Novel form of actin-based motility transports bacteria on the surfaces of infected cells

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Abstract
Enteropathogenic Escherichia coli (EPEC) attach to cells (attachment) lining the intestine and induce a decrease in the number of the cells'microvilli (effacement). This attachment and effacement is followed by diarrhea, which may be explained, at least in part, to the loss of microvilli and the decreased ability of the infected cells to absorb fluids. EPEC also attach to the surfaces of a number of cultured cells including Caco-2, LLC-PK, and PtK2 cells. The extracellular, attached EPEC induce filaments of actin to form in the cytoplasm just underneath the EPEC surface attachment sites. Beneath some of the attached EPEC, the actin filaments become organized into membrane encased columns that extend up to 6 m above the cell surface creating pedestals on which the EPEC rest. The raised pedestals can be readily observed in stereo pairs taken using the Intermediate Voltage Electron Microscope. The concentration of non-muscle isoforms of myosin II and tropomyosin near the base of the pedestals suggests a similarity of these structures to brush border microvilli. Video microscopy indicates that these EPEC pedestals can bend and undulate, alternately growing longer and shorter while remaining tethered in place on the cell surface. Some of the attached EPEC also translocate along the cell surface, reaching speeds up to 0.07 m/sec. Both types of movement are inhibited by cytochalasin D, indicating that actin polymerization in the pedestals is required for the motility of EPEC on the host cell surface. In this respect, EPEC motility on host cells resembles the intracellular motility of Listeria, but there are differences in the actin filament bundles induced by the two different bacteria. The most obvious one is the interposition of the cell membrane between EPEC and the actin filaments in the pedestal in contrast to the close apposition of actin filaments to Listeria. The intensity of fluorescence of rhodamine phalloidin is nearly uniform along most of the length of the pedestals indicating a constant number of actin filaments, whereas the fluorescence intensity decreases along the length of Listeria tails reflecting the disassembly that occurs all along the tails. EPEC's movements may be a hybrid of Listeria filopodia and Aplysia inductopodia movements. This paper is the first report of a microbe attached to the extracellular surface of an infected cell being propelled by an intracellular actin polymerization-dependent mechanism.

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