JBC EDITORS' PICK HIGHLIGHT

In search of regulatory circuits that control the biological activity of vitamin D

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Although the cytochrome P450 CYP27B1 plays a critical role in vitamin D biology, the molecular mechanisms involved in regulation of CYP27B1 have remained undefined. A new study has identified a kidney-specific control module distal to the *Cyp27b1* gene that mediates the basal activity and hormonal regulation of *Cyp27b1*. This work provides a novel mechanism indicating differential regulation of *Cyp27b1* in renal and non-renal cells and has implications for vitamin D biology in multiple sclerosis and perhaps other autoimmune diseases as well.

Vitamin D is critical for calcium homeostasis and is required for the development and maintenance of skeletal integrity. In addition, it has been suggested to have roles in extraskeletal health including cancer, immunity, and autoimmune diseases. Vitamin D undergoes two enzymatic steps to form the active compound 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$).[2] CYP27B1 is a cytochrome P450 enzyme that performs the second step in this process, metabolizing 25-hydroxyvitamin D_3 to $1,25(OH)_2D_3$ (1, 2), and thus controls the biological activity of vitamin D. Inactivating mutations in the gene encoding CYP27B1 cause vitamin D-dependent rickets type 1 (VDDRI) (also known as pseudovitamin D deficiency rickets) despite normal intake of vitamin D (3), indicating the importance of this enzyme for skeletal integrity. CYP27B1 is present predominantly in the proximal straight tubule of the kidney and is also expressed in low levels in other tissues including placenta, immune cells, and malignant epithelia (1). However, whether CYP27B1 serves a function at sites other than the kidney and placenta under normal physiological conditions is a matter of debate (1) that has been difficult to resolve. This has been due at least in part to the fact that the detailed regulatory mechanisms controlling this enzyme are not clear and tools to establish these mechanisms, particularly in a location-sensitive manner, are lacking. A new report by Meyer *et al.* (4) addresses these gaps using ChIP-seq analysis as well as newly generated genetic mouse models to identify a kidney-specific regulatory module that controls basal and hormone-regulated expression of *Cyp27b1*.

CYP27B1 is tightly regulated. A primary signal in mediating induction of $1,25(OH)_2D_3$ in the kidney is elevated parathyroid hormone (PTH). This was demonstrated in early animal studies in which thyroparathyroidectomy resulted in reduced production of $1,25(OH)_2D_3$, whereas administration of parathyroid extract restored $1,25(OH)_2D_3$ production almost to control levels (5). $1,25(OH)_2D_3$ is known to regulate its own production by inhibiting CYP27B1. In addition to $1,25(OH)_2D_3$, the phosphaturic factor fibroblast growth factor 23 (FGF23), which acts as an endocrine factor, also suppresses expression of renal CYP27B1 (1, 2) (Fig. 1). But, what are the molecular mechanisms connecting these hormones to CYP27B1 and each other? Some initial hints have emerged. Early studies showed that $1,25(OH)_2D_3$ treatment could suppress *Cyp27b1* expression in both thyroparathyroidectomy and sham-operated rats, suggesting that activation by PTH and suppression by $1,25(OH)_2D_3$ are two distinct events (6). It was suggested that $1,25(OH)_2D_3$-mediated suppression may not be based on direct binding of the vitamin D receptor to a consensus vitamin D response element in the *Cyp27b1* gene but rather may be indirect (7).

Meyer *et al.* (4) identified, through ChIP-seq analysis, a genomic region distal to *Cyp27b1* as a potential control module to explain the regulation observed. The authors used *in vivo* studies with mice generated via CRISPR/Cas9 genome–editing methods to directly evaluate the functional contribution of this potential regulatory region to control *Cyp27b1* expression. The authors found regions of DNA that mediate PTH induction and $1,25(OH)_2D_3$ and FGF23 suppression of renal *Cyp27b1* expression located in selected introns of the nearby *Mettl1* and *Mettl21b* genes (genes that produce methyltransferase-like proteins). Dissection of these regions pointed to distinct sections responsible for basal expression, PTH induction, and $1,25(OH)_2D_3$- and FGF23-mediated suppression. In mice in which the intronic enhancer located at *Mettl1* (M1-IKO) had been deleted, basal *Cyp27b1* expression was strikingly reduced, and sensitivity to PTH induction (but not to FGF23 and $1,25(OH)_2D_3$ suppression) was lost. Deletion of the *Mettl21b* enhancer (M21-IKO) resulted in mice with a more limited decrease in basal *Cyp27b1* expression that were still sensitive to PTH but insensitive to FGF23 and $1,25(OH)_2D_3$ treatment. Altered vitamin D metabolism and a debilitating skeletal phenotype similar to the *Cyp27b1* KO mouse were observed in

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[2] The abbreviations used are: $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; PTH, parathyroid hormone; MS, multiple sclerosis.

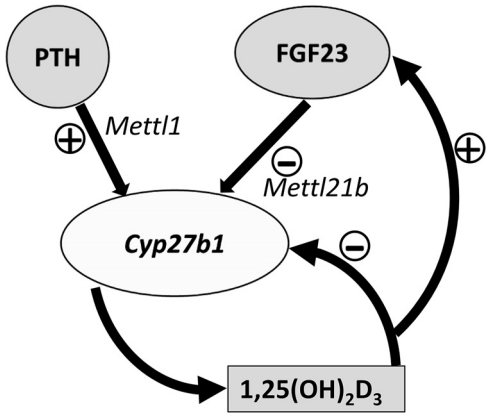

Figure 1. Regulation of renal CYP27B1. Although PTH, FGF23, and 1,25(OH)$_2$D$_3$ play key roles in the regulation of renal CYP27B1, the mechanisms involved have remained undefined. Meyer *et al.* (4) describe for the first time a kidney-specific control module distal to *Cyp27b1* that controls both basal and hormone-regulated expression of *Cyp27b1 in vivo*. The authors found that regions that mediate PTH induction and FGF23 and 1,25(OH)$_2$D$_3$ suppression of renal *Cyp27b1* are located in selected introns of *Mettl1* and *Mettl21b* genes, respectively. Suppression of *Cyp27b1* that includes induction of FGF23 by 1,25(OH)$_2$D$_3$ (or increased P$_i$) is also suggested by the findings of Meyer *et al.* (4).

M1-IKO but not in the M21-IKO mice. The striking phenotype specifically of M1-IKO mice emphasizes the essential role of PTH in the control of bone mineralization and calcium homeostasis.

In additional experiments, Meyer *et al.* (4) explored the localization and hierarchy of this newly discovered circuit. The authors noted that the ability of another known regulator, LPS, to induce *Cyp27b1* in non-renal target cells was unaffected by the enhancer deletion, suggesting a separate mechanism for modulation of locally produced 1,25(OH)$_2$D$_3$. Moreover, because 1,25(OH)$_2$D$_3$ induces FGF23 in bone, the authors tested whether 1,25(OH)$_2$D$_3$ might suppress *Cyp27b1* by inducing FGF23. 1,25(OH)$_2$D$_3$ was indeed found to suppress *Cyp27b1* in kidney within the same time frame as its effect on induction of FGF23 in bone (calcium and P$_i$ were also induced by 1,25(OH)$_2$D$_3$). Although these findings are consistent with suppression of *Cyp27b1* that involves induction of FGF23 by 1,25(OH)$_2$D$_3$ (or by increased P$_i$), further mechanistic studies are needed.

Finally, Meyer *et al.* (4) noted the relevance of their findings to genome-wide association studies (GWAS) that have correlated single-nucleotide polymorphisms to the prevalence of multiple sclerosis. MS-associated single-nucleotide polymorphisms were identified in a region that contains the *CYP27B1* gene as well as *METTL1* and *METTL21B* (8). High expression of *CYP27B1* was also reported to be associated with the MS protective genotype (rs10877013-T allele) (9). The new data from Meyer *et al.* (4) indicating that the regulatory module of renal *Cyp27b1* is located in selected introns of *Mettl1* and *Mettl21b* suggest that multiple sclerosis is associated with renal production of 1,25(OH)$_2$D$_3$. Although further studies examining the mechanisms of regulation of the human CYP27B1 gene are needed, these findings have implications for vitamin D biology in MS and perhaps other autoimmune diseases.

The identification of a kidney-specific multicomponent control module distal to *Cyp27b1* that mediates basal activity and hormonal activation and suppression represents a novel mechanism for the control of *Cyp27b1*. This is an important advance in the vitamin D field that has provided insight for the first time using a genome-wide perspective on the mechanisms that control *Cyp27b1* expression. These findings provide evidence for distinct regulation of *Cyp27b1* for the control of mineral metabolism and for the control of other pleiotropic actions of vitamin D. These pioneering studies, including novel mouse models, will enable future studies related to the identification of transcription factors and exact sites of hormonal regulation of *Cyp27b1*. In addition, the impact of locally produced 1,25(OH)$_2$D$_3$ at sites other than the kidney under normal physiological conditions and in disease (including autoimmune disease and cancer), which has been a matter of debate (1), can now be determined.

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