Fractionation of Chick-Embryo Transfer Ribonucleic Acid

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The tRNA isolated from chick embryo at two stages of development was fractionated on a DEAE-Sephadex column to find the number of isoaccepting tRNA species and their variation, if any, during development. The tRNA samples were prepared from 14- and 20-day-old embryos, at which stages the tRNA content/g of tissue is the highest and the lowest respectively. They were labelled separately with three radioactive amino acids, namely arginine, leucine and serine, by using aminoacyl-tRNA synthetase prepared from chick embryo. Each of the samples was fractionated on a DEAE-Sephadex column by a salt gradient in the presence of 7.0M-urea, and the radioactivity in each fraction was measured. The tRNA from 20-day-old chick embryo, labelled with arginine, separated into two major and three minor peaks, which appeared in the early fractions. The same tRNA, when labelled with serine, gave four radioactivity peaks in the middle fractions. The tRNA from 14-day-old chick embryo, labelled with leucine, gave five radioactivity peaks, which were distributed throughout. When [^{14}C]arginine-labelled tRNA from 20-day-old embryos and [^3H]arginine-labelled tRNA from 14-day-old embryos were chromatographed together, the ^{14}C and ^3H radioactivity peaks did not coincide, showing that the isoaccepting species of arginine tRNA from the two stages of development are not identical.

An Inhibitor of Ribonucleic Acid Polymerase from *Neurospora crassa*

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The cell extract from *Neurospora crassa* grown for 9h showed the presence of an inhibitor of DNA-dependent RNA polymerase from *Escherichia coli* and *N. crassa*. Characterization of the partially purified preparation and incorporation of labelled amino acids indicated the inhibitor to be a protein. Experimental evidence showed that there was binding of the proteins to DNA, thereby blocking the template availability for transcription *in vitro* by RNA polymerase. The protein appeared to be present only at some specific growth period in the life cycle of the fungus. The significance of such a protein in the gene function of the organism will be discussed.

Thymine Starvation and Deoxyribonucleic Acid Synthesis in *Escherichia coli*

By A. K. PILGAOKAR, D. S. PRADHAN and A. SREENIVASAN (*Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-85, India*)

It is known that in certain thymine-requiring strains of *Escherichia coli* the rate of DNA synthesis is enhanced two- to three-fold if the cells are pre-starved of thymine for about 90 min. To elucidate the events underlying this phenomenon, the rate of DNA synthesis, as determined by giving a 1min pulse of [^3H]thymine, was followed at various intervals during a synchronous cell division of *E. coli* 15T $^-$ (557). It was found that in the normal synchronous cell population, after a period at a steady rate, the DNA synthesis spurts up to three times the initial rate. When such a synchronous cell culture is starved of thymine for 90 min and then supplemented with thymine, this spurt (the magnitude of which is also three times the initial rate) is seen earlier. This implies that the increase in DNA-synthetic rate seen in a thymine-pre-starved asynchronous cell population is probably not the consequence of an increased rate of DNA synthesis per replication point. Rather the overall stimulation in DNA-synthetic rate appears to be a reflection of initiation of new replicating points occurring earlier. These results will be discussed in relation to current hypotheses on the regulation of DNA replication.

Induction of Phosvitin Synthesis in Chicken Liver by Oestrogens: Evidence for Gene Duplication

By BANSI JAILKHANI and G. P. TALWAR (*Department of Biochemistry, All India Institute of Medical Sciences, New Delhi-16, India*)

Oestrogenic hormones induce the synthesis of the egg-yolk proteins phosvitin and lipovitellin in the liver of roosters. There is a lag period of about 24h between administration of the hormone and commencement of the phosvitin synthesis. Among the relevant metabolic activities stimulated by the hormone are the following: (1) RNA synthesis is stimulated within 30min of hormone administration, reaching a maximum value at 4h; (2) the synthesis of early-phase and late-phase RNA and proteins is followed by a period during which an activation of DNA synthesis, as measured by [^3H]thymidine incorporation, is noticed. The activation, which is maximum at between 11½ and 12h, is essentially of the nuclear DNA and not that of the mitochondria. The hormone-induced activation of RNA and DNA syn-

thesis is an obligatory prerequisite for the ability of the tissue to synthesize phosphovitin. Mitomycin C, sufficient to block DNA synthesis by about 90%, when given between 6 and 11 h after oestradiol administration, inhibits phosphovitin synthesis. However, if the antibiotic is given at a time after the phase of active DNA synthesis is over no such inhibition is noticed. The possible toxicity effects of mitomycin C under the experimental conditions are excluded on the grounds that other metabolic activities of liver, including the incorporation of radioactive amino acids into proteins, are not affected by the antibiotic. Nor is there a lowering of the concentration of serum albumin, a protein synthesized by this organ.

The increase in DNA synthesis on administration of the hormone is not large enough to indicate a clonal proliferation of hormone-sensitive cells. The results are compatible with the duplication or amplification of limited genetic segments in cells, a probable mechanism by which this organ is geared by the hormone for the synthesis and excretion of a protein needed in large amounts for the manufacture of eggs.

Changes in the Ribosomal Ribonucleic Acid Content during Development of the Chick Embryo

By UMA V. RAO and J. D. CHERAYIL (*Department of Biochemistry, Indian Institute of Science, Bangalore-12, India*)

In our laboratory it has been demonstrated that the tRNA content/g of tissue in developing chick embryo does not remain constant but varies with development (Maya Sundari *et al.*, 1970). It was decided to investigate whether the rRNA content also changes with development. Fertilized eggs were incubated at 37°C, and at various days of development they were taken for isolation of RNA. The embryos were homogenized with buffer in the presence of sodium dodecyl sulphate and bentonite, and the homogenate was deproteinized by repeated treatment with phenol. The phenol layer was re-extracted with buffer for quantitative recovery of RNA. The total RNA was precipitated with ethanol and was checked for DNA contamination. The RNA was fractionated on a Sephadex G-100 column and the rRNA fraction was determined quantitatively in a spectrophotometer. It was further characterized by column chromatography on methylated albumin on kieselguhr.

The amount of rRNA/g of tissue remains constant up to day 10 of development, and then there is a rise until day 16, at which time it is almost twice the day-10 value. After day 16 there is a decrease in the rRNA content, and on day 21 it is more or less the same as that on day 10. The base compositions of the

rRNA at three stages were determined and were found to be almost identical in all cases.

Maya Sundari, R., Cherayil, J. D. & Jacob, T. M. (1970) *Abstr. Conv. Chem., Indian Institute of Technology, Madras*, p. 144

Thionucleotides in the Transfer Ribonucleic Acids of *Pseudomonas aeruginosa* and *Escherichia coli*

By Y. S. PRASADA RAO, B. THIMMAPPAYYA and J. D. CHERAYIL (*Department of Biochemistry, Indian Institute of Science, Bangalore-12, India*)

The presence of thionucleotides in the tRNA of *Pseudomonas aeruginosa* has been demonstrated in our laboratory (Thimmappayya & Cherayil, 1970). A comparison of the number and types of thionucleotides from this source was made with that of *Escherichia coli* tRNA by a column-chromatographic method. tRNA labelled with radioactive sulphur was isolated from cells grown in a synthetic medium containing [³⁵S]sulphate and was digested with alkali. The nucleotides were converted into nucleosides by treatment with excess of bacterial alkaline phosphatase and were chromatographed on a phosphocellulose column with a salt gradient. The extinction at 260 nm and the radioactivity of the fractions were determined. On the basis of the extinction at 260 nm, four peaks, identified as uridine, guanosine, adenosine and cytidine, were obtained. With *Ps. aeruginosa* tRNA the radioactivity resolved into six well-separated peaks with possibly one more minor peak. *E. coli* tRNA on the other hand gave only five radioactivity peaks. From the elution pattern it appears that all the five radioactivity peaks with *E. coli* tRNA are present with *Ps. aeruginosa* tRNA also. 4-Thiouridine and 2-thiocytidine were detected and identified in both cases by using authentic samples. 4-Thiouridine and 2-thiocytidine contained approx. 22 and 6% of the total radioactivity in *P. aeruginosa* tRNA, and were 48 and 3% respectively in *E. coli* tRNA.

Thimmappayya, B. & Cherayil, J. D. (1970) *Proc. 39th. Annu. Meet. Soc. Biol. Chem. India*, p. 5

Pyrimidine Clusters in the Deoxyribonucleic Acid of *Azotobacter vinelandii*

By S. A. KHAN, S. S. KRANDIKAR and T. M. JACOB (*Department of Biochemistry, Indian Institute of Science, Bangalore-12, India*)

Azotobacter vinelandii was grown in Burk's medium under vigorous aeration and DNA was isolated from it by a modification of Marmur's (1961)