[HOK], A NEW YEAST NON-MENDELIAN TRAIT, ENABLES A REPLICATION-DEFECTIVE KILLER PLASMID TO BE MAINTAINED

REED B. WICKNER[1] AND AKIO TOH-E[2,3]

[1] *Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Building 4, Room 116, Bethesda, Maryland 20205, U.S.*

[2] *Department of Fermentation Technology, Faculty of Engineering, Osaka University, Yamada-kami, Suita-shi, Osaka 565, Japan*

Manuscript received March 24, 1981
Revised copy accepted November 13, 1981

ABSTRACT

The K_1 killer plasmid, [KIL-k_1], of *Saccharomyces cerevisiae* is a 1.25×10^6 dalton linear double-stranded RNA plasmid coding for a protein toxin and immunity to that toxin. The [KIL-sd_1] plasmid is a replication-defective mutant of [KIL-k_1] that depends on one of the recessive chromosomal superkiller (*ski*$^-$) mutations for its maintenance (TOH-E and WICKNER 1979). This report concerns a means by which [KIL-sd_1] can be stably maintained in a *SKI*$^+$ host. Strains carrying a plasmid we call [HOK] (*h*elper of *k*iller) stably maintain [KIL-sd_1]. [HOK] segregates 4 [HOK]:0 in meiotic crosses and is efficiently transferred by cytoplasmic mixing (heterokaryon formation). [HOK] depends for its maintenance on the products of *PET18*, *MAK3*, and *MAK10*, three chromosomal genes needed to maintain [KIL-k_1], but is independent of 10 other *MAK* genes and of *MKT1*. [HOK] is not mitochondrial DNA and is unaffected by agents which convert ψ^+ strains to ψ^-. [HOK] is also distinct from the previously described plasmids [URE3], 20S RNA, 2 μ DNA, and [EXL]. Strains lacking [HOK] consistently have a four-fold lower copy number of *L* double-stranded RNA than strains carrying [HOK].

THE killer viruses (or plasmids) of *S. cerevisiae* provide a means of studying the viral and cellular components involved in viral maintenance, replication, and expression in a eukaryotic cell. Studies to date have defined 38 chromosomal genes and six plasmids involved in various ways in these processes. These components show a variety of interactions: exclusion of one plasmid by another, suppression of some chromosomal mutations by other chromosomal mutations, suppression of plasmid mutations by chromosomal mutations and *vice versa*, interference by defective virus mutants with normal virus replication, and others.

Killer yeast secrete a protein toxin which kills sensitive strains. The K_1 killer phenotype is due to a 1.25×10^6 dalton linear double-stranded (ds) RNA genome (called M_1 ds RNA) present in intracellular virus-like particles. M_1

[3] Present address: Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, 3-8-2 Sanda-Mashi, Hiroshima-shi, Hiroshima, Japan, 730.

codes for the toxin protein and immunity to the toxin. A second larger linear ds RNA (called *L*) codes for the major particle protein and is present in most yeast strains whether killers or not (SOMERS and BEVAN 1969; BEVAN, HERRING and MITCHELL 1973; BUCK, LHOAS and STREET 1973; VODKIN, KATTERMAN and FINK 1974; HERRING and BEVAN 1974; SWEENEY, TATE and FINK 1976; MITCHELL, HERRING and BEVAN 1976; HOPPER *et al.* 1977; HARRIS 1978; PALFREE and BUSSEY 1979; BOSTIAN *et al.* 1980; BOSTIAN, STURGEON and TIPPER 1980). The killer system has been recently reviewed (BRUENN 1980; WICKNER 1981). Recently, the linear DNA killer plasmid of Kluyveromyces has been transferred to Saccharomyces (GUNGE *et al.* 1981; GUNGE and SAKAGUCHI 1982).

Components necessary to maintain the M_1 ds RNA killer plasmid include at least one killer plasmid function, defined by the [KIL-sd$_1$] mutants (TOH-E and WICKNER 1979) and at least 29 chromosomal genes (*MAK1*, *MAK3–MAK28*, *PET18*, and *SPE2*) (SOMERS and BEVAN 1969; WICKNER 1974, 1978a, 1979; WICKNER and LEIBOWITZ 1976, 1979; LEIBOWITZ and WICKNER 1978; COHN *et al.* 1978; GUERRY-KOPECKO and WICKNER 1980). The killer plasmid's requirement for the function defective in the [KIL-sd$_1$] mutants is bypassed by mutation of any one of the four chromosomal *SKI* genes (TOH-E and WICKNER 1979). The *ski*$^-$ mutants were first detected by their *superkiller* phenotype (TOH-E, GUERRY and WICKNER 1978) and were subsequently found to suppress the killer plasmid's need for many of the *MAK* products (TOH-E and WICKNER 1980).

We now report a new non-Mendelian genetic element detected by its ability to supply a substitute for the function missing in the replication-defective [KIL-sd$_1$] plasmid mutant. This new element is called [HOK] for *h*elper of *k*iller. Strains carrying [HOK] have an increased level of *L* ds RNA.

MATERIALS AND METHODS

Strains: The nomenclature for the killer system is shown in Table 1. Many of the yeast strains used are listed in Table 2.

Media: Media have been described (WICKNER 1978).

Assays of killing and resistance: Colonies to be tested for killing ability were replica-plated to MB medium which had just been seeded with a lawn of the appropriate sensitive strain (0.5 ml of a 1 OD_{650}/ml suspension spread on each plate and allowed to dry in). 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 × 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° for two to three 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 about six, does not permit the toxin to function (PALFREE and BUSSEY 1979; YOUNG and YAGIU 1978). Diploids were isolated utilizing the complementary nutritional requirements of their parents. Sporulation and dissection were by the usual methods (see MORTIMER and HAWTHORNE 1975, for references).

Cytoduction: A cytoplasmic genome can be transferred from one haploid strain to another without diploidization or other change of nuclear genotype by heterokaryon formation (cytoplasmic mixing, cytoduction) using the *kar1* mutant, defective in nuclear fusion (CONDE and

TABLE 1

Nomenclature

Phenotypes	
K_1^+ or K_1^-	ability or inability to kill a lawn of a $K_2^+R_2^+$ (or a K-R-) strain, but not a lawn of another $K_1^+R_1^+$ strain
K_1^{++}	superkiller phenotype; increased toxin production
K_2^+ or K_2^-	ability or inability to kill a lawn of a $K_1^+R_1^+$ (or a K-R-) strain, but not a lawn of another $K_2^+R_2^+$ strain
R_1^+ or R_1^-	resistance or sensitivity to the K_1 toxin
R_2^+ or R_2^-	resistance or sensitivity to the K_2 toxin
Helper or nonhelper	*SKI*+ [KIL-o] or [KIL-k_2] strains are tested for their "helper" ability by mating with a *ski*- [KIL-sd_1] strain (Toh-e and Wickner 1979) and examining 10–50 diploid single colonies for K_1 killing ability. If all diploid single colonies are $K_1^+R_1^+$, then helper activity is present; if diploids are K-R-, then the strain is a nonhelper. When [KIL-k_2] strains are mated with [KIL-k_1] (Naumova and Naumov 1973) or [KIL-sd_1] strains, the diploids are all $K_2^-R_2^-$ (exclusion of [KIL-k_2]), so that helper activity can be easily scored independent of [KIL-k_2].
Genotypes	
[KIL-o]	absence of killer plasmid
[KIL-k_1]	normal K_1 killer plasmid
[KIL-sd_1]	mutant killer plasmid derived from [KIL-k_1]. Depends for its maintenance on a host *ski*- mutation (Toh-e and Wickner 1979) or on the presence of a new plasmid [HOK] (this work). Cells carrying [KIL-sd_1] are $K_1^+R_1^+$.
[HOK]	plasmid which allows [KIL-sd_1] to replicate in a *SKI*+ host (*h*elper of *k*iller)
[HOK-o]	absence of [HOK] plasmid
[EXL]	plasmid which excludes [KIL-k_2], but not [KIL-k_1] or [KIL-sd_1]
[NEX]	plasmid that prevents [EXL] from excluding [KIL-k_2]
ρ°	the absence of mitochondrial DNA
SKI	chromosomal gene mutation which produces the superkiller (K_1^{++}) phenotype. There are four *SKI* genes. The *ski*- mutants are recessive to wild type.
MAK	chromosomal gene needed for [KIL-k_1] maintenance
PET18	chromosomal gene needed for growth and for maintenance of [KIL-k_1], the mitochondrial DNA, and [HOK].
ds RNA	
M_1	1.25×10^6 dalton linear ds RNA identified as [KIL-k_1]. Codes for K_1 toxin.
M_2	1.0×10^6 dalton linear ds RNA identified as [KIL-k_2]
L	3×10^6 dalton linear ds RNA present in most Saccharomyces. Codes for major protein of particles containing *L* and *M* ds RNAs. High *L* and low *L* refer to the relative cellular content of *L* ds RNA in various strains, shown here to be correlated with the presence and absence, respectively, of [HOK].
L-o	the absence of *L* ds RNA

Plasmids are always given in brackets, [ ], while chromosomal genes are presented in italics. Dominant alleles of chromosomal genes are in upper case letters; recessive alleles are lower case.

TABLE 2

Yeast strains

Designation	Killer phenotype	Genotype	Reference
AT193	K_1++R_1+	α *ade ski2–2* [KIL-sd$_1$1]	Toh-e and Wickner 1979
1405	K_1++R_1+	**a** *ade1 ski2-2* [KIL-sd$_1$3]	Toh-e and Wickner 1979
1406	K_1++R_1+	α *ade1 ski2–2* [KIL-sd$_1$3]	Toh-e and Wickner 1979
1415	K_1++R_1+	**a** *leu1 ski4–1* [KIL-sd$_1$1]	Toh-e and Wickner 1979
1416	K_1++R_1+	α *leu1 ski4–1* [KIL-sd$_1$1]	Toh-e and Wickner 1979
AN33	K-R- nonhelper	α *arg1 thr1* [EXL] [KIL-o] [HOK-o]	Toh-e and Wickner 1979
1020	K-R- nonhelper	α *his4 kar1–1* [KIL-o] [EXL-o]	Conde and Fink 1976
1089	K-R- nonhelper	**a** *leu1 kar1–1* [EXL-o] [KIL-o] [NEX-o] [HOK-o] ρ°	Conde and Fink 1976
1428	K_2+R_2+ nonhelper	α *met13 can1 cyh2 mkt1* [KIL-k$_2$] [EXL-o] [NEX-o] [HOK-o]	Wickner 1980
200	K-R- helper	**a** *lys10 mkt1* [KIL-o] [EXL] [NEX-o] [HOK]	
201	K-R- helper	α *lys10* [KIL-o]	
1385	K_2+R_2+ helper	α *lys1* [KIL-k$_2$] [NEX] [HOK]	Wickner 1980
1387	K_2+R_2+ helper	**a** *ura1* [KIL-k$_2$] [NEX-o] [HOK]	Wickner 1980
2683-1D	K_2+R_2+ helper	**a** *kar1–1 his1 ade5* [KIL-k$_2$] [NEX] [HOK]	
1598	K-R- nonhelper	α *ura1 ilv3 can1 mak10–1* [KIL-o] [HOK-o]	
1633	K-R- nonhelper	α *arg4 aro7 mak3–1* [KIL-o] [HOK-o]	
1066	K-R- nonhelper	α *trp1 pet18–1* [KIL-o] [HOK-o]	
JM6	K-R- nonhelper	**a** *ade2–1 lys1–1 his4–580 met8–1 SUP4–3* [ψ+] *L-o M-o* [HOK-o]	Vodkin 1977

Fink 1976). Usually recipient cells were ρ° (mitochondrial DNA eliminated by growth on ethidium bromide), and donor cells were ρ+. Recipient and donor cells mate on YPAD plates for 6 hr, 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 are 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, helper, etc.

ds RNA: ds RNA was purified by cellulose chromatography and analyzed on agarose gels as previously described (Toh-e, Guerry and Wickner 1978) or by the rapid method described by Fried and Fink (1978). Briefly, spheroplasts produced by zymolyase were lysed by sodium dodecyl sulfate, and the extract was digested with proteinase K, phenol-extracted, and ethanol-precipitated; ds RNA 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 ethanol-precipitated and analyzed on 1% agarose gels.

RESULTS

"Helper" activity shows non-Mendelian inheritance: When a *ski*$^-$ [KIL-sd$_1$] $K_1{}^{++}R_1{}^+$ haploid is crossed with certain *SKI*$^+$ [KIL-o] K^-R^- haploid strains, the diploids formed are K^-R^- and have lost M_1 ds RNA, because the *ski*$^-$ mutations are recessive and the [KIL-sd$_1$] plasmid depends for its replication on its host being *ski*$^-$ (Toh-e, Guerry and Wickner 1978; Toh-e and Wickner 1979; Table 3). However, when the same *ski*$^-$ [KIL-sd$_1$] strains were mated with certain other *SKI*$^+$ [KIL-o] strains, the diploids were found to be uniformly $K_1{}^+$ $R_1{}^+$ (Table 3). We call this the "helper" phenotype of these *SKI*$^+$ [KIL-o] strains. For example, strains 200 and 1385 are helpers, while AN33 and 1089 are nonhelpers.

To determine the genetic basis for their difference, helper strains (*e.g.*, 200, which is [KIL-o] and lacks M_1 ds RNA) were crossed with nonhelpers (*e.g.*, AN33). Each meiotic segregant was tested for its helper activity by crossing it with a *ski*$^-$ [KIL-sd$_1$] strain and examining 10–50 diploid single colonies for the K_1 phenotype. All meiotic segregants had the helper activity originally present in only one of the parents (Table 4). This 4 helper:0 nonhelper segregation indicates that the helper activity is due to a plasmid (nonchromosomal genetic element) present in the helper strains and absent from nonhelper strains. This was confirmed by showing that helper activity was transferred by cytoduction (Table 4). We call this non-Mendelian element [HOK] for *h*elper *o*f *k*iller.

In all cases examined, *SKI*$^+$ diploid strains which retained [KIL-sd$_1$] because they had [HOK] also retained the M_1 ds RNA, while those which lost [KIL-sd$_1$] because they were *SKI*$^+$ and [HOK-o] had also lost M_1 *ds* RNA (sample data shown in Figure 1).

Meiotic analysis of a cross of the type [HOK] [KIL-o] × *ski*$^-$ [KIL-sd$_1$] results in 2 $K_1{}^{++}$: 2 $K_1{}^+$ segregation (Table 4, crosses 2651 and 2655) presumably because [HOK] is supporting the maintenance of the replication-defective [KIL-sd$_1$] plasmid in the *SKI*$^+$ segregants. This contrasts with the 2 $K_1{}^{++}$: 2$K_1{}^-$ segregation found by Toh-e and Wickner (1979) for crosses which we now know were of the type [HOK-o] × *ski*$^-$ [KIL-sd$_1$].

[HOK] requires MAK3, MAK10, *and* PET18 *for its maintenance*: [KIL-k$_1$] requires, for its maintenance and replication, the products of at least 29 chromosomal genes, *MAK1, MAK3-MAK28* (*MAK* = *ma*intenance of *k*iller plasmid), *PET18*, and *SPE2*. Mutants in several of these genes were mated with *MAK*$^+$ [HOK] strains, and the meiotic segregants were tested for helper activity (Table 5). In most cases, 4 helper : 0 segregation was observed, indicating that unlike [KIL-k$_1$], [HOK] does not depend on the products of 10 *MAK* genes tested.

However, in the case of *mak3–1*, *mak10–1*, and *pet18–1*, the segregation observed was 2 *MAK*$^+$ helper:2 *mak*$^-$ nonhelper. Thus, [HOK] requires *MAK3*, *MAK10*, and *PET18* for its maintenance. Because [KIL-k$_1$] requires at least 10 *MAK* genes that [HOK] does not require, [HOK] differs from [KIL-k$_1$].

[HOK] is distinct from several other cytoplasmic genetic elements: Elimination of the mitochondrial genome by growth of cells in ethidium bromide (Goldring *et al.* 1970) did not eliminate [HOK] from strains 200, 201, or 1385, indi-

TABLE 3

Helper and nonhelper strains

	SKI+ parent		*ski*⁻ [KIL-sd] parent		
			1405 **a** *ski2–2* [KIL-sd_1]	1405 ρ°	1415 **a** *ski4–1* [KIL-sd_1]
Nonhelpers	AN33	α [KIL-o] [EXL]	K-R-		K-R-
	AN33 ρ°	α [KIL-o] [EXL] ρ°	K-R-		K-R-
	1020	α [KIL-o] [EXL-o]	K-R-		
	1428	α *mkt1* [KIL-k_2] [NEX-o] [EXL-o]	K-R-		K-R-
	2520–1D	α [EXL] [KIL-o] [NEX-o]	K-R-	K-R-	K-R-
	2520–1D ρ°		K-R-	K-R-	
Helpers	201	α *lys10* [KIL-o]	K_1+R_1+		K_1+R_1+
	201 ρ°	α *lys10* [KIL-o] ρ°	K_1+R_1+		K_1+R_1+
	1385	α [KIL-k_2] [NEX]	K_2-R_2⁻ K_1+R_1+		K_2-R_2⁻ K_1+R_1+

TABLE 3—Continued

	SKI$^+$ parent		*ski*$^-$ [KIL-sd] parent					
			AT193 α *ski2–2* [KIL-sd$_1$]	1406 α *ski2–2* [KIL-sd$_1$]	1406 ρ° α *ski2–2* [KIL-sd$_1$] ρ°	1407 α *ski2–2* [KIL-sd$_1$]	1407 ρ°	1416 α *ski4–1* [KIL-sd$_1$]
Nonhelpers	1427	**a** *mkt1–1* [KIL-k$_2$] [NEX-o]	K–R–	K–R–		K–R–		K–R–
	1039	**a** ρ° [KIL-o] [NEX-o] [EXL-o] *mkt1–1*	K–R–	K–R–		K–R–		
	2520–1C	**a** [KIL-o] [EXL]	K–R–	K–R–	K–R–			
	2520–1C ρ°			K–R–	K–R–			
Helpers	200	**a** [KIL-o] [EXL]	K$_1$+R$_1$+	K$_1$+R$_1$+	K$_1$+R$_1$+	K$_1$+R$_1$+	K$_1$+R$_1$+	K$_1$+R$_1$+
	200 ρ°	**a** [KIL-o] ρ° [EXL]	K$_1$+R$_1$+	K$_1$+R$_1$+	K$_1$+R$_1$+	K$_1$+R$_1$+	K$_1$+R$_1$+	
	1472	**a** *mkt1–1* [KIL-o] [NEX]	K$_1$+R$_1$+	K$_1$+R$_1$+		K$_1$+R$_1$+		K$_1$+R$_1$+
	1472 ρ°			K$_1$+R$_1$+	K$_1$+R$_1$+	K$_1$+R$_1$+	K$_1$+R$_1$+	
	1387	**a** [KIL-k$_2$] [NEX-o]	K$_1$+R$_1$+ K$_2$–R$_2$–	K$_1$+R$_1$+		K$_1$+R$_1$+		K$_1$+R$_1$+
	1423	**a** [KIL-o] [EXL-o] [NEX-o]	K$_1$+R$_1$+	K$_1$+R$_1$+		K$_1$+R$_1$+		K$_1$+R$_1$+

TABLE 4

Non-Mendelian inheritance of helper activity

A. Meiotic segregation

Cross	Parents		Segregation
2666	200 helper	× AN33 nonhelper	4 helper:0 (10 tetrads)
2801	1385 helper	× 1089 nonhelper	4 helper:0 (12 tetrads)
2802	1385 helper	× 1466 nonhelper	4 helper:0 (11 tetrads)
2779	1385 helper	× 1387 helper	4 helper:0 (18 tetrads)
2768	AN33 nonhelper	× 1089 nonhelper	4 nonhelper:0 (9 tetrads)
2651	200 helper	× AT193 *ski2–2* [KIL-sd_1]	2 K_1++R_1+:2 K_1+R_1+ (12 tetrads)
2655	1472 helper	× AT193 *ski2–2* [KIL-sd_1]	2 K_1++R_1+:2 K_1+R_1+ (12 tetrads)

B. Cytoduction

	Donor	Recipient	Cytoductants Number	Phenotype
C10	2674–4A *kar1* helper	→ AN33 ρ° nonhelper	28	all helpers
C11	2647–6D *kar1* helper	→ AN33 ρ° nonhelper	24	all helpers
C56*	2683–1D helper [NEX]	→ 1428 [KIL-k_2] [NEX-o] ρ° *mkt1–1* nonhelper	8	all helpers K⁻R⁻
C60*	2685–4D helper [NEX]	→ 1428 [KIL-k_2] [NEX-o] ρ° *mkt1–1* nonhelper	8	all helpers K⁻R⁻
C58*	2689–3C helper [NEX]	→ 1428 ρ° *mkt1–1* nonhelper	8	all helpers K⁻R⁻

* In cytoductions C56, C58, and C60, the cytoductants all lost [KIL-k_2] because they were *mkt1–1* and [NEX] was introduced; [KIL-k_2] can be maintained in an *mkt1–1* strain only in the absence of [NEX] (WICKNER 1980). However, the helper trait was transferred to the cytoductants and maintained there, indicating that [HOK] does not require *MKT1* in the presence of [NEX]. Strain 200 has the genotype **a** *lys10 mkt1* [HOK] [NEX-o] indicating that [HOK] can be maintained in an *mkt1* cell in the absence of [NEX].

cating that [HOK] is not a mitochondrial gene (Table 3). The ψ plasmid increases the efficiency of ochre suppression (Cox 1965); ψ^+ strains are converted to ψ^- by growth in high osmotic strength media (SINGH, HELMS and SHERMAN 1979) or by growth in the presence of 5 mM guanidine · HCl (TUITE, COX and DOBSON, personal communication). Strains 200, 1385, and 1475 (each carrying [HOK]) were grown to single colonies on YPAD containing 5 mM guanidine · HCl. Fifteen colonies of each strain were tested, and all retained helper activity. Growth of the [HOK-o] strain AN33 under the same conditions also did not affect its lack of helper activity. Finally, the ψ plasmid does not require *PET18* for its replication (LEIBOWITZ and WICKNER 1978) while [HOK] does.

The [URE3] plasmid allows strains to utilize exogenous ureidosuccinate to bypass a *ura2* defect and has other effects on nitrogen metabolism (LACROUTE 1971; AIGLE and LACROUTE 1975). *pet18–1* strains do not lose [URE3] (LEIBOWITZ and WICKNER 1978) so [HOK] is distinct from [URE3]. Moreover, [URE3] does not segregate 4:0 as shown above for [HOK]. The 2 μ DNA plasmid of yeast is also not lost from *pet18–1* strains (LIVINGSTON 1977) or *mak3–1* or *mak10–1* strains (unpublished), and [HOK-o] strains have normal amounts of 2 μ DNA (see below), so [HOK] is not coded by 2 μ DNA.

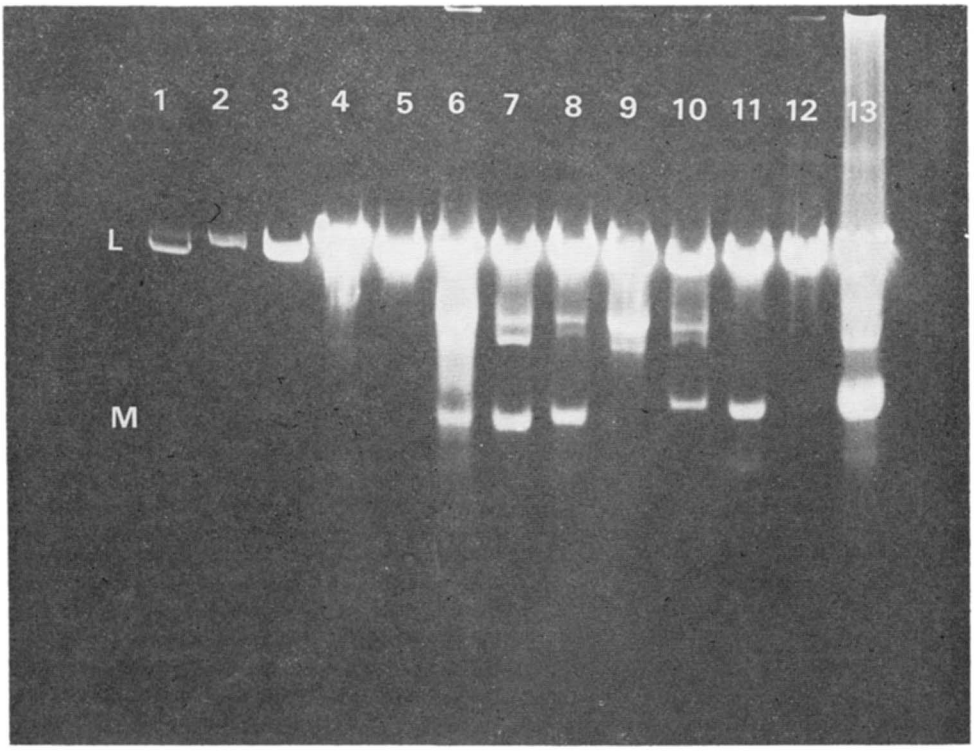

FIGURE 1.—Agarose gel electrophoresis of ds RNA purified by cellulose chromatography from [HOK] and [HOK-o] strains. The amounts loaded in each track do not represent equal numbers of cells.

1. AN33 [HOK-o] [KIL-o]
2. 1089 [HOK-o] [KIL-o]
3. 1466 [HOK-o] [KIL-o]
4. 200 [HOK] [KIL-o]
5. 201 [HOK] [KIL-o]
6. 1387 [HOK] [KIL-k_2]
7. 1384 [HOK] [KIL-k_2]
8. 1385 [HOK] [KIL-k_2] [NEX]
9. 1472 [HOK] [KIL-o] [NEX]
10. 1384 [HOK] [KIL-k_2] × 1405 *ski2–2* [KIL-sd_1] : K_1+R_1+ diploid
11. 200 [HOK] [KIL-o] × 1406 *ski2–2* [KIL-sd_1] : K_1+R_1+ diploid
12. 1089 [HOK-o] [KIL-o] × 1406 *ski2–2* [KIL-sd_1] : K^-R^- diploid
13. 1406 *ski2–2* [KIL-sd_1]

While strains 200 and X2180–1B lack the 20 S RNA plasmid (GARVIK and HABER 1978), both carry [HOK]. Also, *pet18–1*, *mak3–1*, and *mak10–1* strains lose [HOK] (Table 5) but do not lose the 20 S RNA plasmid (GARVIK and HABER 1978). The non-Mendelian element that prevents maltose utilization in some ρ^0 strains (SCHAMHART *et al.* 1975) has not been studied in relation to [HOK].

Two other non-Mendelian genetic elements affect [KIL-k_2] replication (WICKNER 1980). These are called [EXL] (*excl*uder of [KIL-k_2] and [NEX] (makes [KIL-k_2] *non*exc*l*udable by [EXL]). [EXL] requires *MAK1* (unlike [HOK]), and strains AN33 and 1466 carrying [EXL] both lack [HOK].

TABLE 5

[HOK] depends on MAK3, MAK10, *and* PET18, *not on other* MAK *genes*

Cross	Parents		Meiotic segregation*
2793, 2794	200 [HOK]	× 1618 *mak1-1*	4 [HOK]:0 (22)
2810	200 [HOK]	× 1633 *aro7 mak3-1*	2 [HOK] *MAK3*:2 [HOK-o] *mak3-1* (11)†
2786	2666-1C [HOK]	× 748 *mak4-1*	4 [HOK]:0 (10)
2792	2666-1C [HOK]	× 971 *mak5-1*	4 [HOK]:0 (24)
2789	200 [HOK]	× 923 *mak6-1*	4 [HOK]:0 (19)
2787	200 [HOK]	× 1599 *can1 mak10-1*	2 [HOK] *MAK10*:2 [HOK-o] *mak10-1* (18)‡
2796	200 [HOK]	× 1066 *pet18-1*	2 [HOK] *PET18*:2 [HOK-o] *pet18-1* (11)§
2800	200 [HOK]	× 2572-1A *mak14-1*	4 [HOK]:0 (7)
2797	200 [HOK]	× 1096 *mak15-1*	4 [HOK]:0 (15)
2798	200 [HOK]	× 1172 *mak16-1*	4 [HOK]:0 (11)
2791	200 [HOK]	× 1513 *mak19-1*	4 [HOK]:0 (12)
2799	200 [HOK]	× 1518 *mak26-1*	4 [HOK]:0 (11)
2687, 2688	2747-4A [HOK]	× 1369 *mak27-1*	4 [HOK]:0 (19)

* Meiotic segregants were tested for helper activity ([HOK]) by mating with the tester strains 1405, 1406, 1407, or 1408, all of which are *ski2-2* [KIL-sd_1]. Diploids formed were streaked for single colonies on minimal medium, and the killer phenotype tested. The numbers in parentheses indicate the numbers of tetrads examined.

† The 2 [HOK]:2 [HOK-o] segregation in this cross was due to the *mak3-1* mutation as shown by the expected linkage of [HOK-o] to *aro7* (PD = 10, T = 1, NPD = 0).

‡ The 2 [HOK]:2 [HOK-o] segregation in this cross was due to the *mak10-1* mutation as shown by the expected linkage of [HOK-o] to *can1* (PD = 16, T = 2, NPD = 0).

§ The 2 [HOK]:2 [HOK-o] segregation in this cross was due to the *pet18-1* mutation since in each tetrad, the [HOK-o] segregants were also temperature-sensitive and respiration-deficient, two other phenotypes of the *pet18-1* mutation (LEIBOWITZ and WICKNER 1978).

[HOK] is also independent of *MKT1* (C56, C58, C60; Table 3), a chromosomal gene required for maintenance of [KIL-k_2] when [NEX] is present (WICKNER 1980). The relationship of [HOK] and [NEX] is presently under investigation.

Relationship of [HOK] and L *ds RNA*: The dependence of [HOK] on three *MAK* genes and its ability to supply a replication function to a mutant killer plasmid suggest that [HOK] might have a ds RNA genome.

To test this, ds RNA purified by CF11 cellulose from [HOK] and [HOK-o] strains was compared by electrophoresis on agarose (Figure 1) and acrylamide gels. No band was found that was always present in [HOK] strains and always absent in [HOK-o] strains. [HOK] strains lacking M_1 and M_2 ds RNA included strains 200, 201, and 1472. Both [HOK] and [HOK-o] strains had normal amounts of 2 μ DNA. Electrophoresis of whole nucleic acid preparations also failed to reveal bands unique to [HOK] strains.

However, when meiotic segregants from a cross of the type *mak10-1* [HOK-o] × *MAK*$^+$ [HOK] were analyzed by the method of FRIED and FINK (1978) for *L* ds RNA, it was noted that the *MAK*$^+$ [HOK] segregants invariably had substantially higher levels of *L* ds RNA than the *mak10-1* [HOK-o] segregants (Figure 2 and Table 6). If [HOK] were a variant of *L* that was

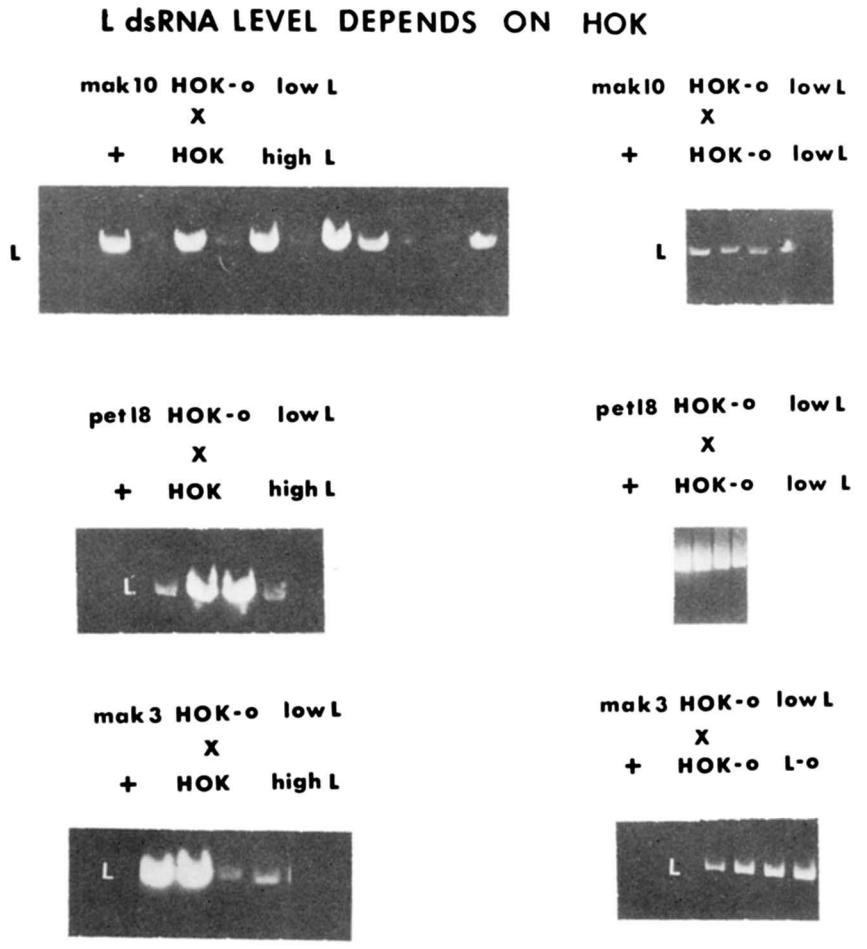

FIGURE 2.—[HOK] strains have higher levels of *L* ds RNA than [HOK-o] strains. Equal weights of cells grown on YPAD plates were used to make crude preparations of ds RNA (FRIED and FINK 1978). Equal aliquots were analyzed by electrophoresis on 1% agarose gels as described (TOH-E *et al.* 1978). This figure shows the *L* ds RNA of meiotic segregants of crosses shown in Table 6. The genotypes of the parents are shown along with the *L* ds RNA from one or more complete tetrads.

both dependent on *MAK10* and could help [KIL-sd$_1$], it might be expected to segregate mitotically relative to the *L* present in [HOK-o] strains, and this might require prolonged mitotic growth. After segregation, some tetrads should be [HOK-o] in all segregants and have equal amounts of *L* ds RNA, while other tetrads should show complete absence of *L* from the *mak*$^-$ segregants. However, this was not observed and the same results were found if the diploids formed in this cross were subcloned 20 times in succession prior to sporulation (about 450 generations total growth).

To confirm and quantitate the difference between the high and low levels, *L* ds RNA was isolated by the CF11 method (TOH-E, GUERRY and WICKNER 1978)

from equal wet weights of the four spore clones of a tetrad of a cross *mak10–1* [HOK-o] × *MAK*$^+$ [HOK]. The amount of *L* ds RNA was measured by optical density. The purified *L* ds RNA was recovered in the ratio of 1.0:1.4:4.0:4.0, with the two lower values for the *mak10–1* segregants and the two higher values for the *MAK*$^+$ segregants. Crossing *mak3–1* [HOK-o] or *pet18–1* [HOK-o] strains with *MAK*$^+$ [HOK] strains likewise produced 2 *MAK*$^+$ [HOK] segregants with high levels of *L* and *2 mak*$^-$ [HOK-o] segregants with low levels of *L* ds RNA in each tetrad (Figure 2 and Table 6). In contrast, crossing *mak3–1*, *mak10–1*, or *pet18–1* [HOK-o] strains with [HOK-o] *L*$^+$ or [HOK-o] *L-o* strains produced segregants all of which lacked [HOK] and had the same low level of *L* ds RNA. Crosses of *mak1–1*, *mak4–1*, or *mak16–1* strains with *MAK*$^+$ [HOK] strains produced segregants all carrying [HOK] and all showing the same high level of *L* ds RNA.

In all these crosses, there is a direct correlation of the presence of [HOK] with an increase in *L* ds RNA. The *L ds* RNA present in crosses lacking [HOK] does not show dependence on *MAK3*, *MAK10*, or *PET18*. While [HOK] is found in about 60% of our nonkiller strains, the five *L-o* strains we have tested all lack [HOK]. This finding, too, is consistent with [HOK] being a double-stranded RNA the size of *L*.

DISCUSSION

[HOK], a new non-Mendelian genetic element, is defined by its ability to provide a helper function to [KIL-sd$_1$], a replication-defective killer plasmid. It is found in about 60% of our nonkiller strains. [HOK] is distinct from ρ, ψ, 2 μ DNA, [URE3], [KIL-k$_1$], [KIL-k$_2$], [EXL], and 20 S RNA. However, it is conceivable that [HOK] is an altered form of one of these elements such that its replication requirements for chromosomal genes or its sensitivity to curing agents has changed, like the [KIL-b$_1$] variant of [KIL-k$_1$] that replicates independent of several *MAK* genes (Toh-e and Wickner 1980).

[HOK] requires the products of *MAK3*, *MAK10*, and *PET18* for its maintenance or replication, but not those of ten other *MAK* genes or *MKT1*. Its dependence on *MAK3*, *MAK10*, and *PET18* suggests that it might be a ds RNA plasmid. The presence of [HOK] is clearly correlated with a four-fold increase in the cellular level of *L* ds RNA, and none of several *L-o* strains tested have [HOK]. These results are compatible with either of two hypotheses:

(*A*) [HOK] is a variety of *L* ds RNA that is compatible with (does not segregate mitotically relative to) another form of *L* ds RNA present in [HOK-o] strains. This would explain the difference in amount of *L* ds RNA in [HOK] versus [HOK-o] strains.

That a form of *L* ds RNA might have a helper function for a replication-defective *M* ds RNA is already suggested by the fact that *L* codes for the major protein of the particles in which *M* is found (Hopper *et al.* 1977; Harris 1978; Bostian, Sturgeon and Tipper 1980).

(*B*) [HOK] is not a ds RNA plasmid, but the presence of [HOK] results in a

TABLE 6

Segregation of L *ds RNA concentration in crosses with and without [HOK]*

Cross*			Segregation
mak3-1	[HOK-o] low *L* × *MAK*+ [HOK]	high *L* →	2 *MAK*+ [HOK] high *L*:2 *mak3-1* [HOK-o] low *L* (10)†
mak3-1	[HOK-o] low *L* × *MAK*+ [HOK-o]	low *L* →	4 [HOK-o] low *L*:0 (3)
mak3-1	[HOK-o] low *L* × *MAK*+ [HOK-o]	*L-o* →	4 [HOK-o] low *L*:0 (2)
mak10-1	[HOK-o] low *L* × *MAK*+ [HOK]	high *L* →	2 *MAK*+ [HOK] high *L*:2 *mak10-1* [HOK-o] low *L* (19)‡
mak10-1	[HOK-o] low *L* × *MAK*+ [HOK-o]	low *L* →	4 [HOK-o] low *L*:0 (6)
mak10-1	[HOK-o] low *L* × *MAK*+ [HOK-o]	*L-o* →	4 [HOK-o] low *L*:0 (2)
pet18-1	[HOK-o] low *L* × *MAK*+ [HOK]	high *L* →	2 *MAK*+ [HOK] high *L*:2 *pet18-1* [HOK-o] low *L* (6)
pet18-1	[HOK-o] low *L* × *MAK*+ [HOK-o]	*L-o* →	4 [HOK-o] low *L*:0 (3)
mak4-1	[HOK-o] low *L* × *MAK*+ [HOK]	high *L* →	4 [HOK] high *L*:0 (3)
mak1-1	[HOK-o] low *L* × *MAK*+ [HOK]	high *L* →	4 [HOK] high *L*:0 (5)
mak16-1	[HOK-o] low *L* × *MAK*+ [HOK]	high *L* →	4 [HOK] high *L*:0 (2)

* The *mak3-1*, *mak10-1*, *pet18-1*, *mak1-1*, *mak4-1*, and *mak16-1* strains used were strains 1633, 1598, 1066, 1616, and 1618, 748, and 1172, respectively. The *MAK*+ [HOK], *MAK*+ [HOK-o], and *MAK*+ [HOK-o] *L-o* strains were 200, 1089, and JM6, respectively. In all the crosses shown here, both parents were [KIL-o]. Spore clones were grown on YPAD plates, approximately equal amounts of each segregant were suspended in 50 mM EDTA, and ds RNA extracted by the method of FRIED and FINK (1978) without the LiCl precipitation step. The extracted material was analyzed on 1% agarose gels.

† The number of tetrads examined is shown in parentheses. The low *L* segregants were identified as the *mak3-1* segregants by the linkage of low *L* with *aro7* (PD = 9, T = 1, NPD = 0) as expected for *mak3-1* (WICKNER and LEIBOWITZ 1976).

‡ The low *L* segregants were identified as the *mak10-1* segregants by the linkage of low *L* with *can1* (PD = 18, T = 1, NPD = 0) as expected for *mak10-1* (THIEVENDIRARAJAH and BEVAN, unpublished; WICKNER and LEIBOWITZ 1976).

higher cellular concentration of *L* ds RNA. [HOK] is a helper for the [KIL-sd$_1$] plasmid and for *L* ds RNA.

At present, our data do not distinguish between these two explanations.

The dependence of [HOK] on *MAK3*, *MAK10*, and *PET18*, but not on ten other *MAK* genes, is also remarkable because mutations in these same three *MAK* genes constitute the group that are suppressed by the *ski1–1* mutation, but not by mutations in *ski2*, *ski3*, or *ski4* (TOH-E and WICKNER 1980).

It was previously shown that the cellular level of *L* depended on *MAK3* (WICKNER and LEIBOWITZ 1979). That result presumably was due to the presence of [HOK] in the cross.

LITERATURE CITED

AIGLE, M. and F. LACROUTE, 1975 Genetic aspects of [URE3] a nonmitochondrial, cytoplasmically inherited mutation in yeast. Mol. Gen. Genet. **136:** 327–335.

BEVAN, E. A., A. J. HERRING and D. J. MITCHELL, 1973 Preliminary characterization of two species of ds RNA in yeast and their relationship to the "killer" character. Nature **245:** 81–86.

BOSTIAN, K. A., J. E. HOPPER, D. T. ROGERS and D. J. TIPPER, 1980a Translational analysis of the killer-associated virus-like particle ds RNA genome of *Saccharomyces cerevisiae*: *M* ds RNA encodes toxin. Cell **19:** 403–414.

BOSTIAN, K. A., J. A. STURGEON and D. J. TIPPER, 1980b Encapcidation of yeast killer double-stranded RNAs: dependence of *M* on *L*. J. Bacteriol. **143:** 463–470.

BRUENN, J. A., 1980 Virus-like particles of yeast. Ann. Rev. Microbiol. **34:** 49–68.

BUCK, K. W., 1980 Viruses and killer factors of fungi, pp. 329–375. In: *The Eukaryotic Microbial Cell*. Edited by G. W. GOODAY, D. LLOYD and A. P. J. TRINCI. Society for General Microbiology Symposium 30. Cambridge University Press, Cambridge, England.

BUCK, K. W., P. LHOAS and B. K. STREET, 1973 Virus particles in yeast. Biochem. Soc. Trans. **1:** 1141–1142.

COHN, M. S., C. W. TABOR, H. TABOR and R. B. WICKNER, 1978 Spermidine or spermine requirement for killer double-stranded RNA plasmid replication in yeast. J. Biol. Chem. **253:** 5225–5227.

CONDE, J. and G. R. FINK, 1976 A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. Proc. Natl. Acad. Sci. U.S.A. **69:** 2846–2849.

COX, B. S., 1965 ψ, a cytoplasmic suppressor of super-suppressor in yeast. Heredity **20:** 505–521.

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.

GARVIK, B. and J. E. HABER, 1978 New cytoplasmic genetic element that controls 20S RNA synthesis during sporulation in yeast. J. Bacteriol. **134:** 261–269.

GOLDRING, E. S., L. I. GROSSMAN, D. KRUPNICK, D. R. CRYER and J. MARMUR, 1970 The petite mutation in yeast. Loss of mitochondrial deoxyribonucleic acid during induction of petites with ethidium bromide. J. Mol. Biol. **52:** 323–335.

GUERRY-KOPECKO, P. and R. B. WICKNER, 1980 Isolation and characterization of temperature-sensitive *mak* mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **144:** 1113–1118.

GUNGE, N. and K. SAKAGUCHI, 1981 Intergeneric transfer of DNA killer plasmids, pGKl1 and pGKl2, from *Kluyveromyces lactis* into *Saccharomyces cerevisiae* by cell fusion. J. Bacteriol. **147:** 155–160.

GUNGE, N., A. TAMARU, F. OZAWA and K. SAKAGUCHI, 1981 Isolation and characterization of linear DNA plasmids from *Kluyveromyces lactis* and the plasmid-associated killer character. J. Bacteriol. **145:** 382–390.

HARRIS, M S., 1978 Virus-like particles and double-stranded RNA from killer and nonkiller strains of *Saccharomyces cerevisiae*. Microbios **21:** 161–176.

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.

HOPPER, J. E., K. A. BOSTIAN, L. B. ROWE and D. J. TIPPER, 1977 Translation of the *L*-species ds RNA genome of the killer-associated virus-like particles of *Saccharomyces cerevisiae*. J. Biol. Chem. **252:** 9010–9017.

LACROUTE, F., 1971 Non-Mendelian mutation allowing ureidosuccinic acid uptake in yeast. J. Bacteriol. **106:** 519–522.

LEIBOWITZ, M. J. and R. B. WICKNER, 1978 *PET18*: a chromosomal gene required for cell growth and the maintenance of mitochondrial DNA and the killer plasmid of yeast. Mol. Gen. Genet. **165:** 115–121.

LIVINGSTON, D. M., 1977 Inheritance of the 2 μm DNA plasmid from *Saccharomyces*. Genetics **86:** 73–84.

MITCHELL, D. J., A. J. HERRING and E. A. BEVAN, 1976 The genetic control of ds RNA virus-like particles associated with *Saccharomyces cerevisiae* killer yeast. Heredity **37:** 129–134.

MORTIMER, R. K. and D. C. HAWTHORNE, 1975 Genetic mapping in yeast. pp. 221–233. In: *Methods in Cell Biology*, Vol. 11. Edited by D. M. PRESCOTT, Academic Press, New York.

NAUMOVA, T. I. and G. I. NAUMOV, 1973 Comparative genetics of yeast XII. Study of antagonistic interrelations in Saccharomyces yeast. Genetika **9:** 85–90.

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.

SCHAMHART, D. H. J., A. M. A. TEN BERGE and K. W. VAN DE POLL, 1975 Isolation of a catabolite repression mutant of yeast as a revertant of a strain that is maltose negative in the respiratory deficient state. J. Bacteriol. **121:** 747–752.

SINGH, A., C. HELMS and F. SHERMAN, 1979 Mutation of the non-Mendelian suppressor, ψ^+, in yeast by hypertonic media. Proc. Natl. Acad. Sci. U.S.A. **76:** 1952–1956.

SOMERS, J. M. and E. A. BEVAN, 1969 The inheritance of the killer character in yeast. Genet. Res. **13:** 71–83.

SWEENEY, T. K., A. TATE and G. R. FINK, 1976 A study of the transmission and structure of double-stranded RNAs associated with the killer phenomenon in *Saccharomyces cerevisiae*. Genetics **84:** 27–42.

TOH-E, A., P. GUERRY and R. B. WICKNER, 1978 Chromosomal superkiller mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **136:** 1002–1007.

TOH-E, A. and R. B. WICKNER, 1979 A mutant killer plasmid whose replication depends on a chromosomal "superkiller" mutation. Genetics **91:** 673–682. ——, 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.

VODKIN, M., 1977 Induction of yeast killer factor mutations. J. Bacteriol. **132:** 346–348.

VODKIN, M., F. KATTERMAN and G. R. FINK, 1974 Yeast killer mutants with altered double-stranded ribonucleic acid. J. Bacteriol. **117**: 681–686.

WICKNER, R. B., 1974 Chromosomal and nonchromosomal mutations affecting the "killer character" of *Saccharomyces cerevisiae*. Genetics **76**: 423–432. ——, 1978a Deletion of mitochondrial DNA bypassing a chromosomal gene needed for maintenance of the killer plasmid of yeast. Genetics **87**: 441–452. ——, 1978b Twenty-six chromosomal genes needed to maintain the killer double-stranded RNA plasmid of *Saccharomyces cerevisiae*. *Genetics* **88**: 419–425. ——, 1979 Mapping chromosomal genes of *Saccharomyces cerevisiae* using an improved genetic mapping method. Genetics **92**: 803–821. ——, 1980 Plasmids controlling exclusion of the K_2 killer double-stranded RNA plasmid of yeast. Cell **21**: 217–226. ——, 1982 Killer systems in *Saccharomyces cerevisiae*. In press. In: *The Molecular Biology of the Yeast Saccharomyces*. Edited by J. N. STRATHEM, E. W. JONES and J. BROACH, Cold Spring Harbor Press, Cold Spring Harbor, New York.

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. ——, 1979 *mak* mutants of yeast: mapping and characterization. J. Bacteriol. **140**: 154–160.

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.

Corresponding editor: F. SHERMAN