads are shown. Nucleic acids extracted according to Fried and Fink (8) were electrophoresed on a 1% agarose gel. (B) Comparison of the amounts of M_1 dsRNA in *ski*, *mks1*, and *MKS50* mutants with their wild-type parents. M_1 was introduced by cytoduction, and nucleic acids were analyzed as described in (A). (C) *ski3-14* and *ski3-15* mutants carrying M_2 and L-A-H are only slightly cold sensitive and have about threefold lower L and M_2 dsRNA than do the same strains into which L-A-HN has been introduced by cytoduction. These cytoductants are fully cold sensitive. (D) *ski*$^-$ [KIL-k_1] strains have more M_1 dsRNA than do isogenic *ski*$^-$ [KIL-sd_1] strains, Isogenic pairs of *ski*$^-$ strains carrying [KIL-k_1] or [KIL-sd_1] were constructed by cytoduction. Nucleic acids were analyzed as described in (A). The multiple M bands and variable size of single M bands are due to the high rate of size variation of M dsRNA (S. S. Sommer and R. B. Wickner, submitted for publication).

a strain lacking [HOK] does not enable the strain to maintain [KIL-sd_1].

Recessive suppressors. The haploid isolation method produced both dominant and recessive suppressor mutations. The recessive suppressors fell into at least eight complementation groups, three of which were the same as *ski2*, *ski3*, and *ski4* (Table 3). Representatives of each complementation group showed 2+:2− segregation in crosses with an isogenic *mkt1* [NEX] [KIL-o] strain. Three of the new complementation groups had the properties of *ski* mutations, namely, the superkiller phenotype with M_1 dsRNA and an increase in copy number of M dsRNA (Table 3 and Fig. 1B). These genes are thus denoted *ski6*, *ski7*, and *ski8*. Mutants defective in the other two new genes, denoted *mks1* and *mks2*, are not superkillers with M_1 dsRNA. That *mks1-1* and *mks2-1* mutants define distinct genes was confirmed by showing that

TABLE 2. *Saccharomyces cerevisiae* strains

Designation	Nuclear genotype	Cytoplasmic genotype	dsRNAs	Reference
1478	α *his4 mkt1*	[KIL-o] [HOK] [NEX]	L-(BC), L-A-HN	25
1426	**a** *ade5 cyh2 can1 mkt1*	[KIL-k_2] [NEX-o]	L-(BC), L-A-H, M_2	25
SP322	α *leu1 mkt1 MKS50-3*	[KIL-k_2] [NEX]	L-(BC), L-A-HN, M_2	This work
S331	α *mkt1 ura1 met13 can1 cyh2*	[HOK] [NEX] [KIL-k_2]	L-(BC), L-A-HN, M_2	This work
S637	α *mkt1 ura1 met13 can1 cyh2 ski2-11*	[HOK] [NEX] [KIL-k_2]	L-(BC), L-A-HN, M_2	This work
S368	α *mkt1 ura1 met13 can1 cyh2 ski3-11*	[HOK] [NEX] [KIL-k_2]	L-(BC), L-A-HN, M_2	This work
S521	α *mkt1 ura1 met13 can1 cyh2 ski4-11*	[HOK] [NEX] [KIL-k_2]	L-(BC), L-A-HN, M_2	This work
S640	α *mkt1 ura1 met13 can1 cyh2 ski6-1*[a]	[HOK] [NEX] [KIL-k_2]	L-(BC), L-A-HN, M_2	This work
S641	α *mkt1 ura1 met13 can1 cyh2 ski7-1*	[HOK] [NEX] [KIL-k_2]	L-(BC), L-A-HN, M_2	This work
S369	α *mkt1 ura1 met13 can1 cyh2 ski8-1*	[HOK] [NEX] [KIL-k_2]	L-(BC), L-A-HN, M_2	This work
S642	α *mkt1 ura1 met13 can1 cyh2 mks1-1*	[HOK] [NEX] [KIL-k_2]	L-(BC), L-A-HN, M_2	This work
S442	**a** *lys2 can1 cyh2 mkt1*	[HOK] [NEX]	L-(BC), L-A-HN	This work
1480	**a** *mkt1-1 lys1*	[HOK] [NEX]	L-B, L-A-HN	17
S140	**a** *mkt1-1 lys1*	[HOK-o] [NEX-o]	L-B	17
SP278	α *thr1 mkt1 ski2-2*		L-(BC)	This work
SP275	α *thr1 mkt1 ski2-2*	[EXL]	L-(BC), L-A-E	This work
SP79	α *thr1 mkt1 ski2-2*	[KIL-k_1] (from A364A) [HOK] [NEX]	L-(BC), L-A-HN, M_1	This work
SP81	α *thr1 mkt1 ski2-2*	[KIL-sd_1] [EXL]	L-(BC), L-A-E, M_1	This work
SP423	α *thr1 mkt1 ski2-2*	[HOK] [NEX] (from A364A)	L-(BC), L-A-HN	This work
SP426	α *thr1 mkt1 ski2-2*	[KIL-sd_1] [HOK] [NEX] (from A364A)	L-(BC), L-A-HN, M_1, ? L-A-E	This work
SP242	**a** *leu1 lys10 kar1-1 ski2-2 mkt1*	[EXL]	L-(BC), L-A-E	This work
SP245	**a** *leu1 lys10 kar1-1 ski2-2 mkt1*		L-(BC)	This work
1383	**a** *leu1 lys10 kar1-1 ski2-2 mkt1*		L-(BC)	19
1475	α *ade5 MKT*$^+$	[KIL-k_2] [HOK] [NEX]	L-(BC), L-A-HN, M_2	25
1090	α *his4 kar1-1 mkt1*		L-(BC)	7
2176	α *his4 kar1-1 mkt1*	[HOK] [NEX]	L-(BC), L-A-HN	This work
SP299	α *his4 kar1-1 mkt1*	[KIL-k_1] [HOK] [NEX]	L-(BC), L-A-HN, M_1	This work
SP226	**a** *ade1 ski2-2 mkt1*		L-(BC)	This work

[a] The new *ski* genes described here begin with *ski6* because Bussey et al. (H. Bussey, O. Steinmetz, and D. Saville, Curr. Genet., in press) have described *ski5*, a gene controlling a cell surface protease that degrades the killer toxin.

they segregate randomly relative to each other in meiosis (parental ditype = 4, nonparental ditype = 8, tetratype = 26).

***ski$^-$* [KIL-k] strains are cold sensitive for cell growth.** Examination of the mutants obtained by the haploid selection method revealed that at least 60 of 62 independent mutants in *ski2*, *ski3*, *ski4*, *ski6*, *ski7*, and *ski8* were unable to grow on YPAD plates at 8°C. This cold sensitivity (CS) cosegregated in meiosis with the suppression of [NEX]-*mkt* exclusion of M_2 (Table 3). These results indicate that the cold sensitivity was due, in each case, to the *ski* mutation. Cold sensitivity was not seen in the case of *mks1* or *mks2* mutants or the dominant *MKS50* mutants from the diploid selection procedure.

A *ski2-2*/+ heterozygous diploid carrying [KIL-k_1] (L-A-HN + M_1 dsRNA) instead of [KIL-k_2] also showed 2 CS *ski*$^-$:2 cold resistance (CR) *SKI*$^+$ cosegregation of cold sensitivity for growth and *ski2-2* (SP226 × SP299; 10 of 10 complete tetrads). One isogenic diploid carrying only L-A-HN and another carrying neither L-A nor M both showed only 4 CR:0 segregation (SP226 × 2176 and SP226 × 1090; 11 tetrads analyed from each diploid), even though *ski2-2* was segregating 2:2 in these tetrads. Likewise, diploids heterozygous for *ski3-1* or *ski8-2* and each carrying only L-A-HN showed only 4 CR:0 segregation (seven and four tetrads, respectively). The results suggest that M was needed for the expression of this cold sensitivity and that neither L-A-HN nor the L-B or L-C present in all of these diploids could alone cause the cold sensitivity.

That a cytoplasmic element was involved in the cold sensitivity of *ski* mutants was also indicated by finding that crossing *ski3-15 mkt1* [KIL-o] [NEX-o] CR and *ski3-14 mkt1* [KIL-k] [NEX] CS strains produced only 4 CS:0 segregation (12 tetrads). Likewise, introduction of cytoplasm by cytoduction from various killer strains into a *ski2-2* [KIL-o] [NEX-o] CR strain produced only CS cytoductants (Table 4).

To critically test what cytoplasmic elements were involved in the cold sensitivity, two types of experiments were carried out. In one, heat curing at 37°C was used to eliminate M_1 (23) and L-A-HN (16) from a *ski2-2* [KIL-k_1] strain. The K^- single colonies were tested for [HOK] by mating with a *ski4-1* [KIL-sd_1] strain. The diploids formed are *SKI*$^+$ and, if [HOK] is present, K_1^+. If [HOK] is absent, the diploids are K^-. It was found that of 127 purified colonies examined, 21 were K_1^+ and still CS (like the parent), 75 were K^- [HOK-o] and CR, and 31 were K^- [HOK] and CR. No K^+ CR or K^- CS colonies were observed. These results indicate that the M_1 dsRNA in these strains were necessary for cold sensitivity. Although the copy number of L-A-HN in the K^- [HOK] colonies is ca. 100-fold more than that in the K^+ colonies (S. G. Ball, C. Tirtiaux, and R. B. Wickner, submitted for publication), these colonies that have lost M_1 and retained L-A-HN are not CS.

The second approach to identifying the cytoplasmic element conferring the cold sensitivity to *ski* mutants was to select CR revertants from a *ski2-2* [KIL-k_1] strain (SP79). The results of analysis of 46 CR revertants showed that all were either nonkillers (18 of 46) or unstable killers (28 or 46). The pure nonkiller clones and the nonkiller subclones of the unstable killers were all CR. The stable killers subcloned from the unstable killers were CS. These results again indicate that M_1 dsRNA is necessary for the cold sensitivity phenotype of *ski2*$^-$ strains.

One exceptional subclone was an unstable killer and largely CR and will be discussed in detail below.

TABLE 3. Properties of mutations suppressing *mktl*-[NEX] exclusion of M_2

Mutation	$2^+:2^-$ segregation of suppression[a]	Growth at 8°C[b] dsRNA content: L-A-HN, M_1	L-A-HN, M_2	L-A-HN	–	K_1 superkilling[c]	Maintenance of [KIL-sd_1]	Increased M_2 dsRNA at 20°C	Increased M_1 dsRNA at 30°C	No. of alleles[d]
None		+	+	+	+	–	–	–	–	
ski2-2		–		+	+	++	+	+	+	25
ski2-3	11/11									
ski2-11	8/8	–	–	+	+	++	+	+	+	
ski2-12		–	–	+	+				+	
ski3-11	26/26	–	–	+	+	++	+	+	+	22
ski3-12	5/5		–							
ski3-13	4/4		–		+					
ski4-11	6/6	W[e]		+	+	+			+	2
ski6-1	5/5	–	–			+			+	6
ski6-2	6/6	–	–			++			+	
ski6-3			–			+				
ski7-1	7/7	–	–	+	+	++	+	+	+	2
ski8-1	5/6	–	–		+	+	+		+	8
ski8-2	5/5	–	–	+	+			+		
mks1-1	6/6		+			–			–	1
mks2-1	5/5		+			–			+/–	1
MKS50-1	6/6						–		–	3
MKS50-2	11/11		+			–				
MKS50-3	5/6		+			–				
Uncharacterized recessive										23
Uncharacterized dominant										29

[a] Mutant strains isolated by the haploid procedure were crossed with isogenic strain S442. *MKS50* mutants were isolated by the diploid procedure, which involved mating the mutants with strain 1426.
[b] Growth at 8°C was tested on YPAD medium. Cold sensitivity with M_2 was shown to cosegregate with suppression of exclusion. Strains carrying L-A-HN and M_1, L-A-HN alone, or neither L-A nor M were variously constructed by curing with heat or cycloheximide, by cytoduction, or by meiotic crosses. All strains shown carry L-(BC).
[c] The M_1 from strain A364A was introduced by either cytoduction or meiotic crosses.
[d] This indicates the number of alleles of each gene among 119 mutants isolated by the haploid method and 3 isolated by the diploid method.
[e] W, Weak growth.

To determine why M_1 was necessary for the cold sensitivity, M_1 dsRNAs from various wild-type strains and mutant derivatives were introduced by cytoduction into *ski2* and *ski3* strains initially lacking L-A and M dsRNAs (Table 3). All wild-type M_1 dsRNAs conferred cold sensitivity as did a neutral (K_1^- R_1^+) mutant and several suppressive mutants. The suppressive mutants are deletion mutants of M_1 dsRNA lacking large parts of the coding region of the molecule and conferring neither ability to produce the toxin nor resistance to the toxin (8). These results indicate that the cold sensitivity is not related to toxin production or to the resistance function and suggest that carrying an M_1-related replicon in a *ski*$^-$ strain confers cold sensitivity.

Nevertheless, that M alone is not sufficient to confer cold sensitivity was indicated by the finding that *ski3-14* or *ski3-15* M_2 L-A-H strains ([NEX-o]) were not fully cold sensitive but that introduction of L-A-HN by cytoduction made these strains become fully cold sensitive. This was accompanied by an increase in both total L and in M_2 copy number of about threefold (Fig. 1C). This point will be further treated below.

***ski*$^-$ [KIL-sd_1] strains have L-A-E + M_1 dsRNAs.** All wild-type K_1 killers examined ([KIL-k_1] strains) carry L-A-HN and M_1 dsRNAs and do not show evidence of [EXL] (27). In *ski*$^-$ [KIL-sd_1] strains, the maintenance of M_1 dsRNA is dependent on the *ski* mutation (19). When, through mating or cytoduction, the host environment is changed to *SKI*$^+$, M_1 is lost. [HOK] counteracts this effect, enabling M_1 from a [KIL-sd_1] strain to persist even in a *SKI*$^+$ host (31). Since the *ski*$^-$ [KIL-sd_1] strains all arose from A364A, which carries [HOK], these *ski*$^-$ [KIL-sd_1] strains must have lost [HOK]. However, we find that *ski*$^-$ strains stably maintain L-A-HN introduced by crosses or cytoduction. We crossed *ski*$^-$ [KIL-sd_1] strains with *SKI*$^+$ [HOK-o] [NEX-o] [EXL-o] [KIL-o] strains and tested the *SKI*$^+$ K^- meiotic segregants for [EXL], [NEX], and [HOK]. All had [EXL], including segregants derived from three different [KIL-sd_1] strains. None had [NEX], and of course, none had [HOK]. The source of [EXL] in these strains is probably strain AN33 (L-A-E + L-C), with which the original *ski* mutants were first crossed (18). Thus, L-A-E is adequate to maintain M_1 in a *ski*$^-$ host but not in a *SKI*$^+$ host, hence the *ski*$^-$ dependence of [KIL-sd_1].

That L-A-E (or some L-A) is necessary for stable mainte-

TABLE 4. Cold sensitivity of *ski* mutants depends on an M-derived genome

Donor	Recipient	Cytoductants					
		Nuclear genotype	Source of S, M, and L-A	dsRNAs	Cold sensitivity	K, R	No. tested
ski2							
1020	SP226	*ski2-2*		L-(BC)	CR	$K^- R^-$	16
1019	1372	*ski2-3*		L-(BC)	CR	$K^- R^-$	16
2176	SP226	*ski2-2*	A364A	L-(BC), L-A-HN	CR	$K^- R^-$	8
2181	1372	*ski2-3*	18	L-(BC), L-A-HN	CR	$K^- R^-$	8
1101	SP226	*ski2-2*	A364A	L-(BC), L-A-HN, M_1	CS	$K_1^+ R_1^+$	24
1074	1372	*ski2-3*	A364A	L-(BC), L-A-HN, M_1	CS	$K_1^+ R_1^+$	16
1488	SP226	*ski2-2*	18	L-(BC), L-A-HN, M_1	CS	$K_1^+ R_1^+$	8
SP108	SP226	*ski2-2*	A8209B	L-(BC), L-A-HN, M_1	CS	$K_1^+ R_1^+$	8
2134	SP226	*ski2-2*	A364A	L-(BC), L-A-HN, M_{nl}[a]	CS	$K^- R_1^+$	8
1494	1372	*ski2-3*	A364A	L-(BC), L-A-HN, M_{nl}[a]	CS	$K^- R_1^+$	8
1204	SP226	*ski2-2*	18	L-(BC), L-A-HN, S732[b]	CS	$K^- R^-$	8
SP106	SP226	*ski2-2*	A8209B	L-(BC), L-A-HN, S3[b]	CS	$K^- R^-$	4
SP107	SP226	*ski2-2*	A8209B	L-(BC), L-A-HN, S4[b]	CS	$K^- R^-$	4
ski3							
1019	1375	*ski3-3*		L-(BC)	CR	$K^- R^-$	8
1074	1375	*ski3-3*	A364A	L-(BC), L-A-HN, M_1	CS	$K^+ R_1^+$	8
SP180	1375	*ski3-3*	A8209B	L-(BC), L-A-HN, M_1	CS	$K_1^+ R_1^+$	8
1494	1375	*ski3-3*	A364A	L-(BC), L-A-HN, M_{nl}[a]	CS	$K^- R_1^+$	8
SP176	1375	*ski3-3*	A8209B	L-(BC), L-A-HN, S1[b]	CS	$K^- R^-$	8

[a] M_{nl} is a neutral mutant of the M_1 dsRNA from strain A364A; that is, it confers resistance but not toxin production ($K_1^- R_1^+$).

[b] S1, S3, and S4 are suppressive deletion mutants of the M_1 dsRNA of strain A8209B (8), whereas S732 is a suppressive deletion mutant of the M_1 of strain 18 (14).

nance of M_1 in a *ski2* strain was indicated by a detailed examination of the exceptional unstable K_1^+ *ski2* CR subclone (called 46B) selected as a CR revertant of a *ski2* [KIL-k_1] strain (see above). The two K^- mitotic segregants of 46B examined lacked both [EXL] and [HOK]. Whereas mating 46B with a SKI^+ [HOK] strain gave mostly killer diploids, mating 46B with a SKI^+ [HOK-o] strain gave no killers at all. (Stable K^+ CS subclones mated with either SKI^+ [HOK] or SKI^+ [HOK-o] strains gave virtually all killer diploids.) Thus, 46B lacks [HOK], and, except for its instability, has a phenotype like [KIL-sd_1]. Mating 46B with a *ski2* [EXL] strain (SP242) produced mostly killer diploids (281 K^+ of 294) which on subcloning proved stable (168 K^+ of 168). Mating 46B with a *ski2* [EXL-o] L-A-o strain (SP245, isogenic to SP242) produced virtually all nonkillers (254 K^- of 262) or unstable killers (7 of 262). Haploid meiotic segregants from the mating of 46B with the *ski2* [EXL] strain SP242 behaved as standard, stable *ski2* [KIL-sd_1] strains. The absence of the L-A markers [EXL] and [HOK] indicates that strain 46B lacks L-A completely since the only L-As in the ancestors of 46B were L-A-HN and L-A-E.

The fact that many isolates of revertant 46B were indeed killers despite the apparent lack of L-A suggests strongly that in *ski2* strains M_1, dsRNA can be replicated and maintained, although inefficiently, without L-A. L-A-E stabilizes M_1 in the *ski2* background but not in a $SKI2^+$ background. L-A-HN or L-A-HE stabilizes M_1 in either a $SKI2^+$ or *ski2* background.

***ski⁻* [KIL-sd_1] strains are only slightly cold sensitive for growth.** When compared with isogenic CS ski^- [KIL-k_1] and CR ski^- [KIL-o] strains, ski^- [KIL-sd_1] strains are only slightly cold sensitive for growth (Fig. 2). Of a total of 48 colonies of strain 1405 (*ski2-2* [KIL-sd_1]) grown at 37°C and then purified at 30°C, the 13 K^- colonies were all cold resistant, whereas the 35 K^+ colonies were all weakly cold sensitive.

The copy number of M_1 dsRNA in a *ski2-2* [KIL-sd_1] strain was also intermediate between the high copy number of an isogenic *ski2-2* [KIL-k_1] strain and the lower copy number of SKI^+ [KIL-k_1] strains (Fig. 1D). Thus, in these strains and in other ski^- strains, cold sensitivity is roughly proportional to the M_1 copy number.

***lts5* = *mak6*.** The *lts5* mutation is linked to *aro7* (parental ditype = 15, nonparental ditype = 0, tetratype = 34) and causes both cold sensitivity for growth (15) and loss of M_1 dsRNA (30). We have examined mutations in 12 *mak* genes, and of these, only *mak6-1* showed cold sensitivity for growth at 8°C cosegregating with the mak^- phenotype in all 24 tetrads examined. The *mak6-1* mutation is also on chromosome XVI near *aro7* (29). Complementation tests between *lts5-1* and *mak6-1* strains showed that both are recessive for cold sensitivity but do not complement each other. The mutations are thus in the same gene.

DISCUSSION

We found that *ski2* and *ski3* mutations prevent the exclusion of M_2 dsRNA that generally accompanies introduction of L-A-HN into an *mkt1* host at 30°C. An array of new mutations suppressing the exclusion defined at least three new *ski* genes (*ski6*, *ski7*, and *ski8*) and three other genes which do not make cells superkillers (*mks1*, *mks2*, and *MKS50*). The *mks1*, *mks2*, and *MKS50* mutations define a new class of genes affecting the killer systems.

In analyzing the new *ski* mutations, we found that they confer cold sensitivity for growth but only if the cell carries M_1 or M_2 dsRNA. The degree of cold sensitivity seems to be correlated with the copy number of M: ski^- [KIL-sd_1] strains have an M_1 copy number intermediate between that of ski^- [KIL-k_1] strains and that of SKI^+ [KIL-k_1] strains, and their cold sensitivity is also intermediate. Also, ski^- L-A-H M_2

strains have a threefold lower M_2 copy number than do *ski*$^-$ L-A-HN M_2 strains, and the former are only slightly cold sensitive, whereas the latter are fully cold sensitive. *ski2-2* L-A-HN L-(BC) M-o strains have over 10-fold more dsRNA than do the same strains carrying M_1 (Ball et al., submitted for publication), and yet the M-o strain is cold resistant, whereas the strain carrying M_1 is cold sensitive. Thus, a high level of dsRNA is not sufficient to cause cold sensitivity.

The correlation of cold sensitivity with M_1 copy number and the existence of a gene, *LTS5* (=*MAK6*), whose product is needed both for cell growth at 8°C (15) and for M_1 dsRNA replication at 30°C (30), suggests that *ski* mutations cause cell cold sensitivity through increasing M copy number. The increased M dsRNA might divert most of the *LTS5* product, making the cell cold sensitive for growth. The *MAK6* product is not needed by L-A (31). Thus, a high copy number of L-A should not (and does not) cause cold sensitivity.

All wild-type K_1 killer strains examined to date carry L-A-HN in addition to M_1 dsRNA, and various lines of evidence indicate that L-A-HN is normally essential for M_1, probably

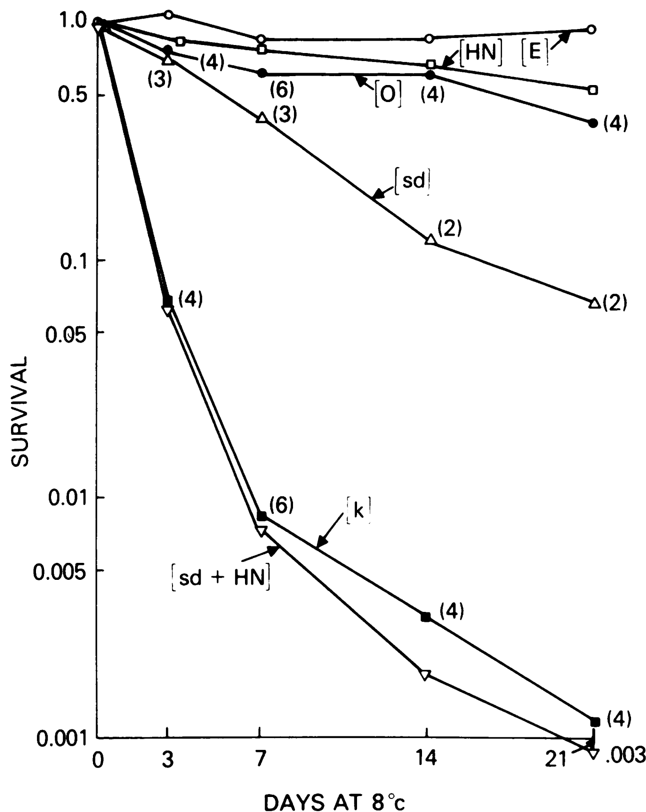
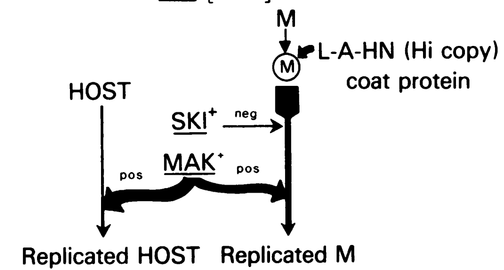
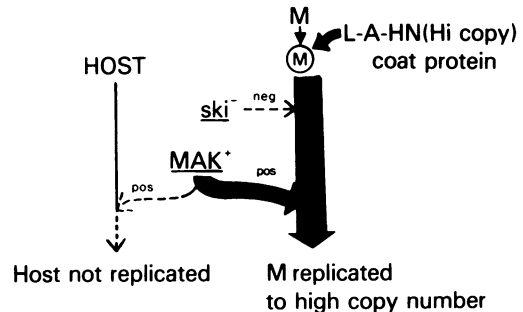


FIG. 2. Effects of various dsRNA plasmids on viability of a *ski2* strain after exposure to cold. All strains were constructed by cytoducing the various plasmids into the same *ski2-2* strain. Strains were plated onto YPAD medium; after the indicated number of days of incubation at 8°C, the plates were further incubated at 30°C for 3 or 4 days, and colonies were then counted. (Increasing the length of incubation at 30°C to 6 days after 14 to 21 days at 8°C increased the number of SP79 colonies by only 2%.) The numbers in parentheses next to points indicate the number of experimental values averaged; averages shown are harmonic means. If no number follows a point, it represents a single value. Symbols: ●, SP278, [KIL-o] [EXL-o] [HOK-o][NEX-o]; ■, SP79, [KIL-k_1][HOK][NEX]; △, SP81, [KIL-sd_1] [EXL]; □, SP432, [HOK-NEX]; ○, SP275, [EXL]; ▽, SP426, [KIL-sd] [HOK-NEX] [EXL?].

WILD-TYPE HOSTS SKI$^+$ [KIL-k] ARE NOT COLD-SENSITIVE



ski$^-$[KIL-k] hosts are cold-sensitive and have ↑ M dsRNA



ski$^-$[KIL-o] hosts are not cold sensitive

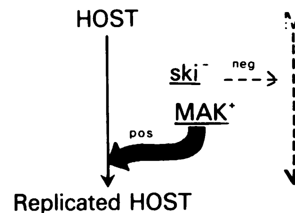


FIG. 3. Model to explain the cold sensitivity of growth and its relation to *MAK* genes (e.g., *LTS5*), *SKI* genes, M dsRNA, and L-A dsRNA. The model assumes that the product of a *MAK* gene (*LT55*), needed for both low temperature growth and M replication, is diverted away from its essential cellular function by high M copy number. M copy number is controlled, in turn, by *SKI* products and L-A dsRNA. Broken lines indicate an absent or defective product or process. pos is positive control and neg is negative control or influence of the indicated gene product on the indicated process.

because it provides the coat protein in which M_1, like L-A, is encapsidated (9, 10, 16, 17). *ski*$^-$ [KIL-sd_1] strains, however, lack L-A-HN and have instead the closely related L-A-E dsRNA and also a lower M_1 copy number than do *ski*$^-$ [KIL-k_1] strains, which have L-A-HN. This may be because L-A-HN has a higher copy number than does L-A-E (16) and thus may provide more coat protein to M_1 than does L-A-E.

The factor that *ski*$^-$ [KIL-sd_1] strains die more slowly at 8°C than do *ski*$^-$ [KIL-k_1] strains may explain how [KIL-sd_1] strains were first isolated. The original *ski* (L-A-HN M_1) mutants were each crossed with strain AN33 (L-A-E, M-o) (18, 19); therefore, the meiotic segregants probably had a mixture of L-A-E and L-A-HN. The *ski*$^-$ (L-A-HN, L-A-E, M_1) cells were stored at 4°C, and most of the cells died. Those that lost M_1 were killed by the high levels of toxin present. Those that lost L-A-HN (due to its mitotic segregation relative to L-A-E) were still K_1^+ R_1^+ but now were partially cold resistant and had a selective advantage. Thus,

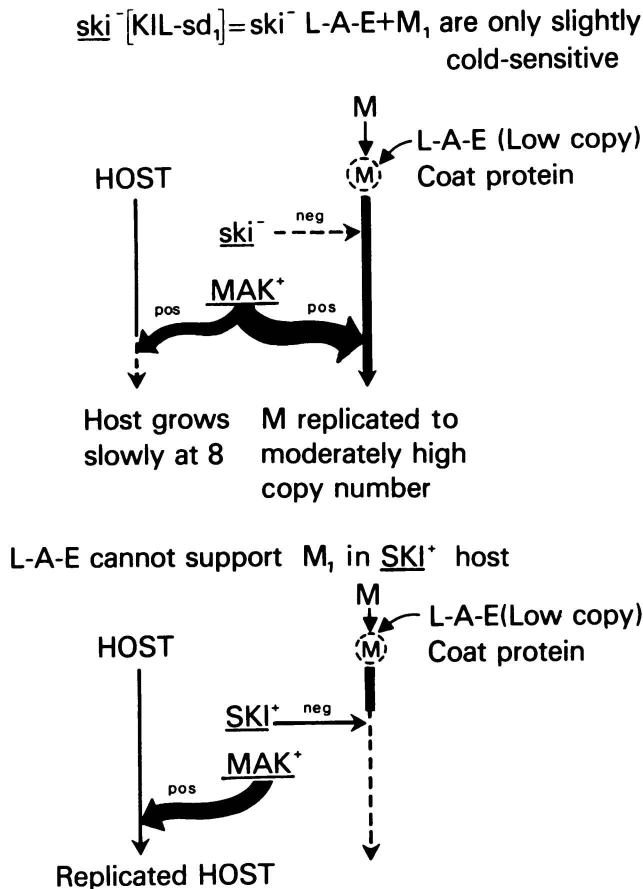


FIG. 4. Extension of the model of Fig. 3 to explain the properties of [KIL-sd_1]. L-A-E is assumed to provide only a limited amount of or a qualitatively different coat protein such that, under the negative influence of the *SKI* products, M disappears from the cell.

[KIL-sd_1] is probably the combination of L-A-E + M_1, and [KIL-k_1] is L-A-HN + M_1.

Earlier work had suggested that [KIL-sd_1] was a mutant of M_1 dsRNA (19); a *ski*$^-$ [KIL-sd_1] strain was mated with a *SKI*$^+$ [KIL-kd] strain (whose M_1 dsRNA had a small deletion serving as a marker not affecting toxin, resistance, or replication). The M_1 from the [KIL-sd_1] parent was preferentially lost from the diploids. Since we did not know about [HOK] at that time, we concluded that the *SKI*$^+$ diploids had lost the M_1 from the [KIL-sd_1] parent because M_1 was the location of the [KIL-sd_1] mutation. But the *SKI*$^+$ [KIL-kd] strain, we now know, had [HOK], and so the loss of the M_1 from the [KIL-sd_1] strain may have been unrelated to the *ski* dependence. Although the M_1 in [KIL-sd_1] strains might be abnormal, a sufficient explanation for the [KIL-sd_1] defect is that L-A-HN has been replaced by L-A-E. L-A-E can support M_1 in a *ski*$^-$ host but not in a *SKI*$^+$ host.

Thus, [HOK] is the function of L-A (presumably the coat protein) which is necessary for M_1 maintenance. Some L-As (e.g., L-A-E) produce a coat protein, but it is inadequate for M_1 maintenance because of either its lower amount or different structure.

Model relating *SKI*, *LTS5*, M_1, L-A-HN, L-A-E, and [KIL-sd_1]. Figures 3 and 4 show a model which relates many of the findings presented here and elsewhere. Two major premises of this model, suggested by our data but not proven, are as follows. (i) The essential role of L-A in M replication or maintenance is to antagonize the negative effects of the *SKI*$^+$ products. Perhaps a higher supply of coat protein from L-A-HN (compared with that from L-A-E) makes M_1 replication or maintenance more resistant to the *SKI*$^+$ products. Another possibility is that L-A itself competes for the *SKI*$^+$ products, making them less available to lower M_1 copy number. In any case, a relation of L-A and *SKI*$^+$ products is indicated by the fact that whether a particular L-A is sufficient to maintain M_1 depends on whether the host is *ski*$^-$. (ii) The cold sensitivity of *ski*$^-$ [KIL-k_1] strains is due to their increasing M_1 copy number; M_1 then siphons off so much of the *LTS5* (*MAK6*) gene product that not enough remains for cell growth at 8°C.

One prediction of this model is that DNA clones selected by their ability to reverse the cold sensitivity of a mutant defective in one *ski* gene should include clones carrying (and overproducing) *LTS5*. Such clones should suppress the cold sensitivity of all *ski* gene mutations, as well as the *lts5* defect.

[NEX], *MKT*, and *SKI* genes. One model to explain the exclusion of M_2 by L-A-HN is that these two dsRNAs compete for a limited supply of the *MKT1* gene product and that M_2 has a lower affinity for the *MKT1* product. The lower copy number L-A-H (Fig. 1C) leaves enough *MKT1* product, even in *mkt1* strains, for M_2 to be maintained. The *ski* mutants, by derepressing M_2 replication upstream, may make more M_2 available to compete with L-A-HN for the *MKT1* supply. Alternatively, the *SKI* products may act by repressing *MKT1* so that *ski* mutants may increase the supply of *MKT1*, with the result that there is enough for both L-A-HN and M_2. This model predicts that (i) elimination of M should increase L-A-HN copy number and (ii) L-A-HN copy number should depend on *mkt1* independent of the presence or absence of M. The first of these predictions has been verified (Ball et al., submitted for publication).

LITERATURE CITED

1. **Adler, J., H. A. Wood, and R. F. Bozarth.** 1976. Virus-like particles from killer, neutral, and sensitive strains of *Saccharomyces cerevisiae*. J. Virol. **17**:472–476.
2. **Bevan, E. A., A. J. Herring, and D. J. Mitchell.** 1973. Preliminary characterization of two species of dsRNA in yeast and their relationship to the "killer" character. Nature (London) **245**:81–86.
3. **Bostian, K. A., J. E. Hopper, D. T. Rogers, and D. J. Tipper.** 1980. Translational analysis of the killer-associated virus-like particle dsRNA genome of S. cerevisiae: M dsRNA encodes toxin. Cell **19**:403–414.
4. **Brennan, V. E., L. Field, P. Cizdziel, and J. A. Bruenn.** 1981. Sequences at the 3′ ends of yeast viral dsRNAs: proposed transcriptase and replicase initiation sites. Nucleic Acids Res. **9**:4007–4021.
5. **Bussey, H.** 1981. Physiology of killer factor in yeast. Adv. Microbiol. Physiol. **22**:93–121.
6. **Chattoo, B. B., F. Sherman, D. A. Azubalis, T. A. Fjellstedt, D. Mehnert, and M. Ogur.** 1979. Selection of *lys2* mutants of the yeast *Saccharomyces cerevisiae* by the utilization of α-aminoadipate. Genetics **93**:51–65.
7. **Conde, J., and G. R. Fink.** 1976. A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. Proc. Natl. Acad. Sci. U.S.A. **73**:3651–3655.
8. **Fried, H. M., and G. R. Fink.** 1978. Electron microscopic heteroduplex analysis of "killer" double-stranded RNA species from yeast. Proc. Natl. Acad. Sci. U.S.A. **75**:4224–4228.
9. **Harris, M. S.** 1978. Virus-like particles and double-stranded RNA from killer and non-killer strains of *Saccharomyces cerevisiae*. Microbios **21**:161–176.
10. **Herring, A. J., and E. A. Bevan.** 1974. Virus-like particles

associated with the double-stranded RNA species found in killer and sensitive strains of the yeast *Saccharomyces cerevisiae*. J. Gen. Virol. **22**:387–394.

11. **Hopper, J. E., K. A. Bostian, L. B. Rowe, and D. J. Tipper.** 1977. Translation of the L-species dsRNA genome of the killer-associated virus-like particles of *Saccharomyces cerevisiae*. J. Biol. Chem. **252**:9010–9017.
12. **Mortimer, R. K., and D. C. Hawthorne.** 1975. Genetic mapping in yeast, p. 221–233. *In* D. M. Prescott (ed.), Methods in cell biology. Academic Press, Inc., New York.
13. **Palfree, R., and H. Bussey.** 1979. Yeast killer toxin: purification and characterization of the protein toxin from *Saccharomyces cerevisiae*. Eur. J. Biochem. **93**:487–493.
14. **Ridley, S. P., and R. B. Wickner.** 1983. Defective interference in the killer system of *Saccharomyces cerevisiae*. J. Virol. **45**:800–812.
15. **Singh, A., and T. R. Manney.** 1974. Genetic analysis of mutations affecting growth of *Saccharomyces cerevisiae* at low temperature. Genetics **77**:651–659.
16. **Sommer, S. S., and R. B. Wickner.** 1982. Co-curing of plasmids affecting killer dsRNAs of *Saccharomyces cerevisiae*: [HOK], [NEX], and the abundance of *L* are related and further evidence that M_1 requires *L*. J. Bacteriol. **150**:545–551.
17. **Sommer, S. S., and R. B. Wickner.** 1982. Yeast L dsRNA consists of at least three distinct RNAs; evidence that the non-Mendelian genes [HOK], [NEX], and [EXL] are on one of these dsRNAs. Cell **31**:429–441.
18. **Toh-e, A., P. Guerry, and R. B. Wickner.** 1978. Chromosomal superkiller mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **136**:1002–1007.
19. **Toh-e, A., and R. B. Wickner.** 1979. A mutant killer plasmid whose replication depends on a chromosomal "superkiller" mutation. Genetics **91**:673–682.
20. **Toh-e, A., and R. B. Wickner.** 1980. "Superkiller" mutations suppress chromosomal mutations affecting double-stranded RNA killer plasmid replication in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **77**:527–530.
21. **Vodkin, M., F. Katterman, and G. R. Fink.** 1974. Yeast killer mutants with altered double-stranded ribonucleic acid. J. Bacteriol. **117**:681–686.
22. **Wesolowski, M., and R. B. Wickner.** 1984. Two new double-stranded RNA molecules showing non-Mendelian inheritance and heat inducibility in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:181–187.
23. **Wickner, R. B.** 1974. "Killer character" of *Saccharomyces cerevisiae*: curing by growth at elevated temperatures. J. Bacteriol. **117**:1356–1357.
24. **Wickner, R. B.** 1978. Twenty-six chromosomal genes needed to maintain the killer double-stranded RNA plasmid of *Saccharomyces cerevisiae*. Genetics **88**:419–425.
25. **Wickner, R. B.** 1980. Plasmids controlling exclusion of the K_2 killer double-stranded RNA plasmid of yeast. Cell **21**:217–226.
26. **Wickner, R. B.** 1981. Killer systems in *Saccharomyces cerevisiae*, p. 415–444. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), Molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Wickner, R. B.** 1983. Killer systems in *Saccharomyces cerevisiae*: three distinct modes of exclusion of M_2 double-stranded RNA by three species of dsRNA, M_1, L-A-E, and L-A-HN. Mol. Cell. Biol. **3**:654–661.
28. **Wickner, R. B.** 1983. Genetic control of replication of the double-stranded RNA segments of the killer systems in *Saccharomyces cerevisiae*. Arch. Biochem. Biophys. **222**:1–11.
29. **Wickner, R. B., and M. J. Leibowitz.** 1976. Chromosomal genes essential for replication of a double-stranded RNA plasmid of *Saccharomyces cerevisiae*: the killer character of yeast. J. Mol. Biol. **105**:427–443.
30. **Wickner, R. B., S. P. Ridley, H. M. Fried, and S. G. Ball.** 1982. Ribosomal protein L3 is involved in replication or maintenance of the killer double-stranded RNA genome of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **79**:4706–4708.
31. **Wickner, R. B., and A. Toh-e.** 1982. [HOK], a new yeast non-Mendelian trait, enables a replication-defective killer plasmid to be maintained. Genetics **100**:159–174.
32. **Young, T. W., and M. Yagiu.** 1978. A comparison of the killer character in different yeasts and its classification. Antonie van Leeuwenhoek J. Microbiol. Serol. **44**:59–77.