pyrG of Aspergillus nidulans, meiotic mapping, marker interactions and growth response

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Abstract
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).
All these mutants are being deposited at FGSC (Table 2) and additional detailed information will be made available to anyone interested in analyzing them further (their investigation is being discontinued).

Table 2.  New mus strains available from FGSC

| Gene  | Allele   | Prototroph Level of backcross | Simple requiring strains | Faulkner no. | Marker | Faulkner no. |
|-------|----------|-------------------------------|--------------------------|--------------|--------|--------------|
| mus-7 | FK116    | II(A) I(a)                    | --                       | 6401 6402    | leu-3  | R156 6405   |
|       |          |                              |                          |              | nic-2  | 43002 6407  |
| mus-9 | FK129    | IV                            | --                       | 6403 6404    | pan-1  | 5531 6411   |
|       |          |                              |                          |              | lys-1  | 33933 6412  |
| mus-21| FK121    | VI                            | --                       | 6414 6415    | trp-1  | 10575 6416  |
|       | FK120    | II                            | trp-1  acr-2 10575 KH5   | 6418 6419    | AC2    | --           |
| mus-11| FK117    | IV                            | --                       | 6409 6410    | leu-1  | Y3757 6436  |
|       |          |                              |                          |              | leu-1  | Y3757 6436  |
| mus-28| FK118    | IV                            | --                       | 6434 6435    | arg-5  | 27947 6430  |
|       |          |                              |                          |              | nic-2  | T28M2 6432  |
| mus-29| FK119    | IV                            | --                       | 6438 6439    | trp-2  | 41 6440 6441|
|       |          |                              |                          |              | ylo-1  | Y30539y 6442|
| mus-30| FK115    | IV                            | --                       | 6444 6445    | pan-1  | 5531 6446  |
|       |          |                              |                          |              | met-2  | K43 6448 6449|
| mus(FK125)|         | IV                            | --                       | 6450 6451    | rib-1  | 51602(t) 6452|
| mus(FK128;)|        | IV                            | --                       | 6457        | lys-5  | DS6-85 6458 |
| mus(FK131)|         | II                            | --                       | 6459 6460    | trp-2  | 41 6461 6462|
| mus(FK132)|         | II                            | rib-1 51602(t) 6465 6466| 6463 6464    |        |              |
| mus(FK133)|         | II                            | met-1 M105 6469 6470     | 6467 6468    |        |              |

Tests of mus(FK115), (FK119) and (FK123) for allelism to recently mapped genes by Dr. H. Inoue are gratefully acknowledged. This work was supported by NSERC of Canada. --- Biology Dept., McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, H3A 1B1, Canada

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pyrG of Aspergillus nidulans, meiotic mapping, marker interaction 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 NH4Cl 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 ppyrG fpaB types reduces only one of the two potential double crossover categories; this can create a sufficient bias to apparently reverse the order of ppyrG and galD (see A, Table 1).

However, the main problem in establishing the orientation of this pair of markers is not specific to ppyrG 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 |
|---------------------|-----------------------------|
|                     | Sets | Total No. | %  | Total No. | %  |
|                     | A  | (1) + (2) |    |   |    |
| Parentals < r       | 60 + 132 = 192 | 95 |
| +                  | 307 | 307 |
| + - +              | 140 |
| Single crossovers:  |     |          |    |   |    |
| r - +              | 10 + 9 = 19^c | 18^c |
| + + -              | 86 | 86 |
| + + -              | 67 | 67 |
|     | 21.7 |
| Single or double    |     |          |    |   |    |
|                     | 9 + 10 = 19 | 41 | 8.7 | 2 | 0.65 |
| or - -              | 9 + 13 = 22 |
| Double crossovers:  |     |          |    |   |    |
|                     | 4 + 1 = 5^c | 3^c |
| + + +              | 36 | 36 |
| + + +              | 4 | 4 |
|     | 1.3 |
| Totals             | 470 | 308 |
| Distances fpaB - pyrG | (86+36)/470 | 26.0 | (67+4)/308 | 23.0 |
| in cMo 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: 7/71 (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 % ± SEM) in groups of closely related crosses heterozygous for pyrG and galD and various outside markers.

| Outside markers | No. of crosses | Intervals | Total tested |
|-----------------|----------------|-----------|--------------|
| SUA             | 3(1*)          | 2.4±0.4   | 10.4±2.6     |
| fpaB-suA        | 3              | 17.7±5    | 9.1±3.4      |
| uvsF - - suA    | 4(2*)          | 22±3      | 12.5±2.5     |
| uvsF, fpaB - suA| 5              | 11.0±1.3  | 4.1±0.9      |
| Total number    | 18             | 20.5±0.3  | 25.7±1.4     |

* 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. --- Dept. of Biology, McGill University, Montreal, Canada H3A 1B1; Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030

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Effect of inositol "analogues" on the production of myo-inositol-1-phosphate synthase in Neurospora crassa slime strain.

The myo-inositol-1-phosphate synthase (MIPS, E.C. 5.5.1.4) synthesis is regulated by at least two regulatory genes, inl^ts and opi-1. Zsindely et al. (1983 Biochem. Biophys. Acta 741:273-278) assumed that inl^ts is a positive regulatory gene, responsible for the production of a thermosensitive protein. Mutations in the inl^ts gene dramatically decrease or completely turn off MIPS production (unpublished results), and a mutation of the regulatory gene opi-1 derepresses MIPS production (Kiss et al. 1986 Fungal Genetics Newsletter 33:29-30).

It has been presumed that non--metabolized inositol analogues might influence the MIPS synthesis. The effect of gamma- and delta-hexachlorocyclohexane (HCH) was examined upon MIPS production. The Neurospora crassa slime (FGSC 1118 inl^+) variant growing in the form of spheroplasts was applied in the experiments.