Truncated Prorenin Comes Up . . . Short

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In the present issue of Hypertension, Xu et al.1 report on a pivotal set of experiments that test the contribution of a key component to the action of the putative “intracellular renin-angiotensin system” (RAS; iRAS) in its normal physiological setting. In contrast to the classic circulating RAS, the iRAS has been proposed to be wholly contained within cells, where it produces angiotensin peptides that trigger noncanonical signaling (reviewed in Reference 2). The results reported by Xu et al1 suggest that an intracellular form of active prorenin cannot compensate for the loss of the classic secreted form of renin in some of the primordial roles of the RAS: directing kidney development, blood pressure regulation, and hematopoesis.

The average mammalian cell makes many thousands of different proteins, each of which has to be delivered to the appropriate destination to exercise its function. The first major triage comes at the level of the secretory apparatus: all of the proteins that are destined to be released from the cell, expressed on the cell surface, or retained in one of the several vacuolar structures within the cell (secretory pathway, lysosomes, etc) first enter the membrane “bags” of the secretory pathway. This is accomplished by the recognition of a signal peptide on the newly made protein by a complex that docks the synthesis machinery on the membranes of the endoplasmic reticulum and ensures the extrusion of the protein into the lumen of the membrane sac. Proteins without a signal peptide are made within the cell cytoplasm, where they will either remain to exercise their function or where they are retargeted to other locations within the cell, such as the nucleus. Thus, whereas proteins both within the secretory pathway and in the cytoplasm are, by definition, intracellular, they remain physically separated from one another by a membrane barrier.

All of the components of the classic RAS (angiotensinogen, prorenin, angiotensin-converting enzyme, and the angiotensin receptors) are proteins that contain signal peptides and, as such, they transit the cell inside the membranes of the secretory pathway. These proteins are subsequently either liberated from the cell (angiotensinogen and renin) or delivered by the secretory pathway to the cell surface (angiotensin-converting enzyme and angiotensin receptors). However, in the course of mapping gene transcription starts sites for prorenin in the rat, Lee-Kirsch et al3 noticed a second transcription start site within the renin gene that was highly enriched in the brain. This secondary transcription site was predicted to result in the synthesis of a shorter form of prorenin that would lack the signal peptide and the first third of the prosegment. The missing part of the prosegment performs an important function: it contains the “glue” that keeps the prosegment attached to renin, and thereby represses its enzymatic activity.4 Thus, this “truncated” prorenin would not only be predicted to be located in the cytoplasm (because of the missing signal peptide), it would also be enzymatically active (Figure). With this finding and strengthened by earlier reports that angiotensin II could elicit biological responses when injected into the cytoplasm of cells,5 an entirely new concept of an iRAS was born. This concept has even led to the proposal that a new class of medications that specifically target the iRAS might offer additional benefits over current therapy.6

Nevertheless, the concept of a cytoplasmic prorenin that drives the iRAS poses several problems. First, the prosegment is not only important for repressing the enzymatic activity of renin, it is also necessary for the proper folding of the protein. In transfected cells, a prorenin protein with an engineered removal of the prosegment is only secreted at 1% of the level of the intact protein, although it is enzymatically active.4 Second, truncated prorenin would be in a compartment of the cell that is separated by a membrane barrier from the other components of the RAS produced in the secretory pathway (Figure). Even if the cell were to take up RAS components from its surroundings, such proteins would be encapsulated in the membranes of the endosomes, and they would not be readily accessible to the cytoplasmic truncated prorenin (Figure). Third, the synthesis and action of angiotensin peptides within the cytoplasm would also require the expression of a cytoplasmic form of angiotensinogen (not yet described) and peculiar forms of angiotensin-converting enzyme and the angiotensin receptor with the peptide-binding domains pointing inward toward the cytoplasm (the opposite of normal). That is starting to add up to a lot of biological oddities!

In spite of this conundrum, this group had previously obtained evidence for the in vivo activity of cytoplasmic truncated prorenin using transgenic animals.6 They first made a line of transgenic mice that targeted the expression of human angiotensinogen to astrocytes. Although these animals produced human angiotensinogen in the brain, they were asymptomatic, because the endogenous mouse renin does not cleave the human substrate. However, when these animals were bred to another line of mice expressing human renin in brain astrocytes, they became hypertensive and exhibited increased thirst, both of which are symptoms of increased brain angiotensin II. Surprisingly, they showed the same symptoms when bred to mice engineered to express the
truncated form of human prorenin in astrocytes, suggesting that somehow the angiotensinogen in the secretory pathway and the truncated prorenin in the cytoplasm were able to meet and produce angiotensins. Although these experiments provide support for the role of cytoplasmic truncated prorenin, they are still an imperfect reflection of the normal physiological situation where expression of the putative truncated prorenin is predicted to be at very low levels and where astrocytes do not make both angiotensinogen and renin.

To address the question in a more physiological context, this group has now taken the clever approach of deleting the “normal” transcription start site in the prorenin gene, resulting in a line of mice that can only make truncated prorenin. By comparing these engineered mice with their normal counterparts, they were able to assess the contribution of the truncated form of prorenin in its normal cellular setting and in the absence of the overexpression that occurs in transgenic mice. In fact, the engineered mice exhibited severe postnatal renal damage, high preweaning mortality, anemia, and hypertension, traits that are identical to those seen when the entire prorenin gene is deleted (resulting in a loss of both the secreted and truncated forms). Their conclusion is that truncated, cytoplasmic prorenin is unable to replace the normal secreted form of prorenin/renin, at least in these critical roles.

The “readout” of postnatal renal lesions may be the most appropriate measure of the activity of truncated prorenin for a couple of reasons. First, it is angiotensin II production in the brain that is most closely tied to the renal development problems, because restoration of angiotensin II exclusively in the brain of mice lacking an RAS eliminates the observed hydronephrosis. Second, on the basis of the relative abundance of the secondary transcription start site, the brain is one of the organs with the highest likelihood of making truncated prorenin. Thus, if truncated prorenin contributes to angiotensin II formation, this should be evident in the brain, and this should result in a preservation of normal renal development. Of course, truncated prorenin might make more subtle contributions to RAS physiology, and this group is clearly pursuing such a possibility. For now, however, it appears that the role of truncated prorenin and, by extension, the iRAS must remain firmly in the realm of the hypothetical.

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References
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