

## LETTER TO THE EDITOR

### Note on the Availability of *Phycomyces* Mutants Deficient in $\beta$ -Carotene Biosynthesis

*Sir:* The purpose of this letter is to draw attention to the availability of *Phycomyces* mutants deficient in enzymes for  $\beta$ -carotene biosynthesis. These mutants should be most helpful in the study of still unclarified steps of  $\beta$ -carotene biosynthesis.

Beta-carotene is the principal pigment in wild type of *Phycomyces* and accounts for over 95% of the colored carotenes. Little is known about the enzymes which are involved in the dehydrogenation and cyclization steps. The dehydrogenation steps probably involve a water soluble cofactor and the cyclizations a participation of protons, whilst the carotenes themselves are water insoluble. These reaction steps therefore presumably take place at lipid-water interfaces and involve enzymes that are membrane bound. In connection with a search for the receptor pigment in the light sensitive sporangiophores of *Phycomyces* several  $\beta$ -carotene deficient mutants were isolated and partially characterized.

Similar to other organisms three main mutant blocks have been located in *Phycomyces*: a block decreasing the production of phytoene and resulting in a low overall concentration of carotenes (albino 5, albino 12); a failure of the dehydrogenation enzymes, resulting in the accumulation of high amounts of phytoene but low concentrations of the less saturated successors (albino 1, albino 10); and a defect of the cyclization step, leading to a replacement of  $\beta$ -carotene by lycopene (albino 12, R1).

Formation of artificial heterokaryons between some of the mutants and strikingly different effects of diphenylamine on some of the mutants yielded suggestive but as yet inconclusive results regarding the mechanisms of these dis-

turbances. Heterokaryons between R1 and albino 5 and between albino 10 and albino 12 complement giving a carotene composition similar to that of wild type. The results are understandable assuming that enzymes missing in one mutant are supplied by the other. Diphenylamine (15  $\mu$ g/ml medium) produces in albino 5 a carotene pattern that is a precisely scaled down version of the pattern induced by diphenylamine in wild type. Beta-carotene synthesis is suppressed by about a factor of 100 with a concomitant accumulation of the more saturated precursors. The lycopene mutants, on the other hand, are much less affected by diphenylamine than are wild type or albino 5. Lycopene is still the principal pigment in albino 12. In R1 the absorption spectrum of petroleum ether extracts shows that lycopene,  $\xi$ -carotene and phytofluene are present in about equal amounts, and lycopene itself is reduced by about a factor of 10. These observations suggest that diphenylamine interferes predominantly with the cyclizations, while dehydrogenations are relatively mildly affected. A puzzling feature is the finding that diphenylamine permits the synthesis of lycopene in the lycopene mutants but not in the wild type. Details of this work are described elsewhere (Heisenberg and Cerdá-Olmedo, in press; Meissner and Delbrück, *Plant Physiol.*, in press).

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