

to unbound DNA fragments is of importance for comparison and internal control. In order to save the flow-through we designed a simple attachment for a commercially available filtration apparatus which allowed the recovery of DNA fragments which passed the filter. DNA from phage λ was given to us by Dr H. Bujard (University of Heidelberg). Chloroplast DNA from spinach, and also spinach chloroplast DNA fragments in pBR 322 (from Dr M. Hartley, Coventry) were isolated according to standard procedures⁷. *E. coli* RNA polymerase (holoenzyme) and restriction endonucleases were purchased from Boehringer, Mannheim. Restriction endonuclease digestion and electrophoresis were performed as described⁷. The filtration apparatus as outlined was built by a local workshop, a first version was made without difficulty in the laboratory workshop. The device was designed to replace the top plate of a 10-fold filtration apparatus (DNA-10, New Brunswick) and could hold a total of 20 cellulose nitrate filters.

The assays contained between 0.04 and 0.1 pmole of DNA. The amount of DNA was measured in principle as described by Reis⁸ using the densitometric comparison of the DNA to be estimated with a standard dilution. The DNA was digested in 5–10 μ l total volume with various restriction endonucleases and after dilution 0.2–4 pmole RNA polymerase holoenzyme was added. After 10 min at 37°C poly rI (20–150 μ g/ml final concentration) or heparin (100–500 μ g/ml final concentration) in prewarmed buffer solution was added and the incubation was continued for another 10 min.

The assay solution was pipetted into the cavity of the filtration device (fig. 1, A). It was then filtered slowly through the cellulose nitrate filter (fig. 1, C) which was held in place by a rubber plate (fig. 1, B). The flow-through, which was collected in the cavities of the lower perspex plate (fig. 1, E), could easily be obtained since the top plate (fig. 1, D) was removable. The whole apparatus was evacuated through the bottom part (fig. 1, G) of a commercially available filtration apparatus (New Brunswick, filtration apparatus DNA-10).

The DNA in the solution which passed the filter was precipitated in the presence of 0.2 M K-acetate and 0.01 M $MgCl_2$ by 2.5 vol. of ethanol at $-80^\circ C$ for 60 min. DNA retained by the filters was eluted at low temperatures by shaking the filters in $2 \times 200 \mu$ l 0.01 M Tris/HCl pH 8.0, 1 mM EDTA, 0.2% SDS⁹ and the DNA was precipitated. DNA fragments were electrophoretically separated in 0.5–1.8% agarose or 4–8% polyacrylamide gels^{7,9}.

The figure 2a gives an example. A cloned DNA fragment of spinach chloroplast DNA (pSoc S15) was digested using the restriction endonucleases EcoRI and Sal I and the DNA fragments were incubated with RNA polymerase at 37°C in a ratio of 1:12. In the 5 individual assays the KCl concentration was adjusted to 200 mM and the assays were then incubated at 15°C for 0–20 min to demonstrate the

varying stability of the DNA-RNA polymerase complexes at low temperature and high salt concentration. After filtration the DNA fragments were separated on an agarose gel. The fragments labelled p 2.41 and p 0.42 respectively, are derived from the cloning vector pBR 322 and show the molecular weight of these subfragments in 10^6 dalton. It is obvious that no strong RNA polymerase binding site comparable to the chloroplast DNA sites exists on pBR 322. The promoter for the plasmid DNA would be expected¹⁰ on the subfragment p 2.41 which is in fact slightly bound. This weak promoter becomes evident only at much higher polymerase/DNA ratios. The region of the binding site on the (1.28×10^6) dalton DNA fragment from the chloroplast DNA contains some very AT-rich segments which by their arrangement most probably account for the strong polymerase binding (to be published).

Similar experiments, using the phage λ , have shown that binding occurs to DNA fragments on which promoters have been located by others^{9,11,12}. The figure 2b shows the DNA fragments produced by a Bam HI/Eco RI double digestion. The strongly bound fragment '9.7' contains the λ promoters P_L , P_{rm} , P_R and P_O , the bound fragment '6.9–2' contains promoter P_R , and '7.5–2' contains the strongest promoters of the λ b_2 region^{9,11,12}.

The function of the several *E. coli* RNA polymerase binding sites on the chloroplast DNA insert pSoc S15 is not yet known. We were however able to show that the binding site on the (1.28×10^6) dalton Sal I/Eco RI subfragment (fig. 2) is located within 120 base pairs upstream of a 32,000 dalton protein gene on the chloroplast DNA (to be published elsewhere). The filtration device described permitted easy and highly reproducible monitoring of such DNA-protein interactions.

- 1 The technical assistance of Christine Michalowski is gratefully acknowledged. Supported by the Deutsche Forschungsgemeinschaft.
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PRAEMIA

Prize Biochemical Analysis 1982

The German Society for Clinical Chemistry awards the prize Biochemical Analysis every two years at the conference 'Biochemische Analytik' in Munich. The prize of DM 10,000.– is donated by Boehringer Mannheim GmbH for outstanding and novel work in the field of biochemical analysis or biochemical instrumentation or for significant contributions to the advancement in experimental biology especially relating to clinical biochemistry.

Competitors for the prize 1982 (conference 27–30 April 1982) should submit papers concerning one theme, either published or accepted for publication between 1 October 1979 and 30 September 1981, in triplicate before 15 November 1981 to: Prof. Dr I. Trautschold, Secretary of the prize Biochemical Analysis, Medizinische Hochschule Hannover, Karl-Wiechert-Allee 9, D-3000 Hannover 61, Federal Republic of Germany.

PRAEMIA

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CONGRESSUS

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Guildford, Surrey, 7–11 September 1981

This international symposium on nephrotoxicity, organized by the Robens Institute of Industrial and Environmental Health and Safety and Department of Biochemistry, will provide a multi-disciplinary forum to discuss: – the assessment of renal function by in vivo and in vitro techniques, – the pathogenesis of a wide range of chemically-induced nephropathies (including heavy metals, therapeutic agents, natural toxins, environmental and industrial chemicals), – the value of experimental models in relation to human toxicity, – the most important areas of future research.

Closing date for registration, 15 July 1981. Further information by the Secretary, Nephrotoxicity Symposium, Department of Biochemistry, University of Surrey, Guildford GU2 5XH, Surrey, England.

Great Britain**7th international symposium on 'Pteridines and folic acid derivatives'**

St. Andrews (Scotland), 21–24 September 1982

The symposium will be based at the Chemistry Department, University of St. Andrews. A series of consecutive scientific sessions covering the chemistry, biology, biochemistry, enzymology and clinical applications of folic acid derivatives and pteridines is planned. Information by: Prof. H.C.S. Wood, Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, Scotland.

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