SOD1 is a cause of the fatal, paralytic disorder ALS. Although mechanisms underlying mutant SOD1 neurotoxicity remain uncertain, this protein associates with mitochondria. In this issue of Neuron, Israelson et al. show that mutant SOD1 binds and inhibits the mitochondrial channel VDAC1. This finding sheds light onto possible molecular links between mutant SOD1, mitochondrial dysfunction, and spinal motor neuron degeneration in inherited ALS.

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive paralytic disorder of middle and late life characterized mainly by a loss of cortical motor neurons and lower motor neurons in the brainstem and spinal cord (Pasinelli and Brown, 2006). There is no apparent genetic linkage in about 90% of ALS patients, but in the remaining cases, the disease is inherited (Pasinelli and Brown, 2006). Among the individuals with familial ALS, roughly one-fifth map to chromosome 21, where there are mutations in the gene for the antioxidant enzyme superoxide dismutase 1 (SOD1) (Pasinelli and Brown, 2006). In transgenic mice carrying the SOD1<sup>G93A</sup> mutation, overexpression of wild-type SOD1 failed to modify the expression of the disease (Bruijn et al., 1998), supporting the notion that the mutated allele gives rise to a gain-of-function phenotype. Although in vitro studies indicate that mutant SOD1 exerts its deleterious effects via a combination of cell-autonomous and non-cell-autonomous processes, as illustrated in Nagai et al. (2007), the actual nature of the acquired adverse property remains to be established. Relevant to this outstanding issue, Israelson and collaborators have now performed a comprehensive set of investigations in the mutant SOD1 model of ALS from which has emerged, as shown in this issue of Neuron, an exciting and novel hypothesis: a mitochondrial channelopathy underpins neurodegeneration in this disease.

SOD1 is known to be essentially a cytosolic enzyme, but a portion of mutant SOD1, especially when overexpressed, has been identified in mitochondria (Pasinelli and Brown, 2006). This physical association has led researchers to posit that mislocalized mutant SOD1, by affecting mitochondrial functions, may contribute to the degeneration of motor neurons in this familial form of ALS. Consistent with this view, reduced respiratory chain activity, abnormally high release of apoptogenic mitochondrial molecules such as cytochrome c, and impaired mitochondrial movement have all been documented in transgenic mice expressing mutant SOD1 (Kirkinezos et al., 2005; Magrane et al., 2009; Pasinelli and Brown, 2006).

One way that mislocalized mutant SOD1 could impact on mitochondrial biology is through aberrant interactions with essential proteins associated with this organelle. For example, mutant SOD1 has already been shown to bind to Bcl-2 (Pasinelli et al., 2004) and lysyl-tRNA synthetase (Kawamata et al., 2008). Now, Israelson et al. (2010) report the remarkable finding that mutant SOD1 also interacts with the mitochondrial voltage-dependent anion channel-1 (VDAC1) (VDAC1/porin-1). They found that VDAC1 (but not its related homolog VDAC2) coimmunoprecipitates with the catalytically active SOD1<sup>G93A</sup> and inactive SOD1<sup>H46R</sup> mutants but not with the wild-type SOD1 protein in solubilized mitochondrial lysates from rat spinal cords. Surprisingly, no evidence of an interaction between VDAC1 and mutant SOD1 is detected in protein extracts prepared from brain tissues despite the fact that the latter contains copious amounts of mutant SOD1 protein. The authors suggest that this regional specificity might be explained by the higher content of the known VDAC1 interactor, hexokinase-1 (Azoulay-Zohar et al., 2004), in brain compared to spinal cord, which may outcompete mutant SOD1. Yet, to support this proposal, additional experiments are still needed. For example, this proposal would be strengthened by (1) immunocytochemical studies showing that ALS-resistant ocular motor neurons express more hexokinase-1 than ALS-susceptible spinal motor neurons and (2) in vitro competition experiments showing that addition of hexokinase-1 recombinant attenuates the magnitude of SOD1/VDAC1 interaction.

By using an antibody that binds to a disease-specific epitope inaccessible on correctly folded SOD1, the authors were able to demonstrate that the VDAC1/SOD1 interaction involves, at least in part, misfolded SOD1 protein, which may represent the actual toxic species. Misfolded SOD1 protein—and consequently its interaction with VDAC1—is detected only in spinal cord mitochondrial fractions of transgenic mutant SOD1 rats and only in symptomatic animals. These results suggest that the binding of the noxious misfolded SOD1 conformer to VDAC1 parallels the time course of the disease and is restricted to areas most affected in this ALS animal model.

Next, to examine the potential functional significance of the VDAC1/SOD1 interaction, Israelson et al. (2010) measured ion channel conductance of purified VDAC1 reconstituted into a planar lipid bilayer by voltage clamp, as before...
uptake of ADP by mitochondria isolated in a genuine setting, the authors assessed the function of VDAC1, but this time in a more controlled manner. To assess another important protein, SOD1, they wanted to determine the impact on normal mitochondrial functions and consider the possibility of a loss of VDAC1. Of note, high concentrations of the hexokinase reaction product glucose 6-phosphate can reopen VDAC1 (Azoulay-Zohar et al., 2004). Therefore, whether glucose 6-phosphate is able to reopen VDAC1 or if the channel is closed due to another factor, the authors aimed to explore these possibilities.

In vivo significance of a loss of VDAC1 function in ALS was discussed. Although the work of Israelson et al. (2010) opens a compelling new way of thinking about the neurobiology of ALS, at this point, it seems that the jury is still out as to whether the loss of channel conductance plays a pathogenic role in this inherited form of ALS. VDAC1 plays a key role in mitochondrial-dependent apoptosis (Mannella and Kinnally, 2008), a form of cell death that is thought to drive the ultimate demise of motor neurons in ALS (Guégan and Przedborski, 2003). Furthermore, VDAC1 is a major component of mitochondria-associated endoplasmic reticulum (ER) membranes (MAM); these are zones of physical interaction between mitochondria and the ER. Interestingly, the MAM has pivotal roles in a host of cellular processes, including Ca²⁺ signaling, lipid transport, energy metabolism, and cellular survival (Hayashi et al., 2009), and, in addition to mitochondria, mutant SOD1 also localizes to the ER (Kikuchi et al., 2006). Therefore, whether mutant SOD1-associated VDAC1 alterations promote neurodegeneration here is a critical area of future research.

In summary, the authors aimed to explore the role of VDAC1 in ALS and to determine the impact of SOD1 mutations on mitochondrial function. Their findings suggest that abrogation of VDAC1 should have instead extended lifespan of animals, but this is still under investigation. The authors conclude that future studies are needed to better understand the role of VDAC1 in ALS pathology.