Towards a molecular understanding of hair loss and its treatment

George Cotsarelis and Sarah E. Millar

Most common forms of hair loss (alopecia) are caused by aberrant hair follicle cycling and changes in hair follicle morphology. However, current treatments for alopecia do not specifically target these processes. We are now beginning to identify the molecules and molecular pathways that control normal hair follicle formation, cycling and growth. In parallel, new techniques are being developed for delivering molecules to hair follicles. Here, we outline the characteristics of common hair loss diseases, and discuss ways in which recent advances in hair follicle biology could be translated into effective therapies for these conditions.

Although hair disorders are not life threatening, their profound impact on social interactions and on patients' psychological well being is undeniable. The demand for treatments for hair loss fuels a multi-billion dollar industry. Despite this, most currently marketed products are ineffective, evidenced by the fact that the FDA has approved only two treatments for hair loss. Recently there have been dramatic advances in our understanding of the molecules and pathways regulating hair follicle formation and hair growth. Here we review these findings and discuss how they might stimulate the development of new, effective therapies.

Introduction to the hair follicle

The end product of hair follicle proliferation and differentiation is the hair shaft, which, together with its surrounding root sheaths, is derived from epithelial cells. The dermal papilla, a cluster of mesenchymal cells at the base of the follicle, also plays an essential role in hair growth (Fig. 1). In humans the formation of hair follicles takes place during embryogenesis, and no new hair follicles form after birth. However, the character of individual follicles can change drastically over time. Thicker and darker hairs replace fine lightly pigmented hairs in the beard at puberty. Conversely, thick scalp hairs convert into fine small hairs later in life. Paradoxically, both processes occur in response to the hormone testosterone.

The hair follicle remodels itself during cyclical periods of growth (ANAGEN, see Glosssary), regression (CATAGEN), rest (TELOGEN) and shedding (EXOGEN)[1] (Fig. 2). During catagen, much of the follicle undergoes programmed cell death (apoptosis)[2], reducing its size as it enters telogen. Follicular regeneration at the onset of the next anagen phase requires the activation of rarely cycling epithelial stem cells located in the permanent, BULGE region of the follicle[3]. Stem cell progeny form a new follicle matrix during early anagen, and the hair shaft and inner root sheath are derived from these relatively undifferentiated matrix cells[4]. The size and length of the hair shaft correspond to the size of the hair follicle and to the duration of anagen, respectively. These characteristics vary considerably with body site, and change as a result of disease. Pigmentation of the hair shaft depends on hair follicle melanocytes, which reside in the hair follicle BULB and deposit melanin into the growing hair shaft. Proliferation of melanocytes occurs during early anagen[5], and is probably regulated by the factors that control the hair growth cycle.

Common disorders of hair growth

ALOPECIA, a generic term for hair loss, results from a diminution of visible hair. The most common forms of alopecia include ANDROGENETIC ALOPECIA or common baldness, TELOGEN EFFLUVIUM, chemotherapy-induced alopecia, and ALOPECIA AREATA[1].

Androgenetic alopecia

In androgenetic alopecia, hair follicles located in specific patterns over the male and female scalp diminish in size over time until they produce effete and cosmetically insignificant hairs (Fig. 3). Testosterone is required, along with a genetic predisposition, for androgenetic alopecia to develop in men[6]. In women, there is no consensus on whether pattern hair loss is truly androgen-dependent, although both male- and female-pattern alopecia result in a decrease in hair follicle size accompanied by a decrease in the duration of anagen and an increase in the percentage of hair follicles in telogen. In addition, several months can transpire between hair shedding and regrowth, a lag period that is absent or fleeting in normal individuals (Fig. 3)[7]. These changes result in very short hairs and follicles devoid of hair shafts. Miniaturized hairs also lack pigmentation. In advanced androgenetic alopecia some follicles disappear and are replaced by fibrous tracts (Fig. 3).

Based on this knowledge, the goals for treating androgenetic alopecia include prolonging anagen, converting telogen follicles to anagen, reversing miniaturization and possibly generating new follicles. Blocking the conversion of testosterone to its more active metabolite, dihydrotestosterone, through administration of the pharmacologic agent finasteride accomplishes some of these goals and clearly benefits patients with early androgenetic alopecia[1]; however, even drastic forms of testosterone

George Cotsarelis*
Dept of Dermatology

Sarah E. Millar
Depts of Dermatology and Cell and Developmental Biology, University of Pennsylvania School of Medicine, M8 Stellar-Chance Laboratories, 422 Curie Blvd, Philadelphia, PA 19104, USA.
*e-mail: cotsarel@mail.med.upenn.edu

  PII: S1471-4914(01)02027-5

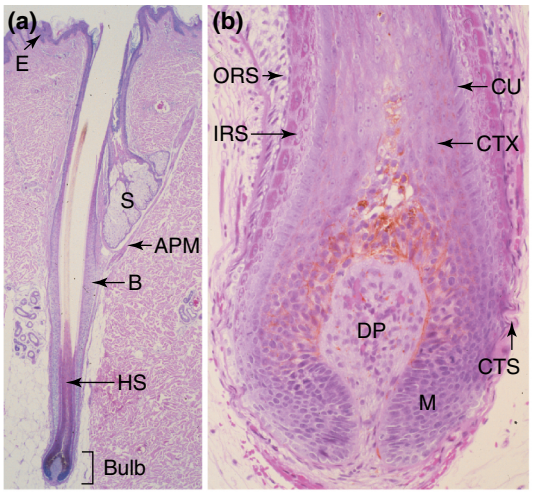

Fig. 1. Light photomicrographs showing structure of sectioned anagen stage human scalp hair follicle stained with hematoxylin and eosin. (a) A full-length longitudinal section of a follicle photographed at low magnification. (b) Higher-magnification photograph of the bulb region of a hair follicle. Abbreviations: APM, arector pili muscle; B, bulge; CTS, connective tissue sheath; CTX, cortex of hair shaft; CU, cuticle of hair shaft; DP, dermal papilla; E, epidermis; HS, hair shaft; IRS, inner root sheath; M, matrix; ORS, outer root sheath; S, sebaceous gland. Photomicrographs courtesy of Edward Chan.

Glossary

Alopecia: Abnormal hair loss.
Androgenetic alopecia: Loss of hair caused by miniaturization of genetically predisposed follicles in a male pattern (frontal recession and thinning at the vertex) or female pattern (loss of hair over the crown with sparing of the frontal hair line).
Alopecia areata: Hair loss in patches caused by an autoimmune inflammatory response to the follicle.
Anagen: Growing stage of the hair follicle cycle.
Bulb: Lowermost portion of the anagen follicle containing rapidly proliferating cells that generate the hair.
Bulge: Putative site of epithelial stem cells within the follicle outer root sheath.
Catagen: Regression stage of the hair follicle cycle.
Club hair: Dead hair possessing thickened base that anchors it in the follicle during telogen.
Exogen: Stage of the hair follicle cycle when hair is shed from the follicle.
Telogen: Resting stage of the hair follicle cycle.
Telogen effluvium: Excessive shedding of hair caused by synchronous entry of many hair follicles into exogen.

reduction (e.g. castration) do not result in complete reversal of miniaturization. Thus more effective treatments are required for the treatment of advanced androgenetic alopecia.

Telogen effluvium

Telogen effluvium, another extremely common form of alopecia, manifests as excessive shedding of hair. Several different mechanisms can cause telogen effluvium, but all result from the synchronous entry of many follicles into exogen[8]. For example, during pregnancy hair follicles tend to remain in anagen, but delivery causes synchronous entry of follicles into telogen, soon followed by exogen (shedding). A different type of telogen effluvium is observed soon after starting medications such as minoxidil, or in response to a rapid change in the duration of daily exposure to sunlight. In these cases existing telogen follicles simultaneously enter a premature exogen phase, involving an increase in the shedding of CLUB HAIRS within weeks of the precipitating event[8]. This observation suggests that club hairs that are normally retained in the follicle can be actively shed. Finally, metabolic events such as fevers or medications can cause premature entry of follicles into telogen.

Although one might argue that therapies for telogen effluvium are not necessary because the follicles remain intact and generate new hairs after several months, the psychological trauma from hair shedding should not be underestimated. One treatment strategy would be to inhibit exogen, so that hair shafts would be retained in their follicles until the new hair grew to an acceptable length. This type of treatment would also benefit patients with androgenetic alopecia, which can initially present as telogen effluvium.

Chemotherapy-induced alopecia

Chemotherapy disrupts the proliferation of matrix keratinocytes in the anagen bulb that produce the hair shaft (Figs 1 and 2). This forces anagen follicles to enter a dystrophic catagen stage in which the integrity of the hair shaft is compromised and the hair then breaks and falls out. Because more than 90% of scalp follicles are in anagen at any one time, these hairs are rapidly lost after chemotherapy, and thus the alopecia is rapid and extensive. Hair loss is one of the most feared side effects of chemotherapy among patients with cancer. However, hair lost following chemotherapy does eventually re-grow, presumably because the rarely cycling follicular stem cells are relatively unaffected by chemotherapy and generate a new hair follicle and hair.

Botchkarev *et al.* have shown that p53 is necessary for the development of chemotherapy-induced alopecia, as p53 knockout mice treated with chemotherapeutic agents remarkably do not lose their hair[9]. Because chemical inhibitors of p53 already exist, testing their efficacy for local inhibition of p53 in hair follicles provides a realistic possibility for preventative therapy. In an exciting recent development, Davis *et al.* showed that inhibition of the cell cycle regulator cyclin-dependent kinase 2 (CDK2) using a novel, topically applied CDK2 inhibitor, prevents chemotherapy-induced alopecia in rats[10]. Human skin maintained as grafts on immunodeficient mice was also sensitive to the actions of the CDK2 inhibitor, suggesting that this compound will be effective for preventing chemotherapy-induced hair loss in human patients.

Alopecia areata

Alopecia areata is an autoimmune disorder in which cells of the anagen hair bulb are attacked by lymphocytes (Fig. 4). In a process similar to that following chemotherapy, anagen follicles enter dystrophic catagen and the hair shaft breaks off. In most patients hair loss occurs in scattered patches over the scalp; however, some lose all scalp and body hair (called alopecia totalis and universalis, respectively). Gilhar[11] demonstrated that alopecia areata is a T-cell

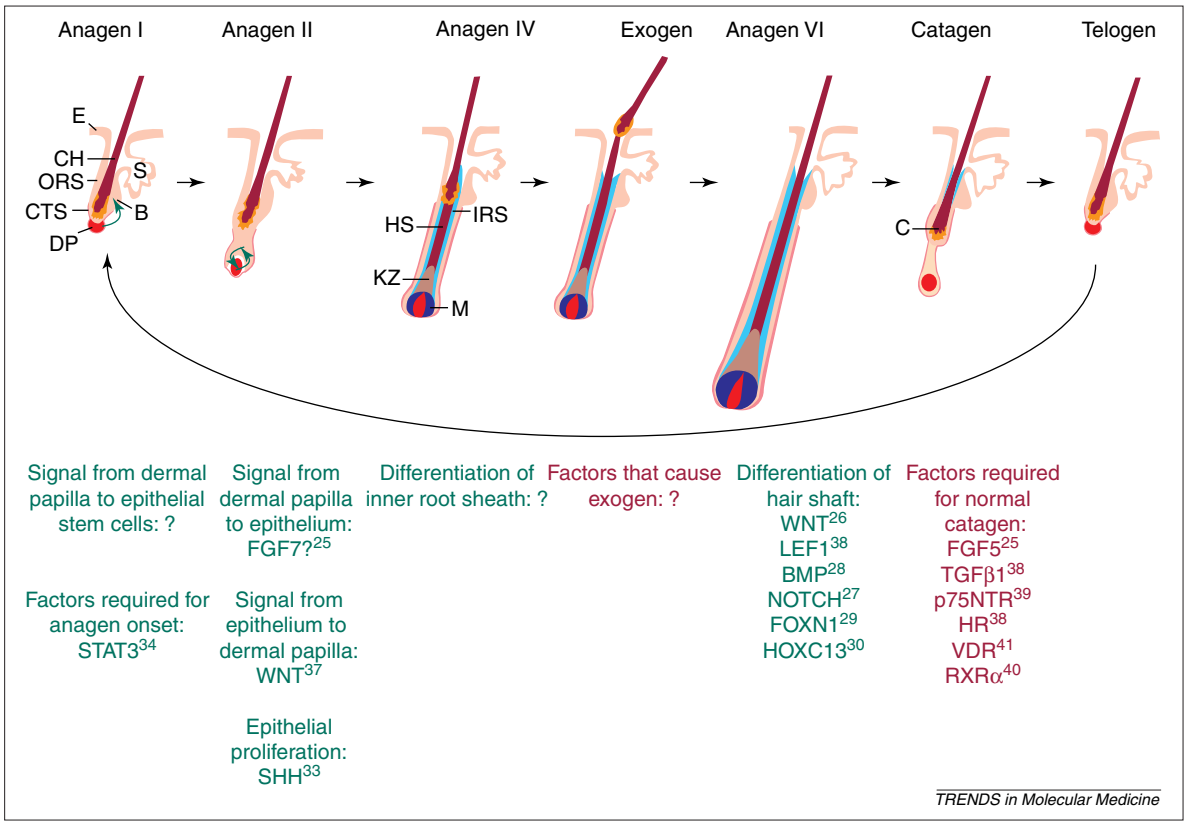

Fig. 2. Factors regulating hair growth and control of the hair follicle cycle. At anagen onset (anagen I) an unknown signal from the dermal papilla is thought to direct transient proliferation of stem cells in the bulge (green arrow). During anagen, signals from the dermal papilla regulate the proliferation of hair matrix cells, whereas epithelial cells maintain the inductive properties of the dermal papilla (green arrows in base of anagen II follicle). A new hair shaft is produced during anagen, and the old hair is released from the follicle as the new shaft develops (anagen IV/exogen). Lateral signaling between differentiating cells might maintain the separate pathways of differentiation of hair shaft and inner root sheath cells during anagen. During catagen the lower two thirds of the epithelial follicle are destroyed but the dermal papilla remains associated with the regressing follicle. The hair develops a club structure at its base, which retains the hair in the follicle. The follicle then enters the resting, telogen phase until a new growth cycle is initiated. Abbreviations: B, bulge; BMP, bone morphogenetic protein; C, club signal transducer and activator of transcription 3 (STAT3); CH, club hair; CTS, connective tissue sheath; DP, dermal papilla; E, epidermis; FGF, fibroblast growth factor; FOXN1, formerly WHN, winged helix nude; HOXC13, homeobox gene C13; HR, hairless; HS, hair shaft; IRS, inner root sheath; LEF1, lymphocyte enhancer factor 1; KZ, keratogenous zone (differentiating cells); ORS, outer root sheath; p75NTR, p75 neurotrophin receptor; RXRα, retinoid X receptor-α; S, sebaceous gland; SHH, sonic hedgehog; M, matrix; TGFβ1, transforming growth factor β1; VDR, vitamin D receptor.

mediated disease by injecting T-cells from patients into human skin grafted to immunodeficient mice. This reproduces the peri-bulbar inflammation and hair loss. Possible targets of the immune attack include matrix keratinocytes, dermal papilla cells and melanocytes. Although a genetic predisposition for this disease is supported by HLA linkage studies, whether the underlying defect lies within the hair follicle, the immune system, or both, is not known. The hair loss is potentially reversible, even after years without hair, confirming the non-scarring nature of the inflammatory process, which spares the stem cell-rich bulge area (Fig. 4).

By contrast, in scarring alopecias such as lichen planopilaris or discoid lupus erythematosis, inflammation occurs higher up in the follicle and generally involves the bulge (Fig. 4). This leads to fibrosis and permanent loss of the follicle. The inciting events in scarring alopecia are unknown, but could involve aberrant functioning of the sebaceous gland, which appears necessary for normal detachment of the inner root sheath from the hair shaft[12] causing follicle rupture followed by an abnormal fibrotic wound healing process that leads to deletion of the follicle[1].

Possible treatment strategies

Identification of the genes involved in both androgenetic alopecia and alopecia areata will be facilitated by microarray technology and by the availability of human and mouse genome sequences, and might ultimately reveal novel therapeutic targets. In the cases of alopecia areata and scarring alopecia, rodent models have been identified that will aid in the localization of susceptibility loci, and the screening of new treatments[13,14]. A rodent model is not currently available for androgenetic alopecia. However, human skin grafted to immunodeficient mice provides a potential system for investigating the molecular basis of this process, and a non-human primate model, the stump-tailed macaque (Macaca artoides), has been used to test the efficacy of various therapies[15].

Treatments for both alopecia areata and inflammatory scarring alopecias must modulate the inflammation or protect the follicle from attack by the autoimmune process. A potential approach for

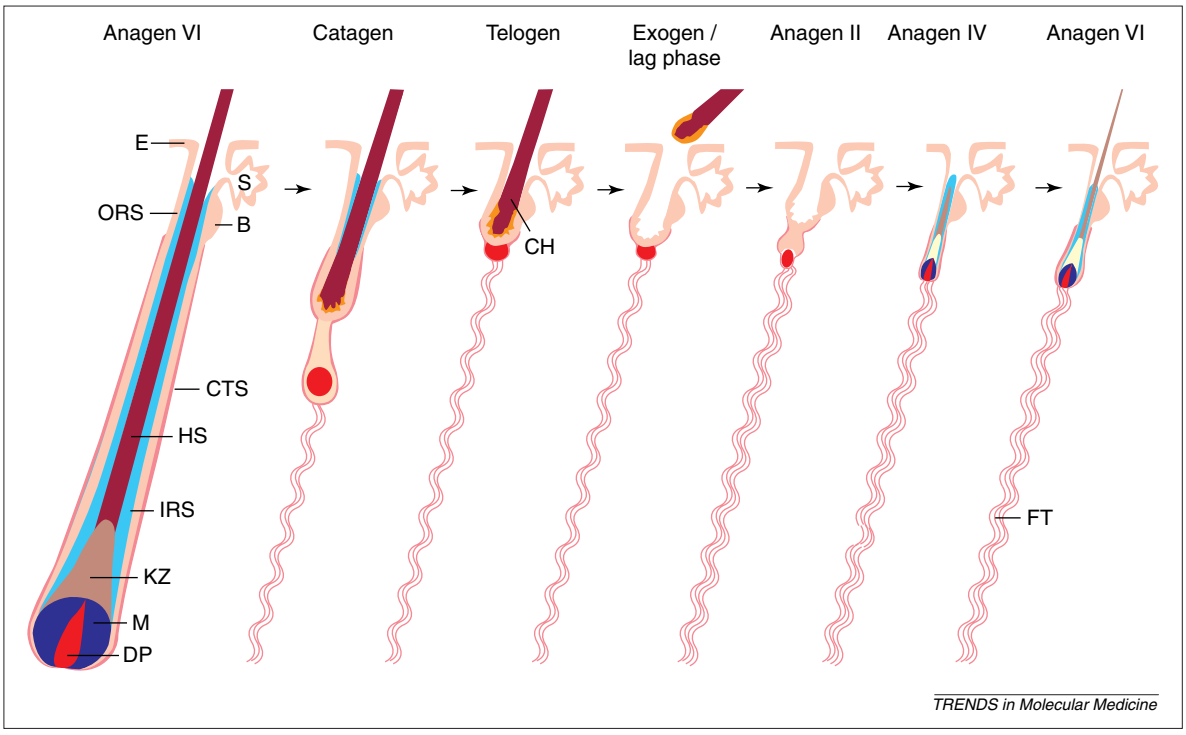

Fig. 3. Miniaturization of hair follicles in androgenetic alopecia. As the follicle regresses during catagen, it is trailed by a collagenous fibrous tract. Following telogen, follicles undergoing miniaturization enter an abnormal, prolonged lag phase, during which the hair shaft is shed. The fibrous tract becomes abnormally thick and could impede subsequent growth of the follicle. Abbreviations: B, bulge; CH, club hair; CTS, connective tissue sheath; DP, dermal papilla; E, epidermis; FT, fibrous tract; HS, hair shaft; IRS, inner root sheath; KZ, keratogenous zone; M, matrix; ORS, outer root sheath; S, sebaceous gland.

developing the latter type of therapy would be to identify primary antigenic targets in the follicle bulb and induce tolerance in the corresponding population of T-cells. Ideally treatments for scarring alopecia would also involve recreating hair follicles at sites of destruction. This might require treatment with anti-fibrotic agents as well.

Molecular mechanisms regulating hair follicle morphogenesis

Formation of hair follicles in the embryo requires interactions between cells of the surface epithelium and the underlying dermis[16]. Hair follicle precursors are first visible as thickenings or placodes in the otherwise uniform surface epithelium (Fig. 5). Placodes form in a regular array by a process that probably requires the competing activities of placode-inducing and placode-repressing molecules[17]. Bone morphogenic protein 2 (BMP2), a repressor of placode formation, and several candidate placode-promoting molecules are initially expressed uniformly in the surface epithelium and subsequently become localized to placodes, consistent with a reaction-diffusion model of pattern formation[17–20].

Once hair follicle placodes form, epithelial signals, possibly including WNT proteins[21], pass from the placodes to the underlying dermis, causing the clustering of a group of cells (the dermal condensate) that will eventually form the dermal papilla (Figs. 1 and 5)[16]. Formation of the dermal papilla requires the secreted signaling molecules sonic hedgehog (SHH)[22,23] and platelet derived growth factor-A (PDGF-A)[24].

A 'second dermal' signal from the dermal condensate to the follicular epithelium directs the proliferation and downgrowth of follicular epithelial cells into the dermis, a process that also requires SHH expression in the epithelium (Fig. 5)[16,22,23]. Mutations in follistatin (FS) and in TGFα and its receptor, epidermal growth factor receptor (EGFR), cause abnormal follicle architecture and wavy hair, suggesting essential roles for these factors in regulating follicle shape[25].

Formation of the inner root sheath, which functions to mold the hair shaft, and differentiation of the hair shaft itself are processes likely to be regulated by signals from the dermal papilla, and by lateral signaling between epithelial cells at different positions in the follicle relative to the dermal papilla[26,27]. WNT, BMP and Notch intercellular signaling molecules and the LEF1, FOXN1 and HOXC13 transcription factors have been implicated in the regulation of hair shaft differentiation[26–30].

Ectopic expression of either SHH or a stabilized form of β-catenin in the epidermis causes the formation of additional follicles[31], raising the exciting possibility that these factors might form a component of therapies aimed at recreating hair follicles

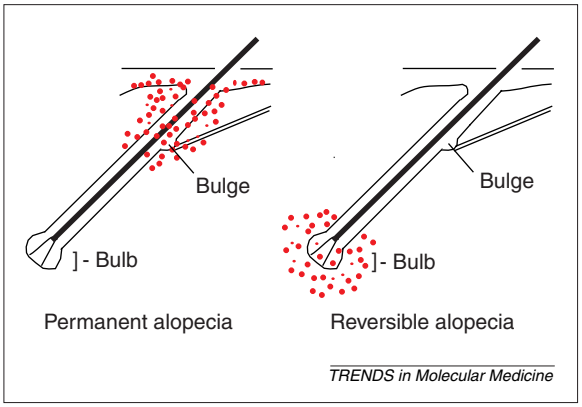

Fig. 4. Model of inflammatory alopecias. The integrity of stem cells in the bulge might determine the follicle's ability to re-form after injury. In 'permanent alopecias,' such as lichen planopilaris and discoid lupus erythematosis, infiltrating immune cells, depicted as red dots, concentrate around the upper portion of the follicle, including the bulge. In 'reversible' alopecias, such as alopecia areata, inflammation generally spares the bulge region and the follicle can regenerate. Intriguingly, in androgenetic alopecia, the hair follicle bulge area is often surrounded by T cells, suggesting a pathogenetic role for the inflammation[69].

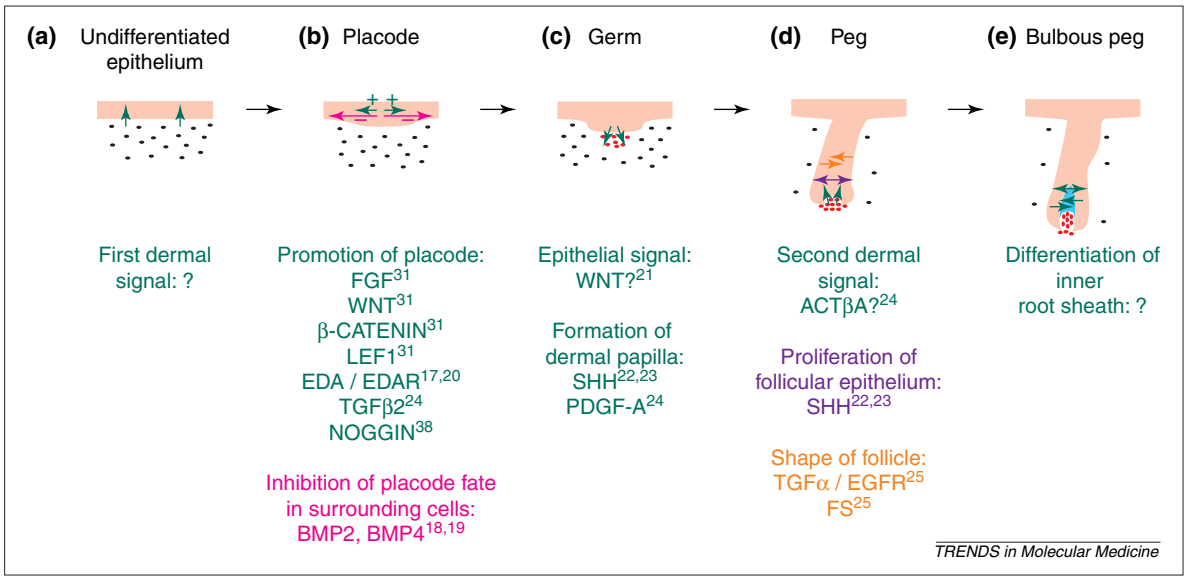

Fig. 5. Intercellular signals operating in hair follicle morphogenesis. (a) Formation of hair follicle placodes is initiated by an unknown signal (green arrows) from the dermis (black dots) to the surface epithelium (pink). (b) Hair follicle placode formation is promoted by fibroblast growth factor (FGF); WNT, acting via its effectors β-catenin and lymphocyte enhancer factor 1 (LEF1); ectodysplasin (EDA) and ectodysplasin receptor (EDAR); transforming growth factor β2 (TGFβ2); and Noggin (green arrows with 'plus' signs). Placode formation is inhibited by bone morphogenic proteins (BMPs) (dark pink arrows with 'minus' signs). For simplicity, placode promoting and repressing signals are diagrammed only in the epithelium. In reality, however, noggin and BMP4 are expressed in follicular mesenchyme, and β-catenin, LEF1, TGFβ2 and BMP2 are expressed in both epithelium and mesenchyme. (c) Formation of the dermal condensate, shown as red dots, is induced by an epithelial signal (green arrows), possibly including a WNT. Development of the dermal condensate into a dermal papilla requires the activity of sonic hedgehog (SHH) and platelet derived growth factor-A (PDGF-A). (d) A second dermal signal, possibly including activin βA (ACTβA) (green arrows), instructs the follicular epithelium to grow down into the dermis. Proliferation of the follicular epithelial cells is regulated in part by SHH (blue arrows). Transforming growth factor α (TGFα), epidermal growth factor receptor (EGFR) and follistatin (FS) are required for normal follicle architecture (orange arrows). (e) Differentiation of inner root sheath precursor cells (pale blue) is regulated by unknown signals (green arrows).

destroyed in scarring alopecia or advanced androgenetic alopecia. It should be noted, however, that activation of SHH or β-catenin signaling in the epidermis also causes basal cell carcinoma or pilomatricoma, a tumor of the hair follicle matrix, respectively[31]. Future therapies based on hair follicle-inducing molecules might therefore require the application of a delicate balance of inducers with inhibitors that limit the actions of these powerful molecules, mimicking the situation in normal morphogenesis.

Molecular mechanisms regulating the hair growth cycle

Anagen onset

Signaling molecules important for hair follicle morphogenesis might be re-used in postnatal life to control follicular cycling. The factors that normally trigger the proliferation of bulge cells at the onset of anagen have not been identified, but they are thought to arise from the juxtaposed dermal papilla (Fig. 2)[3]. Exogenous SHH is capable of initiating anagen in telogen follicles[32]. However, the effects of a neutralizing anti-SHH antibody on hair growth suggest that SHH is normally required for the proliferation of epithelial cells slightly later in anagen, but not for conversion of telogen follicles to anagen[33]. Mutation of mouse signal transducer and activator of transcription 3 (STAT3) prevents normal progression of telogen follicles into anagen[34]. Anagen onset in mice is also blocked by estradiol and promoted by antagonists of estrogen receptor (ER) and parathyroid hormone responsive protein (PTHrP)[35,36]. Whether these factors play a role in human hair follicle cycling is not yet known.

Anagen

It is well accepted that the volume of the dermal papilla determines the number of matrix cells and size of the resulting hair shaft[16]. Because the size of the follicle is determined during the early stages of anagen, this could be a critical time for hair follicles undergoing miniaturization in androgenetic alopecia or for follicles that are enlarging, for instance in the beard area of an adolescent boy. Some factors (e.g. hormones, drugs, morphogens) might act by enhancing or preventing miniaturization only during this window of time at anagen onset. Therefore, they will require long periods of time to alter a significant number of follicles. This might partially explain why the process of miniaturization takes years to both develop and treat.

As anagen proceeds, signals from the dermal papilla to the follicular epithelium are necessary to maintain epithelial proliferation (Fig. 2). FGF7, which is expressed in the dermal papilla and has strong mitogenic effects on epithelial cells, might play a role in this process. However, FGF7 knockout mice exhibit a mild hair phenotype, indicating that FGF7 is at least partially redundant with other factors[25]. The inductive properties of isolated dermal papilla cells are lost after several passages in culture,

Fig. 6. Human hair follicle at anagen onset, following topical application of a plasmid DNA/liposome mixture. Hair progenitor cells in the matrix express the β-galactosidase reporter gene (blue cells, arrow).

indicating that a signal from the follicular epithelium is required to maintain these properties. Interestingly, dermal papilla cells cultured in the presence of WNT protein maintain their inductive abilities over many rounds of culture, suggesting that the epithelial signal is comprised of one or more WNT family members[37].

Anagen–catagen transition

The normal timing of the anagen to catagen transition depends upon the presence of FGF5[25]. FGF5 mutant mice display a striking 'angora' phenotype of long hair due to prolongation of the anagen stage. By contrast, overexpression of either of the anti-apoptotic genes *Bcl-*X_L or *Bcl-2* in the outer root sheath decreases the duration of anagen, suggesting that regulation of cell survival in the outer root sheath might also play a role in control of the hair growth cycle[38].

Catagen

Although it is known that catagen involves massive programmed cell death, the means by which this is achieved are poorly understood at the molecular level. Analysis of mutant mice has revealed roles for transforming growth factor β1 (TGFβ1) and p75 neurotrophin receptor (p75NTR) in the timing of catagen[38,39]. In mice lacking the hairless (HR) protein, a putative transcription factor, the dermal papilla fails to move upward during catagen[38]. As a consequence, contact between the dermal papilla and stem cells in the bulge is lost, and subsequent hair growth cycles fail to occur. Mice lacking retinoid X receptor-α (RXRα) in the epidermis also display separation of the proximal and distal portions of the hair follicle and fail to initiate the first postnatal anagen[40]. The phenotypes of mice and humans carrying mutations in the vitamin D receptor (VDR)[41] are strikingly similar to those of HR and RXRα mutants suggesting that these proteins might be components of the same pathway important for catagen.

The findings summarized above suggest several potential therapeutic opportunities. Inhibition of FGF5 could possibly be used to prolong anagen and produce longer hair, whereas PTHrP antagonists and SHH might be effective for inducing anagen onset in androgenetic alopecia. Treatments involving SHH must be designed to minimize the dose and duration of exposure to this molecule, as expression of ectopic SHH in the skin can induce the formation of basal cell carcinoma[42]. The finding that WNTs can maintain the inductive properties of cultured dermal papilla cells raises the exciting possibility of cloning and expanding such cells in culture and reintroducing them to the scalp to induce the formation of new hair follicles. Possibly, allogeneic dermal cells could be used because male dermal sheath cells were able to generate hair follicles in a female recipient[43]. Given that hairs in different regions of the scalp are differentially affected by androgenetic alopecia, and this property appears to be intrinsic to the hair follicle, it might prove possible to use this method to generate relatively testosterone-resistant follicles. It will clearly be important to determine whether testosterone sensitivity is innate to the dermal or epithelial component of the follicle, and to demonstrate whether or not epithelial cells in bald scalp are competent to generate large terminal hair follicles in response to an inductive dermal signal. Techniques for effective transfer of cultured dermal papilla cells into bald skin would also have to be developed for this approach to be successful.

If bald scalp is not competent for hair follicle induction by dermal papilla cells, an alternative approach for *de novo* generation of hair follicles would be to engineer hair follicles *in vitro*, perhaps by combining hair follicle stem cells with dermal papilla cells. This approach would enable one to alter the characteristics of the cells by gene therapy. Such a scenario requires the ability to isolate hair follicle stem cells. Potential markers for these cells include cytokeratins 15 and 19, β1 integrin, α6 integrin and lack of proliferation markers such as transferrin receptor[44–46].

Gene therapy

The potential power of gene therapy technology has become more apparent recently with the identification of many molecules important in hair biology, and with the cloning of genes mutated in several inherited human hair diseases (Table 1). Li and Hoffman pioneered the introduction of foreign DNA into hair follicle cells using topical lipoplexes (DNA–liposome mixtures) by successfully transducing mouse hair follicle keratinocytes

in vivo[47]. To date, this approach has been used to correct the albino mutation in mouse hairs[48], to immunize mice against hepatitis B virus[49] and to express a reporter gene in human hair shaft progenitor cells[50] (Fig. 6). These studies demonstrate that different cell populations within the follicle, including melanocytes and antigen-presenting cells (Langerhans cells) in addition to keratinocytes, can be targeted using a relatively straightforward topical technique. In the future, specific cell populations could be targeted by changing the composition of the liposomes or by using transgenes with promoters that are active in distinct cell types. For example, stem cells in the bulge might be targeted by the use of the cytokeratin 15 promoter[45]. The timing of expression could also be controlled by addition of inducible promoter elements to the transgenes.

The type of gene therapy could be tailored to the type of alopecia. For example, in genetic disorders of the hair shaft, caused by single gene mutations (see Table 1), long-lasting correction of the hair follicle genotype would be necessary. Stem cells would be ideal targets in this case because of their long lifespan[44]. Theoretically, transgenes integrated into the bulge cell genome would result in long-term expression in bulge cells and their progeny. By using RNA–DNA oligonucleotides single base pair mutations can be permanently repaired both *in vitro* and *in vivo*. Retroviral vectors can also be used to permanently alter genotype[51]. Although the efficiency of repair using these approaches is low, optimization, perhaps using adjunctive peptides to increase cellular and nuclear uptake, could overcome this problem[52,53].

By contrast, short-term expression might be preferred for the treatment of polygenic disorders in which the primary goal is modulation of the hair cycle. Transient and controlled expression of morphogenic and regulatory factors should minimize adverse effects. Transient expression can be achieved by targeting hair follicle progenitors with plasmid DNA[50] or adenoviral vectors[32] at the onset of anagen. Because the characteristics of the lower follicle determine the characteristics of the new hair, the transient action of the plasmid might be sufficient to cause prolonged changes in the appearance of the hair. This strategy might be particularly appropriate for treating androgenetic alopecia with genes that might enlarge the follicle (such as SHH) or for the treatment of alopecia areata, in which most follicles are arrested at anagen onset and should be susceptible to transfection. Perhaps introducing genes that encode for anti-inflammatory cytokines or other immune modulators could protect the follicle against the autoimmune attack.

Table 1. Single gene mutations affecting human hair growth

Disease	Affected gene	Role of encoded protein	Hair phenotype	Ref.
Monilethrix	hHb6 or hHb	Hair keratin (structural protein)	Thin fragile hair with a beaded appearance under light microscopy	55
Netherton's syndrome	SPINK5	Serine protease inhibitor LEKT1	Defective hair shaft differentiation ('bamboo hair')	56
Generalized atrichia with papular lesions	Hairless (HR)	Putative transcription factor	Failure of first postnatal hair growth cycle	57
Generalized atrichia with papular lesions	VDR	Vitamin D receptor	Failure of first postnatal hair growth cycle	58
Human nude	WHN	Transcription factor	Hair shafts fail to emerge from skin	59
X-linked hypohidrotic ectodermal dysplasia	Ectodysplasin (EDA)	Intercellular signaling molecule	Sparse hair	60
Autosomal hypohidrotic ectodermal dysplasia	Ectodysplasin receptor (EDAR)	Receptor for ectodysplasin	Sparse hair	61
Ectodermal dysplasia/familial incontinentia pigmenti	IKK-gamma (NEMO)	Kinase required for activation of the transcription factor NF-κB	Sparse hair	62
Naxos disease	Plakoglobin	Adhesion molecule	Woolly hair	63
Ectodermal dysplasia/skin fragility syndrome	Plakophilin 1	Desmosomal adhesion molecule	Sparse hair	64
Menkes' disease	ATP7a	Copper-transporting P-type ATPase	Hair loss; abnormal hair texture	65
Tricho-rhino-phalangeal syndrome type I	TRPS I	Zinc finger protein, putative transcription factor	Sparse and unruly scalp hair	66
X-linked dominant chondrodysplasia punctata	EBP	Δ(8),Δ(7) sterol isomerase emopamil-binding protein	Coarse hair, alopecia	67
Giant axonal neuropathy	Gigaxonin (GAN)	Unknown	Curly or kinky hairs	68

Outstanding questions

- What is the initial dermal signal directing hair follicle placode formation?
- What are the inductive signals produced by dermal papilla cells?
- What factors regulate hair follicle size?
- What are the molecular mechanisms by which testosterone influences hair follicles?
- What is the inciting event in alopecia areata?

Although an understanding of hair follicle biology at the cellular level will undoubtedly lead to many new molecular targets for the treatment of alopecias, as each hair follicle traverses through its own hair cycle, essentially independent of its neighbors, a high degree of complexity emerges when attempting to predict the effects of cycle–altering compounds on the appearance of the entire scalp. Computer models of human hair growth that simulate the appearance of the scalp as it changes over time in response to treatment or disease are needed[54]. These types of models will aid in pinpointing which parameters are most important for the overall appearance of the hair.

A wealth of recent discoveries, combined with the production of conditional and inducible knockout mice, the development of microarray technologies for analysis of the expression profiles of large numbers of genes under different conditions, the availability of human and mouse genomic sequence information, and advances in gene therapy techniques, combine to make this an exciting time for hair biologists. Our current challenge is to utilize these advances for the development of safe and effective therapies for hair disease.

References

1 Paus, R. and Cotsarelis, G. (1999) The biology of hair follicles. *N. Engl. J. Med.* 341, 491–497
2 Cotsarelis, G. (1997) The hair follicle: dying for attention. *Am. J. Pathol.* 151, 1505–1509
3 Cotsarelis, G. *et al.* (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* 61, 1329–1337
4 Oshima, H. *et al.* (2001) Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* 104, 233–245
5 Sugiyama, S. *et al.* (1995) Proliferating activity of the hair follicular melanocytes at the early and anagen III stages in the hair growth cycle: detection by immunocytochemistry for bromodeoxyuridine combined with DOPA reaction cytochemistry. *J. Dermatol.* 22, 396–402
6 Hamilton, J.B. (1942) Male hormone stimulation is a prerequisite and an incitant in common baldness. *Am. J. Anat.* 71, 451–480
7 Guarrera, M. and Rebora, A. (1996) Anagen hairs may fail to replace telogen hairs in early androgenic female alopecia. *Dermatology* 192, 28–31
8 Headington, J.T. (1993) Telogen effluvium. New concepts and review. *Arch. Dermatol.* 129, 356–363
9 Botchkarev, V.A. *et al.* (2000) p53 is essential for chemotherapy-induced hair loss. *Cancer Res.* 60, 5002–5006
10 Davis, S.T. *et al.* (2001) Prevention of chemotherapy-induced alopecia in rats by CDK inhibitors. *Science* 291, 134–137
11 Gilhar, A. *et al.* (1998) Autoimmune hair loss (alopecia areata) transferred by T lymphocytes to human scalp explants on SCID mice. *J. Clin. Invest.* 101, 62–67
12 Stenn, K. *et al.* (1999) Hair follicle biology, the sebaceous gland and scarring alopecia. *Arch. Dermatol.* 135, 973
13 McElwee, K.J. *et al.* (1998) Experimental induction of alopecia areata-like hair loss in C3H/HeJ mice using full-thickness skin grafts. *J. Invest. Dermatol.* 111, 797–803
14 Zheng, Y. *et al.* (1999) Scd1 is expressed in sebaceous glands and is disrupted in the asebia mouse. *Nat. Genet.* 23, 268–270
15 Sundberg, J.P. *et al.* (1999) Androgenetic alopecia: *in vivo* models. *Exp. Mol. Pathol.* 67 (2), 118–130
16 Hardy, M. (1992) The secret life of the hair follicle. *Trends Genet.* 8, 55–61
17 Barsh, G. (1999) Of ancient tales and hairless tails. *Nat. Genet.* 22, 315–316
18 Noramly, S. and Morgan, B.A. (1998) BMPs mediate lateral inhibition at successive stages in feather tract development. *Development* 125, 3775–3787
19 Jung, H.S. *et al.* (1998) Local inhibitory action of BMPs and their relationships with activators in feather formation: implications for periodic patterning. *Dev. Biol.* 196, 11–23
20 Headon, D.J. and Overbeek, P.A. (1999) Involvement of a novel Tnf receptor homologue in hair follicle induction. *Nat. Genet.* 22, 370–374
21 DasGupta, R. and Fuchs, E. (1999) Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. *Development* 126, 4557–4568
22 Chiang, C. *et al.* (1999) Essential role for Sonic hedgehog during hair follicle morphogenesis. *Dev. Biol.* 205, 1–9
23 St-Jacques, B. *et al.* (1998) Sonic hedgehog signaling is essential for hair development. *Curr. Biol.* 8, 1058–1068
24 McElwee, K. and Hoffmann, R. (2000) Growth factors in early hair follicle morphogenesis. *Eur. J. Dermatol.* 10, 341–350
25 Millar, S.E. (1997) The role of patterning genes in epidermal differentiation. In *Cytoskeletal-Membrane Interactions and Signal Transduction* (Cowin, P. and Klymkowsky, M., eds), pp. 87–102, Landes Bioscience
26 Millar, S.E. *et al.* (1999) WNT Signaling in the Control of Hair Growth and Structure. *Dev. Biol.* 207, 133–149
27 Lin, M.H. *et al.* (2000) Activation of the Notch pathway in the hair cortex leads to aberrant differentiation of the adjacent hair-shaft layers. *Development* 127, 2421–2432
28 Kulessa, H. *et al.* (2000) Inhibition of Bmp signaling affects growth and differentiation in the anagen hair follicle. *EMBO. J.* 19, 6664–6674
29 Meier, N. *et al.* (1999) Whn and mHa3 are components of the genetic hierarchy controlling hair follicle differentiation. *Mech. Dev.* 89, 215–221
30 Godwin, A.R. and Capecchi, M.R. (1998) Hoxc13 mutant mice lack external hair. *Genes Dev.* 12, 11–20
31 Oro, A.E. and Scott, M.P. (1998) Splitting hairs: dissecting roles of signaling systems in epidermal development. *Cell* 95, 575–578
32 Sato, N. *et al.* (1999) Induction of the hair growth phase in postnatal mice by localized transient expression of Sonic hedgehog. *J. Clin. Invest.* 104, 855–864
33 Wang, L.C. *et al.* (2000) Regular articles: conditional disruption of hedgehog signaling pathway defines its critical role in hair development and regeneration. *J. Invest. Dermatol.* 114, 901–908
34 Sano, S. *et al.* (2000) Two distinct signaling pathways in hair cycle induction: Stat3-dependent and -independent pathways. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13824–13829
35 Smart, R.C. *et al.* (1999) Effects of 17-β-estradiol and ICI 182 780 on hair growth in various strains of mice. *J. Invest. Dermatol. Symp. Proc.* 4, 285–289
36 Holick, M.F. *et al.* (1994) A parathyroid hormone antagonist stimulates epidermal proliferation and hair growth in mice. *Proc. Natl. Acad. Sci. U. S. A.* 91, 8014–8016
37 Kishimoto, J. *et al.* (2000) Wnt signaling maintains the hair-inducing activity of the dermal papilla. *Genes Dev.* 14, 1181–1185
38 Stenn, K.S. and Paus, R. (2001) Controls of hair follicle cycling. *Physiol. Rev.* 81, 449–494
39 Botchkarev, V.A. *et al.* (2000) A role for p75 neurotrophin receptor in the control of apoptosis-driven hair follicle regression. *Faseb. J.* 14, 1931–1942
40 Li, M. *et al.* (2001) RXR-α ablation in skin keratinocytes results in alopecia and epidermal alterations. *Development* 128, 675–688
41 Sakai, Y. and Demay, M.B. (2000) Evaluation of keratinocyte proliferation and differentiation in vitamin D receptor knockout mice. *Endocrinology* 141, 2043–2049
42 Oro, A.E. *et al.* (1997) Basal cell carcinomas in mice overexpressing sonic hedgehog. *Science* 276, 817–821
43 Reynolds, A.J. *et al.* (1999) Trans-gender induction of hair follicles. *Nature* 402, 46–47
44 Cotsarelis, G. *et al.* (1999) Epithelial stem cells in the skin: definition, markers, localization and functions. *Exp. Dermatol.* 8, 80–88

45 Lyle, S. *et al.* (1998) The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle stem cells. *J. Cell. Sci.* 111, 3179–3188
46 Tani, H. *et al.* (2000) Enrichment for murine keratinocyte stem cells based on cell surface phenotype. *Proc. Natl. Acad. Sci. U. S. A.* 97, 10960–10965
47 Li, L. and Hoffman, R.M. (1995) The feasibility of targeted selective gene therapy of the hair follicle. *Nat. Med.* 1, 705–706
48 Vitali, A. *et al.* (2000) Localized *in vivo* genotypic and phenotypic correction of the albino mutation in skin by RNA-DNA oligonucleotide. *Nat. Biotechnol.* 18, 43–47
49 Fan, H. *et al.* (1999) Immunization via hair follicles by topical application of naked DNA to normal skin. *Nat. Biotechnol.* 17, 870–872
50 Domashenko, A. *et al.* (2000) Efficient delivery of transgenes to human hair follicle progenitor cells using topical lipoplex. *Nat. Biotechnol.* 18, 420–423
51 Ghazizadeh, S. *et al.* (1999) *In vivo* transduction of mouse epidermis with recombinant retroviral vectors: implications for cutaneous gene therapy. *Gene Ther.* 6, 1267–1275
52 Rothbard, J.B. *et al.* (2000) Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation. *Nat. Med.* 6, 1253–1257
53 Subramanian, A. *et al.* (2000) Nuclear targeting peptide scaffolds for lipofection of nondividing mammalian cells. *Nat. Biotechnol.* 17, 873–877
54 Halloy, J. *et al.* (2000) Modeling the dynamics of human hair cycles by follicular automaton. *Proc. Natl. Acad. Sci. U. S. A.* 97, 8328–8333
55 Winter, H. *et al.* (1997) A new mutation in the type II hair cortex keratin hHb1 involved in the inherited hair disorder monilethrix. *Hum. Genet.* 101, 165–169
56 Chavanas, S. *et al.* (2000) Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat. Genet.* 25, 141–142
57 Ahmad, W. *et al.* (1998) Alopecia universalis associated with a mutation in the human hairless gene. *Science* 279, 720–724
58 Zhu, W. *et al.* (1998) Hereditary 1,25-dihydroxyvitamin D-resistant rickets due to an opal mutation causing premature termination of the vitamin D receptor. *J. Bone Miner. Res.* 13, 259–264
59 Frank, J. *et al.* (1999) Exposing the human nude phenotype. *Nature* 398, 473–474
60 Kere, J. *et al.* (1996) X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. *Nat. Genet.* 13, 409–416
61 Monreal, A.W. *et al.* (1999) Mutations in the human homologue of mouse dl cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. *Nat. Genet.* 22, 366–369
62 Zonana, J. *et al.* (2000) A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in IKK-γ (NEMO). *Am. J. Hum. Genet.* 67, 1555–1562
63 McKoy, G. *et al.* (2000) Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet* 355, 2119–2124
64 Norgett, E.E. *et al.* (2000) Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. *Hum. Mol. Genet.* 9, 2761–2766
65 Pena, M.M. *et al.* (1999) A delicate balance: homeostatic control of copper uptake and distribution. *J. Nutr.* 129, 1251–1260
66 Momeni, P. *et al.* (2000) Mutations in a new gene, encoding a zinc-finger protein, cause tricho-rhino-phalangeal syndrome type I. *Nat. Genet.* 24, 71–74
67 Ikegawa, S. *et al.* (2000) Novel and recurrent EBP mutations in X-linked dominant chondrodysplasia punctata. *Am. J. Med. Genet.* 94, 300–305
68 Bomont, P. *et al.* (2000) The gene encoding gigaxonin, a new member of the cytoskeletal BTB/kelch repeat family, is mutated in giant axonal neuropathy. *Nat. Genet.* 26, 370–374
69 Jaworsky, C. *et al.* (1992) Characterization of inflammatory infiltrates in male pattern alopecia: implications for pathogenesis. *Br. J. Dermatol.* 127, 239–246

Gene therapy strategies for urological dysfunction

Michael B. Chancellor, Naoki Yoshimura, Ryan Pruchnic and Johnny Huard

Novel molecular techniques such as conventional and *ex vivo* gene therapy, and tissue engineering have only recently been introduced to the field of urology. The lower urinary tract is ideally suited for minimally invasive therapy, and also *ex vivo* approaches would limit the risk of systemic side effects. Muscle-derived stem cells have been used successfully to treat stress incontinence, and rats with diabetic bladder dysfunction benefited from nerve growth factor (NGF)-based gene therapy. Nitric oxide synthase and capase-7 might provide suitable gene therapy targets for erectile dysfunction and benign prostatic hyperplasia, respectively.

Until recently, there has been little research on gene therapy for the urinary tract. Most of the previous work focused on urinary tract malignancies such as prostate and bladder cancer. However, this situation has changed radically in the past two years. What is stirring this interest in gene therapy for urinary tract dysfunction? First, the pharmaceutical industry is beginning to realize the significance of these disabilities and the potential market size that urological diseases represent. Second, the lower urinary tract is ideally suited for minimally invasive molecular medicine therapy as it implies a lower risk of systemic toxicity. All of the lower urinary tract can be reached either percutaneously or through endoscopy.

In this review, we will focus on gene therapy strategies for three important urological conditions: urinary incontinence, erectile dysfunction and benign prostatic hyperplasia. We will discuss how both viral and nonviral gene therapy might be applicable. In addition, we will present some studies that illustrate the feasibility of muscle-derived stem cell injection that might be a foundation not only for tissue engineering but also *ex vivo* gene therapy.

Bladder dysfunction and urinary incontinence

Urinary incontinence is a serious medical and social condition in the USA and all over the world. It has recently become a hot research topic in the pharmaceutical industry for several reasons. First, the tremendous number of patients suffering

http://tmm.trends.com 1471-4914/01/$ – see front matter PII: S1471-4914(01)02003-2