
Nominated by Olaf Andersen
Editor, Journal of General Physiology
Cornell University
spare@med.cornell.edu

Question: Do the subconductance levels of K⁺ channels result from heteromeric pore conformations?

Background: The ability of nerve cells to communicate with one another and with other cells is derived from ion channels: membrane proteins that control membrane potential and regulate the flow of specific inorganic ions (Ca²⁺, Na⁺, K⁺, Cl⁻). Ion channels in a closed permeation state are induced by membrane depolarization or ligand binding to stochastically alternate to the open permeation state. The authors had previously demonstrated that a voltage-gated K⁺ (Kv) channel, which consists of four identical subunits, has subconductance levels that are hypothesized to represent heteromeric conformational states where only some of the subunits support ion permeation. However, although the open and closed levels occur in milliseconds, the available techniques could not resolve the much faster subconductance levels.

Observations: Using site-directed mutagenesis, Chapman and VonDongen constructed a mutant dimer K⁺ channel from two subunits with different kinetics, single-channel conductance, and voltage dependence. Electrophysiological experiments revealed the existence of the characteristic open-close behavior when strongly depolarized. However, when the membrane potential was depolarized in between the activation thresholds of the two subunits, two subconductance states were revealed.

Significance: These results represent an important step toward understanding sublevels in Kv channels and support the hypothesis that subconductance levels result from heteromeric pore conformations. It will be interesting to see if the subconductance states of other voltage-gated ion channels also result from heteromeric pore conformations.


Nominated by Michael Caplan
Yale University School of Medicine
michael.caplan@yale.edu

Question: Does the olfactory system amplify incoming signals similarly to the visual system?

Background: GTP-binding (G) proteins act as molecular switches in a number of cellular processes. They are active when GTP is bound, and this activity is regulated by other proteins that influence the dissociation of GDP and the rate of GTP hydrolysis. In retinal cells, photon activation of a single rhodopsin molecule activates many heterotrimeric G proteins, resulting in signal amplification. On this basis, other G protein pathways have been hypothesized to amplify in a similar manner.

Observations: Bhandawat et al. used a technique that allowed precise control of the exposure time and number of odoriferous molecules used to activate G protein olfactory receptors along with long-term measurement of a cell’s response to determine the effects of a single odorant molecule. Quantal analysis of responses to odorant ligands suggests that an activated receptor has a low probability of stimulating even a single G protein molecule. The most probable explanation for this phenomenon is that the odorant-receptor interaction was extremely short in duration (<1 ms).

Significance: These findings raise issues about the hypothesis that the process of smell converts odors into brain signals in a similar manner to how vision converts light into brain signals. In contrast to the amplification observed in phototransduction, signal amplification in olfactory transduction appears to be distinct. Signal amplification could still be achieved in the olfactory system by increasing the probability of G protein activation. This could occur from a high level of receptor expression on cells allowing low concentrations of odorants to bind, by the repetitive binding of a ligand to a receptor, or by many cells expressing the same receptor. Although this is the first of such reports, it will be interesting to see whether other G protein pathways share a similar mechanism.


Nominated by Michael Caplan
Yale University School of Medicine
michael.caplan@yale.edu

Question: Are neuregulin pathways necessary for neuromuscular junction formation?

Background: The molecular mechanism by which a neuron forms a synapse and regulates subsynaptic membrane differentiation has been hypothesized to involve reciprocal interactions between the agrin receptor complex [agrin/muscle-specific tyrosine kinase (MuSK)] and neuregulin signaling pathways. Genomic and proteomic approaches have demonstrated that agrin is sufficient to induce a fully functional postsynaptic-like membrane. Unlike agrin, however, a few obstacles have hindered the elucidation of the precise role of neuregulin signaling in synapse formation. For example, mice lacking the neuregulin-1 gene (nrg-1) or its receptors (erbB2 or erbB4) do not survive past the embryonic stage. Moreover, neuregulin signaling pathways are essential for the survival and differentiation of Schwann cells, which regulate motor neuron function.

Observations: Using the formation of the neuromuscular junction as an experimental paradigm, Escher et al. selectively mutated erbB2 and erbB4 in muscle so that their expression in Schwann cells was unaffected. A role for these receptors in muscle growth was reported, although, unlike agrin, the neuregulin signaling pathways were determined to be largely irrelevant to neuromuscular synapse development and maintenance. This was corroborated by the fact that receptor mutations did not affect acetylcholine receptor cluster formations, timing of innervation, maturation of endplates, regulatory reciprocal signals from muscle, or the development of a functional ectopic endplate.
Significance: These results suggest that activated MuSK does not need to recruit neuregulin/ErbB signaling pathways to induce the expression of synaptic genes, which is in contrast to the previously reported effects of disrupting neuregulin signaling on neuromuscular junction formation. Nonetheless, the previously observed effects of neuregulin may be a consequence of interactions with Schwann cells that surround the neuromuscular junction and indirectly affect development.

Improved muscular efficiency displayed as Tour de France champion matures.

Nominated by Jerry Dempsey
Editor, Journal of Applied Physiology
University of Wisconsin-Madison
jdempsey@wisc.edu

Question: What accounts for the increased physical endurance of a highly trained athlete?

Background: Maximal oxygen uptake (VO2max), blood lactate threshold (LT), and muscular efficiency are central to determining endurance abilities. In general, one can improve endurance by increasing their maximum oxygen capacity and/or by increasing their efficiency. Although several studies have compared endurance abilities between athletes, few have measured the effects of continued endurance training within an individual.

Observations: Measurements of VO2max, LT, and muscular efficiency were conducted at five time points over a seven-year span in the well-known cancer survivor, Lance Armstrong, during which time he won the Tour de France six times. The improvements in performance observed over his career were determined to be a function of an 8% improvement in muscular efficiency. Perhaps most important is that these findings provide some insight and inspiration with regard to recovery of function and performance physiology after successful surgeries and chemotherapeutic treatments for advanced cancer.

Heme regulates allosteric activation of the Slo1 BK channel. Horrigan FT, Heinemann SH, and Hoshi T. J Gen Physiol 126: 7–21, 2005.

Nominated by David Gadsby
Associate Editor, Journal of General Physiology
Rockefeller University
gadsby@mail.rockefeller.edu

Question: What is the mechanism by which heme modulates the large-conductance Ca2+-dependent potassium (Slo1 BK) channel?

Background: Heme usually exists bound as the nonprotein iron-containing portion of hemoglobin, cytochrome, soluble guanylate cyclase, and other molecules wherein the iron is in the ferrous (Fe2+) state. However, there is increasing evidence that unbound heme exists and is transported intracellularly, where it acts as a signaling molecule. In fact, the Hoshi faction recently demonstrated that heme binds to the cytoplasmic domain of the Slo1 BK channel, which is activated by depolarization, divalent ions, or synergistically by the two combined.

Observations: Using gating, macroscopic, and single-channel currents, Horrigan et al. explored the mechanism by which hemin (the oxidized form of heme) affects the Slo1 BK channel. When divalent ions were not present, heme caused a decrease in ionic currents, but gating currents were largely unaffected. Simulations suggest that these effects resulted from a decrease in the strength of allosteric coupling between the voltage sensor and activation gate, which stabilized the open state. When divalent ions were present, the efficiency of heme remained; i.e., the probability of the channel opening increased in the presence of heme, which suggests that the channels are less sensitive to divalent cations when heme is bound.

Significance: These results suggest that under various physiological conditions heme is a potent regulator of allosteric coupling in Slo1 BK channels and provides a useful model to guide future experimentation for elucidating the gating mechanism of BK channels. Importantly, these results may be pertinent to mitigating the damage caused by heme following a traumatic event, such as a stroke or a heart attack, which results in a dramatic increase in intracellular heme concentrations affecting vasorelaxation and oxygen sensing.


Nominated by Baruch Kanner
Hebrew University Hadassah Medical School
kannerb@cc.huji.ac.il

Question: What can the three-dimensional (3-D) structure of a Na+/Cl–-dependent transporter illuminate about transport mechanisms?

Background: Na+/Cl–-dependent transporters exploit electrochemical potentials to regulate the translocation of a diverse array of substrates across a membrane against a concentration gradient. Encoded by a family of genes, Na+/Cl– transporters are essential for terminating neurotransmission of monoamines (dopamine, norepinephrine, and serotonin), amino acids (glutamate and GABA), and osmoles. Although a great deal of research has explored their function, structural data underlying sodium ion selectivity as well as sodium ion-substrate coupling and transport mechanisms have been deficient. The recently discovered bacterial transporters also belong to the Na+/Cl– membrane protein family and in this report are crystalized to elucidate their 3-D structure.

Observations: Yamashita et al. describe the crystal structure of a bacterial Na+/Cl– transporter homolog (LeuTα), which transports leucine. Their data imply that the mechanism of transport includes three conformational states: an open state that allows access for substrate binding from the extracellular milieu; an intermediate, occluded, substrate-bound state; and a state with open access to the intracellular milieu, which completes substrate translocation. Additionally, they
describe determinants of substrate binding and Na\(^+\) ion selectivity. Finally, the data suggest that ion-substrate coupling is a result of direct (or nearly direct) interactions, as their binding sites are juxtaposed in the 3-D structure.

**Significance:** This work provides us with our first indication of a neurotransmitter sodium symporter structure, the binding sites for ions and substrates, and the gating mechanism. The important contribution of this work is underscored by the fact that dysfunction of Na\(^+\)/Cl\(^-\) transporters contributes to multiple disorders, including Parkinson disease and epilepsy, and are important pharmacological targets for the treatment of several disorders ranging from depression to hypertension. Knowing the molecular principles of these transporters will help in designing better drug treatments.

**Background:** Voltage-dependent ion channels are essential for controlling heart rate, regulating hormone secretion, and generating electrical impulses in the nervous system. The structure of voltage-dependent K\(^+\) (Kv) channels has been based on prokaryotic K\(^+\) channels because of the ease with which they can be expressed at high levels in _Escherichia coli_. Although eukaryotic Kv channels share some similarities with prokaryotic channels, they also possess many unique features.

**Observations:** The two reports by Campbell et al. elucidate the crystal structure and describe the voltage-sensing mechanism of a mammalian Kv-\(\beta\)-subunit complex (the \(\beta\)-subunit regulates mammalian Kv channels in vivo). During the crystallization process, they found that it was essential to use a mixture of lipids and detergents and to minimize oxidation for optimal results. The pore of the channel, which is similar in structure to prokaryotic channels, is open. Large side portals are described that allow ions to flow between the cytoplasm and the pore. The electrostatic properties of these side portals along with the positions of the T1 domain and \(\beta\)-subunit are consistent with inactivation gating mechanisms and regulation by the \(\beta\)-subunit. The voltage sensors, which contain four transmembrane segments (S1–S4), are independent domains in the membrane layer and affect the state of the gate via the S4–S5 linker helices, which are positioned intracellularly.

**Significance:** This description of the Kv channel is the first to recognize separate voltage-sensing domains within the membrane. Moreover, the mechanism by which the conformational changes of the voltage sensors are transmitted via the S4–S5 linker helices to the pore is exceptional in its simplicity. Also noteworthy is the technique used to crystallize the channel, as this may be applicable to the study of other membrane protein structures. Although there are several unresolved questions about the Kv channel, this structure represents an important step in answering them.

---


**Question:** What is the structural basis of electromechanical coupling in voltage-dependent K\(^+\) channels?

**Background:** Voltage-dependent ion channels are integral membrane proteins that catalyze the rapid and selective flow of inorganic ions across cell membranes and are essential for controlling heart rate, regulating hormone secretion, and generating electrical impulses in the nervous system. The structure of voltage-dependent K\(^+\) (Kv) channels has been based on prokaryotic Kv channels because of the ease with which they can be expressed at high levels in _Escherichia coli_. Although eukaryotic Kv channels share some similarities with prokaryotic channels, they also possess many unique features.

**Observations:** The two reports by Campbell et al. elucidate the crystal structure and describe the voltage-sensing mechanism of a mammalian Kv-\(\beta\)-subunit complex (the \(\beta\)-subunit regulates mammalian Kv channels in vivo). During the crystallization process, they found that it was essential to use a mixture of lipids and detergents and to minimize oxidation for optimal results. The pore of the channel, which is similar in structure to prokaryotic channels, is open. Large side portals are described that allow ions to flow between the cytoplasm and the pore. The electrostatic properties of these side portals along with the positions of the T1 domain and \(\beta\)-subunit are consistent with inactivation gating mechanisms and regulation by the \(\beta\)-subunit. The voltage sensors, which contain four transmembrane segments (S1–S4), are independent domains in the membrane layer and affect the state of the gate via the S4–S5 linker helices, which are positioned intracellularly.

**Significance:** This description of the Kv channel is the first to recognize separate voltage-sensing domains within the membrane. Moreover, the mechanism by which the conformational changes of the voltage sensors are transmitted via the S4–S5 linker helices to the pore is exceptional in its simplicity. Also noteworthy is the technique used to crystallize the channel, as this may be applicable to the study of other membrane protein structures. Although there are several unresolved questions about the Kv channel, this structure represents an important step in answering them.

---


**Question:** Can the RNA interference (RNAi) screening approach identify human kinases involved in the cellular
process of endocytosis?

**Background:** An essential function of eukaryotic cells, endocytosis is the molecular mechanism by which cells internalize material and distribute it to other cellular compartments. This process involves nascent small membrane-engulfed vesicles from the cell surface (endosomes), which surround the matter. Subsequently, the vesicles deliver the cargo by fusing with the target membrane of the appropriate organelle. However, of the thousands of molecules in the cytosol only a few have been documented to regulate this process.

**Observations:** Using an RNAi screening approach, Pelkmans et al. explored the role of the entire collection of the human protein, lipid, and carbohydrate kinases in the endocytic process. Out of 590 kinases screened, 210 were determined to be involved in the endocytosis of two viruses [vesicular stomatitis virus (VSV) and simian virus 40 (SV40)], which enter cells via clathrin-mediated and caveoleae- or lipid raft-mediated endocytosis, respectively. Interestingly, some of the kinases exerted opposing effects on VSV and SV40 endocytic mechanisms, which may represent a homeostatic balancing of signals.

**Significance:** This is the first use of siRNAs to target an entire class of genes and explore how they organize endocytic mechanisms. The results suggest that endocytic processes are more highly regulated and tightly coupled by signaling pathways than previously appreciated. Finally, this work demonstrates how this methodological approach can provide novel mechanistic insights and could potentially lead to novel therapeutic targets.


antiporter has been implicated in mediating hypertrophic responses associated with cardiac dysfunction. Thus this work may provide novel insight into therapeutic approaches for the treatment of heart failure via antiporter inhibition.


Nominated by Michael Welsh
University of Iowa College of Medicine
michael-welsh@uiowa.edu