ON THE NATURE OF THE POLYMERASE RESPONSIBLE FOR THE TRANSCRIPTION OF RELEASED BACTERIAL DNA IN PLANT CELLS

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SUMMARY: After plants have been in contact with a suspension of bacteria, bacterial DNA and the synthesis of large amounts of bacterial RNA are found in the plant cells. It appears that the presence of bacterial DNA-dependent RNA polymerase is necessary for the transcription of bacterial DNA.

INTRODUCTION

After plants have been in contact with a suspension of bacteria, one notices in their cells: i) the replication of bacterial DNA (13, 14) ; ii) the synthesis of large amounts of bacterial RNA (10 - 14) linked to the partial or total shut-off of plant DNA transcription (11).

However, when bacterial DNA is extracted, purified and given to plants instead of living bacteria, although the bacterial DNA penetrates into the cells and replicates (9), it is not transcribed in our experimental conditions; only plant RNA is synthesized (12).

The presence of a bacterial polymerase has been postulated (14) in order to explain the transcription in plant cells of DNA released from bacteria.

In the present work we have tried to determine if the polymerase responsible for the transcription of released bacterial DNA in plant cells is of vegetal or of bacterial nature.

MATERIAL AND METHODS

In order to determine the nature of the polymerase responsible for the transcription of released bacterial DNA in plant cells we have used rifamycin SV (Lepetit Laboratory, Milan), an inhibitor of DNA-dependent RNA polymerase. We took into account the different rifamycin sensitivities of the plant and bacterial DNA-dependent RNA polymerases.

The bacteria used were either Escherichia coli (strain B) or Agrobacterium tumefaciens (strain B_6) which are DNA-dependent RNA polymerase rifamycin sensitive (5,11), or Escherichia coli (strain K 12, RFS 524) which is rifamycin resistant (7).

The plants and the bacteria in 0.1 ssc (sodium chloride 0.015 M., sodium citrate 0.0015 M.) were incubated for 10 hours with different concentrations of rifamycin in order to determine their in vivo rifamycin sensitivities in our experimental conditions. The ability of bacteria and of whole cells, chloroplasts or mitochondria in plants to incorporate ^3H uridine into RNA, after the rifamycin treatment, was taken as an indication of their in vivo RNA polymerase activity.

Cut shoots of two month old eggplants (Solanum melongena) were dipped for 42 hours in a suspension of bacteria (1×10^9 bacteria / ml of 0.1 ssc) and then sterily washed (hypochlorite 5%, alcohol 70%, sterile water). Let us remind that the electron microscope observations show that, after such a sterile treatment, bacteria are only present in the xylem vessels (14). In order to eliminate the bacteria the central cylinder, including the xylem vessels, was removed. The cortex was dipped in 0.1 ssc or in different concentrations of rifamycin for 10 hours before 0.2 mCi of

^{3}H uridine was applied for three hours. Thus by discarding the bacteria before the antibiotic treatment it was possible to study the effect of the rifamycin on the RNA newly-synthesized in plant cells only.

Supplementary controls were made in order to check that the labelled RNA extracted from the plant cortex was not partly due to some remaining bacteria having survived the sterile treatment:
1) Autoradiographs of frozen sections (2) showed that no bacteria labelled or unlabelled were present around the cortex (we used the cryostat cut sections rather than the classic paraffin imbedding system where some bacteria might be washed in the different dehydrating baths).
2) The cortex of bacteria treated plants were washed sterilly in the same way as the plants of our main experiment. They were gently crushed in such a way to preserve any remaining bacteria. This homogenate was put at 25° C or 37° C on agar medium highly favourable for the growth of our species of bacteria. The plates remained sterile (we thank Dr. E. Schorer, head of the Laboratory of Bacteriology of the Institute of Botany, University of Geneva, for having performed these sterility tests).
3) 200 µg/ml of colimycin or of chloramphenicol which are, in this concentration, harmless for plants but fatal for our three strains of bacteria were applied instead of rifamycin. While rifamycin acts on the DNA-dependent RNA polymerase, colimycin acts as surface active compound and chloramphenicol blocks the protein synthesis by fixation on the 50 S ribosomial subunit.

The newly synthesized plant and bacterial RNA in plant cells was characterized by in vitro RNA-DNA hybridization (4).

The different extraction of bacterial DNA (6), bacterial RNA

(1), plant DNA (6) and plant RNA (3) were done by methods already described. The purity of the separation of chloroplasts (8) and of the mitochondria (8) were determined by electron microscope observations.

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

RESULTS AND DISCUSSIONS

Table I shows the marked differences between the rifamycin sensitivities of DNA-dependent RNA polymerases of *E. coli* (strain B) and of *A. tumefaciens* (strain B_6) and of the polymerases of the whole cells or of the chloroplasts or of the mitochondria in plants. Rifamycin was found to inhibit most of RNA synthesis at a concentration of

Table 1

Effect of rifamycin on the incorporation *in vivo* of ^3H uridine into RNA of bacteria, plant cells, chloroplasts, mitochondria

Material	Percent of incorporation of ^3H uridine into RNA after an incubation in different concentrations of rifamycin			
	10 µg/ml	30 µg/ml	50 µg/ml	100 µg/ml
E. coli in 0.1 ssc	100	24	15	4
A. tumefaciens in 0.1 ssc	21	3	7	3
E. coli (strain K 12, RFS 524) in 0.1 ssc	100	100	100	100
whole cells) in plants	100	100	93	83
chloroplasts) in plants	100	100	87	75
mitochondria) in plants	100	100	91	67

10 ug/ml in the case of A. tumefaciens and of 30 ug/ml in the case of E. coli (strain B), whereas at a much higher rifamycin concentration the RNA synthesis of the plants was only partially reduced. As expected, E. coli (strain K 12, RFS 524) is not sensitive to rifamycin.

In Figure I, A we observe that the transcription of A. tumefaciens DNA in eggplants is blocked by 10 ug/ml of rifamycin. Moreover, with the same 10 ug/ml of rifamycin there is a restoration of plant DNA transcription which had been partially shut off in the

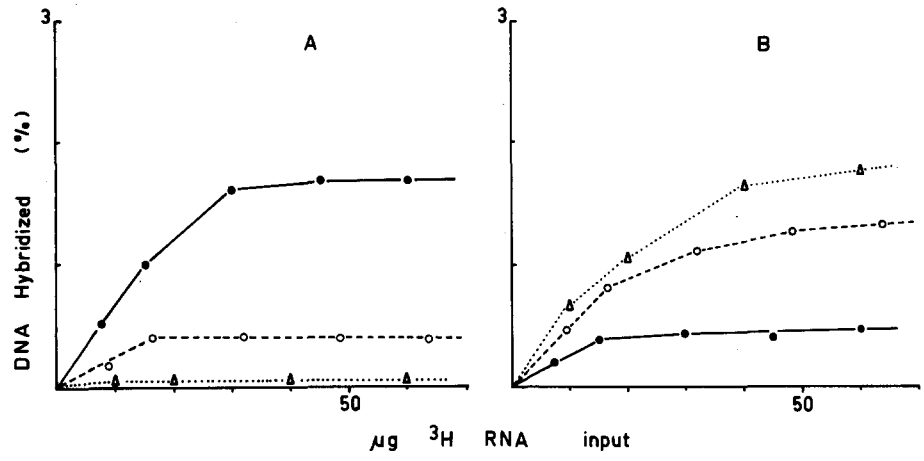

Figure I: Saturation curves with ^{3}H RNA extracted from plants dipped for 52 hours in 0.1 ssc Δ·····Δ or for 42 hours in A. tumefaciens and for 10 hours in 0.1 ssc ●——● or for 42 hours in A. tumefaciens and for 10 hours in 10 µg/ml of rifamycin o– – – – – o and then labelled for 3 hours with ^{3}H uridine. In A) 36 µg of A. tumefaciens DNA is trapped on the filters and in B) 32 µg of eggplant DNA is trapped on the filters.

presence of bacterial RNA (Figure I, B). It should be stressed that plants dipped for 42 hours in A. tumefaciens and for 10 hours in colimycin or in chloramphenicol behave as plants for 42 hours in A. tumefaciens and for 10 hours in 0.1 ssc.

In Figure II, A we observe that plants that have been dipped in a suspension of E. coli (strain K 12, RFS 524), which is rifamy-

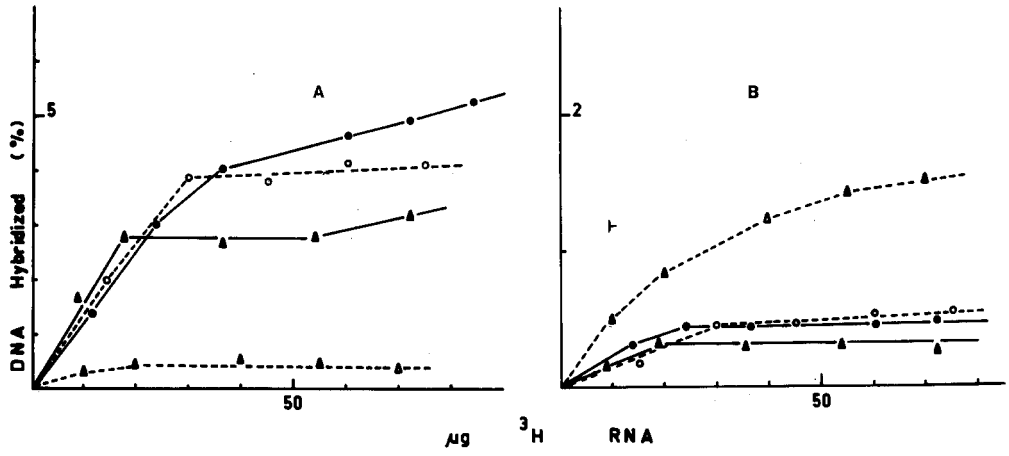

Figure II: Saturation curves with ^{3}H RNA extracted from plants dipped for 42 hours in E. coli (strain B) and for 10 hours in 0.1 ssc ▲——▲ or for 42 hours E. coli (strain B) and for 10 hours in 10 µg/ml of rifamycin ▲- - -▲ or for 42 hours in E. coli (strain K 12, RFS 524) and for 10 hours in 0.1 ssc ●——● or in E. coli (strain K 12, 524) and for 10 hours in 50 µg/ml of rifamycin o- - - - - o and then labelled for 3 hours with ^{3}H uridine. A) 32 µg of E. coli DNA is trapped on the filters and in B) 30 µg of eggplant DNA is trapped on the filters.

cin resistant, continue to synthesize bacterial RNA even after a strong treatment of rifamycin, while the transcription of E. coli, (strain B), which is rifamycin sensitive, is already blocked by 10 µg/ml of rifamycin in plant cells. Let us remark that no difference has been observed with plants dipped for 42 hours in E. coli K or B and then dipped for 10 hours in 0.1 ssc or in colimycin or in chloramphenicol.

In the case of the control experiments with ^{3}H RNA from plants dipped only in 0.1 ssc for 52 hours the percent of hybrization with E. coli DNA is less than 0,1 % and with eggplant DNA is between 1,5 % and 2,1 %.

From these results we can conclude that the transcription of bacterial DNA in plant cells is not performed by any of the plant polymerases. Indeed: i) the polymerase responsible for the trans-

cription of the released bacterial DNA in plant cells is blocked by a concentration of rifamycin which is harmless to the polymerases of eggplants ; ii) the shut-off of the eggplant DNA transcription in the presence of bacterial RNA synthesis (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) is restored by the same concentration of rifamycin which blocks the released bacterial DNA transcription.

On the other hand, there are good reasons to believe that the transcription of bacterial DNA in plant cells is performed by its own DNA-dependent RNA polymerase. Indeed, the polymerase sensitivity or resistance towards the rifamycin is the same for the bacterial DNA transcription in both bacteria and in plant cells. The difference noted in the polymerase sensitivity towards rifamycin for the transcription of E. coli (strain B) DNA in plant cells and for DNA transcription in E. coli (strain B) in culture can be explained if we assume that the polymerase of E. coli (strain B) is present in the plant cells. E. coli (strain B) DNA transcription is blocked in vitro by 10 µg/ml or less of rifamycin (5), and, in our experiment conditions, in vivo by 30 µg/ml of rifamycin. The higher amount of rifamycin necessary in vivo is due to a problem of the wall permeability of this bacteria towards rifamycin (5). But if E. coli (strain B) poly merase has been released from E. coli into plant cells it should be inhibited - which is the case - by the same amount of rifamycin necessary to block in vitro transcription.

From our results it appears that the released bacterial DNA, to be transcribed in plant cells, has to be coupled with it's own DNA-dependent RNA polymerase. This polymerase might either enter the

plant cells with the bacterial DNA or be synthesized subsequently. This latter possibility seems less likely since, in our experimental conditions, purified extracted bacterial DNA is not transcribed in plants although it enters the cells and replicates.

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