Characterization of *Neurospora crassa* albino mutants that were previously unassigned to locus

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Neurospora crassa mutant hunts by various groups have identified albino (*al*) mutants that map to the *al-1* - *al-2* region on the right arm of linkage group I. The cloning of the *al-1* and *al-2* genes (Schmidhauser *et al*. 1990 Mol. Cell. Biol. **10**:5064-5070, Schmidhauser *et al*. 1994 J. Biol. Chem. **269**:12060-12066) allows assignment of locus to the above mutants by DNA mediated transformation. Analysis of phytoene desaturases from different organisms indicates at least three types of enzymes as defined by the number of desaturation steps carried out (Sandmann 1994 J. Plant Physiol. **143**:444-447). The *N. crassa* phytoene desaturase, the *al-1* gene product, introduces four double bonds converting phytoene to lycopene. Of the three intermediates in this reaction sequence two are colored. The occurrence of visibly distinguishable albino alleles in *N. crassa* has been noted (Perkins 1989 Fungal Genetics Newsl. **36**:63). Assignment of locus to *N. crassa* albino alleles represents a first step in the functional characterization of *N. crassa* carotenogenic loci.

Our aim was a rapid assignment of albino mutations to the *al-1* or *al- 2* locus. Albino mutant strains used in this study were obtained from the Fungal Genetics Stock Center. DNA-mediated transformations were performed that should identify albino mutations as alleles of *al-1*, *al-2* or unidentified loci. Competent spheroplasts were prepared according to the protocol of Royer and Yamashiro (1992 Fungal Genetics Newsl. **39**:76-79). Spheroplasts were transformed with plasmid pSV50 DNA and either an *al-1*+ plasmid DNA, pTJS342, or an *al-2*+ plasmid DNA, pTJS542. Transformants were selected on Vogel s FGS media supplemented with benomyl at a final concentration of 1.5 µg/ml. Transformants that broke the surface of the agar were scored as white, indicating no albino complementation; or yellow or orange, indicating albino complementation. For all twenty strains tested complementation was observed following co-transformation with DNA containing a specific functional albino gene (Table 1).

Table 1. Assignment of locus to *N. crassa* albino alleles

Allele and mating type	FGSC #	Assigned locus
85201 a	381	al-1
7-32 A	912	al-1
1500-08 A	1138	al-1
1500-09 A	1139	al-1
1500-010 A	1140	al-1
1500-011 A	1141	al-1
1500-012 A	1142	al-1
1500-013 A	1143	al-1
JH9698 a	801	al-1

RES6 A	2152	al-1
Y256M231 A	909	al-1
Y602 a	797	al-1
Y2170 a	796	al-1
Y2171 a	795	al-1
alC a	800	al-1
alM a	798	al-1
alS A	827	al-2
B102 a	799	al-2
CN1 a	1107	al-2
RES100SUE A	2154	al-2

DNA-mediated transformation of albino mutants provides an easy and specific assay for locus. Genetic crossing is more time consuming due, in part, to the close proximity of the *al-1* and *al-2* loci. Sixteen strains or 80% of those tested in this study are alleles of *al-1*. The FGSC has 30 albino mutants in which the mutation had previously been assigned to locus. Among these 30 albino mutants 18 are *al-1* alleles (including the former *age-3*) and 12 are *al-2* alleles. The *al-1* and *al-2* transcription units are approximately equal in size. *al-2* mutants accumulate steroids while *al-1* mutants accumulate the colorless carotenoid phytoene (Goldie and Subden 1973 Biochem. Genet. **10**:275-284). However, no difference in viability between different albino mutants and wild-type strains has been noted. Goldie and Subden (1973, ref. cit.) found that the RES6 strain accumulates phytoene suggesting a defect in *al-1*. Our results confirm their observation.

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