Chapter 6

Signal Transduction Via Membrane Peptidases

DAGMAR RIEMANN[#], TANJA BLOSZ[#], JENS WULFÄNGER*, JÜRGEN LANGNER[#] and ALEXANDER NAVARRETE SANTOS*

[#]*Institute of Medical Immunology, Martin-Luther-University Halle-Wittenberg, D-06097 Halle/Saale, Germany; *ACGT Pro Genomics AG, Weinbergweg 22, D-06120 Halle/Saale, Germany*

1. INTRODUCTION

Human aminopeptidase N (APN)/CD13 is a type-II transmembrane molecule which belongs to a group of ectoenzymes with a ubiquitous expression and a broad functional repertoire. Other members of this group of membrane enzymes, which often are colocalized and which can cooperate in peptide cleavage are neprilysin/CD10, dipeptidyl peptidase IV (DPIV)/CD26, glutamyl aminopeptidase and angiotensin converting enzyme/CD143. Not only do these membrane peptidases hydrolyse small peptide mediators, resulting in activation or inactivation, but they also function as receptors and as molecules participating in cell motility and in adhesion to extracellular matrix. Membrane peptidases may play a key role in the control of growth and differentiation of various cellular systems by modulating the activity of peptides involved in growth and differentiation of cells and by regulating their access to adjacent cells. As an example, neprilysin can modulate proliferation of bronchial epithelial cells by cleavage of mitogenic bombesins (Ganju *et al* 1994). Expression of neprilysin is inversely correlated with proliferation in bronchial epithelial cells and lung cancer cells (Shipp *et al* 1991). It is also well known that the expression of DPIV is up-regulated following mitogenic activation (Schön *et al* 1985). Last not least, recent observations suggest direct involvement of

Ectopeptidases, edited by Langner and Ansorge
Kluwer Academic/Plenum Publishers, New York, 2002

membrane peptidases in signal transduction processes. The enzymatic activity is obviously not necessary for all of these different functions. This article summarizes current knowledge on signal transduction via membrane peptidases focussing on our results with APN and data from literature to APN, DPIV and neprilysin. Furthermore, evidence is outlined for an involvement of multimeric protein complexes and for the necessity of the presence in special membrane microdomains (rafts, caveolae) for the signalling capacity of membrane peptidases.

2. AMINOPEPTIDASE N

2.1 Expression and functional aspects of APN

APN (EC 3.4.11.2) is a zinc-dependent metallopeptidase of the superfamily of gluzincins (Hooper *et al* 1994). The enzyme cleaves preferentially neutral amino acids from the unsubstituted N-terminus of oligopeptides with Ala>Phe>Leu>Gly and hydrolyses a broad spectrum of oligopeptides (reviewed in Turner 1998; Lendeckel *et al* 1999; Riemann *et al* 1999). Bradykinin and substance P are known inhibitors of the enzyme in micromolar concentrations (Xu *et al* 1995). APN has a widespread distribution, occurring on fibroblasts, epithelial cells, and endothelial cells, with main sources being brush border membranes of kidney proximal tubule cells (George *et al*, 1973) and of enterocytes (Louvard *et al*, 1973). With respect to haematopoietic cells, APN has been considered to be a marker molecule for the myeloid lineage, since monocytes/macrophages and granulocytes but not peripheral blood lymphocytes or lymphocytes in spleen and tonsils express this enzyme. However, $CD13^+$ T cells can be found in the synovial fluid of patients with various forms of arthritis (Riemann *et al* 1993), on tumour-infiltrating lymphocytes (Riemann *et al* 1994) or on pericardial fluid T cells, especially of patients undergoing thoracic surgery for heart valve replacement (Riemann *et al* 1994).

Attempts to crystallize APN have so far been unsuccessful. APN cDNA clones predict a type II integral membrane protein (intracellular amino terminus, extracellular carboxyl terminus) of 967 amino acids with a short cytoplasmic domain *(AKGFYISK, Olsen et al 1988)*, and a 24-residue transmembrane hydrophobic region. The large extracellular domain contains the catalytically active site with the pentapeptide signature sequence His-Glu-Leu-Ala-His revealing the metalloprotease nature of APN. The human enzyme exists as a heavily glycosylated non-disulfide-linked homodimer

with an apparent subunit molecular weight of 140-160 kDa, carbohydrate accounting for at least 20 % of the mass (Turner 1998).

The function of APN varies dependent on its location. In gut and kidneys, the enzyme has been discussed to be involved in the terminal peptide degradation and amino acid scavenging (Kenny *et al* 1987). Otherwise, APN inactivates biologically active peptides, or has been implicated in antigen presentation, trimming peptides protruding out of the binding groove of MHC class II molecules (Larsen *et al* 1996). Soluble APN protein (derived from the membrane-bound form through proteolytic digestion) can be found *in vivo* in various body fluids. In healthy humans, APN in serum seems to exhibit some variations proposed to be related to age, sex, and smoking behaviour (Sanderink *et al* 1988). Soluble APN can induce chemotactic migration of human lymphocytes as shown for APN in bronchoalveolar lavage fluid (Tani *et al* 2000).

Biologically active peptides hydrolysed by APN include neuropeptides, such as enkephalins and endorphins (Matsas *et al* 1985; Miller *et al* 1994), vasoactive peptides, such as kallidin (Kokkonen *et al* 1999) and angiotensin III (Palmieri *et al* 1989), or chemotactic peptides, such as the cytokine MCP-1 (monocyte chemotactic protein 1) (Weber *et al* 1996). Interestingly, most of these substrates signal via G-protein-coupled heptahelical receptors. Signal transduction via these receptors involves kinase cascades commonly used by growth factors or during adhesion via integrins. One can consider that APN – by activating or inactivating biologically active peptides – could indirectly influence these signalling pathways. Otherwise, binding of a substrate as extracellular signal could directly be translated into signals for gene activation as schematised in Fig.1.

2.2 APN induces signal transduction in monocytes

Mitogenic activation of cells can be associated by an up-regulation of APN expression (Kohno *et al* 1985). Otherwise, the inhibition of APN enzyme activity by low-molecular weight inhibitors, mAbs (Löhn *et al* 1997; 2001) or antisense strategies (Wex *et al* 1997) can inhibit cell growth of different cells. One could expect that these observations are due to the enzymatic cleavage of peptides associated with proliferation. However, observations made by MacIntyre *et al* in 1987 pointed to a more complex picture with APN possibly directly involved in signal transduction process: CD13-specific monoclonal antibodies (mAbs) can trigger an increase in the concentration of free cytoplasmic Ca^{2+} in the monocytic cell line U937. Experiments of our group confirmed this result, showing a two-phase calcium increase with a small-belled $[Ca^{2+}]_i$ rise due to the release of calcium

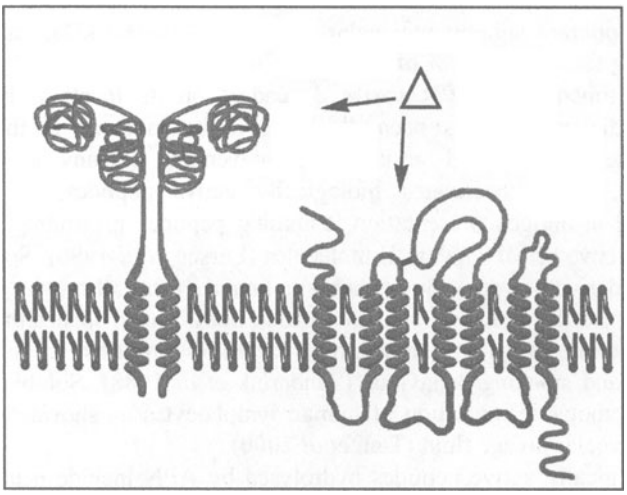

Figure 1: Hypothesis for the involvement of membrane peptidases in signal transduction. The peptide substrate (triangle) could bind to its specific G-protein-coupled receptor and signal via this receptor. The peptidase-heptahelical receptor-complex is likely a functional unit in which the membrane peptidase may directly modulate substrate signalling by cleavage of the peptide substrate, resulting in activation or inactivation or a changed receptor binding capacity. Furthermore, by binding of the peptide to the membrane peptidase, a direct signal could be transduced into the cell. Ligand binding to the G-protein-coupled receptor could induce internalisation of the complex of receptor and peptidase as could ligand binding to the membrane peptidase. Thereby, membrane peptidases could regulate local concentrations of substrate receptors, and vice versa.

from intracellular stores and a more sustained plateau due to the influx of calcium from the extracellular environment with the dye system fluo-3/SNARF-1 and flow cytometry (Navarrete Santos *et al* 2000a). APN-specific mAbs differed with respect to the signalling capacity, with the enzyme activity-inhibiting clones WM-15 and My7 inducing a distinct calcium signal directly, whereas with SJ1D1 and WM47 (non-inhibiting clones), crosslinking with a secondary goat anti-mouse (GAM) antibody was necessary for a similar high signal. Control experiments using only the GAM, or an IgG1 isotype control together with a secondary antibody, or a CD33-specific mAb (as another transmembrane molecule on monocytes) did not trigger a calcium response. Calcium signalling via APN-specific mAbs was not restricted to the U937 cell line, because we obtained similar results with monocytes of pericardial fluid and the more mature cell lines THP-1 and Mono-Mac-6.

Depletion of calcium from cytoplasmic stores by pretreatment of cells with thapsigargin caused a rapid increase in $[Ca^{2+}]_i$. Further addition of APN-specific mAbs failed to induce any significant Ca^{2+} signal, confirming our assumption that the release of calcium from endoplamic reticulum (ER)

is mainly responsible for the early Ca^{2+} rise elicited by APN-specific mAbs. Caffeine as an inhibitor of the inositol 1,4,5-trisphosphate (IP_3)-dependent Ca^{2+} release (Parker *et al* 1991) also reduced the CD13-mAb-induced rise in $[Ca^{2+}]_i$. This finding is in accordance with the assumption that in non-excitable cells the slow IP_3-mediated pathway of calcium release predominates (Ehrlich *et al* 1994). The binding of ligands to G-protein-coupled or tyrosine kinase linked receptors in the plasma membrane activates phospholipase C to generate IP_3. Binding of IP_3 to its receptor in the ER activates a calcium release from the ER lumen into the cytoplasm, generating complex calcium signals including temporal oscillations and propagating waves (reviewed in Mak *et al* 1998). Multiple mechanisms appear to operate in different cells, among them second-messenger-operated calcium influx, phosphorylation of calcium channels, and store-operated calcium entry. Tyrosine kinase inhibitors (genisteine as inhibitor of various protein tyrosine kinases, and PP1 as selective inhibitor of the Src family tyrosine kinases) were able to attenuate the sustained rise in $[Ca^{2+}]_i$ following the initial small-belled peak. Inhibitors of the phosphatidylinositol 3 (PI 3)-kinase, such as wortmannin and LY294002 (Vlahos *et al* 1994), also decreased the calcium rise provoked by APN-specific mAbs by 80 to 90 %. This observation was not unexpected since PI 3-kinase has been implicated in the transmission of activation signals, especially when receptors that have no intrinsic tyrosine kinase domain are involved (Nakanishi *et al* 1995). Inhibitors of APN, such as actinonin (100 μM), probestin (50 μM; kindly provided by Prof. Aoyagi from the Institute of Microbial Chemistry in Tokio) and bestatin (50 μM) were not able to inhibit the increase in free $[Ca^{2+}]_i$ evoked by APN-specific mAbs (Navarrete Santos *et al* 2000b).

Using immunoblot analysis, we could show for the first time that APN-specific mAbs provoke phosphorylation of the mitogen-activated protein (MAP) kinases ERK (extracellular signal-regulated kinase) 1/2, p38, and JNK (c-Jun N-terminal kinase) (Navarrete Santos *et al* 2000a). Phosphorylation of MAP kinases was already evident 1 min after the incubation with the APN-specific mAb, phosphorylation persisted for 20 min and faded after 30 min. Ligation of U937 cells by the SJ1D1 clone, without crosslinking with a secondary antibody, sufficed to induce phosphorylation of MAP kinases. An inhibitory effect on ERK and p38 phosphorylation in cells stimulated via APN-specific mAbs could be found with various inhibitors, among them wortmannin and LY294002 as PI 3-kinase inhibitors, PP1 as an inhibitor of Src phosphotyrosine kinases, PD98059 as well-characterized inhibitor of the gateway tyrosine kinase MEK-1 (Dudley *et al* 1995), and SB203580 as specific inhibitor of the p38α and p38ß MAP kinases (Cuenda *et al* 1995). Caffeine as inhibitor of the IP_3-dependent Ca^{2+} release attenuated phosphorylation of ERK but not of p38 MAP kinase. Calphostin as protein kinase C (PKC) inhibitor inhibited

neither ERK nor p38 phosphorylation after crosslinking U937 cells with APN-specific mAbs.

Since cytokine synthesis is a late event in cell activation, we determined interleukin-8 (IL-8) mRNA synthesis under the influence of APN ligation. Using competitive RT-PCR, we found that under these conditions the mRNA level of the chemotactic cytokine IL-8 was up-regulated up to 23 fold. Maximum stimulation was reached after a 2-hour incubation of the cells with the mAbs. Monocytic cells internalised the APN molecule resulting in a decreased APN expression. 24 hours after antibody ligation of APN with the WM-15 clone in comparison to a mAb specific to CD33, changes in cell form and adhesive properties were observed: WM15 ligation resulted in more adhesive cells and – in the case of U937 cells – in cells with a polarized appearance.

2.3 Signal transduction via APN in synoviocytes

To investigate whether signalling via APN-specific mAbs does occur also in other APN expressing cells, we tried to stimulate fibroblast-like synoviocytes (SFC) prepared from the pannus tissue of patients suffering from rheumatoid arthritis. These cells do express huge amounts of the membrane peptidase. APN-specific mAbs induced also in these cells an increase in free $[Ca^{2+}]_i$ (Navarrete Santos *et al* 2001), as shown both in cell suspensions of SFC using Fluo-3/Snarf-1 (2 μM/0.4 μM) (Fig. 2) as well as in adherent SFC using 4 μM Fura-Red and an inverted microscope device with a 12 Bit CCD camera and the Axon Instruments Image Capture and Analysis Software (Fig. 3). With this dye the increase in $[Ca^{2+}]_i$ can be observed as the decrease in emission fluorescence at 660 nm. Investigating Erk 1/2 activation in synoviocytes after crosslinking APN, we found no specific increase, possibly because in untreated synoviocytes these MAP kinases were already highly phosphorylated.

Summarizing our results: Ligation/crosslinking of APN in monocytes and SFC can lead to a rapid activation of tyrosine kinases that phosphorylate a variety of – up to now – insufficiently elucidated signal-transducing proteins. These in turn activate manifold signalling pathways, including the activation of MAP kinases, a rise in intracellular calcium, and an increase in cytokine synthesis. In non-excitable cells, calcium influx regulates such diverse processes as motility, exocytosis, enzyme regulation, cell proliferation, and apoptosis. Unravelling of each of the respective pathways is difficult due to their insufficiently understood interdependence.

The exact mechanism responsible for APN-mediated stimulation of cells is unclear, particularly in view of the fact that APN has only a short cytoplasmic domain without intrinsic protein tyrosine kinase function or

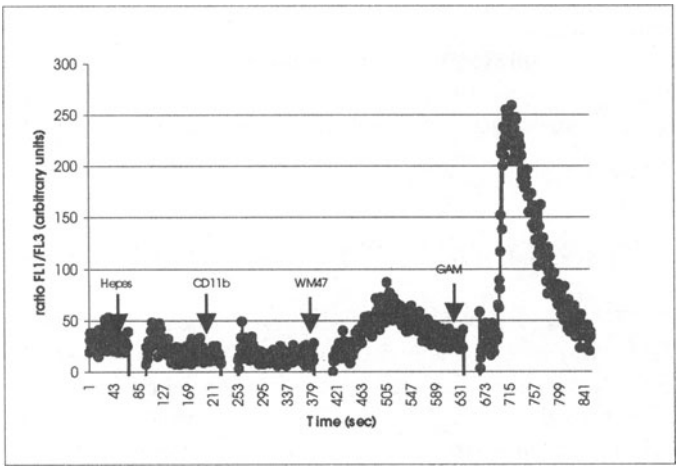

Figure 2: Increase in $[Ca^{2+}]_i$ evoked by an APN-specific mAb (WM47) but not by a CD11b-specific mAb in synoviocytes in suspension. Cells were loaded in Hank's buffer with the dyes Fluo-3 and Snarf-1 (2 μM and 0.4 μM) at room temperature for 30 min and measured by flow cytometry after a rest period of 15 min. The ratio of FL1/FL3 was calculated with the FCS Press 1.1 software. Crosslinking by a goat anti-mouse antibody (GAM) strongly amplifies the induced calcium signal.

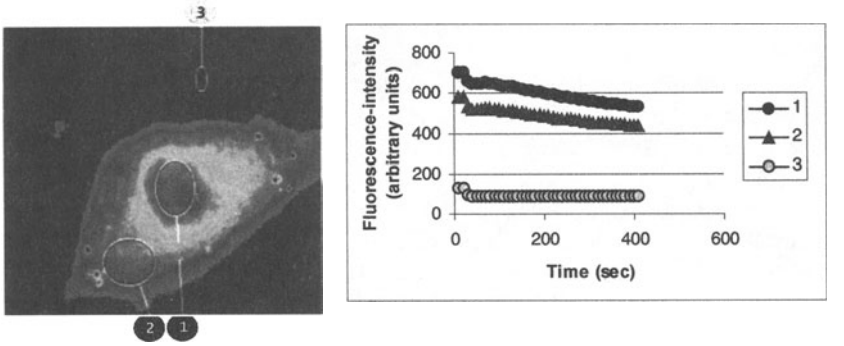

Figure 3: Increase in $[Ca^{2+}]_i$ evoked by the APN-specific mAb clone WM-15 in adherent synoviocytes. Cells were loaded with the Fura-Red dye (4 μM) and imaged with an excitation of 488 nm on an inverted microscope with a CCD camera device (SensiCam) and the Axon Instruments Image Capture and Analysis Software. At 660 nm emission wave length, a decrease in fluorescence intensity was measured in a single cell both in regions in cytoplasm and over the nucleus (a). The time course of the emission fluorescence intensity is shown in (b).

known binding motifs for tyrosine kinases. Signalling via membrane peptidases could be mediated via neighbouring membrane-associated proteins. The ligands which can induce APN-mediated signalling pathways *in vivo* remain to be determined (see chapter 5 of this article). Interestingly,

in monocytes as well as in SFC, APN is a constituent of special membrane microdomains which provide a platform for a number of membrane and intracellular proteins involved in signal transduction, including the IP3-receptor for Ca^{2+}-signalling and signal transducing kinases (see chapter 6 of this article).

3. SIGNALLING VIA DIPEPTIDYL PEPTIDASE IV

DPIV/CD26 (EC 3.4.14.5) as a membrane-associated serine-dependent ectopeptidase releasing dipeptides from oligopeptides with proline or alanine as the penultimate amino acid was the first enzyme for which a signalling capability has been described. DPIV occurs as a transmembrane homodimer with a total molecular mass of 220 - 240 kDa and is associated with adenosine deaminase (ADA, EC 3.5.4.4.) (Kameoka *et al* 1993) on the cell surface of T cells. Similar to APN, DPIV exopeptidase activity is supposed to play a role in the activation or inactivation of biological peptides (Hoffmann *et al* 1993; Mentlein 1999; De Meester *et al* 2000; see also chp. 9 in this book by De Meester *et al*), and substrates of DPIV bind to G-protein-coupled receptors. For a number of chemokines, modification or proteolytic removal of the first few N-terminal amino acids leads to significant changes in receptor selectivity, a lower chemotactic activity and impaired signalling effects (Baggiolini 1998).

Different groups have discussed the involvement of DPIV in the regulation of T cell differentiation and T cell growth (Schön *et al* 1986; Hegen *et al* 1990; Dang *et al* 1991). Though DPIV as a type II transmembrane glycoprotein consists of only a short cytoplasmic tail without any common signalling motifs, CD26 has been known as a costimulatory molecule in T lymphocytes since several years, for a detailed list of the functions of DPIV in T cell costimulation see: Kähne *et al* 1999 and Gorrell *et al* 2001.

Activation of resting T cells requires two independent signals, the first from recognition of the processed antigen in complex with major histocompatibility complex (MHC) molecules on antigen-presenting cells (APC) by the T cell receptor/CD3 complex, and the second costimulatory signal provided by special ligand-receptor interactions between T cell and APC. Crosslinking of DPIV with CD3 using immobilized mAbs induces T cell activation and IL-2 production (Morimoto *et al* 1998) and results in an internalisation of the surface peptidase, an effect which is partially mediated by the interaction of DPIV with the mannose 6-phosphate/insulin-like growth factor II receptor/CD222 (M6P/IGFRII) (Ikushima *et al* 2000). The DPIV internalisation leads to an enhanced proliferative response to anti-CD3

or anti-CD2 stimulation (Hegen *et al* 1990). Also crosslinking of DPIV alone with mAbs can trigger Ca^{2+} signals and T cell activation (for review see von Bonin *et al* 1998). It is well established that several phosphorylation steps are integral parts of DPIV signalling. Ligation of DPIV induces phosphorylation of ERK1/2, ZAP70, p56Lck and CD3ζ (Munoz *et al* 1992; Madueno *et al* 1993; Hegen *et al* 1997; von Bonin *et al* 1998). Thus, DPIV mediated signalling involves many of the same substrates as a signal via the T cell receptor. However, activation of T cells via DPIV seems not to be mediated by the T cell receptor associated adaptor proteins LAT (linker for activation of T cells) and TRIM (T cell receptor interacting molecule) (Huhn *et al* 2000).

Experiments with C-terminal deletion mutants of the human DPIV molecule demonstrated that most of the extracellular part of DPIV (also the active center) can be deleted without affecting its costimulatory activity (Huhn *et al* 2000). However, the necessity of DPIV enzyme activity for its signalling capacity in human lymphocytes has been discussed controversially (Tanaka *et al* 1993; Steeg *et al* 1995). Soluble DPIV causes an enhancement in the stimulus-induced T-cell proliferation (Tanaka *et al* 1994), and synthetic inhibitors and inhibiting mAbs of DPIV can suppress DNA synthesis as well as cytokine production of stimulated human T cells (Tanaka *et al* 1993; Reinhold *et al* 1997b). Also inhibitors of DPIV enzymatic activity induce intracellular signals transmitted by various kinases and p38 MAP kinase, though DPIV inhibitors are not capable themselves of inducing a calcium flux in T cells (Kähne *et al* 1998). This signalling can lead very quickly to a block of anti-CD3-induced signalling pathways, including calcium mobilisation (phospholipase activation), phospholipid kinase and MAP kinase (Erk1/2) activation and results in the induction of transforming growth factor (TGF)-ß1 expression and secretion (Reinhold *et al* 1997a; Kähne *et al* in chp. 8 in this book).

The signalling capacity of DPIV is not restricted to T cells. Outside the haematopoietic cell system, in SFC binding of DPIV to plasmin(ogen) carbohydrate chains is mediated by a lektin-like region in DPIV (Gonzalez-Gronow *et al* 1998). The authors discuss that the increase in free intracellular Ca^{2+} induced after plasminogen cleavage by receptor bound urokinase could be mediated via interaction with the integrin αIIb/ß3 and DPIV. Similarly, in prostate cancer cells the highly sialylated 2γ, 2δ, and 2ε glycoforms of plasminogen bind primarily to DPIV via their carbohydrate chains and induce a calcium signalling cascade which - in the case of plasminogen 2ε leads to stimulation of matrix metalloproteinase (MMP)-9 (Gonzalez-Gronow *et al* 2001). In hepatocarcinoma cells, occupancy of DPIV induces tyrosine phosphorylation within 15 min and provokes apoptotic signals (Gaetaniello *et al* 1998).

Until now neither the complete signalling mechanisms nor all of the molecules participating in signal transduction via DPIV are known. Many observations point to an important role of the highly glycosylated stalk region of DPIV for signal transduction. DPIV could become part of multimolecular complexes as has been shown for M6P/IGFRII which binds to DPIV after phosphorylation of mannose residues of the DPIV molecule (Ikushima *et al* 2000). This receptor complex binds - in a mannose 6-phosphate-independent manner - insulin growth factor II and urokinase-type plasminogen activator receptor/CD87 (Nykjaer *et al* 1998). Moreover, the receptor complex of urokinase receptor/CD87 and M6P/IGFRII can simultaneously recruit the ligands urokinase and plasminogen and controls urokinase-mediated activation of plasminogen to plasmin (Godar *et al* 1999). Similarly, the receptor is involved in the activation of latent TGF-ß by plasmin (Godar *et al* 1999). There exist other observations on the localization of DPIV in multimolecular complexes: In syncytiotrophoblast microvillous membranes of the placenta, DPIV is colocalized with integrins α5 and αv, α-actinin, transferrin receptor, transferrin, placental alkaline phosphatase and monoamine oxidase A (Kertesz *et al* 2000). The authors describe that integrins and DPIV are involved in the inhibition of the proliferation of endothelial cells by syncytiotrophoblast microvillous membranes, whereby the DPIV enzyme activity is not necessary for the effect.

4. SIGNALLING VIA NEPRILYSIN

Neprilysin/CD10 (EC 3.4.24.11) also referred to as neutral endopeptidase 24.11, enkephalinase or CALLA (common acute lymphoblastic leukemia antigen), is another ubiquitously occurring membrane-bound metallopeptidase with a broad functional repertoire (reviewed in LeBien *et al* 1989). In 1974, Kenny and coworkers purified the peptidase to homogeneity from rabbit kidneys. Neprilysin belongs to the peptidase family M13, also known as the Neprilysin subfamily, including Kell blood group protein and endothelin converting enzyme (Turner *et al* 2001). The enzyme shares a characteristic pentapeptide consensus sequence with APN and other zinc metallopeptidases that has been implicated in both zinc binding and catalysis. Neprilysin has a specificity for cleaving oligopeptides (up to about 40 amino acids in length) preferentially on the amino side of hydrophobic residues (Phe, Leu, Met). Amyloid-ß-peptide, the pathogenic agent of Alzheimer's disease, is a neprilysin substrate (Shirotani *et al* 2001). Furthermore, targeted disruption of the NEP locus in mice results in

enhanced lethality to endotoxin shock with a pronounced gene-dosage effect (Lu *et al* 1996).

In most species neprilysin appears to exist as a non-covalently associated homodimer. The molecular weight ranges from about 85 to 100 kDa, depending on tissue-specific differences in its posttranslational modifications. Molecular cloning of neprilysin revealed it as a type II integral membrane protein of 742 residues with a short (27 amino acids) cytoplasmic domain, and a 23-residue transmembrane hydrophobic region. The large extracellular domain contains the catalytically active site characterized by the zinc binding consensus sequence and 12 cysteine residues involved in disulfide bridges stabilizing the conformation of the active enzyme. The cytoplasmic tail of neprilysin contains two potential binding domains for protein kinase CKII (formerly known as casein kinase II), a ubiquitously occurring serin/threonine kinase integrated in proliferation and differentiation (Guerra *et al* 1999). Protein kinase CKII has been discussed to be involved in the down-regulation of neprilysin surface expression by phosphorylation of the intracellular part of the protein (Ganju *et al* 1996).

Neprilysin expression has been described in most types of cells, including endothelial, epithelial, reticular, and osteoblast-like cells and fibroblasts. In haematopoiesis, the expression of surface peptidases is a characteristic of several distinct developmental stages of lymphocytic maturation, and neprilysin has been used for years in the typing of leukemia or lymphoma cells (reviewed in LeBien *et al* 1989). The expression of membrane peptidases on bone marrow and thymic stromal cells seems to be an important feature for the maturation of lymphatic precursors and the regulation of their membrane peptidase expression. Accordingly, maturing B cells grow in a microenvironment where neprilysin is expressed both on stromal cells and on the precursor cells in close contact with the stromal layer (Ishii *et al* 1995). After inhibition of neprilysin, a delayed thymocyte maturation has been observed in fetal thymus organ cultures (Guerin *et al* 1997). Until now, no physiological peptide substrate of neprilysin has been clearly implicated in growth or maturation of lymphatic precursors. Interestingly, ligation of neprilysin is sufficient to activate intracellular signalling, as shown for B-cell precursors, where crosslinking neprilysin with mAbs leads to tyrosine phosphorylation of several proteins, among them the 56-kDa Src-related kinase Lyn (Ganju *et al* 1996; Angelisova *et al* 1999). Thus, neprilysin could be integrated in haematopoiesis not only by substrate cleavage, but also directly by influencing cellular signalling pathways.

A decrease in neprilysin expression in prostatic cancer cells may contribute to tumour progression by allowing bombesin-like peptides and

endothelin-1 to bind to their receptors providing signals which stimulate growth and promote cell migration (Papandreou *et al* 1998). Co-immunoprecipitation experiments in prostatic epithelial cells showed an association of neprilysin with tyrosine-phosphorylated Lyn kinase and the p85 subunit of PI 3-kinase (Sumitomo *et al* 2000). This complex of at least three proteins blocks the interaction of PI 3-kinase with the cytoplasmic tyrosine kinase focal adhesion kinase ($p125^{FAK}$), thereby inhibiting $p125^{FAK}$ phosphorylation and cell migration. This shows that neprilysin can inhibit cell migration via a protein-protein interaction and independently of its catalytic functions. Interestingly, $p125^{FAK}$ is a point of convergence between adhesion and peptidase substrates, because this kinase is activated both by ligation of integrins as well as by G-protein-coupled receptors (Zachary *et al* 1992). An interesting parallel was described in melanoma cells: loss of DPIV expression is coupled with an increased invasive potential of the cells. Transfection of melanoma with DPIV inhibits the invasiveness. Neither the protease activity nor the cytoplasmic domain of DPIV is required for this anti-invasive activity (Pethiyagoda *et al* 2000).

5. LIGANDS OF MEMBRANE PEPTIDASES

Ligation and crosslinking by mAbs are no physiologically relevant signals. Therefore, on the one hand extracellular signals have to be identified which are the first chain links of the signalling cascade. These ligands could be substrates, inhibitors or other effectors of membrane peptidases. Soluble substrates for membrane peptidases are mainly neuropeptides, kinins and chemotactic peptides. Membrane peptidases can actually function as receptor molecules, as has recently been shown for insulin-regulated aminopeptidase, which is an angiotensin IV receptor (Albiston *et al* 2001). Otherwise, ligands for membrane peptidases could be located on other cells and become associated only during cell-cell contact, as has been discussed for the thromboxane A2 receptor on monocytes which is an enzyme inhibiting ligand for DPIV on lymphocytes (Wrenger *et al* 2000).

On the other hand, one has to look for binding partners for membrane peptidases able to help in signal translation. Several hints point to a special importance of the extracellular stalk region of membrane peptidases for the interactions with this kind of expected ligands. Therefore, ligands should be membrane proteins which can come in close contact to this domain of peptidases. As one possibility, the G-protein-coupled heptahelical receptors for peptidase substrates can be expected to be located near the membrane peptidase in the plasma membrane. As one example, the DPIV-CXCR4

complex has indeed been shown to be a functional unit in human lymphocytes (Herrera *et al* 2001): The chemokine stromal derived factor (SDF)-1α induces not only a rapid down-regulation of its receptor CXCR4/CD184 but also of DPIV. One has to consider that ligands for membrane peptidases may vary in different cell types, resulting in an varying signalling capacity. As an example, ß3-integrin has been shown to be a ligand for DPIV in SFC but not in prostate carcinoma cells (Gonzalez-Gronow *et al* 2001). Whereas various ligands involved in the signalling via DPIV have become known, information on possible partners for APN and neprilysin are still scarce.

5.1 Ligands for APN

Substrates as well as effectors of APN could function as natural peptidase ligands. For microbial APN inhibitors, various signal transducing effects have been observed, though it seems not trivial to differentiate between signals following APN enzyme inhibition and signals after a possible ligation of the APN molecule irrespective of its enzyme activity. Furthermore, special transporter systems can exist which possess signalling capacity, as an example bestatin as an inhibitor for various aminopeptidases can get access to the inside of a cell via a H^+-coupled energy-dependent dipeptide transporter (Lee 2000). Bestatin has been shown to modulate PKC in the myeloid cell line K562 (Kumano *et al* 1992) and to enhance pp60/c-Src tyrosine kinase activity in the U937 cell line (Murata *et al* 1994). Probestin and actinonin can induce an elevation of erk2-mRNA levels in KARPAS-299 cells (Lendeckel *et al* 1998). Whereas the results of our group show Erk activation already 10 min after ligation of APN with specific mAbs, Lendeckel *et al* demonstrate the maximum of phosphorylation of Erk2 only 8 hours after inhibitor administration. Future investigations have to deal with the signals occurring before the inhibitor-induced activation of Erk2. As a first step in this direction, Lendeckel and coworkers show by gel-shift analysis that the APN-specific inhibitor RB3014 provokes an induction of the transcription factor Sp1 in human peripheral T cells within 30 - 60 min, which could be involved in the up-regulation of TGF-ß (Lendeckel *et al* chp. 2 of this book).

Also coronaviruses could be taken as a ligand, since APN is a major receptor for coronaviruses of the serogroup I, which cause respiratory and enteric diseases (Yeager *et al* 1992, Delmas *et al* 1992). Virus particles have been suggested to act as crosslinkers of APN, thereby inducing endocytosis and enrichment in large intracellular vesicles (Hansen *et al* 1998). APN can be one of several receptors for human cytomegalovirus (Söderberg *et al* 1993), which can downregulate the expression of APN and neprilysin by

various independent mechanisms (Phillips *et al* 1998). Cytomegalovirus binding to monocytes induces cellular activation with IL-1 secretion (Yurochko *et al* 1999), however future studies have to show whether APN is really involved in this signalling. Furthermore, galectin-4 has been found to be a ligand of APN in enterocytes (Danielsen *et al* 1997). Colorectal galectin-4 may be involved in crosslinking the lateral cell membranes of the surface-lining epithelial cells, thereby reinforcing epithelial integrity against mechanical stress exerted by the bowel lumen (Wasano *et al* 1999). As a new APN ligand the CNGRC peptide has been described which mediates the selective homing into APN-positive endothelial cells of blood vessels in angiogenesis (Pasqualini *et al* 2000), therefore NGR-containing proteins could be APN ligands. Upon treatment of monocytes with CNGRC peptides (kindly provided by Prof. Ruoslahti, La Jolla) we observed inconsistent calcium signals (data not shown).

With respect to the transmembrane molecules colocalized with APN and interacting in signal translation, information is scarce. MacIntyre and coworkers suggested the calcium release induced via APN crosslinking in U937 cells to be mediated partially by aggregation of APN with F_c receptor molecules, such as the F_c receptor for IgG, type III/CD16 (MacIntyre *et al* 1989). Otherwise, G-protein-coupled heptahelical receptors for APN substrates could become colocalized with APN, similar to the situation with CXCR4 and DPIV.

5.2 Ligands for DPIV

Inhibitors of DPIV enzymatic activity induce intracellular signals transmitted by various kinases, though DPIV inhibitors are not capable on their own of inducing a calcium flux in T cells (Kähne *et al* 1998). Peptides containing the N-terminal sequence XXP inhibit DPIV enzyme activity and could be regarded as potential ligands for DPIV: The HIV-1 transactivator protein Tat has been shown to bind to DPIV (Subramanyam *et al* 1993) because of its N-terminal structure (Wrenger *et al* 1997) as well as the thromboxan A2 receptor molecule (Wrenger *et al* 2000). Signal transduction by these molecules independent from enzyme inhibition would have to be shown in future experiments. Several other ligands have been described for DPIV, known since several years has been , e. g., the interaction with ADA (Kameoka *et al* 1993) or with the transmembrane molecule tyrosine phosphatase CD45 (Torimoto *et al* 1991). ADA has been proposed to have catalytically independent functions: ADA/CD26 interaction results in co-stimulatory signals in T cells (Martin *et al* 1995; Franco *et al* 1998). HIV gp120 interacts with DPIV and disrupts ADA binding (Herera *et al* 2001). The authors describe that incubation of T cells with gp120 at 37 °C for 30

min induces the formation of pseudopodia with a redistribution and co-localization of DPIV, CD4 and the chemokine receptor CXCR4 in these pseudopodia.

A remarkable property of DPIV is its affinity to proteins of the extracellular matrix. DPIV has been known as a collagen-binding protein since several years (Bauvois *et al* 1988), though it cleaves only denaturated fibrillar collagens (Bermpohl *et al* 1998). The putative collagen binding site of DPIV is located in the cysteine-rich domain of the extracellular stalk region (Löster *et al* 1995). These authors find no hint for fibronectin binding to DPIV, however, tumour cell surface-associated, polymeric fibronectin has been shown to bind to DPIV and this ligand/receptor pair mediates lung vascular arrest and metastasis of rat breast cancer cells (Cheng *et al* 1998). Plasminogen and plasmin have been shown to be ligands for DPIV in SFC (Gonzalez-Gronow *et al* 1998). Various plasminogen glycoforms also bind to DPIV in prostate cancer cells (Gonzalez-Gronow *et al* 2001).

DPIV can form heterodimers with seprase/fibroblast activation protein (FAP)-α, a member of the DPIV family of serine peptidases (Rettig *et al* 1993). Type I collagen substratum induces the association of α3β1 integrin with seprase (and DPIV?) as a complex on invadopodia as membrane extensions of aggressive tumour cells (Mueller *et al* 1999). Various integrins have been found in close contact to DPIV as demonstrated for β3 integrin in SFC (Gonzalez-Gronow *et al* 1998). M6P/IGFRII binds to DPIV after phosphorylation of mannose residues of the DPIV molecule (Ikushima *et al* 2000), so DPIV could get in contact to other ligands of this receptor molecule, such as plasminogen, plasmin, urokinase receptor and latent TGF-β. In syncytiotrophoblast microvillous membranes of the placenta, DPIV is colocalized with integrins α5 and αv, α-actinin, transferrin receptor, transferrin, placental alkaline phosphatase and monoamine oxidase A (Kertesz *et al* 2000).

6. LOCALIZATION OF MEMBRANE PEPTIDASES IN MEMBRANE MICRODOMAINS

Over the past few years it has become increasingly clear that the lipid bilayer of the plasma membrane of many types of cells contains microdomains rich in glycosphingolipids and cholesterol. These microdomains are thought to exist as relatively ordered (l_o phase) membrane patches surrounded by more fluid, liquid-crystalline (l_c phase) membrane regions composed mainly of glycerophospholipids, and to provide a functional platform for the interactions of different types of particular proteins, including various receptors, membrane transporters, structural

proteins and signal transducers (Brown *et al* 1989, Simons *et al* 1997). The proposed functions of microdomains comprise cholesterol transport, endocytosis, exocytosis, transcytosis, membrane trafficking and signal transduction. Microdomains serve as privileged sites where receptors [such as $F_{c\varepsilon}$ receptor I, T cell receptor, B cell receptor, insulin receptor, EGF (epidermal growth factor) receptor] and proximal signalling molecules optimally interact (Simons *et al* 1997). If receptor activation takes place in microdomains, the signalling complex is protected from enzymes located outside, such as membrane phosphatases. Numerous signalling molecules have been found there, including Src family protein-tyrosine kinases, heterotrimeric and monomeric Ras-like G proteins, and molecules involved in calcium flux (for review see Simons *et al* 2000). There is compelling evidence that the entire Ras/Raf/Mek/Erk cascade is compartmentalized in microdomains (Liu *et al* 1997). The most recent advance in T cell signalling has been the discovery that microdomains represent structural and functional organizers of the "immune synapse" (reviewed in Ilangumaran *et al* 2000).

In most cell types, microdomains exist at least in two forms: plain membrane islets, the so called rafts, and flask-shaped membrane invaginations, so called caveolae, wherein membrane lipids may be organized by the cholesterol-binding protein caveolin (Okamoto *et al* 1998). Rafts are too small to be resolved by standard light microscopy. Marker molecules of rafts are gangliosides, such as G_{M1} ganglioside, and glykosylphosphatidylinositol (GPI)-anchored proteins, such as 5'nucleotidase/CD73 and the folate receptor. The number of proteins in each raft is probably not more than 10-30 proteins, therefore a raft can statistically contain only a subset of all availabe raft proteins (Simons *et al* 2000).Caveolae differ from rafts both in their protein composition and in their functions (Sowa *et al* 2001; Oh *et al* 2001), with caveolins (caveolin-1, -2, and -3) as mostly negative regulator proteins (for review see Schlegel *et al* 2001). Originally described on the basis of their morphology by Palade in the 1950s (Palade 1953), caveolae are particularly abundant in adipocytes, fibroblasts, type 1 pneumocytes, endothelial and epithelial cells as well as in smooth and striated muscle cells. Haematopoietic cells lack caveolae (Stan *et al* 1997); for review see Anderson 1998). The heterotrimeric G proteins target different microdomains, with G_q specifically concentrating in caveolae, and G_i and G_s concentrating much more in lipid rafts (Oh *et al* 2001).

Post-translational acylations of proteins can increase a protein's affinity for membrane microdomains, the most important being membrane-proximal cysteine residue palmitoylation (reviewed in Milligan *et al* 1995). Furthermore, GPI modification directs proteins into rafts (Horejsi *et al* 1999). In non-lymphoid cells, it has been reported that N-glycans on

membrane proteins can also mediate raft association through binding to resident lektin-like molecules within lipid rafts (Benting *et al* 1999). Recent observations indicate that phosphorylation is a prerequisite for the raft targeting of the CD3ξ chain (Ardouin *et al* 1999), whereas mutation analysis demonstrated a role for the transmembrane segment of CD44 in microdomain targeting (Oliferenko *et al* 1999). Otherwise, inducible association with lipid rafts upon ligand binding, oligomerizing or mAb crosslinking has been demonstrated for several surface molecules, such as the Fcε receptor I as a heterotetrameric complex on mast cells and basophils (Field *et al* 1997), CD2 on T lymphocytes and natural killer cells (Yang *et al* 2001), and the CD20 tetraspan protein of B cells (Deans *et al* 1998).

Removal of cholesterol from plasma membranes profoundly perturbs the physical state of the membrane microdomains (Ilangumaran *et al* 1998) and compromises their function (Keller *et al* 1998). Filipin treatment of cultured lymphocytes, as an example, inibits T cell receptor-mediated Ca^{2+} mobilization and protein tyrosine phosphorylation (Xavier *et al* 1998). Thus, the compartmentalization of key signalling molecules in membrane microdomains appears necessary to provide rapid and efficient propagation of extracellular stimuli to intracellular targets.

In intestinal epithelial cells, the membrane peptidases APN and DPIV are constituents of rafts (Danielsen *et al* 1995). Our own observation implicate APN to be partially (35 - 40 % of the total protein) a raft component also in monocytes (Navarrete Santos *et al* 2000c). To show this, we used immunofluorescence and laser scanning microscopy as well as sucrose gradient centrifugation of membrane fractions after treatment with Triton-X100 at 4 ^{0}C. Raft fractions were characterized by the presence of G_{M1} ganglioside and by the high level of tyrosine-phosphorylated proteins. Furthermore we could show that cholesterol depletion of monocytes by methyl-β-cyclodextrin greatly reduced raft localization of APN without affecting APN enzyme activity. Further experiments of our group in cooperation with the group of E.M. Danielsen (Institute of Biochemistry, Panum Institute, Kopenhagen, Denmark) showed the partial presence of APN, DPIV and neprilysin in rafts and in caveolae of SFC, with APN > Neprilysin >> DPIV (Riemann *et al* 2001).

At the ultrastructural level, APN was found in particular in intracellular, yet surface connected vesicle-like structures and 'rosettes' made up of several caveolae. In addition, clusters of ectopeptidases were seen at the cell surface in flat patches of about 100 nm width. Cholesterol depletion of synoviocytes by methyl-β-cyclodextrin disrupted > 90 % of the caveolae and greatly reduced the raft localization of APN. Neprilysin has been found to be raft-associated in early B cells (Angelisova *et al* 1999) and in both CHO and Hek 293 cells transfected with the neprilysin gene in a green fluorescent protein (GFP) containing vector (our own observations, Fig. 4). Results from

literature demonstrate a raft association also for DPIV in T cells (Ilangumaran *et al* 1996), at least after T cell activation (Ishii *et al* 2001), whereas in the enterocyte-like tumour cell line Caco-2 DPIV was not found to be enriched in rafts (Garcia *et al* 1993).

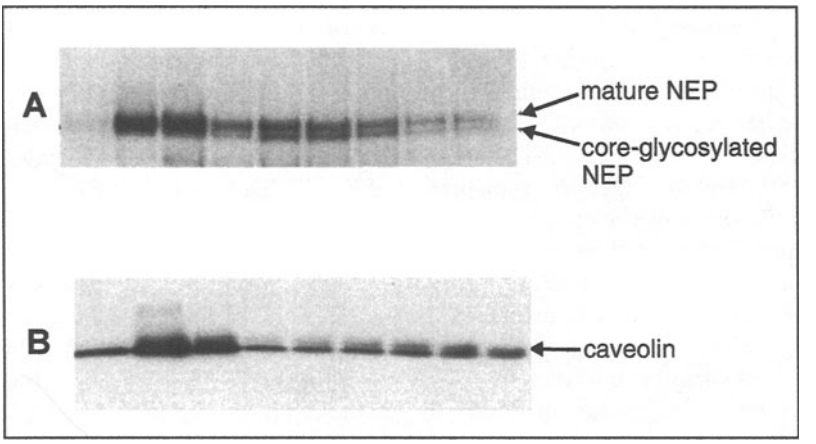

Figure 4: Detection of neprilysin in membrane microdomains. CHO cells were transfected with a neprilysin-containing pEGFP-C3 vector (Clontech) using lipofectamine. After 24 hours, cells were lysed in MNE buffer (25 mM MES, pH 6.5; 150 mM NaCl; 2 mM EDTA) containing 1 % Triton-X100. The homogenate was fractionated on a 5 - 40 % discontinuous sucrose gradient. Nine fraction were collected from the top of the tube and analysed for the presence of neprilysin and caveolin as marker molecule for caveolae by Western blotting.

Besides the possible changes of raft composition in tumour cells, one has to consider that rafts do not represent static membrane components. The interactions that drive raft assembly are dynamic and reversile. Proteins can enter and leave rafts, depending on at the moment rather incompletely understood signals. The movement of raft proteins can be influenced by interaction with cytoskeletal elements (Harder *et al* 1999). In T cells, only a minor percentage of DPIV is raft-associated in resting cells, however, ligation of DPIV by mAbs increases the recruitment of DPIV molecules to rafts (Ishii T *et al* 2001). Crosslinking of DPIV induces the aggregation of rafts, co-localizing DPIV with the protein tyrosine phosphatase CD45RO. The signals which can trigger transport of membrane peptidases into microdomains *in vivo* remain to be elucidated. Acylation of these enzymes has not been described yet. N-glycosylation of membrane peptidases could contribute via binding of up to now unknown raft-associated lektins. It seems that APN and neprilysin show a rather constitutive expression in membrane microdomains, whereas DPIV may need a ligating signal. Further work is necessary to characterize this mechanism as well as possible special functions of microdomain-localized peptidases in more detail.

Outside of membrane peptidases several different enzymes are known constituents of membrane microdomains. The GPI-linked membrane glykoprotein urokinase plasminogen activator receptor/CD87 as well as its ligand urokinase (Stahl *et al* 1995) are examples. Tissue factor/CD142 as cellular receptor for the active serine protease clotting factor FVII becomes similarly caveolae-associated after ligand binding, e.g. in stimulated endothelial cells (Sevinsky JR *et al*, 1996). Recent reports have shown that FVIIa binding to tissue factor can influence a number of biological functions, such as angiogenesis and cancer metastasis. Tissue factor also seems to play an important role in cell adhesion and migration. The intracellular signalling is independent of downstream activation of the blood coagulation cascade (Siegbahn 2000). Various ectonucleotidases are constituents of membrane microdomains, such as the GPI-anchored 5'nucleotidase/CD73 (Strohmeier *et al* 1997) and the palmitoylated ATP diphosphohydrolase/CD39 (Koziak *et al* 2000).

Our knowledge on the importance of peptidase localization in membrane microdomains is still in its early stages. Since membrane compartmentalization in rafts is required for many different cellular functions, peptidase association with microdomains could be associated with various functions. As an example, the presence of APN in intracellular vesicles of synoviocytes could be a hint for an endocytosis/exocytosis process. Caveolae are sites where small and large molecules are internalized at a rather low speed by a process called potocytosis: molecules and ions are concentrated in the sequestered space created when caveolae seal off from the extracellular environment (Mineo *et al* 2001). Therefore, a possible function of APN in synoviocytic caveolae could be the processing of hormonal signals within these vesicles, indeed receptors for peptidase substrates/inhibitors can be found in caveolae, as shown for the bradykinin receptor B_2 (Haasemann *et al* 1998) or the cholecystokinin receptor (Roettger *et al* 1995). Membrane peptidases have been found in the membrane of secreted vesicles, as shown for APN (Arienti et al 1997), DPIV (Schrimpf *et al* 1999), and neprilysin (Renneberg *et al* 2001) in prostasomes. However, there remains the question why so many different signalling molecules are targeted to caveolae as endocytic device. We prefer the presumption that membrane peptidases via their association with microdomains can get involved in signal transduction processes.

How can ligand binding to a membrane peptidase be translated into signals for gene activation? Transmembrane signalling requires modular interactions between signalling proteins, phosphorylation or dephosphorylation of the interacting protein partners and the temporary formation of supramolecular structures to convey the molecular information from the cell surface to the nucleus. In the case of growth factors and most cytokines, ligand binding promotes homodimerization which facilitates modular interactions between the cytoplasmic domains and intracellular

signalling and adaptor proteins (Pawson *et al* 1997). In pathways where receptors have no intrinsic kinase activity, intracellular non-receptor protein tyrosine kinases (i.e. Src family) are recruited to the cytoplasmic domain of the engaged receptor. Results with GPI-anchored proteins could be helpful in our understanding of the signalling capability of membrane peptidases. Despite being restricted to the outer leaflet of the plasma membrane, most GPI-anchored proteins transduce cellular activation signals (Robinson 1991) via a functional link with Src family kinases (Stefanova *et al* 1991). Crosslinking the GPI-anchored proteins or gangliosides on the cell surface has been shown to result in the aggregation of Src kinases and in tyrosine phosphorylation of proteins on the cytoplasmic face of the aggregated rafts (Harder *et al* 1999). How the two leaflets of the plasma membrane communicate with each other remains unclear up to now. The microdomain environment has been suggested to maintain the Src kinases Lck and Fyn in a state of higher activation, or the specialized lipid microenvironment of microdomains itself may allosterically activate the Src-family kinases (Hoessli *et al* 2000). The genes activated by the signalling cascade can vary between different cells depending on interacting molecules.

7. CONCLUSIONS AND FUTURE PROSPECTS

Though membrane peptidases have been explored with respect to their structure, enzyme kinetics and substrate specificity for many years, results obtained only very recently revealed the completely new aspect that these enzymes can be signal transduction molecules in various cells. Signalling via membrane peptidases is not restricted to haematopoietic cells and uses common structures of signal transduction, such as multimeric complexes and special membrane microdomains. According to Hoessli (2000), the biochemical problem of transmembrane signalling seems chiefly to be one of defining how lipids and proteins interact within the distinct phases of plasma membrane, forming complexes that are transient and dynamic. Membrane microdomains serve as privileged sites where signalling platforms are assembled leading to an optimal interaction between receptors and proximal signalling molecules.

Future experiments have to reveal the mechanisms how membrane peptidases as transmembrane proteins can associate with microdomains and which ligands trigger initiation of signalling pathways. Cell-specific multimeric complexes containing membrane peptidases have to be elucidated. There remains the question whether there exist differences between the signalling of membrane peptidases associated with rafts in comparison to caveolae. Despite the many and extensive descriptions of

these enzymes, our knowledge of the detailed functioning of membrane peptidases is still in its early stages.

ACKNOWLEDGEMENTS

We wish to thank Thomas Schlueter from the Biochemical Institute of the Otto-von-Guericke University Magdeburg who contributed to the calcium measurements with adherent cells. Furthermore, we are greatly indebted to Prof. Ruoslahti (La Jolla) for providing us with CNGRC peptide. We also thank Grit Helbing and Sandra Fuhrmann for technical assistance and Cornelius Hempel and Alexander Stange for help with figures.

REFERENCES

Albiston, A.L., McDowall, S.G., Matsacos, D., Sim, P., Clune, E., Mustafa, T., Lee, J., Mendelsohn, F.A., Simpson, R.J., Connolly, L.M., Chai, S.Y., 2001, Evidence that the angiotensin IV (AT4) receptor is the enzyme insulin regulated aminopeptidase. *J. Biol. Chem.* In press.

Anderson, R.G.W., 1993, Caveolae: Where incoming and outgoing messengers meet. *Proc. Natl. Acad. Sci. USA*, **90:** 10909-10913.

Anderson, R.G.W., 1998, The caveolae membrane system. *Ann. Rev. Biochem.* **67:** 199-225.

Angelisova, P., Drbal, K., Horejsi, V., Cerny, J., 1999 Association of CD10/neutral endopeptidase 24.11 with membrane microdomains rich in glycosylphosphatidylinositol-anchored proteins and lyn kinase. *Blood*, **93:** 1437-1439.

Ardouin, L., Boyer, C., Gillet, A., Trucy, J., Bernard, A.M., Nunes, J., Delon, J., Trautmann, A., He, H.T., Malissen, B., Malissen, M., 1999, Crippling of CD3-zeta ITAMs does not impair T cell receptor signaling. *Immunity* **10:** 409-420.

Arienti, G., Carlini, E., Verdicchi, R., Cosmi, E.V., Palmerini, C.A., 1997, Prostasome to sperm transfer of CD13/aminopeptidase N (EC 3.4.11.2). *Biochim. Biophys. Acta* **1336:** 533-538.

Baggiolini, M., 1998, Chemokines and leukocyte traffic. *Nature* **392:** 565-568.

Bauvois, B., 1988, A collagen-binding glycoprotein on the surface of mouse fibroblasts is identified as dipeptidyl peptidase IV. *Biochem. J.* **178:** 64-72.

Benting, J.H., Rietveld, A.G., Simons, K., 1999, N-Glycans mediate the apical sorting of a GPI-anchored, raft-associated protein in Madin-Darby canine kidney cells. *J. Cell. Biol.* **146:** 313-320.

Bermpohl, F., Löster, K., Reutter, W., Baum, O., 1998, Rat dipeptidyl peptidase IV (DPPIV) exhibits endopeptidase activity with specificity for denaturated fibrillar collagens. *FEBS Lett.* **428:** 152-166.

Brown, D.A., London, E., 1998, Function of lipid rafts in biological membranes. *Ann. Rev. Cell. Dev. Biol.* **14:** 111-136.

Cheng, H.C., Abdel-Ghany, M., Elble, R.C., Pauli, B.U., 1998, Lung endothelial dipeptidyl peptidase IV promotes adhesion and metastasis of rat breast cancer cells via tumor cell surface-associated fibronectin. *J. Biol. Chem.* **273:** 24207-24215.

Cuenda, A., Rouse, J., Doza, Y.N., Meier, R., Cohen, P., Gallagher, T.F., Young, P.R., Lee, J.C., 1995, SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. *FEBS Lett.* **364:** 229-233.

Dang, N.H., Torimoto, Y., Shimamura, K., Tanaka, T., Daley, J.F., Schlossman, S.F., Morimoto, C., 1991, 1F7 (CD26): A marker of thymic maturation involved in the differential regulation of the CD3 and CD2 pathways of human thymocyte activation. *J. Immunol.* **147:** 2825-2832.

Danielsen, E.M., 1995, Involvement of detergent-insoluble complexes in the intracellular transport of intestinal brush border enzymes. *Biochemistry*, **34:** 1596-1605.

Danielsen, E.M., van Deurs, B., 1997, Galectin-4 and small intestinal brush border enzymes form clusters. *Mol. Biol. Cell.* **8**: 2241-2251.

Deans, J.P., Robbins, S.M., Polyak, M.J., Savage, J.A., 1998, Rapid redistribution of CD20 to a low density detergent-insoluble membrane compartment. *J. Biol. Chem.* **273:** 344-348.

Delmas, B., Gelfi, J., L'Haridon, R., Vogel, L.K., Sjöström, H., Noren, O., Laude, H., 1992, Aminopeptidase N is a major receptor for the entero-pathogenic coronavirus TGEV. *Nature* **357:** 417-420.

De Meester, I., Durinx, C., Bal G., Proost, P., Struyf, S., Goossens, F., Augustyns, K., Scharpe, S., 2000, Natural substrates of dipeptidyl peptidase IV. *Adv. Exp. Med. Biol.* **477:** 67-87.

Dudley, D.T., Pang, L., Decker, S.J., Bridges, A.J., Saltiel, A.R., 1995, A synthetic inhibitor of the mitogen-activated protein kinase cascade. *Proc. Natl. Acad. Sci. USA*, **92:** 7686-7689.

Ehrlich, B.E., Kaftan, E., Bezprozvannaya, S., Bezprozvanny, I., 1994, The pharmacology of intracellular Ca(2+)-release channels. *Trends Pharmacol. Sci.* **15:** 145-149.

Field, K.A., Holowka, D., Baird, B., 1997, Compartmentalized activation of the high affinity immunoglobulin E receptor within membrane domains. *J. Biol. Chem.* **272:** 4276-4280.

Franco, R., Valenzuela, A., Lluis, C., Blanco, J., 1998, Enzymatic and extraenzymatic role of ecto-adenosine deaminase in lymphocytes. *Immunol. Rev.* **161:** 27-42.

Gaetaniello, L., Fiore, M., DeFilippo, S., Pozzi, N., Tamasi, S., Piguata, C., 1998, Occupancy of dipeptidyl peptidase IV activates an associated tyrosine kinase and triggers an apoptotic signal in human carcinoma cells. *Hepatology*, **25:** 934-942.

Ganju, R.K., Shpektor, R.G., Brenner, D.G., Shipp, M.A., 1996, CD10/neutral endopeptidase 24.11 is phosphorylated by casein kinase II and coassociates with other phosphoproteins including the lyn src-related kinase. *Blood*, **88:** 4159-4165.

Ganju, R.K., Sunday, M., Tsarwhas, D.G., Card, A., Shipp, M.A., 1994, CD10/NEP in non-small cell lung carcinomas. Relationship to cellular proliferation. *J. Clin. Inves.* **94:** 1784-1791.

Garcia, M., Mirre, C., Quadroni, A., Reggio, H., Le Bivic, A., 1993, GPI-anchored proteins associate to form microdomains during their intracellular transport in Caco-2 cells. *J. Cell. Sci.* **104:** 1281-1290.

George, S.G., Kenny, J., 1973, Studies on the enzymology of purified preparations of brush border from rabbit kidney. *Biochem. J.* **134:** 43-57.

Godar, S., Horejsi, V., Weidle, U.H., Binder, B.R., Hansmann, C., Stockinger, H., 1999, M6P/IGFII-receptor complexes urokinase receptor and plasminogen for activation of transforming growth factor-ß1. *Eur. J. Immunol.* **29:** 1004-1013.

Gonzalez-Gronow, M., Weber, M.R., Shearin, T.V., Gawdi, G., Pirle-Shepherd, S.R., Pizzo, S.V., 1998, Plasmin(ogen) carbohydrate chains mediate binding to dipeptidyl peptidase

IV(CD26) in rheumatoid arthritis human synovial fibroblasts. *Fibrinolysis Proteol.* **12:** 366-374.

Gonzalez-Gronow, M., Grenett, H.E., Weber, M.R., Gawdi, G., Pizzo, S.V., 2001, Interaction of plasminogen with dipeptidyl peptidase IV initiates a signal transduction mechanism which regulates expression of matrix metalloproteinase-9 by prostate cancer cells. *Biochem. J.* **355:** 397-407.

Gorrell, M.D., Gysbers, V., McCaughan, G.W., 2001, CD26: a multifunctional integral membrane and secreted protein of activated lymphocytes. *Scand. J. Immunol.* **54:** 249-264.

Guerin, S., Mari, B., Fernandez, E., Belacene, N., Toribio, M.L., Auberger, P., 1997, CD10 is expressed on human thymic epithelial cell lines and modulates thymopentin-induced cell proliferation. *FASEB J.* **11:** 1003-1011.

Guerra, B., Issinger, O.G., 1999, Protein kinase CK2 and its role in cellular proliferation, development and pathology. *Electrophoresis* **20:** 391-408.

Haasemann, M., Cartaud, J., Muller-Esterl, W., Dunia, I., 1998, Agonist-induced redistribution of bradykinin B2 receptor in caveolae. *J. Cell. Sci.* **111:** 917-928.

Hansen, G.H., Delmas, B., Besnardeau, L., Vogel, L.K., Laude, H., Sjöström, H., Noren, O., 1998, The coronavirus transmissible gastroenteritis virus causes infection after receptor-mediated endocytosis and acid-dependent fusion with an intracellular compartment. *J. Virol.* **72:** 527-534.

Harder, T., Simons, K., 1999, Clusters of glycolipid and glycosylphosphatidylinositol-anchored proteins in lymphoid cells: accumulation of actin regulated by local tyrosine phosphorylation. *Eur. J. Immunol.* **29:** 556-562.

Hegen, M., Niedobitek, G., Klein, C.E., Stein, H., Fleischer, B., 1990, The T cell triggering molecule Tp103 is associated with dipeptidyl aminopeptidase IV activity. *J. Immunol.* **144:** 2908-2914.

Hegen, M., Kameoka, J., Dong, R.P., Schlossman, S.F., Morimoto, C., 1997, Crosslinking of CD26 by antibody induces tyrosine phosphorylation and activation of mitogen-activated protein kinase. *Immunology*, **90:** 257-264.

Herrera, C., Morimoto, C., Blanco, J., Mallol, J., Arenzana, F., Lluis, C., Franco, R., 2001, Comodulation of CXCR4 and CD26 in human lymphocytes. *J. Biol. Chem.* **276:** 19532-19539.

Hoessli, D.C., Ilangumaran, S., Soltermann, A., Robinson, P.J., Borisch, B., Nasir-Ud-Din., 2000, Signaling through sphingolipid microdomains of the plasma membrane: the concept of signaling platform. *Glycoconj. J.* **17:** 191-197.

Hoffmann, T., Faust, J., Neubert, K., Ansorge, S., 1993, Dipeptidyl peptidase IV (CD26) and aminopeptidase N (CD13) catalyzed hydrolysis of cytokines and peptides with N-terminal cytokine sequences. *FEBS Lett.* **336:** 61-64.

Hooper, N.M., 1994, Families of zinc metalloproteinases. *FEBS Lett.* **354:** 1-6.

Horejsi, V., Drbal, K., Cebecauer, M., Cerny, J., Brdicka, T., Angelisova, P., Stockinger, H., 1999, GPI-microdomains: a role in signalling via immunoreceptors. *Immunol. Today* **20:** 356-361.

Huhn, J., Ehrlich, S., Fleischer, B., von Bonin, A., 2000, Molecular analysis of CD26-mediated signal transduction in T cells. *Immunol. Lett.***72:** 127-32.

Ilangumaran, S., Arni, S., Chicheportiche, Y., Briol, A., Hoessli, D.C., 1996, Evaluation by dot-immunoassay of the differential distribution of cell surface and intracellular proteins in glycosylphosphatidylinositol-rich plasma membrane domains. *Anal. Biochem.* **235:** 49-56.

Ilangumaran, S., Hoessli, D.C., 1999, Effects of cholesterol depletion by cyclodextrin on the sphingolipid microdomains of the plasma membrane. *Biochem. J.* **335:** 433-440.

Ilangumaran, S., He, H.T., Hoessli, D.C., 2000, Microdomains in lymphocyte signalling: beyond GPI-anchored proteins. *Immunol. Today* **21:** 2-7.

Ikushima, H., Munakata, Y., Ishii, T., Iwata, S., Terashima, M., Tanaka, H., Schlossman, S.F., Morimoto, C., 2000, Internalization of CD26 by mannose 6-phosphate/insulin-like growth factor II receptor contributes to T cell activation. *Proc. Natl. Acad. Sci. USA* **97:** 8439-8444.

Ishii, E., Greaves, A., Grunberger, T., Freedman, M.H., Letarte, M., 1995, Tumor formation by a human pre-B leukemia cell line in scid mice is enhanced by matrigel and is associated with induction of CD10 expression. *Leukemia* **9:** 175-184.

Ishii, T., Ohnuma, K., Murakami, A., Takasawa, N., Kobayashi, S., Dang, N.H., Schlossman, S.F., Morimoto, C., 2001, CD26-mediated signaling for T cell activation occurs in lipid rafts through its association with CD45RO. *Proc. Natl. Acad. Sci. USA,* **98:** 12138-12143.

Kähne, T., Neubert, K., Faust, J., Ansorge, S., 2000, Early phosphorylation events induced by DPIV/CD26-specific inhibitors. *Cell. Immunol.* **189:** 60-66.

Kähne, T., Lendeckel, U., Wrenger, S., Neubert, K., Ansorge, S., Reinhold, D., 1999, Dipeptidyl peptidase IV: a cell surface peptidase involved in regulating T cell growth (review). *Int. J. Mol. Med.* **4:** 3-15.

Kameoka, J., Tanaka, T., Nojima, Y., Schlossman, S.F., Morimoto, C., 1999, Direct association of adenosine deaminase with a T cell activation antigen, CD26. *Science* **261:** 466-469.

Keller, P., Simons, K., 1998, Cholesterol is required for surface transport of influenza virus hemagglutinin. *J. Cell Biol.* **140:** 1357-1367.

Kenny, A.J., Stephenson, .LS., Turner, A.J., '?87, Cell surface peptidases. In: Mammalian Ectoenzymes (Kenny A.J and Turner A.J, eds.); Amsterdam: *Elsevier Science Publishers*, pp. 169-210.

Kerr, M.A., Kenny, A.J., 1974, The purification and specificity of a neutral endopeptidase from rabbit kidney brush border. *Biochem. J.* **137:** 477-488.

Kertesz, Z., Linton, E.A., Redman, C.W., 2000, Adhesion molecules of syncytiotrophoblast microvillous membranes inhibit proliferation of human umbilical vein endothelial cells. *Placenta* **21:** 150-159.

Kohno, H., Kanno, T., 1985, Properties and activities of aminopeptidases in normal and mitogen-stimulated human lymphocytes. *Biochem. J.* **226:** 59-65.

Kokkonen, J.O., Kuoppala, A., Saarinen, J., Lindstedt, K.A., Kovanen, P.T., 1999, Kallidin- and bradykinin-degrading pathways in human heart: degradation of kallidin by aminopeptidase M-like activity and bradykinin by neutral endopeptidase. *Circulation*, **99:** 1984-1990.

Koziak, K., Kaczmarek, E., Kittel, A, Sevigny, J., Blusztajn, J.K., Schulte Am Esch, J. 2nd, Imai, M., Guckelberger, O., Goepfert, C., Qawi, I., Robson, S.C., 2000, Palmitoylation targets CD39/endothelial ATP diphosphohydrolase to caveolae. *J. Biol. Chem.* **275:** 2057-2062.

Kumano, N., Sugawara, S., 1992, Ubenimex (Bestatin), an aminopeptidase inhibitor, modulates protein kinase C in K562 cells. *J. Biol. Regul. Homeost. Agents* **6:** 116-120.

Larsen, S.L., Pedersen, L.O., Buus, S., Stryhn, A., 1996, T cell responses affected by aminopeptidase N (CD13)-mediated trimming of major histocompatibility complex class II-bound peptides. *J. Exp. Med.* **184:** 183-189.

LeBien, T.W., McCormack, R.T., 1989, The common acute lymphoblastic leukemia antigen (CD10)--emancipation from a functional enigma. *Blood* **73:** 625-635.

Lee, V.H., 2000, Membrane transporters. *Eur. J. Pharm. Sci.* **11:** S41-50.

Lendeckel, U., Kaehne, T., Arndt, M., Frank, K., Ansorge, S., 1998, Inhibition of alanyl aminopeptidase induces MAP-kinase p42/ERK2 in the human T cell line KARPAS-299. *Biochem. Biophys. Res. Commun.* **252:** 5-9.

Lendeckel, U., Arndt, M., Frank, K., Wex, T., Ansorge, S., 1999, Role of alanyl aminopeptidase in growth and function of human T cells (review). *Int. J. Mol. Med.* **4:** 17-27.

Liu, P., Ying, Y., Anderson, R.G., 1997, Platelet-derived growth factor activates mitogen-activated protein kinase in isolated caveolae. *Proc. Natl. Acad. Sci. USA* **94:** 13666-13670.

Löhn, M., Mueller, C., Thiele, K., Kähne, T., Riemann, D., Langner, J., 1997, Aminopeptidase N-mediated signal transduction and inhibition of proliferation of human myeloid cells. *Adv. Exp. Med. Biol.* **421**: 85-91.

Löhn, M., Mueller, C., Langner, J., 2001, Cell cycle retardation induced by aminopeptidase N (CD13). *Leukemia Lymphoma* in press

Löster, K., Zeilinger, K., Schuppan, D., Reutter, W., 1995, The cysteine-rich region of dipeptidyl peptidase IV/CD26 is the collagen-binding site. *Biochem. Biophys. Res. Commun.* **217:** 341-348.

Louvard, D., Maroux, S., Baratti, J., Desnuelle, P., 1973, On the distribution of enterokinase in porcine intestine and on ist subcellular localization. *Biochim. Biophys. Acta.* **309:** 127-137.

Lu, B., Gerard, N.P., Kolakowski, L.F. Jr, Finco, O., Carroll, M.C., Gerard, C., 1996, Neutral endopeptidase modulates septic shock. *Ann. N. Y. Acad. Sci.* **780:** 156-163.

MacIntyre, E.A., Jones, H.M., Roberts, P.J., Tidman, N., Linch, D.C., 1987, Identification of signal transduction molecules on monocytic cells. In: Leucocyte Typing III: White cell differentiation antigens. McMichael AJ, Beverly PCL, Cobbold S and Crumpton MJ. Eds. *Oxford University Press*, pp. 685-688.

MacIntyre, E.A., Roberts, P.J., Jones, M., Van der Schoot, C.E., Favalaro, E.J., Tidman, N., Linch, D.C., 1989, Activation of human monocytes occurs on crosslinking monocytic antigens to an Fc receptor. *J. Immunol.* **142:** 2377-83.

Madueno, J.A., Munoz, E., Blazquez, V., Conzalez, R., Aparicio, P., Pena, J., 1993, The CD26 Antigen is coupled to protein tyrosine phosphorylation and implicated in CD16-mediated lysis in natural killer cells. *Scand. J. Immunol.* **37:** 425-429.

Mak, D.O., McBride, S., Foskett, J.K., 1998, Inositol 1,4,5-trisphosphate activation of inositol trisphosphate [correction of tris-phosphate] receptor Ca^{2+} channel by ligand tuning of Ca^{2+} inhibition. *Proc. Natl. Acad. Sci. USA*, **95:** 15821-15825.

Malfroy, B., Kuang W.J., Seeburg, P.H., Mason, A.J., Schofield, P.R., 1988, Molecular cloning and amino acid sequence of human enkephalinase (neutral endopeptidase). *FEBS Lett.* **229:** 206-210.

Martin, M., Huguet, J., Centelles, J.J., Franco, R., 1995, Expression of ecto-adenosine deaminase and CD26 in human T cells triggered by the TCR-CD3 complex. Possible role of adenosine deaminase as costimulatory molecule. *J. Immunol.* **155:** 4630-4643.

Matsas, R., Stephenson, S.L., Hryszko, J., Kenny, AJ., Turner, A.J., 1985, The metabolism of neuropeptides. Phase separation of synaptic membrane preparations with Triton X-114 reveals the presence of aminopeptidase N. *Biochem. J.* **231:** 445-449.

Mentlein, R., 1999, Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regulatory Peptides* **85:** 9-24.

Miller, B. C., Ackroyd, A., Hersh, L.B., Cottam, G.L., 1994, Methionine enkephalin metabolism by murine macrophage ectopeptidase(s). *Regul. Pept.* **50:** 87-98.

Milligan, G., Parenti, M., Magee, A.I., 1995, The dynamic role of palmitoylation in signal transduction. *Trends Biochem. Sci.* **20:** 181-187.

Mineo, C., Anderson, R.G., 2001, Potocytosis. *Histochem. Cell Biol.* **116:** 109-118.

Morimoto, C., Schlossman, S.F., 1998, The structure and function of CD26 in the T-cell immune response. *Immunol. Rev.* **161:** 55-70.

Mueller, S.C., Ghersi, G., Akiyama, S.K., Sang, Q.X., Howard, L., Pineiro-Sanchez, M., Nakahara, H., Yeh, Y., Chen, W.T., 1999, A novel protease-docking function of integrin at invadopodia. *J. Biol. Chem.* **274:** 24947-24952.

Munoz, E., Blazquez, M.V., Madueno, J.A., Rubio, G., Pena, J., 1992, CD26 induces T-cell proliferation by tyrosine protein phosphorylation. *Immunology* **77:** 43-50.

Murata, M., Kubota, Y., Tanaka, T., Iida-Tanaka, K., Takahara, J., Irino, S., 1994, Effect of ubenimex on the proliferation and differentiation of U937 human histiocytic lymphoma cells. *Leukemia* **8:** 2188-2193.

Nakanishi, S., Yano, H., Matsuda, Y., 1995, Novel functions of phosphatidylinositol 3-kinase in terminally differentiated cells. *Cell Signal*, 7: 545-57.

Navarrete Santos, A., Langner, J., Herrmann, M., Riemann, D., 2000a, Aminopeptidase N/CD13 is directly linked to signal transduction pathways in monocytes. *Cell. Immunol.* **200:** 22-32.

Navarrete Santos, A., Langner, J., Riemann, D., 2000b, Enzymatic activity is not a precondition for the intracellular calcium increase mediated by mAbs specific for aminopeptidase N/CD13. *Adv. Exp. Med. Biol.* **477:** 43-47.

Navarrete Santos, A., Röntsch, J., Danielsen, E. M., Langner, J., Riemann, D., 2000c, Aminopeptidase N/CD13 is associated with raft membrane microdomains in monocytes. *Biochem. Biophys. Res. Commun.* **269:** 143-148.

Navarrete Santos, A., Danielsen, M., Riemann, D., 2001, CD13-specific mAbs induce calcium signals in fibroblast-like synoviocytes. *Signal Transduction* **1:** 67.

Nykjaer, A., Christensen, E.I., Vorum, H., Hager, H., Petersen, C.M., Roigaard, H., Min, H.Y., Vilhardt, F., Moller, L.B., Kornfeld, S., Gliemann, J., 1998, Mannose 6-phosphate/insulin-like growth factor-II receptor targets the urokinase receptor to lysosomes via a novel binding interaction. *J. Cell. Biol.* **141:** 815-828.

Oefner, C., D'Arcy, A., Hennig, M., Winkler, F.K., Dale, G.E., 2000, Structure of human neutral endopeptidase (Neprilysin) complexed with phosphoramidon. *J. Mol. Biol.* **296:** 341-349.

Oh, P., Schnitzer, J.E., 2001, Segregation of heterotrimeric G proteins in cell surface microdomains. G(q) binds caveolin to concentrate in caveolae, whereas G(i) and G(s) target lipid rafts by default. *Mol. Biol. Cell.* **12:** 685-698.

Okamoto, T., Schlegel, A., Scherer, P.E., Lisanti, M.P., 2001, Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. *J. Biol. Chem.* **273:** 5419-5422.

Oliferenko, S., Paiha, K., Harder, T., Gerke, V., Schwarzler, C., Schwarz, H., Beug, H., Gunthert, U., Huber, L.A., 1999, Analysis of CD44-containing lipid rafts: Recruitment of annexin II and stabilization by the actin cytoskeleton. *J. Cell. Biol.* **146:** 843-854.

Olsen, J., Cowell, G.M., Konigshofer, E., Danielsen, E.M., Moller, J., Laustsen, L., Hansen, O.C., Welinder, K.G., Engberg, J., Hunziker, W., Spies, M., Sjöström, H., Noren, O., 1988, Complete amino acid sequence of human intestinal aminopeptidase N as deduced from cloned cDNA. *FEBS Lett.* **238:** 307-314.

Palade GE, 1953, The fine structure of blood capillaries. *J. Appl. Phys.* **24:** 1424.

Palmieri, F. E., Bausback, H.H., Ward, P.E., 1989, Metabolism of vasoactive peptides by vascular endothelium and smooth muscle aminopeptidase M. *Biochem. Pharmacol.* **38:** 173-180.

Papandreou, C.N., Usmani, B., Geng, Y., Bogenrieder, T., Freeman, R., Wilk, S., Finstad, C.L., Reuter, V.E., Powell, C.T., Scheinberg, D., Magill, C., Scher, H.I., Albino, A.P., Nanus, D.M., 1998, Neutral endopeptidase 24.11 loss in metastatic human prostate cancer contributes to androgen-independent progression. *Nat. Med.* **4:** 50-57.

Parker, I., Ivorra, I., 1991, Caffeine inhibits inositol trisphosphate-mediated liberation of intracellular calcium in Xenopus oocytes. *J. Physiol.* **433:** 229-240.

Pasqualini, R., Koivunen, E., Kain, R., Lahdenranta, J., Sakamoto, M., Stryhn, A., Ashmun, R.A., Shapiro, L.H., Arap, W., Ruoslahti, E., 2000, Aminopeptidase N is a receptor for tumor-homing peptides and a target for inhibiting angiogenesis. *Cancer Res.* **60**: 722-727.

Pawson, T., Scott, J.D., 1997, Signaling through scaffold, anchoring, and adaptor proteins. *Science* **278:** 2075-2080.

Pethiyagoda, C.L., Welch, D.R., Fleming, T.P., 2000, Dipeptidyl peptidase IV (DPPIV) inhibits cellular invasion of melanoma cells. *Clin. Exp. Metastasis* **18:** 391-400.

Phillips, A.J., Tomasec, P., Wang, E.C., Wilkinson, G.W., Borysiewicz, L.K., 1998, Human cytomegalovirus infection downregulates expression of the cellular aminopeptidases CD10 and CD13. *Virology* **250:** 350-358.

Reinhold, D., Bank, U., Bühling, F., Täger, M., Born, I., Faust, J., Neubert, K., Ansorge, S., 1997a, Inhibitors of dipeptidyl peptidase IV (DP IV, CD26) induces secretion of transforming growth factor-beta 1 (TGF-beta 1) in stimulated mouse splenocytes and thymocytes. *Immunol. Lett.* **58:** 29-35.

Reinhold, D., Kähne, T., Täger, M., Lendeckel, U., Bühling, F., Bank, U., Wrenger, S., Faust, J., Neubert, K., Ansorge, S., 1997b, The effect of anti-CD26 antibodies on DNA synthesis and cytokine production (IL-2, IL-10 and IFN-gamma) depends on enzymatic activity of DP IV/CD26. *Adv. Exp. Med. Biol.* **421:** 149-155.

Renneberg, H., Albrecht, M., Kurek, R., Krause, E., Lottspeich, F., Aumuller, G., Wilhelm, B., 2001, Identification and characterization of neutral endopeptidase (EC 3. 4. 24. 11) from human prostasomes--localization in prostatic tissue and cell lines. *Prostate* **46:** 173-183.

Rettig, W.J., Garin-Chesa, P., Healey, J.H., Su, S.L., Ozer, H.L., Schwab, M., Albino, A.P., Old, L.J., 1993, Regulation and heteromeric structure of the fibroblast activation protein in normal and transformed cells of mesenchymal and neuroectodermal origin. *Cancer Res.* **53:** 3327-3335.

Riemann, D., Schwachula, A., Hentschel, M., Langner, J., 1993, Demonstration of CD13/aminopeptidase N on synovial fluid T cells from patients with different forms of joint effusions. *Immunobiology*, **187**: 24-35.

Riemann, D., Göhring, B., Langner, J., 1994, Expression of aminopeptidase N/CD13 in tumour-infiltrating lymphocytes from human renal cell carcinoma. *Immunol. Letters*, **42**: 19-23.

Riemann, D., Kehlen, A., Langner, J., 1999, CD13 – not just a marker in leukemia typing. *Immunol. Today*, **20**: 83-88.

Riemann, D., Hansen, G.H., Niels-Christiansen, L.-L., Thorsen, E, Immerdal, L., Navarrete Santos, A., Kehlen, A:, Langer, J., Danielsen, M., 2001, Caveolae/lipid rafts in fibroblast-like synoviocytes: Ectopeptidase-rich membrane microdomains. *Biochem. J.* **354:** 47-55

Robinson, P.J., 1991, Phosphatidylinositol membrane anchors and T-cell activation. *Immunol. Today* **12:** 35-41.

Roettger, B.F., Rentsch, R.U., Pinon, D., Holicky, E., Hadac, E., Larkin, J.M., Miller, L.J., 1995, Dual pathways of internalization of the cholecystokinin receptor. *J. Cell. Biol.* **128:** 1029-1041.

Rothberg, K.G., Ying, Y.S., Kamen, B.A., Anderson, R.G., 1990, Cholesterol controls the clustering of the glycophospholipid-anchored membrane receptor for 5-methyltetrahydrofolate. *J. Cell. Biol.* **111:** 2931-2938.

Rothberg, K.G., Heuser, J.E., Donzell, W.C., Ying, Y.S., Glenney, J.R., Anderson, R.G.W., 1992, Caveolin, a protein component of caveolae membrane coats. *Cell*, **68:** 673-682.

Sanderink, G.J., Artur, Y., Schiele, F., Gueguen, R., Siest, G., 1998, Alanine aminopeptidase in serum: biological variations and reference limits. *Clin. Chem.* **34:** 1422-1426.

Schlegel, A., Lisanti, M.P., 2001, The caveolin triad: caveolae biogenesis, cholesterol trafficking, and signal transduction. *Cytokine Growth Factor Rev.* **12:** 41-51.

Schön, E., Mansfeld, H.W., Demuth, H.U., Barth, A., Ansorge, S., 1985, The dipeptidyl peptidase IV, a membrane enzyme involved in the proliferation of T lymphocytes. *Biomed. Biochim. Acta* **44:** K9-15.

Schön, E., Eichmann, E., Grunow, R., Jahn, S., Kiessig, S.T., Volk, H., Ansorge, S., 1986, Dipeptidyl peptidase IV in human T lymphocytes. An approach to the role of a membrane peptidase in the immune system. *Biomed. Biochem. Acta* **45:** 1523-1528.

Schrimpf, S.P., Hellman, U., Carlsson, L., Larsson, A., Ronquist, G., Nilsson, B.O., Identification of dipeptidyl peptidase IV as the antigen of a monoclonal anti-prostasome antibody. *Prostate* **38:** 35-39.

Sevinsky, J.R., Rao, L.V.M., Ruf, W., 1996, Ligand-induced protease receptor translocation into caveolae: A mechanism for regulating cell surface proteolysis of the tissue factor-dependent coagulation pathway. *J. Cell. Biol.* **133:** 293-304.

Shipp, M.A., Tarr, G.E., Chen, C.Y., Switzer, S.N., Hersh, L.B., Stein, H., Sunday, M.E., Reinherz, E.L., 1991, CD10/neutral endopeptidase 24.11 hydrolyzes bombesin-like peptides and regulates the growth of small cell carcinomas of the lung. *Proc. Natl. Acad. Sci. USA*, **88:** 10662-10666.

Shirotani, K., Tsubuki, S., Iwata, N., Takaki, Y., Harigaya, W., Maruyama, K., Kiryu-Seo, S., Kiyama, H., Iwata, H., Tomita, T., Iwatsubo, T., Saido, T.C., 2001, Neprilysin degrades both amyloid beta peptides 1-40 and 1-42 most rapidly and efficiently among thiorphan- and phosphoramidon-sensitive endopeptidases. *J. Biol. Chem.* **276:** 21895-21901.

Siegbahn, A., 2000, Cellular consequences upon factor VIIa binding to tissue factor. *Haemostasis* **30 S2:** 41-47.

Simons, K., Ikonen, E., 1997, Functional rafts in cell membranes. *Nature*, **387:** 569-572.

Simons, K., Toomre, D., 2000, Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell. Biol.* **1:** 31-39.

Söderberg, C., Giugni, T.D., Zaia, J.A., Larsson, S., Wahlberg, J.M., Möller, E., 1993, CD13 (human aminopeptidase N) mediates human cytomegalovirus infection. *J. Virol. 67:* 6576-6585.

Sowa, G., Pypaert, M., Sessa, W.C., Distinction between signaling mechanisms in lipid rafts vs. caveolae. *Proc. Natl. Acad. Sci. USA* **98:** 14072-14077.

Stahl, A., Mueller, B.M., 1995, The urokinase-type plasminogen activator receptor, a GPI-linked protein, is localized in caveolae. *J. Cell. Biol.* **129**: 335-344.

Stan, R.V., Roberts, W.G., Predescu, D., Ihida, K., Saucan, L., Ghitescu, L., Palade, G.E., 1997, Immunoisolation and partial characterization of endothelial plasmalemmal vesicles (caveolae). *Mol. Biol. Cell* **8:** 595-605.

Steeg, C., Hartwig, U., Fleischer, B., 1995, Unchanged signaling capacity of mutant CD26/dipeptidylpeptidase IV molecules devoid of enzymatic activity. *Cell. Immunol.* **164:** 311-315.

Stefanova, I., Horejsi, V., Ansotegui, I.J., Knapp, W., Stockinger, H., 1991, GPI-anchored cell-surface molecules complexed to protein tyrosine kinases. *Science* **254:** 1016-1019.

Strohmeier, G.R., Lencer, W.I., Patapoff, T.W., Thompson, L.F., Carlson, S.L., Moe, S.J., Carnes, D.K., Mrsny, R.J., Madara, J.L., 1997, Surface expression, polarization, and functional significance of CD73 in human intestinal epithelia. *J. Clin. Invest.* **99:** 2588-2601.

Subramanyam, M., Gutheil, W.G., Bachovchin, W.W., Huber, B.T., 1993, Mechanism of HIV-1 Tat induced inhibition of antigen-specific T cell responsiveness. *J. Immunol.* **150:** 2544-2553.

Sumitomo, M., Shen, R., Walburg, M., Dai, J., Geng, Y., Navarro, D., Boileau, G., Papandreou, C.N., Giancotti, F.G., Knudsen, B., Nanus, D.M., 2000, Neutral endopeptidase inhibits prostate cancer cell migration by blocking focal adhesion kinase signaling. *J. Clin. Invest.* **106:** 1399-1407.

Tanaka, T., Kameoka, J., Yaron, A., Schlossman, S.F., Morimoto, C., 1993, The costimulatory activity of the CD26 antigen requires dipeptidyl peptidase IV enzymatic activity. *Proc. Natl. Acad. Sci. USA* **90:** 4586-4590.

Tanaka, T., Duke-Cohan, J.S., Kameoka, J., Yaron, A., Lee, I., Schlossman, S.F., Morimoto, C., 1994, Enhancement of antigen-induced T-cell proliferation by soluble CD26/dipeptidyl peptidase IV. *Proc. Natl. Acad. Sci. USA* **91:** 3082-3086.

Tani, K., Ogushi, F., Huang, L., Kawano, T., Tada, H., Hariguchi, N., Sone, S., 2000, CD13/aminopeptidase N, a novel chemoattractant for T lymphocytes in pulmonary sarcoidosis. *Am. J. Respir. Crit. Care Med.* **161:** 1636-1642.

Torimoto, Y., Dang, N.H., Vivier, E., Tanaka, T., Schlossman, S.F., Morimoto, C., 1991, Coassociation of CD26 (dipeptidyl peptidase IV) with CD45 on the surface of human T lymphocytes. *J. Immunol.* **147:** 2514-2517.

Turner, A.J., 1998, Membrane alanyl aminopeptidase. In *Handbook of proteolytic enzymes* (A. J. Barrett, N. D. Rawlings, and J. F. Woessner eds.), Academic Press, London, pp.996-1000.

Turner, A.J., Isaac, R.E., Coates, D., 2001, The neprilysin (NEP) family of zinc metalloendopeptidases: genomics and function. *Bioessays* **23:** 261-269.

Vlahos, C.J., Matter, W.F., Hui, K.Y., Brown, R.F., 1994, A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J. Biol. Chem.* **269**: 5241-5248.

Von Bonin, A., Huhn, J., Fleischer, B., 1998, Dipeptidyl-peptidase IV/CD26 on T cells: analysis of an alternative T-cell activation pathway. *Immunol. Rev.* **161**: 43-53.

Wasano, K., Hirakawa, Y., 1999, Two domains of rat galectin-4 bind to distinct structures of the intercellular borders of colorectal epithelia. *J. Histochem. Cytochem.* **47:** 75-82.

Weber, M., Uguccioni, M., Baggiolini, M., Clark-Lewis, I., and Dahinden, C.A., 1996, Deletion of the NH_2-terminal residue converts monocyte chemotactic protein 1 from an activator of basophil mediator release to an eosinophil chemoattractant. *J. Exp. Med.* **183**: 681-5.

Wex, T., Lendeckel, U., Reinhold, D., Kahne, T., Arndt, M., Frank, K., Ansorge, S., 1997, Antisense-mediated inhibition of aminopeptidase N (CD13) markedly decreases growth rates of hematopoietic tumour cells. *Adv. Exp. Med. Biol.* **421**: 67-73.

Wrenger, S., Hoffmann, T., Faust, J., Mrestani-Klaus, C., Brandt, W., Neubert, K., Kraft, M., Olek, S., Frank, R., Ansorge, S., Reinhold, D., 1997, The N-terminal structure of HIV-1 Tat is required for suppression of CD26-dependent T cell growth. *J. Biol. Chem.* **272:** 30283-30288.

Wrenger, S., Faust, J., Mrestani-Klaus, C., Fengler, A., Stockel-Maschek, A., Lorey, S., Kähne, T., Brandt, W., Neubert, K., Ansorge, S., Reinhold, D., 2000, Down-regulation of T cell activation following inhibition of dipeptidyl peptidase IV/CD26 by the N-terminal part of the thromboxane A2 receptor. *J. Biol. Chem.* **275:** 22180-22186.

Xavier, R., Brennan, T., Li, Q., McCormack, C., Seed, B., 1998, Membrane compartmentation is required for efficient T cell activation. *Immunity* **8:** 723-732.

Xu, Y., Wellner, D., Scheinberg, D.A., 1995, Substance P and bradykinin are natural inhibitors of CD13/aminopeptidase N. *Biochem. Biophys. Res. Commun.* **208**: 664-674.

Yeager, C.L., Ashmun, R.A., Williams, R.K., Cardellichio, C.B., Shapiro, L.H., Look, A.T., Holmes, K.V., 1992, Human aminopeptidase N is a receptor for human coronavirus 229E. *Nature* **357**: 420-422.

Yurochko, A.D., Huang, E.S., 1999, Human cytomegalovirus binding to human monocytes induces immunoregulatory gene expression. *J. Immunol.* **162:** 4806-4816.

Zachary, I., Rozengurt, E., 1992, Focal adhesion kinase (p125FAK): a point of convergence in the action of neuropeptides, integrins, and oncogenes. *Cell* **71:** 891-894.