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18. W. M. Brieher, A. S. Yap, B. M. Gumbiner, *J. Cell Biol.* **135**, 487 (1996).
 19. S. Chappuis-Flamant, E. Wong, L. D. Hicks, C. M. Kay, B. M. Gumbiner, *J. Cell Biol.* **154**, 231 (2001).
 20. W. S. Shan et al., *J. Cell Biol.* **148**, 579 (2000).
 21. M. Kitagawa et al., *Biochem. Biophys. Res. Commun.* **271**, 358 (2000).
 22. R. B. Troyanovsky, J. Klingelhofer, S. Troyanovsky, *J. Cell Sci.* **112**, 4379 (1999).
 23. M. Ozawa, R. Kemler, *J. Cell Biol.* **111**, 1645 (1990).
 24. A. S. Yap, W. M. Brieher, M. Prusky, B. M. Gumbiner, *Curr. Biol.* **7**, 308 (1997).
 25. A. S. Yap, C. M. Niessen, B. M. Gumbiner, *J. Cell Biol.* **141**, 779 (1998).
 26. H. Takeda, Y. Shimoyama, A. Nagafuchi, S. Hirohashi, *Nature Struct. Biol.* **6**, 310 (1999).
 27. S. M. Troyanovsky, *Curr. Opin. Cell Biol.* **11**, 561 (1999).
 28. K. Miyaguchi, *J. Struct. Biol.* **132**, 169 (2000).
 29. L. A. Staehelin, *Int. Rev. Cytol.* **39**, 191 (1974).
 30. A. W. Koch, D. Bozic, O. Pertz, J. Engel, *Curr. Opin. Struct. Biol.* **9**, 275 (1999).
 31. Single-letter abbreviations for the amino acid resi-
- dues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
32. S. Sivasankar, B. Gumbiner, D. Leckband, *Biophys. J.* **80**, 1758 (2001).
 33. C. M. Niessen, B. Gumbiner, *J. Cell Biol.* **156**, 389 (2002).
 34. H. Tanahara, K. Sano, R. L. Heimark, T. St. John, S. Suzuki, *Cell Adhes. Commun.* **2**, 15 (1994).
 35. S. V. Evans, *J. Mol. Graph.* **11**, 134 (1993).
 36. A. Nicholls, K. Sharp, B. Honig, *Proteins* **11**, 281 (1991).
 37. Collaborative Computational Project No. 4, *Acta Crystallogr. D* **50**, 760 (1994).
 38. R. A. Laskowski, M. W. MacArthur, D. S. Moss, J. M. Thornton, *J. Appl. Crystallogr.* **26**, 283 (1993).
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Materials and Methods

Supporting Text

figs. S1 through S6

tables S1 through S5

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Vitamin D Receptor As an Intestinal Bile Acid Sensor

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The vitamin D receptor (VDR) mediates the effects of the calcemic hormone $1\alpha,25$ -dihydroxyvitamin D₃ [$1,25(\text{OH})_2\text{D}_3$]. We show that VDR also functions as a receptor for the secondary bile acid lithocholic acid (LCA), which is hepatotoxic and a potential enteric carcinogen. VDR is an order of magnitude more sensitive to LCA and its metabolites than are other nuclear receptors. Activation of VDR by LCA or vitamin D induced expression in vivo of CYP3A, a cytochrome P450 enzyme that detoxifies LCA in the liver and intestine. These studies offer a mechanism that may explain the proposed protective effects of vitamin D and its receptor against colon cancer.

A contributing factor to the deleterious effects of a high-fat diet is an associated increase in the excretion of fecal bile acids (1), the most toxic of which is the secondary bile acid LCA (Fig. 1A). Unlike the primary bile acids, chenodeoxycholic acid (CDCA) and cholic acid (CA), LCA is poorly reabsorbed into enterohepatic circulation and passes into the colon. At high concentrations, LCA induces DNA strand breaks, forms DNA ad-

ducts, and inhibits DNA repair enzymes (1–3). LCA can also promote colon cancer in animals (4), and its concentration is higher than other secondary bile acids in patients with colorectal cancer (5).

In contrast to the positive correlation among high-fat diets, LCA, and colon cancer, dietary intake of vitamin D and calcium is related to a reduced incidence of colorectal cancer (6). Furthermore, vitamin D supplementation inhibits colon carcinogenesis induced by either high-fat diets or intrarectal instillation of LCA (7, 8). One route for LCA elimination is through its catabolism by the enterohepatic cytochrome P450, CYP3A, a putative target gene of vitamin D (9, 10). Expression of CYP3A in the liver is regulated by the nuclear xenobiotic and pregnane X receptor (PXR, also called SXR), which can be activated by high concentrations ($\geq 100 \mu\text{M}$) of LCA (11, 12). Primary bile acids (in particular, CDCA and CA) are also ligands for the farnesoid X receptor, FXR (13, 14). However, neither PXR nor FXR responds to vitamin D, and LCA-induced expression of CYP3A is still present in PXR-null animals.

This suggests another LCA-dependent pathway for inducing CYP3A expression (11).

To determine if bile acids could act on the vitamin D receptor (VDR) to induce CYP3A expression, we used a ligand-screening assay based on the ligand-induced interaction of a nuclear receptor with its coactivator (14). The receptor-interacting domain of the coactivator SRC-1 was fused to the DNA binding domain of the yeast transcription factor GAL4, and various nuclear receptors were fused to the transactivation domain of the herpes virus VP16 protein. Expression plasmids for GAL4-SRC-1 and VP16-nuclear receptor were transfected with a GAL4-responsive luciferase reporter plasmid into human embryonic kidney (HEK293) cells and examined for luciferase expression after LCA treatment. LCA ($30 \mu\text{M}$) induced a ligand-dependent interaction between VDR and SRC-1 (Fig. 1B). As previously reported (14), LCA also activated FXR. However, no other nuclear receptors were activated by LCA (Fig. 1B) (15), including PXR, which required higher LCA concentrations ($\geq 100 \mu\text{M}$) to be activated. To further investigate the ligand specificity of VDR and FXR, we tested various primary, secondary, and conjugated bile acids in this assay (Fig. 1C). We performed these experiments in the presence or absence of the ileal bile acid transporter (IBAT), because hydrophilic bile acids such as CA and conjugated bile acids require transport across cell membranes (16). As expected, treatment of cells with the vitamin D hormone $1,25(\text{OH})_2\text{D}_3$ activated VDR but not FXR (Fig. 1C). Conversely, the primary bile acids CDCA, CA, and their conjugated metabolites were effective ligands for FXR but not VDR. FXR was also activated by the secondary bile acids, deoxycholic acid, LCA, and their conjugated metabolites (Fig. 1C). However, the only bile acids that activated VDR were LCA and its major metabolites 3-keto-LCA (Fig. 1A), glyco-LCA, and 6-ke-

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to-LCA (Fig. 1C, shaded area). The 6-keto metabolite of LCA is of further interest because it did not activate FXR. Hence, although VDR and FXR both serve as bile acid receptors, they have distinct specificity profiles.

We also compared the dosage dependency of bile acids to activate human VDR, FXR, and PXR. LCA and 3-keto-LCA activated human VDR with median effective concentration (EC_{50}) values of 8 μM and 3 μM , respectively (Fig. 1D). These bile acids also activated FXR, but effective concentrations were 2 to 3 times greater than those for VDR (Fig. 1E). Although CDCA was an FXR agonist ($EC_{50} = 7 \mu\text{M}$), it

was not effective on VDR at any concentration. LCA and 3-keto-LCA were also equally effective at activating full-length mouse or human VDR [see below and (15)]. The xenobiotic receptor PXR also is activated by LCA (11, 12). However, compared to VDR, PXR required a concentration at least 10 times greater than that of either LCA (Fig. 1F) or 3-keto-LCA (15) for activation. Therefore, the concentration at which these bile acids activate VDR is below the pharmacologic range that activates FXR and PXR. These data suggest that VDR is a more sensitive receptor for bile acids than are FXR and PXR, specifically for LCA and its major metabolite 3-keto-LCA.

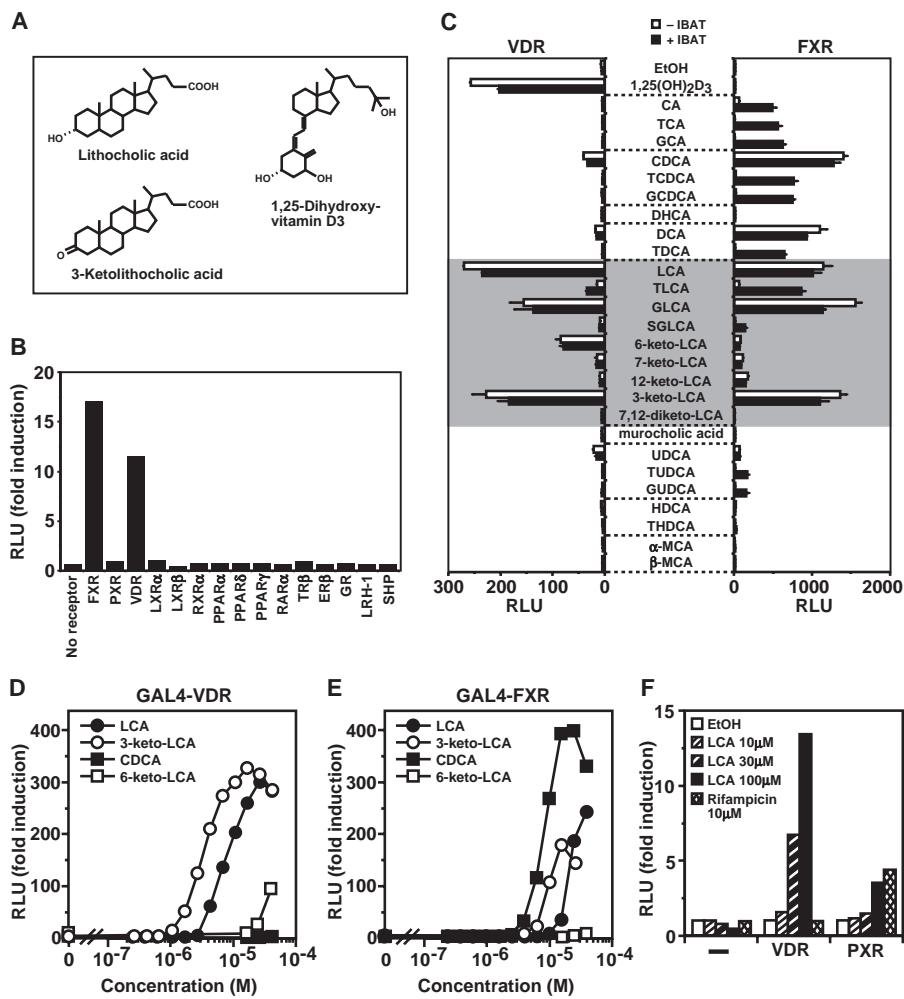


Fig. 1. LCA and its metabolites are VDR agonists. **(A)** Structures of VDR agonists. **(B)** Receptor-specific activation by LCA. Various nuclear receptors were expressed in HEK293 cells and screened for activation by 30 μM LCA with a mammalian two-hybrid GAL4-SRC-1 and VP16-receptor luciferase assay. **(C)** Ligand specificity of VDR- and FXR-activation by bile acids. VDR and FXR were screened for activation by 1,25(OH)₂D₃ (0.1 μM) or various bile acids (30 μM), as in Fig. 1B. The screen was performed on transfected HEK293 cells expressing VDR or FXR in the presence (black bars) or absence (white bars) of IBAT to facilitate uptake of CA and the conjugated bile acids (16). The shaded area identifies LCA-specific metabolites. **(D)** and **(E)** Comparative dose response of human VDR and FXR to bile acids using a GAL4-receptor luciferase assay. **(F)** Comparative dose response of VDR and PXR to bile acids. Transfection assay was as in (D) and (E), except that full-length human VDR and PXR were expressed in monkey kidney CV1 cells with a luciferase reporter plasmid containing three copies of the human CYP3A4 ER6 element (see Fig. 3). Rifampicin was used as a positive control ligand for PXR. RLU, relative light units. Fold inductions by various ligands in (B), (D), (E), and (F) are relative to ethanol (EtOH) vehicle used as a control. See supporting online material for additional methods and materials.

To demonstrate that bile acids directly bind VDR as ligands, we performed a competitive binding assay using [³H]1,25(OH)₂D₃ and increasing concentrations of candidate bile acids (Fig. 2). Both LCA and 3-keto-LCA competed effectively with [³H]1,25(OH)₂D₃ for binding to VDR [inhibition constant (K_i) = 29 \pm 6 μM and 8 \pm 3 μM , respectively], with 3-keto-LCA exhibiting an affinity that is 3.5 times greater than that of LCA. A fluorescence polarization assay also showed that LCA, but not other bile acids, induced association with the coactivator peptide (fig. S1).

LCA is catabolized by CYP3A (11, 17), a putative target gene of vitamin D in the intestine (9, 10). To explore the role of LCA and VDR in the activation of CYP3A, we investigated the promoters of the mouse, rat, and human CYP3A genes for potential VDR-RXR heterodimer binding sites (Fig. 3A). All three gene promoters have direct repeats separated by three nucleotides (DR3 elements) similar to those found in other VDR target genes (18). In addition, the human CYP3A4 gene contains an everted repeat separated by six nucleotides (ER6) that has also been reported to be a VDR-RXR response element (10). Each of these elements bound to the VDR-RXR heterodimer (Fig. 3B), competed for the receptor heterodimer with high affinity (Fig. 3C), and was responsive to VDR-dependent transactivation by either LCA or 1,25(OH)₂D₃ (Fig. 3D). It is noteworthy that these elements were previously reported to mediate the xenobiotic response of the PXR-RXR heterodimer in the liver (19). In a comparison to PXR, we found that all three CYP3A promoters were at least 10 times more sensitive to VDR when activated by LCA (15). The human CYP3A elements were the most sensitive and conferred the strongest

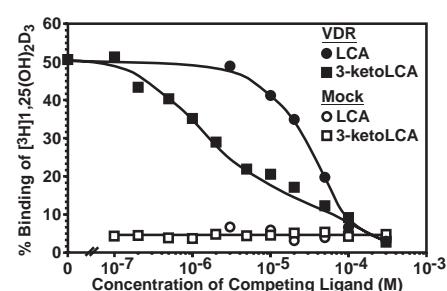


Fig. 2. LCA and 3-keto-LCA directly bind VDR in vitro. Human VDR was expressed in monkey kidney COS-7 cells, labeled with [³H]1,25(OH)₂D₃, and used for competitive binding assays. Results obtained with VDR-containing lysates (closed symbols) or mock lysates transfected with RXR (open symbols) are shown. Data points are representative of three independent experiments using LCA and 3-keto-LCA as competitors. Similar experiments using CDCA, CA, muricholic acid (MCA), and hyodeoxycholic acid (HDCA) showed no competitive binding. See supporting online material for materials and methods.

Fig. 3. CYP3A genes are LCA-dependent VDR target genes. (A) VDR response element (VDRE) sequences from mouse (m), rat (r), and human (h) CYP3A genes. The numbers indicate positions in the gene promoter relative to the transcription start site. (B) VDR-RXR heterodimers bind to CYP3A VDREs. In vitro synthesized RXR and VDR were used in electrophoretic mobility band shift assays (24) with [³²P]-oligonucleotides shown in (A). The arrowheads depict DNA bound VDR-RXR and free probe. (C) CYP3A VDREs compete for

VDR-RXR binding to the high-affinity VDRE from the mouse osteopontin gene, a known VDR target (18). Band shifts were performed with the ³²P-labeled osteopontin VDRE and analyzed for competitive binding to 10- and 100-fold excesses of the indicated CYP3A VDREs (lanes 3 to 10). Lane 1, no VDR-RXR protein; lane 2, no competitor oligonucleotide. (D) LCA stimulates VDR-dependent transcription of CYP3A VDREs. Three copies of the indicated CYP3A VDREs were inserted into a luciferase plasmid, transfected with VDR and RXR expression plasmids into HEK293 cells, and assayed for ligand-dependent transactivation as described in Fig. 1. RLU, relative light units. EtOH, ethanol vehicle. TK-LUC, control thymidine kinase luciferase reporter plasmid.

response (Fig. 3D). These data further suggest that VDR mediates LCA-dependent induction of CYP3A gene expression.

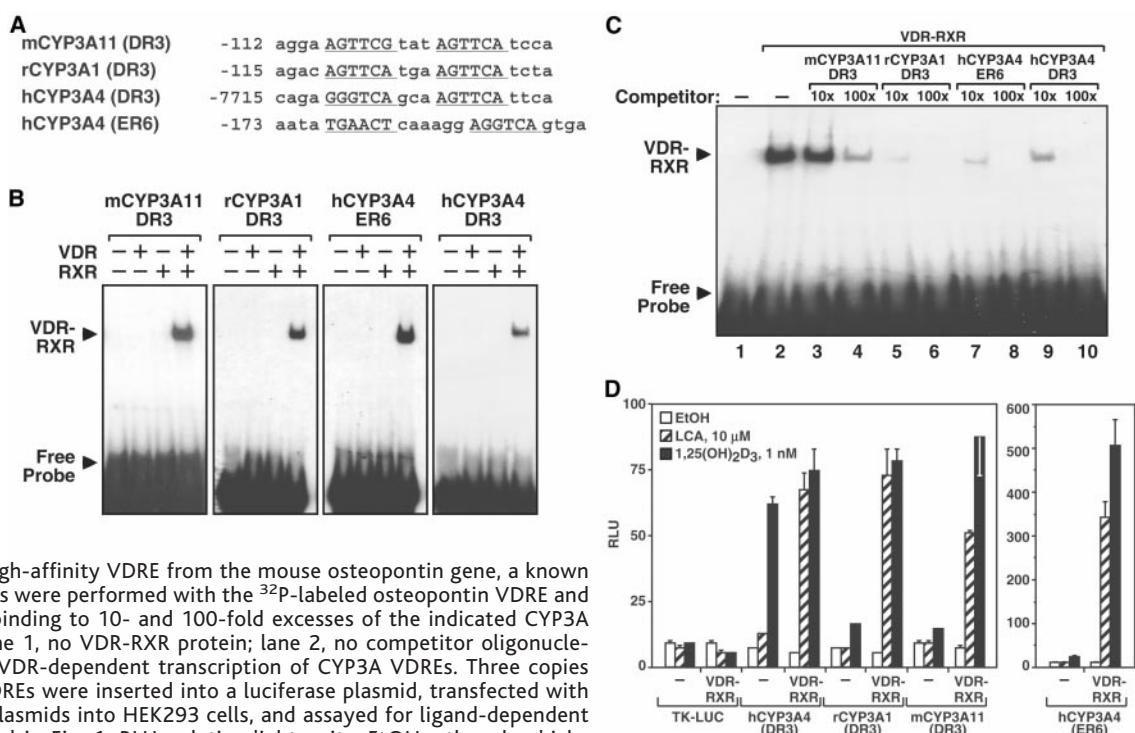
To confirm the effects of VDR on induction of CYP3A expression in vivo, the transactivation of the CYP3A11 gene was determined after treating mice with agonists for VDR, FXR, or PXR. For these experiments, we used 1 α -hydroxyvitamin D₃ (1 α (OH)D₃) (8) and EB1089 (20) as synthetic VDR agonists, pregnenolone-16 α -carbonitrile (PCN) as a PXR-selective agonist (19), and LCA as a panagonist for all three receptors. CYP3A11 mRNA expression in the intestine was increased in response to both VDR- and PXR-specific ligands, as well as LCA. In contrast, the VDR-specific target gene calbindin 9k (18) was activated by LCA and the VDR-selective agonists 1 α (OH)D₃ and EB1089, but not by PCN, indicating that LCA can function as a VDR agonist in vivo. Likewise, the FXR target gene, ileal bile acid binding protein (14), was transactivated by LCA, but not by VDR or PXR selective ligands. None of the compounds altered the expression of VDR (Fig. 4A). To demonstrate that LCA- and VDR-dependent activation in vivo does not require PXR, the expression of CYP3A11 was examined in PXR^{-/-} mice and PXR^{+/+} control mice (Fig. 4, B and C). As expected (11, 12), CYP3A11 mRNA expression in response to PCN was eliminated in the liver and intestine of PXR-null mice. However, CYP3A11 mRNA expression was still induced by both the VDR-selective ligands and LCA. This dem-

onstrates that VDR can function as an LCA sensor in vivo, resulting in increased expression of CYP3A.

Taken together, these results point to VDR as a potential bile acid sensor in the enteric tract, where elevated concentrations of LCA may bind to VDR. This “adopted orphan” function of VDR (21) complements its endocrine role in the small intestine as a high-affinity [dissociation constant (K_d) = 0.1 to 1 nM] receptor for 1,25(OH)₂D₃ to promote calcium and phosphate absorption, which ensures proper mineralization of bone (18). The regulation of the LCA/VDR metabolic cascade is strikingly similar to that mediated by other evolutionarily related nuclear receptors [e.g., PXR, constitutive androstane receptor (CAR), FXR, and liver X receptor (LXR)] that function as lipid sensors and mediate detoxification of their ligands (21). By binding to VDR, both LCA and vitamin D may activate a feed-forward catabolic pathway that increases CYP3A expression and leads to the detoxification of LCA. These findings suggest a model to explain how the enteric system could protect itself from the potentially harmful effects of LCA and why vitamin D is protective against colon cancer under normal physiologic conditions. Protection provided by VDR activation may become compromised when the detoxification pathway is overwhelmed (e.g., by increased levels of LCA due to sustained high-fat diets) or under clinical conditions of vitamin D deficiency (e.g., rickets/osteomalacia). Consistent with this model, there is an epidemiologic relation between the incidence of colon cancer and Western-style, high-fat diets (22), and the highest death rates from colon cancer occur in areas with a high prevalence of rickets (6). Furthermore, mice lacking VDR not only have rickets but also display enhanced cellular proliferation in the colon (23). Thus, this work should provide the impetus for further studies addressing the role of diet, bile acids, and vitamin D in colorectal cancer.

References and Notes

1. F. M. Nagengast, M. J. Grubben, I. P. van Munster, *Eur. J. Cancer* **31A**, 1067 (1995).
2. K. Hamada, A. Umemoto, A. Kajikawa, M. J. Seraj, Y. Monden, *Carcinogenesis* **15**, 1911 (1994).
3. A. Ogawa, T. Murate, M. Suzuki, Y. Nimura, S. Yoshida, *Jpn. J. Cancer Res.* **89**, 1154 (1998).
4. T. Narisawa, N. E. Magadha, J. H. Weisburger, E. L. Wynder, *J. Natl. Cancer Inst.* **53**, 1093 (1974).
5. R. W. Owen, M. Dodo, M. H. Thompson, M. J. Hill, *Nutr. Cancer* **7**, 73 (1987).
6. C. F. Garland, F. C. Garland, E. D. Gorham, *Ann. N.Y. Acad. Sci.* **889**, 107 (1999).
7. B. C. Pence, F. Buddingh, *Carcinogenesis* **9**, 187 (1988).
8. A. Kawaura, N. Tanida, K. Sawada, M. Oda, T. Shimojima, *Carcinogenesis* **10**, 647 (1989).
9. P. Schmidlin-Ren et al., *Mol. Pharmacol.* **51**, 741 (1997).
10. K. E. Thummel et al., *Mol. Pharmacol.* **60**, 1399 (2001).
11. W. Xie et al., *Proc. Natl. Acad. Sci. U.S.A.* **98**, 3375 (2001).
12. J. L. Staudinger et al., *Proc. Natl. Acad. Sci. U.S.A.* **98**, 3369 (2001).
13. D. W. Russell, *Cell* **97**, 539 (1999).
14. M. Makishima et al., *Science* **284**, 1362 (1999).
15. M. Makishima, T. Lu, D. Mangelsdorf, data not shown.
16. C. J. Sippel, P. A. Dawson, T. Shen, D. H. Perlmuter, *J. Biol. Chem.* **272**, 18,290 (1997).



REPORTS

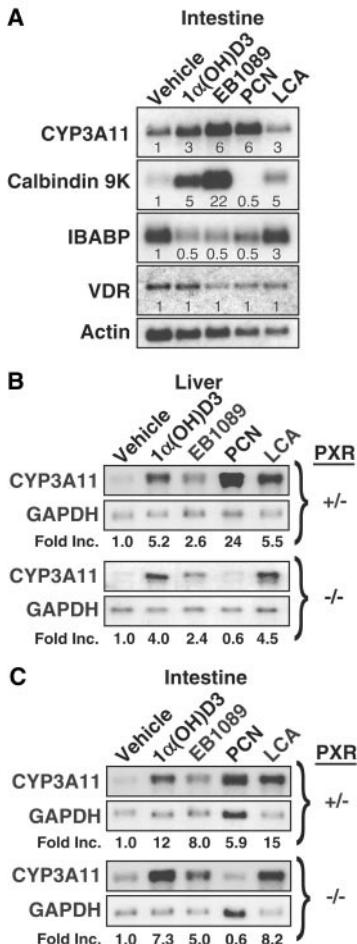


Fig. 4. LCA activation of VDR target genes in vivo. (A) Regulation of VDR, PXR, and FXR target gene expression in small intestine. Mice were treated with the indicated ligands or vehicle (corn oil) for 3 days, and small intestine was harvested for Northern blot (RNA) analysis (25) using 5 µg pooled mRNA ($n = 6$ animals). (B and C) Regulation of CYP3A11 expression in liver and intestine of PXR^{-/-} and PXR^{+/-} mice. Northern blot analysis was performed on liver of mice treated as in (A). Representatives of three independent experiments are shown. The numbers under the lanes indicate fold increases in expression relative to the vehicle and were standardized against actin or glyceraldehyde phosphate dehydrogenase (GAPDH) controls. 1 α D₃, 1 α -hydroxy-vitamin D₃.

17. Z. Araya, K. Wikvall, *Biochim. Biophys. Acta* **1438**, 47 (1999).
18. M. R. Haussler et al., *J. Bone Miner. Res.* **13**, 325 (1998).
19. S. A. Kliewer et al., *Cell* **92**, 73 (1998).
20. A. M. Kissmeyer et al., *Biochem. Pharmacol.* **53**, 1087 (1997).
21. A. Chawla, J. J. Repa, R. M. Evans, D. J. Mangelsdorf, *Science* **294**, 1866 (2001).
22. M. Lipkin, B. Reddy, H. Newmark, S. A. Lamprecht, *Annu. Rev. Nutr.* **19**, 545 (1999).
23. E. Kallay et al., *Carcinogenesis* **22**, 1429 (2001).
24. T. T. Lu et al., *Mol. Cell* **6**, 507 (2000).
25. Male mice (129sv wild type, PXR^{+/-}, or PXR^{-/-}) were gavaged daily with vehicle (corn oil), 1.5 µg 1 α (OH)D₃ (gift from M. Pechet, Research Institute

for Medicine and Chemistry, Cambridge, MA), 1.5 µg EB1089 (gift from L. Binderup, Leo Pharmaceutical Products, Copenhagen), 1.5 mg PCN, or 8 mg LCA. Mice were killed, and mRNA from the intestine and liver were isolated for Northern blot analysis (24) using the indicated gene-specific cDNA probes.

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Supporting Online Material

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Material and Methods

fig. S1

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Heterotopic Shift of Epithelial-Mesenchymal Interactions in Vertebrate Jaw Evolution

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Genes involved in late specification of the mandibular arch, the source of the vertebrate jaw, are expressed with similar patterns in the oral regions of chick and lamprey embryos. However, morphological comparisons indicate that apparently orthologous homeobox genes were expressed in different subdivisions of the ectomesenchyme in the two species. Therefore, the homology and gene expression of the oral region are uncoupled during the transition from agnathan to gnathostome; we conclude that a heterotopic shift of tissue interaction was involved in the evolution of the jaw.

Conventional comparative anatomy assumes that the vertebrate jaw was acquired through modification of the mandibular arch, the most rostral element of the pharyngeal arches (Fig. 1A): In primitive vertebrates, the arches resembled one another, showing no specialization. The mandibular arch was then dorsoventrally subdivided and enlarged to differentiate the upper and lower jaw cartilages (Fig. 1A, right). However, the larval oral apparatus in the lamprey, a jawless vertebrate (agnathan), exhibits well-differentiated upper and lower lips for which morphologic homologies (1) with the jaws are not certain (2) (Fig. 1B). Moreover, the early embryonic pattern of the lamprey is very similar to that of the jawed vertebrates (gnathostomes): Homologous patterns are seen in the global deployment of crest cells (3), in the configura-

tion of the mesoderm (4), and even in the expression of some regulatory genes, including *Otx*, a gene involved in gnathostome jaw development (5, 6).

During development of the amniote mandibular arch, *Dlx* and *Msx* homeobox genes are expressed in an overlapping fashion in the crest-derived ectomesenchyme, and they prefigure the proximal-distal axis of the mandibular arch (Fig. 1C) (7, 8). These genes are regulated through epithelial-mesenchymal interactions (9), downstream of growth factors secreted by the epidermis (7, 8). In the chick late pharyngula, a growth factor-encoding gene, *cFgf8*, is expressed in the perioral epidermis (Fig. 1D), and its target gene, *cDlx1*, is expressed in the subadjacent ectomesenchyme (Fig. 1E) (10). Another growth factor, *cBmp4*, is expressed in the distal epidermis of the upper and lower jaws (Fig. 1F); its downstream gene, *cMsx1*, is expressed in the distal ectomesenchyme (Fig. 1G).

We isolated the above gene cognates from a cDNA library of the Japanese lamprey, *Lampetra japonica*, and observed their expression patterns in the embryonic oral region. Molecular phylogenetic analyses have indicated that the isolated genes,

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