Bacterial RNA Synthesis in Frog Auricles After Intraperitoneal Injection of Bacteria

Plant or animal organs which were dipped in a suspension of bacteria, synthesize bacterial DNA and bacterial RNA[1-8]. This phenomenon, which we called 'transcession'[9], is due to the spontaneous release of DNA from bacteria[10] into cells of higher organisms.

In the present work we tried to see if the same phenomenon could take place in more natural conditions such as occur, for instance, in living animals after a bacterial infection.

Materials and methods. Frogs were used for their high resistance to septicemia. To detect the transcription of bacterial DNA, we chose their auricles for the following reasons: a) This organ, in a suitable medium, can easily live on its own for more than two days: it is thus possible to see if it has survived the bacterial treatment. b) This neuromuscular tissue is very compact without veins, arteries or extracellular spaces where bacteria could get trapped. c) No pinocytosis has been reported in such material.

Adult frogs (*Rana esculenta*) of about 2 years were injected i.p. with 1 ml of *E. coli* (strain K 12) or *A. tumefaciens* (strain B_6) containing 108 bacteria. 20 h later the animals were separated into 2 groups. In one series, ^{3}H uridine (1 mCi) was injected i.p. into each animal. 3 h later the animals were killed and their auricles were taken out. In the other group the auricles were taken out and dipped for 2 h in Ringer's solution[11] containing 800 µg/ml of colimycin and 800 µg/ml of penicillin. These concentrations would eradicate any hypothetical bacteria. The auricles were then labelled in vitro for 3 h (2 mCi of ^{3}H uridine in 10 ml of Ringer's solution) in the presence of the antibiotics (200 µg/ml of colimycin and 200 µg/ml of penicillin). The controls were treated in the same way, except that they were injected i.p. with 1 ml of sterile Ringer's solution. At the end of the labelling, the auricles of all series were thoroughly washed. A sample of each auricle was prepared for autoradiography[12,13]. The auricles were frozen and the RNA extracted[14]. The newly synthesized RNA was characterized by in vitro RNA-DNA hybridization[15]. The bacterial DNA necessary for the hybridization was prepared by the MARMUR method[16]. The specificity of the RNA-DNA hybridization was checked by competition experiments using RNA of different bacteria.

All radioactivity measurements were carried out in a Beckman tricarb scintillator.

Results and discussion. We found the infected frogs to be in poor condition by the time we extracted their hearts. The leukocytes were numerous in the blood and many contained phagocytized bacteria. The frogs which were not killed died 2 to 4 days later.

Typical data on RNA-DNA hybridization presented in the Figure show the presence of bacterial RNA in the auricles of bacteria-infected frogs which have been labelled in vivo as well as in vitro.

It should be stressed that, in competition experiments, non-labelled RNA extracted from the same strain of bacteria displaces about 70% of the ^{3}H RNA hybridized. On the other hand, *Agrobacterium tumefaciens* RNA does not compete with ^{3}H RNA extracted from auricles of frogs injected with *E. coli* and *E. coli* RNA does not compete with ^{3}H RNA extracted from auricles of frogs injected with *A. tumefaciens*. Moreover, when salmon sperm DNA is trapped on the filter instead of bacterial DNA, no hybridization is observed. Therefore there can be no doubt about the specificity of the hybridization.

In these experiments the autoradiographic studies failed to detect any labelled bacteria. The bacterial RNA extracted from the auricles cannot be due to some rare labelled bacteria which we would have missed with the auto-

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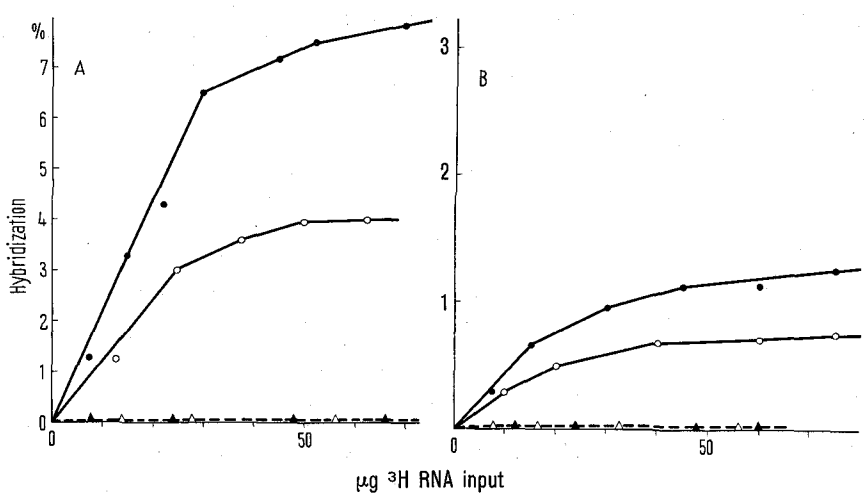

Increasing quantities of ^{3}H RNA extracted from frog auricles are hybridized with bacterial DNA. Saturation curves with ^{3}H RNA extracted from auricles labelled in vivo (●—●) or in vitro (○—○) of frogs infected with bacteria and from auricles labelled in vivo (▲--▲) and in vitro (△--△) of control frogs. The bacteria injected i.p. were in A) *E. coli* (strain K 12) and in B) *A. tumefaciens* (strain B_6). In A) 21 µg of *E. coli* DNA and in B) 17 µg of *A. tumefaciens* DNA were trapped on the filter. The results express the relation, in percent, between the number of µg of denatured DNA trapped on the filter and the amount of ^{3}H RNA hybridized).

radiographic technique. Indeed the amount of radioactivity we should find in the contaminating bacteria should represent at least one to several percent of the total radioactivity present in the tissues. In fact it should amount to even more since over 90% of ^{3}H RNA extracted from bacteria cultured in Ringer's solution and labelled for 3 h is ribosomial ^{3}H RNA. Now the rate of hybridization of ribosomial bacterial RNA with bacterial DNA is always very low. This means, to account for our results, the amount of labelled contaminating bacteria should be in such quantity that they could not be missed. It should be stressed that when frog auricles are bathed in a suspension of labelled bacteria[7] and not treated with antibiotics these bacteria can be easily detected by autoradiography even if they are in such small quantities as, for instance, 5 bacteria per auricle section. Moreover similar results are obtained when the labelling takes place in vivo or in vitro after an antibiotic treatment. Therefore the ^{3}H RNA extracted from the auricles of the infected animals has necessarily been synthesized by animal cells.

Our results show that the phenomenon we had previously observed when separate organs were dipped in a suspension of bacteria, can also occur when an animal suffers a general bacterial infection.

The implications are difficult to evaluate. It is, however, tempting to postulate that this penomenon might be linked to some immunological reactions as we know many different kinds of compounds can be antigenic. It would also be worth investigating whether this mechanism might not play some part in causing the pathological effects on the heart seen after such infections as scarlet fever and rheumatic fever.

Résumé. Des organes de plantes ou d'animaux mis dans une suspension bactérienne synthétisent du RNA bactérien. Ce phénomène que nous avons appelé «transcession» est dû au transfert de DNA spontanément cédé par les bactéries vivantes aux cellules des organismes supérieurs. Dans le présent travail, il est démontré que le même phénomène peut avoir lieu naturellement lorsqu'une grenouille est sujette à une infection bactérienne.

P. Anker, M. Stroun and Jacqueline Laroche[17]

Laboratoire de Physiologie végétale de l'Université, 20, Boulevard d'Yvoy, CH-1211 Genève (Switzerland), 3 November 1971.

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Zone Electrophoretic Studies on Lactate Dehydrogenase Isoenzymes in South American Cichlids (Teleostei, Percomorphi)

Comparative biochemical data have often been suggested as sources of taxonomic information. Electrophoretic studies on animal proteins are increasingly used in confirming and supplementing taxonomic relationships derived from classical criteria[1–4]. Proteins which show little intraspecific variation, might be well suited for uncovering the phylogeny of closely related species. We have compared tissue-specific patterns of proteins in South American Cichlids (Teleostei, Percomorphi) by starch gel electrophoresis. Among various proteins investigated, the isoenzymes of lactate dehydrogenase (LDH; E.C.1.1.1.27) exhibited little intragenergic variation; however, striking differences became apparent by comparison of LDH isoenzyme patterns of species from different genera.

Mitochondria-free supernatant fractions of tissue homogenates were subjected to starch gel electrophoresis, using the vertical electrophoresis apparatus from Buchler Instruments (Fort Lee, N.J., USA). 12% starch gels (Connaught, starch-hydrolysed) were prepared in a continuous triple buffer system (ethylenediamine tetraacetic acid, boric acid, *tris*[5]), pH 8.6. Electrophoresis was conducted at 8 V/cm for 16 h. All preparations were done at 4 °C. LDH isoenzymes were visualized by incubation of gel slices at 30 °C for 30 min in 45 ml 0.2 *M tris*, pH 8.0, containing 120 mg NAD, 0.4 ml L-lactate (1 *M*), 1.2 mg PMS and 8 mg Nitro-BT. Blanks contained no lactate solution. Most animals which were used in this study, were bred at the Gesellschaft für Strahlenforschung mbH, Munich, and originated from commercial breeders.

LDH exists in multiple molecular forms in vertebrates[6]. The LDH polypeptides in teleostean fish are encoded in at least 3 codominant loci[7–9], and are designated A, B and E[7]. These polypeptides are usually expressed tissue-specifically because of differential activities of LDH genes, which code for polypeptides A, B and E. Four polypeptides associate to form enzymatically active tetrameric lactate dehydrogenases[10]. If more than one polypeptide type is synthetized in one cell, a series of heterotetrameric LDH isoenzymes may be formed by random association of different polypeptides[10]. However, the isoenzyme patterns in those fish-tissues where more than one LDH polypeptide is expressed, often deviate from the expected binominal distribution of homo- and heterotetrameric LDHs. Mainly the homopolymers are found[8].

Figure 1 shows a LDH-zymogram of 2 species of *Cichlasoma: C. citrinellum* and *C. spilurum*. The tissue-specific patterns of LDH isoenzymes are virtually identical in both cichlids. Skeletal muscle contains only LDH A_4, extracts from heart- and liver homogenates only LDH B_4. Brain tissues contain both A_4 and B_4 LDH. Traces of heteropolymers from A and B subunits are observed on the zymograms of this tissue at positions intermediate between the isoenzymes A_4 and B_4. Lactate dehydrogenase

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