

invited review

Modulation of membrane traffic by mechanical stimuli

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Apodaca, Gerard. Modulation of membrane traffic by mechanical stimuli. *Am J Physiol Renal Physiol* 282: F179–F190, 2002.—All cells experience and respond to mechanical stimuli, such as changes in plasma membrane tension, shear stress, hydrostatic pressure, and compression. This review is an examination of the changes in membrane traffic that occur in response to mechanical forces. The plasma membrane has an associated tension that modulates both exocytosis and endocytosis. As membrane tension increases, exocytosis is stimulated, which acts to decrease membrane tension. In contrast, increased membrane tension slows endocytosis, whereas decreased tension stimulates internalization. In most cases, secretion is stimulated by external mechanical stimuli. However, in some cells mechanical forces block secretion. External stimuli also enhance membrane and fluid endocytosis in several cell types. Transduction of mechanical stimuli into changes in exocytosis/endocytosis may involve the cytoskeleton, stretch-activated channels, integrins, phospholipases, tyrosine kinases, and cAMP.

membrane tension; stretch; mechanical forces; exocytosis; endocytosis; secretion; cytoskeleton; mechanosensors; signal transduction

ALL CELLS, WHETHER INDIVIDUALLY or in a tissue, experience and respond to intracellular and extracellular mechanical stimuli (43). Cell-associated forces include osmotic pressure and the forces generated by the cytoskeleton as it pushes and pulls against the plasma membrane and the intracellular organelles (58). External forces can be static, incremental, or cyclical and include hydrostatic pressure, shear stress, twisting, compression, and high-frequency vibrations (5). The cell responds to these stimuli by modifying its rate of division, death, differentiation, movement, signal transduction, gene expression, secretion, and endocytosis (6, 7, 21, 24, 27, 43, 62, 84, 107). The effects of mechanical forces on cell growth, signal transduction, gene expression, and ion transport have been the focus of several recent reviews (24, 27, 43, 107). This review is an examination of how endomembranous traffic is modulated by mechanical stimuli.

ENDOCYTOSIS, EXOCYTOSIS, AND PLASMA MEMBRANE TENSION

Endocytosis is a diverse set of processes whereby patches of membrane are invaginated and budded off of specialized domains of the plasma membrane (87). A small amount of fluid is trapped in the forming endocytic vesicles. Types of endocytosis include clathrin dependent (also known as receptor-mediated endocytosis), caveolar dependent, non-clathrin dependent, macropinocytosis, and phagocytosis (87). Endocytosed material is delivered to endosomes and can be recycled back to the plasma membrane, delivered to the trans-Golgi network, sent to lysosomes (where it is degraded), or in polarized epithelial cells it can be delivered to the opposite cell surface in a process termed transcytosis (87). In exocytosis, intracellular vesicles fuse with the plasma membrane, delivering vesicle membrane proteins and releasing secretory cargo in the process (48). Exocytic vesicles come in the form of constitutive cargo released from the Golgi, or specialized secretory vesicles that are also formed at the Golgi but fuse with the plasma membrane in response to external stimuli. Endosome-derived components in-

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cluding recycling vesicles, secretory lysosomes, and transcytotic vesicles also undergo exocytosis (1, 87).

Plasma Membrane Tension

One intrinsic feature of the plasma membrane is its associated membrane tension (21, 84). As will be described below, plasma membrane tension has a significant impact on exocytosis and endocytosis. For a simple thin-walled sphere, Laplace's law defines the in-plane tension as tension = $\frac{1}{2}$ (pressure) \times (radius of curvature). The in-plane tension of a cell's plasma membrane is more complicated to discern as the plasma membrane is attached to the underlying cytoskeleton, and this adhesion contributes significantly to the apparent membrane tension (115). Other factors affecting plasma membrane tension include hydrostatic pressure across the membrane and effects due to local membrane curvature (e.g., regions of membrane associated with microvilli) (115).

Previously, mechanical deformation was used to study the physical nature of the plasma membrane (20). A newer technique employs laser tweezers (see Fig. 1) (120). In these studies, antibody-, lectin-, or extracellular matrix-coated latex beads are allowed to bind to the plasma membrane. The bead is trapped by a laser tweezer, a device that depends on the small pressure generated as focused laser light refracts through the transparent bead, pushing the bead toward the focal point of the laser light. The trapped bead and the cell are pulled from one another at a constant velocity by a motorized stage, whereby the attached membrane is pulled into a thin long membrane tether that remains cell associated but is free of cytoskeletal attachment. The in-plane membrane tension, bending stiffness of the membrane, and cytoskel-

etal adhesion all act to pull the membrane tether back onto the cell (20, 120). In doing so, a tether force is generated that can be calculated by measuring the displacement of the bead in the laser trap. The bending stiffness of the membrane is thought to be relatively constant as is the in-plane membrane tension. Under most conditions, changes in tethering force are thought to result from changes in membrane-cytoskeleton attachment (9, 21). Membrane tension, which can be calculated from the tether force, is ~ 0.02 – 0.12 mN/m across all regions of the plasma membrane (22, 23, 62, 84, 95).

The plasma membrane is largely inelastic and can increase in area only 2–3% before rupture occurs (lytic tensions are in the range of 1–12 mN/m) (21, 84). When plant or animal cells are placed in hypotonic medium, which induces cell swelling, the plasma membrane tension rises dramatically and then settles at a new but higher steady-state level (20, 22, 62). In some cell types, unfolding of surface membrane specializations (e.g., membrane folds and microvilli) can accommodate some of this cell expansion (94, 118, 121). However, in cells that either lack these reserves of plasma membrane or deplete them, cell swelling is accompanied by a rise in capacitance (a measure of membrane surface area where $1 \mu\text{F} \approx 0.5$ – 1 cm^2 of surface area) and cell volume (22, 54). The increase in capacitance is the result of exocytosis, which acts to decrease plasma membrane tension. In general, increases in plasma membrane tension are followed by increases in exocytosis (43, 84); however, this is not always the case (see the discussion below). In the case of guard cell protoplasts, the changes in capacitance occur in a stair-step manner, which may reflect the fusion of discrete packets of exocytic cargo (55). When the cells are returned

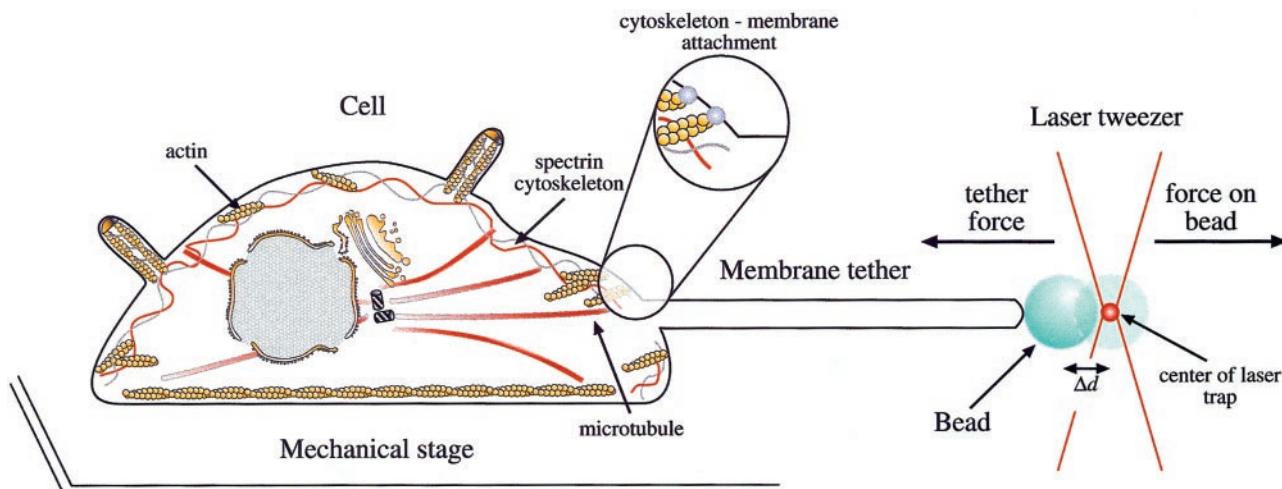


Fig. 1. Use of laser tweezers to probe plasma membrane tension. An antibody-, lectin-, or extracellular matrix-coated bead is bound to the plasma membrane of the cell. The bead is trapped by a laser tweezer and then pulled away from the cell at a constant velocity by a mechanical stage. Attached to the bead is a thin membrane tether that remains associated with the plasma membrane but is free of the cytoskeleton. The in-plane membrane tension, bending stiffness of the membrane, and cytoskeleton attachment pull the bead toward the cell and contribute to the tether force. The tether force is calculated by measuring the displacement of the bead from the center of the laser (Δd) and calibration of the trap. An apparent membrane tension can be calculated from the tether force (116). The figure is redrawn from Ref. 116.

to isotonic conditions, the tension rapidly drops and the plasma membrane is reduced by endocytosis (22, 54, 67).

In addition to modulating exocytosis, membrane tension may also regulate endocytosis. In rat basophilic leukemia cells, stimulation of secretion is associated with a decrease in membrane tension (the result of secretory vesicle exocytosis), which is followed by a rapid rise in endocytosis (23). In HeLa cells, membrane tension increases during mitosis, and endocytosis is inhibited in this phase of the cell cycle (95). This inhibition was previously ascribed to cell cycle-dependent modulation of endocytic machinery, e.g., phosphorylation of regulatory Rab proteins (3). Interestingly, treatment of mitotic cells with agents that decrease membrane tension (DMSO, deoxycholic acid, or ethanol) causes a rapid rise in the endocytic rate (95). These and other observations have led to the hypothesis that tension, which is dependent on membrane traffic and membrane-cytoskeleton adhesion, regulates the rate of endocytosis (23, 62, 84, 95, 115, 138). The underlying mechanism of how tension regulates endocytosis is unknown, but it may reflect the physical nature of the endocytic process. Endocytosis requires deformation of the membrane and detachment of membrane from the cortical cytoskeleton. This process is similar to tether formation (115). When membrane tension is high it would counteract the force necessary to deform the membrane and decrease endocytic rate (115).

By adding or removing plasma membrane, exocytosis and endocytosis act to modulate plasma membrane tension. Moreover, exocytosis and endocytosis can be accompanied by local changes in the cortical actin cytoskeleton (2, 130). Because membrane tension is dependent on cytoskeleton-membrane adhesion, these changes may significantly impact membrane tension. How the cell senses membrane tension or determines its set point is presently unknown. Nor is it understood how alterations in the set point are transduced into changes in membrane traffic. Possible mechanosensors and mechanotransduction pathways are described at the end of this review.

MECHANICAL STIMULATION OF SECRETION

Experimental Simulation of Mechanical Stimuli

Experimental manipulations that mechanically deform cells cause changes in plasma membrane tension and alter secretion. In addition to osmotic stretch described above, other techniques used to mimic physiologically relevant mechanical stimuli include passing fluid over cells plated on solid supports (e.g., cultured endothelial cells), which generates shear stress, or passing fluid through a tubule (e.g., an isolated nephron segment), which generates both hydrostatic pressure and shear stress (see Table 1 for use of these methods). Alternatively, cells (e.g., cardiac myocytes) are grown on flexible supports such as silicone and then subjected to cyclical distension by a vacuum pulled under the support film and then released (Table 1). This technique can be mechanized and computer

controlled. Lung cells can be grown in foam matrices and these organotypic cultures are then mechanically elongated by a computer-controlled stretching device (Table 1). A summary of these and other methods can be found in a recent review by Brown (12).

Stimulation of Exocytic Traffic by Mechanical Stimuli

When cells are exposed to various mechanical manipulations, exocytic traffic is stimulated in several systems, including adult and fetal lung cells, endothelial cells, cardiac myocytes, smooth muscle cells, skeletal muscle cells, kidney mesangial cells, kidney tubular epithelial cells, astrocytes, bladder umbrella cells, fibroblasts, plant guard cells, mammary gland cells, neuronal cells, osteocytes, pleural mesothelial cells, retinal pigment epithelial cells, and toad bladder cells (see Table 1 for details). Stimulated secretion of extracellular matrix proteins, surfactant proteolipid, proteinases, growth factors such as platelet-derived growth factor, nerve growth factor and transforming growth factor- β , and hormones such as atrial natriuretic factor (ANF), angiotensin II, and endothelin 1 is observed in stretched cells (Table 1). Moreover, stretch stimulates the release of small molecules such as ATP, prostacyclin, nitric oxide (NO), and the cytosolic basic fibroblast growth factor, which is secreted by a nonclassic secretory pathway (Table 1). Secretion of angiotensin II is stimulated within 1 min of the introduction of the mechanical force (108, 110), whereas in other cases stimulation is observed after several hours of force application (75, 77, 91, 113, 127, 129, 134). In the latter case, it is likely that the observed effects reflect stretch-regulated enhancement of gene expression and may not represent direct effects on membrane traffic.

Inhibition of Exocytic Traffic by Mechanical Stimuli

Although increased membrane tension stimulates exocytosis in many cells, inflation of mast cells (to 4 times their resting volume) prevents degranulation (118), and secretion of renin by juxtaglomerular cells and production of gelatinases by mesangial cells are inhibited by mechanical stretch (4, 15, 33, 142). Also, hypotonic swelling of HeLa or COS cells causes a block in anterograde transport of cargo from the endoplasmic reticulum to the Golgi (71). The underlying mechanism of this block is unclear, but it may reflect a disruption in COPI coat function. In contrast, retrograde transport (between the Golgi and the endoplasmic reticulum) is not blocked in these cells and as a result the Golgi collapses into the endoplasmic reticulum. Surprisingly, the Golgi reappears after 3–6 h, and this reappearance requires a protein kinase C (PKC)-dependent pathway. Because recovery does not require protein synthesis, it is thought that PKC may play a role in activating a volume-recovery mechanism that facilitates Golgi reassembly. Apparently, treatments that seemingly would increase membrane tension do not always lead to increases in exocytic traffic. This could reflect the specialized physiology of certain cell

Table 1. *Mechanical stimulation of secretion*

Cell Type	Secretory or Exocytic Product	Mechanical Stimulus	Reference No(s).
Alveolar type II	Surfactant proteolipid	Distension of cells grown on elastic membranes	28, 102, 136
Fetal lung	Platelet-derived growth factor- β ; glycosaminoglycans and proteoglycans; macrophage inflammatory protein-2	Elongation of organotypic cultures	77, 86, 139
Endothelial	NO; prostacyclin; ^{35}S -labeled proteins; endothelin-1; tissue plasminogen activator	Pulsatile flow (shear stress); cyclical strain; distension of cells grown on elastic membranes	16; Reviewed in 24, 57, 68, 79, 104, 122, 123
Cardiac myocyte	ANF; angiotensin II; basic fibroblast growth factor; endothelin-1; vascular endothelial growth factor; adrenomedullin	Perfused atria/heart; hypotonic swelling; distension of cells grown on elastic membranes; electrical field stimulation	35, 37, 60, 61, 63–65, 70, 74, 106; Reviewed in 107, 110, 112, 114, 127, 140, 141
Smooth muscle	Heparin-binding epidermal growth factor; parathyroid hormone-related protein; collagen; nerve growth factor; platelet-derived growth factor	Distension of cells grown on elastic membranes	18, 75, 89, 91, 119, 134
Skeletal muscle	Insulin-like growth factor	Distension of cells grown on elastic membranes	90
Kidney mesangial	Transforming growth factor- β ; matrix molecules (fibronectin, laminin, collagen types I, III, and IV); vascular permeability factor; vascular endothelial growth factor; prostoglandin (irPGE ₂)	Distension of cells grown on elastic membranes	38, 39, 47, 52, 99–101
Kidney tubular epithelial	Transforming growth factor- β ; NO	Distension of cells grown on elastic membranes	83
Astrocyte	Endothelin-1	Distension of cells grown on elastic membranes	88
Bladder umbrella	Increased membrane capacitance (fusion of discoidal vesicles?)	Osmotic stretch; hydrostatic pressure	73
Fibroblast	Membrane-type matrix metalloproteinase and tissue plasminogen activator; internalized fluid-phase markers	Distension of cells grown on elastic membranes; pulling of membrane with capillary	41, 129
Guard cell protoplast	Increased membrane capacitance (fusion of membrane vesicles)	Osmotic stretch	54; Reviewed in 62
Mammary gland	ATP, UTP, UDP	Touch	30
Neuronal	Synaptic vesicle exocytosis; increased membrane capacitance	Bending of stereocilia; touch; osmotic stretch	22, 34, 40, 43, 56, 84, 135
Osteocyte	Prostacyclin; prostaglandin E and E ₂ ; NO	Mechanical load; pulsatile flow	66, 96
Pleural mesothelial	Endothelin-1	Shear stress; distension of cells grown on elastic membranes	132
Retinal pigment epithelial	Vascular endothelial growth factor	Distension of cells grown on elastic membranes	113
Toad urinary bladder	Granule exocytosis	Distension of tissue	11

NO, nitric oxide; ANF, atrial natriuretic factor; irPGE₂, immunoreactive PGE₂.

types or differences in how each cell type responds to alterations in tension set point. Because the cell has multiple pathways for exocytosis, it is also possible that these pathways are differentially affected by changes in plasma membrane tension.

Transport Steps Altered by Mechanical Forces

Although the membrane trafficking step altered by mechanical force is not defined in all systems, it is known in some. In mechanically sensitive neuronal cells (e.g., hair cells in the inner ear), synaptic vesicle fusion with the presynaptic membrane is stimulated as a result of stretch-induced membrane depolarization (34, 40, 56). A single cycle of stretch and relaxation is sufficient to promote the exocytosis of lamellar bodies in type II pneumocytes (136). These membranous organelles contain whorls of lipid and associated proteins

that, when secreted, function to reduce surface tension in the alveoli. When isolated toad bladders are stretched, a large increase in the fusion of subapical secretory granules with the apical plasma membrane of the granular cells is noted (11). This leads to a corresponding decrease in the number of secretory granules within the cell. In cardiac myocytes, angiotensin II is found in dense core granules, as is ANF (110, 126). These cargo vesicles are thought to fuse with the plasma membrane in response to stretch. Umbrella cells, which line the mucosal surface of the mammalian bladder, contain an abundant population of vesicles that, depending on species, have a discoidal or fusiform appearance. It is hypothesized that they fuse with the apical membrane as the bladder fills, thereby increasing the available surface area of the bladder (51, 73, 82).

Stretch-Regulated Exocytosis in Bladder Umbrella Cells

The vesicle fusion hypothesis is based on the observation that fewer vesicles are found in the umbrella cells of filled bladders compared with the number observed in cells from contracted bladders (82) and on the demonstration that osmotic stretch or hydrostatic pressure increases membrane capacitance in isolated uroepithelium (73). In our own studies, we measured stretch-induced changes in the surface areas associated with discoidal vesicles, the apical plasma membrane, or the basolateral plasma membrane (Truschel S, Wang E, and Apodaca G, unpublished observations). Epithelial tissue, dissected of underlying musculature, was placed in a modified Ussing chamber and then either left unstretched or bowed toward the serosal side by applying hydrostatic force until a pressure of 8 cm H₂O was generated. Under control unstretched conditions, the membrane surface area per umbrella cell associated with vesicles (~7,200 μm^2) was about three times that of the apical surface area (~2,900 μm^2). The basolateral surface area was ~4,600 μm^2 . After a 5-h period of stretch, which mimics the long storage phase of bladder filling, the apical surface area was increased to ~4,300 μm^2 (an ~50% increase), whereas no significant change in the basolateral surface area was measured. Concordantly, the amount of uroplakin III (a vesicle membrane protein) at the apical surface increased by ~65% after stretch. The membrane area associated with vesicles significantly decreased from ~7,200 to ~1,000 μm^2 . The magnitude of the loss in vesicle surface area (~6,200 μm^2) was significantly greater than the amount added to the apical membrane (~1,400 μm^2). As described below, this is the result of stretch-regulated endocytosis and membrane turnover.

MODULATION OF ENDOCYTOSIS BY EXTERNAL MECHANICAL STIMULI

Because the plasma membrane's composition and surface area are regulated by both endocytosis and exocytosis, it is not unexpected that endocytosis would also be affected by mechanical forces. In fact, exposure of cultured endothelial cells to shear stress is sufficient to enhance endocytosis of extracellular fluid-phase markers (25), and mechanical forces stimulate endocytosis in killifish epithelial cells and bladder umbrella cells (32).

Endocytosis in Response to Cell Shrinkage

When osmotically swollen cells are returned to isotonic conditions, they rapidly recover exocytosed membrane (22, 55, 67, 84). According to the tension hypothesis, this would be the consequence of decreased membrane tension (23, 62, 84, 95, 115, 138). Within minutes of a return to isotonic conditions, tubules are formed that rapidly branch and dilate to form what are called vesicular-like dilations (VLDs) (22, 81, 84). VLDs are observed in several but not all cell types.

They form at contacting surfaces, are reversible, and reform at the same locations when cells are exposed to cycles of swelling and shrinking (81, 97). They can be 10 μm across and penetrate deep into the cytoplasm, and their cytoplasmic face is associated with actin and spectrin (49, 50, 81). Treatment with actin-disrupting agents has no effect on VLD formation, implicating some other mechanism in their generation (perhaps one involving spectrin) (49, 97). Initially, VLDs are contiguous with the plasma membrane, but they are eventually reabsorbed by endocytosis and formation of intracellular vacuoles (81, 97). VLDs may also play a role in regulatory volume decrease, a process by which some cells recover their original volume after a sudden exposure to hypotonic conditions. When kidney tubule cells derived from the medullary thick ascending limb are exposed to hyposmotic medium, they rapidly swell and then are volume regulated (19). This volume regulation is accompanied by the formation of VLDs, presumably as a mechanism to recover surface area and decrease cell volume (19).

Endocytosis During Development

Forces generated during development may also impact membrane recovery. During embryogenesis of the killifish, a cap of epithelial cells on the embryo's animal pole migrate and cover the embryo to form an enveloping layer, which will later form the yolk sac. This enveloping process, termed epiboly, involves morphological transitions that include breaking and forming cell junctions and attendant changes in cell shape. When the apical membrane of the enveloping layer is labeled with fluorescent lipid or lectins, the apical membrane internalizes most rapidly at the sites of cell-cell contact (32). Endocytosis in these cells continues in a centripetal fashion so that membrane turnover occurs at the periphery of the cell first and then proceeds toward the cell center as development continues. Interestingly, endocytosis at sites of cell-cell contact is also observed when, postepiboly, embryos are subjected to mechanical deformation, experimentally induced by pressing a slide on the embryo (32). Similarly, decreasing the surface tension of *Xenopus laevis* embryos by explanting pieces of epithelial tissue is also accompanied by increased membrane turnover (7, 8). The underlying mechanism of this turnover is unknown; however, it is likely to reflect regulation of plasma membrane tension.

Stretch-Regulated Endocytosis in Umbrella Cells

A useful model by which to study stretch-regulated endocytosis is the bladder umbrella system described above (51). When this system was analyzed, it became apparent that the amount of vesicle-associated membrane far exceeded the amount of membrane added to the apical surface during stretch. This led us to examine the hypothesis that stretch stimulates membrane turnover in this system (Apodaca G, Truschel S, and Wang E, unpublished observations). In fact, we find that stretch is accompanied by rapid endocytosis; es-

essentially 100% of labeled apical membrane proteins is endocytosed within 5 min. It is also observed that filling excised, but otherwise intact, bladders is sufficient to stimulate internalization of fluorescently labeled lectins. Like the killifish system described above (32), endocytic vesicles are most prominent near the sites of cell-cell contact. Consistent with previous morphological observations that vesicle membrane is found in multivesicular bodies and lysosomes (93), it is observed that the majority of endocytosed membrane is directed to lysosomes, where it is degraded.

Although it may seem counterintuitive that exocytosis and endocytosis are occurring simultaneously in stretched umbrella cells, exocytosis and endocytosis occur constitutively and simultaneously in all cells, even under resting conditions (48, 87). In synapses, compensatory endocytosis is necessary to recover membrane exocytosed as a result of neurotransmission (59). Coupled exocytosis-endocytosis may allow the umbrella cell to fine-tune its apical surface area and allow for turnover of membrane already exposed to urine. At first glance, the stretch-induced endocytosis observed in the killifish and umbrella cell models seems incompatible with the tension hypothesis; manipulations that are likely to increase surface tension are stimulating endocytosis. However, in both cases endocytosis is accompanied by membrane turnover (i.e., exocytosis). Because exocytosis decreases membrane tension, the simultaneous endocytosis would act as a compensatory mechanism to maintain plasma membrane tension.

MECHANOTRANSDUCTION AND REGULATION OF EXOCYTIC AND ENDOCYTIC TRAFFIC

One goal of present research is to understand how mechanical forces are sensed by the cell and then transduced into downstream cellular events such as exocytosis. The first step in this process is the activation of a mechanosensor that is able to sense changes in membrane tension or alterations in the underlying cytoskeleton. This initial signal is transduced via secondary messenger cascades into downstream cellular events including exocytosis and endocytosis. Several different mechanosensors have been identified (43, 107). Those that regulate membrane traffic are described below and are shown in Fig. 2. It is important to note that a single mechanical stimulus may activate multiple mechanosensors and that each cellular event may be regulated downstream of multiple mechanosensors (43, 80, 107, 108). Alternatively, some mechanosensors may selectively regulate only a subset of downstream events (43, 107, 109).

Role of the Cytoskeleton

The cytoskeleton, composed of actin, microtubules, and intermediate filaments, plays an important role in mechanotransduction (43, 58, 84). Deformation of the plasma membrane is accompanied by a rapid and global reorganization of the cytoskeleton to counteract

the external force. Because the cytoskeleton is attached to the plasma membrane, alterations in the cytoskeleton can affect membrane tension and thereby affect membrane traffic (21, 115). Changes in the cytoskeleton directly alter mechanotransduction by mechanisms involving integrins and stretch-activated ion channels (17, 44, 135). Both of these mechanosensors are attached to the actin cytoskeleton, and disruption of this cytoskeletal interaction can change their activities (43). The cytoskeleton performs other functions that are important to mechanotransduction. It serves as a scaffolding that coordinates the organization of signaling complexes, which modulate cellular events like exocytosis-endocytosis (58). Additionally, the cytoskeleton ensures efficient transport of membranous cargo within the cell, and both exocytosis and endocytosis require access to regions of plasma membrane generally free of cortical cytoskeleton (2, 128). It is not surprising, therefore, that exocytosis and endocytosis are significantly altered by agents that perturb the normal assembly and turnover of the cytoskeleton (2, 130).

Role of Ion Channels

One class of mechanosensors common to most cells includes stretch-activated and -inactivated ion channels (43). Several classes of these channels have been described, including nonselective cation channels, some of which conduct Ca^{2+} and can induce Ca^{2+} release from intracellular stores (42, 43, 45). Increased intracellular Ca^{2+} triggers exocytosis in many cell types, and entry of Ca^{2+} through plasma membrane channels can also regulate endocytosis (10, 117). A nonselective cation channel underlies mechanosensory transduction by the hair cell of the inner ear (34, 56). Bending of the stereocilia activates a nonselective cation channel that depolarizes the cell. Concomitant with this depolarization is the activation of voltage-sensitive Ca^{2+} channels that raise intracellular Ca^{2+} , which in turn stimulates synaptic vesicle exocytosis. Moreover, stretch-activated secretion of ANF is blocked by gadolinium, a rare earth metal that inhibits many stretch-activated nonselective cation channels (69). Recently, a nonselective cation channel was identified in vertebrates. This protein is called the vanilloid receptor-related osmotically activated channel (VR-OAC) and, like its OSM-9 homolog in *Caenorhabditis elegans*, is thought to be important in sensing osmotic stretch (76). Any role that this channel plays in secretion has yet to be defined.

Other mechanosensitive channels conduct Cl^- , K^+ , or Na^+ (43). Examples of the latter two include maxi-K channels and the epithelial sodium channel, the activities of which have recently been shown to be upregulated in isolated kidney cortical collecting ducts subjected to flow stimulation (111, 137). The epithelial sodium channel is homologous to the *C. elegans* family of degenerin proteins (mec-4, mec-10, and deg-1) (125). These are thought to form a mechanosensitive ion

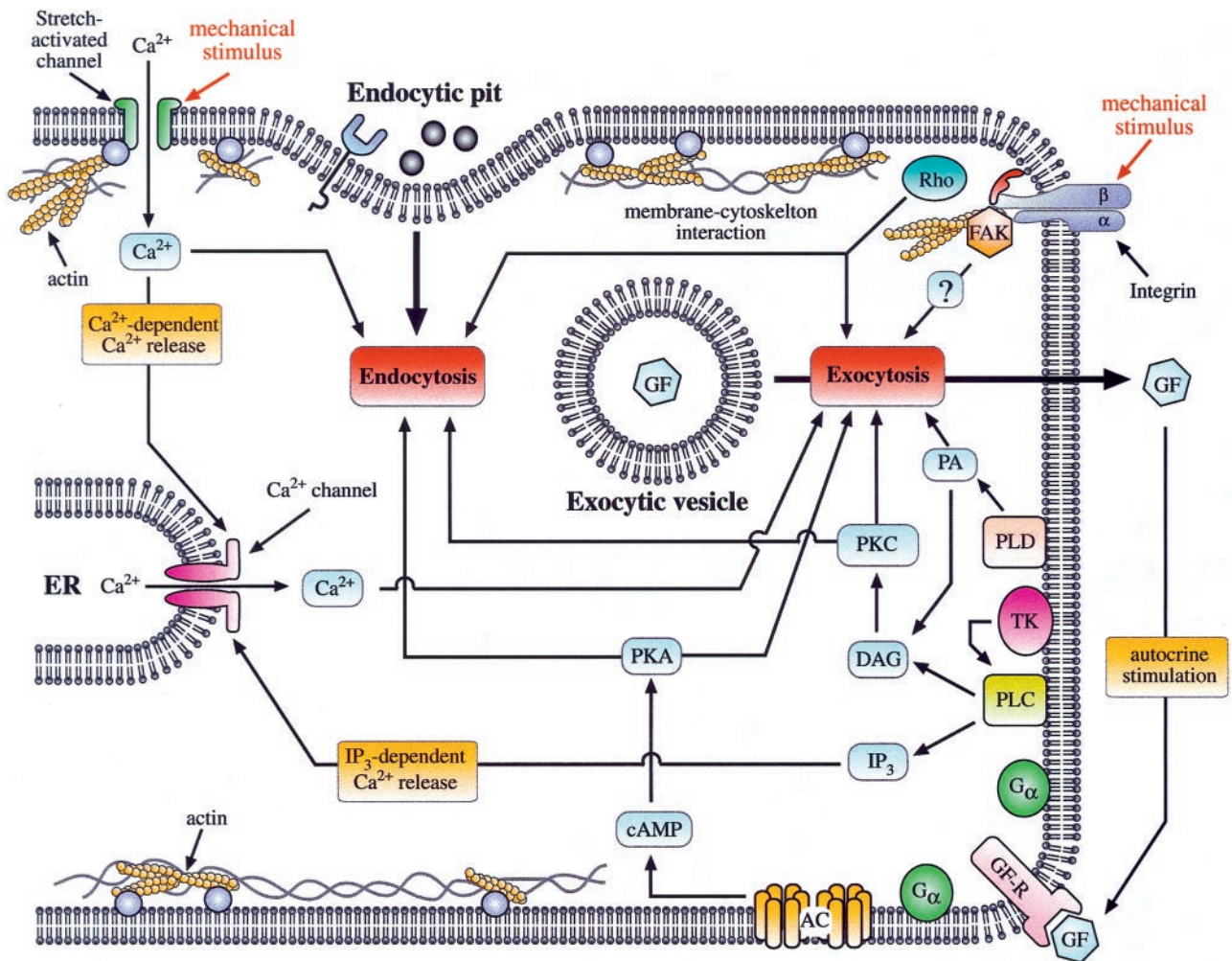


Fig. 2. Signaling pathways coupling mechanical stimuli to changes in endocytosis and exocytosis. See the text for descriptions and details. AC, adenylyl cyclase; DAG, diacylglycerol; ER, endoplasmic reticulum; FAK, focal adhesion kinase; G_α, heterotrimeric G protein α-subunit; GF, growth factor/hormone; GF-R, growth factor/hormone receptor; IP₃, inositol 1,4,5-trisphosphate; PA, phosphatidic acid; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; Rho, Rho family GTPase; TK, tyrosine kinase (e.g., pp60^{src}).

channel that, in a larger complex, couples touch sensation to neurotransmission (125). It remains to be determined whether maxi-K or epithelial sodium channels have any role in mechanotransduction. Finally, stretch-induced ANF secretion also requires the activity of K_{ATP} and Ca²⁺ channels and is blocked by inhibitors of these channels (63).

Role of Integrins

Integrins, which link extracellular matrix molecules to the intracellular actin cytoskeleton, are another class of mechanosensors (58). Magnetic beads coated with an integrin ligand are capable of transmitting mechanical stress to the underlying cytoskeleton, whereas beads specific for nonadhesion receptors have no effect (131). The α-subunit, together with the β-subunit, specifically binds extracellular ligands, and the β-subunit forms interactions with several molecules, including talin and α-actinin (both of which can interact with actin filaments) and focal adhesion kinase (13,

58). Focal adhesion kinase, in turn, interacts with a number of additional molecules including pp60^{src}, Fyn, Grb2, and phosphatidylinositol-3 kinase (13, 58). These molecules further modulate other secondary messenger cascades, including those in the p21ras, mitogen-activated protein kinases, Rho/Rac/CDC-42, and PKC pathways (13, 58). The Rho family of GTPases is of significant interest, as members of this family regulate the formation of focal adhesions/focal complexes, the organization of the actin cytoskeleton, and exocytosis and endocytosis (36, 98). Of the 20 or more integrins known, approximately half bind to the sequence Arg-Gly-Asp (R-G-D in single-letter amino acid code) (105). R-G-D peptide inhibits integrin binding to the extracellular matrix and, in frog skeletal muscle, it inhibits stretch-induced release of neurotransmitters from the motor nerve terminal (17). R-G-D peptide also inhibits production of platelet-derived growth factor by smooth muscle cells that have been exposed to cycles of stretch and relaxation (135).

Role of Tyrosine Kinases and Phospholipases

Other molecules that may play a role in mechanotransduction are the receptor and nonreceptor tyrosine kinases (78, 107, 108) and phospholipases C and D (108). The receptor tyrosine kinases have transmembrane domains whereas nonreceptor kinases such as pp60^{src} are anchored via NH₂-terminal myristoylation. It is possible that membrane stretch causes a conformational change in tyrosine kinases that results in their activation. In cardiac myocytes, stretch leads to an almost instantaneous (within 5 s) rise in tyrosine phosphorylation that is followed by a rise in intracellular Ca²⁺ (108). The tyrosine kinase inhibitor laven-dustin A inhibits stretch-induced ANF secretion (124), implicating tyrosine kinases in the regulation of mechanical stimuli-induced secretion. Furthermore, stretch-induced secretion of vascular permeability factor in mesangial cells is inhibited by genistein and herbimycin A (inhibitors of tyrosine kinases) and a specific peptide inhibitor of pp60^{src} (38).

Mechanical stretch of cardiac myocytes activates phospholipase C (within 1 min) (108), which in turn generates diacylglycerol (DAG) and inositol 1,4,5-trisphosphate. DAG activates PKC, a known regulator of endocytic and exocytic traffic (14, 26, 53, 85). Inositol 1,4,5-trisphosphate promotes Ca²⁺ release, which stimulates exocytosis in many cell systems (10). In fetal lung cells, a mechanical stimulus results in a rapid membrane translocation of pp60^{src}, where it activates phospholipase C- γ (78). The subsequent production of DAG activates PKC. In mechanically stimulated cells, phospholipase D generates phosphatidic acid (108), which has also been implicated in regulating membrane trafficking events including exocytosis (103). Phosphatidic acid can be converted to DAG, which in turn activates PKC signaling pathways (31).

Role of cAMP

The generation of cAMP may also play an important role in mechanotransduction. Mechanical stretch stimulates production of cAMP in some cell types including uroepithelium (72, 133) (Apodaca G, Truschel S, and Wang E, unpublished observations). Agents that raise cAMP (e.g., forskolin) can have significant effects on cellular function including stimulation of exocytic and endocytic traffic (29, 46, 92). H-89, an inhibitor of protein kinase A and the principal downstream target of cAMP, blocks stretch-activated discoidal vesicle exocytosis in umbrella cells (Apodaca G, Truschel S, and Wang E, unpublished observations). In contrast, forskolin causes a significant stimulation of discoidal exocytosis even in the absence of stretch. Under these conditions, there is an ~120% increase in apical surface area over untreated control cells. Forskolin treatment has no effect on endocytosis. This is surprising, as greater degrees of exocytosis would likely decrease membrane tension, and the tension hypothesis predicts that endocytosis would be stimulated (23, 62, 84, 95, 115, 138). The implication of this finding will have

to await direct measurements of membrane tension under these conditions.

Growth Factors, Hormones, and Other Signaling Molecules

Finally, mechanical stimuli can also enhance the production and/or secretion of multiple growth factors and hormones (see Table 1) that, in an autocrine-paracrine fashion, can stimulate multiple secondary messenger systems downstream of a stretch response (107, 110). Within 1 min of stretch, angiotensin II is secreted (108, 110). On binding its receptor, it activates numerous downstream signaling pathways including activation of phospholipase C. As described above, the generation of inositol 1,4,5-trisphosphate and DAG regulate membrane traffic. Other molecules involved in mechanotransduction include ATP, heterotrimeric G proteins, prostaglandins, and NO. Their roles in this process are described elsewhere (24, 27, 43, 107).

CONCLUDING COMMENTS AND FUTURE DIRECTIONS

Although it is clear that mechanical stimuli modulate endomembranous transport, several aspects of this regulation are poorly understood. For example, membrane tension is important, yet it is unclear how the cell determines its set point or how changes in tension are perceived. Exocytosis clearly impacts membrane tension, yet there has been little attempt to systematically explore how the various transport steps in the secretory pathway are modulated by mechanical forces. Moreover, it is possible that different types of forces may differentially affect these transport steps. There are few model systems that analyze external force regulation of endocytosis. The umbrella cell model described above is likely to shed light on this area of inquiry. Endocytosis occurs via a number of different pathways, and postendocytic traffic, like secretory traffic, involves sequential transport between a variety of compartments. The impact of mechanical stimuli on these different forms of endocytosis and transport steps is largely unexplored. The initial mechanosensing mechanism for both exocytosis and endocytosis remains to be defined in many cell systems and is likely to vary among cell types. Furthermore, the pathways for signal transduction are only loosely defined, and, in the case of stimulated endocytosis, very little is understood. Finally, the targets of these secondary messenger cascades and their impact on the vesicular trafficking machinery require further inquiry.

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