Electron-Dense Particles Resembling Ribosomes in Mesosomes of *Bacillus subtilis*

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Thin-sectioning of *Bacillus subtilis* ATCC 6633 protoplasts and cells has revealed electron-dense particles resembling cytoplasmic ribosomes in mesosomal tubules and vesicles.

An ultrastructural study of protoplasts of *Bacillus subtilis* ATCC 6633 by using thin-section methods has revealed the presence of electron-dense particles in dangling tubules and vesicles. Tryptose broth (Difco) cultures of this organism were incubated at 37 °C on a shaker rotating at 160 rev/min. After 12 hr, a 1:20 dilution was made in fresh broth. Cells were harvested after 1 hr and 45 min, washed once with stabilizing medium (SM) consisting of 0.2 M sucrose and 0.02 M MgCl₂ in 0.02 M sodium phosphate buffer, pH 7.0, and suspended to a density of 10⁶ to 2 × 10⁶ colony-forming units per ml. Protoplast conversion was complete within 30 min of incubation at 37 °C with 500 μg of egg white muramidase per ml.

Specimens were fixed by the addition of an equal volume of chilled 12.5% glutaraldehyde buffered to pH 7.0 with 0.05 M sodium phosphate and containing 0.2 M sucrose. After 2 hr the specimens were pelleted by centrifugation, embedded in 2% buffered agar, washed overnight with phosphate buffer, and placed in 2% osmium tetroxide in Millonig's buffer (5) for 1 hr. After dehydration in alcohol and two 15-min washes with propylene oxide, the specimens were infiltrated with an Epon 812 medium devised by R. P. Smith (*personal communication*) which consisted, on a volume basis, of 50% Epon 812, 46% Nadic Methyl Anhydride, and 4% dibutyl phthalate. Just before use, eight to nine drops of benzylidinemethyamine were added as accelerator for 12 ml of the above mixture. Propylene oxide-plastic mixtures at ratios of 2:1, 1:1, and 1:2 were used for 1-hr infiltrations. Sections, stained with uranyl acetate and alkaline lead citrate, were examined in an AEI model EM 6B electron microscope.

Numerous tubules were found exterior to the protoplasts (Fig. 1). They were periodically inflated into small and large vesicles (Fig. 4). Cylindrical segments (50 nm in diameter) were also present in the tubules (Fig. 3). Electron-dense particles (P) were in the vesicles (Fig. 3, 4), side-by-side in tubules (Fig. 4), and at the juncture between tubule and protoplast (not shown).

The "string of pearl" morphology and small vesicular structures have been implicated as the accepted native forms of the mesosome (7, 8, 10). The tubular and vesicular structures observed in the present study are assumed to be mesosomal in nature in spite of the fact that the larger vesicles and tubular segments are the dominant features in cells as well as in protoplasts prepared as described. Membrane-bound vesicles containing electron-dense particles were shown to be associated with transverse septa of cells which had been washed and incubated in SM (Fig. 2) and of cells not treated with SM (unpublished data). Ellar et al. (1) observed a large vesicle in the mesosome of *Bacillus megaterium* growing synchronously in a defined medium and concluded that it was peculiar to a stage of division preceding the development of a new cross wall. Using the freeze-etch technique, Remens (9) demonstrated large vesicles in mesosomes of young, unfixed *B. subtilis* cells which had been grown in complex medium. The diameter of the cylindrical segments, seen here in thin sections, is comparable with that reported by Ryter and co-workers (11) for similar mesosomal structures observed in negative stains.

Evidence that electron-dense particles are present in mesosomes of dividing cells has been reported by other investigators. Granboulan and Leduc (4) called attention to electron-dense "dots" in the center of mesosomal vesicles of *B. subtilis* cells embedded in glycol-
FIG. 1. Protoplasts of Bacillus subtilis embedded in Epon 812. Sections were stained for 30 min with saturated aqueous uranyl acetate followed by 2 min with lead citrate. A flagellum (F) is present on the surface of one protoplast. Numerous tubules and vesicles are present. Parallel arrays of ribosomes are in the cytoplasm of the protoplasts.

FIG. 2. Cells harvested from Tryptose broth, washed with stabilizing medium and fixed after 30 min. Large and small membrane sacs containing ribosomes are associated with septum formation. None of these cells showed obvious signs of plasmolysis or eversion of tubules into periplasmic spaces.

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methacrylate, but they did not know their significance. Nanninga (6), by the freeze-etch method, noted "a little knob in the center of each vesicle cup" of B. subtilis mesosomes. The particles are assumed to be ribosomes because of their size and staining characteristics. Ghosh and Murray (2) and Reaveley and Rogers (8) detected ribonucleic acid in chemical analyses of mesosomes separated from Listeria monocytogenes and B. subtilis, respectively. The latter investigators considered it to be contaminant material because of experimental variability even though one of their determinations showed that ribonucleic acid constituted almost 29% of the dry weight.

Some unanswered questions concerning mesosomal ribosomes are: (i) what is their origin, and (ii) are they involved in the synthesis of enzymes such as penicillinase (3, 14)? Investigations under a variety of environmental conditions may contribute significantly to identifying the physiological function(s) of this organelle in prokaryotic organisms.

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