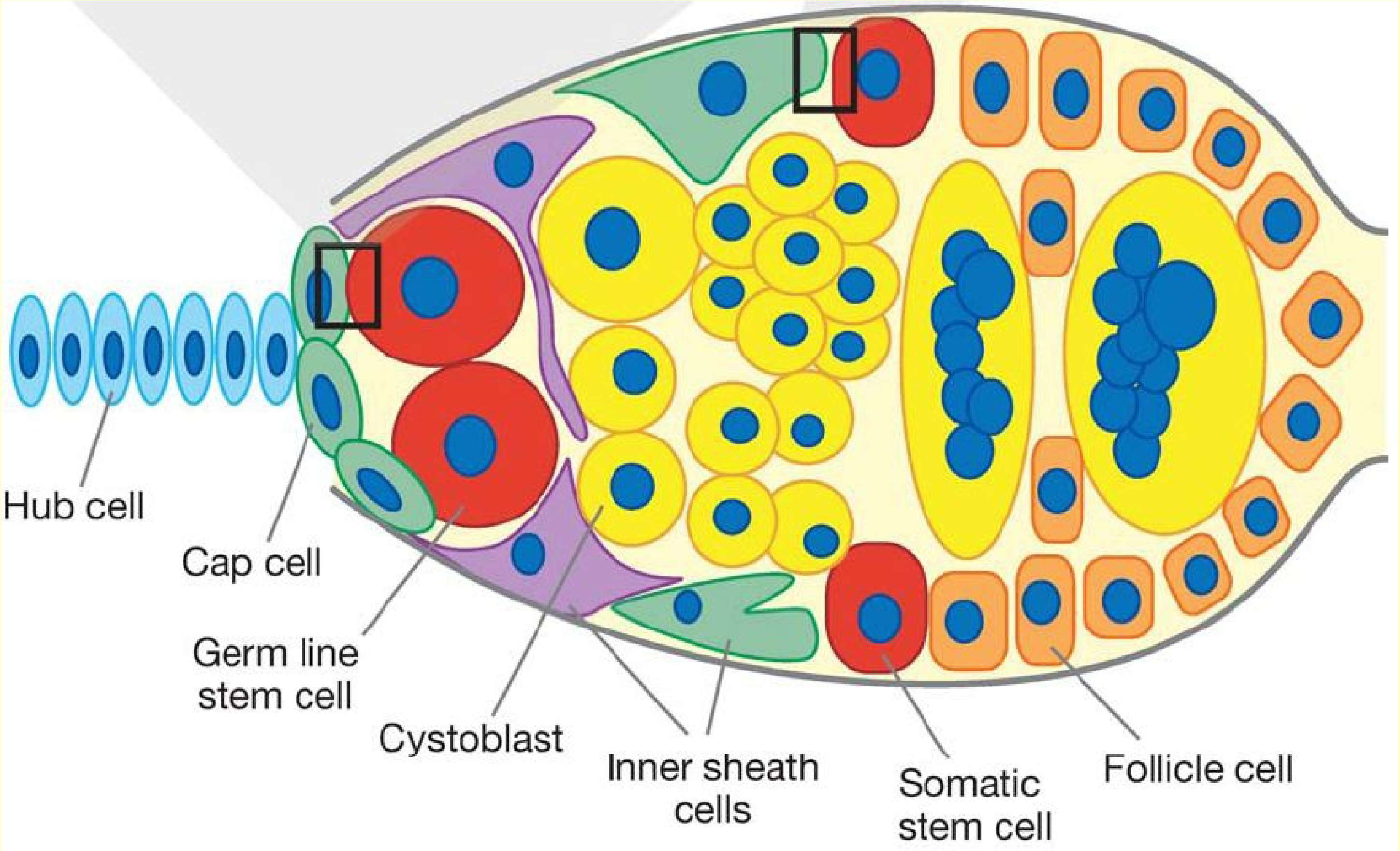
Genomics & Medicine

http://biochem118.stanford.edu/

Stem Cells

http://biochem118.stanford.edu/Stem Cell.html



Doug Brutlag, Professor Emeritus of
Biochemistry & Medicine (by courtesy)
Stanford University School of Medicine

Causal Mutation Homework Assignment

Most of the SNP variations associated with diseases in genome-wide association studies do not cause the disease, but instead, these SNPs serve as genetic markers that are linked to genes which are involved in the disease. Ongoing research is attempting to sequence these genes in patients and in controls to find the actual variations in these genes that do in fact, cause the disease.

For this assignment I would like you to choose a simple Mendelian inherited disease other than those mentioned in class (Huntingtons, diabetes, Parkinsons, cystic fibrosis, sickle cell, etc.) and describe what is known about the genetic variations that cause that disease.

You may search OMIM, dbSNP, dbVAR, HGMD, HGVS, ClinVar, SwissVar and other database of genome variations that are associated with specific diseases to find an example of the kinds of mutations associated with the disease. Please describe how each of these variations cause the disease.

Is it by:

1) mutating the coding region of the protein

2) altering the gene expression by affecting the promoter

3) altering gene expression by affecting a transcription factor binding site

4) altering gene expression indirectly by mutating a transcription factor itself

5) altering copy number, hence changing gene expression levels

6) altering other regulatory sites (miRNA targets)

7) altering splice signals

etc.

Often there will be several types of mutations that can cause the disease. Please comment on all types that are known for your chosen disease.

Henry Stewart Talks

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Home / Cell Biology / Stem Cells

Series: **Stem Cells**

Recent advances in understanding and utilizing

TOPICS COVERED

- Embryonic stem cells in perspective
- The advent of direct reprogramming
- Embryonic stem cells: derivation and properties
- Pluripotency and disease modeling: insights into reprogramming mechanisms
- Stem cells and regeneration: The physiological function of mesenchymal stem cells
- Niche regulation of stem cell function: stem cells and tissue homeostasis
- RNA regulation and stem cells: microRNA regulation of pluripotecy
- The aging of mitotic cells: regeneration and aging
- Stem cells and cancer: lineage tracing in normal stem cells and cancer
- Stem cells derived from amniotic fluid and placenta
- Cord blood stem cells
- Mesenchymal stem cells derived from bone marrow
- Hematopoietic stem cells
- Stem cells derived from peripheral blood
- Stem cells derived from fat
- Skeletal muscle stem cells
- Epithelial skin stem cells
- Stem cells and heart disease
- Islet cell therapy and pancreatic stem cells
- Cell therapy of liver disease: from hepatocytes to stem cells

HumBio 157
The Biology of Stem Cells

HUMBIO 157: The Biology of Stem Cells (DBIO 257)

The role of stem cells in human development and potential for treating disease. Guest lectures by biologists, ethicists, and legal scholars. Prerequisites: HumBio 2A and 3A, or the equivalent in the BioCore in Biological Sciences.

Terms: Spr | Units: 3 | UG Reqs: WAY-SMA | Grading: Letter or Credit/No Credit

Instructors: Fuller, M. (PI) ; Nusse, R. (PI)

Schedule for HUMBIO 157

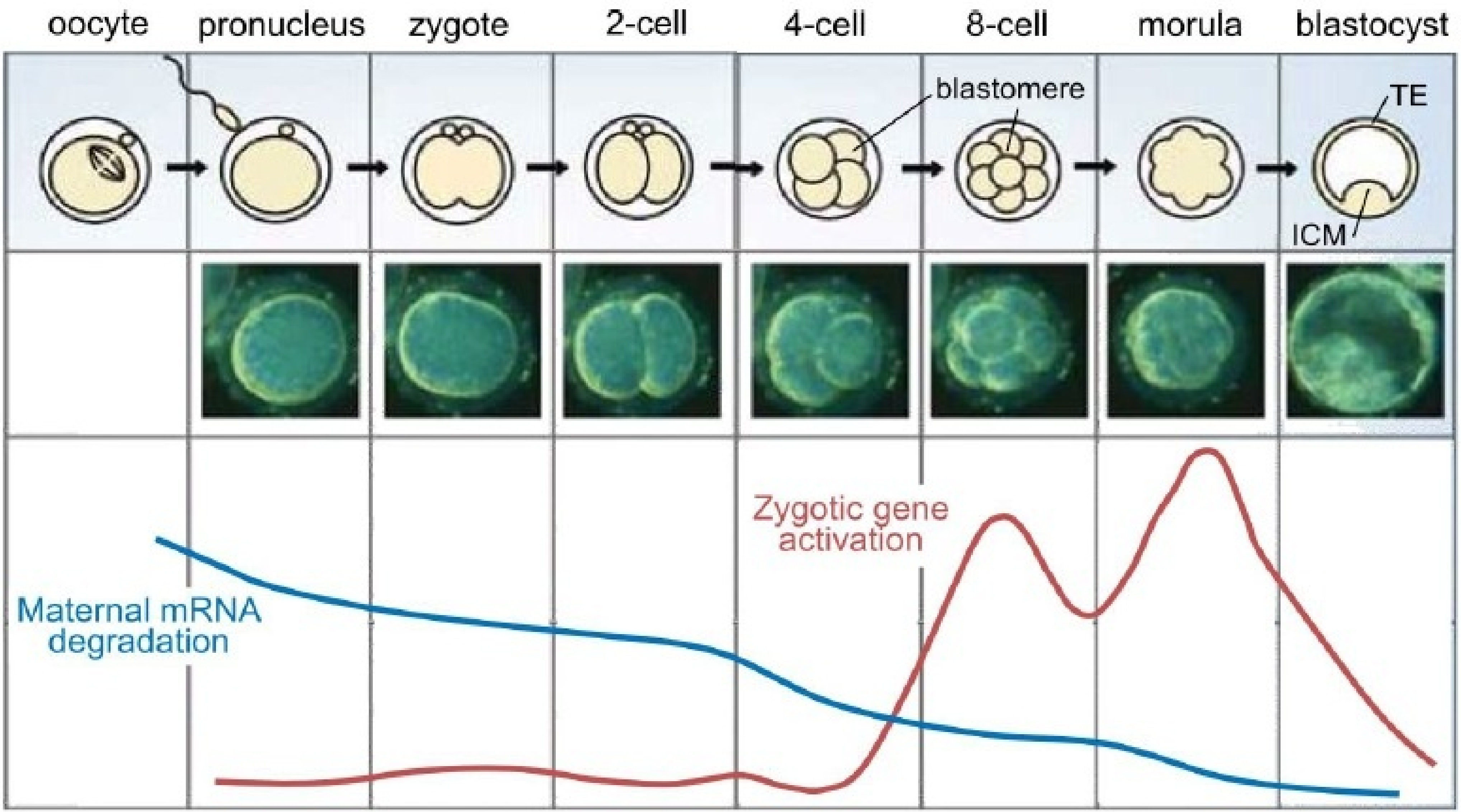
2014-2015 Spring

HUMBIO 157 | 3 units | UG Reqs: WAY-SMA | Class # 20511 | Section 01 | Grading: Letter or Credit/No Credit | LEC

03/30/2015 - 06/03/2015 Tue, Thu 2:15 PM - 3:45 PM with Fuller, M. (PI); Nusse, R. (PI)

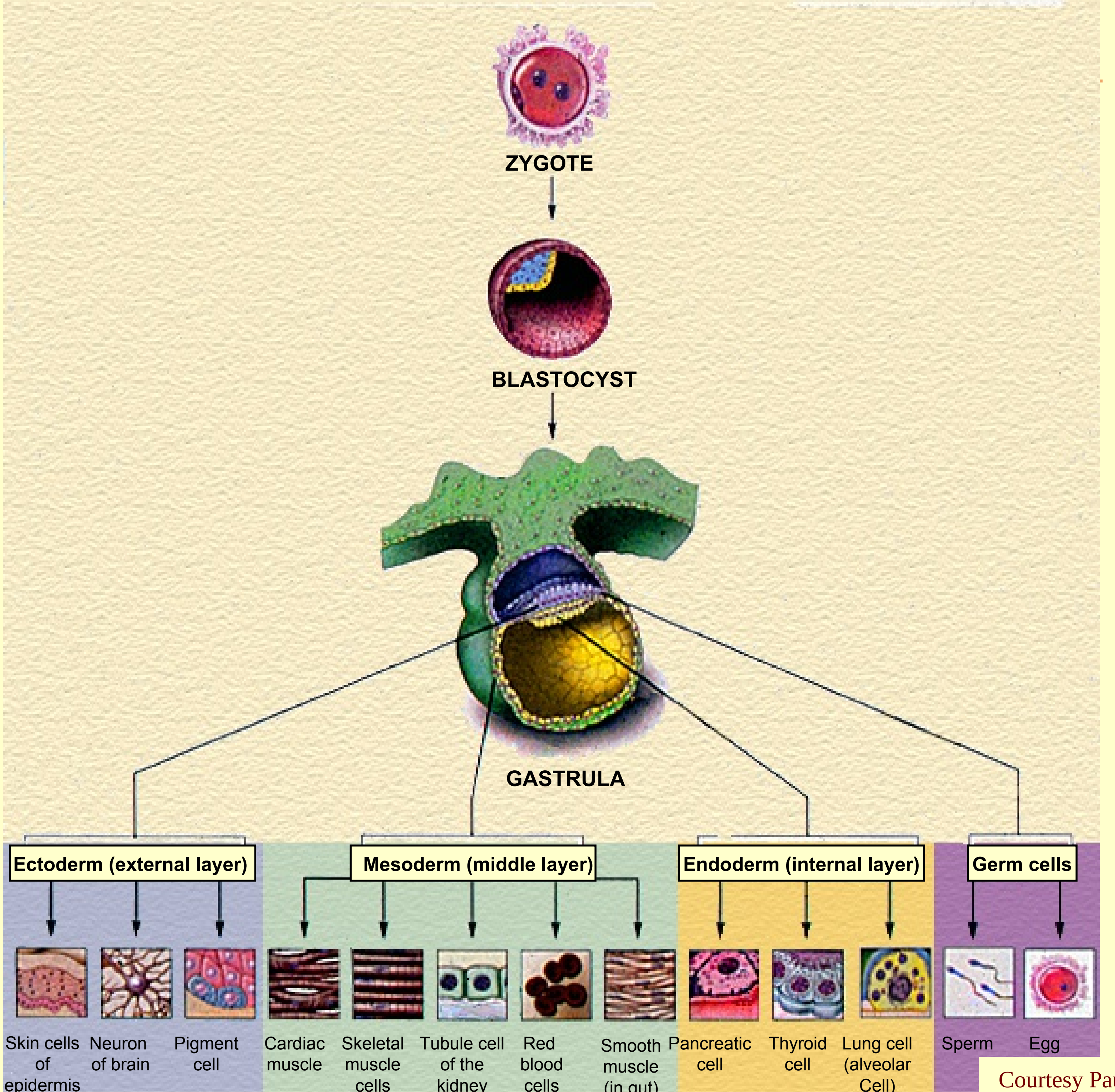
Instructors: Fuller, M. (PI); Nusse, R. (PI)

Early Embryo Development



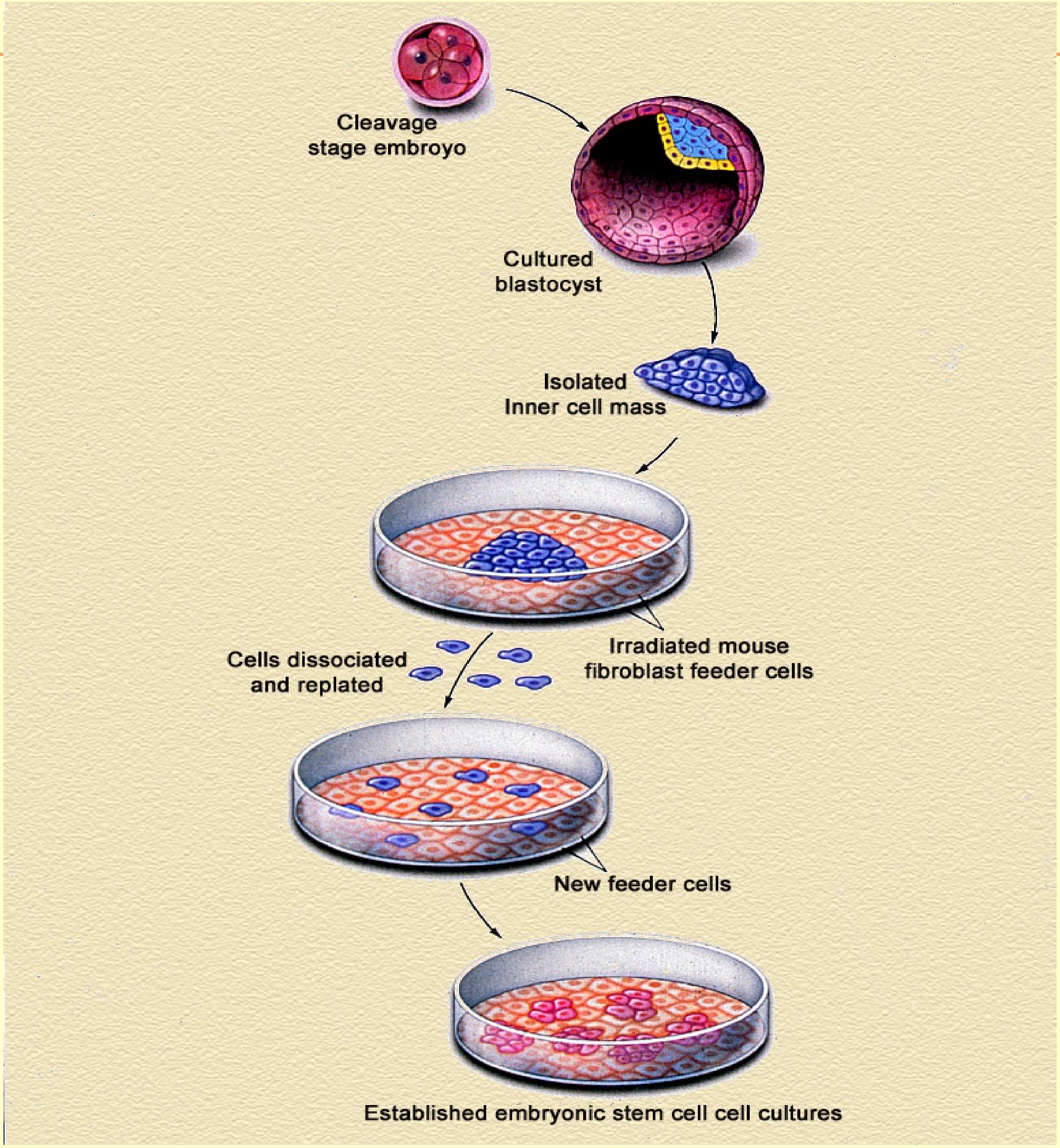
Wong et al. 2010 Nat. Biotech. 28; 115-1121

Differentiation of Human Tissues



Courtesy Paul Berg

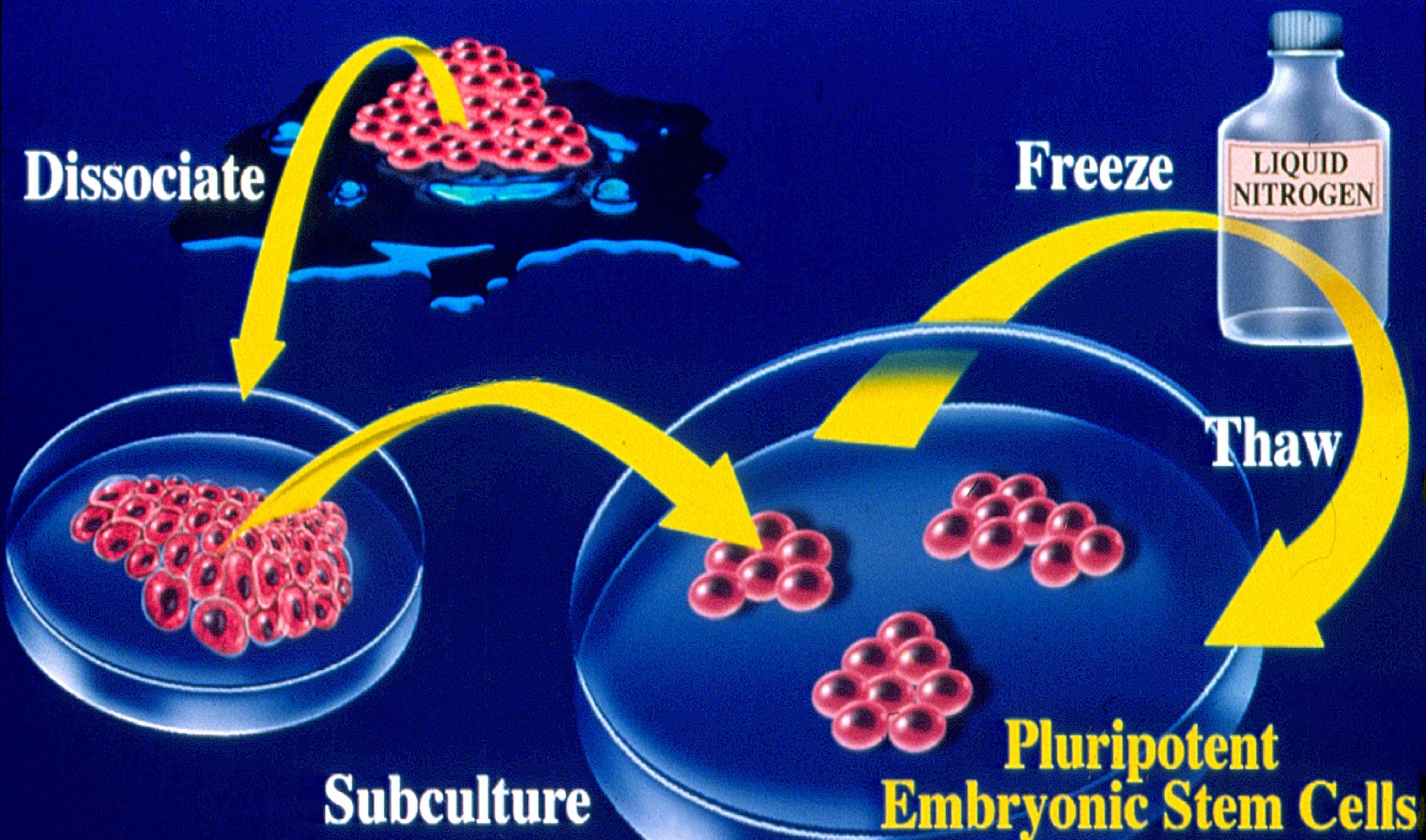
Embryonic Stem Cell Cultures



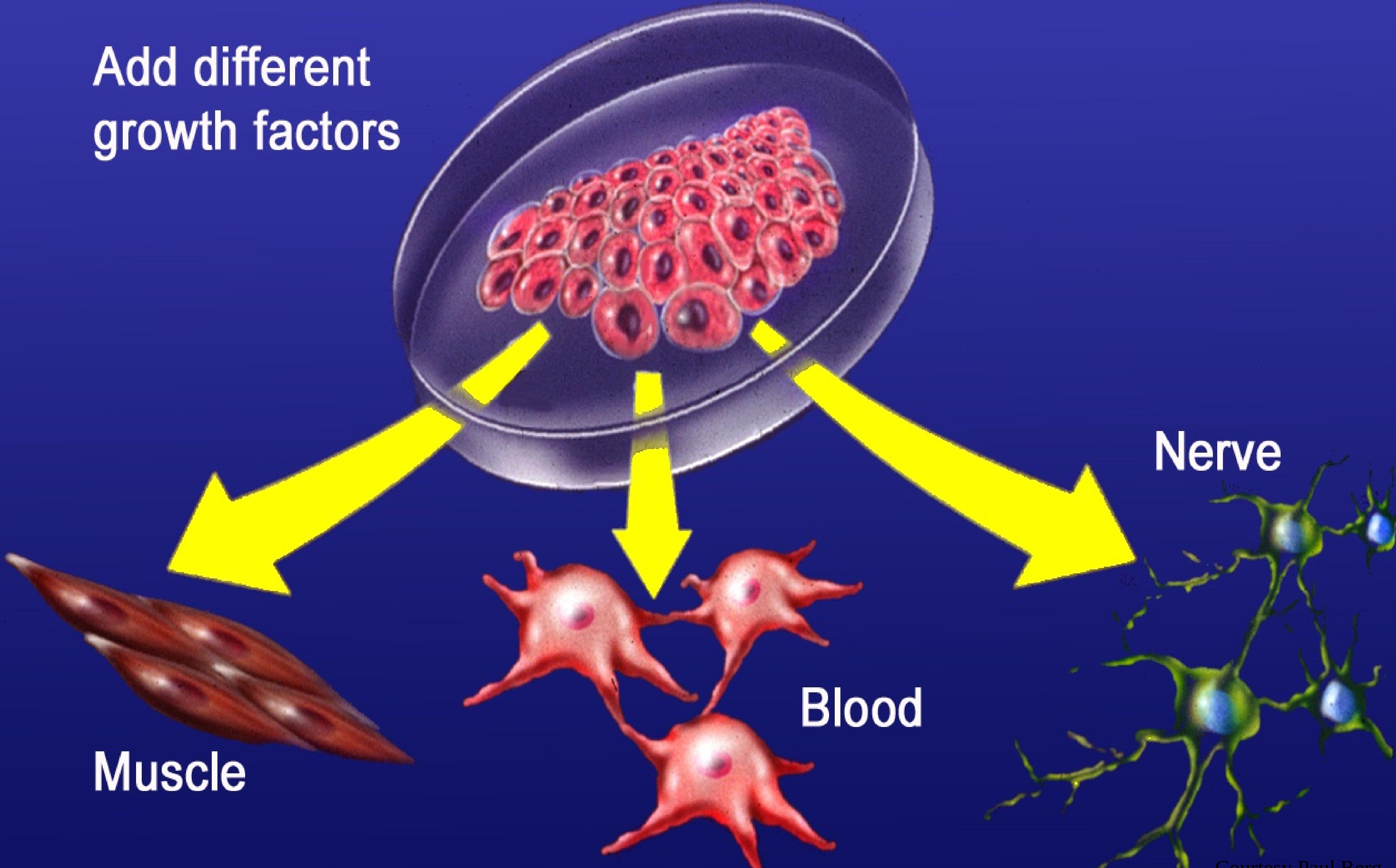
James A. Thomson, Science 282, 1145 (1998)

Courtesy Paul Berg

Inner Cell Mass Cells Continue to Proliferate Indefinitely in Culture



Pluripotent Stem Cells Differentiate into many Cell Types



Courtesy Paul Berg

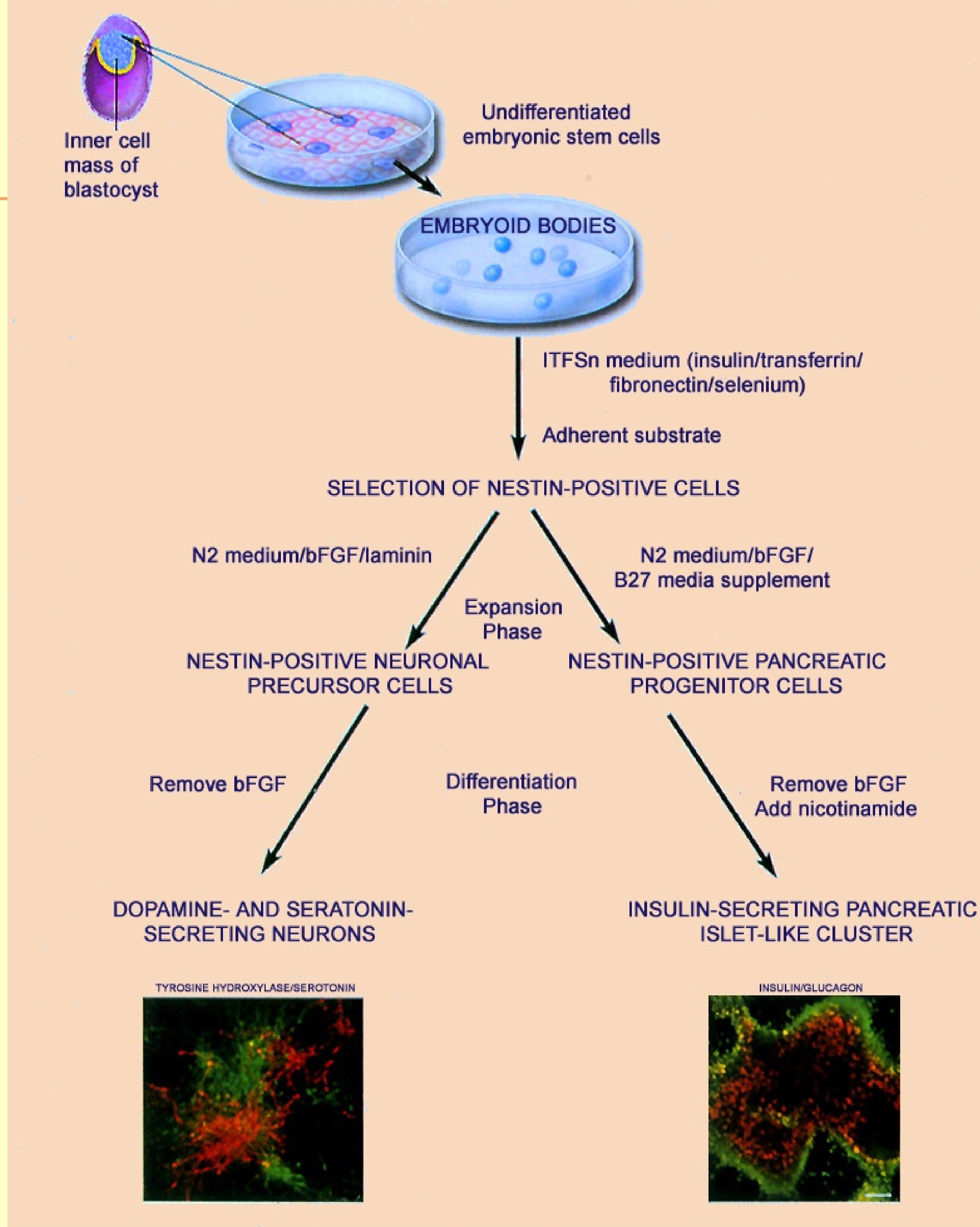
Basic Problems of Stem Cell Therapy

- HOW TO DIRECT DIFFERENTIATION OF CELLS DOWN SPECIFIC PATHWAYS?
  e.g. all into muscle or all into nerve; different "cocktails" of growth factors

- HOW TO OVERCOME IMMUNE REJECTION?
  e.g. alter histocompatibility genes; therapeutic cloning for "customized" lines

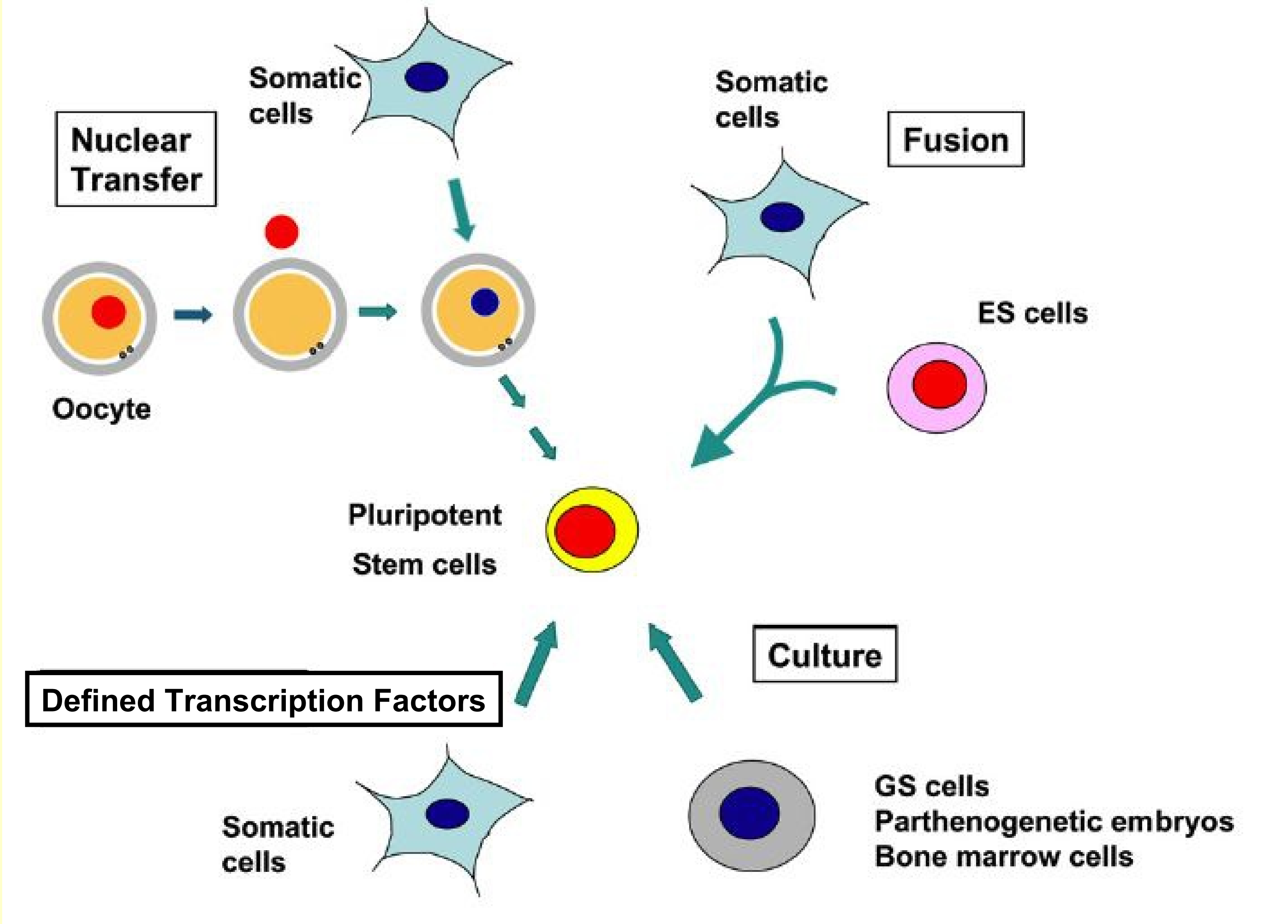
- HOW TO MAKE AN ORGAN?
  e.g. combine different cell types in three dimensional arrangements.

Courtesy Paul Berg



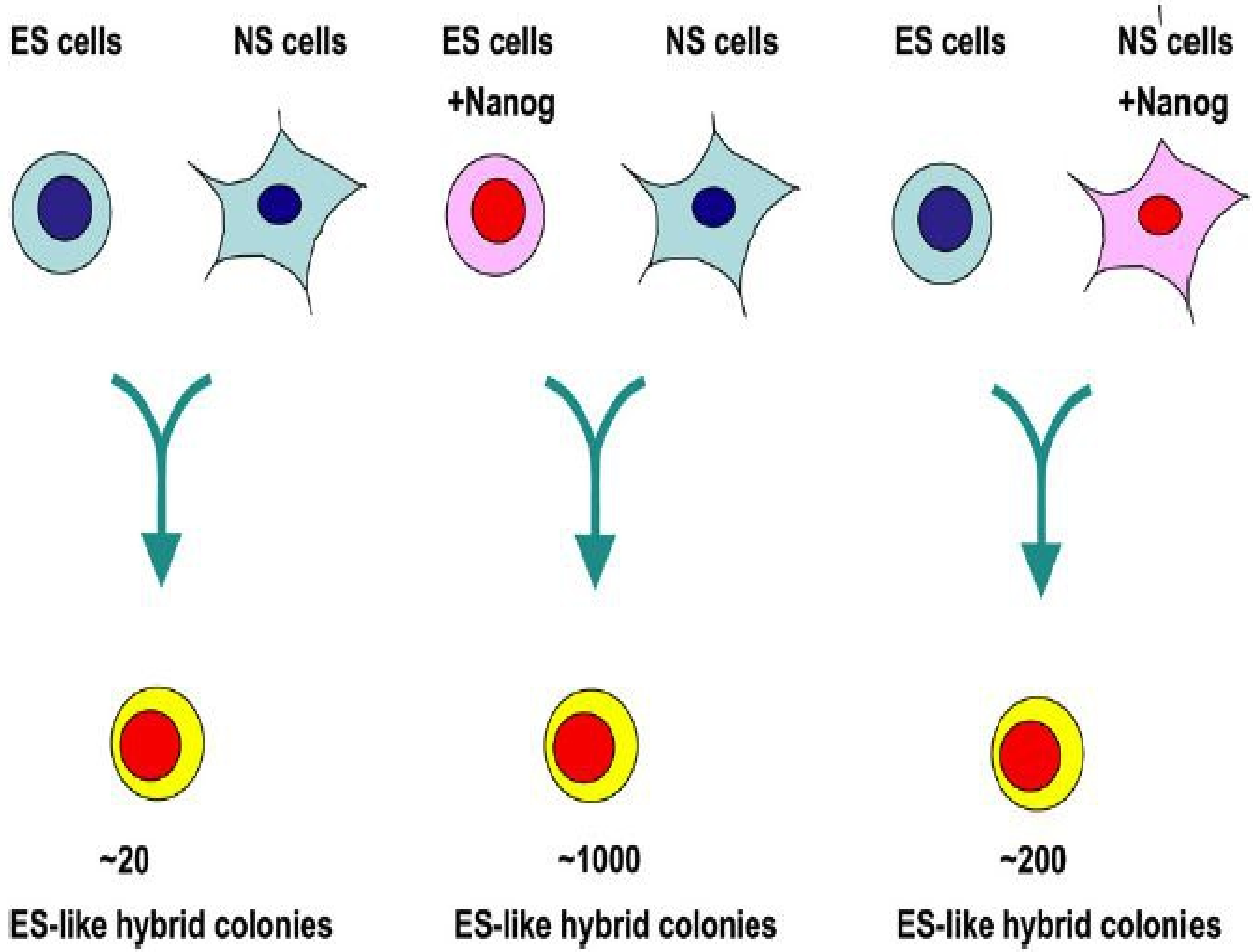
Courtesy Paul Berg

Methods to Generate Pluripotent Stem Cells



Yamanaka. (2007) Cell Stem Cell Vol 1, pp 39-49.

Nanog-Mediated Enhancement of Reprogramming by Fusion



Yamanaka. (2007) Cell Stem Cell Vol 1, pp 39-49.

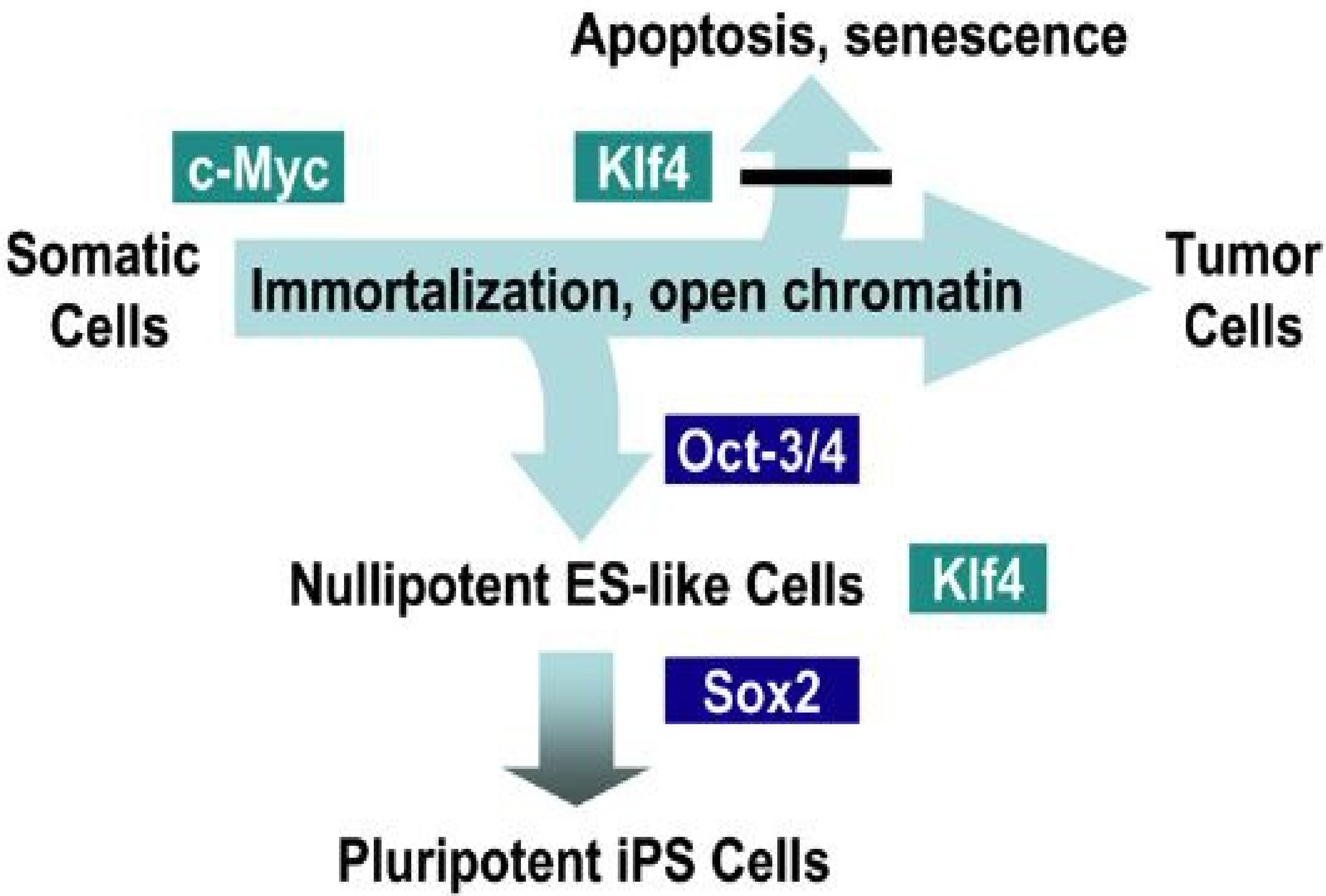
Five Transcription Factors Needed to Maintain Pluripotency

Table 1. Comparison of the Five Factors in the Phenotype of Loss-of-Function and Gain-of-Function Experiments

	Knockout ES Cells	Knockout Embryos	Overexpression in ES Cells
Oct-3/4	Cannot be established	No epiblast	Induces differentiation
	Niwa et al., 2000	Nichols et al., 1998	Niwa et al., 2000
Sox2	Cannot be established	No epiblast	Does not induce differentiation
	Masui et al., 2007	Avilion et al., 2003	Does not induce LIF independency
			M. Nakagawa and S.Y., unpublished data
c-Myc	Can be established	Normal epiblast	Does not induce differentiation
	Normal self-renewal		Induces LIF independency
	Davis et al., 1993	Davis et al., 1993	Cartwright et al., 2005
KLF4	Not reported	Normal epiblast	Does not induce differentiation
		Katz et al., 2002	Induces LIF independency
			Y. Tokuzawa, M. Nakagawa, and S.Y., unpublished data
Nanog	Can be established	No epiblast	Does not induce differentiation
	Spontaneous differentiation		Induces LIF independency
	Mitsui et al., 2003	Mitsui et al., 2003	Chambers et al., 2003; Mitsui et al., 2003

Yamanaka. (2007) Cell Stem Cell Vol 1, pp 39-49.

Induction of Pluripotent Stem Cells (iPS) from Somatic Stem Cells



Yamanaka. (2007) Cell Stem Cell Vol 1, pp 39-49.

Adipose Tissue Provides iPSC Efficiently

'Liposuction leftovers' easily converted to iPS cells, study shows

BY KRISTA CONGER

Globs of human fat removed during liposuction conceal versatile cells that are more quickly and easily coaxed to become induced pluripotent stem cells, or iPS cells, than are the skin cells most often used by researchers, according to a new study from Stanford's School of Medicine.

Steve Fisch Photography

Joseph Wu, Ning Sun and Michael Longaker collaborated on research that showed stem cells found in fat tissue could easily be converted into iPS cells.

"We've identified a great natural resource," said Stanford surgery professor and co-author of the research, Michael Longaker, MD, who has called the readily available liposuction leftovers "liquid gold." Reprogramming adult cells to function like embryonic stem cells is one way researchers hope to create patient-specific cell lines to regenerate tissue or to study specific diseases in the laboratory.

Sun et al, Proc Natl Acad Sci U S A. 2009 Sep 15;106(37):15720-5.

Using CRE – Recombinase to Remove Viral Transforming DNA from iPSCs

Parkinson's Disease Patient-Derived Induced Pluripotent Stem Cells Free of Viral Reprogramming Factors

Frank Soldner,[1,4] Dirk Hockemeyer,[1,4] Caroline Beard,[1] Qing Gao,[1] George W. Bell,[1] Elizabeth G. Cook,[1] Gunnar Hargus,[3] Alexandra Blak,[3] Oliver Cooper,[3] Maisam Mitalipova,[1] Ole Isacson,[3] and Rudolf Jaenisch[1,2,*]
[1]The Whitehead Institute, 9 Cambridge Center, Cambridge, MA 02142, USA
[2]Department of Biology, Massachusetts Institute of Technology, 31 Ames Street, Cambridge, MA 02139, USA
[3]Udall Parkinson Disease Research Center of Excellence, Center for Neuroredegeneration Research, McLean Hospital/Harvard Medical School, Belmont, MA 02478, USA
[4]These authors contributed equally to this work
*Correspondence: jaenisch@wi.mit.edu
DOI 10.1016/j.cell.2009.02.013

Soldner et al. Cell. 2009 Mar 6;136(5):964-77.

Cre-Lox Recombination to Remove Viral DNA

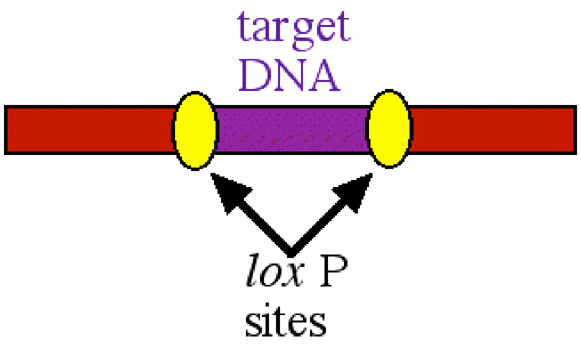


Figure 1. A pair of *lox* P sites (yellow ovals) flanking the target DNA (purple) to be deleted.

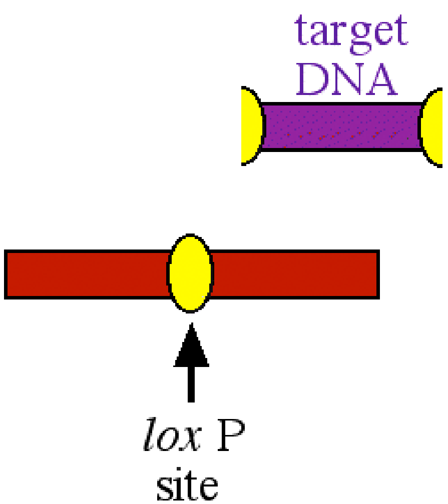


Figure 2. After the cre enzyme has excised the target DNA, one lox P site is left behind and the two flanking fragments of DNA are spliced together. The target DNA is excised and degraded.

Inducing iPSCs using Transcription Factor Proteins

Cell PRESS

Cell Stem Cell
Brief Report

Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins

Dohoon Kim,[1,5] Chun-Hyung Kim,[1,5] Jung-Il Moon,[1] Young-Gie Chung,[3] Mi-Yoon Chang,[1] Baek-Soo Han,[1] Sanghyeok Ko,[1] Eungi Yang,[1] Kwang Yul Cha,[4] Robert Lanza,[3,*] and Kwang-Soo Kim[1,2,4,*]
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[2]Harvard Stem Cell Institute
115 Mill Street, Belmont, MA 02478, USA
[3]Stem Cell and Regenerative Medicine International, 381 Plantation Street, Worcester, MA 01605, USA
[4]CHA Stem Cell Institute, CHA University, 606-16 Yoeksam 1-dong, Gangnam-gu, Korea
[5]These authors contributed equally to this work
*Correspondence: rlanza@advancedcell.com (R.L.), kskim@mclean.harvard.edu (K.-S.K.)
DOI 10.1016/j.stem.2009.05.005

Direct conversion of mouse fibroblasts to self-renewing, tripotent neural precursor cells

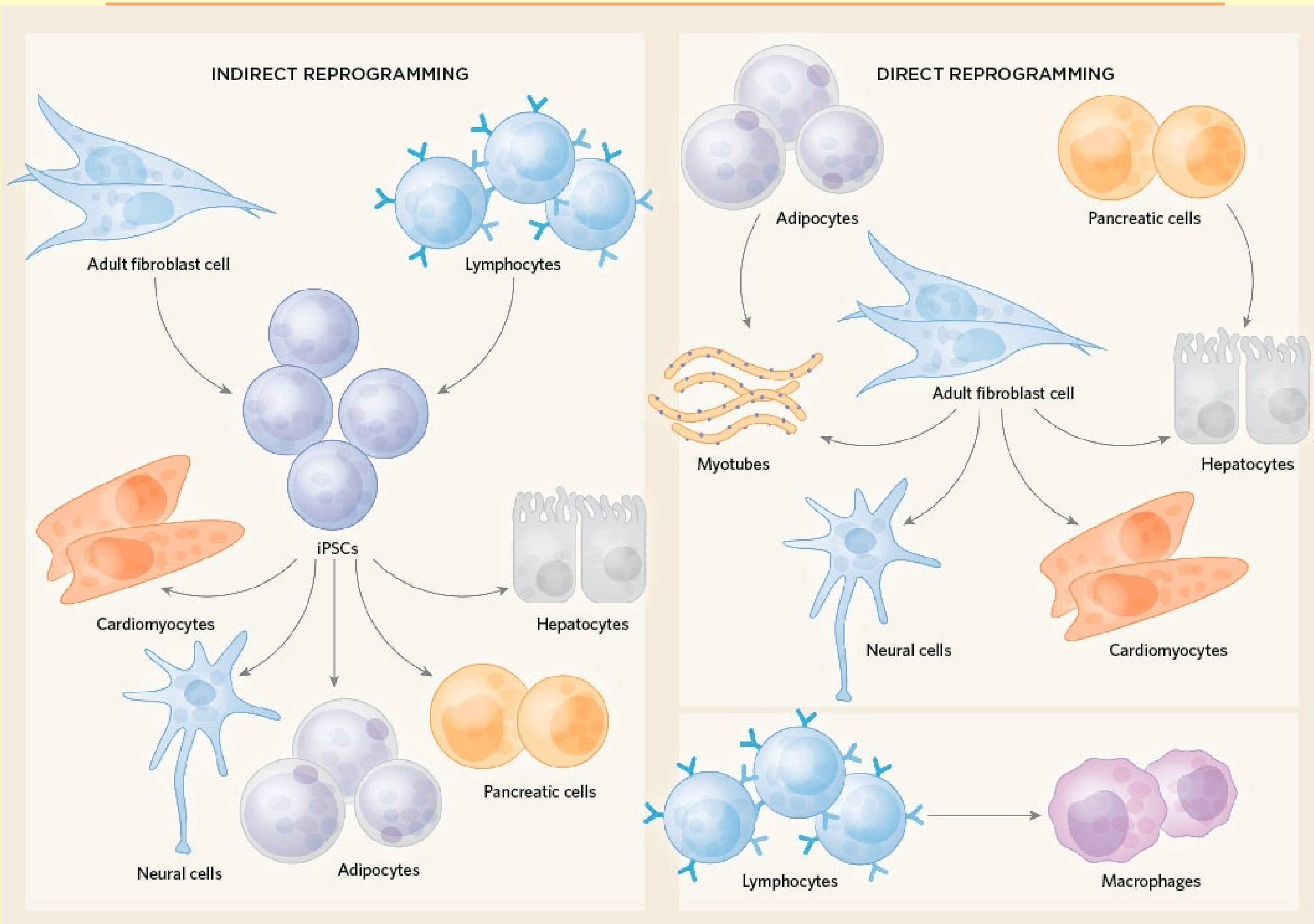
Ernesto Lujan[a,b], Soham Chanda[a,c], Henrik Ahlenius[a,d], Thomas C. Südhof[c,e,1], and Marius Wernig[a,d,1]

[a]Institute for Stem Cell Biology and Regenerative Medicine, Departments of [d]Pathology, [b]Genetics, and [c]Molecular and Cellular Physiology, and [e]Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305

We recently showed that defined sets of transcription factors are sufficient to convert mouse and human fibroblasts directly into cells resembling functional neurons, referred to as “induced neu- ronal” (iN) cells. For some applications however, it would be de- sirable to convert fibroblasts into proliferative neural precursor cells (NPCs) instead of neurons. We hypothesized that NPC-like cells may be induced using the same principal approach used for generating iN cells. Toward this goal, we infected mouse embry- onic fibroblasts derived from Sox2-EGFP mice with a set of 11 transcription factors highly expressed in NPCs. Twenty-four days after transgene induction, Sox2-EGFP+ colonies emerged that expressed NPC-specific genes and differentiated into neuronal and astrocytic cells. Using stepwise elimination, we found that Sox2 and FoxG1 are capable of generating clonal self-renewing, bipotent induced NPCs that gave rise to astrocytes and functional neurons. When we added the Pou and Homeobox domain-contain- ing transcription factor Brn2 to Sox2 and FoxG1, we were able to induce tripotent NPCs that could be differentiated not only into neurons and astrocytes but also into oligodendrocytes. The tran- scription factors FoxG1 and Brn2 alone also were capable of in- ducing NPC-like cells; however, these cells generated less mature neurons, although they did produce astrocytes and even oligoden- drocytes capable of integration into dysmyelinated Shiverer brain. Our data demonstrate that direct lineage reprogramming using target cell-type–specific transcription factors can be used to induce NPC-like cells that potentially could be used for autologous cell transplantation-based therapies in the brain or spinal cord.

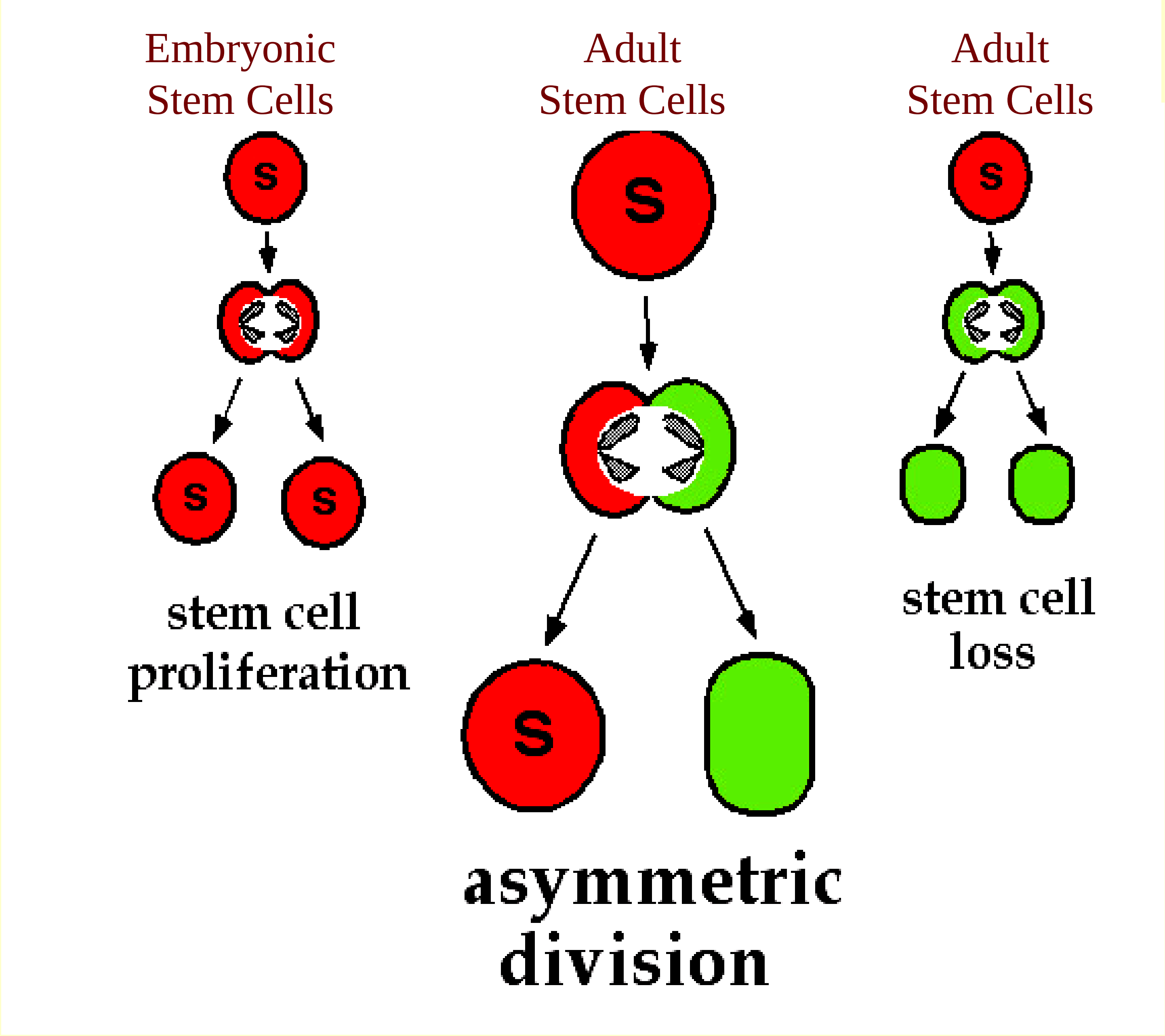
Direct Cell Reprogramming *in vivo* & *in vitro*

http://www.the-scientist.com/?articles.view/articleNo/39241/title/A-Twist-of-Fate/



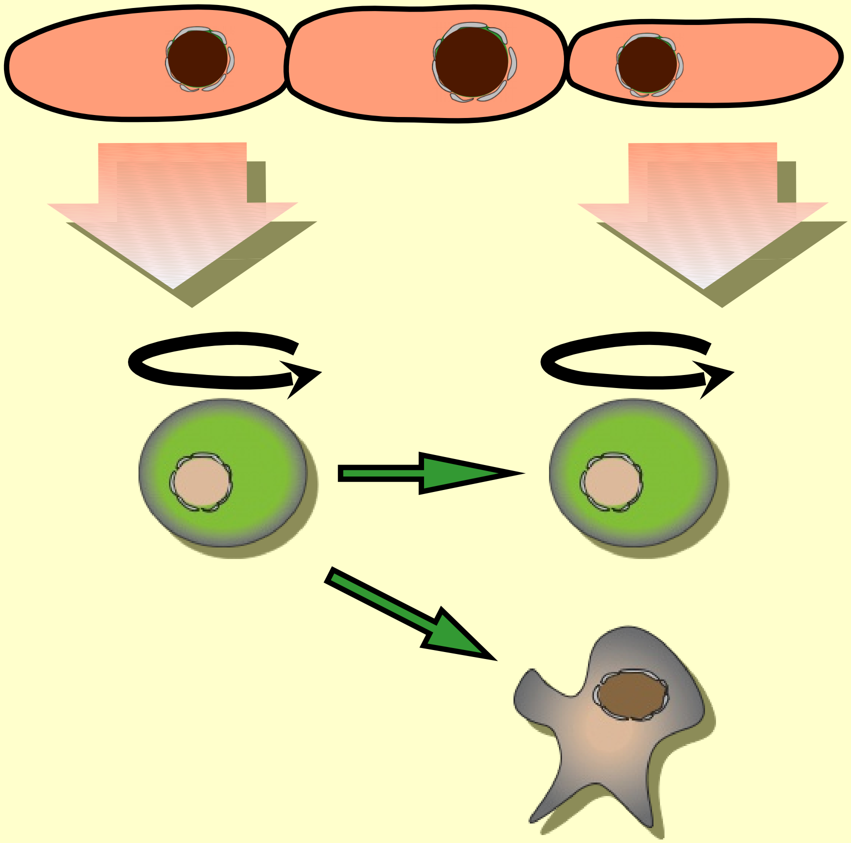
http://www.the-scientist.com/?articles.view/articleNo/39241/title/A-Twist-of-Fate/

Alternate Stem Cell Fates



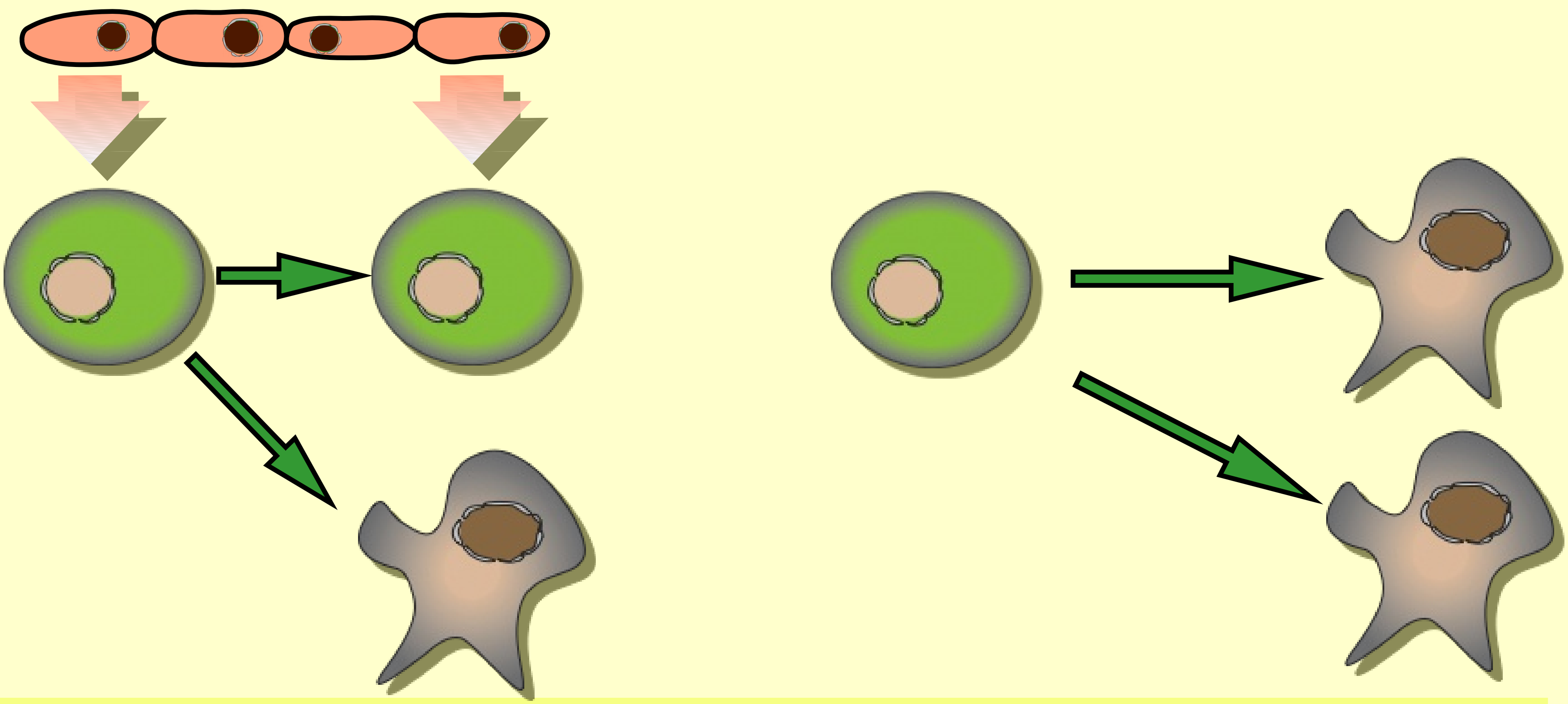
Courtesy of Minx Fuller

signals from niches maintain adult stem cells and tissues



Courtesy of Roel Nusse

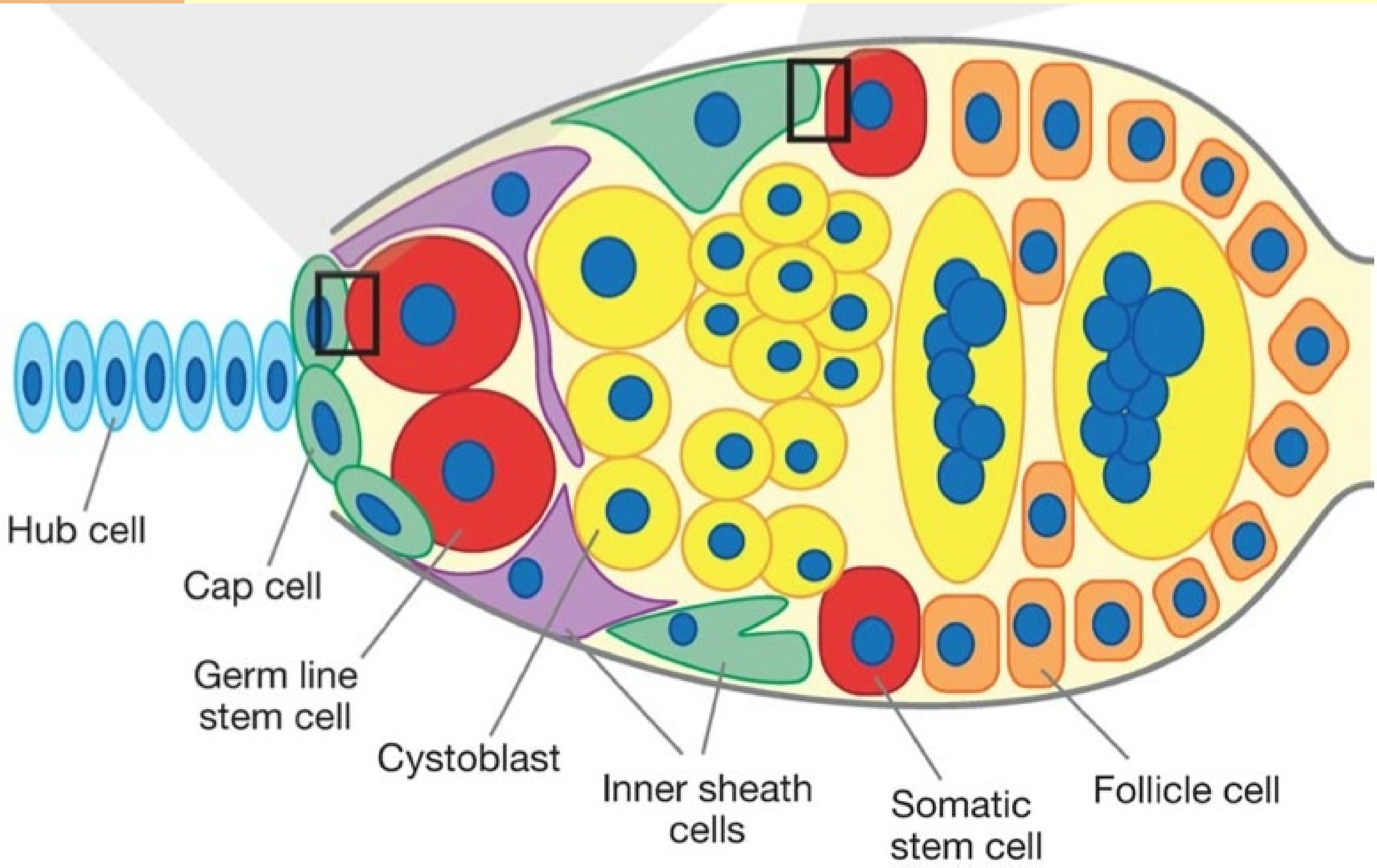
In the absence of niche signals, adult stem cells will differentiate, by default



1. Self-renewal is proliferation coupled to blocking differentiation, controlled by signals.
2.Signals are local; niches have a limited capacity and cells compete for the signals
3. The signals control tissue homeostasis, also after damage

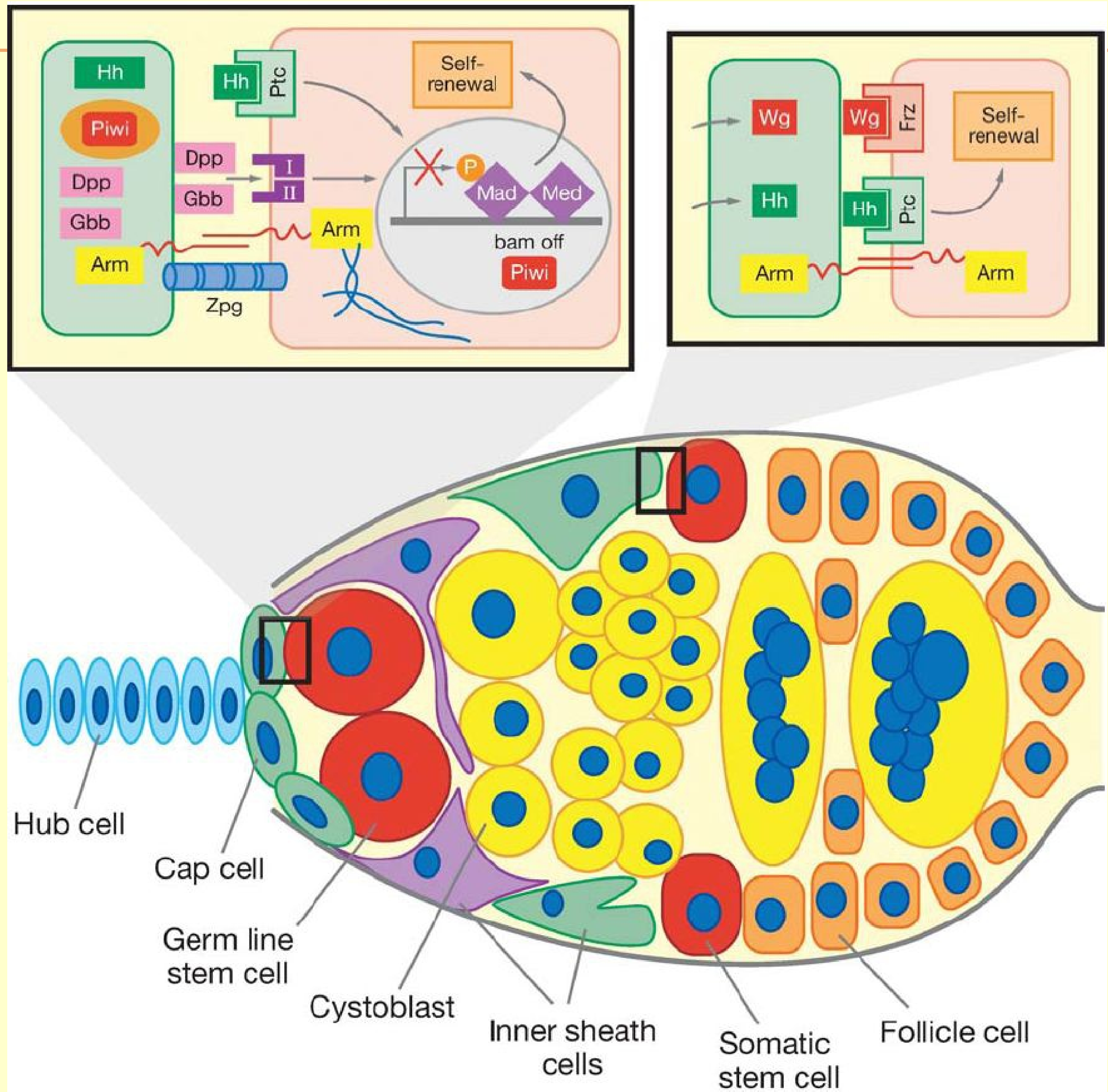
Courtesy of Roel Nusse

Drosophila Oocyte Stem Cells



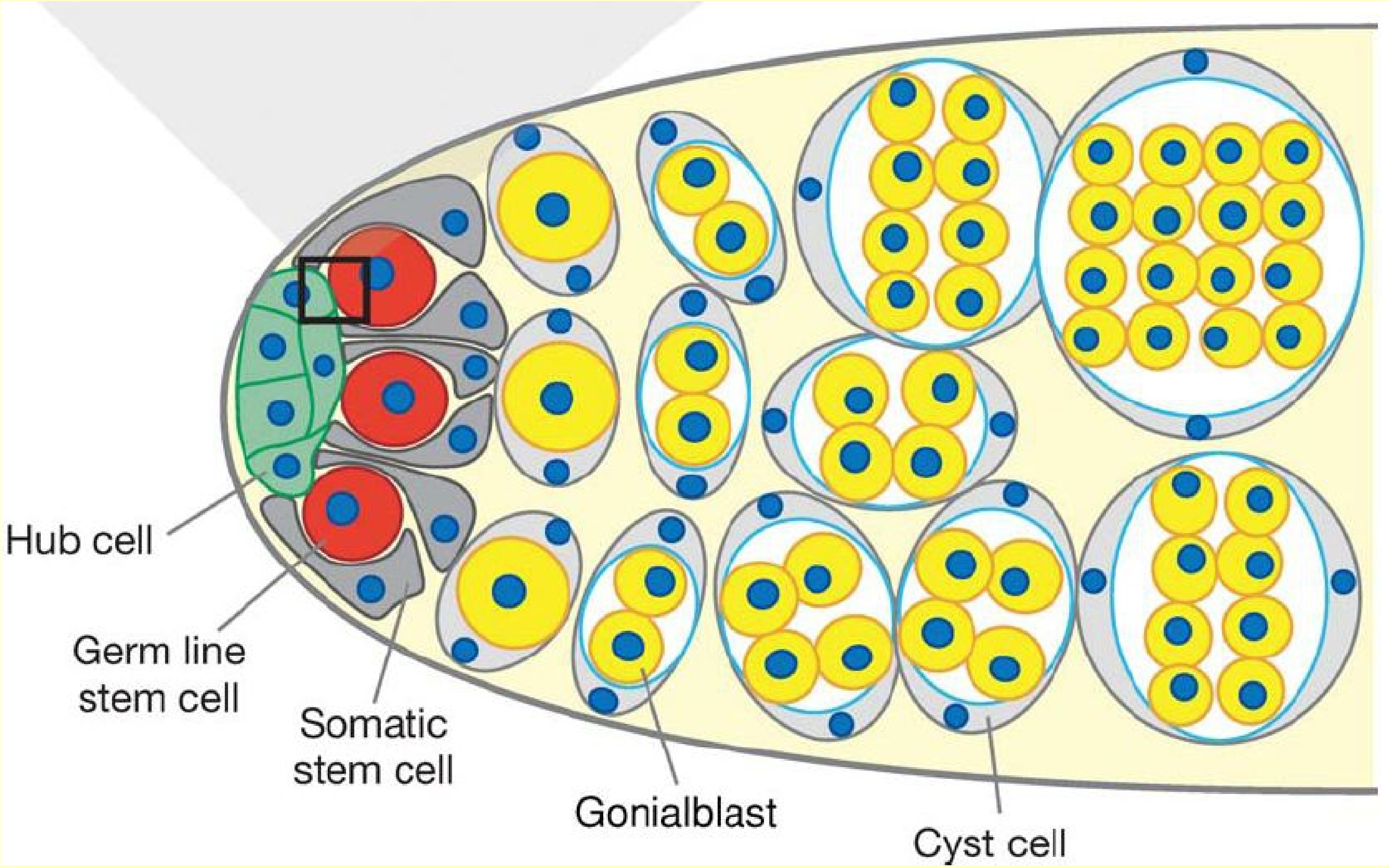
Li and Xie, Ann. Rev. Dev. Biol. 2005, 605-663

Cell-Cell Interactions at Oocyte Niche



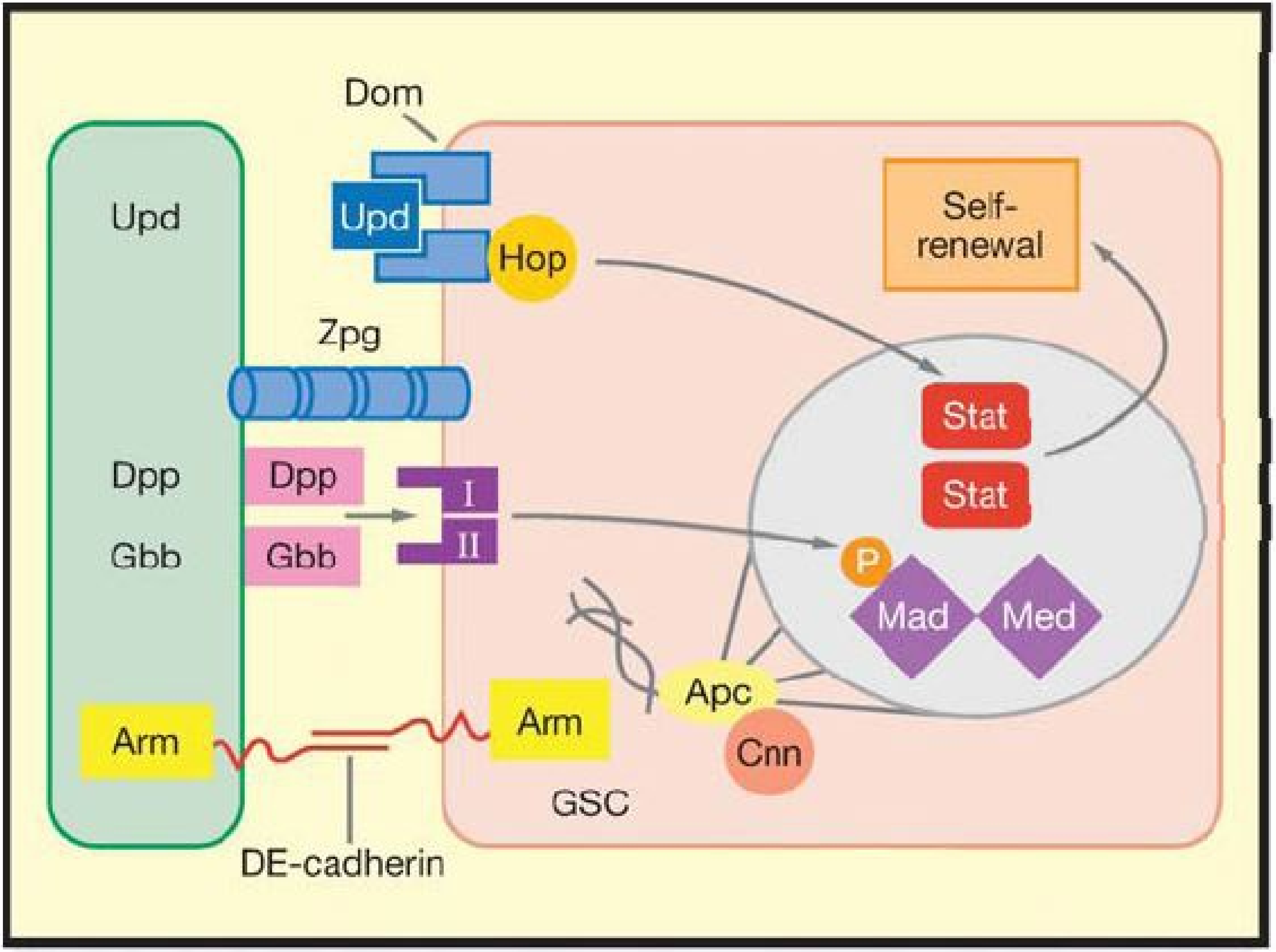
Li and Xie, Ann. Rev. Dev. Biol. 2005, 605-663

Drosophila Spermatogonial Niche



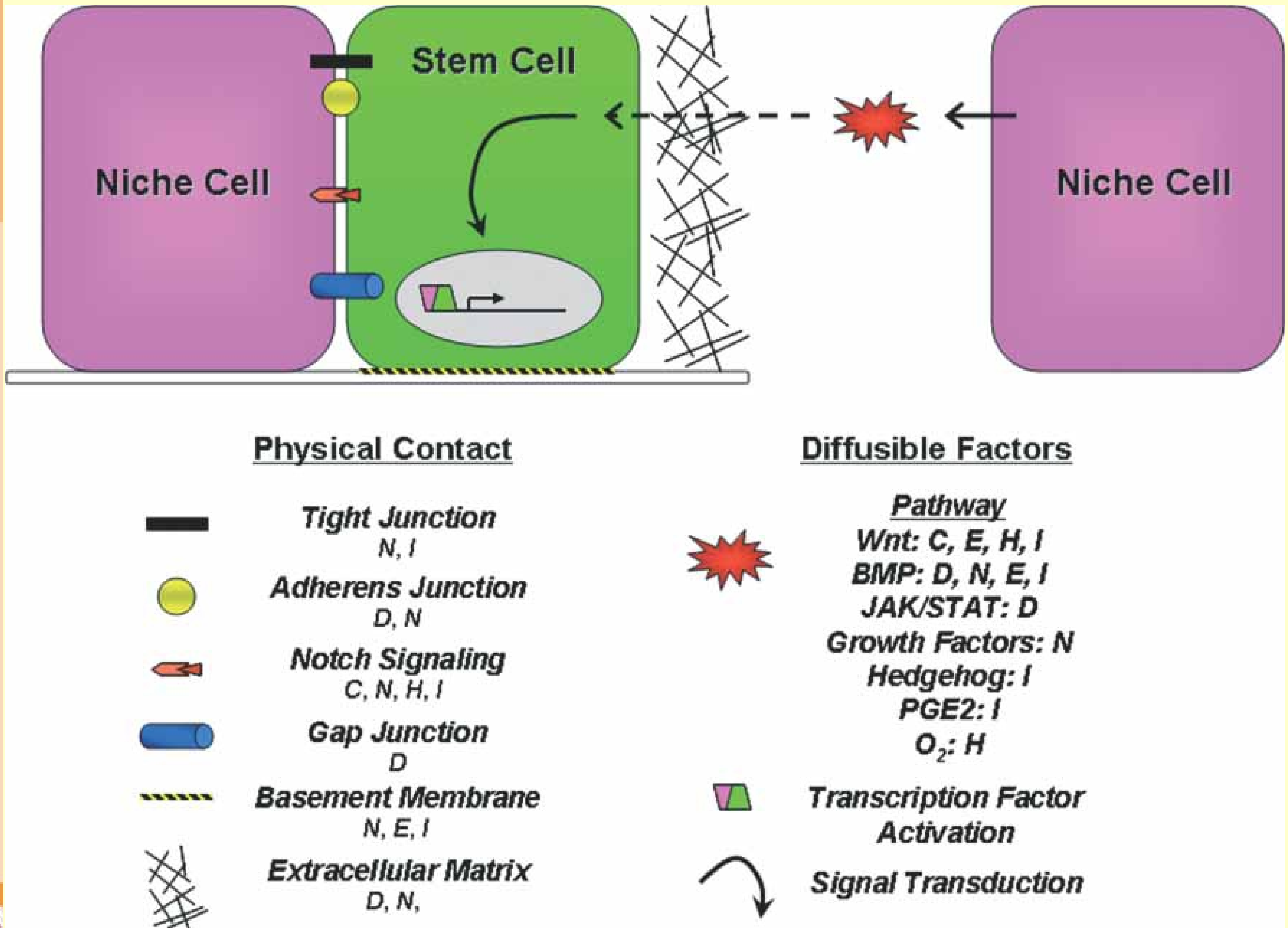
Li and Xie, Ann. Rev. Dev. Biol. 2005, 605-663

Cell-Cell Interactions at the Spermatogonial Niche

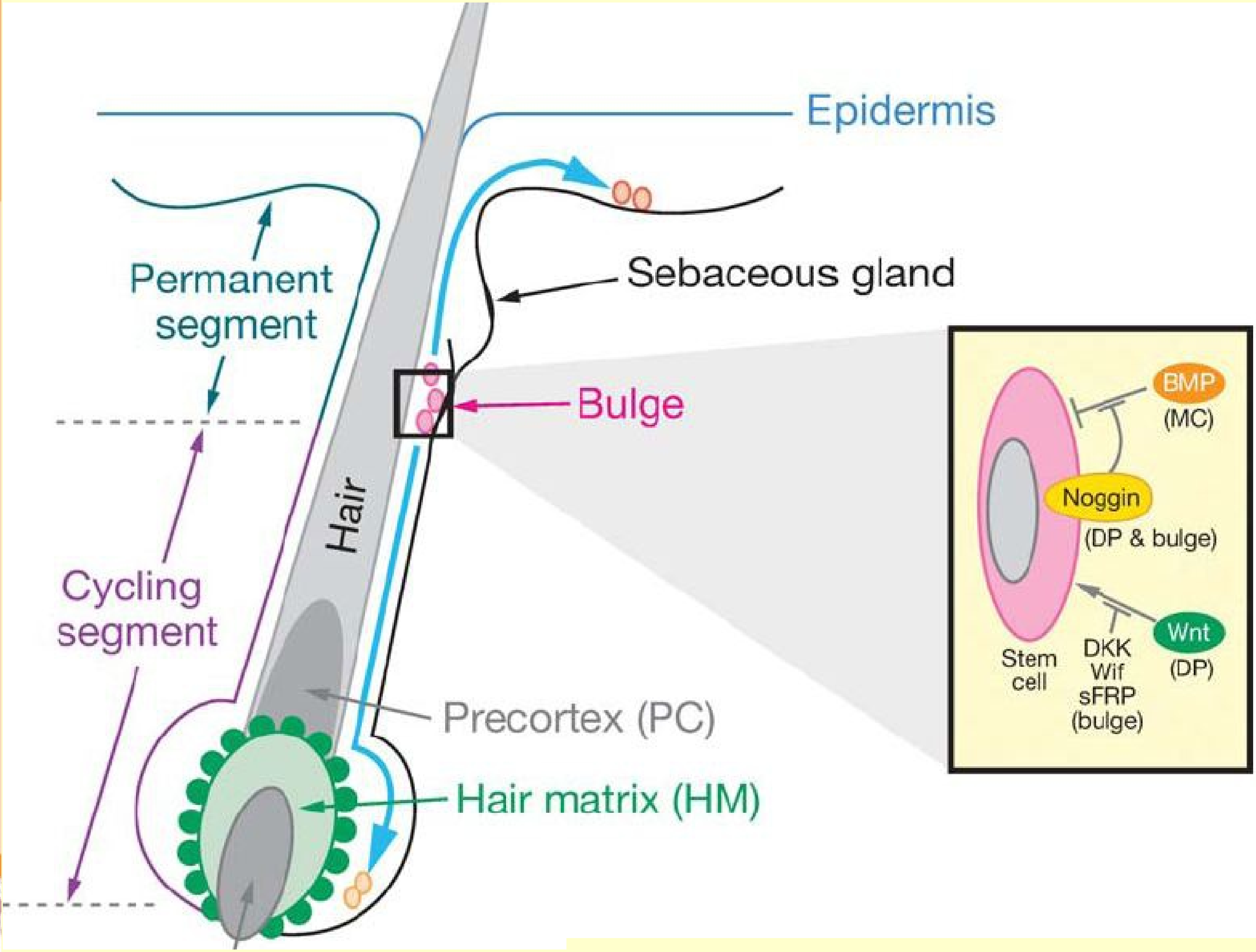


Li and Xie, Ann. Rev. Dev. Biol. 2005, 605-663

Summary of Stem Cell Niche Signals



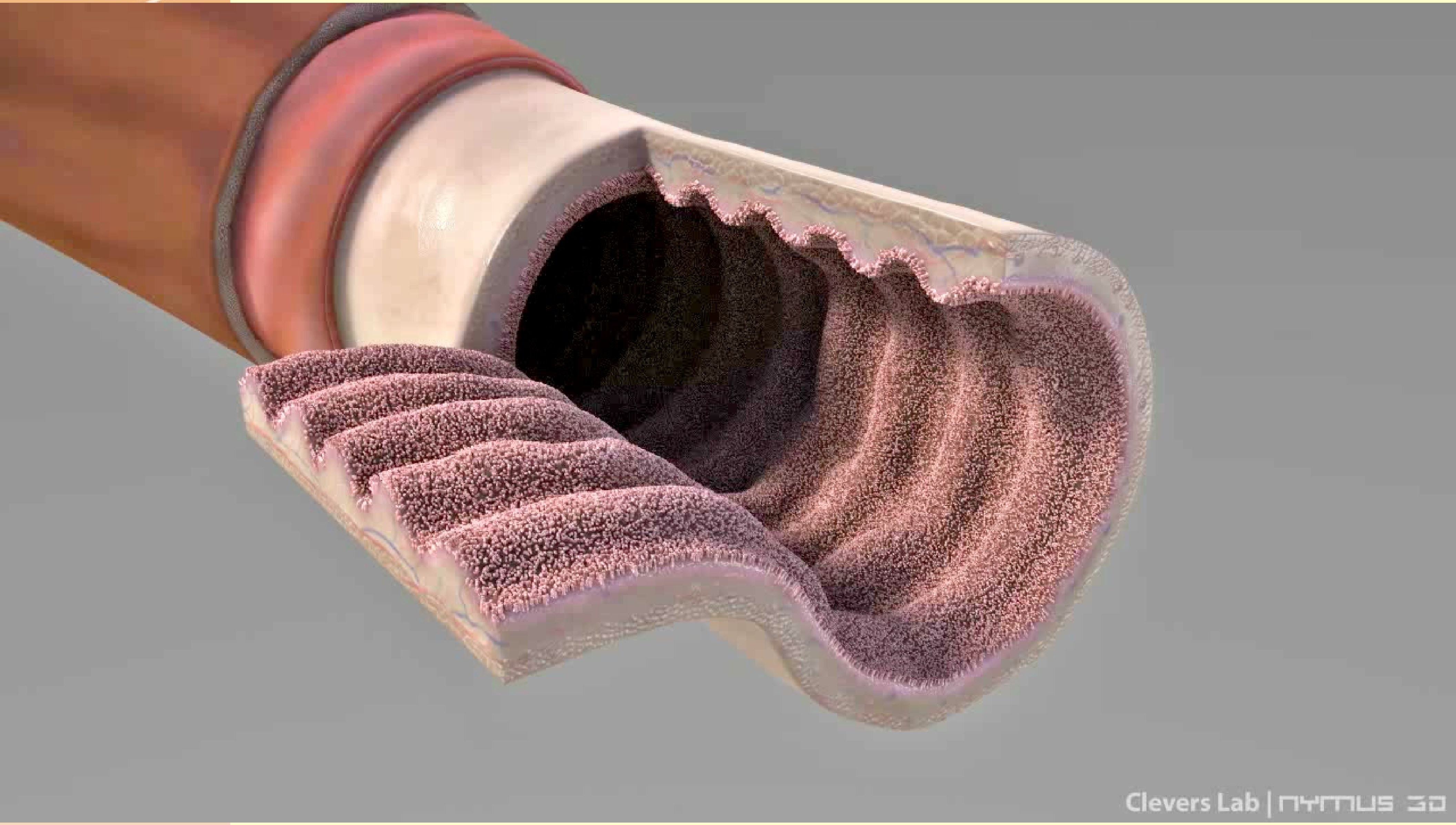
Hair Follicle Niche



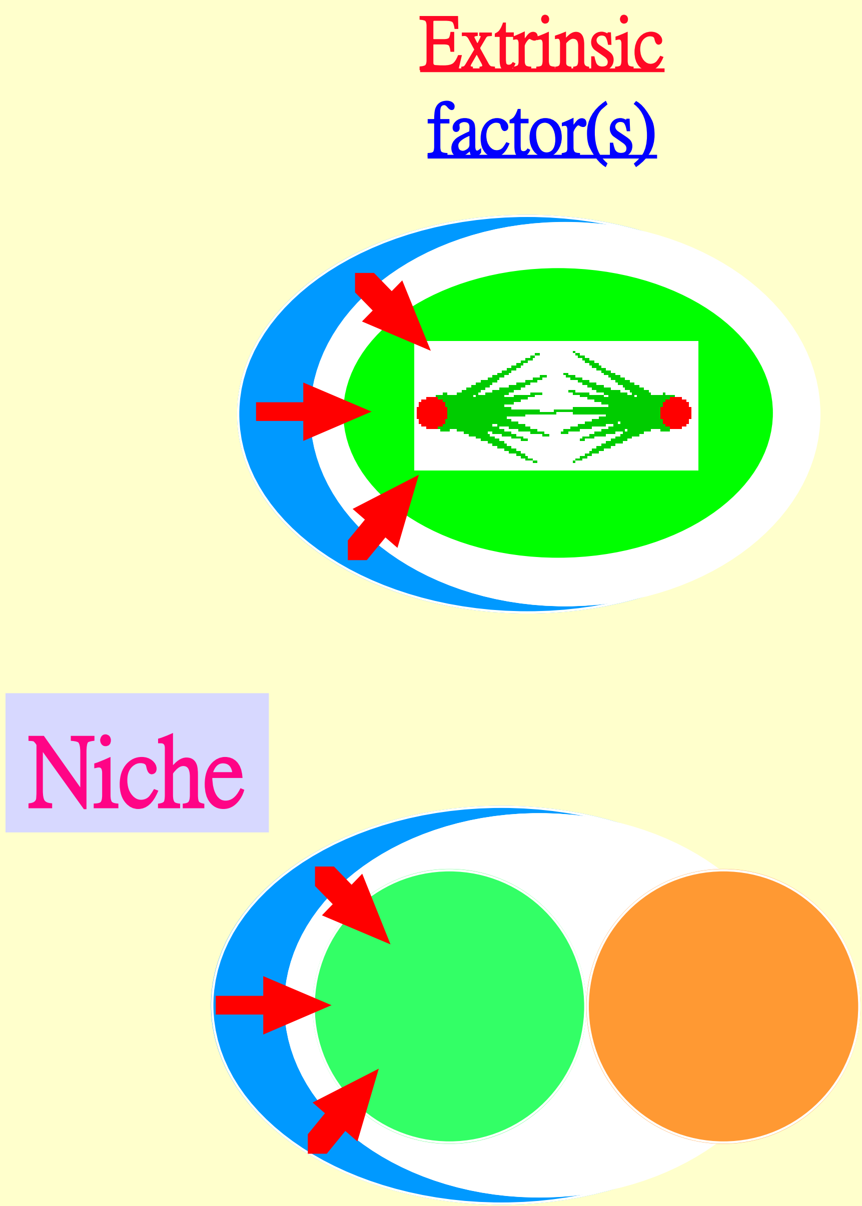
Li and Xie, Ann. Rev. Dev. Biol. 2005, 605-663

Intestinal Stem Cells in Crypts

Rainbow Villi



Asymmetric stem cell divisions



John Cairns: The Immortal Parental Strands

Nature Vol. 255 May 15 1975 **197**

review article

Mutation selection and the natural history of cancer

John Cairns*

Survival of the rapidiy renewing tissues of long-lived animals like man requires that they be protected against the natural selection of fitter variant cells (that is, the spontaneous appearance of cancer). This article discusses three possible protective mechanisms and shows how they could explain various features of the natural history of certain common cancers of man.

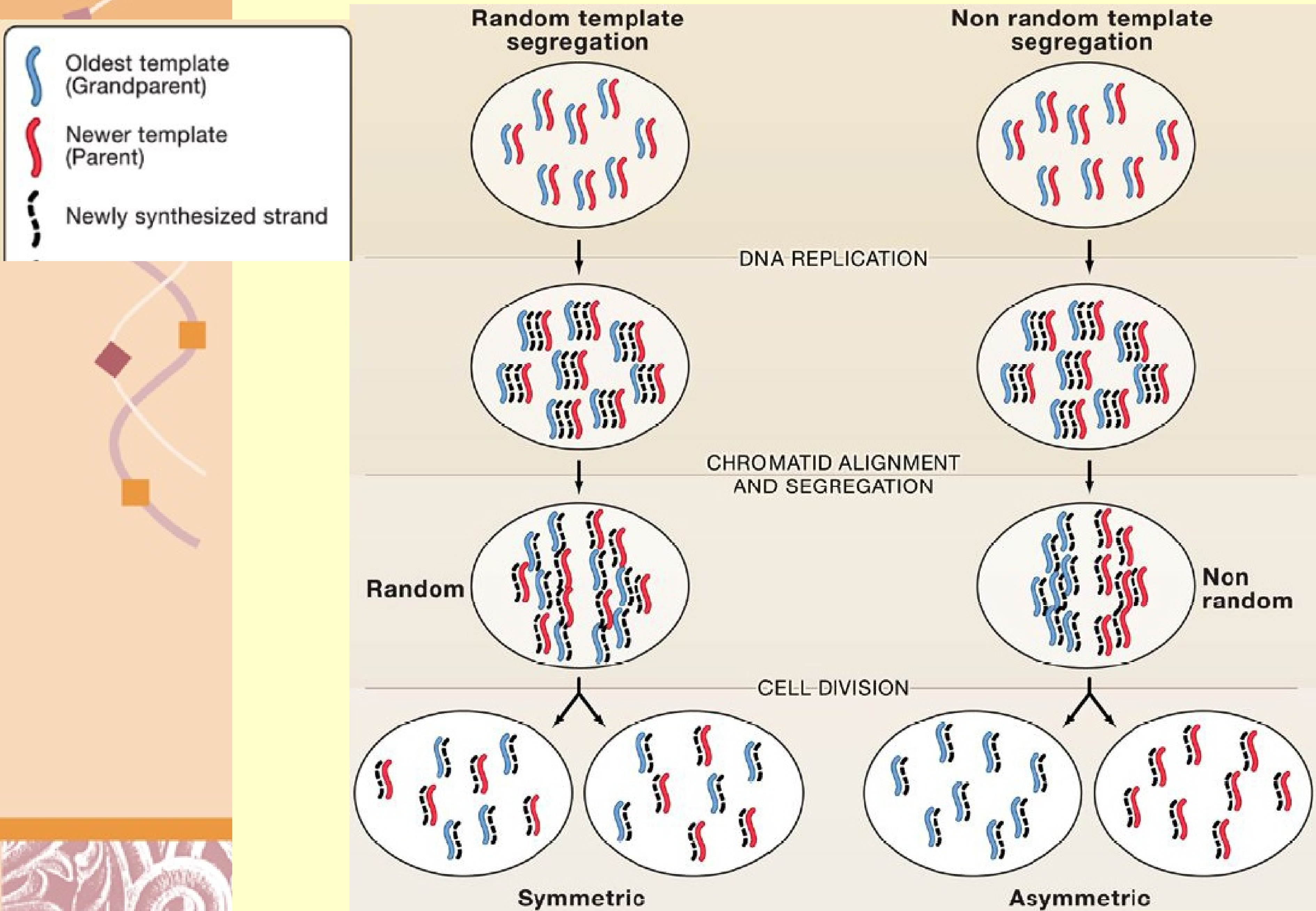
Cairns (1975) Nature **255, 197**

Motivation for Asymmetric Strand Segregation

- Adult rat contains 6×10^{10} cells
- In its small intestine, a rat sheds over 10^{13} epithelial cells during its lifetime.
- Requires 10^3 symmetric cell doublings from embryo to adult followed by 10^{13} asymmetric cell doublings during its lifetime
- How do epithelial cells minimize mutations that lead to cancer?

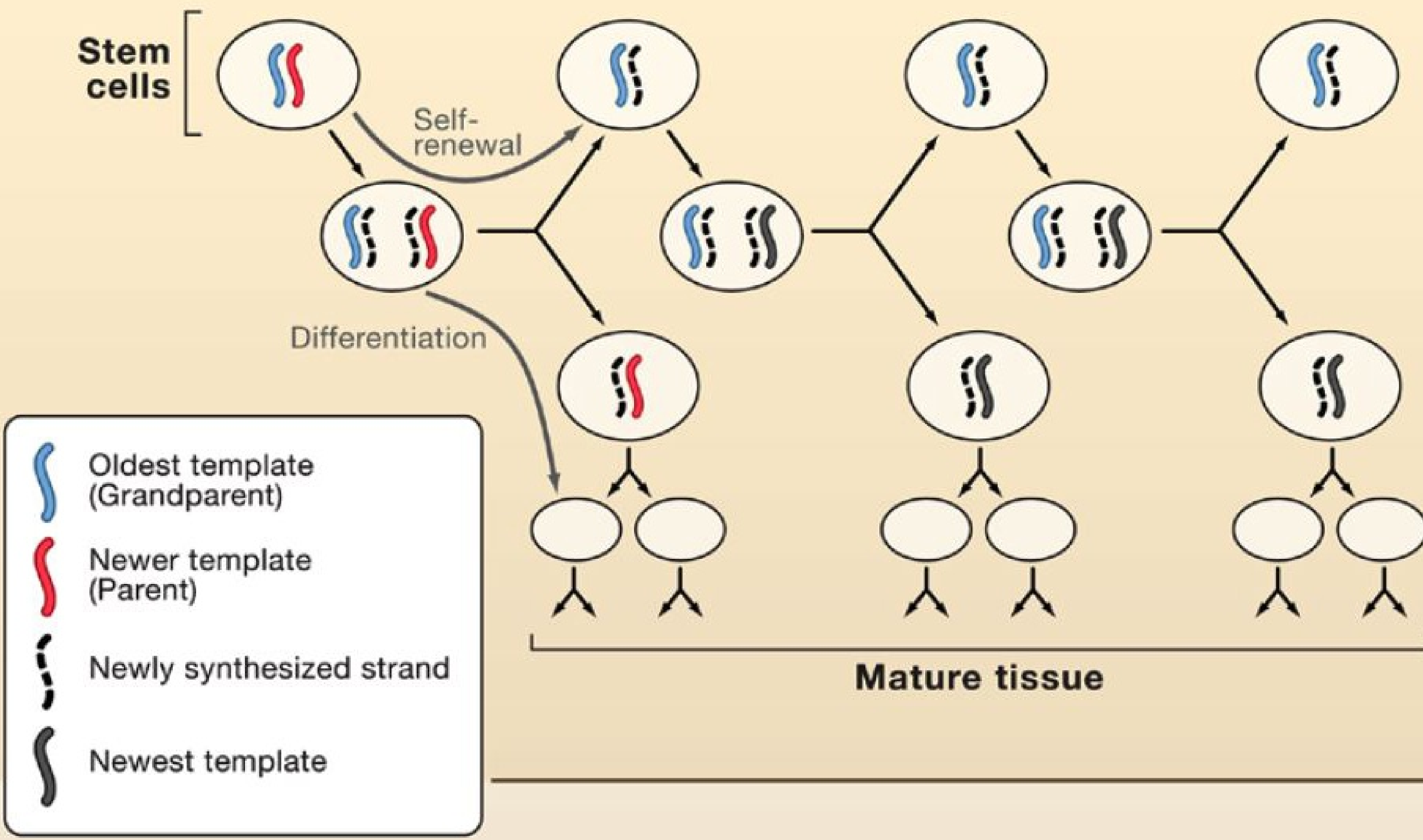
Cairns (1975) Nature **255, 197**

Asymmetric Segregation of Parental DNA Strands



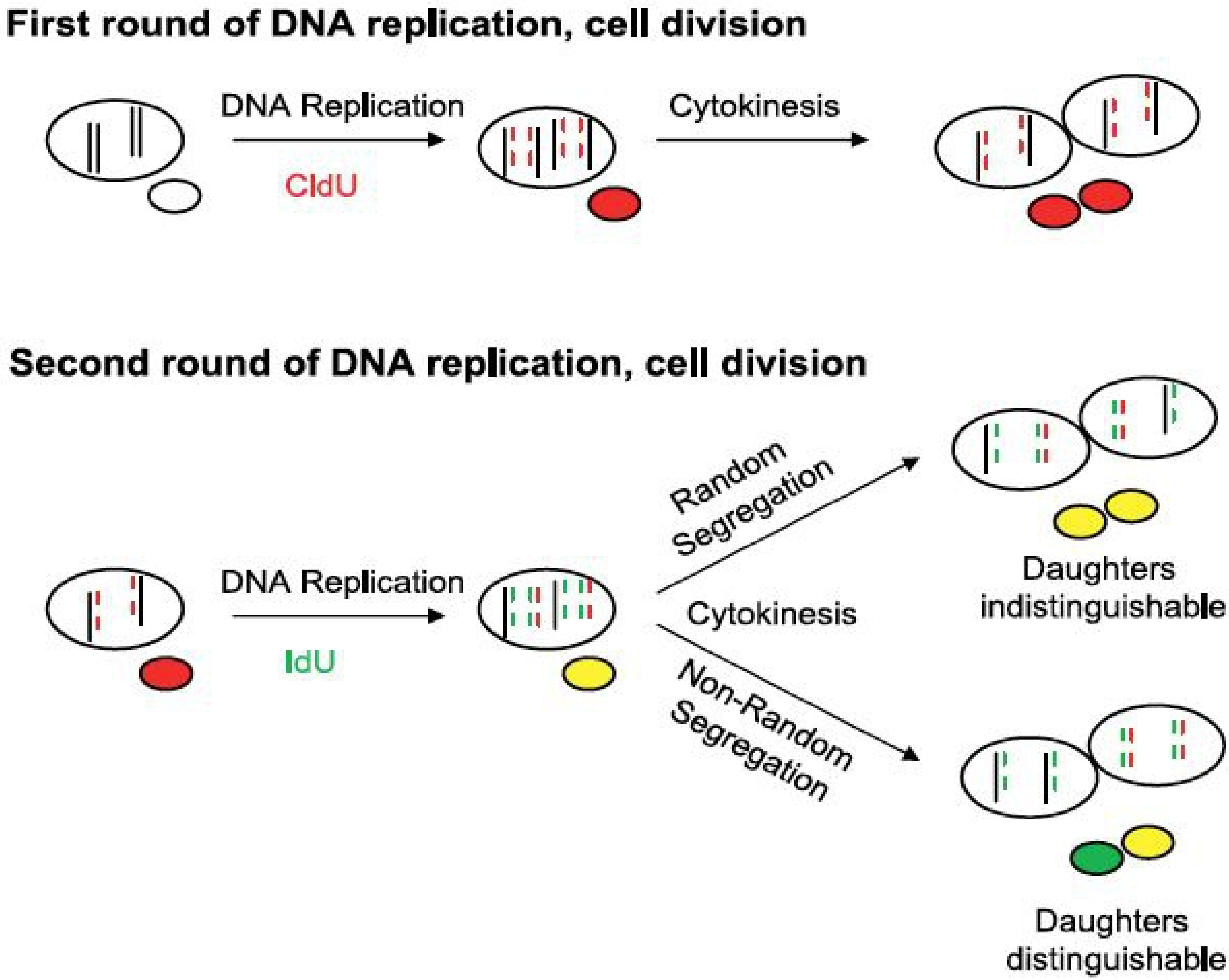
Rando (2007) Cell 129 1239

Asymmetric Stem Cell Growth with Asymmetric Parental Strand Segregation



Rando (2007) Cell 129 1239

Asymmetric DNA Labeling Patterns



Rando (2007) Cell 129 1239

Duplicating Muscle Cell Pairs Display Asymmetric DNA Labeling Patterns

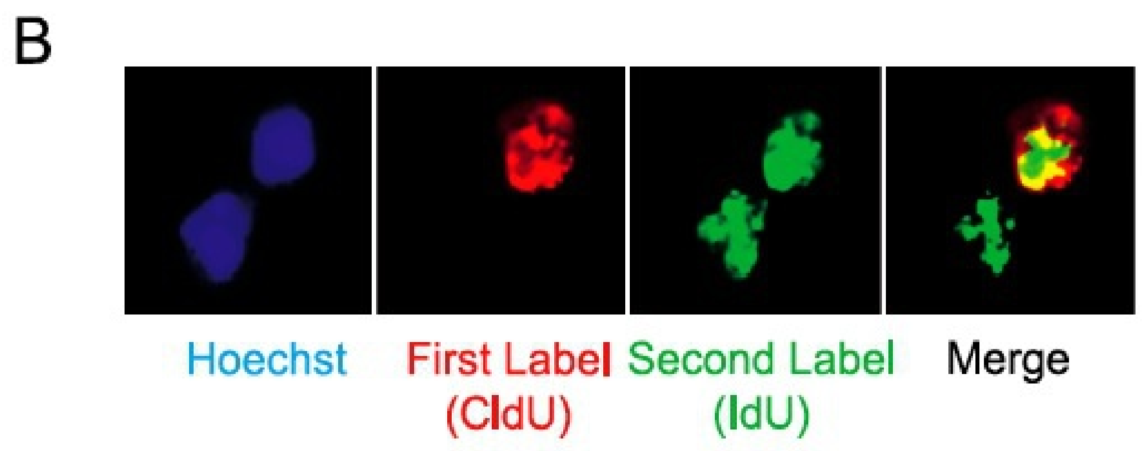
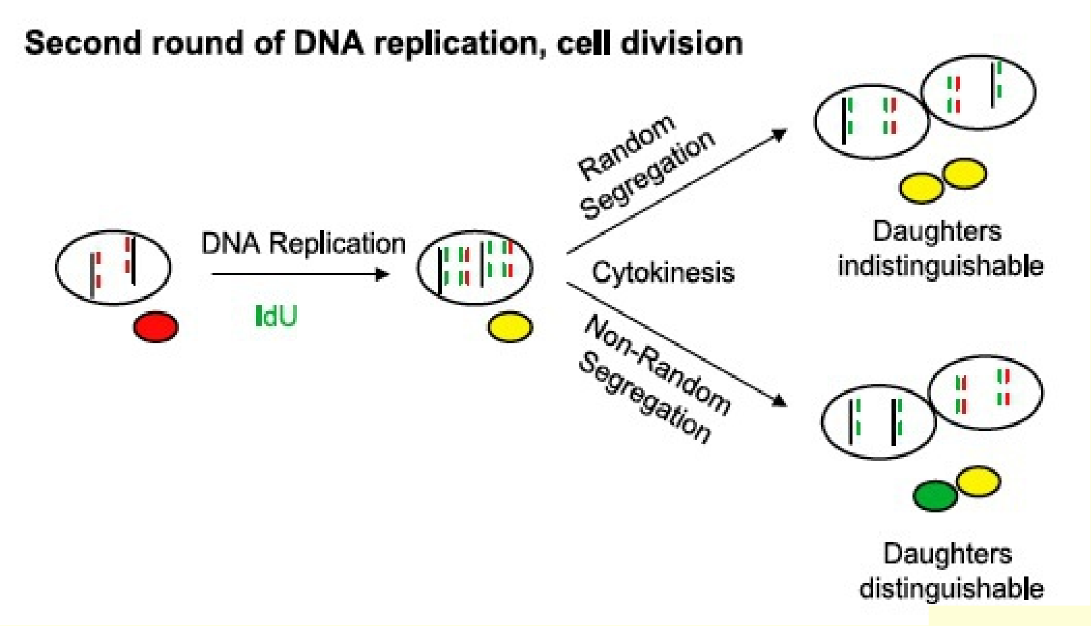
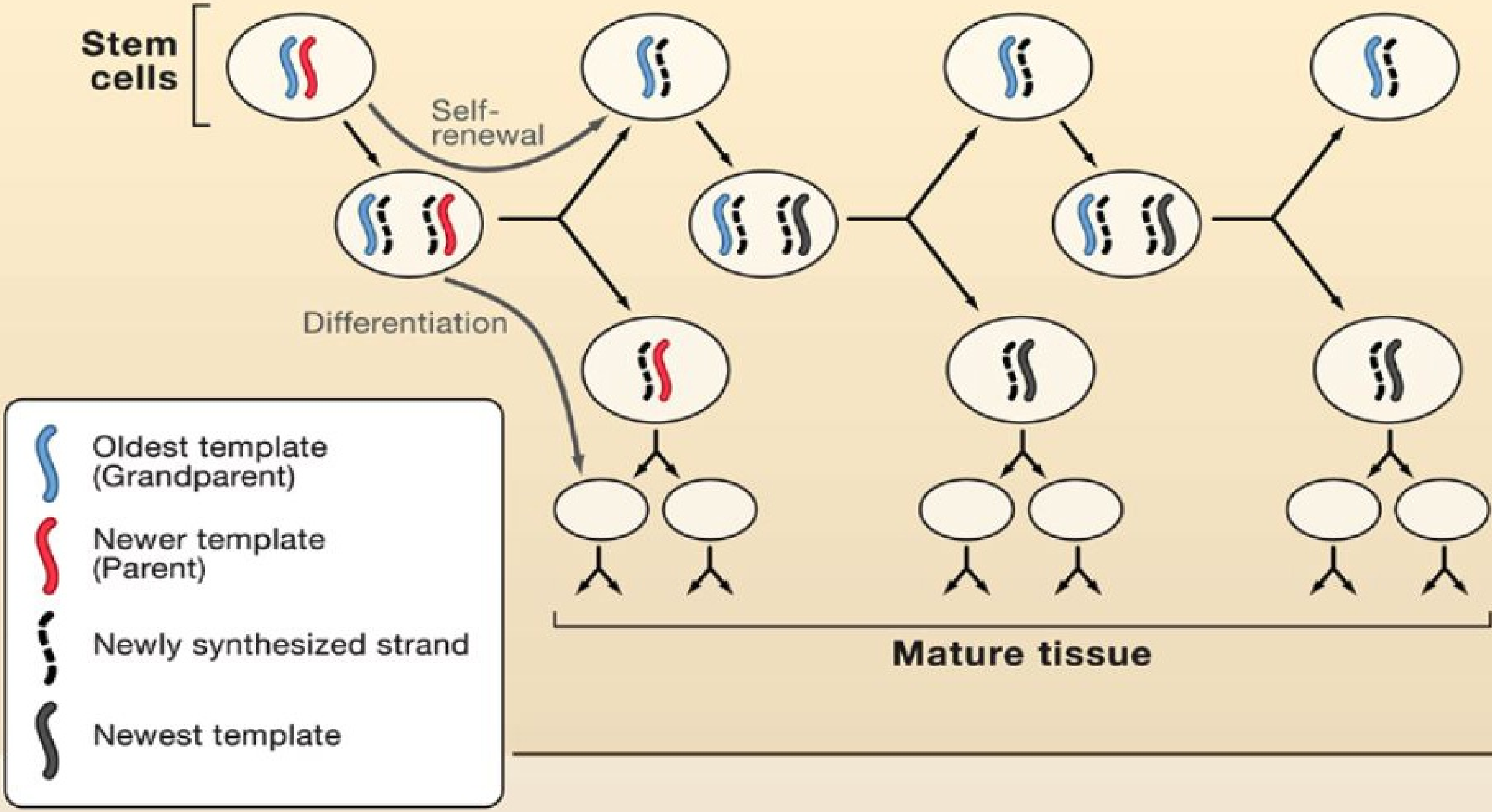


Figure 2. Evidence of Co-Segregation of DNA Template Strands during Muscle Progenitor Cell Division
(B) Cell pairs were immunostained for CldU and IdU. Shown is a representative photograph of an immunostained pair of cells, in which both daughter cells were labeled with the second label, IdU (green), but only one daughter inherited the first label, CldU (red).



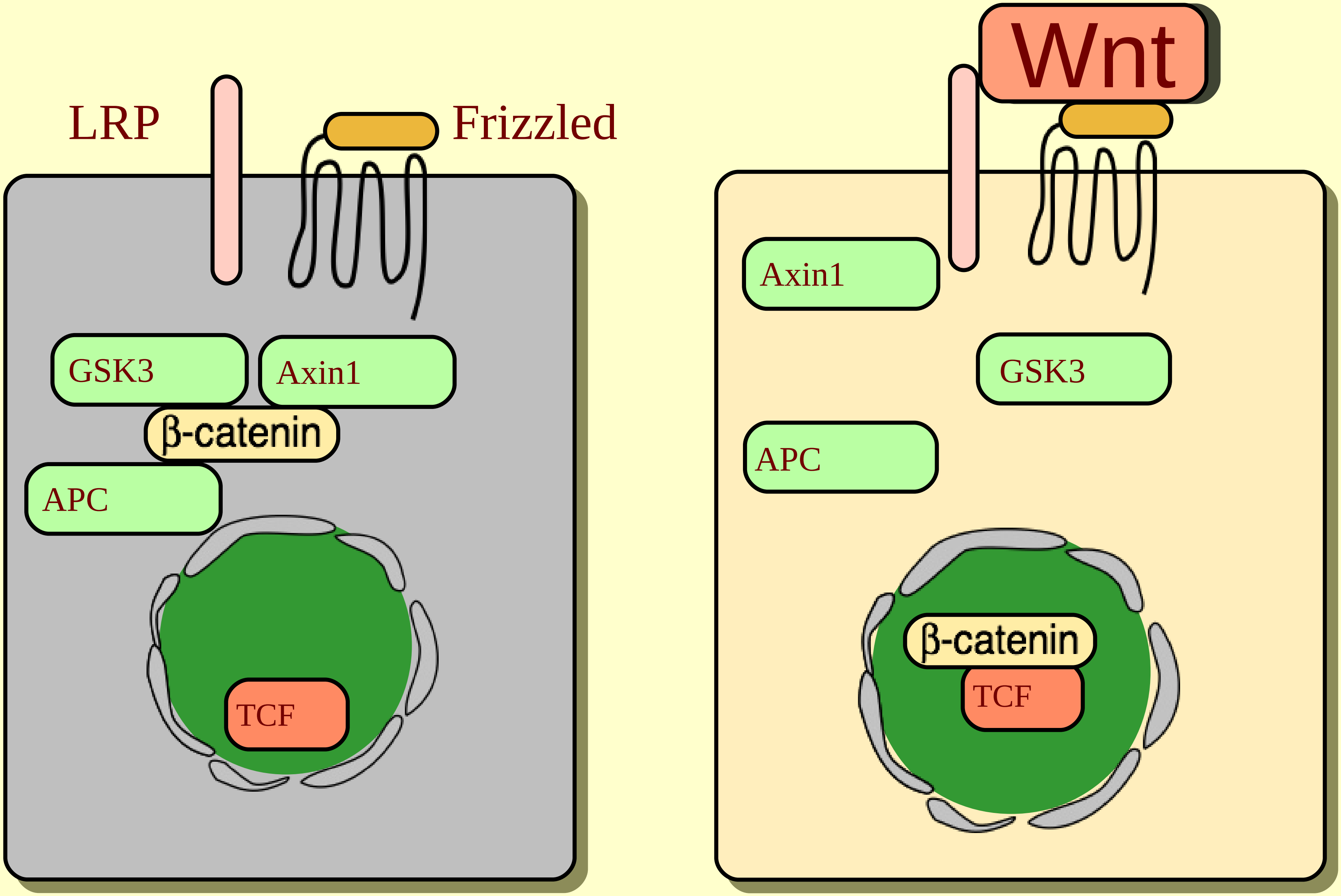
Conboy et al, PLOS Biology (2007)

Asymmetric Stem Cell Growth with Asymmetric Parental Strand Segregation



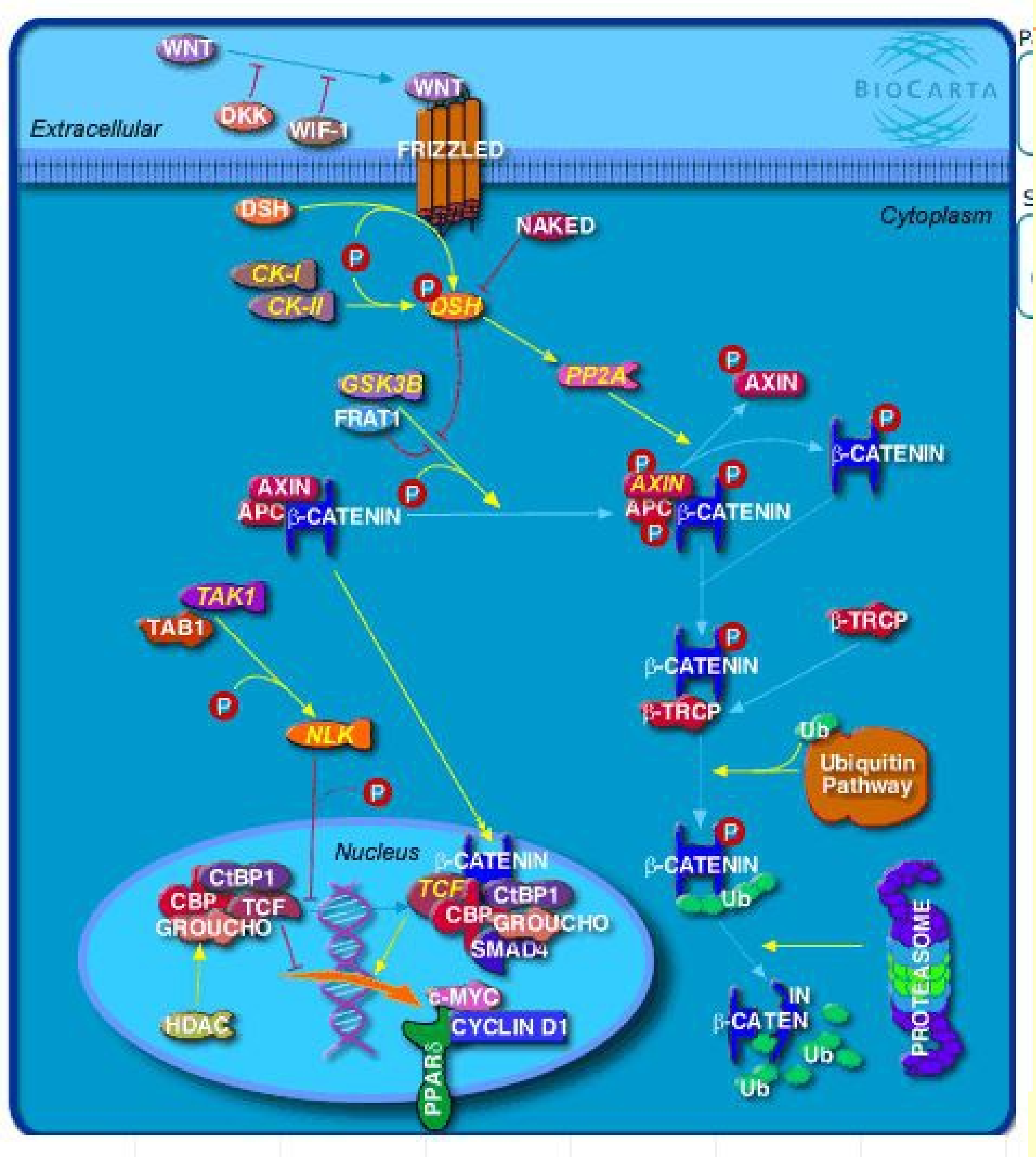
Rando (2007) Cell 129 1239

Wnt signaling



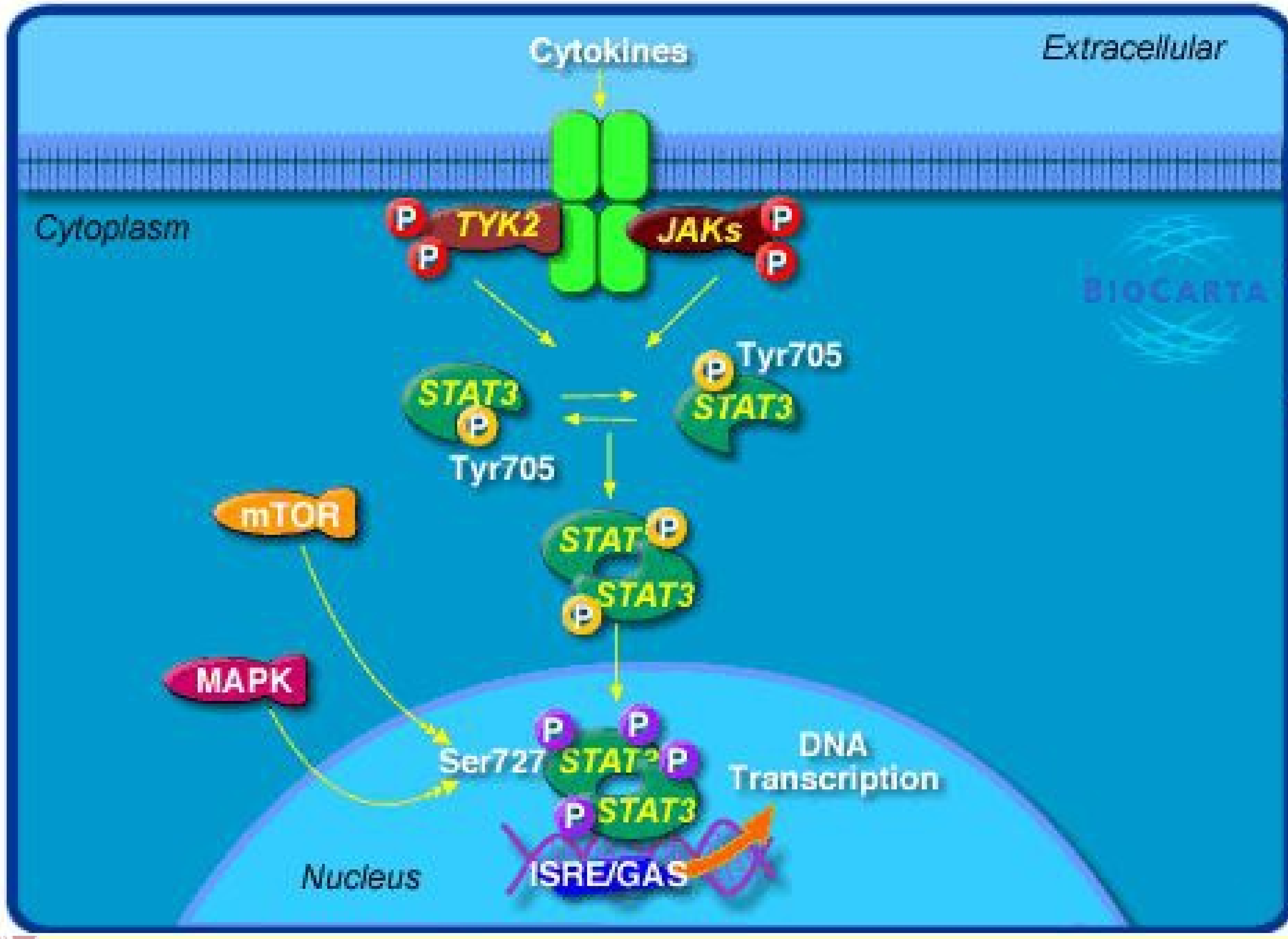
Courtesy of Roel Nusse

Wnt Signaling Pathway



Jak Stat Pathway

http://www.biocarta.com/pathfiles/h_stat3Pathway.asp



The Hedgehog pathway

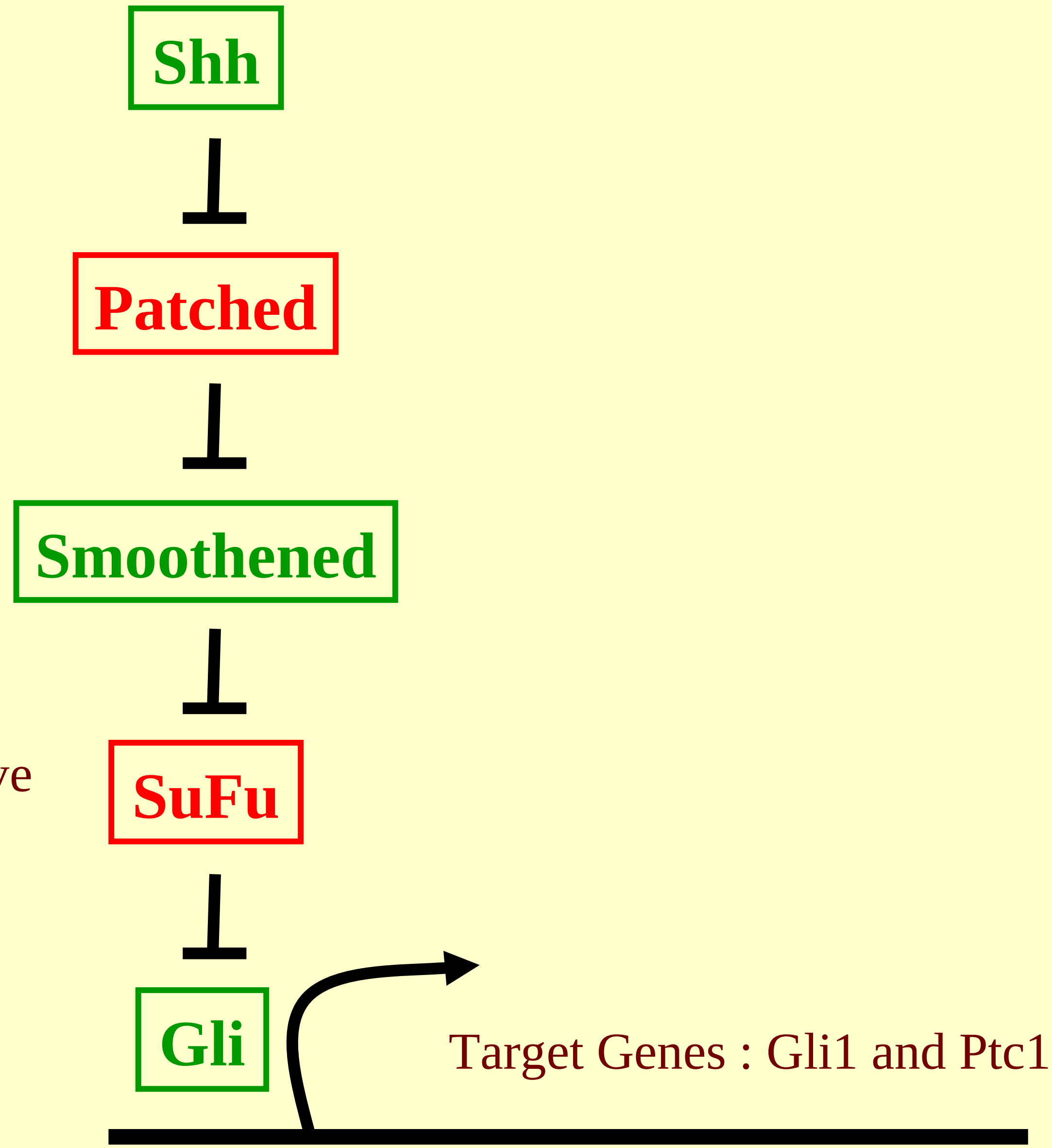
Ligand

Receptor

7-pass membrane transducer

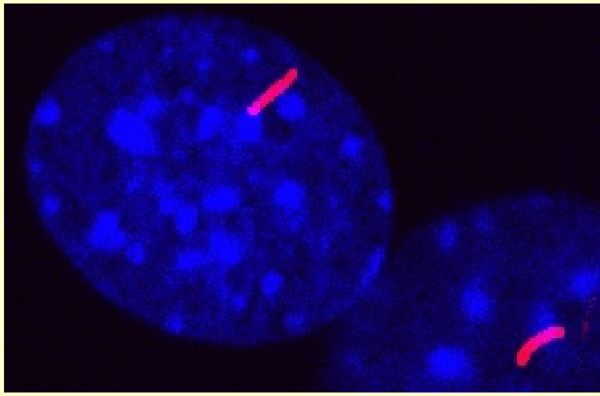
Cytoplasmic Negative Regulator

Transcription Factor

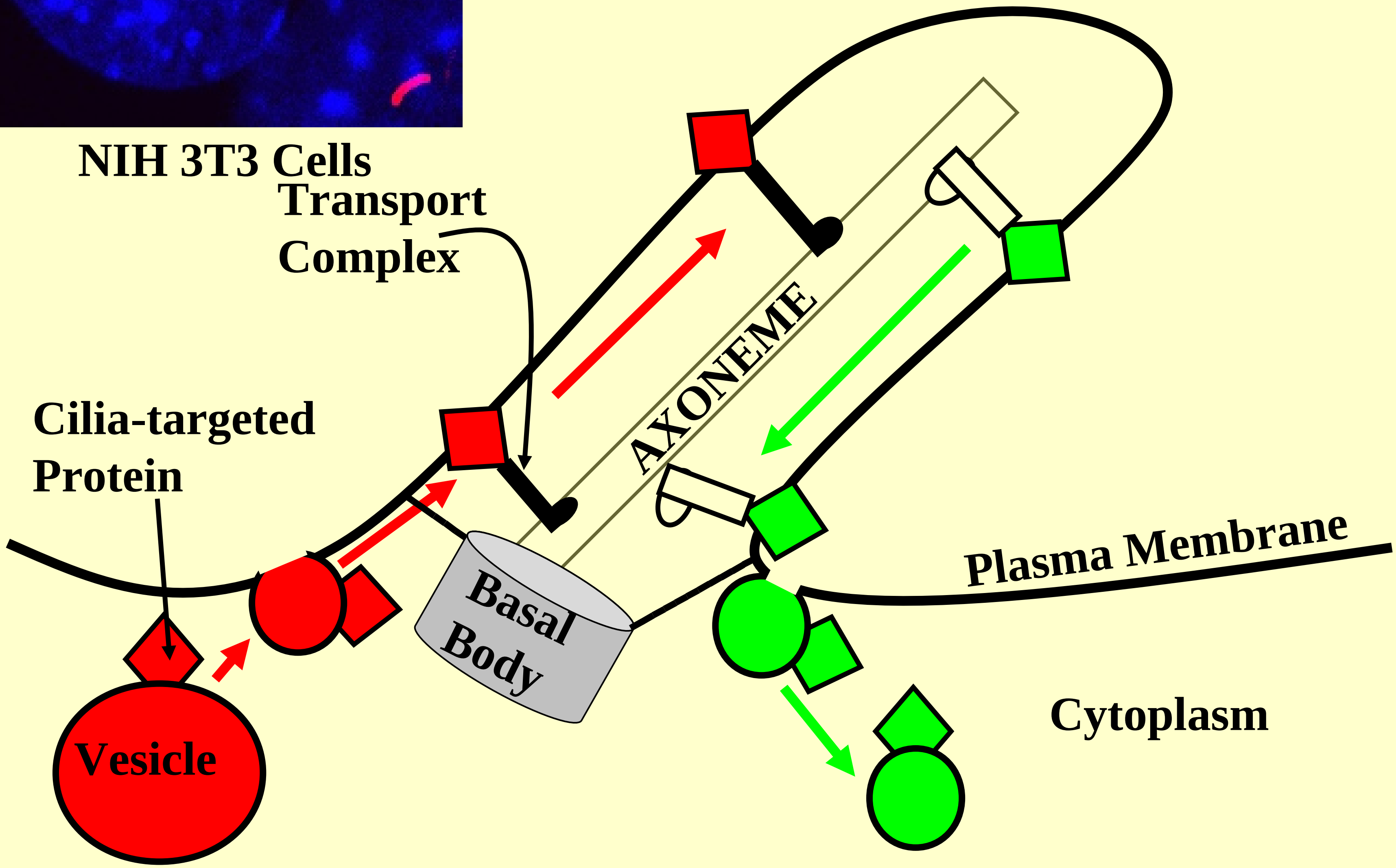


The primary cilium: A specialized compartment for signal transduction

Nucleus Cilium

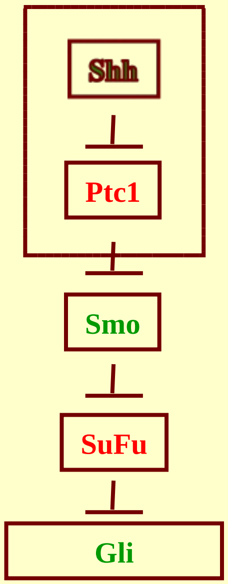


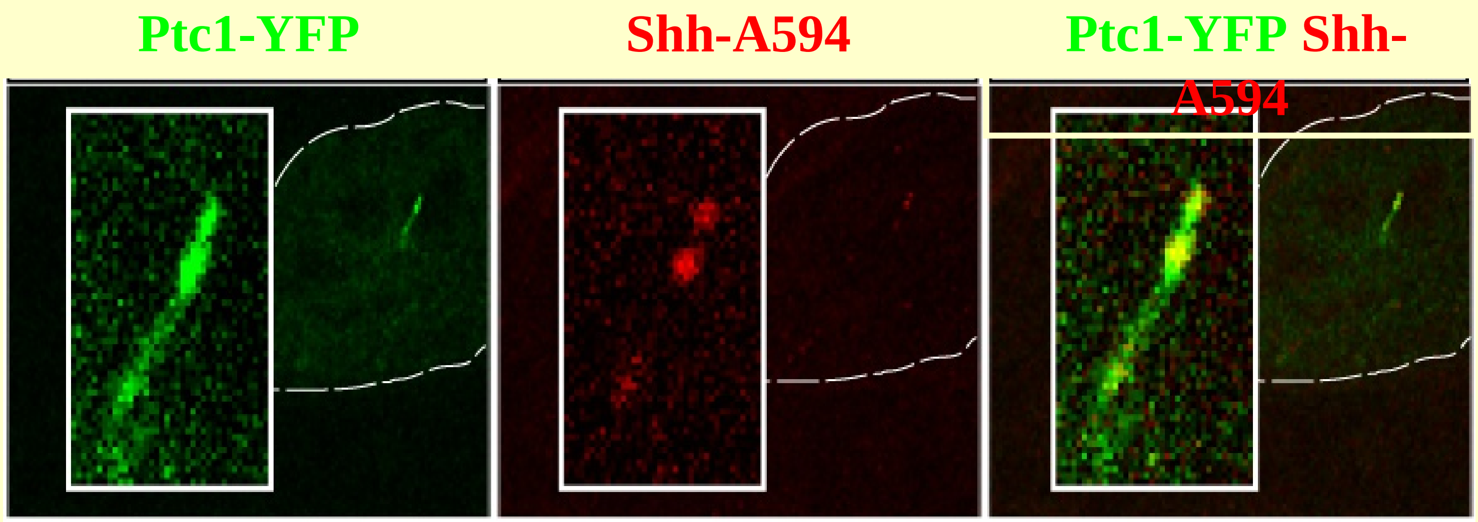
NIH 3T3 Cells



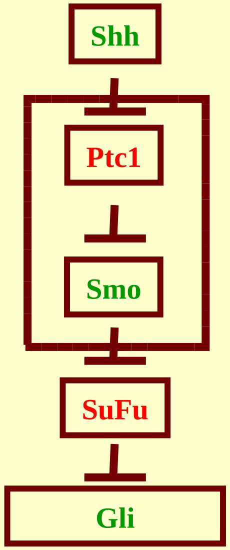
Rosenbaum and Witman, *Nature Reviews Mol Cell Biol (2002)*

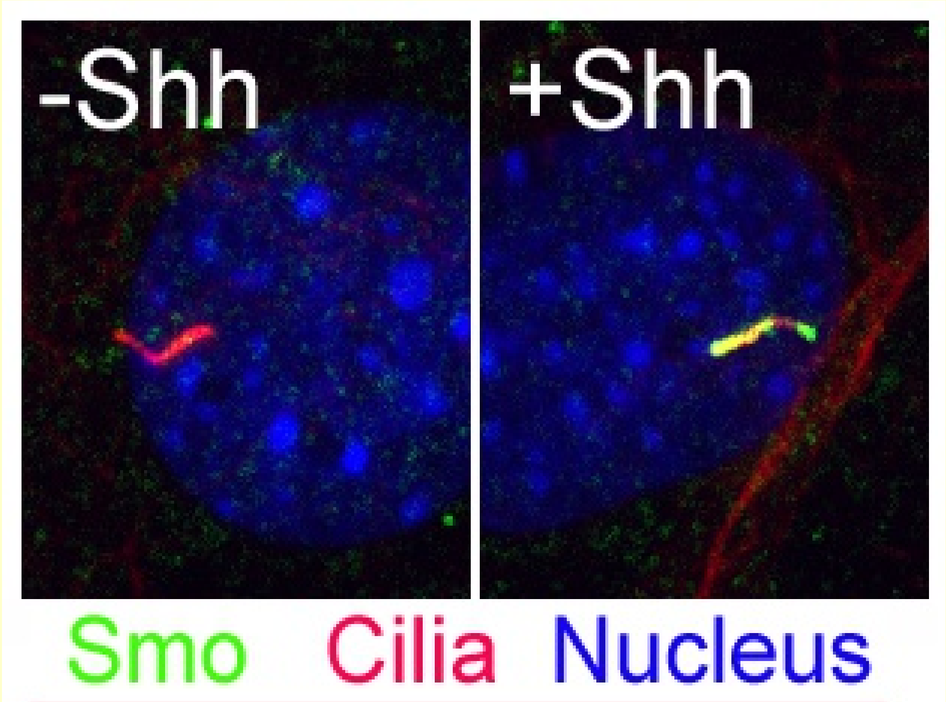
Cilia as sensors for Shh: Shh binds to its receptor Patched1 at primary cilia in live cells



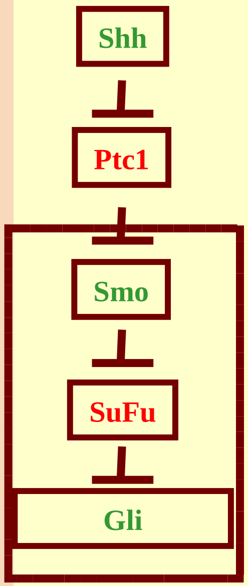


Smo moves to cilia and when the Hedgehog pathway is activated

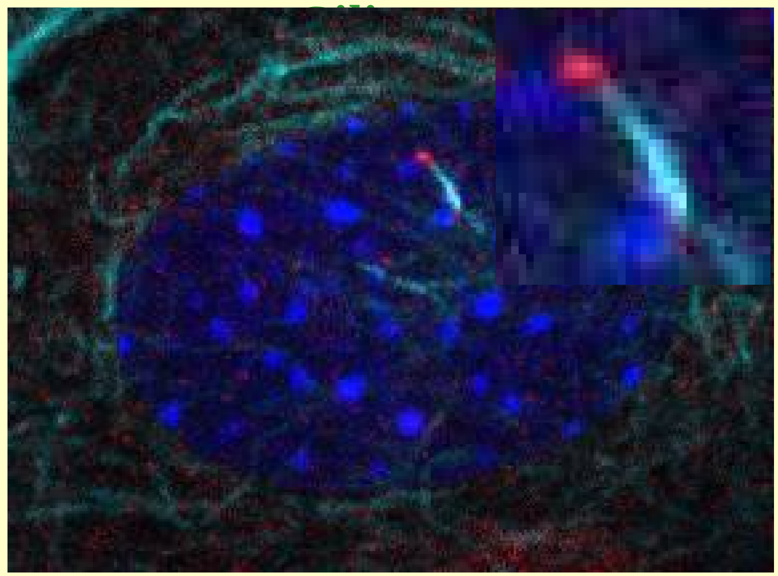




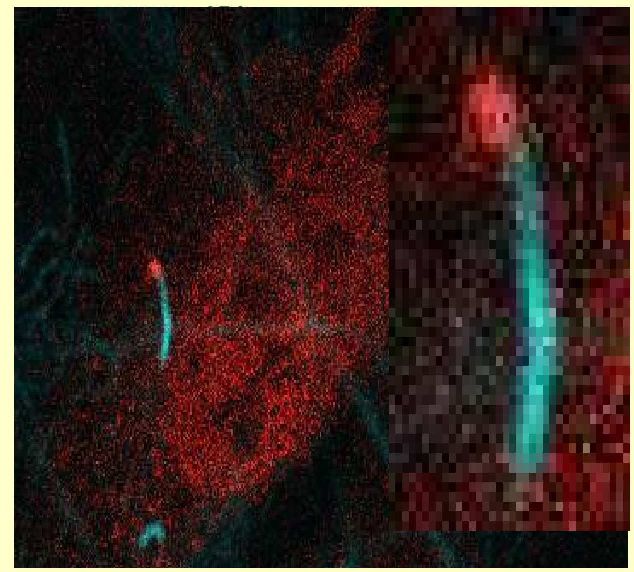
Smo activates downstream signaling components in cilia



SuFu

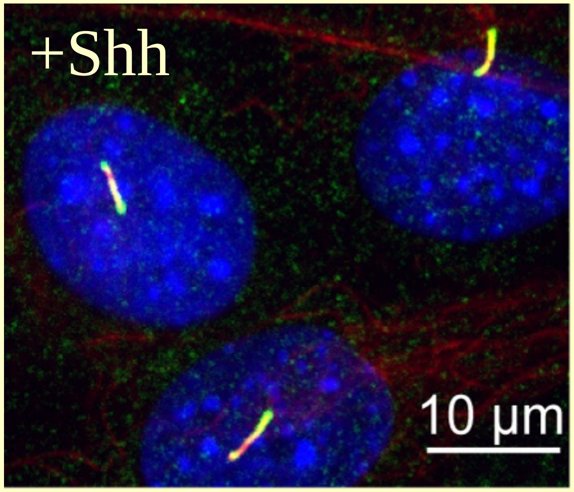


Gli2-YFP

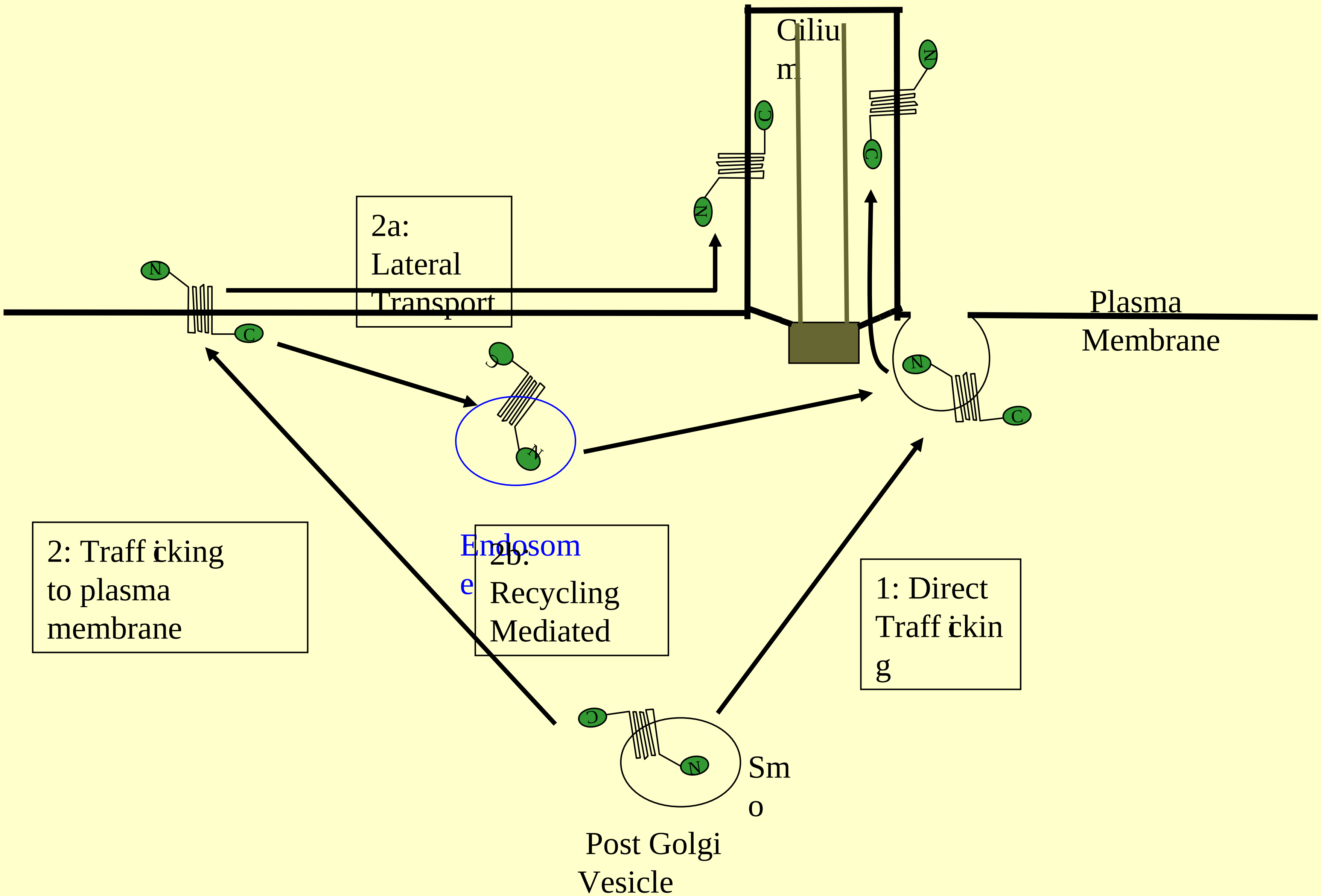


Smo as a model for signal-regulated protein transport at primary cilia

Smo Cilia Nucleus

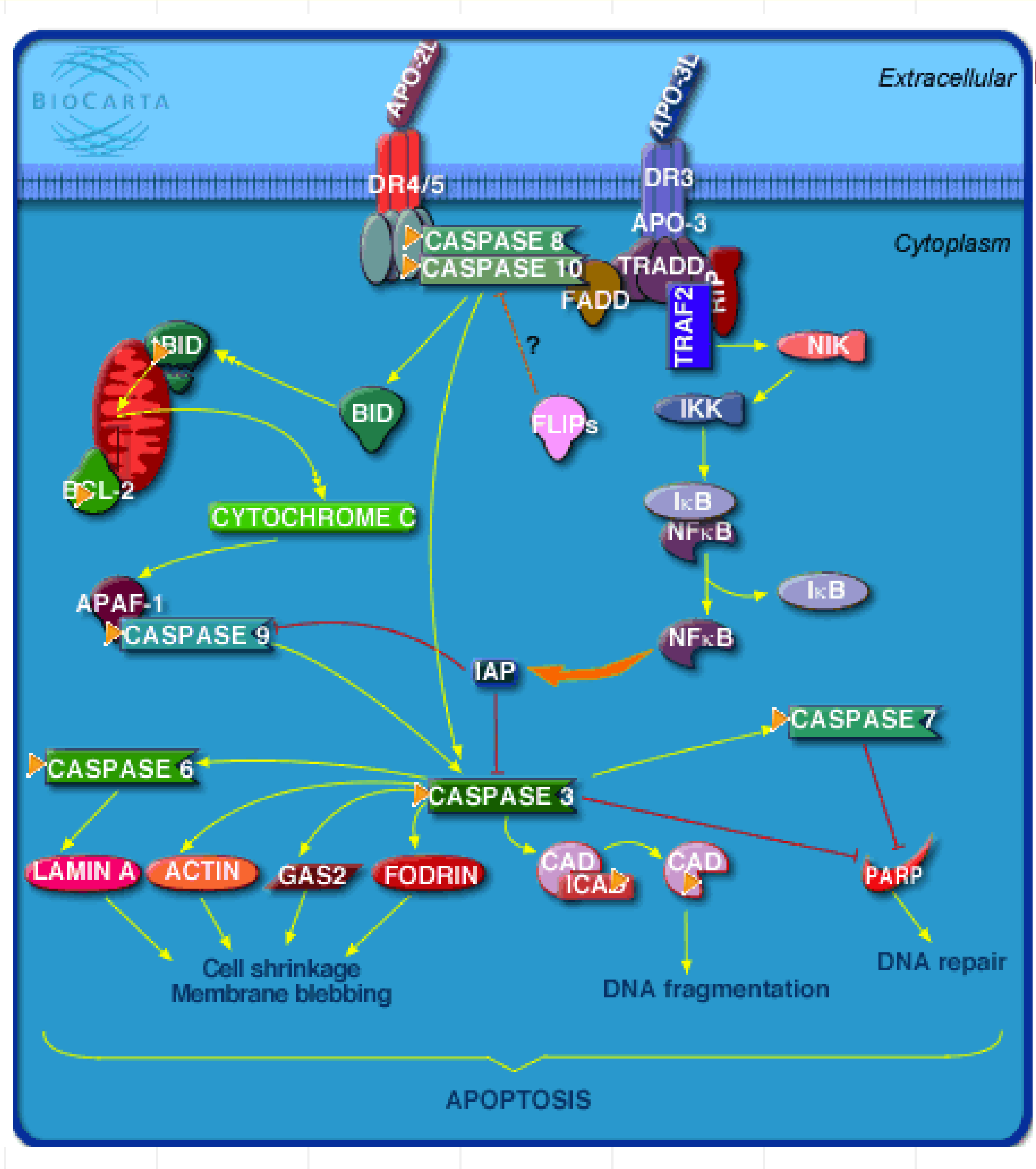


Models for ciliary protein transport

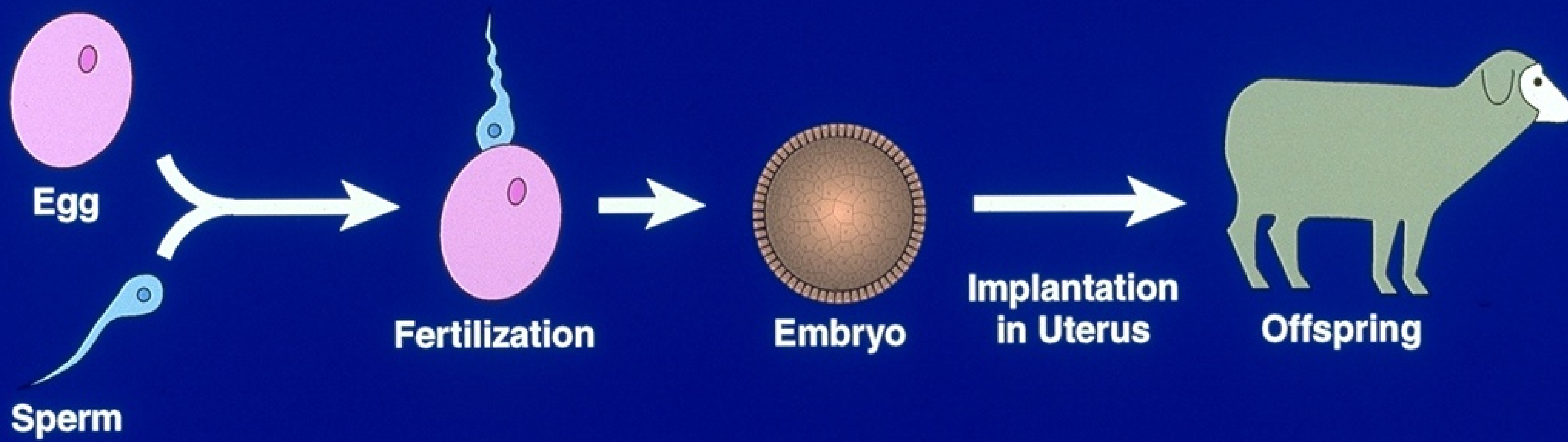


Induction of Apoptosis via DR3 and DR4/5 Death Receptors

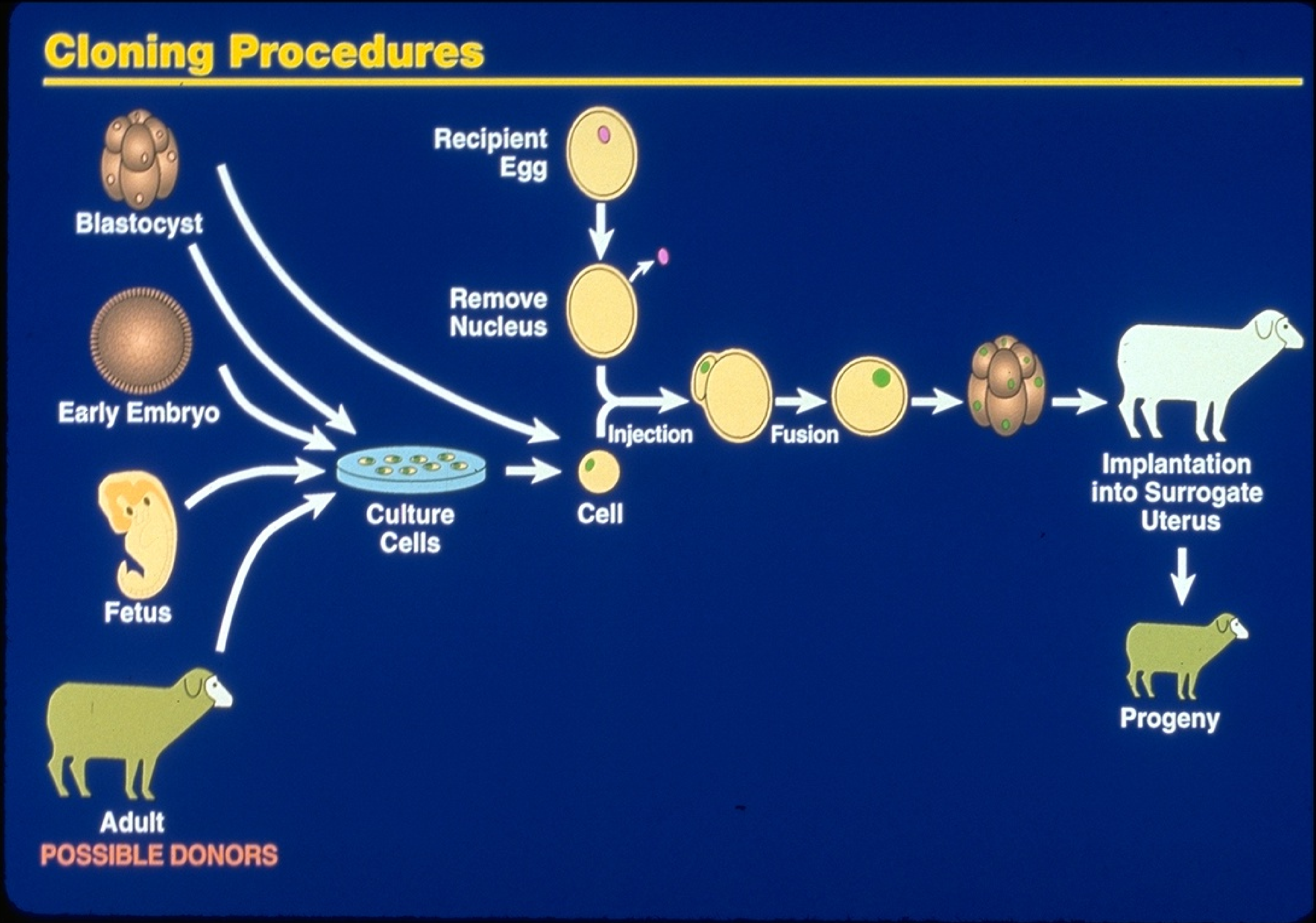
http://www.biocarta.com/pathfiles/h_deathPathway.asp



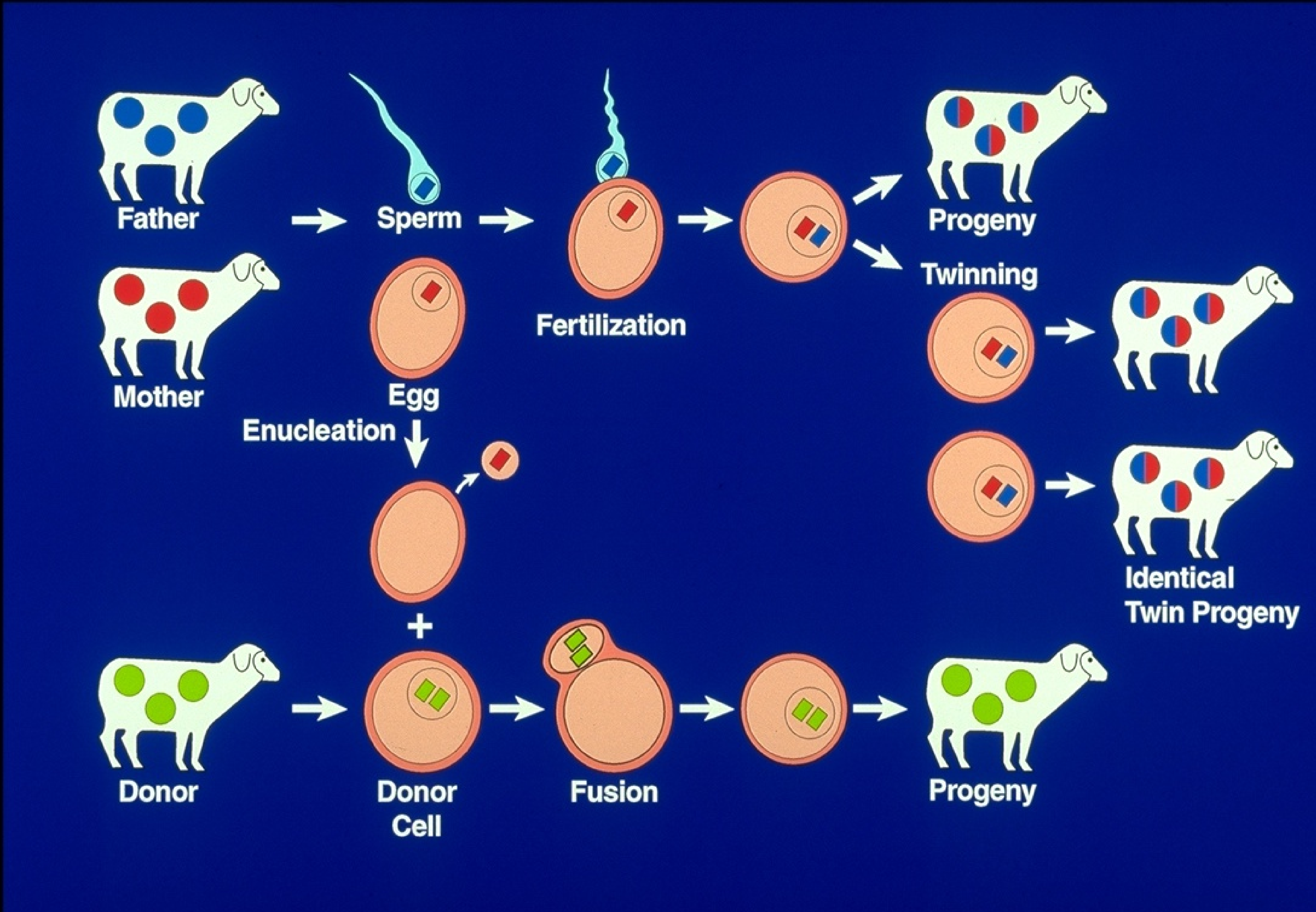
Normal Reproduction



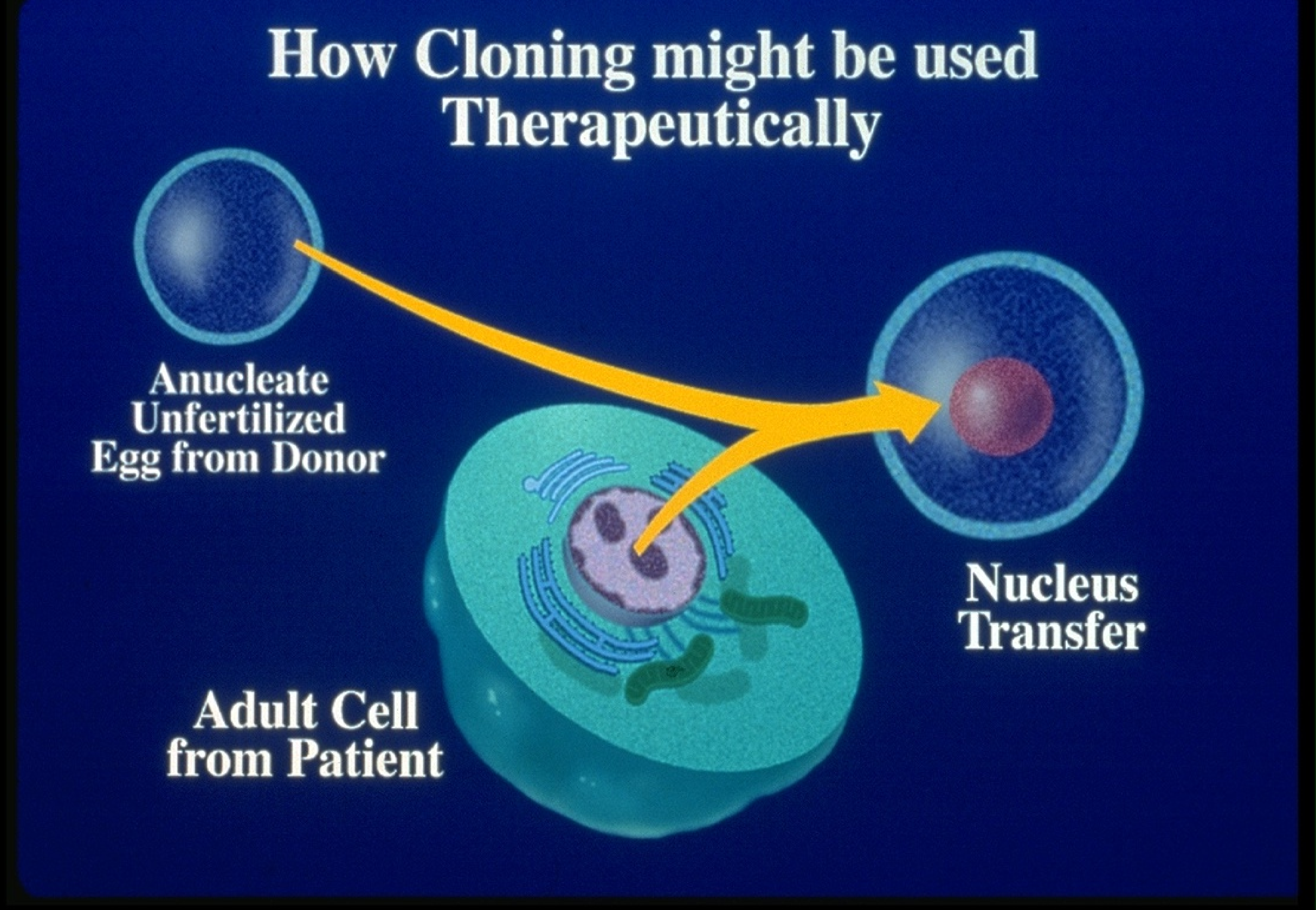
Wilmut et al. Nature. 1996 Mar 7;380(6569):64-6.



Wilmut et al. Nature. 1996 Mar 7;380(6569):64-6.



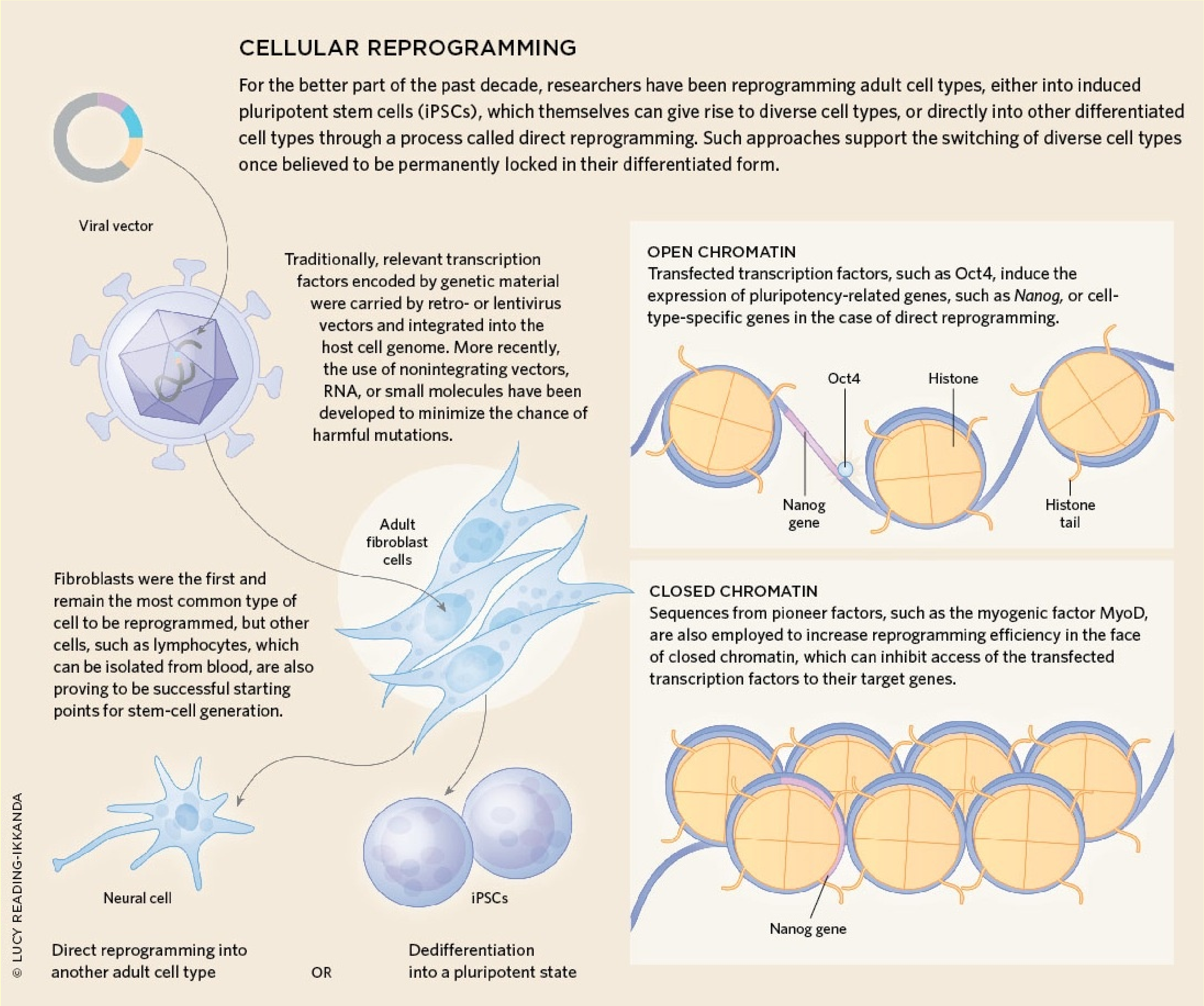
Courtesy Paul Berg



Courtesy Paul Berg

Direct versus indirect Cell Reprogramming

http://www.the-scientist.com/?articles.view/articleNo/39241/title/A-Twist-of-Fate/



http://www.the-scientist.com/?articles.view/articleNo/39241/title/A-Twist-of-Fate/