Biochemistry to the Rescue: A ClC-2 Auxiliary Subunit Provides a Tangible Link to Leukodystrophy

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DOI 10.1016/j.neuron.2012.02.012

ClC-2 is a broadly distributed chloride channel with an enigmatic neurophysiological function. In this issue of *Neuron*, Jeworutzki et al. (2012) use a biochemical approach to identify GlialCAM, a protein with a defined link to leukodystrophy, as a ClC-2 auxiliary subunit.

Auxiliary subunits of ion channels are central players in the exquisite electrical tuning of the central nervous system. While they do not directly form ion-channel pores, auxiliary subunits can substantially alter channel properties through interaction with the pore-forming subunits. The effects of these interactions include modulation of sensitivity to ions and signaling molecules, alteration of voltage dependence and activation/inactivation kinetics, and changes in localization and trafficking. The combination of these effects amplifies the functional diversity of ion channels. Discovery of auxiliary subunits has occurred through diverse avenues, from early biochemical approaches tied to modern mass spectrometry. Biochemical approaches tied to modern mass spectrometry.

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Neuron 73, March 8, 2012 ©2012 Elsevier Inc. 855
Pusch, and colleagues use a biochemical approach to identify ClC-2 as the crucial GlialCAM binding partner, thus reinigorating the link between cystic leukencephalopathies and ClC-2 (Jeworutzki et al., 2012). Their ensuing discovery that GlialCAM targets ClC-2 to cell contacts together with the phenotype of the ClC-2 knockout mouse strongly supports the hypothesis that altered ion flux across oligodendrocyte membranes leads to myelin vacuolization in MLC.

The expression of GlialCAM and ClC-2 in oligodendrocytes is consistent with the major pathology of MLC, but how could loss of ML1, which is not expressed in oligodendrocytes, cause a similar phenotype? Genetic defects in ML1, GlialCAM, and ClC-2 induce similar glial and myelin pathologies in both humans and mice, suggesting that all three proteins contribute to a common functional process. GlialCAM traffics both ClC-2 and ML1 to cell-cell junctions and has a robust effect on ClC-2 electrophysiological function; however, no biochemical or functional interaction between ClC-2 and ML1 could be detected, and ML1 expression and localization are not affected in the ClC-2 knockout mouse. Nevertheless, it remains possible that ML1 and ClC-2 could interact indirectly. Indeed, an indirect interaction through GlialCAM could juxtapose ML1 and ClC-2 across astrocyte-oligodendrocyte cell contacts (Figure 1), thus bringing ML1 to the site of major pathology in the disease. But by what mechanism does the disease occur? It is known that ion movement through the glial syncytium is in delicate balance. Upsetting this balance by disruption of either gap junctions (which facilitate intraglial ion movement) or Kir4.1 potassium channels (which facilitate glial-extracellular ion movement) leads to myelin vacuolation. Thus, it is likely that ClC-2, in parallel to Kir4.1, contributes to ion homeostasis in the narrow extracellular spaces. While the precise mechanism of myelin vacuolation has not been defined, it probably arises from osmotic imbalances associated with the defect in ion homeostasis (Brignone et al., 2011). But what is the function of ML1? Is it an ion channel as well? This remains a mystery and will require further study of ML1 and investigations of how loss of ML1 influences ion permeability across membranes of individual astrocytes and the glial syncytium.

In addition to changing ClC-2 localization, GlialCAM has an amazing effect on ClC-2 currents. In heterologous expression systems, coexpression of GlialCAM and ClC-2 results in large currents that retain ClC-2’s characteristic anionic selectivity, but lack its signature rectification and slow activation by hyperpolarization. The increase in current is due solely to an effect on gating, as surface expression is unchanged (the cells used for recording lack cell-cell contacts), and no increase in current is observed in cells expressing only GlialCAM or GlialCAM plus ClC-5. While the effect of GlialCAM on ClC-2 currents in astrocytes is milder than in the heterologous expression systems (either because of lower relative GlialCAM expression or some other cellular difference), the observed increase in current and decrease in rectification could be physiologically important for bidirectional chloride transport. Regardless of whether the change in electrophysiological properties is important for glial physiology and myelin maintenance, GlialCAM is a fascinating new tool for investigating the biophysics of ClC-2 gating.

GlialCAM is the third CLC auxiliary subunit to be discovered. The other two, Barttin (a ClC-K partner) and Ostm1 (a ClC-7 partner), were identified through their genetic links to disease. Though the genetics approach failed to identify ClC-2 binding partners, the Estevez lab’s success using a biochemical approach here provides hope that additional CLC auxiliary subunits may soon be discovered. Such findings hold promise for clarifying our understanding of the diverse physiology displayed by CLC family members. For example, GlialCAM is expressed only in the brain, but ClC-2 is expressed ubiquitously. Though ClC-2 is functional in the absence of GlialCAM, evidence for the role of ClC-2 in cell junctions outside the CNS (Nighot et al., 2009) hints that new ClC-2 auxiliary proteins remain to be discovered. More intriguing and controversial is the possibility that ClC-3 auxiliary subunits might close the gap between seemingly irreconcilable reports on ClC-3 physiology. ClC-3 is in the branch of the CLC family that localizes to intracellular membranes and consists of chloride-proton antiporters (not channels). In accord with this classification, ClC-3 has been found to play physiological roles in endosomes and synaptic vesicles (Jentsch, 2008). However, ClC-3 has also been variously reported as a plasma-membrane channel that is regulated by cell volume (Xiong et al., 2010; Yang et al., 2011), CamKII (Cuddapah and Sontheimer, 2010; Wang et al., 2006), and acid (Matsuda et al., 2010), in a wide variety of cell types. While it has seemed doubtful that these findings could all be reconciled by auxiliary subunits (Ciapham, 2001), the strong transformation of ClC-2’s localization and electrophysiological properties by GlialCAM perhaps render this possibility
How Do Neurons Sense a Spike Burst?

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DOI 10.1016/j.neuron.2012.02.013

In this issue of Neuron, Xu et al. (2012) show that knock down of Syt1, a major Ca2+ sensor, impairs synaptic transmission similarly in different brain regions but with unexpected, region-specific behavioral outcomes.

Several decades ago, I used to listen to rock and roll by tuning in to Radio Free Europe with a small headphone, basically a magnetic coil and a metal diaphragm, so that the neighbors could not suspect my illegal activities. That of course was not the same thing as being in a concert hall, enjoying the entire frequency spectrum and perceiving the pitch, melody, harmony, and timbre content of the music but despite the high-pass filtering properties of the low quality earphone the rhythm and other remnant features of the broadcasted music made the experience still enjoyable. As engineers know, high-pass frequency filtering of signals makes communication poorer but not hopeless. Now suppose that we introduce high-pass filters in the communication lines between neurons in the brain. This is exactly what Xu et al., (2012) have accomplished, using molecular biological tools. They find that after such manipulation neuronal transmission becomes sluggish but is not completely abolished. For some structures and tasks, such as the hippocampus-dependent contextual fear learning task, high-pass filtering is tolerated, whereas for a prefrontal cortex-dependent remote memory recall, sluggishness of spike communication leads to a serious behavioral impairment.

Let’s examine first how communication between neurons was achieved. Neurons communicate electrochemically. The upstream neuron generates a spike, which is broadcasted to all or most of its presynaptic terminals. Here, electricity is converted to chemically mediated synaptic transmission. This conversion process can be perturbed in multiple ways. For example, tetanus toxin (TetTox) can block transmitter release and thus completely eliminate synaptic communication. Other interventions can produce a more subtle interference. Synaptotagmin-1 (Syt1), together with other vesicle proteins, is essential for the docking and/or fusion of synaptic vesicles with the presynaptic plasma membrane following depolarization and Ca2+ influx in presynaptic bouton. Eliminating or interfering with Syt1 also impairs synaptic transmission to single, isolated spikes yet when high enough amount of Ca2+ enters the terminal in response to high-frequency spike activity chemical transmission is resumed, although it remains sluggish due to the asynchronous release of the transmitter (Maximov and Südhof, 2005). Put simply, interfering with Syt1 amounts to the introduction of a high-pass frequency filter: no or poor...