CHAPTER 1

Historical Perspective

HECTOR F. DELUCA Department of Biochemistry,
University of Wisconsin-Madison, Madison, Wisconsin

I. DISCOVERY OF THE VITAMINS

A. Early Nutritional Views

The field of nutrition was largely dominated in the nineteenth century by German chemists, led by Justus von Liebig [1]. They taught that adequacy of the diet could be described by an analysis of protein, carbohydrate, fat, and mineral. Thus, a diet containing 12% protein, 5% mineral, 10–30% fat, and the remainder as carbohydrate would be expected to support normal growth and reproduction. This view remained largely unchallenged until the very end of the 19th century and the beginning of the 20th century [2–5]. However, evidence opposing this view began to appear. One of the first was the famous study of Eijkman who studied prisoners in the Dutch East Indies maintained on a diet of polished rice [6]. A high incidence of the neurological disorder beri-beri was recorded in these inmates. Eijkman found that either feeding whole rice or returning the hulls of the polished rice could eliminate beri-beri. Eijkman reasoned that polished rice contained a toxin that was somehow neutralized by the rice hulls. Later, a colleague, Grijns [7], revisited the question and correctly demonstrated that hulls contained an important and required nutrient that prevented beri-beri.

Other reports revealed that microorganic nutrients might be present. The development of scurvy in mariners was a common problem. This disease was prevented by the consumption of limes on British ships (hence, the term "Limey" to describe British sailors) and sauerkraut and fruits on other ships. This led Hoist and Frohlich to conclude that scurvy could be prevented by a nutrient present in these foods [8]. Experiments by Lunin, Magendie, Hopkins, and Funk showed that a diet of purified carbohydrate, protein, fat, and salt is unable to support growth and life of experimental animals [2–5]. This suggested that some unknown or vital factor present in natural foods was missing from the purified diets. Hopkins developed a growth test in which natural foods were found to support rapid growth of experimental animals whereas purified materials could not [3]. Funk had found similar results for the prevention of neuritis and reasoned that there were "vital amines" present in foods from natural sources and actually provided the basis for the term "vitamins" used later to describe essential micronutrients [5].

B. McCollum and Osborne and Mendel's Discovery of Vitamin A and B Complex

A key experiment demonstrating essential micronutrients was one carried out at the Wisconsin Agricultural Experiment Station, engineered by Stephen Moulton Babcock and carried out by E. B. Hart supported by McCollum and Steenbock [9]. Herds of dairy cows were maintained on a diet composed individually only of corn, oats, or wheat or were fed a mixture of all of these grains, all receiving the same amount of carbohydrate, protein, fat, and salts and all providing equal analysis according to the German chemists [1]. The animals on the corn diet did very well, produced milk in large amounts, and reproduced normally. Those on the wheat diet failed to thrive and soon were unable to reproduce or lactate. The oat group was found to be intermediate between the corn and wheat groups, and the mixture approximated the growth and reproduction found with corn. Yet all these diets had the same proximate analysis.

The conclusion of the Wisconsin Experiment Station study was that there are unknown nutrients present in corn and not found in wheat that are essential for life and reproduction. This led E. B. Hart, Chairman of Biochemistry at Wisconsin, to conceive that a search for these nutrients must begin. Professor McCollum was asked to search for these nutrients using small experimental animals. McCollum and Davis demonstrated there was present in butter fat a substance that prevented xerophthalmia and was also required for growth. They termed this "a lipin-soluble growth factor" [10]. McCollum later named this factor "vitamin A" [11].

VITAMIN D, 2ND EDITION
FELDMAN, PIKE, AND GLORIEUX,

This substance was absent from lard and other fats but was found in large amounts in cod liver oil. In constructing the diets, McCollum obtained the carbohydrates and salts from milk whey, which, unknown to him, supplied the vitamin B complex group of micronutrients that permitted him to observe a vitamin A deficiency. McCollum at Wisconsin [11] and Osborne and Mendel [12] at the Connecticut Experiment Station carried out experiments in which cod liver oil was used as a source of fat in the diet, but the minerals were supplied from pure chemicals mixed to approximate the mineral composition of milk. Starch or sugar was used as the carbohydrate. These animals developed a different group of symptoms, namely, neuritis, which could be cured by the provision of the milk components. McCollum and Osborne and Mendel correctly concluded that this activity was due to a different micronutrient called "vitamin B." This ushered in the concept of the organic micronutrients known as vitamins.

C. History of Rickets

The disease rickets was very likely known in antiquity but was described in the 15th century as revealed by later writings. Whistler first provided a clear description of rickets in which the skeleton was poorly mineralized and deformed [13]. Rickets undoubtedly in ancient times appeared only on rare occasions and hence was not considered a problem. However, at the end of the 19th century, the industrial revolution had taken place: A highly agrarian population had become urbanized, and smoke from the industrial plants polluted the atmosphere. Thus, in low-sunlight countries such as England, rickets appeared in epidemic proportions. In fact, it was known as "the English disease" [14]. Some reports of the beneficial action of cod liver oil had appeared. However, they were not given scientific credence.

With the discovery of the vitamins, Sir Edward Mellanby in Great Britain began to reason that rickets might also be a disease caused by a dietary deficiency [15]. Mellanby fed dogs a diet composed primarily of oatmeal, which was the diet consumed where the incidence of rickets was the highest (i.e., Scotland). McCollum inadvertently maintained the dogs on oatmeal indoors and away from ultraviolet light. The dogs developed severe rickets. Learning from the experiments of McCollum, Mellanby provided cod liver oil to cure or prevent the disease. Mellanby could not decide whether the healing of rickets was due to vitamin A known to be present in the cod liver oil or whether it was a new and unknown substance. Therefore, the activity of healing rickets was first attributed to vitamin A.

D. Discovery of Vitamin D

McCollum, who had moved to Johns Hopkins from Wisconsin, continued his experiments on the fat-soluble materials. McCollum used aeration and heating of cod liver oil to destroy the vitamin A activity or the ability to support growth and to prevent xerophthalmia [16]. However, cod liver oil treated in this manner still retained the ability to cure rickets. McCollum correctly reasoned that the activity in healing rickets was due to a new and heretofore unknown vitamin that he termed "vitamin D." On the basis of the experiments of McCollum and of Mellanby, vitamin D became known as an essential nutrient.

II. DISCOVERY THAT VITAMIN D IS NOT A VITAMIN

At the same time that Sir Edward Mellanby was carrying out the experiments in dogs, Huldshinsky [17] and Chick *et al.* [18] independently found that rickets in children could be prevented or cured by exposing them to sunlight or to artificially induced ultraviolet light. Thus, the curious findings were that sunlight and ultraviolet light somehow equaled cod liver oil. These strange and divergent results required resolution.

Steenbock and Hart had noted the importance of sunlight in restoring positive calcium balance in goats [19]. At Wisconsin, with McCollum carrying out experiments in small experimental animals (i.e., rats), Steenbock was required to work with larger animals. Steenbock then began to study goats because they would consume less materials and could serve as better experimental animals than cows. Steenbock began to study the calcium balance of lactating goats and found that those goats maintained outdoors in the sunlight were found to be in positive calcium balance, whereas those maintained indoors lost a great deal of their skeletal calcium to lactation [19]. Steenbock and Hart, therefore, noted the importance of sunlight on calcium balance. This work then undoubtedly led Steenbock to realize that the ultraviolet healing properties described by Huldschinsky might be related to the calcium balance experiments in goats. By irradiating the animals and diets, Steenbock and Black found that vitamin D activity could be induced and rickets could be cured [20]. A similar finding was reported soon thereafter by Hess and Weinstock [21]. Steenbock then traced this to the nonsaponifiable fraction of the lipids in foods [22]. He found that ultraviolet light activated an inactive substance to become a vitamin D–active material. Thus, ultraviolet light could be used to irradiate foods, induce vitamin D activity, and fortify foods to eliminate rickets as a major

medical problem. This discovery also made available a source of vitamin D for isolation and identification.

III. ISOLATION AND IDENTIFICATION OF NUTRITIONAL FORMS OF VITAMIN D

From irradiation of mixtures of plant sterols, Windaus and colleagues isolated a material that was active in healing rickets [23]. This substance was called "vitamin D_1," but its structure was not determined. Vitamin D_1 proved to be an adduct of tachysterol and vitamin D_2, and thus vitamin D_1 was actually an error in identification. The British group led by Askew was successful in isolating and determining the structure of the first vitamin D, vitamin D_2 or ergocalciferol, from irradiation of plant sterols [24]. A similar identification by the Windaus group confirmed the structure of vitamin D_2 [25]. Windaus and Bock also isolated the precursor of vitamin D_3 from skin, namely, 7-dehydrocholesterol [26]. Furthermore, 7-dehydrocholesterol was synthesized [27] and converted to vitamin D_3 (cholecalciferol) as identified by the Windaus group [28]. Thus, the structures of nutritional forms of vitamin D became known (Fig. 1). Windaus and Bock, having isolated 7-dehydrocholesterol from skin, provided the presumptive evidence that vitamin D_3 is the form of vitamin D produced in skin, a discovery that was later confirmed by the chemical identification of vitamin D_3 in skin by Esvelt *et al*. [29] and of a previtamin D_3 in skin by Holick *et al*. [30]. Synthetic vitamin D as produced by the irradiation process replaced the irradiation of foods as a means of fortifying foods with vitamin D and was also rapidly applied to a variety of diseases including rickets and tetany and in the provision to domestic animals such as chickens, cows, and pigs.

Windaus' group provided chemical syntheses of the vitamin D compounds, confirming their structures and thus ending the era of the isolation and identification of nutritional forms of vitamin D and making them available for the treatment of disease. For his contributions, Windaus received the 1938 Nobel Prize in chemistry.

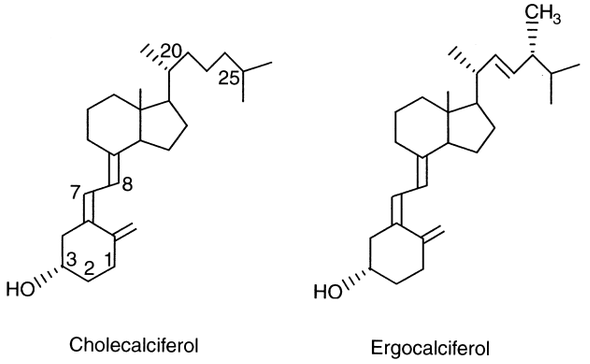

FIGURE 1 Nutritional forms of vitamin D.

IV. DISCOVERY OF THE PHYSIOLOGICAL FUNCTIONS OF VITAMIN D

A. Intestinal Calcium and Phosphorus Absorption

Besides bone mineralization, the earliest discovered function of vitamin D is its important role in the absorption and utilization of calcium. The first report of this finding was in the early 1920s by Orr and colleagues [31]. Kletzien *et al.* [32] demonstrated that vitamin D plays an important role in the utilization of calcium from the diet, and a number of experiments were carried out on the utilization of calcium and phosphorus from cereal diets. Nicolaysen was responsible, however, for demonstrating unequivocally the role of vitamin D in the absorption of calcium and independently of phosphorus from the diet [33]. Nicolaysen also followed the early work of Kletzien *et al.* [32] in which animals adapted to a low calcium diet were better able to utilize calcium than were animals on an adequate calcium diet. This work was confirmed by Nicolaysen, who postulated the existence of an "endogenous factor" that would inform the intestine of the skeletal needs for calcium [34]. This endogenous factor later proved to be largely the active form of vitamin D, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] [35]. Strong support for this concept was provided by the studies of Ribovich *et al*. [36] that showed animals maintained on a constant exogenous source of $1,25(OH)_2D_3$ are unable to change intestinal calcium transport in response to changes in dietary calcium levels.

B. Mobilization of Calcium from Bone

For many years, investigators have attempted to show that vitamin D plays a role directly on the mineralization process of the skeleton. However, early work by Rowland and Kramer [37], later work by Lamm and Neuman [38], and more recent work by Underwood and DeLuca [39] demonstrated very clearly that vitamin D does not play a significant role in the actual mineralization process of the skeleton but that the failure to mineralize the skeleton in vitamin D deficiency is due to inadequate levels of calcium and phosphorus in the plasma. Thus, the action of vitamin D in mineralizing

the skeleton and in preventing hypocalcemic tetany is the elevation of plasma calcium and phosphorus [40]. These discoveries laid to rest the concept of a role of vitamin D in mineralization. However, Carlsson [41] and Bauer *et al.* [42] were the first to realize that a major function of vitamin D is to induce the mobilization of calcium from bone when required. Thus, in animals on a low-calcium diet, the rise in serum calcium induced by vitamin D is the result of actual mobilization of calcium from bone [43]. This important function is known to be essential for the provision of calcium to meet soft-tissue needs, especially those of nerves and muscle, on a minute-to-minute basis when it is in insufficient supply from the diet. It is likely that the function of vitamin D in mobilizing calcium from bone is an osteoclastic-mediated process [44]. It is clear, however, that both vitamin D and parathyroid hormone are required for this function [45]. Furthermore, it is clear that vitamin D plays an important role in osteoclastic-mediated bone resorption [46], which is certainly the first event in bone remodeling and an essential event in bone modeling [47].

C. Renal Reabsorption of Calcium and Phosphorus

A final site of vitamin D action to elevate plasma calcium is in the distal renal tubule. Although experiments were suggestive of a role for vitamin D in increasing renal tubule reabsorption of calcium, a clear demonstration of this did not occur until the 1980s at the hands of Yamamoto *et al.* [48]. The renal tubule reabsorbs 99% of the filtered calcium even in the absence of vitamin D. However, reabsorption of the last 1% of the filtered load requires both vitamin D and parathyroid hormone. Thus, these agents work in concert in the renal reabsorption of calcium as well as in the mobilization of calcium from bone. Both agents are required to carry out this function.

D. Discovery of New Functions of Vitamin D

With discovery of the receptor for the vitamin D hormone (described in Section V,G below) came the surprising result that this receptor could be found in a variety of tissues not previously appreciated as targets of vitamin D action. It localizes in the distal renal tubule cells, enterocytes of the small intestine, bone lining cells, and osteoblasts in keeping with its known role in calcium metabolism [49,50]. However, its appearance in tissues such as parathyroid gland, islet cells of the pancreas, cells in bone marrow (i.e., promyelocytes), lymphocytes, and certain neural cells raised the question of whether the functions of vitamin D might be broader than previously anticipated [49,50]. As a result of those findings, new functions of vitamin D have been found. For example, vitamin D plays a role in causing differentiation of promyelocytes to monocytes and the subsequent coalescing of the monocytes into multinuclear osteoclast precursors and ultimately into active osteoclasts [51,52]. Suppression of parathyroid cell growth and suppression of parathyroid hormone gene expression represent other new vitamin D actions [53,54]. In keratinocytes of skin, vitamin D appears to play a role in suppression of growth and in cellular differentiation [55]. Likely, discoveries of many new functions of $1,25(OH)_2D_3$ will be made and are well on their way, as described in later chapters of this volume.

V. DISCOVERY OF THE HORMONAL FORM OF VITAMIN D

A. Early Work of Kodicek

The true pioneer of vitamin D metabolism was Egan MA Kodicek working at the Dunn Nutritional Laboratory in Cambridge U.K. Kodicek used a bioassay at first to study the fate of the vitamin D molecule and found that much vitamin D was converted to biologically inactive products [56]. Clearly, however, this approach of assaying vitamin D activity following administration of known doses of vitamin D was of limited value in determining metabolism.

B. Radiolabeled Vitamin D Experiments

Professor Kodicek then began to synthesize radiolabeled vitamin D_2. Unfortunately, the degree of labeling was not sufficient to permit the administration of truly physiological doses of vitamin D. Nevertheless, Professor Kodicek continued investigations into this important area. At the conclusion of 10 years of work, he concluded that vitamin D was active without metabolic modification and that the metabolites that were found were biologically inactive [57]. This conclusion was reached even as late as 1967, when it was concluded that vitamin D_3 itself was the active form of vitamin D in the intestine [58]. However, chemical synthesis of vitamin D_3 of high specific activity in the laboratory of the author proved to be of key importance in the demonstration of biologically active metabolites [59].

By providing a truly physiological dose of vitamin D, it could be learned that the vitamin D itself disappeared and instead polar metabolites could be found in the target tissues before those tissues responded [60]. The polar metabolites proved to be more biologically active and acted more rapidly than vitamin D itself [61]. Thus, presumptive evidence of conversion of vitamin D to active forms had been obtained as early as 1967.

C. Isolation and Identification of the Active Form of Vitamin D

By 1968, the first active metabolite of vitamin D was isolated in pure form and chemically identified as 25-hydroxyvitamin D_3 ($25OHD_3$) [62]. Its structure was confirmed by chemical synthesis [63] that provided it for study to the medical and scientific world. For a couple of years, $25OHD_3$, was visualized as the active form of vitamin D. However, when it was synthesized in radiolabeled form, it was found to be rapidly metabolized to yet more polar metabolites [64]. By this time, the Kodicek laboratory reawakened their interest in metabolism of vitamin D and began to study the metabolism of lα-tritium-labeled vitamin D [65]. Furthermore, polar metabolites of vitamin D were found by Haussler, Myrtle, and Norman [66]. The Wisconsin group labeled these metabolites as peak 5 [64], the Norman group called it peak 4B [66], and Lawson, Wilson, and Kodicek described it as peak P [65]. Kodicek *et al.* claimed that the metabolite of vitamin D found in intestine was deficient in tritium at the 1-position [65]. However, Myrtle *et al.* reported that peak 4B did not lose its tritium [67]. Thus, the suggestion of a modification at the 1-position could not be confirmed. The DeLuca group, however, isolated the active metabolite from intestines of 1600 chickens given radiolabeled vitamin D, and, by means of mass spectrometric techniques and specific chemical reactions, the structure of the active form of vitamin D in the intestine was unequivocally demonstrated as $1,25(OH)_2D_3$ [68]. Of great importance was the finding of Fraser and Kodicek that the peak P metabolite could be produced by homogenates of chicken kidney and that anephric animals are unable to produce the peak P metabolite [69]. They correctly concluded that the site of synthesis of the active form of vitamin D is the kidney. The Wisconsin group then chemically synthesized both $1\alpha25(OH)_2D_3$ [70] and $1\beta,25(OH)_2D_3$ [71] and provided unequivocal proof that the active form is $1\alpha,25(OH)_2D_3$. Furthermore, this group was able to synthesize $l\alpha OHD_3$, an important analog that assumed great importance as a therapeutic agent throughout the world [72].

D. Proof That $1,25(OH)_2D_3$ Is the Active Form of Vitamin D

Proof that $1,25(OH)_2D_3$ and not $25OHD_3$ is the active form was provided by experiments in which anephric animals respond to $1,25(OH)_2D_3$ by increasing intestinal absorption of calcium and bone calcium mobilization, whereas animals receiving $25OHD_3$ at physiological doses did not [73–75]. Furthermore, the experiment of nature, namely, vitamin D–dependency rickets type I, an autosomal recessive disorder, provided final proof [76]. This disease could be corrected by physiological doses of synthetic $1,25(OH)_2D_3$, whereas large amounts of vitamin D_3 or $25OHD_3$ were needed to heal the rickets. The exact defect in this disease is now clearly known and is described elsewhere in this volume. $25OHD_3$ at pharmacological doses likely acts as an analog of the final vitamin D hormone, $1,25(OH)_2D_3$ (Fig. 2).

E. Discovery of the Vitamin D Endocrine System

Immediately after the identification of $1,25(OH)_2D_3$ as the active form of vitamin D came studies in which it could be shown that animals on a low-calcium diet produce large quantities of $1,25(OH)_2D_3$, whereas those on a high-calcium diet produce little or no $1,25(OH)_2D_3$ [77]. A reciprocal arrangement was found for the metabolite $24R,25(OH)_2D_3$. Thus, when calcium is needed, production of $1,25(OH)_2D_3$ is markedly stimulated and the 24-hydroxylation degradation reaction is suppressed. When adequate calcium is present, production of $1,25(OH)_2D_3$ is shut off and the 24-hydroxylation reaction is turned on. This discovery also satisfactorily provided evidence that $1,25(OH)_2D_3$ is the likely endogenous factor originally described by Nicolaysen *et al.* [34].

The next important step was the demonstration that it is parathyroid hormone that activates 1α-hydroxylation of $25OHD_3$ in the kidney [78]. Thus, parathyroidectomy eliminates the hypocalcemic stimulation of 1α-hydroxylation and suppression of 24-hydroxylation, whereas administration of parathyroid hormone restores that capability. Fraser and Kodicek also provided evidence that, in intact chickens, injection of parathyroid hormone stimulated the 1α-hydroxylation reaction [79]. Thus, the basic vitamin D endocrine system was largely discovered and reported in the early 1970s, being completed by 1974.

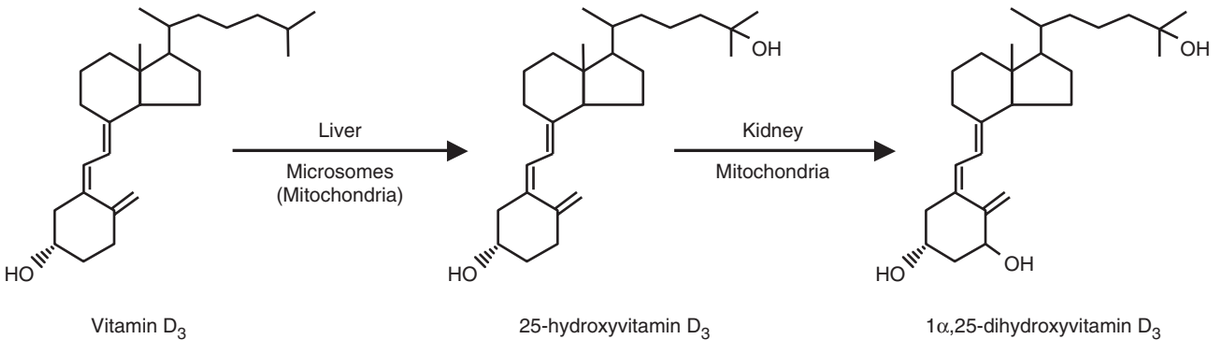

FIGURE 2 Activation of the vitamin D molecule.

F. Other Metabolites of Vitamin D

During the course of identification of $1,25(OH)_2D_3$, $21,25(OH)_2D_3$ was reported as a metabolite, as was $25,26(OH)_2D_3$ [80,81]. However, the identification of $21,25(OH)_2D_3$ was in error and was corrected to $24,25(OH)_2D_3$, with the correct stereochemistry as $24R,25(OH)_2D_3$ [82]. Over the late 1970s and early 1980s, as many as 30 metabolites of vitamin D were identified [83]. These are covered in other chapters in this volume. Of great importance was the use of the fluoro derivatives of vitamin D to illustrate that the only activation pathway of vitamin D is 25-hydroxylation followed by 1-hydroxylation [84]. Thus, 24-difluoro-$25OHD_3$ supported all known functions of vitamin D for at least two generations of animals [85]. 24-Difluoro-$25OHD_3$ cannot be 24-hydroxylated. Furthermore, other fluoro derivatives such as 26,27-hexafluoro-$25OHD_3$ [86] and 23-difluoro-$25OHD_3$ [87] are all fully biologically active, illustrating that 26-hydroxylation, 24-hydroxylation, and 23-hydroxylation are not essential to the function of vitamin D.

G. Discovery of the Vitamin D Receptor

Zull and colleagues provided evidence that the function of vitamin D is blocked by transcription and protein inhibitors [88]. Thus, it became clear very early that a nuclear activity is required for vitamin D to carry out its functions. This work confirmed and extended the earlier work of Eisenstein and Passavoy [89]. With the discovery of the active forms of vitamin D came new attention to the idea that vitamin D may work through a nuclear mechanism. Thus, Haussler *et al.* reported vitamin D compounds to be associated with chromatin [66]. However, these experiments did not exclude the possibility that the vitamin D compounds might be bound to the nuclear membrane. The first clear demonstration of the existence of a vitamin D receptor was at the hands of Brumbaugh and Haussler [90]. Furthermore, the experiments of Kream *et al.* [91] provided strong and unequivocal evidence of the existence of a nuclear receptor for $1,25(OH)_2D_3$. Intense efforts toward purification of the receptor and its study appeared with the knowledge that it is a receptor protein with a molecular weight of approximately 55,000. In 1987, a partial cDNA sequence for the chicken vitamin D receptor was determined [92]. This was followed by isolation of the full coding sequence for the human [93] and rat [94,95] receptors.

Cloning of the cDNAs encoding the vitamin D receptor in a human and mouse permitted the isolation of the gene encoding the vitamin D receptor [96–98]. The human gene was completely described and the mouse promoter was isolated and shown to be a TATA-less Sp1-driven promoter [98]. The human gene appears to have alternate promoters [96,97]. Two groups have prepared receptor Null mutant mice, permitting extensive experiments with vitamin D receptorless animals [99,100].

From a historical point of view, one of the most important discoveries was vitamin D-dependency rickets type II [101], which is now known to be due to a defect in the receptor gene [102,103] (discussed in Chapter 11). This discovery essentially provided receptor knockout experiments in humans, allowing unequivocal proof of the essentiality of the vitamin D receptor for the functions of vitamin D. The nature of the receptor and how it functions are described in subsequent chapters along with current thinking on the molecular mechanism of action of $1,25(OH)_2D_3$.

However, the discovery of vitamin D responsive elements in the osteocalcin, osteopontin, preproparathyroid, and 24-OHase genes represent important

historical developments [104–107]. This led to a consensus sequence and, most important, the development of the 3, 4, 5-rule of Umesono *et al*. [108]. It is now clear that vitamin D–responsive elements represent two imperfect repeat sequences separated by three nonspecified nucleotides. The vitamin D receptor will bind to these response elements, but it requires the presence of another nuclear factor, which proved to be the retinoid-X receptor (RXR) [109,110]. It is quite clear that the vitamin D receptor forms a heterodimer on the vitamin D responsive elements with the RXR protein on the 5′ arm of the responsive element and the vitamin D receptor on the 3′ segment [111]. The work of Rosenfeld, Glass, and colleagues [112] has demonstrated that the RXR protein when complexed with the vitamin D receptor on the responsive elements will not accept an RXR ligand, thus acting as a silent partner. Details of what is known concerning the role of the vitamin D receptor in transcription are described fully in subsequent chapters.

Acknowledgment

This work was supported in part by a fund from the Wisconsin Alumni Research Foundation.

References

1. von Liebig J 1957 Animal Chemistry or Organic Chemistry in Its Application to Physiology and Pathology. In: Glass HB (ed) A History of Nutrition. The Riverside Press, Cambridge, MA.
2. Lunin N 1881 Über die Bedeutung der anorganischen Salze für die Ernährung des Tieres. Z Physiol Chem **5**:31–39.
3. Hopkins G 1912 Feeding experiments illustrating the importance of accessory food factors in normal dietaries. J Physiol **44**:425–460.
4. Magendie F 1816 Ann Chim Phys. **3**:86–87. In: Glass HB (ed) 1957 A History of Nutrition by McCollum EV. The Riverside Press, Cambridge MA.
5. Funk C 1911 The chemical nature of the substance that cures polyneurities in birds produced by a diet of polished rice. J Physiol (London) **43**:395–402.
6. Eijkman C 1897 Ein beri-beri Anliche der Hühner. Virchows Arch **148**:523–527.
7. Grijns G 1957 Concerning polyneuritis geneeskundig gallinarum tijschrift voor ned indie 1901. In: McCollum EV, Glass HB (eds) A History of Nutrition. Houghton Mifflin, Boston, p. 216.
8. Hoist A. Frolich T 1907 Experimental studies relating ship-beriberi to scurvy. II. On the etiology of scurvy. J Hyg **7**: 634–671.
9. Hart EB, McCollum EV, Steenbock H, Humphrey GC 1911 Physiological effect on growth and reproduction of rations balanced from restricted sources. Wis Agric Exp Sta Res Bull **17**:131–205.
10. McCollum EV, Davis M 1913 The necessity of certain lipins in the diet during growth. J Biol Chem **25**:167–175.
11. McCollum EV, Simmons N, Pitz W 1916 The relation of the unidentified dietary factors, the fat-soluble A and water-soluble B of the diet to the growth promoting properties of milk. J Biol Chem **27**:33–38.
12. Osborne TB, Mendel LB 1917 The role of vitamins in the diet. J Biol Chem **31**:149–163.
13. Whistler D 1645 De morbo puerli anglorum, quem patrio ideiomate indigenae vocant "the rickets" (Lugduni, Batavorum 1645). As cited by Smerdon GT,1950 Daniel Whistler and the English Disease. A translation and biographical note. J Hist Med **5**:397–415.
14. Hess A 1929 The history of rickets. In: Rickets, Including Osteomalacia and Tetany. Lea & Febiger, Philadelphia, pp. 22–37.
15. Mellanby E 1919 An experimental investigation on rickets. Lancet **1**:407–412.
16. McCollum EV, Simmonds N, Becker JE, Shipley PG 1922 An experimental demonstration of the existence of a vitamin which promotes calcium deposition. J Biol Chem **53**:293–298.
17. Huldshinsky K 1919 Heilung von Rachitis durch Künstlich Hohen-sonne. Deut Med Wochenschr **45**:712–713.
18. Chick H, Palzell EJ, Hume EM 1923 Studies of rickets in Vienna 1919–1922. Medical Research Council, Special Report No. 77.
19. Steenbock H, Hart EB 1913 The influence of function on the lime requirements of animals. J Biol Chem **14**:59–73.
20. Steenbock H, Black A 1924 Fat-soluble vitamins. XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultraviolet light. J Biol Chem **61**:405–422.
21. Hess AF, Weinstock M 1924 Antirachitic properties imparted to lettuce and to growing wheat by ultraviolet irradiation. Proc Soc Exp Biol Med **22**:5–6.
22. Steenbock H, Black A 1925 Fat-soluble vitamins. XXIII. The induction of growth-promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. J Biol Chem **64**:263–298.
23. Windaus A, Linsert O 1928 Vitamin D1 . Ann Chem **465**:148.
24. Askew FA, Bourdillon RB, Bruce HM, Jenkins RGC, Webster TA 1931 The distillation of vitamin D. Proc R Soc **8107**:76–90.
25. Windaus A, Linsert O, Luttringhaus A, Weidlich G 1932 Crystalline-vitamin D_2. Ann Chem **492**:226–241.
26. Windaus A, Bock F 1937 Über das Provitamin aus dem Sterin der Schweineschwarte. Z Physiol Chem **245**:168–170.
27. Windaus A, Lettre H, Schenck F 1935 7-Dehydrocholesterol. Ann Chem **520**:98–107.
28. Windaus A, Schenck F, von Werder F 1936 Über das antirachitisch wirksame Bestrahlungs-produkt aus 7-Dehydrocholesterin. Hoppe-Seylers Z Physiol Chem **241**:100–103.
29. Esvelt RP, Schnoes HK, DeLuca HF 1978 Vitamin D_3 from rat skins irradiated *in vitro* with ultraviolet light. Arch Biochem Biophys **188**:282–286.
30. Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts JT Jr, Anderson RR, Blank IH, Parrish JA, Elias P 1980 Photosynthesis of previtamin D_3 in human skin and the physiologic consequences. Science **210**:203–205.
31. Orr WJ, Holt LE Jr, Wilkins L, Boone FH 1923 The calcium and phosphorus metabolism in rickets, with special reference to ultraviolet rat therapy. Am J Dis Children **26**:362–372.
32. Kletzien SWF, Templin VM, Steenbock H, Thomas BH 1932 Vitamin D and the conservation of calcium in the adult. J Biol Chem **97**:265–280.
33. Nicolaysen R 1937 Studies upon the mode of action of vitamin D. III. The influence of vitamin D on the absorption of calcium and phosphorus in the rat. Biochem J **31**:122–129.
34. Nicolaysen R, Eeg-Larsen N, Malm OJ 1953 Physiology of calcium metabolism. Physiol Rev **33**:424–444.

35. Boyle IT, Gray RW, Omdahl JL, DeLuca HF 1972 Calcium control of the *in vivo* biosynthesis of 1,25-dihydroxyvitamin D_3: Nicolaysen's endogenous factor. In: Taylor S (ed) Endocrinology 1971. Heinemann Medical Books, London, pp. 468–476.
36. Ribovich ML, DeLuca HF 1975 The influence of dietary calcium and phosphorus on intestinal calcium transport in rats given vitamin D metabolites. Arch Biochem Biophys **170**:529–535.
37. Rowland J, Kramer B 1921 Calcium and phosphorus in the serum in relation to rickets. Am J Dis Children **22**:105–119.
38. Lamm M, Neuman WF 1958 On the role of vitamin D in calcification. Arch Pathol **66**:204–209.
39. Underwood JL, DeLuca HF 1984 Vitamin D is not directly necessary for bone growth and mineralization. Am J Physiol **246**:E493–E498.
40. DeLuca HF 1967 Mechanism of action and metabolic fate of vitamin D. Vit Horm **25**:315–367.
41. Carlsson A 1952 Tracer experiments on the effect of vitamin D on the skeletal metabolism of calcium and phosphorus. Acta Physiol Scand **26**:212–220.
42. Bauer GCH, Carlsson A, Lindquist B 1955 Evaluation of accretion, resorption, and exchange reactions in the skeleton. Kungl Fysiograf Sallskapets I. Lund Forhandlingar **25**:3–18.
43. Blunt JW, Tanaka Y, DeLuca HF 1968 The biological activity of 25-hydroxycholecalciferol, a metabolite of vitamin D_3. Proc Natl Acad Sci USA **61**:1503–1506.
44. Morony S, Capparelli C, Lee R, Shimamoto G, Boone T, Lacey DL, Dunstan CR 1999 A chimeric form of osteoprotegerin inhibits hypercalcemia and bone resorption induced by IL-1β, TNF-α, PTH, PTHrP, and 1,25$(OH)_2D_3$. J Bone Miner Res **14**:1478–1485.
45. Garabedian M, Tanaka Y, Holick MF, DeLuca HF 1974 Response of intestinal calcium transport and bone calcium mobilization to 1,25-dihydroxyvitamin D_3 in thyroparathyroidectomized rats. Endocrinology **94**:1022–1027.
46. Raisz LG, Trummel CL, Holick MF, DeLuca HF 1972 1,25-Dihydroxycholecalciferol: A potent stimulator of bone resorption in tissue culture. Science **175**:768–769.
47. Frost HM 1966 Bone Dynamics in Osteoporosis and Osteomalacia. Henry Ford Hospital Surgical Monograph Series. Thomas, Springfield, IL.
48. Yamamoto M, Kawanobe Y, Takahashi H, Shimazawa E, Kimura S, Ogata E 1984 Vitamin D deficiency and renal calcium transport in the rat. J Clin Invest **74**:507–513.
49. Stumpf WE, Sar M, Reid FA, Tanaka Y, DeLuca HF 1979 Target cells for 1,25-dihydroxyvitamin D_3 in intestinal tract, stomach, kidney, skin, pituitary and parathyroid. Science **206**:1188–1190.
50. Stumpf WE, Sar M, DeLuca HF 1981 Sites of action of 1,25 $(OH)_2$ vitamin D_3 identified by thaw-mount autoradiography. In: Cohn DV, Talmage RV, Matthews JL (eds) Hormonal Control of Calcium Metabolism. Excerpta Medica, Amsterdam, pp. 222–229.
51. Suda T, Takahashi N, Martin TJ 1992 Modulation of osteoclast differentiation. Endocr Rev **13**:66–80.
52. Suda T 1992 The role of 1α,25-dihydroxyvitamin D_3 in the myeloid cell differentiation. Proc Soc Exp Biol Med **191**:214–220.
53. Demay MB, Kiernan, MS, DeLuca HF, Kronenberg HM 1992 Sequences in the human parathyroid hormone gene that bind the 1,25-dihydroxyvitamin D_3 receptor and mediate transcriptional repression in response to 1,25-dihydroxyvitamin D_3. Proc Natl Acad Sci USA **89**:8097–8101.
54. Silver J 1994 Regulation of parathyroid hormone production by 1α,25-$(OH)_2D_3$ and its analogues. Their therapeutic usefulness in secondary hyperparathyroidism. In: Vitamin D and Its Analogues. The Second International Forum on Calcified Tissue and Bone Metabolism. Chugai Pharmaceutical, Tokyo, pp. 60–63.
55. Smith EL, Walworth NC, Holick MF 1986 Effect of 1α,25-dihydroxyvitamin D_3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions. J Invest Dermatol **86**:709–714.
56. Kodicek E 1956 Metabolic studies on vitamin D. In: Wolstenholme GWE, O'Connor CM (eds) Ciba Foundation Symposium on Bone Structure and Metabolism. Little, Brown, and Co., Boston, pp. 161–174.
57. Kodicek E 1960 The metabolism of vitamin D. In: Umbreit W, Molitor H (eds) Proceedings of the Fourth International Congress of Biochemistry. Pergamon, London, Vol. 11, pp. 198–208.
58. Haussler MR, Norman AW 1967 The subcellular distribution of physiological doses of vitamin D_3. Arch Biochem Biophys **118**:145–153.
59. Neville PF, DeLuca HF 1966 The synthesis of [1,2-^{3}H]vitamin D_3 and the tissue localization of a 0.25 μg (10 IU) dose per rat. Biochemistry **5**:2201–2207.
60. Lund J, DeLuca HF 1966 Biologically active metabolite of vitamin D_3 from bone, liver, and blood serum. J Lipid Res **7**:739–744.
61. Morii H, Lund J, Neville PF, DeLuca HF 1967 Biological activity of a vitamin D metabolite. Arch Biochem Biophys **120**:508–512.
62. Blunt JW, DeLuca HF, Schnoes HK 1968 25-Hydroxycholecalciferol. A biologically active metabolite of vitamin D_3. Biochemistry **7**:3317–3322.
63. Blunt JW, DeLuca HF 1969 The synthesis of 25-hydroxycholecalciferol. A biologically active metabolite of vitamin D_3. Biochemistry **8**:671–675.
64. DeLuca HF 1970 Metabolism and function of vitamin D. In: DeLuca HF, Suttie JW (eds) The Fat-Soluble Vitamins. Univ. of Wisconsin Press, Madison, pp. 3–20.
65. Lawson DEM, Wilson PW, Kodicek E 1969 Metabolism of vitamin D. A new cholecalciferol metabolite, involving loss of hydrogen at C-l, in chick intestinal nuclei. Biochem J **115**:269–277.
66. Haussler MR, Myrtle JF, Norman AW 1968 The association of a metabolite of vitamin D_3 with intestinal mucosa chromatin *in vivo*. J Biol Chem **243**:4055–4064.
67. Myrtle JF, Haussler MR, Norman AW 1970 Evidence for the biologically active form of cholecalciferol in the intestine. J Biol Chem **245**:1190–1196.
68. Holick MF, Schnoes HK, DeLuca HF, Suda T, Cousins RJ 1971 Isolation and identification of 1,25–dihydroxycholecalciferol. A metabolite of vitamin D active in intestine. Biochemistry **10**:2799–2804.
69. Fraser DR, Kodicek E 1970 Unique biosynthesis by kidney of a biologically active vitamin D metabolite. Nature **228**: 764–766.
70. Semmler EJ, Holick MF, Schnoes HK, DeLuca HF 1972 The synthesis of 1α,25-dihydroxycholecalciferol—A metabolically active form of vitamin D_3. Tetrahedron Lett **40**:4147–4150.
71. Paaren HE, Schnoes HK, DeLuca HF 1977 Synthesis of 1α,25-dihydroxyvitamin D_3. J Chem Soc Chem Commun, 890–892.
72. Holick MF, Semmler EJ, Schnoes HK, DeLuca HF 1973 1α-Hydroxy derivative of vitamin D_3: A highly potent analog of 1α,25-dihydroxyvitamin D_3. Science **180**:190–191.
73. Boyle IT, Miravet L, Gray RW, Holick MF, DeLuca HF 1972 The response of intestinal calcium transport to 25-hydroxy and 1,25-dihydroxyvitamin D in nephrectomized rats. Endocrinology **90**:605–608.

74. Holick MF, Garabedian M, DeLuca HF 1972 1,25-Dihydroxycholecalciferol: Metabolite of vitamin D_3 active on bone in anephric rats. Science **176**:1146–1147.
75. Wong RG, Norman AW, Reddy CR, Coburn JW 1972 Biologic effects of 1,25-dihydroxycholecalciferol (a highly active vitamin D metabolite) in acutely uremic rats. J Clin Invest **51**:1287–1291.
76. Fraser D, Kooh SW, Kind HP, Holick MF, Tanaka Y, DeLuca HF 1973 Pathogenesis of hereditary vitamin D dependent rickets: An inborn error of vitamin D metabolism involving defective conversion of 25-hydroxyvitamin D to 1α,25-dihydroxyvitamin D. N Engl J Med **289**:817–822.
77. Boyle IT, Gray RW, DeLuca HF 1971 Regulation by calcium of in vivo synthesis of 1,25-dihydroxycholecalciferol and 21,25-dihydroxycholecalciferol. Proc Natl Acad Sci USA **68**:2131–2134.
78. Garabedian M, Holick MF, DeLuca HF, Boyle IT 1972 Control of 25-hydroxycholecalciferol metabolism by the parathyroid glands. Proc Natl Acad Sci USA **69**:1673–1676.
79. Fraser DR, Kodicek E 1973 Regulation of 25-hydroxycholecalciferol-1-hydroxylase activity in kidney by parathyroid hormone. Nature (New Biol) **241**:163–166.
80. Suda T, DeLuca HF, Schnoes HK, Ponchon G, Tanaka Y, Holick MF 1970 21,25-Dihydroxycholecalciferol. A metabolite of vitamin D_3 preferentially active on bone. Biochemistry **9**:2917–2922.
81. Suda T, DeLuca HF, Schnoes HK, Tanaka Y, Holick MF 1970 25,26-Dihydroxycholecalciferol, a metabolite of vitamin D_3 with intestinal calcium transport activity. Biochemistry **9**:4776–4780.
82. Tanaka Y, Frank H, DeLuca HF, Koizumi N, Ikekawa N 1975 Importance of the stereochemical position of the 24-hydroxyl to biological activity of 24-hydroxyvitamin D_3. Biochemistry **14**:3293–3296.
83. DeLuca HF, Schnoes HK 1983 Vitamin D: Recent advances. Annu Rev Biochem **52**:411–439.
84. Brommage R, DeLuca HF 1985 Evidence that 1,25-dihydroxyvitamin D_3 is the physiologically active metabolite of vitamin D_3. Endocr Rev **6**:491–511.
85. Brommage R, Jarnagin K, DeLuca HF, Yamada S, Takayama H 1983 1- but not 24-hydroxylation of vitamin D is required for skeletal mineralization in rats. Am J Physiol **244**: E298–E304.
86. Tanaka Y, Pahuja DN, Wichmann JK, DeLuca HF, Kobayashi Y, Taguchi T, Ikekawa N 1982 25-Hydroxy-26,26,26,27,27,27-hexafluorovitamin D_3: Biological activity in the rat. Arch Biochem Biophys **218**:134–141.
87. Nakada M, Tanaka Y, DeLuca HF, Kobayashi Y, Ikekawa N 1985 Biological activities and binding properties of 23,23-difluoro-25-hydroxyvitamin D_3 and its lα-hydroxy derivative. Arch Biochem Biophys **241**:173–178.
88. Zull JE, Czarnowska-Misztal E, DeLuca HF 1965 Actinomycin D inhibition of vitamin D action. Science **149**:182–184.
89. Eisenstein R, Passavoy M 1964 Actinomycin D inhibits parathyroid hormone and vitamin D activity. Proc Soc Exp Biol Med **117**:77–79.
90. Brumbaugh PF, Haussler MR 1973 lα,25-Dihydroxyvitamin D_3 receptor: Competitive binding of vitamin D analogs. Life Sci **13**:1737–1746.
91. Kream BE, Reynolds RD, Knutson JC, Eisman JA, DeLuca HF 1976 Intestinal cytosol binders of 1,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3. Arch Biochem Biophys **176**: 779–787.
92. McDonnell DP, Mangelsdorf DJ, Pike JW, Haussler MR, O'Malley BW 1987 Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. Science **235**:1214–1217.
93. Baker AR, McDonnell DP, Hughes M, Crisp TM, Mangelsdorf DJ, Haussler MR, Pike JW, Shine J, O'Malley BW 1988 Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proc Natl Acad Sci USA **85**: 3294–3298.
94. Burmester JK, Maeda N, DeLuca HF 1988 Isolation and expression of rat 1,25-dihydroxyvitamin D_3 receptor cDNA. Proc Natl Acad Sci USA **85**:1005–1009.
95. Burmester JK, Wiese RJ, Maeda N, DeLuca HF 1988 Structure and regulation of the rat 1,25-dihydroxyvitamin D_3 receptor. Proc Natl Acad Sci USA **85**:9499–9502.
96. Miyamoto K, Kesterson RA, Yamamoto H, Nishiwaki E, Tatsumi S, Taketani Y, Morita K, Pike JW, Takeda E 1997 Structural organization of the human vitamin D receptor chromosomal gene and its promoter. Mol Endocrinol **11**:1165–1179.
97. Jehan F, DeLuca HF 1997 Cloning and characterization of the mouse vitamin D receptor promoter. Proc Natl Acad Sci USA **94**:10138–10143.
98. Jehan F, DeLuca HF 2000 The mouse vitamin D receptor is mainly expressed through an Sp1-driven promoter *in vivo*. Arch Biochem Biophys **377**:273–283.
99. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, Kawakami T, Arioka K, Sato H, Uchiyama Y, Masushige S, Fukamizu A, Matsumoto T, Kato S 1997 Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet **16**:391–396.
100. Li YC, Pirro AE, Amling M, Delling G, Baron R, Bronson R, Demay MB 1997 Targeted ablation of the vitamin D receptor: An animal model of vitamin D-dependent rickets type II with alopecia. Proc Natl Acad Sci USA **94**:9831–9835.
101. Brooks MH, Bell NH, Love L, Stern PH, Orfei E, Queener SF, Hamstra AJ, DeLuca HF 1978 Vitamin D-dependent rickets Type II. Resistance of target organs to 1,25-dihydroxyvitamin D. N Engl J Med **298**:996–999.
102. Hughes MR, Malloy PJ, Kieback DG, Kesterson RA, Pike JW, Feldman D, O'Malley BW 1988 Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets. Science **242**:1702–1706.
103. Wiese RJ, Goto H, Prahl JM, Marx SJ, Thomas M, Al-Aqeel A, DeLuca HF 1993 Vitamin D–dependency rickets Type II: Truncated vitamin D receptor in three kindreds. Mol Cell Endocrinol **90**:197–201.
104. Demay MB, Gerardi JM, DeLuca HF, Kronenberg HM 1990 DNA sequences in the rat osteocalcin gene that bind the 1,25-dihydroxyvitamin D_3 receptor and confer responsiveness to 1,25-dihydroxyvitamin D_3. Proc Natl Acad Sci USA **87**:369–373.
105. Noda M, Vogel RL, Craig AM, Prahl J, DeLuca HF, Denhardt DT 1990 Identification of a DNA sequence responsible for binding of the 1,25-dihydroxyvitamin D_3 receptor and 1,25-dihydroxyvitamin D_3 enhancement of mouse secreted phosphoprotein 1 (Spp1, osteopontin) gene expression. Proc Natl Acad Sci USA **87**:9995–9999.
106. Demay MB, Kiernan MS, DeLuca HF, Kronenberg HM 1992 Sequences in the human parathyroid hormone gene that bind the 1,25-dihydroxyvitamin D_3 receptor and mediate transcriptional repression in response to 1,25-dihydroxyvitamin D_3. Proc Natl Acad Sci USA **89**:8097–8101.
107. Zierold C, Darwish HM, DeLuca HF 1995 Two vitamin D response elements function in the rat 1,25-dihydroxyvitamin D 24-hydroxylase promoter. J Biol Chem **270**:1675–1678.

108. Umesono K, Murakami KK, Thompson CC, Evans RM 1991 Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D_3 receptors. Cell **65**:1255–1266.
109. Yu VC, Delsert C, Andersen B, Holloway JM, Devary OV, Naar AM, Kim SY, Boutin J-M, Glass CK, Rosenfeld MG 1991 RXRβ: A coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. Cell **67**:1251–1266.
110. Munder M, Herzberg IM, Zierold C, Moss VE, Hanson K, Clagett-Dame M, DeLuca HF 1995 Identification of the porcine intestinal accessory factor that enables DNA sequence recognition by vitamin D receptor. Proc Natl Acad Sci USA **93**:2796–2799.
111. Jin CH, Pike JW 1996 Human vitamin D receptor-dependent transactivation in *Saccharomyces cerevisiae* requires retinoid X receptor. Mol Endocrinol **10**:196–205.
112. DiRenzo J, Soderstrom M, Kurokawa R, Ogliastro M-H, Ricote M, Ingrey S, Horlein A, Rosenfeld MG, Glass CK 1997 Peroxisome proliferator-activated receptors and retinoic acid receptors differentially control the interactions of retinoid X receptor heterodimers with ligands, coactivators, and corepressors. Mol Cell Biol **17**: 2166–2176.