6. Doppelstunde am 11.11.2015

1.1.4. Molekulare Regulation der Selbsterneuerung

1.1.4.1. Intrinsische Faktoren (zellautonom)
- Transkriptionsfaktor Netzwerke,
DNA-RNA Polymerase II
und Telomerase

1.1.4.2. Extrinsische Faktoren (parakrin)
-Signalübertragungsmechanismen

1.1.4.3. Stammzell-Nischen (bereits besprochen)
-Zell-Zell Wechselwirkungen; humorale und metabolische Einflüsse und topologische Aspekte

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1.1.4. Molekulare Regulation der Selbsterneuerung

1.1.4.1. Intrinsische Faktoren (zellautonom)

1.1.4.1.1.	Transkriptionsfaktor Netzwerke und
1.1.4.1.2.	**DNA-RNA Polymerase II** und
1.1.4.1.3.	Telomerase

Transkriptionelle Kontrolle der Selbsterneuerung und Differenzierung durch DNA-RNA Polymerase II

Pausieren der DNA-RNA Polymerase II nach Transkriptionsstart auf Genen die den Zellzyklus regulieren und die Signalübertragunsfaktoren (Mediatoren) kodieren.

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Allg.:

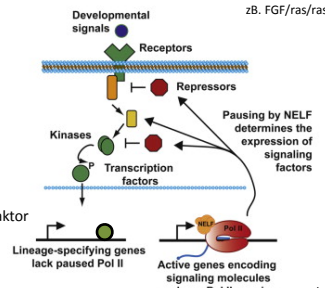
Signal

Rezeptor

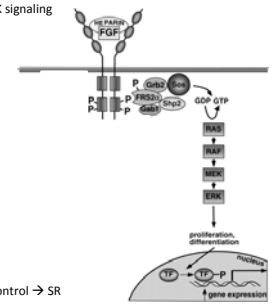
Mediator 1
.
.
.
Mediator n

Effektor = zB: Transkriptionsfaktor

Role for pausing of Pol II in Embryonic Stem Cells



zB. FGF/ras/ras/Mek /ERK signaling



… and cell cycle control → SR

NELF … negative elongation factor

Pausing of RNA Polymerase II Regulates Mammalian Developmental Potential through Control of Signaling Networks

Lucy H. Williams , George Fromm , Nolan G. Gokey , Telmo Henriques , Ginger W. Muse , Adam Burkholder , David C. ...

Molecular Cell, Volume 58, Issue 2, 2015, 311 – 322 http://dx.doi.org/10.1016/j.molcel.2015.02.003

Plasticity of ESCs to maintain SR and be prepared for Differentiation ~ naive state (2i + LIF) (Xa Xa)
Poised state = Zustand indem etwas (ESCs) für etwas (Differentiation) bereit ist. ~
~ primed state (Foetal serum + LIF) (Xa Xi)

From wikipedia

Negative elongation factor
From Wikipedia, the free encyclopedia
In molecular biology, **NELF** (negative elongation factor) is a four-subunit protein (NELF-A, NELF-B, NELF-C/NELF-D, and NELF-E) that negatively impacts transcription by RNA polymerase II (Pol II) by pausing about 20-60 nucleotides downstream from the transcription start site (TSS).[1]

Structure
The NELF-A subunit is encoded by the gene WHSC2 (Wolf-Hirschhorn syndrome candidate 2). Microsequencing analysis demonstrated that NELF-B was the protein previously identified as the protein encoded by the gene COBRA1, and was shown to interact with BRCA1.[2] It is unknown whether or not NELF-C and NELF-D are peptides resulting from the same mRNA with different translation initiation sites, possibly differing only in an extra 9 amino acids for NELF-C at the N-terminus, or peptides from different mRNAs entirely. A single NELF complex consists of either NELF-C or NELF-D but not both. NELF-E is also known as RDBP.[2][3]
NELF binds in a stable complex with DSIF and RNA polymerase II together, but not with either alone. P-TEFb (positive transcription elongation factor b) inhibits the effect of NELF and DSIF on Pol II elongation, via its phosphorylation of serine-2 of the C-terminal domain of Pol II, and the SPT5 subunit of DSIF, causing dissociation of NELF.[2] NELF homologues exist in some metazoans (e.g. insects and vertebrates) but have not been found in plants, yeast, or nematode (worms).[2]

References
Adelman, Karen; Lis, John T. (18 September 2012). "Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans". *Nature Reviews Genetics* **13** (10): 720–731. doi:10.1038/nrg3293. Retrieved 18 December 2014.
Narita, Takashi; Yamaguchi, Yuki; Yano, Keiichi; Sugimoto, Seiji; Chanarat, Sittinan; Wada, Tadashi; Kim, Dong-ki; Hasegawa, Jun; Omori, Masashi; Inukai, Naoto; Endoh, Masaki; Yamada, Tomoko; Handa, Hiroshi (15 March 2003). "Human Transcription Elongation Factor NELF: Identification of Novel Subunits and Reconstitution of the Functionally Active Complex". *Molecular and Cellular Biology* **23** (6): 1863–1873. doi:10.1128/MCB.23.6.1863-1873.2003. PMC 149481. PMID 12612062. Retrieved 18 December 2014.
Yamaguchi, Yuki; Takagi, Toshiyuki; Wada, Tadashi; Yano, Keiichi; Furuya, Akiko; Sugimoto, Seiji; Hasegawa, Jun; Handa, Hiroshi (April 1999). "NELF, a Multisubunit Complex Containing RD, Cooperates with DSIF to Repress RNA Polymerase II Elongation". *Cell* **97** (1): 41–51. doi:10.1016/S0092-8674(00)80713-8. PMID 10199401. Retrieved 18 December 2014.

FRS2α … **F**ibroblast growth factor **r**eceptor **s**ubstrat 2
Grb2 … **G**rowth factor **r**eceptor **b**inding protein 2
SOS … **S**on **o**f **s**evenless homolog 1
Raf … *rapidly **a**ccelerated **f**ibrosarcoma oder **Ra**t **f**ribrosarcoma*
*Ras … Ra*t sarcoma
MEK … **M**itogen-activated protein kinase kinase (MAPKK)
ERK1/2 … **E**xtracellular-signal regulated *kinases 1 and 2 (MAPK)*

1.1.4. Molekulare Regulation der Selbsterneuerung

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1.1.4.1.2. DNA-RNA Polymerase II und
1.1.4.1.3. Telomerase

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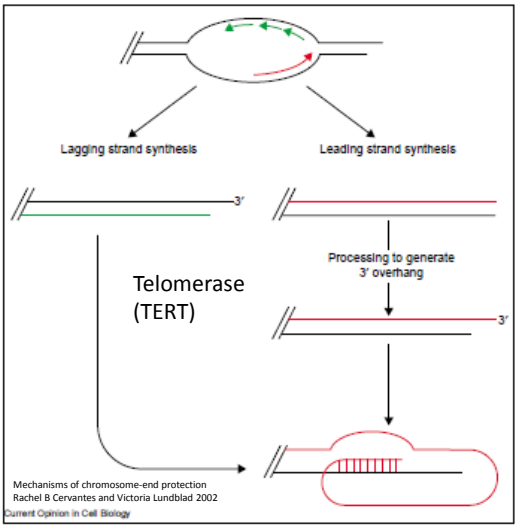
1.1.4.1.3. **Erhaltung der Selbsterneuerungsfähigkeit: Funktionsweise der Telomerase**



Semiconservative DNA replication generates two structurally distinct chromosome termini. Processing of the leading strand terminus through a yet unknown mechanism generates a 3' overhang, thereby rendering it accessible to the same regulatory mechanisms that act on the lagging-strand telomere, such as the formation of a t-loop. Whether the lagging strand is processed further, prior to t-loop formation is unknown. In the absence of end protection, the blunt-ended leading-strand terminus may be subject to end-to-end fusions; whether this implies an additional mechanism for protection of the leading-strand terminus that is not employed by the lagging-strand terminus is unclear.

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6. Doppelstunde am 11.11.2015

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Die Selbsterneuerungsfähigkeit wird beinflusst durch:

Intrinsische Faktoren: Transkriptionsfaktoren („Stemness Factors"):
Oct 3/4
Nanog
Sox2

Transkirptionelle Kontrolle durch DNA-RNA Polymerase II

Extrinsische Faktoren: Wachstumsfaktoren:

IL6 Familie	(JAK / STAT Signaltransduktionsweg)
FGF Familie	(Ras / Raf Signaltransduktionsweg)
Wnt Familie	(ß-catenin Signaltransduktionsweg)
TGFß/BMB Familie	(Smad Signaltransduktionsweg)
IGF	(PI3K / Akt Signaltransduktionsweg) (bei hESCs)

Zell-Zell und Zell-Matrix Wechselwirkungen

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Extrinsische Signale für die Selbsterneuerung (und Differenzierung) von Stammzellen

LIF - JAK/STAT Signaling

FGF – Ras/Raf Signaling

Tgf-ß – Smad Signaling

Wnt – ß-Catenin Signaling

IGF/Insulin – PI3K Signaling

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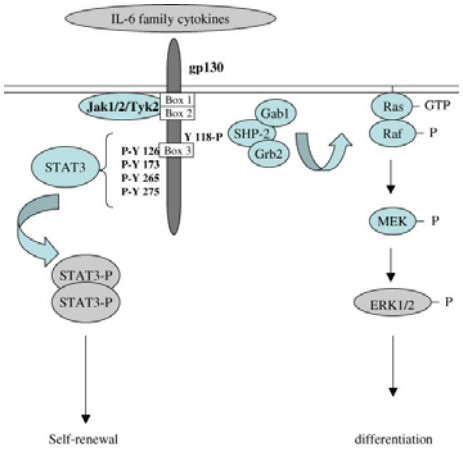
Molekulare Grundlagen der Selbsterneuerung
von embryonalen Stammzellen

Signalübertragungswege der IL6 Familie

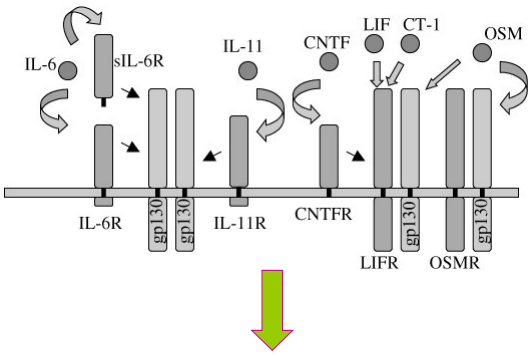
Was passiert in der Zelle,
wenn LIF an den LIFR bindet?

LIF Signalübertragung



Y … thyrosine

Heterogenität der IL6-Familie Rezeptoren



Pleiotrope und redundante Funktionen

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Heterogenität der IL6 Familie

?

- LIF
- IL6
- IL11 (+12)
- OSM Oncostatin M
- CT1 Cardiotropin 1
- CNTF ciliar neurotrphic factor

Interleukin 6 Familie
der Zytokine

- LIFR
- gp130 Glykoprotein 130kDa
- sIL6R
- IL6R
- IL11R
- OSMR
- CNTFR

Zytokinrezeptoren
Klasse 1

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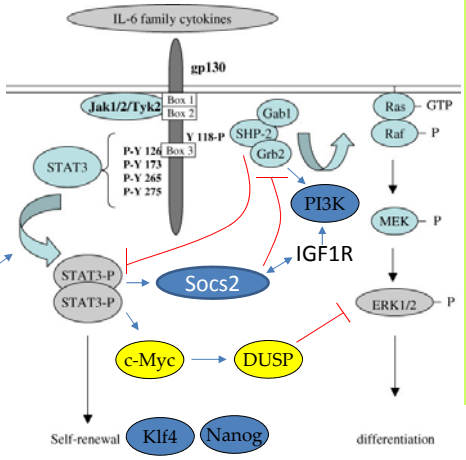
Verbindung zwischen LIF und FGF Signalübertragung

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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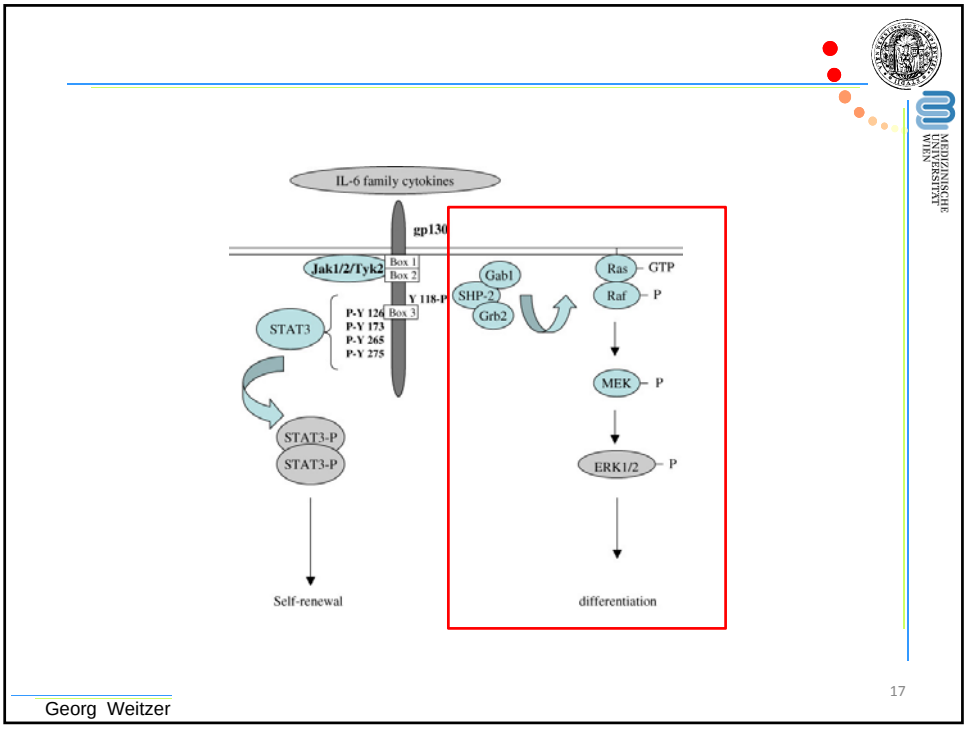
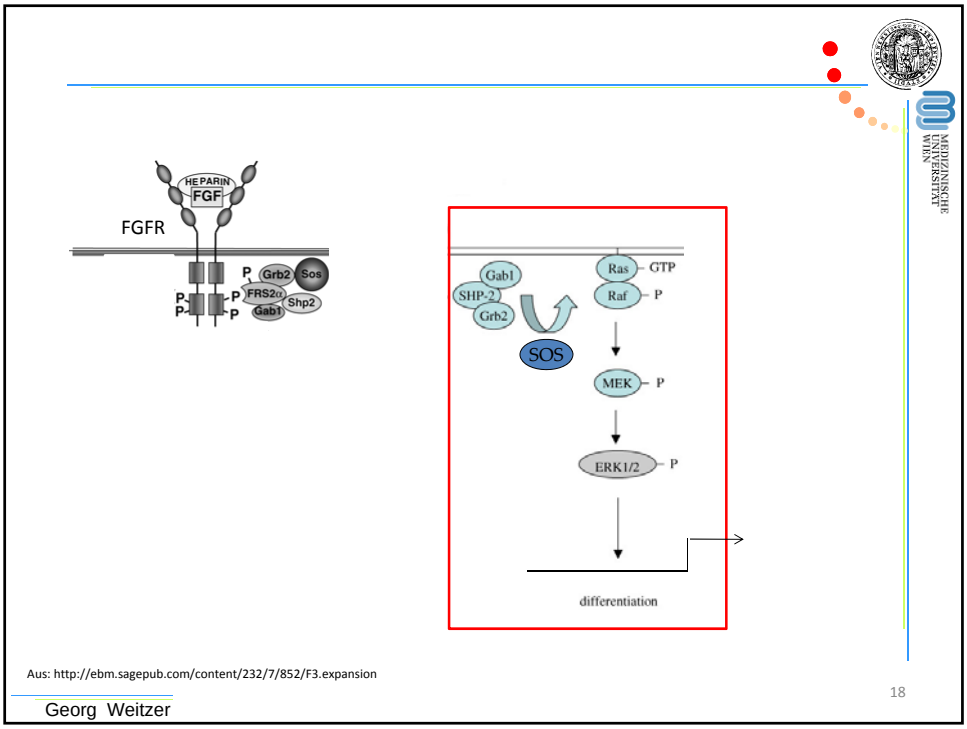
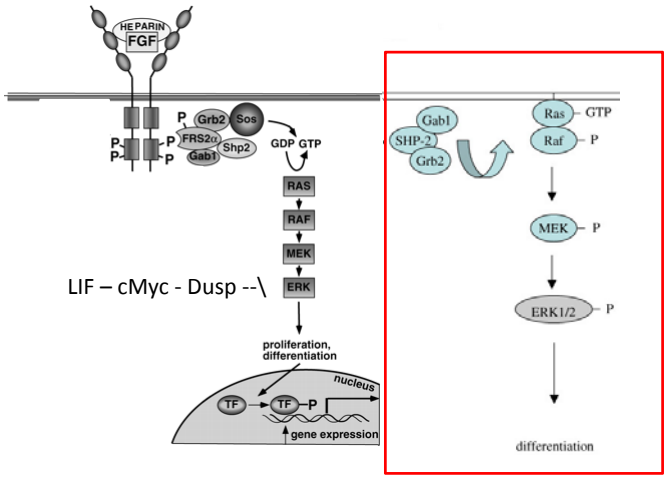
Signalübertragungswege der FGF Familie





Aus: http://ebm.sagepub.com/content/232/7/852/F3.expansion



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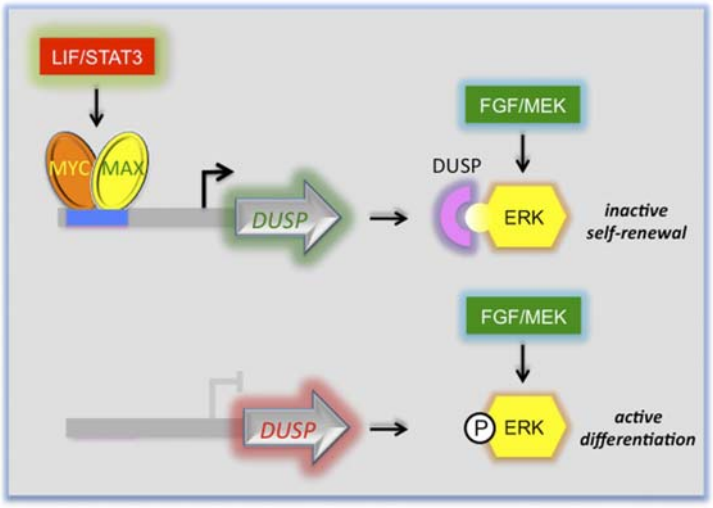
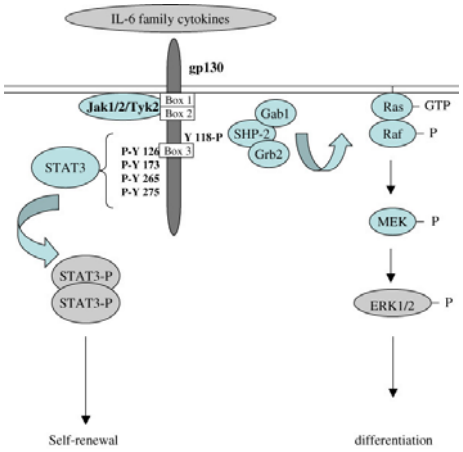
A model for ERK regulation by MYC/MAX complexes in murine pluripotent cells.



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

LIF Signalübertragung



Y … thyrosine

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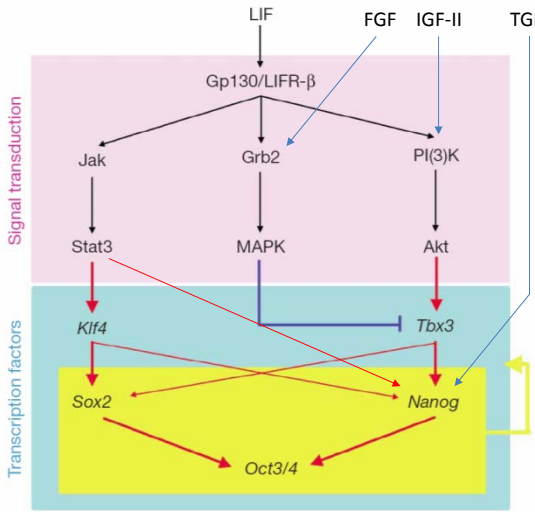
A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells

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



TGFßR / ALK

Smad 1 / 2

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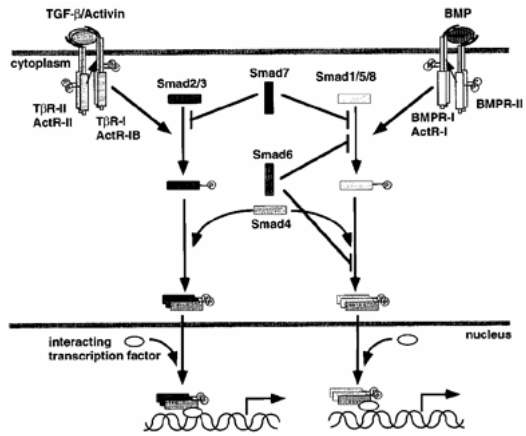
Signalübertragungswege der Tgf-ß Familie



Moustakas, A., and Heldin, C. H. (2005). Non-Smad TGF-beta signals. J Cell Sci *118*, 3573-3584.

Piek, E., Heldin, C. H., and Ten Dijke, P. (1999). Specificity, diversity, and regulation in TGF-beta superfamily signaling. Faseb J *13*, 2105-2124.

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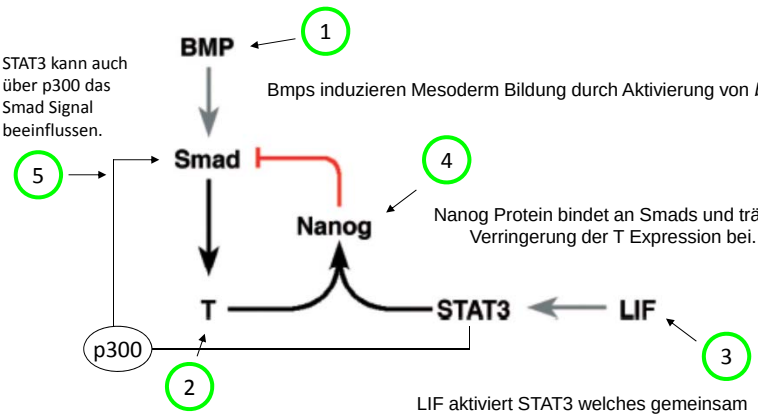
von Astrid Aufinger

Negative feedback mechanism by which Nanog blocks BMP-induced T expression in the presence of LIF/STAT3 signaling

Suzuki, A., Raya, A., Kawakami, Y., Morita, M., Matsui, T., Nakashima, K., Gage, F. H., Rodriguez-Esteban, C., and Izpisua Belmonte, J. C. (2006).
Nanog binds to Smad1 and blocks bone morphogenetic protein-induced differentiation of embryonic stem cells.
Proc Natl Acad Sci U S A *103*, 10294-10299.



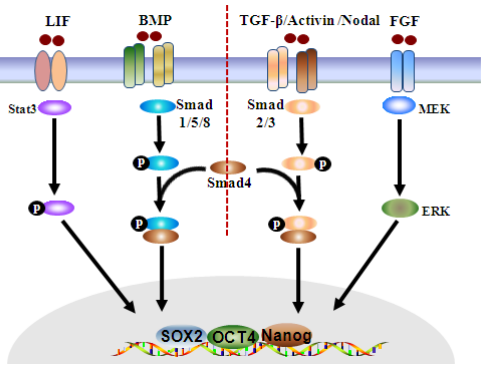
STAT3 kann auch über p300 das Smad Signal beeinflussen.

Bmps induzieren Mesoderm Bildung durch Aktivierung von *brachyury* (T).

Nanog Protein bindet an Smads und trät so zur Verringerung der T Expression bei.

LIF aktiviert STAT3 welches gemeinsam mit T das *nanog* Gen aktiviert.

Brachyury löst in Gegenwart von LIF einen negativen feedback loop aus.



DOI: 10.5772/54365