
Nominated by Olaf Andersen
Editor, Journal of General Physiology
Cornell University
sparre@med.cornell.edu
Communicated by Lawrence Palmer
Cornell University
Ilgualm@med.cornell.edu

Question: Are connexins necessary for Ca2+ homeostasis and cataract prevention in lenses?

Background: The lens of an eye has a surface epithelial layer, which goes through cell division in such a way that the new cells get pushed toward the equator of the lens, where they are internalized and eventually differentiate into mature fibers (MF). All of these cells are interconnected by gap junction channels that are formed from the family of proteins called connexins. The lens possesses internal circulating currents, including a Na+ current, which enters the lens extracellularly and then flows toward the lens center. As Na+ flows inward, it is transported across fiber cell membranes into fiber cells, where it then reverses its direction and flows toward the lens equatorial surface via gap junctions between cells. To prevent cataract formation, lenses need to maintain low intracellular Ca2+. Because surface cells have Ca2+-ATPase activity and Na+/Ca2+ exchange to expel Ca2+ from the lens, whereas MF do not, Ca2+ is hypothesized to mediate nucleotide-dependent proteolysis (and therefore cataract formation): when gap junction coupling modulates Ca2+ flow, which blocks the efflux of Ca2+, inflow of Ca2+ causes accumulation in central cells, activating Ca2+-dependent proteases that cleave cytoplasmic proteins, which aggregate and scatter light.

Observations: Using an innovative technique, Gao et al. examined Ca2+ concentration gradients in connexin knockout (KO) and knock-in (KI) lenses, which have down- or upregulated coupling conductance, respectively. The KO mice developed a cataract that was associated with an increase in internal Ca2+ in the center of the lens. The KI mice had transparent lenses similar to wild-type and lower internal Ca2+ in the center of the lens. Evidence for the connexin-dependence of Ca2+ support by the presence of a Ca2+-diffusion gradient (high in the center, low at the surface), which is mediated by gap junction coupling conductance.

Significance: The authors develop and describe a likely mechanism by which gap junction coupling regulates Ca2+-mediated proteolysis (and therefore cataract formation): when gap junction coupling of MF cells is attenuated, which blocks the efflux of Ca2+, inflow of Ca2+ causes accumulation in central cells, activating Ca2+-dependent proteases that cleave cytoplasmic proteins, which aggregate and scatter light.
resistant F344 rats. This allowed the identification of the lymphopenia locus and the cloning of a novel immune-associated nucleotide gene 5 (Ian5) that lacks a C nucleotide, which results in a truncated protein product.

**Observations:** Michalkiewicz et al. report the use of an in vivo transgenic approach to verify directly the functional consequences of the mutation in the Ian5 gene. A transgenic rat strain was produced by microinjecting the pronuclei of fertilized eggs from an F344 x LYP x LYP congenic strain with a genomic clone containing the wild-type allele of the Ian5 gene from a Brown Norway rat. The resulting transgenic offspring exhibited a correction of the Ian5 protein expression, and the level of T cells was restored to levels seen in normal rats. Complementary recombination studies by this group excluded the possibility that other genes of the Ian family (Ian7, Ian1, Ian6, and Ian3) contributed to the lymphopenia.

**Significance:** These findings provided direct in vivo evidence that the single nucleotide mutation of the Ian5 gene is responsible for the lymphopenia in the diabetic BB strain of rats. This work demonstrates proof of concept that transgenic rescue in the rat is a practical and definitive method for revealing the function of a novel gene, and it provides direct evidence of the involvement of a novel Ian5 gene in the normal development of T cells.


Nominated by Eve Marder
Editor, Journal of Neurophysiology
Brandeis University
marder@brandeis.edu

**Question:** What domain of the Kv2.2 subunit dictates voltage-gated-potassium current density in mature neurons?

**Background:** The excitability of developing neurons is highly plastic, which is a corollary of differential ion channel expression and function. This phase of plasticity in the electrical membrane properties of neurons is followed by a stable phase, which persists throughout adult life. Voltage-gated potassium current (Ik) density follows a similar pattern of expression, being significantly increased early on and stable when neurons mature. The molecular underpinnings of this plasticity are elusive, but Kv1, Kv2, and Kv3 channels contribute to Ik. Therefore, differential transcriptional mechanisms may be responsible for the increase in Ik in immature neurons and the Ik set point in mature neurons.

**Observations:** Blaine et al. microinjected Kv1, Kv2.1, and Kv2.2 RNA into Xenopus embryos to determine the effects of varying their levels of expression. The in vivo overexpression of Kv1.1, Kv2.1, and Kv2.2 subunits increased Ik density in young neurons, whereas only the Kv1.1 and the Kv2.1 subunits could increase Ik density in mature neurons. Further efforts were directed at determining the region of the Kv2.2 subunit that regulates whether or not Ik density increases upon overexpression of Kv2 in the mature neuron. Intuitively, the focus was on the region of greatest heterogeneity, the cytoplasmic carboxy tail. A domain in the cytoplasmic proximal carboxy terminal, proxC, was mediating Ik density upon chimeric Kv2 subunit overexpression in mature neurons.

**Significance:** These data suggest that the transcriptional mechanism that underlies a neuron’s ability to develop from a fluctuating to a stable Ik density is a function of the K+ channel subtype expressed. It is noteworthy that the proxC domain mediated developmental ion channel functions, as this is the first report to identify a channel’s subunit in the regulation of Ik density, rather than regulation by transcription or RNA turnover rates.


Nominated by Heini Murer
Physiologisches Institut
hmurer@access.unizh.ch

**Question:** What mechanistic insights can be gained from the structural characterization of an ammonium transporter?

**Background:** For many years, gases were thought to flow (leak) nearly unimpeded through plasma membranes. Recently, however, some cells were found to have only moderate inherent permeability to gases. This important discovery allowed the expression of channels in these impervious membranes and the ensuing demonstration that channels can mediate the intracellular translocation of gases. Subsequently, gases such as NH3 were determined to access the intracellular milieu via ammonium transporters (Amt) but, the precise molecular interactions that govern NH3 and/or NH4+ membrane crossing has been elusive.

**Observations:** Khademi et al. determined the crystallographic structure of a bacterial ammonia transmembrane channel (AmtB), which was structurally distinct from transporter proteins. AmtB exists as a trimer of proteins with eleven transmembrane-spanning a-helices (M1–M11). At either end of the channel there are vestibules interconnected by a narrow hydrophobic nonpolar pore element, which allows the passage of NH3 but not NH4+. The extracellular vestibule is acidic and structurally organized to attract NH4+ whereas the pore is alkaline and lined with three sites to promote the translocation of NH3. Thus transport does not cause the transfer of protons or affect membrane potential.

**Significance:** Khademi et al. elegantly integrate data from other groups to describe a feasible ammonium transport model. Given the diverse functions of ammonia in physiological processes, this work represents a significant advancement in our understanding of the mechanisms that underlie ammonia transport in humans, because paralogs of AmtB, Rh-related proteins, were predicted to function in a similar manner.

Question: Does activation of the fetal hypothalamic-pituitary-adrenal axis (HPAA) precede increased estradiol and prostanoids (PGs), or do increased placental PGs stimulate the fetal HPAA?

Background: It has been hypothesized that the timing of birth involves activation of the fetal HPAA, which leads to increased PGs and a change in the ratio of estradiol to progesterone (E:P). Subsequently, estrogen increases PG production in the uterus and protein expression in the uterine wall, initiating labor and birth. However, recent studies have shown that ifusin the fetus with PGs increases cortisol levels, and blockade of PG synthesis prevents the late-gestational increase in cortisol. The fact that preconceptional maternal undernourishment (PCU) causes a premature activation of the HPAA and preterm birth suggests that the former hypothesis is correct, but undernutrition may have altered PG synthesis, leading to birthing processes. Thus whether PCU-induced premature birth is preceded by the increase in PGs and the E:P ratio or if the rise in cortisol precedes or follows these increases is unknown.

Observations: The present study determined that an increase in fetal cortisol occurs before an increase in PGs or the maternal E:P ratio in all animals, regardless of early nutrition or timing of delivery. This suggests that activation of the fetal HPAA is the initial (or at least of this group of factors, the first) event in the timing of labor.

Significance: In addressing an interesting phenomenon aimed at delineating some of the mechanisms by which decreased food intake can influence birth timing, the study by Kumaraamy and colleagues answers at least one important question in the mystery of the timing of labor. Interestingly, the data did not provide any clue as to why only half the ewes in the early undernutrition group delivered early or why the events entailed by the decreased nutrition result in precocious activation of the HPAA. As a big step in establishing sequence, this study certainly helps point where to begin looking.


Nominated by Ulrich Pohl
Ludwig-Maximilians-Universitat Munchen
upohl@lmu.de

Question: Does CD73/ecto-5'-nucleotidase (CD73)-induced adenosine formation have a role in regulating the aggregation of blood factors (thromboregulation) or anti-inflammatory responses?

Background: Expressed on the vascular endothelium, CD73 is a cell surface molecule that catalyzes the conversion of 5'-AMP to adenosine. Adenosine is implicated in physiological and pathophysiological events, and activation of the adenosine A2A receptor causes a vasodilatation of coronary arteries, in addition to inhibiting aggregation via a cAMP-dependent pathway. Because a CD73-induced alteration in extracellular adenosine contributes only minimally to total cardiac adenosine production, it is unknown if it contributes to thrombo-regulation and anti-inflammatory responses.

Observations: Knocking out CD73 in mice caused a loss of CD73 activity and a significant decrease in liver and heart AMP hydrolysis. Basal coronary flow was also significantly impaired in the isolated perfused heart of the mutant, which was shown to be an unlikely consequence of altered adenosine receptor density/coupling. Moreover, AMP-induced dilation was shown to require AMP hydrolysis to adenosine formed predominantly from CD73. In a measure of platelet thrombosis formation, cd73−/− mice were more susceptible to vessel obstruction and platelet cAMP was reduced, which is indicative of reduced plasma adenosine.

The role of CD73-produced adenosine in a vascular inflammatory response was evaluated. Although there was no change in blood flow, the mutant mice had a significant increase in the number of adherent leukocytes in carotid arteries under basal conditions and in response to an ischemic insult. Finally, the constitutive role of CD73 in anti-inflammatory vasoprotection was determined in monocyte-perfused carotid arteries. This produced an increase in the adhesion of monocytes to the artery.

Significance: This work represents the first demonstration that CD73-generated adenosine is a fundamental pathway that modulates the inflammatory vascular response, prevents platelet function and activation of leukocytes, and modulates vascular tone.


Nominated by Ulrich Pohl
Ludwig-Maximilians-Universitat Munchen
upohl@lmu.de

Question: How can a single nucleotide polymorphism (SNP) in the promoter region of the nos-3 gene (a C/T transition at position −786 from the first coding triplet of the gene) alter endothelial shear stress-induced NO release, thereby increasing the risk for coronary heart disease (CHD)?

Background: Atherosclerosis, whose major risk factors include high cholesterol, hypertension, smoking, diabetes, and a family history for atherosclerotic disease, underlies CHD. A dysfunctional endothelium in affected arteries, which is influenced by environmental factors and intrinsic impairments in the expression of endothelial gene products, may increase susceptibility to atherosclerosis. Nitric oxide (NO) synthase (NOS-3) is an endothelial gene product critical not only to vascular tone homeostasis but also to...
maintaining the functional and structural integrity of vessel walls. One of the major physiological stimuli of this enzyme is shear stress. Because individuals homozygous for the C variant of the gene have been characterized to have a higher risk to develop CHD, the functional relevance of a T-to-C SNP in the nos-3 gene promoter was explored with regard to shear stress sensitivity.

**Observations:** The CC genotype was associated with a higher risk of CHD development. Basal protein expression was comparable in endothelial cells with either genotype, whereas mRNA levels were significantly lower in cells with the CC genotype. Additionally, shear stress induced an increase in mRNA and protein levels of NOS-3 in TF- and Cφ-derived human endothelial cells, whereas nos-3 expression remained basically unchanged in CC cells. The use of a decoy oligonucleotide determined that, in the C variant, shear stress-induced nos-3 expression is also possible but is normally blocked by an inhibitory DNA-binding protein. Moreover, a decrease in NO-dependent relaxation was observed in blood vessels from patients homozygous for the C variant of the gene.

**Significance:** The data indicate that the means by which a C/C nos-3 genotype at position –786 might increase the risk for developing CHD is possibly due to binding of an inhibitory transcription factor to the C-type promoter, blocking shear stress-dependent NOS-3 expression, and thus impairing the capacity of NO formation in the affected endothelium.


**Question:** What role does an increase in astrocyte 
Ca2+ have in the control of brain microvasculature?

**Background:** The most abundant glial cells in the brain, astrocytes, remove excess neurotransmitter from the synaptic cleft, send out processes that contact both neurons and blood vessels (forming the blood-brain barrier), express neurotransmitter receptors, and respond to neuronal activity. Recent data revealed an unexpected role of astrocytes, i.e., their capacity to cause dilation of the local microvasculature depending on a Ca2+-activated release of prostaglandins.

**Observations:** Mulligan and MacVicar employed an elegant technique that allowed a selective increase in astrocyte intracellular Ca2+ concentrations ([Ca2+]i) without affecting neurons or the microvasculature itself. An increase of [Ca2+]i in the astrocyte endfeet was found to precede a constriction of adjacent blood vessels. The magnitude of this constriction was dependent on the breadth of the propagated Ca2+ wave, such that the more endfeet affected, the greater portion of vessels constricted. They also provide evidence that the activation of phospholipase A2 by Ca2+ triggers the release of arachidonic acid, causing the generation of a vasoconstrictive metabolite (20-HETE) by a cytochrome P-450 enzyme subtype, which induces the vasoconstriction.

**Significance:** This work demonstrates an additional role of astrocytes on brain microvasculature, i.e., their capacity to induce vasoconstriction. Future experiments should clarify the physiological conditions leading astrocytes to release either vasodilating or vasoconstricting agents or both, addressing the possibility that microvessels from different brain regions (cortex vs. hippocampus) respond differently to astrocyte signals. Results from these experiments will provide neuroscientists with a better understanding of the enigmatic astrocyte’s influence on local blood flow and may offer novel alternatives to treat or prevent cerebral insults caused by ischemia.
birth. Together, these two studies quantified the cellular components of the epithelial compartment and the smooth muscle of the interstitial compartment in rhesus monkeys at 5 days and 1, 2, 3, and 6 mo of age. The chief finding of these two studies was that the epithelium establishes an organization and density proportional to airway size, whereas smooth muscle abundance remains constant with increasing airway size. Such relationships are maintained regardless of age. Subtle differences in the orientation of smooth muscle in the most distal bronchioles occur with age, likely due to alveolarization.

**Significance:** Although active proliferation and differentiated function are normally inversely related, these studies show that proliferation and differentiation occur simultaneously in the maturing airway, implying that the abundance and distribution of airway components are tightly coordinated. The overall goal of these studies was to establish a basis for mechanistic studies of disease in which injury and repair occur during lung growth and development. Few studies of postnatal development of the conducting airways have been completed, yet numerous epidemiological studies of humans and basic studies of animals have established that once the lung is injured during an active period of growth (e.g., by factors such as environmental pollutants), normal lung development and repair of acute injury are compromised despite continued lung growth. In such cases, the drive for growth overrides that for repair, likely because of disruption of the tight regulation of airway components.


**Question:** Is heart tube fusion a prerequisite of prenatal cardiac development?

**Background:** It has been postulated that the earliest stages of cardiac morphogenesis proceed in a predetermined sequential order. First the bilateral precardiac mesoderm migrates to the midline, where it fuses together to form the heart tube (fusion); this is followed by cardiac development (chamber identity and left-right cardiac asymmetry) and looping morphogenesis. Models of defective heart tube fusion (cardia bifida) suggest that fusion is necessary for the subsequent stages of development, although they also implicate extracardiac abnormalities in ventral morphogenesis and embryonic turning that may be responsible for these congenital cardiovascular defects. The fox gene Foxp4 is expressed in the developing mouse embryo. Its role in cardiac morphogenesis was evaluated in a mutant murine model.

**Observations:** Perhaps unexpectedly, Li et al. found that the Foxp4 mutants developed two hearts with proper chamber septation, left-right chamber specification, and looping morphogenesis, while exhibiting normal embryonic turning. However, although ventral morphogenesis was largely unperturbed, a defect in the development of the anterior foregut was observed and hypothesized to underlie cardia bifida. Interestingly, the transcription factor Pitx2, which is normally expressed only on the left side of the heart, was expressed in the left heart but not in the right. In contrast, while eHAND is also normally expressed selectively in the left side of the developing heart and eHAND and FGF10 are expressed primarily on the right side, this pattern of expression was conserved in both hearts.

**Significance:** The Foxp4 mutant mouse model of cardia bifida provided an opportunity to demonstrate a dissociation between cardiac development and the requirement for bilateral heart tube migration and fusion. These data force us to reframe our model of cardiac morphogenesis and may have implications for understanding congenital heart disease.


**Question:** What is the role of S6 kinase 1 (S6K1) in obesity and insulin resistance?

**Background:** Obesity-induced insulin resistance is associated with hyperglycemia, hyperlipidemia (high levels of lipids in the blood), and hyperaminoacidemia (high levels of amino acid oxidation). S6K1 not only phosphorylates ribosomal protein S6, it also plays a significant role in body weight homeostasis as it acts to integrate signals from nutrients and insulin. S6K1-deficient mice (S6K1-/-) have low blood insulin (hypoinsulinemia), are glucose intolerant, and have reduced pancreatic β-cell (insulin-synthesizing and -secreting cells) mass. However, they exhibit normal glucose during fasting, suggesting hypersensitivity to insulin. In this study, the impact of knocking out S6K1 on the animal’s metabolism and thus body weight as a function of age and diet were studied.

**Observations:** Um et al. found that the S6K1 deficiency increased insulin sensitivity and attenuated the age-associated increase in body weight. This mitigation was due to decreased fat accumulation, which was a result of an increase in lipolysis and metabolism. The difference in the metabolism of S6K1-/- mice was the result of a shift from storing triglycerides to metabolizing them in white adipose tissue (WAT). This is supported by an increase in WAT mitochondrial number and size and the induction of genes that regulate the oxidative phosphorylation pathway. Similarly, S6K1-/- mice exposed to a high-fat diet were also less susceptible to fat accumulation due to an enhanced metabolic rate and lipolysis. Further evidence is provided that suggests that S6K1 inhibits insulin signaling downstream from the insulin receptor and that in heredity- or diet-induced obesity, S6K1 mediates insulin resistance via activation of insulin receptor substrates.