THE NATURAL RELEASE OF NUCLEIC ACIDS FROM BACTERIA INTO PLANT CELLS AND THE TRANSCRIPTION OF HOST CELL DNA

Maurice STROUN*
Department of Plant Genetics,
Weizmann Institute of Science, Rehovot, Israel

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1. Introduction

Bacterial RNA has been recovered from tomato plant cells after the shoots had been dipped in a suspension of bacteria [1, 2]. The percentage of *in vitro* hybridization between the bacterial DNA and the ^{3}H-RNA extracted from plants dipped in the bacterial suspension is higher than that betweeen this DNA and the ^{3}H-RNA from the same bacteria grown in culture [1, 2]. The presence of bacterial RNA in the cells of plants dipped in a suspension of bacteria is a general phenomenon, not necessarily related to the virulence of a bacterial strain toward the plant [2].

The ^{3}H-RNA extracted from plant cells – used for *in vitro* hybridization with bacterial DNA – has been distinguished from the RNA in living bacteria around the plant tissues by employing: a) biochemical techniques which break up the plant cells without breaking up the bacteria around the plants [1]; b) autoradiographic and electronmicroscope techniques which showed that, in plants dipped in a bacterial suspension and transferred to a solution of ^{3}H-uridine, bacteria are only present in the xylem and on the epidermis without being labelled, while most plant cells from all tissues except the xylem vessels were labelled [2]. It should be stressed that also no viruses could be detected in tomato cells [2].

The presence of bacterial ^{3}H-RNA in plant cells could be due either to RNA coming from bacteria into tomato cells or to DNA released from bacteria into plant cells and then transcribed.

* *Present address:* Department of Plant Physiology, University of Geneva, Switzerland.

In the present work we study the relationship between the presence or the disappearance of bacterial ^{3}H-RNA in plant cells and transcription of the host cell DNA.

2. Material and methods

The experiments were conducted with either *Agrobacterium tumefaciens* (strain B_6), virulent for tomato plants, and *Escherichia coli* (strain B), to which tomato plants are not sensitive.

Cut shoots of tomato plants were washed under sterile conditions and dipped in suspensions of bacteria (1×10^9 bacteria/ml of 0.1 SSC:sodium chloride 0.015 M, sodium citrate 0.0015 M). After 41 hr they were transferred in ^{3}H-uridine (0.2 mCi/ml) for 7 hr. Control plants were treated identically but without bacteria in the first solution.

The extraction of bacterial DNA [3] and RNA [4], of plant DNA [3] and RNA [5], and the *in vitro* DNA-RNA hybridizations [6] were performed by techniques already described.

When we wanted to free the xylem vessels and the epidermis of plants dipped in a bacterial suspension from bacteria, we added to the suspension 10 μg/ml (for *A. tumefaciens*) or 30 μg/ml (for *E. coli*) of rifamycin – harmless to the plant.

The effect of bacterial RNA in plant cells on the formation of plant ribosomal and transfer RNAs was determined by ultracentrifugation in a sucrose gradient. The synthesis of tomato DNA in presence of bacterial RNA was studied – after using ^{3}H-thymidine

instead of ^{3}H-uridine – either by autoradiography [7] or after extraction of plant DNA [3] by counting its specific activity.

All radioactivity measurements were carried out in toluene-based scintillation solution in a Beckmann triCarb counter.

3. Results and discussion

Since the results with *A. tumefaciens* are similar to those with *E. coli* we shall present data from either one.

Fig. 1 shows the high percentage of hybridization between the DNA of *E. coli* and the ^{3}H-RNA extracted from plants dipped in *E. coli* suspension (A), and the shut off of the transcription of the tomato DNA (B). Let us stress that in such conditions the synthesis of tomato DNA or ribosomal and transfer RNAs are not blocked.

It should be noted that if the shut off of transcription of tomato DNA is due to the presence of bacterial RNA in the plant cells, it also depends to a certain extent on the concentration of the bacterial suspension in which the plants are dipped. Whereas with two suspensions of *E. coli* of different concentrations (1×10^9/ml and 1×10^7/ml) the same percentage of hybridization with the bacterial DNA is obtained (fig. 2A), the shut off of transcription of tomato DNA is only partial in the case of plants dipped in the weaker bacterial suspension (fig. 2B).

In order to find out which ^{3}H-RNA was present in the plant cells once the xylem vessels and the epidermis were free from bacteria, we made the following experiment after checking that plants dipped in *A. tumefaciens* suspension for 24 hr or 41 hr present the same phenomena with regard to the amount of bacterial and plant RNA in tomato cells. Before being labelled with ^{3}H-uridine for 7 hr, plants were dipped in *A. tumefaciens* suspension a) for 41 hr; b) for 41 hr with the last 5 hr in presence of rifamycin (10 μg/ml); c) for 41 hr with the last 17 hr in presence of rifamycin (10 μg/ml). In the presence of rifamycin, a decrease in the percentage of hybridization between ^{3}H-RNA, extracted from plant cells, and bacterial DNA (fig. 3A) is observed together with a restoration of transcription of tomato DNA (fig. 3B).

The shut off of tomato DNA transcription in the presence of bacteria could be explained: a) by bacterial toxins blocking, directly or indirectly, the synthesis of plant messenger RNA; b) by a phenomenon – similar to an extreme case of viral infection – where most of the host cell metabolism is essentially taken over by the foreign nucleic acids.

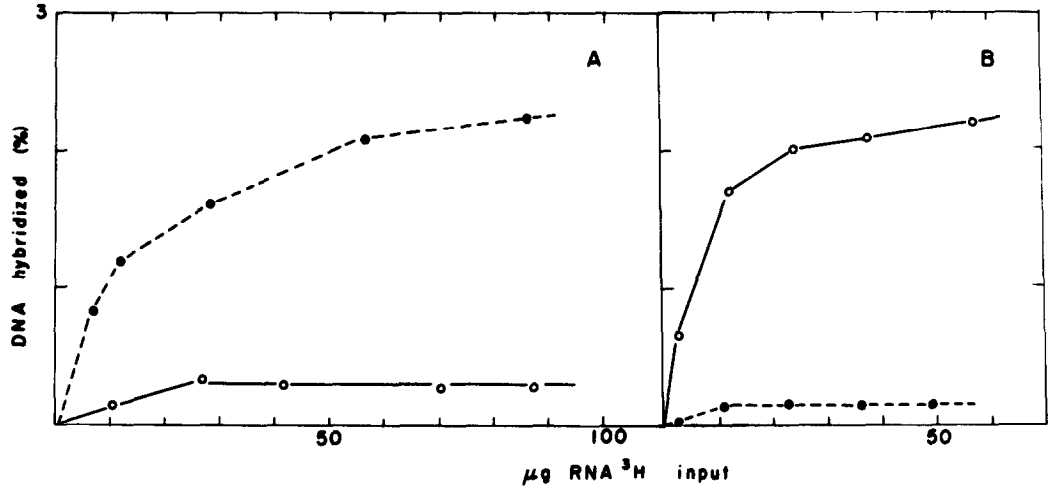

Fig. 1. Saturation curves with ^{3}H-RNA extracted from plants dipped for 41 hr in 0.1 SSC o—o or in *E. coli* suspension •- - -• and then labelled with ^{3}H-uridine. In A) 60 μg of *E. coli* DNA is trapped on the filters and B) 10 μg of tomato DNA is trapped on the filters.

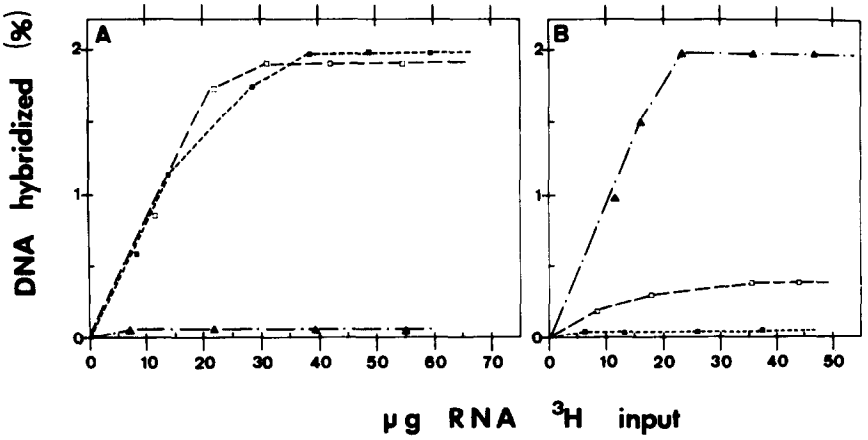

Fig. 2. Saturation curves with ^{3}H-RNA extracted from plants dipped for 41 hr in 0.1 SSC ▲—·—·▲ or in *E. coli* suspension (1×10^9/ml) ■- - - -■ or in *E. coli* suspension (1×10^7/ml) □- - - -□ and then labelled with ^{3}H-uridine. In A) 60 µg of *E. coli* DNA is trapped on the filters and in B) 10 µg of tomato DNA is trapped on the filters.

The hypothesis of unspecific toxins upsetting the cellular metabolism so radically that synthesis of the tomato messenger RNA is blocked hardly fits in with the fact that the synthesis of plant DNA or RNA is not blocked. The production of a toxin specific for transcription of plant DNA would imply the production of the same toxin by two different bacteria such as *E. coli* and *A. tumefaciens*. In support of the second hypothesis we must bear in mind the very close relation which seems to exist – in presence of rifamycin – between the progressive decrease of bacterial RNA in the plant cells and the progressive restoration of transcription of plant DNA.

Research is being undertaken to identify the mechanisms of these phenomenon and their relationship to one another.

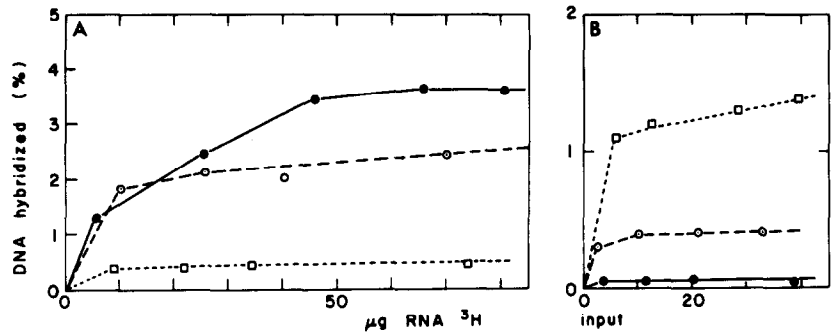

Fig. 3. Saturation curves with ^{3}H-RNA extracted from plants dipped for 41 hr in *E. coli* suspension without rifamycin ●——●, with the last 5 hr in presence of rifamycin ⊙- - - -⊙, with the last 17 hr in presence of rifamycyn □- - - -□, and then labelled with ^{3}H-uridine. In A) 60 µg of *E. coli* DNA is trapped on the filters and in B) 10 µg to tomato DNA is trapped on the filters.

Acknowledgement

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