

Lethality or improved growth as a result of interaction in DNA repair double mutants of *Aspergillus*

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Genetic analysis recently identified nine new DNA repair genes of *Aspergillus nidulans*, *musK - musS*, which map on several different chromosomes (Käfer 1994, preceding article in this Newsletter). Such *mus* mutants are sensitive to certain chemical mutagens, but not sensitive or only slightly sensitive to UV and gamma-radiation. To identify epistatic interactions with members of the 4 *Uvs* groups, double mutants strains were isolated (Käfer and Chae 1994 Curr. Genet. 25:223-232). However, some *mus;uvs* double mutants could not be analyzed because they grew too poorly or were lethal (showing "synthetic enhancement in gene interaction"; Guarente 1993 Trends Genet. 9:362-366). The opposite effect was also found; namely interaction which led to improved recovery and growth (or "rescue") as documented here.

Double *mus;uvs* mutants were isolated as haploid segregants from heterozygous diploids when genes mapped on different chromosomes, or by random ascospore analysis of intercrosses in cases of linkage. Heterozygous diploids usually carried markers linked in repulsion to *mus* and *uvs* mutations. Samples of haploid segregants (50 to >300) were tested for nutritional markers and for growth on MMS, to which all mutants except *uvsI* are sensitive. In most cases, these tests identified the 3 mutant types, including the double mutant segregants which showed increased mutagen sensitivities in several cases (25% each ; Table 1).

In some cases recovery was poor even for single mutants (especially for *musL* or *uvsB* segregants). To obtain sufficiently large samples of poorly viable segregants, linked color markers or associated phenotypes were used to "select" the missing types (e.g., segregants were isolated preferentially for *musL*, *musQ*, *musK* and *uvsC*, which are linked to *yA*, *wA*, or to *fwA* and *chaA*, respectively; similarly, *uvsB* and *D*, or *musO*, could be recognized because they show dark mycelial color, or poor conidiation). In such selected samples, only single and double mutant segregants are expected (50% each; Table 2).

When marker segregation suggested that double-mutant segregants were much rarer than expected, additional haploids from at least two diploids were analyzed (one case with an aberration as the cause of low recovery was identified in this way; *musQ;uvsH*, missing in Table 4). In some cases, very rare segregants with markers expected of double mutants were crossovers, especially when recovery of single mutants was normal. Such exceptions, when checked on benomyl, occasionally turned out to be diploid (e.g., *musO/+;uvsH/H*, which was useful for the isolation of *musO;uvsH* double mutants; Table 2). In all cases, rare presumptive

doubles and cases with ambiguous phenotypes were tested by outcrossing or by complementation to confirm the double mutant genotype.

For some pairs of *mus* and *Uvs* mutations which showed meiotic linkage, double mutant crossover types were obtained by selecting against linked markers used in repulsion (especially in crosses of the III;VII translocations associated with *musO* or S to *UvsI* which maps on chromosome III; Table 3). Double *mus;UvsI* mutants from such crosses were identified as MMS-sensitive progeny which also showed the high UV sensitivity typical for *UvsI* (Chae and Käfer 1993 *Curr. Genet.* 24:67-74).

All types of isolated segregants are listed in Tables 1-3, and the recovery of double mutants is shown in percent relative to the "less viable" single mutant type. In general, only double mutants with good growth showed close to 100% recovery (e.g. *mus;UvsI* doubles), because of the competition between different segregant types on the benomyl plates during haploidization. "Lethal" interactions are assumed when practically no double mutants but significant numbers of both single mutant parents were obtained (e.g., in Table 1, for *UvsF* when combined with *musN*, or in Tables 1 and 2, for *UvsF;musQ* doubles). In random samples, certain *musL* and *musO* double mutants also could not be recovered, mainly because single mutants showed very poor recovery. However, when single mutants were selected, a significant fraction of doubles were obtained among them (compare results in Table 1 with those in Table 2).

The relative recoveries of double mutants from all the data are combined in Table 4. Clearly the majority of lethal interactions were found for *mus* mutations interacting with *UvsF* (5 of the 6 observed cases); only one other case is completely lethal (*musO* with *UvsC*). Unexpectedly, none of the *mus;UvsH77* double mutants was completely lethal and only two showed very poor recovery, even though *UvsH* is a member of the *UvsF* epistatic group. In contrast, the two members of the *UvsB* group showed very similar results. Both *UvsB* and *D* affected viability of double mutants in all cases, but in two opposite ways for different *mus* mutations. Double mutants either were growing more poorly than *UvsB* (or *D*) singles and were produced with very low frequencies; or they showed better growth than *UvsB* (or *D*) and much increased recovery (>200 - 400 % for *musN*, *P* and *R* double mutants).

Lethal interactions between *Uvs* mutations had been found previously appeared to be the rule for mutations of different epistatic groups in *Aspergillus* (Käfer and Mayor 1986 *Mutat. Res.* 161:119-134). However, viable double mutants of *UvsI* with other *Uvs* mutations have been identified recently, even for intergroup pairs (Chae and Käfer 1993 *Curr. Genet.* 24:67-74) as also found here for the many cases of *mus;UvsI* doubles. The latter results resemble the findings for double *rad* mutants of budding yeast, which usually are viable in all combinations. Only a few "synthetic lethals" have been found for radiation-sensitive mutants of this species; e.g., for certain *rad52* mutations, when combined with unusual alleles of the excision repair gene *RAD3*, or of the topoisomerase gene *TOP1* (Montelone et al. 1988 *Genetics* 119:289-301; Levin et al. 1993 *Genetics* 133:799-814). However, for many other important mutants, interacting mutations which lead to enhanced effects and lethality have recently been reported, and selective systems have even been devised (Bender and Pringle 1991 *Mol. Cell. Biol.* 11:1295-1305).

An interesting finding of the opposite type is the interaction of certain *mus* mutations with UvsB group mutations; namely, in double mutants with *musN*, *P* and *R*, suppression of the low growth rate and poor viability typical for UvsB group mutants was found. Such double mutants were also recovered more frequently (3-8x) and showed lower MMS sensitivity than single *uvsB* (or *D*) segregants (but only at low concentrations; Käfer and Chae 1994, ref. cit.). Furthermore, the same effect was observed in triple mutant strains. Growth rate and recovery improved (2-4 x) when *musN* was incorporated into double *uvs* strains containing a UvsB group mutation (e.g., from one such diploid, 18 *musN;uvsC;uvsD*, plus 66 *musN;uvsD* segregants were recovered, compared to 1 *uvsC;D* plus 19 *uvsD* not containing *musN*). In addition, triple mutant *musN;uvsC;uvsD* strains conidiate and grow much better than *uvsC;uvsD* double mutants which have extremely poor growth and conidiation. These findings are analogous to those known for certain *recA* mutants of *E. coli* in which the recBCD enzyme recklessly degrades DNA, while *recA;recB* double mutants do not show such effects.

Acknowledgement: This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

Table 1. Recovery of haploid double mutant segregants from diploids *mus / uvs* for unlinked mutations: Relative frequency of the double- compared to the less viable single-mutant types in random samples (in %)

UvsF group:	<i>uvsF</i>					<i>uvsH</i>						
	4 haploid types [Nos.]				Total [No.]	Doubles Percent recovery	4 haploid types [Nos.]				Total [No.]	Doubles Percent recovery
	Doubles <i>mus</i>	Singles <i>uvs</i>	++				Doubles <i>mus</i>	Singles <i>uvs</i>	++			
<i>musL</i>	— ^a					0 ^b	8 ^b	64	97	[169]	(0) ^b	
<i>musN</i>	0	67	92	60	[219]	0	2 ^c	99	102	[338]	2	
<i>musO</i>	1 ^{b,c}	6 ^b	65	52	[124]	15	0 ^b	12 ^b	26	[113]	(0) ^b	
<i>musP</i>	0	92	107	134	[333]	0	12 ^c	55	26	[187]	46	
<i>musQ</i>	0 ^b	12 ^b	13	21	[46]	0	— ^d					
<i>musR</i>	— ^b						9	12	11	[42]	82	
<i>musS</i>	18	20	29	37	[104]	90	4	4	3	[16]	100	

UvsC group:	<i>uvsC</i> (or <i>uvsE</i> ^e)					UvsB group: <i>uvsB</i> (or <i>uvsD</i> ^e)						
	4 haploid types [Nos.]				Total [No.]	Doubles Percent recovery	4 haploid types [Nos.]				Total [No.]	Doubles Percent recovery
	Doubles <i>mus</i>	Singles <i>uvs</i>	++				Doubles <i>mus</i>	Singles <i>uvs</i>	++			
<i>musL</i>	2 ^b	3 ^b	15	23	[43]	67	0 ^b	2 ^b	15	110	[127]	(0) ^b
<i>musN</i>	— ^b						50 ^f	128	40	112	[330]	125
	74	135	61	157	[427] ^e	>100	77 ^f	128	19	76	[300] ^e	400
<i>musO</i>	0	14	26	40	[80]	0	4 ^c	53	14	61	[132]	29
<i>musP</i>	42 ^b	55	50 ^b	54	[201]	84	66 ^f	104	24	86	[280]	275
<i>musQ</i>	2	9	11	23	[45]	22	3 ^c	135	90	152	[380]	3
<i>musR</i>	— ^b						8 ^f	34	2	39	[83]	400
<i>musS</i>	— ^b						3 ^c	11	8	35	[57]	38

^a *musL* and *uvsF* are both in linkage group I; from the intercross 0 / 10 *musL* progeny were doubles

^b For selected cases, including all *musK* double mutants, see Table 2; for *uvsI* doubles, see Table 3

^c Poorly growing strains

^d *musQ* / *uvsH* diploid not available

^e Alternate member of the same epistatic group

^f Cases of "rescue"; improved growth and therefore improved recovery

Table 2. Recovery of *mus;uvs* double mutant strains compared to single mutant types (in %) among samples of selected *mus*, or *uvs*, haploid segregants from doubly heterozygous diploids

Relevant diploid genotype In bold: Selected chromosome	Double mutants No / Total	Recovery %	Relevant diploid genotype In bold: Selected chromosome	Double mutants No / Total	Recovery %
<i>musK</i> - R, with <i>uvsF</i>			<i>musL</i> - O, with <i>uvsH</i>		
<i>fwA musK</i> <u>+</u> <u>+</u> + + <i>uvsF pabaA</i>	0 / 50	0	<i>fwA musK</i> <u>+</u> <u>+</u> + + <i>uvsH pyroA</i>	1 / 98	1
<i>ActA musO</i> <u>+</u> <u>+</u> + + <i>uvsF pabaA</i>	7 ^b / 63	13	<i>yA musL</i> <u>+</u> <u>+</u> + + <i>uvsH pyroA</i>	30 / 56	~100
<i>wA musQ</i> <u>+</u> <u>+</u> + + <i>uvsF pabaA</i>	0 / 65	0	<i>ActA musO</i> <u><i>uvsH</i></u> <u>+</u> + + <i>uvsH pyroA</i>	16 / 55	41
<i>musR ActA</i> <u>+</u> <u><i>adE</i></u> + + <i>uvsF</i> +	19 / 43	79	<i>musK</i> and <i>L</i> , with <i>uvsB</i>		
<i>mus L</i> - S, with <i>uvsC</i> (or <i>E</i>)			<i>fwA musK</i> <u>+</u> <u>+</u> + + <i>uvsB pyroA</i>	7 / 33	27
<i>yA musL</i> <u>+</u> <u>+</u> + + <i>uvsC chaA</i>	7 / 73	11	<i>chaA musK</i> <u>+</u> <u>+</u> + + <i>uvsB pyroA</i>	<u>12 / 48</u>	<u>33</u>
<i>fwA musK</i> <u>+</u> <u><i>nicA</i></u> + + <i>uvsE</i> <u>+</u>	5 / 15	50	Total:	19 / 81	31
<i>musN</i> <u>+</u> <u>+</u> <u>+</u> + <i>choA</i> <i>uvsC</i> <i>chaA</i>	88 / 170	100	<i>yA musL</i> <u>+</u> <u>+</u> + + <i>uvsB pyroA</i>	18 / 157	13
<i>musP</i> <u>+</u> <u>+</u> <u>+</u> + <i>choA</i> <i>uvsC</i> <i>chaA</i>	43 / 93	86			
<i>musR ActA</i> <u>+</u> <u>+</u> + <i>ActA</i> <i>uvsC</i> <i>chaA</i>	4 / 7	~100			
<i>musS</i> <u>+</u> <u>+</u> <u>+</u> + <i>choA</i> <i>uvsC</i> <i>chaA</i>	20 / 75	36			

Table 3. Recovery of *mus;uvrI* double mutant strains (in % of the less viable single mutant type)

A Haploid segregants from <i>mus/uvrI</i> diploids, heterozygous for unlinked mutations (as in Tables 1 and 2)										
	Random isolates					Selected cases among <i>fwA musK</i> haploids				
	4 haploid types		[Nos.]	Total	Doubles	2 haploid types		[Nos.]	Total	Doubles
	Doubles	<u>Singles</u>	+	+	[No.]	Percent recovery	Doubles	Singles (<i>musK</i>)	[No.]	Percent recovery
	<i>mus</i>	<i>uvr</i>								
<i>musL</i>	3	0	46	81	[130]	>100	10	35	[45]	29
<i>musN</i>	56	87	75	60	[231]	75				
<i>musQ</i>	2	5	11	22	[40]	40 ^a				
<i>musP</i>	31	32	41	46	[150]	97				

B Random ascospore progeny from <i>mus x uvrI</i> intercrosses (mutations of Linkage group III)										
<i>mus</i> mutations	Genotype of parental strains				Doubles among identified <i>mus</i> progeny					
	<i>mus</i>	x	<i>uvrI</i>	and relevant linked markers of Lg. III	Selected markers ^b	Doubles / Total [Nos.]	Percent recovery ^c			
<i>musO226</i>	<i>musO</i> T2(III;VII)	x	<i>uvrI</i>	actA	None	1 / 11	(T-linkage ^c)			
<i>musR223</i>	<i>meaB adl musR</i>	x	<i>uvrI</i>		mea ⁺	10 / 10	(CO selected)			
<i>musS224</i>	<i>actA musS</i> T3(III;VII)	x	<i>meaB uvrI</i>		actA mea ⁺	2 / 16	(T-linkage ^c)			

^a Small sample, not significantly different from expected

^b *mus* progeny with certain markers were identified and only these were tested for UV sensitivity, i.e., *uvrI*

^c Percent recovery is uncertain, because linkage of *uvrI* to the translocations was not measured accurately

Table 4. Pattern of lethal or ameliorating interactions between *mus* mutations and *uvs* of different epistatic groups, as indicated by very low or very high recovery of double mutant strains (%)

<i>mus</i> mutations	Uvs epistatic groups and representative <i>uvs</i> mutations used				
	UvsF		UvsC	UvsI	UvsB
	<i>uvsF201</i> %	<i>uvsH77</i> %	<i>uvsC114</i> or <i>E182</i> %	<i>uvsI501</i> % ^b	<i>uvsB221</i> or <i>D153</i> %
<i>musK228</i>	0	1	33	23	28
<i>musL222</i>	0	54	35	>100	12
<i>musN227</i>	0	2	100	75	>200
<i>musO226</i>	13	31	0	(10) ^b	29
<i>musP234</i>	0	46	46	78	>200
<i>musQ230</i>	0	---	22	43	3
<i>musR223</i>	79	75	50	(100) ^b	>200
<i>musS224</i>	90	100	39	(12) ^b	37

^a Percent recovery of double mutants based on data of Tables 1 and 2, and for *uvsI* of Table 3

^b Double mutants of *uvsI* all showed good viability and recovery, but in some cases linkage data were not accurate enough to estimate expected frequency and % recovery (Table 3)