cell cycle progression induced by exogenous ligands and activity of K^+ channels has recently been demonstrated in the latter (*1, 4, 5, 8*). Similarly, the function, if any, of second messengers in the regulation of K^+ conductances in lymphocytes is unclear. Increase of free intracellular Ca^{2+} (*26*) as well as membrane depolarization (*27*) have been shown to occur during the early stages of B-cell activation, and the K^+ channel described here is a good candidate for mediating the last phenomenon. A physiological role for the modulating effects of cAMP is more difficult to define because, on one hand, the level of cAMP has been shown to increase during B-cell triggering (*22*) and, on the other hand, exogenous elevation of intracellular cAMP has been reported to inhibit both lymphocyte proliferation (*21, 22*) and the early events occurring during activation (*28*). Taken together, these data support the fact that cAMP exerts opposite effects during B-cell cycling (*22*). It remains to be established whether some of these different cAMP-modulated stages of cell cycling are related to activity of K^+ channels. The fact that K^+ currents are also modulated by cAMP in early pre-B cells suggests that at least this cyclic nucleotide has the potential to control K^+ channel activity during the developmental pathway of this lineage.

Because currents similar to those we have seen in B cells have been observed in T cells and their precursors (*5*), it is likely that these K^+ channels develop before the divergence of discrete B- and T-cell lineages, and it would be of importance to determine if T-cell channels are also controlled by cAMP.

REFERENCES AND NOTES

1. T. E. DeCoursey, K. G. Chandy, S. Gupta, M. D. Cahalan, *Nature (London)* **307**, 465 (1984).
2. D. R. Matteson and C. Deutsch, *ibid.*, p. 468; T. E. DeCoursey, K. G. Chandy, S. Gupta, M. D. Cahalan, *J. Neuroimmunol.* **10**, 71 (1985).
3. Y. Fukushima, S. Hagiwara, M. Henkart, *J. Physiol. (London)* **351**, 645 (1984).
4. S. C. Lee, D. E. Sabath, C. Deutsch, M. B. Prystowsky, *J. Cell Biol.* **102**, 1200 (1986).
5. L. Schlichter, N. Sidell, S. Hagiwara, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5625 (1986).
6. O. P. Hamill, A. Marty, E. Neher, B. Sackmann, F. J. Sigworth, *Pfluegers Arch.* **391**, 85 (1981).
7. N. Sidell, L. C. Schlichter, S. C. Wright, S. Hagiwara, S. H. Golub, *J. Immunol.* **137**, 1650 (1986).
8. C. Deutsch, D. Krause, S. C. Lee, *J. Physiol. (London)* **372**, 405 (1986).
9. M. Kuno, J. Goronzy, L. M. Weyand, D. Gardner, *Nature (London)* **323**, 269 (1986).
10. Y. Fukushima, S. Hagiwara, R. E. Saxton, *J. Physiol. (London)* **355**, 313 (1984).
11. J. Andersson, F. Melchers, C. Galanos, O. Luderitz, *J. Exp. Med.* **137**, 943 (1973).
12. E. J. Siden, D. Baltimore, D. Clark, W. E. Rosenberg, *Cell* **16**, 389 (1979).
13. L. A. Whitlock, S. F. Ziegler, L. J. Treiman, J. I. Stafford, O. N. Witte, *ibid.* **32**, 903 (1983).
14. D. Levitt and M. D. Cooper, *ibid.* **19**, 667 (1980); E. J. Siden, F. W. Alt, L. A. Shinefeld, V. L. Sato, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1823 (1981).
15. P. D. Burrows, G. B. Beck, M. R. Wabl, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 564 (1981).
16. M. D. Cahalan, K. G. Chandy, T. E. DeCoursey, S. Gupta, *J. Physiol. (London)* **358**, 197 (1985).
17. P. Bregestovski, A. Redkozubov, A. Alexeev, *Nature (London)* **319**, 776 (1986).
18. B. Hille, *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA, 1984), pp. 303–328.
19. Y. Fukushima and S. Hagiwara, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2240 (1983).
20. I. Inoue, *J. Gen. Physiol.* **78**, 43 (1981).
21. J. Watson, *J. Immunol.* **117**, 1656 (1976).
22. J. Ohara *et al.*, *ibid.* **129**, 1227 (1982).
23. K. B. Seamon, W. Padgett, J. W. Daly, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3363 (1981).
24. P. J. Pfaffinger, J. M. Martin, D. D. Hunter, N. M. Nathanson, B. Hille, *Nature (London)* **317**, 536 (1985).
25. For a review, I. B. Levitan, *J. Membr. Biol.* **87**, 177 (1985).
26. M. K. Bijsterbosch, C. J. Meade, G. A. Turner, G. G. B. Klaus, *Cell* **41**, 999 (1985).
27. H. Kiefer, A. J. Blume, H. R. Kaback, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2200 (1980).
28. J. G. Monroe and J. C. Cambier, *J. Exp. Med.* **158**, 1589 (1983); K. M. Coggeshall and J. C. Cambier, *J. Immunol.* **134**, 101 (1985).
29. M. D. Cooper, D. Mulvaney, A. Coutinho, P. A. Cazenave, *Nature (London)* **321**, 616 (1986).
30. BALB/c spleen cell suspensions were first treated with ammonium chloride to lyse red cells and second with antiserum to Thy-1 and complement to eliminate T lymphocytes. The resultant cell population (more than 95% of the cells were Ig = positive) was cultured in RPMI-1640 supplemented with L-glutamine, specillin G2 (10^3 U/ml), streptomycin (10^3 U/ml) (Specia), 10% fetal calf serum and 2×10^{-5} 2-mercaptoethanol in the presence of 50 μg/ml of LPS from *Salmonella typhimurium* (Difco Laboratories) for 72 hours.

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Molecular Cloning of Complementary DNA Encoding the Avian Receptor for Vitamin D

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Vitamin D_3 receptors are intracellular proteins that mediate the nuclear action of the active metabolite 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. Two receptor-specific monoclonal antibodies were used to recover the complementary DNA (cDNA) of this regulatory protein from a chicken intestinal λgt11 cDNA expression library. The amino acid sequences that were deduced from this cDNA revealed a highly conserved cysteine-rich region that displayed homology with a domain characteristic of other steroid receptors and with the *gag-erbA* oncogene product of avian erythroblastosis virus. RNA selected via hybridization with this DNA sequence directed the cell-free synthesis of immunoprecipitable vitamin D_3 receptor. Northern blot analysis of polyadenylated RNA with these cDNA probes revealed two vitamin D receptor messenger RNAs (mRNAs) of 2.6 and 3.2 kilobases in receptor-containing chicken tissues and a major cross-hybridizing receptor mRNA species of 4.2 kilobases in mouse 3T6 fibroblasts. The 4.2-kilobase species was substantially increased by prior exposure of 3T6 cells to 1,25$(OH)_2D_3$. This cDNA represents perhaps the rarest mRNA cloned to date in eukaryotes, as well as the first receptor sequence described for an authentic vitamin.

VITAMIN D_3 UNDERGOES METABOLIC activation in liver and kidney to 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], the active form principally responsible for the regulation of calcium and phosphorus homeostasis in higher vertebrates (*1*). The biologic response to 1,25$(OH)_2D_3$ in birds and mammals occurs through a complex series of events that are mediated by an intracellular receptor protein and that parallel those of the classical steroid hormones (*2*). Thus, the kinetics of induction of messenger RNAs (mRNAs) for the chicken and rat vitamin D_3–dependent calcium-binding proteins (*3, 4*), for example, are consistent with a genomic action of 1,25$(OH)_2D_3$, although control of the expression of these genes by a direct interaction of the 1,25$(OH)_2D_3$ receptor (VDR) with the nucleus has not been demonstrated. Apart from the classical roles assigned to 1,25$(OH)_2D_3$, it has been shown to participate in regulating such diverse functions as cellular proliferation and differentiation, secretion of polypeptide hormones, and induction of catabolic enzymes for the vitamin and modulating certain aspects of the immune system (*5*). These and certain other pleiotropic effects of the activated vitamin are under the control of VDR (*6*).

Chicken 1,25$(OH)_2D_3$ receptors (cVDR) are polypeptides of approximately 60 kD that selectively bind the active vitamin with high affinity and interact with nuclei and DNA both in vitro and in vivo (*7*). Nevertheless, limited structural and functional insight into this macromolecule has accumulated, primarily because its intracellular con-

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centration is extremely low (0.001%), even in primary target tissues such as intestine. This concentration is less than one-tenth the cellular concentration of steroid receptor molecules (*8*). The purification of the chicken intestinal VDR, however, enabled the development and application of monoclonal antibodies reactive with both the chicken and mammalian isoforms (*9–11*). We used two of these high-affinity monoclonal antibodies, 9A7γ and 4A5γ, to recover VDR complementary DNA (cDNA) clones from a chicken intestinal λgt11 cDNA expression library. These clones provided important preliminary information about the structural organization of the VDR polypeptide and have been used to examine the protein's tissue-specific expression and features of its cellular regulation.

Western blot analysis of chicken intestinal cytosol (Fig. 1A) reveals a major signal that migrates electrophoretically at 60 kD and is clearly dependent on the presence of either the 9A7γ or 4A5γ antibody. This band and a less abundant species at 58 kD have been shown to correspond to cVDR species by their tissue-specific distribution, by their ability to coelute during DNA-cellulose chromatography with 1,25$(OH)_2[^3H]D_3$–receptor complex, and through comigration with purified cVDR (*12*). Proteolytic mapping studies have localized the epitopes for these two monoclonal antibodies to a general region proximal to the DNA binding domain of cVDR (*13*). The recognition patterns of these two monoclonal antibodies (9A7γ apparently cross-reacts with an unknown protein species of >200 kD) (Fig. 1A), coupled with the fact that the antibodies were obtained from hybridomas derived from separate spleen cell–myeloma fusions and display different receptor affinities (*14*), suggest that they are directed against different epitopes. We used 9A7γ for the primary screening of λgt11 cDNA libraries and 4A5γ for specific cross-screening of clones that reacted with 9A7γ.

Two λgt11 expression libraries were independently constructed from chicken intestinal cDNA with polyadenylated RNA primed with oligo(dT) or randomly primed with calf thymus DNA pentamers and hexamers (*15*). These intestinal cDNA libraries contained 10^7 and 10^8 members, respectively, each with more than 90% primary recombinants. A single random primed clone (λVDR4) was identified during the primary screening of approximately 10^7 recombinants from these libraries. Subsequent secondary screening showed that the expression product of λVDR4 was immunoreactive with both the 9A7γ and 4A5γ antibodies (Fig. 1B). λVDR4 was subcloned into plasmid pGEM (Promega Biotech, Madison, Wisconsin) and was designated pGVDR4. Its 310-bp insert was used to rescreen an additional 10^7 recombinants of the randomly primed cDNA library through hybridization. A single cross-hybridizing clone (λVDR5) of 890 bp was obtained. The library concentration of authentic cVDR clones was less than 0.001%, in close agreement with the measurable concentration of VDR itself. To our knowledge, no mRNA in lower cellular concentration has been cloned to date (*16*).

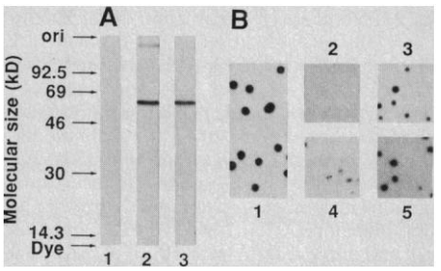

Fig. 1. Immunological detection of chicken intestinal vitamin D receptor or vitamin D receptor–related epitopes. (**A**) Chicken intestinal cytosol [10% (w/v) containing approximately 1 ng of cVDR] was subjected to electrophoresis as described by Laemmli (*30*) on 11% gels and transferred to nitrocellulose (*12*). The Western blot analysis was carried out as described (*12, 23*). Strips of nitrocellulose were incubated with (lane 1) no primary antibody, (lane 2) 9A7γ at 4 μg/ml, or (lane 3) 4A5γ at 4 μg/ml. Autoradiography was performed on Kodak XAR film for 24 hours at −70°C with a Cronex Hiplus intensifying screen. (**B**) *Escherichia coli* Y1090 cells were infected with either plaque-pure λVDR4 (panels 2 to 4) or a mix of pure λVDR4 with an unrelated clone (panel 1), plated on agar plates, and incubated for 4 hours at 42°C. Synthesis of the β-galactosidase-VDR4 fusion protein was induced by overlaying the plates with nitrocellulose filters presoaked in 10 m*M* isopropylthiogalactoside (IPTG) for 6 hours. Solid phase radioimmunoassay was performed as in (A). Filters were incubated with 9A7γ at 4 μg/ml (panels 1 and 3), no primary antibody (panel 2) or 4A5γ at 4 μg/ml (panel 4), and at high stringency with 9A7γ at 4 μg/ml in buffered 1*M* NaCl (panel 5).

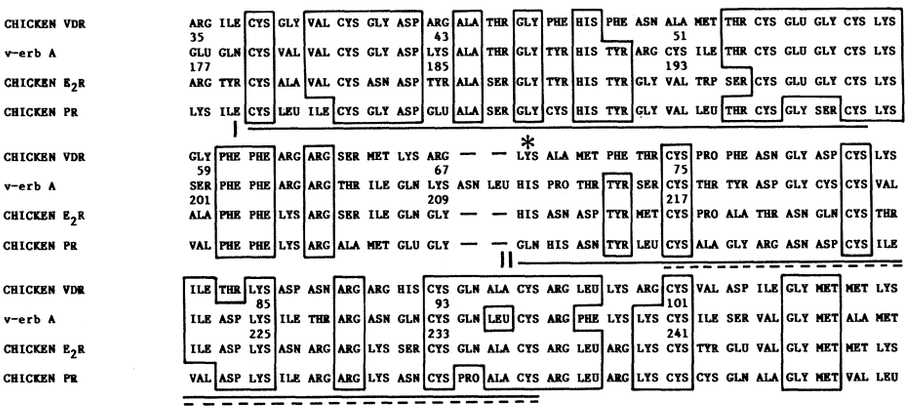

Fig. 2. Comparison of the deduced amino acid sequence of cVDR with v-*erbA* and other selected members of the steroid receptor family. A two–amino acid insertion was allowed in v-*erbA* to permit its exact alignment with each receptor protein; amino acids common to three or more proteins are boxed. Those regions corresponding to two putative DNA binding "fingers" are underlined (I and II). An alternative second-finger structure is indicated by the dashed line. An asterisk indicates a nonconserved residue in one of the possible second-finger structures. A numerical designation as indicated for cE_2R and v-*erbA* has not been assigned to cVDR or cPR as the orientation of these clones with respect to their encoded products has yet to be confirmed.

The deduced amino acid sequence of these cDNA clones revealed a cysteine-, lysine-, and arginine-rich region having high homology with both the steroid receptor family and the avian erythroblastosis *gag-erbA* gene product (*17, 18*). Specifically, this homologous cVDR region exhibited an overall homology of 46% with the chicken estrogen receptor (cE_2R) and the v-*erbA* product (*18, 19*) and 40% with the chicken progesterone (cPR) (*20*) and glucocorticoid (*17*) receptor proteins (Fig. 2). Moreover, nine cysteine residues in the cVDR clones were located in the same relative position as identical residues that are invariant in the other members of the steroid receptor family. It has been proposed that this highly conserved region encodes the DNA-binding domain of this gene family and may be integral to the formation of DNA binding "fingers" similar to those proposed to be coordinated around Zn^{2+} atoms in the 5*S* transcription factor TFIIIA (*21*).

The amino acids constituting the first finger structure proposed for the steroid receptors and v-*erbA* are clearly conserved in cVDR (sequence I in Fig. 2). A second finger structure is also possible in this region comprising at least two alternative sequences (sequences II in Fig. 2, shown as solid or dashed lines). In view of the absence of a conserved histidine in cVDR, the second alternative is likely favored. The presence of essential cysteines in the DNA bind-

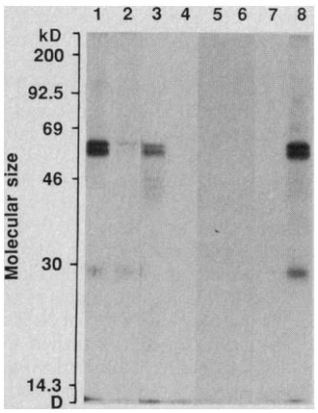

Fig. 3. Hybrid-selected translation of chick intestinal VDR mRNA with pG-VDR4. Total chick intestinal RNA was obtained by the method of Chirgwin *et al.* (*31*), and polyadenylated RNA was selected via oligo(dT)-cellulose (*32*). Filter hybridizations were performed by a slight modification of the method of Ricciardi *et al.* (*33*). pG-VDR4 or pGEM DNA alone was denatured, bound to nitrocellulose filters, and hybridized in 50% formamide, 0.7*M* NaCl, 10 m*M* Pipes (*p*H 6.4), and 4 m*M* EDTA with 50 μg of mRNA at 37°C, for 8 hours. After extensive washing, the selected mRNA was eluted from the filters in boiling water and translated in vitro by using a rabbit reticulocyte lysate (Promega) and added [^{35}S]methionine (New England Nuclear, Boston, Massachusetts). Translation in vitro, immunoprecipitation with monoclonal antibody to the receptor, gel electrophoresis, and fluorography were as described (*23*). Each lane represents protein immunoprecipitated from the following translated RNAs: (lanes 1 and 2) total chick intestine mRNA, (lanes 3 and 4) mRNA hybrid selected with pG-VDR4 or (lanes 5 and 6) with pGEM, and (lane 7) mRNA remaining unselected after hybridization with pG-VDR4 or (lane 8) with pGEM. VDR-specific bands were demonstrated by their absence when blocked from immunoprecipitation with free excess monoclonal antibody (lanes 2, 4, and 6). VDR bands were not easily distinguishable from background radiolabeled proteins in the absence of precipitation with antibodies.

ing domain of cVDR is consistent with earlier biochemical work and with the recent discovery that Zn^{2+} stabilizes the DNA binding capacity of the receptor (*22*). However, it should be emphasized that the Zn^{2+} finger structure is hypothetical for steroid receptors at present and must be clarified by future experimentation.

Since the epitopes for both 9A7γ and 4A5γ have been physically (*12, 13*) and functionally mapped on cVDR to a position in or near this DNA binding domain, the recovery by these antibodies of cDNA that encodes a steroid receptor gene provides strong preliminary evidence for the authenticity of cVDR cDNA. To confirm the authenticity of the putative VDR clone, we used pG-VDR4 hybridization to select an mRNA encoding cVDR. Nitrocellulose-immobilized pG-VDR4 was hybridized with total polyadenylated mRNA isolated from chick intestinal mucosa, and both the selected and unselected mRNAs were evaluated by cell-free translation. Receptor-specific immunoprecipitation of protein translated from selected mRNA led to the identification of two fluorographic bands (lane 3 in Fig. 3) that exhibited electrophoretic mobilities identical to those obtained from cell-free translation of total intestinal polyadenylated mRNA (lane 1 in Fig. 3). These species at 60 and 58 kD are not immunoprecipitable with excess free 9A7γ present (lane 2 in Fig. 3) and correspond to identical bands observed by Western blot analysis (*12, 13, 23*). The minor species at 58 kD is believed to arise through post-translational modification, proteolysis, or as a result of alternate initiation sites during translation. The pool of unselected mRNAs did not direct synthesis of receptor-specific protein (lane 7 in Fig. 3). The specificity of the hybridization between pG-VDR4 and VDR mRNA is demonstrated by hybrid-selected translation with the pGEM vector alone. VDR mRNA did not hybridize to the control pGEM DNA (lanes 5 and 6 in Fig. 3) but instead remained in the unselected mRNA pool (lane 8 in Fig. 3). These data provide strong support for the authenticity of the recovered cDNA clones.

We next performed Northern blot hybridization analysis with polyadenylated RNA from a series of chicken tissues by means of a 510-bp λVDR5 fragment (containing the complete region of homology to v-*erbA*) as a probe. Two mRNA species of 2.6 and 3.2 kb were identified in intestine and kidney; these mRNAs occurred in lower abundance in oviduct and heart and were absent in liver (Fig. 4). The transcripts detected by Northern analysis comigrated exactly with cVDR mRNA fractionated on a neutral sucrose gradient and subjected to cell-free translation (*24*). A single mRNA of 3.2 kb is present in polyadenylated RNA from brain (Fig. 4). VDR has been demonstrated previously in selected brain neurons through immunocytochemical (*25*) and autoradiographic (*26*) studies, although the significance of a single 3.2-kb mRNA in this tissue has yet to be clarified. Nevertheless, it is clear that several VDR mRNAs are present in polyadenylated RNA derived from VDR-containing tissues and that the expression of these transcripts is consistent with the profile developed through $1,25(OH)_2D_3$-binding analysis (*1, 2, 7*).

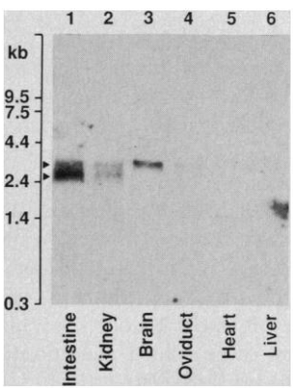

Fig. 4. Northern blot analysis of polyadenylated mRNA isolated from chicken tissue. Chicken intestine, kidney, brain, heart, oviduct, and liver polyadenylated RNA (2.5 μg each) were isolated as described in Fig. 3, subjected to electrophoresis on a 1% agarose-formaldehyde gel, and electrotransferred to a nylon membrane. After an initial prehybridization of the nylon filter for 2 hours, the filter was probed with a 510-bp nick-translated fragment of λVDR5 (10^6 cpm/ml) in new hybridization buffer (*34*) at 42°C for 16 hours. Autoradiography was performed as above for 48 hours.

Considerable evidence has accumulated to suggest that $1,25(OH)_2D_3$ is capable of positively regulating the level of VDR in mammalian cultured cells (*11, 23, 27*). While the mechanism of this regulation is complex, its sensitivity to actinomycin D suggests that VDR may regulate its own gene expression. In view of the considerable homology among species for a given steroid receptor (*18*), we expected cross-reactivity of the cDNA for cVDR with mammalian VDR and sought to probe this mechanism at the level of mRNA. The mouse 3T6 fibroblast was used as a model system, in which treatment of confluent monolayers of these cells with $1,25(OH)_2[^3H]D_3$ leads to a progressive puromycin-sensitive accumulation of $1,25(OH)_2[^3H]D_3$-binding activity (Fig. 5A). The amount of $1,25(OH)_2D_3$ binding in intact cells observed in the presence of puromycin is equivalent to the basal amount of receptor detectable in vitro after preparation of 3T6 cytosol (*28*). Although hormone-binding activity decays at 24 hours in these experiments because of cell age, Western blot analysis of nonconfluent 3T6 cells treated continuously with $1,25(OH)_2D_3$ for periods up to 72 hours revealed a dramatic increase in receptor protein concentration (Fig. 5A, inset). Identically treated cells likewise exhibit an appropriate five- to tenfold increase in receptor mRNA activity over control, as assessed by in vitro translation of isolated polyadenylated mRNA (Fig. 5B). We used Northern blot analysis to detect 3T6 receptor mRNA and to assess qualitatively the effects of vitamin administration on receptor mRNA concentration. Mouse VDR mRNA cross-hybridizes with the λVDR5 probe and displays two transcripts, a major species at 4.2 kb and a minor species at 2.6 kb (Fig. 5C).

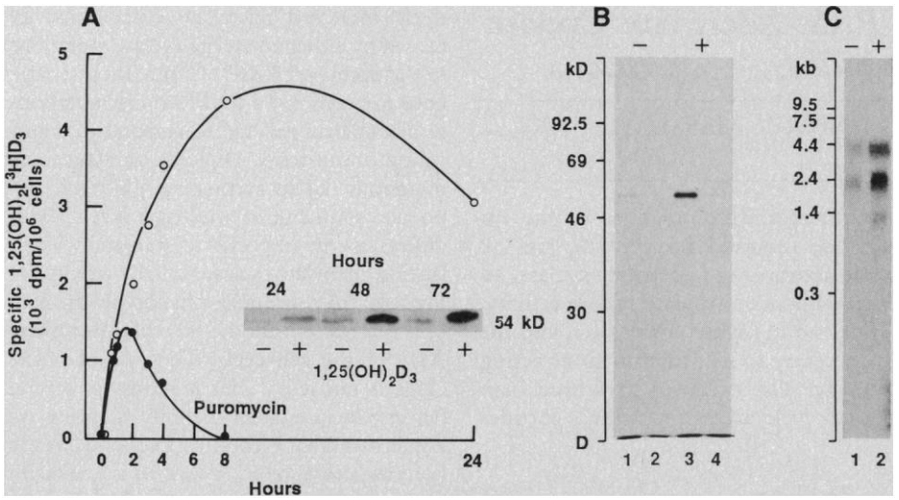

Fig. 5. Regulation of mouse VDR by $1,25(OH)_2D_3$. (**A**) Mouse 3T6 fibroblasts were seeded into 24-well Costar plates and grown for 72 hours at 37°C in a humidified atmosphere of 95% air and 5% CO_2 to a density of 1.1×10^6 cells per well. Cell medium [Dulbecco's modified Eagle's medium (DMEM) and 10% fetal calf serum (FCS)] was removed by aspiration and replaced with 0.2 ml of DMEM containing 2% FCS and either 2 n*M* $1,25(OH)_2[^3H]D_3$ (90 Ci/mmol) alone or with 200 n*M* $1,25(OH)_2D_3$. Cells also were treated as above with puromycin (final concentration 50 μ*M*) added in 0.01 ml of sterile distilled water. At the appropriate times, cells were cooled on ice, washed twice with phosphate-buffered saline (PBS), and lysed in 0.5% Triton X-100. $1,25(OH)_2[^3H]D_3$-receptor complex was assayed by adsorption to DE-81 filter disks as described previously (*28*). Specific binding, as shown, represents the difference between total and nonspecific binding and is the average of triplicate assays at each point. Inset: 3T6 cells were seeded onto Costar plates and treated at 0, 24, and 48 hours with and without 10^{-8}*M* $1,25(OH)_2D_3$. Cells were harvested at the appropriate times, washed with PBS, and sonicated directly in Laemmli denaturing buffer (*30*) at a concentration of 10^7 cells per milliliter; 5×10^5 3T6 cell equivalents were analyzed directly by Western blot analysis as in Fig. 1. (**B**) Polyadenylated RNA (2 μg) was isolated from 3T6 cells grown (lanes 1 and 2) in the absence or (lanes 3 and 4) presence of 10 n*M* $1,25(OH)_2D_3$ and translated in vitro, immunoprecipitated, electrophoresed, and fluorographed for 48 hours. The specificity of the immunoprecipitation (described in Fig. 3) is seen in lanes 2 and 4. Other methods were as described in Fig. 3. (**C**) Northern blot analysis of the RNA used in (B) isolated from 3T6 cells grown (lane 1) in the absence or (lane 2) presence of 10 n*M* $1,25(OH)_2D_3$ and probed with nick-translated 510-bp fragment of λVDR5 as described in Fig. 4.

Treatment of 3T6 cells with 10^{-8}*M* $1,25(OH)_2D_3$ for 48 hours leads to an obvious increase in the apparent concentration of the VDR mRNA signal (Fig. 5C). This increase qualitatively corresponds to that seen at the protein and mRNA activity level and thus corroborates our hypothesis that $1,25(OH)_2D_3$ "upregulates" its receptor via a direct increase in receptor mRNA. Thus, $1,25(OH)_2D_3$ may be essential in the maintenance of basal cellular levels of its cognate receptor.

The primary structural configuration of cVDR within its DNA-binding domain is apparently similar to that of other steroid receptors, implying that its direct interaction with vitamin D–regulated genes is most likely analogous. This strengthens the notion that $1,25(OH)_2D_3$ belongs to the class of true steroid hormones. The high degree of homology between cVDR and the avian erythroblastosis *gag-erbA* fusion peptide in this region suggests that both arise from a primordial gene, as was originally proposed for the human glucocorticoid receptor (*17*). The fact that its greatest homology is with the v-*erbA* product may suggest that the cVDR is one of the more primitive steroid receptor molecules. Importantly, the cDNAs we described have been useful in characterizing cVDR and mouse VDR mRNAs, identifying their tissue-specific expression, and clarifying the mechanism of VDR autoregulation by the $1,25(OH)_2D_3$ ligand in mouse 3T6 fibroblasts. Studies with full-length VDR cDNA will permit a systematic evaluation of structure-function correlates through studies involving mutagenesis and transfection into eukaryotic host cells. Of equal importance, the availability of these cDNAs will permit determination of the genetic basis for tissue resistance observed in human type II vitamin D–dependent rickets, a heterogenous disease whose origins have been traced to dysfunctional VDR domains (*29*).

REFERENCES AND NOTES

1. M. R. Haussler and T. A. McCain, *N. Engl. J. Med.* **297**, 974, 1041 (1977); H. F. DeLuca and H. K. Schnoes, *Annu. Rev. Biochem.* **52**, 411 (1983).
2. M. R. Haussler and A. W. Norman, *Proc. Natl. Acad. Sci. U.S.A.* **62**, 155 (1969); P. F. Brumbaugh and M. R. Haussler, *J. Biol. Chem.* **250**, 1588 (1975); P. F. Brumbaugh, M. R. Hughes, M. R. Haussler, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4871 (1975); M. R. Haussler, S. C. Manolagos, L. J. Deftos, *J. Biol. Chem.* **255**, 5007 (1980).
3. G. Theofan, A. K. Hall, M. W. King, A. W. Norman, in *Vitamin D_3: Chemical, Biochemical, and Clinical Update*, A. W. Norman, K. Schaefer, H.-G. Grigoleit, D. von Herrath, Eds. (de Gruyter, Berlin, 1985), p. 333.
4. C. Perret, C. Desplan, J. M. Dupre, M. Thomasset, in *ibid.*, p. 365.
5. E. Abe *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4990 (1981); Z. Bar-Shavit *et al.*, *ibid.* **80**, 5907 (1983); D. J. Mangelsdorf, H. P. Koeffler, C. A. Donaldson, J. W. Pike, M. R. Haussler, *J. Cell Biol.* **98**, 391 (1984).
6. M. R. Haussler *et al.*, in *The Role of Receptors in Biology and Medicine*, A. M. Gotto, Jr., and B. W. O'Malley, Eds., (Raven, New York, 1986), p. 91.
7. J. W. Pike, *Nutr. Rev.* **43**, 161 (1985); M. R. Haussler, *Annu. Rev. Nutr.* **6**, 527 (1986).
8. W. T. Schrader *et al.*, *Recent Prog. Horm. Res.* **37**, 583 (1981).
9. J. W. Pike and M. R. Haussler, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5485 (1979); M. C. Dame, E. A. Pierce, H. F. DeLuca, *ibid.* **82**, 7825 (1985).
10. J. W. Pike, C. A. Donaldson, S. L. Marion, M. R. Haussler, *ibid.* **79**, 7719 (1982); J. W. Pike, S. L. Marion, C. A. Donaldson, M. R. Haussler, *J. Biol. Chem.* **258**, 1289 (1983).
11. J. W. Pike, in *Vitamin D_3: Chemical, Biochemical, and Clinical Update*, A. W. Norman, K. Schaefer, H.-G. Grigoleit, D. von Herrath, Eds. (de Gruyter, Berlin, 1985), p. 95; E. A. Allegretto and J. W. Pike, *J. Biol. Chem.* **260**, 10139 (1985); J. W. Pike and N. M. Sleator, *Biochem. Biophys. Res. Commun.* **131**, 378 (1985).
12. J. W. Pike, N. M. Sleator, M. R. Haussler, *J. Biol. Chem.* **262**, 1305 (1987).
13. E. A. Allegretto, J. M. Pike, M. R. Haussler, *ibid.*, p. 1312.
14. J. W. Pike, *ibid.* **259**, 1167 (1984).
15. R. A. Young and R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1194 (1983).
16. H. Fukushima, J. R. DeWet, J. S. O'Brien, *ibid.* **82**, 1262 (1985).
17. S. M. Hollenberg *et al.*, *Nature (London)* **318**, 635 (1985); C. Weinberger, S. M. Hollenberg, M. G. Rosenfeld, R. M. Evans, *ibid.*, p. 670.
18. A. Krust *et al.*, *EMBO J.* **5**, 891 (1986); D. P. McDonnell, B. L. Maxwell, O. M. Conneely, B. W. O'Malley, in *Expression of Neural Genes*, F. Wong, Ed. (Liss, New York, 1987).
19. B. L. Maxwell *et al.*, *Mol. Endocrinol.*, in press.
20. O. M. Conneely *et al.*, *Science* **233**, 767 (1986); J. M. Jeltsch *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5424 (1986).
21. J. Miller, A. D. McLachlan, A. Klug, *EMBO J.* **4**, 1609 (1985); J. M. Berg, *Science* **232**, 485 (1986).
22. J. W. Pike, *Biochem. Biophys. Res. Commun.* **100**, 1713 (1981); K. Yamaaoka, J. W. Pike, M. R. Haussler, *J. Bone Miner. Res.* **1**, 406 (abstr.) (1986).
23. D. J. Mangelsdorf, J. W. Pike, M. R. Haussler, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 354 (1987).
24. D. J. Mangelsdorf *et al.*, *J. Bone Miner. Res.* **1** (suppl. 1) 407 (abstr.) (1986).
25. T. L. Clemens, X. Y. Zhou, J. W. Pike, M. R. Haussler, R. S. Sloviter, in *Vitamin D_3: Chemical Biochemical, and Clinical Update*, A. W. Norman, K. Schaefer, H.-G. Grigoleit, D. von Herrath, Eds. (de Gruyter, Berlin, 1985), p. 95.
26. W. E. Stumpf, M. Sar, S. A. Clark, H. F. DeLuca, *Science* **215**, 1403 (1982).
27. E. M. Costa, M. A. Hirst, D. Feldman, *Endocrinology* **117**, 203 (1985); E. M. Costa and D. Feldman, *Biochem. Biophys. Res. Commun.* **137**, 742 (1986).
28. J. W. Pike and M. R. Haussler, *J. Biol. Chem.* **258**, 8554 (1983).
29. S. J. Marx *et al.*, *Recent Prog. Horm. Res.* **40**, 589 (1983).
30. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
31. J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, R. J. Rutter, *Biochemistry* **18**, 5294 (1979).
32. H. Aviv and P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1408 (1972).
33. R. P. Ricciardi, J. S. Miller, B. E. Roberts, *ibid.* **76**, 4927 (1979).
34. T. Maniatis, E. F. Fritch, J. Sambrook, in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).

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