PERSPECTIVES: SIGNAL TRANSDUCTION

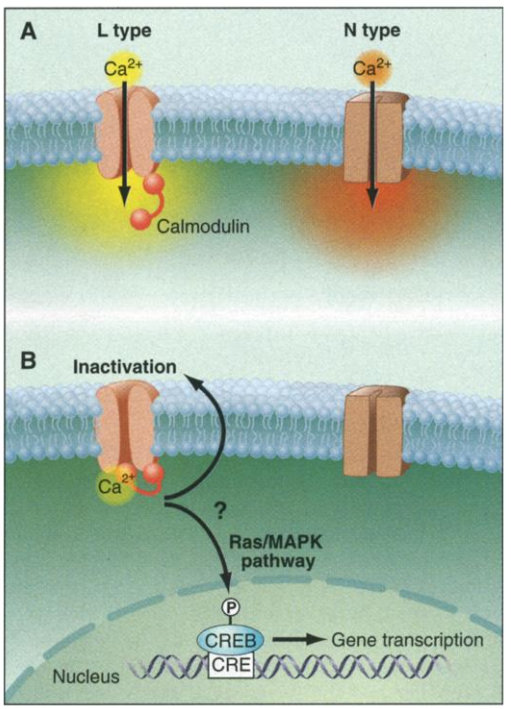
Calcium Channels—Link Locally, Act Globally

Stephen R. Ikeda

Most voltage-gated ion channels produce membrane potential changes in neurons by facilitating the flow of ions across the plasma membrane. The behavior of voltage-gated Ca^{2+} channels (VGCCs), however, is more akin to that of cell surface receptors, which are coupled to intracellular signal transduction pathways. But instead of detecting the presence of extracellular ligands, VGCCs sense the membrane potential and react to depolarization of the neuronal membrane by opening a gate that allows Ca^{2+} to enter the interior of the cell. The resulting rise in the intracellular Ca^{2+} concentration triggers such important events as neurotransmitter release, the opening and closing (gating) of ion channels, kinase activation, and gene transcription. On page 333 of this issue, Dolmetsch *et al.* (*1*) demonstrate that an event local to the L-type Ca^{2+} channel provides the triggering mechanism for a distal event, nuclear gene transcription, with global consequences.

Pharmacological blockade of discrete classes of VGCCs prevents the transduction of membrane depolarization into cellular responses despite the presence of multiple classes of VGCCs in the same cell (*2–5*). Thus, both a rise in intracellular Ca^{2+} and the specific type of channel through which the Ca^{2+} enters the cell are crucial. How does a neuron determine the specific portal through which a small diffusible molecule like Ca^{2+} has entered? The most obvious explanation is the spatial arrangement of the VGCC with its effector molecules. For example, neurotransmitter release is triggered by the opening of N- and P/Q-type VGCCs ($Ca_v2.x$) because these channels are colocalized with, and are most likely tethered to, the neurotransmitter release apparatus found in presynaptic nerve endings (*6*). This intimate association also explains the tight temporal coupling (<1 ms) between Ca^{2+} entry and neurotransmitter release. But spatial colocalization cannot explain signaling between specific VGCCs and distant targets such as the nucleus.

Dolmetsch *et al.* (*1*) provide evidence that a complex between the Ca^{2+} binding protein, calmodulin, and an L-type VGCC triggers a signaling cascade from the plasma membrane to the nucleus. These investigators monitored the depolarization-induced phosphorylation of the cAMP response element binding protein (CREB), a nuclear transcription factor that controls a number of genes involved in neuronal development and plasticity (*7*). In rat cortical neurons, the sustained (but not the transient) phosphorylation of CREB was inhibited by nimodipine, a dihydropyridine (DHP) antagonist of L-type ($Ca_V1.x$) VGCCs. This was surprising given the presence of other pharmacologically distinct VGCCs, such as N and P/Q type, that are the major contributors to increases in intracellular Ca^{2+} after depolarization. Their results corroborate previous findings demonstrating that Ca^{2+} entering specifically through L-type VGCCs (but not bulk increases in intracellular Ca^{2+}) specify Ca^{2+}-dependent CREB phosphorylation (*3, 7, 8*). Next, epitope-tagged L-type VGCCs that were mutated to render them DHP-resistant were transfected into cortical neurons. In the presence of nimodipine, only neurons that expressed the modified VGCCs displayed sustained CREB phosphorylation and CREB-dependent reporter gene transcription in response to membrane depolarization.



Calmodulin acts as a sensor for "local" Ca^{2+} entry. (**A**) L- and N-type voltage-gated Ca^{2+} channels open in response to membrane depolarization, resulting in Ca^{2+} entry. Calmodulin, tethered to the L-type (but not N-type) VGCC, acts as a sensor for localized Ca^{2+} entry. The relatively low affinity of calmodulin for Ca^{2+} spatially restricts Ca^{2+}/calmodulin complex formation to the immediate vicinity of the channel pore. (**B**) Ca^{2+}/calmodulin tethered to the carboxyl terminus of the L-type VGCC triggers a conformational change that leads to both inactivation of the L-type VGCC and activation of the Ras/MAPK cascade. The latter pathway produces prolonged phosphorylation of CREB in the nucleus and consequently gene transcription.

Research on a seemingly unrelated topic—Ca^{2+}-dependent channel inactivation—is yielding clues to the mechanism of this signaling specificity. Ca^{2+} flowing through L-type VGCCs inactivates the channels, thus providing a form of negative feedback (*9*). Deletion of a putative IQ-type calmodulin binding motif abolished Ca^{2+}-dependent channel inactivation (*10*), suggesting that calmodulin bound to the carboxyl terminus of L-type VGCC α subunits is a sensor for Ca^{2+}-dependent channel inactivation. Shortly thereafter, several laboratories demonstrated binding of calmodulin to the IQ-motif and showed that mutations within the IQ-motif disrupted both calmodulin binding and Ca^{2+}-dependent inactivation (*11–13*). Interestingly, expression of calmodulin mutants defective in Ca^{2+} binding (*14*) abrogated Ca^{2+}-dependent inactivation. Thus, this process may be mediated by calmodulin tethered to the channel, rather than by free calmodulin (*15*).

Because both Ca^{2+}-dependent channel inactivation and Ca^{2+}-dependent gene transcription appear to share a similar requirement (Ca^{2+} flowing through L-type VGCCs), Dolmetsch *et al.* (*1*) explored whether the two processes might be connected. They found that mutations within or deletions of the IQ-motif in L-type VGCC α subunits resulted in disruption of channel-to-nucleus signaling. Moreover, expression of the same calmodulin mutants, which could not bind Ca^{2+}, disrupted Ca^{2+}-dependent inactivation and also attenuated CREB-dependent transcription. Thus, identical molecular events—binding of a tethered Ca^{2+}-calmodulin complex to the IQ-motif of an L-type Ca^{2+} channel α subunit—appear to initiate two completely different response pathways (channel inactivation versus gene transcription), in different locations (membrane versus nucleus), and on different time scales (milliseconds versus minutes or hours).

The Dolmetsch *et al.* study raises several questions. First, what is the point of linking

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Ca^{2+}-dependent gene transcription to activation of a specific type of VGCC? Recent work (*8*) suggests that the unique biophysical properties of L-type VGCCs enables them to discriminate between synaptic potentials (excitatory postsynaptic potentials) and action potentials. Clearly, such filtering would be useful for fine-tuning the coupling between Ca^{2+}-mediated gene transcription and different forms of neural stimulation (for example, orthodromic versus antidromic). Second, what steps lie between Ca^{2+} entry and gene transcription? The Dolmetsch work (*1*) implies that activation of the mitogen-activated protein kinase (MAP kinase) cascade is one such intermediate step. However, it is unclear how MAP kinase activation is connected to the rapid translocation of calmodulin to the nucleus after stimuli that open L-type VGCCs (*16*). Finally, does this general mechanism for specifying membrane-to-nucleus signaling apply to other types of VGCCs and Ca^{2+} binding proteins? When P/Q-type VGCCs interact with Ca^{2+}/calmodulin, their biophysical properties change in a manner reminiscent of the Ca^{2+}-dependent inactivation of L-type VGCCs (*17, 18*). The entry of Ca^{2+} through P/Q-type VGCCs may specify the transcription of unique sets of genes. Given that the sequencing of the human genome has identified more than 80 proteins related to calmodulin (*19*), many with functions as yet undetermined, a rich repertoire of potential interactions between this class of proteins and VGCCs remains to be explored.

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PERSPECTIVES: CARBON CYCLE

Potential Impacts of CO_2 Injection on Deep-Sea Biota

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The potential for global warming has spurred the development of various strategies to control the concentrations of greenhouse gases, particularly CO_2, in the atmosphere. Technologies for carbon capture, storage, and sequestration to reduce greenhouse gas concentrations are receiving increasing attention (*1*). Because of its enormous volume, the ocean is an attractive site for possible storage of CO_2. First proposed nearly 25 years ago (*2*), CO_2 disposal in the ocean is now being actively explored (*3*, *4*).

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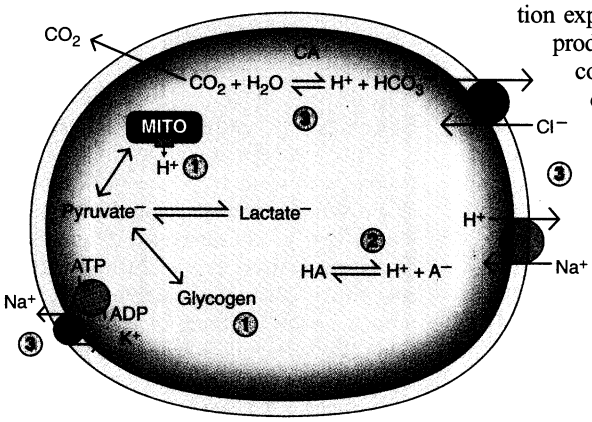
Recent modeling studies indicate that CO_2 must be released at great depths to avoid substantial outgassing (*5*). Direct studies of the biological consequences of CO_2 injection are in their infancy (*4*), but a large literature on the physiology of deep-living animals indicates that they are highly susceptible to the CO_2 and pH excursions likely to accompany deep-sea CO_2 sequestration. Microbial populations may be highly susceptible as well. The impacts of ocean sequestration on deep-sea biota and the biogeochemical cycles dependent on their metabolism are therefore of great concern. Increased CO_2 results in decreases in seawater pH. Primary responses of organisms to the consequent internal acid-base imbalance include metabolic production and consumption of acid-base equivalents, passive chemical buffering of intra- and extracellular fluids, and active ion transport (*6*, *7*) (see the figure). CO_2 and proton transport by extracellular respiratory proteins such as hemoglobin are also important for maintaining acid-base balance in some animal groups.



Regulation of intracellular pH in an animal cell. (1) Metabolic interconversion of acids and bases. (2) Buffering; HA represents a weak acid or base with a dissociation constant in the physiological pH range. (3) Transport of acids and bases across cell membranes; carbonic anhydrase (CA) catalyzes the hydration of CO_2 to yield H_2CO_3, which then dissociates to H^+, HCO_3^-, and CO_3^{2-} (an abbreviated reaction is shown). MITO, mitochondrion.

Failure to control pH within physiological limits due to sequestered CO_2 will have important consequences for the health of aquatic organisms, as has been demonstrated for the effects of acid rain on freshwater fish (*8*). Acid-base imbalances can lead to dissolution of exoskeletal components such as calcareous shells, metabolic suppression (a condition expected to retard growth and reproduction), reduced activity, loss of consciousness due to disruption of oxygen-transport mechanisms, and, if persistent, death.

Over the last 30 years, in situ and laboratory studies of the oxygen consumption rates of deep-sea animals have shown that deep-living animals—both fishes and invertebrates—have low metabolic rates (*9*). The metabolic rates of some deep-living animal species are nearly three orders of magnitude lower than those of their shallow-living relatives after correction for temperature differences (*10*). Metabolism in the deep sea appears to be reduced in part because selection for locomotory capacity is relaxed due to light-limitation on predator-prey interactions. It is reduced further by cold temperatures (*9*) and, in at least some instances, by limited food supply (*11*, *12*).

The reduction in metabolic rates with increasing depth is found to varying degrees in all phyla and all regions studied to date and extends to the deepest depths of the ocean (*9*). Microbial activity is also greatly reduced in the deep sea (*12*). Metabolic pathways in living cells are tightly regulated such that the production and consumption of metabolic end products are in balance.

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