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

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Diana Condrea, Sirine Souali-Crespo, Betty Féret, Muriel Klopfenstein, Sylvain Faisan, et al.. Retinoic Acid Receptor Alpha Is Essential in Postnatal Sertoli Cells but Not in Germ Cells. 22nd European Testis Workshop, Jun 2023, Montreux (Switzerland), Switzerland. hal-04254836

HAL Id: hal-04254836

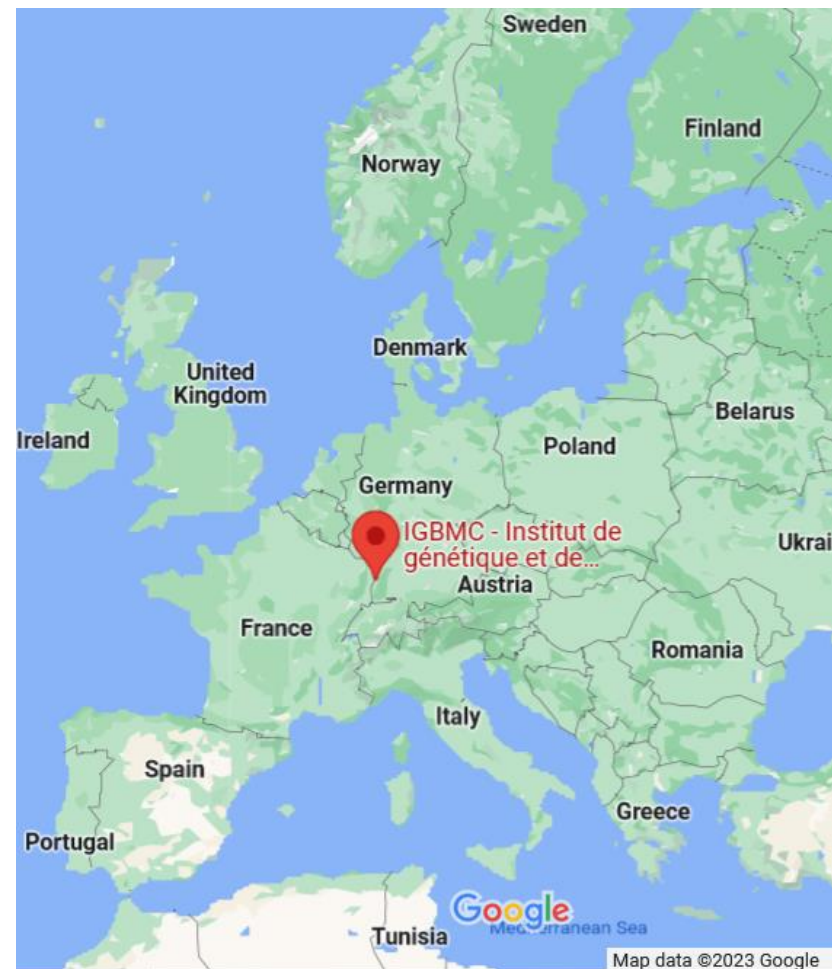
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Submitted on 23 Oct 2023

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ETW 22
June 2023



Institute of Genetics and Molecular and Cellular Biology

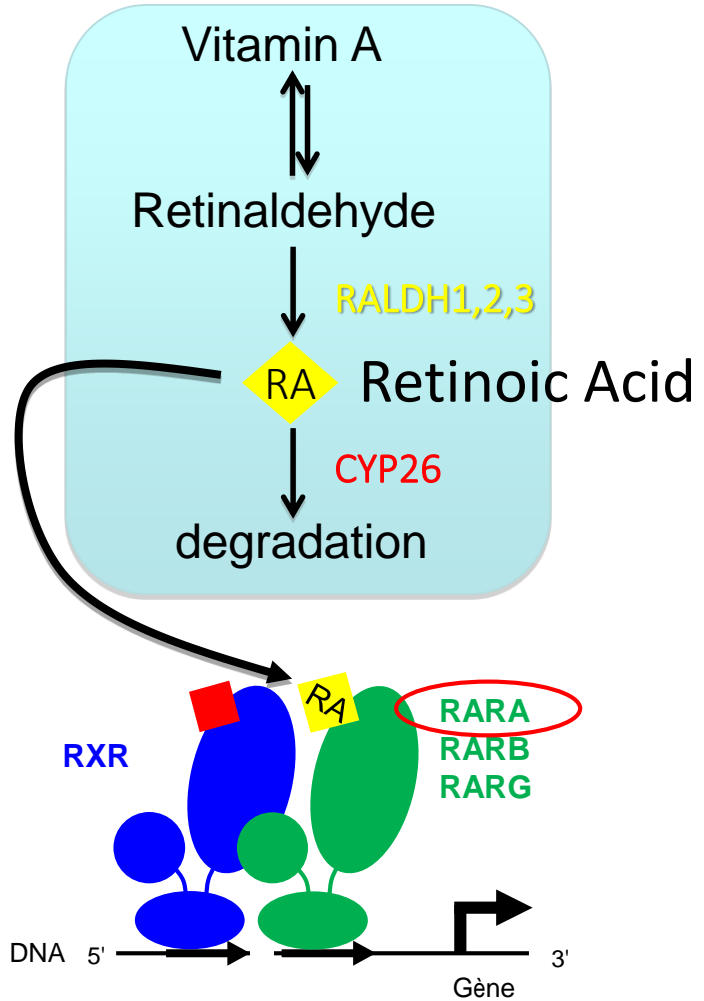
igbmc , Strasbourg, FRANCE
institut de génétique et de
biologie moléculaire et cellulaire

**Retinoic acid receptor alpha is essential in postnatal Sertoli cells,
but not in germ cells**

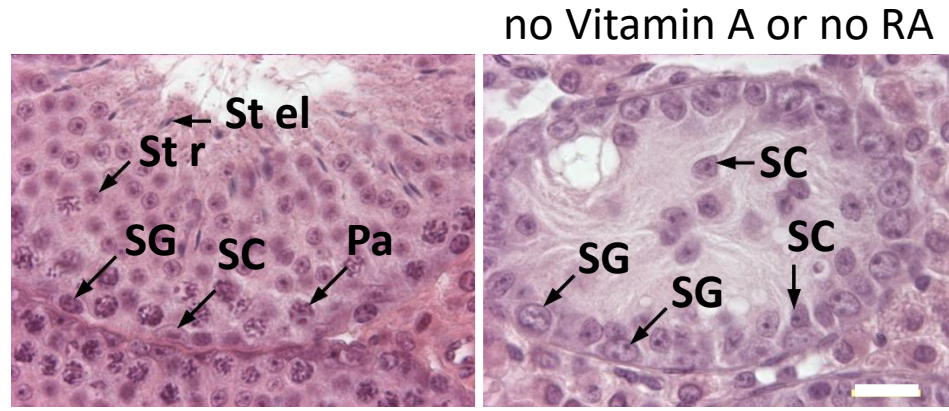


Retinoic Acid signalling pathway

ETW 22
June 2023



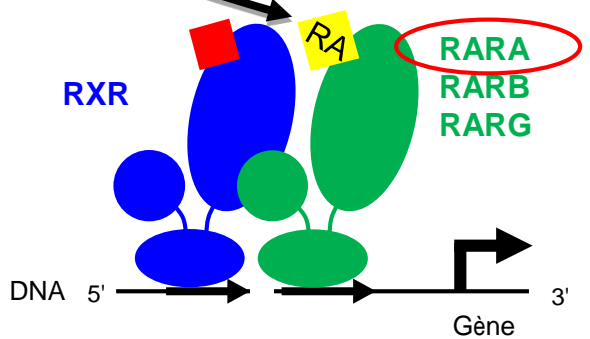
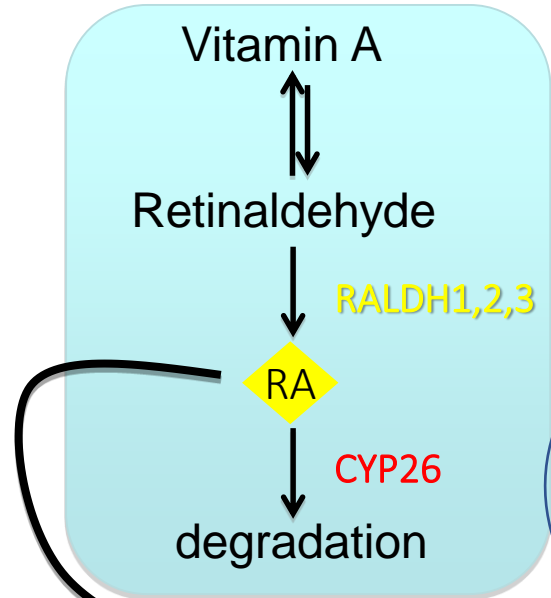
Dietary vitamin A deficiency
Inhibiting RA synthesis
lead to testis degeneration



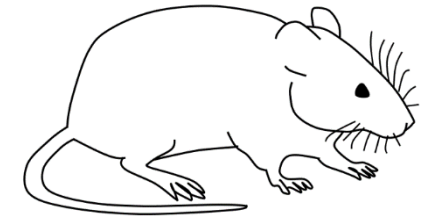
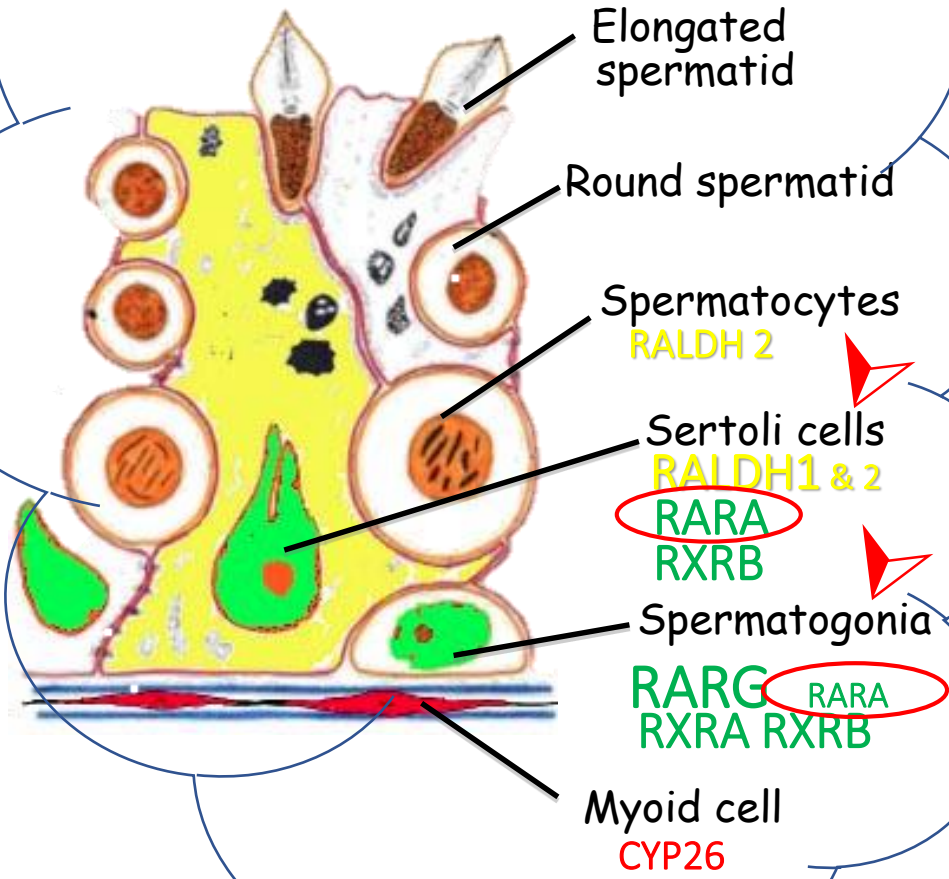
Inactivation of *Rara* gene
also leads to testis degeneration



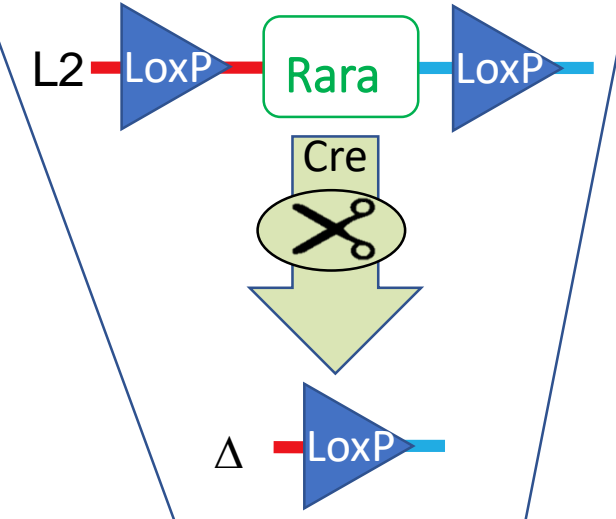
Retinoic Acid signalling pathway



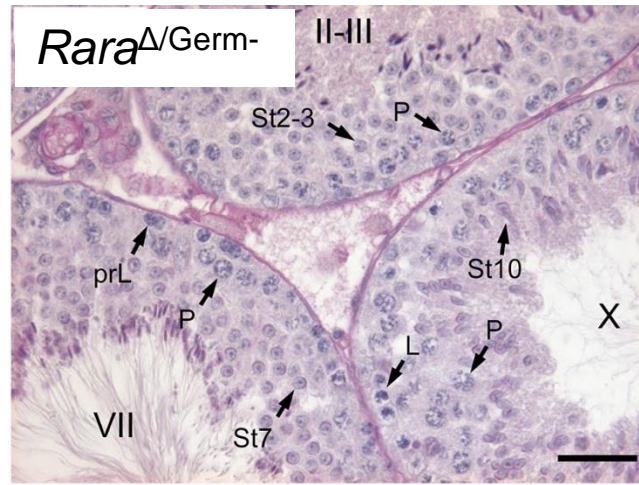
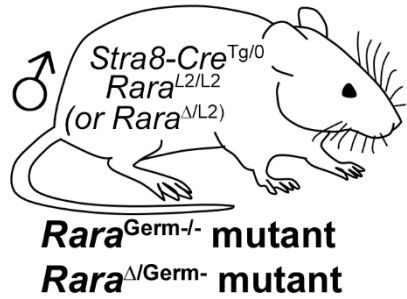
Localisation of retinoic acid signalling pathway members



The methodological approach : somatic mutagenesis

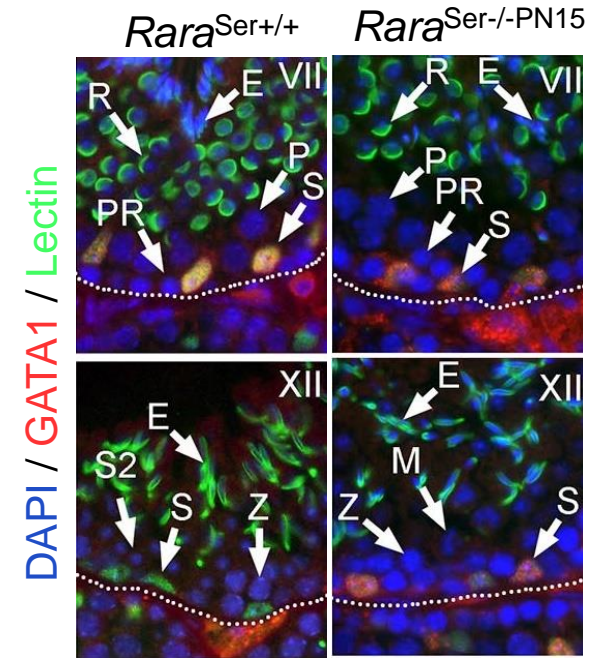
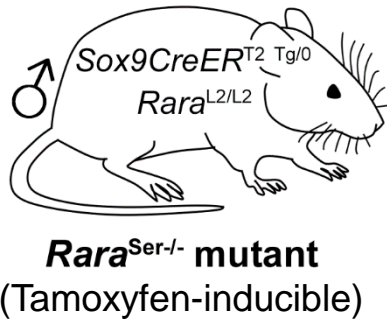


I Mutant mice lacking Rara selectively in germ cells

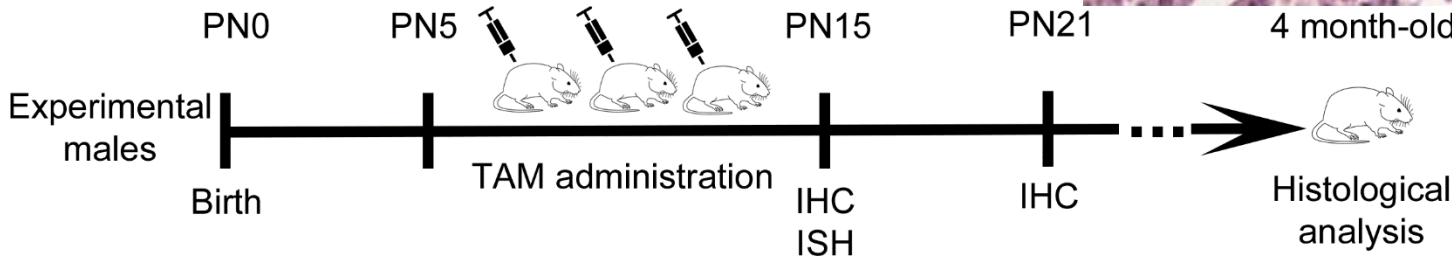


Rara^{Δ/Germ}-</sup> males are fertile without spermatogenesis defect.

II Mutant mice lacking Rara selectively in Sertoli cells



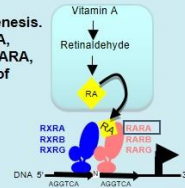
Rara^{Δ/Germ}-</sup> males are infertile
Sertoli cells lose their cyclical changes in gene expression and testis degenerate.





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.



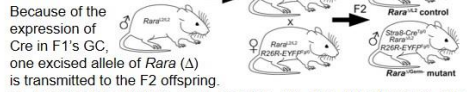
Objectives:

- > 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.

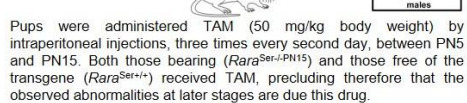
To generate *Rara^{ΔGerm}* mutants, in which the RARA gene was excised only in GC, mice carrying *loxP*-flanked alleles of the *Rara* gene was crossed with mice bearing the *Stra8-Cre* transgene³, which is active only in germ cells from postnatal day 3 onward.



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

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 Harris 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.

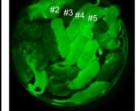
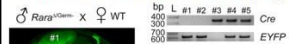
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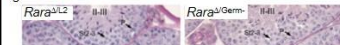
Results

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.



EYFP positive pups observed by ultraviolet light illumination

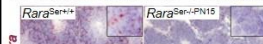


9-month-old PAS & Harris

> *Rara^{ΔGerm}* 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.



PN15 ISH

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



PN21 IHC

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.



4-month-old IHC



4-month-old H&E

> *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.

Data published in: Condrea D, Souali-Crespo S, Féret B, Klopfenstein M, Faisan S, Mark M, Ghyselinck NB, Vernet N. *Cells*. 2022; 11(5):891.
Funded by grants: ANR-17-CE14-0010-01 and ANR-20-CE14-0022.
Supported in part by the grants: ANR-10-LABX-0030-INRT and ANR-10-IDEX-0002-02.

Other poster from the lab
P82 by Giulia Perrotta



Nadège Vernet

