What is the function of MrpL32 and do all of its activities require *m*-AAA proteolysis? Nolden et al. (2005) show that mitochondrial translation is diminished to a similar extent in the absence of either MrpL32 or a functional *m*-AAA protease. This indicates that the *m*-AAA protease is likely to control mitochondrial translation through processing of MrpL32 (Nolden et al., 2005), although this conclusion was not formally demonstrated. The reason for the drastic impact on mitochondrial translation is unclear. Processing of MrpL32 by the *m*-AAA protease results in a very tight association of MrpL32 with the mitochondrial inner membrane, even though no transmembrane domains are predicted. The association of MrpL32 with the inner membrane might serve to localize active ribosomes to the membrane, which may be important for efficient translation. Not all of the functions of MrpL32 require that it be processed given that large ribosomes still assemble without unprocessed MrpL32, in mitochondria with defective *m*-AAA protease. In contrast, in the absence of MrpL32, ribosomes, both large and small, fail to assemble. This implies that the incorporation of mature MrpL32 into the large ribosome is critical for translation in mitochondria. Alternatively, processed MrpL32 may exist in a complex independent of the large ribosome but still essential for mitochondrial translation. This latter possibility is supported by the observation that processed MrpL32 only partially comigrates with large ribosomal subunits in sucrose gradients.

Finally, Nolden et al. (2005) demonstrate the conservation of MrpL32 processing by showing that the mouse *m*-AAA protease can process yeast MrpL32 and vice versa. Moreover, in liver mitochondria from mouse cells lacking paraplegin, an apparently unprocessed form of MrpL32 accumulates, although processed MrpL32 is readily detected. The fact that there is some processing of MrpL32 in mitochondria lacking paraplegin clearly indicates that in both mice and humans, there may exist unidentified AAA-protease subunits that can pair with Afg3L2 and partially replace paraplegin or other unrelated proteases, absent in yeast, compensating for loss of paraplegin. Strikingly, the incomplete processing of mouse MrpL32 in mitochondria lacking paraplegin correlates with a decrease in mitochondrial translation. However, it must be pointed out that a similar experiment using muscle biopsies obtained from HSP patients harboring a deleted paraplegin gene failed to reveal any defect in mitochondrial translation (Atorino et al., 2003).

In the end, the lack of processing of MrpL32 by *m*-AAA protease may not be the critical event that causes the pathology of HSP. Instead, what the work reported by Nolden et al. (2005) reveals is the possibility of a more general pathogenic mechanism involving the absence of proteolytic activation of additional substrates of the *m*-AAA protease, which may account for HSP. This mechanism does not mutually exclude the involvement of an accumulation of nonassembled proteins in the mitochondrial inner membrane. In fact, one could argue that such proteins may act to clog compensatory proteases, effectively competing with substrates that require proteolysis for their activity. The critical question that still remains is why is it specifically the neurons with the longest axons that are affected by the mutation of a mitochondrial protein with an apparent housekeeping function? The revelation by Nolden et al. (2005) of a new mechanism for HSP associated with a defect in paraplegin should aid in the resolution of this fundamental question.

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ADAM and Eph: How Ephrin-Signaling Cells Become Detached

Ephrin ligands presented on one cell surface associate with their receptors on the surface of a juxtaposed cell, often resulting in cell-cell repulsion. In this issue of *Cell*, Janes et al. (2005) show that the ephrin ligand can be proteolytically released from its membrane tether by a complex on the opposing cell composed of the ephrin receptor and an ADAM metalloprotease.

Ephrins are plasma membrane bound proteins that function as signaling ligands for a large family of receptor tyrosine kinases, the Eph receptors. Ephrin signaling guides cell migration and determines the path of cellular protrusions by influencing cell adhesion and organization of the cytoskeleton. Ephrin signaling has been well characterized in the nervous system where it has been shown to guide growing neuronal processes to their targets. For example, in the visual system, ephrin gradients regulate the spatial mapping of retinal

ganglion cells to higher brain centers (Brown et al., 2000). However, ephrins have also been implicated in functions outside the nervous system, for example, in cell morphogenesis, tissue patterning, and angiogenesis (for a review on ephrin receptor signaling, see Pasquale, 2005).

Ephrins are characterized by an N-terminal extracellular receptor binding domain that adopts a globular β-barrel structure with "Greek-key" folding topology. Attachment of this domain to the membrane is preceded by a linker region of ~40 amino acids. There are two subfamilies of ephrins: the A subclass, which is GPI anchored to the cell surface, and the B subclass, which is anchored by a single transmembrane segment followed by a short conserved cytoplasmic region ending with a PDZ binding motif (reviewed in Barton et al., 2004). The human genome encodes six ephrin-A and three ephrin-B ligands.

Vertebrates express ten ephrin-A and six ephrin-B receptors, which show a high degree of conservation within each class. Extracellular regions of these receptors have a conserved architecture consisting of an N-terminal ligand binding domain with a "β-jelly roll" fold, a cysteine-rich region containing an EGF-like motif, and two fibronectin type III repeats (see Figure 1). The cytoplasmic organization of these single-pass transmembrane proteins is also conserved and consists of a tyrosine kinase domain and a C-terminal sterile α motif (SAM), a small domain thought to be involved in protein-protein interactions (Barton et al., 2004). Furthermore, a multitude of receptor variants may be produced by alternative splicing.

With a few exceptions, receptor-ligand interactions are class restricted (reviewed in Kullander and Klein, 2002). Thus, ephrin-A ligands activate EphA receptors, and likewise for the B subclass. However, within each subclass, ligand-receptor interactions are promiscuous, although binding affinities can vary substantially for different ligand/receptor pairs. Nonetheless, cognate ephrin/receptor complexes typically form with nanomolar affinity. Furthermore, after the initial binding event, ligand/receptor pairs oligomerize to form large signaling aggregates (Barton et al., 2004). This poses a paradox: The tight binding of a membrane bound receptor to a ligand tethered to an opposing cell would be expected to favor adhesion, so how can this be reconciled with the typically repulsive activity of the ephrins? A solution to this paradox began to emerge a few years ago when Flanagan and colleagues elegantly showed that ephrin-A2 forms a stable complex with the metalloprotease Kuzbanian (KUZ), the *Drosophila* homolog of ADAM10 (Hattori et al., 2000).

ADAM (a disintegrin and metalloprotease) proteins are membrane-anchored metalloproteases that mediate a wide variety of specific proteolytic events at the cell surface (reviewed in Blobel, 2005). For example, ADAMs catalyze the regulated ectodomain shedding of TGF-α, TNF-α, and the notch ligand Delta, a process that is critical to their signaling function. Furthermore, in *Drosophila*, KUZ/ADAM10 is required for the normal extension of axons (Fambrough et al., 1996).

The large family of ADAM proteases has a characteristic domain organization. The extracellular region of the mature protein is comprised of an N-terminal metalloprotease domain, a disintegrin domain followed by a cysteine-rich region, and an EGF-like domain (see Figure 1). A single-pass transmembrane segment leads to a cytoplasmic domain that often contains signaling motifs such as proline-rich regions and phosphorylation sites. Interestingly, although ADAMs exhibit specificity in the choice of their substrates, this specificity does not appear to reside in the proteolytic domain, which is able to cleave polypeptide chains without obvious preference for primary sequence (Blobel, 2005).

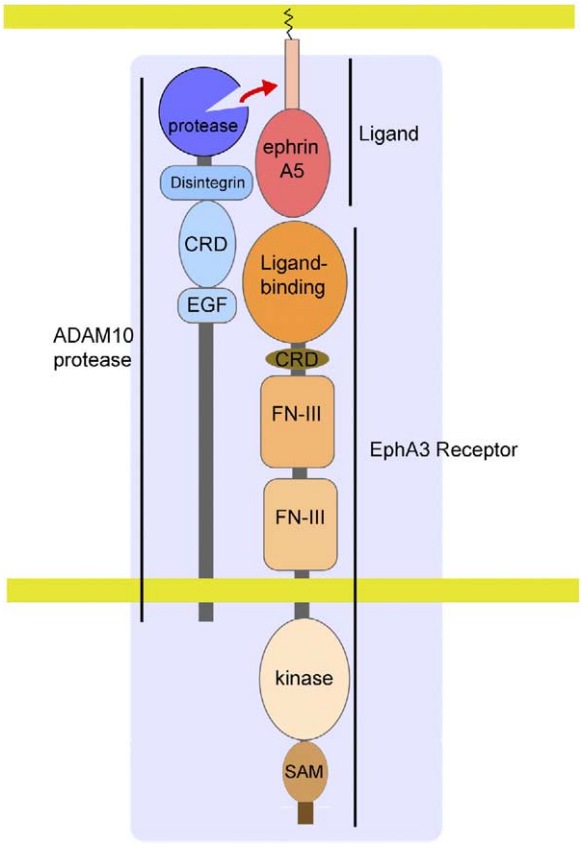

Figure 1. The Association between the ADAM10 Protease and the Ephrin-A5/EphA3 Ligand-Receptor Signaling Complex

Depicted is the plasma membrane (yellow) of two juxtaposed cells, one presenting the ephrin ligand, and the other presenting the ephrin receptor in constitutive association with the protease. Binding of the ephrin ligand to the receptor-protease complex activates proteolysis (red arrow) to remove the ephrin ligand from its membrane tether. Interactions between the cysteine-rich domain of ADAM10 and the receptor-ligand pair appear to confer a specificity that may result in activation of the protease. Subsequently, the entire complex (light blue box) can be taken up by endocytosis by the cell expressing the receptor.

Flanagan and colleagues showed that, upon formation of the ephrin-A2/EphA3 signaling complex, KUZ catalyzed the proteolytic shedding of ephrin-A2 from its membrane tether. Furthermore, cultured neurons expressing a mutant ephrin-A2, which is competent for signaling but that cannot be proteolytically cleaved, failed to exhibit normal repulsive axon withdrawal effects (Hattori et al., 2000). It is likely that the defect in

this withdrawal function is due, at least in part, to an impairment of axon detachment.

In the current issue of *Cell*, Janes et al. (2005) shed new light on the mechanism by which ADAM10 interacts with an ephrin-signaling complex and reveal an unexpected twist. These authors present a detailed functional mutagenesis study of ADAM10, with readouts for the binding, cleavage, and internalization of the ephrin-A5/EphA3 complex. Their work is also enriched by crystallographic analysis of the ADAM10 disintegrin and cysteine-rich domains.

Janes et al. (2005) show, surprisingly, that ADAM10 constitutively associates with the ephrin binding domain of the EphA3 receptor, and this association is enhanced by the presence of the ephrin-A5 ligand. This contrasts, without necessarily contradicting, the prior work on KUZ, the *Drosophila* homolog of ADAM10. KUZ was shown by coimmunoprecipitation experiments to associate with the ephrin-A2 ligand, rather than its EphA3 receptor. Interestingly, fragments of ADAM10 bind to ephrin-A2 and -A1 but not to ephrin-A5. Nonetheless, for both ephrin-A2 and ephrin-A5, complex formation with the EphA3 receptor is required for ephrin cleavage. Furthermore, the current work reports functional dissection of the interaction between ADAM10 and EphA3, revealing that the ligand binding domain of the EphA3 receptor is responsible for binding to ADAM10. Whereas ADAM10 constitutively associates with EphA3, a high-affinity binding site within the cysteine-rich domain of ADAM10 binds to the ephrin-A5/EphA3 complex but to neither component alone. This suggests the potential basis for a conformational "switch" activated only upon complex formation, providing a possible structural basis for the regulation of proteolysis. This model also provides an explanation for the selectivity of cleavage by ADAM despite the lack of primary sequence specificity observed for the protease domain alone.

The crystal structure of the cysteine-rich domain and disintegrin domain fragment from ADAM10, also presented in the report by Janes et al. (2005), reveals an acidic surface pocket in the cysteine-rich domain that could serve as a binding site for the ephrin/Eph complex. Disruption of the acidic character of this pocket by site-directed mutagenesis greatly diminishes binding to the complex, whereas the constitutive association with the EphA3 receptor alone remains unperturbed, suggesting a second site of interaction.

A key finding of the current report is that ADAM10 cleaves ephrin-A5 from its membrane tether only in *trans*. Thus, consistent with its constitutive association with the EphA3 receptor, ADAM10 must be presented by the juxtaposed cell. This is in contrast to other characterized ADAM-mediated proteolytic events, which so far have been shown only to occur in *cis,* that is, when both enzyme and substrate are expressed within the same cell (Blobel, 2005). Thus, for ephrin-A5/EphA3, both initiation and termination of ephrin signaling proceed as intercellular events, dependent on the apposition of the cells expressing ligand and receptor. Although proposed previously (Blobel, 1997), ADAM10 appears to be the first example of a protease that cleaves its substrate in a manner that is cell nonautonomous.

In the ephrin system, the cell harboring the EphA3 receptor has the machinery to both initiate (via the receptor) and terminate (via the protease) the signal. Proteolysis in *trans* ensures that cleavage of the ephrin will occur only upon binding to its receptor on another cell. The proteolytic event that detaches ephrin-A5 from its membrane tether releases the adhesive bond between the two cells, allowing the cells to move apart. Furthermore, the postcleavage ephrin-A5/EphA3 complex is then endocytosed by the cell expressing the receptor.

Given this example of ADAM-mediated proteolysis between cells that are attached through the interaction of ephrin-A5 and the EphA3 receptor, the questions arise: How general is this mechanism? Do other ephrin/receptor pairs share the same fate? Do other ADAM proteases act on other ephrin pairs? Finally, as ADAM proteases appear to have many specific substrates despite the apparent lack of specificity in their protease domains, might these other ADAMs use a similar substrate recognition and activation mechanism involving the cysteine-rich domain as Janes et al. propose? It remains to be understood, for example, how the protease exerts its function in the context of oligomerized signaling pairs at the intercellular interface. Future investigations may begin to answer these questions and may also provide insight into the apparent requirement for higher-order clustering of signaling components.

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