REFRESHER COURSE | Cellular Homeostasis

In the beginning, there was the cell: cellular homeostasis

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Romero, Michael F. In the beginning, there was the cell: cellular homeostasis. *Adv Physiol Educ* 28: 135–138, 2004; doi:10.1152/advan.00048.2004.—In the past 5 years, the biomedical, scientific community has sequenced the genomes of several organisms (including *Homo sapiens*), has cloned entire organisms and has determine the molecular structures for several membrane proteins. These advances combined with the advances in technology enabling high-throughput drug screening, gene expression readout using DNA chips and evolving proteomic techniques, make it imperative that physiologist and biomedical professionals understand the basis of cellular function and homeostasis. The Cellular Homeostasis Refresher Course at Experimental Biology 2004 in Washington, DC, was designed to fulfill this need. The specific topics covered were 1) generation of membrane potential, 2) an update on cellular mechanisms of ion homeostasis, channels and transporters, and 3) cellular volume homeostasis, and regulation of intracellular pH.

Intracellular pH; cellular buffering

Several years ago, the decision was made to incorporate newer elements of modern biological and medical education into the Refresher course. This approach becomes particularly important in our “postgenomic” age. The first trial of this approach was in 2000 with a Refresher Course on “Molecular Biology.” Another very timely topic that is traditionally not well handled by many physiology texts is Cellular Physiology. As a start, the Education Committee (http://www.the-aps.org/education/edu_misc/refresh.htm) decided to hold a Refresher Course devoted to some of the cornerstones of Cellular Physiology, i.e., Cellular Homeostasis. The following written renditions of the presentation from Experimental Biology in Washington, DC (April 2004), represent both basic principles as well as new frontiers in our understanding of these fundamental processes.

In the beginning, there was the cell: cellular homeostasis. The Cellular Homeostasis Refresher Course, we had four presentations covering these basic building blocks of cellular function:

1. generation of membrane potential
2. ionic homeostasis: channels and transporters
3. volume homeostasis
4. pH homeostasis

GENERATION OF MEMBRANE POTENTIAL

Membrane potential ($V_m$), simply put, is the voltage produced across a membrane when charges are separated. Stephen H. Wright, PhD (University of Arizona, Tucson, AZ) assembled this presentation based on his own experience teaching graduate and medical students. Dr. Wright is a transport physiologist but not explicitly an electrophysiologist. Thus my reasoning for inviting Dr. Wright was to simplify the teaching approach so that one does not need to be an electrophysiologist, physicist, or mathematician to understand and convey appropriate concepts to students. However, Dr. Wright was kept from the meeting by a chronic back problem and the talk was actually given by an electrophysiologist—me.

Why is $V_m$ fundamental? A common misconception is that only excitable cells like nerve and muscle have membrane potential and therefore voltage-driven processes. This concept is short-sighted on at least two fronts. First, all living cells have a membrane potential. This list of course includes nerve and muscle but also includes epithelial cells, fibroblasts, red blood cells, skin (except the cornified layer which is no longer living), osteoblasts/osteoclasts (not the crystallized bone), oocytes, sperm, stems, roots, leaves, bacteria, fungi, etc. Thus, in general, a membrane-bound compartment that separates charge will result in a corresponding $V_m$. Dr. Wright discusses the
ionic generation and some of the simple mathematical representations of this quantity in his section.

A second misconception of many students and biologists is that \( V_m = 0 \text{ mV} \) implies that \( V_m \) somehow “cancels out,” has no value or is otherwise unimportant. However, as developed by Dr. Wright, \( V_m \) is a vector not scalar quantity, meaning that \( V_m \) has direction. Zero is merely the point where “+” moves to “−” or vice versa. In other words, 0 mV is more positive than −60 mV, a “typical” animal cell membrane potential. That is, a cellular process may be activated by depolarizing the cell to 0 mV. A good example of this “0 mV activation” is the firing of an action potential resulting from the successive opening and closing of ion channels and transporters.

In his paper, Wright presents ionic details that create and maintain \( V_m \) and the equations that describe \( V_m \). In addition, the value and application of the Nernst equation (a specialized state of the electrochemical potential across a membrane for a particular ion) as well as the Goldman-Hodgkin-Katz equation are discussed.

ION HOMEOSTASIS: CHANNELS AND TRANSPORTERS

How and why do cells maintain ions homeostasis? The “why” is that for cellular processes to run efficiently, cells have evolved to use environmental chemistry to their advantage. The cell by virtue of being a compartment, has an outside (extracellular environment) and an inside (intracellular environment). Cells need to make energy in the form of ATP. And, to make energy, cells need substrates, such as glucose, pyruvate, amino acids, peptides, inorganic ions, etc. Thus one mechanism for maintaining homeostasis is to use ATP hydrolysis or the electrochemical energy of ion and solute gradients to selectively and often specifically move these substrates from the extracellular world to the intracellular world. Many original papers, reviews, journals, and books have been devoted to transport activities, transport molecular entities, and transport regulation. Some useful recent resources discuss these transport process, molecular entities and cellular processes:

1. Solute carrier tables (http://www.bioparadigms.org/slct/menu.asp) and the February 2004 issue of Pflügers Arch 447, issue 5 (9)
2. Physiology of Membranes. In: Medical Physiology (1)
3. Cellular Physiology (2)

George R. Dubyak, PhD (Case Western Reserve University, Cleveland, OH), head of the medical section “Cell Homeostasis” at Case Western, focused his talk of “ion homeostasis” on the following emerging and “murky” areas of cellular physiology:

- Ion Transport Proteins as Channels versus Transporters: Not as different as we think
- Interactions of Ion Transport Proteins with Adapter Proteins: No transporter is an island
- Interactions of Ion Transport Proteins with Local Lipids: The bilayer as more than a low dielectric permeability barrier
- Interactions between Ion Transport Proteins and Modulator Proteins: Cell-specific context explains all

While his presentation was not intended to be comprehensive, Dr. Dubyak highlighted cutting edge cellular physiology teaching and research. As we move farther into the “postgenomic era,” in which many more genomes are sequenced, genes discovered, and protein interactions revealed, we as physiologists and teachers can incorporate rationale for cellular compartments and drug design even into our basic science graduate and professional school lectures. The knowledge that channels are not merely opening and closing gates and that transporters do not merely bind substrates and “kerchunk” solutes across a membrane changes the established models that are still being taught. Soluble domains of channels and transporters are now appreciated as regulators, signaling systems, or both in their own right. Likewise, it is necessary for these transport entities to be targeted to specific membranes. Cells have evolved helper proteins to directly transport proteins to the membrane (cytoskeletal) and away from the membrane (Nedd proteins and ubiquination) or to anchor them in place (often cytoskeletal adapter proteins). Finally, not only can our transporters/pumps/channels behave in different modalities, but the surrounding membrane lipids and proteins can directly influence and control function moment to moment.

CELLULAR VOLUME HOMEOSTASIS

A more global form of cellular homeostasis is the concept of cell size maintenance, i.e., cell volume homeostasis. Why maintain cell size? In the extreme, an ever-expanding cell will lyse, resulting in the outside world and the inside world being the same, i.e., cell death. Conversely, if the outside environment has “too little water,” i.e., high osmolality like sea water (organism level) or the renal inner medulla (tissue level), cells will shrink (lose cell water) unless they protect themselves. Thus cells also tightly regulate their size by moving either solutes or water.

Kevin Strange, PhD (Vanderbilt University, Nashville, TN), developed these concepts with traditional physiology examples (red cell swelling and shrinking) as well as with exciting new data from model organisms. These less complex model organisms (yeast, Caenorhabditis elegans, etc.) have allowed novel volume-sensing pathways, signaling pathways, and gene interactions to be appreciated. Why do these paths and signal matter? First, Dr. Strange presented the fundamentals of water and solute movement through transport systems (transporters and channels) in response to changes in the transmembrane osmotic gradient. Once the driving force was discussed, he introduced the concept of “cell sensing” with respect to volume sensing and provided a few examples. After a volume change is sensed, the cell must respond. This response is traditionally characterized in two parts: an early response via transport systems and a long-term response via altered transporter/channel gene expression. More recently, a midpoint to this osmotic response, altered kinase activities and expression was shown to be at the cutting edge of cellular volume homeostasis.

An additional note should be made at this point. Dr. Strange’s lab, using C. elegans, is discovering new sensors, new effectors, new signaling paths and new phenotypes (5, 10, 12). Some of these findings were mentioned in the Experimental Biology presentation (see http://www.the-aps.org/education/edu_misc/refresh.htm) but have not been reiterated here because they are truly cutting edge. The integrative physiology aspect of this work is clearly beyond the scope of this Refresher Course but was recently reviewed.
(13). Perhaps a Refresher Course on importance and benefits of nonmammalian models will appear in the future!

**CELLULAR PH HOMEOSTASIS**

The final presentation of the morning Cellular Homeostasis Symposium/Refresher course was “Cellular pH Regulation.” Why regulate pH? (Bad question to ask an acid-base physiologist, myself and the speaker, unless you are prepared for the encyclopedia response.) Cells, like body fluids and compartments, use the chemistry of enzymes, organic and inorganic molecules, to carry out normal function. Many cells maintain a cytoplasmic pH (pHcyt) of 7.0–7.3 ([H+]cyt = 50–100 nanomolar). Within this narrow range of pHcyt, i.e., [H+], many cellular enzymes have their “pH optimal activity.” In particular, cellular processes associated with metabolism, DNA replication, and cell division seem to be suppressed or activated within a few tenths of pHcyt change. In addition, protons (H+) are also tricky to think about in solution because every amino acid, and therefore every protein, changes its chemistry depending on the degree of protonation. That is, proteins as well as other organics and inorganics can act as H+ buffers. Thus to regulate or change pHcyt is not merely a matter of transmembrane movements of acids or bases, but also the capacity for H+ to be absorbed by buffers.

Walter F. Boron, MD, PhD (Yale University, New Haven, CT, one of our past APS Presidents), delivered a comprehensive lecture on the fundamentals of pHcyt regulation and homeostasis. Dr. Boron used the very simple-to-understand analogy of temperature regulation and heating and cooling expenditures in a house to illustrate the basic principles of cellular pHcyt regulation and cellular pH buffering. In typical “Boronian” fashion, the analogy included detailed equations and calculations of heat-cold fluxes (acid-base fluxes) and heat capacity (cellular buffering capacity). This particular temperature example has the advantage that even nonbiologists and nonscientists have a concept of how one makes a house hotter or colder using a house thermostat. While some may find the analogy presented in the following text extensive, there is sufficient detail of temperature regulation and pHcyt regulation that this outline and examples could be used for even advanced graduate or modeling classes. After Dr. Boron laid the foundation of the fundamentals of temperature regulation in a house, all of the concepts were expressed in the terms of cellular pHcyt regulation.

Boron made his discussion of pHcyt regulation and buffering current by 1) the explicit discussion of molecular entities now known to participate in this regulation in various cells, and 2) the discussion of cellular sensors and integrated pHcyt regulation. In this regard, the house temperature analogy is quite helpful. This is particularly true because the ortholog pH concepts of the consequences of house temperature regulation and heat capacity are not all able to be studied or modeled in cells using present technology. That is, parallels with components of the temperature system may be used to predict and test novel aspects of cellular pHcyt regulation.

It should be noted that Dr. Boron’s laboratory has been pivotal to the molecular cloning of many acid-base transporters, especially bicarbonate transport systems (4, 6–8, 11, 14, 15). Dr. Boron’s laboratory has also developed major experimental tools for studying intracellular pH and pHcyt regulation, including the NH4+ prepulse (3) and out-of-equilibrium CO2/HCO3 solutions (16).

**CELL PHYSIOLOGY: MINI-LAB WORKSHOP**

The afternoon session of the 2004 Cellular Homeostasis refresher course was a series of mini-labs wonderfully organized by Jeffrey C. Freedman, PhD [State University of New York (SUNY) Upstate Medical University]. For this Pedagogy Workshop, there were three presentations featuring hands-on experience to illustrate some of the principles and methods covered in the morning Symposium.

- **Mini-lab I:** Understanding diffusion potentials using a pH electrode. Jeffrey C. Freedman, PhD (SUNY Upstate Medical University)
- **Mini-lab II:** Computer simulation of equilibrium and action potentials. Michael J. Davis, PhD (Texas A&M University)
- **Mini-lab III:** Fluorescent probe monitoring of cardiac electrical activity. Arkadii M. Perzov, PhD (SUNY Upstate Medical University); Jeffrey Dzubay, Molecular Probes, Inc. (http://www.probes.com/)

In conclusion, four building blocks of “Cellular Homeostasis” were presented at Experimental Biology 2004 in Washington, DC, highlighting the following basic concepts and/or cutting-edge experimentation: 1) the generation of membrane potential; 2) new players in cellular ion homeostasis, 3) cellular volume control, and 4) cellular pH (and domestic temperature) regulation. The presentations were educational and thought provoking. The corresponding articles in this issue of Advances in Physiology Education should provide the reader with great examples as well as an outline for teaching basic concepts and emerging areas that will be crucial for the next generation of graduate and professional biomedical students.

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**REFERENCES**


