THE DNA OF *ESCHERICHIA COLI* CAN BE DISPLAYED IN A SINGLE BAND IN POLYACRYLAMIDE GELS

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1. Introduction

A variety of electrophoretic techniques using polyacrylamide, agarose or composite gels, are currently being used for a plethora of studies concerning DNA. For instance a report appeared where composite polyacrylamide gel electrophoresis was used for the isolation of mouse satellite DNA [1]. Here we show that the total content of the *E. coli* cell can be layered on a polyacrylamide gel and all or most of the DNA can be concentrated in a single band after electrophoresis. This method is very useful for physiological studies, where a large number of samples have to be analysed and the DNA can be used as a yardstick by which other cellular components can be quantitatively compared. This technique might be also helpful in the analysis of various aspects of DNA metabolism.

2. Results and discussion

While we were engaged in studying macromolecules from ^{32}P-labeled *Escherichia coli* cells, we noticed that occasionally after electrophoresis in a gel consisting from 2% polyacrylamide and 0.5% agarose, a band appeared which migrated slower than 30 S RNA. After a number of trials we found that by reducing the level of acrylamide in the gel to 1.5% this slow migrating ^{32}P-labeled material, referred to here as the 40 S band, appeared with regularity (fig.1).

In order to assess the nature of this band, material from long-term, ^{32}P-labeled cells was incubated with RNAase and DNAase prior to electrophoresis. As can be seen in fig.1, when untreated material is analysed, three major bands appear in the main part of the gel while some material is retained in the origin. The second and third bands correspond to 23 S and 16 S rRNA, since they appear when labeled purified ribosomes are analysed in the same manner. When the material is treated with DNAase the origin and the slower moving band disappear while the rRNA remains (fig.1, lane 2). On the other hand when the material is treated with RNAase the 23 S and 16 S rRNA bands disappear while material in the origin and the first band are retained (lane 3). As expected, no material was retained in the origin or in any of the three bands, when cell extracts were treated with RNAase and DNAase prior to electrophoresis (lane 4). Thus, this experiment suggested that the slow migrating band consists of DNA. Very similar results were obtained with ^{32}P-labeled mouse L-cells (Gill and Apirion, unpublished observations). The same pattern of bands is observed whether the cells are treated as described in the legend to fig.1, or the total cells were opened in a buffer containing sodium dodecyl sulfate [2] and directly layered on the gel.

When various strains of *E. coli* were labeled for a long term with ^{32}Pi (1–4 doublings) and the gels were quantitated [2,3] the amount of ^{32}P in the DNA band was 15–20% of the amount of ^{32}P in the rRNA bands. Since the ratio of DNA to rRNA in the *E. coli* cell is about 1:6 [4] these measurements suggested that most of the cellular DNA is included in this band. To further ascertain this proposition, mutants defective in DNA synthesis at elevated temperatures were tested together with their parental strains. Mutants in

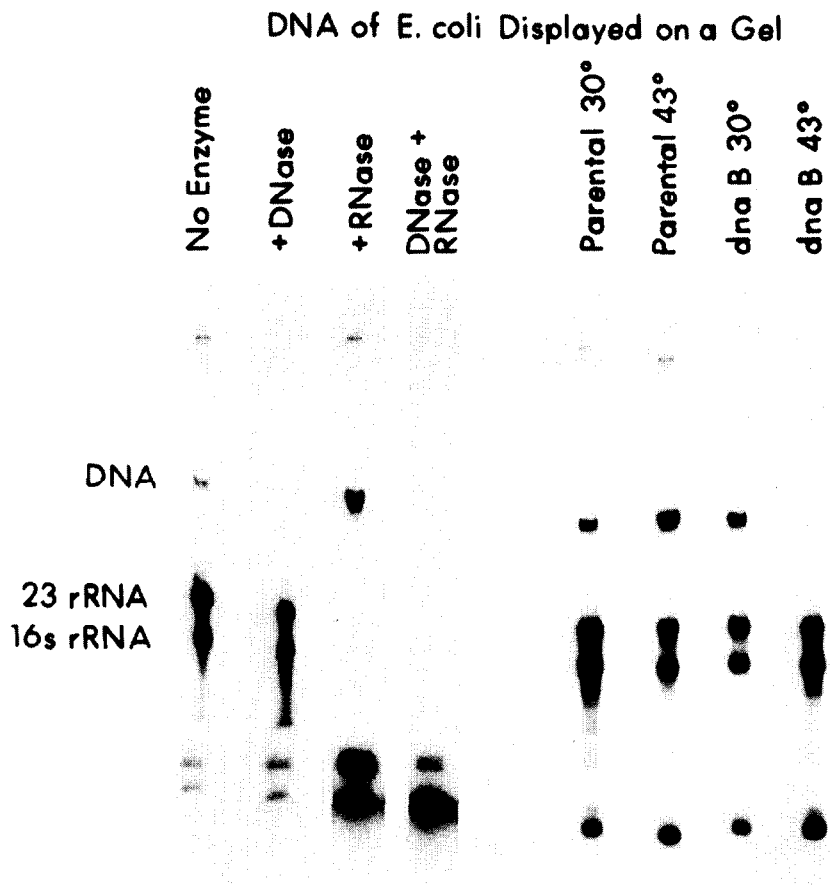

Fig.1. A 30 ml culture of the *E. coli* strain D10 (Met⁻RNAase I⁻) were grown in low phosphate Tris-base medium [2] and labeled with 3 μCi/ml $^{32}P_i$ for 2 h at 37°C with vigorous aeration. At A_{560} nm of about 0.5 cells were collected in a vol./vol. equivalence of killing buffer (80 μg/ml nalidixic acid, 80 μg/ml KCN, 80% ethanol and 0.2% diethylpyrocarbonate). The samples were kept on an ice-water bath until all samples were collected. Samples were spun for 10 minutes at about 6000 × *g* at 0–4°C. The supernatant was poured off and the pellets were resuspended in lysis-buffer at a desired volume (0.5% sodium dodecyl sulfate (SDS), 10 mM Tris–HCl, pH 7.0, 10 mM Na_2EDTA, 20% sucrose and 0.05% bromophenol blue. pH was adjusted to 7.0 prior to the addition of SDS). Samples were heated in lysis-buffer for 3–4 min in a boiling-water bath and then quenched on ice-water (for enzymatic analysis the cracking-buffer did not contain sucrose and bromophenol blue). To the opened cells NaCl was added to 0.4 M and the samples remain in the ice-bath for 15 min for protein-precipitation. Extracts were spun at 10 000 × *g* in the cold for 15 min. The supernatant was removed and 2.1 vol of 95% ethanol were added to it and it was stored overnight at −20°C, spun at 15 000 × *g* in the cold for 15 min. The pellet was resuspended in the dialysis buffer and dialysed (1:1000) against Tris–Mg buffer (Tris–HCl 0.01 M, pH 7.5, $MgCl_2$ 0.01 M). Three samples, each comprising one quarter of the whole cell lysate were treated with DNAase, RNAase, and both DNAase and RNAase. The enzymatic reactions contained 200 μg/ml RNAase (pancreatic) or DNAase 1 (both from Worthington) and incubated at 37°C for 90 min. The samples for lanes 5–8 were derived from the labeling of a *dna B* mutant and its parental strain (a non temperature-sensitive strain). They both were labeled in 2 ml cultures, in a low phosphate medium with 5 μCi/ml of $^{32}P_i$. The cells were labeled for 2 h at each temperature. For labeling at 43°C, cells were grown at 30°C transferred to 43°C and labeled one hour later for 2 h. The cells were lysed as described. Samples were analysed on a 1.5% polyacrylamide–0.5% agarose gel, containing 0.2% SDS, for 2.5 h at 80 V. The gel was dried and autoradiographed for 24 h [2,3]. The details for the gel are exactly as described by Gegenheimer and Apirion [2] for the 2% polyacrylamide–0.5% agarose gel, with the exception that 1.5 rather than 2% polyacrylamide was used. (Occasionally the bottom centimeter of the gel contained a retaining-layer consisting of 3.5% polyacrylamide–0.5% agarose.)

the *dna* loci *A, B, C, D, G* and *H* were analysed with similar results. One example with a *dna B* mutant strain is presented in fig.1. When the parental strain was labeled at 30°C or 43°C, the customary three bands were observed (fig.1, lanes 5 and 6). However when cells from the *dna B* strain were analysed, the DNA band as well as ^{32}P-labeled material in the origin appeared only at the permissive temperature while they were missing in cells labeled at the non-permissive temperature; the rRNA bands appeared as expected (fig.1, lanes 7 and 8). Thus, this experiment shows very clearly, in agreement with the previous experiments, that the origin and the first band contain most of the cellular DNA.

In order to ascertain that all or most of the cellular DNA appears either in the origin of the gel or in the 40 S band, cells were labeled with [^{14}C]thymidine and subjected to similar type of analysis. As can be seen in fig.2 material from cells labeled with [^{14}C] thymidine shows DNA in the origin and in the 40 S band (lanes 1 and 2) while in material treated with DNAase only nucleotides or oligonucleotides were observed (lane 3). After treatment with RNAase, material is retained in the origin and the 40 S band, while no nucleotides appeared (lane 4). Treatment with both RNAase and DNAase shows, as expected, similar results to treatment with DNAase by itself (lane 5). Thus this experiment substantiates the notion that most of the cellular DNA is either retained in the origin or is migrating in a single sharp band after electrophoresis. We observed that the DNA band seemed to migrate slightly further after treating ^{32}P-labeled material with RNAase. This can be rather easily observed with material from [^{14}C]thymidine-labeled cells, (compare lanes 1, 2 and 4, fig.2). This suggests that the DNA band contains RNA as well as DNA. This could be expected since Richardson [5] showed that RNA molecules can be attached by hydrogen bonds to superhelical DNA when the transcription complex is treated with denaturing agents such as sodium dodecyl sulfate.

In order to quantitate the level of RNA in the DNA band, cells were labeled (long-term, 3–4 doubling) with uracil and the material from the DNA band was eluted (by electrophoresis) and digested by perchloric acid to bases and chromatographed (fig.3). Twenty to 25% of the label was recovered as uracil. Since the absolute amount of uracil and thymine in the DNA

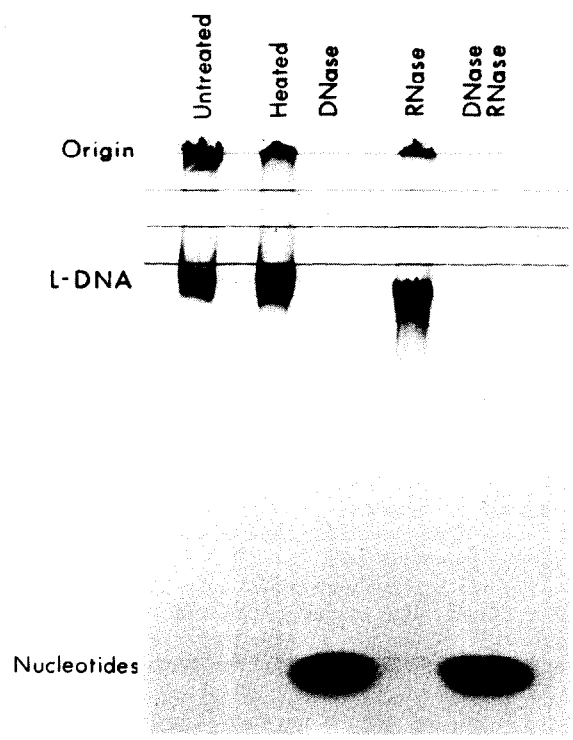

Fig.2. A 10 ml culture of strain D10 was labeled (4 μCi/ml of [^{14}C]thymidine) for 4 h at 37°C, with rapid agitation, and harvested at an $A_{560\,nm}$ of 0.75. Cells were grown and labeled in a minimal glucose (0.1%) medium containing 200 μg/ml 2-deoxyadenosine. The cells were lysed and processed as described in the legend to fig.1. Material was treated with DNAase, RNAase, DNAase and RNAase and incubated without addition of enzyme. The samples were analysed in a 1.5% polyacrylamide–0.5% agarose gel (see fig.1). The gel was dried and autoradiographed for 72 h. A photograph of the autoradiograph is presented.

band was determined by using double auxotraph strains of *E. coli* (*ura*$^-$*thy*$^-$) it was possible to assess that about 3% of the material in the 40 S band (after long-term labeling, two doublings) consists of RNA.

To find out whether or not the DNA retained in the origin is similar or different from the DNA migrating into the gel, DNA from the origin and DNA from the 40 S band were extracted and re-run. Both were redistributed like the original material between the origin and the band, indicating that there is no basic difference between DNA in the origin or in the band. When material is treated with 0.2 N NaOH (on ice) prior to the electrophoresis, virtually all the DNA

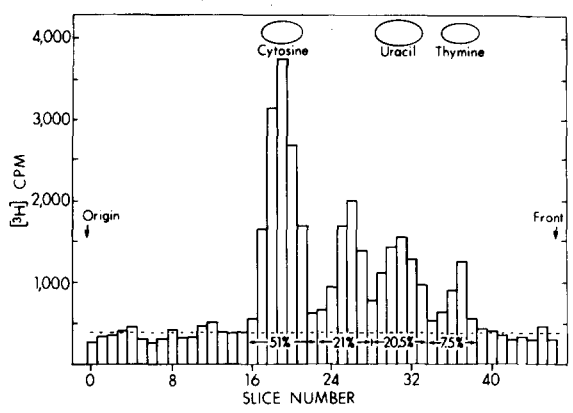

Fig.3. Ten milliliters of strain D10 were grown in minimal medium at 37°C containing 5 μCi/ml [5,6-^{3}H]uracil. The cells were lysed as described in the legend to fig.1 the semipurified material was layered on a 1.5% polyacrylamide–0.5% agarose gel and electrophoresis carried out at 90 V for 2.5 h. The origin and the 40 S band were excised and placed in a gel-elution chamber. Slices were electrophoresed for 2 h at 200 V. Material collected was dialyzed against water overnight, lyophilized and resuspended in 30 μl 70% $HClO_4$ and incubated at 100°C for 1 h. Water, 120 μl, was added and the mixture was ground with a glass rod and centrifuged at 10 000 × *g* for 10 min. About 50 μl of the supernatant were spotted on Whatman No. 3 chromatographic paper and analysed in the following buffer system: 65 ml peroxide-free isopropanol, 16.7 ml 12 N HCl and 18.3 ml water. Descending chromatography was carried out for 36 h. Cytosine, uracil and thymine markers were present. Lanes containing radioactivity were cut into 5 mm sections, incubated overnight at 60°C in 0.5 ml 30% hydrogen peroxide and subsequently counted in 5 ml Instagel scintillation counting fluid. The peak between cytosine and uracil has an R_F value which corresponds to that of 5-methyl cytosine. In other experiments this peak was relatively lower and corresponded to 10–15% of the total counts applied.

enters the gel and again migrates in a single but somewhat more difused band.

To assess the homogeneity of the material in the DNA band, electrophoresis was carried out up to 9 h (the regular runs are for 2.5 h, figs 1 and 2). During this period rRNA migrates to the very bottom of the gel while the DNA, still in a sharp band, migrates through most of the gel (0.8 of the distance).

While it is not clear from these experiments why bacterial DNA (*E. coli*) or mammalian (mouse L-cell) DNA (Gill and Apirion, unpublished observations) behaves in this way, this technique which allows for a fast and quantitative display of DNA from whole cells, could be very useful both for the analysis of a variety of physiological phenomena, where the DNA can be used as a measuring stick, for the assessment of other molecules and for the analysis of the process of DNA synthesis.

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