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"Killer" Character Does Not Influence the Transmission of Mitochondrial Genes in *Saccharomyces cerevisiae*

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Three cytoplasmic genetic elements have been shown to be separate from mitochondrial deoxyribonucleic acid (DNA), [*rho*], in *Saccharomyces cerevisiae*: the killer character [*k*], omicron-DNA, and psi [ψ]. Griffiths has suggested genetic interactions between [*VEN*^{R}] and [*TET*^{R}] mutants possibly located on omicron-DNA and mitochondrial genetic markers, but possible interactions between the best characterized of the three, the killer character, and mitochondrial DNA have not been investigated. To test this we isolated cycloheximide-induced nonkiller segregants (NKS) of killer cells with suitable genetic markers and mated them in [*k*] × [*k*], [*k*] × (NKS), (NKS) × [*k*], and (NKS) × (NKS) combinations. No differences in quantitative mitochondrial marker transmission between these groups were found in crosses illustrating the mitochondrial phenomena of bias, polarity, and suppressiveness. Our studies show that no intercellular interactions between [*k*] and (NKS) cells influence mitochondrial transmission genetics. Intracellular interactions between the smaller double-stranded ribonucleic acid of [*k*] and mitochondrial DNA also were not detected.

The transmission genetics of mitochondrial genes in the single-celled eukaryote *Saccharomyces cerevisiae* is currently a very active area of investigation in many laboratories (reviewed in 14 and in C. W. Birky, Jr., *in* C. W. Birky, P. S. Perlman, and T. J. Byers [ed.], *First Ohio State University Biosciences Colloquium*, in press). Several distinct phenomena have been reported, including suppressiveness (of petites) (10–12), recombinational polarity (4, 7, 9), and transmissional bias (9, 27); however, the cellular and molecular bases of each of these phenomena remain to be elaborated.

The genetic nomenclature used in this paper is taken from Sherman and Lawrence (23). At least three cytoplasmic genetic elements have been found to be separate from mitochondrial deoxyribonucleic acid, [*rho*]; these are the [*VEN*^{R}] and [*TET*^{R}] mutants found by Lancashire and Griffiths (18, 19), which are possibly located on omicron-deoxyribonucleic acid (6, 16; D. E. Griffiths, *in* C. W. Birky, P. S. Perlman, and T. J. Byers [ed.], *First Ohio State University Biosciences Colloquium*, in press), the cytoplasmic suppressor of super-suppressor [ψ] (8, 29, 30), and the killer character [*k*] described by Bevan and Somers (3, 24). Of these three the killer character is the best characterized (1, 2, 13, 25, 28). Cells with the killer character [*k*] have the phenotype of killing sensitive (*s*) cells which lack the killer factor; killer cells are themselves immune to the action of other killers (3, 24). The immunity and toxin functions are associated with two species of double-stranded ribonucleic acid, a heavy component (L) and a lighter component (M). The M component appears to control toxin production (1, 2, 25). Killing is mediated through a diffusable substance that kills sensitive cells and is under the control of at least one nuclear gene (28).

Griffiths has suggested that genetic interactions occur between the [*VEN*^{R}] and [*TET*^{R}] mutants and the mitochondrial genetic markers (*In* Birky et al. [ed.], in press). Possible interactions between each of the others and mitochondrial deoxyribonucleic acid have not been investigated; this study was undertaken to assess whether any interactions might occur between the killer character and mitochondrial deoxyribonucleic acid. These could involve (i) interaction between cells or (ii) interactions between genomes within a cell. In the first case it is possible that the toxin produced by the killer parent might alter a sensitive mating partner physiologically and thus interfere with processes required for normal mitochondrial interactions. This possibility merits serious attention, because it is already known that the cellular physiology of mating partners (15) and cell-cell

interactions during mating involving diffusable mating factors (C. A. Demko, Master's thesis, The Ohio State University, Columbus, 1975) both have significant effects on quantitative mitochondrial marker transmission (bias). In the second case different cytoplasmic genomes could interact by competing for substrates, enzymes, or replication sites.

In this paper we describe our studies, which eliminate the possibility that interactions between killer and sensitive cells are a factor in bias, polarity, and suppressiveness. To accomplish this, we have isolated nonkiller, sensitive (NKS) segregants of killer cells having suitable mitochondrial genetic markers and have mated them in [*k*] × [*k*], [*k*] × (NKS), (NKS) × [*k*], and (NKS) × (NKS) combinations. Since these crosses are identical except for the killer phenotype we could detect killer-induced alterations in the transmission of mitochondrial genes. (Without more detailed characterization it cannot be stated that the NKS segregants are of the genotype [*s*]; however they are clearly nonkillers that are killed by killers and so could not affect a mating partner via the killer toxin.)

MATERIALS AND METHODS

Strains. The strain designations, nuclear and mitochondrial genotypes, and sources of strains used in this study are listed in Table 1. E10-10/10 is a cytoplasmic petite mutant induced by ethidium bromide mutagenesis of strain IL16-10B from Slonimski's collection.

Media. The basic medium contains 0.2% yeast extract, 0.1% KH_2PO_4 (pH 6.5), 0.1% $(NH_4)_2SO_4$, 0.024% $MgSO_4$, and 0.05% NaCl. One percent dextrose was added for YD medium, and 4% glycerol was added for YG medium. For solid media, 2% agar was added. Minimal medium with 1% dextrose (MD) was prepared as described in reference 26. Cells were tested for resistance/sensitivity to antibiotics on YG plates supplemented with one or more drugs at the following concentrations: 0.5% erythromycin (ERY), 0.3% chloramphenicol (CHL), 0.0002% oligomycin (OLI), or 0.3% paromomycin (PAR). Low pH plates containing methylene blue and YG plates containing cycloheximide were made as described by Fink and Styles (13).

Killer phenotype test. Strains were scored for the killer phenotype by the method of Fink and Styles (13). Strains were tested by spotting a suspension of cells (10^6) onto a lawn of 5×10^6 cells; in every case strains were tested with lawns of killer or sensitive cells of the same cellular mating type. Sensitive cells on a killer lawn turn blue, whereas killer cells on a sensitive lawn produce a clear halo surrounded by a ring of blue.

Curing of killer. Killer strains were cured by treatment with cycloheximide on solid media as described by Fink and Styles (13). Those strains which we will refer to as NKS are cycloheximide-induced nonkillers. In all respects they resemble those sensitive cells described by Fink and Styles and by Vodkin et al. (13, 25). Our data show each strain used to be lacking in immunity, ability to kill, and the suppression of killer phenotype; this eliminates the possibility that any of these cycloheximide nonkillers (NKS) might be any of the types of killer mutants described by Vodkin et al. (25).

Mating conditions. Exponential cells grown on YG were mated in YD at 10^7/ml; they were mated at 30 C with shaking until zygotes were observed (usually 2 to 3 h) and then were plated onto MD medium for selection of prototrophic diploids. After 3 days of incubation at 30 C, the cells were suspended in 1 mM ethylenediaminetetraacetic acid, diluted, and replated onto MD medium; after 2 to 3 days, the resulting colonies (random diploid progeny of the cross) were replica plated onto drug plates and scored

TABLE 1. *Strains of S. cerevisiae used in this study*

Strain designation	Phenotype	Nuclear genotype	Mitochondrial genotype					Source
			[*ome*]	[*CHL*]	[*ERY*]	[OLI_1]	[*PAR*]	
2-36-1	Killer	α *try-1*	+	R1	R1	S	R1	R. Kleese
4810-1	Killer	a *lys-1*	+	S	S	S	R1	R. Kleese
D-22-5	Killer	a *ade-2*	+	S	S	S	S	D. Wilkie
(32)1-1/1	Sensitive	α *ade-2*	−	R321	S	R4	S	P. Perlman
E10-10/10	Sensitive (neutral)[a]	α *his*	−	R321	S	0[b]	0	P. Perlman
A364-A[c]	Killer	α *ade-1, ade-2, his-7, lys-2, tyr-1, ura-1*	+	S	S	S	S	L. Hartwell
D243-4A[c]	Sensitive	a *ade, lys*	+	S	S	S	S	R. Criddle
D6[c]	Sensitive	α *arg, met*	+	S	S	S	S	D. Wilkie

[a] This petite strain was isolated from a wild-type parent (IL16-10B) that is sensitive to killer; the petite, however, is not killed under conditions where a sensitive is. It is not genetically neutral.

[b] The allele designation "0" signifies the absence of the locus from the petite mitochondrial genome.

[c] Used as tester strains in the phenotype test only.

for resistance or sensitivity to each of the drugs. In the crosses involving the petite mutant, it was grown to exponential phase in YD and mated as above; the resulting zygotic clones were then scored for respiratory competence by the tetrazolium overlay method (20). Only pure petite clones were scored as petite. For each mating experiment, controls were performed to test for revertants for the auxotrophic nuclear markers, spontaneous petites in the parental cultures, and retention of the killer character.

Experimental design. The basic experiment was to cross two strains isogenic but differing in the killer character with two similarly isogenic strains. All four possible combinations are thus made: [*k*] × [*k*], [*k*] × (NKS), (NKS) × [*k*], and (NKS) × (NKS). Each cross was then analyzed for the quantitative transmission of mitochondrial markers. If there were an effect of the killer character, we expect the progeny of [*k*] × (NKS) and (NKS) × [*k*] crosses to differ between themselves and between [*k*] × [*k*] and (NKS) × (NKS) crosses.

Chemicals. Chloramphenicol, cycloheximide, erythromycin, oligomycin (15% oligomycin A and 85% oligomycin B), and 2,3,5-triphenyl tetrazolium chloride were purchased from Sigma Chemical Co., St. Louis, Mo. Paromomycin sulfate was donated by Parke, Davis and Co., Detroit, Mich.

RESULTS AND DISCUSSION

Upon screening of our collection of yeast strains, several were found to be killer (Table 1). These were cured to form isogenic (NKS) strains (Table 2). These stocks were then used to assess the effect of killer on bias, polarity, and suppressiveness.

Bias is the preferential transmission of all mitochondrial markers from one parent in a cross, regardless of their linkage to their [*ome*] allele (9, 27; Birky, *in* Birky et al., in press). This can be most easily observed in homopolar crosses in which both parents have the same [*ome*] allele. The results of one set of homopolar crosses are presented in Table 3. This cross utilizes the strains 2-36 and 4810, which are both [*ome*+]. Drug resistance markers for chloramphenicol and erythromycin resistance/sensitivity were used to assess mitochondrial transmission. No significant systematic differences were found among the four matings in terms of recombination frequency or level of marker transmission. This cross is an unbiased (or weakly biased) one according to Slonimski's

TABLE 2. *Cycloheximide curing of killer*

Strain	Cycloheximide concn (mg/liter)[a]	Phenotype of treated cells			% Curing of [*k*]	Phenotype of control cells	
		[*k*]	[*k*] + (NKS)[b]	(NKS)		[*k*]	(NKS)
2-36	0.665	4	2	16	67	11	1
	0.332	9	0	11	55		
4810	0.332	5[c]	1	6	50	12	0
D22-5	0.0066	2	0	10	83	12	0

[a] Since different yeast stocks varied in their sensitivity to cycloheximide, it was necessary to use a different concentration in each case.

[b] Some treated cells yielded mixed progeny and were subcloned further.

[c] One of these subclones were found to have become diploid; all subclones used in these studies were shown to be haploid.

TABLE 3. *Homopolar cross 4810 α[ome+ CHL^S ERY^S PAR^R] × 2-36 α[ome+ CHL^R ERY^R PAR^R]*

Parental phenotypes	Genotypes (%) [*CHL ERY*]					% Recombinants	Single marker transmission	
	N	R R	S S	R S	S R		[*CHL^R*]	[*ERY^R*]
4810 [*k*] 2-36 [*k*]	303	57.8	35.0	3.6	3.6	7.2	6.14 ± 5.5[a]	61.4 ± 5.5
4810 [*k*] 2-36 (NKS)	549	49.7	41.5	4.6	4.2	8.8	54.3 ± 4.2	53.9 ± 4.2
4810 (NKS) 2-36 [*k*]	1,206	46.4	44.9	3.4	5.4	8.8	49.8 ± 2.8	51.8 ± 2.8
4810 (NKS) 2-36 (NKS)	973	45.5	47.8	2.8	3.9	6.7	48.3 ± 3.1	49.4 ± 3.1

[a] Ninety-five percent confidence interval.

definition; the presence of killer in one parent did not induce any bias in the cross, compared to the (NKS) × (NKS) cross. Diploid progeny of [*k*] × [*k*], [*k*] × (NKS), and (NKS) × [*k*] crosses were found to be all killer, whereas those of the (NKS) × (NKS) cross were found to have the sensitive phenotype.

To confirm and extend these results, another homopolar cross was analyzed as shown in Table 4. This cross utilizes a third strain, D22-5, and a third mitchondrial marker (for paromomycin resistance/sensitivity). This cross is highly biased for transmission of markers from the α parent, 2-36. Again, no differences were found among the four crosses, and we conclude that killer is not a factor in determining the mitotic segregation or recombination of mitochondrial drug resistance/sensitivity markers, and it does not result in bias. Whereas it has been shown that the alpha mating type locus may result in bias when its effect on crosses is isolated (5), these data clearly show that bias is more complex than that; in crosses shown in Tables 3 and 4 the same alpha parent was used in each case, but there was bias only in one set of crosses.

Polarity is a different transmissional phenomenon from bias. As first shown by Slonimski's group (4, 7, 9) and later confirmed by Linnane's (17) and ours (21), polarity is controlled primarily by the mitochondrial locus [*ome*], which exists in two allelic forms, [*ome*+] and [*ome*−]. In a heteropolar cross ([*ome*+] × [*ome*−]) preferential recovery of markers from the [*ome*+] parent is observed, which is greatest for the [*ome*+]-linked [*CHL*] allele, less for the [*ome*+]-linked [*ERY*] allele, and is absent for the [*ome*+]-linked [*OLI*] and/or [*PAR*] alleles; this gradient of marker transmission has been interpreted as being a consequence of the degree of linkage of these markers to [*ome*+] (4, 7, 9). Recombinant progeny containing the [*ome*−] [*CHL*] allele are rare, and for each recombinational interval only one of the two possible recombinants, the one with the [*ome*+]-linked [*CHL*] allele, is recovered primarily. To test the effect of killer on this phenomenon, a heteropolar cross was analyzed and the results are presented in Table 5. In these crosses strain 4810 was crossed with [*ome*−] strain 1-1/1, and transmission of the markers [*CHL*], [*OLI*], and [*PAR*] was measured. Since an effect of killer would be seen by comparing the [*k*] × (NKS) and (NKS) × (NKS) crosses, the additional crosses with an [*ome*-] killer strain were not done in this case. No difference was found in either the single-marker transmission levels or polarity of recombination. On the basis of these

TABLE 4. *Homopolar cross 2-36 α[ome+ CHL^R ERY^R PAR^R] × D-22-5 a[ome+ CHL^S ERY^S PAR^S]*

Parental phenotypes	Genotypes (%) [*CHL ERY PAR*]									% Recombinants	Single marker transmission		
	N	RRR	SSS	RSS	SRR	RRS	SSR	RSR	SRS		[*CHL^R*]	[*ERY^R*]	[*PAR^R*]
D-22-5-[*k*] 2-36-[*k*]	609	14.8	63.1	3.3	1.3	9.4	5.1	1.0	2.1	22.2	26.6 ± 3.5[a]	25.8 ± 3.5	22.5 ± 3.3
D-22-5-[*k*] 2-36-(NKS)	613	13.5	69.7	1.6	1.5	5.9	5.5	0.7	1.6	16.8	22.2 ± 3.3	22.0 ± 3.3	21.2 ± 3.2
D-22-5-(NKS) 2-36-(*k*)	629	10.7	62.8	4.1	2.5	7.6	5.9	2.7	3.7	26.5	25.1 ± 3.4	24.5 ± 3.4	20.2 ± 3.1
D-22-5-(NKS) 2-36-(NKS)	433	17.1	60.3	3.7	1.2	6.7	6.5	1.6	3.0	22.6	30.0 ± 4.3	27.3 ± 4.2	26.3 ± 4.1

[a] Ninety-five percent confidence interval.

TABLE 5. *Heteropolar cross 4810 a[ome+ CHL^S OLI^S PAR^R] × 1-1/1 α[ome− CHL^R OLI^R PAR^S]*

4810 Phenotypes	Genotypes (%) [*CHL OLI PAR*]									Single marker transmission		
	N	SSR	RRS	SRS	RSR	SSS	RRR	SRR	RSS	[*CHL^R*]	[*OLI^R*]	[*PAR^R*]
Killer	552	18.3	22.6	44.9	0.0	7.6	0.5	5.8	0.2	23.4 ± 3.5[a]	73.9 ± 3.8	75.3 ± 3.6
NKS	624	14.7	20.5	51.3	0.0	6.7	0.6	5.6	0.5	21.6 ± 3.2	78.0 ± 3.3	79.0 ± 3.2

[a] Ninety-five percent confidence interval.

results we conclude that killer is not a factor in polarity. This cross is also a biased one in which high transmission of nonpolar markers from the [*ome*−] parent (1-1/1) was observed; each nonpolar marker was transmitted to the same extent, whereas transmission of the [*ome*−] [*CHL*] allele was reduced.

Suppressiveness is the recovery of petite progeny from a cross of a mitochondrial deoxyribonucleic acid-containing petite with a wild type. The greater the suppressiveness of the petite parent, the greater the proportion of petite progeny (10, 12). The effect of killer on this phenomenon was tested using the suppressive petite E10-10/10; suppressiveness was scored as described above and was expressed using the equation of Sherman and Ephrussi (22). Killer does not interact with the suppressive factor (Table 6).

Conclusions. We have presented an analysis of the possible effects of the non-Mendelian killer factor on the transmission of mitochondrial genes in *S. cerevisiae*. We conclude that intercellular interactions between killer and sensitive cells do not influence the phenomena of mitochondrial transmission illustrated in the crosses made.

We cannot unambiguously rule out intracellular interactions. Vodkin et al. (25) have shown that all cycloheximide nonkiller segregants which they tested lacked the M component of double-stranded ribonucleic acid but have increased amounts of the L component. Therefore intracellular interaction between the smaller double-stranded ribonucleic acid and the mitochondrial genome has been eliminated. If our strains resemble those described by Vodkin et al. (25), then the large component was present in our (NKS) strains but in increased amount; therefore, the ratio of this component to the mitochondrial genome varied in the [*k*] × [*k*] and [*k*] × (NKS) crosses. Since mitochondrial gene dosage alterations of this magnitude do affect the quantitative transmission of mitochondrial genetic markers (Demko, Master's thesis, The Ohio State University, Columbus, 1975), we then conclude, tentatively, that the L component does not interact with mitochondrial genetic processes; however, studies with strains lacking the L component would be required to strengthen this conclusion.

TABLE 6. *Suppressive cross [rho+]4810a [ome+ CHL^S PAR^R] × [rho−]E10-10/10α[ome− CHL^R PAR^O]*

4810 Phenotype	N	% Suppressiveness
Killer	506	39.2 ± 4.3[a]
NKS	517	41.8 ± 4.3

[a] Ninety-five percent confidence interval.

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