TRANSCRIPTION IN NUCLEI ISOLATED FROM TOBACCO TISSUES

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1. Introduction

An advantage of using isolated nuclei for the study of transcription is the presence of endogenous RNA polymerases. Moreover, in the nuclei, the native state of the chromatin, including regulatory proteins, is maintained. Transcription in nuclei from various plant sources, e.g., soybean hypocotyls [1] and tobacco callus [2] has been studied. Using tobacco callus nuclei as an in vitro system we did not find any effect of hormones on transcription [3]. However, one should be aware that nuclei isolated from different tissues of the same plant species may behave differently. Therefore, we have compared the characteristics of nuclei isolated from tobacco stem pith, pith callus and leaves.

Clear differences between the properties of the nuclei were found in the amounts of RNA synthesized per μg DNA, in the contents of free RNA polymerases, and in the stimulation of RNA synthesis by heparin. Exogenous DNA of various origins was transcribed to a different extent by the nuclei studied.

2. Materials and methods

2.1. *Explants*

The culture of explants was as in [2].

2.2. *Isolation of nuclei*

Nuclei from freshly isolated tobacco stem pith and from tobacco pith callus were isolated as described for callus tissue [3]. Nuclei from leaves were isolated in the medium of [4]; 2.5% Ficoll, 5% Dextran-T40, 0.25 M sucrose, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM 2-mercaptoethanol and 25 mM Tris (pH 7.8). Fully expanded leaves, deprived of the mid-rib, were cut in slices, dipped in ice-cold ether for 30 s and immediately taken up in the isolation medium (5 ml/g tissue). After homogenization in a Waring blendor for 45 s, the brei was filtered through 4 layers of nylon cloth (pore size 500 μm) and, with intermittent vibration, through 4 stainless steel sieves with pore sizes of 250, 106, 45 and 10 μm, successively (Retsch 'Vibro', FRG). The filtrate was centrifuged for 10 min at 650 $\times$ *g* and the pellet treated for 5 min with 0.4% Triton X-100 in isolation buffer. After centrifugation for 10 min at 650 $\times$ *g* the pellet was washed twice, taken up in 6.5 ml isolation buffer and layered on a discontinuous sucrose gradient (15 ml 60% sucrose over 15 ml saturated sucrose solution in buffer at 4°C). After centrifugation for 10 min at 10 000 $\times$ *g* and, without braking, 45 min at 70 000 $\times$ *g* the nuclei were collected from the top of the sucrose cushion, washed with isolation buffer and taken up in buffer A, as used in the isolation of callus nuclei [3]. The DNA contents of nuclei were measured as in [2].

2.3. *DNA isolation*

DNA from *Helianthus tuberosus* L. and *Nicotiana tabacum* L. was isolated from the nuclei according to [5].

DNA and Ti-plasmid from *Agrobacterium tumefaciens*, strain B6S3 were gifts from Dr G. Ooms (Biochemical Laboratory, University of Leiden).

2.4. *RNA synthesis*

RNA synthesis in nuclei was studied under the

optimal conditions for RNA polymerases I and II [3], which were found to be the same for nuclei isolated from stem pith, callus and leaves of tobacco. For the study of polymerase II activity under low-salt conditions $(NH_4)_2SO_4$ was decreased from 175 mM to 50 mM.

3. Results and discussion

There was a great difference in the rates of incorporation of [^{3}H]UMP/μg DNA by the three nuclear preparations. Measured under optimal conditions for polymerase II these values were 0.031, 0.015 and 0.18 pmol [^{3}H]UMP/μg DNA/min for nuclei from leaves, stem pith and callus, respectively. These values, found after 1 h incubation, were somewhat dependent on the purity of the nuclear preparations: the purer the nuclei, the higher the incorporation, variation $\leqslant 30\%$. Nuclei isolated from actively growing callus tissue showed a much (6–12-times) higher transcriptional activity than those from pith tissue or leaves.

Heparin effectively inhibits binding of RNA polymerase to DNA and thus inhibits initiation of RNA synthesis [6]. This polyanion can also be applied to determine the transcriptional activity of the RNA polymerase transcription complexes already present in the nuclei, since it is known to cause decondensation of the chromatin and, therefore, to stimulate the rate of RNA synthesis [7]. In our experiments heparin had quantitatively different stimulative effects on the RNA synthesis under optimal conditions for polymerase II activity in nuclei isolated from leaves and callus. The effects also depended on the ionic strength of the incubation medium (fig.1). In leaf nuclei heparin stimulated the RNA synthesis by 300% at high, and by 1000% at low ionic strength. For callus nuclei these values were 40% and 275%. Tobacco pith nuclei showed results comparable to those for leaf nuclei.

The influence of heparin on nuclear transcription under optimal conditions for polymerase I activity was small. Because of the instability of tobacco polymerase I we used shorter incubation times (15 min instead of 1 h) and doubled the specific activity in the incubation medium.

From the data of fig.1 it appears that callus nuclei were in a highly active transcriptional state since at high ionic strength they could hardly be stimulated by heparin. On the other hand, RNA synthesis in leaf and pith nuclei could be stimulated strongly even under high-salt conditions. Therefore, these nuclei are more suitable for the quantitative study of transcription. Since we often got poor nuclear preparations from tobacco pith tissue, we continued our studies with nuclei from callus and leaves.

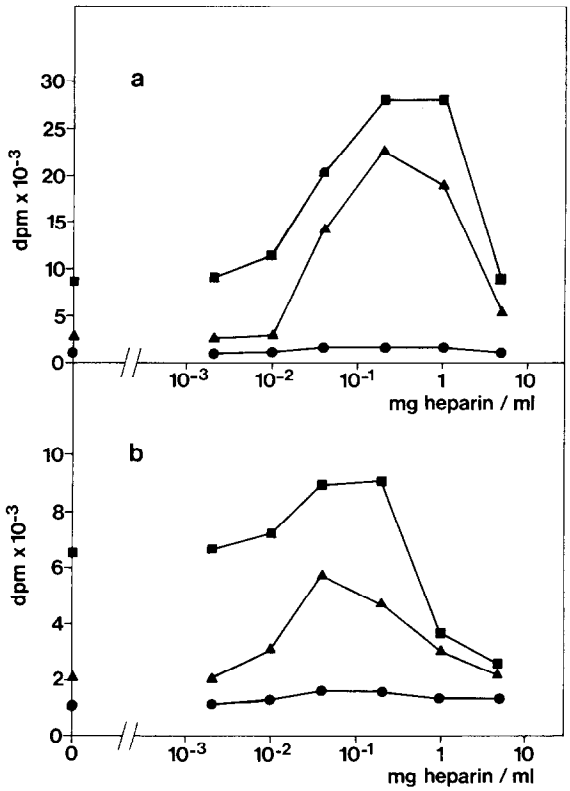

Fig.1. Influence of heparin on [^{3}H]UMP incorporation in (a) leaf and (b) callus nuclei. The incubation medium (0.25 ml) contained: 0.04 M Tris–HCl (pH 7.9), 0.1 mM EDTA, 0.1 mM dithiothreitol (freshly prepared), 0.125 mg bovine serum albumin, 0.14 mM ATP, GTP and CTP, 0.028 mM UTP, 10 pmol [^{3}H]UTP (50 μCi/nmol), and 20 μl nuclear suspension. Polymerase I activity was measured after 15 min incubation at 26°C with the extra addition of 1 μg/ml of α-amanitin, 0.5 mM $MnCl_2$ and doubling of the specific activity of the label (●——●). Polymerase II activity was measured after 1 h incubation at 26°C with the extra addition of 10 mM $MgCl_2$ and 175 mM $(NH_4)_2SO_4$ (optimal conditions (■——■)) or 50 mM $(NH_4)_2SO_4$ (low-salt conditions (▲——▲)).

The same conclusion arises from another set of experiments. Actinomycin D (10 μg/ml) effectively blocks the activity of endogenous nuclear template-bound RNA polymerases (engaged enzymes) [8]. Thus, the presence of RNA polymerase molecules that do not take part in RNA synthesis (free enzymes) can be shown by the extra addition of a template that lacks deoxyguanosine, like poly[d(AT)], which does not react with actinomycin D and is effective in directing RNA synthesis [8]. Table 1 shows the striking difference between leaf and callus nuclei. A high transcription activity on the synthetic template was only seen with leaf nuclei, whereas callus nuclei had a low free polymerase activity. The activity of free polymerase enzymes on poly[d(AT)] was strongly reduced by the addition of the initiation inhibitor heparin.

The relative amount of free RNA polymerases in isolated nuclei can also be estimated from the increase in UMP incorporation upon addition of exogenous DNA. Values obtained at the saturation level can be taken as a measure for the total amount of free RNA polymerases. As shown in fig.2, the highest saturation levels were reached with leaf nuclei as compared with callus nuclei. Apparently the amount of free RNA polymerases was highest in nuclear preparations from leaves.

The specificity of transcription on added DNA was shown by using Ti-plasmid. RNA isolated after addition of Ti-plasmid to callus nuclei was hybridized on blotted *Sma*I restriction enzyme fragments of Ti-plasmid [9]. The autoradiographs showed hybridization of a number of distinct fractions some of which differed from those found by using *E. coli* RNA polymerase [10]. Work on the differences in transcription of Ti-plasmid by *E. coli* and plant RNA polymerases is in progress (H. B., G. Ooms, in preparation).

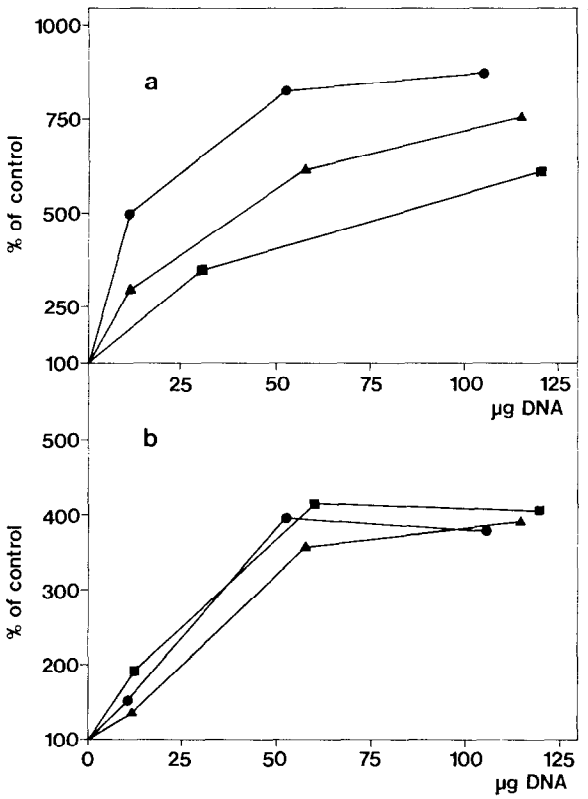

Fig.2. Influence of added DNA on RNA synthesis in (a) leaf and (b) callus nuclei. Incubation was for 1 h under low-salt conditions for polymerase II activity; (●——●) tobacco DNA; (▲——▲) Jerusalem artichoke DNA; (■——■) *Agrobacterium tumefaciens* DNA.

Table 1
Engaged and free RNA polymerase activities as measured in tobacco leaf and callus nuclei after 1 h incubation under low-salt conditions for polymerase II activity

Incubation of	Incorporation of [^{3}H]UMP (dpm) into	
	Leaf nuclei	Callus nuclei
1. nuclei only	2360	4420
2. as 1 + actinomycin D 10 μg/ml	660	570
3. as 2 + poly[d(AT)] 200 μg/ml	3810	1420
4. as 3 + heparin 100 μg/ml	800	1015

For explanation see text

Similar results as described above were obtained with nuclei isolated from tubers and tuber explant cultures of Jerusalem artichoke (*Helianthus tuberosus*). The nuclei from tubers, which were less active in the incorporation of [^{3}H]UMP/μg DNA and showed a strong stimulation with heparin as well as an enhanced RNA synthesis upon addition of the same DNAs as mentioned in fig.2, resembled tobacco leaf nuclei. Nuclei from tuber explants grown for some weeks behaved more like callus nuclei.

In conclusion, tobacco pith callus nuclei and, in particular, tobacco leaf nuclei are appropriate in vitro transcription systems to study the effects of various factors on RNA synthesis.

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