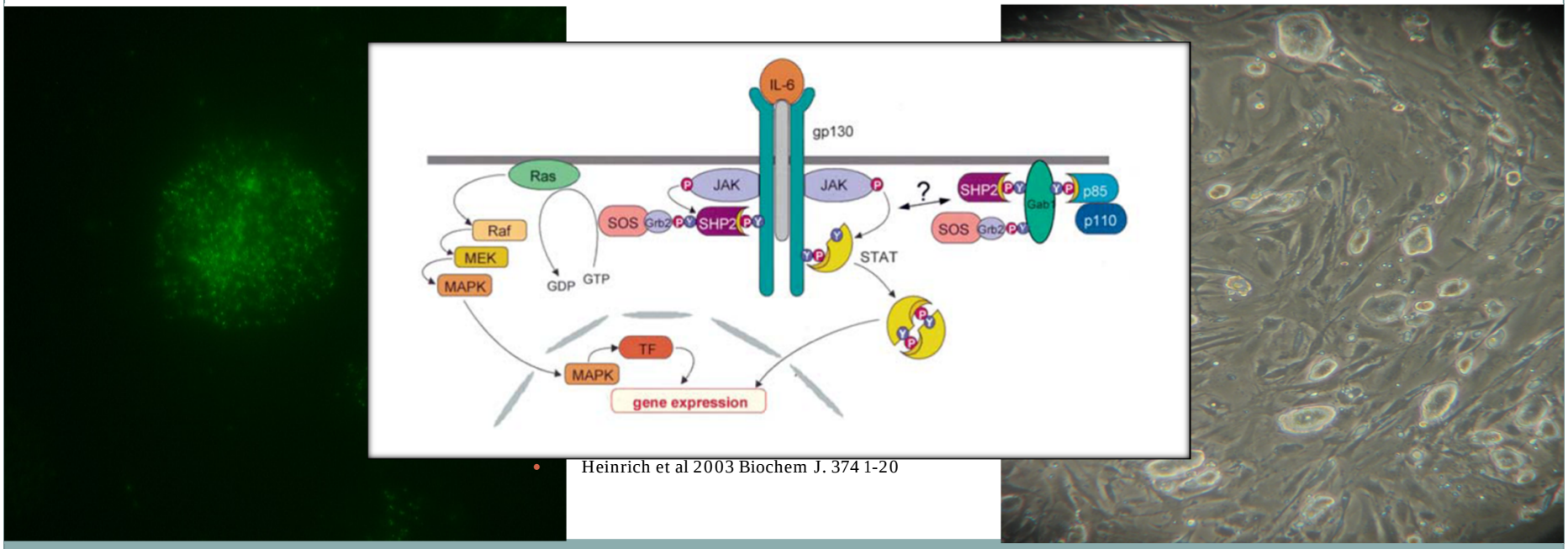
Stem Cells
Regenerative Medicine
Signal Transduction

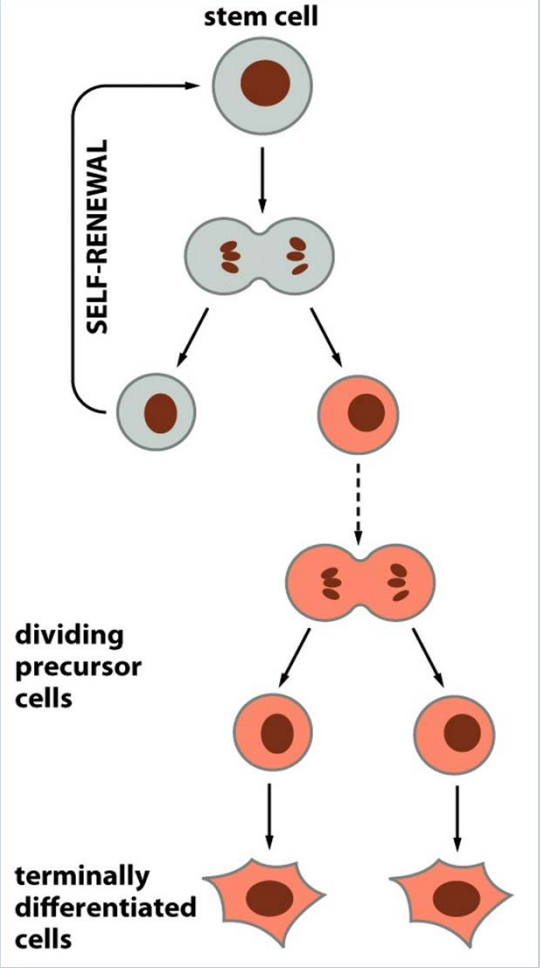
AMY SPROWLES, PHD
LECTURER, DEPT. OF BIOLOGICAL SCIENCES



- Heinrich et al 2003 Biochem J. 374 1-20

What are stem cells?

- Clonal
- self renewing entity
- multipotent and thus can generate multiple cell types.

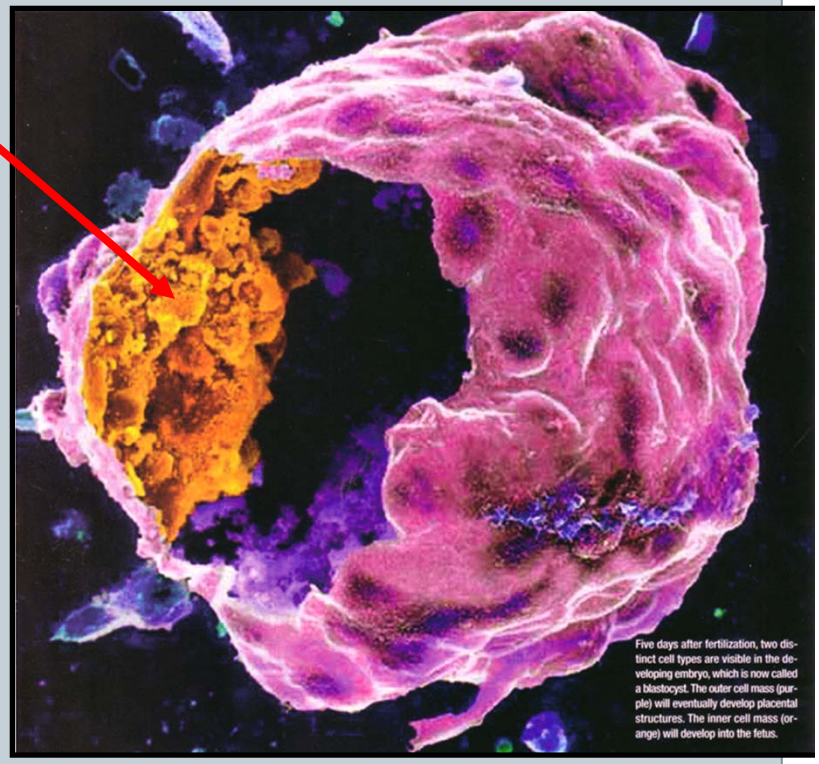


What kinds of stem cells are there?

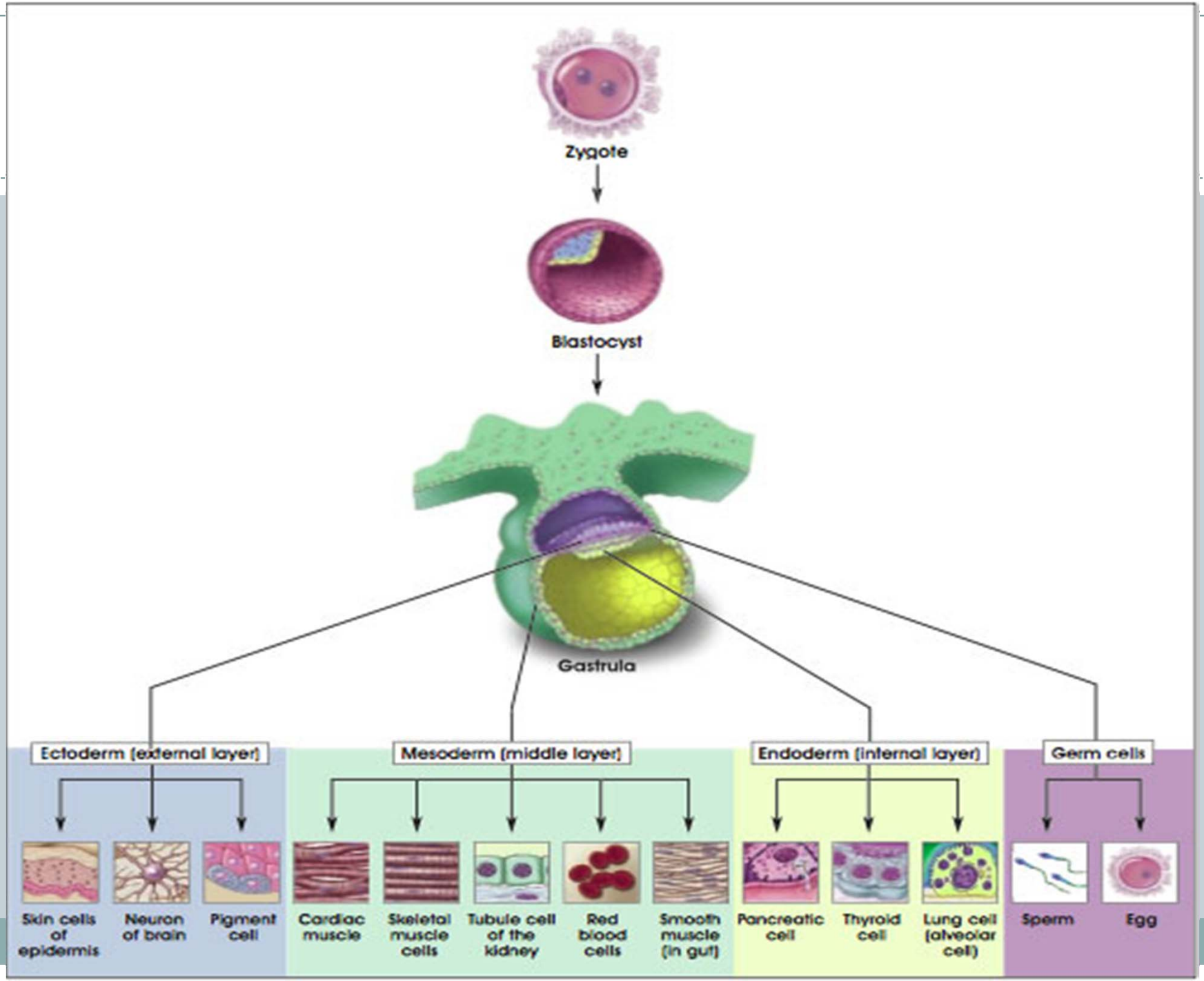
- Embryonic stem cells
- Adult stem cells
- Embryonic Germ Cells
- Somatic Cell Nuclear Transfer (SCNT)
- Induced pluripotent stem cells

Embryonic Stem Cells

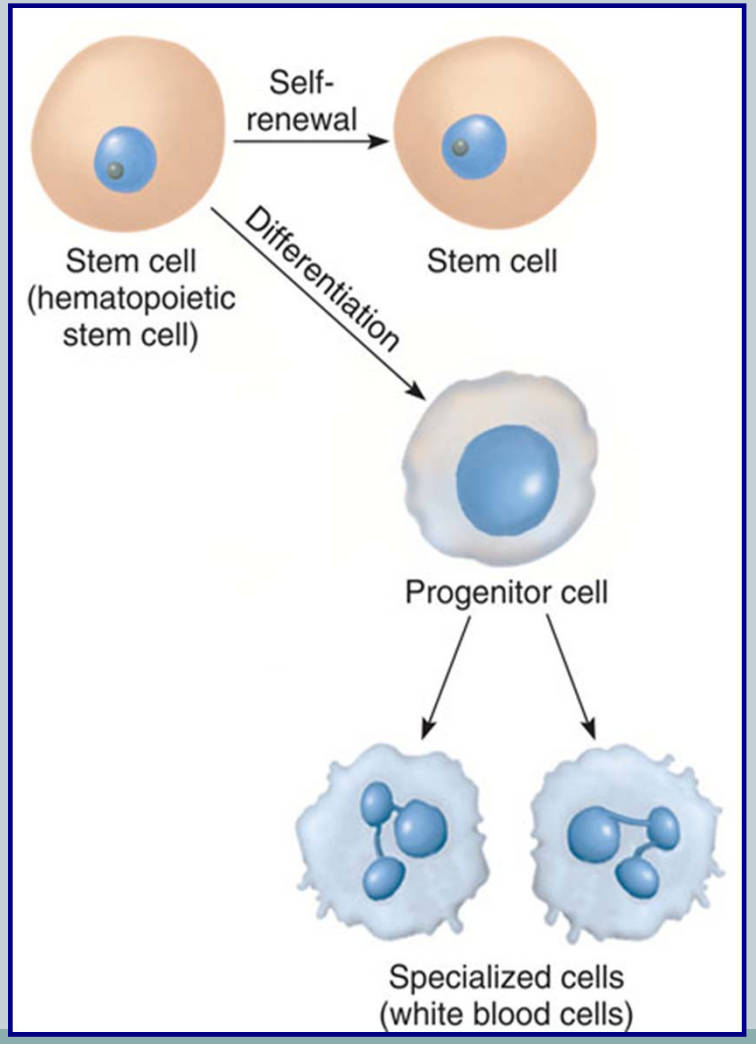
- Appear day 5 as inner cell mass of blastocyst
- Pluripotent: able to differentiate into any of the three cellular germ layers:
- Will ultimately form entire embryo



http://embryology.med.unsw.edu.au/Notes/images/wk2.gif


Zygote
Blastocyst
Gastrula
Ectoderm (external layer)
Mesoderm (middle layer)
Endoderm (internal layer)
Germ cells
Skin cells of epidermis
Neuron of brain
Pigment cell
Cardiac muscle
Skeletal muscle cells
Tubule cell of the kidney
Red blood cells
Smooth muscle (in gut)
Pancreatic cell
Thyroid cell
Lung cell (alveolar cell)
Sperm
Egg

Adult Stem Cells

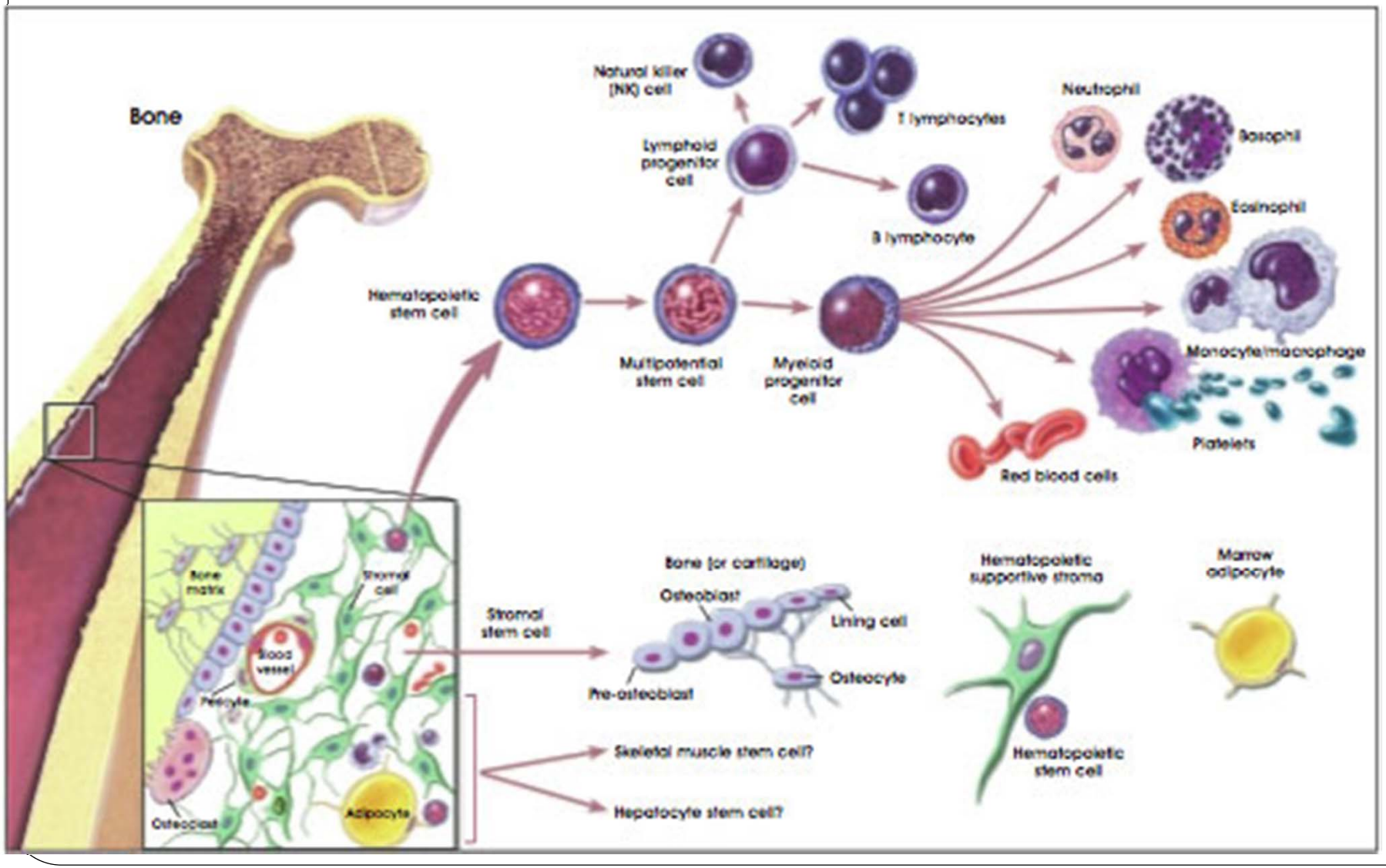


- Single Cell that self renews and generates differentiated cells of multiple types
- Found in multiple tissue and organ types
  - Bone marrow
  - Muscle
  - Brain
  - Internal Organs
  - Skin
  - Fat
  - Eye
- Activated in response to tissue damage

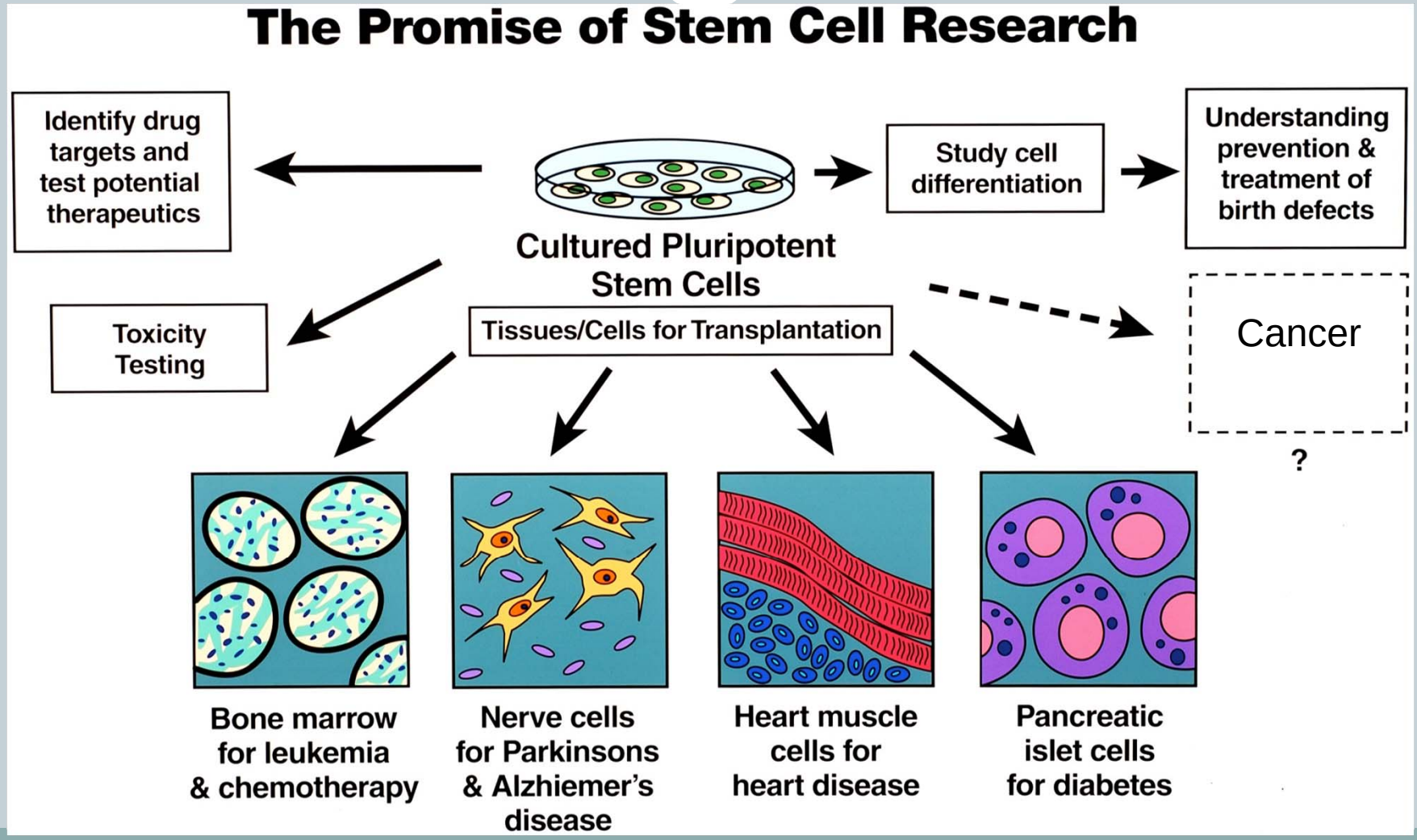
Reservoirs of Post Natal Stem Cells

Tissues of Rapid Self Renewal	Tissues of limited self renewal
• Blood	• Muscle
• Gut	• Dental Pulp
• Skin	• Fat
	• Neural

5 Billion Blood Cells Produced/Day



Regenerative Medicine:



http://stemcells.nih.gov/info/media/

In Vitro Culture and Control of Stem Cells Required for therapy

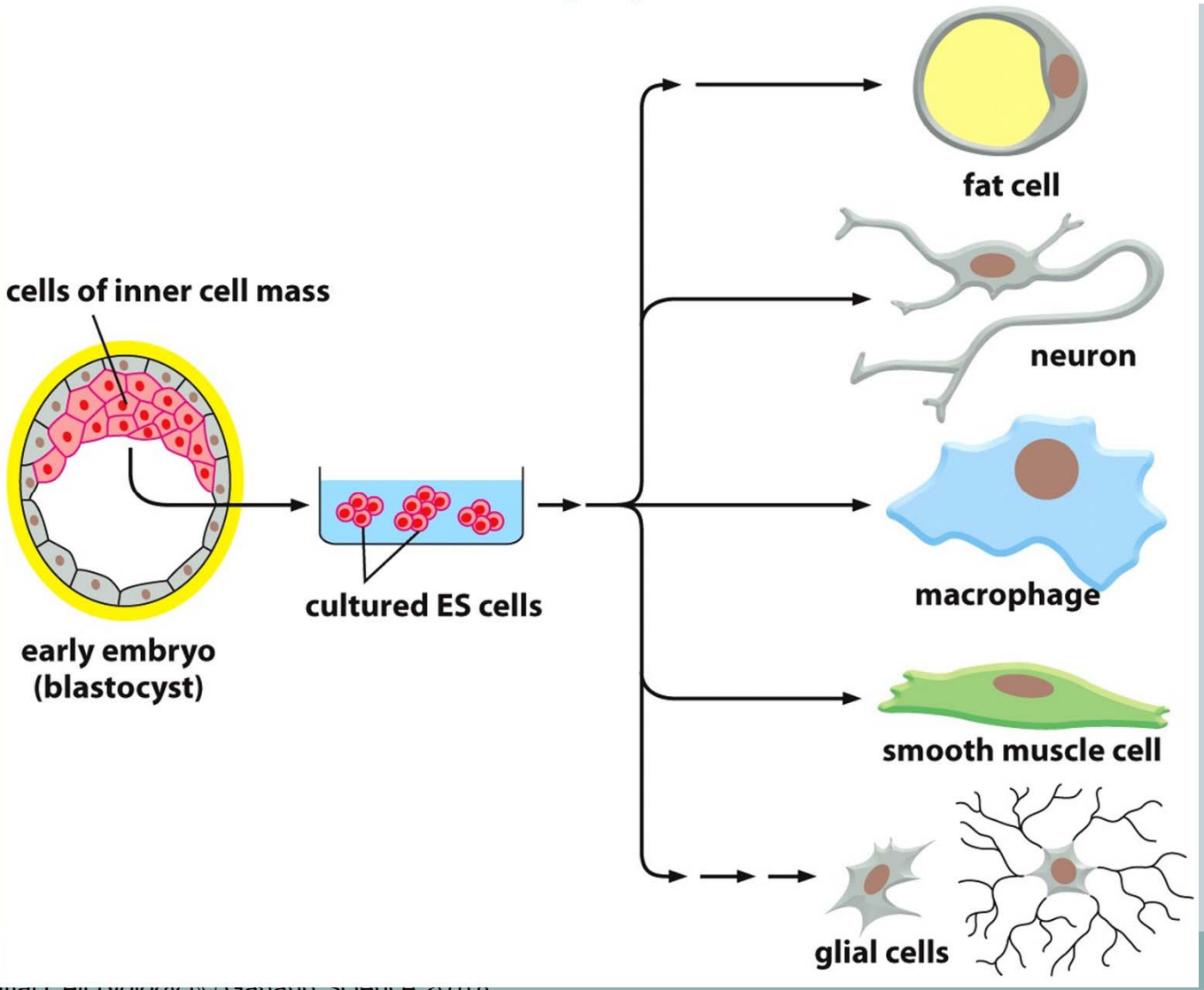


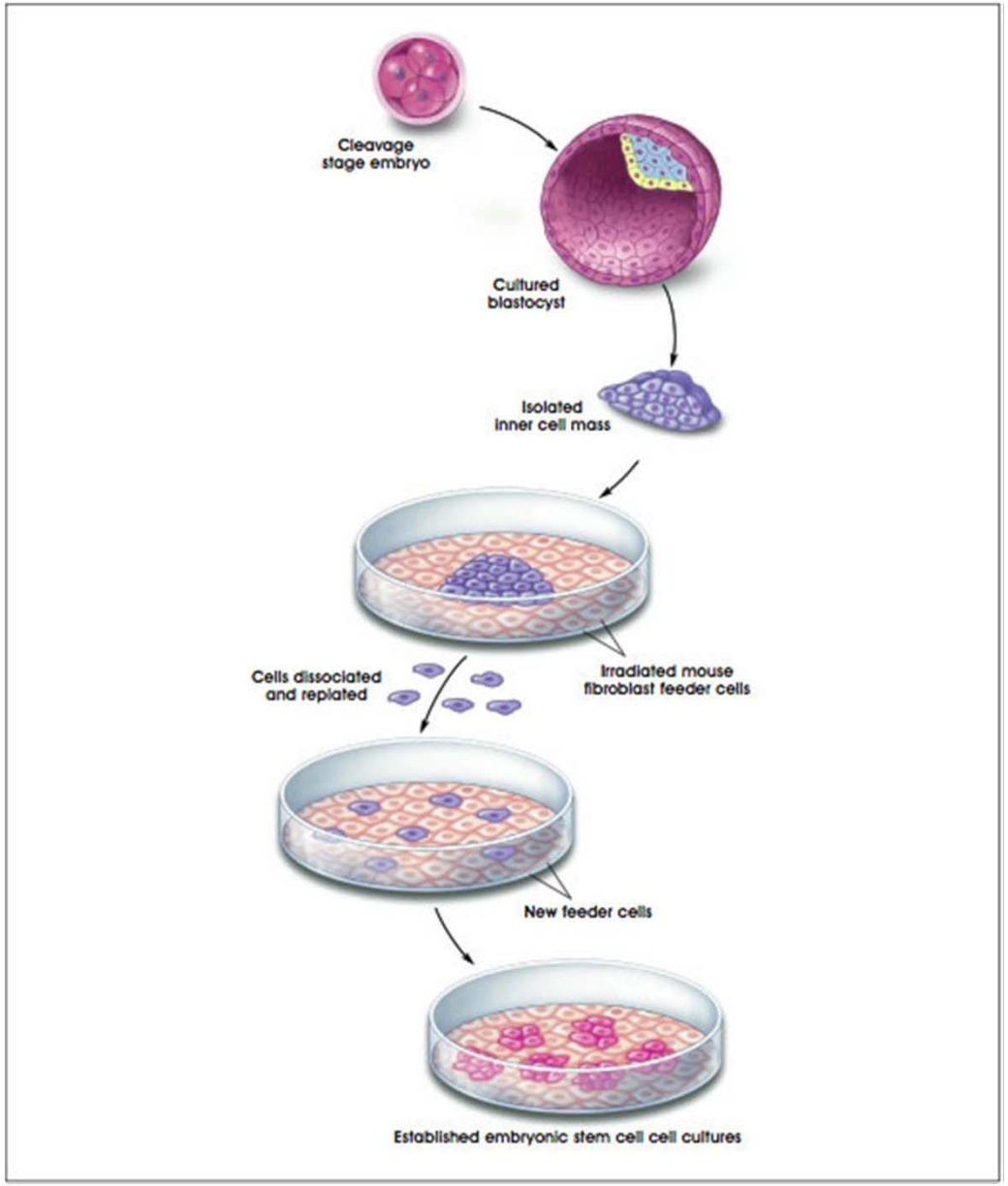
Figure 20-35 *Essential Cell Biology* (© Garland Science 2010)

Why Embryonic Stem Cells?

- Difficult to obtain and expand most adult stem cell populations
- Difficult to procure eggs for SCNT and ethical concerns about cloning
- IPS cells may not be good candidates for regenerative medicine
- Need to increase genetic diversity among existing stem cell pool
- Need to understand basic developmental biology

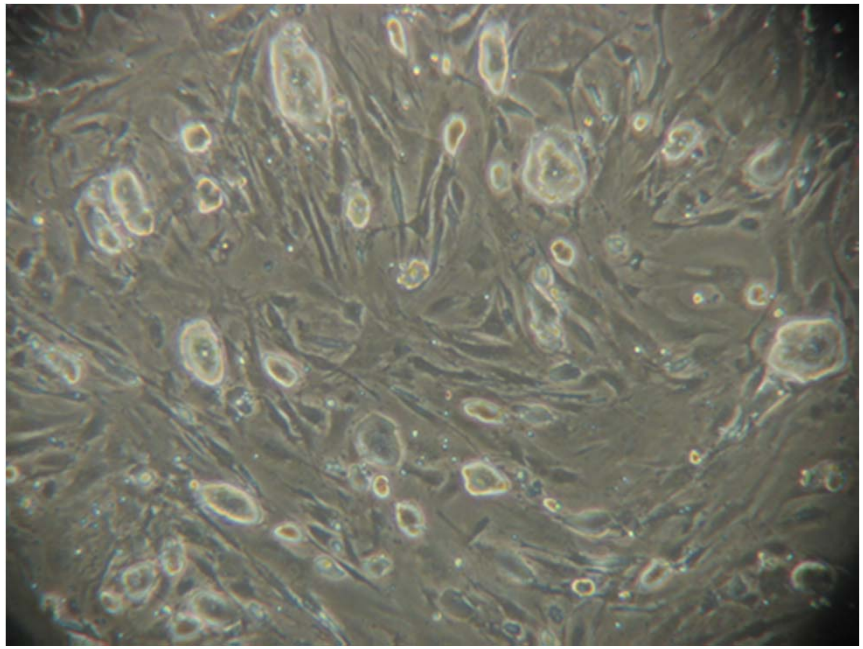
Embryonic Stem Cell Culture

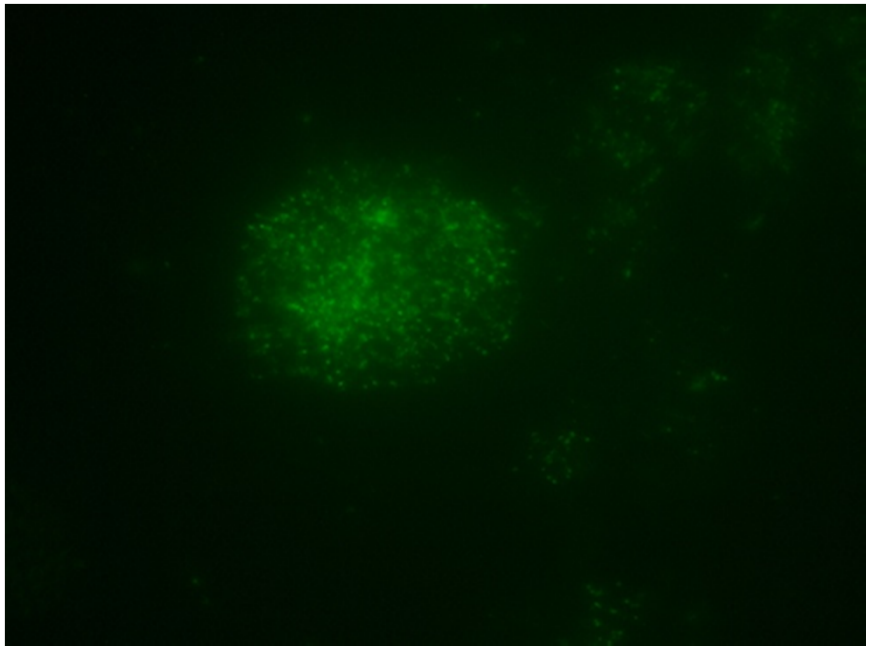
- 1984: Sir Martin Evans cultures mouse embryonic stem cells
- 1998: James Thomson cultures first human embryonic stem cell from the inner cell mass of a day 5.o human blastocyst
- 2008: Human Embryonic Cultures Isolated from a single blastomere



http://stemcells.nih.gov/StaticResources/info/scireport/images/figurec1.jpg

J1 Murine Embryonic Stem Cell Cultures from the BIOL480 Stem Cell Class





Cell Stem Cell
Correspondence

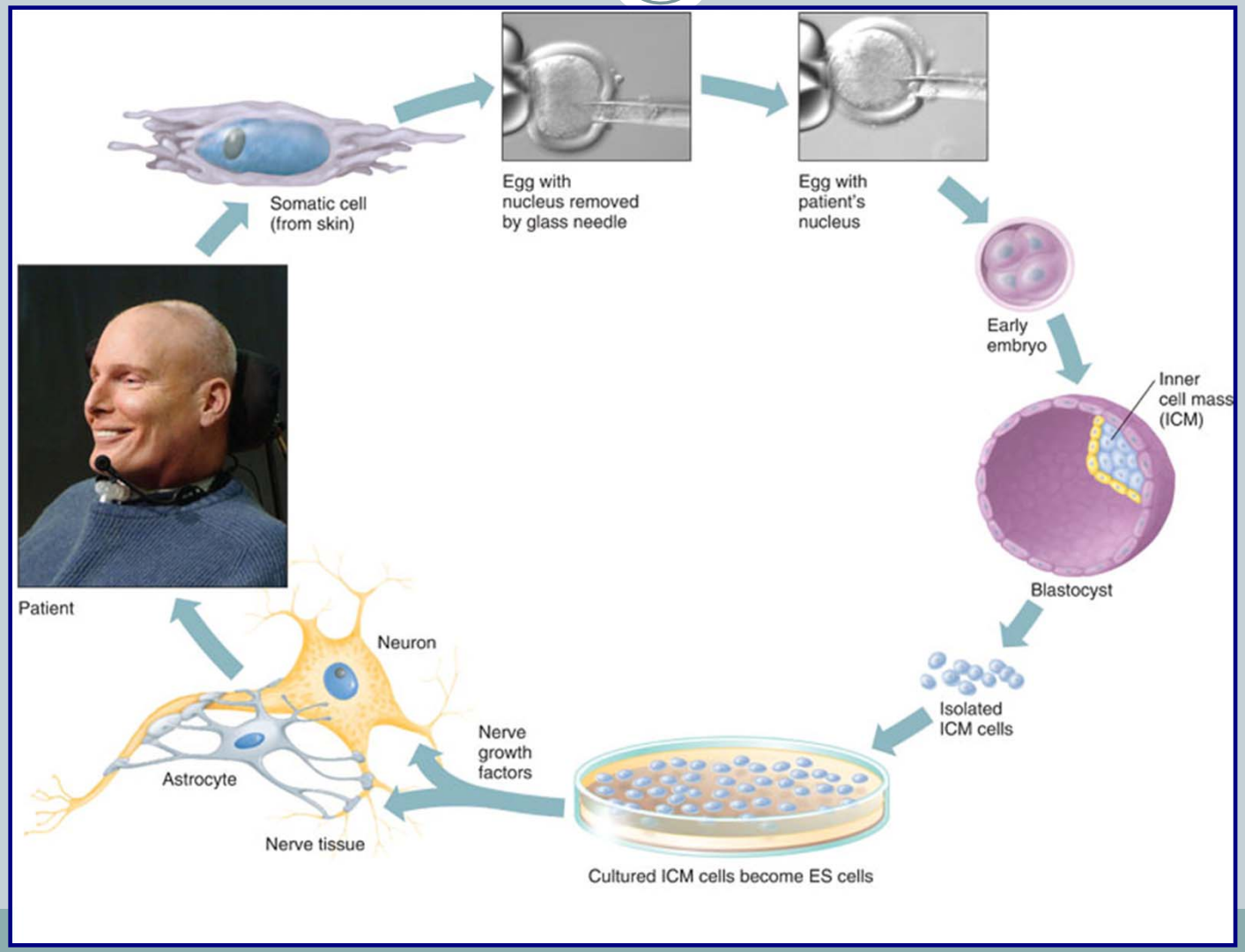
Human Embryonic Stem Cell Lines Generated without Embryo Destruction

Young Chung,[1,6] Irina Klimanskaya,[1,6] Sandy Becker,[1] Tong Li,[1] Marc Maserati,[1] Shi-Jiang Lu,[1] Tamara Zdravkovic,[2] Dusko Ilic,[3] Olga Genbacev,[2] Susan Fisher,[2,4] Ana Krtolica,[3] and Robert Lanza[1,5,*]

http://www.scientificamerican.com/media/inline/65A632E6-0816-AD4F-AE94975827A78F8E_1.jpg

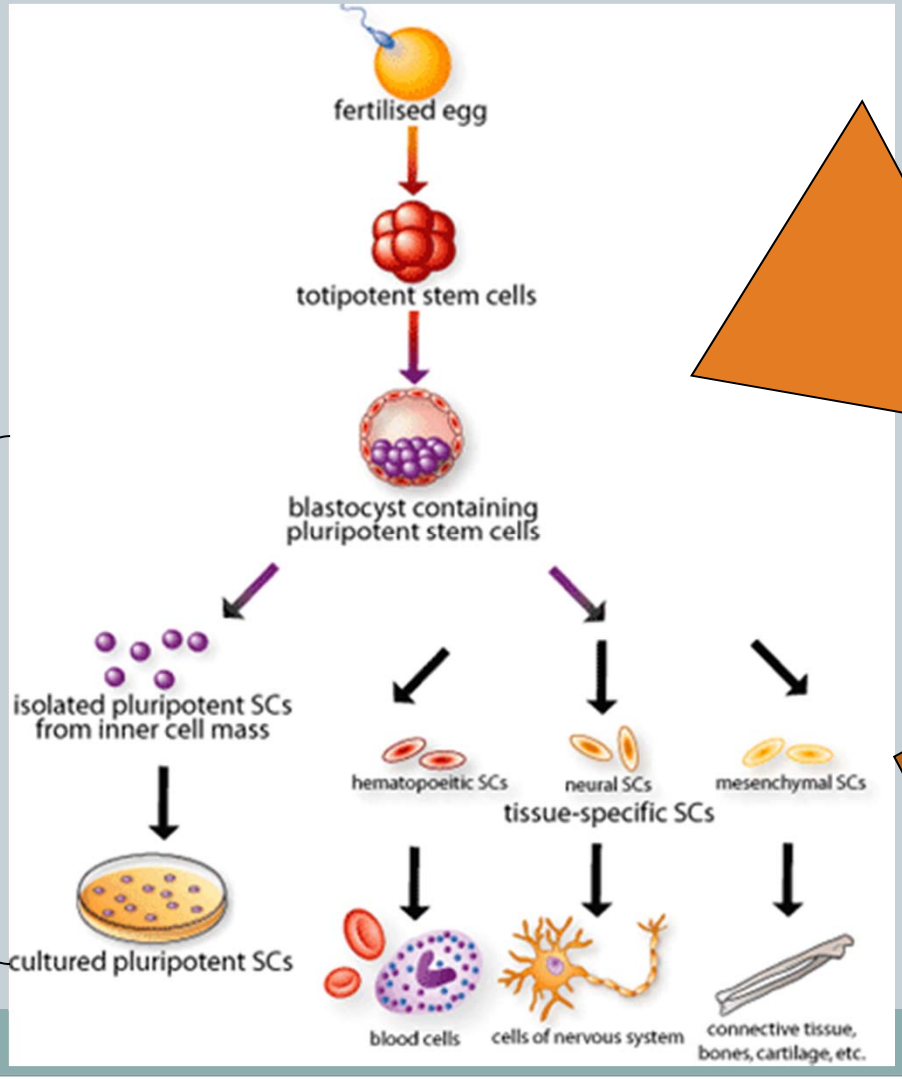
Cell Stem Cell 2008

SCNT + Stem Cell Technology



Induced Pluripotent Stem Cells:

Expression of factors uniquely expressed in ESC in terminally differentiated, somatic cells can induce them to become pluripotent

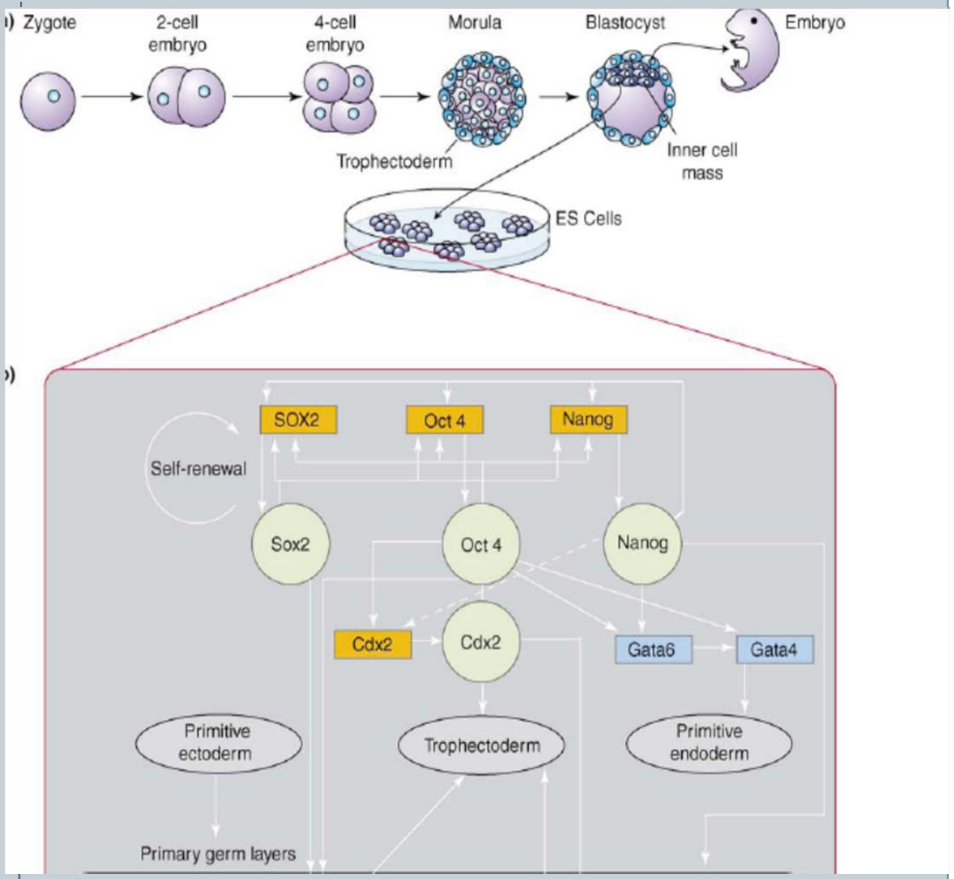


Embryonic Stem Cells

iPSC

Genetic Programs are Associated with Embryonic Stem Cells

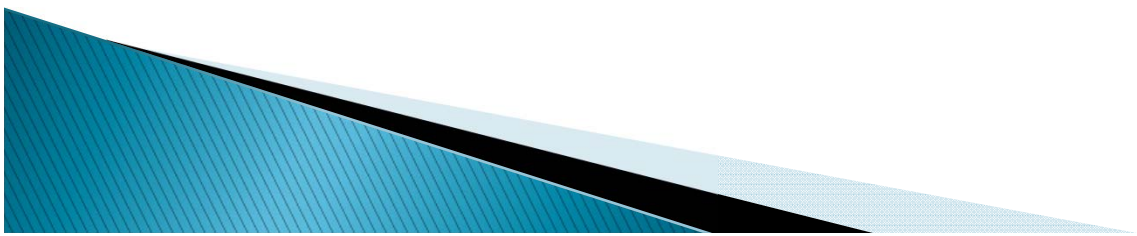
- "Pluripotency Genes" regulate self renewal and potency state of embryonic stem cells



Current Opinion in Genetics & Development 2006, 16:455–462

ESC Behaviors are Regulated by a complex balance of antagonistic signaling pathways

- Self Renewal
- Mainenance of Pluripotency
- Proliferation
- Inhibition of differentiation



Signal Transduction Cascades Regulate Cellular Behaviors

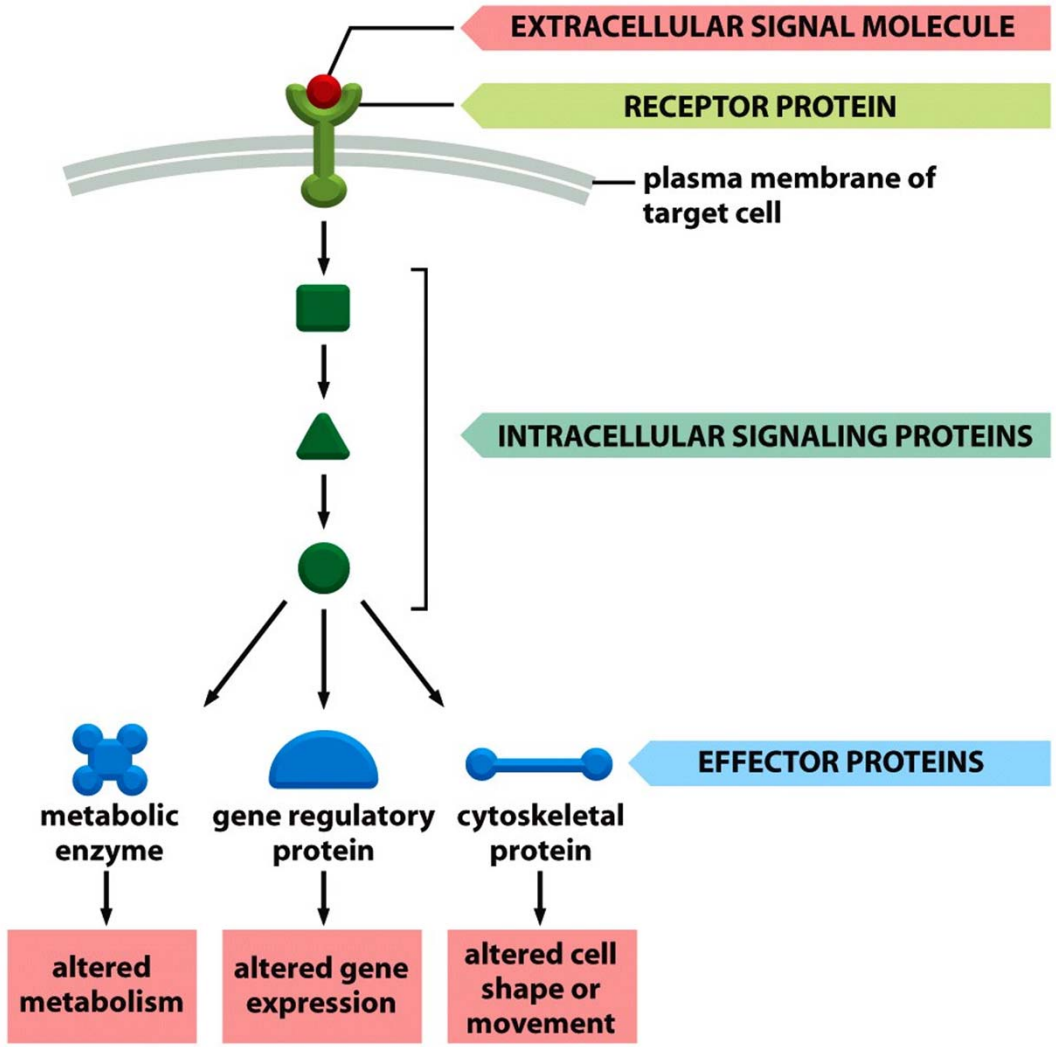
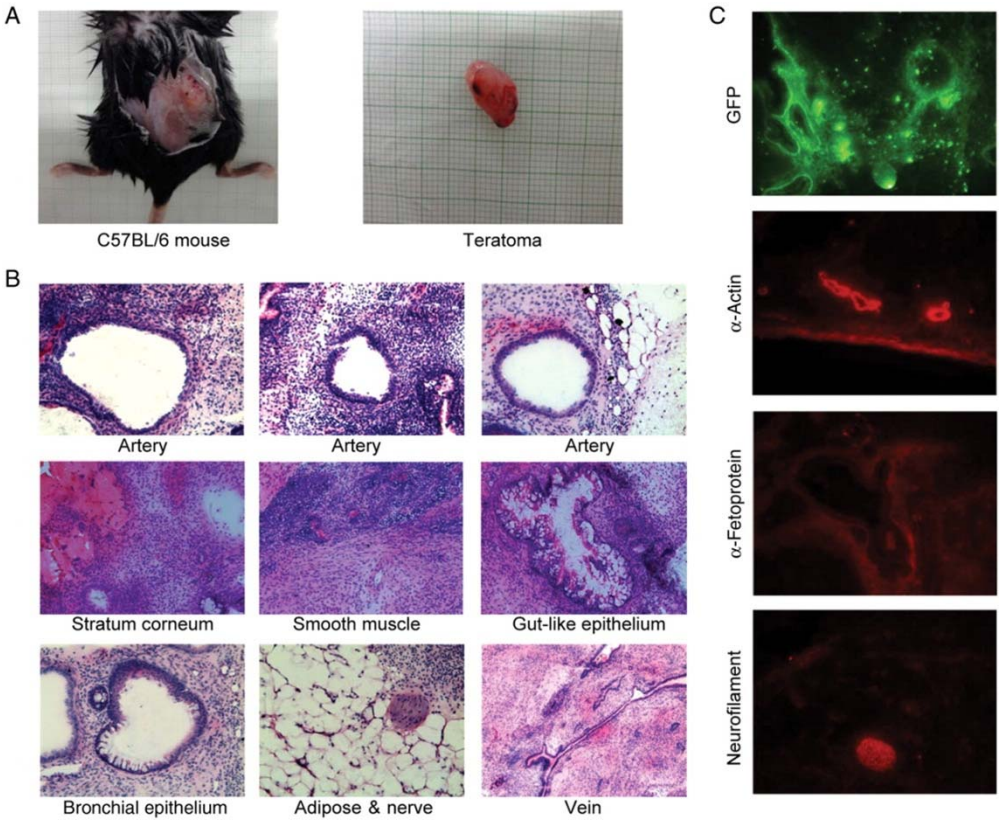


Figure 15-1 *Molecular Biology of the Cell* (© Garland Science 2008)

Uncontrolled Self Renewal/Cell Fate leads to Heterogeneous Tumors

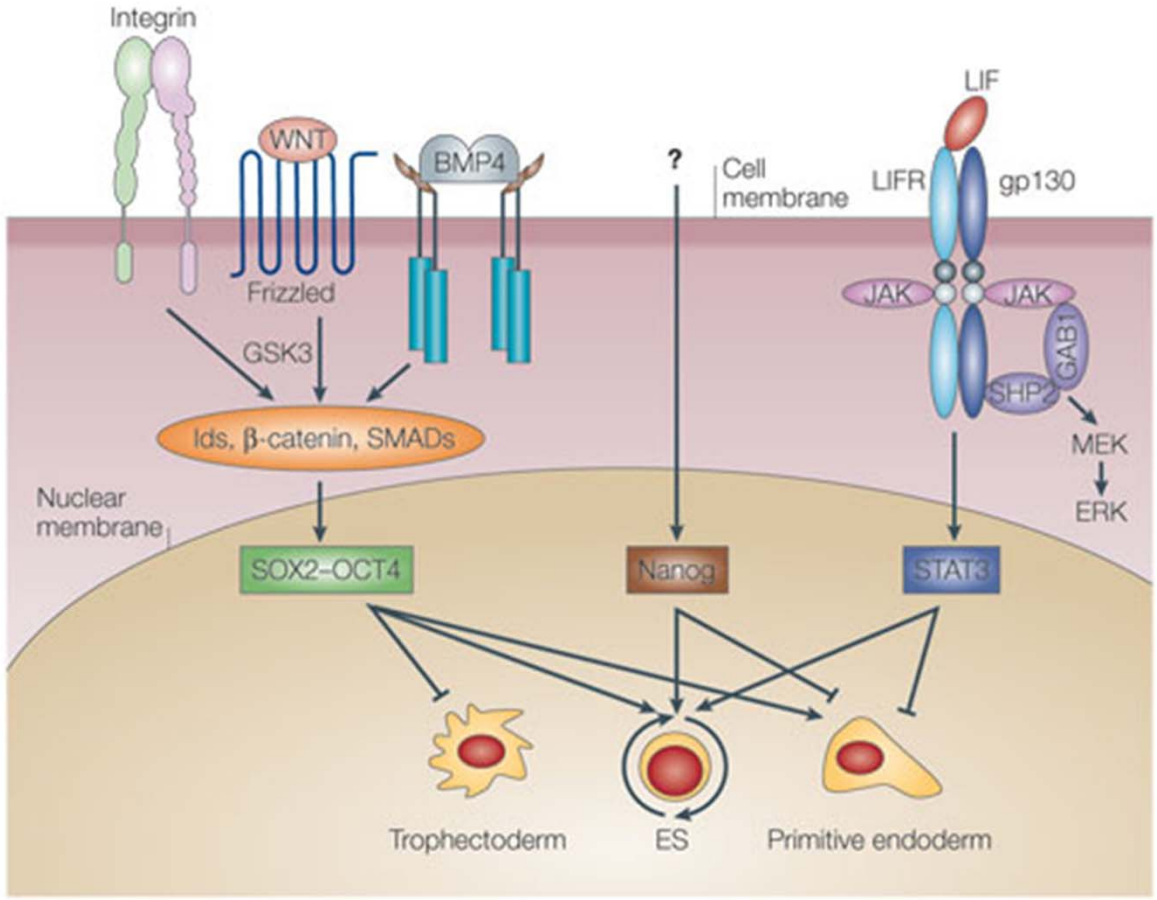
Teratoma formation of BM-M-iPS cells derived from 21-month-old C57BL/6 mice.



Cheng Z et al. J Mol Cell Biol 2011;jmcb.mjq044

Journal of Molecular Cell Biology

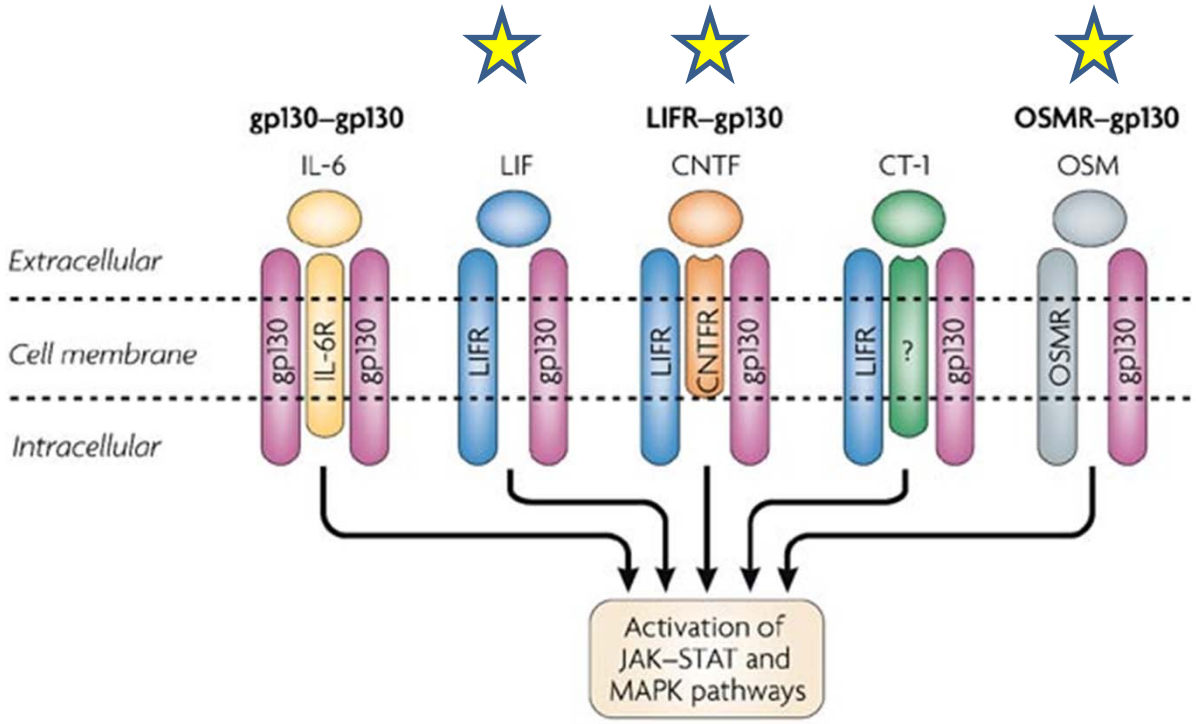
Evidence Implicates Multiple Signaling Cascades in ES Self Renewal



Stat 3 Activity Regulated at Many Levels

- Receptor Availability
- JAK Activation
- SHP2 (Src2 Homology Containing phosphatyrosine phosphatase protein)
- SOCS(Suppressor of Cytokine Signaling)
- PIAS3 (Protein inhibitor of activated Stat3)

Gp 130 Activation is required to maintain pluripotency of murine ESC in vitro



Bauer *et al. Nature Reviews Neuroscience* **8**, 221–232 (March 2007) | doi:10.1038/nrn2054

nature REVIEWS NEUROSCIENCE

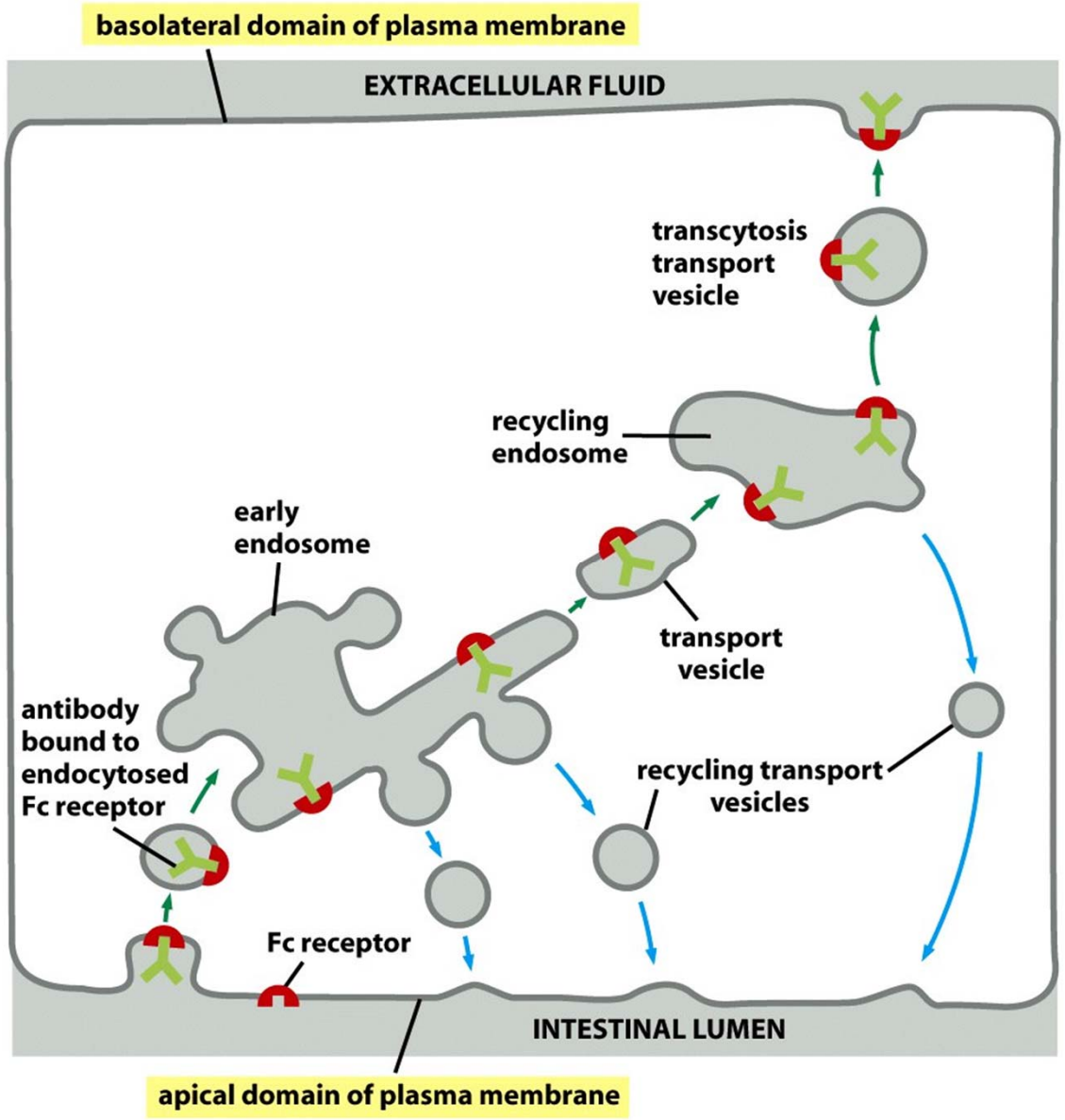


Figure 13-60 *Molecular Biology of the Cell* (© Garland Science 2008)

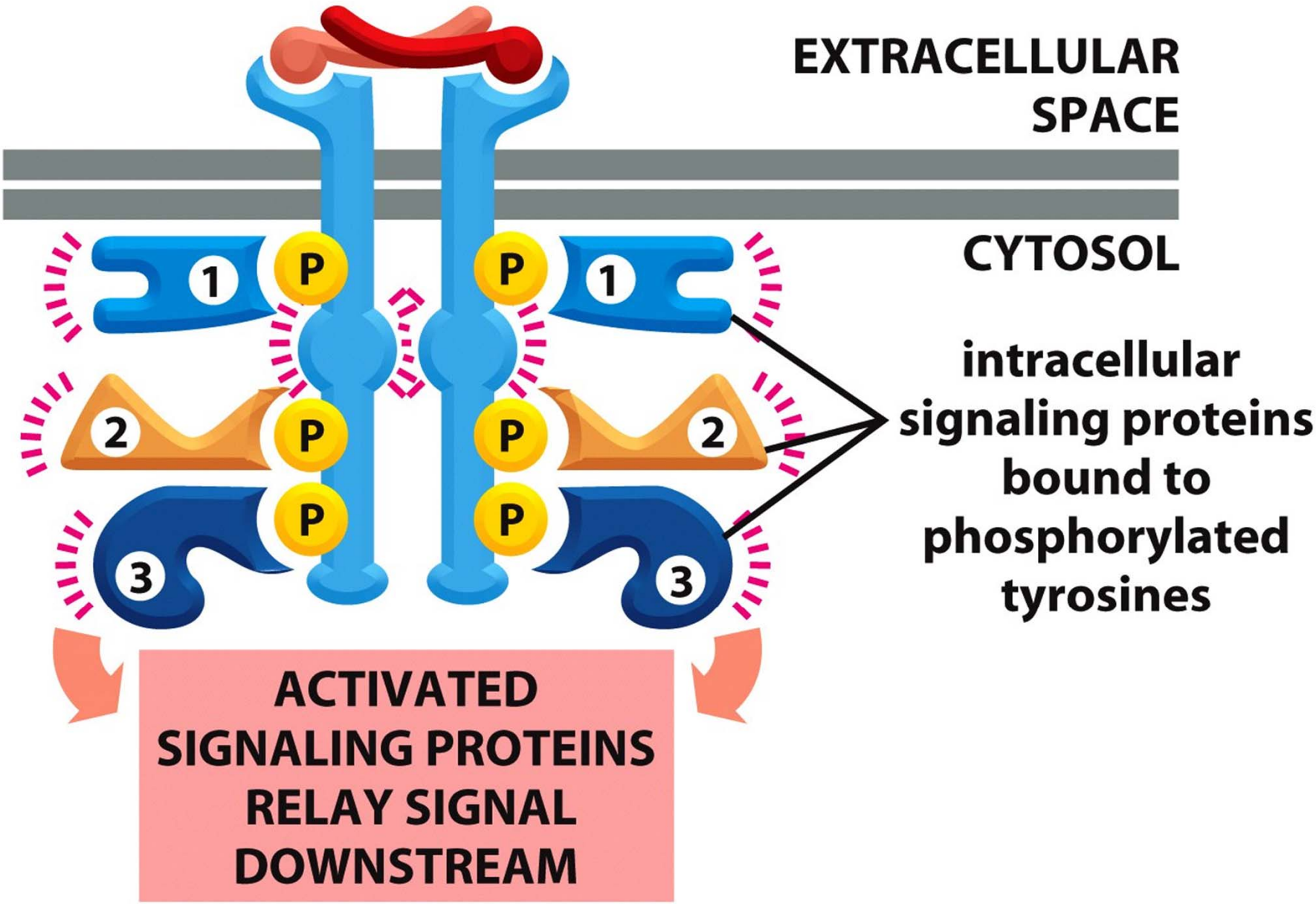
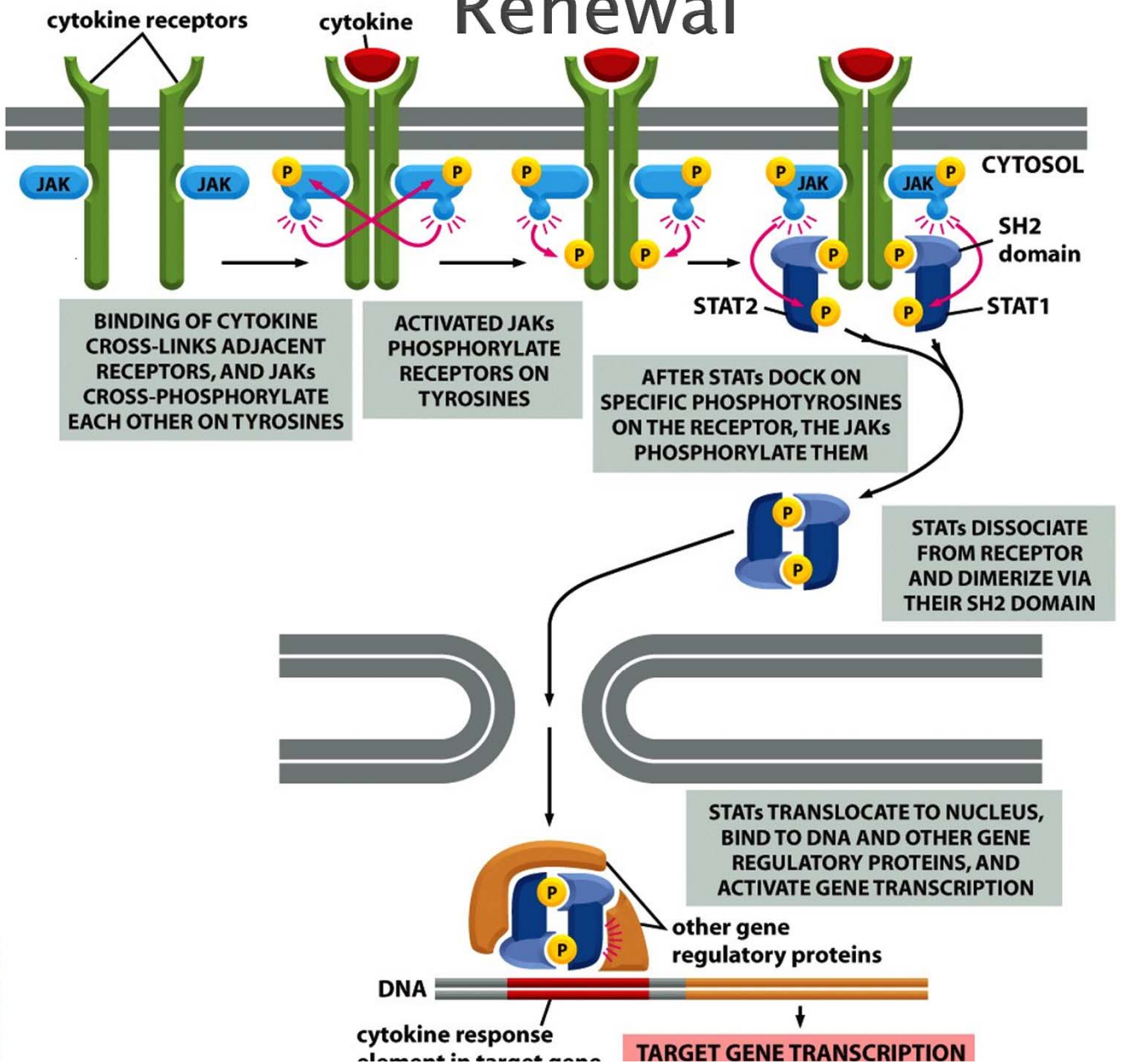
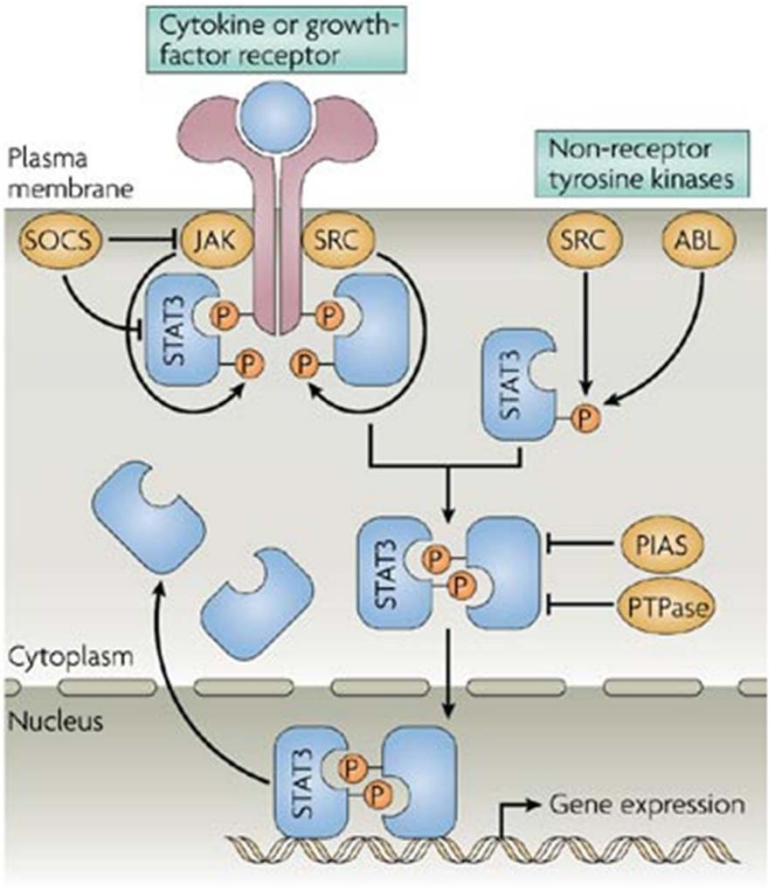


Figure 15-54 *Molecular Biology of the Cell* (© Garland Science 2008)

JAK/STAT Pathway Promotes Self Renewal

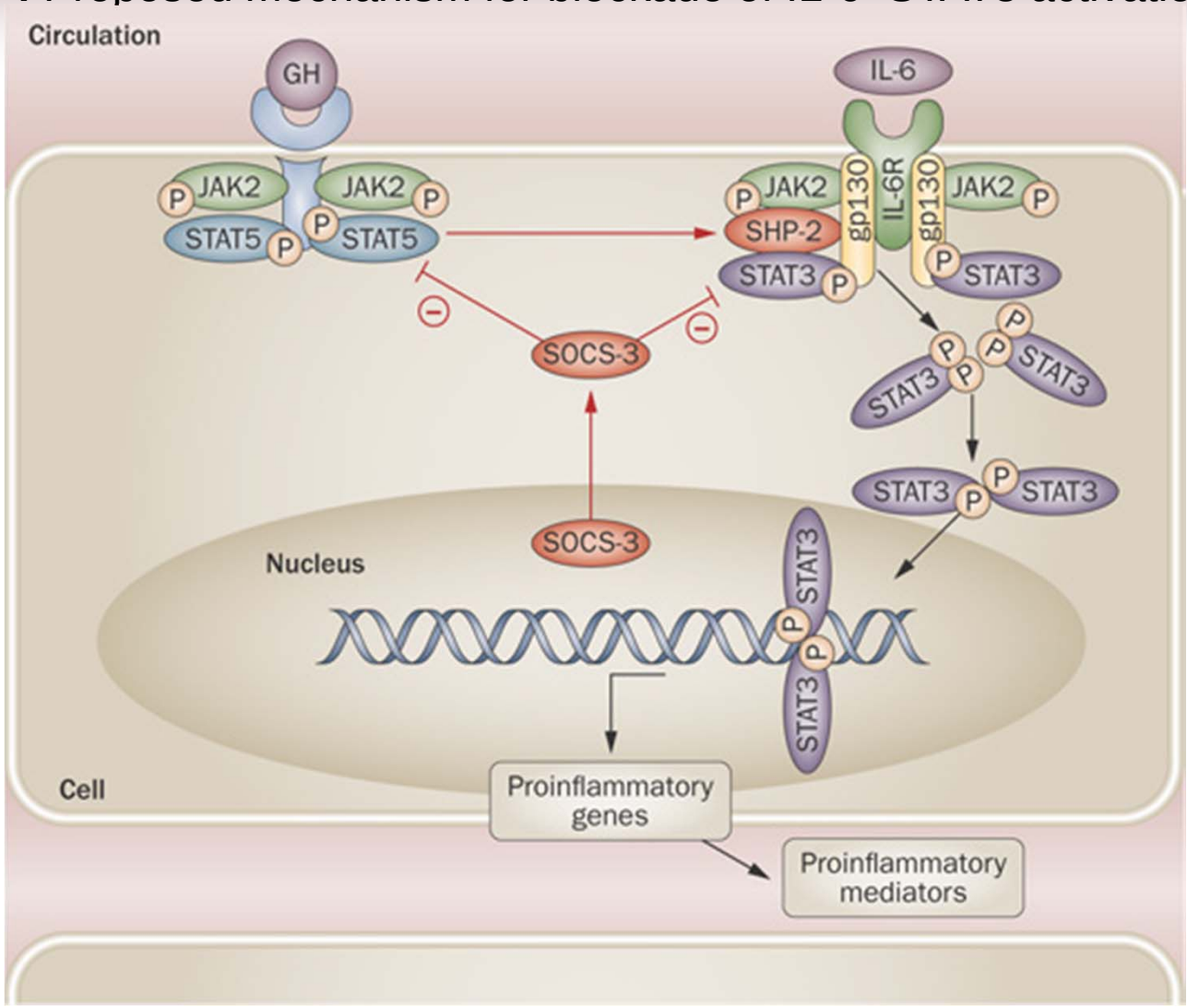


Activation of Stat3 by Tyr 705 Phosphorylation Maintains ESC Self Renewal



Yu *et al*. *Nature Reviews Immunology* **7**, 41–51 (January 2007) | doi:10.1038/nri1995

Figure 4 Proposed mechanism for blockade of IL-6–STAT3 activation by GH



Walters, T. D. and Griffiths, A. M. (2009) Mechanisms of growth impairment in pediatric Crohn's disease
Nat. Rev. Gastroenterol. Hepatol. doi:10.1038/nrgastro.2009.124

nature REVIEWS GASTROENTEROLOGY & HEPATOLOGY

How is Stat3 Regulatory Activity Controlled to regulate ESC Self Renewal?

Sensitivity Analysis of Intracellular Signaling Pathway Kinetics Predicts Targets for Stem Cell Fate Control

Alborz Mahdavi[1,2], Ryan E. Davey[2], Patrick Bhola[2], Ting Yin[1], Peter W. Zandstra[1,2*]

1 Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada, **2** Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada

Homo or Heterodimerization of gp130 Activates two Signaling Cascades

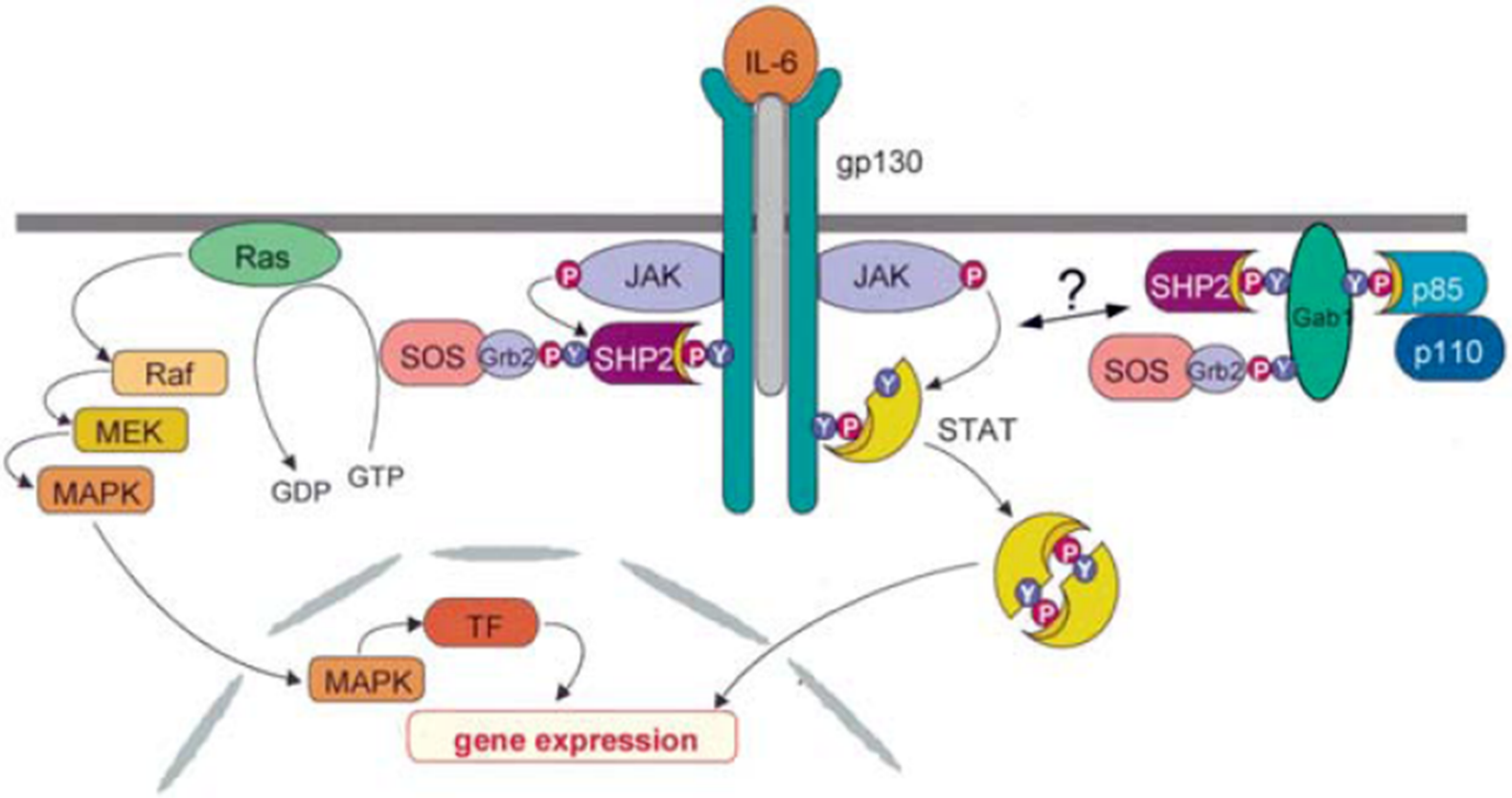
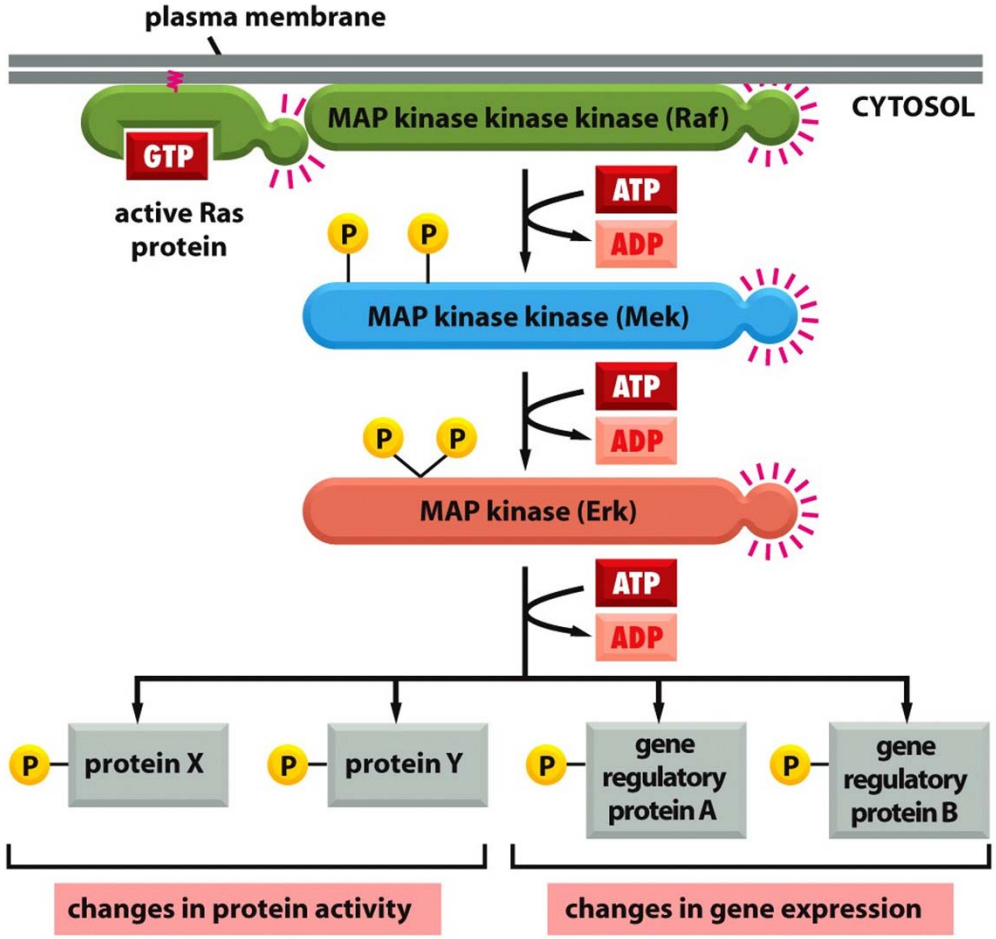


Figure 1 IL-6 activates the JAK/STAT pathway and the MAPK cascade

Representation of the two major pathways activated by IL-6-type cytokines. TF, transcription factor.

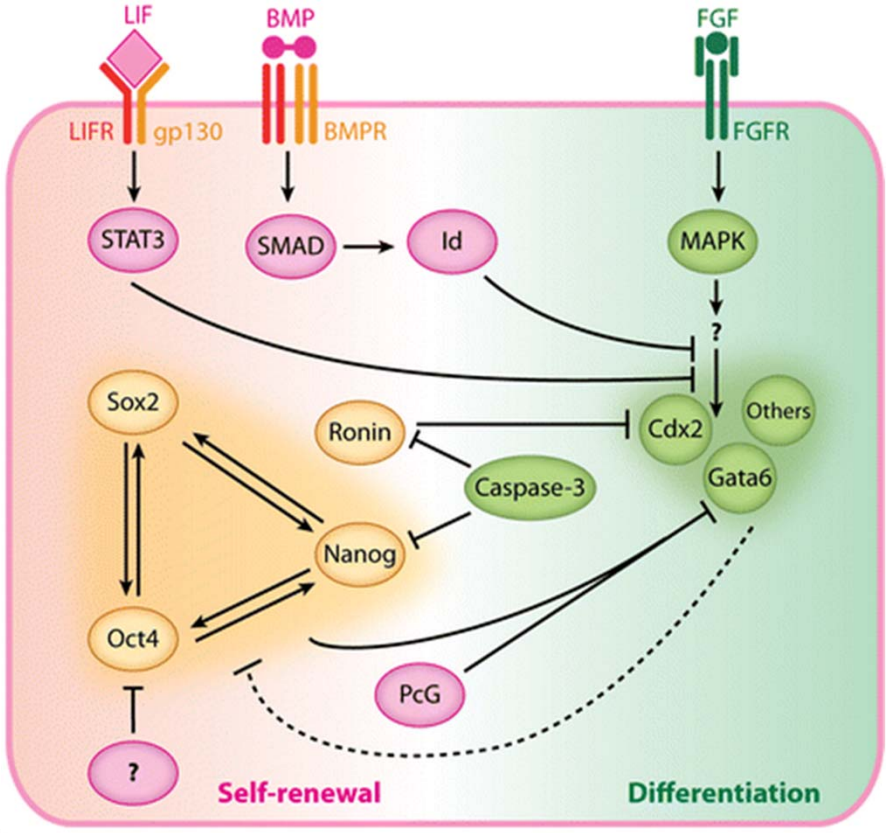
Heinrich et al 2003 Biochem J. 374 1–20

MAPK Signaling SHP/ERK Signaling Inhibits Self Renewal and Required for Differentiation



How are these Signaling Networks Controlled to Regulate Self Renewal/vs. Differentiation?

What is the consequence of loss of control?

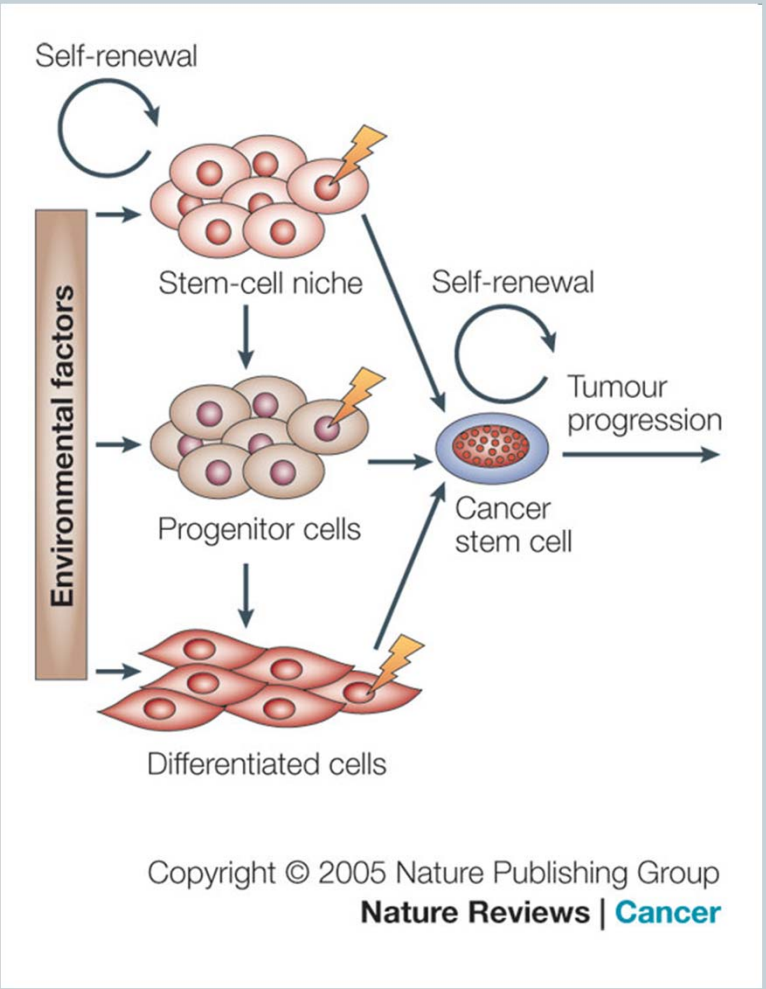


He S, et al. 2009.
Annu. Rev. Cell Dev. Biol. 25:377–406

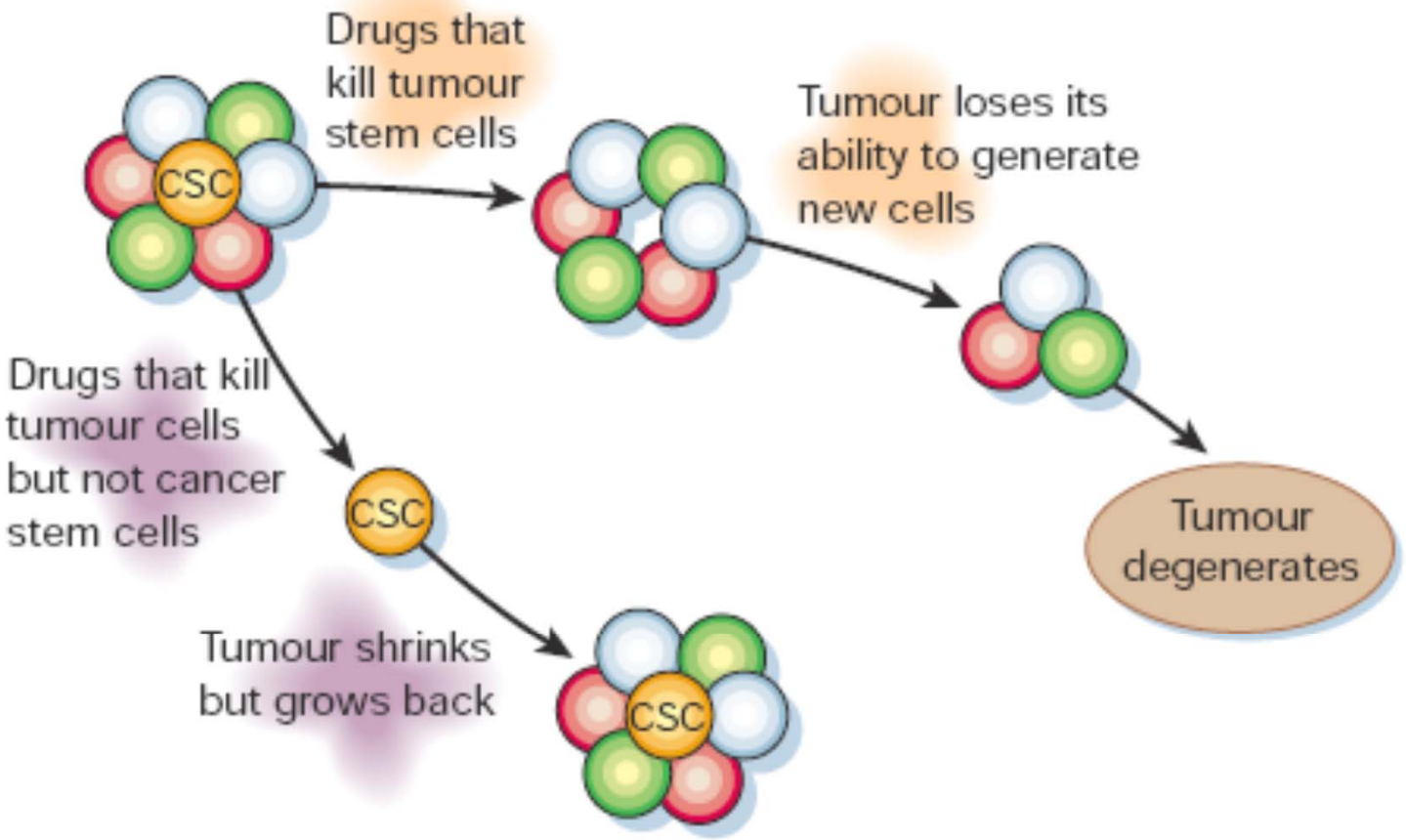
The Cancer-Stem Cell Hypothesis

Each tumor contains a subset of cancer stem cells that are uniquely responsible for tumor growth, heterogenetity, and metastasis

Cancer Stem Cell is a single cancer cell that can form a tumor following transplantation

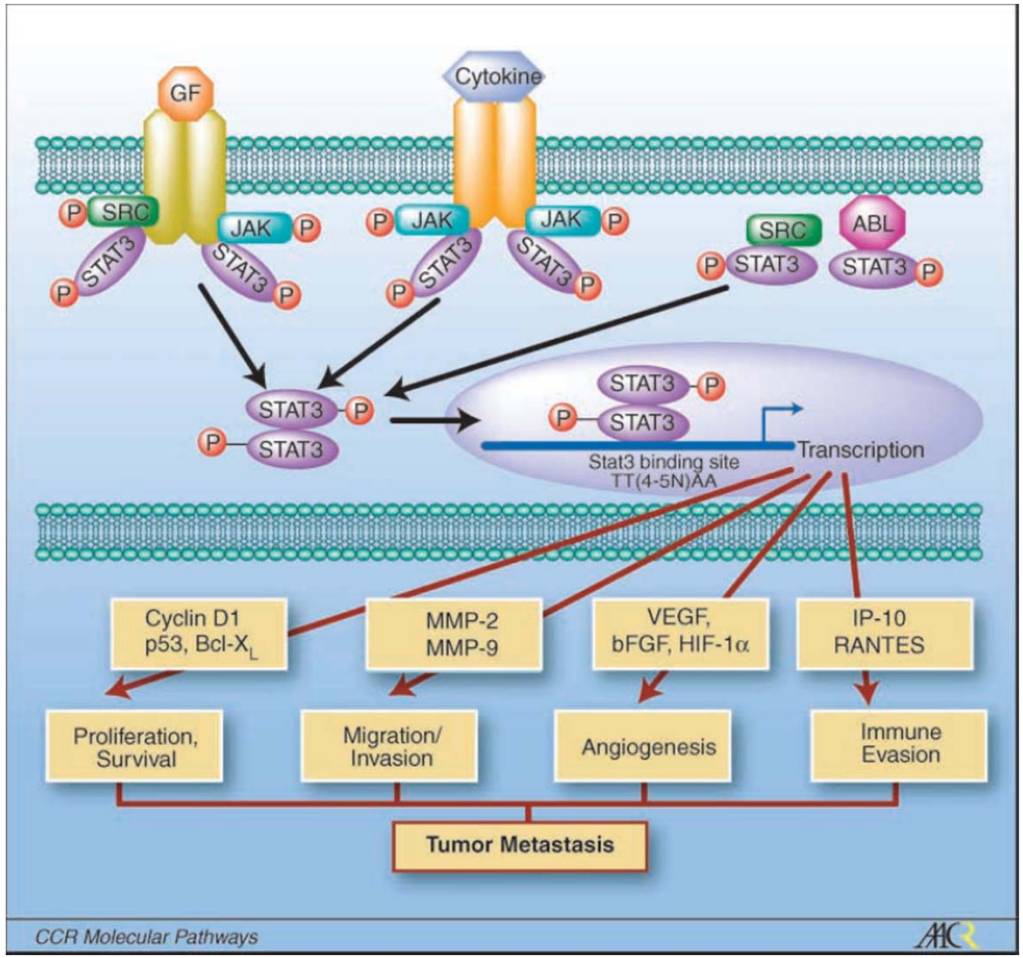


Identification and Treatment of Cancer Stem Cells Could have Profound Clinical Impacts

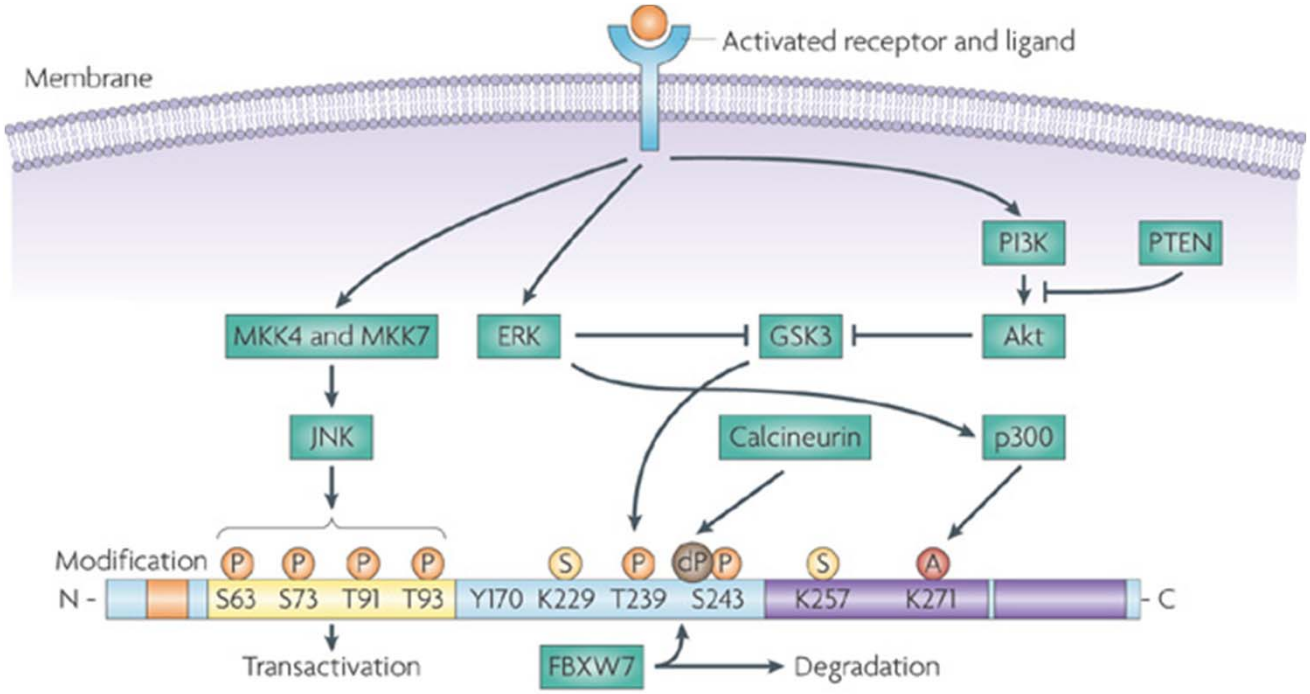


Reya et al. Nature 2001 vol:414 iss:6859 pg:105

STAT3 Promotes Tumor Metastisis



Many components of MAPK signaling are oncogenes

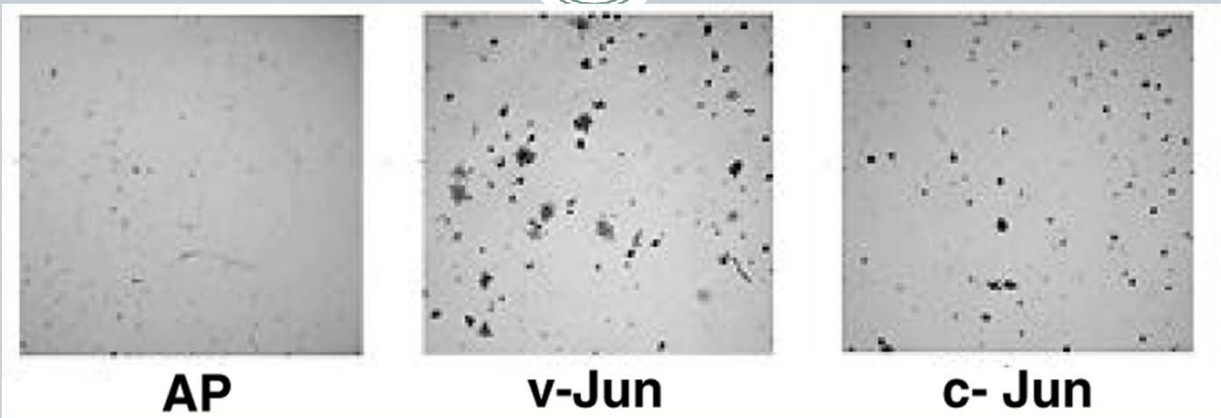


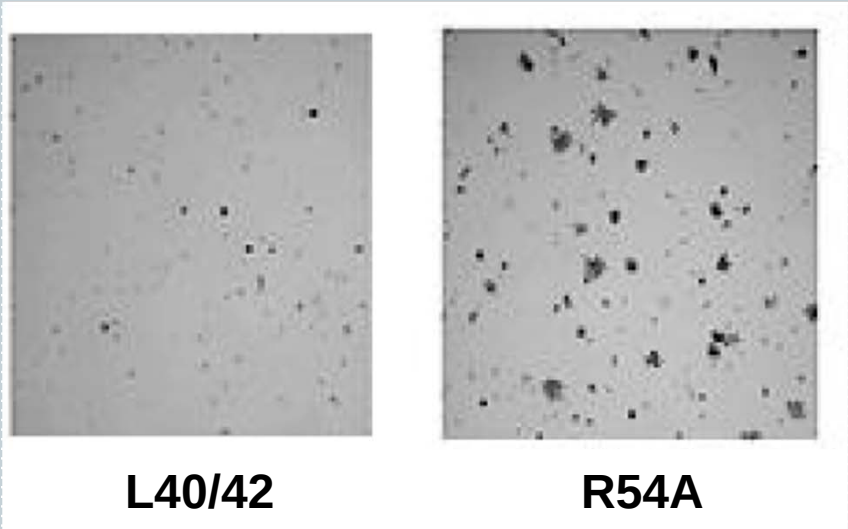
Nature Reviews | Cancer

cJun

- An immediate early gene instrument in initiating the cell cycle primarily through the up-regulation of genes like Cyclin D1.
- Discovered as oncogenic variant, vJun
- It functions in many pathways involved in stem cell self renewal and cellular proliferation

Jun is a Protooncogene





Sprowles and Wisdom 2003

Research Objectives

Test the role of cjun in embroyonic stem cell pluripotency and differentiation

Natalie Grace, Faith Gomes, Diqui Lapenta, Kelly Roelf

Test the role of pluripotency markers in cells overexpressing cJun variants

Lora Davis, Bryan Machado, Nate Meyer, Tawny Neal

Test the role of cjun in embroyonic stem cell pluripotency and differentiation

Hypothesis:

Oncogenic variants of the c Jun oncogene will help maintain the pluripotent state of murine embryonic stem cells

Alternative Hypothesis:

Oncogenic variants of the c Jun oncogene will lead to cellular differentiation of murine embryonic stem cells.

Methods

- Generate stable murine embryonic stem cell lines overexpressing cJun oncogenic and suppressive mutants
- Characterize the pluripotent state of each cell line as compared to normal murine embryonic stem cells
  - Morphology
  - Expression of alkaline phosphotase
    - Expressed in EMS cells
  - Expression of genes expressed in pluripotency
    - Oct 4 & Nanog
- Characterize the differentiation potential of these cell lines

Test the role of pluripotency markers in cells overexpressing cJun variants

- Hypothesis 1:

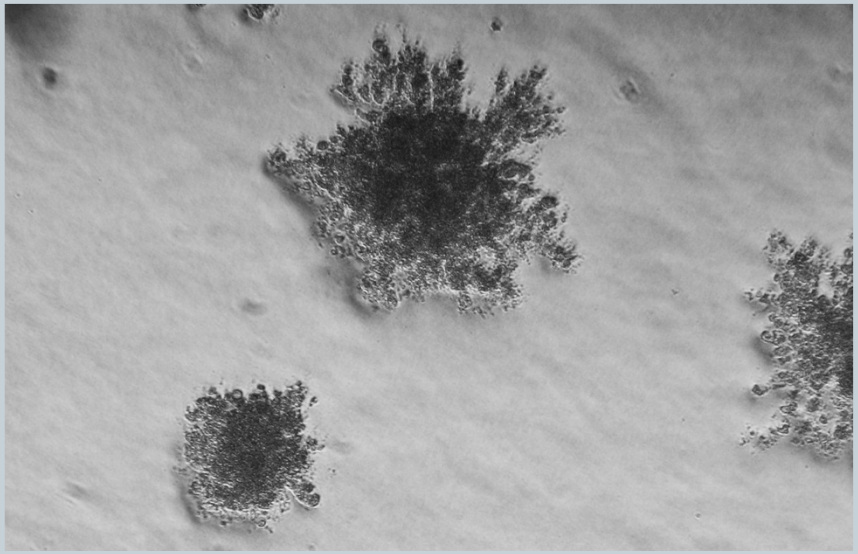
  Cells transformed by cJun will show upregulation of pluripotency markers

Hypothesis 2:

Cellular transformation by cJun requires expression of pluripotency markers

Methods

- Transform CEF cells with cJun variants
- Test for pluripotency markers
  - Zic3
  - cMyc
  - Oct4
  - Nanog
  - Telomerase
- Test role of pluripotency markers in cellular transformation



Nate Meyer 2010