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Review

# Molecular insights into the multilayered regulation of ADAM17: The role of the extracellular region☆

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ABSTRACT

In contrast to many other signalling mechanisms shedding of membrane-anchored proteins is an irreversible process. A Disintegrin And Metalloproteinase (ADAM) 17 is one of the major sheddases involved in a variety of physiological and pathophysiological processes including regeneration, differentiation, and cancer progression. Due to its central role in signalling the shedding activity of ADAM17 is tightly regulated, especially on the cell surface, where shedding events take place. The activity of ADAM17 can be subdivided into a catalytic activity and the actual shedding activity. Whereas the catalytic activity is constitutively present, the shedding activity has to be induced and is tightly controlled to prevent pathological situations induced by the release of its substrates. The regulation of the shedding activity of ADAM17 is multilayered and different regions of the protease are involved. Intriguingly, its extracellular domains play crucial roles in different regulatory mechanisms. We will discuss the role of these domains in the control of ADAM17 activity. This article is part of a Special Issue entitled: Proteolysis as a Regulatory Event in Pathophysiology edited by Stefan Rose-John.

## 1. Introduction

Intercellular signalling is a vital process within multicellular organisms. A crucial step in many of these pathways is the proteolytic release of membrane-bound molecules from the cell surface, such as ligands or decoy receptors. In contrast to many other signalling mechanisms, proteolysis is an irreversible step and is tightly controlled to prevent excess or insufficient release of signal transmitting ectodomains, which is associated with pathogenic processes.

A Disintegrin And Metalloproteinase 17 (ADAM17) is involved in many signalling pathways controlling physiological and pathophysiological processes such as development, regeneration, immunity, (chronic) inflammation or tumourigenesis [1–5]. This variety of processes is based on a wide range of different ADAM17 substrates. So far, more than 70 substrates have been identified, including receptor ligands such as the pro-inflammatory cytokine tumour necrosis factor (TNF)-α [1,2] and epidermal growth factor receptor (EGFR) ligands, which are needed to promote immune response and regeneration, respectively [5,6]. ADAM17-mediated shedding of cytokine receptors can result in the desensitising of cells to their ligands. The generated soluble ectodomains of receptors can either act as antagonist of signalling, such as the interleukin-1 receptor II (IL-$1R_{II}$) [7] or as agonist, via *trans*-signalling, such as the interleukin-6 receptor (IL-6R) [8,9]. An excess of ADAM17 mediated shedding results in an increased release of EGFR ligands, which can drive tumour progression. In contrast, low ADAM17 shedding activity causes problems in development and regeneration due to diminished signalling via the EGFR [3]. In the past years, many attempts have been undertaken to clarify the aspects of ADAM17 regulation, but a complete picture is still missing. Since it was shown that the cytoplasmic region is not required for the induction of shedding we will focus on the ectodomains and their role in the regulation of the shedding process [6,7,10].

ADAM17 is a type-I transmembrane protease with a large extracellular multidomain region. This ectodomain consists of a prodomain, a catalytic-, a disintegrin- and a membrane-proximal domain (MPD), as well as a short juxtamembrane segment called Conserved Adam seventeeN Dynamic Interaction Sequence (CANDIS) (Fig. 1A) [11–14]. Based on a structural model, derived from the snake venom metalloproteinases (SVMPs), which are homologs of mammalian ADAMs, it has been suggested that the ectodomain forms a C-shaped structure. This C-shape structure would result in a compact molecule in which the catalytic domain is in close proximity to the MPD (Fig. 1B) (unpublished).

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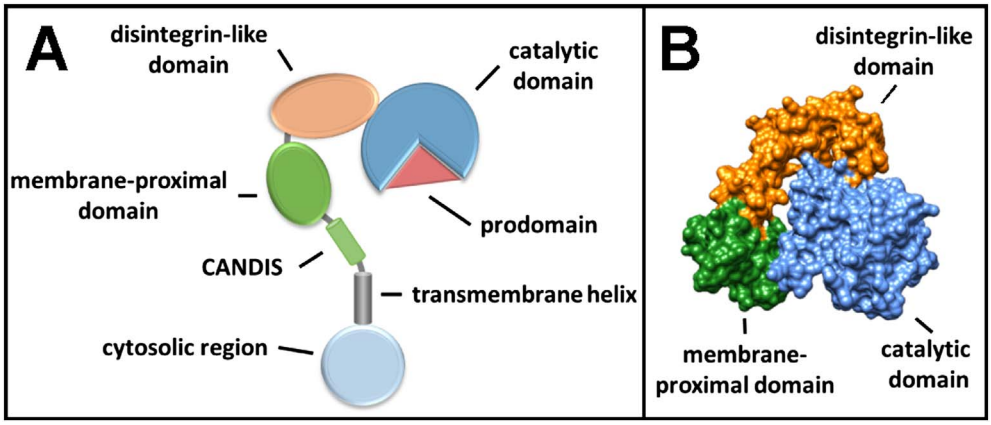


**Fig. 1.** (A) Schematic drawing of the domain structure of ADAM17. (B) A structural model of the extracellular region of ADAM17.

Since all cleavage sites in substrates of ADAM17 are very close to the membrane the catalytic domain has to be moved down towards the membrane to be able to shed molecules (Fig. 4). This requires, beside the C-shape-like structure, a certain limberness either between the domains or of the complete ectodomain relative to the membrane.

The activity of ADAM17 can be subdivided into two categories: the catalytic activity and the actual shedding activity. The catalytic activity is of course located in the catalytic domain, where the catalytic centre of ADAM17 and its close surrounding recognise the cleavage site of the substrate. Other parts of the protease recognise so-called exosite regions of the substrates outside the cleavage site and/or regulate the shedding process itself. In this regard shedding activity means the actual cleavage of substrates and the release of their ectodomains. Accordingly, the catalytic domain is capable of processing small peptides, which is due to its catalytic activity, while at the same time no shedding of membrane-bound substrates occurs [15].

The shedding activity of ADAM17 is regulated on several levels by its extracellular part, for example; i) β1-integrin binding and/ or interaction with tissue inhibitor of metalloproteinases-3 (TIMP3), ii) the conformation (disulphide isomerisation) of the MPD, which iii) facilitates the phosphatidylserine (PS) binding, and iv) the availability of CANDIS for substrate binding. Here, we describe the role and the interplay of the different extracellular domains in the regulation of the ADAM17 shedding activity.

## 2. The prodomain

The prodomain of ADAM17 includes a consensus cysteine switch motif (PKVCGY186), which can ligate the zinc ion in the catalytic centre and keep ADAM17 inactive on its way from the ER to the Golgi apparatus. Mutation of the cysteine-residue into alanine-residue did not abrogate the inhibitory effect completely. An alanine-scan of the surrounding residues had also no dramatic effect on the inhibitory effect of the prodomain. These results demonstrated that regions of the prodomain other than the cysteine switch are responsible for the binding of this domain to the catalytic domain.

In the Golgi apparatus, the prodomain is cleaved off by the Golgi resistant protein convertases such as furin. In addition to its inhibitory capacity, the prodomain was also described to be important for the correct folding and processing of ADAM17 [16]. A construct of ADAM17 lacking the prodomain was proteolytically digested in the ER, probably by self-digesting, showing the protective role of the prodomain for ADAM17 [16].

The fate of the prodomain of ADAM17 after cleavage is completely unclear. Even if it is cleaved off by protein convertases, this does not necessarily mean that it has to dissociate from ADAM17, since the affinity ($IC_{50}$) of the isolated domain to inhibit mature ADAM17 is relatively high at 145 nM [17]. However, this would raise the following questions: what triggers the release and where does it happen? These issues have not been addressed so far.

To date, the three-dimensional structure of the prodomain of ADAM17 is still not known. In fact, there exists no structure of a prodomain of any ADAM. Not even the exact length of this domain is known and which parts are mandatory for the inhibitory effect. In silico fold recognition predictions suggested that the prodomain itself consists of two single domains which constitute a so called Tudor domain tandem repeat (unpublished). The fold of the Tudor domains mainly consists of β-sheets, which is, in case of the prodomain of ADAM17, in good agreement with secondary structure predictions and the circular-dichroism spectra of the isolated domain [17]. Nevertheless, an experimentally derived structure will be of great benefit to understand the inhibitory mechanism and will help in designing muteins with an increased inhibitory capacity. Such muteins might be used as selective inhibitors in the treatment of pathophysiological situations where a specific inhibition of ADAM17 is desired.

## 3. The catalytic domain

ADAM17 belongs to the family of metalloendopeptidases. The three-dimensional structure of the catalytic domain of ADAM17 was resolved by X-ray crystallography [18] and the structure revealed a fold and a catalytic zinc environment resembling that of the snake venom metalloproteinases, identifying this domain in these days as a member of the adamalysin/ADAM family (Fig. 2A). Nowadays, the catalytic domain of ADAM17 is grouped into the family of metzincins. The typical catalytic centre of the metzincins consists of three histidine residues and one glutamic acid residue coordinating a $Zn^{2+}$ ion. The labile water molecule, which is responsible for the nucleophilic attack of the substrate peptide bond, is located between the carboxyl group of the glutamic acid residue and the $Zn^{2+}$ ion. In addition, metzincins share a so-called met-turn, a tight 1,4-turn located directly below the zinc-binding site, which is structurally as well as spatially conserved and possesses a conserved methionine at position three.

The specificity of the catalytic centre for the substrate cleavage sites is mainly determined by the P1–P3 and P1′–P3′ sites. The preferred amino acids at these positions in the substrates were determined by Q-PICS (Quantitative Proteomics for the Identification of Cleavage Sites) analysis and revealed that the P1′ site might be the most important one and valine the most preferred amino acid residue at this position [19]. This is in good agreement with ADAM17 cleavage sites recently identified in native substrates [20].

Two natural inhibitors of the catalytic activity are known. The first is the prodomain of ADAM17 (see above), the second is the tissue inhibitor of metalloproteinases-3 (TIMP3). The later one is not solely specific for ADAM17, but inhibits also other metzincins, such as ADAM12, ADAM19, ADAM33, ADAMTS1, ADAMTS4, ADAMTS5 and ADAMTS2 (weak) [21]. TIMP3 binds with its N-terminus into the catalytic centre of ADAM17 (Fig. 2B and C) with high affinity [22,23].