PROTEINASE-ACTIVATED RECEPTORS

Abstract
The discovery of the proteinase-activated receptors (PARs) resulted in the establishment of a new paradigm for ligand–receptor interaction mechanisms leading to cellular signaling in that proteolysis rather than classical ligand binding activates these receptors. The PARs were originally regarded as cellular signaling receptors for the coagulation serine proteinase thrombin. However, the cloning of additional PARs led to the identification of other proteinase activators, including trypsin, mast cell tryptase, and more recently nonserine proteinases such as MMP-1. PARs are widely distributed in the human lung, and recent evidence indicates a role for PARs in the pathophysiology of lung inflammatory and fibrotic responses.

Introduction
Proteinase-activated receptors (PARs) belong to the family of seven transmembrane domain G-protein-coupled receptors and derive their name from their unique mechanism of activation. Unlike other G-protein-coupled receptors, which are activated by direct ligand binding, activation of the PARs involves the proteolytic unmasking of a cryptic ligand that is already tethered to the receptor. Proteolysis is mediated by certain proteinases, of which thrombin and upstream proteinases of the extrinsic pathway of coagulation, as well as trypsin and tryptase are the most well recognized.

The human gene for PAR1 (formerly known as the thrombin receptor) was discovered in 1991 by Shaun Coughlin and colleagues at the University of California–San Francisco at the end of an intense research effort aimed at identifying the cellular mechanism by which thrombin mediates platelet aggregation. The same year also saw the cloning of the hamster PAR1 gene by Van Obberghen-Schilling and coworkers in Strasbourg. Following the discovery of the thrombin receptor and its unique activation mechanism, it appeared possible that other receptors with a similar mechanism of activation might exist. For example, platelets obtained from PAR1 null mice surviving to adulthood still responded strongly to thrombin. The search for additional thrombin receptors culminated in the discovery of two further thrombin-sensitive receptors, PAR3 and PAR4. Genomic screening led to the identification of a further receptor, PAR2, with a similar mechanism of activation by the more broad-spectrum proteinase trypsin but not thrombin.

Structure
The four PAR genes are between 3.5 and 3.7 kb long and share a similar two-exon structure encoding around 400 amino acids (Table 1). Human PAR1, PAR3, and PAR4 cluster together on band q13 on chromosome 5, suggesting that they may have arisen by gene duplication from a single ancestral gene. In contrast, PAR4 is located separately at position p12 on chromosome 19. The sequence homology between human PARs is between 27% and 33%; with the greatest difference noted between PAR4 and the other three PARs in terms of both the N- and C-termini and the cleavage site. Comparison of amino acid sequence alignment between species revealed that the PARs are highly conserved between humans and mice and that homologs of these genes are present in amphibians.

The predicted protein structure of the PARs share several features with classical seven transmembrane G-protein domain-linked receptors with their signature configuration consisting of seven helical hydrophobic transmembrane regions that in turn give rise to three intra- and three extracellular loops, a C-terminal intracellular tail, and a long N-terminal extracellular domain (Figure 1).

Regulation of Production and Activity
Activation of the PARs is mediated via limited proteolysis of the N-terminus resulting in the cleavage of around 40 amino acids (Figure 1 and Table 1). This leads to the unmasking of a tethered ligand sequence that interacts with the second extracellular loop of the receptor to induce a conformational change allowing the receptor to interact and signal via heterotrimeric G-proteins, which in turn trigger a variety of downstream signal transduction pathways. There is little doubt that thrombin is an important physiological activator of PAR1, PAR3, and PAR4. The upstream coagulation proteinase, factor Xa, as well as the more potent tissue factor–factor VIIa–factor Xa complexes, activate both PAR1 and PAR2, depending on cell type. PAR4, PAR2, and PAR4 can also be activated by trypsin, whereas mast cell tryptase is thought to be a physiological activator of PAR2. A number of other proteinase activators have been described and are listed in Table 1. These include nonserine proteinases, such as the matrix metalloproteinase MMP-1. Recently, nonendogenous proteinases, including proteinases released by house dust mites and certain bacteria, have also been
| PAR₁  | 425 | Thrombin | Trypsin, TF/FVIIa/ FXa, granzyme A, plasmin, trypsin IV, MMP-1 | R⁺¹SLFLRN | SFLLRN-NH₂, TFLLRN-NH₂ | RWJ56110, RWJ58259 | Cathepsin G, neutrophil proteinase-3, elastase, chymase, Der p1 | Airways, blood, brain, bone, breast, cardiovascular system, endometrium, immune system, intestine, lung parenchyma, lymph node, nervous system, skin | Astrocytes, epithelial cells, endothelial cells, fibroblasts, hematopoietic progenitor cells, keratinocytes, macrophages, mast cells, natural killer cells, neuronal cells, platelets, smooth muscle cells, T cells |
| PAR₂  | 397 | Trypsin, trypase, trypsin II, trypsin IV | Matriptase/ MT-SP1, TF/FVIIa/ FXa, proteinase-3, Der p1, Der p3, Der p9 | R⁺³SLIGKV | SLIGKV-NH₂ | None to date | Elastase, chymase | Airways, blood, brain, cardiovascular system, GI tract, immune system, intestine, nervous system, pancreas, skin, testes, urogenital tract, eye | Endothelial cells, eosinophils, epithelial cells, fibroblasts, keratinocytes, mast cells, macrophages, monocytes, neuronal cells, neutrophils, platelets, smooth muscle cells, T cells |
| PAR₃  | 374 | Thrombin | Trypsin, Factor Xa | K⁺³TFRGAP | None known | – | Cathepsin G | Immune system | Megakaryocytes of the bone marrow, platelets, T cells |
| PAR₄  | 385 | Thrombin, trypsin | Cathepsin G, | R⁺⁷GYPGQV | GYPGQV-NH₂, AYPGKF-NH₂ | YD-3 | Unknown | Airway, blood, cardiovascular system | Epithelial cells, smooth muscle cells, endothelial cells, platelets, fibroblasts |

Der p₁, 3, and 9, house dust mite *Dermatophagoides pteronyssinus* proteinase 1, 3, and 9.
MMP-1, matrix metalloproteinase-1.
MT-SP1, Membrane-type serine protease 1.
NH₂, amide.
Letters denote amino acid sequences in one letter code; arrow denotes cleavage site.
recently shown to activate PARs in vitro. However, confirmation for the importance of these enzymes in activating PARs in vivo is still lacking.

Delineating the signaling pathways and cellular responses elicited by PAR activators has been greatly aided by the use of peptide agonists, which mimic the tethered ligand sequence unmasked following receptor cleavage. Current evidence suggests that PAR1, PAR2, and PAR4 act as signaling receptors, whereas PAR3 is thought to act as a thrombin-docking receptor for efficient presentation of the proteinase to PAR4 at low concentrations. Following PAR activation, signal transduction is mediated via heterotrimeric G-proteins. PAR1 is relatively promiscuous in its ability to couple to multiple G-proteins, including Go1i, Gq, and G12/13. This enables the receptor to mediate its pluripotent effects via various signaling pathways, including amongst others phospholipase c/protein kinase c (PLC/PKC), mitogen-activated protein-kinase (MAPK), c-Jun NH2-terminal kinase (JNK), and nuclear factor kappa B (NF-kB) pathways. Fewer signaling studies have been performed for the other three PARs. Current evidence suggests that PAR2 interacts with Gq/11 and possibly Go1i. As mentioned above, PAR3 does not appear to signal, whereas PAR4 has been shown to activate Gi, G12/13, and Gq pathways.

In terms of the regulation of PAR signaling, the mechanisms involved are similar to those employed by other members of the seven transmembrane domain G-protein-coupled receptors. This includes classical receptor desensitization by phosphorylation at the C-terminus followed by endocytosis and degradation. Cell responsiveness is re-established via the appearance of new receptors cycled to the cell surface from pre-existing intracellular stores and de novo protein synthesis. Several proinflammatory cytokines, as well as the activating proteinases themselves (e.g., thrombin), have been reported to induce PAR gene expression. Finally, PAR signaling may also be controlled by inactivation by certain proteinases as a result of proteolysis at nonactivating sites (Table 1).

Biological Function

The clearest physiological role for the PARs is in the activation of platelets by thrombin, one of the key events involved in blood clotting. In humans, this response is mediated by PAR1 and PAR4, whereas murine platelet responses appear to involve binding to PAR3 and subsequent cleavage of PAR4. Therefore, there are important differences in receptor utilization between humans and mice and this needs to be borne in mind when extrapolating results obtained in experimental models to human physiology and pathophysiology.

Intense research efforts into the role of PARs (in particular PAR1 and PAR2) in other cell types has revealed that these receptors influence a wide range of cellular responses via both direct effects and their ability to induce the synthesis and release of a host of secondary mediators, including growth factors, chemokines, lipid mediators, and potent proinflammatory cytokines. It is therefore not surprising that these receptors are capable of influencing a wide range of cellular responses, including promoting cellular proliferation, differentiation, cytoskeletal reorganization, apoptosis, migration, and extracellular matrix production. In vivo evidence for the importance of these responses in the regulation of inflammation, vascular tone, angiogenesis, tissue and blood vessel repair, immune responses, as well as cell invasion in both pathological and nonpathological conditions is rapidly accumulating.

PARs in Respiratory Disease

Fibroproliferative Lung Disease

Interest in the role of PARs in fibrotic lung disease was fuelled by the observation that activation of the coagulation cascade is a characteristic feature of both chronic and acute lung injury. In the normal uninjured lung, the alveolar hemostatic balance is generally antithrombotic and profibrinolytic. However, in both acute lung injury and chronic fibrotic lung disease, there is good evidence that this balance is shifted in
favor of increased procoagulant activity and decreased fibrinolytic capability. Extravascular intra-alveolar accumulation of fibrin, often evident as hyaline membranes, is commonly observed in the lungs of patients with acute lung injury/acute respiratory distress syndrome (ALI/ARDS) and chronic fibrotic lung disease. Levels of tissue factor, the initiator of the extrinsic coagulation pathway is highly upregulated in the lungs of patients with idiopathic pulmonary fibrosis, interstitial pneumonia associated with systemic sclerosis and in idiopathic bronchiolitis obliterans with organizing pneumonia. Bronchoalveolar lavage fluid (BALF) from patients with ARDS also contains tissue factor/factor VII/VIIa complexes and levels of active thrombin have been shown to be increased in the lungs of patients with pulmonary fibrosis associated with systemic sclerosis and in pulmonary fibrosis associated with chronic lung disease of prematurity.

Thrombin BALF levels are also elevated in animal models of lung injury (e.g., bleomycin) and pharmacological inhibition of the coagulation cascade has been shown to be protective against lung collagen accumulation in these models. There is extensive in vitro evidence that activation of PAR1 by thrombin exerts both proinflammatory and profibrotic effects on a number of cell types present in the lung. For example, activation of PAR1 by either thrombin or factor Xa leads to the release of a host of secondary proinflammatory mediators, increases endothelial cell permeability, influences inflammatory cell trafficking, and promotes fibroblast proliferation and transformation into activated myofibroblasts, the main cell type responsible for extracellular matrix deposition in the fibrotic lung. PAR1 immunoreactivity is increased both in patients with pulmonary fibrosis and in the bleomycin model of this condition. This raises the possibility that the coagulation proteinases may contribute to the pathogenesis of these disorders, at least in part via PAR1-dependent pathways. Support for a pivotal role for PAR1 in this model was recently obtained for both the inflammatory and fibrotic phases of this injury model. Lung collagen accumulation in response to bleomycin injury is attenuated by up to 60% and is preceded by similar reductions in inflammatory cell recruitment and microvascular leak. This protection is associated with a reduction in a number of PAR1-inducible genes, including the chemokine monocyte chemotactic protein factor 1 (MCP-1) and the profibrotic mediators connective tissue growth factor and transforming growth factor beta (TGF-β1). Antagonists are currently being developed as potent antithrombotic agents and may therefore prove useful for interfering with the cellular effects associated with excessive activation or recurrent activation of the coagulation cascade in response to both acute and chronic lung injury.

### Asthma and Airway Remodeling

Thrombin levels are increased in BALF from patients with asthma and have recently been shown to correlate with levels of interleukin-5 (IL-5), TGF-β, and inflammatory cell numbers after segmental challenge in asthmatic patients. Several functional responses elicited by thrombin observed in in vitro and ex vivo studies might contribute to the pathology of this condition. Thrombin acts as a bronchoconstrictor, exerts mitogenic effects for airway smooth muscle cells and fibroblasts, and, as mentioned above, releases a host of proinflammatory cytokines by a number of cell types, including airway and alveolar epithelial cells. Current in vitro and ex vivo evidence suggests an important role for PAR1 in mediating a number of these effects but confirmation of the importance of this receptor in this disease setting in vivo is still lacking. In contrast, evidence for a role for PAR2 in this syndrome is rapidly accumulating. Mast cell tryptase, a known PAR2 activator, has been used as a marker of allergic inflammation for many years. Tryptase inhibitors have been shown to be effective in animal models and, albeit to a lesser extent, in clinical trials. PAR2 protein expression by epithelial cells is further increased in asthmatic patients. Experimental evidence suggests several mechanisms by which activation of PAR2 might contribute to this syndrome. Similar to PAR1, activation of PAR2 in vitro leads to the release of potent proinflammatory mediators from a number of cell types present in the airway, and may therefore contribute to airway inflammation. This is consistent with the observation that the early inflammatory response to allergen inhalation is attenuated in PAR2-deficient mice. These mice also produce lower serum IgE levels, so it is possible that PAR2 may also play a role in the sensitization process. Of interest, PAR3 has been reported to trigger dendritic cell development suggesting a mechanism by which this receptor may influence this process in mice. The recent finding that the house dust mite allergens Der p1 (cysteine proteinase), Der p3 (serine proteinase), and Der p9 (serine proteinase) induce the release of the proinflammatory cytokines IL-6 and IL-8 from respiratory epithelial cells in a PAR2-dependent manner suggests another mechanism by which this receptor may contribute to the asthma syndrome. Finally, trypsin and PAR2 activators also influence airway smooth muscle contraction and relaxation in vitro but current in vivo evidence suggests that this receptor exerts a predominantly bronchoprotective/bronchodilator effect. PAR3 is
therefore emerging as a receptor that may exert deleterious effects by contributing to airway inflammatory responses but that may also play important roles in airway homeostatic mechanisms. This will need to be borne in mind when targeting PAR2 for therapeutic intervention in any respiratory condition.

**Chronic Obstructive Pulmonary Disease**

Tryptase levels are detectable in patients with COPD and in smokers, and intratracheal administration of trypsin to rats reproduces some of the cardinal features of COPD. Expression of PAR2 is increased in airway vessels of patients with bronchitis, raising the possibility that PAR2 may also be important in this disease setting.


**Further Reading**


**Proteoglycans** see Extracellular Matrix: Matrix Proteoglycans; Surface Proteoglycans.

**Proteome**

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**Abstract**

Proteomics can be defined as the studies of protein properties on a large scale to obtain a global view of biological processes at the protein level. Essentially, proteomics requires protein separation and identification, and in many cases quantification. The cornerstones in proteomics are protein/peptide separation by gel electrophoresis and/or different chromatographic techniques, and identification by mass spectrometry followed by bioinformatic and biological interpretation of data. By combining these different separation techniques and mass spectrometry it is now possible to identify low abundant proteins with 10–1000 copies per cell. Today, substantial research efforts in proteome studies of the lung are focused on obtaining new diagnostic markers, as well as fingerprinting disease mechanisms.