Stockholm: 7 December, 2012.

The egg and the nucleus: a battle for supremacy

J. B. Gurdon

Cambridge, England.

Content

Background

Attack by the egg

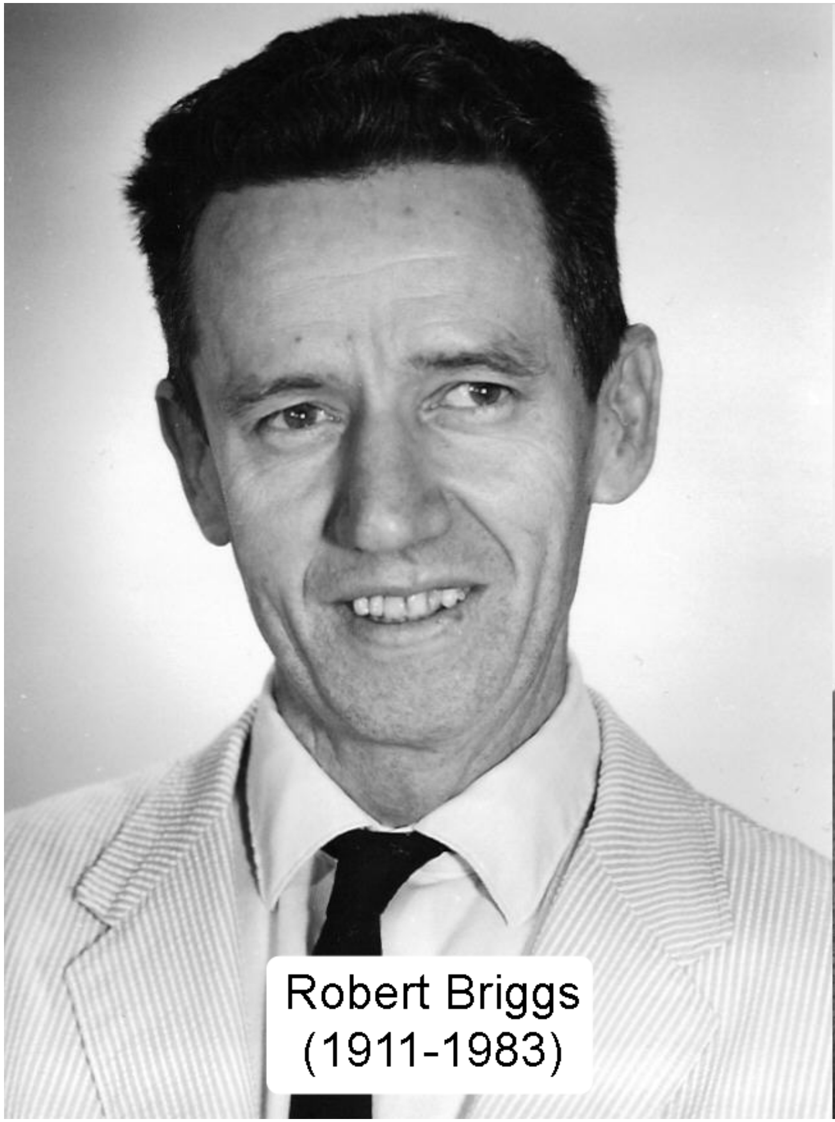
Defense by the nucleus

Prospects

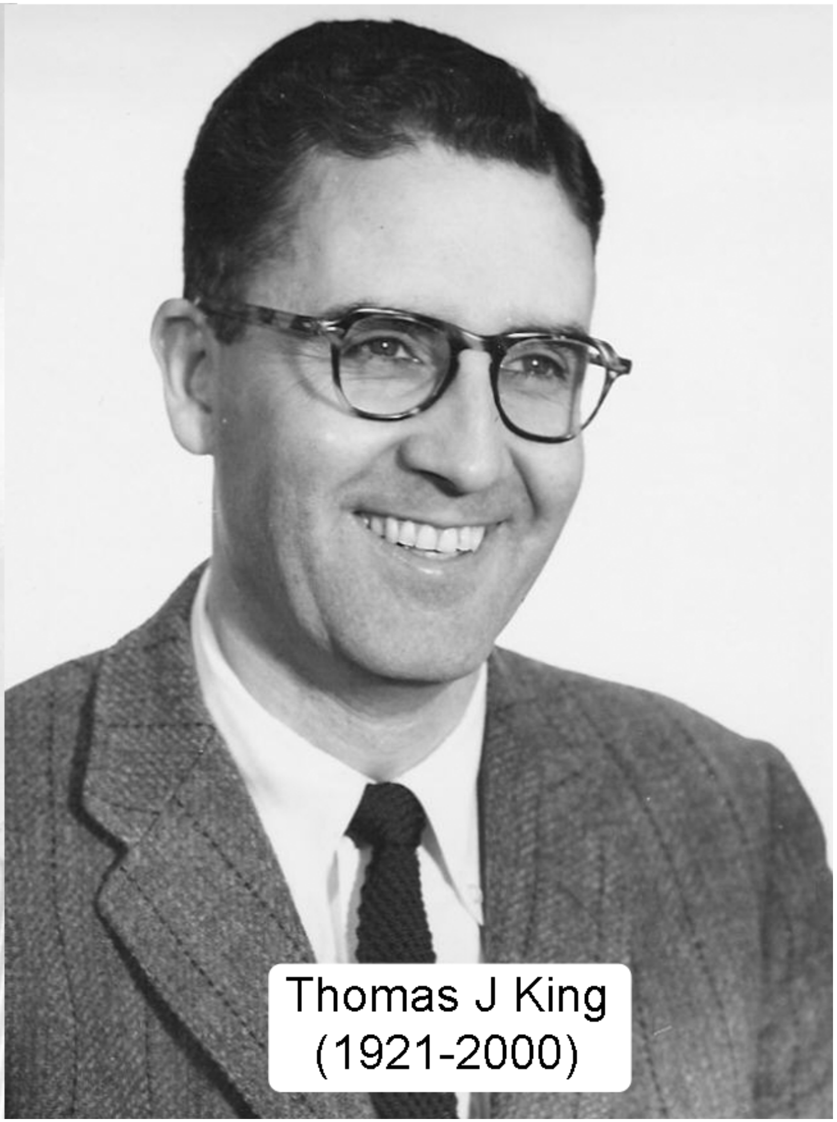
Background

The original question

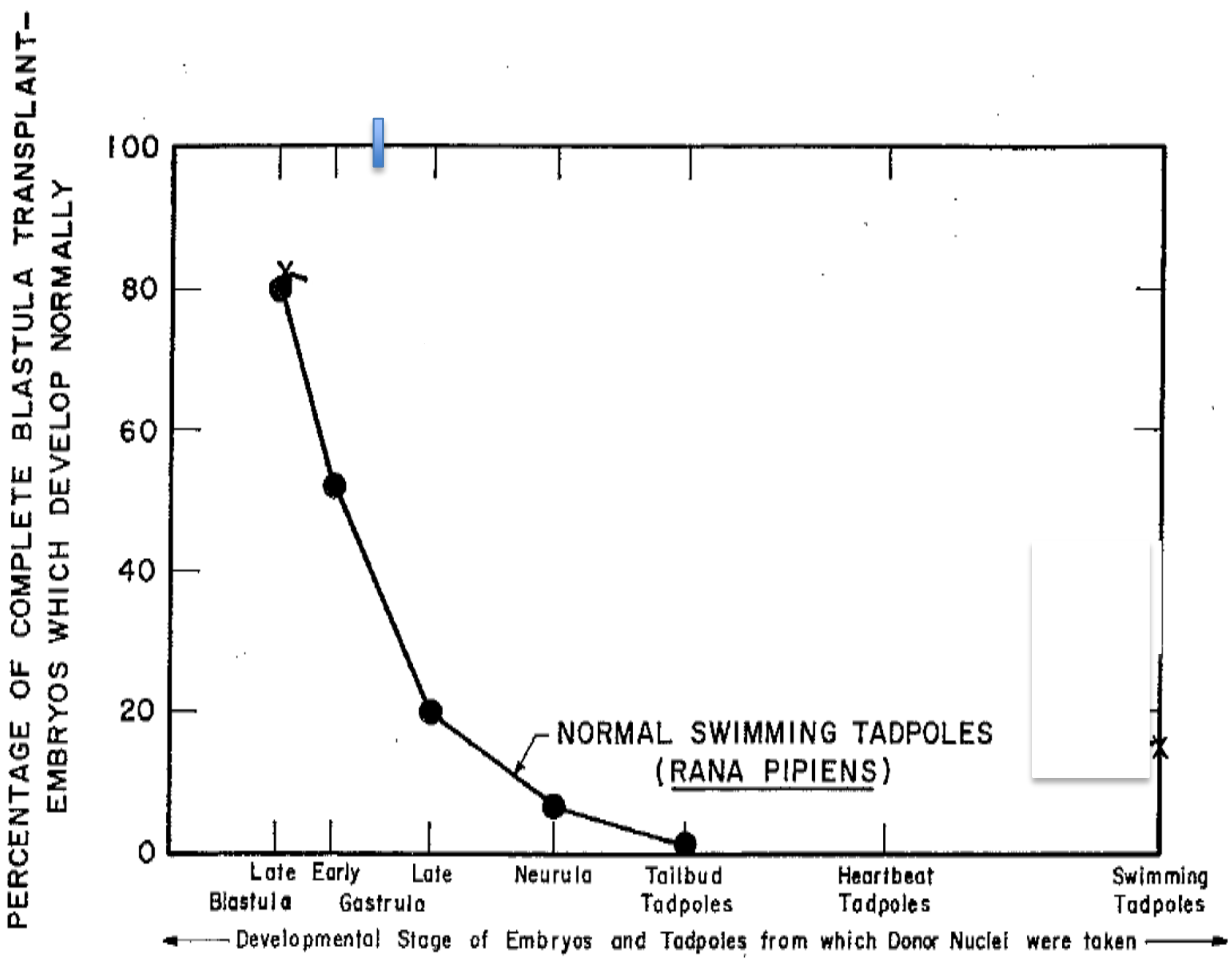
Do all cells in the body have the same sets of genes?



Robert Briggs
(1911-1983)

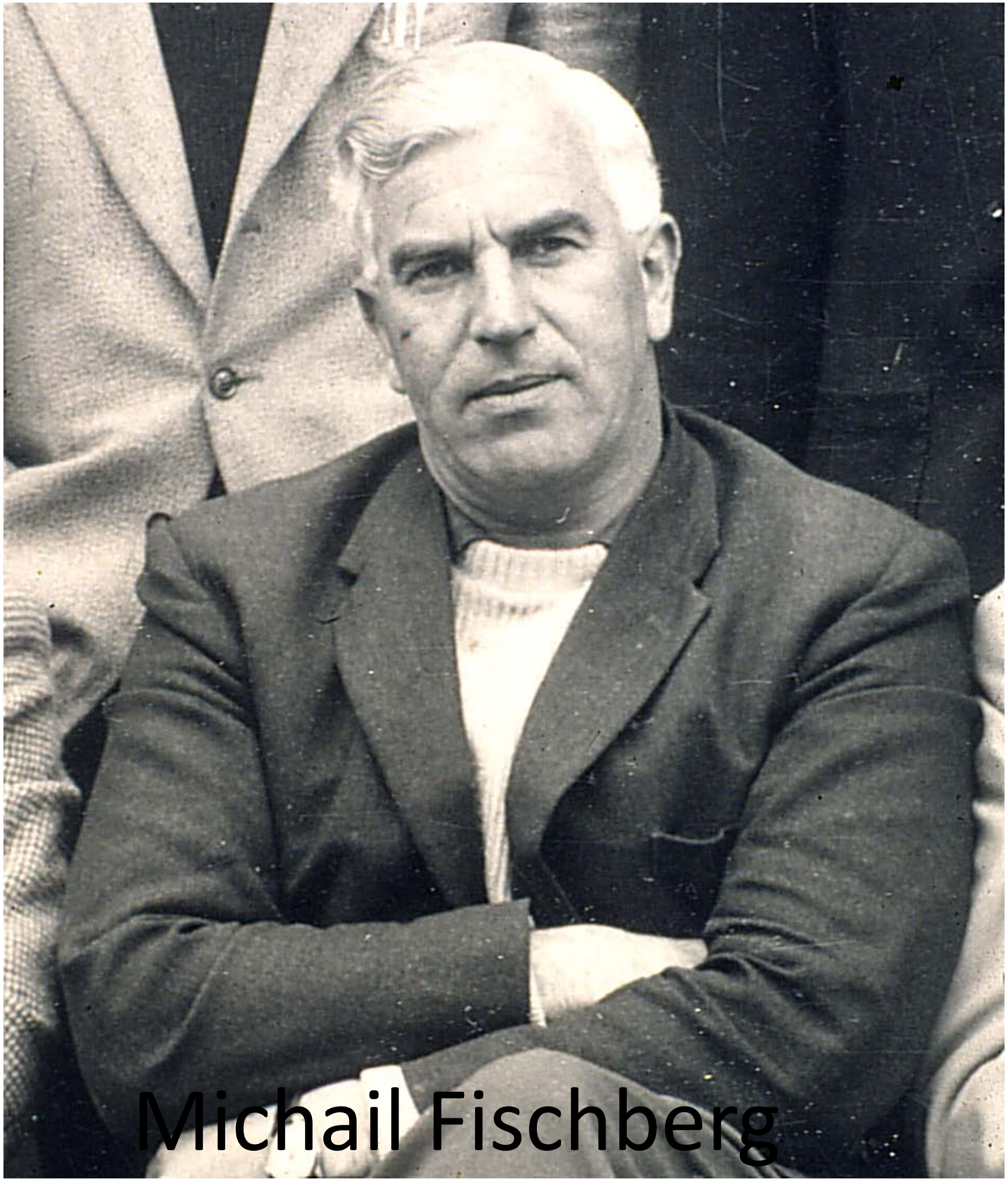


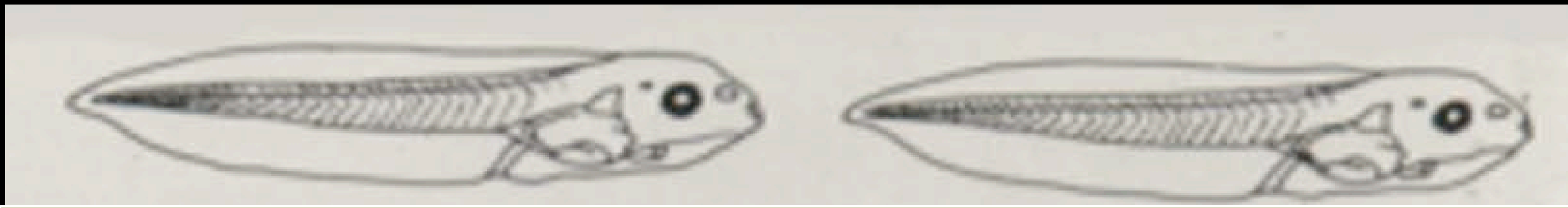
Thomas J King
(1921-2000)



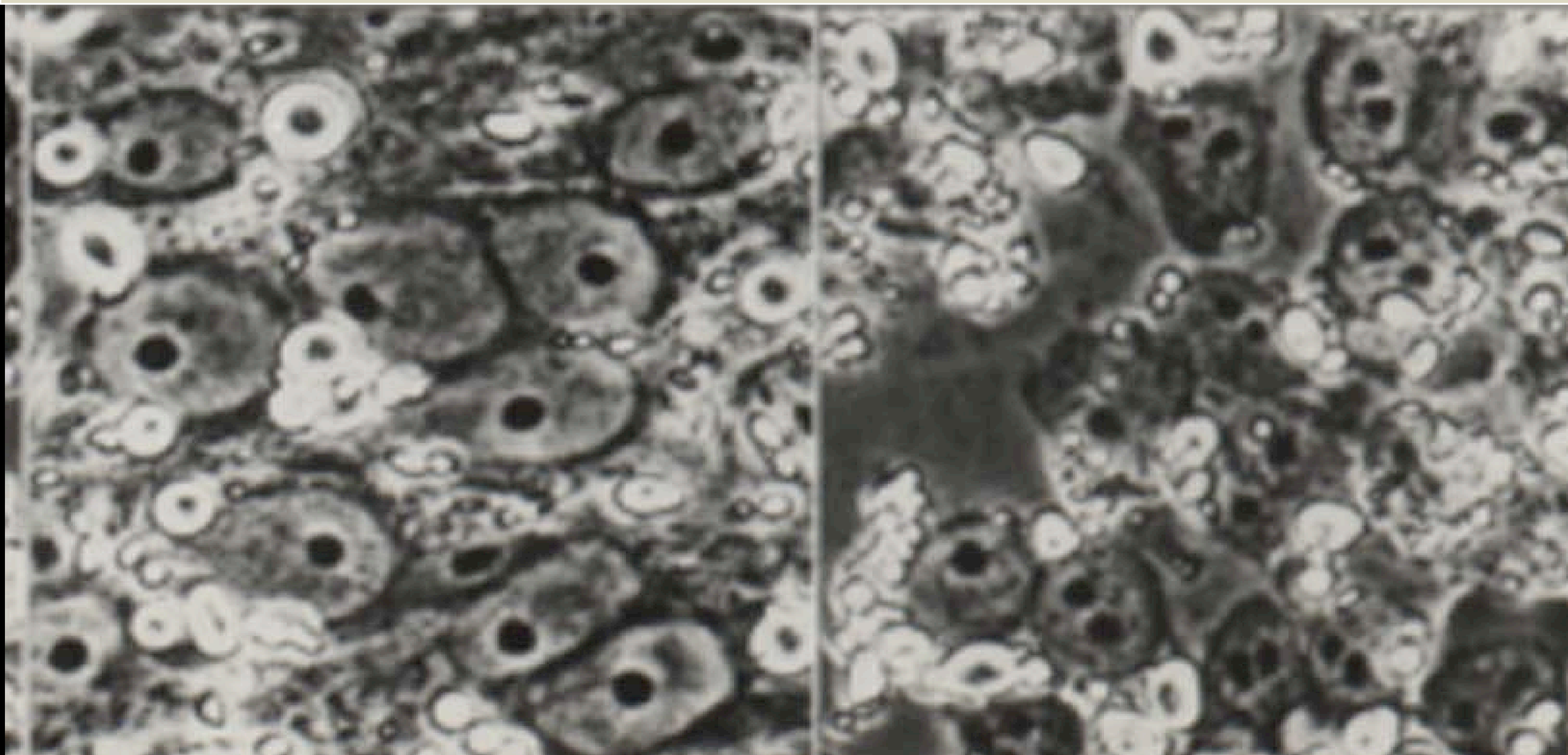
Briggs and King, 1952. Proc. Nat. Acd. Sci., USA

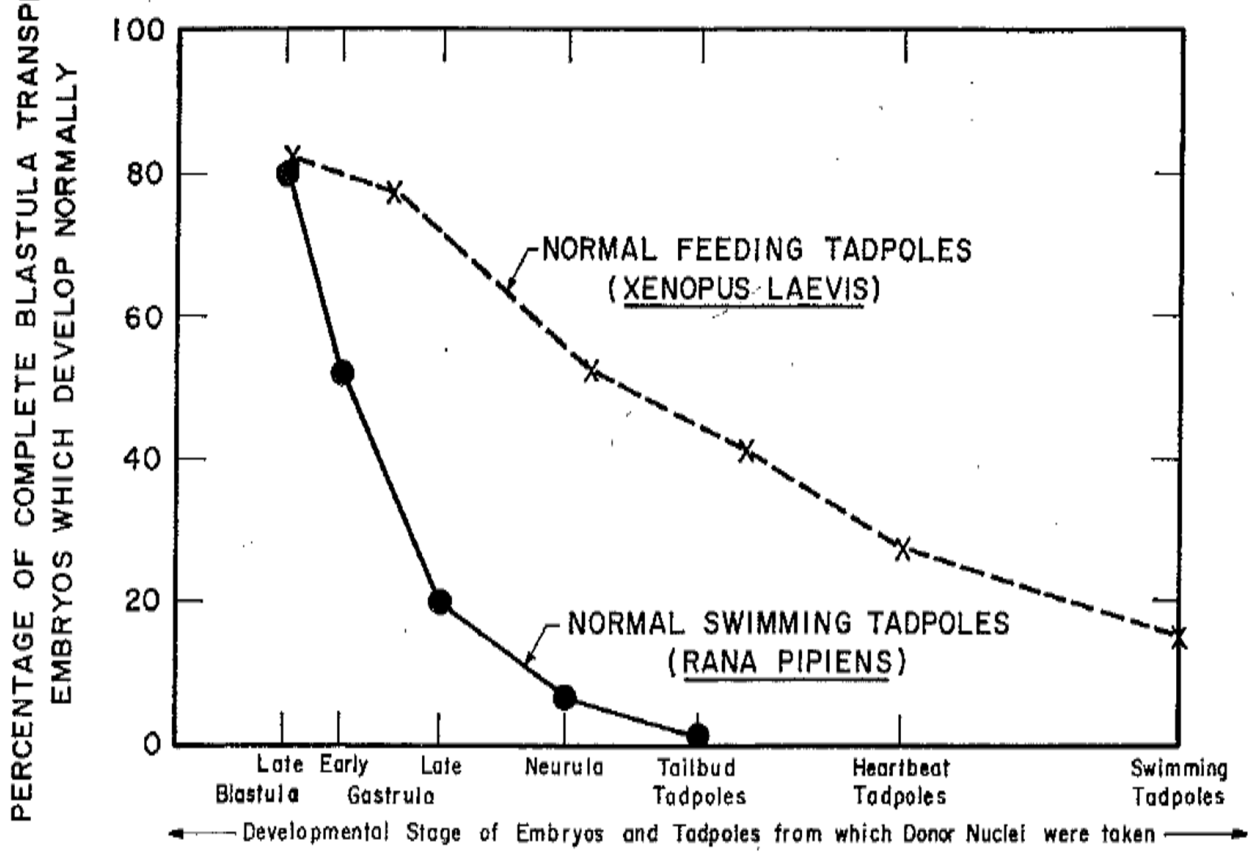
Cloned adult vertebrate (1958)


Michail Fischberg



1-nucleolus　　　　2-nucleolus

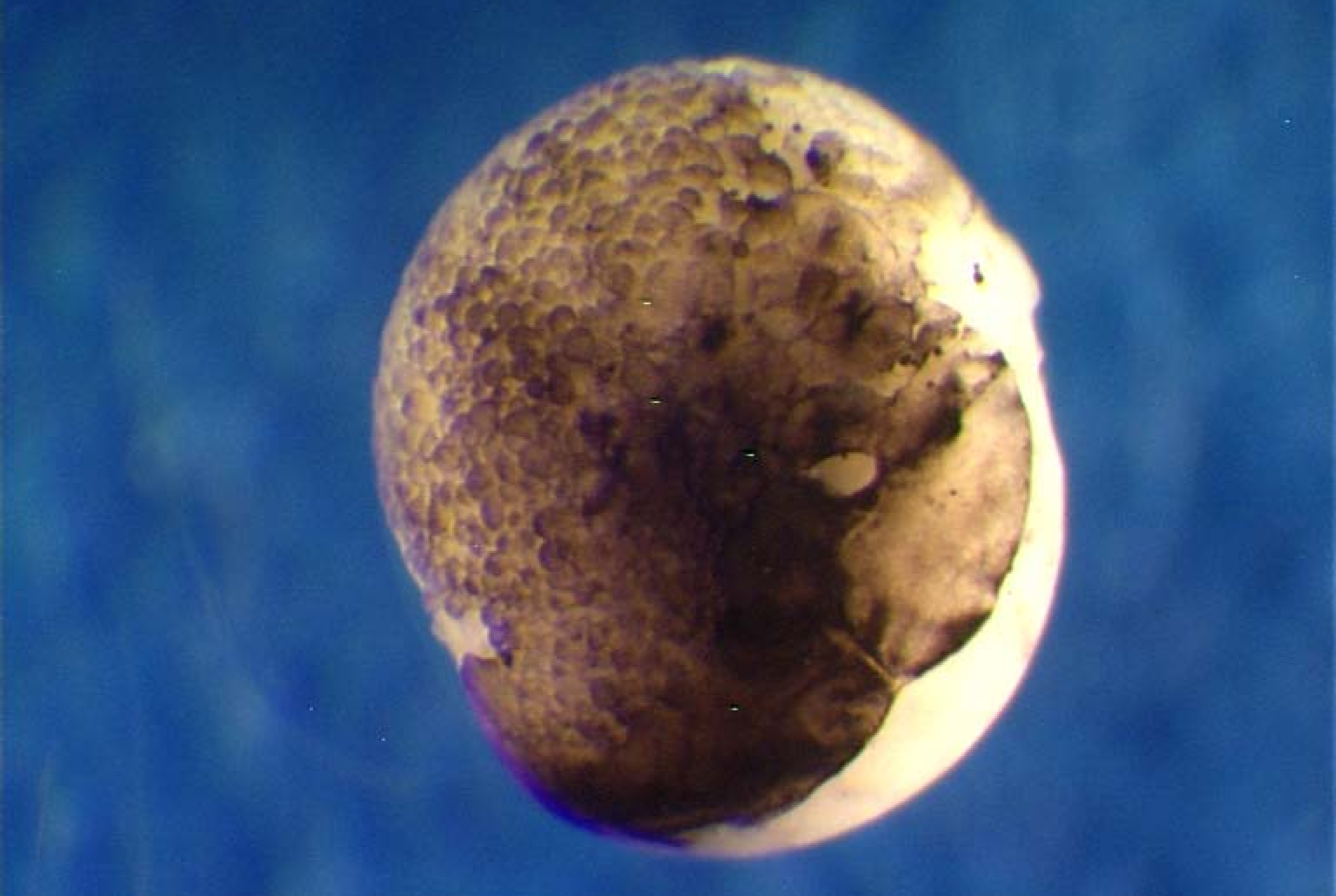



PERCENTAGE OF COMPLETE BLASTULA TRANSPLANT-EMBRYOS WHICH DEVELOP NORMALLY
100
80
60
40
20
0
NORMAL FEEDING TADPOLES
(XENOPUS LAEVIS)
NORMAL SWIMMING TADPOLES
(RANA PIPIENS)
Late Blastula
Early Gastrula
Late
Neurula
Tailbud Tadpoles
Heartbeat Tadpoles
Swimming Tadpoles
Developmental Stage of Embryos and Tadpoles from which Donor Nuclei were taken

Intestinal Tract of Feeding Tadpole

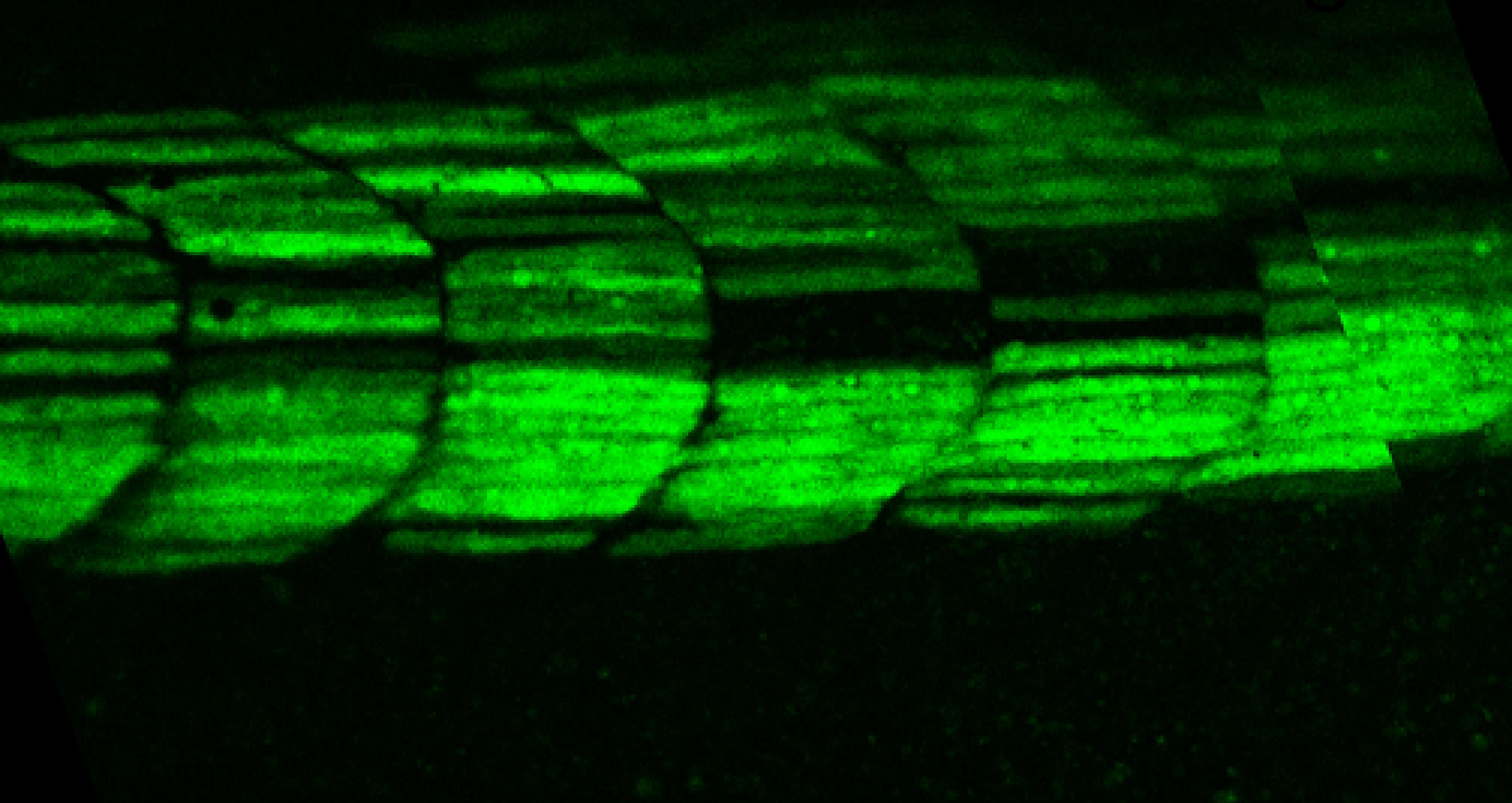
(GFP-marked))

Partial blastula



Nuclear Transplant Embryo Graft

GFP-muscle derived from intestine nuclei



Efficiency of nuclear reprogramming by nuclear transfer to eggs

Switch between cell-types:
e.g. intestinal epithelium to muscle and nerve.

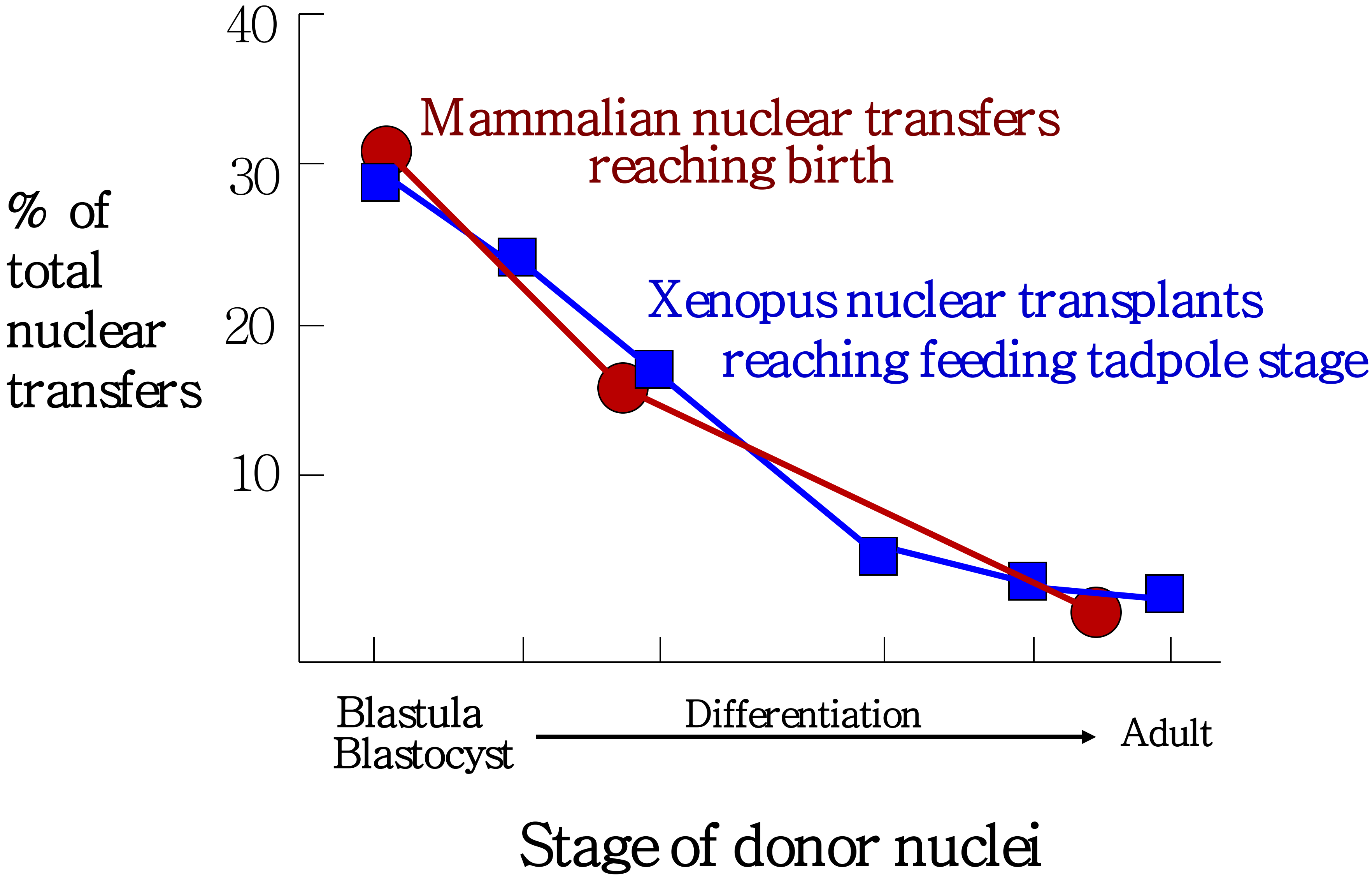
First nuclear transfers……………..……..…15%
Serial nuclear transfers…………..………..….7%
Grafts from nuclear transfer embryos……….8%

Total: 30%

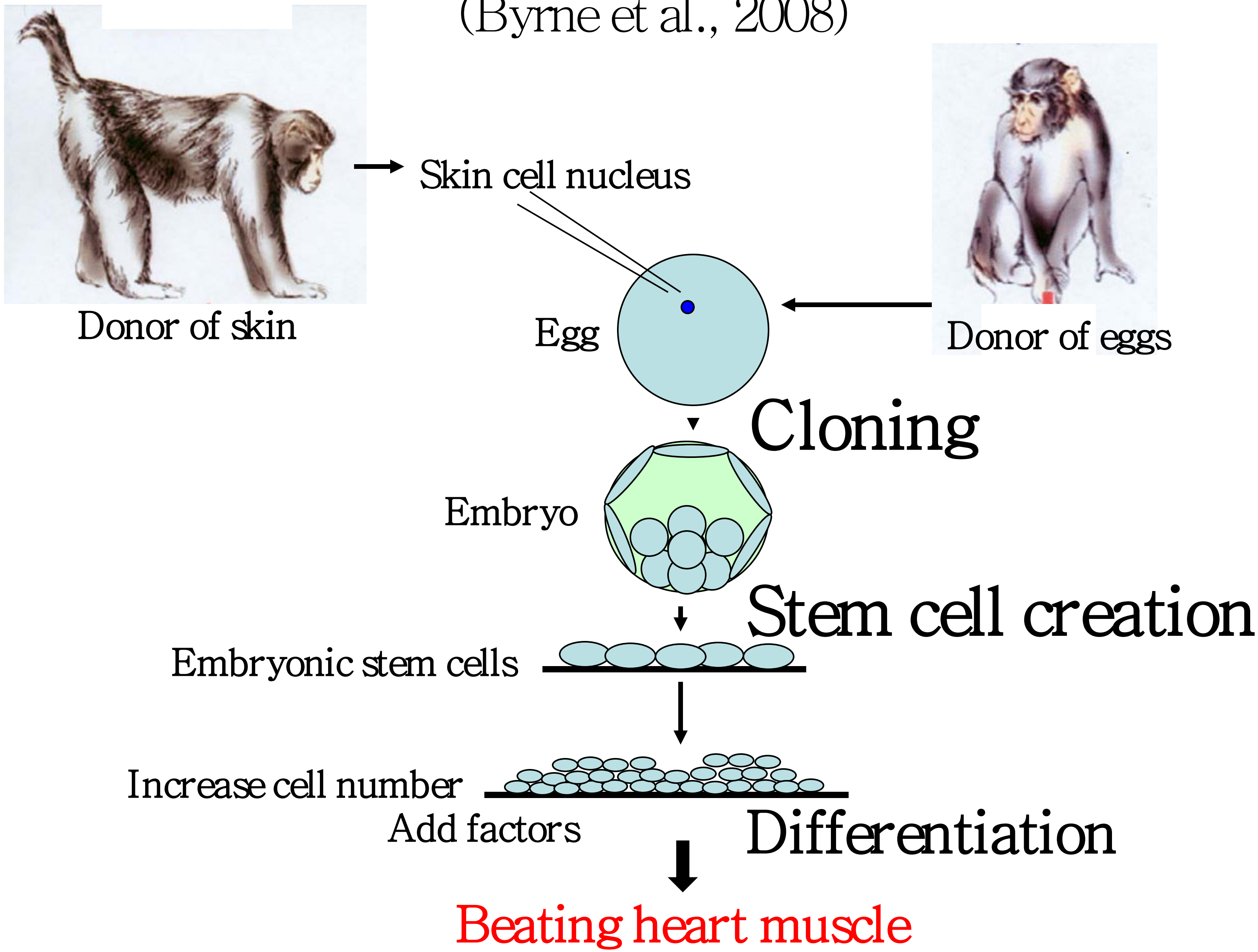
The cloning of sheep

Wilmut, Campbell et al 1996 and 1997.

Nuclear transfer success decreases as donor cells differentiate



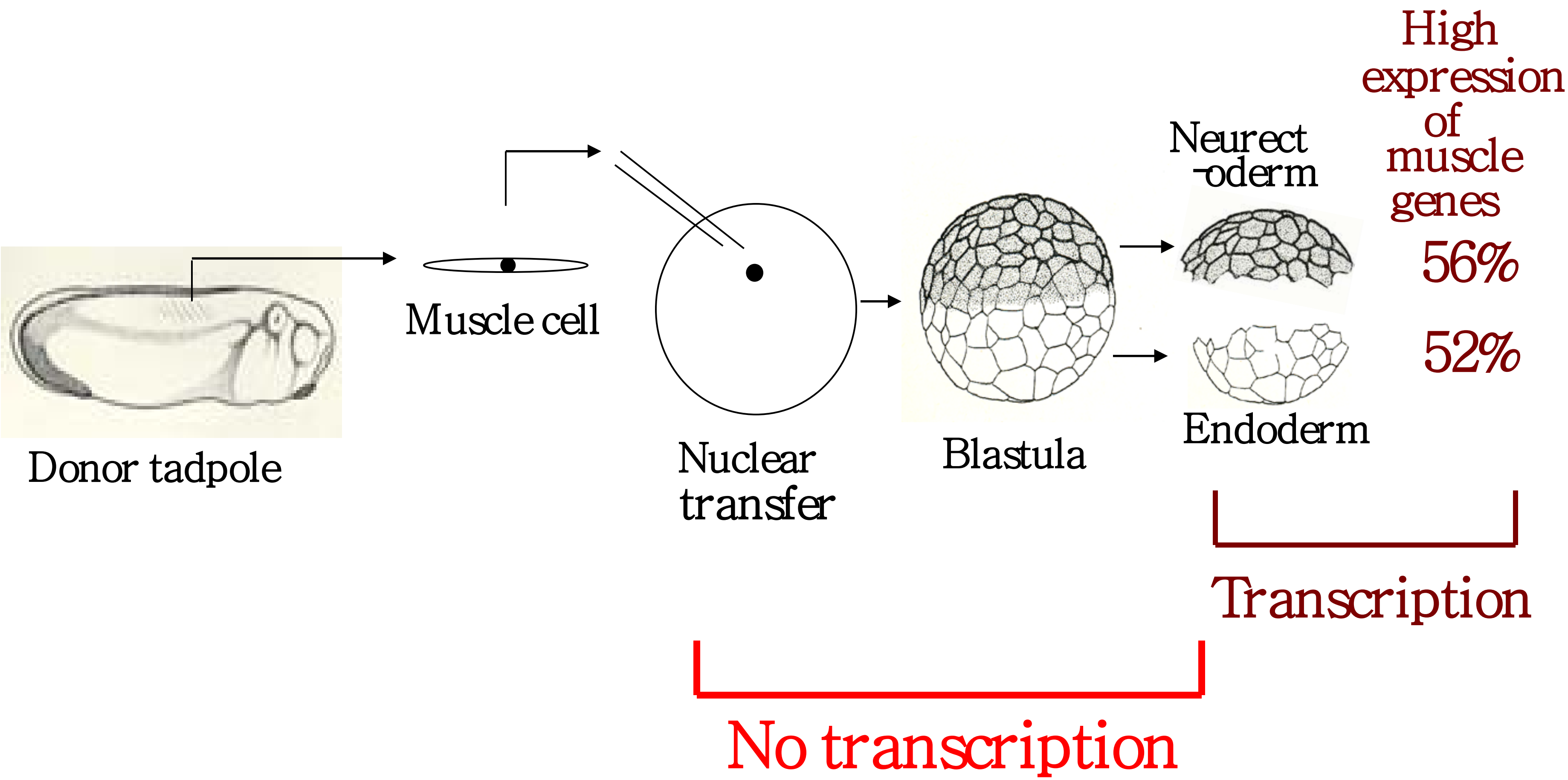
Derivation of functional heart from adult monkey skin
(Byrne et al., 2008)



Differentiation of CRES-2 cells into cardiac tissues

Epigenetic memory

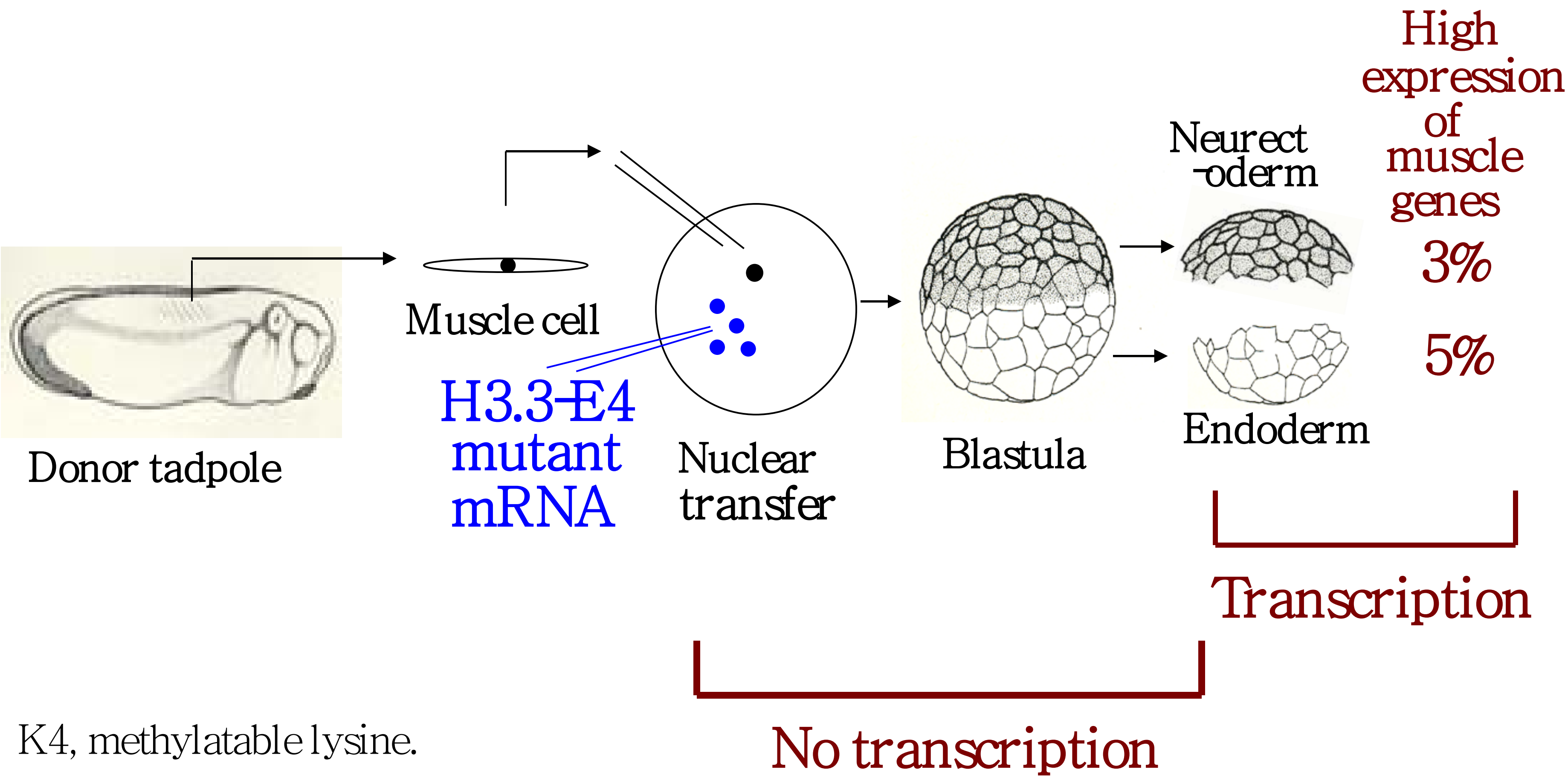
Embryos derived from muscle nuclei remember their origin even in their nerve and endoderm cells



Nature Cell Biol. 2006

H3.3 is required for epigenetic memory.

Elimination by H3.3 mutated from K4 to E4.



K4, methylatable lysine.

E4, gutamine

EPIGENETIC MEMORY

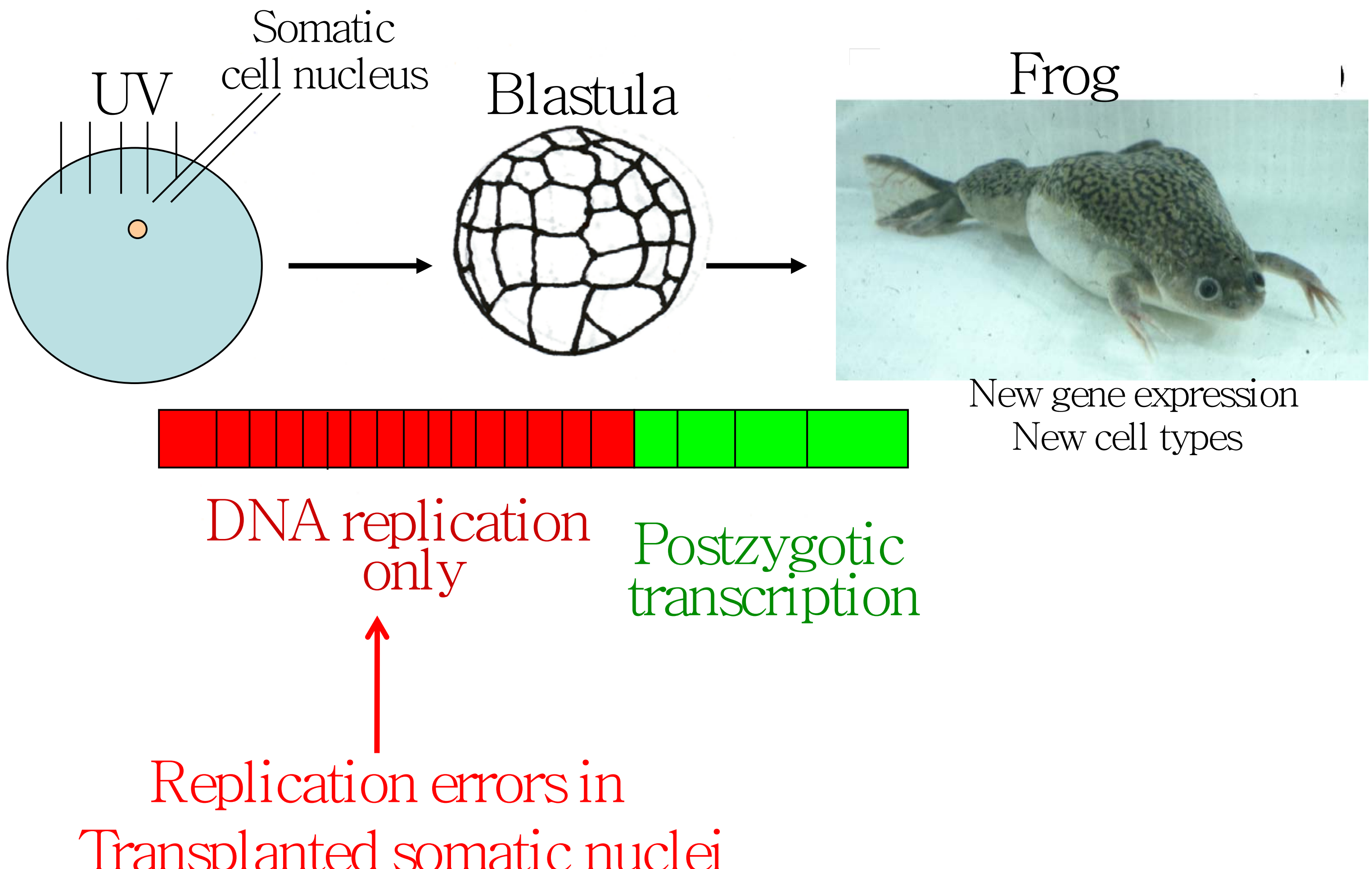
Can be explained if:

1. H3.3 promotes continuing transcription of active genes, and if
2. Egg cytoplasm reverses gene transcription with a 50% efficiency.

First meiotic prophase oocytes

to analyse the mechanism of nuclear reprogramming

Single nuclear transfer to eggs in second meiotic metaphase

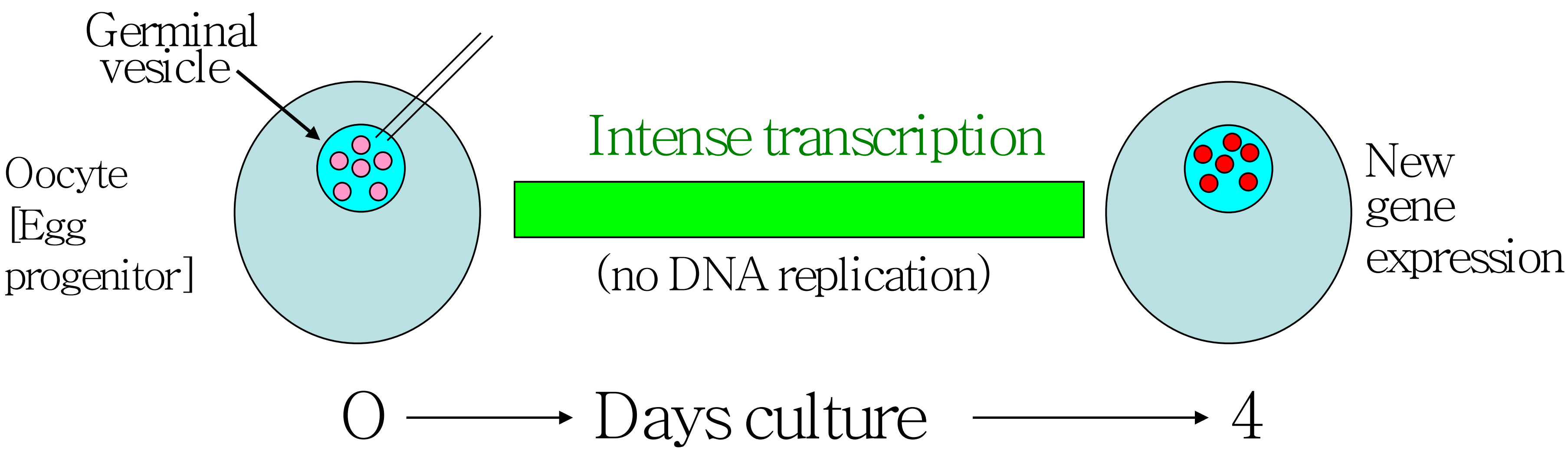


Incomplete DNA replication

damages somatic nuclei

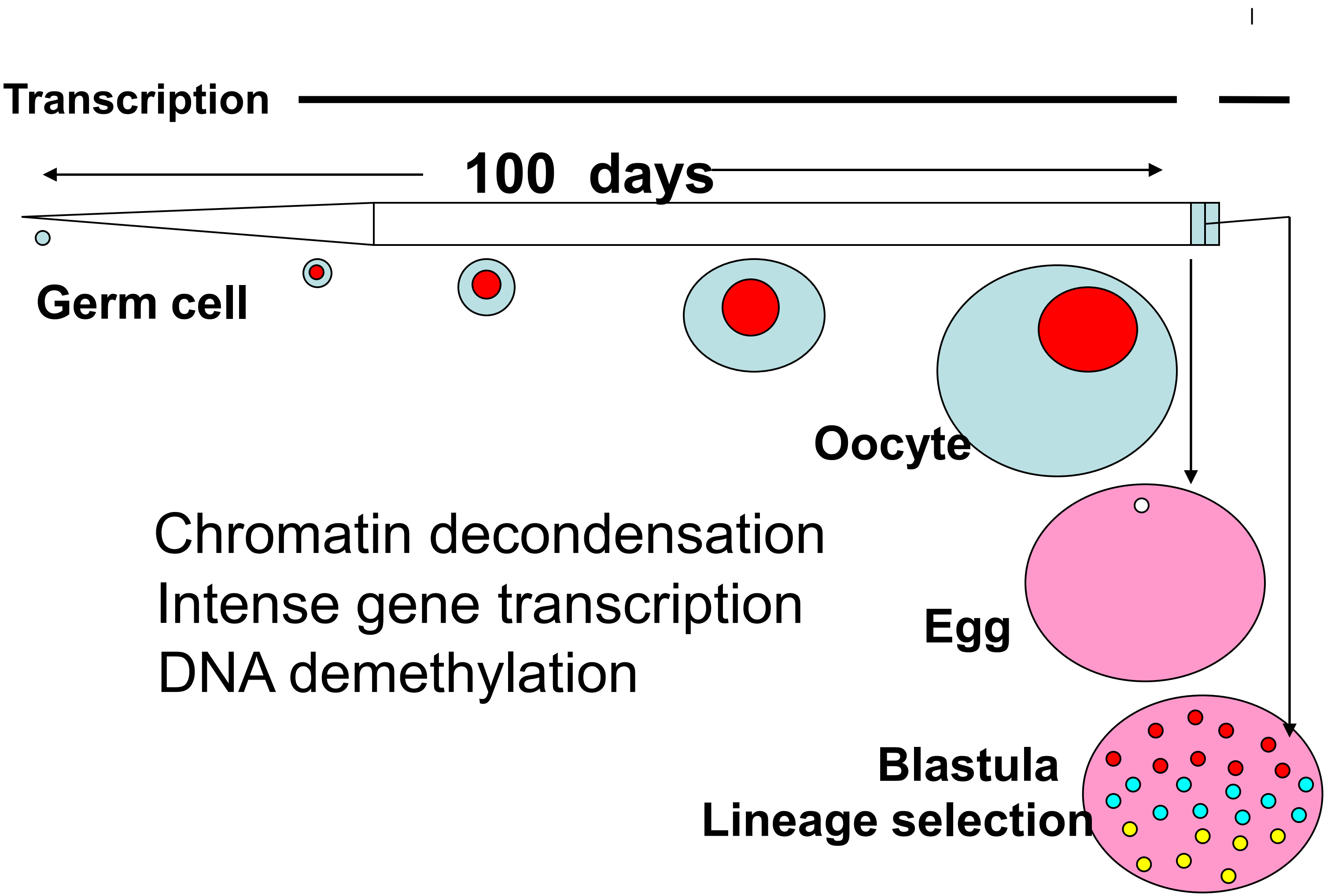
transplanted to eggs

Multiple nuclei transferred to growing oocytes in first meiotic prophase

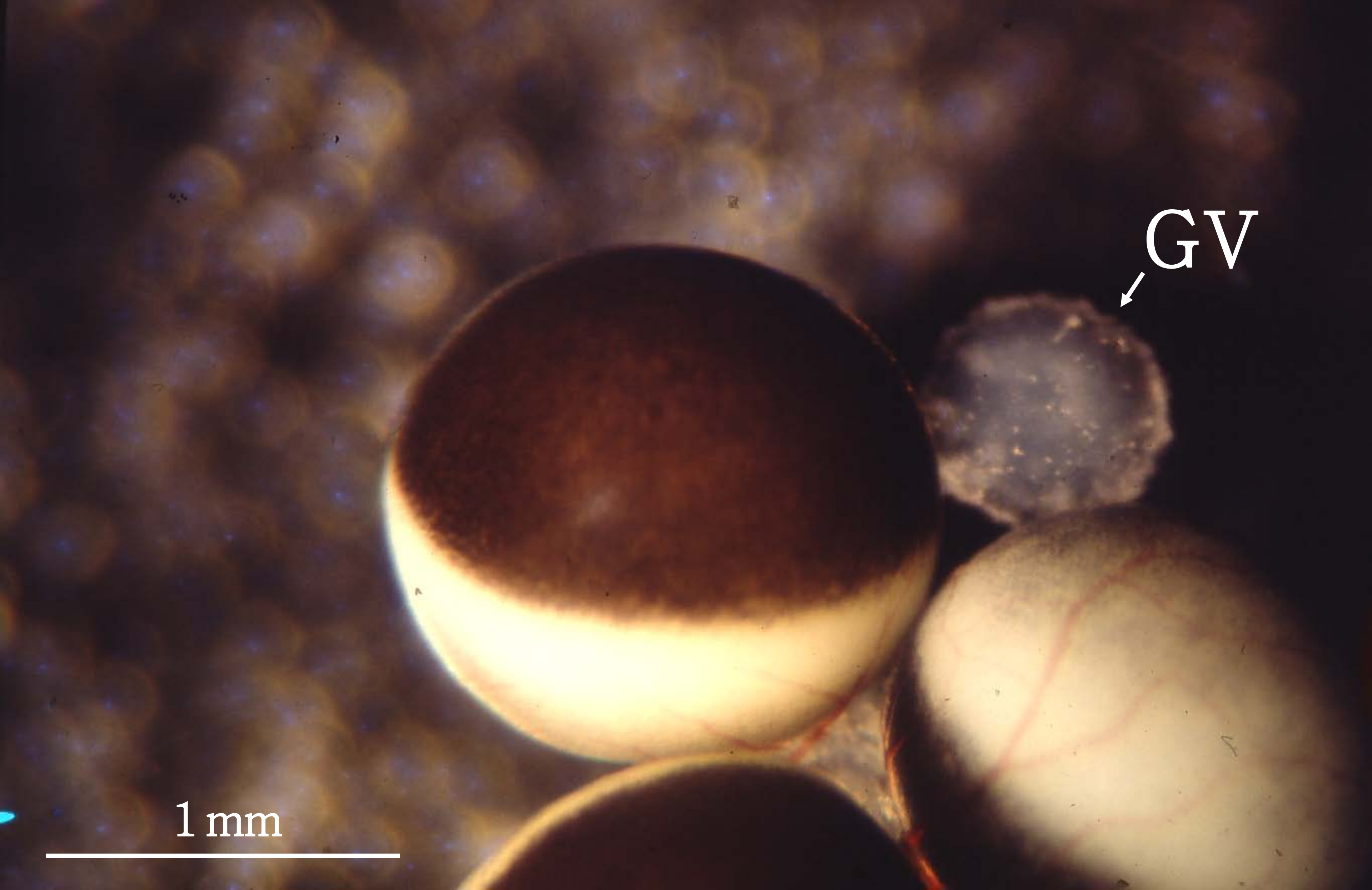


No new cell type

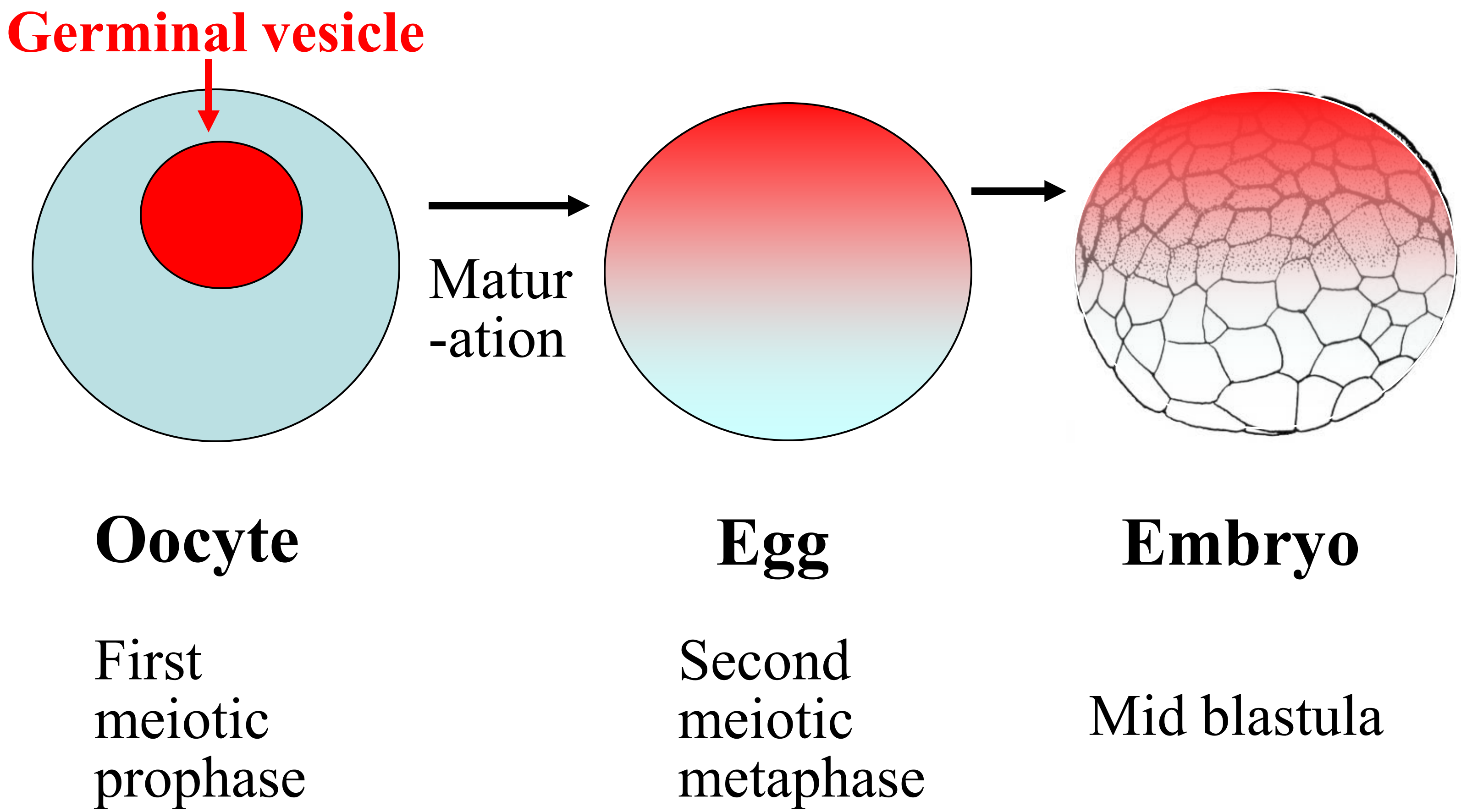
Oocyte formation prepares the egg for develpment



Xenopus oocyte and germinal vesicle

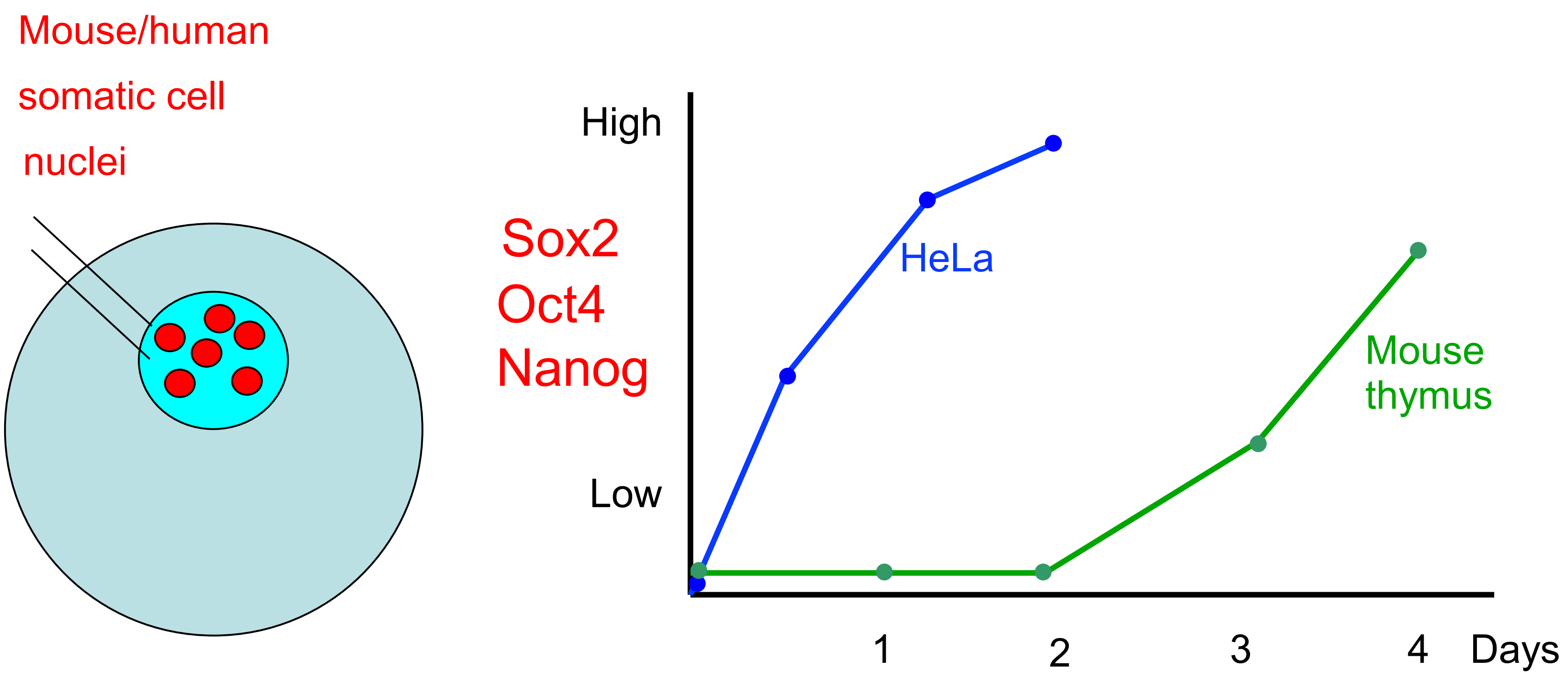


The oocyte germinal vesicle contents contribute to post-fertilization development



Mammalian stem cell genes are rapidly activated in mammalian nuclei transplanted to Xenopus oocytes

Nuclei of differentiated cells are reprogrammed slowly.



Oocyte transcription assay

Living oocyte transcription assay

1. Multiple somatic nuclei in one oocyte.

2. Linear accumulation of new transcripts.

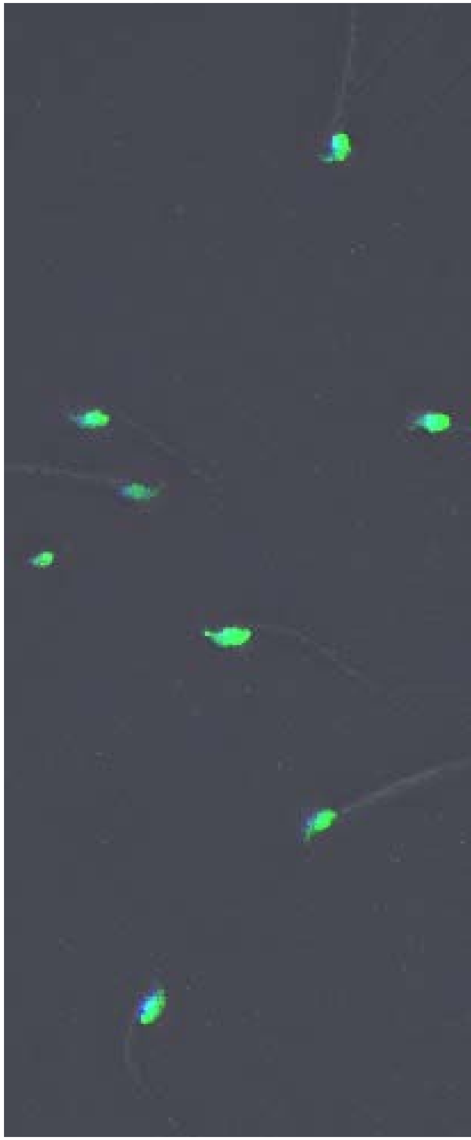
3. Multiple initiations of transcription per gene per day.

4. Oocyte injections show resistance

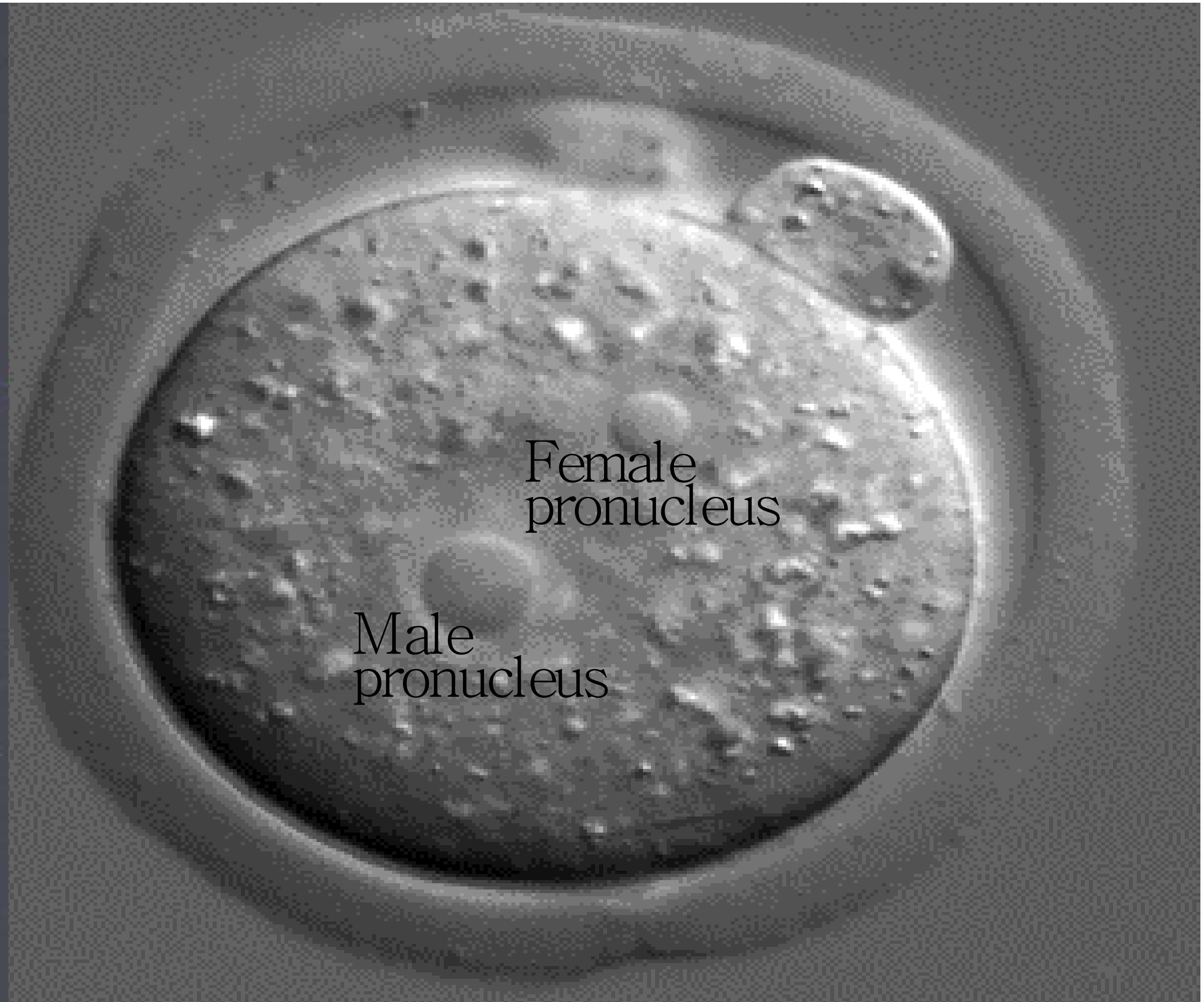
Transcriptional activation:

attack by egg cytoplasm

Mouse sperm

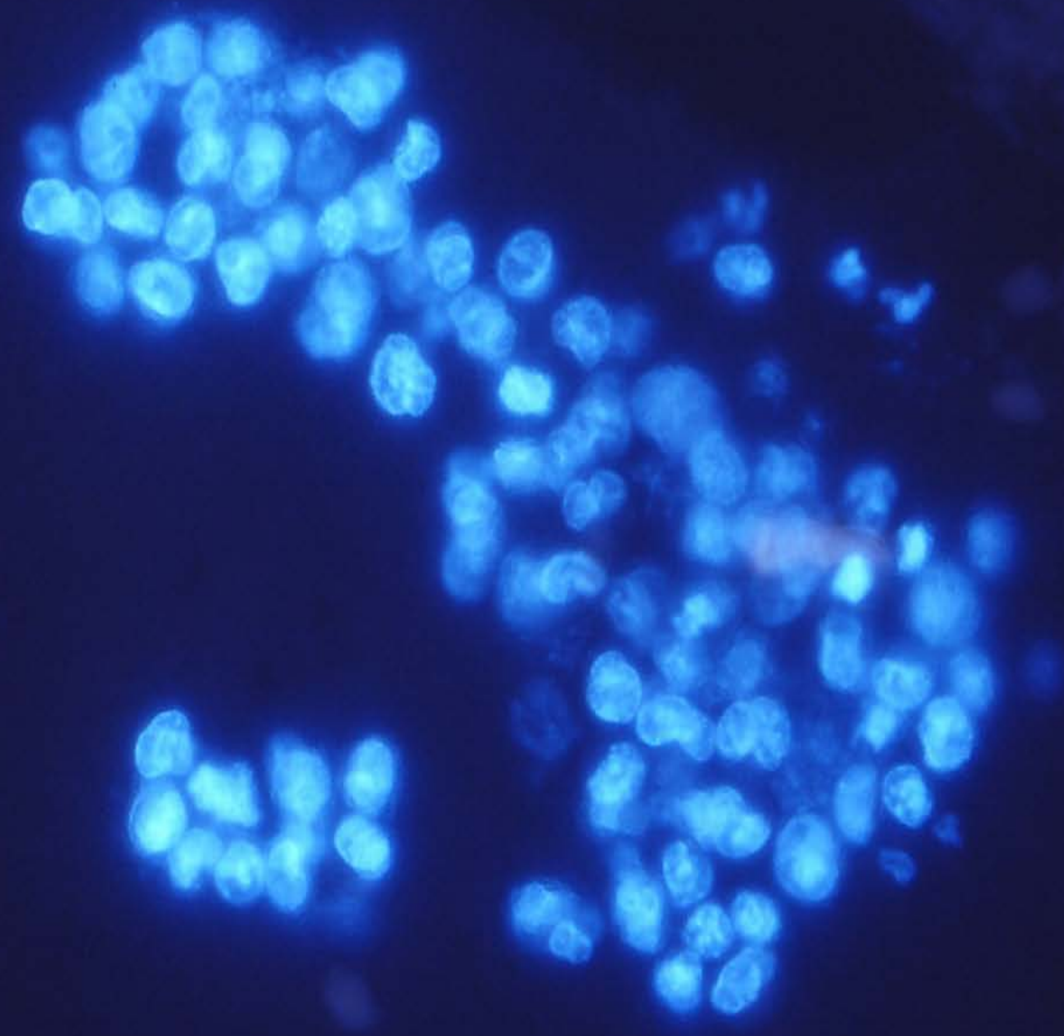


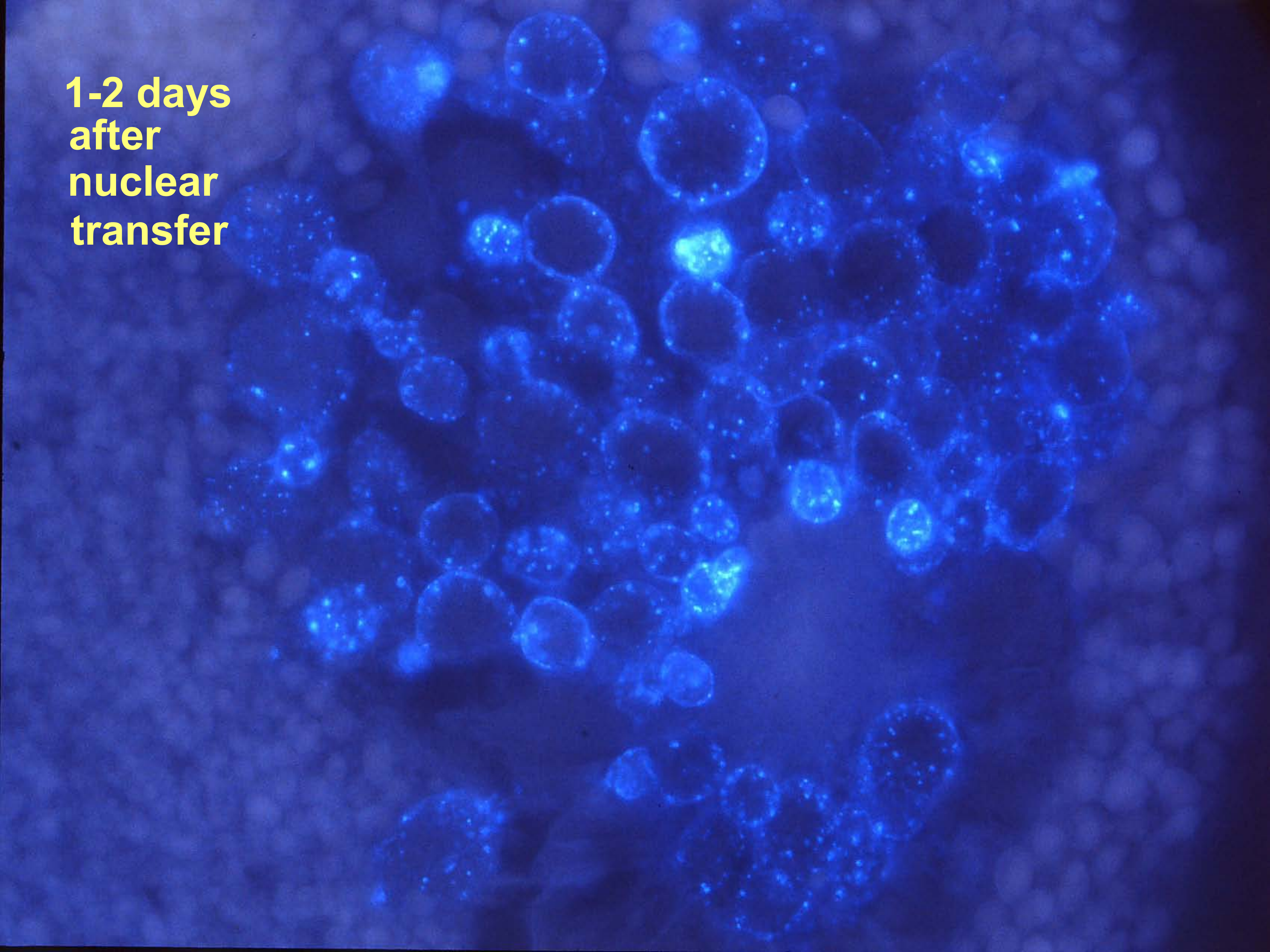
Fertilized mouse egg



Mammalian cultured cell nuclei:

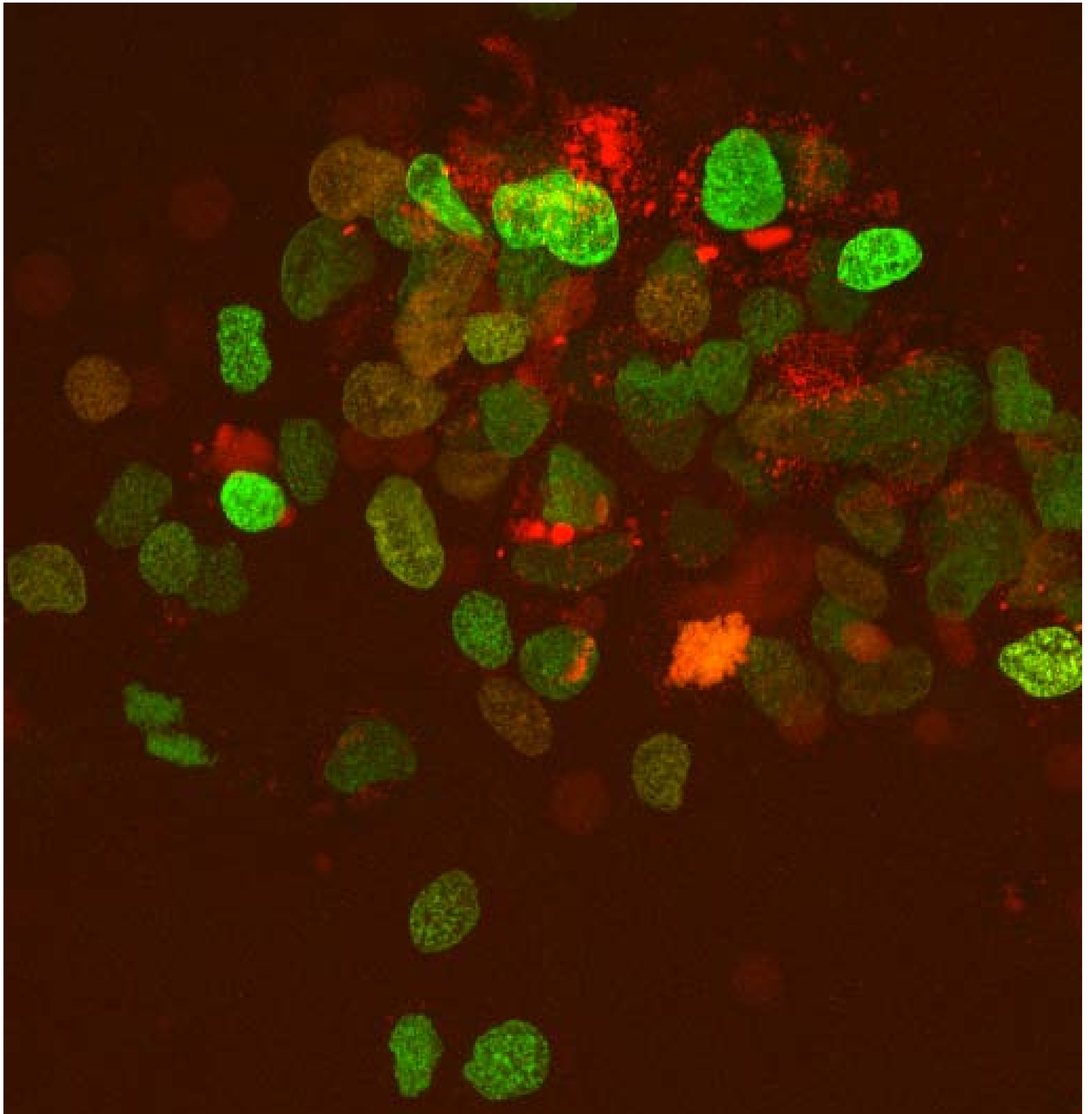
Just after injection




1-2 days
after
nuclear
transfer

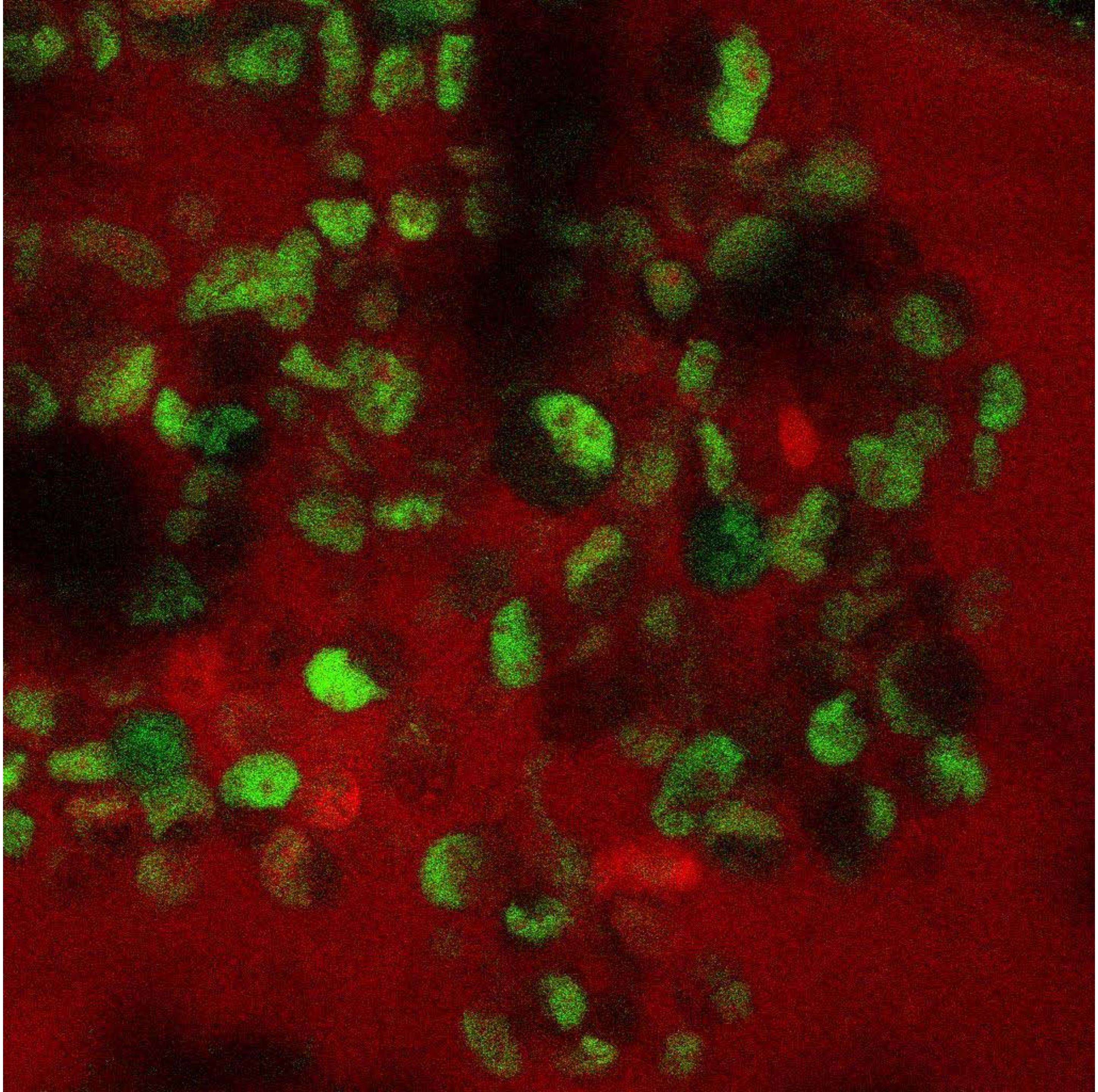
Histone replacement in transplanted nuclei.

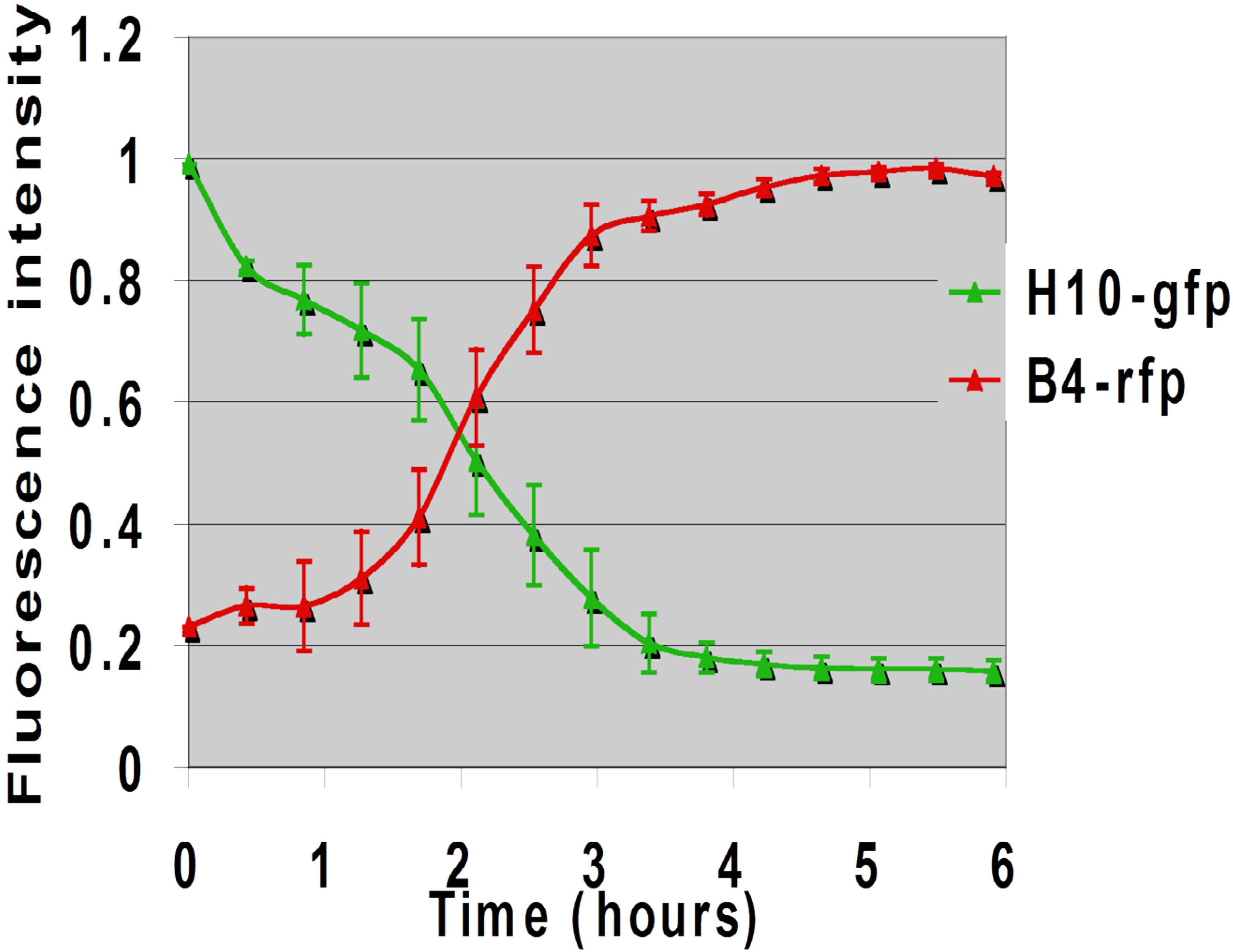
Cultured cell
nuclei in
oocyte GV



Somatic
H1o histone-GFP
replaced by
oocyte
B4 histone-RFP

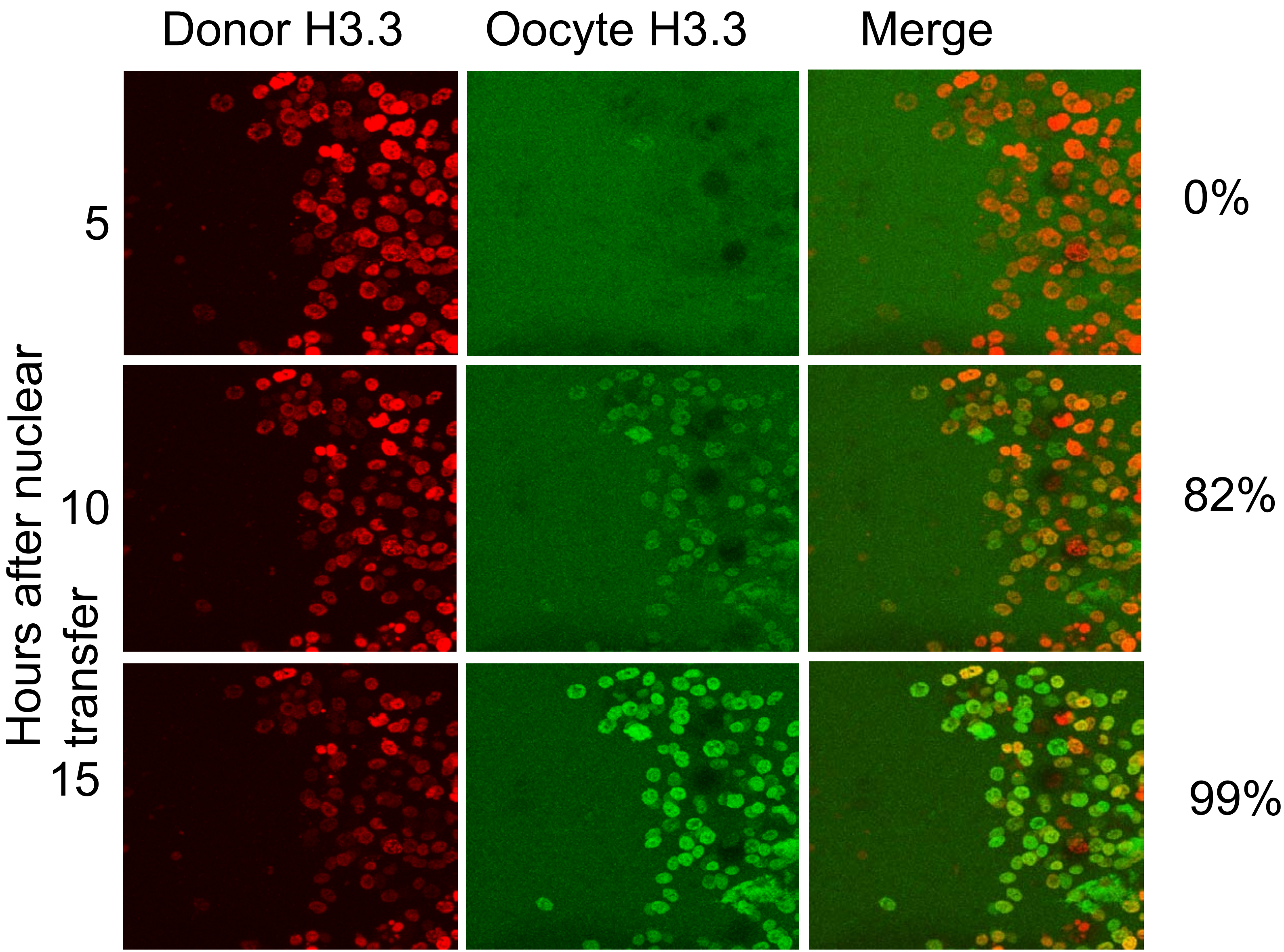
Jerome Jullien




Fluorescence intensity
1.2
1
0.8
0.6
0.4
0.2
0
H10-gfp
B4-rfp
0
1
2
3
4
5
6
Time (hours)

.

Histone H3.3 is incorporated into translanted nuclei



Uptake of linker histone and pol II correlates with reprogramming

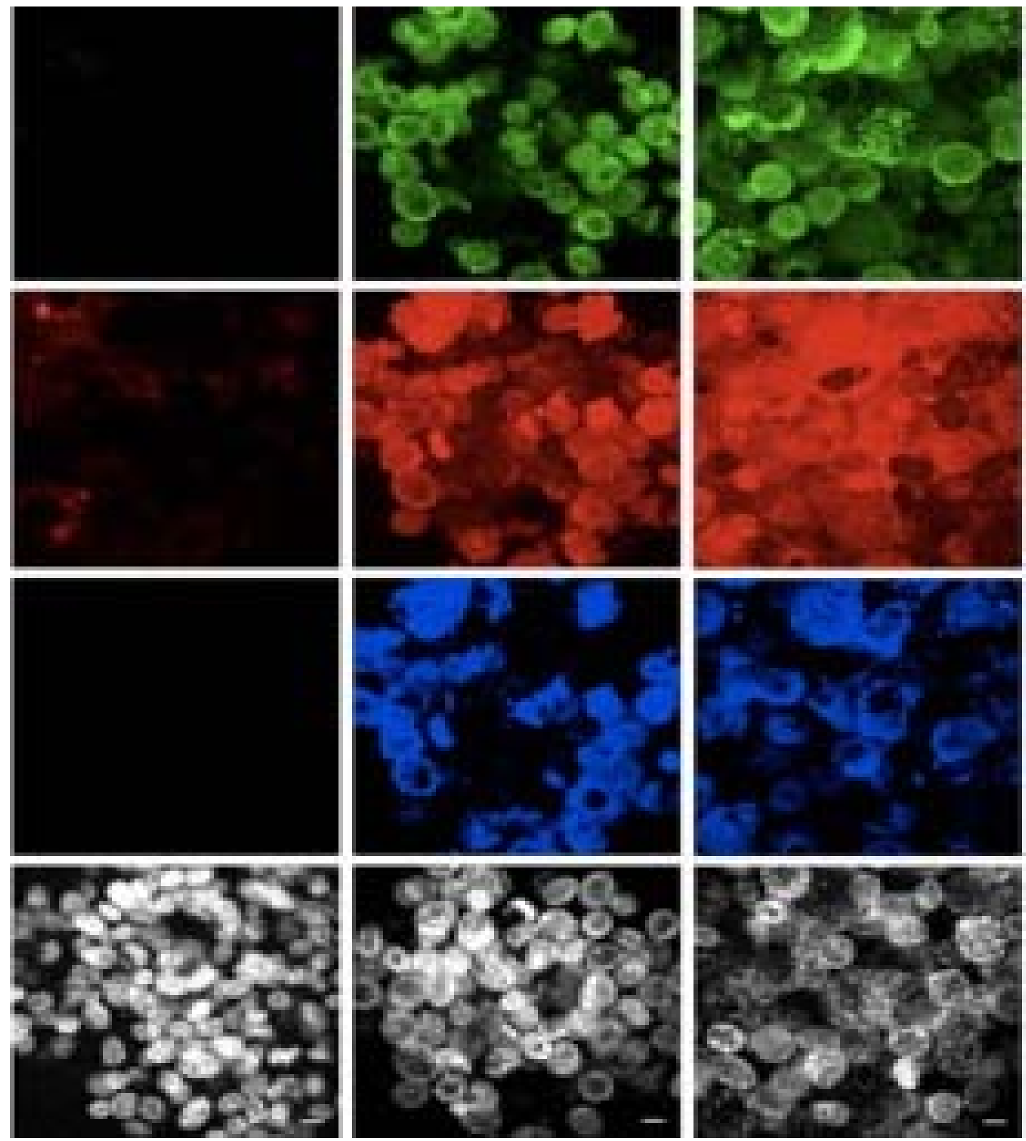
1 hour | 24 hours | 48 hours

Linker histone B4 (oocyte origin)

Polymerase II (unphosphorylated)

Polymerase II (serine 5 phosph.)

DAPI



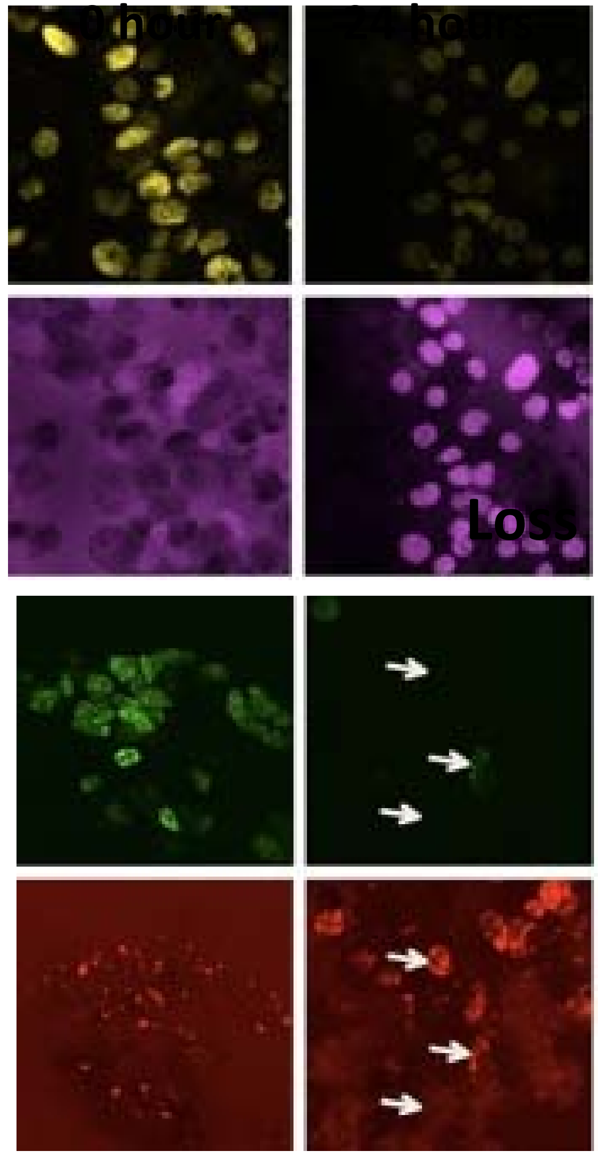
Uptake and loss of chromosomal proteins

YFP-RPB1
(RNA polymerase)

H2B-cherry
(core histone)

GFP-TBP
(TATA-binding protein
of somatic cells)

TBP2 -cherry
(TATA-binding protein
of oocytes)



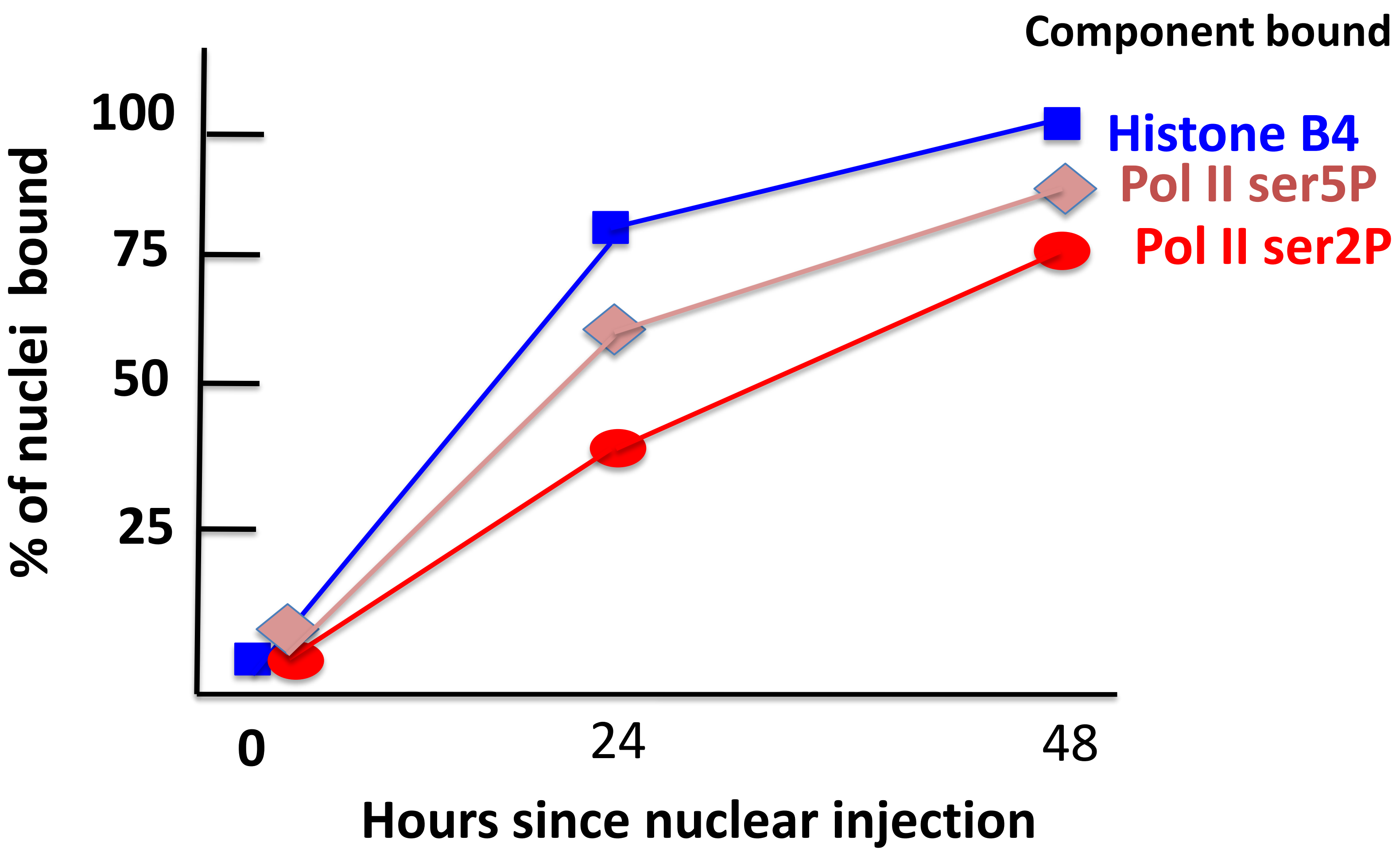
Loss

Gain

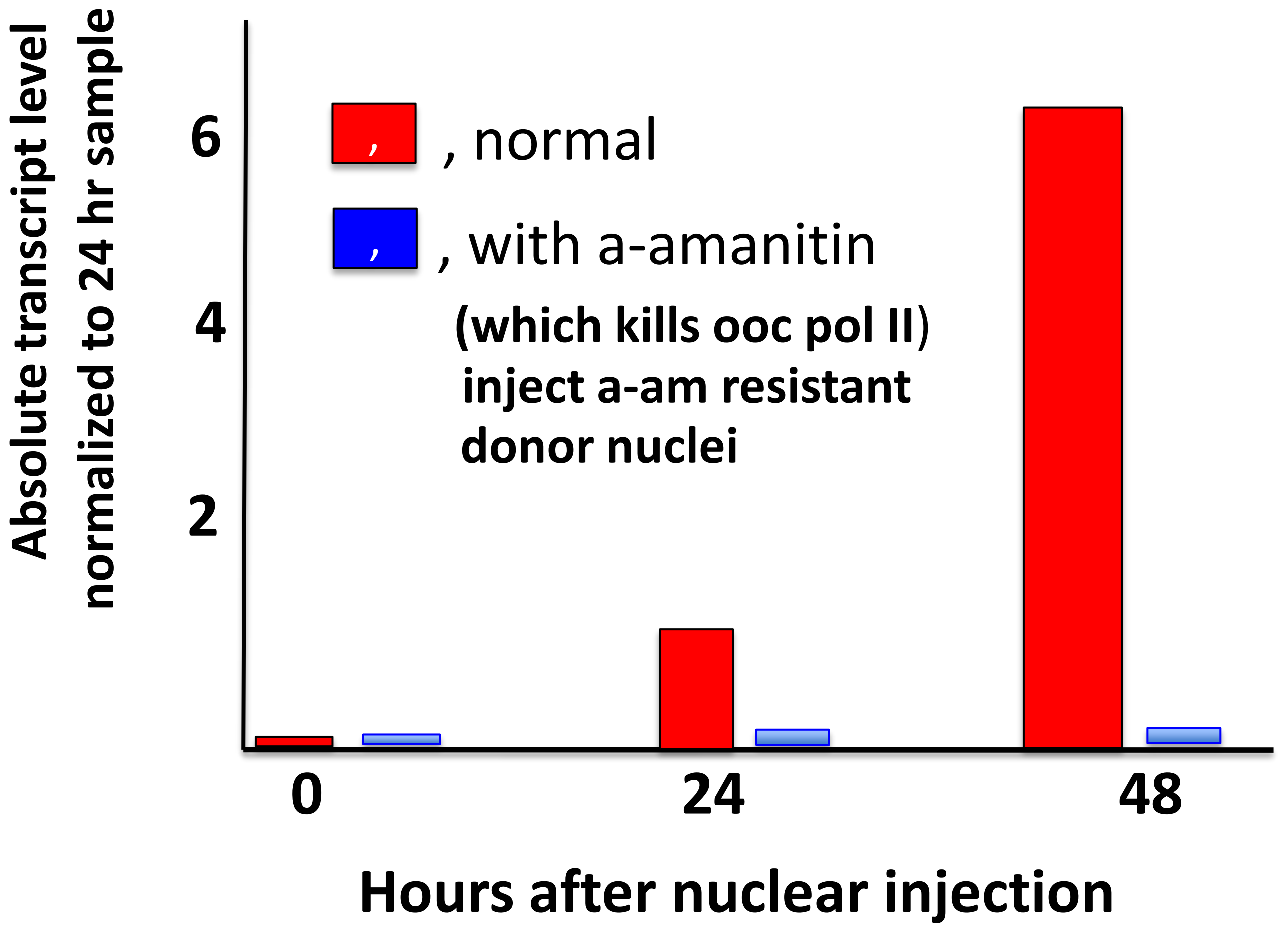
Loss

Gain

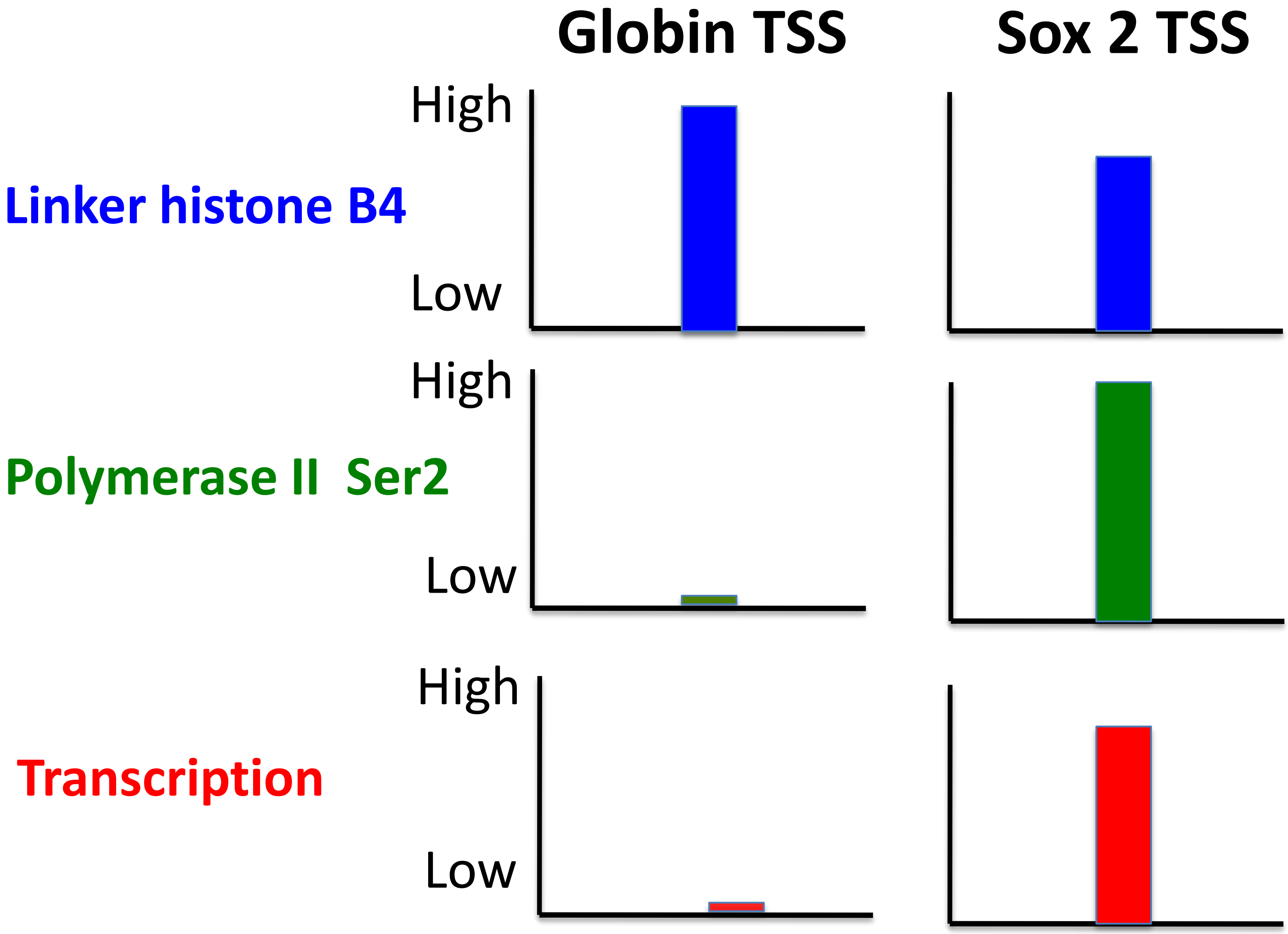
Time sequence of polymerase II components binding to genes



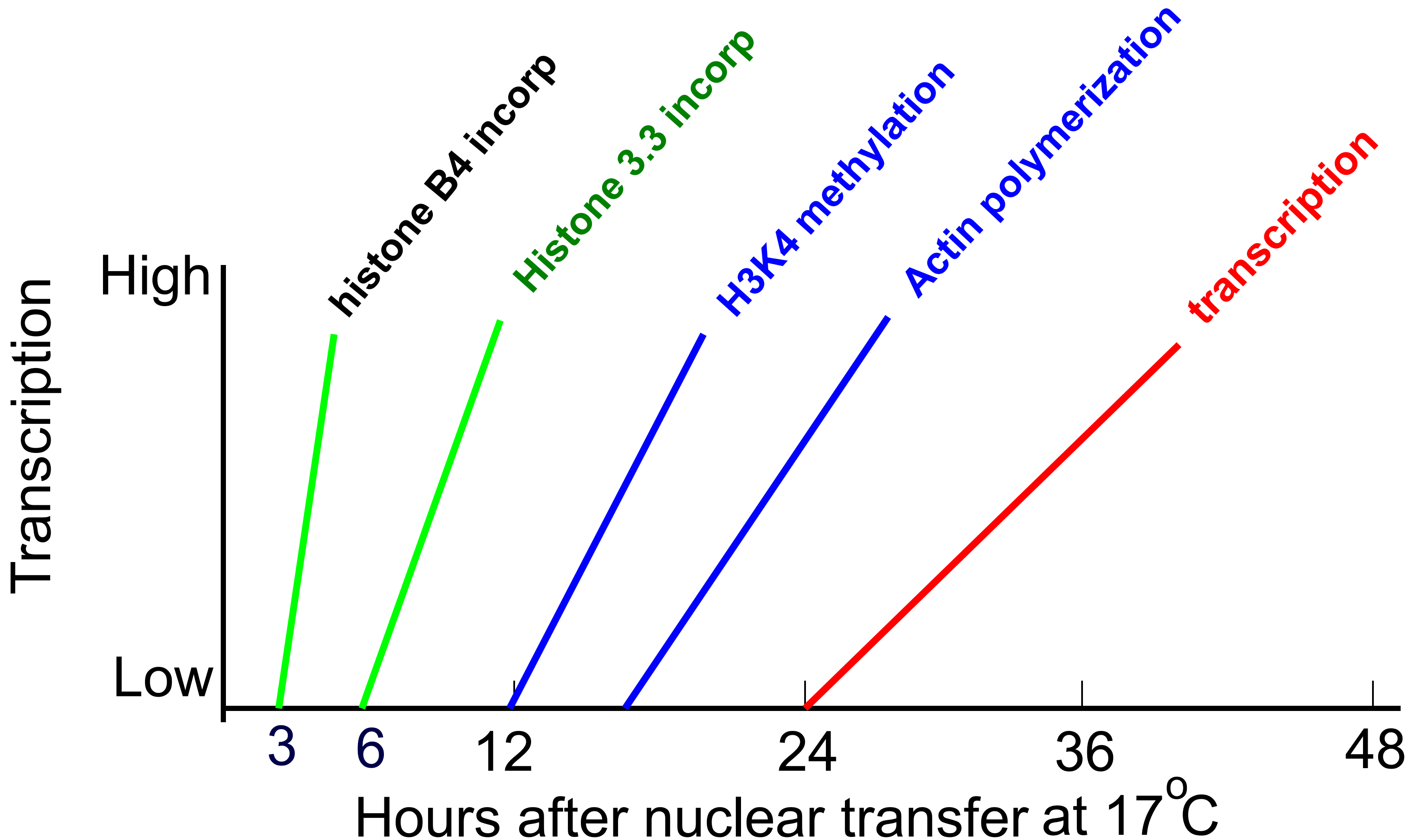
Transcriptional reprogramming depends on polymerase II of oocyte origin



Reprogramming is selective at the level of polymerase II



Time course of transcriptional activation of somatic cell nuclei by oocytes

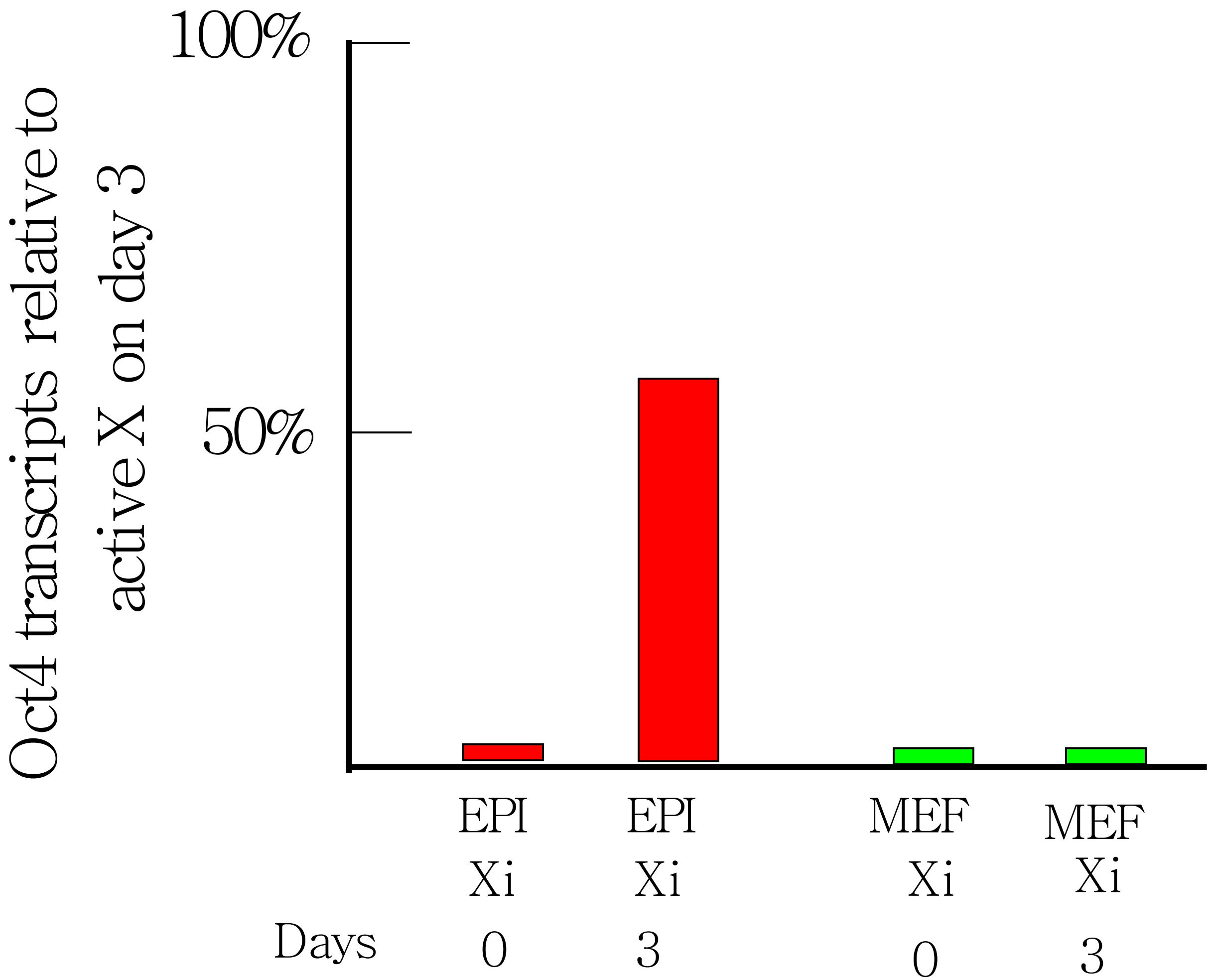


Resistance to reprogramming:

defence by the nucleus

Repressed Xi in female mammals

Epiblast-Xi, but not MEF-Xi, genes are strongly reactivated in injected oocytes



MacroH2A is knocked down by inhibitory RNA,

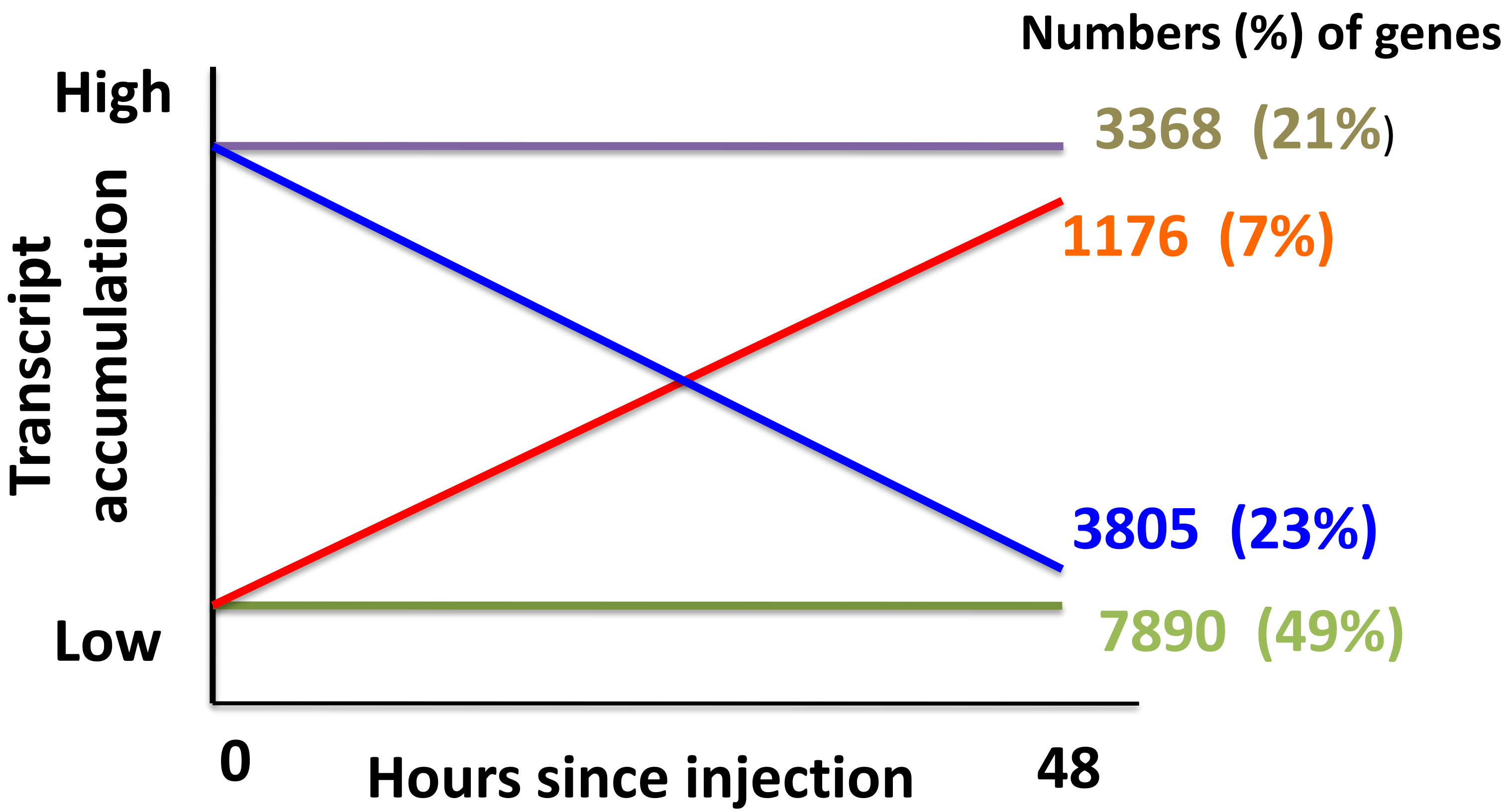
and induces Oct4 and Sox2 in MEF-Xi cells.

MacroH2A helps to explain resistance to reprogramming

Conclusion

macroH2A marks embryonic differentiation and acts as an epigenetic resistance to nuclear reprogramming

Selective gene transcription 48 hours after nuclear transfer to Xenopus oocytes

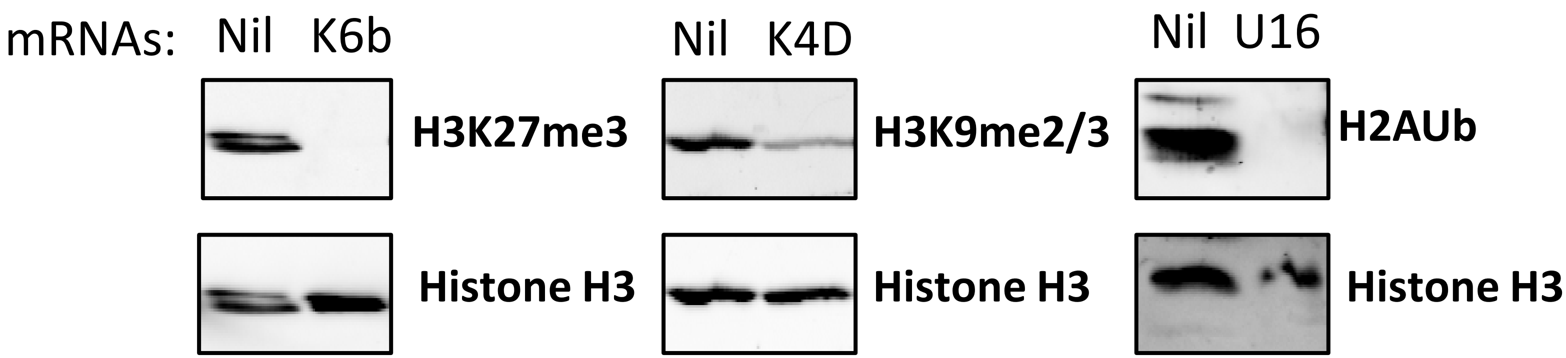


Resistance is gene and cell-type specific

Chromatin modification

Histone modifications in nuclei can be changed after transfer to oocytes

Inject mRNs on day 1.   Transplant nuclei on day 2.
Reisolate transplanted nuclei on day 3 for Western analysis



K6b, H3K27me3, H3K27 demethylase.

K4D, H3K9 demethylase.

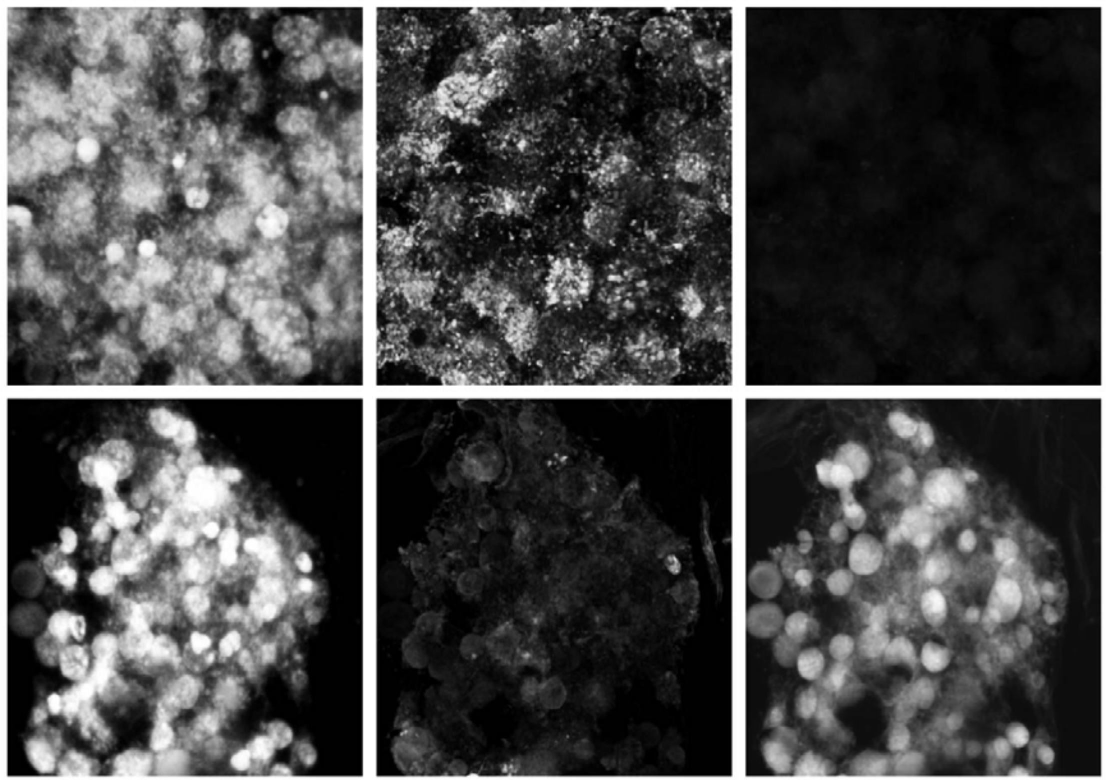
U116, H2A deubiquitinase.

Histone modifiers overexpressed in the oocyte efficiently modify transplanted nuclei chromatin

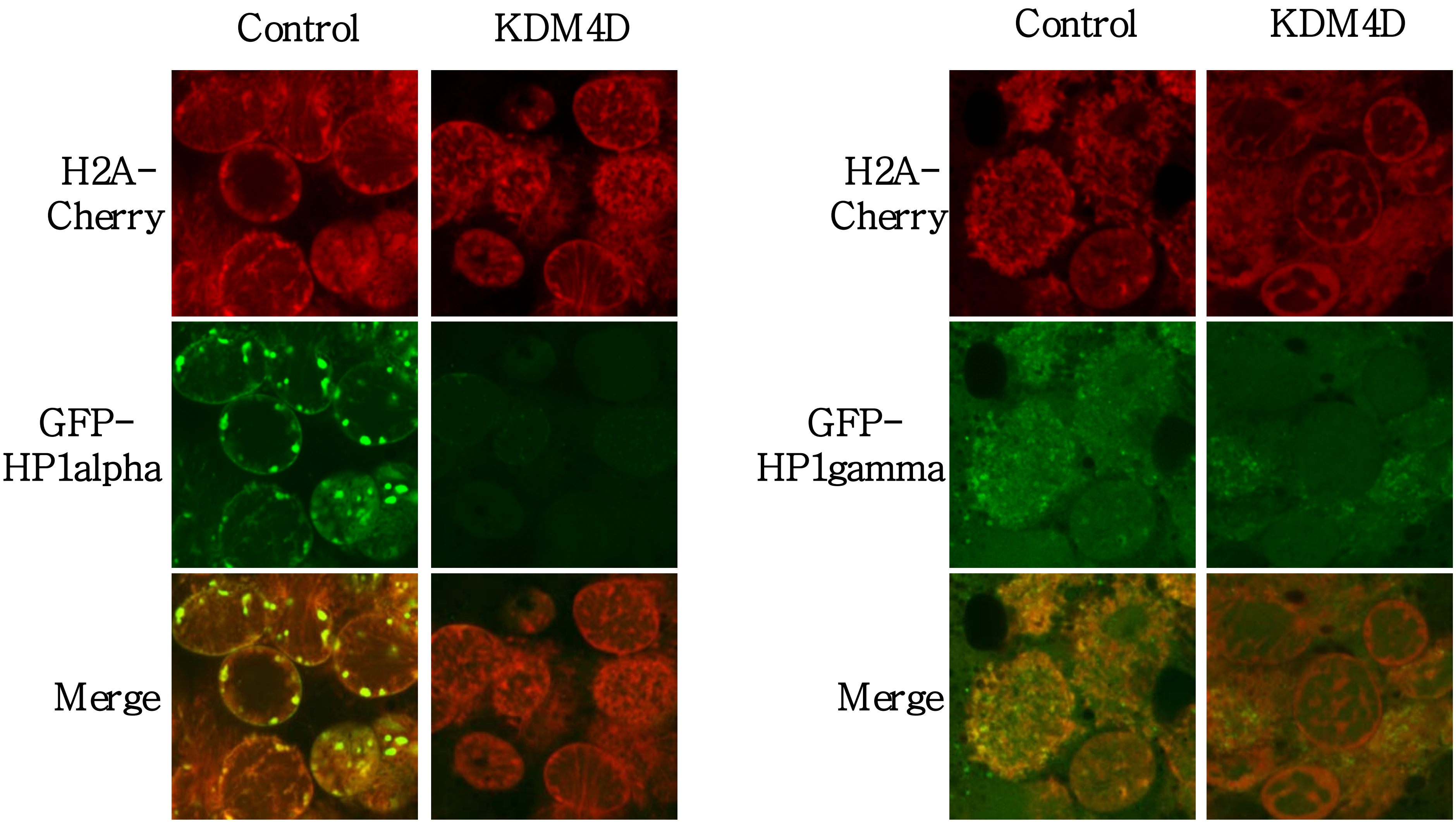
DAPI | H3K9Me3 methylation | Anti-HA

No overexpression

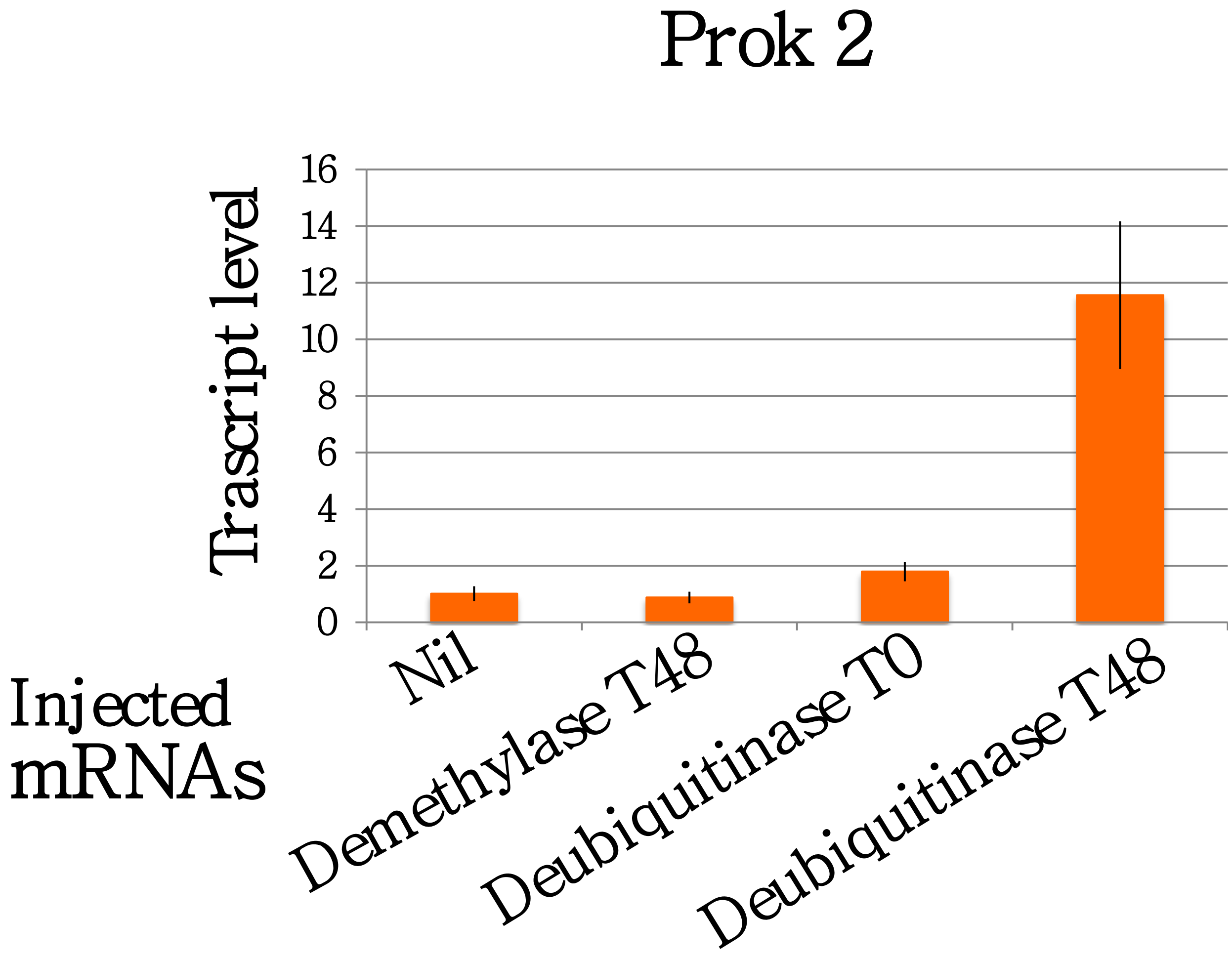
Overexpressed HA-histone demethylase



Loss of HP1 alpha binding to transplanted chromatin after lysine demethylase overexpression



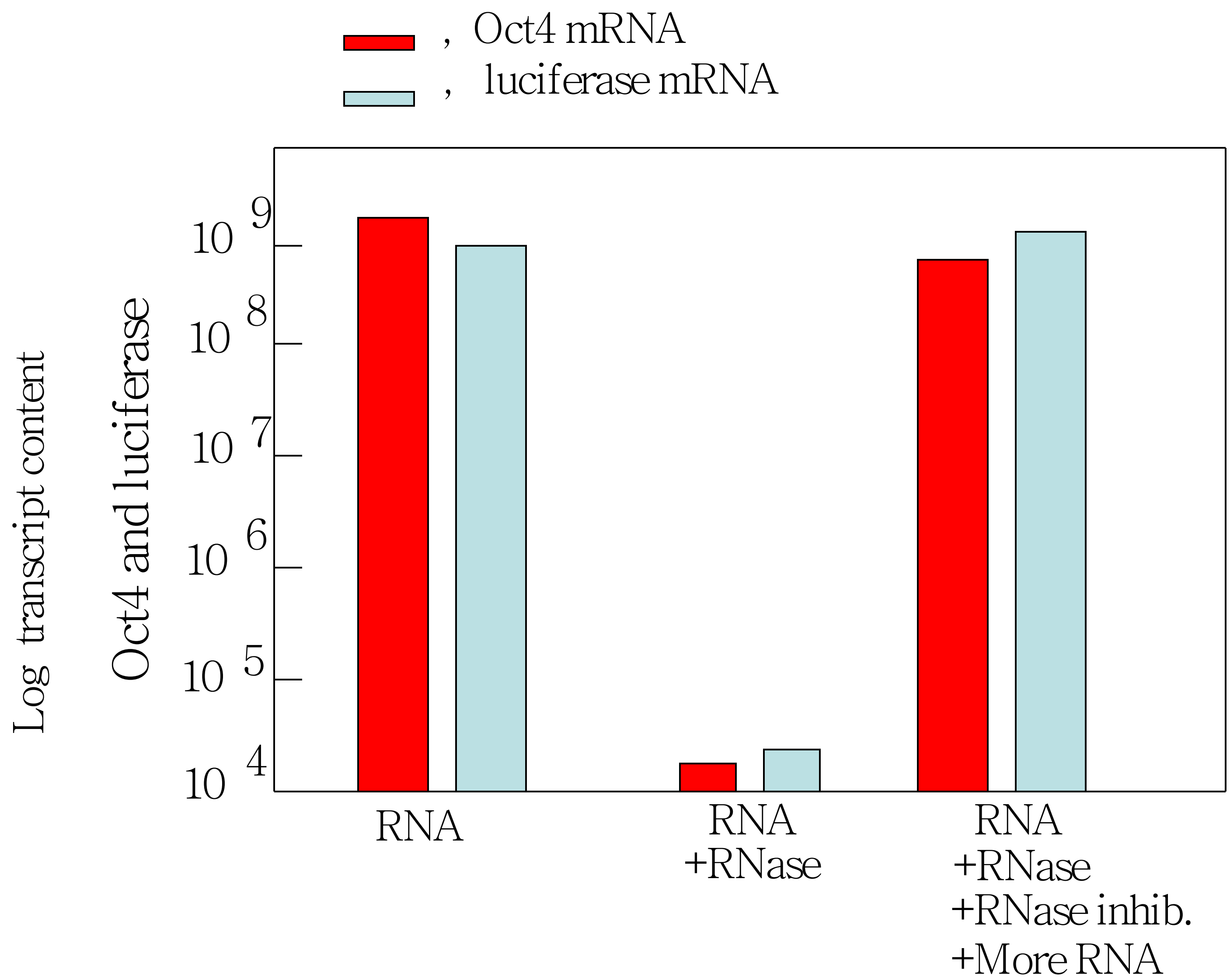
Overexpression of histone 2A deubiquitinase removes resistance



Chromatin depletion

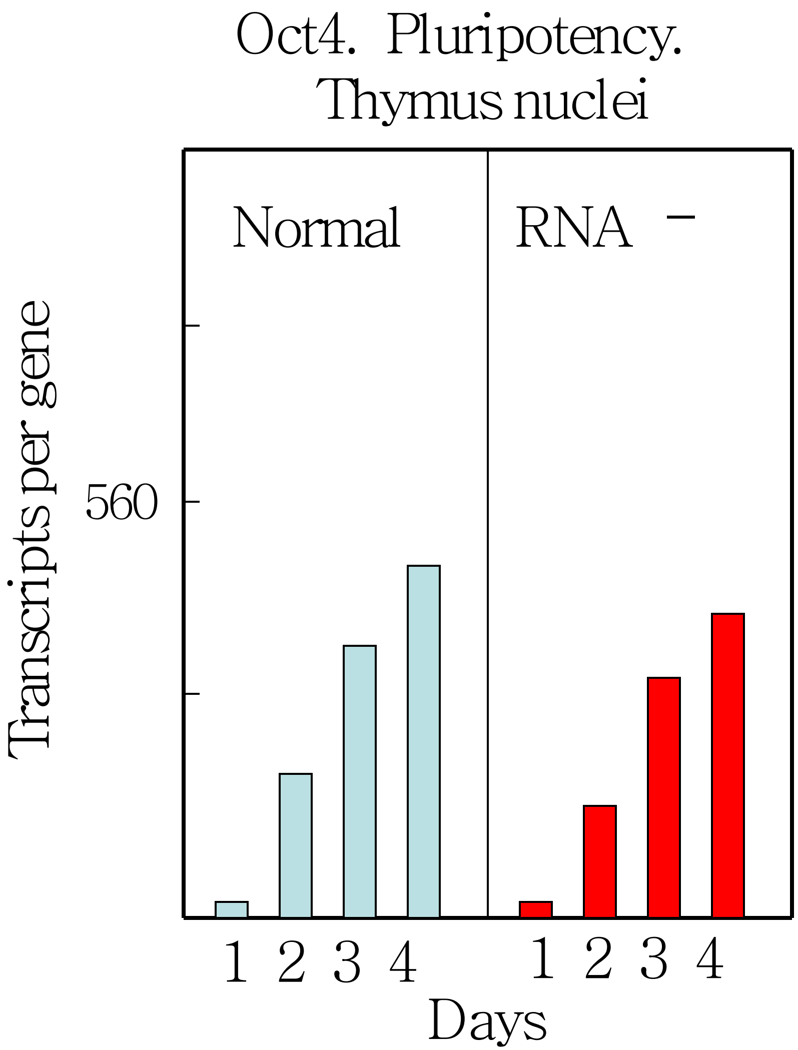
.

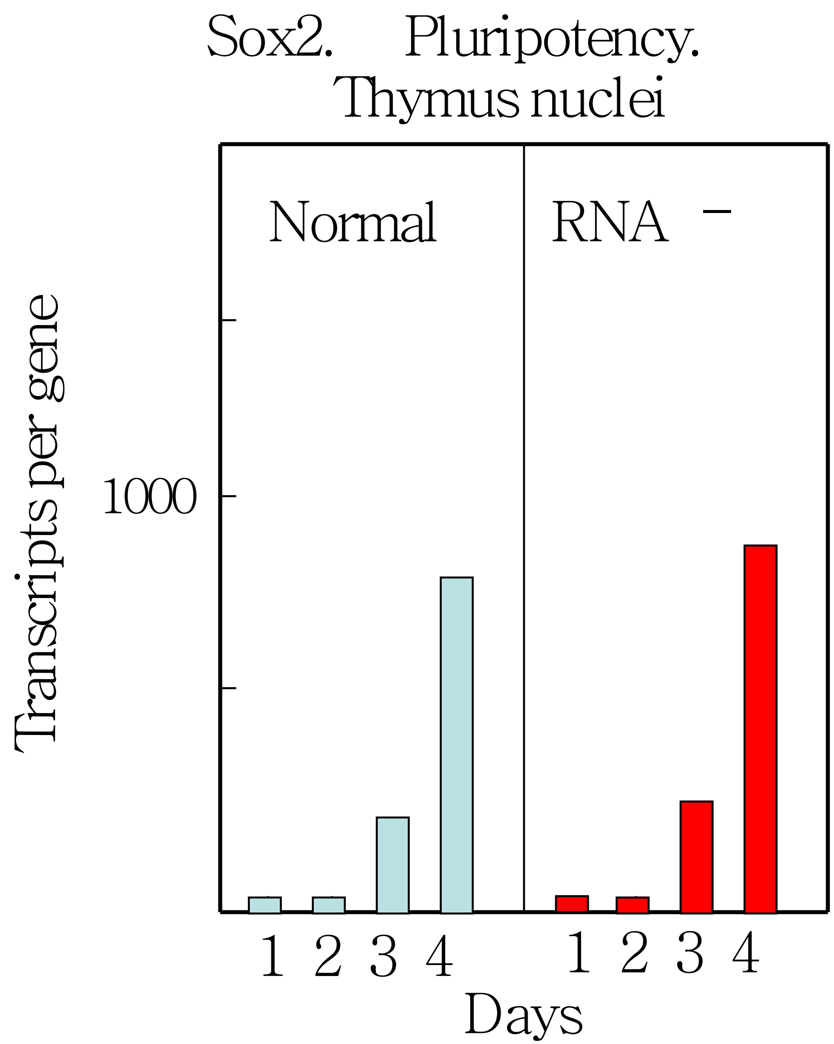
RNA is removed, replaced, and quantitated by RT-PCR



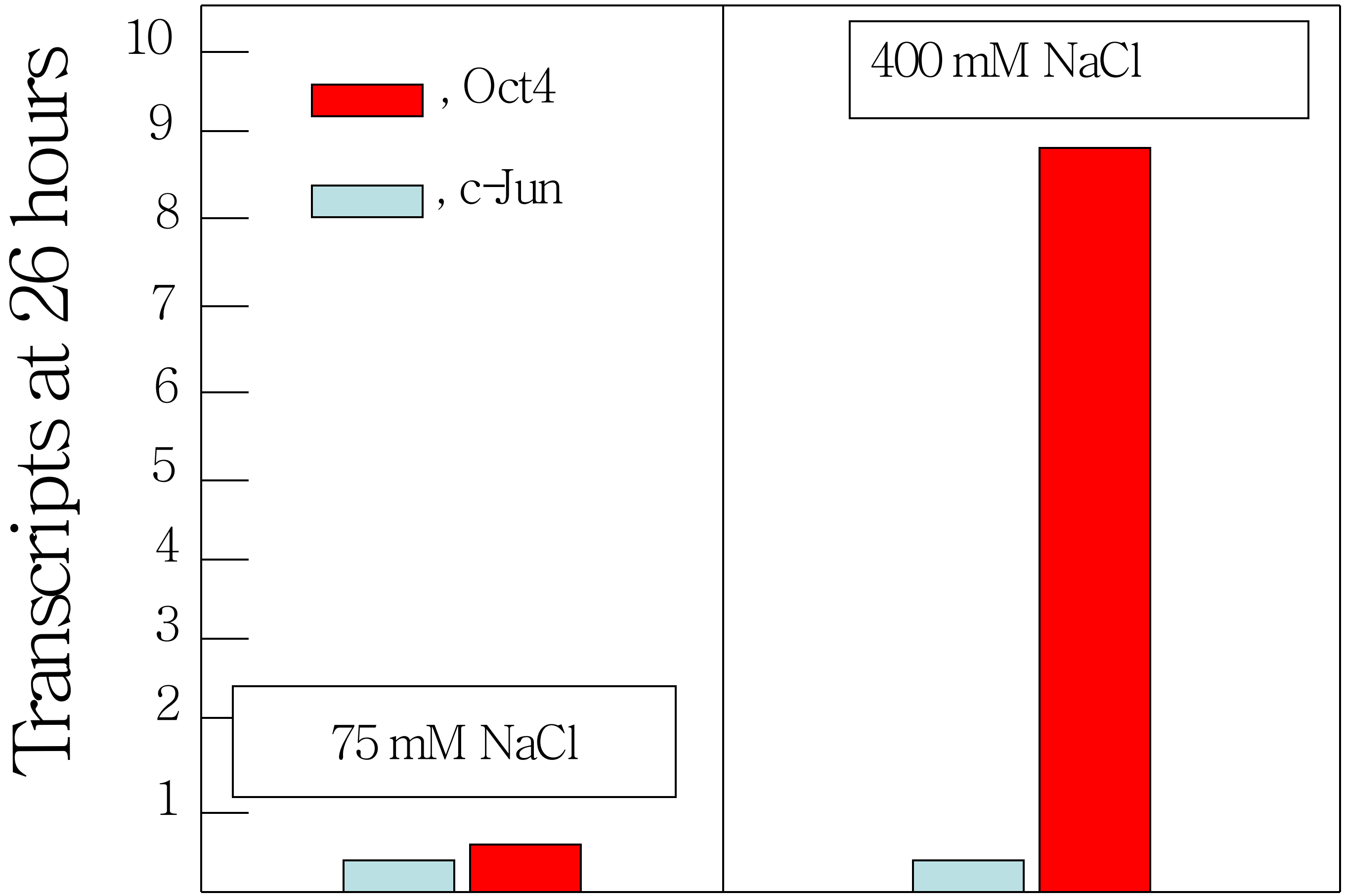
Resistance of nuclei transplanted to oocytes

RNA depletion from donor nuclei does not affect rate or extent of reprogramming



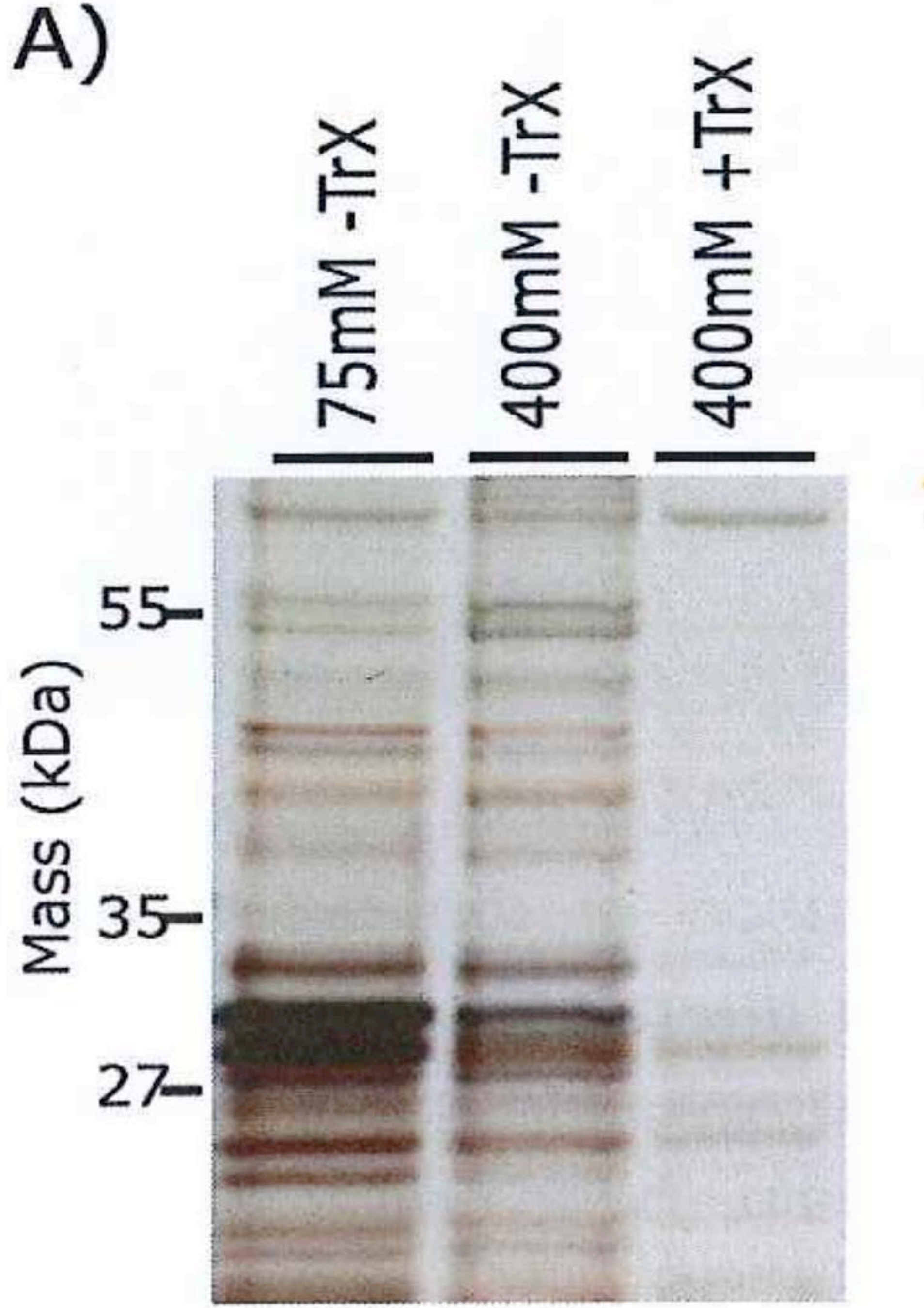


Protein depletion in somatic nuclei removes memory and enhances transcription

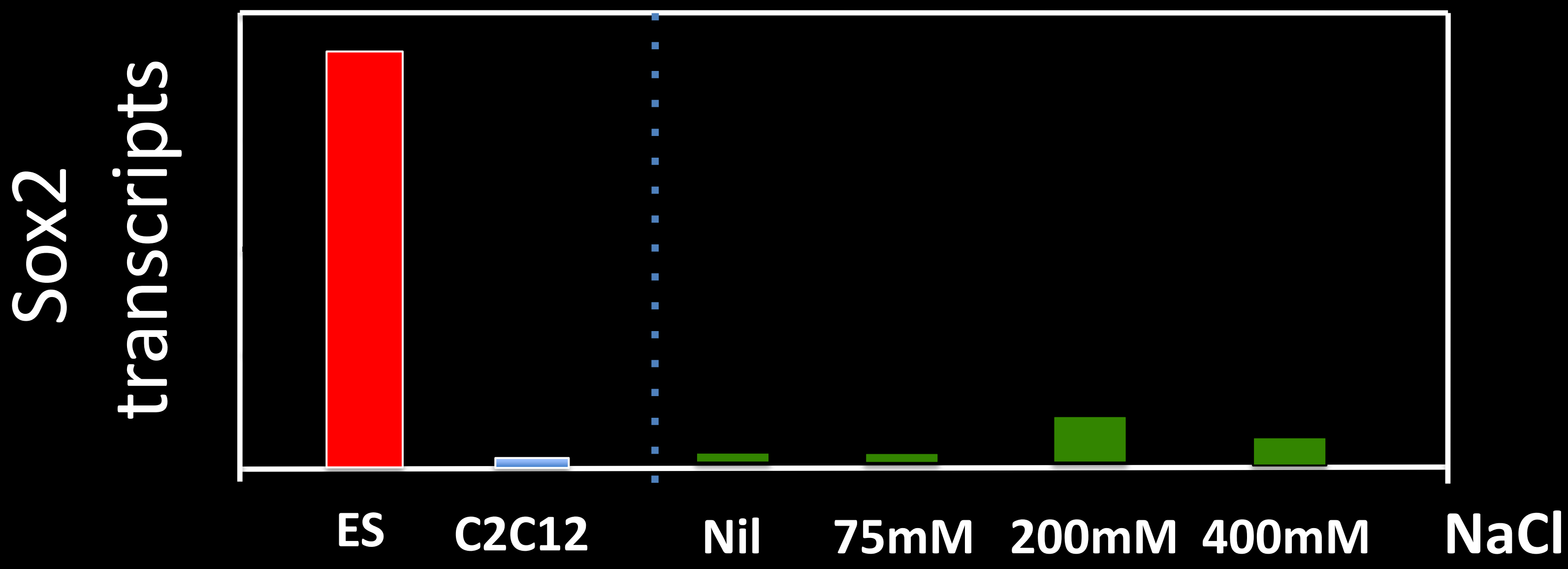


R. Halley-Stott

Protein removal from nuclei by salt and Triton



Resistance to reprogramming is maintained at high salt concentrations



Levels of chromosomal proteins after salt treatment

HMG	10	10	3	1
EED	10	10	8	0
BMi1	10	10	5	0
HP!	10	10	9	0
Brg1	10	10	6	1
Pol II	10	10	9	0
H2A	10	10	10	10

The battle for supremacy

The egg	The nucleus
Designed to transform sperm to an embryo active nucleus	Designed to maintain the same pattern of gene expression
Tries to do the same for somatic nuclei	Tries to resist any change

Prospects

To defeat resistance and win efficient cell replacement

Acknowledgements

Gurdon lab

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Kei Miyamoto

Patrick Narbonne

Vincent Pasque

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Charles Bradshaw (bioinformatics)

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Valerie Moar

James Byrne
Stina Simonsson
Carolina Astrand

Present colleagues

Jerome Jullien
Kei Miyamoto
Rick Halley-Stott
Vincent Pasque
Marta Teperek
Eva Hoermanseder
Stan Wang
Celia Delahay

MEDICAL RESEARCH COUNCIL
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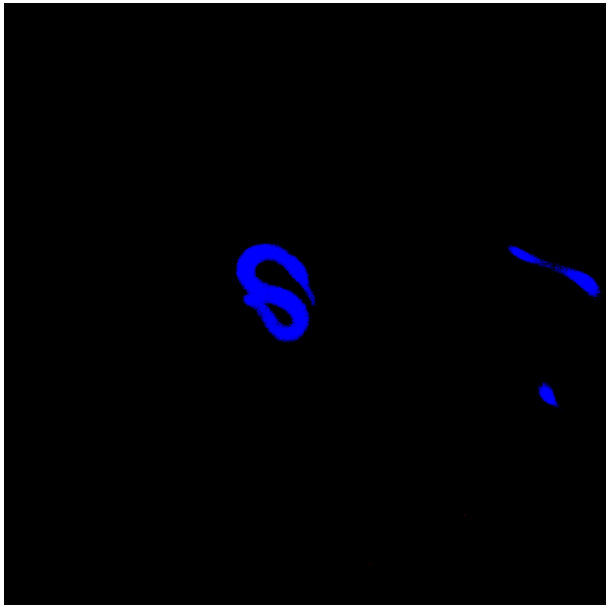
END

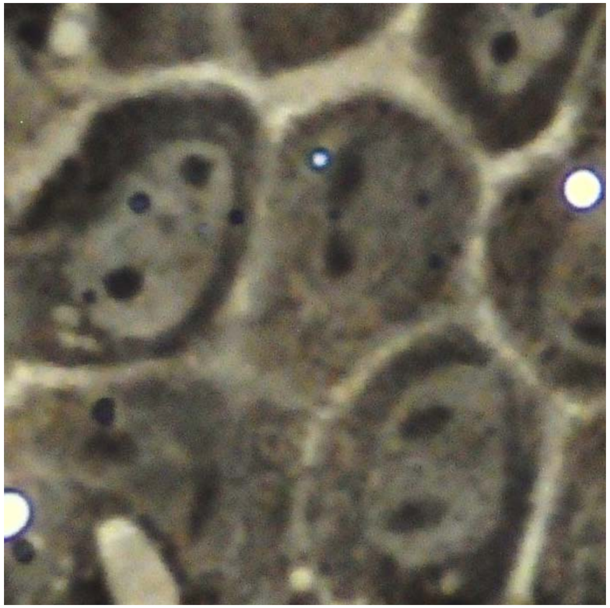
A sperm nucleus is specially designed to yield normal development

Sperm cell



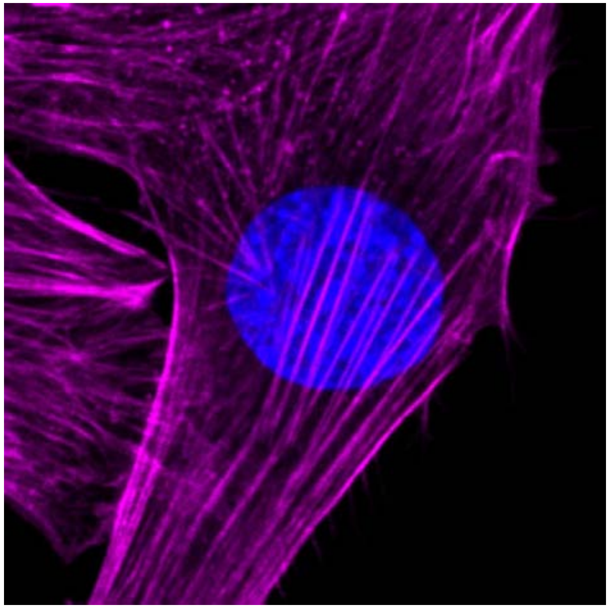
99%

Embryonic cell



35%

Specialised cell



1%

% of normal development after nuclear transfer (to a feeding tadpole)

Images from
Dr Kei Miyamoto
Marta Teperek

Conclusions

1. Some cells (endoderm) undergo a very early stable commitment to their lineage pathway.

2. Stable comitment can be reversed by nuclear transfer to eggs.

3. Nuclei from diferentiated cells show a strong resistance to reprogramming.

4. Resistance is strongly cell-type and gene specific.

5. Resistance depends on histone modifications and on other stable chromosomal components.

Acknowledgements

Nuclear reprogramming

Jerome Jullien (B4)

Kei Miyamoto Polym. Actin)

Vincent Pasque (Xi)

Richard Halley-Stott (Trn)

Kazutaka Murata (Histone mods)

Marta Teperek (Sperm)

Other Laboratories

Welcome Trust

G. Crabtree (Stanford)

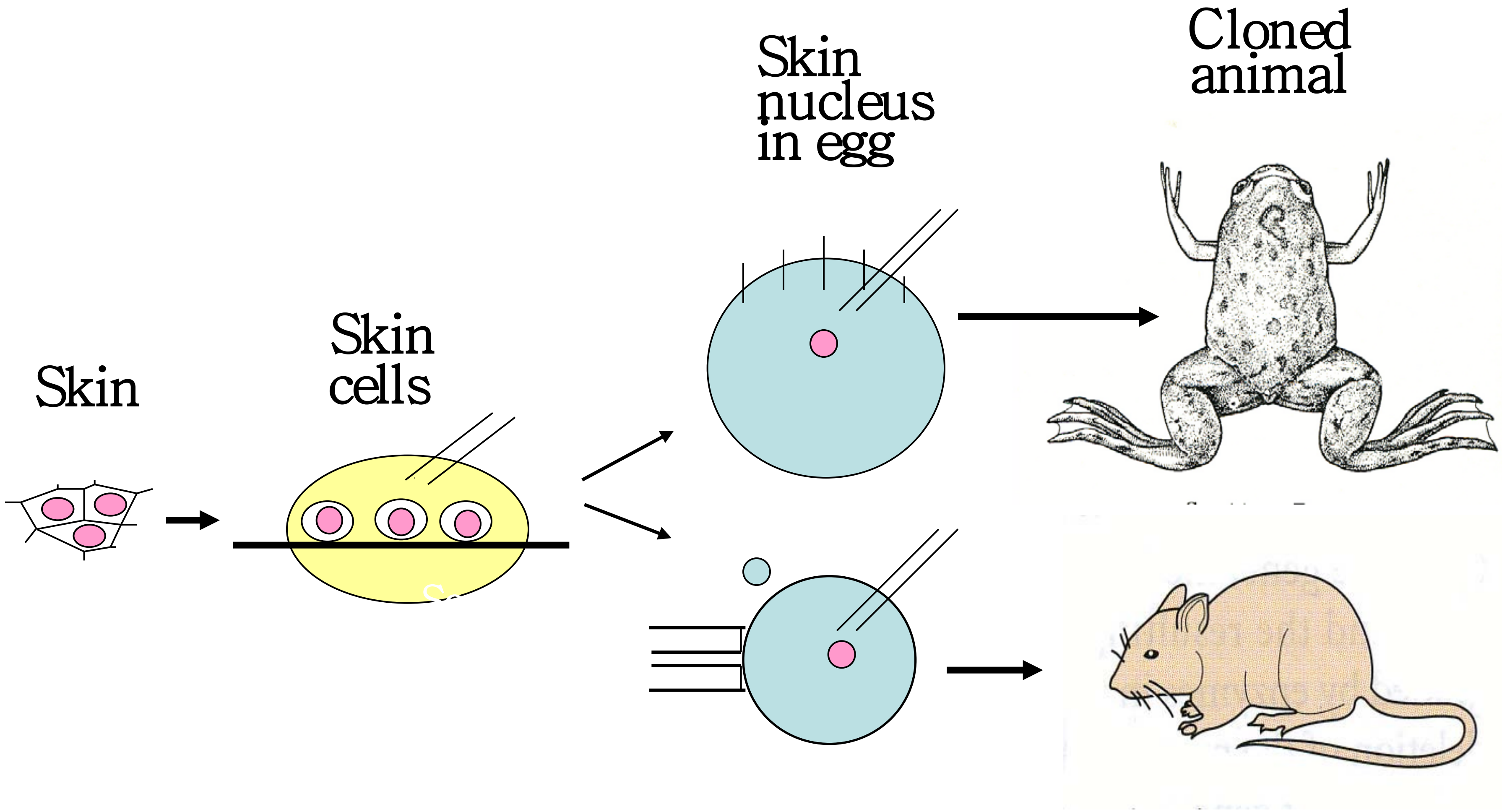
K. Ohsumi (Nagoya)

G. Almouzni (Paris)

K.Shinkai (Kyoto)

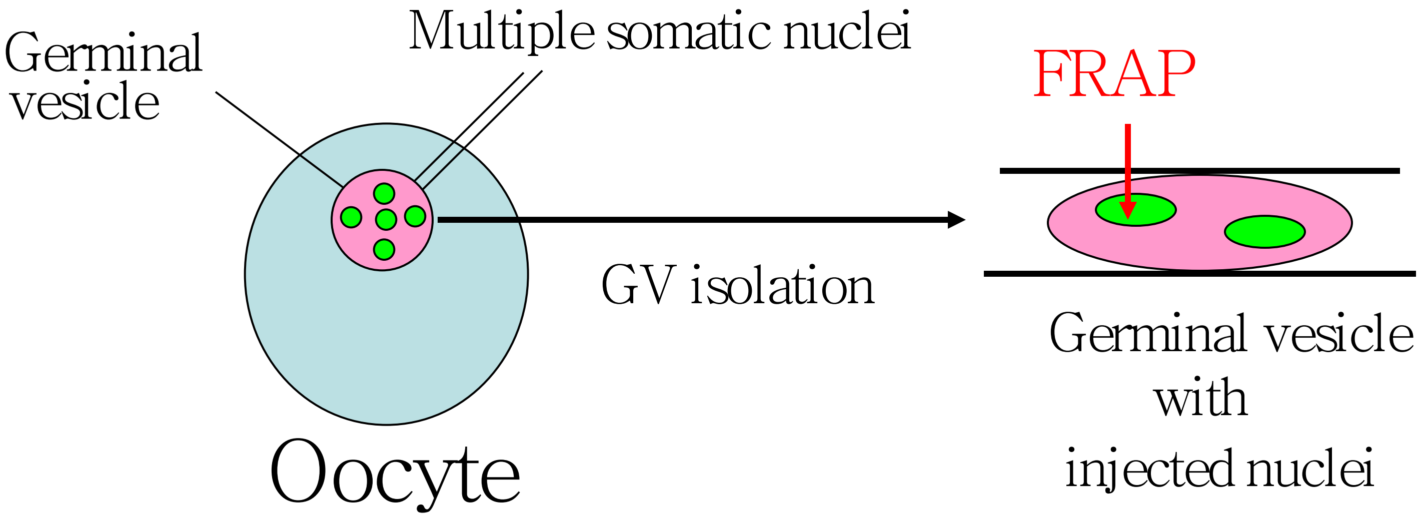
Medical Reseacrh Council

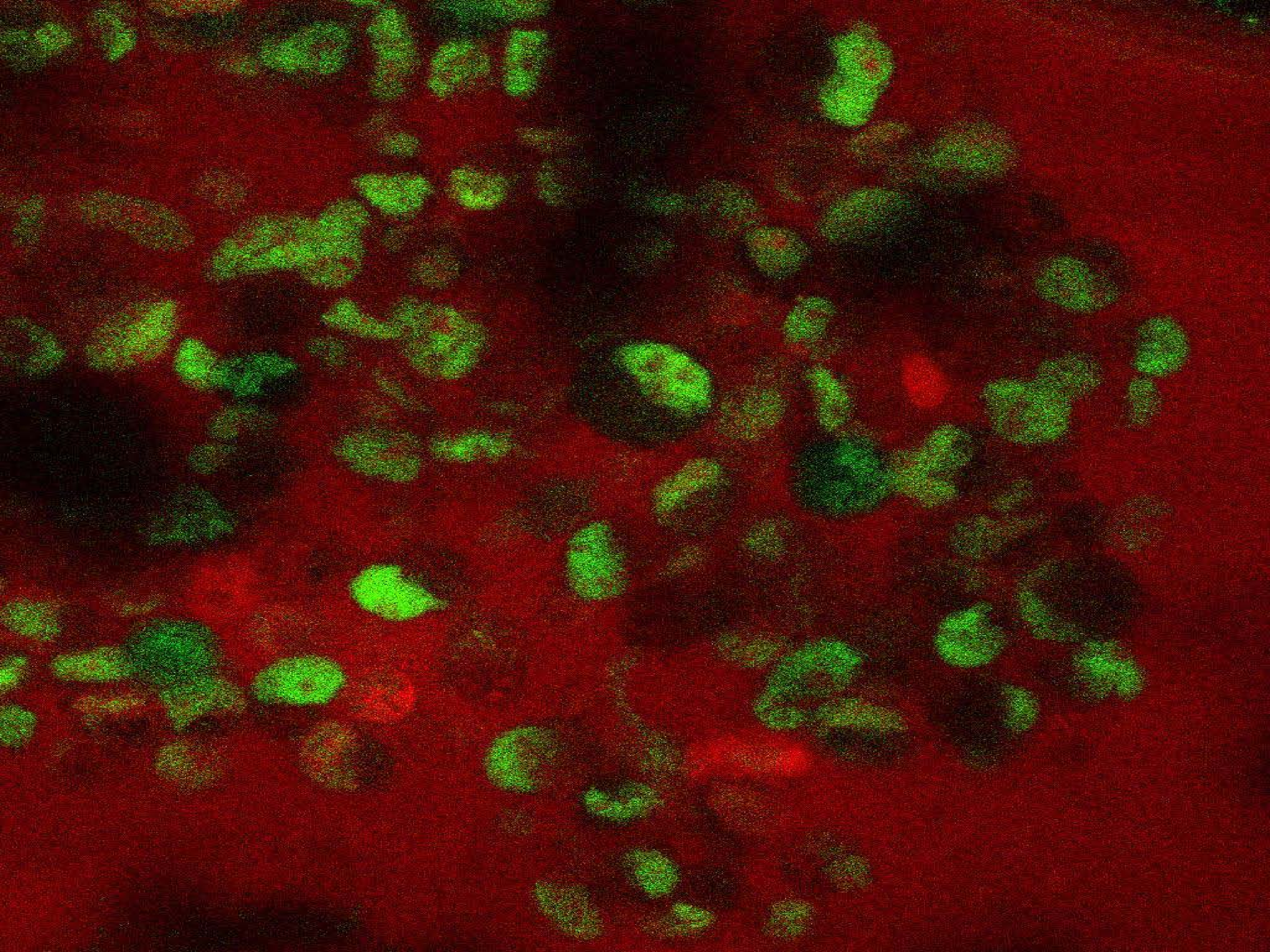
Single nuclear transfer to unfertilized eggs



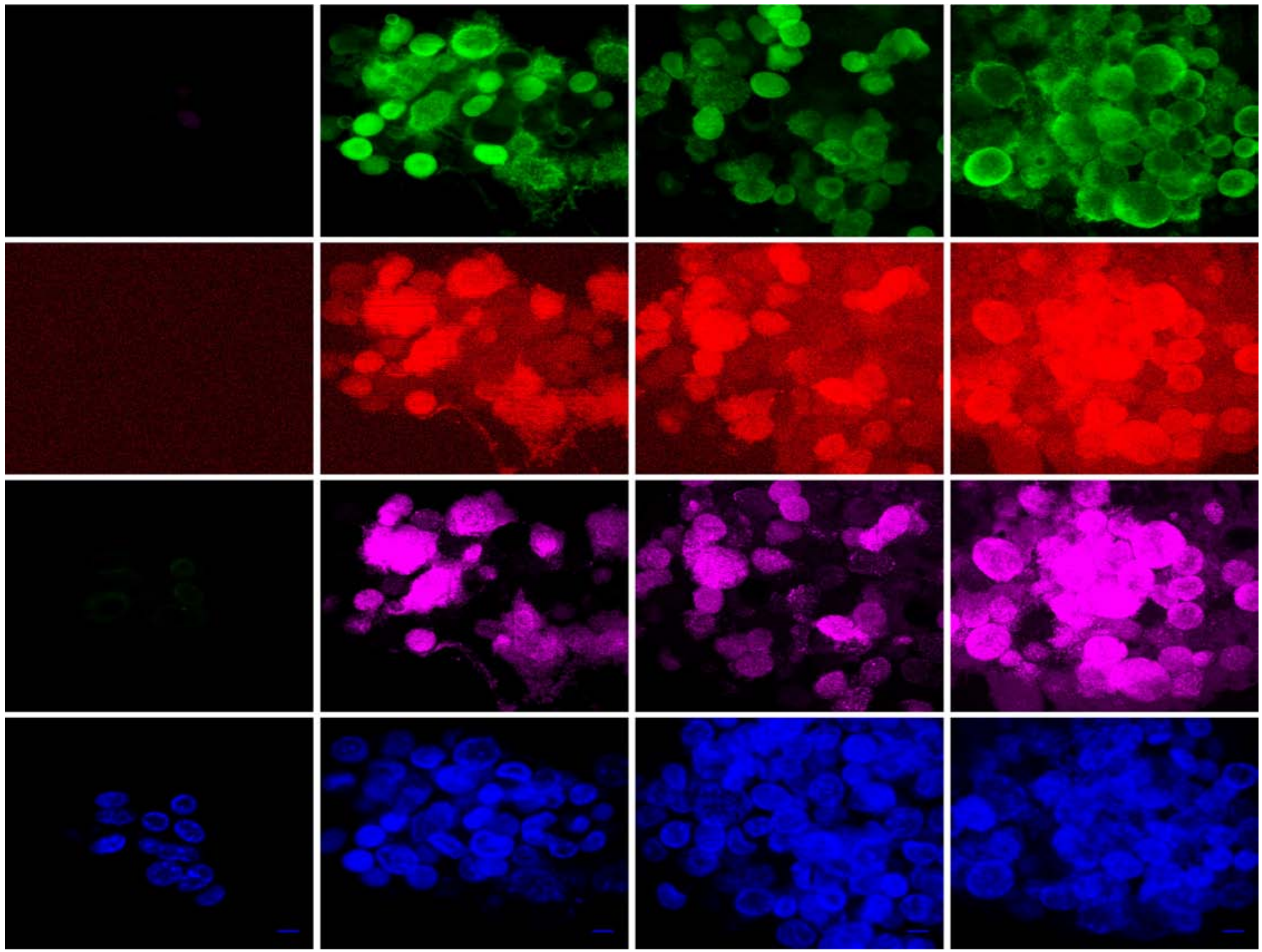
Fluorescence recovery after photobleaching

To determine the exchange rate of a defined protein
in transplanted nuclei



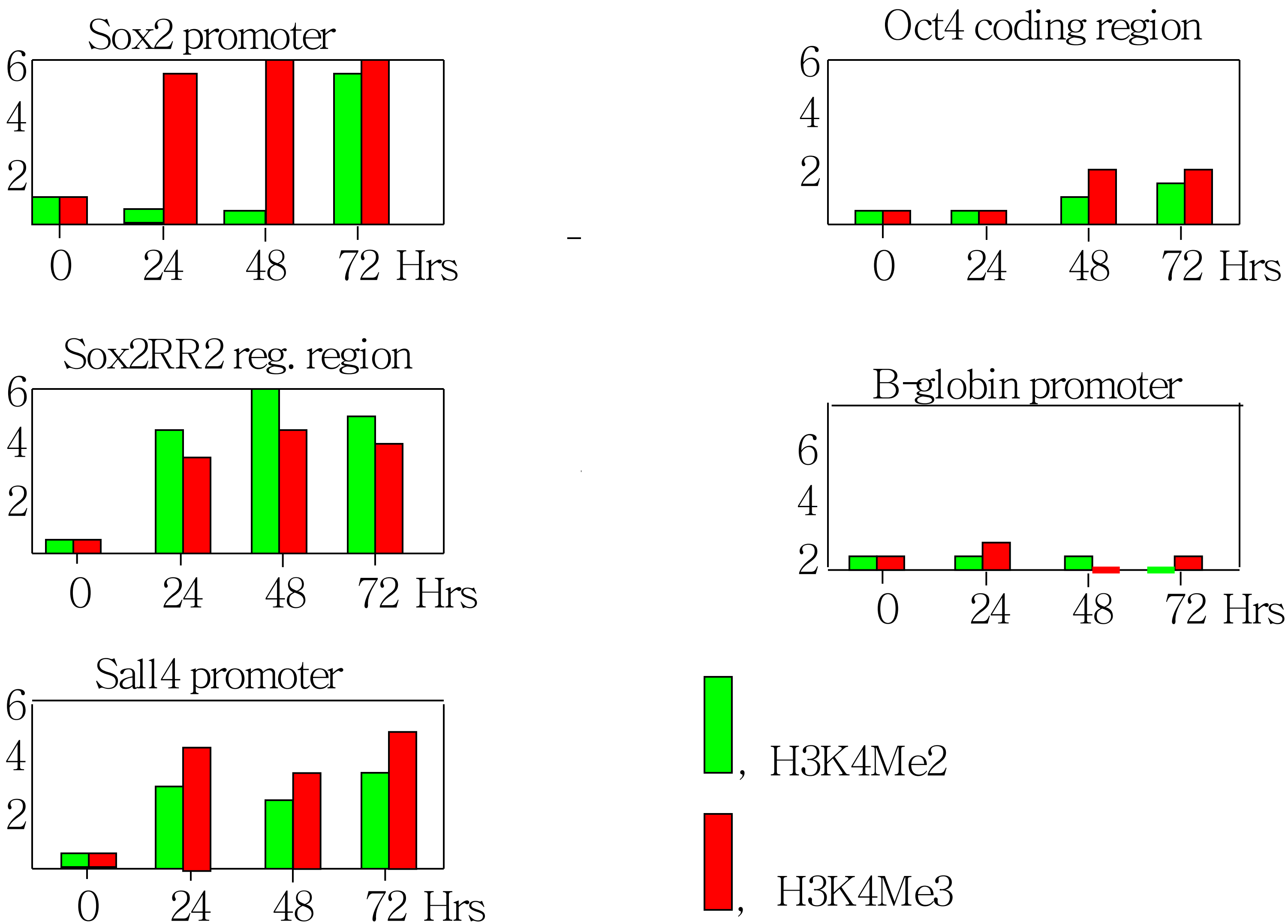


Increase in polymerase II after nuclear transfer



Histones in gene control regions are methylated – Chip analysis

Nuclei from retinoic acid treated ES cells



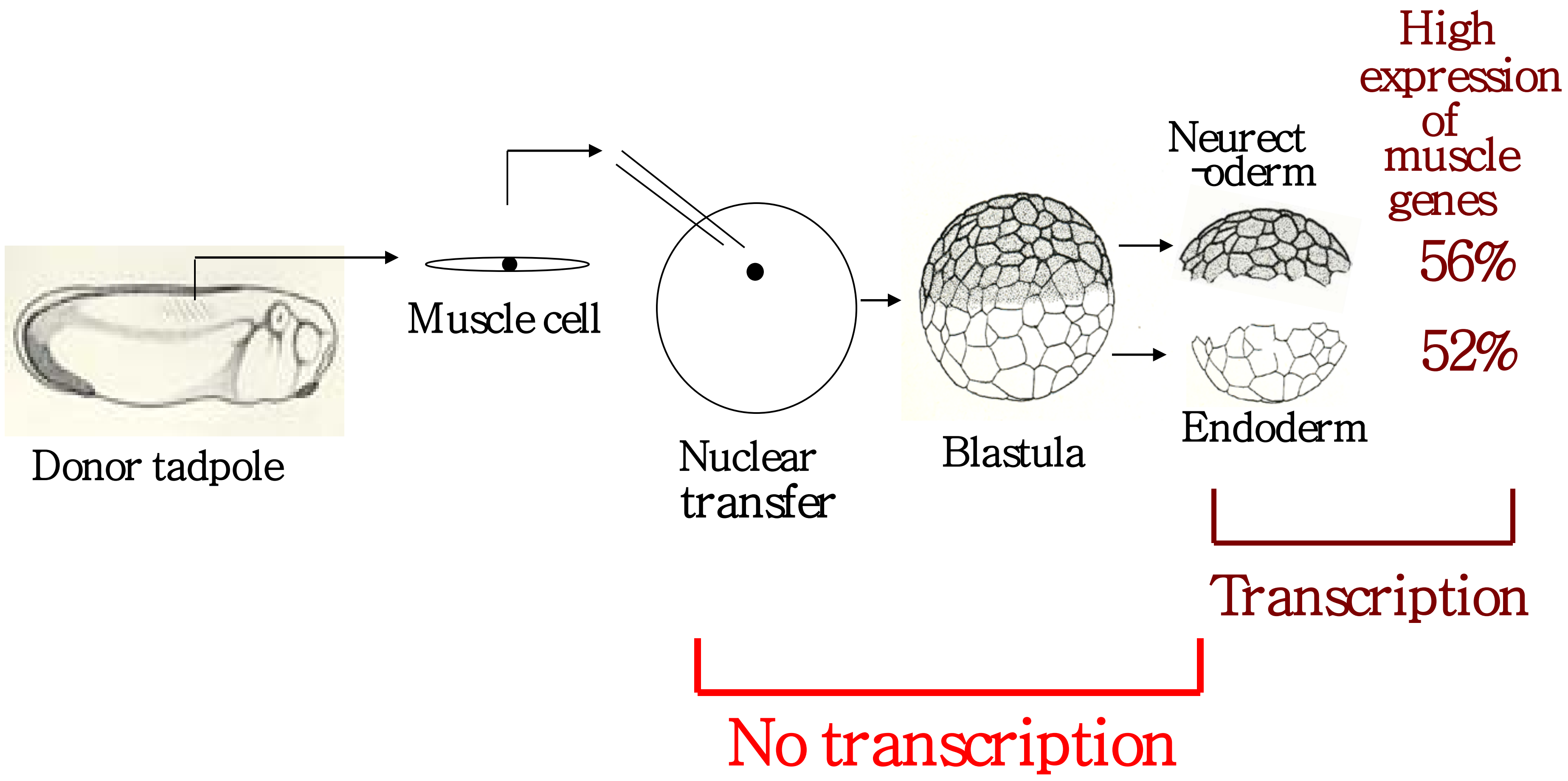
Epigenetics and chromatin, 2010.

MacroH2A helps to explain resistance to reprogramming

MacroH2A is high on MEF-X:i resists reprogramming.
but absent from EPI-Xi: is reprogrammed.

MacroH2A is knocked down by inhibitory RNA,
and induces Oct4 and Sox2 in MEF-Xi cells

Epigenetic memory



Nature Cell Biol. 2006

The resistance of MEF Xi nuclei to reprogramming

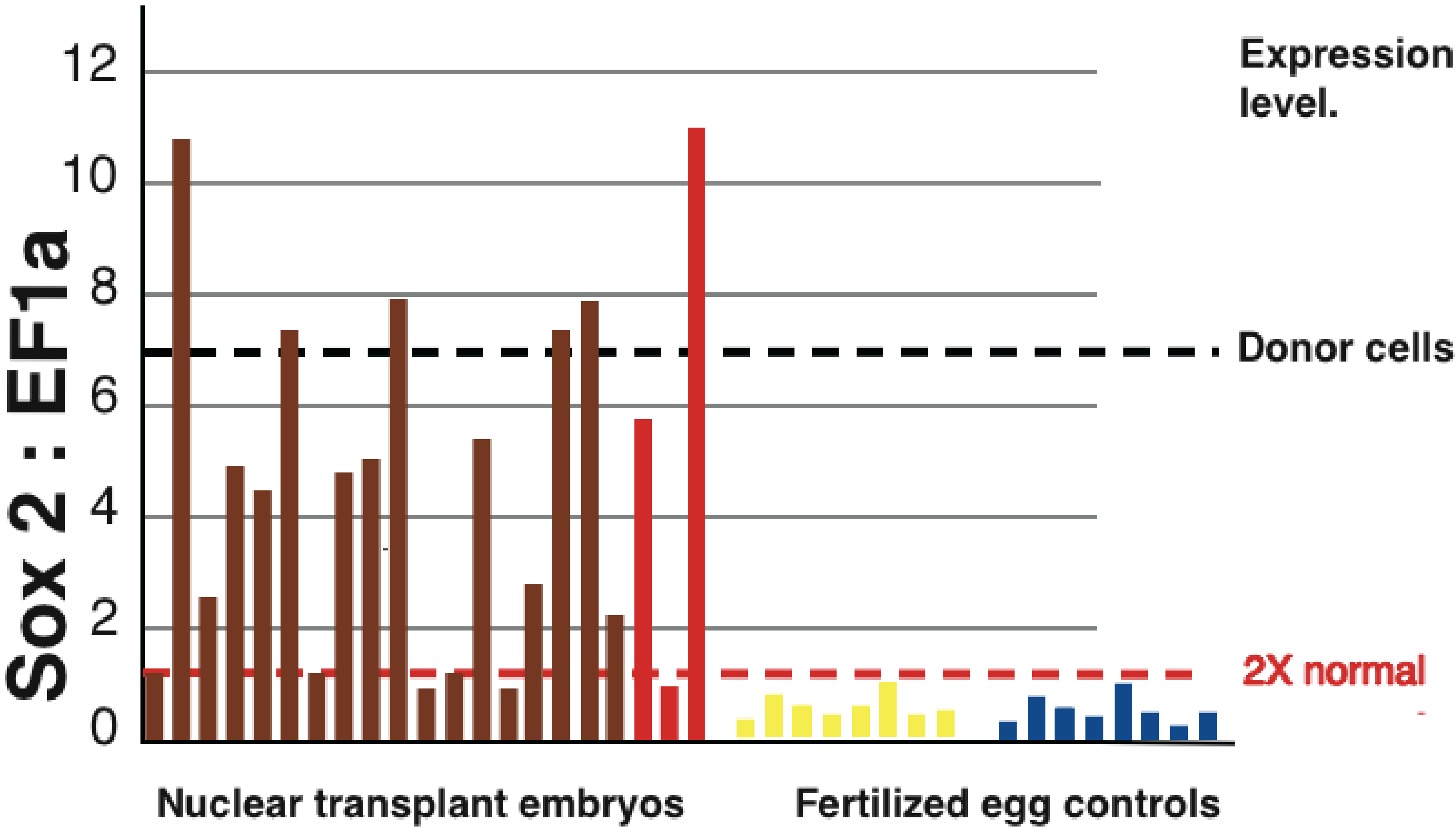
by oocytes is not explained by

DNA methylation or by

histone H3K27 me

Memory of cell type gene expression persists through nuclear transfer.

Neurectoderm nuclei (neural marker Sox2)
and Sox2 expression in endoderm nuclear transfer cells.



Nature Cell Biol.2007

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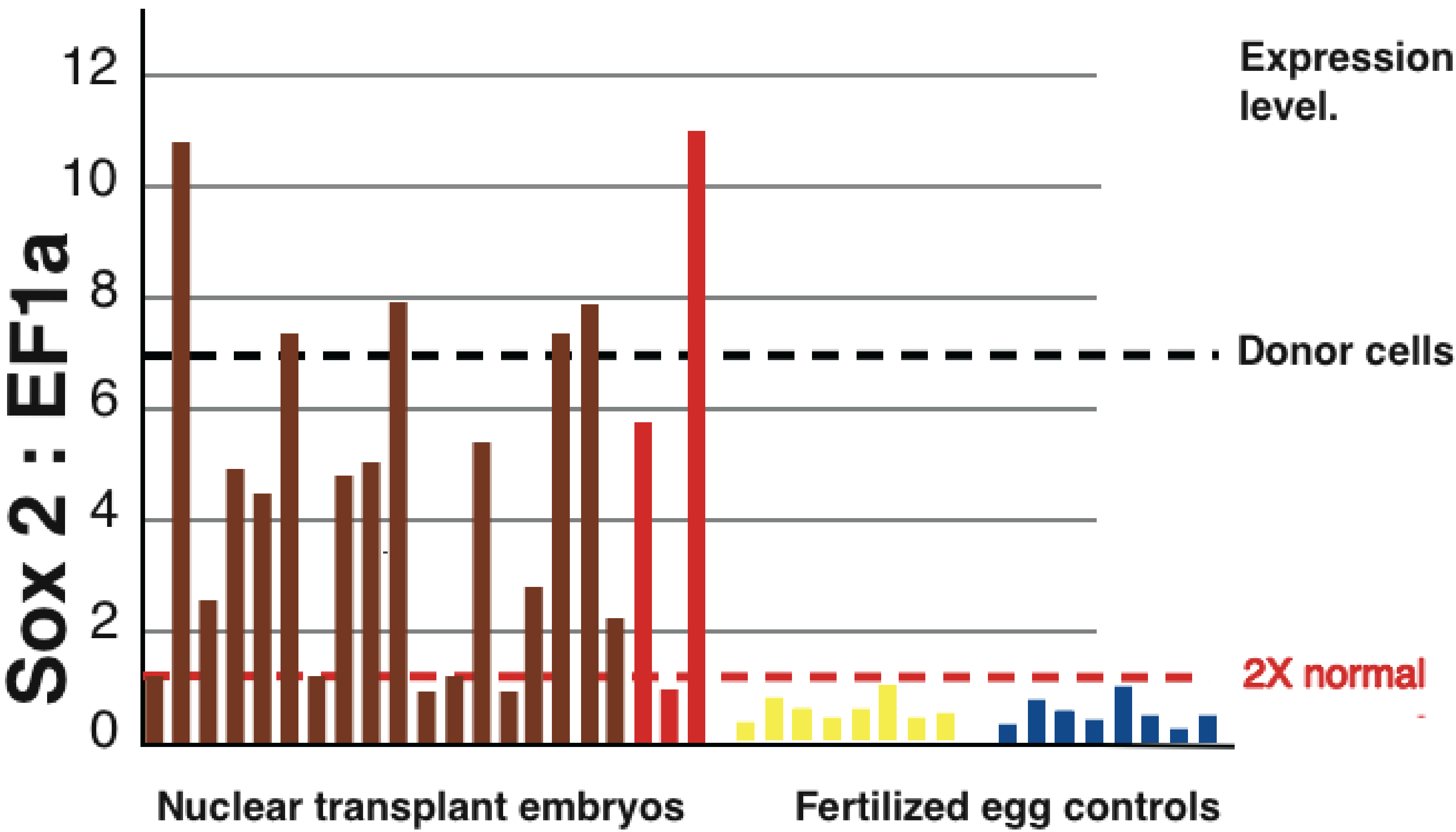
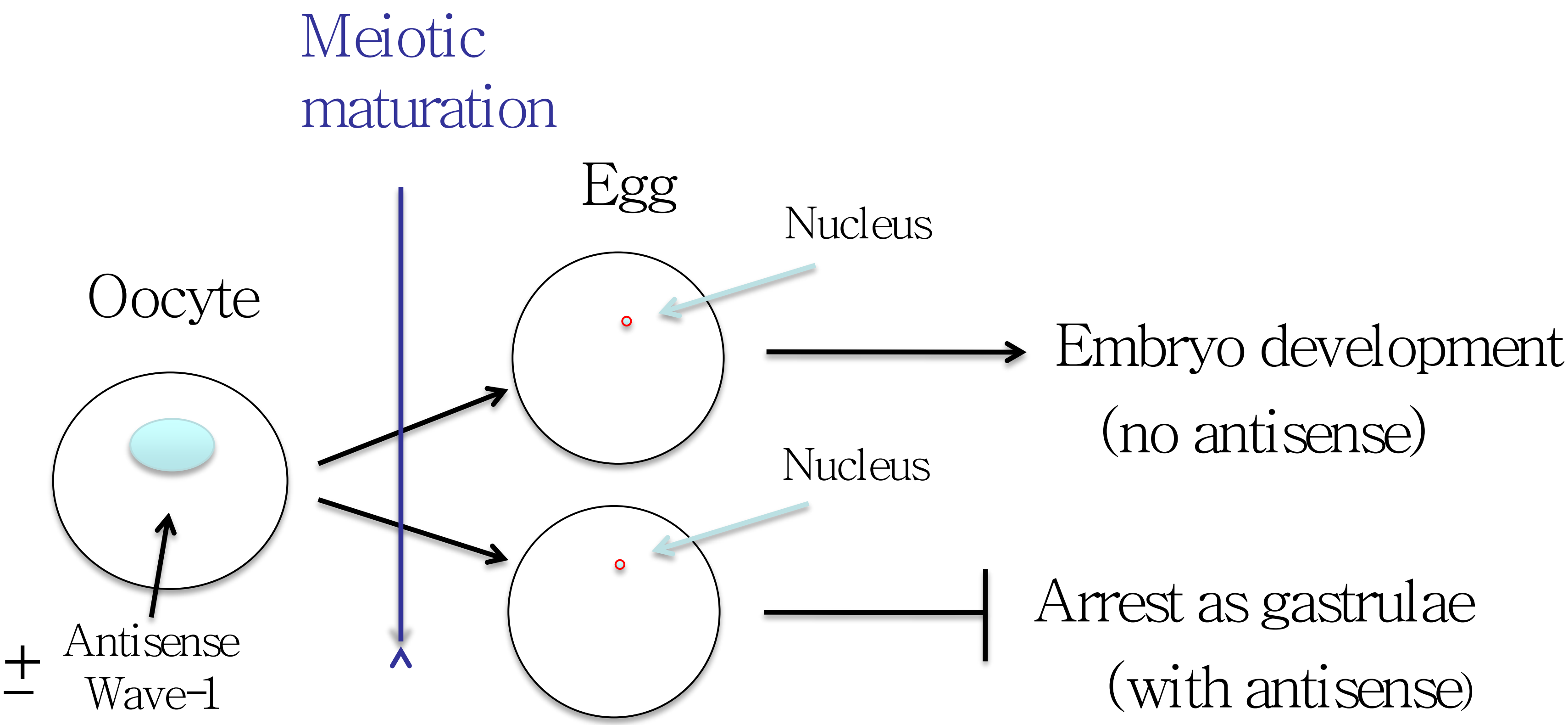


Nature Cell Biol.2007

WAVE-1 is required for zygotic genome activation and embryonic development

(Wiskott-Aldrich syndrome)



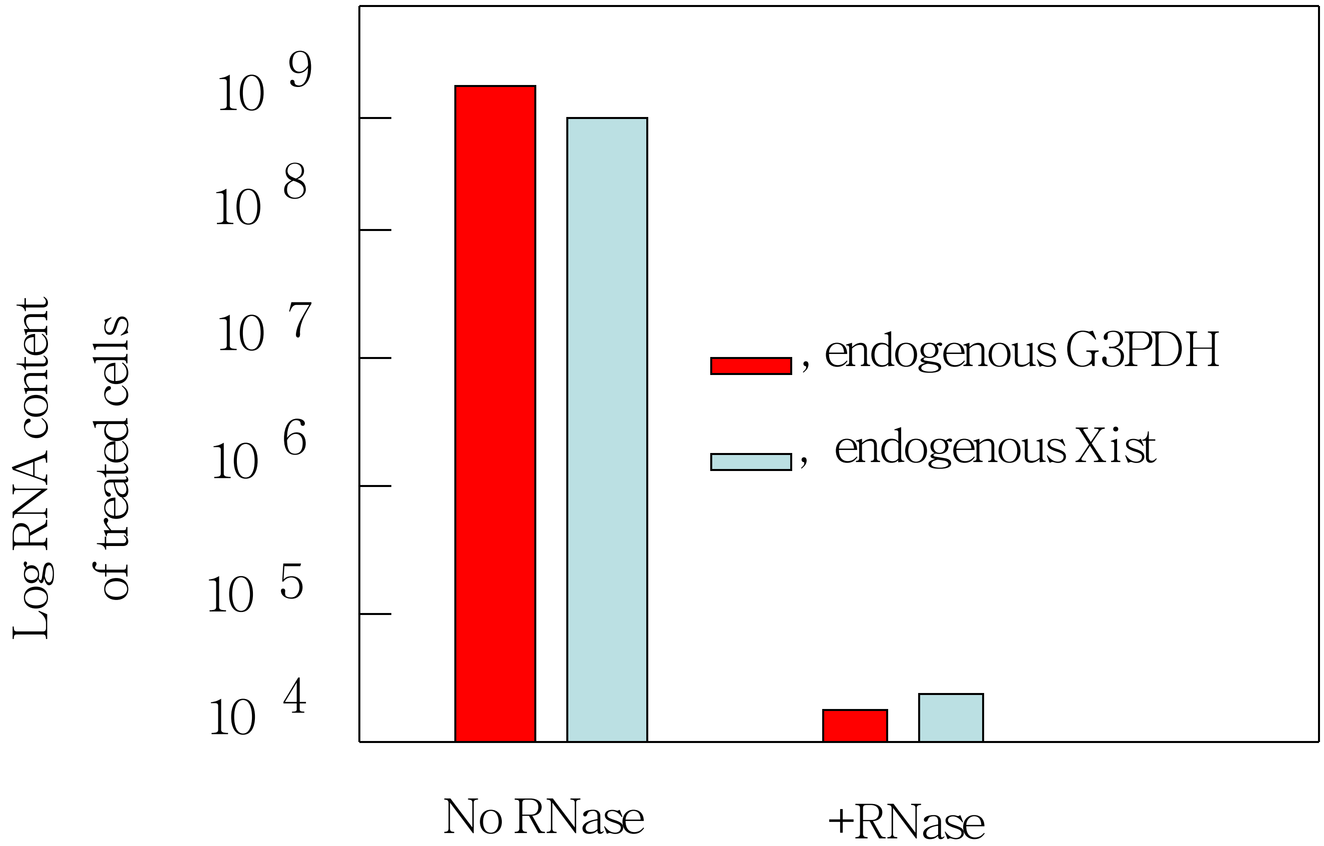
Histone modifiers overexpression in the oocyte:

-H3K9 demethylase KDM4D efficiently removes H3K9Me2/3 from transplanted nuclei and leads to loss of HP1 alpha

-H2A deubiquitinases (USP16&21) reduce ubiquitinated H2A level in transplanted nuclei

RNA can be depleted from donor nuclei by RNase.

Permeabilized cells, containing RNA, are treated with RNase, then assayed for residual RNA.



Rick Halley Stott

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Past colleagues

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Donald Brown

Laurence Korn

Eduardo De Robertis

Marvin Wickens

Alan Colman

Ann Jewkes

Valerie Speight

Present colleagues

Jerome Jullien

Kei Miyamoto

Rick Halley-Stott

Vincent Pasque

Marta Teperek

Eva Hoermanseder

Stan Wang

Celia

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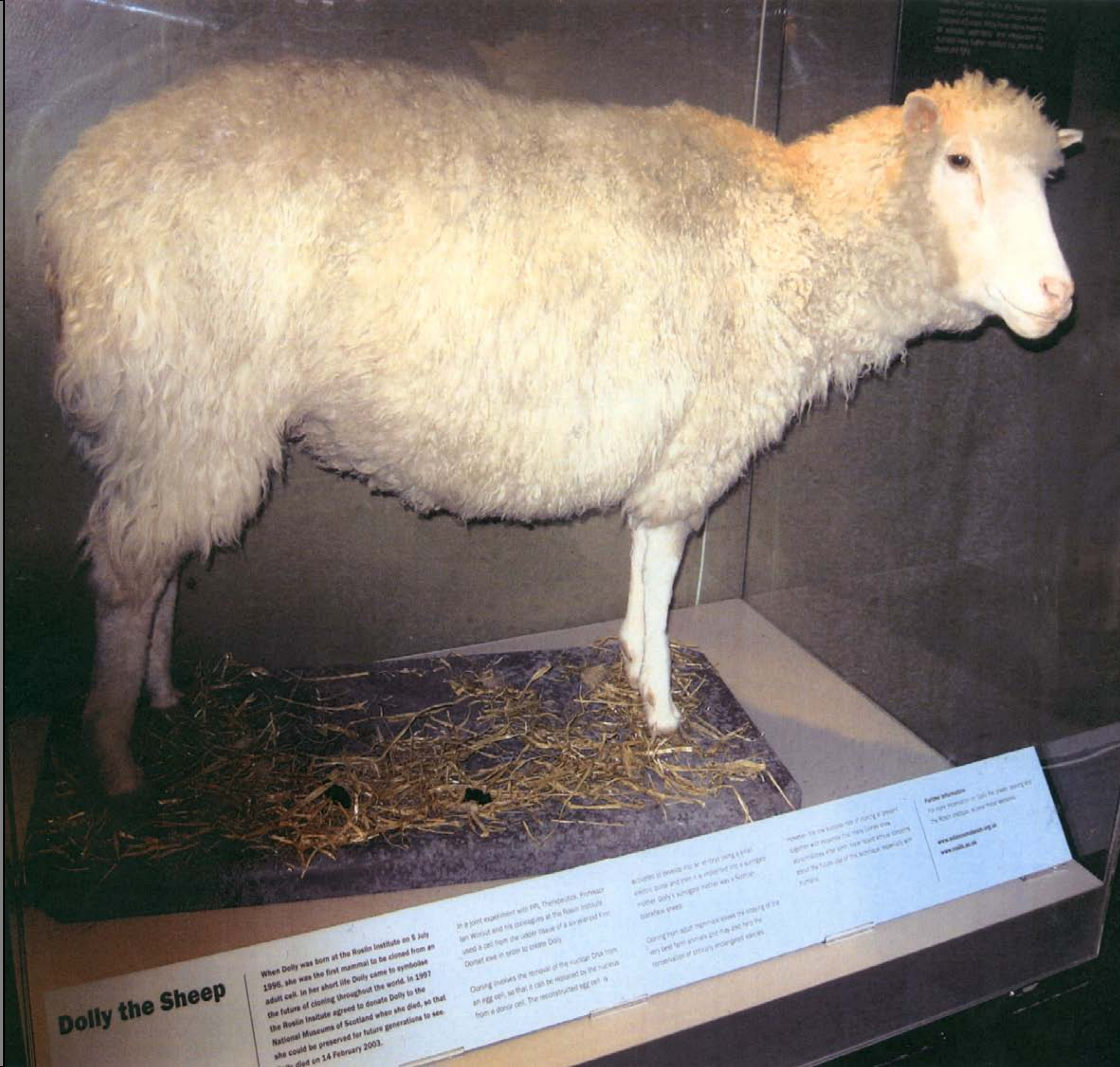
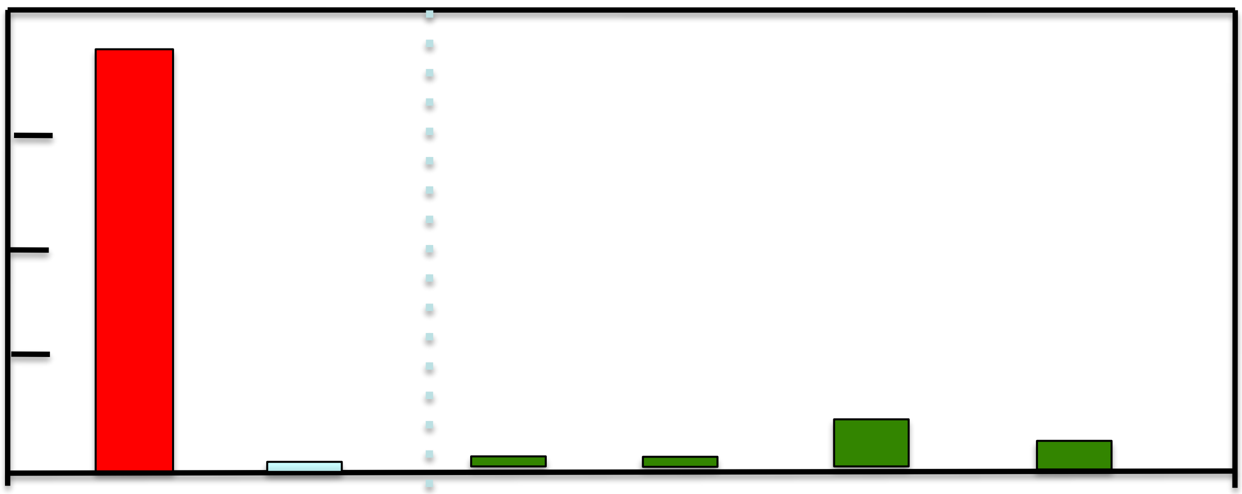
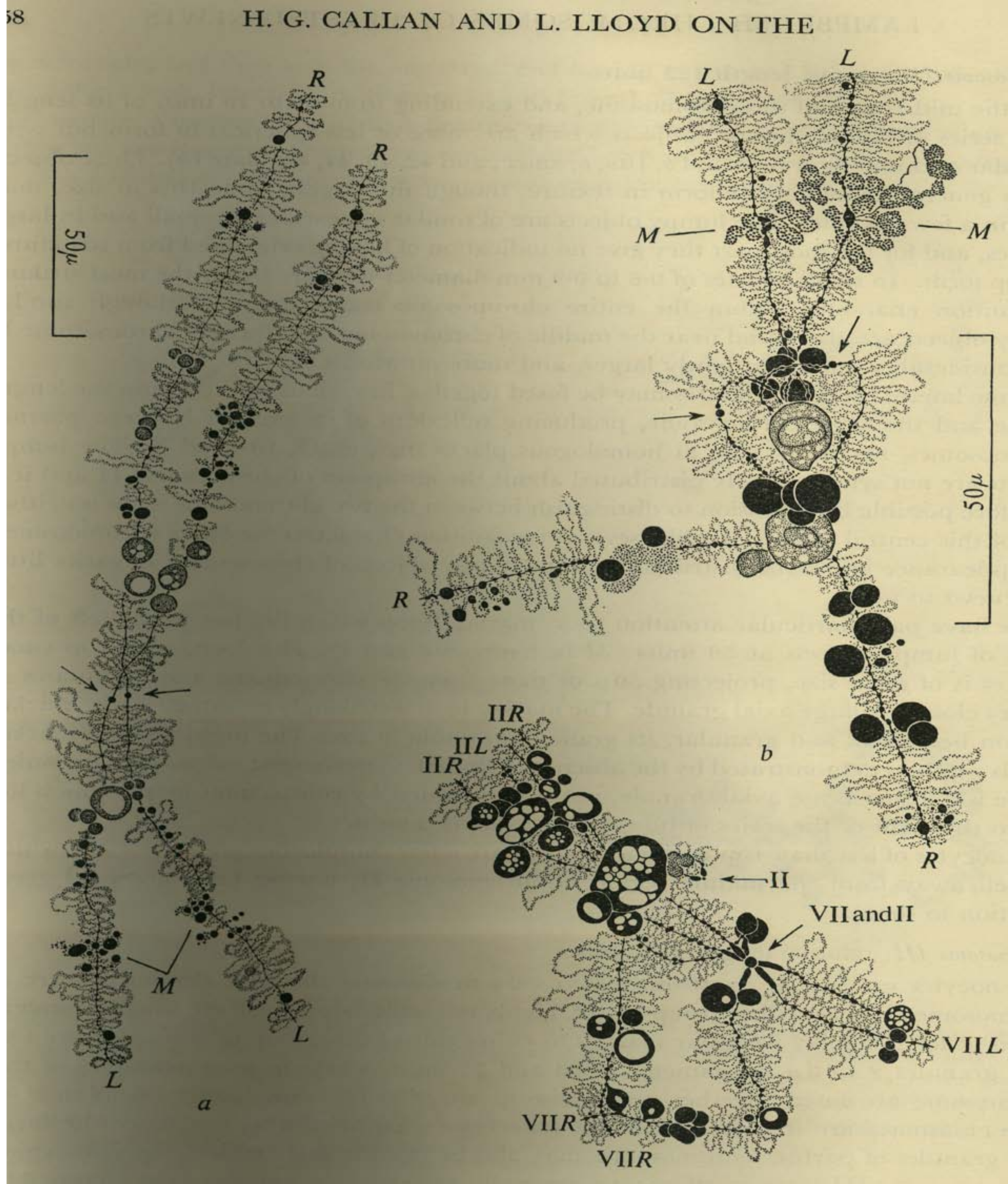
CANCER RESEARCH CAMPAIGN


Dolly the Sheep
When Dolly was born at the Roslin Institute on 5 July 1996, she was the first mammal to be cloned from an adult cell. In her short life Dolly came to symbolise the future of cloning throughout the world. In 1997 the Roslin Insitute agreed to donate Dolly to the National Museums of Scotland when she died, so that she could be preserved for future generations to see.
died on 14 February 2003.



Newt chromosome II.

Amphibian lampbrush chromosomes



Oogenesis and development in the mouse

Oogenesis | Development

Meiotic divisions

Mouse

Germ cell — Mature oocyte: 17 days

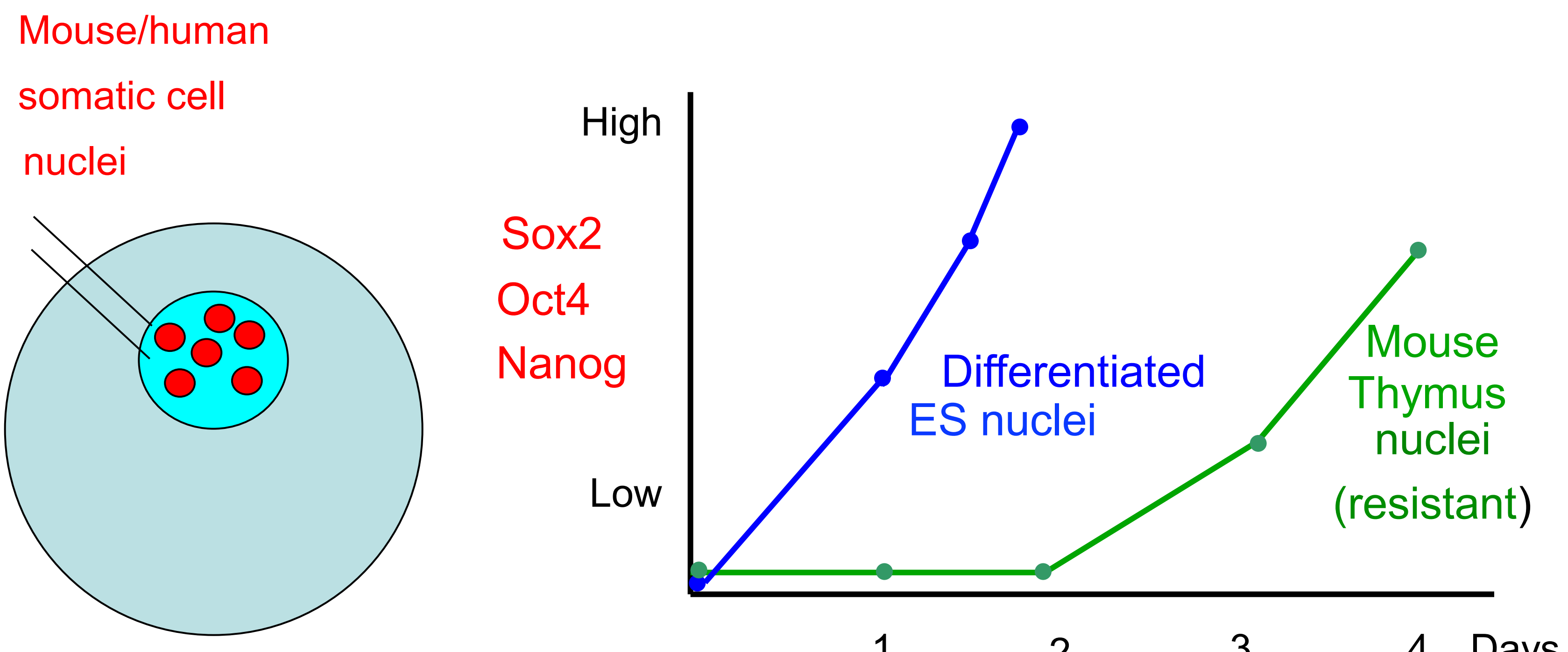
Zygote — Muscular response: 10 days

Major events in nuclear reprogramming

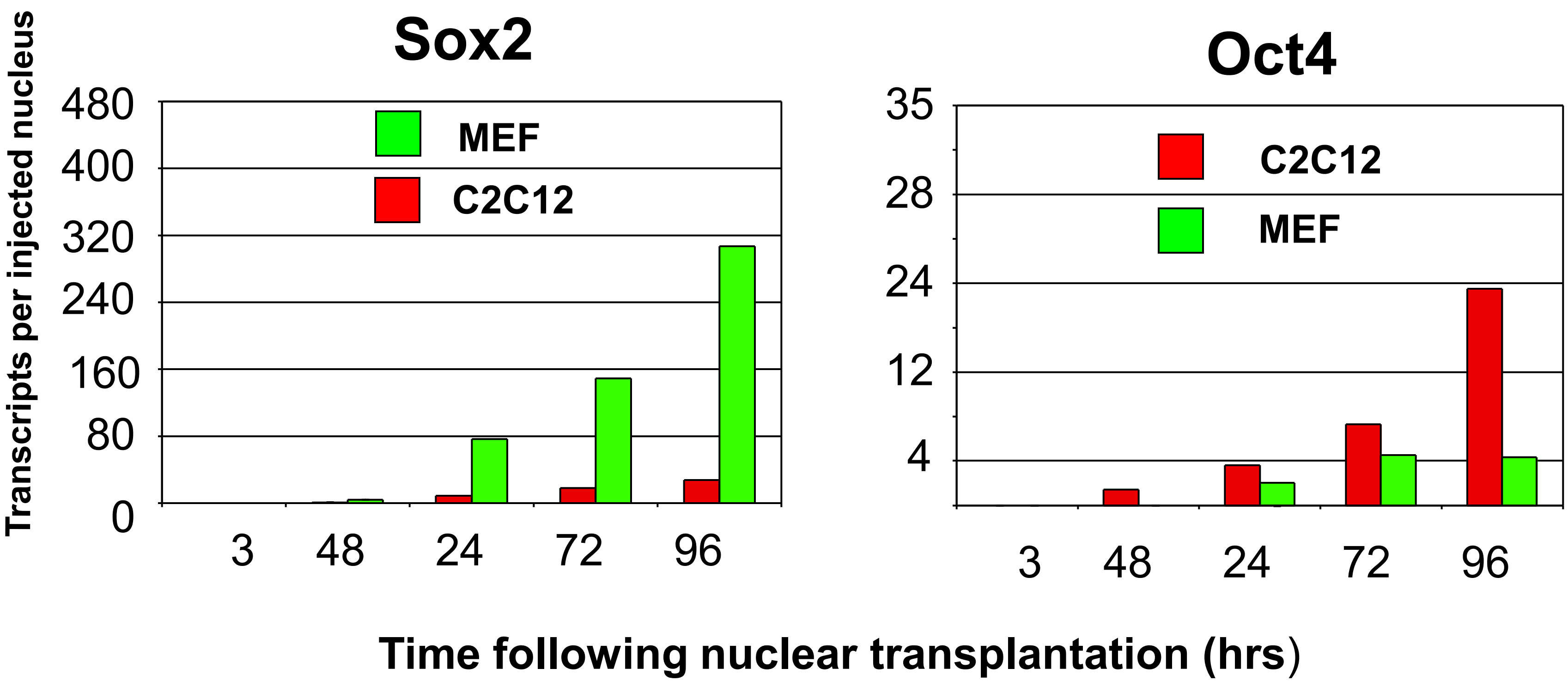
Events	Amphibia
Chromatin decondensation New (pluripotency) gene expression DNA demethylation	Oocyte in meiotic prophase
DNA demethylation DNA replication, cell proliferation Repression of unwanted genes (lineage selection)	Eggs and embryos

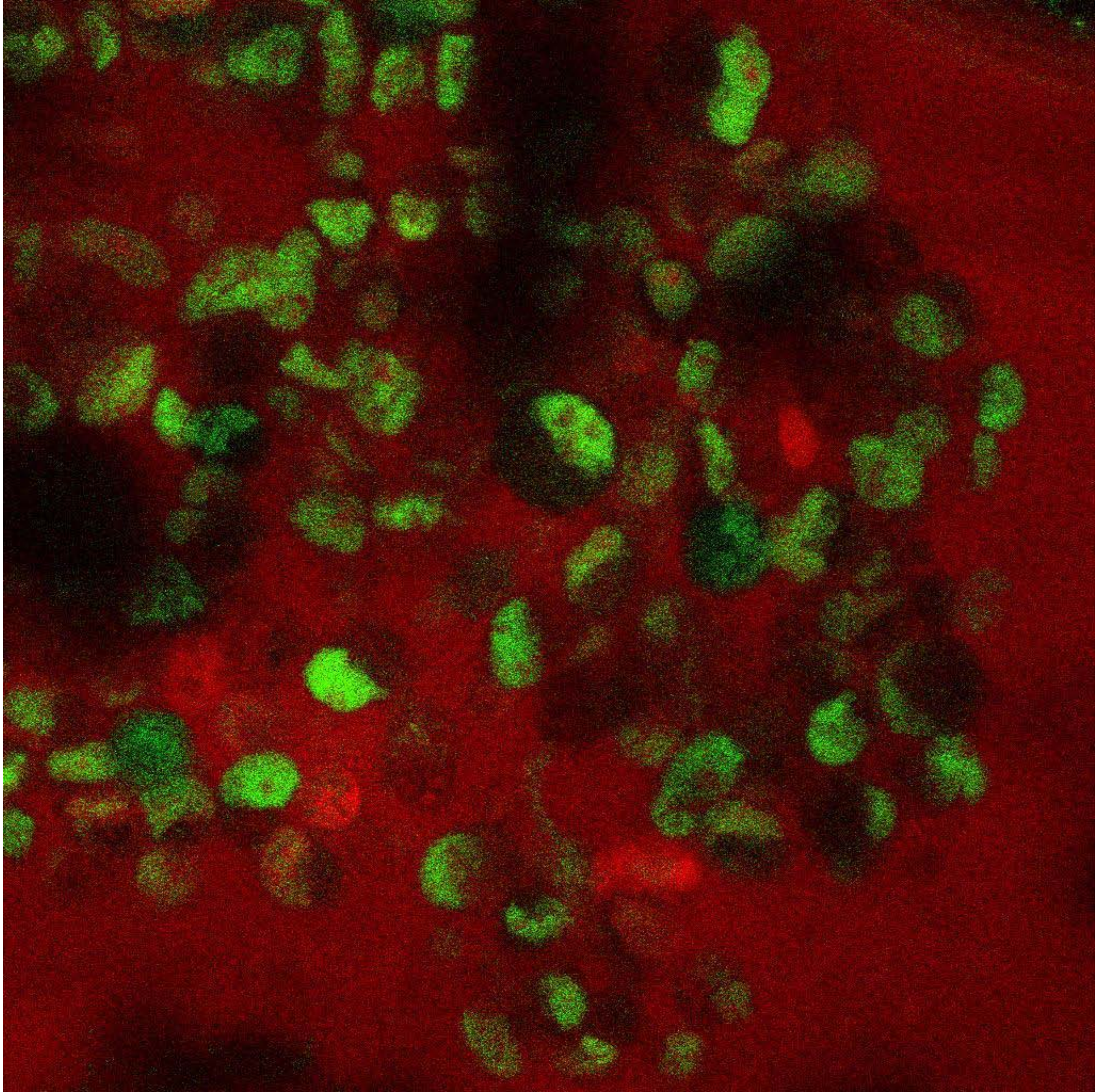
Stem cell genes are rapidly activated in mammalian nuclei transplanted to Xenopus oocytes

Nuclei of most differentiated cells resist reprogramming.

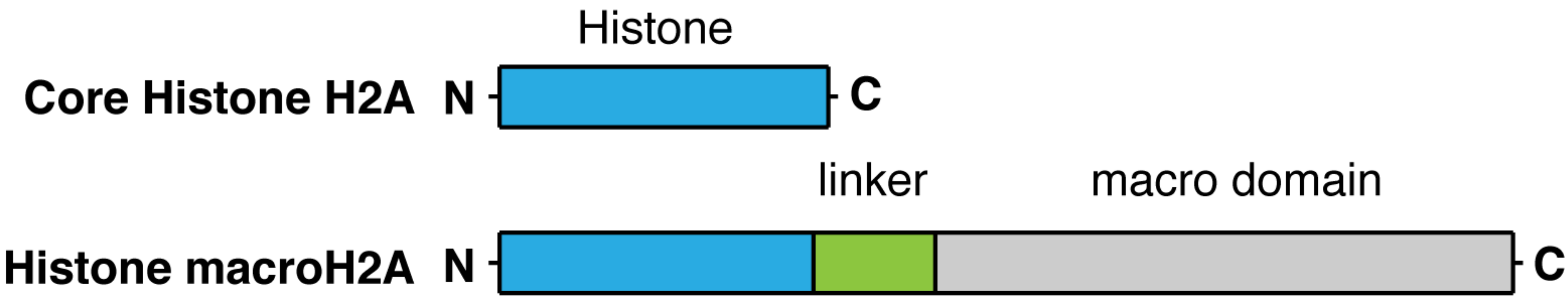


Resistance to reprogramming is pronounced when comparing different donor cell-types. [by up to 50X]



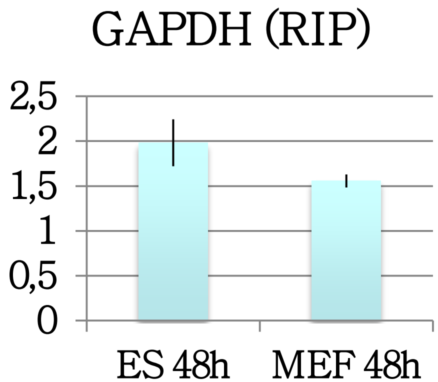


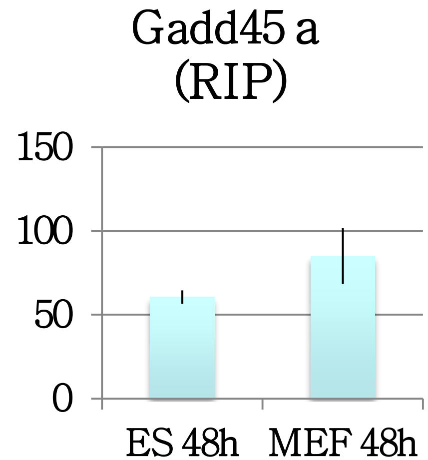
Histone variant macroH2A

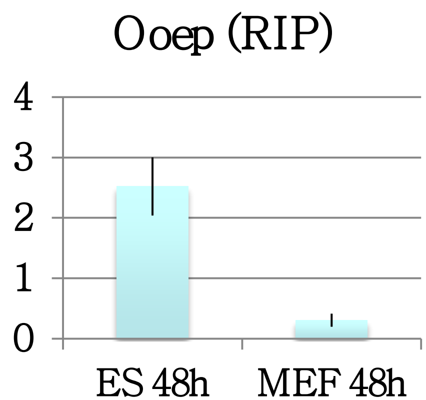


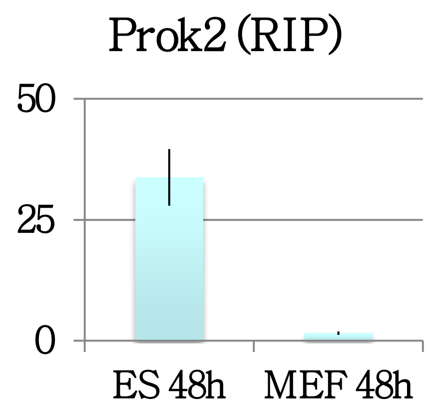
- macro domain = 2/3 of macroH2A
- vertebrate-specific variant
- ‘hallmark’ of vertebrate heterochromatin

Examples of genes with restricted expression in MEF nuclei after transplantation to *Xenopus* oocytes



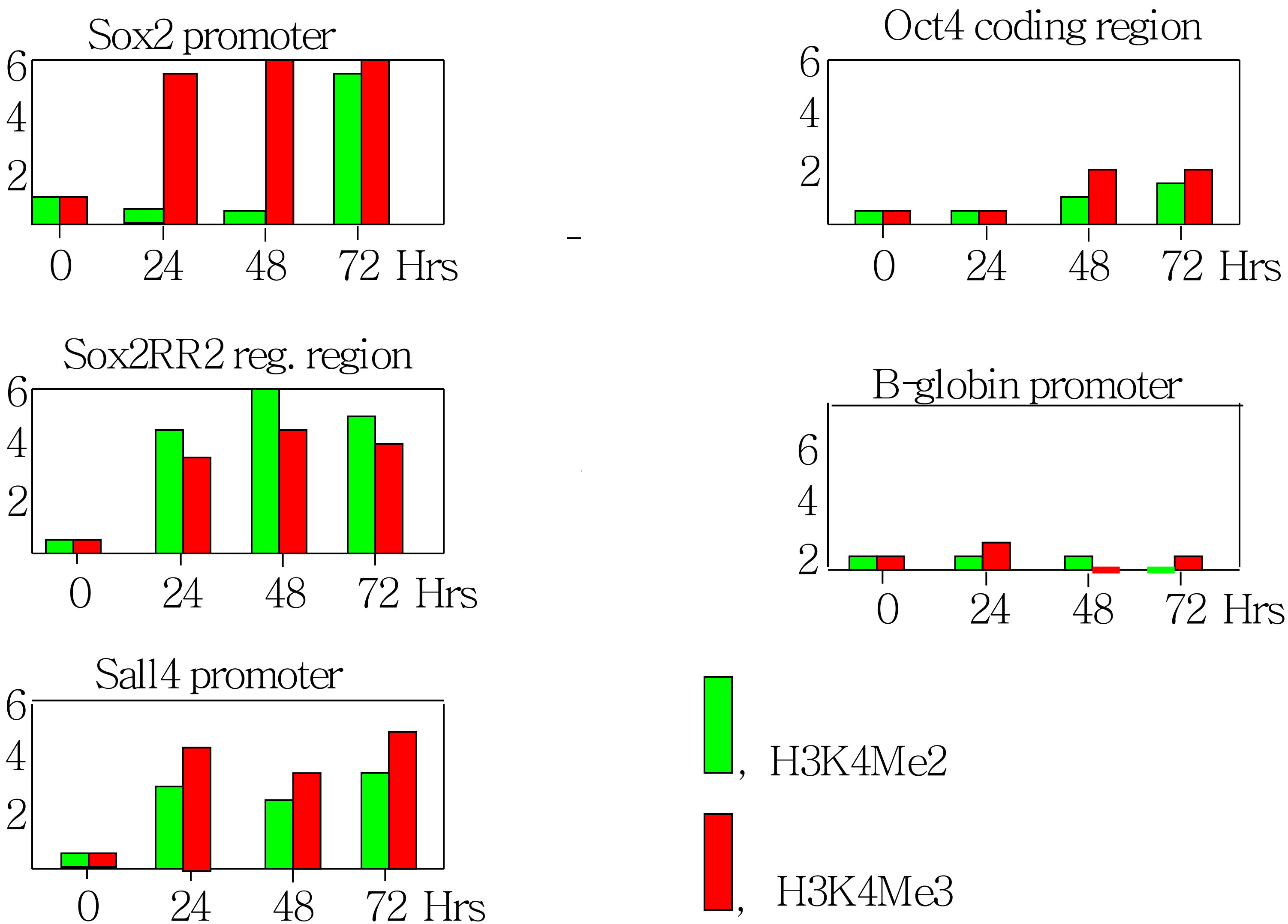






Histones in gene control regions are methylated – Chip analysis

Nuclei from retinoic acid treated ES cells



Epigenetics and chromatin, 2010.

Gene activation in somatic nuclei transplanted to oocytes is selective

		Number	%
Expresssed in MEFs, But NOT in transplanted MEF nuclei,	Repressed	7113	41
NOT expressed in MEFs, BUT in transplanted MEF nuclei	Activated	1176	9
Expressed in MEFs and in transplanted MEF nuclei	No change	3308	29

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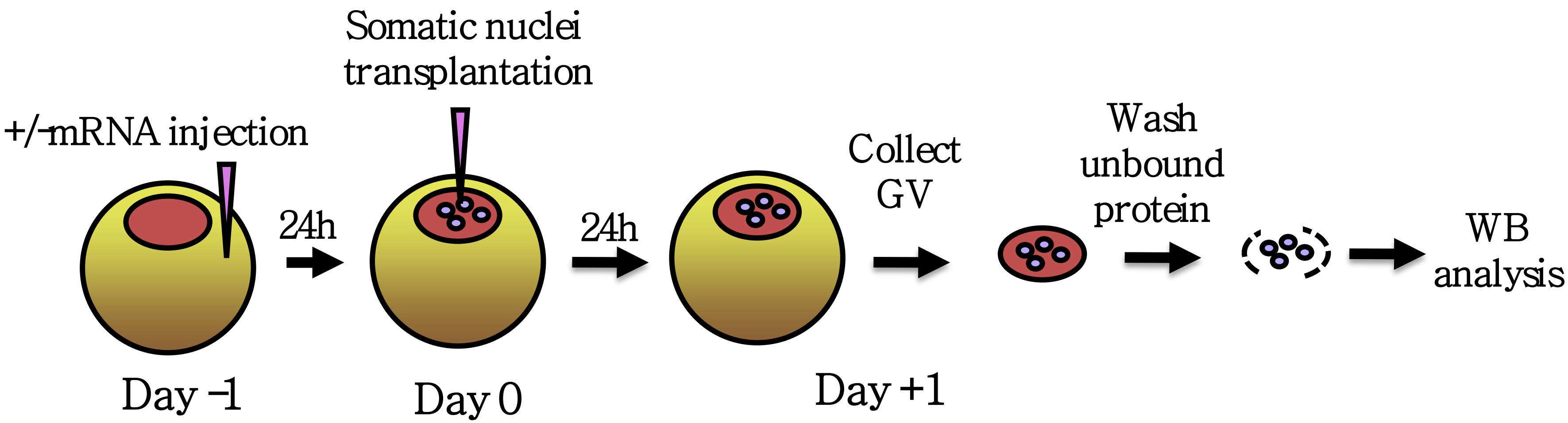
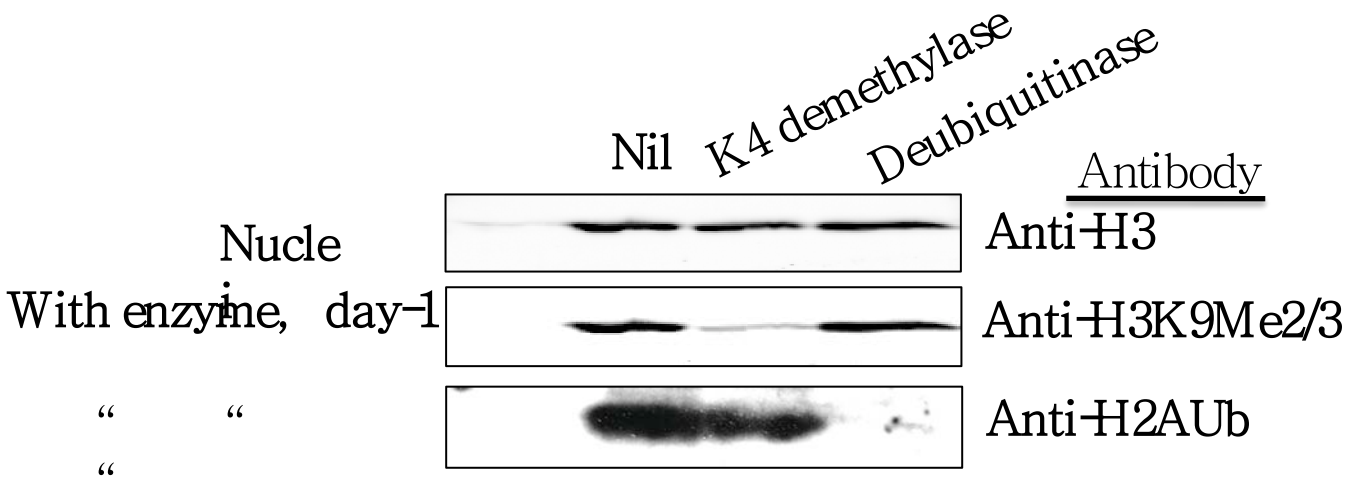
Histone modifiers overexpressed in the oocyte efficiently modify transplanted nuclear chromatin





Chromatin modifiers that alter the epigenetic state of transplanted nuclei

	enzymes	specificity
demethylase	Kdm1a	H3K4me2/1 H3K9me2/1
	Kdm2b	H3K36me2/1 H3K4me3
	Kdm3a	H3K9me2/1
	Kdm4d	H3K9me3/2
	Kdm5	H3K4me3/2
	Kdm6a	H3K27me3/2
deubiquitinase	Usp16	H2Aub
	Usp21	H2Aub
acetylase	Elp3	H3/H4
	Tip60	H3/H4

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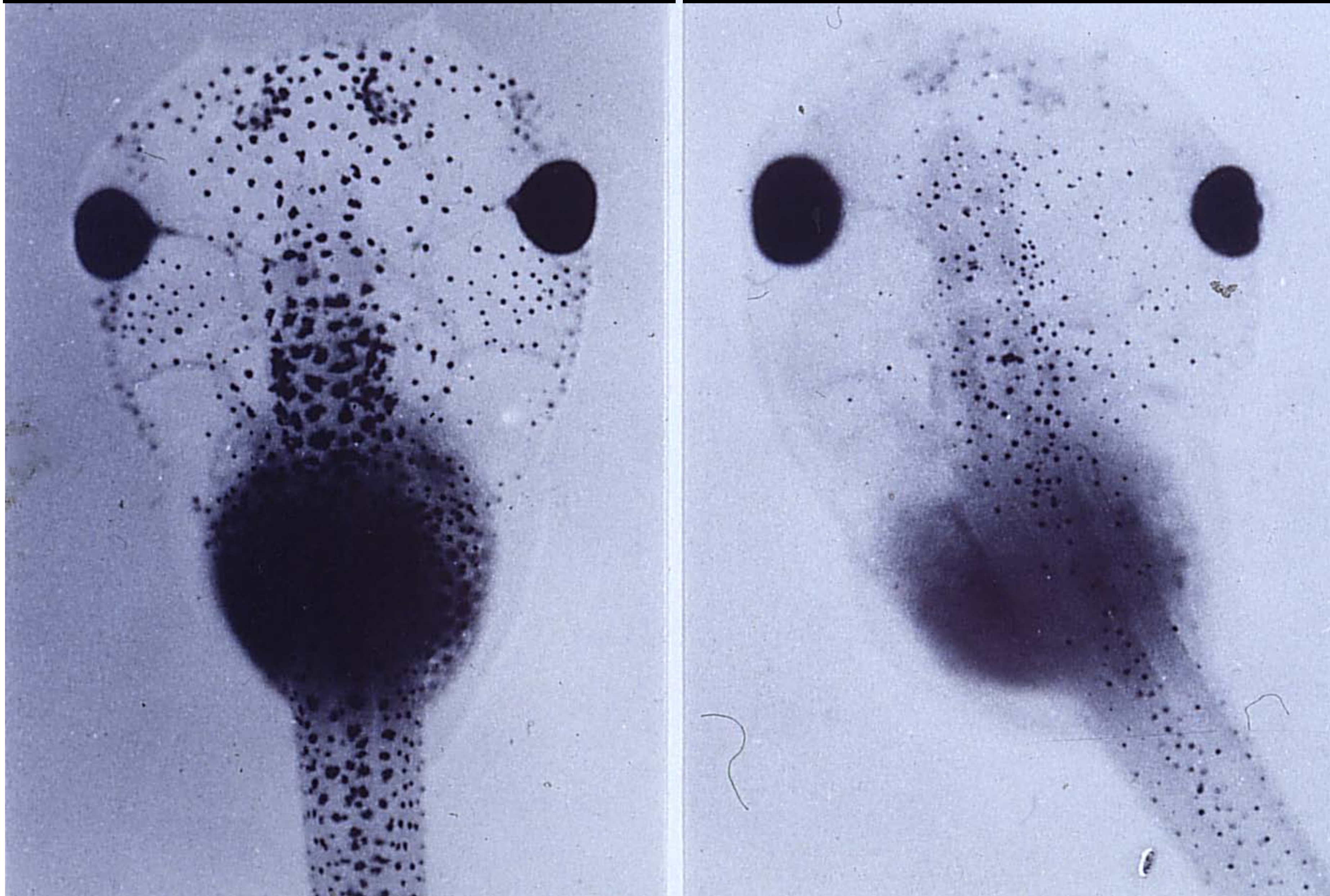
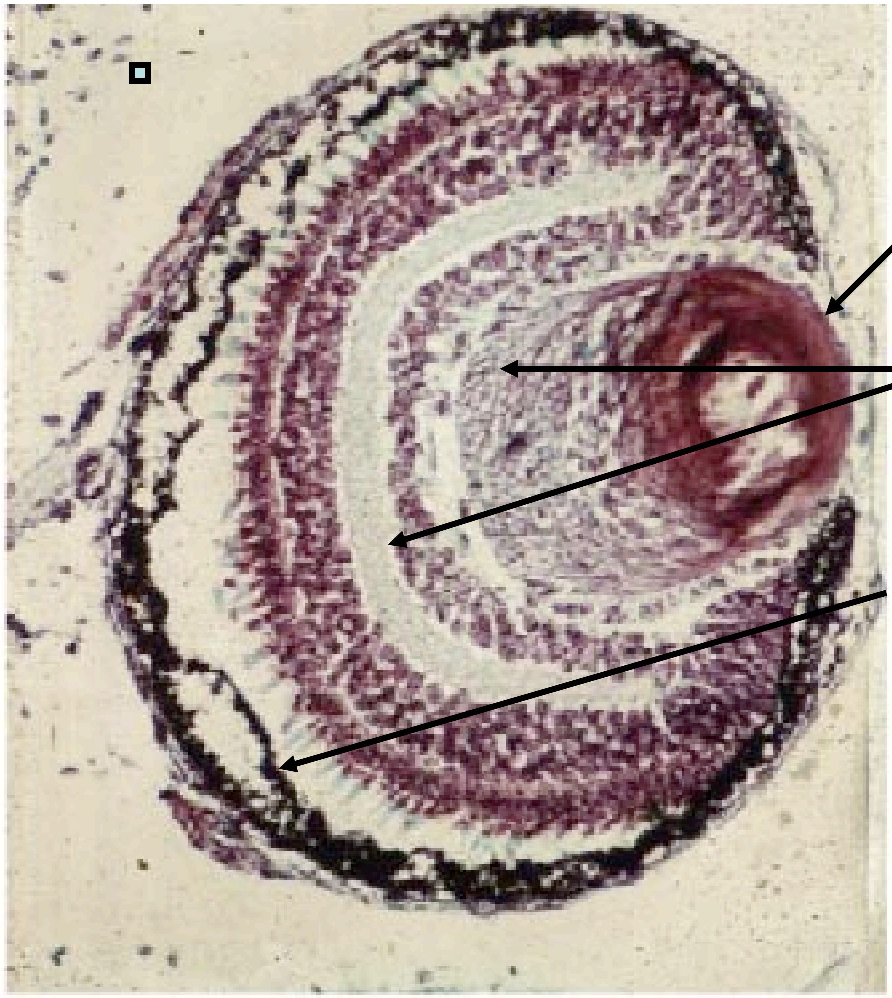
Kazutaka Murata (Histone mods)	Japan
Marta Teperek (Sperm progenitors)	Poland
Stan Wang	USA

Welcome Trust Medical Research Council

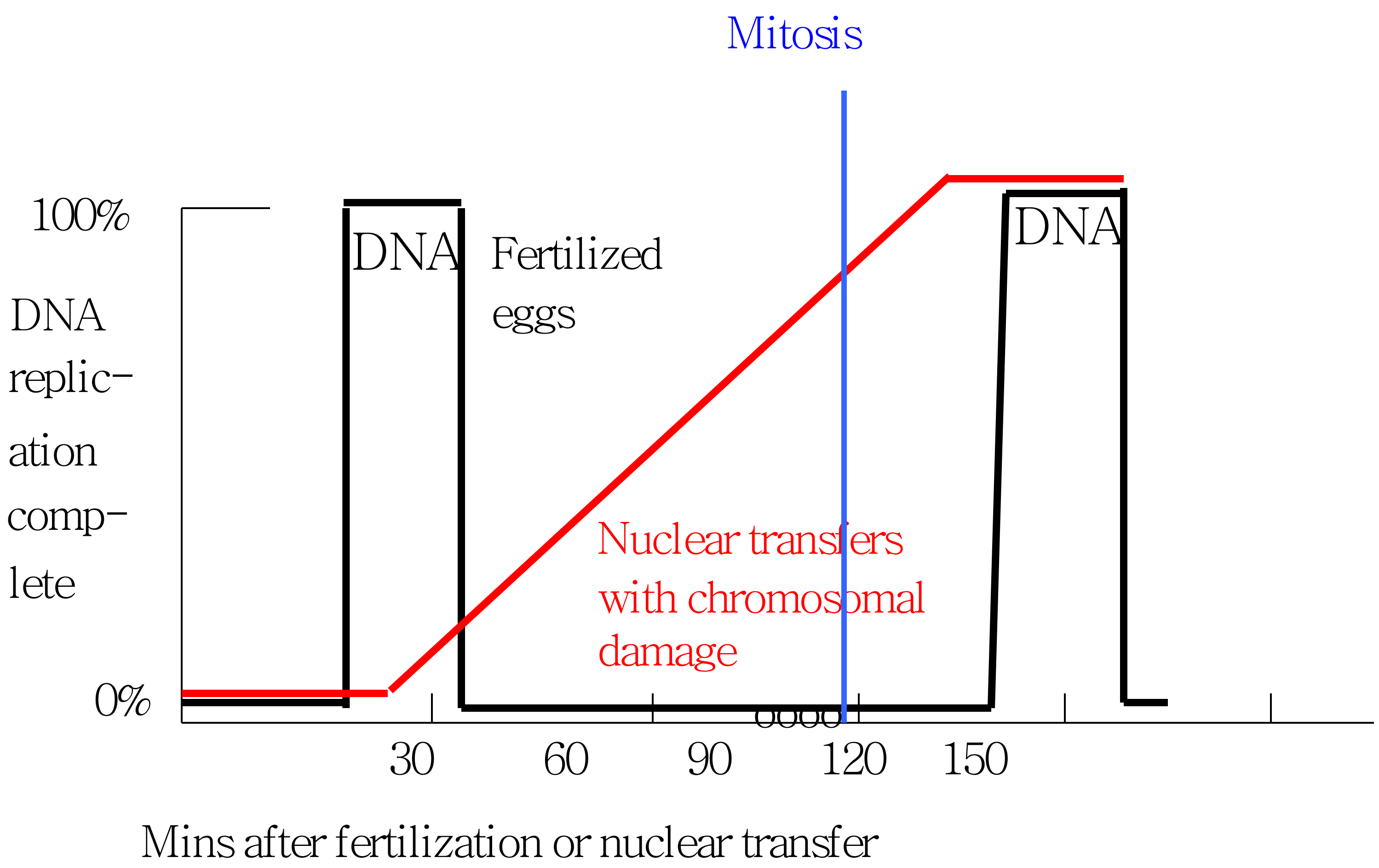
Tadpole from fertilized egg | Tadpole cloned from a muscle cell



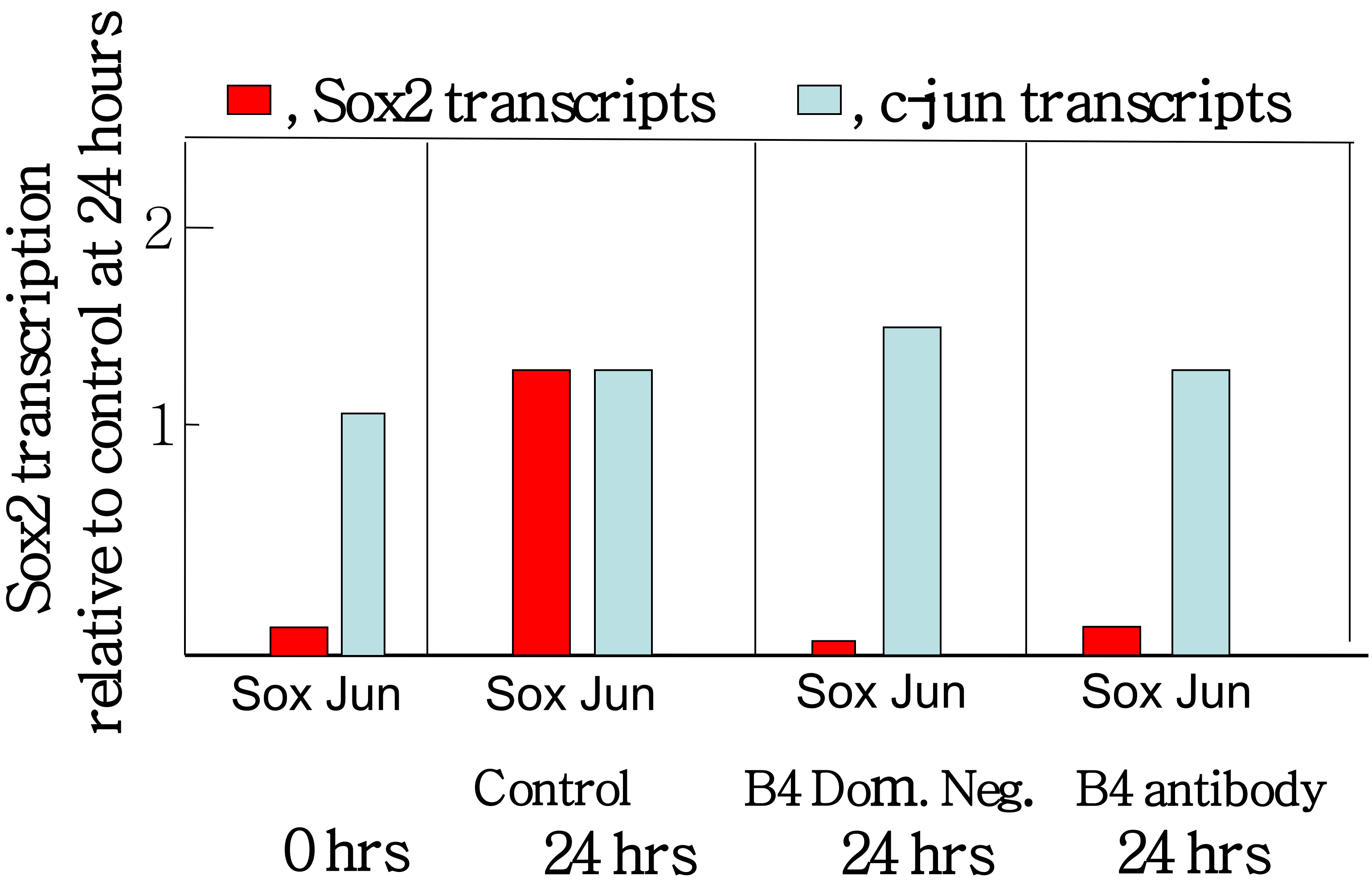
Normal eye
by cloning from a muscle cell



DNA replication is retarded in Amphibian somatic cell nuclear transfers

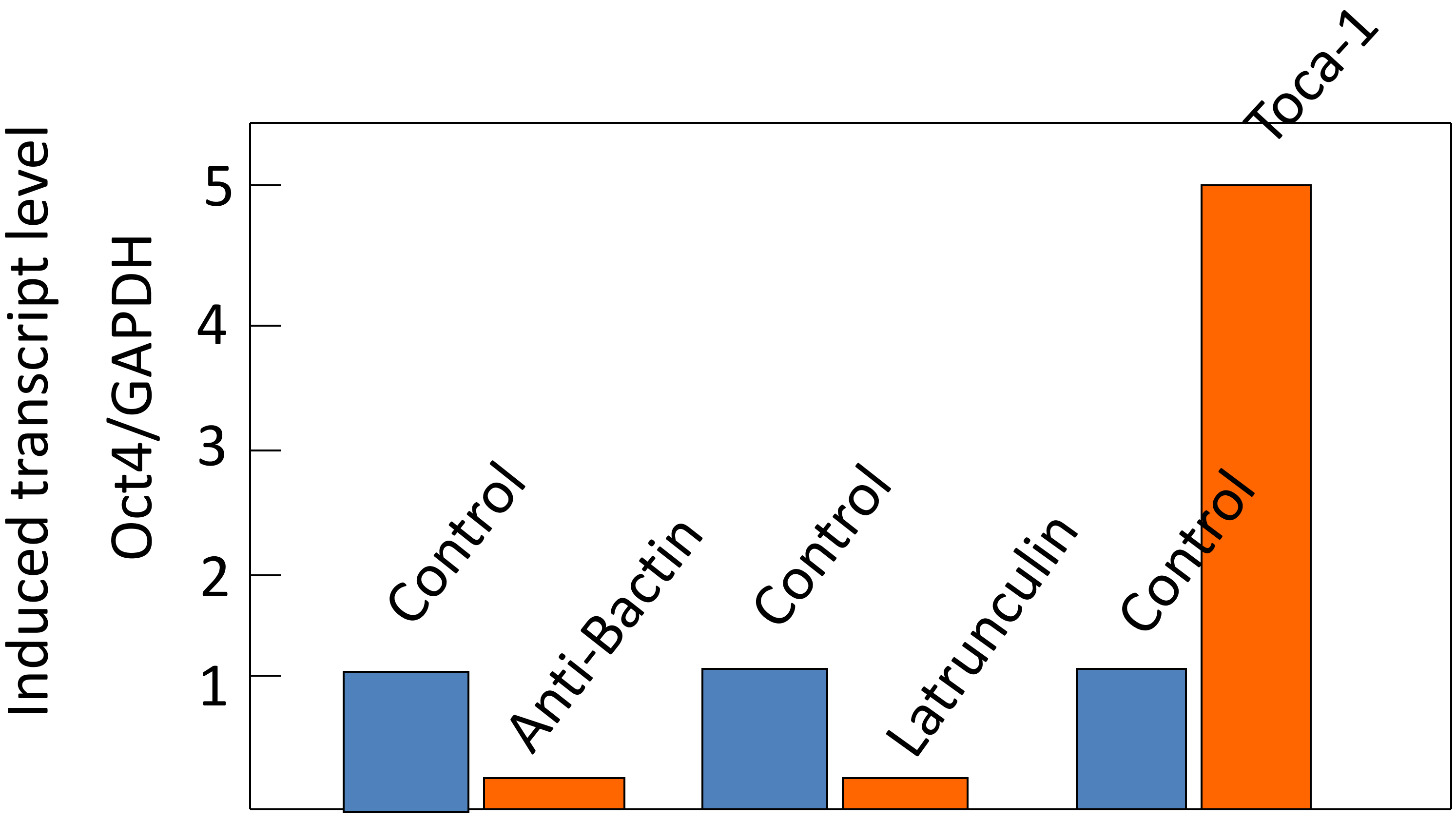


B4 histone is required for gene activation in oocytes



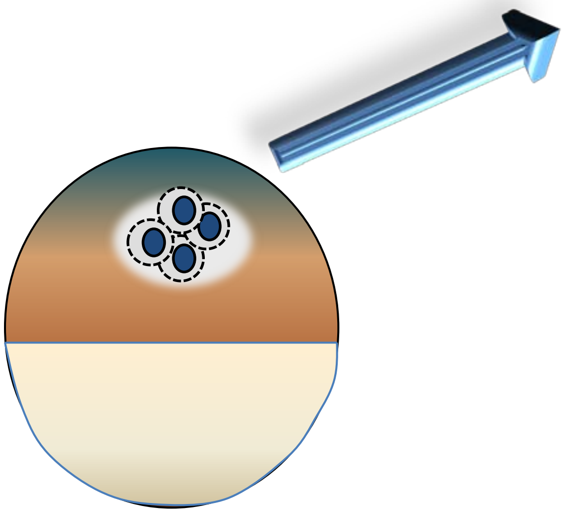
Jerome Jullien

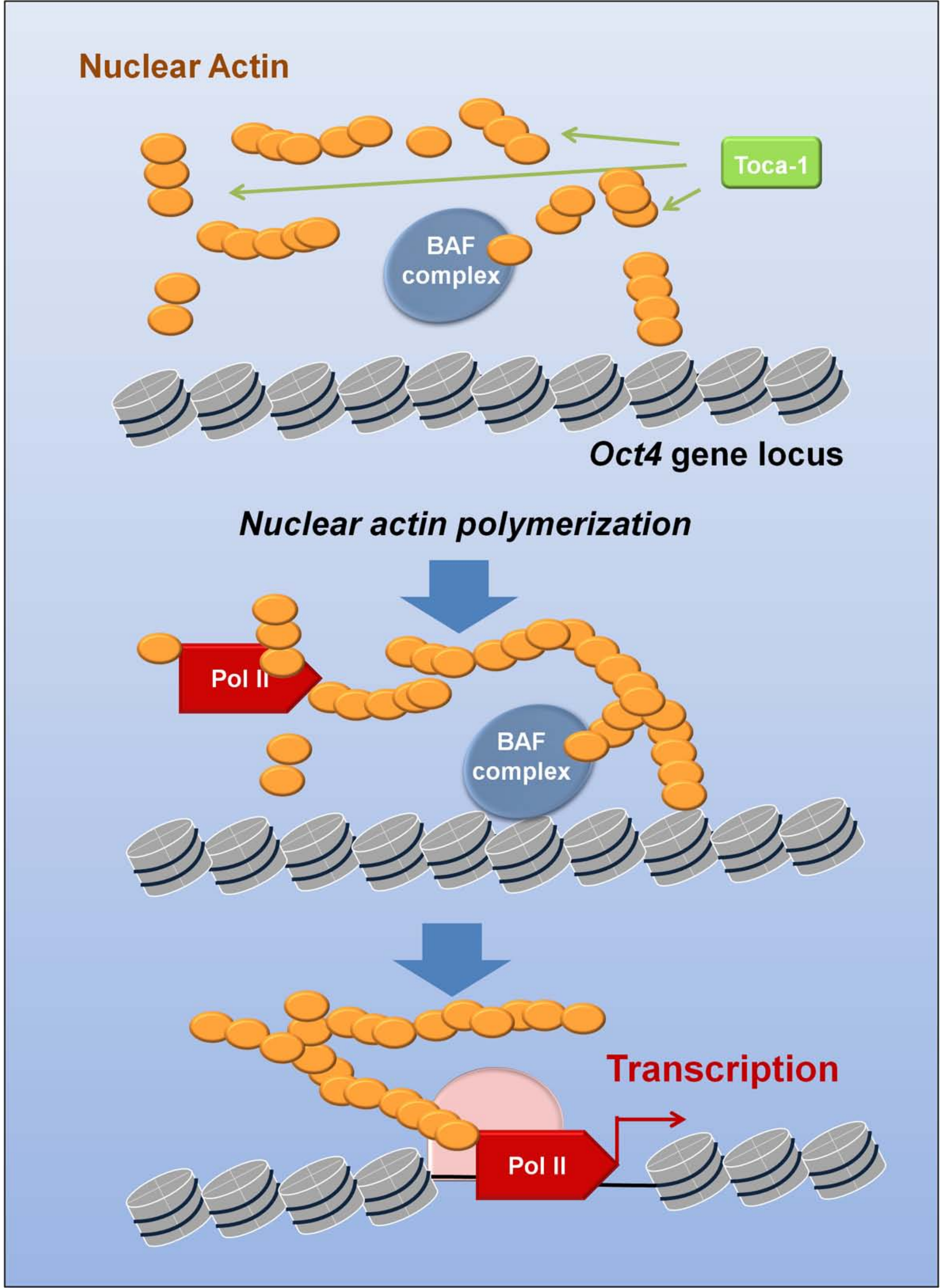
Transcription is enhanced by actin polymerization



K. Miyamoto. Gen.Devel.2011.

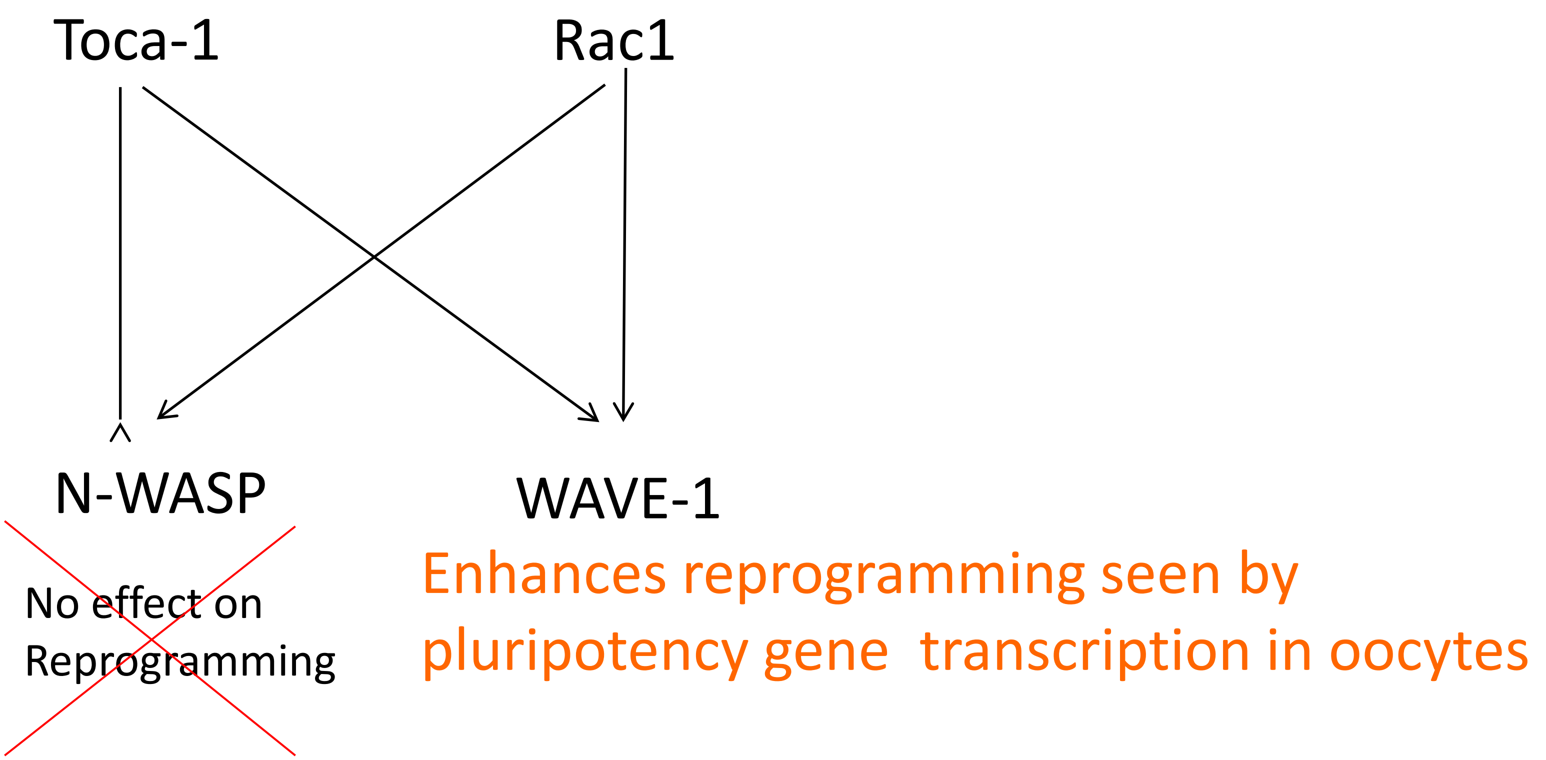
A model of nuclear actin function





Transcriptional activation is much enhanced by WAVE-1
(Wiskott-Aldrich syndrome)

Actin polymerization



WAVE-1 is required for zygotic genome activation and embryonic development

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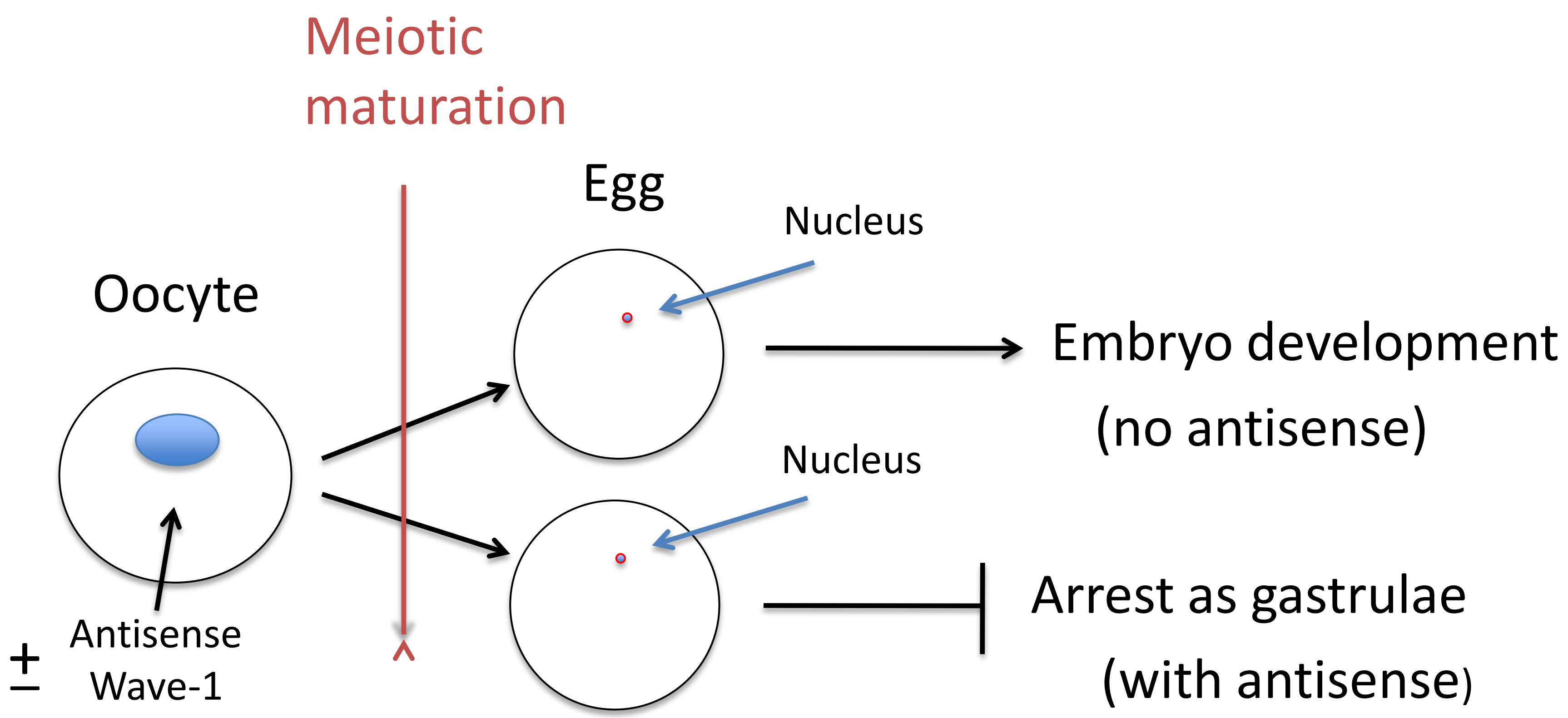
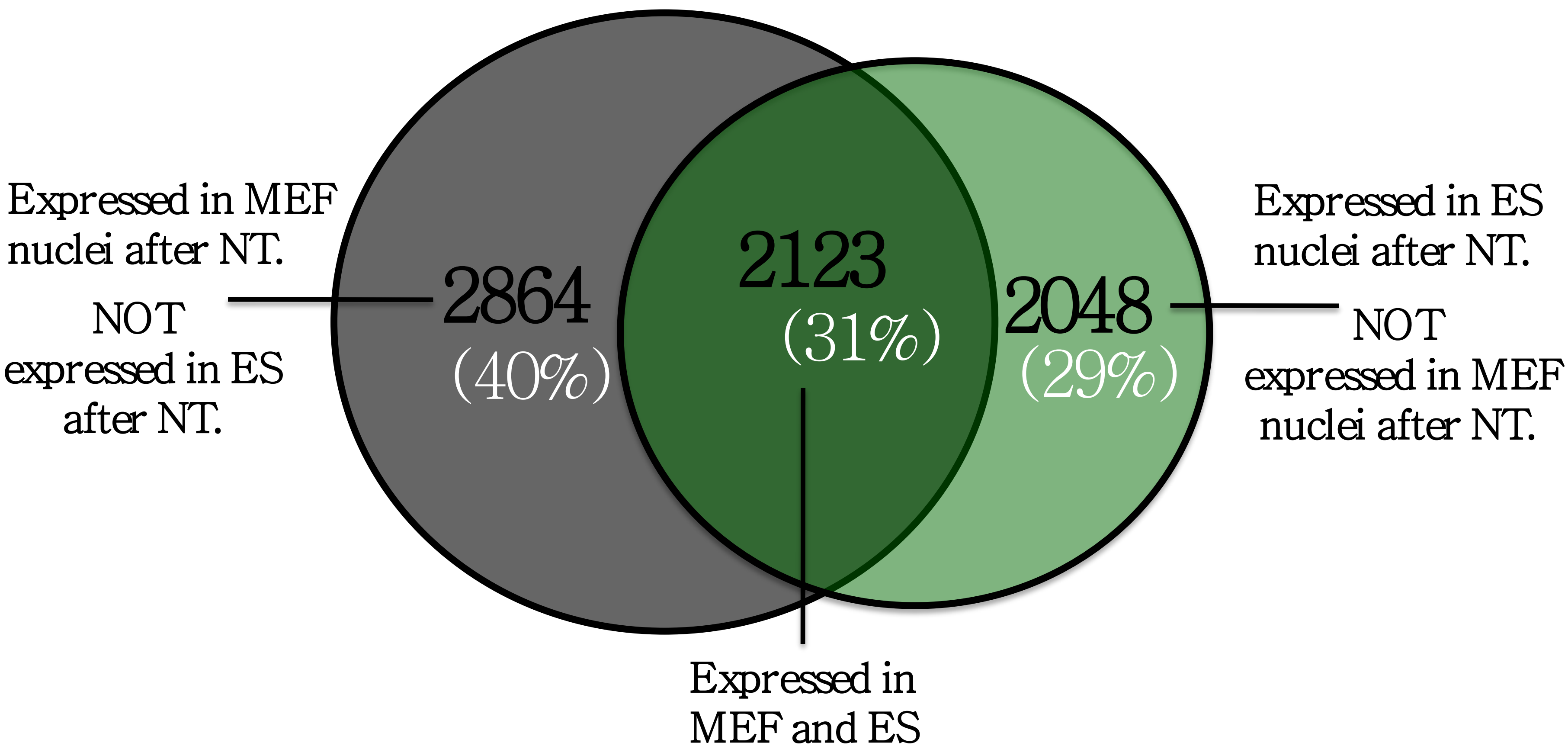


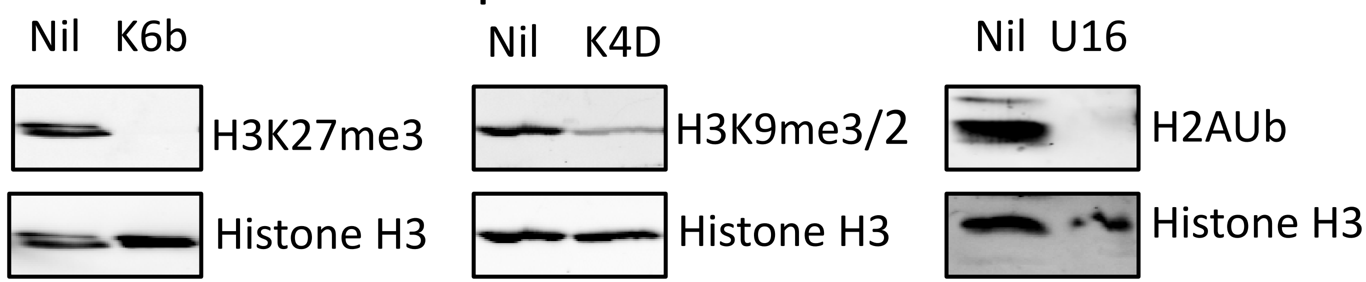
Genes with restricted expression in MEF or ES nuclei after transplantation to *Xenopus* oocytes



Histone modifications in nuclei can be changed after transfer to oocytes

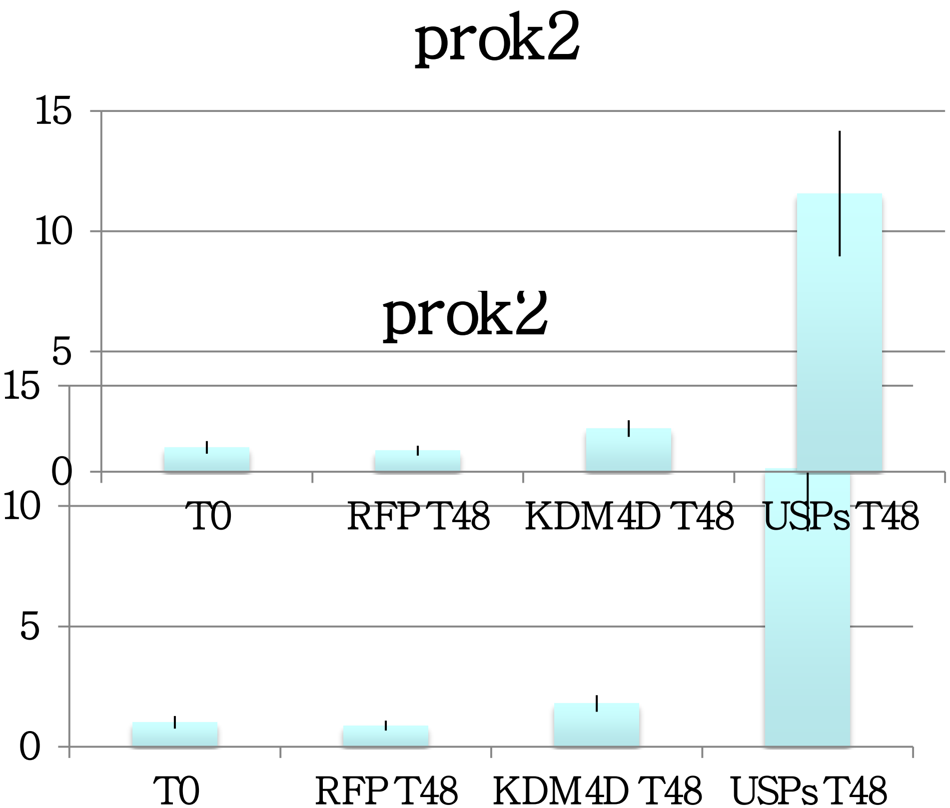
0 hour: mRNA injections. 24 hours: nuclear injections.
48 hours:reisolation of injected nuclei and Western analysis.

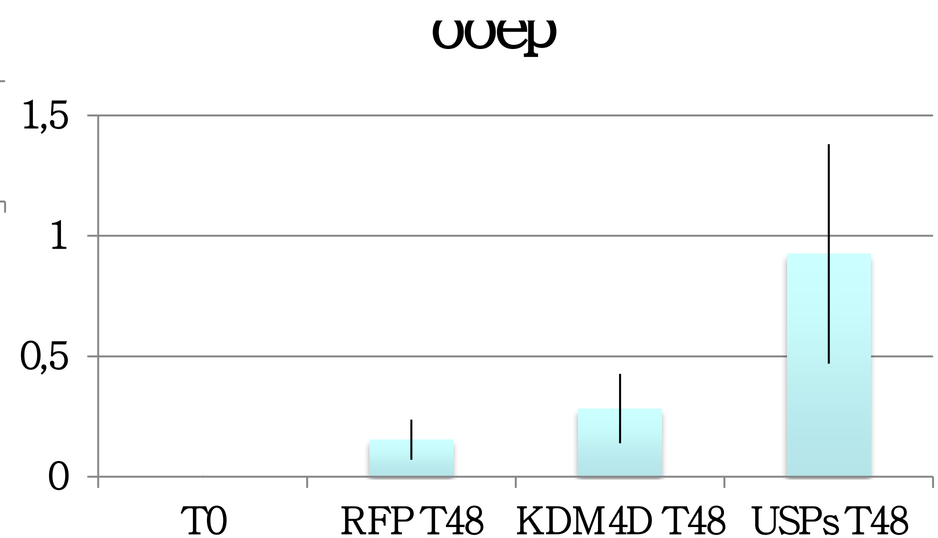
Overexpressed mRNAs



K6b, H3K27 demethylase. K4D, H3K9 demethylase. U16, H2A deubiquitinase.
Western blots to show loss of histone modifications 48 hours after mRNA injection.

Overexpression of H2A deubiquitinases removes restriction





Transcriptional reprogramming

CONCLUSIONS

Eggs and oocytes have a very high content of histone H3.3.

Histone H3.3 prolongs transcription of somatic nuclei in oocytes.