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Review Article

The biological actions of estrogens on skin

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Abstract: There is still extensive disparity in our understanding of how estrogens exert their actions, particularly in non-reproductive tissues such as the skin. Although it has been recognized for some time that estrogens have significant effects on many aspects of skin physiology and pathophysiology, studies on estrogen action in skin have been limited. However, estrogens clearly have an important function in many components of human skin including the epidermis, dermis, vasculature, hair follicle and the sebaceous, eccrine and apocrine glands, having significant roles in skin aging, pigmentation, hair growth, sebum production and skin cancer. The recent discovery of a second intracellular estrogen receptor (ERβ) with different cell-specific roles to the classic estrogen receptor (ERα), and the identification of cell surface estrogen receptors, has provided further challenges to understanding the mechanism of estrogen action. It is now time to readdress many of the outstanding questions regarding the role of estrogens in skin and improve our understanding of the physiology and interaction of steroid hormones and their receptors in human skin. Not only will this lead to a better understanding of estrogen action, but may also provide a basis for further interventions in pathological processes that involve dysregulation of estrogen action.

M. J. Thornton

Department of Biomedical Sciences, University of Bradford, Bradford, West Yorkshire, UK

Key words: estrogen receptors – skin – hair follicle – sebaceous gland – melanocytes

Dr M. J. Thornton, Department of Biomedical Sciences, University of Bradford, Bradford, West Yorkshire, BD7 1DP UK
Tel.: +44 1274 235517
Fax: +44 1274 309742
e-mail: M.J.Thornton@bradford.ac.uk

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Mechanism of estrogen action

The estrogen receptor (ER) belongs to a superfamily of nuclear receptors including those for steroid and thyroid hormones, vitamin-D_3 and retinoic acid (1). The inclusion of proteins with a striking homology to these receptors, but with no known ligands, termed orphan receptors, has expanded the family to now include approximately 150 different proteins (2). Orphan receptors that are members of this superfamily include peroxisomal proliferator activator receptors (PPARs) (3), testicular receptor (TR4) (4), and steroidogenic factor 1 (SF-1) (5).

In the absence of ligand, ER is associated with an inhibitory heat shock protein (HSP) (6). When the ligand binds to the receptor a conformational change occurs, resulting in the dissociation of the HSP and the formation of stable estrogen receptor dimers (7). These ligand-activated receptor dimers tightly associate with specific consensus DNA sequences or estrogen response elements (EREs), which consist of 15 base pair inverted palindromes located within the regulatory region of the target genes. The steroid receptor complex then interacts with other cellular components to either activate or suppress transcription of the target gene in a promoter and cell-specific manner (8).

However, recent studies from a number of laboratories have led to significant changes in the understanding of the mechanism of estrogen action. In line with other members of the nuclear receptor superfamily, ER has a modular structure consisting of distinct functional domains (see Fig. 1) (9). The DNA-binding domain (DBD) is required for binding to the ERE, while the ligand-binding domain (LBD) harbors a nuclear localization signal in addition to an activation function AF2, which is involved in dimerization and tran-

Abbreviations: ER: estrogen receptor; ERβ, estrogen receptor beta; ERα, estrogen receptor alpha; AF1: activation function 1; AF2: activation function 2; PPAR: peroxisomal proliferator activator receptor; TR4: testicular receptor; SF-1: steroidogenic factor 1; VEGF: vascular endothelial growth factor; HSP: heat shock protein; ERE: estrogen response elements; 5α-DHT: 5α-dihydrotestosterone; DHEA: dihydoepiandrosterone; 3β-HSD: Δ-5,3β-hydroxysteroid dehydrogenase; 17 β-HSD: 17β-hydroxysteroid dehydrogenase; SERM: selective estrogen receptor modulators; MAPK: mitogen-activated protein kinase.

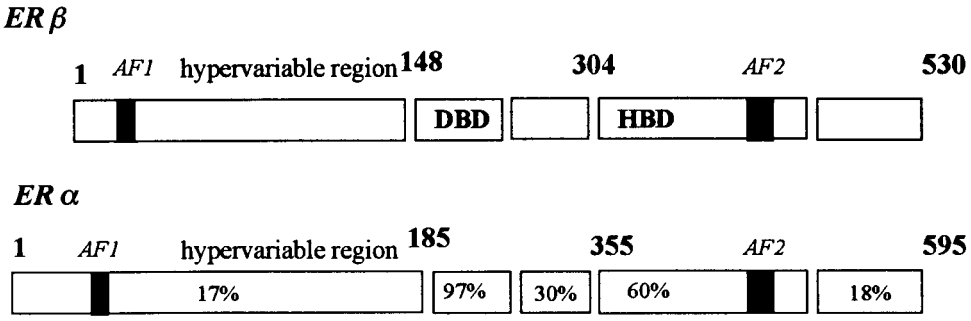

Figure 1. The molecular structure of the two estrogen receptors. The molecular structure of ERβ and ERα illustrates the distinct functional domains. DBD is the DNA-binding domain and HBD is the hormone-binding domain. Numbers within the boxes represent amino acid identity between ERβ and ERα. Numbers at the end of each receptor indicate the total length of the protein. The activation function 2 (AF2) core sequences are identical, but there is no significant region of homology between the regions containing activation function 1 (AF1).

scriptional activation (10). AF1, a second activation function is located in the less conserved amino terminal domain (see Fig. 1). Recent studies have demonstrated that the liganded ER does not only signal through classic ERE, but may also signal via AF1 elements, indicating that ER does not necessarily have to bind to DNA but may bind to other transcription factors which are in contact with DNA (11). Furthermore, mutation of a conserved tyrosine adjacent to AF2 generates ER proteins that stimulate transcription in the absence of ligand (12).

The two estrogen receptors, ERα and ERβ

Since the estrogen receptor was cloned in 1986 (13) it was universally accepted that only one such receptor existed. However, a second gene coding for an estrogen receptor was independently cloned from rat prostate (14) and human testis (15) in 1996. Due to the striking homology of its sequence with the classic estrogen receptor this newly discovered receptor was termed ERβ; the classic ER is now referred to as ERα (see Fig. 1).

The two estrogen receptors are not generated from alternate transcription sites of the same gene, as is the progesterone receptor (16), but are distinct proteins encoded by separate genes located on different chromosomes. ERβ is localized on human chromosome 14, while ERα is found on chromosome 6 (17). Thus, ERα and ERβ represent two separate gene products, that both share a relationship with other members of the steroid receptor superfamily. The ERα and ERβ proteins have ~60% conservation of the residues in the ligand binding domain, yet each bind 17β-estradiol with nearly equal affinity and exhibit a very similar binding profile for a large number of natural and synthetic ligands (18). However, there are striking differences with respect to their tissue distribution (19,20), the phenotype of the corresponding knockout mice and their transcriptional activities (21).

Recent studies using PCR and immunostaining have attempted to compare the expression of ERα and ERβ in a number of tissues (19,20,22). Mammalian tissues known to contain detectable levels of ERα include the male and female reproductive tissues, the female mammary gland and in both sexes, the cardiovascular system, bone and regions of the brain (23). However, ERβ appears to be more widely expressed and has shown to be present in both male and female reproductive tissues in addition to non-reproductive tissues including the lung, bladder, heart, adrenal, thymus, kidney, pituitary, hypothalamus and skin (19, 20, 22, 23). In the ovary, ERβ is more abundant and exhibits a distinct pattern of expression compared with ERα (20, 22), while in the uterus ERα is the predominant ER (22). Furthermore, in the male reproductive tract the pattern of expression of ERβ closely parallels that of the androgen receptor (20) and is strongly expressed in the prostate. The expression of two different ERs in a variety of tissues suggests that there are different, cell-specific roles for the two receptors, which may each modulate the expression of different genes. However, in some tissues, both receptors are expressed suggesting that there may be some interplay between the two ERs. Recently, studies have shown that ERβ and ERα can heterodimerize *in vitro*, indicating the possibility of cooperative, synergistic or inhibitory actions between the two receptors (24, 25, see Fig. 2). Therefore, the discovery of ERβ has introduced a further level of complexity to the mechanism of estrogen signaling and physiology.

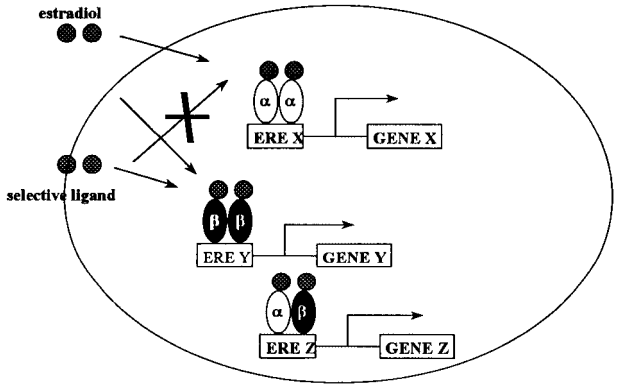

Figure 2. 17β-estradiol can activate different gene expression via two receptors. 17β-estradiol binds to both ERα and ERβ with a similar affinity. Upon ligand binding, the steroid receptors form homodimers, which interact with specific estrogen response elements (ERE) upstream of the target gene. In cells that express both estrogen receptors heterodimerization may occur, resulting in different gene expression. The development of ligands that are selective for only one estrogen receptor (e.g. ERβ) will enable selective gene expression.

Cell surface receptors

In addition to the classic genomic pathway of estrogen signaling, rapid effects in response to estrogens have been documented in a number of different tissues (reviewed 26). There is now increasing evidence that cell surface forms of estrogen receptors exist that are coupled to cytosolic signal transduction proteins. Indeed estrogens have been shown to rapidly initiate a range of signaling cascades involving conventional second messengers (reviewed 27). Estradiol has been shown to activate the mitogen-activated protein kinase (MAPK) signaling cascades within seconds; it can also cause the rapid stimulation of calcium flux, the generation of cAMP and IP3 and the activation of phospholipase C (reviewed 26). This all provides strong evidence for the existence of a plasma membrane form of the estrogen receptor. However, although the existence of such a receptor is now widely accepted, its precise molecular structure is still unclear. Studies have shown that plasma membrane binding sites for estradiol react specifically with antibodies for ERα, suggesting that the structure of this membrane receptor must be similar to that of ERα (28). Furthermore, the transfection of ERα and ERβ into cells which do not express these genes, results in the production of both nuclear and membrane estrogen receptors with a similar affinity for estradiol, suggesting that both nuclear and cell membrane receptors are derived from a single transcript (29). In contrast, other studies have suggested that estrogen membrane binding receptors are different to the nuclear receptors, but are structurally related, sharing some of the domains of the nuclear receptor, while others support the existence of a membrane receptor that is both genetically and pharmacologically distinct from the nuclear receptor (reviewed 27). To add further to the complexity of estrogen signaling, it is now emerging that estradiol interacting with membrane receptors and their subsequent signaling cascades allows for interaction with growth factors and their receptors. Cross-talk between estrogens and growth factors has been reported in a number of tissues and include IGF-1 (30, 31), EGF (32) and TGF-α (33). All these recent studies demonstrate that the classic model of genomic action of estrogen is incomplete and must be extended to include membrane receptors for estrogen signaling (see Fig. 3) and their interactions with other intracellular signaling cascades.

Estrogens and skin

Skin is a heterogeneous tissue that has multiple functions including protection against infection and ultraviolet light, temperature regulation, controlling water balance and excretion. Another important function of skin is that it acts as an endocrine organ. There is sufficient evidence according to receptor distribution and related actions to show that estrogens exert major direct influences on all elements of skin. However, despite the

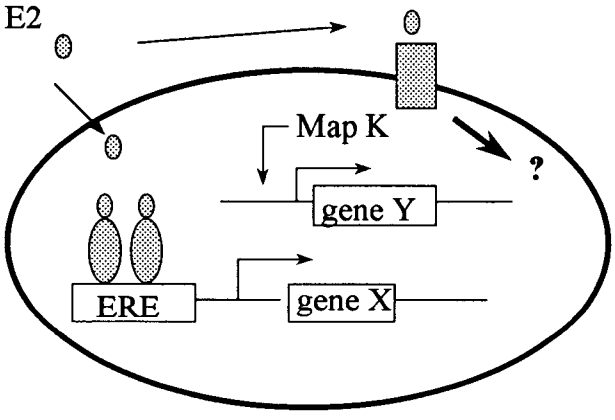

Figure 3. Estrogens can mediate their actions via both cell surface and intracellular receptors. 17β-estradiol can enter the cell and bind with intracellular nuclear receptors that dimerize and interact with estrogen response elements (ERE) to initiate gene transcription (gene X). Alternatively, it can interact with a plasma membrane receptor that in turn interacts with cell signalling pathways including the MAP Kinase pathway and activate a different set of genes (gene Y). This second, non-genomic signaling mechanism results in a rapid (seconds) cellular response to estrogens. Via this plasma membrane receptor, estrogens can also interact with other intracellular signalling pathways (?).

knowledge that estrogens have such important effects on various components of skin, the cellular and subcellular sites and mechanisms of estrogen action are still poorly understood.

Animal skin

It has been recognised since the 1960s that estrogens stimulate the synthesis, maturation and turnover of collagen in rats (34) and guinea pigs (35) and significantly increase the synthesis of hyaluronic acid in mouse skin (36). Estrogens have also been shown to increase mitotic activity in the epidermis of mice (37), which correlates with epidermal thickening, although prolonged administration of estrogen has been reported to reduce epidermal thickness in the rat (38). In the 1970s, autoradiographical studies demonstrated that estradiol was localized in the skin of mice after injection with tritiated estradiol (39). Radioactivity was localized to epidermis, dermal fibroblasts and hair follicle, suggesting that these are all targets for estrogen action. Furthermore, these autoradiographic studies also reported some variation in the amount of radioactivity localized in the epidermis between perineal and dorsal rodent skin. Later biochemical binding studies revealed estrogen-binding sites in minced mouse back skin, demonstrating the presence of an estrogen receptor (40). Binding of ^{3}H-estradiol to this protein was inhibited by estrogens and an antiestrogen, but not by testosterone, corticosterone or progesterone.

Another study using whole rat skin homogenates has demonstrated variations in estrogen binding that coincide with changes in the hair cycle (41). Estrogens also have significant effects on the hair follicle and sebaceous glands (see next sections), so studies of estrogen action on whole skin may well reflect activity in these appendages. In this study, it was found that maximum binding of estradiol occurred when hair follicles were in telogen; there was also some variation in estrogen metabolism with conversion of estradiol to estrone being most active when hair follicles were in anagen. More recent studies have also shown a significant correlation with the phase of the underlying hair follicle and the proliferation of basal epidermal cells in mouse skin with the administration of topical estrogen (42).

Interestingly, it would also appear that estrogens offer some degree of protection against skin photoaging. In rats, decreased estrogen levels due to ovariectomy enhance the UVB sensitivity of the skin, resulting in an acceleration of photoaging in terms of an increased formation of significantly deep wrinkles, decreased skin elasticity and marked damage and advanced curling of the dermal elastic fibers at an early stage (43).

Estrogens may also be important modulators of epidermal carcinogenesis as they have been shown to markedly enhance the development of squamous cell carcinomas in mice and basal cell carcinomas in rats, induced by the long-term administration of a chemical carcinogen (44). In the same study both hypophysectomy and gonadectomy were shown to inhibit the progression of carcinogenesis.

Human skin

For many years it has been recognized that estrogens are important in the maintenance of human skin. They improve collagen content and quality, increase skin thickness and enhance vascularization, features highlighted by the changes that occur in the skin of postmenopausal women (45). Estrogens have been shown to increase mitotic activity in the epidermis of women (46) and the presence of an estrogen receptor in human skin has also been demonstrated that exhibits similar binding parameters to that described in mice (47, 48). Furthermore, the number of estrogen receptors has been reported to vary in different parts of the body, with receptor levels higher in facial skin than in skin from the thigh or breast (47). However, it must be taken into account that these studies were carried out on whole skin homogenates and did not distinguish between the different components within the skin.

In postmenopausal women ($n = 148$) a decrease in the collagen content of thigh skin at rate of 2% per postmenopausal year has been observed for up to 15 years in women not on hormone replacement therapy (49). The daily administration of topical estradiol to the skin of postmenopausal women has shown that the amount of hydroxyproline is significantly increased during treatment (50). Furthermore, in the same study, electron microscopic examination of such skin showed a morphologic improvement in elastic and collagen fibers. Other workers have shown that systemic hormone replacement therapy results in a significant increase in the amount of collagen fibers after only 6 months, as assessed through computerized image analysis (51). Although they did not see any correlation between estrogen treatment and elastic fibers, perhaps the short treatment length of 6 months combined with the low turnover rate of elastic fibers may offer an explanation.

Recently, it has also been shown that the rate and quality of wound healing in skin is estrogen dependent (52), while the delay in wound healing

in elderly patients of both sexes can be significantly reduced by topical estrogen (53).

Although estrogens have been implicated in the progression of basal cell carcinoma in rodents, immunohistochemical studies have failed to show the presence of the estrogen receptor in human basal cell carcinomas (54). However, for several years the only antibodies for estrogen receptors that existed were directed against ERα, while antibodies against ERβ have only very recently become available. Although a number of binding studies with ^{3}H-estradiol have demonstrated estrogen-binding sites in human skin, because estradiol binds to both ERα and ERβ with similar affinity, these studies do not distinguish between the two estrogen receptors. Furthermore, because ERα and ERα share such a close structural resemblance, it is possible that some antibodies used in previous immunohistochemistical studies could recognize both proteins and should therefore be interpreted with caution. Using specific antibodies that distinguish between ERα and ERβ, we have recently demonstrated by immunhistochemistry that ERβ is the predominant estrogen receptor in adult hu-

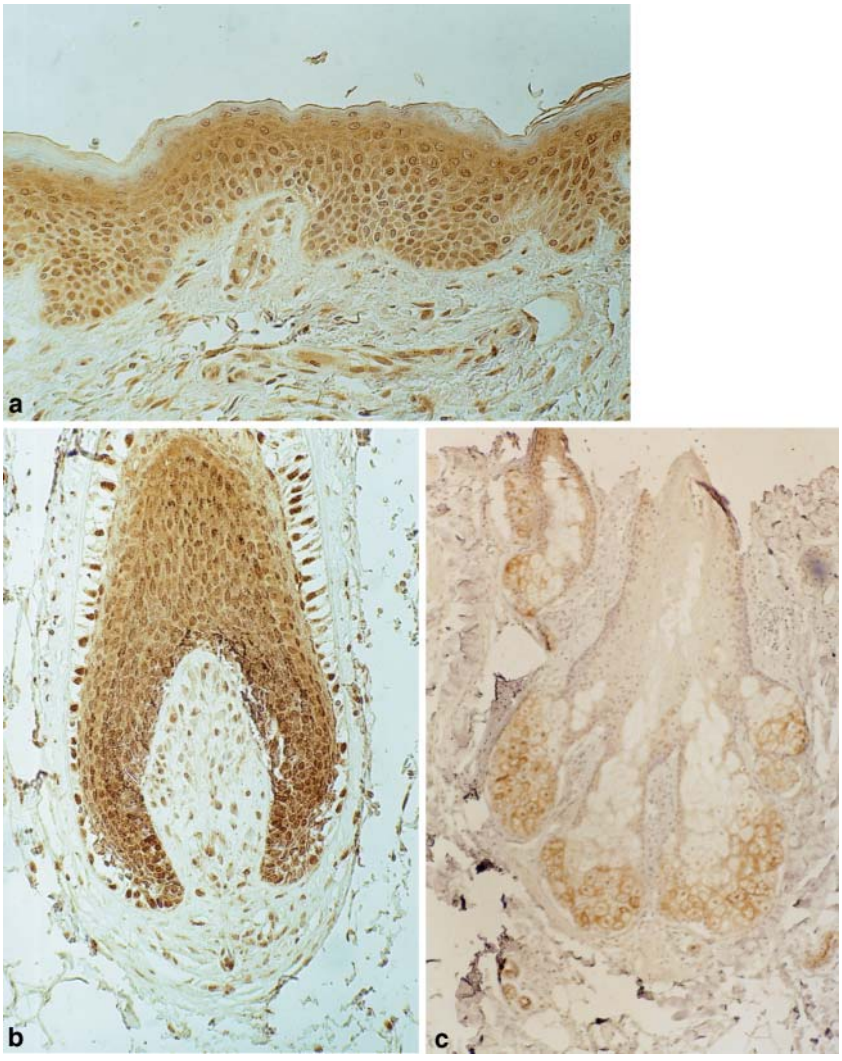

Figure 4. The immunolocalization of ERβ in human skin. Immunohistochemical localization of ERβ in human non-balding scalp skin. Bound antibodies were visualized with diaminobenzadine (DAB) and seen as brown nuclear staining. Sections were counterstained with hematoxylin. *(a)* Strong ERβ-staining was restricted to the keratinocytes of the stratum basale and stratum spinosum, while the cells of the stratum granulosum were less immunoreactive. The stratum corneum was devoid of immunoreactivity for ERβ. In addition to the staining seen in the epidermis, the underlying fibroblasts of the papillary dermis also showed strong nuclear staining for ERβ. *(b)* ERβ was widely expressed in the hair follicle bulb. Specific nuclear staining was seen in both the mesenchymal dermal papilla and connective tissue sheath cells and also in the epithelial hair matrix and outer root sheath cells. (c) ERβ was expressed in the partially differentiated sebocytes of the sebaceous gland. In these cells staining was not restricted to the nucleus, but was also present in the cytoplasm, particularly concentrated around the cell membrane.

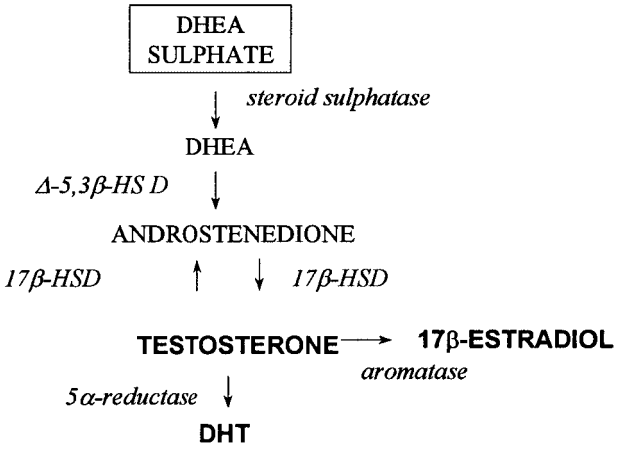

Figure 5. The peripheral conversion of active androgens and estrogens from adrenal precursors. The active androgens are testosterone and 5α-dihydrotestosterone (DHT), which both bind to the androgen receptor with a high affinity and the active estrogen is 17β-estradiol, which binds to both estrogen receptors with similar affinity. However, humans and some primates also secrete large amounts of the precursor steroids dihydoepiandrosterone (DHEA) sulphate and androstendione from the adrenal cortex, which can be converted, to the more potent androgens and estrogens by the appropriate enzymes. Δ-5,3β-HSD; Δ-5,3β-hydroxysteroid dehydrogenase. 17β-HSD; 17β-hydroxysteroid dehydrogenase. The importance of 17β HSD is illustrated by the occurrence of different enzymes with predominantly reductive or oxidative activity. Seven human 17β-HSDs have been cloned, sequenced and characterized. These exhibit different substrate specificity and the potential for different regulatory factors. Therefore, estrogen action in the skin may be locally or systemically regulated.

man scalp skin (55, 56). Strong ERβ expression was seen in human epidermis, with cells situated in the stratum basale and stratum spinosum being more immunoreactive than those in the stratum granulosum, while the stratum corneum was devoid of immunoreactivity (see Fig. 4a). In contrast, there was no specific nuclear staining for ERα. Strong nuclear staining for ERβ was also seen in the papillary dermis and the blood vessels. An independent study using semiquantitative RT-PCR has confirmed that ERβ mRNA is expressed in the skin of the mid-gestational human fetus (22).

Cell and tissue culture

Although significant advances in recent years have been made in both the organ culture of skin (57, 58) and the cell culture of the different cellular components of skin, including epidermal keratinocytes (59), melanocytes (60) and dermal fibroblasts (61), little progress has been made in the investigation of estrogen action in skin tissues.

Cultured human epidermal keratinocytes have been shown to express an estrogen receptor (62, 63), while the human keratinocyte cell line HaCaT is able to metabolize 17β-estradiol to estrone (64). Normal human melanocytes become enlarged and dentritic in culture, following a 2-day incubation with estradiol (65). More recently, cultured human epidermal melanocytes have been shown to contain estrogen receptors by ligand-binding studies (66). Furthermore, these workers were able to demonstrate a biological effect of 17β-estradiol on these cells, demonstrating that estrogens can increase epidermal melanocyte cell numbers, while decreasing melanin content and tyrosinase activity. In contrast, other studies have reported that estradiol significantly increases melanin synthesis and tyrosinase activity in normal human skin melanocytes in culture (67).

Several studies have demonstrated that cultured human dermal fibroblasts express aromatase (68–73), the enzyme responsible for the conversion of testosterone to estradiol (see Fig. 5). It has also been reported that cultured mouse dermal fibroblasts increase collagen synthesis by about 76% in response to estrogens (74), while recent studies have shown that human dermal fibroblasts express ERα and that there is an age-related increase in receptors in women not on hormone replacement therapy (75).

Other skin types including endothelial cells and macrophages are also probably estrogen responsive, possibly via membrane receptors as studies have shown that estradiol can induce a rapid rise in intracellular free calcium concentrations in macrophages (76) and induce the MAP kinase pathway in endothelial cells (77, 78). Indeed, the proliferation of skin hemangioma vascular endothelial cells in culture is stimulated by estradiol (79).

The hair follicle

Hair follicles have a built in rhythm of activity that results in the periodic growth of new hairs and the moulting of old ones. This rhythm appears to be intrinsic, although it can be influenced by hormonal or other systemic factors including gonadal, adrenal and thyroid hormones. It has been known for a number of years that ovarian hormones can significantly influence the hair cycle.

Animal hair follicles

In 1933, Dawson (80) reported that the regrowth of hair in clipped guinea pigs was higher in spayed females than in intact virgin or breeding females. Hooker & Pfieffer were among the early workers to demonstrate that estrogens inhibit hair growth (38). They noted that administration of large

amounts of estradiol for sufficient periods of time was followed by the failure of hair regrowth in rats. This was later confirmed by the work of Johnson (81) who showed that removal of the ovaries in rats accelerated the passage of the moult, increased the rate of hair growth and length of hairs, and accelerated the loss of club hairs. Treatment of ovariectomized rats with estradiol delayed the initiation of the wave, slowed its passage, reduced the rate of growth and definitive length and delayed the loss of the telogen club hairs. Spayed female rats shed more than 80% of telogen club hairs within 2 weeks of the start of anagen; implantation of estradiol delayed this process by 3–4 weeks. Therefore, estradiol can act as a brake by delaying the initiation of anagen and lengthening the duration of the resting period. The initiation of anagen has been shown to be delayed by more than 5 weeks in the ventral hair follicles of rats (81). Later Hale & Ebling demonstrated that estrogen reduces both the rate of growth and the ultimate length of spontaneously erupting hairs, by shortening the anagen period (82) while ovariectomy of rats tended to advance the spontaneous eruption of successive generations of hairs by shortening each complete hair cycle (83).

More recent studies by Smart and colleagues (42, 84, 85) have shown a direct cutaneous effect of estrogens and antiestrogens on mouse skin. The administration of topical estradiol in three different strains of mice maintains the hair follicle in telogen, blocking its transition into anagen, resulting in the inhibition of hair growth. Furthermore, administration of an antiestrogen stimulates the telogen follicle into anagen earlier than the corresponding control mice.

In animals such as rats and mice, hair follicle activity starts in one region and moves in a specific pattern over the body. Thus at any one time adjacent follicles appear synchronized over recognizable areas. In contrast such patterns are not recognizable in some species such as the guinea pig therefore hair follicle activity has been assumed to be random or mosaic. Closer examination of the types of hairs in guinea pigs has also shown a measure of synchrony, at least in the young animal and guinea pigs also respond to estrogens in a similar manner to the rat, with estradiol significantly decreasing the rate of growth in both ovarectomized and intact animals (86). In pregnant guinea pigs, Dawson reported a definite increase in the length of telogen making the rate of growth slower (80) and postulated that this shedding condition seen in mammals may be related to nest making, similar to a condition seen in some birds.

Administration of tritiated estradiol to shaved rat skin (87) and systemic administration has shown that the follicular target for estrogens in the rat by autoradiography is the dermal papilla (39). In all areas of the skin examined the radiolabel was always localized to the dermal papilla. There was also some localization to outer root sheath cells of the hair follicle, but in parallel with the epidermis there was some variation between different areas of the skin (39). However, it was not stated whether this variation was related to the hair cycle. Another study using whole rat skin homogenates has reported that there is some variation of estrogen binding with the hair cycle (41).

Immunohistochemical studies with mouse skin have localized ERα to the dermal papilla of telogen follicles (42), while molecular biological studies have identified the presence of ERα and not ERβ transcripts in mouse skin homogenates (85). However, other workers have found ERα to be poorly expressed in rat skin by immunohistochemistry, while immunostaining for ERβ was observed in the hair follicle of the rat (88).

Human hair follicles

A striking difference between human hair growth and most animal hair growth is a complete lack of synchrony between human hair follicles. However, in the neonatal human scalp and within each De Meijere trio group, there is some measure of synchrony, suggesting that perhaps human follicles do not cycle completely independently of one another. A striking phenomenon seen in women is postpartum alopecia, a condition in which large numbers of telogen club hairs are shed during the period after pregnancy, leading to a transient thinning of the hair. In 1960, Lynfield (89) was the first to suggest that there is an increase in the number of hairs in anagen during pregnancy (95% compared with 82% before pregnancy). Postpartum, this excess number of anagen follicles enters telogen resulting in an increase of hair shedding or postpartum effluvium. However, it is difficult to accredit these changes entirely to the increase in plasma estrogens as there are also other hormonal changes associated with pregnancy including rises in plasma progesterone and prolactin, which have also been shown to influence hair growth (90).

In adult human non-balding scalp skin from both sexes, a comparison of the expression of the two estrogen receptors by immunohistochemistry has shown that in contrast to ERα, ERβ is widely expressed in the hair follicle, with strong nuclear staining for ERβ evident in dermal papilla cells, connective tissue sheath cells, epithelial matrix cells and outer root sheath cells (see Fig. 4b). It is noteworthy that ERβ, and not ERα, is expressed

in the cells of the specialized bulge region of the outer root sheath (55, 56). This bulge region of the hair follicle is believed to contain the stem cells for both the epidermal and hair follicle keratinocytes (reviewed 91). There is some evidence that epithelial stem cells give rise to cutaneous tumours, that the hair follicle is an important source for skin cancers and that estrogens may promote this process. For example, it is thought that basal cell carcinomas originate from the hair follicle outer root sheath, possibly from the bulge region (91) and 17β-estradiol has been reported to markedly change the onset and development of both basal cell carcinoma and squamous cell carcinoma in rodents (44). These observations suggest that ERβ might mediate the 17β-estradiol-dependent modulation of epidermal carcinogenesis and that both skin cancers may have an ERβ-dependent component in their initiation or progression.

The expression of aromatase, the enzyme responsible for the conversion of testosterone to estradiol (see Fig. 5) also seems to have an important role in the hair follicle. Studies of plucked hair follicles from male scalp and pubic skin have demonstrated that they have the capacity to aromatize androstenedione to estrone, albeit the levels were low (92). When biopsies from men and women with androgenetic alopecia were compared, it was reported that aromatase levels were higher in hair follicles from occipital scalp when compared with those from the frontal scalp (93). Furthermore, the same study found that aromatase levels in the frontal hair follicles from women were approximately six times higher than those from male patients. In man and some primates the gonads are not the only source of estrogens. The adrenal cortex also secretes large quantities of the precursor steroids dihydoepiandrosterone (DHEA) sulphate and androstenedione, which can be metabolized to androgens and/or estrogens in peripheral target tissues (see Fig. 5). However, the conversion of these steroids depends exclusively upon the presence of the appropriate enzymes, which include steroid sulphatase, Δ-5,3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD), 5α-reductase and aromatase. To date, seven human 17β-HSDs have been cloned, sequenced and characterized with predominantly reductive or oxidative activity (94), which means that they can either increase or decrease the available estradiol depending on the properties of the enzyme.

Human hair follicles do not only possess the aromatase enzyme, but seemingly all of these steroid metabolizing enzymes (see Fig. 5), including steroid sulphatase (95), 3β-HSD, 17β-HSD and 5α-reductase (reviewed 96).

Hair follicle cell and tissue culture

Advances in cell and tissue culture techniques have allowed the study of the individual cell types that comprise the hair follicle (97–100). The organ culture of human scalp hair follicles has shown that 5 ng/ml estradiol has an inhibitory effect on hair growth (101). Conversely, the culture of outer root sheath keratinocytes and interfollicular keratinocytes from human scalp skin has demonstrated that incubation with pharmacological levels of estradiol above 180 nmol/l significantly increased thymidine incorporation in both cell types, although lower physiological levels had no effect (102). In the same study it was also demonstrated that the proliferation of dermal papilla cells in response to similar levels of estradiol was significantly higher than that of corresponding dermal fibroblasts. More recent studies have localized estrogen receptors to the dermal papilla cells and outer root sheath keratinocytes of human occipital scalp hair follicles both *in vivo* and *in vitro*, although it is not specified which of the two estrogen receptors is expressed (103). In addition, these workers were able to demonstrate that estradiol increases the secretion of vascular endothelial growth factor (VEGF) by cultured dermal papilla cells. Furthermore, this same group using RT-PCR have demonstrated that both cultured dermal papilla cells and outer root sheath keratinocytes express mRNA for aromatase (103).

Sexual hair growth

Although it is generally thought that adrenal androgens are responsible for the growth of pubic and axillary hair in human females (see Fig. 5), ovarian hormones may also play a role. Pubic hair can still develop in the presence of preadrenarchal levels of adrenal androgens in girls with precocious puberty or primary adrenal insufficiency (104). Females with primary ovarian insufficiency have very sparse pubic and axillary hair, if any, which can be stimulated to grow with adequate and prolonged estrogenic therapy (105). Although lack of female sexual hair is often attributed to inadequate adrenal androgens, many patients without pubic hair have adequate levels of adrenal androgens (104), demonstrating that sufficient secretion of DHEA sulphate or androstenedione does not ensure normal sexual hair growth. By treating patients with gonadal dysfunction with estrogens, pubic hair growth can be increased without affecting circulating levels of adrenal androgens (106). This implies that estrogens either alone or together with androgens act directly at the level of the hair follicle in pubic skin to stimulate hair growth.

Further evidence to suggest that estrogens may be important in pubic hair growth comes from case reports of infants that have been accidentally treated with estrogen-containing creams. The use of dermal ointments containing estrogens resulted in the growth of pubic hair in both male and female infants ranging from 4 months to 2 years (107). However, patients with complete testicular feminization have inactive androgen receptors and do not grow pubic and axillary hair, despite signs of estrogen effects in other tissues (108), which indicates that estrogens cannot promote sexual hair growth in the absence of a functional androgen receptor.

Interdependence of estrogen and androgen signaling pathways

It is now becoming apparent that tissues traditionally thought to be responsive to one class of steroids, contain receptors for other classes, and there is growing evidence that steroid receptors can cross-talk with one another (109). The presence of ERβ in the prostate and testis, classic androgen-target tissues, and experiments with estrogen receptor knockout mice has clearly indicated that ERβ plays an important role in prostate and testis development (110). This is further supported by the fact that prostate cancer often responds favourably to estrogen treatment due to interaction with androgen-dependent signaling pathways (111). In addition, there are numerous reports that estrogen and androgen metabolites can interact with both receptor subtypes. For example DHEA, testosterone and 5α-dihydrotestosterone (DHT) can interact with estrogen receptors in MCF-7 human breast cancer cells (112), while 5α-androstane-3β, 17β-diol, a metabolite of 5α- DHT interacts with estrogen receptors in the prostate (113). 17β-Estradiol also appears to be a natural ligand for androgen receptors in the prostate (114) and either androgens or estrogens can activate the non-genomic signaling pathways of ERα, ERβ and the androgen receptor (115). Taken together, this indicates that the roles of estrogens and androgens in the control of skin physiology and pathophysiology are probably interdependent and mechanistically complex (reviewed 56).

The sebaceous, eccrine and apocrine glands

Animal sebaceous glands

It has been known for many years that sebaceous gland activity is stimulated by androgens, but inhibited by estrogens (116, 117). Furthermore, the subcutaneous administration of estrogen causes a reduction in both size and number of sebaceous glands in the rat (38). Animal analogs including the rat preputial gland and the hamster costovertebral gland similarly demonstrate androgenic stimulation and estrogenic inhibition (118). In the male rat, preputial gland estrogens not only appear to suppress the uptake of testosterone *in vitro*, but they also suppress the conversion of testosterone to 5α-DHT (119). However, although estrogens also suppress the uptake of testosterone in castrated rats, they have no effect on the conversion of testosterone to 5α-DHT. In contrast, studies by Ebling demonstrated that while antiandrogens can block the effect of androgens at the point of action by inhibiting the uptake of tritiated testosterone in the rat sebaceous gland, estrogens do not, which suggests that estrogens must reduce sebaceous secretion by another pathway that is not mediated by the androgen receptor (120). Furthermore, estrogens can suppress androgen-stimulated sebaceous activity in doses lower than those required for antiandrogens (121, 122) and if estrogens are given together with an antiandrogen their effect is increased (120). However, the mechanism of action of estradiol in the sebaceous gland is distinct from that of an antiandrogen, in that it does not seem to decrease mitosis, instead appearing to act by inhibiting intracellular lipid synthesis to reduce sebum production (120). Although there has been much interest in the localization of androgen receptors in the sebaceous gland (55, 123, 124), there have been comparatively few studies on the localization of estrogen receptors. In rat skin, immunostaining for ERβ has shown that it is expressed in the sebaceous gland (88).

Human sebaceous glands

In humans the sebaceous glands enlarge at puberty, with increased production of sebum in both sexes, although sebum production is generally lower in females than males (125). In line with the effects seen in animals, large amounts of estrogens administered systemically can both reduce the size and inhibit sebaceous gland secretion in both sexes (125). Likewise, the administration of testosterone to female-to-male transsexuals results in an increase in sebum production, whereas the administration of an antiandrogen and estrogen to male-to-female transsexuals significantly reduces sebum production (126). After the menopause, sebaceous activity gradually decreases in the female, while it remains unaltered in men until the 7th or 8th decade before a decrease is seen (125). Conversely, a comparison of biopsies from the preauricular area of the cheek of premenopausal and postmenopausal women did not show any differences in the *in*

vitro metabolism of estrogens in the sebaceous gland based on unit weight (127). However, it must be noted that there was a large amount of variation between samples and the sample number was low. Nonetheless, it is probably more likely that the changes seen in postmenopausal women are due to the diminished plasma estrogen levels rather than changes in sebaceous gland metabolic activity. Although compounds that can reduce sebum production have important therapeutic potential, the use of estrogens as a treatment for acne has major drawbacks for men and the potential for unwanted side-effects in women. Nevertheless, the site of action of estrogens in the sebaceous gland is of considerable importance. Immunostaining for ERβ has shown that it is expressed in the human sebaceous gland (88), while a recent comparison of the expression of ERα and ERβ in human skin has shown that both receptors are expressed in basal and differentiating sebocytes (55, 56). Because *in vitro* studies have shown that ERα and ERβ can heterodimerize (24, 25), this supports the possibility of cooperative, synergistic or inhibitory actions between the two receptors (see Fig. 2), enhancing the potential for biological diversity of estrogen action in the sebaceous gland.

Sebaceous gland cell and tissue culture

Recently, the human sebaceous gland has successfully been cultured, maintaining an *in vivo* rate of lipogenesis and normal sebocyte differentiation for over 7 days in organ culture (128, 129). Furthermore these studies were able to demonstrate that physiological levels of estradiol significantly decreased lipogenesis without affecting the rates of cell division, supporting the earlier work by Ebling in the rat.

The apocrine gland

Similar to the sebaceous gland, the apocrine gland also develops from the outer root sheath of the hair follicle and remains attached to it, increasing in size and activity with sexual maturity. In contrast to the sebocytes, which undergo holocrine secretion, the epithelial cells of this specialized gland release their secretions by exocytosis. The rabbit has specialized apocrine glands in the submandibular, inguinal and anal regions, which enlarge at puberty and are heavier and more active in the male than the female (130). Prepubertal castration results in lighter and less active glands in the adult male, which can be reversed by the administration of testosterone. Severe reduction in both the weight and activity of the apocrine gland below the level of castrated animals can be produced by the administration of estradiol either when given alone to intact animals or when given together with testosterone to castrated animals (130). In the rabbit the submandibular glands appear to be the most sensitive to the effects of estradiol, while the inguinal glands are the least sensitive. Therefore, it would appear that estradiol acts directly at the site of the gland and not by the suppression of androgen production. It is interesting that two epidermal glandular derivatives with distinctly different characteristics, the sebaceous and apocrine glands, show remarkably parallel responses to control by androgens and estrogens. In humans, hidradenitis suppurativa, a disease presumed to involve the apocrine glands may also improve with estrogen treatment. A recent study of women with papillary hidradenitis demonstrated that approximately 90% of anogenital glands were positive for ER by immunostaining, while typical eccrine and apocrine glands were devoid of ER immunoreactivity, suggesting that these specialized glands may indeed be controlled by estrogens (131).

The eccrine gland

In contrast to the apocrine glands, the sweat or eccrine glands develop from the superficial epidermis and remain independent of the hair follicle, although they also employ a merocrine mode of secretion. In parallel to the sebaceous and apocrine glands they have also been reported to contain androgen receptors (55, 132, 133), while the role of estrogens is less clear. Recently, immunohistochemical studies by two separate laboratories have demonstrated the presence of ERβ, but not ERα in human eccrine glands (55, 88).

Pigmentation

Animal epidermal melanocytes

A number of studies have shown that epidermal melanocytes are estrogen responsive, although the responses are often contradictory and difficult to interpret. In female guinea pigs, after ovariectomy the melanin content of epidermal melanocytes decreases; many become smaller in size and exhibit shortened dendritic processes (134). Furthermore, ovariectomized animals that were treated with estradiol either locally or systemically showed an increase in melanin both inside and outside the melanocytes in all regions examined. In Syrian golden hamsters pigmented melanocytes are restricted to the hair bulbs and occasional networks surrounding the pilosebaceous unit. These melanocytic networks are seen as small pigment spots resembling melanocytic naevi seen in humans and occur more

frequently in the female hamster than the male (135). When male hamsters were subjected to subcutaneous implants of estrogens an increase in both size and number of the small pigmented spots was seen, although it was not determined whether this was due to the production and migration of new melanocytes or the accumulation of pigment in pre-existing ones (135). The melanocytes in scrotal skin are controlled by androgens and are often distributed in a manner that allows qualitative measurements (136). A study of intact male Syrian hamsters demonstrated that estradiol produced a dose-related decrease in the number of scrotal skin melanocytes (137). Furthermore, in the same study, the 5α-DHT-induced increase in pigmentation of the costovertebral spot hair follicles was reversed by estradiol.

A link between ovarian activity and alterations in the pigmentation of sex skin in female non-human primates has also been known for a number of years (138, 139). After ovariectomy, the sex skin of female rhesus monkeys is paler. A more recent study (140) has shown that the administration of either testosterone or estradiol significantly increased redness in the sex skin area of male rhesus monkeys, while 5α-DHT had no effect. However, the aromatase inhibitor fadrozole decreased redness, suggesting that estrogen regulates skin color in males via the aromatization of testosterone. Because there was no increase in serum estradiol, the effect must be due to local metabolic processes giving rise to an enhancement of estrogen action in the skin.

Human epidermal melanocytes

In humans, an increase in cutaneous pigmentation due to an increase in ovarian and/or pituitary hormones is also common during pregnancy with regression using occurring after parturition. Chloasma is a well-characterized form of hyperpigmentation of the face commonly seen in pregnant women that may often be accompanied by increased pigmentation in other areas including the areolae, linea alba and perineal skin, all of which usually fade after parturition. There is also some evidence that fluctuations of these hormones during the menstrual cycle may effect epidermal pigmentation in some women. In one study, 62% of women consistently noticed darkening of the periocular skin towards the end of their menstrual cycle immediately prior to menstruation (141). There have also been reports of estrogen-containing oral contraceptives causing hyperpigmentation of the face in 8–29% of women (142). The use of dermal ointments containing estrogens has also resulted in the intense pigmentation of the genitals, mammary areola and linea alba of the abdomen in both male and female infants ranging from 4 months to 2 years (107).

Hair follicle melanocytes

In addition to the epidermal melanocytes, another population of melanocytes are those found in the hair follicle, which are distinct to those found in the epidermis (143). It has been reported that estrogens do not appear to have a definite effect on the hair color of guinea pigs, suggesting that the two types of melanocytes respond differently to this steroid at least in this species (144). However, it must be noted that the methods of assessment in this study were different to the earlier study on epidermal melanogenesis in the guinea pig by the same group (134). In the epidermis, the morphology of the melanocytes and surrounding keratinocytes was studied, while only the hairs and not the follicular melanocytes were examined in this study. Therefore, any morphologic changes in follicular melanocyte activity in response to estradiol could have been overlooked. In contrast to the guinea pig, studies with C56Bl/6 mice have shown that hair pigmentation is seen earlier after the topical application of an antiestrogen when compared with control mice (84), suggesting that estrogens may inhibit follicular melanocytes. Further evidence for this comes from the generation of aromatase knockout mice, who produce a significantly darker coat than their wildtype or heterozygote counterparts due to an increase in total melanin and eu-melanin (145). Therefore it would seem that estrogens inhibit hair pigmentation, at least in mice. However, whether estrogens act directly on follicular melanocytes, or indirectly via the activation of other follicular cells remains to be established. Nonetheless, it is interesting that normal epidermal and follicular melanoctyes appear to exhibit opposing responses to estrogens. Interestingly, although women report changes in skin pigmentation during pregnancy, there appear to be no reports of hair color changes. However, the length of anagen on the human scalp is considerably longer than in any animal commonly used to investigate hair growth, therefore the relative length of pregnancy may well be too short to see any significant changes.

Malignant melanoma

Understanding the normal physiology of epidermal melanocytes is important in understanding the pathogenesis of malignant melanoma. Although there is little direct evidence of a role for estrogens in this disorder, there is sufficient epidemiological

evidence to suggest that estrogens may have an important function in this disease as females appear to have a sex survival advantage (reviewed 146 and references therein), although there are other studies that oppose this view (147, 148). Nonetheless, the presence of estrogen-binding proteins has been shown both in malignant melanoma hamster cell lines (149, 150) and human melanoma cell lines (151), although other melanoma cell lines reportedly do not bind estrogens (150, 151). However, the choice of assay conditions appears critical when characterizing estrogen binding in pigmented cells, as the radio-labeled by-products of the tyrosinase catalysed oxidation of tritiated estradiol are not absorbed by dextran-coated charcoal, normally used to separate bound from free ligand (152).

Studies on the hormonal growth regulation of melanoma *in vitro* are also confusing and inconclusive. There are some reports that estradiol seems to have no effect on the proliferation of human melanoma cell lines in culture (151, 153), while others report an inhibition of ^{3}H-thymidine uptake in human metastatic melanoma cell lines incubated with estradiol which can be counteracted by an antiestrogen (154) and a dose-related inhibition of the growth of a malignant hamster melanoma cell line by estradiol (150). Furthermore, estradiol can inhibit the invasion of human melanoma cells through fibronectin (153), and athymic mice carrying an estrogen-receptor positive human melanoma cell line show a sex-dependent increase in tumor latency and an overall inhibition of tumor growth after treatment with estradiol (151).

There is also some discrepancy between the presence of specific estrogen binding in human melanomas and a lack of a significant response to endocrine therapy. Although one study detected binding activity in over 100 patients with melanoma, there was no correlation with a response to endocrine manipulation (155). In addition, pure-antiestrogen agents do not appear to have an antiproliferative effect on melanoma cells lines (146). Furthermore, although biochemical assays have shown estrogen-binding proteins to be present in malignant melanoma, estrogen receptors, or at least ERα have not been detected by immunohistochemistry (156). These conflicting data suggests the mechanism of estrogen action in the melanocyte is complex. The role of ERβ has yet to be explored, as does the role of other signaling pathways that can be regulated by estrogens. Some of the apparent contradictions in estrogen action on normal and malignant melanocytes may be clarified by the study of the actions of the emerging class of selective estrogen receptor modulator (SERM) compounds.

Concluding remarks

The skin is an important, albeit generally overlooked, estrogen-responsive endocrine tissue. Not only can it respond to estrogens via specific receptors, but it also has the capacity to synthesise active steroids from inactive adrenal precursors. However, in order to understand the effects of estrogens on skin aging, pigmentation, hair growth, sebum production and skin cancer, there is an overwhelming need to widen our understanding of the physiology and interaction of steroid hormones and their receptors in human skin. Clearly estrogens have an important role in many components of human skin including the epidermis, dermis, vasculature and pilosebaceous unit, yet we still do not understand the molecular processes involved. The discovery of a second estrogen receptor (ERβ) that appears to have different cell-specific roles to the classic estrogen receptor (ERα) and its presence in human skin indicates the potential for enhanced diversity in the mechanism of estrogen action. Furthermore, the emerging degree of interdependence between estrogens and androgens in some tissues suggests that such interactions may also be vital in human skin. Although it has been recognized for some time that androgens have an important role in the regulation of the pilosebaceous unit, the exact interaction of estrogens and androgens in the pilosebaceous unit is at present unclear. Once we start to understand and unravel these complex interactions between steroid hormones and their receptors we will have a better understanding of skin physiology and pathophysiology

With new advances in molecular biology and cell and tissue culture techniques, perhaps it is now time to readdress many of the outstanding questions regarding the role of estrogens in skin. Not only will this lead to a complete and better understanding of estrogen action, but may also provide a basis for further interventions in pathological processes that involve disregulation of estrogen action.

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