Wiederholung der wichtigsten Punkte der 1. und 2. Doppelstunde

A. Grundlagen der Stammzellbiologie – Was ist eine Stammzellen?

Die Ontogenese der Stammzellen

1. Die Eigenschaften von Stammzellen
   - 1.1. Was unterscheidet eine Stammzelle von somatischen Zellen?
   - 1.2. Was ist eine embryonale Stammzelle?
   - 1.3. Was ist eine adulte Stammzelle?

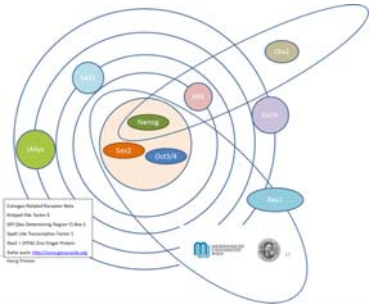
1.1. Eine Stammzelle hat in geeigneter Umgebung das unbegrenzte Potenzial zur phänotypisch stabilen Selbsterneuerung, zum Ruhen, und zur Hervorbringung von somatischen Zellen.
Sie sind selbsterneuernd, klonal, ruhefähig, umweltabhängig und entwicklungsfähig.

1.2. Embryonale Stammzellen befinden sich in der Inneren Zellmasse der Blastozysten und können daraus als stabile Zelllinie isoliert und unbegrenzt erhalten werden.

1.3. Somatische Stammzellen befinden sich in Nischen der Organe adulter Organismen und haben alle, wenn auch quantitativ unterscheidbare Attribute der embryonalen Stammzellen. Sie haben ein stark gedämpftes Selbsterneuerungs- und eingeschränktes Entwicklungspotenzial und ein vermutlich erhöhtes Ruhepotenzial.

A. Grundlagen der Stammzellbiologie – Was ist eine Stammzellen?

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



A. Grundlagen der Stammzellbiologie – Was ist eine Stammzellen?

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   - 3 .1. Intrinsische Faktoren - Transkriptionsfaktor Netzwerke
   - 3 .2. Extrinsische Faktoren – Signalübertragungsmechanismen
   - 3 .3. Stammzell-Nischen

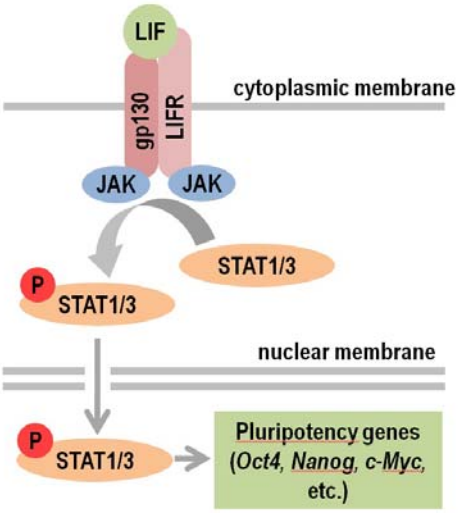
3.2. Extrinsische Faktore - Signalübertragunswege

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

3.2.1. LIF Signalübertragung (Interleukin 6 Familie

Maus ESCs:



2021:
OSM+OSMRß
kann LIF +LIFR
ersetzen!
IL6+ sIL6R auch

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

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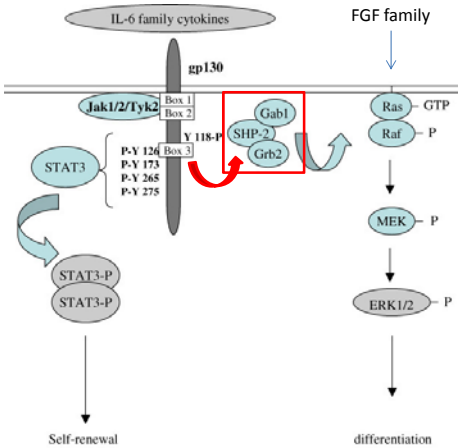

IL … Interleuckin
JAK … Janus activated kinase
STAT … Signal transducer and activator of transcription
ERK … extracellular signal related kinase

Grb2 … growth factor receptor bound 2
Gab1 … GRB2-Associated Binding Protein 1
SHP-2 … Src-homology 2 domain (SH2)-containing protein (Phosphatase)

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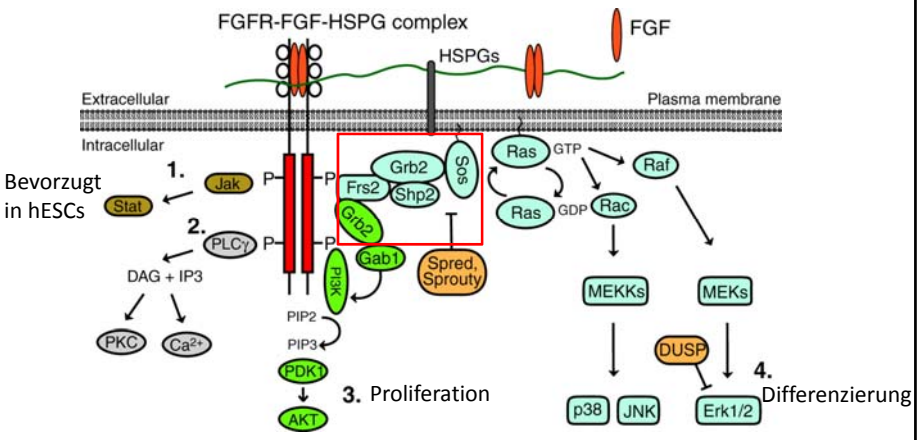
3.2.2. FGF Signalübertragung

FGF signaling in stem cells



HSPG, Heparan sulphate Proteoglycans
PKC, Protein Kinase C
PI3K, Phosphoinositid-3-Kinasen
PDK1 … 3-phosphoinositide-dependent protein kinase-*1*
Sos … Son of sevenless

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

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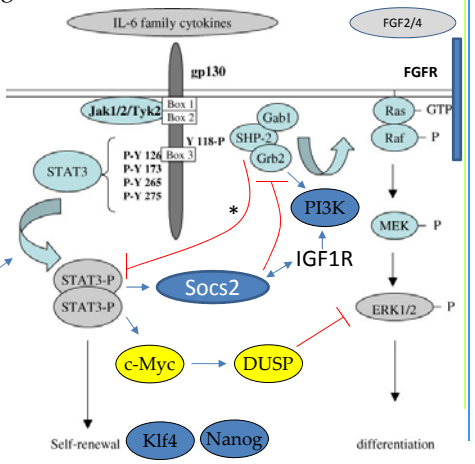
Verbindung zwischen LIF und FGF Signalübertragung

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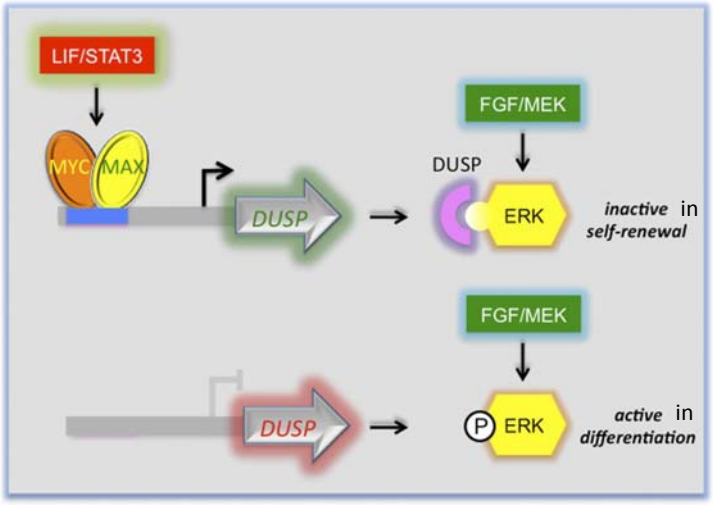
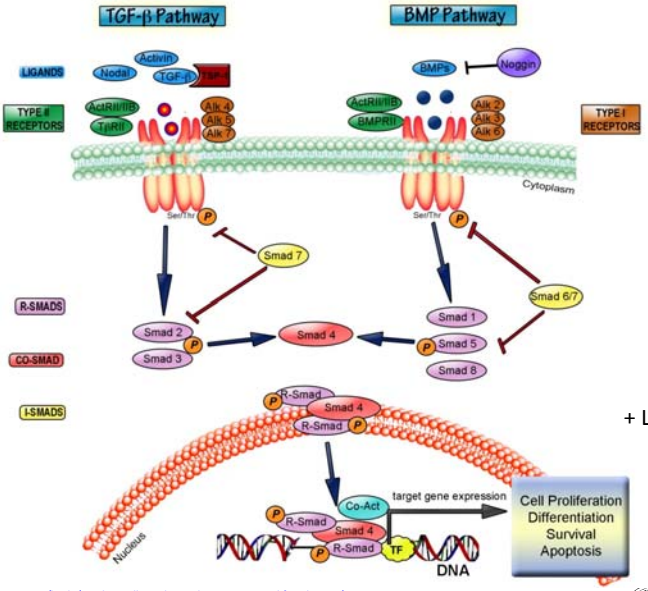
A model for ERK regulation by MYC/MAX complexes in murine pluripotent cells.



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
Brachyury
…

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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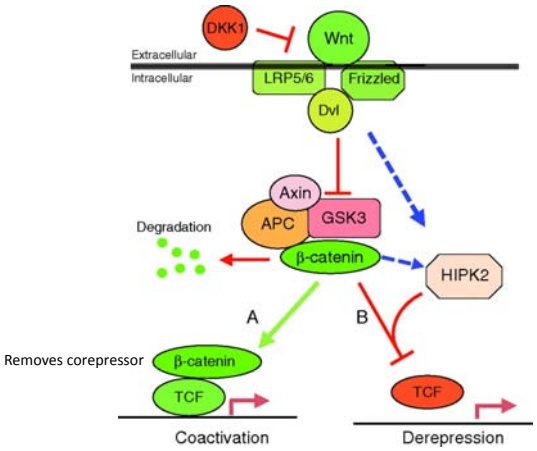
3.2.4. Wnt Signalübertragung

Wnt – ß Catenin – Signaling in ESCs

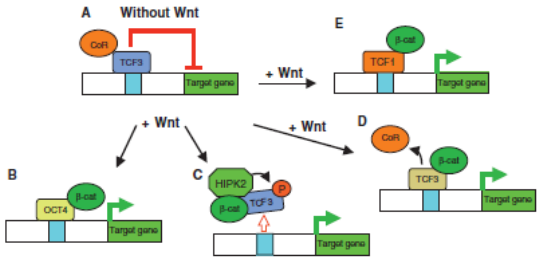


Siehe auch:
Maintaining embryonic stem cell pluripotency with Wnt signaling by Sergei Y. Sokol
Development 2011 138: 4341-4350; doi: 10.1242/dev.066209

TCF3, Transcription factor 3, auch T-cell factor 3
LRP5 … Low-density lipoprotein receptor-related protein 5
HIP2K … Homeodomain-interacting protein kinase 2
APC … Adenomatous polyposis coli-Protein

3.2.4. Wnt Signalübertragung

Wnt – ß Catenin – Signaling in ESCs - einfaches Aktivierungsmodell

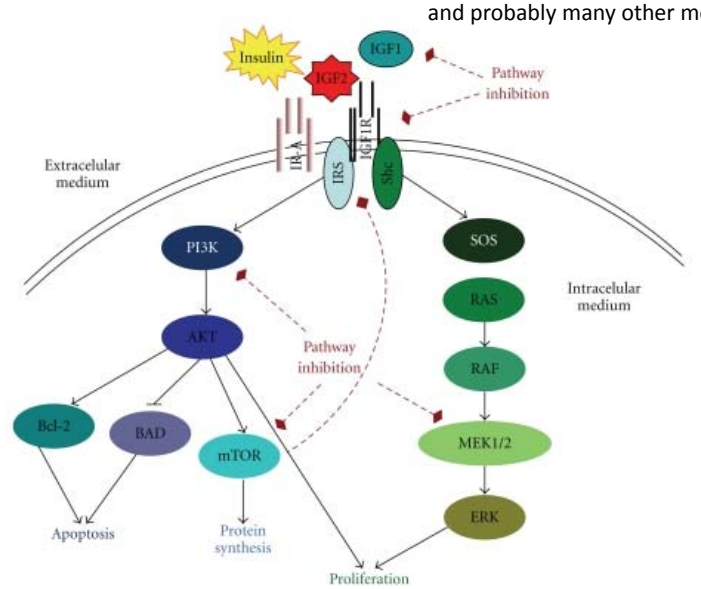


TCF3 = repressor
TCF1 = actovator
CoR, Corepressor

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 +Methabolic regulation via Glucose and Glutamine and probably many other metabolites

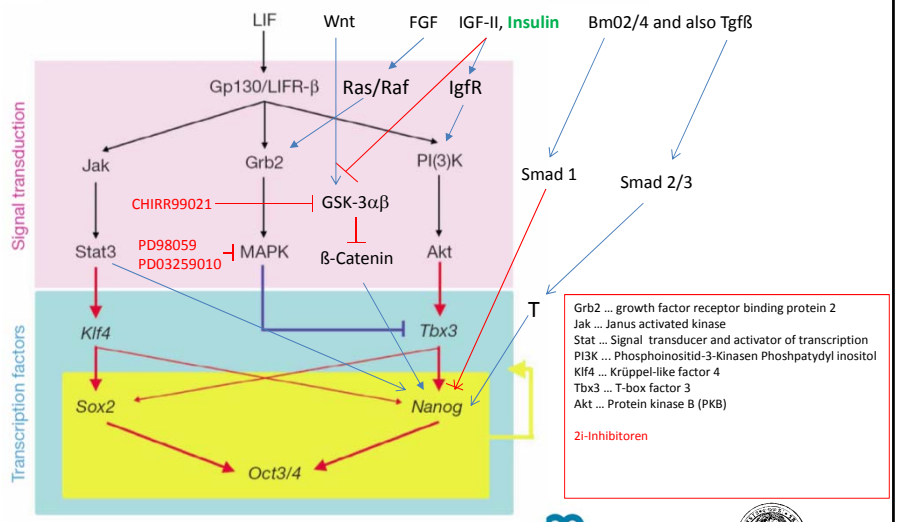


https://www.researchgate.net/figure/A-simple-schema-of-the-IGF-pathway-and-approaches-to-its-inhibition-Insulin-IGF2-and_fig1_50851552

A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells (and hESCs) … and explaining the function of 2i

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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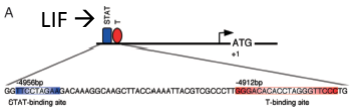
Nanog as gatekeeper of pluripotency



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

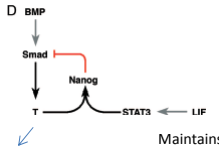
Ren-He Xu,[1,2,*] Tori L. Sampsell-Barron,[2] Feng Gu,[1] Sierra Root,[1] Ruthann M. Peck,[2] Guangjin Pan,[3] Junying Yu,[3,4] Jessica Antosiewicz-Bourget,[3,4] Shulan Tian,[5] Ron Stewart,[5] and James A. Thomson[3,4,5,6,*]

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

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

Atsushi Suzuki*†‡, Ángel Raya*§¶, Yasuhiko Kawakami*, Masanobu Morita*†‡, Takaaki Matsui*¶, Kinichi Nakashima*†‡, Pedro A. Sancho*, Juan Carlos Izpisúa Belmonte*, and Atsushi Ogura*†‡

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



Maintains self-renewal

Differentiation

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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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

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:

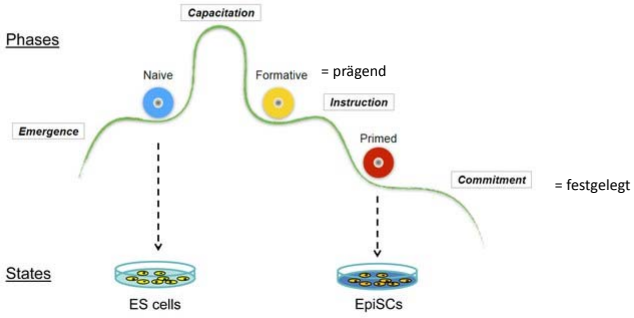
Naive - poised - formative – primed – commited state of embryonic stem cells

zu allen fähig- ausgeglichen – prägend –präpariet - festgelegt

Landscape of Pluripotency

(from Austin Smith 2018)

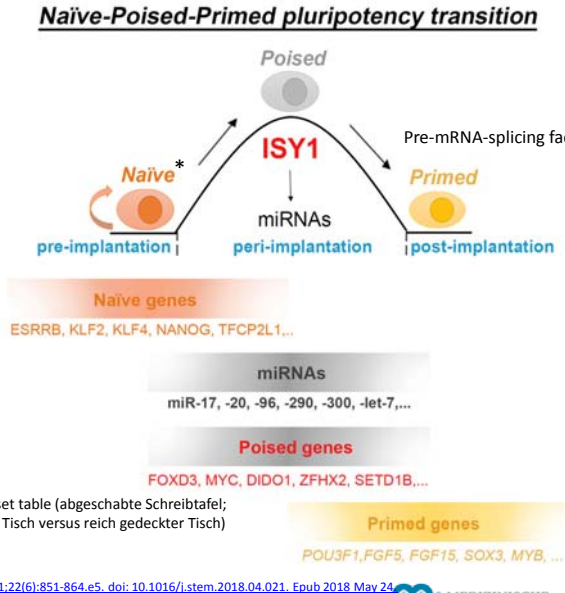
Poised = schwebend, ausgeglichen



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)

Modell wurde notwendig, um die Entstehung von PGCs und somatic stem cells erklären zu können

Gene die bevorzugt in den unterschiedichen Zuständen exprimiert werden:



Pre-mRNA-splicing factor ISY1 homolog

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

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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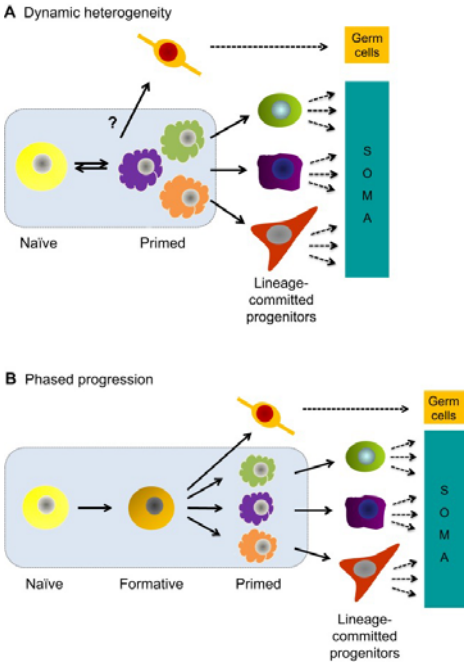
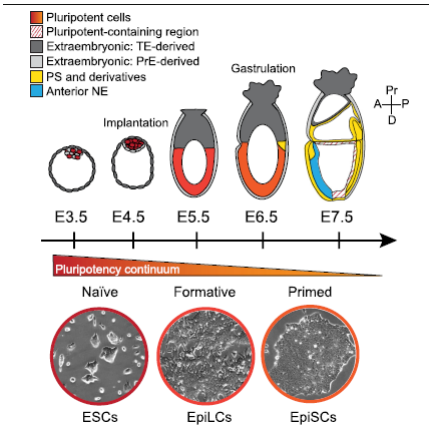
2 Modelle:





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

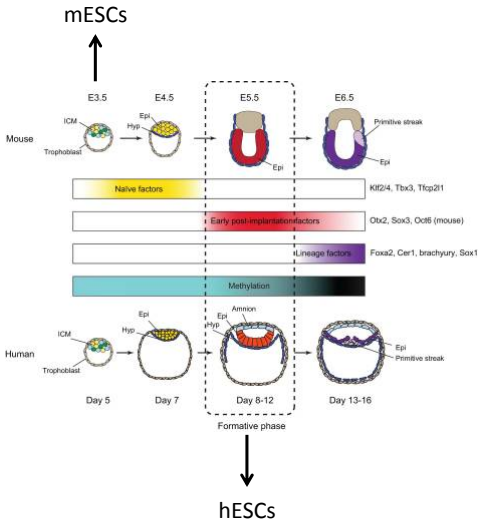
BMC Developmental Biology

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*]

2.3.8. Unterschiedliche Entwicklungsstadien zwischen murinen und humanen embryonalen Stammzellen



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

Wurde möglich, weil
„Tankyrase = Poly-ADP-ribosyltransferase drives hESCs into the primed state"
(siehe http://www.uniprot.org/uniprot/O95271) (durch ADP-riboslyierung von Proteinen) erkannt wurde.

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

→ **3i + LIF für hESC ausreichend**

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.