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COMMENTARY

Calnexin revealed as an ether-a-go-go chaperone by getting mutant worms up and going

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The role of ion channels in cell excitability was first revealed in a series of voltage clamp experiments by Hodgkin and Huxley in the 1950s. However, it was not until the 1970s that patch-clamp recording ushered in a revolution that allowed physiologists to witness how ion channels flicker open and closed at angstrom scale and with microsecond resolution. The unexpectedly tight seal made by the patch pipette in the whole-cell configuration later allowed molecular biologists to suck up the insides of identified cells to unveil their unique molecular contents. By refining these techniques, researchers have scrutinized the surface and contents of excitable cells in detail over the past few decades. However, these powerful approaches do not discern which molecules are responsible for the dynamic control of the genesis, abundance, and subcellular localization of ion channels. In this dark territory, teams of unknown and poorly understood molecules guide specific ion channels through translation, folding, and modification, and then they shuttle them toward and away from distinct membrane domains via different subcellular routes. A central challenge in understanding these processes is the likelihood that these diverse regulatory molecules may be specific to ion channel subtypes, cell types, and circumstance. In work described in this issue, Bai et al. (2018. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.201812025) begin to shed light on the biogenesis of UNC-103, a K^+ channel found in *Caenorhabditis elegans*.

In recent years, geneticists have leveraged behavioral genetics with suppressor screens to shine light into the dark area of ion channel biogenesis and regulation. They start with a model animal carrying an overactive ion channel that causes the animal to behave conspicuously. They then use an unbiased genetic approach, mutating nearly every gene at random, until they find a mutation that appears to "fix" the animal's odd behavior. These mutations often break molecules that have a normal role in up-regulating the ion channel via biogenesis, transport to the plasma membrane, and/or insertion, or molecules that modify the channel into a less-active form. By lowering levels of the abnormally overactive ion channel, the animal can behave normally.

The work described in this issue offers another golden example of such a suppressor screen on the nematode *Caenorhabditis elegans* (Bai et al.). Bai et al. (2018) generated a transgenic worm that carries an overactive orthologue of the human ether-a-go-go (hERG) potassium channel. The hERG channel is vitally important for many excitable cells including those in the heart, where it provides final repolarization of the ventricular action potential at just the right time to maintain steady synchronous beating. The channel's odd name originates from yet another genetic study that found that fruit flies lacking the equivalent potassium channel appear to dance in the style of go-go dancers from the 1960s (Drysdale et al., 1991). Worms expressing an overactive orthologue of the hERG channel called UNC-103 throughout their nervous system display extreme sluggishness. Overactive mutant versions of *unc-103* were previously found in genetic screens for mutant worms defective in locomotion and mating (Brenner, 1974; Garcia and Sternberg, 2003). Any signal of motivation percolating from the worm's "brain" to the locomotor circuitry is quashed by hyperpolarizing ERG potassium current throughout the nervous system. This renders the ERG/UNC-103 gain-of-function transgenic worm stuck at square one for most of its life. Perhaps worse, the depressed nervous system cannot easily allow the worm to contract muscles to lay eggs; thus, retained progeny hatch and feast inside their mother, eventually killing her. An equivalent immobilizing mutation in other animals including fruit flies, zebrafish, and mice would probably prove lethal because it would prevent them from finding food and a mate to sire progeny. The hermaphroditic reproductive system of *C. elegans*, however, allows even a couch potato to flourish. The female worm harbors both egg and sperm, so there is no need to find a mate. Moreover, immobile worms do not starve as long as their bacterial food is piled on top of them. Even the severe defects in egg laying caused by the overactive ERG channel do not prevent strain propagation. Trapped progeny explode out of the mother

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worm in a way that is reminiscent of the movie *Alien*. Thus, *C. elegans* is an optimal model to study overactive ion channels.

When scanning hundreds of plates of pathetic, overactive ERG/UNC-103 mutant worms, Bai et al. (2018) spied rare individuals that crawled actively. Using genetic mapping and whole-genome sequencing techniques, they discovered that every one of the reanimated suppressor mutants carried loss-of-function mutations in the gene *cnx-1*, which encodes an orthologue of mammalian calnexin. Their unbiased search mutated virtually each gene in the genome, and yet, four out of four of their suppressor mutants had unique loss-of-function mutations in only a single gene. This finding strongly suggests that *cnx-1* represents the most important single gene for positively regulating CNX-1 channels. Other genes likely contribute to ERG regulation, but they either cause lethality or sterility when mutated, or they have a lesser role than calnexin. Bai et al. (2018) recently identified one such gene: *dnj-1*, an orthologue of a DNaJ protein previously suspected of chaperoning ion channels through the ER (Li et al., 2017). Whereas the *cnx-1* single mutant moves and lays eggs in the same way as wild-type worms, the *dnj-1* mutant shows gross motor and egg-laying defects. This demonstrates that CNX-1/calnexin has a more specific role in ERG/UNC-103 regulation than DNJ-1 in *C. elegans*.

Although other studies have used behavioral suppressor screens for ion channels in *C. elegans*, the Bai et al. (2018) study is admirable for applying multiple approaches to substantiate a conserved role for calnexin in chaperoning functional ERG channels from the ER to the plasma membrane. After finding that loss of calnexin suppressed the sluggishness caused by overactive transgenic ERG/UNC-103 channel, they first quantified the in vivo abundance and localization of the potassium channels by literally looking in the worm. The transparency of the *C. elegans* body affords simple visualization of transcriptional reporters and fluorophore-tagged molecules in the nervous system, muscle, and even identified cells. Fewer ERG/UNC-103 channels were observed in *cnx-1* and *dnj-1* single mutants, and hardly any were seen in the *cnx-1;dnj-1* double mutants. This simple epistasis result demonstrates that the CNX-1 and DNJ-1 molecules positively regulate ERG/UNC-103 channel abundance in parallel pathways. This parallel functional relation was confirmed at the protein level by measuring tagged ERG/UNC-103 channels in Western blots, at the behavioral level by quantifying rates of movement and egg laying, and at the physiological level by recording whole-cell currents in an identified neuron isolated from worms in culture. In all cases, the *cnx-1;dnj-1* double mutant was worse off than either single mutant, suggesting that the two molecules positively regulate ERG/UNC-103 in parallel rather than in the same pathway.

Bai et al. (2018) pushed on to test whether their worm results would hold up in human cells. Indeed, they found that tagged calnexin and hERG colocalized when transfected in HEK cells. The calnexin and hERG interaction was preserved in reciprocal pulldown immunoaffinity assays. Importantly, they found that knockdown of calnexin expression reduced hERG current when recorded in vitro. Overall, Bai et al. (2018) outline a successful strategy starting with *C. elegans* to identify and validate novel conserved molecular chaperones for ion channels.

Other studies have leveraged a similar approach as Bai et al. (2018) to reveal novel and uncharacterized molecular chaperones, subunits, and modifiers of different ion channels. The laboratories of Hongkyun Kim and Zhao-Wen Wang have focused on the large-conductance calcium- and voltage-activated big potassium (BK) potassium channel called SLO-1 in *C. elegans* (Wang et al., 2001). They cleverly used worms expressing an overactive SLO-1 channel. Worms carrying the SLO-1 gain-of-function mutations are sluggish and lay few eggs due a widely hyperpolarized nervous system. Both laboratories have screened for new suppressor mutations that enable the *slo-1* gain-of-function mutant worm to crawl better and lay more eggs. For instance, Oh et al. (2017) identified a suppressor mutation in the novel protein ERG-28 that resides in the ER. Analogous to CNX-1, ERG-28 promotes the trafficking of SLO-1 BK channels from the ER to the plasma membrane. Oh et al. (2017) went on to determine that EGR-28 accomplishes this by shielding SLO-1 from premature degradation by proteases including the aspartic protease DDI-1. In separate studies, the Wang laboratory identified a calcium and calmodulin-dependent kinase and a BK-associated peptide, BKIP-1, that when mutated suppress the overactive SLO-1 channel (Liu et al., 2007; Chen et al., 2010a). BKIP-1 also interacts with a distinct type of SLO channel called SLO-2 that is gated by voltage and cytosolic chloride and calcium (Niu et al., 2017).

The Kim and Wang laboratories also used suppressor screens to reveal unexpected roles for molecular components of the dystroglycan complex, which were first hinted at in a physiological study (Carre-Pierrat et al., 2006). They independently found that aside from the well-known structural role that dystrophin has in maintaining muscle integrity, dystrophin also appears to localize BK channels near to calcium channels (Kim et al., 2009). This occurs at least in part through physical linkage between dystrophin and SLO-1 via a novel subunit dubbed ISLO-1 (Kim et al., 2009). Their suppressor screens eventually revealed independent evidence that dystrophin also functionally interacts with α-catulin to help localize BK channels near to calcium channels (Abraham et al., 2010; Chen et al., 2010b, 2011; Sancar et al., 2011). In muscle membranes, the SLO-1 channels form a beautiful lattice arrangement when tagged with GFP. Without certain dystrophin-related proteins, BK channels float away from their paired calcium channels in the plasma membrane and remain mostly inactive without the synergistic boost in activation by local microdomains of high calcium. These studies are exciting because they reveal that the BK channel may represent a target to treat forms of muscular dystrophy that may be more druggable than intracellular dystrophin-related proteins. The studies also suggest that natural variation in dystrophin-related genes may also contribute to alcohol-use disorders as BK channel activity is directly modulated by ethanol, and modifying any of these genes alters behavioral sensitivity to alcohol (Davies et al., 2003; Bettinger and Davies, 2014; Oh et al., 2017; Scott et al., 2017). In time, the suppressor approach that Bai et al. (2018) have used to study hERG regulation may reveal more surprising discoveries for hERG as similar studies have revealed for BK channels.

Researchers have spent decades accumulating minute details about how ion channels such as hERG and BK operate using the versatile patch pipette. Recent studies show that simple

but powerful genetic approaches can reveal which intracellular molecules deliver, modify, shuttle, and position specific ion channels in different cell types. These previously intractable molecules will almost certainly represent candidate genes for common disease such as heart arrhythmia (Long QT syndrome), diabetes, seizures, and more. Given that ERG channels are often associated with fatal drug interactions, this genetic approach may also yield new insight into the prevention of dangerous side effects of drugs.

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