The Soma and the Germline

Separation of soma and germline

The Weismann barrier is the strict distinction between the "immortal" germ cell lineages producing gametes and "disposable" somatic cells. In more precise terminology, hereditary information moves only from germline cells to somatic cells (that is, classical somatic mutations are not inherited).

The germ line:soma relationship coordinates metazoan evolution.
Basic terms

- **Primordial germ cells (PGCs):** Primordial germ cells are the founder cells for the germline. They divide symmetrically and all their descendents are germline stem cells. In many organisms, primordial germ cells are motile and migrate to the somatic gonad.

- **Germline stem cells (GSCs):** Germline stem cells are PGC descendents that have acquired the ability to both self-renew and generate daughters that begin gametogenesis.

- **Germ plasm/granules/bodies:** Large ribonucleoprotein complexes typically found around the nuclei of germ cells and predicted to function in posttranscriptional gene regulation. These compartments give germ cells the ability to differentiate while maintaining a pluripotent genome.

Two Ways for PGC Determination

- **Autonomous Specification ('preformation mode')**
  - Maternal cytoplasmic determinants
  - asymmetrically partitioned ‘Germ Plasm’
  - Nematodes, flies, fish and frogs

- **Conditional Specification ('induction mode')**
  - Signals from surrounding cells
  - PGCs arise later from pluripotent progenitors
  - Majority of sexually reproducing organisms
  - Including mammals

Despite these differences in PGCs origin, a common theme has emerged from studies in *Drosophila, C. elegans, and mice*: PGCs’ specification depends on mechanisms that inhibit the expression of somatic genes.
Marcella and Theodor Boveri (1899)

Germ plasm, chromosome diminution.

First two cleavages of *Parascaris* zygote

Boveri’s Experiment:

Germ plasm rearrangement by gentle centrifugation.
Chromatin diminution

**Ascaris suum:**
- 13% of 43 Mb is lost in both sexes from somatic cells
- Tandem repeats + 700 protein coding genes were eliminate
- 52 breakpoints on the chromosomes
- Holocentric chromosomes
- No exact consensus sequences
- No short RNA-driven elimination
- The molecular mechanisms that define the genomic regions to be eliminated or maintained remain completely mysterious.
- Instead of refusion, the remaining pieces of DNA gain new telomeres

**Evasion: Programmed DNA elimination (PDE)**

**Two types of PDE:**
- Chromosome elimination when entire chromosomes are lost.
- Chromatin diminution when chromosomes break and regions of the chromosomes are lost.

The role of chromatin diminution is to remove from the soma germline-specialized paralogs and other germline-specific genes.
Formation of different expression pattern between germline and soma

A) Remove from the soma germline-specialized paralogs and other germline-specific genes by chromatin diminution or chromosome elimination

B) Silencing the soma-specific genes in the germline.

Switching from soma to germline

PIE-1 protein is a conserved zinc-finger (CCCH) protein and localizes to the germline blastomeres both in the nucleus and in the cytoplasmic P-granules.

When pie-1 activity is lost, an increase in RNA transcription is observed in the germ cell, which adopts somatic cell fate.
Apart from the granules, the germ plasm also contains PGCs determinants.

**Perinuclear localization of P granules**

**Composition of P granules**
- GermLine Helicases (GLH 1-3)
- P Granule abnormality (PGL1, 3)
- Dicer, RdRP, Argonautes, Wago
- nos2, pos1, skn1, par1, mex1, gdl1
Germ-line helicases (DEAD box RNA helicases)

Components of P-granules – transcriptome control; translation initiation
Loss of GLH-1 function causes sterility is due to reduced germ-cell proliferation and impaired formation of both sperm and oocytes.

A more sophisticated method: transcriptional silencing in germline

CTD of RNAPII:
- tens of heptad repeat: YSPTSPS
- TFIIH initiation factor: Ser5 → PSer5
- P-TEFb elongation factor: Ser2 → PSer2

In Caenorhabditis PGCs:
- Ser5 is underphosphorylated
- PIE-1 inhibits the phosphorylation of Ser2
- No transcription in PGCs

Nakamura and Seydoux, Development, 2008
Transcriptional gene silencing in some and germline

Argonautes: Members of RISC, RNase-H
NRDE: Nuclear RNAi-Deficient
HMT: histone methyltransferase
HRDE: heritable RNAi-Deficient
HPL-2: heterochromatin protein-like -2
PRG-1: piwi-related gene-1
RdRP: RNA-dependent RNA polymerase
CSR-1: Chromosome segregation and RNAi Deficient Mutator foci

Germ plasm: necessary and sufficient? Or?

Normal, but sterile animals.
Normal and fertile animals.
Germ (pole) plasm a specialized cytoplasm at the posterior of the embryo is probably necessary and sufficient for induction of the germ cell progenitors, the pole cells. An estimated 200 maternal mRNAs are enriched within it and then inherited by the pole cells, where they direct production of proteins required for specification of germline fate and for germline development.

**Polar granules in *Drosophila* egg**

**Composition of polar granules**
- Oskar, Tudor (RNA binding)
- Vasa (GermLine Helicase)
- Pge (Polar Granule Component - transcriptional repressor)
- Pumilio (translational regulator)
- PIWI, Aubergine, Dicer 1
- osk, nos, gcl, pgc, cyclin B (200+ mRNA)

**Composition of germ plasm**
- Gcl, PIWI, Dicer1/2 proteins

Posterior group determinants
- Oskar, Nanos

PG are 500nm big granules enriched with polysomes, indicating that they are a site of dynamic translational activity.
Germ plasm assembly

Short Osk protein recruits other components of the germplasm such as Vasa, Tudor and Aubergine. Symmetric dimethylation of Arginine (sDMA) in Vasa and Aubergine by the methyltransferase Capsuleen allow binding to Tudor domain proteins. This together with liquid to gel phase transitions could provide a possible basis for the dense assembly of polar granules.

Capsuleen: Arg-methyltransferase Tudor domains may serve as ‘docking platforms’ for polar granule assembly.
Valois is required for the synthesis and/or stability of Oskar and the localization of Tudor Aubergine: an Argonaute-protein

The Silence of the Lambs

Heritable transposome silencing in Drosophila.
Mechanism of transcriptional silencing in germline

RNA PolIII: CTD: heptad repeat: YSPTSPS
TFIIH initiation factor: Ser5 → PSer5
P-TEFb elongation factor: Ser2 → PSer2

In Drosophila PGCs:
Ser5 is underphosphorylated ???
Pgc inhibits the phosphorylation of Ser2
No transcription in PGCs

Silencing of zygotic genes in two steps

• As for mechanisms, nothing is known about how the phosphorylation of Ser5 is blocked in pole cells.

• Pgc downregulates the *zen* and *tailless* genes which are responsible for control of histogenesis and formation of posterior end of larva, respectively.
Balbiani body

The Balbiani body or mitochondrial cloud is a transient structure, containing mitochondria, Golgi, endoplasmic reticulum (ER) and RNA, that forms in the young (previtellogenic) oocytes of insects and vertebrates.

Bucky ball, as the creator of the germ plasm

dazl: (Deleted in AZoospermia) gene family encodes potential RNA binding proteins that are expressed in prenatal and postnatal germ cells of males and females; a Balbiani body marker.
Germ plasm and the formation of PGCs in frog

mc: mitochondrial cloud (≡ Balbiani body)

gp: germ plasm
cf: cleavage furrow

n: nucleus

vg: vegetal pole

The major germ granule classes

A: P granule in a germ cell of C. elegans. The crest (≡ P granule; white arrow) and base (≡ nuage; arrowhead) overlying a cluster of nuclear pores (black arrows).

B: Balbiani body in a mouse oocyte. Black arrow points to mitochondria clustered around Golgi membranes

C: In the Drosophila, the sponge bodies (asterisks) intermingled with mitochondria (m) and ER cisternae (arrows). Id, lipid droplet.

D: Polar granules (pg) in the cortical cytoplasm of a Drosophila embryo. Polysomes (arrows) extend from the surface of the granule.
In words:

In Drosophila, Xenopus, Caenorhabditis and zebrafish, germ granules are present continuously throughout development, with the exception of mature sperm. In those organisms, germ granules are transmitted from oocyte to embryo as part of the germ plasm, a specialized cytoplasm that segregates with the germ lineage.

What about Mammals?

Conditional Specification ('induction mode')
- Signals from surrounding cells
- PGCs arise later from pluripotent progenitors
- Majority of sexually reproducing organisms

In mammals, germ granules are not detected in oocytes or early embryos, but are formed de novo in primordial germ cells shortly after their specification.

Balbiani body is present in the oocytes and it probably gets involved in the polarization of oocyte

No germ plasm, of course. Only one report says, but it seems to be a tautology. (Fox et al., Dev. Biol., 2007)

Transplantation experiments:
Pluripotent from epiblast → Primordial germ cells
Of Mice and Men

ExEc: BMP4/8
VEn: Wnt/β-catenin → Brachyury → Blimp1, Prdm4 → Hox genes

Summary of formation of PGCs in diverse animal embryos

Forget the parents!

Epigenetic reprogramming in the germline resets genomic potential and erases epigenetic memory.

The global demethylation observed in human PGCs leads to a dramatic loss of almost all DNA methylation at CpG islands, transcription start sites, gene bodies, and surrounding intergenic regions, compared to somatic cells. 

Meyenn and Reik, Cell, 2015

Genomic imprinting

- Modification of specific genes during gametogenesis so that only the paternal or maternal allele is expressed after fertilization, i.e. “Parent of origin” gene expression.
- A small subset of the total genes behave in this way.
- Seen in placental mammals and in plants.
- Affects the expression but not transmission of alleles
- Two gene copies present
- One gene copy active functional haploidy
Prader-Willi / Angelman syndrome

Genomic imprinting – 15th chromosome

Genetic background of PWA syndrome
Imprinted gene clusters in the mouse genome

<table>
<thead>
<tr>
<th>Cluster name</th>
<th>Chromosome mouse/human</th>
<th>ICE (gametic methylation imprint)</th>
<th>Cluster size (kb)</th>
<th>Gene number in cluster</th>
<th>Parental expression M/P</th>
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<tbody>
<tr>
<td>Igf2r</td>
<td>17/6</td>
<td>Region 2 (M)</td>
<td>490</td>
<td>4</td>
<td>3 M (pc)</td>
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<tr>
<td>Kcnq1</td>
<td>7/11</td>
<td>KvdMR1 (M)</td>
<td>780</td>
<td>12</td>
<td>11 M (pc)</td>
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<td>Pws</td>
<td>7/15</td>
<td>Sper-CGI (M)</td>
<td>3700</td>
<td>&gt;8</td>
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<td>Gnas</td>
<td>2/20</td>
<td>NePas DMR (M)</td>
<td>80</td>
<td>7</td>
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<td>Grb10</td>
<td>11/7</td>
<td>Meg1/Grb10 DMR (M)</td>
<td>780</td>
<td>4</td>
<td>2 M (pc)/</td>
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<tr>
<td>Igf2</td>
<td>7/11</td>
<td>H19-DM (P)</td>
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<td>3</td>
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<td>Dlk1</td>
<td>9/14</td>
<td>IG-DMR (P)</td>
<td>830</td>
<td>&gt;5</td>
<td>4 P (pc)</td>
</tr>
</tbody>
</table>

- Approx. 150 imprinted genes
- In 16 clusters
- 3-12 genes / cluster

Igf2: Insulin-like growth factor 2 (receptor)
Kcnq1: potassium voltage-gated channel, KQT-like subfamily
Pws: Prader-Willi syndrome
Gnas: G-protein coupled receptor
Grb10: Growth factor receptor-bound protein
Dlk1: Delta-like 1 homolog

The imprinting mechanism

NI: nonimprinted
IG: imprinted, protein-coding
IG-NC: imprinted, noncoding
ICE: imprint control element
Mat: maternal
Pat: paternal

experimental deletion of the GDMR:

Barlow and Bartolomei, CSHP, 2014
Silencing mechanisms at imprinted gene clusters

CTCF: Transcriptional (enhancer) regulator
E: enhancer
H19: gene for IncRNA Ins2: insulin

H19 transcription only from maternal allele
H19 maternal knock out: non-lethal, overweighted
H19 maternal overexpressed: lethal

Direct proof for genomic imprinting

Two hypotheses

**Parental conflict** (Moore and Haig 1991):
Embryonic growth is dependent on one parent, but influenced by an embryo whose genome comes from two parents. Paternally expressed imprinted genes are proposed to increase embryonic growth, thereby maximizing the fitness of an individual offspring bearing a particular paternal genome. Maternally expressed imprinted genes are proposed to suppress fetal growth. This would allow a more equal distribution of maternal resources to all offspring and increase transmission of the maternal genome to multiple offspring, which may have different paternal genomes.

**Trophoblast defense** (Varmuza and Mann 1994):
This proposes that the maternal genome is at risk from the consequences of being anatomically equipped for internal reproduction should spontaneous oocyte activation lead to full embryonic development. Because males lack the necessary anatomical equipment for internal reproduction, they do not share the same risks should spontaneous activation of spermatozoa occur. Imprinting is thus proposed to either silence genes on the maternal chromosome that promote placental development or to activate genes that limit this process.

*Sex chromosomes*
Suppression of recombination between the sex chromosomes, associated with degeneration of the non-recombining region of the Y chromosome, results in the morphological and genetic differentiation of sex chromosomes.

Sex determination systems (4)

Polygenic sex determination systems:

- **Single-locus polygenic**
  - **Mus minutoides**
  - **Metriaclima pyrsonotus**
At least three different solutions have independently evolved in mammals, flies and the worm C. elegans. These strategies, grouped under the term ‘dosage compensation’, appear different at a first glance but present remarkable similarities. In mammals, dosage compensation between XY males and XX females occurs by the transcriptional silencing of one randomly chosen X chromosome in each female cell. In flies, XY males increase transcription from most X-linked genes. Hermaphrodite worms (XX) continue to transcribe both X chromosomes, but at half the rate of the single male X.

Meyer B. Wormbook, 2005
Dosage compensation in C. elegans

**XSEs:** X-signal elements  
**ASEs:** autosomal signal elements  
**Xol-1:** XO lethal 1 ("master switch")  
**SDC:** sex determination and dosage compensation

Two mechanisms of xol-1 repression are used: repression at the level of transcription and pre-mRNA splicing.

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Dosage compensation in C. elegans

X0l-1 represses the transcription of sdc genes  
(Sex determination and Dosage Compensation defective)  

*When the level of X0L-1 is very low*

- High level of SDC1 and SDC2 in XX animals
- DCCs (Dosage Compensation Complex) appear
- Repression of her-1 gene (20x) and X chromosome (0.5x)
Dosage compensation in C. elegans

Recruitment and spreading model

REX: Recruitment Element on X

Condensin-like complexes (dosage compensation complexes) are gathered on rex elements and close partly the sequences on X chromosomes.

Dosage compensation in D. melanogaster

- MSL proteins make a complex and bind to X chromosome
- Absent of one of MSL protein inhibits the upregulated transcription
- \textit{maleless (mle)}: ATP dependent DNA/RNA-helicase
- \textit{male specific lethal 1, 2, 3, (msl1, 2, 3)}: only in males, both msl1 and 2 have SXL binding sites on their UTR
- \textit{roX1, 2 (RNA on X 1, 2)}: small RNAs (4,1 and 0.6 kb) without ORF, binding to X. Absent of roX no MSL complex assemble.
- Transcripts of many X-linked genes have SXL binding site.

MSL-complexes on polytene X chromosome
Establishment, maintenance, and erasure of genomic imprints

ZFP57: zinc finger protein
PGC7: primordial germ cell
TET1: methylcytosine dioxygenase

LncRNAs in X-chromosome inactivation

PRC2: Polycomb repressive complex 2
The End