A. Grundlagen der Stammzellbiologie – Was ist eine Stammzellen?

2. Die Entstehung der Stammzellen im Laufe der Evolution (eine Hypothese) - Warum gibt es Stammzellen?
3. Molekulare Regulation der Stammzelleigenschaften - Welche Teile der genetischen Information kodieren die Stammzelleigenschaften?
   - 3 .1. Intrinsische Faktoren - Transkriptionsfaktor Netzwerke
   - 3 .2. Extrinsische Faktoren – Signalübertragungsmechanismen
   - 3 .3. Stammzell-Nischen

Nachtrag zu 3.1.: Nanogs Funktionen als gatekeeper

NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-019-09041-z

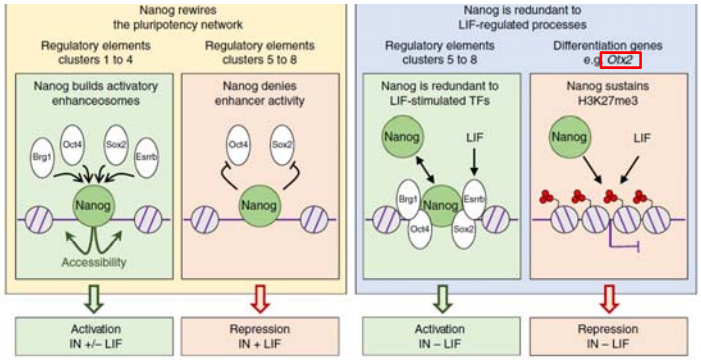


Fig. 6 Nanog is a versatile TF impacting the pluripotency network and epigenome. The function of Nanog at stereotypical clusters of regulatory elements targeted by the pluripotency network, as well as at differentiation genes, is shown. Briefly, Nanog displays four major behaviours (left to right): 1/ recruitment of other factors (Oct4, Sox2 and Esrrb, together with Brg1) to promote chromatin accessibility and activate gene transcription; 2/ inhibiting enhancer activity, leading to gene repression either by blocking Oct4/Sox2 recruitment (shown) or by other mechanisms (not shown for simplicity; see text for details); 3/ complementing enhancer activity redundantly with other factors which are controlled by LIF (such as Esrrb)—in this case, its activatory role can only be appreciated in the absence of LIF; 4/ Nanog and LIF act in parallel to sustain H3K27me3 at differentiation genes such as *Otx2*. This latter role of Nanog is particularly important in the context of Nanog-mediated, LIF-independent self-renewal

Otx2 expression setzt die Entstehung von primitiven Ektoderm in Gang.

Nachtrag zu 3.1.: Nanogs Funktionen als gatekeeper

„**Bivalent domains**“ auf Chromosomen: Promotoren von Diff.-induzierneden Genen sind aktiv, weil Nukleosomen mit H3K4me3 angereichert und gleichzeitig ist die Umgebung der Gene durch Anreicherung der Nukleosomen mit H3K27me3 stillgelegt.

e.g. Nanog keeps H3K27me3 hoch am Otx2 Gens, welches, wenn exprimiert die Bildung von primitven Ektoderm einleitet.

(Nanog bindet an 27.782 regulierende DNA Elemente!)

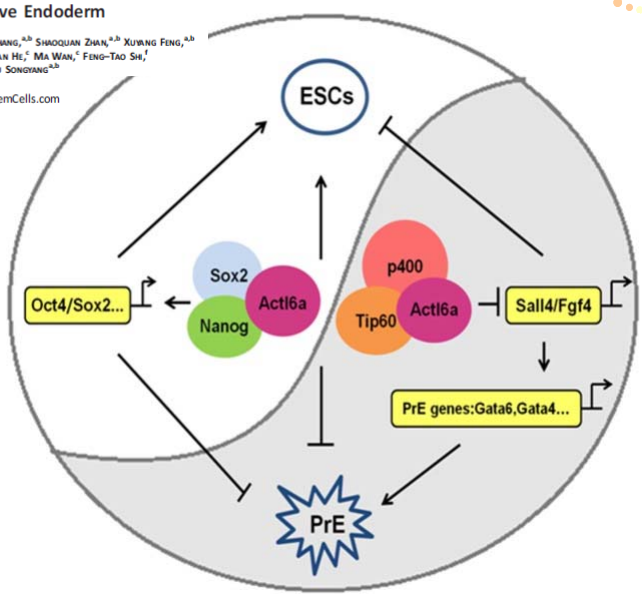
Actl6a as gatekeeper of pluripotency

Actl6a Protects Embryonic Stem Cells From Differentiating Into Primitive Endoderm

Weisi Lu,[a,b,c] Lekun Fang,[d] Bin Ouyang,[e] Xiya Zhang,[a,b] Shaoquan Zhan,[a,b] Xuyang Feng,[a,b] Yaofu Bai,[a,b] Xin Han,[a,b] Hyeung Kim,[c] Quanyuan He,[c] Ma Wan,[c] Feng-Tao Shi,[f] Xin-Hua Feng,[g] Dan Liu,[c] Juniu Huang,[a,b] Zhou Songyang[a,b]

Stem Cells 2015;33:1782–1793 www.StemCells.com

Actl6a = Baf 53a = Actin-like-6a, a component of BAF (SWI/SNF) complexes



3.2. Extrinsische Faktore - Signalübertragunswege

Ad Molekulare Grundlage der Selbsterneuerung von (embryonalen) Stammzellen

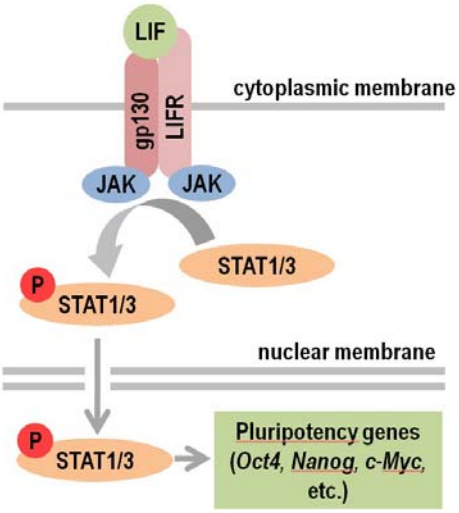
3.2.1. LIF Signalübertragung
3.2.2. FGF Signalübertragung
3.2.3. Tgf-ß Signalübertragung
3.2.4. Wnt Signalübertragung
3.2.5. IGF / Insulin Signalübertragung
3.2.6. Das Zusammenspiel der Signalübertragungswege bei der SR
3.2.7. Die unterschiedlichen Zustände von ESCs
3.2.8. Unterschiede zwischen ESCs von Mensch und Maus

Forschung ist gefährlich: man könnte etwas Neues entdecken.

Gerhard Kocher

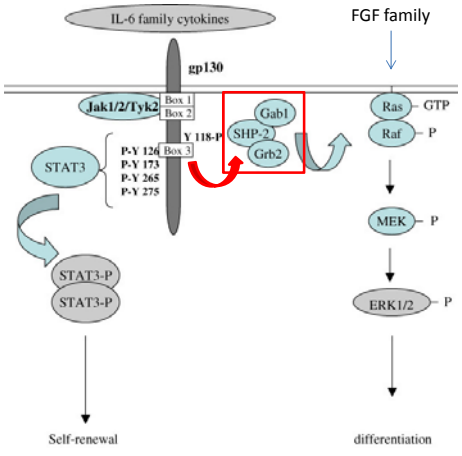
3.2.1. LIF Signalübertragung (Interleukin 6 Familie

Maus ESCs:



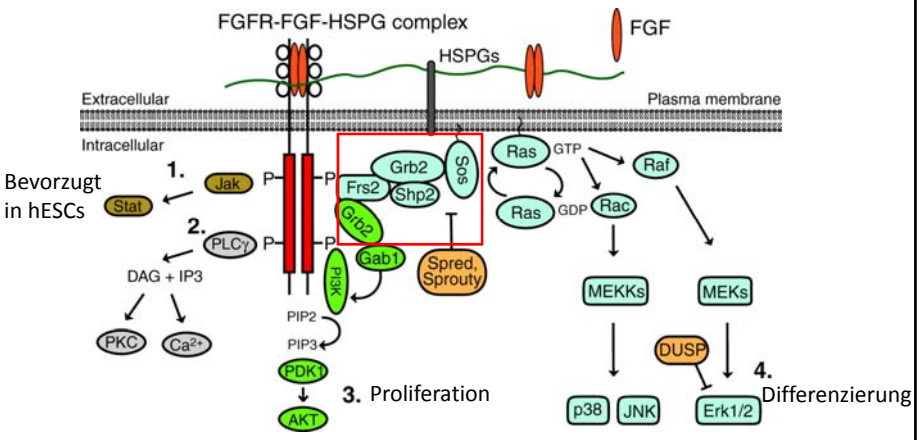
http://www.biodiscoveryjournal.co.uk/Archive/A9.htm

2021:
OSM+OSMRß könnne LIF +LIFR ersetzen!



3.2.2. FGF Signalübertragung

FGF signaling in stem cells



HSPG, Heparan sulphate Proteoglycans
PKC, Protein Kinase C
PI3K, Phosphoinositid-3-Kinasen

http://dev.biologists.org/content/137/20/3351

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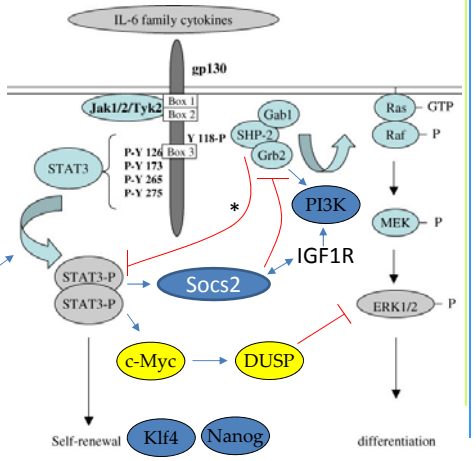
Verbindung zwischen LIF und FGF Signalübertragung

LIF kann auch zu einer Förderung der Differenzierung von ESCs beitragen.

LIF kann auch zu einer Inhibierung der Phopho-STAT3 vermittelten Proliferation von ESC beitragen.

* …weil die Phosphatase SHP2, wenn sie an Y118 des gp130 bindet aktiviert wird (durch Phosphorylierung durch JAKs) und so, die stady-state concentration von Phospho-STAT3 reduziert.

Desynchronisierung der Differenzierung durch Hes 1



Socs2 … Suppressor of cytokine signaling 2
Grb2 … growth factor receptor bound 2
Gab1 … GRB2-Associated Binding Protein 1
SHP-2 … Src-homology 2 domain (SH2)-containing protein (Phosphatase)
DUSP … Dual specific phosphatase

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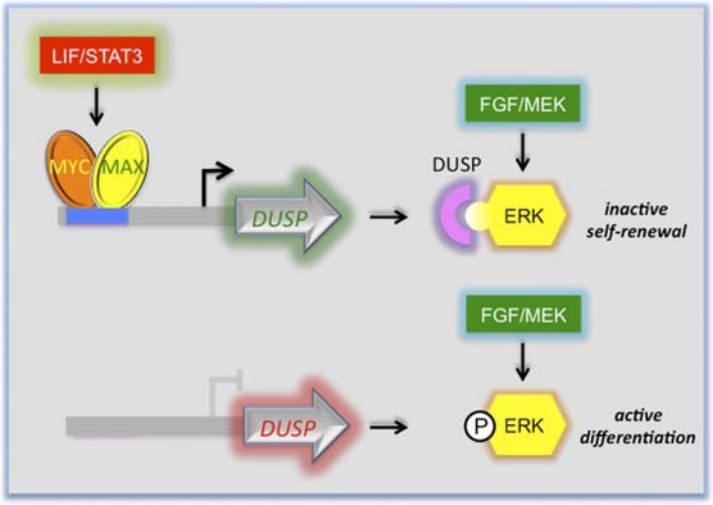
A model for ERK regulation by MYC/MAX complexes in murine pluripotent cells.

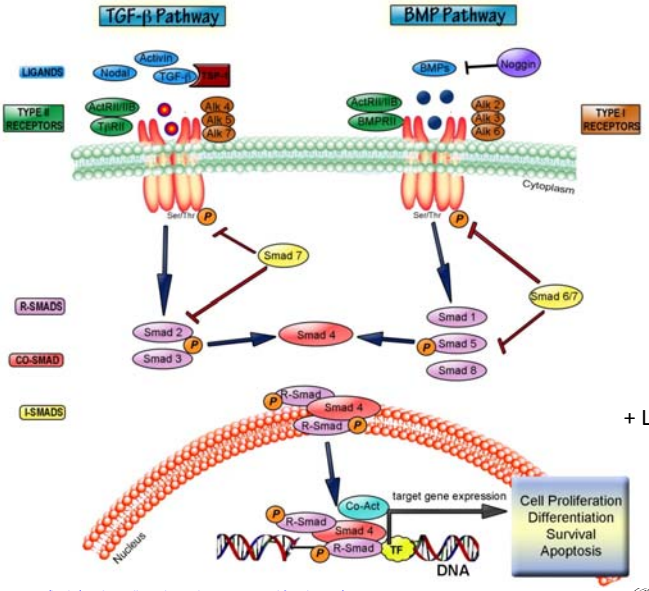


James Chappell et al. Genes Dev. 2013;27:725-733

3.2.3. Tgf-ß Signalübertragung (TGf-ß1-3 / Activin / Nodal / Bmp Familie)

Mensch
hESCs

+ FGF2



Maus
mESCs

+ LIF (~ FGF2)

Nanog
Id1, 2, 3
?

https://www.intechopen.com/books/trends-in-cell-signaling-pathways-in-neuronal-fate-decision/role-of-tgf-signaling-in-neurogenic-regions-after-brain-injury

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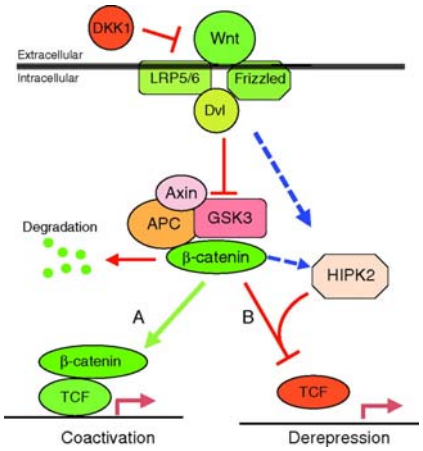
3.2.4. Wnt Signalübertragung

Wnt – ß Catenin – Signaling in ESCs



Siehe auch:
Maintaining embryonic stem cell pluripotency with Wnt signaling by Sergei Y. Sokol
Development 2011 138: 4341-4350;
doi: 10.1242/dev.066209

CR, Corepressor
TCF3, Transcription factor 3, auch T-cell factor 3

3.2.4. Wnt Signalübertragung

Wnt – ß Catenin – Signaling in ESCs - einfaches Aktivierungsmodell

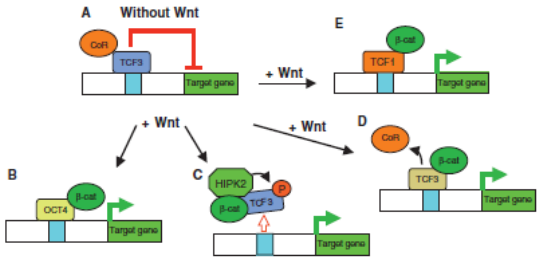
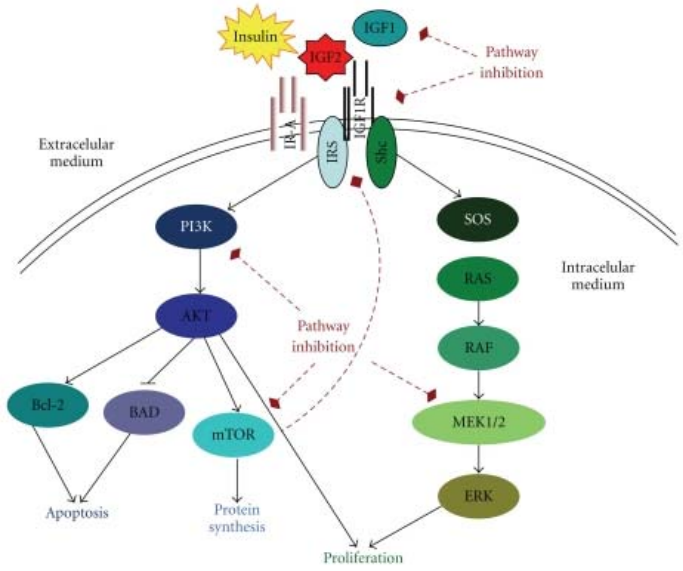


Fig. 4. Models for how Wnt signaling maintains pluripotency in ES cells. (**A**) In the absence of Wnt signaling, β-catenin (βcat) is degraded, and TCF3 in complex with transcriptional co-repressors (CoR) constitutively represses Wnt target genes. (**B-E**) Upon Wnt pathway activation, several alternative models leading to pluripotency are possible: (B) stabilized β-catenin associates with OCT4 to activate OCT4-dependent transcription; (C) HIPK2 is activated by Wnt signaling, associates with β-catenin and phosphorylates TCF3; this phosphorylation results in the removal of TCF3 from target promoters, leading to transcriptional derepression; (D) stabilized β-catenin associates with TCF3, causing the removal of the co-repressors resulting in target derepression (this model predicts that TCF3 is still bound to the promoter but no longer represses its gene targets); and (E) the TCF switch model, in which TCF3 repressor is replaced by TCF1 activator, leading to target activation and pluripotency. OCT4, octamer-binding transcription factor 4.

Siehe auch:
Maintaining embryonic stem cell pluripotency with Wnt signaling by Sergei Y. Sokol
Development 2011 138: 4341-4350;
doi: 10.1242/dev.066209

2.3.5. IGF-II / Insulin Signalübertragung



https://www.researchgate.net/figure/A-simple-schema-of-the-IGF-pathway-and-approaches-to-its-inhibition-Insulin-IGF2-and_fig1_50851552

2.3.6. Verbindung der Signalübertragungswege

… in Bezug auf die Regulierung von Core-Stemmess TFs
Siehe auch folgenden Seite

LIF –LIFR/gp130-JAK-STAT-cMyc / Klf4 – Oct4 / Esrrb Achse

FGF/ FGFR - Ras Raf MEK ERK Nanog Achse

Tgfß / Activin / Nodal / BMP - ALK /ActR-I/II– Smad – Nanog – Brachyury Achse

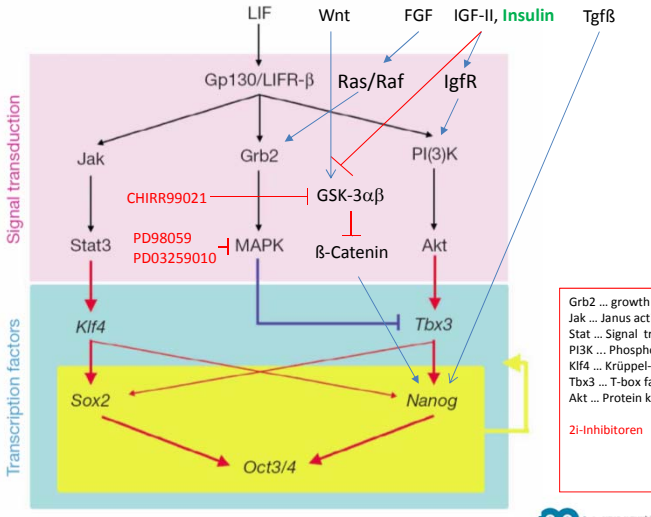
Wnt - LRP6 - Freezled - GSK3a,b – ß-Catenin- Tcf3- Tfcp2l1-Sall4 –Sox2 Achse

IGF2/Insulun - IGF2R/InsulinR – PI3K – AKT – Tbx3 – Nanog Achse

A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells (and hESCs)

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



Grb2 … growth factor receptor binding protein 2
Jak … Janus activated kinase
Stat … Signal transducer and activator of transcription
PI3K … Phosphoinositid-3-Kinasen Phoshpatydyl inositol
Klf4 … Krüppel-like factor 4
Tbx3 … T-box factor 3
Akt … Protein kinase B (PKB)

2i-Inhibitoren

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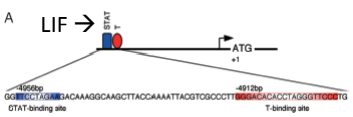
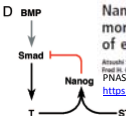
Nanog as gatekeeper of pluripotency





Nanog binds to Smad1 and blocks bone morphogenetic protein-induced differentiation of embryonic stem cells

PNAS July 5, 2006 103 (27) 10294-10299; first published June 26, 2006; https://doi.org/10.1073/pnas.0506945103

NANOG Is a Direct Target of TGFβ/Activin-Mediated SMAD Signaling in Human ESCs

Ren-He Xu,[1,2,*] Tori L. Sampsell-Barron,[2] Feng Gu,[1] Sierra Root,[1] Ruthann M. Peck,[2] Guangjin Pan,[3] Junying Yu,[3,4] Jessica Antosiewicz-Bourget,[3,4] Shulan Tian,[5] Ron Stewart,[5] and James A. Thomson[3,4,5,6,*]

Cell Stem Cell 3, 196–206, August 7, 2008 ©2008 Elsevier Inc.

Self-renewal of human embryonic stem cells (ESCs) is promoted by FGF and TGFβ/Activin signaling, and differentiation is promoted by BMP signaling, but how these signals regulate genes critical to the maintenance of pluripotency has been unclear. Using a defined medium, we show here that both TGFβ and FGF signals synergize to inhibit BMP signaling; sustain expression of pluripotency-associated genes such as *NANOG*, *OCT4*, and *SOX2*; and promote long-term undifferentiated proliferation of human ESCs. We also show that both TGFβ- and BMP-responsive SMADs can bind with the *NANOG* proximal promoter. *NANOG* promoter activity is enhanced by TGFβ/Activin and FGF signaling and is decreased by BMP signaling. Mutation of putative SMAD binding elements reduces *NANOG* promoter activity to basal levels and makes *NANOG* unresponsive to BMP and TGFβ signaling. These results suggest that direct binding of TGFβ/Activin-responsive SMADs to the *NANOG* promoter plays an essential role in sustaining human ESC self-renewal.

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3.2.7. Die unterschiedlichen Zustände von ESCs

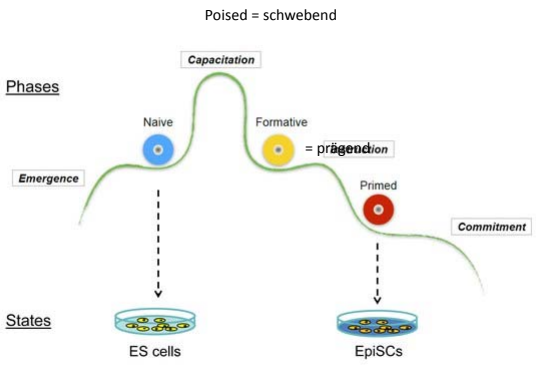
Die zuvor besprochenen Transkriptionsfaktoren und Signalübertragungswege tragen zum Entstehen der unterschiedlichen Entwicklungsstufen der embryonalen Stammzellen bei.

Diese sind

Naive - poised - formative – primed – commited state of embryonic stem cells.

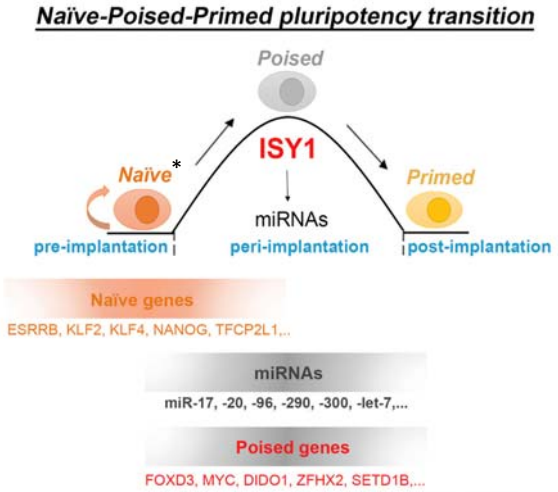
Landscape of Pluripotency

(from Austin Smith 2018)



z.B.: Oct4 Gene has 2 enhanchers, one active in naive mESCs and the other in primed mESCs.
(Choi et al., Stem Cell Report 2016)

Gene die bevorzugt in den unterschieldichen Zuständen exprimiert werden:



*Tabula rasa versus richly set table (abgeschabte Schreibtafel; metaphorisch leergefegter Tisch versus reich gedeckter Tisch)

Pre-mRNA-splicing factor ISY1 homolog

Siehe: Cell Stem Cell. 2018 Jun 1;22(6):851-864.e5. doi: 10.1016/j.stem.2018.04.021. Epub 2018 May 24.

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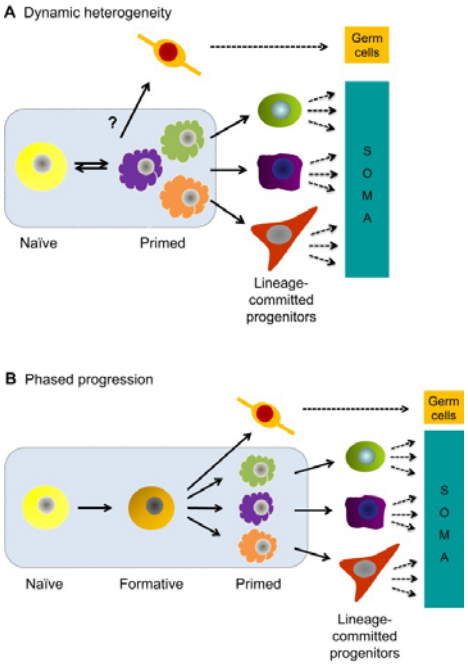
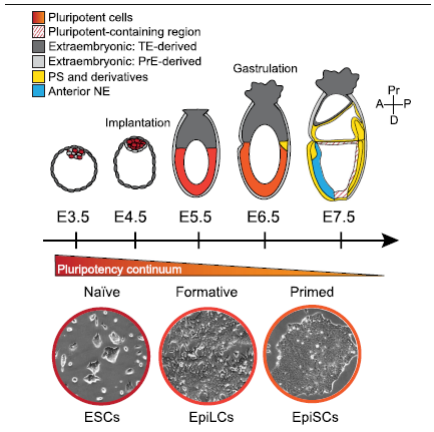




Morgani et al. BMC Developmental Biology (2017) 17:7
DOI 10.1186/s12861-017-0150-4

BMC Developmental Biology

REVIEW Open Access

The many faces of Pluripotency: in vitro adaptations of a continuum of in vivo states

Sophie Morgani[1,2], Jennifer Nichols[2] and Anna-Katerina Hadjantonakis[1*]

Naive Pluripotent Stem Cells Derived Directly from Isolated Cells of the Human Inner Cell Mass (Guo et al., Stem Cell Report 2016)

„Tankyrase = Poly-ADP-ribosyltransferase drives hESCs into the primed state" (siehe http://www.uniprot.org/uniprot/O95271)

Tankyrase inhibition *with XAV939* promotes a stable human naïve pluripotent state with improved functionality (Zimmerlin et al., Development 2016)

→ **3i + LIF für hESC ausreichend**

Was ist eine embryonale Stammzelle?

Der Blickwinkel ist entscheiden:

Siehe Geozentrischens versus Heliozentrisches Weltbild:

See also https://www.youtube.com/watch?v=waexG16WZrE

Tankyrase inhibition promotes a stable human naïve pluripotent state with improved functionality

Zimmerlin et al., 2016

Abstract

The derivation and maintenance of human pluripotent stem cells (hPSCs) in stable naïve pluripotent states has a wide impact in human developmental biology. However, hPSCs are unstable in classical naïve mouse embryonic stem cell (ESC) WNT and MEK/ERK signal inhibition (2i) culture. We show that a broad repertoire of conventional hESC and transgene-independent human induced pluripotent stem cell (hiPSC) lines could be reverted to stable human preimplantation inner cell mass (ICM)-like naïve states with only WNT, MEK/ERK, and tankyrase inhibition (LIF-3i). LIF-3i-reverted hPSCs retained normal karyotypes and genomic imprints, and attained defining mouse ESC-like functional features, including high clonal self-renewal, independence from MEK/ERK signaling, dependence on JAK/STAT3 and BMP4 signaling, and naïve-specific transcriptional and epigenetic configurations. Tankyrase inhibition promoted a stable acquisition of a human preimplantation ICM-like ground state via modulation of WNT signaling, and was most efficacious in efficiently reprogrammed conventional hiPSCs. Importantly, naïve reversion of a broad repertoire of conventional hiPSCs reduced lineage-primed gene expression and significantly improved their multilineage differentiation capacities. Stable naïve hPSCs with reduced genetic variability and improved functional pluripotency will have great utility in regenerative medicine and human disease modeling.

2.3.8. Unterschiede zwischen murinen und humanen embryonalen Stammzellen

mESCs benötigen:		hESC benöteigen:
LIF (STAT3)		FGF2 (MEK) + STAT3
Bmp2/4		TGFß / Activin / Nodal,
		Noggin (a Bmp and TGFß antagonist),
	gemeinsam:	
Wnt (ß-Catenin)		Wnt (ß-Catenin)
Insulin		Insulin

Warum dieser Unterschied?

Weil zwei verschiedene Spezies, die entwicklungsgeschichtlich zu weit weg sind?

Sind ESC, durch die Isolierungsmethode bedingte Artefakte?

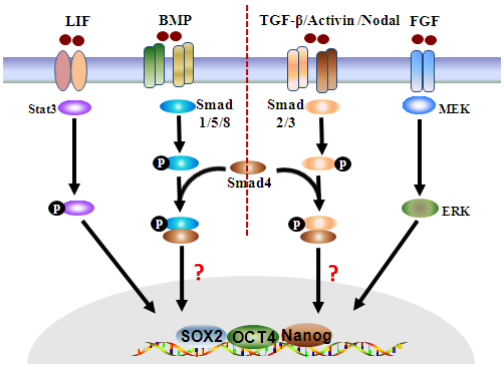
Es haben doch alle Stammzellen die gleiche „stemness“ oder „ trinity“ Transkriptions-faktoren Gene als Ziel-Gene dieser Signalübertragungswege.

Könnten unterschiedliche Entwicklungsstadien sein. →

Zusammenfassung der Unterschiede zwischen mESCs und hESCs

Maus:
mESCs

+ LIF (~ FGF2)



Mensch:
hESCs

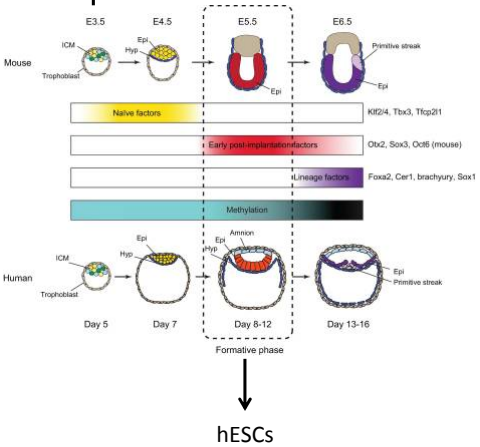
+ FGF2

ICM-like cells primitive Ectoderm-like cells

Ground state (naive state) → poised state → primed state

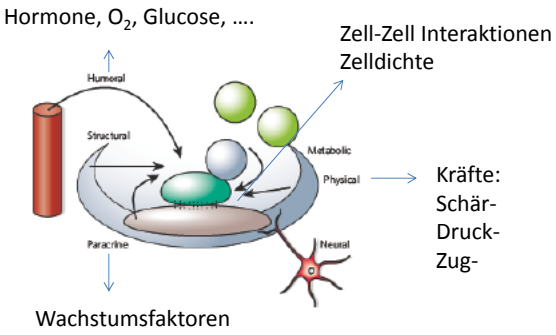
2.3.8. Unterschiedliche Entwicklungsstadien zwischen murinen und humanen embryonalen Stammzellen

mESCs



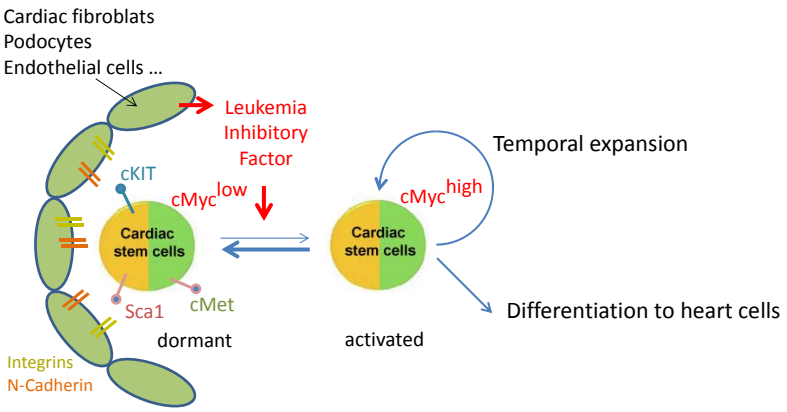
hESCs

3.3. Stammzellnischen



The stem-cell niche as an entity of action
David T. Scadden
Nature **volume 441**, pages 1075–1079 (2006)

The still hypothetic cardiac stem cell niche



Notch signaling and cell-cell interaction in the niche