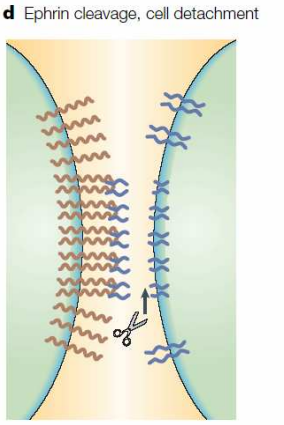
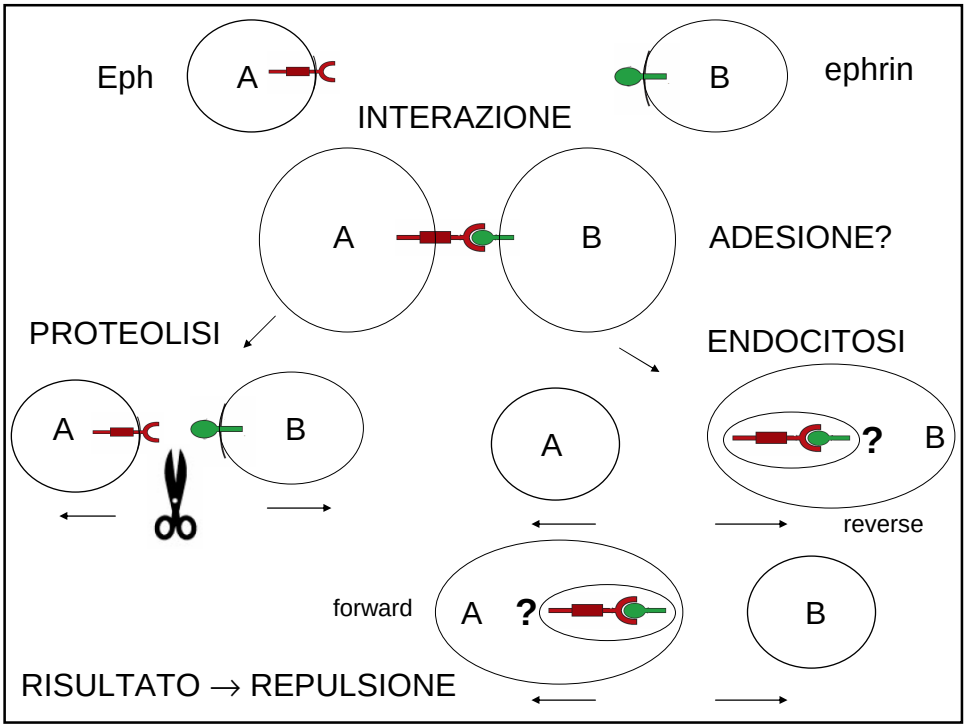
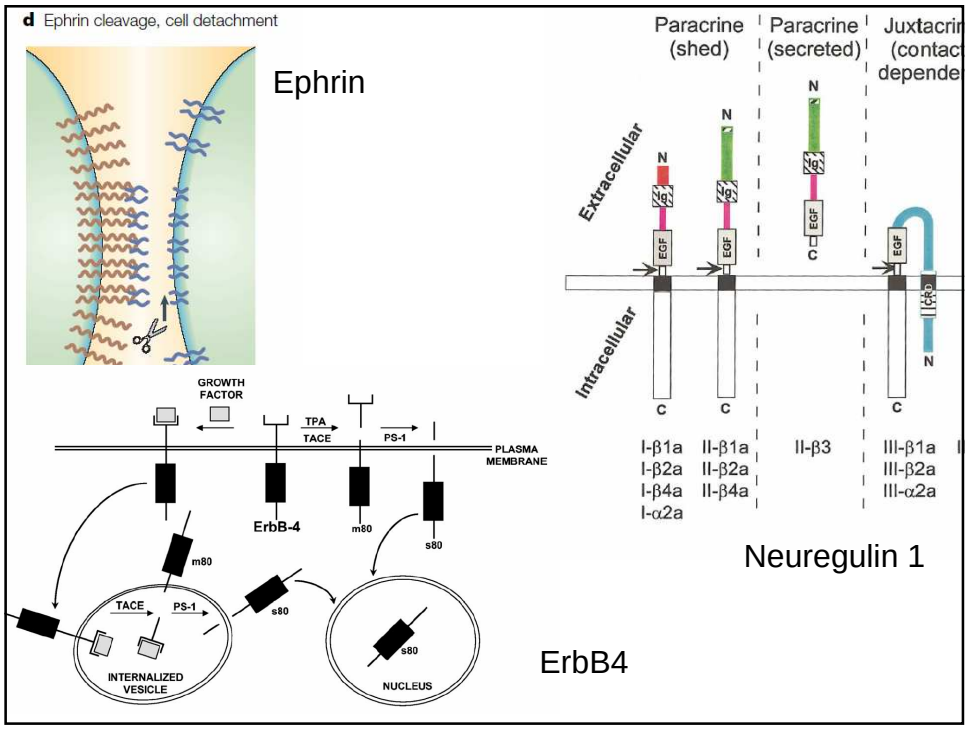
“ADAM FAMILY”

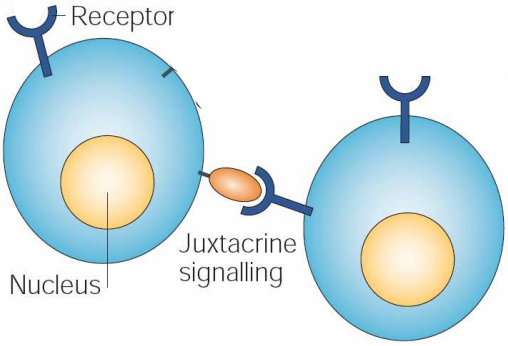
- a family of membrane-anchored metalloproteases that are known as

‘A Disintegrin And Metalloprotease’ proteins and are key components in protein ectodomain shedding



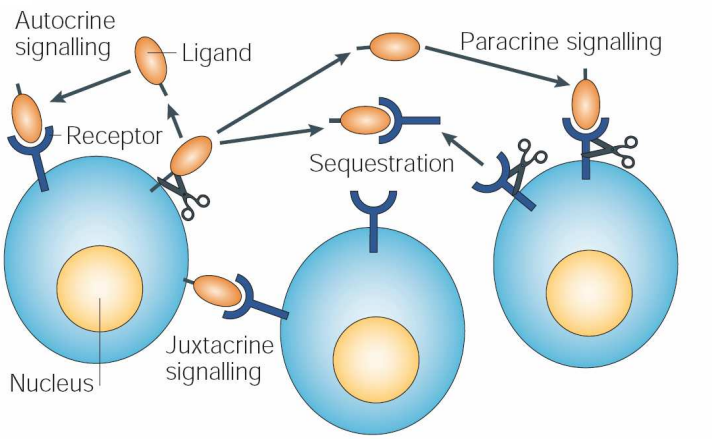






• in the absence of shedding, a membrane-anchored ligand might only engage its receptor in a juxtacrine fashion

• autocrine fashion could be possible, if there aren't impediments to autocrine receptor stimulation, such as improper orientation of the ligand and receptor



• for autocrine stimulation or to reach a receptor at a distance and to participate in paracrine signalling, a membrane-anchored ligand must be shed

• receptors might also be shed, which could result in their activation or inactivation

• shedding might also produce a soluble decoy receptor that could sequester a ligand

ADAM: A DISINTEGRIN And Metalloprotease protein

- disintegrin domain: high sequence similarity to snake-venom disintegrins

-disintegrins: short, soluble proteins, many of which contain an Arg–Gly–Asp (RGD) integrin-binding consensus motif

- with the exception of human ADAM15 none of the ADAMs contain a corresponding RGD sequence

- several studies have implicated ADAMs in cell–cell interactions

- the disintegrin domain and cysteine-rich region can also have a role in substrate targeting and can facilitate the removal of the pro-domain from the catalytic domain

A Disintegrin And METALLOPROTEASE

- metalloprotease: a peptidase that depends on a coordinated metal ion (Zn^{2+}) for its catalytic mechanism

• membrane-anchored metalloproteases

• process and shed the ectodomains of membrane-anchored growth factors, cytokines and receptors

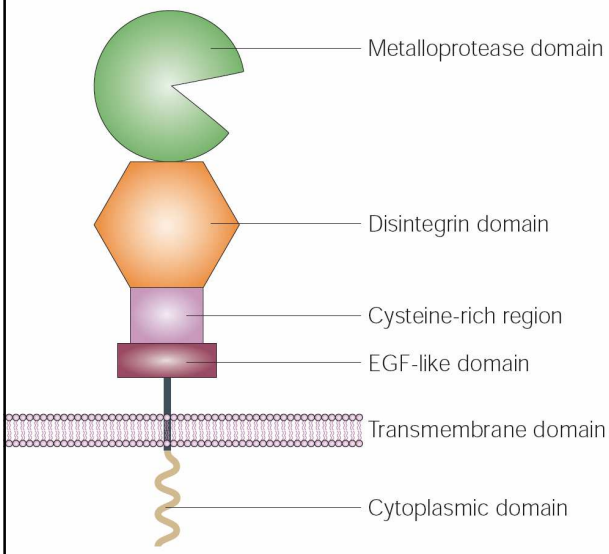
• essential roles in:

- fertilization
- angiogenesis
- neurogenesis
- heart development
- cancer

PROTEIN ECTODOMAIN SHEDDING

- proteolytic processing and release of membrane proteins
- a post-translational switch that regulates the activity of the cleaved substrate
- might activate or inactivate the substrate protein, or substantially change its functional properties

ADAM domain structure

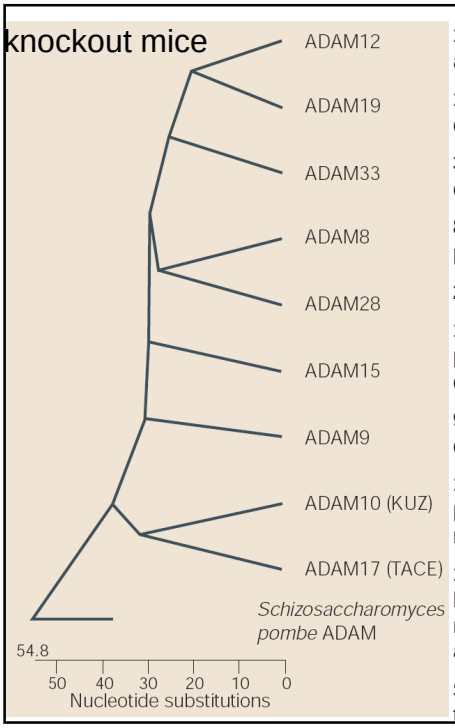


A typical ADAM protein has the following domain organization: the extracellular domain contains an N-terminal metalloprotease domain, a disintegrin domain, a cysteine-rich region and an epidermal growth factor (EGF)-like domain; the cytoplasmic domain frequently contains signalling motifs such as phosphorylation sites or proline-rich regions, which bind src-homology-3 (SH3) domains. ADAMs also contain an N-terminal signal sequence and a pro-domain, which are not shown.

• ADAM1-ADAM2: the two subunits of the heterodimeric protein fertilin (the first ADAMs to be recognized)

• many ADAMs (>33) have been identified in various species, including *Schizosaccharomyces pombe* (but not in *Saccharomyces cerevisiae)*, *Caenorhabditis elegans*, *Drosophila melanogaster* and in vertebrates (the identification numbers are assigned in the order in which ADAMs have been discovered)

knockout mice



12. 30% embryonic lethality, defects in brown adipose tissue

19. 80% postnatal lethality 1–3 days after birth, defects in cardiovascular morphogenesis

33. Viable and fertile, no major histopathological defects

8. Viable and fertile, no evident spontaneous pathological or developmental phenotypes

28. N/A

15. Viable, fertile, no evident spontaneous pathological or developmental phenotypes, but defects in pathological neovascularization

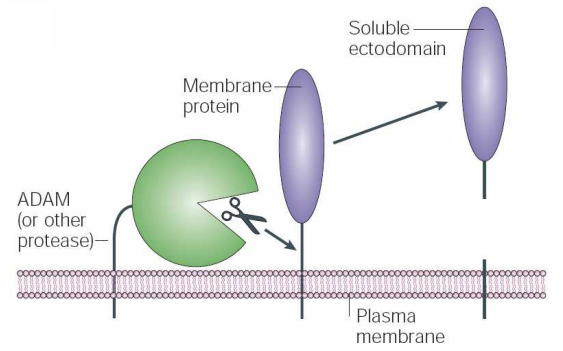
9. Viable, fertile, no evident pathological or developmental phenotypes

10. Early embryonic lethality (embryonic day 9.5), probably owing to defects in angiogenesis; resembles Notch1 knockout

17. Perinatal lethal, probably owing to defects in heart development; phenotype resembles that of mice that are knockout for EGFR,TGFα, HB-EGF or amphiregulin

Sp. Defect in formation of the spore envelope, but this function does not depend on the catalytic activity

Protein ectodomain shedding



• protein ectodomain shedding = the proteolytic release of the ectodomain of a membrane protein that is usually triggered by a cut adjacent to the plasma membrane

• ectodomain shedding affects many structurally and functionally diverse molecules, such as the pro-inflammatory cytokine TNFα, all EGFR ligands, receptors such as TNF receptor-I and -II, ErbB2, ErbB4-JMa, and a number of other proteins such as Delta, the amyloid precursor protein and L-selectin

• 2–4% of the proteins on the cell surface are subjected to ectodomain shedding

Regulated Intramembrane Proteolysis - RIP

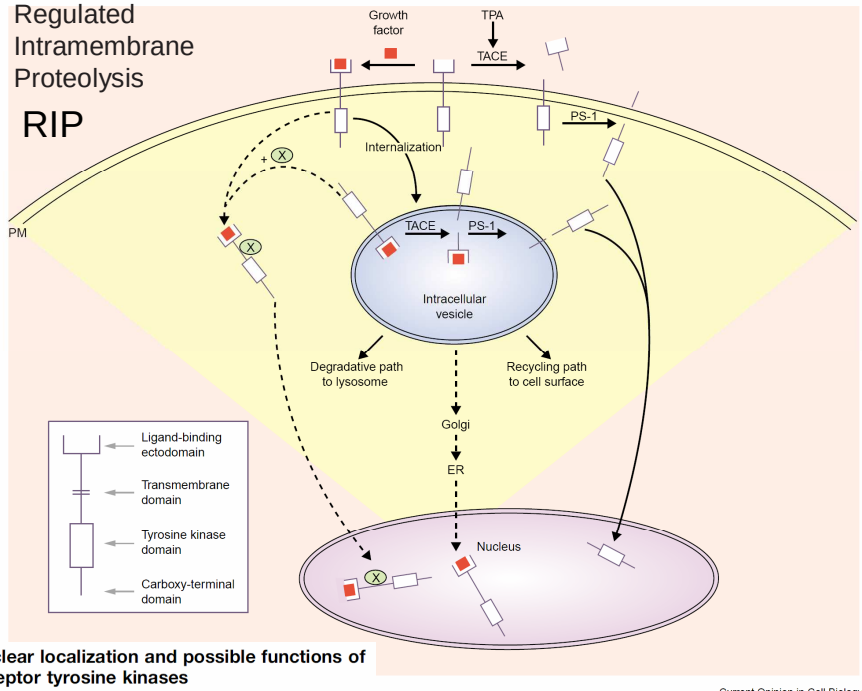
• ectodomain shedding can also activate receptors or ligands

• signalling through Notch and ErbB4-JMa are examples of a role for proteolysis in activating a receptor

• signalling through NRG1 is an example of a role for proteolysis in activating a ligand

• a membrane-proximal cleavage by an ADAM triggers a second (presenilin-dependent) cleavage, which is referred to as Regulated Intramembrane Proteolysis (RIP)

• RIP releases the cytoplasmic domain from its membrane anchor, and allows it to enter the nucleus and participate in the transcriptional regulation of specific target genes



INTRACRINE SIGNALING

Wiley, H. S. *et al.*

Removal of the membrane-anchoring domain of epidermal growth factor leads to intracrine signaling and disruption of mammary epithelial cell organization.

J. Cell Biol. **143**, 1317–1328 (1998)

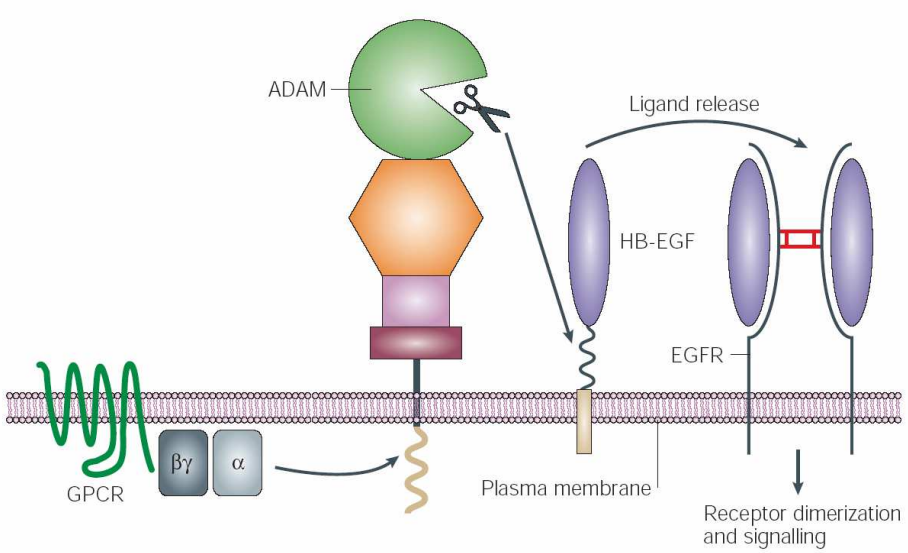
One of the first clues that proteolysis affects EGFR signalling emerged from a study in which EGF was overexpressed in cells as a soluble protein - that is, without its membrane anchor.

Whereas signalling that is elicited by overexpressed EGF with a membrane anchor could be blocked by an anti-EGFR antibody, stimulation of the EGFR by overexpressed soluble EGF could not be inhibited by this antibody.

The authors concluded that the membrane anchor of EGF somehow prevents 'intracrine' stimulation of the EGFR during biosynthesis in the secretory pathway of the EGF-expressing cells.

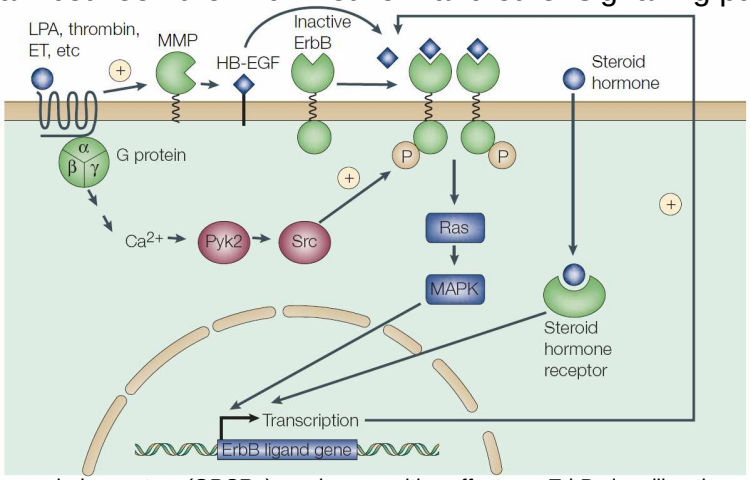
Crosstalk between GPCRs and EGFR

"triple-membrane-passing signal"- TMPS



- evaluation of the role of ADAM proteins in the crosstalk that occurs between G-protein-coupled receptors (GPCRs) and the receptors for epidermal growth factor (EGF) is a fertile area of research
- 1999: initial discovery that GPCR–EGFR crosstalk involved metalloprotease-dependent shedding of EGFR ligands
- the term 'triple-membrane-passing signal' (TMPS) was coined to describe this unexpected means of crosstalk, in which a GPCR activates an ADAM, which, in turn, releases an EGFR ligand (such as HB-EGF) to activate the EGFR

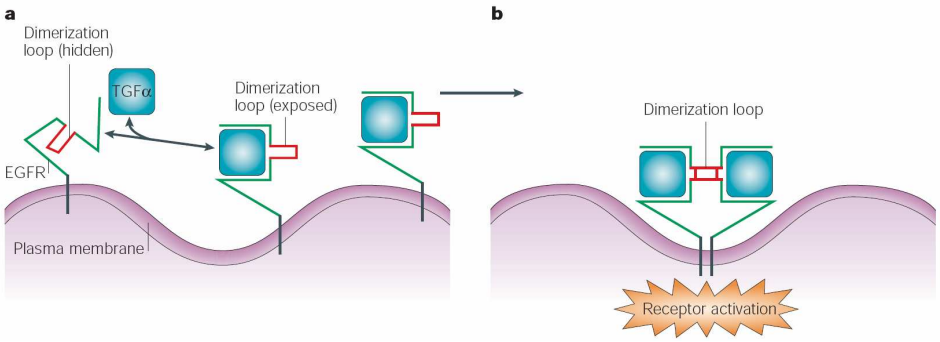
Crosstalk between the ErbB network and other signalling pathways



G-protein-coupled receptors (GPCRs) can have positive effects on ErbB signalling through two mechanisms. **1**. they can activate matrix metalloproteinases (MMPs), which cleave membrane-tethered ErbB ligands (such as heparinbinding EGF-like factor, HB-EGF), thereby freeing them to bind to ErbBs. **2**. GPCRs indirectly activate Src (perhaps via Pyk2), which phosphorylates the intracellular domains of ErbBs on tyrosine residues. Steroid hormones can have a positive effect on ErbB signalling by activating the transcription of genes encoding ErbB ligands. Finally, ErbB activation can activate a positive feedback loop through the Ras–MAPK (mitogen-activated protein kinase) pathway, which also activates transcription of ErbB ligand genes.

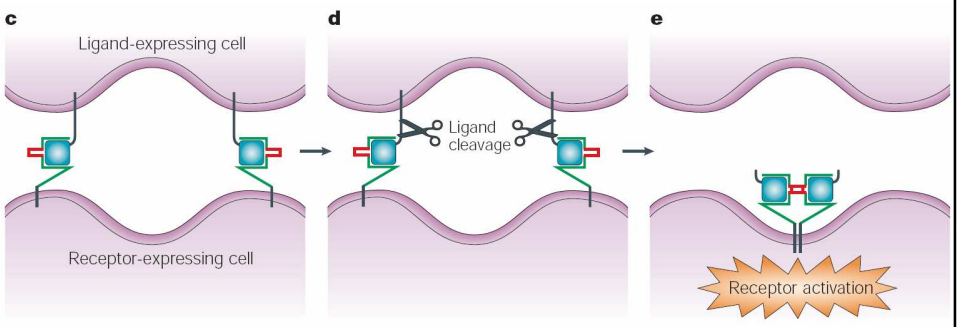
EGFR dimerization and shedding

The epidermal growth factor receptor (EGFR) has an unusual method of dimerization. Its ligands are monomeric, and are therefore not directly responsible for dimerization. Instead, the receptor dimerizes through a loop that is only exposed once the ligand has docked.



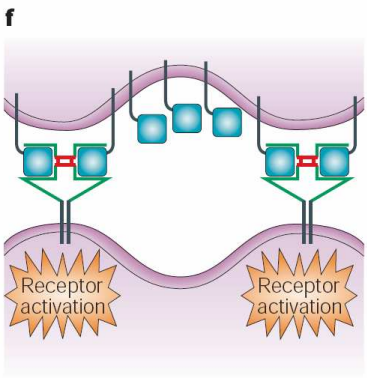
- **a** and **b** show EGFR dimerization that is induced by a soluble ligand.

A possible explanation for the crucial role of shedding has emerged in the context of membrane-anchored substrates (**c**–**e**): a receptor with a bound ligand might be less mobile if the ligand is still tethered to an adjacent cell (**c**) than if the ligand has been cleaved (**d**,**e**).



• overexpression of EGFR ligands could allow juxtacrine signalling, even by uncleavable ligands (**f**)

• an uncleavable EGFR ligand might even enhance signalling if its expression is high enough to allow EGFR dimerization, because it could also prevent internalization and downregulation of the EGFR

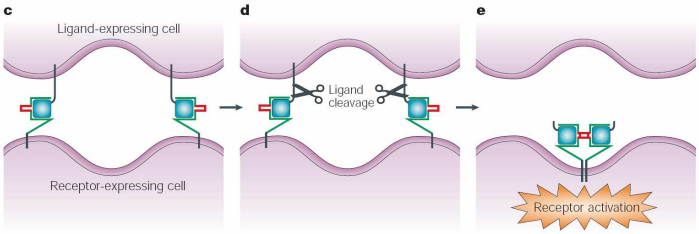


ANTI-CANCER DRUGS

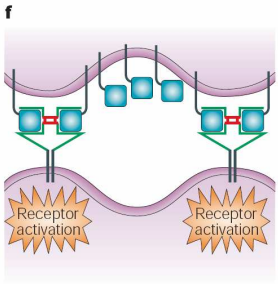
• as the EGFR pathway is a validated target for anti-cancer drugs, upstream activators of EGFR ligands — their sheddases — and regulators of these sheddases might now enter the spotlight as potential new drug targets in the EGFR pathway

• metalloprotease inhibitors could be used in the blocking of EGFR signalling

• blocking EGFR signalling with metalloprotease inhibitors might be beneficial when the ligand is expressed at low levels or if an ADAM is overexpressed, because this would decrease the chances of receptor dimerization

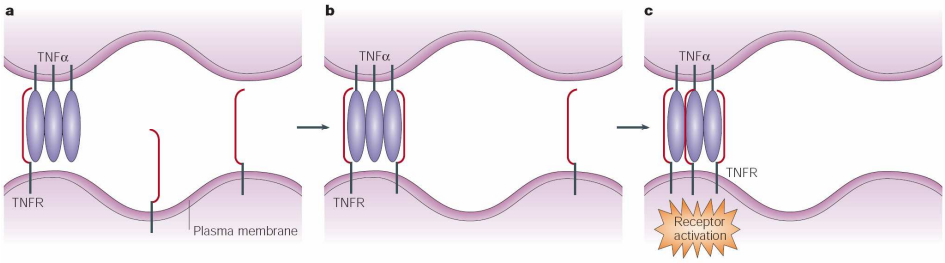


• however, metalloprotease inhibitors might have the opposite effect and stimulate EGFR signalling at higher ligand concentrations



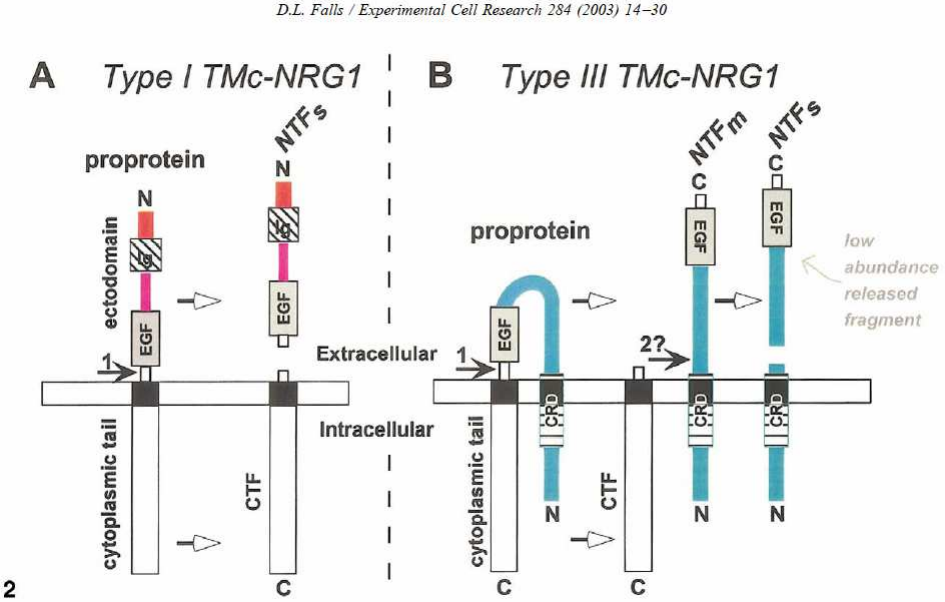
• targets for inhibition or regulation of EGFR signalling include ADAM17 and ADAM10

Juxtacrine signalling through TNFα



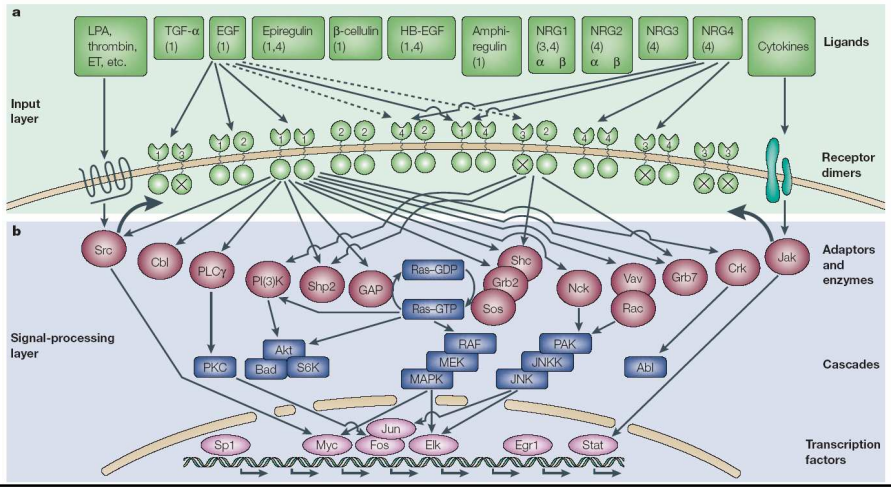
Tumour-necrosis factor (TNFα) is synthesized as a trimer. TNFα receptors (TNFRs) are therefore clustered by binding to TNFα, regardless of whether or not TNFα is anchored to the membrane. The binding of three TNFR monomers to a membrane-anchored TNFα trimer can therefore trigger a signal, as depicted in **a**–**c**.

The shedding of TNFα would theoretically only be required for paracrine signalling, but not for juxtacrine signalling.

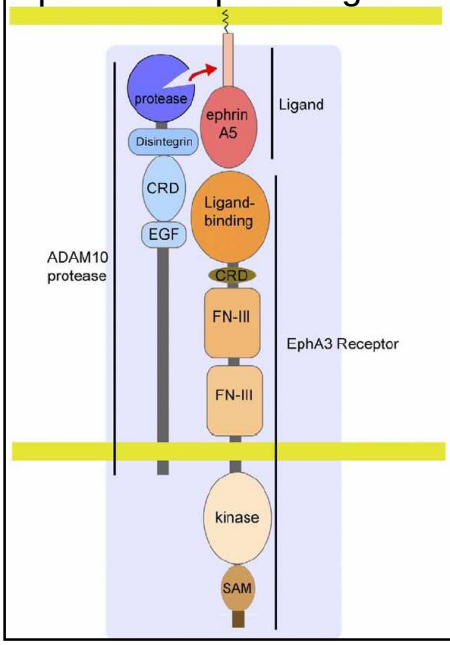


• all EGFR ligands are made as membrane-anchored precursors that can be proteolytically released from cells

• ADAMs have been implicated in the shedding of six out of the seven known EGFR ligands (TGFα, EGF, HB-EGF, betacellulin, epiregulin and amphiregulin) and of several ErbB4 ligands NRGs



The Association between the ADAM10 Protease and the Ephrin-A5/EphA3 Ligand-Receptor Signaling Complex



Binding of the ephrin ligand to the receptor-protease complex activates proteolysis (red arrow) to remove the ephrin ligand from its membrane tether.

Interactions between the cysteine-rich domain of ADAM10 and the receptor-ligand pair appear to confer a specificity that may result in activation of the protease.

Subsequently, the entire complex (light blue box) can be taken up by endocytosis by the cell expressing the receptor.

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Cytoplasmic Relaxation of Active Eph Controls Ephrin Shedding by ADAM10

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