Effect of different combinations of β-ionone and TA on carotenogenesis in B. trispora (–)

Addition		Carotene/ g dry wt. (μg)	Increase compared to control (units)*	
β-ionone (mM)				
2.45×10^{-1}		87.50	3.85	
2.95×10^{-1}		110.00	6.10	
3.92×10^{-1}		145.00	9.6	
TA (μM)				
1.05×10^{-1}		85.00	3.6	
2.09×10^{-1}		125.00	7.6	
2.61×10^{-1}		147.5	9.85	
TA (μM)	+ β-ionone (mM)			Probable value if additive effective
2.61×10^{-1}	2.45×10^{-1}	151.52	10.25	13.7
2.09×10^{-1}	2.95×10^{-1}	217.11	16.81	13.7
1.05×10^{-1}	3.92×10^{-1}	130.41	8.14	13.2

* A unit is defined as an increase of 10 μg of β-carotene per g dry wt.

activity of one of the stimulators was inhibited by the other stimulator which confirmed the competitive nature of β-ionone and TA mediated stimulations. Only in the case of one combination (2.09×10^{-1} μM TA + 2.95×10^{-1} mM β-ionone), stimulators did not inhibit each other's activity. A possible explanation could be that the concentrations of β-ionone and TA were so low that both together made saturation level.

Similarity between β-ionone and TA mediated stimulation of carotenogenesis is that there is an increase in the production of sterol as well as carotenoids in minus and mated cultures indicating that biosynthesis of intermediates of isoprenoid pathway is stimulated[14]. In each case, this effect is inhibited by cycloheximide[6,8]. As the site of β-ionone action is known, TA might be derepressing enzyme or enzymes involved in conversion of 5-phosphomevalonate to dimethyl allyl pyrophosphate. These steps result in the formation of isoprene unit, the building block of sterols and carotenoids.

14 J. D. Bu'Lock and D. J. Winstanley, J. Gen. Microbiol. *69*, 391 (1971).

Effect of the growth-promoting alpha-globulin (GPAG) on in vitro incorporation of exogenous DNA into mammalian cells

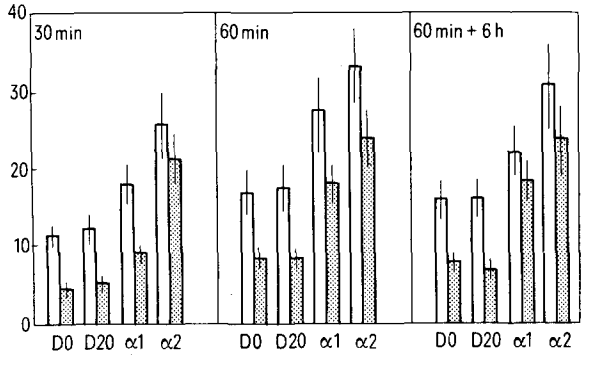
J. Marec, Jarmila Keprtová, Věra Spurná, Eva Minářová and J. Michl

Institute of Biophysics, Czechoslovak Academy of Sciences, Brno (612 65 Brno 12, Czechoslovakia); and Institute of Physiology, Czechoslovak Academy of Sciences, Praha (142 Praha 4, Krč, Czechoslovakia), 18 May 1976

Summary. The uptake of exogenous ^{3}H-DNA by mammalian cells is increased in the presence of a specific serum protein complex – growth-promoting alpha-globulin (GPAG). ^{3}H-DNA is retained in the cell nucleus in a quantity 3 times higher than for control cultures without GPAG even after 47 h of additional cultivation in the medium without ^{3}H-DNA.

The great interest in the incorporation of exogenous DNA into mammalian cells and their nuclei[1] is stimulated by the potential possibility of DNA utilization for introduction of new genetic determinants into the cells. One of the problems is to find proper agents which enhance the incorporation of exogenous DNA and protect it from digestion in the cells. Incorporation of DNA into host cells is stimulated by polyornithine, polyarginine, polylysine, spermine, DEAE-dextran, latex particles, $CaCl_2$ and amphotericin B[2–4]. However, many of these substances may damage the cells as they are not natural components of the cell environment in the organism.

The present study was carried out on a specific serum protein complex – the growth-promoting alpha-globulin (GPAG) – which has been demonstrated to enter the cells by pinocytosis; GPAG has simultaneously a strong binding capacity for some precursors of cell macromolecules. During a co-incubation of GPAG and ^{3}H-thymidine, ^{3}H-uridine, ^{3}H-lysine or $^{32}PO_4'''$, gradual binding of the precursor to protein takes place; subsequently the complex of the 2 components is taken up from the medium by cultivated cells. High pinocytic activity is specifically connected with GPAG and the substitution of serum albumin for GPAG results in a sharply reduced formation of pinosomes[5]. We have therefore studied, in analogous experiments, incorporation of exogenous DNA pre-incubated with GPAG into the cells.



Average number of grains over L-cells (empty column) or their nuclei (solid column) after incorporation of exogenous ^{3}H-DNA or ^{3}H-DNA+GPAG in dependence on incubation period of exogenous ^{3}H-DNA with cells (30 or 60 min) and postincubation without ^{3}H-DNA (60 min incubation + 6 h postincubation).
DO, ^{3}H-DNA not preincubated (control); D 20, ^{3}H-DNA preincubated for 20 h at 37 °C; α_1, ^{3}H-DNA+GPAG (calf); α_2, ^{3}H-DNA+GPAG (sheep). Standard errors of the mean are given for each value.

1 P. M. Bhargava and G. Shanmugam, Progress nucl. acid Res. nucl. Biol. *11*, 103 (1971).
2 J. Keprtová and E. Minářová, Neoplasma *20*, 673 (1973).
3 V. B. Kumar, G. Medoff, G. Kobahashi and D. Schessinger, Nature, Lond. *250*, 323 (1974).
4 F. E. Farber, J. E. Melnik and J. S. Butel, Biochim. Biophys. Acta *390*, 298 (1975).
5 J. Michl and V. Spurná, Exp. Cell Res. *93*, 39 (1975).

Material and methods. Donor ^{3}H-labelled DNA was isolated from L-cells grown in the medium containing ^{3}H-thymidine (3 μCi/ml, 20 h) using the modified method of Marmur, employed already in earlier studies[6] (spec. activity of isolated ^{3}H-DNA 5.64 μCi/mg, m.w. 3.15×10^7 daltons). The ^{3}H-DNA was preincubated for 20 h at 37 °C with GPAG prepared from calf or sheep sera[7]; subsequently the complex of ^{3}H-DNA+GPAG was added to Eagle's MEM in a concentration of 25 μg ^{3}H-DNA/ml of medium and 0.8 mg GPAG/ml of medium. As the host cells, L-strain mouse fibroblasts grown in a monolayer in Eagle's MEM supplemented with 10% calf serum were used. For experiments, cells were plated on cover-slips placed in Petri dishes; in the logarithmic phase of the growth, cover-slips containing cell layer were washed with Hanks' solution and cells grown for additional 30 or 60 min in Eagles's MEM without serum, containing ^{3}H-DNA + GPAG or ^{3}H-DNA preincubated for 20 h at 37 °C. As a control, non-preincubated ^{3}H-DNA was used. After this period, cultures were rinsed and transferred for 30 min into DNAse solution (37 °C, 20 μg/ml DNase N.B.C. 2x cryst. in Hanks' solution, pH 7.0). One set of cultures from each experimental series was fixed immediately (ethanol and concentrated acetic acid 3:1); the second set was cultivated for an additional 6, 24 or 48 h in Eagle's MEM supplemented with 10% calf serum. After this period, cells were fixed, the acidosoluble cell material removed by 1% perchloric acid, and the preparations further processed using autoradiographic technique (Stripping film KODAK AR 10, exposure of 2 to 3 weeks).

Post-incubation (h)	No. of grains/cell		No. of grains/nucleus	
	^{3}H-DNA	^{3}H-DNA+GPAG	^{3}H-DNA	^{3}H-DNA+GPAG
0	15.32	21.45	10.27	16.52
24	11.23	14.33	5.32	11.16
48	6.86	9.51	2.05	7.30

0:60 min incubation of cells with exogenous ^{3}H-DNA or ^{3}H-DNA+GPAG. 24 (48): 60 min incorporation of cells with exogenous ^{3}H-DNA or ^{3}H-DNA+GPAG and subsequent 24 (48) h postincubation without exogenous ^{3}H-DNA. (GAPG was prepared from calf serum.)

Results and discussion. The results of our experiments showed that the uptake of isologous exogenous ^{3}H-DNA into L-cells is markedly stimulated by GPAG. Stimulatory effect of GPAG prepared from sheep serum was higher than that of calf serum GPAG (figure). Preincubation of ^{3}H-DNA at 37 °C did not affect the labelling of cells, which proved that no significant ^{3}H-DNA degradation occurred. That the ^{3}H-DNA retains its macromolecular structure in the host cell is also supported by the pattern of labelling, and especially by the identical ratio of grain distribution between the cytoplasm and the nucleus of cells grown in the presence of control and preincubated ^{3}H-DNA.

In accordance with results described previously[5], the uptake of exogenous ^{3}H-DNA by host cells is increased in the presence of GPAG; at the same time, intracellular transport of ^{3}H-DNA into the nucleus is accelerated. Under the conditions given, radioactivity in the cell nucleus is retained simultaneously in a quantity three times higher than for control cultures even after 48 h of additional incubation in the medium without ^{3}H-DNA (table). Increased quantities of incorporated DNA in nuclei, which are caused by GPAG, thus creates better conditions for DNA integration with recipient cell genome and may be properly utilized in model experiments on exogenous DNA incorporation into mammalian cells under physiological circumstances.

6 J. Keprtová, Neoplasma *20*, 671 (1973).
7 J. Michl, Exp. Cell Res. *23*, 324 (1961).

A plant species of suspected accumulator behaviour

R. A. NADKARNI and S. B. CHAPHEKAR

Department of Chemistry, Cornell University, Ithaca (N. Y. 14853, USA), and Department of Botany, Institute of Science, Bombay 400 032 (India), 1 June 1976

Summary. 17 plant species growing near Bombay, India, were analysed for 26 trace elements by neutron activation analysis. Out of these only *Alternanthera sessilis* was found to be an accumulator plant for Al, V, Ti and Sc.

Accumulator organisms are characterized by their capacity to absorb and store large amounts of specific elements which are not taken up by the population at large growing in the same environment. They are also called indicator or collector species. Several examples of such species have been given by BOWEN[1] and UNDERWOOD[2]. These plants also serve as useful geochemical indicators for identification of the underground mineral deposits.

We recently undertook a study to determine the elemental concentrations of a number of plant species and to compare the effects of air pollution on trace element content of the plants. During the course of this study, we came across some plant species which looked promising as accumulator species.

Materials and methods. The plant samples were collected from the Institute of Science Botanical Gardens, Bombay. The leaves, stems and where possible roots, inflorescence and fruits were collected. The material was washed, dried at 85 °C and powdered. The analysis for trace elements was performed by the technique of neutron activation analysis, the details of which are given elsewhere[3].

Results and discussion. 17 plant species were analyzed for 26 trace elements. Most of the species were within a close range for a given element. Only *Alternanthera ses-*

1 H. J. M. BOWEN, in *Trace Elements in Biochemistry* (Academic Press, London 1966).
2 E. J. UNDERWOOD, in *Trace Elements in Human and Animal Nutrition* (Academic Press, New York 1971).
3 R. A. NADKARNI and G. H. MORRISON, Anal. Chem. *45*, 1957 (1973).