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Question: What allows bats to use echolocation at rates ≥160 calls/s to attack prey?

Background: Some bats use echolocation to accurately map their environment, including detecting and subsequently attacking flying insects. As bats detect insects, their echolocation rates progressively increase through approach until they are maximized during the terminal buzz stage, which is associated with emission rates of ≥160 calls/s. Maximum echolocation rates have been hypothesized to be limited by the ability to produce or the ability to process the echoes, or both.

Observations: To determine the rate-limiting factor of echolocation rates, Elemans et al. first measured when each prey echo began and ended in freely flying bats. They found that there was no overlap between when an individual echo ended and the subsequent one began, which eliminates the possibility that echo-processing limits call repetition rates. Interestingly, however, when they dissected the process of how these calls were produced, they found that bat laryngeal muscles were not typical skeletal muscles but rather the rarely identified superfast muscles that can produce work at >100 Hz.

Significance: Although superfast muscles had been previously identified in several species, this is the first report to have characterized them in a mammal. This evolutionary adaptation was likely critically important for bats to succeed as nocturnal aerial predators. Thus bats have superfast muscles that can power movements at rates ≥190 calls/s that also limit the maximum call rate during the terminal buzz stage.


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Question: Can electrospray mass spectrometry preserve an intact ATPase?

Background: ATPases consist of two reversible molecular motors: an ion domain that mediates the transport of protons across intracellular and plasma membranes of cells and the chemical domain that mediates the generation/consumption of ATP. Although structural details concerning the mechanical coupling of the two domains have been reported, they have been derived from isolated subcomplexes of the two domains. However, a recently established methodology allows intact membrane complexes to be propelled (electrosprayed) into a mass spectrometer (MS) preserving soluble and membrane subunit interactions in vacuum. This suggests that obtaining information about cross talk between subunits could be feasible.

Observations: Indeed, electrospray MS of ATPases from Thermus thermophilus (TtATPase) and Enterococcus hirae (EhATPase) allowed Zhou et al. to compare the subunit stoichiometries and the identity of tightly bound lipids within the membrane rotors of the two complexes. The TtATPase was found to have a 12-subunit ring that formed six dimers, each with a single tightly bound lipid. The EhATPase had 10 monomers with 10 bound lipids. Subsequently, dissociation patterns in the gas phase revealed the regulatory effects of nucleotide binding on TtATP hydrolysis and proton translocation.

Significance: Linking specific regulatory binding events and conformational changes...
between lipids and nucleotides associated with dynamic processes using MS in the gas phase should further our understanding of how biological membranes function under normal and pathological conditions. Thus, since the binding of specific lipids and nucleotides can be linked with distinct regulatory roles, these findings may have important implications for the development of specific ATPase inhibitors as novel treatments of various pathological conditions.

**Question:** How does the heart of a Burmese python increase in size after consuming a large meal?

**Background:** Burmese pythons eat infrequently because they can fast for up to a year after consuming prey that is twice their size. This provides a unique model of extreme metabolic regulation for scientists to study because the python’s metabolism increases by >40-fold. This increase in postprandial metabolic rate is accompanied by increased systemic nutrient transport and organ growth, including the heart, which increases in mass by 40%. Although the cardiac hypertrophy has been well defined, the underlying molecular and cellular mechanisms have not been determined.

**Observations:** Riquelme et al. sought to determine the mechanisms regulating this cardiac enlargement. Employing gas chromatography they analyzed both fasted and fed python blood plasma after feeding and found that, one day after feeding, the amounts of three fatty acids (myristic acid, palmitic acid, and palmitoleic acid) increased by >50-fold. Additionally, they found increased activity of superoxide dismutase, a cardio-protective enzyme, but no evidence of fat deposition in the heart. Subsequently, they increased heart growth of fasting pythons by injecting them with blood plasma from fed pythons. Finally, they confirmed their findings in a mammal by injecting mice with either fed python plasma or the fatty acid mixture, which significantly increased cardiomyocyte size but not cardiac fibrosis.

**Significance:** This study shows that a combination of fatty acids can induce beneficial heart growth. Although exercise can also result in beneficial heart growth, cardiac diseases often prevent people from exercising. Thus these results suggest that fatty acid supplementation may provide a novel mechanism for improving cardiac gene expression and function in people with cardiac disease.


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**Question:** What is the mechanism by which hyperglycemia contributes to diabetic cardiomyopathy (DCM)?

**Background:** Diabetes mellitus is commonly associated with cardiomyopathy and death of cardiac myocytes. Persistent hyperglycemia is most commonly caused by diabetes mellitus and contributes to myocardial cell injury. Moreover, oxidative stress has been implicated as a key contributor to DCM, and hyperglycemia is associated with enhanced generation of reactive oxygen species (ROS). However, the mechanisms for these ROS-induced diabetic complications are not well understood.

**Observations:** In this study, Maalouf et al. examined NADPH oxidase Nox4 as a source of ROS in the development of DCM by treating streptozotocin-induced diabetic rats with antiseptic (AS) constructs directed at Nox4. The diabetic rats had increased NADPH-oxidase activity, ROS generation, and Nox4, but not but Nox1 or Nox2, expression in the left ventricle along with markers of hypertrophy and myofibrosis; treatment with the Nox4 AS constructs attenuated such changes. The authors also used a cell culture model and found that treatment of cardiac myocytes with an adenoviral vector that contained a dominant-negative NOX4 prevented the increase in NADPH oxidase activity, Nox4 expression, and markers of cardiac injury that followed incubation with 25 mM glucose.

**Significance:** These results show that Nox4 is a key source of cardiac ROS in models of DCM. DCM is known to have a high prevalence in diabetes, and these findings suggest that Nox4 is a potential therapeutic target to prevent the progression to congestive heart failure.


**Question:** What is the molecular mechanism by which increased expression of the GLUT1 glucose transporters enhances activity of the mammalian target of rapamycin (mTOR)?

**Background:** Diabetic nephropathy is the leading cause of chronic kidney disease in the United States. Diabetic nephropathy initially manifests in glomerular cells, including mesangial cells, of the kidneys. Expression of the facilitative glucose transporter, GLUT1, is increased in the renal cortex, renal tubule segments, and glomeruli of diabetic rodent models. GLUT1 transporters are thought to regulate mesangial cell glucose metabolic flux. mTOR is thought to have a significant role in the pathogenesis of diabetic nephropathy, and GLUT1 expression is enhanced by mTORC1 activation in several types of cells.

**Observations:** Buller et al. sought to determine whether increased GLUT1 expression enhances activation of mTORC1 in mesangial cells via an AMPK-dependent pathway. Surprisingly, they found that
GLUT1 expression enhances mTORC1 activity but not through an AMPK-dependent or a tuberous sclerosis complex (TSC) 2-dependent mechanism. Instead, enhanced mTORC1 activity occurred through its activator, Rheb, interacting with glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

**Significance:** This study suggests that increased GLUT1 expression in mesangial cells leads to increased glucose flux and mTOR activity via a metabolic effect on GAPDH. Thus a feed-forward mechanism appears to cause persistent GLUT1 overexpression and mTOR activation in diabetic glomeruli. Importantly, treatments that decrease GLUT1 expression or inhibit mTOR activation may mitigate the progression of diabetic nephropathy.

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**Question:** What is the molecular mechanism by which taurine modulates gating of gap-junction channels?

**Background:** Gap junctions are aggregates of specialized intercellular channels that connect the cytoplasmic compartments of coupled cells and allow small cytoplasmic molecules and ions to pass between them. The channels are oligomers of connexin (Cx) proteins. The functional properties of the channels, such as permeability to second messengers and modulation by cytoplasmic factors and voltage, are determined by the isoform composition of the channels. There are 21 connexin isoforms in the human genome. The Harris laboratory recently found that cytoplasmic taurine directly regulates Cx channel activity at low pH. However, the molecular mechanisms of how this regulation is achieved have not been clearly identified.

**Observations:** Locke et al. found that the COOH terminus (CT) of connexin26 (Cx26) binds the cytoplasmic loop (CL) in a pH-dependent manner. Peptide NMR showed that taurine binds to the CL of Cx26 and not the CT. Thus, at low pH, taurine facilitates channel closure by competing with the CT for CL, leading to pore occlusion. A cytoplasmic taurine-sensitive site of Cx26 was confirmed by blocking the taurine transporter.

**Significance:** These studies provide a detailed biophysical channel mechanism for modulation of connexin channels by cytoplasmic factors, such as taurine, and reveal that taurine disrupts a pH-driven cytoplasmic interdomain interaction in Cx26-containing channels, causing the channel to close. In addition, these studies revealed that the CT of Cx26 has a modulatory role in Cx26 function. These studies further elucidate mechanisms of connexin channel function, thus providing insight into the cellular and molecular processes that underlie intercellular signaling in the nervous system.

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**Nominated by Alicia McDonough**

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**Question:** Is there an easy way to visualize the information needed to generate peptide-directed antibodies?

**Background:** Our expanding view of the physiological mechanisms at a cellular level has largely resulted from genome sequencing projects. These sequencing projects have provided the necessary information for generating antibodies to further study the identified proteins and the cellular pathways they affect. Although peptide-derived antibodies are widely used tools in numerous biomedical disciplines, the development of reliable antibodies has been problematic and often based on trial-and-error approaches.

**Observations:** Pisitkun et al. describe a novel web-based software tool (NHLBI-AbDesigner) to assist researchers in choosing epitopes for antibody generation in a fast and reliable manner. NHLBI-AbDesigner analyzes the amino acid sequence of a given protein to identify optimal immunizing peptides to produce antibodies. The software allows the user to choose an immunizing peptide based on the trade-offs between immunogenicity, antibody specificity, multi-species conservation, and posttranslational modifications.

**Significance:** The software associated with this manuscript is a major advance for physiologists seeking to generate peptide-directed antibodies. The AbDesigner should also prove to be useful for those who may not need to generate antibodies but simply wish to learn more about the properties of a given protein. The web-based software tool can be found at http://helixweb.nih.gov/AbDesigner/.

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**Nominated by Jeff jsands@emory.edu Sands**

**Question:** What protein regulates podocyte cell shape and membrane dynamics?

**Background:** Glomerular podocytes are highly specialized epithelial cells of the kidney that have foot processes that aborize into smaller actin-based secondary foot processes. These secondary foot processes have filtration slits that establish the selective permeability of the glomerular filtration barrier. In proteinuric kidney diseases, the actin cytoskeleton of podocytes are rearranged, and their foot processes are retracted or effaced. However, the mechanisms and regulatory proteins underlying foot process effacement are not fully understood.

**Observations:** Akilesh et al. found that the RhoA-activated Rac1 GTPase-activating protein (Rac1-GAP), Arhgap24, was upregulated in podocytes as they differentiated, both in vitro and in vivo. Subsequently, they determined that Arhgap24 inactivates Rac1 in mouse podocytes. They also sequenced the DNA for CL, leading to pore occlusion. A cytoplasmic taurine-sensitive site of Cx26 was confirmed by blocking the taurine transporter.
from patients with focal segmental glomerulosclerosis (FSGS; a proteinuric kidney disease) and determined that there was a loss-of-function mutation in the \( \text{ARHGAP24} \) gene.

**Significance:** These results suggest that cytoskeletal regulation of kidney podocytes is mediated by the Arhgap24 protein. In addition, dysregulation of Arhgap24, which causes an imbalance of the RhoA and Rac1 signaling pathway, is implicated in the proteinuric kidney disease FSGS. Collectively, these findings suggest that modulating Arhgap24 function may provide a novel therapeutic approach to treating proteinuric kidney disease.

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**Question:** Do ion channels have a role in regulating the ability of embryonic stem cells to indefinitely proliferate?

**Background:** Embryonic stem (ES) cells are undifferentiated and pluripotent; thus ES cells have the unique ability to replicate indefinitely and differentiate into any adult cell type. A variety of factors have been reported to govern the propagation of undifferentiated ES cells. ES cells are known to express voltage- and ligand-gated ion channels, although they have been thought to be nonexcitable. Voltage-gated \( \text{Ca}^{2+} \) channels are known to regulate numerous cellular processes including proliferation, differentiation, and apoptosis; however, they have not been studied in detail in ES cells.

**Observations:** Rodriguez-Gomez et al. first determined that Cav3.2 voltage-gated \( \text{Ca}^{2+} \) channels are expressed in mouse ES cells. Subsequently, they provide biophysical and pharmacological evidence, including use of siRNA, to show that Cav3.2 channels contribute to proliferation and self-renewal of mouse ES cells. They also found that Cav3.2 \( \text{Ca}^{2+} \) channel expression in ES cells is induced during the late G1 phase of the cell cycle.

**Significance:** These findings suggest that Cav3.2 \( \text{Ca}^{2+} \) channels modulate \( \text{Ca}^{2+} \) entry into ES cells and thereby contribute to self-renewal and the maintenance of the undifferentiated state. Characterizing the signaling pathways that underlie the ability of ES cells to self-renew and maintain pluripotency should allow their therapeutic and scientific value to be fully realized.