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## Abstract

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# Relationships between pyrimidine metabolism and sensitivity to alkylating agents in *Aspergillus nidulans*

Studies of DNA repair in *Aspergillus nidulans* have resulted in the isolation of a number of strains showing increased sensitivity to a range of mutagenic treatments (e.g. Jansen 1970 Mut. Res. 10:21-32: Fortuin 1971 Mut. Res. 11:149-162). Recently, the isolation of strains sensitive to damage induced by alkylating agents (Swirski et al, manuscript submitted) has introduced the possibility of identifying particular eukaryotic DNA repair enzymes. The development of a high frequency transformation vector (pDJB3) for *A. nidulans* (Ballance and Turner 1985 Gene 36:321-331) now makes feasible the cloning of genes coding for such enzymes. The plasmid pDJB3 relies on complementing a defect in pyrimidine metabolism of the "target" strain (G191) for recognition of transformants. In *Ustilago madis*, some strains which are sensitive to radiation and alkylating agent damage can also exhibit deficiencies in pyrimidine metabolism (Moore 1975 Mut. Res. 28:355-366). Consequently, the cloning of genes affecting DNA repair in *A. nidulans* using genomic DNA banks in pDJB3 may prove impractical if mutations affecting pyrimidine metabolism also affect response to alkylating agents.

We report here a preliminary study of the effects of mutations at two loci (*pyrE* and *pyrG*) controlling pyrimidine biosynthesis (Palmer and Cove 1975 MGG 138:243-255) upon sensitivity to the alkylating agent MNNG (N-methyl-N-nitro-N-nitrosoguanidine).

Initially, in growth tests on solid media containing MNNG, G191 (*pyrG89 pabaA1 fwA1 uaY9*) was found to be slightly more sensitive than wild-type, and G190 (*pyrE8 anA1 luA1 yA2 cnxH5*) very sensitive. In order to test whether the mutations at the *pyrE* or *pyrG* loci affected response to alkylating agents, normal wild-type activity was restored in two ways. Two transformants of G191 (*pyrG*) were selected following Ballance and Turners (1985) technique, whilst some nineteen revertants of G190 (*pyrE*) were selected following UV irradiation. Both the transformant class and the revertant class had functional *pyrG* and *pyrE* activity restored respectively and could grow in the absence of pyrimidine supplementation. Sexual progeny of one such *pyrE* revertant tested showed no segregation of *pyrE*, confirming that true reversion had occurred at the *pyrE* locus and not in some suppressor system (Palmer and Cove, 1975). When tested for MNNG response, both revertant (*pyrE^+*) and transformant (*pyrG^+*) classes showed sensitivities indistinguishable from their parental (G190 and G191) strain responses in growth tests on solid media. Thus the increased sensitivity to MNNG of strains G190 and 6191 could not be the result of mutations at either the *pyrE* or *pyrG* loci.

A recombinant strain harboring *pyrG* and two mutations conferring sensitivity to alkylating agents, *saA1* and *sagC3* (Swirski et al, manuscript submitted) showed no change in MNNG response with change in the level of pyrimidine supplementation.

Additionally, when a heterozygous dipoid between G190 and a strain containing *sagC3* was constructed, it showed a wild-type level of resistance to MNNG confirming that the sensitivity of G190 was not due to mutation of the *sagC3* locus, although *sagC3* maps closely to *pyrE*.

In conclusion, genomic banks of pDJB3 may be used to screen for complementation of mutations conferring sensitivity to alkylating agents, as *pyrG* has no detectable effect upon response to MNNG. The MNNG sensitivity of G190, however, suggests that complex stock strains may harbor mutations other than those for which they were selected. While the

present study has failed to show an interaction between pyrimidine metabolism and sensitivity to alkylating agents, the number of loci involved in both systems in A. nidulans (Palmer and Cove, 1975; Swirski et al, manuscript submitted) may still make such relationships of relevance to DNA repair in Aspergillus. - - - Dept. of Genetics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, United Kingdom