ADAM13 is a member of the disintegrin and metalloprotease protein family that is expressed on cranial neural crest cells surface and is essential for their migration. ADAM13 is an active protease that can cleave fibronectin in vitro and remodel a fibronectin substrate in vivo. Using a recombinant secreted protein containing both disintegrin and cysteine-rich domains of ADAM13, we show that this “adhesive” region of the protein binds directly to fibronectin. Fibronectin fusion proteins corresponding to the various functional domains were used to define the second heparin-binding domain as the ADAM13 binding site. Mutation of the syndecan-binding site (PPRR→PPTM) within this domain abolishes binding of the recombinant disintegrin and cysteine-rich domains of ADAM13. We further show that the adhesive disintegrin and cysteine-rich domain of ADAM13 can promote cell adhesion via β1 integrins. This adhesion requires integrin activation and can be prevented by antibodies to the cysteine-rich domain of ADAM13 and β1 integrin. Finally, wild type, but not the E/A mutant of ADAM13 metalloprotease domain, can be shed from the cell surface, releasing the metalloprotease domain associated with the disintegrin and cysteine-rich domains. This suggests that ADAM13 shedding may involve its own metalloprotease activity and that the released protease can interact with both integrins and extracellular matrix proteins.

ADAMs1 are transmembrane glycoproteins that contain a disintegrin and a metalloprotease domain (1). To date, 33 ADAMs have been identified in worms, insects, amphibians, and mammals (2). They can be classified in two major subgroups depending on the presence or absence of a putative active site (HEGXHXXGXHD) in the metalloprotease domain (3). ADAM proteins have been implicated in many biological processes including fertilization (4, 5), myogenesis (6), neurogenesis (7), axon extension and pathfinding (8), and neural crest cell migration (9). Although the number of potential functions involving ADAMs increases, the function of individual ADAM protein domains remains poorly understood.

The ADAM metalloprotease domain appears to be involved mostly in shedding cell surface molecules (10). Among these molecules are growth factors (tumor necrosis factor-α, transforming growth factor-α, heparin binding-epidermal growth factor), other signaling molecules (Notch, Delta, ephrin), and cell adhesion molecules (selectin, L1-Cam). These cleavages allow cells to send and/or receive a signal as well as change their adhesion to the surrounding cells or substrate. Recently, ADAM13 was shown to cleave fibronectin, one of the major components of the extracellular matrix (ECM) that lies in all cell migration pathways (9).

The disintegrin and cysteine-rich domains compose the adhesive part of the ADAM protein. A small loop within the disintegrin domain, known as the disintegrin loop, can bind to several receptors of the integrin family (11). The best example of this is the interaction of ADAM2 and -3 on the mature mouse sperm with the α6β1 integrin on the egg surface (12–14). In vitro studies of cell lines have also shown that recombinant disintegrin domains can serve as adhesive substrates. One characteristic of ADAM binding to integrins is that dissimilar disintegrin loops can bind to the same integrin. This is the case for ADAM2, -3, and -9, which all bind to the α6β1 integrin despite differences in their disintegrin loop sequences (13–15). This promiscuity makes it hard to predict which disintegrin domain may bind to which integrins. In most of these interactions, the disintegrin domain was shown to be responsible and reagents (antibodies and peptides) to the disintegrin loop were sufficient to interfere with the adhesion. Recently, the ADAM12 cysteine-rich domain was shown to interact with another adhesion molecule, the heparan sulfate proteoglycan syndecan (16). This shows that both the disintegrin and cysteine-rich domains are potentially adhesive to cell surface proteins. Together with the structural data, this suggests that the disintegrin and cysteine-rich domains may in fact represent one functional entity: the “adhesive” domain.

Another level of complexity is that splice variants have been identified for several ADAM proteins. These splice variants encode soluble forms lacking the transmembrane and cytoplasmic domain (17). In most cases the soluble forms contain the metalloprotease domain with or without the inhibitory prodomain, and the disintegrin and cysteine-rich domains. So far it is unclear how these soluble forms function and whether their specificity is the same as their membrane counterparts.
Studies on a closely related family of proteins, the ADAM-TS (soluble proteins containing a disintegrin and a metalloprotease with thrombospondin repeats), may help to elucidate how soluble forms of ADAMs work (18). ADAM-TS proteins are secreted and incorporated in the ECM via their thrombospondin repeats (19). These motifs were initially characterized in the thrombospondin proteins and are known to bind to other components of the ECM. One secreted ADAM-like protein, MIG-17, was shown to influence germinal cell migration in the worm Caenorhabditis elegans (20). The function of the protein was shown to depend on the proteolytic activity and the localization of the protein, via the disintegrin domain, to the basement membrane of the gonads. Similarly, the secreted ADAM12 protein, also called ADAM12 short, was initially reported to bind laminin in a yeast two-hybrid screen (17). These results suggest that adhesive domain of ADAM may interact with extracellular matrix components in a manner similar to that for thrombospondin repeats in ADAM-TS proteins.

Among the various proteins that compose the extracellular matrix, fibronectin is one of the best studied. This large protein promotes cell adhesion and migration as well as signals that are essential for cell survival. Cells use two major families of transmembrane proteins to interact with the various domain of fibronectin. Integrins bind to the central cell-binding domain as transmembrane proteins to interact with the various domain of fibronectin. Integrins bind to the central cell-binding domain as well as to the variable (V) region. These receptors are responsible for most of the cell adhesion, cell migration, and cell survival response to fibronectin (21–23). Syndecan-4 is a proteoglycan that has been shown to interact with the second heparin-binding domain (Hep II), which is located just N-terminal to the V-region. This interaction appears to be essential for the formation of stable adhesion structures called focal contacts (24). It is striking that the two families of receptors involved in cell binding to fibronectin are also known to interact with ADAM proteins.

We have previously shown that ADAM13 can cleave fibronectin in vitro and that cells overexpressing ADAM13, but not those overexpressing a protease-dead point mutant, can remodel a fibronectin substrate (9). In this report we show that ADAM13-DC domain can bind to the Hep II domain of fibronectin. We also show that mutation of the syndecan-binding site within this domain is sufficient to abolish ADAM13-DC binding. Cell adhesion assays suggest that the interaction of ADAM13-DC with the Hep II domain of fibronectin does not prevent syndecan binding to this site. Furthermore, DC13 recombinant proteins support cell adhesion via β1-containing integrins. Finally, ADAM13 metalloprotease domain can be shed from the cell surface in association with the disintegrin and cysteine-rich domains (MDC). Altogether, these results suggest that the adhesive (DC) domain of ADAM13 may play a role in binding to one of ADAM13’s proteolytic substrates: fibronectin. This interaction may “present” the substrate to the metalloprotease domain or localize the shed MDC to the extracellular matrix.

**MATERIALS AND METHODS**

**DNA Constructs—**Primers corresponding to the 5’ end of the ADAM13 disintegrin domain, the cysteine-rich domain, the 3’ end of the disintegrin, and the cysteine-rich domains were used in PCR amplification with Pfu polymerase (Stratagene) to generate the different constructs. SpeI and XhoI sites were added to the sense and antisense primers, respectively, for cloning into the pMT/BipV5-HisA vector (Invitrogen). The ADAM13 signal sequence was inserted into the BamHI and Clal sites of CS2-SSMT (25) to generate the CS2-SS-MT plasmid. XhoI and XbaI cleavage sites were added to the sense and antisense primers, corresponding to each domain, respectively, for cloning into the CS2-SS-MT plasmid. The mutant mGST-12.15V0 was generated from the original GST-12.15V0 fusion protein (26), using the QuickChange site-directed mutagenesis kit (Stratagene) according to the manufacturer’s instructions.

**Cell Culture—**F9 and TKO cell lines were a generous gift of C. Damsky (27). COS-7 (ATCC, CRL1651), F9, and TKO cell lines were grown as a monolayer in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen). Xenopus XTC cells were grown in 67 L15 medium (Sigma) supplemented with 10% fetal bovine serum. Drosophila S2 cells (28) were grown in Schneider’s insect medium (Biomedia) supplemented with 10% fetal bovine serum or 10% Prolifix S6 (Biomedia). Cells were spun down for 5 min at 1000 × g, and the conditioned supernatant was collected and filtered through a 0.22-μm filter. Conditioned medium was dialyzed against 100 mM Tris, pH 8, 200 mM NaCl overnight at 4 °C prior to the incubation (overnight) with Ni-NTA-agarose (Qiagen). The Ni-Ni slurry was washed with 100 bed volumes of 100 mM Tris, pH 8, 100 mM NaCl, 1% Triton X-100, 10 mM imidazole, 2 mM phenylmethylsulfonyl fluoride and with 50 bed volumes of 100 mM Tris, pH 8, 150 mM NaCl. Elution was performed with 100 mM Tris, pH 8, 150 mM NaCl, 400 mM imidazole. The purified protein was dialyzed against PBS, its concentration was determined by measuring absorbance at 280 nm (ε = 0.62), and the purity of the preparation was estimated by silver staining after SDS-PAGE (Fig. 1B). This recombinant protein was called D-DC13, as it is produced in the Drosophila S2 cell line.

**Antibody Preparation—**A histidine-tagged fusion protein corresponding to the disintegrin and cysteine-rich domain of X-ADAM13 (myc-DC13) was produced by transient transfection of CS2-SS-MT-DC13 in COS-7 cells. The secreted fusion protein was purified, as described above, from culture supernatant from cells grown in serum-free media complemented with Prolifix S6, using Ni-NTA-agarose (Qiagen). Monoclonal antibodies 7C9 and 8E8 were obtained, after intraperitoneal injection of BALB/c mice (Jackson Laboratories), using standard techniques (30). Integrin β1 function-blocking antibody mAb HA2/11 (31) was a generous gift of Dr. Ann Sutherland.

**Cell Surface Labeling and Protein Extraction—**Cell surface biotinylation was performed as previously described (9) using EZ-Link Sulfo NHS-LC-Biotin (Pierce). Labeled cells were washed with 100 mM glycine in MBS (XTC) or PBS (COS-7) and extracted with TBS, 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, 5 mM EDTA. Cellular debris was removed by centrifugation at 13,000 × g at 4 °C for 30 min. Protein extracts were incubated with streptavidin-agarose (Sigma) for 1 h at room temperature, and the unbound fraction was then incubated with canavalin A-agarose (Vector Laboratories) overnight at 4 °C (32). The canavalin A and streptavidin) were washed three times with TBS plus 1% Triton X-100 and once with TBS. Proteins were eluted in Laemml buffer, separated on 8% SDS-PAGE, and blotted with the various antibodies as described below.

**SDS-PAGE and Western Blotting—**Protein samples with reducing agent (5%, β-mercaptoethanol, Sigma) were separated alongside prestained molecular size standards (Bio-Rad) through SDS-PAGE gels (32) and transferred to nitrocellulose membrane (Schleicher & Schuell). Proteins were visualized using 0.2% Ponceau Red in 3% trichloroacetic acid prior to Western blotting. Membranes were blocked with 5% nonfat dry milk in TBS, 0.1% Tween 20 (TBST). Purified antibodies and horseshadish peroxidase-conjugated secondary antibody (Jackson) were diluted in the same buffer. Detection was performed using ECL (Amersham Biosciences) and Kodak Biomax films.

**Spot Blots—**Purified bovine fibronectin (1 μg, Sigma), EHS-laminin (1 μg, Sigma), type IV collagen (1 μg, Sigma), and BSA (1 μg, Intergen) were spotted onto nitrocellulose membrane (Schleicher & Schuell). After staining with 0.2% Ponceau Red in 3% trichloroacetic acid prior to Western blotting, Membranes were blocked with 5% nonfat dry milk in TBS, 0.1% Tween 20 (TBST). Purified antibodies and horseshadish peroxidase-conjugated secondary antibody (Jackson) were diluted in the same buffer. Detection was performed using ECL (Amersham Biosciences) and Kodak Biomax films.
ADAM13 Binds Fibronectin

Fig. 1. mAb 7C9 and 8E8 recognize the ADAM13 cysteine-rich domain. A, schematic representation of the recombinant DC13 proteins. Myc-DC13 can be produced in COS-7 and XTC cells. D-DC13 is produced in Drosophila S2 cells. Culture supernatants from nontransfected COS-7 (lanes 1), transfected S2 (lanes 2), and transfected COS-7 (lanes 3) were purified on concanavalin A-agarose. Proteins were separated on 10% SDS-PAGE, transferred to nitrocellulose, and blotted with mAb 9E10 or 7C9. mAb 9E10 only recognizes the myc-DC13 protein (lanes 3), whereas mAb 7C9 recognizes both D-DC13 (lanes 2) and myc-DC13 (lanes 3). B, silver staining of proteins separated on a 12% SDS-PAGE. Total supernatant from transfected S2 cells (10 μl, S) or purified D-DC13 (5 μl, P) were loaded side by side. The major D-DC13 is indicated with an arrowhead, whereas a minor larger protein (potential dimer) is indicated with an arrow. C, Western blot of total protein extract from nontransfected COS-7 (lane 1) and cells transfected with either myc-DC13 (lane 2), myc-D13 (lane 3), and myc-C13 (lane 4) were probed using mAb 9E10, 7C9, and 8E8. Both 7C9 and 8E8 recognize the cysteine-rich (C) domain of ADAM13 (4).

Results

ADAM13 is essential for the migration of cranial neural crest cells. This role is thought to involve remodeling of the fibronectin substrate present in the migration pathways (9). Ongoing studies in our laboratory also suggest that the metalloprotease domain itself may not be responsible for the specificity of ADAM function but that the DC domain of ADAM13 (DC13) carries at least part of the functional specificity. We have therefore investigated whether the adhesive disintegrin and cysteine-rich domain of ADAM13 can interact with ECM proteins and in particular with fibronectin.

ADAM13-DC Binds to ECM Proteins—To investigate the binding of ADAM13-DC to ECM proteins, we generated a secreted form by combining the signal sequence, the disintegrin domain itself may not be responsible for the specificity of ADAM function but that the DC domain of ADAM13 (DC13) carries at least part of the functional specificity. We have therefore investigated whether the adhesive disintegrin and cysteine-rich domain of ADAM13 can interact with ECM proteins and in particular with fibronectin.

Pull-down Experiments—GST fusion proteins were purified exactly as described previously (26). Total bacterial protein extracts or purified proteins (10 μg) were mixed with S2 cell culture supernatant containing the D-DC13 recombinant protein and incubated overnight at 4 °C. Glutathione-agarose beads (Sigma) were added and incubated for 1 h at room temperature. Bound proteins were washed three times with PBS supplemented with 250 mM NaCl, 1% Triton X-100, and once with TBS, eluted with reducing Laemmli buffer, separated on 12% SDS-PAGE, transferred to nitrocellulose, and blotted with mAb 9E10 or 7C9. mAb 9E10—recognizes the myc-DC13 protein (lanes 3), whereas mAb 7C9 recognizes both D-DC13 (lanes 2) and myc-DC13 (lanes 3). B, silver staining of proteins separated on a 12% SDS-PAGE. Total supernatant from transfected S2 cells (10 μl, S) or purified D-DC13 (5 μl, P) were loaded side by side. The major D-DC13 is indicated with an arrowhead, whereas a minor larger protein (potential dimer) is indicated with an arrow. C, Western blot of total protein extract from nontransfected COS-7 (lane 1) and cells transfected with either myc-DC13 (lane 2), myc-D13 (lane 3), and myc-C13 (lane 4) were probed using mAb 9E10, 7C9, and 8E8. Both 7C9 and 8E8 recognize the cysteine-rich (C) domain of ADAM13 (4).

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produce two monoclonal antibodies (7C9 and 8E8) that were used in this study. Although both monoclonal antibodies recognize the recombinant DC13, 7C9 recognizes best the reduced/denatured DC13 protein in Western blotting experiments, whereas 8E8 gives better results for the native protein (immunofluorescence). Interestingly, both monoclonal antibodies recognize the cysteine-rich domain of ADAM13 (Fig. 1C). In all experiments the recombinant proteins produced in COS-7 and insect cells behave similarly and the presence of the large poly-Myc tag did not interfere with our assays. We also noticed that native fibronectin (FN) and laminin (LN) but not to native collagen IV (Col.) Denaturation and saturation of free cysteine residues with iodoacetamide (IAM) do not prevent binding, whereas reduction of disulfide bonds with β-mercaptoethanol (BME) completely abolishes the interaction.

Purified ECM proteins (Sigma) were blotted onto nitrocellulose either immediately or after different treatment (see Materials and Methods). Blotted proteins were then incubated with the myc-DC13 recombinant protein. BSA was used as a negative control for each experiment. In this assay, myc-DC13 bound to native fibronectin and EHS laminin, but not to native BSA, collagen IV, or the reduced fibronectin (Fig. 2). This interaction was not prevented by pre-incubation of fibronectin with reagents that block free cysteine (iodoacetamide). Surprisingly, denaturation of fibronectin by boiling the protein in SDS did not prevent myc-DC13 interaction. We next asked which conditions at the same time. Using the various fusion proteins, we found that DC13 binds to GST-1.9, GST-12.12, GST-12.15, and GST-12.15V0. In contrast, no binding was observed for GST alone, GST-9.11, and GST-V. A representative example of the pull-down results is shown in Fig. 3C. Interestingly, the GST-1.9 and all the C-terminal fusion proteins that bound to DC13 have in common a domain known to interact with heparin (Fig. 3A). In particular, the smallest fusion protein that bound to DC13 (GST-12.15V0) contains Hep II (33), which includes a short sequence responsible for the binding of heparan sulfate proteoglycans like syndecan-4 (24). For simplicity, we will refer to GST-12.15V0 as the Hep II fusion protein.

ADAM13-DC Binding to Hep II Requires an Intact Syndecan-binding Site—In Xenopus fibronectin the Hep II site contains the sequence PPRRPR and mutation of the two arginines into a threonine and a methionine (RR → TM) abolishes cell binding to this site (26). We have generated a similar mutation in the Hep II fusion protein and called the resulting protein mGST-12.15V0 or mHep II (mutated Hep II, Fig. 3A). We tested the effectiveness of the mutation by assaying the ability of various fusion proteins to bind to dextran-agarose beads, which mimic the heparan sulfate proteoglycan group present on syndecan. As expected only the intact Hep II fusion protein was able to bind to dextran sulfate beads (Fig. 3B). Similarly, only the intact Hep II, but not the mutated fusion protein, promoted embryonic cell attachment (Fig. 4A). When pull-down experiments were repeated using wild type and the mutant Hep II fusion proteins, D-DC13 only bound to the wild type Hep II protein (Fig. 3C). This result demonstrates that an intact syndecan-binding site is required for D-DC13 binding to the Hep II domain of fibronectin. This also suggests that syndecan and DC13 may cooperatively bind or compete for this binding site on fibronectin.
In this assay, F9 and TKO cells bound equally well to GST-12.15V0 (Hep II), whereas the TKO cells did not bind to the mutated mGST-12.15V0 (mHep II) fusion protein (Fig. 4B). This difference in behavior can be explained by the presence in both fusion proteins of an α6β1 binding site (15th type III, AVDPS), which can account for the binding of the F9 cells to the mutated form of the Hep II fusion protein. In contrast, the TKO cells are dependent on the heparin binding site as they express only syndecan but not β1-containing integrin. Interestingly, the TKO cells, although they express at least one syndecan and bind to both the Hep II domain of fibronectin and the cysteine-rich domain of ADAM12 (data not shown), did not bind to the recombinant ADAM13-DC protein (Fig. 4B). These results suggest that ADAM13-DC does not interact with the syndecans expressed by the embryonic animal cap (syndecan 1 and 2 (Ref. 34)) and TKO cells.

Many ADAM disintegrin domains have been shown to support cell adhesion through integrin binding. We have used the mouse F9 and the TKO cells (27) to investigate whether ADAM13-DC has similar binding capacity. Although our previous results showed no cell binding to D-DC13 in the presence of calcium and magnesium, addition of manganese during the assay allowed F9 cells to attach to D-DC13 (Figs. 4B and 5).

The adhesion was clearly due to the presence of the recombinant D-DC13 protein as the two monoclonal antibodies (7C9 and 8E8), directed against the cysteine-rich domain of ADAM13, prevented cell adhesion to the substrate (Fig. 5A). In contrast, the TKO cells, which lack all β1-containing integrins, were unable to attach to DC13 under the same conditions. Similarly, a β1 function-blocking antibody completely inhibited F9 cell adhesion to the DC13 substrate in the presence of manganese. Although PMA alone had very little effect on cell adhesion to DC13 in the presence of calcium and magnesium, we found that PMA increased cell binding in manganese-containing medium. Together, these results show that DC13 can interact with activated β1-containing integrins on the cell surface.

**ADAM13-DC Interaction with the Hep II Domain of Fibronectin Does Not Prevent Cell Binding**—We next asked whether DC13 interactions with fibronectin affected cell adhesion to this protein, for example, by preventing syndecan recognition of the PPRPRP sequence. Substrates of fibronectin and the various GST-fibronectin fusion proteins were coated onto plastic and were used in adhesion assays either directly, or after pre-incubation with the D-DC13 protein. Our results show that DC13 binding to the Hep II domain of fibronectin did not inhibit binding of embryonic ectoderm cells (data not shown). Similarly, we found that binding of F9 and TKO cells to either fibronectin or Hep II was not significantly affected by the presence of D-DC13, even when a 10-fold molar excess of the recombinant DC13 was used (Fig. 5B). Similar results were obtained when the D-DC13 protein was left in the medium after the addition of cells. These results suggest that DC13 binding to the Hep II domain of fibronectin does not prevent further binding of syndecan at that site. It is unclear whether syndecan and DC bind to the same sequence, as suggested by the mutagenesis result, or whether the mutation of the syndecan-binding site perturbs the structure of the Hep II domain so that it can no longer interact with the DC domain of ADAM13.

**ADAM13 MDC Is Shed from the Cell Surface**—Because of the presence of both syndecan and members of the integrin family of molecules on the available cell types, we have not been able to analyze whether the adhesive domain of ADAM13 can interact with fibronectin and/or the integrin in the context of the integral ADAM13 protein. In addition, it is
possible that, like other ADAMs, ADAM13 may be present as a soluble form in vivo. To address this possibility, we have transfected COS-7 cells with various construct of ADAM13 and analyzed the conditioned media for released protein (Fig. 6). Transfection of Myc-tagged wt- and E/A-ADAM13 in COS-7 cells results in the expression of a 140-kDa pro form and 120-kDa metalloprotease active form (Fig. 6A). The six-Myc epitope tag accounts for 20 kDa of this apparent molecular mass (Fig. 6, compare A and D). Cell numbers are estimated by reading the optical density (OD) at 595 nm after staining with crystal violet. Cell adhesion to fibronectin (FN) was used as a positive control and BSA as negative control. The TKO cells attached to the Hep II-containing fusion protein (12.15V0) via syndecan. This attachment is inhibited by the mutation (mHep II) RR → MT in m12.15V0. In the same conditions no attachment of TKO cells was measured on D-DC13. In contrast, F9 cells can use integrins to attach to both wt- and the mutated Hep II fusion proteins. Some attachment was also observed on D-DC13.

The size of the shed ADAM13 protease (MDC) varies with the absence or presence of serum in the culture media (Fig. 6, compare A and B). In the presence of serum, the 53-kDa band decreases in intensity whereas multiple larger bands starting at 180 kDa appear. These larger proteins are likely to represent forms of MDC cross-linked to 2-macroglobulin (2M) as described previously for ADAM12 (36). Accordingly, transfected cells incubated with purified 2M produce immunoreactive polypeptides of the same size as those produced in the presence of serum (Fig. 6C). To determine whether the shed form of ADAM13 was only found following overexpression in COS-7 cells, we analyzed protein in conditioned medium from Xenopus XTC cells expressing endogenous ADAM13 (Fig. 6D). Our results show that a similar immunoreactive polypeptide is cleaved from the XTC cell surface. The slightly smaller apparent molecular mass observed for this protein may represent minor glycosylation differences or a difference in the cleavage site in ADAM13 between mammalian and amphibian cells.

These results show that wt-ADAM13 can be cleaved into a soluble active metalloprotease. This metalloprotease can form an inactive complex with 2M similarly to soluble ADAM12 protein. The absence of a soluble form generated from the
protease-dead E/A mutant suggests that ADAM13 metalloprotease activity may be involved in the generation of the soluble MDC protease. Interestingly, transfection of COS-7 cells with a mutant form of ADAM13 lacking the transmembrane and cytoplasmic domain results in an inactive pro-form that is never processed, suggesting that membrane anchorage is required for ADAM13 processing (data not shown).

Collectively, our results show that the ADAM13-DC can interact both with fibronectin, in an ion-independent manner, and with manganese-activated \(\beta_1\) integrin on the cell surface. Furthermore, proteolytic processing of ADAM13 results in a soluble form of the protein containing the metalloprotease domain associated with the DC domain. This form could potentially interact both with the ECM and with integrins at the cell surface.

**DISCUSSION**

ADAM13 is a metalloprotease essential for cranial neural crest cell migration. It can bind and cleave fibronectin in vitro and remodel a fibronectin substrate in vivo. ADAM13 metalloprotease domain can be shed from the cell surface in association with the disintegrin and cysteine-rich domains. The DC domain of ADAM13 is able to bind to either ECM proteins like fibronectin and laminin or to activated \(\beta_1\)-containing integrins on the cell surface. This binding may represent how ADAM13 proteases can choose and hold on to their substrates. Alternatively, ADAM13-DC binding to ECM proteins may be important for its localization in the cellular environment, where its metalloprotease activity could cleave, expose, and/or release other proteins associated with the ECM. Finally, this dual binding to either integrin or ECM may play a role in modulating cell adhesion during cell migration.

ADAM13 appears to be unique among ADAM family members, both because of its expression pattern and by its biochemical features. Our results show that ADAM13 can be shed from the cell surface as an active protease associated with the adhesive DC domain. Although other ADAMs can also generate soluble forms, this is typically through changes in mRNA splicing and not by proteolysis like ADAM13 (17). Furthermore, we have generated “artificial” forms of ADAM13 that do not code for the transmembrane and cytoplasmic domain to mimic the short forms of ADAM, but, unlike ADAM12 and ADAM17, ADAM13 short is never processed and remains inactive. These results suggest that ADAM13 processing requires insertion of the protein into the membrane.

Together with the adhesion data, the shedding of ADAM13 suggests that the adhesive part of the soluble MDC can interact with both fibronectin and \(\beta_1\) integrins on the cell surface. Although ADAM13-DC binding to fibronectin is strong under physiological conditions, only activated integrins can bind to this domain. Unfortunately, we have not been able to demonstrate that ADAM13-DC in combination with the protease domain (MDC) also binds to the same proteins. Our inability to show binding of shed MDC13 to fibronectin may be explained either by a difference in conformation of MDC13 versus DC13 or by the possibility that shed MDC13 may already be associated with a partner (possibly fibronectin) when it is released from the cell. This latter hypothesis is currently being tested using exclusion chromatography of conditioned culture media.

Another question that remains is whether the integral
ADAM13 protein interacts with fibronectin. This has not yet been addressed, as we have not been able to find a cell line lacking both syndeacan and integrins, both of which are strong receptors for the same domain of fibronectin as ADAM13-DC. However, the fact that immunodetection of ADAM13 with a cytoplasmic domain antibody reveals its co-localization with fibronectin at the intesosmatic boundary (future muscle) suggests that the membrane-bound form of ADAM13 may indeed interact with fibronectin at this site (38).

**What Is the Role of ADAM13-DC Domain in the Function of the Protein?**—One possibility is that ADAM13 is normally shed, and that the DC domains act, like the TSP motifs of ADAM-TS, to localize the active protease in the ECM. We should then expect to find shed MDC13 associated with the ECM of Xenopus XTC cells and in the migration pathways of neural crest cells. Using two monoclonal antibodies to the cysteine-rich domain of ADAM13, we have not been able to find endogenous MDC in the embryo; however, we have detected it in Xenopus XTC culture supernatant. The absence of detectable MDC13 in the extracellular matrix may be explained by the fact that the ability of both mAb 7C9 and 8E8 to recognize the recombinant DC13 protein decreases greatly upon binding to fibronectin. It is therefore possible that MDC13 may not be seen by these monoclonal antibodies once it has become associated with a complex ECM network. Finally, it is also possible that the shed ADAM13 protease is unstable and therefore difficult to detect in vivo.

**ADAM13 Control of Cell Migration**—We previously showed that ADAM13 is required for the proper migration of cranial neural crest cells in the branchial and hyoid arch, and we presented a model in which ADAM13 functions to open or widen migration pathways by modifying the extracellular matrix (9). Our current results suggest that the extracellular domain of ADAM13 may be incorporated into the matrix. The protease domain remains active in this soluble form and could still cleave components of the ECM. Additionally, the adhesive domain could be used as a substrate by activated β3-containing integrins. The presence of this adhesive clue in the migration pathways could be interpreted by the migrating cells, to enter or avoid certain directions. In our hands, DC13 was unable to promote cranial neural crest cell migration in vitro, suggesting that it does not act as a substrate for migration.

Consistent with our observations with embryonic ectodermal and F9 cells, DC13 interaction with the Hep II domain of fibronectin does not perturb cranial neural crest cell migration, even though this region of fibronectin is essential for normal cranial crest migration.

Recently, the shedding of the L1 adhesion molecule by ADAM10 was shown to enhance Chinese hamster ovary cell migration (39). The shed extracellular domain of L1 bound to the αβ1 integrin at the Chinese hamster ovary cell surface, sending a signal that stimulated cell migration. This stimulation was blocked both by αβ1 function-blocking antibodies and by reagents that prevented L1 shedding. Similarly, the shed extracellular domain of ADAM13 could bind to an integrin on the cranial neural crest cell surface and promote cell migration. Finally, the αβ1 integrin has been reported to be an essential component of the adhesive repertoire used by migrating neural crest cells (40, 41). This integrin binds to the connecting segment region called ICICS or V (variable) as well as to a similar site in the Hep II domain of fibronectin (42, 43). In native plasma fibronectin the CS-1 adhesion site is masked but can be exposed under partial proteolysis with cathepsin D (44). Because the ADAM13 adhesive domain can bind to the second heparin-binding domain of fibronectin and the ADAM13 metalloprotease domain can cleave fibronectin, it will be of interest to investigate whether this cleavage can also expose the CS-1 site. This could be one way to modify the migration pathways and to present a new adhesion site to following neural crest cells. To date it is unclear whether the αβ1 integrin plays any role in Xenopus neural crest cell migration. Although the mRNA is present at that developmental stage, the protein has not been detected prior to neural crest cell migration (45).

ADAM13 binds and cleaves fibronectin. The proteolytic activity is essential for proper cranial neural crest cell migration. To date, the precise mechanism by which ADAM13 may affect cell migration is not known, but the understanding of individual ADAM13 domain functions is a necessary step toward this goal. The identification of other ADAM13 partners and substrates, whether they are cytoplasmic or extracellular, will provide valuable information and help design reagents that interfere with endogenous ADAM13 function during embryonic development.

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3. A. Gaultier and D. Alfandari, unpublished data.
4. A. Gaultier and D. Alfandari, unpublished data.
5. D. Alfandari, B. G. Hoffstrom, and D. W. DeSimone, unpublished data.
ADAM13 Binds Fibronectin

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