Einführung in die Stammzell- und Embryonenforschung II (ESF-II/9) WS2022/23

Zur Herstellen von Lebewesen aus einer Stammzelle

Biologische Grundlagen – Stand der Forschung – Gesellschaftliche Auswirkungen

5. Doppelstunde

2.1.3. Embryoids - Synthetische Embryonen – Wiederholung der 4. Doppelstunde

1. Synthetische Embryonen entwickeln sich in wenigen Prozent der Fälle bis zum Beginn der Organogenes unter geeigneten Umweltbedingungen.

2. Die Plazentaentwicklung kann ex vivo noch nicht nachgestellt werden.

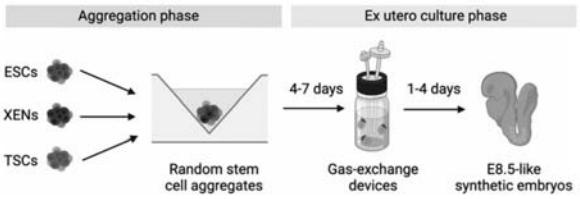


FIG. 1. Synthetic embryos cultured until an E8.5-like stage. In the first phase, embryonic and extraembryonic stem cells are combined into aggregates, which are cultured to promote the formation of self-organized synthetic embryos resembling E5.5–6.5 stages. In the second phase, successfully aggregated structures are selectively picked and transferred into gas-exchanging bioreactors, where they fully develop through gastrulation and into early neurulation and organogenesis.

DOI: 10.1089/cell.2022.0111

Ethische Dimension dieser Experimente : → siehe 7. Doppelstunde

Potenciality	Potenzialität
Autonomy	Autonomie
Contingency	Bedingtheit

Teil 2 Herstellung von Lebewesen - Stand der Forschung (3. bis 6. Doppelstunde)

2.1. Ex vivo Embryonen aus einer pluripotenten Stammzellen

2.1.1. Blastoide - Herstellung von Blastozysten aus Stammzellen
2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten
2.1.3. Embryoids - Synthetische Embryonen

2.2. Ex vivo Keimzellen aus pluripotenten Stammzellen

2.2.1. Der weibliche und männliche Reproduktionszyklus in vivo und ex vivo
2.2.2. Herstellung von Zygoten - In vitro Fertilisation und Klonen
2.2.3. Herstellung von künstlichen Plazenten aus Stammzellen
(siehe auch 2.1.3., 4. Doppelstunde)
2.2.4. Herstellung von Mäusen aus Stammzellen in Leihmüttern
(siehe Einleitung zu 2.1.3., 4. Doppelstunde)

2.2. Zur Herstellung von Lebewesen

- Über die Möglichkeit Lebewesen aus einer diploiden Zelle herzustellen.

Es geht hier nicht um die Herstellung von Leben an sich!

Es geht um die Herstellung von autonom vermehrungsfähigen komplexen Organismen , ohne Zeugung durch Eltern.

2.2.1. Die Herstellung von primäre Keimzellen (Primordial gern cells (PGCs)

Der männliche Reproduktionszyklus in vivo und ex vivo (2016 und 2021)
Der weibliche Reproduktionszyklus in vivo und ex vivo (2016)

2.2.2. Die Herstellung von Zygoten

Ausgangspunk dieser Unternehmens:

Reproduktionsforschung und assistierende Reproduktionsmedizin bis hin zur In vitro Fertilisation (IVF) als Resultat von circa 100 Jahren Gameten-, Befruchtungs- und Embryonenforschung an Tieren und Menschen.

Intension: Erfüllung des Kinderwunsches
Sehnsucht nach langem Leben und Unsterblichkeit
Die Überwindung des Todes
… als Motive für die Forschung.

Resumée

Herstellung von Lebewesen aus diploiden oder haploiden Zellen.

Vorgeschichte:

1.1. aus diploide (2n) Zellen (ESCs + iPSCs + ntESCs = PSCs) -alle habe Methoden derzeit unüberwindlichen Barrieren.

PSCs → Blastozysten + Placentoide (künstl. Plazenten) (Homunkulus / Retortenbaby)

PSCs → EBs → Primordial Germ cells (PGCs) Meose notwendig!

PGCs → Gonadiode → Ovaroide / Testiculoide → Oocyten/Spermine … Nischenbedingungen unbekannt!

1.2. aus haploide (1n) Zellen

1. Partenogenetische PSCs → 1n ESCs[1] → PGCs/Keimzellen. [1]Martin Leeb bzw. Urich Elling, 2011; hpESC (2016)
2. Androgenic PGC-like ahESCs aus Spermien und entkernten Oocyten (2012) ABER: Alle sind 19+X und nicht 19+Y !

→Bi-parental Mäuse und auch Bi-maternal Mäuse (2018)

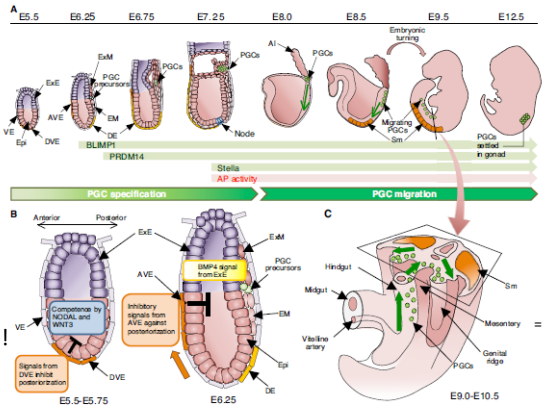
1. Was sind Keimzellen (Primordial Germ cells) und wie entstehen diese?

Vorgeschichte:

Epigenetic reprogramming in mouse pre-implantation development and primordial germ cells

Der Ursprung der Gameten (Keimzellen) sind die Primordial Germ Cells. Primordial Germ Cells entstehen in der Maus am Tag 6.5 - 7.25 (Mensch: 3-4 Woche 21 - 28 dpf) im kaudalen primitiven extra-embryonalen Ektoderm und wandern dann in dem *Mesonephros (Urnieren) Region ein.* *Zur Erinnerung: [Pronethros (Vorniere) - Mesonephros (Urniere) - Metanethros (eigendliche Niere)]*

AVE und DVE !



= Gekröse

Fig. 2. Specification and migration of mouse primordial germ cells. (**A**) A schematic of germ cell specification and migration in developing mouse embryos (prospective anterior is towards the left). Primordial germ cell (PGC) precursors (E6.25) and PGCs are shown as green circles in embryos from E6.25 to E12.5, and the direction of PGC migration is denoted by a green arrow. The timing of expression of key genes (*Blimp1*, *Prdm14* and stella) and alkaline-phosphastase activity is shown below. (**B**) Signalling activities for PGC specification at E5.5-E5.75 and at E6.25. (**C**) A detailed view of PGC migration from the hindgut through the mesentery to genital ridges at E9.0-E10.5. The direction of PGC migration is denoted by a green arrow and anterior is towards the top. Al, allantois; AVE, anterior visceral endoderm; DE, distal endoderm; DVE, distal visceral endoderm; EM, embryonic mesoderm; Epi, epiblast; ExE, extra-embryonic ectoderm; ExM, extra-embryonic mesoderm; PGCs, primordial germ cells; Sm, somite; VE, visceral endoderm.

Mammalian primordial germ cell specification

Grace V. Hancock[1,2,3], Sissy E. Wamaitha[1,3], Lior Peretz[1] and Amander T. Clark[1]

doi:10.1242/dev.189217

Unterschied zwischen Nagetieren und Primaten.

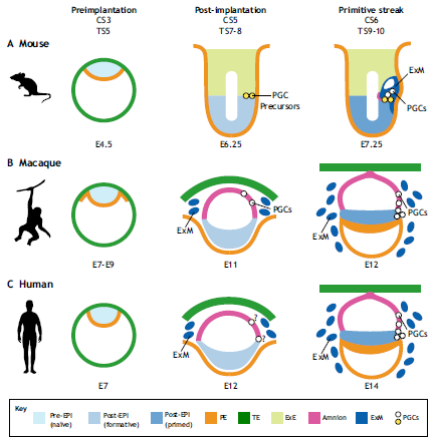


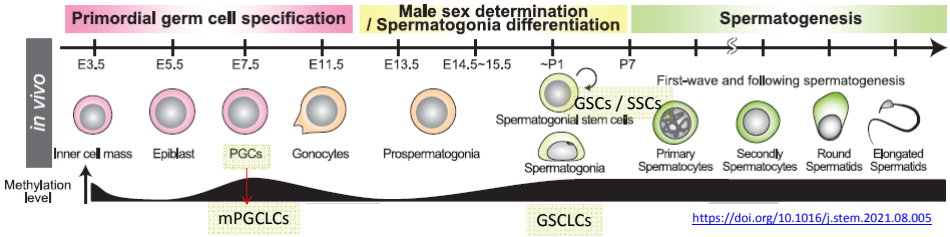
Fig. 1. Representation of peri-implantation embryo development in mammals. Mouse (A), monkey (B) and human (C) development. Formative pluripotency is described only in mouse and is therefore inferred in the other species. (A) In mouse embryos, the egg cylinder stages begin at Theiler stage (TS) 7. A pre-streak embryo is shown at embryonic day E6.25, which can be assigned to late TS8 with the specification of Blimp1+ primordial germ cell (PGC) precursors (yellow). Primitive streak formation begins at TS9, with mPGC specification from the precursors occurring late in TS9 and continuing in TS10. (B) In the monkey (cyno), cyPGCs are first identified at E11 [Carnegie stage (CS) 5c] in the amnion, with morphological evidence of primitive streak formation occurring in CS6. (C) In humans, *ex vivo* culture indicates that hPGCs can be identified before primitive streak formation at E12. '?' indicates that the cell layer of origin is not yet known for humans. At CS6, cyPGCs are found in the posterior primitive streak. In the mouse embryo, bone morphogenetic protein 4 (BMP4) and BMP8b are produced from extra-embryonic endoderm (ExE) to induce mPGC specification. In the nonhuman primate embryo, BMP4 is reported as being produced by amnion cells and possibly by extra-embryonic mesoderm (ExM). In the human embryo, the cell of origin is not yet known, and the source of BMP for inducing PGC specification is also unclear. Supporting embryonic and extra-embryonic tissue types are depicted as: preimplantation epiblast (pre-EPI), represented in culture by the naïve state; post-implantation epiblast (post-EPI), represented by the formative and primed states; primitive endoderm (PE); and trophectoderm (TE).

2.2.1. Der weibliche und männliche Reproduktionszyklus in vivo

2.2.1.1. Der männliche Reproduktionszyklus in vivo

First Meosis
Siehe nächste Folie

Spermatogenese:

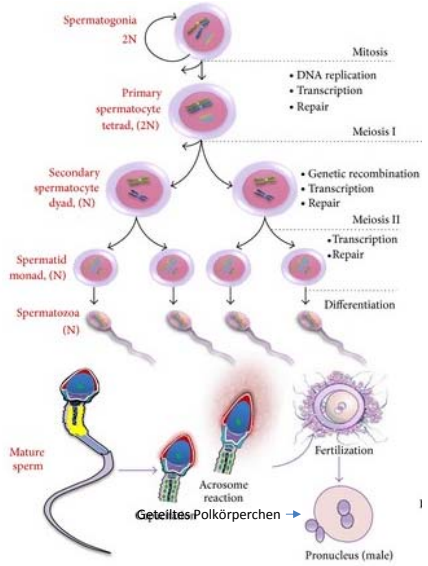


https://doi.org/10.1016/j.stem.2021.08.005

Male germ-cell development consists of three distinct phases.

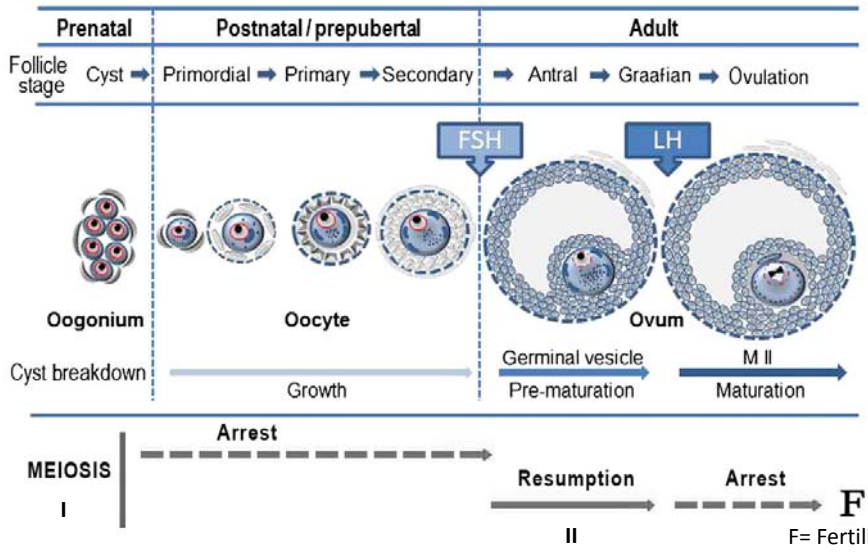
1. The specification of primordial germ cells (PGCs) and their differentiation into gonocytes with epigenetic reprogramming.
2. Male sex determination(i.e., gonocyte-to-prospermatogonium differentiation) and the ensuing differentiation of spermatogonia/spermatogonial stem cells (SSCs) with the acquisition of an androgenetic epigenome. This phase proceeds during the mitotic arrest.
3. Spermatogenesis from spermatogonia/SSCs to haploid spermatozoa through meiosis and spermiogenesis which continues throughout the life period

2.2.1.1. Der männliche Reproduktionszyklus in vivo nach der Geburt und Pupertät



https://www.researchgate.net/figure/Schematic-diagram-showing-the-cellular-genetic-and-chromatin-changes-during_fig1_259450365

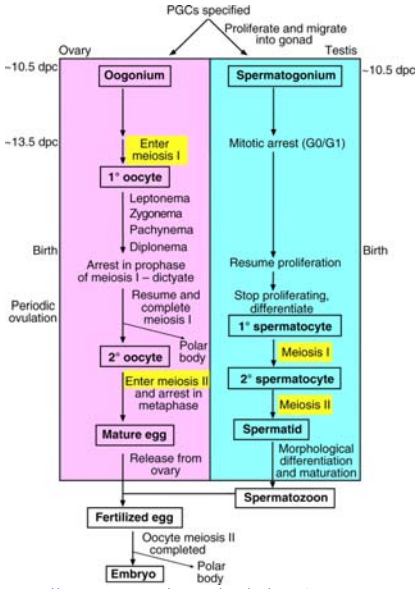
2.2.1.2. Der weibliche Reproduktionszyklus in vivo vor der Geburt und ab der Pupertät



https://embryologistmedia.weebly.com/patients/what-is-a-mature-egg-reasons-why-you-may-not-obtain-mature-eggs-in-your-art-cycle

Vergleich der Meiose in Keimzellen beider Geschlechter:

Weibchen



Männchen

http://dev.biologists.org/content/134/19/3401.figures-only

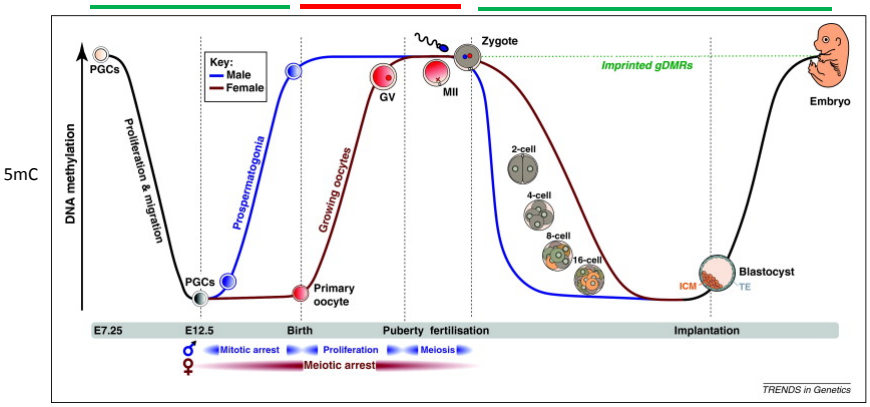
Vergleich:

Die Primordial germ cells (PGCs) entwickeln sich bei weiblichen und männlichen Organismen unterschiedlich.

Gametenentwicklung während der Foetalentwicklung

Geburt bis Geschlechtsreife

Embryogenese

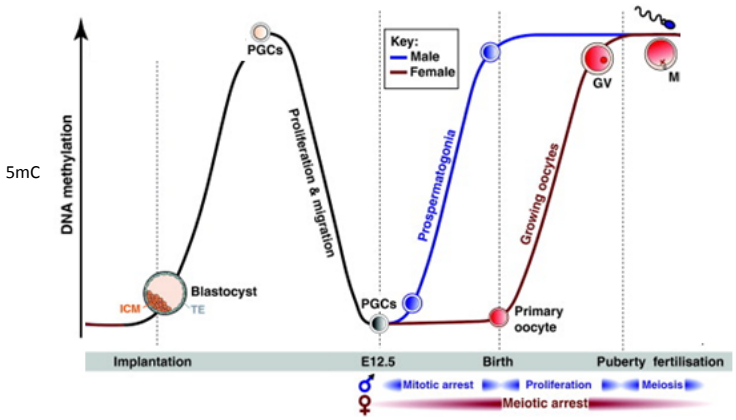


https://www.sciencedirect.com/science/article/pii/S0168952511001582

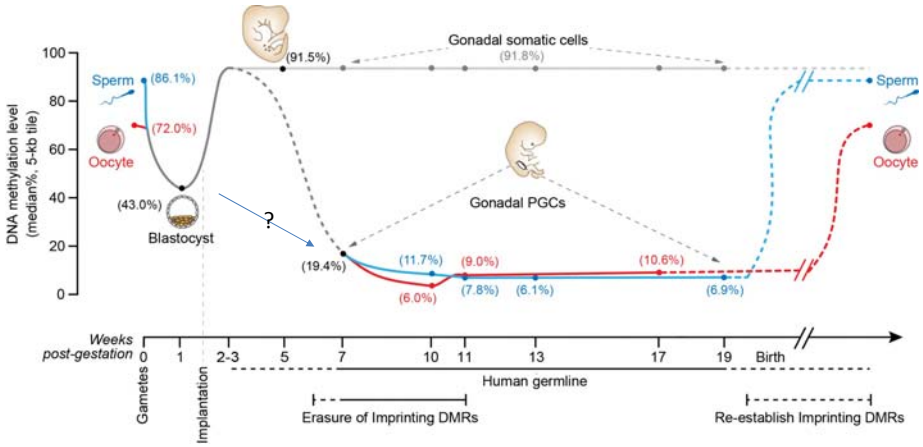
Legende zu vorhergehenden Abbildung:

Figure 1. DNA methylation changes during developmental epigenetic reprogramming. Primordial germ cells (PGCs) emerge in embryos at E7.5 and, concomitant with their proliferation and migration towards the genital ridge, DNA methylation is globally erased (black line). Following sex-determination, new DNA-methylation landscapes are established in germ-cell precursors in an asymmetrical fashion in male and female embryos. In the male embryo (blue line), *de novo* methylation takes place before meiosis in mitotically arrested cells (G1-phase; prospermatogonia) and is completed before birth. In the female embryo (red line), primary oocytes enter meiosis and arrest in prophase-I (diplotene stage); DNA methylation is established after birth during the follicular/oocyte growth phase. At puberty, under specific endocrine triggers, fully-grown germinal vesicle (GV) oocytes resume the first meiotic division. After extrusion of the first polar body, oocytes arrest in metaphase of the second meiotic division (MII oocytes) and meiosis is completed only upon fertilisation. Following fertilisation, a new wave of DNA demethylation takes place that is distinct on the parental genomes. In the zygote, DNA methylation of the paternal genome is rapidly erased by an active mechanism (blue line). Demethylation of the maternal genome is slower (red line) and is dependent on DNA replication (passive demethylation). These post-fertilisation demethylation events do not include imprinted gDMRs (green dotted line), resulting in parental-allele-specific methylation of these elements in early embryos and consequent parental-allele-specific expression of associated imprinted genes. Concomitant with blastocyst implantation and cell-lineage determination, new methylation landscapes become established, associated with cellular differentiation.

Es sind zwei unterschiedliche Wellen von DNA Methylierung notwendig, um aus ESCs Keimzellenherzustellen.



DNA Methylierung während eines ganzen Reproduktionszyklus



http://biopic.pku.edu.cn/english/content.aspx?id=260 nicht mehr verfügbar 11.11.2020

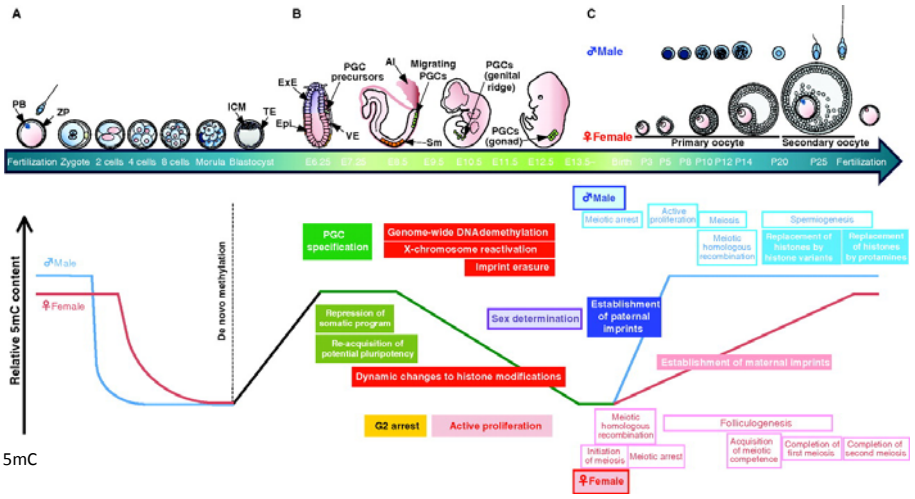
Kann man diese Prozesse auch gesteuert in vitro ablaufen lassen?

Legende zu vorhergehenden Abbildung:

The epigenetic genome-wide reprogramming cycle involves two phases of DNA erasure in the mouse (from [[91],[161],[162]]). (1) A first wave of DNA demethylation takes place in the male (blue curve) or female (red curve) primordial germ cells (PGC) of the F1 individuals; this occurs throughout the genome, including the imprinted genes (embryonic day (E10.5-13.5). (2) Then, the genome of the gametes undergoes de novo methylation, with maternal methylation marks established at a later stage (ovulation) than paternal marks (E14). (3) A second wave of DNA demethylation takes place after fertilization in the F2 zygote (E0.5), with a more rapid demethylation in the paternal than the maternal genome. However, the paternal and maternal imprinted genes maintain their methylation pattern throughout this preimplantation reprogramming (dotted curves), allowing the inheritance of parent-specific monoallelic expression in somatic tissues of the F2 individual. (4) Finally, genome-wide remethylation occurs in both parental genomes at about the time of implantation (E3.5). Altogether the very early embryonic development corresponds to an epigenomic reprogramming step, during which the new epigenetic marks are more prone to being impacted by the environment. This explains why the environment experienced during early development has a greater impact on the adult phenotype than that experienced later in life [163]. Moreover, the timing of the two global DNA demethylation and remethylation waves differs between male and female genomes, possibly explaining why they may be differently impacted by a stress applied during these stages [91,164].

Epigenetic reprogramming in mouse pre-implantation development and primordial germ cells
Mitinori Saitou, Saya Kagiwada, Kazuki Kurimoto Development 2012 139: 15-31; doi: 10.1242/dev.050849

DOI: 10.1242/dev.050849



Legende zu vorhergehenden Abbildung:

Fig. 1. A schematic of mouse pre-implantation and germ cell development. (Top) A schematic of pre-implantation and germ-cell development in mice. (**A**) Pre-implantation development stages; (**B**) post-implantation embryonic development, following blastocyst implantation at around E4.5; and (**C**) postnatal germ cell development and maturation. Primordial germ cell (PGC) precursors (E6.25) and PGCs are shown as green circles in embryos from E6.25 to E12.5. (Bottom) Key genetic and epigenetic events are shown that are associated with pre-implantation and germ cell development, together with relative levels of 5-methylcytosine (5mC) at different developmental stages. Al, allantois; Epi, epiblast; ExE, extra-embryonic ectoderm; ICM, inner cell mass; PB, polar body; PGCs, primordial germ cells; Sm, somite; TE, trophectoderm; VE, visceral endoderm; ZP, zona pelucida.

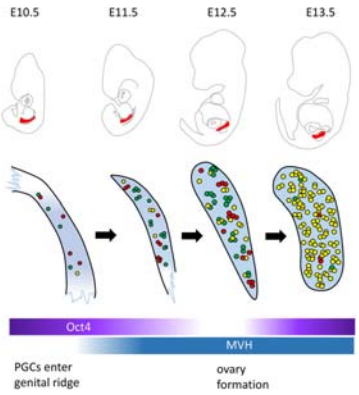
Visualization of X chromosome reactivation in mouse primordial germ cells in vivo

Yoshikazu Haramoto [1], Mino Sakata [1], Shin Kobayashi

2021 Apr 15;10(4):bio058602. doi: 10.1242/bio.058602.

Spezifische Reaktivierung, z.B. PGK-1 locus wird vor dem Hprt locus auf dem X Chromosom reaktiviert.

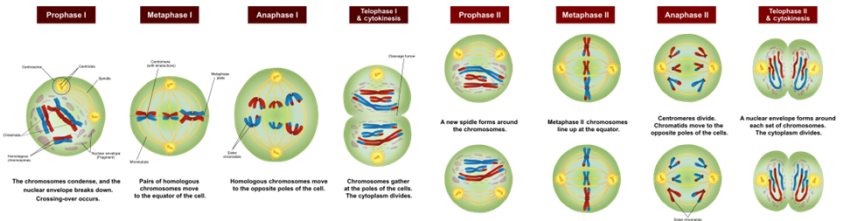
Parallel dazu werden imprinted autosomale genes wieder aktiviert.



DOI: 10.1242/bio.058602

Fig. 4. Schematic diagram of PGC proliferation and X chromosome reactivation during embryogenesis. PGCs in the genital ridge are evenly distributed. Each PGC proliferates and forms clusters. Reactivation at the *Pgk1* locus starts at ∼E10.5 and is almost complete at E13.5 (reactivated cells are shown in yellow; >85% of total cells). The XCR of PGCs progresses simultaneously with proliferation. The whole image of the embryo is shown on a modified diagram (top panel), based on Kaufman's Atlas of Mouse Development (Kaufman, 1992). The location of the GR analyzed is shown in red.

Zur Erinnerung: Meose



https://en.wikipedia.org/wiki/Meiosis

2. BASICS OF THE MEIOTIC CELL CYCLE: ANOTHER LAYER OF COMPLEXITY (NACHTRAG ZU 2.4. MEIOSE)

Meiosis is restricted to germ line cells and has features of cell division that simply do not exist in somatic cells. One striking unique feature, for example, is that there are two metaphase segregation events that occur without an intervening round of DNA synthesis. Another aspect of meiosis that distinguishes it from mitosis is the behaviour of sister chromatids during the first meiotic division. That is, in mitosis, the sister chromatids separate in the single phase of chromosome segregation, while in meiosis, sister chromatids do not separate from one another until the second round of segregation—the first stage involves segregation of homologous chromosomes. In addition, it is critical that meiosis-unique processes, including the synapsis or pairing of homologous chromosome, recombination and formation of chiasmata, be strictly coordinated with cell cycle progression, as it would be catastrophic for the gamete cell to attempt to move into the cell cycle without these other processes having been completed.

Higher organisms are characterized by having sexually dimorphic gametes. While male and female germ cells have stages of cell cycle regulation in common, including a mitotic proliferative stage, entry into meiosis, completion of a reductive division and entry into a quiescent state prior to fertilization, the timing of these events and the stage of development at which they occur differ in the two sexes (reviewed in Wolgemuth *et al*. 1995, 2002; Handel & Eppig 1998). In the mouse model, the germ line is specified early in embryonic development, probably as early as embryonic day 6.0–6.5. The progenitor germ cells migrate from the proximal epiblast to the gonadal ridge, at which point sexual determination based upon genotype occurs. The germ cells then follow either a male pathway, in which the cells enter into a mitotic arrest, or alternatively, a female pathway, in which they enter into pre-meiotic DNA synthesis and meiotic prophase. Thus, germ cells of both sexes undergo mitotic divisions in the embryonic gonad, but the female germ cells enter meiosis during foetal development, whereas this is a postnatal event in the male. Once the male germ cell has entered meiosis, the process continues without interruption until the haploid sperm is produced. In contrast, the oocyte is arrested in the diplotene stage of meiotic prophase I, where it can remain for months or years depending on the species. Following a growth period, which begins at puberty, the oocyte resumes meiosis, but arrests a second time, at metaphase II. Fertilization then triggers the completion of meiosis and extrusion of the second polar body. Given these striking differences in the sequence of mitotic and meiotic events, it is almost given that the genetic programme underlying this regulation will be distinct between the male and female and will be reflected in a sexual dimorphism in the genes involved in regulating these processes (Handel 1998; Wolgemuth *et al.* 2002). See **Regulating mitosis and meiosis in the male germ line: critical functions for cyclins. 2012** D.J. Wolgemuth[1]and S.S. Roberts Philos Trans R Soc Lond B Biol Sci. 365(1546):1653–1662. doi: 10.1098/rstb.2009.0254

Wir suchen für Mitte 2023 eine n Studierende n die/der seine Masterarbeit bei uns machen will.

Anfragen ab sofort persönlich (bitte keine Emails) in meinem Büro 2.118 oder Labor 2.522.