PERSPECTIVES: CELL BIOLOGY

Bacterial Spelunkers

Matthew A. Mulvey and Scott J. Hultgren

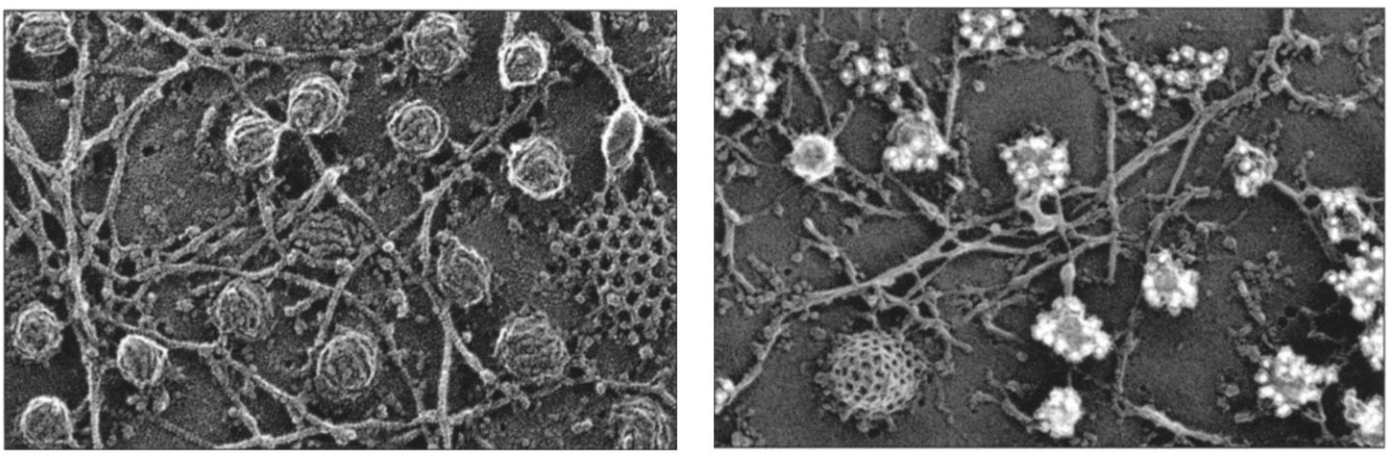
The uptake of bacterial pathogens (and their fate once inside a host cell) is directly influenced by interactions between bacterial adhesin molecules and host cell receptors. These interactions activate signal transduction cascades in the host cell and in so doing stimulate preexisting endocytic pathways, leading to internalization of the adherent microbe. Internalization often benefits an invading microbe, providing it with a safe haven and facilitating its dissemination within and across host tissue barriers (*1*). But, internalization is also an effective component of the host defense system—pathogens engulfed by professional phagocytic cells often enter a phagolysosome pathway where they may be rapidly destroyed. The ultimate fate of engulfed bacteria depends on which endocytic pathway they end up in.

It is well established that mast cells (a type of phagocytic cell) recognize and internalize *Escherichia coli* bacteria expressing the FimH adhesin (*2*–*6*). Present on the surface of *E. coli* and many other bacteria, FimH is a component of filamentous adhesive organelles called type 1 pili (*7*, *8*). The FimH of *E. coli* interacts with its receptor, CD48—a glycosylphosphatidylinositol (GPI)–anchored protein—on the surface of mast cells (*4*). CD48 and other GPI-anchored proteins do not have a transmembrane or cytoplasmic domain and, thus, it has been unclear how they transmit intracellular signals that direct internalization of adherent *E. coli*. Now, on page 785 of this issue, Shin and colleagues (*9*) propose that the uptake of *E. coli* bound to mast cell CD48 depends on specialized host membrane microdomains called caveolae. This mode of internalization may promote survival of the microbes within mast cells.

The term "caveolae," meaning "little caves," was first coined by the electron microscopist Yamada nearly half a century ago to describe flasklike invaginations of the plasma membrane (*10*). These structures, which are distinct from clathrin-coated pits, vary in size from 50 to 100 nm and have been identified in numerous types of cells (see the figure). Biochemical and microscopic data have shown that caveolae are enriched in glycosphingolipids, cholesterol, and caveolin, an integral membrane protein (*11*). From a biochemical perspective, caveolae resemble other detergent-insoluble membrane domains commonly referred to as lipid rafts—microdomains within eukaryotic cell plasma membranes that may be platforms for concentrating and sorting signal proteins and other molecules. Lipid rafts do not necessarily contain caveolin or have the flasklike morphology ascribed to caveolae (*11*, *12*). The incorporation of caveolin into lipid rafts, along with the presence of cholesterol, has been proposed to facilitate the formation of caveolae (*12*, *13*), although it has been argued that not all caveolae-like domains contain caveolin or assume a flasklike caveolar appearance (*11*).

Much is known about the structure and composition of caveolae, but it is still not clear what these membrane structures do in the cell. Numerous proteins involved in signal transduction appear to be localized in caveolae-like domains, and caveolin itself interacts directly with many signaling molecules (*12*). On the basis of such observations, it has been suggested that caveolae are centers from which multiple signaling pathways originate (*11*, *12*, *14*). In addition, caveolae have been implicated in endocytic events, including the transcytosis of macromolecules across cell layers and potocytosis, a potential method for "pumping" small molecules into cells (*11*, *14*). Caveolae and caveolae-like domains also may regulate the internalization of particulate agents such as viruses and bacteria (*15*, *16*).

Morphologically distinct caveolae have not been observed in mast cells, T and B cells, macrophages or other hematopoietic cells (*13*). But, the identification of caveolin in some macrophage cell lines (*17*, *18*) suggests that caveolin-rich, caveolae-like membrane domains may be important in certain types of effector immune cells. Shin *et al.* (*9*) now report that caveolae-like elements within mast cells are recruited to plasma membrane sites where *E. coli* are attached through FimH. These elements then fuse with other caveolae-related domains at these attachment sites, forming massive caveolae-like vesicles that encapsulate and internalize the adherent bacteria. Compounds that



Exploring caves. High-resolution, quick-freeze, deep-etch electron micrographs of caveolae. **(Left)** Multiple caveolae with characteristic striated coats are interspersed among cytoskeletal protein filaments and a honeycombed clathrin-coated pit in the plasma membrane (cytoplasmic face) of a cultured human fibroblast. **(Right)** The caveolae have been specifically labeled with gold beads attached to antibodies to caveolin-1.

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disrupt caveolae effectively block bacterial uptake. The mast cell CD48 receptor also appears to be intrinsically associated with caveolin- and cholesterol-rich domains during the internalization process. Interactions between cholera toxin B and its receptor G_{M1}, an integral component of caveolae (*19*), specifically interferes with bacterial uptake, apparently by usurping caveolae-like domains in the host plasma membrane. These findings suggest that cholera toxin B and *E. coli* that express FimH are internalized in similar ways, despite the fact that they adhere to different host cell GPI-anchored receptors. The Shin report indicates that caveolae-like regions in the mast cell plasma membrane are highly dynamic elements and that GPI-anchored receptors can transmit signals and activate host cell endocytic pathways through association with caveolae-like membrane domains.

The potential of FimH-expressing *E. coli* to co-opt caveolae-mediated endocytic pathways through interactions with CD48 implicates caveolae-related domains in the pathogenesis of certain bacteria. CD48 and other GPI-anchored proteins belong to an expanding class of receptors for various viruses, bacteria, and bacterial toxins. The entry of simian virus 40 into host cells and the uptake of the bacterium *Campylobacter jejuni* by cultured intestinal epithelial cells both depend on caveolae-like membrane domains (*15*, *16*). Caveolae do not appear to fuse with endocytic vesicles (*20*), and so internalization of bacteria through caveolae-like domains could conceivably facilitate their intracellular survival. Indeed, uptake of FimH-expressing *E. coli* by macrophages (a process that is also dependent on CD48 and possibly caveolae) seems to enhance bacterial survival within these immune effector cells (*17*).

The consequences of FimH-dependent interactions of bacteria with mast cells in vivo are unclear. Mast cells are long-lived, heterogeneous immune cells that are strategically situated at sites of microbial entry and are thought to be important for innate host defense. Upon recognizing invading microbes, mast cells become activated and release bactericidal compounds and proinflammatory molecules. The interactions of FimH with CD48 and caveolae-like membrane domains possibly could direct bacteria to nonbactericidal compartments within mast cells, providing them with an obvious survival advantage. Paradoxically, it has been reported that FimH-expressing *E. coli* can specifically enhance mast cell bactericidal activity and the release of inflammatory mediators (*4*, *21*). It will be interesting to learn whether FimH-expressing pathogenic bacteria, once inside the host cell, can modulate its activation and so potentially dampen the antimicrobial response. The importance of caveolin and caveolae-like membrane domains in the uptake of FimH-expressing *E. coli* by mast cells and other cell types awaits further clarification. It appears that the mysterious recesses of caveolae will keep researchers spelunking further into the depths of these fascinating cellular domains for years to come.

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PERSPECTIVES: STRUCTURE

Rhodopsin Sees the Light

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Seven-helix transmembrane receptors are signal detectors that activate heterotrimeric GTP-binding proteins (G proteins) in response to extracellular stimuli. Genes encoding this huge family of G protein–coupled receptors (GPCRs) occupy a hefty 5% of the worm genome and perhaps 3% of our own. GPCRs have cornered much of the signal-transducing market because their shared three-dimensional (3D) architecture, based on a transmembrane bundle of seven α helices, can be adapted to detect diverse extracellular stimuli—hormones, neurotransmitters, odorants, even photons. These receptors transmit signals specific for each extracellular stimulus across the membrane lipid bilayer by selectively activating different G proteins. On page 739 of this issue, Palczewski *et al.* (*1*) report the first 3D structure of a GPCR at 2.8 Å resolution. The x-ray crystal structure of rhodopsin—the light-detecting GPCR found in rod cells of the retina that signals through the G-protein trimer, G_t—is sure to evoke widespread excitement among investigators who want to know how GPCRs transduce signals.

In a number of GPCRs the activating extracellular stimulus (ligand) has been found to occupy a binding pocket within the bundle of seven α helices, in the plane of the lipid bilayer. Somehow, ligand occupancy of the pocket induces rearrangements of the α helices, which in turn alter the shape of the receptor's cytoplasmic surface, thus activating the appropriate G proteins. The rhodopsin structure brings these events into sharp focus. Until now, bouncing notions back and forth about how GPCRs work was like playing tennis without a net. With the new net provided by Palczewski *et al.* the game will be harder, but also more fun.

Rhodopsin, unlike most GPCRs, binds its ligand (retinal) covalently (to lysine-296 in helix VII), both in the inactive (dark) state (represented by the new 3D structure) and also after photoactivation. Spectroscopic observations have shown that a photon causes the inactive ligand, 11-*cis*-retinal, to change into the all-*trans* isomeric form, which activates the rhodopsin receptor. Now we can see the chromophore's inactive form cradled in a pocket (see the figure) formed by transmembrane helices and by an elaborate, multilayered plug that comprises most of the receptor's extracellular domain. All elements of this domain contribute to the plug, which in fact contacts the chromophore. It is already established that the α-helix bundle forms the walls of the retinal-binding pocket, but an extracellular plug blocking exit from the pocket is a real surprise. Similar plugs will probably not be found in most other GPCRs because their ligands (which are reversibly bound) enter and leave the binding pocket in milliseconds.

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