

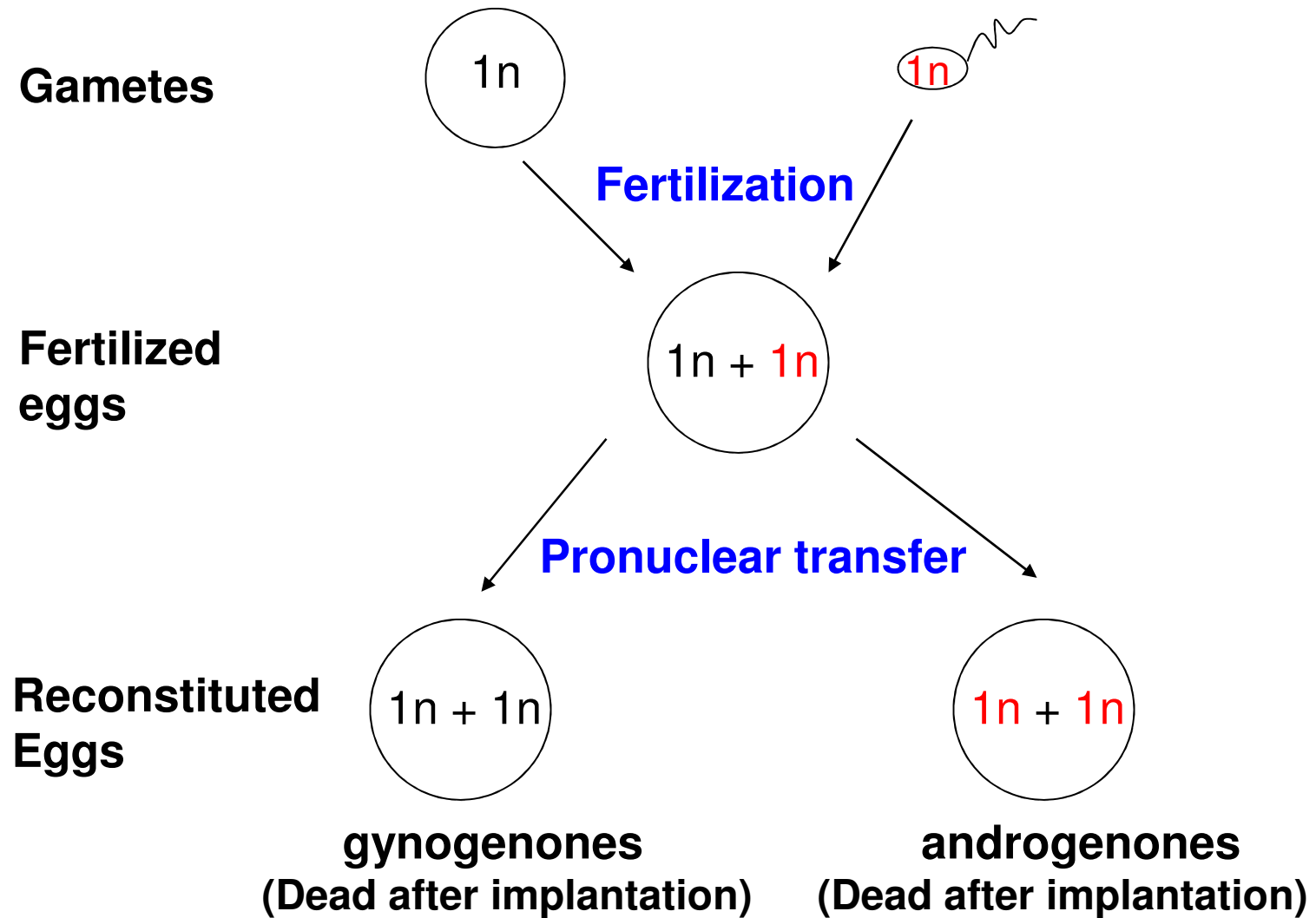
**A maternal-zygotic effect gene maintains genomic
imprinting in embryos**

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Discovery of genomic imprinting

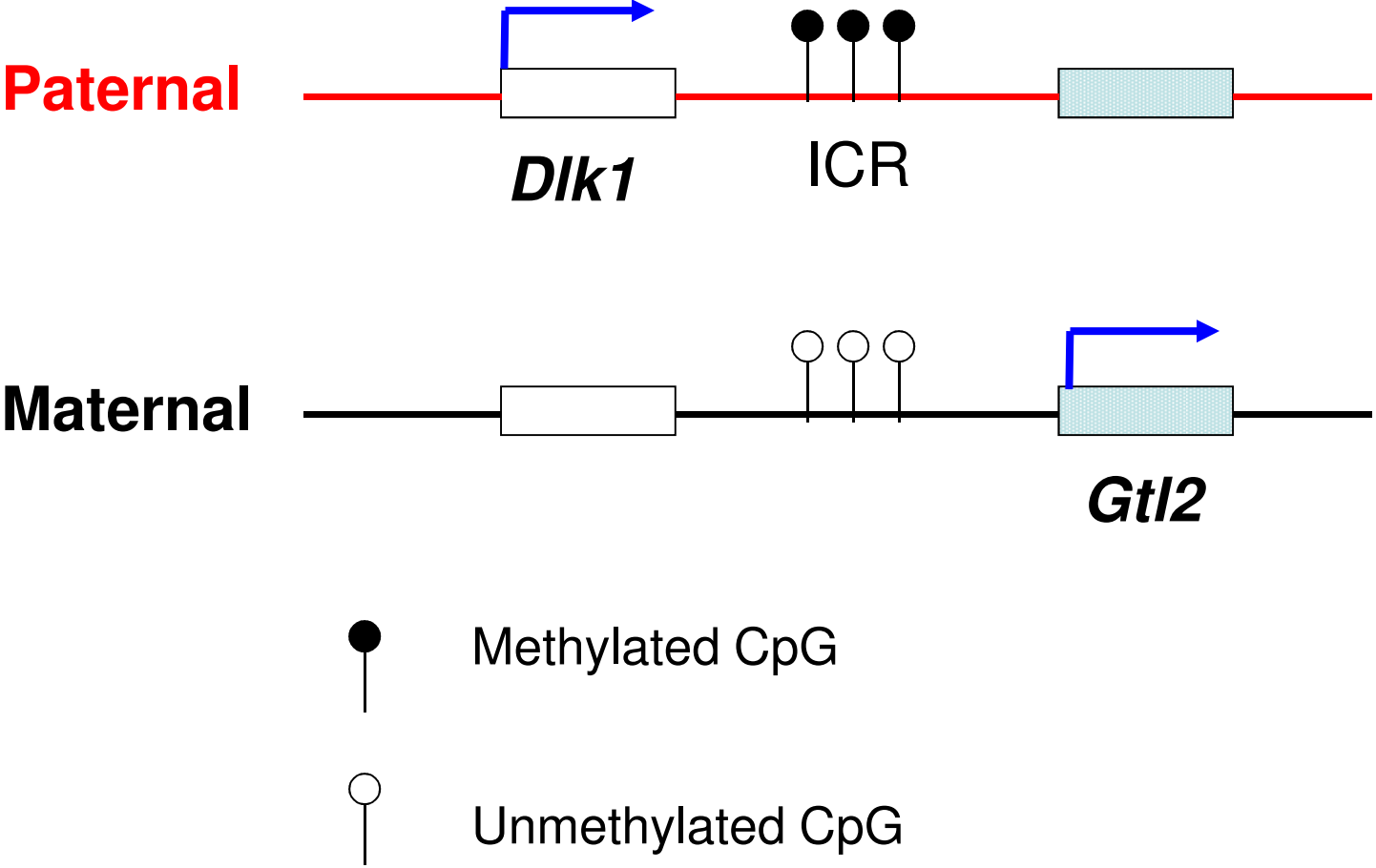


(Adapted from Surani A. et al., *Nature* 308: 548-549, 1984.
& McGrath and Solter, *Cell* 37: 179-183, 1984.)

Parental effect mediated by genomic imprinting

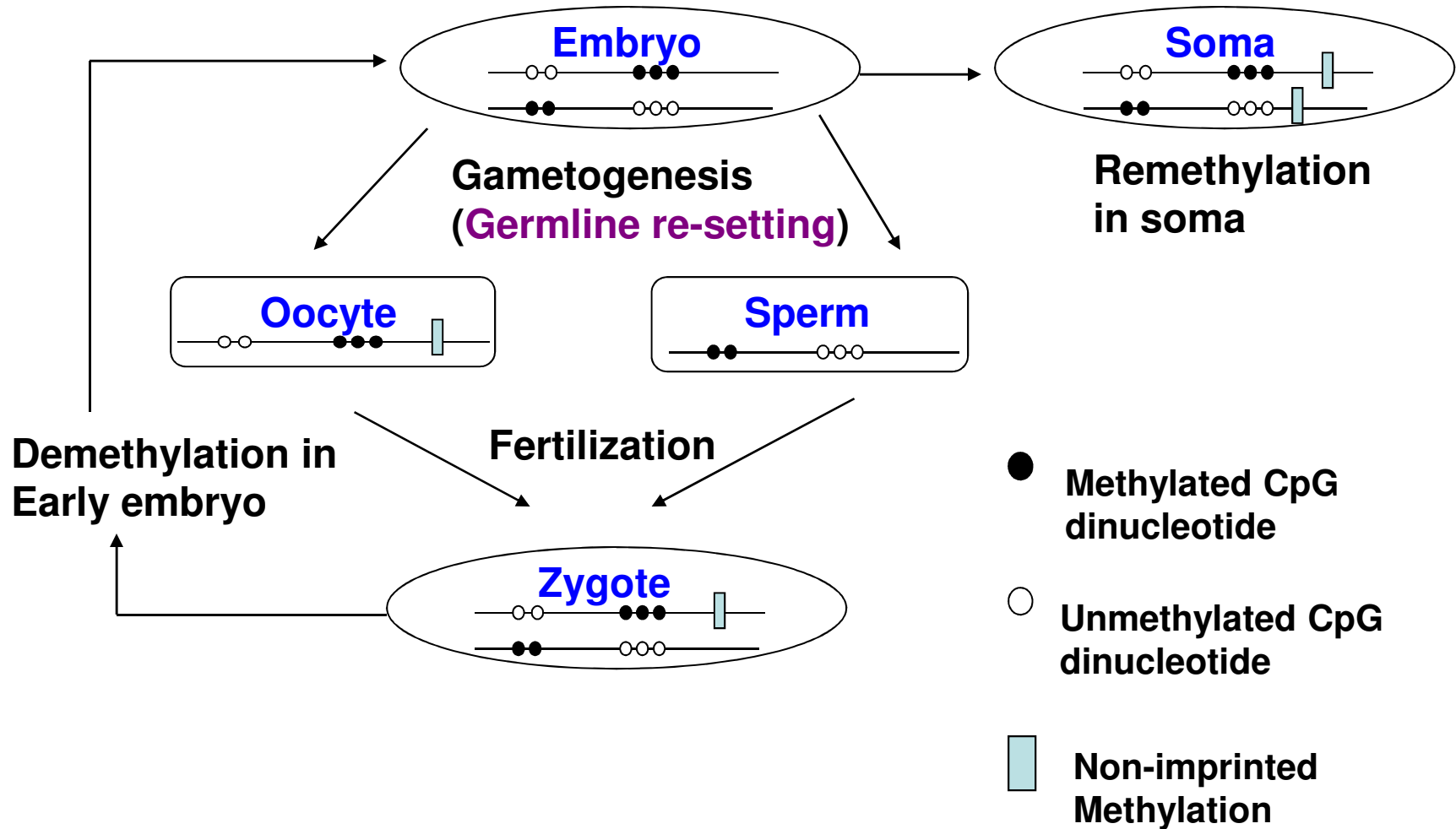
1. Discovered by Dr. Surani and Dr. Solter 25 years ago
2. Essential for embryonic development
3. Observed in eutherian and marsupial mammals, and plants
4. **Parental origin-specific** expression of imprinted genes
5. Cis-acting imprinting control region (**ICR**)
6. Germline-derived differentially methylated region (**DMR**)
7. Imprinting-related diseases (cancer, diabetes, etc.)

Parental origin-specific expression of imprinted genes

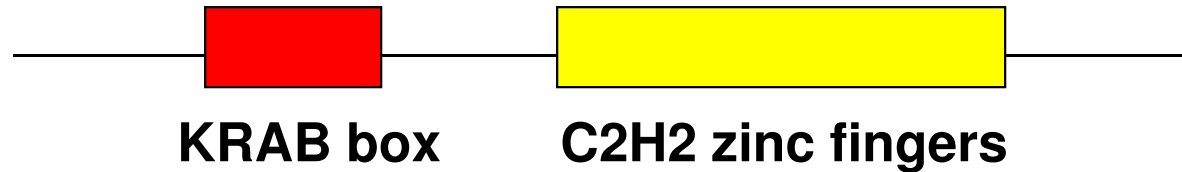


The ontogeny of genomic DNA methylation imprint

(Adapted from Tilghman S. *Cell* 96:185-93, 1999)



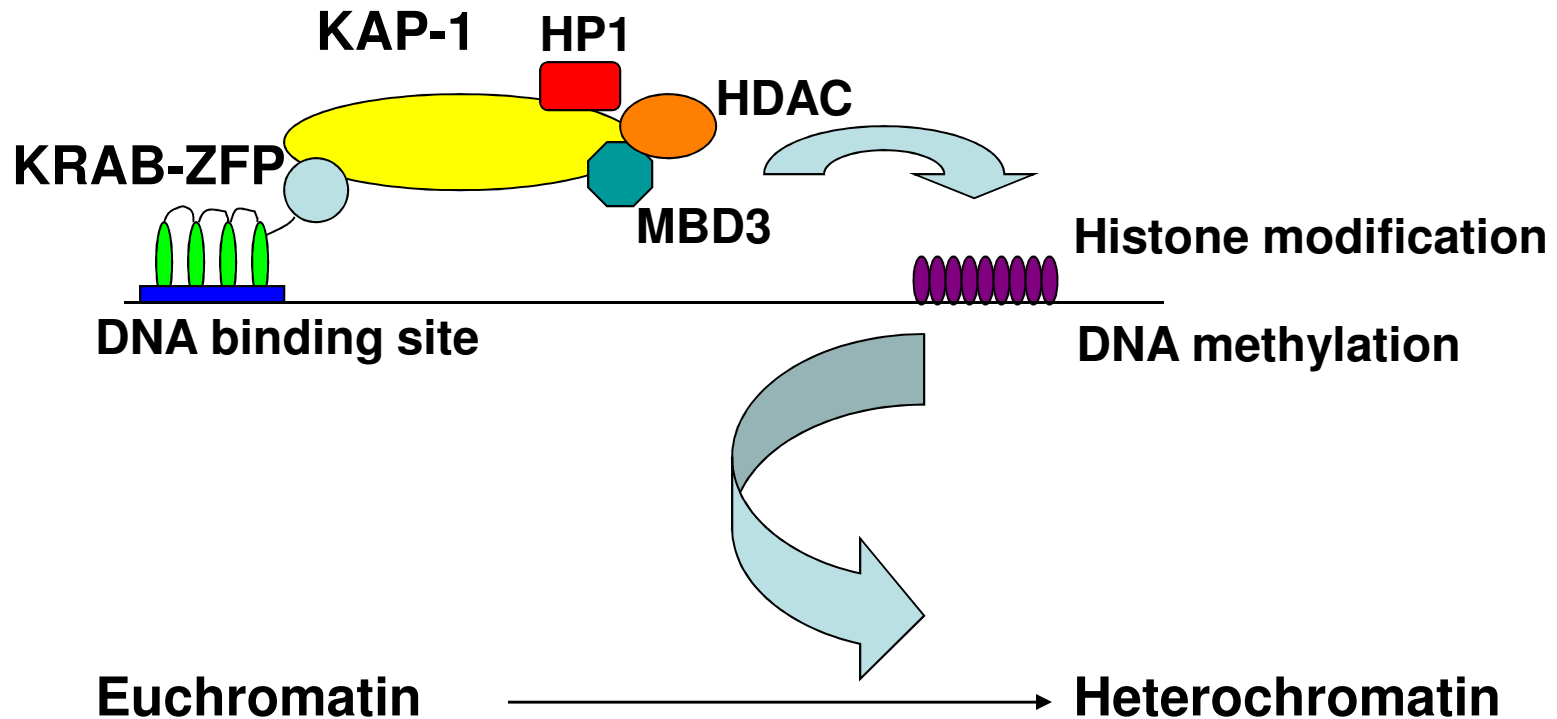
***Zfp57* encodes a putative KRAB zinc finger protein**



Features of the KRAB zinc finger proteins:

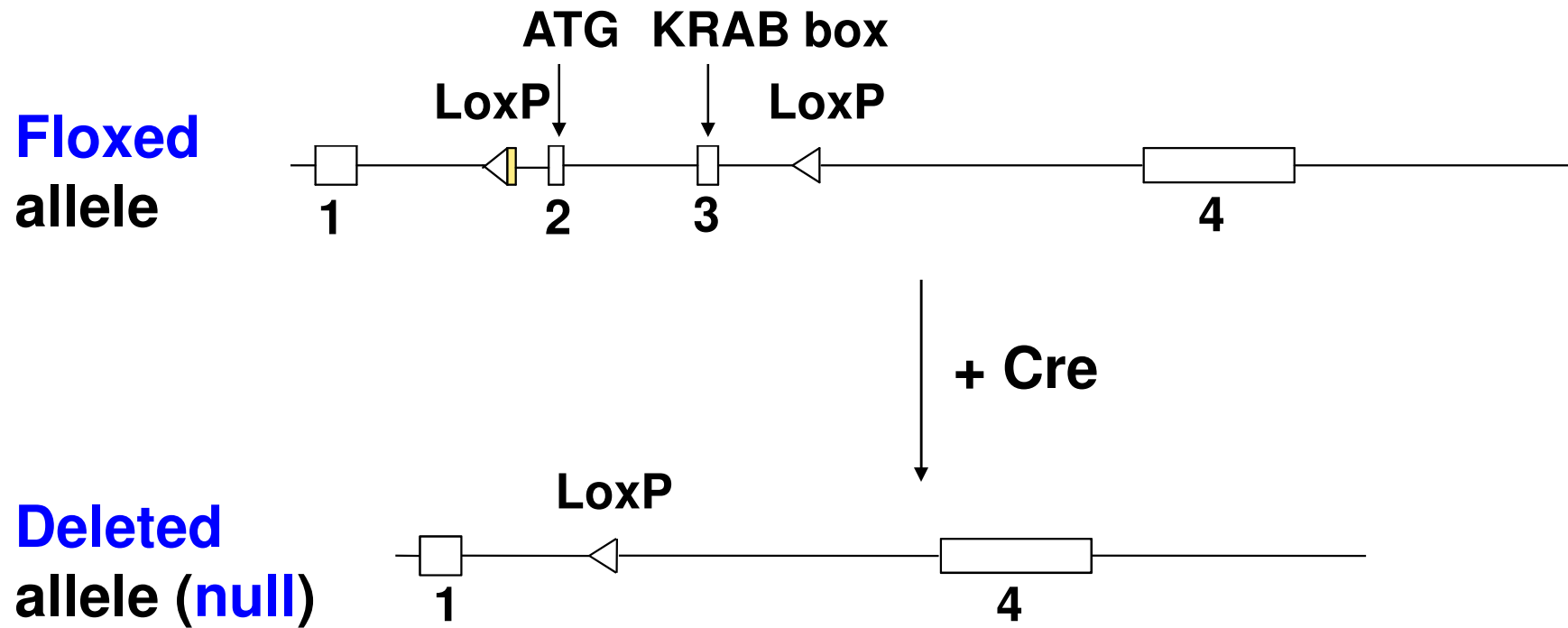
- 1. KRAB zinc finger proteins are unique to vertebrates.**
- 2. There are over 300 members in the human genome.**
- 3. KRAB zinc finger proteins interact with the co-repressor KAP-1/TIF1 β /Trim28 via KRAB box.**

KRAB-ZFP proteins target HP1, HDACs and MBD3 to initiate repression



Adapted from Dr. Frank Rauscher's talk at the AACR meeting

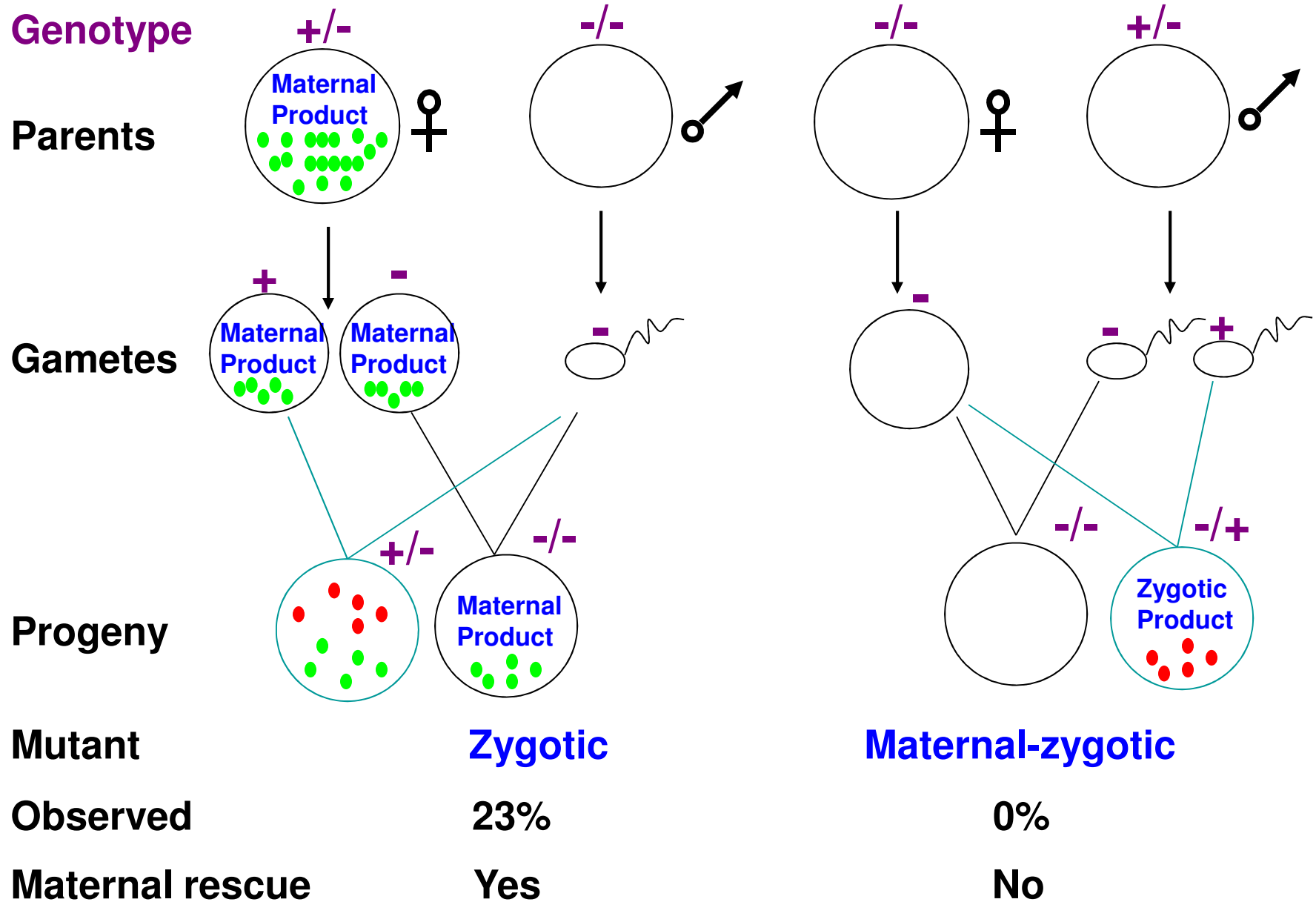
Conditional knockout allele (floxed allele) and deleted (null) allele were constructed for *Zfp57*



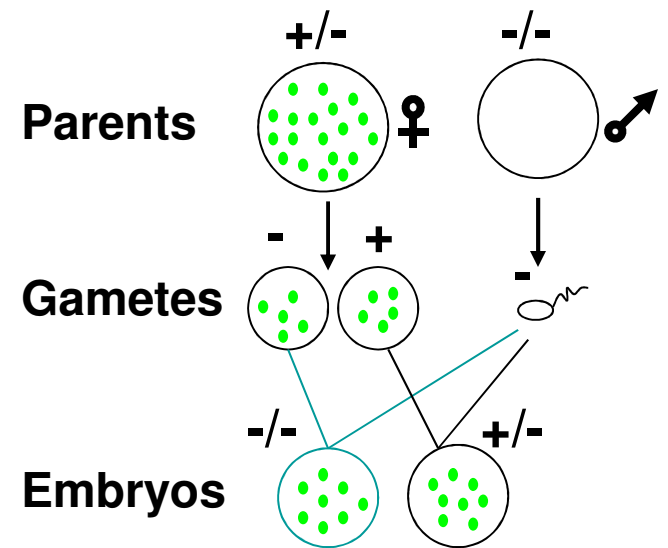
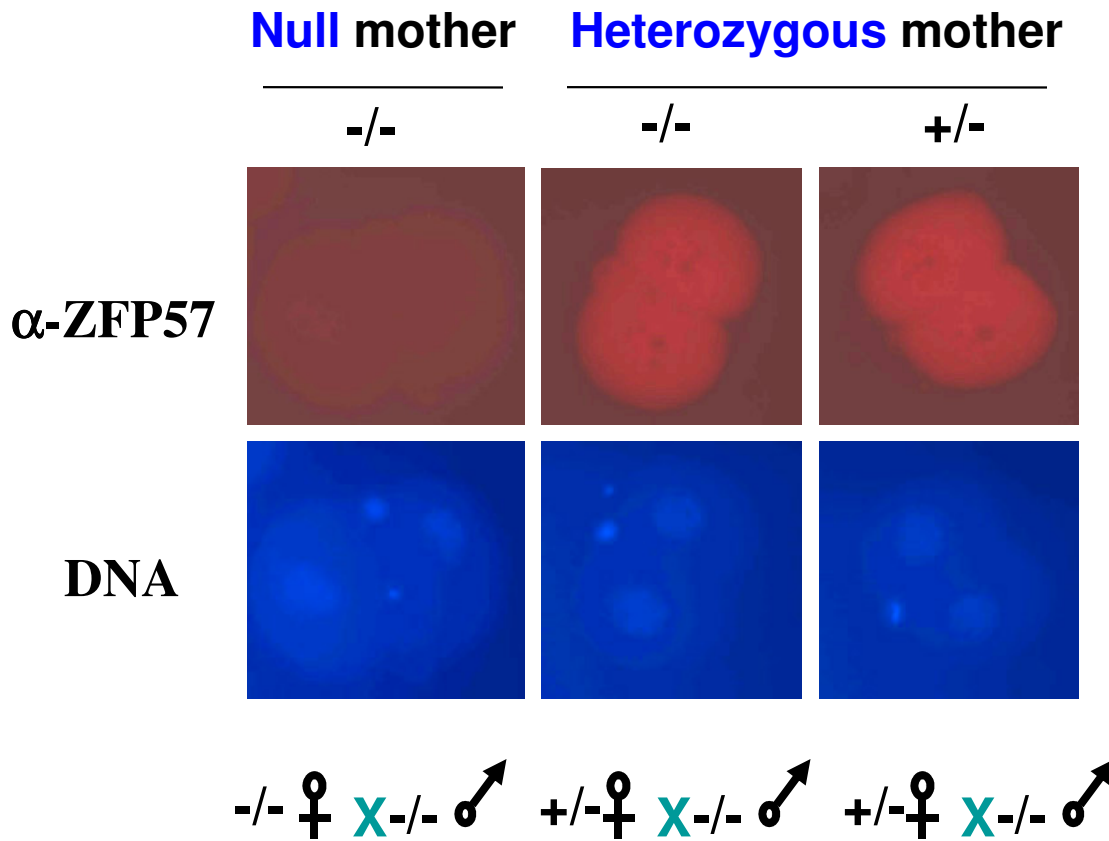
***Zfp57* mutant has reduced viability**

Cross		Expected %	Observed %
♀	♂	of mutants	of mutants
+/-	+/-	25%	11%
+/-	-/-	50%	23%
-/-	+/-	50%	0%

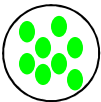
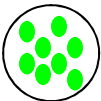
Zfp57 has both **maternal** and **zygotic** functions

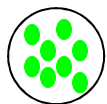


Maternal gene product of *Zfp57* is deposited in early embryos

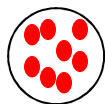


***Zfp57* mutants displayed **perinatal** and **neonatal** zygotic lethality**

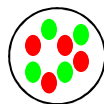
Cross		Stage	% of dead mutant/total mutant
♀	♂		
	+/-	+/-	E18.5 17% (n=18)
			P1 pup 45% (n=20)
	+/-	-/-	E18.5 0% (n=12)
			P1 pup 40% (n=43)



Mat



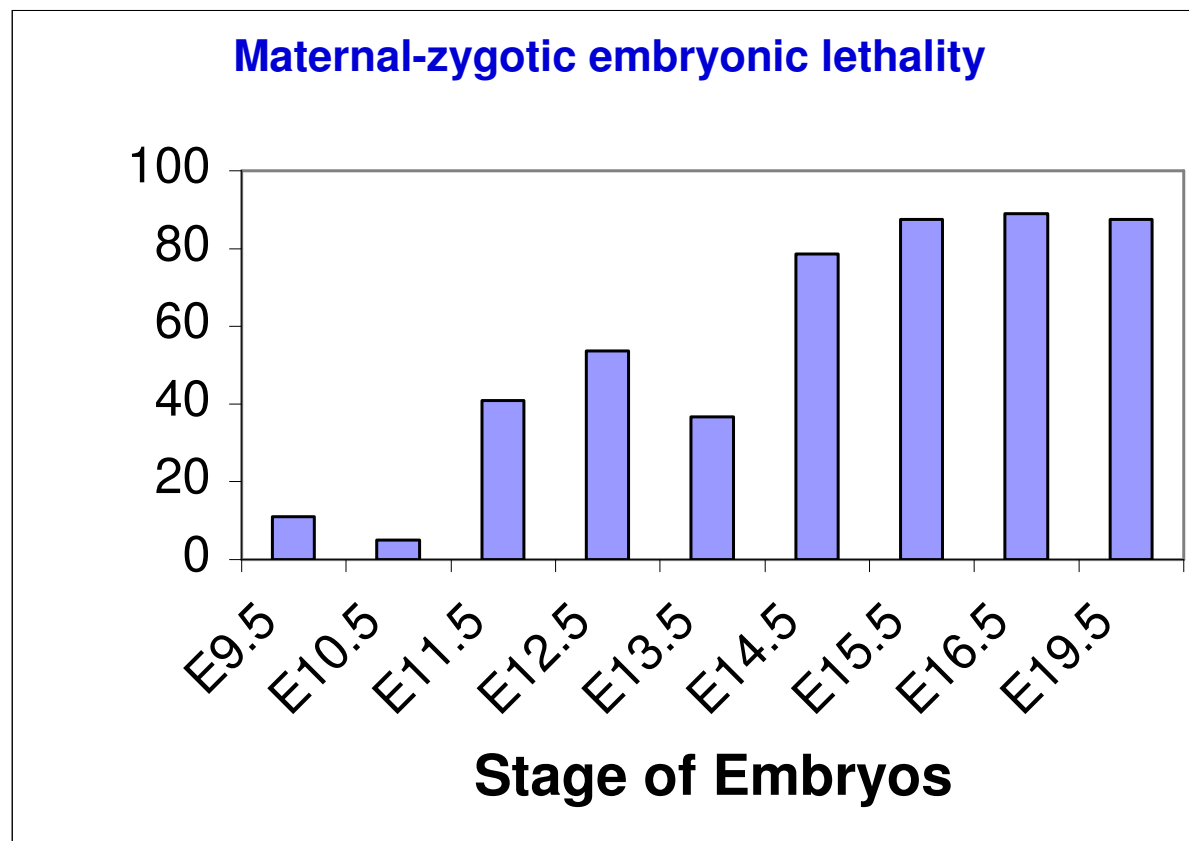
Zyg



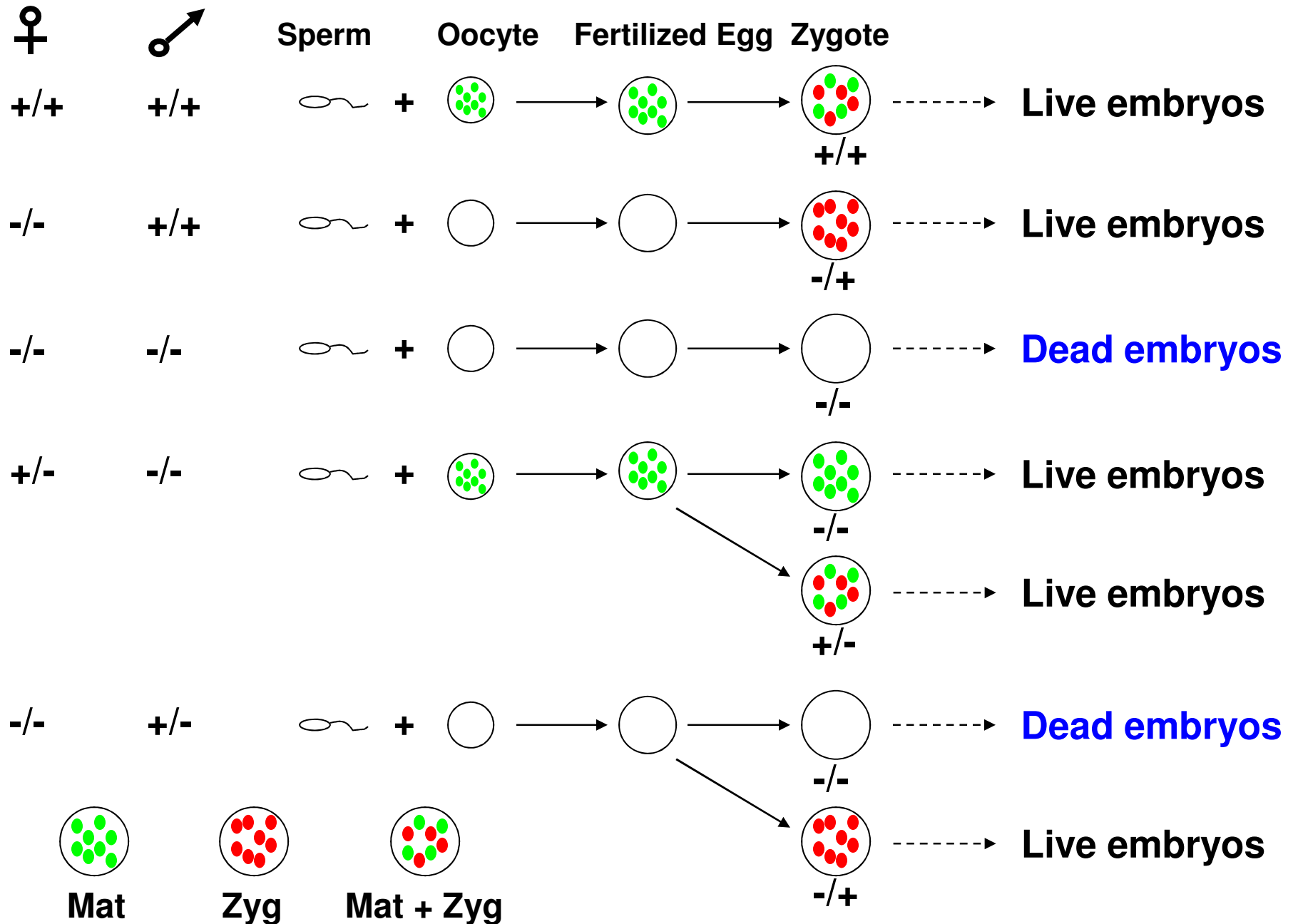
Mat + Zyg

Loss of both maternal and zygotic *Zfp57* results in **maternal-zygotic** lethality around midgestation

Cross: -/- ♀ X -/- ♂



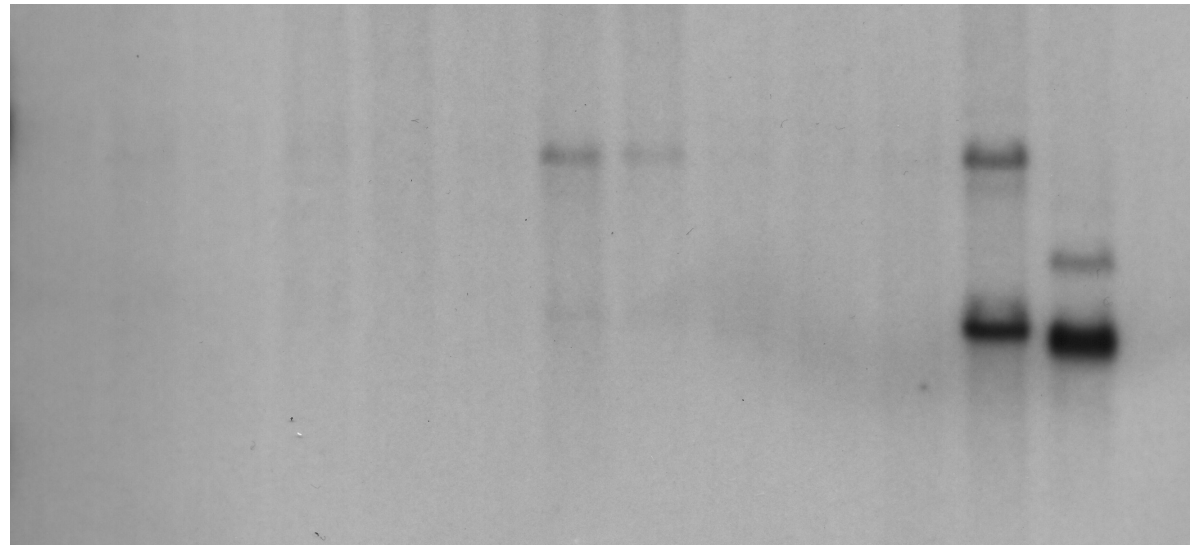
Zfp57 is a **maternal-zygotic** effect gene



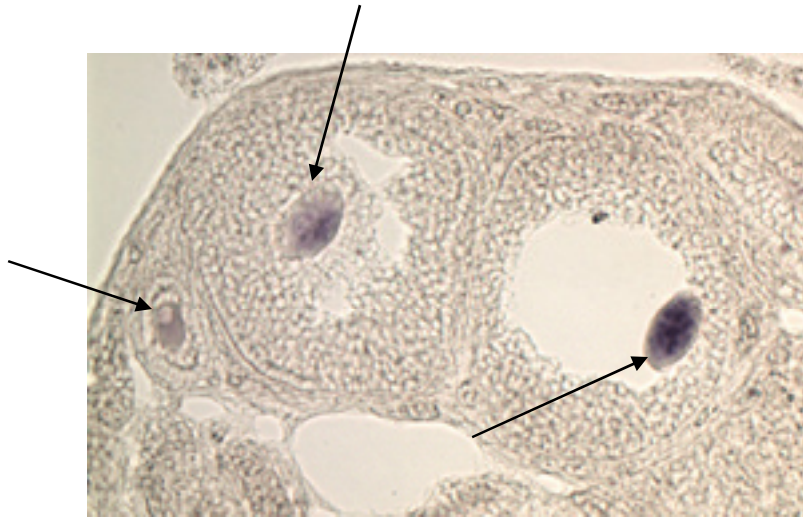
Zfp57 is primarily expressed in the **germline**
in a polyA Northern blot

S: Stomach
LI: Large Intestine
L: Liver
SP: Spleen
T: Thymus
K: Kidney
LG: Lung
C: Cerebrum
H: Heart
M: Muscle
MG: Mammary Gland
O: Ovary
TE: Testis
SV: Seminal Vesicle

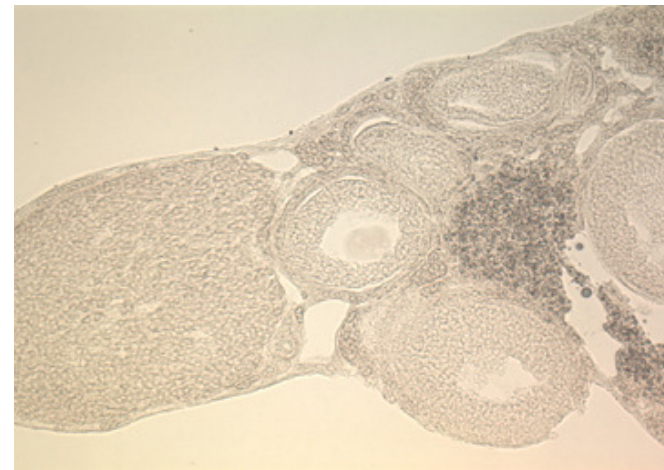
S LI L SP T K LG C H M MG O TE SV



***Zfp57* is specifically expressed in the oocytes by RNA in-situ hybridization**



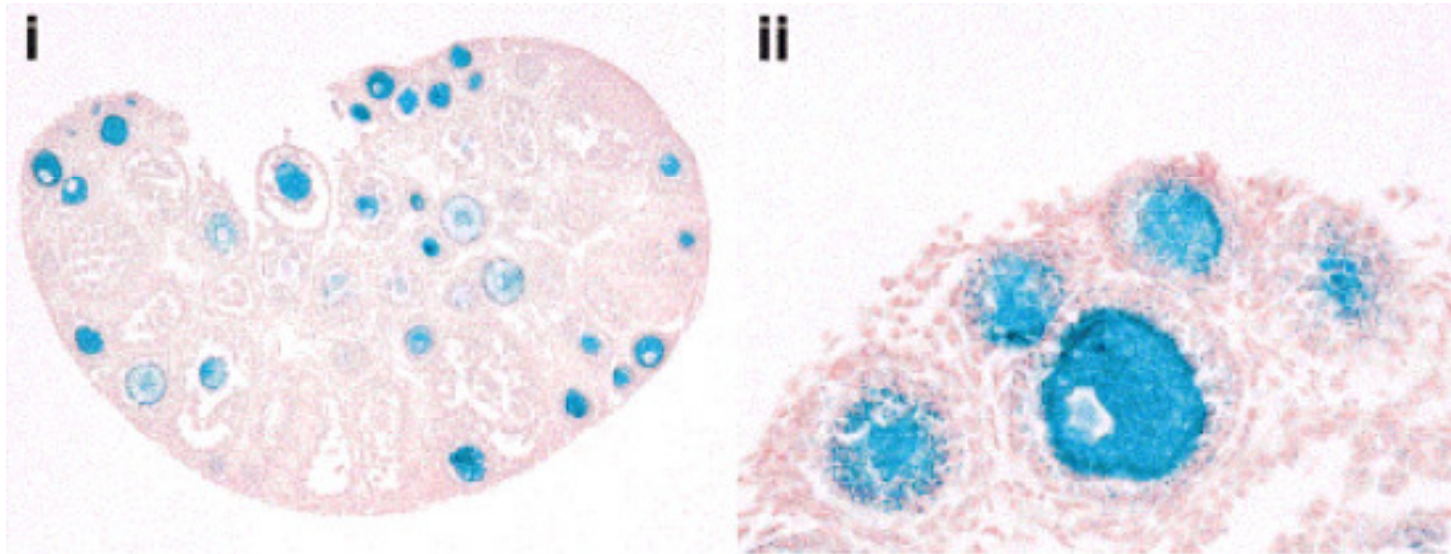
Antisense probe



Sense probe

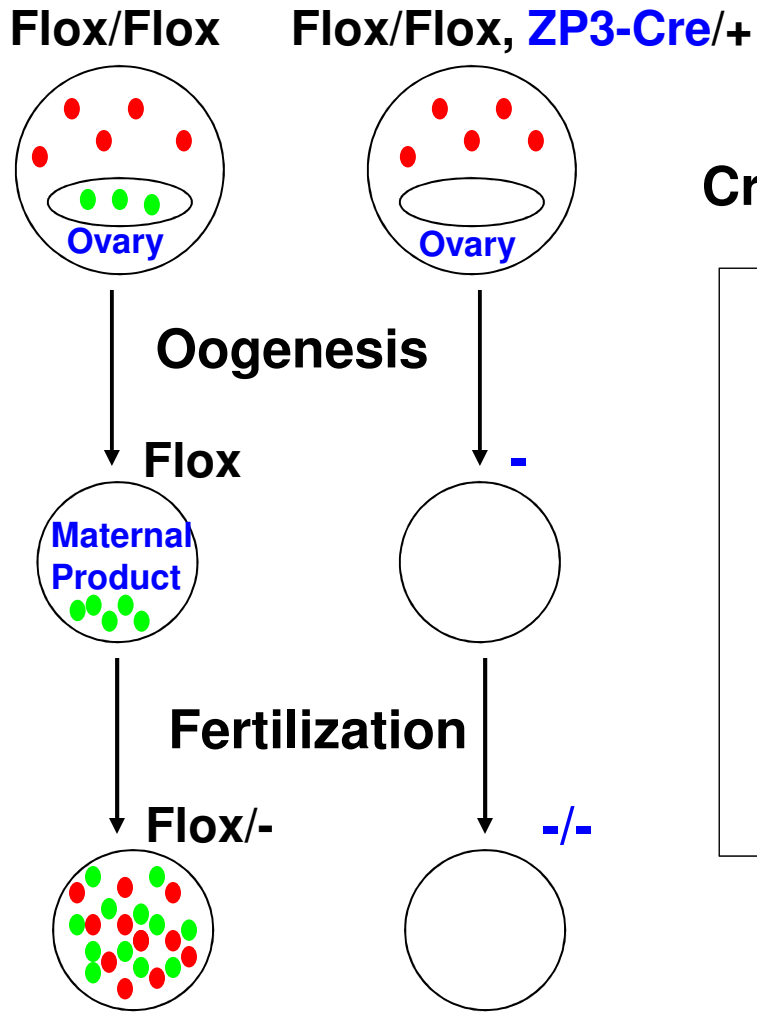
A *ZP3-cre* transgene can be used to study maternal effect genes

LacZ reporter mouse X *ZP3-Cre* transgenic mouse

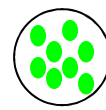
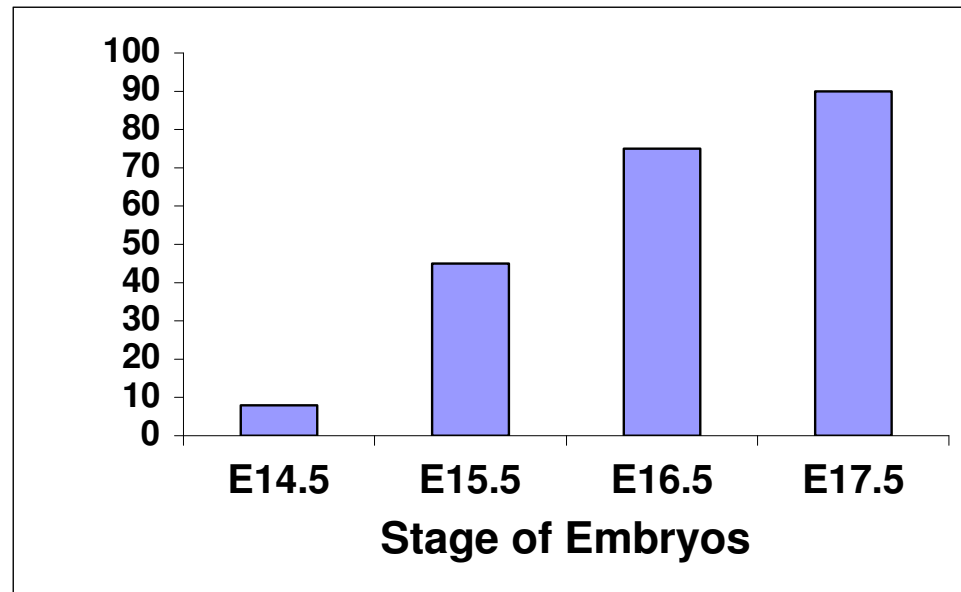


Adapted from de Vries, W. et al, *Genesis* 26:110-112, 2000.

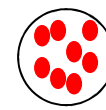
Ablating maternal *Zfp57* in the oocytes can cause maternal-zygotic embryonic lethality



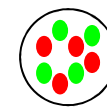
Cross: Flox/Flox, ZP3-Cre/+ ♀ X -/- ♂



Mat



Zyg

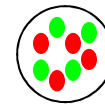


Mat + Zyg

Two possible mechanisms for the maternal-zygotic effect of *Zfp57*

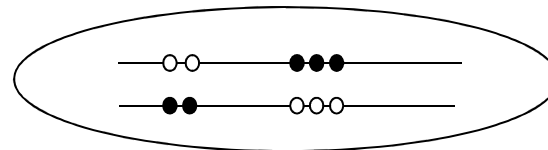
1. Deposition of maternal cytoplasmic factors

Redundant maternal and zygotic products



2. Heritable maternal nuclear determinants

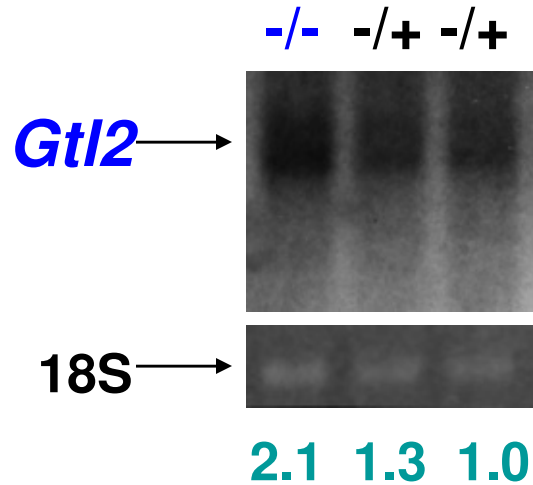
Genomic imprinting



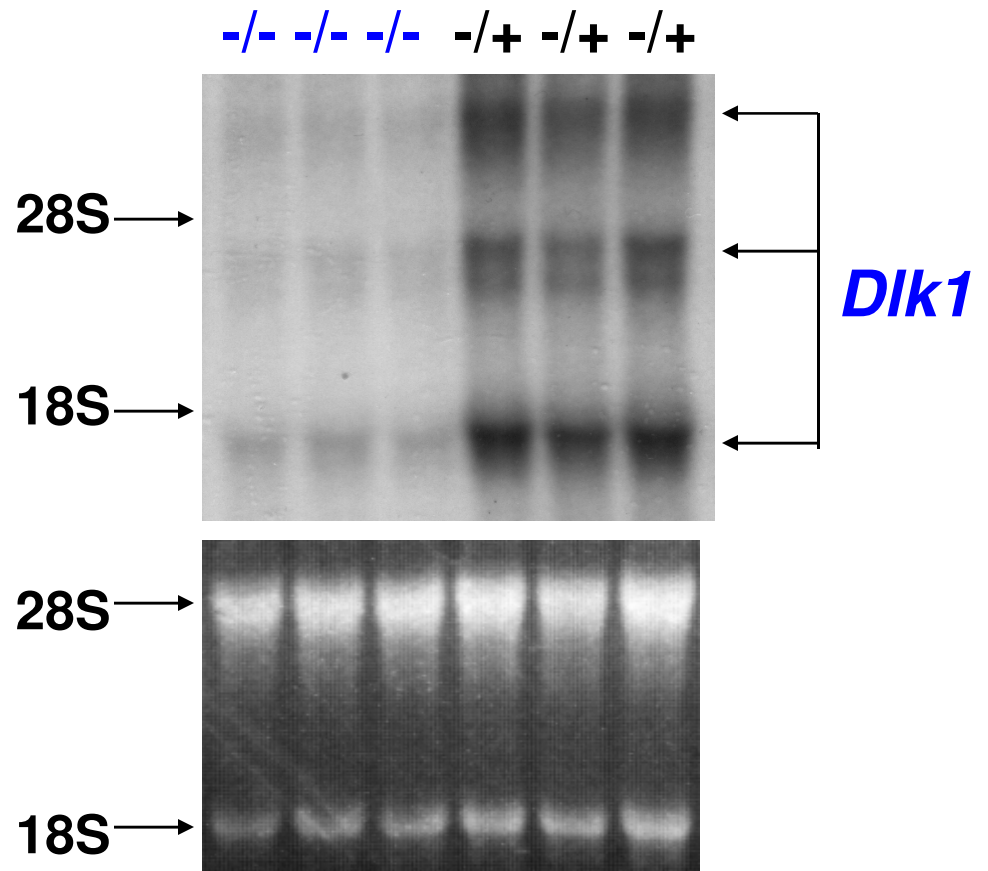
Loss of *Zfp57* causes opposite effects on expression of the co-regulated imprinted *Dik1* and *Gtl2* genes

Cross: $-/-$ ♀ X $+/-$ ♂

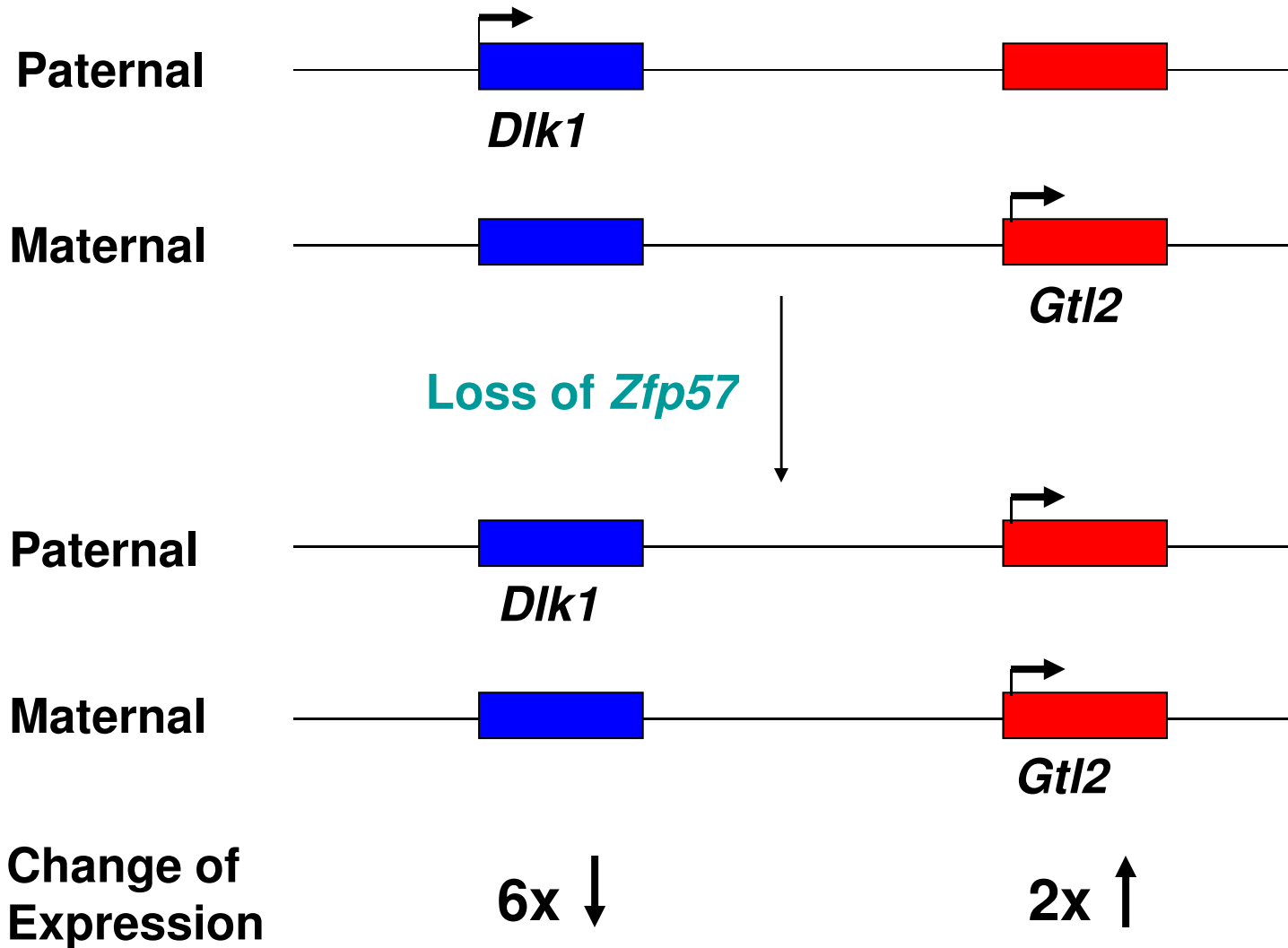
E11.5 embryos



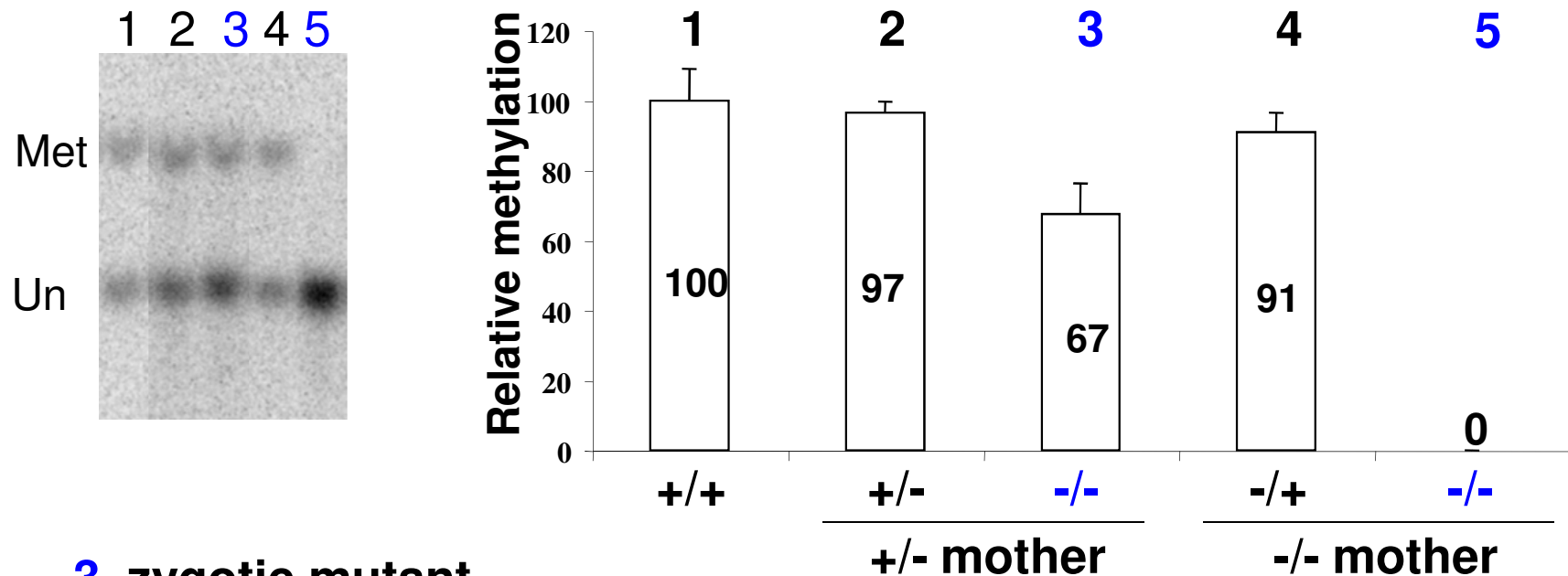
PolyA Northern blot



Loss of *Zfp57* perturbs the imprinting status at the *Dlk1-Gtl2* domain



Maternal-zygotic effect of *Zfp57* on DNA methylation imprints



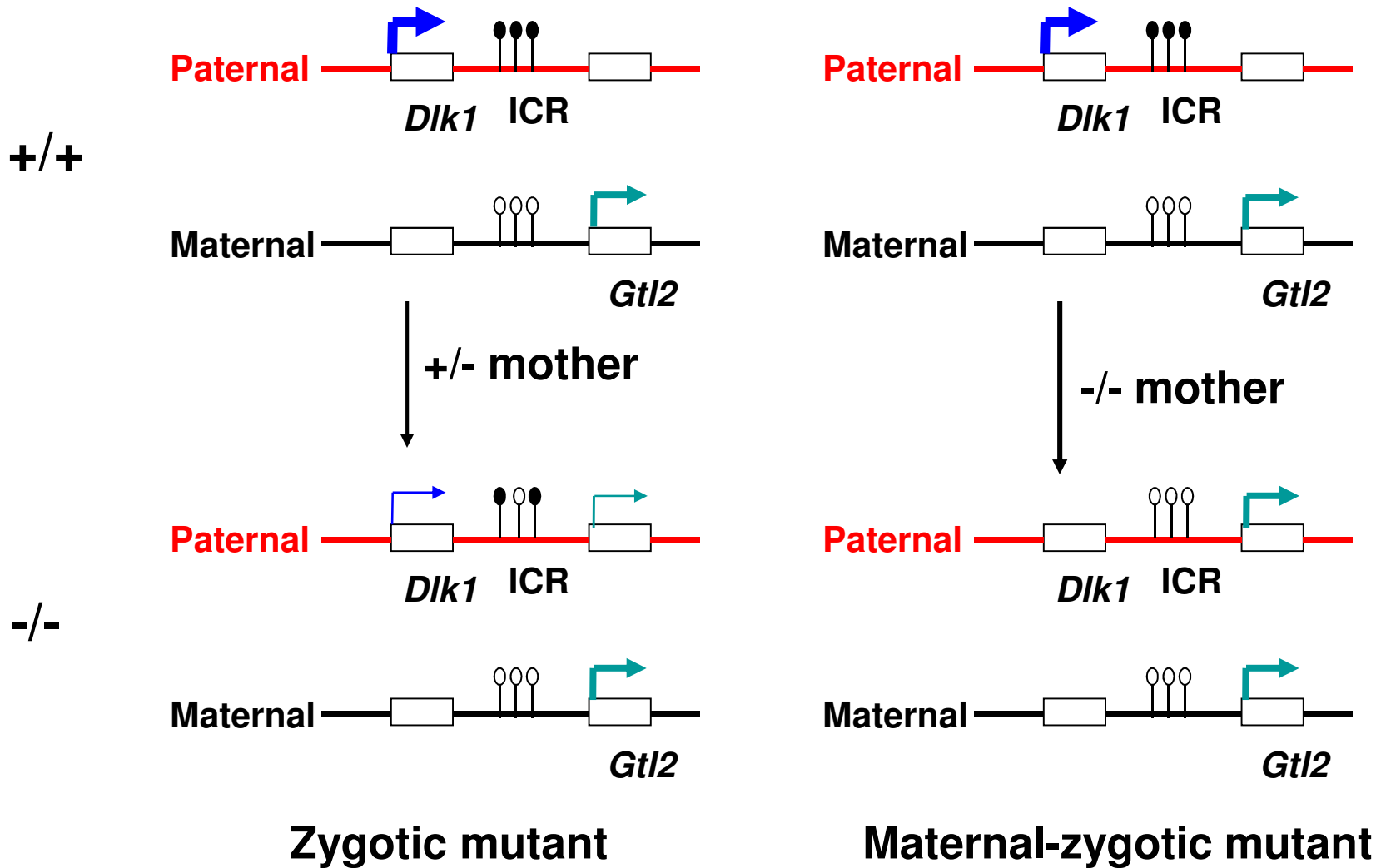
3, zygotic mutant

5, maternal-zygotic mutant

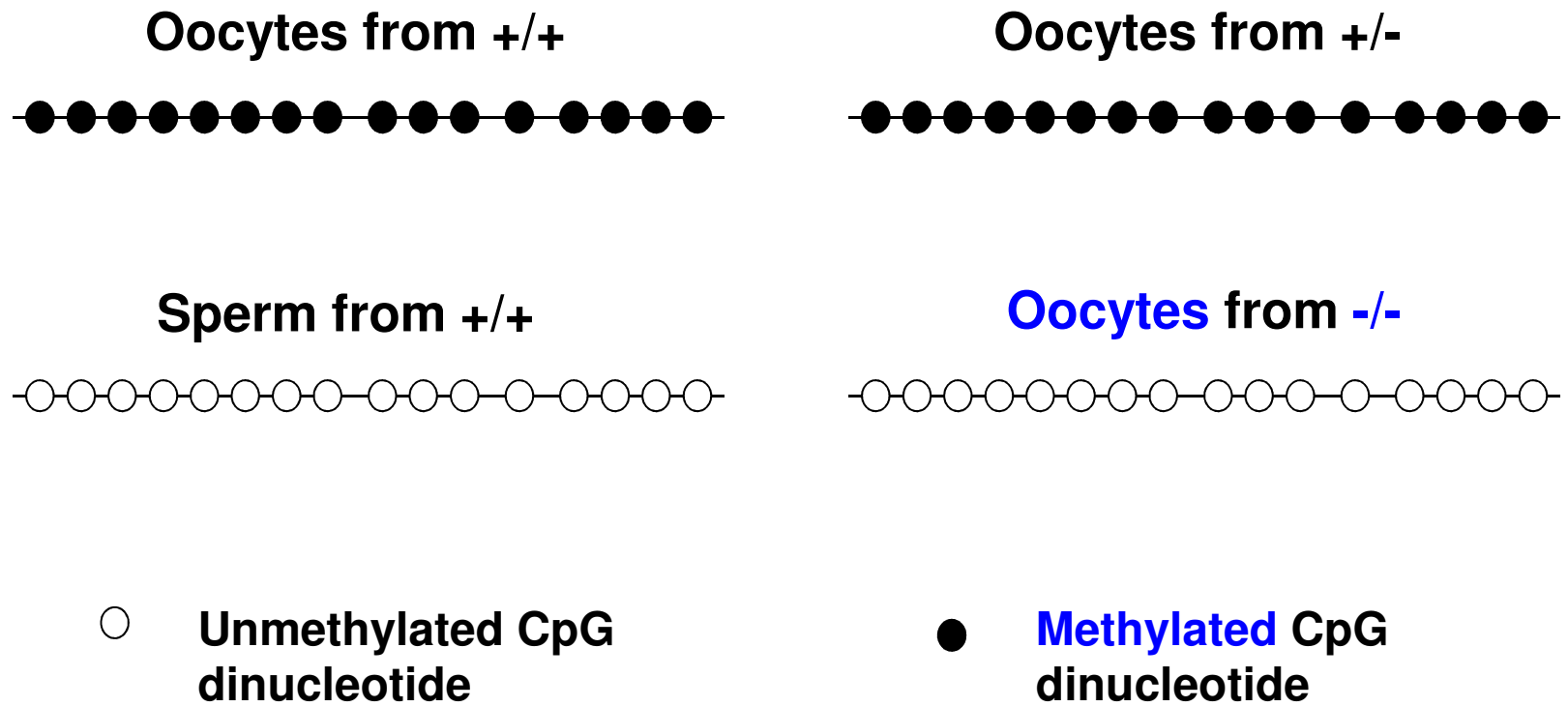
Met, methylated

Un, unmethylated

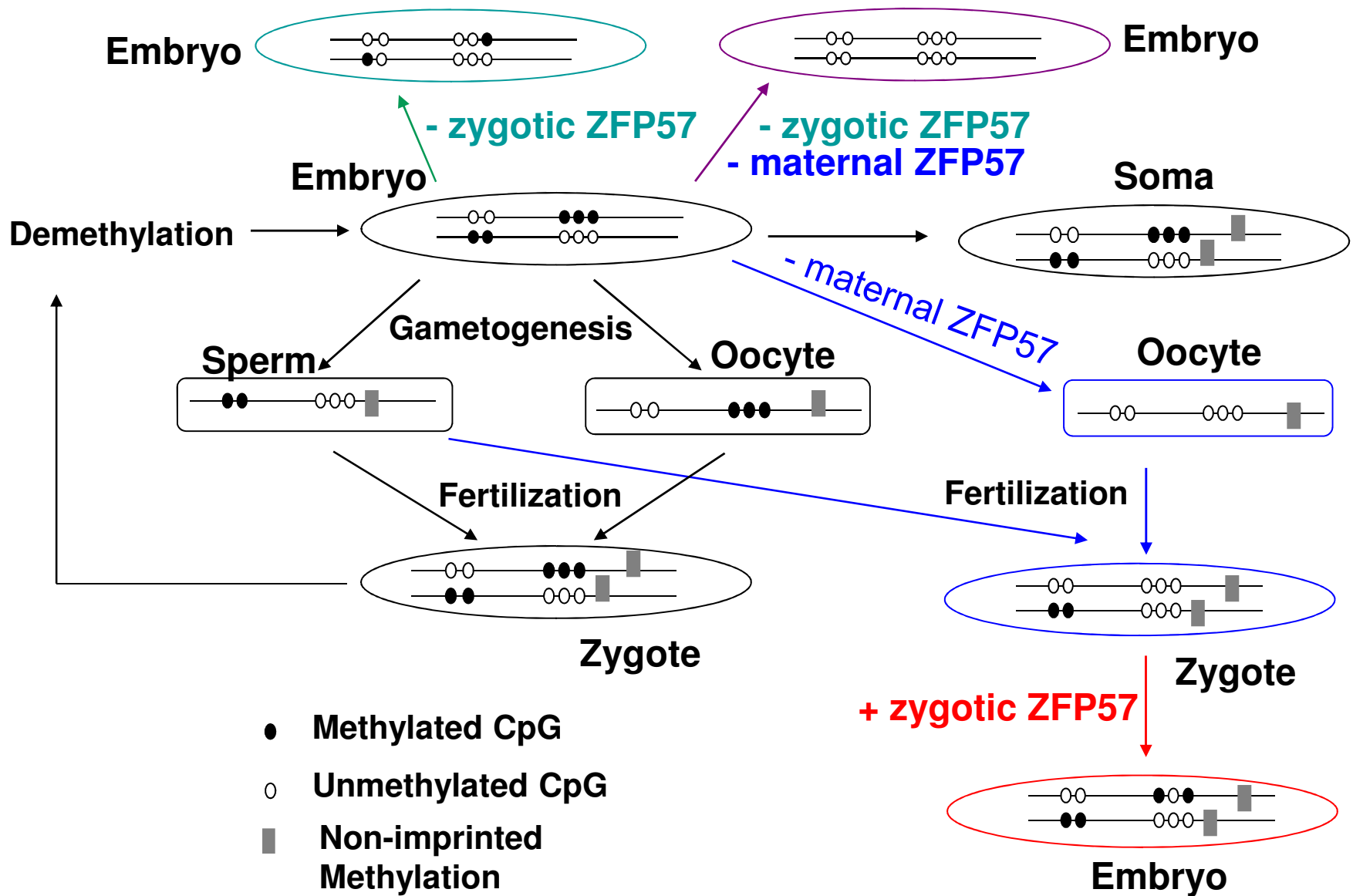
Loss of *Zfp57* causes loss of DNA methylation imprints



DNA methylation at the *Snrpn* DMR is absent in the **oocytes** derived from null female mice



Zfp57 is involved in the maintenance and acquisition of DNA methylation imprints



Summary

- 1. *Zfp57* is an essential maternal-zygotic effect gene.**
- 2. *Zfp57* is involved in the acquisition and maintenance of DNA methylation imprints.**
- 3. *Zfp57* maintains a large subset of paternal and maternal methylation imprints.**

Acknowledgments

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Jipo Sheng

Xiaopan Zuo

Helen Hay Whitney Foundation

Black Family Stem Cell Institute

American Heart Association

National Institutes of Health (in process)

Purpose of my trip:

- 1. Learn novel things**
- 2. Make new friends**
- 3. Establish good collaborations**
- 4. Visit historic places**
- 5. Recruit talented students**