in brain areas known to be involved in AD.

Multiple lines of transgenic animals engineered to develop AD-like amyloid-β plaque formation have been developed. Yet despite the presence of abundant senile plaques in these mice, there appears to be little or no neuronal cell loss (*15*, *16*). This suggests that amyloid deposits alone may be insufficient to cause neurodegeneration. In contrast, the formation of tau tangles accompanied by axonal and/or neuronal loss has been detected in several transgenic mouse models of tauopathies (*17*, *18*). Indeed, a recent study from Feany's group showed that transgenic flies overexpressing wild-type and mutant forms of human tau accumulate tau protein, resulting in progressive neurodegeneration and eventual neuronal loss in an age-dependent manner (*19*). Intriguingly, the wild-type and mutant fly models of tauopathy did not develop the neurofibrillary tangles characteristic of rodent and human AD, although aggregates of tau were detected. An earlier study in lamprey fish demonstrated that expression of wild-type tau in lamprey neurons resulted in tau-rich tangles and degeneration of the neurons containing them (*20*). Because the development of tau tangles is an invariant feature of both the rare familial form and the common sporadic form of AD, it is tempting to speculate that the formation of tau tangles or aggregates may be a necessary prerequisite for neurodegeneration in AD (see the figure). The identification of a number of neurodegenerative diseases characterized by tau tangles (either with or without other pathologies) suggests that tangle formation may initiate as well as contribute to the final step in the relentlessly progressive brain degeneration that characterizes AD. Future experiments with Lewis's Tau/APP mice could test this hypothesis by determining whether brain regions that have more tangles than plaques also have increased neuronal loss.

The discovery of possible interactions between amyloid-β deposits and tau tangles and the availability of transgenic mouse models containing both pathologies will facilitate efforts to develop more effective AD therapies. Indeed, the success of several emerging therapeutic approaches that target the production, aggregation, and clearance of amyloid-β peptide deposits is likely to depend on the severity or stage of AD brain degeneration and the extent of tangle formation. Thus, eliminating amyloid-β deposits by administering an amyloid-β vaccine may improve cognition in AD patients who have few tau tangles, but may have little or no effect on late-stage AD patients who have already developed significant tau pathology. It would be wise for future AD therapies to combine targeting of amyloid-β deposits with strategies for eliminating tau tangles.

References

1. J. Lewis *et al.*, *Science* **293**, 1487 (2001).
2. J. Götz, F. Chen, J. van Dorpe, R. M. Nitsch *Science* **293**, 1491 (2001).
3. A. Goate *et al.*, *Nature* **349**, 704 (1991).
4. R. Sherrington *et al.*, *Nature* **375**, 754 (1995).
5. E. Levy-Lahad *et al.*, *Science* **269**, 973 (1995).
6. D. Sheuner *et al.*, *Nature Med.* **2**, 864 (1996).
7. D. R. Borchelt *et al.*, *Neuron* **17**, 1005 (1996).
8. M. Citron *et al.*, *Nature Med.* **3**, 67 (1997)
9. D. Games *et al.*, *Nature* **373**, 523 (1995).
10. P. Poorkaj *et al.*, *Annal. Neurol.* **43**, 815 (1998).
11. M. Hutton *et al.*, *Nature* **393**, 702 (1998).
12. M. G. Spillantini *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 7737 (1998).
13. K. Hsiao *et al.*, *Science* **274**, 99 (1996).
14. K. Duff *et al.*, *Neurobiol. Dis.* **7**, 87 (2000).
15. M. C. Irizarry *et al.*, *J. Neuropathol. Exp. Neurol.* **56**, 965 (1997).
16. M. C. Irizarry *et al.*, *J. Neurosci.* **17**, 7053 (1997).
17. T. Ishihara *et al.*, *Neuron* **24**, 751 (1999).
18. J. Lewis *et al.*, *Nature Genet.* **25**, 402 (2000).
19. C. W. Wittman *et al.*, *Science* **293**, 711 (2001).
20. G. F. Hall, B. Chu, G. Lee, J. Yao *J. Cell Sci.* **113**, 1373 (2000).

PERSPECTIVES: CELL BIOLOGY

Caveolae—Not Just Craters in the Cellular Landscape

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Caveolae—flask-shaped invaginations in the plasma membrane—are present on many types of mammalian cells, including endothelial cells, smooth muscle cells, and adipocytes. Like lipid rafts (their close relatives), caveolae are plasma membrane assemblies of glycosphingolipids and cholesterol that are associated with specific molecules including signaling proteins (*1*, *2*). Often they contain the protein caveolin and have proteins anchored to glycosylphosphatidylinositol (GPI), a plasma membrane phospholipid. But exactly why cells have caveolae or lipid rafts is puzzling. They are believed to be involved in cholesterol transport, the transport of solutes across endothelial cells, tumor suppression, and signal transduction through immune and growth factor receptors. In addition, it seems they have been commandeered by several species of viruses, parasites, and bacteria (and even bacterial toxins) to enable these pathogens to enter host cells (*3*–*13*). Classic endocytosis (uptake of extracellular agents) depends on clathrin-coated pits and involves an intracellular pathway in which lysosomes fuse with internalized vesicles, degrading their contents. In contrast, and clearly of benefit to pathogens, caveolae-dependent endocytosis does not feed into the lysosome pathway and does not result in the degradation of the contents of caveolar vesicles.

If taken up into host cells by classic endocytosis, intracellular pathogens must avoid degradation in the endosome-lysosome pathway, either by escaping from their endocytic vacuoles (phagosomes) into the cytoplasm (before lysosomes fuse with phagosomes) or by actively neutralizing the microbicidal agents inside the phagolysosome after fusion. Smart pathogens and bacterial toxins can avoid this problem by binding to caveolae and triggering endocytosis through a pathway that avoids lysosomes altogether. *Escherichia coli* bacteria that express the FimH antigen use caveolae to invade phagocytic cells such as macrophages (*3*, *4*). Under serum-free conditions in culture, FimH-expressing *E. coli* are internalized by phagocytes and, strikingly, remain viable within phagosomes, which fail to fuse with lysosomes (*3*). Known disrupters of caveolae formation block uptake of FimH-expressing *E. coli* but not of opsonized *E. coli* (bacteria coated in antibody) that are taken up into clathrin-coated pits and enter the classic endosome-lysosome pathway (*4*). Apparently, caveolae-dependent endocytosis is triggered when bacterial FimH binds to its GPI-anchored receptor CD48 in the caveolae of the host cell (*4*). Once bound to caveolae, the bacteria become encapsulated within caveolar vesicles characterized by specific markers (cholesterol, caveolin, and G_{M1}) (*4*).

A broad range of pathogens (or their products) prefer caveolae- or lipid raft–mediated endocytosis, including: mycobacteria (*5*), simian virus 40 (SV40) (*2*), *Toxoplasma gondii* and *Plasmodium falciparum* (malaria) parasites (*6*, *7*), and toxins of *Vibrio cholerae* and *Helicobacter pylori* bacteria (*8*, *9*). Caveolae are also important in pathogen trafficking within the cell after endocytosis, as exemplified by the fact that they shuttle SV40 virus to the endoplasmic reticulum (*2*) and cholera toxin to the Golgi apparatus (*8*). In some cases, the endocytosed microbial cargo is directly transported across the cell (transcytosed), as exemplified by human im-

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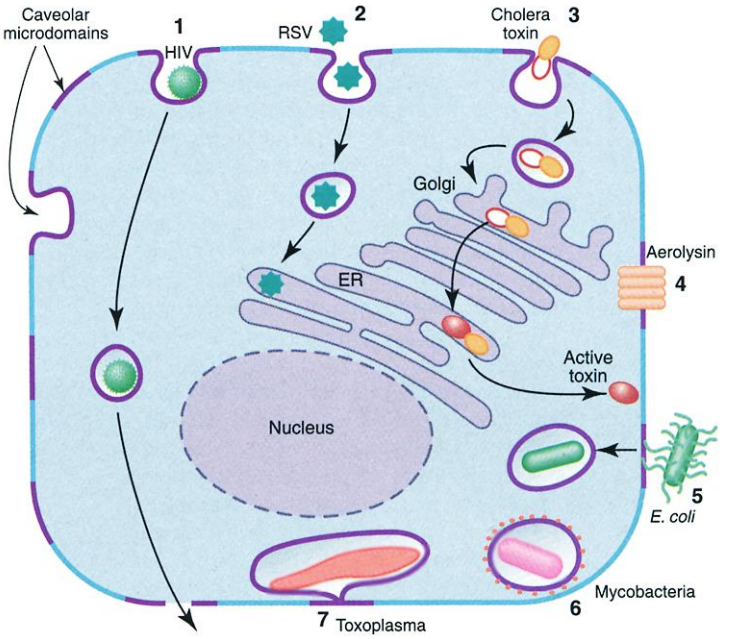
munodeficiency virus (HIV) (*10*). Occasionally, the intrinsic biophysical properties of caveolae or lipid rafts, rather than their endocytic properties, are co-opted by pathogens. For example, the bacterial toxins aerolysin (*11*) and streptolysin O use caveolae (lipid rafts) to facilitate their targeting and assembly as discrete pores in the host cell plasma membrane (see the figure).

Cumulatively, these findings point to the existence of a highly versatile caveolae-dependent endocytic pathway in mammalian cells that protects microbes from being degraded by lysosomes. Of course, mammalian cells have not developed caveolae as an aid to pathogen invasion. Caveolae-dependent endocytosis is valuable to mammalian cells because many macromolecules must be routinely imported and transcytosed while remaining intact and biologically active. Emerging evidence indicates that several of these macromolecules, including folate (*2*), chemokines (*12*), and immunoglobulin A (*13*), are indeed translocated, apparently without being degraded, after caveolae-mediated (clathrin-independent) endocytosis. It is ironic that caveolae have now been implicated in endocytosis, because their discovery precedes that of clathrin-coated endocytic vesicles by at least 10 years.



One smart way to avoid destruction. (**1**) HIV particles bind to glycosphingolipid galactosyl ceramide receptors in caveolae and are directly transported across epithelial cells (transcytosis). (**2**) Respiratory syncytial virus (RSV) is internalized by caveolae and directly carried to the endoplasmic reticulum of the host cell. (**3**) Cholera toxin binds to G_{M1} molecules within caveolae and is translocated in caveolar vesicles to the Golgi apparatus. (**4**) Aerolysin and streptolysin O bacterial toxins are assembled at caveolae and form channels spanning the plasma membrane. (**5**) FimH-expressing *E. coli* bind to the GPI-anchored moiety CD48 in caveolae, triggering recruitment of caveolae and endocytosis. (**6**) Mycobacterial species enter phagocytes through caveolae and initiate the aggregation of a distinct host protein, which prevents subsequent fusion of the phagosome with lysosomes. (**7**) *T. gondii* and *P. falciparum* actively induce the formation of intracellular vacuoles rich in caveolar components that fail to fuse with lysosomes.

Many intriguing questions arise about caveolae-mediated endocytosis and trafficking of macromolecules and microorganisms through the cell. How can particulate and soluble agents—so different in size and in the host cell receptors to which they bind—adopt the same caveolae-dependent endocytic machinery to gain entry to the cell? How do caveolar vesicles inside the cell avoid fusion with lysosomes and the destruction of the pathogenic or macromolecular cargo that they carry? And what determines whether cargo-carrying caveolar vesicles will be targeted to particular cellular sites or transported across the cell? Studying microbes in vitro may yield some answers. For example, studies of microbes and bacterial toxins that use caveolae indicate that the receptors to which they bind are localized in caveolae (*4, 8*). Thus, a criterion for caveolae-mediated uptake of a microorganism is that the putative receptor for that pathogen is either a constituent of caveolae, or moves into caveolae once there is contact between the pathogen and the host cell.

Once inside the cell, how do caveolae vesicles avoid fusing with lysosomes and move to the correct intracellular sites? The molecular composition of caveolae is dynamic, and the signaling molecules arranged along the cytoplasmic face of caveolae are likely to be crucial for preventing lysosomes from fusing with caveolar vesicles. Such molecules include those that mobilize the cellular cytoskeleton and so modulate both endocytosis and trafficking events. Indeed, both endocytosis through caveolae and subsequent trafficking of caveolar vesicles are closely tied to actin polymerization (*14*). Clustering of receptors, lipids, and signaling molecules in caveolae creates an invagination suitable for endocytosis, and presumably the tight association of this site with the cytoskeleton obviates the need for the membrane-deforming contribution of clathrin coat proteins.

Not all pathogens use caveolae in a passive manner. Certain long-term intracellular pathogens appear to actively tailor the natural composition of their caveolar capsules, presumably to facilitate intracellular residency and to blunt their trafficking to inhospitable terrain. For example, *T. gondii* and *P. falciparum* modulate the composition of their parasitophorous vacuoles by preferentially enriching the vacuole membranes with GPI-anchored and cytoplasmic proteins derived from caveolae, while simultaneously excluding specific molecules that direct fusion with lysosomes (*6, 7*). Intracellular *Mycobacterium bovis* appears to accumulate a distinct host protein (TACO) in the caveolar vesicle membrane, which prevents lysosomes from fusing with the vesicle (*5*). It is not yet clear whether any of these pathogen-directed modifications of caveolar capsules affect intracellular trafficking.

Clearly, we need more information about the molecular pathways directing caveolae-mediated endocytosis and intracellular trafficking. Studying the endocytosis and transport of pathogens is beneficial because their size makes them easy to trace within host cells. Because microbes discovered caveolae at least 100 million years ago and have adapted so that they can co-opt them during host cell invasion, it is not unreasonable to surmise that microbes will help us to understand why mammalian cells have caveolae in the first place. Such microbial studies are not only of intrinsic interest—they will help us to design better ways to deliver drugs to cells and more efficacious therapeutic strategies for blocking pathogen infection.

References and Notes

1. K. Simons, E. Ikonen, *Nature* **387**, 569 (1997).
2. R. G. Anderson, *Annu. Rev. Biochem.* **67**, 199 (1998).
3. D. M. Baorto *et al.*, *Nature* **389**, 636 (1997).
4. J. S. Shin, Z. Gao, S. N. Abraham, *Science* **289**, 785 (2000).
5. J. Gatfield, J. Pieters, *Science* **288**, 1647 (2000).
6. S. Lauer *et al.*, *EMBO J.* **19**, 3556 (2000).
7. D. G. Mordue, N. Desai, M. Dustin, L. D. Sibley, *J. Exp. Med.* **190**, 1783 (1999).
8. W. I. Lencer, T. R. Hirst, R. K. Holmes, *Biochim. Biophys. Acta* **1450**, 177 (1999).
9. V. Ricci *et al.*, *Mol. Biol. Cell* **11**, 3897 (2000).
10. M. Bomsel, A. Alfsen, *Mol. Biol. Cell* **11**, 2576 (2000).
11. L. Abrami, M. Fivaz, F. G. van der Goot, *Trends Microbiol.* **8**, 168 (2000).
12. J. Middleton *et al.*, *Cell* **91**, 385 (1997).
13. G. H. Hansen *et al.*, *Gastroenterology* **116**, 610 (1999).
14. A. L. Rozelle *et al.*, *Curr. Biol.* **10**, 311 (2000).
15. We thank M. Kuehn and J. Hart for helpful discussions.