The kidneys fulfill a variety of functions, such as elimination of waste products of metabolism, regulation of fluid volume, and electrolyte concentrations and acid-base balance. Besides these excretory and homeostatic functions, the kidneys are endocrine organs essentially involved in the regulation of bone mineralization, blood pressure, and erythropoiesis. Thereto, the kidneys produce and secrete the hormones calcitriol, erythropoietin, klotho, and renin.

Klotho, which exists in a membranous and a secreted form, regulates calcium and phosphate homeostasis (67, 83, 129), thus contributing to bone mineralization. Membrane klotho functions mainly as a coreceptor for fibroblast growth factor (FGF) 23 (85, 178), which causes phosphaturia (85) and decreases circulating calcitriol levels (187) and renal phosphate resorption, resulting in reduced plasma phosphate levels. In its soluble form (66, 110), klotho mediates enhanced Ca\(^{2+}\) (re)absorption in the kidney and in the intestine, by activating TRPV5 and TRPV6 (21, 23) channels. Missense mutations or deficiency of klotho result in hyperphosphatemia, hypercalcemia, and vascular calcification (64, 84, 95, 157).

Calcitriol (34, 35, 138), the physiologically active form of vitamin D, contributes to bone mineralization by regulating homeostasis of calcium and phosphate through acting on the intestinal tract and the kidneys. Besides its role in regulation of calcium and phosphate homeostasis, calcitriol contributes to erythropoiesis (5, 48), as well as the activity of monocytes and macrophages (30). In contrast, proliferation and activity of T-lymphocytes and, thus, the adaptive immunity are suppressed by calcitriol (30). Vitamin D deficiency results in osteomalacia (59), and, in addition, calcitriol inhibits renin expression and, thus, modulates the activity of the renin-angiotensin-aldosterone system (RAAS) (99).

The aspartyl protease renin is the key regulator enzyme of the RAAS, which controls blood pressure and fluid-electrolyte balance (56, 180) by regulating salt transport, salt appetite, and thirst (6, 43, 106, 116, 147). Renin cleaves the decapetide ANG I from liver-derived angiotensinogen. ANG I is further cleaved by the angiotensin-converting enzyme ACE to the octapeptide ANG II (163), which binds to two distinct receptors, the AT\(_1\) and the AT\(_2\) receptor (17, 20). The majority of actions of ANG II are mediated via the AT\(_1\) receptor. The AT\(_1\) receptor-mediated signaling cascades result in vasoconstriction and an increase of extracellular fluid volume, an increase of renal tubular sodium reabsorption, and stimulation of aldosterone release (4, 20). These effects lead to an increase of blood pressure. The AT\(_2\) receptor is considered to oppose the actions of ANG II via the AT\(_1\) receptor, by mediating vasodilatation via formation of NO, prostanoids, and bradykinin (1, 16, 17, 33,
The glycoprotein hormone erythropoietin (EPO) is the primary humoral regulator of red blood cell formation in mammals (77–79, 81, 149). It is essential to maintain normal blood hemoglobin concentration (70, 182). Binding of EPO to high-affinity receptors (EPO-R) on the surface of cells belonging to the committed erythroid lineage (13) in the bone marrow, activates predominantly the JAK/STAT signaling cascade in the target cells (31, 32, 60), resulting in survival, proliferation, and differentiation of immature erythroid precursors to mature erythrocytes (12, 41, 54, 76, 168). Insufficient production of EPO, such as in states of chronic kidney disease, results in anemia (70, 182).

The aforementioned endocrine functions of the kidneys are provided by different cell types (Fig. 1). Klotho is expressed by distal tubule cells (118, 122), and calcitriol is activated in the mitochondria of proximal tubule cells by the action of 1α-hydroxylase (127, 172). EPO is expressed by interstitial fibroblast-like cells of the renal cortex (7, 78, 113). In the adult kidney, renin is produced by juxtaglomerular (JG) cells that are located in the media layer of afferent arterioles at the entrance into the glomerulus (56, 151).

It is known that under specific circumstances, cells can (reversibly) transform into another cell type with different protein expression patterns and characteristics (36, 82, 139). Also for renal renin and EPO-expressing cells, transformation into another cell type and, hence, cell plasticity is known (15, 24, 49, 101, 165). For example, if the RAAS is challenged, additional cells transform into renin-producing cells (15, 90). A similar recruitment phenomenon is also known for EPO-producing cells in the kidney. Moreover, during development of interstitial kidney fibrosis, EPO-expressing cells undergo a phenotype switch into myofibroblasts, which stop producing EPO, resulting in the well-known renal anemia. As in the case of renin-expressing cells, the transformation of EPO-expressing cells into another cell type is reversible, at least to some extent (165). Recently, the first evidence was provided for an endocrine transformation of renin-expressing cells into EPO-producing cells, indicating a novel aspect of renal endocrine plasticity (89).

In this review, we focus on the characteristics of renin- and EPO-producing cells, especially their origin, structure, and localization, their reversible transformations, and the mediators, which are responsible for transformation. Finally, we will discuss a possible interconversion of renin and of EPO expression.

**Plasticity of Renin Production**

**Development of renin cells.** In the adult kidney, renin cells are located in the distal parts of afferent arterioles just at the entrance into the glomerulus (8, 56, 151, 175), thus coining their name, juxtaglomerular cells. These cells, which have a cuboid-like shape, are an integral part of the media layer of afferent arterioles, thereby forming the walls of the vessels entering the glomeruli. This typical juxtaglomerular position of renin cells in the adult kidney is the final result of a highly dynamic spatiotemporal renin expression pattern during nephrogenesis (151). During embryonic kidney development, renin expression is occasionally found already in the undifferentiated metanephric mesenchyme (102) in a state before vessel formation has begun. It is thought that renin-expressing cells derive from FoxD1-mesenchymal cells (100), which are also precursors for vascular smooth muscle cells, mesangial cells, pericytes, and fibroblasts (101). Once renal vascularization has begun, renin-expressing cells are found as wall cells of the developing interlobar and arcuate arteries (50, 75). With ongoing nephrogenesis and maturation of the arterial tree, when cortical radial arteries branch off and spread out from arcuate arteries and afferent arterioles spread out from cortical radial arteries, renin-expressing cells appear as mural cells covering and stabilizing the newly formed vessels. In this context, renin expression appears to be highest in the youngest and immature preglomerular vessels. With ongoing vessel

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**Fig. 1.** Schematic overview of the adult kidney cortex with different cell types expressing calcitriol, klotho, renin, and erythropoietin (EPO). Calcitriol is produced by proximal tubular cells (PT), and klotho is produced by distal tubular cells (DT). Renin is produced by juxtaglomerular epithelioid cells (indicated by arrowheads), which are located in the media layer of afferent arterioles (aff. art.) at the entrance into the glomerulus (G). EPO is produced by interstitial fibroblast-like cells (indicated by arrow), which are located between proximal tubules. cTAL, cortical thick ascending limb; CCD, cortical collecting duct; cort. rad. art., cortical radial artery.
maturation, renin expression in these segments ceases, and the cells acquire a smooth muscle-like phenotype. As a result, developmental renin expression has a wave-like character flushing over the preglomerular arteriolar tree. At the end of development, in the adult kidney, only few renin-expressing cells are left over at the classical juxtaglomerular position (8, 56, 151, 175). These cells form the interface between the mural vascular smooth muscle cells of afferent arterioles on the one side and the mesangial cells on the other, especially the extraglomerular mesangial (EGM) lacis or Goormaghtigh cells (167). Therefore, it is easy to speculate that the JG cells, as the normal physiological site of renal renin production, are developmentally related to both smooth muscle and EGM cells.

Structure of juxtaglomerular renin-producing cells. Juxtaglomerular renin-producing cells are morphologically characterized by large electron-dense vesicles that occupy up to 40% of the extranuclear cell volume (169). These vesicles belong to the family of lysosome-related organelles (LRO) (177). As lysosomes, they have an acidic content (137), and they contain proteins, such as lysosomal integral membrane protein 2 (LIMP-2), lysosomal-associated membrane protein 1 (LAMP-1), and LAMP-2 (98, 152), which are important for directing proteins to lysosomes (148). At the level of the Golgi apparatus, glycosylated prorenin is sorted to these lysosome-related organelles. Within the vesicles prorenin is proteolytically cleaved to enzymatically active renin, which remains stored in the vesicles until it is released by controlled exocytosis, which resembles the mode of compound exocytosis (169). Hereby, vesicles fuse intracellularly to huge vesicular networks, which occasionally fuse with the plasma membrane to release their contents into the extracellular space. Notably, the development of the prominent vesicles is dependent on (pro)renin accumulation. If prorenin is not directed to those lysosome-related organelles, but instead is constitutively secreted, only very few and small vesicles are found in JG cells (169). Another remarkable structural characteristic of juxtaglomerular cells is the high density of gap junctions, which connect renin-producing cells with neighboring smooth muscle and endothelial and mesangial cells (2, 57, 63, 176).

Markers and other genes conferring identity of renin-producing cells. Gene expression profiling of renin cells (136) revealed strong enrichment of Akrt1b7 (aldo-keto reductase family 1, member 7), an enzyme involved in NADPH-dependent oxidoreduction of carbonyl groups (71). Immunohistochemistry confirmed that Akrt1b7 expression in the adult kidney is restricted to the juxtaglomerular renin-expressing cells (14, 103). Another gene, that is enriched in the JG cells compared with total kidney cortex, is the gap junction (GJ) protein connexin 40 (Cx40), which has already been shown in ultrastructural and immunohistochemical studies to be highly expressed in juxtaglomerular renin-expressing cells. Additionally, Cx40 is expressed in endothelial cells of the afferent arterioles and in mesangial cells. These two cell types, together with renin-producing cells, form a network of cells that are interconnected via Cx40 gap junctions (2, 57, 63, 176). Obviously, Cx40 and, hence, cell-to-cell communication is important for the correct positioning, as well as for the identity of renin-expressing cells in the kidney (92, 179). The gene expression analysis identified other enriched genes in the JG renin cells, such as RGS5 (regulator of G protein signaling 5) and CRIP1 (cysteine-rich intestinal protein) (14). Their exact roles concerning the identity of renin-expressing cells is still elusive. Another factor that has been discovered to be important for renin cell identity is the RNase III endonuclease Dicer (162). Dicer is responsible for the processing of a pre-miRNA to a mature miRNA, which are small noncoding RNAs that regulate gene expression at the posttranscriptional level. Renin cell-specific deletion of Dicer results in an almost complete loss of renin-expressing cells and a thinner and smaller morphology of the residual renin cells. Further analysis revealed the two miRNAs miR-330 and miR-125b-5p to be relevant for the identity of renin-expressing cells with opposite actions (117).

In this context, several candidate transcription factors have been suggested to be of higher relevance for the identity of renin-producing cells. Among these, one is the transcription factor “recombination signal binding protein for immunoglobulin κ region” (RBP-J), which is the main transcriptional mediator of the Notch signaling pathway that regulates cell fate. Conditional deletion of RBP-J in the renin cell lineage results in a strongly decreased number of renin-expressing cells, thus emphasizing the impact of this transcription factor for renin cell identity (144). Besides RBP-J, the transcription factor CREB (cAMP response element-binding protein) has been found enriched at the corresponding binding site in renin-expressing cells (14). Indeed, the importance of the cAMP/CREB signaling pathway concerning the renin cell identity has already been shown in several studies (51, 125). cAMP mediates its effects via CREB, which acts in association with the histone acetyl transferases CBP and p300. Renin cell-specific knockouts of CBP and p300 resulted in a markedly diminished number of renin-expressing cells, indicating their importance for renin cell identity (51). Also renin cell-specific deletion of Gsα, which mediates activation of adenylate cyclase and, thus, cAMP formation, resulted in a nearly complete abolition of renin-expressing cells in the mouse kidney (125). In addition to RBP-J and CREB, more transcription factors have been found that might determine the renin cell phenotype, such as KLF2 (Krueppel-like transcription factor 2) and NR2F2 (nuclear receptor subfamily 2) (14). Further studies should determine their exact contributions to the renin cell identity.

Recruitment of renin-expressing cells. Chronic challenges of the RAAS, such as reduced renal perfusion pressure, salt restriction, or pharmacological inhibition of the RAAS, result in an enhancement of renin expression. Elevated renin expression is caused by an increased number of renin-expressing cells (9, 49, 52, 153, 166) rather than by gradual increases of renin mRNA in existing renin-producing cells (104). Recruitment of renin-expressing cells (Fig. 2) occurs mainly via reversible metaplastic retransformation of EGM cells (known as hyperplasia of the juxtaglomerular apparatus) and of smooth muscle cells along the media layer of afferent arterioles into renin-producing cells (26, 101, 102, 131). In addition, cell division of renin-expressing EGM has been reported, suggesting that recruitment of renin cells may also include limited renin cell hyperplasia (131). Notably, only vascular smooth muscle (VSM) cells and mesangial cells that originate from renin-expressing precursors, can undergo the reversible metaplastic retransformation into the renin-expressing endocrine state, when homeostasis is threatened (101). Obviously, those smooth muscle and mesangial cells, which had already ex-
pressed renin during nephrogenesis, retain the memory to reenact the developmental program. The retransformation of VSM cells and, hence, recruitment of renin-expressing cells along the afferent arterioles occurs in a retrograde direction starting from the juxtaglomerular position back to interlobar arteries (15, 50). At the same time, when VSM cells redifferentiate into renin-expressing cells, genes that are typically expressed in JG cells, such as Akr1b7 (14, 103) and Cx40 (88), are expressed by the recruited renin cells, underlining the phenotype switch of VSM into renin-expressing cells. Parallel to the appearance of Akr1b7 and Cx40, as markers of the renin cell phenotype, the VSM cell-specific markers Cx45, α-smooth muscle actin (αSMA), smoothelin, and myosin heavy chain disappear due to the redifferentiation from the contractile phenotype of VSM cells to the endocrine phenotype of renin-expressing cells (88, 136). This characteristic change of gene expression during recruitment of preglomerular smooth muscle cells into renin-producing cells does not occur in EGM cells, which strongly express Cx40 but not α-smooth muscle actin already in the resting renin negative state (91, 171, 189). This leads to the impression that two quite different cell populations are operating under RAAS stress with the common goal of producing renin.

In vivo factors determining recruitment of renin expression. The number of renin cells is essentially regulated by the systemic and intrarenal blood pressure, by the sodium balance of the organism, and more directly by the activity of the RAAS itself (9, 52, 153, 166). Longer-lasting states of low arterial blood pressure lead to recruitment of renin cells, while states of (non-renin-dependent) hypertension cause downregulation of the number of renin-producing cells. These effects are mediated by the activity of the sympathetic nervous system on the one hand, which favors renin cell recruitment (61) and by an intrarenal mechanism on the other. How this intrarenal pressure-related mechanism works to determine the number of renin cells, is not understood in detail. It is conceivable that the macula densa could play a mediator function in this context (58, 120, 130). The macula densa cells express cyclooxygenase 2 (COX-2), and some lines of evidence suggest an involvement of COX-2 and prostaglandin E₂ (PGE₂), which should favor recruitment of renin cells. COX-2 and PGE₂ are also thought to mediate renin cell recruitment in states of sodium depletion (40), while sodium overload downregulates the number of renin cells.

Genetic loss-of-function defects of the RAAS cause a massive overformation of renin cells in the kidney (53, 170, 174). The development of this special form of cell hyperplasia is independent of the affected component of the RAAS (105). This suggests that renin cell hyperplasia is triggered by parameters dependent on the activity of the RAAS. In agreement with this hypothesis, inhibitors of the RAAS, such as direct renin inhibitors, ACE-inhibitors, and AT₁ receptor inhibitors, cause a compensatory strong recruitment of renin cells in adult kidneys of humans and laboratory animals (90). Recruitment of renin cells, thereby, involves hypertrophy of the juxtaglomerular apparatus due to retransformation of EGM cells and retransformation of smooth muscle cells of afferent arterioles. In addition, limited proliferation of renin-expressing cells can occur in this context. Retransformation of smooth muscle cells, but not of extraglomerular mesangial cells, appears to be dependent on the availability of nitric oxide, in particular, endothelium-derived nitric oxide (126).

Intracellular signaling pathways involved in renin cell recruitment. Some genes/factors that confer or contribute to the identity of adult JG renin-expressing cells have also a strong impact on the ability for recruitment of renin-expressing cells. The enzyme Akr1b7 has been shown to be coregulated with renin, meaning that recruited renin-expressing cells also express Akr1b7. Since identity and plasticity of renin-expressing cells in Akr1b7 knockout mice are not affected (103), it is
suggested that Ak1b7 plays no important role for the integrity and plasticity of renin-expressing cells. Cell-to-cell communication via the gap junction protein Cx40, which couples renin-expressing cells to each other, to endothelial cells, and to mesangial cells, is indispensable for the correct positioning of adult JG cells, as well as for the ability to recruit renin cells when the RAAS is challenged. Even during prolonged stimulation of renin expression/secretion by severe sodium depletion, Cx40-deficient renin-expressing cells are not recruited in preglomerular arterioles. Notably, these mutant dislocated renin cells escape the physiological negative feedback control by pressure (92, 179). Renin cell-specific deletion of Dicer, the RNase III endonuclease that produces microRNAs, results in a decreased number of renin-expressing cells, showing the importance of posttranscriptional control for the maintenance of renin-expressing cells. It is suggested, that two miRNAs balance the endocrine/VSM cell phenotype of the JG cells: under normal conditions, miR-125b-5p is expressed in VSM and in JG cells, to ensure their contractility. However, when homeostasis is threatened, miR-125b-5p expression decreases in the VSM cells, thereby, allowing them to regain the endocrine phenotype. But in the JG cells, miR-125b-5p is still expressed. Additionally, miR-330 is expressed in the JG cells, which inhibits the expression of vascular smooth muscle genes in favor of an endocrine phenotype. Besides the aforementioned genes, two transcription factors have been identified, which are crucial for identity, as well as for recruitment of renin-expressing cells: CREB and RBP-J. In the absence or impaired activation of these transcription factors in the renin cell lineage, recruitment of renin-expressing cells due to homeostatic challenge is lost (19, 135).

Plasticity of EPO Production

**Development of EPO cells.** During embryonic development, EPO is predominantly produced by the liver, mainly by hepatocytes surrounding the central veins (79). Besides hepatocytes, nonparenchymal perisinusoidal cells, identified as Ito cells, have also been shown to be EPO producers (111, 154). With ongoing maturation of the kidney, which occurs during late gestation in humans or at birth and shortly after birth in rodents, EPO expression switches from the liver to the kidneys as the main production site (38, 121, 188), with the kidneys accounting for about 85–90% of serum EPO levels during normal erythropoiesis (46, 77). In adults, the liver retains its ability to produce EPO to some extent in response to challenge (73, 119, 128) but cannot compensate for the loss of kidney-derived EPO (68, 173). In the kidneys, EPO is produced by peritubular fibroblast-like interstitial cells. So far, only data for the distribution of EPO-producing cells in postnatal kidneys are available. They show that in the unstressed kidney EPO-expressing cells are located in the deep renal cortex (predominantly juxtaglomerular region) and outer medulla (7, 113, 133). Notably, in the continuously hypoxic area of the inner medulla, no EPO-expressing cells are found (97). The origin of EPO-expressing cells is not fully clear. According to their fibroblast-like character, a descent from FoxD1-positive cells of the renal mesenchyme would be plausible (62, 100). Because some neuronal marker proteins have also been detected in EPO-producing cells, a descent from the neural crest has been discussed (128). Tracing of the neural crest lineage with myelin protein zero-Cre (P0-Cre) in the kidney revealed that almost 90% of the fibroblast-like EPO-expressing cells belonged to this lineage (3). The spatiotemporal development of EPO-producing cells in the developing kidney has not yet been investigated in detail. There is, however, evidence that in the adult kidney, potential EPO-producing cells exist, which have not produced EPO before, but can be activated in the adult kidney for the first time (186). This suggests the existence of a silent reserve pool of cells capable of producing EPO.

**Structure of EPO cells.** EPO-producing cells in the kidney do not display special morphologic characteristics. They appear as elongated cells with several extensions that are in close neighborhood to basolateral surfaces of tubuli (7, 134). Similar to pericytes, the cells are in close contact to capillary endothelial cells (7), leading to the early suggestion that EPO-producing cells might be endothelial cells (94).

**Markers and other genes conferring identity of EPO cells.** The majority of EPO-producing cells expresses CD73 (ecto-5'-nucleotidase) (7, 45, 113, 128, 133, 134) and PDGFR-β (the β-receptor for platelet-derived growth factor) (134), both typically being expressed by fibroblasts (25, 47, 164). Another study showed positive staining of the renal EPO-producing cells for the neural markers, such as MAP2 (microtubule-associated protein 2) and NF-L (neurofilament light polypeptide), suggesting that the EPO-producing cells originate from the neural crest (128).

EPO secretion is directly linked to transcription of the EPO gene via the transcription factor HIF-2 (hypoxia-inducible factor 2) (55, 133, 140, 156). The hypoxia-inducible transcription factors (HIFs) belong to the PAS (Per-ARNT-Sim) family of heterodimeric basic helix-loop-helix transcription factors, which consist of the constitutively expressed HIF-1β (ARNT) subunit, and one of the either O2 labile HIF-α subunits HIF-1α, HIF-2α, and HIF-3α (114, 115, 159, 183). The HIFs bind to promoter or enhancer sequences of target genes, with HIF-1 inducing transcription of genes involved in, for example, anaerobic glycolysis (115), and HIF-2 as the main regulator of EPO production (55, 140, 155). By generating transgenic mice carrying EPO gene constructs of a different length, it turned out that EPO expression in the liver and kidney is differently regulated. The same conclusion was reached by the expression of gene constructs incorporating different sections of the EPO gene and its flanking sequence in cell lines. The regulatory elements for hypoxic induction in the liver are between 0.4 kb upstream and 0.7 kb downstream of the EPO gene-coding sequences, while an essential regulator element for renal EPO expression lies between 12 and 8 kb in the 5′-region (160, 161). Besides HIF-2, the transcription factors GATA-2 and NF-κB are also involved in the control of EPO expression. It was shown in this context, that GATA-2 binds to a GATA element, which is located at −30 bp of the 5′-promoter region, thereby inhibiting the promoter activity and, hence, the expression of the EPO gene (65). Another in vitro study found that, besides GATA-2, NF-κB has also been shown to inhibit the promoter activity of the EPO gene and, hence, EPO production, thus providing a mechanism for downregulation or switch-off of EPO expression during inflammatory diseases (93). It was, furthermore, found in vivo that inhibition of the EPO promoter via GATA-2 is essential for tissue-specific EPO gene expression in the way that GATA-2 constitutively represses ectopic EPO expression, such as in distal tubules and...
collecting ducts of the kidney (128). Regarding the switch of EPO production from the liver to the kidney, the possibility has to be considered that the different regulation of EPO expression could result from trans-acting elements, such as GATA factors, that are themselves expressed in a defined temporal-spatial pattern.

**Recruitment of EPO-expressing cells.** During normoxia, renal fibroblasts, hepatocytes, and hepatic Ito cells express EPO, but these cells are only few in number (37, 79, 80). Both liver and kidneys respond to hypoxia, with strongly increased expression of EPO mRNA. Kidneys respond to insufficient oxygen supply by recruiting additional EPO-producing fibroblasts (Fig. 3). Notably, the number of EPO-producing cells increases almost exponentially with the severity of hypoxia in the kidneys (37, 80). EPO-producing cells were found almost exclusively in the renal cortical labyrinth, with recruitment of EPO-expressing cells—depending on the degree of hypoxia—from the juxtamedullary cortical areas up to the midcortical zone, and finally to the superficial cortex (37). It was also found in this context that during hypoxia stimulation, the amount of EPO mRNA expression per cell was not markedly changed, suggesting that elevated EPO expression during hypoxia mainly results from a recruitment of EPO-producing interstitial cells (37, 80). This phenomenon is very similar to that seen for recruitment of renin expression (104). It is not known whether the recruitment of EPO-producing cells requires de novo formation of cells, or leads to a transformation of preexisting cells into EPO producers, or is merely due to a switch-on of EPO gene transcription in preexisting silent cells. Since massive recruitment of EPO-expressing cells induced by acute hypoxia or pharmaceutical prolyl-hydroxylase inhibitors occurs in very few hours (37, 133), a de novo formation of EPO-producing cells appears to be a less likely explanation. There is, in fact, evidence for a reserve pool of EPO-producing cells, that even may be activated in the adult kidney for the first time (186). Hypoxia-related regulation of EPO expression in the liver is to some extent different from that in the kidney. It is commonly known that during hypoxic conditions, there is limited recruitment of EPO-expressing hepatocytes and Ito cells in the liver. However, above a certain degree of hypoxia, higher amounts of EPO are not produced by a further recruitment of cells, but rather by an elevated EPO production per cell (79).

**In vivo factors determining recruitment of EPO expression.** The number of EPO-expressing cells in the healthy kidneys is essentially determined by the arterial oxygen tension, the oxygen carrying capacity of the blood, and the oxygen affinity of hemoglobin (182). Finally, all of these parameters determine the essential control factor of EPO expression, namely, the pericellular oxygen tension in the interstitium of the renal cortex. Thus, low oxygen tension in the arterial blood, low hemoglobin concentrations, and an increased oxygen affinity of hemoglobin to lower tissue oxygen tensions cause recruitment of EPO-producing cells. Notably, renal perfusion (pressure) is not a physiological regulator of EPO cell recruitment (42, 132). Changes of renal blood flow lead to parallel changes of oxygen delivery and oxygen consumption, because the energy-consuming workload of kidney tubules is determined by renal perfusion and glomerular filtration. As a consequence, tissue oxygen tensions in the renal cortex remain stable even when renal perfusion varies, at least within the physiological range.

Prolyl-hydroxylase inhibitors (PHD-I), which are currently developed for therapeutic use, also cause a strong recruitment of EPO-producing cells (133).

Derecruitment of EPO-producing cells is frequently seen during chronic kidney disease, particularly, in states of interstitial kidney fibrosis. The reasons for this are that potential
EPO producers have irreversibly transformed into cell types not capable of expressing EPO (3, 165). Another possibility is that potential EPO producers do not receive the proper signal to turn on EPO expression (10).

Intracellular signaling pathways involved in EPO cell recruitment. An essential process for the recruitment of renal EPO-producing cells in response to tissue hypoxia is stabilization of the transcription factor HIF-2 (133), according to the currently accepted concept of oxygen sensing for the control of gene expression (27). Whether HIF-2 alone is sufficient to recruit EPO-producing cells is yet unknown. HIF-2 certainly also acts as an enhancer of EPO gene transcription in the kidney. Renal EPO gene regulation is still less understood than the well-characterized regulation of EPO gene transcription in hepatocytes (140). The regulatory elements for hypoxic induction in the liver are between 0.4 kb upstream and 0.7 kb downstream of the EPO gene-coding sequences, while an essential regulator element for renal EPO expression lies between 12 and 8 kb in the 5’-region (160, 161). Whether HIF-2-related events or other hypoxia-induced events are required in addition to render EPO gene transcription susceptible in recruited renal cells, is also unknown. This lack of knowledge is mainly due to the lack of suitable in vitro models, to study the regulation of renal EPO gene expression at the cellular level. Although successful isolation and culture of retino-cortical interstitial fibroblasts has been described, these cells stop producing EPO under cell culture conditions (69, 134, 158).

In most forms of chronic renal disease, the patients suffer from anemia, which results from a relative deficiency of renal EPO production (18, 29, 39, 124). Chronic kidney disease finally results in fibrosis, with the severity of renal anemia depending on the progression of fibrosis (124, 143). Several studies have shown that interstitial changes involve proliferation and increased size of fibroblasts, as well as altered expression profiles of these cells (112). Interstitial fibroblasts, especially those in the cortical labyrinth, express substantially less CD73, but express instead desmin and αSMA, which are markers of myofibroblasts (3, 112). At the same time, the number of EPO-producing cells declines due to renal injury. Recently published data suggest that most of the myofibroblasts in the diseased kidneys result from a transformation of EPO-producing cells into non-EPO-producing myofibroblasts (165). It is known that inflammatory cytokines, such as TNF-α inhibit EPO expression (44, 93, 123) and that the TGF-β-Smad signaling pathway is responsible for myofibroblast transformation (74, 150). Their involvement in transformation of EPO-producing fibroblasts into non-EPO-producing myofibroblasts has been investigated. It has been described that NF-{\kappa}B signaling is responsible for repression of EPO expression and that the Smad signaling pathway promotes the transformation of EPO-expressing cells (165). Notably, there appears to exist a reversible transition state between EPO-producing cells and myofibroblasts (165). Those cells that still exist in chronically diseased kidneys are probably able to turn on the EPO gene in response to episodes of acute hypoxia, even if the daily production of EPO is inappropriately low to maintain normal blood cell counts (11, 22).

Interconversion of Renin- and EPO-Producing Cells

Recent evidence suggests that the relation between renin and EPO-producing cells might be even closer than previously thought and that at least renin-producing cells can convert into EPO-producing cells under certain conditions. This surprising observation was made in experiments in which HIFs were stabilized (114) in the renin cell lineage by protecting them from proteasomal degradation. Such a stabilization of HIFs in renin cells was achieved by deletion of the von Hippel-Lindau protein (pVHL) from cells expressing Cre under the control of the endogenous renin-1d promoter and their descendants (101). It turned out that this maneuver increased HIF-2, but not

![](http://ajpregu.physiology.org/)

![Diagram](http://ajpregu.physiology.org/)
HIF-1, protein in the renin cell lineage in the juxtaglomerular vessel walls and JG cells (89), suggesting that renin cells use HIF-2 rather than HIF-1 as the main hypoxia-inducible transcription factor. A similar preferential use of HIF-2 has already been described for interstitial fibroblasts and endothelial and mesangial cells of the kidneys (133, 145, 146), whereas tubular kidney cells preferentially use HIF-1 (133, 145). Notably, deletion of pVHL and concomitant stabilization of HIF-2 in the renin cell lineage suppressed renin expression in the JG area (89). The typical juxtaglomerular cells appeared flat and agranular in the pVHL-deleted state. This shift of the cell morphology was accompanied by the disappearance of marker proteins for renin cells, such as Akr1B7 and Cx40, and by the appearance of novel markers, such as collagen I, CD73, and PDGFR-β (86), suggesting that JG cells had transformed into a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like appearance of novel markers, such as collagen I, CD73, and PDGFR-β (86), suggesting that JG cells had transformed into a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell. Strikingly, these cells now expressed EPO (Fig. 4), leading to increased plasma EPO concentrations and a fibroblast-like cell.

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DISCLOSURES
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AUTHOR CONTRIBUTIONS
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