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The Attachment of the Bacterial Chromosome to the Cell Membrane

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I. Introduction

Prokaryotic cells are small, do not contain organelles limited by independent membrane systems and, instead of a complex nucleus, possess a pleomorphic central region of condensed DNA, the nucleoid. Nucleoids do not divide by mitosis and, at least in *Escherichia coli*, consist of single chromosomes. The DNA is in the form of a circular duplex molecule, and its replication takes place in both directions from a distinct starting point.

Bacteria have the ability to respond to changes in environmental conditions by grossly altering their size and macromolecular composition. Thus, as a response to changes in the kind of nutrients provided, *growing* bacteria may vary in size or in RNA content by a factor of 10 or more. They change from one physiological state to another in a remarkably efficient and rapid manner. A review of

these attributes appears in a book by Maaløe and Kjeldgaard (1966). Bacteria are efficient and structurally simple; consequently they make multiple uses of cellular structures. In this vein, the synthesis and regulation of several macromolecules have been shown to be related to the behavior of the cell membrane. The subject of this article is one of these relationships, the connection of the bacterial chromosome with the cell membrane.

Concern for this subject originated with a proposal by Jacob, Brenner, and Cuzin for the control of DNA replication in bacteria. They formally termed the unit of DNA replication the *replicon*, and proposed that initiation of replication is controlled by diffusible gene products (Jacob *et al.*, 1963; Jacob and Brenner, 1963): A structural gene produces an initiator which acts upon a region of the chromosome at a specific site, the *origin*. Replication begins at the origin and proceeds linearly until the entire chromosome has been duplicated. Included in the model is the proposition that the chromosome is attached to the bacterial membrane. The model suggests that the DNA-synthesizing complex is fixed to the bacterial membrane and that the DNA moves through this complex. The membrane is thought to provide a mechanism for the segregation of the daughter replicons by growth of the cell surface between their sites of attachment. The bacterial membrane would thereby perform the function of the mitotic apparatus of higher organisms, as well as being the site of DNA synthesis. This model predicts the existence of membrane components that recognize specific sites on the chromosome.

The replicon model stimulated a search for the association between the bacterial chromosome and the cell membrane. In this article we present morphological, genetic, and biochemical evidence for this association. We attempt to provide tentative state-of-the-art answers to the following questions: (1) Is DNA attached to the membrane? (2) If so, at how many sites? (3) If there are several sites, are they alike in function? (4) Is attachment possible along any region of the genome? (5) Is the membrane unique at the site of attachment? Answers to these questions are tentative, because the necessary methodology is in an early state of development. In very few cases have findings been confirmed by unrelated techniques. Even more difficult are the following questions: (6) Do all attachment points exist at the same time? (7) At what time in the cell cycle are attachment points born? (8) Do attachment points remain at their site of birth?

Many researchers have interpreted their data in terms of a connection between the bacterial chromosome and the cytoplasmic mem-

brane. We feel that much of this work is only tangentially related to the subject of this article. For this reason we have selected evidence that comes closest to providing answers to the questions listed above, and which we feel deals directly with the issue of whether or not attachment exists. In addition, we have omitted work that is relevant to this field but which appears to lie beyond the conceptual framework of this article. The special topic of the attachment of bacteriophage DNA to the bacterial cell membrane has been reviewed recently (Siegel and Schaechter, 1973).

II. Morphological Considerations

A. The Nucleoid

Perhaps the most convincing proof for the existence of nucleoids comes from observations of living *E. coli* with the phase-contrast microscope. Nucleoids can be seen when cells are grown in media of high refractive index. In time-lapse motion pictures nucleoids are first seen to change in conformation as they divide, and then to segregate into daughter cells prior to the completion of cell division (Adler *et al.*, 1969). There has never been any demonstration of a membrane separating nucleoids from the cytoplasm, nor is there evidence of any of the elements of a mitotic apparatus.

The DNA of *E. coli* consists of a single circular duplex molecule about 1100 μm in length (Cairns, 1963). Since the apparent volume of the nucleoid of this cell is about 0.1 μm^3, it follows that the chromosome must be folded on itself and exist in a phase state quite unlike that of DNA in solution. Although this represents a very high concentration of DNA, the nucleoid is considerably less dense than its surrounding cytoplasm.

The degree of condensation of the nucleoid observed in the electron microscope varies with the method of fixation. Freeze-etched preparations of unfixed bacteria reveal no clear distinction between the nucleoid and cytoplasm, but typical nucleoids are seen occasionally if cells are fixed with osmium tetroxide (Nanninga, 1968). The Ryter–Kellenberger (R-K) procedure is currently the most widely used fixation method for ultrathin sectioning of bacteria. It employs osmium tetroxide for both prefixation and fixation. With this fixation nucleoids are most frequently seen in the central portion of the cell, and they show bundles of fibers with dimensions similar to those of DNA (Kellenberger *et al.*, 1958). Prefixation in glutaraldehyde followed by fixation with osmium tetroxide (G-O fix-

tion) causes the nucleoplasm to appear in a dispersed configuration rather than as a centrally located body (Margaretten *et al.*, 1966; McCandless *et al.*, 1968).

Recent work by M. L. Higgins and L. Daneo-Moore (personal communication) suggests that degradation of RNA, which takes place during R-K but not G-O fixation, may lead to further condensation of the nucleoids. Despite the uncertainties introduced by these studies, R-K fixation results in sections which conform to what is expected from studies on living bacteria with the phase microscope.

B. The Membrane

The cytoplasm of bacteria is bounded by a trilaminar membrane, about 8–10 nm thick. Outside this membrane is the cell wall and outside it, in gram negative cells, is a membranelike outer layer. The inner membrane has intracytoplasmic involutions termed *mesosomes*, which vary in complexity among taxonomic groups (Fitz-James, 1960; van Iterson, 1961; Glauert *et al.*, 1961; Glauert, 1962). A comprehensive review of these structures has recently appeared (Reusch and Berger, 1973). The mesosomes of gram-positive bacteria appear as extensions of the cytoplasmic membrane forming saclike structures (outer mesosomal membranes) filled with vesicles, tubules, and/or lamellae (internal mesosomal membranes) (e.g., *Bacilli.* Ryter and Jacob, 1966, van Iterson, 1961, 1965, Fitz-James, 1960; Holt and Leadbetter, 1969; *Listeria monocytogenes:* Edwards and Stevens, 1963; *Mycobacteria:* Imaeda and Ogura, 1963; *Streptomyces:* Glauert, 1962). These internal structures are considered in turn to be invaginations of the sac, or the outer mesosomal membrane (Fitz-James, 1960; Ryter and Jacob, 1966).

Mesosomes are also found in gram-negative bacteria (*E. coli:* Kaye and Chapman, 1963; Steed and Murray, 1966; *Pseudomonas aeruginosa:* Hoffmann *et al.*, 1973; *Spirillum serpens:* Steed and Murray, 1966; *Caulobacter:* Stove Poindexter and Cohen-Bazire, 1964). Ultrathin sections usually reveal that these mesosomes are uncomplicated structures, most often containing lamellae which apparently result from delicate foldings of the plasma membrane. Mesosomes in gram-negative bacteria are probably devoid of tubules. As with gram-positive bacteria, there is variation in structure among taxonomic groups.

There is disagreement on the true morphology of mesosomes and on their number and location in the cell (Remsen, 1968; Nanninga, 1968; Highton, 1969, 1970a,b; Burdett and Rogers, 1970; Rogers, 1970). It must be emphasized that the morphology of the mesosome

is altered markedly by the conditions of fixation (e.g., Burdett and Rogers, 1970).

In ultrathin sections mesosomes are seen to be touching the nucleoids of dividing cells, and to be continuous with division septa. There is evidence to implicate these structures in DNA replication (Higgins and Daneo-Moore, 1972), in the segregation of chromosomes (Ryter and Jacob, 1963), in the location of membrane and cross-wall synthesis and prespore septation (Ellar *et al.*, 1967; Steed and Murray, 1966; Chapman and Hillier, 1953; Fitz-James, 1960, 1967; Freese, 1973), in subcellular degradative activities (lysosomal functions) (Reusch and Berger, 1972), and in oxidative function (van Iterson and Leene, 1964; Ferrandes *et al.*, 1966).

The morphological development of mesosomes was followed in synchromously dividing *Bacillus megaterium* by Ellar *et al.* (1967). Mesosomes develop by an initial concentric infolding of the cytoplasmic membrane and eventually assume a saclike shape. Cross wall formation begins at the base of these mesosomes which are located at the center of the cells. This implicates them in the initiation of cross wall synthesis. Later, the mesosome is seen on both sides of the developing cross wall, which suggests that it is also involved in the synthesis of cross walls. These central mesosomes are often associated with nucleoids, as are other mesosomes located at the poles.

From these morphological considerations it seems likely that mesosomes are responsible for thickening of the cell wall prior to cell separation, and for initiation and synthesis of the cross wall. This has not yet been borne out by fractionation studies, since mesosomes have been found not to be particularly rich in enzymes and precursors involved in membrane or wall synthesis (Patch and Landman, 1971; Reusch and Berger, 1972). However, Nanninga (1968) showed differences in the freeze-etched surface structure of mesosomes and cytoplasmic membranes and concluded that mesosomes may in fact differ from the rest of the cytoplasmic membrane. This subject has been reviewed by Reusch and Berger (1973).

C. Morphological Evidence for the Association between Nucleoids and the Membrane

In ultrathin sections the nucleoid is located in a central region of the cell and is not in obvious contact with the peripheral membrane. For this reason the morphological association between them escaped detection for many years. Upon closer examination the nuclear regions and mesosomes of both gram-positive and gram-negative

FIG. 1. Ultrathin sections of growing *B. subtilis* showing the association of mesosomes (M) with nucleoids (N). Fixation by the R-K method. (From Jacob *et al.*, 1966, reproduced with permission from the publishers, The Royal Society, and the authors.)

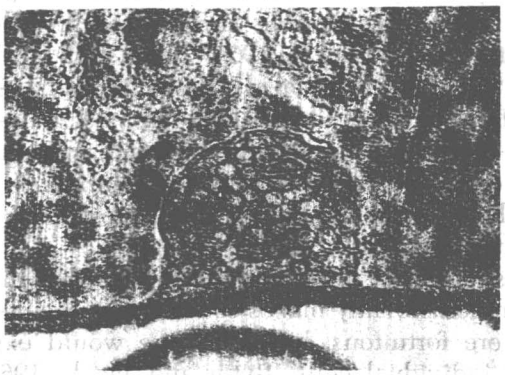

FIG. 2. Ultrathin section of a mesosome in *B. subtilis* after prefixation with the G-O method. ×90,000 (From Ryter, 1968, reproduced with permission from the publishers, The American Society for Microbiology, and the author.)

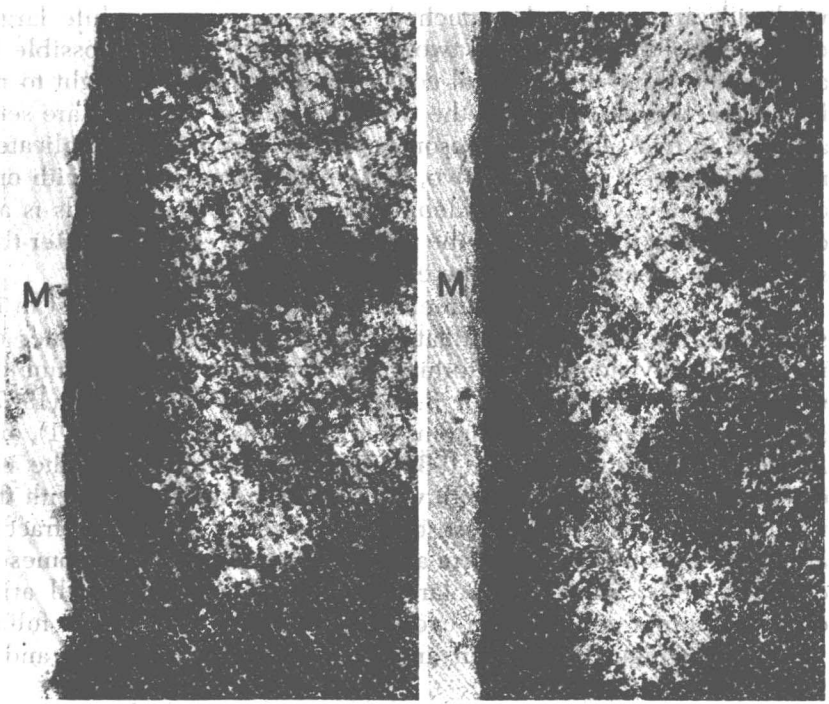

FIG. 3. Mesosomes (M) of *E. coli*, which appear as delicate folds of membrane in contact with the bacterial nucleoid. ×85,600. (From Ryter and Jacob, 1966, reproduced with permission from the publishers, Masson et Cie., Editeurs, and the authors.)

cells can nearly always be shown to be touching (see Figs. 1–3). They usually have considerable surface contact and often penetrate one another (van Iterson, 1961; Ryter and Jacob, 1964, 1966; Ellar *et al.*, 1967; Pontefract *et al.*, 1969; Remsen, 1968; Hoffmann *et al.*, 1973). The contact is less dramatic in most gram-negative cells, because their mesosomes are smaller. In fact, in a mutant of *E. coli* which forms extensive intracytoplasmic membranes, the contact of the DNA with these membranes is readily evident (Altenburg and Suit, 1970; Altenberg *et al.*, 1970).

Offhand it should not be surprising to find that the pleomorphic nuclear region occasionally makes contact with the mesosome. If the association were fortuitous, however, one would expect great disparity among individual cells. Ryter and Jacob (1964) determined that the nucleoid and mesosomes of *Bacillus subtilis* were visibly linked *in each* of 20 serially sectioned cells which included all stages of the cell cycle. The nucleoid was associated with either one or two mesosomes, depending on the stage of growth (Ryter, 1968). Smaller nucleoids appeared to be attached to one mesosome, while larger ones were often attached to two. Consequently, it was possible to arrange the three-dimensional constructs in an order thought to reflect the cell cycle. Initially, the two nucleoids in each cell are seen attached to two separate mesosomes; as the chromosome replicates, mesosomes seem to split in two, each maintaining contact with one of the two newly formed nucleoids; segregation of nucleoids is accomplished by the growth of the membrane between them; after this segregation process begins, the cell septum starts to form.

There is considerable disagreement with this model, at least in its simplest form. Several investigators have found that mesosomes do not arise by division but are formed *de novo* at the site of septum formation. This was reported for *B. megaterium* by Ellar *et al.* (1967), *Streptococcus faecalis* by Higgins and Shockman (1970a, 1971), and *E. coli* by Pontefract *et al.* (1969). There is evidence that the nucleoid is always associated both with a polar mesosome and with the newly synthesized, septal mesosome (Ellar *et al.*, 1967; Pontefract *et al.*, 1969). Mesosomes that form at septa become polar mesosomes in daughter cells. Since two polar mesosomes within one cell arise during different cell division cycles, it should be possible in future work to distinguish between an old segregation apparatus and a new one.

There are also indications that nucleoids may not always be associated with mesosomes. For instance, Highton (1970b) found that

multinucleated *B. subtilis* cells contain fewer mesosomes than nucleoids. There is evidence that in *S. faecalis* mesosomes may not participate in nucleoid segregation. This spherical bacterium has an equatorial band on the external surface of the wall which marks the site of new cross wall synthesis and the boundary between old and new wall. Upon initiation of wall synthesis these bands split, double in number, and move to a subequatorial position. Each daughter cell has an equatorial band from the preceding generation which marks the initiation site of wall growth for that generation. Mesosomes are usually seen just beneath an equatorial wall band on the cell surface, and are attached to the base of the septal membrane by a membranous *stalk* (Higgins and Shockman, 1970b, 1971; see Fig. 4). Mesosomes located near the septum are most often seen penetrating the nuclear mass. Mesosome formation precedes cross wall formation. The mesosome appears to maintain direct contact with the septum only during the early part of the cell cycle. The septal connection is lost prior to the completion of the cross wall at the time the nucleoid appears to have divided into two masses. Two new mesosomes are now found beneath wall bands in the developing daughter cell (Higgins and Shockman, 1971; Higgins and Daneo-Moore, 1972).

The effect of selective inhibition of DNA, RNA, and protein synthesis on the development of mesosomes was studied in *S. faecalis* by Higgins and Daneo-Moore (1972). It has been shown that the cross-sectional area of mesosomes increases rapidly during amino acid starvation (Higgins and Shockman, 1970b). These authors proposed that the increase in mesosome size might be related to continued DNA synthesis since, during amino acid starvation, RNA synthesis is shut off and the rate of protein synthesis decreases (Ziegler and Daneo-Moore, 1971). They suggest that the termination of DNA replication might result in activation of the regions of the envelope involved in segregation to form a site for the formation of a new mesosome. They suggest that mesosomes in this organism are necessary for the initiation of the cross wall and for DNA replication, but not for cross wall formation or nuclear segregation. Segregation would take place through direct attachment to the cytoplasmic membrane.

It is not known if these discrepancies in the behavior of mesosomes are due to differences among various species of bacteria. It is likely that they are due to a combination of many factors, including fixation artifacts and differences in the physiological state of the

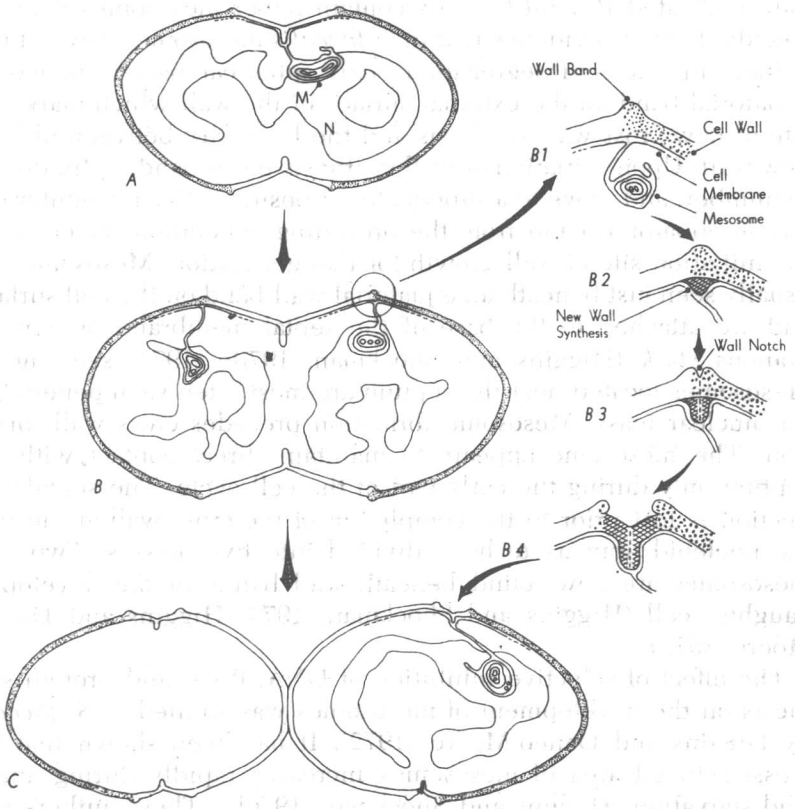

FIG. 4.—Diagrammatic representation of the cell division cycle for *Streptococcus faecalis*. The model proposes that linear wall elongation is a unitary process which results from wall synthetic activity at the leading edges of the nascent cross wall. The diplococcus in A is in the process of growing new wall at its cross wall and segregating its nuclear material to the two nascent daughter cocci. In rapidly growing exponential phase cultures before completion of the central cross wall, new sites of wall elongation are established at the equators of each of the daughter cells at the junction of old, polar wall (stippled) and new equatorial wall beneath a band of wall material that encircles the equator (B). Beneath each band a mesosome is formed while the nucleoids separate and the mesosome at the central site is lost. The mesosome appears to be attached to the plasma membrane by a thin membranous stalk (B1). Invagination of the septal membrane appears to be accompanied by centripetal cross wall penetration (B2). A notch is then formed at the base of the nascent cross wall which creates two new wall bands (B3). Wall elongation at the base of the cross wall pushes newly made wall outward. At the base of the cross wall, the new wall peels apart into peripheral wall, pushing the wall bands apart (B4). When sufficient new wall is made so that the wall bands are pushed to a subequatorial position (e.g., from C to A to B) a new cross wall cycle is initiated. Meanwhile the initial cross wall centripetally penetrates into

cells. We feel that precise knowledge of the number and location of mesosomes awaits a detailed analysis of synchronously dividing cells growing at different rates.

Linkage of the nucleoid to mesosomes has been reported to persist throughout the first stages of sporulation (Ryter and Jacob, 1964). In the first stage the contact appears to be mediated through one of the polar mesosomes which eventually participates in the development of the spore membrane. Later, the nucleoid migrates to a peripheral position in the spore cytoplasm, the mesosome disappears, and vesicles suggestive of mesosome tubules are found along the spore membrane. In the last stage of sporulation, the nucleoid is connected directly to the spore membrane. It is not clear if this represents the initial contact between the nucleoid and mesosome seen in the first stage of sporulation, or if a new contact point is formed.

The sequence of events in nuclear division has also been studied in spore germination (Ryter, 1967). Early in germination of *B. subtilis* spores, the nucleoid assumes a central position, becomes an axial filament, and is connected to the spore membrane by a huge mesosome. Later, the number and size of mesosomes vary, and they are not always in contact with the nucleoid. Nonetheless, the nucleoid is linked to the membrane at two sites, either through mesosomes, by direct attachment to the membrane, or both. The distance between the attachment points increases as the cell elongates, but the distance from each attachment point to the pole of the cell seems to remain the same. This suggests that the membrane grows by the deposition of new material at the equator of the cell. Occasional sections reveal the presence of small nonmesosome structures in the membrane to which the nuclear fibrils are attached (Ryter, 1967).

Mesosomes are evaginated when cells are plasmolyzed in hypertonic medium or when spheroplasts (wall-less or wall-deficient cells) are prepared. In such cases the nucleoid is found at the periphery of the cell, as if it had been dragged toward the cell surface by its attachment to the membrane (Ryter and Landman, 1964, 1967; Ryter and Jacob, 1964, 1966). One would expect that upon extrusion of the mesosome the chromosome would be linked to the portion of the membrane that was the cytoplasmic surface of the mesosome. In

the cell, dividing it into two daughter cocci. At all times the body of the mesosome appears to be associated with the nucleoid. Doubling of the number of mesosomes seems to precede completion of the cross wall by a significant interval. Nucleoid shapes and the position of mesosomes are based on projections of reconstructions of serially sectioned cells. (From Higgins and Shockman, 1971, with permission of CRC Press, Inc., and by courtesy of the authors.)