MECHANISMS OF CELL VOLUME REGULATION

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Summary

In animal organisms, cell volume undergoes dynamic changes in many physiological and pathological processes. Since animal cells do not possess a rigid cell wall, as seen in plant cells, swelling and shrinkage jeopardize their structural integrity. Moreover, even relatively small volume changes adversely affect cellular functions via alterations in precisely balanced enzyme and substrate concentrations. Therefore, cell survival critically depends on volume regulatory mechanisms. Cell volume regulatory machinery

consists of (i) a hypothetical cell volume sensor (or multiple sensors), (ii) intracellular signaling cascades, which transfer information from the volume sensor to (iii) the volume sensitive transporters, which regulate intracellular osmolarity via uptake or release of osmotically active substances. Volume-dependent ion transporters are evolutionary ancient proteins that are similar in various cell types across evolutionarily distant species. These transporters contribute not only to volume homeostasis but also to a variety of other cellular processes including proliferation, apoptosis, transepithelial solute transport, modulation of neurotransmission, and regulation of liver cell function by insulin and glucagon. Impaired or inefficient cell volume regulation underlies many pathological processes and in particular contributes to sickle cell anemia, diabetes mellitus, and brain damage from hyponatremia and ischemia.

1. Introduction

Surface (plasma) membranes of animal cells are highly permeable to water but possess very limited permeability towards inorganic ions and the vast majority of small organic molecules, collectively termed osmolytes. If the total concentration of osmolytes (*osmolarity*) is not equal outside and inside the cell, it leads to the net-movement of water along an osmotic gradient resulting in cell swelling or shrinkage. Changes in cell volume are detrimental for two major reasons. First, swelling and shrinkage compromise tissue architecture and function, and excessive swelling leads to cell lysis. Second, even relatively small volume changes strongly affect the physical and chemical properties of the cytoplasm and alter otherwise precisely balanced concentrations of enzymes and substrate molecules. Swelling and shrinkage compromise intracellular biochemical reactions, energetic metabolism, and/or trigger programmed cell death (apoptosis). Lower animal organisms permanently confront osmotic stress whereas higher animals tightly regulate the osmolarity of their extracellular fluids. Therefore the majority of animal cells (with the exception of several types of epithelial cells and blood cells passing kidney capillaries) do not encounter significant osmotic gradients. Nonetheless even in higher animals, cell volume is subject to frequent changes resulting from variations in intracellular osmolarity. Cell metabolism involves constant conversion of high molecular weight polymers to their osmotically active low molecular weight precursors and metabolites and vise versa. This metabolic activity can potentially cause changes in cytoplasm osmolarity. In addition, the unbalanced transmembrane transport of organic and inorganic solutes also results in variations of intracellular osmolarity. For example, insulin induces swelling of hepatocytes via stimulation of Na^+,K^+,$2Cl^-$ co-transport and Na^+/H^+ exchange, while activation of K^+ channels by glucagon in hepatocytes, or by bradykinin in endothelial cells causes cell shrinkage.

To protect their structural integrity and to maintain an optimal intracellular milieu, animal cells have developed specialized mechanisms for active accumulation or extrusion of osmotically active molecules, allowing for intracellular osmolarity adjustments and cell volume regulation. These mechanisms may be superficially divided into three groups: (1) steady-state cell volume regulation mainly due to work of the Na^+,K^+-ATPase (see *section 2*); (2) “fast” volume regulation due to rapid activation of membrane ion transporters (see *sections 4.1 and 4.2*); and (3) “slow” adaptation to chronic changes in extracellular osmolarity involving modifications in gene expression

and intracellular organic osmolyte content (see *section 4.3*). To date there are several unresolved aspects of cell volume regulation, including the identity of the cellular structure(s) responsible for sensing volume changes (discussed in *section 5*), and the nature of intracellular signaling pathways that link the hypothetical cell volume sensor to activation of volume-dependent ion transporters (discussed in *section 6*).

Under steady-state conditions (middle panel) osmotic forces are generated by high intracellular concentration of organic molecules (the so-called Donnan effect). To compensate for water accumulation cells pump out inorganic ions. Na^+,K^+-ATPase (depicted by **1**) transports $3Na^+$ out of the cell in exchange for $2K^+$. Since the plasma membrane has a higher permeability for K^+ over Na^+ and Cl^-, an outward K^+ leak creates a negative membrane potential. In turn, this negative potential drives Cl^- out of the cell, compensating for the presence of impermeable inorganic anions.

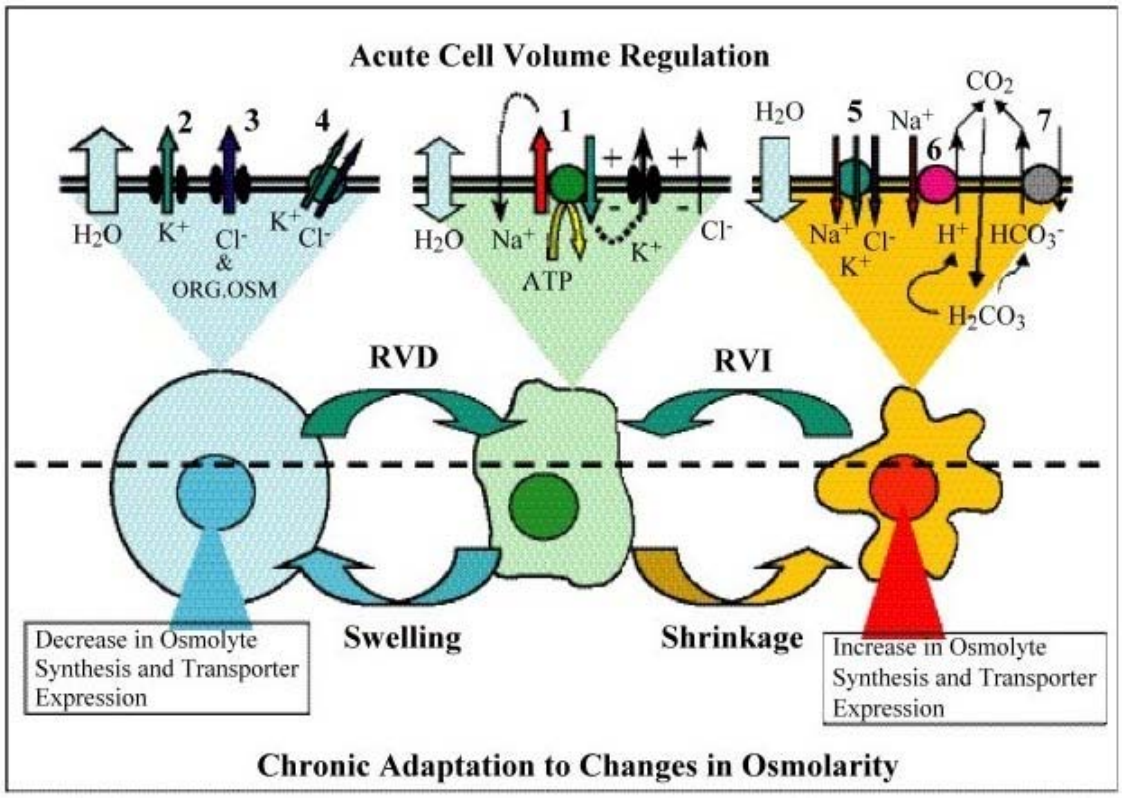

Figure 1. Schematic representation of cell volume homeostasis. Acute cell volume regulation is mediated by the activation of membrane ion transporters (upper panel), whereas chronic adaptation to changes in extracellular and/or intracellular osmolarity is contributed by the regulation of organic osmolyte transport and synthesis (lower panel).

In swollen cells (left panel), regulatory volume decrease (**RVD**) is accomplished by simultaneous activation of volume-sensitive potassium (**2**) and anion (**3**) channels and/or electroneutral K^+,Cl^- co-transport (**4**). Volume-sensitive anion channels are permeable to Cl^- and low molecular weight organic osmolytes (**ORG.OSM**.), such as amino acids etc. Chronic adaptation to hypo-osmolarity is mediated by a decrease in

organic osmolyte content due to decreased expression of enzymes involved in osmolyte synthesis and accumulation.

In shrunken cells (right panel), regulatory volume increase (**RVI**) involves activation of the Na^+,K^+,2Cl^- co-transporter (**5**) and the Na^+/H^+ exchanger (**6**). The Na^+/H^+ exchanger is functionally coupled to the Cl^-/HCO_3^- anion exchanger (**7**), which itself is insensitive to cell volume changes. Cooperative work of the latter two transporters accumulates NaCl inside the cell and extrudes H_2CO_3. Outside of the cell H_2CO_3 is converted to CO_2, which diffuses back to the cell and replenishes the pool of H^+ and HCO_3^-. Long-term adaptation to hyper-osmolarity is accomplished via increased expression of organic osmolyte transporters and enzymes involved in osmolyte synthesis.

2. Factors determining cell volume under steady-state conditions

Even under conditions of tightly controlled osmolarity of the extracellular milieu, high intracellular concentrations of impermeant organic molecules (proteins, amino acids, nucleic acids, carbohydrates, and others) generate significant osmotic force between extracellular and intracellular compartments; this is known as the Donnan effect. To compensate for the accumulation of water caused by the presence of non-permeable organic osmolytes, cells pump out inorganic ions, predominantly Na^+ and Cl^-. A key role in this process belongs to the Na^+,K^+-pump, which transports 3Na^+ out of the cell in exchange for 2K^+, partially compensating for the presence of non-permeable molecules in the cytoplasm. Since the plasma membrane typically has a higher permeability for K^+ over Na^+ and anions, an outward-directed K^+ leak creates a negative membrane potential. In turn, this negative membrane potential drives inorganic anions, mostly Cl^-, out of the cell, additionally compensating for the presence of impermeable organic anions. As a result, cells achieve dynamic asymmetric distribution of organic and inorganic ions known as the Gibbs-Donnan equilibrium. It is important to understand that the mechanisms of steady-state volume maintenance are principally different from cell volume regulation under non-steady-state conditions described in *section 4*.

3. Physiological and pathological causes of non-balanced cell volume changes

A large variety of physiological reactions and pathological states can disrupt the fragile Gibbs-Donnan equilibrium and cause acute changes in cell volume. Changes in extracellular osmolarity present the simplest reason for cell volume alterations. However**,** in higher animal organisms such changes are rather unusual and have a predominantly pathological nature. For instance, hyponatremia—a decrease in plasma sodium concentration—is typically caused by hormonal disorders leading to alterations in kidney function. The reasons for hyponatremia include overproduction of antidiuretic hormone (ADH), glucocorticoid deficiency, and renal failure. Conversely, acute dehydration, osmotic diuresis and drop in ADH production may initiate hypernatremia—an increase in plasma sodium concentration.

A far more common reason for cell volume alterations are changes in intracellular osmolarity. Cell swelling may be linked to increased Na^+-dependent uptake of organic osmolytes such as glucose and amino acids, as seen in hepatocytes and intestinal mucosal cells. Non-compensated Na^+ uptake during activation of sodium or non-

selective cation channels by excitatory neurotransmitters, glutamate and aspartate, causes neuronal volume increase. Insulin swells hepatocytes via stimulation of Na^+/H^+ exchange and $Na^+,K^+,2Cl^-$ co-transport, leading to solute accumulation. An opposite process of solute efflux and cell shrinkage is caused by the stimulation of potassium channels by glucagon in hepatocytes or by ATP in endothelial cells. In pulmonary epithelium cells, volume decrease is associated with cyclic guanosine monophosphate(cGMP)-dependent activation of Cl^- channels belonging to the cystic fibrosis transmembrane conductance regulator (CFTR) family.

In astrocytes and many other cell types, an increase in extracellular K^+ concentration promotes slow cell swelling because of the plasma membrane depolarization followed by (K^+ + Cl^-) influx due to new electrochemical gradients for K^+ and Cl^- ions. Muscle cells swell during exercise due to accumulation of lactate which leads to acidification of the cytoplasm and activation of the Na^+/H^+ exchanger. Inhibition of energetic metabolism generally causes cell swelling resulting from a loss of Na^+,K^+-ATPase function and subsequent accumulation of Na^+, membrane depolarization and Cl^- influx.

4. General mechanisms of cell volume regulation under non-steady-state conditions

Animal cells have developed “emergency” systems of rapid cell volume autoregulation in order to protect themselves from excessive cell volume perturbations caused by various factors described in the preceding section. Mechanisms of “fast” cell volume regulation under non-steady-state conditions are principally similar in cells from various tissues as well as between evolutionary distant species. They include (i) a hypothetical sensor(s) of cell volume, (ii) intracellular signaling systems coupled to this sensor(s), and, finally, (iii) membrane ion transporters mediating the release/uptake of osmotically active compounds to compensate for cell swelling/shrinkage. The release of osmolytes and compensatory cell shrinkage in response to acute cell swelling is termed *regulatory volume decrease* (RVD). An opposite process of osmolyte accumulation and compensatory cell swelling in the response to cell shrinkage is termed *regulatory volume increase* (RVI).

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Bibliography

Burg M.B., Kwon E.D., and Kultz D. (1996). Osmotic regulation of gene expression. *FASEB J.* 10, 1598-1606. Kultz D. and Burg M. B. (1998) Intracellular signaling in response to osmotic stress. *Contributions to Nephroogy* 123, 94-109. [Two reviews discussing mechanisms and physiological roles of the volume-dependent gene expression]

Chamberlin M.E. and Strange K. (1989). Anisosmotic cell volume regulation: a comparative view. *American Journal of Physiology* 257, C159-C173. [A review covering comparative aspects of cell volume regulation]

Hoffmann E.K. and Dunham P.B. (1995). Membrane mechanisms and intracellular signaling in cell volume regulation. *International Review of Cytology* 161, 173-262. [Detailed discussion of membrane mechanisms and intracellular signaling involved in cell volume regulation]

Lang F., Busch G.L., Ritter M., Volkl H., Waldegger S., Gulbins E., and Haussinger D. (1998). Functional significance of cell volume regulatory mechanisms. *Physiol Rev.*, 78, 247-306. [The most comprehensive to-date review on cell volume regulation discussing most aspects of this problem]

Kirk K. and Strange K. (1998). Functional properties and physiological roles of organic solute channels. *Annu. Rev. Physiol.*, 60, 719-739. [This review provides a good compilation on the role of anion/organic osmolyte channels in cell volume regulation and other cell functions]

Mongin A. A. and Orlov S.N. (2001). Mechanisms of cell volume regulation and possible nature of the cell volume sensor. *Pathophysiology*, 8: 77-88. [An attempt to analyze systematically current hypotheses on the nature of the cell volume sensor]

Biographical Sketches

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