From Telogen to Exogen: Mechanisms Underlying Formation and Subsequent Loss of the Hair Club Fiber

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The hair follicle has the unique capacity to undergo periods of growth, regression, and rest before regenerating itself to restart the cycle. This dynamic cycling capacity enables mammals to change their coats, and for hair length to be controlled on different body sites. More recently, the process of club fiber shedding has been described as a distinct cycle phase known as exogen, and proposed to be an active phase of the hair cycle. This review focuses on the importance of the shedding phase of the hair cycle and, in the context of current literature, analyzes the processes of club fiber formation, retention, and release, which may influence progression through exogen, particularly in relation to human hair.

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INTRODUCTION

During morphogenesis, embryogenesis of hair follicles (HFs) occurs, and is predominantly controlled through reciprocal interactions between the mesenchymal and epithelial components of the skin (Hardy, 1992). Throughout its adult life, the HF renews itself in a cyclic manner, going through periods of growth (anagen), regression (catagen), and rest (telogen) (Dry, 1926; Chase, 1954; Kligman, 1959).

Molecular factors regulating the transition between these key phases in hair cycling are well documented in the literature (Paus *et al.*, 1999; Muller-Rover *et al.*, 2001; Stenn and Paus, 2001; Alonso and Fuchs, 2006; Blanpain and Fuchs, 2006). However, more recently, a fourth phase in the hair cycle was proposed, and is the least-studied aspect of HF activity. Although considered to be independent from the rest of the hair cycle, this phase was termed ''exogen'' by Stenn, in accordance with accepted hair terminology (Stenn *et al.*, 1998; Milner *et al.*, 2002; Stenn, 2005). This phase relates to the loss of club fiber from the follicle, with exogen describing an active process relating both to the release of club fiber and to any signaling or structural changes preceding club fiber release, which is thought to regulate the process. Therefore, exogen as a phase ends when the club fiber has been shed. This review will address the evidence of exogen as an independent cycle phase, and crucially as one that is actively regulated. In addition to exogen, the terms teloptosis and kenogen have also been introduced into hair cycle terminology. Teloptosis, derived from the Greek word for ''falling off'', refers solely to the release of club fiber from the follicle because of a loss of cellular adhesion, with Rebora considering club fiber shedding to be a passive process, whereas kenogen describes an empty follicle in telogen, after the club fiber has been shed (Pierard-Franchimont and Pierard, 2001; Rebora and Guarrera, 2002, 2004).

In rodents, it is typical for club fibers to be retained from previous growth cycles, and for the growing fiber to be observed adjacent to them. Each fiber sits within its own silo within the follicle; hence, a growing fiber and a club hair can share a follicle, although they are in anagen and exogen silos, respectively. In mouse pelage, club fiber shedding of overfur is coupled with anagen (Figure 1, $Exogen_B$). Large numbers of underfur hairs are also shed during anagen, although many are also shed during the subsequent telogen (Milner *et al.*, 2002). One follicle in which exogen is a relatively predictable event is the rat vibrissa follicle, in which the club fiber is retained throughout the next anagen phase, but lost before the onset of catagen in a highly regulated manner (Figure 1, $Exogen_C$) (Dry, 1926; Ibrahim and Wright, 1975). As the timing of club fiber release can vary between follicle types, it shows that exogen occurs independently of the other hair cycle phases (Stenn *et al.*, 1998; Milner *et al.*, 2002; Stenn, 2005).

Is exogen clinically relevant in man? In the human scalp, the actual timing of exogen in relation to the activity of the HF is less clear. It is uncommon for club fibers to be retained through subsequent anagen phases (Rook and Dawber, 1982), and, in the majority of follicles, exogen is believed to occur before or during the transition from telogen to anagen (Figure 1, $Exogen_A$). Phototrichogram studies do reveal a cycle stage when the telogen follicle is empty and, in this case, exogen must occur without the coincident entry into anagen (Rebora and Guarrera, 2002, 2004). Although rare, it is still possible to find club fibers retained during anagen in the human skin, when the loss of the club fiber probably occurs before the new tip of the anagen fiber reaches the skin surface (Rook and Dawber, 1982).

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Abbreviations: HF, hair follicle; IRS, inner root sheath; ORS, outer root sheath; TE, telogen effluvium

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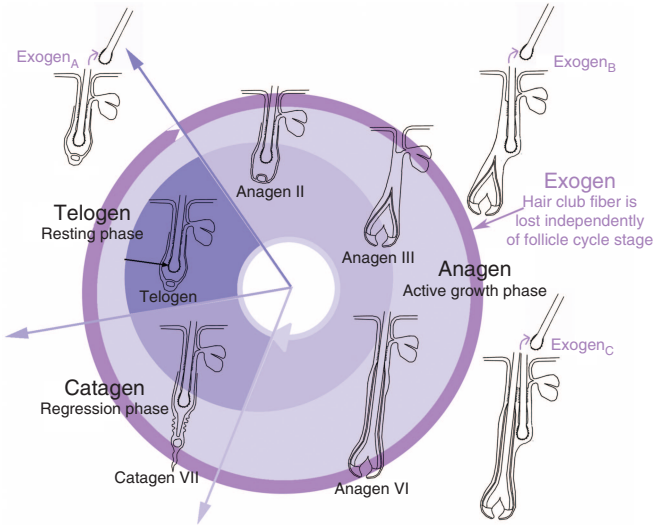

Figure 1. Exogen: The fourth phase of the hair cycle. Exogen can be postulated to occur in three ways: (i) exogen occurs independently of entry into anagen (Exogen$_A$); (ii) exogen occurs at some time after the follicle has re-entered anagen, and indeed, club hairs may be retained through a subsequent one or even two cycles, (Exogen$_B$); (iii) exogen occurs late in anagen of the following hair cycle (Exogen$_C$).

The nature of this ''club'' fiber is reportedly similar whether hair is shed normally or during periods of effluvium (Kligman, 1961). This suggests that, although exogen is the process required to complete the hair cycle for the fiber, it is the cycle dynamics that dictate the number of hairs being shed and the subsequent thinning and baldness.

EXOGEN: ACTIVE OR PASSIVE?

Although the loss of club fibers occurs in the vast majority of hairy mammals, very little is known about this cycle phase. Moreover, there are two schools of thought on the process of hair shedding. The first is that shedding is a passive process caused by a physical force exerted by the new growing fiber that dislodges the club fiber (Segall, 1918; Kligman, 1961; Rebora and Guarrera, 2002, 2004). Evidence supporting this comes in part from the observation that, in mice (Dry, 1926), several small fibers can be present in a follicle at one time, whereas with larger fibers, fewer are present, indicating that there is limited space in the epithelial sac.

A second and more compelling proposal is that exogen is an active process with specific signals controlling the breakdown of cellular adhesion between the club fiber and its surrounding epithelial sac, leading to the release of club fiber (Stenn *et al.*, 1998; Milner *et al.*, 2002; Stenn, 2005). Evidence for this includes the absence of club fibers in telogen follicles (kenogen) leaving them empty in man (Courtois *et al.*, 1994, 1995; Guarrera *et al.*, 1998; Rebora and Guarrera, 2002; Guarrera and Rebora, 2005), in several breeds of domestic sheep (Auber and Burns, 1947), and in the back skin of mice (Plonka *et al.*, 2008). These observations suggest that more than just a mechanical stimulus from the new growing fiber is required to trigger exogen. This argument is also based on the observation that the timing of exogen can be controlled, as in the seasonal molt of animals.

Exogen can also be entered prematurely as seen in cases of immediate telogen effluvium (TE) (Headington, 1993), a type of TE observed with treatments such as minoxidil (Bamford, 1987; Bardelli and Rebora, 1989). It is assumed in the latter case that the entry of the follicle into anagen triggers the release of club hairs.

SUBDIVISION OF THE EXOGEN PHASE

Exogen was proposed by Stenn *et al.* (1998) to refer to a phase that is independent from the cycle phase of the follicle, with club fibers being regarded as in exogen from the moment of their complete formation through to their release. Thus, exogen refers to the signaling and adhesion factors involved in both active retention and active release, and this terminology can be used to describe club fibers in all types of follicles, enabling a distinction between the status of the club fiber and the status of the follicle.

Earlier work analyzing the shed club fibers in humans indicated variations in the amounts of adherent material attached to club ends. Kligman (1961) concluded that the majority of club fibers that were shed appeared to have no sheath attached, although a few were seen with epithelial sacs. The latter were regarded as prematurely shed fibers, whereas those with no sheath attached were considered to be mature clubs, ready for natural shedding. More recently, differences between club fibers have been characterized, with distinctions between retained and shed club fibers being identified and described in both human scalp and mouse pelage follicles (Milner *et al.*, 2002; Van Neste *et al.*, 2007). Exogen clubs, as described by these authors, are analogous to Kligman's mature club fibers.

More recently, work in our laboratory has used the vibrissa model, wherein club fibers can be selected at different time points before their release from the follicle, to show that the adhesion of the fully formed club fiber to its surrounding epithelial material decreased progressively, with the progression through to its release (Higgins *et al.*, 2009). This work led to the proposal that the exogen phase could be subdivided into two stages, with new terminology proposed to define each stage: ''early exogen'' (referring to an actively retained club fiber) and ''late exogen'' (referring to a club fiber still in the follicle but ready to be shed).

THE PHYSIOLOGICAL IMPORTANCE OF CLUB FIBER RETENTION

In 1972, Johnson described the molting properties of small animals in relation to hormonal and seasonal factors. It is clear from this work that a stable telogen phase in the absence of hair shedding is important in mammals with a seasonal molt, wherein the retention of the club hair in the skin between molts is essential as this forms the majority of the coat, protecting the animal from heat loss, trauma, infestation, and sunlight (Johnson, 1972; Stenn and Paus, 2001). Johnson (1972) proposed that, in rodents, the passage between stable telogen and active anagen was under the control of adrenal and gonadal hormones, as well as the

presence of an intrinsic rhythm within the skin. Paus and Foitzik (2004) also speculate on the existence of an innate ''hair cycle clock''.

In laboratory rats and mice, which lack a seasonal influence, club fiber shedding and hair growth cycles are observed in waves along the length of the body and are continuous throughout the year (Chase and Eaton, 1959; Ebling and Johnson, 1964; Plikus and Chuong, 2008). In contrast, in humans, an irregular mosaic shedding pattern is observed, wherein each individual follicle cycles independently of those surrounding it (Chase, 1954; Kligman, 1959). Although a molt wave is obvious in animals, seasonal shedding has also been observed in humans, with a spring and autumn molt being observed on the thigh in some individuals and one predominant molt cycle, during autumn, being observed on the scalp (Randall and Ebling, 1991). The mosaic growth and shedding pattern in human scalp, rather than a noticeable synchronized molt, means that humans lose club hairs diffusely from all over the scalp, so that they appear to retain a full head of hair (Harrison and Sinclair, 2002). This is important because, in humans, hair is a social signature and required for both sun protection and camouflage (Stenn and Paus, 2001).

In humans, especially on the scalp, the retention of club hairs is of little consequence until the factors driving hair loss conditions, such as male and female pattern hair loss, lead to excessive hair shedding and development of hair loss. Diffused hair loss is the result of TE: the loss of telogen hairs with insufficient replacement. TE was originally described by Kligman (1961), and again by Headington (1993), who divided TE into five subtypes, characterized by some form of synchronization of the hair cycle.

The role of hormones has been implicated in TE, after some shedding phenomena were observed, such as the retention of telogen club hairs and release during a seasonal molt (Johnson, 1972), or the delay of entry into telogen as observed during pregnancy (Lynfield, 1960), whereas oral retinoid therapy is described as increasing shedding by synchronizing the cycle through the shortening of anagen (Berth-Jones *et al.*, 1990). However, Berth-Jones did speculate that individuals treated with oral retinoids, who shed hair in large numbers, may have a defect in telogen anchorage. Hyper- and hypo-thyroidism have also been implicated in TE (Rook, 1965; Rook and Dawber, 1982). Clearly hormones are central to follicle progression between rest and activity, but only recently has evidence for a relationship between hormones responsible for male pattern balding and the local factors responsible for hair cycle regulation been proposed in man. Dihydrotestosterone has been proposed to be responsible for the shortening of anagen and the miniaturization seen in pattern balding (Hibino and Nishiyama, 2004). However, despite much evidence supporting the role of hormones in transforming HFs between cycle stages and between terminal and vellus types, there is no evidence that such factors directly influence exogen processes, merely that they shift the dynamics of the hair cycle either by shortening anagen or delaying telogen.

Premature hair loss and/or excessive hair shedding in humans can have negative psychological and psychosocial effects (Cash *et al.*, 1993; Cash, 1992, 1999; Williamson *et al.*, 2001; Schmidt, 2003; Hadshiew *et al.*, 2004; Hunt and McHale, 2005). Understanding the retention mechanisms of the telogen club fiber and the controls and triggers for exogen could prove invaluable in identifying the therapeutic routes for stabilizing the shedding process and lessen suffering and concern. Such strategies would focus on retaining the club hair for a longer duration in the telogen follicle until, at least, the follicle has re-entered anagen. Thus, understanding how a club hair is retained, what triggers its release, and how these link to the follicle cycle are essential new areas for research.

THE PHYSIOLOGICAL EFFECT OF CLUB FIBER LOSS

A well-known observation has been associated with the removal of club fiber in mice and rats. Plucking of the club fiber from the follicle during telogen seems to act as a cycle break and initiates follicular activity and re-entry into the anagen phase (Johnson and Ebling, 1964; Hale and Ebling, 1975; Ebling, 1976). Premature removal of the club fiber from pelage skin, as seen in this situation, also results in removal of the innermost cell layer that surrounds and is adhered onto the club (Silver *et al.*, 1969; Ito and Kizawa, 2001). This results in apoptosis in the cells that surround the club fiber, including the bulge region (Matsuo *et al.*, 2003). A new, albeit traumatic, anagen phase is initiated subsequent to mitotic activity in the secondary hair germ, followed by repopulation of the bulge from the secondary hair germ (Ito *et al.*, 2002, 2004). It is interesting that, in the desmoglein-3 knockout mouse, which prematurely loses its club fiber and the innermost cell layer surrounding the club, initiation of anagen is not observed after club fiber loss. Instead, re-entry into anagen is delayed by up to 8 days (Koch *et al.*, 1998). Adding another level of complexity to the issue are the recent results published by Plikus *et al.* (2008), indicating that anagen initiation after plucking is only observed when the follicle is in, what is described as, competent telogen, as opposed to refractory telogen. It seems that this observed effect is linked to the presence of bone morphogenic proteins in the dermal macroenvironment during refractory telogen, which confers inhibitory effects on anagen initiation (Plikus *et al.*, 2008). It seems that the trauma related to the premature plucking of the club fiber is not enough to induce anagen, but the macroenvironment surrounding the follicle also plays an important role.

Comparatively, in man, the same phenomenon has not been observed, and there is no evidence of plucking-induced anagen initiation in human hairs. The observation of follicles in kenogen (Rebora and Guarrera, 2002), when the telogen follicle is empty, would suggest that normal club shedding does not necessarily result in, or occur because of, concomitant initiation of anagen.

It is more difficult to link the effect of physiological exogen (versus mechanical) on anagen initiation, as it is believed that physiological exogen occurs during anagen of the subsequent hair cycle during anagen VI in mice (Milner *et al.*, 2002).

In man, a ''normal'' exogen is presumed to occur without causing trauma to the follicle, as club fibers are removed cleanly from the club hair silo without any cells attached (Kligman, 1961; Milner *et al.*, 2002; Van Neste *et al.*, 2007). Therefore, it is perhaps less likely that normal exogen in human scalp would have an effect on the regulation of the follicular cycle.

FORMATION AND RETENTION OF THE CLUB FIBER

The stimulus for formation of the telogen club hair can occur several months before final club fiber shedding occurs. In human scalp follicles, the telogen phase is generally considered to last approximately 3 months (Price and Griffiths, 1985), which is brief when compared with the duration of the anagen phase in the scalp, which lasts 2–6 years (Kligman, 1961). This is in contrast to trunk follicles in humans, in which the duration of the telogen phase can equal, or last longer than, the period of growth (Saitoh *et al.*, 1970; Seago and Ebling, 1985). If not to be shed, but to be actively retained, club fibers are firmly anchored in the follicle, with the force required to remove them being similar to that required to extract an anagen fiber from its follicle (Chapman, 1992; Roersma *et al.*, 2001).

A developing club hair is characterized by a brush-like appearance at its proximal end, which is because of formation of an electron-dense non-nucleated layer, containing ''trichilemmal keratin'' forming the base of the fiber (Maurer, 1895). The trichilemmal keratin is not derived from the club fiber itself, but rather forms an envelope around the base of the club fiber, and is described as being ''fused'' onto the club fiber (Vandevelde and Allaerts, 1984). As seen in Figure 2, electron-dense processes of the trichilemmal keratin layer extend outwards and between the surrounding keratinocytes of the outer root sheath (ORS), and, along with desmosomal contacts in this location, are thought to firmly secure the developing club fiber in place (Pinkus, 1969; Pinkus *et al.*, 1981).

The origin of the trichilemmal keratin layer attached to the club fiber is unclear. Early work by Pinkus showed the trichilemmal keratin of the club fiber stained bluish white with thioflavin T, a histology stain that normally stains the keratinized inner root sheath (IRS) material yellow. Pinkus concluded that the electron-dense layer surrounding the club fiber was derived from the ORS, and gave it the name ''trichilemmal keratin'' (Pinkus, 1969; Pinkus *et al.*, 1981), with this remaining the dogma ever since. Pinkus indicated that the trichilemmal keratin of the club fiber is derived in the same manner as the trichilemmal keratin of the anagen-growing follicle, hence the same name derived from ''trichilemma'' meaning ORS. The trichilemmal keratin of the anagen follicle is detected in the isthmus region, where the ORS directly abuts onto the hair shaft after sloughing of the IRS. It is formed when the ORS keratinizes without forming an intermediate granular layer (Pinkus *et al.*, 1981).

This sometimes leads to confusion, with many researchers extrapolating information about the trichilemmal keratin of the anagen follicle to the electron-dense layer that surrounds the club fiber, even though there are unique differences between these two layers. One key example is with thioflavin T staining, originally used by Pinkus to stain the club fiber trichilemmal keratin bluish white. This does not stain the trichilemmal keratin of the anagen follicle (Honda *et al.*, 1997). An interesting observation is the innermost layer of the ORS cells that surround the trichilemmal keratin of the club fiber. We and others have observed that these cells stain for companion layer-specific markers such as Keratin 6, Plasminogen Activator Inhibitor-2, Calretinin, and the S100 calcium-binding protein, A6 (Lavker *et al.*, 1998; Winter *et al.*, 1998; Ito and Kizawa, 2001; Bernot *et al.*, 2002; Poblet *et al.*, 2005; Gu and Coulombe, 2007). This leads us to propose that this layer is the companion layer of the club, or the companion^{CL} (CL, club layer) (Figure 3). The development of this layer during late catagen and the processes by which the cells of the companion^{CL} keratinize remain unclear but may be related to the function of the companion layer in anagen.

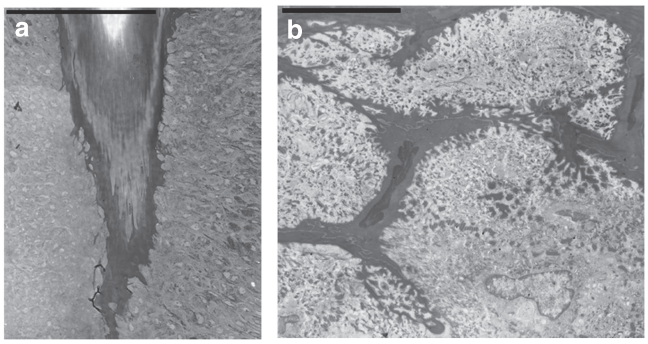

Figure 2. Trichilemmal keratin surrounding the club fiber—a vibrissa example. (**a**) Toluidine blue staining of semithins highlights the trichilemmal keratin layer surrounding vibrissa club fibers (dark gray). The trichilemmal keratin is characterized by numerous anchoring protrusions, believed to secure the club fiber in place (scale bar 100 μm). (**b**) Electron microscopy highlights the complex interdigitations between the trichilemmal keratin and the surrounding ORS (outer root sheath) (scale bar 5 μm).

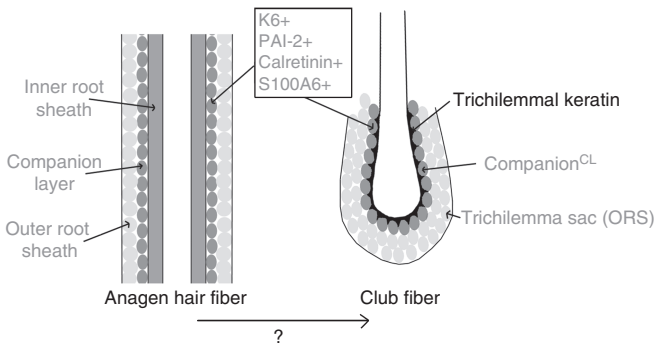

Figure 3. Similarities between the companion layer of the anagen hair and the companion^{CL} of the club fiber. Markers detected in the companion layer surrounding the anagen fiber are also detected in the innermost cell layer surrounding the trichilemmal keratin of the club fiber, here termed the companion^{CL}. It has not yet been shown whether one is a cycle manifestation of the other. CL, club layer; PAI, Plasminogen activator inhibitor; K6, keratin-6; ORS, outer root sheath.

MOUSE MODELS OF DEFECTIVE EXOGEN

In laboratory mice, the waves of hair growth that occur over the body mean that any changes in the timing of the hair cycle are easily detected. A range of mouse models have been described with aspects of exogen affected (Table 1), with several of these mice exhibiting a cyclic hair loss and regrowth, with the hair loss initiated in the catagen or telogen stages of the growth cycle corresponding with club fiber formation. These mouse models can be characterized as altered in formation of the club fiber, retention of the club fiber, or release of the club fiber (Table 1).

Several mouse models have been described that show defective club fiber formation, resulting in premature club fiber loss. This includes a Notch-1 transgenic model that shows delayed IRS differentiation and an absent trichilemmal keratin layer, which leads to defective retention and early club fiber loss (Uyttendaele *et al.*, 2004). Cathepsin-L knockout mice also have a poorly formed trichilemmal layer

Table 1. Summary of mouse models giving an insight into club fiber formation, retention, and release

Mouse model	Function of protein	Relevance to club fiber	Aspect of club fiber affected	References
Cathepsin-L knockout	Proteolytic function. Important in IRS differentiation	Knockout causes defective trichilemmal keratin formation resulting in defective retention and premature loss of the club fiber.	Formation	(Roth *et al.*, 2000; Tobin *et al.*, 2002)
Notch-1 transgenic	Function regulating epidermal differentiation. Within hair follicle regulates IRS differentiation	Overexpression driven by the involucrin promoter delays IRS differentiation. Club fibers do not possess a trichilemmal keratin layer and therefore have defective retention in the ORS.	Formation	(Uyttendaele *et al.*, 2004)
Msx-2 knockout	Involved in signal transduction (HOX). Required for hair shaft and cuticle differentiation	Premature hair loss is observed in the knockout mouse. No visible defects are seen in shed fibers from this mouse although the knockout affects club fiber formation.	Formation	(Ma *et al.*, 2003)
Stratifin mutation	Regulates epidermal differentiation	Knockout results in defective club fiber retention and club fiber release is observed corresponding with club fiber formation.	Formation	(Herron *et al.*, 2005; Li *et al.*, 2005)
Hairless mutation	Transcriptional corepressor. Regulates terminal differentiation in the epidermis and IRS	Club fibers in this mouse have no trichilemmal keratin layer as a result of the continued presence of undifferentiated IRS surrounding the fiber, preventing trichilemmal keratin formation.	Formation	(Orwin *et al.*, 1967; Panteleyev *et al.*, 1999; Panteleyev *et al.*, 1998)
Myelin Protein zero-like 3 mutation	Cell adhesion molecule	Club fiber shedding corresponds with club fiber formation at d 18 postnatally.	Formation, retention	(Hayashi *et al.*, 2004; Cao *et al.*, 2007)
Desmoglein 3 knockout	Desmosomal component role in cellular adhesion	Club fiber appears normal in this mouse, although the innermost layer of cells surrounding the fiber lack functional desmosomes resulting in splitting between the cells and early release of the club fiber.	Retention	(Koch *et al.*, 1998)
Bcl-x_L transgenic	Anti-apoptotic function	Expression driven by the Keratin 14 promoter causes delayed release of the club fiber-potentially by preventing cell death in the cells surrounding the club fiber.	Release	(Pena *et al.*, 1999)

IRS, inner root sheath, ORS, outer root sheath.

that lacks desmosomal contacts and anchoring protrusions that usually secure the fiber in place. This seems to be because of the persistence of Huxley's layer of the IRS surrounding the club fiber, which fails to keratinize (Tobin *et al.*, 2002). The hairless mutant mouse has no trichilemmal keratin layer, but instead has the continued presence of a thick undifferentiated IRS surrounding the club fiber (Orwin *et al.*, 1967; Panteleyev *et al.*, 1998, 1999). Thus, it would seem that the mechanisms in place for IRS differentiation during anagen also play a role during club fiber formation during catagen and telogen. These mice would be useful tools with which to analyze the signaling pathways and molecular controls important in the formation of the trichilemmal keratin layer that anchors the club fiber.

Another mouse that prematurely loses its club fiber is the desmoglein-3 knockout. A loss of functional desmosomes in the innermost layer of cells surrounding the club fiber leads to splitting in the ORS, resulting in early release of the club fiber from its epithelial sac (Koch *et al.*, 1998). This indicates the importance of adhesion molecules in the retention of the club fiber. One mouse model exhibiting a longer retention of club fibers is the Bcl-$_{XL}$ transgenic model (Pena *et al.*, 1999). Expression of the anti-apoptotic gene, Bcl-$_{XL,}$ driven by the K14 promoter, seems to cause longer retention of club fibers in these mice, which suggests that programed cell death may be involved in the release of the club fiber from its epithelial sac.

ANALOGIES BETWEEN EPIDERMAL DESQUAMATION AND EXOGEN

In 1895, Maurer first identified what we now know as the trichilemmal keratin layer of the ORS, initially describing it as the stratum corneum layer of the HF (Maurer, 1895). The trichilemmal keratin layer may act in a similar manner to the stratum corneum and provide a sloughable barrier between the hair and the proximal follicle. To maintain a consistent thickness of the stratum corneum and its integrity as a barrier, there is a delicate balance between the rate of proliferation of corneocytes and the rate of desquamation (Ya-Xian *et al.*, 1999; Egelrud, 2000). This dynamic turnover is not, however, a feature of club hair ''desquamation'', as shedding is a singular event within each follicle, whereas stratum corneum desquamates continuously as the epidermis is renewed. However, the key processes that regulate differentiation and desquamation of the epidermis may have parallels in club fiber maturation and release. In 1984, Vandevelde and Allaerts showed that there was an increasing thiol-disulfide conversion in the epithelial sac surrounding the club fiber, trichilemmal keratin, coinciding with the onset of the succeeding hair generation, and proposed this as a contributory factor in the loosening of the old club fiber from its epithelial sac (Vandevelde and Allaerts, 1984). They likened this process to cornification of epidermal cells as a similar thiol-disulfide conversion is associated with epithelial keratinization before desquamation (Broekaert *et al.*, 1982).

Although corneocytes are ''dead'', in the sense that they have no active protein synthesis, and in this way are similar to the trichilemmal keratin layer of the club hair, proteolysis of intercellular adhesive structures is a key process in desquamation, facilitating the reduction of cell cohesion so that the corneocyte is easily dislodged (Bisset *et al.*, 1987; Egelrud *et al.*, 1988; Lundström and Egelrud, 1988; Chapman and Walsh, 1990; Egelrud, 2000). A similar proteolytic mechanism has been suggested for club fiber cleaving from its surrounding epithelial sac. Spontaneously shed club fibers are distinct in that they lack cytological details, such as prominent nuclei and an abundant cytoplasm, which are seen in prematurely plucked club fibers. This led Milner *et al.* (2002) to propose that the cells surrounding spontaneously shed club fibers have undergone proteolytic degradation, leading to the cleaving of the fiber from its surrounding epithelial sac. Moreover, plasminogen activator inhibitor 2, detected in the granular layer of the epidermis and with a role regulating epidermal differentiation (Dano *et al.*, 1985; Lyons-Giordano *et al.*, 1994), is also detected in a single layer of keratinocytes that border the trichilemmal keratin of the club fiber in both mice and humans (Lavker *et al.*, 1998; Jensen *et al.*, 2000). This is consistent with Maurer's analogy of the trichilemmal keratin layer as equivalent to the stratum corneum, as the cell layer adjacent to the trichilemmal is similar to the granular layer of the epidermis.

Supporting a possible link between desquamation and club shedding are clinical observations of altered hair shedding in cases of severe dandruff and seborrheic dermatitis, disorders exhibiting altered epidermal desquamation. Pierard-Franchimont *et al* (2006) found a positive correlation of the severity of dandruff with the number of club fibers in plucked trichogram counts. This may be because of a shift in the anagen:telogen ratio toward an increased number of telogen follicles or, alternatively, may be indicative of an increase in the length of time that a club is retained before shedding, thereby increasing retained club hairs. This would suggest that delayed exogen occurs with dandruff but with the cycle of the follicle unaffected. Related to this suggestion is the finding that antidandruff treatments have been proposed to decrease hair shedding and increase the proportion of follicles in anagen as measured by plucked trichograms (Pierard-Franchimont *et al.*, 2002). Dandruff is a symptom of disturbed epidermal differentiation. If the factors that influence such a disturbance are also able to affect the differentiation processes that control exogen, then retention of the club hair is a possibility. If antidandruff treatments increase the proportion of follicles in anagen, then the number of telogen follicles with hairs available for shedding decreases, explaining a decrease in hair shedding as a result of treatment.

CONCLUSIONS

It is essential for animals to grow new hairs before the old club fiber falls out to ensure that they are never naked or without a key sensory apparatus, such as the whisker. In the majority of cases, premature hair loss would be disastrous as the hair fiber not only provides camouflage but protects against heat and cold. In the human scalp, the presence of follicles that have lost their club fibers in the absence of the mechanical stimulus from a new growing fiber indicates that exogen does not always

occur merely as a result of an exclusion force. Evidence supports exogen as an active process relating to both the signaling and structural changes before club fiber release and to the final liberation of the shaft.

The importance of the exogen phase in the context of HF biology is becoming more widely recognized, although more comprehensive research is required to understand the molecular mechanisms controlling this cycle phase. New insights into regulation of club fiber formation, retention, and the final release will reveal whether these are potentially useful targets for therapeutic intervention. Delaying the exogen phase of the hair cycle could provide immediate relief to individuals with excessive hair shedding and the retention of long terminal hairs could be of benefit for those with early pattern hair loss and follicle miniaturization. On the other hand, inducing exogen club release could be a novel hair reduction strategy for unwanted body hair.

Our understanding of exogen may be furthered by drawing upon systems that are better understood, such as desquamation in the epidermis and mouse models with altered exogen hair loss. It seems that the club fiber is retained at a cellular level in the epithelial sac through inter-cellular adhesion complexes; however, currently, there is no information on any local signal or signals that initiate exogen in animals or man, nor is there evidence that it can be spontaneously triggered or indeed delayed or prevented. Further research is required into this new phase of HF biology to develop our understanding of the hair cycle as a complete process, from growing fiber formation through to club fiber release.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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