THE GENETIC CONTROL OF DS-RNA VIRUS-LIKE PARTICLES ASSOCIATED WITH *SACCHAROMYCES CEREVISIAE* KILLER YEAST

DIANE J. MITCHELL, A. J. HERRING and E. A. BEVAN

*Department of Plant Biology and Microbiology, Queen Mary College,
University of London, London E1 4NS*

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SUMMARY

Previous results have indicated that laboratory strains of yeast possessing two types of dsRNA virus-like particles, V1 and V2, are capable of producing a killer toxin which kills sensitive strains. This paper reports on genetic crosses which correlate the presence of these particles both with a host nuclear gene $\pm/mak_1^*$ and with the production of a killer toxin. The results show that:

- (i) V1 particles are inherited independently of the $\pm/mak_1$ gene.
- (ii) V2 particles are present only in combination with those of V1 and the $\pm$ allele of the $\pm/mak_1$ nuclear gene.

1. INTRODUCTION

KILLER strains of yeast release a toxin into the growth medium to which they are themselves immune but which can kill sensitive strains of yeast under appropriate conditions (Bevan and Makower, 1963; Woods and Bevan, 1968; Bussey, 1972). These killer strains possess two viruses containing different, high molecular weight species of dsRNA (Berry and Bevan, 1972; Bevan, Herring and Mitchell, 1973; Vodkin and Fink, 1973; Herring and Bevan, 1974). The higher molecular weight species of dsRNA (m. wt 2.5×10^6) has been designated P1 and the lower molecular weight species (m. wt 1.4×10^6) as P2 (Bevan, Herring and Mitchell, 1973). P1 and P2 dsRNA species have been shown to be components of separately encapsidated viruses (Herring and Bevan, 1974). The P1 dsRNA containing virus has been termed the V1 particle and P2 dsRNA containing virus the V2 particle.

The results of a strain survey and the analysis of 5-fluorouracil-induced sensitive mutants (Mitchell, Bevan and Herring, 1973) indicated that the killer strains possess both V1 and V2 particles, since both P1 and P2 dsRNA were present, whilst sensitive strains either possess V1 alone or neither particle.

These findings led naturally to a series of genetic crosses, reported in this paper, which were undertaken to elucidate the relationships between the two particles V1 and V2 and the nuclear maintenance gene $\pm/mak_1$ ($\equiv M/m$; Somers and Bevan, 1969). Killer and sensitive strains, previously characterised as to their V1 and V2 content, were crossed and the segregation patterns of the dsRNA species amongst the progeny were established. Since it had already been shown (Herring and Bevan, 1974) that the presence of the dsRNA species P1 and P2 is indicative of the presence of V1 and V2, the

* Known previously as *M/m*.

characterisation of the progeny was based both on the analysis of their dsRNA content and the determination of their phenotypes with regard to the killer character.

2. MATERIALS AND METHODS

The haploid auxotrophic and prototrophic strains of *Saccharomyces cerevisiae* used were:

Genotype	VLP content		Phenotype	Designation
± α <i>arg</i> ₉	V1	V2	Killer	K10
± <i>a ade</i> _{2,8}	V1	V2	Killer	K22.704
± <i>a arg</i> ₉	V1	V2	Killer	K7
± α <i>ade</i> _{2,5}	V1	V2	Killer	K12
± <i>a try</i> ₅ <i>leu</i> ₁	V1	V2	Killer	JK19
± <i>a ade</i> _{2,1}	—	—	Sensitive	SX
<i>mak</i> ₁ α	—	—	Sensitive	S3
<i>mak</i> ₁ <i>a arg</i> ₉	—	—	Sensitive	R9.4C
± <i>a ade</i> _{2,8}	V1	—	Sensitive	SK22
<i>mak</i> ₁ <i>a ade</i> _{2,1}	V1	—	Sensitive	S9
<i>mak</i> ₁ <i>a</i>	V1	—	Sensitive	S5
± <i>a arg</i> ₉	V1	—	Sensitive	SSK7
<i>mak</i> ₁ α <i>ade</i> _{2,5}	V1	—	Sensitive	SR18
± <i>a his</i> ₄	V1	—	Sensitive	JN5
<i>mak</i> ₁ α <i>arg</i> ₉	V1	—	Sensitive	SCol.3
<i>mak</i> ₁ α <i>ade</i> _{2,8}	V1	—	Sensitive	R7.2B
<i>mak</i> ₁ α <i>ade</i> _{2,8}	V1	—	Sensitive	R7.6C
<i>mak</i> ₁ <i>a ade</i> _{2,8}	V1	—	Sensitive	R7.6D

All the strains with the exception of one were stock cultures, or their derivatives, of this laboratory. The exception was K22.704 which originated from D. Wilkie's laboratory (University College, London).

The media used for mating and growth were: (a) Wickerman's defined minimal medium and (b) complete medium (1 per cent yeast extract, 1 per cent bacto peptone, 2 per cent glucose and 1.5 per cent agar. Acetate medium was used to induce sporulation (Pomper, Daniels and McKee, 1954).

Crosses involving the auxotrophic strains were carried out by complementation on minimal medium. Those involving the prototrophic strain S3 were undertaken on complete medium. Single diploid cells were isolated, grown into a colony and the cells sporulated.

Tetrad analyses were carried out after the asci walls had first been dissolved using either helicase* or mushroom enzyme (Bevan and Costello, 1964). The tetrads were separated using a Singer micromanipulator (Barer and Saunders-Singer, 1948), in conjunction with a Wild binocular microscope (M11-37919) magnifying $\times 225$ and with the open plate method of micromanipulation (Kemp and Bevan, 1959) adapted for yeast (Bevan and Woods, 1963). Microloops of *c.* 70 μ diameter were made on a De Fonbrunne microforge (De Fonbrunne, 1949).

Details of the phenol extraction technique used for nucleic acid preparations, and the subsequent separation and characterisation of these nucleic

* Suc d'helix pomatia—Industrie Biologique Française.

acids by polyacrylamide gel electrophoresis, are given elsewhere in Bevan, Herring and Mitchell (1973).

3. RESULTS

(i) *Killer* $\times$ *Sensitive* and *Sensitive* $\times$ *Sensitive* crosses

All crosses shown in table 1 gave a normal segregation of the killer phenotype with the $\pm/mak_1$ gene, *i.e.* in a $\pm(K)\dagger \times mak_1(S)\dagger$ cross the progeny segregated 2K:2S and in a $\pm(K) \times \pm(S)$ the progeny segregated 4K:0S.

TABLE 1

Killer $\times$ *Sensitive* crosses undertaken to establish a correlation between the *Killer/Sensitive* phenotype and VLP content

Cross No.	Strains crossed				No. of tetrads analysed	Killer/Sensitive phenotype and VLP content
	Designation	Genotypes and phenotypes				
R21	K10 $\times$ SK22	$\pm$ V1	V2 (K) $\times$ $\pm$	VI - (S)	10	4 V1 V2 (K):0 (S)
R1	K10 $\times$ SX	$\pm$ V1	V2 (K) $\times$ $\pm$	- - (S)	7	4 V1 V2 (K):0 (S)
R2	K10 $\times$ S9	$\pm$ V1	V2 (K) $\times$ <i>mak</i> ₁	VI - (S)	14	2 V1 V2 (K):2 V1 - (S)
R3	K10 $\times$ R7.6D	$\pm$ V1	V2 (K) $\times$ <i>mak</i> ₁	V1 - (S)	5	2 V1 V2 (K):2 V1 - (S)
R8	K10 $\times$ S5	$\pm$ V1	V2 (K) $\times$ <i>mak</i> ₁	V1 - (S)	5	2 V1 V2 (K):2 V1 - (S)

VLP: Virus-like particle.

$\pm/mak_1$: Nuclear maintenance gene.

V1 and V2: VLPs containing P1 and P2 dsRNA species respectively.

K and S: Killer and Sensitive phenotypes respectively.

Similarly the progeny from the *Sensitive* $\times$ *Sensitive* crosses, table 2, segregated normally 0K:4S.

TABLE 2

Sensitive $\times$ *Sensitive* crosses undertaken to establish a correlation between the *Killer/Sensitive* phenotype and VLP content

Cross no.	Strains crossed		No. of tetrads analysed	Killer/Sensitive phenotype and VLP content
	Designation	Genotypes and phenotypes		
R18	SSK7 $\times$ SR18	$\pm$ V1 - (S) $\times$ <i>mak</i> ₁ V1 - (S)	4	0 (K):4 V1 - (S)
R22	JN5 $\times$ S3	$\pm$ V1 - (S) $\times$ <i>mak</i> ₁ - - (S)	4	0 (K):4 V1 - (S)
R5	SX $\times$ SCol.3	$\pm$ - - (S) $\times$ <i>mak</i> ₁ V1 - (S)	3	0 (K):4 V1 - (S)
R6	S9 $\times$ S3	<i>mak</i> ₁ V1 - (S) $\times$ <i>mak</i> ₁ - - (S)	5	0 (K):4 V1 - (S)

VLP: Virus-like particle.

$\pm/mak_1$: Nuclear maintenance gene.

V1 and V2: VLPs containing P1 and P2 dsRNA species, respectively.

K and S: Killer and Sensitive phenotypes, respectively.

The V1 particle was present in all the progeny listed in tables 1 and 2, whether of $\pm$ or mak_1 genotype. Thus, it is concluded that V1 is inherited cytoplasmically and independently of $\pm/mak_1$. The V2 particle was found only in combination with V1 and in the presence of the $\pm$ allele, *i.e.* V2 was

$\dagger$ See key to table 1.

present in all the $\pm$ progeny if one of the parents was a $\pm$ V1 V2 killer, but was found to be absent in all the mak_1 progeny.

(ii) *The action of the mak_1 allele*

The tetrad progeny of the Killer $\times$ Sensitive cross R7, segregated $2 \pm$ V1 V2:2 mak_1 V1 - (table 3).

TABLE 3

Further crosses undertaken to establish a correlation between the Killer/Sensitive phenotype and VLP content

Cross no.	Strains crossed		No. of tetrads analysed	Killer/Sensitive phenotype and VLP content
	Designation	Genotypes and phenotypes		
R7	K22.704 $\times$ S3	$\pm$ V1 V2 (K) $\times$ mak_1 - - (S)	7	2 V1 V2 (K):2 V1 - (S)
R12	SX $\times$ R7.6C	$\pm$ - - (S) $\times$ mak_1 V1 - (S)	3	0 (K):4 V1 - (S)
R17	K12 $\times$ R9.4C	$\pm$ V1 V2 (K) $\times$ mak_1 - - (S)	3	2 V1 V2 (K):2 V1 - (S)
R20	JK19 $\times$ S3	$\pm$ V1 V2 (K) $\times$ mak_1 - - (S)	2	2 V1 V2 (K):2 V1 - (S)
R10	K7 $\times$ R7.2B	$\pm$ V1 V2 (K) $\times$ mak_1 V1 - (S)	4	2 V1 V2 (K):2 V1 - (S)
R11	R10.5A $\times$ S3	$\pm$ V1 V2 (K) $\times$ mak_1 - - (S)	4	2 V1 V2 (K):2 V1 - (S)

VLP: Virus-like particle.

$\pm/mak_1$: Nuclear maintenance gene.

V1 and V2: VLPs containing P1 and P2 dsRNA species, respectively.

K and S: Killer and Sensitive phenotypes, respectively.

A possible explanation for this segregation is that the expression (*i.e.* the detectable presence) of V2 is suppressed by the mak_1 allele. This hypothesis was tested by analysing the results of the cross R12, between $\pm$ - - and mak_1 V1 - (table 3), the latter strain having arisen from R7. If mak_1 suppressed the expression of V2 in mak_1 V1 -, then one would have expected to recover $\pm$ V1 V2 killers from $\pm$ - - $\times$ mak_1 V1 - (R12). It will be seen from table 3, however, that all the progeny were in fact V1 - sensitives. This means that the mak_1 allele does not act by suppressing V2 but that V2 is not perpetuated in the presence of mak_1 .

(iii) *The segregational pattern of an exceptional killer strain*

The results of the Killer $\times$ Sensitive cross R9, between K7 ($\pm$ V1 V2 (K)) and S3 (mak_1 - - (S)) were at variance with those arising from all other crosses of the class $\pm$ V1 V2 (K) $\times$ mak_1 - - (S). Nine tetrads from R9 were examined and ratios of $2 \pm$ V1 V2 (K):2 mak_1 - - (S), whereas R7, R17 and R20 (table 3) gave $2 \pm$ V1 V2 (K):2 mak_1 V1 - (S) segregations. Four other crosses between different killer strains and mak_1 - - (S) strains have since been carried out and initial results show that their segregations are the same as those for R7, R17 and R20.

The sensitive parent, S3, in all all four crosses, R9, R7, R17 and R20, was the same. Therefore the difference in segregational patterns was presumably due to the killer parents; this difference could either be a difference in the host genotype or a cytoplasmic difference. To test the hypothesis that the difference was due to a nuclear gene difference, the exceptional killer strain, K7, was crossed to a "normal" killer strain K22.704 and each of the four spores in one resultant tetrad was crossed to a

*mak*₁ -- sensitive strain and the progeny analysed. A total of 28 tetrads from the four crosses were analysed, with the result that all the sensitive progeny were demonstrated as being *mak*₁ V1 -. This is inconsistent with a nuclear gene difference between K7 and K22.704 being responsible for the difference in their behaviour when each is crossed to *mak*₁ -- (S) but is consistent with a cytoplasmic difference. Further crosses are underway in an attempt to elucidate the nature of this cytoplasmic difference.

4. DISCUSSION

The results of investigations described here confirm that V1 particles are inherited cytoplasmically and that their maintenance is independent of the action of the nuclear gene $\pm/mak_1$. On the other hand, the V2 cytoplasmic particles are dependent on both the presence of V1 particles and the $\pm$ allele of this gene. These results confirm those of Herring and Bevan (1974), whose biochemical studies indicate that V1 and V2 particles are genetically distinct in that they are associated with different dsRNA species.

The release of the killer toxin by cells is dependent on the presence of V2 particles. Those lacking V2 particles are always sensitive to the toxin irrespective of the presence of V1 particles or the action of the $\pm/mak_1$ gene.

Crosses between Killers and Sensitives where K7 was the parent killer yielded segregations not consistent with those of other such crosses. The abnormal results obtained support the hypothesis that K7 differs cytoplasmically from the other killer strains. Whether or not such a cytoplasmic difference can be equated with mutant V1 or V2 particles remains to be elucidated by further investigations.

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