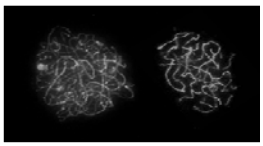
Current understanding
of primordial germ cell (PGC) biology



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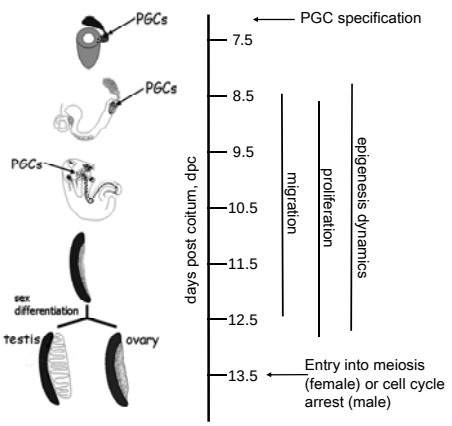
Who are primordial germ cells (PGCs) ?

PGCs are the embryonic precursors of oocytes and spermatogonia

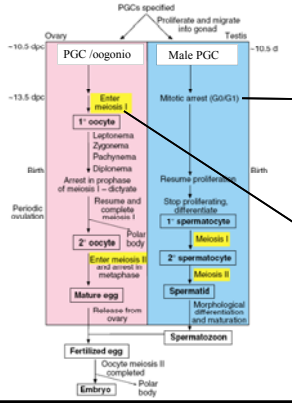
PGC sex differentiation: entry into meiosis

Looking for a role of Stimulated by Retinoic Acid 8 (STRA8) in mouse germ cell development

PGC development in the mouse embryo
(our experimental model)



Germ cell sex and differentiation in the mouse



How is the germ cell sex-specific fate determined?

Prevention of meiosis in male *PGCs*:

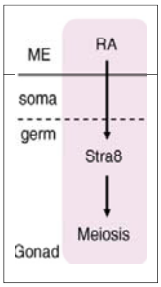
1. Is due to the lack of Meiosis Inducing Substance (MIS)
2. Is due to the action of Meiosis Preventing Substance (MPS) secreted by the surrounding somatic cells

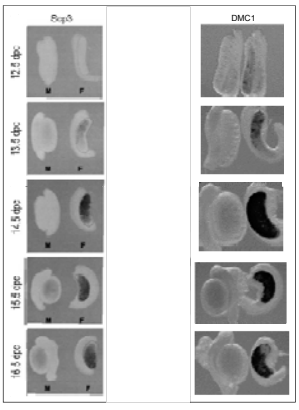
Entry into meiosis in female *PGCs*/oogonia:

1. Occurs spontaneously, perhaps regulated by a molecular clock
2. Is induced by surrounding somatic cells (MIS)

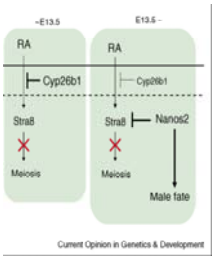
Modified from Bowles J. and Koopman P., 2007

The onset of meiosis in female PGCs is induced by RA





CYP26B1 is the meiosis–preventing substance (MPS)



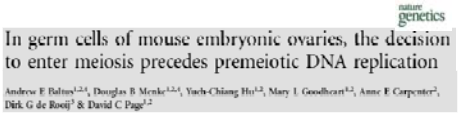
Cyp26b1 encodes an enzyme that metabolizes RA

It is expressed in male gonads from 12.5 dpc and downregulated in female gonads at the same time (Koubova et al., 2006; Bowles et al., 2006)

In *Cyp26b1* $^{-/-}$ mice, male germ cells enter into meiosis to subsequently undergo apoptosis (MacLean et al., 2007)

In the fetal testis, endogenous RA is cleared by CYP26B1 ensuring that onset of meiosis in male germ cells is delayed until after birth

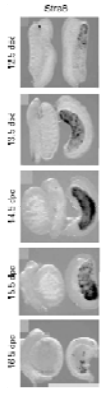
How does Retinoic Acid induce meiosis? STimulated by Retinoic Acid8 (STRA8) is a primary RA effector


nature genetics

In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication

Andrew E Baltus[1,2,5], Douglas B Menke[1,2,5], Yueh-Chiang Hu[1,2], Mary L Goodheart[1,2], Anne E Carpenter[2], Dirk G de Rooij[3] & David C Page[1,2]

- *Stra8* -/- female mice are infertile
- *Stra8*-/- female PGCs do not undergo pre-meiotic DNA replication and are blocked in the pre-leptotene stage of meiotic prophase (chromosomes do not: condense, make synapsis and recombination)



From Menke et al., 2003

Stra8 was originally identified as an RA-responsive gene in ES and EC cells (Oulad-Abdelghani et al., 1996) and it is expressed at relatively high levels in male and female premeiotic germ cells

Stra8 expression begins at ~12.5 dpc in the anterior part of the gonad and extinguishes at ~16.5 dpc at the posterior end

The molecular functions of this protein in the meiosis are unknown.

Is RA really indispensable for meiosis entry in female PGCs?

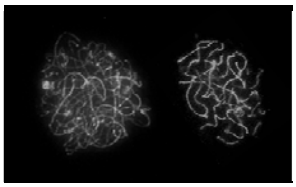
ARTICLE

Sex-specific timing of meiotic initiation is regulated by Cyp26b1 independent of retinoic acid signalling

- Stra8 expression in the fetal ovary does not require RA signaling
- RA is not required for induction of meiosis

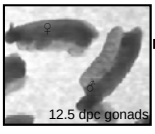
We use an *in vitro* culture system that was devised in our laboratory:

Purified premeiotic PGCs enter and progress through the different stages of meiotic prophase I in the absence of somatic cells and with kinetics similar to the in vivo condition as determined by cytospreads and SYCP3 staining

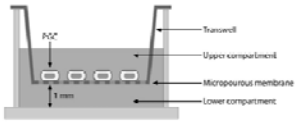


Isolation and purification of PGCs

The mouse PGCs were purified from male and female 12.5 dpc gonads with immunomagnetic sorting (MiniMACS)



In vitro culture method for purified 12.5 dpc PGCs onto Transwell chambers (Farini et al., 2005)



Complete Medium (CM): **15% FCS**, 0.5 mg/ml N-acetyl-L-cysteine, Forskolin, and growth factors (SCF, bFGF, BMP-4, SDF-1a)

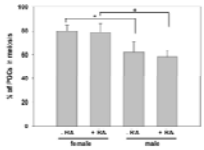
TRANSWELL MEMBRANE ⇨

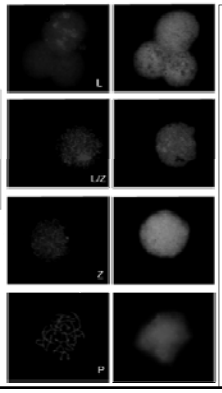
GROWTH FACTORS
- adhesion
- low apoptosis
- proliferation
- **meiosis (SYCP3 staining)**

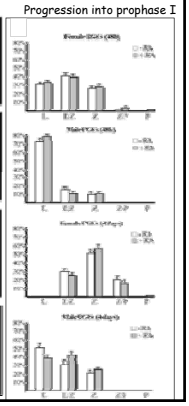
Both 12.5 dpc female and male PGCs are able to enter and progress through meiotic prophase I without the addition of RA to the culture medium

Progression into prophase I

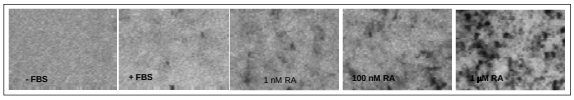
Entry into meiosis



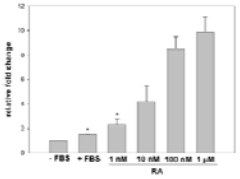




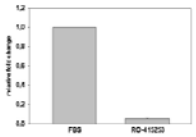
Low concentration of RA present in serum is able to directly stimulate *Stra8* expression in PGCs



The RA concentration in the FBS is ≤ 1 nM as evaluated using F9-RARE-lacZ cells, a cell line stably expressing a retinoic acid inducible response element (RARE) driving the *lacZ* gene

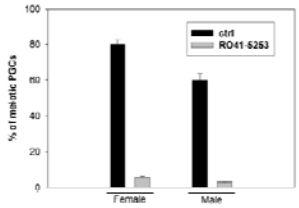


FBS and RA stimulates *Stra8* expression in a dose-dependent manner

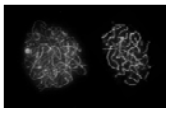


The RA receptor antagonist, RO 41-5253, reverts the stimulatory effect of FBS on *Stra8* expression

The RA receptor antagonist RO 41-5253 inhibits the entry into meiosis of PGCs in a FBS-containing medium



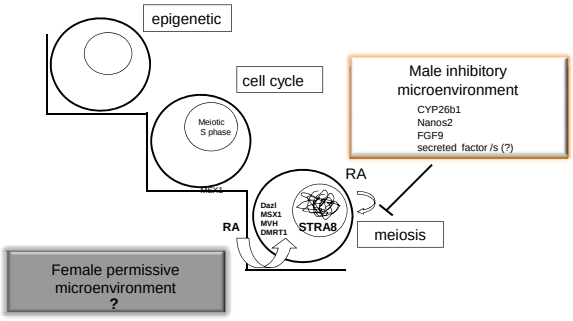
RA may exert its meiotic promoting action directly on PGCs at very low concentrations



CONCLUSIONS 1

Very little amount of RA (around 1 nM) is sufficient to induce *Stra8* expression and entry into meiosis both in female and male PGCs

At 12.5 dpc, male PGCs can still be induced by the low amount of RA to express significant amounts of *Stra8* and to enter meiosis

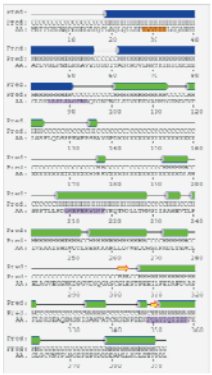


PGC sex differentiation: entry into meiosis

Looking for a role of Stimulated by Retinoic Acid 8 (STRA8) in mouse germ cell development

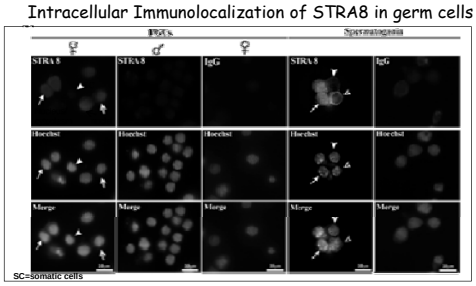
The N-terminal HLH domain is a well conserved region of the *Stra8* sequence

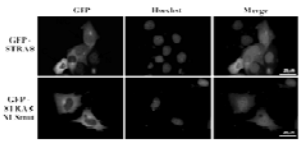
(NCBI GeneID 20899, aa 17–84)



A PSIPRED Server was used for secondary structure prediction of mouse STRA8

Intracellular Immunolocalization of STRA8 in germ cells

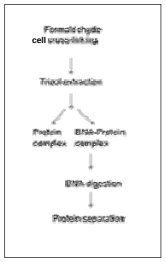


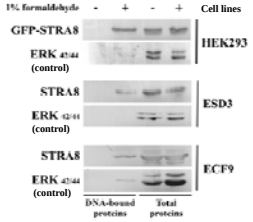


The NLS sequence within the HLH domain is responsable for STRA8, N-C shuttling

Tedesco et al., 2009

STRA8 can associate with DNA

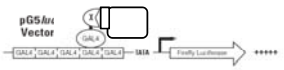




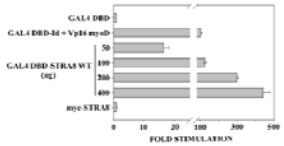
After cross-linking, both transiently expressed (*upper panel*) and endogenous STRA8 (*lower panel*) were recovered in the DNA-bound protein fractions and also in the total protein fractions

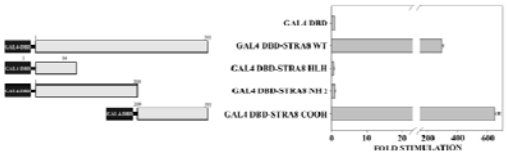
Transcriptional activity of STRA8 in HEK293 cells

One-hybrid Transcription Activation Assay



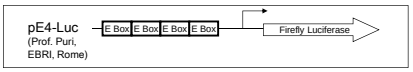
STRA8 fused to the yeast GAL4-DNA binding domain caused a concentration-dependent increase in the transcription of the GAL4 reporter gene

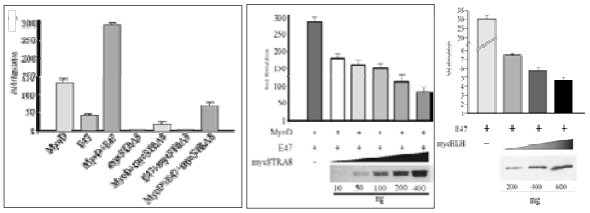




Transactivation domain of STRA8 is located in the COOH –terminal region

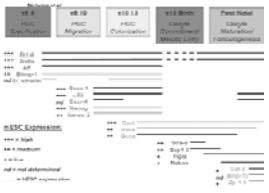
STRA8 negatively influences the E-Box-mediated transcription



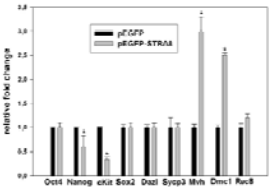


STRA8, through HLH domain, inhibits E-Box- mediated transcription in a dose-dependent manner.

STRA8 can affect the expression of key genes of germline when overexpressed in ESC

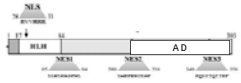


From Nicholas, C. R. et al. Endocr Rev 2009;30:264-283



Quantitative expression of genes in ES cells transfected with pEGFP or pEGFP-STRA8 plasmids. Results were normalized to levels of *GAPDH* gene expression.

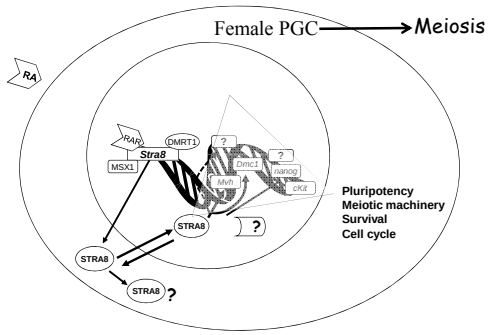
Conclusions 2



STRA8 shuttles between nucleus and cytoplasm

Stra8 is able to associate directly /indirectly to DNA and possesses an activatory or inhibitory transcriptional activity

Stra8 can regulate the expression of specific germ cell and meiotic associated genes




Thank you
Prof. M. De Felici
Dr. Tedesco M.
Dr. Desimio MG
Dr. Klinger F.