PHYSIOLOGY 22: 231–233, 2007; 10.1152/physiol.00021.2007

Block of Cav1.2 channels by Gd^{3+} reveals preopening transitions in the selectivity filter. Babich O, Reeves J, Shirokov R. *J Gen Physiol*; doi:10.1085/jgp.200709733.

Ca^{2+}-dependent inactivation of Cav1.2 channels prevents Gd^{3+} block: does Ca^{2+} block the pore of inactivated channels? Babich O, Matveev V, Harris AL, Shirokov R. *J Gen Physiol*; doi:10.1085/jgp.200709734.

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Question: How do Ca^{2+} channels regulate their own activity?

Background: Traditionally, ion channels are thought to have a selectivity filter that discriminates between incoming ions, and an intracellular gate that undergoes conformational changes associated with the open-closed transitions that regulate the flow of ions. However, accumulating evidence suggests that a binary description is incomplete and that the selectivity filter of K^+ channels can undergo conformational changes that contribute to channel gating. The details of Ca^{2+} channel conduction, selectivity, and ion binding are not clear because the crystal structure has not yet been resolved and the molecular organization of Ca^{2+} channels is distinct from K^+ channels.

Observations: Shirokov's group, in two separate reports, describe molecular events that reveal structural rearrangements in the selectivity filter of L-type Ca^{2+} channel ($Ca_V1.2$). Babich et al. found that submicromolar Gd^{3+} produces a tonic block and accelerates the current decay of the L-type channel by binding at the same site, potentially the extracellular entrance of the selectivity filter. During channel activation, a 10-fold decrease in Ca^{2+} affinity appears to occur, which allows Gd^{3+} to gain access to the site and produce the use-dependent block. Thus Gd^{3+} unmasks a voltage-dependent gating transition in the Ca^{2+} pore before activation, which is thought to involve structural changes in the selectivity filter. In the second report, they determined that Gd^{3+} block prevents Ca^{2+}-dependent inactivation, and inactivated channels become resistant to Gd^{3+} block.

Significance: Babich and colleagues reinterpret the Gd^{3+} use-dependent block to be a consequence of an increased off-rate of the permeant ion for the same site as opposed to a higher affinity of the blocker for the open, compared with the closed, state. Moreover, their findings suggest that an extracellular low-affinity binding site flanks the high-affinity ion binding site of the selectivity filter and that gating and permeation merge. Finally, they propose that Ca^{2+} is binding to the same site as Gd^{3+} and therefore suggest that ion flux of Ca^{2+}-inactivated channels is abolished by a subtle turning of a binding site in the pore and not by an intracellular gate. Overall, these data provide exciting new details about how Ca^{2+} channels are regulated and allow the authors to propose a new model of Ca^{2+} channel gating.

A selective activity-dependent requirement for dynamin 1 in synaptic vesicle endocytosis. Ferguson SM, Brasnjo G, Hayashi M, Wolfel M, Collesi C, Giovedi S, Raimondi A, Gong LW, Ariel P, Paradise S, O'toole E, Flavell R, Cremona O, Miesenbock G, Ryan TA, De Camilli P. *Science* 316: 570–574, 2007.

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Question: Is dynamin I essential for the formation of functional synapses?

Background: Dynamins are GTPases involved in endocytosis in eukaryotic cells. Following invagination of the plasma membrane, dynamins participate in the pinching of the bud neck, through GTP hydrolysis, to form a synaptic vesicle. Three different dynamin genes have been identified in mammals. Dynamin I is expressed primarily in neurons; dynamin II is ubiquitous; and dynamin III is expressed in the testis, heart, brain, and lung tissue. Although dynamin I is thought to be critically involved in the fission reaction of synaptic vesicle endocytosis, this theory has not been directly tested.

Observations: Ferguson et al. produced dynamin I knockout mice and found that they contained functional synapses, which is surprising given the role dynamin I was thought to play in vesicle formation. They also demonstrate that dynamin I is crucial for synaptic vesicle endocytosis at high stimulation frequency but that, at lower levels of stimulation, mechanisms independent of dynamin I support synaptic vesicle endocytosis. Ultrastructural analysis of dynamin I knockout neurons revealed synaptic vesicles that were larger than wild-type and with an increased number of clathrin-coated vesicular profiles.

Significance: These novel findings reveal that, contrary to current hypotheses, dynamin I is not essential for synaptic vesicle biogenesis or synapse formation. Thus a major role of dynamin I appears to be to sustain presynaptic endocytosis during high levels of neuronal activity, and other dynamin isoforms appear to contribute to synaptic vesicle endocytosis during periods of little release.

Neuronal competition and selection during memory formation. Han JH, Kushner SA, Yiu AP, Cole CJ, Matynia A, Brown RA, Neve RL, Guzowski JF, Silva AJ, Josselyn SA. *Science* 316: 457–460, 2007.

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Question: Do neurons compete during the formation of an auditory conditioned fear memory?

Background: Auditory conditioned fear occurs when mice experience a footshock associated with a tone, and they then become frightened by the tone alone and freeze when they here it. Learning this association involves the lateral amygdala (LA), which has ~70% of its neurons capable of encoding the memory but only ~25% of the neurons actually displaying this plasticity. This suggests that a competition exists between neurons. The transcription factor CREB has been implicated in neuronal competition in the developing brain, but until now no one has explored its potential role in competing neurons in the adult brain.

Observations: Consistent with the proportion of LA neurons that show plasticity associated with memory, Han et al. found that CREB was phosphorylated in ~25% of neurons in the LA following auditory fear conditioning. Utilizing the activity-regulated cytoskeleton-associated protein (*Arc*) gene, which is expressed 5–15 minutes after a neuron is active, they found neurons that

expressed CREB also expressed Arc, and those with reduced CREB showed less Arc expression.

Significance: These results demonstrate that LA neurons with more CREB have a competitive advantage over neighboring neurons in producing auditory fear conditioning memory formation. The authors speculate that the more CREB a neuron expresses, the more likely it is to be excited by the conditioned fear paradigm. Thus, not only is competition necessary for refining neural circuits during development, it is also important for the formation of memories in the adult brain.

Chemotaxis in the absence of PIP3 gradients. Hoeller O, Kay RR. *Curr Biol* 17: 813–817, 2007.

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Question: Are phosphatidylinositol-(3,4,5)-trisphosphate (PIP3) gradients necessary for chemotaxis?

Background: Type-1 phosphoinositide 3-kinases (PI3K) that generate PIP3 are localized to the front of the cell, whereas the PTEN phosphatase that metabolizes PIP3 is found at the posterior of the cell. For more than a decade, this arrangement has been thought to define the molecular framework of cell signaling that controls chemotaxis and possibly cell motility in general. Although it has been widely accepted that PIP3 gradients direct cell migration, there has been some controversy as to whether cause and effect were established in this model, in part because PIP3 gradients could not be definitively abolished with experimental approaches that are routinely employed.

Observations: In this report, Hoeller and Kay create a multiple gene knockout in the *Dictyostelium* amoebae that lacks all five type 1 PI3Ks and the PTEN phosphatase, all of which could potentially produce PIP3 gradients in the plasma membrane. In the multiple knockout mutants, there was no detectable PIP3 signaling or gradients. Remarkably, the mutant was able to chemotax almost as well as the wild-type. Moreover, the mutants were able to trigger actin polymerization, a characteristic occurrence during chemotaxis. However, the mutants display a defect in movement speed, especially random movements.

Significance: Although it is possible that PIP3 gradients are involved redundantly with another signaling pathway, these findings suggests that an intracellular PIP3 gradient is not essential for either the establishment of cell polarity required for chemotaxis or the regulation of motility during chemotaxis. In short, this study implies that there is a signaling pathway that guides cells independently of PIP3 polarization. Future efforts will undoubtedly be focused on elucidating the unknown guidance pathway and the question of whether PIP3 signaling plays a redundant or only a modulator role.

Muscle metabolism during graded quadriceps exercise in man. Helge JW, Stallknecht B, Richter EA, Galbo H, Kiens B. *J Physiol* (March 22, 2007); doi:10.1113/jphysiol.128348.2006.

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Question: Does the way muscle metabolizes fat change when whole body exercise and quadriceps exercise are compared?

Background: Under most conditions, exercise causes skeletal muscle to utilize substrates from carbohydrates and fat stored in extramuscular and intramuscular sources. During whole body exercises, as the intensity of exercise is increased, plasma glucose and muscle glycogen (carbohydrate) utilization also increase. In contrast, whole body fat oxidation plateaus during moderate exercise intensity (55–65% maximal oxygen uptake). This difference has been attributed to the neuroendocrine system because the neuroendocrine response is increased as exercise intensity is increased, which may reduce blood flow to adipose tissue and/or fatty acid (FA) reesterfication, ultimately decreasing plasma FA uptake and oxidation.

Observations: Helge and colleagues sought to determine how exercise intensity affects muscle substrate utilization without inducing a neuroendocrine response. Utilizing a leg exercise that minimally elicits an autonomic neuroendocrine response, they found that total fat oxidation was not influenced by exercise intensity. However, there was a progressive increase in plasma fatty acid oxidation as contraction intensity increased.

Significance: In contrast to previous findings that utilized whole body measurements of fat oxidation, these results suggest that, with increasing exercise intensity, oxidation of plasma free FA increases. This highlights the importance of extramuscular factors in the regulation of muscle metabolism during whole body exercises.

Molecular basis for PP2A regulatory subunit B56 alpha targeting in cardiomyocytes. Bhasin N, Cunha SR, Mudannayake M, Gigena MS, Rogers TB, Mohler PJ. *Am J Physiol Heart Circ Physiol* (April 6, 2007); doi:10.1152/ajpheart.00059.2007.

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Question: Does ankyrin-B play a role in targeting protein phosphatase 2A (PP2A) to subcellular sites?

Background: Ankyrin-B is a membrane protein with a conserved NH_2-terminal membrane-binding domain, a spectrin binding domain (SBD) and a COOH-terminal regulatory domain. Recently, the ankyrin-B SBD has been implicated in human cardiac disease. Specifically, SBD COOH-terminal variant E1425G is the cause of ankyrin-B-associated cardiac arrhythmia. PP2A has many functional roles and molecular targets in the heart; however, little is known regarding the cellular pathways that localize PP2A isoform activities to subcellular sites.

Observations: In an effort to determine novel proteins that associate with the SBD of ankyrin-B, Bhasin et al. screened a human heart library and identified a targeting/regulatory subunit of PP2A (B56α) as a binding partner. They determined that a unique 13 amino acid domain on B56α is necessary for this association to occur. They also demonstrated that ankyrin-B plays a critical role in targeting B56α to specialized membranes in cardiomyocytes.

Significance: These findings demonstrate a novel role for ankyrin-B in targeting a new class of key signaling proteins, phosphatases, to specialized subcellular domains in cardiomyocytes. Given the critical roles

PP2A and ankyrin-B are known to play in heart function, these findings could have implications for understanding a myriad of cardiac disease phenotypes.

Paraventricular opiods alter intake of high-fat but not high-sucrose diet dependeing on diet preference in a "binge" model of feeding. Naleid AM, Grace MK, Chimukangara M, Billington C, Levine AS. *Am J Physiol Regul Integr Comp Physiol* (April 11, 2007); doi:10.1152/ajpregu.00675.2006.

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Question: Do opioids modulate intake of calories or of a preferred food?

Background: The endogenous opioid system is known to play a role in regulating feeding behavior. Some studies suggest that opioids specifically regulate fat intake, as opposed to carbohydrate and protein. However, other studies have provided evidence that opioids modulate intake of an animal's preferred food, regardless of the nutrient content. The paraventricular nucleus (PVN) produces many hormones and plays a central role in feeding behaviors. Therefore, Naleid et al. hypothesized that opioids injected into the PVN would modulate intake of calories rather than of a preferred food.

Observations: Animals were first divided into sucrose- or fat-preferring groups and then injected either centrally (intra-PVN) or peripherally with opioid agonist and antagonist. They found that a centrally administered opioid agonist stimulated fat intake only in fat-preferring animals. However, although a centrally administered antagonist inhibited fat intake in both sucrose and fat consumers, the total number of calories was significantly decreased only in the fat consumers. They also determined that peripheral injection of an opioid antagonist inhibited sucrose intake.

Significance: This study suggests that there is a complex role for PVN opioids in food intake and that there is a difference in the PVN opioid system of animals that prefer fat versus those that prefer sucrose. In addition, these results suggest that opioid control of sucrose intake is mediated outside of the PVN. Understanding the role of the opioid reward system in feeding behavior is important since it may lead to new insights regarding harmful human feeding behaviors, such as anorexia nervosa, bulimia nervosa, and eating-induced obesity. ■