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Forum

Hair Coloration by Gene Regulation: Fact or Fiction?

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The unravelling of hair pigmentation genetics and robust delivery systems to the hair follicle (HF) will allow the development of a new class of colouring products. The challenge will be changing hair colour from inside out by safely regulating the activity of target genes through the specific delivery of synthetic/natural compounds, proteins, genes, or small RNAs.

Genetics of HF Pigmentation and Greying

Pigmentation is one of the most striking phenotypic traits in humans. It has great social and psychological relevance and the possibility of controlling it has immense resonance for any individual or ethnic group. The colour of human hair, skin, and eyes is determined by the amount, type, and tissue distribution of melanin. Melanin is a complex mixture of pigmented indole-rich biopolymers that are synthesised by specialised cells called melanocytes. The main types of melanin polymer are the black-to-brown eumelanin (highly polymerised) and the yellow-to-reddish-brown pheomelanin (lighter, less polymerised, and containing sulfur) [1]. Black hair has the highest eumelanin-to-pheomelanin ratio while red-to-yellow hairs have the lowest ratio; grey or white hairs have insignificant or no melanin at all. For more information on melanin production by melanocytes, see Box 1.

Pigment synthesis, storage, and transport occur in lysosome-related organelles known as melanosomes. Although the

biochemical synthesis of melanin is common to all pigmented tissues, melanogenesis is regulated differently among them. In hair, melanogenesis occurs only during the anagen (active growth) phase of the hair growth cycle in melanocytes located exclusively in the hair bulb, while in skin, for instance, melanin is constitutively produced. An interesting study [2] demonstrated that a SNP previously associated with blond hair in northern Europeans is responsible for a 20% reduction in the activity of a tissue-specific regulatory enhancer affecting KITLG gene transcription in the HF only. Different regulatory mechanisms underlie the discrepancy between the pigmentation phenotypes of hair, skin, and eyes commonly seen in an individual. Knowing how to interfere

with melanogenesis in a tissue-specific manner will thus be an invaluable cosmetic tool.

Several approaches have contributed to identifying genes involved in melanin synthesis and in the biogenesis, transport, and distribution of melanosomes, as well as genes regulating those processes (Box 1) [3,4]. These approaches include comparative genomics of candidate genes such as those identified in animal models. By October 2011, the International Federation of Pigment Cell Societies database had described 378 putative pigmentation loci for mice and their human and zebrafish homologues (http://www.espcr.org/micemut/). Despite the many genes already implicated in melanogenesis, those

Box 1. HF Pigmentation

Among the various cells that comprise the HF, the relevant differences seen in gene expression between pigmented and grey HFs can be related to melanocyte biology (Table I and Figure I). Microarray analysis is a powerful technique that allows a global perspective on what is occurring in a cell, tissue, or organism at a particular moment through the comparison of the levels of all mRNAs corresponding to the genes being expressed: the transcriptome. Such analysis was conducted by a research group on pigmented and white human HFs [12].

Table I. Mean Fold Changes in the Expression of a Selected Group of Genes that were Upregulated in Pigmented HFs Compared with White HFs [8]

| Gene Symbol	Gene Name	Mean Fold Change
TYRP1	Tyrosinase-related protein 1	116.58
SILV	Silver homologue (mouse)	36.21
TYR	Tyrosinase (oculocutaneous albinism IA)	26.36
MLANA	Melan-A	25.02
TRPM1	Transient receptor potential cation channel, subfamily M, member 1	8.58
SLC45A2	Solute carrier family 45, member 2	5.21
GPR143	G protein-coupled receptor 143	3.88
CAPN3	Calpain 3 (p94)	3.68
PLXNC1	Plexin C1	3.64
KIT	v-kit Hardy–Zuckerman 4 feline sarcoma viral oncogene homologue	2.81
PAX3	Paired box 3	2.23
OLFM1	Olfactomedin 1	2.06
MET	Met proto-oncogene (hepatocyte growth factor receptor)	2.04
HPS1	Hermansky–Pudlak syndrome 1	1.86
OSTM1	Osteopetrosis-associated transmembrane protein 1	1.66
EDNRB	Endothelin receptor type B	1.65

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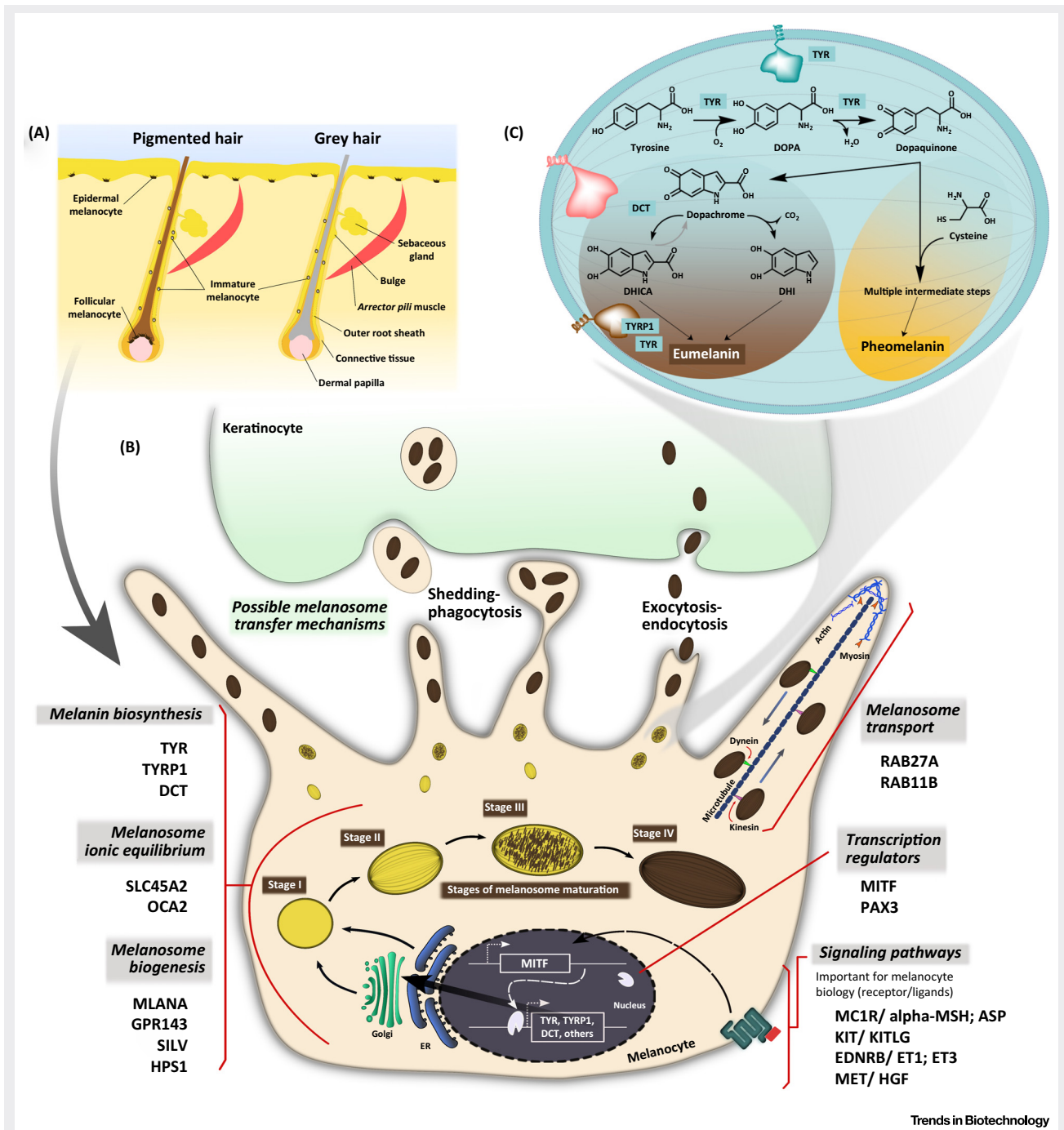
Figure I. Melanocyte Function in HF Pigmentation. (A) Schematic drawing of a pigmented and a grey HF, where some structures and cells are highlighted. (B) Differentiated melanocyte showing its typical cellular processes and some of the most frequently associated genes. (C) Biochemical synthesis of both types of melanin, which occurs inside specialised organelles called melanosomes. Abbreviation: HF, hair follicle.

associated with natural human hair colour variation are rather few [4]. A research group has created a model with very good predictive accuracy for hair-colour categories based on no more than 45 SNPs related to only 12 different genes [5]. We think that the already reported genes, included those in the hair-colour prediction model, are an excellent starting point to develop cosmetic strategies to modulate gene activities, changing hair colour from the follicle (Box 2). However, any

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Box 2. New Cosmetics

The necessary conditions to start a new era in hair cosmetics are now being met. In the available state of the art, a relevant group of genes and their respective proteins were identified whose activities, when altered by DNA polymorphisms, are associated with a particular natural colour of hair within a human population. However, in past decades researchers have accomplished important advances in the development of specific nanocarriers for HF delivery. The combination of the acquired knowledge will soon allow the safe and specific delivery of molecules capable of modelling the activity of these genes and their proteins in the cells of the hair bulb, simulating what occurs in the presence of DNA polymorphisms (Figure I).

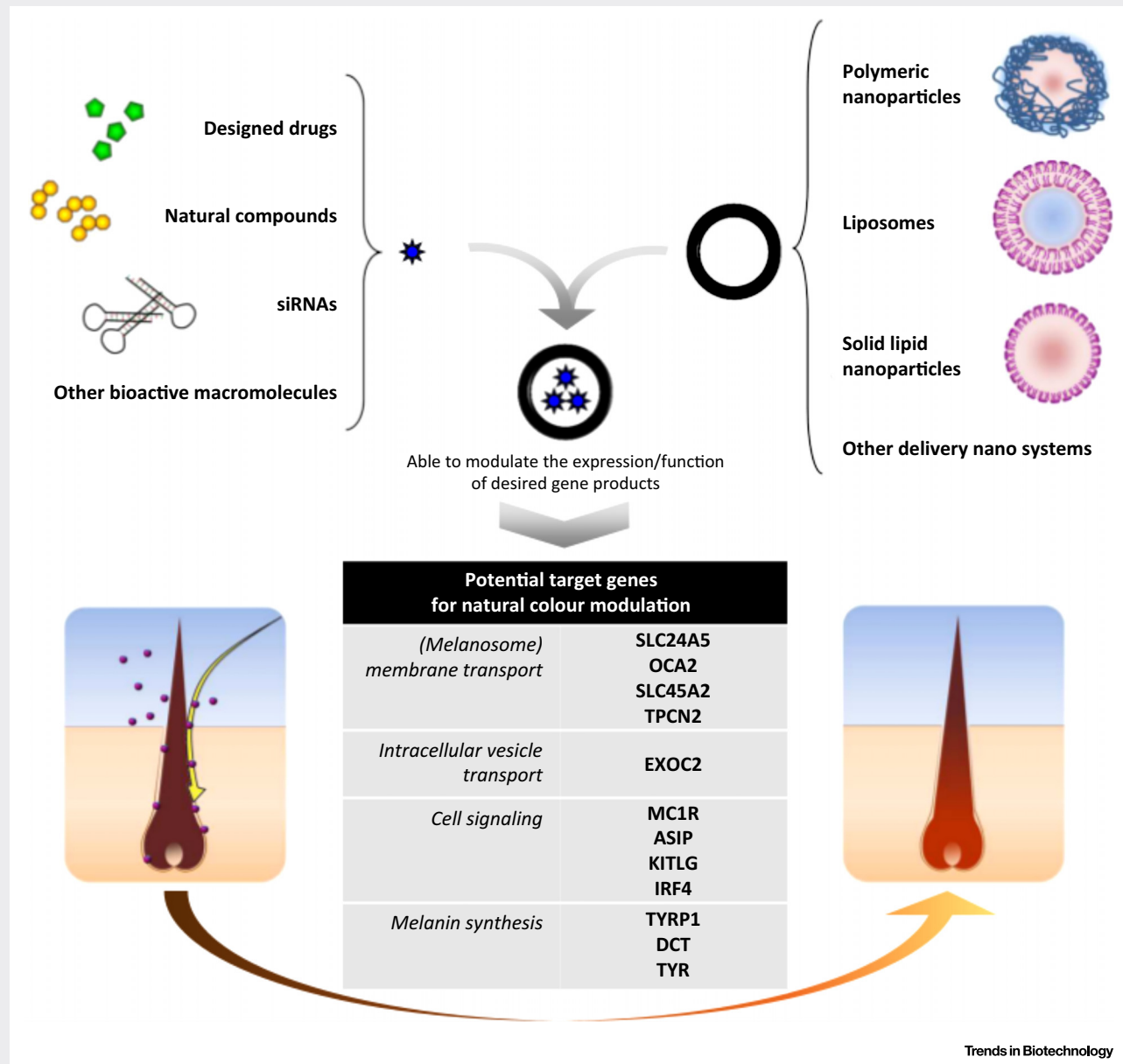| Potential target genes for natural colour modulation	
(Melanosome) membrane transport	SLC24A5 OCA2 SLC45A2 TPCN2
Intracellular vesicle transport	EXOC2
Cell signaling	MC1R ASIP KITLG IRF4
Melanin synthesis	TYRP1 DCT TYR

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Figure I. Scheme for Innovative Approaches to Hair Coloration Based on the Modulation of Genes.

gene acting directly or indirectly in melanogenesis is a potential candidate.

The cellular mechanisms that synchronise melanin synthesis with the active growth phase of the HF remain unclear. Hair greying represents the striking uncoupling of those two events. Intense research on hair greying indicates age-related oxidative stress in the HF [6,7]. Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and their neutralisation. ROS are produced during normal cell metabolism (melanin production involves massive

ROS production) or are caused exogenously by exposure to UV, pollution, inflammation, or even emotional stress [7]. The loss of pigment from the scalp HFs is therefore a direct consequence of the shutdown of melanin synthesis, as confirmed by comparative transcriptome analysis between grey and pigmented HFs (Box 1). This shutdown can have several causes acting at different levels and HF locations [6,7], coordinately or independently: (i) cessation of melanin production; (ii) apoptosis of follicular differentiated melanocytes; and (iii) incapacity to maintain the homeostasis of the melanocyte stem cell niche.

The key observation common to all of these processes is oxidative damage. It is unclear whether this damage is a cause or a consequence of an 'earlier'-acting element determining the timing of hair greying, often a heritable trait. If it exists, this element must be genetic in nature.

The varying susceptibility of melanocytes to oxidative stress within the HF and the independent ageing of HFs throughout the scalp and body indicate a high level of autonomous physiological regulation [6]. The particular crosstalk between melanocytes– keratinocytes and melanocytes–dermal papilla cells in scalp HFs must be important in determining the faster ageing of scalp HFs compared with HFs elsewhere. We believe that in the near future the molecular basis of the cellular events behind hair greying will be deciphered. The ongoing identification of molecular targets will allow the development of efficient antigreying cosmetics.

HFs as a Target for Nanodelivery Systems

The HF is a privileged target for topical delivery. The components of nano/microdelivery systems are known to accumulate preferentially in HFs compared with elsewhere in the skin. Bioeffective doses of compounds can be efficiently delivered to HFs [8,9]. Strategies under development almost invariably include drug encapsulation since it presents advantages over direct application of free drugs. Nanosized carriers address two relevant points in HF-targeted drug delivery [9]: increased guidance into HFs (reducing the exposure of non-target tissues and skin irritation) and increased retention (signifying fewer applications and less-prolonged exposure to the drug). Tuning the physicochemical characteristics of particles allows the control of their penetration depth, protects encapsulated molecules from degradation (particularly high-molecular-weight molecules like proteins or nucleic acids), and improves formulation aesthetics and product stability. In practice, cosmetic care routines are performed repeatedly (several times per week); therefore, continuously applying and retaining an active ingredient in the HF to fulfil its purpose is feasible.

The most common types of nanocarriers for drug delivery to HFs include polymeric and liposomal aggregates (Box 2). Polymer-based micro/nanoparticles are the most interesting for HF delivery because of their more advantageous features: higher fluidity, more pleasant touch, transparency, and a non-oily sensation that is desirable for a leave-on hair-care product. The specific delivery to the hair bulb of a reporter gene in a liposome formulation was successfully demonstrated in 1995, by Lingna Li and Robert Hoffman, as a model for HF gene therapy. Since then, the HF has gained the attention of the scientific community as a target *per se* [10]. The range of macromolecules interesting for HF therapies is continuously growing and includes growth factors, signalling molecules, peptides, and antioxidant enzymes [9]. Despite having a therapeutic purpose [8], we consider that all of the acquired knowledge can be translated to the development of new cosmetic approaches for hair care, centred on its live component: the follicle. The clarification of melanosome-transfer mechanisms to keratinocytes and the identification of the molecular players involved in their recognition by the keratinocyte cell membrane will allow the design of nanosystems specifically targeting keratinocytes to supply melanin efficiently and directly to a greying HF, recycling an old idea by Lingna Li and Robert Hoffman (1993).

The Future of Hair Care: Topical Modulation of HF Biology

Greying reversal will most probably require that undifferentiated melanocytes be recruited to the HF matrix and activated, as cyclically occurs on normal new, pigmented HF formation. There is evidence that, in grey HFs, there is a population of amelanotic melanocytes located at the outer root sheath [6,7]. As indicated by others, the fact that differentiated follicular melanocytes are not associated with melanoma suggests that greying is a protective mechanism and care must be taken when developing antigreying strategies. However, unintentional greying reversal, as consequence of clinical chemical or physical therapeutics or inflammation, puts the subject into a new perspective.

We believe that, if early events responsible for the maintenance and differentiation of the stem pool of melanocytes are clearly identified, it will become possible, by supplying the appropriate stimuli to the follicle, to activate a cascade of events that will culminate in the safe recruitment of active melanocytes to the HF matrix during the transition to a new hair cycle. Obviously, it would be preferable if hair-greying reversal could occur immediately after the application of a formulation, regardless of the hair cycle stage. The migration of melanocytes in an already formed follicle would not be as easy but is possible, since these cells are capable of movement as seen *in vitro* and in vitiligo treatment. We believe that, in the future, it will be possible and safe to recruit undifferentiated melanocytes to repopulate the hair bulb and to produce pigment. Taking into account published works, induction of tyrosinase (Tyr), the major enzyme responsible for melanin production, stability and activity, as well as the exogenous application to HFs of

protective antioxidant enzymes (e.g., catalase, superoxide dismutase) or antioxidants (e.g., curcumin) to protect melanin-producing melanocytes from ROS generation, is also a feasible approach.

The ability to regulate gene activity through small RNA oligonucleotides has opened a new range of opportunities for hair care and treatment. Topical delivery of miRNAs or siRNAs, naked or formulated, has become feasible [9]. The recent work of Hardman *et al.* describes the stimulation of human melanogenesis in *ex vivo* HFs and *in vitro* cultured HF melanocytes by siRNA silencing of two molecular clock genes associated with ROS homeostasis, BMAL1 and PER1 [11].

Changing the colour of a pigmented hair will require not the differentiation or movement of melanocytes but 'only' redirecting a biochemical pathway towards enrichment in one of the possible products. The modulation of natural hair colour by interfering with gene activity in the follicle is, in our opinion, a promising and as-yet-unexplored field of hair-care research. As clearly demonstrated *in vivo* by Guenther *et al.* [2], small reductions in the activity of a HF-specific enhancer were sufficient to change the mouse coat phenotype. This is an extremely important finding for future cosmetic applications, such a modest variation in gene expression levels being sufficient to cause a visible phenotypic change. We expect that producing a formulation specific for HF delivery of siRNA against the KITLG gene will affect human hair colour in the same way, shifting it to a lighter shade.

In terms of natural hair-colour modulation, in our opinion it would be important to decipher the 3D structure of Tyr and tyrosinase-related protein 1 (Tyrp1). Since Tyr is the key enzyme in melanogenesis and the total amount of melanin is dependent on its activity and stability, and since Tyrp1 expression is important for Tyr stability, a drug-design approach could be undertaken to specifically inhibit Tyrp1 catalytic activity without affecting either its expression or the catalytic activity of Tyr. Tyr catalyses the synthesis of dopaquinone, a key precursor of both eumelanin and pheomelanin. While eumelanogenesis requires the direct intervention of two other enzymes, Tyrp1 and Tyrp2, pheomelanogenesis requires only the initial activity of Tyr. By inhibiting Tyrp1, we would expect a shift towards pheomelanin synthesis and therefore a lighter shade of hair colour.

Hair colour science is experiencing exciting times; we expect promising findings for hair care that only a few years ago seemed like science fiction.

Concluding Remarks
Innovative hair care will be grounded in the most recent advances in HF biology and in follicle-targeted delivery for the development of new products that consumers can safely use at home. Major challenges remain, however: the complete understanding how to interfere with the eumelanin-to-pheomelanin ratio at the genetic level and the efficient delivery of molecules to HF cells to safely modulate gene activity. Overcoming those limitations will, in our opinion, take hair care to a new level: changing hair colour from inside out.

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