Purkinje cells and its receptor Robo2 by granule cells (Marillat et al., 2002), Guan et al. suggest that the repulsive action of Slit-2 prevents the premature migration of EGL cells to the IGL. Therefore, Slit-2 may be potentially involved in controlling the transition of granule cell migration from tangential migration to radial directions. In this case, Slit-2 expression in the Purkinje cells or Robo2 expression on the granule cells would need to be downregulated at the time that the granule cells begin migrating radially. Alternatively, Slit-Robo signaling could be silenced by crosstalk with other guidance signaling pathways (Grunwald and Klein, 2002). Regardless of these outstanding questions, the exciting findings on long-range Ca^{2+} signaling reversing neuronal polarity and migration have opened up a door for more studies that will provide a better understanding of the developmental events that pack billions of neurons into highly organized structures for complex functions and how their failure contributes to human migration disorders.

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The Sodium "Leak" Has Finally Been Plugged

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Most electrophysiologists generally do not speak highly of leak currents. In reality, these conductances represent a crucial functional mechanism by which neurons control resting membrane potentials. A new study in *Cell* by Lu et al. has surprisingly confirmed the identity of the long-sought voltage-insensitive sodium leak conductance to be encoded by the third branch of the voltage-gated sodium and calcium channel family.

Nerve cell resting membrane potentials are set by a complex interplay of ionic pumps, transporters, and channels, most of which have been well characterized at the molecular level. Missing from this molecular description has been the identification of the voltage-insensitive background sodium "leak" conductance that helps to maintain resting potentials depolarized to the ~−90 mV potassium equilibrium potential. While the background sodium conductance has variously been speculated to result from voltage-gated sodium channels, "leaky" potassium channels, transporters, and TRP channels, until a recent report in *Cell* (Lu et al., 2007) there has been no definitive evidence one way or the other as to the molecular nature of this conductance so crucial to setting membrane resting potential.

To set the story up, between the late 1980s and into the 1990s, molecular cloning and exogenous expression studies revealed that a family of 20 four-domain type α subunits encode the known voltage-gated sodium ($Na_v1.1$–1.9 and Nax) and calcium ($Ca_v1.1$–1.4, $Ca_v2.1$–2.3, $Ca_v3.1$–3.3) channels (Figure 1). Much subsequent work went into defining the structure-function relationships concerning sodium and calcium channel permeation, gating, modulation, and trafficking. However, a 1999 report of the existence of a third branch on the four-domain voltage-gated ion channel genetic tree left the somewhat

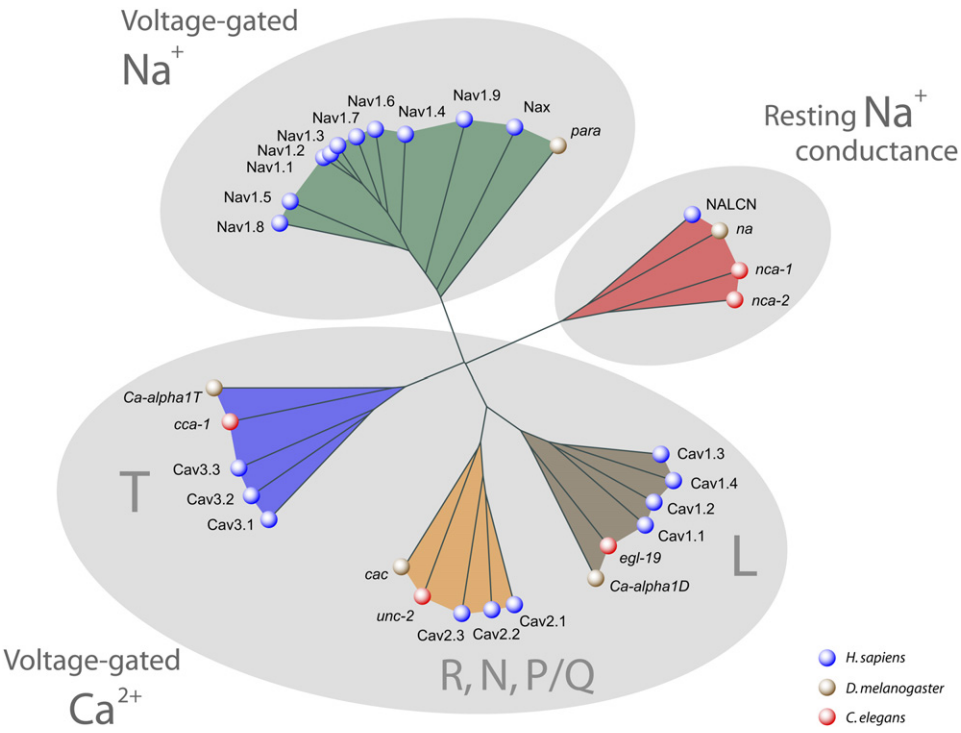

Figure 1. Identity Tree of Pore-Forming α Subunits for the Four-Domain Ion Channel Family, Comparing Human, *D. melanogaster*, and *C. elegans*

Ten α subunit genes encode the mammalian family of voltage-gated sodium channels ($Na_v1.1$–1.9, Nax), ten α_1 subunit genes encode the mammalian voltage-gated calcium channels ($Ca_v1.1$–1.4, $Ca_v2.1$–2.3, $Ca_v3.1$–3.3) while the third major branch encodes the newly identified resting sodium conductance that helps to set nerve-cell resting membrane potentials (NALCN). Figure provided by Dr. John Tyson.

uncomfortable feeling that something important was missing from the big picture (Lee et al., 1999). The third branch encoded a related but structurally distinct four-domain channel (now called NALCN) that is widely expressed in mammalian neurons, but until now there had not been a report of its physiological or pharmacological properties. In a convincing study published in the April 20, 2007, issue of *Cell*, Lu et al. have finally been able to elucidate the permeation characteristics and some of the biophysical and pharmacological properties of this channel. Utilizing heterologous expression in HEK293 cells, the authors find the NALCN channel to encode a constitutively activated, nonselective cation channel that forms the long-sought neuronal background sodium "leak" conductance. Similar to that for the native conductance, the NALCN current is voltage insensitive and both TTX and Cs^+ resistant. That the NALCN channel is not voltage gated is perhaps not surprising since the S4 transmembrane segment in each of the four domains contains a reduced number of positively charged residues compared to the strongly voltage-activated calcium and sodium channels. In the same way, the distinct permeation properties of NALCN are consistent with the unique residues in its selectivity filter (EEKE) that are a "hybrid" between that for the sodium (DEKA) and calcium (EEEE or EEDD) channels. Indeed, that NALCN actually forms the channel itself is supported by the fact that when Lu and coworkers mutated the wild-type NALCN selectivity filter from EEKE to EEKA the selectivity ratio for calcium decreased from $P_{Ca} = 0.5$ to $P_{Ca} = 0.1$.

Homozygous NALCN gene knockout mice present a severely disrupted respiratory rhythm associated with increased periods of apnea and leading to neonatal death of all animals within 24 hr of birth. The disrupted respiratory patterns in the mutant animals are likely due to a defect in neuronal function, as C4 nerve root electrical recordings showed a loss of the normal rhythmic bursting pattern associated with control of diaphragm contraction. Further analysis of hippocampal neurons from NALCN mutant mice suggests that this channel is the major contributor to resting sodium conductance in central neurons. Mutant neurons exhibit a resting membrane potential about 10 mV more hyperpolarized than wild-type (~−71 mV versus ~−61 mV), an effect that clearly contributes basally to hippocampal excitability.

The study of Lu et al., 2007, is the first piece in a puzzle, and plenty of questions remain concerning understanding the contributions of NALCN toward overall neuronal physiology. Some of these may be addressed by construction of a conditional knockout to determine the physiological roles of NALCN in adult populations of neurons. Additional interesting clues

already come from studies performed in *D. melanogaster* and *C. elegans.* The *D. melanogaster* NALCN ortholog called *na* (for narrow abdomen) and two orthologous genes in *C. elegans*, *nca-1* and *nca-2*, are also known to be expressed in neurons (Nash et al., 2002; Humphrey et al., 2007). In *Drosophila*, *na* protein is concentrated in synaptic regions but not in areas of the brain occupied primarily by cell bodies or axonal tracts. Some hypomorphic mutations of *na* in *Drosophila* induce a strong resistance to halothane, while contrastingly the depletion of *nca-1* and *nca-2* in *C. elegans* results in hypersensitivity to halothane (Nash et al., 2002; Mir et al., 1997). It has been reported that halothane significantly reduces the amplitude of nerve-evoked excitatory junctional currents but not miniature excitatory junctional currents in wild-type *D. melanogaster* neuromuscular junction. Halothane has no effect in the mutant har38 and har85 nerve-evoked excitatory neuromuscular junction currents (Nishikawa and Kidokoro, 1999). These results suggest that the NALCN channel could have a presynaptic function at the glutamate-mediated synapses of the neuromuscular junction in *D. melanogaster*. It should be of interest to examine the possibility of a presynaptic localization of NALCN in mammalian central neurons.

Why other groups over the past 8 years were unsuccessful in functionally expressing the cloned NACLN channel remains a mystery. Another and perhaps related issue is whether the NALCN α subunit protein functions alone or as part of a multisubunit complex. Considering the situation for the other four-domain voltage-gated channels (Catterall et al., 2006), a multisubunit complex is likely, and further molecular and biochemical experiments are needed. This is also hinted at in both *D. melanogaster* and *C. elegans*, where Humphrey et al. (2007) have recently reported the existence of a large cytosolic gene product (called *unc-79*) that modulates the expression of ortholog NALCN protein levels via a posttranscriptional mechanism. The depletion of *unc-79* protein in these organisms results in similar locomotion and anesthetic sensitivity phenotypes to those observed for the NALCN ortholog mutants. Interestingly, homozygous gene knockout of the mouse ortholog of *unc-79* also results in a phenotype similar to that reported by Lu and coworkers for NALCN, namely initial activity just after birth but followed shortly thereafter by neonatal lethality (Nakayama et al., 2006).

In the human genome, NALCN is localized on chromosome 13q32.3, a region known to contain a susceptibility locus for bipolar disorders (Hayden and Nurnberger, 2006). Interestingly, in *Drosophila*, the *na* hypomorphic mutation affects the neuronal output of the circadian pacemaker, presumably by an abnormal release of the key neuropeptide PDF (Lear et al., 2005). The involvement of NALCN in the circadian rhythm of *Drosophila* may be of importance since bipolar disorders are often associated with altered diurnal behavior (McClung, 2007). Further investigations are needed to determine any possible contributions of NALCN in bipolar or related psychiatric disorders.

In summary, Lu and coworkers have at last revealed some of the biophysical, pharmacological, and neurophysiological properties of the last member of the four-domain type of ion channel. Describing NALCN as the sodium "leak" channel implies this conductance to be passive, understated, and perhaps even somewhat annoying from the electrophysiologist's experimental perspective. In fact, as NALCN critically contributes to setting neuronal resting potential, its function and regulation are likely to be crucially relevant to the multitude of downstream physiological processes dependent upon such a fundamental activity. The description "sodium resting conductance" is perhaps a more apt name for this fundamental ionic channel.

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