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Linear forms of plasmid DNA are superior to supercoiled structures as active templates for gene expression in plant protoplasts

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Abstract

Introduction of the plasmids pUC8CaMVCAT and pNOSCAT into plant protoplasts is known to result in transient expression of the chloramphenicol acetyl transferase (CAT) gene. Also, transfection with the plasmid pDO432 results in transient appearance of the luciferase enzyme. In the present work we have used these systems to study the effect of DNA topology on the expression of the above recombinant genes. Linear forms of the above plasmids exhibited much higher activity in supporting gene expression than their corresponding supercoiled structures. CAT activity in protoplasts transfected with the linear forms of pUC8CaMVCAT and pNOSCAT was up to ten-fold higher than that observed in protoplasts transfected by the supercoiled template of these plasmids. This effect was observed in protoplasts derived from two different lines of *Petunia hybrida* and from a *Nicotiana tabacum* cell line. Transfection with the relaxed form of pUC8CaMVCAT resulted in very low expression of the CAT gene.

Northern blot analysis revealed that the amount of poly(A)$^+$ RNA extracted from protoplasts transformed with the linear forms of the DNA was about 10-fold higher than that found in protoplasts transformed with supercoiled DNA.

Southern blot analysis revealed that about the same amounts of supercoiled and linear DNA molecules were present in nuclei of transfected protoplasts. No significant quantitative differences have been observed between the degradation rates of the various DNA templates used.

Introduction

The topological state of DNA may play a crucial role in regulating gene expression. In prokaryotic cells it has been clearly demonstrated that processes such as genetic recombination, transcription and DNA replication are greatly influenced by the extent of DNA supercoiling [24, 26]. From recent experiments [11, 27] it appears that DNA topology is an important parameter in controlling gene expression in animal cells. Thus, transferring of supercoiled DNA containing the gene for chloramphenicol acetyltransferase (CAT) into cultured cells resulted in much higher levels of CAT expression than that of its corresponding linear form [27]. Similarly, injection of supercoiled DNA templates into frog oocytes led to a high level of gene expression [11]. On the other hand, when the linear forms of the same DNA molecules were injected, very little or no transcripts were observed indicating that these linear

forms are poor substrates for the transcription machinery [11].

These results strongly suggest that the role of the supercoiling is very similar to that of enhancer sequences, namely to increase the efficiency with which the DNA templates are used. It has been suggested [27] that the supercoiling enhance the ability of a transcription factor to activate a given promoter following binding of such factor to the DNA molecule.

Needle microinjection of SV40-DNA molecules into cultured cells was also used to study the correlation between the topological forms of the DNA molecule and its expression [9]. Experiments with this system indicated that the linear form of the SV40-DNA is less active than the supercoiled form when both were microinjected into cell cytoplasm. However, when the linear and the supercoiled templates were microinjected into cell nuclei they were equally expressed [9]. Southern blot analysis revealed that the linear form of the SV40-DNA seems to be digested in the cell cytoplasm much faster than its corresponding supercoiled template. These results may, in part, explain the differences observed between the biological activity of these DNA structures. From all these experiments it appears that in animal cells, supercoiled DNA molecules are much more active than their counterpart linear forms.

Different results were obtained when the effect of DNA topology on gene expression was studied, following stable transformation of animal cells [5]. In these systems linear forms were found to be more active than supercoiled structure in supporting gene expression. This was attributed mainly to the ability of linear forms to integrate better than supercoiled structures into the chromosomal DNA [5]. Recently [21] it was also reported that stable transformation of plant cells with linearized plasmids was two- to ten-fold more efficient as compared to the efficiency obtained with circular structures probably due to integration into the recipient cell DNA [21].

However, as opposed to the large body of information accumulated during the past few years regarding the effect of DNA topology on transient gene expression in animal cells, practically nothing is known about it in plant cells. Evidently transient gene expression is the best tool to study, in a direct way, the effect of DNA topology on gene expression without the involvement of processes such as DNA recombination.

In plant cells, plasmids containing the bacterial CAT and the firefly luciferase genes were used to study transient gene expression [7, 16]. In the present work, these plasmids have been used to study the effect of DNA topology on the expression of these genes in petunia and tobacco protoplasts. Our results clearly show that, as opposed to observations in transfected animal cells, transient gene expression in plant protoplasts is better supported by linear forms of the above plasmids than by supercoiled structures.

Materials and methods

Preparation of plasmid DNA templates

The plasmids pUC8CaMVCAT and pNOSCAT were a generous gift from Dr V. Walbot (Department of Biological Sciences, Stanford University).

Both plasmids contain the coding region of the bacterial chloramphenicol acetyltransferase (CAT) gene and the polyadenylation site of the nopaline synthase (NOS) gene at the 3′ end. The CAT sequences are linked at its 5′ end either to the cauliflower mosaic virus (CaMV) 35S promoter (pUC8CaMVCAT) (M. Fromm, personal communication, and [18]) or to the NOS promoter (pNOSCAT) [7]. The plasmid pDO432 was a generous gift from Dr S. H. Howell (University of California, San Diego). This plasmid contains the firefly luciferase coding region linked to the CaMV 35S promoter at the 5′ end and to the NOS polyadenylation region at the 3′ end [16].

The plasmids were propagated and isolated as described before [14]. The isolated DNA was purified twice by CsCl gradient centrifugation. About 80% of the DNA thus isolated appeared to be in the supercoiled form (see Results).

The relaxed form of pUC8CaMVCAT was obtained by incubating this plasmid with the *Crithidia* nicking enzyme as described before [22]. The nicking enzyme was kindly provided by Dr J. Shlomai (The Hebrew University of Jerusalem, Hadassah

Medical School). This treatment converts fully supercoiled DNA templates to their relaxed forms by creating one nick per molecule [22]. Fully relaxed forms were purified by electro-elution after electrophoresis on 0.8% agarose gel.

Linear forms were obtained by digesting the plasmids containing the bacterial CAT gene with the restriction enzyme *Cla* I (New England Biolabs). The pUC8CaMVCAT and pNOSCAT possess a single restriction site located either immediately downstream to the NOS polyadenylation region or upstream to the NOS promoter respectively. The plasmid pDO432 was linearized with *Nde* I (New England Biolabs).

All the various topological forms of plasmid DNA were purified by phenol:chloroform and chloroform.

Isolation of plant protoplasts

Petunia hybrida protoplasts (lines 3704 and 10619-14F) were isolated from exponentially growing cells by incubating the cells (25% w/v) in cpw solution [6] (10 mM $CaCl_2$, 0.2 mM KH_2PO_4, 2 mM $MgSO_4$, 1 mM KNO_3 supplemented with 10% mannitol) containing 2% cellulase R-10, 0.3% macerozyme R-10 and 0.01% pectolyase Y-23 for 15 h at 26 °C [23]. The protoplasts obtained were separated from large debris by filtration through 125 μm mesh nylon screen, and washed three times in MS "150" medium [23].

Nicotiana tabacum L. cv. Wisconsin-38 cells were harvested and incubated in cpw (supplemented with 13% mannitol) containing 1.5% cellulase, 0.1% macerozyme and 0.01% pectolyase for 3 h at 37 °C [20]. The protoplasts were then centrifuged through a cushion of 23% sucrose (600 × *g* for 10 min). The top layer was collected and washed three times with 13% mannitol.

Cellulase R-10 and macerozyme R-10 were purchased from Yakult Pharmaceutical Industry Co., Ltd. (Nishinomiya, Japan). Pectolyase Y-23 was obtained from Seishin Pharmaceutical Co., Ltd. (Chiba, Japan).

Transformation of protoplasts with plasmid DNA molecules

Transfer of the various plasmid DNA molecules into plant protoplasts was performed essentially as described by Krens *et al.* [12] and in our previous work [1]. Briefly, samples of 4×10^6 protoplasts were pelleted and then resuspended in 1 ml of solution T (30 mM $CaCl_2$, 13% mannitol w/v). Unless otherwise specified, 25 μg of plasmid DNA, 85 μg of calf thymus DNA (carrier DNA) each in a volume of 25 μl and 0.4 ml of polyethylene glycol (PEG) 1500 (40% in solution T) were added to protoplasts sequentially. After mixing, the resulting suspension was incubated for 30 min at 26 °C with gentle shaking. After dilution with 30 ml of cold mannitol (13%), and 30 min incubation in the ice, the protoplasts were collected by centrifugation (300 × *g* for 5 min). The pellet obtained was then resuspended in 9 ml of modified MS "150" (MS "150" medium in which 0.5 M mannitol is substituted by 0.3 M glucose) and the protoplasts were cultured for 20 h at 26 °C.

Unless otherwise stated, transformation was performed with *P. hybrida* line 3704.

Determination of CAT activity

After transformation the cultured protoplasts were pelleted and resuspended to give a concentration of 50% (v/v) in a solution containing 0.25 M Tris-HCl, pH 7.8, 1 mM EDTA, 0.5 mM PMSF. The suspension obtained was sonicated and following centrifugation (10000 × *g* for 5 min) the supernatant was heated at 60 °C for 10 min and assayed for CAT activity as previously described [8]. The assay mixture contained protoplast extract, 0.6 μCi [^{14}C] chloramphenicol (CM) (53 mCi/mmol), 0.5 mM acetyl coenzyme A in a final volume of 350 μl of 0.25 M Tris-HCl, pH 7.8. The reaction products were separated by thin-layer chromatography (TLC) and autoradiographed. The CAT activity is expressed as the counts present in the spots of the acetylated forms of CM as a percentage of the counts present in the spots of non-acetylated plus the acetylated forms of CM [1].

Measurement of the firefly luciferase activity

The protoplasts were harvested and assayed for luciferase activity essentially as described before [4]. Briefly, 20 h after transformation, the protoplasts were pelleted by centrifugation and resuspended to give 50% (v/v) in a buffer containing 0.2 M sodium phosphate pH 7.5 and 2 mM dithiothreitol (DTT). The protoplasts were lysed by three times freezing in liquid nitrogen and thawing at 37 °C and then centrifuged (10000 × *g*) for 5 min at 4 °C. Aliquots of the extract were assayed for luciferase activity exactly as described before [4].

The light produced (light units, LU) during the first 10 s of the enzymatic reaction was measured by an LKB Luminometer.

A standard titration curve of the luciferase activity was obtained by using a commercial preparation of the firefly luciferase (Sigma). (The enzyme was diluted in 0.1 M sodium phosphate pH 7.5 containing 5 mM DTT and 0.2% bovine serum albumin.)

Isolation of poly(A)$^+$ RNA and Northern blot analysis

Total RNA was extracted from 2 × 10^7 transformed protoplasts, using the guanidinium thiocyanate and CsCl gradient centrifugation method [2]. Poly(A)$^+$ RNA was isolated by oligo(dT) cellulose chromatography as described before [3]. For Northern blot analysis, 1–2 μg of poly(A)$^+$ RNA were electrophoresed on a 1.2% agarose gel containing 6% formaldehyde. The gel was blotted onto nitrocellulose and hybridized with ^{32}P-labeled DNA fragment containing the CAT or the luciferase gene (see legends to figures).

Extraction of DNA from isolated nuclei and Southern blot analysis

Nuclei were isolated from 1.2 × 10^7 transformed protoplasts as described before [17]. DNA was extracted by incubating the nuclei in a buffer (20 mM Tris-HCl pH 7.4, 10 mM NaCl, 25 mM EDTA) containing 1% SDS and 0.5 mg/ml proteinase K, for 2 h at 37 °C. At the end of the incubation period 5 M NaCl (1/10 volume of the DNA solution) was added and the DNA was gently extracted by phenol:chloroform and chloroform.

The purified DNA was electrophoresed on 0.9% agarose gel, and after blotting to nitrocellulose filter it was probed with ^{32}P nick-translated plasmid as described [14].

Results

Linearization of supercoiled plasmids containing the CAT or the firefly luciferase genes enhances the expression of these genes in plant protoplasts

The results of Fig. 1 and Table 1 show that linear forms of pUC8CaMVCAT was much more active in supporting gene expression in pentunia protoplasts than the corresponding supercoiled or relaxed struc-

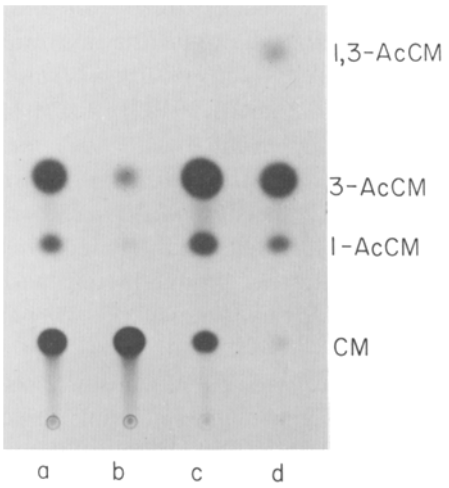

Fig. 1. Transformation of petunia protoplasts with the various topological structures of pUC8CaMVCAT: effect on CAT activity.

Transformation of petunia protoplasts was performed with 25 μg of the following forms of the plasmid pUC8CaMVCAT: (a) supercoiled, (b) relaxed, (c) linear. The linear and the relaxed forms were obtained by digestion with the restriction endonuclease *Cla* I and *Crithidia* nicking enzyme respectively as described in Materials and methods. The protoplasts were lysed 20 h after transfection and assayed for CAT activity as described in Materials and methods. (d) CAT activity of *Anabaena* cell free extract carrying the plasmid pRL6 which possesses the CAT gene [28]. CM, chloramphenicol; AcCM, acetylated CM.

Table 1. Linear forms of plasmid DNA molecules are better expressed than the supercoiled counterparts in protoplasts of *Petunia hybrida* and *Nicotiana tabacum* cell lines.

Plasmid template	Acetylated CM (%)		
	P. hybrida 3704	*P. hybrida* 10619-14F	*N. tabacum* 'Wis 38'
Exp. I.			
pUC8CaMVCAT supercoiled	20.0	11.5	14.3
pUC8CaMVCAT linear (*Cla* I)	79.0	33.6	65.0
pNOSCAT supercoiled	9.7	-	-
pNOSCAT linear (*Cla* I)	70.0	-	-
Exp. II			
	Light units		
None	0		
pDO432 supercoiled	1.3×10^5		
pDO432 linear	8.7×10^5		

Protoplasts were transformed as described in Materials and methods except that the number of tobacco protoplasts was 1.5×10^6/system and these protoplasts were cultured in 9 ml of MS "150". Percentages of the AcCM and LU were measured as described in Materials and methods.

tures. This was inferred from quantitative determination of the CAT activity following transformation of protoplasts with the various topological forms of the plasmid. Interestingly, the poorest CAT activity was observed in protoplasts transformed with the relaxed form of the plasmid (Fig. 1).

Similar results were obtained upon transformation of protoplasts prepared from either two lines of *Petunia hybrida* or from tobacco protoplasts (Table 1). These results suggest that the high expression obtained by the linear form was not limited to one kind of plant protoplasts. Furthermore, our results (Table 1) show that a second plasmid, pNOSCAT, exhibited the same characteristics. Our experiments (Table 1, Exp. II) also demonstrated that the linear form of the plasmid pDO432 was much more active in supporting gene expression than its corresponding supercoiled structure. Transformation of petunia protoplasts with this plasmid resulted in synthesis of the firefly luciferase enzyme. These observations may support the view that the phenomenon observed might be of general implication and is not restricted to a certain kind of promoter, gene or protoplast.

The activity of the linear form was not dependent on the site of linearization, as long as the recombinant gene is intact. Protoplasts transformed with pUC8CaMVCAT which was cleaved either by *Cla* I (which cuts once in the vector sequence) or by *Hind* III (which cuts twice, at both ends of the recombinant CAT gene) resulted in much higher CAT activity than that observed following introduction of the supercoiled form of the plasmid (Table 2). On the other hand, transformation with pUC8CaMVCAT which was cleaved by *Bam* HI (which cuts between the 5′ end of the CAT gene and the CaMV 35S promoter) resulted in only negligible CAT activity. Based on these results, it should be inferred that the linear form of the plasmid is not sub-

Table 2. Transformation of petunia protoplasts with pUC8CaMVCAT DNA linearized with different restriction enzymes: Effect on CAT activity.

pUC8CaMVCAT cleaved with	Acetylated CM (%)
None	20.4
Cla I	81.0
Hind III	62.0
Bam HI	4.3

pUC8CaMVCAT DNA was cleaved with *Cla* I, *Hind* III or *Bam* HI as described in Material and methods. The restriction products obtained were used to transform petunia protoplasts. CAT activity and the percentage of actylated chloramphenicol was estimated, as described.

jected to a process of intracellular ligation and evidently the CaMV 35S promoter is required for expression of the CAT gene.

Transformation of petunia protoplasts with the supercoiled and linear forms of the pUC8CaMVCAT and PDO432 plasmids: Kinetic studies

Kinetic studies (Fig. 2A) revealed, as expected, that the CAT activity observed in protoplasts transformed with either forms of the plasmids was transient, leading to the appearance of maximum CAT activity within 15–30 h after transformation. These studies (Fig. 2A) also showed that (a) at early periods of incubation (5–20 h) the activity observed in protoplasts transformed with the linear form of the plasmid was up to ten-fold higher than that observed with the supercoiled form; (b) a certain degree of CAT activity could be observed as early as 2 h after transformation (2% of acetylated chloramphenicol) with the linear form of the plasmid; at this time period no activity could be detected in protoplasts transformed by the supercoiled form (Fig. 2A); (c) relatively high CAT activity could still be detected even at 60 h after transformation with the linear form. Essentially the same results were obtained after transfection of petunia protoplasts with pDO432 plasmid (Fig. 2B).

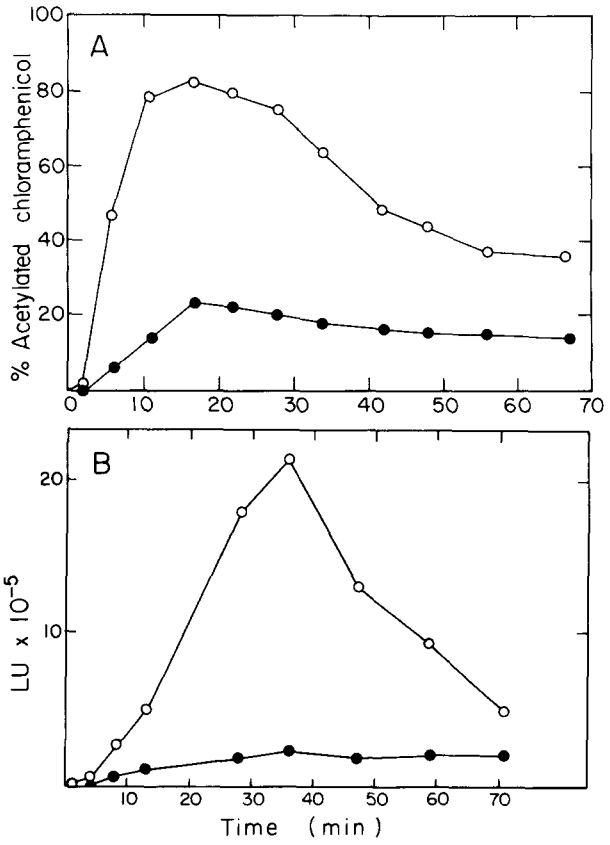

Fig. 2. Transformation of petunia protoplasts with supercoiled and linear plasmids: kinetic studies.

Samples of 4 × 10^6 protoplasts were transformed with 25 μg of the supercoiled (●) or the linear (○) form of the plasmids pUC8CaMVCAT (A) or pDO432 (B) as described in Materials and methods. The protoplasts were cultured at 26 °C and at the indicated times were pelleted and assayed for CAT (A) or luciferase (B) activity.

Transformation with the linear form of pUC8CaMVCAT resulted in high levels of CAT activity at wide range of DNA concentrations (10–100 μg DNA/system) (data not shown).

Effect of DNA topology on the transient appearance of specific transcripts

Our Northern analysis shows that specific transcripts were detected in protoplasts transformed with the plasmids pUC8CaMVCAT or pDO432 of 1.6 kb and 2.3 kb respectively (Figs. 3 and 4). This analysis clearly shows that the appearance of the poly(A)$^+$ RNA molecules was transient and that its level in protoplasts transformed with the linear form was significantly higher than that observed after transformation with the respective supercoiled structure (Figs. 3 and 4). The highest amount of transcripts was detected 3–4 hours after transformation (Fig. 4).

From the results obtained it is clear that with both plasmids and at the various time periods studied, the amount of intact transcripts found in protoplasts transformed by linear forms was about 10-fold higher than that detected in protoplasts transformed by the supercoiled structures.

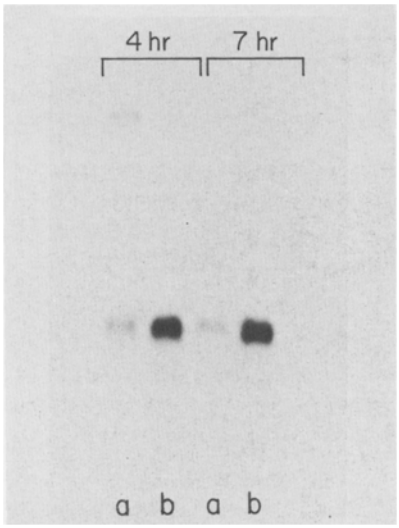

Fig. 3. Northern blot analysis of the CAT poly(A)$^+$ RNA in transformed plant protoplasts.

Transformation of *Petunia hybrida* protoplasts was performed with 40 μg of the plasmid pUC8CaMVCAT in a supercoiled (a) or linear (b) form. At the indicated times, RNA was isolated from 2×10^7 protoplast as described in Materials and methods. Poly(A)$^+$ RNA (2 μg) was electrophoresed on 1.2% agarose gel blotted onto nitrocellulose filter and hybridized to ^{32}P-labeled *Hind* III fragment of pUC8CaMVCAT containing the CAT gene.

Ribosomal RNA (28S and 18S) stained with ethidium bromide was used as a size marker.

The intracellular fate of the various topological forms of the plasmid pUC8CaMVCAT

Southern blot analysis of DNA extracted from nuclei of protoplasts transformed with the supercoiled template revealed the presence of all the three topological forms of the pUC8CaMVCAT plasmid, namely the supercoiled, relaxed and linear structures (Fig. 5Aa). On the other hand, DNA extracted from nuclei of cells transformed with the linear template did not contain any other topological forms beside the linear structure (Fig. 5Ab). The various topological forms of the plasmid (supercoiled, relaxed, linear) gradually disappeared from nuclei of the transformed cells due probably to degradation processes (Fig. 5B). Quantitative analysis revealed that the "disappearance rate" of the different topological forms appears to be similar (Fig. 5Ba).

It is also evident from the results in Fig. 5B that about the same amount of radioactive probe was found to be associated with DNA molecules extracted from nuclei of protoplasts transformed with either the linear or the supercoiled forms of the plasmid. (Compare the total amount of radioactive material associated with the DNA in Fig. 5Ba to that

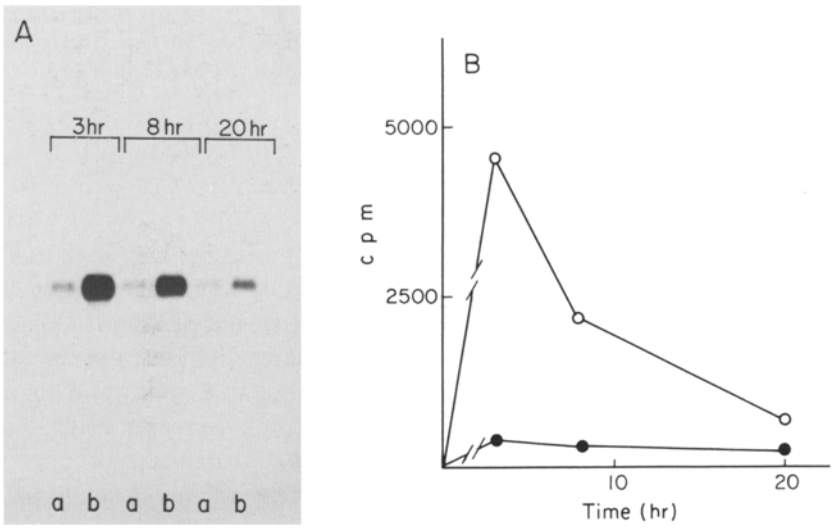

Fig. 4. Northern blot analysis of the luciferase poly(A)$^+$ RNA in transformed plant protoplasts.

Transformation of petunia protoplasts was performed with 40 μg of supercoiled (a) or linear (b) pDO432. All other experimental conditions were as described in the legend to Fig. 3 and in Materials and methods. Hybridization was performed with ^{32}P-labeled *Bam* HI fragment of pDO432 containing the luciferase gene.

A. An autoradiogram of the Northern blot.

B. The bands seen in A were cut out from the nitrocellulose filter and the radioactivity was counted. ●, supercoiled; ○, linear pDO432.

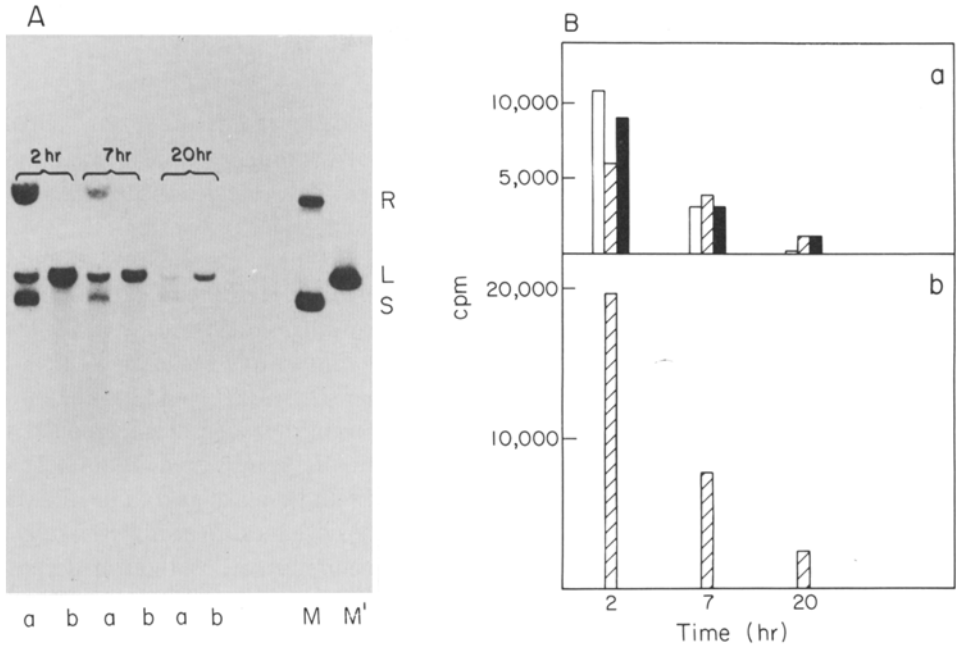

Fig. 5. The intracellular fate of the plasmid pUC8CaMVCAT: Southern blot analysis of DNA extracted from nuclei of protoplasts transformed with supercoiled or linear DNA.

Samples of 4×10^6 petunia protoplasts were transformed with 30 μg of supercoiled (Aa) or linear (Ab) pUC8CaMVCAT DNA. The protoplasts were cultured at 26 °C and at the indicated time (A), nuclei were isolated from 1.2×10^7 protoplasts. DNA was extracted from the nuclei and samples of 20 μg were electrophoresed on 0.9% agarose gel, blotted to nitrocellulose and hybridized with ^{32}P-labeled pUC8CaMVCAT DNA, as described in Materials and methods.

A. An autoradiogram as described above. S, supercoiled; L, linear; R, relaxed forms of DNA. M and M′ show the supercoiled and the linear forms respectively of the plasmid preparations used in the transformation experiments. Note that the supercoiled DNA preparation contains a certain amount of the relaxed form.

B. The bands seen in A were cut out from the nitrocellulose and the radioactivity was counted. (a) and (b) refer to transformation with supercoiled and linear forms respectively. Black bars: supercoiled; hatched bars: linear; white bars: relaxed.

in Fig. 5Bb.) From these results it should be inferred that no quantitative differences could be detected in the level of plasmid DNA present in nuclei of cells transformed with either linear or supercoiled DNA.

Analysis of DNA molecules extracted from nuclei of cells which were transformed by the relaxed form of the plasmid (Figure 6) showed also the appearance of the linear form. It appears, therefore, that similar to the supercoiled template also the relaxed form is converted intracellularly to its linear form. Furthermore, quantitative analysis (Fig. 6B) clearly showed that the degradation rate of the relaxed form was similar or very close to that of the linear structure.

Discussion

Three approaches have been used to study the relationship between DNA topology and its ability to support gene expression.

(A) The extent of DNA supercoiling in intact living cells was altered mainly by manipulating the activity of DNA topoisomerases [10]. This was achieved by the use of inhibitors of these enzymes such as novobiocin which was found to strongly influence transcription and expression of various genes in eucaryotic cells [10].

(B) *In vitro* transcription systems have also been used to study the dependency of the transcription process on DNA typology showing specifically requirement for supercoiled structures. Recent experi-

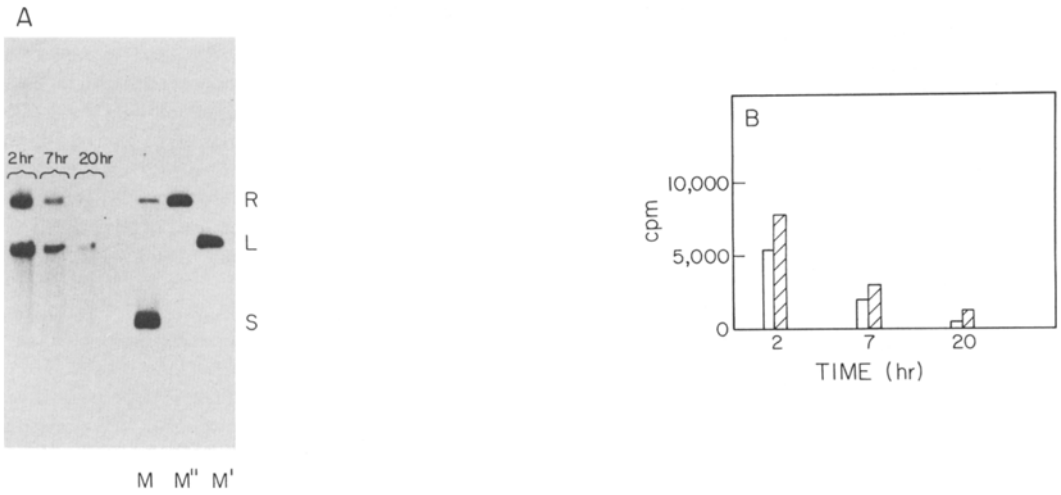

Fig. 6. The intracellular fate of the relaxed form of pUC8CaMVCAT DNA: Southern blot analysis of DNA extracted from nuclei of transformed protoplasts.

Transformation of petunia protoplasts was carried out with 30 μg of the relaxed form of the plasmid pUC8CaMVCAT. At the indicated time periods (A), nuclei were isolated and the DNA was extracted and analysed as described in Materials and methods and in the legend to Fig. 4.

A. An autoradiogram. M, M′ and M′′ show the supercoiled, linear and the relaxed templates respectively used in the transformation systems.

B. A histogram of the radioactive counts present in each band, determined as described in the legend to Fig. 4. S, R and L are supercoiled, relaxed and linear forms of the plasmid respectively. Hatched bars: linear; white bars: relaxed.

ments with chloroplast transcription system have demonstrated the requirement for supercoiled template for expression of chloroplast genes [13, 25]. (C) Gene transfer into cultured cells have served as an excellent experimental tool to study the correlation between DNA topology and gene expression. Transfection experiments in animal cells clearly demonstrated the requirement for supercoiled template for efficient gene expression [27].

Gene transfer systems allow the analysis of different recombinant DNA molecules containing various combinations of structural genes and transcription regulatory sequences in different cell species. In such systems, transient gene expression results from the expression of non-chromosomal, free DNA sequences [19] thereby eliminating the effect of processes such as DNA recombination on gene expression.

The results of the present work show that introduction of the linear form of the plasmids pUC8CaMVCAT and pNOSCAT into petunia and tobacco protoplasts resulted in much higher CAT enzymatic activity than introduction of the corresponding supercoiled structure. Essentially the same results were obtained following transformation of petunia protoplasts with the plasmid pDO432 bearing the firefly luciferase gene.

Kinetic studies as well as experiments using various DNA concentrations clearly revealed that the linear forms of the plasmids were up to ten-fold more active than the respective supercoiled forms. The possibility that linear forms are better introduced than supercoiled structures into plant protoplasts seems unlikely from Southern blot analysis. About the same amounts of DNA molecules were observed in nuclei of protoplasts transformed with either forms of the plasmids.

Quantitative determination of specific transcripts of the CAT and the luciferase genes clearly demonstrated that the high expression observed with the linear forms indeed takes place at the transcription level. This may suggest that in certain cases RNA polymerases of plant cells can interact more efficiently with linear structure than with supercoiled

form of DNA. Another possibility is that the linearization increases the efficiency of RNA termination processes and this enhances the transcription efficiency.

The possibility that the expression observed with the supercoiled and the relaxed forms is due to an activity of linear molecule formed intracellularly from the above structures, cannot be excluded. Evidently our results suggest that the relaxed forms are the poorest substrate of the RNA polymerase or alternatively that intracellularly the relaxed forms are converted into non-functional, inactive linear forms.

Our results contradict those obtained in animal cells in which it was well established that the linear form of specific DNA templates are either inactive or much less effective than their corresponding supercoiled structure [9, 11, 27].

It is noteworthy that preliminary results in our laboratory clearly showed that microinjection of the plasmids pUC8CaMVCAT and pNOSCAT into frog oocyte cytoplasm resulted in the appearance of the CAT enzyme. As opposed to our observations in plant protoplasts and similar to the results obtained in animal cells, the linear forms of the above plasmids were much less active in supporting gene expression following its microinjection into oocytes than their corresponding supercoiled structure. These results clearly show that the biological activity of certain DNA structures is dictated mainly by the host cell and not by the DNA molecule itself.

Acknowledgements

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