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Abstract

We have produced mutants defective in tyrosinase activity using the RIP procedure (Selker et al. 1987 Cell 51:741-752, Marathe et al. 1990 Mol. Cell. Biol. 10:2638-2644). The tyrosinase gene has been shown to be a useful reporter in gene expression experiments (Kothe et al. 1993 FGN 40: 43-45). The availability of tyrosinase mutants will assist in these studies and will also be of use for future protein engineering experiments on tyrosinase (Fuentes et al. 1993 FGN 40:38-39)

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A qa-2 arom-9 al-2 strain of N. crassa was cotransformed with the qa-2-containing plasmid pRAL-1 (Akins and Lambowitz 1987, Cell 50:331-345) and the plasmid GRG-1/TYR103 (Kothe et al. 1993 FGN 40:43-45), which contains the tyrosinase gene sequences. The transformed strain was crossed with the wild-type strain 74-OR23-1A. Mutants defective for tyrosinase activity were isolated and used as males in crosses with am132 mutants of both mating types. Several progeny containing both mutations (am132and T-) were isolated and analysed by genomic Southern blotting (Fig. 1).

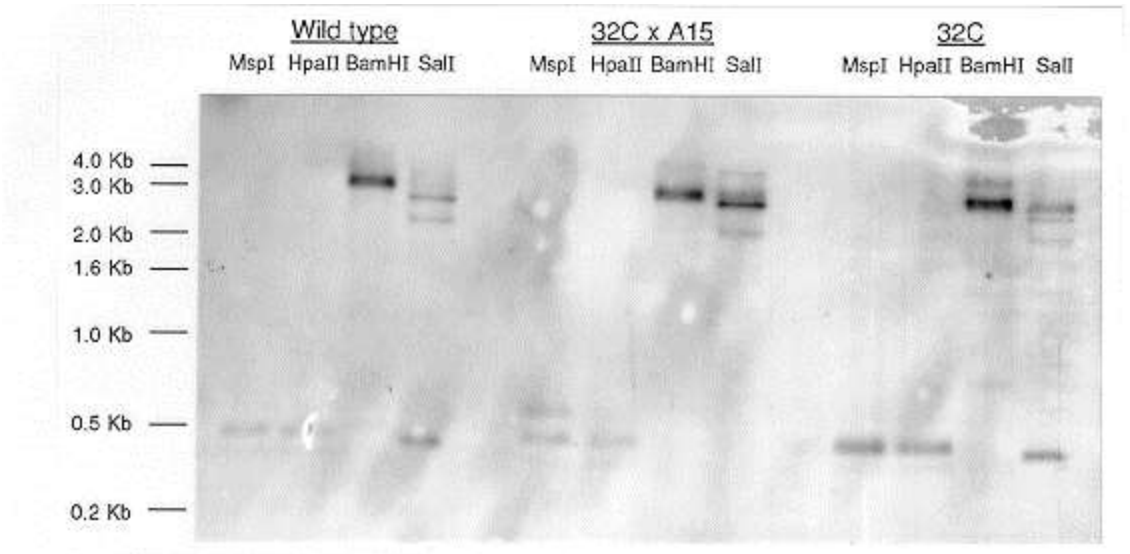

Figure 1. Southern transfers of genomic DNAs of Neurospora crassa digested with MspI, HpaII, BamHI and SalI, probed with a 1.2 kb PCR fragment spanning the mature region of the T gene. Wild-type (74-OR23-1A), 32CxA15 and 32C. The internal ~450 bp SalI fragment present in the restriction digests of the wild-type and parental DNAs is noticeably missing in 32CxA15 (am132 T- al-2).

A comparison of the progeny DNA with the wild-type DNA shows that isolate 32C (an isolate from the mating of a transformant with 74-OR23-1A) has an extra copy of the T gene as

demonstrated by the SalI and BamHI digestion patterns shown in Fig. 1. The T gene hybridizing sequence in isolate 32CxA15 (a T- mutant isolate from the mating of 32C with the am mutant) has lost the ~450 bp SalI fragment. This has probably arisen due to the methylation of the internal cytosine residue of the SalI recognition site which prevents its cleavage. The differential restriction pattern observed for the isochizomeric enzyme pair MspI/HpaII in this strain also indicates the presence of cytosine methylation. These methylation events are often accompanied by the base pair changes associated with the RIP phenomenon (Cambareri et al 1989 Science 244:1571-1575).

From a number of sexual crosses (>100) it is clear that all of our tyrosinase defective isolates are female sterile. The T- mutants are unable to complete protoperithecial development. The isolates can, however, be used as the male partner in genetic crosses. The regulatory mutant ty-1 which is defective in tyrosinase induction is also female sterile. However, unlike the ty-1 mutants which have a velvet morphology the T- mutants exhibit a normal growth phenotype. The segregation of the tyrosinase defective phenotype can be followed either by directly assaying for tyrosinase activity or by following the female sterility phenotype.

Isolates with mutant tyrosinase genes (am132 T- al-2 and T- al-2 mutants) have been deposited with the FGSC, which will be of value to investigators using the tyrosinase gene as a reporter in transformation experiments.

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