This Week in The Journal

How Signaling Proteins Reach Their Ciliary Destination

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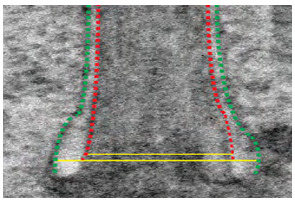
(see pages 7514–7531)

Primary cilia fulfill critical cellular functions—most famously moving fluids past the cell membrane—but they also play key sensory and signal transduction roles, requiring a specific complement of proteins to be localized to cilia. Those proteins get to their destination thanks to a bult-in cilia localization sequence (CLS), but little is known about how CLSs help proteins target specific types of cilia. In this week's *Journal*, Chadha et al. combine clever transgenic cell line techniques with powerful microscopy to show how proteins reach their various targets.

Cilia-targeted proteins include the G-protein-coupled receptors rhodopsin and the rhodopsin-like somatostatin receptor 3 (SSTR3). The authors had previously identified a CLS in the C-terminus of rhodopsin, and another in an intracellular loop of SSTR3. Here, they examined protein localization in three distinct cell types: the simple IMCD3 cell line; the more complex hTERT-RPE1, in which cilia emerge from a specialized "pocket"; and perhaps the most complex of ciliated cells, rod photoreceptors. Both proteins localized to the cilia of IMCD3 and RPE1 cells, with SSTR3 showing slightly more specificity than rhodopsin for cilia localization. To determine the localization of the two proteins in mouse photoreceptors, the researchers performed subretinal electroporation in wild-type mice and found, surprisingly, that both proteins localized to photoreceptor cilia equally well. The findings indicated that whereas rhodopsin was more highly specialized to localize to cilia in photoreceptors, SSTR3 seemed capable of finding cilia in a range of cell types.

The authors next looked for a new CLS in SSTR3 by using a series of proteins with progressive deletions of the 428 aa C terminus, which severely reduced but did not eliminate ciliary localization of SSTR3. Conversely, addition of the SSTR3 C terminus to rhodopsin increased its ciliary localization in IMCD3 cells. Addition of candidate CLSs from SSTR3 to the single-pass transmembrane protein CD8 resulted in robust ciliary localization, demonstrating its sufficiency to confer targeting. Structured illumination microscopy allowed for discrimination between the ciliary and periciliary membranes, revealing more granular subcellular localization and showing evidence for a CLS specific for localization to the periciliary membrane. The study reveals a novel CLS in SSTR3 and advances the understanding of how proteins reach their ciliary end points.



Transmission electron microscopy of a central longitudinal section of the base of a cilium from an hTERT-REP1 cell distinguishes between the cilium (red) and periciliary, or pocket, (green) membranes. Yellow lines demark the diameter of the cilium (210 nm) and pocket (315 nm).

Peripheral Calcium Channels Mediate Heat Pain Hypersensitivity

Daniel M. DuBreuil, Eduardo Javier Lopez Soto, Simon Daste, Remy Meir, Daniel Li, et al.

(see pages 7546–7560)

Voltage-gated calcium channels control the presynaptic release of neurotransmitters; accordingly, block of $Ca_V2.2$ or N-type channels in the spinal cord can dampen some types of chronic neuropathic pain, the type caused by nerve damage. Unfortunately, the ubiquity and necessity of $Ca_V2.2$ channels in the nervous system make them a poor target for analgesics. Now, DuBreuil, Lopez Soto, et al. show that a specific splice variant of the channel expressed in peripheral nerves mediates a particular type of pain, potentially revealing a path to development of new painkillers.

$Ca_V2.2$ channels play a clear role in pain transduction, which the researchers confirmed by showing that transgenic mice lacking $Ca_V2.2$ channels had longer withdrawal latencies from heat and higher mechanical withdrawal thresholds than wild-type mice. Similarly, optogenetic activation of sensory neurons containing the transient receptor potential vanilloid 1 (TRPV1) channel elicited pain-withdrawal behaviors in mice that were attenuated in mice lacking $Ca_V2.2$ channels. These behavioral readouts were paralleled by electrophysiological findings that optogenetically evoked postsynaptic currents were smaller in neurons from mice lacking $Ca_V2.2$ compared with wild-type mice.

Next, the team wanted to determine whether $Ca_V2.2$ channels were responsible for the hallmark pain hypersensitivity elicited by capsaicin, the pungent chemical found in chili peppers that activates TRPV1. Intradermal injection of capsaicin induced robust heat pain withdrawal behaviors in wild-type mice that were absent in mice lacking $Ca_V2.2$. In contrast, mechanical withdrawal behaviors were indistinguishable between wild-type and $Ca_V2.2$ knock-out mice, indicating that $Ca_V2.2$ channels are required for heat but not mechanical capsaicin-evoked hypersensitivity. Transgenic mice expressing only the e37b splice variant of $Ca_V2.2$ channels also displayed an attenuated pain hypersensitivity response to capsaicin, suggesting that the e37a variant is required for maximal expression of hypersensitivity.

The researchers next wanted to determine *where* $Ca_V2.2$ channels were required for hypersensitivity, so they made intraplantar injections of ω-CgTx MVIIA, a marine snail-derived conotoxin that blocks $Ca_V2.2$. Remarkably, although capsaicin induced acute pain behaviors in all animals, ω-CgTx MVIIA-treated mice displayed significantly reduced prolonged heat pain hypersensitivity. Further experiments showed that $Ca_V2.2$ channels were also required for the release of ATP and interleukin 1, proinflammatory mediators of pain hypersensitivity.

The results strongly suggest that peripheral—not spinal—$Ca_V2.2$ channels mediate heat pain hypersensitivity. The accessibility of such channels at skin nerves hints at $Ca_V2.2$ as an attractive, safe target for new analgesics against certain types of chronic pain.

This Week in The Journal was written by *Stephani Sutherland, Ph.D.*

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