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EFFECT OF THE EXTENT OF DNA TRANSCRIPTION OF PLANT CELLS AND BACTERIA ON THE TRANSCRIPTION IN PLANT CELLS OF DNA RELEASED FROM BACTERIA

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1. Introduction

After plants have been in contact with a suspension of bacteria, one observes in plant cells: i) the replication of bacterial DNA [1], and ii) the synthesis of large amounts of bacterial RNA [2–4].

The present work deals with the effect of the extent of DNA transcription of plant cells and bacteria on the transcription in plant cells of DNA released from bacteria.

2. Materials and methods

The bacteria used were either *Escherichia coli* (strain B), *Agrobacterium tumefaciens* (strain B_6) or *Pseudomonas fluorescens* (strain B_{49}).

Cut shoots of young (5 weeks) or old (12 weeks) eggplants were washed sterile and dipped for 24 or 48 hr in either 0.1 SSC (sodium chloride 0.015 M, sodium citrate 0.0015 M) or in a suspension of bacteria (1×10^9 bacteria/ml of 0.1 SSC). After another sterile washing the central cylinder, including the xylem vessels, was removed and 0.2 mCi of ^{3}H-uridine was applied for three hours. Electron microscope observations combined with light autoradiography show that after plants were dipped in a suspension of bacteria and given a sterile washing, bacteria are present only in the xylem vessels [1, 3]. Therefore, by eliminating the bacteria with the xylem after the bacterial incubation and just prior to the labelling, we can be sure that the labelled RNA has been synthesized in the plant cells only [1].

In some experiments bacteria in nutrient broth were grown in the presence of ^{3}H-uridine for three generations in order to label the bacterial RNA.

The extraction of bacterial DNA [5], bacterial RNA [6], plant DNA [5] and plant RNA [7] was done by methods already described.

We compared the percentage of DNA transcribed in plant cells after the different treatments by the technique of in vitro RNA–DNA hybridization [8].

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

3. Results

3.1. *Ability of cells to transcribe their own DNA and bacterial DNA*

The typical data in fig. 1A show that the percentage of hybridization between the DNA of eggplant and the ^{3}H-RNA extracted from young plants dipped in 0.1 SSC is higher after 24 hr than it is after 48 hr. The extension of plant DNA transcription is greater in young plants than in old ones. In fig. 1B we can also note that the percentage of hybridization between bacterial DNA and the ^{3}H-RNA extracted from

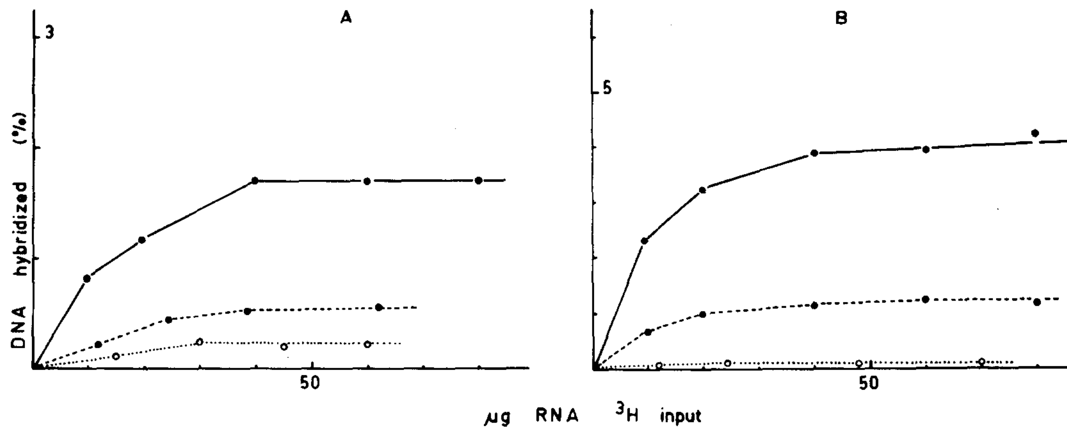

Fig. 1. Saturation curves with ^{3}H-RNA extracted in A) from young plants dipped in 0.1 SSC for 24 hr •——• or 48 hr •– – – –•, or from old plants dipped in 0.1 SSC for 24 hr o-----o and then labelled for 3 hr with ^{3}H-uridine; in B) from young plants dipped in *A. tumefaciens* for 24 hr •——• or 48 hr •– – – –•, or from old plants dipped in *A. tumefaciens* for 24 hr o------o and then labelled for 3 hr with ^{3}H-uridine. In A) 32 µg of eggplant DNA is trapped on the filters and in B) 30 µg of *A. tumefaciens* DNA is trapped on the filters.

young plants dipped in *A. tumefaciens* suspension for 24 hr is higher than that resulting from a 48 hr immersion. There is very little bacterial synthesis in the old plants which were dipped in the bacterial suspension. Similar results were obtained using *E. coli* or *P. fluorescens.*

The differences observed cannot be attributed to differences in DNA uptake of the various plant types. In fact, one finds replicating bacterial DNA in both young and old plants which have been dipped for 24 or 48 hr in a suspension of bacteria [1].

These results indicate that the ability of plant cells to transcribe bacterial DNA depends on their capacity to transcribe their own DNA. Some of the factors which are necessary for an active plant DNA transcription are also used by the invading DNA.

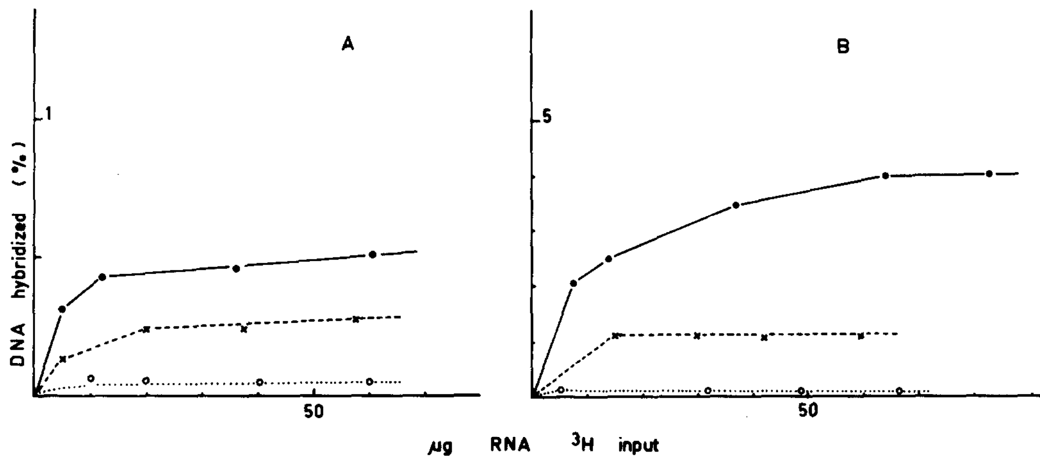

Fig. 2. Saturation curves with ^{3}H-RNA extracted in A) from *P. fluorescens* labelled for three generations with ^{3}H-uridine x-----x or from plants dipped for 24 hr in *P. fluorescens* •——• or in 0.1 SSC o-------o and then labelled for 3 hr with ^{3}H-uridine; in B) from *E.coli* labelled for three generations with ^{3}H-uridine x-----x or from plants dipped for 24 hr in *E. coli* •——• or in 0.1 SSC o-------o and then labelled for 3 hr with ^{3}H-uridine. In A) 25 µg of *P. fluorescens* DNA is trapped on the filters and in B) 32 µg of *E. coli* DNA is trapped on the filters.

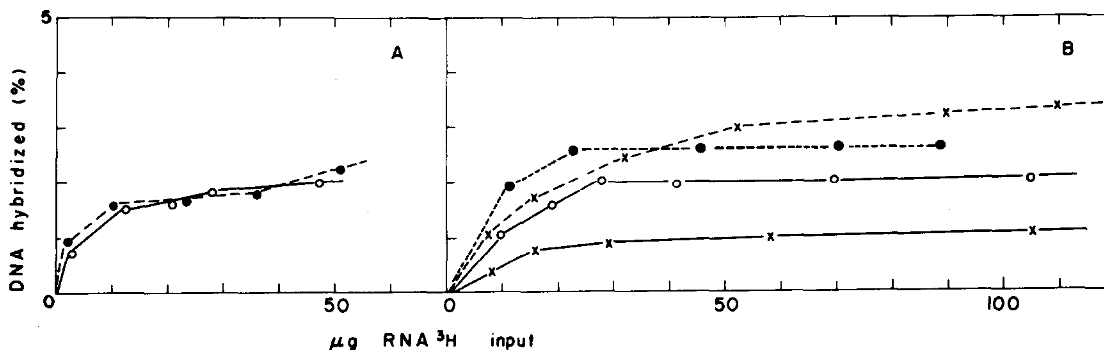

Fig. 3. Saturation curves with ^{3}H-RNA extracted in A) from plants dipped for 24 hr in 0.1 SSC o——o or in 0.1 SSC in the presence of 100 µg of chloramphenicol •------•; in B) from *E. coli* suspension x——x or from *E. coli* suspension with 100 µg of chloramphenicol x-----x or from plants dipped for 24 hr in *E. coli* o——o or from plants dipped for 24 hr in *E. coli* in the presence of 100 µg of chloramphenicol •-----•; in A) 16 µg of eggplant DNA is trapped on the filters and in B) 32 µg of *E. coli* DNA is trapped on the filters.

3.2. *Bacterial DNA transcription in bacteria and in plant cells*

In fig. 2 we can observe that the precentage of hybridization between *E. coli* DNA and ^{3}H-RNA extracted from *E. coli* in culture is higher that that between *P. fluorescens* DNA and ^{3}H-RNA extracted from *P. fluorescens* in culture. It should be stressed that similar differences are observed when bacteria are placed in 0.1 SSC instead of nutrient broth. The extension of bacterial DNA transcribed in plant cells is also higher when plants are dipped in *E. coli* than when they are dipped in *P. fluorescens.*

The difference in the extent of *E. coli* DNA and *P. fluorescens* DNA transcribed in plant cells is not due to the fact that *E. coli* has a shorter division cycle than *P. fluorescens.* In point of fact, although *A. tumefaciens* has a shorter division cycle than *E. coli*, the percentage of hybridization between the respective DNA and ^{3}H-RNA extracted from these bacteria in culture is similar, and the extent of bacterial DNA transcribed in plants which have been dipped in *A. tumefaciens* or in *E. coli* is also identical. Moreover, if 100 µg of chloramphenicol is added to a culture of *E. coli*, protein synthesis is blocked, resulting in a decrease in the rate of bacterial divisions but an increase in the rate of RNA synthesis [9]. In the cells of plants dipped in *E. coli* with 100 µg of chloramphenicol, harmless to the plant, there is a larger part of the bacterial genome transcribed (fig. 3).

These results indicate that the part of the bacterial genome transcribed in plant cells is dependent on the part transcribed in the bacteria. It would be tempting to postulate that the DNA naturally released from living bacteria [10] consists of extra copies or working genes [11] which have been used for transcription and are discarded during the renewal of DNA.

Further research based on these results is being carried out to attempt to determine which are the biochemical factors of competence in both donor and acceptor cells.

Acknowledgements

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