Functional Assessment of Two Vitamin D-responsive Elements in the Rat 25-Hydroxyvitamin D₃ 24-Hydroxylase Gene*

Yoshikiko Ohyama‡‡, Keiichi Ozono‡, Motoyuki Uchida, Michiko Yoshimura‡, Toshimasa Shinke**, Tatsuo Suda**, and Osamu Yamamoto‡

From the ‡Graduate Department of Science, Faculty of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739, Japan, †Osaka Medical Center for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 590-02, Japan, ‡Bio-Medical Research Laboratories, Kureha Chemical Industry Company, Ltd., 3-26-2 Hyakunin-cho, Shinjuku-ku, Tokyo 169, Japan, and **Department of Biochemistry, School of Dentistry, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan

Two vitamin D-responsive elements (VDRE-1 and VDRE-2) were recently identified in the 5′-upstream region of the rat 25-hydroxyvitamin D₃ 24-hydroxylase gene at −151/−137 and −259/−245, respectively. We studied the transcriptional regulation of this gene by vitamin D by means of mutational analysis. Introducing mutations into VDRE-1 and VDRE-2 in the native promoter −291/+9 reduced vitamin D-dependent chloramphenicol acetyltransferase activity by 86 and 41%, respectively. Mutation of the direct repeat base pairs upstream of VDRE-1 also caused 50% decrease of chloramphenicol acetyltransferase activity. Connection of the element −169/−155 to VDRE-1 enhanced the vitamin D responsiveness of VDRE-1 5-fold through the heterologous β-globin promoter. The fragment −291/−102 containing the two VDREs showed two shifted bands in the presence of the vitamin D receptor and retinoid X receptor in gel retardation analysis, and the appearance of the slower migrating band indicates that two sets of receptor complexes bind to this fragment simultaneously. These results demonstrate that VDRE-1 is a stronger mediator of vitamin D function than VDRE-2 due to the presence of the accessory element −169/−155 located adjacent to VDRE-1, although VDRE-2 exhibits a smaller dissociation constant for the vitamin D receptor-retinoid X receptor complex than VDRE-1.

Vitamin D₃ exerts its biological activity after conversion into 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) by successive hydroxylations at C-25 in the liver and C-1 in the kidney. 1,25-(OH)₂D₃ mediates various biological activities, including calcium homeostasis, bone remodeling, cell growth and differentiation, and immune responsiveness (1–5).

One of the targets of 1,25-(OH)₂D₃ is the vitamin D 24-hydroxylase gene. The enzyme expressed by this gene catalyzes 24-hydroxylation of 25-hydroxyvitamin D₃ and 1,25-(OH)₂D₃ into 24,25-dihydroxyvitamin D₃ and 1,24,25-trihydroxyvitamin D₃, respectively (6). 24-Hydroxylation is considered an inactivation process of 1,25-(OH)₂D₃ (3, 7–9). We have isolated rat 24-hydroxylase cDNA and determined the gene structure (10, 11). The expression of 24-hydroxylase mRNA was strikingly increased by vitamin D in vivo and in vitro (9, 12, 13). Reinhardt and Horst (14) have suggested that the self-induced metabolism of 1,25-(OH)₂D₃ reduced the 1,25-(OH)₂D₃ occupancy of VDR and the target tissue responsiveness. Therefore, 24-hydroxylase may play an important role in regulating the functions of 1,25-(OH)₂D₃ in the target cells.

The vitamin D receptor (VDR) is a member of a steroid and thyroid hormone receptor superfamily (15, 16). The VDR occupied with 1,25-(OH)₂D₃ forms a heterodimer with the retinoid X receptor (RXR) (17, 18) and modulates the expression of target genes by binding to the vitamin D-responsive element (VDRE) in the promoter region. Several VDREs have been identified in the genes of human (19) and rat osteocalcin (20–22) and mouse osteopontin (23). These VDREs consist of a direct repeat of two hexanucleotides separated by 3 base pairs.

Three groups have independently identified two functional VDREs in an antisense orientation in the rat 24-hydroxylase gene promoter; at −151/−137 (VDRE-1) (24, 25) and −259/−245 (VDRE-2) (26). Zierold et al. (27) examined the interaction between the two VDREs by means of a reporter gene assay using heterologous promoters and by a gel mobility shift assay. They concluded that the two VDREs synergistically increased binding affinity for the receptor complex to elicit powerful promoter activity.

In this study, we assessed the functions of the two VDREs in more detail using a native promoter containing site-specific mutations at the VDREs and determined their affinity for the receptor complex by gel retardation assays. We found that the two VDREs work additively. VDRE-1 coupled with the 3-base upstream segment of −169/−155 accounted for most of the vitamin D responsiveness.

EXPERIMENTAL PROCEDURES

All enzymatic manipulations proceeded according to standard procedures (28).

Materials—Restriction and modifying enzymes were purchased from Toyobo Inc. (Osaka, Japan) and Takara Shuzo (Kyoto, Japan). γ-[³²P]ATP (111 TBq/mmol) and [¹⁴C]butyryl-coenzyme A (148 MBq/mmol) were obtained from DuPont NEN (Boston, MA).

Construction of Reporter Plasmids—To characterize half-sites in the flanking region of VDRE-1, eight fragments of −169/−127, −169/−134, −164/−134, VDRE-1, −169/−152, (−169/−150)M1, (−169/−153)M2, and (−169/−154)M3 (depicted in Fig. 1A) with XhoI and SauI overhangs were synthesized and ligated into the SaI site of a pGCaT vector containing the β-globin promoter upstream of the chloramphenicol acetyltransferase (CAT) gene (29). Sequences of the top strands for VDRE-2 and (−169/−152)-VDRE-2 shown in Fig. 1B were as follows...
Two VDREs in the Vitamin D 24-Hydroxylase Gene

(VDRE-2 is underlined): VDRE-2, 5'-GCACGCCACCCGCCTGAGC- CACCGCTGAAACC. These fragments were synthesized with XhoI and SacI overhangs and also ligated into the SacI site of the 5' end of the pGEM vector. The specific mutations of VDREs in the native promoter were introduced by polymerase chain reaction-based site-directed mutagenesis using p(−291/+9) containing the fragment −291/+9 at the XhoI site of pCAT-basic vector as a template (24). To construct pM-VDRE-2, a set of P1 and P3 primers (P1, 5'-TACGCCGCTGAGGAGTTAGCTG-3', corresponding to the vector sequence at the 5'-end of the insert; P3, 5'-GGGTCGAGCCCGAGAAGGCCTG-3', corresponding to −256/−232; the lower case letters indicate the mutation sites) were used for polymerase chain reaction. The amplified fragment was digested with HindIII and BanII, and the corresponding region of p(−291/+9) was replaced by pM-VDRE-1, a set of P2 and P4 primers (P2, 5'-GCATCCTGTCAGAGTCAC-3', corresponding to the vector sequence at the 3'-end of the insert; P4, 5'-GTTGTCGTCGTCAGGCCGCCGGC- CCGCTTCAGTAC-3', corresponding to −173/−142; the lower case letters indicate the mutation sites) were used for amplification. The amplified fragment was digested with XhoI and BstEII and the corresponding region of p(−291/+9) was replaced. Plasmid pM(−169/−150) was constructed by the same procedure using a set of P2 and P5 primers (P5, 5'-GTTGTCGTCGTCAGGCCGAAACCCCG, corresponding to −173/−153; the lower case letters indicate the mutation sites). Plasmid pM-VDRE-1.2 was prepared by polymerase chain reaction of pM-VDRE-1 using the primer set P1 and P3 as described above. Plasmid pM-VDRE-2(−169/−155) was also constructed by polymerase chain reaction using the primer set P1 and P3 and pM(−169/−155) as a template. A truncated mutant (pM-truncation) was prepared by digesting p(−291/+9) with BanII and BstEII, filling in with a Klenow fragment, and self-ligating. All mutation constructs were sequenced, and the base substitutions were confirmed.

Cell Culture and DNA Transfection—LLC-PK1 cells were maintained in medium 199 (Life Technologies, Inc.) containing 5% dextran-coated charcoal-stripped fetal calf serum and plated at a density of 3 × 10^5 cells/60-mm dish the day before transfection. Transfections were performed by means of calcium phosphate precipitation with 2 μg of reporter plasmid (in the experiment in Fig. 2, 10 μg of reporter plasmid was used) and 2 μg of reference plasmid (pSV-β-galactosidase control vector from Promega, Madison, WI) as an internal control to correct for variations in transfection efficiency. Four hours after transfection, the cells were treated with 15% glycerol and were then incubated for 2 days with 1,25-(OH)₂D₃ (or vehicle) at a final concentration of 10 nM. The cell were harvested and the activities of CAT and β-galactosidase were measured by means of a diffusion assay (30) and a β-galactosidase assay kit from Promega, respectively. Each set of experiments was repeated at least three times, and the results are presented in terms of fold induction with the means ± S.E.

Preparation of VDR and RXR—The plasmid pFX-hVDR containing a full-length copy of the human VDR cDNA (a gift from Dr. J. W. Pike, Ligand Pharmaceuticals, San Diego, CA) and pSG6RXR-β containing the coding region of rat RXR-β cDNA (a gift from Dr. S. Kato, Tokyo University of Agriculture) were linearized by enzyme digestion and used to produce capped RNA from the SP6 and T7 promoters, respectively. The resulting RNAs were translated in the reticulocyte lysate system according to the directions of the manufacturer (Promega). The VDR concentration was determined by a ligand binding assay (1–2 fmol/μl of lysate).

Cell Mobility Shift Assay—Mobility shift assays were basically performed as described (19). The fragments −291/−102, (−291/−102-M-VDRE-1, (−291/−102-M-VDRE-2, (−291/−102-M-VDRE-1.2, and (−291/−102-M-truncation were prepared from the corresponding reporter genes by digesting with PstI (at −291 bp) and BsuUI (at −102 bp), respectively. Ten picomoles of the fragments were end-labeled with [γ-³²P]ATP using T4 polynucleotide kinase. To determine the specific activity of the probes, a portion of the reaction mixture was loaded onto an 8% polyacrylamide gel in Tris-glycine buffer to separate it from the [γ-³²P]ATP (normally 5 × 10^8 cpm/pmol of probe). After purifying the remaining reaction mixture using a QIAquick nucleotide removal kit (Qiagen, Hilden, Germany), the probe concentration was calculated by its specific activity. The reticulocyte lysate containing translated VDR (1 μl) and RXR (1 μl) were incubated in the presence or absence of 500 nM 1,25-(OH)₂D₃ for 20 min in a buffer containing 25 mM Tris- HCl, 15 mM Hepes, pH 7.9, 40 mM NaCl, 5 mM KCl, 3 mM MgCl₂, 4.5 mM EDTA, 6% glycerol, 0.08% Tween 20, 1 mM dithiothreitol, and 1 μg of poly(dI-dC). One microliter of DNA probe (10⁶ cpm/reaction) was then incubated with the reaction mixture (total, 20 μl) for 20 min at room temperature, and then the mixture was resolved by electrophoresis on a 5% polyacrylamide gel in 50 mM Tris/ 380 mM glycine buffer, pH 8.5, at 200 V at 4 °C. The gels were dried on DE-81 paper (Whatman, Kent, United Kingdom) and visualized by autoradiography overnight. All Kₚ values were determined by the gel retardation assay after changing the probe concentration (0.1–4 nM). The receptor-DNA complex and free probe were quantified with a Fujix autoradiography overnight. All Kₚ values were determined by the gel retardation assay after changing the probe concentration (0.1–4 nM). The receptor-DNA complex and free probe were quantified with a Fujix Bio Image Analyzer (Fuji Film, Tokyo, Japan).

RESULTS

Localization of Two VDREs in the Rat 24-Hydroxylase Gene—Two VDREs have been identified at −151/−137, (VDRE-1) and −259/−245 (VDRE-2) in the promoter region of the rat 24-hydroxylase gene (24–26). One and two half-sites resembling the consensus sequence (AGGTCA) were found downstream and upstream of VDRE-1, respectively (Fig. 1A). Another half-site was found 6 base pairs downstream of VDRE-2 (31).

Characterization of the Flanking Region of VDRE-1—The region −169/−127 has a characteristic structure consisting of five half-sites, which are side by side just 3 bases apart, and a pair of half-sites was identified as a VDRE (VDRE-1). The three other half-sites may be important in enhancing VDRE-1 function (27). To evaluate the function of the half-sites flanking VDRE-1, eight fragments were synthesized, and CAT activity was assayed using the heterologous β-globin promoter (Fig. 1A). The deletion of a half-site at the 3' end did not affect the
responsive to 1,25-(OH)₂D₃. On the other hand, deletions of one or two of the half-sites from 5'-end markedly decreased vitamin D-dependent activation. The two half-sites located up-stream of VDRE-1 appear to be a direct repeat 3 motif (16), although this direct repeat -169/-155 as such did not mediate any vitamin D responsiveness in LLC-PK1 cells (Fig. 1A). To analyze the function of this direct repeat in more detail, we introduced mutations either into the half-sites or into the spacer (Fig. 1A, -169/-134)-M1, (-169/-134)-M2, and (-169/-134)-M3). Mutations in each half-site greatly hampered the inducibility, whereas that in the spacer scarcely affected the induction of CAT activity by 1,25-(OH)₂D₃. Connecting the fragment -169/-155 to 3 base pairs upstream of VDRE-2 enhanced the vitamin D responsiveness of VDRE-2 about 2.5-fold (Fig. 1B). These results suggest that the segment -169/-155 functions as an accessory element that elevates 1,25-(OH)₂D₃ responsiveness, although it did not respond to vitamin D by itself. Therefore, VDRE-1 coupled with the accessory element -169/-155 was more powerful than VDRE-2 for transcriptional activation by 1,25-(OH)₂D₃ in the heterologous β-globin promoter.

Comparison of VDREs in the Native Promoter—To elucidate the induction mechanism regulated by the two VDREs, we introduced specific mutations into VDRE-1, VDRE-2, and the accessory element -169/-155 in the native promoter of -291/+9 by means of polymerase chain reaction-based site-directed mutagenesis and then assayed CAT activity. As shown in Fig. 2, the mutation in VDRE-1 caused a striking decrease in the vitamin D-dependent CAT induction from 25.6- to 3.7-fold. On the other hand, introduction of the mutation into VDRE-2 retained 15.2-fold induction. The mutation in -169/-155 significantly affected the vitamin D-dependent responsiveness (13.0-fold induction). Double mutations in both VDRE-1 and VDRE-2 completely abolished the vitamin D response. The construct of double mutations in VDRE-2 and -169/-155 showed only 4.0-fold induction, which was almost identical to that of the construct of pmVDRE-1. These results indicated that VDRE-1 and VDRE-2 exhibit almost the same responsiveness when measured separately. However, VDRE-1 coupled with the accessory element -169/-155 elicited a stronger vitamin D response than VDRE-2. Deleting the region -243/-166, which contains a putative half-site 6 base pairs downstream of VDRE-2 from -291/+9 did not affect the vitamin D-dependent CAT induction.

**DISCUSSION**

There are reportedly two VDREs in the promoter region of rat 25-hydroxyvitamin D₃ 24-hydroxylase at -151/-137 (VDRE-1) and -259/-245 (VDRE-2) (24-26). Both consist of a direct repeat of hexanucleotides with a 3-base pair spacing. Characteristically, they reside on the antisense strand, which differs from other known VDREs. According to the definition of enhancers, the direction of the response element is not important to elicit its function. However, the preferential binding of RXR to the upstream half-site and VDR to the downstream half-site may influence the trans-activation function depending on the direction of the VDRE (33, 34).

In this study, we characterized the responsiveness of these elements 1,25-(OH)₂D₃ in the context of homologous and heterologous promoters. VDRE-1 and VDRE-2 conferred almost the same response to 1,25-(OH)₂D₃ through the heterol-
TABLE I

\(K_d\) values of the VDR : RXR complex to DNA fragments containing VDREs in the presence of 1,25-(OH)\(_2\)D\(_3\)

| DNA fragments                      | \(K_d\) (nM) |
|------------------------------------|-------------|
| -291/-102                          | 1.52 ± 0.11 |
| (-291/-102)-M-(-169/-155)         | 1.07 ± 0.13 |
| (-291/-102)-M-VDRE-1               | 0.24 ± 0.03 |
| (-291/-102)-M-VDRE-2               | 0.25 ± 0.06 |
| (-291/-102)-M-truncation           | 0.29 ± 0.02 |
| (-291/-102)-M-(-169/-155)         | 0.43 ± 0.03 |
| (-291/-102)-M-truncation           | 0.21 ± 0.03 |

**Fig. 3. Gel mobility shift analysis of VDREs.** Sequences of the fragments are described under “Experimental Procedures.” A, VDR and RXR, 1 \(\mu\)l of the reticulocyte lysate containing in vitro translated VDR or RXR, was used. 1,25D, 1 \(\mu\)l of 1,25-(OH)\(_2\)D\(_3\) (10 \(\mu\)M) was applied (+). In all experiments, the final volume of the reaction mixture was 20 \(\mu\)l. VDRE-1 and VDRE-2 formed complexes with VDR in the presence of RXR. The intensity of the shifted band was greatly enhanced by adding 1,25-(OH)\(_2\)D\(_3\). B, the fragment of -291/-102 contains VDRE-1 and VDRE-2. Numbers in the lanes for VDR and RXR indicate the volume of reticulocyte lysate containing in vitro translated receptors. Complexes 1 and 2 contain one and two VDR-RXR dimers, respectively. The intensity of bands was acquired by Bio Image Analyzer as arbitrary units. C, the fragment -291/-102 containing specific mutations was used in the analyses. This fragment was prepared from the corresponding reporter plasmids shown in Fig. 2 by digestion with PstI at -291 and BstUI at -102. The experiments were performed with 1 \(\mu\)l of the reticulocyte lysate containing in vitro translated VDR and 1 \(\mu\)l of RXR in the presence of 1,25-(OH)\(_2\)D\(_3\).

Two VDREs in the Vitamin D 24-Hydroxylase Gene

Constant amounts of in vitro translated VDR and RXR were used in gel mobility shift assays with varying concentrations of labeled probes. The DNA-protein complex and the free probe were quantified as described under “Experimental Procedures” and analyzed by Scatchard plots. Values represent the means ± S.E. from three independent experiments. The sequences of VDRE-1, VDRE-2, -291/-102, (-291/-102)-M-(169/-155), (-291/-102)-M-VDRE-1, (-291/-102)-M-VDRE-2, and (-291/-102)-M-truncation were described in the legend to Fig. 3. Apparent \(K_d\) values for -291/-102 and (-291/-102)-M-truncation were determined for complex 1 under conditions in which the intensity of complex 2 was less than 5% of that of complex 1.

The DNA-protein complex and the free probe were quantified as determined for complex 1 under conditions in which the intensity of complex 2 was less than 5% of that of complex 1.

The DNA-protein complex and the free probe were quantified as determined for complex 1 under conditions in which the intensity of complex 2 was less than 5% of that of complex 1.

The DNA-protein complex and the free probe were quantified as determined for complex 1 under conditions in which the intensity of complex 2 was less than 5% of that of complex 1.

The DNA-protein complex and the free probe were quantified as determined for complex 1 under conditions in which the intensity of complex 2 was less than 5% of that of complex 1.

The DNA-protein complex and the free probe were quantified as determined for complex 1 under conditions in which the intensity of complex 2 was less than 5% of that of complex 1.

The DNA-protein complex and the free probe were quantified as determined for complex 1 under conditions in which the intensity of complex 2 was less than 5% of that of complex 1.

The DNA-protein complex and the free probe were quantified as determined for complex 1 under conditions in which the intensity of complex 2 was less than 5% of that of complex 1.
Two VDREs in the Vitamin D 24-Hydroxylase Gene

30385

region – 169/–155 nor mediated the vitamin D effect through this region with the heterologous promoter (24). It is under investigation what kind of factor(s) bind(s) to this accessory element.

In this study, we have three enhancer sequences, including two distinct VDREs in the rat 24-hydroxylase gene promoter. This is the first example for the VDRE, although multiple copies of glucocorticoid and estrogen elements have been found in several genes (36). In general, multiple enhancer elements are supposed to permit synergistic gene expression (37). Synergistic interactions of the VDR with SP1, AP-1, and VDR have been found in an artificial arrangement of the elements in the promoter (36). The mechanism of the synergistic interaction in trans-activation may, at least in part, implicate cooperative binding resulting from the specific protein-protein interaction. In the natural promoter, VDRE is juxtaposed to an AP-1 site (19) and the sodium butyrate response element (38) in the human osteocalcin and mouse calbindin D28k promoter, respectively.

In the rat 24-hydroxylase promoter, however, no cooperative binding of receptors was observed in this study. Quantification of the intensity of retarded bands indicates that VDRE-RXR complexes bind to VDRE-1 and VDRE-2 independently but not cooperatively (Fig. 3B), since the band intensity of complex 2 was not stronger than that of complex 1. The higher affinity of the long fragment –291/–102 containing both VDREs was maintained even when the mutations were introduced into VDRE-1 or -2, indicating that there is no interaction between the two VDREs. In concert with the results obtained by binding assay, no obvious synergistic effect of the two VDREs was observed in the trans-activation function of 1,25-(OH)2D3. Mutants of VDRE-1 and VDRE-2 exhibited 3.7- and 15.2-fold induction in response to 1,25-(OH)2D3 (Fig. 2). Because the sum of the -fold induction (18.9-fold) of individual activation in response to the circulating levels of 1,25-(OH)2D3.

In conclusion, we found that the two distinct VDREs function additively in the promoter of the rat 24-hydroxylase gene. VDRE-1 appears to be a stronger mediator of vitamin D function than VDRE-2 regardless of its lower affinity for the receptor complex. This is due to the presence of the accessory element – 167/–155 located adjacent to VDRE-1.

REFERENCES

1. DeLuca, H. F. (1988) PASES J. 2, 224–236
2. Minghetti, P. P., and Norman, A. W. (1988) PASES J. 2, 3043–3053
3. Haussler, M. R., Mangelsdorf, D. J., Koom, B. S., Terpening, C. M., Yamaoka, K., Allegretto, E. A., Baker, A. R., Shine, J., McDonnell, D. P., Hughes, M., Wengel, N. L., O'Malley, B. W., and Pike, J. W. (1988) Recent Prog. Horm. Res. 44, 263–305
4. Suda, T., Shinkii, T., and Takahashi, N. (1990) Annu. Rev. Nutr. 10, 195–211
5. Pike, J. W. (1991) Annu. Rev. Nutr. 11, 189–216
6. Ohyama, Y., and Okuda, K. (1991) J. Biol. Chem. 266, 8680–8685
7. Tanaka, Y., and DeLuca, H. F. (1974) Science 183, 1198–1200
8. Brommage, R., and DeLuca, H. F. (1985) Endocrinol. Res. 6, 491–511
9. Shinkii, T., Jin, C. H., Nishimura, A., Nagai, Y., Ohyama, Y., Noshiro, M., Okuda, K., and Suda, T. (1992) J. Biol. Chem. 267, 13757–13762
10. Ohyama, Y., Noshiro, M., and Okuda, K. (1991) FEBS Lett. 278, 195–198
11. Ohyama, Y., Noshiro, M., Eggerstens, G., Gotoh, O., Kato, Y., Bjerkhem, I., and Okuda, K. (1993) Biochemistry 32, 76–92
12. Nishimura, A., Shinkii, T., Jin, C. H., Ohyama, Y., Noshiro, M., Okuda, K., and Suda, T. (1994) Endocrinology 134, 1794–1799
13. Iida, K., Shinkii, T., Yamaguchi, A., DeLuca, H. F., Kurokawa, K., and Suda, T. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 6112–6116
14. Reinhardt, T. A., and Horst, R. L. (1989) J. Biol. Chem. 264, 15917–15921
15. Evans, R. M. (1988) Science 244, 889–895
16. Umesono, K., Murakami, K. K., Thompson, C. C., and Evans, R. M. (1991) Cell 65, 3001–3011
17. Sone, T., Kerner, S., and Pike, J. W. (1991) J. Biol. Chem. 266, 23296–23305
18. Yu, V. C., Delserc, C., Andersen, B., Holloway, J. M., Devary, O. V., Naar, A. M., Kim, S. Y., Boutin, J.-M., Glass, C. K., and Rosenfeld, M. G. (1991) Cell 67, 1251–1266
19. Ozono, K., Liao, J., Kerner, S. A., Scott, R. A., and Pike, J. W. (1990) J. Biol. Chem. 265, 21881–21888
20. Demay, M. B., Gerardi, J. M., DeLuca, H. F., and Kronenberg, H. M. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 369–373
21. Markose, E. K., Stein, J. L., Stein, G. S., and Lian, J. B. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 1701–1705
22. Macdonald, P. N., Haussler, C. A., Terpening, C. M., Galligan, M. A., Reeder, M. C., Whitefield, G. K., and Haussler, M. R. (1991) J. Biol. Chem. 266, 18808–18813
23. Noda, M., Vogel, R. L., Craig, A. M., Prahl, J., DeLuca, H. F., and Denhardt, D. T. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 9995–9999
24. Ohyama, Y., Ozono, K., Uchida, M., Shinkii, T., Kato, S., Suda, T., Yamamoto, O., Noshiro, M., and Kato, Y. (1994) J. Biol. Chem. 269, 10545–10550
25. Hahn, C. N., Kerry, D. M., Omdahl, J. L., and May, B. K. (1994) Nucleic Acids Res. 22, 2410–2416
26. Zierold, C., Darwish, H. M., and DeLuca, H. F. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 900–902
27. Zierold, C., Darwish, H. M., and DeLuca, H. F. (1995) J. Biol. Chem. 270, 1675–1678
28. Sambeirok, J., Freitsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
29. Kato, S., Torr, L., Yamauchi, J., Masushige, S., Bellard, M., and Chambon, P. (1992) Cell 68, 731–742
30. Neumann, J. R., Morency, C. A., and Russian, K. O. (1987) BioTechniques 5, 444–448
31. Kahlen, J.-P., and Carlberg, C. (1994) Biochem. Biophys. Res. Commun. 202, 1366–1372
32. Macdonald, P. N., Sherman, D. R., Dowd, D. R., Jefcoat, S. C., Jr., and DeLisle, R. K. (1995) J. Biol. Chem. 270, 4748–4752
33. Perlman, T., Ramarajang, P. N., Umesono, K., and Evans, R. M. (1993) Genes Dev. 7, 1411–1422
34. Kurokawa, R., Yu, V. C., Noshiro, M., and Kato, Y. (1994) J. Biol. Chem. 269, 14323–14325
35. Scarlett, C. O., and Robins, D. M. (1995) Mol. Endocrinol. 9, 413–423
36. Liu, M., and Freedman, L. P. (1994) Mol. Endocrinol. 8, 1593–1604
37. Herschlag, D., and Johnson, F. B. (1993) Genes Dev. 7, 173–179
38. Gill, R. K., and Christakos, S. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 2984–2988
39. Chen, K.-S., and DeLuca, H. F. (1995) Biochem. Biophys. Acts 1263, 1–9