PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 530th. Meeting of the Society was held at the University of Surrey, Guildford, on Tuesday, Wednesday and Thursday, 18, 19 and 20 July 1972, when the following papers were presented:

COLLOQUIUM ON 'ENZYME INDUCTION'

Induction of Bacterial Enzymes

By PATRICIA H. CLARKE (*Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.*)

Many bacterial species are capable of growth on a wide variety of organic compounds, and the necessary catabolic enzymes may be subject to several types of control. Enzyme induction is a phenotypic response of a culture to the presence of the appropriate chemical signal in the environment, resulting in an increase of a 1000-fold or more in the synthesis of enzymes so controlled. The earlier physiological experiments in this field showed that a catabolic enzyme appeared to be induced by its substrate, and it was thought that a series of enzymes of a metabolic pathway might be sequentially induced by the intermediates of that pathway that would be the substrates and inducers of subsequent enzymes. The rate of synthesis of many catabolic enzymes was also observed to alter considerably in the presence of other carbon or nitrogen compounds and to vary with the growth rate of the culture. In general, high growth rates and the presence of rapidly metabolizable carbon compounds resulted in a decrease in the rate of synthesis of inducible enzymes, and this effect was termed catabolite repression. During the last 20 years detailed biochemical and genetic investigations have suggested how these events may be interpreted at the molecular level. Bacteria are particularly suitable for studying enzyme control systems, since they have a very short mean generation time, they respond rapidly to changes in environmental conditions, mutants can be isolated and methods are available for genetic analysis.

Two enzyme control systems that have been studied in great detail are those concerned with the utilization of lactose and arabinose by *Escherichia coli*: the *lac* operon and the *ara* operon. The operon theory of Jacob & Monod (1961) postulated that the synthesis of an induced enzyme was under the negative control of a regulator gene, which produced a macromolecular repressor that acted on the operator gene *o* and prevented the expression of one or more enzyme structural genes. This implied that the inducer need not be the enzyme substrate, and this clear separation of inducer and substrate roles has been demonstrated in many systems. The cytoplasmic repressor produced

by the *i* gene of the *lac* operon was later isolated and shown to be a tetrameric protein that interacts both with inducers of β -galactosidase and *lac*-operon DNA (Gilbert & Müller-Hill, 1966). Compounds that are inducers *in vivo* are also able to inhibit repressor-operator binding *in vitro* (Riggs *et al.*, 1970). The promoter *p* is the binding site for RNA polymerase and initiation of transcription, and appears to be also the site concerned with catabolite repression (Ippen *et al.*, 1968; Silverstone *et al.*, 1969). *In vivo*, catabolite repression of β -galactosidase synthesis by glucose can be relieved by the addition of cyclic AMP (adenosine 3':5'-cyclic monophosphate) (Perlman & Pastan, 1968). A cyclic-AMP-binding protein (CRP) was isolated from wild-type strains, and a similar protein with lower affinity for cyclic AMP was isolated from mutants pleiotropically negative for the utilization of several sugars. A cell-free system containing *lac*-operon DNA and the subcellular components required for protein synthesis is capable of transcription and translation. This is inhibited by the *lac* repressor and stimulated by cyclic AMP and the cyclic-AMP-binding protein (Chen *et al.*, 1971). The *lac* operon thus appears to be under negative control by the *i* gene and under positive control by the *crp* gene. A positive control of the *ara* operon was suggested from genetic evidence, and gene *araC* is thought to determine a protein required for initiation of the expression of the structural genes for the arabinose enzymes. The *ara* operon is also subject to catabolite repression, and a cell-free system is stimulated by cyclic AMP and the cyclic-AMP-binding protein (Zubay *et al.*, 1971; Greenblatt & Schleif, 1971).

Another group of bacteria that have been particularly useful in studying the regulation of enzyme synthesis are the *Pseudomonas* species. The wide metabolic diversity of pseudomonads is dependent on induced enzymes that are subject to complex regulatory controls that are not encountered in the more restricted metabolism of *E. coli* (Ornston, 1971). *Pseudomonas aeruginosa* and *Pseudomonas putida* can grow on many aromatic compounds, which are converted eventually into intermediates of the tricarboxylic acid cycle. The enzymes can be assigned to a number of separately controlled regulatory units, and enzymes under a common control can be identified by co-ordinate induction and repression. The overall pattern is of sequential induction, in which

the first group of enzymes is induced by one or more of the substrates and the later groups of enzymes are induced by intermediate metabolites. This allows growth on intermediates of the pathway, since they are effective as inducers of subsequent enzymes; the earlier enzymes would not be synthesized. For several of the enzymes involved in the metabolism of aromatic compounds the inducer is the product of the enzyme and not its substrate. These enzymes are also subject to repression by products of the pathways, so that the rates of synthesis are balanced according to the metabolic demands of the culture and the nutrients available in the environment.

The aliphatic amidase produced by *Ps. aeruginosa*, and those produced by other species of *Pseudomonas*, exhibit differences in inducer and substrate specificities that are reflected in differences in the amide growth range. Various classes of amidase mutants that have been used to investigate the regulatory controls for the synthesis of amidase include constitutive mutants, altered-inducibility mutants and mutants producing amidases with altered substrate specificities (Clarke, 1970).

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Analysis of Microsomal Hydroxylase Induction in Cell Culture

By HARRY V. GELBOIN, FRIEDRICH J. WIEBEL and HAYDEN G. COON (*Chemistry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014, U.S.A.*)

The mammalian microsomal enzyme system aryl hydrocarbon hydroxylase is highly inducible in a variety of cells in culture. On the addition of inducer there is a 35min lag, after which the enzyme activity increases up to 100-fold during a 16h period. The half-life of the induced enzyme is 3–4h, and the presence of the inducer has a stabilizing effect on the

enzyme and increases its half-life by about 50%. The rise in enzyme activity during induction is prevented by cycloheximide throughout the induction and by actinomycin D only when added within 1–2h after the inducer. After 2h the further rise in enzyme activity for 6h is insensitive to actinomycin D. Thus RNA synthesis is required only very early in induction. The cells pass from actinomycin D-sensitivity to -insensitivity in the presence of cycloheximide, indicating that the induction-specific RNA may be synthesized in the absence of protein synthesis. The synthesis of the induction-specific RNA is also entirely dissociable from rRNA synthesis, since a low concentration of actinomycin D that inhibits the latter by more than 90% has no effect on enzyme induction. The induction-specific RNA is most probably present in the heterogeneous cytoplasmic RNA, and its synthesis and transfer to the cytoplasm are not dependent on rRNA synthesis. Also, sequential suppression of rRNA synthesis by cycloheximide and actinomycin D does not prevent enzyme induction.

In other studies intraspecific hybrids were made between cells that differ many-fold in base-line activity and inducibility of aryl hydrocarbon hydroxylase. Two hamster cell lines (OBP and BHK), which differ 15-fold in both base-line activity and inducibility, yielded hybrids whose basal and induced activities were as low as or lower than the less-active parent (BHK). Similar results were obtained in hybrids of human cell lines JEG-3 and VA-2. The hybrids of these cells exhibited activity similar to that of the low-activity parent.

Hybrids were formed between the mouse fibroblast line A9, which has no detectable base-line or induced aryl hydrocarbon hydroxylase activity, and 3T3.4C2, which has moderate basal and induced activities. In the A9–3T3.4C2 hybrids both the basal and induced activities were two- to five-fold higher than in the 3T3.4C2 parent. Thus in this hybrid there are increases in both control and induced enzyme activities. No examples were found of a dissociation of basal and induced aryl hydrocarbon hydroxylase activities, suggesting a linkage between the regulation of the basal and induced enzyme activities.

Regulation of Enzyme Amounts in Developing Liver

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The main purpose of our studies of enzyme inductions in immature organs has been to elucidate some of the mechanisms involved in biochemical differentiation. The first step was to identify a series of