AGROBACTERIUM TUMEFACIENS RNA IN NON-TUMOROUS TOMATO CELLS

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Summary

Agrobacterium tumefaciens, strain B_6 (virulent), RNA has been found in non-tumorous tomato cells after the plants have been dipped in a bacterial suspension. The percentage of in vitro hybridization between the A. tumefaciens DNA and the RNA extracted from plants dipped in the bacterial suspension is higher than between this DNA and the RNA from bacteria grown in culture.

On uptake of Agrobacterium tumefaciens DNA by tomato plants, the DNA reaches the cell nuclei retaining its original composition and structure (Stroun, et al., 1966; Stroun and Anker, 1968). This bacterial DNA replicates in association with the tomato DNA (Stroun, Anker and Ledoux, 1967). Moreover, the presence of A. tumefaciens DNA has been detected in the tomato cells at the time of crown gall induction by the living bacteria (Stroun, Anker and Ledoux, 1967). It has also been found in tobacco tumor tissues (Schilperoort et al., 1967; Quatier, Huguet and Guillé, 1969), and Milo and Srivastava (1969) have been able to detect some A. tumefaciens RNA in crown gall tobacco tumors.

The present work deals with the appearance of A. tumefaciens, strain B_6 (virulent), RNA in non-tumorous tomato cells after the plants have been dipped in a bacterial suspension.

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Materials and Methods

A. tumefaciens, strain B_6, (kindly provided by Dr. Manigault, Pasteur Institute, Paris) were grown for 24 hours in a nutrient broth. Bacterial cells were collected by centrifugation and used for treating the plants and for isolation of DNA by the Marmur procedure (1961). For labelling A. tumefaciens RNA, bacteria were grown in the presence of H^3 uridine for three generations. The RNA was extracted by the Bautz and Hall technique (1962).

After being washed in 5% sodium hypochloride, 70% alcohol and sterile water, the upper shoots of the plants - without their top end - were dipped in a suspension of A. tumefaciens B_6 (1×10^9 bacteria/ml) in 0.1 x ssc (1 x ssc:sodium chloride 0.15 M, sodium citrate 0.015 M) for 41 hours and for additional 7 hours in a solution of H^3 uridine (0.2 mC/ml). Control plants were dipped in the same solution without bacteria. The extraction of plant tissue RNA was carried out by the procedure of Gigot, Phillips and Hirth (1968) discarding the portion of the cells directly in contact with the bacteria via the cut top of the shoots. Under such conditions no crown gall developed on the plants dipped in the A. tumefaciens suspension.

Salmon sperm DNA used in some experiments was obtained from Worthington Biochemical Corporation and repurified by the Marmur procedure (1961).

Denaturation of DNA, its immobilization on nitrocellulose filters, formation of RNA-DNA hybrids and post-annealing ribonuclease treatment, were carried out as described by Gillespie and Spiegelman (1965). Sixty micrograms of denatured DNA per filter were used in all experiments. Retention of DNA on nitrocellulose filters during immobilization was about 90% and no measurable loss of DNA during annealing was noted. One DNA filter and a blank filter were incubated for 20 hours at 67^0 in each scintillation vial containing various quantities of radioactive RNA in 2 ml of 6 x ssc. All radioactivity measurements were carried out in toluene-based scintillation solution in a Packard Tricarb counter.

Results and Discussion

Typical data on RNA-DNA hybridization presented in Figure I shows a higher percentage of hybridization between the DNA of A. tumefaciens and the H^3 RNA extracted from plants dipped in the bacterial suspension, than between this DNA and H^3 RNA from bacteria grown in nutrient broth.

When salmon sperm DNA is trapped on the filters instead of A. tumefaciens DNA, no hybridization is observed. When bacterial H^3 RNA is extracted by the

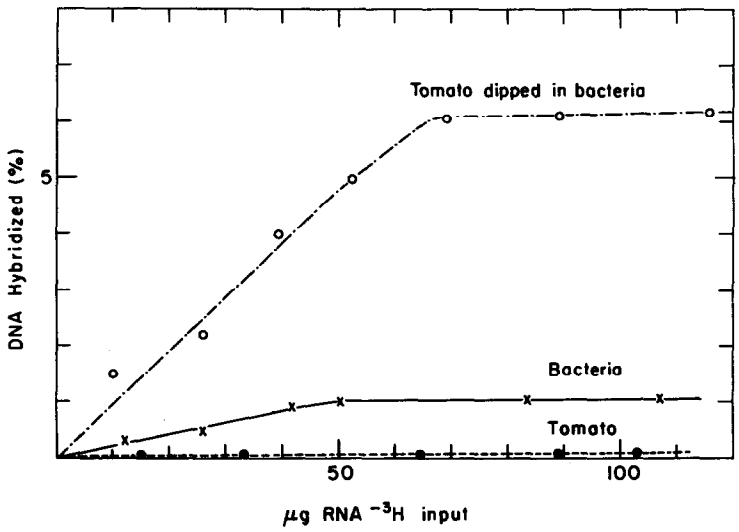

Figure I. Saturation curves with H^3 RNA extracted from A. tumefaciens, tomato shoots and tomato shoots dipped in A. tumefaciens. 60 µg of A. tumefaciens DNA are trapped on the filters. The bacteria were labelled with H^3 uridine for three generations and the tomato shoots for 7 hours.

technique used for the plants (Gigot, Philipps and Hirth, 1968) in the presence of non-labelled tomato plants the percentage of hybridization is the same as when RNA was extracted by the Bautz and Hall (1962) procedure. Moreover, non-labelled RNA from A. tumefaciens competes up to 90% with the H^3 RNA of plants dipped in the bacterial suspension, whereas non-labelled E. coli RNA does not.

The high percentage of hybridization obtained when plants dipped in bacterial suspension in 0.1 x ssc as compared to that obtained with bacteria grown on rich media, might have been attributed to the wider expression of the bacterial genom in such conditions. However, when the bacterial suspension in which the plants had previously been dipped was labelled for 7 hours with H^3 uridine, the percentage of hybridization between the bacterial H^3 RNA and A. tumefaciens DNA was less than 1%.

When the bacteria in or on the plants were counted (upper shoots were gently crushed in 0.1 x ssc and plated), calculating on the basis of the number of bacteria necessary to obtain 1 mg of bacterial RNA, it was found that the percentage of RNA derived from living bacteria did not exceed 2% of the total RNA extracted from the plants dipped in the bacterial suspension. This low percentage of RNA derived from living bacteria cannot account for the high percentage of A. tumefaciens DNA hybridized. The 6.1% of DNA hybridized at the saturation point (Figure I) was

obtained with 70 μg input of H^3 RNA of which 3.6 μg formed the hybrid; not more than 1.4 μg (2% of 70 μg H^3 RNA) could be accounted for by RNA coming from living bacteria.

In order to find out whether the transcription of the bacterial DNA took place in the plant cells, we used the following technique to separate living bacteria from plant cell RNA extract: The plants were crushed in buffer solution with 0.5% of triton x-100. The extract was centrifuged at 16.300 x g for 20 min. The supernatant was kept (Solution I) and the pellet suspended in the same amount of buffer with 2% sodium lauryl sulphate (Solution II). RNA was extracted from the supernatant and the pellet in about equal quantities. In the control experiments (Table I) carried out with non-labelled plants to which labelled bacteria (with H^3 thymidine or H^3 uridine were added, it was found that Solution I contained 1.3% - 2.1% of the total labelled nucleic acids content of the bacteria whereas Solution II contained 24% - 30.4% of labelled material. The fact that only part of the bacteria were broken is due to the thick walls of A. tumefaciens, so that a much more drastic technique is needed to obtain a good yield of nucleic acid from this material. If the high percentage of hybridization was due to living bacteria outside the plant cells we should obtain a higher percentage of hybridization with RNA from the pellet than from the supernatant at any rate for the same amount of H^3 RNA input. On the other hand, if the transcription of bacterial RNA took place in the plant cells, the

TABLE I

Percentage of radioactivity found in tomato plants extracted in presence of labelled Agrobacterium tumefaciens bacteria with triton or with sodium lauryl sulfate

Conditions	BACTERIA LABELLED WITH			
	H^3 thymidine		H^3 uridine	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
	%	%	%	%
Supernatant of plants extracted with triton x-100 (Solution I)	1.8	1.2	1.7	2.1
Supernatant of the pellet extracted with SLS (Solution II)	28	24.7	30.2	30.4

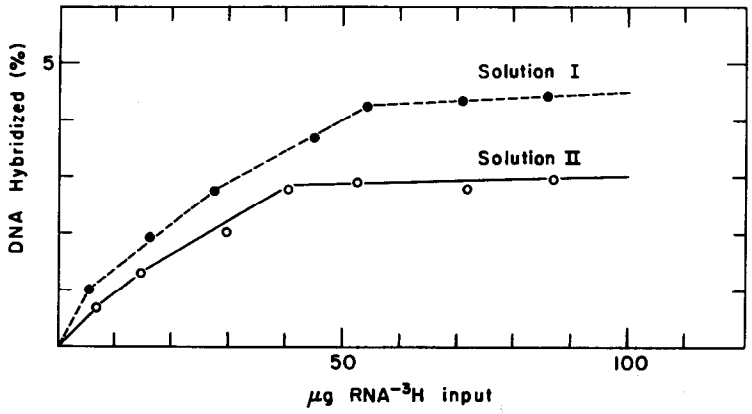

Figure II. Saturation curves with H^3 RNA extracted from tomato dipped in A. tumefaciens suspension extracted with triton x-100 (Solution I) or sodium lauryl sulphate (Solution II). 60 μg of A. tumefaciens DNA are trapped on the filters. The plants were labelled with H^3 uridine for 7 hours.

percentage of hybridization should not be lower with the RNA from the supernatant than with that from the pellet. In fact, as Figure II shows, there is even a tendency for a higher percentage of hybridization with the RNA from the supernatant (Solution I), indicating that the synthesis of the A. tumefaciens RNA has indeed taken place in the plant cells. However, whether the transcription is due to living bacteria in the plant cells or to DNA released by them is still in question; in any event, the accumulation of large quantities of messenger RNA in such conditions is different from messenger RNA synthesis in free bacteria.

Experiments are in progress to determine in which part of the plant cells the transcription of bacterial DNA takes place and if it is performed using the bacterial or the plant machinery.

Acknowledgement

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