

Primordiální zárodečné buňky - PGC (primordial germ cell)

- PGC se u myši objevují již 6 dpc, pravděpodobně je jejich vznik indukovaný v průběhu gastrulace, a to vnějšími signály, zejména BMP (na rozdíl od žab, *Drosophily* a *C. elegans*).
- 6 - 7.5 dpc migrují vně vlastní embryo, později (8.5 dpc <) migrují podél zadního střeva embrya do vytvořené zárodečné lišty.
- PGC zanikají po usazení v zárodečné liště (10-13 dpc u myši), stávají se z nich zárodečné buňky. Prodělají ještě 2-3 mitózy a u samců vznikají prospermatogonie zastavené v G0/G1 fázi mitózy. U samic vstupují do meiotické profáze (obojí > 12.5 dpc).
- Podobně jako ICM a ES buňky mají vysokou hladinu alkalické fosfatázy (ale ne TNAP, ale GCAP/TNAP), také exprimují *Oct4*, *Nanog*, ..
- Významné (pro specifikaci) jsou zdá se zejména *Stella*, *Fragilis*, *Nanog*,....
- PGC nejsou pluripotentní, jsou unipotentní!
- PGC mají omezený počet dělení, u myši napočítáno kolem 1000 buněk, rozdíly v závislosti na imbrední lini (případně metodě 😊)



Další klíčové geny ve specifikaci PGC

Stella (DPPA - developmental pluripotent associated 3)

Od oocyty (maternalní) po epiblast, později jen u PGC, podílí se na udržení jejich fenotypu, po jejich usazení v zárodečné liště jeho exprese vymizí. Také u ES buněk a některých nádorů. Represor transkripce. PCG protein, regulace methylace genomu.

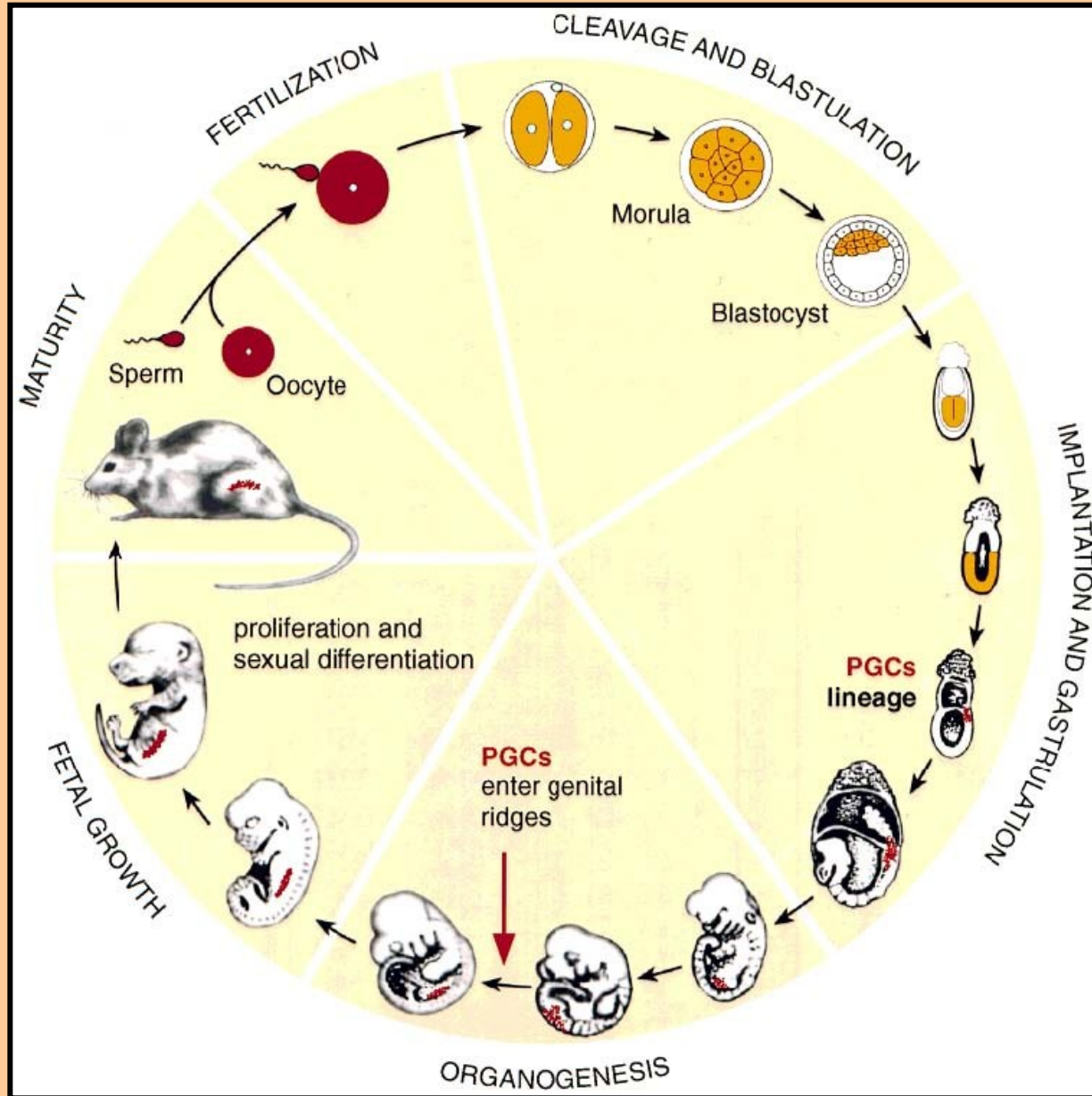
Fragilis (IFITM3 - Interferon induced transmembrane protein 3)

Z rodiny IFN indukovaných genů, je silně exprimován při formování PGC, s jejich migrací jeho exprese klesá.

Transmembránový protein, narušuje intracelulární metabolismus cholesterolu a tím modifikuje vlastnosti buněčných membrán.

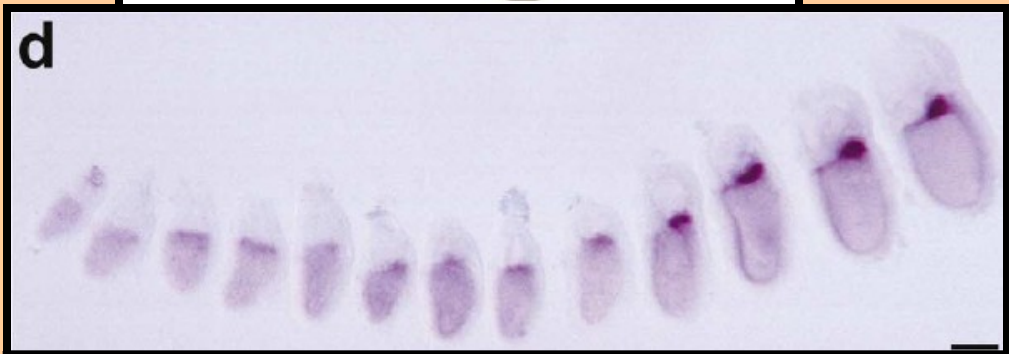
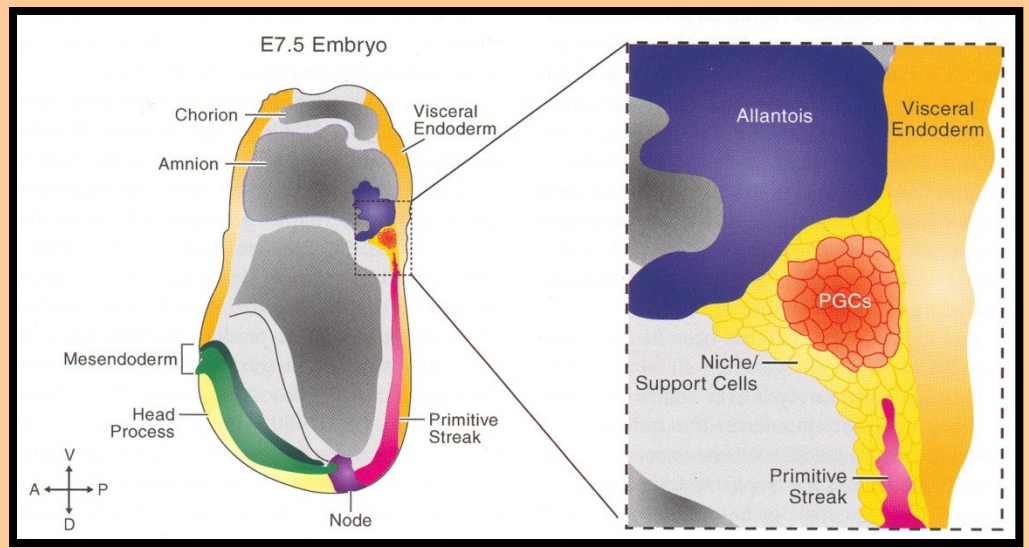
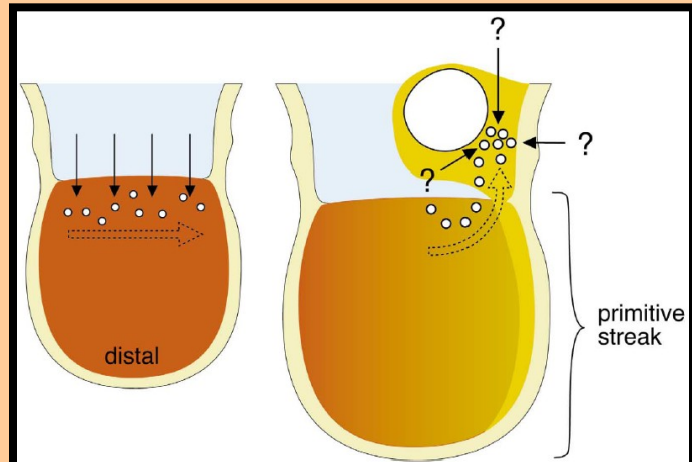


Cyklus zárodečných buněk u myši



PGC

primordiální zárodečné buňky



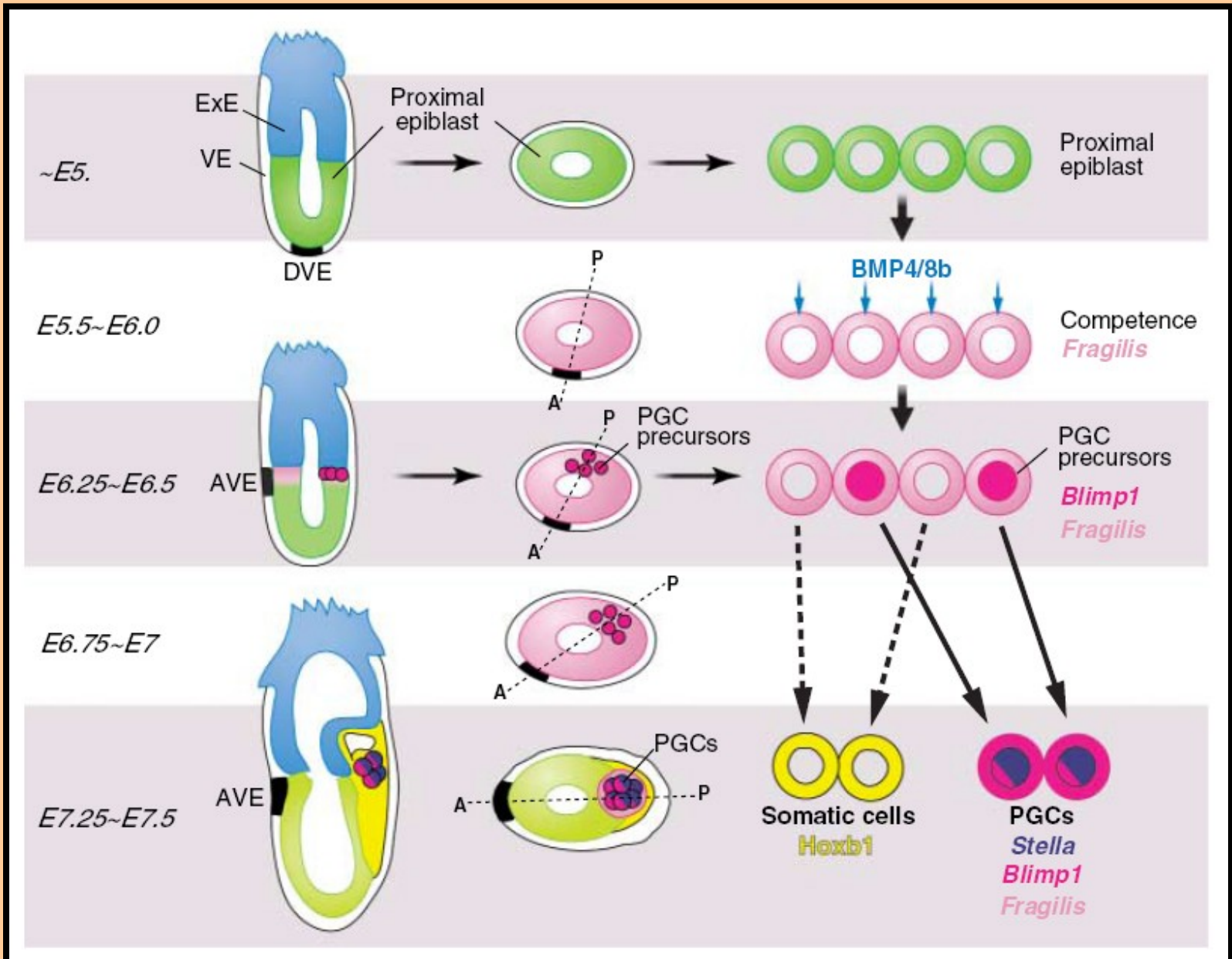
Expresse Fragilis mezi 6 – 7 dpc

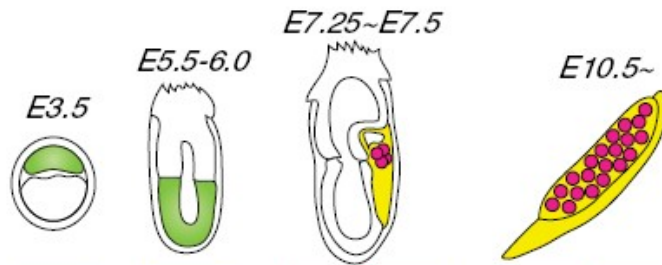


Expresse Oct-4 v PGC usazených v zárodečné liště



Mechanismus vzniku PGC



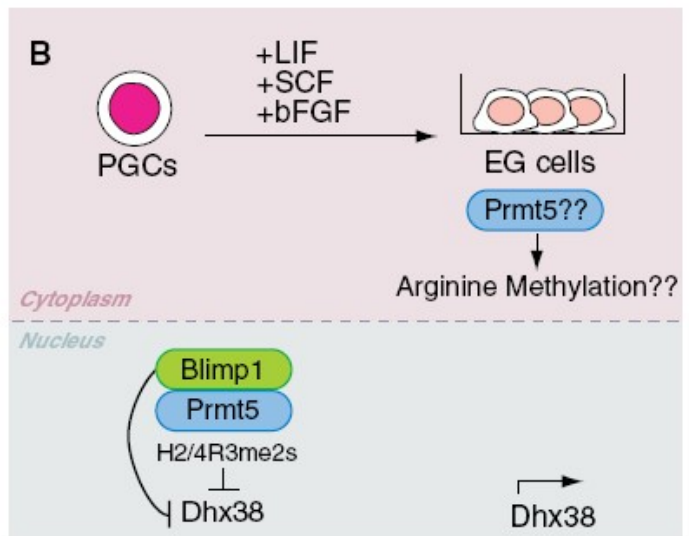
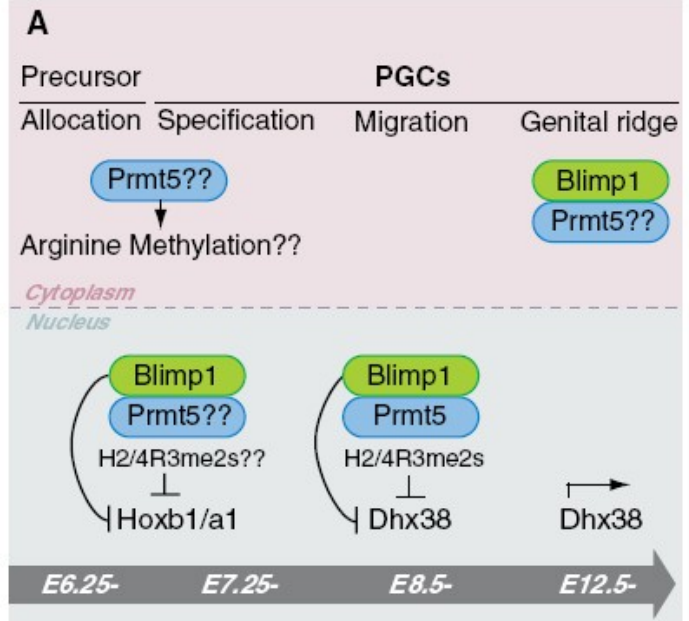


| | | E3.5 | E5.5-6.0 | E7.25~E7.5 | E7.25~E7.5 | E10.5~ | E10.5~ |
|---------------------------|-----------------|------|----------|------------|------------|--------|--------|
| Pluripotent | <i>Oct4</i> | + | + | + | - | + | - |
| | <i>Sox2</i> | + | +/- | + | - | + | +/- |
| | <i>Nanog</i> | + | - | + | - | + | - |
| Germ cell-enriched | <i>Blimp1</i> | - | - | + | - | + | +/- |
| | <i>Stella</i> | + | - | + | - | + | - |
| | <i>Fragilis</i> | + | +/- | + | +/- | + | +/- |
| | <i>Nanos3</i> | - | - | + | - | + | - |
| | <i>Dnd</i> | -? | -? | + | +/- | + | +/- |
| | <i>Dazl</i> | +? | -? | - | - | + | - |
| | <i>Mvh</i> | - | - | - | - | + | - |
| Somatic | <i>Hoxa1</i> | - | - | - | + | - | +/- |
| | <i>Hoxb1</i> | - | - | - | + | - | +/- |
| | <i>T</i> | - | +/- | - | + | - | +/- |

+/- The expression depends on cells, regions or tissues

? Data are preliminary

| Epigenetic | | E7.25~E7.5 | E10.5~ |
|-------------------|-----------|------------|--------|
| | H3K4me2/3 | +++ | + |
| | H3K9me2 | + | +++ |
| | H3K27me3 | +++ | + |
| | 5meC | + | +++ |



Embryonální zárodečné buňky - EGC (Embryonic germ cells)

- EGC jsou odvozeny z primordiálních zárodečných buněk (PGC - primordial germ cell).
- Podobně jako ES buňky je lze expandovat *in vitro*, a jsou pluripotentní, jak dokazuje jejich schopnost diferencovat do buněk všech tří zárodečných listů jak *in vitro* (EB), tak *in vivo* (chiméry a teratomy).
- Z epigenetického pohledu (DNA metylace) jsou však více podobné PGC než ES buňkám
- Rozdíly v metylaci DNA se týkají zejména imprintovaných genů v závislosti na pohlaví, u EGC izolovaných z pozdějších embryonálních stádií se tento rozdíl zmenšuje. Tyto „imprinting-free“ PGC, však již netvoří zdravé chimerické jedince.
- U myši lze EGC izolovat z PGC mezi 8.5 - 12.5 dpc, později to již nelze
- PGC a následně EGC lze izolovat *in vitro* z ES buněk (lidských i myších)
- m/hEGC jsou závislé na LIF, během časně kultivace i na FGF2 a Stem cell faktoru (SCF; + feeder & FCS/SR).
- Exprimují podobné markery jako ES buňky, lidské EGC jsou fenotypem více podobné mES než hES (morfologie + exprese SSEA-1!;
u hES se SSEA-1 exprimuje až s jejich diferenciací)

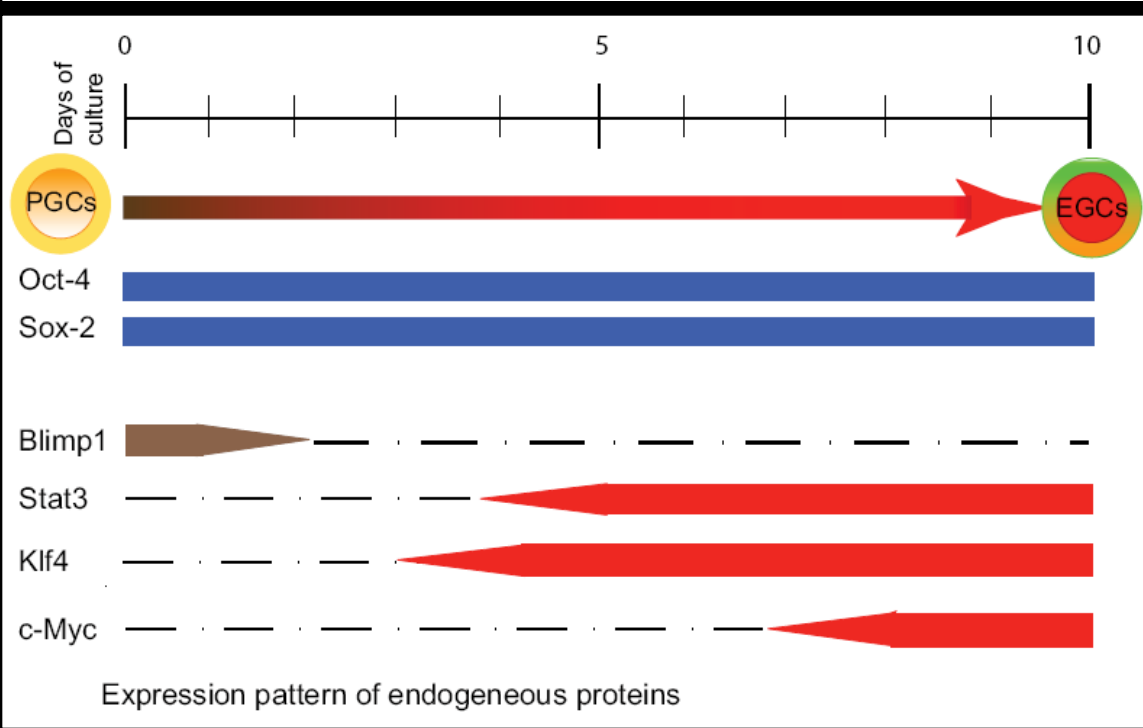
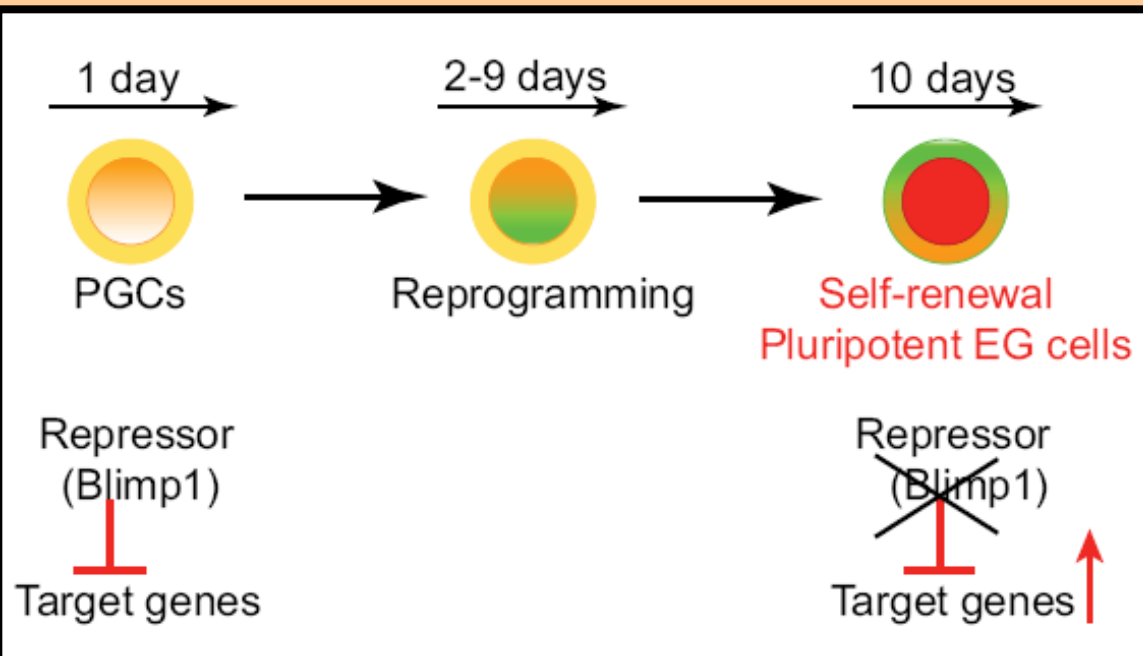


Vznik EGC z PGC

| Gene | 8.5 dpc PGCs | | | | EG cells | | | |
|----------------|--------------|---|----|----|----------|---|---|---|
| | 5 | 7 | 14 | 20 | 1 | 2 | 3 | 6 |
| Group I | | | | | | | | |
| Oct-4 | + | + | + | + | + | + | + | + |
| Sox-2 | + | + | + | + | + | + | + | + |
| nanog | + | + | + | + | + | + | + | + |
| Dppa5(Esg-1) | + | + | + | + | + | + | + | + |
| Sall4 | + | + | + | + | + | + | + | + |
| mRif1 | + | + | + | + | + | + | + | + |
| E-cadherin | + | + | + | + | + | + | + | + |
| TNAP | + | + | + | + | + | + | + | + |
| Rex1 (Zfp42) | + | + | + | + | + | + | + | + |
| Utf1 | + | + | + | + | + | + | + | + |
| Dppa3 (Stella) | + | + | + | + | + | + | + | + |
| Sox15 | + | + | + | + | + | + | + | + |
| Grb2 | - | + | + | + | + | + | + | + |
| β-catenin | - | + | + | + | + | + | + | + |

| Gene | 8.5 dpc PGCs | | | | EG cells | | | |
|-----------------|--------------|---|----|----|----------|---|---|---|
| | 5 | 7 | 14 | 20 | 1 | 2 | 3 | 6 |
| Group II | | | | | | | | |
| <i>c-Myc</i> | - | - | - | + | + | + | + | + |
| <i>Stat-3</i> | - | - | - | - | + | + | + | + |
| <i>Klf-4</i> | - | - | - | - | + | + | + | + |
| Dnmt3L | - | - | - | - | + | + | + | + |
| ERas | - | - | - | - | + | + | + | + |
| Fthl-17 | - | - | - | - | + | + | + | + |
| Dppa2 | - | - | - | - | + | + | + | + |
| Dppa4 | + | - | - | - | + | + | + | + |
| Ecat1 | - | - | - | - | + | + | + | + |
| Ecat8 | - | + | + | - | + | + | + | + |
| Gdf3 | + | + | + | + | + | - | - | - |
| Fbxo15 | - | + | - | + | + | + | + | + |
| Tcl1 | - | + | - | - | + | + | + | + |

Group I – genes in this group were expressed in both PGCs and EG cells.
 Group II – genes were expressed differentially in PGCs and EG cells, and shown as as positive (+) and negative (-).
 E8.5 PGCs samples tested for expression are referred to as. 5. 7. 14. 20.



Porovnání transkriptomů mES, mPGC a některých tkání

(Mise et al., 2008)

Table 1 Pairwise comparisons of gene expression profiles

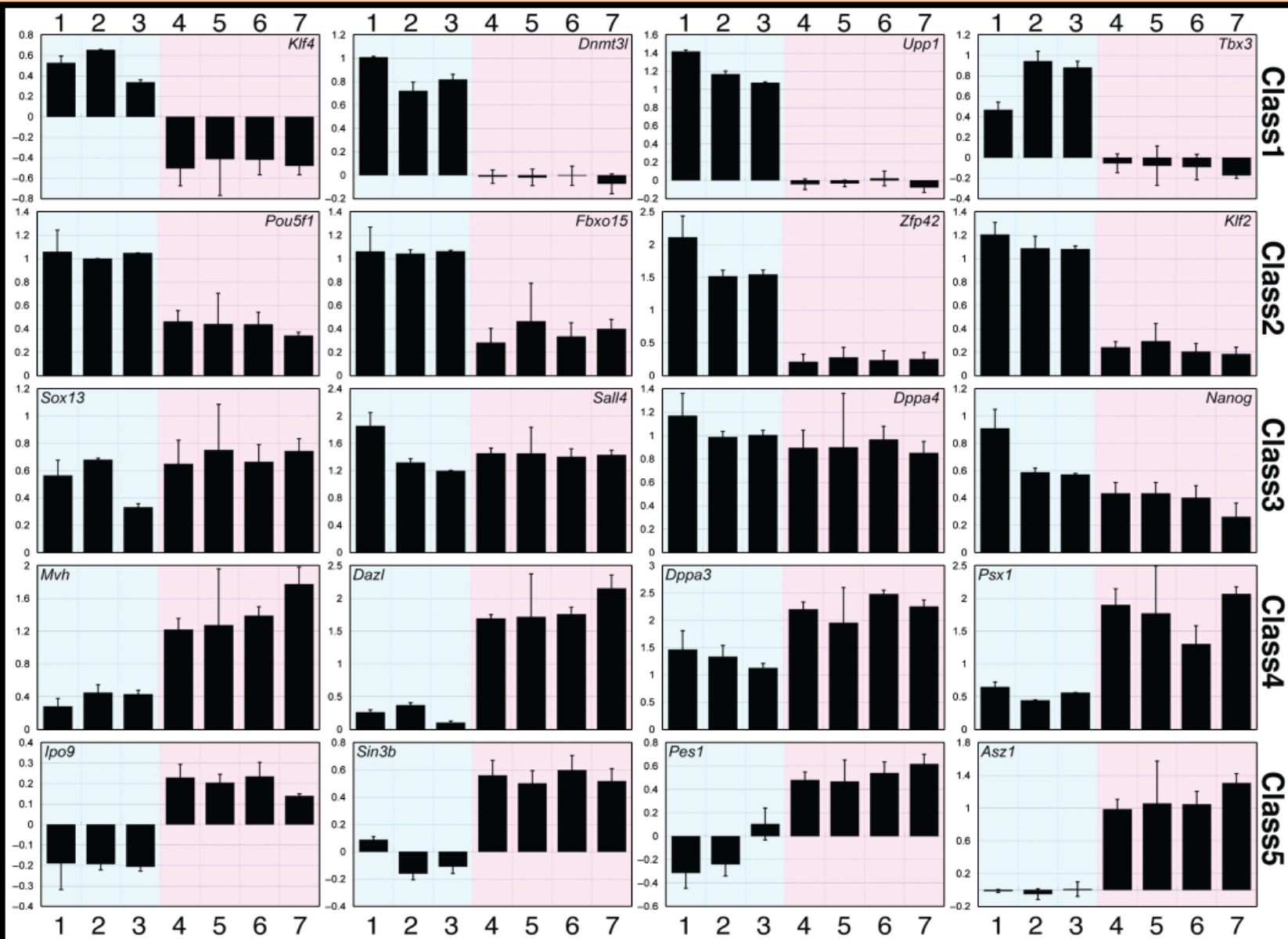
| | R1 | E14tg2a | TMA5 | TMA55G | TMA58G | <i>in vitro</i> PGC_LacZ | <i>in vitro</i> PGC_GFP | 11.5 male PGC | 11.5 female PGC | 12.5 male PGC | 12.5 female PGC | 13.5 male PGC | 13.5 female PGC | GS | Testis | Heart | Brain | Liver | Spleen | Thymus |
|--------------------------|-------|---------|-------|--------|--------|-----------------------------|----------------------------|------------------|-----------------------|---------------------|-----------------------|---------------------|-----------------------|-------|--------|-------|-------|-------|--------|--------|
| R1 | | 0.878 | 0.709 | 0.742 | 0.684 | 0.651 | 0.639 | 0.515 | 0.662 | 0.613 | 0.654 | 0.629 | 0.591 | 0.533 | 0.454 | 0.150 | 0.203 | 0.203 | 0.213 | 0.278 |
| E14tg2a | 0.878 | | 0.715 | 0.754 | 0.643 | 0.572 | 0.615 | 0.439 | 0.619 | 0.561 | 0.634 | 0.582 | 0.562 | 0.489 | 0.459 | 0.109 | 0.201 | 0.183 | 0.238 | 0.309 |
| TMA5 | 0.709 | 0.715 | | 0.867 | 0.598 | 0.520 | 0.460 | 0.424 | 0.612 | 0.492 | 0.547 | 0.525 | 0.489 | 0.431 | 0.606 | 0.274 | 0.135 | 0.179 | 0.201 | 0.300 |
| TMA55G | 0.742 | 0.754 | 0.867 | | 0.672 | 0.614 | 0.555 | 0.517 | 0.696 | 0.604 | 0.649 | 0.627 | 0.598 | 0.505 | 0.602 | 0.287 | 0.155 | 0.193 | 0.218 | 0.297 |
| TMA58G | 0.684 | 0.643 | 0.598 | 0.672 | | 0.874 | 0.652 | 0.645 | 0.745 | 0.800 | 0.708 | 0.794 | 0.733 | 0.664 | 0.505 | 0.396 | 0.304 | 0.401 | 0.294 | 0.320 |
| <i>in vitro</i> PGC_LacZ | 0.651 | 0.572 | 0.520 | 0.614 | 0.874 | | 0.686 | 0.644 | 0.692 | 0.741 | 0.660 | 0.747 | 0.706 | 0.620 | 0.468 | 0.379 | 0.275 | 0.380 | 0.249 | 0.257 |
| <i>in vitro</i> PGC_GFP | 0.639 | 0.615 | 0.460 | 0.555 | 0.652 | 0.686 | | 0.585 | 0.611 | 0.647 | 0.630 | 0.635 | 0.593 | 0.531 | 0.406 | 0.280 | 0.313 | 0.342 | 0.236 | 0.257 |
| 11.5 male PGC | 0.515 | 0.439 | 0.424 | 0.517 | 0.645 | 0.644 | 0.585 | | 0.760 | 0.724 | 0.700 | 0.758 | 0.714 | 0.623 | 0.419 | 0.319 | 0.350 | 0.311 | 0.245 | 0.300 |
| 11.5 female PGC | 0.662 | 0.619 | 0.612 | 0.696 | 0.745 | 0.692 | 0.611 | 0.760 | | 0.898 | 0.908 | 0.901 | 0.855 | 0.723 | 0.536 | 0.290 | 0.224 | 0.291 | 0.262 | 0.320 |
| 12.5 male PGC | 0.613 | 0.561 | 0.492 | 0.604 | 0.800 | 0.741 | 0.647 | 0.724 | 0.898 | | 0.909 | 0.950 | 0.881 | 0.755 | 0.501 | 0.344 | 0.270 | 0.341 | 0.289 | 0.297 |
| 12.5 female PGC | 0.654 | 0.634 | 0.547 | 0.649 | 0.708 | 0.660 | 0.630 | 0.700 | 0.908 | 0.909 | | 0.899 | 0.917 | 0.726 | 0.495 | 0.242 | 0.226 | 0.247 | 0.260 | 0.298 |
| 13.5 male PGC | 0.629 | 0.582 | 0.525 | 0.627 | 0.794 | 0.747 | 0.635 | 0.758 | 0.901 | 0.950 | 0.899 | | 0.900 | 0.797 | 0.519 | 0.339 | 0.281 | 0.334 | 0.289 | 0.315 |
| 13.5 female PGC | 0.591 | 0.562 | 0.489 | 0.598 | 0.733 | 0.706 | 0.593 | 0.714 | 0.855 | 0.881 | 0.917 | 0.900 | | 0.791 | 0.524 | 0.294 | 0.281 | 0.292 | 0.279 | 0.317 |
| GS | 0.533 | 0.489 | 0.431 | 0.505 | 0.664 | 0.620 | 0.531 | 0.623 | 0.723 | 0.755 | 0.726 | 0.797 | 0.791 | | 0.500 | 0.282 | 0.288 | 0.307 | 0.264 | 0.303 |
| Testis | 0.454 | 0.459 | 0.606 | 0.602 | 0.505 | 0.468 | 0.406 | 0.419 | 0.536 | 0.501 | 0.495 | 0.519 | 0.524 | 0.500 | | 0.453 | 0.299 | 0.356 | 0.333 | 0.381 |
| Heart | 0.150 | 0.109 | 0.274 | 0.287 | 0.396 | 0.379 | 0.280 | 0.319 | 0.290 | 0.344 | 0.242 | 0.339 | 0.294 | 0.282 | 0.453 | | 0.437 | 0.568 | 0.333 | 0.347 |
| Brain | 0.203 | 0.201 | 0.135 | 0.155 | 0.304 | 0.275 | 0.313 | 0.350 | 0.224 | 0.270 | 0.226 | 0.281 | 0.281 | 0.288 | 0.299 | 0.437 | | 0.434 | 0.260 | 0.343 |
| Liver | 0.203 | 0.183 | 0.179 | 0.193 | 0.401 | 0.380 | 0.342 | 0.311 | 0.291 | 0.341 | 0.247 | 0.334 | 0.292 | 0.307 | 0.356 | 0.568 | 0.434 | | 0.411 | 0.409 |
| Spleen | 0.213 | 0.238 | 0.201 | 0.218 | 0.294 | 0.249 | 0.236 | 0.245 | 0.262 | 0.289 | 0.260 | 0.289 | 0.279 | 0.264 | 0.333 | 0.333 | 0.260 | 0.411 | | 0.675 |
| Thymus | 0.278 | 0.309 | 0.300 | 0.297 | 0.320 | 0.257 | 0.257 | 0.300 | 0.320 | 0.297 | 0.298 | 0.315 | 0.317 | 0.303 | 0.381 | 0.347 | 0.343 | 0.409 | 0.675 | |

R1, E14tg2a, TMA5 – linie mES buněk

TMA55G (male), TMA58G (female) – EGC

GS – germ stem cell (derived from spermatogonia)

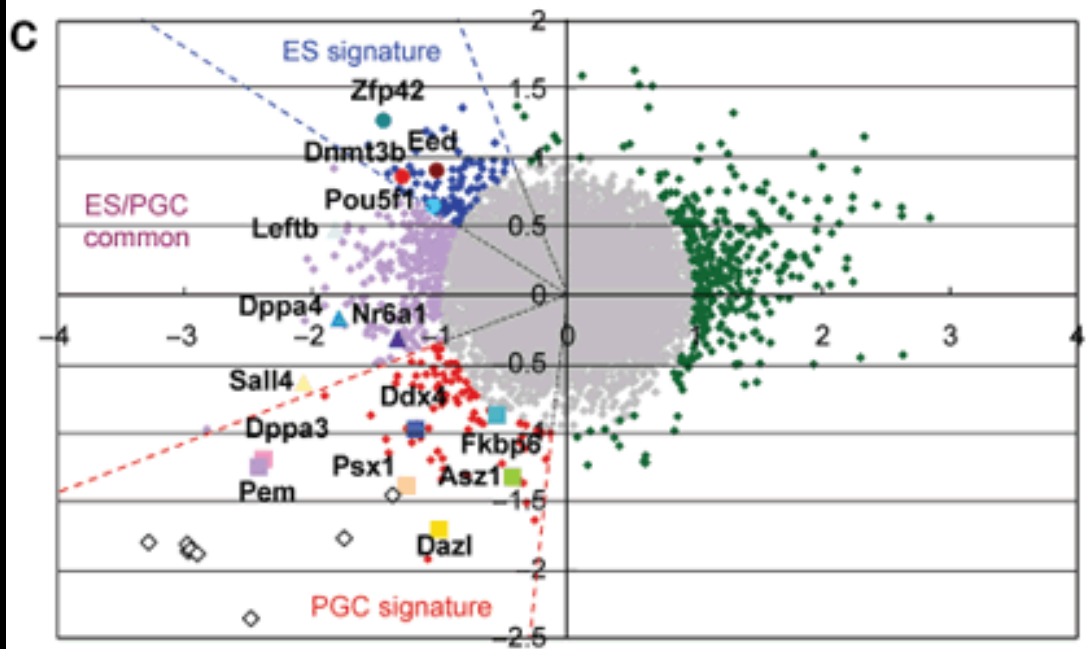
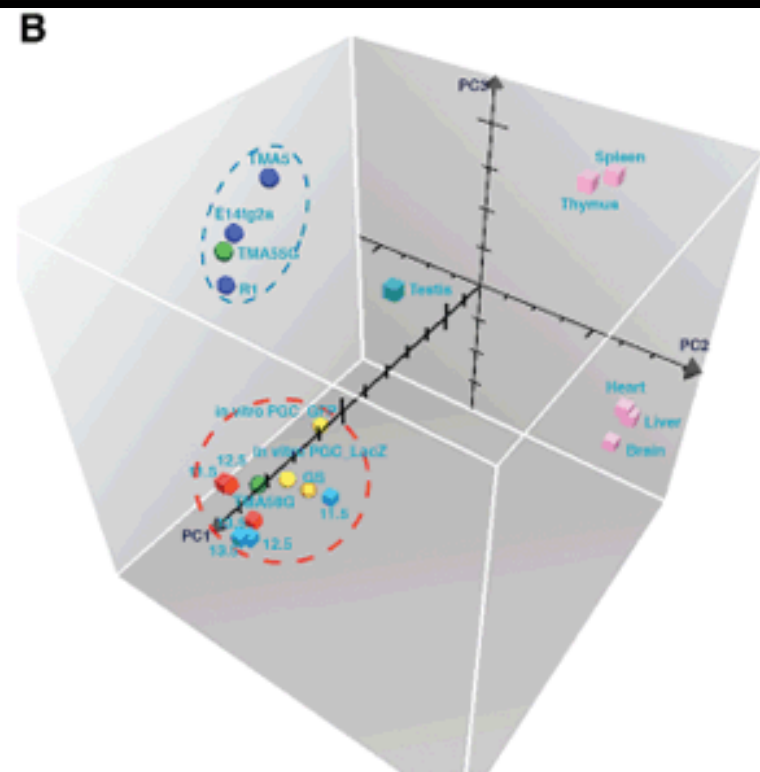
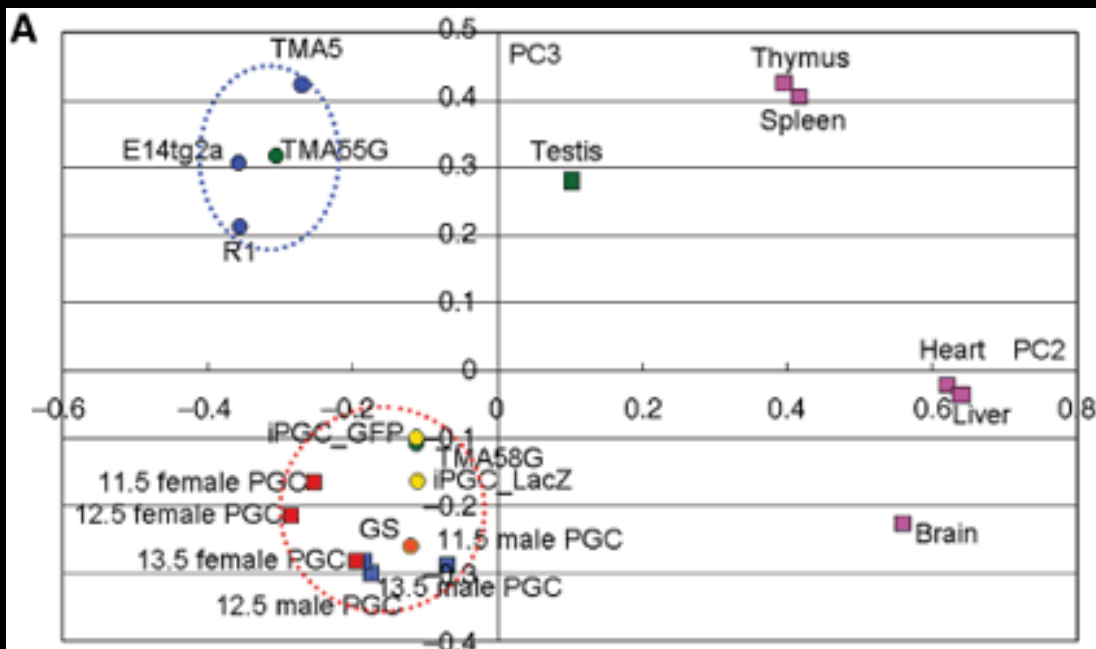




- | | |
|------------|--------------------|
| 1. R1 | 4. 12.5 male PGC |
| 2. E14tg2a | 5. 12.5 female PGC |
| 3. TMA5 | 6. 13.5 male PGC |
| | 7. 13.5 female PGC |



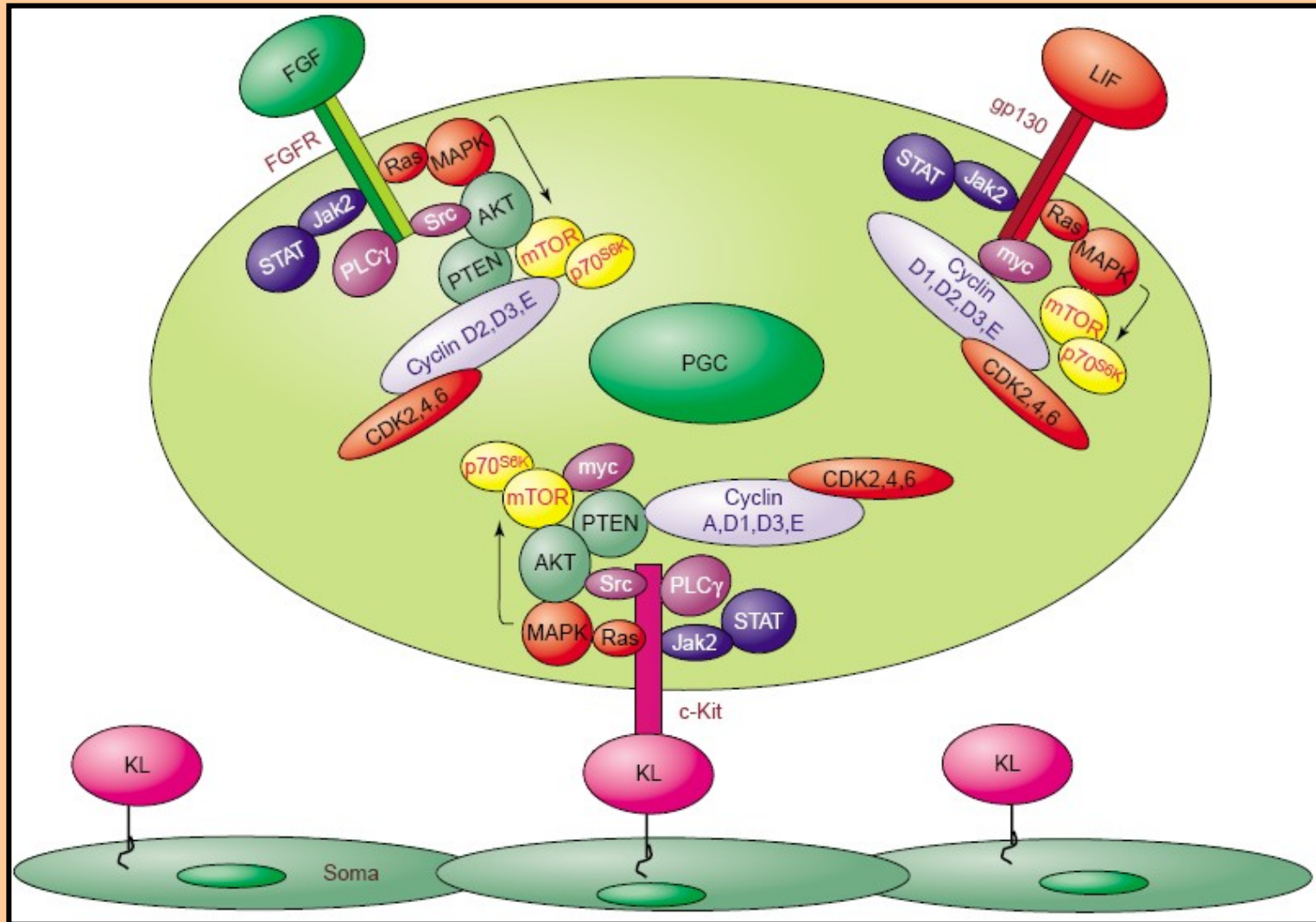
Analýza hlavních komponent expresních profilů



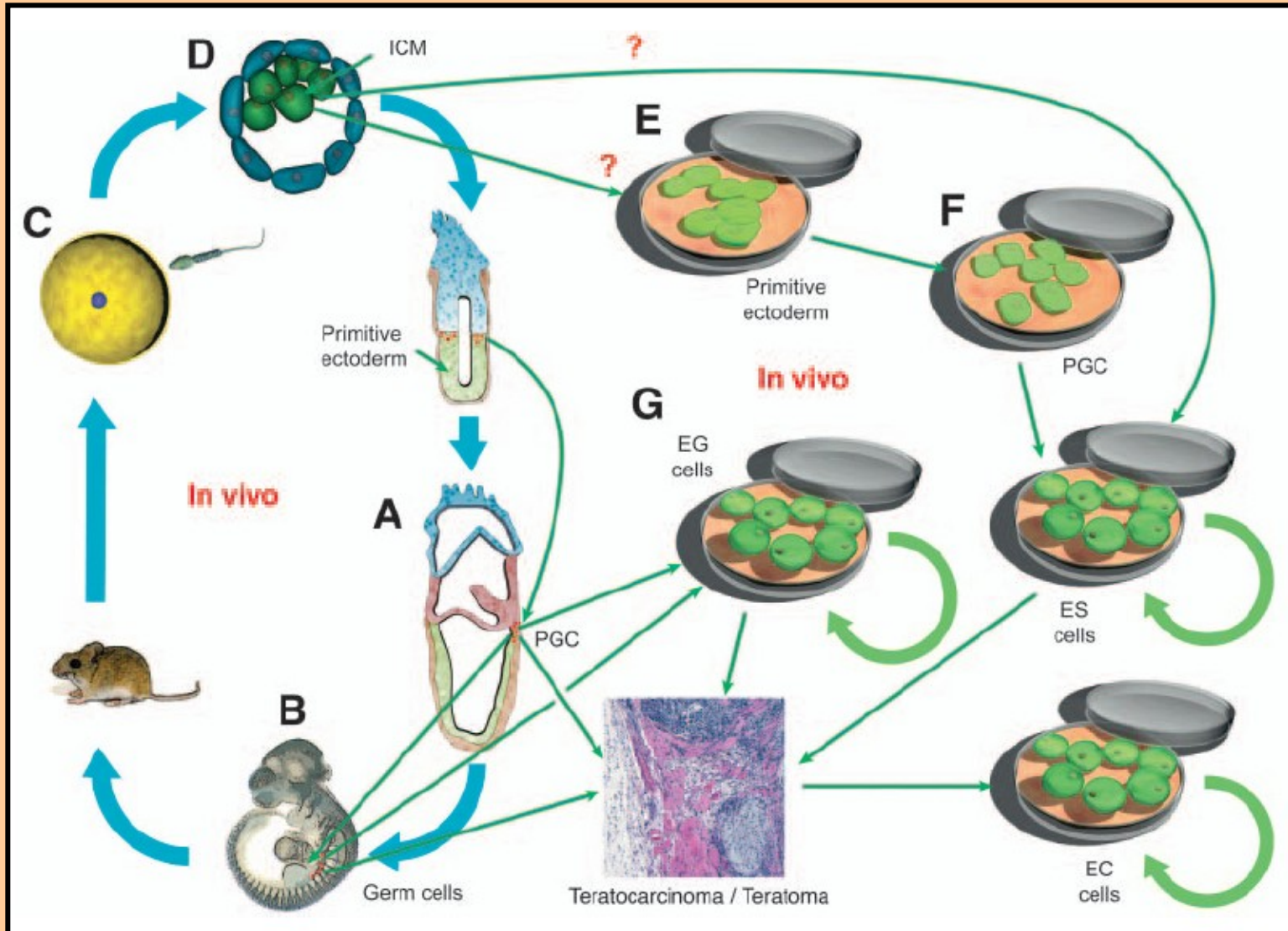
blue – ES specifický patern
 red – PGC specifický patern
 purple - ES/PGC specifický patern
 green – nevýznamné pro specifikaci ES/PGC



Schema předpokládaného zapojení FGF, LIF a KL (c-Kit ligand = Steel factor (SF)/ stem cell factor (SCF) v regulaci sel-renewal EG buněk



Není původ ES buněk v PGC ???



Není původ ES buněk v PGC ???

| Gene | Species | ES | EGC | LGC | ICM | PE |
|--|---------|----|-----|-----|-----|-----|
| <i>Pou5f1</i> (Pesce and Scholer, 2001) | M | + | + | + | + | + |
| <i>Nanog</i> (Chambers et al., 2003) | M | + | + | + | + | + |
| <i>Dppa3</i> (Saitou et al., 2002) | M | + | + | + | + | + |
| <i>Ifitm3</i> (Saitou et al., 2002) | M | + | + | + | + | + |
| <i>Kit</i> (Horie et al., 1991) | M | + | + | + | - | N/D |
| <i>DAZL</i> (Clark et al., 2004) | H | + | + | + | - | N/D |
| <i>Ddx4</i> (Toyooka et al., 2003) | M | - | - | + | - | - |
| <i>Akp2</i> (Chiquoine, 1954) | M | + | + | + | + | + |
| <i>Zfp42</i> (Rogers et al., 1991) | M | + | N/D | N/D | + | - |
| <i>Fgf5</i> (Haub and Goldfarb, 1991; Hebert et al., 1991) | M | - | N/D | N/D | - | + |
| <i>Gbx1</i> (Chapman et al., 1997) | M | + | N/D | N/D | + | - |

Zwaka, 2005

M- myš; H – člověk; N/D – netestováno; ES – embryonální kmenové buňky; EGC – časné primordiální zárodečné buňky (!); LGC – pozdní primordiální zárodečné buňky; ICM – vnitřní buněčná masa; PE – primitivní ectoderm/epiblast

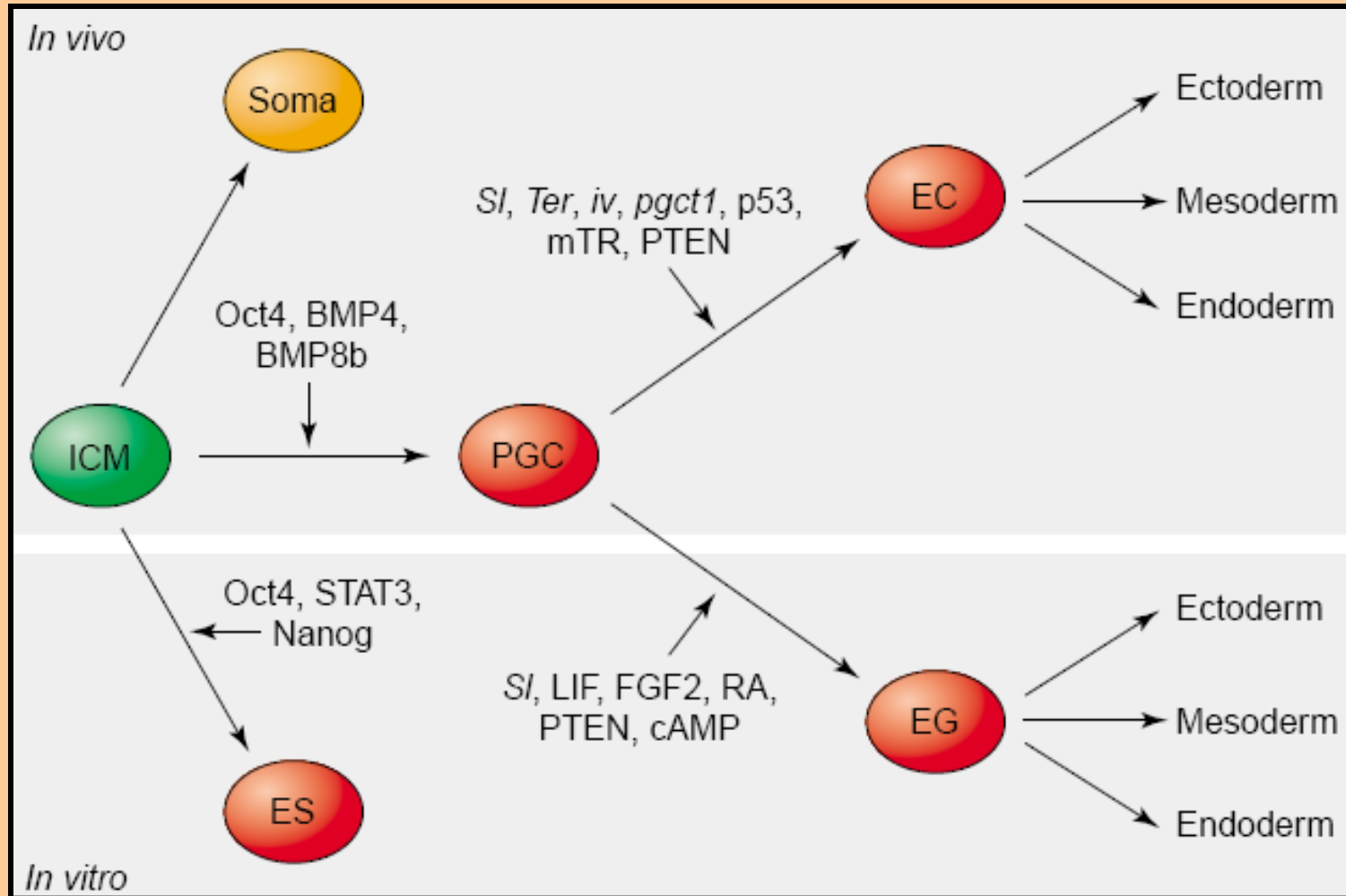


Kmenové buňky teratomu / teratokarcinomu - ECC Embryonální nádorové buňky (Embryonal carcinoma cells)

- Izolované rozkultivováním a klonální selekcí buněk teratomu / teratokarcinomu
- Lidské spontánně, myší indukované transplantací časných embryonálních buněk do dobře vyživované tkáně (varlata, ledviná kapsa, břišní dutina,...), musí být imunotolerance
- Podobné vlastnosti jako ES buňky, ale méně závislé na specifických růstových faktorech (+)
- Tvoří také chiméry, ale nedokonalé, většinou hynou v průběhu embryogeneze (-)
- Většinou snižená schopnost pluripotence jak *in vivo*, tak *in vitro* (-)
- Obecně nestabilní genotyp a časté aneuploidie (-)
- Modelové studie genetické nestability a diferenciací, vzniku teratomů
- Levnější alternativa k ES buňkám, lepší stabilita v experimentálních systémech jak ES (+)



Vztahy mezi pluripotentními buňkami a některé klíčové regulační komponenty těchto buněk



Sl – Steel locus, *Ter* – Teratoma locus, *pgct1* – primordial germ cell tumor susceptibility locus, PTEN – Phosphatase and tensin homologue deleted on chromosome 10, mTR (mTOR) - serine-threonine kinase mammalian target of rapamycin



[Nature](#). 2013 Oct 30. doi: 10.1038/nature12745. [Epub ahead of print]

Derivation of novel human ground state naive pluripotent stem cells.

[Gafni O](#), [Weinberger L](#), [Mansour AA](#), [Manor YS](#), [Chomsky E](#), [Ben-Yosef D](#), [Kalma Y](#), [Viukov S](#), [Maza I](#), [Zviran A](#), [Rais Y](#), [Shipony Z](#), [Mukamel Z](#), [Krupalnik V](#), [Zerbib M](#), [Geula S](#), [Caspi I](#), [Schneir D](#), [Shwartz T](#), [Gilad S](#), [Amann-Zalcenstein D](#), [Benjamin S](#), [Amit I](#), [Tanay A](#), [Massarwa R](#), [Novershtern N](#), [Hanna JH](#).

Source

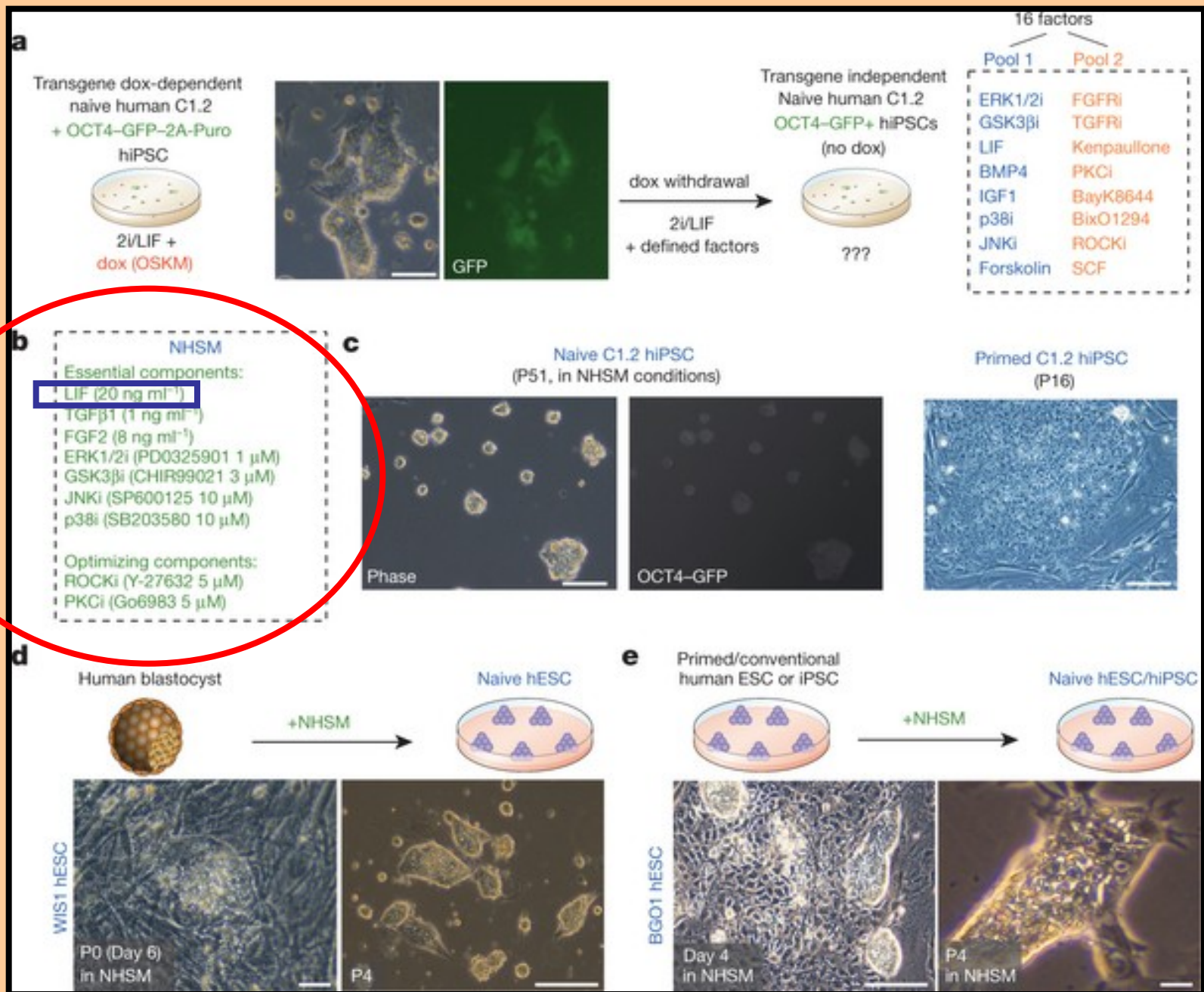
1] The Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, Israel [2].

Abstract

Mouse embryonic stem (ES) cells are isolated from the inner cell mass of blastocysts, and can be preserved in vitro in a naive inner-cell-mass-like configuration by providing exogenous stimulation with leukaemia inhibitory factor (LIF) and small molecule inhibition of ERK1/ERK2 and GSK3 β signalling (termed 2i/LIF conditions). Hallmarks of naive pluripotency include driving Oct4 (also known as Pou5f1) transcription by its distal enhancer, retaining a pre-inactivation X chromosome state, and global reduction in DNA methylation and in H3K27me3 repressive chromatin mark deposition on developmental regulatory gene promoters. Upon withdrawal of 2i/LIF, naive mouse ES cells can drift towards a primed pluripotent state resembling that of the post-implantation epiblast. Although human ES cells share several molecular features with naive mouse ES cells, they also share a variety of epigenetic properties with primed murine epiblast stem cells (EpiSCs). These include predominant use of the proximal enhancer element to maintain OCT4 expression, pronounced tendency for X chromosome inactivation in most female human ES cells, increase in DNA methylation and prominent deposition of H3K27me3 and bivalent domain acquisition on lineage regulatory genes. The feasibility of establishing human ground state naive pluripotency in vitro with equivalent molecular and functional features to those characterized in mouse ES cells remains to be defined. Here we establish defined conditions that facilitate the derivation of genetically unmodified human naive pluripotent stem cells from already established primed human ES cells, from somatic cells through induced pluripotent stem (iPS) cell reprogramming or directly from blastocysts. The novel naive pluripotent cells validated herein retain molecular characteristics and functional properties that are highly similar to mouse naive ES cells, and distinct from conventional primed human pluripotent cells. This includes competence in the generation of cross-species chimaeric mouse embryos that underwent organogenesis following microinjection of human naive iPS cells into mouse morulas. Collectively, our findings establish new avenues for regenerative medicine, patient-specific iPS cell disease modelling and the study of early human development in vitro and in vivo.



Lidské „naivní“ pluripotentní kmenové buňky (human naive hESC = ekvivalent mESC)



Dospělé multipotentní zárodečné kmenové buňky (AMGDSC Adult multipotent germ-derived stem cell)

- odvozeno ze spermatogonií
- fenotyp velmi podobný ES buňkám
- oproti ES buňkám snižená schopnost pluripotence – jen chiméry
- pluripotence také *in vitro*

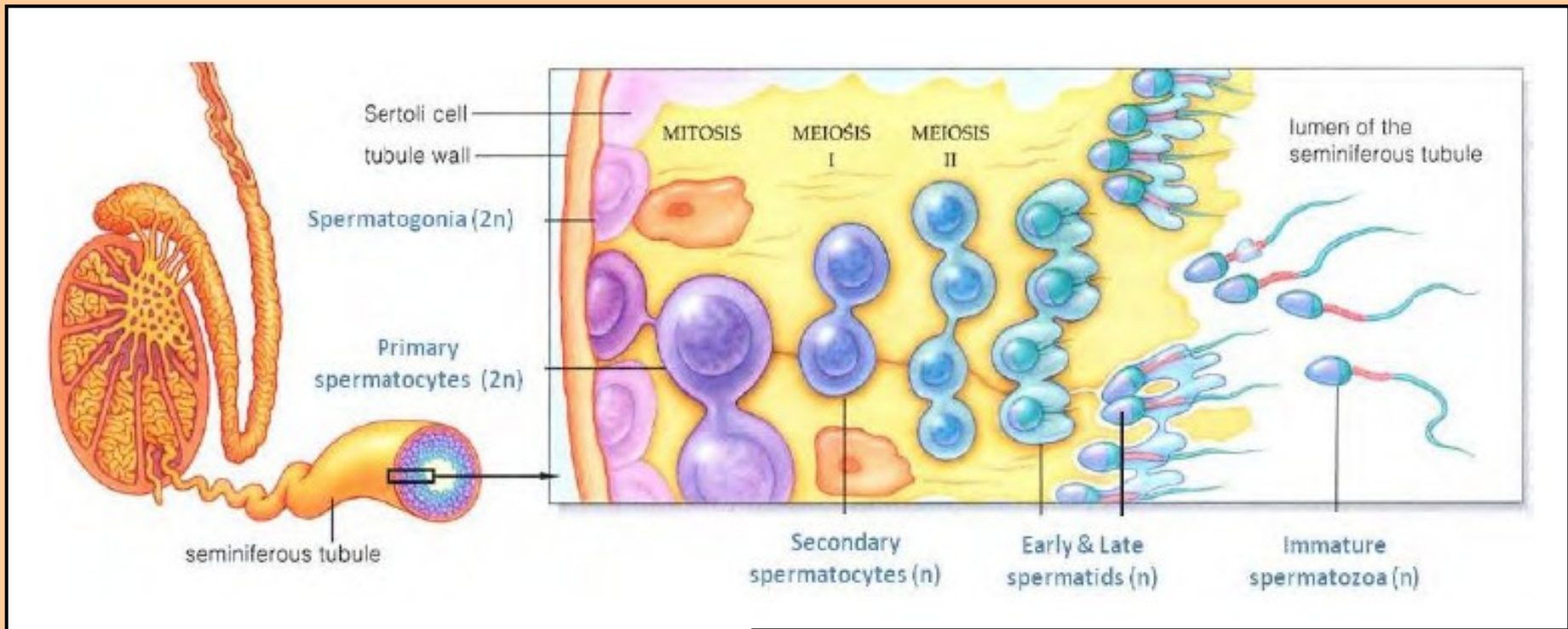


Image modified from (Hammack's universe of ideas. 2



Pluripotency of spermatogonial stem cells from adult mouse testis.

Guan K, Nayernia K, Maier LS, Wagner S, Dressel R, Lee JH, Nolte J, Wolf F, Li M, Engel W, Hasenfuss G. Nature. 2006 Apr 27;440(7088):1199-203.

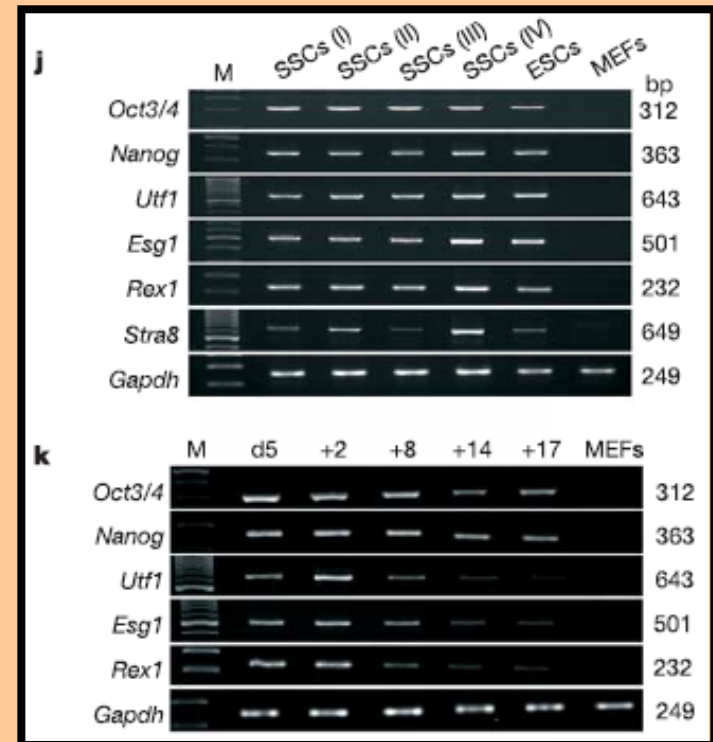
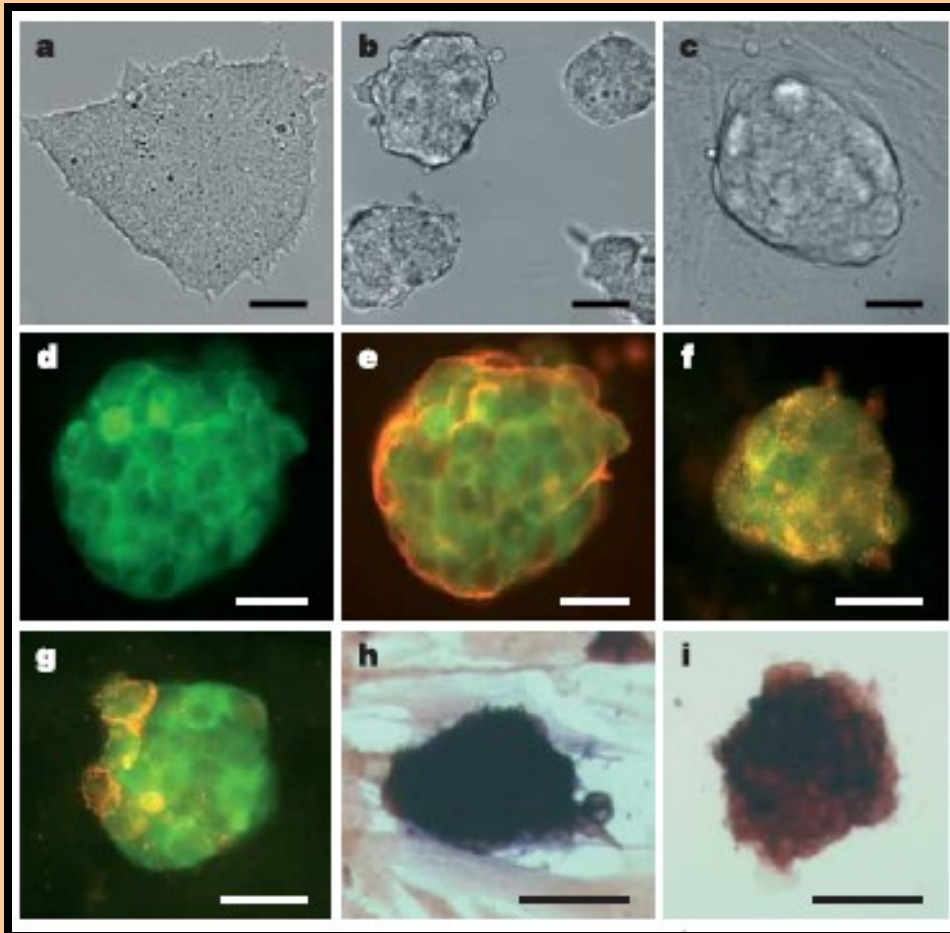


Figure 1 | Cellular and molecular characterization of cultured SSCs and maGSCs. **a**, Epiblast-like colony formed under culture condition I. **b**, ESC-like colonies appeared under culture condition II. **c**, A typical colony of established culture under condition IV at passage 30. **d–g**, Double immunostaining of maGSCs in culture condition IV (**d–f**) or condition II (**g**) with antibodies against GFP (green, **d–g**) and SSEA-1 (red, **e**), Oct4 (red, **f**) or SSEA-3 (red, **g**). **h, i**, Alkaline phosphatase staining. SSCs cultured under condition IV (maGSCs, **h**) are strongly positive for alkaline phosphatase, whereas SSCs under condition II (**i**) show a mixed colony phenotype. **j, k**, RT-PCR analyses of transcription factors essential for undifferentiated cells in SSCs cultured under conditions I, II, III and IV (**j**) and during differentiation of embryoid bodies after plating at day 5 (**k**). M, 100-bp DNA markers; numbers to the right indicate the sizes of resolved DNA fragments (in bp). Scale bars, 50 μ m (**a–c, h, i**), 25 μ m (**d–g**).

Condition II & IV + LIF

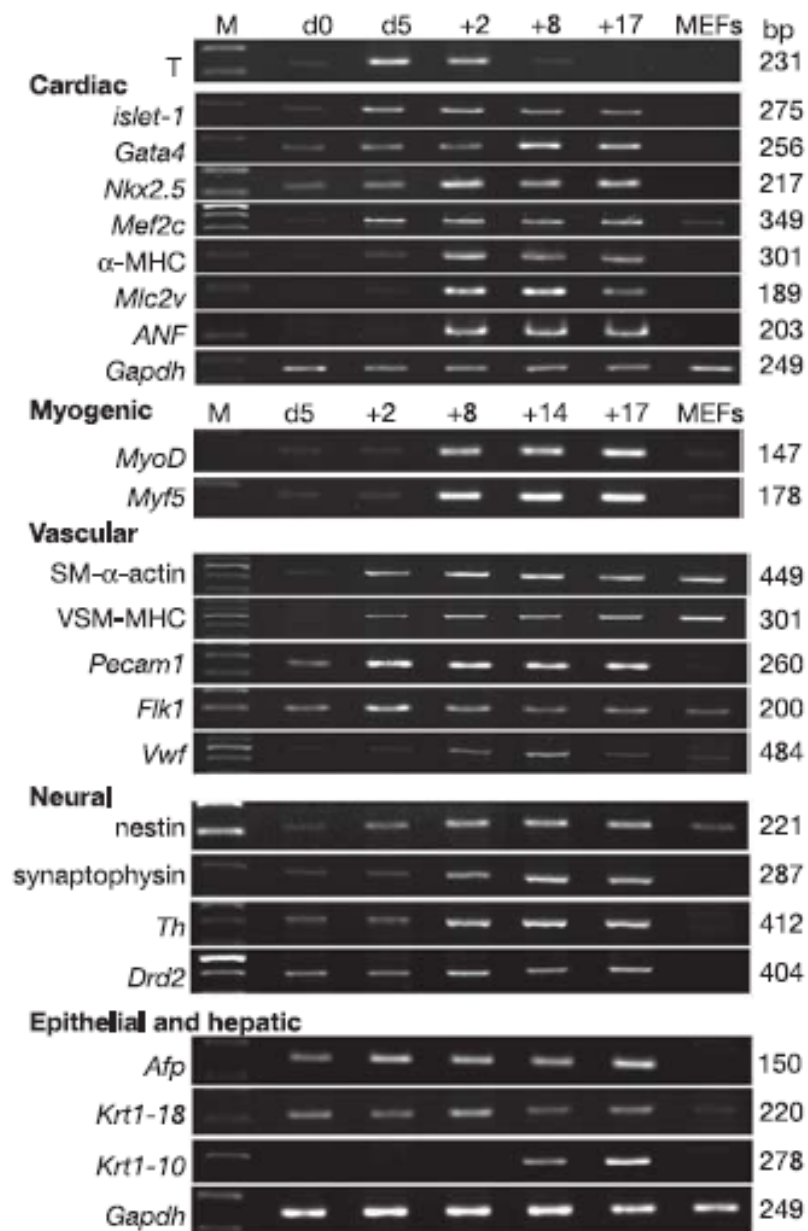


Figure 2 | RT-PCR analysis of lineage-specific transcription factors and genes. Analyses were performed at different stages during the differentiation of embryoid bodies after plating at day 5 (d5). M, 100-bp DNA markers. d0, maGSCs before embryoid body formation.



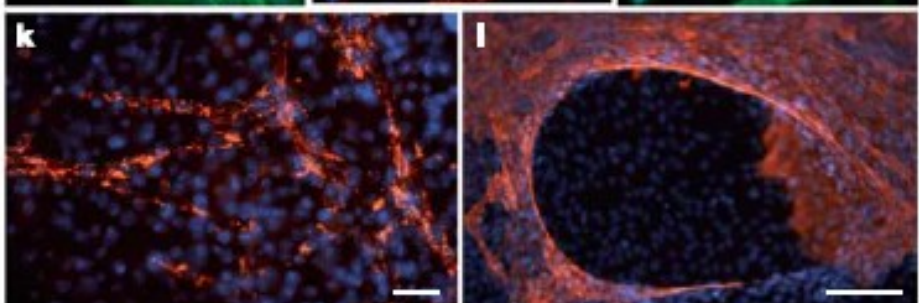
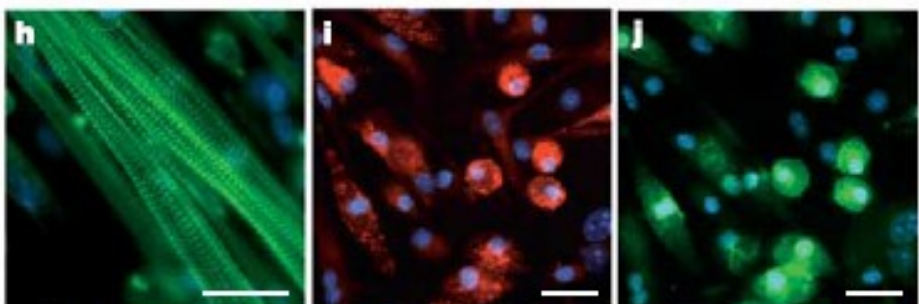
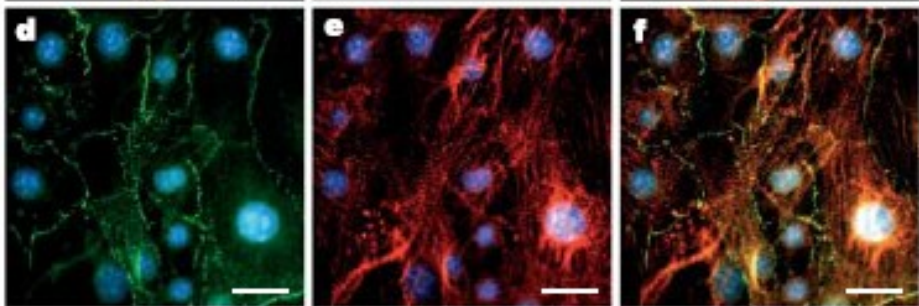
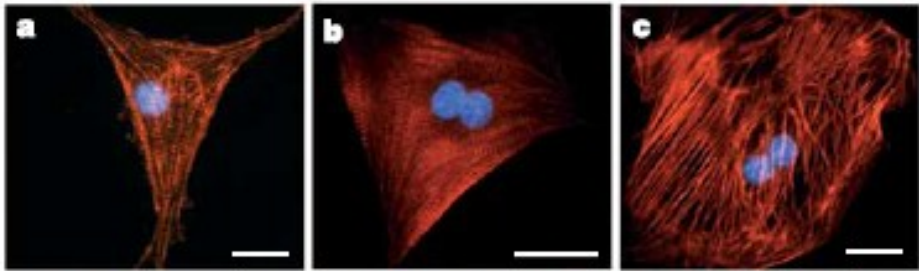


Figure 3 | Mesoderm differentiation of maGSCs. **a–c**, Organization of the sarcomeric proteins α -actinin (**a**), sarcomeric MHC (**b**) and cardiac troponin T (**c**) in isolated cardiomyocytes at day 5 + 7. **d–f**, Connexin 43 staining (**d**, green) in a cluster of uninucleate cardiac cells stained for sarcomeric α -actinin (**e**, red), with an overlay of **d** and **e** shown in **f**. Nuclei are stained with DAPI. **g**, Original traces of ventricle-like action potentials in a cardiomyocyte derived from maGSCs. **h**, Nebulin-positive myotubes at differentiation day 5 + 23. **i, j**, Dil-acLDL uptake (**i**, red) and lectin binding (**j**, green) of endothelial cells in embryoid body outgrowths at day 5 + 14. **k**, Vwf-positive endothelial cells (red) at day 5 + 17. **l**, Smooth muscle α -actin-positive cells (red) of tube-like structure in embryoid body outgrowths at day 5 + 14. Scale bars, 25 μ m (**a–f, h–k**), 100 μ m (**l**).



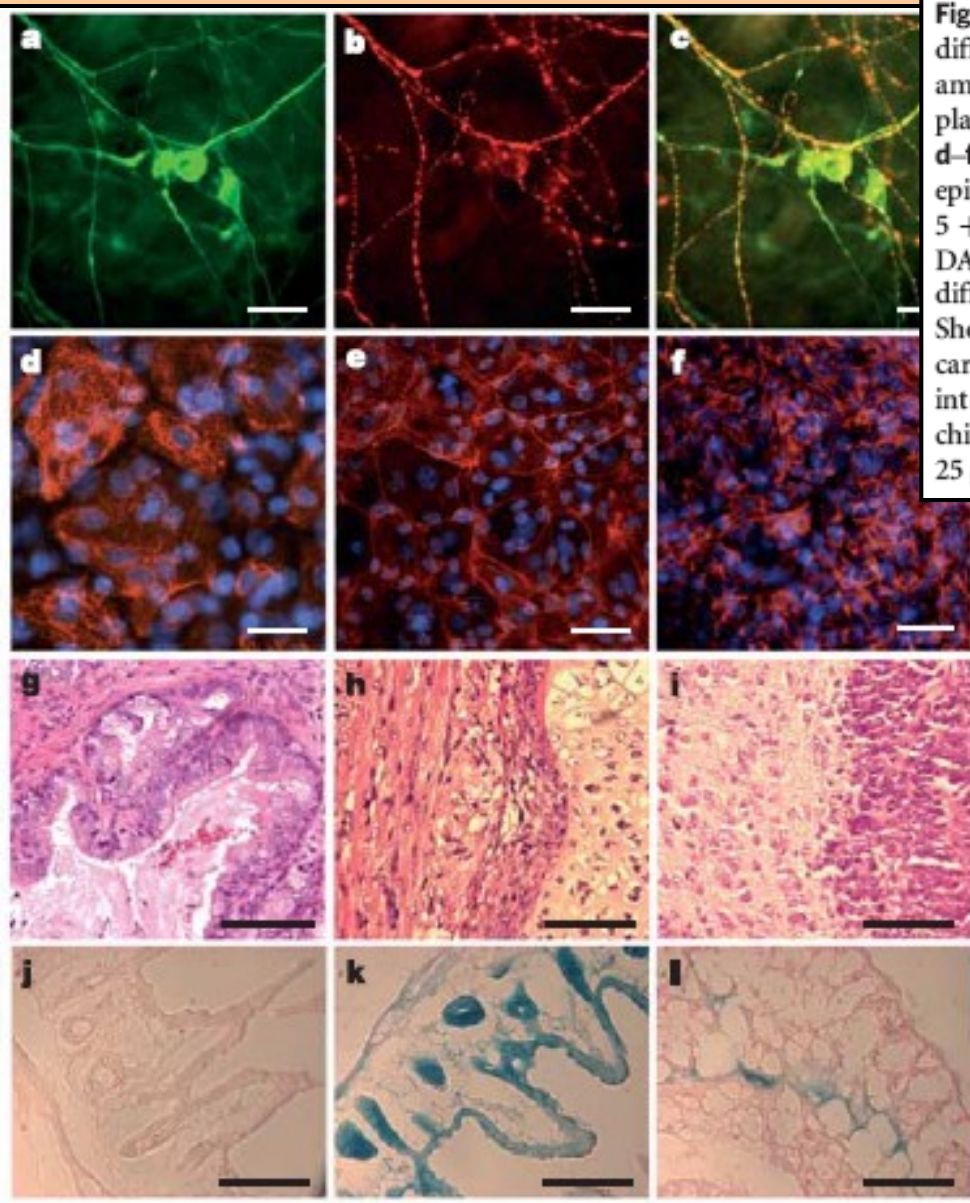
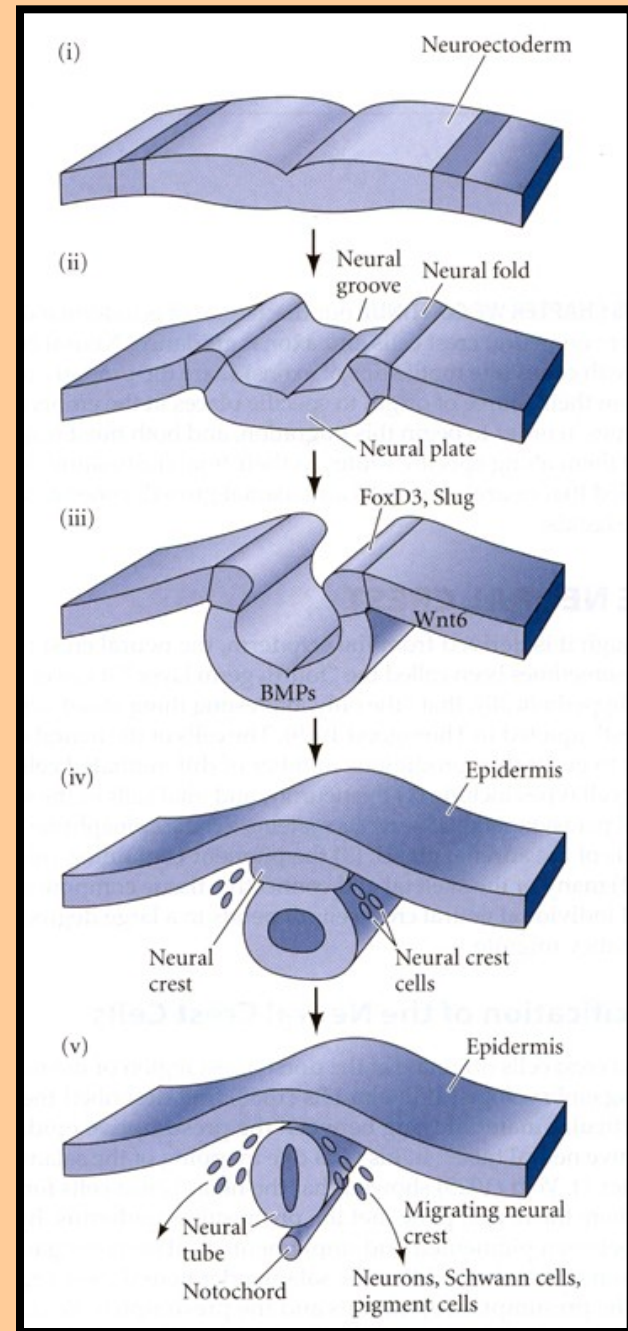
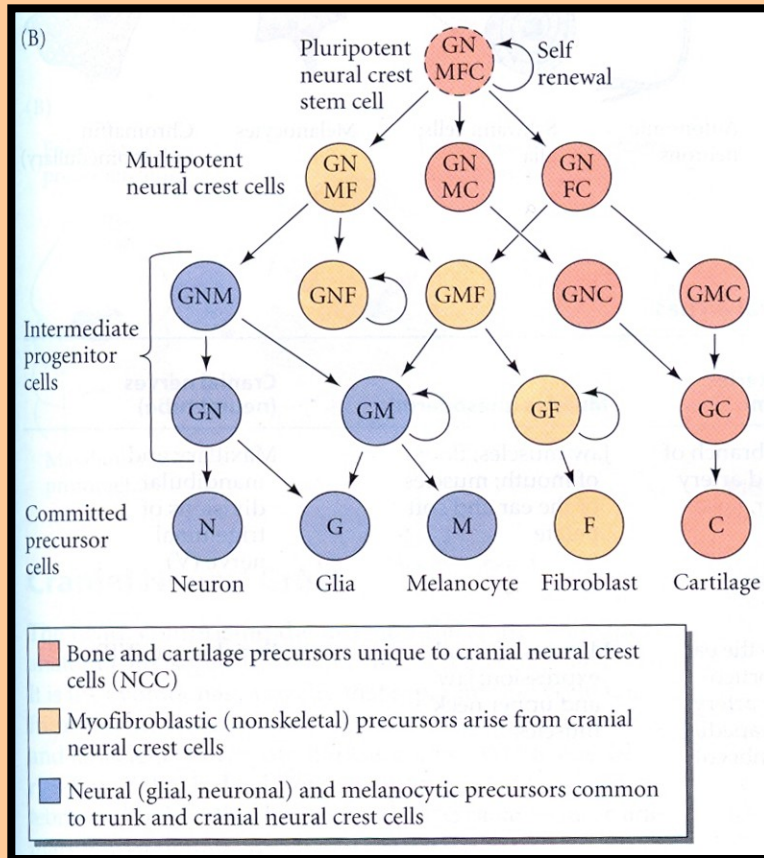


Figure 4 | Differentiation of maGSCs *in vitro* and *in vivo*. **a–c**, Neuronal differentiation of maGSCs. TH-positive dopaminergic neurons (**b**) appeared among neurofilament protein M (NFM)-positive neurons (**a**) on day 12 after plating of embryoid bodies at day 5 (day 5 + 12). **c**, Overlay of **a** and **b**. **d–f**, Epithelial cell/hepatocyte differentiation. **d**, Pan-cytokeratin-positive epithelial cells on day 5 + 14. **e**, CK18-positive large epithelioid cells on day 5 + 17. **f**, CK7-positive bile duct cells on day 5 + 14. Nuclear staining with DAPI. **g–i**, Teratomas from maGSCs. The tumours contained abundant differentiation of advanced derivatives of all three embryonic germ layers. Shown are epithelium with intestinal differentiation (**g**), striated muscle, cartilage (**h**) and neural tissue (**i**). **j–l**, Representative images of LacZ-stained intestine tissue from a wild-type mouse (**j**), a mouse with high grade of chimaerism (**k**) and a mouse with low grade of chimaerisms (**l**). Scale bars, 25 μ m (**a–f**), 50 μ m (**g–i**), 100 μ m (**j–l**).

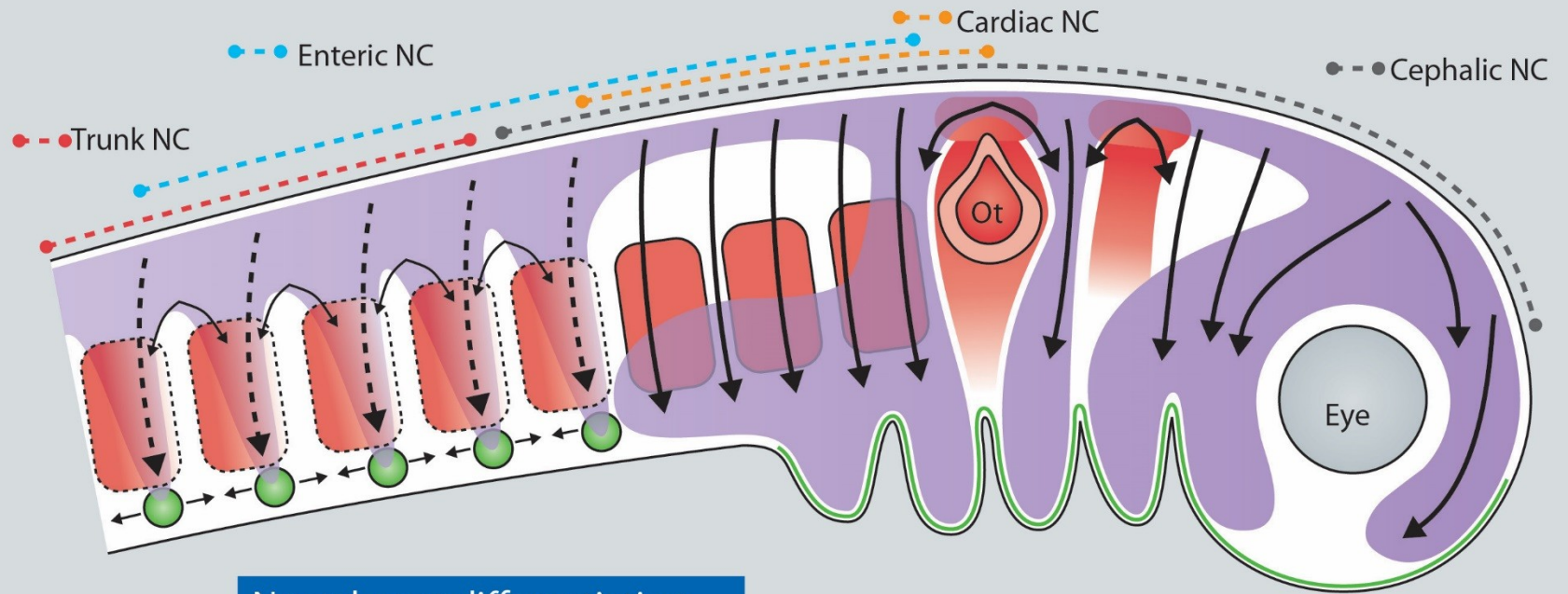
V současnosti ale pochybnosti



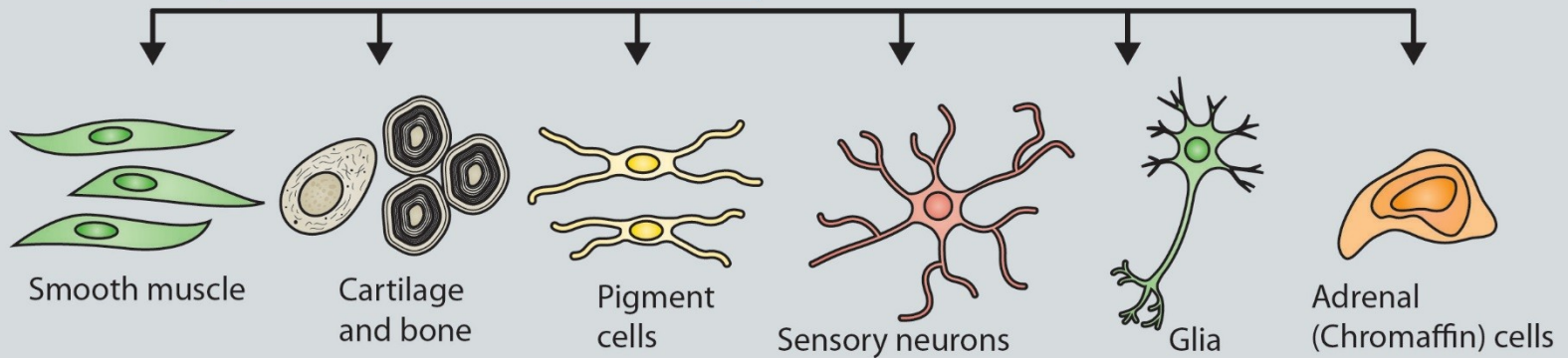
Neurální lišta a kmenové buňky neurální lišty



Neural crest migration



Neural crest differentiation



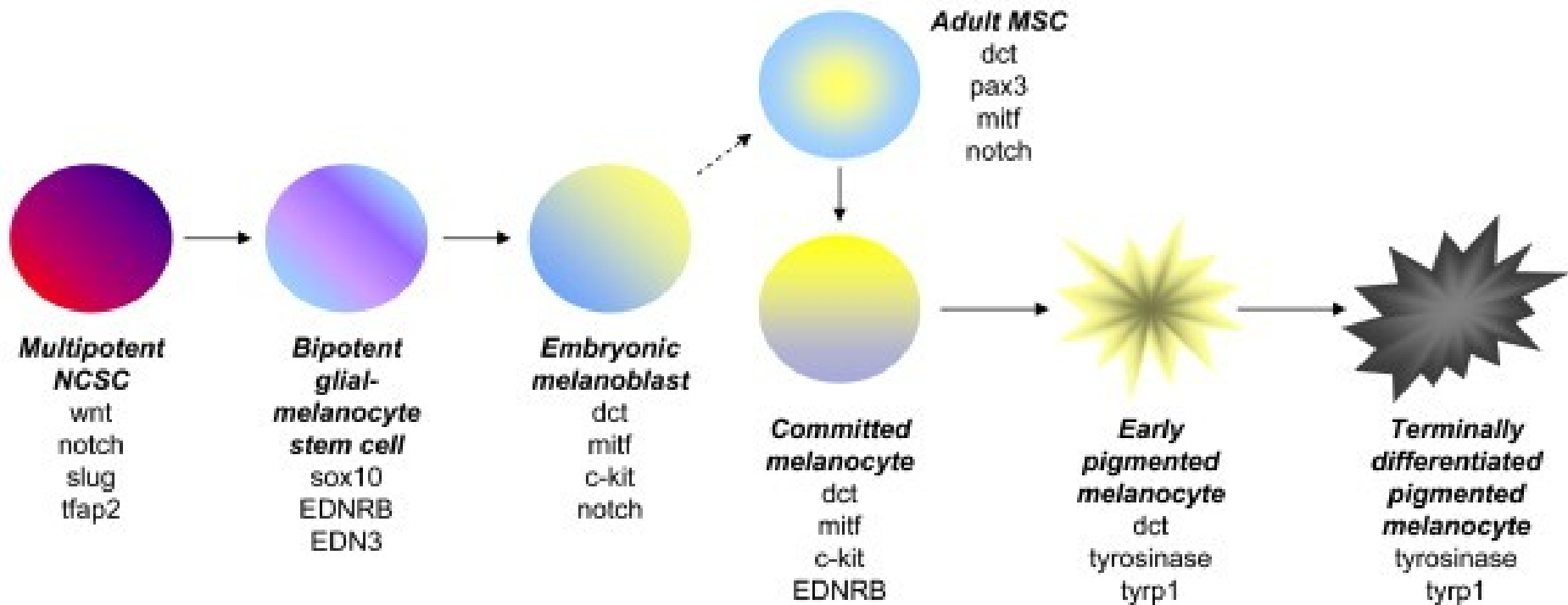
Neural crest stem cell - NCSC

Sebeobnova

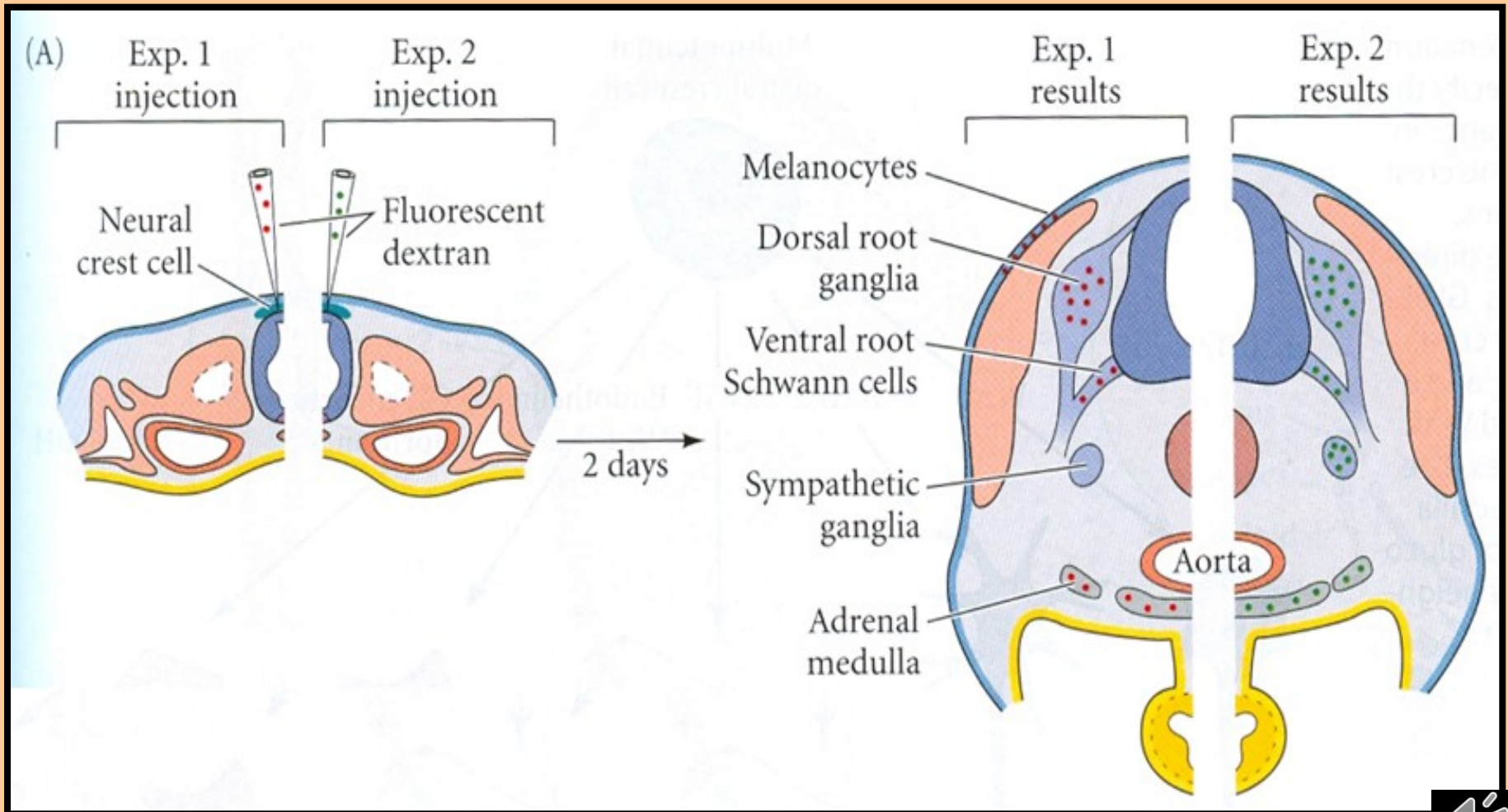
Růstové faktory - FGF2, Notch, Wnt

Transkripční faktory - Slug (Snai2), Sox10

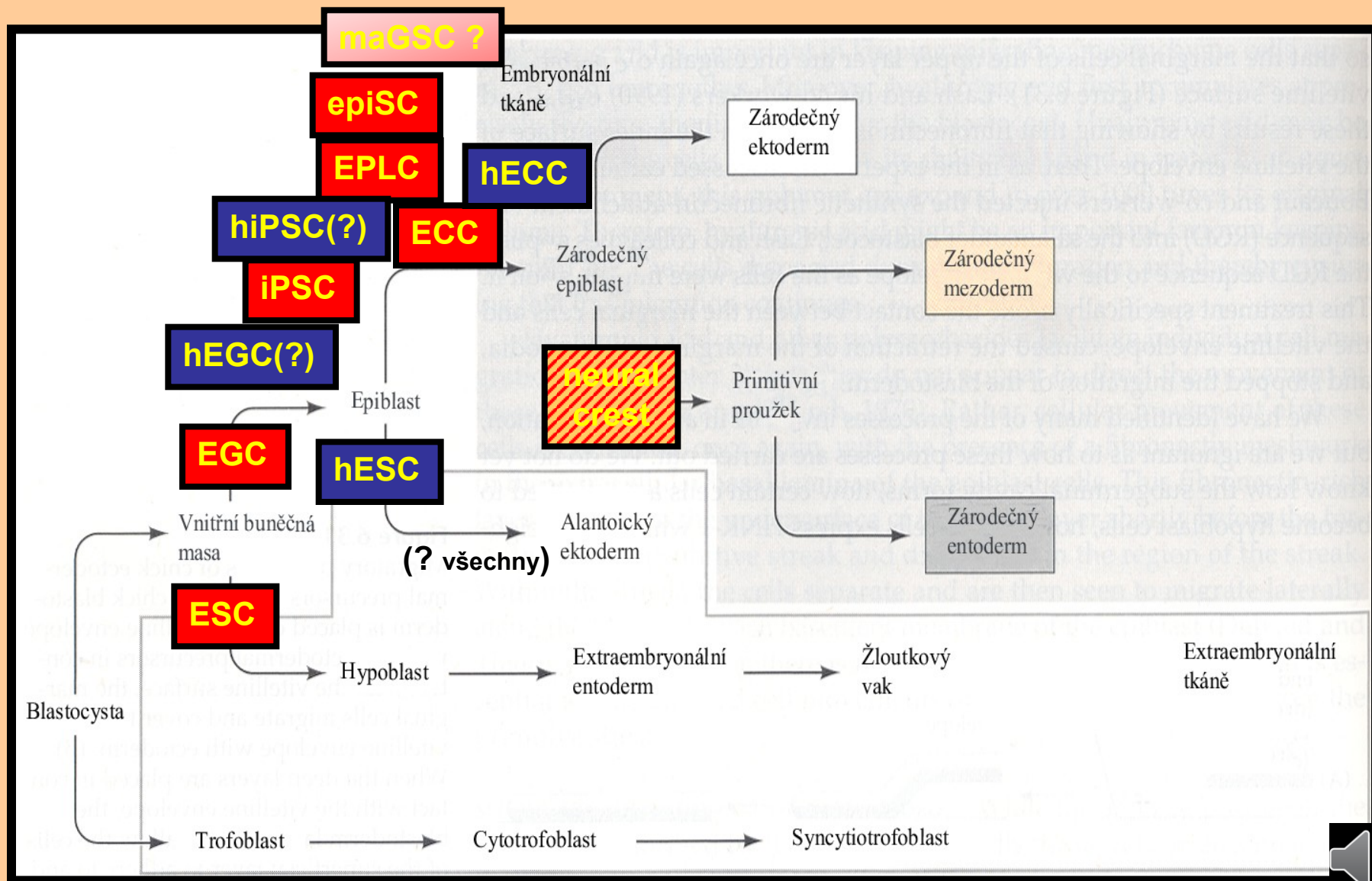
Fenotyp - blízký NSC - exprese Nestinu (protein intermediálních filament)



Experiment potvrzující pluripotenci buněk neurální lišty



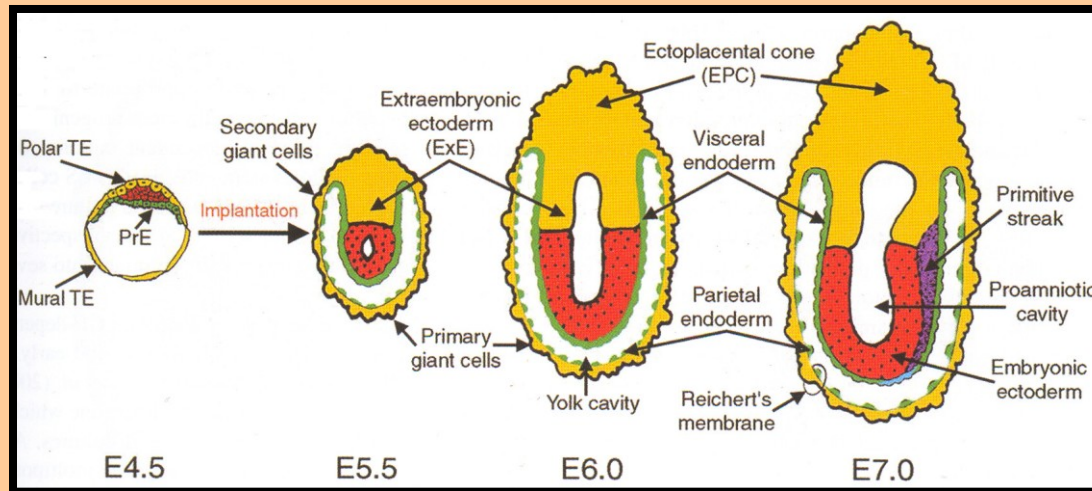
Schopnost pluripotence – tvorba chimér a teratomů



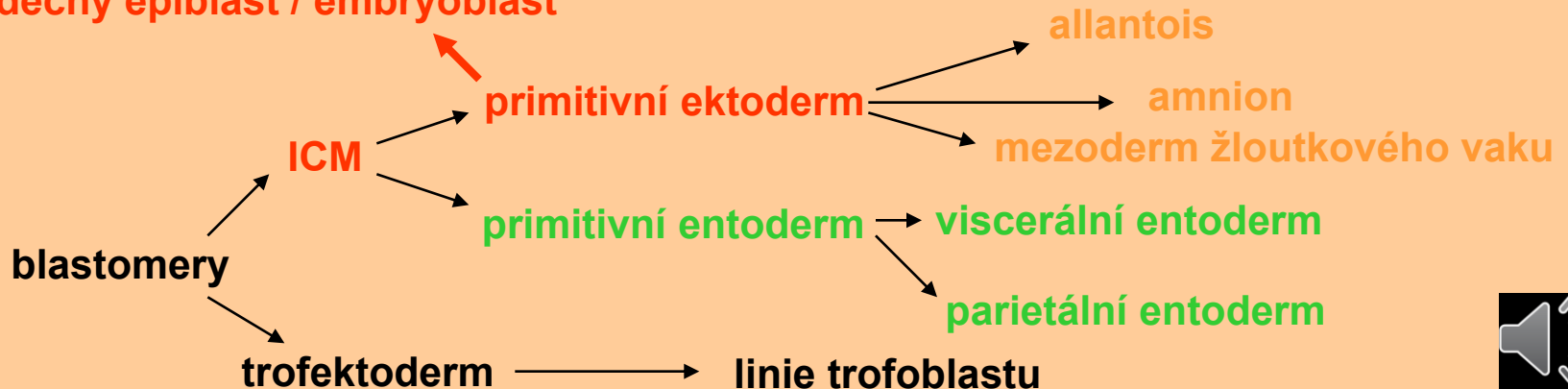
Kmenové buňky extraembryonálních tkání

A) Kmenové buňky trofektodermu (trofoblastu)
FGF-dependent (FGF4, FGFR2); *Cdx2*, *Eomes*, *Errβ*

B) Kmenové buňky primitivního entodermu (hypoblastu)
 XEN – buňky extraembryonálního entodermu



zárodečný epiblast / embryoblast



Regulace kmenových buněk trofoblastu signály z epiblastu

