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Relationship Between Cyclic AMP Level and Accumulation of Carotenoid Pigments in *Neurospora crassa*

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Abstract. Dark grown mycelial cells of *Neurospora crassa* bearing mutant genes *crisp-I* or *frost* and having a decreased level of cyclic adenosine 3′,5′-monophosphate contained more carotenoid pigments than the cells with wild alleles of these genes. A transient decrease of the cyclic AMP occurred following photoinduction of carotenoid synthesis during its lag-period. Its intensity correlated with the increase of carotenoid pigmet level due to photoinduction. No correlation in the content of cyclic guanosine 5′-phosphate with both constitutive level of carotenoids and its photoinduced increase was observed.

Key words: *Neurospora crassa* – *Crisp-I* mutant – *Frost* mutant – Cyclic AMP – Cyclic GMP – Carotenogenesis – Photoinduction

In the mycelial cells of *Neurospora crassa*, like in some other microorganisms, the carotenoid synthesis can be induced by light (for references: Harding and Shropshire 1980). Photoreceptor mechanism regulates, most likely at the transcriptional level, expression of the genes responsible for the formation of proteins involved in enzyme reactions of synthesis and dehydrogenation of phytoene (Harding and Mitchell 1968; Harding et al. 1969; Subden and Bobowski 1973; Spurgeon et al. 1979; Harding and Turner 1981). This conclusion was supported by observation, that in a similar photoinduction system in *Fusarium aquaeductuum*, different populations of polyA-rich RNA (mRNA) are synthesized before and after illumination (Schrott and Rau 1977; Rau 1980).

Since cyclic AMP and cyclic GMP are known to play an important role in the regulation of genome expression, we have made an attempt to reveal a possible relation of cyclic nucleotides to the mechanisms controlling accumulation of carotenoids in *N. crassa*. We investigated the capacity of the mutants with genetically disturbed cyclic nucleotides synthesis for the constitutive accumulation of carotenoids, as well as the effect of light on the cellular level of cyclic nucleotides in the course of photoinduced accumulation of carotenoids. Combination of the above experimental approaches has made it possible to reveal the negative correlation between the content of cyclic AMP (not of cyclic GMP) and the level of carotenoid accumulation both at constitutive and photoinduced syntheses.

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Materials and Methods

Neurospora Strains. The following strains of *Neurospora crassa* were used: RL3-8 (wild type); *nada* (allele *100*) deficient in NAD(P)-glucohydrolase and identical to the wild type with respect of the carotenoid accumulation; *frost* (*fr*) (allele B110); *crisp* (*cr-1*) (allele B123); *albino* (*al*-3) (allele RP100). All these strains were kindly provided by the Fungal Genetics Stock Center, Humboldt State University, California, USA. A double mutant *cr*-1, *al*-3 was obtained from the above mentioned strains *cr*-1 and *al*-3 in our laboratory according to the routine hybridization technique (Davis and de Serres 1970).

Cultivation and Illumination of Mycelium. The mycelium was grown from conidia ($2.5 \cdot 10^5$ cells per ml) in 750 ml flasks containing 200 ml of Vogel's liquid medium (Vogel 1956) plus 2% sucrose upon rotatory shaking (200 rpm) in the dark at 28°C. Growth curves were plotted for the strains studied; at the end of exponential growth the cells were harvested for the determination of the dark level of the carotenoid pigments and cyclic nucleotides, as well as for the photoinduction experiments. The mycelium was filtered from the medium and carefully washed with cold water. For the photoinduction the mycelium was resuspended in distilled water (1 g wet weight per 20 ml of water), preincubated in the dark at room temperature upon continuous stirring, and then exposed to luminescent light (illumination in the spectrum area of 380–520 nm was 2,000 erg $\cdot$ cm$^{-2} \cdot$ s^{-1}). The control portions were incubated under the above conditions in the dark.

Measurement of Carotenoid Pigments. The pigment content was estimated by optical density (OD) at 475 nm in hot absolute ethanol extracts from mycelium. The photoinduction intensity was determined as an increment of OD_{475} after a 4-h light exposure.

Measurement of Cyclic Nucleotides. Cyclic nucleotides were extracted according to Scott and Solomon (1975). The nucleotide concentration was determined using Cyclic AMP Assay Kit and Cyclic GMP RIA Kit, Amersham, England. The cyclic nucleotide content in each studied sample of mycelium was expressed as a mean of at least three individually grown portions of cells; in each extract at least six determinations were made.

Results and Discussion

Correlation Between the Dark Levels of Cyclic AMP and Carotenoids in Neurospora Crassa Mutants. The relationship between the content of cyclic nucleotides in the cells of

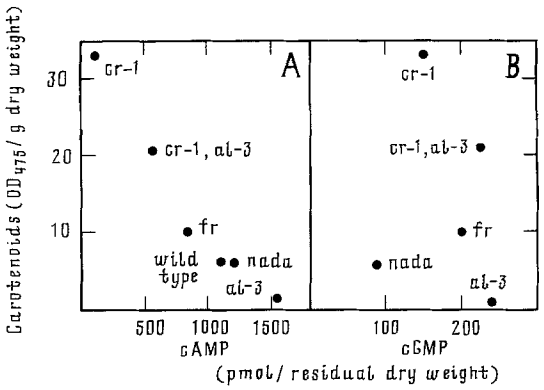

Fig. 1 A, B. Cyclic nucleotides and carotenoid pigments levels in the dark grown mycelium cells of *Neurospora crassa* mutants. The names of mutations are designated at the symbols on the Figure. **A** Cyclic AMP. **B** Cyclic GMP

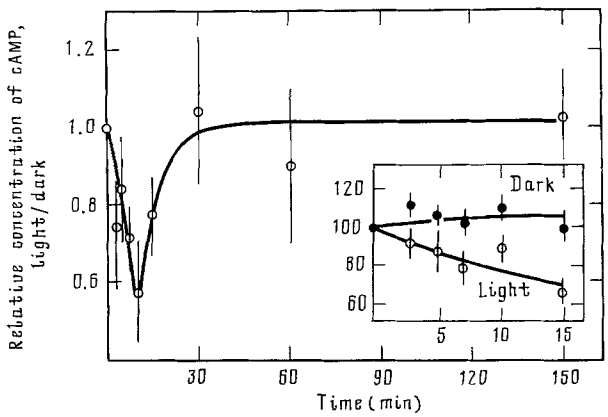

Fig. 2. Light-to-dark ratios of the cyclic AMP content in the mycelium of *Neurospora crassa* (*nada* allele *100*) in the course of photoinduction. The symbols illustrate the ratios of mean values of cyclic AMP content in the light incubated cells to the dark grown ones. The bars show the mean error of the ratios. *Insert:* The cyclic AMP level in light and dark incubated cells. The symbols illustrate mean values of the cyclic AMP content, per cent of initial. ○, light; ●, dark

Neurospora crassa and their capacity for accumulation of carotenoid pigments under the conditions of the constitutive, i. e. dark synthesis, was studied using some mutants with normal and decreased content of cyclic AMP. The cells of mutant *crisp*-I contained genetically damaged adenylyl cyclase (Terenzi et al. 1974, 1976) and in mutant *frost* a decrease in the adenylyl cyclase reaction rate was caused by an impaired cell membrane (Scott 1976). They both had a diminished level of cyclic AMP, if compared with the strains bearing wild alleles of these genes, and, respectively, a higher constitutive level of carotenoid pigments (Fig. 1A).

Since among various *N. crassa* mutants with a blocked carotenoid synthesis the *albino*-3 mutant can be considered as a leaky one (Harding and Turner 1981), we explored the possibility of its "curing" in respect pigment accumulation, by lowering the level of cyclic AMP. For this purpose a double mutant *cr*-I, *al*-3 was obtained; it represented a characteristic *crisp* phenotype and had a lower cyclic AMP content, than that of the parental *al*-3 mutant. Its cells, however, were capable of accumulating more carotenoid pigments, than the cells of mutant *al*-3 under conditions of constitutive synthesis (Fig. 1A).

The relationship between the content of cyclic AMP and the level of carotenoid pigments in dark grown cells of different mutants can be obviously demonstrated by a high absolute value of correlation coefficient (r) equal to -0.97 ± 0.02 (Bailey 1959). This gives evidence for a close negative correlation between the level of cyclic AMP and the capacity for a constitutive accumulation of carotenoids in the *N. crassa* cells. The content of cyclic GMP in the studied mutants did not correlate with the level of carotenoid pigments ($r = -0.17$) (Fig. 1B).

Influence of Light on the Cyclic AMP Level During Photoinduction. The changes in the level of cyclic AMP at the photoinduction were investigated using the strain *nada*, which is employed in our laboratory for the studies of photoregulation mechanisms in *N. crassa* and practically does not differ from wild type cells by its capacity for either dark or light induced carotenoid accumulation. During the first few minutes of light exposure, in the course of lag-period of photoinduced carotenoid synthesis, the cyclic AMP level in the illuminated cells diminished in comparison with the dark incubated ones (Fig. 2). A maximum decrease occurred within 10–15 min after the onset of illumination; at that time the cyclic AMP level dropped to about 60 % (on average from 16 experiments) of that in the dark control cells. It is important, that the cyclic AMP level decreased at that period when the photoinduction system maintained its complete sensitivity to translation inhibitor cycloheximide (Kritsky and Chernysheva 1980), and the synthesis of enzymes catalyzing the formation of carotenoids has not yet started. After a 30 min light exposure, i. e. by the end of a lag-period, the cyclic AMP levels in both the illuminated and non-illuminated portions of mycelium were essentially identical. At a later stage of photoinduction process, when the induced cells were intensively accumulating carotenoid pigments, no distinct difference was found between cyclic AMP content in the illuminated and non-illuminated cells.

It should be noted that in the individual portions of mycelium the maximum change of the cyclic AMP level during the first few minutes of illumination was not always the same. In parallel, the amount of carotenoids that accumulated as a result of induction also varied from experiment to experiment. With a substantial diminishment in the cyclic AMP level, when it decreased in 15 min of illumination by 40 – 60 % as compared to the initial, the pigment content increased 6 – 12-fold. At a lesser decline of the cyclic AMP content, the quantity of carotenoid accumulated was somewhat lower; when no change of the cyclic AMP content or even its slight increase was observed in some experiments, the carotenoid level practically did not change following photoinduction (Fig. 3). The correlation coefficient between the change of the cyclic AMP level after 15 min of illumination and the increase of the carotenoid pigment content after 4 h of photoinduction was 0.83 ± 0.08. This fact is indicative of the negative relationship between the light dependent change in the cyclic AMP level and the intensity of photoinduced carotenoid accumulation in mycelial cells of *N. crassa*. The cyclic GMP level did not show statistically significant changes during the lag-period of photoinduction.

The results presented suggest that in *N. crassa* there exists a negative relationship between the cellular level of cyclic AMP and the rate of accumulation of carotenoids, both at the

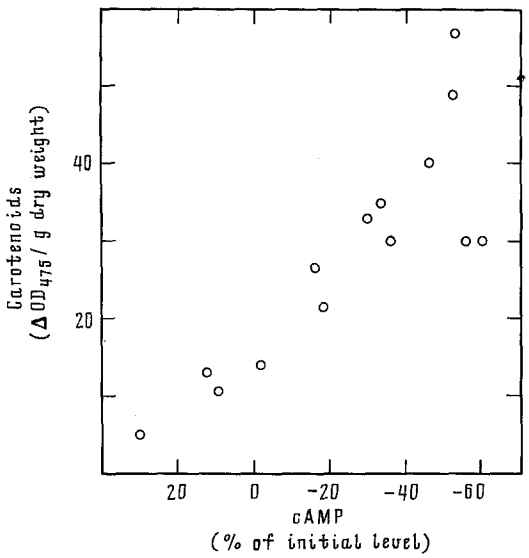

Fig. 3. Relationship between the change of cyclic AMP content during a lag-period of photoinduction (after a 15 min of illumination) and the increase of the carotenoid level (OD_{475}) following photoinduction (after a 4 h illumination)

constitutive (dark) and the light induced syntheses. This observation is consistent with the data indicating the inhibitory effect of a high extracellular concentration of cyclic AMP (10^{-3} M or higher) on the photoinduced carotenoid accumulation in *N. crassa* (Harding 1973; Turian and Khandjian 1973). Thus, there are reasons to believe that the cyclic AMP level in the *N. crassa* cells may be one of the factors of negative control over the expression of the genome loci responsible for the formation of proteins participating in the catalysis or/and regulation of carotenoid synthesis.

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