Prorenin and Its Ancient Receptor

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Prorenin, the precursor of renin, has for a long time been considered to be functionally inert, despite high circulating concentrations. Plasma levels of prorenin usually exceed renin levels and are even further increased under pathological conditions, such as in diabetes. First support of a functional significance of prorenin came from studies in transgenic rats, which overexpress a renin gene in the liver resulting in high circulating prorenin levels. Without systemic renin–angiotensin system activation and with normal blood pressure, these animals developed a vascular phenotype reminiscent of malignant hypertension. Moreover, in a series of elegant experiments, Ichihara et al.2–4 supported the concept of prorenin as a relevant factor in the pathophysiology of diseases, such as hypertension and diabetes, by using a peptide that binds and inhibits the activation of the precursor. Thus, this peptide attenuated nephropathy in streptozotocin-induced diabetes2 and cardiac fibrosis in stroke-prone spontaneously hypertensive rats.3 By successfully repeating the streptozotocin experiment in mice deficient for the angiotensin II type 1a (AT1a) receptor, they consider the observed effects as being angiotensin II independent.

In this issue of Hypertension, Saris et al.5 contribute further evidence for an angiotensin-independent action of prorenin on cells by showing that neonatal cardiomyocytes respond to purified prorenin by p38 mitogen-activated protein (MAP) kinase phosphorylation and broad changes in gene expression partially also caused by the MAP kinase activation. Because the authors had shown before that these cells are not able to synthesize angiotensin II after the addition of prorenin, they consider the observed effects as being angiotensin II independent.

Both groups, Ichihara et al.2–4 and Saris et al.5 suggest that the effects of prorenin observed in their experiments are mediated by the recently discovered (pro)renin receptor6 RR/ATP6ap2; however, they do not formally prove this notion. RR/ATP6ap2 has been cloned by a group who had already pioneered the idea of direct, angiotensin-independent effects of renin on cells. Ten years ago, Nguyen et al7 reported that human recombinant renin induced thymidine incorporation and increased plasminogen activator inhibitor type I synthesis in renal mesangial cells independent of angiotensin II generation. This effect was confirmed recently and extended to other factors involved in matrix remodeling by another group.8 By looking for the responsible receptor, Nguyen et al.6 cloned a protein, which fulfilled all expectations. It bound prorenin and renin and induced intracellular signaling, that is, p42/p44 MAP kinase activation. Furthermore and somehow unexpectedly, binding of prorenin to this receptor induced its activation presumably without cleavage of the profragment. Accordingly, we could show recently that transgenic overexpression of RR/ATP6ap2 in smooth muscle cells leads to increased prorenin binding in the vascular wall and a slowly progressing elevation of blood pressure in rats.9

RR/ATP6ap2 is a single transmembrane domain protein of 350 amino acids, with a large unglycosylated and highly hydrophobic N-terminal domain and a short cytoplasmic tail of ~20 amino acids (Figure). It does not display domain homology either with known protein families in general or with cell surface receptors in particular. However, the search for evolutionarily conserved sequences in the protein is informative. Orthologs of RR/ATP6ap2 are present in vertebrates and invertebrates (Figure). The N-terminal part of the protein corresponding with the extracellular domain displays a high amino acid sequence identity exclusively in vertebrates. In contrast, the C-terminal part of the protein is strikingly conserved in invertebrates and vertebrates raising the proposal that the 2 domains might have a divergent evolutionary fate; a recently acquired renin binding capacity for the N-terminal domain and a more conserved ancestral function for the C-terminal domain. Accordingly, we could attribute renin binding capacity to the isolated N-terminal domain of RR/ATP6ap2 in coprecipitation experiments (C. Burcklé, unpublished data, 2006). In contrast, RR/ATP6ap2, through its evolutionarily conserved C-terminal domain, might be involved in processes unrelated to the renin–angiotensin system that does not exist in the fruit fly. At least in vertebrates, the 2 domains are linked by the putative proprotein processing site of RxR300 (Figure), implicating that the 2 parts of the protein may even act separately.

In fact, other functions for RR/ATP6ap2 have already been suggested by independent descriptions of the same protein causing confusion in names and subcellular locations assigned to it. A truncated part of RR/ATP6ap2, originally called M8-9, was first identified as copurifying with the V0 domain of vacuolar H+-ATPase in bovine adrenal chromaffin membranes.11 Interestingly, this protein is identical with the evolutionarily conserved C-terminal domain and, except 3 lacking amino acids, also with the theoretically predicted smaller processing product (Figure). After a nomenclature revision, it was renamed ATP6ap2 (adapt protein type II for vacuolar H+-ATPase). A lysosomal location was proposed for the protein, but experimental evidence was not provided.
A yet unpublished entry in Genbank (AY038990) calls the full-length protein CAPER (endoplasmic reticulum [ER]-localized type I transmembrane adaptor precursor C) suggesting its location in the ER. Saris et al.5 use specific antibodies to detect RR/ATP6ap2 in neonatal cardiomyocytes, and their data clearly support a mainly intracellular location of the protein with a minor portion on the cell surface. However, the intracellular compartment carrying the protein was not specified.

Examination of the amino acid sequence of the cytoplasmic tail reveals 2 theoretical targeting signals, both compatible with the proposed locations. There is a tyrosine-based motif Y335DSI and a C-terminal dibasic motif (K346IRMD; Figure). The tyrosine-based sorting signal, YxxØ (where x is any amino acid, and Ø is a large hydrophobic amino acid), is used for protein sorting to endosomes and lysosomes through interaction with adaptor protein complex.12 Conventional dibasic signals are K(x)Kxx or R(x)Rxx and function as ER retention/retrieval signals.13 In vertebrates, K346IRMD, although optimally located with lysine being 5 amino acids apart from the C-terminus of the protein, is an uncommon sequence. Therefore, the relevance of these motifs is still hypothetical and requires functional testing.

Nevertheless, an involvement of RR/ATP6ap2 in renin-independent basic cellular functions is strongly supported by the following observations. Mouse embryonic stem cells deficient for RR/ATP6ap2 failed to generate chimeras when injected into blastocysts, suggesting an essential function of the protein in cell proliferation, differentiation, or survival (own unpublished observations).

Mutant zebrafish for RR/ATP6ap2 display early developmental abnormalities, including eye and body hypopigmentation, as well as neuronal cell death, and die early in development.14 This phenotype resembles the one of other zebrafish mutants for well-characterized subunits of the vacuolar H+/H1 ATPase14 supporting, but by far not proving, the interaction of RR/ATP6ap2 with this complex.

Ramser et al.15 described recently a family with X-linked mental retardation, in which a splicing enhancer for the inclusion of exon 4 into the mature mRNA of RR/ATP6ap2 is deficient, leading to a partial truncation of the protein. (Pro)renin binding is conserved in these patients, but p42/p44 MAP kinase activity is modestly impaired. Possibly not only this minor defect is causative for the disease, but an impairment of the more basic and probably neuronal actions of the protein may also explain the pathology.

In conclusion, RR/ATP6ap2 may be a protein with 2 functionally distinct domains, an evolutionarily old part consisting of the transmembrane domain and the intracellular
tail being essential for cell survival and a vertebrate-specific extracellular domain involved in (pro)renin binding and signaling. Further studies are necessary to clarify the physiological functions of this bipartite protein. Its involvement in the angiotensin II–independent actions of prorenin and renin in the cardiovascular system is particularly important in light of the fact that renin inhibitors are just coming into clinical use, and the possible effects of this novel class of drugs on the interaction of (pro)renin with its receptor may be of therapeutic relevance.

Disclosures
None.

References
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