hypodermic syringe through the arthrodial membrane of the chelate leg. Six different timings were chosen to cover the 24 h period of the day choosing 3 specimens for each time. The experiment was repeated for 3 consecutive days to see whether the pattern of activity levels remained the same in all 3 days. The levels of blood glucose and hepatopancreatic glycogen was assayed using the methods of MENDEL, KEMP and MYERS[7] and KEMP and HEIJNINGER[8] respectively.

Results and discussion. The mean ± standard deviation values of the blood glucose and hepatopancreatic glycogen levels at different times of the day are shown in the figure (Figure). The amount of the blood glucose ranges from 7.99 ± 0.48 (08.00 h) to 27.32 ± 0.63 mg. (20.00 h) of glucose/100 ml of blood and hepatic glycogen from 7.84 ± 0.56 (20.00 h) to 17.21 ± 0.37 mg (08.00 h). From the Figure it is evident that through out the light period and into the early part of the dark period, glycogen stores decline to a minimum at 20.00 h. The blood glucose levels rose steadily during that time to reach a maximum at 20.00 h. As glycogen stores then increased (20.00 h to 04.00 h), blood glucose levels fell. This pattern of activity was seen on all the 3 days proving clearly the existence of diurnal rhythm in the levels of metabolites like glucose and glycogen.

A rhythmic pattern has been shown to occur in various activities like locomotion, poison secretion[9] and neurosecretion[10] besides the rate of heart beat[1] and choline esterase activity on the heart muscle[2] in the scorpion. In the present study, as evident from the results, the variations in the hepatic glycogen bear an inverse relation to the corresponding variations in the blood glucose level, while glycogen declines to a minimum (20.00 h) the blood glucose level shoots up to a maximum (20.00 h) and vice-versa. A similar trend was also found in the rate of heart beat[1] and acetylcholine activity[2] in the heart muscle.

The variations noticed in the blood glucose of *Heterometrus fulvipes* at different intervals of the day indicate variation in its glucose utilisation rate and hence in its metabolic rate during those periods. These variations in its metabolic rate is directly proportional to the variations in the internal physiological activities like synthesis of enzymes and regulatory mechanism of different organs in the body. The high amount of choline esterase activity in the heart muscle[2] of the scorpion at 20.00 h and the maximum rate of heart beat[1] of the scorpion at the same time, from the earlier findings suggested the need for high amount of energy during those hours of the day. The necessary energy is perhaps made available through increased metabolic degradation of blood glucose.

That hepatic glycogenolysis is the predominant source of blood glucose is evidenced by the trend of variation of hepatopancreatic glycogen relating to blood glucose (Figure). The following observations provide further evidence for this. As the scorpions were starved prior to these estimations, dietary carbohydrates could not have been the source of blood glucose.

From these findings it is tempting to suggest the difference in the levels of blood glucose in our findings reflect its varying levels of utilization to meet the energetic and synthetic demands, such as synthesis of acetycholine, in accordance with their pattern of activity, thus showing a regular circadian rhytm like that of heart beat[1] and choline esterase activity[2].

Zusammenfassung. Experimenteller Nachweis einer reziproken Tagesrhythmik von Blutzucker und Leberglykogen beim Skorpion, *Heterometrus fulvipes*.

D. CHENGAL RAJU, M. D. BASHAMOHIDEEN and C. NARASIMHAM

Department of Zoology, S. V. Arts College, Tirupati (A. P. India); and Department of Zoology, S. V. University, Tirupati (A. P. India),
1 September 1972.

[7] B. MENDEL, A. KEMP and D. K. MYERS, Biochem. J. *56*, 639 (1954).
[8] A. KEMP and HEIJNINGEN A. J. M. KITSVAN, Biochem. J. *56*, 646 (1954).
[9] T. GOPALAKIRHSNA REDDY, Dissertation submitted to Sri Venkateswara University, Tirupati (1966).
[10] Md. HABIBULLAH, Dissertation submitted to Sri Venkateswara University, Tirupati (1962).

Evidence for Actinomycin D Inhibition of Transcription of Carotenoid Loci in *Neurospora*

Most micro-organisms that produce carotenoids do so in response to light induction[1-3]. Compounds such as β-ionone (4-(2,6,6-trimethyl-2-cylo, hexen-1-yl) 3 buten-2-one)[4] or diphenylamine[5] inhibit carotenogenesis at the protein level while cycloheximide[6] and chloramphenicol[7] have been found to inhibit at the level of translation in eukaryotes and prokaryotes respectively. To date no evidence has been produced to demonstrate that induction proceeds by some photo-induced mechanism resulting in the specific transcription of hitherto repressed or low level constitutive genes that are responsible for the production of the carotenogenic proteins. This paper presents evidence for the existence of a photosensitive control molecule (either a repressor or inducer) which mediates the specific transcription of carotenogenic cistrons.

The action spectrum for carotenoid induction in *Neurospora*, plateaus at 450–480 nm[8] and tails off towards both ends of the visible spectrum although one recent report has shown some induction at 254 nm[9]. Using a *Neurospora* wild type strain[10] we observed that the carotenoid induction potential was greatest in the late log or stationary phase of growth and that induction is stoichiometrically dose dependent i. e. dark conditions subsequent to light induction do not repress carotenogenesis and the amount of carotenoid formed is proportional to the amount of incident light. The constitutively synthesized dark phase carotenoids were kept at a constant low level by the addition of Tween 80 (15–20 ppm) or some other wetting agent.

[1] H. C. RILLING, Biochem. biophys. Acta *60*, 548 (1962).
[2] W. RAU and C. ZEHENDER, Arch. Mikrobiol. *32*, 423 (1959).
[3] C. D. CHICHESTER, P. S. WONG and G. MACKINNEY, Pl. Physiol. *29*, 238 (1954).
[4] G. MACKINNEY, T. NAKAYAMA, C. BUS and C. CHICHESTER, J. Am. Chem. Soc. *74*, 3456 (1952).
[5] G. TURIAN, Physiologia Pl. *10*, 667 (1957).
[6] R. W. HARDING, Archs Biochem. Biophys. *128*, 814 (1968).
[7] H. HOWES and P. BATRA, Archs Biochem. Biophys. *137*, 175 (1970).
[8] M. ZALOKAR, Archs Biochem. Biophys. *56*, 318 (1955).
[9] E. DE FABO, personal communication.
[10] Isolate No. 74A-OR23-1A gratefully obtained from Fungal Genetics Stock Centre Humboldt College, California, USA.

Actinomycin D (AM) inhibits DNA dependent RNA polymerase activity with varying success in *Neurospora*[11–13]. We found, using growth rate as a crude parameter of transcriptional inhibition, that 5.0 μg AM/ml medium[14] at 35 °C or 3.0 ug/ml potentiated by 0.1 μ*M* ethylenedinitrilo-tetraacetic acid, disodium salt (EDTA) in 0.1 μ*M* *tris* buffer pH 8.0 at 30 °C were optimal effective treatments. Some weight loss was noted after the addition of AM (Figure 1).

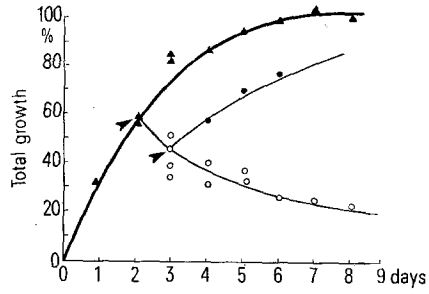

Fig. 1. Growth rates (dry weight). ▲—▲, untreated cultures; ○—○, 5 μg AM/ml added after 48 h; ●—●, AM removed after 24 h of AM treatment.

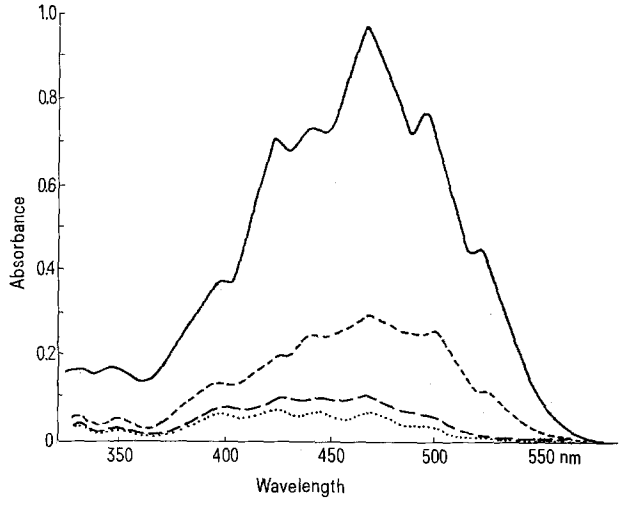

Fig. 2. Absorption spectra from total carotenoid extracts. —, light induced control; – – –, dark, uninduced control; - - - - -, 5 μg AM/ml prior to induction; . . . . . , 2 μg cycloheximide/ml prior to induction.

Absorption maxima from 2-fold dilutions of total carotenoid extracts from 0.65 g (fresh weight) of mycelia

Wavelength (nm)	Induced control	Uninduced control	AM (5 μg/ml)	Cycloheximide (2 μg/ml)
330	0.17	0.04	0.06	0.04
348	0.17	0.03	0.06	0.03
398	0.37	0.07	0.17	0.05
424	0.71	0.10	0.20	0.07
442	0.73	0.10	0.25	0.07
469	0.97	0.10	0.29	0.06
497	0.77	0.05	0.21	0.03
525	0.45	0.00	0.11	0.00

Cycloheximide inhibits translation by 80S ribosomes and as previously noted[6] inhibits carotenoid synthesis if added 8 min before light induction.

AM was obtained from Nutritional Biochem Corp. of Cleveland and cycloheximide from Calbiochem of Los Angeles. All other compounds and solvents were reagent grade. Light induction was performed by exposing cultures to an incandescent light bank giving an incident 0.2×10^6 lux/sec for the photoactive wave lengths as measured on a YSI radio-meter model 65.

Dark grown, 3 day 100 ml cultures of a wild type strain were treated with 5.0 μg/ml. AM for 1 h before induction then incubated for 4 or 6 h in the dark to complete carotenoid synthesis. The mycelia were then collected on cheesecloth, washed twice with distilled water then disrupted in a Waring Blender containing 200 ml of acetone and 1 g acid washed sand. The preliminary extraction was followed by a further phase separation into petroleum ether bp 30° – 49° + 10 % benzene. Cell debris, particulate matter and trace quantities of one carotenoid (neurosporaxanthin) remained in the hypophase. All other carotenoids remained in the epiphase which was washed with distilled water 5 times to remove traces of acetone then saponified with a methanol:water:KOH (90:10:20, v/v/w) solution to remove fatty acids. The epiphase was taken off, left overnight at –17 °C, filtered then treated with 0.9 μg/ml digitonin[6] to precipitate the 3-β-hydroxysteroids. The supernatant was taken down to dryness in vacuo under a nitrogen stream and resuspended in redistilled petroleum ether. Estimates of total 'late' carotenoid content were made by using the absorption maxima at 469 nm as read on a Unicam SP800 spectrophotometer using $E^{1\%}_{1\,cm} = 2500$. A more accurate estimation of the carotenoid content can be seen in Figure 2. Qualitative estimations of carotenoids were accomplished by using thin layer chromatography on silica gel F254 plates from Desaga using light petroleum ether with a diethyl ether gradient[15] as solvent. Carotenoid standards for co-chromatography were gifts from Dr. O. ISLER of Hoffmann-La Roche, Basel.

All work was done under red light to prevent incidental induction or isomerization of pigments in solution. AM is known to be photolabile but analysis of maxima (422 nm, $E^{1\%}_{1\,cm} = 19.6$) from absorption spectra of ethanolic extracts from the medium before and after induction showed little or no AM degradation from the amount of light used for induction.

A comparison of the absorption maxima from the Table shows that the dark grown controls produced up to 10 % of the carotenoids produced by the induced controls. Cycloheximide added 1 h before induction resulted in absorption spectra that were similar but slightly lower than the dark grown cultures. AM added 1 h before induction permits carotenoid synthesis up to 29 % of the induced controls. Carotenoid extracts from cultures exposed to AM 15 min after induction are the same as the induced control. If the AM is added 1 h before induction then removed immediately after or 1 h after induction, the carotenoid content is again similar to that of the induced controls. Concentrations of AM up to 50 μg/ml failed to significantly decrease inducible carotenoid

[11] R. E. TOTTEN and H. BRANCH HOWE JR., Biochem. Genet. *5*, 521 (1971).
[12] J. TURNER, K. TERRY and W. MATCHETT, J. Bact. *103*, 370 (1970).
[13] W. KERSTEN, H. KERSTEN and H. M. RAUEN, Nature *187*, 60 (1960).
[14] M. WESTERGAARD and H. K. MITCHELL Am. J. Botany *34*, 573 (1947).
[15] A. H. GOLDIE and R. E. SUBDEN, in press.

synthesis. It should also be noted (Figure 2) that the carotenoids produced in the dark grown, cycloheximide, and AM inhibited cultures are the 'early' polyenes while the induced controls had proportionately more of the 'later' carotenoids; neurosporene, γ-carotene and some lycopene.

These results can be interpreted by assuming that *Neurospora* constitutively synthesizes a low level of dark synthesized carotenogenic proteins which can be increased 10 fold by photoinduction. The results indicate that if there is a photosensitive control molecule, it must persist in its effective state for at least 1 h.

Cycloheximide is a potent translation inhibitor and completely blocks the synthesis of newly induced carotenogenic proteins. AM on the other hand inhibits only 92% of the transcriptional activity of *Neurospora crassa*[11] and presumably the residual 8% is responsible for the incomplete inhibition of carotenogenesis.

These results do indicate however, that carotenogenesis proceeds by the de novo transcription of new m RNA rather than the translation of pre-existing m RNA as is the case with some other regulatory systems[16].

Wheter the photosensititive control moiety is a photosensitive repressor using light as a deactivating agent (similar to the galactoside allosteric inhibition of the *lac* repressor according to JACOB and MONOD[17]), or whether light acts as activating agent for a photosensitive control moiety responsible for initiation of transcription for specific gene sequences or batteries of genes (according to BRITTEN and DAVIDVON[18]), cannot be resolved at this time[19].

Résumé. Neurospora crassa produit constitutivement un faible taux de caroténoïdes. Celui-ci peut être élevé dix fois par photoinduction. Nous avons trouvé que l'actinomycine D inhibe cette synthèse photoinduite des caroténoïdes probablement au niveau de la transcription des protéinse caroténogènes.

R. E. SUBDEN and G. BOBOWSKI

Department of Botany and Genetics, University of Guelph, College of Biological Sciences, Guelph (Ontario, Canada), 8 September 1972.

[16] I. PASTAN and R. L. PERLMAN, J. biol. Chem. *244*, 2226 (1969).
[17] F. JACOB and J. MONOD, Molec. Biol. *3*, 318 (1961).
[18] R. BRITTEN and E. DAVIDSON, Science *165*, 349 (1969).
[19] Financial support of the National Research Council of Canada is acknowledged.

Determination of C-Terminal Group of Aspergillopeptidase Af

The physico-chemical properties, composition of amino acids in isolated extracellular endopeptidase Af from liquid culture *Aspergillus fumigatus* was described. It was also determined that the N-terminal groups of these enzyme are glycine and glutamic acid[1]. On the basis of this work, it may be supposed that the isolated enzyme is a polypeptide compound of 2 chains. The purpose of this report was to analyse the C-terminal amino acids of the enzyme in order to determine the 2 chain structure of this protein.

Material and methods. The material used was lyophillizing enzyme preparation isolated from the Čapka-Dox liquid culture from *Aspergillus fumigatus*. The method of isolation and purification was described earlier[1]. C-terminal amino acid analysis was carried out by the hydrazine method according to BRADBURY[2-4]. 50 mg of enzyme and 130 mg $N_2H_4H_2SO_4$ was dried in room temperature for about 3 h in vacuum. 1 ml of 100% of previously distilled hydrazine was added, the whole sealed in test tube and heated at 60 °C for 16 h. The excess of hydrazine was evaporated over concentrated H_2SO_4 in vacuum.

The remander was dissolved in 0.1 *N* HCl. The solution was shaken with 2.0 ml benzaldehyde for 2 h. The water solution was separated by centrifugation. In 3 ml of the solution, 0.1 ml 3.1 *M* KCl was added, and pH was adjusted to 9.0 by the addition of 40 µmol 0.20 *N* NaOH. The solution was saturated with 1, 2, 4-fluorodinitrobenzene at 40 °C by energetic mixing (by excess reagent) at constant pH 9 for 80 min. The solution was extracted using ether free from peroxide in order to remove FDNB. The remainder was acidized with 5 N HCl. DNP amino acids were extracted 5 times with ether. As a result of extraction, 2 fractions were obtained, ether and water. Both fractions were analyzed to detect the presence of DNP amino acids by placing 200 µl concentrated extract on a sheet of Whatman no 1. Analysis of water and ether

[1] J. KEDZIORA, Acta biochim. pol. *18*, 109 (1971).
[2] J. H. BRADBURY, Nature, Lond. *178*, 912 (1956).
[3] A. L. LEVY, Nature, Lond. *174*, 126 (1954).
[4] A. L. LEVY and I. I. GESCHWIND, J. biol. Chem. *213*, 187 (1955).