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Abstract

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.

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).

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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.

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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:	***uvsF***						***uvsH***					
	4 haploid types [Nos.]				Total	Doubles	4 haploid types [Nos.]				Total	Doubles
	Doubles	Singles mus	Singles uvs	++	[No.]	Percent recovery	Doubles	Singles mus	Singles uvs	++	[No.]	Percent recovery
musL	—[a]					**0**[a]	0[b]	8[b]	64	97	[169]	(0)[b]
musN	0	67	92	60	[219]	**0**	2[c]	99	102	125	[338]	**2**
musO	1[b,c]	6[b]	65	52	[124]	**15**	0[b]	12[b]	26	75	[113]	(0)[b]
musP	0	92	107	134	[333]	**0**	12[c]	55	26	94	[187]	**46**
musQ	0[b]	12[b]	13	21	[46]	**0**	—[d]					
musR	—[b]						9	12	11	10	[42]	**82**
musS	18	20	29	37	[104]	**90**	4	4	3	5	[16]	**100**

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

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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 %
musK - R, with ***uvsF***		
fwA musK / + + + + / *uvsF pabaA*	0 / 50	**0**
ActA musO / + + + + / *uvsF pabaA*	7[b] / 63	**13**
wA musQ / + + + + / *uvsF pabaA*	0 / 65	**0**
musR ActA / + + + *adE* / ***uvsF*** **+**	19 / 43	**79**
mus L - S, with ***uvsC*** (or *E*)		
yA musL / + + + + / *uvsC chaA*	7 / 73	**11**
fwA musK / + + + *nicA* / *uvsE* +	5 / 15	**50**
musN + / + *choA* + + / ***uvsC chaA***	88 / 170	**100**
musP + / + *choA* + + / ***uvsC chaA***	43 / 93	**86**
musR ActA / + *ActA* + + / ***uvsC chaA***	4 / 7	**~100**
musS + / + *choA* + + / ***uvsC chaA***	20 / 75	**36**
musL - O, with ***uvsH***		
fwA musK / + + + + / *uvsH pyroA*	1 / 98	1
yA musL / + + + + / *uvsH pyroA*	30 / 56	**~100**
ActA musO / + + ***uvsH*** + / ***uvsH*** *pyroA*	16 / 55	**41**
musK and ***L***, with ***uvsB***		
fwA musK / + + + + / *uvsB pyroA*	7 / 33	27
chaA musK / + + + + / *uvsB pyroA*	12 / 48	33
Total:	19 / 81	**31**
yA musL / + + + + / *uvsB pyroA*	18 / 157	**13**

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

A Haploid segregants from *mus/uvsI* diploids, heterozygous for unlinked mutations (as in Tables 1 and 2)

	Random isolates						Selected cases among *fwA musK* haploids			
	4 haploid types [Nos.]				Total	Doubles	2 haploid types [Nos.]		Total	Doubles
	Doubles	Singles mus	Singles uvs	++	[No.]	Percent recovery	Doubles	Singles (*musK*)	[No.]	Percent recovery
musL	3	0	46	81	[130]	**>100**	10	35	[45]	**29**
musN	56	87	75	60	[231]	**75**				
musQ	2	5	11	22	[40]	**40**[a]				
musP	31	32	41	46	[150]	**97**				

B Random ascospore progeny from *mus x uvsI* intercrosses (mutations of Linkage group III)

	Genotype of parental strains			Doubles among identified *mus* progeny		
mus mutations	*mus* and relevant linked markers of Lg. III	x	*uvsI*	Selected markers[b]	Doubles / Total [Nos.]	Percent recovery[c]
musO226	*musO* T2(III;VII) actA	x	*uvsI*	None	1 / 11	(T-linkage[c])
musR223	*meaB adI musR*	x	*uvsI*	mea+	10 / 10	(CO selected)
musS224	*actA musS* T3(III;VII)	x	*meaB uvsI*	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., *uvsI*

[c] Percent recovery is uncertain, because linkage of *uvsI* to the translocations was not measured accurately

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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 (%)

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