Protein Ectodomain Shedding

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I. Introduction

Not long ago, the release of the extracellular domain through limited proteolysis was recognized as a general mechanism to regulate the function of transmembrane proteins. This type of limited proteolysis is currently known as ectodomain shedding and affects a surprisingly large group of transmembrane proteins. In fact, all structural and functional categories of transmembrane proteins include members susceptible to shedding. Thus, ectodomain shedding can potentially regulate most cellular functions mediated by transmembrane proteins and, therefore, has attracted the attention of cell biologists focused on different problems such as cell adhesion or signal transduction, or certain pathologies such as Alzheimer's disease or cancer, to mention a few.

Ectodomain shedding occurs at or near the cell surface and is a regulated process; although it occurs in nonstimulated cells (and is then known as basal shedding), it can be dramatically activated by several independent mechanisms. Use of phorbol esters, well-characterized nonphysiological compounds with the ability to activate protein kinase C (PKC), is the most common way to activate ectodomain shedding. Typically, soon after phorbol ester addition (~10 min) cells shed the ectodomains of a considerable fraction of cell surface molecules (~2%). Several independent lines of evidence indicate that phorbol esters do not activate many shedding enzymes with restricted specificities. In contrast, it appears that few common factors are required for the shedding of the majority of susceptible proteins. Since this proteolytic machinery has the ability to act on such a large number of cell surface molecules, it seems reasonable to suppose that it is under strict control. Although there have been some advances in the identification of the components of the shedding machinery, as well as in the characterization of the mechanisms that regulate their activity, the picture is still far from clear.

Substrates of the regulated shedding machinery are sometimes susceptible to cleavage within the transmembrane domain by other proteolytic systems. Thus, it appears that, in addition to the shedding machinery, additional proteases act in a coordinated fashion to alter the structure and function of proteins that follow the secretory pathway. Although these additional proteolytic cleavages are not the main subject of this review, they will also be briefly discussed.

II. Functional Consequences of Shedding

Given the diversity of proteins that undergo shedding, the functional outcome of this proteolytic event obviously depends on the particular protein considered. We will, therefore, discuss separately the shedding of transmembrane proteins grouped in different functional categories. Since there are several recent excellent reviews on different aspects of the substrates of ectodomain shedding,[1−8] we will try to focus on aspects not primarily covered in those; it is advisable to consult the mentioned reviews to learn more on aspects not found below.

A. Transmembrane Growth Factors and Related Molecules

Cells exchange growth and differentiation signals through diffusible polypeptides collectivelly known as growth factors that bind and activate specific receptors in target cells. Certain growth factors and cytokines, which include the well-characterized EGF (epidermal growth factor) and TNF (tumor necrosis factor) families, are synthesized as transmembrane forms that, through ectodomain shedding, release the

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receptor-binding domain into the extracellular medium (Figure 1B).[9] Early experiments performed with transforming growth factor (TGF)-α, a prototypical growth factor belonging to the EGF family (Figure 1A), indicated that the membrane-anchored form (named proTGF-α) is able to activate its cognate receptor, the epidermal growth factor receptor (EGFR),

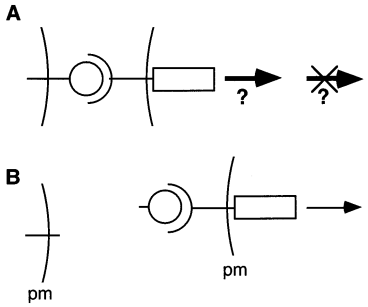

Figure 1. Mechanisms of action of transmembrane growth factors. Transmembrane growth factors are represented as open circles, and the tyrosine kinase domain of the receptors is represented as open squares. The plasma membrane (pm) is indicated. (A) Juxtacrine signaling. Apparently conflicting results suggest that growth factors activate their cognate receptors, transducing a strong signal, or that transmembrane growth factors are inactive. (B) After ectodomain shedding the soluble version of the growth factor can reach cells located at a distance and activate EGFRs.

expressed in adjacent cells.[10,11] Therefore, according to these reports, proTGF-α is a biologically active form and not a mere precursor of the soluble form (known as TGF-α). This mode of signaling, mediated by the interaction of two transmembrane proteins expressed in neighboring cells, is named juxtacrine. Although some recent reports support the importance of juxtracrine signaling mediated by EGF-like growth factors, others cast doubts about the activity of transmembrane proTGF-α and related transmembrane growth factors. According to some papers, the active transmembrane form of certain growth factors can be potentiated by CD9, a protein that belongs to the family of tetraspanins (proteins with four membrane-spanning domains). CD9 physically interacts with proheparin-binding EGF-like growth factor (proHB-EGF), another member of the EGF family that also binds to the EGFR.[12] Using a coculture of cells expressing proHB-EGF, alone or in the presence of CD9, and cells expressing EGFR, CD9 was found to potentiate the juxtacrine mitogenic effect of proHB-EGF.[12] CD9 has been recently shown to bind also to proTGF-α.[13] This interaction induces a hyperactivation of EGFR by increasing the expression of proTGF-α at the cell surface.[13] Another type of evidence also highlights the importance of juxtacrine signaling; human colon carcinoma cell lines that show a defective shedding of proTGF-α have been found to promote a higher level of EGFR activation than that of corresponding equivalent amounts of soluble TGF-α, indicating a higher activity of proTGF-α.[14] Therefore, defective shedding of proTGF-α could be a mechanism whereby malignant cells can obtain a growth advantage.[14] In contrast to these reports, it has been found that juxtacrine signaling mediated by proTGF-α and other EGFR ligands is abolished by inhibitors known to block ectodomain shedding,[15] indicating that soluble rather than transmembrane forms mediate the biological effect of proTGF-α and other EGFR ligands. In support of this conclusion, mice genetically deficient in the protease responsible for the shedding of proTGF-α show, in addition to a lack of production of soluble TGF-α, a phenotype similar to that of

proTGF-α knock-out mice.[16−18] In view of these apparently contradictory reports, it seems that further experimental approaches should be used to clarify the possible function of EGF-like transmembrane growth factors in different pathophysiological situations.

Growth factor ectodomain shedding has been recently found responsible for the integration of different signaling pathways. Although it is well-known that stimulation of G-protein-coupled receptors (GPCRs) leads to transactivation of the EGFR, the mechanism supporting this cross-talk has remained elusive until a recent report[19] showed that activation of GPCRs induces the shedding of proHB-EGF (Figure 3). The soluble ectodomain, HB-EGF, then binds and activates the EGFR.[19] Activation of GPCRs does not seem to specifically enhance only the shedding of proHB-EGF; the shedding of c-Met, a cell surface receptor, has also been recently found to be enhanced by GPCRs.[20] Thus, ectodomain shedding likely represents a mechanism used to integrate the different signals that simultaneously reach a single cell.

The functional significance of the shedding of another type of membrane-anchored ligand has been recently shown. To form circuits, neurons extend growing axons (growth cones) under the influence of attractive and repulsive molecular cues. The ephrin membrane-anchored ligands form a large family of axon guidance molecules with the ability to bind receptor tyrosine kinase of the Eph family in a juxtacrine fashion.[21] Although initally ephrin binds to Eph receptors supporting cell adhesion, as well as intercellular communication, soon after contact has taken place the growth cone expressing Eph receptors surmounts adhesion and breaks away from the ephrins.[21] A recent report shows that ephrin-A2 can be shed from the cell surface and that mutations that inhibit the shedding of ephrin-A2, without affecting its binding to Eph, delay axon withdrawal, indicating that the shedding of the ectodomain of ephrins could be the mechanism that mediates axon detachment from the ephrin-coated cell surface.[22]

In summary, although some questions, such as the role of the transmembrane forms of certain EGF-like growth factors, remain unsolved, the shedding of the ectodomain of growth factors has been recently recognized as a novel and versatile regulatory step. It is an effective means to regulate the function of certain EGF-like growth factors and a mechanism to establish cross-talk between different signaling pathways and a switch of the activity of ephrins.

B. Membrane Receptors

It has long been known that the soluble domains of receptors generated by shedding can modulate the function of ligands by preventing (antagonists) or favoring (agonists) the formation of active signaling complexes (reviewed in ref 23). Recent reports have focused on the function of the transmembrane/cytoplasmic domain that remains bound to the cell following receptor ectodomain shedding in normal and pathological situations (Figure 2).

Tyrosine kinase receptors, such as EGFR, transduce signals controlling cell growth, survival, motility, and differentiation and seem to be crucial for the development of some tumors.[24] Several lines of evidence indicate that, at least in certain instances, the transmembrane/cytoplasmic domains devoid of the extracellular ligand-binding domain may exhibit constitutive kinase activity and, thus, enhanced signaling potency. An engineered deletion of HER2, a receptor belonging to the EGFR family, lacking the extracellular domain, shows an increased tyrosine kinase activity and transforming efficiency.[25,26] Several retroviral oncogenes code for receptors that lack most of the extracellular domain, resulting in the production of constitutively active, membrane-bound receptor fragments.[27] Mutant EGFRs with truncations in the extracellular domain found in several human carcinomas have enhanced oncogenic activity.[28,29] Similarly, the shedding of another member of the EGFR family, HER4, results in the formation of a phosphorylated truncated fragment that has tyrosine kinase activity, and may act as a membrane dock for signaling molecules with Src-2 homology domains.[30] Therefore, although in many of the mentioned instances the transmembrane/cytoplasmic domain does not arise through ectodomain shedding, they show the potential autonomous signaling ability of this portion of the receptor. Thus, the activation

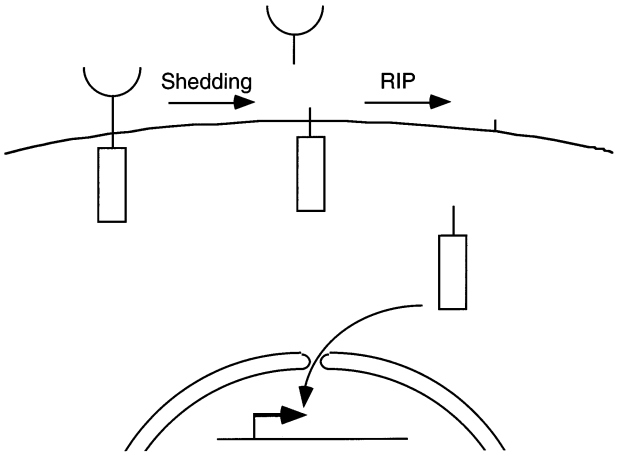

Figure 2. Certain receptors sequentially undergo ectodomain shedding and RIP. While shedding releases the ectodomain to the extracellular media, RIP releases the intracellular domain that, in the case of certain receptors, can move into the nucleus where it modulates the transcription of target genes.

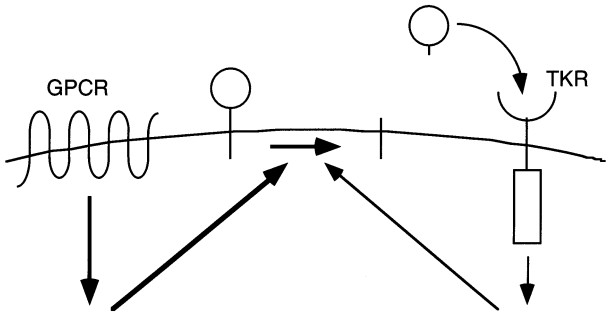

Figure 3. Ectodomain shedding mediates the cross-talk between different signaling pathways. Activation of G-protein-coupled receptors leads to the activation of the shedding of the ectodomain of proHB-EGF (see the text) that can activate tyrosine kinase receptors. Since tyrosine kinase receptors also activate ectodomain shedding, a positive feedback loop can be established.

of tyrosine kinase receptors of the EGFR family by shedding of the ectodomain is an attractive hypothesis for many authors. Furthermore, the shedding of the neurotrophin receptor and that of certain cell adhesion molecules with well-characterized ability to transduce signals produce signaling-competent transmembrane/cytoplasmic fragments.[31,32]

The shedding of tyrosine kinase receptors may also be relevant for the development of tumors. Around 30% of human breast cancers overexpress HER2.[33] This increase in HER2 levels has been associated with enhanced tumor aggressiveness as well as a high risk of relapse and death.[33] High concentrations of the products of HER2 shedding, p110 (soluble ectodomain) and p95 (membrane-bound truncated receptor) (ref 34 and references therein), have been found in human breast cancer and are associated with a more aggressive behavior,[35] indicating that an excessive shedding of HER2 can contribute to the development of a malignant phenotype. Humanized monoclonal antibodies against HER2 have demonstrated their effectiveness in the therapy of HER2 overexpressing breast cancers by largely unknown mechanisms.[36] These anti-HER2 monoclonal antibodies have been shown to inhibit the shedding of HER2, opening the possibility that this inhibition represents one of the mechanisms whereby these antibodies block tumor progression.[37]

A third aspect related to the shedding of receptors has been recently recognized: after the HER4 receptor has undergone shedding, the remaining membrane-anchored part of the receptor is cleaved within its transmembrane domain by a second proteolytic system.[38] This type of proteolytic intramembranous cleavage is known as RIP (regulated intramembrane proteolysis).[39] RIP is not a unique property of HER4, for it also affects other transmembrane molecules such as Notch, a receptor that specifies cell fate decisions during embryonic development.[40] In fact, the RIP of Notch has been intensively studied in recent years, and it has been found that the Notch intracellular domain that appears after the RIP of Notch is transported to the nucleus, where it directly regulates gene transcription[40] (Figure 2). Although it has also been shown that the intracellular domain of HER4 moves to the nucleus and has a weak transcriptional activity when fused to a reporter, it remains to be definitively established whether in vivo this portion of HER4 acts as a direct regulator of gene transcription.

In summary, although initially the shedding of transmembrane receptors was initially viewed as a means to release ligand-binding proteins that regulate the access of circulating ligands to their cognate receptors, recent findings indicate that the transmembrane cytoplasmic fragment left behind after shedding has a relevant functional role. This fragment is in some cases the substrate of a proteolytic system that releases the cytoplasmic moiety that is transported to the nucleus where it can modulate the expression of target genes.

C. Adhesion Molecules

The extracellular domain of cell adhesion molecules (CAMs) supports cell−cell and cell−extracellular matrix interactions. The shedding of the ectodomains of CAMs, therefore, represents a crucial point in the dynamic regulation of these types of interactions. Several reports on prototypic cell adhesion molecules seem to confirm this viewpoint.

The selectins, one of the five major classes of CAMs, bind to carbohydrate epitopes present in endothelial cells and mediate transient cell−cell adhesion in the bloodstream (for a recent review, see ref 41). One of the members of this family, L-selectin, was initially described as a homing receptor, which mediates the interaction of lymphocytes with high endothelial venules of lymph nodes and their migration into peripheral lymph nodes.[42] The phenotype of L-selectin knock-out mice confirmed its critical role in these events.[43] In addition, L-selectin participates in neutrophil extravasation into inflamed or injured areas,[44] by mediating the initial attachment followed by slow rolling of neutrophils along the vascular endothelium.[45] Once this initial contact has taken place, integrins, another major class of CAMs, mediate the subsequent tightening of the adhesion and transendothelial migration of neutrophils.[44] The expression of L-selectin at the cell surface of lymphocytes and neutrophils can be rapidly down-regulated through ectodomain shedding triggered by different activators or L-selectin cross-linking.[46−48] It has been proposed that the shedding of L-selectin regulates the rolling velocity of loosely adherent leukocytes along the endothelium, since inhibitors of ectodomain shedding decreased the rolling velocity.[49,50] Furthermore, it has been recently shown that the shedding of L-selectin also regulates the firm adhesion and transmigration by promoting leukocyte activation via outside-in signaling that regulates interaction mediated by integrins.[51] Therefore, the shedding of L-selectin would have a direct role in the initial rolling and also an indirect but crucial role by regulating the interactions that lead to firm adhesion and transmigration.[51]

L1 is a type I membrane glycoprotein, consisting of six immunoglobulin-like domains and five fibronectin type III repeats, expressed in neural, hematopoietic, and some epithelial cells.[52] L1 promotes homotypic L1−L1 binding, sometimes potentiated by other cellular proteins,[53] or heterotypic binding with different CAMs and several integrins (ref 54 and references therein). Expression of L1 enhances the migration of cells on fibronectin and laminin through an $\alpha v\beta 5$ integrin-dependent mechanism.[55] Inhibitors of ectodomain shedding block the L1-dependent enhanced migration, indicating that the shedding of L1 is necessary to promote migration. The effect of inhibitors can be overcome by adding soluble L1 ectodomain.[55] Therefore, as in the case of L-selectin, the shedding of L1 is required to regulate cell migration.

The shedding of certain CAMs seems to participate in the regulation of the availability of signaling ligands. The syndecans are a family of transmembrane heparan sulfate proteoglycans (HSPGs) with capacity to bind a wide variety of soluble and insoluble ligands including extracellular matrix components, cell adhesion molecules, growth factors,

cytokines, proteinases, lipid metabolism proteins, and microbial pathogens.[56] The soluble ectodomains of syndecans accumulate especially following injury or inflammation[57] and facilitate the formation of signaling complexes by acting as coreceptors, modulating ligand activities.[56] For example, soluble syndecan ectodomains have been reported to antagonize fibroblast growth factor 2 (FGF-2).[58] However, the regulation of the activity of FGF-2 by syndecans seems to be a complex process given that recent reports show that partial degradation of soluble syndecans by platelet heparanase produces fragments, found in wound fluids, that agonize with FGF-2.[58] Soluble syndecans have also been suggested to play a role in diverse situations such as the regulation of feeding behavior[59] or the modulation of the activity of certain extracellular enzymes. For example, soluble syndecans reduce the affinity of cathepsin G and elastase for their physiological inhibitors, α1-antichymotrypsin and α1-proteinase inhibitor, respectively.[60] Finally, it has been shown that syndecan-1 ectodomains enhance bacterial virulence in newborn mice.[61]

As in the case of receptors for cytokines and growth factors, recent evidence indicates that the shedding of CAMs is not merely a way to produce soluble binding partners with the ability to modulate protein−protein interactions. The transmembrane/cytoplasmic portion left behind after the shedding of certain cell adhesion molecules has taken place seems to have the ability to transduce signals. The platelet−endothelial cell adhesion molecule PECAM-1, another glycoprotein of the immunoglobulin superfamily, prevents endothelial cell apoptosis through homotypic interactions.[62] However, PECAM-1 is shed during apoptosis,[63] and the resulting truncated transmembrane/cytoplasmic domain seems to have signaling abilities since it binds with higher affinity to signal transducers such as γ-catenin and SPH-2 than the full-length form.[31] Supporting this possibility, the transmembrane/cytoplasmic domain of PECAM-1 also decreases cell proliferation probably through activation of caspase-8 and p38/JNK phosphorylation.[31] On the other hand, as described for Notch and HER-4, the RIP of the transmembrane/cytoplasmic domain of CD44 releases an intracellular fragment that translocates to the nucleus and activates transcription-potentiating transactivation mediated by the transcriptional coactivator CBP/p300.[64] Thus, RIP seems to be a general mechanism that acts not only on transmembrane receptors but also in cell adhesion molecules.

D. β-Amyloid Precursor Protein

The β-amyloid (βA) peptide is the main component of the proteinaceous filaments that form the amyloid plaques, a type of brain lesion found in patients with Alzheimer's disease (AD) (recently reviewed in ref 65). The social relevance of this disease has led to an enormous effort to understand how the β-amyloid peptide is generated. The transmembrane domain of the β-amyloid precursor protein, a type I membrane protein, contains the C-terminal moiety of the β-amyloid peptide, which spans 28 amino acids outside the cell membrane. At least three different proteolytic activities (collectively known as secretases) control the production of βA. α-Secretase prevents the formation of the βA peptide by cleaving within it, 12 amino acids from the transmembrane domain (Figure 4).[66] The cleavage produced by α-secretase shares many characteristics with that produced by ectodomain shedding in other transmembrane proteins.[67] β- and γ-secretases produce the β-amyloid peptide by cleaving at the N- and C-termini, respectively. γ-Secretase catalyzes an unusual and heterogeneous proteolytic event within the transmembrane domain to produce the ∼4 kDa βA (reviewed in ref 68). This cleavage is equivalent to the RIP recently described for transmembrane receptors (see above). The longer and more hydrophobic form of βA is 42 amino acids long (βA42) and tends to form fibrils.[69] In fact, the main component of the plaques found in patients with AD is this βA42.[70] Mutations that cause a familial form of AD are located near the β- and γ-secretase cleavage sites and lead to an elevated production of βA.[71−73] Collectively, these results strongly suggest that βA42 is involved in the pathogenesis of Alzheimer's disease and indicate that a reasonable therapeutic approach could be to treat patients with specific inhibitors of the β- and γ-secretases[74] or activators of the shedding of βAPP (α-secretase activity).[75]

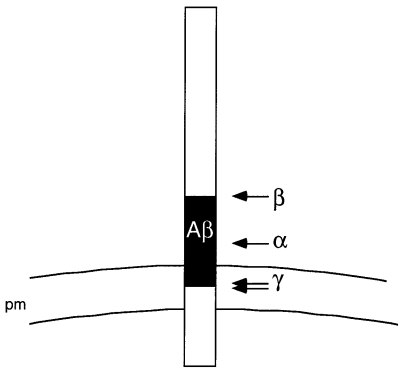

Figure 4. Secretase activities acting in βAPP. See the text for a description.

The metalloproteases involved in α-secretase activity will be discussed in the next section. The identity of the protease responsible for the β-secretase activity was recently identified and named BACE (β-site βAPP cleaving enzyme[76−78]). BACE is a widely expressed transmembrane protein that belongs to the family of aspartyl proteases. Currently, specific inhibitors are being developed and tested for their potential in the prevention of cellular production of the βA peptide.[79] The identity of proteases with γ-secretase activity is still controversial. Several lines of evidence indicate that presenilins, two multipass membrane proteins initially identifed as the products of genes responsible for most cases of familial AD,[80−82] tightly control the intramembranous cleavage of βAPP in the γ-site. Furthermore, presenilins seem to control the RIP of Notch and probably other transmembrane proteins.[74] Using a variety of biochemical and pharmacological criteria, different authors have suggested that presenilins are the catalytic component of the γ-secretase activity (the

evidence supporting this conclusion has been specifically reviewed recently[74]). However, this hypothesis has been recently challenged by results showing that fibroblasts from presenilin knock-out mice do not produce the intracellular fragment of Notch but do produce normal levels of the βA.[83] In addition, the RIP of Notch and that of the βAPP can be pharmacologicaly distinguished.[84] Therefore, the mechanism used by presenilins to control the RIP of βAPP is still unclear and awaits further experiments.

Although the pathological role of βAPP seems to be widely accepted, the normal function of the β-amyloid precursor protein is unknown. Several features indicate that its metabolism is strikingly similar to that of the Notch receptor. A C-terminal fragment, possibly representing a product of RIP proteolysis that occurs after ectodomain shedding, has been recently found in cells. This fragment, known as AICD (βAPP intracellular domain), forms a complex with Fe65 and the histone acetyl transferase Tip60 with the ability to stimulate transcription.[85,86] Although these are very recent results, they clearly indicate that, as happens with several transmembrane receptors and cell adhesion molecules, βAPP is able to transduce signals. Future reports will surely test this interesting hypothesis.

III. Proteases Involved in Ectodomain Shedding

A. Metalloprotease Disintegrins

In 1994 three different groups simultaneously showed that hydroxamic acid-based inhibitors of zinc-dependent metalloproteases block the shedding of the protumor necrosis factor-α (proTNF-α) in vitro and in vivo.[87−89] Soon after these reports appeared, and using the same inhibitors, it became apparent that metalloproteases also mediate the shedding of a functional and structural variety of transmembrane proteins.[90,91] Since then, hydroxamic acid-derived inhibitors have been routinely used to characterize the shedding of most molecules analyzed to date, and invariably, these inhibitors are shown to prevent ectodomain shedding. Thus, the vast majority, if not all, of the shedding events are mediated by zinc-dependent metalloproteases. An independent line of evidence indicated that relatively few components were responsible for the shedding of most transmembrane proteins. Somatic Chinese hamster ovary (CHO) cell mutants initially isolated for their lack of the phorbol ester-induced ectodomain shedding of proTGF-α were also unable to shed the ectodomains of proTNF-α and other unrelated proteins such as βAPP, L-selectin, and a variety of anonymous cell surface proteins.[67,90,92−94] Two independently isolated mutant CHO cell lines showing the same generalized defect in ectodomain shedding were found to belong to the same complementation group,[90] suggesting the existence of few essential components of a general shedding machinery regulated by phorbol esters.

The first protease shown to be responsible for a particular shedding event to be purified and identified was the so-called tumor necrosis factor-α converting enzyme (TACE).[95,96] T-cells derived from mice genetically deficient in the zinc-binding domain of TACE (tace−/− cells) showed a 80−90% reduction in proTNF-α shedding and a distinct increment in cell surface proTNF-α expression compared with T-cells obtained from wild-type animals.[95] Thus, these data strongly suggested that TACE is responsible in vivo for the shedding of proTNF-α. TACE (also known as ADAM17) belongs to the family of metalloprotease disintegrins (also known as ADAM (a disintegrin and metalloprotease) or MDC (metalloprotease disintegrin containing)).

Figure 5. Domain organization of ADAM proteases: SP, signal peptide; Prod, prodomain; Metall, metalloprotease domain; EGF, EGF-like domain; TM, transmembrane domain. The cleavage site for proprotein convertases is shown.

Table 1. Proteins with a Defective Activated Shedding in Tace−/− Cells

functional category	protein	ref
growth factors	proTNF-α	95, 96
	proTGF-α	18
	proNRGa-2C	128
	proHB-EGF	94
	proamphiregulin	129
	fractalkine	130, 131
receptors	p75 TNF-α RII	18
	p55 TNF-α R	18
	CD30	132
	IL-6 R α	133
	IL-1R II	126
	GHR	134
	HER-4	135
	Notch	112
cell adhesion molecules	L-selectin	18
others	βAPP	99

Metalloprotease disintegrins are modular type I transmembrane proteins (Figure 5) that, in addition to the catalytic domain, contain a disintegrin and an EGF-like domain which is apparently involved in protein−protein interactions.[97] To date 33 metalloprotease disintegrins have been identified (a regularly updated table can be accessed at http://www.people.Virginia.EDU/~jag6n/Table_of_the_ADAMs.html); 18 of them are predicted to function as proteases, while the rest do not contain the consensus Zn-binding domain, indicating that they are not active proteases. Considering the diversity of proteins susceptible to undergoing ectodomain shedding, an obvious hypothesis would be that several metalloprotease disintegrins are involved in ectodomain shedding and that each one is endowed with a restricted specificity. According to this hypothesis, TACE would be responsible for the shedding of proTNF-α and related molecules. However, the activated shedding of a wide variety of structurally and functionally diverse transmembrane molecules is severely impaired in (tace−/− cells) (see Table 1), indicating that TACE is a common protease required for the shedding of many more proteins than expected.

Table 2. Proposed Substrates of Different Metalloprotease Disintegrins

metalloprotease	proposed substrate	ref
ADAM9	proHB-EGF	101
	βAPP	102
ADAM10	βAPP	99
	ephrin-A2	22
	L1	55
	proHB-EGF	136
ADAM12	proHB-EGF	137
ADAM19	neuregulin-β1 and -4	138

In contrast with the number of reports suggesting a prevalent role of TACE in ectodomain shedding, comparatively few reports have suggested the involvement of other metalloprotease disintegrins in particular shedding events (see Table 2). These substrate−protease matchings are generally based on experiments that involved cotransfections of the substrates with the suspected protease or a dominant negative version of it. While overexpression of the metalloprotease disintegrin assayed leads to an augmented level of shedding of the corresponding substrate, that of the dominant negative form inhibits it. The recent development of ADAM9 knock-out mice[98] indicates that, at least in certain instances, this approach does not necessarily mirror the in vivo situation in all cell types. Upon phorbol ester addition, fibroblasts isolated from these mice (adam9−/− cells) show levels of proHB-EGF or βAPP indistinguishable from those observed in wild-type fibroblast, indicating that despite expectations, ADAM9 does not have a major role in the shedding of these molecules, at least in these cells.[98] In contrast, tace−/− fibroblasts show an apparent defect in the phorbol ester-activated shedding of both molecules[92,94,99] (see Table 1), suggesting that, at least in this cell line, TACE is reponsible for the activated shedding of proHB-EGF and βAPP and MDC9 does not play a major role. Supporting this conclusion, the somatic CHO cell mutants defective in the activated shedding of proTGF-α, βAPP, and several other transmembrane proteins are also defective in the activated shedding of proHB-EGF.[94] These cells have been recently shown to have a specific defect in the activation of TACE that does not affect ADAM9 or ADAM10.[100] Thus, this constitutes independent evidence that also suggests that TACE and not ADAM9 or ADAM10 is responsible for the phorbol ester-activated shedding of proHB-EGF and βAPP. A possible explanation that would reconcile the results of cotransfection and those observed in knock-out cell lines would be that different metalloprotease disintegrins display a cell-type-specific specificity: ADAM9 could be responsible for the shedding of proHB-EGF in Vero cells[101] and βAPP in Cos cells,[102] while TACE is responsible for the shedding of these molecules in embryonic fibroblasts and CHO cells. Alternatively, transient overexpression of certain active metalloprotease disintegrins or their dominant negative versions could lead to an artifactual situation where the overexpressed protease could act on substrates that are not normally shed by the endogenous counterpart, and the dominant negative version could sequester a common component necessary for the activity of several metalloprotease disintegrins, including TACE. A third possibility, which does not exclude the others, is that several ADAMs can act on a single substrate: TACE could be responsible for the phorbol ester-induced shedding of many proteins (Table 1), while ADAM10 or/and other metalloprotease disintegrins would be responsible for basal shedding of some of them. This last possibility seems particularly plausible in the case of βAPP since tace−/− cells or the somatic cell mutants show a detectable level of basal βAPP shedding that cannot be augmented by phorbol esters.[67,99]

Although a preponderant role of TACE in ectodomain shedding seems well documented, several shedding events have been shown to be independent of TACE. In addition to a normal level of basal shedding of βAPP, tace−/− cells show a normal shedding of the ectoenzyme ACE or the cytokine TRANCE.[103,104] Furthemore, it has been recently shown that addition of compounds that in vitro induce the autocatalytic activation of certain metalloproteases activate the shedding of proTGF-α, presumably by a PKC-independent mechanism, showing the existence of metalloprotease(s) alternative to TACE with the ability to shed proTGF-α.[94] On the other hand, mutational analysis indicates that the basal shedding of L-selectin is mediated by a protease different from TACE. Proline substitution mutations around the cleavage site completely block the shedding induced by phorbol esters without affecting basal cleavage; therefore, it has been proposed that, while TACE is responsible for the activated shedding of L-selectin, a different protease is responsible for its basal shedding.[105] In summary, although a preponderant role of TACE in activated ectodomain shedding seems widely accepted, other metalloproteases participate in ectodomain shedding. Nonetheless, further studies will be necessary to confirm the protease−substrate pairs that have already been suggested to exist.

B. Matrix Metalloproteases

MMPs are a large family of zinc-dependent metalloproteases with a multidomain structure including prodomains and catalytic domains similar to those of the ADAMs (for a recent review on MMPs see ref 106). As in the case of metalloprotease disintegrins, several lines of evidence including experiments with inhibitors, transient overexpression of certain MMPs and putative substrates, and experiments with cell lines derived from knock-out mice indicate a role of certain MMPs in the shedding of transmembrane proteins.

In addition to TACE, MMP7 (also known as matrylisin) has been proposed to participate in the basal shedding of proTNF-α because conditioned media of macrophages from MMP-7−/− mice generate a substantially reduced amount of TNF-α compared with macrophages from wild-type mice, despite the fact that the level of cell surface proTNF-α is comparable in both types of macrophages.[107] Interestingly, TACE is active in MMP-7−/− mice since the regulated shedding of proTNF-α seems to be intact in these mice.[107] The results reinforce the notion that certain cell surface molecules, such as proTNF-α, are sub-

strates of more than one protease. Perhaps MMP-7 could be responsible for a basal level of proTNF-α shedding in certain cell lines, such as macrophages, and TACE would cleave proTNF-α in response to different activators.

The membrane type (MT)-MMPs is a subfamily of the MMPs; as their name implies, MT-MMPs are membrane anchored.[106] The shedding of TRANCE, a proTNF-α family member, is sensitive to the tissue inhibitor of metalloproteases (Timp)-2, a hallmark of MT-MMPs, and is dramatically augmented by overexpression of MT1-MMP.[104] Similar experiments indicate that MT1-MMP participates in the shedding of the cell adhesion molecules CD44.[108] Since MT1-MMP knock-out mice have been recently developed,[109] the analysis of the shedding of these molecules in cells derived from them would help to confirm the involvement of MT1-MMP in these shedding events.

IV. Regulation of Ectodomain Shedding

A. Proteolytic Activation of Proteases Involved in Ectodomain Shedding

Metalloprotease disintegrins are synthesized as zymogens containing a prodomain that keeps them in an inactive state. Proteolytic removal of the prodomain leads to the activation of the proteolytic activity of the metalloprotease disintegrins (first shown for ADAM12[110]). Between the prodomain and the catalytic domain, metalloprotease disintegrins bear a typical cleavage site for furin-type proprotein convertases (R−X−R/K−R), indicating that this type of proteases is involved in processing of the prodomain.[111] Proprotein convertases are a family of serine proteases of at least seven members; four of them (furin, PC7, PACE4, and PC6) are widely expressed and are, therefore, candidates to process ubiquitously expressed metalloprotease disintegrins. To test this possibility, a variety of approaches, including the use of specific inhibitors, cotransfection of certain metalloprotease disintegrins with individual proprotein convertases, and the use of cell lines defective in furin activity, have been used. As a result, it has been shown that ADAM10 is processed by a proprotein convertase(s) different from furin, probably PC7.[75] The processing of TACE is blocked by α1-antitrypsin Portland, a protein inhibitor of proprotein convertases,[112] and is partially inhibited in mutant cells devoid of furin activity,[100] indicating that, in addition to furin, other proprotein convertases can process the prodomain of TACE. The processing of ADAM9 is also likely due to proprotein convertases since furin correctly processed ADAM9 in vitro.[113] Therefore, all evidence at hand strongly suggests a decisive role of furin and other proprotein convertases in the proteolytic activation of TACE and other metalloprotease disintegrins possibly involved in ectodomain shedding. Furthermore, a similar mechanism activates MT1-MMP that, as all MMPs, is also synthesized as a zymogen containing an inactivating prodomain.[114] MMP7 does not contain a furin cleavage site and thus, to date, would be the only known metalloprotease with the ability to shed cell surface molecules that escapes the control of proprotein convertases.

Processing by proprotein convertases takes place in the secretory pathway and is generally considered a constitutive process. However, several reports indicate that it can be a regulatory step in certain situations. Overexpression of PC7 leads to an up-regulation of the shedding of βAPP, consistent with the hypothesis that an enhanced removal of the prodomain of metalloprotease disintegrins leads to an enhanced level of ectodomain shedding.[75] Conversely, in cells devoid of furin, the activated shedding of βAPP is impaired.[115] Thus, up- or down-regulation of the proprotein convertase activity correlates with equivalent modifications in the activities responsible for ectodomain shedding. Keeping in mind the recent finding that aggressive tumors show elevated furin expression,[116] it is tempting to speculate that, in tumors, the activity of TACE and other metalloprotease disintegrins can be up-regulated, leading to the overproduction of soluble growth factors of the EGF family that can contribute to the development of tumors that overexpress EGFRs. In agreement with this view, it has recently been shown that expression of a furin inhibitor results in absent or decreased invasiveness and tumorigenicity of human cancer cells.[117]

B. Regulators of Ectodomain Shedding

It has long been known that shedding of the ectodomain of certain proteins is a regulated process that occurs slowly in unstimulated cells but can be dramatically activated by different independent mechanisms, the best characterized of which involves PKC.[9] Early experiments indicated that PKC activates the shedding of many different proteins, and confirming this notion, the shedding of all substrates of TACE analyzed to date have been shown to be shed in response to phorbol esters, which is the most common way to activate ectodomain shedding in the laboratory.

Several physiological activators of ectodomain shedding such as chemotactic peptides, cytokines, and growth factors have also been described.[118−121] The activation of receptor tyrosine kinase and G-protein-coupled receptors induces the activation of the shedding of growth factors that activate tyrosine kinase receptors, establishing a positive feedback loop[19,119] (Figure 3). Upon investigation of the intracellular signaling pathways that up-regulate shedding, it became apparent that although several independent pathways seem to be involved in regulating ectodomain shedding, mitogen-activated protein (MAP) kinases are common regulators of the shedding of proHB-EGF[122] and that of proTGF-α, proTNF-α, L-selectin, L1, syndecan-1 and -4, and the tyrosine kinase c-Met receptor.[20,123−125] Furthermore, specific inhibitors of Erk2 MAP kinase block the shedding of proTGF-α activated by growth factors, while inhibitors of the p38 MAP kinase prevent the basal shedding of proTGF-α in unstimulated cells, indicating that several independent MAP kinase signaling pathways are also involved.[123]

How does PKC or the intracellular MAP kinase cascade enhance the activity of TACE? Despite the efforts in answering this question, it remains unsolved. Transfection of TACE devoid of a cytoplasmic tail into tace−/− cells reconstitutes the activated shedding of proTNF-α and other proteins,[126] indicating that, although TACE and other metalloprotease disintegrins are phosphorylated soon after phorbol ester addition,[113,126] direct phosphorylation of the cytoplasmic domain of TACE is not required for activation. Many substrates of TACE lacking their cytoplasmic domain are shed in response to phorbol esters, indicating that PKC does not act directly on the substrates either (ref 127 and references therein). Apparently, phorbol esters do not enhance the transport of TACE to the cell surface[95] or the processing of the prodomain of TACE (our unpublished observations). Thus, how the activities of TACE and other metalloprotease disintegrins are regulated remains one of the most challenging questions in this field.

V. Conclusions

The recent acknowledgment of protein ectodomain shedding as an important way to modulate the function of all kinds of cell surface molecules has attracted the interest of numerous scientists from diverse fields. In the last few years, it has been well established that metalloprotease disintegrins and certain MMPs are involved in ectodomain shedding. Surprisingly, a particular metalloprotease disintegrin, TACE, seems to play a central role in ectodomain shedding and has been proposed as responsible for the shedding of more than a dozen proteins with diverse structures and functions. Many questions in the field remain to be answered: Does the activation of TACE lead to the simultaneous shedding of such a wide variety of cell surface molecules? How many metalloproteases are involved in ectodomain shedding? Are they coordinately regulated with TACE? Presumably the development of knock-out mice for individual metalloproteases or a subset of metalloproteases will help to solve these questions.

Regarding the regulation of ectodomain shedding, it is clear that MAP kinases are likely physiological regulators of TACE and perhaps of other metalloproteases; however, the activation mechanism of TACE remains a mystery. On the other hand, given its lack of specificity, it seems reasonable to suppose the existence of tight cellular controls of TACE activity to prevent unwanted proteolysis. Furthermore, few factors with the ability to regulate metalloproteases have been found to date. Foreseeable future reports will deal with these and related problems.

In summary, in recent years the continuously growing number of proteins that are found to undergo shedding has not been accompanied by significat progress in the understanding of the mechanisms that mediate regulated ectodomain shedding. Several fundamental questions remain unsolved, especially those pertaining to regulation and specifity, and future efforts should be directed to answer them.

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VII. References

(1) Kiessling, L. L.; Gordon, E. J. *Chem. Biol.* **1998**, *5*, R49.
(2) Blobel, C. P. *Cell* **1997**, *90*, 589.
(3) Schlöndorff, J.; Blobel, C. P. *J. Cell Sci.* **1999**, *112*, 3603.
(4) Blobel, C. P. *Curr. Opin. Cell Biol.* **2000**, *12*, 606.
(5) Black, R. A. *Int. J. Biochem. Cell Biol.* **2002**, *34*, 1.
(6) Black, R. A.; White, J. M. *Curr. Opin. Cell Biol.* **1998**, *10*, 654.
(7) Werb, Z.; Yibing, Y. *Science* **1998**, *282*, 1279.
(8) Kheradmand, F.; Werb, Z. *Bioessays* **2002**, *24*, 8.
(9) Massagué, J.; Pandiella, A. *Annu. Rev. Biochem.* **1993**, *62*, 515.
(10) Wong, S. T.; Winchell, L. F.; McCune, B. K.; Earp, H. S.; Teixidó, J.; Massagué, J.; Herman, B.; Lee, D. C. *Cell* **1989** *56*, 495.
(11) Brachmann, R.; Lindquist, P. B.; Nagashima, M.; Kohr, W.; Lipari, T.; Napier, N.; Derynck, R. *Cell* **1989** *56*, 691.
(12) Higashiyama, S.; Iwamoto, R.; Goishi, K.; Raab, G.; Taniguchi, N.; Klagsbrun, M.; Mekada, E. *J. Cell Biol.* **1995**, *128*, 929.
(13) Shi, W.; Fan, H.; Shum, L.; Derynck, R. *J. Cell Biol.* **2000**, *148*, 591.
(14) Yang, H.; Jiang, D.; Li, W.; Liang, J.; Gentry, L. E.; Brattain, M. G. *Oncogene* **2000**, 1901.
(15) Dong, J.; Opresko, L. K.; Dempsey, P. J.; Lauffenburger, D. A.; Coffey, R. J.; Wiley: H. S. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6235.
(16) Luetteke, N. C.; Qiu, T. H.; Peiffer, R. L.; Oliver, P.; Smithies, O.; Lee, D. C. *Cell* **1993** 73, 263.
(17) Mann, G. B.; Fowler, K. J.; Gabriel, A.; Nice, E.; Williams, R. L.; Dunn, A. R. *Cell* **1993** 73, 249.
(18) Peschon, J.; Slack, J.; Reddy, P.; Stocking, K.; Sunnarborg, S.; Lee, D.; Rusell, W.; Castner, R.; Johnson, R.; Fitzner, J.; Boyce, N.; Nelson, C.; Kozlosky, M.; Wolfson, M.; Rauch, C.; Cerretti, D.; Paxton, R.; March, C.; Black, R. *Science* **1998**, *282*, 1281.
(19) Prenzel, N.; Zwick, E.; Daub, H.; Leserer, M.; Abraham, R.; Wallasch, C.; Ullrich, A. *Nature* **1999**, *402*, 23.
(20) Nath, D.; Williamson, N. J.; Jarvis, R.; Murphy, G. *J. Cell Sci.* **2001**, *114*, 1213.
(21) Wilkinson, D. G. *Nat. Rev. Neurosci.* **2001**, *2*, 155.
(22) Hattori, M.; Osterfield, M.; Flanagan, J. G. *Science* **2000**, *289*, 1360.
(23) Rose-John, S.; Heinrich, P. C. *Biochem. J.* **1994** 300, 281.
(24) Yarden, Y.; Sliwkowski, M. X. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 127.
(25) Di Fiore, P. P.; Pierce, J. H.; Kraus, M. H.; Segatto, O.; King, C. R.; Aaronson, S. A. *Science* **1987**, *237*, 178.
(26) Segatto, O.; King, C. R.; Pierce, J. H.; P., D. F. P.; Aaronson, S. A. *Mol. Cell Biol.* **1988**, *8*, 5570.
(27) Rodrigues, G. A.; Park, M. *Curr. Opin. Genet. Dev.* **1994**, *4*, 15.
(28) Moscatello, D. K.; Holgado-Madruga, M.; Godwin, A. K.; Ramirez, G.; Gunn, G.; Zoltick, P. W.; Biegel, J. A.; Hayes, R. L.; Wong, A. J. *Cancer Res.* **1995**, *55*, 5536.
(29) Moscatello, D. K.; Montgomery, R. B.; Sundareshan, P.; McDanel, H.; Wong, M. Y.; Wong, A. J. *Oncogene* **1996** 13, 85.
(30) Vecchi, M.; Carpenter, G. *J. Cell Biol.* **1997**, *139*, 995.
(31) Ilan, N.; Mohsenin, A.; Cheung, L.; Madri, J. A. *FASEB J.* **2001**, *15*, 362.
(32) Cabrera, N.; Diaz-Rodriguez, E.; Becker, E.; Martin-Zanca, D.; Pandiella, A. *J. Cell Biol.* **1996**, *132*, 427.
(33) Slamon, D. J.; Clark, G. M.; Wong, S. G.; Levin, W. J.; Ullrich, A.; McGuire, W. L. *Science* **1987**, *235*, 177.
(34) Pupa, S. M.; Menard, S.; Morelli, D.; Pozzi, B.; De Palo, G.; Colnaghi, M. I. *Oncogene* **1993,** *8*, 2917.
(35) Christianson, T. A.; Doherty, J. K.; Lin, Y. J.; Ramsey, E. E.; Holmes, R.; Keenan, E. J.; Clinton, G. M. *Cancer Res.* **1998**, *58*, 5123.
(36) Baselga, J.; Tripathy, D.; Mendelsohn, J.; Baughman, S.; Benz, C. C.; Dantis, L.; Sklarin, N. T.; Seidman, A. D.; Hudis, C. A.; Moore, J.; Rosen, P. P.; Twaddell, T.; Henderson, I. C.; Norton, L. *J. Clin. Oncol.* **1996**, *14*, 737.

(37) Molina, M. A.; Codony-Servat, J.; Albanell, J.; Rojo, F.; Arribas, J.; Baselga, J. *Cancer Res.* **2001**, *61*, 4744.
(38) Ni, C. Y.; Murphy, M. P.; Golde, T. E.; Carpenter, G. *Science* **2001**, *294*, 2179.
(39) Heldin, C.-H.; Ericcson, J. *Science* **2001**, *294*, 2111.
(40) Fortini, M. E. *Curr. Opin. Cell Biol.* **2001**, *13*, 627.
(41) Ley, K. *Results Probl. Cell Differ.* **2001**, *33*, 177.
(42) Gallatin, W. M.; Weissman, I. L.; Butcher, E. C. *Nature* **1983**, *304*, 30.
(43) Arbones, M. L.; Ord, D. C.; Ley, K.; Ratech, H.; Maynard-Curry, C.; Otten, G.; Capon, D. J.; Tedder, T. F. *Immunity* **1994**, *1*, 247.
(44) Springer, T. A. *Cell* **1994**, *76*, 301−314.
(45) Lawrence, M. B.; Springer, T. A. *Cell* **1991**, *65*, 859−873.
(46) Kishimoto, T. E.; Jutila, M. A.; Berg, E. L.; Butcher, E. C. *Science* **1989**, *245*, 1338.
(47) Chen, A.; Engel, P.; Tedder, T. F. *J. Exp. Med.* **1995**, *182*, 519.
(48) Migaki, G. I.; Kahn, J.; Kishimoto, T. K. *J. Exp. Med.* **1995**, *182*, 549.
(49) Walcheck, B.; Kahn, J.; Fisher, J. M.; Wang, B. B.; Fisk, R. S.; Payan, D. G.; Feehan, C.; Betageri, R.; Darlak, K.; Spatola, A. F.; Kishimoto, T. K. *Nature* **1996**, *380*, 720.
(50) Hafezi-Moghadam, A.; Ley, K. *J. Exp. Med.* **1999**, *189*, 939.
(51) Hafezi-Moghadam A, T. K., Prorock AJ, Huo Y, Ley K. *J. Exp. Med.* **2001**, *193*, 863.
(52) Brummendorf, T.; Kenwrick, S.; Rathjen, F. G. *Curr. Opin. Neurobiol.* **1998**, *8*, 87.
(53) Kadmon, G.; Kowitz, A.; Altevogt, P.; Schachner, M. *J. Cell Biol.* **1990**, *110*, 193.
(54) Blaess, S.; Kammerer, R. A.; Hall, H. *J. Neurochem.* **1998**, *71*, 2615.
(55) Mechtersheimer, S.; Gutwein, P.; Agmon-Levin, N.; Stoeck, A.; Oleszewski, M.; Riedle, S.; Fogel, M.; Lemmon, V.; Altevogt, P. *J. Cell Biol.* **2001**, *155*, 661.
(56) Park, W.; Reizes, O.; Bernfield, M. *J. Biol. Chem.* **2000**, *275*, 29923.
(57) Subramanian, S. V.; Fitzgerald, M. L.; Bernfield, M. *J. Biol. Chem.* **1997**, *272*, 14713.
(58) Kato, M.; Wang, H.; Kainulainen, V.; Fitzgerald, M. L.; Ledbetter, S.; Ornitz, D. M.; Bernfield, M. *Nat. Med.* **1998**, *4*, 691.
(59) Reizes, O.; Lincecum, J.; Wang, Z.; Goldberger, O.; Huang, L.; Kaksonen, M.; Ahima, R.; Hinkes, M. T.; Barsh, G. S.; Rauvala, H.; Bernfield, M. *Cell* **2001**, 105.
(60) Kainulainen, V.; Wang, H.; Schick, C.; Bernfield, M. *J. Biol. Chem.* **1998**.
(61) Park, P. W.; Pier, G. B.; Hinkes, M. T.; Bernfield, M. *Nature* **2001**, *411*, 98.
(62) Bird, I. N.; Taylor, V.; Newton, J. P.; Spragg, J. H.; Simmons, D. L.; Salmon, M.; Buckley, C. D. *J. Cell Sci.* **1999**, *112*, 1989.
(63) Hart, S. P.; Ross, J. A.; Ross, K.; Haslett, C.; Dransfield, I. *Cell Death Differ.* **2000**, *7*, 493.
(64) Okamoto, I.; Kawano, Y.; Muramaki, D.; Sasayama, T.; Araki, N.; Miki, T.; Wong, A. J.; Saya, H. *J. Cell Biol.* **2001**, *26*, 755.
(65) Tanzi, R. E.; Bertram, L. *Neuron* **2001**, *32*, 181.
(66) Sisodia, S. S. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6075.
(67) Arribas, J.; Massagué, J. *J. Cell Biol.* **1995**, *128*, 433.
(68) Esler, W. P.; Wolfe, M. S. *Science* **2001**, *293*, 1449.
(69) Jarrett, J. T.; Berger, E. P.; Lansbury, P. T. J. *Biochemistry* **1993**, *32*, 4693.
(70) Iwatsubo, T.; Odaka, A.; Suzuki, N.; Mizusawa, H.; Nukina, N.; Ihara, Y. *Neuron* **1994** 13, 45−53.
(71) Citron, M.; Oltersdorf, T.; Haass, C.; McConlogue, L.; Hung, A. Y.; Seubert, P.; Vigo-Pelfrey, C.; Lieberburg, I.; Selkoe, D. J. *Nature* **1992**, *360*, 672.
(72) Cai, X. D.; Golde, T. E.; Younkin, S. G. *Science* **1993**, *259*, 514.
(73) Suzuki, N.; Cheung, T. T.; Cai, X. D.; Odaka, A.; Otvos, L. J.; Eckman, C.; Golde, T. E.; Younkin, S. G. *Science* **1994**, *264*, 1336.
(74) Selkoe, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 11039.
(75) Anders, A.; Gilbert, S.; Garten, W.; Postina, R.; Fahrenholz, F. *FASEB J.* **2001**, *15*, 1837.
(76) Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. *Science* **1999**, *286*, 735.
(77) Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. F.; Frigon, N.; Hong, J.; Jacobson-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensaari, S. M.; Wang, S.; Walker, D.; John, V.; et al. *Nature* **1999**, *402*, 537.
(78) Yan, R.; Bienkowski, M. J.; Shuck, M. E.; Miao, H.; Tory, M. C.; Pauley, A. M.; Brashler, J. R.; Stratman, N. C.; Mathews, W. R.; Buhl, A. E.; Carter, D. B.; Tomasselli, A. G.; Parodi, L. A.; Heinrikson, R. L.; Gurney, M. E. *Nature* **1999**, *402*, 533.
(79) Tung, J. S.; Davis, D. L.; Anderson, J. P.; Walker, D. E.; Mamo, S.; Jewett, N.; Hom, R. K.; Sinha, S.; Thorsett, E. D.; John, V. *J. Med. Chem.* **2002**, *45*, 259.
(80) Sherrington, R.; Rogaev, E. I.; Liang, Y.; Rogaeva, E. A.; Levesque, G.; Ikeda, M.; Chi, H.; Lin, C.; Li, G.; Holman, K. et al. *Nature* **1995**, *375*, 754.
(81) Levy-Lahad, E.; Wasco, W.; Poorkaj, P.; Romano, D. M.; Oshima, J.; Pettingell, W. H.; Yu, C. E.; Jondro, P. D.; Schmidt, S. D.; Wang, K.e.a. *Science* **1995**, *269*, 973.
(82) Rogaev, E. I.; Sherrington, R.; Rogaeva, E. A.; Levesque, G.; Ikeda, M.; Liang, Y.; Chi, H.; Lin, C.; Holman, K.; Tsuda, T.; et al. *Nature* **1995**, *376*, 775.
(83) Armogida, M.; Petit, A.; Vincent, B.; Scarzello, S.; Alves da Costa, C.; Checler, F. *Nat. Cell Biol.* **2001**, *3*, 1030.
(84) Petit, A.; Bihel, F.; Alves da Costa, C.; Pourquie, O.; Checler, F.; Kraus, J. L. *Nat. Cell Biol.* **2001**, *3*, 507.
(85) Kimberly, W. T.; Zheng, J. B.; Guenette, S. Y.; Selkoe, D. J. *J. Biol. Chem.* **2001**, *276*, 40288.
(86) Cao, X.; Sudhof, T. C. *Science* **2001**, *293*, 115.
(87) Gearing, A. J. H.; Beckett, P.; Christodoulou, M.; Churchill, M.; Clements, J.; Davidson, A. H.; Drummond, A. H.; Galloway, W. A.; Gilbert, R.; Gordon, J. L.; Leber, T. M.; Mangan, M.; Miller, K.; Nayee, P.; Owen, K.; Patel, S.; Thomas, W.; Wells, G.; Wood, L. M.; Wolley, K. *Nature* **1994**, *370*, 555.
(88) McGeehan, G. M.; Becherer, J. D.; Bast Jr, R. C.; Boyer, C. M.; Champion, B.; Connolly, K. M.; Conway, J. G.; Furdon, P.; Karp, S.; Kidao, S.; McElroy, A. B.; Nichols, J.; Pryzwansky, K. M.; Schoenen, F.; Sekut, L.; Truesdale, A.; Verghese, M.; Warner, J.; Ways, J. P. *Nature* **1994**, *370*, 558.
(89) Mohler, K.; Sleath, P. R.; Fitzner, J. N.; Cerretti, D. P.; Alderson, M.; Kerwar, S. S.; Torrance, D. S.; Otten-Evans, C.; Greenstreet, T.; Weerawarma, K.; Kronhein, S. R.; Petersen, M.; Gerhart, M.; Kozlosky, C. J.; March, C. J.; Black, R. A. *Nature* **1994**, *370*, 218.
(90) Arribas, J.; Coodly, L.; Vollmer, P.; Kishimoto, T. K.; Rosejohn, S.; Massagué, J. *J. Biol. Chem.* **1996**, *271*, 11376.
(91) Crowe, P.; Walter, B. N.; Mohler, K. M.; Otten-Evans, C.; Black, R. A.; Ware, C. F. *J. Exp. Med.* **1995**, *181*, 1205.
(92) Merlos-Suárez, A.; Fernández-Larrea, J.; Reddy, P.; Baselga, J.; Arribas, J. *J. Biol. Chem.* **1998**, *273*, 24955.
(93) Merlos-Suárez, A.; Arribas, J. *Biochem. Soc. Trans.* **1999**, *27*, 243.
(94) Merlos-Suárez, A.; Ruiz-Paz, S.; Baselga, J.; Arribas, J. *J. Biol. Chem.* **2001**, *276*, 48510.
(95) Black, R. A.; Rauch, C. T.; Kozlosky, C. J.; Peschon, J. J.; Slack, J. L.; Wolfson, M. F.; Castner, B. J.; Stocking, K. L.; Reddy, P.; Srjnivasan, S.; Nelson, N.; Bolani, N.; Schooley, K. A.; Gerhart, M.; Davis, R.; Fitzner, J. N.; Johnson, R. S.; Paxton, R. J.; March, C. J.; Carreti, D. P. *Nature* **1997**, *385*, 729.
(96) Moss, M. L.; Jin, C. S.-L.; Milla, M. E.; Burkhart, W.; Carter, H. L.; Chen, W.-J.; Clay, W.-C.; Didsburry, J. R.; Hassler, D.; Hoffman, C. R.; Kost, T. A.; Lambert, M. H.; Laesnitzer, M. A.; McCauley, P.; McGeehan, G.; Mitchell, J.; Moyer, M.; Pahel, G.; Rocque, W.; Overton, L. K.; Schoenen, F.; Seaton, T.; Su, J.-L.; Warner, J.; Willard, D.; Bacherer, J. D. *Nature* **1997**, *385*, 733.
(97) Primakoff, P.; Myles, D. G. *Trends Genet.* **2000**, *16*, 83.
(98) Weskamp, G.; Cai, H.; Brodie, T. A.; Higashyama, S.; Manova, K.; Ludwig, T.; Blobel, C. P. *Mol. Cell Biol.* **2002**, *22*, 1537.
(99) Buxbaum, J. D.; Liu, K. N.; Luo, Y.; Slack, J. L.; Stocking, K. L.; Peschon, J. J.; Johnson, R. S.; Castner, B. J.; Cerretti, D. P.; Black, R. A. *J. Biol. Chem.* **1998**, *273*, 27765.
(100) Borroto, A.; Ruíz-Paz, S.; Borrell-Pagès, M.; Villanueva de la Torre, T.; Merlos-Suárez, A.; Blobel, C. P.; Baselga, J.; Arribas, J. Submitted for publication, 2002.
(101) Izumi, Y.; Hirata, M.; Hasuwa, H.; Iwamoto, R.; Umata, T.; Miyado, K.; Tamai, Y.; Kurisaki, T.; Sehara-Fujisawa, A.; Ohno, S.; Mekada, E. *EMBO J.* **1998**, *17*, 7260.
(102) Koike, H.; Tomioka, S.; Sorimachi, H.; Saido, T. C.; Maruyama, K.; Okuyama, A.; Fujisawa-Sehara, A.; Ohno, S.; Suzuki, K.; Ishiura, S. *Biochem. J.* **1999**, *343*, 371.
(103) Sadhukhan, R.; Santhamma, K. R.; Reddy, P.; Peschon, J. J.; Black, R. A.; Sen, I. *J. Biol. Chem.* **1999**, *274*, 10511.
(104) Schlöndorff, J.; Lum, L.; Blobel, C. P. *J. Biol. Chem.* **2001**, *276*, 14665.
(105) Zhao, L.-c.; Shey, M.; Farnsworth, M.; Dailey, M. *J. Biol. Chem.* **2001**, *276*, 30631.
(106) McCawley, L. J.; Matrisian, L. M. *Curr. Opin. Cell Biol.* **2001**, *13*, 534.
(107) Haro, H.; Crawford, H. C.; Fingleton, B.; Shinomiya, K.; Spengler, D. M.; Matrisian, L. M. *J. Clin. Invest.* **2000**, *105*, 143.
(108) Kajita, M.; Itoh, Y.; Chiba, T.; Mori, H.; Okada, A.; Kinoh, H.; Seiki, M. *J. Cell Biol.* **2001**, *153*, 893.
(109) Holmbeck, K.; Bianco, P.; Caterina, J.; Yamada, S.; Kromer, M.; Kuznetsov, S. A.; Mankani, M.; Robey, P. G.; Poole, A. R.; Pidoux, I.; Ward, J. M.; Birkedal-Hansen, H. *Cell* **1999**, *99*, 81.
(110) Loechel, F.; Gilpin, B. J.; Engvall, E.; Albrechtsen, R.; Wewer, U. M. *J. Biol. Chem.* **1998**, *273*, 16993.
(111) Zhou, A.; Webb, G.; Zhu, X.; Steiner, D. F. *J. Biol. Chem.* **1999**, *274*, 20745.
(112) Brou, C.; Logeat, F.; Gupta, N.; Bessia, C.; LeBail, O.; Doedens, J. R.; Cumano, A.; Roux, P.; Black, R. A.; Israel, A. *Mol. Cell* **2000**, *5*, 207.

(113) Roghani, M.; Becherer, J. D.; Moss, M. L.; Atherton, R. E.; Erdjument-Bromage, H.; Arribas, J.; Blackburn, R. K.; Weskamp, G.; Tempst, P.; Blobel, C. P. *J. Biol. Chem*. **1999**, *274*, 3531.
(114) Yana, I.; Weiss, S. J. *Mol. Biol. Cell* **2000**, *11*, 2387.
(115) Lopez-Perez, E.; Zhang, Y.; Frank, S. J.; Creemers, J.; Seidah, N.; Checler, F. *J. Neurochem*. **2001**, *76*, 1532.
(116) Bassi, D. E.; Mahloogi, H.; Al-Saleem, L.; Lopez De Cicco, R.; Ridge, J. A.; Klein-Szanto, A. J. *Mol. Carcinog.* **2001**, 224.
(117) Bassi, D. E.; Lopez De Cicco, R.; Mahloogi, H.; Zucker, S.; Thomas, G.; Klein-Szanto, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10326.
(118) Lee, R. K.; Wurtman, R. J. *Ann. N. Y. Acad. Sci.* **2000**, *920*, 261.
(119) Baselga, J.; Mendelsohn, J.; Kim, Y.-M.; Pandiella, A. *J. Biol. Chem*. **1996**, *271*, 3279.
(120) Jones, S.; Novick, D.; Horiuchi, S.; Yamamoto, N.; Szalai, A.; Fuller, G. *J. Exp. Med*. **1999**, *189*, 599.
(121) Yabkowitz, R.; Meyer, S.; Black, T.; Elliott, G.; Merewether, L. A.; Yamane, H. K. *Blood* **1999**, *93*, 1969.
(122) Gechtman, Z.; Alonso, J. L.; Raab, G.; Ingber, D. E.; Klagsbrun, M. *J. Biol. Chem*. **1999**, *274*, 28828.
(123) Fan, H.; Derynck, R. *EMBO J*. **1999**, *18*, 6962.
(124) Gutwein, P.; Oleszewski, M.; Mechtersheimer, S.; Agmon-Levin, N.; Krauss, K.; Altevogt, P. *J. Biol. Chem*. **2000**, *275*, 15490.
(125) Fitzgerald, M. L.; Wang, Z.; Park, P. W.; Murphy, G.; Bernfield, M. *J. Cell Biol*. **2000**, *148*, 811.
(126) Reddy, P.; Slack, J. L.; Davis, R.; Cerretti, D. P.; Kozlosky, C. J.; Blanton, R. A.; Shows, D.; Peschon, J. J.; Black, R. A. *J. Biol. Chem*. **2000**, *275*, 14608.
(127) Arribas, J.; López-Casillas, F.; Massagué, J. *J. Biol. Chem*. **1997**, *272*, 17160.
(128) Montero, J. C.; Yuste, L.; Diaz-Rodriguez, E.; Esparis-Ogando, A.; Pandiella, A. *Mol. Cell Neurosci.* **2000**, *16*, 631.
(129) Sunnarborg, S. W.; Hinkle, C. L.; Stevenson, M.; Russell, W. E.; Raska, C. S.; Peschon, J. J.; Castner, B. J.; Gerhart, M. J.; Paxton, R. J.; Black, R. A.; Lee, D. C. *J. Biol. Chem*. **2002**, Jan 31 [electronic publication ahead of print].
(130) Tsou, C.-L.; Haskell, C. A.; Charo, I. F. *J. Biol. Chem*. **2001**, *276*, 44622.
(131) Garton, K. J.; Gough, P. J.; P., B. C.; Murphy, G.; Greaves, D. R.; Dempsey, P. J.; Raines, E. W. *J. Biol. Chem*. **2001**, *276*, 37993.
(132) Hansen, H. P.; Dietrich, S.; Kisseleva, T.; Mokros, T.; Mentlein, R.; Lange, H. H.; Murphy, G.; Lemke, H. *J. Immunol*. **2000**, *165*, 6703.
(133) Althoff, K.; Reddy, P.; Voltz, N.; Rose-John, S.; Müllberg, J. *Eur. J. Biochem*. **2000**, *267*, 2624.
(134) Zhang, Y.; Jiang, J.; Black, R. A.; Baumann, G.; Frank, S. J. *Endocrinology* **2001**, *141*, 4342.
(135) Rio, C.; Buxbaum, J. D.; Peschon, J. J.; Corfas, G. *J. Biol. Chem*. **2000**, *275*, 10379.
(136) Lemjabbar, H.; Basbaum, C. *Nat. Med*. **2002**, *8*, 41.
(137) Asakura, M.; Kitakaze, M.; Takashima, S.; Liao, Y.; Ishikura, F.; Yoshinaka, T.; Ohmoto, H.; Node, K.; Yoshino, K.; Ishiguro, H.; Asanuma, H.; Sanada, S.; Matsumura, Y.; Takeda, H.; Beppu, S.; Tada, M.; Hori, M.; S., H. *Nat. Med*. **2002** *8*, 35.
(138) Shirakabe, K.; Wakatsuki, S.; Kurisaki, T.; Fujisawa-Sehara, A. *J. Biol. Chem*. **2001**, *276*, 9352.

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