MEMBRANE-BOUND DNA FROM *ESCHERICHIA COLI*: EXTRACTION BY FREEZE-THAW-LYSOZYME

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1. Introduction

Folded chromosomes from *Escherichia coli* can be extracted by the gentle lysis procedure described by Stonington and Pettijohn [1] or by the modification of Worcel and Burgi [2,3]. In the latter method, two distinct DNA structures are obtained: a fast sedimenting, membrane-bound DNA structure (3000–4000 S particles) and a slow sedimenting, 'membrane released' structure (1500–2000 S particles). The distribution of the DNA between those fractions depends on the temperature in which lysis is performed and on the physiological properties of the bacterial cultures. It was assumed that the membrane-bound fraction contains replicating DNA molecules.

This communication describes an alternative method for extracting membrane-bound folded chromosomes from *E. coli*. The extraction can be performed quickly, with great convenience and gives consistent results. The method depends on the fact that freezing and thawing in the presence of lysozyme results in gentle lysis of *E. coli* cells [4].

2. Experimental

E. coli K-12 strain DG-76 (supplied by Dr B. J. Bachmann) *thy*$^-$, *leu*$^-$ [5] was grown at 37°C with aeration, in modified Hershey medium [6] supplemented with glucose, 0.2%, leucine, 100 μg/ml, and thymine, 4 μg/ml. Generation time was about 75 min. DNA was labeled with [^{3}H]thymine (New England Nuclear) 4 μCi + 5 μg/ml for 1 generation before harvesting. ^{35}S-labeling was performed by growing the cells for 2 generations in medium containing $Na_2{}^{35}SO_4$ (Nuclear Research Center-Negev, Israel) 0.2 μCi + 1.1 μg/ml.

In a typical experiment 5 ml samples of a culture at 2×10^8 cells/ml were harvested by centrifugation in the cold, 10 min at 15 000 *g*. The pellets were resuspended at 0°C in 0.1 ml of cold sucrose solution, 20% (Mann, RNase free) containing Tris–HCl pH 7.6, 0.01 M; magnesium acetate, 0.015 M, and egg-white lysozyme, 0.5 mg/ml (Sigma Chemical Co.) The suspension was freezed in acetone dry ice. If not used immediately the suspensions could be stored at –20°C. For analysis, the cell suspensions were thawed in ice-cold water until the ice melted. Lysis was completed by a short incubation with 0.1 ml of cold non-ionic detergent solution containing NaCl, 2.0 M; Brij 58, 1%; and EDTA, 0.01 M. As soon as the lysates became viscous, they were spun in a Sorvall centrifuge in the cold at 4000 *g* for 2 min. The pellets were transparent and contained cell debris and a few 'intact' spheroplasts. The supernatant was layered on a 3.7 ml 10–30% linear sucrose gradient, prepared over a 0.4 ml shelf of 50% sucrose. The sucrose solutions contained NaCl, 1 M; Tris–HCl, pH 8.2, 0.01 M; EDTA, 1 mM, and β-mercaptoethanol, 1 mM. In most experiments bacteriophage T_4 ([^{14}C] leucine labeled) was added to the lysate as an internal marker (1000 S) [7]. Centrifugation was carried out in a SW56 rotor in a Beckman ultracentrifuge for 30 min at 17 000 rev/min. Fractions, 3 drops each, were collected after puncturing the bottom of the gradient tubes. 0.1 ml aliquots from each fraction were put onto Whatmann 3 MM filters (25 mm diatmeter). The filters were put into cold TCA (10%), washed with TCA, ethanol–ether (1:1 v/v), ether and

dried. They were counted in toluene in a Packard Tri-Carb scintillation counter.

3. Results and discussion

The results in fig.1 illustrate a typical sucrose gradient profile. In this experiment DNA was extracted from cells doubly labeled in their DNA by [^{3}H]thymine and in their proteins by ^{35}S. By comparison to the Worcel and Burgi method [2,3], the ^{3}H containing peak nearer to the bottom of the gradient contains the fast sedimenting, DNA-membrane particles, and the 'membrane-released' structures sediment at lower sucrose densities. This interpretation is supported by the fact that in the heavy peak the ratio of ^{35}S to ^{3}H counts is higher than in the lighter peak. The presence of ^{35}S counts in the 'membrane-released' DNA fraction might be due to the presence of several proteins such as RNA polymerase [3], although the high level of proteins in this peak does not exclude the possibility that structural proteins are involved. Each of the DNA

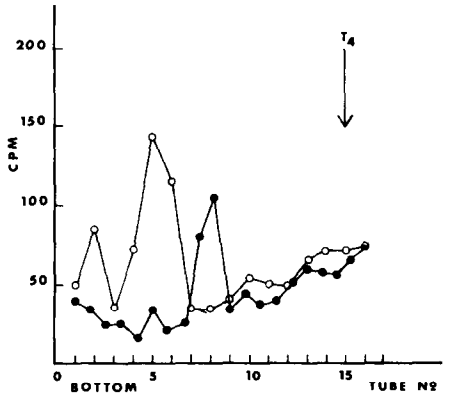

Fig.2. Effect of Sarkosyl on the fast sedimenting material. An exponential culture was labeled with [^{3}H]thymine, lysed and centrifuged as described in the text. The first 6 fractions (out of 18) were collected and pooled. Half of the material (○—○) was rerun on a sucrose gradient and the other half was incubated with Sarkosyl (1%, 15 min in ice) before recentrifugation (●—●).

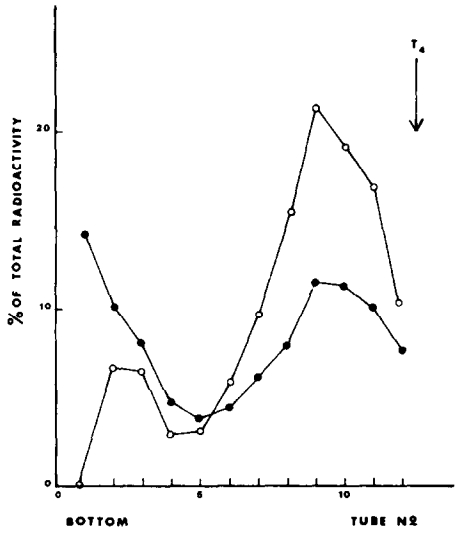

Fig.1. Sucrose gradient centrifugation of DNA from an exponential culture. A culture doubly labeled with [^{3}H] thymine (○—○) and $Na_2{}^{35}SO_4$ (●—●) was lysed and treated as described in the text. The arrow marks the position of T_4. Total radioactivity was 6200 cpm of ^{3}H and 18 000 cpm of ^{35}S.

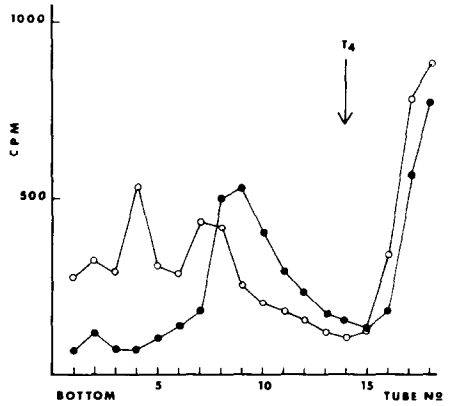

Fig.3. Effect of amino acid starvation on membrane-bound DNA. An exponentially growing culture was labeled with [^{3}H]thymine as described in the text. At 2×10^8 cells per ml part of the culture was harvested and frozen for further analysis (○—○) and the other part was washed twice by centrifugation and resuspended in an equal volume of prewarmed medium lacking leucine and supplemented with [^{3}H] thymine. This culture was incubated at 37°C for one generation time and harvested (●—●). Lysates of cells from both cultures were treated as described in fig.1.

fractions sedimented at the same sucrose density after recentrifugation.

In order to verify the nature of the membrane-bound DNA structures the following experiment was performed. Fractions containing the DNA peaks were collected from a gradient, pooled and incubated with Sarkosyl NL97, a non-ionic detergent known to release the folded chromosome from the membrane, presumably by breaking hydrophobic bonds [3]. After incubation the DNA fractions were recentrifuged, and only the lower peak shifted position (fig.2).

It has been suggested that the membrane-bound DNA might be an artifact of the preparation method [8]. Alternatively, membrane-bound DNA might reflect the state of the replication apparatus [3]. The results in fig.3 support the latter possibility. They indicate that the membrane-bound DNA fraction decreases with starvation for a required amino acid, probably due to termination of chromosome replication [3].

The results presented in this communication indicate that the freeze-thaw-lysozyme method of lysis yields two DNA structures: membrane-bound and free folded DNA. The main advantage of this method is that the cells can be kept frozen for several weeks, allowing one to perform an experiment involving many samples without having to lyse and centrifuge all of them simultaneously. The two DNA fractions are obtained by this method consistently, reproducibly and with a good yield.

References

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