Protease-activated receptors: the role of cell-surface proteolysis in signalling

Graeme S. Cottrell, Anne-Marie Coelho and Nigel W. Bunnett

Department of Surgery, University of California at San Francisco, 521 Parnassus Avenue, San Francisco, CA 94143-0660, U.S.A., and Department of Physiology, University of California at San Francisco, 521 Parnassus Avenue, San Francisco, CA 94143-0660, U.S.A.

Abstract

Certain extracellular proteases, derived from the circulation and inflammatory cells, can specifically cleave and trigger protease-activated receptors (PARs), a small, but important, sub-group of the G-protein-coupled receptor super-family. Four PARs have been cloned and they all share the same basic mechanism of activation: proteases cleave at a specific site within the extracellular N-terminus to expose a new N-terminal tethered ligand domain, which binds to and thereby activates the cleaved receptor. Thrombin activates PAR1, PAR3 and PAR4, trypsin activates PAR2 and PAR4, and mast cell tryptase activates PAR2 in this manner. Activated PARs couple to signalling cascades that affect cell shape, secretion, integrin activation, metabolic responses, transcriptional responses and cell motility. PARs are ‘single-use’ receptors: proteolytic activation is irreversible and the cleaved receptors are degraded in lysosomes. Thus, PARs play important roles in ‘emergency situations’, such as trauma and inflammation. The availability of selective agonists and antagonists of protease inhibitors and of genetic models

1To whom correspondence should be addressed (e-mail: nigelb@itsa.ucsf.edu).
has generated evidence to suggests that proteases and their receptors play important roles in coagulation, inflammation, pain, healing and protection. Therefore, selective antagonists or agonists of these receptors may be useful therapeutic agents for the treatment of human diseases.

**Introduction**

The superfamily of G-protein-coupled receptors (GPCRs) comprises the largest and most functionally diverse groups of signalling molecules. These receptors play essential roles in normal regulation of most biological processes. They are also of great importance in human disease since receptor mutations can cause disease and many of the most commonly prescribed drugs are receptor antagonists or agonists. Although all GPCRs share several structural features, e.g. seven transmembrane domains and conserved motifs, they are able to interact with very diverse agonists, including peptides, lipids, ions and even photons. However, proteases are one of the most intriguing agonists. Certain extracellular proteases, derived from the circulation and inflammatory cells, can specifically cleave and activate protease-activated receptors (PARs), a small, but important, sub-group of the GPCR superfamily. To date, four PARs have been cloned and all share the same basic mechanism of activation: proteases cleave at a specific site within the extracellular N-terminus to expose a new N-terminal tethered ligand domain, which binds to and thereby activates the cleaved receptor (Figure 1).

The concept that a protease cleaves and activates a GPCR raises several questions from the standpoint of receptor regulation, signal transduction and function. What is the molecular mechanism of activation? How can a catalyst, which would ultimately cleave all surface receptors, induce concentration-dependent responses? Since receptor cleavage is an irreversible event, what happens to the cleaved receptors and how do cells recover their ability to respond? Given that PARs are ‘one-shot’ receptors (once cleaved they can no longer be activated by proteolysis) is it likely that they participate in normal regulation, or would they serve to signal under emergency situations during injury or inflammation? If so, would agonists or antagonists of such receptors serve as therapies for human disease?

This brief article, which focuses on the recent advances in our understanding of the mechanism of activation and function of PARs, attempts to address some of these questions. Several recent articles comprehensively review this field [1–4].

**Cell-surface proteolysis initiates signal transduction**

A common theme of signalling by GPCRs is that a single agonist can activate several different receptors, and receptors can respond to many agonists, albeit with varying potencies. Proteases are also capable of activating several distinct PARs. In all cases, the basic mechanism of activation is the same: cleavage at a specific site within the extracellular N-terminus of the receptor exposes a new
N-terminus that serves as a tethered ligand by binding to and activating the cleaved receptor. Many factors markedly alter the efficiency of this mechanism. They include the activation of zymogens, the presence of protease inhibitors, the existence of binding sites for proteases on the receptors or at the cell surface, interactions between PARs, and pre- and post-translational modifications of receptors. Thus, the protease agonists of PARs will have different potencies depending upon the cellular environment in which they are acting.

**Thrombin receptors: PAR1, PAR3 and PAR4**

Thrombin, which is generated in the circulation during activation of the coagulation cascade, converts fibrinogen into fibrin which leads to clot formation (see Chapter 8). However, thrombin has multiple biological effects, including platelet aggregation and endothelial cell proliferation, both of which...
are mediated by PARs. Thrombin activates PAR1, PAR3 and PAR4, although the precise mechanisms of activation vary [1] (Figure 1).

Thrombin activates PAR1 in two stages [5] (Figure 1a). First, thrombin binds to PAR1 on either side of the cleavage site. The downstream site has similarities with the C-terminal sequence of hirudin, an anticoagulant thrombin inhibitor from leech saliva. Secondly, thrombin cleaves PAR1 between Arg41 and Ser42 to expose a new N-terminal tethered ligand domain, SFLLRN. The tethered ligand interacts with domains in extracellular loop 2, which presumably alters the conformation of the receptor to permit coupling to G-proteins. PAR3 also contains the thrombin binding sites and these two receptors have similar EC50 values (approx. 0.2 nM), whereas PAR4 lacks thrombin-binding sites and so only responds to higher concentrations of thrombin (EC50 approx. 5 nM) [1,6]. PAR4 also responds to trypsin with a similar EC50 value.

These differences in the mechanism of activation have interesting functional consequences. For example, human platelets express both PAR1 and PAR4. The existence of two thrombin receptors with different affinities for thrombin allows platelets to respond to graded concentrations of this enzyme in a regulated manner: PAR1 mediates responses to low concentrations of thrombin and PAR4 comes into play at high concentrations. Mouse platelets express PAR3 and PAR4, but not PAR1. Even though mouse PAR3 does contain a binding site for thrombin, mouse PAR3 does not mediate thrombin signalling, even when over-expressed. So how can mouse platelets respond to low concentrations of thrombin if they lack a functional high affinity receptor? The answer lies in an unusual intermolecular interaction between PAR3 and PAR4 (Figure 1b). Thrombin binding to PAR3 transiently anchors the enzyme to the cell surface where it can cleave and activate PAR4.

**Trypsin and tryptase receptor: PAR2**

Although trypsin is usually viewed as a digestive enzyme produced in the pancreas, trypsinogens are widely expressed in endothelial and epithelial cells, leucocytes and neurons, and both pancreatic and extra-pancreatic trypsins can signal to many cell types by cleaving and activating PAR2. The trypsin-like serine protease tryptase is expressed by most human mast cells. This enzyme is released as an active, heparin-bound tetramer, which is capable of cleaving neuropeptides, procoagulant proteins and PAR2. Trypsin and tryptase do not bind to PAR2, but rather cleave directly at Arg36 and Ser37 to expose the tethered ligand SLIGKV [7,8] (Figure 1c). The absence of binding sites may be reflected in their higher EC50 values compared with PAR1 and PAR3 activation by thrombin (EC50 values: trypsin, approx. 1 nM; tryptase, approx. 100 nM).

The ability of proteases to activate PAR2 can depend on the presence of associated proteins that anchor the proteases to the plasma membrane and thereby concentrate them on the cell surface. High concentrations of coagulation factor VIIa are unable to elicit cellular Ca2+ responses [9]. However, in cells that also express tissue factor, an integral membrane protein, factor VIIa is
bound and concentrated to the cell surface and cleaves PAR2 (EC_{50} approx. 3.5 nM), and to a much lesser extent PAR1; however, this concentration is much higher than physiological levels. It is intriguing that addition of physiological concentrations of coagulation factor X, which is converted into its active form (factor Xa) by factor VIIa (see Chapter 8), reduces the concentration requirement of factor VIIa (EC_{50} approx. 8 pM) [9]. Coagulation factor Xa can also activate PAR2 by a mechanism independent of factor VIIa and tissue factor. Human vascular endothelial cells express effector cell protease receptor-1 (EPR-1), a high-affinity binding site for factor Xa. EPR-1 binds, and thereby concentrates, factor Xa at the cell surface to facilitate activation of PAR2 (EC_{50} approx. 30 nM) (Figure 1c) [10].

Membrane-bound proteases are perfectly localized to act upon PARs and one such protease has been identified. Membrane-type serine protease 1 is a type II integral membrane protein, which is capable of signalling through PAR2 but not through PAR1, PAR3 or PAR4. Since membrane-type serine protease 1 and PAR2 have similar tissue distribution, it is conceivable that this protease could be a physiological PAR2 agonist.

Non-mammalian proteases can activate PARs

Recent observations support the intriguing possibility that proteases from parasites and pathogens can ‘hijack’ mammalian PARs and thereby cause disease. Gingipains are bacterial cysteine proteases with a trypsin-like activity from the bacterium *Porphyromonas gingivalis*. Gingipains cleave after arginine and lysine residues, and can cleave PAR1 and PAR4, thereby causing their activation. This activity has potential implications for certain disease states. Dust mite proteolytic allergens can also activate PAR2, which may contribute to asthma.

Pre- and post-translational modifications of PARs alter protease signalling

Modifications of PARs can profoundly alter the capacity of proteases to cleave and activate their receptors. A polymorphic form of human PAR2 exists with a point mutation in extracellular loop 2 (Phe^{204} \rightarrow \text{Ser}) [11]. This variant displays diminished sensitivity to trypsin and SLIGKV, yet enhanced sensitivity to PAR4-derived peptides. It is not known whether there are polymorphic variants of the other PARs and if their expression is associated with human disease.

PARs, like many GPCRs, possess putative glycosylation sites and can be extensively glycosylated, which markedly increases their mass. Recent studies indicate that glycosylation of PAR2 profoundly alters the ability of trypase to activate this receptor [12]. Tryptase is a ‘doughnut-shaped’ tetramer with active sites on the inner surface. The extracellular tail of PAR2 contains a glycosylation site in close proximity to the activation site. The potency with which tryptase (but not trypsin) activates PAR2 is dramatically increased by mutation of this sequon, by enzymic deglycosylation of PAR2, or by expres-
ion of PAR2 in glycosylation-defective cells, such that tryptase becomes almost as potent as trypsin. Perhaps the deglycosylated receptor is more readily accommodated by the active site of tryptase, allowing for efficient cleavage.

**Initiation and termination of signal transduction**

Once activated, PARs can couple to several heterotrimeric G-proteins and thereby trigger a cascade of signalling events that result in marked phenotypic changes. PAR1 can couple to \( G_{12/13} \), \( G_q \) and \( G_i \) families, whereas PAR2 couples to \( G_q \). This coupling activates signalling pathways that alter cell motility, secretion, shape, growth and survival (Figure 2). Low concentrations of a protease could eventually cleave all receptors on the surface of a cell. However, the enzyme concentration determines the rate of receptor activation and thus the rate of second messenger generation, which permits concentration-dependent responses. This dose-response is achieved because
the activated receptors are quickly shut off, terminating their signalling after
the production of a ‘quantum’ or ‘packet’ of second messenger [13]. Thus, the
level of second messenger (or response) generated is proportional to the
number of receptors activated in a given time period and not to the total
number of receptors cleaved by the protease agonist. Even though a low
concentration of enzyme could eventually cleave all surface receptors, it is the
rate at which they are cleaved that determines the cellular response to the
enzyme concentration.

After proteolytic activation, the tethered ligand of a PAR is always avail-
able to interact with the cleaved receptor. However, PAR signalling is rapidly
terminated by mechanisms similar to those used by other GPCRs, namely
uncoupling from heterotrimeric G-proteins. PAR activation triggers receptor
phosphorylation by G-protein receptor kinases and second messenger kinases.
β-Arrestins translocate from the cytosol to the plasma membrane where they
interact with phosphorylated receptors to mediate uncoupling and desensitiza-
tion [14] (Figure 3).

Figure 3. Mechanism and function of agonist-induced trafficking of PAR2
(1) PAR2 agonists cleave the receptor to induce (2) membrane translocation of G-protein recep-
tor kinases (GRKs) and β-arrestins. (3) β-Arrestins interact with GRK-phosphorylated PAR2 to
uncouple the receptor from G-proteins and to terminate the signal; they are also adaptors for
endocytosis (4) at sites of clathrin-coated pits, which pinch off from the plasma membrane in a
dynamin-dependent process. (5) β-Arrestin forms a complex with PAR2, raf-1 and activated
ERK1 and ERK2 (extracellular signal regulated kinases). Cytosolic activated ERK1 and ERK2
phosphorylate cytoskeletal proteins, microtubule associated proteins (MtAPs), and phospholipase
A₂ (PLA₂). (6) Endocytosed PAR2 is targeted to lysosomes. (7 and 8) Resensitization requires
mobilization of PAR2 from Golgi stores or synthesis of new receptors. MEK, mitogen-activated
protein kinase kinase.
Activated PARs internalize into early endosomes at sites of clathrin-coated pits. β-Arrestins couple PAR2 to clathrin, and are thus required for agonist-induced receptor endocytosis. The GTPase dynamin is required for the final stages of endosome formation. Internalized PARs are mostly destined for degradation in lysosomes. Therefore, resensitization of responses to proteases requires either the mobilization of preformed pools of receptors or the synthesis of new receptors (Figure 3).

In addition to mediating PAR uncoupling and endocytosis, β-arrestins also play an important role in signal transduction (Figure 3). β-Arrestin-dependent endocytosis of PAR2 is required for activation of the mitogen-activated protein (MAP) kinase cascade [15]. β-Arrestins serve as molecular scaffolds that recruit and organize various upstream components of the MAP kinase pathway (e.g. raf-1) into endosomes. This process determines the sub-cellular location of activated MAP kinases and thereby governs their specificity and function.

Cell surface proteolysis also contributes to terminating PAR signalling. Certain proteases cleave PARs to remove or destroy the tethered ligand domain, thereby rendering them unresponsive to protease agonists. For instance, neutrophil cathepsin G cleaves PAR1 and PAR3 to form receptors that are unresponsive to thrombin. In a similar manner, mast cell chymase inactivates PAR1 and PAR2.

**Role of PARs in health and disease states**

The appreciation that proteases can serve as signalling molecules has provided new insights into the physiological and pathophysiological functions of these enzymes (Figure 4). However, an understanding of the functions of PARs has been hampered by several obstacles. The first obstacle is the lack of highly selective and potent agonists. Proteases are not absolutely selective for one PAR. Proteases such as thrombin and trypsin can cleave several PARs, as well as other proteins, which may account for their biological actions. Synthetic peptides that correspond to the tethered ligand domains of PAR1, PAR2 and PAR4 can directly activate their receptor and are useful pharmacological tools. However, there is some cross-reactivity (for example, the PAR1 tethered ligand peptide SFLLRN can also activate PAR2). Moreover, these activating peptides are weak agonists that are effective only at micromolar concentrations, where some peptides can have non-specific effects. The second obstacle is that, with the exception of PAR1, there are no selective antagonists of PARs. The use of protease inhibitors is one strategy for understanding the functions of proteases; however, a more selective approach has been the use of genetically modified mice that lack or overexpress PARs.

PARs are disposable ‘one-shot’ receptors, as they are activated in an irreversible manner and then degraded. Given this seemingly wasteful mechanism of activation it is unlikely that PARs mediate routine intercellular signalling. Proteases and PARs instead appear to play important roles in ‘emergency
situations’, e.g. during coagulation or when mast cells degranulate. Indeed, accumulating evidence suggests that PARs play roles in inflammation, hyperalgesia, tissue repair and cancer biology.

**Pro-inflammatory effects**

Agonists of PARs can trigger all of the hallmarks of inflammation: swelling, redness, heat, pain and tissue repair (Figure 4). Thus, thrombin activates PAR1 on endothelial cells to induce vasodilatation, elevated vascular permeability to plasma proteins and increased rolling and adhesion of leucocytes. PAR1 agonists also activate inflammatory cells (mast cells, lymphocytes, neutrophils) to trigger release of chemoattractive agents and inflammatory mediators (e.g. histamine, cytokines, eicosanoids). The availability of thrombin inhibitors, PAR1 antagonists and PAR1\(^{-/-}\) mice has facilitated recent investigations into the role of PAR1 in animal models of disease. Renal inflammation is markedly diminished by the thrombin inhibitor hirudin and is attenuated in PAR1\(^{-/-}\) mice. Hirudin also ameliorates collagen-induced arthritis by inhibiting thrombin.

PAR2 also plays an important pro-inflammatory role. PAR2 is upregulated on endothelial cells by pro-inflammatory agents, and PAR2 agonists, such as tryptase from mast cells, cause increased vascular permeability, systemic hypoten-
sion and bronchoconstriction. PAR2 agonists also induce the rolling, adherence and recruitment of leucocytes in venules by a mechanism that is dependent on platelet-activating factor release. Observations in PAR2 knockout mice suggest a role for this receptor in leucocyte adhesion to venules after surgery.

**Neurogenic inflammation**

Some of the pro-inflammatory effects of PAR1 and PAR2 agonists are mediated by neurogenic mechanisms (Figure 5). Neurogenic inflammation is a form of inflammation that is controlled by the sensory nervous system and that depends on the release of the neuropeptides substance P (SP) and calcitonin gene related peptide (CGRP) from the peripheral endings of sensory nerves. Neurogenic inflammation is characterized by arteriolar dilation and hyperaemia, plasma extravasation, and oedema formation.

**Figure 5. Neurogenic mechanisms of PAR2-induced oedema and hyperalgesia**

1. Tryptase released from degranulated mast cells cleaves PAR2 at the plasma membrane of sensory nerve endings to expose a tethered ligand domain that binds to and activates the cleaved receptor.
2. Activation of PAR2 stimulates the release of CGRP and the tachykinsins, SP and neurokinin A (NKA) from sensory nerve endings.
3. CGRP interacts with the CGRP receptor to induce arteriolar dilatation and hyperaemia.
4. SP interacts with the NK1R on endothelial cells of post-capillary venules to cause gap formation and plasma extravasation. The hyperaemia and plasma extravasation cause oedema.
5. SP may stimulate the degranulation of mast cells, thereby providing positive feedback.
6. Tryptase degrades CGRP and terminates its effects.
7. CGRP inhibits SP degradation by neutral endopeptidase and also enhances SP release, thereby amplifying the effects.
8. Mediators from mast cells and other inflammatory cells stimulate the release of vasoactive peptides from sensory nerves; they also sensitize nerves.
9. Sub-inflammatory doses of PAR2 agonists also induce central sensitization and both thermal and mechanical hyperalgesia, which is likely to depend on enhanced release of SP and activation of the NK1R on spinal neurons.

primary spinal afferent neurons. Certain inflammatory agents trigger the release of these peptides within peripheral tissues such as the skin, airway and intestine, where they interact with neurokinin, receptors (NK1Rs) and CGRP\textsubscript{1}, receptors to cause hyperaemia, plasma extravasation and recruitment of granulocytes. Recent studies suggest that thrombin from the circulation and tryptase from mast cells, which are in close association with sensory nerves, can signal to sensory nerves through PAR1 and PAR2 [16]. A large proportion of small diameter neurons in the dorsal root ganglia, which contain SP and CGRP, also express PAR1 and PAR2. Thrombin and tryptase can directly signal to these neurons to release SP and CGRP. When injected into rat paws, PAR1 and PAR2 agonists cause a severe oedema that lasts for several hours and that is accompanied by an intense granulocyte infiltration. Both PAR1- and PAR2-induced oedema is inhibited by NK1R and CGRP\textsubscript{1} receptor antagonists or by ablating C-fibres with capsaicin. Thrombin and tryptase can similarly signal to the enteric nervous system, which may have marked effects on gastrointestinal secretion and motility.

**Hyperalgesia**

The discovery of receptors for proteases on nociceptive sensory neurons raised the possibility that activation of PAR1 or PAR2 on these neurons would lead to central transmission of a signal, and particularly nociceptive messages. A recent study points to a direct role of proteases and their receptors in somatic pain [17]. Injections into the paw of sub-inflammatory doses of PAR2 agonists in rats and mice induce a prolonged and sustained thermal and mechanical hyperalgesia. Hyperalgesia is not observed in PAR2\textsuperscript{−/−} animals, confirming involvement of this receptor. Additionally, deletion of the NK1R and preprotachykinin A, which encodes SP and neurokinin A, as well as the central administration of NK1R antagonists and cyclo-oxygenase inhibitors attenuates the hyperalgesia. Together, these results suggest that the hyperalgesia depends on the central activation of NK1R and the release of prostaglandins within the spinal cord (Figure 5). Remarkably, the hyperalgesia that follows intraplantar administration of formalin and of compound 48/80, which degranulates mast cells, is also diminished in PAR2\textsuperscript{−/−} mice, suggesting an important role for this receptor in pain transmission. The involvement PAR1, PAR3 and PAR4 in nociception remains to be determined.

**Protection**

In addition to their pro-inflammatory effects, there is evidence in some systems that PAR agonists can have protective roles. Trypsin in the intestinal lumen can signal to enterocytes by cleaving apical PAR2 to release prostaglandins, which have protective functions in the gastrointestinal tract. Agonists of PAR2 can trigger the relaxation of murine airway through the release of prostaglandin E\textsubscript{2}, thereby inducing a strong bronchodilatation, and can diminish infiltration of neutrophils in response to bacterial lipopolysaccharide [18,19]. After myocardial
ischaemia, infusion of PAR2 agonists significantly improves cardiac function and reduces tissue damage [20]. Some of these protective effects may be mediated by a neurogenic mechanism, and there is evidence that sensory nerves and CGRP have protective functions in several tissues. For example, PAR2 agonists induce secretion of mucus by triggering the release of CGRP, and thereby protect the gastric mucosa from experimentally induced ulcera­tion [21].

**Tissue repair and cell proliferation**

Agonists of PAR1 and PAR2 can stimulate proliferation of several cell types, including endothelial cells, myocytes and fibroblasts, which suggests a role for PARs in repair, angiogenesis and wound healing. A role for PAR1 in vascular injury is suggested by the observation that a potent and selective PAR1 antagonist RWJ-58259 markedly reduces neointimal thickness in a model of vascular restenosis induced by balloon injury in rats [22]. Observations in PAR1−/− mice also suggest a role for this receptor in the regulation of extracellular matrix formation and remodelling associated with vascular injury. A role for PAR1 in embryonic development is indicated by the finding that half of PAR1 knockout mice die *in utero*, a defect rescued by targeted overexpression of PAR1 in endothelial cells. Thus, PAR1 may play a role in normal vascular development [23].

Agonists of PARs have been found to have roles in cell proliferation, tumour cell invasion and metastasis. There appears to be a correlation between PAR1 expression and metastatic potential of tumour cells [24]. For example, PAR1 antisense oligonucleotides, which reduce PAR1 expression, also diminish the invasive nature of tumour cells. The molecular mechanism of the PAR1 involvement is thought to involve integrin recruitment to focal adhesion sites, thereby allowing migration of the cells.

There is increasing evidence that trypsin may play a major role in proliferation and cancer progression. Trypsin is produced by many lung, colon and ovarian tumours and also by endothelial cells in close proximity to gastric tumours. Activation of PAR2 by trypsin and the peptide ligand (SLIGKV) induces proliferation of human gastric and colon cancer cell lines.

**Conclusions**

To date, four PARs have been cloned, and there is accumulating pharmacological evidence for the existence of new receptors or new subtypes of existing receptors. The number of proteases known to cleave and activate receptors is growing rapidly. Considerable progress has been made in characterizing the molecular mechanisms of receptor activation and signal transduction. The availability of selective agonists and antagonists, of protease inhibitors and of genetic models has generated evidence to suggest that proteases and their receptors play important roles in coagulation, inflammation, pain, healing and protection.
There is, however, much still to learn. Many of the biological functions of proteases cannot be accounted for by the known PARs, which suggests the existence of additional mechanisms. In many systems, the physiologically important agonists of PARs are unknown. The tools to study proteases and PARs in animal models of disease have not been applied to many systems, and there are no selective antagonists of PAR2, PAR3 and PAR4. Finally, although the experimental evidence in animal models is tantalizing, little is known about the direct role of PARs in human disease.

Summary

- Certain proteases from the circulation and from inflammatory cells can directly signal to cells by cleaving PARs, members of a new sub-family of heptahelical receptors that couple to G-proteins.
- Cleavage exposes a tethered ligand domain that binds to and activates the cleaved receptors. Activated PARs couple to signalling cascades that affect cell shape, secretion, integrin activation, metabolic responses, transcriptional responses and cell motility.
- PARs are 'single use' receptors: proteolytic activation is irreversible and the cleaved receptors are degraded in lysosomes. Thus, PARs play important roles in 'emergency situations' such as trauma and inflammation.
- Proteases and their receptors play important roles in coagulation, inflammation, pain, healing and protection.
- Selective antagonists or agonists of these receptors may be useful therapeutic agents for the treatment of human diseases.

Research in the authors’ laboratory is funded by National Institutes of Health grants DK39957, DK43207, DK57840 and a Focussed Giving Grant from the R.W. Johnson Foundation.

References


