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J. Simmons

P. Chary

D. O. Natvig

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Abstract
Linkage group assignments for two Neurospora crassa catalase genes: the Metzenberg RFLP mapping kit applied to an enzyme polymorphism.

Simmons, J., P. Chary and D.O. Natvig

Linkage group assignments for two *Neurospora crassa* catalase genes: the Metzenberg RFLP mapping kit applied to an enzyme polymorphism

The enzyme catalase, as a scavenger of hydrogen peroxide, is considered one of the primary defenses against the toxic effects of oxygen. The genetic control of *N. crassa* catalase is of special interest to us, because this enzyme is induced by the superoxide-generating compound paraquat to a greater degree than has been reported for any other organism (unpublished results). Extracts from stationary-phase Neurospora mycelia (N broth) typically exhibit three distinct catalase bands on stained (Harris and Hopkinson 1976 Handbook of Enzyme Electrophoresis in Human Genetics) polyacrylamide gels (Fig. 1). These consist of 8 major band and two minor bands, one faster and one slower than the major band. A survey of wild-collected strains revealed natural polymorphism among catalases. In crossing experiments, we demonstrated that variant forms of major and minor catalases exhibit Mendelian segregation. We have employed two strains that exhibit altered catalase mobilities with reference to standard Oak Ridge strains to establish tentative genetic map positions for major and minor catalase loci, which appear to reside on separate chromosomes. One strain, FGSC 2225 (Mauriceville, Texas), possesses a minor band that is "slower" relative to the corresponding band of Oak Ridge. The linkage group assignment for the minor catalase gene was determined by analysis of strains in the Metzenberg *et al.* (1984 Neurospora Newsl. 31:35-39) large RFLP mapping kit, *multicent-2.* The second strain, FGSC 2230 (Welsh, Louisiana) possesses a major band that is "fast" relative to Oak Ridge. Mapping of this major catalase gene was accomplished by analysis of crosses between strain 2230 and alcoy strains (Perkins *et al.* 1969 Genetica 40:247-278). Our results are summarized below. Values for p represent a X^2 test of the hypothesis that two loci exhibit random assortment.

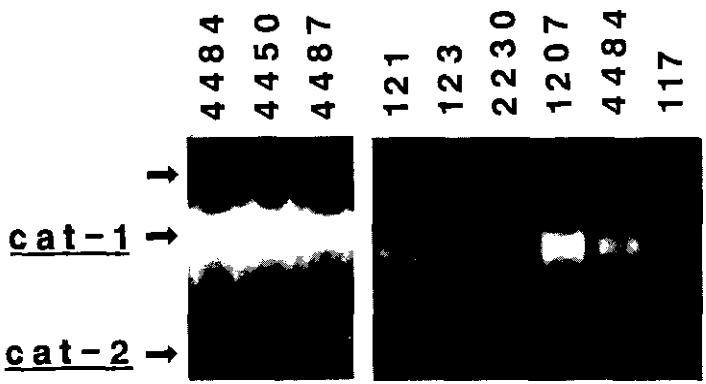

Fig. 1 Catalase bands on acrylamide gels. A. Major (*cat-1*) and minor (*cat-2* and uppermost arrow) bands from *multicent-2* progeny. Note the faster minor band in strain 4450 (Oak Ridge type). B. Fast (first three lanes) and slow major catalase bands in selected strains. Strains 117, 121 and 123 are progeny from the 2230 x 1207 cross.

Major catalase (cat-1)

Strain 2230 (mt=a, fast catalase) was crossed to the alcoy translocation strain 3361 (mt=A, slow catalase) which carries genetic markers for ylo-2, cot-1, csp-2 and al-1 (Perkins and Bjorkman 1979 Neurospora Newsl. 26:9). Thirty-nine ascospore-derived progeny were scored for these markers (only non-albino progeny can be scored for ylo-1), and mycelial extracts were examined for catalase mobility. Results indicated linkage to either chromosome III or VI (data for non-albino progeny: 5 slow, yellow; 20 fast, wild type; 1 slow, wild type; $p<(0.01)$. A second cross of strain 2230 was made to the alcoy follow-up tester strain 1207 which carries non-translocation markers for ylo-1 (L.G. VI) and trp-1 (L.G. III). Progeny scoring from this cross indicated linkage to trp-1 (17% recombination) and therefore chromosome III (13 trp^+, fast; 3 trp^-, fast; 2 trp^+, slow; 11 trp^- , slow; $p<0.025$).

We have designated this major catalase locus cat-1.

Minor catalase (cat-2)

A cross of wild-collected Mauriceville strain to Oak Ridge-derived strain 4488, which carries genetic markers for all linkage groups, was performed by Metzenberg et al. (op cit). Progeny were scored and deposited with FGSC to facilitate the mapping of cloned DNA sequences using restriction-fragment polymorphisms. We scored mycelial extracts from 37 of the 38 progeny from the Metzenberg et al. cross # 2 for the presence of Mauriceville (slow) or Oak Ridge (fast) minor catalase. The best matches were with loci nic-3 (24/37 matches) and ars-1 (27/37 matches, $p<0.01$) indicating that this second catalase locus maps to chromosome VII. Assuming that cat-2 is on the right arm of chromosome VII, our data indicate 23/37 parental progeny, 13/37 single recombinants and l/37 double recombinants (therefore 38% cat-2/nic-3 recombination and 27% cat-2/ars-1 recombination; data not presented).

We have designated this minor catalase cat-2.

Our assumption that the mapped loci represent structural catalase genes is consistent with genetic analyses of catalases from other organisms (e.g. Scandalios 1968 Ann. NY Acad. Sci. 151:272-293). A catalase purified from N. crassa mycelium was comprised of four identical subunits of MW 80,000, making this catalase somewhat larger than, but otherwise similar to, catalases from diverse organisms (Jacob and Orme-Johnson 1979 Biochemistry 18:2967-2974 and references therein). Because there does not appear to be biochemical or genetic evidence for strain-specific post-translational modification of catalases, we tentatively conclude that cat-1 and cat-2 do not represent genes for minor subunits or protein modifiers.

We note that the second minor catalase band (uppermost band on gels) exhibits a reduced migration in strain 2225 that appears to correspond with that of the cat-2 band. this suggests that the two minor catalases are products of the same gene or linked genes. This conclusion is tentative, however, because the uppermost band migrates close to the major band, and it has not been possible to score this upper band consistently on our gels. - - - Dept. of Biology, University of New Mexico, Albuquerque, NM 87131