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REVIEW

Signal transduction by protease-activated receptors

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The family of G protein-coupled receptors (GPCRs) constitutes the largest class of signalling receptors in the human genome, controlling vast physiological responses and are the target of many drugs. After activation, GPCRs are rapidly desensitized by phosphorylation and β-arrestin binding. Most classic GPCRs are internalized through a clathrin, dynamin and β-arrestin-dependent pathway and then recycled back to the cell surface or sorted to lysosomes for degradation. Given the vast number and diversity of GPCRs, different mechanisms are likely to exist to precisely regulate the magnitude, duration and spatial aspects of receptor signalling. The G protein-coupled protease-activated receptors (PARs) provide elegant examples of GPCRs that are regulated by distinct desensitization and endocytic sorting mechanisms, processes that are critically important for the spatial and temporal fidelity of PAR signalling. PARs are irreversibly activated through proteolytic cleavage and transmit cellular responses to extracellular proteases. Activated PAR_1 internalizes through a clathrin- and dynamin-dependent pathway independent of β-arrestins. Interestingly, PAR_1 is basally ubiquitinated and deubiquitinated after activation and traffics from endosomes to lysosomes independent of ubiquitination. In contrast, β-arrestins mediate activated PAR_2 internalization and function as scaffolds that promote signalling from endocytic vesicles. Moreover, activated PAR_2 is modified with ubiquitin, which facilitates lysosomal degradation. Activated PARs also adopt distinct active conformations that signal to diverse effectors and are likely regulated by different mechanisms. Thus, the identification of the molecular machinery important for PAR signal regulation will enable the development of new strategies to manipulate receptor signalling and will provide novel targets for the development of drugs.
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Abbreviations: AP-2, adaptor protein complex-2; APC, activated protein C; EPCR, endothelial protein C receptor; ERK1, 2, extracellular signal-regulated kinases 1 and 2; ESCRT, endosomal-sorting complex required for transport; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; HRS, hepatocyte growth factor-regulated tyrosine kinase substrate; ILVs, intraluminal vesicles; MVBs, multivesicular bodies; PAR, protease-activated receptor; PKC, protein kinase C; Tsg101, tumour suppressor gene 101; VEGF, vascular endothelial growth factor

The family of protease-activated receptors (PARs) is comprised of four members: PAR_1, PAR_2, PAR_3 and PAR_4 (Alexander *et al.*, 2008). PARs are G protein-coupled receptors (GPCRs) that are uniquely activated by proteolysis. PARs are expressed predominantly in vascular, immune and epithelial cells, astrocytes and neurons and transmit cellular responses to coagulant proteases as well as other proteases expressed in distinct tissues (Coughlin, 2005; Russo *et al.*, 2009b). PARs are critical mediators of haemostasis, thrombosis, inflammation, and have been implicated in cancer progression, making this receptor class an important drug target. Several recent studies suggest that different agonists, proteases and synthetic peptide ligands, elicit distinct signalling responses through the activation of the same PAR. The observation that different ligands acting at the same receptor stimulate distinct signalling responses is best characterized for GPCRs and is a process termed 'functional selectivity' or 'biased agonism' (Urban *et al.*, 2007). The mechanism responsible for agonist-induced biased agonism at PARs appears to involve stabilization of distinct active receptor conformations that may be facilitated by receptor compartmentalization in plasma membrane microdomains.

The ability of different agonists to distinctly activate PAR signalling presumably by stabilization of distinct active receptor conformations raises the question of whether a single or multiple processes of signal termination exist for distinctly activated PARs. Similar to other GPCRs, activated PARs are rapidly desensitized by phosphorylation and β-arrestin binding. In addition to rapid desensitization, PAR trafficking is crucial for the temporal and spatial control of receptor

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signalling. The mechanisms responsible for endocytic trafficking of PARs remain poorly understood. Moreover, the efficiency with which PARs are degraded makes this receptor class an excellent model system to investigate the molecular basis of GPCR lysosomal degradation. PAR_2, like many GPCRs, is modified with ubiquitin, which facilitates lysosomal trafficking through the endosomal-sorting complex required for transport (ESCRT) pathway (Jacob *et al.*, 2005; Hasdemir *et al.*, 2007). We recently discovered that activated PAR_1 traffics from endosomes to lysosomes independent of ubiquitination and the ubiquitin-binding components of the ESCRT machinery (Gullapalli *et al.*, 2006; Wolfe *et al.*, 2007). The molecular basis of the novel ubiquitin-independent lysosomal sorting of PAR_1 as well as the identities of the adaptor molecules and processes that control endocytic trafficking remains largely unknown. Here, we discuss the mechanisms of PAR activation, signalling, desensitization, resensitization, endocytic trafficking and crosstalk with other receptors.

PAR activation and signalling

Activation of PARs occurs through an irreversible proteolytic mechanism. Proteases bind to and cleave the extracellular N-terminal domain of PARs at specific sites to unmask a new N-terminus that acts as a tethered ligand that binds to the receptor and triggers intracellular signalling (Figure 1) (Vu *et al.*, 1991a,b). Synthetic peptides that mimic the first six amino acids of the newly formed N-terminus can activate PARs independent of protease and receptor cleavage (Scarborough *et al.*, 1992; Chen *et al.*, 1994; Lerner *et al.*, 1996), except for PAR_3, which is unresponsive to synthetic peptide agonists (Nakanishi-Matsui *et al.*, 2000). After protease cleavage, the N-terminal 41-amino-acid domain of PAR_1 is released into the extracellular milieu and may exert some biological activity, but this remains controversial. Recent work suggests that the released N-terminal region of PAR_1 containing an N-terminal hydrophobic domain and dubbed 'Parstatin', inhibits vascular endothelial cell growth factor (VEGF) and fibroblast growth factor-induced angiogenesis *in vitro* and *in vivo* by regulating intracellular signalling events (Zania *et al.*, 2009). Previous studies have also reported effects of the released N-terminus of PAR_1 at the platelet cell surface that appears to modulate platelet function (Furman *et al.*, 2000a,b). The mechanism by which the cleaved-off N-terminal domain of PAR_1 exerts its effects on cells remains unclear and whether the released N-terminal extracellular regions of other PARs also have biological activities has not been determined.

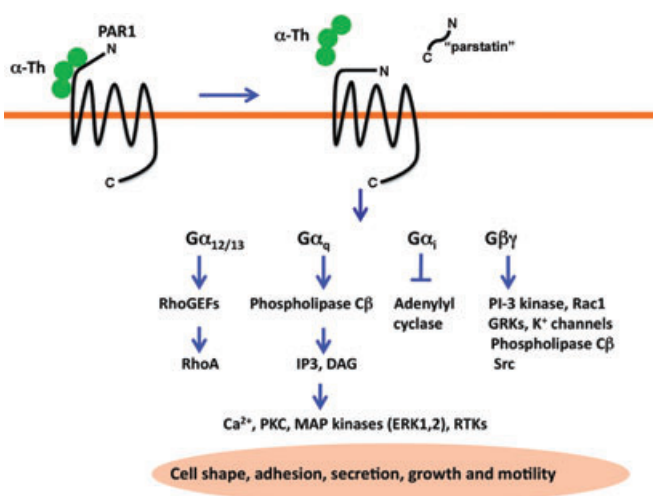

Figure 1 Activation and signalling by protease-activated receptor-1 (PAR_1). PAR_1 is a seven transmembrane G protein-coupled receptor that is irreversibly proteolytically activated by thrombin. Thrombin binds to and cleaves the N-terminus of PAR_1, generating a new N-terminal domain that binds intramolecularly to trigger transmembrane signalling. Recent studies suggest that the cleaved N-terminal domain of PAR_1 is released and exhibits biological activity in certain settings and has been termed 'parstatin'. Activated PAR_1 couples to multiple heterotrimeric G protein subtypes including $G_{12}/_{13}$, G_q and G_i and activates a variety of signalling effectors important for inducing cell shape changes, adhesion molecule expression, secretion of vasoactive factors, cellular growth and motility. The mechanisms that specify PAR_1 coupling to distinct G protein subtypes is not known.

The ligand activation of PARs is likely to induce conformational changes within the transmembrane helices that expose receptor cytoplasmic surfaces important for interaction with the α subunits of heterotrimeric G proteins at the inner leaflet of the plasma membrane (Oldham and Hamm, 2007; 2008). The activation of PARs is thought to occur through peptide ligand interactions with residues residing in the second extracellular loop (Gerszten *et al.*, 1994), unlike most classic GPCRs where ligand binding occurs in a pocket formed by the transmembrane helices. New high-resolution structures of four different GPCRs reveal considerable divergence in the second extracellular loops as well as in the ligand-binding pocket and intracellular loops (Hanson *et al.*, 2009; Rosenbaum *et al.*, 2009), suggesting distinct mechanisms of GPCR activation and signal transduction. Once activated, GPCRs function as guanine nucleotide exchange factors and promote exchange of GDP for GTP on the α subunit leading to βγ subunit dissociation. Both the GTP bound α subunit and βγ subunits signal to various effectors to promote diverse cellular responses (Figure 1) (Oldham and Hamm, 2007; 2008). Activated PAR_1 and PAR_2 couple to multiple heterotrimeric G-protein subtypes including G_i, G_q and $G_{12/13}$ (Coughlin, 2005; Russo *et al.*, 2009b). PAR_4 couples to G_q and $G_{12/13}$ activation, whereas PAR_3 was previously thought not to signal autonomously (Nakanishi-Matsui *et al.*, 2000). However, new studies indicate that thrombin activation of natively expressed PAR_3 can elicit Rho- and Ca^{2+}-dependent release of ATP from lung epithelial A549 cells, a cell type that does not appear to express either PAR_1 or PAR_4 (Seminario-Vidal *et al.*, 2009).

Activated PARs also interact with various adaptor proteins that facilitate signal transduction independent of heterotrimeric G protein coupling. The best-characterized G protein-independent effectors for GPCRs are the β-arrestins, multifunctional adaptor proteins (Table 1) (Lefkowitz and Shenoy, 2005). There are four members of the arrestin family including visual arrestin (arrestin 1) and X-arrestin (arrestin 4), which are expressed primarily in retinal rods or cones, and the highly abundant, ubiquitously expressed β-arrestin 1 and 2 (also known as arrestin 2 and 3). Arrestins are comprised of distinct N and C structural domains linked by a 12-residue polar core that engages receptor-associated phosphates (Hirsch *et al.*, 1999; Gurevich and Gurevich, 2006). Interest-

Table 1 Agonist, signalling and shut-off mechanisms of protease-activated receptors (PARs)

Receptor	*Tethered ligand*	*Activating proteases*	*Signalling effectors*	*Signal termination*
PAR_1	SFLLRN	Thrombin TF-VIIa-Xa or Xa APC-EPCR Trypsin Plasmin MMP-1 Granzyme 1	G_q G_i $G_{12/13}$ Hsp90 Creatine kinase Zyxin	Phosphorylation β-Arrestins Internalization Degradation
PAR_2	SLIGKV	Trypsin Tryptase TF-VIIa TF-VIIa-Xa Matriptase (MT-SP1) Bacterial gingipains Kallikreins Granzyme A	G_q G_i $G_{12/13}$ β-Arrestins Jab1	Phosphorylation β-Arrestins
PAR_3	TFRGAP	Thrombin	G_q	?
PAR_4	GYGQV	Thrombin Trypsin TF-VIIa-Xa Plasmin Cathepsin G Bacterial ginginpains Killikreins MASP-1	G_q $G_{12/13}$	Internalization

ingly, the Vps26 protein, a component of the retromer complex important for retrograde trafficking of cargo from endosomes to the *trans*-Golgi network (Bonifacino and Hurley, 2008), has a structure surprisingly similar to β-arrestins, despite limited primary amino acid sequence homology (Shi *et al*., 2006). Strikingly, Vps26 appears to be more closely related to α-arrestins, a new family of arrestin-like proteins discovered in yeast; humans appear to have six α-arrestin proteins (Alvarez, 2008). The α-arrestins are predicted to harbour distinct N and C domains and to resemble β-arrestins and Vps26 structurally, but unlike these molecules α-arrestins contain proline-sequence (PY) motifs that can bind to WW domains of E3 ubiquitin ligases to promote ubiquitination of cargo proteins (Lin *et al*., 2008; Nikko *et al*., 2008). Thus far, there are no reported studies that have addressed the function of α-arrestin proteins in the regulation of mammalian GPCR signalling and trafficking.

The multifaceted β-arrestins control the magnitude and duration of GPCR-mediated G protein signalling as well as signalling to non-G protein effectors by functioning as scaffolds that form discrete signalling complexes with certain GPCRs (Lefkowitz and Shenoy, 2005; Moore *et al*., 2007). β-Arrestins recruit c-Src to some activated GPCRs to facilitate activation of extracellular signal-regulated kinases 1 and 2 (ERK1, 2). In addition, β-arrestins co-internalize with activated GPCRs and function as scaffolds that bring components of the ERK1, 2 and JNK3 signalling modules together, a process that is regulated by agonist stimulation. Unlike other PARs, activated PAR_2 is the only protease-activated receptor shown to bind and co-internalize with β-arrestins, which facilitates ERK1, 2 signalling from endocytic vesicles to mediate changes in the actin cytoskeleton and cell migration (Defea *et al*., 2000; Ge *et al*., 2004; Stalheim *et al*., 2005). In addition to β-arrestins, PAR_2 has been reported to bind to the Jun activating binding protein-1 (Jab1), a protein that stabilizes complexes of c-Jun or Jun D with the transcription factor activator protein 1 DNA binding sites (Luo *et al*., 2006). Jab1 was identified using PAR_2 in a yeast two-hybrid screen, and has been reported to mediate PAR_2-induced activation of c-Jun and gene transcription (Table 1). Previous studies using the PAR_1 cytoplasmic tail in a yeast two-hybrid cDNA library screen led to the identification of several binding partners including heat shock protein 90 (Hsp90) and creatine kinase (Table 1). PAR_1 interacts directly with creatine kinase and Hsp90, which appears to modulate activated receptor stimulation of RhoA signalling and cytoskeleton rearrangements in neuronal systems (Mahajan *et al*., 2000; Pai *et al*., 2001). A recent study suggests that the PAR_1 cytoplasmic tail domain interacts directly with zyxin, a LIM domain containing protein, which signals to the actin cytoskeleton independent of RhoA activation (Han *et al*., 2009). There are no known reports of G protein-independent effectors that mediate signalling by activated PAR_3 or PAR_4.

PAR desensitization and resensitization

In the classic paradigm, activated GPCRs are rapidly desensitized and uncoupled from G protein signalling by phosphorylation and β-arrestin binding. GPCR phosphorylation occurs predominantly on serine and threonine residues within the cytoplasmic tail and third intracellular loop but rarely on tyrosine residues. G protein-coupled receptor kinases (GRKs) mediate phosphorylation of activated GPCRs, which increases receptor affinity towards β-arrestins and thereby prevents further receptor–G protein coupling and consequent signalling (Lohse *et al*., 1990; Krupnick and Benovic, 1998). The second messenger kinases, protein kinase A and protein kinase C (PKC), can also phosphorylate and desensitize GPCRs regardless of the state of receptor activation, through a process that does not involve β-arrestin recruitment to the receptor (Pitcher *et al*., 1992). In addition, phosphorylation is not absolutely required for agonist-dependent β-arrestin binding and desensitization of certain activated GPCRs (Mukherjee *et al*., 2002; Jala *et al*., 2005). Moreover, some GRKs can uncouple GPCRs from G protein signalling independent of receptor phosphorylation (Ferguson, 2007). Recent work further indicates that GPCRs undergo site-specific phosphorylation that confers important functions and signalling outcomes in distinct cellular contexts (Tobin *et al*., 2008). Taken together, many studies suggest that GPCR signalling is regulated by multiple complex pathways that is likely important for distinct biological functions.

Despite the irreversible proteolytic mechanism of PAR activation that results in the generation of a tethered ligand that cannot diffuse away, signalling by the receptor is rapidly shut off. Previous studies suggest that each activated PAR_1 generates a defined amount of second messenger response, and then shuts off, at least in terms of G_q signalling (Ishii *et al*., 1993). Phosphorylation of activated PAR_1 appears to be important for rapid uncoupling from G-protein signalling as

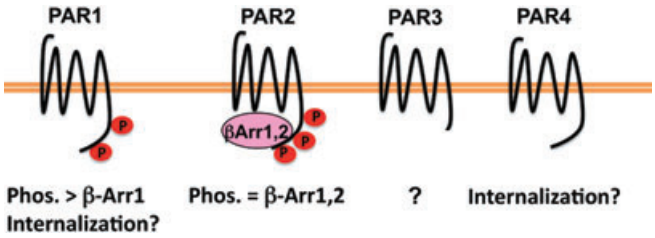

Figure 2 Protease-activated receptor (PAR) desensitization mechanisms. The mechanisms responsible for desensitization of PARs are varied and poorly understood. Phosphorylation of activated by PAR_1 by GRK3 and GRK5 is the predominant mediator of PAR_1 uncoupling from G protein signalling at least to G_q. β-Arrestin 1, but not β-arrestin 2, also contributes to PAR_1 desensitization through a mechanism that does not involve receptor phosphorylation or internalization. The contribution of internalization to the uncoupling of PAR_1 from G proteins signalling is not clearly understood. In contrast, activated PAR_2 is robustly phosphorylated by protein kinase C and other kinases, binds both β-arrestin 1 and β-arrestin 2 and is rapidly uncoupled from G protein signalling at the cell surface. The mechanisms responsible for desensitization of PAR_3 and PAR_4 are largely unknown; however, internalization may contribute to the termination of PAR_4 signalling.

overexpression of either GRK3 or GRK5 enhances PAR_1 phosphorylation and markedly inhibits signalling (Figure 2) (Ishii *et al*., 1994; Tiruppathi *et al*., 2000). A PAR_1 mutant in which all serines and threonines in the cytoplasmic tail are converted to alanines is neither extensively phosphorylated nor inhibited by GRK3 overexpression in multiple cell types. Interestingly, Paing *et al*. previously showed that β-arrestin 1 is more effective than β-arrestin 2 at desensitizing PAR_1 signalling, through a pathway that occurs independent of receptor phosphorylation and internalization (Figure 1) (Paing *et al*., 2002; Chen *et al*., 2004). Moreover, β-arrestin 1 R169E and β-arrestin 2 R170E mutants that bind to activated GPCRs with high affinity independent of receptor phosphorylation were equally effective at promoting desensitization of both PAR_1 wild type and a phosphorylation-defective mutant. Thus, desensitization of PAR_1 signalling is regulated by multiple independent mechanisms including phosphorylation of the cytoplasmic tail and the binding of β-arrestin 1, which occurs independent of receptor phosphorylation.

A role for phosphorylation in desensitization of activated PAR_2 signalling has recently been reported (Ricks and Trejo, 2009). Previous studies showed that pharmacological inhibitors of PKC enhance PAR_2-mediated calcium responses in transformed rat kidney epithelial (KNRK) cells and Berkeley rat intestinal (hBRIE 380) cells, suggesting a role for phosphorylation in PAR_2 regulation (Bohm *et al*., 1996). We recently demonstrated that PAR_2 activation caused a rapid and robust increase in phosphorylation of PAR_2 wild type, but not a mutant receptor in which all serines and threonines in the cytoplasmic tail were converted to alanines, suggesting that the major sites of PAR_2 phosphorylation occur within the cytoplasmic tail (Figure 2) (Ricks and Trejo, 2009). Similar to other GPCRs, phosphorylation of PAR_2 occurred on multiple redundant sites, any of which were sufficient for receptor desensitization or internalization. In addition, a phosphorylation-defective PAR_2 mutant failed to recruit either β-arrestin 1 or β-arrestin 2 and desensitization was markedly impaired compared with wild type receptor (Figure 2). These findings are consistent with previous work showing that β-arrestins are essential for activated PAR_2 desensitization and internalization (Dery *et al*., 1999; Defea *et al*., 2000; Stalheim *et al*., 2005). However, internalization of phosphorylation-defective PAR_2 mutant proceeded through a dynamin-dependent but clathrin- and β-arrestin-independent pathway in both a constitutive and agonist-dependent manner (Ricks and Trejo, 2009). We interpret these findings to suggest that mutations of serines and threonines within the PAR_2 cytoplasmic tail promote constitutive internalization by either diminishing basal receptor phosphorylation and/or altering a distinct receptor conformation important for retention at the plasma membrane. Thus, unlike PAR_1, β-arrestins mediate activated PAR_2 desensitization, presumably through phosphorylation, internalization and signalling to downstream effectors. PAR_4, a low-affinity receptor for thrombin, promotes sustained signalling responses and does not appear to undergo agonist-promoted phosphorylation when overexpressed in Rat1 fibroblasts (Shapiro *et al*., 2000), despite the presence of multiple serines and threonines residues within its cytoplasmic tail. Activated PAR_4 also displays a slowed rate of internalization, through a poorly understood process. The cytoplasmic tail of PAR_3 is considerably shorter than the cytoplasmic tail domains of other PARs, and the regulatory mechanisms responsible for termination of PAR_3 signalling have not been determined (Figure 2).

The proteolytic activation of PARs is unique among GPCRs, and distinct mechanisms mediate the disposal of irreversibly activated receptors and replenish the cell surface with uncleaved receptor critical for cellular resensitization. The redistribution of uncleaved PARs from an intracellular pool to the cell surface permits rapid recovery of protease signalling independent of *de novo* receptor synthesis in certain cell types and is critical for cellular resensitization (Hein *et al*., 1994; Bohm *et al*., 1996). This ensures that endothelial cells, fibroblasts and other cell types that are exposed to proteases repeatedly recover signalling responses in a timely manner. The mechanisms responsible for replenishing the cell surface with uncleaved PAR_1 and PAR_2 appear to be distinct. In endothelial cells and fibroblasts, unactivated PAR_1 is delivered to the cell surface and cycles constitutively between the plasma membrane and an early endosomal recycling compartment (Paing *et al*., 2006), forming a cytoplasmic pool that is protected from protease cleavage. Although PAR_1 internalization is dependent on clathrin and dynamin, it occurs independent of β-arrestins (Trejo *et al*., 2000; Paing *et al*., 2002; Chen *et al*., 2004). Instead, the clathrin adaptor protein complex-2 (AP-2) binds directly to a tyrosine-based motif in the cytoplasmic tail of PAR_1 and is essential for constitutive internalization and cellular recovery following thrombin exposure (Paing *et al*., 2006). The expression of a PAR_1 tyrosine mutant or depletion of AP-2 by RNA interference leads to significant inhibition of PAR_1 constitutive internalization, loss of intracellular uncleaved PAR_1, and failure of endothelial cells and other cell types to regain thrombin responsiveness. In contrast, human platelets, which presumably respond to thrombin only once, the majority of PAR_1 is retained on the cell surface (Molino *et al*., 1997). Thus, platelets lack an internal pool of protected receptors, as recovery of thrombin responsiveness is not a physiological requirement of this cell type.

In contrast to PAR_1, full cellular resensitization to PAR_2 signalling involves mobilization of naïve receptor from the Golgi apparatus as well as *de novo* receptor synthesis (Bohm *et al*., 1996). In KNRK and hBRIE 380 epithelial cells, initial resensitization to trypsin, a potent activator of PAR_2 signalling, remained intact after incubation with brefeldin A, an inhibitor of Golgi protein transport, or cycloheximide, an inhibitor of *de novo* protein synthesis, suggesting that initial resensitization depends upon the existence of a reserve receptor pool (Bohm *et al*., 1996). However, subsequent challenge with agonist required both intact Golgi function and *de novo* synthesis for full PAR_2 responsiveness. A more recent study identified the type I transmembrane protein p24a as an important mediator of PAR_2 transport from the Golgi apparatus to the cell surface and cellular resensitization (Luo *et al*., 2007). P24a is a member of the p24 family of proteins that facilitates protein transport through the Golgi network and binds to the second extracellular loop of PAR_2 retaining it at the Golgi until receptor activation causes receptor dissociation and exocytic transport, a process that involves activation of the small GTPase, ADP-ribosylation factor-1 (ARF1). In addition to ARF1, the small GTPase rab11a, a key regulator of perinuclear, plasma membrane and Golgi endosomal recycling, has also been implicated in PAR_2 exocytic trafficking and cellular resensitization (Roosterman *et al*., 2003). The mechanisms that regulate PAR_3 and PAR_4 trafficking and cellular resensitization have not been determined.

Endocytic trafficking of PARs

The internalization, recycling and lysosomal sorting of PARs are crucial for the spatial and temporal regulation of receptor signalling. Unactivated PAR_1 cycles tonically between the cell surface and endosomes and is important for cellular resensitization (Paing *et al*., 2006), whereas activated PAR_1 internalization and lysosomal degradation is critical for signal termination (Trejo *et al*., 1998; Trejo and Coughlin, 1999). In contrast, activated PAR_2 internalization is required for signal propagation from endocytic vesicles (Defea *et al*., 2000; Stalheim *et al*., 2005). The underlying mechanistic basis for these processes is just beginning to emerge and has been reported in recent studies as discussed below.

The transport of GPCRs within the endocytic pathway is initiated through a highly regulated process that leads to deformation of select regions of the plasma membrane (Marchese *et al*., 2008). Clathrin-coated pits formed at the plasma membrane are largely responsible for recruitment and internalization of most GPCRs. Clathrin-coated pits assemble at sites enriched in phosphatidylinositol (4,5)-bisphosphate, and involve the recruitment of clathrin, adaptor proteins and many other regulatory proteins that coordinate the formation and invagination of clathrin-coated vesicles (Edeling *et al*., 2006). The recruitment of cargo to clathrin-coated pits is mediated by clathrin adaptors that recognize phosphorylated or ubiquitinated cargo or short linear sorting sequences residing within the cytoplasmic domains of cargo proteins. Most activated and phosphorylated GPCRs are recognized by β-arrestins, which facilitate receptor internalization through clathrin-coated pits. The binding of β-arrestins to GPCRs induces a conformational change in β-arrestins exposing C-terminal sequences that bind to clathrin and the β2-adaptin subunit of the clathrin adaptor protein AP-2 (Goodman *et al*., 1996; Laporte *et al*., 1999).

Similar to most classic GPCRs, activated PAR_2 is robustly phosphorylated and recruited to clathrin-coated pits by binding to β-arrestins (Defea *et al*., 2000; Stalheim *et al*., 2005; Ricks and Trejo, 2009). Moreover, activated PAR_2 is phosphorylated on multiple serine and threonine residues positioned at the end of the cytoplasmic tail (Ricks and Trejo, 2009), which increases its affinity for β-arrestins and facilitates PAR_2–β-arrestin complex co-internalization and colocalization on endocytic vesicles. Unlike most classic GPCRs, activated and internalized PAR_2 does not recycle, but instead remains associated with β-arrestins on endocytic vesicles and promotes sustained ERK1, 2 signalling in the cytoplasm (Defea *et al*., 2000; Stalheim *et al*., 2005).

PAR_1 displays both constitutive and agonist-promoted internalization, which proceeds through a clathrin- and dynamin-dependent pathway independent of β-arrestins (Figure 3) (Trejo *et al*., 2000; Paing *et al*., 2002; 2006). We previously showed that the clathrin adaptor AP-2 is essential for constitutive PAR_1 internalization (Paing *et al*., 2006; Wolfe *et al*., 2007). In addition, the post-translational modification of PAR_1 with ubiquitin specifies a distinct clathrin adaptor requirement that mediates activated receptor internalization through clathrin-coated pits independent of AP-2 and β-arrestins (Wolfe *et al*., 2007). Ubiquitin is a 76-amino-acid polypeptide that is covalently linked to lysine residues of substrate proteins by ubiquitin ligases and removed by deubiquitinating enzymes. A role for ubiquitination in GPCR internalization was first demonstrated for the yeast *Saccharomyces cerevisiae* G protein-coupled Ste2 and Ste3 receptors (Hicke and Riezman, 1996; Roth and Davis, 1996). The ubiquitination of PAR_1 is likely a highly dynamic and reversible process, and, the receptor probably exists in both ubiquitinated and deubiquitinated forms at steady state. Remarkably, the major sites of PAR_1 ubiquitination occur within the cytoplasmic tail at lysines residues, which are located within the distal tyrosine-based motif, an important binding site for the μ2 subunit of the AP-2 complex that mediates constitutive receptor internalization (Figure 3) (Paing *et al*., 2006; Wolfe *et al*., 2007). Thus, PAR_1 ubiquitination may preclude AP-2 binding to negatively regulate constitutive receptor internalization. Consistent with this model, an ubiquitin-deficient PAR_1 '0K' mutant displays an enhanced rate of constitutive internalization and the fusion of an ubiquitin moiety to the cytoplasmic tail of PAR_1 '0K' mutant attenuated constitutive internalization. Thus, modification of PAR_1 by ubiquitination switches the trafficking phenotype and the endocytic machinery required to internalize activated receptor. In addition to PAR_1, several other mammalian GPCRs internalize through clathrin-coated pits independent of β-arrestins (Wolfe and Trejo, 2007) and raise the possibility that similar internalization mechanisms exist for other mammalian GPCRs.

Once internalized, GPCRs are sorted within an endosomal tubulo-vesicular compartment to either a recycling or lysosomal degradation pathway. Sorting of GPCRs to a recycling pathway occurs through a default pathway similar to bulk

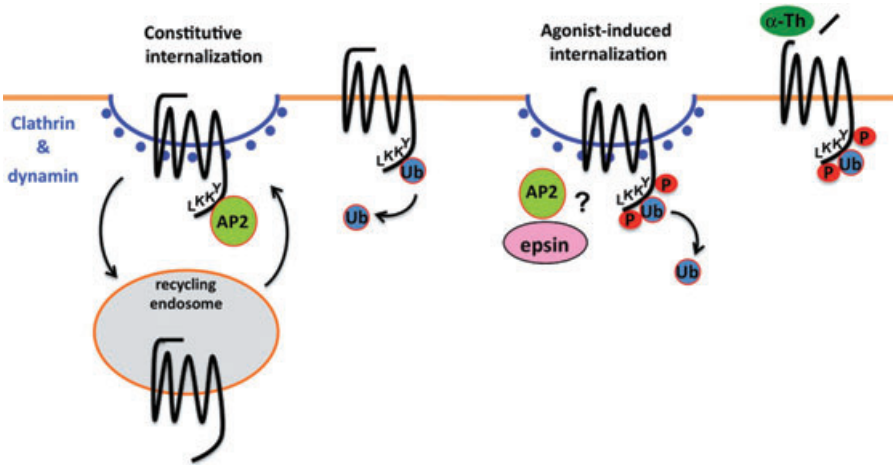

Figure 3 Constitutive and activated protease-activated receptor-1 (PAR_1) internalization are regulated by distinct mechanisms. Unactivated PAR_1 cycles constitutively between the cell surface and an intracellular compartment, which generates an internal pool of uncleaved receptor that functions to rapidly resensitize cells after protease cleavage. In contrast, activated PAR_1 is phosphorylated, internalized and sorted directly to lysosomes for degradation, a process critical for termination of activated receptor signalling. Both constitutive and activated PAR_1 internalization occur through a clathrin- and dynamin-dependent pathway that is independent of β-arrestins. Rather than β-arrestins, the clathrin adaptor (protein) AP-2 binds directly to a tyrosine-based motif at the C-terminus of PAR_1 to mediate constitutive internalization. Interestingly, PAR_1 is basally ubiquitinated and ubiquitination appears to negatively regulate PAR_1 constitutive internalization, perhaps by impeding AP-2 binding. In contrast, ubiquitination of activated PAR_1 specifies a distinct clathrin-dependent internalization pathway that is independent of AP-2. However, both AP-2 and epsin, a clathrin adaptor that binds ubiquitin, mediate activated PAR_1 internalization through clathrin-coated pits but precisely how this occurs remains to be determined. Moreover, deubiquitination of PAR_1 occurs after activation through mechanisms that remain poorly understood.

membrane flow or through a regulated process (Hanyaloglu and von Zastrow, 2008). Trafficking of internalized receptor from endosomes to lysosomes is a major pathway for GPCR degradation or down-regulation. The regulatory mechanisms that control degradation of GPCRs by internalization and lysosomal sorting involve adaptor protein recognition of either structural determinants within the receptor cytoplasmic tail and/or post-translational modification with ubiquitin (Marchese *et al*., 2008).

Several mammalian GPCRs harbour tyrosine-based motifs within their cytoplasmic tails that may function as binding sites for endocytic AP complexes to facilitate membrane trafficking (Marchese *et al*., 2008). In addition to their endocytic function, tyrosine-based motifs have been implicated in targeting transmembrane proteins to lysosomes. The lysosomal targeting tyrosine-based motifs are often found close to the end of the transmembrane domain. The heterotetrameric AP complexes bind directly to tyrosine-based motifs and have distinct functions in membrane trafficking (Bonifacino and Traub, 2003). AP-2 is abundant at the plasma membrane and mediates endocytosis. AP-1 localizes predominantly to the *trans*-Golgi network, whereas AP-3 associates with early-endosome-associated tubules and facilitates endosome-to-lysosome trafficking. AP complexes are composed of α, β, μ and σ subunits; the μ subunit directly binds to tyrosine-based motifs. We previously found that a proximal PAR_1 cytoplasmic tail tyrosine-based motif localized near the end of the seventh transmembrane domain facilitates lysosomal degradation (Paing *et al*., 2004), but whether this involves recognition by distinct AP complexes has not been determined. Moreover, lysosomal sorting of the galanin R1 receptor and M2 muscarinic receptor has also been shown to require a cytoplasmic localized tyrosine-based motif (Goldman and Nathanson, 1994; Xia *et al*., 2008), but how this occurs mechanistically is not known.

The sorting of ubiquitinated cargo from early endosomes to lysosomes involves transit through an endosomal compartment that contains intralumenal vesicles (ILVs) and is termed multivesicular bodies (MVBs). MVBs (also known as late endosomes) fuse with lysosomes, resulting in the degradation of ILVs containing lipids by lipases and proteins by proteases. The ubiquitin-dependent ESCRT machinery is comprised of a complex network of proteins that function coordinately to sort ubiquitinated cargo to a degradative pathway and was discovered in yeast *S. cerevisiae* (Piper and Katzmann, 2007). The ESCRT machinery is conserved in mammalian cells, although mammalian cells have a greater diversity and specialization in endocytic sorting pathways. The ESCRT machinery is comprised of three distinct complexes that recognize ubiquitinated cargo and prevent their recycling or retrograde trafficking and has the capacity to deform the endosomal membrane allowing cargo to be sorted into ILVs of MVBs. The hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) (also known as ESCRT-0) is recruited to endosomes via its FYVE domain, which binds to phosphatidylinositol-3-phosphate, a lipid enriched on endosomal membranes. HRS binds directly to ubiquitinated cargo on endosomes and to tumour suppressor gene 101 (Tsg101), a component of ESCRT-I. ESCRT-II and ESCRT-III complexes are then recruited sequentially to endosomes and coordinate the recruitment and sorting of ubiquitinated cargo to MVBs. Cargo is then deubiquitinated before entry into ILVs of MVBs. The activity of vacuolar protein sorting protein-4, an AAA-ATPase that catalyses the disassembly of the ESCRT components, is critical for ESCRT function.

Several mammalian GPCRs are directly modified with ubiquitin and sorted to lysosomes through an ESCRT-dependent pathway (Marchese *et al*., 2008). Activated PAR_2 is internalized via a β-arrestin, clathrin and dynamin-dependent pathway (Defea *et al*., 2000; Stalheim *et al*., 2005; Ricks and Trejo, 2009), and is directly ubiquitinated by the RING-finger ubiquitin ligase c-Cbl and sorted to lysosomes for degradation (Jacob *et al*., 2005). Activated PAR_2 requires not only ubiquitination but also HRS, a component of the ESCRT machinery, for lysosomal degradation and not for receptor internalization (Figure 4) (Jacob *et al*., 2005; Hasdemir *et al*., 2007). In addition, overexpression of the catalytically inactive deubiquitinating enzymes AMSH and UBPY increase the steady state amount of ubiquitinated PAR_2 and slowed agonist-triggered lysosomal degradation (Hasdemir *et al*., 2009). Although PAR_2 is considered a class B GPCR as defined by its ability to form stable complexes with β-arrestins, rather than transient interactions like class A receptors, the role of β-arrestin ubiquitination and its ability to recruit E3 ubiquitin ligases to the regulation of PAR_2 ubiquitination is not known (Drake *et al*., 2006). However, not all GPCRs that sort rapidly and efficiently to lysosomes require direct ubiquitination (Marchese *et al*., 2008). We previously found that activation of wild type and ubiquitin-deficient PAR_1 expressed in HeLa cells or Rat1 fibroblasts caused a similar extent of receptor degradation, suggesting that PAR_1 ubiquitination is not required for lysosomal degradation (Figure 4) (Wolfe *et al*., 2007). Moreover, activated PAR_1 sorts from endosomes to lysosomes independent of HRS and Tsg101 (Gullapalli *et al*., 2006), components of the ubiquitin-binding ESCRT machinery. These findings strongly suggest that deubiquitinated rather than ubiquitinated PAR_1 transits through the endosomal–lysosomal system independent of the ESCRT proteins HRS and Tsg101. However, the sorting of PAR_1 from early endosomes to lysosomes at an early stage in the pathway is dependent on sorting nexin-1 (SNX1) (Figure 4) (Wang *et al*., 2002; Gullapalli *et al*., 2006). SNX1 associates with the cytosolic face of endosomes and contains a phox homology (PX) domain important for binding to

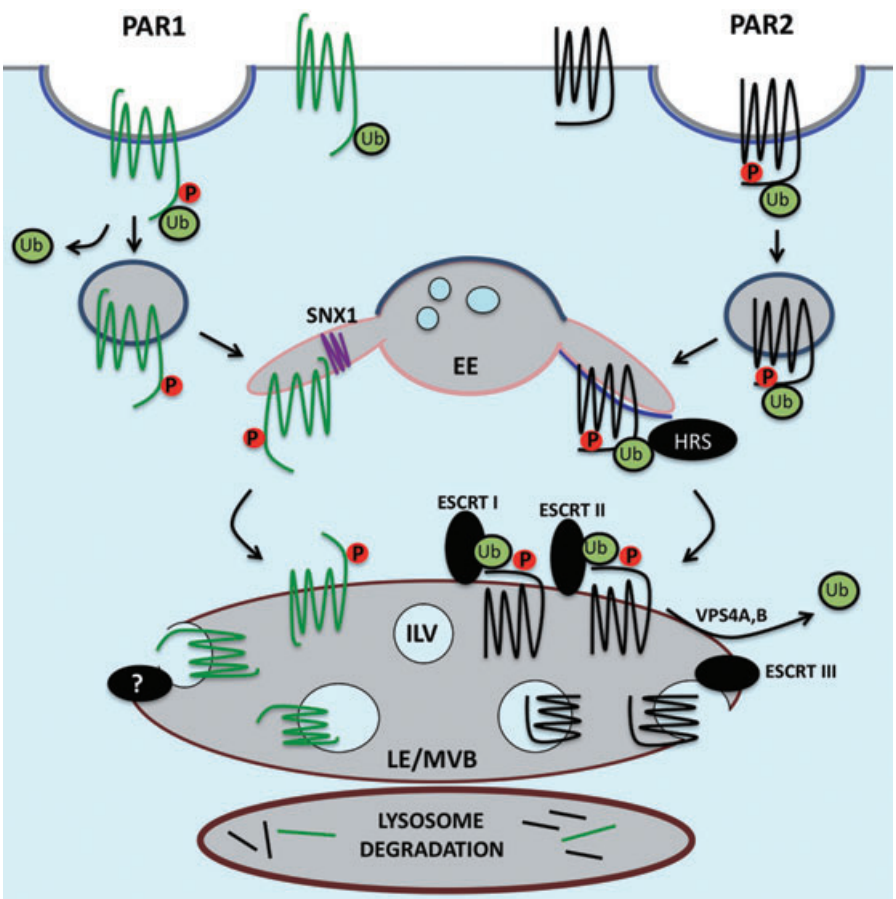

Figure 4 Ubiquitin-dependent and -independent lysosomal trafficking of protease-activated receptors (PARs). Activated PAR_2 is phosphorylated, binds β-arrestins and is then recruited to clathrin-coated pits and internalized from the cell surface. In contrast, internalization of activated PAR_1 occurs via a phosphorylation, clathrin- and dynamin-dependent pathway independent of β-arrestins. Clathrin-coated pits pinch off to form vesicles, which fuse with early endosomes (EE). Internalized PARs are then sorted through endocytic compartments to lysosomes and degraded. Most ubiquitinated cargo are sorted to lysosomes through the well-characterized ubiquitin-dependent endosomal-sorting complex required for transport (ESCRT) pathway. Activated PAR_2 is ubiquitinated and requires hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) and vacuolar protein sorting protein-4 (Vps4) for lysosomal sorting. Remarkably, activated PAR_1 is delivered to lysosomes independent of ubiquitination and the ESCRT machinery – HRS, tumour suppressor gene 101 and Vps4. Trafficking of PAR_1 to lysosomes, however, requires its C-terminal tyrosine-based motif and is mediated by sorting nexin 1 (SNX1). The mechanism by which activated PAR_1 is sorted to intralumenal vesicles (ILVs) of late endosomes (LE)/multivesicular bodies (MVBs) is not known.

endosomal-enriched phosphoinositides. The C-terminal BAR domain of SNX1 forms banana-shaped dimers with different degrees of curvature that sense or induce membrane curvature and is thought to drive tubule formation by preferentially interacting with the narrow diameter of membrane tubules (Carlton *et al*., 2004; Cullen, 2008). Thus, SNX1 facilitates the pinching off of endosomal tubules through a process that involves other as-yet unidentified endocytic proteins. The identities of other endocytic adaptor proteins that mediate ubiquitin-independent lysosomal sorting of PAR_1 and other GPCRs remain to be determined.

Agonist selective PAR signalling

'Functional selectivity' or 'biased agonism' is a process by which distinct ligands acting on the same receptor can elicit different signalling responses by stabilizing distinct active receptor conformations and has been best characterized for GPCRs (Urban *et al*., 2007). In many cases, differences in GPCR signalling have been observed in studies comparing synthetic ligands to natural ligands. Indeed, previous studies suggest that distinct cellular responses could be evoked by PAR_1 when activated proteolytically by its tethered ligand versus activation by untethered 'free' synthetic peptide agonists (Blackhart *et al*., 2000). McLaughlin *et al*. demonstrated that thrombin activation of PAR_1 in human endothelial cells favours activation of $G_{12/13}$ signalling and induction of endothelial barrier permeability over G_q or G_i signalling (McLaughlin *et al*., 2005). In contrast, the activation of PAR_1 by synthetic peptide agonists SFLLRN or TFLLRNPNDK caused preferential coupling to G_q-triggered increases in Ca^{2+} mobilization rather than $G_{12/13}$ signalling, suggesting that tethered versus 'free' peptide ligands can distinctly activate PAR signalling. Consistent with this idea, the activation of PAR_2 by distinct tethered versus 'free' peptide agonist also appears to cause biased receptor signalling. The mutant SLAAA peptide sequence stimulates PAR_2 induction of Ca^{2+} mobilization when tethered to the receptor but failed to elicit signalling responses when presented to cells as a soluble ligand (Al-Ani *et al*., 2004). In new work, Ramachandran *et al*. showed that although the soluble SLAAA peptide ligand could not trigger Ca^{2+} mobilization, it retained the capacity to activate ERK1, 2 signalling comparable to native agonist peptide SLIGKV (Ramachandran *et al*., 2009), suggesting that the agonist peptide SLAAA agonist induces biased signalling.

The unique proteolytic cleavage mechanism of PAR activation that occurs at a defined site in the N-terminus and results in the generation of a specific tethered ligand sequence, suggested that different proteases are unlikely to function as biased agonists for PAR signalling. However, several recent studies suggest that distinct proteases can stabilize different active PAR conformations to elicit distinct cellular responses that may be facilitated by compartmentalization in plasma membrane microdomains (Figure 5) (Russo *et al*., 2009b). Several proteases have been reported to cleave and activate PARs including serine, cysteine and metalloproteases that either function as soluble enzymes or require membrane-associated cofactors that facilitate membrane localization and/or allosterically modulate protease activity (Table 1)

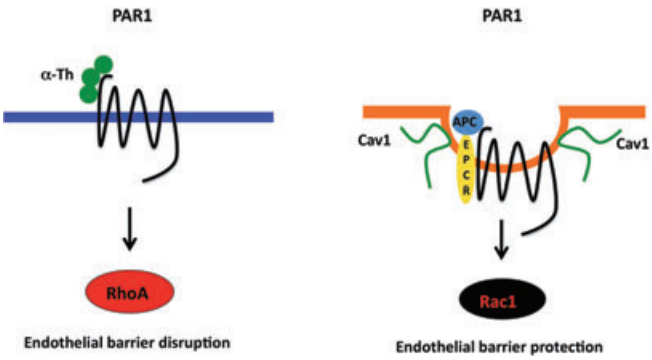

Figure 5 Protease-selective protease-activated receptor-1 (PAR_1) activation and signalling is mediated by compartmentalization in caveolae. Thrombin efficiently binds to and cleaves PAR_1 to elicit preferential activation of $G_{12}/_{13}$ signalling and Rho activation in endothelial cells that results in endothelial barrier disruption. In contrast, the activated protein C (APC) cofactor endothelial protein C receptor, PAR_1 and signalling effectors sequester in caveolae, a subtype of lipid raft, and promote Rac1 activation and endothelial barrier stabilization.

(Russo *et al*., 2009b). Thrombin, the main effector protease of the coagulation cascade, promotes pro-inflammatory responses and disrupts endothelial barrier permeability through the activation of PAR_1 (Komarova *et al*., 2007). In contrast, activated protein C (APC), an anticoagulant protease, elicits anti-inflammatory responses and promotes endothelial barrier stabilization through PAR_1 signalling (Feistritzer and Riewald, 2005; Finigan *et al*., 2005). APC is generated on the endothelial cell surface via activation of protein C by the thrombin–thrombomodulin complex. APC bound to endothelial protein C receptor (EPCR) cleaves and inactivates coagulation factors Va and VIIa diminishing thrombin generation and induces cellular responses through the activation of PAR_1 (Riewald *et al*., 2002; Mosnier *et al*., 2007). These findings raise the question of how two different proteases can activate the same receptor to elicit opposite cellular responses.

Our recent work demonstrates that compartmentalization of PAR_1 in caveolae is essential for APC-dependent cytoprotective signalling in endothelial cells (Figure 5) (Russo *et al*., 2009a). We hypothesized that if the difference between thrombin and APC signalling were due to efficiency in PAR_1 cleavage and activation we would expect to observe quantitative, but not qualitative differences in signalling responses. Thus, to determine whether this was indeed the case we examined the ability of thrombin and APC to activate RhoA and Rac1, small GTPases that differentially regulate endothelial barrier permeability (Komarova *et al*., 2007). We found that thrombin caused robust RhoA signalling but not Rac1 activation, whereas APC stimulated a marked increase in Rac1 activation but not RhoA signalling (Russo *et al*., 2009a), consistent with the opposing functions of these proteases on endothelial barrier integrity. Moreover, one mechanism that contributes to the differential activation of PAR_1 by thrombin versus APC involves localization of the receptor to caveolae, a subtype of lipid rafts (Figure 5). Caveolae are enriched in cholesterol and caveolin-1, a structural protein essential for caveolae formation in endothelial cells (Razani *et al*., 2001). The APC cofactor EPCR, PAR_1 and G_i partition into lipid rafts

and cofractionate with caveolin-1 on sucrose gradients (Bae *et al*., 2007a,b), suggesting that these molecules exist as a preassembled complex poised to signal after APC generation. Several reports indicate that APC's proteolytic activity is required for PAR_1-dependent cytoprotective signalling and that APC cleaves the N-terminus of PAR_1 (Riewald *et al*., 2002; Ludeman *et al*., 2004; Mosnier *et al*., 2004); however, the precise cleavage site of PAR_1 that confers cytoprotective signalling by APC remains to be determined. Together these studies suggest that localization of PAR_1 and EPCR with specific G protein signalling effectors stabilizes a distinct active receptor conformation that favours endothelial barrier protective signalling. Interestingly, caveolae are also required for the activation of PAR_2 by the tissue factor-coagulant protease factor VIIa complex but not by synthetic peptide agonists (Awasthi *et al*., 2008); however, it was not determined whether the different agonists caused biased signalling.

In human platelets, collagen initiates activation of matrix metalloprotease-1 (MMP-1) on the platelet surface; MMP-1 then cleaves and activates platelet PAR_1 (Trivedi *et al*., 2009). Interestingly, MMP-1 cleaves the N-terminus of PAR_1 at a different site from that previously reported for thrombin, but surprisingly MMP-1 signals comparably to thrombin to induce platelet shape change. However, previous studies showed that MMP-1 and thrombin differentially activated gene transcription through PAR_1 activation in endothelial cells to promote angiogenesis (Blackburn and Brinckerhoff, 2008), suggesting that the two proteases elicit distinct cellular signalling responses through the activation of the same receptor. Other metalloproteases such as disintegrin and metalloprotease 17 (ADAM17)/TACE have been shown to cleave PAR_1 at sites that are unproductive and render PAR_1 unresponsive to thrombin signalling (Ludeman *et al*., 2004). Plasmin, a serine protease generated in plasma by urokinase and tissue plasminogen activator cleavage of plasminogen, cleaves PAR_1 at multiple sites, which either activates or incapacitates the receptor depending on the cleavage site (Kuliopulos *et al*., 1999). In astrocytes, plasmin functions as an endogenous activator of PAR_1 and increases Ca^{2+} mobilization, which potentiates *N*-methyl-D-aspartate receptor function in hippocampal neurons (Mannaioni *et al*., 2008). Thus, it remains to be determined whether proteases such as MMPs and/or plasmin cleave and activate PAR_1, which results in the stabilization of an active receptor conformation that is able to induce selective signalling responses that are distinct from thrombin.

PAR crosstalk with other receptors

Although typically proteolytic cleavage leads to activation of the same receptor, there is evidence of crosstalk between different PARs. In murine platelets and transfected COS-7 cells, human PAR_3 binds to thrombin with high affinity but does not appear to signal (Nakanishi-Matsui *et al*., 2000). Instead, PAR_3 localizes thrombin to facilitate activation of PAR_4, a receptor with low affinity for thrombin, to elicit platelet activation (Nakanishi-Matsui *et al*., 2000). In addition, PAR_3 dimerizes with PAR_1 and consequently potentiates thrombin signalling in endothelial cells, suggesting that PAR_3 functions as an allosteric modulator of PAR_1 signalling in certain cell types (McLaughlin *et al*., 2007). Another type of PAR crosstalk occurs in endothelial cells, where the tethered ligand domain of signalling defective cleaved PAR_1 containing a mutation in a critical proline residue in the second extracellular loop, transactivates PAR_2 (O'Brien *et al*., 2000). In addition, during progression of sepsis, a severe inflammatory condition, activated PAR_1 switches from endothelial-disruptive signalling to protective signalling via transactivation of PAR_2, a receptor whose expression is up-regulated in endothelial cells during sepsis (Kaneider *et al*., 2007).

In addition to other PARs, PAR_1 has been shown to modulate signalling of other GPCRs including the sphingosine 1-phosphate (S1P) receptor and platelet-activating factor receptor (PAFR). Interestingly, the endothelial barrier protective effects elicited by APC activation of PAR_1 involve crosstalk with $S1P_1$ receptor, but not the $S1P_3$ receptor (Feistritzer and Riewald, 2005). The sphingolipid S1P is generated intracellularly by sphingosine kinases 1 and 2 (SPK1) and depletion of SPK1 or $S1P_1$ receptor expression by RNAi resulted in the loss of APC-induced barrier protective signalling, a process that requires expression of PAR_1 (Feistritzer and Riewald, 2005; Russo *et al*., 2009a). These findings indicate that S1P is an important mediator of endothelial barrier integrity. In contrast, dendritic cell signalling by PAR_1 induces S1P activation of the $S1P_3$ receptor to promote inflammation in decompensated innate immune response (Niessen *et al*., 2008). In melanoma, PAR_1 expression is important for basal PAF production and PAFR expression, which mediates induction of the melanoma adhesion molecule via activation of the cAMP-responsive element-binding protein and tumour progression (Melnikova *et al*., 2009).

In addition to S1P and PAF, activation of PARs stimulates production and release of a variety of cytokines, chemokines and growth factors including interleukin (IL)-6, IL-8, VEGF, platelet-derived growth factor and modulates the activation of integrins to elicit various cellular responses (Figure 1) (Coughlin and Camerer, 2003; Coughlin, 2005). Transactivation of epidermal growth factor receptors and ErbB2 by PAR_1 and PAR_2 through activation of ADAMs or MMPs, which in turn releases membrane-anchored ligands such as heparin-binding epidermal growth factor and transforming growth factor-α or through intracellular signalling pathways have been reported in both normal and transformed cells (Daub *et al*., 1996; Prenzel *et al*., 1999; Darmoul *et al*., 2004; Melnikova *et al*., 2009). In recent work, PAR_2 has been demonstrated to signal synergistically with Toll-like receptor 4 (TLR4) (Chi *et al*., 2001; Rallabhandi *et al*., 2008). The Toll-like receptors are type I transmembrane glycoproteins that mediate innate immune responses. The coexpression of TLR4 complex with PAR_2 caused synergistic signalling to inflammatory responses that was distinct from PAR_2 or TLR4 signalling alone and required specific TLR4 adaptor components (Rallabhandi *et al*., 2008). Interestingly, unlike other signalling crosstalk the PAR_2 and TLR4 receptor cooperativity appears to be dependent on physical interaction. This is similar to that reported for receptor-activity-modifying proteins (RAMPs), which are type I transmembrane proteins that interact with many GPCRs to modulate receptor signalling and trafficking

(Sexton *et al*., 2009), although RAMPs have not been reported to interact with PARs.

Conclusions

PARs are expressed in a variety of cell types and mediate critical cellular responses important for haemostasis, thrombosis, and inflammatory and proliferative responses associated with tissue injury (Coughlin, 2005). With the exception of coagulant proteases, the specific proteases responsible for activation of PARs expressed in tissues other than the vasculature remain poorly characterized. Several recent studies provide compelling evidence that activation of PARs by different proteases or in complex with other receptors elicit distinct signalling outcomes and raise the possibility that distinct active receptor conformations are differentially regulated. In addition to desensitization, the internalization, recycling and lysosomal sorting of PARs is crucial for the spatial and temporal regulation of receptor signalling and appropriate cellular responses. However, the mechanisms responsible for regulation of PAR signalling when distinctly activated by different proteases or in complex with other receptors remain poorly understood. Indeed, activation of PAR_1 by APC causes minimal receptor internalization and lysosomal degradation (Schuepbach *et al*., 2008; Russo *et al*., 2009a), a process critical for termination of thrombin-activated PAR_1 signalling (Trejo *et al*., 1998). The mechanisms responsible for APC-activated PAR_1 desensitization are not known. The finding that PARs can be differentially activated by distinct ligands also provides new important therapeutic opportunities. Moreover, as alterations in PAR trafficking can promote changes in the magnitude, duration and localization of receptor signalling, it is critical to understand the processes that control sorting of these receptors through the endocytic pathway. An understanding of the mechanisms by which specific adaptor proteins and processes regulate ubiquitin-dependent and -independent PAR lysosomal sorting will enable us to develop new strategies to manipulate receptor signalling and provide novel targets for the development of drugs that can be used in the prevention and treatment of a wide range of human diseases and cancer progression. This is particularly important because there are currently no available drugs that selectively block PAR signalling although a new PAR_1 antagonist compound based on the natural product himbacine is currently undergoing Phase III clinical trials for acute coronary syndrome (Chackalamannil *et al*., 2008). Finally, the development of other drugs such as allosteric modulators that could promote PAR internalization independent of cellular signalling or cause sustained signalling without receptor down-regulation could also be potentially useful in certain disease scenarios.

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Conflict of interest

The authors state no conflict of interest.

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