morphologically distinct modes of migration, such as the radial migration of cortical pyramidal cells (Marin et al., 2006).

How cell migration, a complex process involving a multitude of interactions between signaling molecules, can be regulated by transcription factors is not well understood. In this context, the regulation of PAK3 expression by the transcription factors Dlx1/2 is particularly interesting, because studies addressing the regulation of this type of intracellular signaling molecules usually focus on the control of their activity by protein-protein interactions. An additional layer of regulation at the transcriptional level may be important to produce a precise amount of active protein, which could be critical if the protein has different functions at different levels of activity. Cobos et al. (2007) show that a high level of PAK3 promotes the growth of neuronal processes but is detrimental to neuronal migration. However, it is possible that PAK3 also has a positive role in migration of cortical interneurons when expressed at a lower level. PAK proteins are involved in the motility of fibroblasts and other cell types, where they stabilize microtubules and the actin cytoskeleton at the leading edge of migrating cells (Bokoch, 2003). Although the role of PAK proteins in neuronal migration is less well understood, PAK activity has been implicated in the migration of cerebellar neurons, and significantly, overexpression of wild-type PAK in these cells has been shown to inhibit extension of the leading process, suggesting that PAK must be expressed at a correct level to support neuronal migration (Sakakibara and Horwitz, 2006). An attractive hypothesis is therefore that *Dlx* genes are acting throughout cortical interneuron development to establish the different expression levels of PAK3 and other cytoskeleton regulatory proteins that are required for their various functions. By bridging the fields of transcription and signal transduction, the work of Cobos et al. (2007) takes us an important step closer to understanding how neuronal migration is regulated.

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TRP Channel Structural Biology: New Roles for an Old Fold

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DOI 10.1016/j.neuron.2007.06.011

The capsaicin receptor, TRPV1, contributes to thermal and chemical sensitivity of primary afferent neurons of the pain pathway, but many aspects of its regulation remain elusive. In this issue of *Neuron*, Lishko et al. describe a high-resolution structure of a TRPV1 domain, providing insight into the molecular basis of channel modulation while revealing new functions for a widely expressed protein interaction fold.

Members of the transient receptor potential (TRP) superfamily of cation channels have emerged as primary molecular sensors in diverse physiological pathways (Clapham, 2003). This is well illustrated in the mammalian somatosensory system, where TRP channels expressed by sensory neurons of trigeminal and dorsal root ganglia function as the initial detectors of noxious chemical and thermal stimuli (Jordt et al., 2003). One example is TRPV1, which serves as the receptor for capsaicin, the main pungent ingredient in "hot" chili peppers. In addition

to pepper extracts, TRPV1 is activated by a host of endogenous inflammatory agents, such as extracellular protons and bioactive lipids, as well as by pain-producing (noxious) heat (Jordt et al., 2003). Currently, an outstanding challenge is to determine how these disparate chemical and thermal stimuli modify channel gating.

Studies of both native and cloned TRPV1 channels have revealed several mechanisms that modulate their responsiveness to noxious stimuli. For example, a single prolonged application of capsaicin leads to a gradual reduction in current over the course of seconds to minutes, a calcium-dependent process referred to as desensitization. A related regulatory mechanism, known as tachyphylaxis, occurs when multiple separate challenges with an agonist evoke progressively weaker channel currents (Tominaga et al., 1998). Presumably, desensitization and tachyphylaxis both operate to tune down TRPV1 responsiveness so that pain-producing conditions do not permanently activate the channel and overload the neuron with toxic levels of calcium. In fact, long-term tachyphylaxis may also contribute to the paradoxical analgesic properties of topically applied capsaicin by rendering the sensory neurons temporarily indifferent to a spectrum of noxious thermal or chemical stimuli. The calcium sensor calmodulin has been implicated as a factor required for TRPV1 desensitization (Tominaga and Tominaga, 2005), but it is unclear how calmodulin acts on the channel to alter its gating. In this issue of *Neuron*, the Gaudet laboratory (Lishko et al., 2007) has revealed an important and unexpected piece of this puzzle. Using a combination of biochemical, functional, and crystallographic methods, the authors show that calmodulin controls channel tachyphylaxis by displacing ATP from a novel multiligand binding site within the intracellular N terminus. Aside from illuminating specific mechanistic details of TRPV1 regulation, these studies may provide more general insight into the structural underpinnings of TRP channel gating and novel biochemical roles for ankyrin repeat domains.

TRP channels possess six transmembrane domains, with a membrane-reentrant pore loop between helices 5 and 6 plus large intracellular N and C termini. Although this basic topology is common to many ion channel families, such as voltage-gated K+ channels, TRP and K+ channels show relatively little sequence homology, particularly within the intracellular domains. Most notably, many TRP channels possess long N-terminal stretches of modular protein-protein interaction motifs known as ankyrin repeats, a feature that appears to be absent from the voltage-gated channel family. Despite the fact that ankyrin repeats serve as structural signatures for many TRP channels, their role in channel modulation, trafficking, or interaction with auxiliary factors remains elusive. In the case of voltage-gated channels, X-ray crystallographic studies have provided high-resolution maps of intact channels or bits thereof, illuminating the structural underpinnings of channel permeation, gating, and modulation. In contrast, TRP channels remain relatively enigmatic in this regard, although high-resolution glimpses of channel segments are beginning to appear (Jin et al., 2006; McCleverty et al., 2006). The Gaudet lab has now crystallized the ankyrin repeats of TRPV1, yielding a surprising result. Unlike other known ankyrin repeat domains, a molecule of ATP is complexed to a noncanonical nucleotide binding pocket within the TRPV1 ankyrin fold (Lishko et al., 2007), providing unexpected insight into the physiological regulatory mechanism underlying channel tachyphylaxis.

Although proteins often bind ATP through canonical "Walker box" motifs, it is clear from the new structure that no such motif exists in the TRPV1 N terminus. Rather, ATP is held within a concave surface of the ankyrin repeat domain, where it is stabilized via interactions with several charged and hydrophobic residues. To prove that this pocket represents a bona fide ATP interaction site, the authors individually mutated residues within the pocket and assessed ATP interaction using an in vitro binding assay. While the ankyrin repeat domain from the wild-type channel robustly bound ATP, mutation of key residues within the pocket completely eliminated nucleotide interaction. Curiously, the authors found that binding of ATP with the wild-type channel was inhibited by divalent cations, contrasting with more classical ATP binding pockets in polymerases or protein kinases, for example, where divalents often stabilize protein-nucleotide interactions.

What role does this newly identified ATP interaction site play in TRPV1 regulation? Previous electrophysiological studies showed that high intracellular ATP concentrations antagonize channel tachyphylaxis (Kwak et al., 2000; Liu et al., 2005). The authors therefore asked whether their interaction site was required for the functional effects of ATP on TRPV1, and expected that their mutations would produce a channel that undergoes tachyphylaxis regardless of cellular ATP concentration. Instead, these TRPV1 mutants showed a striking reduction in tachyphylaxis that persisted even in the absence of intracellular ATP. To explain these seemingly contradictory results, the authors hypothesized that the ATP binding module must also interact with another cellular factor that promotes tachyphylaxis. If so, then mutation of this site would abolish interaction with the inhibitory factor as well as with ATP, thereby hindering the ability of TRPV1 to undergo tachyphylaxis. Indeed, the authors found that calmodulin is required for efficient tachyphylaxis, and that it influences channel gating by competing with ATP for binding to the N terminus. Exactly how this molecular competition is specified at the atomic level remains unknown and further crystallographic studies will be required to more completely delineate the TRPV1-calmodulin interaction surface, which may involve other regions of the channel besides the ankyrin repeat domains.

Thus, the data from Lishko et al. suggest that ATP and calmodulin compete for a novel binding site in the TRPV1 N terminus, and that the effectiveness of these interactions determines the extent of channel tachyphylaxis. The authors propose that in the wild-type

channel, ATP is bound to the ankyrin repeats, thereby occluding calmodulin and preventing inappropriate desensitization. Upon TRPV1 activation, calcium permeates the channel, binding to and activating calmodulin. Activated calmodulin then displaces ATP and downregulates channel function. One question posed by this model is whether calmodulin can effectively antagonize ATP binding when the cellular concentrations of nucleotide exceed that of calmodulin by at least three orders of magnitude. Perhaps calmodulin binds to the channel with much higher affinity compared with ATP, a possibility that can be tested through direct biochemical measurements.

The authors also ask whether the ATP/calmodulin interaction is affected by the membrane phospholipid PIP_2. Why is this relevant to TRPV1 physiology, and to TRP channel regulation in general? When tissue is damaged, diffusible factors such as extracellular protons, bioactive lipids, peptides, and neurotrophins are released at the injury site. These factors either activate TRPV1 directly, or, alternatively, interact with their own receptors, many of which couple to phospholipase C (PLC) signaling pathways, with consequent hydrolysis of plasma membrane PIP_2 (Jordt et al., 2003). Activation of PLC leads to potentiation of TRPV1, rendering the channel more sensitive to both chemical and thermal stimuli, a phenomenon that likely contributes to inflammatory hyperalgesia.

Mechanisms underlying PLC-evoked TRPV1 sensitization remain controversial. Robust channel activation is observed with application of recombinant PLC or a PIP_2-sequestering antibody to excised patches from TRPV1-expressing HEK293 cells or sensory neurons, suggesting that TRPV1 modulation is positively correlated with a reduction in cellular PIP_2 levels, and that channel potentiation results from PIP_2 depletion (Chuang et al., 2001). Consistent with this model, mutations within a putative PIP_2 binding site in the TRPV1 C terminus endow the channel with abnormally high basal activity and insensitivity to PLC-mediated potentiation (Prescott and Julius, 2003). On the other hand, some studies, including the one here, have found that increasing PIP_2 levels via direct application of PIP_2 to the intracellular face of the channel causes channel activation (Stein et al., 2006). It has also been reported that TRPV1 activation and desensitization leads to PIP_2 hydrolysis, and that PIP_2 replenishment via metabolism of ATP is required for efficient recovery from desensitization (Liu et al., 2005; Lishko et al., 2007). Indeed, many of these issues are still being debated for the canonical *Drosophila* TRP channel that mediates depolarization of the photoreceptor cell following light-induced activation of PLC-coupled rhodopsin in the fly eye.

The authors suggest that PIP_2 competes for calmodulin binding to TRPV1 (Hardie et al., 2001), but whether this reflects interactions of lipid and calmodulin with sites at the N terminus, C terminus, or both remains to be determined. In any case, how might PIP_2 antagonism of calmodulin-dependent TRPV1 tachyphylaxis be reconciled with the model of PIP_2-mediated channel inhibition? One could propose dual actions of PIP_2 during the lifetime of channel opening and closing. Tonic PIP_2 inhibition could be required to keep the channel closed in the basal state, a property exploited by some PLC-coupled receptors to effect TRPV1 potentiation in the setting of inflammation. However, PIP_2 might also play a permissive function that returns the channel to the resting state following desensitization or tachyphylaxis (Liu et al., 2005). Appropriate channel behavior could require both activities of PIP_2, a scenario that may also apply to TRP channel regulation in *Drosophila* photoreceptors (Hardie et al., 2001). However, it is important to consider the possibility that the inhibitory effect of ATP on TRPV1 tachyphylaxis occurs solely via antagonism of calmodulin binding, irrespective of any role of ATP in PIP_2 resynthesis. Future experiments will help to determine whether positive and negative PIP_2 regulatory mechanisms involve distinct interactions within the N and C termini or a single C-terminal interaction site.

The model presented in this study leads to some new questions concerning mechanisms of channel activation. For example, how does binding of ATP or calmodulin to the TRPV1 N terminus couple to channel gating? Pore opening must involve conformational changes in the transmembrane and possibly C-terminal regions of TRPV1. It will be interesting to explore the mechanism by which the N terminus can "communicate" with other regions of the channel. Further structural analysis, conducted on the full-length TRPV1 channel or possibly a C-terminal fragment, will also clarify questions about the relative orientation of N- and C-terminal domains within the cytoplasm and the location of the N terminus relative to the pore and putative intracellular gate. Interestingly, a related TRP channel, TRPA1, has been shown to respond to environmental irritants, such as mustard oil, via covalent modification of cysteines within its ankyrin-rich cytoplasmic N terminus (Hinman et al., 2006; Macpherson et al., 2007). Such studies, along with the one by Lishko et al., raise the possibility that for many TRP channels the intracellular N terminus may play a more critical role in gating than was previously appreciated.

Overall, the present study provides strong evidence that the ankyrin repeat domains in TRPV1 are involved in gating, a result that may have implications throughout the TRP channel superfamily. The structure also provides a framework for interpreting structure-function studies that could provide additional insights into the regulatory functions subserved by this region of the channel. In a larger sense, this study is a compelling demonstration of the power of structural biology to help neuroscientists formulate and test hypotheses about ion channel physiology. Indeed, a role for ankyrin repeats as ATP binding motifs would have been missed by bioinformatics-based approaches due to the lack of homology with canonical ATP binding sequences. In the future, we can look forward to additional TRP family structures that will allow us to better understand the fundamental physiological properties of these important cellular sensors.

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I_A in Play

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DOI 10.1016/j.neuron.2007.06.008

Everyone agrees about how long-term potentiation (LTP) is induced—NMDA receptor activation—but much remains to be learned about how the increase in the strength of a synaptic connection between two neurons is expressed. In this issue of *Neuron*, Kim et al. report a new form of NMDAR-dependent plasticity that may contribute to LTP: internalization of postsynaptic Kv4.2 potassium channels that mediate transient I_A-type outward current in dendrites.

An increase in the strength of the excitatory connections between principal neurons is the predominant means by which an engram is stored during the learning process. It is widely accepted that synaptic strength is increased when strong, temporally correlated presynaptic activity produces both glutamate release and a sufficiently large depolarization of the postsynaptic cell's membrane potential to relieve the block of the NMDA-type glutamate receptor (NMDAR) by Mg^{2+} ions. Ca^{2+} ions thereby enter the postsynaptic dendritic spine, and the resulting elevation of the free intracellular Ca^{2+} concentration triggers a cascade of enzymatic pathways that ultimately results in the strengthening of the synapse. The nature of the change (or changes) in the connection between the two cells that is responsible for the increase in synaptic strength remains highly contentious. There is now overwhelming evidence that an increase in the number of AMPA-type glutamate receptors in the postsynaptic plasma membrane is induced in long-term potentiation (LTP) of excitatory synaptic transmission, a heavily studied form of NMDAR-dependent synaptic plasticity (Malenka and Bear, 2004). The increase in receptor number results in a larger depolarization of the postsynaptic cell in response to a given amount of presynaptic glutamate release. There is also evidence that factors "downstream" of direct synaptic modulation also contribute to the overall increase in the strength of a synaptic connection during learning, such as changes in the intrinsic ionic currents of the postsynaptic cell. In this issue of *Neuron*, Kim et al. (2007) report an exciting new form of NMDAR-dependent plasticity and suggest that this process contributes to synaptic potentiation: internalization of transient A-type outward current mediated by Kv4.2 potassium channels.

The dendrites of pyramidal cells are endowed with a variety of voltage-dependent channels that sculpt the voltage changes induced in the postsynaptic cell in response to synaptic activation. One particularly important dendritic current is the A-type potassium current (I_A). This outward current is activated by even small depolarizations from the cell's normal resting membrane potential. It has the biophysical characteristic of strong inactivation. That is, it activates rapidly in response to depolarization, but the channels stop passing current within