1 Kapitel: Eigenschaften von Stammzelllinien

Was ist eine Stammzelllinie?

1.1. Selbsterneuerungsfähigkeit (self renewal capacity)

= nicht endend wollende Teilung zu Tochterzellen (TZ)

mit identischen Potenzial und Eigenschaften wie Mutterzelle (MZ).

Derzeit Passagen (P) > 150; somatische Zellen werden spätestens bei Passage P=80 senescent.

1.2. Klonalität, Klonierbarkeit (clonality)

= Fähigkeit als einzelne Zelle zu überleben, sich zu vermehren,und den Mutterphänotyp zu erhalten. (In vivo sind bei den Stammzellen wohl immer Helfer- oder Nischenzellen notwendig dabei.)

1.3. Differenzierungspotenzial (potency of differentiation)

= Anzahl der Möglichkeiten in somatische Zellen zu differenzieren

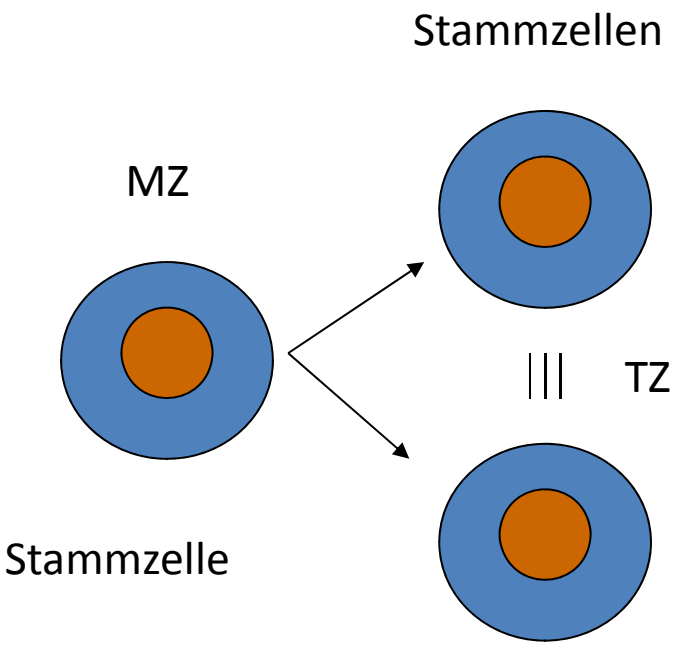
dazu kommt die Differenzierungskontrolle (große Unterschiede bei den Stammzellarten)

1.4. Ruhezustand (dormancy, hibernation)

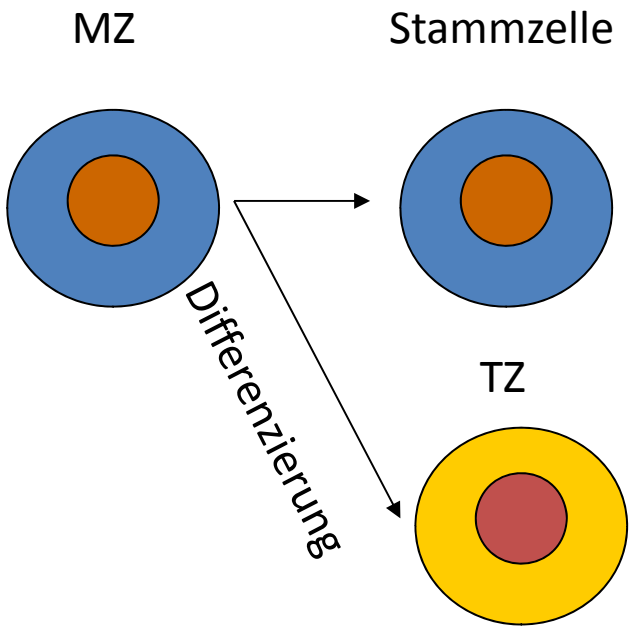
Stammzellen überleben auch ohne Teilung

1.1. Selbsterneuerungsfähigkeit von Stammzellen

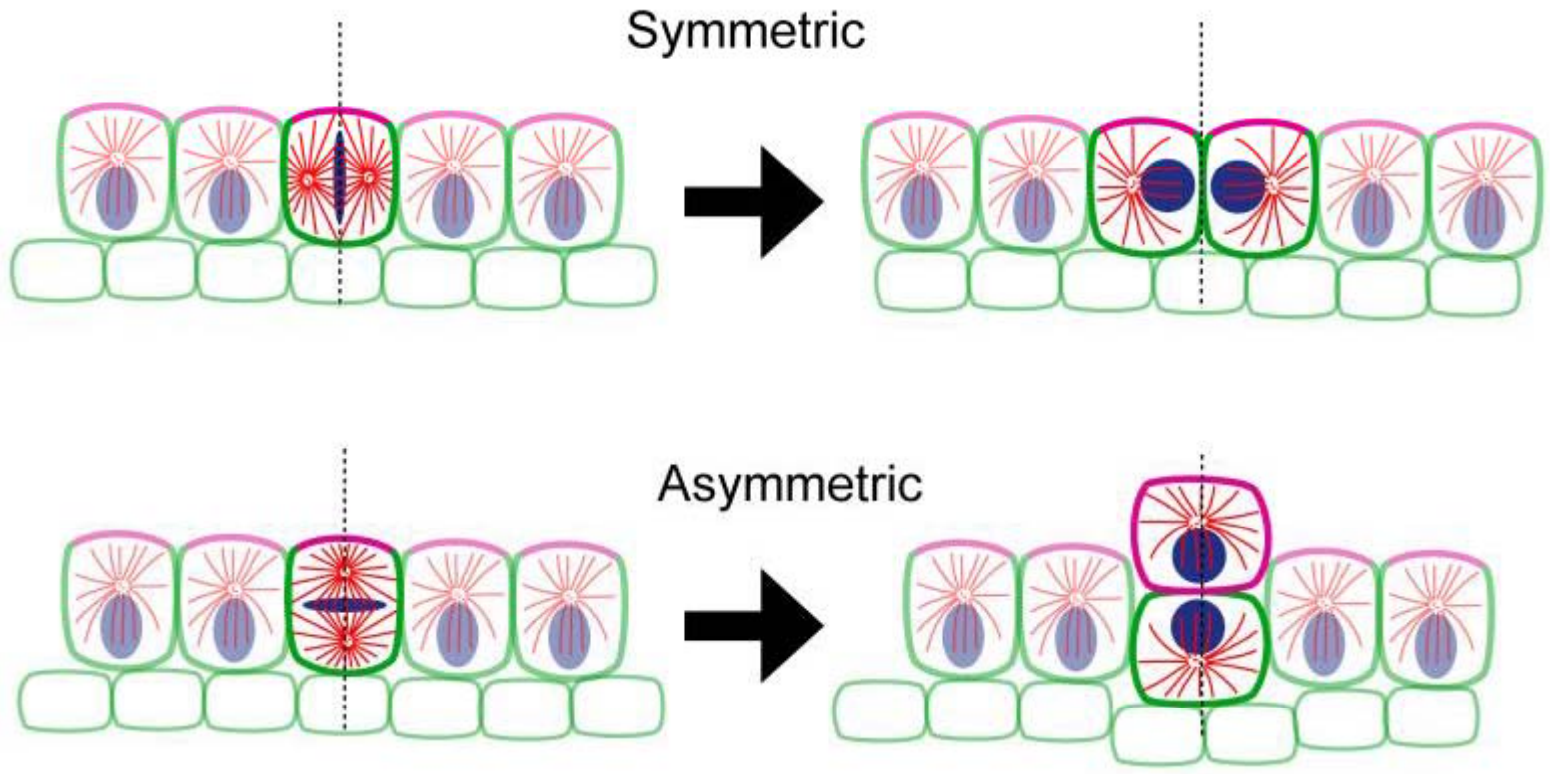
Symmetrische Zellteilung



Asymmetrische Zellteilung



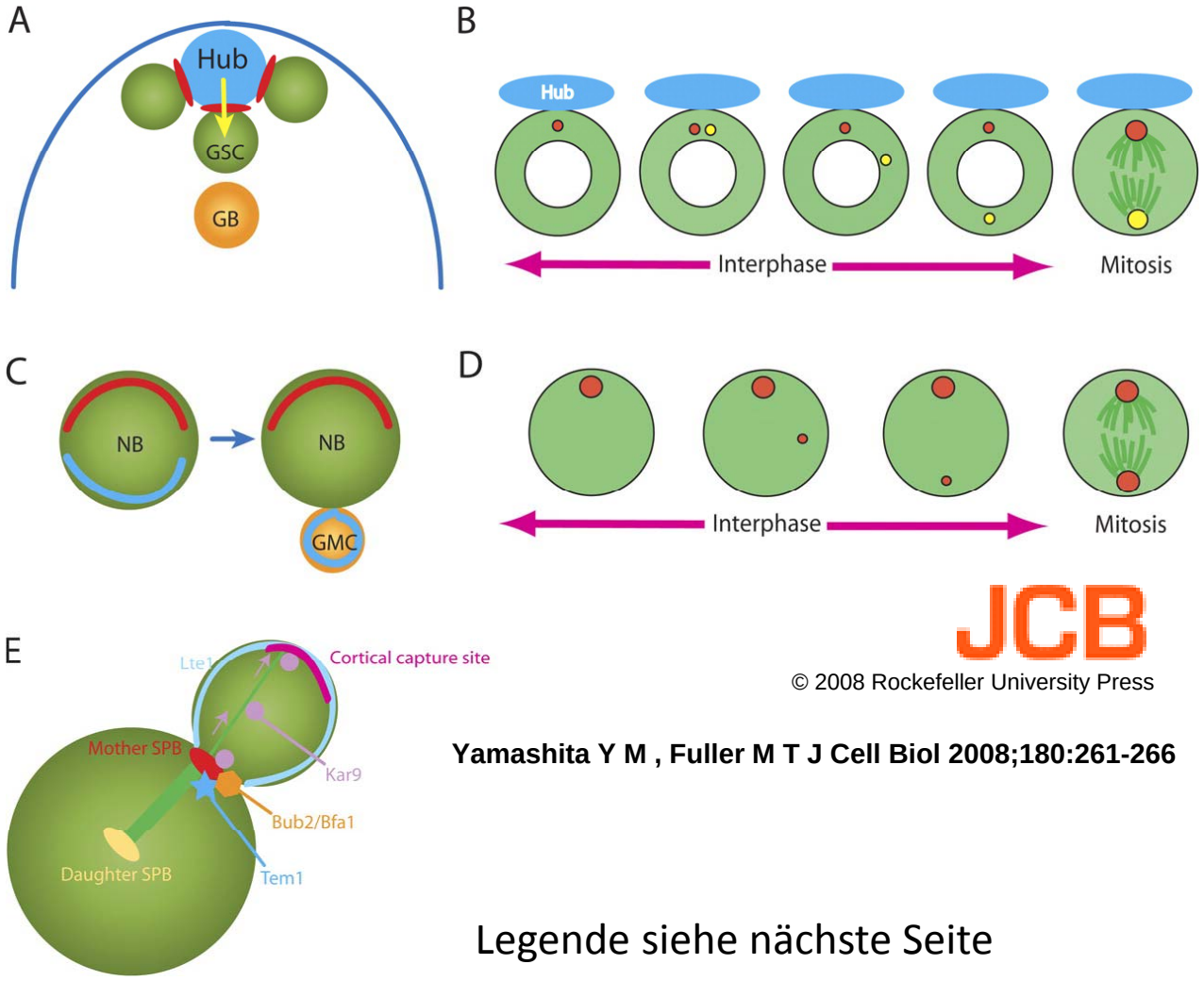
Bedingt durch Anordnung der Spindelpole (Centrosomen)



http://sites.google.com/site/manuelthery/Home/cell-division

Von **Manuel Théry**

Asymmetric centrosome/SPB behavior during asymmetric cell divisions.



JCB

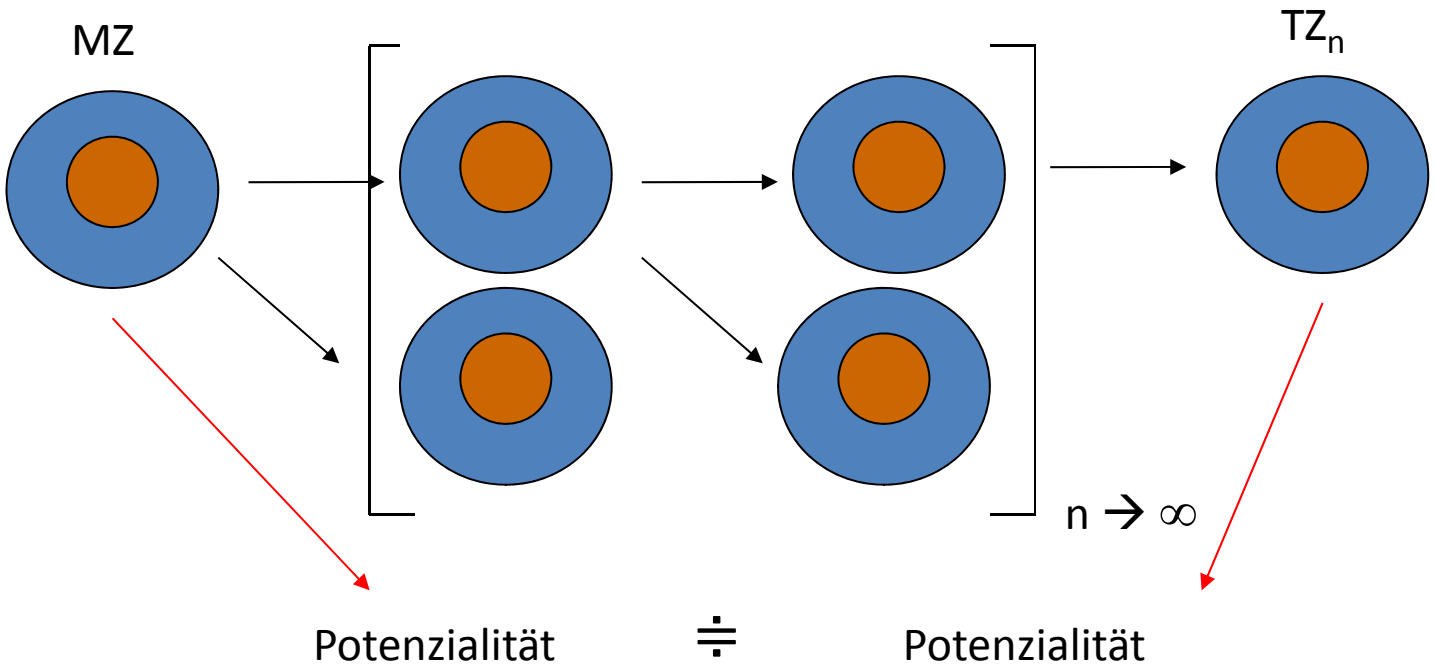
Yamashita Y M , Fuller M T J Cell Biol 2008;180:261-266

Legende siehe nächste Seite

Figure 1. **Asymmetric centrosome/SPB behavior during asymmetric cell divisions.** (A) Structure of the stem cell niche in the *Drosophila* male germline. Somatic hub cells are the major components of the niche for male GSCs. The GSC attaches to the hub via adherens junction (red lines) so that it can receive the signaling ligand, Upd, from the hub (yellow allow) to activate the JAK–STAT (Janus kinase–signal transducer and activator of transcription) pathway. The stem cell daughter that is displaced away from the hub (gonialblast; GB) starts differentiation. (B) Consistent centrosome positioning orients the mitotic spindle in male GSCs. The mother centrosome (red dots) is always located close to the hub, whereas the daughter (yellow dots) migrates toward the opposite side of the cell to set up orientation of the mitotic spindle. (C) Asymmetric division of the *Drosophila* neuroblast (NB). The neuroblast divides asymmetrically by segregating fate determinants. Apical protein complexes (red lines) include Par6, Baz, and atypical PKC, and basal fate determinants (blue lines) include Numb, Miranda, and Prospero (for a more comprehensive set of asymmetrically localized proteins, see the review Yu et al., 2006). (D) Consistent centrosome behavior orients the mitotic spindle in the neuroblast. The apical centrosome (large red dots) retains MTOC activity throughout the cell cycle, whereas the other centrosome (small red dots) becomes active only at the G2-M transition. The inactive centrosome migrates toward the basal side during interphase. Pins might be functioning to provide a cortical cue to anchor active MTOC. (E) Spindle orientation in budding yeast. The mother SPB is normally delivered to the bud cell, where it is captured by the bud tip cortex. This process is mediated by astral microtubules emanating from the mother SPB and Kar9 protein. Cell cycle regulators such as Tem1 and Bub2/Bfa1 are specifically localized to the bud-directed SPB (normally the mother SPB) to coordinate spindle position and cell cycle progression.

Siehe auch http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2712604/

1.2. Klonalität von Stammzellen



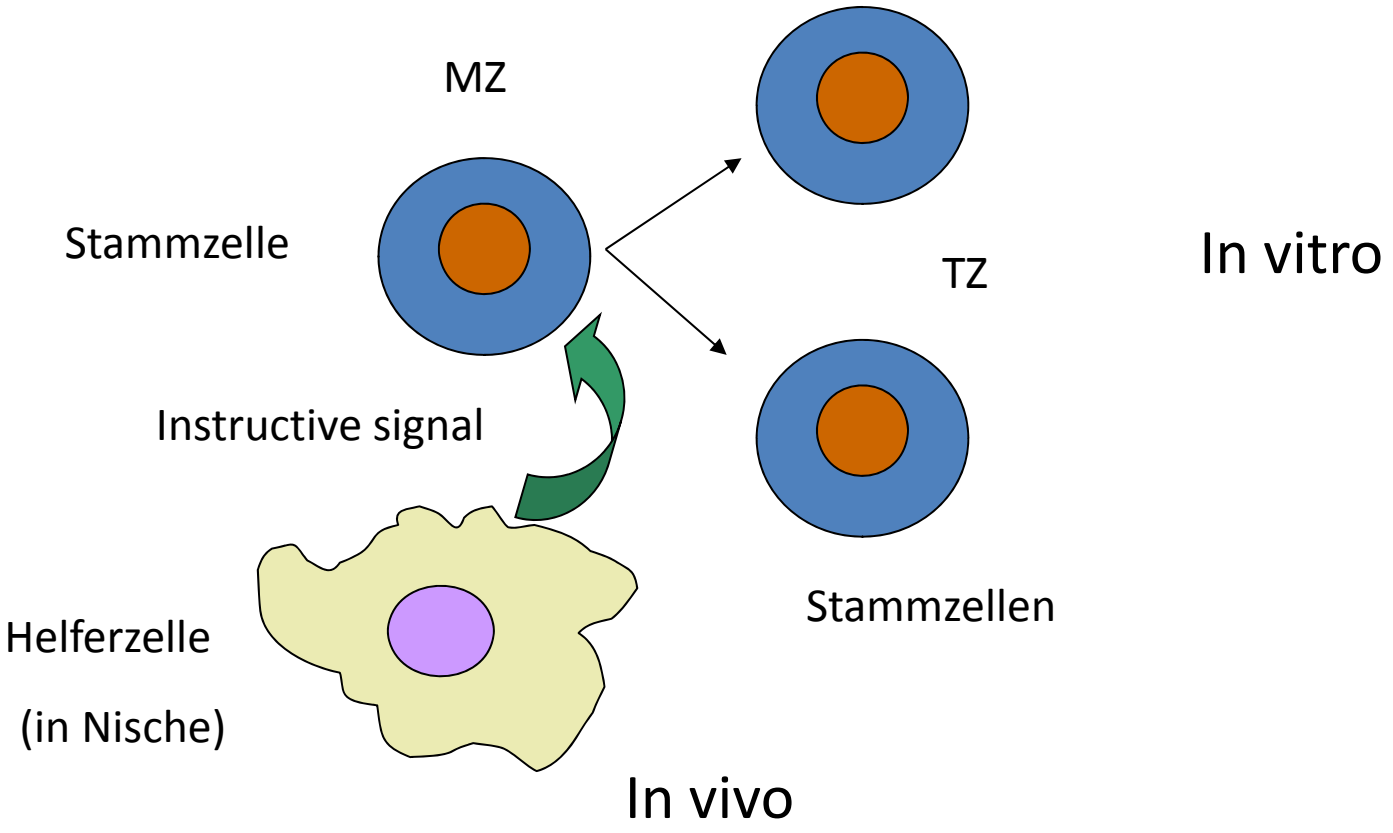
Wenn Zelllinie klonal, muss die **Potenzialität** von MZ und TZ_n identisch sein.

Nachweis der gleichen Potenzialität:

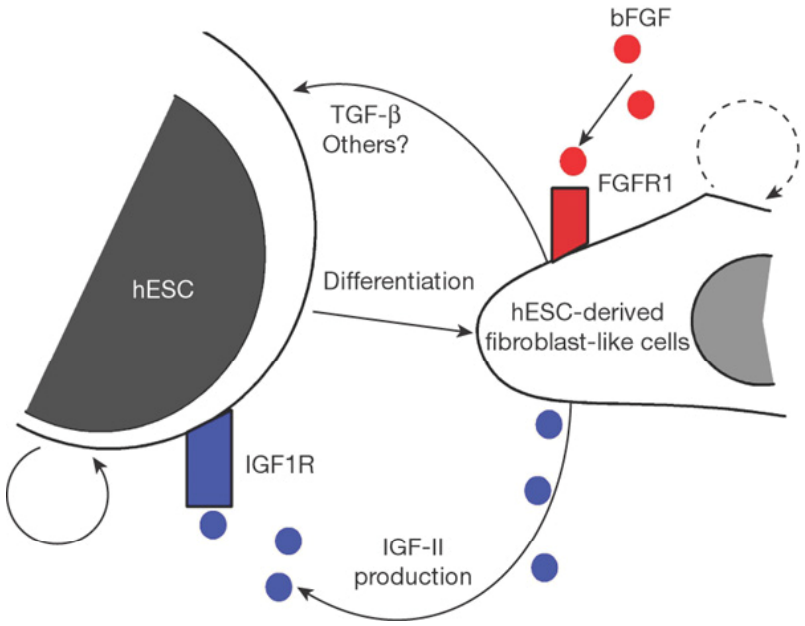
Herstellung von Chimären
Tetraploidaggregation
In vitro Differenzierung

Nischen

tragen zum Erhalt des Selbsterneuerungspotenziales und der Klonalität von Stammzellen bei.



Nischenbildung und Bedingungen für hESCs



To maintain culture homeostasis within its autologously produced niche, human ES cells (hESCs) spontaneously and continuously differentiate into hdFs, providing a continuous source of endogenous human ES cell supportive factors, including IGF-II and a host of TGF- family of factors and other ligands.

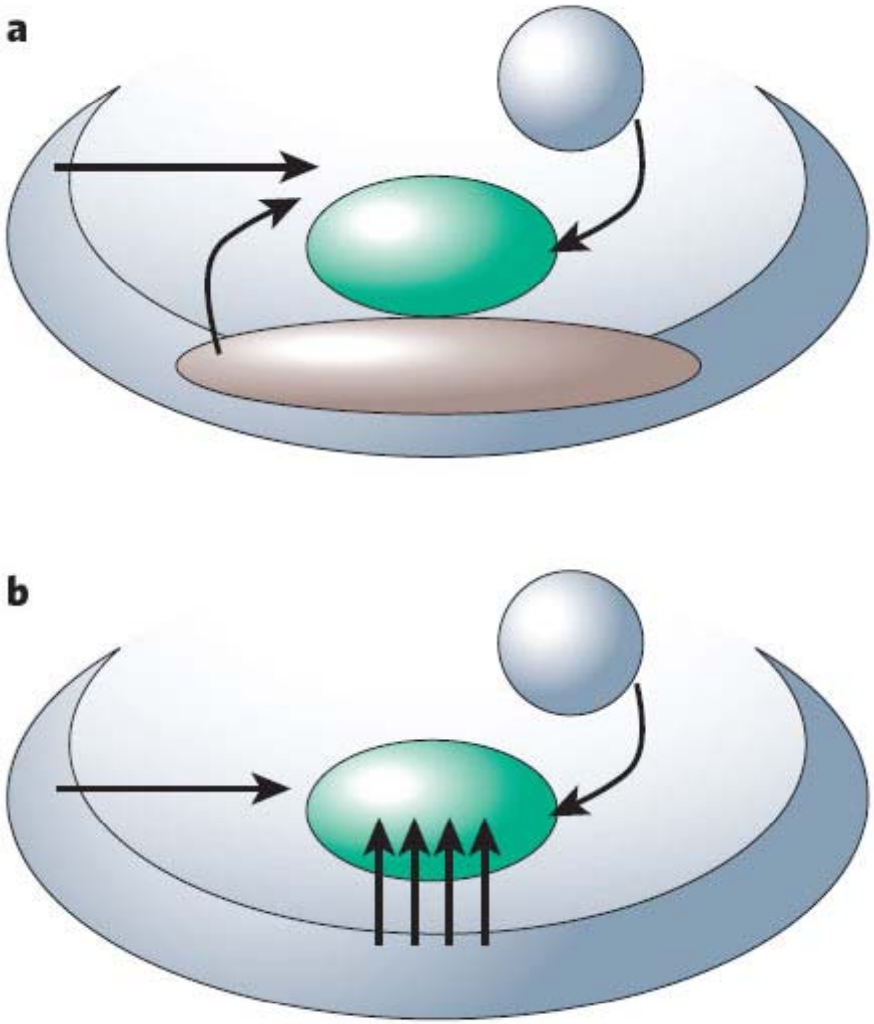
From the following article:

IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells *in vitro*

Sean C. Bendall, Morag H. Stewart, Pablo Menendez, Dustin George, Kausalia Vijayaragavan, Tamra Werbowetski-Ogilvie, Veronica Ramos-Mejia, Anne Rouleau, Jiabi Yang, Marc Bossé, Gilles Lajoie & Mickie Bhatia

Nature 448, 1015-1021(30 August 2007)

doi:10.1038/nature06027



From the following article:

The stem-cell niche as an entity of action

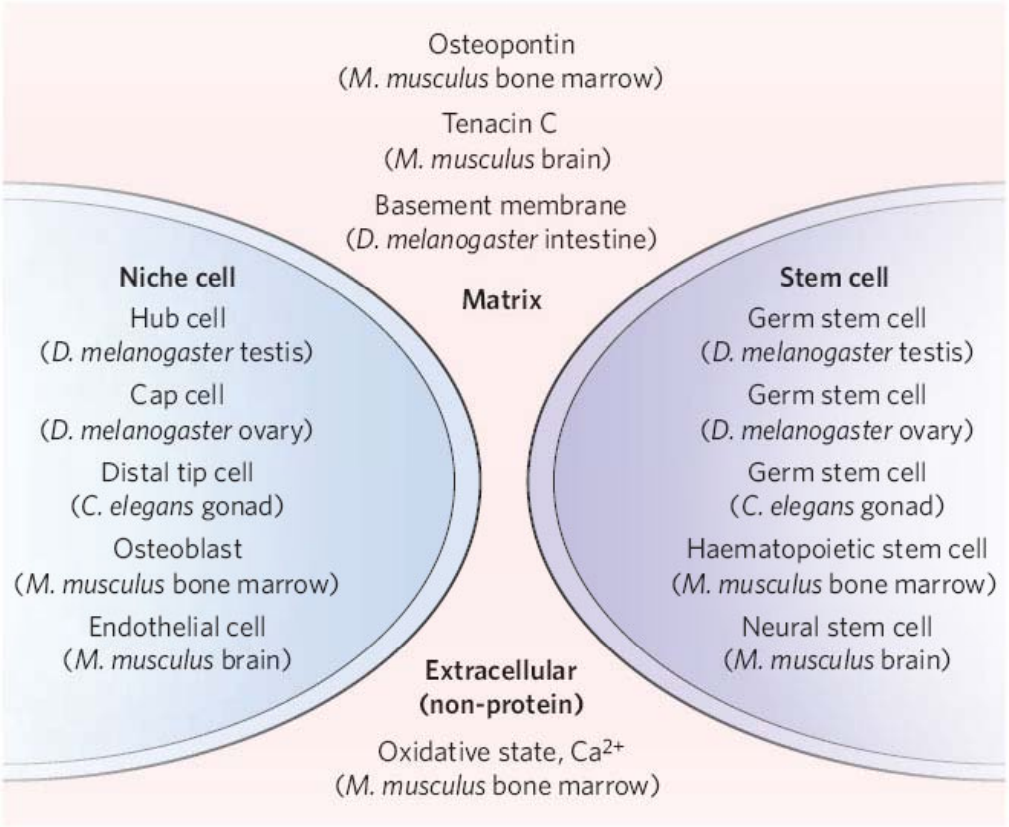
David T. Scadden

Nature 441, 1075-1079(29 June 2006)

doi:10.1038/nature04957

Figure 1: Refining elements necessary for an adult stem-cell niche.

a, Early studies provided evidence that heterologous cell types created a three-dimensional structure in which stem cells reside. **b**, Recent data raises the possibility that a regulatory microenvironment might include stem cells simply resident on the basement membrane with homologous cell–cell interactions. Stem cells are shown in deep green and more mature offspring are represented by a lighter shade.



Intentionally excluded are the complex molecular interactions that are present at the interface between the stem cell and its niche cell counterpart. *C. elegans*, *Caenorhabditis elegans*; *D. melanogaster*, *Drosophila melanogaster*; *M. musculus*, *Mus musculus*.

From the following article:
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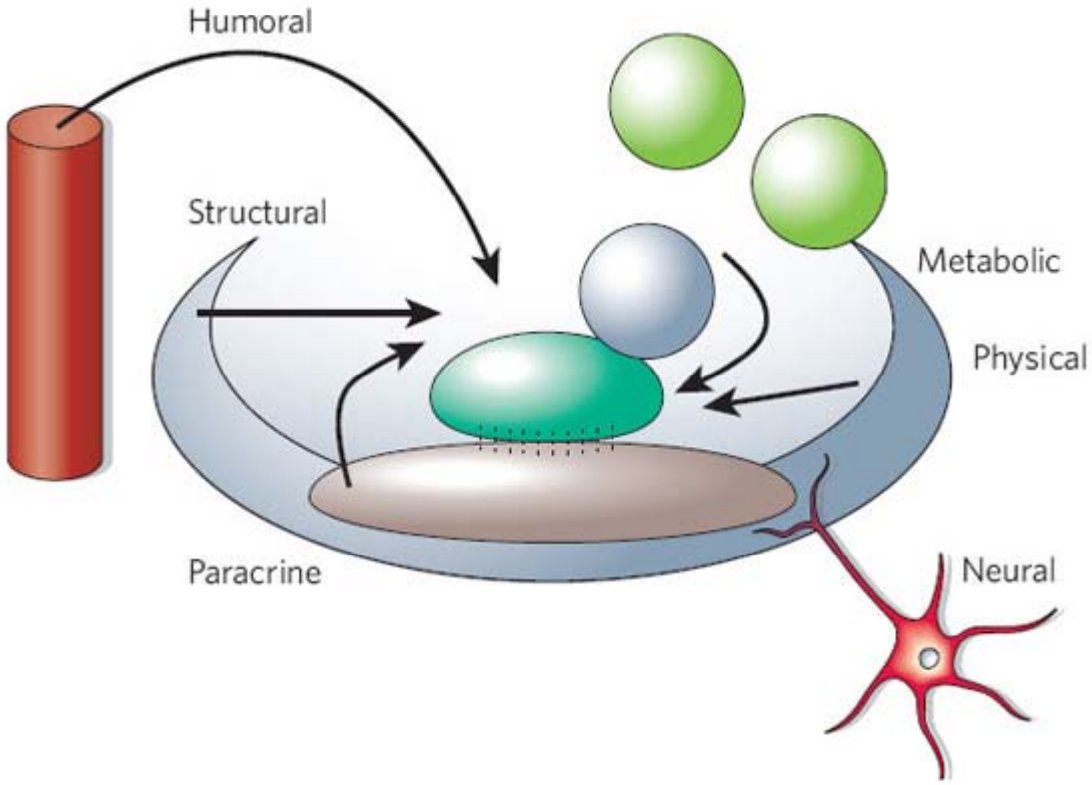


Figure 3: Inputs feeding back on stem-cell function in the niche.

Elements of the local environment that participate in regulating the system of a stem cell in its tissue state are depicted. These include the constraints of the architectural space, physical engagement of the cell membrane with tethering molecules on neighbouring cells or surfaces, signalling interactions at the interface of stem cells and niche or descendent cells, paracrine and endocrine signals from local or distant sources, neural input and metabolic products of tissue activity.

Regulation des naiven Grundzustandes von Stammzellen

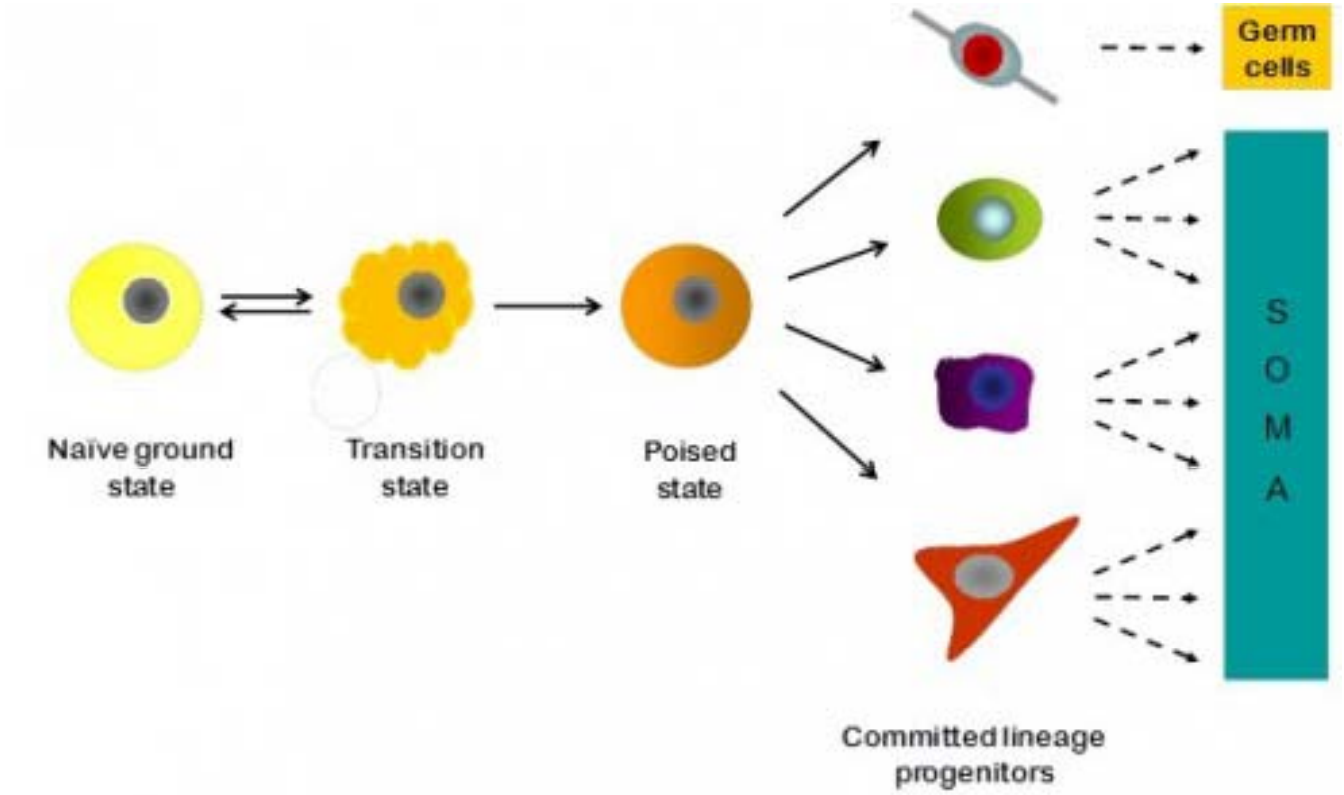


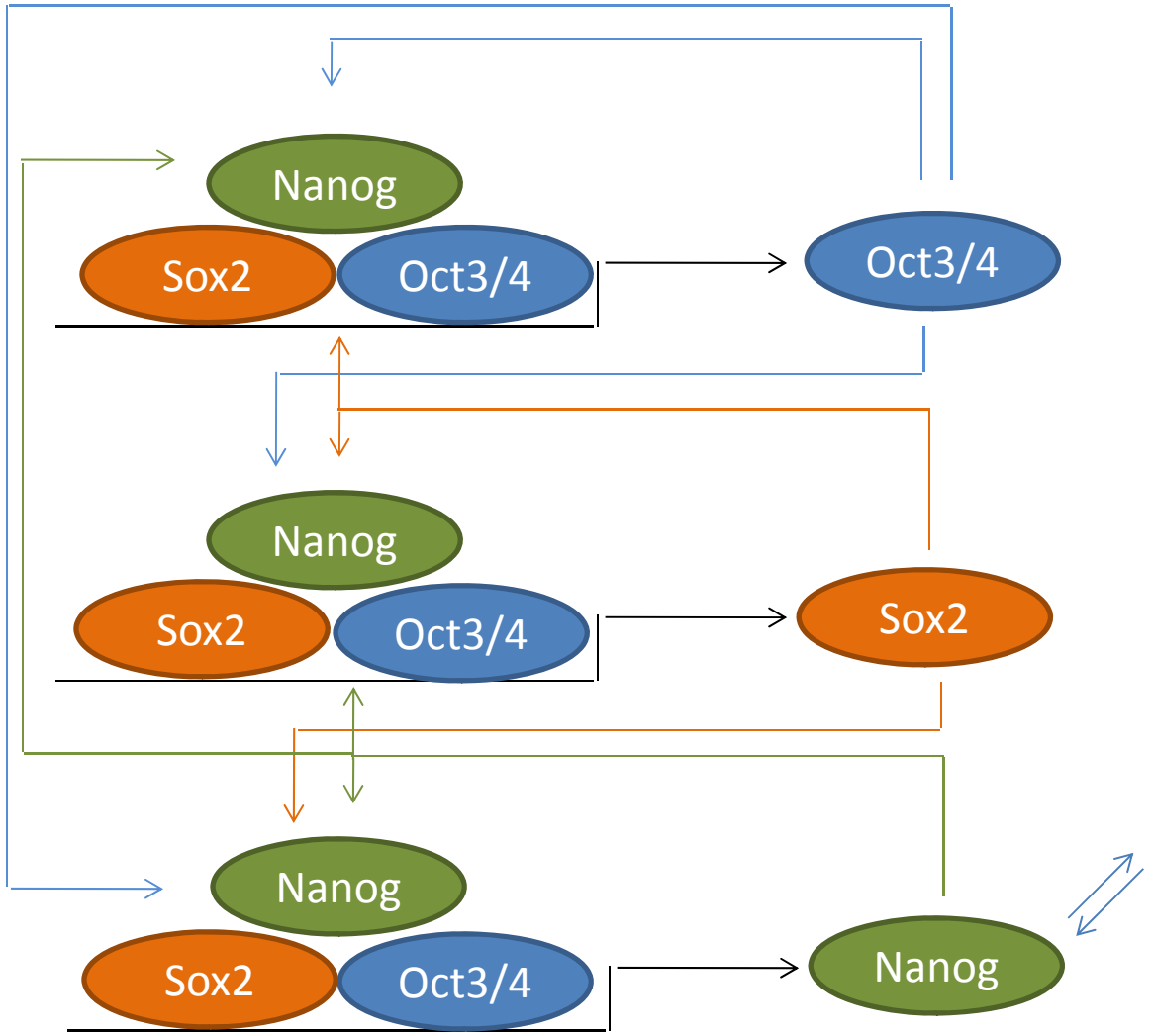
Figure: Hypothetical scheme for loss of pluripotency and lineage commitment.

From the homepage of Austin Smith
URL:http://www.stemcells.cam.ac.uk/researchers/principal-investigators/pressor-austin-smith

Die Selbsterneuerungsfähigkeit wird beinflusst durch:

Intrinsische Faktoren:	Transkriptionsfaktoren („Stemness Factors“): Oct 3/4 (Gen: Pou5f1) Nanog Sox2
Extrinsische Faktoren:	Wachstumsfaktoren: IL6 Familie (JAK / STAT Signaltransduktionsweg) Wnt Familie (ß-catenin Signaltransduktionsweg) IGF bei hESCs Zell-Zell und Zell- Matrix Wechselwirkungen Humorale und metabolische Einflüße

Das Netzwerk der „trinity factors“ zur Erhaltung der Pluripotenz



Netzwerk
von Differen-
zierungsfaktoren
z.B.: Brachyury
Tgfß, Bmps
Smads
Wnts, ßCatenin
Tcfs, Lefs

doi:10.1038/74199
Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells
Hitoshi Niwa Jun-ichi Miyazaki & Austin G. Smith
Nature Genetics **24**, 372 - 376 (2000) doi:10.1038/74199

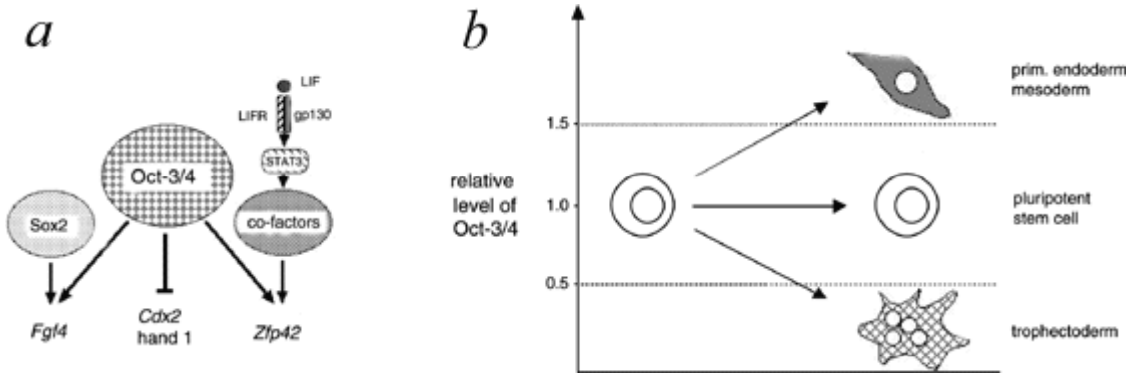
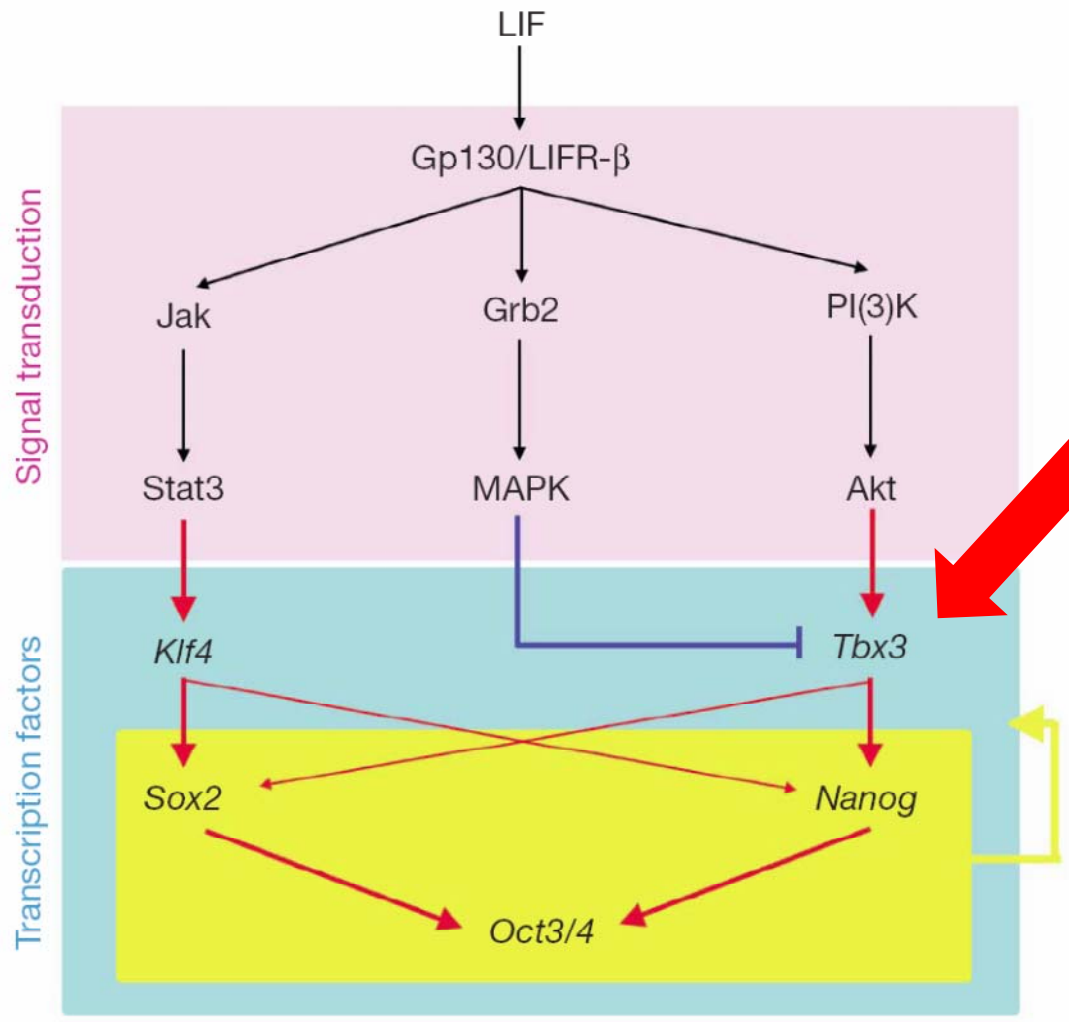


Figure 6. Functions of Oct-3/4 in pluripotent stem cells.
a, Model for Oct-3/4 and Stat3 cooperative function. Self-renewal requires transcriptional functions of both Oct-3/4 and LIF-stimulated Stat3. Oct-3/4 directly or indirectly represses trophectodermal determination genes such as *Cdx2* and *Hand1* and thereby blocks differentiation to trophectoderm. Oct-3/4 also activates target genes in stem cells through interaction with a set of coactivators. We hypothesize that a subset of such partners, including Rox-1, are regulated by Stat3. Sequestration of such co-activators by excess Oct-3/4 or their downregulation following LIF withdrawal would thus result in a similar loss of expression of targets such as *Zfp42*. ***b***, Relationship between Oct-3/4 expression level and stem-cell fate. To maintain the undifferentiated stem-cell phenotype, Oct-3/4 expression must remain within plus or minus 50% of normal diploid expression. If Oct-3/4 expression is increased beyond the upper threshold level, differentiation is triggered into primitive endoderm or mesoderm. If Oct-3/4 expression is decreased, stem cells are redirected into the trophectoderm lineage.

A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells

Hitoshi Niwa[1,2,3], Kazuya Ogawa[1], Daisuke Shimosato[1,2] & Kenjiro Adachi[1]

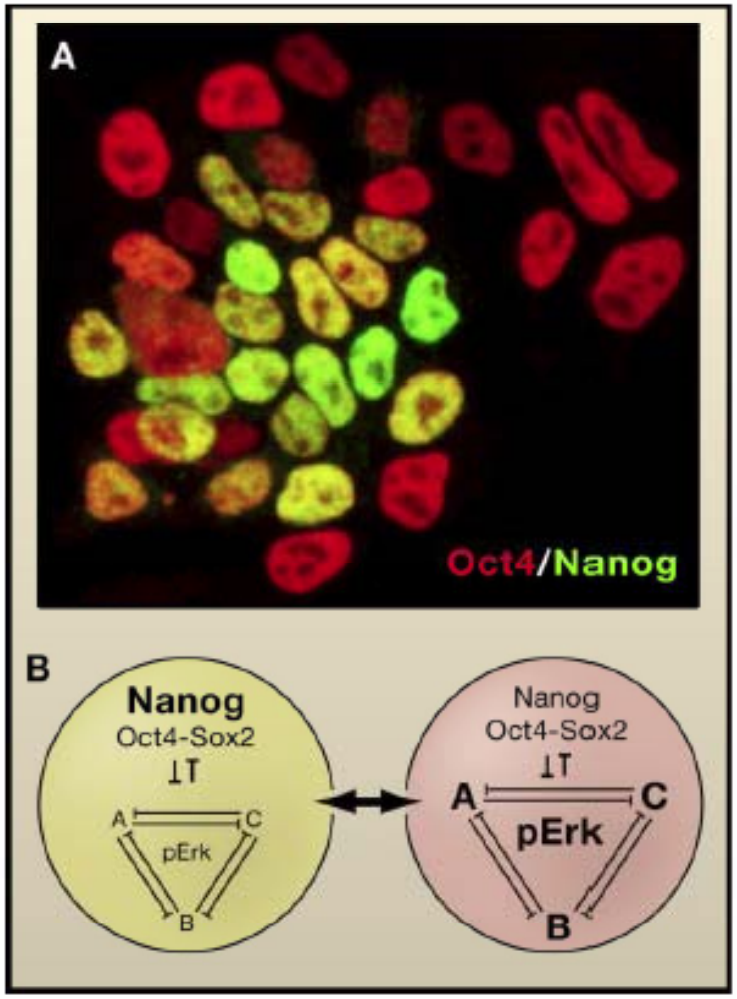
nature Vol 460|2 July 2009|doi:10.1038/nature08113



Bindeglied zwischen LIF und Oct4

MEDIZINISCHE UNIVERSITÄT WIEN

Ist der Expressionsraum in Stammzellen eine Tabula rasa oder ein überreich gedeckter Tisch?



A Metastable Coalition

The transcription factor Nanog secures self-renewal of ES cells, and cell-to-cell variation creates the possibility for differentiation. (A) Embryonic stem (ES) cells are heterogeneous. Immunostaining shows highly variable levels of Nanog protein in Oct4-positive undifferentiated ES cells. (B) In our model, lineage-associated transcriptional circuits (A, B, and C) are maintained below threshold levels due to mutual antagonism and suppression by the three transcription factors Oct4, Sox2, and Nanog. A destabilized transitional state arises when downregulation of Nanog coincides with increased activation of Erk. Phosphorylated Erk (pErk) may activate inductive signaling pathways or directly promote lineage-affiliated transcriptional networks. The fluctuations in network activities generated by pErk confer an opportunity to establish a new stable cell state. However, if Nanog levels rise before commitment is effected, the actions of pErk are neutralized, the metastable ground state is restored, and the gate is closed. Photo courtesy of J. Silva and A. Smith.

Published as: *Cell*. 2008 February 22; 132(4): 532–536.

Capturing Pluripotency

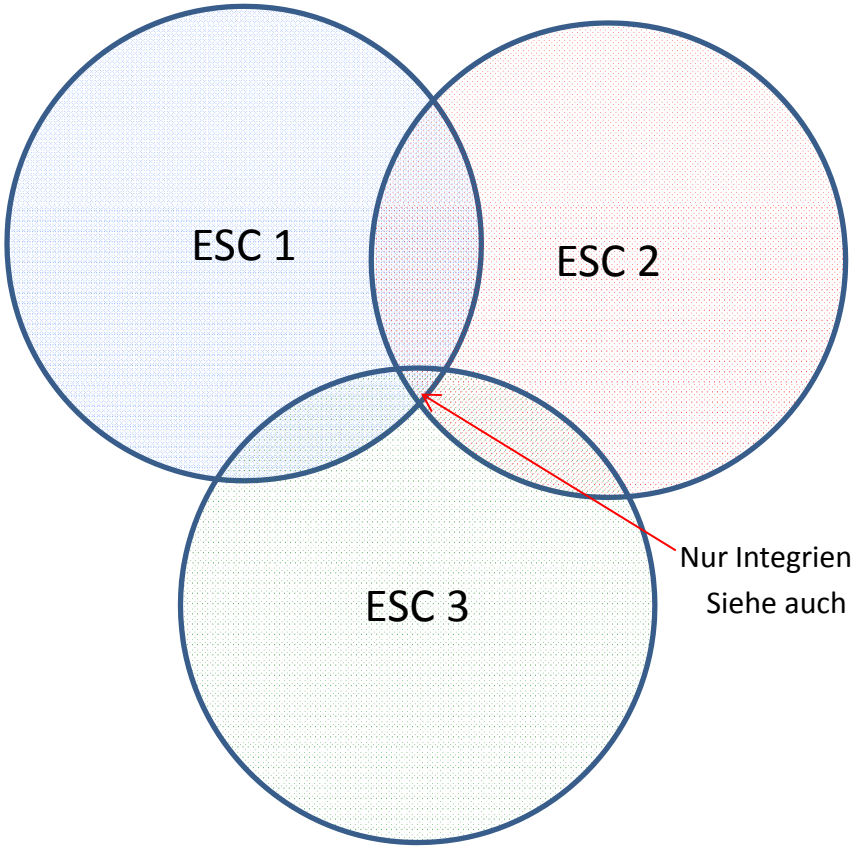
Jose Silva[1] and **Austin Smith**[1]*

[1]*Wellcome Trust Centre for Stem Cell Research and Department of Biochemistry, University of Cambridge, Cambridge CB2 1QR, UK.*

Abstract

In this Essay, we argue that pluripotent epiblast founder cells in the embryo and embryonic stem (ES) cells in culture represent the ground state for a mammalian cell, signified by freedom from developmental specification or epigenetic restriction and capacity for autonomous self-replication. We speculate that cell-to-cell variation may be integral to the ES cell condition, safe-guarding self-renewal while continually presenting opportunities for lineage specification.

Expressionsräume von ähnlichen Stammzelllinien unterscheiden sich sehr stark



Stochastische Genexpression kann auch als Phänomen der Natur-immanenten Kontingenz betrachtet werden!
→ Siehe letzte Vorlesungseinheit

Nur Integrien 6 alpha war gemeinsam exprimiert → ???
Siehe auch Fortunel et al., 2003 Science, 302, 393b.

Gibt es überhaupt Genom-weite Gemeinsamkeiten zwischen verschiedenen Arten von Stammzellen?

Weiterführende Primär-Literatur von

Austin Smith

Hitoshi Niwa

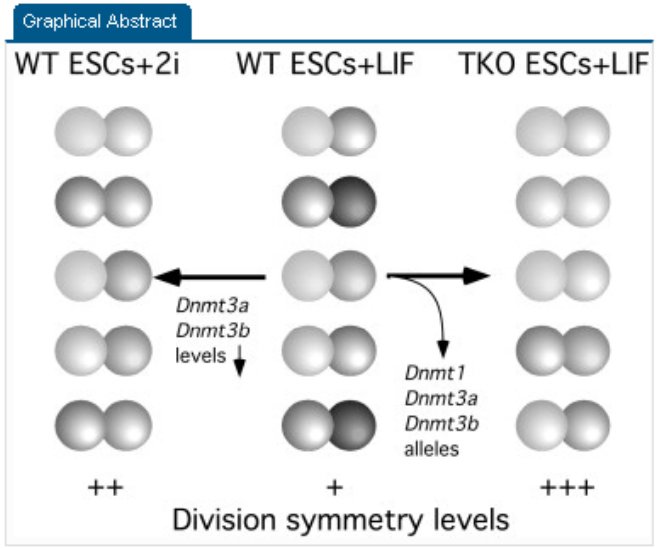
Dov Zipori

Nachtrag zur symmetrischen Stammzellteilung:

Identifying Division Symmetry of Mouse Embryonic Stem Cells: Negative Impact of DNA Methyltransferases on Symmetric Self-Renewal

Stem Cell Reports, Volume 1, Issue 4, 360-369, 26 September 2013

10.1016/j.stemcr.2013.08.005



Authors

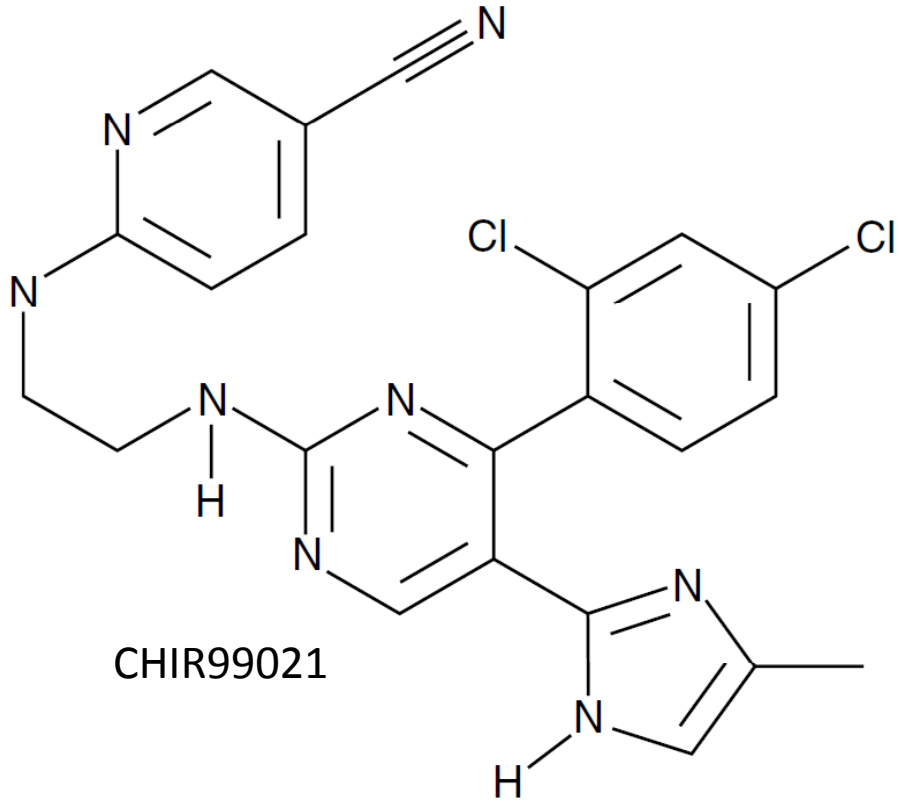
Lukasz Jasnos, Fatma Betül Aksoy, Hersi Mohamed Hersi, Slawomir Wantuch, Tomoyuki Sawado
See Affiliations

Highlights

- ESC division symmetry was characterized through high-throughput RNA analyses
- Pluripotent ESCs displayed considerable gene expression diversity between sister cells
- Similarity between sister ESCs is significantly elevated at ground-state pluripotency

Summary

Cell division is a process by which a mother cell divides into genetically identical sister cells, although sister cells often display considerable diversity. In this report, over 350 sister embryonic stem cells (ESCs) were isolated through a microdissection method, and then expression levels of 48 key genes were examined for each sister cell. Our system revealed considerable diversities between sister ESCs at both pluripotent and differentiated states, whereas the similarity between sister ESCs was significantly elevated in a 2i (MEK and GSK3b inhibitors) condition, which is believed to mimic the ground state of pluripotency. DNA methyltransferase 3a/3b were downregulated in 2i-grown ESCs, and the loss of DNA methyltransferases was sufficient to generate nearly identical sister cells. These results suggest that DNA methylation is a major cause of the diversity between sister cells at the pluripotent states, and thus demethylation per se plays an important role in promoting ESC's self-renewal.



CHIR99021

A Glycogen-Synthase-Kinase 3ß(GSK3ß) Inhibitor mimicking Wnt signaling by activating the translocation of ß-catenin into the nucleus of cells.

A Mitogen–activated-Protein Kinase Kinase (MEK) inhibitor which prevents phosphorylation and nuclear localisation of Extracellular –Signal-Related-Kinases (ERK1/2), thus inhibiting differentiation promoting signals such as FGF4.

PD98059