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Induction of Yeast Killer Factor Mutations

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Two related killer strains of *Saccharomyces cerevisiae* were mutagenized and screened for nonkiller variants. About 20% of the mutants derived from one strain lacked all detectable double-straned ribonucleic acid (dsRNA). About 70% of the mutants from the other strain lacked one of the dsRNA species normally associated with the killer factor and had in its place another species of dsRNA with a lower molecular weight.

Killer strains of the yeast *Saccharomyces cerevisiae* release a toxin that is lethal to sensitive strains of the same species (3, 16). Cells having the killer factor contain two distinct species of double-stranded ribonucleic acid (dsRNA) with molecular weights of 2.5×10^6 (L) and 1.1×10^6 (M) (1, 12, 13), which are encapsulated in viruslike particles (5). Genetic and physical evidence suggests that M dsRNA is necessary for ability to kill (K^+) as well as for immunity to the toxin (R^+) (12, 13). It has been difficult to ascribe functions to L dsRNA, because almost all wild-type strains of yeast investigated contain it. Moreover, nonkiller strains derived by mutagenesis retain L dsRNA as well as the viruslike particles. It is possible that L dsRNA codes for the capsid proteins of the viruslike particles.

Mutations that affect ability to kill can be classified into two categories: those with a Mendelian inheritance and those with a non-Mendelian inheritance (2, 4, 11). The former class includes ten *mak* genes, which are essential for the maintenance or replication of M dsRNA (2, 15), and two *kex* genes, which are necessary for the expression of killing (14). The latter class includes: temperature-sensitive killers, presumably point mutations of the M dsRNA; spontaneous or cycloheximide-induced nonkillers, which result from a loss of M dsRNA; suppressive nonkillers, which may be partial deletions of M dsRNA leading to a novel S dsRNA; and super killers, which have two to three times more M dsRNA than ordinary killer strains (12, 13). Strains that lack all detectable dsRNA (6, 7) are rare and do not appear in standard mutagenesis runs.

This report describes the isolation of mutants with loss of one or both dsRNA's. The absence of both dsRNA's shows a non-Mendelian mode of inheritance. Presumably the deletions should follow a similar inheritance pattern, though this parameter has not been extensively investigated.

FM11 a *ade2-1 lysl-1 his4-580 met8-1 SUP4-3[PSI$^+$]* and FM811C a *ade2-1 lysl-1 SUP4-2 [PSI$^+$]* were described previously by Rasse-Messenguy and Fink (9). PSI is an element that segregates in a non-Mendelian manner and increases the efficiency of ochre nonsense suppressors. SUP4-2 is a temperature-sensitive ochre suppressor, and SUP4-3 is a temperature-sensitive amber suppressor; SUP4-2 and 4-3 are allelles. The adenine and lysine mutations are ochre suppressible. Both strains secrete toxin, show resistance to it, and contain L and M dsRNA indistinguishable from those of other killer strains. When the above two strains are crossed to either killer or sensitive strains, the resulting diploids and their meiotic progeny have the predicted phenotypes. A8207B (K^+ R^+) and its (K^- R^-) derivatives were described previously by Vodkin et al. (13).

Growth of yeast cells and their mutagenesis by ethyl methane sulfonate were performed as described previously (10). Mutagenesis with ultraviolet light was accomplished by exposing the cells on solid media for 1.5 min to a 30-W General Electric germicidal bulb at a distance of 50 cm. At that dose, 90% of the cells were killed. The mutagenized cells were grown in darkness for 3 days before they were subjected to the screening tests. Mutagenized cells were allowed to grow on solid media (about 100 colonies per plate) for 48 to 72 h at 30°C. Each plate was printed to two others with media buffered at pH 4.8, each with a sensitive lawn (4). One replica of that series was incubated at 23°C and the other was incubated at 30°C. Variants that showed a difference in the diameter of the killing zone at one or both temperatures were picked and purified twice from contaminating cells that did not show the aberrant phenotype.

JM series of mutants. Seventy-eight var-

iants of the killer phenotype were derived from FM11 by mutagenesis with ethyl methane sulfonate. An average of 1,000 colonies were screened for each varient picked. Sixty-eight did not kill at either 30 or 23°C, eight killed poorly or not at all at 30°C but had almost normal activity at 23°C, and one was a super killer.

All of the JM nonkillers were crossed to A8207B (K^+ R^+), A8207B NKI (K^- R^-), and A8207B NK5 (K R). All diploids with A8207B as a parent were killers; only 3 of 68 nonkillers yielded diploid killers when crossed to the latter two strains. Since A8207B NKI and A8207B NK5 contain all the nuclear genes necessary for maintenance and expression of the killer phenotype, the three strains were probably defective in one of the *kex* genes. The other 65 strains could have a mutation in either one of the *mak* genes or in the dsRNA itself.

To characterize these mutants further, dsRNA from each strain was extracted and analyzed. Table 1 summarizes the gel patterns of a number of JM strains that were examined. The results agree with earlier reports in the literature (13). All strains that kill (including weak killers and the super killer) have both L and M dsRNA. All strains that contained only L dsRNA had grossly altered quantities of it, as compared to the parental killer strain.

In addition to the nonkillers with altered dsRNA composition, there is a class lacking all detectable dsRNA. Mitchell et al. (6) have also reported that such mutants can be induced with 5-flurouracil. When these strains lacking dsRNA are crossed to our tester killer strain, A8207B, the diploid retains the ability to produce toxin. Genetic analysis of such diploids from crosses of two of these independent nonkillers revealed a 4:0 killer-nonkiller ratio. Thus, the [KIL-o] strains had all the nuclear genes necessary for the maintenance of the M dsRNA, and the original lesion was either the deletion of dsRNA's of their inability to replicate.

TABLE 1. *Summary of gel patterns from JM strains*[a]

Strain	Growth in YPG[b]	Killing	L band	M band
JM 6, 7, 9, 15, and 17	+	–	–	–
JM 1	–	–	–	–
JM 2, 11, 20, 25, 40 and A8207B NK5	+	–	Light	–
JM 33	–	–	Light	–
JM 12, 14, 37	–	–	+	+
JM 22 and 27	–	–	+	+
JM 3, 10, 16, 29, and 30	+	Weak	+	+
A8207B and FM11	+	+	+	+
JM 13	+	Super	+	+
JM 4, 18, 21, 26, 28 and A8207B NKI	+	–	Dark	–
JM 32	–	–	Dark	–

[a] dsRNA was extracted from 1.5 g (wet weight) of cells and analyzed by electrophoresis on 5% polyacrylamide gels (13). The intensity of staining of the L band was visually compared with our three tester strains, A8207B, A8207B NKI, and A8207B NK5, which contain x, $3x$, and $0.33x$ amounts of L dsRNA, respectively. The amount of L dsRNA in the parent strain of this study, FM11, could not be visually distinguished on gels from that of A8207B.

[b] YPG is a medium with glycerol as the sole carbon source. It selects against the growth of cells lacking mitochondrial function.

LO series of mutants. Twenty-three variants of the killer were derived by ultraviolet light mutagenesis from FM811C. An average of 600 colonies were screened for each variant picked. Nineteen failed to kill at either 30 or 23°C; four killed poorly or not at all at 30°C and had normal or reduced activity at 23°C. When dsRNA was extracted from these strains and analyzed, a very high proportion (about 70%) showed the S dsRNA with its reduced molecular weight. The gel patterns are summarized in Table 2. The molecular weights of the S RNA varied from 3.7×10^5 to 10×10^5 based on their migration in polyacrylamide gels. When these strains were crossed to the tester killer, many of the diploids segregated nonkillers at a high frequency. These dsRNA's resemble the suppressive dsRNA's, which are "dominant" to the wild-type killer.

Nesterova et al. (8) have reported that strains selected for ochre suppressors lose the ability to kill. FM811C carries a temperature-sensitive ochre suppressor and retains the ability to kill. It is possible to select from FM811C strains that show temperature-independent suppression. All 10 colonies selected on minimal medium at 30°C failed to kill, whereas all 12 selected at 30°C on minimal medium supplemented with adenine and lysine retained the ability to kill. It is possible that either the more

TABLE 2. *Summary of gel patterns from LO series*

Strain	Growth in YPG[a]	Killing	L band	M band	S band
LO 2, 4, 6, 9, 12, 16, 17, 21, 23, and 24	+	–	+	–	+
LO 14, 18, and 19	–	–	+	–	+
LO 15	+	–	+	Light	+
LO 1, 13, and 22	–	–	Light	–	–
LO 11 and 20	+	–	+	+	–
LO 5	–	–	+	+	–
FM811C	+	+	+	+	–
LO 10	+	–	Heavy	–	–

[a] See footnote *b*, Table 1.

efficient suppression per se or the stress to protein synthesis caused the loss of ability to kill.

Two related strains have been mutagenized, and the nonkillers resulting from mutagenesis were analyzed for their dsRNA composition. In both cases, a very high frequency of the mutants showed alterations in the dsRNA itself. The frequency of this event was much higher than that found in previous studies. For instance, none of the hundreds of nonkillers derived from A8207B with ethylmethane sulfonate in the same way as in this study lacked both dsRNA's. Suppressive nonkillers have been obtained from A8209B – at a frequency of about 10 to 15%. In this study they represent 67% of the nonkillers derived from FM811C.

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