# Highlights From The Literature

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**Visual cycle: dependence of retinol production and removal on photoproduct decay and cell morphology.** Ala-Laurila P, Kolesnikov AV, Crouch RK, Tsina E, Shukolyukov SA, Govardovskii VI, Koutalos Y, Wiggert B, Estevez ME, and Cornwall MC. *J Gen Physiol* doi:10.1085/jgp.609557.2006.

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**Question:** What accounts for the different rates of recovery of the visual pigments in rods and cones?

**Background:** Light is perceived by two distinct photoreceptors in the vertebrate retina: the rods and the cones. In both rods and cones, the first photochemical event of vision occurs when a visual pigment (rhodopsin) is hit by light, which initiates a cis-to-trans isomerization of the retinal chromophore (the moiety that causes a conformational change of the molecule when hit by light) and results in activation of the rhodopsin (metarhodopsin). Metarhodopsin dissociates, producing opsin and all-trans-retinal. All-trans retinal is then reduced to all-trans retinol by retinal dehydrogenase (RDH), which is cleared from the outer segments and enzymatically converted to 11-cis retinol and back to 11-cis retinal. Finally, 11-cis retinal is condensed with opsin to regenerate rhodopsin. This rate of recovery is known as the "visual cycle" and occurs in rods and cones at different rates.

**Observations:** Ala-Laurila et al. hypothesized that the differential rates of recovery between rods and cones is due to differences in rates of retinal liberation following metapigment decay and rates of RDH. These studies characterized the time courses of metarhodopsin decay and all-trans retinol production in different types of rods and cones. Their data indicates that the rate of retinol production is defined by either the decay rate of the metapigment or the RDH reaction rate, which depended on the cell type and the outer segment region.

**Significance:** This work provides a mechanistic basis for understanding the differential rates of recovery between cones and rods. This represents an important step forward toward the elucidation of rod and cone physiology, which has remained elusive for many years.

**Outer membrane active transport: structure of the BtuB:TonB complex.** Shultis DD, Purdy MD, Banchs CN, and Wiener MC. *Science* 312: 1396–1399, 2006.

**Structure of TonB in complex with FhuA, *E. coli* outer membrane receptor.** Pawelek PD, Croteau N, Ng-Thow-Hing C, Khursigara CM, Moiseeva N, Allaire M, and Coulton JW. *Science* 312: 1399–1402, 2006.

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**Question:** How do Gram-negative bacteria move essential nutrients intracellularly?

**Background:** Gram-negative bacteria have inner cell walls that are surrounded by an outer membrane. Porins are β barrel proteins that exist in the outer membrane and function as pores. However, these pores do not permit the diffusion of necessary molecules like iron and cobalamins (cobalt-containing compounds). Thus an active transport mechanism has evolved that requires an inner membrane multiprotein complex, an inner membrane proton-motive force (pmf), and a member of the family of outer membrane (OM) transport proteins, which all adopt a barrel-like architecture and have one domain that serves as a plug. Although the inner membrane protein, Ton-B, is known to couple the pmf to the Ton-box region of the OM transporter and unplug the transporter, how a nutrient is moved through the barrel is unknown.

**Observations:** These two papers describe how the pmf of the inner membrane is coupled to the translocation of complexes ($Fe^{3+}$-siderophore and vitamin B12) across the OM. Pawelek et al. describe the structure of the TonB-dependent OM receptor with FhuA (the iron transporter), whereas Shultis et al. describe the structure of the TonB-dependent OM receptor with BtuB (the transporter of cobalamin) and the energy-transducing TonB protein. In both cases, TonB induces the Ton-box portion of the OM plug to form a β strand, which is then converted into an interprotein beta sheet.

**Significance:** Bacteria are remarkably resilient and are constantly evolving, which can lead to antibacterial drug resistance. The increasing presence of bacteria that are drug resistant has created a need for new pharmacological approaches. These papers represent a major advance in understanding how Gram-negative bacteria use a proton gradient to transport life-sustaining nutrients across the OM and thus may be important for identifying novel antibacterial drug targets.

**Neuronal pathway from the liver modulates energy expenditure and systemic insulin sensitivity.** Uno K, Katagiri H, Yamada T, Ishigaki Y, Ogihara T, Imai J, Hasegawa Y, Gao J, Kaneko K, Iwasaki H, Ishihara H, Sasano H, Inukai K, Mizuguchi H, Asano T, Shiota M, Nakazato M, and Oka Y. *Science* 312: 1656–1659, 2006.

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**Question:** What role does the peroxisome proliferator-activated receptor (PPAR)-γ2 have in regulating metabolic signals from the liver to peripheral tissues?

**Background:** Energy balance is a dynamic phenomenon that is controlled by several tissues, which produce compensatory metabolic and/or behavioral changes when the amount of energy taken in or exerted is altered. The liver is a key player in regulating metabolism, because it sends metabolic signals to adipose tissues. However, how the liver communicates with other tissues is unknown. PPARs are a group of transcription factors that induce the proliferation of peroxisomes in cells and are intimately connected to the cellular metabolism of carbohydrates, lipids, and proteins. Thus PPARs were hypothesized to be part of the mechanism by which the liver affects metabolism in peripheral tissues.

**Observations:** Uno et al. show that hepatic PPAR-γ2 overexpression in mice leads to the abnormal synthesis and breakdown of triglyceride fats in the liver while simultaneously decreasing the amounts of peripheral adipose tissue, increasing energy expenditure and improving systemic insulin sensitivity. Similarly, hepatic vagotomy or selective blockage of the afferent vagus resulted in similar phenotypes, evidence that the effects of PPAR-γ2 involve the afferent vagal nerve. They also showed that this pathway was enhanced by a PPAR-γ2 agonist.

**Significance:** That the nervous system plays an important role in relaying metabolic information is not a novel concept; this paper supports that idea and provides new directions for elucidating the mechanisms

that regulate metabolism. These findings suggest that the liver communicates with peripheral adipose tissue by means of a neuronal pathway consisting of the afferent vagus nerve from the liver and efferent sympathetic nerves to adipose tissues to regulate metabolism. This pathway may be important for balancing metabolic disturbances caused by excess fat storage and represent a possible therapeutic target for treating the metabolic syndrome.

**CRACM1 is a plasma membrane protein essential for store-operated $Ca^{2+}$ entry.** Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, and Kinet JP. *Science* 312: 1220–1223, 2006.

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**Question:** What protein is responsible for the regulation of store-operated $Ca^{2+}$ influx?
**Background:** Intracellular stores of $Ca^{2+}$ are released via channels upon activation. To replenish theses stores, calcium release-activated calcium (CRAC) channels open, allowing $Ca^{2+}$ transport across the plasma membrane. Although CRAC channels have been biophysically characterized in detail, the molecular identity of the channels and the mechanism by which it is activated were unknown until now.
**Observations:** Vig et al. completed a high-throughput genome-wide RNA interference (RNAi) screen and a subsequent patch-clamp screen in *Drosophila* to identify novel proteins required for CRAC channel function. Two candidate membrane proteins, CRAC modulators 1 and 2 (CRACM1 and CRACM2), were determined to be necessary components for CRAC channel activity. CRACM1 was found to have a human homolog, and, although overexpression did not affect CRAC currents, RNAi-mediated knockdown disrupted its activity. Further experiments demonstrate CRACM1 localizes to the plasma membrane.
**Significance:** CRACM1 is definitively shown to play a role in store-operated $Ca^{2+}$ influx via CRAC channels, although the exact function of the CRACM1 protein is unclear. Nonetheless, this is an important step forward toward understanding the phenomenon of CRAC-mediated $Ca^{2+}$ entry across the plasma membrane, which is a fundamental process in several physiological events, such as gene transcription.

**Transcapillary fluid balance consequences of missing initial lymphatics studied in a mouse model of primary lymphoedema.** Karlsen TV, Karkkainen MJ, Alitalo K, and Wiig H. *J Physiol* 574: 583–596, 2006.

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**Question:** How does inactivation of vascular endothelial growth factor receptor-3 (VEGFR-3) signaling disrupt tissue fluid balance?
**Background:** Lymph is the interstitial fluid that originates from the filtration of capillary blood plasma. The lymphatic or immune system, which collects and filters the interstitial fluid, has a major role in the body's ability to defend against pathogens. Lymphoedema is a condition that occurs when lymph drainage is compromised, resulting in localized fluid retention. The VEGF-3 mediates lymphangiogenesis (the formation of lymphatic vessels), but its role in lymphoedema is unclear. Karelsen et al. thus sought to determine how dysfunctional lymphangiogenesis contributes to lymphoedema.
**Observations:** Karlsen et al. investigated the physiological consequences of disrupting the VEGFR-3 in genetically engineered Chy mice, which is a model of congenital lymphoedema. The physiological consequences of lymphatic aplasia were assessed in relation to transcapillary fluid balance parameters before or after intravenous fluid load. Although lymphatic capillaries were absent in all areas examined, only a few had visible edema. Although the interstitial colloid osmotic pressure (ICOP) was increased in all areas, interstitial fluid pressure (IFP) and interstitial fluid volume (IFV) were increased only in visibly swollen tissues. Upon intravenous fluid load, Chy mice displayed increased IFV and IFP.
**Significance:** These data help to resolve some of the controversial issues in this field. Lymphoedema appears to result in a high protein concentration in interstitial fluid, but this does not induce an inflammatory response. Moreover, this work further highlights the importance of the VEGFR-3 in tissue fluid homeostasis. Finally, these findings are relevant to understanding a number of disease processes such as the spread of cancer, infection and inflammation, asthma, transplant rejection, and of course Milroy disease, a form of hereditary lymphoedema.

**Continued divergence in $Vo_{2\ max}$ of rats selected for running endurance is mediated by greater convective blood $O_2$ delivery.** Gonzalez NC, Kirkton SD, Howlett RA, Britton SL, Koch LG, Wagner H, and Wagner PD. *J Appl Physiol* (June 15, 2006); doi:10.1152/japplphysiol.01527.2005.

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**Question:** Do central and/or peripheral mechanisms continue to evolve and account for the increased exercise capacity of low- versus high-capacity runner rats?
**Background:** Increasing evidence supports the contention that aerobic exercise capacity is a predictor of overall general health. Interactions between environmental and genetic factors influence aerobic capacity. The genetic component includes genes that underlie inherited aerobic capacity and the genes that adapt to aerobic exercise. In an effort to determine the relative contribution of these genetic factors, sedentary rats have been artificially selected as low- (LCR) or high-capacity runners (HCR). After seven generations (G7), it was previously determined that the HCR had higher maximal $O_2$ uptake ($Vo_{2\ max}$), which was not due to differences in cardiac output (central $O_2$ delivery mechanisms) but resulted from changes in peripheral mechanisms that transport and utilize $O_2$.
**Observations:** Gonzalez et al. hypothesized that the increase in peripheral functions observed in G7 HCR would have to be accompanied by a concomitant increase in central functions to maintain $Vo_{2\ max}$. Therefore, systemic $O_2$ transport was analyzed in G15 HCR and LCR during maximal exercise. They observed a similar but greater difference in tissue $O_2$ diffusive conductance than was previously found between G7 HCR and LCR. However, in contrast to their previous results, they also determined that this was accompanied by increased cardiac output, which led to there being no difference in the overall tissue $O_2$ extraction between the two strains.

**Significance:** These intriguing findings suggest that the different mechanisms that control an organism's given phenotype may evolve at different rates. Perhaps there is an important lesson to be learned from this: When determining the relative contribution of central versus peripheral mechanisms that underlie aerobic capacity, heed the stage at which the animal model is at evolutionarily.

**Role of reactive oxygen species in contraction-mediated glucose transport in mouse skeletal muscle.** Sandstrom ME, Zhang SJ, Bruton J, Silva JP, Reid MB, Westerblad H, and Katz A. *J Physiol* (June 15, 2006); doi:10.1113/jphysiol.110601.2006.

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**Question:** Do reactive oxygen species (ROS) have a role in contraction-mediated glucose transport in skeletal muscle?
**Background:** Similar to insulin, exercise increases the rate of glucose uptake into contracting skeletal muscles. The mechanism by which exercise regulates this process diverges from insulin's: exercise regulates glucose uptake by controlling the translocation of GLUT-4 glucose transporter proteins. The signaling pathway that mediates the exercise-induced increase of glucose is unclear, although many pathways have been implicated. One such pathway involves AMP-activated protein kinase (AMPK). Recently, ROS, such as $H_2O_2$, have been implicated in the AMPK signaling pathway. In separate studies, $H_2O_2$ was shown to activate AMPK, accelerate glucose transport, and be produced at higher rates in response to contracting muscles.
**Observations:** Sandstrom et al. hypothesized that endogenously produced ROS play an important role in contraction-mediated activation of glucose transport in skeletal muscle. They present evidence that supports the contention that endogenously produced ROS are involved in contraction-mediated glucose transport. Antioxidants selectively diminished contraction-mediated 2-deoxyglucose (2-DG) uptake to a similar extent as antioxidant-induced decreases in the activation and phosphorylation of AMPK. In addition, overexpression of a catalyst that produces $H_2O_2$ increased 2-DG uptake.
**Significance:** This is the first report to demonstrate that endogenously produced ROS are involved in contraction-mediated glucose transport in muscle by a mechanism that involves AMPK. Ultimately, understanding the signaling mechanisms linking exercise with increased glucose transport will have significant clinical value in the management of diabetes, insulin resistance, and the design of new therapeutic interventions.

**Effects of female steroid hormones on A-type $K^+$ currents in murine colon.** Beckett EA, McCloskey C, O'Kane N, Sanders KM, and Don Koh S. *J Physiol* 573: 453–468, 2006.

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**Question:** How does estrogen regulate colonic smooth muscle activity?
**Background:** Several lines of evidence suggest there is a link between levels of female steroid hormones and gastrointestinal function and motility. For example, the incidence of constipation is higher in pregnancy, when estrogen levels are elevated, but gastrointestinal motor function quickly recovers postpartum. Recent studies suggest there is an association between estrogen and down-regulation of A-type currents and Kv4.3 channels in uterine smooth muscle (myometrium). Moreover, A-type currents in colonic myocytes were shown to be predominately encoded by Kv4.3 channels. Thus these studies were designed to assess the effects of female steroid hormones on Kv4.3 channel expression in colonic smooth muscle cells.
**Observations:** Beckett et al. investigated the effects of acute and chronic changes in female steroid hormones on colonic smooth muscle A-type $K^+$ currents in male and female rats, following ovariectomy, with and without estrogen replacement, and during pregnancy. These studies demonstrate that estrogen suppresses functional expression of the A-type $K^+$ current, which previous studies have shown to be encoded by Kv4.3. Although quantitative PCR failed to detect any difference in Kv4.3 transcript expression, expression of the $K^+$ channel interacting protein type 1 was elevated, which could explain the elevated A-type current and altered kinetics of inactivation seen in colonic smooth muscle.
**Significance:** This study is important because it provides a possible link between altered colonic motor function associated with menstrual cycle, pregnancy, and menopause in females. Thus, similar to the important association between Kv4.3 channels and myometrial smooth muscle, Kv4.3 channels appear to also be important for regulating estrogen effects on colonic motility.

**Synaptic amplifier of inflammatory pain in the spinal dorsal horn.** Ikeda H, Stark J, Fischer H, Wagner M, Drdla R, Jager T, and Sandkuhler J. *Science* 312: 1659–1662, 2006.

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**Question:** Does the low-frequency activity that is associated with inflammation result in long-term potentiation (LTP) and, hence, increased pain sensitivity?
**Background:** Tissue inflammation can lead to hyperalgesia (increased sensitivity to pain), but the mechanism by which this occurs is unclear. All previous studies of pain pathways have utilized high-frequency stimulation (HFS) of afferent nerve fibers to induce LTP, which implies that LTP induces hyperalgesia. However, inflammation causes low-frequency afferent nerve activity, which induces long-term depression (LTD). Ikeda et al. hypothesized that a low-frequency afferent barrage differentially induces activation of pain pathways.
**Observations:** Ikeda et al. investigated the effect of low-frequency stimulation (LFS) on synaptic transmission in two major pain pathway projection areas: parabrachial (PB) and periaqueductal grey (PAG). Consistent with previous findings, HFS of primary afferent nerve fibers induced LTP at synapses between C-fibers and spino-PB neurons. LFS, in the typical frequency range of C-fibers during inflammation, had no effect on synaptic strength in this pathway. In contrast, although HFS of primary afferents had no effect on synaptic strength between C-fibers and spino-PAG neurons, LFS induced LTP at these synapses. LFS was determined to induce LTP by increasing intracellular $Ca^{2+}$, which causes amplification of pain-related information.
**Significance:** That a group of neurons in an ascending pain pathway can have their firing patterns enhanced by low-level activity in nociceptive nerve fibers is remarkable. These results suggest that there is a synaptic pain amplifier that is turned on by LFS and by natural noxious stimulation, which caus-

es amplification of pain-related information at the first synapse in pain pathways. Importantly, these findings provide a model that is compatible with known signal transduction pathways that are associated with hyperalgesia.

**Ischemia opens neuronal gap junction hemichannels.** Thompson RJ, Zhou N, and MacVicar BA. *Science* 312: 924–927, 2006.

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**Question:** What ion channel is responsible for the necrosis associated with stroke?

**Background:** A stroke occurs when a blood vessel is blocked, or bursts, resulting in insufficient delivery of oxygen and nutrients to the brain, which leads to necrosis. Although it is accepted that this occurs because of dysregulation of intracellular ion concentrations, the ion channels that are activated are unknown. A connexon, or hemichannel, is an assembly of proteins called connexins [pyramidal cells in the CNS express pannexin 1 (Px1) rather than connexins] that typically forms a bridge with another connexon from an opposing cell, forming a gap junction between the cytoplasm of two adjacent cells. However, in some cells, the hemichannel can function alone as a large-conductance channel between the cytoplasm and the extracellular space that allows flux of ions and molecules. It was hypothesized that a hemichannel contributes to the unregulated ionic fluxes that occur during a stroke.

**Observations:** Thompson et al. provide exciting pharmacological data that hemichannel activation could be responsible for the ionic dysregulation underlying the excitotoxicity that occurs during a stroke. Voltage-clamp recordings of oxygen- and glucose-depleted (OGD) rat hippocampal slices revealed a current with features reminiscent of hemichannel activation. The involvement of hemichannels was confirmed by experiments that demonstrated their selective inhibition blocked channel opening induced by ischemic stress. Finally, prolonged OGD was found to lead to irreversible current activation, neuronal swelling, and breakdown of cell membranes.

**Significance:** These results lead the authors to conclude that ischemia results in the opening of Px1 hemichannels, which contribute to the dysregulation of ionic currents that occur during a stroke. These provocative new findings represent an important step toward understanding the extensive necrosis that occurs in brain regions following a stroke and may be a novel therapeutic target to prevent neuronal death in stroke victims.

**Effects of congestive heart failure on $Ca^{2+}$ handling in skeletal muscle during fatigue.** Lunde PK, Sejersted OM, Thorud HM, Tonnessen T, Henriksen UL, Christensen G, Westerblad H, and Bruton J. *Circ Res* 98: 1514–1519, 2006.

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**Question:** How does congestive heart failure (CHF) lead to skeletal muscle weakness and decreased energy capacity?

**Background:** CHF is a condition in which the heart is not able to pump blood efficiently and thus cannot meet the body's energy demands. Interestingly, skeletal muscle weakness and decreased exercise capacity are symptoms of CHF that are not correlated with the attenuation of heart function. This suggests that intrinsic maladaptive changes occur in skeletal muscles after CHF. To this end, impaired sarcoplasmic reticulum (SR) $Ca^{2+}$ handling and decreased force development has been observed in skeletal muscle cells of a rat CHF model. However, the mechanism(s) that underlie impaired skeletal muscle function following CHF remain unresolved.

**Observations:** Lunde et al. found that CHF rats had clear signs of cardiac dysfunction. They next determined that, at rest, the expression of proteins essential for $Ca^{2+}$ handling, ryanodine receptors and SR $Ca^{2+}$-ATPase, were minimally, but significantly, affected by CHF 6 wk after infarction. Predictably from these findings, myoplasmic free $Ca^{2+}$ and basic contractile function were not affected in resting CHF muscle fibers. Conversely, when muscles were fatigued, there was a decrease in force that was surprisingly not attributable to changes in myoplasmic free $Ca^{2+}$ but rather due to reduced myofibrillar function.

**Significance:** These studies reveal the mechanism responsible for the intrinsic changes that occur in skeletal muscle following CHF in rats. The importance of this work is underscored by the prevalence of CHF in America. Hopefully this will lead to treatments to combat the fatigue people suffer, which can make ordinary daily activities, like going to the grocery store, exhausting.

**Targeted disruption of peroxiredoxin 6 gene renders the heart vulnerable to ischemia reperfusion injury.** Nagy N, Malik G, Fisher AB, and Das DK. *Am J Physiol Heart Circ Physiol* (June 9, 2006); doi:10.1152/ajpheart.003994.2006.

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**Question:** Does the peroxidase peroxiredoxin 6 (PRDX-6) contribute to protecting the heart against ischemia-reperfusion injury?

**Background:** A peroxidase is an enzyme that catalyzes a reaction with organic hydroperoxides such as $H_2O_2$ and lipid peroxides. Glutathione peroxidase (GSHPx) and catalase are important peroxidases that attenuate the inflammation and oxidative damage caused in the heart during ischemia-reperfusion injury. PRDX-6 belongs to a relatively new family of antioxidant enzymes that possess peroxidase activity and is expressed in the heart. Thus Nagy et al. hypothesized that PRDX-6 may play a role in ischemia-reperfusion injury.

**Observations:** PRDX-6 knockout mice (PRDX-$6^{-/-}$) mice were generated. Importantly, GSPHx and catalase levels were unaffected by the absence of PRDX-6 activity. Nonetheless, PRDX-$6^{-/-}$ mice were more susceptible to ischemia-reperfusion injury. The hearts of PRDX-$6^{-/-}$ mice displayed greater vulnerability to cellular injury and increased amounts of oxidative stress when compared with wild-type mice.

**Significance:** This paper demonstrates for the first time that PRDX-6 subserves a non-redundant antioxidant mechanism that confers protection against myocardial ischemic-reperfusion injury. This may represent an important finding, since most of the cardiac surgeries performed today are associated with ischemia and reperfusion injury. Thus PRDX-6 may represent a novel target to mitigate the ischemic and reperfusion injury and, therefore, improve the outcome of operations. ■