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COMMENTARY

Metalloprotease-disintegrins: modular proteins capable of promoting cell-cell interactions and triggering signals by protein-ectodomain shedding

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SUMMARY

Metalloprotease-disintegrins (ADAMs) have captured our attention as key players in fertilization and in the processing of the ectodomains of proteins such as tumor necrosis factor α (TNFα), and because of their roles in Notch-mediated signaling, neurogenesis and muscle fusion. ADAMs are integral membrane glycoproteins that contain a disintegrin domain, which is related to snake-venom integrin ligands, and a metalloprotease domain (which can contain or lack a catalytic site). Here, we review and critically discuss current topics in the ADAMs field, including the central role of fertilin in fertilization, the role of the TNFα convertase in protein ectodomain processing, the role of Kuzbanian in Notch signaling, and links between ADAMs and processing of the amyloid-precursor protein.

Key words: ADAM, Fertilin, TNFα-convertase (TACE), Kuzbanian (KUZ), Notch, Amyloid precursor protein (APP)

INTRODUCTION

Metalloprotease-disintegrins are a family of transmembrane glycoproteins that play roles in cell-cell interaction and in the processing of the ectodomains of proteins such as tumor necrosis factor α (TNFα) and Delta. They are characterized by a conserved domain structure, consisting of an N-terminal signal sequence followed by prodomain, metalloprotease and disintegrin domains, a cysteine-rich region, usually containing an EGF repeat, and finally a transmembrane domain and cytoplasmic tail (Blobel and White, 1992; Wolfsberg and White, 1996; Blobel, 1997; Black and White, 1998). Thus, family members are referred to as ADAMs (for a disintegrin and metalloprotease domain) or MDC proteins (for metalloprotease/disintegrin/cysteine-rich proteins). Almost 30 metalloprotease-disintegrin cDNA sequences have been identified in organisms ranging from *Schizosaccharomyces pombe* to humans.

The extracellular components of metalloprotease-disintegrins bear striking similarity to a group of snake-venom metalloproteases (SVMPs) and disintegrins (Blobel and White, 1992; Wolfsberg and White, 1996; Blobel, 1997; Black and White, 1998). Indeed, the short, soluble disintegrins were first isolated from snake venom (Huang et al., 1987, 1989; McLane et al., 1998). Snake-venom disintegrins bind tightly to the platelet integrin αIIbβ3 and function as competitive inhibitors of platelet aggregation by preventing binding of platelets to fibrin (McLane et al., 1998). The name disintegrin refers to this disruption of integrin binding (Gould et al., 1990). Interestingly, although all ADAMs have a relatively well-conserved metalloprotease domain, only 15 of those identified to date contain the zinc-binding catalytic-site consensus sequence (HEXXH), a hallmark of the metzincin superfamily of zinc peptidases (Jongeneel et al., 1989; Bode et al., 1993; Blundell, 1994; Stocker et al., 1995) (see Fig. 1). Thus, only half of the known ADAMs are predicted to be catalytically active, whereas the others most likely lack metalloprotease activity.

Metalloprotease-disintegrins have been implicated in diverse processes, including sperm-egg binding and fusion, myoblast fusion, protein-ectodomain processing or shedding of cytokines, cytokine receptors, adhesion proteins and other extracellular protein domains. Furthermore, they are necessary for proper axonal guidance, neural and wing development in *Drosophila*, vulval development in *Caenorhabditis elegans*, and epithelial maturation and skin and hair development in the mouse. Insight into these functions has emerged from studies of only a few family members, specifically the sperm proteins fertilin (Primakoff et al., 1987; Blobel et al., 1992; Myles et al., 1994; Almeida et al., 1995) and cyritestin (Heinlein et al., 1994; Linder and Heinlein, 1997; Yuan et al., 1997), TNFα-converting enzyme (TACE, ADAM17) (Black et al., 1997; Moss et al., 1997; Peschon et al., 1998a), Kuzbanian (KUZ, MADM, ADAM10, SUP-17) (Fambrough et al., 1996; Rooke et al., 1996; Pan and Rubin, 1997; Sotillos et al., 1997; Wen et al., 1997) and meltrin α (ADAM12) (Yagami-Hiromasa et al., 1995). For most other family members, a function has yet to be ascribed.

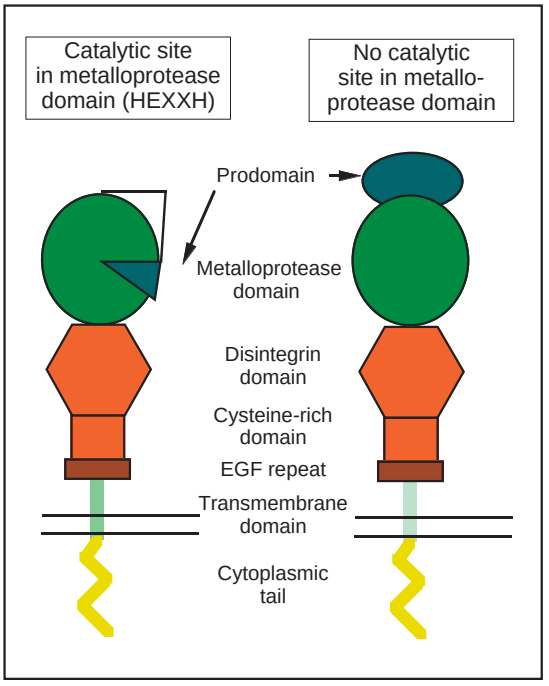

Fig. 1. Metalloprotease-disintegrin domain organization. Two models of the domain organization of membrane anchored ADAMs (a disintegrin and metalloprotease) are shown. On the left is an ADAM with a catalytic site consensus sequence (HEXXH) in its metalloprotease domain; the prodomain is depicted as an inhibitor. The model on the right is of an ADAM that does not have a catalytic site in its metalloprotease domain, although the protein sequence of this domain as a whole is conserved compared with ADAMs that have a catalytic site consensus. The overall domain organization of all membrane anchored ADAMs identified to date includes an N-terminal signal sequence (not shown), a prodomain, which is followed by a metalloprotease domain (with or without a catalytic site consensus), a disintegrin domain and cysteine-rich region, a transmembrane domain and a cytoplasmic tail. Most, but not all, membrane-anchored ADAMs also include a membrane-proximal EGF repeat. Three frequently updated websites provide access to the GenBank accession numbers and sequence alignments of known ADAMs: (1) www.people.Virginia.EDU~jag6n/adams.html; (2) www.uta.fi/~loiika/HADAMs.htm; and (3) www.gene.ucl.ac.uk/users/hester/metallo.html.

From a mechanistic point of view, the functions of ADAMs in cell-cell adhesion have been attributed to the activity of the disintegrin domain as an integrin ligand, whereas those involving protein ectodomain processing have been attributed to the metalloprotease domain. For other functions of metalloprotease-disintegrins, such as their role in muscle fusion, the underlying molecular mechanisms remain obscure. In addition, the importance of the incorporation of both metalloprotease and disintegrin domains into a single molecule is not yet known.

Here, we highlight several exciting recent developments in the field and outline what is currently known about the various functions of metalloprotease-disintegrins. First, we evaluate the role of fertilin and other ADAMs in cell-cell interactions; we then discuss the catalytic activity of ADAMs such as TACE and KUZ, and their roles in protein-ectodomain processing and signaling. We also consider intriguing links between the functions of ADAMs and presenilins in the sequential processing of Notch and the amyloid-precursor protein (APP). In addition, we present and discuss new insights and models that emerge from these recent developments. (For other recent reviews, see Huovila et al., 1996; Wolfsberg and White, 1996; Blobel, 1997, 1999; Myles and Primakoff, 1997; Black and White, 1998; McLane et al., 1998; Evans, 1999.)

FERTILIN AND SPERM-EGG BINDING

The first metalloprotease-disintegrins to be recognized in cells were the α and β subunits of the guinea-pig sperm protein fertilin (Primakoff et al., 1987; Blobel et al., 1992). Fertilin, initially named PH-30, was isolated after a functional screen of monoclonal antibodies raised against sperm surface proteins. A monoclonal antibody against PH-30 recognizes an antigen present on the posterior head (PH) of mature sperm and blocks sperm from fusing to eggs that lack the zona pellucida (a glycoprotein coat that surrounds the egg) (Primakoff et al., 1987). Support for the proposed role of fertilin in sperm-egg fusion came from the observation that fertilin α contains a short stretch of hydrophobic amino acids that can be modeled as an amphipathic α-helix, which is reminiscent of viral fusion peptides (Blobel et al., 1992). White and co-workers hypothesize that such a peptide inserts into the target membrane as part of a process that ultimately results in membrane fusion (White, 1992; Huovila et al., 1996).

Both subunits of fertilin are metalloprotease-disintegrins, but only fertilin α possesses a catalytic-site consensus sequence (HEXXH) in its metalloprotease domain (Blobel et al., 1992; Wolfsberg et al., 1993). During sperm maturation in the testis and epididymis, both subunits of fertilin undergo a series of proteolytic processing events, which leads to the removal of their prodomains and metalloprotease domains (Blobel et al., 1990; Phelps et al., 1990; Lum and Blobel, 1997). In the resulting fertilin heterodimer on fertilization-competent sperm, the disintegrin domain is at the N-terminus of both subunits (Blobel et al., 1992; Lum and Blobel, 1997). Because these disintegrin domains resemble the known integrin ligands found in snake venoms, fertilin was proposed to bind to an integrin(s) on the egg through the peptide sequences present where the RGD sequence lies in snake venom disintegrins (Blobel and White, 1992; Blobel et al., 1992).

Several lines of evidence now support this hypothesis. Peptides mimicking the predicted binding sequence of guinea-pig fertilin β inhibit fertilization in a concentration-dependent manner, whereas peptides containing the same amino acids in a random order do not (Myles et al., 1994). Similarly designed peptides corresponding to the sequences found in fertilin β from other species, as well as to several other metalloprotease-disintegrins expressed in testis, also inhibit fertilization (Almeida et al., 1995; Evans et al., 1995, 1997a,b; Shilling et al., 1997; Yuan et al., 1997).

Investigators have used different approaches to attempt to overcome the limitations of peptides in assessing the importance of fertilin in fertilization. Various domains of fertilin α and β, expressed in bacteria and refolded after reduction, have properties consistent with a role in sperm-egg binding (Evans et al., 1997a,b, 1998). Chen et al. (1999)

incubated beads coated with an anti-fertilin-β-cytoplasmic-tail antibody in lysates of fertilization-competent sperm. Beads incubated in sperm extract were able to bind to eggs, whereas control beads were not, and binding of the coated beads was inhibited by a peptide corresponding to the predicted integrin-binding loop of fertilin β.

Current evidence suggests that the α6β1 integrin is the egg's receptor for sperm. Although both α6β1 and αvβ3 integrins are expressed on the surface of eggs, a function-blocking antibody against α6 but not against αvβ3 inhibits sperm binding (Almeida et al., 1995). The most direct evidence for an interaction through the fertilin β predicted integrin-binding site is that an iodinated peptide mimicking this site can be specifically crosslinked to an α6 integrin on the egg surface (Chen and Sampson, 1999). The α6β1 integrin appears to be capable of taking on distinct ligand-binding states in which binding to fertilin is favored in the presence of Ca^{2+}, and binding to laminin is favored in the presence of Mn^{2+} (Chen et al., 1999).

It will now be important to examine the ability of purified, eukaryotically expressed, recombinant forms of fertilin to bind to the surface of eggs. Such an approach should allow for a more definitive analysis of the functions of the various domains of the fertilin subunits in sperm-egg binding and fusion. The importance of the predicted integrin-binding site could then be elucidated through the generation of point mutations. This should also help resolve the question of whether the α6 integrin, other integrins or perhaps even different proteins on the oocyte are involved in these interactions.

TARGETED DELETION OF THE GENE THAT ENCODES FERTILIN β IN MICE HAS EXPECTED AND UNEXPECTED CONSEQUENCES

The targeted deletion of fertilin β has provided additional information about the function of fertilin in sperm and raised intriguing new questions about the role of this protein in sperm maturation and sperm function (Cho et al., 1998). Male mice lacking fertilin β are infertile, but are viable and appear to mature normally. Analysis of sperm from these animals demonstrates that their sperm are defective in at least three distinct steps of fertilization. There is an eight-fold decrease in the levels of binding of sperm to zona-free eggs, which is consistent with one of the major proposed roles of fertilin β, although sperm-egg fusion was reduced by only 50%. When sperm lacking fertilin β did fuse with the egg, egg activation proceeded normally. The residual binding between eggs and sperm lacking fertilin might be mediated by additional sperm disintegrins or other proteins.

The modest decrease in the level of sperm-egg fusion seen in fertilin-β-knockout mice might be because of the continued presence of fertilin α, the levels of which were reduced but which is not absent in testicular cells lacking fertilin β. Although this observation is consistent with the hypothesis that, in the absence of one subunit of the heterodimer, the other is rapidly degraded, it remains to be shown that murine fertilin is a heterodimer as the guinea-pig and bovine fertilins are. To assess the contribution of fertilin to sperm-egg membrane fusion, it will now be necessary to generate mice lacking both fertilin α and fertilin β.

Besides the defect in sperm-egg binding, which is consistent with the predicted role of fertilin, fertilin-β-deficient sperm also show two phenotypes that were quite unexpected (Cho et al., 1998). First, these sperm have a defect in their ability to migrate from the uterus to the oviduct, although sperm number and motility are not affected by the lack of fertilin β. Perhaps fertilin is required for adhesion of sperm to the uterotubal junction and isthmus, where they are briefly stored prior to their continued migration. Second, fertilin-β-deficient sperm cannot bind to the zona pellucida.

An intriguing explanation for some of the unexpected phenotypes of sperm lacking fertilin β could be that the absence of fertilin β also leads to a functional defect in fertilin α activity. As mentioned above, testicular spermatogenic cells lacking fertilin β also show a significant decrease in the levels of the precursor form of fertilin α. It is tempting to speculate that the putative metalloprotease function of fertilin α is necessary for the proper processing of other sperm surface proteins, which might in turn be required for the proper migration or zona binding of sperm. Alternatively, fertilin β may have a direct role in binding to cells in the oviduct and/or in binding to the zona pellucida. Finally, a specific complement of proteins might have to be present in a given sperm membrane domain for sperm to function, and removing one of these proteins could indirectly affect the functions of others.

In summary, numerous in vitro studies initially uncovered a role of fertilin in sperm-egg binding and fusion. The initial analysis of fertilin-β-deficient sperm indicates that fertilin functions in sperm-egg binding in vivo but also plays an important role in other aspects of sperm function, including migration and zona adhesion. We look forward to learning more about the role of fertilin β in these processes. The importance of the α subunit of fertilin, as well as of other sperm metalloprotease-disintegrins, in sperm function and fertilization must also be examined.

DO OTHER ADAMS INTERACT WITH INTEGRINS?

Most of our understanding of ADAM-mediated cell-cell interactions has emerged from studies of their roles in fertilization; however, two recent studies provide the first example of a metalloprotease-disintegrin that has a role in somatic cell-cell interactions. Both focus on human MDC15, the only metalloprotease-disintegrin known to date to contain an RGD sequence in the predicted integrin-binding loop of the disintegrin domain (Krätzschmar et al., 1996; Herren et al., 1997). Zhang et al. (1998) generated a bacterial GST-fusion protein containing only the disintegrin domain of MDC15. CHO cells expressing αvβ3, but not those expressing several other integrins, were able to bind to the fusion protein, and this binding was inhibited by an antibody against αvβ3. Furthermore, αvβ3, but not αIIbβ3, from cell lysates bound to the MDC15 fusion protein in vitro.

Nath et al. (1999) took a different approach by expressing the extracellular domain of human MDC15 as an Fc-fusion protein in COS-7 cells. This fusion protein could interact with cells expressing αvβ3 or α5β1 integrins. The interactions were competitively inhibited by peptides corresponding to the RGD loop of MDC15 and were dependent on divalent cations and temperature, which is reminiscent of known integrin-ligand

interactions. Antibodies against the respective integrins inhibited binding of cells to MDC15 Fc, and the fusion protein bound to purified αvβ3 and α5β1 integrins in a solid-phase-adhesion assay.

The RGD-sequence-containing MDC15 can therefore interact with αvβ3. It remains to be determined whether the apparent murine homologue of MDC15, which contains the sequence TDD in place of the RGD sequence in the putative integrin-binding loop, can also bind to integrins (Lum et al., 1998). A peptide mimicking the human MDC15 binding-site sequence coupled to Sepharose beads supports cell adhesion and binding of αvβ3, as expected, whereas a peptide mimicking the mouse sequence does not (L. Lum and C. Blobel, unpublished observation). Future studies of MDC15 and other metalloprotease-disintegrins will help elucidate the extent to which integrin binding is a general characteristic of this protein family.

Interestingly, the genome of *S. pombe*, but not that of *Saccharomyces cerevisiae*, includes an ADAM gene that encodes a protein that has a catalytic site and a remarkably conserved disintegrin domain (GenBank accession number Z98849). This protein appears to be most closely related to KUZ/ADAM10 and TACE (see below). The presence of an ADAM in *S. pombe*, which is not known to have integrin-type receptors, raises the question of whether the disintegrin domain and the adjacent cysteine-rich region of ADAMs in general have other functions besides that of binding to integrins. Given the known function of ADAMs in fertilization and protein-ectodomain processing in higher eukaryotes, elucidation of the functions of an ADAM in *S. pombe* should be exciting.

TACE, A PROTEIN-ECTODOMAIN SHEDDASE THAT HAS CRITICAL FUNCTIONS IN DEVELOPMENT AND IN THE ADULT ANIMAL

In addition to their roles as potential integrin ligands, metalloprotease-disintegrins are involved in proteolytic processing of several proteins that have interesting roles in development and in physiological and pathological processes. The release of extracellular domains of transmembrane proteins, a process known as ectodomain shedding, has been demonstrated for cytokines, growth factors, receptors, adhesion proteins and other proteins, such as APP (Ehlers and Riordan, 1991; Massague and Pandiella, 1993; Arribas et al., 1996; Hooper et al., 1997) (see Fig. 2). Prior to the seminal discovery that TNFα-converting enzyme (TACE) is an ADAM (Black et al., 1997; Moss et al., 1997) (see below), inhibitor studies had implicated metalloproteases in several shedding events, including the release of transforming growth factor α (TGFα) (Arribas et al., 1996) and TNFα (Gearing et al., 1994; McGeehan et al., 1994; Mohler et al., 1994). Furthermore, given that several shedding events could not be blocked by two tissue inhibitors of matrix metalloproteases (MMPs), TIMP-1 and TIMP-2, Black et al. (1996) predicted that shedding depends on a class of metalloproteases that is distinct from the MMPs.

Two groups reported the identification of TACE as an ADAM simultaneously (Black et al., 1997; Moss et al., 1997). To validate that the proper convertase had been isolated, both studies showed that recombinant TACE cleaves both recombinant TNFα and peptides spanning the cleavage site directly. T cells that have a targeted disruption of the exon that encodes for the Zn^{2+}-binding domain of TACE (*Tace*$^{\Delta Zn}$) show a severe defect in TNFα release, which confirms that TACE is probably the major TNF-converting activity in vivo (Black et al., 1997). This genetic confirmation is critical in view of the fact that other metalloproteases, including ADAM10, can cleave TNFα in vitro and are expressed in cells known to shed TNFα (Lunn et al., 1997; Rosendahl et al., 1997).

Analysis of *Tace*$^{\Delta Zn/\Delta Zn}$ mice indicates that TACE has a role in the shedding of numerous other substrates besides TNFα and thus a more general role than initially anticipated (Peschon et al., 1998a). A large majority of *Tace*$^{\Delta Zn/\Delta Zn}$ mice die between embryonic day 17.5 and the first day of birth. The severe phenotype was unexpected in light of the predicted role of TACE in TNFα shedding, because animals that lack either TNFα or the TNF receptors p55 TNFR and p75 TNFR are viable and appear normal (Pasparakis et al., 1996; Marino et al., 1997; Peschon et al., 1998b). The *Tace*$^{\Delta Zn/\Delta Zn}$ animals demonstrate a failure to fuse their eyelids, lack a conjunctival sac, and have thinned corneas and several epidermal and hair defects. These defects are reminiscent of those seen in mice bearing a disruption of the TGFα gene (Luetteke et al., 1993; Mann et al., 1993). Additional defects were observed in the epithelial maturation of multiple organs and in the spongiotrophoblast layer of the placenta. The epithelial defects are similar to those described in animals bearing null alleles of a gene that encodes the epidermal-growth-factor receptor (EGFR) (Miettinen et al., 1995; Sibilia and Wagner, 1995; Threadgill et al., 1995). Various EGFR ligands, which include amphiregulin (Brown et al., 1998; Vecchi et al., 1998), EGF (Dempsey et al., 1997), heparin-binding EGF-like growth factor (Higashiyama et al., 1991, 1992; Izumi et al., 1998) and TGFα (Arribas and Massague, 1995; Arribas et al., 1996), are synthesized as transmembrane proteins and subsequently undergo metalloprotease-mediated ectodomain shedding. The proteolytic processing of at least one of these ligands, TGFα, is absent in fibroblasts derived from *Tace*$^{\Delta Zn/\Delta Zn}$ animals, whereas recombinant TACE is able to cleave a peptide spanning the known TGFα-cleavage site in vitro (Peschon et al., 1998a). Thus, genetic and biochemical evidence indicates that TACE is responsible for the shedding of at least one EGFR ligand, TGFα.

Analysis of thymocytes and myeloid cells from TACE-deficient mice indicates that TACE is also involved in the proteolytic processing of both L-selectin and the p75 TNFR (Peschon et al., 1998a). Separate studies of embryonic fibroblasts from TACE-deficient animals indicate that TACE is required for the increase in APP α-secretase activity observed upon PMA stimulation by phorbol myristate acetate (PMA) (Buxbaum et al., 1998). As in the cases of TNFα and TGFα, peptides spanning the cleavage sites of L-selectin and APP are cleaved by recombinant TACE at the correct position. However, TACE is not responsible for all protein-ectodomain shedding: release of the angiotensin-converting enzyme (ACE) from the plasma membrane is not significantly affected in *Tace*$^{\Delta Zn/\Delta Zn}$ fibroblasts, and ACE serum levels are close to normal in *Tace*$^{\Delta Zn/\Delta Zn}$ mice (Sadhukhan et al., 1999).

One initial concern about *Tace*$^{\Delta Zn/\Delta Zn}$ mice and cells was that a mutant TACE$^{\Delta Zn}$ lacking the catalytic site could potentially also have a dominant negative effect. The specific mutation in TACE$^{\Delta Zn}$ is predicted to remove only a part of the metalloprotease domain. This type of deletion within an

extracellular protein module can often interfere with protein folding and maturation, especially if cysteine residues are involved. Therefore, TACE$^{\Delta Zn}$ probably does not fold correctly and is retained in the endoplasmic reticulum for degradation. Functionally, there is no indication that TACE$^{\Delta Zn}$ has a dominant negative effect in heterozygous animals and cells (Peschon et al., 1998a). More importantly, overexpression of recombinant TACE$^{\Delta Zn}$ in COS-7 cells from cDNA cloned from *Tace*$^{\Delta Zn/\Delta Zn}$ mice has no detectable effect on shedding of TNFα, L-selectin or p75 TNFR (J. Peschon and R. Black, personal communication). All available evidence therefore suggests that the *Tace*$^{\Delta Zn}$ mutation leads to a loss of function instead of a dominant negative effect.

Analysis of *Tace*$^{\Delta Zn/\Delta Zn}$ mice and cells thus addresses a key question about the function of membrane-anchored cytokines that are cleaved by a protease such as TACE: is the signaling activity conveyed by the membrane-bound form in a juxtacrine fashion, by the soluble protein in a paracrine manner, or by both? Although the answer to this question could depend on the particular substrate in question, the soluble forms of at least some of these growth factors, such as TGFα, appear to play a critical role in development that cannot be provided by the membrane-anchored precursor protein. Furthermore, EGFR-dependent cell proliferation and migration also appear to depend primarily on the soluble forms of EGFR ligands, given that both processes can be blocked by hydroxamic-acid-type metalloprotease inhibitors (Dong et al., 1999). Future analysis of the shedding of other EGFR ligands in the *Tace*-knockout mice will probably bring more insight into the relative functions of soluble and membrane-bound forms of these proteins.

THE METALLOPROTEASE ACTIVITY OF KUZBANIAN IS REQUIRED FOR NOTCH-MEDIATED SIGNALING

In the development of both the central and peripheral nervous systems, an initially identical population of cells must be instructed to adopt distinct fates (Jan and Jan, 1994; Artavanis-Tsakonas et al., 1995; Greenwald, 1998). Two classes of *Drosophila* genes play sequential roles in this process. The first set, the proneural class, is responsible for inducing a proneural potential onto clusters of cells. The second set, the neurogenic gene class, ensures that only a single cell within each proneural cluster adopts a neural potential; a process termed lateral inhibition, whereby the developing neural cell issues an inhibitory signal to its neighbors, prevents their adopting a similar fate. Thus, loss of function of a neurogenic gene leads to an excessive number of cells adopting a neural fate at the expense of epidermal lineages.

Rooke et al. (1996) isolated the *Drosophila* metalloprotease-disintegrin KUZ in a screen for neurogenic genes. KUZ-deficient embryos show an increase in the proportion of cells adopting a neural fate (Rooke et al., 1996; Pan and Rubin, 1997). Loss-of-function mutations in KUZ give rise to many of the same defects as do mutations in Notch, a primary mediator of lateral inhibition. Notch is a transmembrane protein that acts as a receptor for the lateral-inhibition signal, which is generally thought to be provided by members of the DSL (Delta/Serrate/Lag-2) family of transmembrane proteins (Artavanis-Tsakonas et al., 1995; Greenwald, 1998). Epigenetic analysis in flies demonstrated that KUZ is required upstream of, or in parallel to, Notch (Pan and Rubin, 1997; Sotillos et al., 1997). Similar analysis in *C. elegans* revealed an analogous relationship between SUP-17, a likely homologue of KUZ, and LIN-12, a member of the Notch family (Wen et al., 1997). In both organisms, the ADAM acts in a cell-autonomous manner, i.e. its function is required in the cell that receives the inhibitory signal. Thus, the genetic data are consistent with a model in which KUZ is required for the formation of an activated form of the Notch receptor (see below).

Pan and Rubin (1997) first illustrated the significance of the metalloprotease domain of KUZ in mediating its function in lateral inhibition. They found that mutant forms of KUZ containing either a point mutation predicted to abrogate protease function, or lacking the protease domain entirely, act in a dominant negative manner in flies. Furthermore, a truncated form of the mouse homologue of KUZ, ADAM10, which lacks the metalloprotease domain, acted as a dominant negative in *Drosophila* and in the *Xenopus* embryo, which suggests that the function of KUZ is evolutionarily conserved.

Recent insights into the processing of Notch suggest that Notch undergoes up to three proteolytic cleavages, one of which might depend on KUZ (see Fig. 3). First, the extracellular domain of Notch is constitutively processed in the secretory pathway by a furin-like proprotein convertase (Blaumueller et al., 1997; Logeat et al., 1998). This yields a ~200 kDa extracellular fragment which remains associated with a ~100 kDa fragment consisting of the remainder of the extracellular domain, the transmembrane domain and the cytoplasmic domain. Second, a presumably KUZ-dependent cleavage event is hypothesized to occur in the extracellular domain of the 100 kDa fragment upon ligand binding (Logeat et al., 1998). Third, a proteolytic cleavage occurs within the transmembrane domain of Notch (Schroeter et al., 1998; Struhl and Adachi, 1998) and depends on a presenilin (see below) (De Strooper et al., 1999; Struhl and Greenwald, 1999). This final cleavage releases the cytoplasmic fragment of Notch, which then can translocate to the nucleus in conjunction with a member of the CSL family of transcription factors and activate expression of downstream target genes (Schroeter et al., 1998; Struhl and Adachi, 1998). The finding that CSL-dependent Notch signaling requires functional KUZ, as well as translocation of the cytoplasmic fragment to the nucleus, is consistent with a model in which cleavage of Notch by KUZ is required to trigger the third cleavage, in the transmembrane domain, which releases the cytoplasmic tail (see Fig. 3).

As in the case of Notch, one of its ligands, Delta, also undergoes several proteolytic processing events (Klueg et al., 1998). Recently, Qi et al. (1999) implicated KUZ in cleavage of Delta, which releases a soluble extracellular form of this Notch ligand. This form of Delta binds to cells expressing Notch, and, in a neurite-retraction assay used to assess Notch signaling, it appears to act as a Notch agonist. Overexpression of KUZ in cells also expressing Delta increases processing of Delta, whereas the dominant negative form of KUZ inhibits processing. Furthermore, the extracellular fragment of Delta is not detected in embryos lacking both maternal and zygotic KUZ. Although metalloprotease inhibitors inhibit the release of extracellular Delta, in vitro biochemical data will be needed to confirm that KUZ can cleave Delta directly.

In *Drosophila*, the KUZ phenotype can be interpreted as an

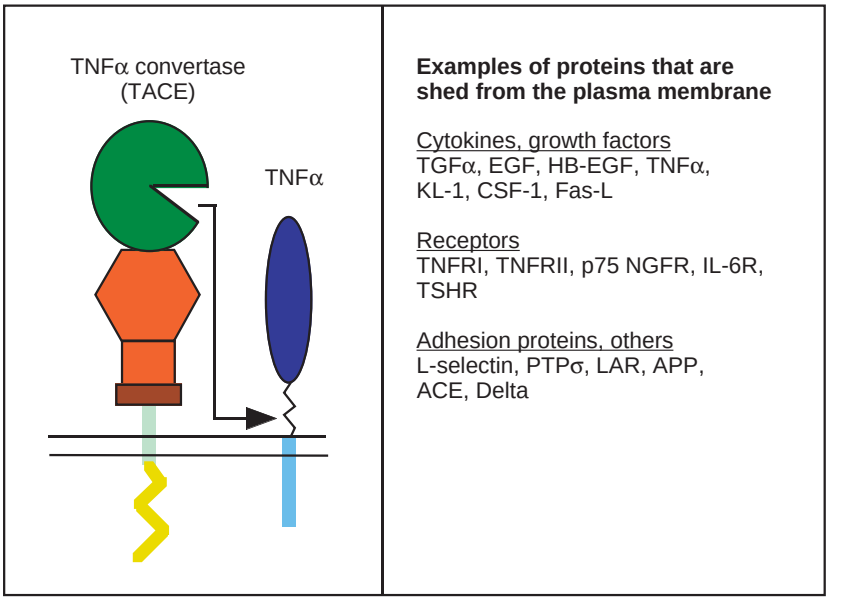

Fig. 2. Cleavage of tumor necrosis factor α (TNFα) by TNFα convertase (TACE). The membrane-anchored pro-inflammatory cytokine TNFα is cleaved by TACE in a membrane-proximal position to release the soluble cytokine (Black et al., 1997; Moss et al., 1997). Examples of other proteins that are made as membrane-anchored precursors and are released from the plasma membrane, in many cases by a metalloprotease, are shown on the right: transforming growth factor α (TGFα) (Massague and Pandiella, 1993; Arribas and Massague, 1995), epidermal growth factor (EGF) (Dempsey et al., 1997), heparin-binding EGF-like growth factor (HB-EGF) (Izumi et al., 1998), TNFα (Black et al., 1997; Moss et al., 1997), c-Kit-ligand-1 (KL-1) (Huang et al., 1992), colony-stimulating factor 1 (CSF) (Stein and Rettenmier, 1991), Fas-ligand (Fas-L) (Tanaka et al., 1998), TNF receptor I (TNFRI, p60 TNFR) (Mullberg et al., 1995), TNF receptor II (TNFRII, p80 TNFR) (Porteu and Nathan, 1990; Porteu et al., 1991), p75 nerve growth factor receptor (p75 NGFR, low-affinity NGFR) (DiStefano and Johnson, 1988), interleukin 6 receptor (IL-6R) (Mullberg et al., 1994, 1995), thyroid-stimulating hormone receptor (TSHR) (de Bernard et al., 1999), L-selectin (Kahn et al., 1994; Migaki et al., 1995), protein tyrosine phosphatase σ (PTPσ), protein tyrosine phosphatase LAR (LAR) (Serra-Pages et al., 1994; Aicher et al., 1997), amyloid precursor protein (APP) (Buxbaum et al., 1990; Selkoe, 1998), angiotensin-converting enzyme (ACE) (Ramchandran and Sen, 1995), Delta (Klueg et al., 1998; Qi et al., 1999).

inability to produce active Delta. In *C. elegans*, however, genetic evidence indicates that SUP-17 has additional functions in LIN-12 signaling (Wen et al., 1997). Specifically, in *C. elegans* and flies, ligand-independent, intracellular forms of LIN-12 and Notch signal in the absence of functional SUP-17 or KUZ; however, SUP-17 is required for the activity of at least one ligand-independent LIN-12 mutant in *C. elegans*. Further analysis will be needed to determine the relative contributions of defects in KUZ-mediated cleavage of Delta and Notch to explain the KUZ phenotype.

Fambrough et al. (1996) identified KUZ independently in a genetic screen for genes involved in axonal guidance in *Drosophila*. *Kuz* mutant embryos show a defect in the axonal guidance of a subset of neurons within the central nervous system. A separate study has found murine KUZ expressed at high levels in nerve fibers as they converge on their targets (Yavari et al., 1998). In PC12 cells, expression of a dominant negative murine KUZ that lacks the metalloprotease domain induces several phenotypic changes that are characteristic of neuronal differentiation, including neurite extension. This last observation is interesting in view of the apparent role of Delta-Notch signaling in inducing neurite retraction in cortical neurons (see above). It will be important to determine whether the role of KUZ in axonal guidance is due to its role in Notch signaling or whether, similarly to TACE, KUZ is involved in the proteolytic cleavage of several proteins responsible for distinct developmental and cell biological processes.

COMMON AND DISTINCT FEATURES OF NOTCH AND β-AMYLOID-PRECURSOR-PROTEIN PROCESSING

The complex proteolytic cleavage of Notch has an intriguing resemblance to the processing of another well-studied transmembrane protein, APP (Selkoe, 1998). APP, which is processed at several distinct sites by at least three different proteolytic activities, termed α-, β- and γ-secretase (Buxbaum et al., 1992; Caporaso et al., 1992; Haass et al., 1992; Shoji et al., 1992), has been linked to the pathogenesis of Alzheimer's disease. Both α-secretase and β-secretase cleave APP in the extracellular domain, whereas γ-secretase acts on the transmembrane domain of APP (see Fig. 3). These secretase activities generate several different peptides. Cleavage of APP by the β- and γ-secretases gives rise to Aβ40 or Aβ42, depending on the exact cleavage site of the γ-secretase. These Aβ peptides are the major component of amyloid plaques, which are considered to be of central importance in the pathogenesis of Alzheimer's disease. Cleavage by the α-secretase leads to the release of a soluble extracellular fragment (APPs), the precise function of which remains to be elucidated in vivo (Selkoe, 1998). The α-secretase can be considered to be a protective factor against Alzheimer's disease, given that processing of APP at the α-secretase site precludes cleavage by the β-secretase and thus formation of Aβ (Caporaso et al., 1992).

As noted above, TACE has been implicated as a regulated α-secretase activity (Buxbaum et al., 1998). More recently, Lammich et al. (1999) presented evidence that KUZ/ADAM10 can also function as an α-secretase. A perplexing question raised by these two studies is whether TACE, ADAM10 or both are physiological α-secretases.

In vitro, both TACE and KUZ can cleave an APP peptide at the same position that is used by the major PMA-dependent α-secretase of most cells (Buxbaum et al., 1992, 1994, 1998; Caporaso et al., 1992; Lammich et al., 1999). However, the lack of PMA-dependent α-secretase activity in TACE-deficient fibroblasts points towards TACE as the major PMA-inducible α-secretase in these cells (Buxbaum et al., 1998). Note that constitutive α-secretase activity is not significantly affected in

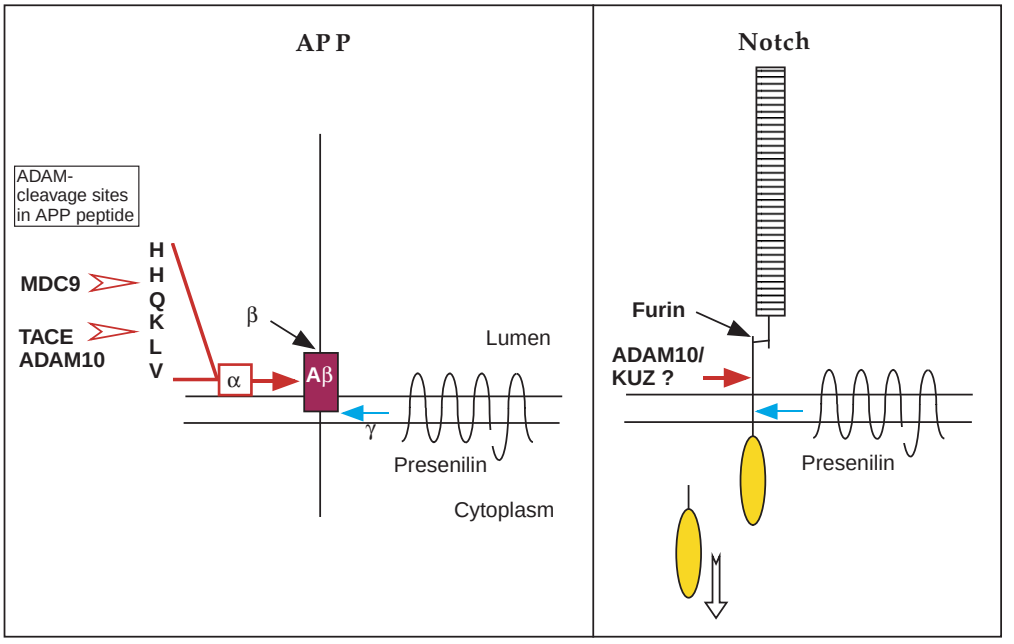

Fig. 3. Parallels in the proteolytic processing of amyloid-precursor protein (APP) and Notch. Proteolytic processing of the APP (left panel), and predicted and known processing events of Notch (right panel). APP can be subjected to at least three distinct processing steps by an α-, β- or γ-secretase (Selkoe, 1998). Sequential processing by the β- and γ-secretases produces the Aβ fragment (brown box). Cleavage of APP by the α-secretase prevents Aβ formation. Recent results implicate TACE and ADAM10 as α-secretases (Buxbaum et al., 1998; Lammich et al., 1999). Both cleave an APP α-secretase-cleavage-site peptide in the position used by the major α-secretase activity in nonneuronal cells (open arrowhead). Furthermore, recombinant MDC9 cleaves the α-secretase-cleavage-site peptide in a position that is also utilized in hippocampal neurons (open arrowhead) (Simons et al., 1996; Roghani et al., 1999). The γ-secretase cleaves APP within its transmembrane domain. Presenilin is required for the γ-secretase activity, and, recently, Wolfe et al. (1999) have proposed that presinilin is the γ-secretase. Notch contains a furin-cleavage site in its extracellular domain and is processed at this site before it emerges on the cell surface (Logeat et al., 1998). Notch signaling through the CSL (CBF1, Su(H), Lag-1) family of transcription factors depends on the release of the Notch cytoplasmic tail from the plasma membrane (Struhl and Adachi, 1998). Functional presenilin is required for cleavage of Notch within its plasma-membrane domain (De Strooper et al., 1999; Struhl and Greenwald, 1999). Given that mutations in KUZ/ADAM10 affect Notch signaling, Chan and Jan (1998) propose that KUZ/ADAM10 is required for a ligand-dependent cleavage of Notch in a membrane-proximal region. To explain the genetic data, the cleavage by ADAM10/KUZ would have to be a prerequisite for the cleavage of Notch by presenilin. ADAM10/KUZ might also affect Notch signaling by cleaving the Notch ligand Delta (Qi et al., 1999).

TACE-deficient fibroblasts. One of the main pieces of evidence for a role of ADAM10 as an α-secretase is that HEK cells overexpressing ADAM10 have an increased constitutive and PMA-stimulated α-secretase activity (Lammich et al., 1999). However, because ADAM10 can cleave the APP peptide, we must consider the possibility that this effect represents a nonphysiological function of ADAM10 that is only apparent when ADAM10 is overexpressed. In comparison, inhibition of constitutive and PMA-dependent APP processing by the dominant negative ADAM10 (ADAM10 DN) construct is more compelling evidence for a physiological role of ADAM10 in APP processing. Several mechanisms for how ADAM10 DN exerts its dominant negative effect are conceivable, including binding and sequestering of a substrate, interfering with an activator or inhibitor of the protease, and/or interfering with its proper subcellular localization or targeting. Alternatively, expression of ADAM10 DN could have an impact on the function of other ADAM proteases, such as TACE, if these other proteases share a common regulatory factor with

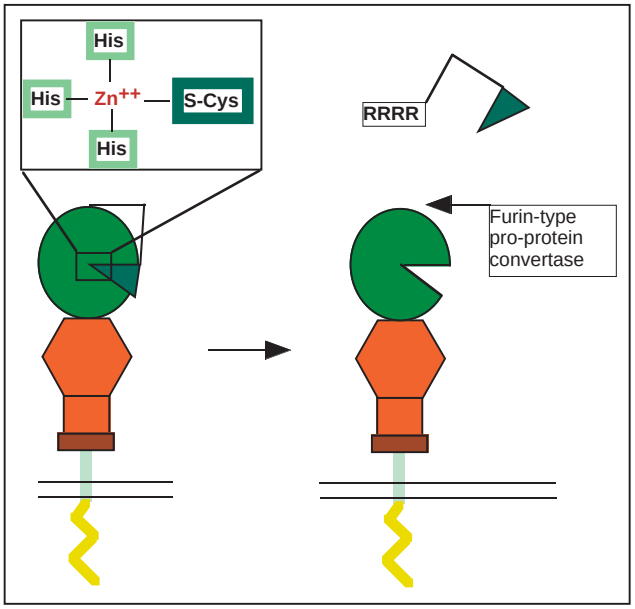

Fig. 4. Cysteine-switch mechanism in a metalloprotease-disintegrin. Metalloprotease-disintegrins that have a catalytic-site consensus sequence contain an odd-numbered cysteine residue in their prodomain that has been proposed to function as a cysteine switch (Van Wart and Birkedal-Hansen, 1990). The Zn^{2+} is coordinated by three histidines in the catalytic site; the free sulfhydryl group of the cysteine residue provides a fourth coordination site. This keeps the protease inactive until the prodomain is removed. For several ADAMs, including MDC9, ADAM12 and MDC15, a proprotein convertase such as furin plays a role in prodomain removal in the *trans*-Golgi network (Loechel et al., 1998; Lum et al., 1998; Roghani et al., 1999). Given that protein-ectodomain shedding is regulated, but prodomain processing by furin is constitutive, there are probably other means of regulating the protease activity following prodomain removal.

ADAM10. There is actually evidence for a common regulator of protein-ectodomain shedding that affects the function of TACE (discussed below), although it is not known whether this regulator is also involved in ADAM10 function.

Clearly, more work needs to be done to resolve the identity of the α-secretase, including analysis of cells lacking ADAM10. If a dominant negative TACE that interferes with TNFα shedding can be designed, for example, by deletion of the entire TACE metalloprotease domain, it will be important to evaluate its effects on α-secretase activity. Other possible explanations for the discrepancy include the fact that the major PMA-dependent α-secretase in fibroblasts is TACE and in HEK cells is ADAM10. Note also that the effect of ADAM10 DN on APP expression in HEK cells was not documented; a decrease in APP expression in cells transfected with ADAM10 DN must therefore be excluded as the cause of the decrease in APP release. Finally, given that most studies of α-secretase activity are performed on nonneuronal cells, it will be important to confirm that candidate α-secretases also functional in neuronal cells. Additional α-secretase activities, besides those that have been described in nonneuronal tissue culture models such as fibroblasts (see below), apparently exist in neurons (Simons et al., 1996). Ultimately, it will be important to evaluate the role of α-secretase(s) in animal models of Alzheimer's disease, in which they are postulated to prevent Aβ formation. If the targeted deletion of a protease results in embryonic lethality, as is the case with TACE, this will require a conditional knockout that, for example, affects only the brain or brain areas involved in memory, such as the hippocampus.

Notch and APP are also subjected to an unusual cleavage within the transmembrane domain (Schroeter et al., 1998; Selkoe, 1998; Struhl and Adachi, 1998). In both cases, this intramembranous cleavage depends on presenilins, integral membrane proteins containing eight membrane-spanning domains. Hardy (1997) first linked presenilins to Alzheimer's disease by identifying mutations in patients who had familial Alzheimer's disease. The mutations increase the γ-secretase activity, which leads to an increase in the level of Aβ42 formed relative to that of Aβ40 (Aβ42 appears to possess a greater potential for forming amyloid plaques and for cellular toxicity). A very exciting recent report by Selkoe et al. provides evidence that presenilin is indeed the γ-secretase, or might at least provide γ-secretase access to APP (Wolfe et al., 1999). Targeted disruption of murine presenilin 1 leads to a strong decrease in γ-secretase activity (De Strooper et al., 1998), whereas overexpression of a mutant presenilin 1 linked to Alzheimer's disease increases γ-secretase cleavage of APP, which results in Aβ42 formation (Duff et al., 1996).

Interestingly, a link between the presinilin SEL-12 and Notch/LIN-12 signaling was initially observed in *C. elegans* (Levitan and Greenwald, 1995). A recent report demonstrates that neurons isolated from wild-type mice are able to cleave a truncated form of Notch within the transmembrane domain, whereas neurons isolated from mice lacking presenilin are not (De Strooper et al., 1999). Genetic analysis in *C. elegans* demonstrates a need for presenilin/SEL-12 for the activity of a form of membrane-anchored LIN-12 that is constitutively active because of a mutation in the extracellular domain (Levitan and Greenwald, 1998). *sel-12* mutants, however, fail to reduce signaling via a constitutively active intracellular fragment of LIN-12 that lacks a membrane anchor. Finally, CSL-dependent Notch signaling in *Drosophila* is abolished in flies that lack presenilin, which provides strong evidence for the idea that presenilin-mediated intramembranous cleavage is essential for Notch signaling (Struhl and Greenwald, 1999).

Sequential proteolytic processing thus appears to be critical for Notch signaling and for the pathogenesis of APP. Although these proteins have distinct functions, they appear to rely on at least some common components such as presenilins and potentially ADAMs. In contrast, the extracellular and intramembranous proteolytic processing of the sterol-regulatory-element-binding protein (SREBP) involves proteases that are distinct from those implicated in processing of Notch and APP (Rawson et al., 1998; Sakai et al., 1998). Sequential proteolytic cleavage of membrane proteins therefore appears to represent a regulatory mechanism that has evolved independently at least twice.

MDC9 MAY BE INVOLVED IN THE ECTODOMAIN SHEDDING OF HB-EGF

Whereas TACE apparently mediates processing of at least one EGFR ligand, TGFα, MDC9 might cleave another EGFR ligand, the heparin-binding EGF-like growth factor (HB-EGF) (Izumi et al., 1998). As in the case of TNFα shedding, HB-EGF shedding is upregulated by treatment of cells with phorbol esters, known activators of protein kinase C (PKC), and a dominant negative form of PKCδ inhibits this upregulation. PKCδ binds to the cytoplasmic tail of MDC9 and can phosphorylate it in vitro (Izumi et al., 1998), and MDC9 is phosphorylated in cells upon phorbol-ester treatment (Roghani et al., 1999). Furthermore, overexpression of full-length MDC9 increases HB-EGF shedding, whereas overexpression of the cytoplasmic domain of MDC9 decreases TPA-induced release of HB-EGF (Izumi et al., 1998). Although the dominant negative effect of an overexpressed MDC9 cytoplasmic domain might be due to sequestration of PKCδ, the dominant negative effect of MDC9 constructs that have mutations in the metalloprotease domain is more difficult to interpret. The particular point mutation used in this study (mutation of zinc-chelating histidines in the HEXXH catalytic site) is known to impair folding and transport of a *Leishmania* metalloprotease to the cell surface (McGwire and Chang, 1996), and deletion of only a part of the metalloprotease domain might have a similar effect (see above). Despite the caveats raised by the dominant negative mutants, these results implicate MDC9 in the ectodomain shedding of HB-EGF. The fact that both HB-EGF (Das et al., 1994) and MDC9 (Olson et al., 1998) are highly expressed at the site of blastocyst implantation, a process that depends on HB-EGF (Raab et al., 1996) is consistent with such a role.

As with the identification of other enzyme-substrate partners, certain criteria must still be met if we are to conclude that MDC9 is the protease responsible for HB-EGF release. Demonstration that MDC9 is capable of directly cleaving HB-EGF at the correct position will require in vitro biochemical analysis. Overexpression of MDC9 could lead to an increase in the amount of HB-EGF released from cells without being the protease that normally cleaves HB-EGF in cells. Therefore, the analysis of animals that lack MDC9 is necessary if we are

to determine whether MDC9 is the protease responsible for cleavage in vivo or whether another, perhaps related, protease is in fact the primary enzyme responsible for the release of HB-EGF.

WHAT DETERMINES SUBSTRATE SPECIFICITY IN METALLOPROTEASE-DISINTEGRINS?

TACE appears to be responsible for the cleavage of several unrelated substrates, including both type I and type II transmembrane proteins (Black et al., 1997; Moss et al., 1997; Buxbaum et al., 1998; Peschon et al., 1998a). ADAM10 cleaves pro-TNF at the physiological site in vitro (Rosendahl et al., 1997); yet analysis of TACE-deficient mice indicates that ADAM10 cannot compensate for TACE-mediated TNFα cleavage in vivo (Black et al., 1997; Peschon et al., 1998a). These findings bring new urgency to the question of how substrate specificity is achieved by metalloprotease-disintegrins. Earlier studies on proteins that undergo ectodomain shedding have indicated that mutations in the primary peptide sequence surrounding the cleavage site have little effect on processing (Ehlers and Riordan, 1991; Sisodia, 1992; Mullberg et al., 1994; Chen et al., 1995; Migaki et al., 1995; Arribas et al., 1997; Hooper et al., 1997). The consensus emerging from these studies is that proteases responsible for ectodomain shedding appear to require at least an 8-10-residue long stretch of presumably unfolded peptide surrounding the target bond, and that the distance from the transmembrane domain also affects the site of proteolysis. Because these experiments were performed in cell-based assays, in which many proteases might be active, they do not necessarily address the question of whether or not a given ADAM has a preference for certain cleavage sites.

The recent determination of the crystal structure of the catalytic domain of TACE by Maskos et al. (1998) has brought insight into the mechanism of enzyme-substrate recognition. The core structure of the metalloprotease domain shows remarkable similarity to the snake-venom metalloprotease adamalysin II (Gomis-Rüth et al., 1993). A notable difference is the presence of several loops within the catalytic domain that form distinct surface projections. The authors speculate that these unique features play a role in recognition of structural features of substrates of TACE (Maskos et al., 1998). Given the apparent lack of sequence specificity immediately surrounding the substrate-cleavage site, these surface projections might contact with areas of the substrate considerably removed from the actual target-peptide bond. ADAM10, whose sequence specificity is very similar to that of TACE in in vitro peptide-cleavage assays, is the only other identified member of the metalloprotease-disintegrin family that includes these potential loop sequences (Maskos et al., 1998).

Although the cleavage specificity of ADAMs in cells is difficult to assess because there are several ADAMs in any given cell, a comparison of the cleavage specificities of purified soluble TACE and MDC9 in vitro indicates that these two ADAMs have distinct substrate specificities, at least with respect to soluble substrate peptides (Roghani et al., 1999). Kinetic analysis shows that cleavage of the APP peptide by MDC9 is remarkably efficient and comparable to the efficiency of cleavage of the TNFα peptide by TACE (Roghani et al., 1999). However, the MDC9-cleavage site in the APP peptide is different from that of ADAM10 and TACE (see above, and Fig. 3). Interestingly, the hippocampus contains an α-secretase activity that cleaves APP at the peptide bond preferred by MDC9 (Simons et al., 1996), whereas most nonneuronal cells and cell lines display an α-secretase activity that cleaves APP only at the position cut by TACE and ADAM10 in vitro (Buxbaum et al., 1998; Lammich et al., 1999) (see Fig. 3). Future genetic and biochemical analysis must establish whether there is indeed a correlation between cleavage site selection on peptides in vitro and cleavage-site selection in cells.

Recently, Lum et al. (1999) used the in vitro substrate specificity of TACE as one criterion to determine whether TACE plays a role in the ectodomain shedding of TRANCE, a member of the TNF family that plays a role in dendritic-cell survival and osteoclast differentiation. Purified TACE can cleave a recombinant TRANCE fusion protein and a cleavage-site peptide at the position at which TRANCE is cleaved in vivo. This suggests that TACE, or a protease that has a similar cleavage-site specificity, is responsible for shedding TRANCE from cells. Researchers must now evaluate TACE-deficient cells for potential defects in TRANCE shedding.

Other parts of ADAMs, such as the disintegrin and/or the cysteine-rich domains, might also contribute to targeting of the catalytic domain to its substrate (Blobel, 1997). Models include direct targeting, in which domains other than the metalloprotease bind to potential substrates either membrane-proximal or distal to the cleavage site, and indirect targeting, in which these domains bind to a third protein, which in turn presents the substrate protein to the protease. Either of these models can be adapted to include the possibility that the substrate and protease are expressed on the same cell (in *cis*), or that the substrate is present on an adjacent cell or in the surrounding matrix (in *trans*). Future comparison of the abilities of the isolated TACE catalytic domain and the catalytic domain in conjunction with its disintegrin and cysteine-rich domains to recognize and cleave substrates will help address the importance of these domains in substrate recognition. Additionally, now that potential substrates have been identified for family members other than TACE, we can address these questions through the use of mutagenesis and domain-swapping experiments.

ECTODOMAIN SHEDDING IS REGULATED

The regulation of substrate cleavage remains a pressing issue in the field of ectodomain shedding. Physiological stimuli, including acetylcholine in the case of APP (Buxbaum et al., 1992; Nitsch et al., 1992) and lipopolysaccharide (LPS) in the case of TNFα (McGeehan et al., 1994), or synthetic compounds, such as phorbol esters and calcium ionophores, dramatically increase the release of numerous shed proteins (Massague and Pandiella, 1993; Hooper et al., 1997). The identification of metalloprotease-disintegrins as the proteases responsible for several of these shedding events has focused attention on regulation of their catalytic activities.

During biosynthesis, catalytically active ADAMs such as MDC9, MDC15 and meltrin α are made as larger, inactive precursors in which the prodomain is attached to the catalytic

domain (Loechel et al., 1998; Lum et al., 1998; Roghani et al., 1999). This is thought to involve a cysteine-switch mechanism, in which a sulfhydryl group of an odd-numbered cysteine in the prodomain interacts with Zn^{2+} in the catalytic site of the metalloprotease, thereby inhibiting the enzyme (Van Wart and Birkedal-Hansen, 1990), is thought to operate (see Fig. 4). Besides its role as an inhibitor of the protease domain, the prodomain appears to be important for the proper maturation and intracellular transport of at least some metalloprotease-disintegrins (Loechel et al., 1999; Roghani et al., 1999). Although prodomain removal is probably a prerequisite for protease activity, this processing appears to be mediated constitutively by a furin-type proprotein convertase in the *trans*-Golgi network (Loechel et al., 1998; Lum et al., 1998; Roghani et al., 1999). Yet the observation that shedding is regulated strongly suggests that ADAMs are subjected to other forms of regulation once the prodomain has been removed.

A recent study demonstrated that TIMP-3, but not TIMP-1, TIMP-2 or TIMP-4, is a potent inhibitor of TACE (K_i'=182 pM) (Amour et al., 1998). TIMPs were first identified as proteins that bind to and inhibit the catalytic domains of matrix metalloproteases (Werb, 1997). The finding that TIMP-3 also inhibits TACE might explain why TIMP-3, but not TIMP-1 or TIMP-2, inhibits ectodomain shedding of TNFα (Smith et al., 1997), L-selectin (Borland et al., 1999) and perhaps also the interleukin 6 (IL-6) receptor (Hargreaves et al., 1998). Future studies will help determine whether TIMP-mediated inhibition is a general principle of metalloprotease-disintegrin regulation, whether other types of inhibitors of ADAMs can bind after the prodomain has been removed, and whether the prodomain can function as a reversible inhibitor.

In order to attempt to identify the proteins involved in regulation of ectodomain shedding of TGFα, Arribas et al. (1996) mutagenized CHO cells stably expressing a tagged form of TGFα and selected clones that failed to shed surface TGFα in response to phorbol esters. They found that two independently isolated, stable cell lines in which PMA-induced TGFα shedding was defective act identically to wild-type cells in all other aspects of TGFα synthesis, maturation and transport to the cell surface. Cell-fusion experiments indicated that both cells harbor a recessive mutation in the same complementation group. The mutant cells also fail to shed several other cell-surface proteins, including APP, L-selectin, the IL-6 receptor and TNFα (Arribas and Massague, 1995; Arribas et al., 1996). Although the defect in pro-TNFα and TGFα cleavage indicates that TACE activity is affected, transfection of TACE into these cells failed to rescue the processing defect (Merlos-Suarez et al., 1998). Fusion of these mutant cells to TACE-deficient cells restored the PMA-inducible release of TNFα, TGFα and APP. Thus, the mutation does not appear to be in TACE itself, although it does affect the function of TACE. Analysis of the various known isoforms of PKC failed to uncover any global defect in PKC function (Merlos-Suarez et al., 1998). Furthermore, the mutant cell line also fails to shed TGFα in response to the calcium ionophore A23187 or serum (Arribas and Massague, 1995). Taken in context with other studies that indicate that phorbol esters and calcium ionophores activate distinct pathways leading to the ectodomain shedding of various proteins, it appears that these cell lines harbor a defect at a point where these different pathways converge but upstream of TACE and perhaps other ADAMs (Merlos-Suarez et al., 1998). Clearly, the molecular identification of the defect in these cells represents an important goal in advancing our understanding of the regulation of shedding.

Additional lines of evidence suggest that ADAMs are regulated by their cytoplasmic domains. The dominant negative effect of a KUZ construct that lacks a metalloprotease domain depends at least in part on a conserved sequence within its cytoplasmic tail (Pan and Rubin, 1997). Similarly, the cytoplasmic domain of MDC9 appears to act as a dominant negative mutant in HB-EGF-shedding experiments, and PKCδ binds to and phosphorylates the cytoplasmic tail of MDC9 in vitro (Izumi et al., 1998). Finally, proline-rich SH3-ligand motifs are found in the cytoplasmic tails of a significant number of metalloprotease-disintegrins (Yagami-Hiromasa et al., 1995; Krätzschmar et al., 1996; Weskamp et al., 1996; Black et al., 1997; Moss et al., 1997; Inoue et al., 1998). Which proteins interact with the cytoplasmic domains of metalloprotease-disintegrins, and which of these modulate the sheddase activity of ADAMs? The cytoplasmic signaling motifs in ADAMs might also affect other functions of these proteins besides their catalytic activity, such as signaling pathways triggered by binding of ADAMs to integrins or other extracellular proteins.

Although evidence suggests that the cytoplasmic domain of the protease regulates ectodomain shedding, there are conflicting reports about the role of the cytotails of the substrates in targeting them for proteolytic processing. In the cases of both TGFα and APP (Arribas et al., 1997), the extracellular juxtamembrane sequence, and not the transmembrane or cytoplasmic tail sequences, appear to be necessary and sufficient to target an unrelated protein for phorbol-ester-induced shedding. In contrast, the cytoplasmic domain of L-selectin seems to play a role in regulating its susceptibility to ectodomain shedding (Kahn et al., 1998). The extent to which the intracellular domain(s) of sheddase substrates are involved or required for the proper regulation of their proteolytic processing remains to be seen.

Taken together, several observations point to the importance of the cytoplasmic domains of ADAMs in upregulation of ectodomain shedding in response to appropriate stimuli. Although the mechanism by which intracellular signaling pathways lead to an increase in proteolytic activity has not yet been established, several potential models can be proposed. The sheddase might be sequestered in a location where it is isolated from its potential substrates. In response to intracellular signaling events, the subcellular localization of the protease could change such that it comes into contact with and then cleaves its substrates. Alternatively, intracellular signaling events might lead to the disruption of a protease-inhibitor complex. It is also possible that a conformational change in the cytoplasmic tail of the metalloprotease-disintegrin induces a complementary change in the structure of the extracellular domains, which leads to protease activation. Currently, we know little about the molecular mechanism through which activators of shedding, such as PKC activators, calcium ionophores and tyrosine phosphatase inhibitors, function. Furthermore, we must determine to what extent phorbol-ester-triggered protein ectodomain shedding reflects the regulation of ADAMs in response to a physiological trigger.

POTENTIAL ROLES FOR ADAMS IN DEVELOPMENT

Two ADAMs, TACE and KUZ, have been shown to have essential roles in development (see above). In contrast, little is known about the functions of other ADAMs in development. Meltrin α is a catalytically active metalloprotease-disintegrin (Loechel et al., 1998) that has a role in myoblast fusion (Yagami-Hiromasa et al., 1995) and is expressed during early mouse development in mesenchymal cells that give rise to skeletal muscle, bones and visceral organs (Kurisaki et al., 1998). Meltrin α might therefore play a role in muscle development. Besides the full-length precursor form of meltrin α (termed ADAM12-L), an alternatively spliced soluble form of the protein lacking the transmembrane and cytoplasmic tail sequences (termed ADAM12-S) exists (Gilpin et al., 1998). When rhabdomyosarcoma cells expressing a truncated form of ADAM12-S (which lacked the prodomain and metalloprotease domain) were injected into nude mice, the resulting tumors were found to contain a pattern of ectopic muscle-cell formation, which was not seen in tumors formed by parental cells (Gilpin et al., 1998). These ectopic muscle cells were derived from the host mouse, and not from the tumor cells. Thus, the soluble form of meltrin α appears to play a role in recruiting cells for muscle formation, although the mechanism by which this occurs remains to be elucidated.

Several other ADAMs are expressed in early development; some of these have interesting expression patterns, including expression of ADAM11a and ADAM13 in neural-crest cells (Alfandari et al., 1997; Cai et al., 1998). Elucidation of the expression patterns of these metalloprotease-disintegrins is a first step in identifying their function in development. Future insights into the role of other ADAMs during development will probably come from analysis of mice carrying targeted disruptions of genes that encode metalloprotease-disintegrins, or transgenic expression of dominant negative forms of the proteins. Furthermore, genetic manipulations in *Drosophila* and *C. elegans* should lead to the identification of genes capable of suppressing or enhancing the effect of mutations in metalloprotease-disintegrins.

ADAMS WITHOUT A MEMBRANE ANCHOR

Besides the membrane-anchored ADAMs discussed above, several metalloprotease-disintegrins lack membrane anchors. These soluble ADAMs, which will not be discussed in any detail here, appear to fall into two groups: those expressed solely without a membrane anchor, such as ADAMTS-1 (Kuno et al., 1997), aggrecanase-1 (ADAMTS-4) (Tortorella et al., 1999), and decysin (Mueller et al., 1997); and those for which the soluble form represents a splice variant of a membrane-bound form, as is the case for ADAM11 (Emi et al., 1993) and ADAM12 (Gilpin et al., 1998). Whereas the models for metalloprotease-disintegrin function in cell-cell interactions and ectodomain shedding have relied on the fact that these proteins are membrane-anchored, the existence of metalloprotease-disintegrins that lack membrane anchors suggests several other possible roles for this family of proteins. Their disintegrin domains could function in a manner analogous to soluble snake-venom disintegrins, mediating de-adhesion by binding to integrins. Alternatively, the presence of novel domains such as thrombospondin (TS) motifs in the ADAMTS proteins might be used to target substrates or anchor these proteins to the extracellular matrix. In such a context, the disintegrin domain could act as a matrix ligand for integrins or other cell surface proteins. Finally, a soluble metalloprotease might be capable of diffusing and cleaving substrates at a distance from the cell where it is produced.

CONCLUSION

Since the discovery of the first metalloprotease-disintegrins less than a decade ago (Yoshida et al., 1990; Blobel et al., 1992; Perry et al., 1992; Emi et al., 1993; Heinlein et al., 1994; Weskamp and Blobel, 1994), almost 30 additional family members have been identified. On the basis of the known functions of only a few of these proteins, potential roles for the metalloprotease-disintegrin family in general have been proposed. Functional studies on fertilin indicate that the disintegrin domain is likely to act in mediating cell-cell adhesion, although the recent description of the fertilin-β-knockout mouse indicates that our understanding of this heterodimeric protein is still incomplete. Biochemical studies of MDC15 and the related SVMPs and soluble disintegrins corroborate the proposed involvement of metalloprotease-disintegrins in mediating adhesion. Future studies in this area should increase our understanding of the binding partners of the disintegrin and cysteine-rich regions, and of the structural basis for ligand specificity. Elucidation of mechanisms that regulate the adhesion properties of the proteins, and of whether disintegrin-mediated adhesion can lead to the activation of intracellular signaling pathways via the cytoplasmic tail, should prove interesting. New insights into the common and distinct functions for the disintegrin and cysteine-rich domains in catalytically active and inactive members of the metalloprotease-disintegrin family are also awaited.

Several of the catalytically active metalloprotease-disintegrins are now implicated in the fascinating process of ectodomain shedding and ligand-dependent protein processing. What are the substrates of the other catalytically active family members? A better understanding of what determines the substrate specificity of these proteases, including the potential role of the disintegrin domain, cysteine-rich region and EGF repeat in this process, and of whether and how the catalytic activity is regulated are also of great importance. Finally, the reason for the evolutionarily conserved pairing of different ADAM protein modules remains a central question, as does the extent to which cooperation between these domains is important for the diverse functions of metalloprotease-disintegrins.

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