Effect of retinoid status on α , β and γ retinoic acid receptor mRNA levels in various rat tissues

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We have investigated the effects of retinoids, vitamin D and thyroid hormone on the levels of retinoic acid receptor (RAR) α , RAR β and RAR γ mRNAs in intact animals. Although vitamin A deficiency caused no significant changes in the levels of RAR α and RAR γ mRNAs, the level of RAR β transcripts was greatly decreased in various tissues of vitamin A-deficient rats, but was restored rapidly to a normal level after administration of retinoic acid. Retinol also restored the RAR β mRNA level, but the magnitude and kinetics of the induction differed from those by retinoic acid. The use of specific inhibitors demonstrated that this autoregulation of RAR β gene expression *in vivo* occurred at the transcriptional level. In addition, from these results it was postulated that the maintenance of the normal RAR β mRNA levels seemed to require a threshold serum retinol concentration (about 25 μ g/dl). Moreover, we found that administration of retinol and retinoic acid to normal rats caused the overexpression of RAR β transcripts (2–15-fold) when compared with the control levels of RAR β mRNA, although the levels of RAR α and RAR γ mRNAs were not affected. Vitamin D and thyroid hormone did not modulate the levels of RAR transcripts. These findings clearly indicate the specific ligand regulation of RAR β gene expression in intact animals. The altered levels of RAR β according to retinoid status may affect retinoid-inducible gene expression.

INTRODUCTION

It is well documented that vitamin A (retinol) and its derivative retinoic acid (RA) are essential for a wide range of functions such as vision, reproduction, growth and cellular differentiation in adult animals [1–3]. Moreover, studies have shown that RA is involved in vertebrate development and embryogenesis, probably as a natural morphogen [4–6].

The discovery of three mammalian nuclear retinoic acid receptors (RARs; designated RAR α , RAR β and RAR γ) [7–12] has provided an understanding of the underlying mechanisms of retinoid signal transduction. RARs belong to the steroid/thyroid receptor superfamily and, like other members of this family, function as ligand-dependent transcription factors [13,14]. More recently, a new class of nuclear retinoid receptors, the RXRs (RXR α , RXR β and RXR γ), have also been shown to participate in retinoid signal transduction in response to 9-*cis*-RA [15–18]. Comparison among all mammalian RARs has revealed two conserved regions, i.e. DNA- and ligand-binding regions. However, the N- and C-terminal regions of these proteins are less well conserved, suggesting that each RAR may have a distinct role *in vivo* [7–12]. This hypothesis is also favoured by results of *in situ* hybridization experiments, in which RARs differed in their spatial and temporal patterns of expression during mouse embryogenesis [6,12,19–21]. Hence it is of great interest to determine how the expression of RAR genes is regulated at the transcriptional and post-transcriptional levels. Studies using several kinds of cell lines have shown the occurrence of auto-regulated RAR β gene expression [22,23], and moreover have identified a RA response element (RARE β) located in the 5' upstream region of the mouse and human RAR β genes [24–26]. These RARE β s consist of two tandem repeated A/GGTTCA

motifs, different from canonical inverted repeats [24–26]. Similar types of ligand-response element, having tandem repeated motifs with different spacing, have recently been shown to serve as response elements for vitamin D and thyroid hormone [27]. Although the spacing between the two motifs is postulated to have a crucial role in determining the target specificity of transactivation between RARs, the vitamin D receptor (VDR) and the thyroid hormone receptor (TR), the tandem repeats in naturally occurring sequences are closely related to each other, and some of them are promiscuous for binding of these three receptors [27], suggesting a possible modulation among the receptors upon DNA binding.

One of the most important questions of retinoid action in intact animals is whether the levels of RARs are influenced by retinoid nutritional status as well as other factors, since abnormal retinoid status causes some malfunctions of retinoid-dependent physiological process [1–3], probably based on retinoid-inducible gene expression. It has already been established that the levels of other retinoid-binding proteins are regulated by retinoid status [28–30]. These findings, when taken together with the fact that the autoregulation of RAR β has been demonstrated *in vivo* and *in vitro* [22,23,31–33], suggest that autoregulation of RAR gene expression may occur in adult animals. If so, profound changes in target gene expression through altered levels of RARs may be caused by relatively small changes in the level of the retinoid ligand.

The present study was undertaken to systematically clarify whether retinoids, as well as vitamin D and thyroid hormone, affect the levels of RAR α , RAR β and RAR γ transcripts in whole animals. We found that the RAR β gene expression is dependent on the retinoid status in the intact animal, whereas neither vitamin D nor thyroid hormone affected the levels of the RAR transcripts.

Abbreviations used: RAR, retinoic acid receptor; RXR, retinoid X receptor; RARE, retinoic acid response element; VDR, vitamin D receptor; TR, thyroid hormone receptor; RA, retinoic acid.

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MATERIALS AND METHODS

Animals

Male weanling rats of the Wistar strain (3 weeks old) were randomly divided into two groups (normal and retinol-deficient groups). The retinol-deficient group was depleted of retinoids by feeding them on a vitamin A-deficient diet [34], in which the other nutrients except retinol were sufficient to support normal growth. The composition of the experimental diets was based on the AIN-76 diet. Both the retinol-deficient diet and the retinoid-supplemented diet were prepared fresh weekly. The normal group was fed with the same vitamin A-deficient diet, but were supplemented with 1.38 mg of dietary vitamin A/kg (supplied as retinyl acetate). Serum and liver contents of retinol were determined by h.p.l.c. with authentic retinol as the standard [35]. Animals were judged as being in a retinol-deficient state when serum retinol levels were below 1.8 µg/dl (normal levels are around 40 µg/dl) and the liver content of retinol was depleted to 2.5 µg/g of liver (normal levels are about 30 µg/g of liver). In replenishment experiments, retinol-deficient rats were given intragastrically 100 µg of retinol or 100 µg of RA in 0.2 ml of soybean oil (controls were given 0.2 ml of soybean oil alone), and were killed after the indicated times. Vitamin D-deficient rats were produced by feeding animals on a vitamin D-deficient diet [36] supplemented with vitamins A, E and K for 40 days, and control rats were fed on the same diet but with the addition of vitamin D. Vitamin D deficiency was defined by serum calcium being below 6 mg/dl [36]. Goitrous thyroids were produced by feeding rats on a diet containing 6-propyl-2-thiouracil (1.5 mg/g of diet) for 20 days [37]. The goitres at the final stage weighed 83 ± 6 mg (the thyroid glands of control rats were 16 ± 2 mg).

Animal experiments were conducted in accordance with the principles and procedures as outlined by the NIH Guide for the Care and Use of Experimental Animals.

RNA isolation and Northern blot analysis

Total RNA was isolated from rat tissues by the acid guanidinium thiocyanate/phenol/chloroform method [38]. Poly(A)⁺ RNA was prepared by oligo(dT)-cellulose chromatography. Total RNA (50–100 µg) or poly(A)⁺ RNA (3–5 µg) was electrophoresed through 1% agarose/1.1 M-formaldehyde gels and transferred to nitrocellulose filters (Schleicher and Schuell BAS85) [11]. The full-length cDNAs of mouse RAR α , RAR β and RAR γ were labelled with [³²P]dCTP by the random priming method [9]. Hybridization was carried out at 42–45 °C for 18 h in 5 × SSPE (1 × SSPE = 0.15 M-NaCl/10 mM-NaH₂PO₄/1 mM-EDTA, pH 7.0), 50% formamide, 0.2 mg of denatured salmon-sperm DNA/ml and 1 × Denhardt's reagent. The most stringent wash was performed at 65 °C in 0.1 × SSPE containing 1.0% SDS and 0.03% sodium pyrophosphate [11]. Filters were dehybridized by 15 min treatments in 0.1 × SSPE/0.1% SDS at 90 °C. A 1.5 kb fragment of rat β -actin cDNA [39] was used as a β -actin probe to confirm that the same amounts of RNA from the tissues of both normal and treated animals were loaded on each lane. Autoradiography was usually performed with two different lengths of exposure (1–2 weeks for RAR transcripts and 1–2 days for actin transcripts) at –80 °C with intensifying screens.

All animal experiments, each using more than four rats per group, were repeated at least three times. The data from only one representative series of experiments are shown. To obtain quantitative data on RAR transcripts we used densitometric analysis of the autoradiograms, with actin transcripts as an internal control in each given tissue. The relative abundance of RAR transcripts in a given tissue is shown as the mean $\pm$ S.D. for at

least four samples from each different rat after normalizing to the actin mRNA level.

RESULTS

Differential expression of RAR α , RAR β and RAR γ mRNAs in rat tissues

Northern blot analysis of total RNA or poly(A)⁺ RNA extracted from adult rat tissues, using ³²P-labelled mouse(m) RAR α , mRAR β and mRAR γ cDNA probes, was carried out under stringent conditions. It clearly showed that three receptor genes were differentially expressed (see lanes C in Fig. 1a), as in previous reports [11,12,21]. The mRAR α probe hybridized with two distinct transcripts of 2.8 and 3.8 kb in all tissues examined. With the mRAR β probe, two transcripts of 3.1 kb and 3.6 kb were detected in most tissues, and were particularly abundant in epithelial tissues. A transcript of 3.3 kb was detected by the mRAR γ probe at high levels in skin and lung, and at a trace level in brain and testis.

Vitamin A deficiency causes a decrease in RAR β mRNA

To investigate whether RAR gene expression is affected by retinoid status in whole animals, levels of transcripts for RARs were measured by Northern blot analysis of RNAs extracted from various tissues of rats fed on the normal or retinol-deficient diet. Depletion of retinoids in rats was achieved without overt effect by feeding them on a retinol-deficient diet for 38 days. As shown in Fig. 1(a), the level of RAR β transcripts was clearly decreased in various tissues from rats fed on the retinol-deficient diet [compare lanes C (control) and D (deficiency)]. This decrease in RAR β mRNA levels in various tissues was statistically significant, as estimated by densitometric analysis of the autoradiograms (Fig. 1b).

In some tissues, slight (but not statistically significant) differences in RAR α and RAR γ mRNA levels were observed between control and deficient rats. Such an alteration might be due to a change in the levels of mRNA of one specific isoform [40–43].

To determine the time of onset of the retinol-deficiency-induced decrease in RAR β transcripts, rats fed on the experimental diets were killed every 10 days up to 30 days and the RAR β mRNA and serum retinol were analysed (only the results of lung RAR β transcripts are shown as representative data in Fig. 2). Transcripts for the RAR β gene in the lungs of deficient rats had decreased by day 10 (30% decrease compared with day 0) (Fig. 2a), indicating that the level of RAR β mRNA is very sensitive to the serum retinol level. After 20 days, although the serum retinol concentration of deficient rats was approx. 50% of that in control animals, RAR β transcripts were completely absent (Figs. 2a and 2b). Such a rapid decrease in RAR β mRNA induced by retinol deficiency was observed in all tissues in which the RAR β gene is expressed (results not shown).

Administration of retinol and RA to vitamin A-deficient rats restores the levels of RAR β mRNA

Oral administration of RA (100 µg/rat) to retinol-deficient rats rapidly restored the levels of RAR β mRNA in the lung and liver (see Figs. 2a and 2c) and other tissues (results not shown) within 4 h. Time course studies (Fig. 2a) showed that this single dose of RA transiently induced RAR β gene transcription, and this induction reached a maximum at 4 h after administration. Retinol (100 µg/rat) also induced transient RAR β gene expression, but the magnitude of induction by retinol was less than that by RA. Interestingly, when retinol was administered, the serum retinol level peaked 4 h later, and was thereafter maintained at 50% of the maximum level even after 48 h (Fig. 2b), without a correlation with the RAR β mRNA levels. Taken

Fig. 1. Vitamin A deficiency causes a marked decrease in RAR β mRNA levels

(a) Northern blot analysis. Vitamin A deficiency in rats was achieved by feeding them on the retinol-deficient diet as described in the Materials and methods section. C, Tissues from control rats; D, tissues from vitamin A-deficient rats. Northern blotting, using 5 μ g of poly(A)⁺ RNA (brain, kidney and heart) and 100 μ g of total RNA (lung, liver, spleen, testis and skin) was performed as described in the Materials and methods section. To correct for variations in the abundance of mRNAs between the two groups, the amounts of RNAs were normalized to the level of β -actin in each given tissue (this method was used in all experiments to normalize variation among groups). The exposure times of the autoradiographs were 1 week (tissues except brain) or 2 weeks (brain) for the RAR transcripts, and 2 days (tissues except liver) and 1 week (liver) for the actin transcript. In all experiments, tissues from at least four rats/group were used for Northern blot analysis. One typical sample is shown as a representative result in this figure. (b) Relative abundance of RAR mRNAs. The relative abundance of RAR mRNAs on Northern blot analysis was calculated using the values of densitometric scanning of all the RAR subtype-specific bands (two bands for RAR α and RAR β and one band for RAR γ). The relative values shown in the Figure were normalized by the abundance of rat actin transcript. The values are expressed as means $\pm$ S.D. of samples from at least four different rats.

together with the results of a deficiency study, these observations suggest that there is a threshold serum retinol level (approx. 25 μ g/dl) above which the RAR β transcript levels are constant. To investigate at which level the induction of RAR β by retinoids occurs, a specific inhibitor of transcription (0.15 mg of actinomycin D/rat) or translation (2 mg of cycloheximide/rat) was injected 1 h before administration of retinoid. Actinomycin D completely blocked the 4 h induction of the RAR β mRNAs by retinoids in lung and liver (Fig. 2c) and other tissues (results not

shown), whereas cycloheximide did not inhibit, but in fact slightly enhanced, the level of transcripts in the liver, probably through the stabilization of RAR β mRNA by inhibiting the synthesis of a short-lived RNAase [23]. In the lung the induction of the RAR β gene by retinol was completely inhibited by cycloheximide. Conversion of retinol into RA may require a newly synthesized enzyme. Significant changes in the levels of the RAR α and RAR γ transcripts were not observed in any of the replenishment studies.

Fig. 2. The level of RAR β transcripts is affected at the transcriptional level by retinoid status

(a) Relative abundance of RAR β transcripts. The left panel shows the time course of the RAR β mRNA decline in lung caused by vitamin A deficiency. In the right panel, the recovery of lung RAR β transcripts with time after administration of RA or retinol (ROL) is shown. (b) Concentration of serum retinol. The results in both panels correspond to those in (a). The concentration of serum retinol was measured by h.p.l.c. as described in the Materials and methods section. (c) Relative abundance of RAR β transcripts. The effects of inhibitors on the recovery of RAR β transcripts on retinoid administration were estimated using the transcriptional inhibitor actinomycin D (Act D; 0.15 mg/rat) or the translational inhibitor cycloheximide (CHX; 2 mg/rat). The drug, or control saline, was injected into the retinol-deficient rats, and after 1 h either RA (100 μ g/rat) or retinol (ROL; 100 μ g/rat) was administered intragastrically to the rats. After 4 h the rats were killed and total RNA from the tissues was isolated for analysis. The relative abundance of RAR β lung mRNA on Northern blots using 100 μ g of total RNA was calculated by densitometric scanning as described in Fig. 1. Values are means $\pm$ S.D. of at least four samples from different rats.

Fig. 3. An excess amount of retinoids causes the overexpression of the RAR β gene

Excess amounts of RA (1 mg/rat) or retinol (ROL; 1 mg/rat) were administered intragastrically to normal rats. (C, control) After 4 h the rats were killed and RNAs were isolated from various tissues for analysis. The relative abundance of RAR transcripts in various tissues on Northern blot analysis using 3 μ g of poly(A)⁺ RNA (brain) and 50 μ g of total RNA (lung, testis, liver and skin) was calculated as described in the legend to Fig. 1. Values are relative abundance of mRNAs and are expressed as means $\pm$ S.D. for at least four rats.

RA induces the overexpression of the RAR β gene

Since RA rapidly restored the level of RAR β mRNAs in retinol-deficient rats, we addressed the question of whether RA can enhance the level of RAR β transcripts in normal retinoid status by intragastric injection of 1 mg of RA or retinol to normal rats. Although an excess amount of RA did not significantly modulate the levels of the RAR α or RAR γ transcripts 4 h after administration, it clearly caused the overexpression of the RAR β gene in lung, liver, brain and skin (2–15-fold, as estimated by densitometric scanning of appropriately exposed autoradiographs) (Fig. 3). Interestingly, even retinol, which did not effectively restore the level of lung RAR β transcripts in retinol-deficient rats, significantly and rapidly up-regulated its level (about 2–3-fold) in normal rats. Thus the excess amount of retinoid caused the enhancement of RAR β mRNA levels in tissues in which the RAR β gene is expressed. However, in some specific tissues, such as testis and intestine, where the RAR β gene is not expressed, even an excess amount of retinoid had no effect. In such tissues the RARE upstream of RAR β gene [24] may be strictly suppressed in a tissue-specific manner, in spite of the presence of RAR α , RAR γ or both.

RAR gene expression is not affected by vitamin D or thyroid hormone

The effect of vitamin D and thyroid hormone on the levels of RAR transcripts was investigated in various tissues (only the results in skin for vitamin D and in lung for thyroid hormone are shown). Vitamin D deficiency was achieved by feeding vitamin D-deficient diet, and propylthiouracil was used to induce goitres. Replenishment experiments were performed in intraperitoneal injection of cognate ligands. As shown in Fig. 4, neither vitamin

Fig. 4. Vitamin D and thyroid hormone do not affect the levels of RAR transcripts

(a) Effect of vitamin D. Rats were depleted of vitamin D by feeding them on a vitamin D-deficient diet. In the replenishment experiment, Northern blot analysis of RAR transcripts using 50 μ g of total RNA was performed using tissues from vitamin D deficient-rats given 0.2 μ g of 1,25(OH) $_2$ D $_3$ (D $_3$) 5 h before killing. Only the result from skin is shown. The relative abundance of RAR transcripts was estimated as described in the legend to Fig. 1. C, control rats; D, vitamin D-deficient rats; D $_3$, vitamin D-replenished rats. (b) Effect of thyroid hormone. The goitrous thyroids were produced by 6-propyl-2-thiouracil (PTU) and judged by their weight. Thyroid hormone (T $_3$) (100 μ g/rat) was then given for replenishment, and 18 h later the RAR transcripts were analysed by Northern blotting using 50 μ g of total RNA, and calculated as the relative abundance of RAR transcripts. Only the result in the lung is shown. C, control rats; PTU, PTU-treated rats; T $_3$, thyroid hormone-replenished rats.

D nor thyroid hormone had any effect on the levels of RAR transcripts in skin (vitamin D) or lung (thyroid hormone), or indeed in other tissues (results not shown).

DISCUSSION

As it has been reported that nutritional retinoid status affects the level of cellular retinoid-binding proteins in adult animals [28–30], it is important for a better understanding of retinoid action to determine the role of the specific retinoids in the regulation of RAR gene expression in intact animals. Though the ligand regulation of RAR β gene has already been demonstrated *in vitro* and *in vivo* [22,23,31–33], it was not clear whether such autoregulation of RAR β gene expression occurs in intact animals and, if so, in all tissues. Therefore this study was designed to investigate how nutritional retinoid status affects the levels of the RAR α , RAR β and RAR γ mRNAs.

Retinol depletion, achieved by feeding rats on a retinol-deficient diet, caused a significant decrease in RAR β mRNA levels. Such a decrease in RAR β mRNA was quickly restored at the transcriptional level by administration of retinoid, though the magnitude and kinetics of RAR β gene induction differed depending on whether retinol or RA was administered. From monitoring of the serum retinol levels, it is likely that a threshold serum retinol concentration is required to maintain the normal

level of RAR β transcripts. Moreover, we have shown that administration of retinoid to normal (non-deficient) rats resulted in the overexpression of the RAR β gene. Such ligand regulation was found in all tissues in which the RAR β gene is expressed at normal retinoid status. Thus it is clear from the present study that RAR β gene expression depends on retinoid ligand status in intact rats. These results may account in part for the toxicity of excess amounts of RA in animals [44–47], as the enhanced levels of RAR β may perturb normal retinoid signalling.

Umesono *et al.* [27] have suggested, from the analysis of a number of naturally occurring sequences, that the direct repeated AGGTCA motifs with different spacing may be a new type of consensus ligand response element for RA, vitamin D and thyroid hormone. The spacing between two AGGTCA motifs was proposed to be crucial for achieving target specificity among these three nuclear receptors. However, in some of the naturally occurring elements composed of tandemly repeated motifs the binding specificity of the nuclear receptors to the motifs seems to be permissive [27]. Since the rat RARE β has not yet been identified or tested for its binding specificity for nuclear receptors, it is possible that the TR and VDR might modulate the activity of rat RARE β transactivated by RAR. More recently, RXR has been shown to function as an auxiliary protein for TR, VDR and RAR, also indicating a close relationship among these three receptors with regard to their signal transduction [16,48–50]. Therefore we tested whether vitamin D and thyroid hormone could affect the gene expression of RAR β , as well as of RAR α and RAR γ . As shown in Fig. 4, however, it is clear that vitamin D and thyroid hormone have no effect on the levels of RAR β , RAR α or RAR γ mRNAs. It is most likely that the rat RARE β is identical or closely related to the human and mouse RARE β s, and specifically serves only as a RARE without the binding of TR and VDR to this sequence.

Recently, each subtype of the RAR gene has been shown to transcribe a group of slightly different mRNAs, designated as RAR isoforms [40–42]. Analysis of gene structure suggested that the gene expression of the isoforms is controlled by different promoters. Therefore it is possible that the gene expression of some RAR α and RAR γ isoforms may be affected by retinoids and other ligands in a tissue-specific way. In fact, a RARE activity in embryonic carcinoma cells has been identified in the promoter of one RAR α isoform, RAR α 2 [43], although we could not detect a significant change in the RAR α mRNA level caused by retinoid status in intact animals. The discrepancy between the previous observation [43] and our results might be due to the properties of this RARE, which may act as an enhancer only at embryogenesis. However, it is most likely that the sensitivity of our system is too low to detect a change in one specific RAR isoform transcript such as RAR α 2, since the probe used in the present study hybridizes with all isoforms of a given RAR subtype. In order to obtain a comprehensive understanding of retinoid transduction, it will be of interest to determine how the gene expression of each RAR isoform, as well as of RXR, is regulated in a tissue-specific way by retinoids and the other factors in intact animals.

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REFERENCES

1. Dowling, J. E. & Wald, G. (1960) *Proc. Natl. Acad. Sci. U.S.A.* **46**, 587–608

2. Frolik, C. A. (1984) in *The Retinoids: Metabolism of Retinoids* (Sporn, M. B., ed.), vol. 2, pp. 177–208, Academic Press, New York
3. Wolf, G. (1984) *Physiol. Rev.* **64**, 873–937
4. Maden, M. (1985) *Trends Genet.* **1**, 103–107
5. Eichele, G. (1989) *Trends Genet.* **5**, 246–251
6. Ruberte, E., Dollé, P., Krust, A., Zelent, A., Morris-Kay, G. & Chambon, P. (1990) *Development* **108**, 203–213
7. Petkovich, M., Brand, N., Krust, A. & Chambon, P. (1987) *Nature (London)* **330**, 444–450
8. Giguère, V., Ong, E. S., Segui, P. & Evans, R. M. (1987) *Nature (London)* **330**, 624–629
9. Brand, N., Petkovich, M., Krust, A., Chambon, P., de Thé, H., Marchio, A., Tiollais, P. & Dejean, A. (1988) *Nature (London)* **332**, 850–853
10. Benbrook, D., Lernhardt, E. & Pfahl, M. (1988) *Nature (London)* **333**, 669–672
11. Zelent, A., Krust, A., Petkovich, M., Kastner, Ph. & Chambon, P. (1989) *Nature (London)* **339**, 714–717
12. Krust, A., Kastner, Ph., Petkovich, M., Zelent, A. & Chambon, P. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5310–5314
13. Green, S. & Chambon, P. (1988) *Trends Genet.* **4**, 309–314
14. Evans, R. M. (1988) *Science* **240**, 889–895
15. Mangelsdorf, D. J., Ong, E. S., Dyck, J. A. & Evans, R. M. (1990) *Nature (London)* **345**, 224–229
16. Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J.-Y., Staud, A., Garnier, J.-M., Mader, S. & Chambon, P. (1992) *Cell* **68**, 377–395
17. Levin, A. A., Sturzenbecker, L. J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeisen, C., Rosenberger, M., Lovey, A. & Grippo, J. F. (1992) *Nature (London)* **335**, 359–361
18. Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M. & Thaller, C. (1992) *Cell* **68**, 397–406
19. Dollé, P., Roberte, E., Kastner, Ph., Petkovich, M., Stoner, C. M., Gudas, L. J. & Chambon, P. (1989) *Nature (London)* **342**, 702–705
20. Dollé, P., Roberte, E., Leroy, P., Morris-Kay, G. & Chambon, P. (1989) *Development* **110**, 1133–1151
21. Rees, J. L., Daly, A. K. & Redfern, C. P. F. (1989) *Biochem. J.* **259**, 917–919
22. de Thé, H., Marchio, A., Tiollais, P. & Dejean, A. (1989) *EMBO J.* **8**, 429–433
23. Martin, C., Ziegler, L. M. & Napoli, J. L. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4804–4808
24. de Thé, H., del Mar Vivanco-Ruiz, M., Tiollais, P., Stunnenberg, H. & Dejean, A. (1990) *Nature (London)* **343**, 177–180
25. Hoffmann, B., Lehmann, J. M., Zhang, X.-K., Hermann, T., Husmann, M., Graupner, G. & Pfahl, M. (1990) *Mol. Endocrinol.* **4**, 1727–1736
26. Sucov, H. M., Murakami, K. K. & Evans, R. M. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5392–5396
27. Umesono, K., Murakami, K. K., Thompson, C. C. & Evans, R. M. (1991) *Cell* **65**, 1255–1266
28. Kato, M., Blaner, W. S., Mertz, J. R., Das, K., Kato, K. & Goodman, D. S. (1985) *J. Biol. Chem.* **260**, 4832–4838
29. Sherman, D. R., Lloyd, R. S. & Chytil, F. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3209–3213
30. Blaner, W. S., Das, K., Mertz, J. R., Das, S. R. & Goodman, D. S. (1986) *J. Lipid. Res.* **27**, 1084–1088
31. Rowe, A., Richman, J. M. & Bricknell, P. M. (1991) *Development* **111**, 1007–1016
32. Rossant, J., Zirngible, R., Cado, D., Shago, M. & Giguere, V. (1991) *Genes Dev.* **5**, 1333–1344
33. Riaz-ul-Hag, Pfahl, M. & Chytil, F. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8272–8276
34. Lamb, A. J., Apiwatanaporn, P. & Olson, J. (1974) *J. Nutr.* **104**, 1140–1148
35. Abe, K., Ishibashi, K., Ohmae, M., Kawabe, K. & Katsui, G. (1977) *Vitamins (Japan)* **51**, 275–280
36. Sandgren, M. E. & DeLuca, H. F. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4312–4314
37. Habbab, O., Bhalla, R. C. & Halmi, N. S. (1979) *Biochim. Biophys. Acta* **571**, 233–243
38. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159
39. Spiegelman, B. M. & Farmer, S. R. (1982) *Cell* **29**, 53–60
40. Kastner, P., Krust, A., Mendelsohn, C., Garnier, J. M., Zelent, A., Leroy, P., Staub, A. & Chambon, P. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2700–2704
41. Leroy, P., Krust, A., Zelent, A., Mendelsohn, C., Garnier, J. M., Kastner, P., Dierich, A. & Chambon, P. (1991) *EMBO J.* **10**, 59–69
42. Zelent, A., Mendelsohn, C., Kastner, P., Krust, A., Garnier, J.-M., Ruffenach, F., Leroy, P. & Chambon, P. (1991) *EMBO J.* **10**, 71–81
43. Leroy, P., Nakshatri, H. & Chambon, P. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10138–10142
44. Morriss, G. M. (1972) *J. Anat.* **113**, 241–250
45. Sulik, K. K. & Dehart, D. B. (1988) *Teratology* **37**, 527–537
46. Satre, M. A. & Kochhar, D. M. (1989) *Dev. Biol.* **133**, 529–536
47. Asselineau, D., Bernard, B. A., Bailly, C. & Darmon, M. (1989) *Dev. Biol.* **133**, 322–335
48. Yu, V. C., Delsert, C., Andersen, B., Holloway, J. M., Devary, O., Nàar, A. M., Kim, S. Y., Boutin, J. M., Glass, C. K. & Rosenfeld, M. G. (1991) *Cell* **67**, 1251–1266
49. Zhang, X.-K., Hoffmann, B., Tran, P. B.-V., Graupner, G. & Pfahl, M. (1992) *Nature (London)* **355**, 441–446
50. Klier, S. A., Umesono, K., Mangelsdorf, D. J. & Evans, R. M. (1992) *Nature (London)* **335**, 446–449