The discovery of a “(pro)renin receptor” has renewed the interest in prorenin, the inactive precursor of renin. Prorenin binding to this receptor allows prorenin to display enzymatic activity without the accompanying cleavage of the prosegment that normally occurs in the kidney when prorenin is converted to renin. The underlying concept is that binding to the (pro)renin receptor induces a conformational change in the prorenin molecule, involving unfolding of the propeptide from the enzymatic cleft so that the cleft is now accessible to angiotensinogen (“nonproteolytic activation” of prorenin). Similar conformational changes occur at low pH (acid-activation) and low temperature (cryoactivation), but whether these phenomena are of physiological relevance is uncertain. In contrast, the (pro)renin receptor provides a physiological role for prorenin, explaining how angiotensin production might occur at the tissue level and putting into perspective the relatively high prorenin levels (10-fold those of renin) in the human circulation. These levels are even higher in diabetes mellitus complicated by retinopathy and nephropathy.

Unexpectedly, prorenin binding to its receptor also induces intracellular signaling, independent of angiotensin generation, suggesting that prorenin may act as an agonist of the receptor. Although similar observations were made on renin, prorenin binds with higher affinity to the receptor and, thus, appears to be its endogenous agonist.

The (pro)renin receptor is a 350-amino acid protein with a single transmembrane domain. Although it was first identified on cultured human mesangial cells, the C-terminal part of the receptor had been described earlier by Ludwig et al as an 8.9-kDa fragment associated with a vacuolar H+-ATPase. Consequently, the second name of the protein is AT6ap2 (ATPase, H+-transporting, lysosomal accessory protein 2). Vacuolar H+-ATPases play important roles in the acidification of intracellular compartments and cellular pH homeostasis, eg, in the secretory granules of renin-synthesizing juxtaglomerular cells, where proteases (cathepsin B, prohormone convertases) cleave off the 43-amino acid prosegment to yield renin from prorenin. Vacuolar H+-ATPases are expressed in virtually every cell of the body. They consist of many subunits, encoded for by different genes and sometimes having >1 isoform. Although their location is mainly intracellular, they also occur in the membrane of certain cells, including renal intercalated cells, osteoclasts, and macrophages. In the apical membrane of A-type renal intercalated cells, vacuolar H+-ATPases function in proton secretion in preurine and, hence, in urinary acidification.

Angiotensin II increases the activity of vacuolar H+-ATPase in renal proximal tubule cells, mainly by increasing the apical plasma membrane expression of vacuolar H+-ATPase. This most likely relates to the observation that the vacuolar H+-ATPase B subunit contains binding sites to F-actin so that an interaction between vacuolar H+-ATPase and actin filaments allows trafficking between the cytosol and the cell surface. Because the p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 inhibited such trafficking, it appears that p38 MAPK, after its activation by angiotensin II, plays a role in the control of cytoskeleton proteins. Interestingly, in neonatal rat cardiomyocytes, prorenin also induced stimulation of the p38 MAPK pathway, thereby similarly resulting in alterations in actin filament dynamics. This phenomenon occurred both with and without renin-angiotensin system blockers, indicating that it is angiotensin-independent. Apparently, therefore, both prorenin and angiotensin activate actin filament trafficking in a p38 MAPK-dependent manner, and in the case of angiotensin II, this results in cell surface expression of vacuolar H+-ATPase, thus increasing apical vacuolar H+-ATPase activity.

In the present issue of Hypertension, Advani et al provide data suggesting that renin and prorenin also increase vacuolar H+-ATPase activity, most likely via activation of the (pro)renin receptor. They first demonstrate that the (pro)renin receptor is predominantly expressed in collecting ducts, distal convoluted tubules, and distal tubules. This opposes the early observation by Nguyen et al regarding (pro)renin receptor localization in arteries and mesangium. The authors attribute this discrepancy to a difference in the primary antibodies used. Within the collecting ducts, expression was most abundant in microvilli at the apical surface of A-type intercalated cells. Interestingly, the receptor colocalized markedly with the B subunit of vacuolar H+-ATPase (overlap coefficient: 0.92). Moreover, in cultured collecting duct/distal tubule lineage Madin-Darby canine kidney cells, both renin and prorenin induced extracellular signal-regulated kinase (ERK) 1/2 phosphorylation, and the selective vacuolar H+-ATPase inhibitor bafilomycin prevented this activation. This suggests that ERK1/2 phosphorylation in these cells depends on vacuolar H+-ATPase activity. Because bafilomycin did not prevent renin binding to vascular smooth muscle cells overexpressing the human (pro)renin receptor (Batenburg et al, unpublished observations, 2008), it appears that the increase in vacuolar H+-ATPase activity occurs after renin/prorenin binding to their receptor. Advani et al applied
Neither blockers of p38 MAPK nor of ERK1/2, although both have been implied in (pro)renin-induced signaling. However, the link among p38 MAPK, actin filament dynamics, and vacuolar H^+-ATPase activity observed previously combined with the current observation that ERK1/2 phosphorylation depends on vacuolar H^+-ATPase activity, now suggests that ERK1/2 activation by renin/prorenin is the consequence of the following order of affairs (Figure): renin/prorenin binding to (pro)renin receptor → p38 MAPK activation → actin filament trafficking → vacuolar H^+-ATPase activity ↑ → ERK1/2 phosphorylation. (Pro)renin-induced ERK1/2 activation in mesangial cells resulted in the upregulation of transforming growth factor-β gene expression; the subsequent upregulation of genes coding for profibrotic molecules, eg, plasminogen-activator inhibitor 1, fibronectin, and collagens; and the induction of cell proliferation.

Several questions remain. Most important, virtually all of the studies so far that investigated the direct, angiotensin-independent effects of renin/prorenin (in particular, their induction of ERK1/2 phosphorylation) applied high, nanomolar concentrations of the enzyme. Given the low, picomolar concentrations of both renin (=0.5 pmol/L) and prorenin (=5.0 pmol/L) in blood, the physiological relevance of such observations seems questionable. Advani et al. show that the ERK1/2 phosphorylation in tubule lineage Madin-Darby canine kidney cells already occurred at renin/prorenin concentration as low as 20 pmol/L. This may relate to the fact that, in these cells, not only vacuolar H^+-ATPase but possibly also the (pro)renin receptor has a predominant cell surface location, whereas in other cells (cardiomocytes and smooth muscle cells), their location is almost entirely intracellular. High cell surface expression of the (pro)renin receptor would allow for activation of signaling cascades at low (physiological) renin/prorenin concentrations, whereas an intracellular location might require pharmacological extracellular renin/prorenin concentrations to obtain sufficiently high intracellular renin/prorenin concentrations to stimulate the receptor. Such high concentrations may not normally occur, except perhaps in (pro)renin-producing organs, eg, the kidney, adrenal, ovary, testis, and eye.

When introducing a high level of (pro)renin receptor expression, like in transgenic animals, the location of the receptor might change. This could explain why human (pro)renin receptor transgenic animals become hypertensive in the absence of changes in renin-angiotensin system component levels. Interestingly, Kang et al. have suggested recently that collecting duct principal cells are the source of renin in diabetes mellitus, and, thus, at least at this location, the renin concentrations might be sufficiently high to allow (pro)renin receptor stimulation, even when the receptors are located intracellularly. What is the evidence that the (pro)renin receptor truly mediates the above-described phenomena? The putative (pro)renin receptor antagonist “handle region peptide” was without effect in tubule lineage Madin-Darby canine kidney cells (A. Advani, written communication). Application of (pro)renin receptor small interfering RNA induced a marginal (~10%), albeit significant, decrease in renin/prorenin-induced ERK1/2 phosphorylation. This leaves space for alternative (pro)renin receptors, like the mannose 6-phosphate/insulin-like growth factor II receptor. This receptor not only binds phosphomannosylated proteins like renin and prorenin but is also closely linked to vacuolar H^+-ATPase.

Finally, it now appears that both angiotensin II and renin/prorenin increase vacuolar H^+-ATPase activity. Does this increase in activity underlie the (pro)renin receptor–induced prorenin activation, similar to the acid activation of prorenin? In other words, is the (pro)renin receptor–dependent prorenin activation the consequence of regional H^+ release rather than a binding-induced conformational change? And, if so, do angiotensin II and prorenin exert independent effects that,
when combined, result in additive effects or even synergy? Clearly, the findings of Advani et al provide important clues that stimulate future studies on prorenin and its receptor.

Disclosures
None.

References
(Pro)renin Receptor and Vacuolar H⁺-ATPase
A.H. Jan Danser

Hypertension. 2009;54:219-221; originally published online June 22, 2009;
doi: 10.1161/HYPERTENSIONAHA.109.135236

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/54/2/219

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/