Potocytosis: Sequestration and Transport of Small Molecules by Caveolae

RICHARD G. W. ANDERSON,* BARTON A. KAMEN, KAREN G. ROTHBERG, STEPHEN W. LACEY

THE UPTAKE OF SMALL MOLECULES (LESS THAN 1 KILODALTON) by eukaryotic cells is generally thought to occur by movement across the membrane through water-filled channels, carriers, or transporters (*1*). In contrast, the uptake of macromolecules such as low density lipoproteins, transferrin, and protein toxins occurs through receptor-mediated endocytosis (*2*). Studies on receptor-dependent folate transport (*3–9*) have drawn our attention to the existence of a mechanism for small molecule transport that embodies certain features of receptor-mediated endocytosis. This pathway uses caveolae rather than clathrin-coated pits as an uptake vehicle. Caveolae, working together with glycosylphosphatidylinositol (GPI)-anchored membrane proteins that are able to concentrate select molecules at these sites, may take up a variety of different small molecules. We call this process potocytosis.

Caveolae (*10*) and plasmalemmal vesicles (*11*) are interchangeable terms for the same membrane feature. They are recognized in electron microscopic images as invaginated pits or vesicles that have a uniform diameter of ~50 nm. The cytoplasmic surface of each caveola is decorated with a coat material composed of delicate filaments arranged into striations on the membrane surface (*2, 12*). Both invaginated and noninvaginated caveolae are frequently seen, which suggests that the shape of caveolae can change as a consequence of the mechanical activity of the striated coat (*2*). The organization of the coat is disrupted by sterol-binding drugs (*13*), and the number of visible caveolae is reduced in cholesterol-depleted cells (*9*). In addition, the sterol-binding drug filipin causes the formation of cholesterol precipitates over caveola membrane (*14*), which indicates that there is a high concentration of cholesterol in this membrane domain.

Insight into the role of caveolae in potocytosis was gained from studying the function of the folate receptor. Certain cultured cells express a high affinity receptor for 5-methyltetrahydrofolate on their surfaces (*3*). The cloning of the receptor complementary DNA (*7*) and the biochemical analysis of the pure protein (*15*) have shown that this receptor is an integral membrane protein anchored by GPI. Folate receptors occur in dense clusters on the cell surface in association with caveolae (*8*). Each cluster contains up to 700 receptor molecules with an estimated density of ~30,000 molecules per square micrometer (*8*). Cholesterol-binding drugs such as filipin or nystatin and depletion of cholesterol from the plasma membrane cause the folate receptors to uncluster and lose their association with caveolae (*9*). Thus, the physical forces that maintain the receptors in clustered arrays appear to come from interactions between the lipid portion of the GPI anchor and the surrounding lipids. Both the folate receptor and the caveolae therefore seem to depend on cholesterol for structure and function.

A role for this receptor in 5-methyltetrahydrofolate internalization was determined on studies of folate-depleted MA104 cells (*3*). Half of the membrane receptors in these cells were able to bind folate at 0°C; however, when the cells were incubated continuously at 37°C for 1 hour, both internal and external populations of receptor filled with the ligand (*4*). Occupied external receptors could be distinguished from occupied internal receptors because folate was released from the former by a brief acid wash. Acid-labile receptors exchanged with acid-resistant complexes once every hour at 37°C (*4*), which indicates that all receptors recycle. As soon as the receptor-vitamin (ligand) complex was internalized, the vitamin was delivered to the cytoplasm of the cell. After ~6 hours of incubation, the cell became full (*6*), and accumulation in the cytoplasm stopped, even though normal amounts of receptor-vitamin complex continued to recycle.

The sequestration step increases the amount of folate in caveolae to a concentration that favors movement across the membrane through an organic anion carrier into the cytoplasm (*6*). This occurs within closed caveolae after the folate dissociates from receptors in response to a low pH (*4*) (Fig. 1). Besides the chemical and morphological information that links folate receptor clusters to caveolae, the internalization and recycling kinetics of the receptor (*4*) are similar to the kinetics of bulk-phase sequestration by caveolae (*16*). Also, the folate receptor is not present within any internal endocytic compartments, and when these cells are incubated at 37°C with antibodies to the folate receptor, they do not accumulate immunoglobulin G in endosomes or lysosomes (*8*). Through cyclic phases of opening and closing, caveolae transiently create a sequestration compartment that operates at or near the cell surface without merging with the coated pit, endocytic pathway.

Caveolae and GPI-anchored membrane proteins are common among cells. Moreover, several lipid-anchored membrane molecules have been found to be associated with caveolae. This raises the possibility that GPI anchors and caveolae might work together to mediate the uptake of many different extracellular molecules by potocytosis. There are several ways this might occur.

A number of proteins that are anchored through GPI to the membrane are enzymes. Caveolae could use these enzymes to sequester small molecules and direct their movement into cells. In the open configuration, the caveola is accessible by substrates for the

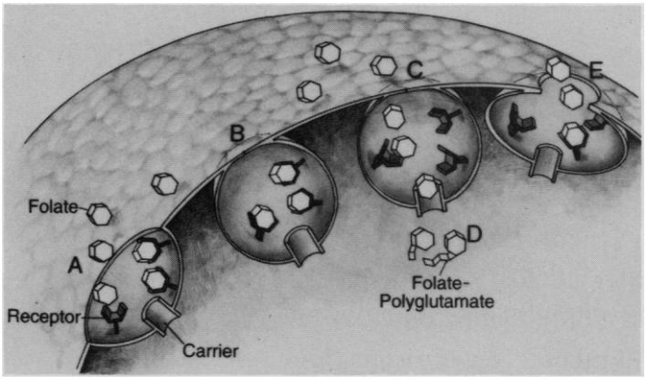

Fig. 1. A model for receptor-mediated potocytosis of folate. Caveolae contain receptors that bind folate when the compartment is open (A). The caveola closes and possibly detaches from the membrane (B), and a proton gradient is generated that causes the folate to dissociate from the receptor (C). The high concentration of folate generated in the caveola space creates a gradient that favors movement across the membrane by an anion carrier. The folate is polyglutamated in the cytoplasm to retain the vitamin within the cell (D). The caveola reopens to initiate another round of folate uptake (E).

R. G. W. Anderson and K. G. Rothberg, Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75235. B. A. Kamen, Departments of Pharmacology and Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX 75235. S. W. Lacey, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75235.

*To whom correspondence should be addressed.

enzyme. After closure, the low molecular weight products that are generated increase to a concentration that favors movement across the membrane by a carrier. Thus, caveolae could act transiently to form vesicular compartments that protect either the substrate or the product and thereby control the direction of molecular movement to benefit the cell.

Caveolae may also control the transport of small molecules into cells by housing a receptor that could bind an extracellular carrier protein bearing a low molecular weight ligand. The caveola would close, the pH or some other parameter would change, and the ligand would be released. Transport across the membrane into the cytoplasm would occur as the concentration increased. Potocytosis offers the cell a different kind of control over the spatial and temporal uptake of the ligand than does receptor-mediated endocytosis, which involves extensive membrane traffic through the cellular endomembrane system.

Another potential function for potocytosis is to receive or to transmit various kinds of cellular signals, such as signaling molecules derived from GPI-anchored membrane molecules. It has been proposed that insulin (*17*), nerve growth factor (*18*), and interleukin-2 (*19*) transmit messages to cells in part through inositol phosphoglycan (IPG) intermediates that are generated from GPI-anchored membrane proteins or lipids. All GPI-anchored molecules appear to be located on the external surface of the plasma membrane (*20*) so that any released IPG would rapidly diffuse away from the cell. Caveolae could solve this problem by controlling the transmembrane movement of either IPG or its metabolite if both the GPI-anchored molecule and an appropriate phospholipase were housed in this compartment. Insulin receptors have been found to be located near adipocyte caveolae (*21*), which place them in an optimal position for stimulating a reaction cascade that leads to the import of IPG intermediates.

As a result of studies of folate internalization and bulk-phase sequestration by caveolae (*16*), certain characteristics can be predicted of a membrane-bound molecule suspected of being involved in potocytosis. (i) The molecule should be associated with caveolae. (ii) Internalization and recycling of this molecule should occur with a cycle time of about 1 hour. (iii) Internalization ought to be relatively temperature-insensitive, as compared to internalization by clathrin-coated pits. (iv) The molecule should not be degraded after internalization. The folate receptor (*3–9*), 5′-nucleotidase (*22*), alkaline phosphatase (*23*), cholera toxin (*24*), and lipoprotein lipase (*25*) are examples of membrane-associated molecules that meet one or more of these criteria. Further work is required to determine if these molecules are involved in potocytosis.

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Superantigens and Endogenous Retroviruses: A Confluence of Puzzles

JOHN M. COFFIN

A SPECIAL PLEASURE OF DOING SCIENCE COMES WHEN long-standing problems in seemingly unrelated areas flow together into a single problem. The association between certain retroviruses—the mammary tumor viruses—and superantigens is one such event that not only provides a solution to an old immunological puzzle and partially solves a virological one, but promises to provide new insights into the interaction of viruses with the immune system.

T cells can be activated or depleted independently of conventional antigens (*1–3*). Bacterial superantigens comprise a set of bacterially produced protein toxins that, when complexed with major histocompatibility complex (MHC) class II molecules, specifically bind to certain subsets of T cell receptor (TCR) β chains (*4*). This binding causes stimulation of T helper cell function as though the cell had been exposed to antigen in the context of a presenting cell.

Endogenous superantigens were first identified as genetic elements that could stimulate T cell proliferation in mixed cultures of lymphocytes from mice that were matched at the MHC. The genes responsible for this effect were named *Mls* (for minor lymphocyte stimulating), and several distinct loci have been identified. Like bacterial superantigens, Mls stimulates only T cells that express a particular one (or a subset) of the 20 or so genes encoding alternative β chains of the TCR (Table 1). But like normal antigens, stimulation requires presentation of the *Mls* product together with MHC class II proteins on the surface of antigen-presenting cells. During development, mice that have *Mls* actively delete those T cells that express the V_β subset or subsets that recognize the specific *Mls* product. The genetics of *Mls* were initially confusing, but eventually it was recognized that there are several distinct *Mls* loci, each of which has two alleles: a positive allele that confers a specific pattern of V_β reactivity and a negative or null allele that confers no reactivity. Although the properties of Mls proteins had been inferred

Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111.