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The Biology of the (Pro)Renin Receptor

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ABSTRACT

The (pro)renin receptor (PRR) binds renin and prorenin, its proenzyme inactive form. Receptor-bound prorenin becomes enzymatically active and binding then activates the MAP kinases ERK1/2 and p38 pathways, leading to upregulation of profibrotic and cyclooxygenase-2 genes independent of angiotensin II generation. These characteristics explain the interest in the potential role of PRR in organ damage in diseases associated with activation of the renin-angiotensin system (RAS), in particular hypertension and diabetes. Although identification of PRR has improved our understanding of the physiology of the tissue RAS, its role in pathology is far from clear. Transgenic animals overexpressing PRR ubiquitously or selectively in smooth-muscle cells develop high BP or glomerulosclerosis, and increased expression of PRR is reported in models of hypertension or kidney damage. However, definitive proof is still lacking for a role for PRR in disease, or by showing improvement of disease by tissue-specific ablation of *PRR* or by administration of a specific PRR antagonist. Furthermore, the early embryonic lethality seen in *PRR*-null mice suggests PRR has additional essential cellular functions we do not understand.

J Am Soc Nephrol 21: 18–23, 2010. doi: 10.1681/ASN.2009030300

Renin is an aspartyl protease that cleaves angiotensinogen into angiotensin I, the rate-limiting reaction in the cascade generating angiotensin. The existence of a receptor for renin and for its inactive precursor, prorenin, was postulated long ago,[1] and a receptor binding renin and prorenin, termed the (pro)renin receptor (PRR), was cloned in 2002.[2] The PRR is a true receptor that is able to activate intracellular signaling, and surprisingly, PRR-bound prorenin is enzymatically active as a result of a conformational change without cleavage of the prosegment.[2] Unlike other components of the renin-angiotensin system (RAS), the *PRR* gene is highly conserved among species, and *PRR* orthologues are found in species as far from mammals as *Caenorhabditis elegans* and *Drospohila melanogaster*,[3,4] which express some of the RAS components but not for hemodynamic functions.[5] This suggests PRR has functions unrelated to the hemodynamic aspects of the RAS. Furthermore, PRR also exists in truncated forms that provide the potential molecular basis for these additional functions.[4]

A great deal of excitement was generated when studies indicated that prorenin activation might play a central role in diabetic nephropathy and cardiac fibrosis, and that tissue damage could be totally prevented by blocking prorenin binding to PRR. Here we summarize our knowledge of the biochemistry of PRR, discuss the variations of PRR in several disease models, and report data supporting a role for PRR in embryologic development and RAS-independent actions.

BIOCHEMISTRY OF THE PRR

PRR Gene and Protein Structure

In humans there is a unique gene encoding PRR on the X chromosome at locus p11.4. The messenger RNA is 2034 bp in length and has a long 3′ untranslated region and no alternative splicing product.[2] The protein is 350 amino acids long and has a single transmembrane domain and a short cytoplasmic domain that has no intrinsic kinase activity (Figure 1).[2] The degree of homology between human, rat, and mice *PRR* is about 95% for the nucleotide sequence and over 80% at the amino-acid level, indicating an extremely conserved protein. A multispecies protein sequence comparison reveals homologues to the human receptor in many species, including rat, mouse, chicken, frog, zebrafish, mosquito, and drosophila, and in species as remote from humans as *C. elegans* and the bacteria, *Ehrlichia chaffeensis*. The highest homology is in the transmembrane and cytoplasmic regions, pointing to an important function for this fragment of PRR.[3,4,6,7]

Published online ahead of print. Publication date available at www.jasn.org.

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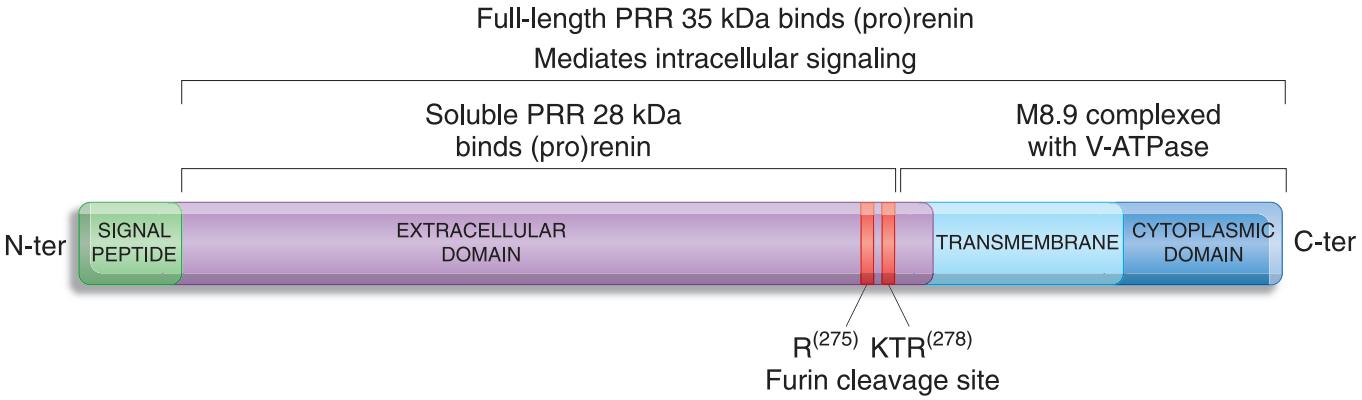

Figure 1. Schematic organization of PRR protein (the indicated 28 kDa molecular weight was determined by SDS-PAGE, and 35 kDa and 8.9 kDa were estimated based on the amino-acid sequence).

Characteristics and Consequences of Renin and of Prorenin Binding

The PRR receptor specifically binds renin and prorenin (collectively named (pro)renin when used interchangeably) with two important consequences: prorenin, the inactive proenzyme form of renin, becomes enzymatically active by a conformational change that does not require cleavage of the prosegment, and PRR activation triggers intracellular signaling pathways. Cross-linking[2] as well as co-immunoprecipitation[8] studies indicate that PRR functions as a dimer on plasma membranes. The affinity of PRR for (pro)renin is in the nanomolar range.[2,9,10] The sequences of the ectodomain responsible for the interaction with (pro)renin have not yet been determined by structure-function studies.

The binding of (pro)renin triggers intracellular signaling and the activation of the mitogen-activated protein (MAP) kinases ERK1/2, leading to upregulation of TGFβ1, PAI1, collagens, fibronectin,[11,12,13] and cyclooxygenase-2,[14] independent of angiotensin (Ang) II generation and EGF receptor transactivation.[15] Definitive proof that ERK1/2 phosphorylation is mediated by (pro)renin binding to PRR is provided by the disappearance of this response after knockdown of PRR expression by transfection with small-inhibiting RNAs.[11] Additionally, PRR activation also triggers a MAP kinase p38-heat shock protein 27 cascade[16,17] and the PI3K-p85 pathway.[8] The latter results in the nuclear translocation of the promyelocytic zinc finger transcription factor that downregulates the expression of PRR, thereby providing a feedback mechanism by which (pro)renin controls the amount of its own receptor.[8,18] However, the negative feedback mechanism has been described mostly *in vitro*, and *in vivo* evidence is scarce.[19] For example, Krebs *et al.*[20] used a model of severe renal ischemia to demonstrate that ischemia leads to a pronounced increase in renal renin and prorenin, but PRR is increased rather than downregulated, underlining that caution is always needed when trying to apply *in vitro* signaling data to every *in vivo* pathologic situation.

Are Binding of (Pro)Renin to PRR and ERK Activation Relevant *In Vivo*?

As discussed by Campbell,[21] the plasma concentration of renin and prorenin is in the picomolar range and the affinity of (pro)renin for PRR is in the nanomolar range. Therefore, one would expect very low receptor occupancy (approximately 1%), suggesting that local Ang II generation dependent on the uptake of circulating (pro)renin is negligible. However, in organs and tissues able to synthesize renin and prorenin, such as kidneys, ovaries, placenta, testes, adrenal glands, and retina, the role of (pro)renin in Ang II generation could be substantial. Unfortunately, studies on PRR occupancy in these tissues are not available and only tissue-specific null animals would help clarify this issue.

On the other hand, intracellular signaling only needs minimal binding and ERK1/2 activation is observed with (pro)renin concentrations as low as 1 pmol/L.[12] The relevance of ERK1/2 activation in animal models will be discussed later in this review. But there is at least one physiologic situation in which activation of ERK1/2 by PRR is essential, namely during brain development, as suggested by X-linked mental retardation and epilepsy observed in a family in which linkage analysis and mutation screening identified only one single mutation in the *PRR* gene. Studies on immortalized lymphocytes of one patient show the mutation is responsible for the absence of ERK1/2 phosphorylation by renin.[22]

Binding and Nonproteolytic Activation of Receptor-Bound Prorenin: Why Is It So Exciting?

Prorenin is the inactive proenzyme form of renin and yet its concentration in human plasma is 7- to 9-fold higher that of renin. Prorenin is inactive because a 43-amino acid prosegment covers the enzymatic cleft and is activated in a proteolytic or nonproteolytic manner.[23] Proteolytic activation is due to the removal of the propeptide by an unidentified proconvertase, for which the process is irreversible and takes place only in the renin-producing cells of the juxtaglomerular apparatus during normal physiology. In plasma, there is a dynamic equilibrium between 2% of prorenin in an open, active form and 98% in closed, inactive conformation. Opening the proseg-

ment *in vitro* is achieved by exposure to low pH (pH = 3.0) or cold temperatures (4°C)[23] and is reversed by neutralizing the pH or increasing the temperature to 37°C. This reversible open-close conformation of prorenin is called nonproteolytic activation and is likely to occur for prorenin bound to PRR, which then transduces enzymatic activity.[2,10,24]

If prorenin activates intracellular signaling after binding to PRR, then prorenin does seem to have a function of its own. Intriguingly, there is local generation of Ang II in tissues able to synthesize prorenin that are not accessible to renin because of the blood brain barrier, such in the brain and eye, as well as correlations between high prorenin levels in diabetes complicated by nephropathy and retinopathy and the occurrence of microvascular complications such as microalbuminuria.[25,26] It is therefore tempting to suspect these high prorenin levels play a role in diabetic nephropathy by stimulating PRR and inducing profibrotic protein syntheses.

How then is one to explain why high prorenin levels during pregnancy are not associated with any poor prognostic outcomes?[27] Furthermore, glomerulosclerosis is not observed in transgenic *ren-2* rats with inducible prorenin expression despite 200-fold higher prorenin levels after induction,[28] nor is cardiac fibrosis or glomerulosclerosis observed in transgenic mice overexpressing native prorenin or active site-mutated prorenin.[29] These data argue against the notion that increases in prorenin are *per se* a primary determinant of fibrotic complications. Rather, the data suggest prorenin may be an amplifier of fibrosis, in addition to high glucose, infection, inflammation, or immunological cytokines.

Can We Block Renin or Prorenin Binding to PRR with Existing Compounds?

The answer is clearly "no" with existing renin inhibitors and likely "no" with a reported PRR blocker. Studies with aliskiren, the available inhibitor of renin, clearly show that blocking the active site of renin and prorenin do not alter their binding to PRR or subsequent ERK1/2 activation.[10,15,30]

The description of a peptide called "handle region peptide (HRP)," which mimics part of the prosegment of prorenin and inhibits prorenin binding to PRR and nonproteolytic activation,[31] initially generated much interest because it suggested there was a PRR antagonist capable of preventing diabetic nephropathy and cardiac fibrosis in stroke-prone spontaneously hypertensive rats,[16,32,33,34] thereby demonstrating a role for PRR in pathology. Alas, enthusiasm was rapidly replaced by skepticism when the *in vivo* data could not be reproduced.[10,15,20,30] It has subsequently been argued that the apparent lack of effect of HRP could be explained by the notion that HRP exerts its effect only in diseases associated with high prorenin and low renin levels because HRP blocks prorenin and not renin binding to PRR. *In vitro* results have also been discrepant. Some groups using straightforward methods such as inhibition of binding of radiolabeled prorenin and ERK activation in the presence of HRP found no inhibitory effects, even at HRP concentrations as high as 10 μmol/L.[10,15,30] Others report that HRP inhibits not only prorenin binding[35] but also renin binding to recombinant PRR,[36,37] or that HRP stimulates ERK1/2 by itself.[38] With these latter findings, it is difficult to understand how HRP could totally inhibit ERK1/2 phosphorylation in the kidney of HRP-treated animals when it could itself stimulate ERK, and why HRP would not be effective in high renin models when it could inhibit renin binding *in vitro*.

In summary, much confusion remains concerning the mode of action of HRP, and the discrepancy between *in vitro* and *in vivo* data cannot be explained at this time. Therefore, it seems reasonable not to call HRP a "PRR blocker" until more clarity emerges.

WHAT DO ANIMAL MODELS TELL US ABOUT THE ROLE OF PRR IN PATHOPHYSIOLOGY?

Evidence that PRR is related to cardiovascular disease comes from studies with transgenic rats overexpressing PRR. In a study where the human *PRR* is overexpressed ubiquitously in transgenic rats, the rats remain normotensive but develop proteinuria and a slowly progressive nephropathy, suggesting a direct pathologic role of PRR in renal damage. The glomeruli of these transgenic rats show a measurable degree of ERK1/2, p38, and c-Jun N-terminal kinase activity, but not EGF receptor phosphorylation compared with controls, and renal levels of Ang II are normal.[39]

A second transgenic model overexpressing the human *PRR* gene exclusively in smooth muscle cells, including vascular smooth muscle cells, provides further insight into the genesis of hypertension. After 6 mo of age, transgenic rats develop a cardiovascular phenotype with elevated systolic blood pressure (BP) and augmentation in heart rate. Kidney function is normal with increased levels of plasma aldosterone and a rise in the aldosterone/renin ratio. These alterations also progressively increase with age.[40] Different levels of transgene expression might account for different phenotypes. In the latter model, the vascular expression of the transgene is not dramatically elevated in the kidney and therefore probably not sufficient to induce proteinuria and glomerulosclerosis. However, in transgenic smooth muscle cells, PRRs show very high levels of expression in lung, which increases their respiratory rate with age and might contribute to the rise in BP.

Altogether, the animal models are disappointing because, if they tend to suggest a role for PRR in cardiovascular and renal diseases, the message is far from clear. To date, the best argument supporting a role for PRR in hypertension is the demonstration of an association between *PRR* gene polymorphism and ambulatory BP in Japanese men.[41]

Several groups have yet to generate a *PRR*-null mouse despite numerous attempts, possibly because $PRR^{-/-}$ embryonic stem cells do not form chimeras after blastocyst injection (Michael Bader, personal communication). *PRR* deletion in *C. elegans*[42] and zebrafish[43] yields embryos that die before the end of embryogenesis, thus supporting an essential but

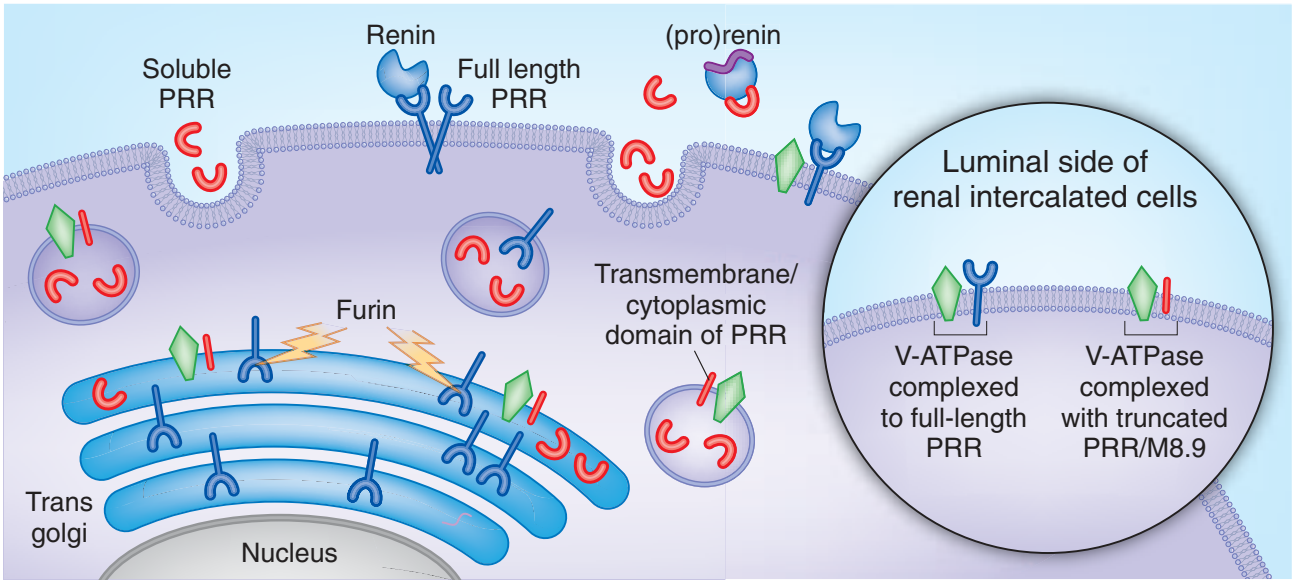

Figure 2. Intracellular processing of PRR. Part of PRR is cleaved by furin in the trans-Golgi to generate soluble PRR, which is secreted, and transmembrane PRR, which remains associated with V-ATPase. Part of PRR remains intact and is addressed to the plasma membrane. The V-ATPase may be complexed with full-length or truncated PRR. Soluble PRR binds renin and prorenin.

unknown cellular function for PRR. It is tempting to speculate this essential function is related to the truncated form of PRR composed of the transmembrane and cytoplasmic domains of PRR that copurify with vacuolar H^+-ATPase (V-ATPase).[44] This V-ATPase plays an essential role in controlling cellular and intracellular vesicle pH.[45] The gene coding this PRR is called *ATP6ap2* (*ATPase-associated protein 2*). Zebrafish embryo mutants for V-ATPase subunits, and for *PRR/ATP6ap2,* display similar phenotypes,[43] suggesting a possible link between the two proteins. The demonstration of a functional link between the two proteins was recently made by Advani *et al.*,[46] who show that PRR colocalizes with V-ATPase in the apical villi of intercalated cells of the distal nephron, and that inhibition of V-ATPase with bafilomycin impairs ERK activation induced by (pro)renin.

CONCLUSIONS AND PERSPECTIVES

The biology of PRR is more complex than expected in several ways. It is a multifunctional protein existing in different molecular forms, and even its cellular localization is intriguing. Indeed, immunofluorescence studies on endogenous[46,47] or transfected PRR[18] show most of the protein is intracellular. We have recently shown that PRR accumulates in the trans-Golgi, where its is cleaved by furin to generate a 10-kD transmembrane/cytoplasmic fragment that likely represents the truncated PRR, which copurifies with the V-ATPase, and a 28-kD soluble form, which is secreted into the conditioned medium of cultured cells (Figure 2); more importantly, this truncated PRR is also found in plasma of humans and rats.[47] The function of this soluble PRR is not yet established; studies are ongoing to determine its concentration in biologic fluids and its variation in different pathophysiological states. The only known mutation in the *PRR* gene primarily affects the central nervous system, and animal studies indicate that PRR may not be an essential determinant of cardiovascular and renal diseases. We still know very little about its other cellular functions beyond serving as a receptor, and it is likely that studies in tissue-specific *PRR*-null mice will give more informative results. Finally, we know very little about the structure of this protein because recombinant PRR is very difficult to generate in native form. It may be wise to not put the cart before the horse in trying to assign functions for this new receptor before we have a clearer understanding of its roles in development, physiology, and pathophysiology.

DISCLOSURES

None.

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