Minireview

Domain integration of ADAM family proteins: Emerging themes from structural studies

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Impact statement

Recent structural advances have provided a deeper appreciation for interdomain relationships that modulate the activity of ADAM proteins in ectodomain shedding and cellular adhesion. Our review covers these new findings, and places them into historical context. The new results make clear that the metalloproteinase domain works in combination with its ancillary domains to execute its biological function. The ADAM ectodomain is dynamic, and accesses conformations that require interdomain movements during its enzymatic "lifecycle." Fundamental questions about ADAM activation and substrate selection, however, still remain unanswered. Elucidating the biochemical and structural basis for ADAM regulation will be an exciting avenue of future research that should greatly advance our understanding of ADAM function in biology and human pathogenesis.

Abstract

ADAM (a disintegrin and metalloproteinase) proteins are type-1 transmembrane and secreted proteins that function in cell adhesion and signal transduction. Here we review the structural features of ADAM proteins that direct their biological functions in ectodomain shedding and cell adhesion.

Keywords: Metalloproteinase, structural biology, biophysics, signal transduction, notch signaling, ectodomain shedding

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Introduction: History and overview of ADAMs

ADAM (a disintegrin and metalloproteinase) proteins are type-1 transmembrane and secreted proteins that function in cell adhesion and signal transduction. PH30α, the founding member of this family, was initially identified as a protein implicated in the process of sperm-egg fusion.[1] It is now evident that ADAM proteins are found throughout the phylum *Chordata*, with expansion of the family in vertebrate evolution producing a total of 21 ADAM genes in humans.[2]

ADAMs are widely expressed in mammalian tissues, and exhibit a wide range of different biological functions. Knockouts of individual ADAM proteins have implicated ADAM function in fertilization, cell differentiation, immunity, angiogenesis, and development of epithelial and nervous tissue. Accordingly, dysfunctional ADAM activity is observed in a variety of pathophysiological conditions such as cancer,[3] infertility,[4] chronic immunity,[5] Alzheimer's disease,[6] asthma, and epilepsy.[7]

Of the 21 human ADAM proteins, 13 are known or predicted to be catalytically active (ADAM8, -9, -10, -12, -15, -17, -19, -20, -21, -28, -30, -33, and -DEC1), whereas 8 ADAMs (ADAM2, -7, -11, -18, -22, -23, -29, and -32) are

predicted to be catalytically inactive.[8] Catalytically active ADAM proteins that are membrane-tethered typically result in ectodomain shedding, the irreversible processing of a membrane-associated protein to release its ectodomain into the extracellular or luminal space.[9]

ADAM10 and ADAM17 are the most extensively studied proteins among ADAM family members. Both of these proteins carry out critical functions in mammalian development. ADAM17 was originally identified by Moss *et al.* as the enzyme that catalyzes cleavage of the TNFα precursor, and dubbed "TACE," short for TNFα-converting enzyme.[10,11] Characterization of ADAM17 knockout mice, however, revealed defects in the developing epithelium and mice born with open eyelids secondary to a loss in EGFR signaling.[12,13] ADAM17 was then shown also to be the primary sheddase of the epidermal growth factor precursor as well as the sheddase for transforming growth factor-alpha (TGFα) and amphregulin.[14,15] ADAM10, in contrast, is the primary sheddase for Notch receptors, and ADAM10 knockout mice show characteristic Notch loss-of-function phenotypes in a variety of different tissues including the nervous and cardiovascular system.[16,17] ADAM10 is also a well-characterized alpha-secretase, catalyzing cleavage of the amyloid-beta precursor protein APP, and it has been proposed that certain mutations in its prodomain are risk factors for late onset Alzheimer's disease.[18]

Aberrant ADAM activity has also been linked to a number of different diseases. For example, ADAM17 has been reported to be capable of processing mutated forms of Notch1 found in T cell acute lymphoblastic leukemia.[19] The ability of a single ADAM, such as ADAM17, not only to process multiple different substrates, but also to substitute for the physiologic catalyst in the cleavage of a mutated substrate of another ADAM, adds an additional layer of complexity to acquiring a complete understanding of how these molecules function in both physiology and disease.

Here we review the structural features of ADAM proteins that direct their biological functions in ectodomain shedding and cell adhesion. Table 1 lists all ADAM family proteins for which structural coordinates have been deposited, along with their PDB ID codes. The reader is referred to many other excellent reviews focused on ADAM biology in development and pathogenesis.[3,8,20,21]

Table 1. ADAM structures and associated PDB ID codes.

ADAM	Domain	PDB	References
ADAM8	M	4DD8	22
ADAM10	DC	2AO7	23
	DC/8C7 F_{ab}	5L0Q	24
	MDC	6BE6	25
	MDC/11G2 F_{ab}	6BDZ	26
ADAM17	M	1BKC	27
	M/N_T-TIMP3	3CKI	28
	C	2M2F	29
ADAM22	MDCE	3G5C	30
	MDCE/LGI1	5Y2Z, 5Y31	31
ADAM33	M	1R54, 1R55	32

ADAM: a disintegrin and metalloproteinase.

Modular domain organization of ADAMs

ADAMs, ADAMTS (a disintegrin and metalloproteinase with thrombospondin repeats), and SVMP (snake venom metalloproteinase) make up the M12B adamalysin subfamily of the Metzincin superfamily of metallopeptidases. An early alternative name for the ADAM family of proteins was MDC, derived from the metalloprotease, disintegrin, and cysteine-rich modules in their ectodomains.[33] Like the ADAMs, both SVMP and ADAMTS proteins have a modular domain organization, but are secreted proteins with additional structural features in the ancillary domains unique to each individual family.

All ADAM proteins are synthesized as an inactive precursor with an N-terminal signal peptide and a prodomain immediately C-terminal to the signal peptide. Metalloproteinase (M), disintegrin (D), cysteine-rich (C), and (with the exception of ADAM10 and ADAM17) epidermal growth factor-like (EGF) domains then follow the prodomain, and precede a transmembrane segment and a cytoplasmic tail of variable length (Figure 1). Maturation of ADAMs occurs as they pass through the secretory pathway, where they are post-translationally modified with N-linked sugars and their prodomain is cleaved.

The cytosolic tails of the ADAMs differ considerably, with variations in their posttranslational modification sites and in other sequences that serve as binding motifs for proteins that control their subcellular localization. For example, ADAM10 contains an ER retention sequence, whereas ADAMs 22, 23, and 11 have PDZ binding motifs that dock onto PSD-95 scaffolding protein in the synaptic cleft.[34,35] One family member, ADAMDEC-1, is secreted as a soluble protein with a short D domain, and lacks the transmembrane region and cytoplasmic tail entirely. Other ADAM family members, including ADAMs 9, 12, and 28 can undergo alternative splicing to generate secreted variants (called 9S, 12S, and 28S), but the function of these secreted forms remains poorly understood.[36,37]

Catalytic domain architecture

Metzincin proteins are characterized by conserved secondary structural and active site features in their M domains. The structure of the ADAM17 M domain, first determined in 1998, revealed a fold resembling a kidney bean (Figure 2), with an extended notch at the catalytic cleft dividing the domain into amino terminal (N_T) and carboxyl terminal (C_T) lobes.[27] At the core of the N_T lobe is a twisted 5-stranded antiparallel β-sheet that is enveloped by four conserved α-helices. The C_T lobe contains a series of loops followed by a conserved α-helix, which is anchored to the N_T lobe by a conserved disulfide bond near the C-terminus.

The catalytic cleft is formed between the N_T and C_T lobe along α-helix α4, generating a narrow canyon for the polypeptide substrate. Typically, metzincin family members contain a consensus **HE**XX**H**XX**G**XX**H** sequence on the fourth α-helix in the N_T lobe. The three histidine residues

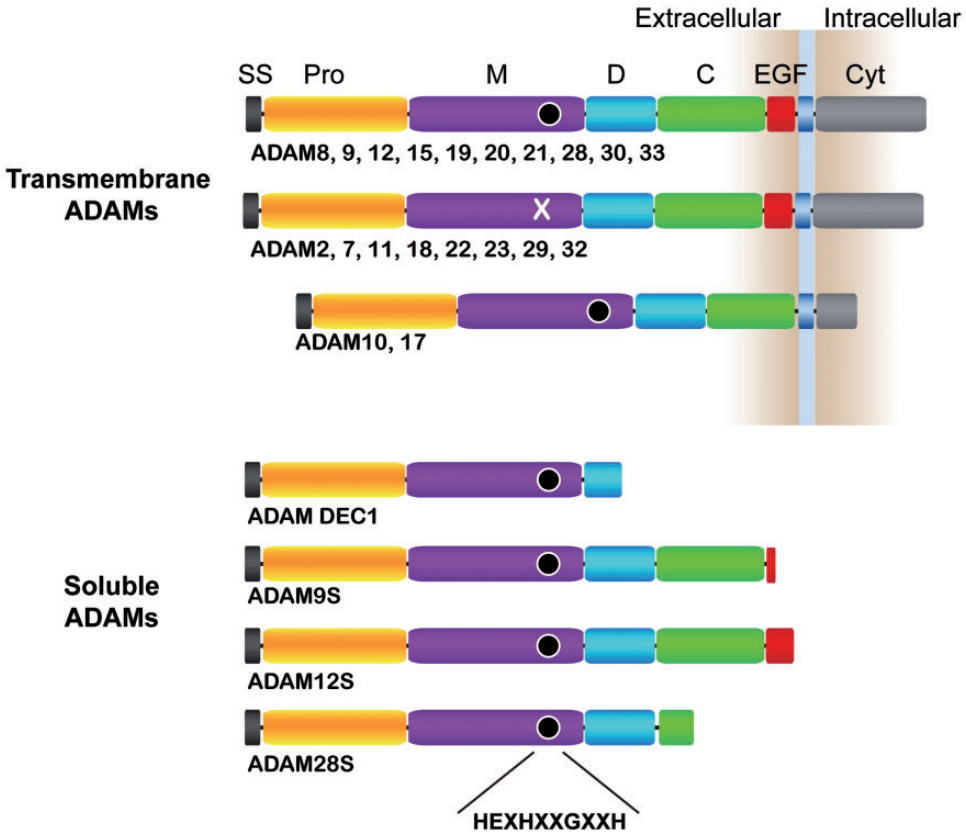

Figure 1. ADAM family domain organization. Domain schematics of human ADAM family members are shown. SS: signal sequence; Pro: prodomain; M: metalloproteinase; D: disintegrin-like; C: cysteine-rich; EGF: epidermal growth factor-like; Cyt: cytoplasmic tail. The transmembrane segment is indicated with a blue box. The conserved active site amino acids are collectively represented using a black circle. ADAMs which do not have the conserved active site sequence have an "X" instead of the black circle in the M domain. (A color version of this figure is available in the online journal.)

coordinate a zinc ion, which together with the conserved glutamate residue, activates a water molecule that is the nucleophile responsible for substrate hydrolysis. Following the active site is a conserved loop in the C_T lobe called the Met-turn, which wraps around the active site to position a methionine residue as a buttress beneath the zinc-coordinating cluster of histidine residues. Eight members of the ADAM family (ADAMs 2, 7, 11, 18, 22, 23, 29 and 32), however, lack the active site glutamate and have other mutations within the active site consensus sequence that almost certainly render them catalytically inactive.

Substrate selectivity of ADAMs

In general, the substrate selectivity of metzincin family members is strongly dependent on the flexibility of the P3-P1 positions to fit into the S3-S1 pockets while permitting the extension of the side chain from the P1′ position to reach into the selective S1′ pocket.[38] Analysis of the cleavage preferences of ADAMs using peptide library approaches has revealed that ADAM17 has a strong preference for small hydrophobic amino acids, such as Leu and Val, in the P1′ position, while ADAM10 can also accommodate larger, aromatic amino acids, including phenylalanine and tyrosine.[39,40] The origins of this selectivity derive from a deeper S1′ pocket in ADAM10 than in ADAM17[25]; this deeper pocket likely contributes to the selectivity of the small molecular weight inhibitor GI254023X for ADAM10. In the ADAM10 ectodomain structure (see below), each ADAM10 molecule in the crystal has a C-terminal hydrolytic peptide product from a neighboring copy in the asymmetric unit fortuitously captured in its active site, even though the P1′ residue was glycine. This observation indicates that an extended conformation and cleavage-site accessibility also exert an important influence on substrate selection. In addition, the D and C domains of both ADAM10 and ADAM17 appear to actively participate in substrate recognition (see below), and efforts have been made to exploit these "exosites" in the design of specific ADAM10 and ADAM17 inhibitors.[41,42]

Disintegrin and cysteine-rich domains

The ADAM disintegrin (D) domain is named for its resemblance to the disintegrins, a family of small proteins, found in the venom of vipers and pit vipers, that block the binding of platelets to adhesion receptors and thereby suppress platelet aggregation.[43] Snake venom disintegrins are classified into three groups (short, medium and long) based on the number of disulfide bonds that serve to rigidify their structures. The D domains of the ADAMs most closely resemble the largest of these snake venom disintegrins, and typically have seven conserved disulfide bonds, except for ADAM5 (a potential pseudogene) and ADAM17, which each have six. In the ADAMs, the D

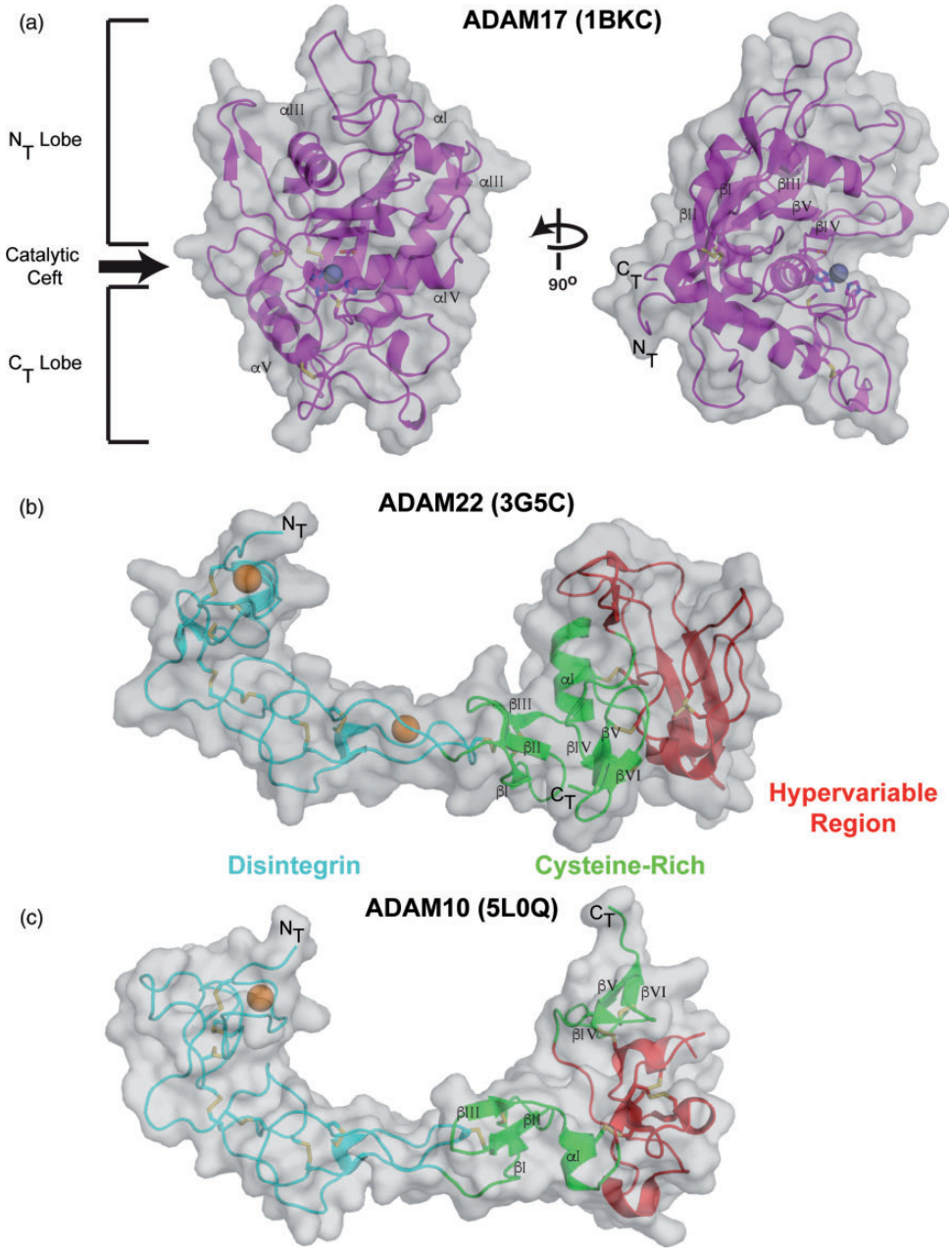

Figure 2. M domain and D + C domain structures. (a) The ADAM17 M domain (PDB: 1BKC) is shown in carton representation with a transparent grey surface. The catalytic cleft and structurally conserved subdomains are indicated on the left. The active site histidine, glutamic acid, and methionine residues are represented as sticks with the bound zinc ion as a grey sphere. The bound inhibitor has been removed in these representations. The D + C domains of (b) ADAM22 (PDB: 3G5C) and (c) ADAM10 (PDB: 5L0Q) are shown in cartoon representation with a transparent grey surface. The D domain is in cyan, the C domain is in green, and the HVR is in red. Conserved structural α-helices and β-strands are labeled in the individual domains and disulfide bonds are represented as sticks. Bound calcium ions in the ADAM22 and ADAM10 D domains are represented as orange spheres. The M and EGF domains of ADAM22 have been removed from this representation. (A color version of this figure is available in the online journal.)

domain can be further rigidified by the presence of two or one structural calcium ions. The residues responsible for coordinating the second calcium ion are not conserved across all ADAM family members, and in these instances a charged side chain, such as Arg525 in ADAM10, can fill in for the calcium ion. Like the disintegrins, certain ADAMs have also been implicated in binding to adhesion receptors,[44–46] though the molecular basis for this interaction has remained obscure. Whereas a conserved loop presents the RGD adhesion recognition sequence in the snake venom disintegrins, all of the ADAMs except for ADAM15 have lost this RGD sequence from their D domains. Even in ADAM15, however, this loop also contains a cysteine residue that forms a disulfide bond linking the D and C domains, likely burying the RGD in the interior of the structure.

The cysteine-rich (C) domain, consisting of 80–150 amino acids, immediately follows the disintegrin domain and exhibits poor sequence conservation across the ADAM family. This domain is primarily stabilized through

a network of six disulfide bonds. The ADAM cysteine-rich domain also contains a segment dubbed the "hypervariable region" (HVR), named for the significant divergence in length and amino acid composition among the different ADAMs in this region.[47] Within the ADAM family, the HVR ranges in length from 37 residues in ADAM17 to 87 residues in ADAM19. Sequence alignment of the cysteine-rich domains also shows the HVRs of ADAM10 and ADAM17 to be much shorter than in the rest of the ADAMs. This decrease in size is associated with a different disulfide bonding pattern in ADAM10 and ADAM17 when compared to ADAM22 (and likely all other ADAM family members as well).

The X-ray structure of the D + C fragment from ADAM10 revealed that the two domains actually form a continuous, elongated cup shape without a clear boundary between them (Figure 2(c)).[23,24] The D domain has an arced, extended overall shape with little secondary structure, stabilized instead by a series of conserved disulfide bonds that staple the loops together and rigidify the domain. The C domain is more globular, flanked by a pair of beta-hairpins at its N- and C-terminal ends.

The C domain contributes to ADAM function by regulating subcellular localization, modulating enzymatic activity, and potentially also by influencing substrate recognition through exosites. In ADAM10, the C domain regulates subcellular localization by binding to one of a subset of tetraspanin proteins with eight cysteines in its ectodomain (tspanC8 proteins). The tspanC8 proteins not only escort ADAM10 to the cell surface but also appear to influence substrate selectivity.[48–50] Whether the C domain of ADAM17 is also targeted by its escort factors, the inactive rhomboid proteinases iRhom1 and iRhom2, is not yet known. Increased surface exposure of phosphatidylserine (PS) on the cell surface, which can be induced by ionomycin stimulation of calcium influx, is associated with increased sheddase activity of ADAM10 and ADAM17 in several cell types.[51,52] The working model for this observation is that the exposed PS headgroup engages the membrane proximal region of the ectodomain to optimize the positioning of the enzyme active site near the cell surface, where cleavage sites for ectodomain shedding are located. The proposed exosite function of the C domain extends influences on substrate selectivity beyond local interactions within the M domain. For ADAM10, published data suggest that an acidic pocket of three aspartic acid residues within the C domain may act as an exosite, recognizing the EphA3 receptor and stimulating the processing of EphA3 upon formation of ligand-receptor complexes.[23]

Interdomain relationships in mature ADAM ectodomains

The first mammalian ADAM for which a mature, full ectodomain structure was reported was the catalytically inactive family member ADAM22.[30] Its overall domain arrangement is compact, with the D + C C-shaped arm serving as an open cup into which the M domain is nestled (Figure 3(a)). This interdomain arrangement is shared with the vascular apoptosis-inducing proteins (VAPs) VAP1

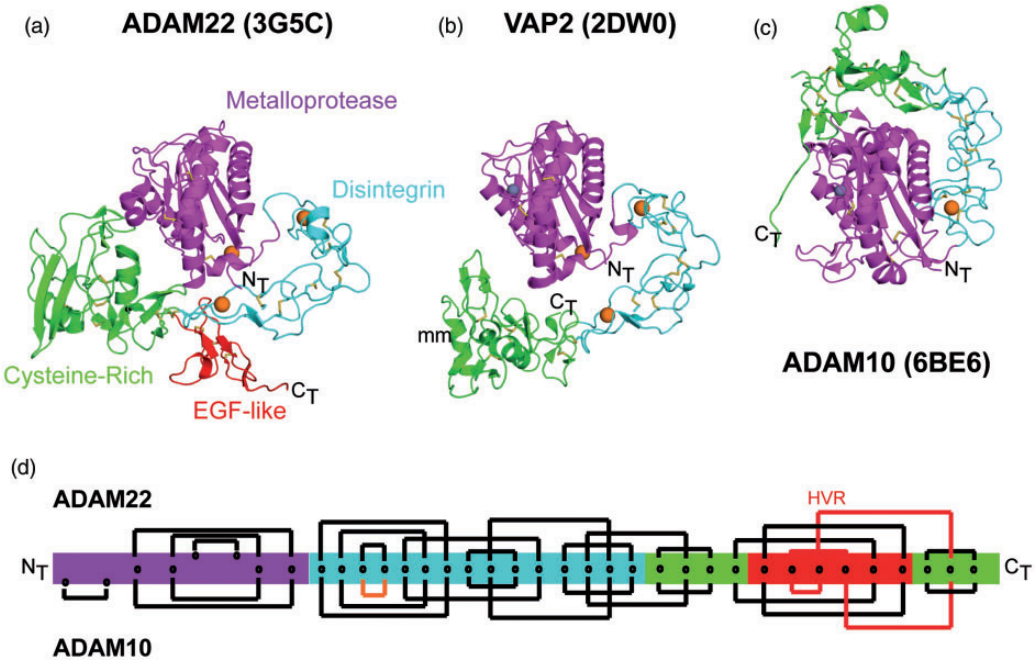

Figure 3. Interdomain relationships in the structures of ADAM22, VAP2, and ADAM10. The ectodomain of (a) ADAM22 (PDB: 3G5C), (b) VAP2 (PDB: 2DW0) and (c) ADAM10 is shown in cartoon representation with amino- (N_T) and carboxy- (C_T) termini labeled. The individual domains are colored according to the schematic representation in Figure 1. Bound calcium and zinc ions are represented as spheres colored orange and grey, respectively. Disulfide bonds are represented in stick form. (d) Disulfide bonding patterns in ADAM22 (Top) and ADAM10 (Bottom) are indicated with closed brackets connecting cysteine residues to their disulfide partner. Non-conserved cysteine residues that form disulfide bonds in only one of the two proteins are offset. The disulfide bond predicted to be missing from the ADAM17 D domain is colored orange. The lines for the disulfide pattern that is mismatched between ADAM22 and ADAM10 in the C domain are colored red. (A color version of this figure is available in the online journal.)

and VAP2, two secreted snake venom toxins that also have the MDC architecture of the ADAMs (Figure 3(b)).[47,53]

In the recently elucidated structure of the mature ADAM10 ectodomain, the overall architecture of ADAM10 is strikingly different from that of ADAM22, even though the isolated M, D, and C domains superimpose well on each other (Figure 3(c)). Superposition of the ADAM10 and ADAM22 M domains shows that the D + C region of ADAM10 wraps along the "side" of the M domain rather than along its base, resulting in the placement of the C domains on opposing surfaces of the M domains in the two structures. The different structural relationship of the D + C region with respect to the M domain in ADAM10 likely results from the shorter linker connecting the M and D domains, which places more limits on the interdomain flexibility of ADAM10.[25]

In the ADAM10 ectodomain structure, the C domain rests at the lip of the catalytic cleft of the M domain, and has a "fail-safe" autoinhibitory role in modulating catalytic activity.[25] Accordingly, the cleavage of a fluorogenic peptide substrate by ADAM10 could be increased several-fold by addition of a monoclonal antibody, 8C7, which overcomes autoinhibition by binding to an epitope that overlaps the interdomain interface between the C and M domains.[24]

The high degree of sequence conservation between ADAM10 and ADAM17, together with prior studies investigating the regulation of ADAM17 activity in cells, suggests that the C domain may have a similar autoinhibitory role in ADAM17. ADAM17-catalyzed ectodomain shedding increases when cells are treated with PMA, a potent PKC pathway activator, and Blobel's group showed that the tight binding inhibitor DPC33 only gains access to the ADAM17 active site in PMA stimulated, but not in quiescent cells.[54] These data led the authors to postulate a model in which the ADAM17 active site was sterically blocked prior to stimulation with PMA, only becoming accessible to the active site inhibitor after compound treatment. In a completely independent line of investigation, the Murphy group developed a cross-domain inhibitory antibody that simultaneously recognizes the M and D + C domains of ADAM17, a finding that is also consistent with the idea that an interdomain interaction occludes the ADAM17 active site.[55] Natural inhibitors targeting ADAM17 likewise show more potency toward the isolated catalytic domain than when the entire ectodomain is present.[56] Based on this combination of structural information, sequence homology, and functional data, it seems likely that the active site in ADAM17 is also autoinhibited by its C domain using an autoregulatory interface analogous to that of ADAM10.

Catalytically inactive ADAMs rely on protein–protein interactions to carry out their functions. The first characterized ADAMs, fertilin-a (ADAM1a) and fertilin-b (ADAM2) are found on the surface of sperm and are required for proper sperm-egg fusion. The mature form of ADAM2 on the sperm surface lacks both its Pro and M domains, using its D + C domains to interact with surface adhesion receptors and facilitate sperm-egg binding.[57]

Unlike the earliest characterized non-catalytic ADAMs, ADAM22 uses its M domain to bind a secreted protein called LGI1, which is characteristically mutated in autosomal dominant partial epilepsy with auditory features (ADPEAF). These proteins appear to play an important role in neuronal development and synapse formation, as genetic disruption of *lgi1*, *adam22*, or *adam23* in mice is postnatal lethal with mice suffering from severe epileptic seizures and peripheral nerve hypomyelination.[26,31,58] Structures of two ADAM22-LGI1 complexes reported recently show that the epitempin-repeat of LGI1, a beta-propeller domain, uses its top face to engage the ADAM22 M domain at the distal end of the ADAM22 catalytic cleft. Structural alignment of ADAM22 in its unbound and bound conformations shows that the Cα atoms of the two states superimpose with a root mean-square deviation of only 0.5 Å. The one notable conformational change occurs in two loops of the metalloproteinase domain at the binding interface, with Trp398, Tyr408, and Tyr409 of ADAM22 flipping out to engage the LGI1 beta propeller (Figure 4(a)[59]). When complexes are formed with ADAM22 using a form of LGI1 that includes both its beta-propeller and leucine-rich repeat domains, the proteins form a domain-swapped 2:2 assembly, which the authors interpret as supportive of *trans* complex formation across the synapse (Figure 4(b)[59]).

Biological inhibition of ADAM proteins by prodomains and TIMPs

After biosynthesis, ADAMs enter the ER as inactive precursors, with latency conferred by the prodomain. The prodomains are then cleaved either by autoproteolysis or by a proprotein convertase, commonly furin, in the trans-Golgi network. ADAM prodomains are considerably larger than those of other metzincin proteins, ranging from 158 to 174 amino acids in length, and have a single conserved cysteine residue that occupies the active site in the latent state.

Although there are no atomic resolution structures yet available for an ADAM prodomain either in isolation or occupying an ADAM catalytic site, structures of matrix metalloproteases MMP1, MMP2, and MMP9 have each been solved with prodomain intact. In these structures, the free cysteine thiol protrudes from the prodomain to complete tetrahedral coordination of the zinc ion at the active site. The flanking residues of the prodomain are situated away from the active site, keeping the prodomain peptide backbone protected from autoproteolysis. Although this "cysteine switch" is considered a defining feature of metzincin family proteases, not all ADAMs contain an odd number of cysteine residues in their prodomains, and recombinant forms of the ADAM17 prodomain harboring a C184A mutation inhibits ADAM17 with comparable potency.[56] These observations indicate that the prodomains use additional points of contact beyond the cysteine at the active site to confer latency. Indeed, certain ADAMs (ADAM10, ADAM17, and ADAM19) contain multiple "shadow" proprotein convertase recognition sites in predicted unstructured regions of

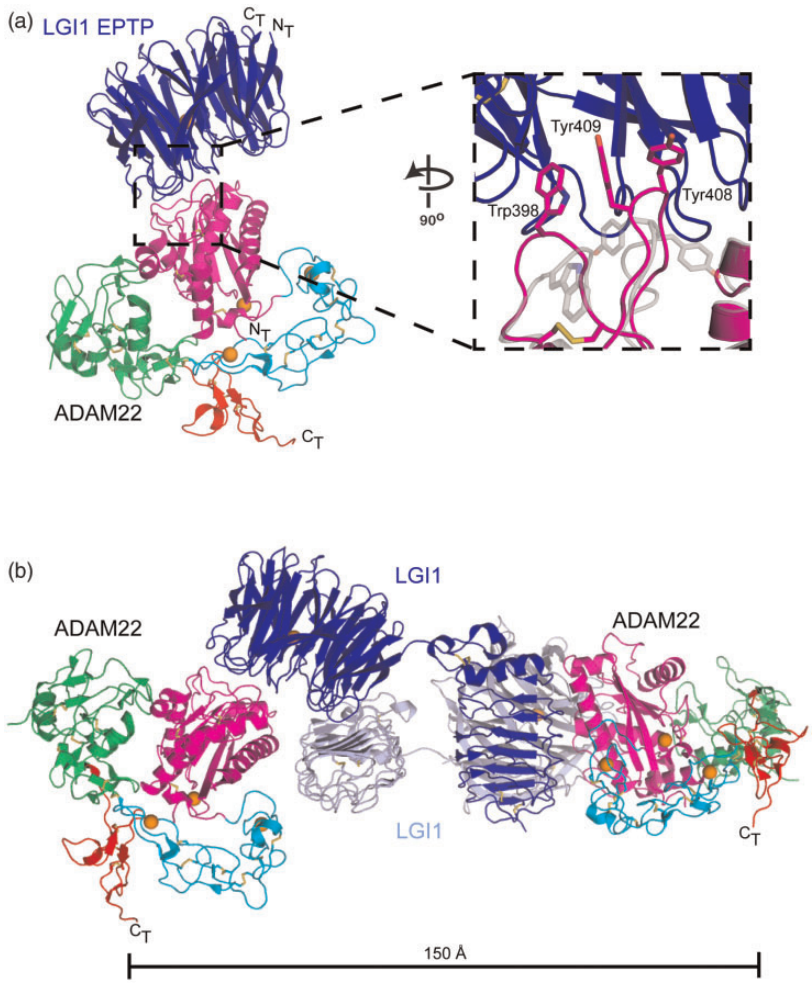

Figure 4. Structure of an ADAM22-LGI1 complex. (a) The ADAM22 ectodomain and bound LGI-1 EPTP domain (PDB: 5Y2Z) are shown in cartoon representation with disulfide bonds as sticks and bound calcium ions as orange spheres. The binding interface between the ADAM22 M domain and the LGI1 EPTP is magnified on the inset to the right. The apo ADAM22 structure (PDB: 3G5C) is superimposed on the ADAM22-LGI1 complex and shown in a transparent grey cartoon representation. The side chains of Trp398, Tyr408 and Tyr409 are represented as sticks. (b) The Cryo-EM structure of the 2:2 complex between ADAM22 and LGI1 (PDB: 5Y31) is shown in cartoon representation. (A color version of this figure is available in the online journal.)

the prodomain and are processed multiple times to enable prodomain release.[60]

In addition to their inhibitory properties, ADAM prodomains also serve as molecular chaperones to ensure transport of the mature ADAM through the secretory pathway to the cell surface. Two mutations in the ADAM10 prodomain, Q170H and R181G, co-segregate with late-onset Alzheimer's disease.[61] These mutations impair the maturation of ADAM10, and transgenic mice bearing forms of ADAM10 with these mutations attenuate ADAM10-dependent APP processing, leading the authors to attribute the increased disease risk from these mutations to a dysfunctional prodomain that has lost its chaperone properties.[18]

The human genome encodes four tissue inhibitor of metalloproteinase (TIMPs 1–4) proteins, originally identified as broad-spectrum inhibitors of MMPs.[28] It has since become clear that TIMPs also inhibit ADAM metalloproteases. TIMP1 and TIMP2 show selective inhibition of ADAM10 and ADAM12, respectively, whereas TIMP3 has a broader spectrum of activity, inhibiting ADAMs 10, 17, 12, 28, and 33.[62]

The TIMPs are small globular proteins that range from 184 to 194 amino acids in length. Structurally, they have two subdomains, referred to as N_T and C_T lobes.[63] The N_T lobe contains three α-helices and a 5-stranded β-barrel that adopts a wedge shape with its edge, consisting of the amino terminus and its AB and CD loops, binding into the catalytic cleft of the metalloproteinase (Figure 5). An intramolecular disulfide bond between the two cysteines of a conserved amino-terminal Cys-X-Cys motif places the N-terminal amine and backbone carbonyl group of Cys1 in position to coordinate the active site zinc, replacing the water molecule used in catalysis. The X-ray structure of TIMP3 in complex with the metalloprotease domain of ADAM17 also shows that the second residue, Thr2, is directed towards the S1′ pocket.[64] Additional contacts from the N_T lobe are observed in the catalytic cleft from the CD and AB loops protruding from the β-barrel. These additional contacts likely contribute to TIMP selectivity as engraftment of TIMP3 residues from these regions onto a TIMP1 scaffold increased the inhibitory activity of the chimeras for ADAM17.[22]

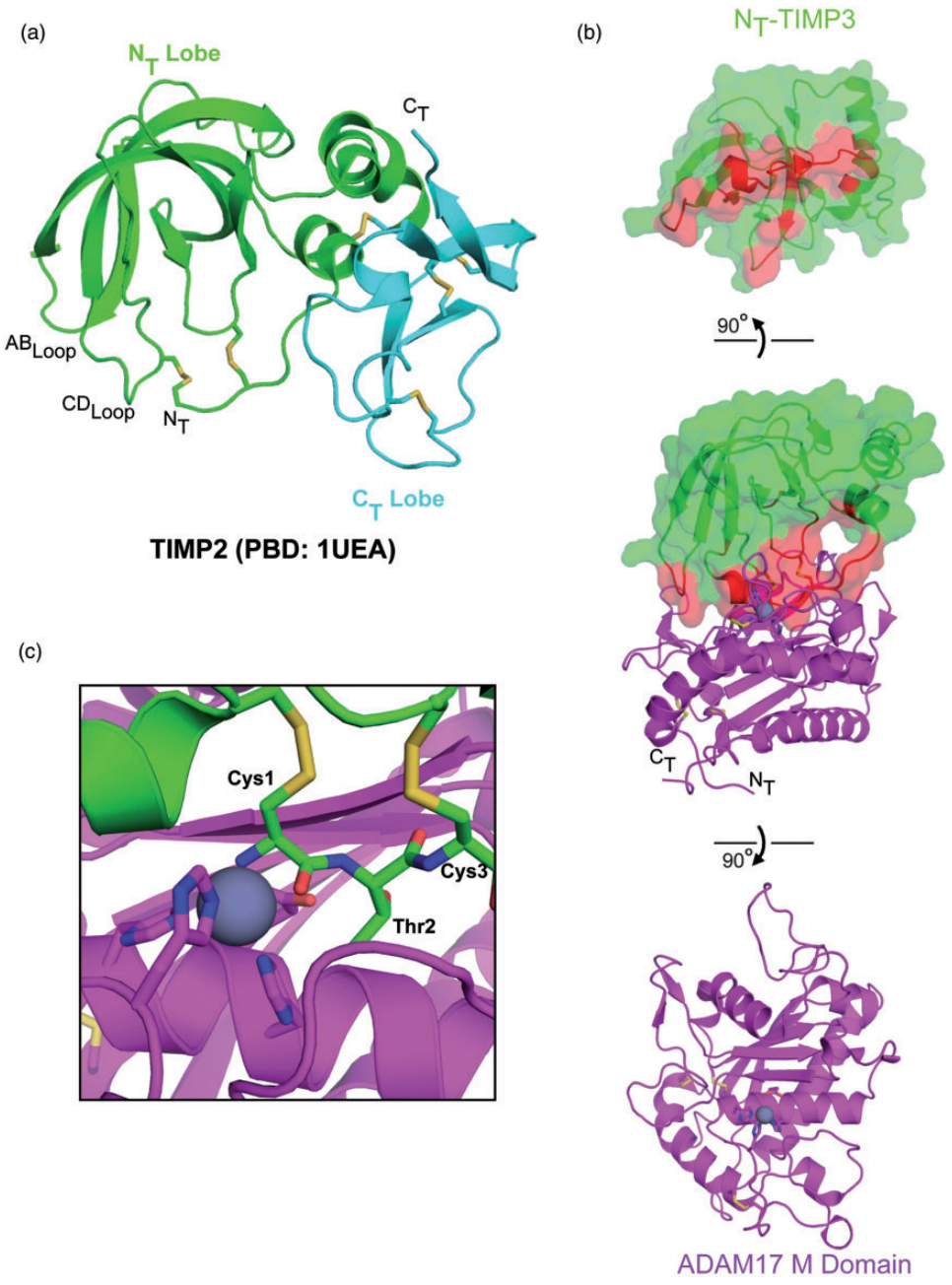

Figure 5. ADAM17-TIMP3 complex. (a) The X-ray structure of full length TIMP1 (PDB: 1UEA) is shown in cartoon representation, with N_T and C_T subdomains are in green and cyan, respectively. Disulfide bonds are represented in stick form. (b) Contact interface of the X-ray structure of the N_T lobe of TIMP3 (green) in complex with the M domain of ADAM17 (purple), shown in "open book" form. Amino acids within 4.5 Å of the M domain are highlighted red on N_T-TIMP3. (c) Magnified view of the N-terminus of TIMP3 bound in the active site of the ADAM17 M domain. The conserved TIMP3 Cys-Thr-Cys sequence and ADAM17 active site amino acids are shown in stick representation. The active site zinc ion is represented as a grey sphere. (A color version of this figure is available in the online journal.)

Concluding remarks

Recent structural advances have provided a deeper appreciation for interdomain relationships that modulate the activity of ADAM proteins in ectodomain shedding and cellular adhesion. It is clear the metalloproteinase domain works in combination with its ancillary domains to execute its biological function. The ADAM ectodomain is dynamic, and accesses conformations that require interdomain movements during its enzymatic "lifecycle." Fundamental questions about ADAM activation and substrate selection, however, still remain unanswered. Maturation of ADAM10 requires association with tetraspanin family C8 proteins, which may also influence substrate selectivity for Notch or APP,[48–50] yet the molecular basis for this role of the C8 tetraspanins is not understood at a structural or biophysical level. Likewise, proper ADAM17 trafficking and activation appears dependent on the iRhom family of proteins,[29,32] but detailed structural information about ADAM17-iRhom complexes is also lacking. Whether other ADAM family members rely on similar maturation or regulatory factors remains unknown, but the ADAM10 and ADAM17 examples suggest that such factors may also

exist. Elucidating the biochemical and structural basis for ADAM regulation will be an exciting avenue of future research that should greatly advance our understanding of ADAM function in biology and human pathogenesis.

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DECLARATION OF CONFLICTING INTERESTS

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