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Induction of δ -Aminolaevulate Synthetase Activity in Cultured Chick-Embryo Liver Cells by Ribonucleic Acid

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AIA* is porphyrinogenic in experimental animals (Goldberg & Rimington, 1954), and specifically induces δ -aminolaevulate synthetase, the initial and rate-limiting enzyme of porphyrin biosynthesis (Granick, 1966).

Hickman, Saunders, Dowdle & Eales (1967) found that high-molecular-weight RNA isolated from the livers of rats rendered porphyric by administration of AIA increased the porphyrin content of segments of chick-embryo liver grown in organ culture. Repetition of these experiments in this laboratory has given inconclusive results. In the present study, however, we have measured aminolaevulate synthetase activity by the method of Dowdle, Mustard & Eales (1967) in cultured chick-embryo liver cell monolayers (Skea & Nemeth, 1969).

Rats were rendered porphyric by intraperitoneal injection of AIA and RNA was prepared from their livers and from the livers of normal rats by the method of Parish & Kirby (1966). Incubation of cell monolayer cultures of 14–15-day-old chick embryo liver with a solution of RNA from porphyric rat liver (0.2mg of RNA/ml of Eagle's minimum essential medium) caused a two- to threefold increase in aminolaevulate synthetase activity within 4h, the enzyme activity returning to the control value by 8h. No increase of aminolaevulate synthetase activity was found in cultures incubated with normal rat liver RNA. This effect is apparently RNA-dependent, since prior incubation of porphyric rat liver RNA with ribonuclease abolished it. Since RNA isolated from normal rat liver homogenate deliberately contaminated with AIA did not increase aminolaevulate synthetase activity in the cultures, the effect was presumably not due to contaminating traces of AIA.

Preliminary fractionation of porphyric-rat liver RNA has been carried out on Sephadex G-100 by the method of Kay & Cooper (1969). By this method 90% of the RNA was excluded from the column, the rest being eluted later in two shallow peaks. The RNA from the three fractions was reprecipi-

tated with ethanol and tested separately at a concentration of 0.2mg of RNA/ml for ability to increase aminolaevulate synthetase activity in chick-embryo liver cultures. All three fractions showed significant inducing activity, but the two lower-molecular-weight fractions were two to three times as active as the Sephadex-excluded fraction. Similar fractions of normal rat liver RNA showed no significant inducing activity.

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Binding of Polylysine and Toluidine Blue to Deoxyribonucleoprotein

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The accessibility of the DNA component of dNP* to molecules of various sizes was studied by measuring its binding to the dye Toluidine Blue, and to poly-L-lysine hydrobromide of three different molecular weights. Mixtures with dNP were made at concentrations such that all the dNP precipitated. Estimates were then made of the excess of unbound dye or polylysine over a range of dNP concentrations.

With Toluidine Blue, about 60% of the DNA phosphate groups in dNP were available for binding. This high value was not attributable to dissociation of the DNA from the protein, nor to any substantial binding to the protein acidic groups.

With polylysine, estimates were made of both completely unused polylysine molecules and of lysyl residues in molecules only partly bound to dNP, i.e. of unused ('wasted') residues. 'Titration' values of available DNA phosphate groups were 46, 38 and approx. 44% respectively for polylysine preparations of mol.wt. 1250, 7790 and 62000 (PL1, PL2 and PL3). In DNA, 100% of the phosphate groups were available. Near the point of mutual charge neutralization with dNP and DNA no lysyl residues were 'wasted', at least for preparations PL1 and PL2. This result and the

* Abbreviation: AIA, allylisopropylacetamide.

* Abbreviation: dNP, deoxyribonucleoprotein.

relatively small length of the PL1 molecule eliminate the possibility of cross-linkage of small free DNA zones in dNP by preparations PL1 and PL2. Thus 38% of the 'free' DNA zones are sufficiently long to accommodate either preparation PL1 or PL2. With preparation PL3, cross-linkage probably does occur and may account for its slightly higher titration value. The even higher values for preparation PL1 and for Toluidine Blue are presumably related to their smaller size.

Previous enzyme studies (R. F. Itzhaki, unpublished work) had shown that there are few, if any, extensive truly free DNA zones in dNP. This and the present result can be explained on the basis that dNP contains zones of phosphate groups unbound but hidden beneath protein. The distribution of basic residues in histones is very irregular (DeLange, Farnbrough, Smith & Bonner, 1969), and model-building studies (Richards & Pardon, 1970) with the F2a1 histone suggest that less than 60% of the DNA phosphate groups in dNP can be neutralized by the basic residues. The remaining phosphate groups would not be estimated as free by the enzyme method because of their associated protein, but would be free to bind dyes and polylysine. The relevance of the results to the role of histones in control of gene transcription will be discussed.

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Investigations into the Nucleotide Sequence of Cysteine Transfer Ribonucleic Acid from Baker's Yeast

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Cysteine tRNA has been isolated from baker's yeast RNA by a modification of the reversed-phase partition-chromatographic system described by Kelmers, Novelli & Stulberg (1965).

Cysteine tRNA represents about 1% of the RNA applied to the column and after chromatography is enriched at least 20-fold in its ability to accept [¹⁴C]cysteine in the standard assay system with a partially purified aminoacyl-tRNA synthetase prepared from rat liver. In our hands the maximum incorporation of [¹⁴C]cysteine corresponded to about 40% of that calculated, assuming mol.wt. 25000 for cysteine tRNA.

Our preparation does not accept significant quantities of serine, phenylalanine, tyrosine, iso-

leucine, proline and alanine. Further, there is no incorporation of radioactivity from a mixture of all ¹⁴C-labelled amino acids lacking cysteine. It is not further resolved by chromatography in other systems nor by countercurrent distribution for 500 transfers in a phosphate-formamide-propan-2-ol system (Apgar, Holley & Merrill, 1962). The elution patterns resulting from enzymic degradation described below are consistent with the view that this RNA preparation is substantially pure.

The nucleotide composition was established by DEAE-cellulose chromatography of an alkaline or ribonuclease T2 digest, with 0.07M-sodium acetate buffer, pH 4.10 or 4.15, as eluent. Oligonucleotides resulting from complete pancreatic and T1 ribonuclease hydrolyses have been isolated and partially characterized. Minor nucleotides identified as constituents of these oligonucleotides include ψ p, Tp, 5-Me-Cp, diMe-Gp and a nucleotide resembling isopentenyl-Ap.

Treatment with ribonuclease T1 at 2°C for 45 min affords two fragments that can be separated by DEAE-cellulose chromatography in 7M-urea at pH 7.9. With a higher enzyme:RNA ratio a more complex series of products is obtained. The sequences of these larger oligonucleotides are still to be determined.

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Chemotaxonomy and Biosynthetic Relationships of Boletineae Pigments

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Morphological taxonomy of the Boletineae is attended with the disadvantage of subjective assessment of essentially qualitative features. The nature of the pigments and the presence or absence of a polyphenol oxidase in eight species of three Boletineae genera have therefore been examined as a possible aid in taxonomy.

T.l.c. of fungal extracts separates polyhydroxy-diphenylbenzoquinones and pulvinic acid derivatives, which have been identified by spectroscopic methods and colour reactions. Some *Boletus* species turn blue on bruising and this has been attributed to enzymic oxidation of variegatic and xerocommic acids, which are pulvinic acid derivatives (Steglich, Furtner & Prox. 1968; Beaumont, Edwards & Elsworth, 1968). In a study of eight Boletineae species four pulvinic acids have been isolated.