Introduction

Graying of hair correlates with chronological aging and occurs in all individuals regardless of sex and race. At least half the hair of half of all white individuals is thought to be gray by the time they are 50 years old. Although premature grayness usually appears with no underlying...
disease, the graying process has been associated with
pernicious anemia, hypothyroidism, osteopenia,2 and a
range of uncommon syndromes. There have been isolated
reports of reversal of graying after epinephrine injections3
and sympathectomy4 and by vasoconstriction or continuous
exposure to low temperatures.

In human skin, at least 2 different populations of
melanocyte are present: those in the epidermis and those
in hair follicles.5,6 Melanogenesis in hair follicles is related
to the stage in the hair growth cycle, whereas epidermal
melanogenesis is continuous7 and can be affected by UV
radiation. Hair follicle melanocytes can be divided into 3
different subpopulations. The first, found in the hair bulb,
only expresses tyrosinase-related protein (TRP) 2, does not
proliferate, and presumably represents the melanocyte stem
cells of the hair follicles. The second, found in the outer
root sheath, expresses TRP2 and to a lesser extent TRP1,
shows proliferative activity during the early and intermediate
anagen phase, and represents differentiated melanocytes.
The third, localized to the hair matrix over the dermal
papilla, expresses TRP1, TRP2, and tyrosinase, proliferates
only during the intermediate anagen phase, produces melanin
during the intermediate and late anagen phase, and
progressively disappears in the catagen phase.8 The last 2
subpopulations express c-kit and depend on stem cell factor
(SCF)/c-kit signaling during the anagen phase.9

The best model for studying the hair growth cycle is a
highly standardized C57BL/6 mouse model, in which
manual depilation induces the synchronous growth of
terminal hair.10 From the skin color of the mice after
depilation, we can readily distinguish the telogen phase
(pink color) from the anagen phase (gray to black color).
This model is widely used and the duration of each phase
of the hair growth cycle and the correlation with clinical
and pathological variables are perfectly well defined.10
Between the telogen and anagen phase, the resting
melanocytes proliferate, differentiate, and migrate within
the hair follicle in synchronous fashion to regenerate and
form a new hair bulb.

Thyroid hormone is essential for homeostasis in cells
derived from the neural crest—indeed, congenital
hypothyroidism causes severe neurological damage. In the
skin, it is known that thyroid hormone plays a role in
epidermal differentiation, in enhancing local response to
growth factors, in the physiology of sebaceous, eccrine, and
apocrine glands, and in hair growth.

In this study, we discuss the possible role of thyroid
hormone in the darkening of gray hair with initial reference
to clinical findings from 2 patients. Furthermore, we
investigated the effect of triiodothyronine (T3) on hair
follicles in a mouse model with a view to defining the effect
of this hormone on melanocyte function. Finally, we
corroborated that T3 stimulates hair shaft growth in an in
vitro experiment with human follicular units. On the basis
of these results and a review of the literature, we formulate
several hypotheses on the role of melanocytes not only in
repigmentation but in the whole process of follicular
homeostasis.

Materials and Methods

Case Reports

Patient 1

A 63-year-old man, who and been progressively graying
over the years and who had a 1-year history of fungoid
mycosis (stage IA) treated with psoralen-UV-A (PUVA)
(3 sessions/wk), went into a myxedema coma of unknown
cause. When he attended the clinic for a PUVA session
accompanied by a family member, he showed a low level
of consciousness, irregular breathing, inflammation,
reddening of the face and both hands, and periorbital edema.
His vital signs were as follows: systolic blood pressure, 110
mm Hg; diastolic blood pressure, 60 mm Hg; pulse rate,
56 beats/min; and body temperature, 35°C. Of note in the
laboratory tests were free T3 levels of 0.4 pg/mL (normal
range, 1.5-4.3 pg/mL), free thyroxine (T4) levels of 1.8
pmol/mL (normal range, 10.5-25.0 pmol/mL), and thyroid
stimulating hormone (TSH) levels of 102 mU/L (normal
range, 0.45-7.0 mU/L). The patient was admitted to the
intensive care unit (ICU) for 10 days, where he received
support measures and intravenous therapy with 0.5 mg of
L-thyroxine. He responded satisfactorily and continued
oral therapy with 0.1 mg of L-thyroxine. He was able to
return home after a month. On discharge from the ICU,
complete repigmentation of all gray hairs of his scalp was
observed and these have retained their coloration during 2
years of follow-up. His underlying disease did not show
significant changes and he still needed specific treatment.
The patient did not receive any other medication while he
was in hospital.

Patient 2

The second patient was a 51-year-old man with Cowden
syndrome and a history of thyroid carcinoma treated by
surgery and thyroid hormone replacement therapy. His hair
had gone progressively gray over time. Two years later, he
developed non-Hodgkin lymphoma of the tonsils and cervical
lymph nodes, which was treated with chemotherapy and
radiotherapy (total dose, 36 Gy). At the time, 60% of his
hair was white. After completing 4 cycles of chemotherapy
(cyclophosphamide, doxorubicin, vincristine, prednisone,
and bleomycin) he presented in the clinic suffering from
nervousness, anxiety, and insomnia. He had lost 15 kg. He
was diagnosed with iatrogenic hyperthyroidism caused by
hormone replacement therapy (0.2 mg/d of L-thyroxine). The laboratory tests performed revealed free T3 levels of 1.62 pg/mL, free T4 levels of 34.4 pmol/L, and undetectable TSH (<0.1 mU/L). During this time, complete repigmentation of his hair occurred. The levels of free T4 were normal 3 months after daily treatment with 0.15 mg of L-thyroxine. The pigmentation of his hair has persisted during the 3 years of follow-up.

Both patients received high doses of thyroid hormone, one due to myxedema coma and the other due to overcompensation during thyroid hormone replacement therapy. Although other causes for the reversal of the graying processes cannot be ruled out, such as for example progression of cutaneous lymphoma or PUVA therapy in the first patient,11 or chemotherapy in the second one,12 hair pigmentation due to thyroid hormone seems the most plausible and reasonable explanation. In the first patient, repigmentation cannot be attributed to psoralsens or UV-A, as this treatment was being performed for months before any affect on hair color was noted.

Mice

The animals were cared for and handled in accordance with the standard protocols. Six-week-old female C57BL/6 mice were used (Jackson Labs, Bar Harbor, USA) with an approximate weight of 20 g. Once acclimatized and after a week in observation, the animals were divided into 2 groups of 5 and the dorsum was depilated with cold wax.10 The experiment was approved by the corresponding animal ethics committee.

T3 was purchased from Sigma (Barcelona, Spain). We used C57BL/6 experimental mice, standardized for studies of the hair growth cycle.10 Specifically, cold wax was applied to the dorsum of the 7-week-old mice. After application and depilation, a pink skin color was noted, indicative that all follicles were in the telogen phase. With a micropipette, 0.5 µg of T3 dissolved in 20 µL of ethanol was applied to the dorsum of all the mice in one of the groups, while 20 µL of ethanol was applied to the other group as control. Application was daily, and the study lasted 10 days.

Culture of Follicular Units

After obtaining the appropriate informed consent, follicular units in the anagen phase were obtained by dissection under a microscope of surplus samples from the occipital region of 2 women (aged 30 and 38 years) undergoing hair transplantation. The follicular units were cultured in Williams E medium (Gibco, Madrid, Spain) with glutamine, fetal bovine serum (1%), insulin (10 ng/mL), transferrin (10 µg/mL), hydrocortisone (10 ng/mL), and sodium selenite (10 ng/mL). To avoid superinfection, the culture medium was treated with penicillin/streptomycin (50 U/mL - 50 µg/mL) and amphotericin B (Fungizone, 2.5 µg/mL).13 The follicular units were divided into 2 groups: a control group and a group with the same culture medium but with 100 nmol/L of T3 added. The follicular units were kept half immersed in 24-well plates in an incubator set to a uniform temperature of 37ºC under an atmosphere of 95% air and 5% carbon dioxide. The medium was changed every 72 hours. Photographs were taken with a digital camera using an inverted binocular microscope at the beginning of culture (baseline), and at 72 and 144 hours. All photographs were taken using the same settings.

Results

Mice

At the age of 7 weeks, the skin of all C57BL/6 mice visible after depilation had the characteristic pink color of the telogen phase (Figure 1). When the mice entered the anagen phase, their hair started to grow again, as reflected by a progressive gray to black pigmentation of the dorsum. After 5 days of treatment with 0.5 µg/d of T3, all study animals had entered the anagen phase (Figure 2). In the control...
group, increased pigmentation—suggestive of the start of
the anagen phase—was observed from day 9, but it was not
observed in all mice until day 15.

After 8 days of treatment, a histological study was
undertaken to confirm induction of the anagen phase.
The skin, stretched and adhered to photographic paper
to prevent creasing, was fixed in formalin, washed with
ethanol, and embedded in paraffin according to standard
techniques. Hematoxylin-eosin and Masson trichromatic
staining was performed (Figure 3). All experiments were
repeated twice.

Cultures

Eight follicular units per group were used from each donor.
The results were very similar in both cases. In one of the
groups, after 72 hours, significant growth was seen in 6
of the 8 follicular units treated with T3 whereas growth
only occurred in 3 of the 8 follicular units in the control
group (Figure 4). During this time, the follicular
architecture was completely conserved. The measurements
were made using hardcopies of uniformly enlarged
photographs. The mean (SD) growth for the group treated
with 100 nM of T3 was 1.2 (0.05) mm at 72 hours
compared to 0.65 (0.05) mm for the control group. At
the 200 nmol/L dose of T3, the differences were not
increased (data not shown).

Figure 2. After 5 days of treatment, a change in the skin
coloration of the mouse treated with topical T3 (right) was
observed compared to the control mouse (left), suggesting
synchronous entry into the anagen phase.

Figure 3. Masson trichromic staining of the biopsy
performed at day 10. (A) Control group: follicles
in the anagen phase (IV) in which the tip of the new hair
shaft still had not reached the epidermis (×100). (B) Topical
T3-treated group: a higher follicular density can be seen
in a more advanced anagen phase (VI), with hair
protruding through the epidermis (×100).
Discussion

Hair grays due to changes (slowing or failure) in the repopulation of the new hair bulb in anagen phase with “fresh” melanocytes derived from the outer root sheath of the hair. Expression of tyrosinase and melanogenic activity can only be detected in the subpopulation of follicular melanocytes close to the papillary dermis. This structure, the papillary dermis, secretes growth factors such as SCF and hepatocyte growth factor, which are probably involved in the conversion of hair follicles to fully differentiated melanin producers.

Several studies have shown that hair graying is not an irreversible process. There have been isolated reports of repigmentation of hair after radiotherapy for cancer or after inflammatory processes such as erythrodermic eczema, erosive candidiasis of the scalp, carbuncle, and furunculosis. There have also been reports of temporary hair darkening after administration of high doses of paraaminobenzoic acid and using drugs such as verapamil, tamoxifen, cyclosporine, etretinate, levodopa, latanoprost, and arsenic. Likewise, anecdotal cases have been published of repigmentation of gray hair after exposure to x-rays and following use of PUVA in a patient with Sézary syndrome, in 2 patients with celiac disease, and in 2 patients with porphyria cutanea tarda.

The skin is a well-known target of thyroid hormones. Low hair density on the scalp, thinning of the outer third of the eyebrows, and decreased body hair have often been associated with hypothyroidism and are due to an increased proportion of follicles in the telogen phase compared to those in the anagen phase. Given that these situations can be corrected after the administration of L-thyroxin, thyroid hormone is implicated in the growth of human hair. The thyroid hormone receptors TR-α and TR-β have been detected in hair follicles, with TR-β being the predominant form. These receptors have been detected in cells of the outer root sheath, cells of the papillary dermis, and fibroblasts surrounding the outer lining of the follicle. In addition, in vitro studies with cells of the outer root sheath and the papillary dermis have shown that thyroid hormone may have a direct effect on the growth of hair follicles through its specific receptors.

Our study showed how topical application of T3 accelerates conversion to the anagen phase in a standard murine model. In an in vivo study with SKH-1 mice (hairless mice due to a genetic mutation) and rats, topical administration of T3 in liposomes was seen to significantly increase the number and size of hairs compared to the control group and another group treated with low doses, although this study did not assess the influence of the intervention on any specific phase of the hair growth cycle. More interesting is a recent study that shows a variable influence of thyroid hormone on the skin according to route of administration (topical or intraperitoneal). Specifically, the mice that received T3 intraperitoneally had an epidermis 10% thinner and a lower hair density (48% lower); in contrast, mice that were administered T3 topically had a 78% thicker epidermis and a higher hair density.

Figure 4. Follicular units at baseline (a-d) and after 72 hours (a’-d’) of in vitro culture. Significant growth of the hair shaft can be seen in samples treated with 100 nmol/L T3 (a, b) compared to control samples (c, d). The images correspond to photographs representative of all experiments.
and cell proliferation by stimulating these melanocytes, some adrenergic drugs enhance melanogenesis. Treatment of certain cases of vitiligo have contained thyroid hormone. Some of the preparations that have proved effective in the homeostasis, both with regard to nevus cells (nevus growth) and epidermal melanocytes in a process as common as maturation and differentiation of astrocytes occurring in the hair shafts in gray hairs is not due to complete loss of all follicular melanocytes; in fact, melanocytes negative for dopa and many other markers have been found in the outer root sheath of senile white hairs. This finding leaves the door open to the possibility of stimulating the migration and differentiation of melanocytes to favor repigmentation of gray hair follicles.

Obviously, thyroid hormone may influence other cell types that are not studied or discussed here but that are present in normal skin, such as keratinocytes and fibroblasts. In addition, an essential point to be verified in the above hypothesis, and something that we have not been able to do because it was impossible for us to obtain depigmented follicular units, is whether in vitro repigmentation by selective culture with T3 is possible. Although gray hairs are not usually present in the occipital region (the region from which donated follicles are taken during hair transplantation), we do hope to have such follicular units available in the near future to confirm the described findings.

In summary:

1. Thyroid hormone stimulates in vitro growth of hair shafts in a culture of follicular units.
2. After topical application of T3 to C57BL/6 mice, follicles in telogen phase entered the anagen phase earlier.
3. In humans, thyroid hormone may reverse graying by repigmentation of terminal hair. Follicular melanocytes may be the target cell for these actions.

This work stemmed from clinical findings concerning the role of thyroid hormone in follicular repigmentation; subsequently, using standard models, we have shown other actions, more or less known, of T3 in the regulation of the hair growth cycle. At this point, we can postulate a possible role for melanocytes not only in repigmentation but in the whole process of follicular homeostasis. It therefore seems logical that these cells with neuronal morphology, derived from the neural crest, may play an essential role in the

(160% greater). These findings suggest that although T3 stimulates keratinocyte proliferation, it also favors release of proliferation inhibitors by dermal fibroblasts. In addition, administration of T3 to hypothyroid dogs increases conversion of hair to the anagen phase compared to euthyroid dogs or untreated hypothyroid dogs.35

In vitro studies of follicular units have shown how transforming growth factor (TGF)-β, phorbol esters (tetradecanoyl phorbol acetate [TPA]), bFGF, and fetal bovine serum reduce the growth of hair shafts and play an important role in inducing the catagen phase of the hair growth cycle.13,36 This effect, of unknown origin, could be explained by the fact that both TGF-β37 and bFGF38 stimulate expression and production of c-kit. Melanocytes can regulate the hair growth cycle, pigmentation, and conversion to and from the anagen and catagen phases through expression of c-kit.39 If expression of c-kit is inhibited with anti-TGF-β antibodies or with selective TGF-β inhibitors, for example, pigmentation of the hair shaft is lost and the anagen phase is extended.40 All these substances (TGF-β, TPA, bFGF, etc) have in common that they favor proliferation and differentiation of melanocytes in pure cultures. Thyroid hormone is also necessary for the culture of melanocytes, as is the case with other cells derived from the neural crest. We can therefore infer that the melanocytes could be one of the cellular targets of all these substances (including thyroid hormone) when they act on hair follicles. Along these lines, we can speculate on the role played by thyroid hormone in melanocyte homeostasis, both with regard to nevus cells (nevus growth) and epidermal melanocytes in a process as common as vitiligo, in which thyroid dysfunction has been documented in as many as 30% of newly appearing cases.42 Specifically, as discussed earlier, thyroid hormones participate in the maturation of cells derived from the neural crest through regulation of β2-adrenergic receptors. Thus, for example, the maturation and differentiation of astrocytes occurring after administration of thyroid hormone is mediated by β2-adrenergic receptors.43 With regard to the culture of melanocytes, some adrenergic drugs enhance melanogenesis and cell proliferation by stimulating these β2 receptors, thereby increasing levels of cyclic adenosine monophosphate.44 In contrast, the use of β-blockers may trigger the onset of vitiligo in predisposed patients.45 Finally, some of the preparations that have proved effective in the treatment of certain cases of vitiligo have contained thyroid hormone.46

On a much more theoretical level, and without getting into the interaction of multiple neuroendocrine factors, we can infer that decreases in thyroid hormone levels affect melanocytes, as occurs with other cells derived from the neural crest. At times, sudden hair whitening may be preceded by a stressful experience or neuropsychiatric disorders, often related with decreased thyroid hormone or increased demand. It is popularly accepted that premature graying over a short period of time is caused by worries and being in a state of tension. Thus, events such as a fright, a stressful episode, and sustained anxiety (situations in which the appearance of alopecia areata or sudden graying of hair is widely documented both historically and culturally) may lead to a sudden increase in demand for T3. In situations in which it is impossible to produce more hormone, the first cells affected may be the follicular melanocytes. Epidermal melanogenesis is constant and may have lower hormonal demands, whereas follicular melanogenesis is cyclic, momentary, and may have a higher hormonal demand.

Melanocytes of the hair follicles reach their peak proliferative capacity during the early and intermediate anagen phase, which is associated with expression of c-kit on the surface of the melanocytes. Loss of pigmentation in the hair shafts in gray hairs is not due to complete loss of all follicular melanocytes; in fact, melanocytes negative for dopa and many other markers have been found in the outer root sheath of senile white hairs.47 This finding leaves the door open to the possibility of stimulating the migration and differentiation of melanocytes to favor repigmentation of gray hair follicles.
regulation of the hair growth cycle and are not just limited to providing hair color. In everyday life, we see how gray hair of the beard and scalp is thicker and more resistant to thinning than pigmented hair. Pharmacological intervention, using melanocytes as new targets, might open up new lines of research in hair growth besides the preferred current ones aimed at blocking miniaturization due to sex hormones.

Conflicts of Interest
The authors declare no conflicts of interest.

References

38. Burger P, Lukey PT, Coetzee S, Wilson EL. Basic fibroblast growth factor modulates the expression of glycoporphin A and