Perkins, D.D.

Photoinduced carotenoid synthesis in perithecial wall tissue of *Neurospora crassa*

Perithecial wall tissue is known to be of haploid maternal origin. This can readily be seen using the *per-1* perithecial color mutants first described by Howe and Benson (Mol. Gen. Genet. 131:79-83, 1974) and by T.E. Johnson (Ph.D Thesis, U. of Washington, 1975). Production of black pigment in the wall tissue is blocked or impaired when the maternal (protoperithecial) parent is *per-1*, even if the paternal (fertilizing) parent carries the wild-type *per-1^+* allele. But perithecia are black like the wild type in the reciprocal cross, *per-1^+* female x *per-1* male.

Alleles of *per-1* fall into two phenotypically distinct classes (Howe and Johnson, 1976). When kept in the dark, Type I perithecia remain white. Perithecia of Type II are white at first but then become relatively dark orange or light brown, with a ring of black pigment around the ostiole.

Several observations have indicated that the orange-brown pigment seen in perithecial walls of *per-1* Type II is not carotenoid. Howe and Benson (1974) reported that the pigment was not noticeably decreased by blocks in carotenoid synthesis when double mutants *per-1;al-1, per-1;al-2* or *per-1;al-3* were used as female parents. Howe and Johnson (1976) observed that accumulation of orange pigment in *per-1* perithecia paralleled temporally the accumulation of black pigment in *per-1^+*, and suggested that the orange pigment might be related to melanin. However, R.W. Harding (personal communication) obtained preliminary evidence in 1974 suggesting the presence of small amounts of carotenoid when orange-brown perithecial walls of *per-1* were extracted. In contrast, no carotenoid was evident from extracts of orange-brown perithecia of genotype *per-1;fl;al-3.* The perithecia used by Harding were provided by H.B. Howe, and the *per-1* allele was Type II.

The present note reports observations with *per-1* Type I, which was not examined for carotenoids in the previous studies. Carotenoid induction is readily observed in Type I *per-1*, presumably because Type I alleles do not produce other wall pigments in appreciable quantity.

I first observed induction of orange perithecial pigment by light when crosses in slants using *fl;per-1* (Type I) as the maternal parent were brought into the light after dark incubation. The light induction was confirmed in *fl;per-1* x *fl* crosses made in petri dishes, where the parents were inoculated on opposite sides or in alternating quadrants of the plate so that they formed a barrage when mycelia of the opposite mating type converged (Figure 1A. See Griffiths and Rieck, Can J. Bot. 59:2610-2617, 1981). In a typical barrage, two lines of perithecia are formed, separated by a clear zone. The barrage. The barrage is presumably spanned by trichogynes. The genotype of perithecial wall tissue in each line is that of the adjoining mycelium. This is demonstrated by barrages between *per-1^+* and *per-1*, where perithecia on the *per^+* side of the barrage are black while those on the *per-1* side are not (Figure 1B).

Different genotypes were observed systematically in crosses either homozygous or heterozygous for *per-1*. When a gene was present that blocked carotenoid synthesis, perithecial, walls of *per-l* Type I did not become orange but remained white after light induction. The following crosses were made by inoculating the parents separately on opposite sides or in alternate quadrants of 10 cm petri dishes containing synthetic cross medium with 2% sucrose, 2% agar:

fl;per-1 A x *fl;per-1 a* (FGSC nos. 3311, 3312)

fl;per-1 al-3 A x *fl;per-1 al-3 a* (FGSC nos. 3960, 3120)

The Type I allele was PBJ1 in the first cross, AR174 in the second. Crosses done in triplicate were incubated 8 days at 25°C and placed 30 cm from a 40W cool-white fluorescent light. Bands of perithecia had developed in both crossas, forming a typical barrage.

All the perithecia in both crosses were snow-white when first brought into the light. An hour later, both lines of perithecia had become distinctly orange in the first cross but not in the second, where they remained white. After six days at 23°, only a trace of pale yellow pigment could be seen in the *per-1 al-3* perithecia of the second cross, whereas perithecia of the first cross were vivid orange.

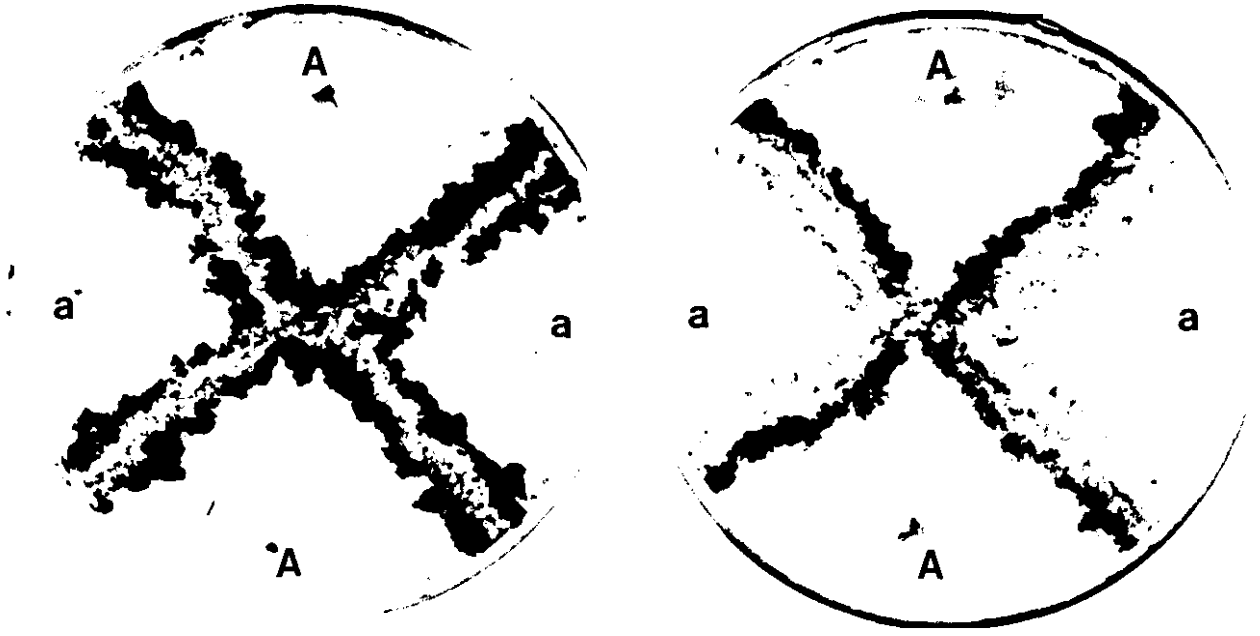

Figure 1. Barrage formation between fl A and fl a. The parents were inoculated in alternate quadrants at the positions marked A or a. Left: Both parents were per-1^+. Right: The fl a parent was per-l (Type I allele PBJl) rather than per-1^+. A barrage the same width as in the other plate separates the black perithecia from a second line of orange perithecia, which appear only as ghosts in this photograph because they are the same color as the mycelial lawn that adjoins them. The orange perithecia on the per-l side were fully as numerous as the wild-type black perithecia opposite them.

The rapid induction of orange pigment in per-1 al^+ perithecia following dark incubation and the absence of induced pigment in per-l al-3 perithecia are typical of carotenoid induction as it is known to occur in mycelia and conidia. Production of carotenoids does not depend on which Type I per allele is used.

The same protocol was used to obtain barrages in which one partner was fl a; per-1 (PBJ1); wc-1 (P829), the other fl; per-1 (PBJ1) A. The mutant white collar allele blocks carotenoid synthesis in mycelia but not in conidia. Carotenoid synthesis was blocked in perithecial walls on the wc-l side of the barrage, while the wc^+ perithecia on the other side were bright orange after induction. In another cross of fl; per-l; wc-1 x f1; per-l al-3, perithecial walls on both sides of the barrage were white and identical in appearance. Clearly, the mutant wc-1 allele blocks carotenoid synthesis in the wall tissue, just as it does in mycelia.

When a Type II per-l allele was used instead of Type I, the experiments were uninformative regarding carotenoid synthesis because the per-l Type II perithecia formed so much orange-brown pigment during the dark incubation that visual detection of carotenoid was precluded. The Type II per-1 allele (UG1837 was used, with and without mutant albino alleles to block carotenoid synthesis. (UG1837 is the original per-1 allele of Howe and Benson. The albino strains were fl; per-l; al-2 a1-1.) In both crosses - with and without the albino genes - the Type II perithecia accumulated orange-brown pigment during the eight-day dark-incubation. Perithecial walls were already so deeply pigmented when the cross plates were brought into the light than an increment comparable to that in the Type I experiments could not have been detected by eye. In the cross where carotenoid synthesis was blocked by al-2 al-l, perithecia were at least as dark as in the cross where wild-type albino alleles were present. The orange-brown pigment of per-l Type II perithecia thus appears to be at least predominantly noncarotenoid, consistent with the observations of Howe, Benson, Johnson and Harding. It is reasonable to assume that carotenoids are also present after induction in perithecial walls of per-l Type II; but that their presence is obscured. - - - Department of Biological Sciences, Stanford University, Stanford CA 94305