SEARCH FOR CELL VOLUME SENSORS: AN UPDATE

Orlov, S.N.1–3 and Grygorszyk, R.1

1 Centre de recherche, Centre hospitalier de l’Université de Montréal (CRCHUM), Montreal, PQ, Canada
2 Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russian Federation
3 Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russian Federation

ABSTRACT

Cell volume changes affect a plethora of cellular functions including macromolecular synthesis and catabolism, cell proliferation, differentiation and death. Because of these critical roles, cells regulate their volume with <1% accuracy via compensatory mechanisms termed regulatory volume increase and regulatory volume decrease. Our review focuses on recent advances in understanding the molecular origin of up-stream sensors triggering cellular responses to volume perturbations. The analysis demonstrates a major role of cytoplasmic hydrogel as well as the crosstalk of polyphosphoinositides and two-dimensional cytoskeleton (membrane carcass) in cell volume sensing.

KEY WORDS: cell volume, membrane stretch, cytoskeleton, biogel.

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Sergei N. Orlov obtained his M.Sc. (1971) and Ph.D. (1975) degrees in biophysics from M.V. Lomonosov Moscow State University, Russia and Doctoral Degree in Biological Science from the Institute of Photobiology, Academy of Science, Belarus Republic. From 1976 to 1989 he was a senior research associate at the Central Research Laboratory of the Ministry of Public Health of the USSR and since 1989 up to now he is a Professor of Biophysics and Head of the Laboratory of Biological Membranes, Faculty of Biology, M.V. Lomonosov Moscow State University. He worked as invited professor in Research Centre, University of Basel Hospital, Switzerland, Department of Physiology, University of Kuopio, Finland and Research Centre, University of Montreal Hospital (CRCHUM), Canada. Since 1996 till 2012 he is Head of Laboratory of Pathophysiology of Ion Transport Disorders at CRCHUM and Professor at the Department of Medicine, University of Montreal, Canada. His current research is focused on the role of ion transporters in cell volume regulation, gene expression, cell proliferation and death. Dr. Orlov was supported by research grants from the Canadian Institutes of Health Research, the Kidney Foundation of Canada and Heart, Stroke Foundation of Canada and currently from Russian Foundation for Fundamental Research and Ministry of Science and Education of the Russian Federation. He holds 2 patents and is author/co-author of over 250 peer-reviewed scientific publications.

Ryszard Grygorczyk obtained his M.Sc. and Ph.D. degrees in solid state physics from Wroclaw University, Poland. From 1982 to 1987 he was a postdoctoral fellow at the Max-Planck Institute of Biophysics in Frankfurt, Germany, from 1989 to 1994 he was a Senior Research Biologist at Merck-Frosst in Montreal, Canada. Since 1996 he is a faculty member in the Department of Medicine, University of Montreal and head of a research laboratory at the Montreal University Hospital Research Centre (CRCHUM). His current research is focused on mechano-purinergic signalling in the lung, abnormalities in fluid and mucin secretion in cystic fibrosis, and cell volume regulation. Dr. Grygorczyk is supported by research grants from the Canadian Institutes of Health Research, Cystic Fibrosis Canada, Natural Sciences and Engineering Council of Canada, and National Institute of Health, USA. He holds 11 patents and is author/co-author of over 70 peer-reviewed scientific publications.

Changes in cell volume frequently occur under physiological and pathological conditions due to alterations in the content of extra- or intracellular osmolytes. When arise, such changes affect a plethora of cellular functions including macromolecular synthesis and catabolism, cell proliferation, differentiation and death. Because of this, cells regulate their volume with accuracy of <1% [1] via ac-
cumulation or loss of intracellular osmolytes. The resulting compensatory processes are termed regulatory volume increase (RVI) and regulatory volume decrease (RVD). The intracellular signaling events that lead to activation of RVI and RVD include dozens of cell-type specific signaling pathways, and incorporate G-proteins, Rho family GTPase, numerous protein kinases, phospholipases, [Ca\textsuperscript{2+}], signaling, etc. The detailed mechanisms of cell volume regulation are considered in several comprehensive reviews [2–11]. The present mini-review is aimed to analyze recent findings concerning the molecular origin of up-stream volume sensors that trigger cell volume regulatory responses.

1. Plasma membrane stretch

The control of animal cell volume has traditionally been modeled as water flux driven by solute fluxes across the plasma membrane [12]. Consistently with this model, the predicted hydrostatic pressure across plasma membrane is calculated from van’t Hoff’s law as $\Delta\Pi = RT\Delta\pi$, where $\Delta\pi$ is the gradient of osmolyte concentration across the plasma membrane and $RT = 24.4$ Atm/M at 25°C. Laplace’s law predicts that the plasma membrane in rounded swollen cells will be exposed to tension $T = \Delta\Pi r/2$, where $r$ is the cell radius, and attenuation of extracellular concentration of NaCl by 5 mM (~10 mM osm) results in elevation of surface area $S = 4\pi r^2$ by ~5%. Importantly, the lipid bilayer can withstand only 3% increase of the S before its collapse [13]. This model suggests that the plasma membrane in swollen cells will be stretched that, in turn, leads to conformational changes in ion channels and other potential membrane-based cell volume sensors.

Numerous studies demonstrated that mechanical sensitivity of ion channels is a property that is as general as voltage sensitivity [14]. Moreover, Callies and co-workers demonstrated that membrane depolarization might be mediated by altered membrane stiffness [15]. Among mechanosensitive channels involved in osmolyte movements most notable are bacterial channels MscL and MscS and members of the transient receptor potential (TRP) vanilloid subfamily (TRPV) whose activity is increased in endothelial and epithelial cells subjected to shear and anisotonic stresses [16, 17]. It should be noted, however, that unlike bacterial channels whose mechanosensation was demonstrated in vivo [18], activation of mammalian channels by stretch has only been mainly observed in patch-clamp experiments using excised membrane fragments incubated at room temperature. Moreover, activation of TRPV4 channels registered by this technique was not detected by measurement of ion current in whole-cell patch-clamp studies [19].

The canonical model considered above assumes that because of the stretch the plasma membrane in swollen cells became stiffer [8]. However, using atomic force microscopy (AFM), Spagnoli and co-workers demonstrated that during osmotic swelling mammalian cells became softer rather than stiffer [20]. These data indicate that 2-dimensional mechanics of Laplace’s low can not be applied to intact cells, and the results also predict that osmotic stresses must be distributed within a 3 dimensional sponge-like cytoplasmic hydrogel (for more details, see Section 7).

Several pharmacological tools are employed to assess the role of membrane stretch in signal transduction. All type of mechanosensitive ion channels studied so far are inhibited by Gd\textsuperscript{3+} [21]. However, side-by-side with channel’s inhibition, Gd\textsuperscript{3+} and other lanthanides compress lipid bilayers and exhibit high affinity binding with all proteins studies so far. Thus, for example, we observed that at concentration of 10–100 µM Gd\textsuperscript{3+} blocks luciferase activity, furthermore its addition to A549 cells activates rather than inhibits swelling-induced ATP release [22]. Sachs and co-workers reported that activity of mechanosensitive channels can be inhibited by GsMTx4, a peptide isolated from tarantula venom. This compound inhibited RVD in rat kidney cell line but had no effect in primary rat astrocytes and Madin-Darby canine kidney (MDCK) cells [23].

Considering 2D model of cell volume sensing, it is important to note that because of the multiple invaginations of the plasma membrane in nucleated cells, generation of significant mechanical forces during swelling or shrinkage seems to be unlikely. Indeed, electrophysiological experiments register no changes in membrane capacitance (a parameter related to total surface area) even upon two-fold increase of cell volume [24]. To explore the issue of membrane reserves, we employed the dual-image surface reconstruction (DISUR) technique. This method allows accurate and simultaneous measurements of cell height, total surface area and volume with temporal resolution of ~100 ms in single substrate-attached cells [25–27]. The size of surface area reserve was determined by transferring cells into extreme hypotonic solution. In this study, 3 different types of nucleated mammalian cells could increase their surface area and volume until membrane rupture by ~3 and 10-fold respectively [27]. Importantly, cells accommodate moderate (2-fold) volume increase by shape changes and plasma membrane unfolding, while significant endomembrane insertion was observed during extreme swelling (Fig. 1). This conclusion is consistent with unfolding of membrane invaginations during modest hypotonic swelling in Ehrlich ascites tumor cells [28] and human erythrocytes [28], detected by scanning electron microscopy and AFM, respectively. Importantly,
in mammalian erythrocytes lacking intracellular membrane reserve, membrane rupture occurs under modest (~70%) volume increase caused by discocyte-spherocyte shape transition [30].

It should be stressed that data considered above do not exclude the possible generation of mechanical stretch within highly specialized cells or membrane segments. Thus, for example, hair cells consist of hundreds of actin-filled stereocilia that form rows of increasing length. In these cells, even minor mechanical deflection of bundles sharply increases mechanical tension and stretches the space limited membrane compartment abundant with ion channels [31]. In substrate-attached cells, space-limited plasma membrane abundant with integrin α/β heterodimers might be considered as another candidate site, where stretch stress could be triggered by cell swelling. Indeed, inhibition of integrin/src kinase signaling blocked RVD in hepatocytes [32, 33]. In myocytes, integrin signaling activates volume-regulated anion channels [34] whereas in renal epithelial cells it is involved in expression of tonicity-responsive enhancer-binding protein (TonEBP) triggered by hyperosmotic environment [35].

Caveolae are specialized plasma membrane invaginations with a diameter around 50 nm detected in majority of animal cells studied so far. Depending on cell type, they are organized by 4 isoforms of specialized proteins, caveolins, and contain high concentration of cholesterol [36]. Recently, it was shown that caveolae act as membrane reserve [37] responding to mechanical stress by rapid disassembly [38]. Trouet and co-workers reported that dominant negative caveolin-1 inhibits VRAC in endothelial cells [39]. On contrast, Eduarsen et al. [40] found that caveolae integrity is not required for cell volume regulation in adipocytes. Thus, additional experiments should be performed to examine the role of caveolae in cell volume sensing.

Numerous studies demonstrated that cell volume perturbations are accompanied by changes in the shape and the curvature of the plasma membrane. Thus, for example, AFM revealed folded membrane relief of human erythrocytes with average wave height of 3–5 nm and average height of 1–2 nm; erythrocyte swelling decreased the height of membrane surface waves by 40% [29]. A priori, changes in membrane curvature can alter the physical properties of lipid bilayer and affects interactions of neighboring proteins and phospholipids [10]. It should be noted that with an exception of liposomes [41] the role of membrane curvature changes in cell volume sensing remains unexplored.
2. Cytoskeleton

The architecture of nucleated cells is supported by a three-dimensional (3D) intracellular cytoskeleton formed by actin microfilaments, microtubules, and intermediate filaments (Fig. 2,a). Several research teams proposed a key role of cytoskeleton network rearrangement in cell volume sensing (for comprehensive reviews, see [2, 5, 7]). In accordance with this hypothesis, cell volume alterations result in rearrangement of cytoskeleton network that, in turn, affects conformation and functional activity of diverse cytoskeleton-associated proteins. This hypothesis is supported by two major observations. First, actin microfilaments have been found depolymerized and polymerized in a variety of nonadherent swollen and shrunken cells, respectively. Second, in several types of cells, additions of F-actin disrupting agents (cytochalasins, latrunculin) and microtubule depolymerization agents (colchicine, vinblastin) suppressed RVI/RVD or other volume-dependent cellular responses (for review see [7, 42–44]). It should be noted, however, that in our recent experiments we did not detect any significant action of vinblastine and cytochalasin B on RVD in hyposmotically swollen A549 cells [45]. Importantly, volume-responsive ion fluxes were detected in mammalian erythrocytes lacking 3D cytoskeleton [46, 47]. These results show that the 3D cytoskeleton can not be considered as a universal sensor of cell volume changes.

3. Cortical cytoskeleton and polyphosphoinositides

Plasma membrane architecture is supported by a two-dimensional (2D) cytoskeletal network that lies directly beneath plasma membrane and is called cortical cytoskeleton. This structure is comprised of spectrin heterodimers, actin bundles and proteins involved in regulation of spectrin/actin interaction and anchoring of this network to membrane integral proteins and acid phospholipids (Fig. 2,b). Nuclear-free mammalian erythrocytes, which are lacking typical 3D cytoskeleton, are commonly used for the analysis of the functional implications of near-membrane cortical cytoskeletal network. Pharmacological tools for these studies have not yet been developed. However, it has been shown that temperature elevation to 49–50 °C leads to irreversible annealing of spectrin heterodimers in human [48] and rat [49] erythrocyte ghosts (Fig. 3,a) [48–50]. We demonstrated that 10-min preincubation of rat erythrocyte at 49 °C abolishes volume dependent regulation of Na⁺,K⁺,2Cl⁻ (Fig. 3,b) and K⁺,Cl⁻ cotransporters [47] thus suggesting involvement of spectrin-based near membrane structures in cell volume sensing.
In contrast to rat blood cells, hyperosmotic shrinkage has negligible impact on the activity of Na⁺,K⁺,2Cl⁻ in control and heat-treated human erythrocytes [51]. Taking this into account, we focused our investigations on K⁺,Cl⁻ cotransport. Similar to rats, 10-min preincubation of human erythrocyte at 49 °C increased baseline activity of this carrier and sharply attenuated its activation by swelling [52, 53]. To further explore the role of 2D cortical cytoskeleton in volume sensing, we employed AFM. This method revealed folded membrane relief of fixed human erythrocytes with average wave height of 3–5 nm covered by globular structures with diameter of 40–50 nm and average height of 1–2 nm. Erythrocyte swelling caused by reduction of medium osmolality decreased the height of membrane surface waves by 40% and increased K⁺,Cl⁻ cotransport by ~6-fold. Both volume-sensitive changes of membrane relief and activity of K⁺,Cl⁻ cotransporter were abolished by 10 min preincubation at 50 °C [29]. These results strongly suggest that volume-dependent reorganization of 2D spectrin-actin network contributes to regulation of the activity of volume-sensitive ion transporters.

It is well-documented that the content of phosphatidylinositol 4,5-diphosphate (PIP₂), located in the inner surface of the plasma membrane phospholipid bilayer, is a major regulator of actin microfilaments polymerization [54]. More than 20 years ago, we demonstrated that hyperosmotic shrinkage of rat erythrocytes sharply increases the content of PIP₂ (Fig. 4,b) [51]. Later on this phenomenon was also observed in nucleated cells of different origin [55–57]. These data allowed to propose a central role for PIP₂ in shrinkage induced reorganization of actin microfilaments [56]. Nielsen and co-workers suggested that accumulation of PIP₂ seen in shrunken Ehrlich ascites tumour cells is caused by elevation of ionic strength rather than by cell volume attenuation [57]. It should be noted that in spite the same elevation of ionic strength triggered by hyperosmotic shrinkage, accumulation of PIP₂ has been detected in rat but not in human erythrocytes (Fig. 4A) lacking shrinkage-sensitive Na⁺,K⁺,2Cl⁻ cotransporter and Na⁺/H⁺ exchanger [51].

Phosphorylation of phosphatidylinositol 4-phosphate (PIP) to PIP₂ is catalyzed by PIP₂-kinase (Fig. 5, step C). Yamamoto and co-workers reported that both elevation of PIP₂ content and reorganization of actin cytoskeleton in shrunken HeLa cells are abolished by depletion of type 1 phosphatidylinositol 4-phosphate 5-kinase β isoform...
Fig. 5. Biochemical pathways of polyphosphoinositide turnover. Enzymes affected by cell shrinkage are shown in red squares. Adopted from Wikipedia, the free encyclopedia (www.en.wikipedia.org)

(PIP₃KIB) using an RNAi approach. They also demonstrated that activation of PIP₃KIBeta is abolished by calycin A, a potent inhibitors of Ser/Thr protein phosphatase 1 [56]. PIP₂ might be further phosphorylated by phosphatidylinositol 3-kinase (PI-3K) to produce phosphatidylinositol 3,4,5-triphosphate (PIP₃). Browe and Baumgarten reported that inhibition of PI-3K by wortmannin and LY294002 abolished activation of volume-sensitive chloride current triggered by integrin-dependent stretch of ventricular myocytes [58]. The role of PI-3K in signaling triggered by cell swelling has not been yet explored.

4. Autocrine release of signalling molecules

Side-by-side with direct modulation of membrane-bound and cytoplasmic proteins, cellular responses triggered by volume-changes can be mediated by autocrine regulation via release extracellular signaling molecules. Hoffmann and co-workers were the first to demonstrate that in several cell types hyposmotic swelling triggers massive production of arachidonic acid. Arachidonic acid is produced from sn-2 position of glycerophospholipids by phospholipase A₂ (PLA₂) and serves as a precursor for synthesis of prostaglandins via activation cyclooxygenases, hydroxy fatty acids, leukotrienes and lipoxins via activa-
tion of lypoxygenases and epoxides via cytochrome P-450. Later on, it was shown that that cell swelling triggers release of leukotrienes LTC4 and LTD4 rather then prostaglandins, suggesting activation of lypoxygenases rather than cyclooxygenases. Importantly, in several types of cells, inhibitors of PLA2 and lypoxygenase-5 block RVD as well as osmolyte transporters activated by cell swelling (for comprehensive review, see [7, 28, 59]).

Wang and co-workers reported that swelling of cultured hepatocytes leads to massive release of intracellular ATP that, in turn, triggers RVD via interaction with P2Y receptor-mediated activation of VRAC [60]. Whereas release of ATP and other nucleotide was documented in all type of swollen nucleated cells studied so far [22, 61], an essential and uniform role of this phenomenon in activation of volume-sensitive ion channels and RVD has not been confirmed [62, 63]. For example, activation of P2Y receptors does not have direct effect on VRAC in rat astrocytes, but rather modulates their activity in swollen cells [64].

In the recent work, we used DISUR technique to explore the influence of purinergic agonists on cell volume in the C11-MDCK cell line resembling intercalated cells from kidney collecting ducts. We found that in contrast to brain astrocytes, ATP and UTP triggered very robust (55–60%) cell shrinkage that lasted up to 2 hr after agonist washout. We also found that 30 min incubation with ATP led to ~5-fold increase in the c-Fos immunoreactivity. Both cell shrinkage and c-Fos expression was sharply attenuated by inhibitor of BKcα channels [65]. These data suggest a key role of cell shrinkage in excitation-transcription coupling and intracellular signaling pathways mediated by diverse c-Fos-sensitive late-response genes. Such coupling may occur in the kidneys, for example, during the exposure of intercalated cells to mechanical stimul, such as shear stress and transepithelial pressure pulses, which have been shown to cause ATP and UTP release from both the apical and basolateral surfaces of epithelial monolayers [66]. Using calcine-loaded cells, Holtzclaw and co-workers reported that 30-min exposure to shear stress led to shrinkage of C11-MDCK cells that was abolished by Ca2+-depletion, inhibition of K+ channels and sharply diminished in cells treated with BKcα-siRNA [67]. In contrast, cell shrinkage in wild-type MDCK cell subjected to shear stress did not utilize Ca2+-mediated signalling pathways including Ca2+-activated K+ channels [68]. Additional studies should be performed to clarify the role of purinergic receptors in shear stress-induced shrinkage of epithelial cells as well as upstream sensor(s) triggering volume-dependent release of ATP and other mediators of autocrine cell volume regulation.

5. Macromolecular crowding

In the 1980s and early 90s, several research group proposed that intramolecular interactions stemming from high concentration of macromolecules in the cytoplasm (so-called macromolecular crowding) crucially affect molecular and cellular functions [69, 70]. Indeed, physical chemistry predicts that because of the extremely high concentration in cytoplasm thermodynamic activity of the macromolecules is an exponential function of their apparent concentration. This thermodynamical model is consistent with numerous in vitro experiments showing dramatic impact of elevated macromolecular crowding on the activity of high molecule solutes such as enzymes and their substrates [71–73]. These data as well as notion that even small changes of intracellular water content affect macromolecular crowding, allowed researchers to propose that changes in macromolecular crowding may serve as a cell volume sensor [74, 75]. Indeed, in dog erythrocytes, shrinkage-activated Na+/H+ exchange and swelling-activated K+,Cl− cotransport was observed when hemoglobin concentration within resealed ghosts exceeds 300 mg/ml that corresponds to hemoglobin concentration in intact cells. Importantly, the set points of activation of both carriers were affected by modulation of intracellular macromolecular crowding rather than cell volume [46, 76].

Summers et al. examined the volume of internally perfused barnacle muscle cells under constant ionic strength, osmolality and membrane stretch [77]. They observed that cell volume attenuation triggered by reduction in intracellular concentration of ovalbumin or a polymer with molecular weight of ~20 kDa was abolished by inhibitors of RVD. To the best of our knowledge, this paper by Summers et al. published more than 10 years ago remains the only evidence implicating macromolecular crowding in volume regulation in nucleated cells. In contrast, several findings do not support a universal role of macromolecular crowding in cell volume sensing. Thus, in contrast to dog red blood cells, elevation of the volume of resealed human erythrocyte ghosts activate K+,Cl− cotransport even though solute concentration was constant and ghosts were virtually free of proteins [53]. Fig. 3b shows that 10-min preincubation of rat erythrocytes at 49 °C does not affect baseline activity of Na+,K+,2Cl− cotransporter and Na+/H+ exchanger but completely abolished their activation by hyperosmotic shrinkage. Because modest heat treatment does not affect macromolecular crowding, these results allowed us to formulate two alternative hypotheses. (i) In rat erythrocytes, macromolecular crowding does not contribute to volume sensing. (ii) Heat treatment inactivates osmosensing prop-
6. Intracellular hydrogel

As mentioned above, it is generally accepted that because plasma membrane is permeable to water, cell volume is under the control of intracellular content of small molecular weight organic osmolytes and major inorganic osmolytes such as K⁺ and Cl⁻. Indeed, elevation of intracellular content of NaCl and KCl and osmotically obliged water triggered by inhibition of the Na⁺,K⁺-ATPase and dissipation of the Donnan equilibrium leads to cell swelling [78]. However, because of the macromolecular crowding and cytoskeleton network-mediated compartmentalization, cytoplasm exists as an aqueous gel [79]. This conclusion was initially supported by data showing that squid axoplasm after extrusion from the giant axon into high-K⁺ solution retains its cylindrical shape [80]. Recently, we demonstrated that permeabilization of the plasma membrane with modest concentrations of digitonin or amphotericin B leads to dissipation of the Donnan equilibrium and cell swelling but does not affect the integrity of nucleated mammalian cells [26]. Unexpectedly, we found that permeabilized cells swell and shrink in hypo- and hypertonic solutions, respectively. Remarkably, osmolarity-induced volume changes were several-fold larger than those observed with intact cells, consistent with the cytoplasm’s high water-binding capacity. Binding or release of large amounts of water may allow rapid modulation of local fluidity, macromolecular crowding and activity of the intracellular environment. These findings are consistent with the cytoplasm’s function as osmosensor.

More recently, we compared cell volume adjustment of intact and permeabilized A549 cells subjected to disruption of microtubules by vinblastine and microfilament disassembly by cytochalasin B. Both compounds decreased the maximal amplitude of cell swelling in hypotonic medium and completely abolished cell volume increment triggered by hyposmotic medium in permeabilized cells. In contrast to swelling, permeabilization did not significantly affect the amplitude of shrinkage evoked by hyperosmotic medium. In both, intact and permeabilized cells, the rate of shrinkage was sharply attenuated by cytochalasin B [45]. We also found that 10-min preincubation of intact and permeabilized cells at 48 °C but not at 44 °C, completely blocked volume changes triggered by cell exposure to anisotonic medium [81]. These data strongly suggest that 3D cytoskeleton plays a key role in the behaviour of cytoplasmic biogel as an osmosensor. They also show that temperature-dependent inhibition of volume-sensitive ion carriers documented in early studies [47] is caused by inactivation of osmosensing behaviour of cytoplasmic biogel rather than biochemical signalling involved in RVD and RVI.

7. Role of intracellular [Na⁺]/[K⁺] ratio

To avoid sustained changes in cytoplasm ionic strength, cells respond to long-term exposure to anisosmotic environment by up- or down-regulation of gene expression those products are involved in the synthesis of compatible organic osmolytes. Thus, for example, hypertonically stressed renal epithelial cells induce a group of genes that encode transporters of organic osmolytes such as BTG1 for betaine, SMIT for inositol and taurine, as well as aldose reductase (AR) that catalyzes conversion of D-glucose to membrane-impermeable sorbitol [82]. It is generally accepted that augmented transcription of SMIT, BGT1 and AR is caused by elevation of ionic strength, i.e. total intracellular concentrations of Na⁺, K⁺ and Cl⁻, that, in turn, leads to binding of the transcription factor tonicity enhancer binding protein (TonEBP, also known as NFAT5) with toxicity enhancer cis-element (TonE) located within 5'-UTR [83, 84].

It should be noted, however, that cell volume perturbations in isosmotic environment are caused by alteration of the [Na⁺]/[K⁺] ratio rather than ionic strength. Thus, for example, shrinkage of C11-MDCK cells triggered by activation of P2Y receptors is mediated by strong reductions in intracellular K⁺ [65]. In the recent study, we identified ubiquitous and tissue-specific [Na⁺]/[K⁺]-sensitive transcriptomes by comparative analysis of differentially expressed genes in vascular smooth muscle cells from rat aorta (RVSMC), the human adenocarcinoma cell line HeLa, and human umbilical vein endothelial cells (HUVEC). To augment [Na⁺], and reduce [K⁺], cells were treated for 3 hrs with the Na⁺,K⁺-ATPase inhibitor ouabain or placed for the same time in the K⁺-free medium. Employing Affymetrix-based technology, we detected changes in expression levels of 684, 737 and 1839 transcripts in HeLa, HUVEC and RVSMC, respectively, that highly correlated between two treatments [85]. Among ubiquitous Na⁺/K⁺-sensitive genes we found ~2-fold increment of NFAT5 and up-to 10 fold elevation of expression of prostaglandin-endoperoxide synthase 2 (PTGS2) and cytochrome P450 CYP1A1, i.e. genes involved in cell volume regulation via PLA₂-mediated pathways (see Section 4). Among the cell-type specific genes involved in volume regulation of endothelial cells we found up to 3-fold elevation of RNA encoding P2Y2 purinergic receptors, ~2 fold attenuation of mRNA encoding catalytic subunits of PI-5-kinase (PIPK1C), up to 5-fold attenuation of regulatory subunits of PI-3-kinase PIK3R4 and PIK3R2, and 10-fold attenuation of expression of a nega-
tive regulator of PI-3 kinase activity PI-3-kinase interacting protein 1 (PIK3IP1) (unpublished results). The role of these transcriptomic changes in long-term volume adjustment is currently investigated in our laboratory.

Conclusions

Data considered in this review strongly suggest that osmosensitive properties of cytoplasmic hydrogel and interaction of cortical 2D cytoskeleton with polyphosphoinositides play an important role in detecting cell volume changes. Recent findings also demonstrate that 3D cytoskeleton contributes to regulation of osmosensing properties of cytoplasmic hydrogel rather than to triggering microfilament- or microtubule-specific volume-dependent signals. In addition to ubiquitous impact of hydrogel and cortical 2D cytoskeleton and polyphosphoinositides, cell volume sensing is mediated by diverse cell type-specific signaling. Other important processes that may be relevant to cell volume sensing include activation of ion channels by membrane stretch which likely involve integrin/cytoskeleton network, autocrine regulation via activation of PLA2 and ATP release in swollen cells. In spite of recent progress in the field, many questions remain unanswered. What is the relative impact of altered ionic strength and [Na+]i/[K+]i ratio on polyphosphoinositide turnover and transcriptomic changes triggered by sustained cell volume perturbations? What is the molecular origin of Na+, and/or K+, sensors generated polyphosphoinositide-mediated signals and transcriptomic changes? We will have to address these and other relevant gaps in knowledge in the forthcoming studies.

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Orlov Sergei N. (✉), Centre de recherche, Centre hospitalier de l’Université de Montréal (CRCHUM), Montreal, PQ, Canada; Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russian Federation; Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russian Federation

Grygorczyk Ryszard, Centre de recherche, Centre hospitalier de l’Université de Montréal (CRCHUM), Montreal, PQ, Canada

✉ Orlov Sergei N., Telephone: (514) 890-8000 Extension 23615; Fax: (514) 412-7638; e-mail: sergei.n.orlov@umontreal.ca