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Somatic segregation of the killer (k) and neutral (n) cytoplasmic genetic determinants in yeast

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1. INTRODUCTION

Strains of the yeast *Saccharomyces cerevisiae* show one of three phenotypes with respect to the killer character, namely killer, sensitive or neutral (Bevan & Makower, 1963). Killer cells release an unstable macromolecular protein, the 'killer factor', into the culture medium, which brings about the death of sensitive cells (Woods & Bevan, 1968). Neutral cells are not killed by killer cells, nor do they kill sensitive cells.

A genetic analysis of the three phenotypes (Somers & Bevan, 1969) has shown that the killer character is under the control of two types of cytoplasmic genetic determinants, (k) and (n). The presence of (k) confers the killer phenotype, and that of (n) the neutral phenotype. (k) and (n) are maintained in the cell only in the presence of the dominant nuclear allele *M*. The genotype of killer cells is therefore represented as *M*(k), and that of neutral cells as *M*(n). The absence of both types of determinant, (o), confers the sensitive phenotype, regardless of the nuclear genotype. Hence the genotype of sensitive cells may be either *M*(o) or *m*(o).

According to this model, a killer by neutral cross $M(k) \times M(n)$ is expected to yield a diploid cell of genotype *MM*(k)(n). If both types of determinant are maintained in the cell and are independent in action, the resulting diploid culture is expected to show the killer phenotype. The presence of (n) determinants would not be directly apparent; their presence gives only immunity to the killer factor, whereas the presence of (k) determinants confers both immunity and the killer phenotype. If during cell division of this diploid culture the (k) and (n) determinants are distributed randomly between daughter cells, a continuous range in the proportions of (k) and (n) in daughter diploid cells is to be expected. Moreover, their random distribution at meiosis may be expected to result in the recovery of the complete range of tetrad phenotypic ratios from 4 killer:0 neutral through to 0 killer:4 neutral. Again, the presence of (n) in spore-derived cultures of the killer phenotype would not be directly apparent.

The results of the killer by neutral crosses performed by Somers & Bevan (1969) showed that diploid cultures of *MM*(k)(n) genotype had the killer phenotype and

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yielded a complete range of tetrad phenotypic ratios: however, no attempt was made to demonstrate the presence of (n) determinants in the killer diploid cultures or in the spore-derived cultures showing the killer phenotype. Such a demonstration would provide strong support for the genetic model already proposed (Somers & Bevan 1969). The aim of the present work is to show that the results of the killer by neutral crosses can be satisfactorily accounted for by the segregation pattern of (k) and (n) cytoplasmic determinants amongst mitotically and meiotically dividing diploid cells.

Since cytoplasmic genetic determinants are not expected to be distributed between daughter cells with the precision of chromosomal genes a culture of killer diploid cells of genotype *MM*(k)(n) may, in addition to yielding *MM*(k)(n) daughter cells, be expected to yield cells of genotype *MM*(k), *MM*(n) and *MM*(o), possessing killer, neutral and sensitive phenotypes respectively. The cells of genotype *MM*(k) would yield killer colonies indistinguishable (at least qualitatively) from those of genotype *MM*(k)(n), except for the ability of the latter to segregate in further divisions.

In the work to be described two killer by neutral crosses were performed. Cells of the parental haploid cultures, the diploid, and the spore cultures obtained, were plated out, allowed to form colonies, and their phenotypes determined.

2. MATERIALS AND METHODS

(i) *Strains.* Wild-type killer strains K_1 and K_2, both of mating type α, and wild-type neutral strain N_1, mating type a (Somers & Bevan 1969), were the parental strains of the two crosses.

(ii) *Media and methods.* The composition of the media used, the methods of genetic crossing, tetrad analyses and phenotype testing were as described by Somers & Bevan (1969).

3. RESULTS

(i) *Genetic crosses and tetrad analyses*

Three-day-old single cell cultures of strains K_1, K_2 and N_1 were used. The results of the two crosses are shown in Table 1. The diploid culture resulting from the cross $K_1 \times N_1$ yielded the complete range of spore phenotypic ratios, whereas that resulting from $K_2 \times N_1$ gave only 4K:0 N and 3 K:1 N ratios. In all tetrads, normal gene segregation occurred with respect to the mating type locus a/α.

Table 1. *The results of killer by neutral crosses* $K_1 \times N_1$ *and* $K_2 \times N_1$

(K = killer phenotype; N = neutral phenotype.)

Parental strains crossed	Diploid phenotype	Total no. of tetrads analysed	No. of each type of tetrad obtained				
			4 K:0 N	3 K:1 N	2 K:2 N	1 K:3 N	0 K:4 N
$K_1 \times N_1$	Killer	26	13	5	1	4	3
$K_2 \times N_1$	Killer	24	22	2	0	0	0

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(ii) *Cell platings to test for somatic segregation*

The results of the cell platings of the diploid resulting from the cross $K_1 \times N_1$ are shown in Table 2. The killer and neutral parental haploid clones gave only killer and neutral colonies respectively, while the killer diploid clone yielded 92·2 % killer and 7·8 % neutral colonies. The presence of (n) determinants amongst

Table 2. *The results of the cell platings from the cross* $K_1 \times N_1$

Clone	Total no. of colonies tested	Colonies obtained with the phenotypes (%)		
		Killer	Neutral	Sensitive
K_1 parent	2348	100	0	0
N_1 parent	2640	0	100	0
Killer diploid	1879	92·2	7·8	0
Spore cultures of one 4 K:0 N tetrad				
A, killer	504	63·9	36·1	0
B, killer	681	84·2	15·8	0
C, killer	606	15·4	84·6	0
D, killer	470	60·2	39·8	0
Spore cultures of one 0K:4 N tetrad				
A, neutral	585	0	100	0
B, neutral	1204	0	100	0
C, neutral	835	0	100	0
D, neutral	859	0	100	0

Table 3. *The results of cell platings from the cross* $K_2 \times N_1$

Clone	Total no. of colonies tested	Colonies obtained with the phenotypes (%)		
		Killer	Neutral	Sensitive
K_2 parent	2417	100	0	0
N_1 parent	2640	0	100	0
Killer diploid	1825	100	0	0
Spore cultures of one 4 K:0 N tetrad				
A, killer	674	20·5	79·5	0
B, killer	603	90·4	9·6	0
C, killer	660	99·7	0·3	0
D, killer	603	96·5	3·5	0

the cells of this killer diploid clone is therefore indicated. Cells from each of the four spore cultures from one of the 4 K:0 N tetrads when plated on complete medium gave percentages of neutral colonies ranging from 15·8 to 84·6. However, it will be noted that no killer colonies were detected when each of the four spore cultures of a 0 K:4 N tetrad were plated out.

The results of the cell platings from the diploid clone resulting from the cross $K_2 \times N_1$ are shown in Table 3. Again, the killer and neutral parental haploid clones gave only killer and neutral colonies respectively. But here no neutral diploid colonies were obtained after plating out the killer diploid clone. However, on

plating out samples of cells from each of the four killer spore cultures of a 4 K:0 N tetrad derived from this diploid, each of them gave a number of neutral colonies, ranging in percentages from 0·3 to 79·5. Thus, here again the presence of (n) determinants in cells of the killer spore cultures is indicated.

No sensitive colonies were detected during these studies.

(iii) *The correlation between diploid phenotype and tetrad phenotypic ratios*

The results given above show that both (k) and (n) determinants are present in cells of killer diploid cultures derived from killer by neutral crosses, and that these determinants undergo somatic segregation during the division of the diploid cells. The results also show that such diploid clones may yield the complete range of tetrad phenotypic ratios from 4 K:0 N to 0 K:4 N. It therefore appears likely

Table 4. *The results of tetrad analysing three diploid colonies derived from a diploid clone obtained from the cross* $K_1 \times N_1$

Phenotype of diploid colony	Total no. of tetrads analysed	No. of each type of tetrad obtained				
		4 K:0 N	3 K:1 N	2 K:2 N	1 K:3 N	0 K:4 N
Strong killer	10	9	1	0	0	0
Weak killer	10	3	1	1	2	3
Neutral	7	0	0	0	0	7

K = killer phenotype; N = neutral phenotype.

that this range in tetrad ratios is a reflexion of the cytoplasmic constitution of each individual diploid cell undergoing meiosis. A diploid cell containing a relatively high proportion of (k) determinants is expected to yield a majority of 4 K:0 N tetrads, whereas a diploid cell containing only (n) determinants would yield only 0 K:4 N ratios. Furthermore, it was observed during phenotype testing of cell platings of killer diploid clones that there were differences in the 'strength' of the killer phenotype amongst different killer colonies: some colonies gave wide killing zones on the sensitive background plates, while others gave intermediate or very weak reactions. Within limits it was therefore possible to distinguish colonies whose cells contained relatively high proportions of (k) determinants (strong killers) from those containing relatively high proportions of (n) determinants (weak killers). Consequently, on further division it was expected that strong killer diploids would give high proportions of killer spores, and weak killer diploids high proportions of neutral spores.

Cells of the diploid resulting from the cross $K_1 \times N_1$ were plated out and from the resulting colonies one strong killer, one weak killer and one neutral diploid colony were selected, their cells sporulated and tetrad analysed. The results are shown in Table 4. It will be seen that the strong killer colony gave only 4 K:0 N and 3 K:1 N tetrads, the weak killer the complete range, and the neutral colony gave only 0 K:4 N ratios.

The corresponding studies on the diploid colonies derived from the diploid

which resulted from the cross $K_2 \times N_1$ (Table 5) again showed the same correlation between the strength of the killer phenotype of the diploid colony and the proportion of killer spores recovered.

A similar range in the strength of the killer phenotype of all the haploid spore cultures obtained from the two types of killer by neutral diploids was also noted. Further, here again, there was a general correlation between the strength of the killer phenotype of any spore culture and the proportion of neutral colonies obtained after cell platings. For example, spore culture C in Table 2 was a weak killer, as was spore culture A in Table 3.

Table 5. *The results of tetrad analysing three diploid colonies derived from a diploid clone obtained from the cross* $K_2 \times N_1$

Phenotype of diploid colony	Total no. of tetrads analysed	No. of each type of tetrad obtained				
		4 K:0 N	3 K:1 N	2 K:2 N	1 K:3 N	0 K:4 N
Strong killer	8	8	0	0	0	0
Weak killer	12	1	4	0	1	6
Weak killer	8	1	1	0	1	5

K = killer phenotype; N = neutral phenotype.

(iv) *Variability between repeated crosses of cells of the same haploid clones*

It has been observed that the diploid clone obtained from the cross $K_1 \times N_1$ gave a number of neutral colonies on plating out, and that the killer spore cultures on further division yielded high proportions of neutral colonies. On the other hand, the diploid clone obtained from the cross $K_2 \times N_1$ gave no neutral colonies on plating out and three of the four killer spore cultures tested gave only a very low proportion of neutral colonies. Further, the former cross gave rise to a higher proportion of neutral spore cultures than the latter. If, as suggested by the results shown in Tables 2 and 3, the cells of the two haploid killer parental strains used in the crosses contained no (n) determinants, then it is expected that the differences between the results of these two crosses are due simply to the relative numbers of (k) and (n) determinants contributed to the original diploid cell by the killer and neutral haploid parental cells respectively. If this is the case, then further matings may show that no over-all differences exist between the results of the crosses $K_1 \times N_1$ and $K_2 \times N_1$.

Table 6 shows the results of the tetrad analyses of four further diploid cultures of each cross, together with the results of the two original crosses reported earlier. Each diploid culture analysed was derived from single diploid cells obtained from separate mating figures. From a total of 116 tetrads derived from $K_1 \times N_1$ crosses, 14 (12·1 %) contained two or more neutral spore cultures. On the other hand, of ninety-six tetrads derived from $K_2 \times N_1$ crosses, none contained two or more neutral spore cultures. It is possible therefore that some difference does exist between the haploid parental killer strains K_1 and K_2, although the possibility remains that the tetrad analyses of more diploids would demonstrate that this

difference is not a real one. However, should it be so, it would indicate that different haploid killer and neutral strains may contain different numbers of cytoplasmic determinants.

Table 6. *The results of tetrad analysing the original and derived killer diploid clones obtained from the crosses* $K_1 \times N_1$ *and* $K_2 \times N_1$ *respectively*

Cross	Diploid clone	Total no. of tetrads analysed	No. of each type of tetrad obtained				
			4 K:0 N	3 K:1 N	2 K:2 N	1 K:3 N	0 K:4 N
$K_1 \times N_1$	1	18	17	1	0	0	0
	2	20	12	4	4	0	0
	3	24	19	4	1	0	0
	4	27	21	5	1	0	0
	Original	26	13	5	1	4	3
$K_2 \times N_1$	1	22	22	0	0	0	0
	2	19	18	1	0	0	0
	3	13	12	1	0	0	0
	4	18	15	3	0	0	0
	Original	24	22	2	0	0	0

K = killer phenotype; N = neutral phenotype.

4. DISCUSSION

Previous genetic studies (Somers & Bevan 1969) indicated that the difference between killer and neutral strains resided solely in the cytoplasm. Twenty-two sensitive strains were analysed and each had a genotype such that they could maintain either both or neither type of cytoplasmic determinant when introduced by crossing. Similar studies of rare sensitive cells which arose spontaneously from killer strains showed that these cells have a nuclear genotype which is subsequently able to express the neutral phenotype when (n) determinants are introduced by crossing (Somers, 1968). To date, therefore, no nuclear gene differences between killer and neutral strains have been detected.

The present studies have shown that the diploid, and haploid spore-derived, cultures resulting from killer by neutral crosses contain cells which give neutral colonies in frequencies ranging from 0·3 to 84·6 %. Such high frequencies of neutral cells in mitotically dividing killer cultures cannot readily be accounted for on the basis of gene mutation, aneuploidy or mitotic recombination. Bearing in mind the results of previous genetic studies, the results of the killer by neutral crosses and cell platings reported here can most readily be accounted for on the basis of somatic segregation of the (k) and (n) cytoplasmic determinants. Thus, a killer by neutral cross results in the formation of a killer diploid of genotype MM(k)(n), and the resulting range in tetrad ratios can be attributed to the somatic segregation of (k) and (n) during mitotic and meiotic cell divisions of the diploid culture.

The fact that no sensitive colonies were observed during the course of these cell platings may indicate either that the replication of (k) and (n) is well synchronized with cell division, or that there is such a large number of cytoplasmic determinants per cell that the chances of neither type of determinant entering a

daughter cell are remote. However, the finding that (k) determinants in cells of genotype *MM*(k)(n) do fail with appreciable frequency to enter daughter cells does not favour the hypothesis that their replication is well synchronized with cell division. Previous studies have shown that sensitive cells arise spontaneously in haploid killer cultures with a frequency of approximately 1 in 2500 cells. However, no sensitive cells have yet been observed to arise spontaneously in neutral haploid cultures. Previous work has also shown that when the nuclear genotype is changed from *M* to *m*, both (k) and (n) determinants begin to be lost by the 5th cell generation (Somers & Bevan, 1969). It therefore appears that the numbers of (k) and (n) determinants in *M*(k) and *M*(n) cultures respectively are approximately equal. It is therefore possible that the replication of (n) is more rigidly controlled by the nuclear allele *M* than the replication of (k). Further studies are necessary to test this hypothesis.

The possible difference between the haploid parental strains K_1 and K_2 suggested in section (iv) may simply be that strain K_1 contains a small number of (n) determinants not detected by the cell platings shown in Table 2 (2348 colonies). If this is so, a contribution of (n) from the parental killer cell to the diploid would result in the diploid culture containing relatively more (n) determinants than diploid cultures obtained from $K_2 \times N_1$ crosses. However, the possibility that the various killer strains differ from each other with respect to their nuclear genotypes cannot be ruled out at this stage.

SUMMARY

When killer and neutral strains of *Saccharomyces cerevisiae* are crossed the resulting diploid clones possess a killer phenotype and when spored yield a complete range of tetrad ratios.

The combined results of analysing tetrads and vegetative cells of diploid clones derived from two different neutral × killer crosses ($K_1 \times N_1$ and $K_2 \times N_1$) demonstrate that the range of tetrad ratios can be accounted for by the occurrence of somatic segregation of killer (k) cytoplasmic determinants prior to sporulation. Such results support the genetic model for the inheritance of the killer character in yeast already proposed (Somers & Bevan, 1969).

During the course of these studies a correlation was found between the strengths of the killer phenotypes of diploid colonies and the proportions of killer spore cultures obtained after sporulation of their cells.

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