

## RESEARCH NOTES

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"E-like" ascospore excision mutants  
in Neurospora tetrasperma resistant  
to either p-DL-fluorophenylalanine  
or methyl benzimidazol-2-yl carbanate.

The amino acid analogue p-DL-fluorophenylalanine (FPA) and the fungicide methyl benzimidazol-2-yl carbanate (MBC) are agents known to affect chromosome segregation. FPA can cause chromosomal nondisjunction in fungi, including N. crassa (Griffiths and DeLange 1977 *Mutat. Res.* **46**: 345). MBC has effects similar to those of FPA. In addition, some MBC resistance mutants have alterations in a structural protein that participates in microtubule formation (Davidse and Flach 1977 *J. Cell Biol.* **72**: 174).

The precise role of microtubules, spindles, and spindle pole bodies in ascospore excision is unknown. The excision process in N. tetrasperma must be exact if normal development is to occur. If mutations conferring resistance to FPA and to MBC affect the components of spore excision, N. tetrasperma should provide a sensitive system for detecting such mutations. Such mutants could provide information on the part played by cytoskeletal elements during development.

The strains used were N. tetrasperma 85A and 85a (recommended reference cultures - FGSC #1270 and 1271). Conidiospores were mutagenized with dimethyl sulfate (DMS). FPA resistance mutations were selected by plating treated conidia on enriched minimal agar plates, a Fries minimal agar supplemented with 1.5% sucrose and 0.5% (w/v) Difco yeast extract, containing 250 µg FPA/ml (EM FPA). Vigorously growing mycelia from plates incubated 48 to 72 h at 22°C were subcultured on a second EM FPA plate. If this retest were positive, a fragment of the resulting colony was removed and grown on a Fries complete agar slant for later analysis. To select MBC resistant mutants, DMS mutagenized conidia were resuspended in liquid Fries minimal medium containing 1.5% sucrose (w/v) and swirled overnight at room temperature. The suspension was plated on Fries minimal agar containing 1.5% sucrose (w/v) with a final concentration of 0.2 µg MBC/ml. Resistant growth was treated as in the FPA procedure.

Resistant mutations were found for both agents, 16 resistant to MBC and 23 resistant to FPA. Two of the FPA resistant mutations arose spontaneously on control EM FPA plates. All the mutants have been backcrossed once and strains reisolated from homokaryotic ascospores.

So far two have shown a developmental pattern different from the wild type strain of N. tetrasperma which typically produces four-spored asci. One isolate was resistant to FPA only, the other to MBC only. When strains carrying the same allele for the FPA resistant mutant were mated, asci with 4, 5, 6, 7, and 8 ascospores were found. However, when this FPA resistant strain was crossed to wild type, normal four-spored asci resulted. For the MBC mutant, the cross resistant by resistant was sterile. When the MBC resistant mutant was crossed to wild type, perithecia contained asci with 4, 5, 6, 7, and less frequently, 8 ascospores. All crosses were made on Difco corn meal agar. Phenocopies of the mutant asci could be obtained by mating wild type N. tetrasperma on corn meal agar containing small amounts of FPA. MBC has not yet been tested thoroughly.

The two resistant mutations resemble the E mutation of Dodge in producing asci with greater than the normal number of ascospores. These two resistance mutations are not allelic. The FPA and MBC resistant cultures are being further characterized genetically and cytologically to determine their relationship to one another and to E.

The above work demonstrates the possibility of selecting a particular class of mutants that alters the mechanism for nuclear pairing and genetic segregation in the ascus of N. tetrasperma. Further experimentation should lead to a better understanding of the possible roles of microtubules and spindle pole bodies in nuclear migration and ascospore delimitation in Neurospora. (Supported by Grants GM 07617-02 and GM 12953 from the National Institute of General Medical Sciences, USPHS.) - - - Section of Genetics and Development, Cornell University, Ithaca, New York 14853.