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REVIEW

# The ADAMs family of metalloproteases: multidomain proteins with multiple functions

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The ADAMs family of transmembrane proteins belongs to the zinc protease superfamily. Members of the family have a modular design, characterized by the presence of metalloprotease and integrin receptor-binding activities, and a cytoplasmic domain that in many family members specifies binding sites for various signal transducing proteins. The ADAMs family has been implicated in the control of membrane fusion, cytokine and growth factor shedding, and cell migration, as well as processes such as muscle development, fertilization, and cell fate determination. Pathologies such as inflammation and cancer also involve ADAMs family members. Excellent reviews covering various facets of the ADAMs literature-base have been published over the years and we recommend their examination (Black and White 1998; Schlondorff and Blobel 1999; Primakoff and Myles 2000; Evans 2001; Kheradmand and Werb 2002). In this review, we will first discuss the properties of each of the domains of the ADAMs. We will then go on to describe the involvement of ADAMs in selected biological processes. Then, we will highlight recent interesting findings suggesting roles for ADAMs in human disease. Finally, we look to the future and discuss some of the open issues in ADAMs function and regulation.

## ADAMs are members of the zinc protease superfamily

Zinc proteases are subdivided according to the primary structure of their catalytic sites and include gluzincin, metzincin, inuzincin, carboxypeptidase, and DD carboxypeptidase subgroups (Hooper 1994). The metzincin subgroup (to which the ADAMs belong) is further divided into serralysins, astacins, matrixins, and adamalysins (Stocker et al. 1995). The matrixins comprise the matrix metalloproteases, or MMPs. These enzymes are the principle agents responsible for extracellular matrix degradation and remodeling, and play important roles in development, wound healing, and in the pathology of diseases such as arthritis and cancer (Chang and Werb 2001). Adamalysins are similar to the matrixins in their metalloprotease domains, but contain a unique integrin receptor-binding disintegrin domain (Fig. 1). It is the presence of these two domains that give the ADAMs their name (a disintegrin and metalloprotease). The domain structure of the ADAMs consists of a prodomain, a metalloprotease domain, a disintegrin domain, a cysteine-rich domain, an EGF-like domain, a transmembrane domain, and a cytoplasmic tail. The adamalysins subfamily also contains the class III snake venom metalloproteases and the ADAM-TS family, which although similar to the ADAMs, can be distinguished structurally (Fig. 1).

## Expression, subcellular location, and domain activity of the ADAMs family

In humans, there are 19 *adam* genes, as shown in Table 1. In the literature, this family is often also referred to as the MDC family, indicating the presence of metalloprotease, disintegrin, and cysteine-rich domains. Furthermore, individual family members often have two or more names. For clarity, in this review, we will use the ADAM nomenclature for each mammalian family member. However, the alternative names of the individual ADAMs are also provided in Table 1. In total, there have been at least 34 *adam* genes described in a variety of species. Up-to-date registries of all ADAMs family members in different species can be found at: http://www.uta.fi/~loiika/ADAMs/HADAMs.htm, http://www.uta.fi/%7Eloiika/ADAMs/MMADAMs.htm, and http://www.people.virginia.edu/%7Ejw7g/Table_of_the_ADAMs.html.

### *Expression*

ADAMs are found in vertebrates, as well as in *Caenorhabditis elegans*, *Drosophila*, and *Xenopus*. They are not present in *Escherichia coli*, *Saccharomyces cerevisiae*, or plants. The fission yeast *Schizosaccharomyces pombe* has what may be an early progenitor of the ADAMs family, although its properties have not been studied. Expression profiles of the ADAMs can vary con-

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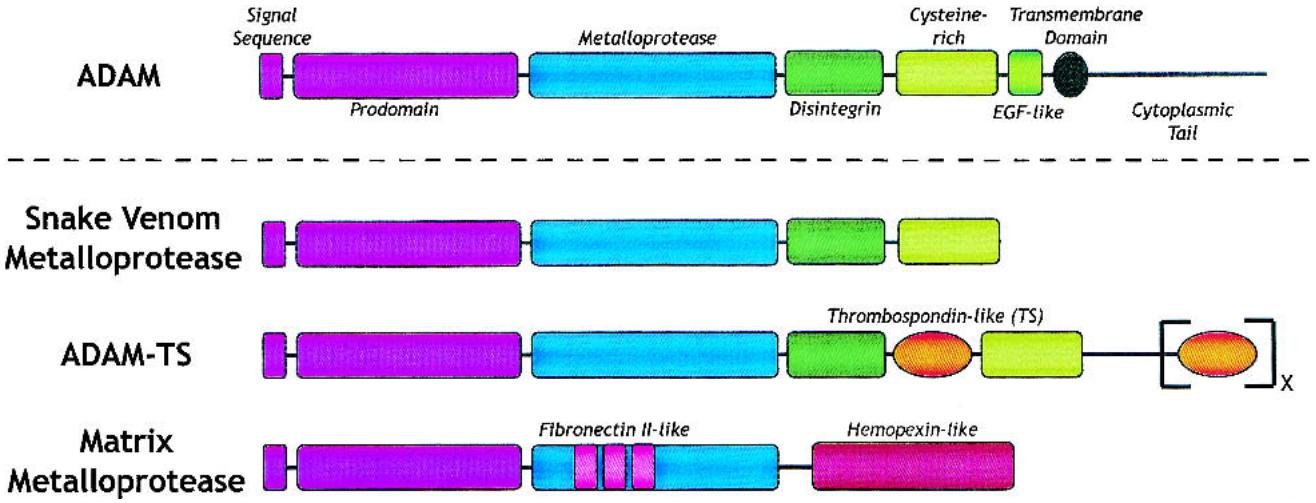


**Figure 1.** The topography of the ADAMs and related metalloproteases. Generalized domain structures of the ADAMs, SVMPs, ADAM-TS, and MMP families are shown. Note that ADAM-TS family members have a variable number of thrombospondin-like (TS) motifs. The MMP shown is of the gelatinase class. Other subclasses of MMPs lack hemopexin-like sequences and/or fibronectin type II-like sequences. The subclass of MT-MMPs have transmembrane domains and cytoplasmic tails in addition to the domains shown.

siderably. In mammals, many of them (including ADAMs 2, 7, 18, 20, 21, 29, and 30) are exclusively or predominantly expressed in the testis and/or associated structures. Other members (ADAMs 8, 9, 10, 11, 12, 15, 17, 19, 22, 23, 28, and 33) show a more broad somatic distribution. Yet, there are clear differences in the pattern of expression of these enzymes (Table 1). ADAMs 9, 12, and 19 were originally cloned from myoblasts (Yagami-Hiromasa et al. 1995), but have been shown subsequently to be more broadly expressed.

Several ADAMs are expressed in multiple splice forms (Table 1). For example, ADAM22 (Sagane et al. 1998), ADAM29 and ADAM30 (Cerretti et al. 1999) have two to three forms that vary in the lengths of their cytoplasmic tails, although no functional differences in these isoforms have been reported. In other cases, alternative splicing produces proteins with markedly different activity. For example, ADAM12 has two splice forms: One, called L, produces a membrane-bound protein, the other, called S, diverges just upstream of the transmembrane domain, which results in a shorter form that is secreted from the cell (Gilpin et al. 1998). Recent studies indicate that ADAM12-S has functional IGFBP-3 and IGFBP-5 protein cleavage activity (Shi et al. 2000). Because ADAM12 is overexpressed during pregnancy, it is possible that ADAM12-S is responsible for increasing the pool of IGF in the bloodstream during pregnancy through IGFBP proteolysis. Alternative splicing of *adam28* yields isoforms with different subcellular localization patterns and tissue expression. Murine ADAM28 may have three forms, two larger ones predicted to encode membrane-anchored proteins and expressed in the epididymis and lung, as well as a smaller one predicted to encode a secreted protein with testis-specific expression (Howard et al. 2000). Meanwhile, human ADAM28 has two forms. In this case, the secreted form is preferentially expressed in the spleen, whereas the membrane-bound form is lymph node specific (Roberts et al. 1999). ADAM9 and ADAM10 are also alternatively spliced and have both secreted and membrane-associated forms (Yavari et al. 1998; Hotoda et al. 2002). Finally, there is evidence that *adam11* and *adam33* genes produce alternative spliced products (Katagiri et al. 1995; Van Eerdewegh et al. 2002).

To date, there have been few studies reporting the phenotypes of mice lacking individual ADAMs family members. However, ADAM2, ADAM3, ADAM9, ADAM10, ADAM17, and ADAM23-deficient mice have been described. Mice lacking either ADAM2 or ADAM3 are viable and healthy with normal development, although male mice are infertile (Cho et al. 1998; Nishimura et al. 2001). The molecular basis for the infertility will be discussed later. Despite the ubiquitous, and in certain tissues high expression of ADAM9, null mice develop normally, are viable and fertile, and do not show any major pathologies (Weskamp et al. 2002). This perhaps suggests redundancy with other members of the ADAMs family. Mice harboring a germ-line mutation in the metalloprotease domain of ADAM17 exhibit perinatal lethality (Black et al. 1997; Peschon et al. 1998). Those born alive usually die within several hours. The more obvious defects in newborns include open eyelids, stunted vibrissae, and wavy hair. Histological studies of mutant fetuses reveal defects in epithelial maturation and organization that impairs the development of the digestive, respiratory, and hormonal systems (Peschon et al. 1998). A gene-trapping analysis of murine genes involved in brain-wiring patterns revealed that homozygous deletion of ADAM23 results in tremor and ataxia (Leighton et al. 2001). There is a recent report describing the targeted disruption of the *adam10* gene (Hartmann et al. 2002). ADAM10--deficient mice die by day 9.5 of embryogenesis with pronounced defects in the neural and cardiovascular systems. ADAM12, ADAM15, and ADAM19 null em-

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**Table 1.** *Human ADAMs*

| ADAM | Common name(s) | Potential functions | Expression | Alternative splicing | Domain function: MP active | Domain function: Integrin binding | Domain function: PxxP |
|---|---|---|---|---|---|---|---|
| 2 | fertilin-β, PH-30β | sperm/egg binding/fusion | testis[1] | | | √ | |
| 7 | EAP1 | | epididymis[2] | | | | √ |
| 8 | MS2, CD156 | | granulocytes/ monocytes[3] | | √(d) | | √ |
| 9 | meltrin-γ, MDC9 | sheddase, cell migration | somatic[4,5] | √(FL,s)[5] | √(d) | √ | √ |
| 10 | Kuz, MADM, SUP-17 | sheddase; cell fate determination | somatic[6] | √(L,S)[7] | √(d) | | √ |
| 11 | MDC | putative tumor repressor | brain[8] | √[9,10] | | | |
| 12 | meltrin-α | sheddase, myoblast fusion | somatic[11,12,13] | √(L,S)[13] | √(d) | √[a] | √ |
| 15 | metargidin, MDC15 | cell/cell binding | somatic[14] | | √(p) | √ | √ |
| 17 | TACE | sheddase | somatic[15] | | √(d) | | √ |
| 18 | tMDCIII | | testis[16] | | | | |
| 19 | meltrin-β, MADDAM | sheddase, dendritic cell dev. | somatic[11,17] | | √(d) | | √ |
| 20 | | | testis[18] | | √(p) | | |
| 21 | | | testis[18] | | √(p) | | |
| 22 | MDC2 | | brain[8,19] | √(γ,δ,ε)[19] | | | √ |
| 23 | MDC3 | cell adhesion/neural dev. | brain[8,20] | | | √ | |
| 28 | MDC-L | immune surveillance | epididymis, lung, lymphocytes[21,22,23] | √(ms,)[21] | √(d) | √ | |
| 29 | | | testis[24,25] | √(α,β,γ)[24] | | | √ |
| 30 | | | testis[24] | √(α,β)[24] | √(p) | | |
| 33 | | genetically linked to asthsma | somatic[26] | √ | √(p) | | √ |

[1](Gupta et al. 1996) [2](Lin et al. 2001) [3](Yoshiyama et al. 1997) [4](Weskamp et al. 1996) [5](Hotoda et al. 2002) [6](Chantry and Glynn 1990) [7](Yavari et al. 1998) [8](Sagane et al. 1998) [9](Katagiri et al. 1995) [10](Wu et al. 1997) [11](Yagami-Hiromasa et al. 1995) [12](Harris et al. 1997) [13](Gilpin et al. 1998) [14](Kratzschmar et al. 1996) [15](Patel et al. 1998) [16](Frayne et al. 2002) [17](Kurisaki et al. 1998) [18](Poindexter et al. 1999) [19](Harada et al. 2000) [20](Cal et al. 2000) [21](Roberts et al. 1999) [22](Howard et al. 2000) [23](Howard et al. 2001) [24](Cerretti et al. 1999) [25](Xu et al. 1999) [26](Yoshinaka et al. 2002)

MP Active refers to either predicted (p) or demonstrated/ published (d) activity based on the amino acid sequence of the catalytic active site.

[a]Reference to the syndecan-binding activity of ADAM12's cytsteine-rich domain (see text).

PxxP refers to the presence of SH3-binding sites in cytoplasmic tail domains (also see Fig. 2).

bryos have been created, but the phenotypes, if any, have yet to be reported (Schlondorff et al. 2001).

### *Subcellular location*

The prevailing data indicate that ADAMs are probably synthesized in the rough endoplasmic reticulum and mature in a late Golgi compartment (Lum et al. 1998; Roghani et al. 1999; Hougaard et al. 2000; Howard et al. 2000; Schlondorff et al. 2000; Kang et al. 2002). Maturation involves the removal of the prodomain from the ADAM precursor protein, which is thought to make the ADAMs metalloprotease competent. However, the intracellular localization in which the recognition and cleavage of ADAMs substrates occurs is still a matter of debate. Where studied, it appears that the bulk of the ADAM protein resides in a region near the nucleus, in some cases colocalizing with Golgi markers (Lum et al. 1998; Lammich et al. 1999; Hougaard et al. 2000; Schlondorff et al. 2000; Kang et al. 2002). However, ADAMs can also be detected on the cell surface. In many cases (e.g., ADAMs 9, 10, 15, 17, and 28), the cell surface form appears to be processed and thus catalytically active (Black et al. 1997; Lum et al. 1998; Lammich et al. 1999; Roghani et al. 1999; Howard et al. 2000; Schlondorff et al. 2000). There is one report describing regulated transition from intracellular compartments to the cell surface. The alternatively spliced short form of ADAM12 (ADAM12-S) transits through the endomembrane system and is secreted, but the large form, ADAM12-L, is retained in the trans-Golgi network (Hougaard et al. 2000; Kadota et al. 2000). Removal of the cytoplasmic domain of ADAM12-L blocks retention and leads to cell surface accumulation, suggesting the presence of a retention signal within a region composed of the transmembrane domain and cytoplasmic tail (Hougaard et al. 2000). It has been speculated that an intracellular pool of ADAM12 could be released in a regulated manner for cell surface expression, and perhaps activity, by removing these constraints. Nevertheless, several ADAMs family members may be active intracellularly. For example, most of the mature form of ADAM15 is resistant to trypsinization treatment, indicating a predominantly intracellular pool (Lum et al. 1998; Howard et al. 2000). Furthermore, the metalloprotease activity of ADAMs 10, 17, and 19 can occur within intracellular compartments

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(Skovronsky et al. 2000; Shirakabe et al. 2001). Such differences in subcellular localization and activity may ultimately depend on the cell type, the ADAM, and the substrate involved.

### *The prodomain*

The N terminus of ADAMs contains a signal sequence that directs ADAMs into the secretory pathway and a prodomain that functions in ADAMs maturation (Fig. 1). Primarily, the prodomain keeps the metalloprotease site of ADAMs inactive, through a cysteine switch (Van Wart and Birkedal-Hansen 1990; Becker et al. 1995). A conserved cysteine residue within the prodomain preferentially coordinates the required active site zinc atom, and thereby sequesters the metalloprotease domain in an inactive conformation. Pharmacological inhibitors of the early secretory pathway like brefeldin A and monensin block the processing of ADAM9 and ADAM15, thus positioning the location of ADAMs processing and activation at the trans-Golgi network (Lum et al. 1998; Roghani et al. 1999; Howard et al. 2000; Kang et al. 2002). This location is consistent with the localization of furin and other proprotein convertases (PCs; Nakayama 1997). PCs cleave the prodomain from the rest of the protein at a conserved Rx(R/K)R motif, effectively releasing the prodomain and switching the zinc coordination to the metalloprotease domain, thereby making it available for catalytic activity. Support for such a mechanism comes from a number of experimental studies.

Firstly, furin cleaves ADAM15 in vitro (Lum et al. 1998). Secondly, overexpression of PCs such as PC7 and furin increase the amount of processed ADAM10 in vivo, and processing is blocked by the addition of a peptide analog of the PC cleavage site (Anders et al. 2001). Thirdly, mutation of PC cleavage sites blocks the processing of ADAM10, ADAM12, and ADAM19 to their mature, active forms (Loechel et al. 1998; Anders et al. 2001; Kang et al. 2002). Together, these data indicate that, in vivo, cleavage of the prodomain is a prerequisite for the generation of an active protease. Further support for the model comes from the observation that, in vitro, the inhibitory effect of a PC cleavage site mutation is overcome by treatment with NEM, a sulfhydryl reactive compound that alkylates the cysteine residue, thereby switching the coordination of zinc to the active site of the metalloprotease and bypassing the cleavage dependence of protease activation (Loechel et al. 1999). Mutation of the cysteine residue in the prodomain of ADAM12 to an alanine or histidine also leads to protease activation independently of prodomain cleavage (Anders et al. 2001). Finally, the application of cysteine switch peptides to cells inhibits ADAM9 and ADAM17, presumably because the peptide competes for zinc coordination in trans (Roghani et al. 1999). It is likely that this mechanism of maturation and activation applies to most ADAMs metalloproteases. However, there are cases in which ADAMs may undergo autocatalytic activation. This is most clearly shown with ADAM8 and ADAM28, in which activity-blocking mutations in the metalloprotease domains produces only the precursor form of the protein in transfected cells (Howard et al. 2000; Schlomann et al. 2002).

The secondary function of the prodomain is to chaperone the proper folding of ADAMs, particularly the metalloprotease domain. This has been suggested by studies showing that the removal of the prodomain of ADAM17 generates a protease-inactive protein (Milla et al. 1999). Similarly, an ADAM10 construct lacking its prodomain is catalytically inactive in vivo. But cotransfection of this form together with a construct expressing just the prodomain of ADAM10 generates protease activity (Anders et al. 2001). Additionally, a form of ADAM12-S lacking a prodomain, unlike wild-type protein, is not secreted from the cell, but instead remains in the early endomembrane system (Loechel et al. 1999). Deletion of both the prodomain and the metalloprotease domain allows for secretion of the protein. Taken together, these data suggest that prodomain deletion constructs are synthesized in an inactive form because they are improperly folded during synthesis. Hence, the prodomain appears to be necessary for maintaining the latency of these enzymes, and it assists in the proper folding of ADAMs, in the structuring of the catalytic active site, and in the proper transit of ADAMs throughout the secretory pathway.

### *The metalloprotease domain*

The crystallization of the metalloprotease domain of several metzincin family members, including ADAM17, has allowed the mechanism of proteolytic activity to be more accurately defined (Maskos et al. 1998). The active site contains zinc and water atoms that are necessary for the hydrolytic processing of protein substrates, and which are coordinated by three conserved histidine residues and a downstream methionine. The methionine lies in a Met turn motif that loops around to face the consensus HExxHxxGxxH site. There is remarkable conservation within this catalytic site among the various metzincins, however, individual proteins within the family have distinguishing structural features that may impart specificity for substrates and protease inhibitors (Stocker et al. 1995). On the basis of this structural definition, 12 of the human ADAMs are predicted to be proteolytically active (Table 1), although in only half of these cases has protease activity been measured.

Is there any selectivity to the protease activities of individual ADAMs? This has not yet been examined directly in vitro, although examination of the substrate specificities of some ADAMs in cell-based assays has suggested that this may be the case. Mixture-based oriented peptide libraries have been used to examine the cleavage site motifs for six members of the related MMP family (Turk et al. 2001). The data obtained suggest that MMPs do have some innate specificity for particular cleavage sites, although it is to be expected that features such as colocalization will also play a role in substrate selection. It will be important to test ADAMs specificity using such peptide libraries.

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Inhibitors of ADAMs metalloprotease activity fall into four broad classes: those that inhibit by denaturation; those that inhibit by Zn-chelation; small molecule inhibitors of catalysis; and proteinacious inhibitors called TIMPs. The first two categories represent nonselective inhibitors such as reducing agents or zinc chelating agents. The third class arose from efforts to develop inhibitors of both MMPs and ADAMs, and comprise hydroxamate-based inhibitors that bind competitively to the active site. These have proved to be useful tools for studying ADAMs and MMPs (for review, see Moss et al. 2001). The crystal structure of ADAM17 bound to a compound called IC-3 suggests that hydroxamate inhibitors replace Zn-coordinating water molecules in the active site (Maskos et al. 1998). Other chemical features, such as a peptide chain that aligns along the side of the active site, and a hydrophobic moiety within an active site pocket, define their fit and potency as metalloprotease inhibitors.

Several hydroxamate-based inhibitors have been tested in preclinical and clinical settings (Hidalgo and Eckhardt 2001). One subset, which includes batimastat and marimastat, were designed to mimic the cleavage site of collagen, an MMP substrate. Other hydroxamate inhibitors include synthetic side chains that maximize fit into the catalytic site (e.g., CGS-27023). However, despite their potency, these small molecule inhibitors are not always selective for MMPs. A direct comparison of the selectivity of some of the more commonly used metalloprotease inhibitors indicates that they are equally potent inhibitors of ADAMs (Roghani et al. 1999; for review, see Moss et al. 2001). Batimastat and Ro-31-9790 inhibit ADAM17 better than several MMPs (Barlaam et al. 1999; Amour et al. 2000). Furthermore, clinical testing of marimastat revealed side effects that may well be due to inhibition of other adamalysins (Hidalgo and Eckhardt 2001). Efforts continue in generating specific and selective metalloprotease inhibitors, and many of these new compounds exhibit better selectivity for either MMPs or ADAMs (Kottirsch et al. 2002; Sawa et al. 2002), and discriminate between various ADAMs-dependent growth factor shedding activities (Parkin et al. 2002). Nevertheless, caution must be used in interpreting studies that use inhibitors as the sole criterion for implicating either MMP or ADAMs in a given biological process.

Tissue inhibitors of metalloproteases, or TIMPs, are endogenous regulators of MMPs (Brew et al. 2000). There are four known TIMPs in vertebrates, all of which exhibit high potency for MMP inhibition. The N-terminal domain of TIMPs fits like a wedge into the catalytic site of MMPs, whereas the C-terminal domain probably imparts binding specificity. However, the TIMPs are not totally selective for MMPs. TIMP-3 also inhibits ADAM17 (Amour et al. 2000) and ADAM12 (Loechel et al. 2000), as well as ADAM-TS4 and ADAM-TS5 (Kashiwagi et al. 2001). ADAM10 is inhibited by both TIMP-1 and TIMP-3 (Amour et al. 2000). But not all ADAMs are sensitive to TIMP3; ADAM8 and ADAM9 processing of myelin basic protein is not inhibited by any TIMP (Amour et al. 2002). As with the hydroxymates, TIMPs with targeted mutations that alter their specificity have been generated. It will be interesting to test these proteins in cell-based assays, as well as their therapeutic potential in preclinical models.

There is also an activator of metalloproteases, 4-aminophenylmercuricacetate or APMA. The mechanism for its activation was originally thought to be through displacement of the prodomain from the metalloprotease domain through preferential association with the key cysteine switch residue. However, mutational studies of MMP prodomains suggest that the activation mechanism may be more complicated and related to prodomain conformational changes (Galazka et al. 1999). APMA also activates ADAMs-dependent growth-factor shedding, presumably through a similar mechanism to that of MMP activation (Milla et al. 1999; Merlos-Suarez et al. 2001).

### *The disintegrin domain*

The disintegrin domain is named for its presence in the snake venom metalloproteases (SVMPs), in which it is involved in binding of platelet integrin receptors. This prevents the association of platelets with their natural ligands such as fibrinogen, and results in a block in platelet aggregation at the wound site. (This disintegrin-mediated interaction of SVMPs along with the breakdown of basement membrane components by their metalloprotease activity leads to the severe hemorrhaging caused by bites from snakes harboring these toxins.)

The disintegrin domain of ADAMs proteins is ~90 amino acids long. Structurally, there is little known about the disintegrin domain of ADAMs, although one can gather information from the structural studies of SVMP crystals and other integrin receptor ligands (Gomis-Ruth et al. 1994). The disintegrin domains of SVMPs mimic the ligand site of matrix proteins like fibronectin for integrin receptors. Like fibronectin, many have an RGD consensus sequence within a 13 amino acid stretch called the disintegrin loop, which projects from the surface of the protein and confers binding to αIIbβ3 and αvβ3 integrin receptors (Blobel et al. 1992). The disintegrin domain of human ADAM15 also has a conserved RGD sequence, and associates with αvβ3 and α5β1 in an RGD-dependent manner (Nath et al. 1999; Eto et al. 2002). Most ADAMs (including murine ADAM15) do not have the RGD sequence in their disintegrin loop, even though the disintegrin domains of many ADAMs do associate with integrin receptors. How does this association take place?

There is a subfamily of integrin receptors, the α4/α9 subfamily, that do not recognize RGD, but instead bind to aspartic acid-containing sequences in proteins like fibronectin, VCAM-1, MadCAM-1, and tenascin-C (Zhu and Evans 2002). The disintegrin loops of each ADAM, except for 10 and 17, also have similar aspartic acid-containing sequences. Many ADAMs share a sequence (Rx6DEVF) in the disintegrin domain that, when mutated, inhibits the association with α9β1integrins (Eto et al. 2002). Consistent with this, all ADAMs tested except

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ADAM10 and ADAM17 can bind to α9β1 (Eto et al. 2002). But ADAMs can also associate with other integrin receptors. For example, ADAM28 binds α4β1 (Bridges et al. 2002), ADAM15 associates with αvβ3 and α5β1 (see previous paragraph), and several ADAMs can associate with α6β1 integrin receptors (Chen et al. 1999a,b; Nath et al. 2000). The structural basis for these associations has yet to be defined.

### *The cysteine-rich and EGF-like domains*

If there were a black box in the study of ADAM domains, it would be the cysteine-rich and EGF-like domains. What is known about these domains does not appear to provide any of the unifying functional themes that characterize the metalloprotease and disintegrin domains. It was originally noted that ADAMs 1, 3, 12, and 14 have a motif in their cysteine-rich domain that is very similar to sequences found in viral fusion peptides (Blobel and White 1992). This, coupled with the observations that ADAMs 1, 3 and 12 participate in cell fusion reactions, led to the proposal that the cysteine-rich domain is involved in membrane fusion. But this hypothesis has not been borne out by experimentation. It is more likely that the cysteine-rich domain complements the binding capacity of the disintegrin domain, and perhaps imparts specificity to disintegrin domain-mediated interactions. For example, the cysteine-rich (and perhaps also the disintegrin) domain of ADAM12 promotes the adhesion of fibroblasts and myoblasts (Zolkiewska 1999). Also, the disintegrin and cysteine-rich domains of ADAM13 bind to both fibronectin and to β1-containing integrin receptors, and binding can be inhibited with antibodies to the cysteine-rich domain (Gaultier et al. 2002).

Perhaps the most compelling piece of data concerning a cysteine-rich domain-specific function is that it acts as a ligand for the cell-adhesion molecule syndecan. This was first discovered in a study showing that the cysteine-rich domain of ADAM12 supports the in vitro binding of several different tumor cell lines, as well as a variety of nontumorigenic cells of bone and muscle origin, through the interaction with cell surface heparan-sulfate proteoglycans (Iba et al. 1999, 2000). Transfection of syndecans renders cells competent for adhesion to the cysteine-rich domain of ADAM12. Furthermore, affinity chromatography experiments suggest an interaction between syndecan-4 and ADAM12 (Iba et al. 2000). Other work has suggested coordination between syndecans and integrins in the ADAMs-dependent mediation of cell attachment and cell spreading respectively.

### *The cytoplasmic tail*

The cytoplasmic tails of the ADAMs family are highly variable both in length and in sequence (Fig. 2). This domain contains specialized motifs that have been postulated to be involved in the inside-out regulation of metalloprotease activity, the outside-in regulation of cell signaling, and/or the control of maturation and subcellular localization. The most common motifs are PxxP binding sites for SH3 domain-containing proteins. These SH3-binding sites are present in human ADAMs 7, 8, 9, 10, 12, 15, 17, 19, 22, 29, and 33 (Fig. 2; Table 2). Several ADAMs also have potential phosphorylation sites for serine-threonine and/or tyrosine kinases. Not only might this regulate ADAM function directly, but the resulting phosphotyrosine residues could also provide ligands for SH2 domain-containing proteins. Consequently, ADAMs may serve adaptor functions to assemble complexes of proteins at critical sites of functional activity. The list of proteins that have been shown to interact with ADAMs either by in vitro or in vivo assays is shown in Table 2.

ADAM15 has an extensive array of protein–protein interaction sites, including eight possible SH3-binding domains and four potential sites for tyrosine phosphorylation/SH2-association (Fig. 2). Perhaps not surprisingly, ADAM15 associates with a number of different proteins including adaptors (endophilin I, SH3PX1, and Grb2), and three Src family tyrosine kinases (Src, Lck, and Hck; Howard et al. 1999; Poghosyan et al. 2001). Most of these associations have only been reported in vitro, although an in vivo association between ADAM15 and Lck was observed in JURKAT cells. ADAM15 is also a substrate for Lck and Hck, and its tyrosine phosphorylation greatly impacts the specificity of its associations. For example, the association of ADAM15 with Hck is stimulated by Hck-dependent ADAM15 phosphorylation. The SH2 domain of Hck can associate with ADAM15, presumably via one of the four tyrosines in the tail of ADAM15. Tyrosine phosphorylation also impacts the association of ADAM15 with Lck and Grb2, although, in this case, it may be more related to conformational changes in the presentation of nearby SH3-binding domains, and not necessarily in the formation of an SH2 domain attachment site. A physiological context for the association of ADAM15 with these proteins has not yet been reported.

ADAM12 has 10 possible SH3-binding domains and 2 potential sites for tyrosine phosphorylation (Table 2). Like ADAM15, ADAM12 has also been reported to associate with Src, the related kinase Yes, and Grb2 (Kang et al. 2000; Suzuki et al. 2000). In C2C12 myoblasts, this is mediated by the SH3 domain of Src and the most membrane proximal of the proline motifs in ADAM12. ADAM12 is also a substrate for Src at its C-terminal tyrosine residue. An association of ADAM12 with the p85α subunit of phosphatidylinositol (PI) 3-kinase has been reported to activate PI-3 kinase (Kang et al. 2001). ADAM12 also associates with α-actinin-1 and 2, through interaction of either the spectrin-like repeats or the C-terminal EF hand-containing region of α-actinin with the membrane proximal portion of the tail of ADAM12 (Galliano et al. 2000).

ADAM9, like ADAM15, binds to endophilin I and SH3PX1 (Howard et al. 1999). In this case, the interactions appear to favor the unprocessed, intracellular forms of these ADAMs. Given the potential function of endophilin I and SH3PX1 in vesicle sorting, it is speculated that these interactions may play a part in the regulation of ADAM maturation and/or subcellular localization.

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**Figure 2.** The cytoplasmic tails of the ADAMs. Shown is an alignment of the cytoplasmic tails of all human ADAMs, made using CLUSTALW. PxxP motifs, predicted to be able to associate with SH3 domains, are shaded in blue.

The membrane proximal region of the tail of ADAM9 associates with the catalytic domain of protein kinase C (PKCδ; Izumi et al. 1998). The tail of ADAM9 can be phosphorylated by PKC in vitro at one or more serine and threonine residues. It is speculated that PKCδ helps to recruit ADAM9 to specific sites on the plasma membrane and upon phosphorylation/activation of ADAM9, HB-EGF shedding occurs (see below).

Yeast 2-hybrid analysis was used to detect an association between MAD2 and ADAM17 and between MAD2β and ADAM9 (Nelson et al. 1999). MAD2 is a component of the spindle assembly checkpoint mechanism. MAD2β shows 25% similarity to MAD2. These associations occur in regions of ADAMs that have PXXP motifs, yet MADs lack discernible SH3 domains. Perhaps these associations are involved in coordinating ADAM function with the cell cycle, or in a regulatory process by which MADs compete for binding of SH3 domain-containing proteins (Nelson et al. 1999).

## The shedding of cytokines and cytokine receptors

Cytokines and their receptors play important roles during development and in inflammatory processes. They are elicited both as a response to infection by microorganisms and also during disease processes such as rheumatoid arthritis and cancer. ADAMs-mediated shedding of both cytokines and cytokine receptors has been reported, and much attention has been given to the possible use of ADAMs inhibitors as therapeutics for various diseases. We will discuss selected examples of cytokine and cytokine receptor shedding below.

### *Cytokines*

Perhaps the most well-studied member of the ADAMs family is TNFα converting enzyme (TACE) or ADAM17 (Black 2002). TNFα is a major immunomodulatory and proinflammatory cytokine. It exists in two forms, a 223 amino acid precursor form anchored to the membrane, and a 146 amino acid soluble form, generated by precursor cleavage and comprising the ectodomain. ADAM17 was originally identified by screening for an activity that functioned in TNFα peptide cleavage assays, and could release TNFα into the culture medium of live cells. Confirmation of its activity came with the observation that TNFα processing was significantly inhibited by targeted disruption of the *adam17* gene (Black et al. 1997; Moss et al. 1997). However, processing of TNFα can still occur in cells derived from ADAM17-deficient mice. This residual sheddase activity is inhibited by the metalloprotease inhibitor IC-3, suggesting that other ADAMs family members may also process TNFα (Reddy et al. 2000). Both ADAM9 and ADAM10 cleave TNFα peptides in

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**Table 2.** *Proteins that associate with the cytoplasmic tails of ADAMs*

| ADAM-associated protein | ADAM | Mechanism | Technique |
|---|---|---|---|
| Abl | ADAM12[1] | SH3 | pd |
| | ADAM13[2] | SH3 | pd |
| | ADAM15[3] | SH3 | pd |
| α-actinin-1 | ADAM12[4] | c-tail (mapped) | fw, pd, tip, col |
| α-actinin-2 | ADAM12[5] | c-tail (mapped) | yh, pd, tip |
| Bcr-Abl | ADAM15[3] | c-tail, substrate | fw |
| endophilin I | ADAM9[6] | SH3 (mapped) | yh, pd |
| | ADAM15[6] | SH3 (mapped) | yh, pd |
| | ADAM19[6] | SH3 | yh |
| ERK | ADAM17[7] | substrate | pd, eip |
| Fyn | ADAM15[3] | SH3 | pd |
| Grb2 | ADAM12[1] | SH3 | pd, eip, col |
| | ADAM15[3] | c-tail (mapped) | pd |
| Hck | ADAM15[3] | SH2, SH3, substrate | pd, tip, fw |
| Lck | ADAM10[3] | c-tail | pd |
| | ADAM15[3] | SH3 (mapped), substrate | pd, eip, fw |
| MAD2 | ADAM10[3] | c-tail | pd |
| | ADAM15[3] | c-tail | pd |
| | ADAM17[3] | c-tail | pd |
| | ADAM17[8] | c-tail (mapped) | yh, tip |
| MAD2β | ADAM9[8] | c-tail (mapped) | yh, pd |
| | ADAM12[8] | c-tail | yh |
| | ADAM15[8] | c-tail | yh |
| PACSIN2 | ADAM13[2] | SH3 | pd, col |
| PI3kinase (p85α) | ADAM12[1] | SH3 | pd |
| | ADAM12[9] | SH3 (mapped) | pd, fw, tip, col |
| PKCδ | ADAM9[10] | c-tail (mapped), substrate | tip, fw |
| | ADAM12[11] | not specified | yh |
| SH3PX1 | ADAM9[6] | SH# (mapped) | yh, pd |
| | ADAM15[6] | SH3 (mapped) | yh, pd |
| Src | ADAM9[12] | SH3 (mapped) | fw |
| | ADAM12[1] | SH3, substrate | pd, eip, col |
| | ADAM12[13] | SH3 (mapped) | pd, fw, tip, eip |
| | ADAM13[2] | SH3 | pd |
| | ADAM15[3] | SH3 | pd |
| Yes | ADAM12[1] | SH3 | pd |

[1](Suzuki et al. 2000) [2](Cousin et al. 2000) [3](Poghosyan et al. 2001) [4](Cao et al. 2001) [5](Galliano et al. 2000) [6](Howard et al. 1999) [7](Diaz-Rodriguez et al. 2002) [8](Nelson et al. 1999) [9](Kang et al. 2001) [10](Izumi et al. 1998) [11](Asakura et al. 2002) [12](Weskamp et al. 1996) [13](Kang et al. 2000)

Mechanism indicates the mechanism/location of the ADAM/protein interaction (c-tail, SH3 domain) and whether there was any attempt at mapping the binding site; substrate indicates that the ADAM is a kinase substrate. Techniques key: yh, yeast 2-hyrid; pd, GST pull down; fw, far Western blot; col, colocalization; tip, immunoprecipitation in transiently transfected cells; eip, endogenous immunoprecipitation.

vitro (Lunn et al. 1997; Rosendahl et al. 1997; Roghani et al. 1999; Amour et al. 2000), although expressing ADAM10 in ADAM17 null cells does not reconstitute full TNFα shedding (Reddy et al. 2000). It is likely that the exact composition of TACE activity is dependent on the expression levels of ADAM17 and other ADAMs, in particular tissues and cell types. Nevertheless, a major role for ADAM17 in cytokine biology is supported by the observation that its expression increases during the inflammatory response (Patel et al. 1998; Colon et al. 2001). Because the production of TNFα has been implicated in the development of several inflammatory diseases, including arthritis and chachexia, understanding the mechanism of shedding of cytokines by ADAMs is of great therapeutic relevance.

Fractalkine is a chemoattractant cytokine that is thought to function in both adhesion (when membrane anchored) and chemoattraction (when soluble) of inflammatory cells bearing its receptor (Rossi and Zlotnik 2000). Fractalkine shedding from cell surfaces is inhibited by GM6001, and is therefore dependent on metalloprotease activity. ADAM17 null cells fail to shed fractalkine in response to a TPA stimulus (Garton et al. 2001), suggesting that ADAM17 is the responsible enzyme.

### *Cytokine receptors*

The activity of TNFα receptors is also controlled by sheddases. Proteolysis of the p75 TNF receptor is blocked by a dominant-negative ADAM17 construct

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lacking its metalloprotease domain. Both the p55 and p75 TNF receptors are processed in ADAM17 null cell lines reconstituted with functional ADAM17, but not in the null cells (Solomon et al. 1999; Reddy et al. 2000; Zhang et al. 2001). Thus, both the cytokine and its receptor appear to be processed predominantly by ADAM17. Because in one case the activity of ADAM17 has a positive effect (by generating active cytokine), whereas in the other case the effect would be to inhibit cytokine action (by removing active receptor from the cell surface), it will be interesting to determine how the relative amounts of these activities impact inflammatory as well as other TNFα-regulated processes.

Interleukin1 (IL-1) receptor-II shedding also does not occur in ADAM17-deficient fibroblasts (Reddy et al. 2000). Reconstitution of these cells with ADAM17, but not ADAM10, restores shedding. A chimera in which the disintegrin, cysteine-rich, and EGF-like domains of ADAM17 are replaced with those of ADAM10 fails to process the IL-1 receptor-II, suggesting that one or more of these domains modulate protease activity. ADAM17 also reconstitutes TPA-inducible shedding of the IL-6 receptor in ADAM17 deficient cells (Althoff et al. 2000). But constitutive shedding of the IL-6 receptor is measurable in ADAM17 null cells, indicating an additional role for a second metalloprotease in IL-6R shedding.

## Growth factor and growth factor receptor processing

The production of peptide growth factors in response to diverse stimuli is frequently controlled at the transcriptional level. But many of these growth factors are then produced in membrane-associated precursor forms. Another level of control is therefore the regulation of the processing of these precursor forms to allow the shedding of active growth factors. This shedding often involves ADAMs family proteins. Growth factor receptors can also be shed from the cell surface, thus terminating signaling. Here too, members of the ADAMs family play a role. In this section, we will describe some cases of both growth factor and growth factor receptor processing.

### *Growth factors*

Many ligands for the EGF receptor family are shed from cell surfaces in response to specific signals. One such ligand is heparin-binding EGF (HB-EGF), which activates the EGF receptor (Massague and Pandiella 1993). The precursor for HB-EGF is composed of signal peptide, heparin-binding, EGF-like, transmembrane, and cytoplasmic domains. Membrane-anchored HB-EGF is biologically active, binding to α3β1 integrin receptors on neighboring cells and transmitting a nondiffusable signal that inhibits cell proliferation (Raab and Klagsbrun 1997). However, HB-EGF is also processed in response to activation of protein kinase C (PKC) by phorbol esters (e.g., TPA), generating a soluble, mitogenically active protein (Gechtman et al. 1999). PKCδ appears to be the enzyme responsible for TPA-stimulation of HB-EGF shedding (Izumi et al. 1998). HB-EGF-dependent activation of the EGF receptor also occurs when G protein-coupled receptors are activated, in a process often referred to as EGF receptor transactivation (Daub et al. 1996; Luttrell et al. 1997). The release of soluble HB-EGF during this EGF receptor transactivation is mediated by metalloproteases. (Prenzel et al. 1999)

Which metalloprotease is involved in the regulated shedding of HB-EGF? ADAM9 has been implicated in some experiments. For example, PKCδ directly associates with, and phosphorylates, the cytoplasmic domain of ADAM9 (Izumi et al. 1998). Overexpression of ADAM9 stimulates HB-EGF shedding in the absence of TPA stimulation, whereas expression of metalloprotease mutants of ADAM9 inhibits TPA stimulated HB-EGF shedding. Yet, it was reported recently that fibroblasts derived from ADAM9 null mice still process HB-EGF (Weskamp et al. 2002). HB-EGF shedding does not occur in cells derived from ADAM17-deficient mice, and ADAM17 can cleave an HB-EGF peptide in vitro (Merlos-Suarez et al. 2001; Sunnarborg et al. 2002). ADAM10 has also been reported to mediate the transactivation of EGF receptor in Cos7 and PC-3 cells (Yan et al. 2002). A case may also be made for ADAM12, which appears to regulate HB-EGF shedding and EGF receptor transactivation in cultured cardiomyocytes (Asakura et al. 2002). Further studies will clearly be needed to determine which ADAMs are responsible for regulating HB-EGF shedding in response to signals such as TPA and G protein-coupled receptor ligands in different cell types.

TGFα is also a ligand for members of the EGF receptor family and is also produced in a membrane-associated precursor form. ADAM17 is thought to be the major sheddase for TGFα. The most compelling evidence supporting this hypothesis comes from an analysis of genetically modified mice. As mentioned earlier, mice harboring a germ-line mutation in the metalloprotease domain of ADAM17 have defects in epithelial maturation and organization (Peschon et al. 1998). Similar defects are observed in mice lacking the EGF receptor and in mice null for TGFα (Luetteke et al. 1993; Miettinen et al. 1995; Sibilia and Wagner 1995; Threadgill et al. 1995). Fibroblasts and keratinocytes derived from ADAM17-deficient mice shed reduced levels of TGFα into the medium, and TGFα is cleaved by ADAM17 in vitro (Peschon et al. 1998; Sunnarborg et al. 2002). ADAM17 can also cleave peptides of many EGF family members including amphiregulin, betacellulin, epiregulin, and HB-EGF (Sunnarborg et al. 2002). However, ADAM17 may not be the only TGFα sheddase, as the metalloprotease activator APMA still stimulates TGFα shedding in ADAM17-deficient cells (Merlos-Suarez et al. 2001).

Neuregulin (NRG) is another member of the family of EGF-like ligands, and acts predominantly on the receptors HER2, HER3, and HER4. It regulates acetylcholine receptor synthesis, Schwann cell growth, and the early development of the heart and central nervous system (Garratt et al. 2000). Several lines of evidence suggest that ADAM19 mediates the shedding of neuregulinβ (NRGβ; Shirakabe et al. 2001). Firstly, the two proteins are coexpressed in dorsal root ganglia during mouse em-

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bryogenesis and during the differentiation of neural precursor cells (Kurisaki et al. 1998). Secondly, ectopic expression of wild-type ADAM19 enhanced the release of NRGβ from transiently transfected L929 cells, whereas expression of ADAM19 metalloprotease-deficient mutants blocked NRGβ shedding (Shirakabe et al. 2001). It is thought that NRG processing occurs in the Golgi compartment because it is sensitive to the ER-to-Golgi transport inhibitor brefeldin A, but is insensitive to the intra-Golgi transport inhibitor monensin. ADAM9 could also process NRGβ when tested in a transfection system, but produced smaller and presumably inactive products (Shirakabe et al. 2001). Whether ADAM19 is the only member of the ADAMs family that is involved in the physiological processing of NRGβ will have to await analysis of cells deficient in ADAM19.

### *Growth factor receptors*

HER4/erbB4 is a receptor tyrosine kinase of the EGFR family. It is predominantly activated by neuregulin, but HB-EGF, betacellulin, and epiregulin are also suitable ligands. HER4 is important for early development of the cardiovascular and central nervous systems, and its expression has also been linked to cancer development, particularly of the breast (Hackel et al. 1999). The HER4 JM-a isoform is shed in response to phorbol ester stimulation, and shedding is inhibited with the metalloprotease inhibitors BB-94, BB-3103, and TAPI-2 (IC-3), (Vecchi and Carpenter 1997; Rio et al. 2000). Shedding is essentially absent in cells lacking ADAM17. Furthermore, ADAM17 re-expression reconstitutes both constitutive and TPA-induced HER4 shedding.

Colony stimulating factor-1 (CSF-1) receptor functions in the survival, proliferation, and differentiation of mononuclear phagocytes. Shedding of the receptor by PKC activators generates a soluble form that is still competent for ligand binding, and down-modulates CSF-1 signaling and inactivates macrophages. CSF-1 receptor shedding is blocked by metalloprotease inhibitors as well as by an antibody to the catalytic site of ADAM17 (Rovida et al. 2001). The reduction of cell surface CSF-1 receptor by TPA treatment is also effectively abolished in ADAM17 deficient monocytes.

The hepatocyte growth factor receptor Met is also a sheddase target. BB-94 blocks EGF and LPA-dependent Met shedding. TIMP3 blocks EGF, LPA, and TPA-dependent Met shedding, but TIMPs 1 and 2 are ineffective. Because all known MMPs, but not ADAMs, are inhibited by TIMP1 or TIMP2, whereas TIMP3 also inhibits some ADAMs, it is likely that either an unknown MMP or an ADAM is responsible for Met shedding (Nath et al. 2001).

The nerve growth factor (NGF) receptor, also known as TrkA, is shed in response to TPA or osmotic stress (Diaz-Rodriguez et al. 2002). Both stimuli result in an activation of MAP kinase pathways, ERKs in the case of TPA, and both ERKs and p38 in the case of osmotic stress. MEK inhibitors partially reduce TPA-induced shedding, as does transfection of a dominant-negative ERK2 construct. TPA-dependent NGF receptor shedding does not occur in ADAM17 deficient fibroblasts and can be reconstituted by transfection of wild-type ADAM17. In contrast, the osmotic pathway is unaffected by ADAM17 expression, thus implicating different metalloproteases. Interestingly, ERK can phosphorylate the cytoplasmic domain of ADAM17 in vitro at threonine 735. Stimulation with TPA, EGF, or NGF also leads to an increase in ADAM17/ERK association and ERK-dependent ADAM17 phosphorylation in vivo, which is blocked by MEK inhibitors, dominant-negative ERK2, and dominant-negative ADAM17.

## The cleavage of other molecules

### *Insulin-like growth factor-binding proteins*

The six members of the (IGFBP) family bind and sequester mitogenic insulin-like growth factors (Ferry et al. 1999). The soluble form of ADAM12 (ADAM12-S) binds to and processes purified IGFBP 3 and 5 (but not other IGFBPs). In keeping with a role for an ADAMs family protease, this processing is inhibited by TIMP-3 (but not the other TIMPs; Loechel et al. 2000; Shi et al. 2000; ). It is likely that ADAM12-S represents the serum protease that is up-regulated during pregnancy and degrades IGFBP-3, thus freeing IGF in its fully active form (Ferry et al. 1999). Because IGFBP-3 is a major IGF-binding protein, its regulation by ADAM12-S also has broad implications in diseases related to low IGF levels such as osteoarthritis and diabetes, as well as perhaps the high levels of IGF-1 seen in some tumors.

### *Prions*

Normal processing of cellular prion precursor yields a product called N1, which is nontoxic. In contrast, aberrant processing of the precursor leads to cerebral deposition of an insoluble, protease-resistant protein, which can lead to neurodegenerative diseases. The formation of N1 in HEK293 cells can be partially inhibited by the metalloprotease inhibitors TAPI and BB-3103 (Vincent et al. 2001). ADAM10-deficient fibroblasts show a marked reduction in both constitutive and TPA-inducible production of N1, whereas ADAM17-deficient fibroblasts show normal constitutive N1 shedding, and only a slight reduction in the inducible pathway. Thus, ADAM10 and ADAM17 may function in prion processing and could have positive effects in preventing formation of insoluble prions.

### *Amyloid precursor protein*

The accumulation of β-amyloid peptides in the cerebral cortex is a crucial step in the pathogenesis of Alzheimer's disease (Gandy and Petanceska 2000). The β-amyloid peptides are formed by stepwise processing of the amyloid precursor protein (APP) by β and γ-secretases. An alternative pathway of APP secretion is through the action of α-secretase activity. This pathway is TPA-induc-

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ible. The soluble APPα (sAPPα) that results from α-secretase activity has positive neurotrophic effects, and opposes the harmful effects of β-amyloid formation.

Overproduction of either membrane bound, or an alternatively spliced and soluble form, of ADAM9 in COS cells enhances sAPPα production in a TPA-dependent and metalloprotease inhibitor-sensitive manner (Koike et al. 1999; Hotoda et al. 2002), suggesting that ADAM9 is an α-secretase. But although purified ADAM9 can process APP more efficiently than other peptide substrates in vitro, the processing is not at the α site (Roghani et al. 1999). Furthermore, there are no differences in APP processing between wild-type fibroblasts and fibroblasts lacking ADAM9 (Weskamp et al. 2002), making it unlikely that ADAM9 is the sole α-secretase. In keeping with this, overproduction of ADAM10 in HEK cells also enhances sAPPα production, and both constitutive and TPA-inducible sAPPα production is inhibited by the metalloprotease inhibitor BB3103 and by a point mutation in the catalytic site of the metalloprotease domain of ADAM10 (Lammich et al. 1999). ADAM10 also cleaves APP in vitro at the α site (Lammich et al. 1999; Amour et al. 2000). However, as with ADAM9, embryonic fibroblasts derived from ADAM10-deficient mice are still capable of sAPPα production (Hartmann et al. 2002). Finally, ADAM17 also has α-secretase activity in vitro (Buxbaum et al. 1998). Furthermore, metalloprotease inhibitors such as TAPI-1 block both constitutive and inducible sAPPα production, overproduction of ADAM17 increases sAPPα production, and ADAM17-deficient cells cannot produce sAPPα (Buxbaum et al. 1998; Slack et al. 2001). It is quite possible that there is redundancy in the regulation of sAPPα secretion, or that individual ADAMs regulate secretion independently. Slack and coworkers argue that ADAM17 is the principle mediator of constitutive sAPPα production and that a different ADAM functions during inducible sAPPα production (Slack et al. 2001). APP expression aligns better with that of ADAM10 than ADAM17 in brain tissue and neurons (Marcinkiewicz and Seidah 2000). In any case, these findings warrant the analysis of ADAMs activators for the possible treatment of Alzheimer's disease.

### *Extracellular matrix components*

It is often thought that degradation of extracellular matrix (ECM) components is the sole purview of the matrix metalloprotease family. But there have been reports of ECM degradation by ADAMs family members. For example, ADAM10 cleaves type IV collagen in vitro (Millichip et al. 1998), ADAM15 cleaves type IV collagen and gelatin in vitro (Martin et al. 2002), and ADAM13-expressing cells degrade fibronectin (Alfandari et al. 2001). It is speculated that such activity may assist in cell migration (see below).

## The regulation and specificity of sheddase activity

As we have seen, ADAM-mediated shedding of diverse membrane proteins occurs both constitutively and in response to a variety of stimuli, including unidentified serum factors, peptide growth factors, changes in intracellular calcium concentration, hypertonicity (osmotic stress) and PKC activation. Given this diversity of signals and substrates for sheddase action, it is possible that shedding is controlled by multiple regulatory mechanisms (Pandiella and Massague 1991). We will consider these mechanisms in this section.

TPA-induced shedding has been widely studied. In the TPA-stimulated processing of HB-EGF, PKCδ is involved, as described earlier (Izumi et al. 1998). Other studies have also implicated the MAP kinase pathway. For example, TPA-treatment of CHO cells leads to temporally coincident increases in ERK phosphorylation and HB-EGF shedding, which is inhibited by a MEK inhibitor (Gechtman et al. 1999). In support of multiple regulatory mechanisms, experiments have shown that calcium-modulating agents fail to impact TPA-induced HB-EGF shedding. Conversely, PKC inhibitors do not affect shedding induced by intracellular calcium-modulating agents (Dethlefsen et al. 1998). Similarly, TPA and pervanadate both induce L1 adhesion protein shedding, but the TPA pathway is insensitive to Src inhibitors, whereas the pervanadate pathway is Src dependent, but unaffected by inhibition of PKC (Gutwein et al. 2000). Further, L-selectin is shed in response to TPA and hypertonicity, but only hypertonic shedding is sensitive to p38 MAPK inhibitors (Rizoli et al. 1999).

Growth factors such as fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and EGF treatment of CHO cells stimulate TGFα shedding (Fan and Derynck 1999). Inhibitors of the corresponding receptors, transient transfections of dominant-negative receptor constructs, and MEK inhibitors all block growth factor-dependent TGFα shedding. In contrast, the constitutive shedding of TGFα is inhibited by a p38 antagonist. The two MAPK pathway inhibitors together completely block both growth factor-dependent and independent shedding processes. Curiously, no shedding activity occurs when cells are in suspension even though TPA-stimulated MAPK activation is completely normal under these conditions (Gechtman et al. 1999; Gutwein et al. 2000). Thus, the MAPK pathway may be necessary for TGFα shedding, but is clearly not sufficient. L-selectin shedding in response to F-MetLeuPhe stimulation of neutrophils is also inhibited by MEK and p38 inhibitors, as is TNFα and L-selectin release from CHO cells in response to FGF, PDGF, serum, and TPA stimulation (Gutwein et al. 2000).

In an attempt to define the molecular components that regulate shedding, a mutational analysis in CHO cells was undertaken, screening for mutants that no longer shed TGFα in response to TPA, calcium, and serum. Two such cell lines were obtained and further analyzed. Interestingly, constitutive shedding was unaffected in these mutants (Arribas and Massague 1995). Remarkably, it was not just the shedding of TGFα that was suppressed, the shedding of APP, L-selectin, and the IL-6 receptor were also inhibited (Arribas et al. 1996). It was later shown that these two mutant cell lines belong to

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the same complementation group; that is, a single gene is affected. These mutant cell lines, called M2, are neither defective in PKC signaling nor can be complemented by ADAM17 transfection, thus indicating that there is some other component regulating inducible shedding processes in these cells (Merlos-Suarez et al. 1998). These data suggest that, even though different shedding responses involve different proteins and pathways, most of these pathways converge at some point on a single protein. Obviously, it will be of great interest to identify this protein.

How is the generalized proteolysis of membrane proteins avoided when ADAMs are activated? Part of the regulation could be imparted by the specificity of the ADAMs for their targets. This was addressed by replacing the extracellular juxtamembrane sequence of betaglycan (which does not undergo regulated shedding) with sequences from the equivalent region of either TGFα or APP. Both of the resulting betaglycan chimeras are shed in response to TPA, increased calcium, and serum (Arribas et al. 1997). Because the juxtamembrane sequences of TGFα and APP are not highly related, it is thought that the secondary structure of the juxtamembrane region is the important recognition element for the sheddase. Presumably, not all membrane proteins share this secondary structure.

There have also been suggestions that the ADAMs have intrinsic substrate specificity, based on a short peptide sequence, which would limit their proteolytic activity on most surface proteins (Hattori et al. 2000). However, as we have described earlier, it is likely that multiple ADAMs family members process a single cell surface molecule, and a single ADAM can process several growth factors, adhesion molecules, and/or cytokines. Regulation of sheddase activity likely depends on the expression profile of ADAMs family members, the stimulus being used, and some innate substrate specificity of the ADAMs.

## Cell fate determination and cell migration

During the development of multicellular organisms, multiple cell types are generated from a population of initially equivalent cells. These cells must then migrate and establish the patterns that ultimately lead to the formation of tissues and organs during embryogenesis. This cell fate determination involves both stimulatory and inhibitory cell–cell interactions, which have been most fully studied in the case of the nervous system. In this section, we will describe the involvement of ADAMs in cell fate determination and cell migration.

### *Delta and Notch*

The Notch/Delta signaling system is involved in the development of the nervous system (Mumm and Kopan 2000). It was first described in *Drosophila*, but it has since been shown that this system is conserved in worms and mammals. During the development of the nervous system, neural precursor cells inhibit adjacent cells from adopting a similar neurogenic fate through a process called lateral inhibition. Both *notch* and *delta* were genetically defined as being involved in this process.

Biochemical analyses have shown that Notch functions as a cell surface receptor for membrane-bound Delta, and other members of the DSL ligand family (Serrate, Lag2 in *Drosophila*) on adjacent cells. The binding of Delta to Notch signals cleavage of Notch in its ectodomain, proximal to the membrane, at a so-called site 2. Once this cleavage has occurred, a presenilin-dependent activity cleaves Notch in the transmembrane region, releasing the active cytosolic fragment of Notch to associate with and activate transcription factors of the CSL family (CBF1, Suppressor of Hairless, Lag1).

Delta can also be a substrate for sheddase activity. For example, transiently transfected Schneider-2 (S2) cells shed Delta constitutively into the growth medium and this shedding also occurs in *Drosophila* embryos (Qi et al. 1999). Shed, soluble Delta, not only serves as a ligand for Notch, but also blocks the aggregation of Notch-expressing cells with Delta-expressing cells, as well the extension of axodendritic neurites from primary cortical neurons.

Various lines of evidence point to the involvement of metalloproteases in the shedding of both Notch and Delta. For example, Delta processing is inhibited with the general metalloprotease inhibitors EDTA and phenanthroline (Qi et al. 1999). Compelling genetic analyses from both *Drosophila*, worms, and mice indicate that either loss of function or deletion mutants in various *adam10* orthologs (*kuz, sup-17,* and *adam10,* respectively) exhibit similar phenotypes to loss-of-function mutations in *notch/lin-12* and *delta* (Rooke et al. 1996; Wen et al. 1997; Hartmann et al. 2002). The similarity between these enzymes is further demonstrated by the neurogenic phenotype elicited in both *Drosophila* and *Xenopus* embryos by a dominant-negative ADAM10 mutant lacking its metalloprotease domain (Pan and Rubin 1997).

How are Kuzbanian, Notch, and Delta related in *Drosophila*? When *notch* mutations are crossed into the *kuzDN* background, phenotypic aggravation is observed (Pan and Rubin 1997). Further genetic interaction studies revealed that a gain-of-function *notch* mutation was epistatic to *kuzDN* indicating that Kuzbanian functions upstream of Notch during signaling. Given the known activity of Kuzbanian as a metalloprotease, it was speculated that Kuzbanian was necessary to generate or mediate a negative neurogenic signal, probably through the processing of Notch. In keeping with this, Notch processing is blocked both in vitro and in vivo by coexpression of kuzDN, and processing does not occur in *kuz* null embryos (Pan and Rubin 1997). More recently, using tagged Notch constructs and genetically modified *Drosophila* strains, it has been shown that Kuzbanian associates with Notch and that RNA interference assays targeted at Kuzbanian block cleavage of Notch (Lieber et al. 2002). All of this provides a compelling argument for the role of Kuzbanian/ADAM10 in Notch processing.

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Nevertheless, it has been noted by other researchers that there are key differences between loss-of-function mutations in either *kuz* and *notch*. The former exhibits a more incomplete neurogenic transformation with no loss in cell proliferation (Sotillos et al. 1997), suggesting that other mechanisms besides Kuz-dependent proteolysis may be operational in Notch signaling. Mammalian cells lacking ADAM10 exhibit normal processing of a truncated dominant-active form of Notch (Mumm et al. 2000). Furthermore, other ADAMs, including ADAM17, can process Notch in transfected Schneider-2 cells, albeit in a way that may not mimic that of Kuzbanian (Lieber et al. 2002). ADAM17 and Notch-processing activity can also be copurified in HeLa cells, and ADAM17 can directly process various Notch-related constructs in vitro (Brou et al. 2000). Finally, reconstitution of ADAM17 into ADAM17-deficient monocyte precursor cells blocks their PMA-induced differentiation into macrophages, a process that Notch is also known to inhibit (Brou et al. 2000). However, this is the only published demonstration linking ADAM17 and Notch in a developmental assay. Despite the strong circumstantial evidence implicating ADAM17 in Notch processing, ADAM17 null mice do not phenocopy *notch* mutants (Peschon et al. 1998), suggesting that the specific ADAM involved may be species, tissue, or developmental stage specific.

To further complicate matters, there are apparent genetic and biochemical links between Kuzbanian and the Notch ligand Delta. Flies carrying three copies of the *delta* gene can suppress kuzDN phenotypes, indicating a possible interaction between Kuzbanian and Delta (Qi et al. 1999). Kuzbanian and Delta coexpression promotes Delta shedding, whereas kuzDN effectively blocks constitutive shedding of Delta in the same cells. Delta processing is also blocked in embryos lacking Kuzbanian. The physiological relevance (in terms of cell fate determination) of Kuzbanian shedding of Delta is not yet established.

### *Axon growth and guidance*

Neurite outgrowth occurs at the tip, or growth cone, of neurons, and depends on proteolytic activity, possibly mediated by ADAMs family members. The most compelling argument for this comes from a study of *Drosophila*, in which it was shown that mutations in *kuz* lead to alterations in central nervous system development that mostly relate to stalling in the outgrowth of longitudinal axon bundles (Fambrough et al. 1996). This stalling is rescued by expression of Kuzbanian from a transgene.

Axonal guidance can be mediated by the chemoattractant netrin-1 and its receptor DCC (Livesey 1999). It is thought that ADAM-dependent shedding of DCC effectively decreases the number of attractive contacts that direct axon outgrowth, and thus leads to a termination of neurite outgrowth. Inhibition of ADAMs would therefore be expected to stimulate outgrowth. The metalloprotease inhibitor IC-3 potentiates neurite outgrowth stimulated by netrin-1 (Galko and Tessier-Lavigne 2000). IC-3-treated dorsal spinal cord explants also have increased staining of the netrin-1 receptor DCC. Furthermore, CHO cells transiently transfected with DCC, as well as untransfected E13 commissural neurons, process DCC in an IC-3-sensitive manner.

Axon guidance also involves contact-dependent repulsion, in a way that ensures the precise coordination of the spatial pattern of neural connections. ADAMs appear to participate in this highly regulated process in conjunction with a family of ligands called ephrins and their membrane receptors the Eph receptors. Treatment of neuroblastoma cells (as well as primary neurons) with a clustered EphA3 receptor construct causes cleavage of endogenous ephrin-A2 and its release into the supernatant (Hattori et al. 2000). ADAM10 may be the responsible sheddase in this case, as ephrin-A2 and ADAM10 can form stable interactions. Furthermore, ephrin-A2 shedding is inhibited by protease-deficient ADAM10, and stimulated by overexpression of wild-type ADAM10. Known ADAM10 substrates such as TNFα, APP, and Delta, as well as the receptor-binding sequences of ephrin-A2 share a conserved motif. A synthetic peptide conforming to this motif stimulates ephrin-A2 shedding, further implicating ADAM10. Cocultures of NIH-3T3 cells expressing GFP-tagged ephrin-A2 and hippocampal neurons allows for the visualization of ephrin-A2 release at sites in which neurites touch the fibroblast cell surface and are repelled. Fibroblasts expressing a noncleavable ephrin-A2 show a marked reduction in the ability to repel neurites, indicating that the ADAMs act to terminate signaling. Thus, the tight regulation of metalloprotease activity may be critical in early axon outgrowth and guidance events within the central nervous system.

### *Cranial neural crest cells*

ADAM13 is highly expressed in the cranial neural crest (CNC) cells of *Xenopus*. These cells give rise to many of the morphological features of the head and face. They are highly migratory, and push out from the neural tube toward developmental sectors within the branchial arches. Embryos injected with a protease-inactive ADAM13 show considerable loss in the formation of the hyoidal and branchial developmental streams, indicating that migration to these regions is impaired (Alfandari et al. 2001). Conversely, overexpression of wild-type ADAM13 results in the appearance of trunk neural crest cells in a lateral pathway in which they are not normally found. In keeping with a role for ADAM13 in migration, grafted CNC cells expressing ADAM13 are capable of migrating to the ventral surface and segmenting in a normal pattern, whereas grafted cells expressing protease-inactive ADAM13 fail to migrate at all. Furthermore, explanted embryonic tissues from embryos overexpressing wild-type ADAM13 show pronounced dispersion when plated on fibronectin-coated cover slips, whereas cells expressing protease-deficient ADAM13 did not disperse. In keeping with this, ADAM13 cleaves fibronectin (Alfandari et al. 2001). Together, these experiments suggest an important role for ADAM13 in potentiating the binding

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of CNC cells to their surrounding matrix, and in generating a channel of matrix clearance into which the cells can migrate.

### *Adhesion molecules*

The L1 adhesion molecule is a type I transmembrane glycoprotein and member of the immunoglobulin superfamily of proteins that contains repeated immunoglobulin and fibronectin domains. L1 mediates cell binding through homotypic interactions and by binding to integrin receptors. It has specialized functions in neural morphogenesis through mechanisms involving interactions with extracellular matrix proteins and activation of signal transduction (Burden-Gulley et al. 1997). The L1 ectodomain is also shed from various tumor cell lines at its membrane proximal region. This process may be mediated by ADAM10, as both a dominant-negative form of ADAM10, as well as several metalloprotease inhibitors, block L1 release and L1-mediated cell migration (Gutwein et al. 2000; Mechtersheimer et al. 2001). L1-transfected CHO cells show increased migration, which is inhibited by L1 antibodies, αvβ5 integrin receptor antibodies, and the metalloprotease inhibitor BB3103. It is thought that cell migration occurs through the action of shed L1 molecules interacting with cell surface αvβ5 integrin receptors to stimulate signal transduction. Curiously, MCD, a drug that decreases membrane cholesterol levels, stimulates L1 release in a manner that is still inhibited by BB3103 (Mechtersheimer et al. 2001). This has also been shown for ADAM10-dependent processing of amyloid precursor protein (Kojro et al. 2001). Whether cholesterol or a cholesterol-associated protein blocks ADAM10 activity, or the higher fluidity of cholesterol-free membranes enhances ADAM10 activity, is currently unknown. Regardless, the ability to cleave the L1 adhesion molecule may be an important avenue of study as it relates to the migrational (metastatic) potential of tumor cells (see below) as well as in the study of nervous system development and regeneration.

L-selectin is a cell surface adhesion molecule found on leukocytes that supports associations with glycosylated proteins of the vascular endothelium (Tedder et al. 1995). These associations trigger the tethering and rolling of leukocytes prior to their migration into lymphoid organs and sites of inflammation. Once tethered to the endothelial surface, there is rapid loss of L-selectin from the cell surface via proteolytic cleavage of its extracellular domain. This shedding of L-selectin is sensitive to synthetic (IC-3, KD-IX-73-4, Ro 31-9790) and natural (TIMP-3) metalloprotease inhibitors in several cell types (Bennett et al. 1996; Walcheck et al. 1996; Kahn et al. 1998; Borland et al. 1999; Faveeuw et al. 2001), suggesting the possible involvement of ADAMs family metalloproteases. It is possible that there may be effects of metalloprotease inhibitors on the rolling and ultimate transendothelial migration of leukocytes causing the arrest of cells in the endothelial lining (Walcheck et al. 1996; Faveeuw et al. 2001). Alhough there is at least one case in which metalloprotease inhibitor treatment does not affect neutrophil adherence, rolling, or migration (Allport et al. 1997). It has been suggested that ADAM17 is the major L-selectin sheddase. L-selectin shedding does not occur in cells with very low expression levels of ADAM17. In addition, thymocytes derived from ADAM17-deficient mice do not show a reduction in surface L-selectin levels in response to TPA stimulation (which has been interpreted as a lack of shedding). Finally, purified ADAM17 can cleave an L-selectin peptide (Peschon et al. 1998; Beer et al. 1999).

### *Other migratory processes*

In a number of renal diseases, including mesangial capillary glomerular nephritis, mesangial cells of the renal glomerulus inappropriately migrate through the mesangial matrix into the peri-capillary space. In model systems, migration can be partially blocked by ADAM15 antibodies, antisense ADAM15 oligonucleotides, and the metalloprotease inhibitor BB-3103 (Martin et al. 2002). ADAM15 can also degrade type IV collagen and gelatin in vitro, leading to the suggestion that ADAM15-driven restructuring (clearance) of the matrix may be able to enhance the ability of cells to migrate, similar to the role of ADAM13 in *Xenopus*. It has not yet been established whether overexpression or hyperactivity of ADAM15 underlies the human pathology.

## Fertilization

Fertilization of an egg by a sperm is initiated by the close adhesion of the two cells, and the fusion of their membranes. ADAMs family members play a key role in this initiation of fertilization. In particular ADAM1, ADAM2 (originally called fertilinα and fertilinβ), and ADAM3 are involved. In mice ADAM1 and ADAM2 form a heterodimer (Evans 2001). Homologous recombination experiments have shown that targeted deletion of ADAM2 (which also prevents the presentation of ADAM1 on the cell surface) renders male mice infertile (Cho et al. 1998). Targeted deletion experiments in mice have also shown an obligatory role in fertilization for ADAM3, another family member expressed on the sperm surface (Nishimura et al. 2001). Curiously, humans have neither a functional *adam1* nor *adam3* gene. Instead, ADAM2 is involved in fertilization (Takahashi et al. 2001). It is intriguing that several other ADAMs are specifically expressed in the testis (Table 1), although their roles in fertilization have not been explored.

Because ADAMs family members have sequences in their cysteine-rich region that resemble those present in viral fusion peptides, it was originally thought that ADAM2 would be required for sperm and egg membrane fusion. However, it was subsequently shown that it is the adhesion step, not fusion, that is defective in these knockout cells.

Because ADAM2 is predicted to be proteolytically inactive, and both the prodomain and metalloprotease domain of ADAM2 are cleaved prior to the acquisition of fertilization competence (Primakoff et al. 1987; Blobel et

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al. 1990), a role for protease activity in fertilization can be ruled out. Instead, the adhesion of sperm and egg requires the disintegrin domain of ADAM2 (Blobel et al. 1992). This has been most clearly demonstrated by the use of inhibitory synthetic peptides specific for this domain. Exactly which integrin receptors ADAM2 binds to on the egg surface is still the subject of some controversy. ADAM2 has an ECD sequence in its disintegrin loop. Mutation of ECD to ECA greatly inhibits egg binding (Bigler et al. 2000; Zhu et al. 2000). However, it should be noted that mutation of virtually any amino acid within this region causes some inhibition, so it is likely that there are constraints on the tertiary structure of the entire disintegrin loop that help to maintain the integrity of this domain as an integrin receptor ligand.

The integrin receptor to which ADAM2 binds is also still the subject of some debate. It was first suggested that α6β1 is the receptor (Almeida et al. 1995), because the disintegrin domain of ADAM2 blocks the ability of α6 antibodies to bind to the egg surface. Furthermore, an antibody specific for α6 blocks sperm-egg fusion and binding of ADAM2 to the egg surface (Chen et al. 1999b; Bigler et al. 2000). However, others could not confirm these findings, and instead noted that β1 antibodies prevented the binding of ADAM2 to the egg surface (Evans et al. 1997; Zhu and Evans 2002). Furthermore, α6 antibodies do not inhibit fertilization of unprocessed, cumulus-intact eggs, and eggs prepared from α6 null mice are still capable of undergoing fertilization (Miller et al. 2000). One reason for these discrepancies may be in the methodologies used for preparing the eggs for such binding assays.

Recently, members of the α4/α9 subfamily of integrin receptors have been implicated in fertilization (Zhu and Evans 2002). A synthetic peptide (MLDG) known to inhibit interactions of α4/α9 subfamily members also blocks ADAM2 binding to the egg surface. Because so many ADAMs are able to bind to α9β1, it may be that this integrin receptor, or another of similar selectivity, serves as a target for ADAMs during fertilization.

### Myoblast fusion

Myoblast cells differentiate (align) and fuse to form long, multinucleated myotubes during the course of skeletal muscle deposition. Proteins involved in this developmental transformation include p21 (a cyclin-dependent kinase inhibitor that leads to cell cycle arrest) and MyoD (a basic helix-loop-helix transcription factor that directs the synthesis of muscle-specific genes). Insulin-like growth factors also positively influence myogenesis, via activation of PI 3-kinase, NF-κB, and nitric oxide synthase (for review, see Perry and Rudnick 2000). Several years ago, three ADAMs family metalloproteases, meltrin α (ADAM12), β (ADAM19), and γ (ADAM9), were cloned from a myoblast source. ADAM12, in particular, showed a restricted spatial-temporal expression pattern that correlated well with early skeletal muscle development (Yagami-Hiromasa et al. 1995; Kurisaki et al. 1998) and muscle regeneration (Borneman et al. 2000; Galliano et al. 2000). Most studies testing the role of ADAM12 in myogenesis have used cultured C2C12 myoblasts, which differentiate into myotubes when serum growth factors are removed from the culture medium (Yagami-Hiromasa et al. 1995; Kang et al. 2000). In this system, antisense ADAM12 constructs inhibit myoblast fusion, suggesting a positive role for this protein in myogenesis (Yagami-Hiromasa et al. 1995). However, overexpression of full-length ADAM12 also inhibits fusion. Because myoblasts often express a form of ADAM12 that lacks the prodomain and metalloprotease domain, the effect of expression of ADAM12 constructs containing a deletion in the metalloprotease domain was also tested. In this case, myoblast fusion is stimulated two- to threefold (Yagami-Hiromasa et al. 1995). This suggests a positive role for the disintegrin, cysteine-rich, and/or EGF-like regions of ADAM12 in myogenesis, and a negative role for the metalloprotease domain.

It has been suggested that the metalloprotease domain may have a negative impact on muscle development via its processing of the growth factor myostatin, which is a member of the TGFβ family of growth factors, and a known inhibitor of muscle development. C2C12 cells treated with the metalloprotease inhibitors TAPI and BB3103 undergo enhanced differentiation concomitant with a block in the processing of myostatin (Huet et al. 2001). Removal of the metalloprotease domain would prevent the processing of myostatin, and might also release the disintegrin and cysteine-rich domains to interact with cell surface C2C12 myoblast proteins to promote attachment and fusion (Zolkiewska 1999). It will be important to determine whether, and under what conditions, the metalloprotease domain of ADAM12 might be cleaved.

Another potential mechanism of regulation of ADAM12-mediated myogenesis is through protein–protein interactions in its cytoplasmic tail, which associates with the SH3 domain of p85α, the PI 3-kinase regulatory subunit. PI 3-k-inase activity is required for myogenesis. Colocalization of ADAM12 and a green fluorescent protein (GFP) fused to a PH domain [which marks the location of membranes enriched in the PI 3-kinase product PI(3,4,5)P3; Kang et al. 2001] has been observed. This colocalization is not seen in the presence of PI 3-kinase inhibitors, nor in cells expressing an ADAM12 protein mutated to ablate p85α association. Thus, ADAM12 may bring PI 3-kinase to the membrane, where its full activity can be manifested.

Another protein with a possible function in myogenesis and with a link to ADAM12 is Src. ADAM12 and Src associate in C2C12 myoblasts by interaction of the PxxP motif of the ADAM12 tail with the SH3 domain of Src (Kang et al. 2000). This association appears to trigger an increase in Src activity, as measured in cells cotransfected with ADAM12 and Src. Curiously, the constitutively high kinase activity of the transforming viral Src protein inhibits the fusion of myoblasts to myotubes (Falcone et al. 1985). Whether the association of cellular Src with ADAM12 would promote or inhibit myoblast fusion is an open question.

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Interaction between the cytoplasmic tail of ADAM12 and the actin-binding proteins α-actinin-1 and 2 may also be important for myogenesis (Galliano et al. 2000). Because α-actinins form a bridge between cytoskeletal and membrane proteins at focal adhesion sites, these associations may explain some of the gross changes in cell morphology that occur during the differentiation of muscle cells.

Transient transfections of the myristylation-tagged, and hence, membrane-bound cytoplasmic tail from ADAM12 blocks fusion of C2C12 myoblasts (Galliano et al. 2000). Presumably, this construct sequesters proteins that would normally associate with ADAM12, and that are required for myotube formation. But it is not yet clear which of the proteins described above that associate with the tail of ADAM12 are most important.

## Human disease

Members of the metzincin family have been implicated in a variety of human disease processes. For example, tumor cells are known to use matrix metalloproteases to promote their growth and metastasis (Chang and Werb 2001). Members of the ADAM-TS family are associated with the breakdown of aggrecan seen in osteoarthritis (Tang 2001). Furthermore, mutations of ADAM-TS13 cause thrombotic thrombocytopenic purpura, due to a failure to proteolytically cleave the clotting protein Von Willebrand factor (Levy et al. 2001). And, as we discussed earlier, ADAM17 plays a major role in the development of a variety of inflammatory processes by regulating the shedding of TNFα. In this section, we will discuss briefly other human diseases in which ADAMs have been implicated recently.

### *Cancer*

As we have seen in the previous sections, ADAMs can mediate the shedding of growth factors and regulate the adhesion and motility of cells. Tumor cells frequently produce autocrine growth factors, and are often highly motile and invasive. Is there any evidence for the dysregulation or overexpression of ADAMs in tumors? The data on hand to date are largely correlative. For example, ADAM12 is overexpressed in a number of carcinoma tissues (breast and colon) and cell lines (Wu et al. 1997; Iba et al. 2000). ADAM10 is also overexpressed in pheochromocytomas and neuroblastomas (Yavari et al. 1998).

Many tumor cells produce growth factors, and their proliferation independently of exogenous growth factors may contribute to their tumorigenicity. Given the involvement of ADAMs family proteins in the regulated shedding of growth factors from normal cells, it seems reasonable to postulate that they might also be involved in this process in tumor cells. To date, there have been few direct tests of this hypothesis. However, there was a report recently of EGF receptor transactivation stimulated by G protein-coupled prostaglandin (PGE2) receptors. PGE2 is mitogenic for gastric epithelial cells and a number of colon cancer cell lines (Pai et al. 2002). Transactivation of the EGF receptor in these cells is blocked by inhibitors of Src, metalloproteases, and the EGF receptor, as well as by antibodies to TGFα. Because prostaglandin levels are increased in colorectal carcinoma tissue, this pathway may represent an important autocrine mitogenic signal.

Given the involvement of ADAMs in migratory processes during development, is it possible that they are involved in the migration of tumor cells? Again, very few direct analyses have been conducted. ADAMs do associate with tumor cell surfaces: ADAM12 and ADAM15 bind to α9β1-expressing Ntera-2 embryonic carcinoma cells (Eto et al. 2000); ADAM15 binds to αvβ3 expressing melanoma cells (Zhang et al. 1998; Iba et al. 1999); ADAM12 binds to a several cancer cell lines through cysteine-rich domain/cell surface syndecan interactions (Iba et al. 1999); ADAM9 binds to α6β1-expressing fibrosarcoma cells (Nath et al. 2000); and ADAM23 binds to NB100 neuroblastoma cells (Cal et al. 2000). In the latter two cases, binding to an ADAM substratum stimulated cell migration of the tumor cells. ADAMs may also be able to bind to integrin ligands such as laminin or fibronectin with a similar effect (Gilpin et al. 1998; Gaultier et al. 2002). Furthermore, Src family kinases and ADAMs may regulate the shedding of L1 adhesion molecule from breast and ovarian carcinoma cells, potentially regulating their migrational capacity (Gutwein et al. 2000).

### *Cardiac hypertrophy*

Cardiac hypertrophy is an adaptive response of the heart to maintain cardiovascular output during early cardiovascular disease. Vasoactivators like angiotensin II, endothelin-1, and phenylephrine all activate G protein-coupled receptors, leading to cellular responses such as gene activation, protein synthesis, and an increase in cell size (Rockman et al. 2002). A new inhibitor of ADAMs family metalloproteases, KB-R7785, not only blocks the shedding of HB-EGF and the transactivation of the EGF receptor during vasoactivation of cardiomyocytes, but also shows some beneficial effects when used to treat chronic cardiac hypertrophy in an animal model (Asakura et al. 2002). KB-R7785 also blocks the formation of soluble HB-EGF in vivo. It appears that the responsible HB-EGF sheddase in this system is ADAM12, as ectopic expression of a metalloprotease deletion mutant of ADAM12 inhibits phenylephrine-induced sheddase activity as well as EGF receptor transactivation.

### *Bacterial infections*

When the lung epithelium is exposed to pathogenic bacteria, mucin production occurs. This anti-bacterial response is usually beneficial to the host, although in cystic fibrosis patients in which mucin induction can obstruct air passages, the mucin layer can protect bacteria from antibiotics. Mucin production is stimulated by the

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activation of G protein-coupled receptors by bacterial lipoteichoic acid, which, in turn, leads to the shedding of HB-EGF, the activation of an EGF receptor-signaling cascade, and an increase in mucin gene expression. Both the metalloprotease inhibitor GM6001 and an anti-sense ADAM10 (but not ADAM17) oligonucleotide blocked EGF receptor transactivation (Lemjabbar and Basbaum 2002). These data suggest that drugs targeting ADAM10 may provide an alternative mechanism to controlling bacterial lung infections.

### *Asthma*

Asthma is characterized by episodes of coughing, wheezing, and breathlessness. Allergen exposure and other environmental factors contribute to the development of asthma, but several studies have also suggested that there is a strong genetic component. In a very recent study, a genome-wide scan was performed on 460 families, and a locus linked to asthma and bronchial hyperresponsiveness was identified on chromosome 20 (Van Eerdewegh et al. 2002). Subsequent analysis of polymorphisms in 23 genes in the p13 region of chromosome 20 led to the identification of *adam33* as a putative asthma-susceptibility gene.

ADAM33 is most closely related to ADAMs 12, 15, and 19, and is predicted to have protease activity. It is expressed in lung fibroblasts and bronchial smooth muscle, and undergoes alternative splicing. Some of the polymorphisms found in the asthma study were in noncoding regions, and may affect splicing or RNA stability. Others involve the transmembrane and the cytoplasmic domain (Van Eerdewegh et al. 2002). Further analyses will seek to determine whether changes in ADAM33 activity or expression underlie airway dysfunction.

## Conclusions

In this review, we have described the involvement of ADAMs in a variety of cellular processes, including processing of proteins, interactions with integrin receptors and with signaling molecules. Figure 3 attempts to summarize much of this information, and shows how ADAMs may play a pivotal role in the transfer of information from the cell to its environment and vice versa. It is clear that ADAMs family members are positioned to play important roles in development, cell signaling, and disease pathologies. But there are many unanswered questions about ADAMs regulation and function, which we will address here.

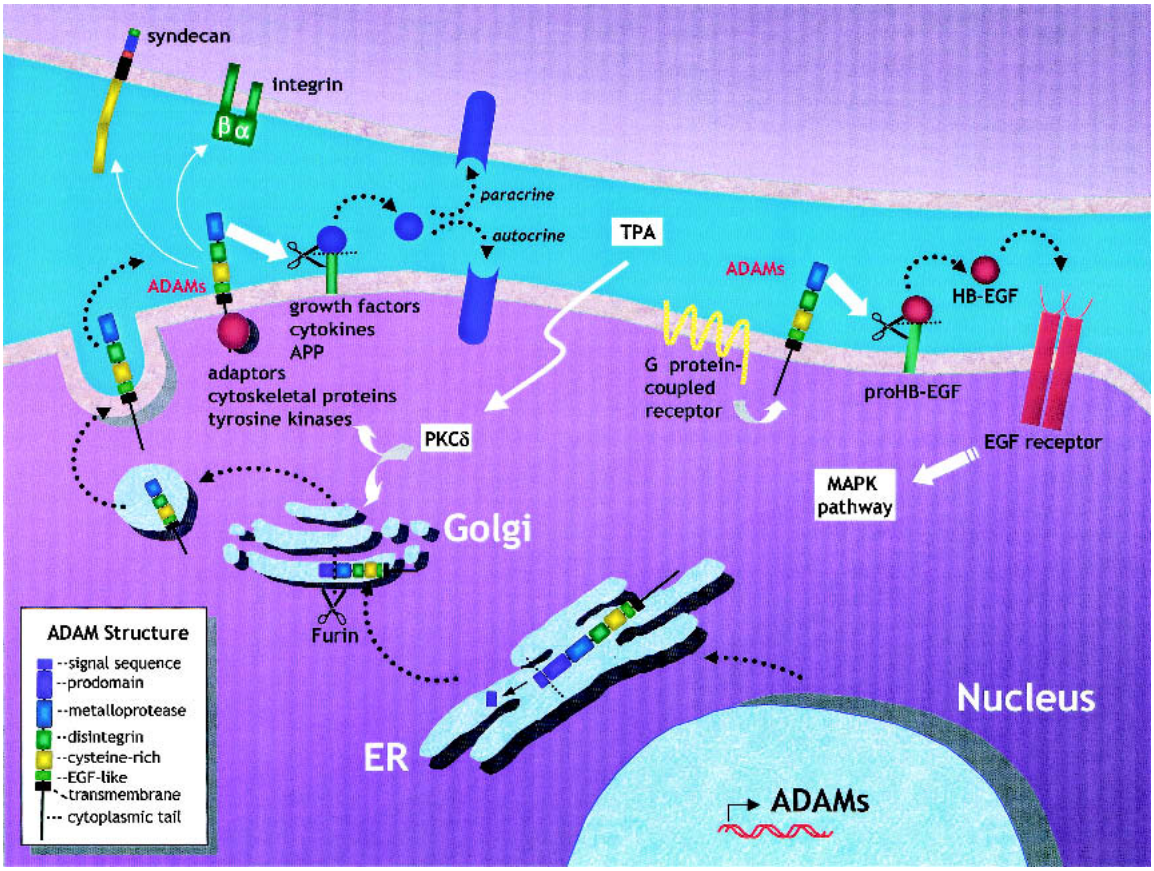


**Figure 3.** An overview of ADAMs synthesis, processing, and function. Several representative activities of the ADAMs are schematized. Note that not all possible functions of the ADAMs are shown. In addition, as discussed in the text, some of the ADAMs may exert their effects in intracellular membranes.

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It is notable that, of the ADAMs expressed outside of the testis, those that are predicted to be proteolytically active usually also contain multiple SH3 domain-binding sites (PxxP motifs) in their cytoplasmic tails (Table 1; Fig. 2). Does this observation have a functional significance? Certainly, overexpression of the cytoplasmic tail of ADAM12 inhibits myoblast fusion (by promoting protease activity?) and similar overexpression of the ADAM9 tail inhibits TPA-induced HB-EGF shedding. These data raise the possibility that proteins associated with the tails of ADAMs can influence the metalloprotease activity. Because it is believed that by the time ADAMs are expressed on the cell surface, where these associations can take place, the prodomain of the ADAM has been cleaved and, therefore, the enzyme is active, it is unlikely that the tail-associated proteins affect enzyme activity directly. Rather, it may be that the associated proteins, which themselves often have multiple protein–protein interaction motifs, may relocate ADAMs to specialized regions of the membrane, or cause the clustering of ADAMs with specific substrates.

Is there any cross-talk between the metalloprotease and the disintegrin domains? There is no evidence for positive interactions between these domains. In fact, where studies have been performed, there have been suggestions that the metalloprotease domain negatively impacts the activity of the disintegrin domain. For example, a form of ADAM12 lacking its metalloprotease domain promotes myoblast fusion, whereas the full-length protein inhibits it. Curiously, removal of the metalloprotease domain of ADAM2 correlates with fertilization competence, even though this domain is proteolytically inactive. Perhaps the metalloprotease domain sterically hinders the disintegrin domain from associating with integrin receptors.

How much redundancy is there among ADAMs family members? Certainly there are several instances in which multiple ADAMs can cleave the same substrate. And, even though ADAM9 has been shown in cell-based systems to be involved in TPA-stimulated HB-EGF shedding, cells derived from an ADAM9 knockout are not defective in this process. We must await reports on the phenotypes of other ADAM knockouts and their crosses, but perhaps redundancy exists among the most similar ADAMs (for example those with multiple SH3-binding sites in their tails). More selective ADAMs inhibitors will also help to address the redundancy issue.

There are many members of the ADAMs family, both testis specific and somatically expressed, that are to date functionally uncharacterized. It will be interesting to determine which other biological processes are also controlled by this interesting family of enzymes.

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# The ADAMs family of metalloproteases: multidomain proteins with multiple functions

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