Review Article

Methods for Imaging Renin-Synthesizing, -Storing, and -Secreting Cells

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Renin-producing cells have been the object of intense research efforts for the past fifty years within the field of hypertension. Two decades ago, research focused on the concept and characterization of the intrarenal renin-angiotensin system. Early morphological studies led to the concept of the juxtaglomerular apparatus, a minute organ that links tubulovascular structures and function at the single nephron level. The kidney, thus, appears as a highly "topological organ" in which anatomy and function are intimately linked. This point is reflected by a concurrent and constant development of functional and structural approaches. After summarizing our current knowledge about renin cells and their distribution along the renal vascular tree, particularly along glomerular afferent arterioles, we reviewed a variety of imaging techniques that permit a fine characterization of renin synthesis, storage, and release at the single-arteriolar, -cell, or -granule level. Powerful tools such as multiphoton microscopy and transgenesis bear the promises of future developments of the field.

1. Renin Cells in the Context of the Juxtaglomerular Apparatus (JGA) and Hypertension

Ever since Golgi published his seminal microdissection studies [1] on mammalian nephrons, our understanding of the JGA has emerged from a tight complementarity between anatomical and functional approaches [2–4]. The recent and fast development of digital confocal fluorescence microscopy [5–7] has considerably reduced the gap between structure and function. Such evolution can be illustrated by the recent "two-photon" fluorescence imaging of both cell structures, intracellular calcium signaling, and an array of regulatory mechanisms in live isolated-perfused JGA [8, 9].

Our knowledge about the nervous, tubular, and vascular structures that constitute a functional JGA unit was obtained by a variety of morphological techniques. They range from transmission electron microscopy (TEM, [3, 10–16]), scanning electron microscopy (SEM, [17–23]), histochemistry [24–27], fluorescence microscopy [6, 28–32], and immunohistochemistry [10, 25, 33–38], to atomic force microscopy [39]. Functional studies were carried out both in vivo and in vitro. They encompass the entire anatomical spectrum from whole kidney to single cell levels. They bring additional layers of complexity into our picture of the JGA with the tubuloglomerular feedback mechanism [40, 41], and cell signaling mechanisms in vessels and tubules [41–51].

The identification of cells with a synthetic (i.e., endocrine) phenotype along the afferent arteriole in close contact to nerve terminals, and located at the glomerular vascular pole near the macula densa led Goormaghtigh, seventy years ago, to the crucial concept that the JGA was a "neuro-myo-endocrine" organ (for historical account see: [10, 24, 52]). These granulated or epithelioid cells were later found to synthesize, store, and release renin [2–4, 10, 44, 53–55], a key enzyme of the renin-angiotensin system, and a key physiological player in the integrative control of blood pressure, glomerular hemodynamics, and in the homeostasis of sodium and electrolytes [41]. A recent review [56] on "renal vascular dysfunction in hypertension" fully confirms these views within the context of hypertension.

At this point, it is important to note that epithelioid cells are specialized smooth muscle cells present along the arteriovenous anastomoses of the rabbit ear vasculature [57].

Epithelioid cells are thus unlikely to be unique to the renal vasculature. Furthermore, renin-producing cells were also found in the media of the abdominal aorta in adult mice [58]. Immunoreactive renin was found in the cytoplasm of media cells in small blood vessels of human pulmonary tumors [59], and in a derived human tumor cell line, CaLu-6, that expresses the human renin gene endogenously [60, 61]. Interstitial fibroblast-like cells were found to express a complete renin-angiotensin system in a murine model of renal fibrosis [62].

Recent studies elegantly showed that renin cells differentiate in situ from progenitors scattered within the mesenchyme of the fetal kidney [25]. Subsequently, they colonize the early renal vasculature from arcuate arteries to afferent arterioles [63]. Most renin cells later adopt a smooth muscle cell phenotype [25, 64]. At early developmental stages, renin cells are associated with the development and branching patterns of the preglomerular vessels [34, 65], and with renal nerves [66]. However, it remains unknown whether renal nerves provide guidance cues and coordinate renal vascular branching, as it has recently been shown in the developing skin vasculature of mice [67], or more generally discussed in a recent review [68].

In the adult kidney, both renin cells and contractile smooth muscle cells populate the media of the afferent arteriole [10]. Under basal conditions, renin cells occupy a short (i.e., 20–40 μm) arteriolar segment near the glomerular vascular pole [10, 36]. Under conditions known to stimulate renin synthesis (e.g., angiotensin I converting-enzyme inhibition, or low sodium diet, [69–71]), new renin-producing cells are recruited via the so-called "metaplastic transformation" of preexisting smooth muscle cells [10, 72–75]. Furthermore, renin cells may exhibit an array of ultrastructurally well-defined phenotypes from fully differentiated cells, at the tip of afferent arterioles, to "intermediate" cells which retain some characteristics of smooth muscle cells [10]. It is interesting to note that within a few days in primary culture, vascular smooth muscle cells shift from a contractile to an epithelioid (i.e., a synthetic) phenotype and loose their contractile proteins [76]. To the best of our knowledge, however, no renin production has been reported in these epithelioid cells in culture. Therefore, the "metaplastic" process leading to renin production appears as a characteristic of renal vascular smooth muscle cells. Alternatively, recent studies on renin cell lineage demonstrate that metaplasia affects cells that momentarily expressed renin during the early phase of the renal vascular development and later transformed into smooth muscle cells [25, 77].

The purpose of the present article is to review a series of techniques that permit the microscopic visualization of renin cells. These techniques can be incorporated into a variety of experimental designs as some of them can be used in live cells (i.e., use of vital dyes), whereas others require tissue processing. Immunohistochemical techniques which allow the assessment of renin cell distribution along renal vessels at a whole-kidney scale will be treated with more details, and illustrated.

The amount of intracellular renin represents a dynamic equilibrium between renin production/capture-storage, and release. As a logical complement, we will also examine techniques that permit the visualization of renin secretion and renin gene expression at the single cell level, though most of the time, these approaches involve enzymatic cell dispersion and a complete disruption of renal anatomy. As a final remark, it was beyond our scope to provide step-by-step protocols and the reader will be referred to the relevant literature for practical details.
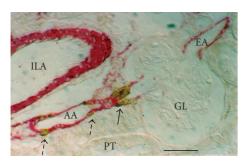
2. Techniques for Visualization of Cellular Renin

2.1. Vital Dyes, Neutral Red, and Quinacrine. Histochemistry was developed early by microscopists. It provides chemical recipes to differentially stain cell organelles. The price to pay for its simplicity and directness is a limited specificity. This limitation can nevertheless be compensated for by using antibody-based approaches in a complementary way. Use of vital dyes is thus far the most direct way to stain intracellular renin granules.

Neutral red (2% solution in saline) accumulates in 1-2 hours within renin granules after its intraperitoneal injection, and subsequent observation is carried out on paraffin sections [24]. Direct cellular uptake of neutral red was recently used to highlight potentially renin-producing cells in the fetal renal mesenchyme [25]. Importantly, it was found that only 15% of neutral red-stained cells contained renin [25]. Of notice, use of neutral red uptake/release was instrumental in documenting stretch-induced secretion of atrial natriuretic peptide in single, isolated rat atrial myocytes [78].

The fluorescent dye quinacrine hydrochloride (Sigma) accumulates within 1 hour within renin granules after its intravenous administration (1 mg/kg), and can be imaged on paraffin sections [28]. This approach is functionally relevant since opposite changes in renin cell granularity and plasmatic levels of C^{14}-labeled quinacrine were documented in circumstances associated with high renin secretion rates (i.e., hemorrhage and ischemia, [29]). In order to visualize both renin cell distribution and reactivity in live afferent arterioles, we recently incorporated quinacrine labeling into the in vitro blood-perfused juxtamedullary nephron preparation [79, 80]. Recently, using isolated-perfused rabbit glomeruli and the high resolving power of two-photon confocal microscopy, Peti-Peterdi et al. [81] were able to record, for the first time, the exocytosis of a single renin granule. Some potential limitations of the latter approach evoked in a recent review [42] will be dealt with in Section 3.6.

2.2. Immunohistochemistry and Photonic Microscopy. Immunohistochemistry has become very popular due to the commercial availability of many antibodies, and easy-to-use staining kits for single- or double-immunolabeling (e.g., staining kits from Vector Laboratories, Burlingame, CA, or from Dako A/S, Glostrup, Denmark). This approach allows the visualization of renin cell distribution in whole kidney sections which preserves topological characteristics. Classic tissue processing involves perfusion-fixation of
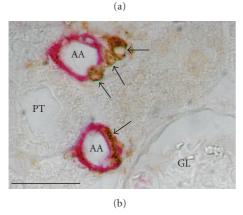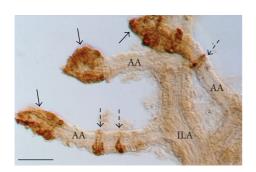
(a)

(b)

FIGURE 1: Microscopic views of paraffin kidney sections from a rat chronically treated with the converting enzyme inhibitor captopril (30 mg per day for 7 weeks per os; Sigma, St Louis, MO). Sections were double-stained against α-smooth muscle actin (red label, monoclonal anti-α-SM actin, clone # 1A4, 1 : 1000 dilution, Sigma), and against renin (brown label, polyclonal anti-rat renin antibody, ref. # 936, 1 : 10,000 dilution, gift from T. Inagami, Vanderbuilt University, Nashville, TN), using Dako En Vision Doublestain System (Ref. K1395, Dako A/S, Glostrup, Denmark). (a) A juxtamedullary glomerulus (GL) with its afferent (AA) and efferent arterioles (EA), close to an interlobular artery (ILA), and proximal tubules (PT). Renin cells accumulate near the glomerular vascular pole (single arrow) and other renin cells were recruited upstream along the afferent arteriole (dashed arrows). (b) Enlarged view of two transversally cut afferent arterioles (AA) near a glomerulus (GL). Arrows point to individual granulated renin cells (brown label) that exhibit a variety of topologies with respect to smooth muscle cells (red label). The renin cell in the lower arteriole is included within the arteriolar wall between smooth muscle cell processes. In the upper arteriole, one renin cell (lower arrow) is part of the arteriolar wall, whereas the upper two renin cells are located on top of the smooth muscle cell layer. Regarding the latter two observations, it is interesting to note that recent studies in connexin 40-deficient mice [82] unveiled a "topological escape" of renin-cells away from afferent arterioles towards the extraglomerular mesangium. Bars: 50 μm.

the kidney, paraffin embedding, sectioning, antigen retrieval, and immunoperoxidase procedures (details can be found in [33, 83]). Examples of immunostaining of renin combined with that of α-smooth muscle actin to highlight blood vessels are given in Figure 1(a). Complex topologies of renin and smooth muscle cells can thus be revealed on tissue sections (Figure 1(b)).

Tissue sectioning, however, limits the researcher's ability to get an integrated view of the distribution of renin cells throughout the preglomerular vasculature. Recent developments in digital image processing [84] would theoretically facilitate reconstruction of renin cell distribution from serial sectioning, but this approach remains time consuming. In fact, similar approach was recently performed on immunostained serial paraffin sections and superbly illustrated the "development of renin expression in the mouse kidney" [63]. More recently, [85] similar reconstruction approach was performed in transgenic mice to assess the impact of altered cyclic AMP pathway on the developmental regulations of vascular renal renin expression. Similarly, studies assessed the impact of connexin 40 in Cx40-deficient transgenic mice on vascular renin cell distribution [82]. Furthermore, as these studies [63, 82, 85] use serial paraffin sections, and since "standard" confocal microscopy currently allows the imaging of three fluorophores [86], additional information to characterize the surrounding renal parenchyma or intestitium (e.g., collagen IV) could be obtained and processed three-dimentionally by using an appropriate additional immunostaining.

In a more straightforward way, we designed a technique that associates isolation of preglomerular vascular trees after HCl hydrolysis, and immunostaining for renin [33]. Dissection of HCl-macerated kidneys yields long segments of the preglomerular vasculature with preserved spatial geometry. Cells full of renin granules refract incident light differently than smooth muscle cells do and can thus be spotted under a dissecting microscope without staining. After immunostaining, vascular distribution of renin cells and their status (i.e., "intermediate" or fully differentiated cells) can be determined precisely under the microscope (Figure 2(a)). Interestingly, an excellent correlation ($r = 0.84$) was found between the relative frequency of afferent arterioles with renin cells recruited along their mid-portion, and renal renin activity [69]. Our approach has been instrumental in various subsequent studies [34, 43, 58, 87]. As illustrated in Figure 2(b), the use of confocal microscopy, with its optical sectioning capability, allows a detailed study of the morphology of individual renin cells within their vascular environment.

2.3. Combining Photonic Microscopy, Microdissected Afferent Arterioles, Primary Cultures of Isolated Juxtaglomerular Renin Cells, and Renin Assay. Several recent studies [88, 89] took advantage of previously developed techniques [90] to obtain primary cell cultures of mouse juxtaglomerular renin-cell cultures, a radioimmunoassay of renin synthesis and renin secretion from these cultures, and combined it with the optical power of fluorescence confocal microscopy. These authors [88, 89] demonstrated colocalization of adenylyl cyclase isoform V and renin within granules and provided important clues on signaling mechanisms and proteins involved in renin release. Previous studies approached the role of adenosine on renin release [91] based on similar renin assay [90] and using isolated superfused juxtaglomerular cells from rat kidneys. Other studies [60] combining primary cell cultures of rat juxtaglomerular cells, human tumor cell

(a)

(b)

FIGURE 2: Portions of the renal preglomerular vasculature comprised of an interlobular artery (ILA) and afferent arterioles (AA) were isolated after HCl hydrolysis in rats chronically treated with captopril (see legend to Figure 1). Renin cells are localized to the AA tip (single arrows); captopril treatment also recruits renin cells upstream (dashed arrows). (a) Single labeling against renin (see Figure 1 legend) with ABC peroxidase kit (brown label, Vector Laboratories, Burlingame, CA). Recruited renin cells are spindle-shaped as smooth muscle cells, whereas renin cells at the tip of AA adopt a more globular shape. (b) Double labeling against renin (green label, fluorescein isothiocyanate-labeled secondary anti body) and against α-SM actin (red label, tetramethylrhodamine-labeled secondary antibody); for primary antibodies see legend to Figure 1. The image was generated as previously detailed [86] using a confocal microscope (Biorad MRC1024, Bio-Rad Life Science Research, Hercules, CA) by projecting 52 serial optical sections. Bars: 50 μm.

line (CaLu-6, [61]), renin enzymatic assay, and a variety of optical techniques provided important clues about the existence of a biomechanical coupling in renin-releasing cells (i.e., the so-called "intrarenal baroreceptor", [92]). Previously, using microdissected rabbit afferent arterioles with or without attached tubules containing the macula densa segment and a renin radioimmunoassay [93] permitted important advances in our understanding of renin release (reviewed in [42, 94]).

2.4. Transmission Electron Microscopy (TEM). TEM is the method of choice to explore cell ultrastructure in tiny volumes of tissue. TEM methodologies were implemented during the fifties. They involve strong aldehyde fixation for proper structural preservation, incorporation of heavy metals to generate contrast, and ultramicrotomy for ultra-thin sectioning. We owe this technique most of our current knowledge about the ultrastructure of renin cells, renin granules, and their cellular processing [2, 3, 10, 53, 95, 96]. Protein antigenicity may nevertheless be preserved by milder fixation, though at the cost of structural definition. Specific antibodies and the protein A-gold technique can then be used to detect renin and/or other proteins within granules (for details and illustrations see [10, 97, 98]).

2.5. Scanning Electron Microscopy (SEM). SEM allows the observation of surface details in whole kidney sections with unprecedented depth of field, and offers a wide range of magnifications (e.g., [17–19]). The development of high resolution field-emission SEM and a variety of cytoplasmic extraction and immunostaining procedures allow a unique, three-dimensional imaging of intracellular organelles (spectacular illustrations can be found in: [99, 100]). Despite such imaging potential, few studies, so far, have attempted to visualize intracellular renin granules by SEM (e.g., [20]).

3. Visualization of Single-Arteriolar, Single-Cell, or Single-Granule Renin Secretion

3.1. The Reverse Hemolytic Plaque Assay. An adaptation of the reverse hemolytic plaque assay allows a microscopic evaluation of renin release at the single cell level [101]. This elegant technique is performed on renal cell suspensions in vitro. Released renin is captured by a specific anti-renin antibody. The renin-antibody complex will then bind to protein A-conjugated sheep erythrocytes which will in turn be hemolyzed by complement attack. Renin secretion is visualized in a semiquantitative way as a circle of hemolyzed erythrocytes around individual renin-producing cells (complete technical description can be found in: [97, 101]).

3.2. The Cell Blot Assay. A very elegant method was developed to image single cell protein release, the so-called cell blot assay [102]. Secreted proteins are irreversibly captured by a polyvinyldiene difluoride transfer membrane on top of which cell suspensions are deposited. Proteins are then recognized by an appropriate primary antibody, and visualized with a secondary antibody as peroxidase [102] or fluorescent halos around secreting cells [103]. This technique is yet to be used with renin cells but might constitute a simpler alternative to the reverse hemolytic plaque assay (Section 3.1).

3.3. Video-Enhanced Differential Interference-Contrast Microscopy. Differential interference-contrast (DIC) microscopy allows thin optical sectioning and detailed imaging of live cells without staining (e.g., [104]). Combined with video-enhancement procedures, DIC microscopy resolves submicrometer details [105]. This

approach has been successfully applied to the direct study of movement and exocytosis of single secretory granules in intact tissue [106] or in dispersed cells [107]. Its application in renin cells is yet to be done.

3.4. Evanescent Wave Fluorescence Microscopy. Evanescent-wave microscopy is based upon the total internal reflection of a laser light beam directed at the interface between a glass coverslip and a cell tightly attached to it. Total internal reflection generates an evanescent light field 100–300 nm thick that will illuminate any fluorescently tagged particle that penetrates it. This very powerful optical approach has been used in neurons [108], chromaffin [109], or pancreatic β cells [110, 111] to document single-vesicle or single-granule approach, docking and exocytosis in real time, optically sorting dense-core vesicles in chromaffin cells according to their age [112], and for single molecule imaging within cells [113]. As techniques are available to isolate and culture granulated renin cells (e.g., [60]), further developments may be expected for renin cells in this field.

3.5. Ultramicro-Radioimmunoassay of Renin Concentration/ Whole-Cell Patch-Clamp Technique. The "quantal" nature of renin release [114], and its relationship with fusion of renin granules with cell membrane [96] were first demonstrated by combining a unique ultramicroradioimmunoassay of renin concentration [115], superfusion of single isolated rat afferent arterioles, and TEM of renin granules in the same superfused single isolated rat afferent arterioles [96]. The same renin assay [115] was later combined with a newly developed isolated perfused rabbit macula densa preparation [104]. For the first time [54], the inverse relationship between tubular fluid sodium chloride concentration at the macula densa and renin release rate of the afferent arteriole was demonstrated. More recently whole-cell patch-clamp techniques were successfully used to demonstrate exocytosis and endocytosis in single mouse juxtaglomerular cells in relationship with known stimuli or inhibitors of renin release [42, 44, 116].

3.6. Two-Photon Confocal Microscopy. As previously mentioned (Section 2.1), the exocytosis of a single renin granule was documented using two-photon confocal microscopy and the in vitro microperfused rabbit afferent arterioles whose renin was stained with the acidotropic fluorophore quinacrine [81]. In the same study, renin granules were stained by perfusion with an acidotropic lysosomal fluorophores, Lyso Tracker-Red (Molecular Probes), and increases in renin activity resulting from renin granule release were probed within the perivascular space with EDANS (Molecular Probes), allowing simultaneous imaging of renin release and activity in a single perfused afferent arteriole [81]. Despite the unprecedented imaging capacity of multiphoton imaging and the multiple possibilities offered to functional approaches in a live, integrated nephrovascular unit (reviewed recently in [8, 117]), one must underline some specific limits to the observation of the behaviour of renin cells and renin granules. Though careful

validations were performed (i.e., colocalization of quinacrine and renin immunolabelling within granules in mouse kidney sections, granule release occurring as burst or quantal release, release occurring in response to isoproterenol, or decrease in arteriolar perfusion pressure, [81]), these studies rely on the assumption that surrogate molecules (i.e., quinacrine, Lyso Tracker-Red, [81]) rather than renin itself are observed. Remarquably, too, an estimated renin release affecting 40% of cell granules disappearing in 10 minutes [81] is substantially higher than 1-2% release rates obtained in dissected afferent arterioles in rats [96, 114] or rabbits [93] based respectively on renin ultramicro-radioimmunoassay or radioimmunoassay. Along these lines, a "relative rarity of exocytosis events" was previously noted in TEM studies in mice renal cortical slices [95] and was recently underlined in a review on renin release [42]. Furthermore, and still poorly appreciated in the renal microcirculation field, interactions between light and fluorophores may generate heat and photochemical processes that may affect protein synthesis in live specimens. The so-called "light-dye" or "laser-dye" effects locally and/or temporarily modify endothelial cell function (e.g., [118, 119]), and the impact of confocal microscopy on cell calcium handling and cell death has recently been emphasized in bovine chondrocyte cultures [120]. Further studies seem therefore warranted to settle these issues linked to "classic" fluorescence, mono-, or multiphoton imaging in live tissues.

4. Visualization of Renin Gene Expression

4.1. In Situ Hybridization. Since circulating renin may be captured by vascular cells [121], it is important to determine, by in situ hybridization, whether renin synthesis occurs in cells that stock renin. Protocols based on the use of radioactive [60, 122] or nonradioactive, digoxigenin-labeled riboprobes [123] are currently available to visualize cellular renin mRNA on kidney sections or cultured cells. One must note that in situ hybridization was combined with the reverse hemolytic plaque assay (i.e., Section 3.1) to visualize both gene expression and peptide secretion in single cells [124].

4.2. Single-Cell Reverse Transcription-Polymerase Chain Reaction Technique. The detection of specific mRNAs within a single-cell is made possible by the reverse transcription-polymerase chain reaction technique initially developed for cultured neurons [125]. This approach requires cell isolation. It has been successfully performed in glomerular podocytes individually aspirated with a micropipet [126], in freshly dispersed smooth muscle cells [52, 127], in renal epithelial cells [128], and in renin cells aspirated from embryonic kidneys [25]. Detection of single cell transcripts is performed on agarose gels. Using the so-called in situ reverse transcription polymerase chain reaction technique [129], transcripts can be visualized within individual cells with digoxigenin staining [130].

4.3. Laser Capture Microdissection. A laser capture microdissection technique was recently developed from Ashkin's

"optical tweezers" [131]. Under microscopic observation, small cell samples or single cells can be dissected with a sharp laser beam from precise areas of thin cryostat or paraffin sections, and optically catapulted into a test tube. Anatomical localization is facilitated by hematoxylin or immunofluorescence staining. In association with single-cell reverse transcription-polymerase chain reaction technique, this technique opens new avenues in the study of spatial distribution of gene expression [132–135].

4.4. Transgenic Mice. Molecular biology and transgenic approaches (e.g., homologous recombination) have introduced new ways to visualize renin gene expression and its regulation in vivo during development, and during adulthood. Various transgenic mice have been recently generated. Green fluorescent protein (GFP) or LacZ reporter genes are fused to the appropriate promoter sequences and allow to follow with high fidelity renin gene expression in the renal vasculature. Imaging may either be performed directly by fluorescence microscopy of live tissues [25, 72, 136], or β-galactosidase activity is detected by X-gal staining after tissue processing [58]. Figure 3 illustrates the afferent arteriolar distribution of cells expressing the Ren1^d gene as revealed by the direct fluorescence study of GFP-expressing cells. In this example, renin synthesis was boosted by a one-week treatment with losartan in cells localized at the glomerular vascular pole (Figure 3, single arrow) and in cells recruited upstream (Figure 3, dashed arrows). These transgenic strains will be crucial for future elucidation of the molecular determinants of gene expression patterns in vivo.

5. Conclusions

A variety of techniques and procedures are now in the researcher's toolbox to visualize renin cells, and more will emerge from researchers' and engineers' relentless inventiveness. Renin gene expression, renin storage/processing, and renin release are three basic aspects of cellular renin processing that can be imaged. Some techniques were listed (i.e., Sections 2.4, 3.2, 3.3, 3.4, 4.3) because they would deserve an application to renin cells. For instance, they have the potential to provide insights into the processing and exocytosis of single renin granules, and may help to approach the "calcium paradox" and the role played by myofilaments in "intermediate" renin cells [95, 137]. Several of these techniques may be or have been combined to assess synthesis, storage, and release at the single cell level and have provided important knowledge on cell pathways leading to renin release (recently reviewed in [42]). The vascular topology or renin cells can be well preserved in various approaches dealing with renin storage and renin gene expression. In the well-studied rat species, it was observed that sodium depletion leads to the recruitment of renin-containing cells along afferent arterioles, whereas renin gene expression, as probed by in situ hybridization, increased near glomerular vascular poles only [73]. This spatial discrepancy between gene expression and protein distribution was interpreted as indicative of either renin uptake, or ephemeral expression
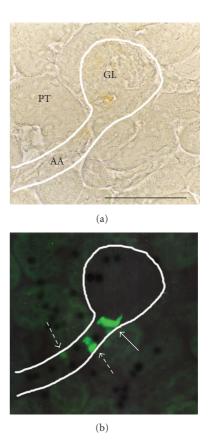

(a)

(b)

FIGURE 3: Light micrographs of a cryostat kidney section. Renal tissue was obtained from a transgenic heterozygous (Ren 1^d-GFP/$^+$) adult mouse in which green fluorescent protein (GFP) was placed under the control of the Ren 1^d renin locus by homologous recombination [25, 72]. Renin synthesis was stimulated by a 7-day-treatment with the angiotensin II type-1 receptor antagonist, losartan (25 mg/kg per day). (a) brightfield view showing the outline of an afferent arteriole (AA) and a glomerulus (GL) among proximal tubules (PT). (b) fluorescence view of the same field (white outline) showing green GFP signal from renin-synthesizing cells at the glomerular vascular pole (single arrow), and from upstream recruited cells (dashed arrows). Bar: 100 μm.

of the renin gene [70]. As a result, the distribution of renin-positive cells along preglomerular vessels will not always reflect renin gene expression in the rat, as is the case during inhibition of the renin-angiotensin system [72, 73]. Future studies will help unfolding the complex nature of the spatio-temporal relationships between renin gene expression and protein processing. The story becomes more complex in some mice strains which may have two cooperative renin genes [72]. The development of transgenic mice whose fluorescent reporter gene accurately mimics the spatio-temporal expression patterns of the endogenous renin gene(s), provides a spectacular and promising new tool for the direct study of renin cells and JGA in situ [72, 136].

As a final remark, technical choices will always be served by a thorough critical review of inherent, though occasionally unsuspected limits, sometimes better appreciated in a nearby

field of research which then becomes a "source" for fruitful new applications.

Acknowledgments

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