Transcription of bacterial DNA by isolated plant nuclei

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Summary. Plant nuclei prepared from protoplasts can be used as a cell-free system for testing the template activity of procaryotic DNA for plant polymerases. We were able to demonstrate that plant polymerases of *Petunia hybrida* are capable of transcribing linear bacterial DNA, whereas supercoiled DNA could not be used as a template.

Recently, attempts to introduce exogenous genetic material into higher plants are intensified[1]. An uptake of fd-DNA[2] and double-stranded *E. coli*-DNA[3] into the cytoplasm of isolated mesophyll protoplasts could be proved. The same holds true for phages[4]. Whether also the nuclei could take up the exogenous gene material, remains an open question[5]. In any case, the mere uptake would be useless without an expression of the transferred gene material. The first step of a successful expression has to be transcription. Therefore, the transcription of foreign DNA by plant enzymes should be studied. There are, however, just a few publications dealing with the use of bacterial DNA by higher plant polymerases[6].

Transcription of bacterial DNA by isolated plant nuclei was studied. Nuclei were prepared by a gentle treatment with triton X-100 from isolated mesophyll protoplasts of *Petunia hybrida*[7]. The method gives a higher yield of nuclei than other techniques[5], and the nuclei show a higher transcription of their own DNA than nuclei prepared by the mechanical procedures used hitherto[7]. The nuclei were incubated with *E. coli* chromosomal resp. plasmid DNA (Col E_1-DNA) and then assayed for transcription with [^{3}H]-UTP. The labelled RNA was extracted[8], and hybridized with chromosomal resp. Col E_1-DNA of *E. coli*, and in control experiments with *Petunia*-DNA, using the filter technique[9]. Chromosomal DNA of *E. coli* was prepared according to Marmur[10], Col E_1-DNA according to[11], and *Petunia*-DNA according to Liebke et al.[3].

First, the nature of the radio-labelled product was analyzed. A RNAse-treatment (125 RNAse A µg/ml) after transcription reduced the incorporation of [^{3}H]-UTP into an acid-insoluble fraction to 11.4% of untreated control. If Actinomycin D (40 µg/ml) was added during transcription, radioactivity in the acid-insoluble fraction was reduced to 11.3% of untreated control. These results prove the RNA-character of the transcriptional product. Thereafter, nuclei were incubated with *E. coli* chromosomal DNA. In the control experiment, the nuclei remained untreated. The samples were further subdivided. In one part, the nuclei were washed before starting the transcription assay, thus removing the excess DNA. In all 4 experiments approximately the same transcription rate, measured by the incorporation of [^{3}H]-UTP into RNA, was found (figure 1). As demonstrated by hybridization, there was *Petunia*-RNA in all the experiments.

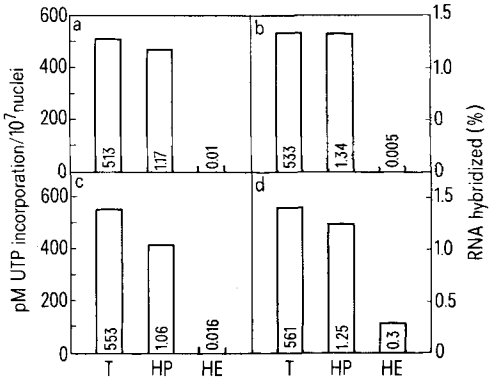

Fig. 1. DNA-RNA hybridization experiments with RNA extracted from nuclei incubated with *E. coli*-DNA before transcription. Isolated nuclei of *Petunia hybrida* were incubated for 2 h at 5 °C with 100 µg *E. coli*-DNA per 10^6–10^7 nuclei (*b*, *d*) or DNA incubation was omitted (*a*, *c*). Nuclei were sedimented by centrifugation and resuspended in a reaction mixture (*a*, *b*) or transcription was performed directly (*c*, *d*). Transcription was carried out at 37 °C for 30 min in the following reaction mixture: 0.01 M Tris/HCl, pH 7.8; 0.25 M sucrose; 0.025 M KCl; 5 mM $MgCl_2$; 1 mm $MnCl_2$; 0.01 M mercaptoethanol; 1 mM each of ATP, CTP, GTP; 0.02 mM of UTP; 5 µCi of [^{3}H]-UTP (49 Ci/mM). RNA was extracted by hot phenol method, and aliquots were taken for measurement of transcription rate by counting TCA insoluble radioactivity. Transcription is expressed as pM UTP incorporated by 10^7 nuclei (T). In control experiments, transcription was stopped at time 0 to detect background radioactivity. Hybridization mixture (nitrocellulose filter hybridization technique) contained one blank filter, one filter with 40 µg filter-bound *Petunia*-DNA and 1 filter with 40 µg filter-bound *E. coli*-DNA. Hybridization rate is expressed as RNA hybridized in percent of input RNA (HP = hybridization to *Petunia*-DNA; HE = hybridization to *E. coli*-DNA.)

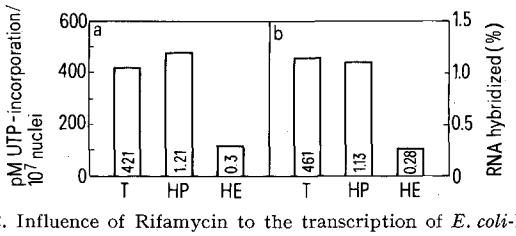

Fig. 2. Influence of Rifamycin to the transcription of *E. coli*-DNA by isolated plant nuclei. Nuclei were incubated with *E. coli*-DNA as described in figure 1. 30 min before starting transcription, Rifamycin SV was added to one sample (*b*) to a final concentration of 50 µg/ml. Transcription and hybridization were performed according to experiment *d* in figure 1. T = Transcription rate; HP = hybridization to *Petunia*-DNA; HE = hybridization to *E. coli*-DNA.

1 D. Hess, in: Plant Cell, Tissue, and Organ Culture, p. 506. Ed. J. Reinert and Y. P. S. Bajaj. Springer, Berlin–Heidelberg–New York 1977.
2 M. Suzuki and I. Takebe, Z. PflPhysiol. *78*, 421 (1976).
3 B. Liebke and D. Hess, Biochem. Physiol. Pflanzen, in press (1977).
4 D. Hess, in: Microbial and Plant Protoplasts, p. 125. Ed. J. F. Peberdy, A. H. Rose, H. J. Rogers and E. C. Cocking. Academic Press, London 1976.
5 B. Liebke, W. Blaschek and D. Hess, Z.PflPhysiol., in press (1977).
6 C. T. Duda, A. Rev. Pl. Physiol. *27*, 119 (1976).
7 W. Blaschek, D. Hess and F. Hoffmann, Z.PflPhysiol. *72*, 262 (1974).
8 K. Scherrer and J. E. Darnell, Biochem. biophys. Res. Commun. *7*, 486 (1962).
9 D. Gillespie and S. Spiegelman, J. molec. Biol. *12*, 829 (1965).
10 J. Marmur, J. molec. Biol. *3*, 208 (1961).
11 N. Godson and R. Sinsheimer, Biochim. biophys. Acta *149*, 489 (1967).

E. coli-RNA could be detected only if the nuclei were not washed before the transcription assay. Otherwise hybridization with *E. coli*-DNA was negligible, as in the controls not treated with *E. coli*-DNA. This result is consistent with a transcription on the surface of the nuclei or in the surrounding medium. Several repetitions gave the same results.

The control experiments permit us to exclude the participation of bacteria. The bacterial DNA was purified in a CsCl density gradient. Therefore its contamination with bacterial RNA-polymerases seems highly improbable. Additionally the transcription experiments were performed in the presence of rifamycin, a potent inhibitor of bacterial RNA-polymerases. Rifamycin was without any effect on the synthesis of bacterial RNA (figure 2), thus indicating that only the *Petunia*-RNA-polymerases were at work. Similar experiments were performed with Col E_1-DNA. The RNA formed was hybridized with single stranded *Petunia*-DNA and Col E_1-DNA[12]. The *Petunia*-DNA was transcribed as before. A transcription of the plasmid DNA, however, could not be detected.

The experiments demonstrate that plant polymerases are capable of transcribing bacterial linear DNA. In the case of supercoiled DNA, however, difficulties arise. There are several possibilities to account for the above results: either the plant RNA-polymerases are unable to recognize the initiation sites in the Col E_1-DNA, or the differences are part of the conformation of DNA (supercoiled, complete native Col E_1-DNA – linear, conditional native *E. coli*-DNA). Further experiments to clarify these questions are in progress. Anyway, plant nuclei prepared from protoplasts offer a simple system for testing the efficacy of plant RNA-polymerases.

12 W. Goebel and W. Schiess, Molec. gen. Genet. *138*, 213 (1975).

Uptake of ferritin from the medium by *Tokophrya infusionum*[1]

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Summary. Tokophrya infusionum, a suctorian, is deprived of a mouth opening. The uptake of ferritin from the medium is accomplished through pits, invaginations of the plasma membrane which are permanent structures. From the pits containing ferritin, flat vesicles are pinched off transporting the ferritin to the cytoplasm.

Tokophrya, as all Suctoria, does not have a cytostome and its feeding apparatus consists of numerous tentacles, long slender tubes extending to the outside of the body and also deep into the cytoplasm[3–5]. At the distal end, the tentacle terminates into a rounded knob covered by the plasma membrane[3]; inside the cytoplasm the proximal end of the tentacle remains open[5]. Each of the tentacles is capable of changing into a mouth when a living ciliate gets attached to the knob of one or more tentacles[4]. The attachment is followed by the rupture of the plasma membrane of the prey which merges with the membrane covering the tentacle and the 2 organisms become united by a continuous common membrane enveloping prey and predator[6]. Immediately thereafter the cytoplasm of the still living prey begins to stream down through the attached tentacle into the predator where it become enclosed into food vacuoles[4]. It was assumed that this way of feeding supplies the predator with all the necessary food and that the medium plays a negligible role as a source of nutrients. However, observations indicated that also the medium influences the life span, the reproduction rate and the survival of clones. Although cultures of *Tokophrya* could grow for some time in a well balanced inorganic medium, or even in distilled water, the conditions for longevity, multiplication and duration of clones improved greatly in a medium containing yeast extract. Apparently for normal growth *Tokophrya* needs some supplements deriving from the medium in addition to the cytoplasm of the prey. A diluted yeast medium has been routinely used for the maintenance of cultures[7]. The mechanism by which nutrients and particularly large molecules enter from the medium to the body of *Tokophrya* was so far unknown and to solve this problem a thorough study of structures covering the organism and experiments with tracers were necessary. The following is an account of such a study.

Tokophrya is covered by 3 unit membranes (figure 2, insert) and beneath them lies the epiplasm, a dense homogeneous layer about 70 nm thick (figure 2). To the outside of the external membrane *Tokophrya* is surrounded by an amorphous coat of low density about 300 nm thick (figures 1 and 2). It seemed therefore that the organism is well shielded and as if isolated from the surrounding medium. A more detailed analysis disclosed however the existance of gaps in some of the membranes and layers. Only the external membrane is continuous and at uneven intervals dips down into the cytoplasm (figures 1 and 2) forming small saccules, so-called 'pits'[6,8]. The pits disrupt the 2 other unit membranes and the dense epiplasm. The pits are about 390 nm deep and their opening is over 100 nm in diameter; the part of the pit located in the cytoplasm is covered by a single membrane (figure 2) and it is about 250 nm wide, bulbous in shape but often irregular forming outpocketings.

The role and function of the pits has been and still is of great interest. They are found throughout the Subphylum Ciliophora to which Suctoria belong[9]. Experiments with tracers performed on ciliates[10] could not give conclusive results because these organisms possess an oral cavity

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3 M. A. Rudzinska, J. Cell. Biol. *25*, 459 (1965).
4 M. A. Rudzinska, Biol. Sci. *23*, 87 (1973).
5 M. A. Rudzinska, J. Protozool. *21*, 721 (1974).
6 M. A. Rudzinska, Trans. N.Y. Acad. Sci., series II, *29*, 512 (1967).
7 M. A. Rudzinska, J. biophys. biochem. Cytol. *4*, 195 (1958).
8 L. L. Millecchia and M. A. Rudzinska, J. Protozool. *19*, 473 (1972).
9 Committee on Taxonomy and Taxonomic Problems, Society of Protozoologists, J. Protozool. *11*, 7 (1964).
10 C. Noirot-Timothee, C. r. Acad. Sci. Paris *267*, 2334 (1968).