
Question: Do cancer cells utilize pyruvate kinase M2 (PKM2) to regulate reactive oxygen species (ROS) levels, and, if so, how?

Background: Pyruvate kinase (PK) catalyzes the final and one of the rate-limiting steps of glycolysis. There are two forms of PK expressed in a non-tissue-specific manner, PKM1 and PKM2. The regulatory properties of the specific isoform expressed in a cell influence whether glucose is metabolized predominantly by the TCA cycle (PKM1) or aerobic glycolysis (PKM2). Recent studies from the Cantley laboratory revealed that PKM2 is utilized by cancer cells to promote the metabolism of glucose, which cancer cells take up at high rates, for biosynthetic processes that support proliferation. Cancer cells also need to deal with stresses, such as excess ROS, produced because of aberrant growth factor signaling. There is ample evidence for oxidation of PKs from bacteria to human cells; however, the consequences of this modification on metabolism have been unclear.

Observations: To interrogate the relevance of PK oxidation on cancer metabolism, Anastasiou et al. subjected lung cancer cells to oxidative stress and observed that oxidants inhibit the activity of PKM2 but not that of PKM1. Using metabolomic analyses, they found that oxidation of PKM2 promoted glucose metabolism via the pentose phosphate pathway, which stimulated the generation of antioxidant molecules (in the form of NADPH) for detoxification of ROS. They also demonstrated that pharmacologically activating PKM2 under conditions of high oxidative stress sensitized the cells to oxidant-induced death and that cells which express a non-oxidizable PKM2 mutant did not form tumors as well as cells with wild-type PKM2.

Significance: This study suggests that cancer cells attenuate PKM2 activity to manipulate glucose metabolism and increase the production of antioxidants, thereby offsetting the toxic effects of ROS. Perhaps more importantly, the ability of the PKM2 activator to interfere with this process suggests that they may improve the efficacy of radiation therapy and chemotherapeutic agents, which are thought to kill cancer cells by inducing excess ROS production.


Question: What is the efflux mechanism of lysosome-recycled nucleosides?

Background: Hematopoietic cells express equilibrative nucleoside transporter (ENT) proteins to take up nucleosides and subsequently produce all the blood cell types, including monocytes and macrophages. Monocytes, produced by bone marrow from hematopoietic cells, migrate through the bloodstream to other tissues where they are differentiated into tissue-specific macrophages. Histiocytes, the tissue macrophages, remove apoptotic cells and recycle their nucleic acids into nucleosides through lysosomal degradation. This study explored the mechanism by which lysosomes export the recycled nucleosides.

Observations: Hsu et al. initially determined that of the four known ENTs, ENT3 had the highest expression levels in macrophages. Subsequently, they generated ENT3 knockout mice (ENT3−/−). The ENT3−/− mice developed splenomegaly as a result of increased macrophage proliferation (histiocytosis). In ENT3−/−-derived macrophage cultures, degradation of apoptotic cells was delayed, which led to nucleoside buildup, elevated intralysosomal pH, and altered macrophage function. Finally, they determined that the lysosomal defects lead to persistent signaling at the macrophage colony-stimulating factor 1 receptor complex, which may underlie increased macrophage proliferation.

Significance: These findings suggest that ENT3 is responsible for nucleoside efflux from lysosomes and that ENT3 is essential for both lysosomal function and macrophage homeostasis. Importantly, these findings provide insight into a potential pathophysiological mechanism by which ENT3 mutations in humans result in histiocytosis.
the time window from the activation of the starvation program until a lack of nutrients limited growth. Under steady-state growth conditions, constitutive expression of the high-affinity transporters did not adversely affect yeast fitness. In contrast, when transitioning from low- to high-nutrient conditions, constitutive expression of the high-affinity transporters was detrimental to yeast growth. However, this was not due to phosphate or zinc toxicity because when they constitutively expressed the low-affinity transporters, transitioning from low- to high-nutrient conditions did not adversely affect growth. The fitness defects during the cells’ recovery from starvation were rescued by constitutive activation of the starvation program.

**Significance:** This study suggests that the dual-transporter system increases tolerance to fluctuating nutrient levels, essentially giving the cells more time to prepare for depleted nutrient conditions. Thus the low-affinity transporter may provide an early intracellular signal that extracellular nutrient conditions are suboptimal, which would increase transcription of the starvation program and mitigate deleterious fitness effects.

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**Question:** What role do Rab3-interacting molecule (RIM)-binding proteins (RBPs) play in neurotransmitter release?

**Background:** Presynaptic neurotransmitter release requires that synaptic vesicles, proteins such as SNARE [SNAP (soluble NSF attachment protein) receptor] and voltage-gated Ca\(^{2+}\)-channels (VGCCs) are properly organized at the presynaptic active-zone (AZ) membranes. The Sigrist laboratory previously found that the Bruchpilot protein (BRP) was an essential building block of the electron-dense projections (the T bar) of the AZ membrane, which mediates clustering of VGCCs and efficient neurotransmitter release. RBPs have also been reported to act as a scaffolding protein at the AZ membrane; however, their role in neurotransmitter release is unclear.

**Observations:** Liu et al. employed stimulated emission microscopy (STED), which drastically increases the resolution of fluorescence microscopy, to determine whether *Drosophila* Rab-interacting molecule-binding protein (DRBP) forms part of the AZ cytomatrix core. They found DRBP localized toward the AZ center and surrounding the central VGCC field. They also found in DRBP mutants that VGCC clustering and Ca\(^{2+}\) influx were impaired and that synaptic release probability was drastically reduced.

**Significance:** These findings suggest that, similar to BPR, DRBP is required for coupling of Ca\(^{2+}\) influx with vesicle fusion. However, they also found that the deficits caused by the BPR mutation might be partially explained by a concomitant loss of DRBP, which implicates DRBP as a more important contributor to presynaptic neurotransmitter release. More notably, genetic defects in the RBP proteins appear to be an increasingly important aspect of autism in humans. Thus this report also may help elucidate the pathophysiology of autism.

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**Question:** How are hormonal signals coordinated across different neuronal cell types?

**Background:** Numerous small nuclei in the ventral part of the diencephalon make up the hypothalamus. This almond-sized part of the brain regulates several autonomic processes, including hunger, by synthesizing and secreting hormones that directly affect the endocrine system via the pituitary gland. Hormonal signals are known to modulate neuropeptide Y (NPY)/Agouti-related protein (AgRP) neurons of the hypothalamic arcuate nucleus and pro-opiomelanocortin (POMC) neurons of the anterior pituitary. For example, the gut-derived orexigenic hormone ghrelin and the adipocyte-derived anorexigenic hormone leptin both differentially affect POMC and AgRP neurons. How these hormonal signals are coordinated was explored by Yang et al.

**Observations:** Yang et al. combined electrophysiological, pharmacological and optogenetic [a temporally (ms) precise manipulation of genetically modified (photo-excitable) cells] approaches to elucidate the influence of excitatory synaptic inputs on the activity of NPY/AgRP neurons. They found that food deprivation induced a persistent upregulation of excitatory synaptic inputs to, and increased firing rates of, NPY/AgRP neurons. This persistent synaptic activity to NPY/AgRP neurons was determined to be initiated by ghrelin but maintained via an AMP-activated kinase (AMPK)-dependent positive feedback loop. In contrast, food deprivation decreased firing rates of POMC neurons. However, the persistent AMPK-dependent synaptic activity was terminated by leptin, which optogenetic results suggest may be mediated by opioids released from POMC neurons.

**Significance:** These findings suggest a novel AMPK-dependent positive feedback loop that functions as a flip-flop memory circuit when exposed to hormones that regulate appetite. Moreover, new evidence is provided that suggests AMPK is important in presynaptic neurons upstream of NPY/AgRP neurons rather than in the NPY/AgRP neurons or the opposing POMC neurons. Thus this resolves a previous puzzle in the field because, although AMPK had been suggested to play an important role in appetite control, knocking it out in NPY/AgRP or POMC neurons had little effect.

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Question: How does exposed phosphatidylserine (PS) on the membrane of erythrocytes increase adhesion to endothelial cells?

Background: Eryptosis, programmed cell death of erythrocytes (red blood cells, RBCs), is characterized by cell shrinkage, irregular bulging of the plasma membrane (blebbing), and exposure of PS on their surface. Eryptosis may result from activation of Ca^{2+}-permeable cation channels that subsequently increase cytosolic Ca^{2+} concentrations. In addition, activation of sphingomyelinase, which catalyzes the hydrolysis of lipids and generates the pro-apoptotic molecule ceramide, may exacerbate the scrambling effect of Ca^{2+}. The transmembrane CXC chemokine ligand 16 (CXCL16) is expressed on human vascular endothelial cells (HUVEC) where it acts as a scavenger receptor that binds phosphatidylserine and promotes adhesion.

Observations: Borst et al. sought to determine the mechanism of PS-exposing RBC adhesion to HUVEC. They found that Ca^{2+} ionophores or glucose depletion both triggered eryptosis and increased adherence to HUVEC under flow conditions at arterial shear rates. In addition, they found that adherence to HUVEC could be modified by coating and functionally neutralizing the erythrocyte-exposed phosphatidylserine. Similarly, antibodies directed against CXCL16 or decreasing CXCL16 expression in HUVEC significantly blunted adherence.

Significance: These experiments add important new information regarding eryptosis by identifying a mechanism for the adhesion of erythrocytes to endothelial cells and perhaps other cells, such as macrophages. Lipid-laden macrophages of atherosclerotic plaques have high levels of expression of CXCL16; thus adherence of RBCs to CXCL16 may contribute to the pathogenesis of thrombo-occlusive diseases. In fact, these findings may have implications for advancing our understanding of numerous additional conditions because excessive eryptosis is associated with everything from sickle cell anemia to malaria.

Finally, they found that tsp4<sup>−/−</sup> isolated myocytes responded normally to stretch.

Significance: That the response of isolated tsp4<sup>−/−</sup> myocytes was normal suggests that TSP4 signaling is required only when matrix is present. Thus this reveals a novel and key role of TSP4 signaling to matrix-myocyte interaction and the adaptive cardiac contractile responses to acute stress. Identifying TSP4 as the coupling mechanism between subacute myocardial stress and contractility highlights it as a potential therapeutic target to further enhance adaptive cardiac stress responses.


Question: Are the prophylactic effects of a protein kinase Ca/β (PKCa/β) inhibitor observed in a rodent model of heart failure preserved in a large animal model?

Background: PKCa is the predominant PKC isoform expressed in human heart and activated by heart failure. PKCa activation is detrimental because it reduces cardiac contractility, ventricular dilation, and secondary hypertrophy. In contrast, mutating or blocking PKCa signaling is cardioprotective against insults and genetic mutations. Similarly, inhibition of PKCa/β enhances cardiac function, augments cardiac contractility and function, reduces mortality, and reduces contractile abnormalities in small animal models of heart failure and disease.

Observations: Although compelling evidence in rodents supports the contention that inhibition of PKCa is cardioprotective, there are several important distinctions between the heart functions and biochemistry of large and small mammals. Thinking toward initiating clinical trials prompted Ladage et al. to determine whether inhibition of PKCa is cardioprotective in a large animal (pig) model of heart failure. They found that administration of ruboxistaurin, a PKCa/β inhibitor, to pigs significantly increased recovery of
cardiac contractility, ejection fraction, and cardiac output 3 mo after myocardial infarction-induced heart failure.

**Significance:** These data further validate the potential of PKCα/β inhibition to protect the heart from failure after injury in humans. In fact, the safety profile of ruboxistaurin has already been established in clinical trials for the treatment of diabetic retinopathy and microvascularopathy. Collectively, the present studies, validating this novel pharmacotherapeutic in a large-animal model of heart failure coupled with the safety profile previously observed during other clinical trials, support further investigation of ruboxistaurin for the treatment cardiac disease in humans.

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**Question:** Does stem cell therapy mitigate the metabolic remodeling of the injured heart?

**Background:** Metabolic remodeling of the heart, which occurs after a myocardial infarction (MI), includes reduced mitochondrial-mediated fatty acid (FA) catabolism and increased glucose catabolism. This metabolic shifting can lead to mitochondrial reductions and dysfunction of oxidative phosphorylation in the remaining mitochondria. Although stem cell therapy following a MI has been shown to improve cell viability, vascularization, and cardiac function, therapeutic effects on the detrimental remodeling of metabolic processes is less clear.

**Observations:** Hughey et al. determined the effects of mesenchymal stem cell (MSC) therapy on metabolic remodeling following MI in mice. They found that MSC transplantation preserved insulin-stimulated FA uptake peri-infarct and inhibited the normally observed increased glucose metabolism in the remote left ventricle. MSC therapy was also found to prevent dysfunction of mitochondrial oxidative phosphorylation. Finally, MSC therapy improved fractional shortening and reduced heart-to-body weight ratios.

**Significance:** This is the first paper to combine a comprehensive in vivo approach for evaluating insulin action, substrate uptake, and cardiac function with MSC therapy following induction of MI in the conscious mouse. These data clearly demonstrate that MSCs exhibit therapeutic potential as a metabolic intervention to attenuate post-MI energetic abnormalities by preserving metabolic flexibility and improving myocardial contractility and function.

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**Question:** What is the role of ATF6 in insulin-secreting pancreatic beta cells?

**Background:** The unfolded protein response (UPR) is activated in response to an accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER) lumen. The UPR transiently inhibits protein translation, activates the ER-associated protein degradation (ERAD) signaling pathway, and upregulates production of ER chaperones involved in protein folding. ATF6, PERK, and IRE1 are the three main ER-membrane-bound transcription factors activated by stress. Activated ATF6 translocates to the Golgi, where it is cleaved by proteases to form an active 50-kDa fragment (ATF6α-p50). Subsequently, ATF6α-p50 translocates to the nucleus, where it binds to promoters that upregulate proteins including the chaperone GRP78. This study sought to determine whether ATF6 has a role in pancreatic beta-cell dysfunction and apoptosis.

**Observations:** Teodoro et al. found that ATF6 may be activated in pancreatic islet beta cells, even under basal conditions, and further increased by ER stress. Depletion of ATF6α-p50 levels in insulinoma cells reduced steady-state levels of GRP78 mRNA and protein levels in nonstressed cells and induced an apoptotic phenotype. Interestingly, however, when ATF6α-p50 levels were depleted, PERK and IRE1 were not activated. Finally, in ATF6α-depleted cells, phosphorylation of the mitogen-activated protein kinases JNK and p38 were elevated in ATF6α-depleted cells, and inhibition of JNK or p38 prevented apoptosis.

**Significance:** This study points out the intriguing possibility that the UPR, via ATF6, may regulate basal cell metabolism and contribute to the retention (or loss) of beta cells that occurs in the pre-diabetic state. Pre-diabetic beta cells compensate for developing peripheral tissue insulin resistance by increasing insulin synthesis and secretion, which stresses protein folding in the ER. The molecular details of the UPR divulged by this study may lead to the development of novel pharmacological treatments that improve beta-cell function before they progress toward apoptosis and the diabetic state.
hypothesis, the CaM binding domain of myoV forms a relatively stiff neck (lever arm) that amplifies motions in the motor head and enables it to move 36 nm along actin with each turnover of ATP. However, the extremely complex intracellular milieu presents a challenge to efficient myoV transport, and how myoV proficiently maneuvers through the cell had been unresolved.

**Observations:** Lewis et al. used single molecule fluorescence polarization and total internal reflection fluorescence (TIRF) microscopy to map the real-time rotational motions of myoV that had either the native-length (6 IQ) or truncated (4 IQ) lever arms. A new type of analysis enabled them to quantify the angular motions of the lever arm, not merely the motions of the fluorescent probe. They found that the lever arm of myoV-6 IQ took primarily straight steps, with occasional azimuthal tilts, along the actin filament. In contrast, the truncated lever arm of myoV-4 IQ displayed an increased frequency of azimuthal steps, although the magnitudes of these steps were nearly identical to those of myoV-6 IQ.

**Significance:** The authors present a new approach to explicitly determine domain orientation using TIRF and fluorescence polarization. By applying this novel method to estimate the local probe angle on the lever arm and thereby transform the probe angles into lever arm angles, they elucidated the processive walk of single myoV molecules along actin filaments. Although the data in the manuscript will be of interest to scientists studying motors, the technique should also have a broad appeal to biophysicists in other areas.