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Review

Solute Transport Controls Membrane Tension and Organellar Volume

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Key Words
Endocytosis • Lysosomes • Membrane tension • pH • TPC • TRPML • VRAC • NHE • TMEM175

Abstract
The regulation of cellular volume in response to osmotic change has largely been studied at the whole cell level. Such regulation occurs by the inhibition or activation of ionic and organic solute transport pathways at the cell surface and is coincident with remodelling of the plasma membrane. However, it is only in rare instances that osmotic insults are experienced by cells and tissues. By contrast, the relatively minute luminal volumes of membrane-bound organelles are constantly subject to shifts in their solute concentrations as exemplified in the endocytic pathway where these evolve alongside with maturation. In this review, we summarize recent evidence that suggests trafficking events are in fact orchestrated by the solute fluxes of organelles that briefly impose osmotic gradients. We first describe how hydrostatic pressure and the resultant tension on endomembranes can be readily dissipated by controlled solute efflux since water is obliged to exit. In such cases, the relief of tension on the limiting membrane of the organelle can promote its remodelling by coat proteins, ESCRT machinery, and motors. Second, and reciprocally, we propose that osmotic gradients between organellar lumens and the cytosol may persist or be created. Such gradients *impose* osmotic pressure and tension on the endomembrane that prevent its remodelling. The control of endomembrane tension is dysregulated in lysosomal storage disorders and can be usurped by pathogens in endolysosomes. Since trafficking and signaling pathways conceivably sense and respond to endomembrane tension, we anticipate that understanding how cells control organellar volumes and the movement of endocytic fluid in particular will be an exciting new area of research.

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Introduction

The control of cell size and volume has been thoroughly studied, and its major underlying mechanisms are becoming well-appreciated. While cell size can vary considerably between cell types and in different tissues, maintaining constancy to cell volume is tightly regulated;

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cells adapt to osmotic changes in their extracellular environment to overcome cellular swelling or lysis [1]. Such regulation occurs by the inhibition or activation of ion transporters, channels, and pumps at the plasma membrane (PM) that orchestrate regulatory volume increase (RVI) or decrease (RVD), causing cellular swelling or shrinking respectively [2]. Because the PM of mammalian cells is made permeable to water through channels like aquaporins, water readily diffuses though the membrane [3, 4]. Due to this permeability, water follows an osmotic gradient either into or out of the cell during RVI and RVD (Fig. 1). Through a combination of active and passive ionic and organic solute transport pathways, together with the movement of water across membranes, cells are therefore broadly capable of counteracting changes in osmotic pressure and high membrane tension [1, 5]. As the PM can experience, but does not maintain, an osmotic gradient between the intracellular and extracellular space, a gross build-up of osmotic pressure on the PM is not predicted.

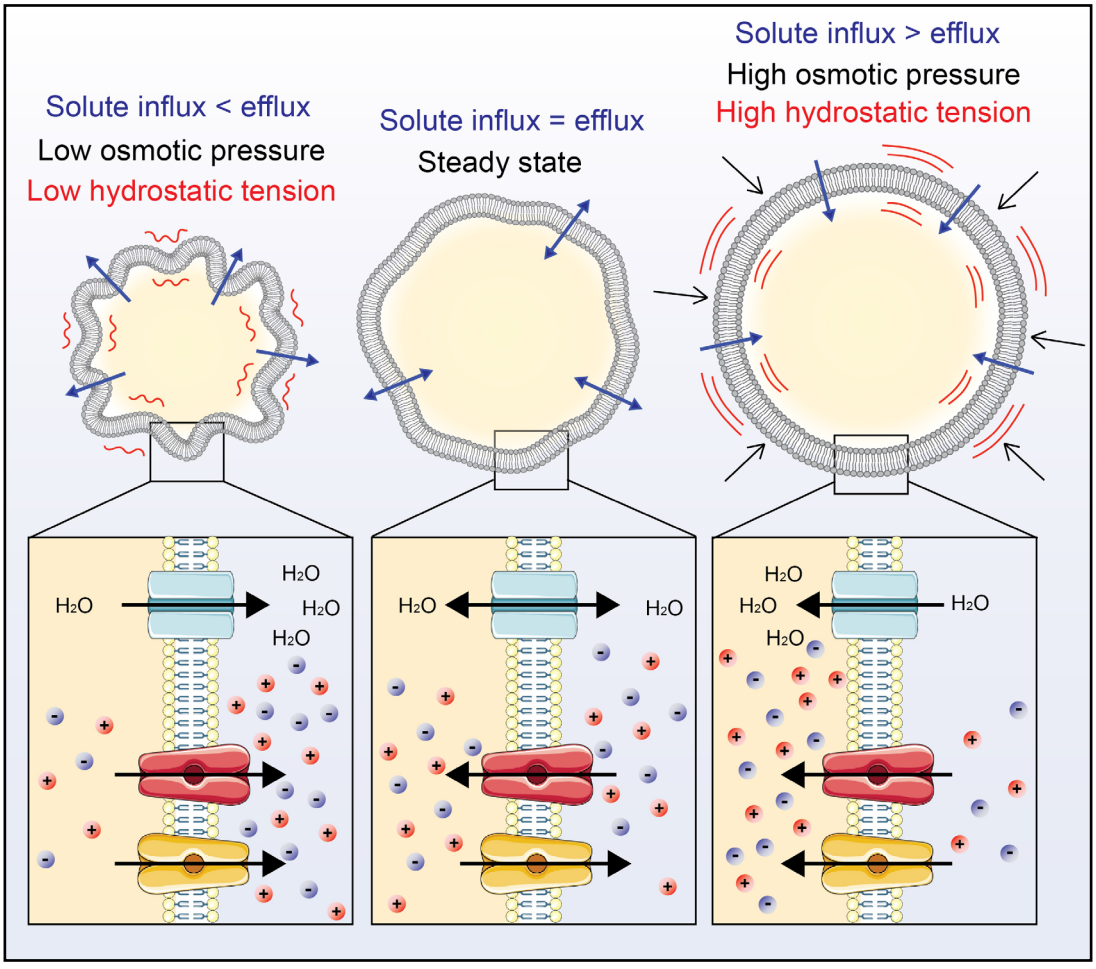

Fig. 1. Solute flux across membranes. Generally, the movement of water (an incompressible fluid) into and out of membrane-bound compartments follows the concentration gradient of solutes/osmolyte on either side of the membrane. Mammalian membranes are permeable to water and at steady-state (centre), the rate of solute influx and fluid across the membrane equals that of solute efflux. As a result, no osmotic gradient is established. When solute influx is greater than efflux (right), water is drawn into the compartment, but only to an extent accommodated by the stretch that the membrane can withstand (3-5%). Beyond that point, osmotic pressure yields the movement of water which generates a perpendicular tension force experienced across the membrane. By contrast, when solute efflux is greater than influx (left), the opposite occurs; osmotic pressure briefly drops and water is drawn out of the compartment. This creates conditions of low membrane tension as volume is lost. Graphics in the lower panels were generated using Smart Servier.

The control of membrane tension by cells is nevertheless an essential and conserved mechanism that goes hand-in-hand with osmotically-imposed or -driven changes in their volume. Tension on the PM undergoes a major global perturbation that precedes RVI and RVD, and while the PM is flexible, like all mammalian membranes it does not typically stretch during such circumstances or beyond 3-5% [6-8]. Instead, the cell rapidly deploys various mechanisms in an effort to remodel the PM and recalibrate its tension. Increased membrane tension due to hydrostatic pressure or mechanical force results in increases in membrane surface area to compensate; membrane reservoirs such as invaginations or microvilli can be flattened or unfurled to decrease tension [9-12] or new membrane can be delivered by fusion from the endocytic pathway [9, 13, 14]. Sudden decreases in membrane tension, on the other hand, result in either the immediate internalization of excess, slack membrane to lower cell surface area or the stabilization of crenations to the membrane [9, 15].

All of the aforementioned principles have classically been applied to the cell surface. However, membrane-bound compartments in the cell (i.e. organelles) must also be regulated in their volume and are subject to much greater changes in solute concentrations and composition than the cell itself. Though remarkably understudied, organellar membranes are ostensibly also highly permeable to water [16-18]. There is evidence that aquaporins (such as AQP2) are indeed present in endocytic organelles and, together with poorly selective channels with large pores, allow for the unimpeded movement of water between the cytosol and the endocytic fluid [19-22]. Mechanisms that govern a net flux of solutes to and from organellar lumens, then, are expected to control osmotic pressure and tension on the endomembrane. The extent to which the limiting organellar membrane is under tension will in turn orchestrate its remodelling and traffic just as occurs at the PM. This is particularly evident in the endocytic pathway of cells where osmolytes are internalized and liberated at different stages of maturation. Moreover, in specialized cellular processes that result in large, quasi-spherical organelles like autophagosomes, phagosomes, and macropinosomes, we have proposed that solute efflux supersedes the events that drive their resolution (e.g. autophagic lysosome reformation or ALR, tubulation, budding, and vesiculation) [23]. Finally, in the secretory pathway of cells that package large cargos like mucins, collagens, or other extracellular matrix proteins, the control of luminal volumes is also expected to feature prominently in fusion, trafficking, and exocytosis. Generally, control of organellar volumes in the endocytic and secretory pathways is ultimately also critical for the maintenance of cell volume. While we focus this review on endocytic pathway in particular, we hope that the principles described herein will serve to broadly highlight connections between organellar volume control and cell volume regulation.

Endocytic pathways and resultant tension on early endomembranes

The mechanisms that drive endocytosis vary considerably, as does the membrane tension of nascent endosomes (Fig. 2). It has long been appreciated that tension on the PM itself regulates endocytic pathways [14, 24, 25]. It follows that endocytosis arising from low PM tension will result in nascent endosomes under low endomembrane tension. For example, clathrin-independent endocytic pathways (CLIC/GEEC) and macropinocytosis both begin with a decrease in PM tension; this membrane slack allows for the invagination of cargo (in the case of CLIC/GEEC) or membrane ruffling (in macropinocytosis) [26-28]. Decreases in PM tension that arise from the rapid delivery of membrane by focal exocytosis reciprocally induce endocytic programs [28-31]. The membrane tension sensors involved in initiating these programs are beginning to be identified and include factors such as the lipid raft-associated lipase, phospholipase D2 [28] and the focal adhesion protein, vinculin [27]. In each of these cases (i.e. CLIC/GEEC and macropinocytosis), the endosomes formed undergo an initial phase of rapid remodeling and recycling that is unopposed by membrane tension.

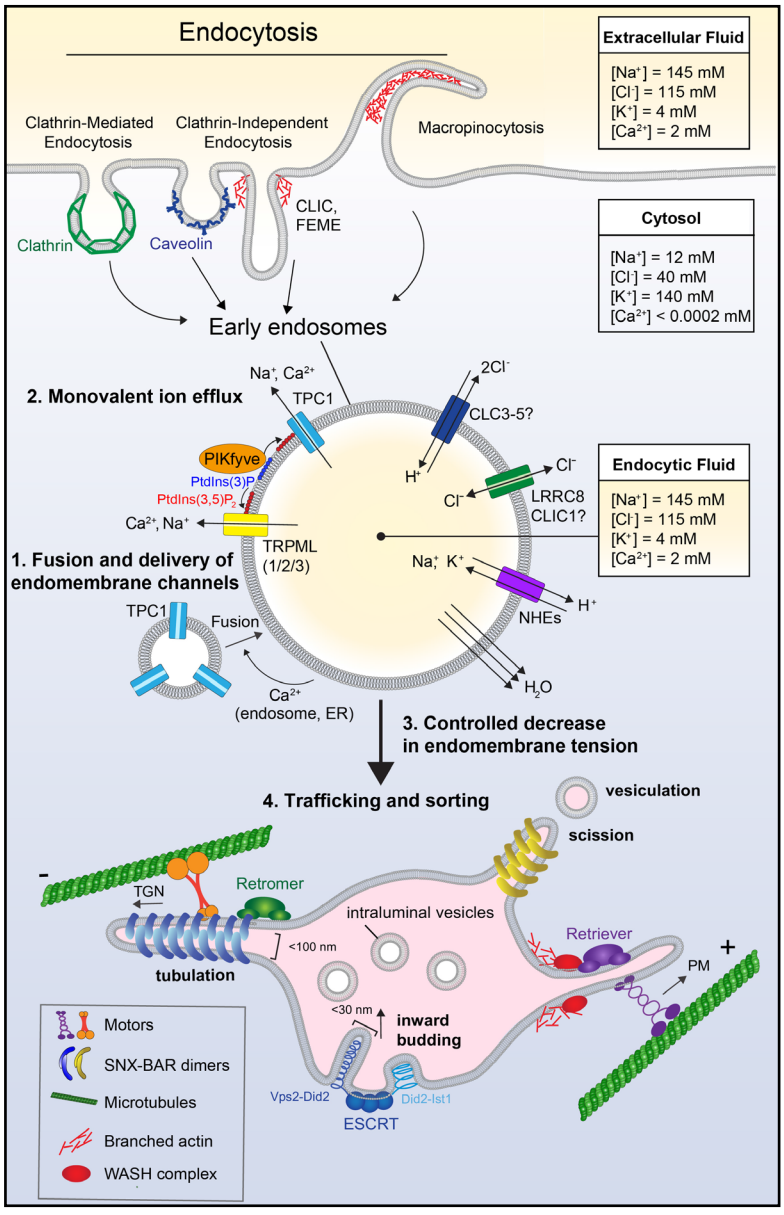

Fig. 2. Endocytosis, ion flux, and membrane dynamics in early endosomes. Extracellular fluid can be internalized through a variety of mechanisms such as clathrin-mediated endocytosis, clathrin-independent endocytosis, or macropinocytosis, forming the nascent early endosome. As the endosome matures, it undergoes Ca^{2+}-mediated homotypic fusion with other (early) endosomes and vesicles containing additional cargo and endomembrane channels (1). Compared to the cytosol, the concentrations of ions such as Na^+, Cl^-, and Ca^{2+} are relatively high in the extracellular fluid and the resultant endosome, which are round and minimize surface:volume. As membrane is recycled back to the surface, these ions are removed from endosomes to decrease their volume and prevent an osmotic pressure that causes membrane tension (2). Monovalent ion flux occurs through the action of lipid-gated cation channels such as TRPML1/2/3 and TPC1, ion co-transporters such as ClCs and NHEs, and potentially other anion channels such as VRAC/CLIC. Lipid-gated cation efflux further relies on the action of PIKfyve, which phosphorylates PtdIns(3)P to form PtdIns(3,5)P_2, to then gate the conductance of cation channels TRPML and TPC. As ions are removed from the early endosome, this leads to osmotically-driven water loss and shrinkage of the compartment. Volume loss decreases endomembrane tension, creating slack in the membrane and allowing its remodeling through tubulation, vesiculation, and budding (3). Endocytic cargo is then sorted; Retromer and Retriever complexes recognize and sort cargo destined for recycling and segregate this cargo into subdomains which undergo tubulation (4). These tubules have a high surface:volume ratio, allowing the effective concentration of membrane-bound cell surface receptors to recycle back to the plasma membrane. The forces needed for tubulation are generated by concerted actions of the actin cytoskeleton, including actin polymerization by WASH, and pulling forces of motor proteins moving along microtubules. Endomembrane tubules are coated and stabilized by curvature-sensing proteins containing with BAR domains These tubules undergo scission and vesiculation, leaving behind cargo destined for degradation. Remaining cargo can be recognized by ESCRT complexes, which also utilize membrane slack in order to deform the membrane and generate inwardly-budding intraluminal vesicles (ILVs). Inward budding is supported by corkscrew-shaped protein complexes which polymerize and pull the membrane into narrow tubules that eventually undergo scission to form ILVs. These ILVs form the basis of the multivesicular late endosome, which later fuses with the lysosome for degradation.

Clathrin-mediated endocytosis, on the other hand, can proceed even under high PM tension, and therefore frequently results in endosomes of higher tension. Tension on the PM can be overcome by clathrin assemblies that apply additional cytoskeletal forces afforded by the local polymerization of actin against the PM to complete pit formation and aid scission [32]. The curvature of the resultant clathrin-mediated endosome may even augment the tension on the endomembrane as compared to the already tensed PM from which it was derived [25, 32-34], but this would also depend on where constriction and scission of the endosomal neck by the GTPase dynamin occurs [35]. Taken together, nascent endosomes formed from diverse endocytic pathways will not all have the same degree of membrane tension, a feature that should garner consideration when examining control of their subsequent remodelling.

Immediately upon their scission from the plasma membrane, all endosomes still undergo rapid recycling of membrane replete with its receptors (Fig. 2). Such remodelling supports "fast" recycling pathways wherein cargo (e.g. the Transferrin Receptor or the neonatal Fc receptor) is returned directly to the membrane by bulk membrane flows [36, 37]. It should be obvious, however, that without a parallel loss of fluid, such membrane recycling would eventually be limited, arresting when hydrostatic pressure against the endomembrane of the organelle builds. Moreover, the substructures that emanate from the parental organelle to facilitate these recycling pathways contain very small amounts of fluid. Indeed, many of the coat proteins that drive recycling bend and shape the endomembrane by inducing or stabilizing remarkably high curvature [38]. These coat proteins help to form tubular structures with high surface-to-volume ratios. This geometric consideration lends itself to maximizing the traffic of surface membrane but requires substantial volume loss. Therefore, an essential parameter and required feature of recycling in the early endocytic pathway is that it is accompanied by the efflux of osmolytes. To understand the mechanisms that drive solute fluxes to and from the endosome, fluid resolution, and ultimately trafficking, one must consider when and how solute transporters are incorporated into the endomembrane and how their activities are controlled therein.

Early endocytic fluid, monovalent ion efflux, and volume loss

Unlike the variances with tension on the nascent endomembrane, the sealed solutes of the endocytosed fluid will equal that of the extracellular fluid in most forms of endocytosis (Fig. 2). Exceptions would of course occur when particulate cargo –that occupies a majority of the endosomal volume– is internalized or if macromolecules are sorted into or out of endosome before its scission. Importantly, the compartment needs to lose volume since the volume of the endocytic pathway and that of the cell remains constant [39]. The shrinkage of large endosomes is in fact connected to regulations of the endomembrane tension: Shrinkage lowers membrane tension and the resultant high curvature and membrane slack serve to recruit curvature-sensing proteins that also function as sorting complexes [16]. In the early endocytic pathway, the mechanisms that drive volume loss are poorly understood but are known to involve monovalent ion efflux followed by osmotically-obliged water. Control of monovalent ion flux from endosomes therefore serves to couple cargo sorting to the formation of the substructures (e.g. tubules and vesicles) into which cargo is sorted (Fig. 2). Some of these recycling pathways actually expel endocytosed fluid directly back to the extracellular medium along with the return of bulk membrane and receptors [40-43], but the narrow lumens of tubules and vesicles do not accommodate large volumes.

Instead, the mechanisms underlying volume regulation of endosomes bear some resemblance to cell volume regulation. In this compartment, fluid and ions are removed as other macromolecules, solutes, and cargo are distilled. The removal of endocytosed fluid requires a rapid decrease in the luminal osmolarity of the endosome, which needs to occur under electrically neutral pathways. Fortunately, the gradients between the major osmolytes of the endocytic fluid and that of the cytosol are steep. Extracellular concentrations of Na^+ and

Cl^- (the relevant monovalent ions) are ~110-130 mM, constituting 80% of the osmoticants of the endocytosed fluid, while that of the cytosol are between 10-20 and 40 mM respectively [44, 45]. The cell takes advantage of these gradients to efflux Na^+ and Cl^- via channels and transporters down their respective gradients from the endosome to the cytosol. The ions and fluid must then be further expelled from the cell by pumps and channels at the plasma membrane. This last step may seem to cost the cell an exorbitant amount of energy, since ATP is at least required to move the Na^+ out of the cell against its concentration gradient via the action of the Na^+/K^+-ATPase. Such efforts indeed are estimated to consume up to 50% of the energy used in the brain [46] and perhaps highly endocytic cells can afford the ATP. Moreover, it has long been appreciated that inhibition of the Na^+/K^+-ATPase with ouabain causes some cells to swell and burst, but with curious variability [47, 48]. Further studies investigating connections between rates of endocytosis and cell volume control by the Na^+/K^+-ATPase will likely be illuminating.

Though the final steps of monovalent flux at the PM are unclear, measurements obtained from recycling endosomes for Cl^- show a substantial drop from the nascent endosome (130 mM) to recycling endosomes (20 mM) [49, 50] supporting a sharp decrease in $[Cl^-]_{endosome}$. Measurements of Na^+ are sorely lacking and experimentally challenging to obtain [51], however, it was recently determined that Na^+ is indeed effluxed alongside with Cl^- from nascent endosomes [16]. This was done using cells that undergo macropinocytosis and therefore readily entrap bulk fluid from their surroundings. Under these experimental conditions, ion substitutions were performed at the time of endosome formation. Notably, removing monovalent ion gradients between the endocytic fluid and the cytosol not only ablates shrinkage of the compartments formed in specialized cell types, it also causes distention of endosomes formed in garden variety fibroblasts and in various tumor cells [16].

Na^+ efflux

Na^+ pumps, transporters, and channels are present in virtually all mammalian membranes including the PM and endomembranes. The relevant pathway for the efflux of Na^+ from the early endosome could therefore conceivably come from the PM from which it is derived or by fusion of the nascent endosome with other endocytic compartments. Importantly, there must be control over such mechanisms since Na^+ is pumped out of the cell at the PM by the Na^+/K^+-ATPase but exchanged inward for H^+ by Na^+-H^+ exchangers (NHEs). The plasma membrane Na^+/K^+-ATPase can be readily endocytosed and, if still active, would serve to increase rather than decrease the luminal concentration of Na^+. Instead, the pump is thought to be inactivated upon internalization [52-54]. While these studies focused on the phosphorylation that led to the internalization of the pump, mechanisms that arrest the pump upon its endocytosis are less clear. Interestingly, the pump is regulated by tonicity; increasing the osmolarity of the medium inhibits the activity of the pump while cell swelling causes its stimulation [55].

NHEs normally found at the PM are also endocytosed, and therefore present in early endosomes, but their endocytosis can be limited by tethering to the cortical actin cytoskeleton [56]. Still, NHE3, for example, has been shown to remain active upon clathrin-mediated endocytosis [57]. Since the normal function of plasma membrane-resident NHEs is to extrude H^+ in exchange for Na^+, they may continue this exchange in the endosome to contribute to its early acidification [57-60]. Of note, however, the exchange activity of NHE3 is regulated by its association with ezrin, a PtdIns(4,5)P_2 and actin binding protein [61]. Given the rapid loss of PtdIns(4,5)P_2 upon scission of nascent endosomes, H^+-exchange by this particular NHE may be limited to very brief periods of time. Importantly, both NHE3 and the Na^+/K^+-ATPase are recycled back to the plasma membrane from the early and sorting endosome [54, 58].

How then is Na^+ fluxed out of the early endosome? Clues have come from investigating the phosphoinositides (PtdIns) that regulate the process altogether. Among those, PtdIns phosphorylated at the D3 position – namely PtdIns(3)P and PtdIns(3,5)P_2 – have been

shown to play an essential role in intracellular [Na^+] regulation, endomembrane trafficking, sorting, and the control of endolysosomal/vacuolar volume [16, 62, 63]. PtdIns(3)P is quickly generated on the cytosolic leaflet of the early endosome by a class III PI3-kinase called vacuolar protein sorting 34 (VPS34). In mammalian cells, the recruitment and activation of the kinase is orchestrated by Rab5, a small GTPase that is reviewed elsewhere [64]. Importantly, PtdIns(3)P attracts effectors that harbour a FYVE domain including the early endosomal marker, EEA1. EEA1 supports the fusion of early endosomes with other compartments, which is critical since these are sources of Na^+ channels (discussed below).

PtdIns(3)P also recruits the FYVE domain containing kinase aptly termed FYVE finger-containing phosphoinositide kinase (PIKfyve). PIKfyve can then phosphorylate PtdIns(3)P to generate PtdIns(3,5)P_2, which is a rare lipid species found on endomembranes [62, 63, 65-67]. The scarcity of PtdIns(3,5)P_2 is largely owed to the fact that PIKfyve is in a complex with the 5-phosphatase, Fig4 [68]. Despite its low abundance, PtdIns(3,5)P_2 is perhaps the master regulator of endosome and lysosome volume: Cells defective in PIKfyve or its homologue in yeast, Fab1 show grossly enlarged endosomes, lysosomes, and vacuoles [69-76]. Specific inhibitors that prevent the activation of PIKfyve in mammalian cells also lead to rapid swelling of endocytic compartments, including early endosomes and lysosomes. PtdIns(3,5)P_2 production is also sensitive to osmotic perturbations to the cell, suggestive of its role in the osmoregulation of the endocytic pathway [63]. In yeast, levels of the phosphoinositide dramatically increase when cells are exposed to hyperosmotic medium. The response of the vacuole is to quickly shrink and undergo fragmentation, processes dependent on PtdIns(3,5)P_2[63, 68].

Given these observations in yeast and the fact that the inhibition of PIKfyve causes swelling of lysosomes even in mammalian cells that do not endocytose large volumes, it seems likely that PtdIns(3,5)P_2 plays distinct roles in early and late compartments. In cells that readily endocytose large volumes (e.g. those employing macropinocytosis), PIKfyve inhibition blocks the resolution of ingested fluid from the early endosomes and even leads to massive volume gain of the cells [16]. With this observation in hand, it would seem plausible that PtdIns(3,5)P_2 is required for monovalent ion efflux.

Indeed, the best characterized effectors or targets of PtdIns(3,5)P_2 are channels found in endomembranes including Transient Receptor Potential Cation Channel, Mucolipin Subfamily (TRPML) and Two Pore Channels (TPCs). TRPMLs are a family of lipid-gated cation channels of the endocytic pathway involved in the efflux of Na^+ and Ca^{2+}, thereby playing a role in endosome volume regulation and trafficking. Very recently, TRPML2 was also identified as being mechanosensitive, outwardly conducting cations in response to osmotic pressure and mechanical strain on the membrane [77].

Mechanical strain caused by swelling on the endomembrane augmented the gating of TRPML2 by PtdIns(3,5)P_2. This affords the cell two means by which endomembrane channels can respond to osmotic shifts: 1) the pressure can mechanically gate endomembrane TRP channels and 2) kinases that sense the ionic strength of the cytosol can generate lipid species that gate these (and other) channels.

The small TPC family contains three TPC isoforms in mammals – TPC1, 2, and 3 – with TPC1 and 2 being the most ubiquitously expressed and widely studied, as TPC3 is not expressed in humans nor mice [78, 79]. TPCs conduct monovalent and divalent cations including Na^+ and to a lesser extent, Ca^{2+}, and are gated by PtdIns(3,5)P_2 and voltage in the case of TPC1 [65, 79, 80]. TPC1 is found throughout the early and late endocytic pathway, while TPC2 is found primarily at the late endosome/lysosome; both transport cations quickly down their concentration gradients [81]. Through cryo-EM studies, the structures of mammalian TPC1 and 2 have been recently determined [65, 80, 81]. Though there is surprisingly little sequence similarity between these isoforms, they share key structural elements: Both form homodimers, with each subunit containing two six-transmembrane domain repeats (6-TM I and 6-TM II), and both undergo PtdIns(3,5)P_2 binding-induced conformational changes to allow ion conductance [65, 81]. When inactive, four pore-lining helices form a "constriction point", preventing the passage of cations; when active, these helices rotate away from the

pore, creating a larger opening and likely exposing the negatively charged side chains of acidic residues in order to further facilitate the passage of cations [65, 81]. Interestingly, while TPC2 contains many of the canonical elements associated with voltage-gated activation, it lacks a key Arg residue found in the TPC1 voltage-sensing domain (replaced by Ile); in fact, a single Ile to Arg substitution confers voltage-gating [81].

These structural studies have also provided important insights into the cation selectivity of TPCs. Initially, TPCs were touted as NAADP (nicotinic acid adenine dinucleotide phosphate)-gated Ca^{2+} release channels [82-84]. Patch-clamp studies of endolysosomal membranes however revealed that mammalian TPCs are primarily gated by PtdIns(3,5)P_2 and while they may conduct Ca^{2+}, they are significantly more selective for Na^+ [65, 66, 85]. Indeed, activating TPCs in isolated endolysosomes using PtdIns(3,5)P_2 alone results in current activation of the channels; by contrast, NAADP did not appear to activate the channels at all. Activation with PtdIns(3,5)P_2 in various cation bath/ion substitution conditions demonstrated that both TPC1 and TPC2 were highly permeable to Na^+ over other cations, and channel activation in isolated lysosomes was also shown to cause significant loss of Na^+ from the lysosome lumen (but not K^+) [66]. This ion selectivity of both TPC1 and TPC2 can be attributed to identical sets of "filter residues"; whether bound to PtdIns(3,5)P_2 or not, a string of Thr-Ala-Asn (filter I) residues in 6-TM I and Val-Asn-Asn (filter II) in 6-TM II from each TPC monomer align to form an extremely narrow pathway through which Na^+ can pass easily, but may exclude other larger cations [65]. Mutating the central Asp residue of filter II in particular can completely abolish its Na^+ selectivity. While Na^+ in the early endocytic fluid is engulfed as part of the extracellular fluid, sources at the lysosome are less clear but could include remnant Na^+ from the extracellular fluid and Na^+ exchanged into the lumen by lysosome-resident NHEs. Functional NHEs on the lysosome would further emphasize the need for Na^+ efflux pathways throughout maturation (see section on lysosomes).

Importantly, for TPC1 to incorporate into nascent endosomes in the first place, fusion with other endosomes and even late endosomes is required. Like all membrane fusion steps, this is expected to necessitate local elevations in cytosolic Ca^{2+}. The source of this Ca^{2+} could come from endocytosed channels like Orai1 and 2 which are activated by endoplasmic reticulum (ER)-resident stromal interaction molecules (STIMs). While connections have been made between ER Ca^{2+} and/or store operated calcium entry (SOCE) and endolysosomal fusion, details of the timing and mechanism of this process are not well understood and will likely be an interesting topic for future research. Another source of Ca^{2+} is endomembrane-resident cation channels including the TPCs and TRPMLs [86]. In this regard, TRPMLs may feature as they are non-selective Ca^{2+} channels found in endosomes and lysosomes [87]. While TRPML1 is found in endolysosomes, TRPML2 and 3 are in fact found in early endosomes [67, 88].

Cl^- efflux

With ongoing extrusion of Na^+, a counter ion flux is required to maintain electroneutrality. While this could come with cation exchange, in order to reduce the osmolarity of the compartment, a parallel efflux of anions would seem advantageous. Indeed, the efflux of Cl^- was shown to be necessary for macropinosomes, large endosomes formed in macrophages, to undergo maturation and osmotically-driven shrinkage [16]. Despite this, the mechanisms of Cl^- exit from the early endosome are poorly understood in comparison to what is known about Na^+. Cl^- efflux could be provided by the ClC family of ion exchangers, which can efflux $2Cl^-$ ions while importing $1H^+$ at near neutral pH [89]. The transport here is an active process; it has been found that ClCs actively pump H^+ into the endosome while transporting Cl^- out, which is mediated by a single glutamate residue in the pore region (E221) [90, 91]. ClCs are present throughout the endocytic pathway; ClC-4 and -5 are found in the early and recycling endosomes [92, 93], ClC-3 and -6 are found in the late endosome [94], and ClC-7 is found in the lysosome [95]. Given their ubiquity, it is perhaps unsurprising that defects in ClCs can have broad consequences in endosomes and control of their volume.

In addition to ClCs, other channels may also conduct Cl^- across the endosomal membrane. Volume-regulated anion channels (VRACs) are activated during RVD and are permeable to Cl^-, in addition to numerous other solutes and osmolytes. In patch clamp studies, VRAC/LRRC8 was shown to conduct Cl^- in hypotonic conditions [96]. Chloride intracellular channels (CLICs) are another possibility; they have been shown to permit Cl^- flux in bone marrow-derived macrophages [97] and, separately, CLIC3 has been proposed to play a role in trafficking from the recycling endosome [98]. Despite their ability to conduct Cl^-, however, neither CLICs nor VRACs have been definitively shown to remove Cl^- from endocytic vesicles and drive osmotically driven shrinkage. Future studies will be needed to identify whether these or possibly other types of Cl^- channels fulfill this role.

Monovalent ion flux and early endocytic traffic

Monovalent ion flux and TPCs/ClCs in particular have been shown to play a wide variety of roles in endocytic traffic, recycling, and volume regulation, suggesting major contributions of such fluxes in whole-cell function [16, 99]. Blocking or inhibiting TPCs *in vivo*, for example, leads to defects in the recycling of integrins as well as defective immune surveillance and responsiveness of resident tissue macrophages [16]. Bone marrow-derived macrophages from *Tpcn1*$^{-/-}$ *Tpcn2*$^{-/-}$ mice also show significant fluid trafficking defects; these mice show delayed macropinosome shrinkage and tubulation, likely owing to a lack of fluid efflux as ions were not removed from the compartments [16]. TPCs also contribute to viral entry and trafficking via the endocytic pathway. Enveloped viruses including Ebola and coronaviruses that are endocytosed and trafficked to acidic organelles prior to fusion, require TPC and PIKfyve activation for infection [100, 101]. Interestingly, *Tpcn2*$^{-/-}$ mice also show a profound susceptibility to fatty liver disease that was attributed to impaired endolysosomal liberation and trafficking of LDL-cholesterol [102]. This was proposed to occur at the level of endosome-lysosome fusion, a process determined (at least in part) by Ca^{2+}.

It seems plausible that in many of these scenarios, defects arise in regulating the tension across the endomembrane rather than fusogenic or tubulation machinery *per se*. In the case of fusion with the limiting host endomembrane by enveloped viruses for example, Ca^{2+} which is abundant in the lumen, is certainly not the issue. Supporting this hypothesis, macropinosomes or lysosomes made to swell by inhibiting PIKfyve or TPCs could be forcibly shrunken by increasing the tonicity of the medium; this promptly leads to tubulation of the compartments. Further lending supporting to this notion, the pharmacological inhibition of PIKfyve blocks endosome fission [75], arrests receptor recycling [103] and trafficking of the V-ATPase [104], impairs retrograde transport of cargoes to the TGN [72], and ultimately arrests the resolution of organelles that form *de novo* in myeloid cells like macropinosomes and phagosomes [105]. All of these processes proceed through membrane deformation events such tubulation, budding, vesiculation, and scission and require low membrane tension to proceed.

In parallel, impairing the chloride fluxes of early endosomes also prevents their normal trafficking and receptor recycling. The loss of ClC-5 in a model of Dent's disease was shown to reduce membrane expression of numerous receptors and transporters, thereby reducing the receptor-mediated endocytosis of their respective ligands [92]. It was later shown that inhibition of ClC-4 or ClC-5 not only impaired trafficking of endocytic cargo, but also impaired "fast" recycling, likely explaining the reduced membrane expression of cell surface receptors seen with ClC-5 knockout. Interestingly, inhibiting these transporters also blocked acidification of the endosome [93]. This appears to be a common element of ClC perturbations; ClC-6-deficient Chinese hamster ovary cells show both Cl^- accumulation and loss of late endosomal acidification, and siRNA knockdown of ClC-7 in the lysosome leads to impaired acidification as well [95]. While the late endosome and lysosome are more acidic than the early endosome, acidification of the early endosome is still imperative, particularly for the dissociation of ligands from internalized receptors [106]. The regulation of endolysosomal pH is mainly attributable to the V-ATPase proton pump, which moves protons into the maturing endosome to acidify it [107]. ClCs have been proposed to act as

Cl^- shunts to maintain electroneutrality for the V-ATPase: Should ClCs be a major source of Cl^- in the early endocytic pathway, the elusive outward anion current becomes even more critical for endocytic fluid resolution and volume control.

Membrane tension regulates endocytic remodeling and traffic

The complexes that orchestrate the formation of tubules and vesicles including Retromer, Retriever, and ESCRT, are all resisted by high endomembrane tension (Fig. 2). Because endomembrane trafficking is made to be highly dynamic by these systems – numerous fusion and fission events occur as endosomes mature, membrane is gained from the cell surface by fusion of vesicles, fission sorts cargo vectorially to its destination, and cargo is also sorted by inward budding to form intraluminal vesicles (ILVs) for degradation– the endomembrane system is highly dependent on osmoregulation and organelle volume control. Moreover, the sorting complexes and coat proteins that interface with tubular endomembranes do so via the recognition and stabilization of high curvature, events that can be mediated by BAR (Bin–Amphiphysin–Rvs) domain-containing proteins [108, 109]. The crescent-shaped BAR domains interact with the membrane either through electrostatic interactions with the negatively-charged surface and/or insertion of an amphipathic helix into the lipid bilayer itself [108-110]. These domains can then oligomerize, forming lateral and/or end-to-end helical assemblies that further deform the membrane, generating tubules. The generation of these tubules also requires the coordination of other components such as the cytoskeleton. The WASH complex, for example, mediates actin polymerization that helps to form tubules, and motor proteins such as dynein-dynactin pull the elongated tubules along microtubule networks. WASH is associated with both Retromer and Retriever and likely assists in cargo sorting and concentration in the early endosome and recycling endosome in addition to its role in tubulation [110].

The ability of BAR domain-containing proteins to induce high membrane curvature is opposed by high tension on the membrane [16, 108, 111, 112]. In conditions of high membrane tension, BAR domain oligomerization and assembly is reduced, thereby preventing tubulation. When membrane tension is decreased, such as in the case of a resolving endosome or the endocytic recycling compartment as solutes and fluids are removed from the lumen, spontaneous BAR domain recruitment and assembly is induced, causing tubulation [16, 108]. Osmotic pressure and tension regulation, then, is an important factor in the fate of early endocytic cargo.

ESCRT and endomembrane tension

As previously mentioned, cargo sorting into ILVs for eventual degradation is regulated by the ESCRT family of protein complexes [17, 113] (Fig. 2). ESCRT-0 primarily associates with the early endosome through the FYVE domain of one of its subunits (Hepatocyte growth factor-regulated tyrosine kinase substrate, Hrs) which binds to PtdIns(3)P [110, 113]. Ubiquitinated cargo recognized and bound by ESCRT-0 is sequestered into a degradative subdomain as ESCRT-0 begins to self-associate and recruit clathrin. ESCRT-I and -II have a lower affinity for ubiquitin binding but also help enrich cargo at these subdomains. ESCRT-III is the last to arrive; while ESCRT-III does not directly bind ubiquitin, it senses the accumulation of ESCRT-II and self-associates to trap cargo and generate inwardly-budding ILVs. These ILVs are therefore highly enriched with cargo destined for degradation and demarcate the beginning of the maturation from early endosomes to multi-vesicular late endosomes [113].

Recently, the effect of ESCRT-III on ILV formation has been directly linked to alterations in membrane tension [17]. The authors evaluated this using fluorescent membrane tension probes, FliptR [114] and Lyso Flipper [115]; these probes insert into the membrane and utilize planarizable fluorescent groups which flip/rotate in response to lateral force (e.g. changes in membrane lipid packing when tension is applied). The rotation of the fluorescent groups leads to alterations in the fluorescence lifetime, which can in turn be measured through FLIM (fluorescence lifetime imaging microscopy) [17, 114, 115]. Hypertonic shock,

which decreases endosome volume, leads to a decrease in fluorescence lifetime of the probe and thereby signals decreased membrane tension; hypotonic shock leads to the opposite. In both cultured cells and vesicles reconstituted *in vitro*, it was demonstrated that decreases in endosomal volume and membrane tension were associated with increased recruitment and polymerization of the ESCRT-III subunit, CHMP4B. This was found to be the case when membrane tension was altered by several methods including incubation of the cells with hyper/hypotonic solutions, distending endolysosomal membranes using L-leucyl-L-leucine methyl ester (LLOMe) that enters and is cleaved in lysosomes to increase their luminal osmolarity, or by physically pulling on the membrane with micropipette aspiration and optical tweezers [116]. Collectively, these results indicate that the effect on ESCRT-III recruitment is directly related to membrane tension. Finally, the authors showed that the low membrane tension-driven recruitment of ESCRT-III triggered the formation of ILVs from endosomes in canonical EGFR-induced endocytic pathways [117]. As with BAR domains, a decrease in endosomal membrane tension likely also provides the membrane slack necessary for ESCRT-III to polymerize and deform the membrane into ILVs (Fig. 2).

Osmoregulation of lysosomes

Early endocytic compartments mature to become late endosomes and eventually terminate in lysosomes. These transitions are chiefly characterized by the exchange of Rab5 for Rab7 and the gross conversion of PtdIns(3)P to PtdIns(3,5)P_2 as reviewed elsewhere [118-121] concomitant with a steep drop in luminal pH. Compared to the early endocytic pathway, the flux of solutes is generally better studied at the lysosome because there has been tremendous interest in the movement of organic solutes across the lysosomal membrane [122]. Despite the intensity of research in the area, there remains precious little information about the osmotic consequences of this transport and the resultant tension on the limiting membrane of lysosomes. Moreover, the major solutes of the lysosome are still ions. While measurements of the steady-state concentrations of ionic solutes are challenging to determine for lysosomes –compartments that are difficult to access by traditional patch-clamping methods and where harsh luminal conditions have unwanted impacts on reporter molecules– some estimations of these solutes in lysosomes have in fact been made. The luminal concentrations of Na^+ and Cl^- are indeed reported to be higher than in early endosomes. For example, $[Cl^-]$ increases from 20 mM in the early endosome to 60 mM in the late endosome/lysosome [45]. The luminal $[Na^+]$ and $[K^+]$ of lysosomes show a broad range of estimates from 20-140 mM for Na^+ and 2-50 mM for K^+ [51, 66]. Still, even the low range for estimates for $[Na^+]_{lysosomes}$ exceeds that of $[Na^+]_{cytosol}$, which is ~12 mM. With caveats and challenges in mind, as we did in the previous section, here we describe the individual solutes of the lysosome, their relative osmotic contributions and transport pathways, and their role in storage disorders.

pH and osmotic considerations

The best appreciated ion transport at the lysosome is that of protons being pumped into the lysosomal lumen by the V-ATPase, an ATP-dependent rotary enzyme [123]. This effectively decreases the pH from ~6.2 in the early endosome to 4.5-5.0 in the endolysosome [44]. Regulation of the proton pump, the buffering power of the lysosomal fluid, and the osmotic consequences of both are remarkably complex. For example, in order to prevent the establishment of an electrochemical gradient, the net increase in positive charge during acidification must be coupled to a counter-ion flux [124]. Some of the counter-ion flux mechanisms have significant osmotic effects. Critically, much of the trafficking and cargo sorting in the late endocytic pathway and even the flux of ionic and organic solutes are closely tied to the activity of the V-ATPase and use a proton motive force. As a result, though lysosomes can have a range in their acidity, the maintenance of a low pH requires ongoing activity of the V-ATPase since protons are constantly exiting via a 'leak' [125, 126].

Taken together, there are four major components that influence the acidification of the lysosome: 1) the activity and copy number of the V-ATPase per unit area/volume of the compartment, 2) the conductance of counter-ions, 3) the rate of the H^+ leak and 4) the buffering capacity (Fig. 3). These factors are interrelated and the net effect of these relationships is expected to impact the osmolarity, volume, and tension on lysosome membranes. For a discussion on the buffering capacity of the lysosome, we refer the reader to a review by Casey et al. [60].

The counter-ion flux that supports the activity of the V-ATPase can come in the form of either a cation efflux, an anion influx, or a combination of the two (Fig. 3). A subgroup of organellar NHEs, namely NHE6-9, can support the former mechanism at different stages of endosome maturation [127-129]. At the lysosome, the proton gradient that drives NHE exchange and proton efflux facilitates the influx of Na^+ and/or K^+. Although electroneutral, NHEs therefore contribute to the H^+ leak and leads to the luminal accumulation of monovalent alkali cations. This may serve to prevent over-acidification of the lysosome, which could occur from a purely thermodynamic perspective in the absence of H^+ leaks; the pump is sufficient to acidify compartments to pH <3.5 when operating without kinetic inhibition [130, 131]. Corroborating this idea, loss of NHE6 alone leads to over-acidification of murine neuron endosomes [128], and overexpression of NHE9 leads to alkalization of murine astrocyte recycling endosomes [129].

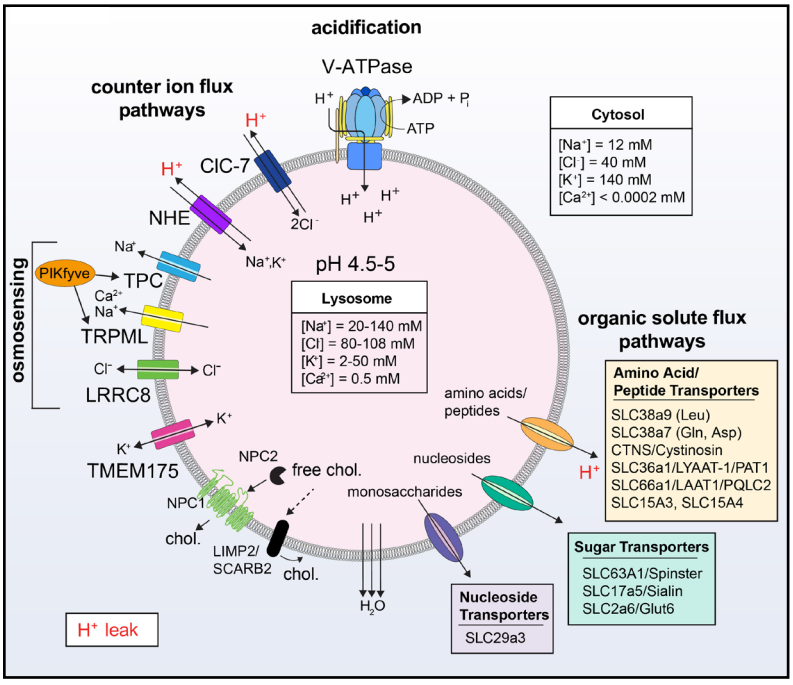

Fig. 3. Ionic and organic solute fluxes of lysosomes. The steady-state pH of lysosomes is maintained by the rate of H^+ influx (mediated by the V-ATPase), the rate of H^+ efflux or 'leak', and the conductance of counter-ions. At a pH of 4-5, lysosomal $[H+]_{lumen}$ can be 1000-fold higher than $[H^+]_{cytosol}$, which is at pH 7.2. The pump can work against this gradient and does not acidify the lysosome further, partly because of the H^+ leak which is closely tied to the activity of solute transporters. These transporters, some of which are dependent on either the H^+ or Na^+ gradient to function, mediate the efflux of newly liberated amino acids, nucleosides, and monosaccharides derived from incoming endocytic cargo. NHEs functioning at the lysosome utilize the H^+ gradient and exchange monovalent cations (e.g. Na^+) into the lumen. As a result, to avoid their accumulation, monovalent cations are expelled from the lysosome via channels including TPC1-2, TRPML1-3, and TMEM175, a bidirectional K^+ channel. These channels are gated by PtdIns(3,5)P_2 and at least in the case of TRPML2, are activated by osmotic pressure and high endomembrane tension. PIKfyve is also stimulated by osmotic stress, suggesting multiple means for osmosensing by lysosomes. To rapidly acidify earlier endocytic compartments, ClC-7 provides a counter-ion force in the form of Cl^- influx. While ClC-7 is another source of the H^+ leak, the net negative charge of the lumen supports the activity of the V-ATPase from an electrical standpoint. Cl^- efflux, on the other hand, involves LRRC8 and other putative transporters/co-transporters. Like TRPML2, LRRC8 also senses the ionic strength of the cytosol. Importantly, monovalent ion and solutes flux dictate hydrostatic pressure and membrane tension on the lysosome and ultimately fission/traffic.

The constitutive action of lysosomal NHEs, on the other hand, now causes an ongoing influx of monovalent cations with which the lysosome must contend, otherwise the V-ATPase would quickly be stunted from an imposing electrogenic gradient. To this end, cation conductance on the lysosome is mediated by channels previously described: TPCs, TRPMLs, and TMEM175 [16, 66, 132, 133], all of which could conceivably provide the counter-ion flux for the V-ATPase, enabling the efflux of cations from the lysosomal lumen. In the absence of this counter-ion force, the activity of the V-ATPase would decrease, which should lead to an increase in lysosomal pH, as the steady-state H^+ leak overtakes the rate of H^+ influx. Indeed, *Tpcn2*$^{-/-}$ mouse skeletal muscle cells display more alkaline lysosomes than in their wildtype counterparts [134]. Interestingly, overexpressing an active form of TPC2 in HeLa cells is sufficient to lead to increased lysosomal tubulation and motility [16], suggesting that cation conductance in the lysosome contributes to membrane remodelling and resolution via the movement of ions and osmotically-obliged water.

Importantly, the pH of endocytic compartments is not always at a steady-state. Transitions in pH, though rarely measured or appreciated, are essential for efficient trafficking and processing in the endocytic pathway. This is well-exemplified in myeloid cells that take up large, often infectious, particles by phagocytosis that need to be degraded in a timely manner. Certain myeloid cells indeed show rapid acidification of nascent phagosomes [135, 136]. For rapid, deep transitions in luminal pH of considerable volumes, cation fluxes alone may be insufficient. Instead, anion influx may also be required. Myeloid cells highly express the lysosomal $H^+/2Cl^-$ exchanger ClC-7. By moving 2 Cl^- in for each H^+ out, ClC-7 has the potential to greatly affect the rate of the V-ATPase by providing a robust anion influx [137]. Defects in ClC-7 in mice and humans lead to osteopetrosis where osteoclasts cannot well-acidify the lacunae [89, 138, 139]. Patients with a mutation in ClC-7 rendering the exchanger constitutively active, on the other hand, have hyper-acidified lysosomes [140].

While Cl^- exchange supports acidification, without mechanisms of control, it is expected to have osmotic consequences on the compartment. The hyper-acidified lysosomes in the ClC-7 gain-of-function patients are large, phase-lucent, and round/distended [140], suggesting that they are fluid-filled and likely experience high membrane tension that precludes the formation of tubules. In the osteoclast lacunae, the space is open and the action of ClC-7 at the plasma membrane does not have an osmotic effect. Macrophages may rapidly acidify nascent phagosomes containing potential pathogens at the cost of some swelling to the compartment.

The efflux of Cl^-, on the other hand, could occur via VRAC/LRRC8. Supporting this idea, VRAC deficient cells have recently been shown to possess osmotically swollen lysosomes [141]. Reciprocally, LRRC8 localized to lysosomes has been recently described to play a role in sensing cytoplasmic ionic strength. In response to low cytoplasmic ion concentrations, LRRC8 conducts a large Lyso-VRAC current *inward*, thereby moving water from the cytoplasm and into the lysosomes which can then expel it through exocytosis [142]. Through this, LRRC8 reduces cellular swelling and prevents lysis in response to hypoosmotic conditions. The parallel, charge-neutralizing transport of cations could come from TMEM175 (a bidirectional K^+ channel) [133] or previously described mechanisms of Na^+ transport.

That osmoregulation is an ongoing phenomenon at the lysosome is supported by the long-standing observation that inhibition of PIKfyve causes swelling of lysosomes. The formation of PtdIns(3,5)P_2 and its gating of cation channels is clearly important for monovalent ion effluxes from the lysosome and in some cell systems, it is unlikely that even a majority of the ions are accounted for via endocytosis. For example, in yeast, the loss of Fab1, its PIKfyve homologue, leads to the gross enlargement of its vacuole [143, 144]. We have also noted that the swelling of lysosomes in macrophages can occur when macropinocytosis is inhibited (S.A.F. unpublished observations). The best evidence, however, that ongoing monovalent ion fluxes regulate lysosome volume is that the swelling phenotype observed when cells are treated with PIKfyve inhibitors is ablated by treating the cells concomitantly with inhibitors of the V-ATPase [75]. Moreover, genetic screens in yeast and mammalian cells have demonstrated that the loss of ClC-7 and cation exchangers also blunt the swelling of

the lysosomes [145, 146]. The simplest explanation for such results is that the source of swelling upon PIKfyve inhibition is the Cl^- and Na^+/K^+ that are continuously supplied into the lysosome lumen by ClC-7 and NHEs which is no longer counter-balanced by the outward flow through TPCs/TRPMLs (or K^+ channels) and unknown Cl^- channels. More elaborate explanations could be proposed including the notion that PtdIns(3,5)P_2 directly influences the activities of NHEs and ClCs, but these would need to be tested.

The liberation of organic solutes

In addition to the dominant ionic solutes of the lysosome, the organelle contains over 50 degradative hydrolases [139], including proteases, lipases, glycosylases, nucleases, sulfatases, and phosphatases, that degrade endocytosed content and cargoes. In so doing, the hydrolases liberate organic solutes which are effective osmolytes including peptides and amino acids, monosaccharides, nucleosides, metals, and lipids that can be broken down into fatty acid components, and phospholipid headgroups. With this constant degradation comes the critical task of contending with the osmotic loads that these organic solutes impose on the compartment and potentially calibrating lysosomal volume and tension accordingly. As we described in earlier sections, the lysosomal membrane will only be able to stretch minimally, so handling the osmotic burden must be achieved by either fusion and the addition of new membrane surface or the efflux of solutes followed by osmotically-obliged water. Fusion may indeed be a fail-safe mechanism for avoiding osmotic pressure build-up. For example, when faced with pathogens that grow and expand in the phagolysosome, macrophages begin to fuse lysosome reserves to accommodate the expansion while containing the threat [147]. In cells where the synthesis of PtdIns(3,5)P_2 is prevented, ongoing fusion of endolysosomes almost certainly prevents severe ruptures of the compartment [148, 149]. But the normal strategy of lysosomes is to flux the liberated organic solutes out of the lysosome to avoid any build up in osmotic pressure. This action maintains membrane dynamics and ensures proper trafficking which is presumably also driven by curvature-sensing proteins at the lysosome, though these remain poorly characterized.

The efflux of organic solutes is orchestrated by a huge family of solute carriers termed SLCs (Fig. 3). Lysosomal membranes are heavily populated by these solute transporters. Perhaps the best studied are the SLCs responsible for the transport of amino acids, solutes liberated from ingested proteins and peptides. Examples include SLC66A4 (cystinosin), a lysosomal H^+/cysteine symporter [150]; SLC66A1 (PQLC-2) [151], a lysosomal lysine/arginine transporter; SLC15A4, an oligopeptide transporter [152]; as well as SLC36A1/2 (PAT-1/2), lysosomal H^+/di-, tri peptide cotransporters [153], mutations in some of which lead to swollen lysosomes and lysosomal storage disorders in patients [150-152]. Other lysosomal amino acid transporters, including SLC38A9 [154] and SLC38A7 (SNAT-7) [155], which mediate the efflux of arginine and glutamine respectively, also impact cell growth and regulate lysosomal volume, although little is currently known about the latter. Additionally, amino acid efflux not only osmotically regulates the lysosome, but also greatly contribute to cell growth via mTOR [156]. mTOR forms major signalling protein complexes that bind to the surface of lysosomes and monitor amino acid abundancy in the cell. This can be conveyed via SLC38A9 [154], as well as SLC36A1 [157], which directly signal to mTOR the presence of free amino acid exiting the lysosome.

In conditions with ample amino acid supply, mTOR promotes cell proliferation and growth [158]. Interestingly, mTOR has been shown to inhibit TPCs, and under starvation conditions where mTOR dissociates from the lysosome, TPCs are constitutively active [159]. Since activation of TPCs cause the efflux of Na^+, lowering membrane tension and leading to tubulation and trafficking, this could be a mechanism wherein mTOR inhibits lysosomal fission such that the compartment does not resolve prior to removing all the amino acids. Whether or not mTOR or signaling pathways involved in cell growth (i.e. PI3K/Akt) control other endomembrane resident ion channels and lysosome fission remains of great interest.

Other than proteins, major macromolecules degraded in the lysosome include carbohydrates. A well-known sugar transporter present on lysosomes is SLC17A5 (sialin),

a sialic acid/monosaccharide transporter that utilizes the H^+ gradient for its activity [160] (Fig. 3). Mutations in the transporter lead to Salla disease, a rare lysosomal storage disorder wherein patient cells incur enlarged lysosomes with impaired trafficking, owing to the build-up of sialic acid in lysosomes [161-163]. Other lysosomal sugar transporters include the SLC63 family SLC63A1-3 (Spns1, Spns2, and Spns3); deficiencies in its *Drosophila* and zebrafish homologues cause lysosomal storage disorders in neurons [164]. In addition, loss of SPSN has been shown to cause enlarged lysosomes in epithelial cells, and also seems to play a role in regulating ALR [165]. SLC2A8 (GLUT8), which maintains glucose efflux in the lysosomes of testis, may also play a role in maintaining lysosomal homeostasis [166].

Lipid efflux from the lysosome is much more complex than sugars and amino acids, largely due to their inherent hydrophobicity. During instances of efferocytosis or phagocytosis, where cells ingest large amounts of bacterial or cell membrane, the phagolysosome is a site where large quantities of lipids are extracted and phospholipid head and tail groups are liberated. These products need to be exported from the lysosome in a timely manner. Some studies have looked at SLC44A1 (CTL-1), a choline and ethanolamine transporter highly expressed in macrophages [167]. These phospholipid headgroups are responsible for the biosynthesis of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), two of the most common phospholipid species in cells, and will likely make up a portion of a macrophage's diet. Little is known, however, about the role of SLC44A1 at the lysosome.

Another source of lipids are those coming in to cells via chylomicrons like low-density lipoproteins (LDLs), which can be ingested via receptor-mediated endocytosis [168]. LDLs, when broken down, liberate a large quantity of cholesterol, which are exported from the lysosome via the Niemann-Pick proteins (Fig. 3). Briefly, the luminal cholesterol binds Niemann-Pick C2 (NPC2), which transports it across the inner glycocalyx and passes it to the N-terminus of the transmembrane protein NPC1. NPC1 forms a multimeric complex similar to a protein tunnel, wherein cholesterol is able to pass through and exit the lysosome [169-171]. Upon export, cholesterol is then contacted by a variety of lipid binding proteins and shuttle it to the ER, mitochondria, or other organellar membranes [172]. In line with other solute transporters, loss of function in either NPC (usually NPC1) lead to the pathogenesis of Niemann Pick type C disease, a rare lysosomal lipid storage disease, characterized by enlarged lysosomes in patient cells [173, 174]. Although this is chiefly caused by cholesterol accumulation and may not be totally osmotic in nature, one can imagine the membrane is likely under high tension, which only exacerbates the impairment of lysosomal remodelling and trafficking seen in these cells.

Finally, in the event of efferocytosis/phagocytosis, another major category of liberated solutes is nucleosides (dephosphorylated nucleotides). Derived from the DNA of ingested species, nucleosides are primarily exported via the lysosomal transporter SLC29A3 (ENT3), deficiencies in which have been implicated in lysosomal storage disorders [175-178]. Lysosomes from SLC29A3$^{-/-}$ mice also are enlarged [179]. Like many of these transporters, SLC29A3 is pH sensitive and requires low pH for its proper function [180, 181].

Do lysosomal storage disorders cause osmotic pressure?

Impairments in the export of liberated amino acids, nucleic acids, lipids, or sugars all lead to enlarged, distended/round, and often perinuclear/clustered lysosomal phenotypes, with some being characterized as lysosomal storage disorders (Fig. 3). Pathogeneses of many lysosomal storage disorders fall under one of two categories; mutations in lysosomal solute transporters or mutations in lysosome hydrolases [182]. In the event of the latter, large macromolecules that enter the endocytic pathway are not digested in the lysosome, leading to their accumulation and swelling. In the former, the liberated solutes are unable to exit the lysosome post enzymatic breakdown. In either case, the accumulation of solutes in the lumen of lysosomes is matched with the osmotically-driven retainment of water. While is it not known how much hydrostatic pressure the lysosomal membrane can withstand before rupture, even a small osmotic imbalance between the cytosol and the lysosome will cause tension on the membrane. It is interesting to postulate that impaired trafficking and fission

observed in lysosomal storage disorder patients are a consequence on the hydrostatic pressure that builds against the limiting membrane of the lysosome as solutes accumulate in the lumen. An effort to quantify the magnitude of this pressure would be a worthy endeavour. Broadly, the control of lysosome volume and potentially osmotic pressure on the organelle in health and disease plays a significant role in the overall cellular activity and metabolism.

Conclusion

The interplay of ion flux, fluid movement, and membrane remodelling collectively regulate many functions along the endocytic pathway. We have focused this review on their control of endomembrane tension and organellar volume, often-overlooked considerations in the field of membrane trafficking and cell volume. The monovalent ion channels and transporters that localize to endocytic compartments are gated by lipids, are themselves osmosensors, and are required for the removal of solutes. The regulation of these channels and transporters occurs temporally and spatially and likely contribute to the cellular decision making for numerous processes including the rate and extent of acidification, endocytic maturation, and resolution, to name a few. Controlled solute efflux is ultimately necessary for the resolution of fluid and orchestrates the fission and fragmentation of organelles. Most germane to this special issue, these fluxes can have significant impact on cellular volume and how these volumes are distributed between the cytosol and organelles. Volume regulation in the secretory pathway, especially for systems that need to accommodate large extracellular matrix proteins, mucins, etc., are expected to have equally complex mechanisms behind their volume regulation. As the field has begun to near fully appreciate regulated volume increases and decreases of cells, we hope that this review has served to highlight how much remains to be determined for the regulation of organellar volume.

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Disclosure Statement

The authors declare they have no conflict of interest.

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