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Lurcher, nPIST, and Autophagy

Previous work has shown that neurodegeneration in the lurcher mouse is due to a mutation in the *GluRδ2* gene that results in a constitutively active glutamate receptor ion channel. Characterization of the cell death pathway in these animals reported by Yue et al. in this issue of *Neuron* provides important insight into the toxicity induced by the abundant transmitter glutamate. Through protein-protein interactions, the GluRδ2^{Lc} mutant channel activates autophagy.

Since the initial studies of Olney and Sharpe in 1969, considerable effort has been directed toward understanding the mechanisms underlying how neruonal injury develops after exposure to the excitatory neurotransmitter glutamate. At least in vitro, this neuronal "excitotoxicity" requires the influx of Ca^{2+} (Choi 1988), with the mitochondria being the suggested intracellular target of this Ca^{2+} influx. Understanding of how glutamate might alter Ca^{2+} influx came with the characterization of a family of receptor channels activated by L-glutamate. In 1997, using the neurological mutant lurcher mouse, the group headed by Nathaniel Heintz at Rockefeller University provided the first direct evidence linking cerebellar Purkinje cell neurodegeneration with the activation of a glutamate receptor channel, the GluRδ2 channel (Zuo et al., 1997). At the time, a speculation was that in addition to being permeable to Na^{+}, the mutant lurcher GluRδ2 channel (GluRδ2^{Lc}) might also allow the entry of Ca^{2+} into Purkinje cells that would trigger the subsequent excitotoxicity (Seeburg, 1997).

Yue et al. now provide strong evidence that lurcher Purkinje cells die via an autophagic pathway and that the GluRδ2^{Lc} channel activates this pathway through protein-protein interactions. Using the yeast two-hybrid approach, this group identified a novel protein (nPIST) capable of interacting with the C-terminal tail of GluRδ2 via a PDZ domain. nPIST cofractionated with GluRδ2 in cerebellar extracts and like GluRδ2, nPIST localized to the postsynaptic density of Purkinje cell:parallel fiber synapses. Taking notice of structural clues from nPIST that this protein might function as an adaptor protein for the assembly of a signal transduction complex, Yue et al. performed a second yeast two-hybrid screen with the coiled coil domain of nPIST as the bait. This screen identified the protein Beclin1 as being able to interact with nPIST. Why did Beclin1 peak their interest? For two reasons. Beclin1 had previously been associated with apoptosis since it can interact with the BH1 domain of Bcl-2 (Liang et al., 1998). In addition, *Beclin1* is the mammalian ortholog of the yeast gene Apg6/Vps30 that has a critical role in autophagy (Kametaka et al., 1998). Thus, these data identify two potential cell death pathways that may contribute to the neurodegeneration seen in lurcher animals. While there is evidence indicating that apoptosis is activated in *lurcher* cerebellar neurons, recent genetic analysis examining the role for Bax in the lurcher neurodegeneration indicated that apoptosis has a minor role in the loss of Purkinje cells, a primary site of neurodegeneration in lurcher (Doughty et al., 2000; Selimi et al., 2000).

Autophagy, like apoptosis, is a form of programmed cell death. It is classically thought of as the cell death pathway activated in yeast in response to nutrient deprivation and involves the degradation of many subcellular components (Klionsky and Emr, 2000). However, evidence is accumulating that autophagy is an important player in neuronal cell death (Pettmann and Henderson, 1998). Thus, upon demonstrating that nPIST and Beclin1 can act synergistically to induce autophagy in HEK 293 cells, Yue et al. searched for evidence of autophagy in GluRδ2^{Lc}-mediated cell death. Morphologically, autophagy is distinguished by the presence of autophagic vacuoles and autophagosomes that are formed by rearranged subcellular membranes (Dunn, 1994). These membrane compartments enclose cytoplasmic constituents and organelles and traffic them to lysosomes for degradation. In HEK 293 cells expressing the constitutively active GluRδ2^{Lc} channel and Beclin1-GFP, Yue et al. found Beclin1-GFP-positive cytoplasmic vesicles. No such structures were seen in cells expressing wild-type GluRδ2 and Beclin1-GFP. The fact that the vesicles in the GluRδ2^{Lc}-transfected cells were positive for Beclin1 is consistent with the suspected involvement of this protein in the subcellular membranes dynamics associated with autophagy. Signs of an autophagic cell death in lurcher Purkinje cells were also found. Electron microscopic analyses of dying lurcher Purkinje cells revealed the presence of membrane bound vesicles containing cytoplasmic material and fragments of cellular organelles. These latter data suggest that autophagy at least contributes to the neurodegeneration seen in lurcher mice in vivo.

While the identification of the autophagic cell death pathway as a mediator of the pathological effects of the GluRδ2^{Lc} mutant channel is of importance in its own right, the results reported by Yue et al. have broader implications for how glutamate-induced excitotoxicity in general might be best viewed. As mentioned earlier, a popular picture of glutamate-induced excitotoxicity is with the mitochondria as the major target of the deleterious effects of a bulk increase in cytoplasmic Ca^{2+}. The ability of Beclin1 to bind to Bcl-2 and the fact that the mitochondria are a target of autophagy indicate that mitochondrial dysfunction does likely occur in lurcher neurons. It is yet to be determined the extent to which the lurcher phenotype and perhaps excitotoxicity in general are due to alterations in mitochondrial function. Since autophagy also effects the function of many subcellular components, it now seems likely that excitotoxicity includes much more than mitochondrial dysfunction. Furthermore, the elucidation by Yue et al. that protein interactions involving the GluRδ2^{Lc} channel, nPIST, and Beclin1 are critical for the induction of autophagy argue against cellular depolarization and a bulk increase in cytoplasmic Ca^{2+} as the trigger of cell death.

Rather, these data strongly suggest Ca^{2+} influx results in very local changes in Ca^{2+} concentrations, at the site of the GluRδ2 signaling complex. This suggests that the cell death pathway triggered by glutamate and the GluR$\delta 2^{Lc}$ channel relies for the most part on the formation of macromolecular signaling complexes and protein-protein interactions. If you will, solid state processes.

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Selected Reading

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Trapping the Sensor

Voltage-gated ion channels open in response to a change in membrane potential. The "sensor," or the channel's molecular entity responsible for the detection of voltage change, is formed by a transmembrane element, rich with basic residues, called the "voltage sensor" or the "S4 domain." The movement of the S4 drives a global conformational change leading to the opening of the permeation pathway and ion conduction. In this issue of *Neuron*, Schönherr and colleagues (2002) show that physical constrains of the "gating canal," or the crevice through which the S4 moves, determines whether voltage-gated potassium channels open quickly or slowly.

Exactly 50 years ago, Hodgkin and Huxley (Hodgkin and Huxley, 1952), in a seminal study, suggested that voltage-dependent channel gating is the consequence of a series of voltage-dependent transmembrane motions of four identical and independent charged "gating particles." In their model, movement of the last gating particle turns on ion conduction. This series of activation transitions is now known to involve independent voltage sensing transitions followed by conformational rearrangements of the S4 segments in each of the four subunits in the potassium channel tetramer. S4 responds to depolarizing membrane potential by displacing some of its basic residues in the outward direction, across the membrane electric field, generating currents known as the "gating currents" (Aggarwal and MacKinnon, 1996; Bezanilla, 2000). These currents precede channel opening, suggesting that the S4 domains must first move in order to open the permeation pathway.

The nonpolar nature of the membrane does not favor a transmembrane α helix rich with basic residues such as the S4. Two mechanisms exist to overcome this limitation. One is to shield the S4 transmembrane domain between other transmembrane α helices (S2, S3, and S5) to effectively shield the positive charges of S4 and, in addition, to physically stabilize and effectively neutralize the S4 positively charged basic groups by providing negatively charged side chains to create salt bridge interactions (Tiwari-Woodruff et al., 1997). The second solution is to decrease the number of effective charges within the low dielectric environment by creating a crevice or a gating canal, $\sim$14 Å thick (compared to a lipid bilayer with average thickness of $\sim$35 Å), placing most of the S4 basic side chains in a cozy watery environment, mainly on the intracellular side at rest (Larsson et al., 1996; Starace and Bezanilla, 2001). Once the membrane is depolarized, the S4 undergoes a rotation of $\sim 180^{\circ}$ (Cha et al., 1999; Glauner et al., 1999), probably accompanied by an axial translation in the outward direction, somewhat like a drill (Glauner et al., 1999). This overall motion, therefore, requires the breaking of the salt bridges formed with the S2 and S3 transmembrane domains in the hyperpolarized resting state of S4 (where the gate is closed) and the formation of new ones in the depolarized activated state (where the gate is open). This intimate interaction of the S4 with the S2-S3 crevice implies that physical constraints in the crevice have the potential of affecting S4 motion during depolarization, much as the motion of a piston would be affected by a change in the shape of its cylinder.

In this issue of *Neuron*, Schönherr and colleagues (2002) explored the molecular mechanism by which magnesium ions affect the gating of the bovine EAG1 potassium channel. EAG channels have a unique ability to switch between two gating speeds: a fast mode that opens the channel in milliseconds and a hyperpolarization-dependent slow mode that takes a sizable fraction of a second and with very sigmoidal opening kinetics. Switching between these two modes depends on external magnesium ions. One magnesium ion interacts with a pair of negatively charged residues in S2 and S3, which are uniquely found in the EAG channel family (Silverman et al., 2000). To determine whether the action of magnesium is directly on S4 motion or on the S4-gate coupling mechanism, Schönherr and colleagues combine three powerful methodologies, steady-state cysteine accessibility mutagenesis, perturbation scanning, and voltage clamp fluorometry (VCF), which together enable a correlation to be made between S4 topology and protein movement in the transitions that precede the opening of the permeation pathway. The authors now provide evidence for a change in the physical dimension of the crevice during channel activation.