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Novel Mechanisms for the Control of Renin Synthesis and Release

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Abstract

Renin is the key regulated step in the enzymatic cascade that leads to angiotensin generation and the control of blood pressure and fluid/electrolyte homeostasis. In the adult unstressed animal, renin is synthesized and released by renal juxtaglomerular cells. However, when homeostasis is threatened, the number of cells that express and release renin increases and extends beyond the juxtaglomerular area; the result is an increase in circulating renin and the reestablishment of homeostasis. The increase in the number of renin cells, a process termed *recruitment*, is achieved by dedifferentiation and re-expression of renin in cells derived from the renin lineage. The mechanisms that regulate the related processes of reacquisition of the renin phenotype, renin synthesis, and renin release are beginning to be understood. Numerous studies point to cAMP as a central common factor for the regulation of renin phenotype. In addition, we are seeing the emergence of gap junctions and microRNAs as new and promising avenues for a more complete understanding of the complex regulation of the renin cell.

Keywords

Juxtaglomerular cells; cAMP; Plasticity; Cell identity; MicroRNAs; Homeostasis

Introduction

The synthesis and release of renin are the key regulated steps of the renin-angiotensin system cascade, which (through the generation of angiotensin II) regulates blood pressure and fluid/electrolyte homeostasis. Renin also plays a fundamental role in kidney development. Although renin was discovered over 100 years ago, only in the past few decades have the mechanisms for its synthesis and release begun to be thoroughly studied.

In adult mammals, the main source of circulating renin is the kidney, where renin is synthesized, stored in granules, and released from juxtaglomerular (JG) cells. JG cells are myoepithelioid granulated cells located in the wall of the afferent arterioles at the entrance to the glomeruli. Occasionally, a few JG cells are found in the efferent arteriole. The term *JG* applies only to the cells of the adult, unstressed mammalian kidney: in nonmammalian species, renin is distributed throughout the arterial tree. Remarkably, the ontogeny of renin expression reenacts the phylogenetic pattern. Because JG cells synthesize renin, are few in number, and have the aforementioned restricted localization in the adult mammal, they have been considered as terminally differentiated. Although this may be true for the “classic” adult JG cell, during early development, renin cells act as precursors for other cell types within the kidney and in extrarenal organs [1]. In fact, during embryonic and fetal life, renin cells are widely distributed

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along the renal arterial tree, in the glomerular mesangium, in the renal interstitium, and in a subset of cells in developing tubules. With maturation, renin cells differentiate into other cell types such as vascular smooth muscle cells, mesangial cells, and a few tubular cells, and they become restricted to the classic JG localization found in the adult unstressed mammal. In response to threats to homeostasis, however, the cells derived from renin cells (such as smooth muscle cells along and upstream of the afferent arterioles) dedifferentiate into renin cells in an attempt to reestablish homeostasis [1]. This process is known as *recruitment* (a better term may be *dedifferentiation*); it suggests reversion to an endocrine phenotype reflected by the synthesis and release of renin and downregulation of the smooth muscle proteins within the wall of the intrarenal arterioles and arteries, resembling the embryonic pattern [2].

In recent years, many advances have been made in understanding the mechanisms that control renin synthesis, renin release, and cell identity. It is now established that cAMP is a fundamental factor not only during physiologic states but also in the phenomenon of recruitment [3–6]. cAMP is critically involved in all three major mechanisms controlling renin synthesis and release: 1) the intrarenal β 1-adrenoreceptor (through renal sympathetic nerves and circulating catecholamine stimulation), 2) the intrarenal baroreceptor, and 3) the macula densa mechanism.

This review will focus primarily on recent advances concerning the role of the cAMP pathway in renin expression and identity of the renin cell. In addition, recent information on gap junctions and the role of microRNAs (miRNAs)—small, noncoding RNAs—is discussed.

The Fundamental Role of cAMP

cAMP is a second messenger used for intracellular signal transduction critical for the control of a diverse range of biologic processes. The cAMP signaling pathway starts by the stimulation of G protein–coupled cell membrane receptors (by extracellular ligands); these trigger the activation of adenylyl cyclases, which in turn generate cAMP from adenosine triphosphate (ATP). cAMP activates protein kinase A, which in turn phosphorylates a number of other proteins that regulate gene expression and hormonal release. cAMP is degraded by phosphodiesterases, which therefore limit cAMP action. The cellular concentration of cAMP is determined by a balance between the rates of cAMP synthesis by adenylyl cyclases and cAMP degradation by phosphodiesterases (Fig. 1).

Factors that increase the levels of cAMP in the JG cells, such as β -adrenergic agonists, prostaglandins E1 and I2, dopamine, and kinins, have a stimulatory effect on renin. Direct stimulation of adenylyl cyclase with forskolin [7], administration of cAMP analogues, or inhibition of the phosphodiesterases also increases renin synthesis and release. We have shown that newborn renal microvessels and isolated single microvascular cells release renin and increase renin mRNA levels in response to activation of adenylyl cyclase with forskolin [6]. The increase in the amount of renin is due to an increase in the number of renin-expressing and renin-secreting cells without changes in the amount of secreted renin per individual cell [6]. These in vitro studies corroborated many reports in whole animals showing that, in response to a challenge to homeostasis, the increase in synthesis and release of renin is due to an increase in the number of renin-expressing cells (“recruitment”).

More recently, our in vitro and in vivo studies have demonstrated the crucial role of the cAMP pathway in the acquisition and maintenance of renin cell identity [8•, 9•]. Studies performed on β 1/ β 2-adrenergic receptor–deficient mice showed a marked reduction of basal renin expression and release [10]. However, the number of cells expressing renin is not changed (unpublished observations by the authors), and the regulatory ability in response to several physiological regulators (variations of salt intake, acute stimulation with furosemide and angiotensin-converting enzyme inhibitors or angiotensin type 1 receptor inhibitors) was not

abrogated, indicating a relevant role for β -adrenergic tone in basal levels of circulating renin and renal renin expression [10].

Recently, Chen et al. [11••] confirmed the fundamental role of $G\alpha$ in renin synthesis and release. For this elegant study, the authors used a cre/loxP conditional deletion approach. Mice with floxed $G\alpha$ were crossed with animals expressing cre recombinase in renin cells [1]. Upon activation of cre recombinase, $G\alpha$ was deleted in renin-synthesizing cells. These mice showed reduced basal levels of renin expression and plasma renin concentration, with consequent hypotension; in addition, the acute release responses of renin to furosemide, hydralazine, and isoproterenol (a β -adrenergic agonist) were practically abolished [11••]. Furthermore, culture studies of JG cells isolated from these mice showed impairment of the cells' ability to secrete renin in response to stimulation with isoproterenol or prostaglandin E2 [11••]. Further developmental studies in mice with renin cell-specific deletion of $G\alpha$ showed that renin expression is also practically absent during embryonic and early postnatal life, and the preglomerular arterial tree is also affected [12]. These studies indicate that the cAMP pathway is involved in the expression of renin during the embryonic development of the kidney.

Culturing Renin Cells and Understanding Renin Cell Plasticity

As mentioned above, cells from the renin lineage have the plasticity to switch back and forth from the renin phenotype. The study of the in-depth molecular mechanisms regulating this process would be more easily addressed using an in vitro cell culture model. However, maintaining renin cells in culture systems has been a major impediment because even if enriched populations of renin-expressing cells are isolated, after only a few days in culture, these cells stop making renin and differentiate into other cell types [13]. Significant advances have been made with the use of tumoral cell lines that constitutively express renin, such as the As4.1 (isolated from a renal tumor in a mouse expressing an SV40 large T antigen under the control of the *Ren2* promoter [14]) and Calu 6 (isolated from a human lung renin-producing carcinoma [5]); these allowed study of the transcriptional and post-transcriptional regulation of the mouse and human renin genes [15,16]. Studies on As4.1 cells identified several transcription factor binding sites in the proximal promoter (from -197 to -50 bp: at least seven sites) and in the enhancer region (from -2866 to -2625 bp: at least 11 sites) [15]. The enhancer sequence is highly homologous in the renin gene of mouse, rat, and human. Its crucial role in the regulation of renin transcription seems to be rather complex. For instance, several binding sites are shared by more than one factor, sometimes with opposite effects: the RAR/RXR binding site stimulates transcription in the presence of its ligand vitamin A and inhibits transcription when bound to vitamin D [17,18]. Interestingly, peroxisome proliferator-activated receptor- γ (PPAR γ) seems to activate renin transcription through sites in both the enhancer and proximal promoter [19,20]. However, among the transcription factor binding sites of the enhancer region, the site where cAMP-responsive element binding protein (CREB) and cAMP-responsive element modulator (CREM) bind, along with the adjacent E-box site, were shown to be the most critical for the expression of the mouse *Renin* gene [15]. Forskolin, an activator of adenylyl cyclase, has been shown to stimulate renin expression in fresh cultures of enriched JG cells, but unfortunately, the As4.1 cells do not respond to forskolin without concomitant inhibition of the phosphodiesterase activity. This limitation may be due to a constitutive overactivation of the cAMP pathway in these tumoral cells [15]. Recently, to overcome these problems, we developed a cell model to study mechanisms that govern the ability of the cells to switch back and forth between renin expression and repression [8••]. First, we generated *Ren1^d-cre/ROSA^{loxP}(CFP)/Ren1^c-YFP* mice to mark smooth muscle cells of the renin lineage with cyan fluorescent protein (CFP) and cells actively transcribing the renin gene with yellow fluorescent protein (YFP) [8••]. These mice showed normal distribution of renin during development and recruitment concomitantly with the appropriate expression of CFP in cells derived from the renin lineage (ie, arteriolar smooth muscle cells) and expression

of YFP within the renin cells. Subsequently, after isolation and culture of renal arteriolar smooth muscle cells (expressing CFP) from those mice, we demonstrated that upon stimulation of cAMP, there is re-expression of renin mRNA (and therefore YFP), confirming that the smooth muscle cells from the renin lineage retain the plasticity to dedifferentiate—an effect that can be achieved even after numerous cell passages. Current efforts in our laboratory are aimed at improving the culture system so that granulopoiesis and release can be better studied.

Because cAMP activates transcription via phosphorylation of CREB followed by the recruitment of coactivators that possess intrinsic histone acetyl transferase (HAT) activity (CBP and/or p300) (Fig. 1), we explored whether activation of renin gene transcription was facilitated by the acetylation of histone tails of the nucleosomes (by CBP and/or p300) close to the CRE site located in the enhancer region of the renin promoter, where CREB binds. Indeed, we demonstrated that the activation of renin transcription that occurs upon cAMP stimulation is in effect mediated by histone 4 acetylation, chromatin remodeling, and binding of CREB at the CRE site in the renin promoter [8••]. These experiments suggested that the cAMP pathway and histone modifications in the renin promoter are involved in the process of reacquisition of the renin phenotype.

Histone Acetyl Transferase Activity in Renin Cells

To further determine *in vivo* the role of the coactivators CBP and p300, which possess HAT activity, we generated mice with conditional deletion of *CBP* and *p300* in the renin-expressing cells. Individual deletion of *CBP* or *p300* in renin-expressing cells did not affect the expression of renin, and the morphology and function of the kidney were well preserved [9••]. However, the simultaneous homozygous deletion of both *CBP* and *p300* in renin-expressing cells showed a striking decrease in the number of cells expressing renin concomitantly with marked structural renal abnormalities, including reduced kidney growth and extensive interstitial fibrosis [9••]. Those abnormalities may be due to the lack of renin cells combined with the lack of CBP and p300 in cells from the renin lineage. The fact that an individual deletion of these coactivators did not affect the expression of renin suggests that the lack of one coactivator is not sufficient to interfere with the transcriptional events; probably the lack of HAT activity from one is compensated for by the presence of the other one. Those studies revealed an essential function for these coactivators in maintaining the renin phenotype (cell identity) and emphasized the role of the cAMP pathway acting at the CRE promoter region of the renin gene [9••].

Altogether, the information in this section clearly suggests that cAMP (via its CREB/CBP/p300 complex) plays a central role in maintaining the renin cell.

The Role of Calcium

Calcium is a direct stimulator of secretion in most secretory cells (with the exception of cells in the parathyroid gland), but renin synthesis and secretion are stimulated by a decrease in extracellular and intracellular calcium, a phenomenon known as the “calcium paradox.” Interestingly, the inhibitory effect of calcium on renin synthesis and release also involves the cAMP pathway [21••, 22••]. Stimulation of calcium-sensing receptors in JG cells decreases cAMP and consequently renin synthesis and release [23]. Recent studies reported that the intrinsic mechanism for inhibition of renin release by calcium depends on the coordinated inhibition by calcium of adenylyl cyclase type V (expressed in JG cells) and the activation of phosphodiesterase 1C by the calcium-sensing receptor [24–26]. The result is not only a decrease in the synthesis of cAMP but also degradation of this second messenger crucial for renin synthesis and release.

It has also been proposed that an increase in intracellular calcium concentration inhibits renin transcription by binding transcription factors (Redox factor 1 [27]) at the renin promoter/enhancer, concomitantly destabilizing renin mRNA by binding proteins at the 3' untranslated regions (UTR) [28]. Interestingly, cAMP also acts at the post-transcriptional level by increasing renin mRNA half-life. This effect is apparently achieved by the action of binding proteins at the 3'UTR of renin mRNA [29].

Thus, it seems clear that calcium does not control renin release directly. Its role consists mainly in modulating enzymatic activities to balance the synthesis and degradation of cAMP and activating regulators that inhibit renin transcription and destabilize renin mRNA [21•, 22•, 28].

Gap Junctions

JG cells connect among themselves and with other cells such as smooth muscle, endothelial, epithelial, and mesangial cells. Gap junctions connect the cytoplasm of adjacent cells and comprise two hemichannels (one contributed per each cell). The hemichannels are homoheptamers or heteroheptamers of connexin proteins. Connexin 40, the most abundant connexin in renin cells, has been shown to connect JG cells not only with each other but also with other cell types in the JG apparatus and upstream in the renal arteriole [30]. Deletion experiments of connexin 40 in mice resulted in an increase of renin expression extending to the interstitium surrounding the JG apparatus. In addition, these mutant mice have hypertension [31,32]. In the absence of connexin 40, the mice not only are less sensitive to the suppression of renin release by angiotensin II but also have an inverted relationship between perfusion pressure and renin secretion, indicating that the baroreceptor does not respond properly to low perfusion pressure; paradoxically, it increases renin release in response to high blood pressure, which aggravates the hypertension [33•]. Another effect observed in mice lacking connexin 40 is disarrangement in the distribution, number, and appearance of renin-expressing cells. Mice with deletion of connexin 40 show an increase in the number of renin-expressing cells, extending beyond the classical JG localization toward the periglomerular interstitium, and they possess fewer and smaller secretory granules [31,32]. Replacement of connexin 40 by connexin 45 (a connexin with a lower conductivity) partially rescues the defect: the location of JG cells is reestablished but the mice remain hypertensive [34].

Overall, those studies suggest an important role for connexins and gap junctions in renin-cell positional information and renin release. The existence of a renal baroreceptor was demonstrated long ago [35], but its precise location and structure remain elusive. The fact that deletion of a connexin results in deregulation of renin secretion in response to perfusion pressure suggests the intriguing possibility that the baroreceptor mechanism at least partially depends on gap junctions. This hypothesis remains to be tested. Experiments in the near future, using available tools for cell-specific and time-specific deletions of single connexins and combinations of connexins, may help to clarify more precisely how gap junctions regulate renin synthesis, distribution, and release in response to mechanical and humoral factors [36].

Dicer and MicroRNAs

Endogenous miRNAs, a group of small, noncoding RNAs 18 to 22 nucleotides in length, have been shown to regulate gene expression through posttranscriptional or translational repression and play important roles in cell differentiation and morphogenesis (Fig. 1) [37–39]. The biogenesis of miRNAs involves the sequential enzymatic cleavage of a primary miRNA precursor (about 100 to 1000 nucleotides in length) by two protein complexes involving RNase III endonucleases: Drosha-DGCR8 in the nucleus and Dicer in the cytoplasm.

Conditional deletion studies of Dicer have proven valuable in determining whether miRNAs are important for the development, differentiation, or function of various cells, tissues, and organs. Recently, to define whether Dicer, the RNase III endonuclease responsible for the generation of mature miRNAs, plays a role in renin cells, we generated mice with a conditional deletion of Dicer in cells of the renin lineage [40]. Deletion of Dicer in the renin cell lineage results in a near absence of JG cells in the kidney and decreased expression of the renin genes and circulating renin, accompanied by hypotension and striking kidney abnormalities, including striped corticomedullary fibrosis. These studies uncovered an essential role of Dicer (and consequently microRNAs) in maintaining renin cells and the morphological integrity and function of the kidney [40]. Recent studies showed that the expression of miRNAs can also be transcriptionally regulated. For example, miR132 was identified as a target of the transcription factor CREB [41]. Therefore, the next challenge will be not only to identify miRNAs (or combinations of miRNAs) that directly affect the expression of renin but also to detect their regulators. Recently, a number of miRNAs have been shown to regulate the expression of genes involved in β -cell exocytosis [42]. It remains to be determined whether similar miRNAs participate in renin release via these unique, noncoding RNAs.

Conclusions

In the past few years, major advances in the understanding of the mechanisms that control renin synthesis have been achieved largely through the availability of renin-expressing tumoral cell lines, fluorescence-marked renal arteriolar smooth muscle cells from the renin lineage, and transgenic mouse models, including a knock-in of cre recombinase in the renin locus. We expect that in the near future, experiments using these and new models will definitely accelerate the pace of discovery in this field.

In summary, renin cell identity, renin synthesis, and renin release depend on multiple events centrally regulated by cAMP at the transcriptional and post-transcriptional levels. In addition, new players such as noncoding RNAs and gap junctions exert additional points of control. Relevant questions that remain to be answered include 1) the identification of the signals that control the developmental pattern of renin expression, 2) the identification of the set of genes that determines the identity of the renin cell, and 3) understanding of the extracellular and cell-cell interactions that regulate renin synthesis and release, during both development and adulthood.

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Fig. 1.

Simplified scheme of the cAMP pathway and the regulation of renin synthesis and release. Activation of a G protein-coupled receptor (GPCR) by its ligand results in a conformational change that is transmitted to a heterotrimeric G protein complex. The G α subunit, through conversion of guanosine triphosphate (GTP) into guanosine diphosphate (GDP), is released from the complex and activates the adenylyl cyclase, which in turn converts adenosine triphosphate (ATP) into the second messenger cAMP (red dots). cAMP binding to the regulatory subunits (R) of protein kinase A (PKA) results in the release of the catalytic subunits (C) that, in the nucleus, phosphorylate the cAMP-responsive element binding protein (CREB). Phosphorylated CREB recruits the coactivators CBP and p300. Upon binding to the cAMP-responsive element (CRE) in the enhancer region of the renin promoter, this complex switches on the transcription of renin mRNA. Posttranscriptional regulation at the 3' untranslated region (UTR) occurs by binding proteins that increase renin mRNA stability, and perhaps by microRNAs (miRNA), which repress renin translation and/or degrade renin mRNA. cAMP also stimulates renin release (*dashed arrow*). Degradation of cAMP occurs by the action of a phosphodiesterase (PDE) that converts it to AMP. The modulating effects of calcium described

in the text are omitted for clarity. *GJ* gap junction, *MEJ* myoendothelial junction, *Pol II* polymerase II