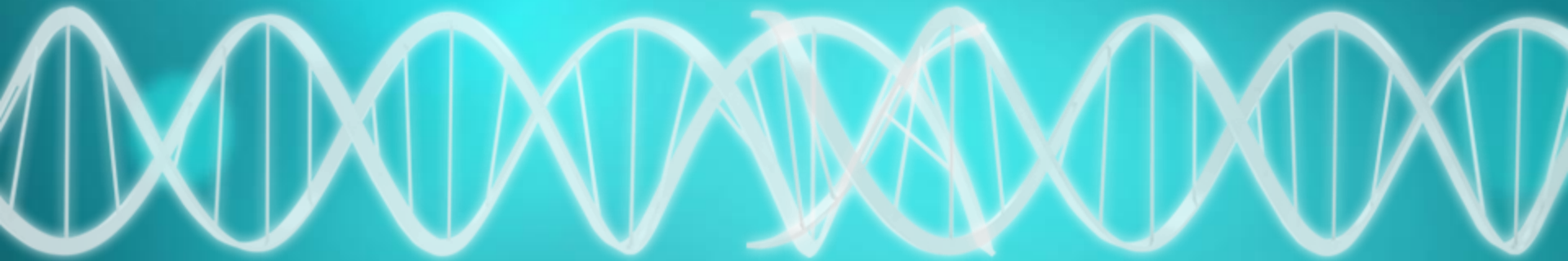


Rasd1 is essential for maturation of mouse oocyte



Youngeun Lee B.A.

Department of Biomedical Science
Cha University



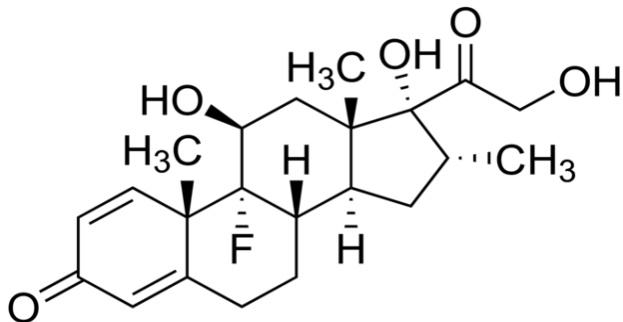
차의과학대학교

Introduction

RASD1

(Dexamethasone-induced Ras-related protein 1)

- encodes a member of the Ras superfamily of small GTPases and is induced by dexamethasone.
- may play a role in dexamethasone-induced alterations in cell morphology, growth and cell-extracellular matrix interactions.
- extremely important in numerous signaling pathways and physiological processes.

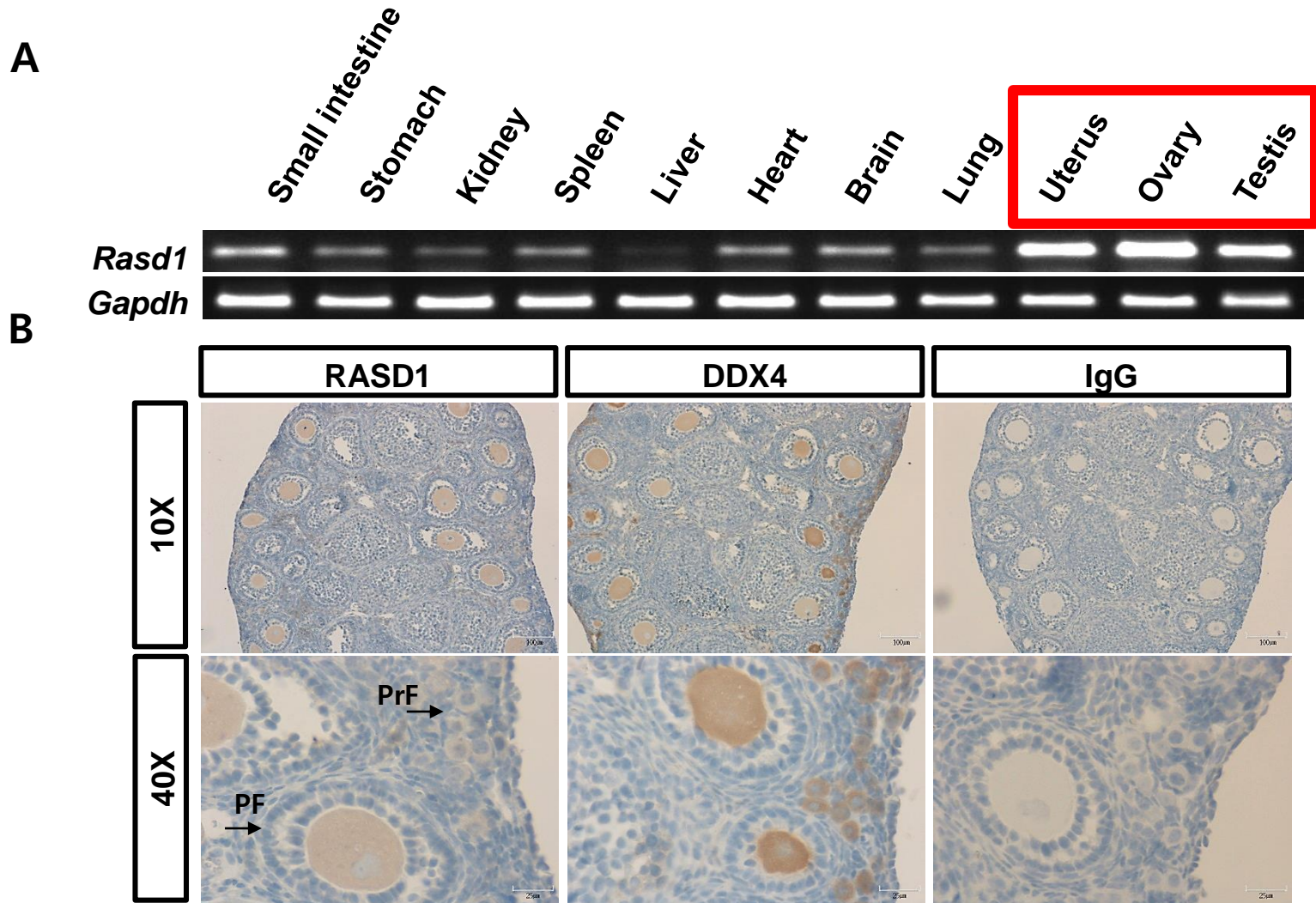


Dexamethasone

An **anti-inflammatory glucocorticoid** with a range of effects on cell survival, cell signaling and gene expression.

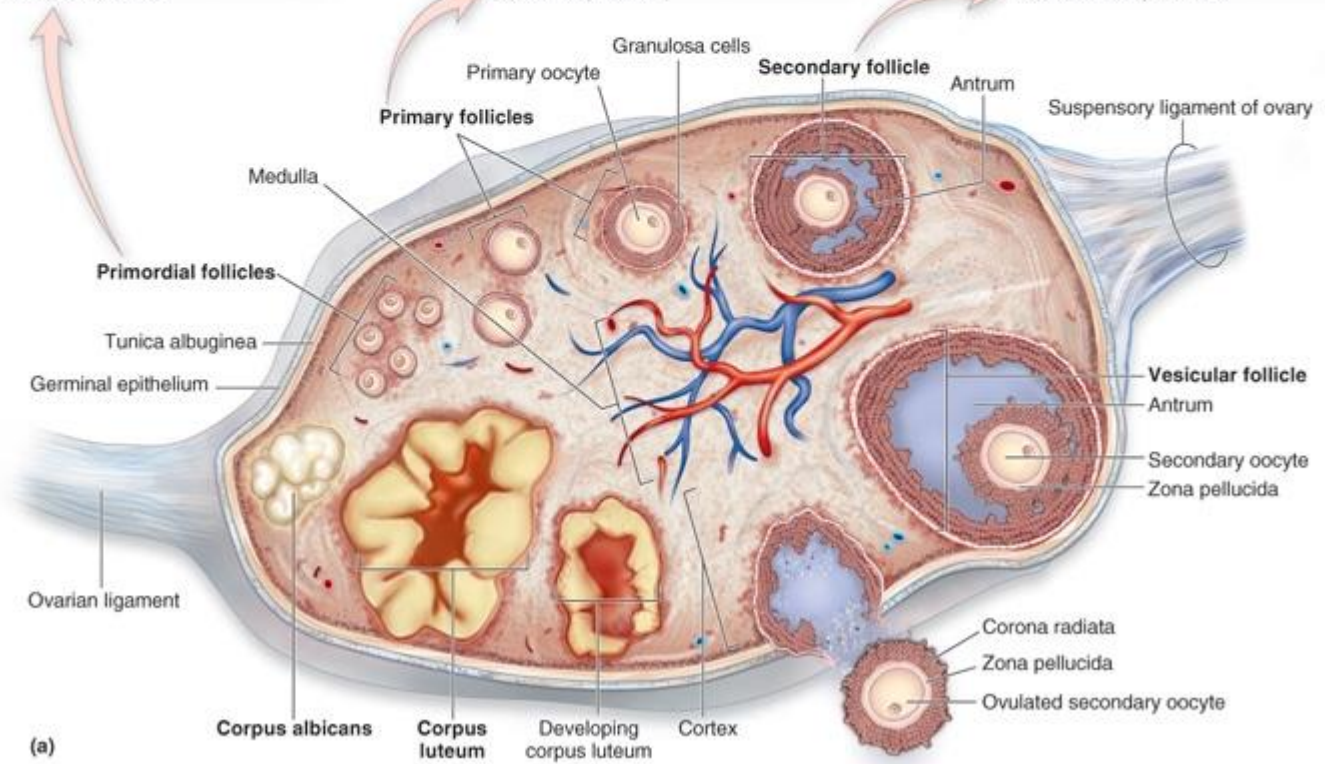
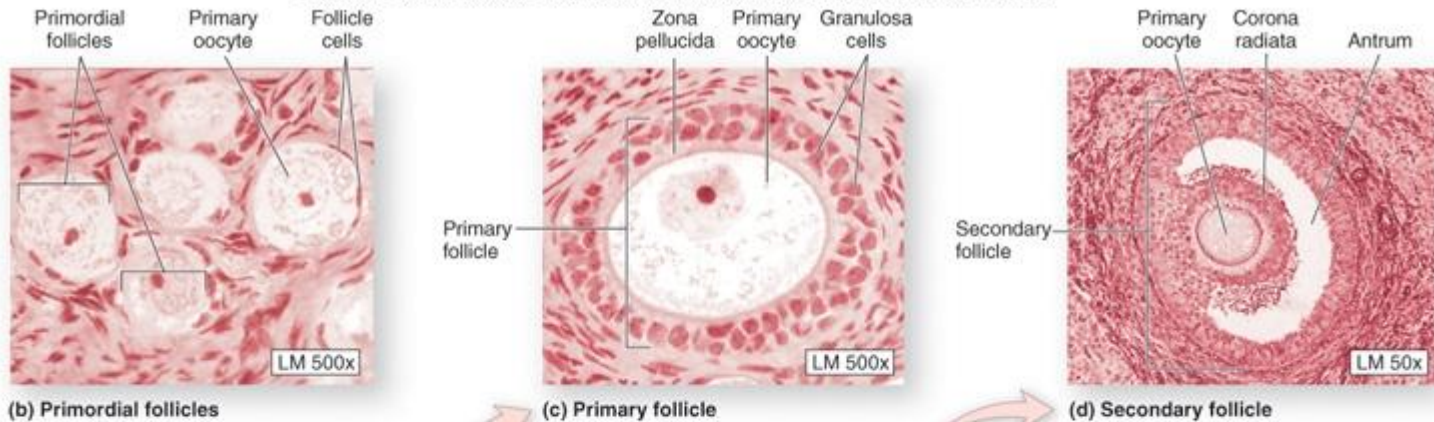
Use to study apoptosis, cell signaling pathways and gene expression.

Introduction

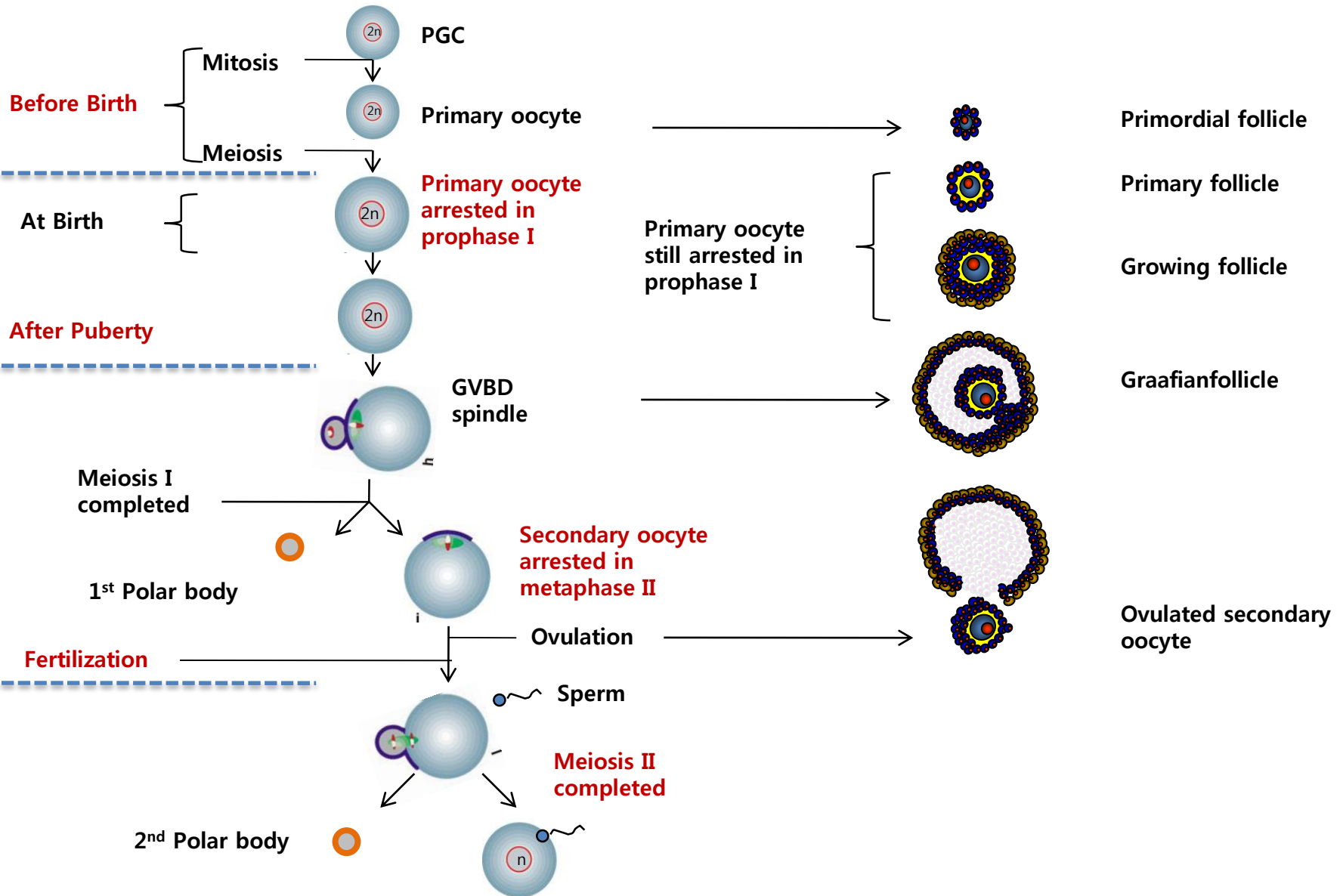


Introduction

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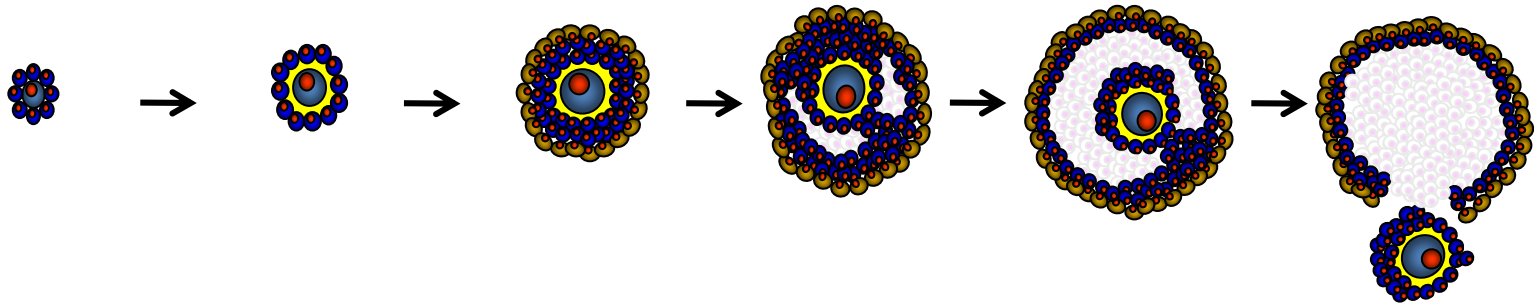


Introduction

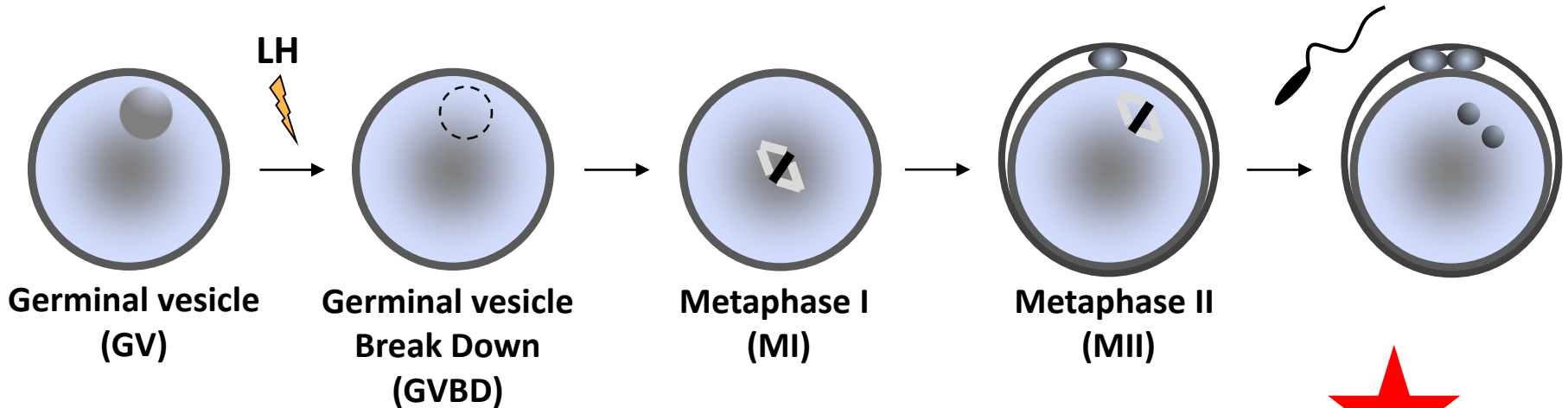


Introduction

Primordial Follicle Primary follicle Secondary follicle Antral follicle Preovulatory follicle Ovulation



Oocyte growth during follicle development



Oocyte maturation



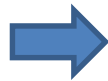
Objective

- **To examine Rasd1 expression pattern during mouse oocyte maturation.**
- **To analyze the phenotype of Rasd1 knockdown in mouse oocytes.**
- **To identify the regulatory mechanism of Rasd1 in mouse oocyte maturation.**

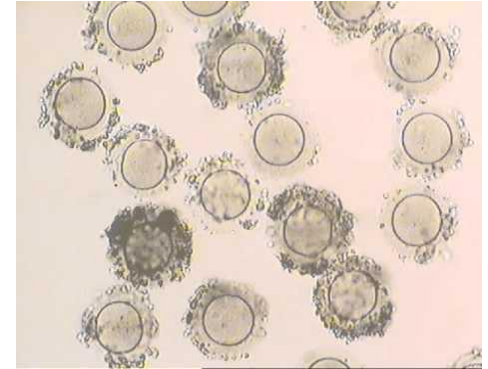
Materials & Methods



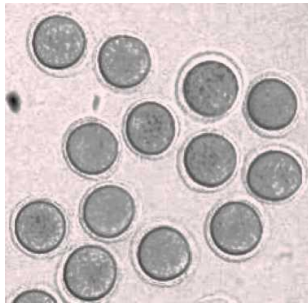
**PMSG injection
(5IU, 100 μ l)**



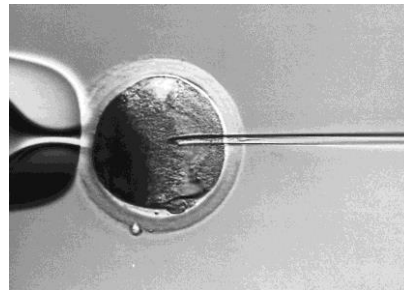
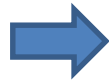
**After 2 days,
Mouse ovary 적출**



**Ovary에서 oocyte
collection**



Oocyte denudation



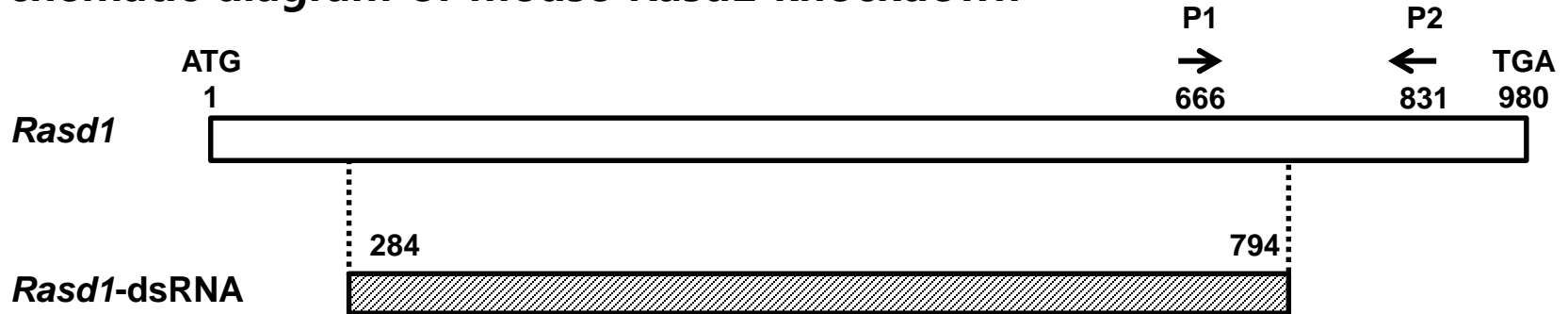
**Oocyte dsRNA
injection**



***In vitro* culture**

Materials & Methods

Schematic diagram of mouse *Rasd1* knockdown



- To make double-strand RNA, a part of *Rasd1* coding region sequence was amplified in ovary cDNA using **PCR and cloned into pGEM-T Easy vector** (Promega, Madison, WI, USA).
- Cloned *Rasd1* was linearized the *SpeI* enzyme and **single-stranded sense and antisense RNA strands were transcribed** in vitro with **T7 polymerase** using MEGAscript kit (Ambion, Austin, TX, USA).
- Each RNA strands **were hybridized/annealed** at 75°C and slowly cooled to 25°C. The dsRNA was digested with DNaseI, RNase and purified and concentrated.

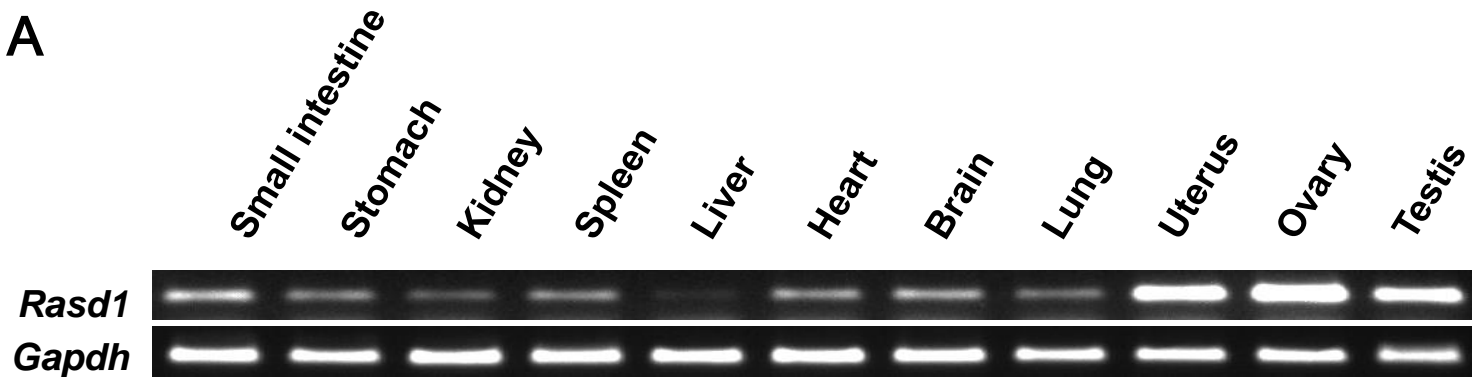
For characterization of *Rasd1*

- **RT-PCR, Quantitative PCR** from *Rasd1* mRNA
- **Immunofluorescence** from *Rasd1* knockdown oocytes

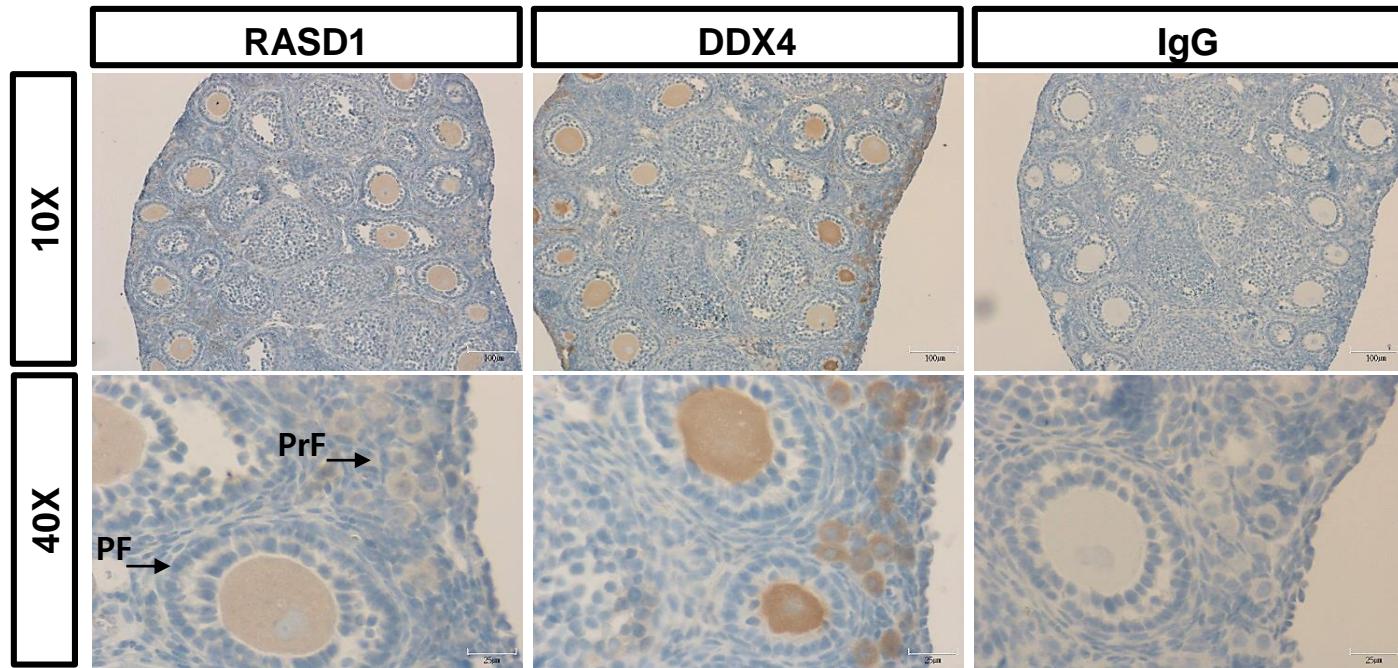
Results

Expression of *Rasd1* mRNA in mouse tissues and localization of Rasd1 in mouse ovary

A

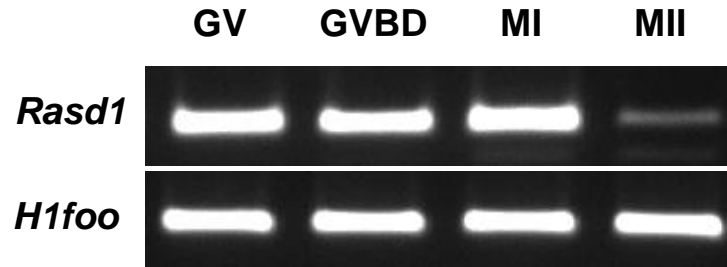


B

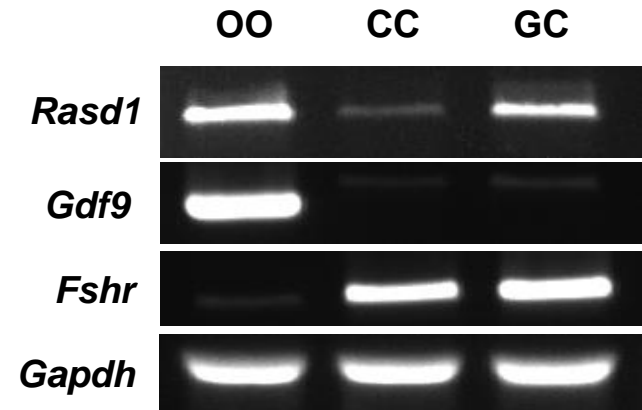


Expression of *Rasd1* mRNA in mouse oocytes and follicular cells

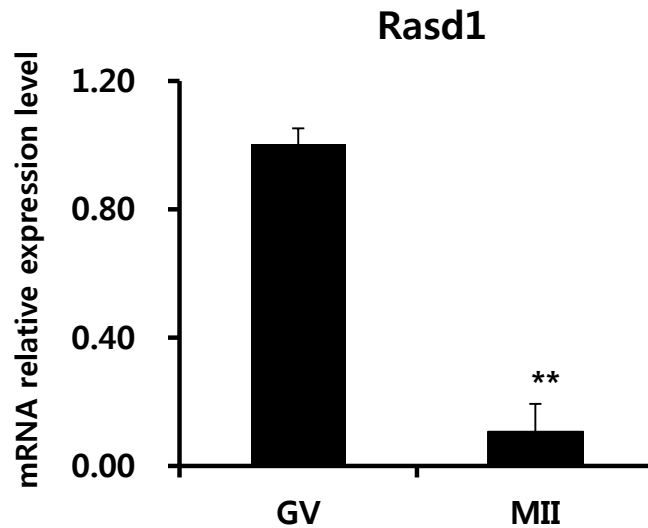
A



B

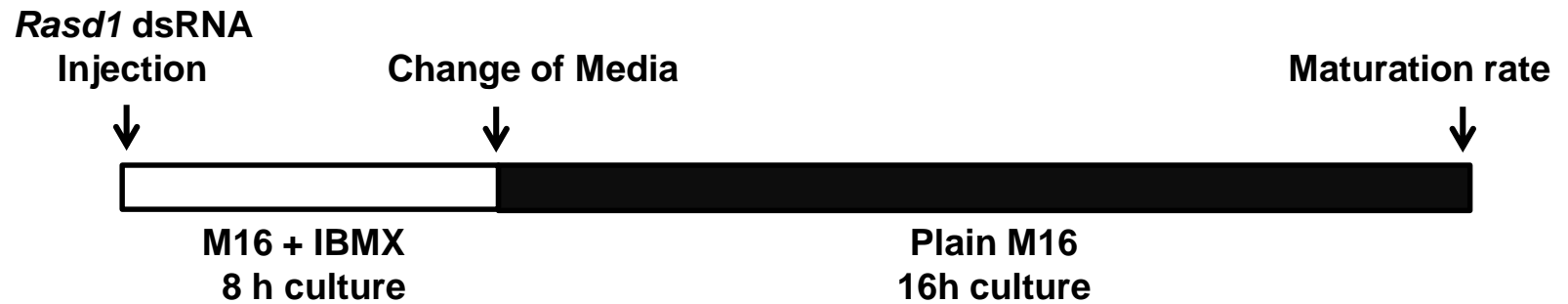


C

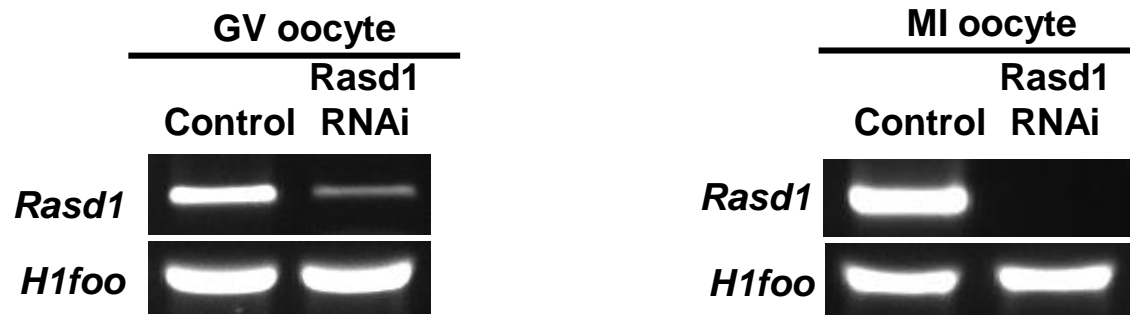


Rasd1 knockdown by RNAi in mouse oocytes

A

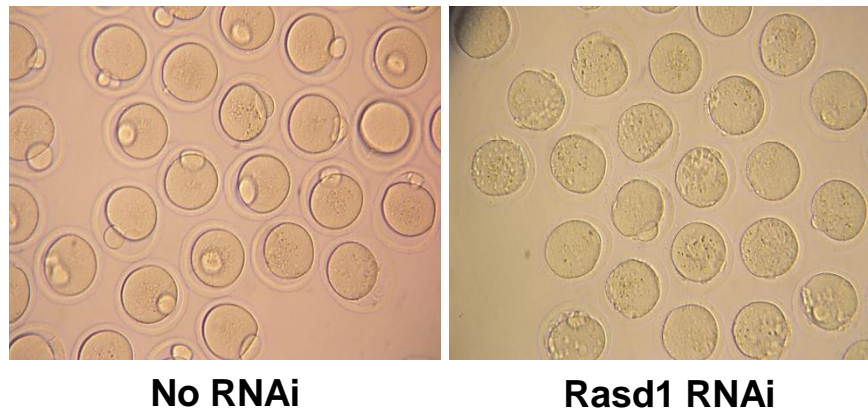


B

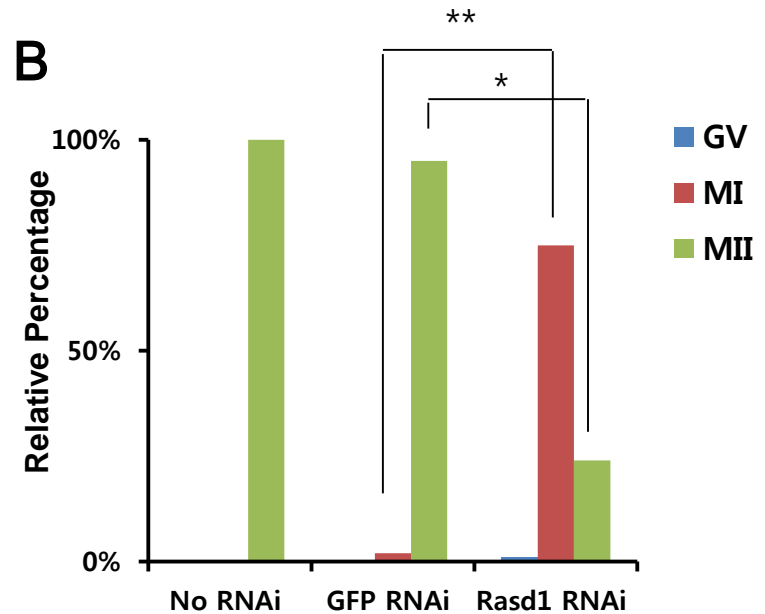


Phenotype of Rasd1 knockdown in mouse oocytes

A



B



C

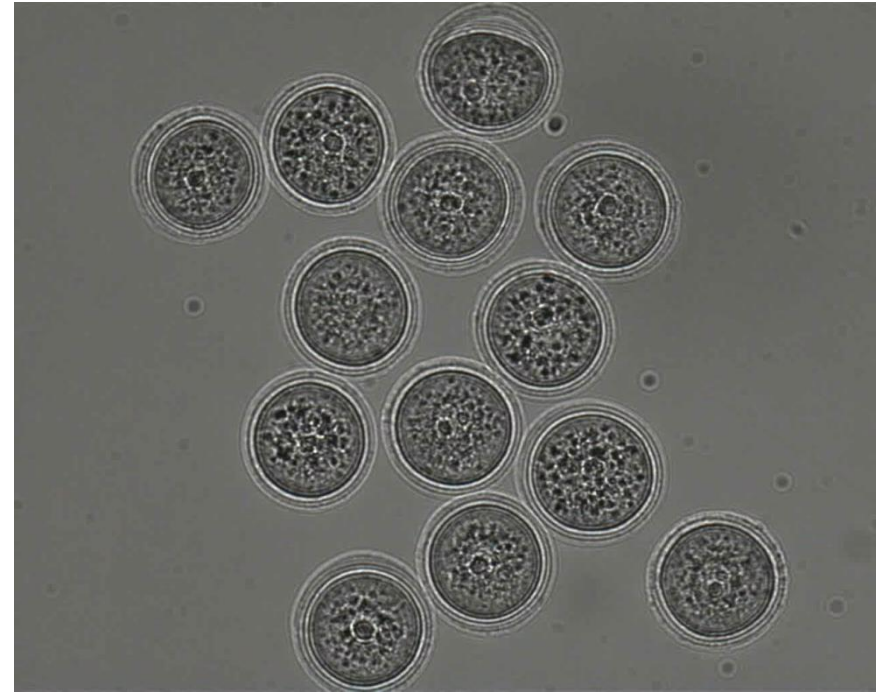
Treatment	No. of oocytes (%)			
	Total	GV	MI	MII
No RNAi	93	0 (0%)	0 (0%)	93 (100%)
GFP RNAi	60	0 (0%)	1 (2%)	57 (95%)
Rasd1 RNAi	216	4 (1%)	161 (75%)	51 (24%)

Phenotype of *Rasd1* knockdown in mouse oocytes

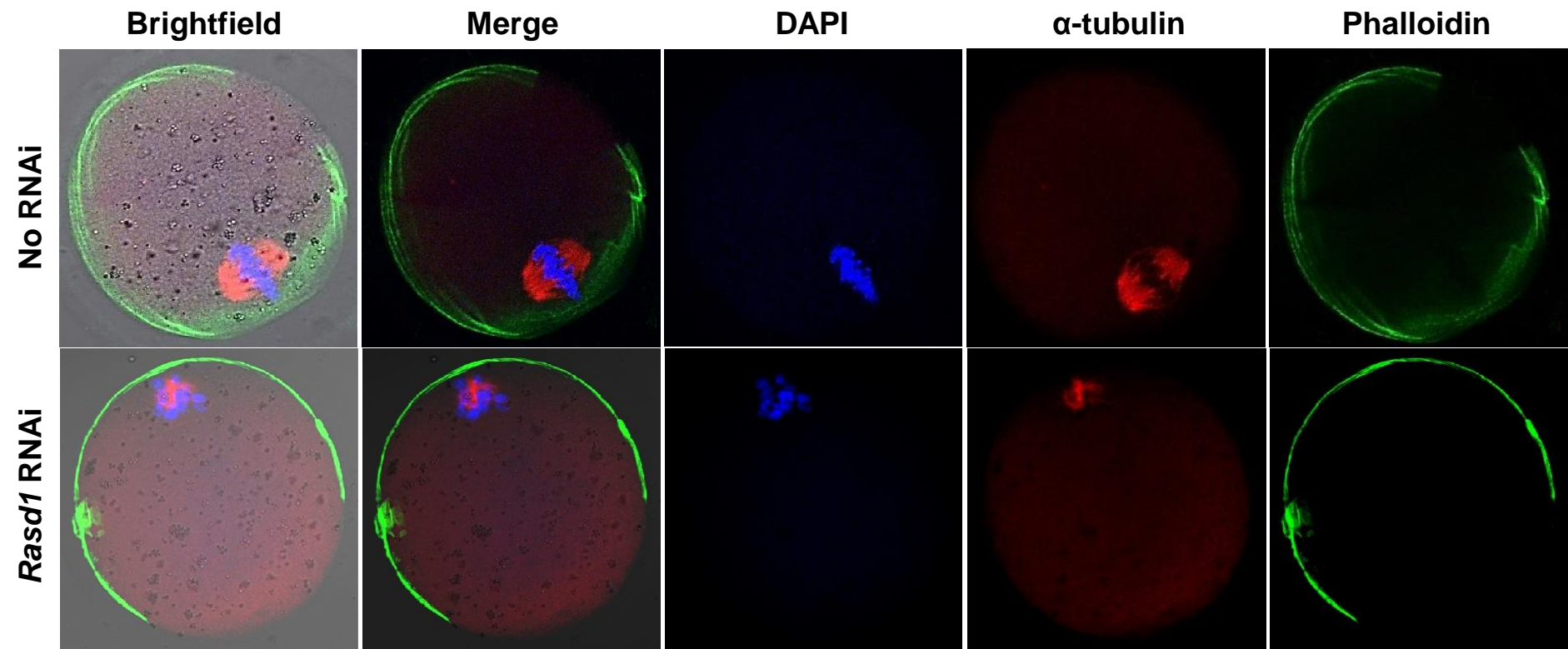
No RNAi(16h)



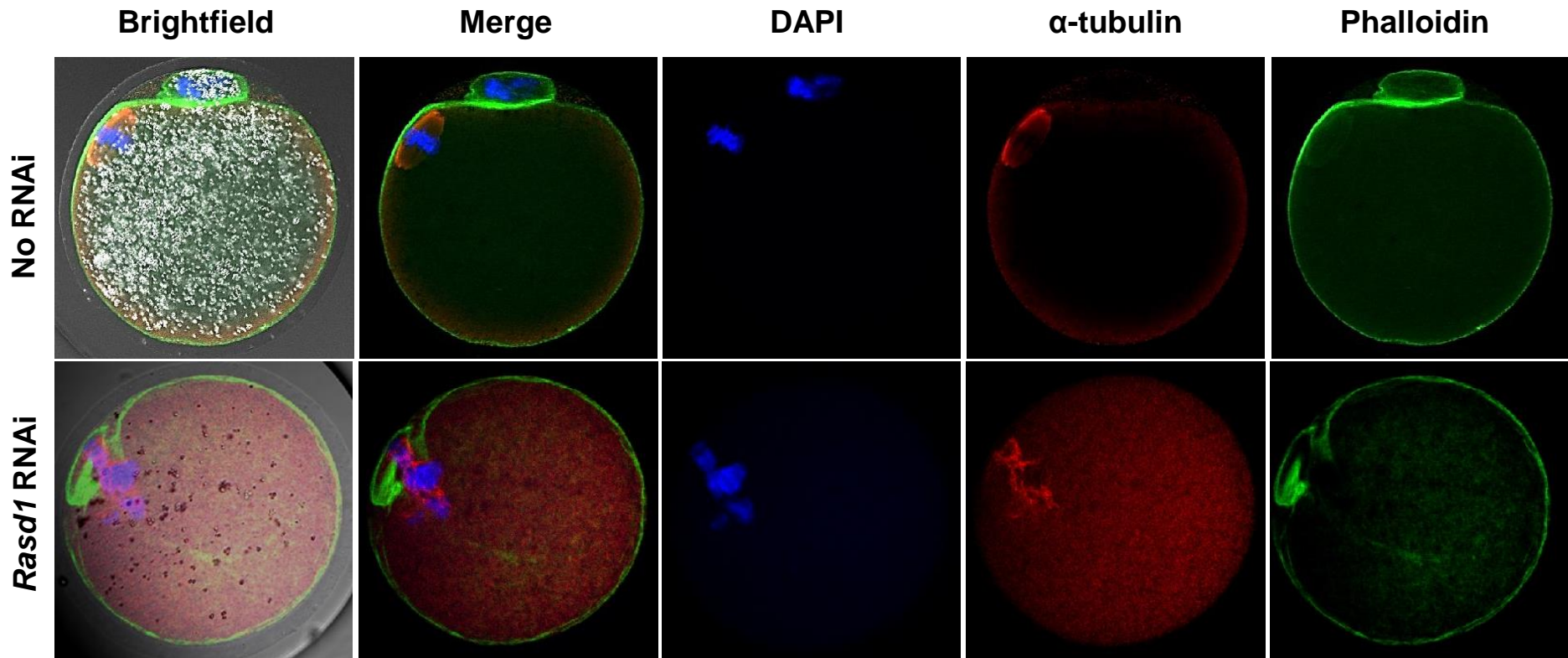
Rasd1 RNAi(16h)



Formation of spindle and chromosomes in MI oocyte



Formation of spindle and chromosomes in MII oocyte

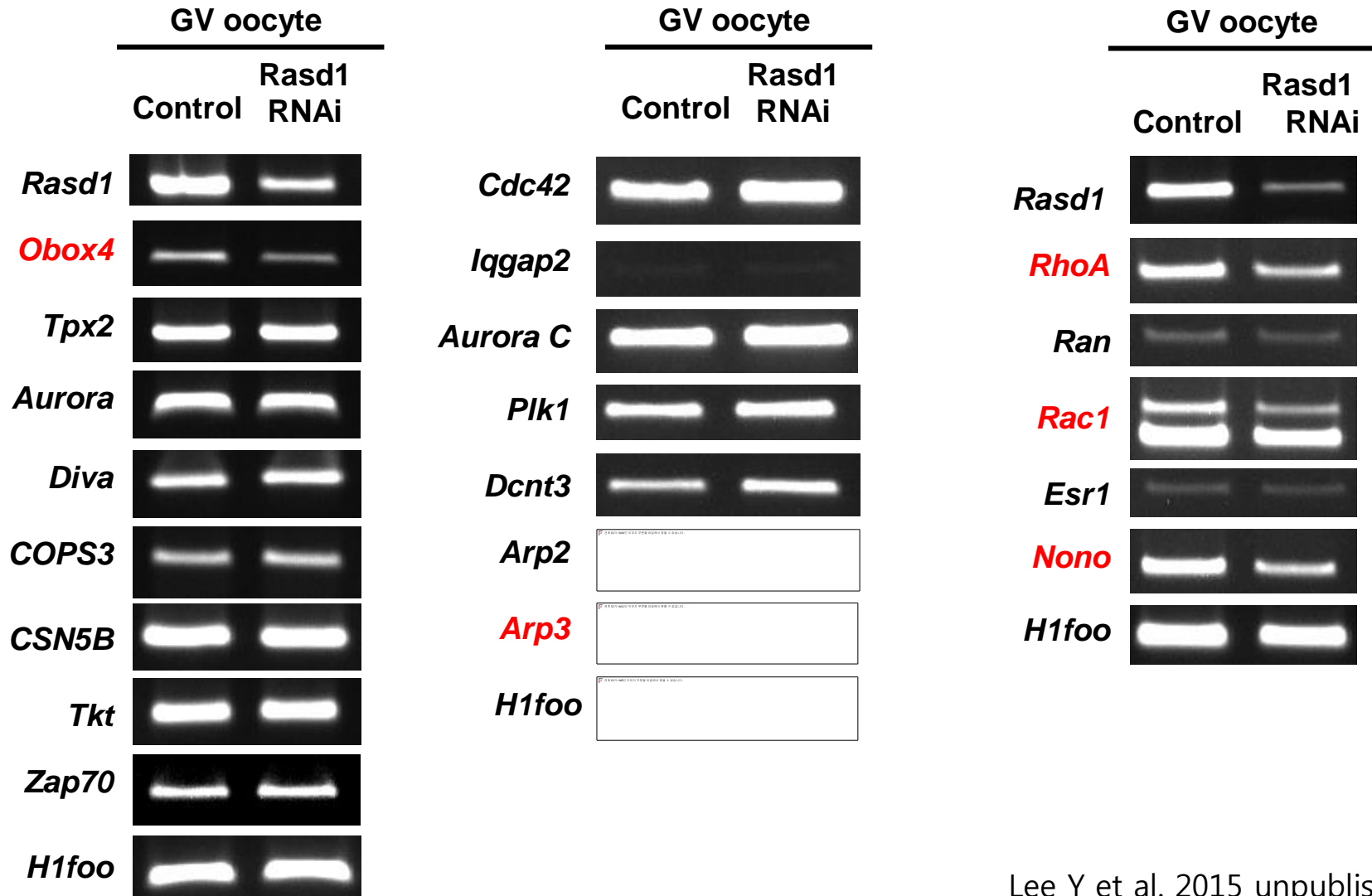


The expression of oocyte maturation related genes by Rasd1 knockdown

MI arrest related factors

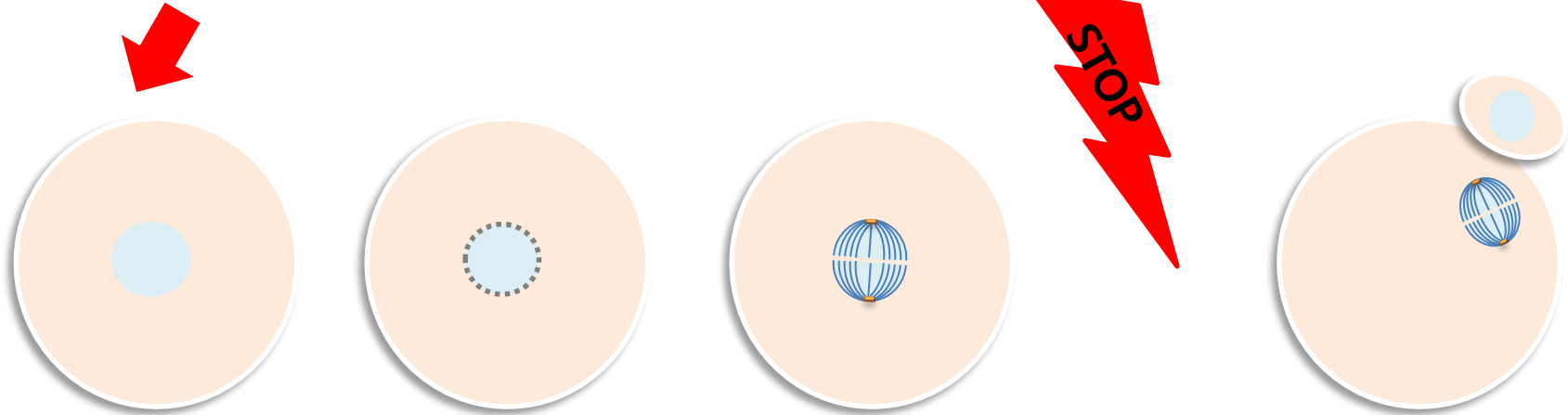
Cytokinesis related factors

Small GTPase related factors



Summary

Rasd1 Knock down

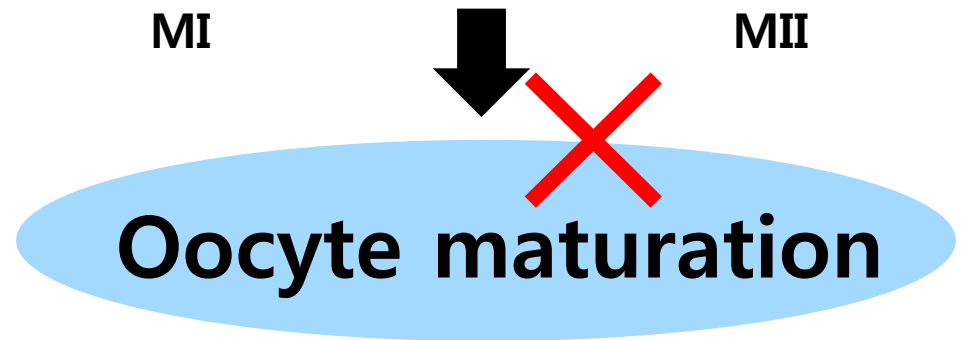


GV

GVBD

MI

MII

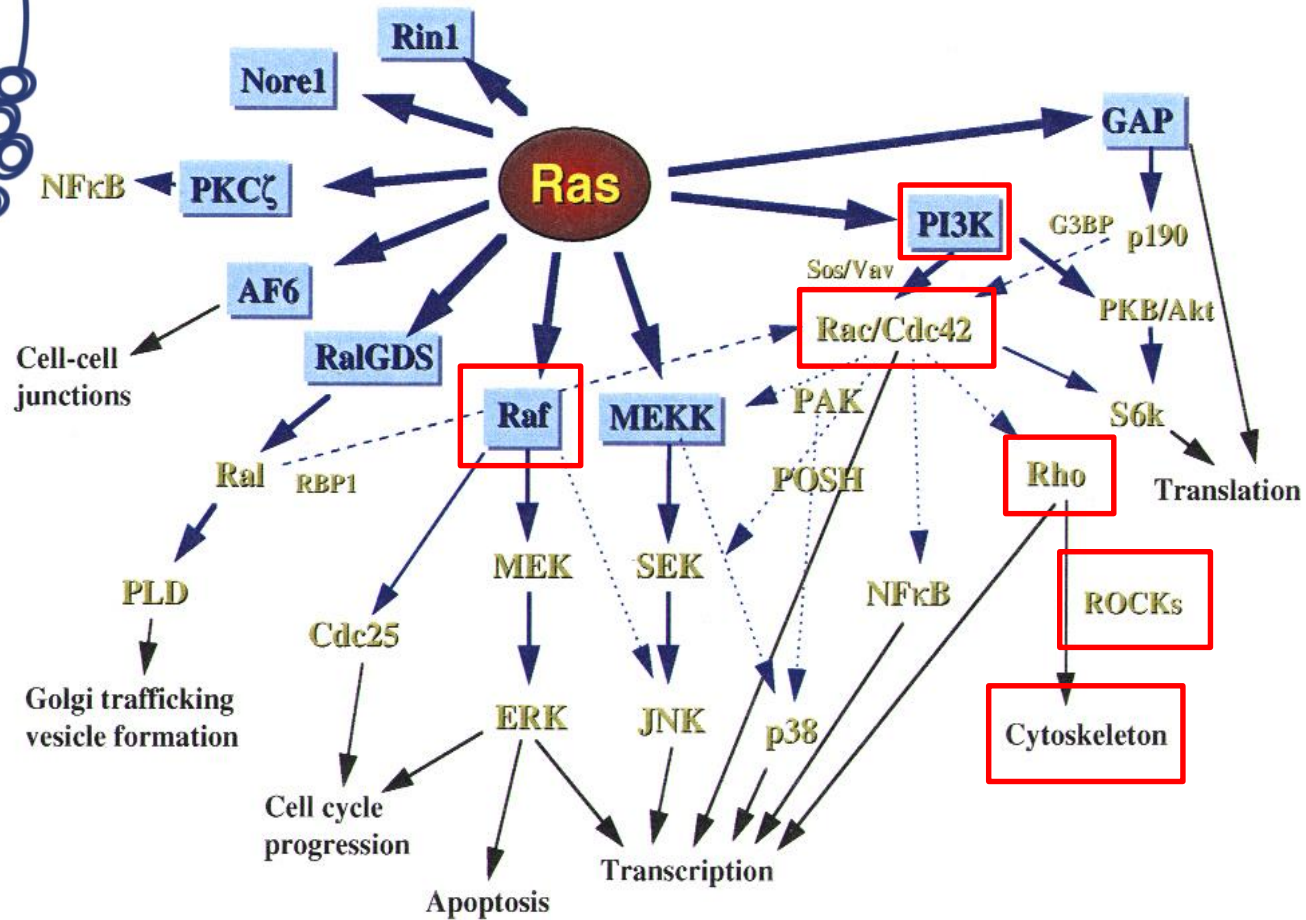
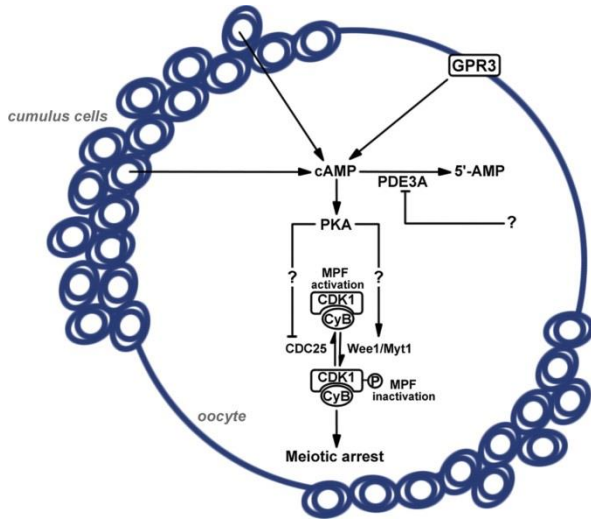


Abnormality of Spindle and Chromosome

Conclusion

- 1. Rasd1 is highly expressed in reproductive organs including mouse ovary.**
- 2. Rasd1 knockdown results in MI arrest during maturation of mouse oocytes.**
- 3. Rasd1 might be essential for spindle formation during oocyte maturation.**
- 4. It needs further study to look into relation between Rasd1 and cytokinesis in MI-MII transition.**

Further study



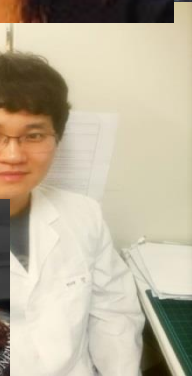
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