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pyrG of Aspergillus nidulans,

meiotic mapping, marker inter-

actions and growth response.

Information of the genetic location of pyrG and its growth response under various conditions has become important with the recent cloning of pyrG (Oakley et al. 1987. Gene 61:385-399) and with the use of pyrG strains as recipients for transformation when pyr-4 cloning vectors are used for A. nidulans libraries (e.g. May et al. 1985 J. Cell Biol. 101:712-719 ; Osmani et al. 1987 J. Cell Biol. 104:1495-1504).

Several problems have surfaced in crosses with pyrG89. Two of them, which are related to the genetic mapping of pyrG and were investigated in detail are the following: 1) pyrG is linked to galD but the distance between these markers and orientation of the linked pair were found to vary in crosses with different outside markers; 2) pyrG mutants interact with the linked distal markers, fpaB and trpB, to give very poorly or non-viable double mutant progeny.

Two further unexpected problems, encountered among pyrG progeny from heterozygous crosses, were only partly analysed and preliminary results have led to the following proposals: 3) pyrG89 is apparently cold sensitive (cs) on the simple yeast extract-glucose supplemented with uridine which is suitable for growth of pyrG recipient strains (YAGU; Osmani et al. 1987, ref. cit.); however, some stocks (e.g., FGSC A576) carry unlinked suppressors which results in 1:1 segregation for cs among pyrG progeny; 4) Two pyrG89 strains (including the Glasgow strain, G191) when crossed to one of two related galD and/or uvsF strains produced a fraction ($>1/4$) of progeny with a new requirement which can be satisfied by NH_4Cl and partly by adenine, but not by nitrate or nitrite. It seems likely that the two cases are related and that the mutations involved are present in stock strains (information about similar observations would be helpful for the assessment and investigation of this intriguing observation).

From our extensive analysis of the first two of these problems we conclude that the originally observed differences in linkage values for pyrG were caused by environmental variation rather than chromosomal aberrations. This is demonstrated in Table 1 where results from repeats of the same cross, carried out in different laboratories, show very large differences. On the other hand, the results summarized in Table 2 are based on original data from many different crosses which were relatively uniform (hence the small SEM). Furthermore, when crosses are grouped according to branches of the pedigrees which might involve presumptive normal vs. potential aberration strains, no significant differences in recombination frequencies are seen.

The difference in orientation may also partly be caused by variations in conditions (media, crowding, temperature), since the variably poor recovery of the recombinant pyrG fpaB types reduces only one of the two potential double crossover categories; this can create a sufficient bias to apparently reverse the order of pyrG and galD (see A, Table 1).

However, the main problem in establishing the orientation of this pair of markers is not specific to pyrG and results directly from the absence of interference in *Aspergillus* crosses (Käfer 1977 Adv. Genet. 19:33-131).

Table 1.
Classification of crossover types from a cross of pyrG89 x fpaB37 galD5^a
A) details of published data(2 sets)^a; B)recent repeat^b.

Segregant genotypes				Frequency of crossover types					
fpaB pyrG galD				A Sets (1) + (2)		Total No.	%	B Total No.	%
Parentals <	r	+	-	60	+	132=192	307	95	235^b
	+	-	+	76	+	39=115		140	
Single crossovers<	r	-	+	10	+	9=19^c	86	18^c	21.7
	+	+	-	28	+	39=67		49	
Single or double cross overs?	r	+	+	9	+	10=19	41	1	0.65
	+	-	-	9	+	13=22		2	
	r	-	-	4	+	1=5^c	36	3^c	1.3
	+	+	+	8	+	23=31		4	
Totals						470			308
Distances in cMo	fpaB	-	pyrG	(86+36)/470		26.0		(67+4)/308	23.0
	fpaB	-	galD	(86+41)/470		27.0		(67+2)/308	22.4
	pyrG	-	galD	(41+36)/470		16.4		(2+4)/308	2.0

a The strains used are GCR2.13 (of GM; pyrG89 pabaA1;tubC2.14;benA22) and FGSC A515 (fpaB37 galD5 suAladE20 riboA1 yA2 adE20;pyroA4;facA303;chaA1; Oakley et al. 1987 ref. cit.).
b Non-random sample, enriched for pyrG segregants (smallish colonies)
c Double mutant strains, fpaB pyrG, show very poor growth (not remedial by uridine supplements).

Analysis of the distribution of crossing over in the "standard" crosses (of Table 2) confirms earlier meiotic data and indicates a random coincidence with no hint of positive interference. [Among 81 confirmed cases of crossing over between galD and pyrG, the following fractions of double crossovers were found for adjacent intervals: 4/54 (7.4% for suA, 6/32 (18.8%) for fpaB, and 15/42 (35.7%) for uvsF which in each case is very close to expectation for random coincidence.] When this is the case, two closely linked markers like galD-pyrG (average 3%, Table 2) can reliably be arranged in sequence only if an outside marker is reasonably close. For example, suAadE, at a distance of less than 10%, mapped closer to pyrG than galD in all crosses. In contrast, the more distant markers fpaB and uvsF usually but not always showed closer linkage to galD in individual crosses. In general, therefore, when markers at suitable distances are not available, mapping results from single crosses and samples of limited size must remain provisional until confirmed (or reversed, as occurred for several published cases, e.g. galD which originally was placed proximal to suAadE).

Table 2
Frequencies of recombination (average % $\pm$ SEM) in groups of closely related crosses heterozygous for pyrG and galD and various outside markers.

Outside markers	No. of crosses	Intervals					Total All types	tested <u>adE</u> $\pm$ <u>suA</u>
		<u>uvsF</u>	<u>fpaB</u>	<u>galD</u>	<u>pyrG</u>	<u>suA</u>		
<u>SUA</u>	3(1*)			2.4 $\pm$ 0.4		8 $\pm$ 2	521	238
					10.4 $\pm$ 2.6			
<u>fpaB</u> - <u>suA</u>	3		17.7 $\pm$ 5	19 $\pm$ 5	1.7 $\pm$ 0.3	9.1 $\pm$ 3.4	467	291
					10 $\pm$ 3			
<u>uvsF</u> - - <u>suA</u>	4(2*)		22 $\pm$ 3	23 $\pm$ 3	1.9 $\pm$ 0.9	9 $\pm$ 2	896	415
					12.5 $\pm$ 2.5			
<u>uvsF</u> , <u>fpaB</u> - <u>suA</u>	5	11.0 $\pm$ 1.3	18.4 $\pm$ 1.5	20.2 $\pm$ 1.3	4.1 $\pm$ 0.9	9.8 $\pm$ 2.0	835	409
			23.2 $\pm$ 2.4		12.4 $\pm$ 2.5			
<u>uvsF</u> or <u>fpaB</u> - <u>suA</u>	3		22	23	3.5 $\pm$ 0.8	7.0 $\pm$ 0.5	781	514
			31.1 $\pm$ 1.3		9.0 $\pm$ 0.8			
Total number	18						3500	1867
Combined %		11.0 $\pm$ 1.3	19.0 $\pm$ 1.3	20.5 $\pm$ 0.3	3.0 $\pm$ 0.3	8.5 $\pm$ 0.8		
			25.7 $\pm$ 1.4		10.7 $\pm$ 1.0			

* Number of crosses not classifiable for segregation of suA1adE20 because adE20 progeny could not be identified.

It may be of interest that in Neurospora crassa these same problems have been encountered when well-backcrossed marker strains were used to map new mutants. ---
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