Substrate specificity and inducibility of TACE (tumour necrosis factor $\alpha$-converting enzyme) revisited: the Ala-Val preference, and induced intrinsic activity


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Abstract

Tumour necrosis factor $\alpha$ (TNF$\alpha$)-converting enzyme (TACE/ADAM-17, where ADAM stands for a disintegrin and metalloproteinase) releases from the cell surface the extracellular domains of TNF and several other proteins. Previous studies have found that, while purified TACE preferentially cleaves peptides representing the processing sites in TNF and transforming growth factor $\alpha$, the cellular enzyme nonetheless also sheds proteins with divergent cleavage sites very efficiently. More recent work, identifying the cleavage site in the p75 TNF receptor, quantifying the susceptibility of additional peptides to cleavage by TACE and identifying additional protein substrates, underlines the complexity of TACE–substrate interactions. In addition to substrate speci-
ficity, the mechanism underlying the increased rate of shedding caused by agents that activate cells remains poorly understood. Recent work in this area, utilizing a peptide substrate as a probe for cellular TACE activity, indicates that the intrinsic activity of the enzyme is somehow increased.

Introduction

Tumour necrosis factor α (TNFα)-converting enzyme (TACE/ADAM-17, where ADAM stands for a disintegrin and metalloproteinase) plays a role in both inflammation and mammalian development. It therefore remains a subject of intensive investigation, particularly with respect to its substrate specificity and the regulation of its activity. These topics will be the focus of this review.

TACE was identified by its ability to cleave TNF at the physiological processing site [1,2]. TNF is a central mediator of inflammation: it is induced by bacterial wall components and by various immune system regulators, and it causes the production of proteases, reactive oxygen species, vasoactive compounds, adhesion proteins and other cytokines. It is critical for host defensive responses to certain infections, but it can also cause severe damage in autoimmune diseases such as rheumatoid arthritis [3]. Full-length TNF is a type II transmembrane protein, with cytokine activity residing in the C-terminal 157 residues [4]. This portion of the protein is released from cells by a proteolytic cleavage between Ala76 and Val77, approx. 20 residues from the predicted transmembrane domain. Both the cell-associated and the released forms of TNF are biologically active, but full inflammatory responses require the soluble form in at least some situations [5].

Studies with cells bearing mutated TACE, lacking its Zn-binding domain (Δ Zn TACE), demonstrated a requirement for this domain for the bulk of TNF release by anti-CD3-stimulated T cells and by lipopolysaccharide (LPS)-stimulated monocytic cells [1]. Surprisingly, however, the phenotype of the knockout mice resembled in several respects the phenotype of transforming growth factor α (TGFα)-deficient mice [6]. TGFα is a ligand of the epidermal growth factor receptor (EGFR) and is required for normal mammalian development. It is made as a type I transmembrane protein, and a soluble form is generated by a proteolytic cleavage nine residues from the predicted transmembrane domain [7]. Approx. 20 N-terminal residues are also sometimes removed by proteolytic processing. The phenotype of Δ Zn TACE mice suggested that TACE is the enzyme that generates soluble TGFα, and that this form is essential for the developmental function of this factor, even though the cell-associated form is active. Subsequent studies confirmed that TACE knockout cells are deficient in the release of soluble TGFα [6,7].

The finding that TACE releases TGFα as well as TNF was surprising, because early studies showed that it did not cleave a TGFα C-terminal processing-site peptide under conditions in which a TNF-based peptide substrate was readily cleaved. However, as discussed in detail below, increasing the amount of enzyme used did result in cleavage of the TGFα peptide, and the P1-P1' sequence is identical with the corresponding sequence in TNF (Ala-Val). What came as an even greater surprise than its role in TGFα processing was the
finding that TACE is required for the shedding of a wide variety of membrane protein ecto-domains, from receptors to adhesion proteins, many with cleavage sites very different from Ala-Val. These findings will be discussed in detail in the first section of the review.

The function of ecto-domain shedding is not known in most cases, although various consequences have been proposed. Possible functions include (i) the generation of growth factors that act at a distance from the releasing cell, or that act in an autocrine fashion; (ii) the conversion of transmembrane into soluble receptors, thereby simultaneously desensitizing cells to growth factors and generating binding proteins that compete with the cell-associated receptors; and (iii) the release of adhesion proteins, thereby modifying the ability of cells to migrate. The importance of shedding (although not necessarily TACE-mediated shedding) has been demonstrated in a number of situations in addition to inflammation (TNF) and epithelial development (TGFβ), including axonal pathfinding (ephrin-A2) [8], lymphocyte migration (L-selectin) [9], enhanced bacterial virulence (syndecan-1) [10] and evasion of immune system surveillance by tumour cells (class I MHC homologues) [11]. In some of these examples, as well as in several cases where the function is less clear, a striking aspect of the shedding is its inducibility. The inducibility of these events is itself an indication of physiological significance. The regulation of TACE activity will therefore be the focus of the second section of the review.

TACE is a 684-residue member of the ADAM family of zinc-dependent metalloproteinases, which falls within the metzincin superfamily along with the matrix-degrading metalloproteinases [12]. ADAMs are multi-domain, transmembrane proteins (Figure 1). These proteins include, in addition to the protease module found in the ecto-domain, a so-called disintegrin domain, which can bind to integrins, and a cytoplasmic domain. The pro-domain must be removed, most probably by furin or a related enzyme, to generate the active

![Diagram of TACE Domain Structure](image-url)

**Figure 1** Domain structure of TACE. Removal of the pro-domain is believed to be required for activity, but it may not be sufficient.

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form of TACE. The crystal structure of the protease module has been determined [13], revealing a rather deep S1' pocket with an unusual tunnel-like connection to the S3' space. Interestingly, while the structure of the core of the enzyme closely resembles the structure of a member of this family found in snake venom, the TACE catalytic domain has a number of prominently protruding loops that are not found in the snake venom enzyme.

**Substrate specificity**

**Peptide substrates based on TNF and EGFR ligands**

Studies with peptide substrates have consistently demonstrated a strong preference of TACE for cleaving at Ala–Val bonds, but also an ability to cleave much less efficiently at a wide variety of sequences. The first indication of specificity was the finding that a 20-residue peptide spanning the cleavage site in TNF (Leu<sup>67</sup>–Asp<sup>86</sup>) was cleaved solely between Ala<sup>76</sup> and Val<sup>77</sup> [14]. This study used partially purified TACE, so no enzyme/substrate ratio was reported. Another early study, in which four matrix metalloproteinases (MMPs) were tested in addition to TACE, demonstrated that this specificity was not due to inaccessibility of other peptide bonds in this sequence [15]. With a 12-residue peptide spanning the TNF processing site (acetyl-SPLAQA↓VRSSSR-NH<sub>2</sub>, where ↓ denotes cleavage), TACE again cleaved solely at the Ala–Val bond, but MMPs-1, -2, -3, -7 and -9 all cleaved at multiple sites.

Experiments investigating the effects of discrete variations in the TNF cleavage site sequence further indicated that TACE is highly discriminating. Replacement of the P1 Ala with Ile in the 12-residue peptide described above completely eliminated cleavage by TACE, while cleavage by MMP-1 and MMP-3 was virtually unaffected [15]. A separate study, employing 10-residue peptides with a fluorophore at the N-terminus and a quenching group on the C-terminus, confirmed that TACE is unable to cleave a TNF-based peptide with Ile substituted for Ala at position P1, and the rate of cleavage was drastically reduced with Val (93% reduction) or Gly at P1 (95% reduction) [16]. Cleavage with Phe or Leu at P1 was reduced by approx. 85% (this figure is based on an adjustment of the reported value, to eliminate consideration of secondary cleavages that occurred with these peptides; since no secondary cleavages are found with peptides lacking modified ends [14], the addition of the fluorophore and quencher may affect the way these peptides bind to the enzyme).

TACE is somewhat less discriminating with respect to the P1’ residue, based on the study with the fluorophore/quencher substrates. Peptides with Leu or Ile at P1’ were cleaved at a rate only about 30% lower than the rate of cleavage with Val at P1’. However, cleavage of a peptide with P1’ Ala was reduced by over 85% (again, adjusting the rate downward to eliminate consideration of secondary cleavages). A peptide that showed inversion of the primary scissile bond, i.e. with Val–Ala instead of Ala–Val, was cleaved only 7% as rapidly as the peptide with the native TNF sequence. This decrease could have been due to the P1 Val rather than (or in addition to) the replace-
ment of Val with Ala at P1, but overall it appears that TACE strongly prefers Val or Leu at P1, and a short P1 side chain.

This conclusion is buttressed by studies of TACE’s ability to cleave peptides containing the cleavage sites found in other proteins known to be TACE substrates. A peptide spanning the cleavage site in mouse pro-TNF, MAQTL↓LRSSR, was cleaved by TACE, although with approx. 5-fold less efficiency than the corresponding human sequence (again, SPLAQA↓VRSSR) (R.A. Black, unpublished work). The decrease in susceptibility to cleavage could be due either to the different P2–P6 residues or to the presence of Thr-Leu instead Ala-Val, but this result at least confirms that Thr and Leu are acceptable substitutes for Ala and Val. (Whereas this experiment was done with human TACE, we found no better cleavage of the mouse TNF-based peptide by mouse TACE.) Moreover, the only other protein known definitively to be a physiological substrate of TACE, TGFα, is shed by cleavage at an Ala–Val bond, in the sequence ADLLA↓VVAAS. In addition, a peptide with this sequence was cleaved by TACE, albeit only 10% as efficiently as the corresponding TNF-based peptide [6]. The generation of mature TGFα also requires cleavage at an N-terminal site between Ala and Val in the sequence shown in Table 1, and TACE cleaved a peptide representing this site about as well as it cleaved the juxtamembrane cleavage-site peptide.

TGFα is one of a family of proteins that bind to the EGFR, all of which have both soluble and membrane-bound forms. It is likely, although not definitely demonstrated, that TACE also releases one or more of these family members in addition to TGFα, because the phenotype of the TACE knockout mouse is more severe than the phenotype of the TGFα knockout, resembling in several respects the phenotype of EGFR knockout mice [6]. The soluble forms of betacellulin and heparin-binding EGF (HB-EGF) are released from their precursors by cleavage, respectively, of Tyr–Leu and Pro–Val bonds (see Table 1). TACE was found to cleave peptides representing these processing-site sequences at the authentic scissile bond, as well as between Arg and Leu in the HB-EGF peptide. The Val and Leu residues at P1 are consistent with a preference of TACE for aliphatic residues in this position. It was found that 20-fold more TACE was required to observe cleavage of these peptides than was required for cleavage of the TGFα juxtamembrane processing-site peptide. This difference could be due to the lack of Ala or Thr in the P1 position or, of course, to one or more of the many other differences between these peptides. Interestingly, peptides representing the N-terminal processing site of two additional EGFR ligands, amphigregulin and epiregulin, are also cleaved by TACE (and by intact cells) prior to Val, fitting the apparent pattern (Table 1).

The substrate requirements of TACE are clearly more complex than the presence of a Val or Leu residue in the P1’ position, however. As already noted, the P1 residue can have considerable influence. In addition, three of the peptides in Table 1 with one or more Val and Leu residues were not cleaved even by a relatively large amount of TACE [betacellulin (BTC) N-terminal, EGF N-terminal and EGF C-terminal peptides]. Conversely, while all the TACE-mediated cleavage of the peptides in Table 1 occurred prior to Val or Leu (with the caveat that the cleavage in the epiregulin C-terminal peptide was not deter-
Table 1 Cleavage by TACE of EGF family processing-site peptides. Peptides representing the cleavage sites of human EGF family members (↓ denotes predicted cleavage site) were incubated with recombinant TACE [1] at 37°C for 4 h and products were analysed by liquid chromatography/MS as described previously [6]. Results of cleavage of TGF\(\alpha\) peptides [6] are included for comparison. Observation of cleavage and the relevant site are indicated. The approximate concentration of TACE required for reaction is indicated. Note that cleavage of TGF\(\alpha\) peptides required approx. 10 times more TACE than cleavage of a TNF peptide [6]. AR, amphigrregulin; EPR, epiregulin; N.D., not determined. Reproduced from [7].

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</thead>
<tbody>
<tr>
<td>TGF(\alpha)</td>
<td>PVAAA↓VVSHF</td>
<td>Yes; A/V</td>
<td>1.4</td>
<td>ADLLA↓VVAAS</td>
<td>Yes; A/V</td>
<td>1.4</td>
</tr>
<tr>
<td>AR</td>
<td>SVRVEQ↓VKPPQ</td>
<td>Yes; Q/V</td>
<td>28</td>
<td>ERCGEK↓SMKTHS</td>
<td>No</td>
<td>28</td>
</tr>
<tr>
<td>BTC</td>
<td>RSPETN↓LLCGDP</td>
<td>No</td>
<td>28</td>
<td>RVDLFT↓LRGDRG</td>
<td>Yes; Y/L</td>
<td>28</td>
</tr>
<tr>
<td>EPR</td>
<td>NPRVAQ↓VSITKC</td>
<td>Yes; Q/V</td>
<td>28</td>
<td>CEHFFL↓TVHQPL</td>
<td>Yes; N.D.</td>
<td>28</td>
</tr>
<tr>
<td>HB-EGF</td>
<td>N.D.</td>
<td>–</td>
<td>–</td>
<td>GLSLPV↓VENRLYTD</td>
<td>Yes; PV, R/L</td>
<td>28</td>
</tr>
<tr>
<td>EGF</td>
<td>HHYSVR↓NSDSEC</td>
<td>No</td>
<td>28</td>
<td>KWWELR↓HAGHGQ</td>
<td>No</td>
<td>28</td>
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mined), the same amount of TACE in the same time period is able to cleave a diverse set of peptides with no similarity at P1 or P1’ to TNF or TGFβ. This finding was made in the course of studies of other shed proteins, discussed in the next section.

**Peptide substrates based on proteins other than TNF and EGFR ligands**

In addition to TNF and the EGFR ligands, there are three other proteins which meet the criteria that (i) their shedding is believed to be of physiological significance and (ii) there is strong evidence that TACE is a major shedding enzyme (see Table 2). L-selectin shedding appears to be important in lymphocyte migration [9]. Thymocytes from Δ Zn TACE mice were found to be markedly deficient in PMA-induced L-selectin shedding [6], and an unpublished study found that neutrophils from mice reconstituted with a Δ Zn TACE haematopoietic system fail to shed L-selectin in response to LPS or fMet-Leu-Phe as well as PMA (R.A. Black, K. Charrier and J.J. Peschon, unpublished work). The reported processing site in mouse L-selectin, QETNR↓SFSKI, is not at an Ala/Thr–Leu/Val bond, but it appears that TACE is able to cleave here nonetheless. Indeed, a peptide representing the corresponding site in human L-selectin (QKLDK↓SFSMI) was cleaved by human TACE, albeit with greatly reduced efficiency compared with its preferred substrates discussed above. Cleavage was observed over the course of a 4 h incubation with 7 μM TACE. Taking into account the amount of TACE used and the rate of cleavage, the reaction was 2250-fold less efficient than the cleavage of a TNF processing-site peptide. The cleavage was, however, at the physiological site (between Lys and Ser).

A second protein, other than TNF and the EGFR ligands, whose shedding is of particular interest is the amyloid-β precursor protein (APP). Cleavage of this protein at the α-secretase site releases the bulk of the ectodomain and, importantly, occurs within the sequence of the amyloid-β peptide that is generated by cleavages at the β and γ sites. Thus shedding of the APP ecto-domain may reduce the production of amyloid-β peptide, a major component of the plaques found in Alzheimer’s disease. Primary embryonic fibroblasts from Δ Zn TACE mice were found to be completely deficient in PMA-induced shedding of APP [17], strongly implicating TACE as an APP α-secretase. Consistent with that conclusion, TACE did cleave a peptide representing the α-processing site, acetyl-VHHQKLVFFA-amide, and it did so at

### Table 2 Cleavage by TACE of processing-site peptides from other proteins. Procedures were as described for Table 1.

<table>
<thead>
<tr>
<th>Shed protein</th>
<th>Cleavage site</th>
<th>Cleavage (by 7–28 μM TACE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-selectin</td>
<td>QETNR↓SFSKI</td>
<td>Yes</td>
</tr>
<tr>
<td>APP</td>
<td>VHHQK↓LVFFA</td>
<td>Yes</td>
</tr>
<tr>
<td>p75 TNFR</td>
<td>MGPSPPAEG↓STGDFA</td>
<td>Yes</td>
</tr>
<tr>
<td>Type II IL-1R</td>
<td>TLRTTVKEASS↓TFSWG</td>
<td>No</td>
</tr>
<tr>
<td>p55 TNFR</td>
<td>PQIEN↓VKGTEDS</td>
<td>Yes</td>
</tr>
<tr>
<td>IL-6R</td>
<td>SLPVQ↓DSSV</td>
<td>No</td>
</tr>
</tbody>
</table>

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the physiological site, between Lys and Leu. The P1' Leu fits the pattern noted above for TNF and EGFR ligands, but the reaction was extremely inefficient, comparable with that found with the L-selectin processing-site peptide.

The third shed protein with both considerable physiological interest and convincing TACE involvement is the p75 TNF receptor (TNFR). Significant amounts of the soluble form of this receptor are found in serum, and it may modulate the effects of TNF by competing with cell-associated receptors. TACE knockout monocytic cells are markedly deficient in PMA-induced shedding of the p75 TNFR, indicating that TACE is probably a physiological sheddase of the receptor [6]. It has been reported that "the major C terminus of the soluble p75 receptor isolated from urine corresponds to Val-[214]" [18] (Val192 if leader sequence is not counted), and some investigators have inferred from this observation that release of the p75 receptor results from cleavage between Val214 and His215 in the sequence SMAPGAVHLQP. However, since the material sequenced came from urine, it may have resulted from secondary cleavages, and, consistent with that possibility, the site is considerably further from the predicted transmembrane domain of the intact protein than is typical for shed proteins. We therefore revisited the issue of the cleavage site that releases the ecto-domain of the p75 TNFR, using material from a short-term culture of PMA-stimulated C127 cells overexpressing the human receptor. Western analysis of the medium showed that these cells released a 46 kDa form of the receptor, consistent with observations made with various other cells [19]. This protein was purified using an immunoaffinity column, and treatment with cyanogen bromide yielded a peptide, GPSPPAEG (with the serine linked to galactose and N-acetylgalactose), which is from the juxtamembrane region. This analysis suggests that the p75 TNFR is shed by cleavage between Gly253 and Ser254, in the sequence MGPSPPAEG-STGDFAL. A peptide with this sequence was cleaved by purified TACE at the apparent physiological site, but with even lower efficiency than observed with the L-selectin and APP processing-site peptides.

Peptides based on the sequences of a number of other shed proteins have also been tested as TACE substrates, even though the biological significance of their shedding is less clear than in the cases discussed thus far. The type II interleukin-1 (IL-1) receptor (IL-1R) is apparently shed by TACE, based on a proteomic analysis of medium conditioned by TACE wild-type compared with Δ Zn TACE monocytic cells stimulated with PMA [20], even though it would serve as a 'decoy' in any case, since it lacks a significant cytoplasmic domain and does not signal upon IL-1 binding. Several years ago we determined the C-terminus of the soluble form by purifying it from the medium of CV-1 cells overexpressing the full-length protein and then analysing the mass of fragments generated by the protease endo-Asp-N. This work suggested that cleavage occurs between Ser and Thr in the sequence TLRTTVKEASS↓TFSWG. A peptide corresponding to this sequence was not cleaved even by 28 μM TACE, the amount required for cleavage of the more resistant peptides described above. This result is consistent with a study of the shedding of various proteins by Δ Zn TACE cells reconstituted with either TACE or a chimaeric protein composed of the TACE catalytic domain and the ADAM-10
This study indicated that shedding of the type II IL-1R, but not of the other proteins tested, requires the TACE disintegrin/cysteine-rich domain [21].

The p55 TNFR and the IL-6R provide two final examples of proteins that have served as the basis of substrate peptides in our work. In neither case is there evidence that the endogenous protein is shed by TACE, but ΔZn TACE fibroblasts co-transfected with these proteins plus TACE release more of the ecto-domains than do cells transfected with the substrate proteins alone [21,22]. Again, however, peptides served as very poor substrates: TACE did cleave a p55 TNFR processing-site peptide and did so at the physiologically correct site, between Asn and Val in the sequence PQIEN↓VKGTEDS, but the reaction was extremely inefficient (comparable with the reaction with the L-selectin- and APP-based peptides). And no cleavage at all was observed with the IL-6R peptide SLPVQ(↓)DSSV.

A recent study, by another group, reported $k_{\text{cat}}/K_m$ values for the reactions between TACE and several of the peptides discussed above [23], and the conclusions were generally consistent with our findings. A peptide representing APP was cleaved correctly by TACE, but with a $k_{\text{cat}}/K_m$ approx. 100-fold lower than the value obtained with a TNF-based peptide as substrate. No cleavage at all was observed with IL-6R or p55 TNFR peptides. The complete lack of cleavage with the p55 TNFR peptide, in contrast with the weak cleavage we observed, could have been due to the use of less enzyme, or to the presence of a dinitrophenol group at the N-terminus of the peptide.

The conclusions from the peptide substrate studies are reasonably clear. (i) TACE has a strong preference for the processing site in TNF, and a lesser but still pronounced preference for the processing site in TGF-β. The TNF-based peptide was cleaved approx. 10-fold more efficiently than the TGF-β peptide, and 100–1000-fold more efficiently than all of the other peptides tested. (ii) The determinants of this specificity are not simple, perhaps including a summation of negative and positive influences of different side chains, but an affinity for Ala/Thr–Val/Leu bonds, and particularly for Val/Leu at the P1' position, appears likely. The results with substitutions in the TNF sequence are consistent with this interpretation. Moreover, in addition to TGF-β, all six of the EGFR ligand peptides that were cleaved by TACE were cleaved prior to Val or Leu, as were the peptides representing APP and the p55 TNFR. However, TACE also cleaved, with similar extremely low efficiency, at sites lacking either Val or Leu (e.g. L-selectin, p75 TNFR), and a Val or Leu was clearly not sufficient for cleavage (note result with BTC and the two EGF peptides in Table 1).

Explanations for TACE's apparent role in the shedding of multiple proteins

These studies raise the question of how the data from TACE-deficient cells indicating a role for TACE in shedding of many proteins can be reconciled with the enzyme's extremely inefficient cleavage of the corresponding processing-site peptides. One possible explanation is simply that interactions with the proteins at sites distal to the cleavage site provide the necessary affinity.
However, we found that TACE also failed to cleave full-length L-selectin, p55 TNFR and p75 TNFR, extracted from cells with detergent (R.A. Black and J. Slack, unpublished work). The detergent could have interfered with the interaction, but the membrane form of TNF extracted under the same conditions was readily cleaved, even by much less TACE than that used with the other proteins [1]. Another caveat in interpreting this experiment, however, is that the transmembrane and cytoplasmic domains, released from the compartmentalization imposed by the plasma membrane, could fold over the cleavage site. Indeed, this complication appears to occur with pro-TGFα extracted from cells. Recent work demonstrated that, while solubilized full-length pro-TGFα is not cleaved by TACE, an ecto-domain construct ending at the transmembrane domain is cleaved, at the physiological processing site [7]. Moreover, it is possible that, in cells, distal interactions are re-inforced by the alignment of TACE and substrate proteins resulting from their membrane anchoring. Another point in favour of the distal-interaction hypothesis is that such interactions have in fact been shown for at least one shed protein (which, however, is apparently not shed by TACE) [24]. In addition, as discussed above, shedding of the type II IL-1R apparently requires the TACE disintegrin/cysteine-rich domain.

Other ways to reconcile the peptide-cleavage data with the cell-based evidence for multiple protein substrates of TACE centre on factors that may alter the conformation of either the enzyme or the substrates in the context of intact cells. Thus it has been proposed that adaptor proteins may increase the affinity of TACE for these substrates [23], and in fact evidence that a protein called ARTS-1 binds to the p55 TNFR and thereby increases its shedding has recently been published [25]. A less interesting possibility is that soluble TACE or detergent-extracted substrate proteins simply lose aspects of their native conformation, even though the enzyme remains active and the receptors still bind their ligands. There is in fact something odd about both recombinant ecto-domain TACE and solubilized native TACE: they are much less active in the presence of physiological salt concentrations than in the absence of salt. As a consequence, all studies with both peptide and solubilized protein substrates have been carried out under low-salt, non-physiological conditions. Moreover, it should be noted that we do not actually know how TACE and its substrates are distributed in the plasma membrane (e.g. they could be concentrated in microdomains), or what the local pH, ionic strength and redox conditions are.

**Regulation of TACE activity**

The shedding of most protein ecto-domains occurs at a basal rate in resting cells, but can be dramatically up-regulated by activating the cells. Many studies of this phenomenon have been carried out with PMA, a non-physiological activator of protein kinase C and also, directly or indirectly, a number of other kinases. However, several physiological stimuli can also induce shedding, including LPS, growth factors, fMet-Leu-Phe and other G-protein-coupled receptor ligands [26]. It should be emphasized that, in many shedding studies, the sheddase involved is not definitively identified, and ADAM-10, the closest homologue of TACE, is probably the major enzyme in some cases (e.g. [8]).
addition, matrix-degrading metalloproteinases can also act as sheddases [27,28]. This review will focus on regulated shedding that is likely to involve TACE.

Some progress has been made in elucidating the signal transduction components in at least some cases of induced shedding. In a study utilizing TGFα transiently expressed in CHO cells, it was found that inhibitors of the mitogen-activated protein kinase (MAP kinase) cascade blocked fibroblast growth factor-induced shedding of TGFα, and constitutively active forms of the MAP kinase extracellular-signal-regulated kinase 2 (ERK2) or MEK1 (MAP kinase/ERK kinase) increased the shedding [29]. Serum- and PMA-induced shedding also apparently required the MAP kinase cascade in this system. Moreover, the authors found that the fibroblast growth factor-, serum- and PMA-induced shedding of transiently expressed TNF and L-selectin in CHO cells was dependent on ERK MAP kinase signalling. That study also investigated the effects of different kinase inhibitors on the basal shedding of these proteins (TGFα, TNF and L-selectin) in transiently transfected CHO cells, and in all three cases the p38 MAP kinase was found to be essential. Finally, L-selectin shedding from human neutrophils in response to fMet-Leu-Phe was blocked by inhibitors of either ERK or p38 MAP kinase.

An independent study, looking at the shedding of the EGFR ligand HB-EGF stably expressed in CHO cells, also presented convincing evidence that PMA- and serum-induced release require the ERK MAP kinase cascade [30]. Interestingly, the investigators in that study observed a considerable lag between the activation of MAP kinase and the induced shedding, suggesting that additional intermediary steps are required. Consistent with this suggestion, shedding did not occur when the cells were placed in suspension even though MAP kinase activation was unimpaired.

It should be noted that by no means all induced shedding involves the ERK MAP kinase cascade. Stress-induced shedding apparently requires the p38 MAP kinase instead [30,31], and we have found that inhibitors of the ERK MAP kinase pathway had at most a partial inhibitory effect on the shedding of the p75 TNFR by U937 cells.

In any event, the mechanism by which the MAP kinase cascade or other intracellular signalling components affect the shedding machinery remains unknown. Direct modification of TACE by a component of the MAP kinase cascade is of course a possibility, and one study showed that, in HEK-293 cells, ERK2 indeed associated with TACE and phosphorylated a threonine in the cytoplasmic domain upon PMA stimulation of the cells [31]. (The growth factors EGF and nerve growth factor also induced the phosphorylation of TACE in these cells, but an involvement of ERK2 was not demonstrated.) It is not clear, however, whether the phosphorylation of TACE is related to the induced shedding observed in these cells, particularly since only the pro-form of TACE appeared to be phosphorylated. Another caveat is that the only shed protein monitored was the TrkA nerve growth factor receptor, and the assay employed looked only at the generation of the cell-associated remnant resulting from cleavage. It seems plausible that PMA could affect the accumulation of this remnant in multiple ways, not just by increasing shedding. In addition, in studies of TACE-deficient fibroblasts co-transfected with constructs encoding TrkA and...
either wild-type TACE or TACE in which the threonine in question had been mutated to alanine, PMA-induced accumulation of remnants was only moderately reduced with the Thr→Ala mutant compared with wild-type TACE. It is thus clear that phosphorylation of this threonine residue is not essential for most of the PMA-induced shedding in these cells. Moreover, in a study by other investigators, the shedding of three other proteins by this cell line was found to be just as inducible by PMA when the cells were reconstituted with a truncated form of TACE lacking the entire cytoplasmic domain as when reconstituted with wild-type TACE [21]. Taken altogether, these results indicate that modification of the TACE cytoplasmic domain is not a general mechanism by which the rate of shedding is increased, although it could contribute in some cases.

Other obvious possible explanations for stimulated shedding also appear to be ruled out. We have found no increase in the amount of surface TACE in response to various cell activators [1,32], nor have we found any increase in the extent of TACE processing. It is possible that TACE is activated by displacement of the pro-domain, without its actual removal, and one paper suggested that NO in fact increases shedding by this mechanism [33]. This mechanism could not be a general means by which shedding is increased, however, because at least some proteins are shed from the cell surface, and no pro-TACE has been found there. An increase in co-localization of TACE with its substrates is yet another possible mechanism for induced shedding, but we have not observed such co-localization (in PMA-stimulated THP-1 cells, at least).

One remaining possibility is that the intrinsic activity of TACE is increased, either by removal of an inhibitor or by engagement of an activator. The activator could be a released soluble factor or a membrane-bound factor. We have investigated this possibility using a peptide-based assay for cellular TACE activity. We find that, indeed, TACE-mediated cleavage of a TNF processing-site peptide is dramatically increased by exposing cells to PMA. Moreover, this increase did not require the TACE cytoplasmic domain (J.R. Doedens, R. Mahimkar and R.A. Black, unpublished work).

Conclusion

From peptide substrate studies, it appears that TACE is highly adapted to cleave TNF, and to a lesser extent TGFβ, yet somehow it apparently cleaves other substrates in the cellular milieu. Exosite interactions between TACE and its protein substrates could explain this paradox, and future studies should attempt to identify such interactions. The extensive surface loops found on TACE, but not on related enzymes, are obvious starting points for such studies. However, it should also be noted that the peptide substrate studies discussed in this review did not actually test TACE under physiological conditions, perhaps in part because TACE is peculiarly sensitive to even modest concentrations of salt. Under physiological conditions, the disparities in TACE’s ability to cleave different substrates might not be so great.

How shedding is activated largely remains a black box. At least some progress has been made in identifying the signalling cascades involved, but very little is known about how these pathways actually alter the shedding machinery.
Several obvious possible mechanisms, such as the classical removal of the pro-domain, do not appear to be involved. One productive area for future research may be an increase in the intrinsic activity of TACE upon cell activation.

References

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