Formation of Active Phosphoenzymes with the Diphosphoglycerate-Dependent Phosphoglycerate Mutases

By H. G. BRITTON,* J. CARRERAS and S. GRISOLIA (*Department of Biochemistry and Molecular Biology and Department of Obstetrics, University of Kansas Medical Center, Kansas City, Kans.* 66103, *U.S.A.*)

It is not clear whether the diphosphoglycerate-dependent phosphoglycerate mutases combine with diphosphoglycerate to give enzyme–diphosphoglycerate complexes or whether phosphoenzymes are formed. In the latter case monophosphoglycerate should be released in stoicheiometric quantity during the reaction. 2,3-Diphosphoglycerate was therefore added to substrate quantities of the enzymes in the presence of enolase, pyruvate kinase, lactate dehydrogenase, ADP and NADH. Monophosphoglycerate determined by the change in E_{340} was formed in an initial burst in the quantity expected for phosphoenzyme formation (2mol of phosphate/mol for the rabbit muscle and pig kidney enzymes; >3mol of phosphate/mol for the yeast enzyme); thereafter there was a steady production of monophosphoglycerate until the diphosphoglycerate was completely hydrolysed. Successive additions of diphosphoglycerate indicated that the lifetime of the phosphoenzymes was of the order of minutes, and the instability of the phosphoenzymes accounted for the diphosphoglycerate phosphatase activity. In further experiments with the rabbit muscle and pig kidney enzymes, the amount of enolase was lowered to rate-limiting quantities and only sufficient diphosphoglycerate was added to titrate the enzyme. The initial rate of reaction was the same as that obtained when an equal quantity of 3-phosphoglycerate was substituted for diphosphoglycerate. Reversible association of the phosphoenzymes with monophosphoglycerate to form enzyme–diphosphoglycerate complexes could therefore be excluded. Since the enzymes show Michaelis–Menten kinetics under these conditions, it follows that the phosphoenzymes must be catalytically active.

Incubation of the enzymes (from rabbit muscle, pig kidney and yeast) with [^{32}P]diphospho[^{14}C]-glycerate followed by gel filtration yielded phosphoenzymes with no bound ^{14}C. After denaturation with sodium dodecyl sulphate, the phosphoproteins from pig kidney and yeast were stable to alkali and unstable to acid, suggesting the involvement of phosphohistidine, as has been shown for the rabbit muscle enzyme (Rose, 1970).

* Present address: Department of Physiology, St. Mary's Hospital Medical School, London W.2., U.K.

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Nucleic Acids Spontaneously Released by Living Frog Auricles

By MAURICE STROUN and PHILIPPE ANKER (introduced by S. R. PELC) (*Laboratoire de Physiologie Végétale, Université de Genève, Geneva, Switzerland*)

Bacteria spontaneously release DNA (Borenstein & Ephrati-Elizur, 1969). The bacterial DNA shed can be taken up by other bacteria (Borenstein & Ephrati-Elizur, 1969) as well as by cells of plants or animals (Stroun *et al.*, 1970; Stroun & Anker, 1971*a*,*b*; Stroun, 1971). In the present paper we report the release of nucleic acids by living frog auricles, which can be maintained for more than 2 days in Ringer solution.

Extracted auricles were placed for various times in sterile Ringer solution containing [^{3}H]thymidine. From the auricles and the Ringer solution DNA was extracted by the Marmur and the Schmidt–Tannhauser techniques. The amount of DNA shed after 24h can represent over 10% of the total DNA of the auricles. This DNA can be hybridized *in vitro* with frog auricle [^{3}H]RNA and sediments after a CsCl-density-gradient centrifugation at the level of frog DNA. Sterility tests have shown that the nucleic acids found in Ringer solution cannot come from bacteria. They are not produced by organelles or organisms such as mycoplasma, since no pellet was found after centrifugation of the Ringer solution at $3\times10^{4}g$ for 4h. The nucleic acids shed cannot come from the wounded cells, since the cut cells resulting from the ablation of the ventricles represent only 0.2% of the total cells. Moreover auricles that have been cut in three do not release more nucleic acids than whole auricles.

The specific radioactivity of the released DNA is 2–3 times that of the DNA extracted from the auricles, indicating that it has been recently synthesized. Moreover the auricles are composed of non-dividing cells. It therefore seems that the released DNA is metabolic DNA.

These results suggest that nucleic acids may circulate from cell to cell in certain conditions. Indeed the DNA released represent more than the totality of the nucleic acids contained by the cells on the surface of the explants. The possibility of finding some circulating DNA completely *in vivo* is being investigated.

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Bacterial Ribonucleic Acid Synthesis in Frog Organs after Intraperitoneal Injection of Bacteria

By PHILIPPE ANKER and MAURICE STROUN (introduced by S. R. PELC) (*Laboratoire de Physiologie Végétale, Université de Genève, Geneva, Switzerland*)

Plant or animal organs dipped in a suspension of bacteria synthesize bacterial RNA (Stroun *et al.*, 1969; Stroun & Anker, 1971*a*,*b*; Anker & Stroun, 1972). This phenomenon, which we called 'transcession', is due to the spontaneous release of DNA from bacteria (Borenstein & Ephrati-Elizur, 1969) into cells of higher organisms.

In the present work we show that the same phenomenon can take place in more natural conditions, such as occur, for instance, in living animals after a bacterial infection.

Adult frogs were injected intraperitoneally with 1ml of a suspension (10^9 bacterial/ml) of *Escherichia coli* (strain B) or *Agrobacterium tumefaciens* (strain B_6). Then 1–72h later 1mCi of [^{3}H]uridine was injected, and 3h later the RNA was extracted from the brains, the heart auricles, the ovaries and the skin. In control animals the suspension of bacteria was characterized by hybridization *in vitro* of [^{3}H]RNA extracted from the animals with bacterial DNA. Samples of each organ were taken for radioautography and sterility tests.

Specific bacterial RNA characterized by RNA–DNA hybridization was found in all organs, even as late as 3 days after infection. The largest amount is found in the brain, which in fact is protected by natural barriers against the bacteria we have used. No bacteria were detected by radioautography. The sterility tests showed no bacteria in the brain and none or few (in too small an amount to be detected by radioautography) in other organs. The number of contaminating bacteria in auricles, ovaries and skin would not yield more than 1 part in 10^6 of the bacterial RNA extracted from these organs. Thus these bacteria cannot account for the extent of hybridization between [^{3}H]RNA extracted from the infected frogs and the bacterial DNA. When auricles of injected frogs were extracted and treated with antibiotics before labelling *in vitro*, bacterial RNA was also found in the same amount. This last result suggests that the bacterial RNA extracted from the animal tissues has been synthesized directly in the cells and does not come from [^{3}H]RNA released from labelled bacteria.

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Biosynthetic Studies of Glycocalyx-Bound Glycoprotein and Glycolipid Components in Isolated Brush Borders of the Rabbit Small Intestine

By P. W. KENT and J. R. COOPER (*Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3OU, U.K.*)

Intestinal brush borders can be regarded as highly differentiated plasma membranes that fulfil a specialized role in the digestion and absorption of foodstuffs. These functions are closely dependent on the intactness of the brush-border structure and hence may be extensively disturbed by factors that change the structure or its rate of biosynthesis. The membranes bear, on their lumenal surface, a filamentous structure, the glycocalyx, which is a zone of localized digestive enzyme activity and which has a strongly positive periodic acid–Schiff histological reaction indicative of the presence of carbohydrate-containing material. A considerable body of evidence suggests that these carbohydrates form part of glycocalyx-bound enzymically active glycoproteins, which can be solubilized by papain (Eicholz, 1968) or butanol (Gitzelmann *et al.*, 1964). The present investigation is concerned with the identification of glycolipids of the glycocalyx and with their isotopic labelling, in comparison with glycoprotein components.

The presence of glycolipids in whole intestinal tissue has been demonstrated by Suzuki *et al.* (1968) and Vance *et al.* (1966), although the exact cellular origin does not appear to have been established. Brush borders have been isolated from rabbit small-intestinal scrapings by graded fragmentation in sucrose–5mM-EDTA (D. S. Parsons, unpublished work) in 60–80% yield [in terms of invertase (β-fructofuranosidase) recovery] and with an eight- to ten-fold enrichment in invertase and lactase activities. Lysis of the brush borders in chloroform–methanol (2:1, v/v) resulted in release of glycolipid (Folch *et al.*, 1957) and denaturation of the bound enzyme. T.l.c. on silica gel G (Brady & Mora, 1970) showed the presence of five glycolipids including trisialoganglioside (GT) and disialogangliosides ($GD1_a$) (Svennerholm, 1963). The glycolipid fraction was shown