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Solution structure of the integral human membrane protein VDAC-1 in detergent micelles. Hiller S, Garces RG, Malia TJ, Orekhov VY, Colombini M, Wagner G. *Science* 321: 1206–1210, 2008.

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Question: What is the structure of the human voltage-dependent anion channel (VDAC)-1?

Background: VDACs are a class of transmembrane protein channels located on the outer mitochondrial membrane. VDAC-1 facilitates ion and metabolite diffusion across the membrane in a voltage-dependent manner. VDAC-1 is also involved in mitochondrial apoptosis. Although the exact mechanisms are not yet fully understood, it seems that VDAC-1 closure is correlated with formation of mitochodrial exit channels allowing the release of apoptogenic proteins, which induce cell death presumably by activating an executioner capase. The anti-apoptotic protein Bcl-x_L opens the VDAC-1 channel to allow the passage of metabolites and concominantly inhibits the release of apoptogenic proteins.

Observations: Utilizing high-resolution nuclear magnetic resonance (NMR), Hiller et al. describe the three-dimensional solution structure of human VDAC-1 reconstituted in detergent micelles. This experimental approach reveals an unusual 19-stranded beta barrel. They also identify the locations of interaction sites with two natural ligands and with Bcl-x_L.

Significance: Although many insights concerning the organization of VDAC have been gained from electron microscopy, biochemical, and biophysical studies, this is the first high-resolution structure of a voltage-dependent anion channel. These new findings on the structure of VDAC-1, along with the existing data, will aid in elucidating the mechanism of VDAC in apoptosis.

Identification of SLEEPLESS, a sleep-promoting factor. Koh K, Joiner WJ, Wu MN, Yue Z, Smith CJ, Sehgal A. *Science* 321: 372–376, 2008.

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Question: What can we learn about sleep regulation from fruit flies?

Background: Although it is known that sleep is an essential physiological process (as evidenced by the fact that, if rats are sleep deprived for long enough, they will die), it is unknown what biological needs are fulfilled. However, sleep is known to be regulated by circadian and homeostatic processes. Circadian rhythms determine when to sleep, whereas homeostatic mechanisms control the drive to sleep. Previous attempts to determine the molecular mechanisms that underlie the homeostatic drive to sleep in *Drosophila* have revealed interesting phenotypes when the Shaker K channel is mutated, which suggest membrane excitability is critical to the sleep process.

Observations: Following up on the findings in mutant *Drosophila*, Koh et al. used a large-scale unbiased genetic screen to identify genes that are essential for baseline sleep and rebound sleep after deprivation. They identified a gene, *sleepless* (*sss*), which encodes a brain-enriched, glycosylphosphatidylinositol (GPI)-anchored membrane protein that can cause an impressive reduction in overall time spent sleeping (>80%) when mutated. Interestingly, they also found that *quiver* (*qvr*), a mutation that causes impairments in the aforementioned gene that encodes the Shaker (Sh) K^+ channel, is an allele of *sss*, and that Sh protein levels are reduced in *sss* mutant flies.

Significance: It is thought that determining the molecular mechanisms that underlie the homeostatic drive to sleep could lead to new approaches to improve the quality of sleep. This work contributes significantly to that goal by identifying SLEPPLESS as a signaling molecule responsible for driving sleep by altering K^+ channel activity. Although there is some doubt that we can relate fruit fly genotype/phenotype to human genotype/phenotype, these findings are nonetheless an important step toward identifying genes responsible for sleep processes in humans.

The structure of an open form of an *E. coli* mechanosensitive channel at 3.45 A resolution. Wang W, Black SS, Edwards MD, Miller S, Morrison EL, Bartlett W, Dong C, Naismith JH, Booth IR. *Science* 321: 1179–1183, 2008.

A structural mechanism for MscS gating in lipid bilayers. Vásquez V, Sotomayor M, Cordero-Morales J, Schulten K, Perozo E. *Science* 321: 1210–1214, 2008.

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Question: What is the molecular basis of mechanosensitive ion channels (Msc) gating?

Background: Msc are found in a variety of tissues and organisms where they act as sensors for a number of systems, including osmotic homeostasis. As such, they play a critical role in transducing turgor pressure at the membrane into an electrochemical response, allowing the efflux of cytoplasmic contents to maintain osmotic balance. However, despite the many years that have been spent attempting to elucidate the structural arrangements of the reversible opening mechanism, it remains unclear. Two reports in *Science*, by Wang et al. and Vásquez et al., provide some insight into how the Msc of small conductance (MscS) overcomes this challenge.

Observations: Using two unique but innovative approaches to overcome the closed state of the MscS that is highly favored in the absence of applied tension, both research groups structurally characterized the open state of MscS. Wang et al. crystallized and characterized the electrophysiological properties of a mutant channel that required greater tension to open, but once the channel was open it formed a stable subconducting state. In contrast, Vásquez et al. obtained electron paramagnetic resonance measurements of wild-type MscS trapped in the open conformation by adding a cone-shaped lipid to the outer bilayer. Both studies revealed some similarities between the structures (e.g., a wider separation between transmembrane TM3 helices, which leads to an increased pore radius). However, there were also clear differences (e.g., whereas one structure suggests that the TM3 helices are almost parallel to the pore axis, the other structure implies these helices are tilted).

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Significance: These findings are the first to reveal a single gated ion channel in open and closed configurations. When the findings of these two studies are compared with the closed state of the structure, which has been previously described, and combined with functional data or the computational analysis, it is possible to model the movements of the transmembrane helices that cause channel opening. Thus the MscS channel is now one of a select few that has structural and biophysical data for multiple states.

Regulation of CFTR trafficking by its R domain. Lewarchik CM, Peters KW, Qi J, Frizzell RA. *J Biol Chem* 283: 28401–28412, 2008.

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Question: Is the trafficking of the cystic fibrosis transmembrane conductance regulator (CFTR) regulated by an internal structural feature of the protein?

Background: The CFTR is an ABC transporter-class anion channel that transports chloride and bicarbonate ions across epithelial cell membranes. Unlike other ABC transporters, CFTR has a regulatory (R) domain with multiple phosphorylation sites, which mediate cAMP-dependent channel activation via protein kinase A. cAMP agonists function to regulate channel activity and modulate CFTR channel density in the plasma membrane. However, the protein interactions and trafficking pathways that underlie CFTR trafficking are not well defined.

Observations: Lewarchik et al. determined the structural basis of CFTR trafficking regulation by inducing agonist-evoked increases in plasma membrane capacitance in CFTR deletion mutants expressed in *Xenopus* oocytes. When the R domain was deleted, the channel had an elevated basal current and did not undergo trafficking when stimulated. Similarly, when an amino acid sequence known as NEG2 was deleted, the channel could not undergo agonist-induced trafficking. Other data supported the idea that NEG2 is essential for the trafficking of CFTR.

Significance: These findings suggest that a structural feature of CFTR, NEG2, permits CFTR to enter a regulated intracellular compartment from which it traffics to the plasma membrane in response to agonist stimulation. The identification of the CFTR component necessary for regulated trafficking could lead to the identification of targets to modulate the density of CFTR mutants. Although there is still much work to be done, the prospect of being able to modulate the density of CFTR mutants holds promise ultimately for treating cystic fibrosis.

Nicotine administration and withdrawal affect survival in systemic inflammation models. Steiner AA, Oliveira DL, Roberts JL, Petersen SR, Romanovsky AA. *J Appl Physiol* 105: 1028–1034, 2008.

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Question: How do acute exposure, chronic exposure, and withdrawal from nicotine affect systemic inflammatory response syndrome (SIRS) and survival rates in mice?

Background: A leading cause of death in hospitalized patients, SIRS is defined as a clinical response to an inflammatory insult of either infectious or noninfectious origin. If it is determined that the SIRS was caused by an infection, it is sepsis. Whatever the cause of SIRS may be, shock, multiple organ failure, and eventually death can occur, which is due, in large part, to the production of pro-inflammatory mediators. Activation of nicotinic acetylcholine receptors is known to inhibit pro-inflammatory cytokine production and inhibit some of the symptoms of SIRS. Hence, the group of researchers at St. Joseph's Hospital (Phoenix, AZ) led by Andrej Romanovsky sought to determine the effect of nicotine on SIRS.

Observations: To mimic a number of human conditions concerning nicotine exposure, Steiner et al. determined the effect of acute and chronic nicotine administration and acute nicotine withdrawal on aseptic and septic systemic inflammation. They found that chronic nicotine exposure did not affect survival in either inflammatory model. In contrast, acute nicotine administration increased survival rate in aseptic inflammation but decreased survival rate in septic inflammation. Finally, nicotine withdrawal increased survival rates in the sepsis model.

Significance: These complex findings on the effect of nicotine on SIRS are intriguing, but become even more complex when one considers that the constant rate of nicotine infusion in these studies differs from how humans typically consume the drug. Nonetheless, because patients in hospital settings are frequently in the withdrawal group, the astute inclusion of a representative cohort for these patients (or of the corresponding group of animals in an experimental study) is a key piece of data that is often overlooked in other studies that characterize inflammatory factors. A final accolade this research group deserves is for addressing the effect of a factor (nicotine) on inflammation resulting from either a septic or aseptic source, which is not typically addressed in studies of inflammation.

12-Lipoxygenase-knockout mice are resistant to inflammatory effects of a high fat western diet. Nunemaker CS, Chen M, Pei H, Kimble SD, Keller SR, Carter JD, Yang Z, Smith KM, Wu R, Bevard MH, Garmey JC, Nadler JL. *Am J Physiol Endocrinol Metab* (September 9, 2008); doi:10.1152/ajpendo.90371.2008.

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Question: Can the inflammatory effects of a high-fat diet be circumvented?

Background: Numerous studies have provided evidence that visceral fat mass is associated with an increased risk of developing insulin resistance and cardiovascular disease. More recently, a growing body of evidence has identified inflammation in mediating, at least in part, these health concerns. Lipoxygenases (LOs) are a family of iron-containing enzymes that are involved in fatty acid metabolism. 12-LO expression/activity is upregulated by hyperglycemia or cytokine-mediated damage, and the downstream products of 12-LO-induced metabolism are known to activate signaling pathways that lead to increased levels of inflammation.

Observations: Based on the physiological role of 12-LO described above, Nunemaker et al. hypothesized that mice lacking 12-LO would be immune from the inflammatory-mediated damage associated with a high-fat diet. As predicted, they found that 12-LO KO mice were able to maintain glucose and

insulin tolerance when fed a high-fat diet. Moreover, proinflammatory cytokines and protective adipokines were not affected in the 12-LO KO mice, which is in contrast to what was found in controls.

Significance: These findings and other data not mentioned here together suggest that 12-LO activation has a role in high-fat diet-induced inflammation. Not withstanding the potential benefits from a high-fat/low-carbohydrate diet, the negative consequences of a high-fat diet are described extensively throughout scientific journals. Although this appears to be having little effect on the way people eat. As such, it may prove beneficial to prevent inflammation-mediated metabolic consequences of excess fat intake. Although inhibiting 12-LO may make this a possibility someday, there is still much work to be done to determine whether 12-LO in one or a combination of tissues is responsible for the protective effects seen during high-fat feeding. Dr. Nadler is currently generating unique models to address these questions and hopes to have the answers in the next few years.

Tissue-specific pyruvate dehydrogenase complex deficiency causes cardiac hypertrophy and sudden death of weaned male mice. Sidhu S, Gangasani A, Korotchkina LG, Suzuki G, Fallavollita JA, Canty JM Jr, Patel MS. *Am J Physiol Heart Circ Physiol* 295: H946–H952, 2008.

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Question: Does glucose oxidation via the tricarboxylic acid (TCA) cycle have a role in maintaining cardiac energy homeostasis?

Background: Pyruvate dehydrogenase complex (PDC) consists of three enzymes that transform pyruvate into acetyl-CoA, which is essential for producing citrate for the complete oxidation of glucose carbons. PDC deficiency is a metabolic disorder associated with an energy deficiency because citrate is the primary substrate of the TCA cycle. Under normal physiological conditions, the majority of ATP in the adult heart is derived from fatty acid oxidation by the combined actions of the β-oxidation pathway and the TCA cycle. Because only a small fraction of the total energy requirements of the heart are derived from glucose oxidation via the TCA cycle under basal conditions, it is not clear whether glucose oxidation via the TCA cycle has an important role in maintaining cardiac energy homeostasis.

Observations: Sidhu et al. examined the cardiac physiology of a mouse model with a knockout of the α-subunit of the pyruvate dehydrogenase component of PDC in heart/skeletal muscle. Remarkably, knockout mice (H/SM-PDCKO) grew normally until weaned. However, after weaning, they found profound effects on mortality of the homozygous males, which did not survive beyond ~7 days. Interestingly, when the male KO mice were weaned onto a high-fat diet they survived, although they developed marked myocyte hypertrophy and left ventricular dysfunction.

Significance: This study highlights just how important glucose oxidation is for optimal cardiac energy homeostasis and function under basal dietary conditions. Although an argument can be made that certain genotypes are better suited to high-fat diets, the unfortunate effects of this particular genotype include left ventricular hypertrophy and dysfunction, which would endanger survival regardless of the diet. As is the case of most research, these findings lead to several other interesting questions such as whether this particular model of cardiac hypertrophy is entirely maladaptive. ■