STEM CELL WRAP-UP

Review Part 1 of Dr. Fuchs' lecture, using study and viewing guide
Review Part 2 of Dr. Fuchs' lecture through the discussion about applications of stem cell research to burn therapy, using study and viewing guide

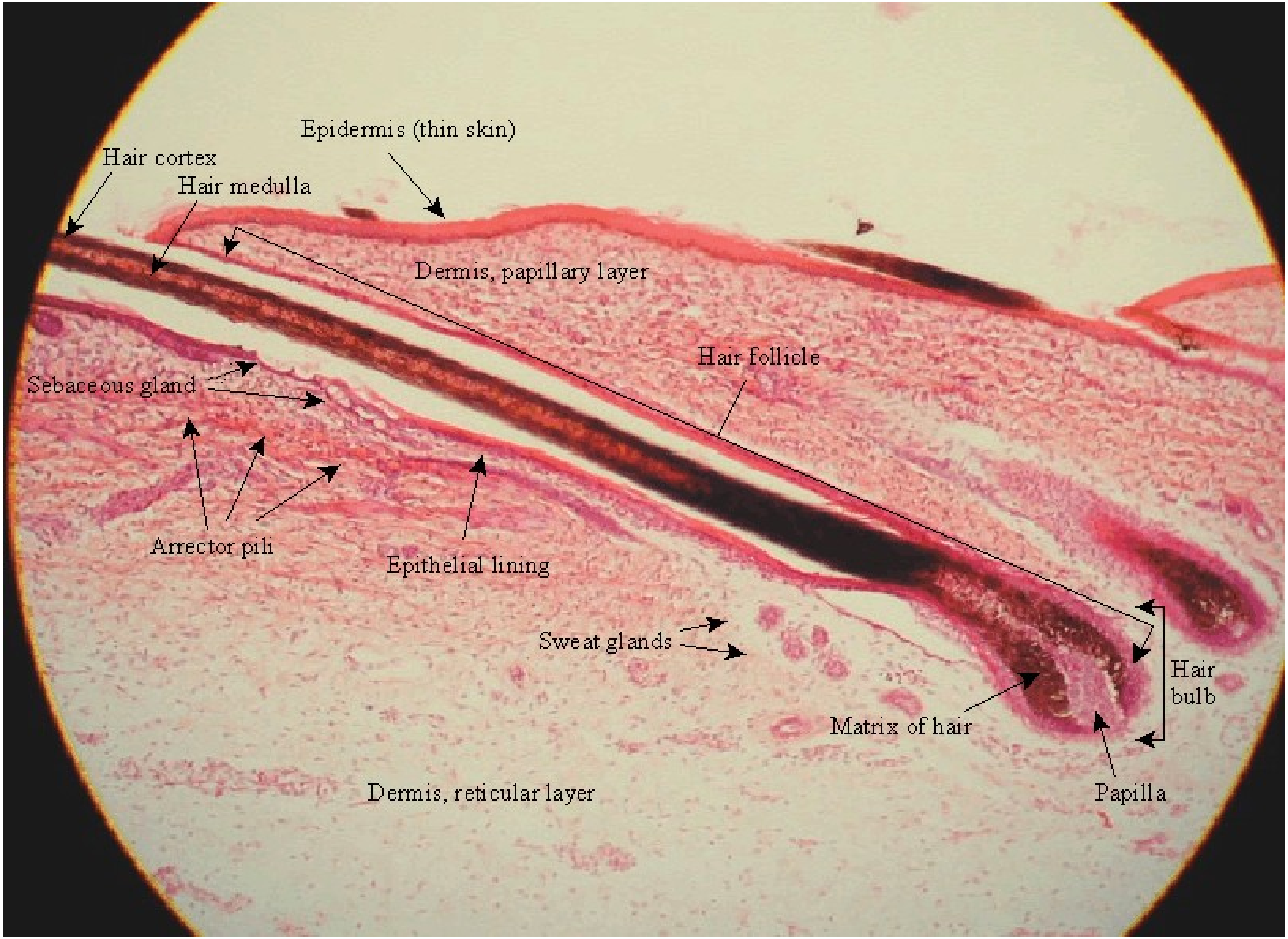
TODAY: Goal is to highlight two important points from the last part of Dr. Fuchs' lecture in Part 2, addressing two basic questions:

Within the epidermis, where are stem cells located?

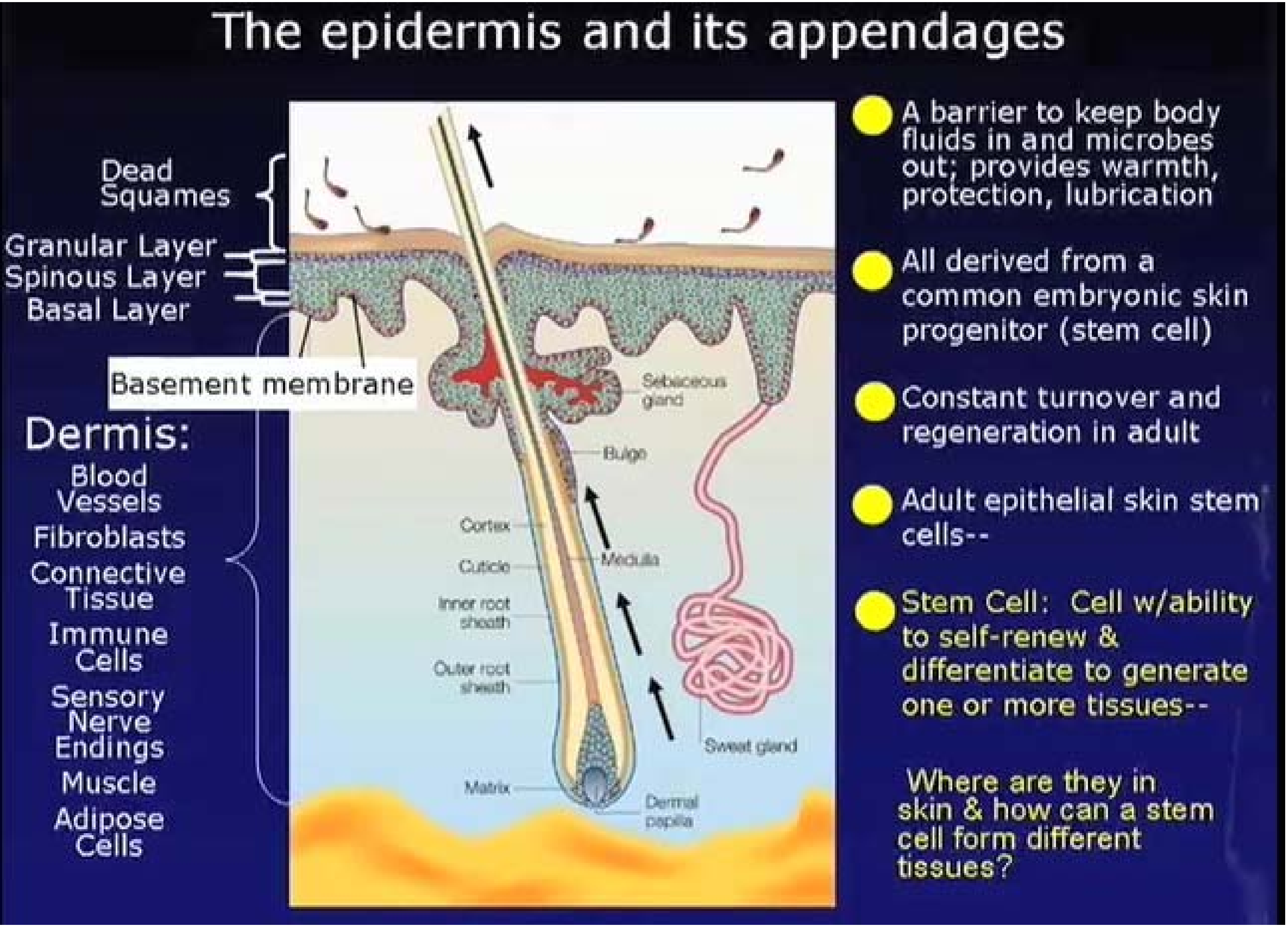
How are adult skin stem cells different from other cells in the epidermis, particularly in what genes are expressed?

We will examine an experimental approach used to obtain some answers to these questions.

www.ascb.org: Fuchs lecture part 2



http://biology.clc.uc.edu/fankhauser/Labs/Anatomy_&_Physiology/A&P201/Integumentary/hair_follicle



www.ascb.org: Fuchs lecture part 2

Skin Cell Highlights:

- About 20 cell types are present
- Main cell type is the **keratinocyte**
- Among the other cell types are:
  - **melanocytes** (produce melanin that helps to protect skin from the effects of UV irradiation)
  - **Langerhans** cells (which are involved in skin immune responses)
  - **Merke**l cells (which detect mechanical pressure forces)
- Keratinocytes produce different variants of the protein **keratin**.

Within the epidermis, where are stem cells located?

How are skin stem cells different from other cells, particularly in what genes are expressed?

GAME PLAN

1) Use an expected characteristic of the stem cell population to distinguish them from other cells in the skin. Based on Dr. Fuchs' studies, expect stem cells to be used sparingly and to divide infrequently. Keratinocytes with the potential to proliferate produce specific keratin proteins.

2) Based on these characteristics, engineer a "**TAG**" onto dividing keratinocytes.

Note that one only want to "TAG" epidermal cells and NOT other types of cells. Also note that as cells divide, there should be a difference in the intensity of the "TAG" that is retained by cells. The "TAG" will be more intense in cells that divide infrequently, but will be diluted out in cells that divide repeatedly.

Therefore, based on what one expects for stem cells, the "TAG" should be retained by stem cells, but lost over time in other cells (dilution effect) (seen in pulse-chase experiments).

Use a method (fluorescence microscopy) to visualize "tagged" cells within the tissue to determine where the stem cells are located.

3) Use a method (FACS) to separate different types of cells from one another based on the presence of the "tag" or not. Collect "tagged" cells into one pot and "untagged" cells into another pot.

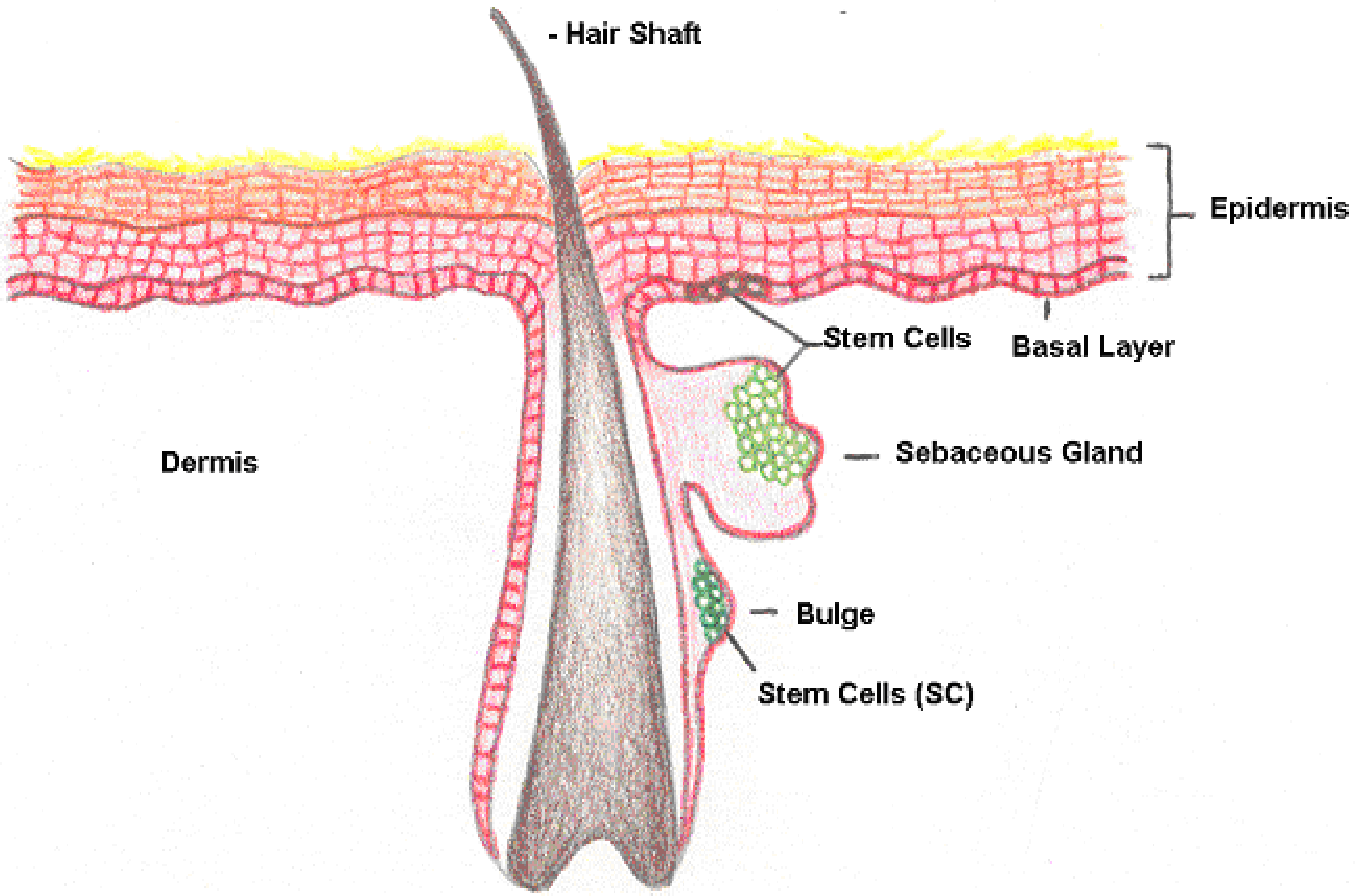
4) Use a method (DNA microarray analysis) to determine what different genes are expressed in the "tagged" cells compared to the "untagged" cells.

Use a characteristic of the stem cell population to distinguish them from other cells in the skin

Stem cells are used sparingly and would be expected to undergo cell division infrequently.

Therefore, is there a way to monitor cell division in skin cells (and ONLY within skin cells and not other cell types)?

And can a change in the RATE of cell division over time be detected in different populations of skin cells?



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First, we want to TAG proliferating keratinocytes AND regulate when that TAG is actually made in the dividing cells .

BASIC ORGANIZATION OF A PROTEIN-ENCODING GENE

What is the TAG?

A DNA packaging protein (in this case, a histone protein).

Cells undergoing new DNA synthesis in preparation for cell division will incorporate the packaging protein onto their DNA.

Add **GFP** to the histone so that all cells that undergo new synthesis (as an indicator of cell proliferation) can be visualized by fluorescence microscopy.

How can the TAG gene be constructed so that it is only expressed in skin cells?

Use a gene promoter that is only activated in skin cells.

Note that the keratin 5 and 14 genes have a keratinocyte-specific promoter, active only in keratinocytes because the proteins required to activate the promoter are only found in keratinocytes, and not other cell types.

How can the TAG gene be turned ON and OFF when needed?

Use a protein that BLOCKS (represses) the expression of the TAG gene.

The repressor only works in the presence of tetracycline (tet).

Repressor + tet → repressor binding to REG and TAG gene expression is BLOCKED.

Repressor – tet → no repressor binding to REG and TAG gene expression is NOT blocked.

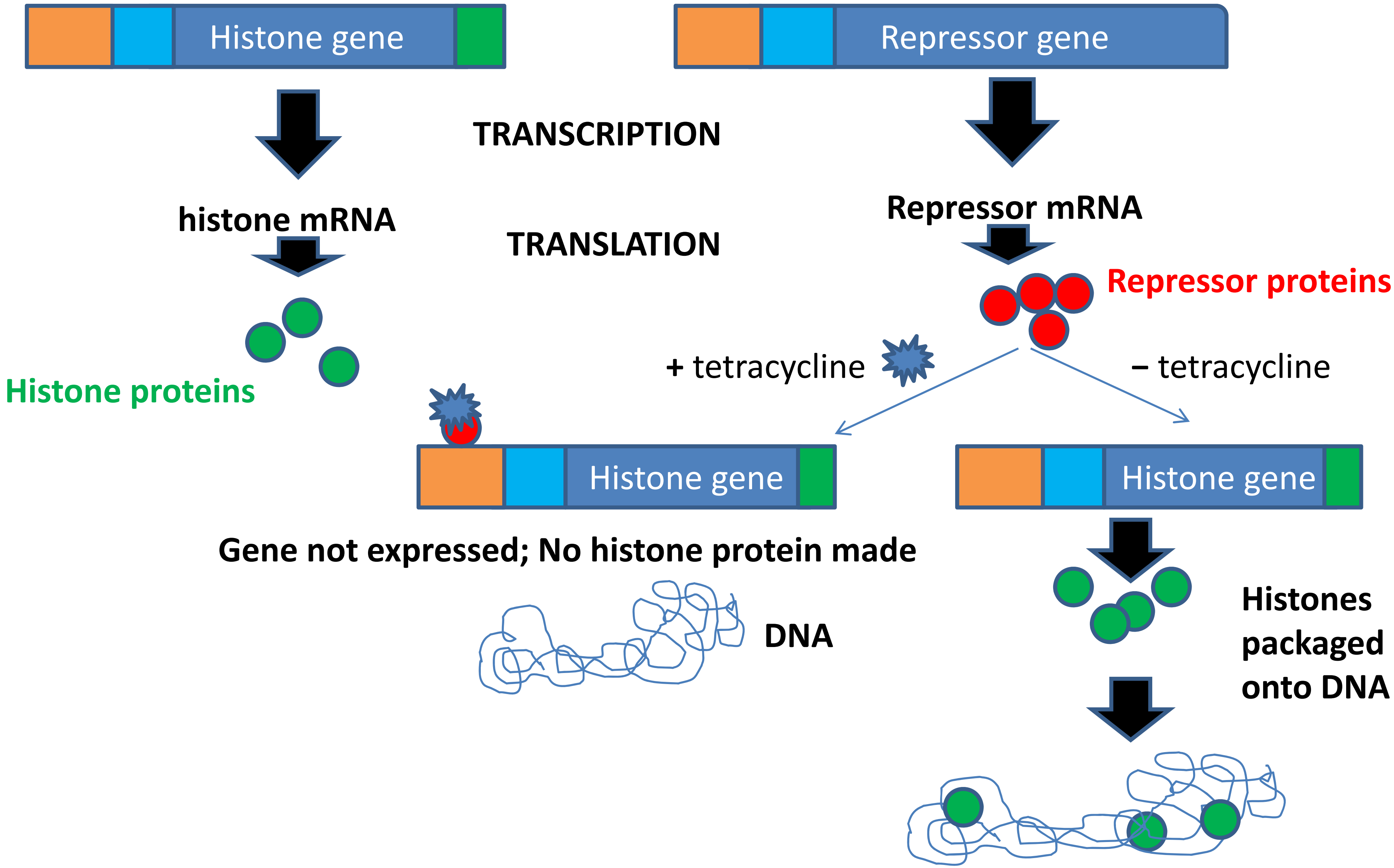
1. MAKE A DNA CONSTRUCT FOR THE TAG

1) Control sequences regulate when the gene is ON or OFF
2) The promoter will only be turned on in keratinocytes
3) The histone protein will be used to package newly synthesized DNA found in the cell nucleus
4) The histone protein itself will be tagged with GFP so that cell nuclei that house newly synthesized DNA can be visualized by microscopy

2. MAKE A SECOND DNA CONSTRUCT THAT ENCODES THE REPRESSOR that BLOCKS TAG GENE EXPRESSION

Note that the REGULATORY SEQUENCES for the REPRESSOR gene and the TAG gene are DIFFERENT. The REPRESSOR protein binds to REG of the TAG gene only in the presence of tetracycline.

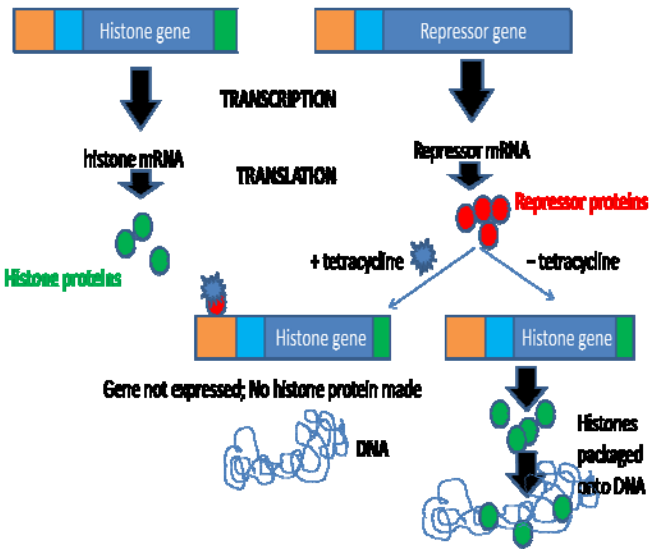
3. INTRODUCE GENES INTO MALE PRONUCLEI TO MAKE TRANSGENIC MICE

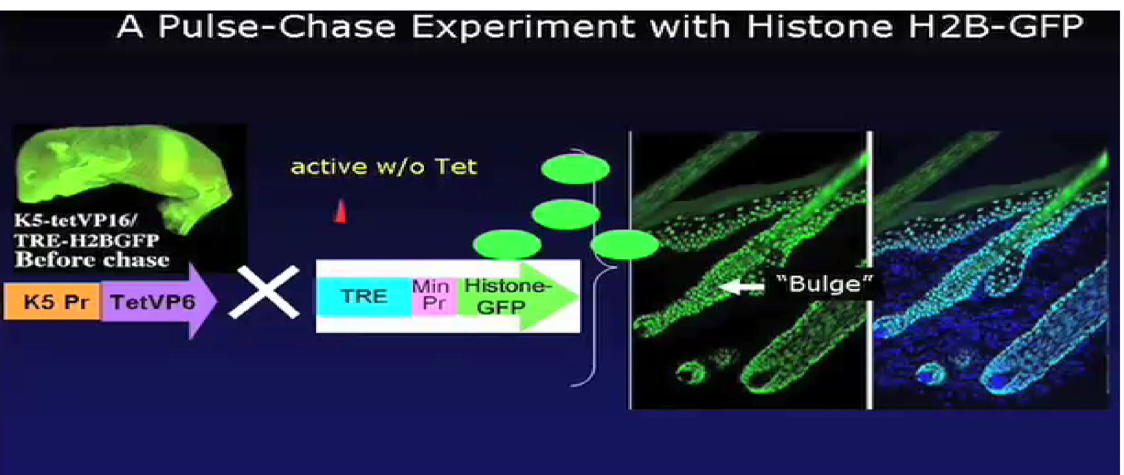

Histone gene
Repressor gene
TRANSCRIPTION
histone mRNA
Repressor mRNA
TRANSLATION
Repressor proteins
Histone proteins
+ tetracycline
– tetracycline
Histone gene
Histone gene
Gene not expressed; No histone protein made
DNA
Histones packaged onto DNA

PERFORM A <u>PULSE-CHASE</u> EXPERIMENT in TRANSGENIC MICE

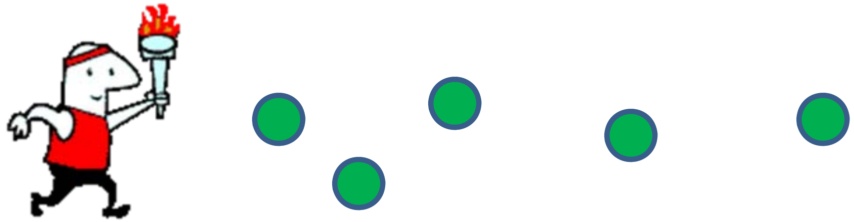
The **PULSE** :

Histone gene expression occurs in the <u>absence of tetracycline</u>, allowing new DNA from dividing keratinocytes to be packaged with the "GREEN" histone. Nuclei in dividing cells fluoresce, showing cells that are actively dividing.

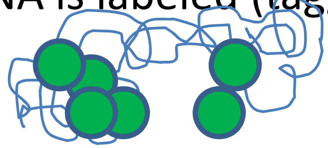




THE CHASE!!!!



Newly synthesized DNA is labeled (tagged) with the GREEN histone protein.



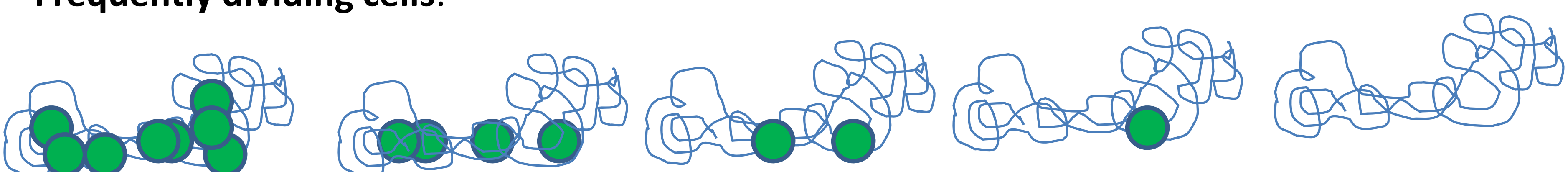
Now TURN OFF EXPRESSION of the HISTONE GENE by adding TETRACYCLINE to the diet of the transgenic mice

No new histone proteins are made

WHERE DO THE PREVIOUSLY MADE GREEN HISTONES GO? (CHASE the TAG)!

Now with each round of DNA replication for cell division, no additional GREEN histones are bound to DNA. Thus, over time, the DNA in dividing cells becomes LESS GREEN (dilution effect) EXCEPT in stem cells where cell division is infrequent!

Frequently dividing cells:

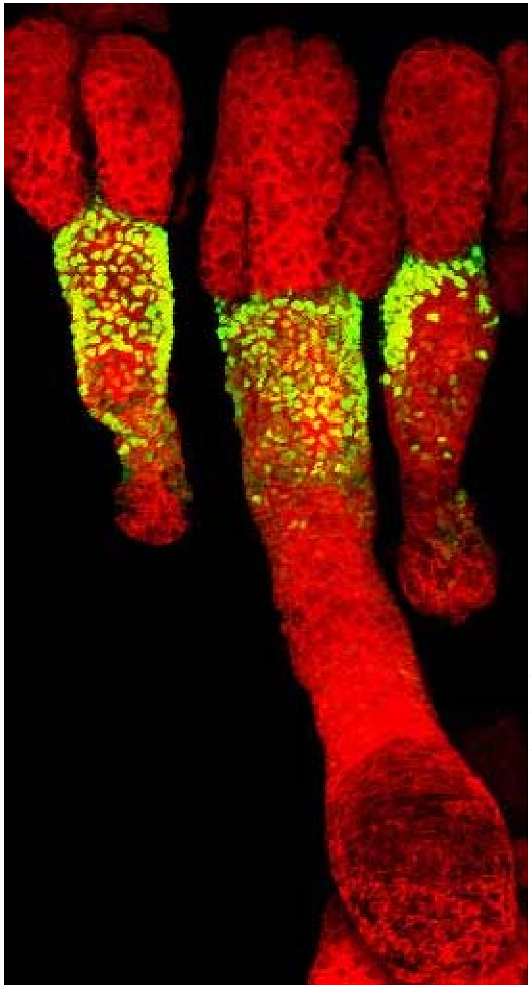


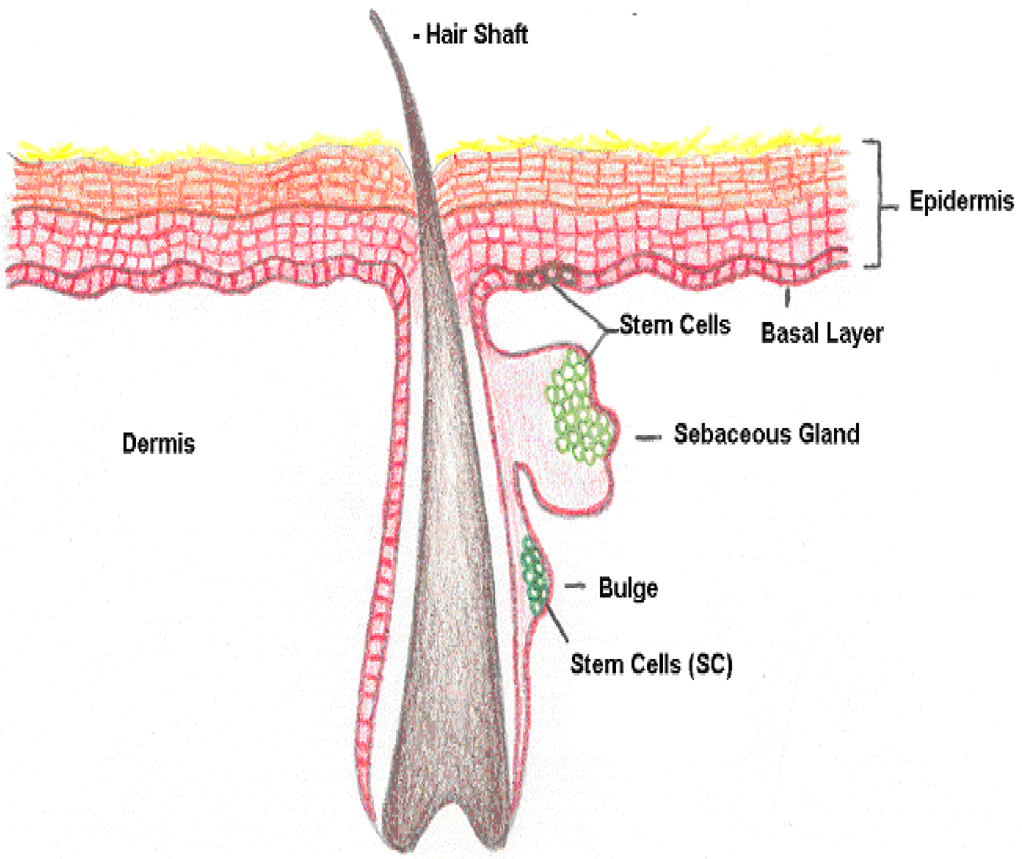
Stem cells:

DAY 1

DAY 28

Green nuclei are obvious in cells in the BULGE area of the hair follicle. In Fuchs' talk, these cells are referred to as "label retaining cells (LRCs)"





Tumbar et al., Science, 2004

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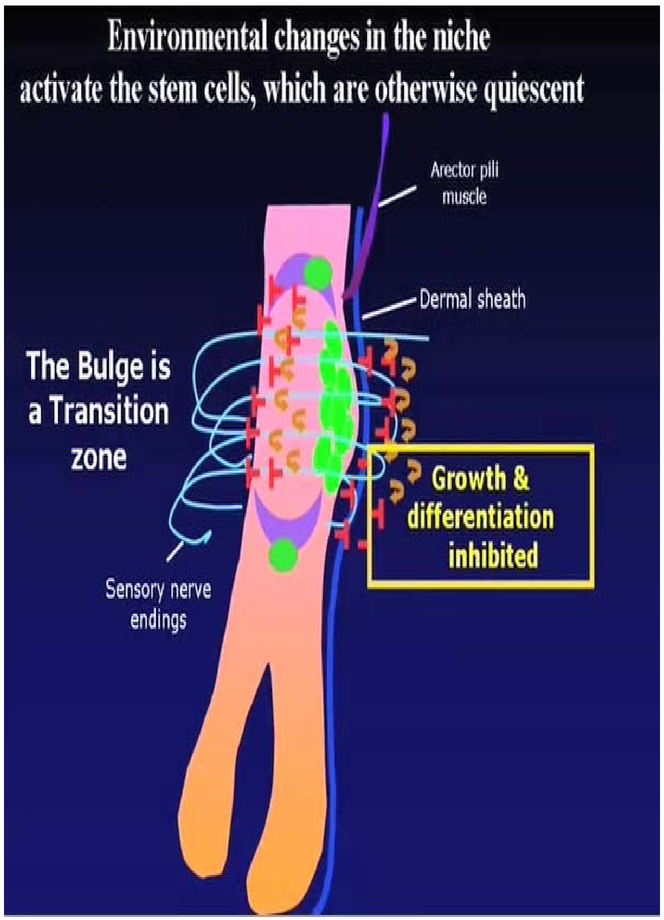
Do the "tagged" bulge cells behave as one would expect for stem cells? YES!

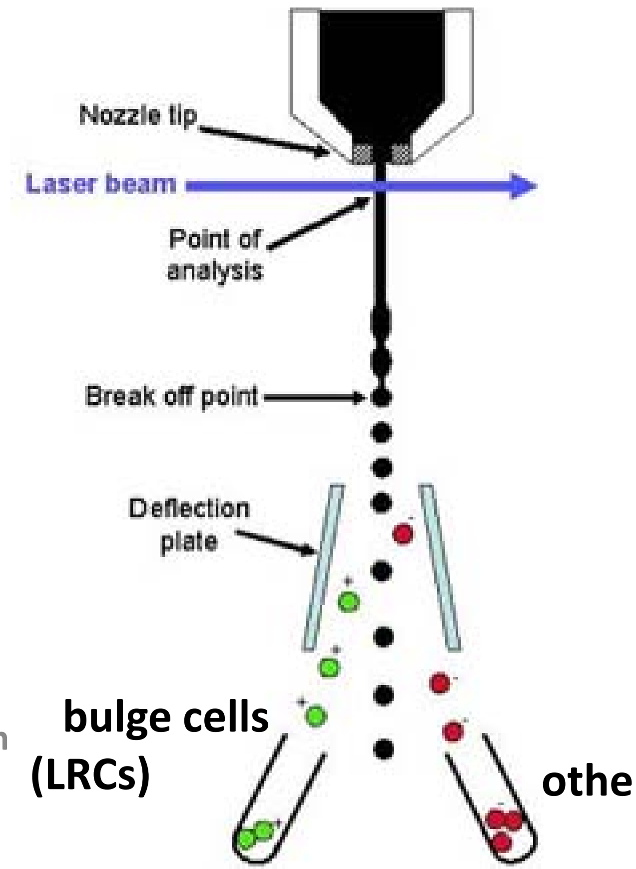


1) Upon wounding of the skin of the transgenic mouse, the bulge cells move <u>upward</u> and divide to repair the skin.

2) With each new hair cycle, a few bulge cells leave the bulge area (niche), <u>exit at the base</u> of the bulge, start dividing rapidly, and differentiate to become the new hair germ. Therefore, these cells have the ability to regenerate the hair follicle.

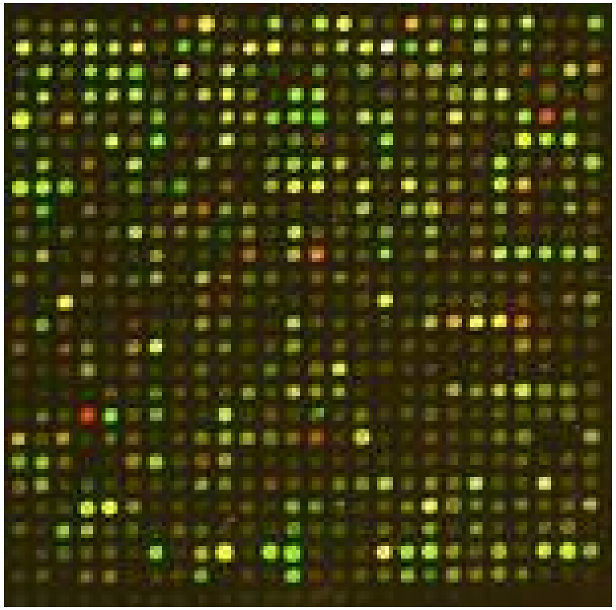
How can bulge cells be separated from other cells so that gene expression patterns can be studied?

1) Use a **fluorescence activated cell sorter (FACS)** to separate GREEN (GFP)- tagged bulge cells from cells that lack a GREEN (GFP) tag (rapidly dividing cells).



www.dundee.ac.uk/.../FACS/cell_sorting.htm

2) Isolate mRNA from the two populations of cells. Then use **DNA microarray analysis** to determine which genes are up-regulated (expressed at a higher level) or down-regulated (expressed at a lower level) in each population of cells.



Which genes are up-regulated in adult skin stem cells compared to their progeny?

Over **150** genes are up-regulated in adult skin stem cells

What are some of the genes that maintain the quiescent state of stem cells in the bulge niche until activation is needed for epidermal wound repair or to make hair follicles?

1) Genes that encode signaling pathway growth factors **TGFβ**, **BMP**
2) Genes that encode **inhibitors** of the **Wnt** pathway. One of the important proteins in the Wnt pathway is **β-catenin**

In their quiescent state, stem cells express TGFβ and BMP as well as inhibitors of the Wnt pathway.

In order to be activated, TGFβ and BMP signaling pathways must be inhibited AND Wnt signaling must be activated. This allows stem cells to proliferate and differentiate into a hair follicle.

What happens when Wnt signaling is manipulated in stem cells?

↑ Wnt signaling (β-catenin)→ excess hair

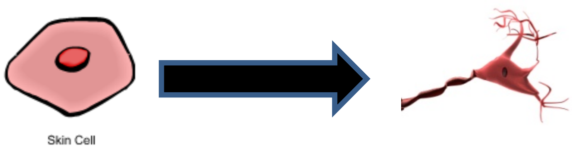
↓ Wnt signaling (β-catenin) → baldness

↑↑ Wnt signaling (β-catenin)→ hair tumors

Changes in the genes that are expressed can alter the fate of an adult skin stem cell:

Skin stem cells can differentiate into hair follicles if Wnt signaling is up-regulated.

Can skin stem cells be manipulated to become other types of cells, such as neurons?



UNLIKELY because the differences in epidermal cells and neurons include more factors than just differences in the types of genes that are expressed. These differences will make it unlikely that one could use adult skin stem cells to produce neurons. The prospect for this accomplishment with embryonic stem cells is more promising.

SUMMARY

1) Many different types of cells are located in the epidermis.
2) Adult skin stem cells are multipotent and remain in a quiescent state (rarely dividing) until activation occurs through wounding , general renewal, or during the hair follicle cycle.
3) Skin stem cells are located in the bulge niche of the hair follicle, as determined by pulse-chase experiments from the Fuchs' laboratory.
4) Skin stem cells can leave the niche, moving upward to repair a wound in the epidermis, for example, or moving downward to make a hair follicle depending on environmental signals in the niche.
5) Activation of different genes causes skin stem cells to respond differently to the environment. The quiescent state is maintained in adult stem cells through activation of over 150 genes, including activation of TGFβ and BMP signaling pathways. Activation of the Wnt signaling pathway (e.g., through β-catenin) causes skin stem cells to start dividing (leaving the quiescent state) and to differentiate into hair follicles.
6) It is unlikely that adult stem cells could differentiate into neurons, for example.

Ode to a Skin Stem Cell
by VCW

There once was stem cell stuck in skin BULGE ‘hood’
Living quietly, undisturbed, but wishing he could
Become something else, a nail, a hair, or some other type
Yet for weeks and weeks, he sulked and uttered this gripe
“Why am I not needed?” to his friends he would say
Isn’t there a wound somewhere, a hair to replace today?
Well, if you really want to move up and out, or down as well,
You must change your tune, for we are here to tell
If you would just stop listening to your friends TGFB and BMP
You surely could divide and leave the hood, for the world to see
That you Wnt on to become a hair, in all of your glee!