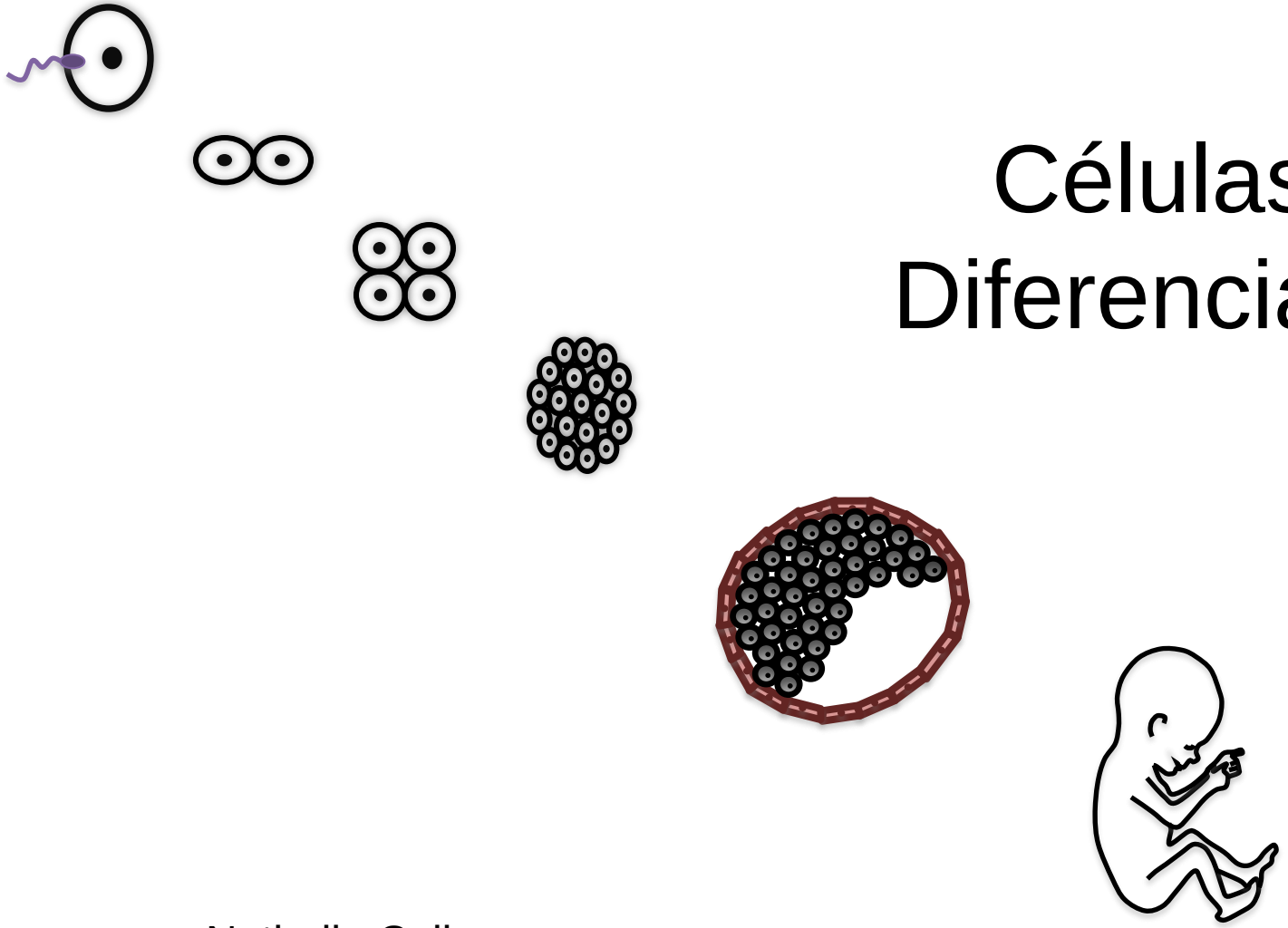
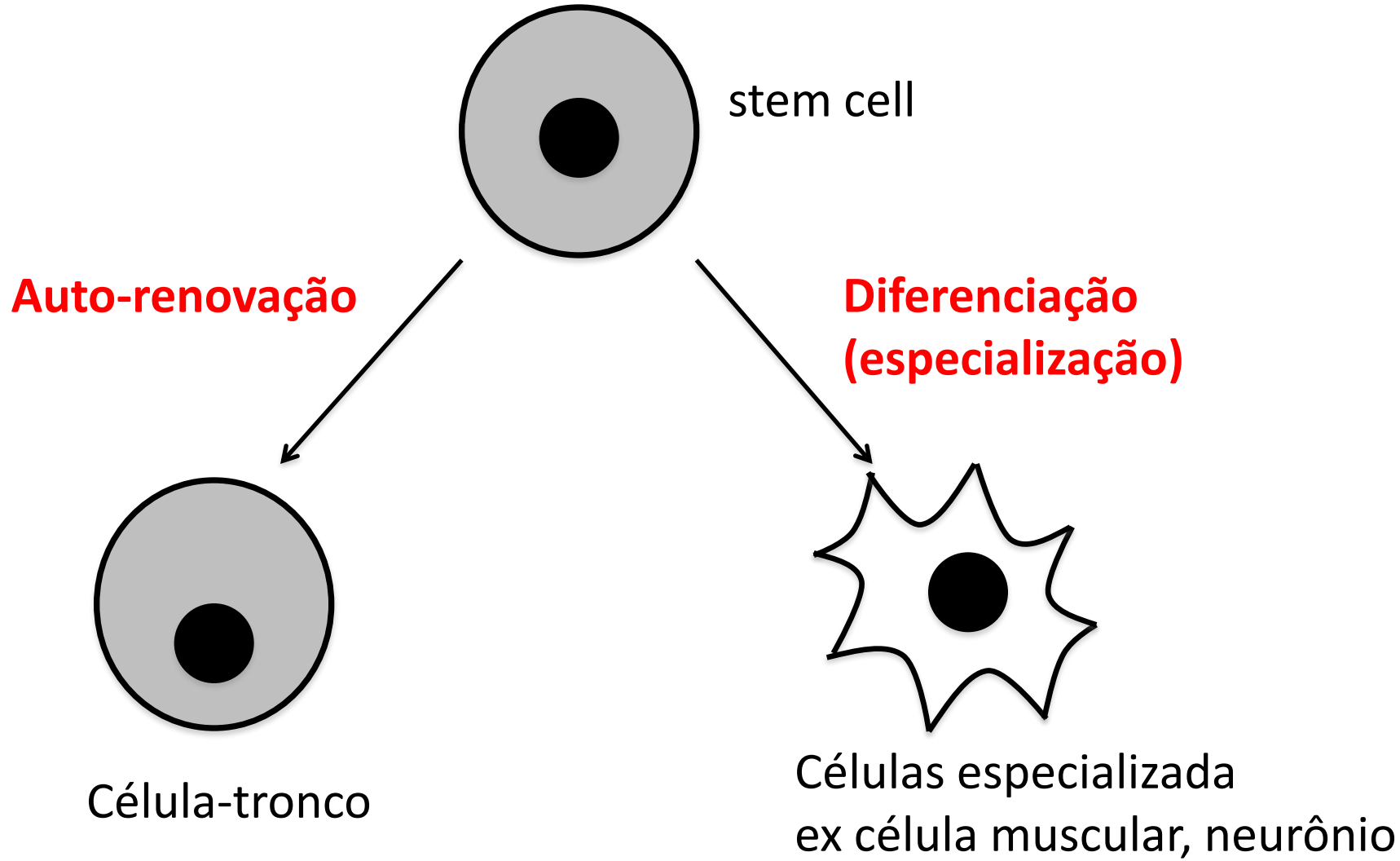


Células-tronco e Diferenciação celular

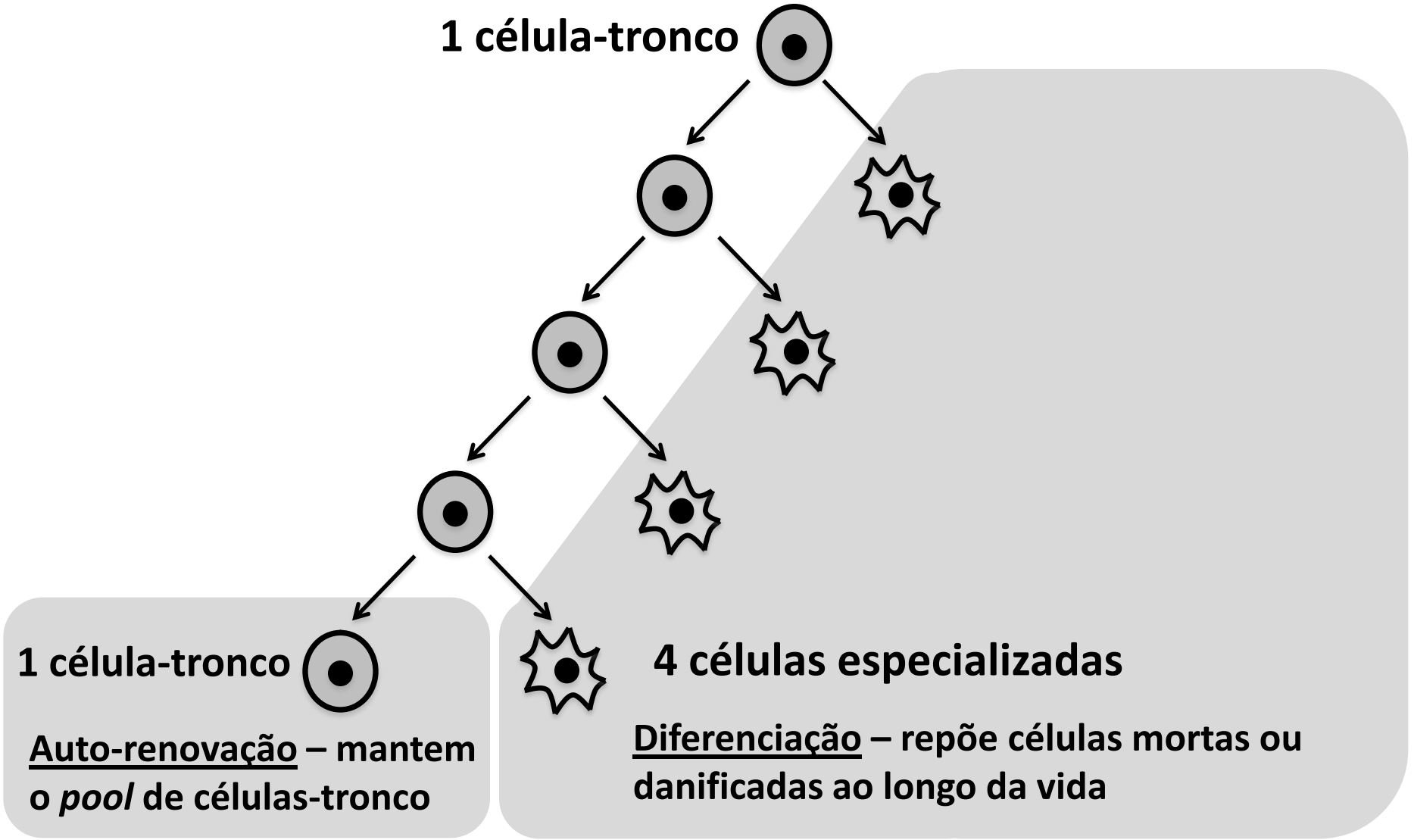
Nathalie Cella
23/05/2019
Departamento de Biologia Celular
e do Desenvolvimento
Instituto de Ciências Biomédicas – USP
ncella@usp.br

O que é uma célula-tronco?



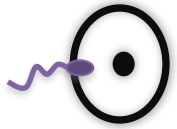
https://www.eurostemcell.org/

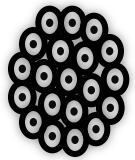
Por que auto-renovação E diferenciação?



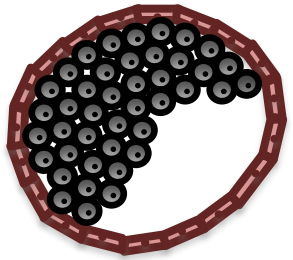
https://www.eurostemcell.org/

Onde as células-tronco são encontradas?





céls-tronco embrionárias
blastocisto



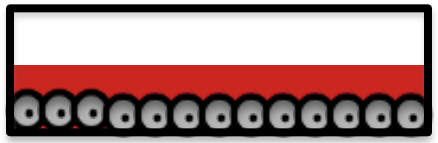
Céls-tronco teciduais
feto, bebê ou adulto

https://www.eurostemcell.org/

Tipos de células-tronco:
1) células-tronco embrionárias

Células-tronco embrionárias (ES cells):

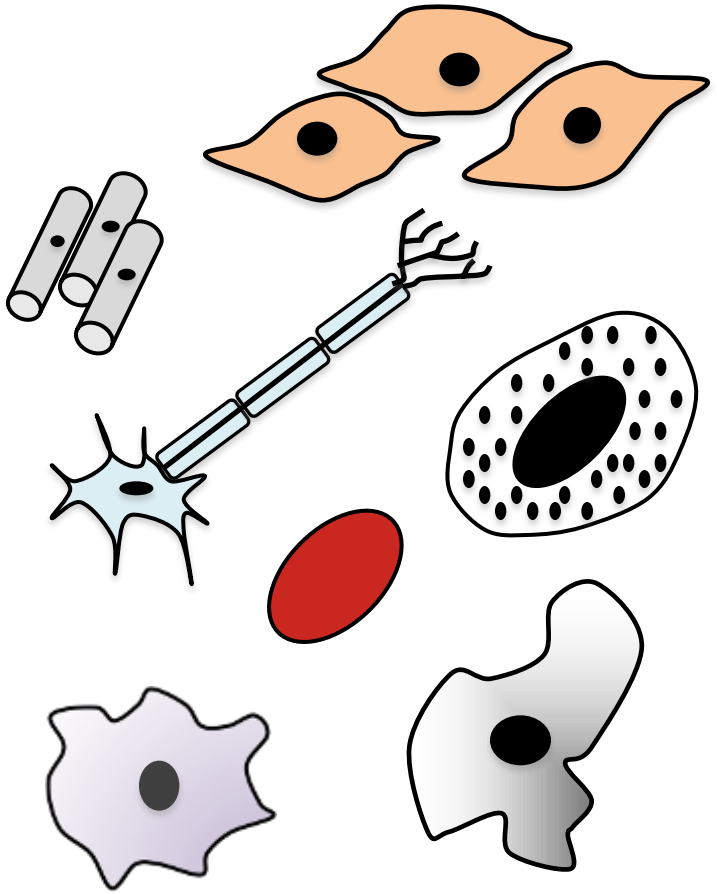
O que elas podem fazer



células-tronco
embrionárias

PLURIPOTENTE

diferenciação

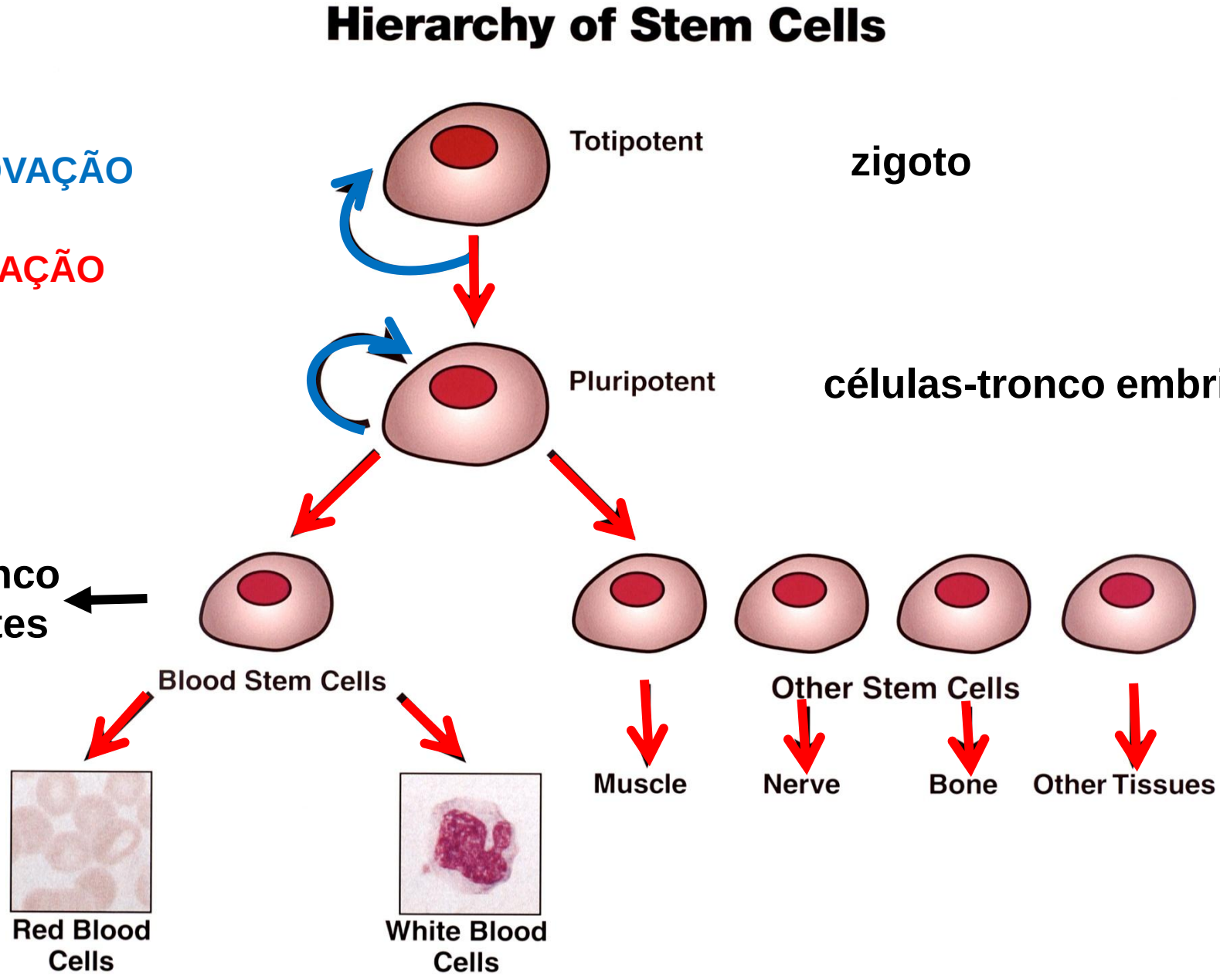


Todos os tipos de células
especializadas

https://www.eurostemcell.org/

AUTO-RENOVAÇÃO

DIFERENCIAÇÃO

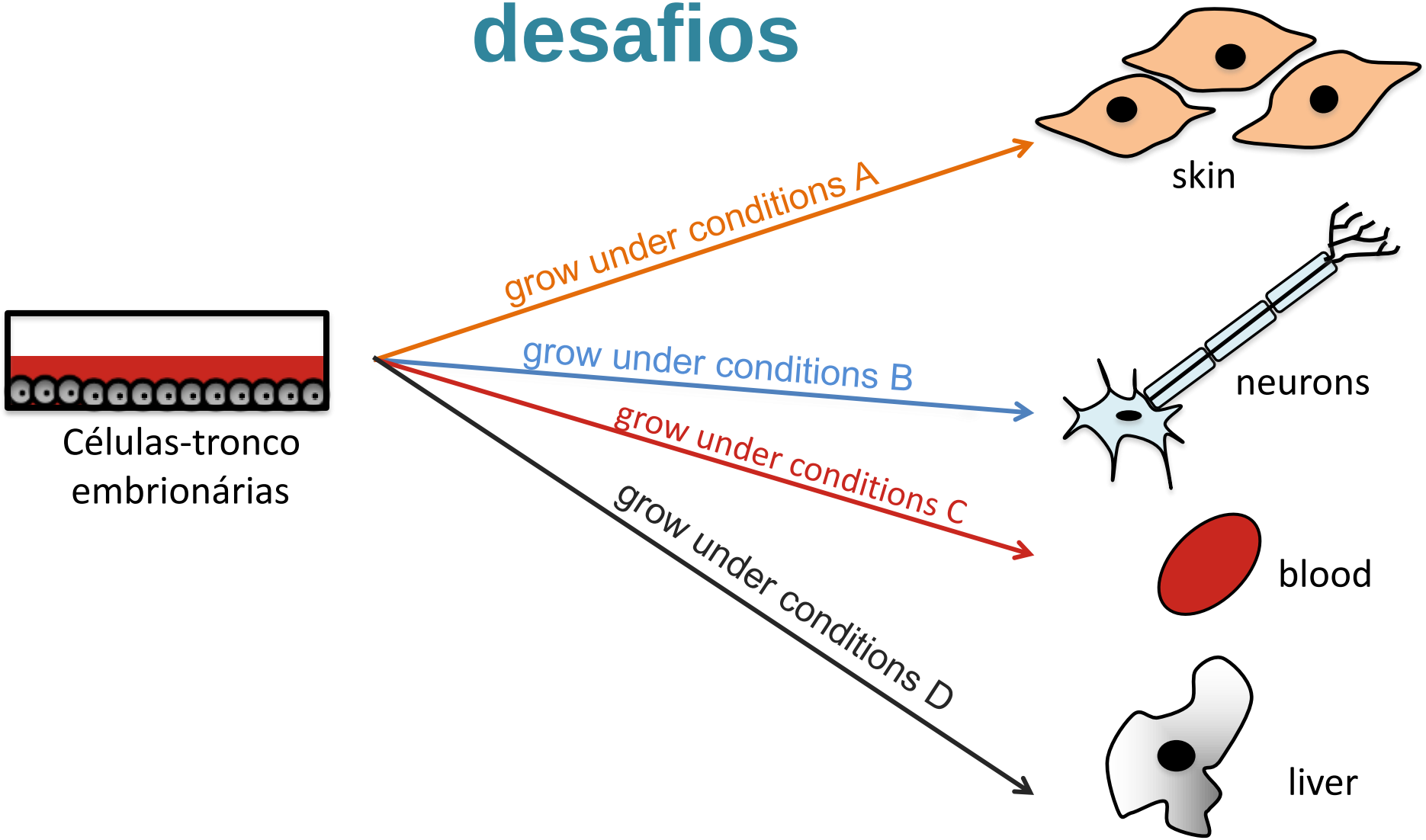


zigoto

células-tronco embrionárias

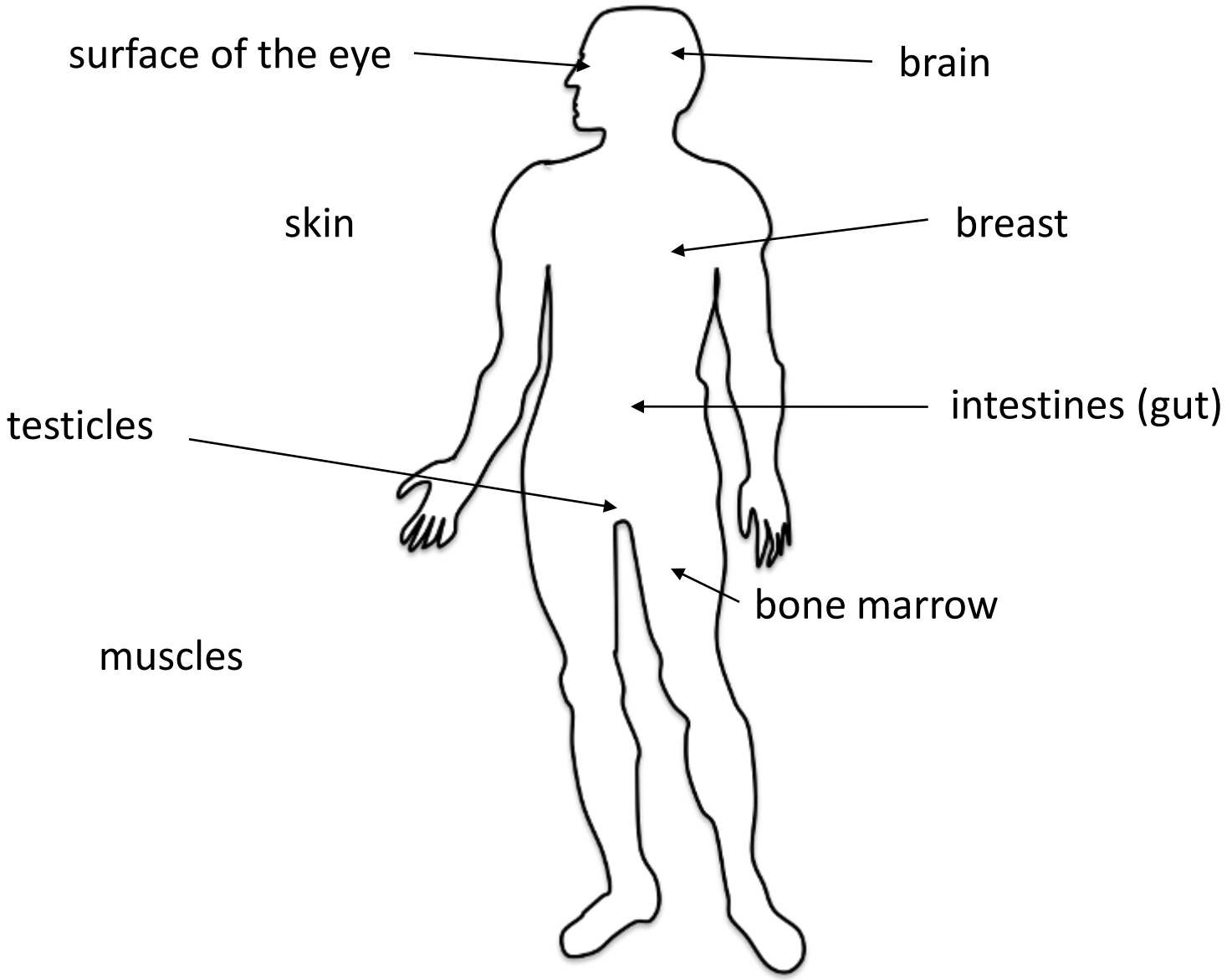
células-tronco multipotentes

Céls-tronco embrionárias:
desafios



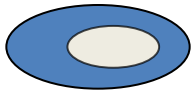
https://www.eurostemcell.org/

Tipos de células-tronco:
2) células-tronco teciduais

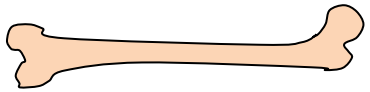

surface of the eye
brain
skin
breast
testicles
intestines (gut)
bone marrow
muscles

Células-tronco teciduais

O que elas podem fazer



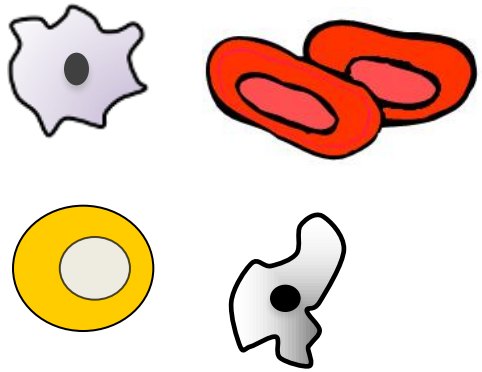
céls-tronco
hematopoiéticas



encontradas na
medula óssea

MULTIPOTENTES

diferenciação



Somente células sanguíneas
especializadas:
hemácias, leucócitos, plaquetas

https://www.eurostemcell.org/

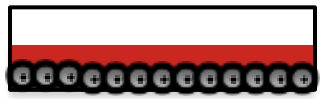
Tipos of células-tronco:
3)Células-tronco pluripotentes induzidas (iPS)

Células-tronco pluripotentes induzidas (iPS)

célula
de adulto

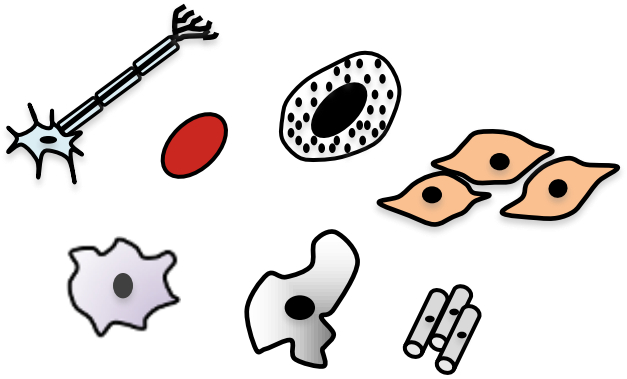
‘reprogramação genética’
= adicionar alguns genes à célula

Células iPS
Comporta-se como uma célula-tronco embrionária



células iPS em cultura

diferenciação

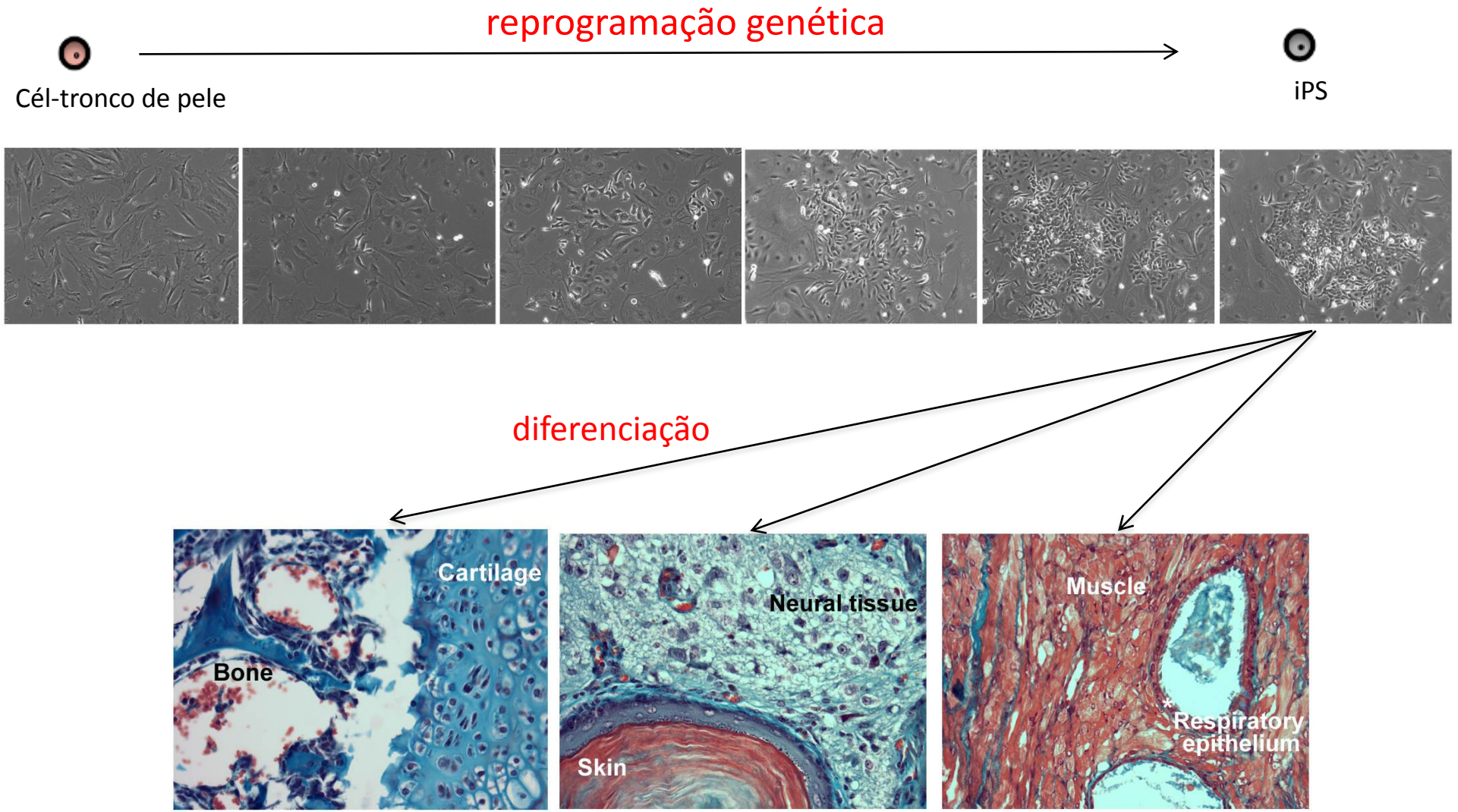


Todos os tipos
de células especializadas

vantagem: não precisa de embriões!

https://www.eurostemcell.org/

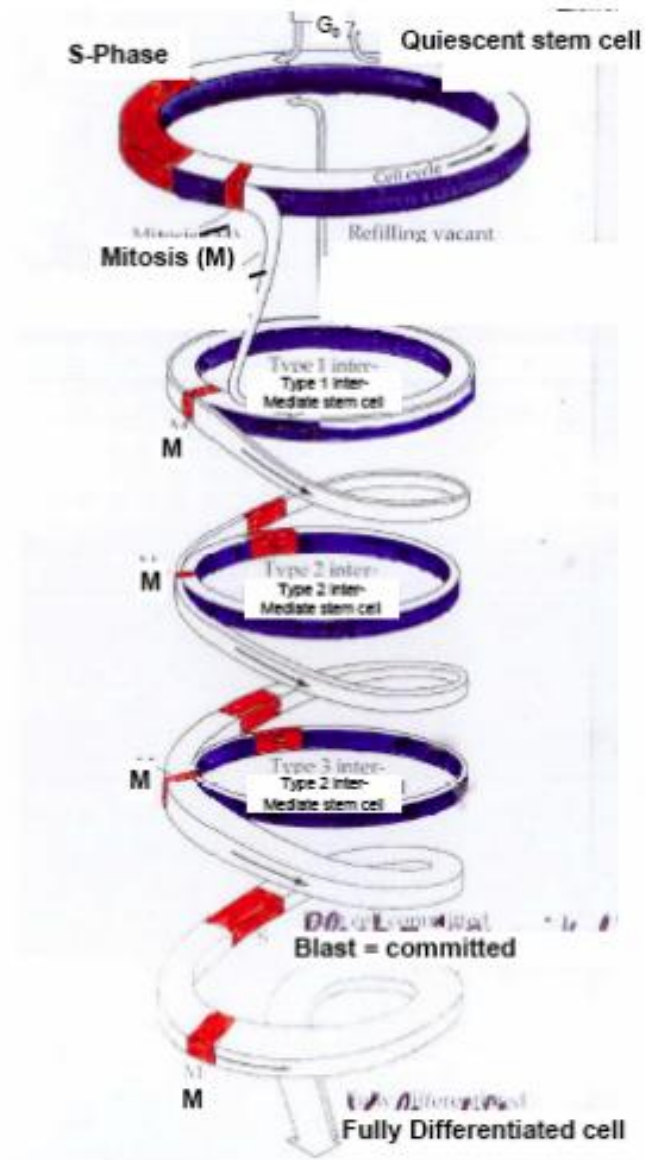
Células-tronco pluripotentes induzidas (iPS)



https://www.eurostemcell.org/

Self renewing cells

Differentiating cells



Cells mostly quiescent, only enter cell cycle to replace self

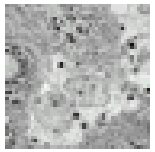
Cells enter the cell cycle, with an increasing propensity to become more committed to a particular lineage

Fully differentiated cells exit the cell cycle

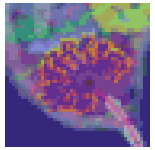
Células quiescentes. Só se dividem para se auto-renovar

Células entram no ciclo celular com crescente propensão a se diferenciar

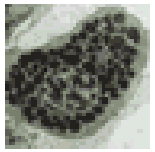
Ectoderm



Skin cells of epidermis

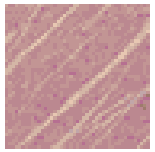


Neuron of brain

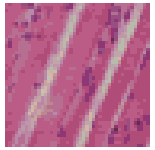


Pigment cell

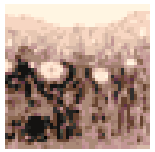
Mesoderm



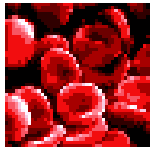
Cardiac muscle



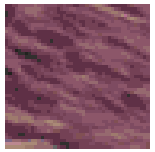
Skeletal muscle cells



Tubule cell of the kidney

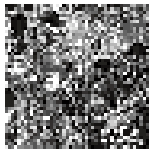


Red blood cells

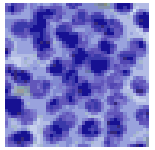


Smooth muscle (in gut)

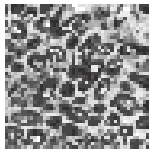
Endoderm



Pancreatic cell



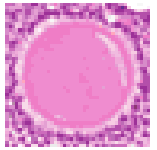
Thyroid cell



Lung cell (alveolar cell)

Germ Cells

Sperm



Egg

Diferenciação Celular

É O MECANISMO ATRAVÉS DO QUAL UMA CÉLULA ADQUIRE A CAPACIDADE DE DESEMPENHAR FUNÇÕES ESPECIALIZADAS

DIFERENCIAÇÃO

CÉLULAS TRONCOS

↓

CÉLULAS TRONCOS PROGENITORAS

↓

CÉLULAS DIFERENCIADAS

TRANSDIFERENCIAÇÃO

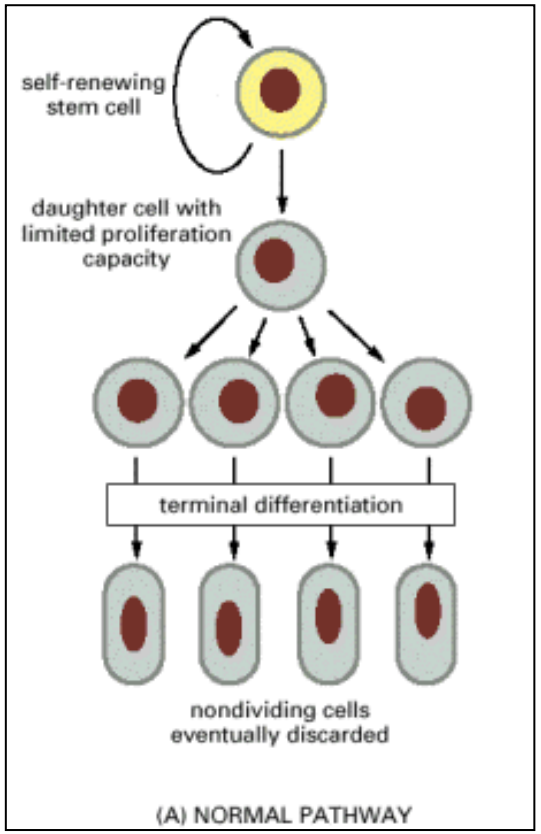
CÉLULAS DIFERENCIADA tipo A

↓

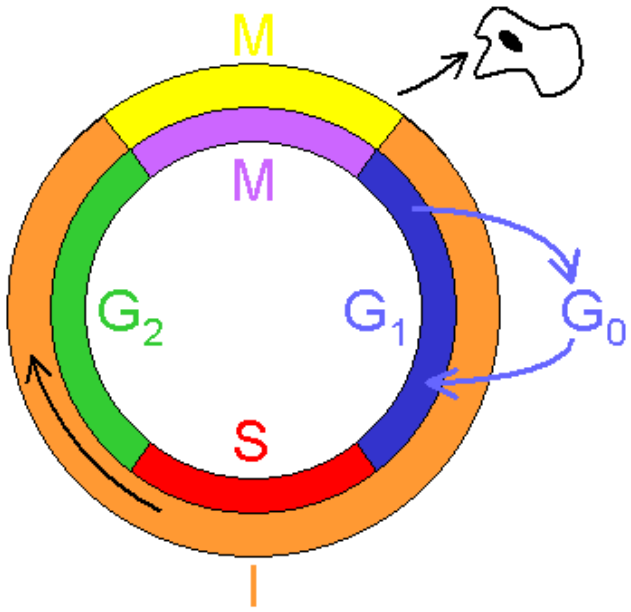
CÉLULAS DIFERENCIADA tipo B

Importância da diferenciação cellular

Possibilita o desenvolvimento de tecidos e órgãos altamente especializados



A diferenciação celular ocorre por que as células passam a expressar genes diferentes **(expressão gênica diferencial)**



SAÍDA DO CICLO CELULAR

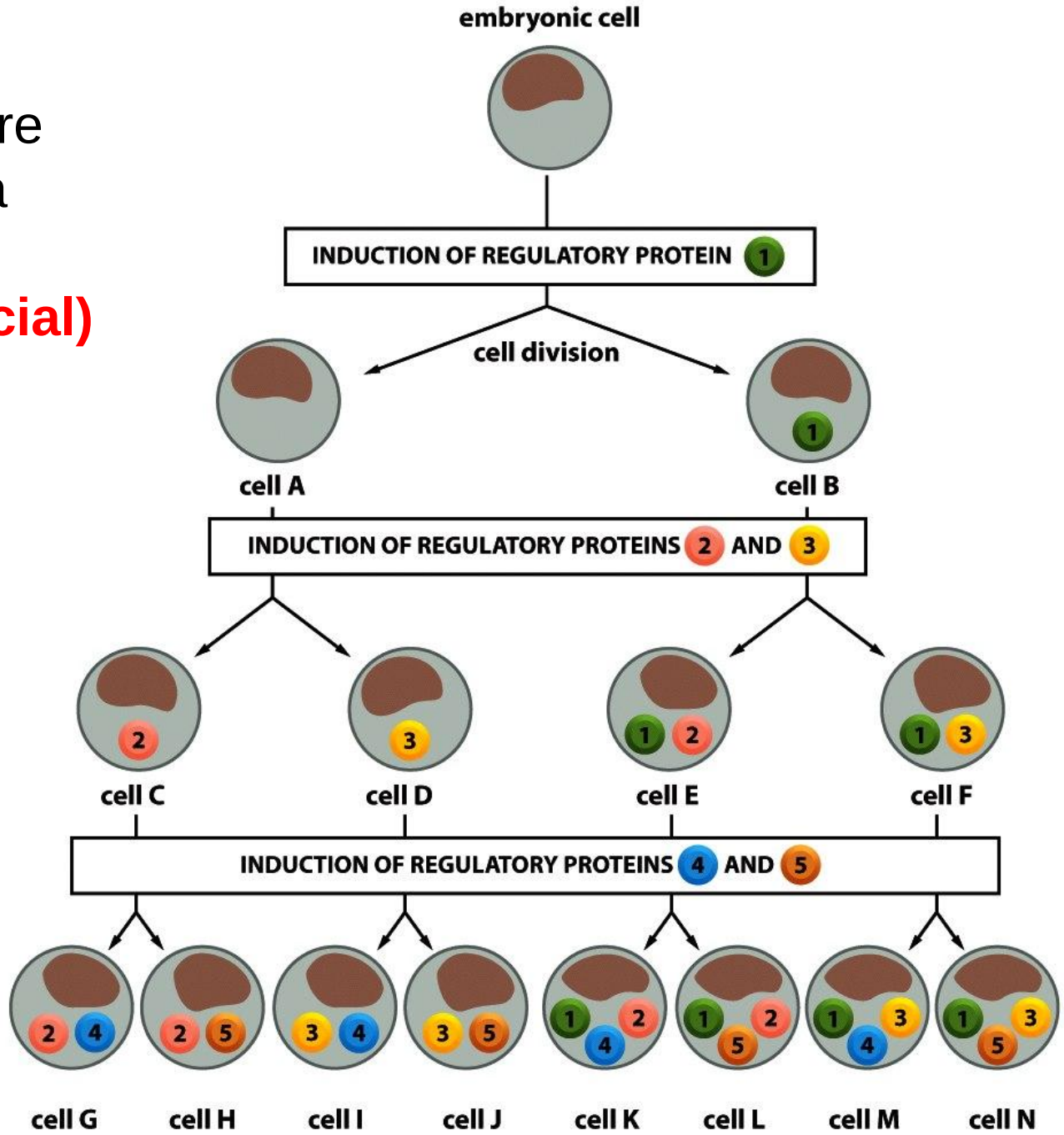
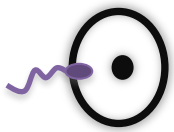
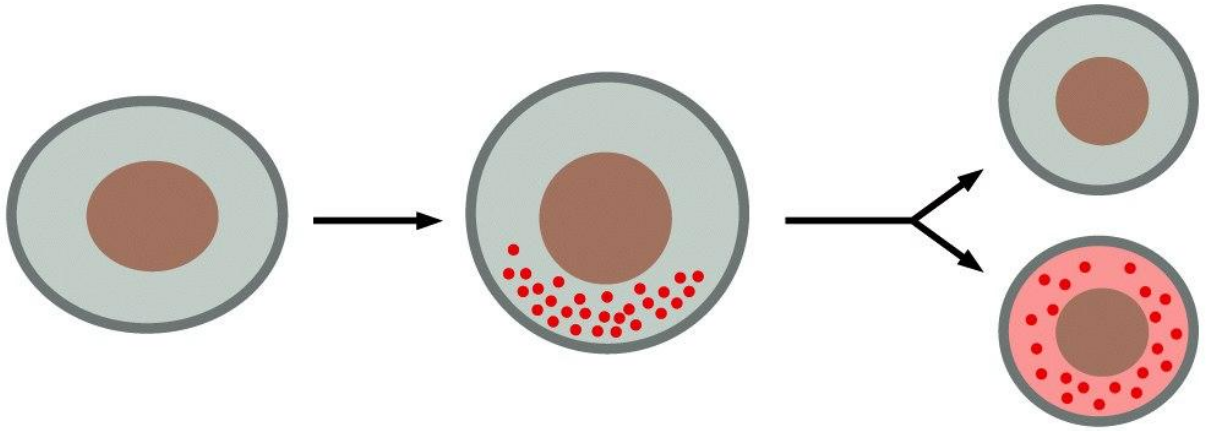


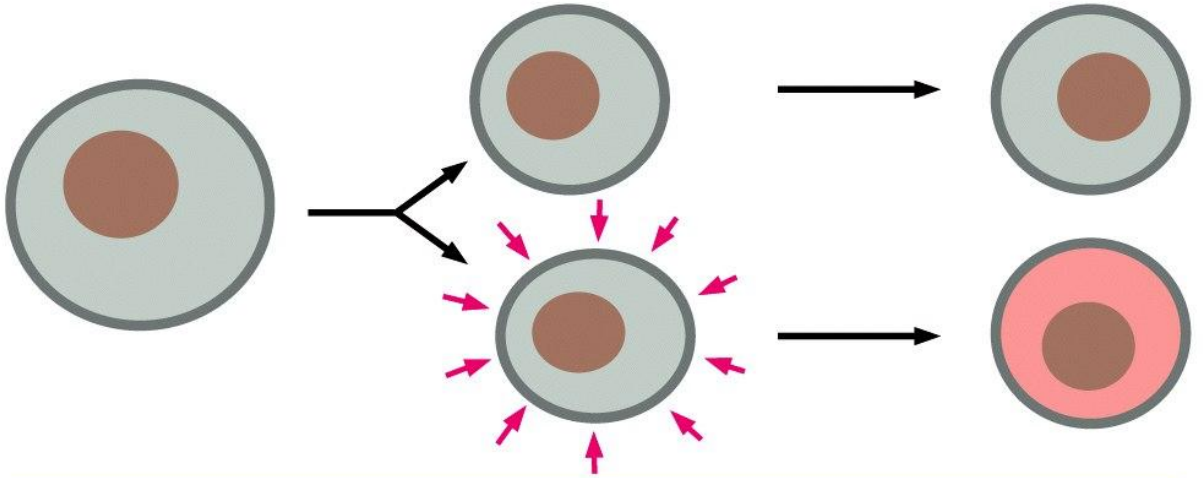
Figure 7-76 Molecular Biology of the Cell 5/e (© Garland Science 2008)



Como ??



1. asymmetric division : sister cells born different



2. symmetric division : sister cells become different as result of influences acting on them after their birth

Figure 22-11 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Inibição Lateral

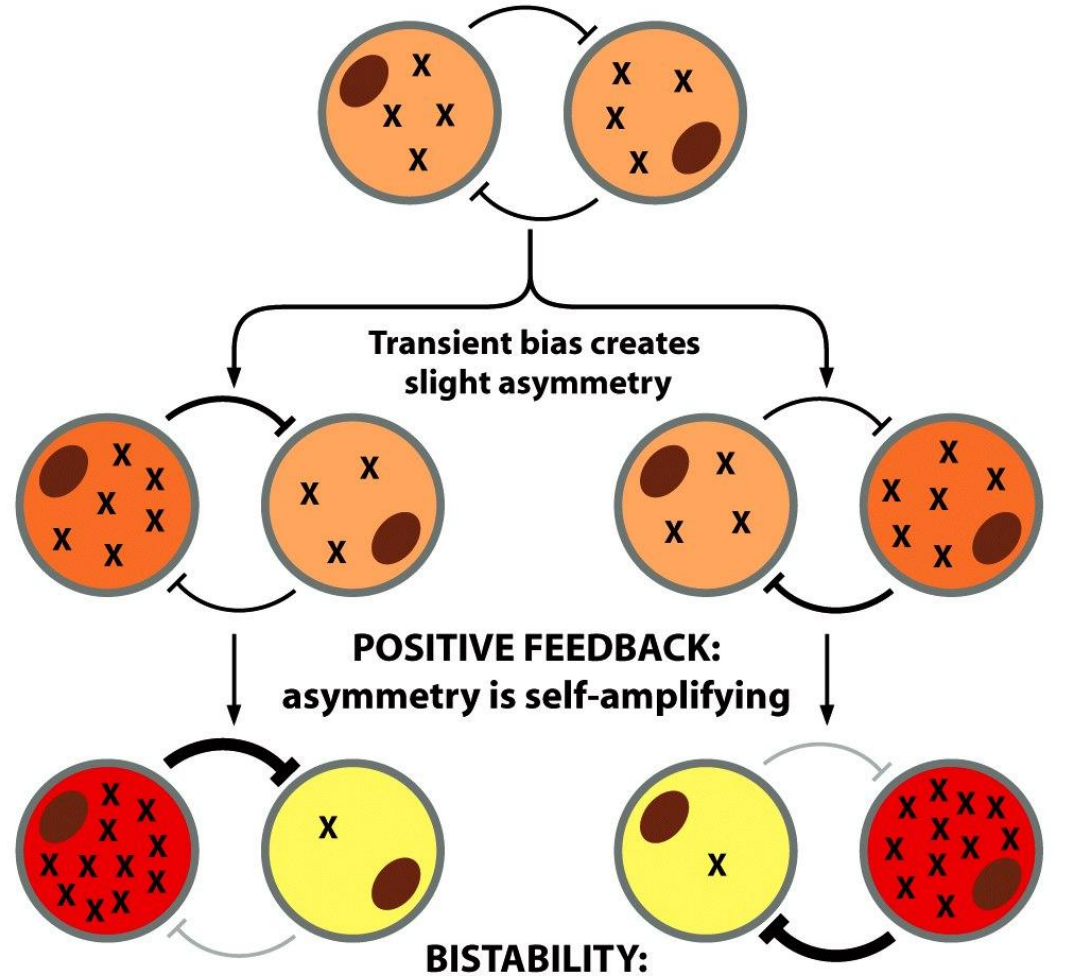


Figure 22-12 Molecular Biology of the Cell 5/e (© Garland Science 2008)

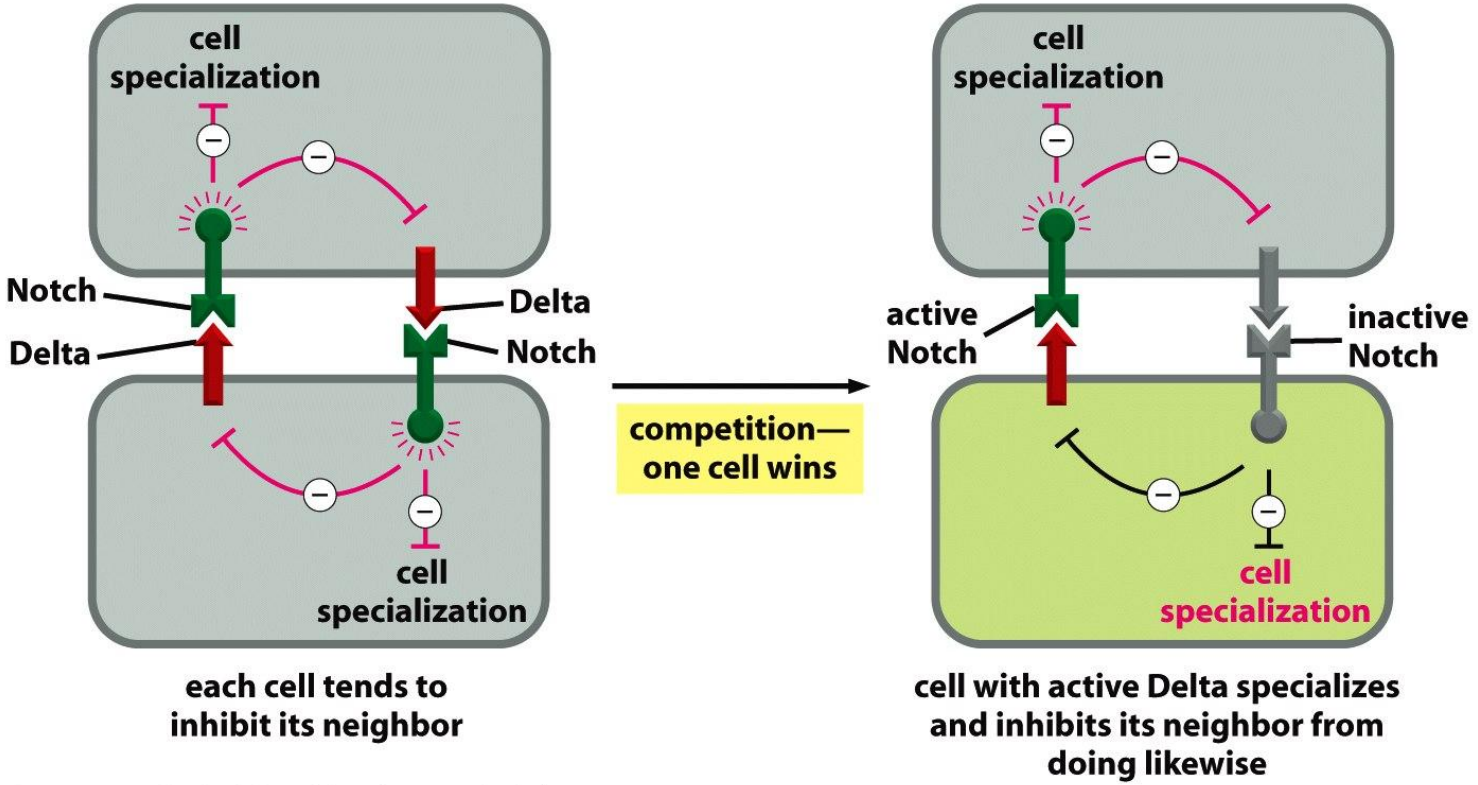


Figure 22-60a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Assim que uma célula ganha alguma vantagem sobre a outra, essa diferença se amplia

Quando uma célula perde a capacidade de se diferenciar, ela também perde a capacidade de inibir a diferenciação da célula adjacente

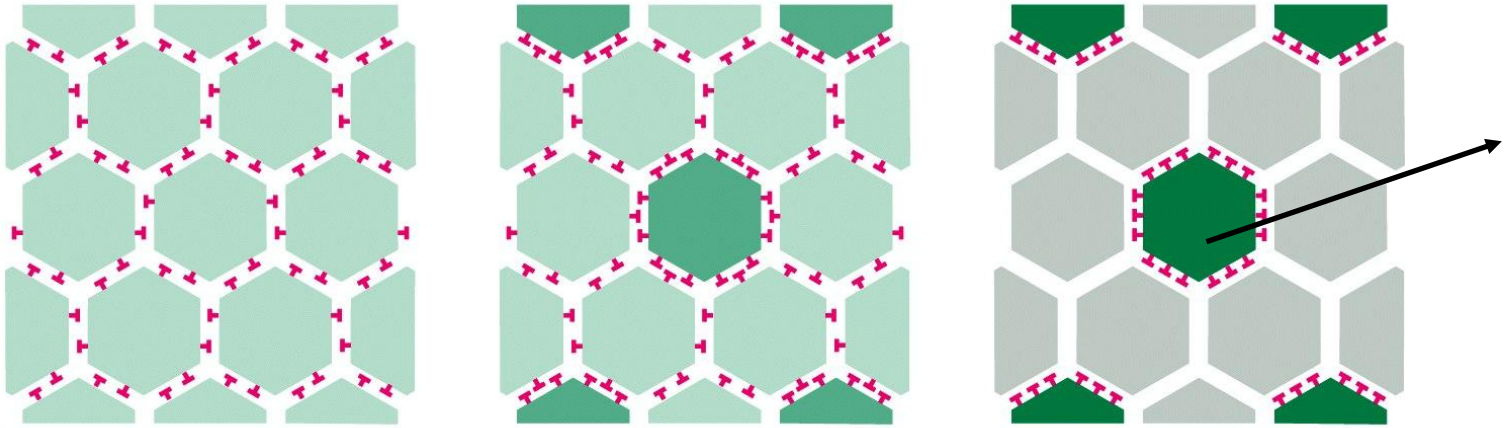


Figure 22-60b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Delta parcialmente inativado

Inibição lateral sofre um“desequilíbrio”

Todas as células da região se tornaram células sensoriais (presença de cílios)

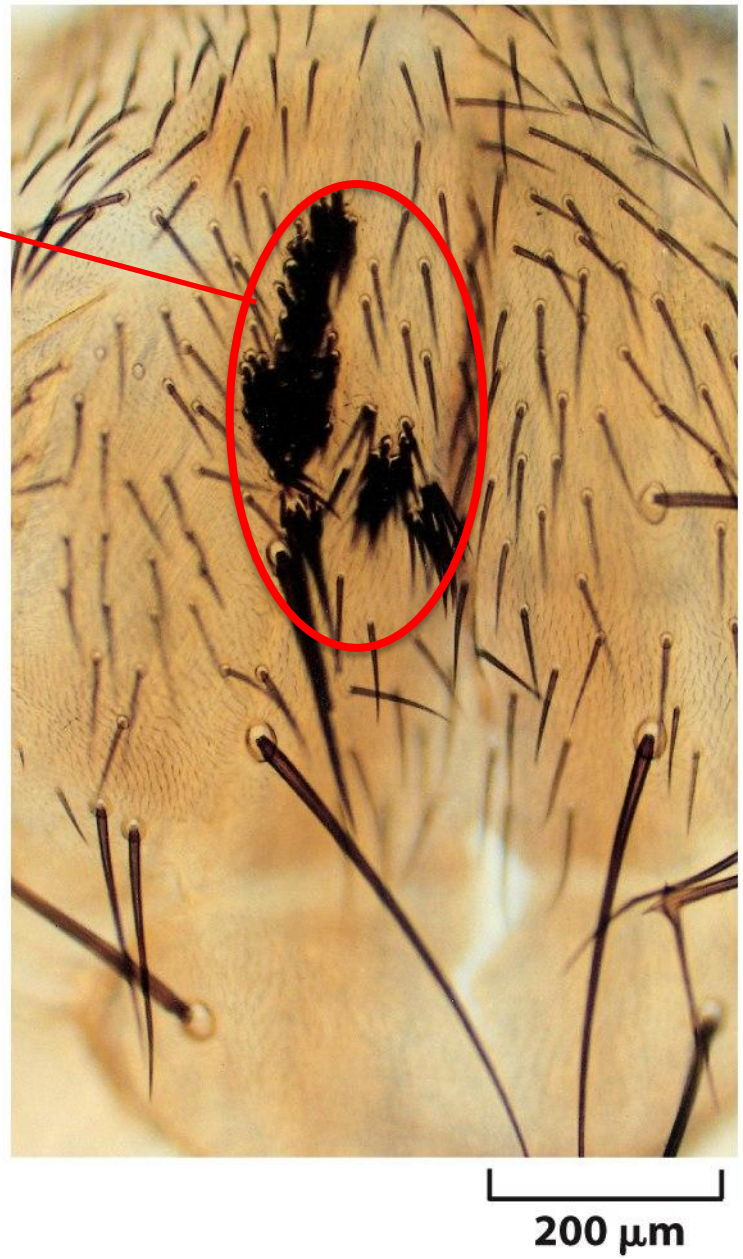
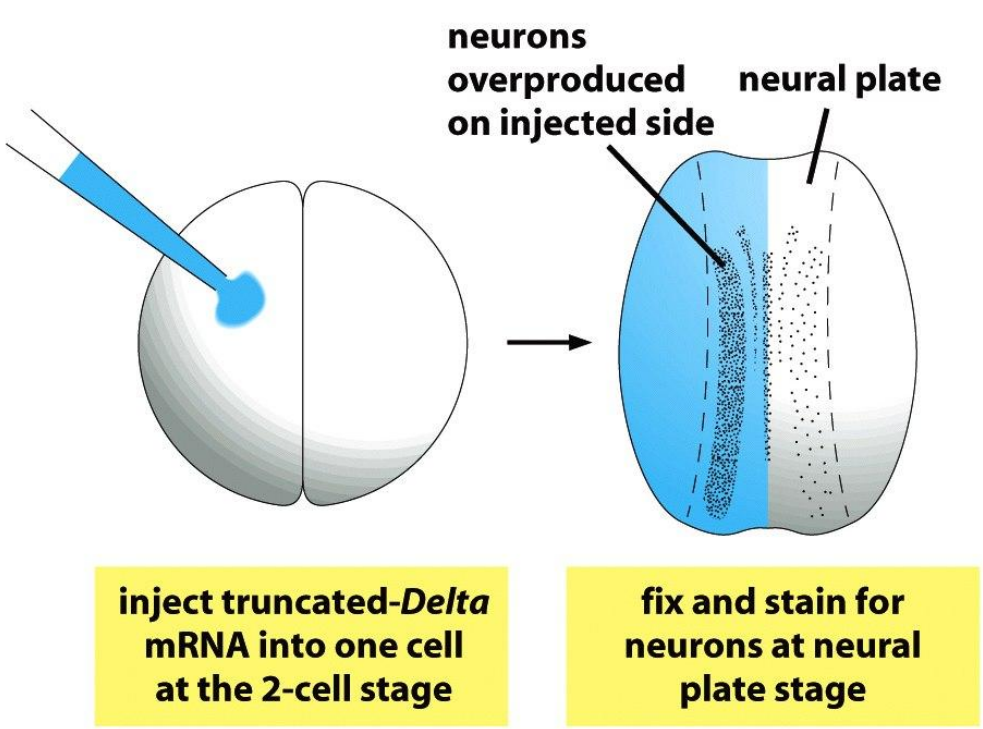
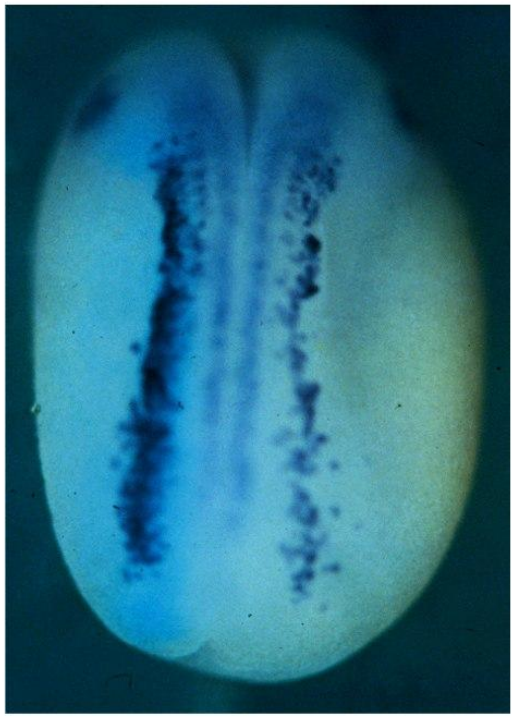
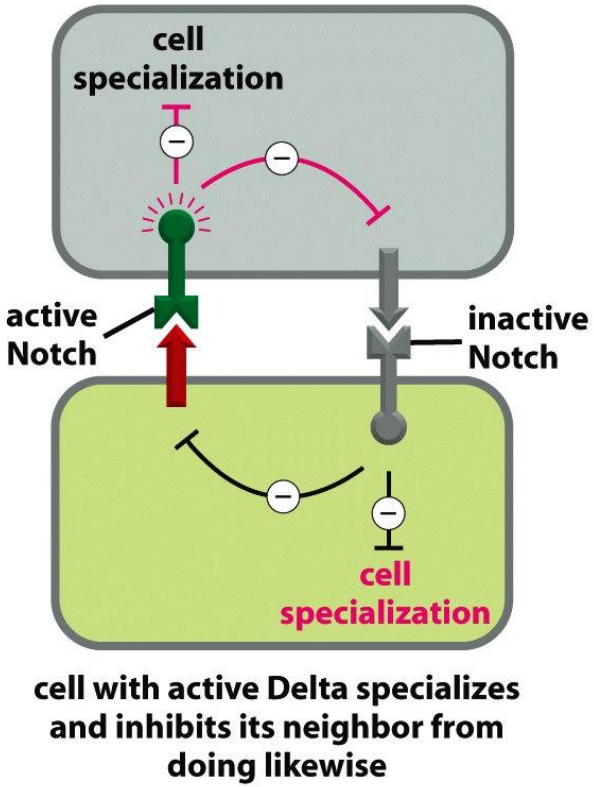


Figure 22-61 Molecular Biology of the Cell 5/e (© Garland Science 2008)

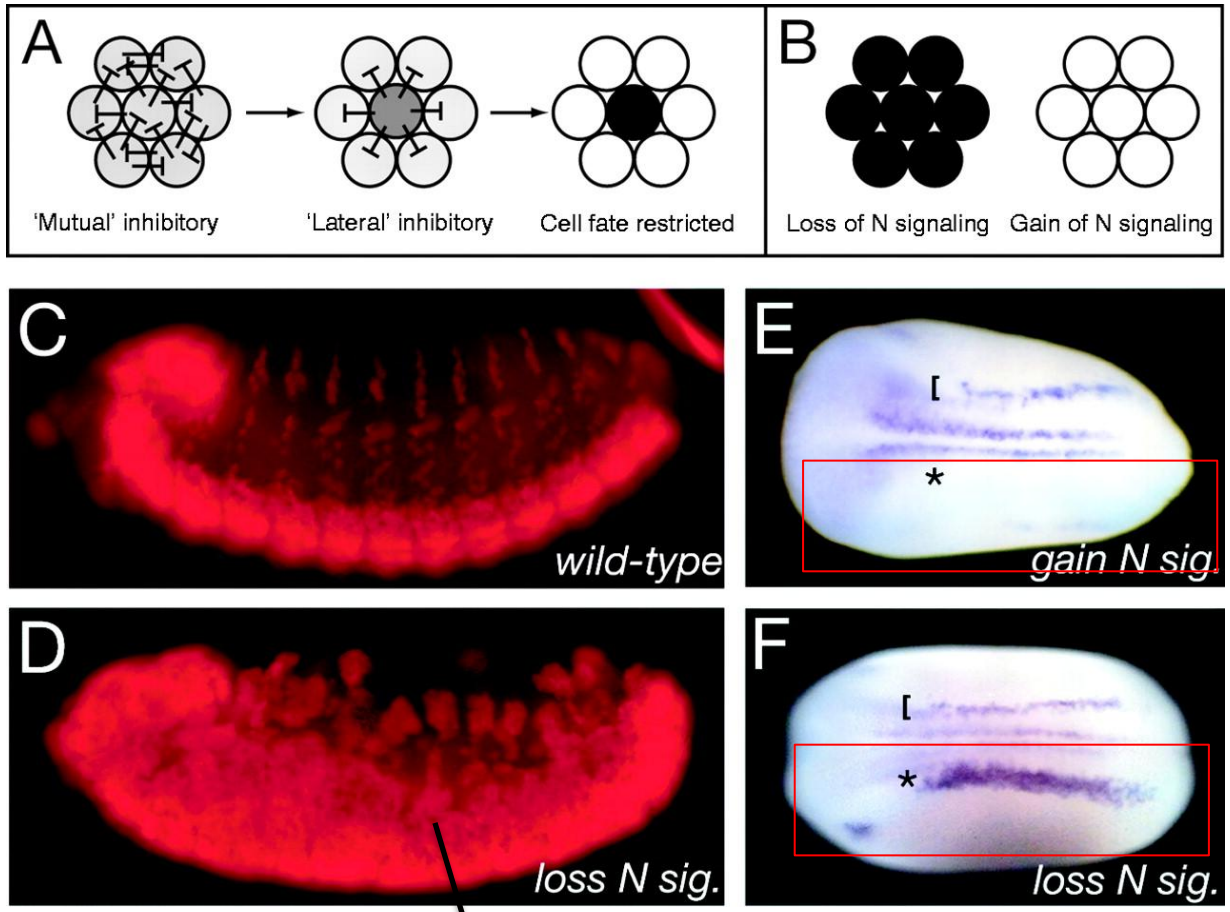






Delta truncado não será capaz de inibir a diferenciação da célula adjacente

O sinal inibitório de Notch (N) inibe a diferenciação de neurônios



Eric C. Lai Development 2004;131:965-973

↑Notch ⟶ ↓neurogênese

↓Notch ⟶ ↑neurogênese

ELAV – marcador de neurônios

↓Notch ⟶ ↑ neurogênese

Development

A célula sensorial-mãe passa a se dividir assimetricamente

Não herdaram Numb, portanto continuam susceptíveis à inibição de Notch, por isso se diferenciam em células não neurais

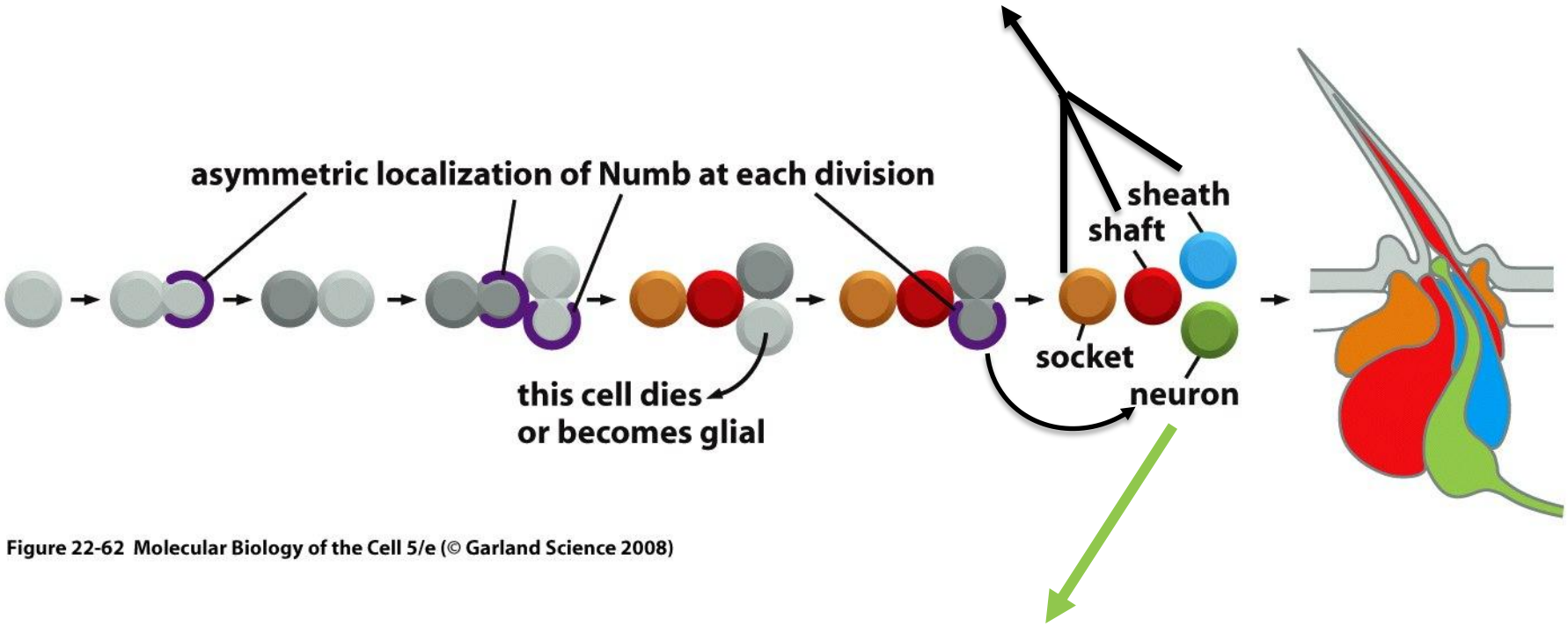
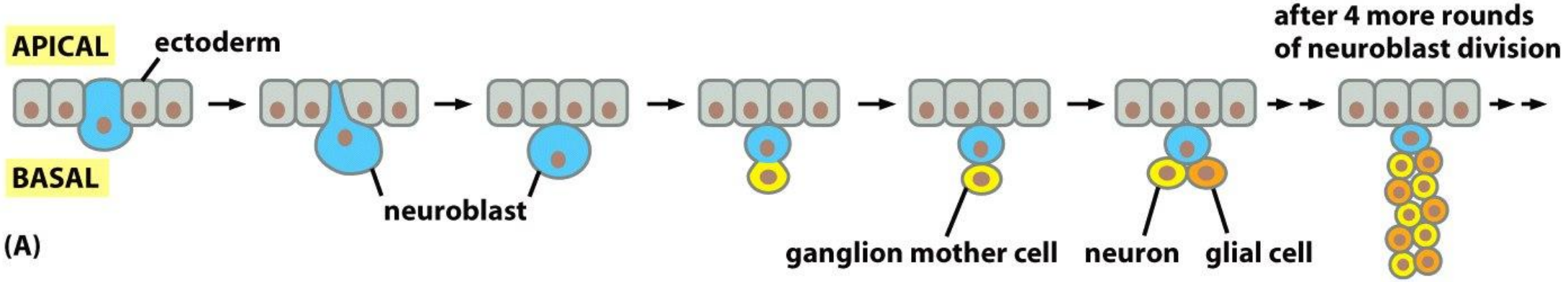
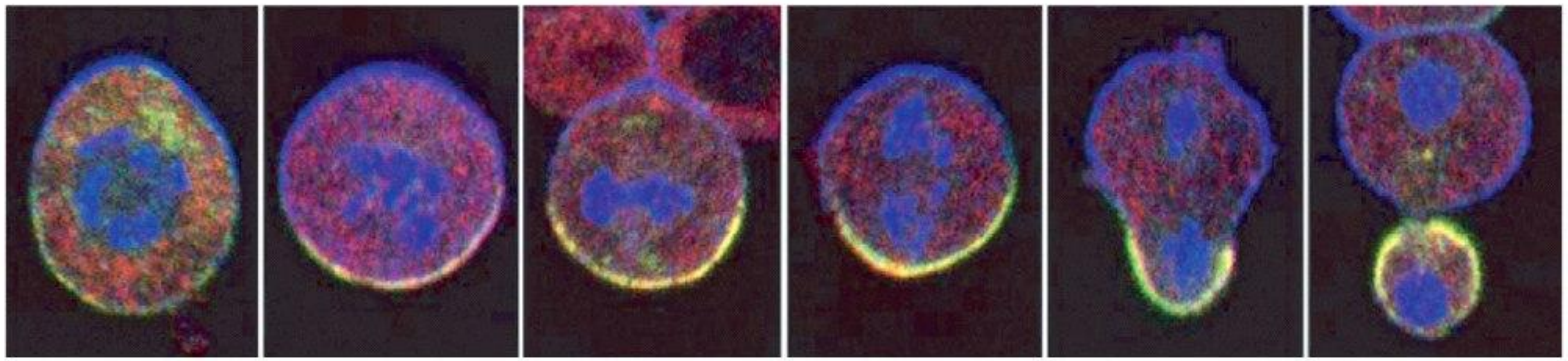


Figure 22-62 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Numb bloqueia o sinal de Notch, garantindo que a célula que estiver com Numb siga na linhagem neural

Divisão assimétrica continua a dar origem a outras células do sistema nervoso





(B)

Figure 22-66 Molecular Biology of the Cell 5/e (© Garland Science 2008)

núcleo
grânulos P

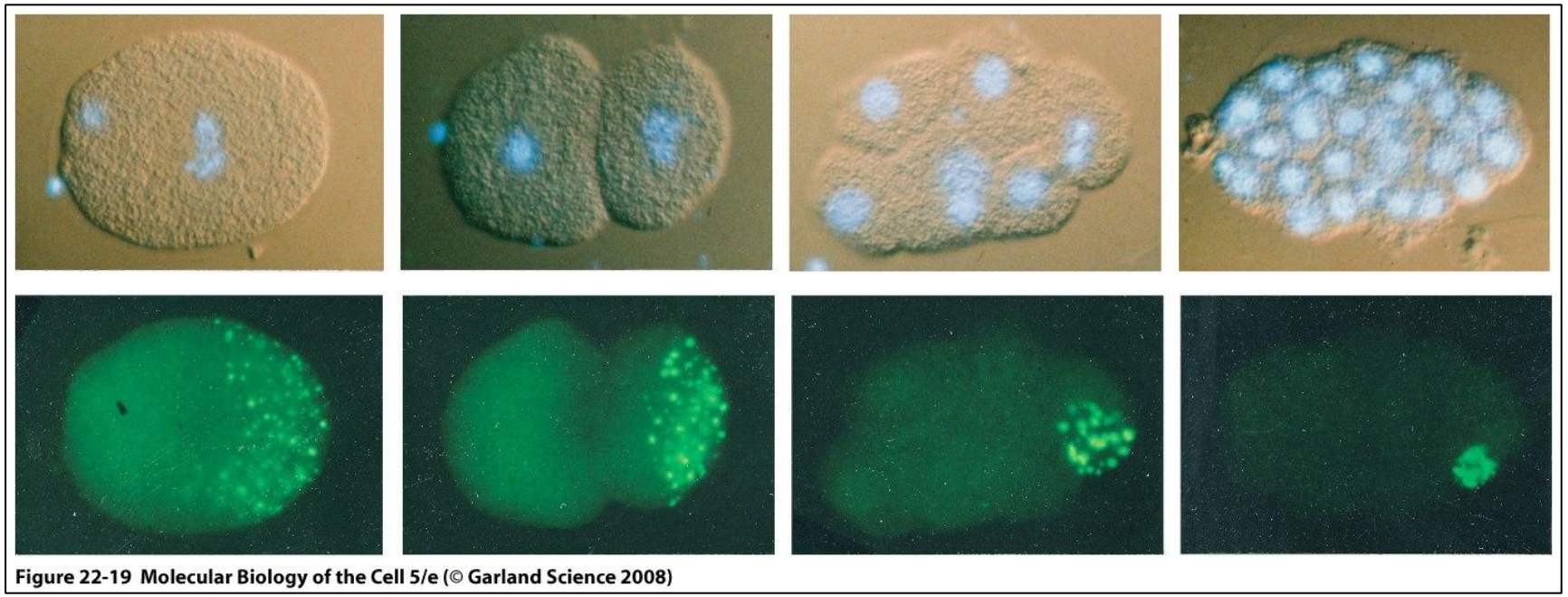
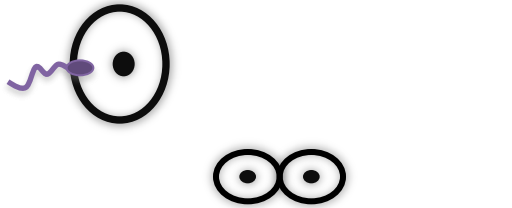
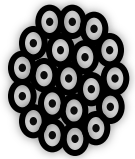


Figure 22-19 Molecular Biology of the Cell 5/e (© Garland Science 2008)

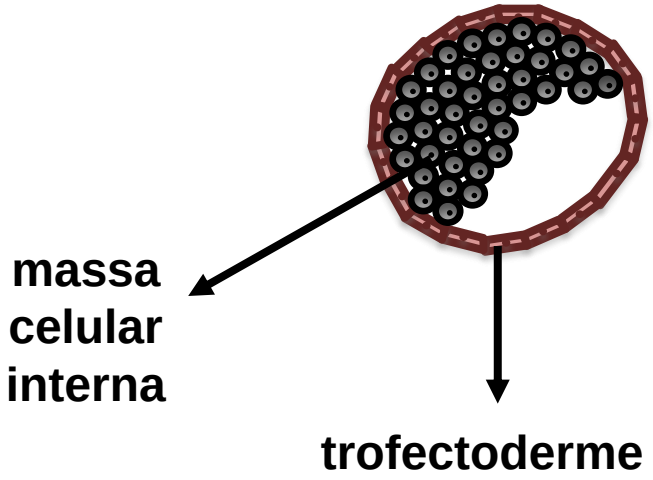
Caenorhabditis elegans

voltando à história da vida..





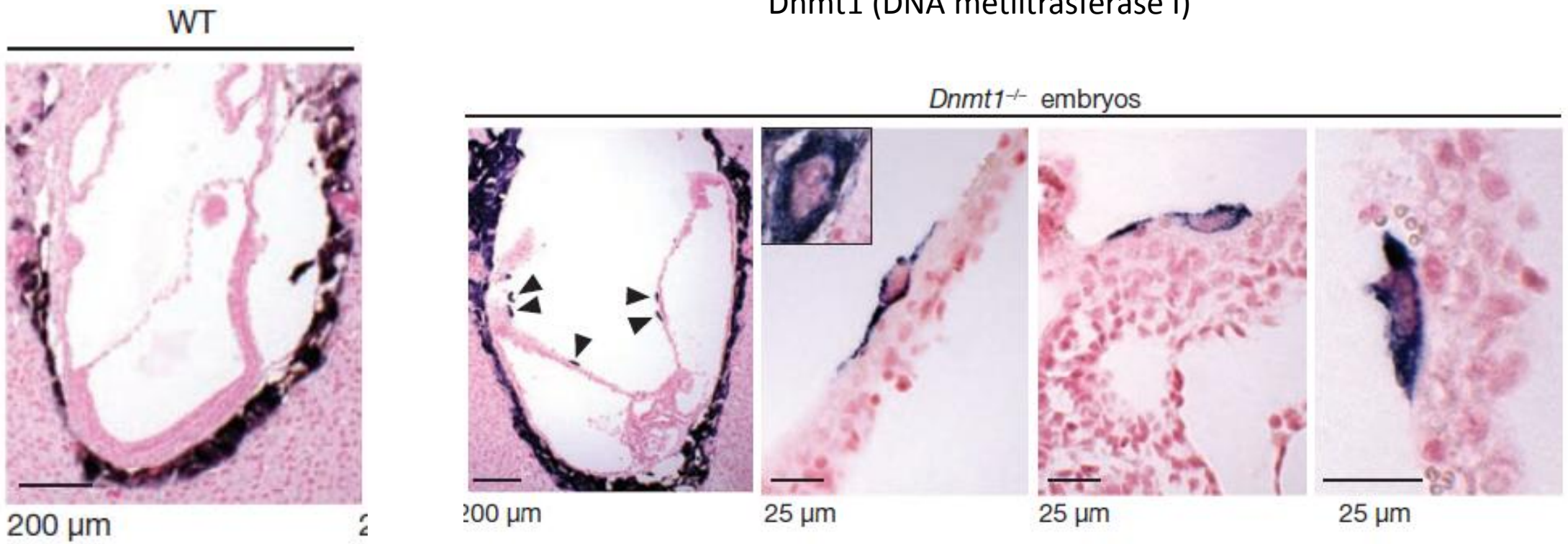
Qual é o PRIMEIRO evento de diferenciação celular na vida ??



https://www.eurostemcell.org/

em azul – marcador de trofectoderme

Embriões que não expressam
Dnmt1 (DNA metiltrasferase I)

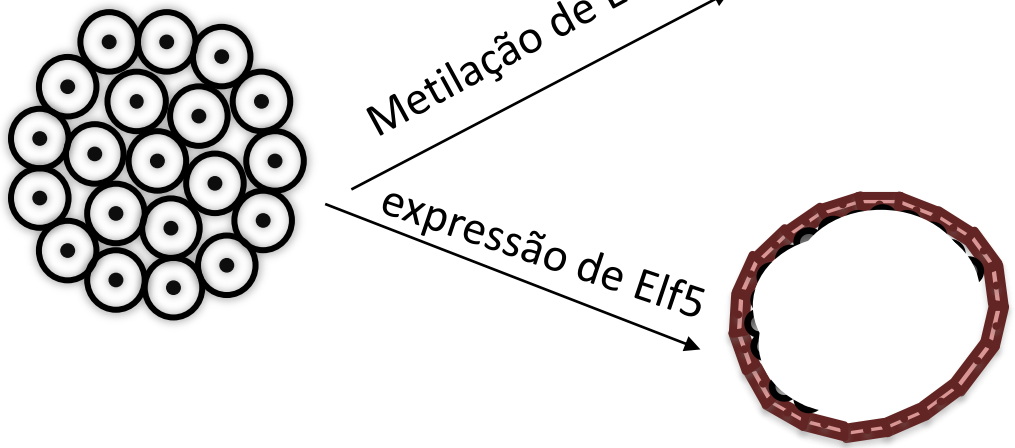


Embriões deficientes em DNA metiltransferase NÃO apresentam diferenciação entre massa celular interna e trofectoderme

Portanto esse evento de diferenciação depende da METILAÇÃO de genes

Ng Rk et al 2008. Epigenetic restriction of embryonic cell lineage fate by methylation of *Elf5.* Nat Cell Biol., 10: 1280-1290

Expressão do fator de *Elf5*



Diferenciação em massa celular interna
(=linhagem embrionária)

Diferenciação em trofectoderme
(=anexos embrionários)

Capítulo só em pdf do Alberts

Chapter 23

Specialized Tissues, Stem Cells, and Tissue Renewal

23

Exemplos usados pelo livro (capítulo 23)

- Pele
- Glândula mamária
- Epitélio olfatório
- Epitélio auditivo
- Retina
- Pulmão e intestino
- Fígado
- Pâncreas
- Vasos
- Células do sangue
- Músculo
- Tecido conectivo – fibroblastos, céls ósseas, adiposas
- Células tronco embrionárias
- Vias de sinalização: Wnt, Notch, Ephrin–Eph, Hedgehog, PDGF, and BMP

ectoderme

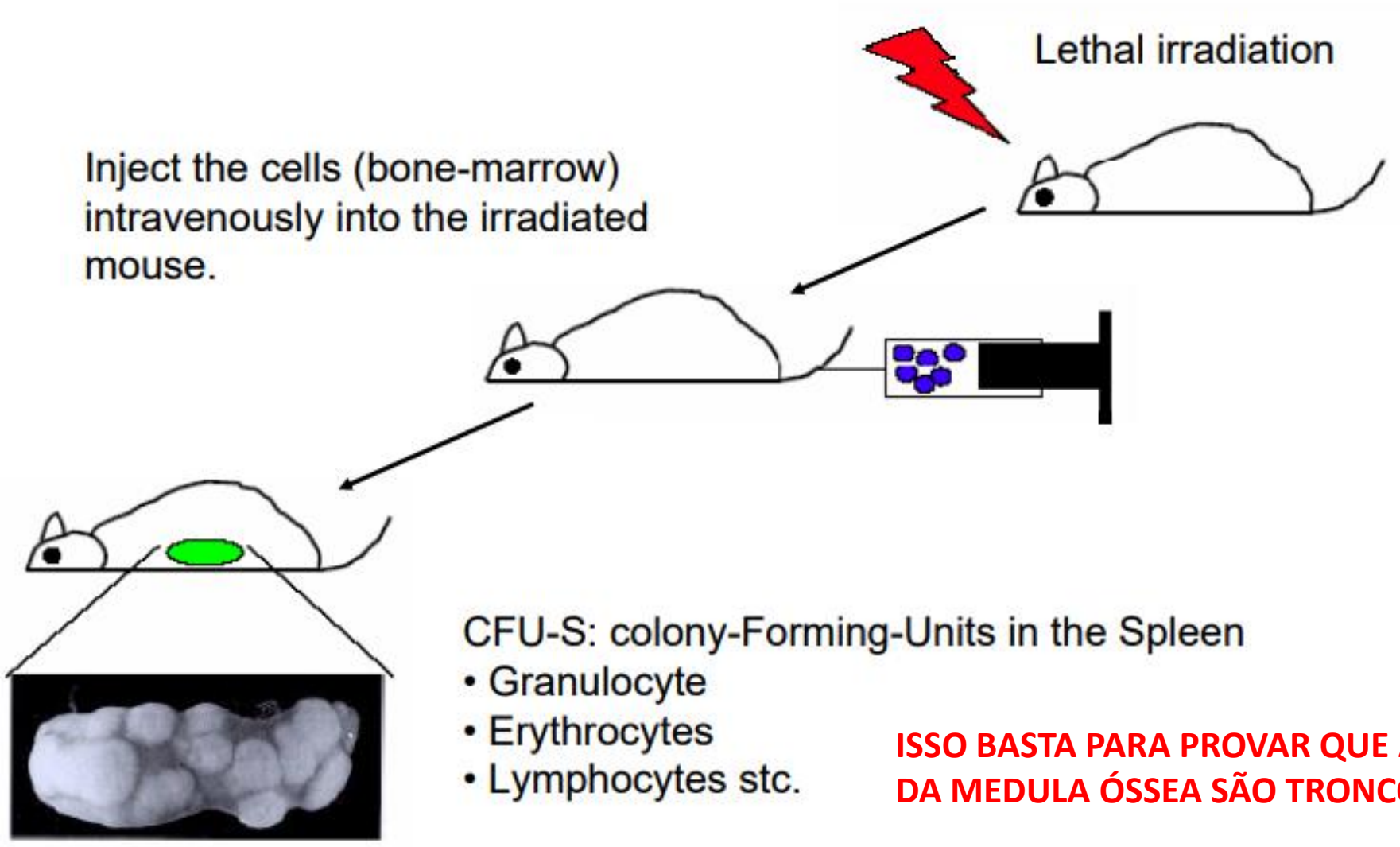
endoderme

mesoderme

- Auto-renovação
- Capaz de se diferenciar em diversos tipos celulares
- Capaz de se dividir ilimitadamente, porém divide-se “pouco”

Como verificar a existência de células tronco ?

Como sabemos se uma célula é ou não tronco??

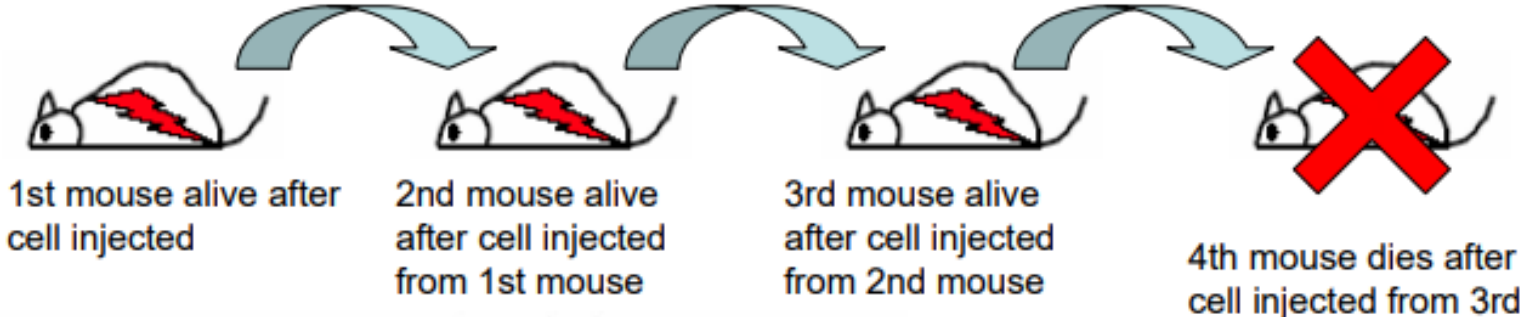


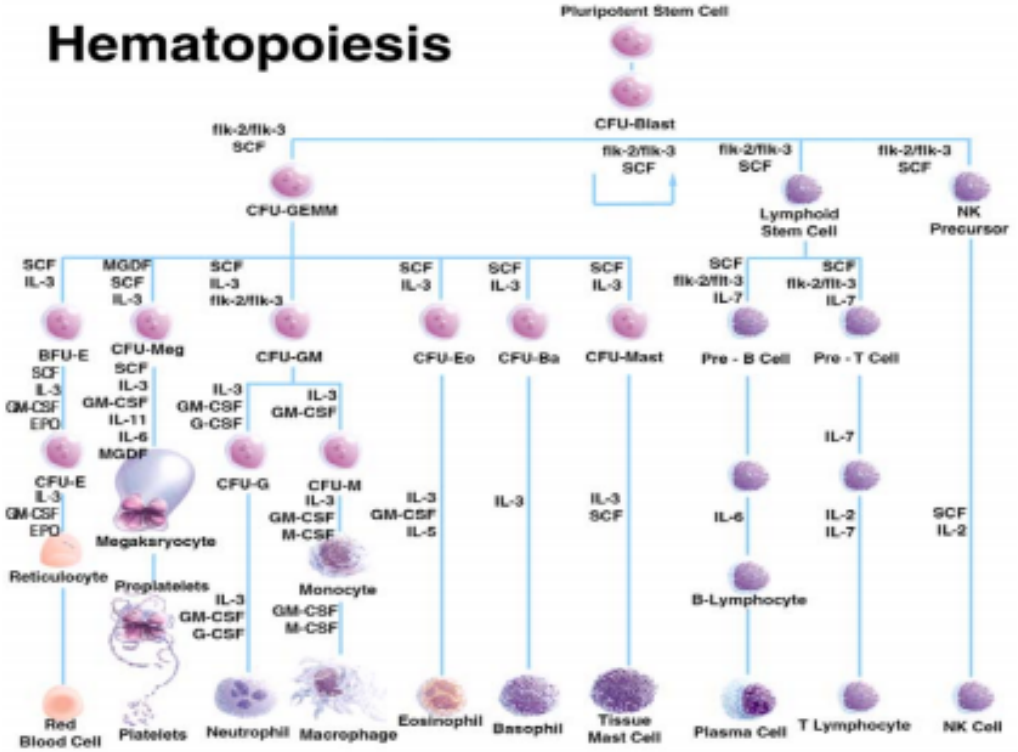
CFU-S: colony-Forming-Units in the Spleen

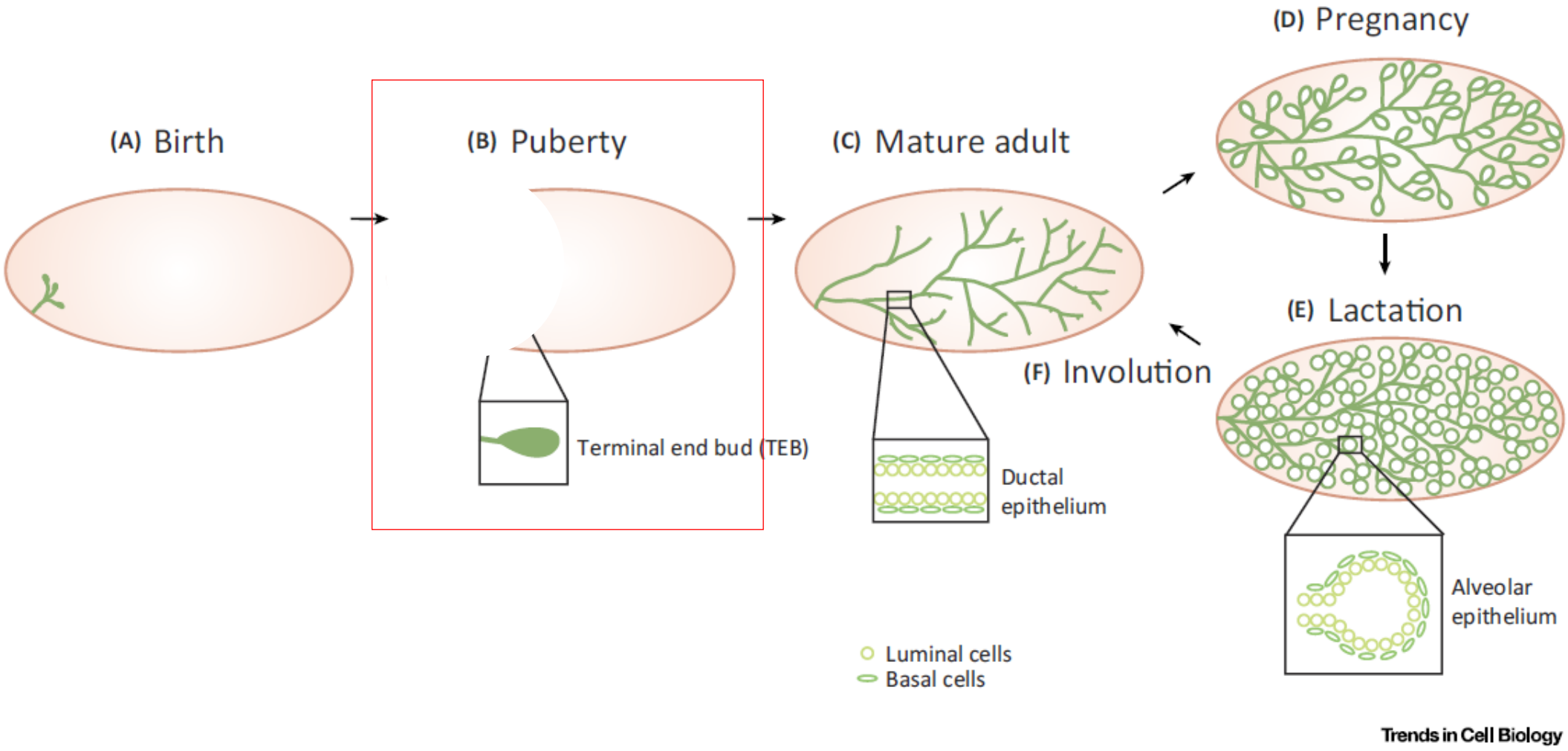
- Granulocyte
- Erythrocytes
- Lymphocytes stc.

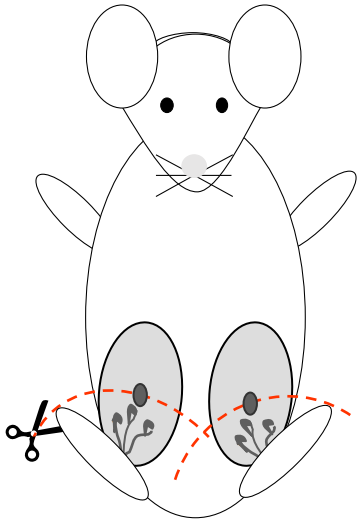
ISSO BASTA PARA PROVAR QUE AS CÉLULAS DA MEDULA ÓSSEA SÃO TRONCO?

Como sabemos se uma célula é ou não tronco??

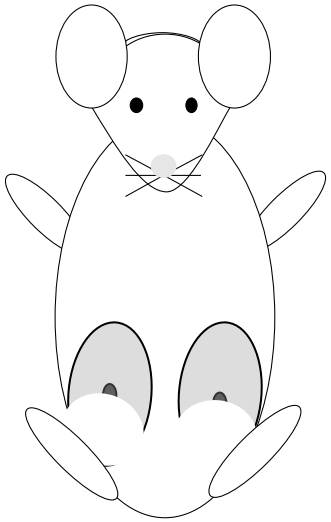




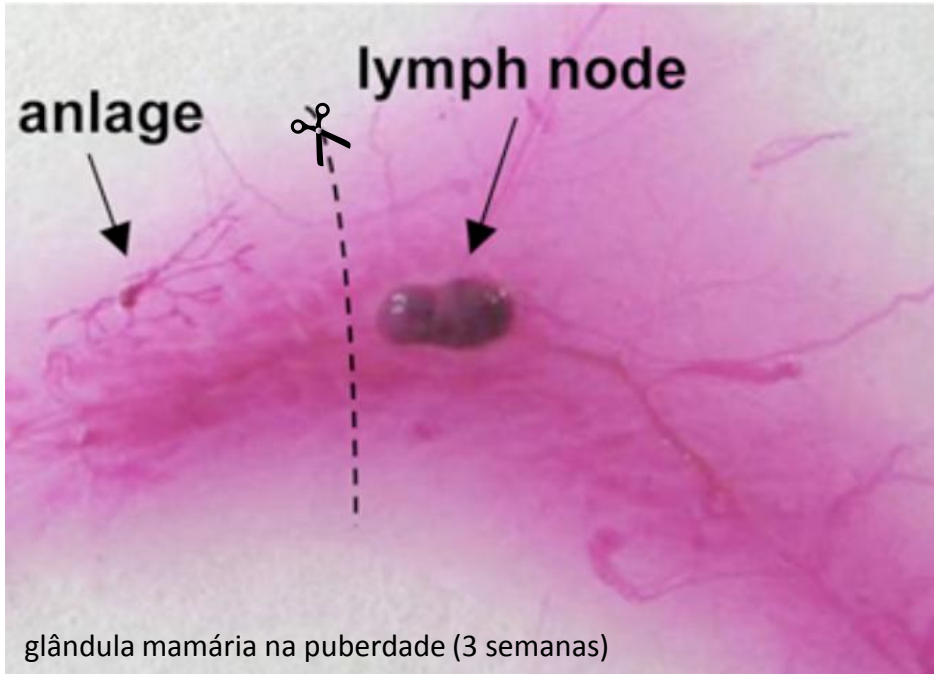

(A) Birth
(B) Puberty
(C) Mature adult
(D) Pregnancy
(E) Lactation
(F) Involution
Terminal end bud (TEB)
Ductal epithelium
Alveolar epithelium
Luminal cells
Basal cells
Trends in Cell Biology



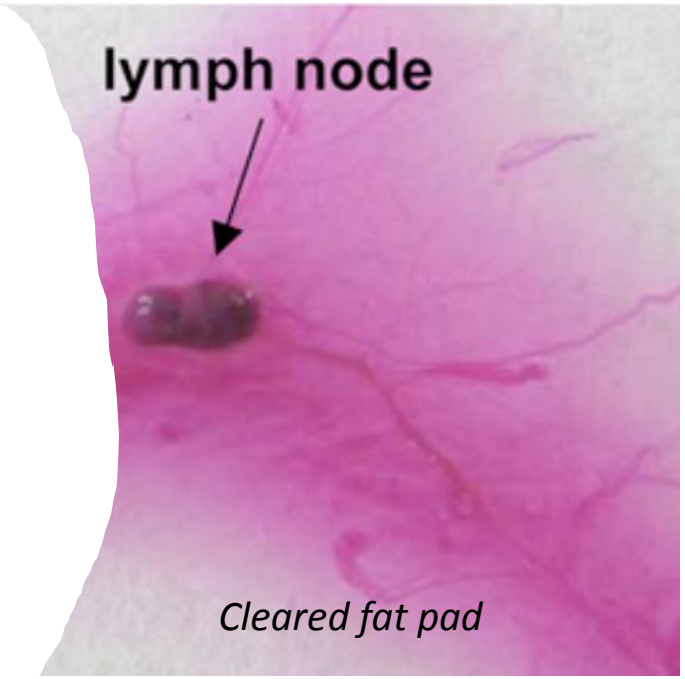
Remoção
do epitélio
insipiente



DeOme, et al, 1959. Cancer Res 19: 515-



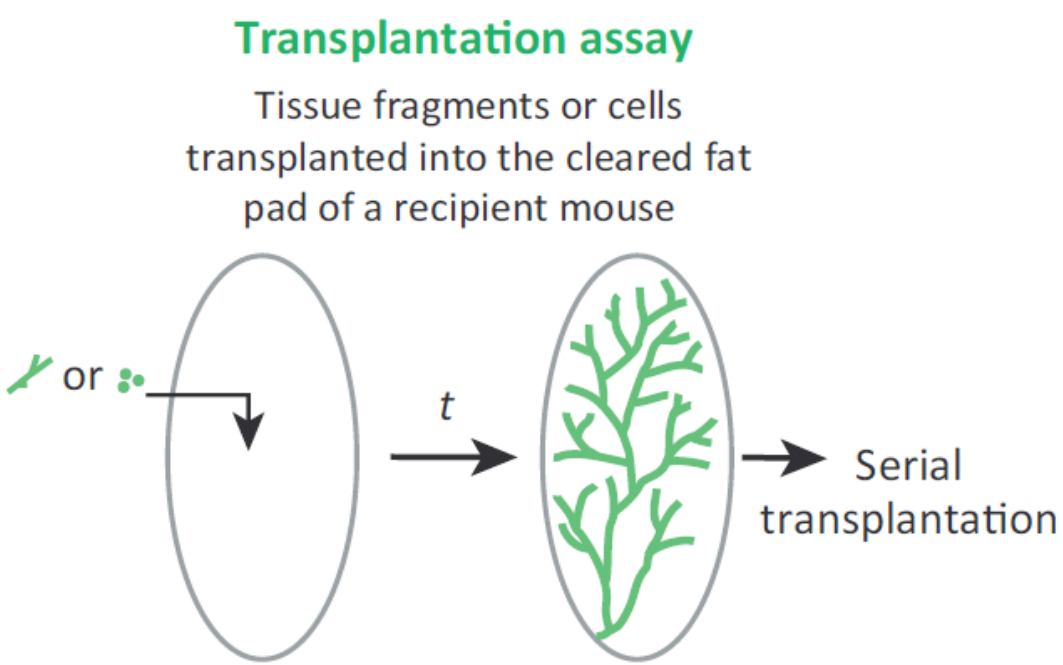
glândula mamária na puberdade (3 semanas)

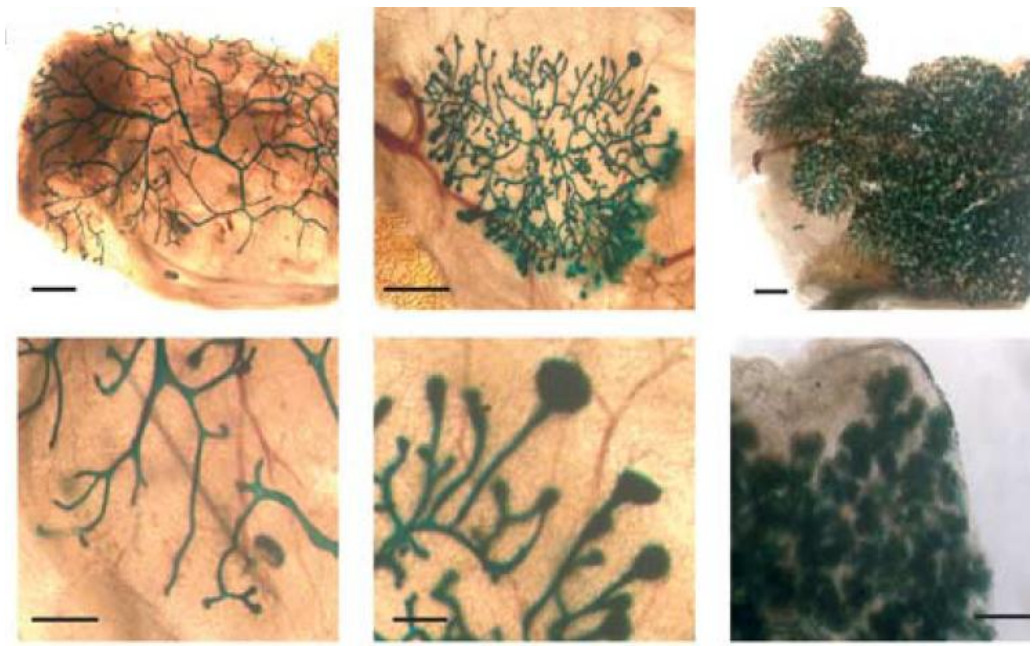


nature Vol 439|5 January 2006|doi:10.1038/nature04372

Generation of a functional mammary gland from a single stem cell

Mark Shackleton[1,2], François Vaillant[1,2], Kaylene J. Simpson[3]†, John Stingl[4,5], Gordon K. Smyth[1], Marie-Liesse Asselin-Labat[1,2], Li Wu[1], Geoffrey J. Lindeman[1,2] & Jane E. Visvader[1,2]



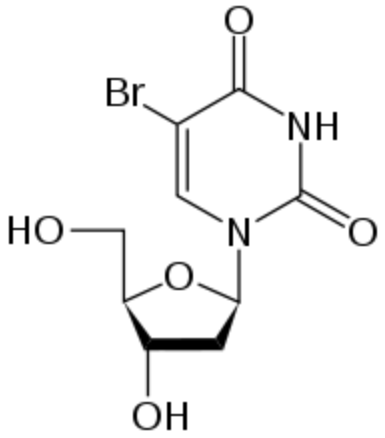


- Auto-renovação
- Capaz de se diferenciar em diversos tipos celulares
- Capaz de se dividir ilimitadamente, **porém divide-se “pouco”**

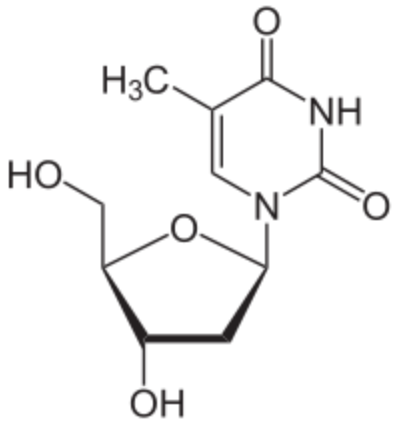
Como verificar se as células se dividem pouco ?

Ensaio de incorporação de BrdU

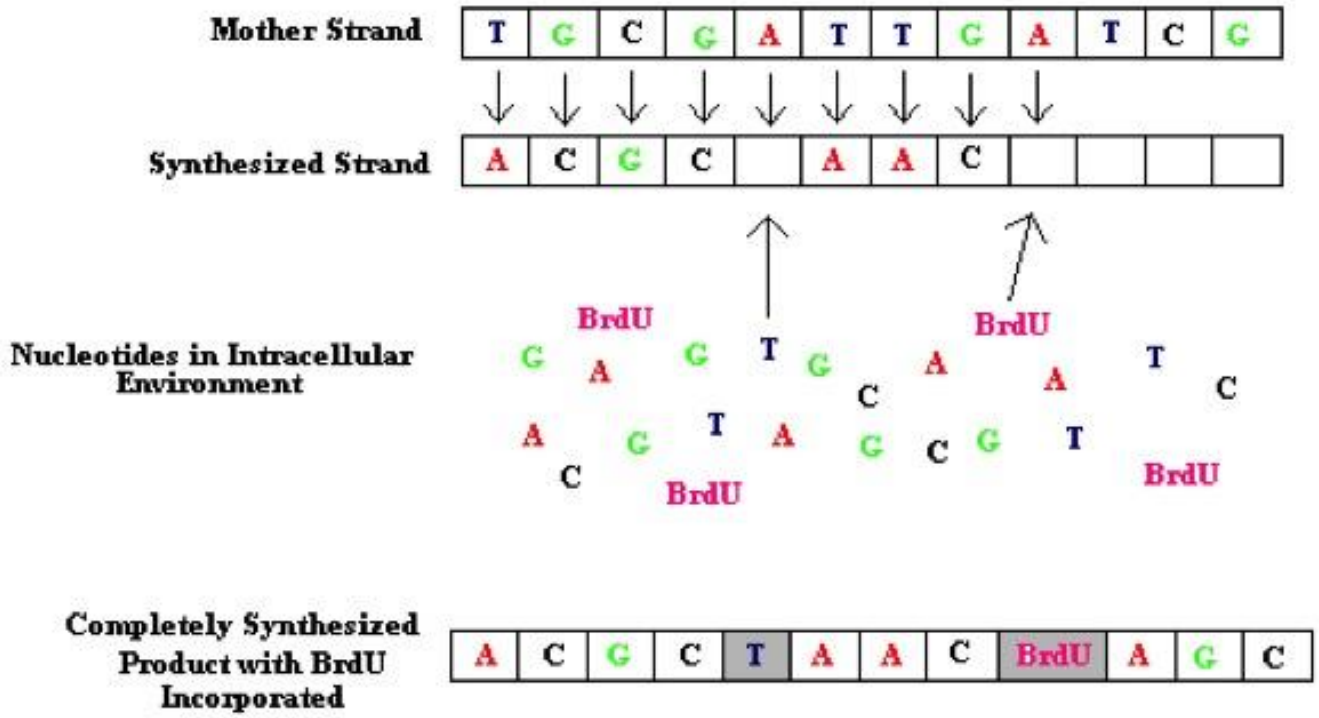
(*bromodeoxyuridine*)



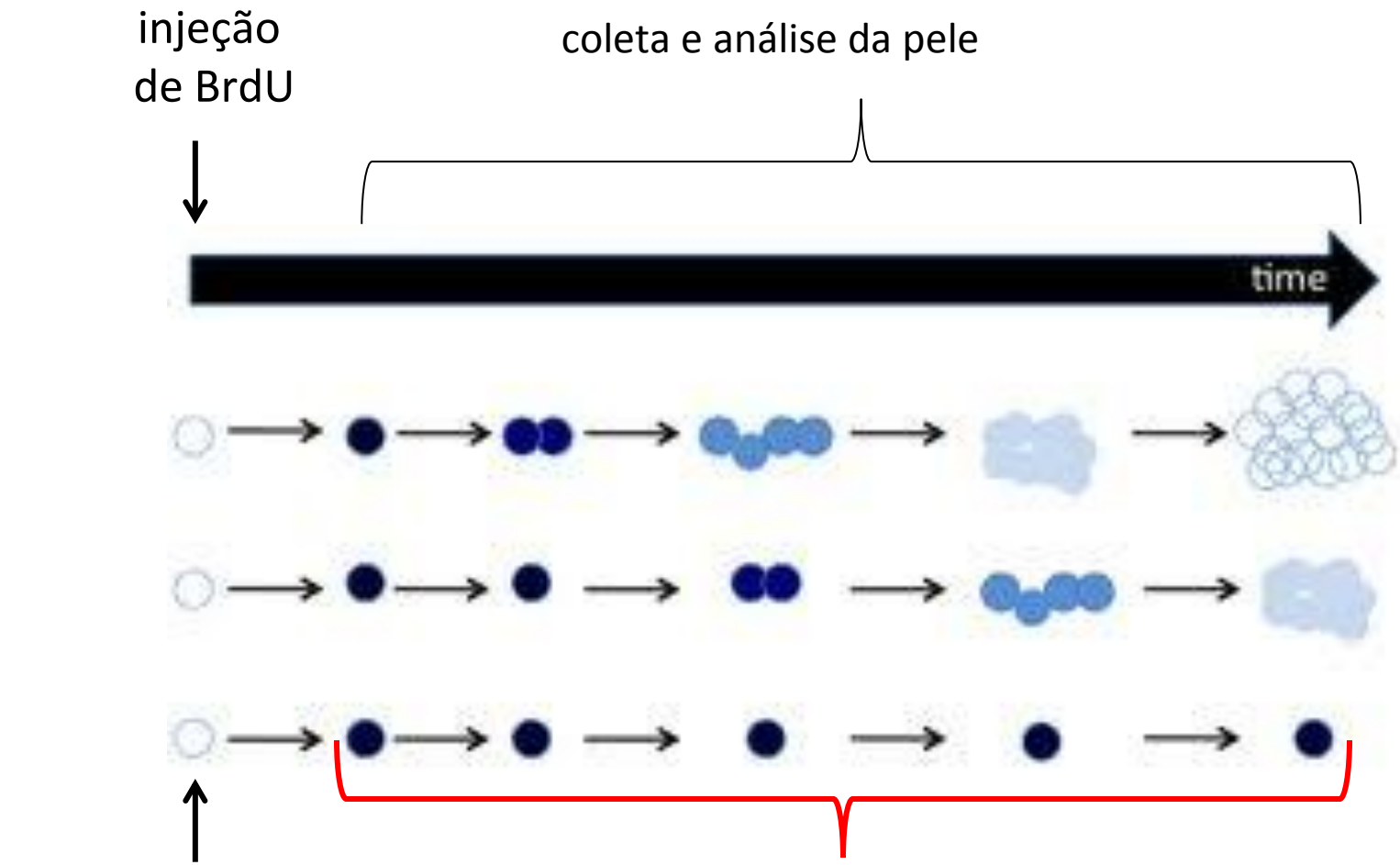
bromodeoxyuridine



thymidine



Ensaio de retenção de marcação



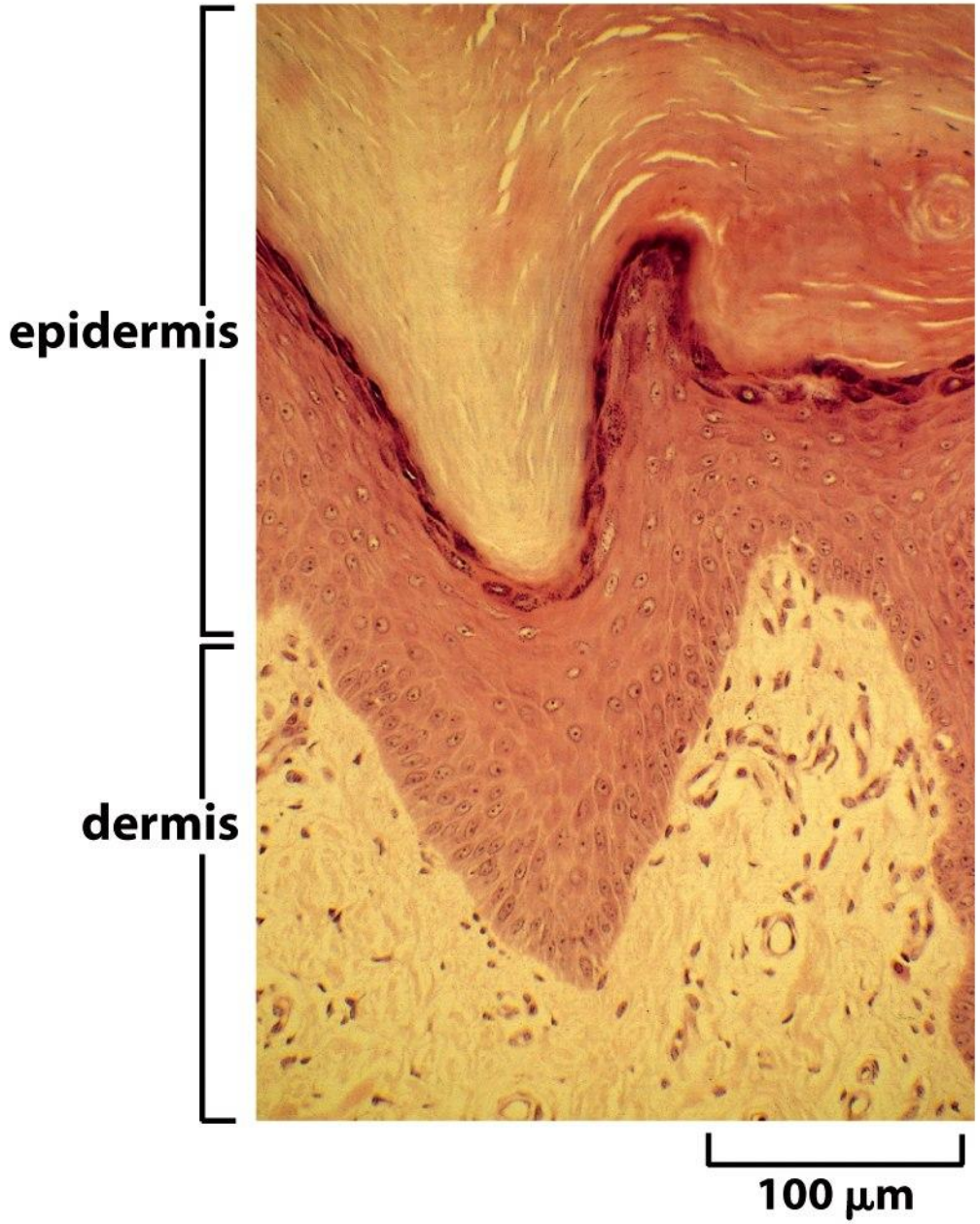


Figure 23-1b Molecular Biology of the Cell 5/e (© Garland Science 2008)

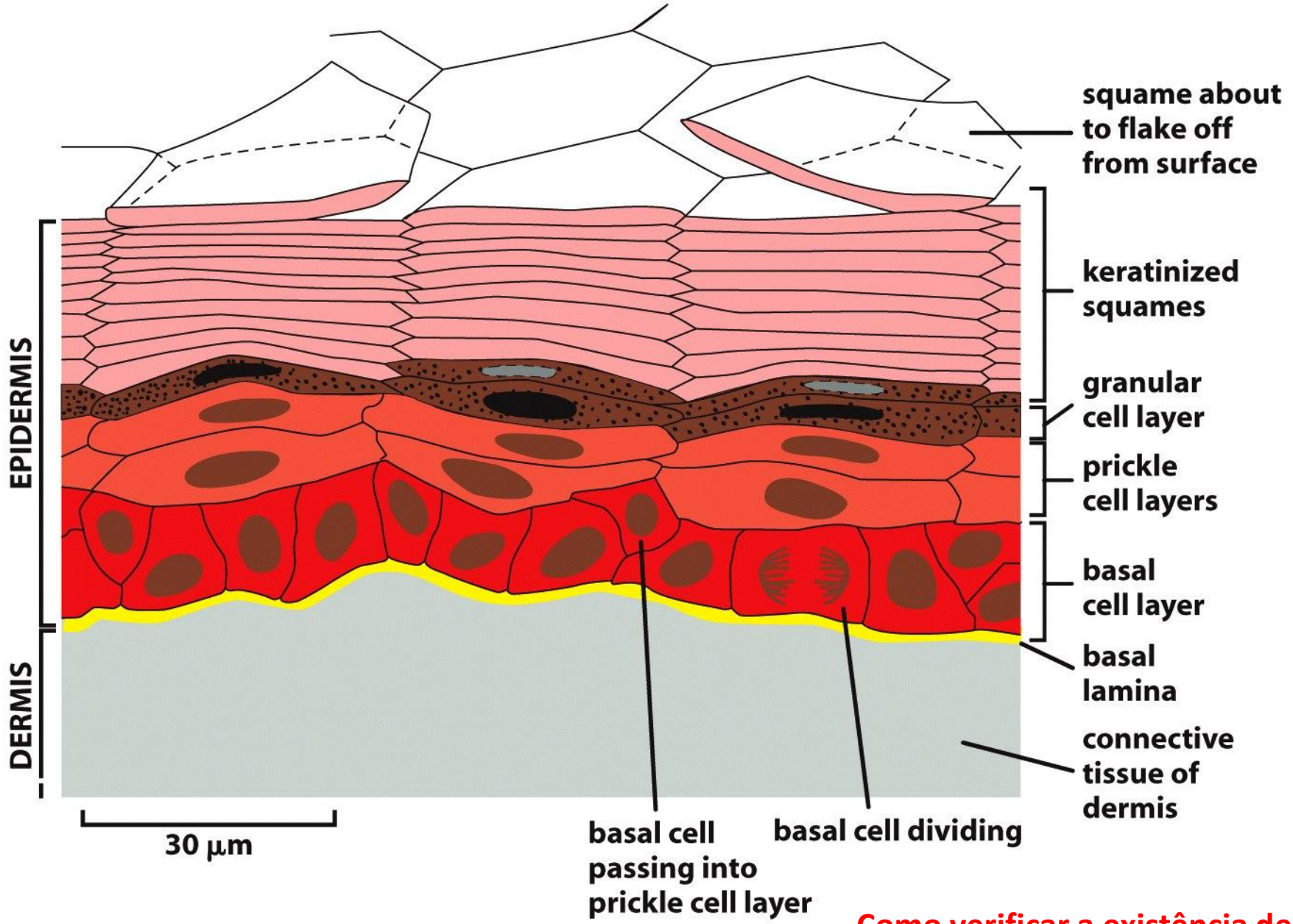


Figure 23-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Como verificar a existência de células tronco ?

Renovação das estruturas da epiderme: o folículo piloso

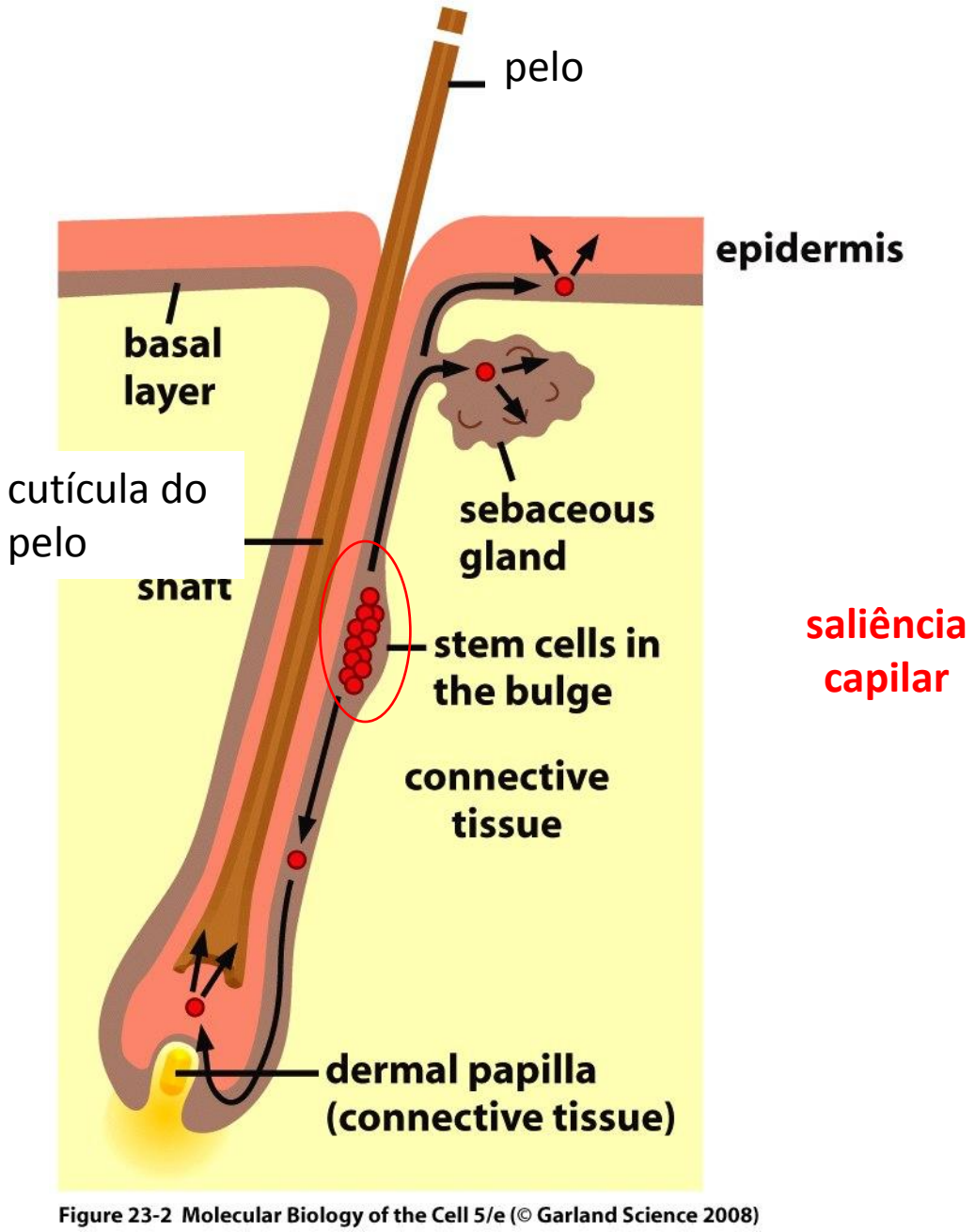
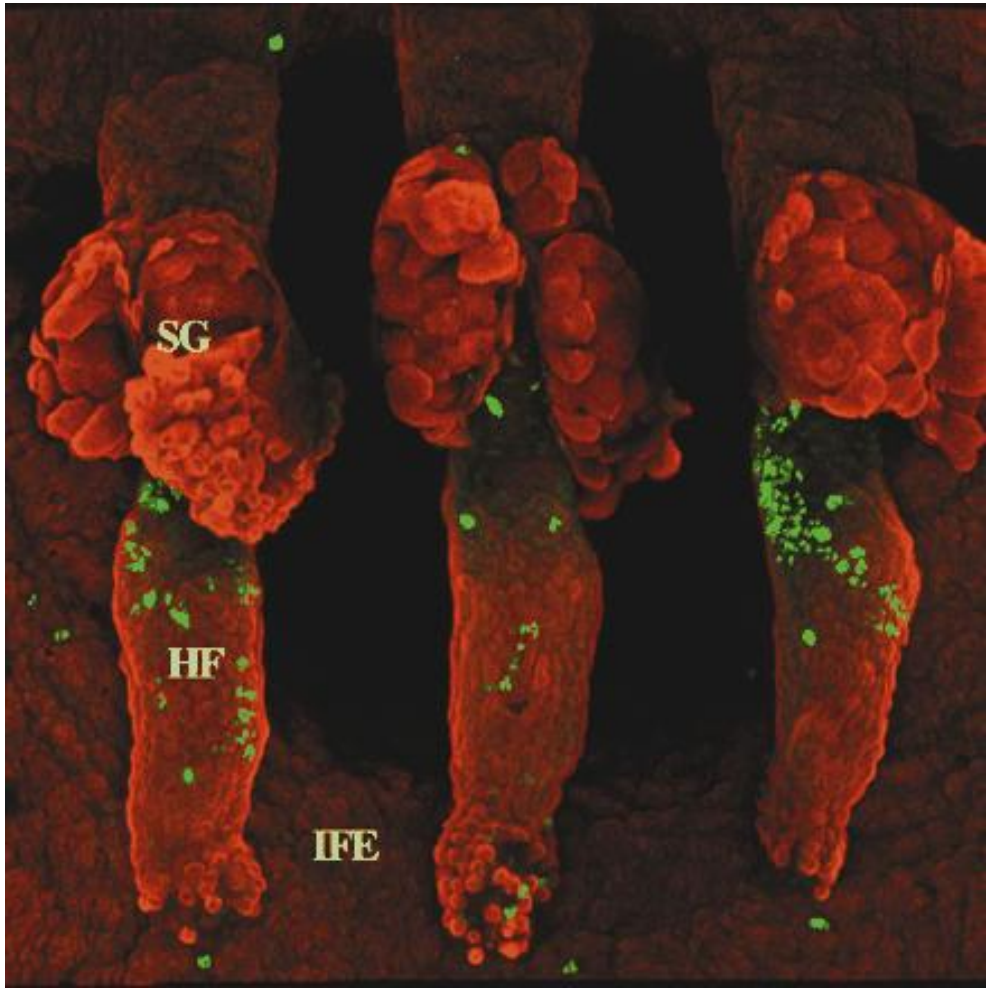


Figure 23-2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

saliência capilar

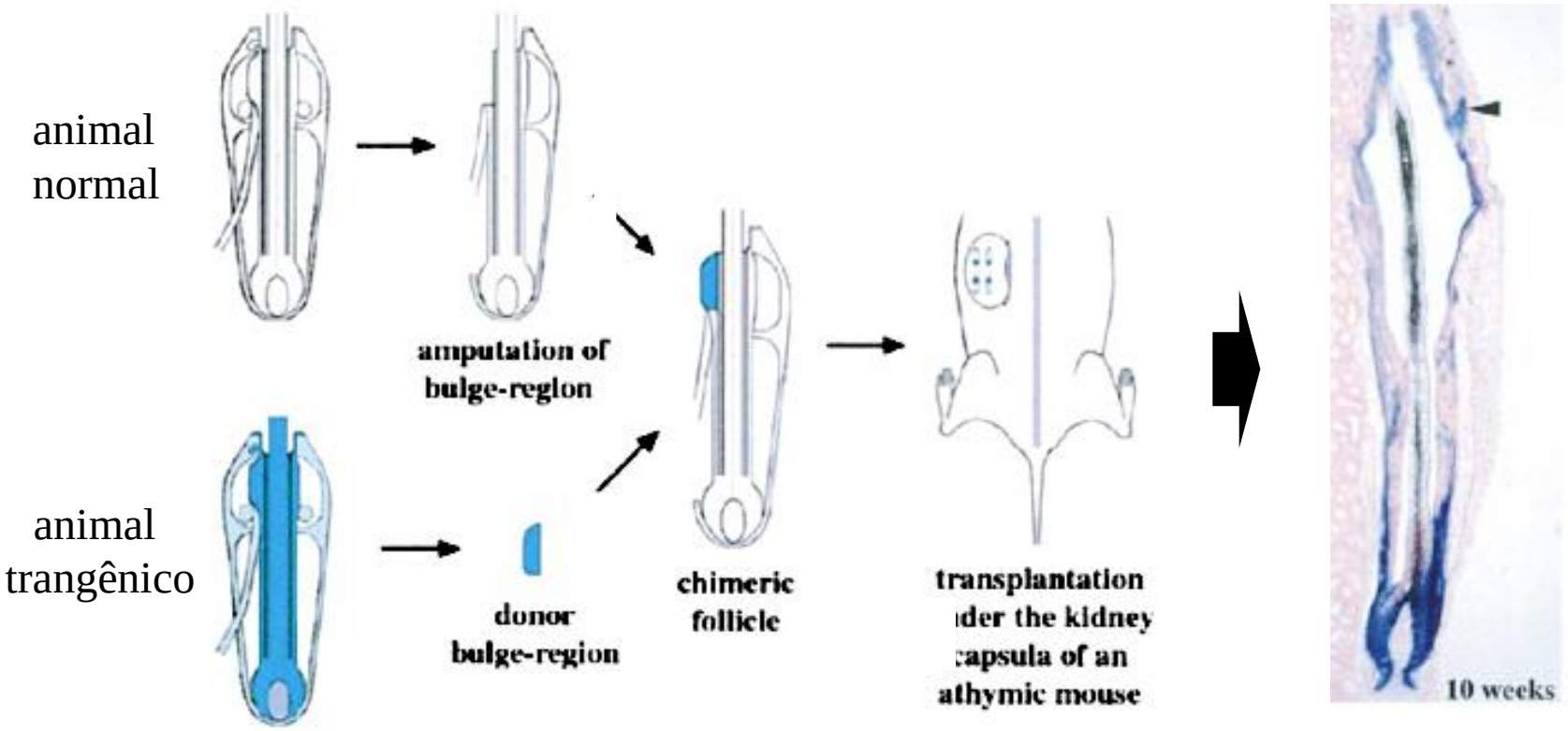
Injeção de BrdU por 48 h → 70 dias → imunofluorescência com anti-BrdU (verde)

Ensaio para verificar células que detêm a marcação de BrdU



HF, hair follicle;
IFE, interfollicular epidermis;
SG, sebaceous gland

Células da saliência capilar dão origem a todas as estruturas da epiderme



Oshima et al, 2001. Cell, 104: 233-245

Há alguma outra interpretação para a retenção de marcação de BrdU ...?

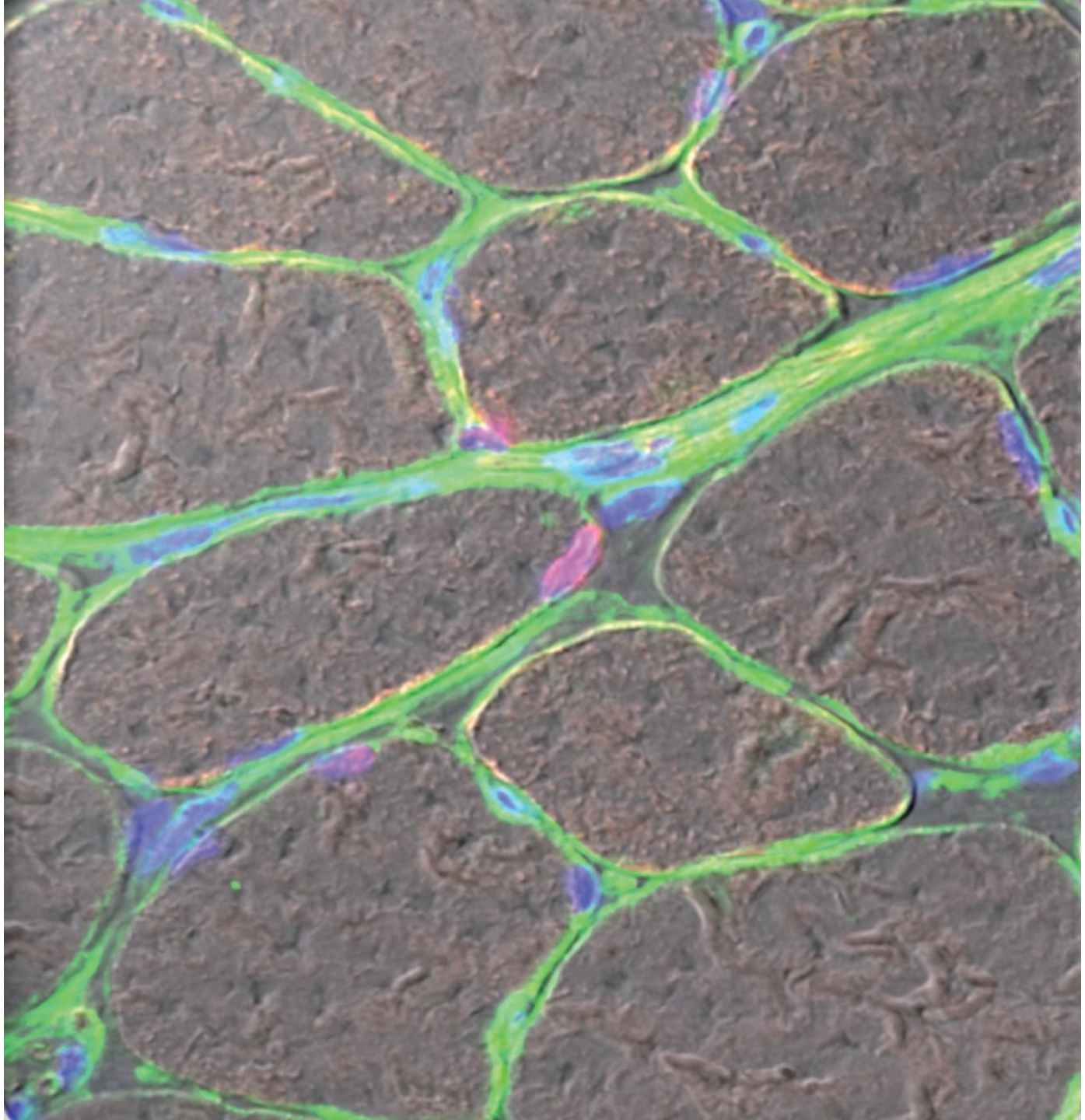
Article abstract

Nature Cell Biology **8**, 677 - 682 (2006)
Published online: 25 June 2006 | doi:10.1038/ncb1425

Asymmetric division and cosegregation of template DNA strands in adult muscle satellite cells

Vasily Shinin[1], Barbara Gayraud-Morel[1], Danielle Gomès[1] & Shahragim Tajbakhsh[1]

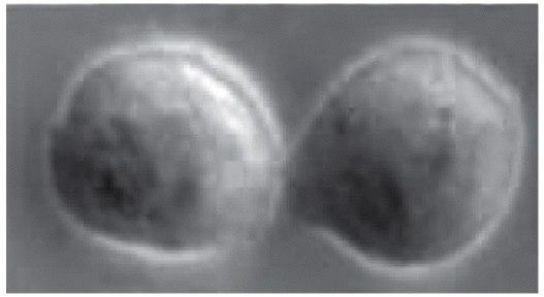
Satellite cells assure postnatal skeletal muscle growth and repair. Despite extensive studies, their stem cell character remains largely undefined. Using pulse-chase labelling with BrdU to mark the putative stem cell niche, we identify a subpopulation of label-retaining satellite cells during growth and after injury. Strikingly, some of these cells display selective template-DNA strand segregation during mitosis in the muscle fibre *in vivo*, as well as in culture independent of their niche, indicating that genomic DNA strands are nonequivalent. Furthermore, we demonstrate that the asymmetric cell-fate determinant Numb segregates selectively to one daughter cell during mitosis and before differentiation, suggesting that Numb is associated with self-renewal. Finally, we show that template DNA cosegregates with Numb in label-retaining cells that express the self-renewal marker Pax7. The cosegregation of 'immortal' template DNA strands and their link with the asymmetry apparatus has important implications for stem cell biology and cancer.



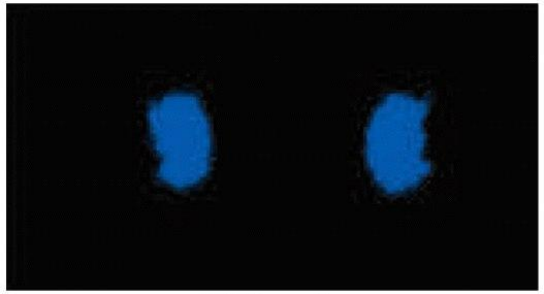
We can see here a section of muscle with the muscle fibers separated by membranes (stained green). The muscle stem cells (satellite cells) are stained red.

Image: Shahragim Tajbakhsh

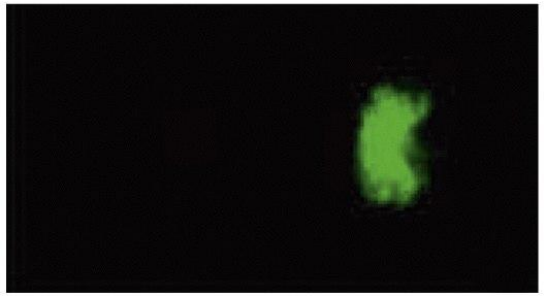
A hipótese da fita de DNA imortal



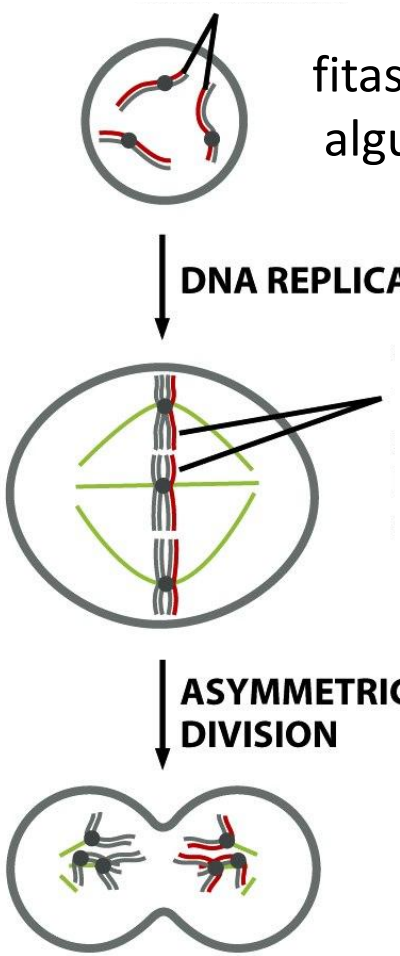
DNA



BrdU



Como ocorreria a herança da fita de DNA imortal (**hipótese**) ?



fitas imortais são de alguma maneira “marcadas”

cromátides que herdaram as fitas são segregadas para um mesmo polo

fita de DNA imortal

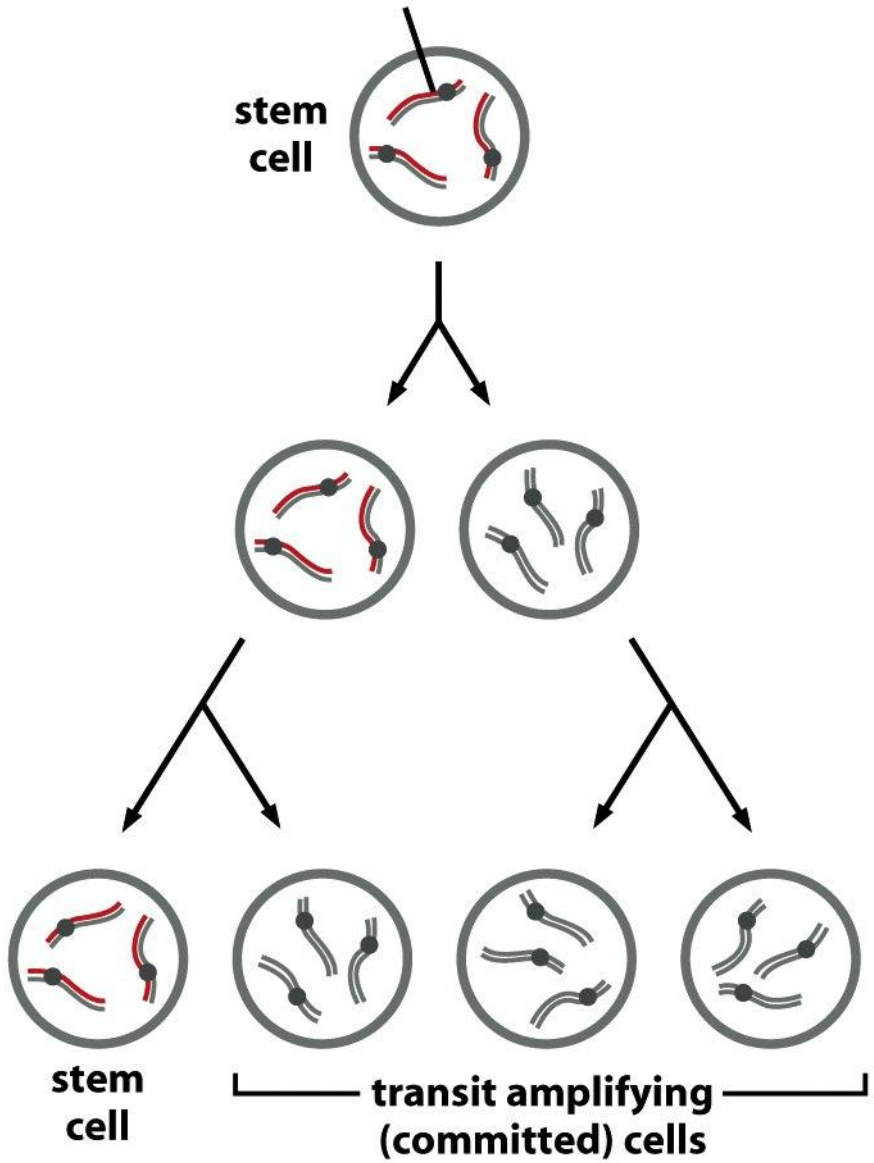


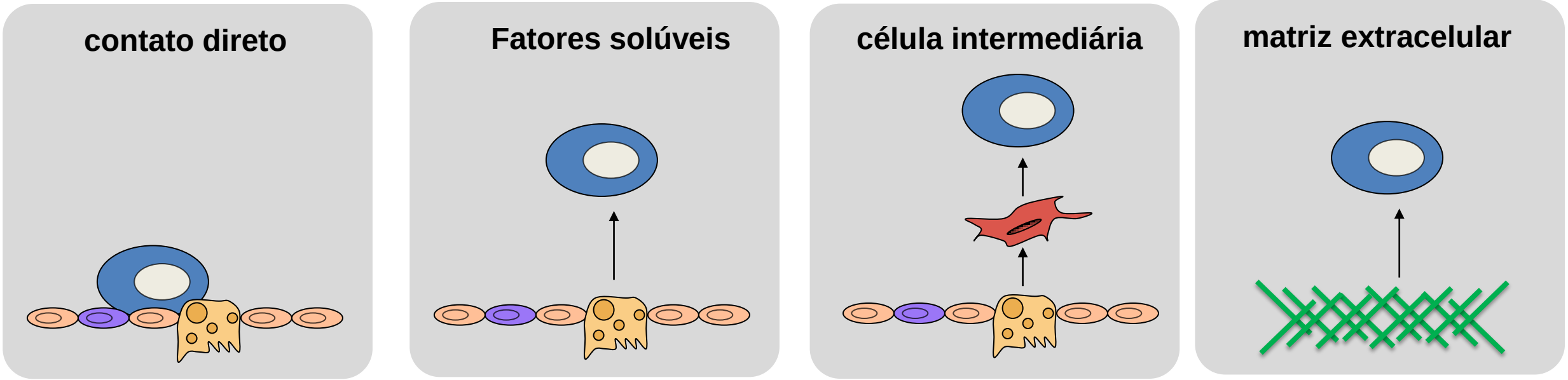
Figure 23-10c *Molecular Biology of the Cell* (© Garland Science 2008)

progenitoras

Nichos das células-tronco

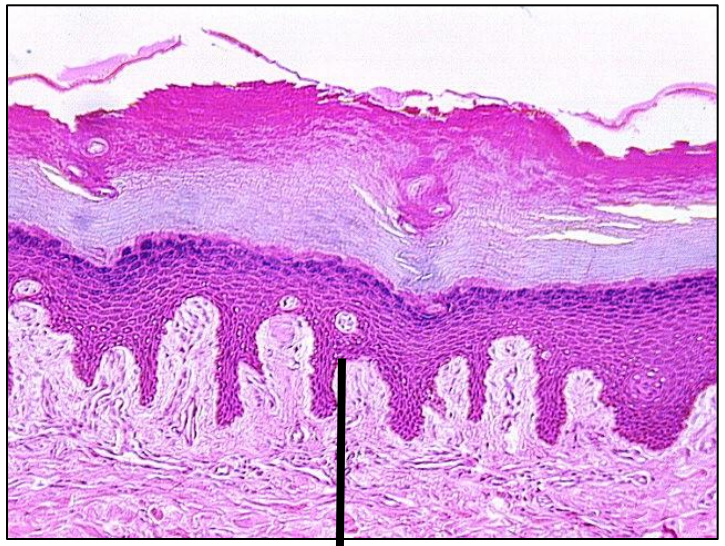
Nicho

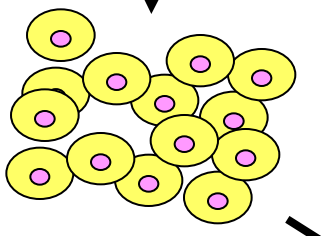
Microambiente que regula a auto-renovação e a diferenciação das células-tronco



Na epiderme, a interação com a matriz extracelular inibe a diferenciação

Na pele, a manutenção
do fenótipo tronco
É mantido
Pelo microambiente
(células em contato
com a lâmina basal
permanecem
indiferenciadas)

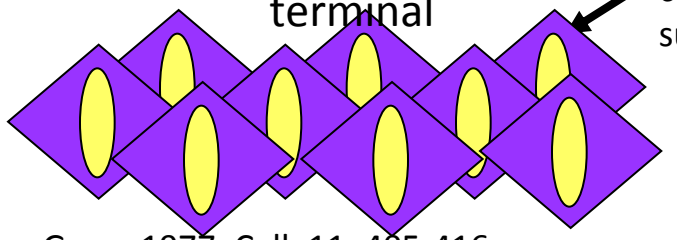




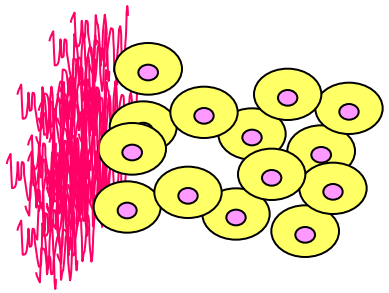
células em
suspensão

\+
matriz

diferenciação
terminal



manutenção do
fenótipo tronco



Geen, 1977. Cell, 11: 405-416

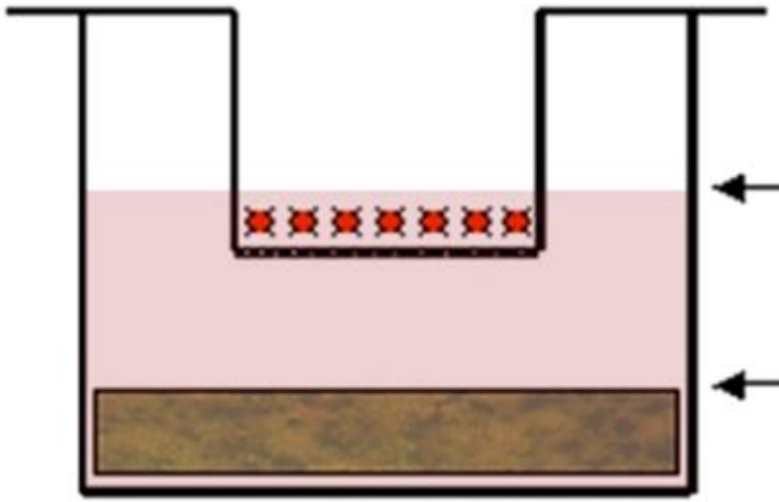
Adams & Watt, 1989. Nature, 340:307-309

nature cell biology

Hematopoietic stem cells convert into liver cells within days without fusion

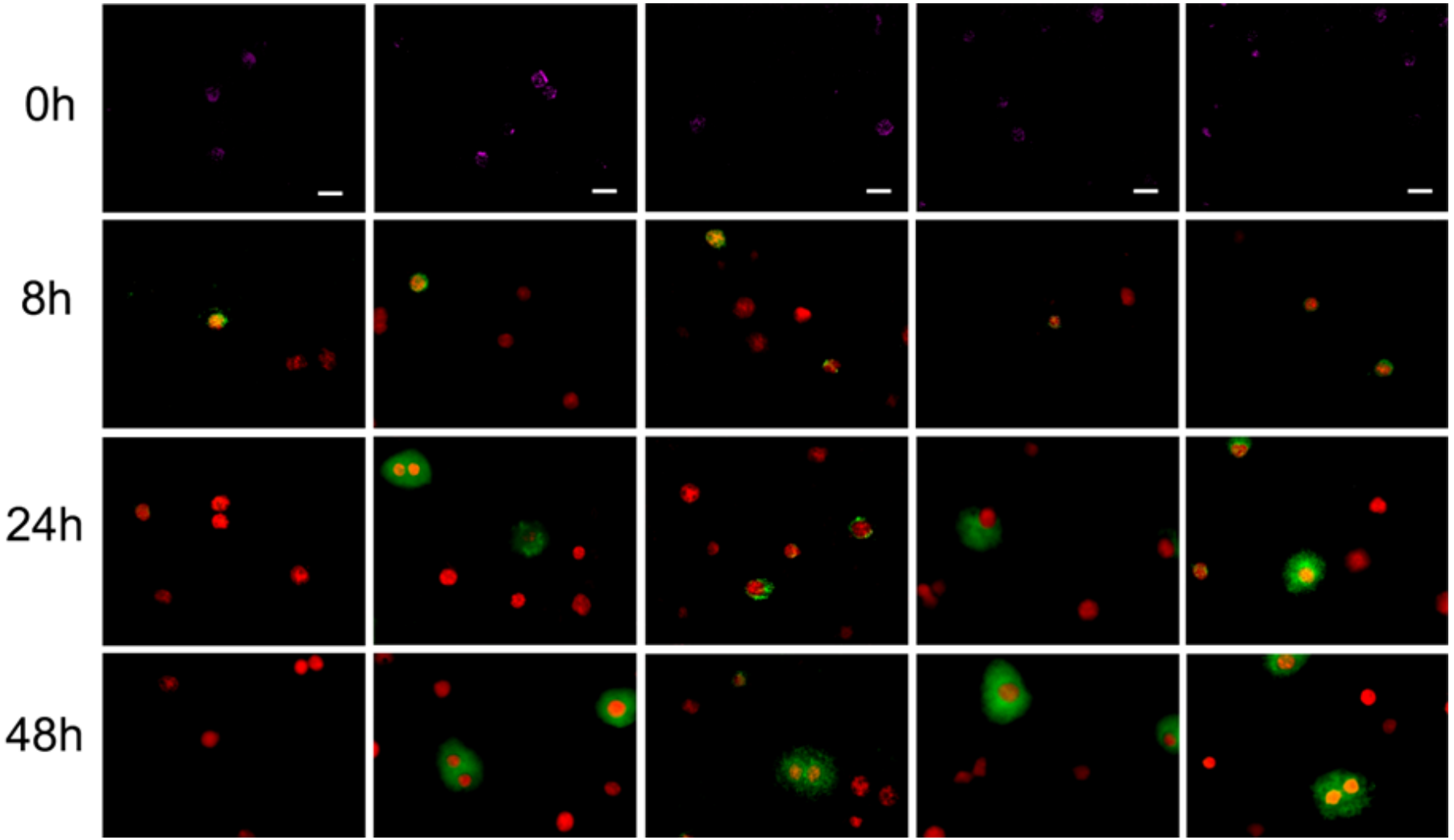
Yoon-Young Jang, Michael I. Collector, Stephen B. Baylin, Anna Mae Diehl & Saul J. Sharkis ✉

Nature Cell Biology **6**, 532–539 (2004) | Received: 23 February 2004



Células-tronco hematopoiéticas isoladas de camundongos machos

Hepatócitos lesionados isolados do fígado de um camundongo fêmea



Verde e vermelho indicam expressão de proteínas de fígado

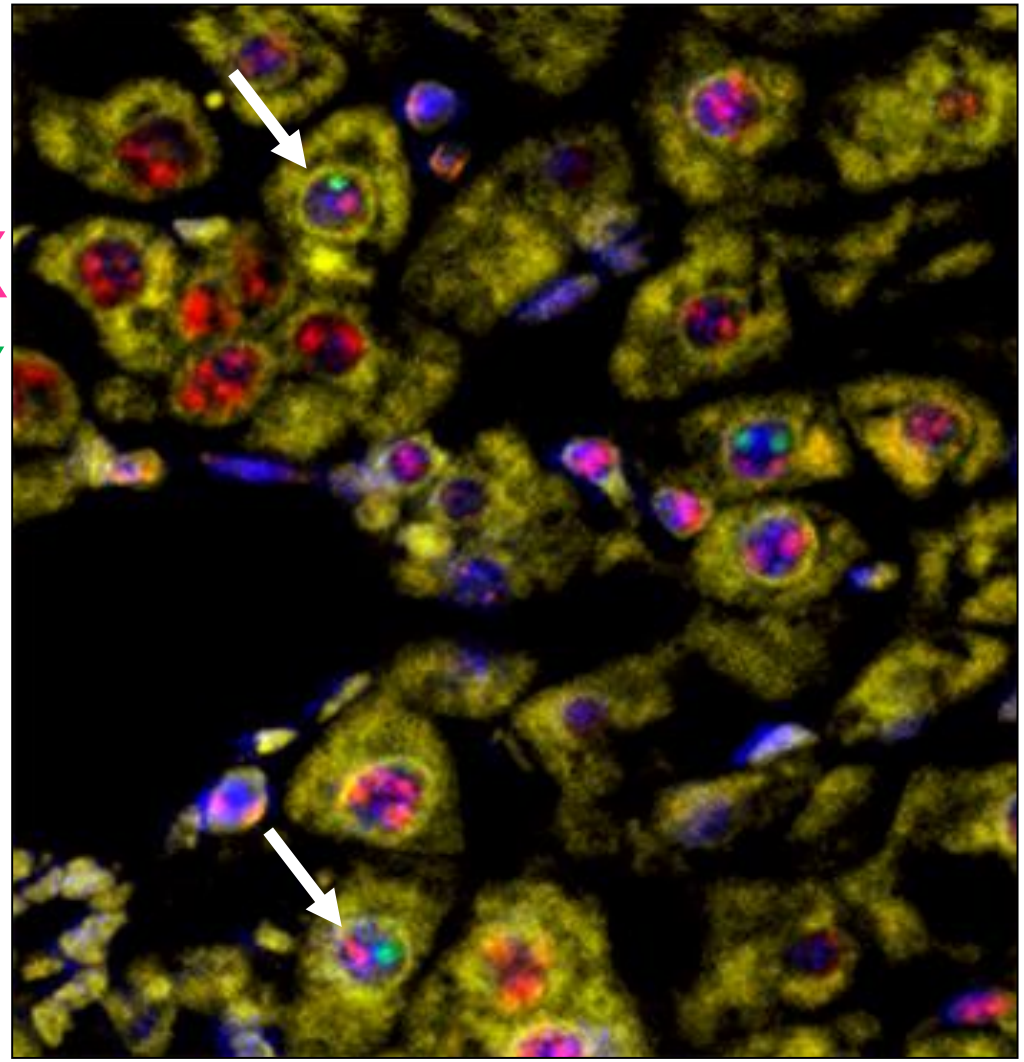
Jang YY, et al. 2004. Nat Cell Biol., 6:532-9.

Se células-tronco da medula óssea podem se diferenciar em hepatócitos em cultura, será que elas fariam o mesmo em um animal?

100.000 células-tronco de medula óssea de um macho foram transplatados em uma fêmea com lesão hepática

Células do fígado da fêmea transplantada após várias semanas:

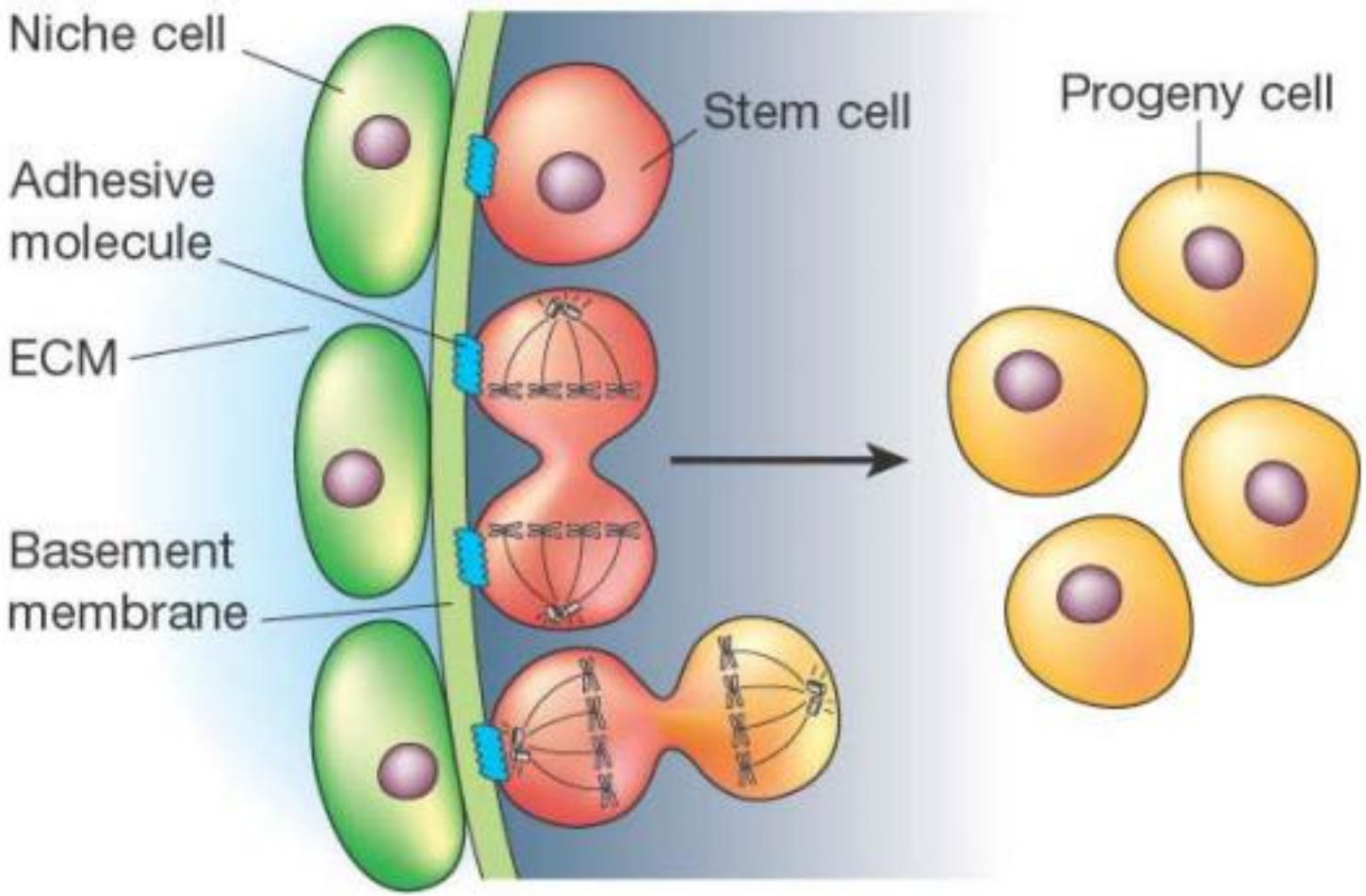
Cromossomo X
Cromossomo Y



Conclusão: fatores secretados pelos hepatócitos foram capazes de induzir a diferenciação de células-tronco hematopoiéticas

Jang YY, et al. 2004. Nat Cell Biol., 6:532-9.

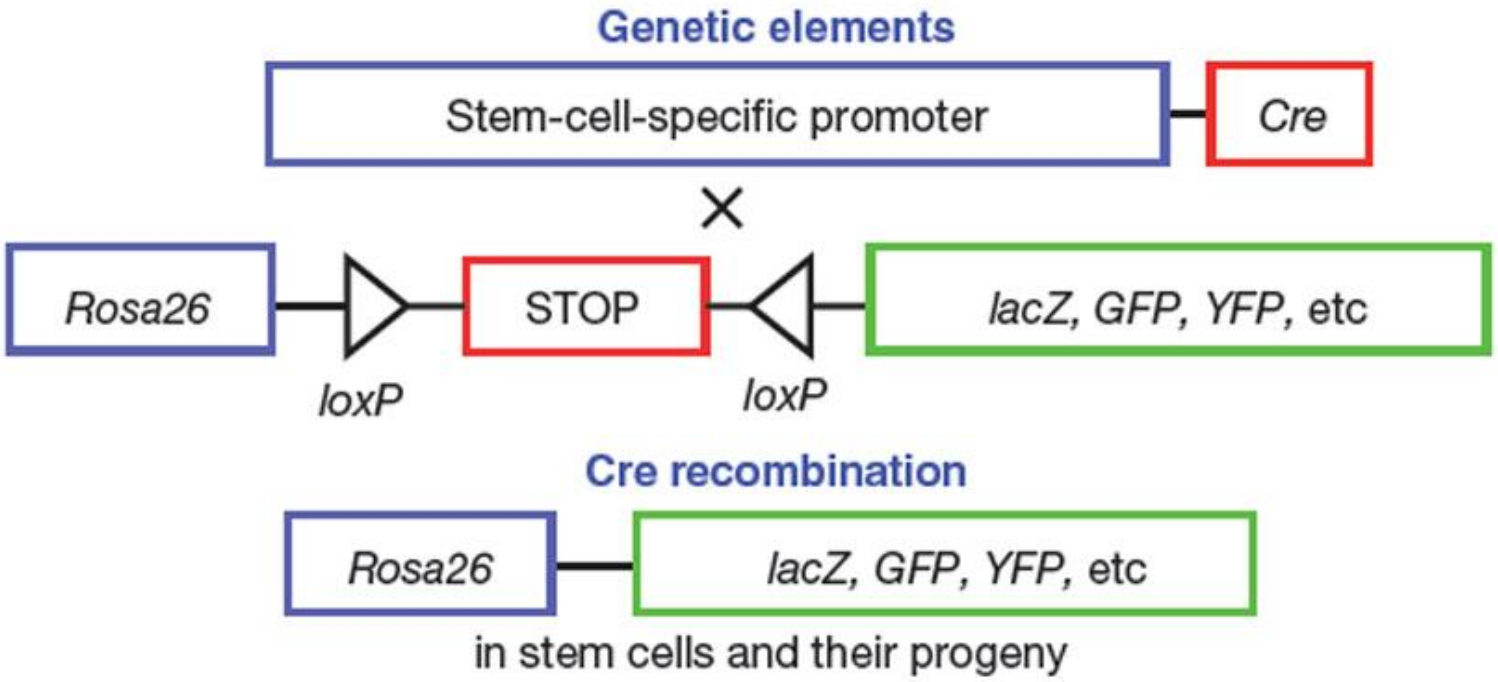
Orientação do fuso mitótico pode definir o destino da célula



É possível comprovar a existência de células-tronco *in vivo*?

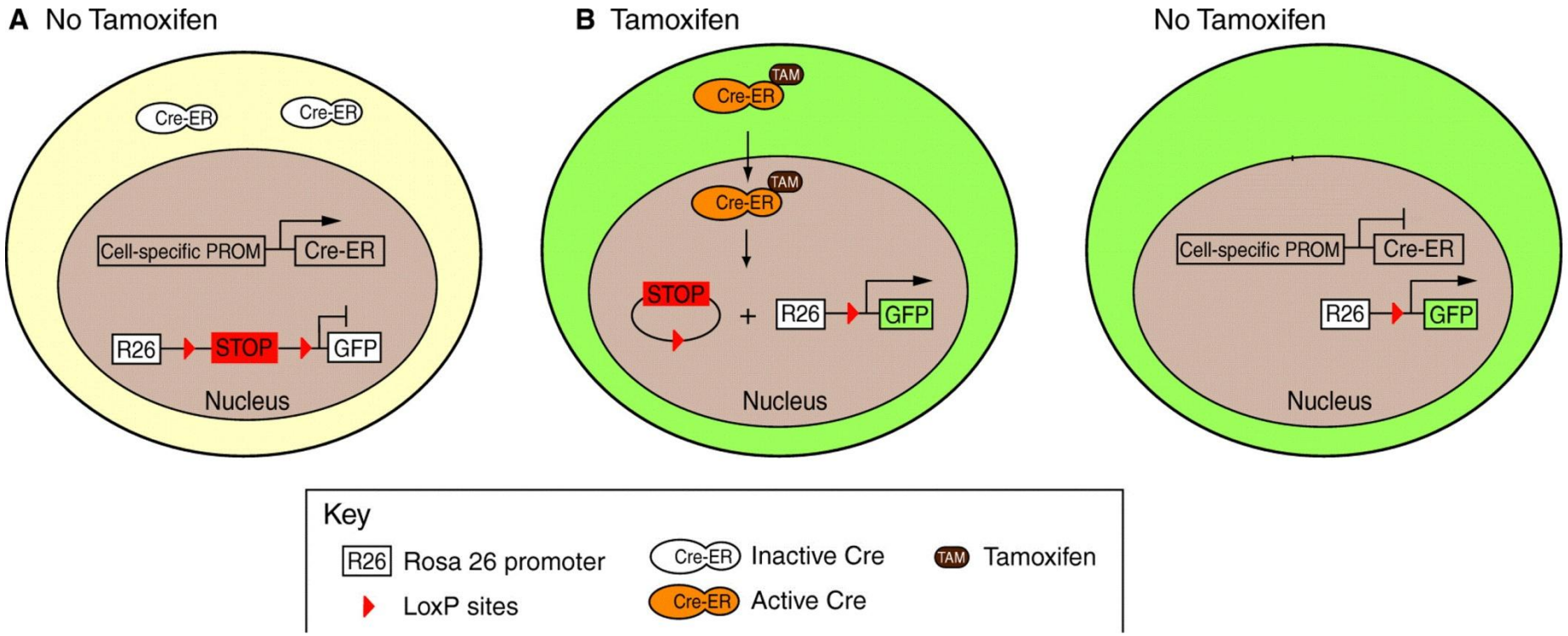
Técnica de rastreamento de linhagem

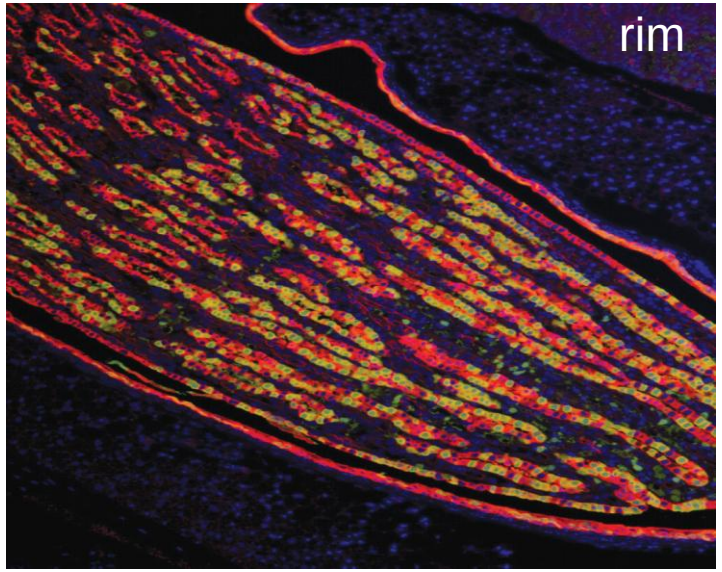
Técnica de rastreamento de linhagem

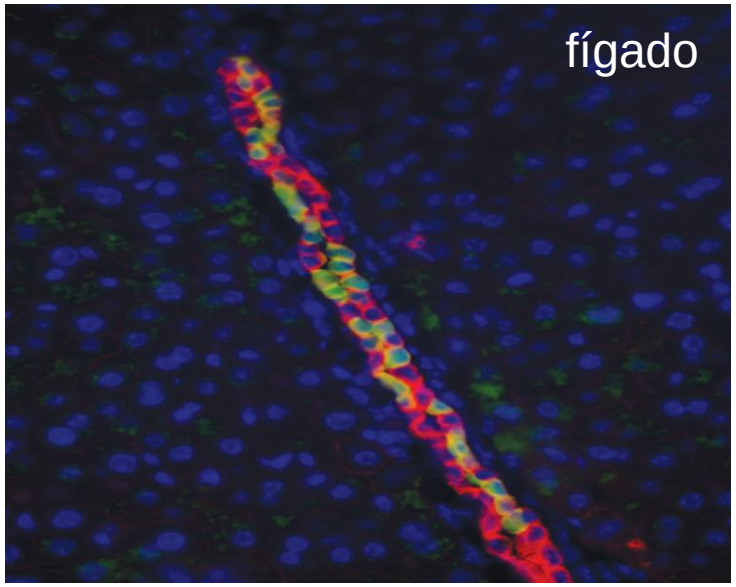


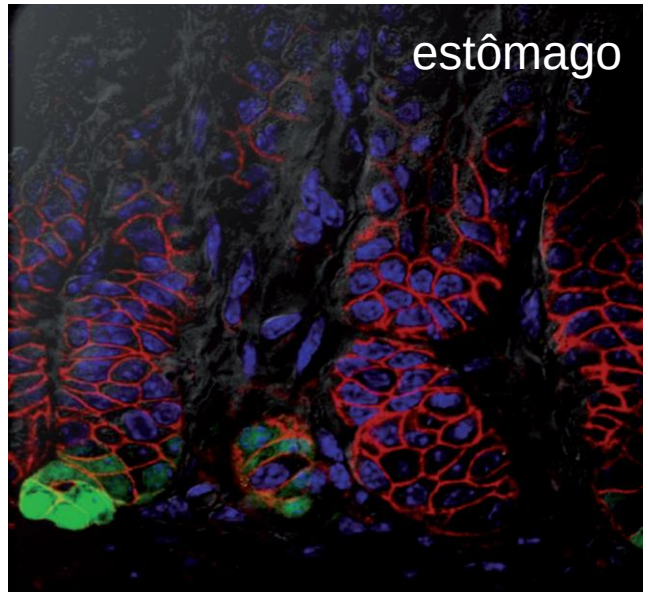
Fuchs & Horsley, 2011. Ferreting out stem cells from their niches. Nat Cell Biol, 13, 513

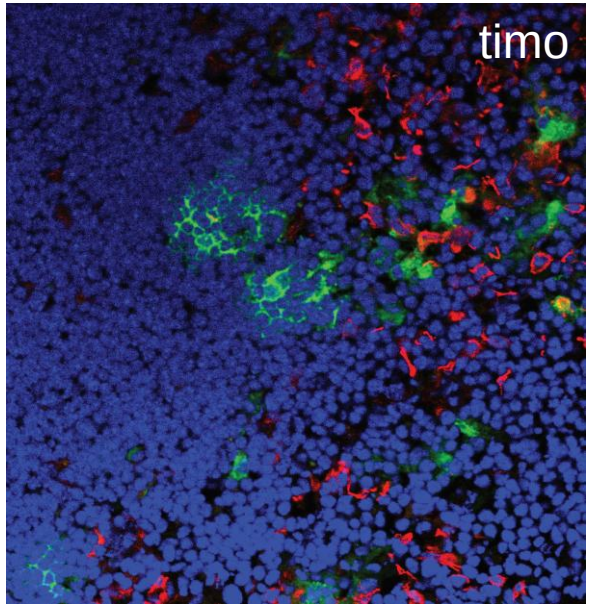
Como podemos rastrear o destino de uma célula no organismo?




rim


fígado


estômago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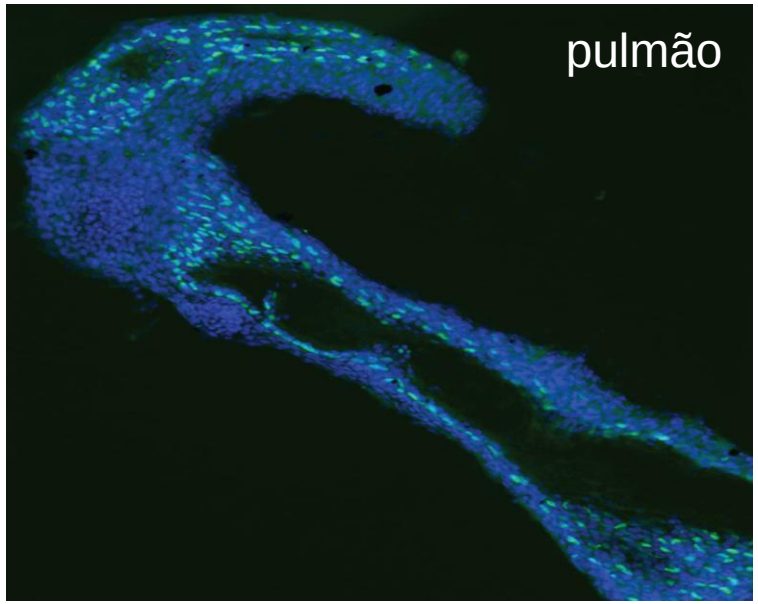
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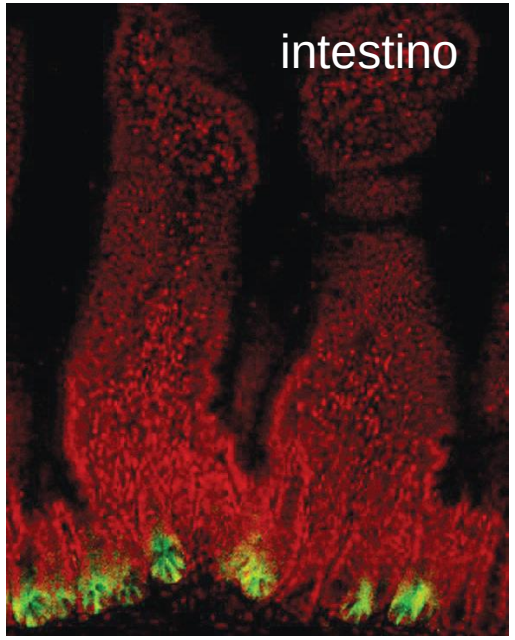
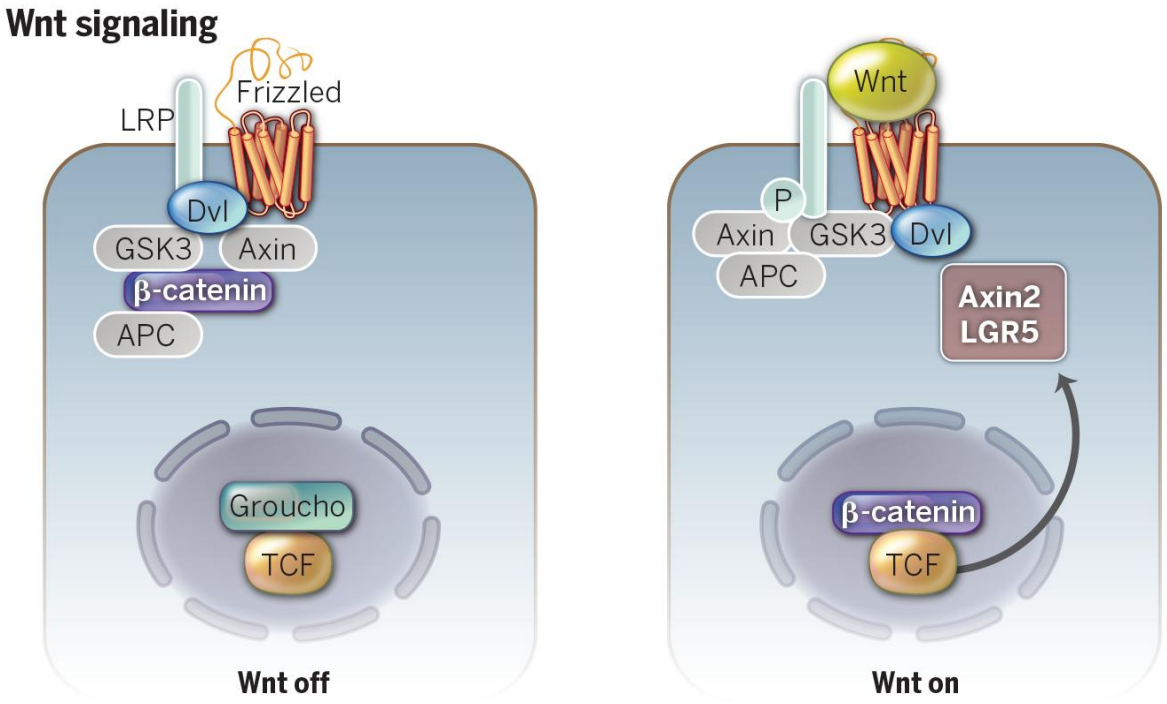
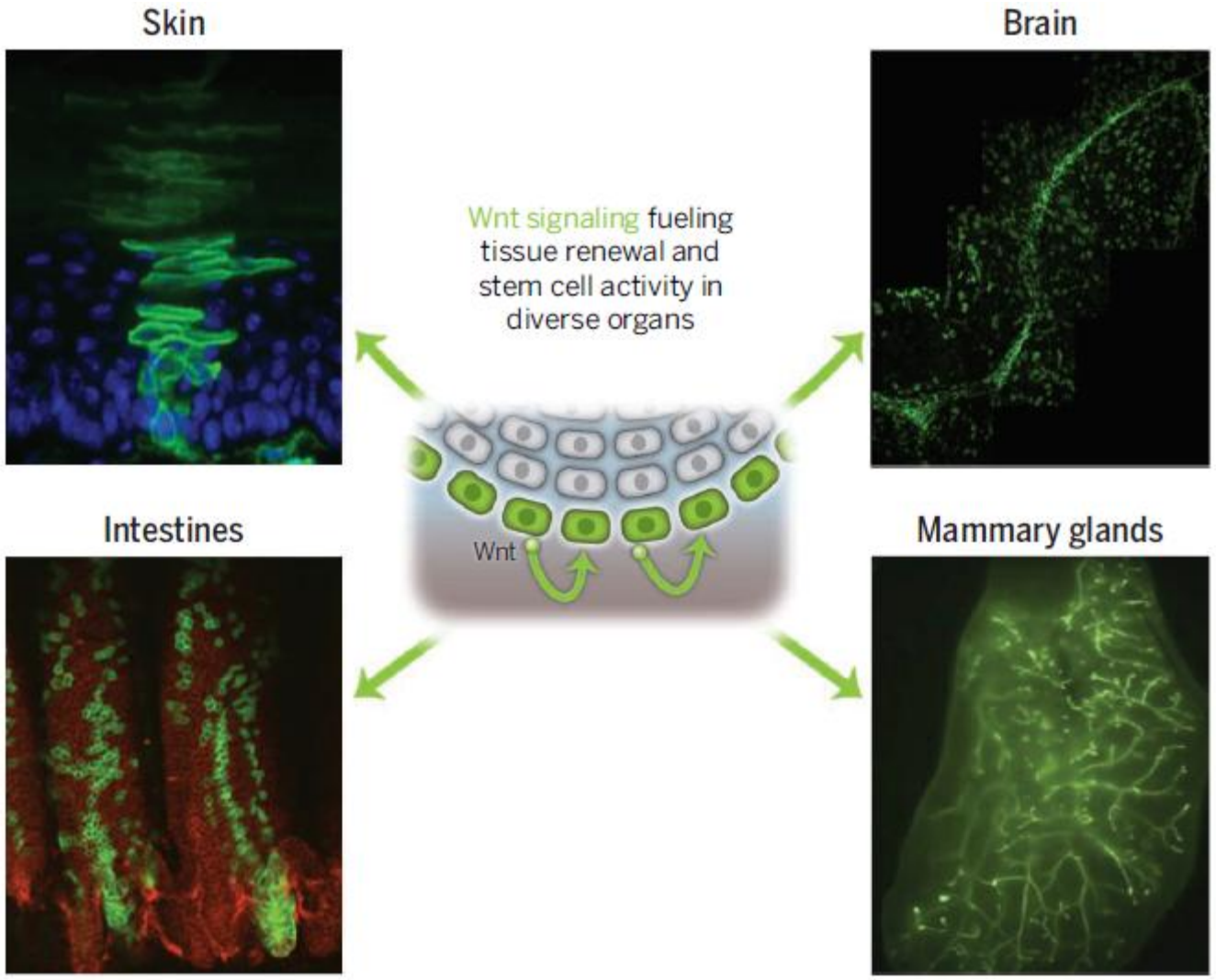

intestino

Quais são os sinais do nicho que controlam a auto-renovação e diferenciação das células-tronco?



Os alvos de β-catenina variam em função do órgão, porém alguns alvos são comuns em todas as células:

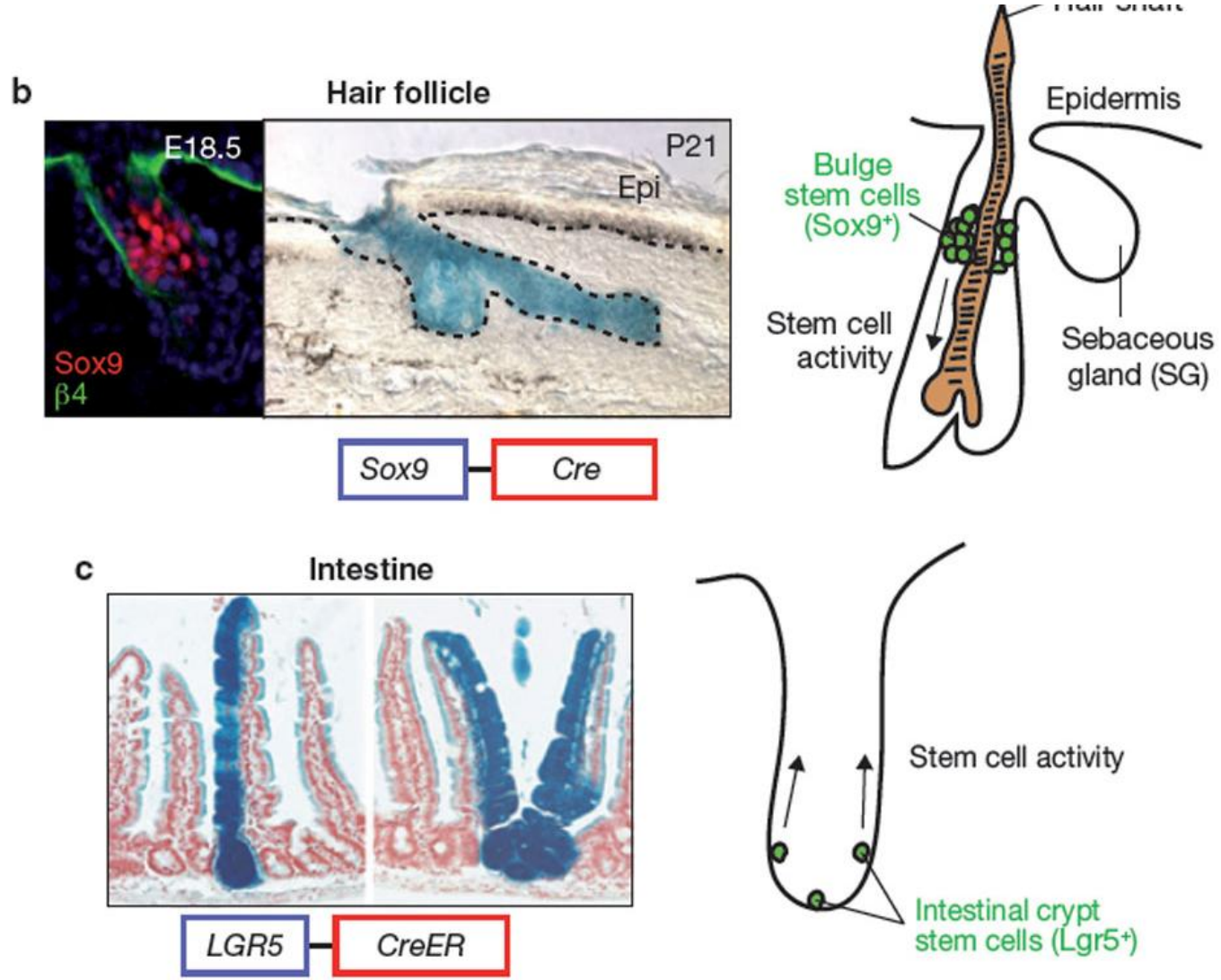
- **telomerase**
- **Axina 2**
- **LGR5**



H. Clevers et al., 2014 Science 346, 1248012

Table 1. Wnt-responsive tissue stem cells identified by means of lineage tracing.

Tissue	Stem cell	Marked by	Reference
Intestine	Crypt base columnar cell	Lgr5	(*25*)
Mammary gland	Basal cell	Axin2, Lgr5	(*24*, *50*–*53*)
Stomach	Basal pyloric cell	Lgr5	(*85*)
Interfollicular epidermis	Basal cell	Axin2	(*44*, *45*)
Central nervous system	Radial glial cell	Axin2	(*98*)
Hair follicle	Outer bulge cell	Lgr5	(*99*)
Kidney	Nephron segment-specific stem cell	Lgr5, Axin2	(*100*, *101*)
Cochlea	Tympanic border	Axin2	(*102*)
Ovary	Hilum ovarian surface epithelial cell	Lgr5	(*103*)
Taste bud	Circumvallate papilla stem cell in posterior tongue	Lgr5	(*104*, *105*)



Fuchs & Horsley, 2011. Ferreting out stem cells from their niches. Nat Cell Biol, 13, 513

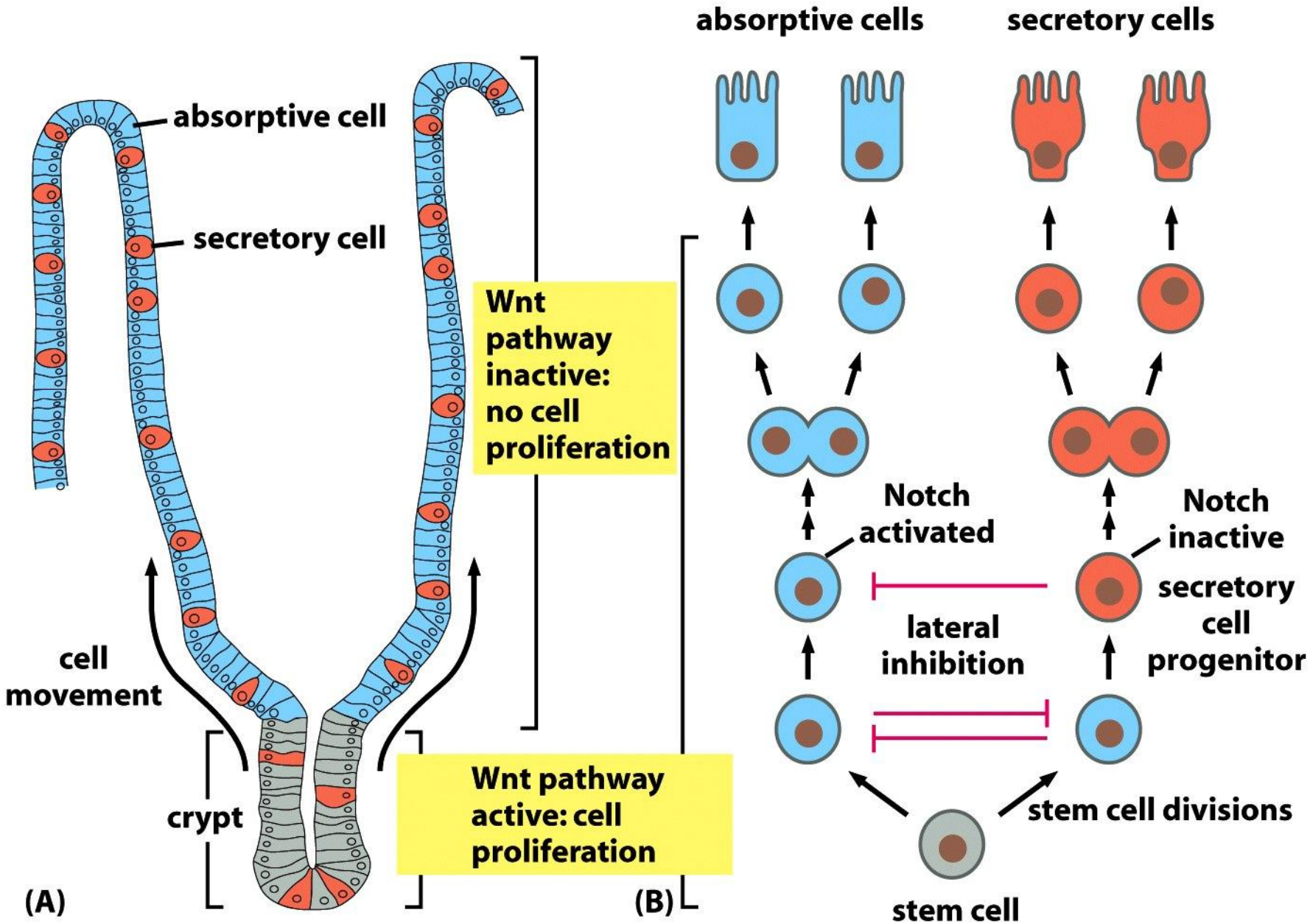


Figure 23-24 Molecular Biology of the Cell 5/e (© Garland Science 2008)

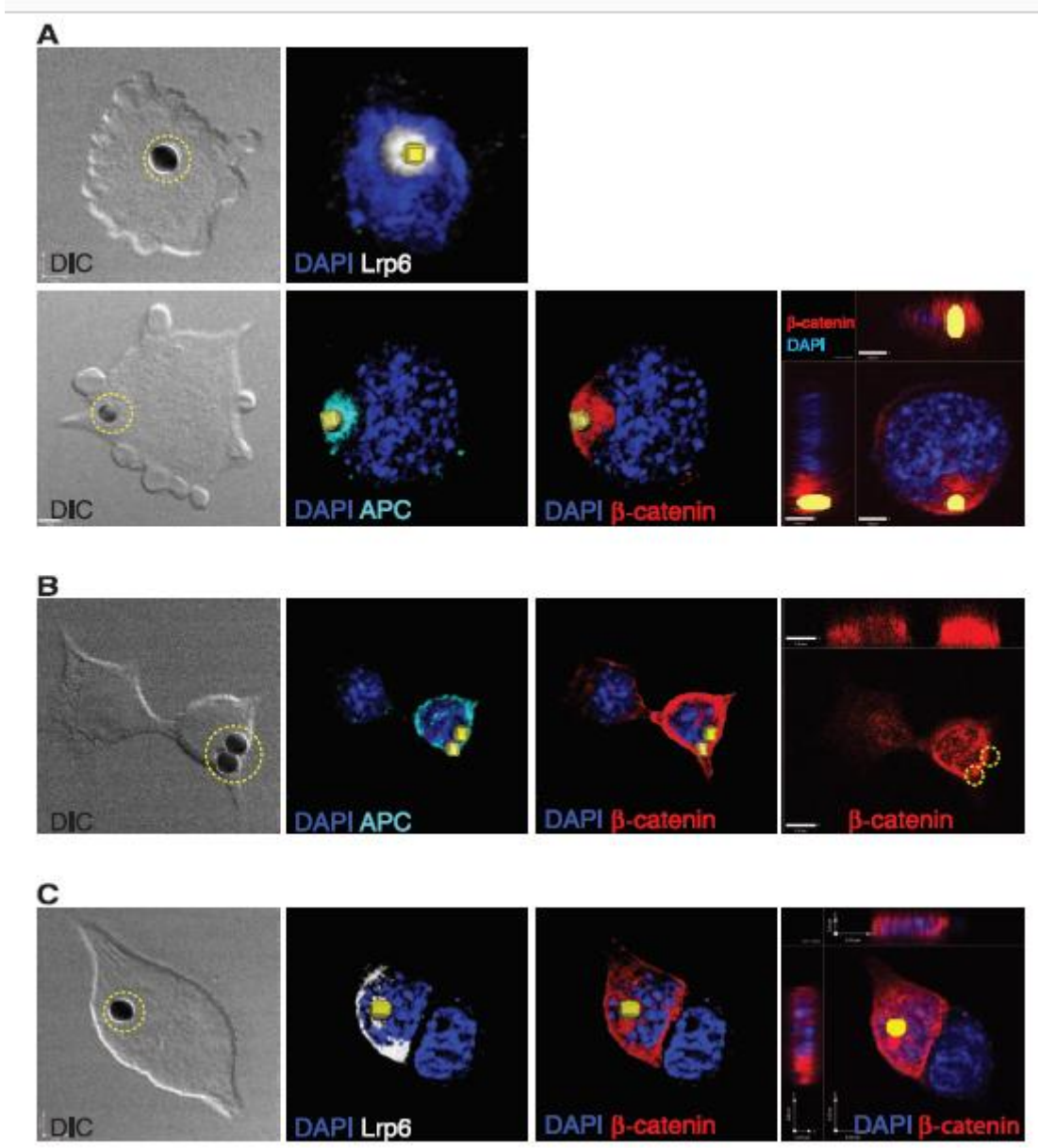
A Localized Wnt Signal Orients Asymmetric Stem Cell Division in Vitro

Shukry J. Habib,[1,2*] Bi-Chang Chen,[2] Feng-Chiao Tsai,[3] Konstantinos Anastassiadis,[4] Tobias Meyer,[3] Eric Betzig,[2] Roel Nusse[1*]

Developmental signals such as Wnts are often presented to cells in an oriented manner. To examine the consequences of local Wnt signaling, we immobilized Wnt proteins on beads and introduced them to embryonic stem cells in culture. At the single-cell level, the Wnt-bead induced asymmetric distribution of Wnt–β-catenin signaling components, oriented the plane of mitotic division, and directed asymmetric inheritance of centrosomes. Before cytokinesis was completed, the Wnt-proximal daughter cell expressed high levels of nuclear β-catenin and pluripotency genes, whereas the distal daughter cell acquired hallmarks of differentiation. We suggest that a spatially restricted Wnt signal induces an oriented cell division that generates distinct cell fates at predictable positions relative to the Wnt source.

S. J. Habib et al., Science 339, 1445–1448 (2013)

Co-cultura de células-tronco embrionárias com beads recobertos com Wnt



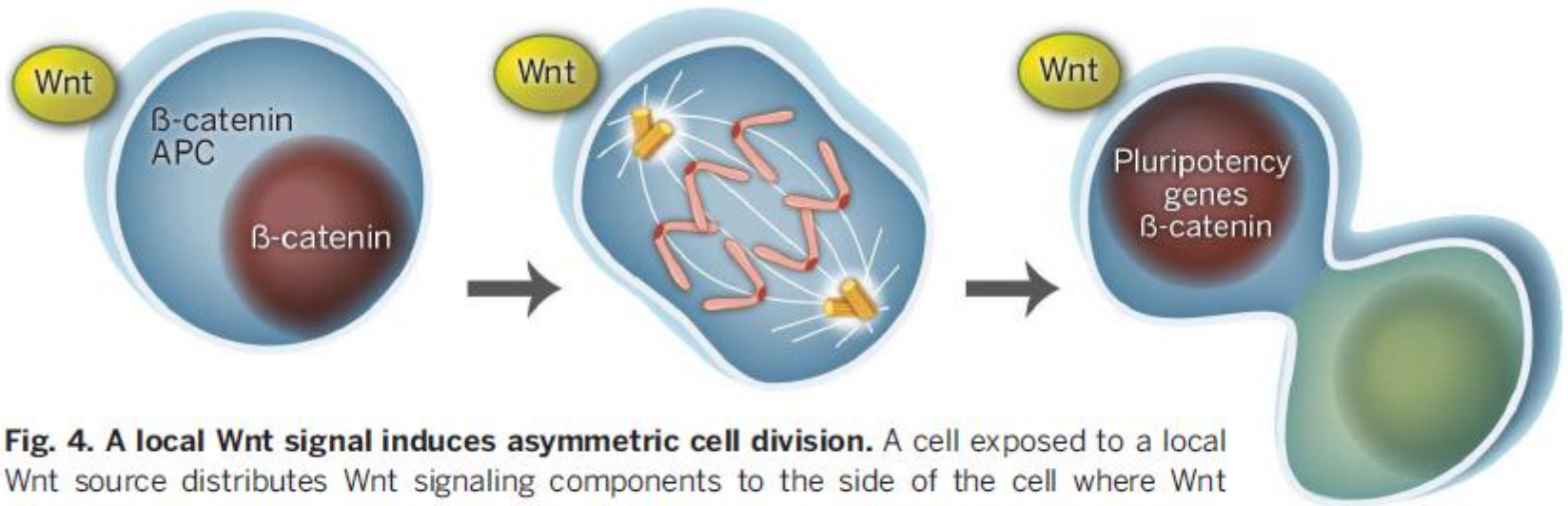
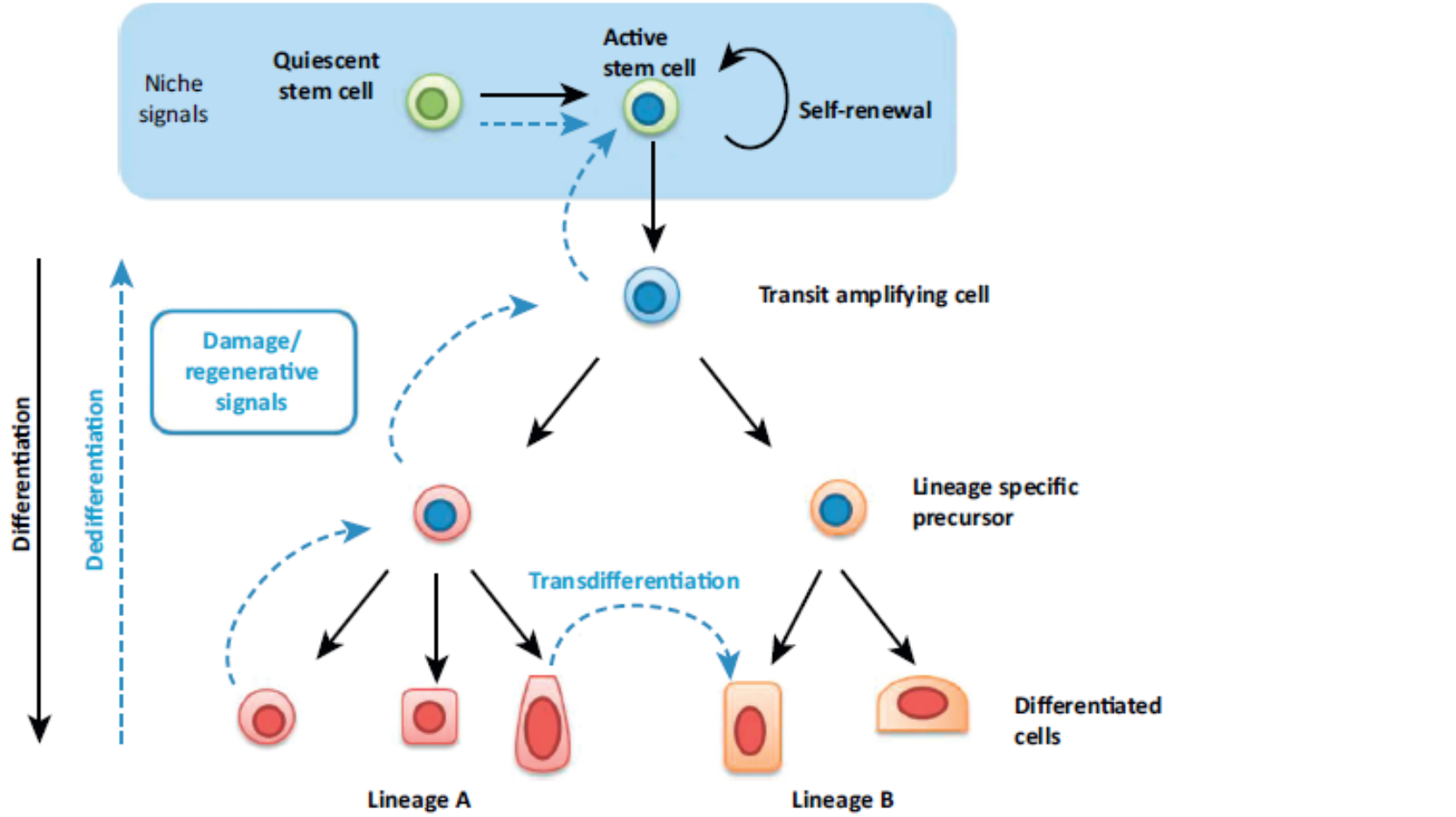


Fig. 4. A local Wnt signal induces asymmetric cell division. A cell exposed to a local Wnt source distributes Wnt signaling components to the side of the cell where Wnt touches. This orients the mitotic spindle and centrosomes during division. The daughter cell close to the Wnt source maintains nuclear β-catenin and stem cell gene expression, whereas the distal cell away from Wnt loses expression of such genes.

A segregação da “fita imortal” de DNA é regulada por Wnt?
Aparentemente NÃO

H. Clevers et al., 2014 Science 346, 1248012

Plasticidade das células tronco normais



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