DEVELOPMENTAL DYNAMICS 208:447–453 (1997)

Class IV Alcohol/Retinol Dehydrogenase Localization in Epidermal Basal Layer: Potential Site of Retinoic Acid Synthesis During Skin Development

ROBERT J. HASELBECK, HWEE LUAN ANG, AND GREGG DUESTER*
The Burnham Institute, La Jolla, California 92037

ABSTRACT **Vitamin A (retinol) plays a signaling role in the development of skin and other epithelial tissues. This is accomplished by a two-step metabolic pathway in which the rate-limiting step is oxidation of retinol to retinal, followed by oxidation of retinal to retinoic acid, which serves as the active ligand to activate nuclear retinoic acid receptors. Previous studies in mouse skin have shown that retinol oxidation is catalyzed by a cytosolic retinol dehydrogenase that may be a member of the alcohol dehydrogenase (ADH) enzyme family. Analysis of the ADH family has shown that class IV ADH is the most efficient isozyme for retinol oxidation but that other isozymes can catalyze this reaction. Here we have examined mouse skin for the expression of genes encoding class I ADH and class IV ADH, the only ADH isozymes in this species able to function as retinol dehydrogenases in vitro. In situ hybridization analysis of mouse skin revealed that class I ADH mRNA was absent, whereas class IV ADH mRNA was abundant and localized in the epidermal basal layer, providing evidence that the skin retinol dehydrogenase previously identified was class IV ADH. Immunohistochemical studies indicated that class I ADH protein was absent in the mouse skin, but class IV ADH protein was detected primarily in the basal layer of the epidermis, with less detection in the spinous layer and no detection in the cornified layer. This apparent down-regulation of class IV ADH expression during keratinocyte terminal differentiation provides evidence that the basal layer of the epidermis may be the primary site of local retinoic acid synthesis needed for retinoid signaling in the skin.** ***Dev. Dyn. 208:447–453, 1997.***

Key words: alcohol dehydrogenase; retinol dehydrogenase; retinoic acid; epidermis; skin; stratum basale; keratinocyte terminal differentiation

INTRODUCTION

Vitamin A deficiency is known to cause defects in many epithelia throughout the body (Wolbach and Howe, 1925; Wilson et al., 1953), including skin defects characterized by hyperkeratinization of the epidermis (Frazier and Hu, 1931; Klein-Szanto et al., 1980). The vitamin A metabolite retinoic acid (RA) has been proposed to regulate the level of keratinization during terminal differentiation of epidermal keratinocytes as they migrate from the basal to cornified layers (reviewed by Darmon, 1991). Administration of RA to vitamin A-deficient animals restores epithelia to their normal state of keratinization, indicating that RA is the active form of vitamin A needed for maintenance of epithelia (Dowling and Wald, 1960). Recent studies have shown that RA serves as a ligand for nuclear retinoic acid receptors (RAR), which are capable of regulating gene transcription (Kastner et al., 1994; Mangelsdorf et al., 1994). RARs have been localized in the epidermis (Elder et al., 1991; Tavakkol et al., 1992) and are able to mediate the retinoid response in cultured keratinocytes (Xiao et al., 1995). Transgenic mice expressing a dominant-negative mutant form of RAR in the epidermis suffer from an inhibition of skin development during late embryogenesis, providing further evidence for the importance of RA signaling in the skin (Saitou et al., 1995; Imakado et al., 1995). Although much progress has been made identifying the receptors involved in skin retinoid signaling, relatively little is known about the enzymatic pathway involved in producing the RA ligand or its spatiotemporal regulation.

RA synthesis occurs by two sequential oxidation steps in which retinol is first oxidized to retinal (the rate-limiting step), followed by oxidation of retinal to retinoic acid (Leo et al., 1989; Napoli, 1986; Kim et al., 1992). Several lines of evidence show that epidermis contains the cellular machinery necessary for endogenous RA production from retinol provided from the blood stream. Keratinocytes have been reported to possess a serum retinol-binding protein receptor that facilitates retinol uptake (Båvik et al., 1995). Epidermis has been found to express cellular retinol-binding protein, which might facilitate the uptake of plasma retinol destined to be converted to either retinyl esters for storage or RA for retinoid signaling (Fisher et al., 1995). Biological activation of retinol has been shown to require its oxidation to RA in both mouse epidermis

Contract Grant sponsor: National Institutes of Health; Contract grant numbers: AA07261 and AA09731.
*Correspondence to: Dr. Gregg Duester, The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037.
Received 15 November 1996; Accepted 18 December 1996

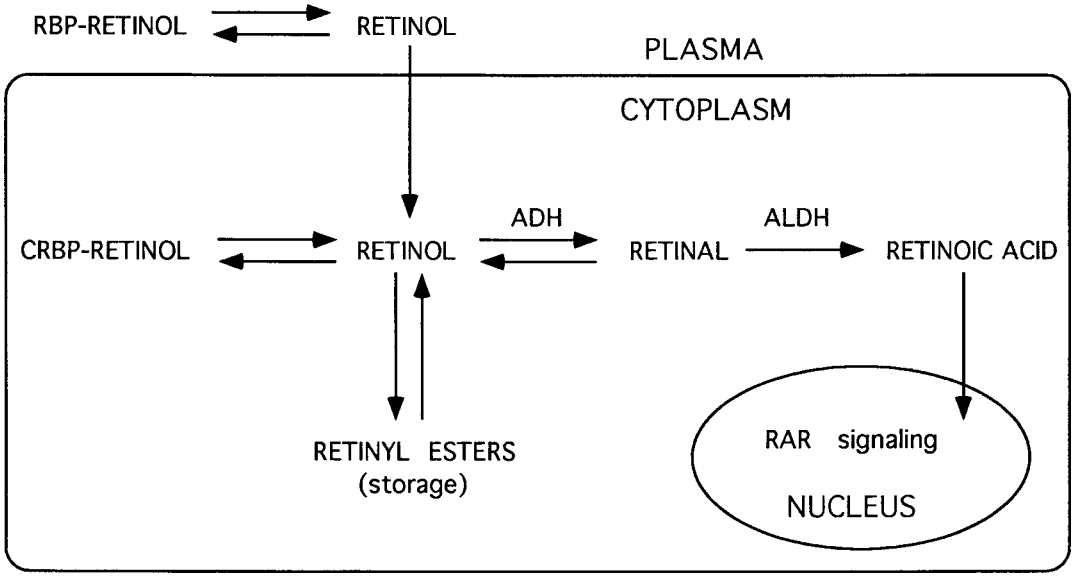

Fig. 1. Proposed retinoic acid synthetic pathway in skin. Retinol is transported in serum bound to retinol-binding protein (RBP) and can be taken up by keratinocytes and bind to cellular retinol-binding protein (CRBP), which may facilitate uptake. Retinol can be converted to retinyl esters for storage or converted to retinoic acid. Synthesis of retinoic acid involves first the oxidation of retinol to retinal by certain isozymes of alcohol dehydrogenase (ADH), followed by the oxidation of retinal to retinoic acid by specific forms of aldehyde dehydrogenase (ALDH). Retinoic acid binds to a nuclear retinoic acid receptor (RAR), which mediates retinoid signaling by directly regulating transcription of key target genes.

(Connor, 1988) and human keratinocytes (Kurlandsky et al., 1994). This metabolic pathway is summarized in Figure 1.

Recent studies suggest that RA is produced locally in the epidermis from retinol and retinal. In mouse epidermis, endogenous RA is below the limit of detection by HPLC analysis; however, application of retinol to mouse skin leads to concomitant detection of RA in epidermis, suggesting that the synthetic pathway is functional in this tissue (Connor and Smit, 1987a). Topical application of retinol to human skin stimulates the same biological effects as topical RA treatment, probably by local metabolism to RA (Kang et al., 1995). Similarly, treatment of human skin with retinal leads to RA biological activity, further suggesting local conversion to RA (Saurat et al., 1994). Measurement of retinoids in cultured human keratinocytes has shown that RA can be detected in these cells (Siegenthaler et al., 1990; Randolph and Simon, 1993).

The retinol dehydrogenase catalyzing retinol oxidation in mouse epidermis has been partially purified and shown to be a cytosolic enzyme having properties of an alcohol dehydrogenase (ADH) isozyme (Connor and Smit, 1987b), although the specific nature of this isozyme remains unknown. The mouse epidermis was also found to have a cytosolic retinal-oxidizing activity (retinal dehydrogenase) with the properties of an aldehyde dehydrogenase isozyme (Connor and Smit, 1987b). Similar studies with human keratinocytes have detected a cytosolic retinol dehydrogenase that is distinct from ethanol-active class I ADH (Siegenthaler et al., 1990). Human keratinocytes also possess a microsomal retinol dehydrogenase activity (Kurlandsky et al., 1996), which may be similar or identical to rat liver microsomal retinol dehydrogenase, which was recently found to be a member of the short-chain dehydrogenase/reductase enzyme family (Chai et al., 1995). However, the enzymatic properties of this enzyme suggest that it may function in the reverse direction as a retinal reductase to convert retinal to retinol for retinoid storage (Duester, 1996).

Enzyme purification and gene cloning have led to the identification of a human ADH gene family consisting of five distinct classes (Jörnvall et al., 1995; Duester et al., 1995). The mouse has conserved only three of these forms, i.e., class I ADH, class III ADH, and class IV ADH (Zgombic-Knight et al., 1995). Enzymatic studies have shown that liver class I ADH is most efficient for ethanol oxidation but is weakly effective with retinol (Boleda et al., 1993; Yang et al., 1994). Liver class III ADH does not function in retinol or ethanol oxidation but instead acts as a glutathione-dependent formaldehyde dehydrogenase (Koivusalo et al., 1989; Holmquist and Vallee, 1991). Class IV ADH catalyzes the oxidation of ethanol very inefficiently and is not present in liver; however, this isozyme isolated from stomach mucosa has a catalytic efficiency for retinol that is approximately 100-fold higher than that of either liver class I ADH (Yang et al., 1994; Kedishvili et al., 1995) or liver microsomal retinol dehydrogenase (Duester, 1996). The spatiotemporal expression pattern of class IV ADH in the mouse correlates with sites of retinoic acid synthesis during embryogenesis (Ang et al., 1996a), spermatogenesis (Deltour et al., 1997), and epithelial differen-

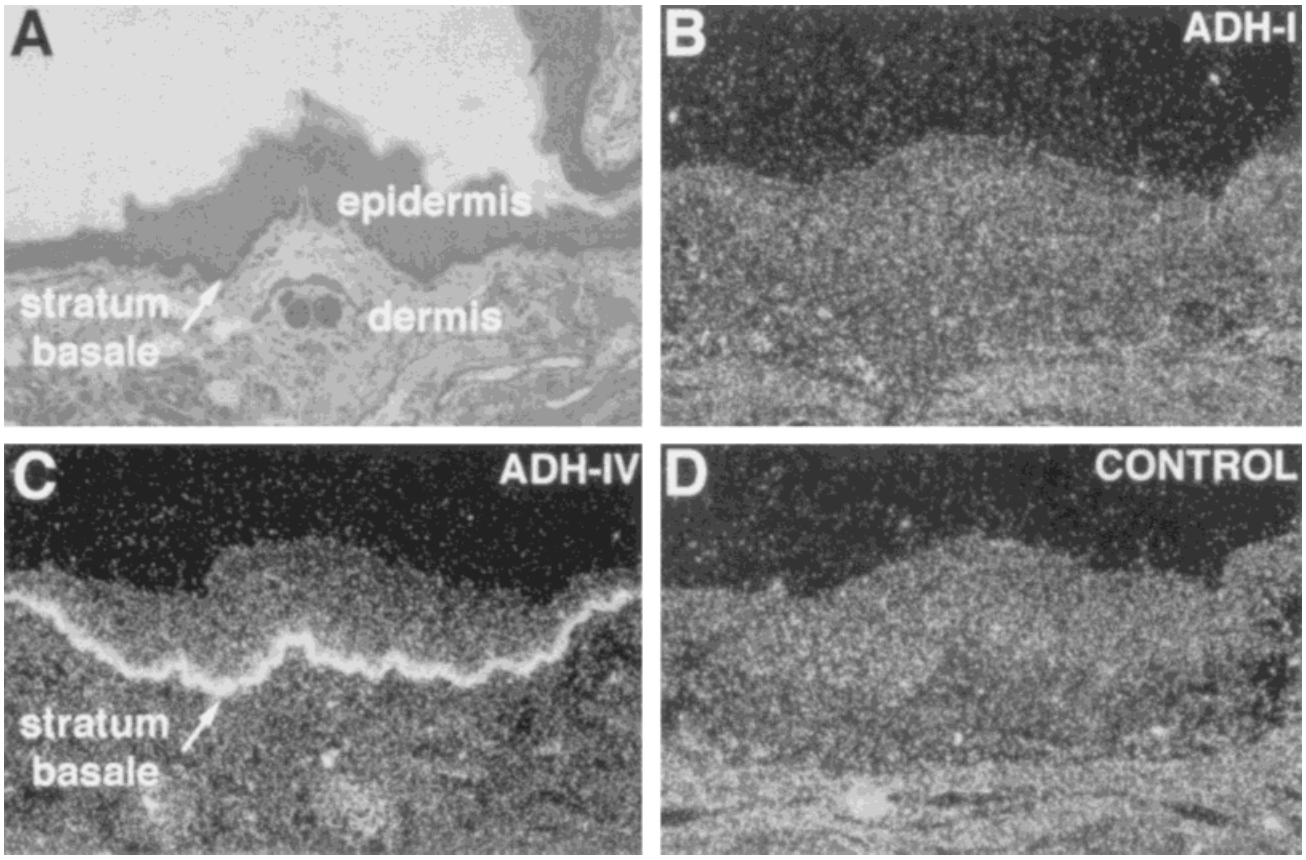

Fig. 2. In situ hybridization analysis of class IV ADH mRNA in mouse skin. **A:** Brightfield view of mouse skin section stained with hematoxylin-eosin. **B:** Darkfield view of mouse skin section following in situ hybridization to a class I ADH riboprobe showing no specific detection. **C:** Hybridization to a class IV ADH riboprobe showing a highly specific signal along the stratum basale in mouse epidermis. **D:** Hybridization to a sense control riboprobe showing no specific detection. ×100.

tiation (Ang et al., 1996b). Thus, it is becoming increasingly clear that class IV ADH may function as the physiological retinol dehydrogenase for many tissues.

In this study we have examined the expression of ADH in mouse skin. Using murine cDNAs encoding class I ADH (Edenberg et al., 1985; Ceci et al., 1986) and class IV ADH (Zgombic-Knight et al., 1995), we have performed in situ hybridization to identify cell types exhibiting ADH mRNA expression. In addition, we have conducted immunohistochemical studies using specific antisera for class I ADH and class IV ADH to identify sites of ADH protein localization in mouse skin. These results provide insight into the mechanism governing spatiotemporal regulation of RA synthesis during epidermal differentiation.

RESULTS

Expression of Class I ADH and Class IV ADH Genes in Mouse Skin

Sections of adult mouse skin were examined by in situ hybridization to detect expression of the genes encoding class I ADH or class IV ADH. A stained section of adult mouse skin indicates dermal and epidermal morphology as well as the location of the stratum basale, the basal layer of epidermal cells lying at the basement membrane adjacent to the dermis (Fig. 2A). Hybridization with an antisense riboprobe for class I ADH indicated no specific signal in the skin (Fig. 2B) compared to a control sense riboprobe (Fig. 2D); the integrity of the class I ADH antisense riboprobe was verified by its ability to detect a specific class I ADH mRNA signal in sections of testis (data not shown). On the other hand, an antisense riboprobe for class IV ADH indicated a distinct zone of detection along the basal layer of the epidermis (Fig. 2C). In comparison to the control, there appeared to be no class IV ADH mRNA detection above background in the outer layers of the epidermis (spinous or cornified layers). Because of the low numbers of cells in the dermis (primarily dermal fibroblasts embedded in extracellular matrix), we could not conclude from these studies whether class I or class IV ADHs were expressed in this tissue.

Immunohistochemical Analysis of Class I ADH and Class IV ADH in Skin

Paraffin sections of adult mouse skin were subjected to immunohistochemical staining using affinity-puri-

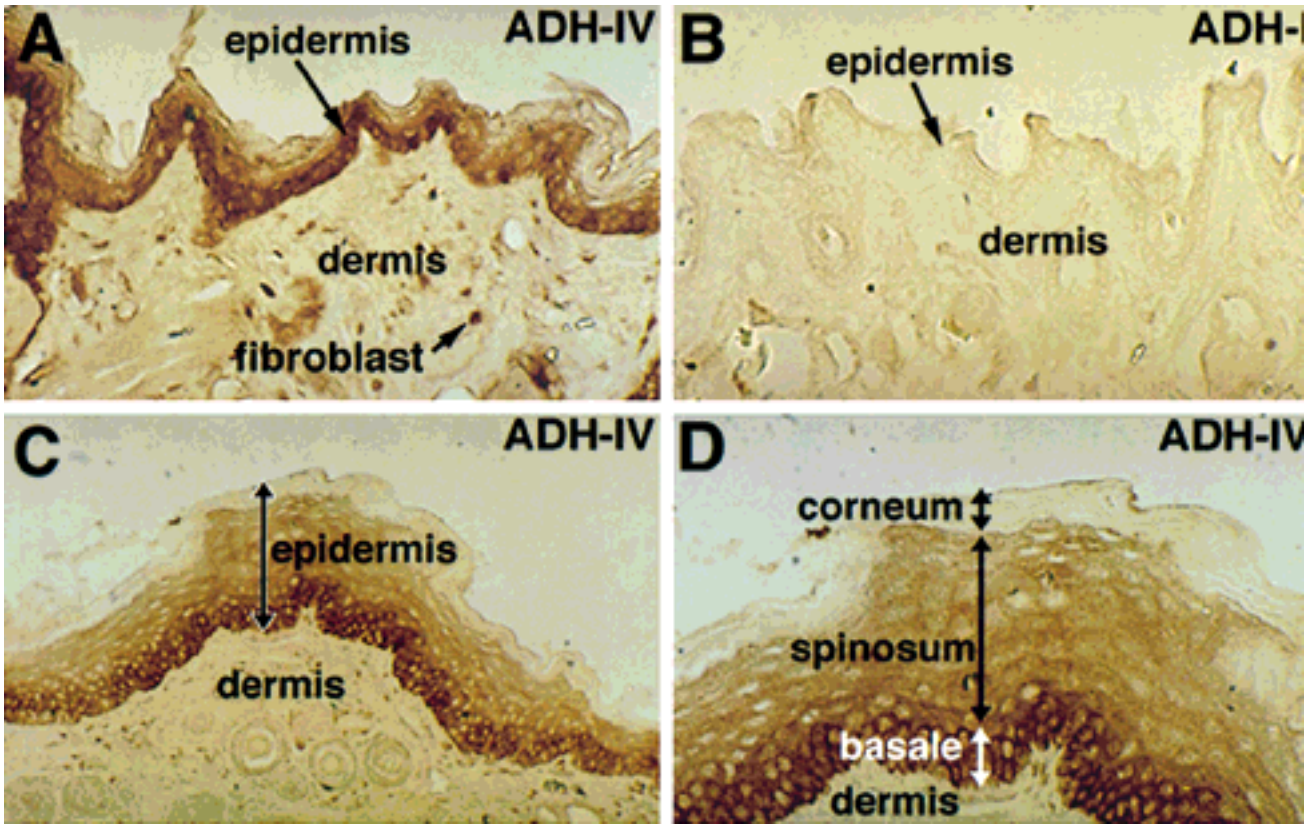

Fig. 3. Immunohistochemical localization of class IV ADH in mouse and human skin. **A:** Immunostain of a section of mouse skin using affinity-purified class IV ADH polyclonal antibodies showing specific protein localization in the epidermis and dermal fibroblasts. **B:** Immunostain of a nearby section using class I ADH affinity-purified polyclonal antibodies indicating an absence of protein detection in the entire skin layer. **C:** Tangential section showing class IV ADH immunodetection across the epidermis. **D:** Higher magnification view of the previous section showing class IV ADH immunodetection in gradient across the epidermis, with highest level of detection in the stratum basale. ×200 (A,B,D). ×100 (C).

fied polyclonal antibodies specific for either class I ADH or class IV ADH. In a transverse section of skin, a high level of immunodetection for class IV ADH protein was observed in the epidermis, with detection also being observed in dermal fibroblasts (Fig. 3A). In contrast, there was no class I ADH immunodetection in either the epidermis or the dermis, indicating an absence of this enzyme in the skin (Fig. 3B). The integrity of the class I ADH antibody was verified by its ability to detect class I ADH in sections of mouse testis (data not shown). Control experiments, in which either the primary antibody was omitted or preimmune serum was used, showed essentially the same result as was seen with the class I ADH antibody (data not shown).

Preferential localization of class IV ADH along the basal layer of the epidermis was more easily observed by immunohistochemical analysis of tangential sections showing a broader layer of epidermal cells (Fig. 3C). A high-magnification view of a tangential section indicates that class IV ADH immunodetection was localized primarily in the basal layer, with much less detected in the spinous layer and essentially none detected in the cornified layer (Fig. 3D).

DISCUSSION

Class I and class IV ADHs function as retinol dehydrogenases in vitro (Connor and Smit, 1987b; Boleda et al., 1993; Yang et al., 1994) and thus may participate in RA synthesis in tissues where they are present. In this study we have sought to determine the presence and specific location of ADH isozymes in mouse epidermis. Our findings provide firm evidence that class IV ADH is abundantly expressed in the adult mouse skin. Both mRNA and protein for class IV ADH were observed in the epidermis. Class IV ADH mRNA in the epidermis was restricted to only the basal layer, whereas protein localization was highest in the basal layer, with less detection in the spinous layer and no detection in the cornified layer. These results indicate that class IV ADH mRNA and protein expression are down-regulated as keratinocytes in the basal layer differentiate and migrate into the spinous and cornified layers. In contrast, neither mRNA nor protein for class I ADH was detected in any skin cell types.

Retinol is locally converted to RA in both mouse (Connor, 1988) and human (Kurlandsky et al., 1994)

epidermis. Localization of class IV ADH in mouse epidermis provides strong evidence that this enzyme is the retinol dehydrogenase participating in RA synthesis. The function of RA in skin development has been proposed to involve an RAR-mediated signaling event controlling keratinocyte terminal differentiation (Darmon, 1991; Fisher and Voorhees, 1996). Expression of RARγ1 mRNA in skin has been found to be uniformly distributed across the epidermis (Tavakkol et al., 1992), suggesting that all layers of the epidermis may be capable of responding to an RA signal. However, the preferential localization of class IV ADH in the epidermal basal layer suggests that RA may be synthesized at a higher rate in this layer relative to the spinous and cornified layers. Thus, the primary role of RA may be to control the differentiation of basal stem cells into suprabasal keratinocytes, such that, subsequent to that event, RA and retinoid signaling is down-regulated as keratinocytes migrate to the surface of the skin. This hypothesis is supported by studies indicating that an excess supply of retinol or RA administered to skin leads to a proliferation of suprabasal cells in the epidermis (Kang et al., 1995) and that a reduction in the retinoid supply during vitamin A deficiency leads to hyperkeratinization, an increase in the size of the cornified layer (Frazier and Hu, 1931; Klein-Szanto et al., 1980).

Recent examination of the entire mouse ADH gene family determined that the mouse possesses only three ADH isoforms, i.e., class I ADH, class III ADH, and class IV ADH (Zgombic-Knight et al., 1995), formerly named ADH-A_2, ADH-B_2, and ADH-C_2, respectively (Holmes, 1978). Previous studies on the enzymology of RA synthesis in mouse skin led to partial purification of a cytosolic retinol dehydrogenase present in epidermis, the kinetic properties of which were found to be similar to ADH-C_2 (Connor and Smit, 1987b). Thus, it is now clear from the studies reported here that the retinol dehydrogenase activity previously identified in mouse epidermis is ADH-C_2, now known as class IV ADH.

Cultured human keratinocytes possess a cytosolic retinol dehydrogenase activity, which was reported to be distinct from ADH insofar as it was not inhibited by ethanol or 4-methylpyrazole (Siegenthaler et al., 1990). In fact, these inhibitors are effective against liver class I ADH (Mezey and Holt, 1971; Vallee and Bazzone, 1983), making it possible to rule out a role for this class of ADH as a keratinocyte retinol dehydrogenase. However, since those findings were reported, there has been a tremendous increase in our knowledge of the ADH family, including discovery of class IV ADH, which is not very sensitive to inhibition by these inhibitors (Parés et al., 1990; Moreno and Parés, 1991). Thus, the cytosolic retinol dehydrogenase present in human keratinocytes may be class IV ADH, the form we have identified in mouse epidermis.

Class IV ADH may play a widespread role in RA synthesis. Expression of class IV ADH in adult tissues is localized in several epithelial cell populations that may require RA for differentiation, including epidermis, stomach and esophageal mucosa, adrenal cortex, seminiferous tubule, and epididymal basal cells (Ang et al., 1996b; Deltour et al., 1997). Expression of class IV ADH in mouse embryos during gastrulation and neurulation coincides spatially and temporally with sites of RA detection, suggesting that this ADH isozyme may play a role in the initiation of RA synthesis during embryogenesis (Ang et al., 1996a; Deltour et al., 1996). Genetic studies with the mouse can be used to examine further the physiological role of class IV ADH in the epidermis as well as in other retinoid target tissues.

EXPERIMENTAL PROCEDURES

Tissue Sectioning

Skin samples from adult female mice (strain FVB/N) were fixed in Bouin's reagent, embedded in paraffin, and sectioned at 6–10 μm using standard histological procedures. Paraffin sections were used for both in situ hybridization and immunohistochemical studies.

In Situ Hybridization

Tissues were processed for in situ hybridization under high-stringency conditions as described previously (Wilkinson and Nieto, 1993). The hybridization probes consisted of ^{35}S-labelled antisense RNAs derived from the full-length cDNAs for mouse class I ADH (Edenberg et al., 1985; Ceci et al., 1986) and class IV ADH (Zgombic-Knight et al., 1995) subcloned in pBluescript II KS (Stratagene Cloning Systems, Inc., La Jolla, CA). Antisense RNAs were synthesized using [α-^{35}S]UTP and T3 or T7 RNA polymerase as described elsewhere (Wilkinson and Nieto, 1993). We have previously shown that there is no cross-hybridization observed between the ADH classes under high-stringency conditions in either Northern or Southern blots, likely resulting from the low ADH interclass sequence identity, which is in the 60% range (Zgombic-Knight et al., 1995). As a control to monitor background detection, we used a sense RNA probe transcribed from the plasmid containing the mouse class I ADH cDNA. For a positive control, we analyzed sections of mouse testis, which has previously been shown to contain mRNAs for both class I ADH and class IV ADH (Ang et al., 1996b; Deltour et al., 1996b). Slides were exposed to emulsion for 2–3 weeks prior to development. Staining of hybridized sections was performed with neutral red, and adjacent nonhybridized sections were stained with hematoxylin and eosin as described previously to observe tissue morphology (Kaufman, 1992). Hybridization results were observed by darkfield microscopy, and stained adjacent sections were observed by brightfield microscopy.

Immunohistochemistry

Antibodies used in this study were affinity purified from rabbit polyclonal antisera raised against mouse class I ADH and class IV ADH expressed as glutathione-

S-transferase fusion proteins in *E. coli* as previously described (Deltour et al., 1997). Tissue sections were subjected to immunohistochemical detection using the Vectastain avidin-biotin-horseradish peroxidase ABC kit (Vector Laboratories, Inc., Burlingame, CA). Diaminobenzidine was used for color detection, and slides were not counterstained prior to mounting and observation by brightfield microscopy. Control experiments included those in which the primary antibody was not used as well as serial dilutions of each specific antibody and preimmune sera as previously described (Deltour et al., 1997). Sections of mouse testis previously shown to contain immunoreactive proteins for both class I ADH and class IV ADH (Deltour et al., 1997) were used as positive controls.

ACKNOWLEDGMENTS

We thank M. Felder for the mouse class I ADH cDNA. This study was supported by NIH grants AA07261 and AA09731.

REFERENCES

Ang, H.L., Deltour, L., Hayamizu, T.F., Zgombic-Knight, M., and Duester, G. (1996a) Retinoic acid synthesis in mouse embryos during gastrulation and craniofacial development linked to class IV alcohol dehydrogenase gene expression. J. Biol. Chem. 271:9526–9534.

Ang, H.L., Deltour, L., Zgombic-Knight, M., Wagner, M.A., and Duester, G. (1996b) Expression patterns of class I and class IV alcohol dehydrogenase genes in developing epithelia suggest a role for alcohol dehydrogenase in local retinoic acid synthesis. Alcohol Clin. Exp. Res. 20:1050–1064.

Båvik, C.O., Peterson, P.A., and Eriksson, U. (1995) Retinol-binding protein mediates uptake of retinol to cultured human keratinocytes. Exp. Cell Res. 216:358–362.

Boleda, M.D., Saubi, N., Farrés, J., and Parés, X. (1993) Physiological substrates for rat alcohol dehydrogenase classes: Aldehydes of lipid peroxidation, omega-hydroxyfatty acids, and retinoids. Arch. Biochem. Biophys. 307:85–90.

Ceci, J.D., Lawther, R., Duester, G., Hatfield, G.W., Smith, M., O'Malley, M.P., and Felder, M.R. (1986) Androgen induction of alcohol dehydrogenase in mouse kidney: Studies with a cDNA probe confirmed by nucleotide sequence analysis. Gene 41:217–224.

Chai, X., Boerman, M.H.E.M., Zhai, Y., and Napoli, J.L. (1995) Cloning of a cDNA for liver microsomal retinol dehydrogenase. A tissue-specific, short-chain alcohol dehydrogenase. J. Biol. Chem. 270:3900–3904.

Connor, M.J. (1988) Oxidation of retinol to retinoic acid as a requirement for biological activity in mouse epidermis. Cancer Res. 48:7038–7040.

Connor, M.J., and Smit, M.H. (1987a) The formation of all-trans-retinoic acid from all-trans-retinol in hairless mouse skin. Biochem. Pharmacol. 36:919–924.

Connor, M.J., and Smit, M.H. (1987b) Terminal-group oxidation of retinol by mouse epidermis: Inhibition in vitro and in vivo. Biochem. J. 244:489–492.

Darmon, M. (1991) Retinoic acid in skin and epithelia. Semin. Dev. Biol. 2:219–228.

Deltour, L., Ang, H.L., and Duester, G. (1996) Ethanol inhibition of retinoic acid synthesis as a potential mechanism for fetal alcohol syndrome. FASEB J. 10:1050–1057.

Deltour, L., Haselbeck, R.J., Ang, H.L., and Duester, G. (1997) Localization of class I and IV alcohol dehydrogenases in testis and epididymis: Potential retinol dehydrogenases for endogenous retinoic acid synthesis. Biol. Reprod. 56:102–109.

Dowling, J.E., and Wald, G. (1960) The biological function of vitamin A acid. Proc. Natl. Acad. Sci. USA 46:587–608.

Duester, G. (1996) Involvement of alcohol dehydrogenase, short-chain dehydrogenase/reductase, aldehyde dehydrogenase, and cytochrome P450 in the control of retinoid signaling by activation of retinoic acid synthesis. Biochemistry 35:12221–12227.

Duester, G., Ang, H.L., Deltour, L., Foglio, M.H., Hayamizu, T.F., and Zgombic-Knight, M. (1995) Class I and class IV alcohol dehydrogenase (retinol dehydrogenase) gene expression in mouse embryos. Adv. Exp. Med. Biol. 372:301–313.

Edenberg, H.J., Zhang, K., Fong, K., Bosron, W.F., and Li, T.-K. (1985) Cloning and sequencing of cDNA encoding the complete mouse liver alcohol dehydrogenase. Proc. Natl. Acad. Sci. USA 82:2262–2266.

Elder, J.T., Fisher, G.J., Zhang, Q.-Y., Eisen, D., Krust, A., Kastner, P., Chambon, P., and Voorhees, J.J. (1991) Retinoic acid receptor gene expression in human skin. J. Invest. Dermatol. 96:425–433.

Fisher, G.J., and Voorhees, J.J. (1996) Molecular mechanisms of retinoid actions in skin. FASEB J. 10:1002–1013.

Fisher, G.J., Reddy, A.P., Datta, S.C., Kang, S., Yi, J.Y., Chambon, P., and Voorhees, J.J. (1995) All-trans retinoic acid induces cellular retinol-binding protein in human skin in vivo. J. Invest. Dermatol. 105:80–86.

Frazier, C.N., and Hu, C.K. (1931) Cutaneous lesions associated with a deficiency in vitamin A in man. Arch. Intern. Med. 48:507–514.

Holmes, R.S. (1978) Electrophoretic analyses of alcohol dehydrogenase, aldehyde dehydrogenase, aldehyde oxidase, sorbitol dehydrogenase and xanthine oxidase from mouse tissues. Comp. Biochem. Physiol. [B] 61:339–346.

Holmquist, B., and Vallee, B.L. (1991) Human liver class III alcohol and glutathione dependent formaldehyde dehydrogenase are the same enzyme. Biochem. Biophys. Res. Commun. 178:1371–1377.

Imakado, S., Bickenbach, J.R., Bundman, D.S., Rothnagel, J.A., Attar, P.S., Wang, X.-J., Walczak, V.R., Wisniewski, S., Pote, J., Gordon, J.S., Heyman, R.A., Evans, R.M., and Roop, D.R. (1995) Targeting expression of a dominant-negative retinoic acid receptor mutant in the epidermis of transgenic mice results in loss of barrier function. Genes Dev. 9:317–329.

Jörnvall, H., Danielsson, O., Hjelmqvist, L., Persson, B., and Shafqat, J. (1995) The alcohol dehydrogenase system. Adv. Exp. Med. Biol. 372:281–294.

Kang, S.W., Duell, E.A., Fisher, G.J., Datta, S.C., Wang, Z.Q., Reddy, A.P., Tavakkol, A., Yi, J.Y., Griffiths, C.E.M., Elder, J.T., and Voorhees, J.J. (1995) Application of retinol to human skin in vivo induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels or irritation. J. Invest. Dermatol. 105:549–556.

Kastner, P., Chambon, P., and Leid, M. (1994) Role of nuclear retinoic acid receptors in the regulation of gene expression. In: "Vitamin A in Health and Disease," Blomhoff, R. (ed). New York: Marcel Dekker, Inc., pp. 189–238.

Kaufman, M.H. (1992) "The Atlas of Mouse Development." San Diego: Academic Press, Inc.

Kedishvili, N.Y., Bosron, W.F., Stone, C.L., Hurley, T.D., Peggs, C.F., Thomasson, H.R., Popov, K.M., Carr, L.G., Edenberg, H.J., and Li, T.-K. (1995) Expression and kinetic characterization of recombinant human stomach alcohol dehydrogenase. Active-site amino acid sequence explains substrate specificity compared with liver isozymes. J. Biol. Chem. 270:3625–3630.

Kim, C.-I., Leo, M.A., and Lieber, C.S. (1992) Retinol forms retinoic acid via retinal. Arch. Biochem. Biophys. 294:388–393.

Klein-Szanto, A., Martin, D., and Pine, A. (1980) Cutaneous manifestations in rats with advanced vitamin A deficiency. J. Cutan. Pathol. 7:260–270.

Koivusalo, M., Baumann, M., and Uotila, L. (1989) Evidence for the identity of glutathione-dependent formaldehyde dehydrogenase and class III alcohol dehydrogenase. FEBS Lett. 257:105–109.

Kurlandsky, S.B., Xiao, J.-H., Duell, E.A., Voorhees, J.J., and Fisher, G.J. (1994) Biological activity of all-*trans* retinol requires metabolic conversion to all-*trans* retinoic acid and is mediated through activation of nuclear retinoid receptors in human keratinocytes. J. Biol. Chem. 269:32768.

Kurlandsky, S.B., Duell, E.A., Kang, S., Voorhees, J.J., and Fisher, G.J. (1996) Auto-regulation of retinoic acid biosynthesis through

regulation of retinol esterification in human keratinocytes. J. Biol. Chem. 271:15346–15352.

Leo, M.A., Kim, C., Lowe, N., and Lieber, C.S. (1989) Increased hepatic retinal dehydrogenase activity after phenobarbital and ethanol administration. Biochem. Pharmacol. 38:97–103.

Mangelsdorf, D.J., Umesono, K., and Evans, R.M. (1994) The retinoid receptors. In: "The Retinoids: Biology, Chemistry, and Medicine, 2nd Ed," Sporn, M.B., Roberts, A.B., and Goodman, D.S. (eds). New York: Raven Press, Ltd., pp. 319–349.

Mezey, E., and Holt, P.R. (1971) The inhibitory effect of ethanol on retinol oxidation by human liver and cattle retina. Exp. Mol. Pathol. 15:148–156.

Moreno, A., and Parés, X. (1991) Purification and characterization of a new alcohol dehydrogenase from human stomach. J. Biol. Chem. 266:1128–1133.

Napoli, J.L. (1986) Retinol metabolism in LLC-PK1 cells: Characterization of retinoic acid synthesis by an established mammalian cell line. J. Biol. Chem. 261:13592–13597.

Parés, X., Moreno, A., Cederlund, E., Höög, J.-O., and Jörnvall, J. (1990) Class IV mammalian alcohol dehydrogenase: Structural data of the rat stomach enzyme reveal a new class well separated from those already characterized. FEBS Lett. 277:115–118.

Randolph, R.K., and Simon, M. (1993) Characterization of retinol metabolism in cultured human epidermal keratinocytes. J. Biol. Chem. 268:9198–9205.

Saitou, M., Sugai, S., Tanaka, T., Shimouchi, K., Fuchs, E., Narumiya, S., and Kakizuka, A. (1995) Inhibition of skin development by targeted expression of a dominant-negative retinoic acid receptor. Nature 374:159–162.

Saurat, J.H., Didierjean, L., Masgrau, E., Piletta, P.A., Jaconi, S., Chatellard-Gruaz, D., Gumowski, D., Masouyé, I., Salomon, D., and Siegenthaler, G. (1994) Topical retinaldehyde on human skin: Biologic effects and tolerance. J. Invest. Dermatol. 103:770–774.

Siegenthaler, G., Saurat, J.-H., and Ponec, M. (1990) Retinol and retinal metabolism. Relationship to the state of differentiation of cultured human keratinocytes. Biochem. J. 268:371–378.

Tavakkol, A., Griffiths, C.E.M., Keane, K.M., Palmer, R.D., and Voorhees, J.J. (1992) Cellular localization of mRNA for cellular retinoic acid-binding protein II and nuclear retinoic acid receptor-gamma1 in retinoic acid-treated human skin. J. Invest. Dermatol. 99:146–150.

Vallee, B.L., and Bazzone, T.J. (1983) Isozymes of human liver alcohol dehydrogenase. Curr. Top. Biol. Med. Res. 8:219–244.

Wilkinson, D.G., and Nieto, M.A. (1993) Detection of messenger RNA by in situ hybridization to tissue sections and whole mounts. Methods Enzymol. 225:361–373.

Wilson, J.G., Roth, C.B., and Warkany, J. (1953) An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation. Am. J. Anat. 92:189–217.

Wolbach, S.B., and Howe, P.R. (1925) Tissue changes following deprivation of fat-soluble A vitamin. J. Exp. Med. 42:753–777.

Xiao, J.-H., Durand, B., Chambon, P., and Voorhees, J.J. (1995) Endogenous retinoic acid receptor (RAR)-retinoid X receptor (RXR) heterodimers are the major functional forms regulating retinoid-responsive elements in adult human keratinocytes. Binding of ligands to RAR only is sufficient for RAR-RXR heterodimers to confer ligand-dependent activation of hRARβ2/RARE (DR5). J. Biol. Chem. 270:3001–3011.

Yang, Z.-N., Davis, G.J., Hurley, T.D., Stone, C.L., Li, T.-K., and Bosron, W.F. (1994) Catalytic efficiency of human alcohol dehydrogenases for retinol oxidation and retinal reduction. Alcohol Clin. Exp. Res. 18:587–591.

Zgombic-Knight, M., Ang, H.L., Foglio, M.H., and Duester, G. (1995) Cloning of the mouse class IV alcohol dehydrogenase (retinol dehydrogenase) cDNA and tissue-specific expression patterns of the murine ADH gene family. J. Biol. Chem. 270:10868–10877.