



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

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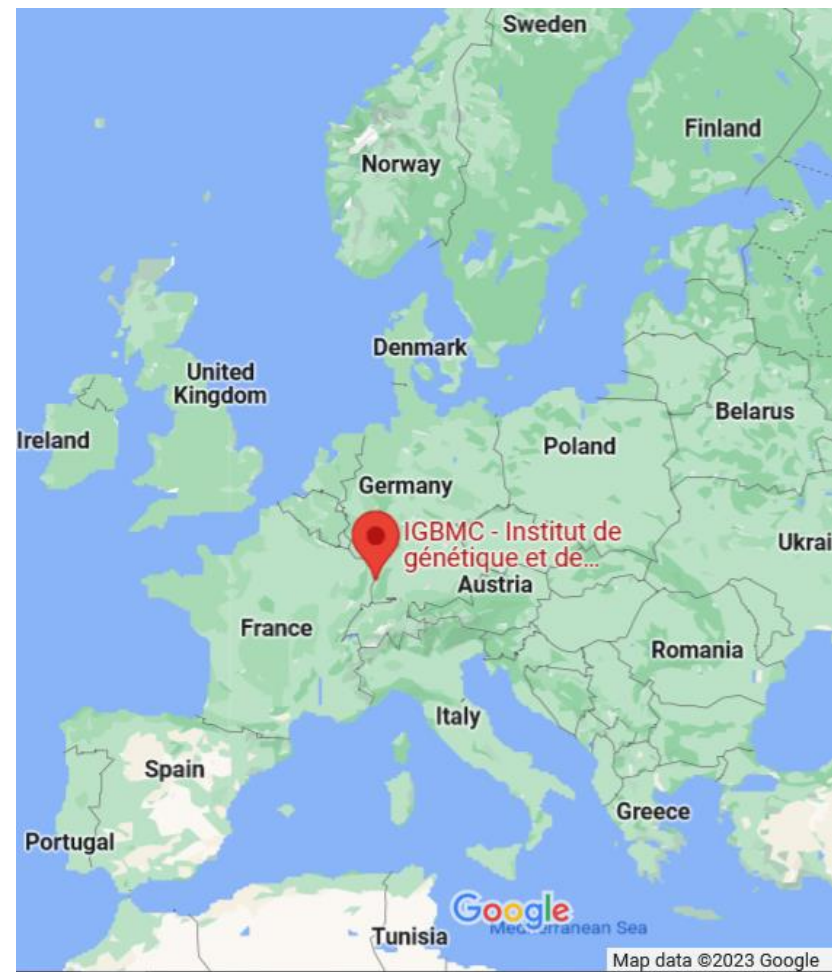
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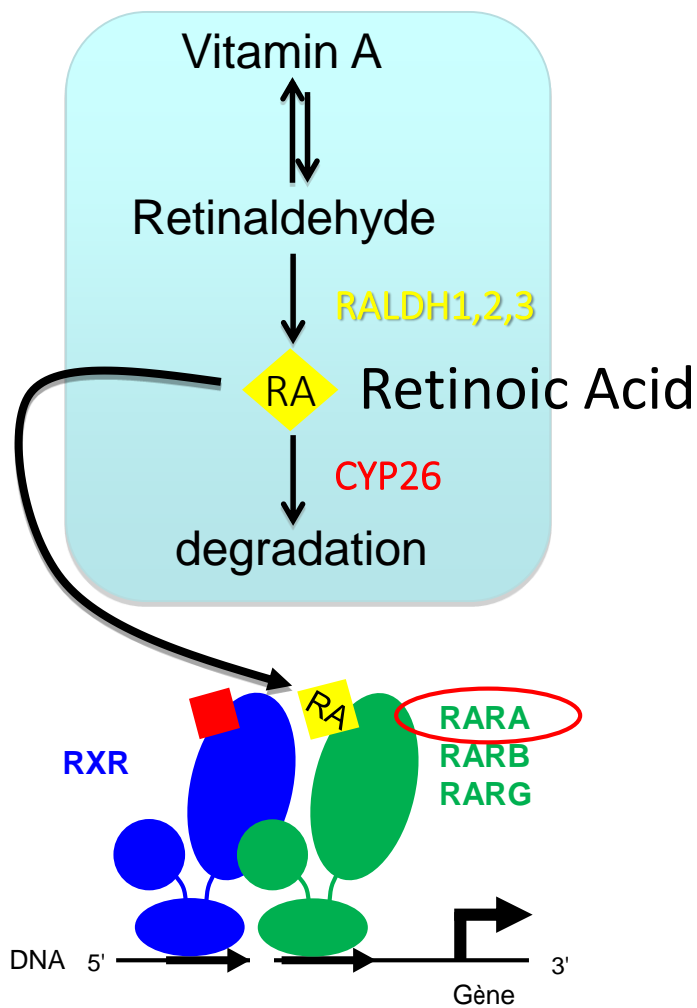
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biologie moléculaire et cellulaire

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

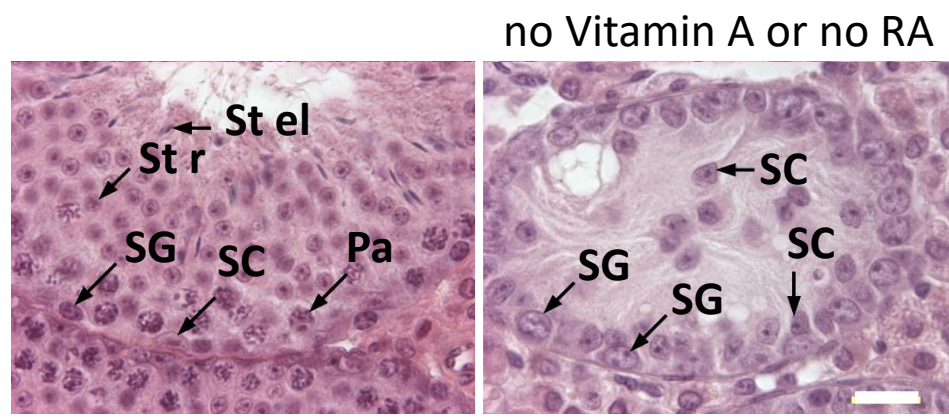


# Retinoic Acid signalling pathway



Dietary vitamin A deficiency  
Inhibiting RA synthesis

lead to testis degeneration

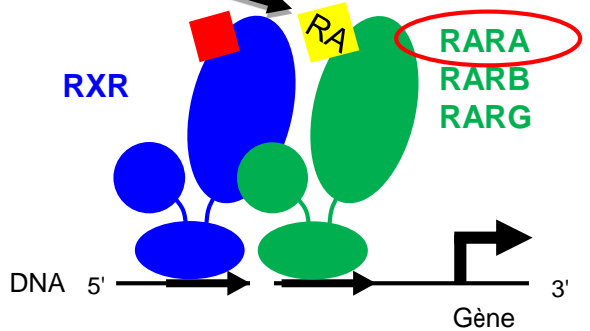
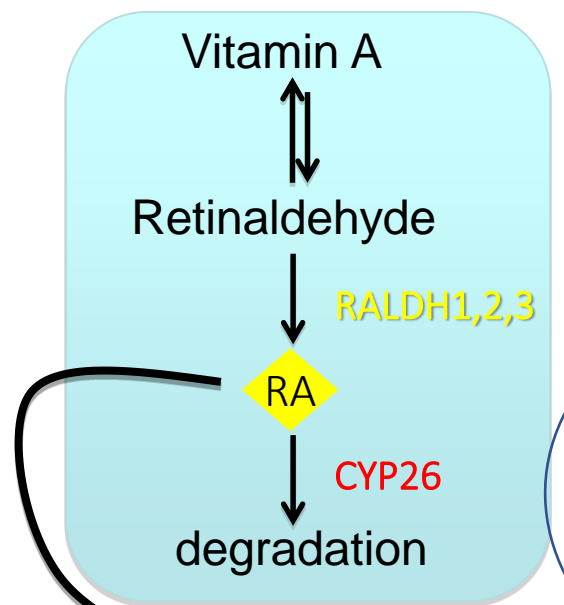


Inactivation of *Rara* gene  
also leads to testis degeneration

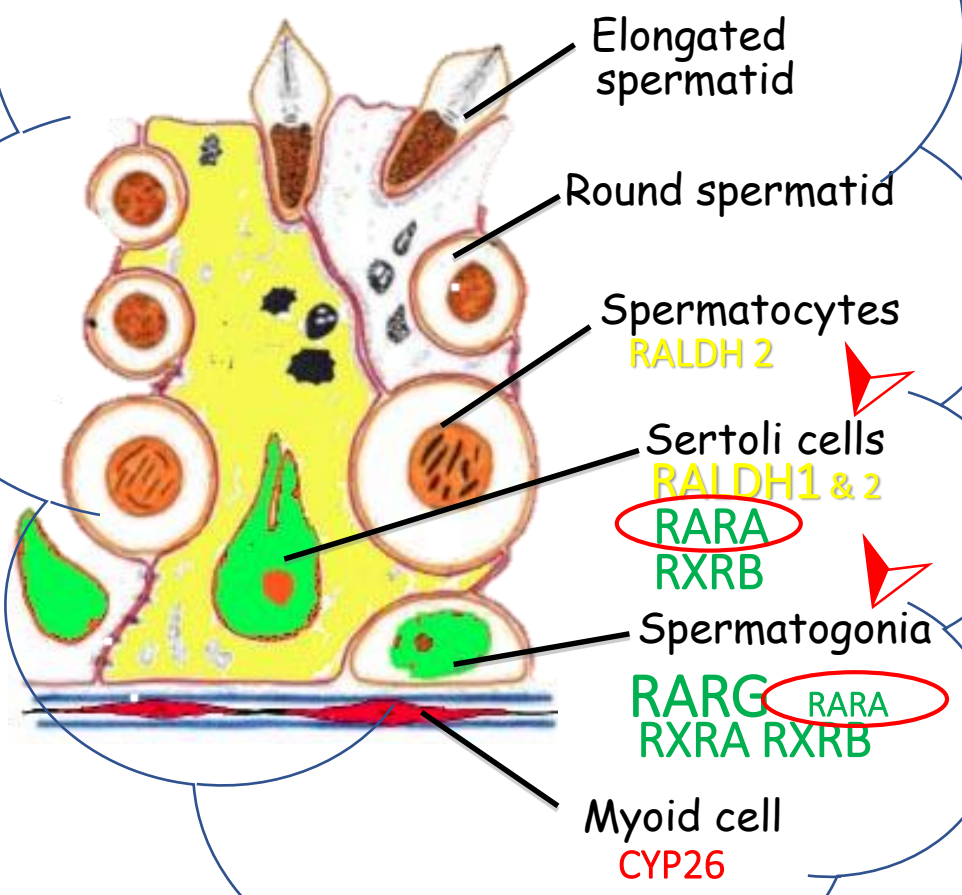


# Retinoic Acid signalling pathway

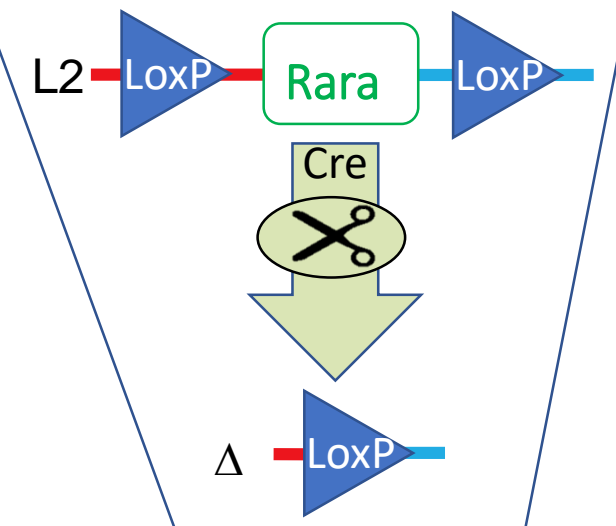
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June 2023



## Localisation of retinoic acid signalling pathway members

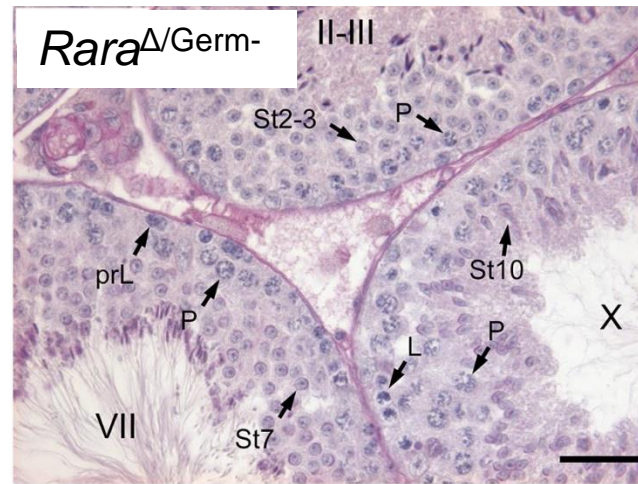
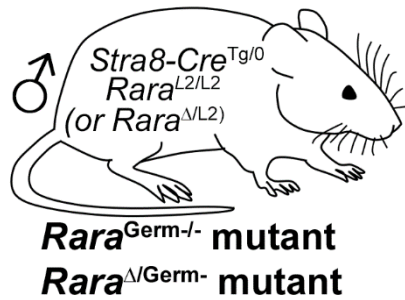


## The methodological approach : somatic mutagenesis



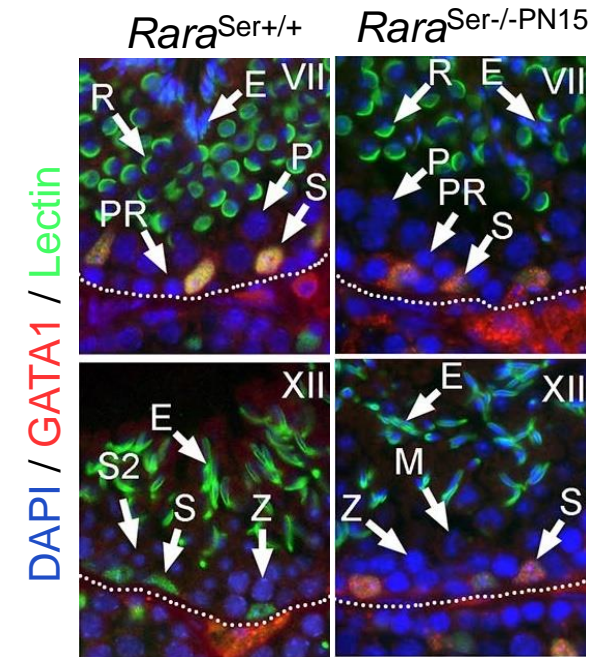
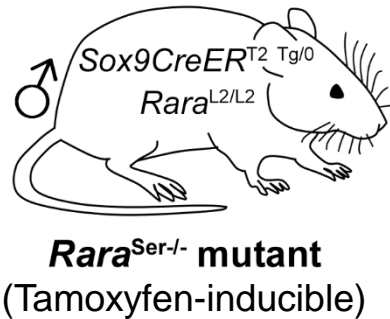


## I Mutant mice lacking *Rara* selectively in germ cells

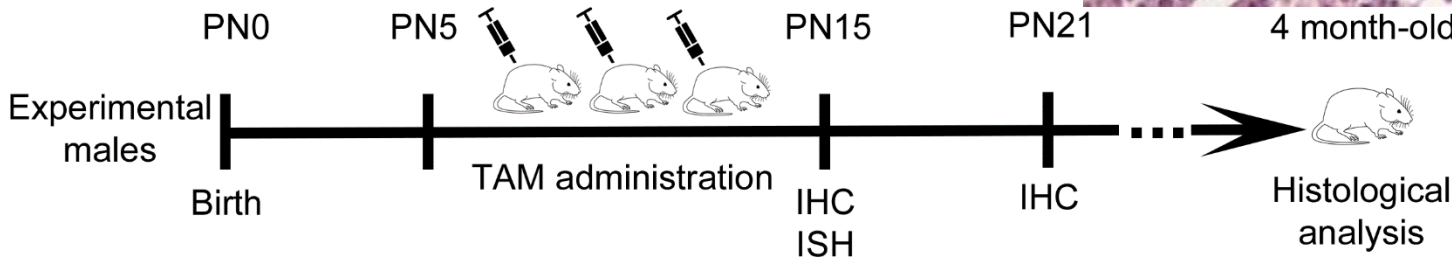


*Rara<sup>Δ/Germ-</sup>* males are fertile without spermatogenesis defect.

## II Mutant mice lacking *Rara* selectively in Sertoli cells



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



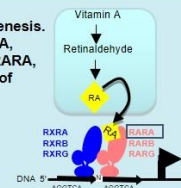


**Introduction/background**

- 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 cells<sup>1</sup>, but a recent paper suggested that RARA may also play a role in germ cells<sup>2</sup>.

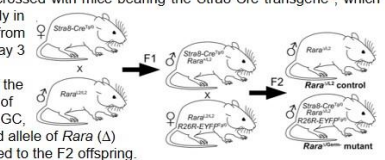
**Objectives:**

- 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**

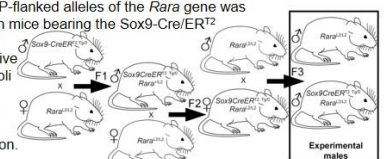
**I Generation of mutant mice lacking all *Rara* selectively in GC.**  
To generate *Rara*<sup>ΔGerm</sup> mutants, in which the RARA gene was excised only in GC, mice carrying loxP-flanked alleles of the *Rara* gene were crossed with mice bearing the Stra8-Cre transgene<sup>3</sup>, which is active only in germ cells from postnatal day 3 onward.



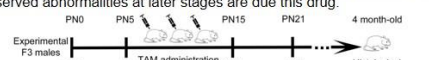
Because of the expression of Cre in F1's GC, one excised allele of *Rara* (Δ) is transmitted to the F2 offspring.

To monitor for efficient gene excision, we also introduced in the breedings the *R26R-EYFP*<sup>4</sup> 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*<sup>Ser-ΔPN15</sup> 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 were crossed with mice bearing the Sox9-Cre/ER<sup>T2</sup> transgene<sup>5</sup>, which is active only in Sertoli cells upon Tamoxifen (TAM) administration.

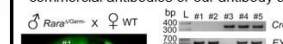


Pups were administered TAM (50 mg/kg body weight) by intraperitoneal injections, three times every second day, between PN5 and PN15. Both those bearing (*Rara*<sup>Ser-ΔPN15</sup>) and those free of the transgene (*Rara*<sup>Ser+/+</sup>) received TAM, precluding therefore that the observed abnormalities at later stages are due this drug.



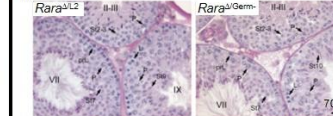
**Results**

**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.



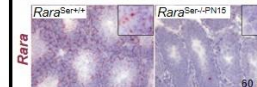
- A mendelian proportion of pups sired by *Rara*<sup>ΔGerm</sup> males were EYFP-positive.
- 94% pups born from these breeding were *Rara*<sup>+/+</sup> and 6% were *Rara*<sup>Δ/Δ</sup>.

➤ Even though we could not evidence RARA loss by IHC, we concluded that *Rara*<sup>ΔGerm</sup> were actually deprived of RARA in 94% of their germ cells.



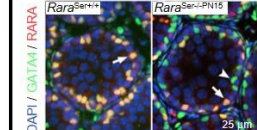
➤ *Rara*<sup>ΔGerm</sup> males were fertile without spermatogenesis defect.

**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*<sup>Ser-ΔPN15</sup> mutants.

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



RARA, detected by IHC in SC using our polyclonal antibodies<sup>6</sup>.

➤ Ablation of the *Rara* alleles occurred in about 65% of the SC from the *Rara*<sup>Ser-ΔPN15</sup> mutants.

**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.

**References**

- Vernet N, Dennefeld C, Rochette-Egly C, Oulad-Abdelghani M, Chambon P, Ghyselinck NB, Mark M. Endocrinology. 2006; 147(1):95-110.
- Peer NR, Law SM, Murdoch B, Goulding EH, Eddy EM, Kim K. Endocrinology. 2018; 159:3409-3420.
- Sadate-Ngachou PI, Payne CJ, Dearth AT, Braun RE. Genesis. 2008; 46(12):738-42.
- Srinivas S, Watanabe T, Lin CS, Williams CM, Tanabe Y, Jessell TM, Costantini F. BMC Dev Biol. 2001; 1:4.
- Kopp JL, Dubois CL, Schaffer AE, Hao E, Shih H, Seymour PA, Ma J, Sander M. Dev. 2011; 138:653-665.
- Gaub MP, Rochette-Egly C, Lutz Y, Ali S, Matthes H, Scheuer I, Chambon P. Exp Cell Res. 1992; 201:335-346.

**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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Other poster from the lab  
**P82** by Giulia Perrotta

