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Endogenous retinoids in the hair follicle and sebaceous gland

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Abstract

Vitamin A and its derivatives (retinoids) are critically important in the development and maintenance of multiple epithelial tissues, including skin, hair, and sebaceous glands, as shown by the detrimental effects of either vitamin A deficiency or toxicity. Thus, precise levels of retinoic acid (RA, active metabolite) are needed. These precise levels of RA are achieved by regulating several steps in the conversion of dietary vitamin A (retinol) to RA and RA catabolism. This review discusses the localization of RA synthesis to specific sites within the hair follicle and sebaceous gland, including their stem cells, during both homeostasis and disease states. It also discusses what is known about the specific roles of RA within the hair follicle and sebaceous gland.

Keywords

Retinoic acid; synthesis; retinoid; hair follicle; stem cells; sebaceous gland

1.1 Introduction

Wolbach and Howe (1925) [1] first found that vitamin A deficiency lead to metaplasia of keratinized epithelia including the hair follicle and atrophy of the sebaceous gland. Follicular hyperkeratosis was also seen in vitamin A deficient humans [2]. Vitamin A deficiency in rodents also leads to a thin hair coat that is frequently seen, but rarely reported [3] (unpublished observation from [4]). Hair loss (alopecia) is a consistent finding during vitamin A toxicity [5–7]. Excess RA also inhibits sebaceous gland function, as is exploited in the treatment of acne [8, 9]. The results from these studies suggest that precise levels of retinoic acid (RA, active metabolite) are needed for optimal function of the hair follicle and sebaceous gland (pilosebaceous unit, PSU). This review will focus on the role of endogenous RA within the PSU.

2.1 Retinoid metabolism in the skin

Plasma atRA was demonstrated to contribute little to endogenous atRA levels in numerous organs/sites in the rat [10]. Thus, atRA synthesis occurs at the site of action and precise levels of atRA are achieved by regulating several key steps in vitamin A metabolism (reviewed by Joe Napoli [11] and Greg Duester [12] in this special issue Figure 1).

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Regulation of retinoid metabolism occurs in part by the regulation of retinoid binding proteins. Vitamin A circulates in the blood in the form of retinol bound to the retinol binding protein (RBP, tentative gene symbol *Rbp4*) [13] and one level of regulation is to affect RBP4 levels (reviewed in [14]). Stra6 (stimulated by retinoic acid gene 6) was recently shown to bind RBP4 on the surface of the cell and facilitate the cellular uptake of retinol [15]. Within the cell, cellular retinol binding proteins (CRBP I-III, argued gene symbols *Rbp1–3*) direct retinol to specific enzymes, as only Lecithin: retinol acyltransferase (LRAT) and some retinol dehydrogenases can utilize retinol bound to CRBP as a substrate [16, 17]. Napoli [17] argued that this CRBP bound retinol is what produces atRA for the activation of RA receptors (RARA, B, G). LRAT can esterify either CRBP-retinol or free retinol for storage [16, 17]. This esterification of retinol limits the amount of retinol availabe for RA synthesis, as the majority of retinol that enters primary keratinocytes is converted to retinyl esters and only a small amount is actually oxidized to RA [18]. In addition, *Lrat* expression and its activity are regulated by atRA in a tissue specific maner, which is one way atRA regulates its own levels [19–21]. Dehydrogenase reductase SDR family member 9 (DHRS9) is one enzyme that catalyzes the oxidation of CRBP-bound, as well as free, retinol to retinal [22] in the epidermis, hair follicle, and sebaceous gland [23, 24]. Retinal is further oxidized to atRA by retinal dehydrogenases 1–3 (ALDH1A1, 2 and 3 [17, 25, 26]). There are three RA binding proteins that direct atRA to different fates. CRABP2 binds atRA, transports atRA to the nucleus, and binds to RARA to achieve efficient channelling of atRA to RARA, which increases its transcriptional efficiency [27–29]. It is assumed that CRABP2 also binds RARB and RARG, but this was not tested. CRABP2 is expressed at many sites of probable atRA synthesis and its presence correlated with times of atRA synthesis [30–32]. When CRABP2 is absent or saturated, atRA was reported to bind fatty acid binding protein 5 (FABP5), which directs atRA to peroxisome proliferators-activated receptor delta (PPARD) [33], although this role of atRA was refuted by two other groups [34, 35]. In contrast, CRABP1 is associated with catabolism of atRA via cytochrome P450 family members CYP26A1, CYP26B1, and CYP26C1 [36–38], although there is still a debate over whether these metabolites are functional or not [39–41]. RARA, RARB and RARG are atRA inducible transcription factors of the nuclear hormone family [42, 43] which regulate the expression of over 500 genes either directly or indirectly [44]. RARs heterodimerize with retinoid X receptor (RXR) [45]. While 9-*cis* RA (9cRA) was initially reported as the ligand for RXR [46], more recent genetic studies argue against 9cRA being the physiological ligand for RXR [47]. Unsaturated fatty acids also bind and activate RXR [48, 49].

When retinol levels exceed the capacity of CRBP a separate system of enzymes is argued by some to be used to clear the potentially toxic free retinol. Ross *et al* (1982) [50] found retinol could be esterified with acyl coenzyme A retinol acyltransferase (ARAT) activity and *Lrat^{tm1kpal}* null mice suggested some retinyl esters were stored in the absence of LRAT [51]. DGAT1 was recently shown to esterify retinol to retinyl esters with ARAT activity [52–54]. Since DGAT1 cannot use CRBP bound retinol [55, 56], it was suggested that it only was physiologically relevant when excessive concentrations of retinol were present. Although analysis of *Dgat1^{tm1Far}, Lrat^{tm1Kpal},* and *Rbp2^{tm1Eli} (Crbp2)* single and double knockout mice demonstrated that in vivo CRBP2 does not block DGAT1 activity within the intestine when physiological doses of retinol are provided [54]. In addition, these studies revealed that yet another enzyme exists with ARAT activity besides DGAT1. Studies with skin specific *Dgat1^{tm1Far}* null mice *(Dgat1^{tm2Far}Tg(KRT14-cre)1AMC)* revealed that while retinol and atRA were elevated, there were no differences between null and wild type mice in retinyl ester levels regardless of dietary vitamin A levels, suggesting that LRAT or another ARAT is present within the skin. Both LRAT activity [20] and mRNA [57] were found in cultured keratinocytes. We also see LRAT protein within the epidermis and hair follicle, but have not fully characterized it within the hair cycle (HB Everts, JP Sundberg, DE Ong, unpublished observation). Overall these data suggest that LRAT acts in the skin to

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store retinyl esters, while DGAT1 acts primarily to clear excess retinol to prevent toxicity (Figure 1). Free retinol can also be oxidized by the medium chain alcohol dehydrogenase family of retinol dehydrogenases (ADH1–4) [58]. Studies with *Adh1^{tm1Gdu}* and *Aldh1a1 ^{tm1Gdu}* null mice suggest that the clearance of excess retinol in the liver involves the oxidation of retinol to retinal by ADH1 followed by the oxidation of retinal to RA by ALDH1A1 [59–61]. Mice that lack *Adh4 tm1Gdu* also have decreased retinol oxidation in the kidney [62, 63], but not in the liver [59] when given a toxic retinol dose. ADH1 and ADH4 are present within the skin (unpublished observation and [64]). It is unclear if either of these enzymes is important for clearing free retinol in the skin when CRBP is saturated. In addition, both ADH1 and ADH4 can oxidize other alcohols to aldehydes and may play a role in general alcohol detoxification as well. Combined, the results of these studies show that retinoid metabolism is complex and the regulation of any one of numerous genes can alter RA activity.

2.2 The hair follicle and sebaceous gland (pilosebaceous unit)

The pilosebaceous unit (PSU) consists of a hair follicle and associated sebaceous gland. Specialized sebaceous glands also exist. The hair follicle contains 5 specific layers of epithelial cells that surround the hair fiber (Figure 2). The outer root sheath is continuous with the basal layer of the epidermis and these layers differentiate as they move inward toward the hair fiber and upward towards the exterior of the skin. The layers include the companion layer, Henle layer of the inner root sheath, Huxley layer of the inner root sheath, and the inner root sheath cuticle. Surrounding the hair follicle is a layer of mesenchymal cells, the connective tissue sheath, which maintains the dermal papilla at the base of the hair follicle. Three types of cells reside in the sebaceous gland (undifferentiated, maturing, and mature sebocytes) [65, 66]. Undifferentiated basal (reserve) cells are located on the entire periphery of the sebaceous gland but only the ones on the bottom are thought to differentiate as they migrate upward (maturing [65]). Lipogenesis occurs as sebocytes differentiate [67]. Mature fully differentiated sebocytes become completely filled with lipid, die, rupture, and release their contents into the hair canal resulting in holocrine secretion of sebum. In cultured cells apoptosis occurred in response to the terminal differentiation marker arachidonic acid [68], suggesting that terminal differentiation and the accumulation of specific lipids may induce apoptosis and aid in the holocrine secretion process. The PSU cycles through four major stages: growth and differentiation (anagen), regression and apoptosis (catagen), rest (telogen), and release of the old hair follicle (exogen) [69].

Hair follicle stem cells are being tested for use in regenerative medicine allowing a patient to be both the donor of the stem cells and the recipient of these newly differentiated cells [70]. Initial studies localized label retaining cells to the bulge region of the hair follicle [71], Figure 2). These bulge cells are quiescent, marked by CD34 and Keratin 15 (Krt15), and express a unique signature of genes [72, 73]. Results from lineage tracing studies suggest that these bulge stem cells produce all of the cells in the hair follicle during the normal hair cycle, but can repopulate the interfollicular epidermis (IFE) during wound healing (reviewed in [74]). Six additional regions of potential stem cells have since been reported (reviewed in [75]). BLIMP1 marks sebocyte stem cells that sit at the edge of the sebaceous gland duct and give rise to all of the cells of the sebaceous gland, but no other cells in the hair follicle or IFE [76]. Four regions of stem cells were found in the upper isthmus area. Leucine-rich repeats and immunoglobulin-like domain protein 1(LRIG1)$^+$ stem cells localized to the junctional zone between the infundibulum and isthmus [77], while the region just below this (called upper isthmus; UI) contained $\alpha 6^L$/CD34$^-$/Sca1$^-$ stem cells [78]. Both LRIG$^+$ and UI stem cells also express MTS24, which was independently found to have stem cell like properties [79]. Both LRIG$^+$ and UI cells produced the hair follicle, sebaceous gland, and IFE in skin reconstitution assays; but during homeostasis LRIG$^+$ stem cells only gave rise to

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sebaceous glands and IFE and UI were not tested. Leucine-rich repeat-containing G protein coupled receptor 6 $(LGR6)^+$ stem cells localized between this region and the bulge [80]. During prenatal development LGR6+ cells gave rise to hair follicle, sebaceous gland, and IFE; but in postnatal and adult skin these stem cells only gave rise to sebaceous gland and IFE cells. Wounding also induced LGR6+ cells to the site of the wound. At the base of the telogen bulge and secondary germ cells sits Leucine-rich repeat-containing G protein coupled receptor 5 (LGR5) stem cells [81]. These $LGR5^+$ cells migrate with the proliferating keratinocytes during early anagen to sit at the base of the bulb and lower outer root sheath. LGR5 localized to the entire outer root sheath during catagen. $LGR5^+$ stem cells produce all of the cells of the hair follicle below the sebaceous gland, including the bulge stem cells. Note that bulge stem cells were also reported to repopulate the secondary germ cells (i.e. $LGR5^+$ cells) at the end of catagen [82]. It was originally believed that the outer root sheath differentiated from the bulge downward, while all other layers of the hair follicle and fiber differentiate from the bulb [83]. The discovery of these LGR5+ stem cells questions this and suggests that the outer root sheath may also differentiate from the bulb. Together the various stem cell populations in the isthmus specify the upper hair follicle and IFE, while the stem cells of the bulge and secondary germ layer specify the cycling portion of the hair follicle during homeostasis. It is still debatable whether all of these cells with stem cell like qualities are truly stem cells or if there is a hierarchy of stem cells like in the hematopoietic system [74, 75]. It was also suggested that all cells of the hair follicle may have stem cell properties in the right environment [84]. When misregulated, these stem cells lead to skin cancers [85].

2.3 Retinoids in the hair follicle

Studies with transgenic mice support a role for RA in the hair follicle. Both a reduction in RA signaling (*Krt14 & Krt5 Cre Rxra*^{tm4Ipc} null mice) and an excess of retinol and atRA (*Krt14 Dgat1*^{tm2Far} null mice) within the basal epidermis and outer root sheath led to progressive alopecia [6, 86, 87]. Blocking RA signalling (*Rxra*^{tm4Ipc} null mice) delayed anagen initiation, while increasing retinol and atRA (*Dgat1*^{tm2Far} null mice) accelerated the transition from telogen to anagen. This increased anagen induction and alopecia could be reduced in *Dgat1*^{tm2Far} null mice by severely reducing dietary vitamin A intake. RXR partners with many nuclear receptors and these effects were originally attributed to its partnering with the vitamin D receptor (VDR), as *Vdr*^{tm1Mbd} null mice have similar progressive alopecia and anagen inhibition [88, 89]. While it is unlikely that the effects of reduced DGAT1 lead directly to altered vitamin D metabolism or signalling, as they could be reversed by altering dietary vitamin A, an indirect effect of excess vitamin A on vitamin D metabolism or signalling in the hair is possible as interactions between these two vitamins have been seen [90–92]. These *Rxra*^{tm4Ipc} null mice also have elevated proliferation and defective differentiation in IFE and increased inflammation, which were not seen in *Vdr*^{tm1Mbd} null mice [93], suggesting other RXR partners are involved. Exogenous atRA also induced catagen in cultured hair follicles [94]. In addition, exogenous atRA with BMP directed the differentiation of embryonic [95] and induced pluripotent stem cells into keratinocytes that when grafted into nude mice produced normal epidermis, hair follicles, and sebaceous glands [96]. Transgenic mice that overexpress a dominant negative *Rara* targeted to the epidermis have aberrant skin, no hair or wrinkles and die shortly after birth [97, 98]. These mice have reduced epidermal barrier function due to defects in lipid metabolism [98, 99]. These effects were not seen in mice that overexpress a dominant negative thyroid receptor [97] or *Rxr* [100] in the epidermis. In addition, VDR responses were not altered by dominant negative *Rara* expression, suggesting that these effects are specific to RAR and RA signaling and not other partners of RXR. A similar defect in lipid metabolism and epidermal barrier function was seen in epidermal ($Krt14CreER^{T2}$) and suprabasal ($CMV\text{-}CreER^{T}$) targeted *Rarg*^{tm3Ipc} null mice [47], although an initial report of

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epidermal (Krt5) targeted *Rara*^{tm3Ipc} and *Rarg*^{tm3Ipc} double null mice failed to see this effect [101]. No studies have examined epidermal or hair follicle specific *Rarb* null mice or triple *Rar* null mice as it was assumed that RARB was not important in the epidermis, as it was not expressed in the nucleus [101] and no skin or hair defects were seen in the original *Rarb*^{tm1Ipc} null mice [102]. Yet weak expression of RARB was seen in the follicle keratinocytes [103] and we saw cytoplasmic only localization of RARB in the basal epidermis, sebaceous gland, outer root sheath, and hair follicle bulge [24]. In addition, RARs may be important in other cells within the PSU that do not express Krt14 or Krt5. Combined, the results from these studies suggest that atRA alters stem cells to regulate the hair cycle at both the telogen to anagen and anagen to catagen transitions, as well as regulate lipid metabolism in the IFE to maintain epidermal barrier function, but not all of the receptors responsible or specific mechanisms have been elucidated.

Several reports have localized components of RA synthesis and signaling to the hair follicle in various or unknown stages of the hair cycle [23, 103–106]. To obtain a more complete picture, we localized a whole system of retinoic acid synthesis and signaling proteins to the hair follicle, including all seven stem cell regions (Figure 3, [24, 107]). This localization pattern changed throughout the hair cycle with a peak occurring during mid-anagen through early catagen. We have confirmed this pattern of RA synthesis during mid-anagen using RA reporter mice (Tg(RARE-Hspa1b/lacz)12Jrt/J made by [108]; Figure 3c). RA synthesis was present in the cells of the sebaceous gland duct, the site of BLIMP1$^+$ stem cells, and throughout the whole isthmus, site of MTS24, LRIG$^+$, UI, and LGR6$^+$ stem cells, during mid-anagen (Figure 3b, Red). Topical atRA induced LRIG$^+$ cells to expand and give rise to cells in the sebaceous gland, infundibulum, and IFE [77]. Thus, endogenous RA synthesized within the isthmus may be important for the maintenance of the sebaceous gland, infundibulum, and IFE. In addition, wounds heal faster when hair follicles are in anagen [109], thus the peak of RA synthesis within the isthmus during anagen may also contribute to better wound healing. RA synthesis also localized to the bulge region during mid-anagen (Fig 3c) and some RA synthesis enzymes and binding proteins remained in the bulge throughout the hair cycle ([24], Fig 3a,b). RA synthesis enzymes (DHRS9 and ALDH1A2) and RARA, B, and G localized to proliferating keratinocytes as they migrated downward during early anagen, and RA synthesis was seen in the outer and lower cells of the bulb, where LGR5$^+$ stem cells also localized [24, 81]. During late anagen/early catagen there was a drop in the expression of RARs within the bulb and an increase in CRBP, suggesting a role in catagen induction. These localization patterns suggest that RA may also play a role in the maintenance of hair follicles and its cycle. Together, the results from these studies suggest that endogenous RA may be important for all of the stem cells in the hair follicle and the maintenance of the hair follicle, sebaceous gland, and IFE, although the specific mechanisms of these effects are yet to be determined.

Excess RA was found to reduce the number and length of hair follicles *in vitro* [110]. Feather specification and dermal condensations were also inhibited by excess RA, which altered the pattern of Hox gene expression and NCAM in the developing feather, a structure similar to the mammalian hair follicle [111]. In addition, *Dgat1*^{tm2Far}*Tg(KRT14-cre)1AMC* null mice have cyclical hair loss that is restored by reducing dietary vitamin A, as discussed above [6]. We found increased expression of RA synthesis enzymes and binding proteins in biopsies from patients with several hair loss diseases and skin from their mouse models, including central centrifugal cicatricial alopecia (CCCA) and alopecia areata (AA; HB Everts, LE King Jr, unpublished observation). Flowers *et al* [112] also found increased retinol, atRA, retinyl esters and mRNA of retinoid binding proteins and target genes in one of these animal models. Primary cicatricial alopecias are a collection of scarring hair loss diseases that result in inflammatory attack of the hair follicle, their stem cells, and permanent hair loss [113]. The cause of this disease is unknown but theories include altered

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sebaceous gland function with reduced sebum [114], reduced PPARG [115], structural defects in the hair follicle [114], and loss of immune privilege in the hair follicle [116]. Alopecia areata is an autoimmune non-scarring hair loss disease that is mediated by $CD8^+$ T cell attack on the lower cycling hair follicle [117–119] and a loss of immune privilege in the hair follicle [120–122]. We also found that reducing dietary vitamin A prevented cicatricial alopecia and delayed the onset of alopecia areata in mouse models (HB Everts, JP Sundberg, unpublished observation). Future studies are needed to better understand the mechanisms of RA's function within the hair follicle to produce better treatments for these hair loss diseases.

2.4 Retinoids in the sebaceous gland

Results from early studies suggested a potential therapeutic role for oral retinyl palmitate in the treatment of psoriasis (reviewed in [123]). But this led to severe hypervitaminoisos A and was abandoned. To produce effective results with fewer side effects topical synthetic atRA (tretinoin, Retin-A) and oral synthetic 13-*cis* RA (13cRA; Accutane) were developed (reviewed in [123]). Retinoids (i.e. Accutane, Retin-A™) are the most effective and first choice for acne treatment [124], but still have significant side effects ranging from teratogenesis [125] to telogen effluvium [7]. Even after 25+ years of use the mechanism of action of retinoids in acne is still unclear. Results from an initial study of acne patients revealed that 13cRA significantly reduced the size of the sebaceous gland and inhibited sebocyte differentiation [126]. Numerous *in vitro* studies have been performed to better understand this mechanism (reviewed in [8, 9]). Lack of vitamin A in cultured human sebocytes resulted in decreased cell proliferation and lipogenesis that could be partially restored with low doses of 13cRA [127]. At concentrations above 10^{-7}M 13cRA and atRA inhibited proliferation and lipogenesis [127–130]. Thus, while low levels of RA are important for sebaceous gland function, excess RA synthesis within the sebaceous gland could lead to atrophy of the gland, and reduced sebum production.

More recent studies suggest that 13cRA treats acne by both RAR dependent and independent mechanisms. 13cRA does not bind to RARs, but was shown to isomerizes to atRA in culture [131] and RARB and/or RARG were reported to mediate the antiproliferative and antidifferentiative affects of retinoids [132]. 13cRA induced apoptosis after 48 and 72 hours independent of RARs [133]. This long time frame suggests an indirect mechanism. In addition to inhibiting genes involved in lipid metabolism [134], 13cRA also inhibits androgen synthesis [135], FGFR2 signaling (reviewed in [136]), and genes involved in arachidonic acid generation [134, 137], which have all been implicated in acne pathogenesis. In contrast, 13cRA induces genes encoding extracellular matrix proteins involved in wound healing and tissue remodeling including collagens, fibronectin, neutrophil gelatinase associated lipocalin, and matrix metalloproteinases [134, 137, 138]. Thus, 13cRA uses multiple mechanisms to reduce sebum and treat acne, future studies will focus on altering these downstream effects of retinoids directly to treat acne with fewer side effects than current retinoid therapies, although an additional option would be to directly regulate RA synthesis within the sebocytes.

We localized RA synthesizing enzymes (DHRS9 and ALDH1a1–3) and cytoplasmic RARB to the basal and early maturing cells with decreased intensity as the sebocytes matured (Figure 3) [24]. Cytoplasmic localization of CRABP2 and RARA were seen in the early maturing sebocytes, while these proteins were nuclear localized in the most differentiated mature cells. This suggests something blocked nuclear localization of CRABP2 and RARA in basal sebocytes, or RA synthesis in the presence of enzymes DHRS9, ALDH1A1, and ALDH1A3. In testis cell lines, PPARA agonist inhibited nuclear localization of RARA, while promoting nuclear localization of PPARA [139]. PPARA, PPARB/D, and PPARG are

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all expressed in sebocytes [140] and PPARG localized to the basal sebocytes in adult scalp skin [141]. Collins and Watt (2008) [142] also saw nuclear localization of CRABP2 in mature sebocytes, as well as FABP5 nuclear localization in the maturing sebocytes. This would suggest that RA made in the maturing sebocytes could activate PPARD. But we also saw cytoplasmic RARB in these RA synthesizing cells suggesting a potential non-genomic effect, as seen in other tissues (reviewed in [143]). Wnt signaling is normally low in the sebaceous gland and ectopic expression of Wnt signaling mediator beta-catenin inhibited CRABP2 and FABP5 expression in the sebaceous gland [142]. The localization of RA synthesis to the sebaceous gland may also be a way to send CRABP2 bound RA to the surface of the epidermis. Vitamin E was delivered to the stratum corneum of the epidermis by this mechanism of holocrine sebum secretion [144]. All the enzymes necessary for Vitamin D metabolism were also found in the sebaceous gland [145]. Thus, sebum may contain numerous fat-soluble vitamins and the secretion of sebum may deliver all of these vitamins to the surface of the skin and help in barrier function. Future studies are needed to distinguish these potential mechanisms of endogenous RA synthesis within the sebaceous gland. Combined, the results from these studies suggest that precise levels of RA are required for optimal sebum production and that this may be achieved by endogenous synthesis of RA within the sebocyte, as all of the necessary enzymes are present.

3.1 Summary and Future Studies

The PSU is a complex mini-organ that is regulated by the same signaling factors that regulate development of other organs. Similar to organogenesis in other sites, RA synthesis enzymes, binding proteins, receptors, and RA were seen in a specific temporal and spatial pattern. This localization pattern and the fact that both vitamin A deficiency and toxicity lead to defects in the PSU suggest that RA is important for its function. Older work in the field laid the groundwork, but the specific mechanisms by which RA regulates the PSU are just beginning to be dissected. Future studies are needed to determine the mechanisms by which: RA alters all of the stems cells in the PSU, RA regulates differentiation of the PSU, and RA regulates sebaceous gland function. In addition, future studies are needed to determine what signaling factors regulate RA synthesis and how modulating RA synthesis can be used to treat various diseases of the PSU such as CCCA, AA, and acne.

Highlights

- The pilosebaceous unit consists of a hair follicle and sebaceous gland.
- The pilosebaceous unit requires precise levels of retinoic acid.
- Retinoic acid synthesis localized to the pilosebaceous unit, including stem cells.
- Mechanisms of action of retinoic acid within the pilosebaceous unit are emerging.
- But much more information has yet to be discovered.

Abbreviations

RA	retinoic acid
atRA	all-trans retinoic acid
9cRA	9-cis retinoic acid
13cRA	13-cis retinoic acid

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PSU	pilosebaceous unit
IFE	interfollicular epidermis

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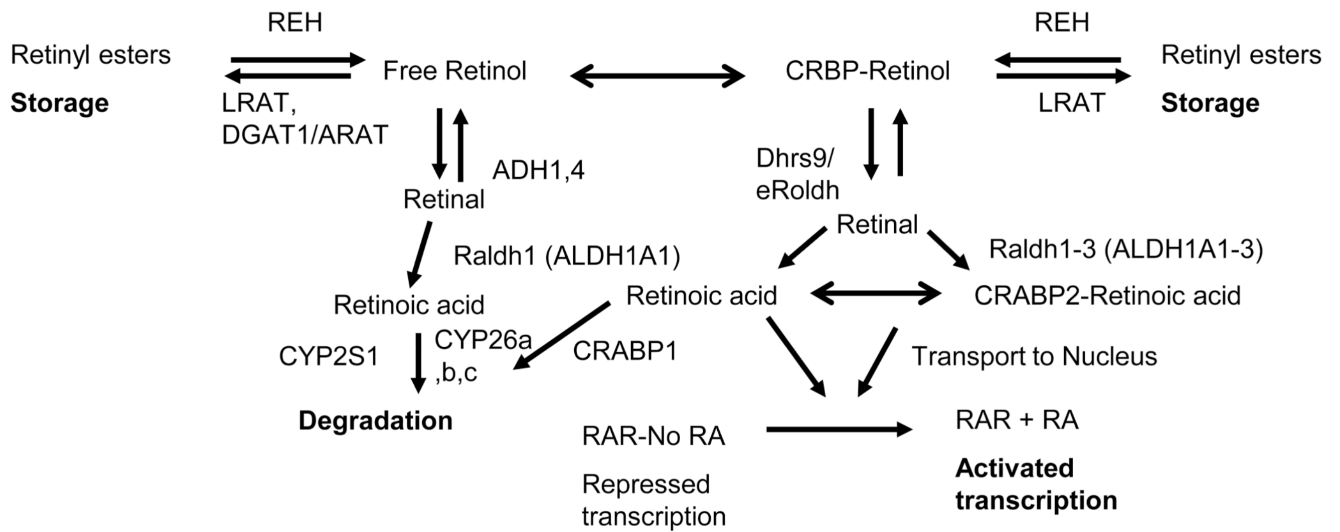

Figure 1. Cellular retinol metabolism in the skin

Once inside the cell, retinol is either stored as retinyl esters, or oxidized to retinoic acid (RA). Several families of enzymes and binding proteins are involved in this process.

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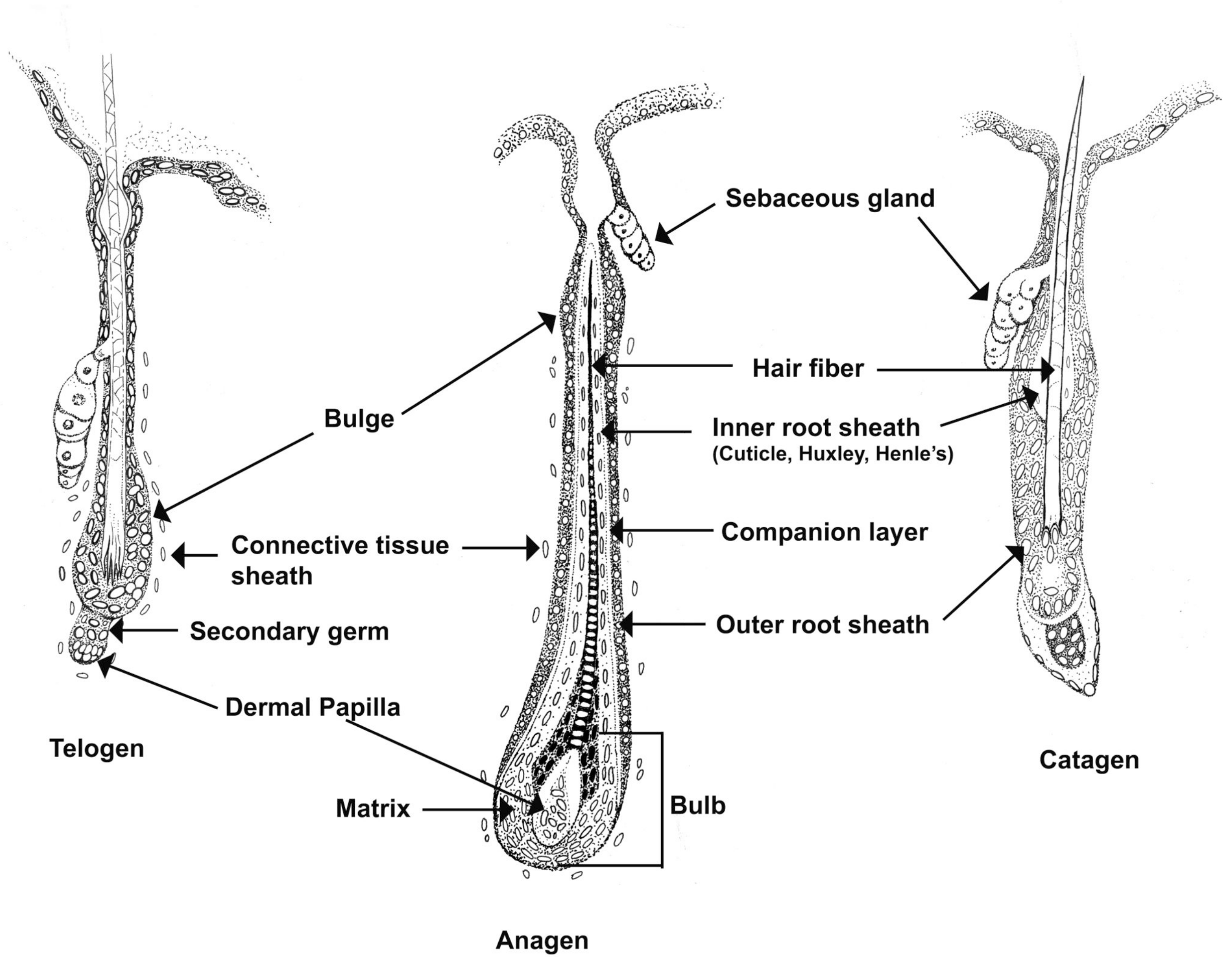

Figure 2. The hair follicle

The hair follicle is illustrated with structures labeled as they change during the hair cycle.

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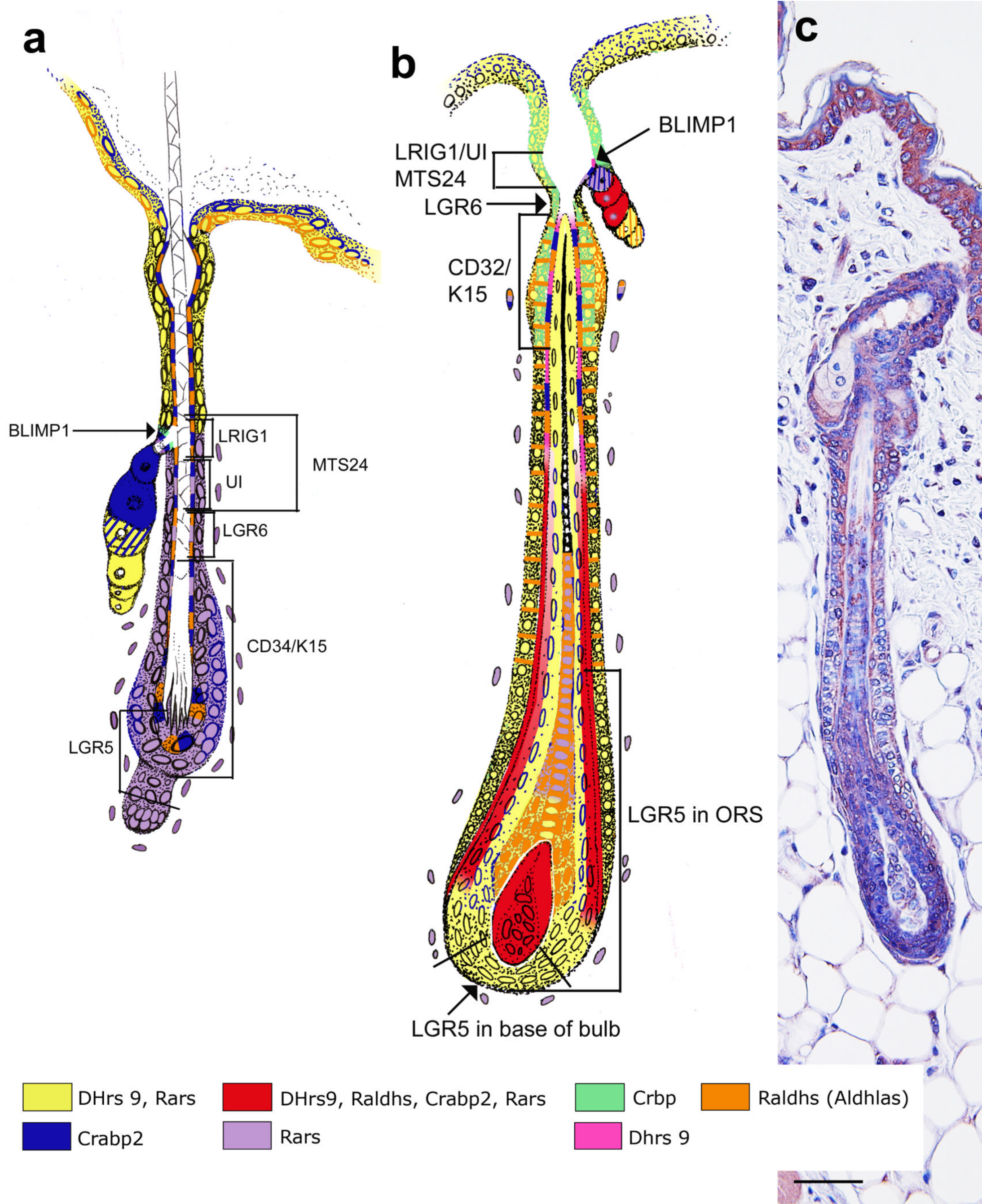

Figure 3. Retinoic acid synthesis in the hair follicle

Telogen (a) and anagen (b) hair follicles are color coded with retinoic acid synthesis components: DHRS9 plus RARs (yellow), DHRS9 alone (pink), RARs alone (purple), CRABP2 (blue), CRBP (green), ALDH1A1, 2, or 3 (orange), a complete system with DHRS9, ALDH1A1, 2, or 3, CRABP2, and RARa, b,or g (red). Stem cell locations are marked in relation to this localization pattern of RA synthesis components. (c) RA synthesis as determined by immunohistochemistry with an antibody against beta-galactosidase in RA reporter mice (Tg(RARE-Hspa1b/lacz)12Jrt/J). Modified from Everts *et al* [24].