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7 "genetic Transformation ... of bacteria by RNA
Belgarski et Marmigault

CELLULAR MODIFICATION AND GENETIC TRANSFORMATION BY EXOGENOUS NUCLEIC ACIDS

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7. "GENETIC' TRANSFORMATION OF BACTERIA BY RNA AND LOSS OF ONCOGENIC POWER PROPERTIES OF AGROBACTERIUM TUMEFACIENS. TRANSFORMING RNA AS TEMPLATE FOR DNA SYNTHESIS

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INTRODUCTION

Transformation of bacteria by deoxyribonucleic acid (DNA) and transfer of hereditary information (1) has been extensively studied since Avery et al (2) showed that the transforming principle was DNA. Ribonucleic acid (RNA) by itself had not been considered capable of provoking a similar response.

In 1971 we presented data showing that hereditary information could be transferred to different bacterial species by a specific RNA from E. coli K-12 Hfr mutant, showdomycin resistant. Transforming RNA was isolated, purified, and characterized (3,4). It induces a massive transformation of recipient cells. RNase completely destroys the transforming RNA potential, while DNase is without effect (3). Transformants exhibit new and stable biochemical and physiological changes (5). Thus, Agrobacterium tumefaciens (oncogenic for plants), once transformed by E. coli RNA, has lost definitely its oncogenic power and acquired new properties (6).

Appearance of stable transformants in the presence of transforming RNA leads us to a particular approach to this problem. We wanted to find out if some appropriate enzymes could use transforming RNA as a template for RNA and for DNA synthesis. In other words, could the transfer of information from RNA be mediated by two distinct enzymes giving two distinct products? We have demonstrated that polynucleotide phosphorylase (PNPase) of E. coli wild type is capable of recognizing the transforming RNA as a template, synthesizing in vitro a poly AGUC which possesses a particular base ratio characteristic of that RNA (7). Second, we have recently found that bacteria (E. coli) contain an enzyme that, like viral reverse transcriptase, uses transforming RNA as a template for in vitro synthesis of complementary DNA.

SOURCE OF TRANFORMING RNA AND PROCEDURE USED FOR TRANSFORMATION

Transforming RNA has been isolated from E. coli: 1. from preparations of DNA after purification under appropriate conditions (episomal RNA), either from wild-type cells or from showdomycin-resistant mutants (4); 2. from the supernatant of showdomycin-resistant E. coli mutant cultures (mutant M 500 sho-R and mutant ML 30 sho-R); 3. after high speed centrifugation.

Both RNA preparations have a particular base ratio (G+A/C+U = 1.72 - 2.0) and are active in transformation of wild-type E. coli bacteria, regardless of their sex (4).

In order to get the transformants, wild-type E. coli is incubated in fresh synthetic medium (2 X 10^7 cells/ml for 1 hour at 37°C), supplemented with 0.1 to 2μg of active RNA/ml. The presence of transformants can be easily shown by determining the ratio:ribose/UV absorption at 260 nm of endogeneously synthesized RNAs (see *Note* to Table I). Thus transformation is expressed by the ratio:ribose/UV, that is, more of AMP and GPM nucleotides in the RNAs (Table I), since the ribose of these two nucleotides reacts in orcinol reaction.

TABLE I

Ribose/UV Ratio of Endogenous RNA of E. coli Transformants and Mutant M 500

Recipient bacteria	Transforming RNA	Ribose/UV ratio	Difference (%)
E. coli K-12 Hfr	no	0.64	–
	+ 0.1 μg	0.90	40
	+ 2.0 μg	0.93	45
	+ 2.0 μg + RNase (20 μg)	0.65	1.5
	+ 2.0 μg + DNase (20 μg)	0.91	42
E. coli-RV (8)	no	0.67	–
	+ 0.1 μg	0.89	32
	+ 0.2 μg	0.95	40
	+ 2.0 μg + RNase	0.69	2.8
	+ 2.0 μg + DNase	0.93	38
	+ 2.0 μg + Pronase[a]	0.96	40
	+ 0.1 μg + r-RNA (10 μg)	0.93	45
E. coli mutant M 500 sho-R	no	0.94	–

Note: Exponentially growing bacteria are collected by centrifugation and reincubated at 37°C (2 x 10^7 cells/ml) in 5 ml of synthetic medium containing glucose and 0.1 to 3 μg of transforming RNA. After 30 min or 2 hr, bacteria are collected and washed three times with 10 ml of 5% TCA solution, and exogeneous RNA extracted by heating the suspension (in 2 ml of 10% TCA) at 100°C for 20 min. After centrifugation supernatant is used for determination of UV absorption at 260 nm and for orcinol reaction (8). Ratio ribose/UV (arbitrary ratio) = divisions read at 670 nm (ribose)/divisions read at 260 nm.

[a] ARN preincubated with 200 μg of pronase for 1 hr.

Figure I shows the quantitative effect of active RNA in transformation of E. coli K-12 Hfr wild type. Transformation does not take place if RNA is pretreated with pancreatic RNase, while DNase has no effect. The modified ratio of endogenous RNA is characteristic of the showdomycin-resistant mutant from which transforming RNA was excreted (Table II). If ribose/UV ratio is close or identical to that of mutant sho-R, all necessary controls are performed with transformants. Ribosomal RNA does not compete with transforming RNA (Table I), and pronase is without effect.

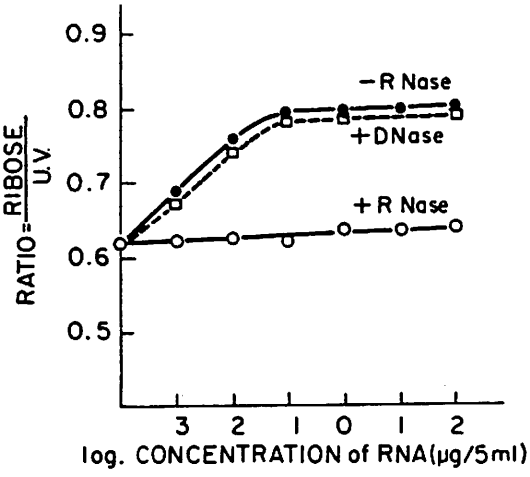

Fig 1. Effect of transforming RNA on change of ribose/UV absorption ratio in E. coli K-12 wild type. Conditions, see *Note* to Table 1.

Transformation of wild-type E. coli can also be achieved by simple incubation of a mixture of growing cultures of 98% wild bacteria and 2% mutant sho-R; both strains have the same growth rate at 37°C. After 10–16 hours, the whole population is transformed, that is, it has the characteristics of mutant sho-R.

TABLE II

Nucleotides Composition of r-RNAs of Agrobacterium tumefaciens, Wild-type B_6 and Transformant B_6-Tr-1

Nucleotide	Transforming ARN E. coli ML 30 sho-R	(mol per 100 mol of nucleotides)			
		wild type		transformant	
		23 S	16 S+17 S	23 S	16 S+17 S
A	30.3	26.0	25.2	30.6	29.3
G	33.5	30.4	29.8	33.3	31.4
C	18.3	24.7	23.5	19.6	20.6
U	17.8	18.9	21.5	16.5	18.7
G+A/C+U	1.76	1.27	1.22	1.77	1.56
G+C/A+U	1.05	1.20	1.16	1.03	1.08

Note: Ribosomal RNA (23 S, 16 S + 17 S) was isolated by the phenol method from 50 S and 30 S ribosomal subunits after separation on sucrose gradient (5). Purified RNA (1 mg) was hydrolyzed (KOH 0.5 N, 16 hr at 37°C) (60,000 cpm) and the nucleotides were analyzed, using a Dowex 1 X 2 column, 200-400 mesh (5). Transforming RNA was isolated as described from labeled ^{32}P E. coli ML 30 sho-R, separated by gel electrophoresis, hydrolyzed with 0.5 N KOH. Nucleotides were separated by Dowex column and the radioactivity determined.

TABLE III

Repartition of ^{14}C-uracil (Transforming RNA) Incorporated by Wild-type E. coli

Analyzed material	^{14}C-uracil, CPM in TCA precipitable material	
Washed bacteria	18.460	
Washed debris bacteria	3.621	
Membrane fractions	4.990	18.151
Ribosomes (70 S)	1.180	
105.000 x g supernatant	8.360	
DNA isolated directly from washed bacteria (18.370 CPM) and purified as described	1.552	

Note: 200 ml of exponentially growing culture (10^8 cells/ml) were incubated at 37°C with shaking in synthetic medium containing glucose (9) in the presence of 40 μg of ^{14}C-uracil labeled transforming RNA (36,640 cpm). After 1 or 2 hrs incubation, cells were collected by centrifugation at 15,000 x g for 30 min and washed with fresh culture medium. The presence of labeled RNA (TCA precipitable) was determined in the supernatant and in different fractions after labeled bacteria were degraded by grinding with alumina, and different fractions separated.

UPTAKE OF C^{14}-URACIL LABELED TRANSFORMING RNA BY RECIPIENT BACTERIA

^{14}C-uracil labeled RNA, excreted by E. coli mutant M 500 sho-R, was isolated (3) and incubated for 1 hour with recipient strain E. coli K-12 Hfr. Table II shows that half of the labeled RNA was incorporated by recipient bacteria. Remaining RNA, which did not penetrate the cells, is totally precipitable by trichloroacetic acid. This shows that labeled RNA was not degraded before entering the cells. Among different constituents obtained after disruption of labeled cells (Table III), ribosomes contain little radioactivity. "Membrane" fraction and 105,000 X g supernatant contain most of the ^{14}C-uracil. Five to 10% of incorporated ^{14}C-uracil is associated with DNA purified under described conditions (4). Although we cannot assert that all of ^{14}C-uracil RNA was active in transformation, we can conclude that radioactive RNA did penetrate the recipient cells. If this RNA was degraded inside the bacteria, most of the radioactivity should be found in ribosomes which contain roughly 70% of bacterial RNA. This is not the case.

MAIN CHARACTERISTICS OF E. COLI TRANSFORMANTS

1. As shown in Table I, the transformants have the same ribose/UV ratio as that of mutant M 500 sho-R, whose population is homogenous (repeated plating and analysis). Clones of transformants are stable and revertants were not obtained.

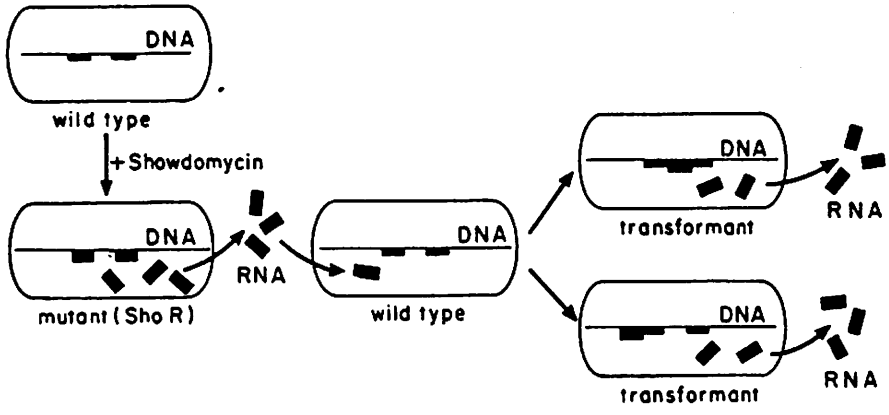

Fig 2. Scheme for excretion of transforming RNA from E. coli mutant sho-R and from transformants.

2. Transformants excrete the transforming RNA into culture medium as does the mutant M 500 sho-R (Fig 2).

3. Both ribosomal RNAs (23 S and 16 S) of transformants contain purine nucleotides in excess over pyrimidines (Table II).

4. The polynucleotide phosphorylase from transformants synthetizes in vitro (from equimolar amount of each of the four XDP) a poly AGUC in which purine nucleotides are in excess over pyrimidine when compared with poly AGUC synthesized by the wild-type enzyme. This is a characteristic of the PNPase of the mutant sho-R (7).

5. Showdomycin resistance is only poorly transferred (4). However, transformants acquire showdomycin resistance much more rapidly than wild-type E. coli.

INTERSPECIFIC TRANSFORMATION

Procedure for Transformation of Agrobacterium tumefaciens

The techniques and criteria used for the measure of the quantitative effect of transforming RNA (RNA excreted by E. coli, ML 30 mutant sho-R), the control of the inhibitory action of RNase and other necessary tests have been done. They are the same as those used for transformation of E. coli, wild type. It should be emphasized that in the case of the Agrobacterium tumefaciens B_6 strain, the incubation time in the presence of transforming RNA takes much longer (6 to 24 hours). The proportion of transformants increases with time, and in certain experiments the whole population was transformed in 16 hours. This looks like "progressive and cumulative transformation" as formulated for tumor induction in plants (10,11). The complete transformants (complete and definitive loss of oncogenic power), plated on solid medium, give colonies twice as large as the wild type. Partial transformants have also been obtained; they are intermediate between those of wild-type and completely transformed cells. Replacement of transforming RNA by a purine-rich nucleotide mixture or synthetic

TABLE IV

Characteristics of Agrobacterium tumefaciens Wild-type B_6 and Transformants

	Conditions of growth		Synthetic growth			
	aerobic	anaerobic	medium 63[a]	medium Stoll[b]	3 keto-lactose formation	Serologic test
Wild type B_6	+	no	no	+	+++	+++
B_6-Tr-4 transformant	+	no	no	+	+++	+–
B_6-Tr-1 transformant	+	no	no	+	+ (delayed)	–
E. coli ML 30 sho-R	+	+	+	no	no	no

Note: Test for 3 keto-lactose considered as specific one for Agrobacterium tumefacients (12) was performed on bacteria grown for 36 hr at 30°C on one large spot on solid Stoll medium containing 2% of lactose. The yellow color (3 keto-lactose) appears around grown colonies. Serological test was done with the anti-B_6 serum.

[a] = synthetic medium 63 routinely used for growth of E. coli (9).

[b] Medium Stoll (13) rather specific for A. tumefaciens.

polyribonucleotides does not lead to the appearance of transformants. Spontaneous transformants, such as those we have obtained in the presence of E. coli RNA, never have been described.

Characteristics of Agrobacterium tumefaciens Transformants

The essential and rather specific tests for Agrobacterium tumefaciens are summarized in Table IV. Transformants have kept certain specific characteristics of Agrobacterium tumefaciens and have acquired some new ones described here and elsewhere (6,14). Some striking biochemical changes of transformants are illustrated by Figure 3. The densitometer tracings of ribosomal RNAs and ribosomal proteins of Agrobacterium tumefaciens, wild type, and those of complete transformants show that the importance of the transformation was profound, indeed. In addition, the nucleotide composition of ribosomal RNA of the transformants strongly differs from that of the wild type (Table II). Consequently, we expected a modification in the synthesis or activities of certain enzymes in these transformants.

L-Asparaginase in Transformants of Agrobacterium tumefaciens

L-Asparaginase, which at pH 5.0 degrades l-asparagine into aspartic acid and ammonia, has been studied particularly in Agrobacterium tumefaciens transformed by E. coli RNA (8). The reason for choosing this enzyme was that l-asparaginase of bacterial origin causes certain mammalian tumors to regress and has an antilymphoma and antileukemic effect (16 - 18). Some of the results described (14) are illustrated by Figure 4. It is clear that partial transformants B_6-Tr-4 contain substantially more l-asparaginase (pH 5.0) than wild-type B_6, while in complete transformants B_6-Tr-1 the amount of l-asparaginase is several times greater. Thus, a correlation appears between

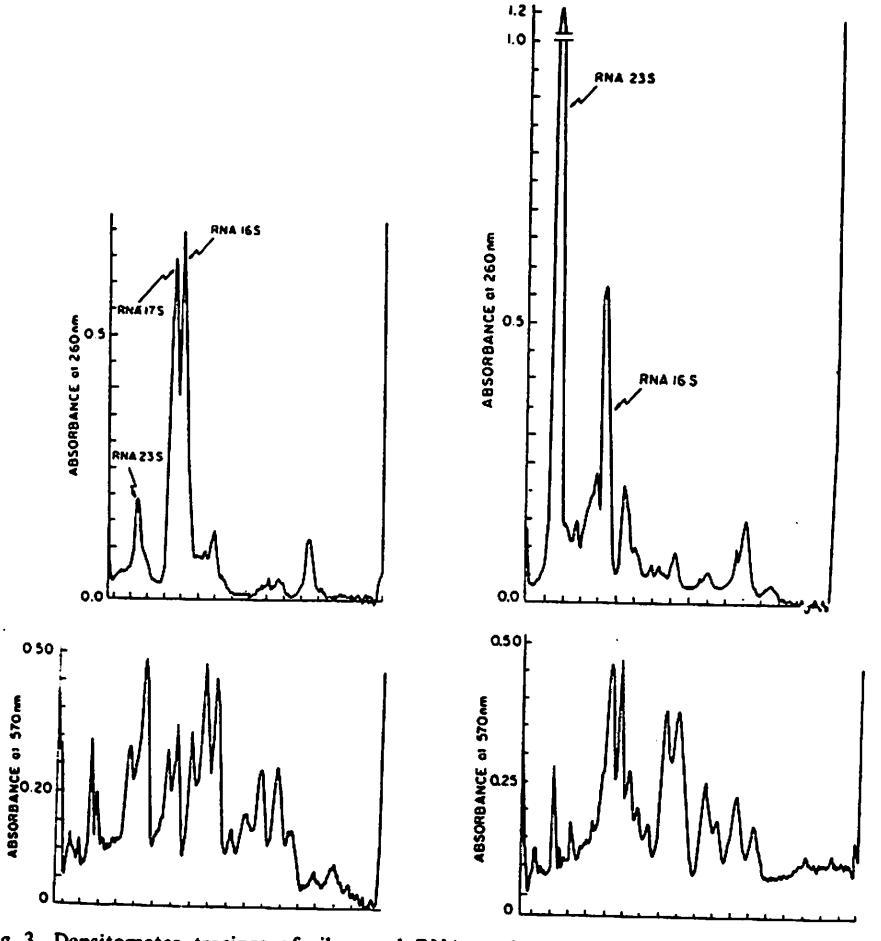

Fig 3. Densitometer tracings of ribosomal RNAs and ribosomal proteins of wild-type B_6 and transformants (A. tumefaciens).

Ribosomal RNA and ribosomal proteins after polyacrylamide gel electrophoresis. Conditions described elsewhere (5). *Above left:* r-RNA of wild-type B_6; *right:* r-RNA of B_6-Tr-1. *Below left:* ribosomal proteins of wild-type B_6; *right:* that of B_6-Tr-1.

loss of oncogenic capacity and great increase of the amount of l-asparaginase in transformants of Agrobacterium t. Commercially purified l-asparaginase, when inoculated with oncogenic strain B_6, causes significant regression (50 - 74%) of tumors in plants. However, one cannot exclude a simple coincidence between loss of oncogenic power and important changes in the activity of l-asparaginase in transformants.

Evidence for Loss of Oncogenic Properties in Agrobacterium tumefaciens Transformed by E. coli RNA

Agrobacterium tumefaciens wild-type B_6, carries a tumor-inducing principle that causes heritable changes in the plant host. Tumors appeared in plants that were inoculated with strain B_6 wild type. The tumor-inducing capacity of partially transformed strain B_6Tr-4A is substantially lower than that of the wild type, as judged by

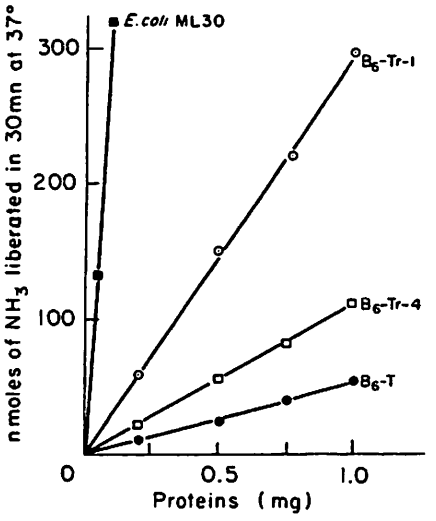

Fig 4. Activity of l-asparaginase at pH 5.0 in extracts of Agrobacterium tumefaciens wild-type B_6, transformants B_6-Tr-4, B_6Tr-1, and E. coli ML 30 sho-R.

L-asparaginase activity was tested as described (14). After aerobic growth, bacterial culture was left without shaking for 4 hrs at 30°. Bacteria were collected, washed, and sonicated. After centrifugation at 105.000 g for 10 min. supernatant was incubated in the presence of l-asparagine and the activity determined.

weight of tumors. Tumors did not appear with complete transformants (B_6-Tr-1) (Fig 5). Clones of that strain confirm the homogeneity of the population.

It was suggested (19) that an RNA fraction may be an essential part of the tumor-inducing principle of Agrobacterium tumefaciens. This conclusion came from the observation that tumors do not appear in the presence of RNase, while they do appear normally in the presence of DNase. Our results suggest that a transforming RNA (episomal RNA) could be excreted by oncogenic A. tumefaciens when a plant is inoculated with that bacteria. This RNA could penetrate the plant cells (if it is not destroyed by RNase) and provoke as an autonomous episome the appearance of modified biochemical and biological properties that become inherited in these cells. Eventually, it might be transcribed into DNA which, in turn, would be integrated into the plant genome.

COULD THE TRANSFORMATION OF BACTERIA BY RNA BE DUE TO DNA SYNTHESIZED ON AN RNA TEMPLATE (REVERSE TRANSCRIPTASE)?

a) Transformation of bacteria by transforming RNA from E. coli leading to heritable changes in transformants raised the following question: Could the transfer of information from RNA be mediated by an RNA-dependent DNA polymerase? In the case of oncogenic viruses (20,21), viral RNA is transcribed into DNA by reverse transcriptase contained in viruses (12,14,25,28). On the basis of our results, the search for an enzyme-like "reverse transcriptase" was justified by the fact that E. coli K-12 Hfr wild type do synthesize the transforming RNA and that the showdomycin-resistant mutant of these bacteria excretes a transforming RNA.

Fig 5. Oncogenic power of A. tumefaciens B_6 wild type. Comparison with transformants B_6-Tr-4 and B_6-Tr-1.

Technique for inoculation of bacteria into wounded plants has been described elsewhere (15). Datura stramonium was wounded and bacterial suspension (equal optical density) introduced into the wound. In routine experiments pea seedlings were used to test the oncogenic capacity of Agrobacterium tumefaciens (15). St = sterile treatment of Datura stramonium (no bacteria).

The polymerization of deoxyribonucleoside-5′-triphosphates (d-XTP) can be easily demonstrated when transforming RNA is incubated with an endogenous nucleic acid-free enzyme preparation. Thus, the passage of a 105.000 X g supernatant through a DEAE column gives an enzyme preparation in the presence of which the polymerization of d-XTP is completely dependent on active RNA (Table V). All four d-XTP are required to get a maximal amount of a H3-d-XTP incorporated into a trichloraacetic acid (TCA) precipitable product. Omission of one or two of the d-XTP leads to a considerable decrease in the product formation. One d-XTP does not seem to be polymerized.

DNA synthesis by E. coli transcriptase was examined in the presence of different RNA preparations and E. coli DNA. It should be emphasized that the RNA preparations used did not contain DNA in detectable amounts, while DNA preparations did contain RNA (3;4). The best template for E. coli transcriptase was (Table VI)

TABLE V

^{3}H-d-ATP, Incorporated (20 mn) into Acid-Precipitable Product under Various Conditions

Reaction mixture	ρ moles	Reaction mixture	ρ moles	Inhibition (%)
Complete	411	complete	418	–
Minus transforming RNA	< 1	+ DNase 5 μg	< 1	99
Minus $MgCl_2$	< 2	+ RNase 20 μg	230	40
Minus d-GTP	136	+ RNase 20 μg preinc.	43	90
Minus d-CTP	125	+ showdomycin 50 μg	105	75
Minus d-GTP, d-CTP, d-TTP	< 1	+ showdomycin, 50 μg (mutant enz.)	420	0

TABLE VI

Activity of E. coli Reverse Transcriptase in the Presence of Different Templates

Reaction mixture	ρ moles of ^{3}H d-ATP (incorporated in 20 min)	ρ moles of ^{3}H d-TTP
Complete with transforming RNA, E. coli M 500	402	656
excreted RNA wild-type E. coli	392	400
ribosomal RNA (23 S + 16 S)	< 1	< 1
bulk t-RNA	< 1	< 1
t-RNA^{met}	0	–
A. faecalis 5.5 S RNA	145	136
Poly AG + poly UC	< 1	< 1
DNA	98	–
DNA treated with RNase	2	–

Note: Reaction mixture contains per 0.2 ml: $MgCl_2$, 2 μM; Tris-HCl buffer pH 7.65, 25 μM; each deoxyribonucleoside-5'-triphosphate 5 n moles + ^{3}H d-ATP or ^{3}H d-TTP (100,000 CPM); transforming RNA, 4 μg; enzyme fraction (DEAE) 60 μg. Incubation 20 min at 37°C. After addition of trichloroacetic acid (TCA) to incubation mixture, the precipitate was filtered on Whatman GC/F glass filter, washed, dried, and radioactivity measured in a Packard spectrophotometer. Enzyme fraction DEAE containing reverse transcriptase activity was obtained by the method described (29). Conditions as described in the note to Table I. Templates 4 μg, ^{3}H d-ATP, and ^{3}H d-TTP (100.000 CPM); DNA was pretreated with 20 μg of pancreatic RNase. Alcaligenes faecalis 5.5 S RNA was isolated as described (30).

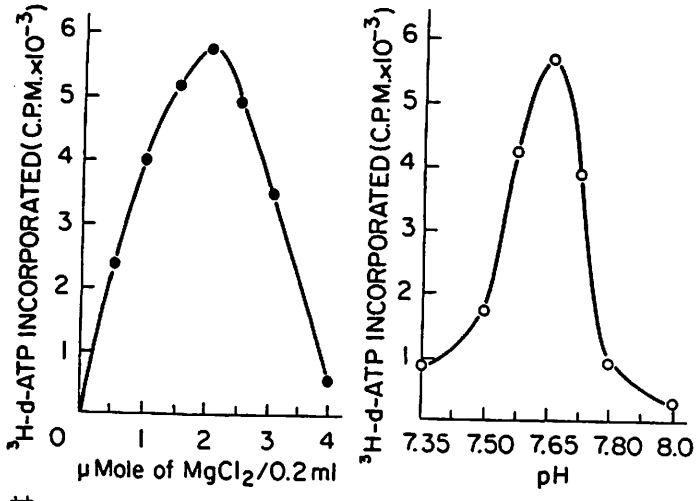

Fig 6. Mg^{++} ions requirement and pH of buffer solution for activity of E. coli reverse transcriptase. Incubation conditions: See *Note* to Table VI.

extracellularly excreted RNA, especially transforming RNA. Ribosomal RNA and transfer RNA are inactive; Alcaligenes faecalis 5.5 S RNA containing rapidly labeled RNA is utilized by E. coli transcriptase for DNA synthesis. Synthetic polyribonucleotides have no effect on enzyme activity. Low enzyme activity observed in the presence of E. coli DNA disappears when the DNA preparation is pretreated with RNase (pancreatic and T_1). Template activity of RNA is destroyed by preincubation with RNase and the synthesis of the deoxypolymer does not take place in the presence of DNase (Table V). Showdomycin inhibits strongly the deoxypolymer formation (Table V).

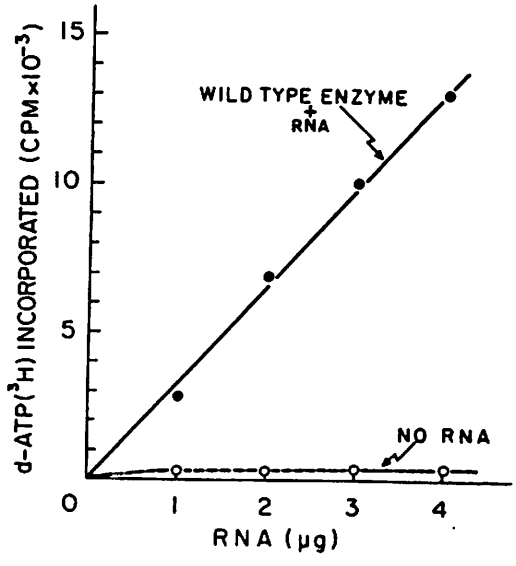

Fig 7. Deoxyribopolymer formation in the presence of different concentrations of transforming RNA.

Conditions: see *Note* to Table VI. Transforming RNA excreted by E. coli mutant M 500 sho-R was used.

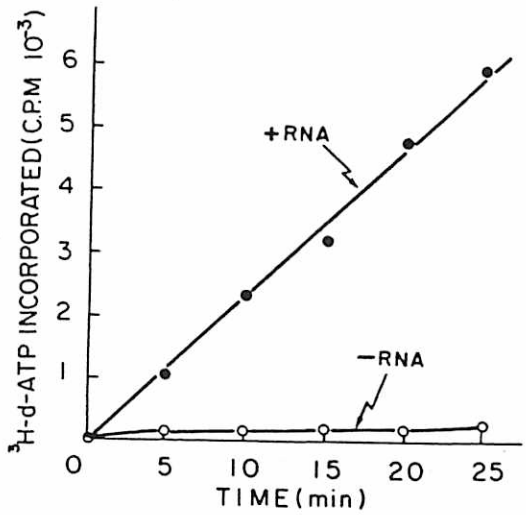

Fig 8. RNA-dependent deoxypolymer formation by E. coli reverse transcriptase in function of time. Conditions as described in the *Note* to Table VI. Transforming RNA from E. coli M 500 sho-R was used.

b) Metal requirements and pH; The maximal incorporation of ^{3}H-product depends upon Mg^{++} ion concentration and on the pH of the buffer (Fig 6). Mn^{++} partially replaces (50%) Mg^{++}.

The formation of ^{3}H deoxypolymer in the absence and presence of different concentrations of transforming RNA and as a function of time are illustrated by Figures 7 and 8. Polymer formation is strictly dependent on RNA acting as template.

c) Properties of synthesized ^{3}H-deoxypolymer: The ^{3}H product synthesized under optimal conditions (20 min.) is not sensitive to RNase. pronase, and KOH. In contrast, it is degraded by pancreatic deoxyribonuclease (35).

TABLE VII

A/U Ratio of Template RNA and dT/dA Ratio of ^{3}H Product Synthesized by E. coli Transcriptase

	mol per 100 mol of nucleotides	A/U ratio	dT/dA ratio of ^{3}H product
E. coli M 500 RNA	A = 29.0 G = 34.0 C = 19.0 U = 18.0	1.57	1.62 (Exp I); 1.54 (Exp II); 1.58 (Exp 3)
A. faecalis 5.5 S RNA	A = 16.2 G = 34.5 C = 32.0 U = 17.3	0.95	0.96 (Exp I); 0.89 (Exp II); 0.99 (Exp 3)

Note: Conditions. see *note* to Table VI.

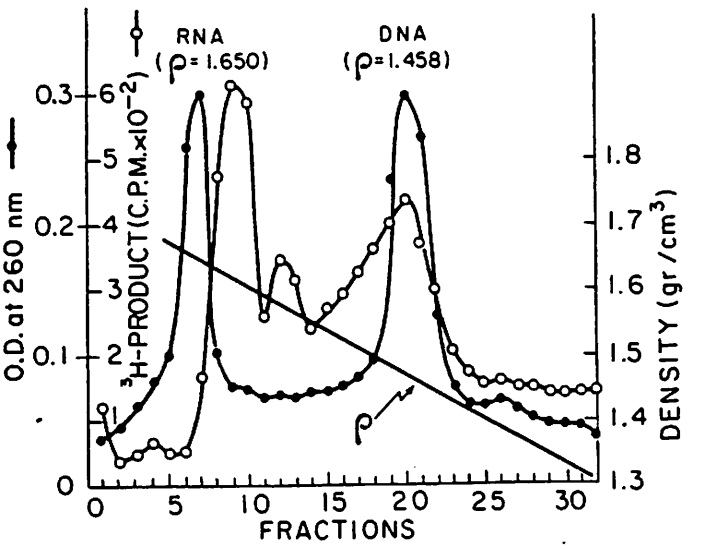

Fig 9. Density of ^{3}H-product synthesized by E. coli reverse transcriptase in the presence of transforming RNA.

^{3}H-product was synthesized as described in the *Note* to Table VI. After 30 min of incubation, the mixture was treated with chloroform (twice), centrifuged, and the aqueous solution dialyzed for 24 hrs at 4°C against 1 l. of Tris buffer 10^{-2} M containing KCl 0.1 M (two changes of buffer solution). Radioactive product is completely precipitable by trichloroacetic acid. Dialyzed ^{3}H product was mixed with saturated C_2SO_4 to a density of 1.550 gr/Cm^3. Internal markers: transforming RNA and E. coli DNA were included and centrifuged at 30,000 rpm at 20°C for 64 hrs in a Spinco SW 39 rotor. Fractions were collected. Absorption at 260 nm and radioactivity were determined.

The data presented in Table VII show the ratio A/U of two template RNAs and that of d-T/d-A of the ^{3}H-deoxypolymer synthesized in vitro by E. coli transcriptase. The ratio d-T/d-A (on average) is identical to that of the A/U of the RNA. On that basis, one can conclude that complementary deoxypolymer was synthesized on the RNA template. To provide evidence that the ^{3}H heterodeoxypolymer is of a DNA nature, we separated it from the enzyme by treatment with chloroform, then dialyzed it and examined it by equilibrium density centrifugation in C_2SO_4. Internal markers (transforming RNA and E. coli DNA) were included with ^{3}H product. Figure 9 shows that the ^{3}H product is overspread in the density regions between RNA and DNA (gradient at pH 7.65). This heterogeneity is expected for RNA-DNA hybrids (23) (27). However, a certain amount of radioactive material is present in the DNA density region, which suggests that some free DNA is present in the gradient. These data and those presented in Table VI show that some specific RNAs, transforming RNA in particular, are the template for the transcriptase by which transfer of information is mediated.

Experiments are in progress to determine if a particular DNA portion can be detected in DNA from wild and mutant bacteria and if this portion is able to hybridize with DNA synthesized by E. coli reverse transcriptase.

CONCLUSIONS

The data presented here summarize our recent work, showing that a transforming RNA excreted by showdomycin-resistant mutants of E. coli penetrates the recipient cells (E. coli wild type) producing a massive transformation. The quantitative effect of transforming RNA on recipient bacteria proves that transformants must arise from the interaction of transforming RNA and bacteria that undergo transformation. Transformants which behave as stable mutants possess new and inherited biochemical characteristics identical to those found in E. coli showdomycin-resistant mutants from which transforming RNA was excreted. However, transfer of the resistance character to recipient cells is very low. It is worth emphasizing that Agrobacterium tumefaciens, transformed by E. coli transforming RNA, has lost completely the oncogenic power for plants and acquired several new properties. In both types of transformants (E. coli and Agrobacterium tumefaciens), we have found a very satisfactory correlation between the chemical properties of transforming RNA (A+G/C+U = 1.75 to 2.0) and those of intracellular RNAs synthesized by transformants (A+G/C+U = 1.75 to 2.0). In addition, as in the E. coli sho-R mutant, the consequence of modified RNAs in transformants is reflected by important changes in ribosomal proteins as well as in certain enzymes. The most intriguing correlation exists between the loss of oncogenic power of transformants and the increase of l-asparaginase pH 5.0 of Agrobacterium t.

The central question raised by our studies is: Is it possible to construct a general picture of the process of transformation of bacteria by RNA?

We propose two possible explanations. First, transforming RNA having particular chemical properties, once inside the recipient cells, could in some way be associated with bacterial DNA in order to be protected as an original "RNA episome," itself (4). This RNA episome can be replicated by a process in which complementarity of bases is not necessary, since the base composition of several RNA species (t-RNA is an exception) in transformants corresponds exactly to that characteristic for transforming RNA. PNPase seems to fulfill this requirement (6), since it has the capacity to replicate in vitro the transforming RNA into a product which has a G+A/C+U ratio close to or identical with that of transforming RNA. Drastic alteration of several RNA species and proteins (ribosomal proteins and enzymes) in transformants obtained by RNA implies that RNA episome must have a strong autonomy, since it escapes the control of the bacterial genome. If this is so, then the RNA episome could be considered as an extrachromosomal determinant. In that regard, it satisfies a number of criteria: it can easily migrate from one cell to another; it induces profound biochemical and physiological changes; and transformants excrete transforming RNA. This last property could easily explain why transformants do not give rise to revertants.

The presence of the RNA episome in small amounts in wild-type bacteria and in large amounts in the showdomycin-resistant mutant (3) strongly suggests that this kind of episome could be considered as a primitive cell determinant which can be expressed only under certain conditions (28).

Another explanation could be that transforming RNA once inside the recipient bacteria can direct DNA synthesis mediated by a reverse transcriptase. Complementary DNA synthesized on the template of transforming RNA could be integrated into a bacterial genome, as suggested by Temin for RNA of oncogenic viruses (2), thus leading to a genetic mutation. This kind of mutation will be drastic if the bacterial

genome contains enough sequences, as specified by transforming RNA (G+A/C+U = 1.70 - 2.0). This view is partly supported by the data we have presented in this paper, showing that E. coli K-12 possesses an enzyme, reverse transcriptase, which in the presence of 4 d-XTP and a template (transforming RNA) synthesizes a deoxyribopolymer characterized as being DNA (35). This does not necessarily mean that such DNA has to be incorporated into the cell genome. DNA synthesized on an RNA template may well be helpful in protecting that RNA (transforming RNA for example) which can be freed when needed. Something of that sort could happen even to messenger RNA when it is transcribed into complementary DNA, as has been shown for hemoglobin-purified messenger RNA (25,32,33). Along these lines it should be pointed out that an RNA-DNA "hybrid" preparation (34) can mediate the transfer of sulfamide resistance to pneumococcus, and that both RNase and DNase destroy the transforming principle. It was suggested that during transformation by the RNA-DNA preparation "RNA may act to preserve activity of DNA or vice versa." In addition, the importance of the RNA-DNA hybrid as a template for polydeoxyribonucleotide synthesis by purified E. coli DNA polymerase I has been emphasized (36,37).

The data presented concerning transformation of bacteria by transforming RNA and RNA-directed DNA synthesis constitute a new approach to the study of extrachromosomal inheritance in bacteria and its implication in evolution. As suggested recently (38), a multienzyme complex may be necessary to direct and regulate the biosynthesis of biologically active DNA and RNA in living cells.

SUMMARY

Transforming RNA released from showdomycin-resistant mutants of E. coli is capable of promoting "genetic transformation" of wild-type E. coli and Agrobacterium tumefaciens. Transformants possess profoundly modified biochemical and physiological properties. Thus certain types of transformants of Agrobacterium tumefaciens have definitively lost their capacity to be oncogenic in plants. Evidence for the existence in bacteria of an RNA-directed DNA polymerase is presented. Its possible role in the transformation of bacteria by RNA is discussed.

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