# A Bald Statement – Current Approaches to Manipulate Miniaturisation Focus only on Promoting Hair Growth

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**Abstract**

Hair plays a large part in communication and society with its role changing through time and across cultures. Most people don't leave the house before combing their hair or shaving their beard and for many hair loss or irregular hair growth can have a significant impact on their psychological health. Somewhat unsurprisingly, according to GMR Data today's global hair care industry is worth an estimated $87 Billion in 2018, with hair loss estimated at $2.8 Billion. Considering that no current hair loss related products can completely reverse hair loss, it is reasonable to believe this market could expand significantly with the discovery of a comprehensive therapy. As such, a great deal of research focuses on overcoming hair loss, and in particular, a common form of hair loss known as Androgenetic Alopecia (AGA) or male pattern baldness. In AGA hair follicles miniaturise in a large step change from a terminal to a vellus state. Within this viewpoint article, we discuss how influx and efflux of cells into and out from the dermal papilla can modulate dermal papilla size during the hair cycle. As dermal papilla size is positively correlated with the size of the hair fibre produced by a follicle, we argue here that therapies for treating AGA should be developed which can alter dermal papilla size, rather than just promote hair growth. We also discuss current therapeutics for AGA, and emphasize the importance of using the right model systems to analyse miniaturisation.

**Key Words**

Androgenic Alopecia, Male Pattern Baldness, Dermal Papilla, Hair Cycling, Hair Follicle

**Hair Today Gone Tomorrow**

The adult hair follicle is mainly comprised of epithelial and mesenchymal tissue types, however, cells within these compartments interact with and are regulated by several other cells types, including neural crest derived melanocytes, immune cells, and even adipocytes [1]. Hair Follicle Stem cells (HFSCs) are located in a region of the epithelium known as the bulge, and when the follicle is actively producing a hair fibre, cells migrate from the bulge giving rise to cells of the outer root sheath (ORS). In humans, the proximal outer root sheath is believed to contain a niche of stem cell progenitors that replenish transit amplifying cells of the matrix which form the growing hair shaft [2,3]. The dermal papilla (DP) and dermal sheath (DS) make up the hair follicle mesenchyme and play a central role in regulating the differentiation pattern of overlying epithelial cells [4,5]. Hair shaft diameter is also strongly correlated to the size of the DP; the more DP cells and the larger the DP volume the thicker the hair shaft produced [6,7]. This is important to note in the context of this viewpoint, as a reduction in DP size is observed with the onset of male pattern baldness [8]. We have therefore focused predominantly on the DP within this piece, as a target to increase hair shaft thickness.

All hair follicles develop prior to birth, except in extreme circumstances such as during wound healing [9]. The process of hair morphogenesis is fairly well understood and a full breakdown can be found in several other reviews [10–12]. All adult hair follicles transition through various stages of the follicular cycle (Table 1).

Hair follicles are extremely heterogeneous across body sites in terms of morphology, cycling, and gene expression profile [13]. Almost all of the human body bears hair follicles, however most of these are tiny, unpigmented, and almost invisible hairs known as vellus hairs. In pattern baldness, we see terminal hairs (thick and pigmented hair shafts) on the scalp transition to a vellus state through a process known as miniaturisation. This gives the appearance of hair loss when in most people (90%) the quantity of hair follicles remains unchanged [14,15]. There is heterogeneity in fibre size but this is between follicles and not within a single fibre [16]. Thus, it seems clear that miniaturisation of the fibre occurs during transitions through the cycle stages, rather than part way

though anagen (Figure 1). Original theories proposed that miniaturisation occurred gradually over several hair cycles, with terminal hair transitioning to a vellus state through a series of shorter and shorter anagen phases. However, in 2001, Whiting argued that a reduction in anagen phase duration over a number of cycles could not be the sole mechanism responsible for miniaturisation as the process would take years to occur, which is significantly longer than clinical observations of miniaturisation (6-12 months) [17]. It is now generally accepted that miniaturisation occurs as an abrupt large step process during one cycle transition instead of over several cycles. The question is now whether miniaturisation is perpetuated as a result of disruption in the anagen-catagen transition or the telogen-anagen transition, or both.

During the hair follicle cycle, there are also cyclic changes in the number of cells within the DP. At the end of anagen there is a decrease in the number of cells in the DP, however with anti-apoptotic factors expressed and little evidence of cell death [18], cell migration from the DP is thought to drive this reduction. Recently, serial imaging of labelled hair follicles in mice during the transition from late anagen to telogen show that DP cells efflux from the DP into the DS. Here it is thought they either undergo apoptosis or incorporate into the hair follicle dermal stem cell (hfDSC) niche [19], while similarly in humans apoptosis has been observed in the DS, reinforcing this suggestion that DP cells apoptose once outside the DP [20]. Transitioning from telogen-anagen there is a small amount of cell division within the DP itself [20], however the majority of the increase in cell number is due to an influx of cells from the dermal sheath, populated by proliferation of hfDSCs in late telogen and early anagen [19,21]. Thus, if miniaturisation occurs during one cyclic transition, either the efflux from the DP is too great or the influx from the DS is too little (Figure 1).

Increased cell efflux in the anagen-catagen transition may be due to loss of cell adhesion molecules such as N-CAM, tenascin or integrin within the DP [22], increased mechanical forces acting on the DP, or a greater migratory response of DP cells attempting to replenish the DS which undergoes apoptosis during catagen [20]. Reduced influx during the telogen-anagen transition may be due to an inability of hfDSCs to proliferate and thus replenish the DP. Alternatively, there may be a

reduction in adhesive, or aggregative cues from the DP which would signal to promote its enlargement.

Hair loss is not entirely dependent on a loss of cells from the DP, and cells within the epithelial portion of the follicle have also been implicated with male pattern baldness. In human scalp follicles highly expressed cytokeratin 15 ($KRT15^{hi}$) is a well described positive marker of bulge stem cells [23–25] while low levels are found within the proximal ORS of anagen follicles and secondary germ in telogen [24]. Analysis of epithelial cells (bulge and progenitor) from balding and non-balding follicles using flow cytometry revealed that while $KRT15^{hi}$ bulge cells were present in the bald scalp at similar numbers to non-balding scalp, $CD200^{hi}Itga6^{hi}$ and $CD34^{hi}$, which are believed to represent progenitor cells were markedly diminished [25]. There are two possible explanations for the loss of progenitor cells in balding scalp which depend on whether the shortcoming is epithelial or mesenchymal. Potentially, the ability of bulge stem cells to convert into progenitor cells is perturbed, either due to an autonomous defect or an inability to respond to signalling initiating the conversion [25]. Alternatively, the DP signal initiating the conversion of bulge cells into progenitors may be altered or weakened. Evidence in support of this second proposal comes from work performed in vitro where balding 'induced' DP cells, unlike non-balding DP cells, were unable to initiate expression of the differentiated follicular keratin, K6hf, in cultured bulge cells [26], suggesting differences in the ability of the two DP types to signal to epithelial cells.

In recent years, other components of the follicle have been suggested to be involved in the onset of AGA. In particular observational studies on the arrector pili muscle (APM) attachment and AGA remarked that miniaturised follicles within a follicular unit have had their APM replaced by fat [27]. This phenomenon is not observed in alopecia areata nor in standard vellus hair follicles, so it poses an interesting question as to whether APM loss is simply a consequence of AGA miniaturisation or a factor in determining it [28]. Lending weight to the idea that there is communication between the follicle bulge and the APM are mouse studies where loss of matrix proteins produced by the bulge resulted in a shift in the site of attachment of the APM [29].

**Getting Testy – Androgens and Company**

The cellular mechanism of how miniaturisation occurs is complicated, however, a clue that androgen action is fundamental to this process lies in the clinical name for male pattern baldness; Androgenic Alopecia (AGA). Somewhat paradoxically androgens are also required for terminal hair maturation during puberty (pubarche). In individuals with testicular feminisation functional androgen receptors fail to develop which results in no pubarche or AGA [30].

The androgen receptor (AR) is a ligand activated transcription factor [31,32], found in both DP and DS [33–35]. The focus on androgens in male pattern baldness has been intense over the years as genetic variability in this receptor was shown to be a pre-requisite for early onset of AGA [36]. Since identifying variants in the AR as pre-disposing for AGA, several other potential loci have been identified in further genetic studies. Most recently, a GWAS assessing AGA was performed which identified 71 possibly causative loci, most notably associated with 3 pathways; the Wnt signalling pathway, apoptosis, and the AR signalling pathway [37,38]. Aside from GWAS, transcriptomic studies, or those analysing serum content in men with AGA have identified a myriad of other factors associated with hair loss [39,40].

A number of androgens can bind to the AR leading to differential binding of the AR to genomic locations regulating gene expression. In balding the necessary androgen is 5a-Dihydrotestosterone (DHT). DHT is a metabolite of testosterone that is converted in the hair follicle mesenchyme by the action of 5$\alpha$-reductase [35]. The capacity of a hair follicle to convert circulating testosterone determines whether or not it will miniaturise, and DP from balding scalp are known to express higher levels of 5a-reductase than non-balding scalp [41,42]. This heterogeneity in 5a-reductase expression in hair follicles explains the classic pattern of balding on the frontal scalp, but also explains how a hair follicle from occipital non-balding scalp can be utilised in hair transplant surgeries and why a balding hair follicle will still miniaturise at the same rate even if transplanted away from the scalp [43]. However, as alluded to earlier, androgens have a paradoxical effect on hair

follicles. DHT is required for hair follicles on the scalp to miniaturise, but it is also required for beard and chest hair follicles to mature [34,44]. So DHT is able to elicit the exact opposite response in different hair follicles. Some clues as to why this might be can be found in expression studies comparing beard DP and occipital scalp DP [45] and whilst the definitive reason for this inverse response is yet to be elucidated it may well hold the key to reversing miniaturised hair follicles.

While androgens seem to initiate miniaturisation, the molecular mechanism by which miniaturisation occurs is not well understood. Recently, elevated expression levels of AR associated Prostaglandin $D_2$ Synthase (PTGDS) and its product prostaglandin $D_2$ ($PGD_2$) were identified in balding scalp skin compared to haired occipital scalp skin [46]. $PGD_2$ is thought to mediate apoptosis of the non-permanent hair follicle keratinocytes through Prostaglandin $D_2$ receptor 2 ($DP_2$).

**Are we Manipulating Miniaturisation or Simply Accelerating the Hair Cycle?**

While much research has been carried out into the causes of hair loss, currently, there are only 2 drugs that have been FDA approved for AGA; finasteride and minoxidil. However, these are viewed as preventative therapies, and neither finasteride, minoxidil nor current drugs under clinical trials have shown the ability to reverse terminally miniaturised hair follicles [47,48] underscoring that we are yet to see a comprehensive therapy for reversing AGA.

Finasteride inhibits the action of 5$\alpha$-reductase and therefore reduces the upstream action of DHT and effectively halts the progression of miniaturisation [49]. Finasteride's effect on the cellular mechanism of miniaturisation is not clear, however, an increase in anagen to telogen ratio is observed in patients receiving finasteride [50] indicating that hair follicles are promoted to either stay in anagen or transition into anagen.

Minoxidil, in its approved topical form is believed to act by prompting hair follicles to transition from telogen to anagen. However, lengthier body hairs in individuals using minoxidil would indicate that anagen duration may also be prolonged [51]. There is confusion as to the exact mechanism by which

minoxidil works; opening potassium ion channels promoting vasodilation, upregulation of VEGF, and upregulation of prostaglandin E2 have all been proposed [52–55]. Regardless, if minoxidil simply accelerates hair cycle progression and does not impact efflux or influx it would only slow the progression of miniaturisation and/or reduce the appearance of baldness by increasing the number of hair follicles in anagen at any given point in time.

Prostaglandin/Prostamide F2a analogs such as latanoprost and bimatoprost have also shown an ability to increase hair growth by stimulating hair follicles to transition from telogen to anagen [56]. As of yet, subsequent research into these analogues for AGA has not led to an approved therapy for AGA but this is perhaps expected as the progression from bench to bedside can take several years [57,58]. Setipiprant is a more recently developed orally administered antagonist of $DP_2$ which is currently undergoing clinical trials (NCT02781311) due to finish mid-2018. $DP_2$ antagonists are proposed to reduce $PGD_2$ mediated apoptosis, effectively delaying catagen.

For the most part manipulating the hair cycle seems to be the mode by which newer therapies look to treat alopecia. The main reason for this could be down to the animal models used to evaluate efficacy of drugs to take forward for trials. The main animal model for research in hair growth revolves around either shaving or depilating female C57BL/6 lab mice at 7 weeks of age [46,56,59]. At this stage, all hair follicles are in telogen and skin appears pink rather than black; depilation induces a homogenous re-entry into anagen which can then be observed and potentially perturbed [60]. Alternatively, if mice are shaven mice one can assess whether anagen re-entry, which would not normally occur for another 4-5 weeks, can be brought forward [61] by application of candidate therapeutics [56,59]. While these mouse models are incredibly useful to observe signalling during hair follicle cycling in mice, a drug's ability to reverse miniaturisation cannot be truly observed.

Probably the most relevant model for AGA is the stump tailed macaque [62,63], due to the similar physiology of androgen induced miniaturisation, however, its use is severely limited due to availability, size, cost and ethical implications. As an alternative, transgenic K5-hAR mice, which

express hAR in the ORS and basal epidermis have also been developed, where cycling is inhibited temporarily by introducing DHT [64]. While this model is androgen dependant, the primary measure would be re-establishing normal cycling. A perhaps more relevant mouse model for studying miniaturisation is the recently utilised K14-Ptgs2 mice, which over expresses PTGS2 resulting in higher $PGD_2$ levels. These mice develop alopecia due to miniaturisation of hair follicles, which have a marked resemblance to human miniaturised follicles [46].

The focus on manipulating the hair cycle is also the case with clinical trials where total anagen hair count is the primary measured outcome. While a prospective drug may be able to accelerate the induction of anagen in hair follicles this does not with certainty indicate that a miniaturised follicle will be resurrected. We see this with current therapies where none are able to restore terminally miniaturised hair follicles.

**Cellular Therapies and Models That Look Beyond the Hair Cycle**

As we have introduced during this viewpoint, there are three main cellular mechanisms which can be possible causes for follicular miniaturisation. Firstly, there may be too much efflux of cells from the DP in the anagen to catagen transition. Secondly, and in combination with the above point, there may be too little influx of cells into the DP in the telogen to anagen transition. Thirdly, there may be an impairment in the conversion of bulge cells into their progenitors. Thus, while research focus is often on manipulating the signalling pathways associated with male pattern baldness, such as AR signalling, we believe that modulating the cellular mechanisms behind miniaturisation will help identify treatments which can reverse AGA in addition to preventing its progression. However, while targeting all three of these mechanisms may help prevent miniaturisation, they will not necessarily reverse it. For example, reducing efflux from an already miniaturised DP in the anagen-catagen transition may result in a gradual increase in DP size over time so long as hfDSCs migrate into the DP, but not a step-change such as the one observed during miniaturisation (Figure 2). We believe the key to reversing miniaturisation lies in the telogen-anagen transition, and influx of cells into the DP. Thus, to increase DP size over and above the increase normally observed during a cycle transition

there has to 1) be an active recruitment by the DP or 2) be an increased number of cells migrating toward the DP. We know DP enlargement is possible, as hirsutism is seen in individuals after taking of certain therapies including oral minoxidil [55,65]. In addition, as mentioned previously beard follicles undergo a reverse miniaturisation process during puberty; transitioning from a vellus state with a small DP to a terminal state with a larger DP (Figure 2). Assuming DP cells do not proliferate significantly during this transition, net migration of cells into the DP must be positive. Possible avenues to promote net migration into the DP may be inspired by our understanding of hair development and hair follicle cycling where WNT, FGF, and BMP signalling all play roles in promoting dermal condensation and maintaining the inductive properties of the DP [10,11,66,67].

Whether or not increasing DP influx will act as a permanent therapy for AGA may depend on whether a miniaturised state is the new normal. DP in mice have been shown to self-regulate their size during transitions through the follicular cycle, returning to their original size in anagen subsequent to partial ablation in a previous anagen [68]. So while promoting influx may increase DP size, intrinsic properties may revert the DP to miniaturised state in a subsequent cycle. Recently, the Hippo pathway has been implicated and proposed as a potential modulator of the hair follicle due to its ability to guide cell proliferation, differentiation and stemness [69,70]. Moreover the hippo pathway is well established in organ size control and tissue homeostasis [71]. While it has not been comprehensively shown to play a role in AGA if indeed Hippo or another signalling pathway enables DP to self-regulate their size or the bulge cells to maintain their stemness, then these may provide intriguing targets through which we can manipulate the size of the fibre produced by a hair follicle.

## Conclusion

In summary, to identify therapeutics to reverse AGA we believe focus should be on modulating the cellular processes perturbed during transitions through the stages of the cycle, rather than the specific stages. Inter-phase migration between the mesenchymal compartments and the capacity of hfDSCs to replenish the DP, either through epithelial signalling from bulge (similar to the placodal signalling seen in development) or through self-modulating mechanisms, can determine whether or

not a follicle will miniaturise. Our viewpoint is that an ideal therapy for AGA is one which can promote influx into the DP from the hfDSCs, resulting in DP enlargement and a step change reversal of miniaturisation.

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**Conflict of Interest**

The authors have no conflicts to declare.

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**Table**

| Phase | Description |
|---|---|
| *Anagen* *(growth phase)* | Characterised by the proliferation and differentiation of epithelial cells, originating directly or indirectly from the bulge, that form the follicle and eventually hair itself. The duration of anagen varies across follicles and determines the length of the hair produced. It can last from a few months in eyelashes to a several years in scalp hairs. |
| *Catagen* *(regression phase)* | Often referred to as the first stage of the hair cycle, catagen is a 2-week period of regression and pre-programmed apoptosis. The hair shaft itself becomes a club hair, which is no longer actively growing. During catagen the DP reduces in size, and there is apoptosis within the DS and epithelium [20], resulting in movement of the DP so it rests beneath the regressing epidermal compartment. |
| *Telogen* *(resting phase)* | After catagen, the follicle transitions through to telogen, a resting stage of low metabolic activity that lasts for around 3 months in scalp follicles. The club hair resides in a trichilemmal sac, or old bulge [72]. It is thought that the DP regulates the return of the follicle back to anagen by signalling the overlying epithelial cells, known as the hair germ, to proliferate and differentiate in a process reminiscent of development [73]. |
| *Exogen* *(shedding phase)* | Exogen is a phase independent of the main hair follicle cycle where the club hair is actively shed from the old bulge [72,74,75]. Exogen refers to the state of the club hair rather than the hair follicle. |
| *Kenogen* *(latent lag phase)* | Kenogen is not observed in all hair follicles, however, frequency and duration increases in scalp follicles of individuals with AGA [76,77]. It refers to a lag phase, or a follicle in telogen which has lost its club hair (exogen) prior to re-entry into anagen. |

Table 1 – Brief overview of stages of the hair cycle in human scalp follicles.

**Figure Legends**

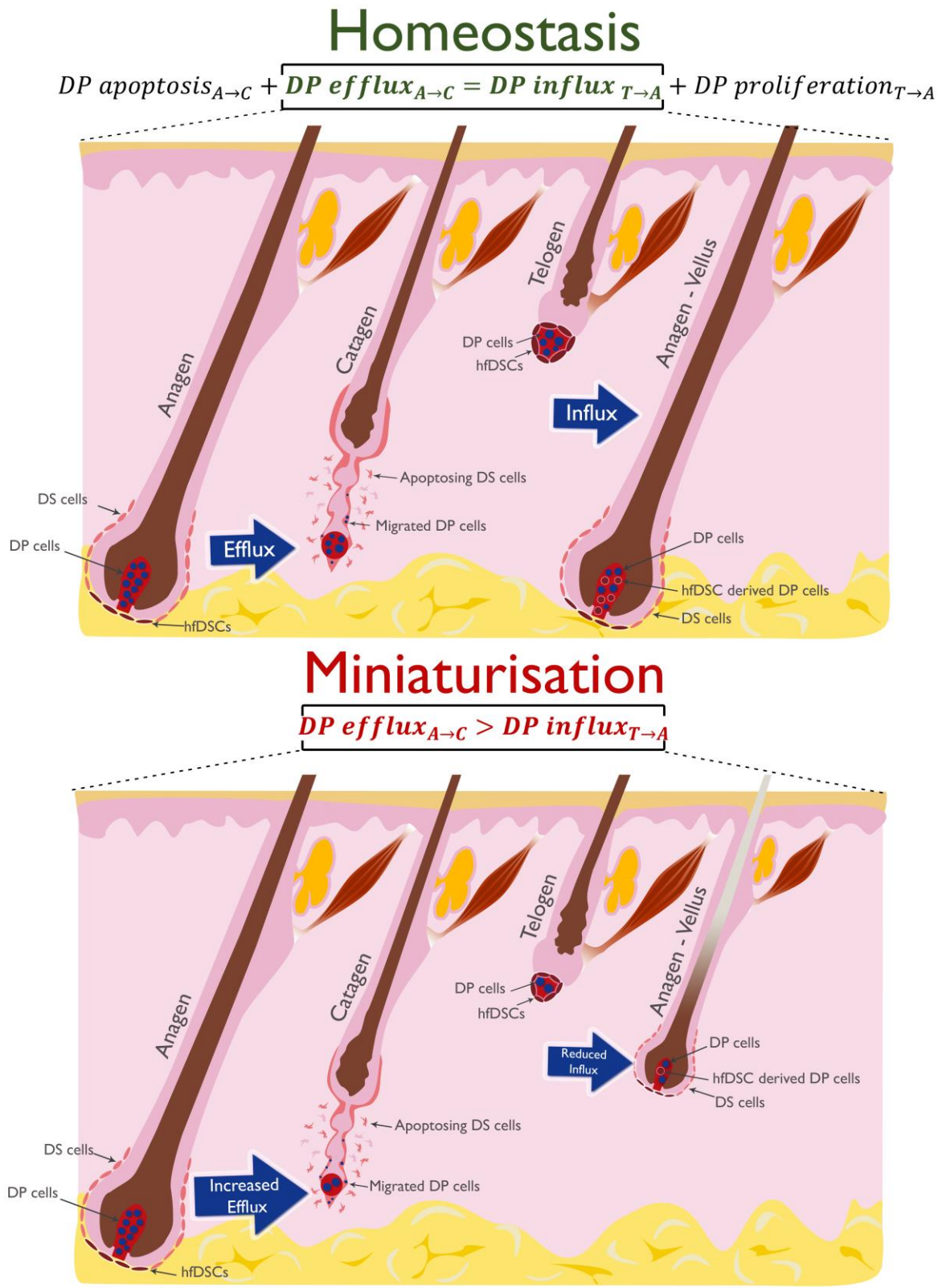

**Figure 1 – Simplified dermal mechanisms underlying terminal follicle cycling and miniaturisation.** In catagen DP cells migrate out of the DP into the DS which is largely degraded. In telogen the dermal compartment of the hair follicle is reduced with a small number of hfDSC surrounding the DP. In homeostasis the DP is fully restored via migration of these hfDSC into the DP during anagen resulting in the same number of cells in the DP as in the previous anagen. In miniaturisation, increased efflux of DP cells may occur in the anagen to catagen transition. Alternatively, or conjointly hfDSCs might not replenish the DP fully in the telogen to anagen transition. Loss of hair follicle homeostasis may be due to external factors such as DHT and/or perturbations to self-regulating modulators.

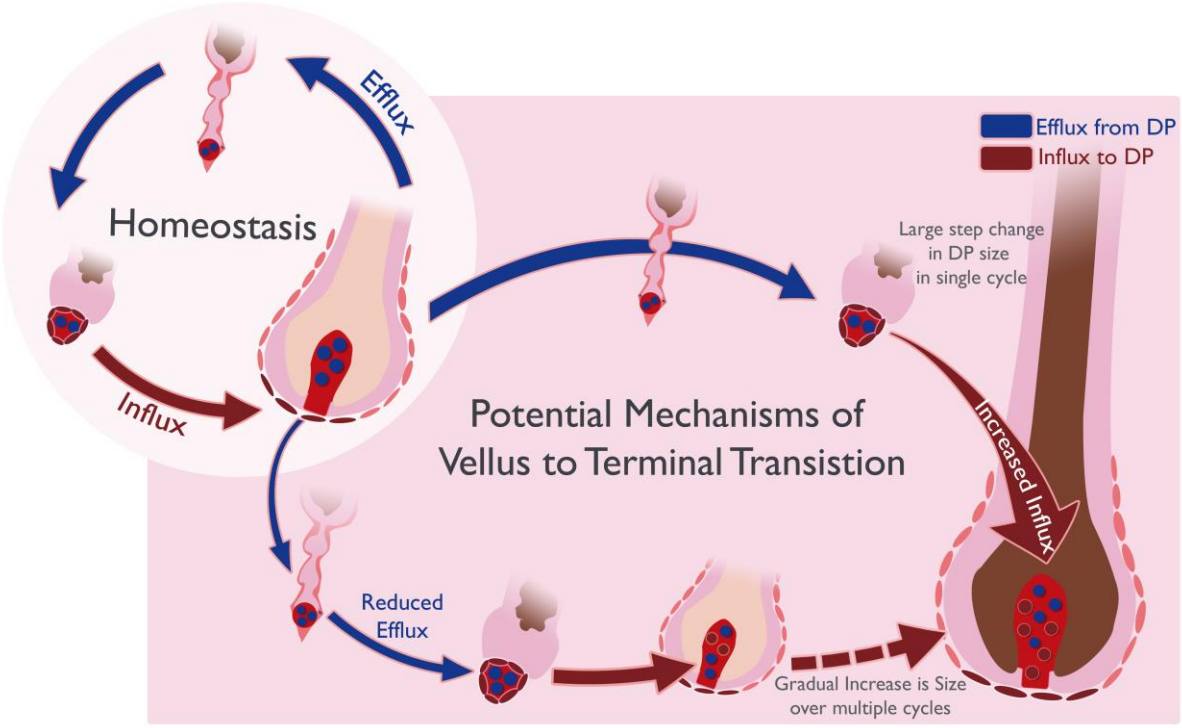


**Figure *2* – Schematic with possible dermal cellular mechanisms modulating a vellus-terminal transition**. Increased influx into the DP during the telogen-anagen transition would trigger a large step change that would take a vellus hair to terminal state in one cycle. Comparatively, reduced DP cell efflux during catagen would over time result in a gradual increase in DP size assuming that influx

of hfDSCs remains unchanged. Alternatively, both of these mechanisms may be required to act in tandem with one another to achieve a reversal of male pattern baldness.