embryonic neurons, this calcium-dependent process may be active only when synapses are being formed. Thus, while a more complicated signaling pathway is needed to cluster nicotinic receptors at neuromuscular junctions, local activity may be sufficient to induce the formation of glycine receptor clusters in neurons. Although this might explain how clustering of a single type of receptor can be controlled, the sorting of different receptor types to specific postsynaptic sites must require even more complex regulation.

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PERSPECTIVES: CELL BIOLOGY

A Cellular Striptease Act

Zena Werb and Yibing Yan

The cell surface is a dynamic place. During its life history the cell alters the repertoire of proteins displayed on its surface many times. Membrane-anchored adhesion molecules, receptors, ligands, and enzymes are removed and replaced as the cell proceeds through development and as its activation state changes. How is this wholesale refurnishing of the cell membrane orchestrated? One key mechanism is proteolytic processing of the ectodomain (extracellular domain) of such membrane proteins. Cleavage or shedding of the ectodomains of plasma membrane proteins—widely observed in cells in culture—is blocked by inhibitors of metalloproteinases (*1, 2*). This result suggests that transmembrane and soluble metalloproteinases, such as matrix metalloproteinases (MMPs) and their relatives, are rate-limiting for cleavage and shedding. Other evidence also implicates serine proteinases in these processing events (*3, 4*).

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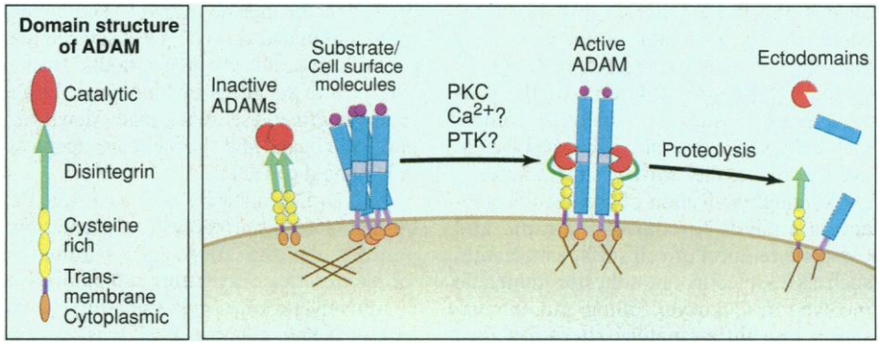
The first such "sheddase" characterized was the tumor necrosis factor–α (TNF-α) converting enzyme (TACE) (*5*). The study by Peschon and colleagues (*6*) on page 1281of this issue now points to TACE's essential role in the shedding of ectodomains during mouse development. The surprise comes from the observation that mice lacking TACE do not show a phenotype indicative of a lack of TNF-α availability. Rather, they show the same phenotype as mice engineered to be without the epidermal growth factor (EGF) receptor—because TACE-mediated proteolysis makes available ligands for the EGF receptor, particularly transforming growth factor–α (TGF-α).

TACE turns out to be a membrane-anchored proteinase that is a member of the ADAM (a disintegrin and metalloproteinase) domain family of proteins that combines features of both cell surface adhesion molecules and proteinases (*8*). ADAMs all have a common domain organization, which endows these proteins with several potential functions—proteolysis, adhesion, signaling, and fusion (see figure below). The proteolytically competent ADAMs, such as TACE (ADAM17), are zinc-dependent metalloproteinases, closely related to the MMP family with which they share small molecule inhibitors and even one tissue inhibitor, TIMP-3 (*9, 10*). Several newly discovered MMPs appear to be hybrids of both MMP and ADAM domains (*11*), indicating that these two types of enzymes are part of one, larger family.

The ADAM proteinases are themselves targets of proteolytic events that ultimately strip off the catalytic domains (*5, 8*). This action could be a mechanism of functionally blunting the effects of the proteinases (see the figure on the next page). These soluble ADAMs may have proteolytic activity, as is the case for snake venom enzymes (*8*), but soluble TACE is much less active than membrane-bound enzyme (*5, 6*). The residual adhesive domains of ADAMs left after cleavage may have regulatory or adhesive functions. In support of this idea, a catalytic domain-deleted mutant of *Kuz* (ADAM10/SUP17), first identified as being required for cleavage of Notch during neural development in *Drosophila*, exerts a dominant negative effect (*8, 12*). During sperm maturation fertilin, a heterodimeric ADAM essential for sperm-egg interaction (*13*), also loses its catalytic domains by proteolytic processing. The remaining adhesive disintegrin domain is then competent to bind integrins.

How does TACE act? TACE is widely expressed in the animal. Mutation of the catalytic domain of TACE (*6*) reveals several distinct functions for this ADAM in development. Ligands for the EGF receptor, which is essential for epithelial development (*7*), are usually made and used locally (*14*). Although the growth factor precursors may have some biological activity (*15*), the new results imply that the membrane-anchored forms are essentially inactive precursors (*6*). TACE also cleaves ectodomains of other receptors and ligands, such as TNF-α, the p75 TNF receptor, and L-se-



Activation of sheddases. The ADAM proteases (as dimers) and substrates are anchored apart in the plane of the membrane. Upon activation (via protein kinases and other pathways) they are brought together and proteolysis takes place, leading to free ectodomains.

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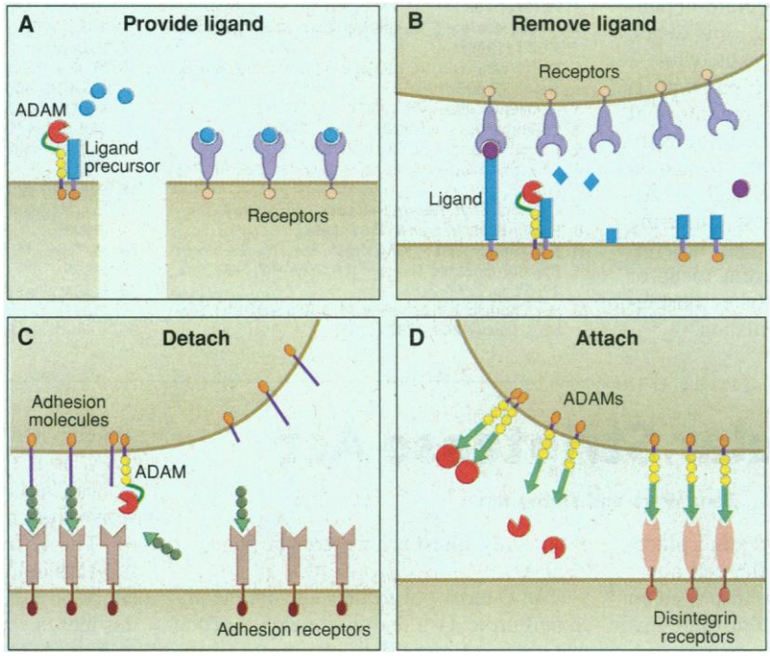
lectin, and thus participates in inflammatory and pathological reactions (*6*).

Processing membrane proteins by the ADAMs and other sheddases requires both the membrane-anchored enzyme and its substrate to be present in cis on the same cell (*6, 8, 12*). This presents several interesting problems. How are the active cell surface proteinases kept separate from their cell surface substrates until shedding is triggered? How do you exert selectivity for only certain ectodomain targets, out of many transmembrane proteins displayed on the cell surface? And how are the proteinase and substrate brought together in a coordinated manner so that all the cell surface substrate molecules can be removed within seconds, as occurs for the adhesion molecules L-selectin and syndecans (*4, 16*)?



Versatile shedding. Sheddases can supply or down-regulate ligands for receptors. Cleavage of adhesion molecules on cell surface or exposure of the disintegrin domain of ADAM regulate cell-cell and cell–extracellular matrix interactions.

Despite nonconserved cleavage sites that may be adjacent to the membrane or further out on the molecule, there are clues that a common strategy may operate in most cases. First, all ectodomain shedding is inhibited in a single mutant cell line (*1*). Second, the proteolysis is regulated in different cell types by activation of protein kinase C (PKC), calcium/calmodulin kinases, or receptor tyrosine kinases (*1, 17*). A model that accounts for these observations requires the processing proteinases and their transmembrane substrates both to be anchored in distinct domains of the plasma membrane, probably through cytoskeletal interactions (see figure on previous page). Upon cell activation, the attachments change and the proteinases and substrates become coclustered and can interact. Alternatively, the signaling cascade could modify the cytoplasmic domains of the proteinases or substrate, producing a conformational change that either activates the enzyme or makes the cleavage site available.

Although activation of the shedding reaction appears to control the rapid and complete removal of cell surface molecules such as L-selectin (an adhesion molecule involved in leukocyte rolling and extravasation into inflammatory sites) for most processing reactions there appears to be a constitutive level of ectodomain shedding. Processing is necessary to make available paracrine growth and survival factors such as TGF-α, EGF, HB-EGF, the kit ligand, and amphiregulin (*18*). This makes sense to allow for the consistent supply of growth factors (see figure above).

Endogenous inhibitors allow even finer control of the action of the shedding enzymes. Recently TACE was shown to be inhibited by TIMP-3, but not by the three other TIMPs that also inhibit MMPs (*10*). If TACE liberates a survival factor, then the presence of TIMP-3 could lead to cell death. This may explain why TIMP-3, but not other TIMPs, induce apoptosis (*19*).

Proteolysis of the ectodomains of growth factor coreceptors such as syndecan provide a second mechanism for regulating growth factor availability. Shedding the ectodomain of syndecan converts it to a potent inhibitor of FGF-2 (*20*). Just as shedding can make growth factor ligands available and control proliferation and survival, cleavage can also control cell death. Membrane-bound Fas ligand induces apoptosis by binding to the Fas receptor. Proteolysis functionally down-regulates the ligand and short-circuits apoptosis in lymphoid cell (*21*).

Cell surface adhesive molecules can also be regulated by proteolysis. An emerging paradigm is that cleavage of adhesive molecules not only alters adhesion, but completely revamps cell signaling. In the case of Notch, cleavage by *Kuz* is required to make it functional as a receptor, promoting adhesion, signaling, and cell lineage choices (*12*). Shedding of L-selectin by TACE or related enzymes inhibits leukocyte rolling and blunts their extravasation to inflammatory sites (*16*). The shedding of the ectodomains of E-cadherin (*22*) and transmembrane protein tyrosine phosphatases such as LAR have profound effects on cell-cell adhesions and also on important signaling pathways (*17*). These changing adhesion receptors and ligands may also be part of the apparatus for pathfinding in the nervous system.

Cells use a limited number of strategies to remodel their microenvironments. It is clear that the shedding process is an ancient, conserved, and fundamental pathway present from worms to humans. Thus, proteolysis by cell surface shedding enzymes provides a mechanism by which the wardrobe of externally displayed molecules can be changed or discarded. Spatial restriction of the enzymes and their substrates allows for either instant action or sustained activity.

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