Best Practice & Research Clinical Endocrinology & Metabolism 25 (2011) 531–541

Contents lists available at ScienceDirect

Best Practice & Research Clinical Endocrinology & Metabolism

journal homepage: www.elsevier.com/locate/beem

1

Regulation of vitamin D metabolism

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Keywords:
vitamin D
25-hydroxyvitamin D_3
1,25-dihydroxyvitamin D_3
24
25-dihydroxyvitamin D_3
metabolism
hydroxylase
CYP2R1′ CYP27B1
CYP24
CYP24A1
parathyroid hormone (PTH)
fibroblast growth factor 23 (FGF23)

Fundamental to understanding the way in which perturbations in the vitamin D endocrine system can affect human health is an appreciation of the steps involved in the production of the well-recognized active hormonal form, 1,25-dihydroxyvitamin D_3. Thus this paper focuses first on the nature and regulation of the two enzymes responsible for the production of 1,25-dihydroxyvitamin D_3, the 25-hydroxylase in the liver and the 1α-hydroxylase in the kidney. The most important regulators of the 1α-hydroxylase in the kidney are 1,25-dihydroxyvitamin D_3 itself, parathyroid hormone and FGF23. The extent and importance of extra-renal, 1,25-dihydroxyvitamin D_3 synthesis is then considered. Finally the features of the 24R-hydroxylase, which produces 24R,25-dihydroxyvitamin D_3inthe kidney and is induced by and inactivated, 1,25-dihydroxyvitamin D_3in target cells are described.

Overview

The entry of vitamin D into the body is through the production of vitamin D in the skin upon exposure to sunlight and through ingestion of either foods containing cholecalciferol (vitamin D_3) or ergocalciferol (vitamin D_2) or dietary supplements of these substances. The cells of the epidermis contain 7-dehydrocholesterol which, upon irradiation, is converted to pre-vitamin D_3, in which ring B of the cholesterol ring system is broken. Following thermal rearrangement of triene system, vitamin D_3(D_3) is released into the blood (Fig. 1). The amount of D_3 made by the skin is determined by the exposure of the epidermal cells to UVB irradiation, which in turn depends on several geographical, physical, and cultural conditions. Geographical conditions leading to greater D_3 production include proximity to the equator and altitude. D_3 production in the skin is decreased in direct proportion to increased skin pigmentation. Culturally based interference of access of the skin to UVB irradiation

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doi:10.1016/j.beem.2011.05.003

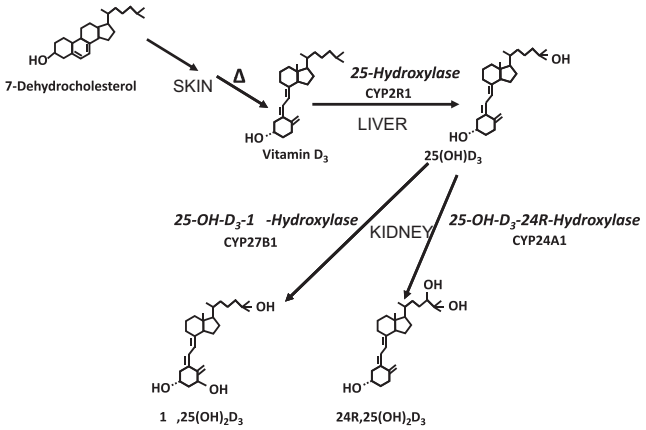

Fig. 1. Metabolism of vitamin D_3 in the skin, liver and kidney. 7-Dehydroxholesterol in the skin is converted in two steps and beginning with the exposure to ultraviolet light to vitamin D_3, which is transported in the blood (mostly protein-bound) to the liver where the side chain is hydroxylated at the C25-position. The resulting $25(OH)D_3$ is the major circulating form of vitamin D. In the kidney, $25(OH)D_3$ is hydroxylated at either the C1- or the C24 position to form either $1\alpha,25(OH)_2D_3$ or $24R25(OH)_2D_3$. The cytochrome P450 (CYP) dependent enzymes responsible for these transformations are, respectively, the 25-hydroxylase (CYP2R1), the 1α-hydroxylase (CYP27B1) and the 24R-hydroxylase (CYP24A1 or CYP24).

includes complete clothing of the body and face and the use of sun screens as a protection against skin cancer.

Once produced in the skin or absorbed from ingestion, D_3 is released into the circulation where the majority is quickly hydroxylated in the liver at the C25-position of the side chain. The product of this enzymatic modification, 25-hydroxyvitamin D_3 ($25(OH)D_3$; $25D_3$) is the major circulating D_3 derivative. The enzyme that catalyzes the 25- hydroxylation of vitamin D is a cytochrome P-450-dependent enzyme, as are the two other enzymes under consideration in this review which carry out hydroxylations at the 1α- and 24R- positions. Cytochrome P450-dependent hydroxylases are integral membrane proteins located in either the mitochondria or smooth endoplasmic reticulum (microsomes) and require electrons from NADPH to reduce molecular O_2 to one hydroxyl group and one water molecule.Although initially characterized in the kidney, both the 1α- and 24R-hydroxylases, have wide tissue distribution representing the breadth of their roles in the endocrine and paracrine functions of the vitamin D endocrine system.

The aim of this paper is to review the current understanding identification, occurrence, and physiological regulation of the three hydroxylase enzymes that are responsible for the production and catabolism of the hormonal form(s) of vitamin D_3. It should be noted that ingested vitamin D2 (ergocalciferol) undergoes similar metabolic transformations, although whether all the details of its regulation and biological activity are identical to those of D_3 remains unclear. The remainder of this article will be devoted to what is known about D_3 and its transformations in mammals, emphasizing, but not limited to humans.

The 25-hydroxylation of vitamin D_3

Identification of the 25-hydroxylase

The major circulating form of D_3, and the form that is currently used as a measure of an organism's vitamin D status, is 25-hydroxyvitamin D_3($25D_3$). The liver was identified as the site of 25-hydroxylation of vitamin D_3 very shortly following the characterization of this metabolite over forty years ago.[1] In the interim, there have been several candidates for the 25-hydroxylase, both mitochondrial and microsomalthat were found to be capable of adding a hydroxyl group to the side chain of D_3,[2] but the question of whether a single specific enzyme dedicated to this function existed and if so, its subcellular

location,was not resolved until recently. A former "orphan" (i.e. unknown endogenous substrate) liver cytochrome P450, CYP2R1, was found to be a highly conserved and substrate-specific microsomal 25-hydroxylase.[3] Its genetic loss in humans leads to deficient 25-hydroxylation of D_3[4] demonstrating its physiological importance, which is further supported by is predominant location in the liver and is not sexually dimorphic. Some residual 25-hydroxylation in this subject may have been due to the mitochondrial CYP27A1, whose main physiological function is the hydroxylation of the side chain of cholesterol in bile acid synthesis, but which can also 25-hydroxylate D_3.

The structural analysis of CYP2R1 revealed that D_3 is bound in an elongated form which provides some rationale for the substrate specificity that the enzyme displays. The hydrophobic pocket in which D_3 is bound places the hydrocarbon side chain in proximity to the heme for the catalytic addition of the hydroxyl group.[5]

Studies of the regulation of hepatic 25-hydroxylation in the context of the vitamin D endocrine system, e.g., by varying vitamin D status, PTH, or dietary calcium or phosphorous levels, have failed to reveal robust effects of these calcium regulatory factors on the production of circulating $25D_3$. In addition to its sensitivity to bile acids (given its prominent role in the formation of these molecules) rat hepatocyte CYP27A1 mRNA can be affected by a variety of factors, including $1,25D_3$,[2] but the physiological significance of these observations, obtained prior to the recognition of CYP2R1 as the primary catalyst of D_3 25-hydroxylation, to calcium homeostasis is not clear.

Occurrence and regulation of D_3 25-hydroxylase in extra-hepatic tissues

The identification of CYP2R1 as the primary physiologically important enzyme catalyzing the 25-hydroxylation of D_3 has allowed further investigation of its occurrence and study of its possible regulation. At the same time that it was identified as the liver 25-hydroxylase, CYP2R1 was also noted to occur in the testis.[4] Recent studies confirm this and note that lower levels of this enzyme in individuals with testicular failure are associated with decreased bone mineral deficiency.[6] CYP2R1 has also been studied in dermal fibroblasts and in prostate cancer LNCaP cells, where it is suppressed by 1,25D, but not by antiepileptic drugs.[7]

Regulation of the synthesis of $1,25D_3$ in the kidney

$1\alpha,25(OH)_2D_3$ ($1,25D_3$) is the primary hormonally active form of vitamin D. It occurs in the kidney, which supplies the circulating hormone and in extra-renal cells. The regulation of the enzyme's activity in these two locations will be considered separately, since they serve different purposes in the organism. The three main influences on $1,25D_3$ production by the kidney are direct negative feedback by $1,25D_3$ itself, parathyroid hormone (PTH) as a signal of calcium status and fibroblast growth factor 23 (FGF23) as a signal of phosphate homeostasis.

Feedback regulation by $1,25D_3$

One of the earliest regulatory influences on the renal 1α-hydroxylation of $25D_3$ to be recognized was the vitamin D status of the animal under study. Whether the formation of $1,25D_3$ was measured *in vivo*, in kidney tissue or mitochondrial fractions thereof from treated animals, or in cultured kidney cells treated with $1,25D_3$, this direct negative feedback effect is universally observed.[8] When the 1α-hydroxylase was cloned and identified as CYP27B1, this negative feedback effect was established as a transcriptional one, mediated by interaction of the steroid hormone, 1,25D with its ligand activated-nuclear receptor, the VDR. In fact it was the unsuppressed levels of 1α-hydroxylase activity in the first line of VDR null mice which allowed the cloning of the gene for the enzyme and provided a key piece of evidence on this point.[9] At approximately the same time, cDNAs encoding 1α-hydroxylase from rats and human were cloned[10,11] all of which have contributed to our molecular understanding of how $1,25D_3$ regulates its own production.

It is likely that $1,25D_3$, having formed a complex with its receptor, the VDR, down-regulates expression of the gene for CYP27B1 through interaction with a negative response element (nVDRE) in or near the promoter region of the gene. Evidence for such an nVDRE about 500 bp upstream from

the transcriptional start site (TSS) has been reported.[12,13] In these and subsequent reports,[14,15] these authors show that binding of the VDR-RXR heterodimer is not directly to the nVDRE, which shows no sequence similarity with other VDRE's, but is through another transcription factor, VDIR (VDR interacting repressor). VDIR is a basic helix-loop-helix transcription factor which itself binds to the nVDRE and activates transcription; this transcriptional activity of VDIR is suppressed by ligand bound VDR-RXR heterodimer; the mechanism of this suppression appears to involve methylation of specific CpG sites in the promoter region. Furthermore, in the absence of VDR-RXR-1,25D_3, the (active) transcriptional complex is characterized by the presence of histone acetylases and in its presence (inactive, repressed complex), these are replaced by histone deacetylases, indicating that the modification of chromatin structure is an integral part of the regulation of CYP27B1 by the hormone 1,25D_3.[15]

Recently[16] an extended analysis of the CYP21B gene revealed 1,25D_3 responsive regions 2.6 and 3.2 kb upstream of the TSS, within an intron of the preceding gene (methyltransferase-like gene, METTL1). In contrast to the nVDRE located closer to the TSS described above, these two distal sites contain classical VDRE sequences that display ligand-dependent direct binding of the VDR-RXR heterodimer, leading to recruitment of protein cofactors and chromatin looping to bring the distal and proximal regions of the promoter into proximity to one another. The fact that these newly recognized nVDRE's resemble positive VDREs in sequence and characteristics is consistent with observations regarding nVDREs of other genes whose expression is down-regulated by 1,25D_3.

While the details of the mechanisms of 1,25D_3 down-regulation of the enzyme that produces it in the kidney it is not yet fully developed, it can be expected that, as understanding of the control of gene expression in general is deepened, greater understanding of the tissue-specific control of CYP27B1 expression will emerge as well.

Regulation of 1,25D_3 synthesis by calcium/PTH

As was the case with the role of vitamin D, through its active hormonal metabolite 1,25D_3, in the regulation of the 1α-hydroxylase, it was clear very early on that the activity of this enzyme is inversely proportional to the dietary and serum calcium status of the organism. Much early work was directed at demonstrating that this regulation is mediated by parathyroid hormone (PTH), that this effect is exerted directly on the kidney cell, and that it involves the second messengers generated by protein kinases A and C.[8,17] Regulation of the ability of the kidney to produce 1,25D_3 by PTH is specific to the proximal cells of the kidney[18] and is exerted at both the transcriptional and post-transcriptional levels. For example, PTH stimulation of 1,25D_3 production declines with age in both humans[19] and rats,[20] but the decreased 1α-hydroxylase activity in response to PTH treatment is not accompanied by decreased CYP27B1 mRNA levels.[21]

The promoter region of the CYP27B1 gene contains a region that renders its transcription sensitive to stimulation by PTH.[22,23] Recently a role for DNA methylation and demethylation in the regulation of CYP27B1 expression by PTH has been reported[15] with demethylation and transcriptional activity resulting from PTH stimulation. Proteins isolated from a complex associated with the VDIR described above, include DNA methyl transferases as well as a glycosylase whose phosphorylation by protein kinase C is implicated in the demethylated mechanism, consistent with the implication of this enzyme in PTH-mediate regulation of CYP27B1.

Regulation of 1,25D_3 by phosphate/FGF23

The role of phosphate in the regulation of renal 1,25D_3 synthesis in mammals was demonstrated in vivo and in vitro very early in the study of the 1α-hydroxylase.[24–26] In both humans and in the *Hyp* mouse model developed to gain insight into human disease, hypophosphatemic disorders are associated with renal phosphate excretion, defective skeletal mineralization, osteomalacia in adults and rickets in children and inappropriately (for the given serum phosphate levels) low serum 1,25$(OH)_2D_3$levels. The signaling pathway involved in this regulation of vitamin D metabolism associated with serum phosphate levels remained an enigma until about a decade ago when the human gene for FGF23, a new member of the fibroblast growth factor family, was identified as the site of

mutations found in autosomal dominant hypophosphatemic rickets,[27,28] as well as the causative factor in tumor induced osteomalacia.[29]

FGF23 levels are increased in X-linked hypophosphatemia (XLH) in humans and in the *Hyp* mouse. Studies in this animal model as well as in mice genetically engineered to over- or under-express FGF 23[30–32] revealed that in addition to the role of FGF23 as the hypophosphatemic agent it also seems to mediate the regulatory effect of serum phosphate levels on vitamin D metabolism, in particular the lowering of 1,25D levels in the blood.[31] The experiments that have led to the recognition of FGF23 as the signal from the bone to the kidney and the parathyroid glands (and, although not considered here, the intestine) regarding the sufficiency or excess of phosphate levels have been the subject of several excellent recent reviews to which the reader is referred for more detail.[33–35]The underlying mechanism by which the synthesis and secretion of FGF23 synthesis in the bone cells is stimulated by elevated phosphate levels has not been elucidated (as indicated by the dotted lines in Fig. 2, see below), but it is clear that FGF23 is integral to maintenance of phosphate homeostasis, one aspect of which is the modulation of vitamin D metabolism.

Fig. 2 shows many of the interrelationships of the hormones involved in calcium and phosphorous homeostasis and, in turn, their interactions with the renal production of $1,25D_3$. As noted in the previous sections, PTH, secreted in response to low serum calcium levels, stimulates the transcription (and possibly post-transcriptional activation) of CYP27B1 and $1,25D_3$ inhibits it. Since $1,25D_3$ also stimulates the movement of phosphate into the blood from the intestine and bone, it is not surprising that an inverse relationship between the synthesis and circulating concentrations of $1,25D_3$ and the serum phosphate levels has been known for decades. Recent studies have shown that the basis of this relationship lies in the inhibition of the transcription of CYP27B1in the kidney by FGF23, which is secreted by bone osteocytes in response to elevated serum phosphate concentrations. Thus, in the kidney FGF23 causes, at the same time, increased phosphate excretion in the urine, through reduction

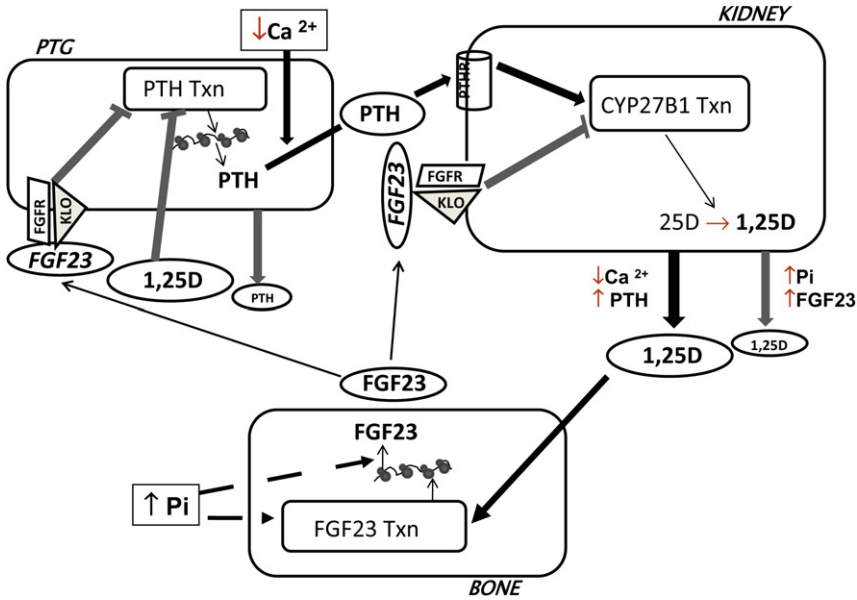

Fig. 2. Hormones that regulate CYP27B1 (1α-hydroxylase) activity in the kidney. All the effects shown are depicted as being on the transcription (TXN) of CYP27B1 mRNA, although additional post-transcriptional effects have not been ruled out. The right hand side of the figure depicts the feedback inhibitory effect of $1,25D_3$ on its own production. In the upper left is shown the parathyroid gland (PTG), from which PTH is secreted to stimulate CYP27B1 when serum calcium levels are low. The synthesis and secretion of PTH is inhibited by $1,25D_3$, as a second negative feedback loop in $1,25D_3$ synthesis. FGF23 produced in the bone (bottom of figure) in response to elevated phosphate levels, inhibits CYP27B1 synthesis, completing a third negative feedback loop involving phosphate. FGF23 is also thought to inhibit PTH secretion.

in the number of sodium-phosphate co-transporters in the brush border membranes[36,37] and decreased $1,25D_3$ synthesis by the proximal renal tubules.[38]

The signaling pathway for regulation of CYP27B1 by FGF23 in the kidney begins with the interaction of the peptide with its receptor, FGFR. The current model for this interaction is that it is only productive if one of the several isoforms of the FGFR that binds FGF23 with low affinity is associated with the membrane protein klotho, which confers upon the FGFR the binding capacity and specificity for FGF23.[39–41] In this regard, FGF23 is part of a small subfamily of FGFs (which also includes FGF 19 and FGF 21). These have been referred to as the endocrine FGFs because they have low affinity for any of the FGF receptors in the absence of the binding cofactor klotho.[42,43]

Numerous studies (reviewed in[34,44] in the past decade have implicated klotho in the aging process, suppressing aging characteristics when it is over-expressed and accelerating them when under-expressed. The collaboration between FGFR and klotho in FGF23 signaling in the kidney was deduced from the observation that klotho KO mice have precisely the same phenotype as FGF23 deficient mice.[42,45] The recent description of a patient carrying a mutation in the *klotho* gene exhibiting severe tumoralcalcinosis and phosphate retention, confirms the applicability of the FGF23/klotho relationship to humans.[46]Although beyond the scope of this review article, this recently identified relationship between klotho and the vitamin D endocrine system suggests new areas of research into the role of vitamin D and aging.[47]

Klotho is a single-pass membrane protein with expression in the kidney highest in, if not limited to, the distal convoluted tubules. This raises the question of how it regulates CYP27B1 in the proximal tubules, a question that has not yet been answered. One hypothesis is that activation of FGFR-Klotho in the distal tubule releases a soluble form of Klotho which then acts in a paracrine fashion in the proximal tubule.[42] Although there is some evidence from animal studies in support of this possibility,[48] this area clearly awaits further investigation. Fig. 1 also shows the negative feedback effect of $1,25D_3$ on its own synthesis through the inhibition of CYP27B1 synthesis as well as its stimulatory effect on FGF23 synthesis in the bone. In both *fgf23* and *Klotho*knockout mice, serum 1,25D levels are high, suggesting that part of the negative feedback loop of 1,25D in the whole organism involves its VDR-dependent stimulation of FGF23 synthesis in the bone.[49–51]

The well-known feedback effect of 1,25D on PTH synthesis and secretion is depicted in Fig. 1, along with a similar regulatory effect FGF23, for which there is evidence in animal studies.[52] The picture in humans is complicated by the observation that in chronic kidney disease in humans high FGF23 and PTH levels co-exist. While availability of klotho may play a role in this disparity, there is not yet clarity on this point or the role of FGF23 in regulating PTH secretion in humans.

Extra-renal 1,25D production

In the early period of the study of vitamin D metabolism, it was believed that the kidney was the only site of $1,25D_3$ production. It is now known that, while the kidney is the major, if not only, endocrine organ that produces $1,25D_3$ in response to other calcium- and phosphate-regulating hormones, the presence of CYP27B1 and the ability to convert $25D_3$ to $1,25D_3$ in fact resides in many other tissues. These are summarized in Table 1, where the emphasis is on studies in humans. In most cases, the presence of 1α-hydroxylase activity has been determined from the presence of the mRNA encoding the protein (M), the protein itself based on immunochemical methods (P) and the ability of the cell or tissue under study to convert the substrate, $25D_3$ to $1,25D_3$ (E).

In general extra-renal 1α-hydroxylase activity is not regulated by the classical calcium and phosphate-regulating hormones (although FGF23 has not been studied as thoroughly in this regard as PTH), but the enzyme's activity may be subject to changes specific to the cell's environment or function. For example, keratinocytes respond to UVB light with increased $1,25D_3$ synthesis which, in turn, stimulates the cells's 24R-hydroxylase activity[53] (see below). The ability of cells of the immune system to produce $1,25D_3$ depends on the cytokines, growth factors, and other immunomodulatory factors to which the cells are exposed.[54] Further studies will undoubtedly lead to an elucidation of the details of these autocrine/paracrine interactions between the peripheral synthesis and the local actions of $1,25D_3$.

Table 1
Extra-Renal 25(OH)D_3-1α-hydroxylase (CYP27B1). In each tissue listed, evidence for the presence of the 25–OH–D_3-1α-hydroxylase was based on the measurement of the mRNA encoding CYP27B1 (M), the detection of the protein by immunochemical or immunohistochemical methods (P), or measurement of the conversion of 25OHD_3 to 1,25$(OH)_2D_3$ (E). In some cases (e.g. skin, breast) several laboratories have reported relevant data and citations are representative, but not exhaustive.

Cell/Tissue	Identification*	Reference
Bone	M, P, E	63–65
Breast (N,C)	M, P, E	66,67
Brain	P	68,69
Colon (N, C)	M, P, E	70,71
Dendritic cell, Macrophage	E	72
Endothelial cells	M, P, E	73
Keratinocytes, skin	M, P, E	74–76
Pancreatic islet	M, P, E	77
Parathyroid (N,C)	M, P	78
Placenta,/Decidua	M, P, E	79
Prostate (N,C)	M, P, E	80–82

25OHD_3- and 1,25D_3-24- hydroxylation

The 24R-hydroxylase, a cytochrome P450-dependent enzyme also known as CYP24A1 or CYP24, is, as are several other hydroxylases involved with the synthesis of steroid hormones, a multicatalytic enzyme.[55] That is, it catalyzes more than one successive step at the same active site of the enzyme. Intermediates, may or may not be released from the enzyme. Furthermore, also in common with other steroid hydroxylases, the enzyme uses either of two substrates, namely 25D_3 or 1,25D_3. The series of reactions catalyzed by CYP24 and the ultimate products from the two substrates in which the side chain has been shortened by four carbons are depicted in Fig. 3.

Renal production of 24,25D_3

As shown in Fig. 1 the kidney produces circulating levels of 24,25D_3 and it is known that renal 24R-hydroxylase activity is inversely related to 1α-hydroxylase activity. This is largely due to the opposing effects of 1,25D_3 on the expression 24R-hydroxylase activity (stimulation) and 1α-hydroxylase expression but it also involves suppressive effects of PTH on the transcription of the CYP24 gene in the kidney.[56]

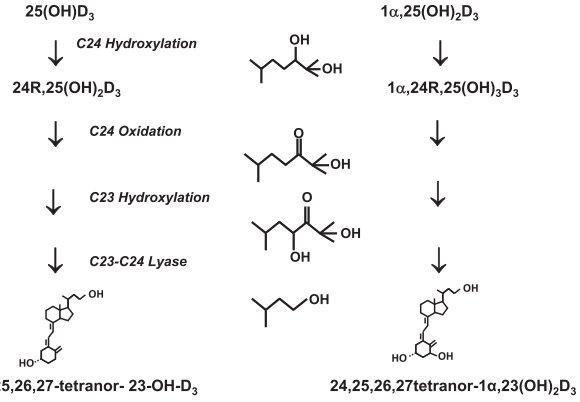

Fig. 3. The reactions catalyzed by the 25D_3-/1,25D_3-24R-hydroxylase, CYP24. Parallel pathways are shown for the two substrates, for which the reactions on the side chain are identical. All four reactions are catalyzed by the same active site on CYP24, with new reducing equivalents required for each reaction. The intermediates may either remain bound to the active site be released.

The physiological significance of 24R,25D_3 has long been the subject of debate in the field of vitamin D endocrinology. Many believe that the only purpose of the enzyme is to inactivate 1,25D_3 in target tissues whereas others believe that there is evidence that 24R,25D_3 has biological properties distinct from 1,25D_3. For example, an avian model of fracture-healing in birds was used to implicate 24,25D_3 in this process.[57] More recently, studies with a mouse model in which the CYP24 gene is inactivated, indicate that 24,25D_3 is also important for bone healing in mammals.[58] Further studies with this and related model systems will further elucidate the biological significance of 24,25-dihydroxyvitamin D_3.

Inactivation of 1,25D_3 in its target tissues

Cyp24 is one of the, if not the, most dramatically activated genes in target tissues of 1,25D_3 with increases of mRNA levels from barely detectable to several hundred or a few thousand fold above basal levels. Indeed, such is the consistency of the presence and magnitude of this response that it is frequently used as a marker of 1,25D_3 or and/or VDR presence within a cell. Clearly the induction of this CYP24, which can carry out the catabolism of 1,25D_3 to the inactive product(s) shown in Fig. 3, is an important element of negative feedback to curtail the hormone's action in its target cells.

Because of the importance of 1,25D_3 in the control of cell growth and its potential role in the processes of malignancy,[59] much attention has been given to the regulation of CYP24. Multiple vitamin D response elements within the powerful CYP24 promoter are thought to mediate the response to1,25D_3; in addition mechanisms involving the metabolism of CYP24 mRNA,[60] its alternative splicing,[61] and epigenetic gene silencing,[62] have also been suggested to contribute to the role 1,25D_3 plays in the regulation of cell growth.

Summary

The current status of our understanding of the regulation of vitamin D metabolism, beginning with the production of vitamin D_3 in the skin has been considered. The liver 25-hydroxylase whose activity has recently been attributed to a specific protein, CYP2R1 will allow more refined studies to determine whether there are conditions under which the conversion of the parent vitamin D to the most abundant form circulating in the blood is regulated. In the kidney, It is now clear that a trio of regulators ensure that (i) 1,25D_3 synthesis is responsive to the calcium needs of the organism through the stimulatory effects of PTH on the 1α-hydroxylase; (ii) 1,25D_3 synthesis is responsive to the phosphate needs of the organism through the inhibitory effects of FGF23 (in response to high serum phosphate levels) on 1α-hydroxylase activity; and (iii) 1,25D_3 synthesis is held in check by the direct feedback inhibition of 1,25D_3 on 1α-hydroxylase activity. It is likely that much of the regulation by these three regulators occurs at the level of transcription of the CYP27B1 gene, although mechanisms have only just begun to be elucidated. An exciting story has been the discovery of the role of FGF23 in vitamin D metabolism; the involvement of klotho in mediating this role opens up such research avenues in vitamin D and aging. The factors that regulate the extra-renal (autocrine/paracrine) synthesis of 1,25D_3 are just emerging, as are detailed molecular mechanism of the regulation of the 24-hydroxylase in the kidney as well as in target cells of 1,25D_3.

Research agenda

1. Investigate the possible regulation of CYP2R1 as a possible step in modulation of serum levels of 25D_3
2. Extend studies of the epigenetic and post-transcriptional regulation of CYP27B1
3. Elucidate the mechanism of FGF23 signaling in the kidney to repress CYP27B1 transcription
4. Study the local control of the activity of extra-renal CYP27B1.

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