BACTERIAL RNA SYNTHESIS IN ANIMAL CELLS FOLLOWING BACTERIAL CONTACT

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1. Introduction

After plants have been in contact with a suspension of bacteria, bacterial DNA [1, 2] and the synthesis of large amounts of bacterial RNA [1–6] are found in the plant cells.

In the present work we have tried to see if animal cells could also synthesize bacterial RNA under the same conditions.

2. Material and methods

We chose frog 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 nor arteries where bacteria could get trapped. (c) No pinocytosis has been reported in such material. The bacteria used were either *Agrobacterium tumefaciens* (strain B6) or *Escherichia coli* (strain B) or *Bacillus subtilis* (strain Caron).

Hearts were sterily extracted from frogs (*Rana esculenta*), the auricles were separated from the ventricles and the aortic bodies. The auricles were dipped for several hours in a suspension of bacteria (1×10^9 bacteria/ml) of Ringer solution [7]. Then, in order to get rid of the bacteria remaining on the perifery of the auricles, the animal material was transferred successively into 10 baths of sterile (1 l) Ringer. Between the different baths the auricles were put in a strainer and washed by pouring Ringer on them. In order to kill any remaining bacteria the auricles were transferred for 2 hr to a solution containing an antibiotic in such a concentration as to be bactericide. 200 μg/ml of colimycin or streptomycin were used in the case of *A. tumefaciens* or *E. coli* and 200 μg/ml of penicilin in the case of *B. subtilis*. A mixture containing 200 μg/ml of colimycin and 200 μg/ml of penicilin was also used with all species of bacteria. The different series of auricles were then labelled for 3 hr with ^{3}H uridine (1 mCi in 10 ml of Ringer containing 10 auricles) in presence of the proper antibiotic. The controls were treated in the same way except they were not dipped in a bacterial suspension but in a sterile Ringer solution.

The extractions of animal RNA [8], bacterial RNA [9], bacterial DNA [10] and the *in vitro* RNA-DNA hybridization [11] were effected by methods already described. All radioactivity measurements were carried out in a Beckmann triCarb scintillator.

In order to check that the labelled RNA extracted from the auricles was not partly due to some remaining bacteria having survived the sterile treatment, the following controls were made:

(i) Sterility controls of the solutions and of the tissues were made after the 2 hr of the first antibiotic treatment and at the end of the labelling. No bacterial colony could be detected.

(ii) Electronmicroscope observations showed that no bacteria were present in or outside the tissues.

(iii) A sample of each auricles was frozen, cut in a cryostat and stripping films were applied [12] (we have used cryostat sections rather than the classic paraffin imbedding system where some bacteria might be washed into the different dehydrating baths). Again no bacteria could be found either labelled or unlabelled in or outside the tissues, while, as in controls, all heart cells were heavily labelled.

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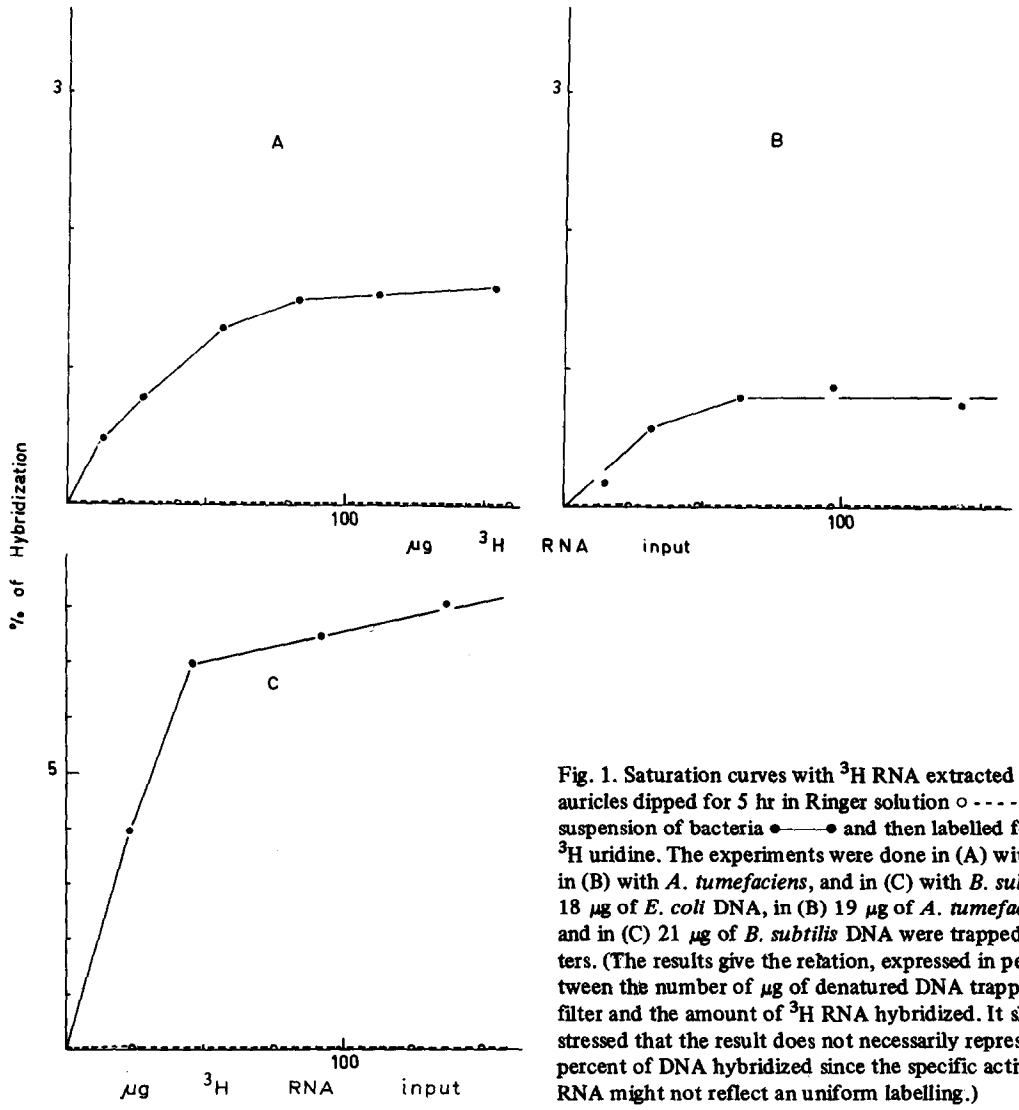

Fig. 1. Saturation curves with ^3H RNA extracted from frog auricles dipped for 5 hr in Ringer solution o - - - - o or in a suspension of bacteria •——• and then labelled for 3 hr with ^3H uridine. The experiments were done in (A) with *E. coli*, in (B) with *A. tumefaciens*, and in (C) with *B. subtilis*. In (A) 18 µg of *E. coli* DNA, in (B) 19 µg of *A. tumefaciens* DNA, and in (C) 21 µg of *B. subtilis* DNA were trapped on the filters. (The results give the relation, expressed in percent, between the number of µg of denatured DNA trapped on the filter and the amount of ^3H RNA hybridized. It should be stressed that the result does not necessarily represent the percent of DNA hybridized since the specific activity of ^3H RNA might not reflect an uniform labelling.)

3. Results

Typical data on RNA-DNA hybridization presented in fig. 1 show a high percentage of hybridization between the bacterial DNA and the ^{3}H RNA extracted from frog auricles dipped in the bacterial suspension. Out of 5 experiments made with *E. coli* the percentages of hybridization were 0.4%, 1%, 1.4%, 2.3%, 2.7%; five experiments with *A. tumefaciens* gave percentages of 0.7%, 0.8%, 1.2%, 3.4% and 5.2%; three experiments with *B. subtilis* gave percentages of 2.8%, 3.3% and 7%.

It should be stressed that in the three series, up to 90% of the hybridizing ^{3}H RNA of frog auricles dipped in a suspension of bacteria is displaced by non-labelled RNA from the same strain. The hybridization is specific since ^{3}H RNA extracted from frog auricles dipped in one strain of bacteria does not hybridize in more than 0.15% with DNA extracted from another strain of bacteria. This percentage can be attributed to some common ribosomal RNA. Let us note that when ^{3}H RNA from bacteria in culture is hybridized with DNA from another strain of bacteria, the same low percentage is found.

The present of hybridization we find in frog auricles can definitely not be due to some rare labelled bacteria which we would have missed with the autoradiographic 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 the ^{3}H RNA extracted from bacteria cultured in Ringer and labelled with ^{3}H uridine for 3 hr is ribosomal ^{3}H RNA. Now the rate of hybridization of ribosomial bacterial RNA with bacterial DNA is always very low. Moreover the specific activity of ^{3}H RNA of these bacteria labelled in the same conditions (1 mCi of ^{3}H uridine for 10 ml of Ringer in presence of auricles) lies in the same range as the specific activity of ^{3}H RNA extracted from frog auricles. This means that, 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 dipped in a suspension of labelled bacteria and not subsequently treated with antibiotics these bacteria are easily detected.

Our results show that the phenomenon we described in plants and called 'trancession' [2] can occur also in animal cells. Though the exact mechanisms are not yet known it appears that elaborate organisms such as bacteria can release their nucleic acids in animal cells which then produce foreign information. Viral infections would then be but one aspect of a more general phenomenon and, as in the case of viruses, bacterial nucleic acids might sometimes act as infectious agents.

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