THE CELL PLATE IN BACTERIAL CYTOKINESIS[1]

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The first phase of bacterial cytokinesis was described by Knaysi (1951) as involving an inward growth of the cytoplasmic membrane to form a septum across the cell. Transverse plasma membranes or boundaries were previously reported by Migula (1894) in *Bacillus oxalaticus*, by Guillermond (1908) in *Bacillus mycoides*, by Knaysi (1930) in *B. mycoides* and *Bacillus mesentericus*, and by Robinow (1942, 1944) in *Escherichia coli*. Robinow (1945) and Knaysi (1951) concluded that cross wall material is formed at the position of the transverse membrane by centripetal growth of the lateral wall; however, the authors differed considerably in details of the process. Bisset (1948) emphasized the differences in the mode of cell division between morphologically smooth and rough forms of bacteria and suggested that cross walls do not "grow" but rather are produced by secretion at the surface or interface of cell membranes. The nature and origin of the "transverse membrane" often reported as initiating cytokinesis in the bacilli are still subject to controversy (Chapman and Hillier, 1953).

Chance (1953*a*, *b*) demonstrated through the use of the crystal violet nuclear stain that a cell plate of nuclear origin forms just prior to cytokinesis in 12 species of cocci and 5 species of bacilli. The cell plate was observed to form within the dividing nucleus and to develop in a manner similar in many respects to cell plate formation during cytokinesis of higher plant cells. The cell plate was shown to be neither a cytoplasmic membrane nor of cell wall material, since the crystal violet nuclear stain does not stain cell wall material, cytoplasm, or cytoplasmic membranes. The stain was shown to be specific for a protein component of the bacterial nucleus (Clark and Webb, 1955), thus suggesting the protein nature of the cell plate. Schaudinn (1902) reported that the first evidence of cell division was the appearance of a highly refractile granule in what later became the plane of division. This granule was always in the long axis of the cell, and gradually broadened centrifugally into a disc perpendicular to the long axis of the cell. This continued until it reached the lateral membrane, grew in thickness, and split to separate the cells. This description is similar to what has been observed in cell plate studies.

The present investigation was undertaken to determine the relationship between cell plate development and cross wall formation during cytokinesis in certain bacteria.

MATERIALS AND METHODS

Corynebacterium pseudodiphtheriticum (University of Oklahoma culture collection) and *Micrococcus pyogenes* var. *aureus* (FDA 209) were studied in some detail. Other cocci and bacilli were studied to a lesser extent for comparative purposes.

Cytokinesis was observed in living cells on slide cultures with an American Optical Company phase microscope equipped with bright contrast medium, dark contrast medium, and B-minus contrast medium objectives. The bright contrast medium objective was found to be superior for these observations.

In order to study the relation between cell plates and cross septation, cells were stained with the crystal violet nuclear stain (Chance, 1952). Excess decolorization with nigrosin was sometimes required to successfully demonstrate cell plates, since if the nucleus was darkly stained, the cell plate was obscured. Cells showing cell plates in various stages of development were located and photographed. The slides were then soaked successively in toluene, water, and 50 per cent ethanol to remove the nuclear stain. The destained smear was stained with the crystal violet, tannic acid, Congo red cell wall stain (Webb,

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1954) and the field relocated by stage micrometer readings and photographed.

RESULTS AND DISCUSSION

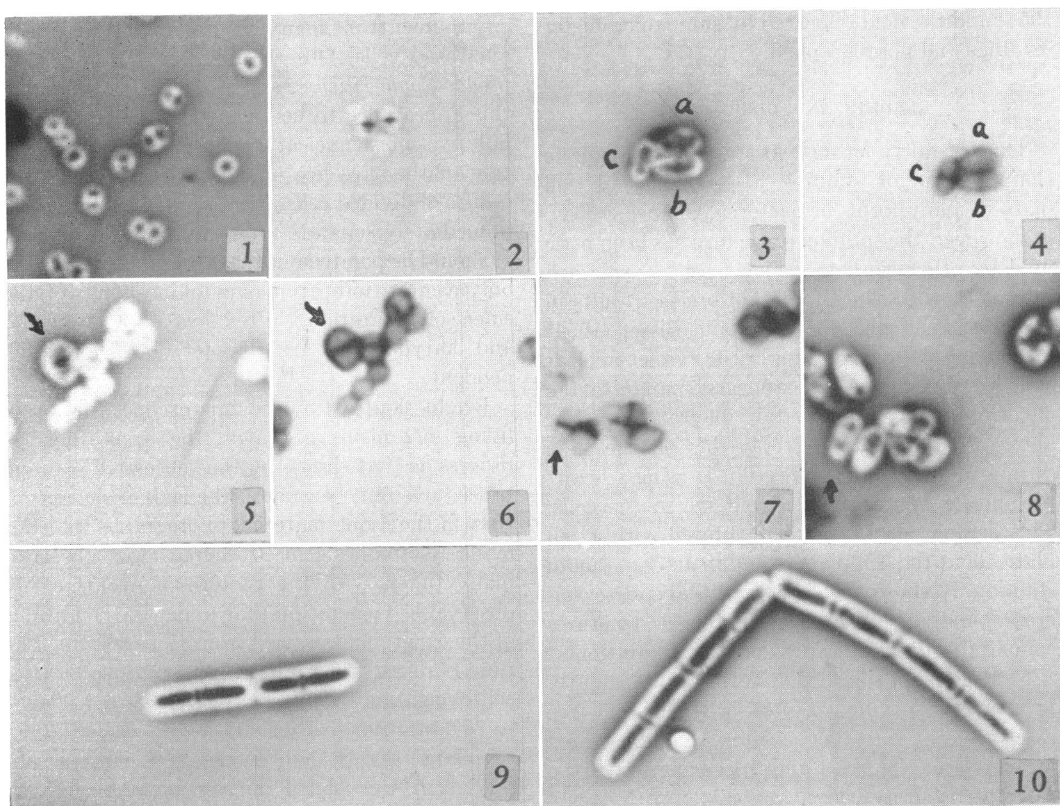
The crystal violet nuclear stain has the unique characteristic of staining a proteinaceous cell plate in many forms of bacteria (Chance, 1953*a*, *b*); however, the staining procedure has been little used by other workers, which may be explained in part by the failure to obtain precise results in the first experience with the stain. Bisset (1954) presents a photograph of a crystal violet nuclear stain preparation which appears similar to the effect obtained when crystal violet at pH 7.5 or above is allowed to partially dry on the slide during the stain procedure, thus causing a heavy precipitate to form on the outside of the cells. This artifact should not be confused with a cell plate. That the cell plate is not an artifact due to staining is shown by the fact that phase microscope studies of living cells reveal structures comparable to those seen in stained preparations.

Cell plates are revealed uniformly and regularly in actively growing cultures, whereas they are rarely present in resting cultures as demonstrated in *M. aureus* (figures 1 and 2). Formation of the cell plate is always closely associated with karyokinesis, but the appearance of the nucleus at this stage may vary in different organisms. Results from successive staining with the crystal violet nuclear stain and a cell wall stain demonstrated that the cell plate differentiates into or is replaced by a double cell wall to complete cytokinesis. The staining reactions of cell plates and cross septa in *C. pseudodiphtheriticum* at various stages of development are shown in figures 3 through 8. In the early stages of development the cell plate was observed to stain deeply with the nuclear stain, but remained unstained by the cell wall stain (figures 3*a* and 4*a*). Both the nuclear and cell wall stains revealed similar structures in mature cells indicating that at this stage the septum is composed of both cell plate and cell wall material (figures 3*b*, 4*b*, 5, and 6). Mature cross septa are not stained by the crystal violet nuclear stain (figures 3*c*, 4*c*, 7, and 8).

The failure to stain mature cross septa, a characteristic of most nuclear stains, results in the misleading appearance of a multinucleated cell. This characteristic has caused some authors to consider the polycelled units, which normally appear under appropriate conditions of age and environment in many bacteria, to be multinucleated cells. Observations on *M. aureus*, *C. pseudodiphtheriticum*, and *E. coli* have suggested this appearance to be an artifact due to (1) the failure of most nuclear stains to give any indication of the presence of cross septa and (2) the failure of divided cells under certain conditions to immediately separate after division is complete. It should be pointed out that cross septation is the only accurate indication of cytokinesis in bacteria, since cell separation is often delayed. Cytokinesis and karyokinesis are distinct, but not independent.

Bright contrast phase microscope studies of living organisms revealed the same general process as that shown by the successive staining procedures but, because of the lack of sharp contrast in the living material, photographs were not made. Cytokinesis in *M. aureus* was observed many times, with the pattern of events easily observable. Generation time under the conditions of the experiments varied from 17 to 30 min. The time required for division to be completed after cell elongation was first observed varied from 7 to 15 min. Prior to division, the cell was observed to enlarge slightly and become less dense as observed in the phase microscope. As the cell elongated, a thin disc formed first near the center of the cell and then extended to the outer cell wall. This disc was comparable to the cell plate which was stained by the crystal violet stain. A heavy ring of cell wall developed at the point of juncture of the disc and outer cell wall and developed inward along the narrow band. The cell wall appeared under bright contrast as a heavy bright band across the cell. After the cross septum reached the center of the cell, it appeared that a double wall was formed; however, the resolution limits of the light microscope make an exact interpretation impossible. In young cultures of *M. aureus* the outer wall often showed no indication of an indentation at the position of the cross septum until the entire structure presented a doubled appearance. Then the two cells usually snapped apart suddenly, forming two separate spherical cells. Goodman and Moore (1956) show electron micrographs of thin sections of *M. aureus* which reveal a structure analogous to the cell plate. The lack of electron density in this structure differentiates it from cell wall material.

Evidence from both the successively stained preparations and phase observations of living



Figures 1–10

Figure 1. Resting cells of *Micrococcus aureus*, crystal violet nuclear stain.

Figure 2. Actively dividing cells of *Micrococcus aureus*, cell wall stain.

Figure 3. Corynebacterium pseudodiphtheriticum, crystal violet nuclear stain showing various stages of division.

Figure 4. Cell wall stain of cells in figure 3.

Figure 5. Corynebacterium pseudodiphtheriticum, crystal violet nuclear stain showing mature cell plate.

Figure 6. Cell wall stain of cells in figure 5.

Figure 7. Corynebacterium pseudodiphtheriticum, cell wall stain showing mature cross septa.

Figure 8. Crystal violet nuclear stain of cells in figure 7.

Figures 9 and 10. Bacillus cereus, crystal violet nuclear stain showing cell plates.

All photographs magnified 3,250 ×.

cultures suggests that cell wall material is formed at the cell plate, cytoplasm interface forming a double cross wall. Chapman and Hillier (1953) show an excellent electron micrograph showing the double cell wall. They also report that "after the transverse cell wall is completed, it appears to thicken and to divide into two layers." The deposition of cell wall material, beginning at the ring of contact of the cell plate with the outer wall and moving inward along the surface of the cell plate, gave the appearance of "centripetal growth" in *M. aureus* and some other cocci. In *C. pseudodiphtheriticum* the deposition of cell wall material appeared to occur evenly over the cell plate in such a way that at no time was centripetal growth of the cross wall suggested. The nature of the formation of the cell wall material in forming the cross septum is unknown. The cell plate apparently affords an interface on which the cell wall is deposited or grown. It is possible that the cell plate may function in a passive role by merely forming this interface, and that the reactions leading to cell wall formation are cytoplasmically controlled. It is also conceivable that

the cell plate is enzymatically active and actively contributes to cross septum formation. In either case, the proteinaceous cell plate disappears after its function is fulfilled, probably by being metabolized by the cell.

The cell plate of the bacillaceae has been studied to a lesser extent. The crystal violet nuclear stain has been applied successfully to only a few bacilli, but in many forms the cell plate is much easier to stain than the nuclear material. Figures 9 and 10 show cell plates in *Bacillus cereus*. The rod-shaped nucleus seen in *B. cereus* is not representative of the true appearance at this stage of division since the nuclear components are not resolved in these photographs.

The findings of Chapman and Hillier (1953), based on excellent thin section preparations, are in many respects consistent with these findings. The electron microscope photographs clearly suggest ingrowth of cross septa from the outer cell wall. The reported function of "peripheral bodies" in initiating and producing the cross septa is less clearly shown. These structures may be artifacts or perhaps remnants of a "transverse membrane" or cell plate which would probably be partially destroyed by the rather drastic fixing procedure required. The peripheral bodies, on the other hand, may represent a method of initiating cross septation different from that of cocci and some bacilli. However, as with stained preparations, electron microscope photographs must be interpreted with caution. The prolonged fixation in 2 per cent osmium tetroxide required in some thin section work (Chapman and Hillier, 1953) increases the possibility of producing artifacts. According to Bahr (1954), in electron microscopy, "... osmium depositions in the tissue will play the decisive role in contrast formation, and consequently, in very thin sections only the distribution of the osmium can be seen." In another study of osmium tetroxide fixation procedures for electron microscopy Bahr (1955) reports: "Osmium fixation, particularly when prolonged, brings about progressive destruction of tissue constituents and results in increased extractability with water." "Supernumerary," transverse cell walls which were reported by Chapman and Hillier (1953) are of unknown function and may represent division anomalies.

SUMMARY

The evidence of the fundamental position of the cell plate in bacterial cytokinesis is summarized as follows: (1) The cell plate is formed during nuclear division. (2) Cell plate formation occurs just prior to cross septation. (3) The cell plate at early stages is stained by the crystal violet nuclear stain which does not stain cell wall material. (4) Mature cross septa are not stained by the nuclear stain. (5) The young cell plate is not stained by the cell wall stains. (6) The mature cell plate is deeply stained by the cell wall stain. (7) Cell plates of intermediate age may be stained lightly by cell wall stains. (8) Phase microscope studies of living cultures show definite evidence of a cell plate which is formed prior to and is replaced by the cross wall septum.

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