A Neurospora crassa mutant which overaccumulates carotenoid pigments

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A Neurospora crassa mutant which overaccumulates carotenoid pigments

Abstract
A *Neurospora crassa* mutant which overaccumulates carotenoid pigments

Authors
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Results: Using this system the incorporation of \([^{35}S]\)methionine into acid-insoluble material increased linearly for 40-60 min. in the endogenous system. The N. crassa lysate subjected to micrococcal nuclease treatment did not support any translation; no increase in incorporation of the label into protein fraction was observed as a function of incubation time. On priming this lysate with Neurospora RNA translation proceeded for about 60 min. At optimal levels of exogenously added RNA, the incorporation of the label approached the level of endogenous translation observed in untreated lysates. Translation of both poly (A)-containing and poly (A-) RNA fractions of Neurospora was supported by this system. Analysis of in vitro translation products of endogenous messengers and exogenously supplied Neurospora mRNA on SDS-polyacrylamide gels revealed a protein profile identical to that demonstrated in extracts of cells pulse-labelled in vivo with \([^{35}S]\)methionine. Polypeptides of up to 200,000 daltons were synthesized in vitro. Pyruvate kinase was detected in the translation products by immunoprecipitation using monospecific antibodies as well as by immunoadsorption on Affigel columns coupled to anti-PK antibody.

It is necessary to establish the optimum K- and Mg-acetate concentration for each type of mRNA as efficiency of translation and its dependence on these salts may vary from message to message. Whereas optimal Mg-acetate requirement for both endogenous and exogenous translation was 0.35 to 0.40 mM endogenous protein synthesis exhibited a sharp K-acetate optimum at 80 mM but the exogenous PK-RNA enriched fraction was translated more efficiently with 20-50 mM.

Another factor that influenced translation was GTP concentration. With 0.125 mM GTP both endogenous exogenously primed protein synthesis proceeded efficiently for only ~10 min. On the other hand with 0.25 mM GTP it was observed to proceed linearly for at least 40 min. This could be due to the stabilization of initiation factors by higher levels of GTP. Heterologous RNA, such as globin mRNA (BRL) was translated efficiently in this system the optimum K- and Mg-acetate concentrations being 250 mM and 4 mM respectively. The translation of exogenous RNA was comparable to that supported by the commercial rabbit reticulocyte lysate (BRL) as witnessed by a similar incorporation of \([^{35}S]\)methionine.

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 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 30 ml hexane/g dry weight of mycelia extracted before absorption spectra were determined.

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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 30 ml hexane/g dry weight of mycelia extracted before absorption spectra were determined.
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 ovc mutant produces higher levels of every neutral carotenoid at 25° C except phytoene. In addition, neurosporaxanthin (the major acidic pigment) is produced by the ovc 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 ovc mutant did not produce any carotenoids not previously identified in wild-type Neurospora.

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

TABLE I

| Carotenoid                     | Treatment and Strain | 6°     | 25°    |
|--------------------------------|----------------------|--------|--------|
|                                |                      | Em5297a| ovc    | Em5297a| ovc    |
|                                | dark light           | dark light | dark light |

Neutral carotenoids

(others than phytoene)

| Carotenoid       | 6° | 25° |
|------------------|----|-----|
| phytofluene      | 0.9| 1.4 |
| θ-carotene       | 1.5| 3.2 |
| α-carotene       | 3.6| 6.3 |
| neurosporaxanthin| 0.6| 2.8 |
| torulene         | 0.1| 0.9 |
| lycopene         | 0.2| 0.9 |
| 3,4-dehydrolycopene| 0 2.3| 0 4.1|

Acidic carotenoids

| Carotenoid            | 6°      | 25°      |
|-----------------------|---------|----------|
| neurosporaxanthin     | 0.6     | 25.3     |
| Total of above        | 3.9     | 45.9     |
| phytoene              | 77.5    | 45.9     |
| Total carotenoids     | 31.4    | 91.8     |

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

Linkage of ovc to met-5 and col-4

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

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.**

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Effect of sorbitol, L-sorbose and inositol on myo-inositol-1-phosphate synthase activity in Neurospora crassa strains.

Protoplasts of Neurospora crassa R2506 carrying the inl 89601 allele are able to regenerate and grow on Vogel's minimal medium containing 1 M sorbitol. We assumed that the defective enzyme which is related to the myo-inositol-1-phosphate synthase (EC 5.5.1.4) and is produced in this strain (Zsindely et al., 1983, Biochim. Biophys. Acta 741: 273), may be activated by sorbitol; therefore, the strain becomes able to synthesize myo-inositol-1-phosphate.

In order to test this assumption the effect of sorbitol, L-sorbose and inositol on inositol-1-phosphate synthase isolated from wild type (RL-3-8A), from the thermosensitive inositol-requiring mutant (inl, 83301(t), FGSC #2257) and from another inositol-requiring strain (89601) was determined. Wild type and thermosensitive inl mutant was grown at 22° C. The active and the defective enzymes were isolated from the different cultures in a highly purified form (Aradi et al., 1982. Prep. Biochem. 12: 137). Enzyme activity was determined according to Barnett et al. (1970, Biochem J 119: 183) with glucose-6-phosphate as substrate, measuring inorganic phosphate released from inositol-1-phosphate by periodate oxidation, as described earlier (Zsindely et al., 1977, Acta Biol. Acad. Sci. Hung. 28: 281). One unit of activity is 1 nmol Pi released during 1 h incubation.

It can be seen from the results presented in Table 1 that the activity of enzymes purified from wild type and the thermosensitive inl mutant was decreased considerably in the presence of sorbitol and L-sorbose, whereas the influence of inositol was significantly smaller. However, the specific activity of the defective enzyme isolated from the inositol-requiring strain became about 2.5 times higher in the presence of sorbitol and L-sorbose.