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Membrane Attachment of Folded Chromosomes of *Escherichia coli*

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By fractionation of the envelope part of membrane-associated folded chromosomes it is shown that only the outer membrane is bound to the DNA. Experiments with the M-band technique suggest the presence of attachment points for the membrane also in membrane-released folded chromosomes.

The DNA of *Escherichia coli* can be isolated in a folded state, the so-called nuclear body or folded chromosome (Stonington & Pettijohn, 1971). The protein and the lipid contents of these particles depend on the lysis conditions: they are very high if lysis of bacterial protoplasts is carried out at 0°C (Dworsky & Schaechter, 1973). Results obtained from sodium dodecyl sulphate/polyacrylamide-gel electrophoresis led to the assumption that the proteins are mainly constituents of the outer cell membrane (Worcel & Burgi, 1974; Worcel *et al.*, 1974). By the M-band technique (Tremblay *et al.*, 1969), which is an indicator for membrane-associated DNA, it was shown that folded chromosomes can be attached to magnesium sarcosylate (*N*-lauroylsarcosinate) crystals (Dworsky & Schaechter, 1973). From all these data it has been concluded that parts of the membrane are bound to nuclear bodies if they were isolated at 0°C.

If the lysis temperature is raised to 25°C a considerable decrease in the lipid and protein contents and in the sedimentation coefficient of the folded chromosomes occurs (Pettijohn *et al.*, 1973). In 'membraneless' nuclear bodies obtained in this way the remaining 5–10% of the proteins mainly consists of RNA polymerase (Worcel & Burgi, 1974). From these results two questions arose, which were studied in this work. (i) Can the membraneless nuclear bodies also be attached to magnesium sarcosylate? (ii) Are membrane parts indeed bound to membrane-associated nuclear bodies, or are membrane proteins bound to them at random?

Materials and Methods

Escherichia coli strain D10 (Gesteland, 1966) was grown in Antibiotica Bouillon (E. Merck, Darmstadt, West Germany) until the culture reached a density of 10^7 cells/ml. Folded chromosomes were isolated as described by Stonington & Pettijohn (1971): cells were converted into spheroplasts by lysozyme and then lysed by detergents. The lysate was fractionated in a 10–30% (w/v) sucrose density gradient.

To study the envelope that is bound to the nuclear bodies, bacteria were labelled with [2-^{14}C]thymidine (0.1 μCi/ml) and either L-[4,5-^{3}H]leucine or L-[2,3-^{3}H]valine (1 μCi/ml) for three generations. Lysis was carried out at 0°C. The fraction corresponding to the DNA peak in the sucrose density gradient was sonicated and then fractionated by isopycnic centrifugation by the method of Osborn & Munson (1974).

For the measurement of the amount of DNA in the M-band, cells were labelled for two generations with [*Me*-^{3}H]thymidine (0.1 μCi/ml). Lysis was carried out at 18° and 25°C. All fractions from a 10–30% (w/v) sucrose density gradient were investigated separately with respect to their binding to magnesium sarcosylate by mixing them with pre-formed crystals of this substance (Dworsky & Schaechter, 1973). All samples were centrifuged in a 15–47% (w/v) sucrose density gradient for 30 min (Tremblay *et al.*, 1969). The crystals with the adhering membrane material and DNA (the 'M-band') were collected from the middle of the gradient. The DNA not attached to the membrane should remain on the top of the gradient.

Results

Estimations of the number of the attachment sites between membrane and the chromosome of *Escherichia coli* that have been made by the M-band technique gave results similar to those obtained by other methods. Purified DNA mixed with membrane material does not bind to magnesium sarcosylate crystals (Dworsky & Schaechter, 1973). Therefore it can be assumed that this method is specific for the attachment sites on the DNA. Since folded chromosomes may no longer bind to magnesium sarcosylate after release from the membrane, this question was investigated here by the M-band technique. However, it was found that more than 90% of the DNA from nuclear bodies obtained by lysis at 25°C could be attached to magnesium sarcosylate crystals.

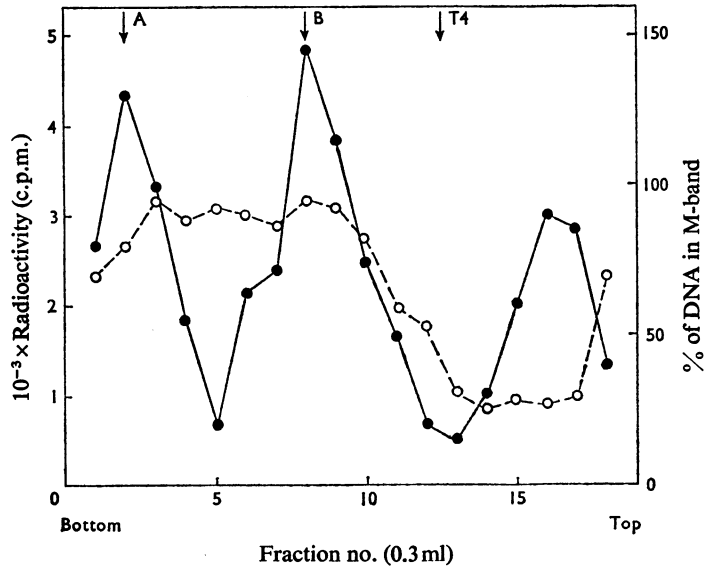

Fig. 1. *M-bands of nuclear bodies*

A lysate, obtained at 18°C from [^{3}H]thymidine-labelled cells, was centrifuged in a 10–30% (w/v) sucrose density gradient at 17000 rev./min in an SW 50L rotor of a Spinco centrifuge for 24 min. T4 bacteriophage, with a sedimentation coefficient of 1025S (Cummings, 1964), was used as a marker. All fractions were M-banded. A, Membrane-associated nuclear bodies; B, membrane-released nuclear bodies. ●, Radioactivity; ○, % of DNA in the M-band.

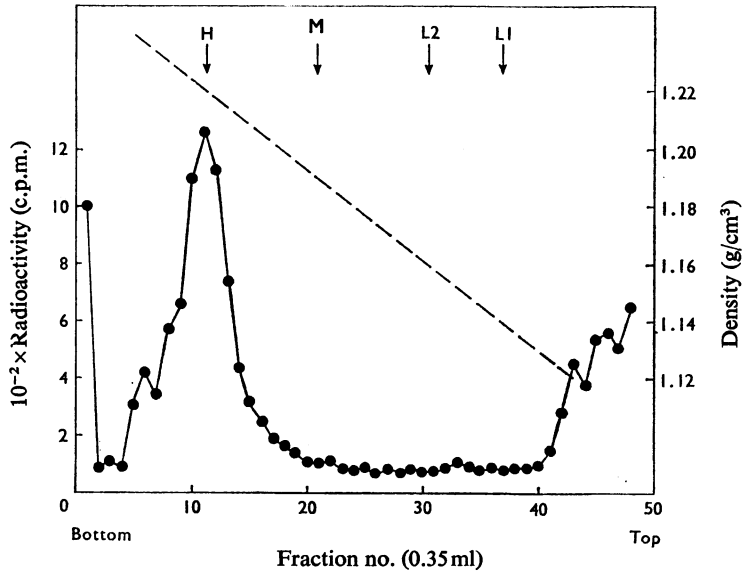

Fig. 2. *Fractionation of the membrane associated with the nuclear bodies by isopycnic sucrose-density-gradient centrifugation*

For experimental details see the text. ●, ^{3}H-labelled amino acids; — —, density of the gradient.

M-banding of nuclear bodies is shown in Fig. 1. In this experiment lysis was performed at an intermediate temperature of 18°C in order to obtain a mixture of membrane-bound and membrane-free nuclear bodies, which were separated by gradient centrifugation. The percentage of DNA in the M-band lay between 80 and 95% for most fractions of the middle and the lower part of the gradient, thus indicating that both kinds of folded chromosomes bind to magnesium sarcosylate. The low values in the upper part of the gradient are due to interference of the detergents of the lysate with the formation of the M-band, and probably also to some material of low molecular weight on the top of the gradient.

Since the binding of the folded chromosomes to the M-band does not depend on the amount of the membrane material present, it should be made certain that there are indeed pieces of membranes attached to these particles. The only evidence for this was electrophoretograms with the highest protein peaks in the molecular-weight range 30000–40000 (Worcel & Burgi, 1974; Worcel *et al.*, 1974), which is similar to the molecular weight of the 'major protein' of the outer membrane of *Escherichia coli* (Braun & Hantke, 1974). These findings were interpreted to mean that mainly outer membranes are attached to the DNA, a result that is rather surprising because the isolation was done from spheroplasts from which much of the cell wall and the outer membrane had been removed. To clarify this point, the envelope part of membrane-associated nuclear bodies was fractionated. Fig. 2 shows that most of the proteins were in the high-density fraction H (the outer membrane). Almost no activity was in the light fractions L1 and L2, or in the medium fraction M. This result proved correct the assumption that the outer membrane is associated with folded chromosomes.

Discussion

The results can be explained in two ways. (i) The number of attachment sites between DNA and membrane is decreased in membrane-free nuclear bodies. This cannot be the only effect of membrane release because the decrease of lipid material to less than 5% (Pettijohn *et al.*, 1973) or 2% (A. Wright, personal communication) corresponds to the loss of all 10–20 attachment sites (Dworsky & Schaechter, 1973). Further, in none of the fractions H, M, L2 and L1 was a peak of radioactivity observed when membrane-free folded chromosomes were subjected to the fractionation procedure of Osborn & Munson (1974). (ii) Membrane material is removed from the attachment sites during lysis at 25°C but still binds to the M-band. There are some indications for this second possibility: from experiments with inhibition of RNA synthesis it was concluded that some of the attachment sites represent points of DNA synthesis and the origin of replication that have been reported to be membrane-bound (Dworsky & Schaechter, 1973). The enzyme involved in this reaction, DNA polymerase III, acts in non-ionic environment, probably in the bacterial membrane (Nüsslein *et al.*, 1971), and therefore may be responsible for binding to lipophilic surfaces as to magnesium sarcosylate crystals.

It cannot be excluded that the binding of the outer membrane to the chromosome of *Escherichia coli* is an artifact caused by association after lysis. *In vivo* such a binding can only be imagined by considering that the process of separation of the newly built nucleoids occurs before cell division. This can be brought about by drawing the nuclear bodies apart by the growing cell wall (Jacob *et al.*, 1963). Therefore an interaction between DNA and cell wall has to be postulated where an involvement of the outer membrane may take place.

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