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Retinoic Acid Receptor Alpha Is Essential in Postnatal Sertoli Cells but Not in Germ Cells

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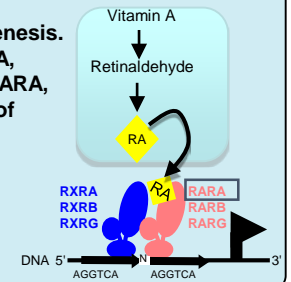
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Introduction/background

- It has long been recognized that vitamin A is indispensable to various processes amongst which spermatogenesis. In cells, successive enzymatic reactions lead to the synthesis of the biologically active derivative of vitamin A, retinoic acid (RA) that regulates the transcription of target genes through binding to nuclear receptor RAR (RARA, RARB and RARG isotypes). It has been shown, notably, that vitamin A deficiency in rodent and inactivation of Rara gene in mice lead to testis degeneration.
- Initial genetic studies established that RARA acts in Sertoli cells¹, but a recent paper suggested that RARA may also play a role in germ cells².



Objectives:

- To reevaluate the role of RARA in germ cells (GC).
- To test whether RARA is required in Sertoli cell (SC) during the fetal period or after birth.

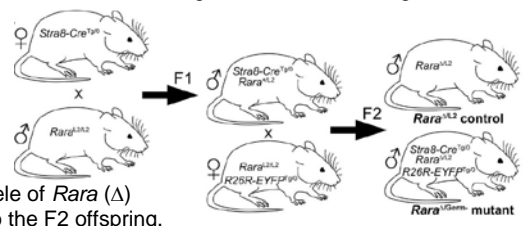
Methodologies

I Generation of mutant mice lacking all Rara selectively in GC.

To generate *Rara^{Δ/Germ-}* mutants, in which the RARA gene was excised only in GC, mice carrying *loxP*-flanked alleles of the *Rara* gene was crossed with mice bearing the *Stra8-Cre* transgene³, which is active only in germ cells from postnatal day 3 onward.

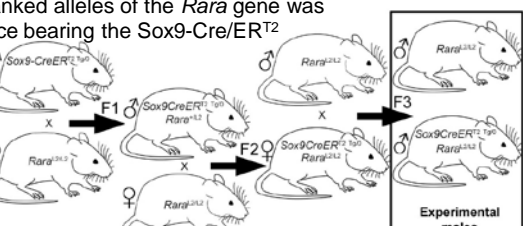
Because of the expression of Cre in F1's GC, one excised allele of *Rara* (Δ) is transmitted to the F2 offspring.

To monitor for efficient gene excision, we also introduced in the breedings the *R26R-EYFP*⁴ transgene that directs the expression of EYFP in cells that have experienced Cre-mediated recombination.

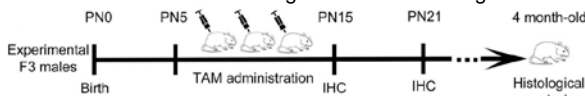


II Generation of mutant mice lacking all Rara selectively in SC.

To generate *Rara^{Ser/-PN15}* mutants, in which the RARA gene was excised only in SC from postnatal day 15 (PN15) onwards, mice carrying *loxP*-flanked alleles of the *Rara* gene was crossed with mice bearing the *Sox9-Cre/ERT2* transgene⁵, which is active only in Sertoli cells upon Tamoxifen (TAM) administration.



Pups were administered TAM (50 mg/kg body weight) by intraperitoneal injections, three times every second day, between PN5 and PN15. Both those bearing (*Rara^{Ser/-PN15}*) and those free of the transgene (*Rara^{Ser+/+}*) received TAM, precluding therefore that the observed abnormalities at later stages are due this drug.



III Histology, immunohistochemistry and in situ hybridization.

For histology, testis samples were fixed in Bouin's fluid for 16 hours and embedded in paraffin. Histological sections were stained with hematoxylin and eosin (H&E) or with periodic acid-Schiff (PAS) and counterstained with Harrys hematoxylin.

For immunohistochemistry (IHC), testes were fixed by intracardiac perfusion of icecold 4% paraformaldehyde in PBS, then kept in the same fixative overnight at 4°C, washed in PBS, dehydrated and embedded in paraffin. 5µm sections were immunolabeled with RARA, GATA4, AR or GATA1 antibodies (see Figure) and with Alexa Fluor 488-conjugated peanut agglutinin (lectin) to label acrosomal systems.

For in situ hybridization (ISH), the BaseScope v2-RED Reagent kit was used according to the manufacturer's instructions (Advanced Cell Diagnostics, ref. 323900). Probe Mm-Rara, ref. 82473.

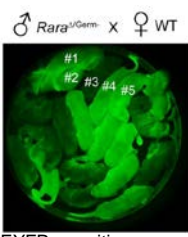
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Results

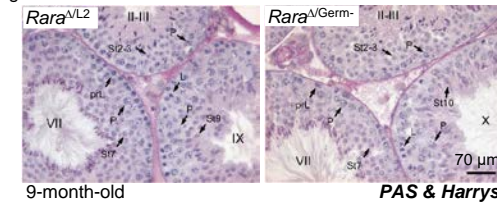
I Spermatogenesis is not altered in males lacking RARA in GC.

RARA could not be detected by IHC in GC from wild type mice using commercial antibodies or our antibody against RARA.



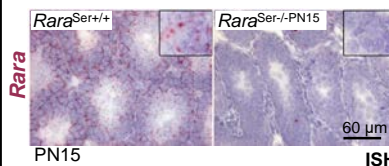
- A mendelian proportion of pups sired by *Rara^{Δ/Germ-}* males were EYFP-positive.
- 94% pups born from these breeding were *Rara^{Δ/+}* and 6% were *Rara^{Δ/Δ}*.

Even though we could not evidence RARA loss by IHC, we concluded that *Rara^{Δ/Germ-}* were actually deprived of RARA in 94% of their germ cells.

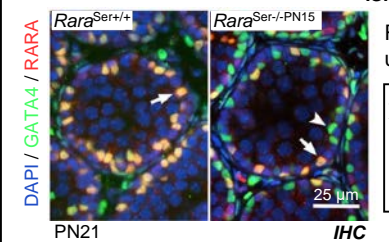


Rara^{Δ/Germ-} males were fertile without spermatogenesis defect.

II Generation of mutant mice lacking all Rara selectively in SC. II.1 Evidence for ablation of Rara gene in postnatal SC.



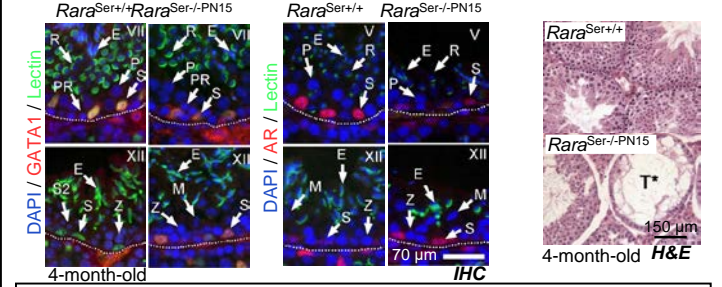
Rara mRNA could not be detected in testis from *Rara^{Ser/-PN15}* mutants.



RARA, detected by IHC in SC using our polyclonal antibodies⁶.

Ablation of the *Rara* alleles occurred in about 65% of the SC from the *Rara^{Ser/-PN15}* mutants.

II.2 Spermatogenesis is affected in males lacking Rara in SC.



Rara^{Ser/-PN15} males were infertile. Sertoli cells loose their cyclical changes in gene expression and testis degenerate.

Conclusion

RARA exerts its functions in Sertoli cells at puberty, but not at fetal stages. RARA plays no crucial role in germ cells.

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