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From ER to Eph Receptors: New Roles for VAP Fragments

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Dominantly inherited mutations in an endoplasmic reticulum protein called VAPB have been found in a subset of patients with a rare familial form of amyotrophic lateral sclerosis (ALS). In this issue, Tsuda et al. (2008) identify a secreted form of VAPB that binds directly to Eph receptors inducing their activation and signaling, providing fresh insights into ALS pathogenesis, including non-neuronal aspects of this disorder.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a progressive loss of upper and lower motor neuron function. Although environmental and genetic factors may contribute to disease susceptibility, 95% of patients do not have an obvious family history of ALS (Schymick et al., 2007). A prevailing hypothesis is that understanding the mechanisms responsible for the relatively rare familial cases may uncover targets for therapeutic intervention in the more common sporadic forms of the disease. Indeed, mutations in a subset of familial ALS patients have been identified in a variety of genes including those encoding superoxide dismutase (SOD1, accounting for approximately 20% of familial ALS) and VAPB, ALS2, DCTN1, SETX, and TARDBP (Pasinelli and Brown, 2006; Sreedharan et al., 2008). The role of these mutations in disease pathogenesis is under intensive investigation. New work in this issue by Tsuda et al. (2008) explores the function of the VAMP-associated membrane protein B (VAPB), a protein located in the endoplasmic reticulum (ER). They demonstrate that VAPB has a secreted form that binds to Eph receptors and activates them, and that a disease-causing mutation in VAPB disrupts secretion of this protein.

A dominantly inherited proline 56 to serine (P56S) missense mutation in the *VAPB* gene was first identified in a large Brazilian family with a slowly progressive and late-onset atypical form of ALS (Nishimura et al., 2004). Interestingly, individuals carrying the same mutation have been described with three distinct conditions ranging from a late-onset slowly progressing form of spinal muscular atrophy (SMA), to an atypical slowly progressing form of ALS (ALS8), to a typical severe and rapidly progressing ALS (Marques et al., 2006; Nishimura et al., 2004). Studies characterizing VAPB carrying the P56S mutation in cultured cells showed that it accumulates in the ER and sequesters the wild-type protein into ubiquitinated inclusions (Nishimura et al., 2004; Teuling et al., 2007). These data suggest that the P56S mutation acts in a dominant-negative fashion to disrupt the normal activity of ER-localized VAPB. Normal functions of VAPB may include regulation of vesicle trafficking, ceramide and sphingomyelin transport and synthesis, and maintenance of ER homeostasis.

The exciting work of Tsuda et al. (2008) identifies a new activity of VAPB that may play an important role in the normal function and maintenance of motor neurons. Working in a fly model, these authors show that the new activity resides in a secreted form of the *Drosophila* VAPB homolog, VAP33A (dVAP), which contains an evolutionarily conserved N-terminal major sperm protein (MSP) domain. Analysis of human serum demonstrated that like dVAP, the MSP domain of VAPB is secreted. The dVAP protein with a P58S mutation (corresponding to the human P56S mutation) is not cleaved or secreted and is retained within ER aggregates, suggesting that the P56S mutation would disrupt the potential activity of secreted VAPB fragments.

Using flies overexpressing either wild-type or P58S dVAP, Tsuda et al. show that secreted dVAP has signaling activity. In previous work, loss of dVAP at the neuromuscular junction has been shown to result in a decrease in the number of synaptic boutons and an increase in their size. In contrast, overexpression of wild-type dVAP in presynaptic neurons increases the number of boutons but decreases their size, leading to a disruption in flight ability (Pennetta et al., 2002). However, Tsuda et al. now demonstrate that overexpression of P58S dVAP in presynaptic neurons has no significant effect on bouton number or size and only a mild impact on flight activity. Interestingly, neuronal overexpression of wild-type dVAP also induces structural abnormalities in flight muscles that were not observed in flies expressing mutant dVAP. These data suggest that loss of dVAP secretion imparted by the P58S mutation prevents these overexpression phenotypes that are at least in part mediated by the MSP domain of secreted dVAP.

The presence of the MSP domain in the VAPB protein suggested that VAPB may share signaling functions with the MSP sperm-specific proteins, which mediate oocyte maturation and ovarian sheath contraction in the worm *Caenorhabditis elegans.* In agreement with this notion, microinjection of worm, fly, or human VAP MSP domains into the MSP-deficient gonad of *C. elegans* rescues the gonadal pheno-

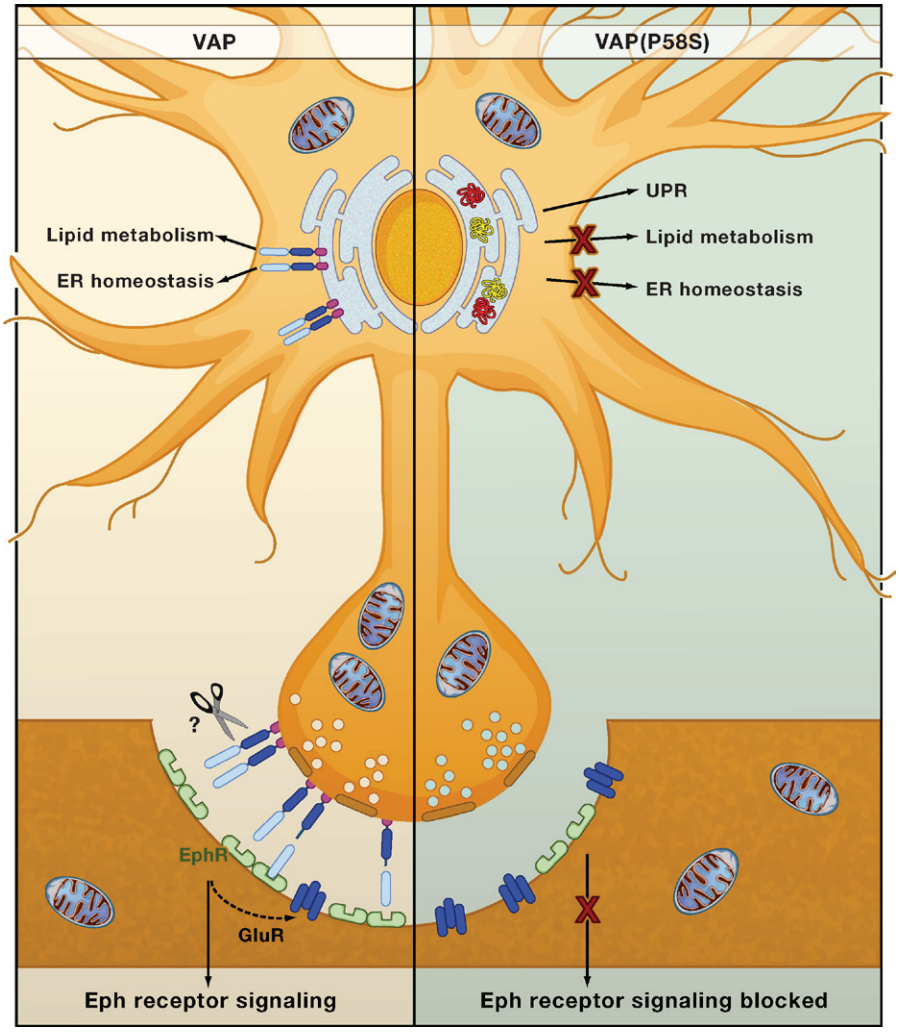

Figure 1. Effects of the ALS-Associated VAP Mutation

VAP (VAMP-associated membrane protein) contains an N-terminal MSP (major sperm protein) domain, a central coiled-coil domain, and a C-terminal transmembrane region. It exists as an endoplasmic reticulum (ER)-localized type II membrane protein and modulates diverse pathways including ER homeostasis and ceramide and sphingolipid metabolism. In addition, VAP is cleaved by an unknown protease, releasing MSP domain-containing fragments that are secreted. Extracellular cleavage is shown here; however, the cellular location of this event remains to be determined; the resulting VAP fragments bind to Eph receptors (EphRs) although again the location is unknown (Tsuda et al. suggest from their fly model that the location of VAP fragment binding to EphRs could be in the *Drosophila* flight muscles). VAP fragments could also act in an autocrine way to modulate neuronal activity, for example, via glutamate receptors (GluRs). The proline 56 to serine (P56S) mutation (P58S in *Drosophila*) in VAPB has been observed in a subset of ALS patients with the rare familial form of the disease. This mutation results in aggregation of the mutant (red) and wild-type (yellow) VAP protein in the ER, upregulation of the unfolded protein response (UPR), and loss of VAP function including Eph receptor signaling and probably other ER-associated activities of VAP.

type (Tsuda et al., 2008). VAP carrying the P56S mutation mimics the wild-type protein in this assay, demonstrating that the mutant protein still retains functional signaling properties. This further suggests that the defects of the mutant protein are primarily a result of aberrant trafficking and a propensity to develop aggregates, not loss of its normal activities.

MSP domains bind to multiple receptors including the *C. elegans* VAB-1 Eph-related receptor protein tyrosine kinase (Calò et al., 2006). Tsuda et al. demonstrate that dVAP also modulates Eph receptor signaling pathways. Both *Drosophila* and *C. elegans* mutants lacking VAP have phenotypes that overlap with those observed in Eph receptor mutants. In addition, loss of function of the *Drosophila* Eph receptor suppresses the muscle phenotypes induced by overexpression of wild-type dVAP. Lastly, the *C. elegans* and mammalian VAP MSP domains bind directly to their respective Eph receptor ectodomains. The similarity between the phenotypes of VAP mutants and Eph receptor mutants suggests that binding of VAP MSP domains to this receptor activates signaling. Whether this ligand/receptor interaction leads to positive or negative regulation of downstream signaling cascades, the nature of these pathways, and whether they are phosphorylation dependent or phosphorylation independent remain open questions. However, the abundance of different Eph receptors, many of which are expressed in the neuromuscular junction and brain, and the observation that the VAP MSP domain can be cleaved and secreted (rather than remaining membrane-bound like the ephrins) raise the possibility that VAPs may act in an autocrine or paracrine fashion, or both. In addition, VAP/Eph receptor binding could have very different effects on signal transduction based on the type of receptors expressed by individual cells. For example, binding to Eph receptors expressed by neurons could stabilize synapses—indeed ephrins and Eph receptors have been shown to impact NMDA glutamate receptor clustering and synaptic plasticity (Calò et al., 2006). Thus, it is possible that defective VAPB-P56S signaling in ALS patients with the rare familial form of the disease and carrying this particular mutation might alter the susceptibility of motor neurons to proposed pathogenic mechanisms such as glutamate excitotoxicity. Moreover, data from the Tsuda et al. study suggest that disrupted secretion by motor neurons of the VAPB MSP domain caused by the P56S mutation could mediate the effects on glia, endothelia, or muscle cells described in ALS patients and in transgenic animal models expressing mutant SOD1 (Pasinelli and Brown, 2006).

The relevance of this VAP signaling activity to human ALS will rest on defining the cellular activities of VAPB that are critical for the normal function and maintenance of motor neurons, and how each is affected by the P56S mutation. This provocative new report by

Tsuda et al. raises at least three possible mechanisms by which VAPB could be involved in motor neuron disease (Figure 1). First, prolonged ER stress induced by the dominant-negative aggregation of mutant and wild-type VAPB proteins might directly mediate cellular toxicity. Second, the loss of VAPB's ability to anchor FFAT-motif-containing enzymes required for lipid metabolism in the ER might predispose motor neurons to cell death (Hanada et al., 2007). Finally, a reduction in the newly described VAP MSP signaling activity through Eph receptors (and possibly other receptors) could result in a non-cell-autonomous activation of motor neuron death. Of course, aspects of each scenario may occur simultaneously. It is interesting to speculate that loss of some or all of these activities might account for the variability in human disease phenotypes observed with VAPB-P56S mutations.

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Golgi Governance: The Third Way

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It is a subject of intense debate whether proteins are transported by vesicles through the membranous stacks of the Golgi or whether the stacks mature, carrying the cargo along. In this issue, Patterson et al. (2008) present evidence for a third model in which the Golgi stacks are a continuous structure and proteins rapidly equilibrate between the layers.

Secretory cargo made in the endoplasmic reticulum transits through the Golgi apparatus, where various posttranslational modifications occur, prior to sorting into different tubulovesicular carriers for transport to the surface of the cell (Farquhar and Palade, 1981). In one model for Golgi function called the vesicular transport model, each stack is static with its own defined structure and enzymes (Figure 1). Cargo is delivered to the stack by fusion of a membrane-bound vesicle. Next, the cargo is sorted out of the stack into a vesicle that in turn fuses to the subsequent stack. Thus, cargo that enters on one face of the Golgi is transported in a series of vesicular transport steps to the other end. A second model, called the maturation model, proposes that it is the stacks themselves that move from one face of the Golgi across to the other. As they move, processing enzymes are sorted out into vesicles that then fuse with a younger stack. In this issue, Patterson et al. (2008) present evidence for a different possibility—that the Golgi stacks are interconnected and that proteins freely and quickly distribute and partition between them.

There are many reported experimental results consistent with either the vesicular transport model or the maturation model, as well as an equally large number of experiments demonstrating that neither model, on its own, sufficiently explains all of the results (Pelham and Rothman, 2000). For example, it is difficult to reconcile the vesicular transport model with the observations that some cargo molecules that are too large to fit into vesicles, such as collagen precursors or cell wall fragments in algae called scales, still make their way through the Golgi (reviewed in Pelham and Rothman, 2000). Likewise, the observations that vesicles at the rims of the Golgi contain only cargo molecules with no enzymes (Orci et al., 2000) are difficult to reconcile with the maturation model.

A seminal review by Pelham and Rothman (2000) proposed a potential resolution of two models for the movement of cargo within the Golgi. They put forward the notion that vesicles carrying small cargo "percolate" in both directions through the stack with rapid transport rates, whereas larger cargo move via slow cisternal move-