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A *Neurospora crassa* mutant which overaccumulates carotenoid pigments.

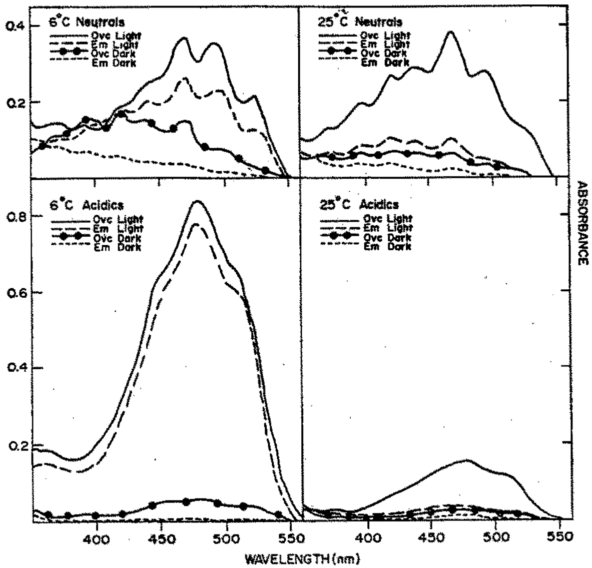

Figure 1. -- Absorption spectra of neutral and acidic carotenoid extracts (in hexane) obtained from dark or light treated wild-type and ovc (S20-16) strains. Treatments were given at 6 vs. 25° C as described in the text. The Em5297a wild-type strain is designated as Em in the figure. The volume of each extract was adjusted to 10 ml hexane/g dry weight of mycelia extracted before absorption spectra were determined.

Previously it was demonstrated that photoinduced carotenoid biosynthesis in *Neurospora crassa* mycelia shows an unusual temperature dependence (R.W. Harding. 1974 Plant Physiol. 54: 142-147). The primary light reaction is independent of temperature as expected, but the amount of pigment which subsequently accumulates in the dark is temperature dependent, and surprisingly the optimum temperature is 6°C. We have isolated mutants which produce more pigment than the wild-type strain at temperatures above 6° C but about the same amount at 6° C. We have designated this type of mutant as *ovc* (overaccumulator of carotenoids). One of these *ovc* mutants (S20-16), isolated after UV mutagenesis of *cot-4* (70007c, FGSC #1177), has been characterized, and the results are presented here. The *ovc* locus was then put into a wild-type background by a series of four backcrosses with Em5297a (FGSC #352).

Fig.1 presents the absorption spectra of neutral and acidic carotenoid extracts obtained from the Em5297a and ovc (S20-16) strains following different light and temperature treatments. The dark-grown mycelia1 pads were harvested and incubated at either 6 or 25° C for 2 h before irradiation for 2 min with blue light plus an additional 24 h incubation in the dark at the same temperature (R.W. Harding and R.V. Turner, 1981 Plant Physiol. 68: 745-749). Corresponding dark controls were carried out for each of these treatments. After a light treatment and dark incubation at 25° C, the *ovc* mutant had accumulated more neutral and acidic carotenoid pigment than the wild-type, but both strains produced less pigment at 25° C than at 6° C. The *ovc* strain also produced significantly more pigment than the wild-type in the dark at both 6° and 25°C.

This dark production of pigment can be readily observed visually. however, the mutant is not fully induced in the dark, since light still produces a dramatic increase in carotenoid production.

To determine which neutral pigment the ovc mutant overaccumulates at 25° C, alumina chromatography (6% water deactivated alumina) of neutral carotenoid extracts was carried out. In Table I, it is shown that the light treated <u>ovc</u> mutant produces higher levels of every neutral carotenoid at 25° C except phytoene. In addition, more neurosporaxanthin (the major acidic pigment) is produced by the <u>ovc</u> strain. At 6° C, the accumulation of each pigment following irradiation (with the exception of α-carotene and 3,4-dehydrolycopene) is comparable in the two strains. The <u>ovc</u> mutant did not produce any carotenoids not previously identified in wild-type Neurospora.

Mapping of the ovc locus was carried out. From backcrosses of <u>ovc</u>, <u>col-4</u> double mutants with wild-type, the <u>ovc</u> and <u>col-4</u> loci were found to be linked. In a subsequent cross of <u>ovc</u>, <u>col-4</u>$^+$, X <u>ovc</u>$^+$ <u>col-4</u>, a re-combination frequency of 14% (133 progeny scored) was determined. Subsequently ovc was-shown to be linked to <u>met-5</u> (9666, FGSC #141) as expected, since the <u>met-5</u> locus is about 23 map units to the right of <u>col-4</u> on linkage group IVR (A. Radford, 1972 Neurospora Newsletter <u>19</u>: 25-26). The order of these loci was determined by the cross shown in Table II. These results show that the gene order is <u>col-4</u>, <u>ovc</u>, <u>met-5</u> with a recombina-tion frequency between <u>col-4</u> and <u>ovc</u> of approximately 10% and between <u>ovc</u> and <u>met-5</u> of about 14%.

TABLE I

Level of neutral and acidic carotenoids in wild-type and <u>ovc</u> strains following different light and temperature treatments

Carotenoid	Treatment and Strain							
	6°				25°			
	Em5297a		<u>ovc</u>		Em5297a		<u>ovc</u>	
	dark	light	dark	light	dark	light	dark	light
	(μg carotenoid/g dry weight mycelia)							
<u>Neutral carotenoids</u> <u>(other than phytoene)</u>								
phytofluene	0.9	1.4	1.1	1.2	0.3	0.8	0.4	1.7
θ-carotene[1]	1.5	3.2	4.1	3.6	0.6	0.7	2.3	6.3
α-carotene	0	0.	0	1.2	0	0	0	0.6
neurosporene	0.6	1.9	2.8	2.1	0.2	0.9	1.8	3.7
torulene	0	0	0	0	0	0.1	0	0.9
lycopene	0.3	1.8	0.9	2.0	0	0.1	0	3.8
3,4-dehydrolycopene	0	2.3	0	4.1	0	0.3	0	3.9
<u>Acidic carotenoid</u>								
neurosporaxanthin	0.6	25.3	2.4	33.2	0.5	1.9	0.8	8.3
Total of above	3.9	45.9	11.3	48.0	1.6	4.8	5.3	29.2
phytoene	77.5	45.9	72.2	44.0	54.2	81.5	59.9	76.0
Total carotenoids	31.4	91.8	83.5	92.0	55.8	86.3	65.2	105.2

1Shown to be a mixture of ζ-carotene and asymmetrical ζ-carotene (B. H. Davies et al. 1974 Phytochemistry <u>13</u>: 1209-1217).

TABLE II

Linkage of ovc to met-5 *and* col-4

Zygote genotype and % recombination	Parentals	Recombination Singles Region I	Recombination Singles Region II	Recombination Doubles Regions I and II
I 11				
col 4 *ovc* +	38	1	4	0
+ + met-5	31	8	9	0
9.9 14.3				

The biochemical basis for the overaccumulation of carotenoids by the ovc mutant at temperatures above 6° C is unknown at present. the ovc strain described has been submitted to the Fungal Genetics Stock Center (see ovc, FGSC #4503). - - - Smithsonian Environmental Research Center, Smithsonian Institution, Rockville, MD 20852. Part of this research was carried out to partially satisfy the requirements for a Master's Degree, Department of Botany, Howard University, Washington, D.C. 20059. **B.Z.D. was supported by Office of Fellowships and Grants, Smithsonian Institution.