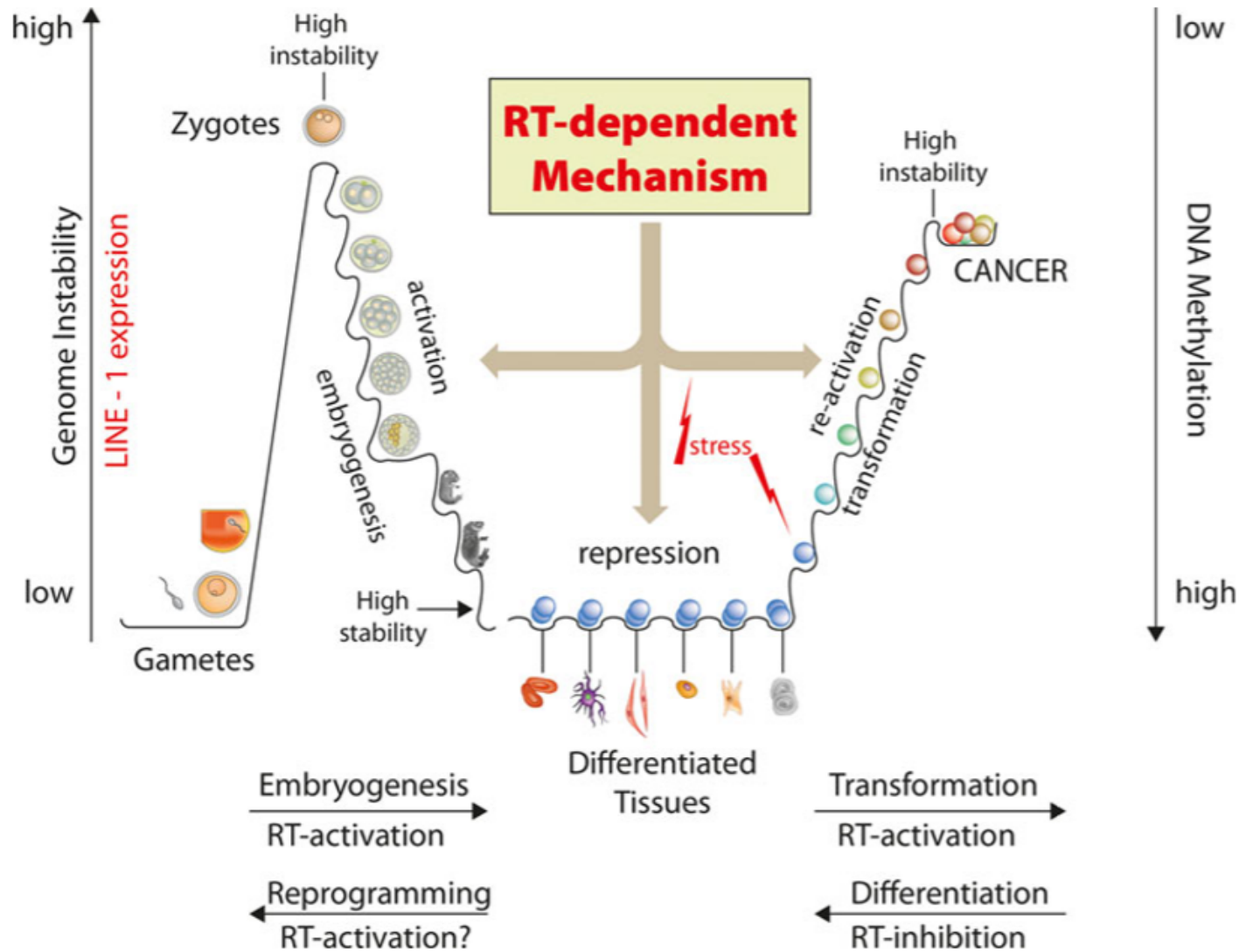


## ***PSEUDOGENE lncRNAs EXAMPLE 1***

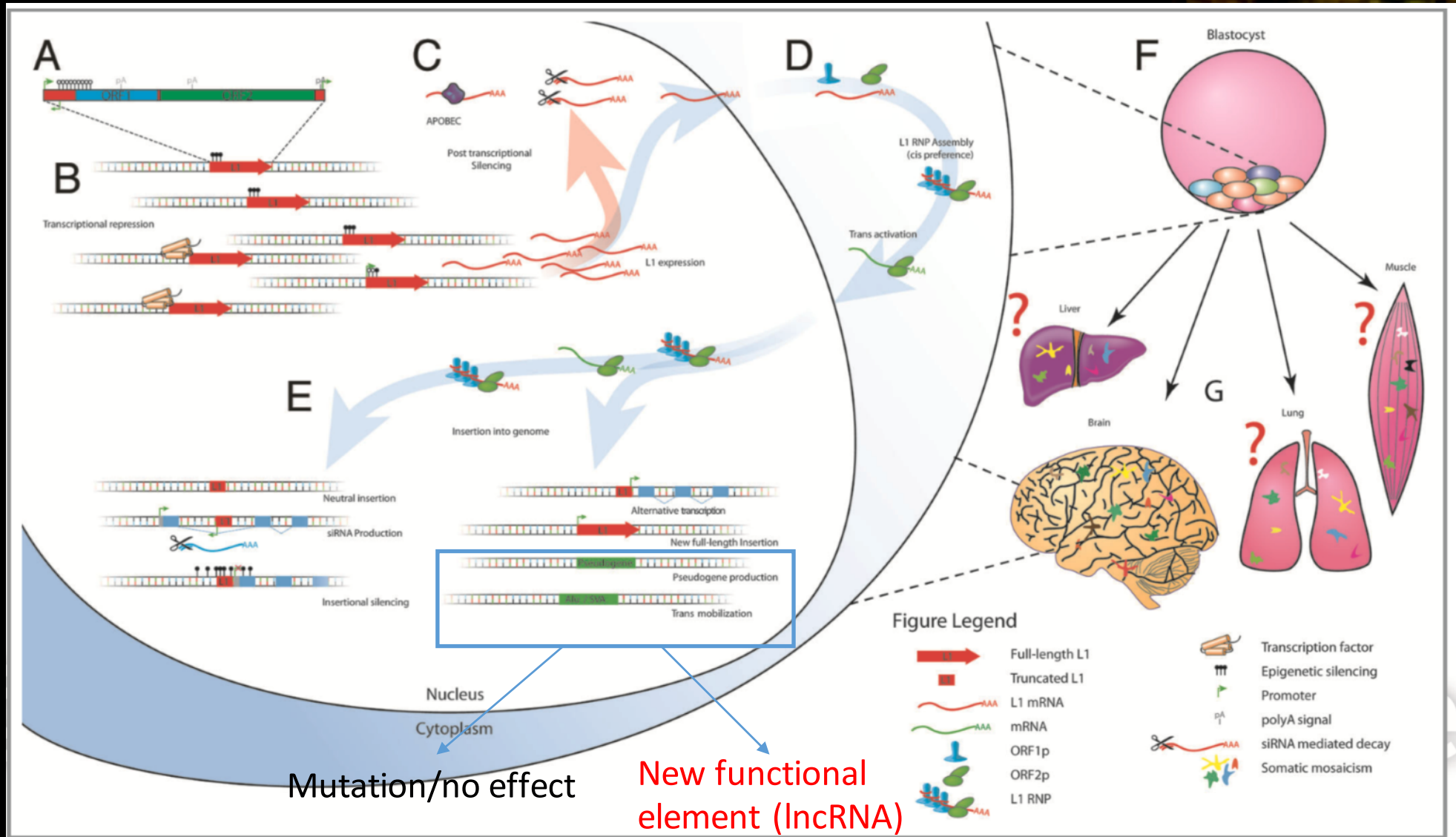
***Pseudogene lncRNA that controls embryonic stem cell  
Self-renewal***

***A Oct4P4 pseudogene derived lncRNA silences the  
ancestral Oct4 gene in trans***

# Retrotransposon activity during development

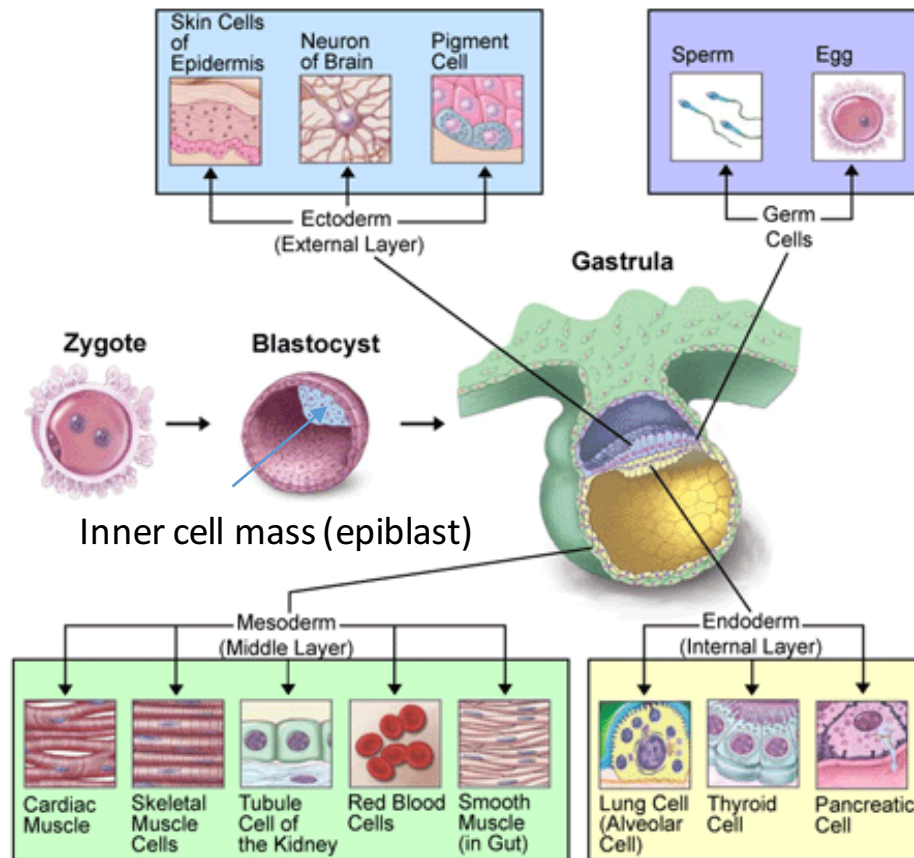


# Retrotransposons can change genetic Context → mosaic somatic tissues

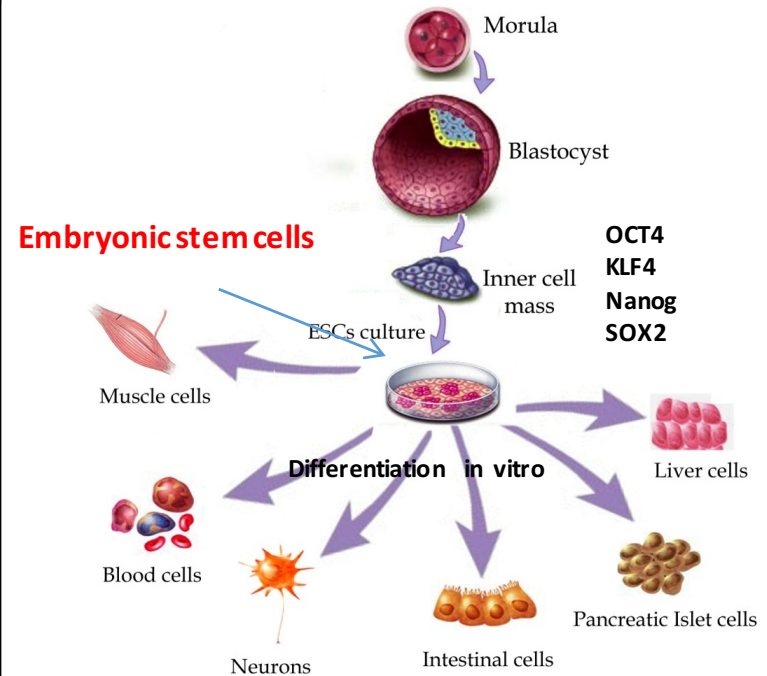


# The inner cell mass of the blastocyst are the source of pluripotent embryonic stem cells

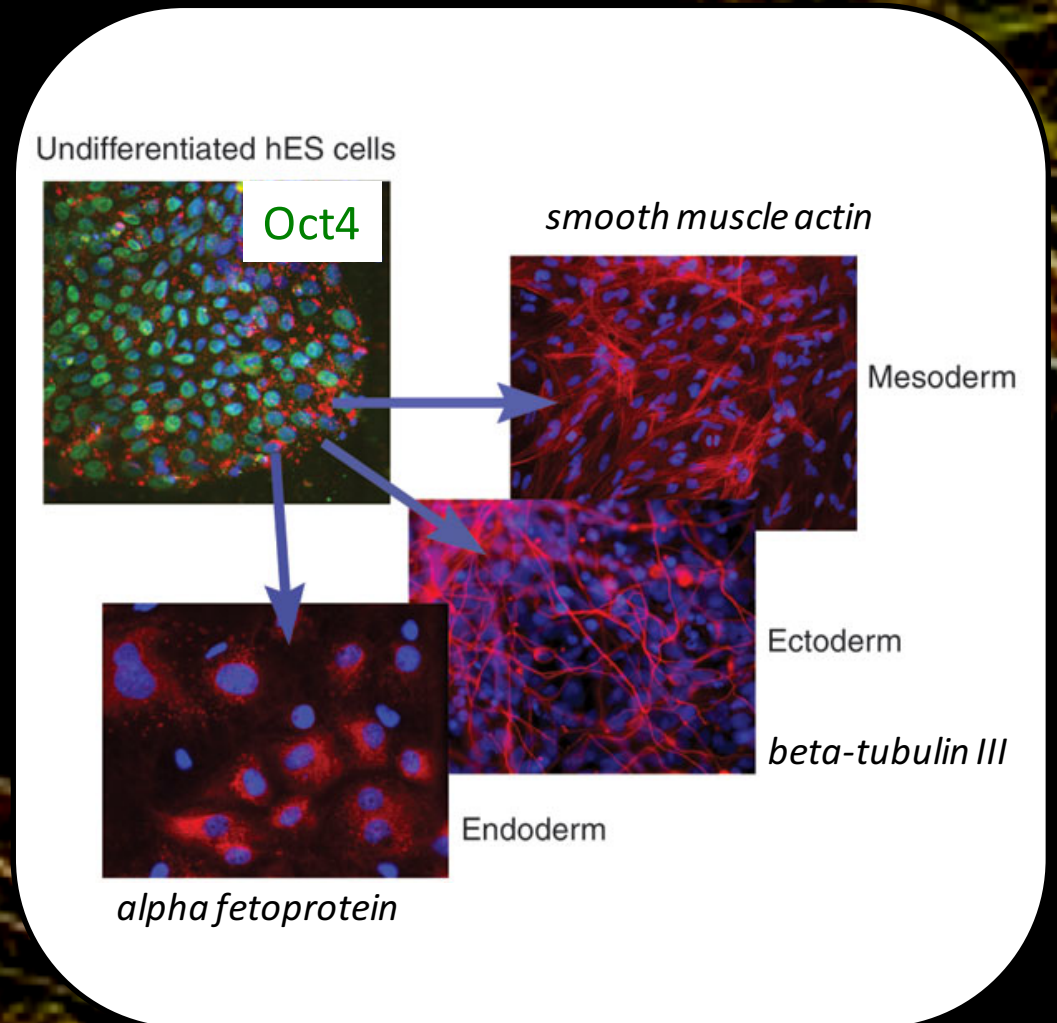
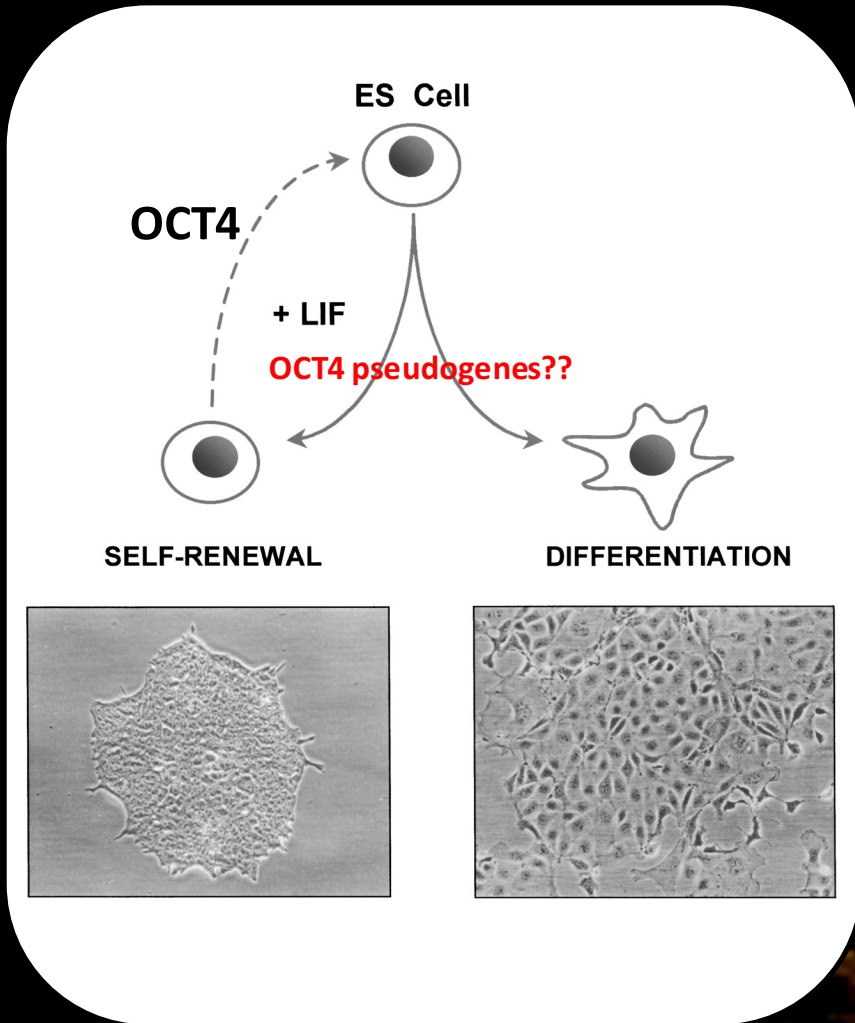
Blastocyst cells give rise to all organs and cell types



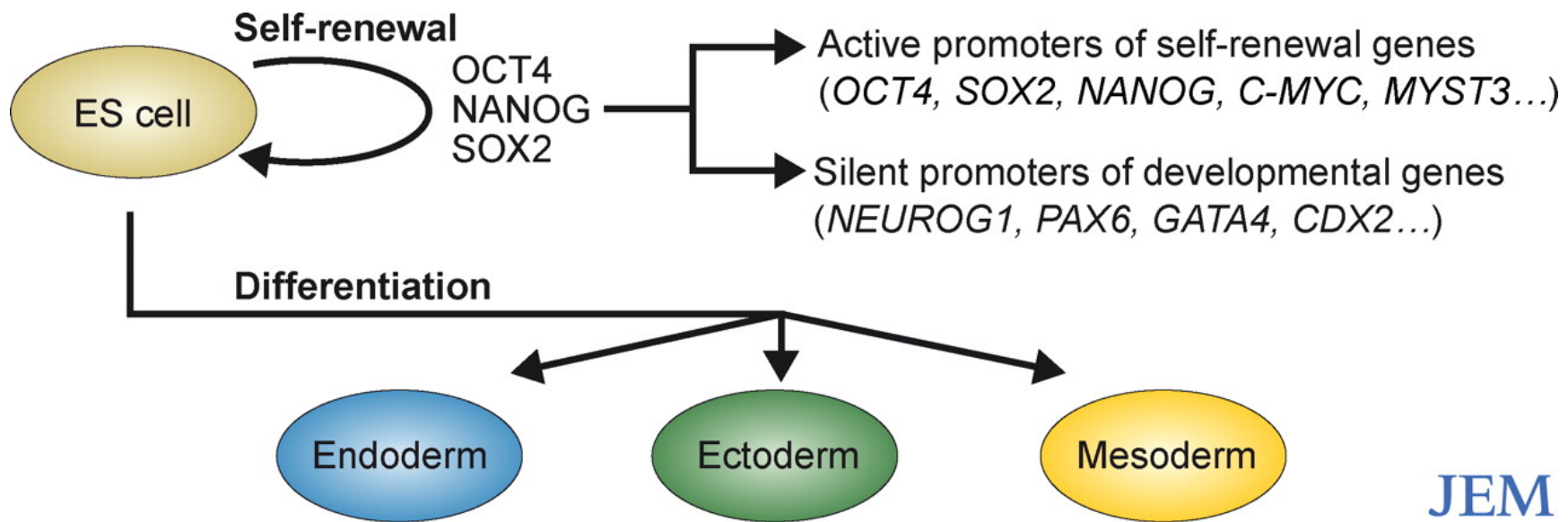
Blastocyst cells can be Isolated and cultivated



# OCT4 expressing ES cells have self-renewing and differentiation potential in vitro

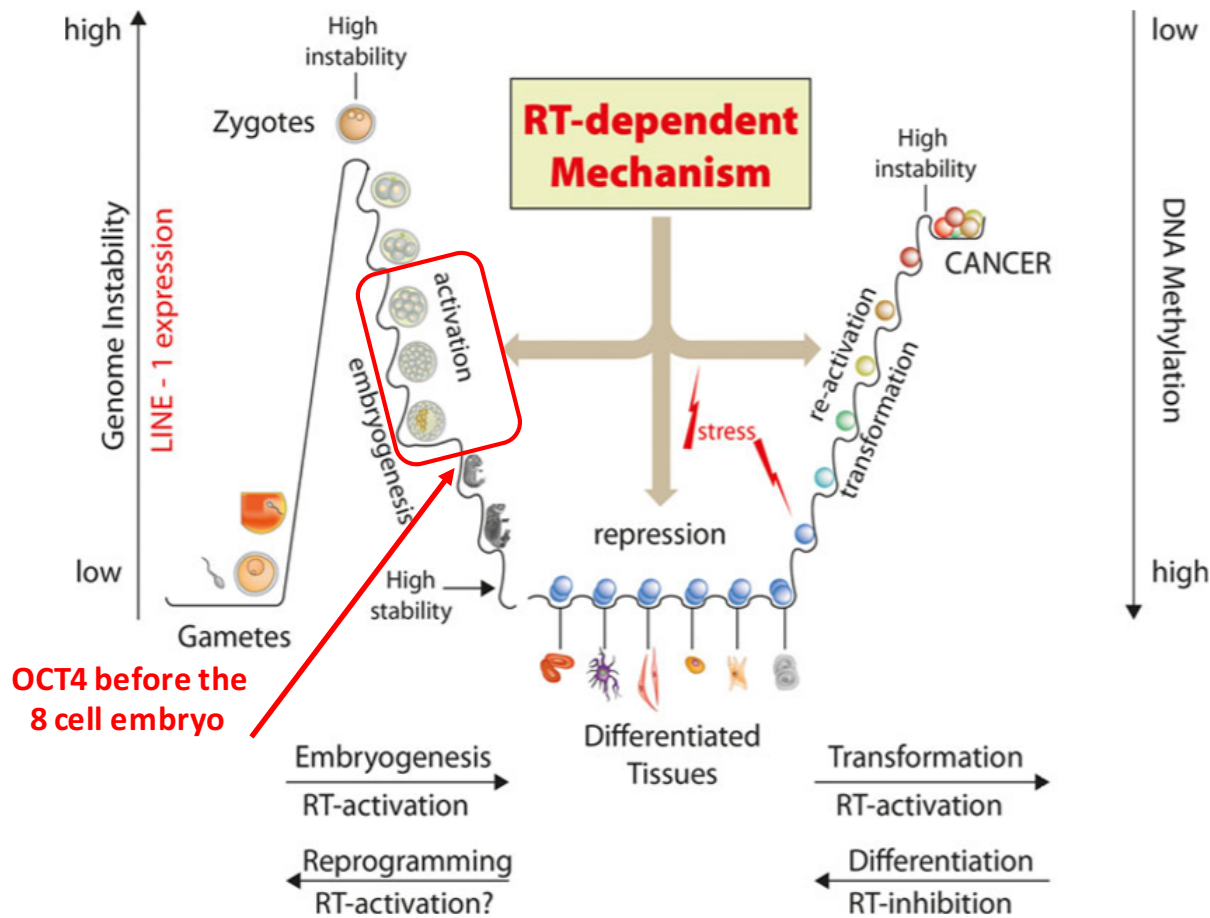


# The self-renewal transcription factor Oct4 is essential for embryonic stem cell self-renewal



Nicolaj Strøyer Christophersen, and Kristian Helin J Exp Med 2010;207:2287-2295

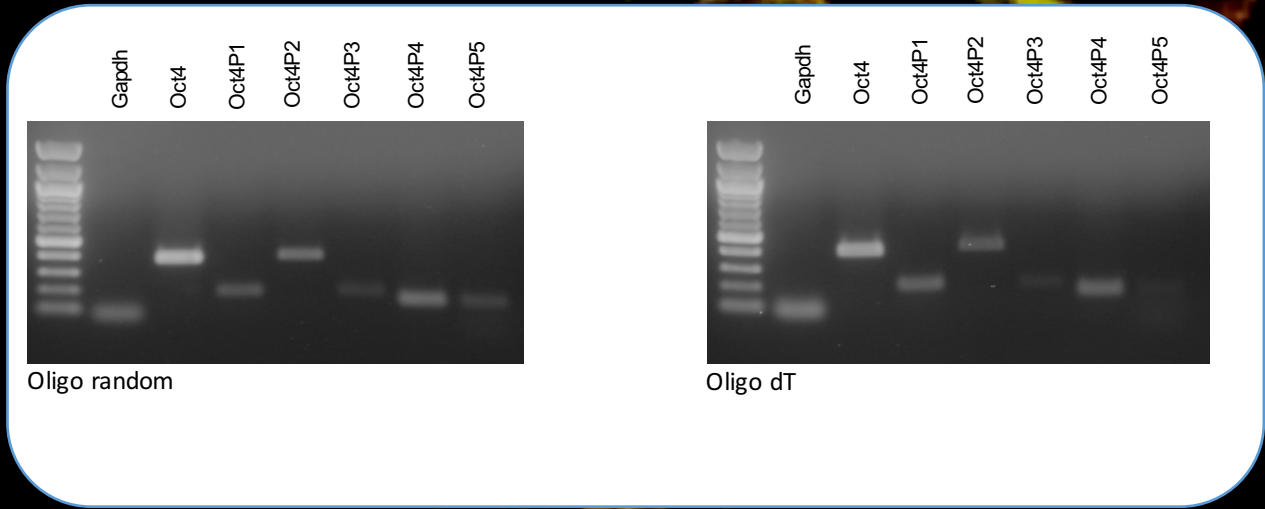
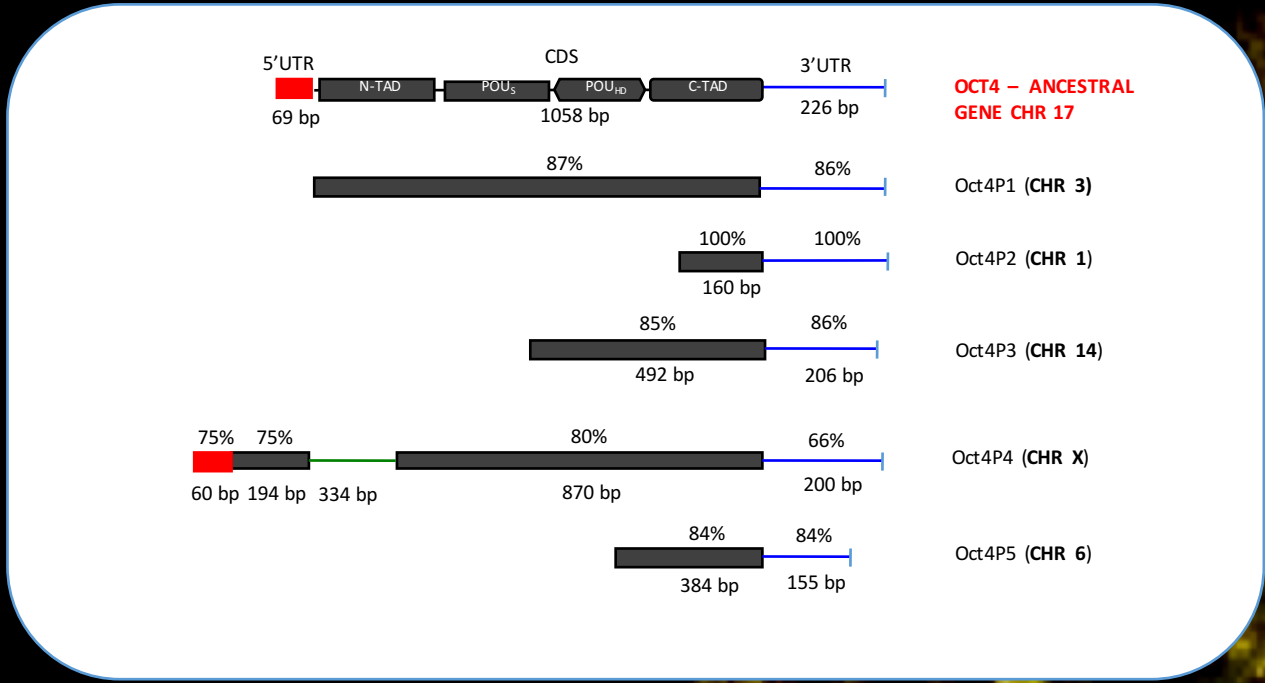
# Mouse and human contain several processed OCT4 pseudogenes



**Human:**  
 1 ancestral OCT4  
 7 processed OCT4  
 pseudogenes

**Mouse:**  
 1 ancestral Oct4  
 5 processed OCT4  
 pseudogenes

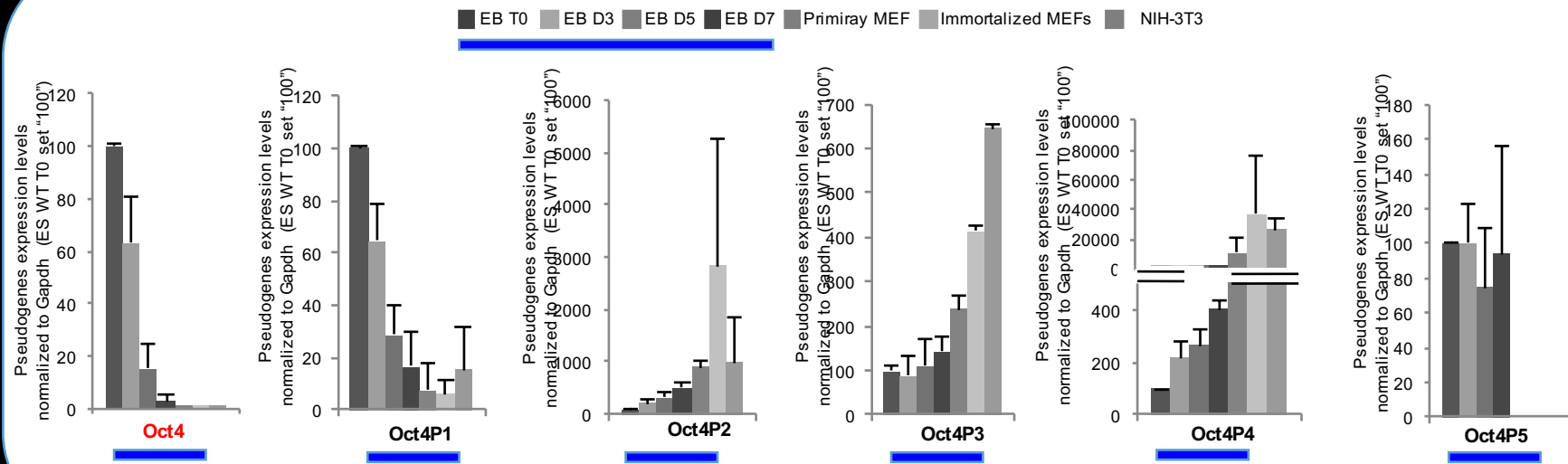
# Ancestral OCT4 gave rise to 5 processed pseudogenes that are expressed in mESCs



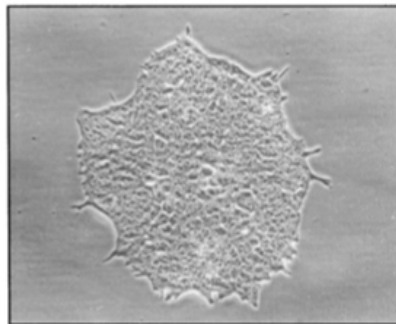


# Oct4 pseudogenes are tightly controlled during the differentiation of mESCs

EB: embryoid body differentiation  
(a model for ES differentiation)

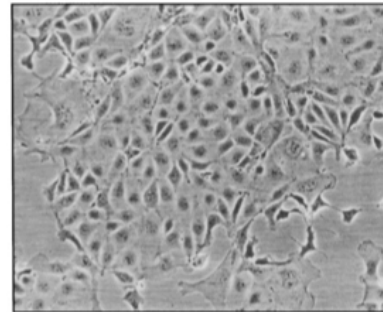


SELF-RENEWAL



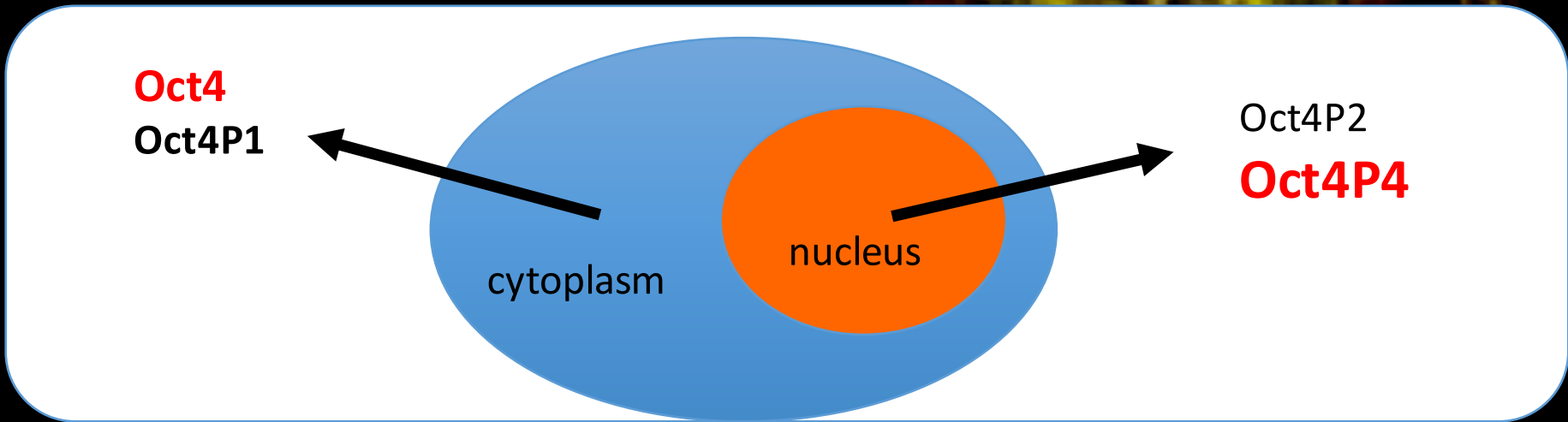
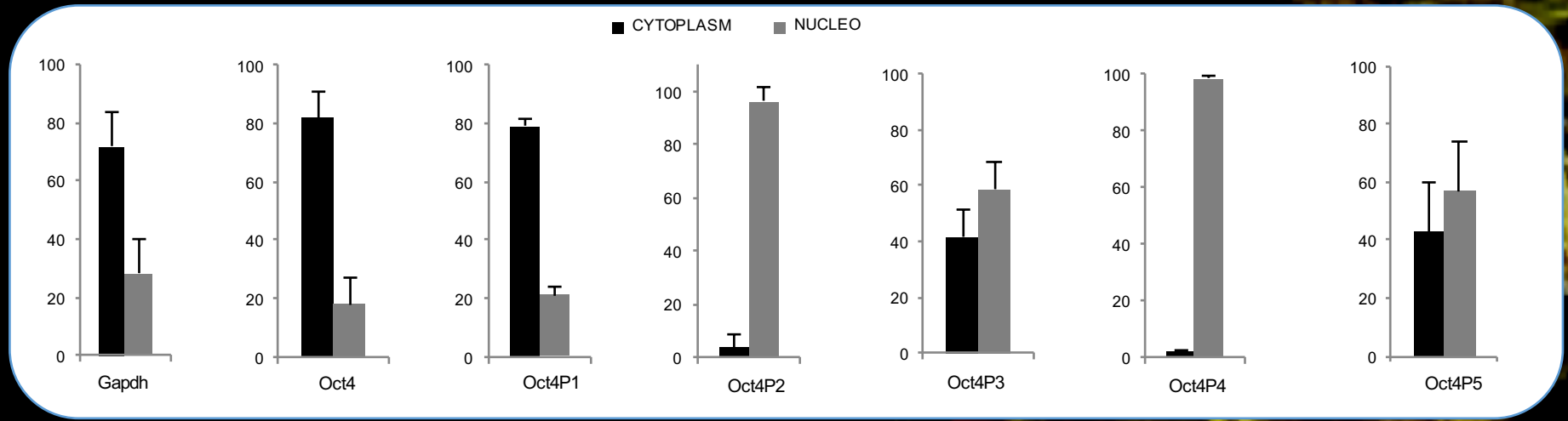
Oct4  
Oct4P1 (-10X)

DIFFERENTIATION

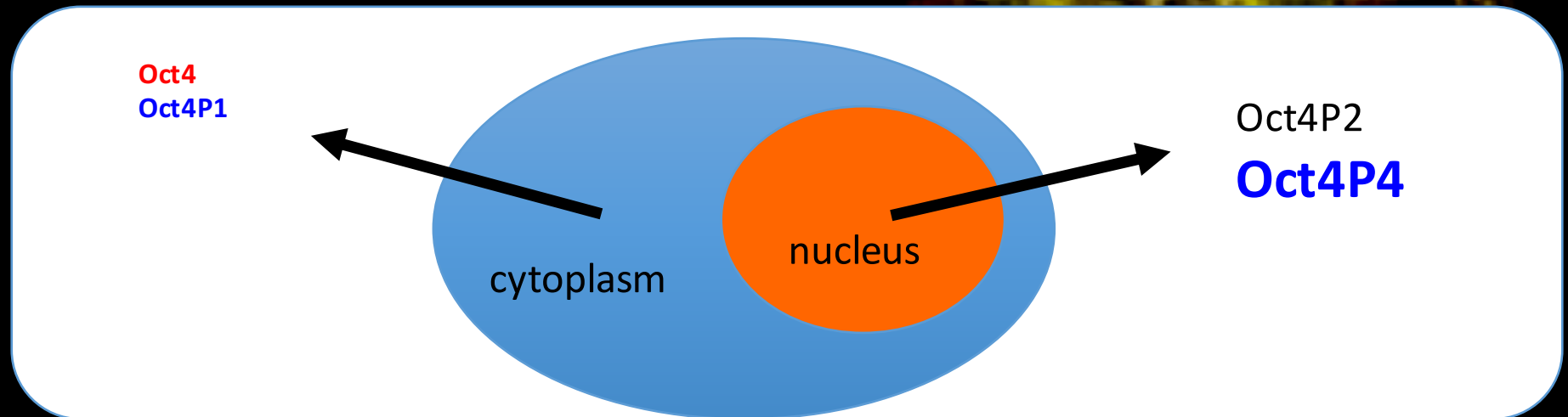
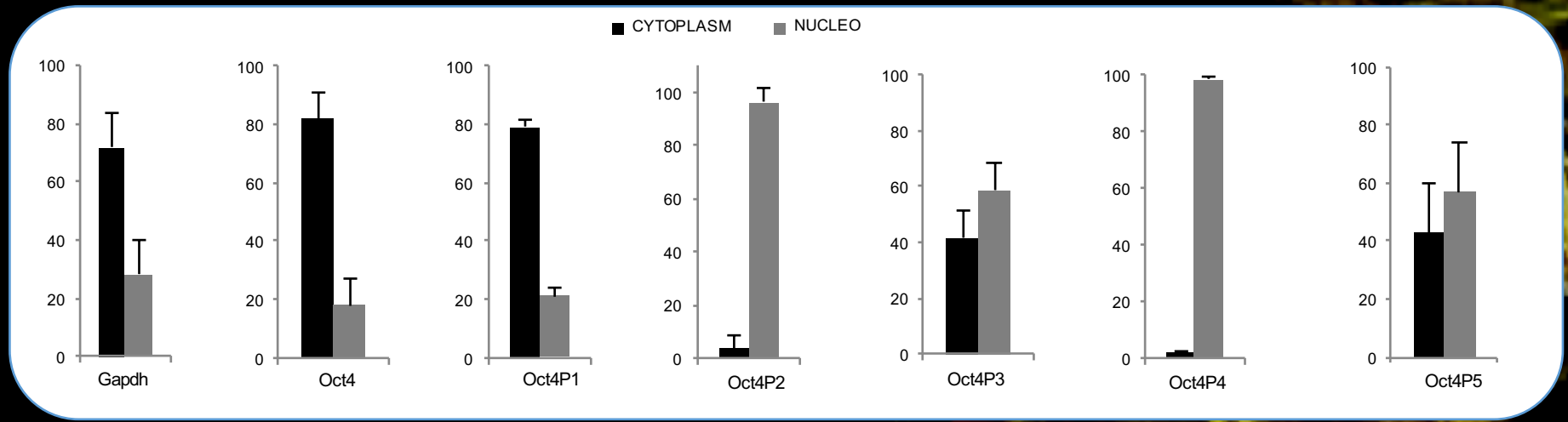


Oct4P2 (+9x)  
Oct4P3 (+2x)  
Oct4P4 (+4x; Fibrobl. +200x)

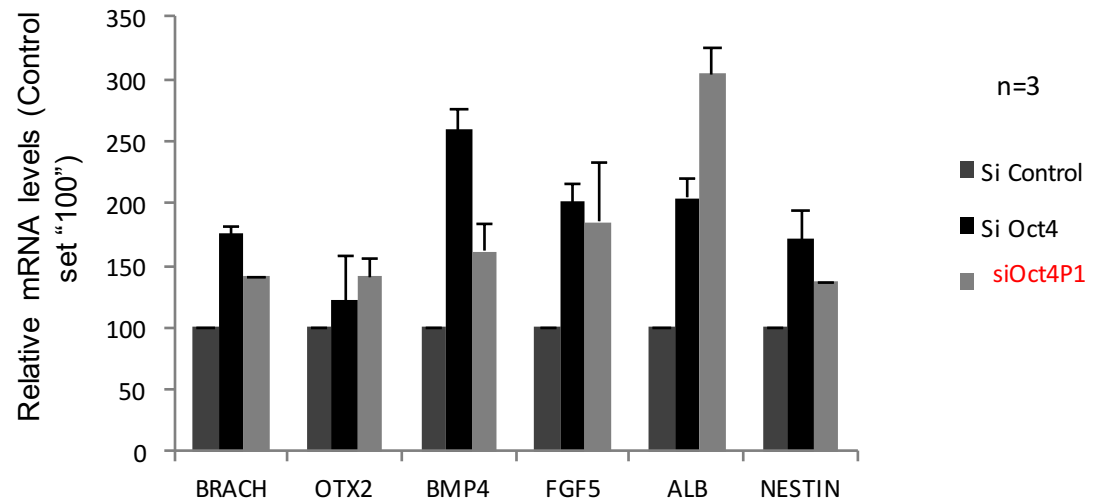
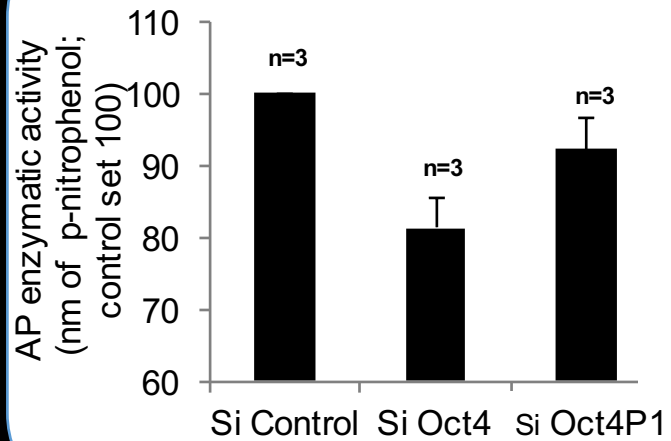
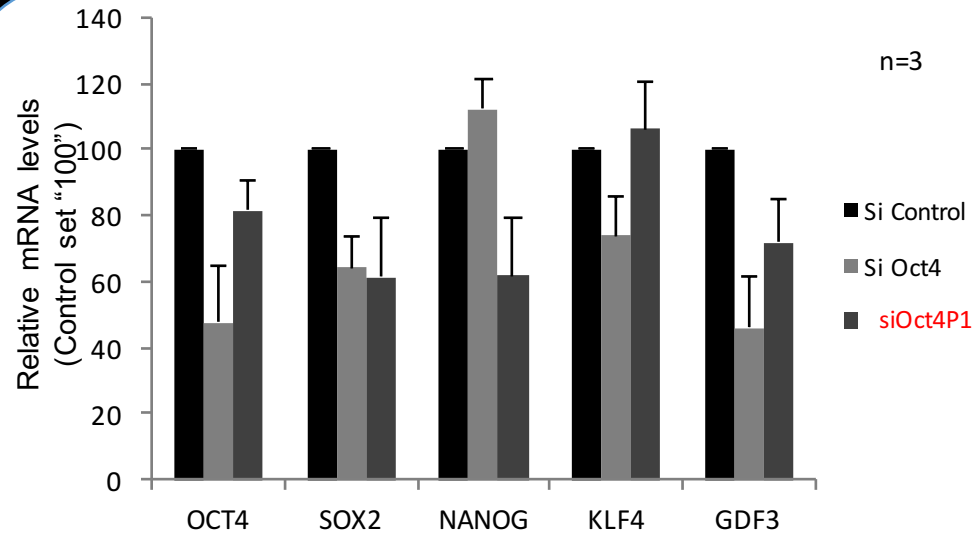
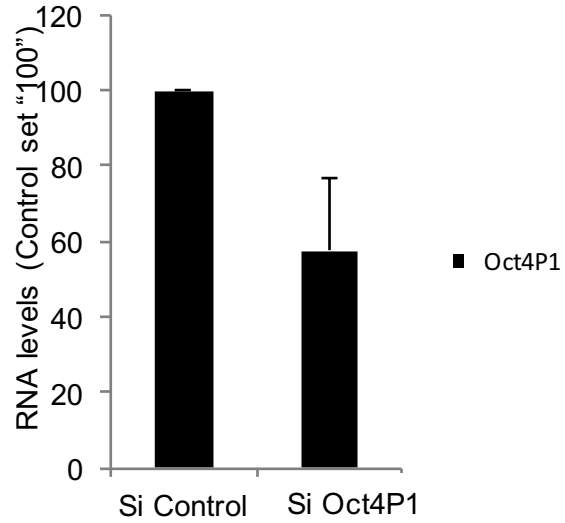
# OCT4 pseudogenes are localized to nucleoplasm or cytoplasm



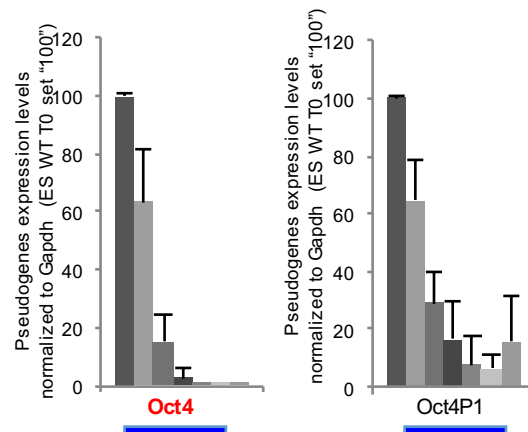
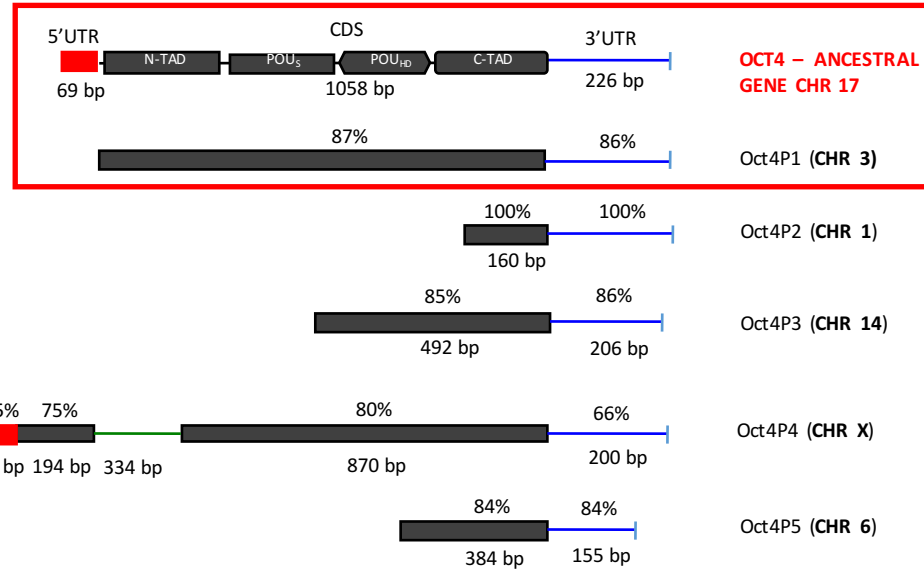
# OCT4 pseudogenes are localized to nucleoplasm or cytoplasm



# Cytoplasmatic OCT4P1 promotes mESC self-renewal



# Ancestral OCT4 gave rise to 5 processed pseudogenes that are expressed in mESCs



siOct4: high in ESC

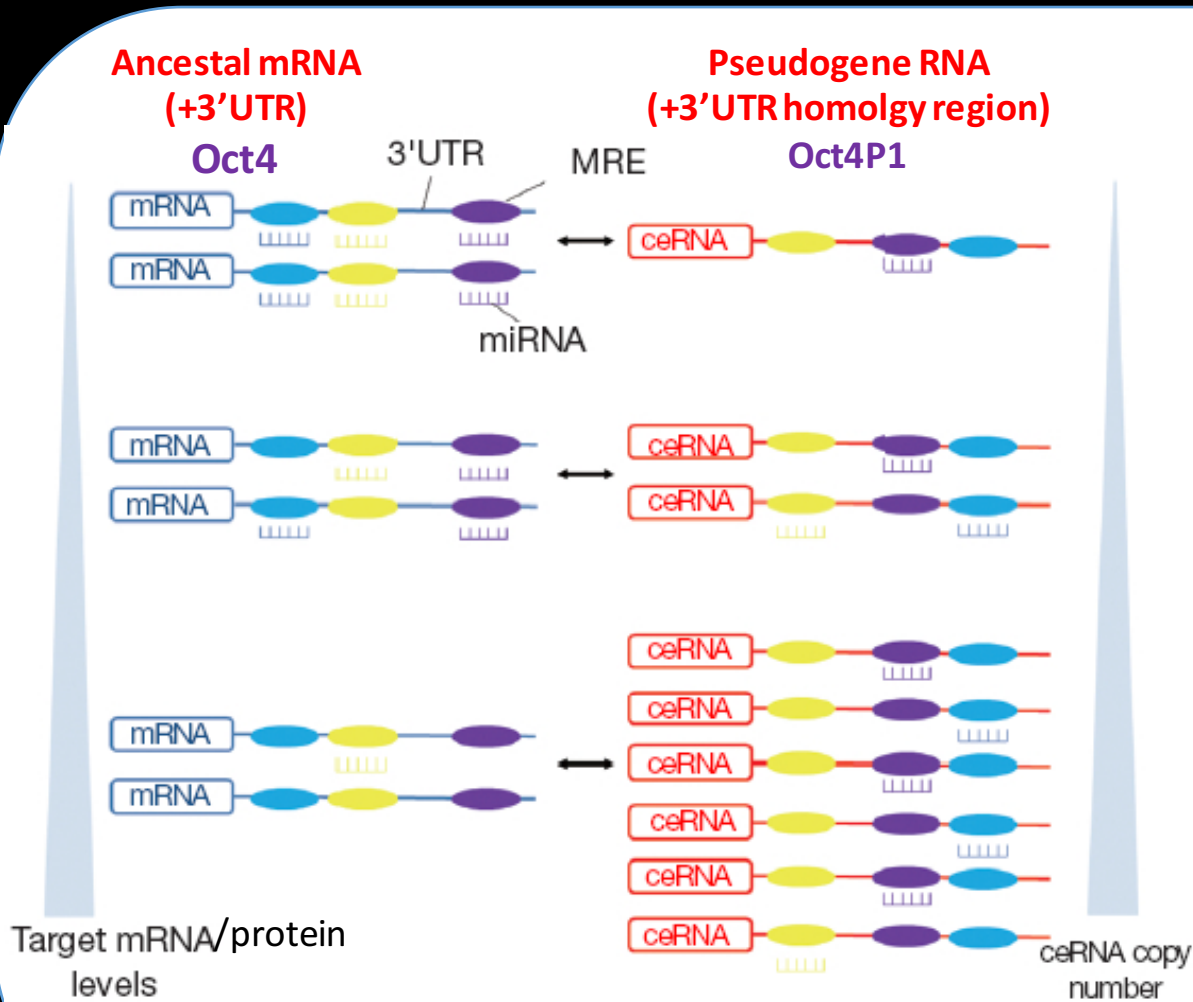
siOct4P1: high in ESC

siOct4: differentiation

siOct4P1: differentiation

Oct4P1 needed to keep high Oct4 expression in self renewing ESC??

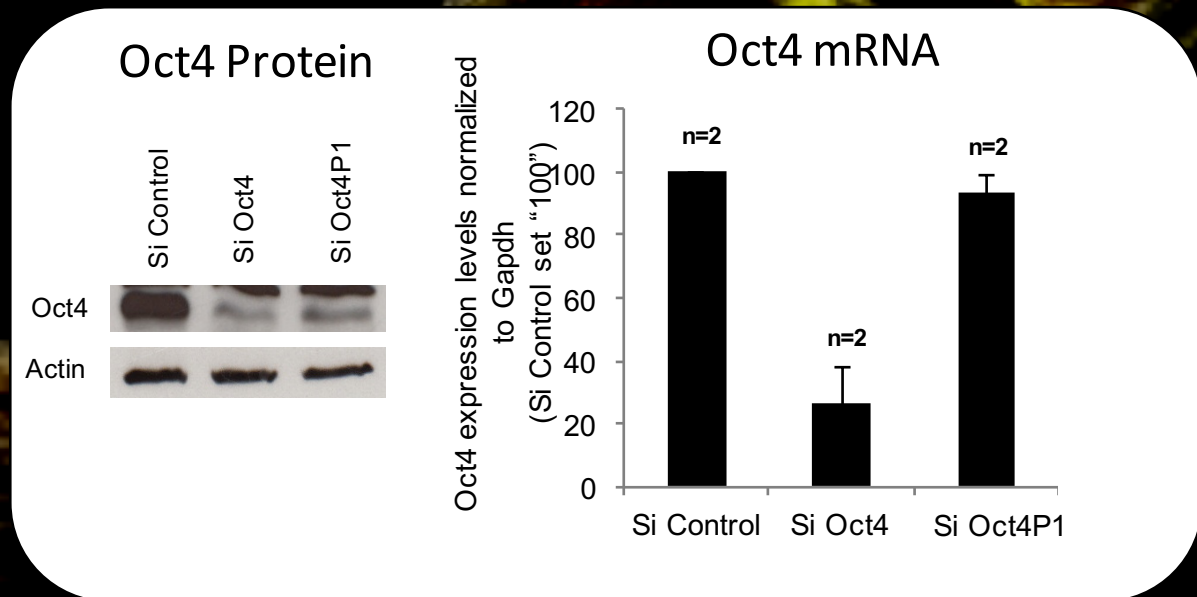
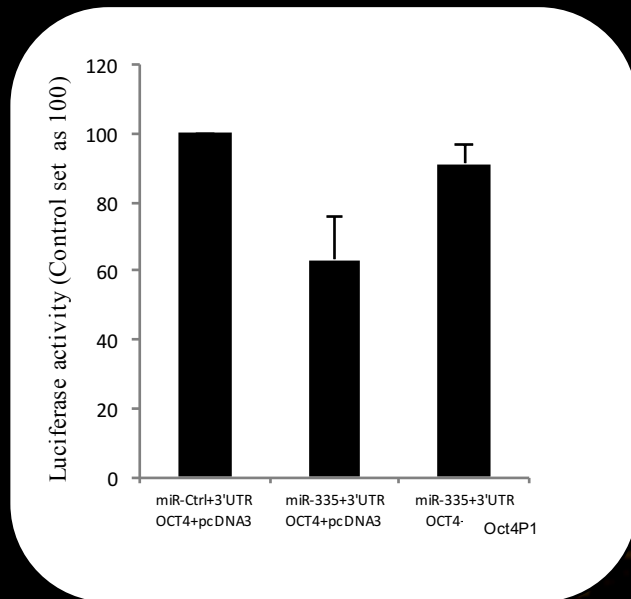
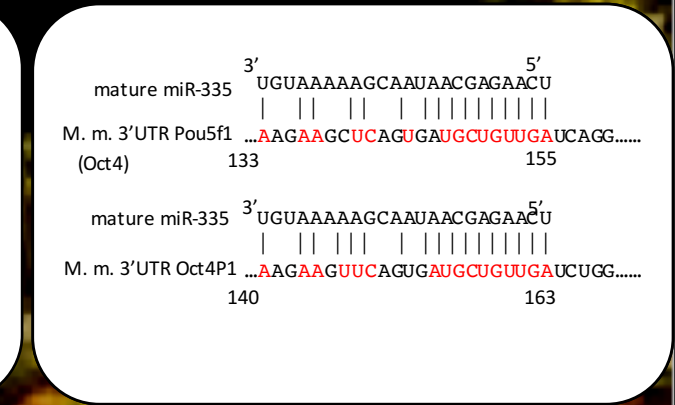
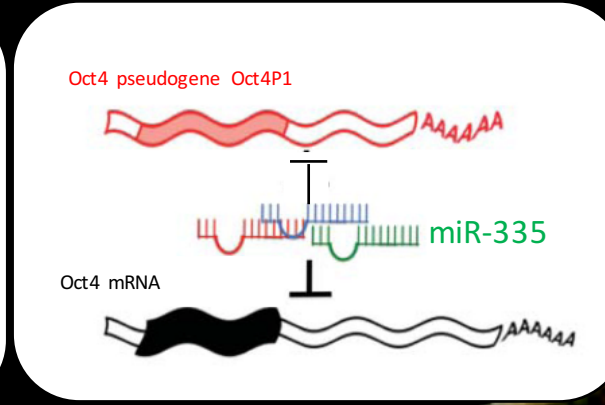
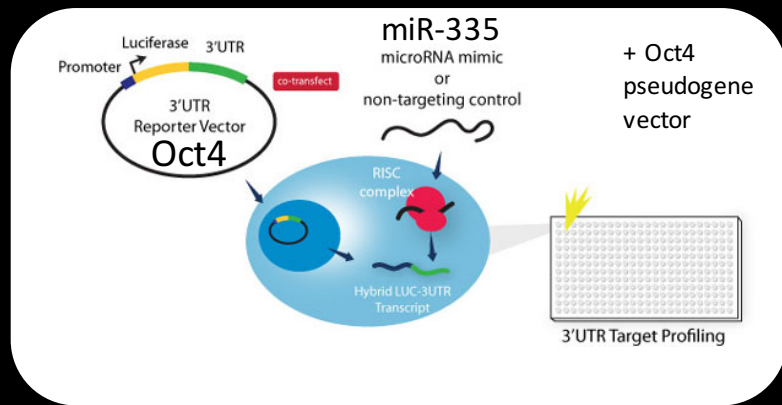
# Pseudogene sponge miRNAs that target the ancestral gene



miRNA that targets Oct4: i.e. miR-335  
MRE: miRNA response element

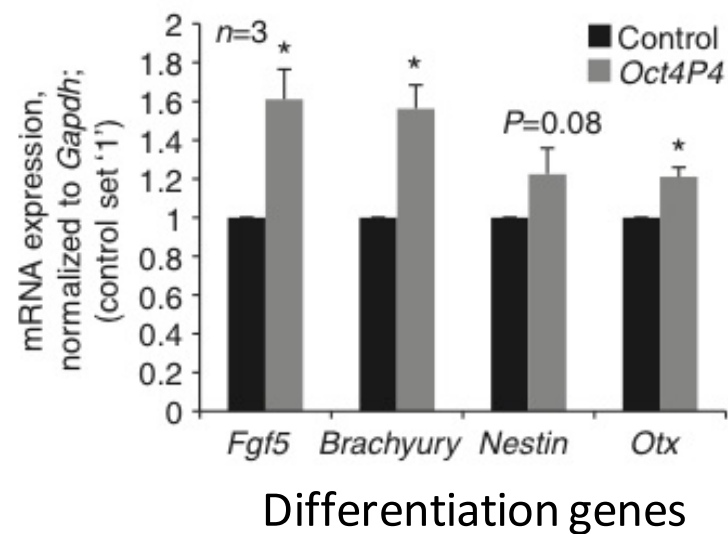
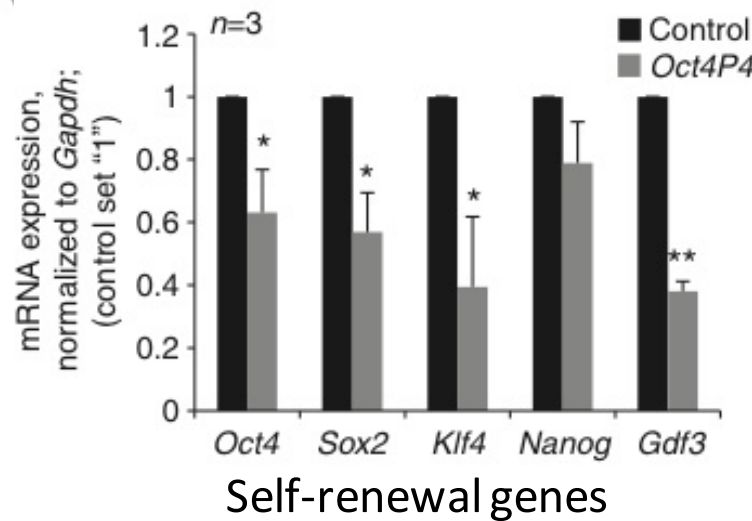
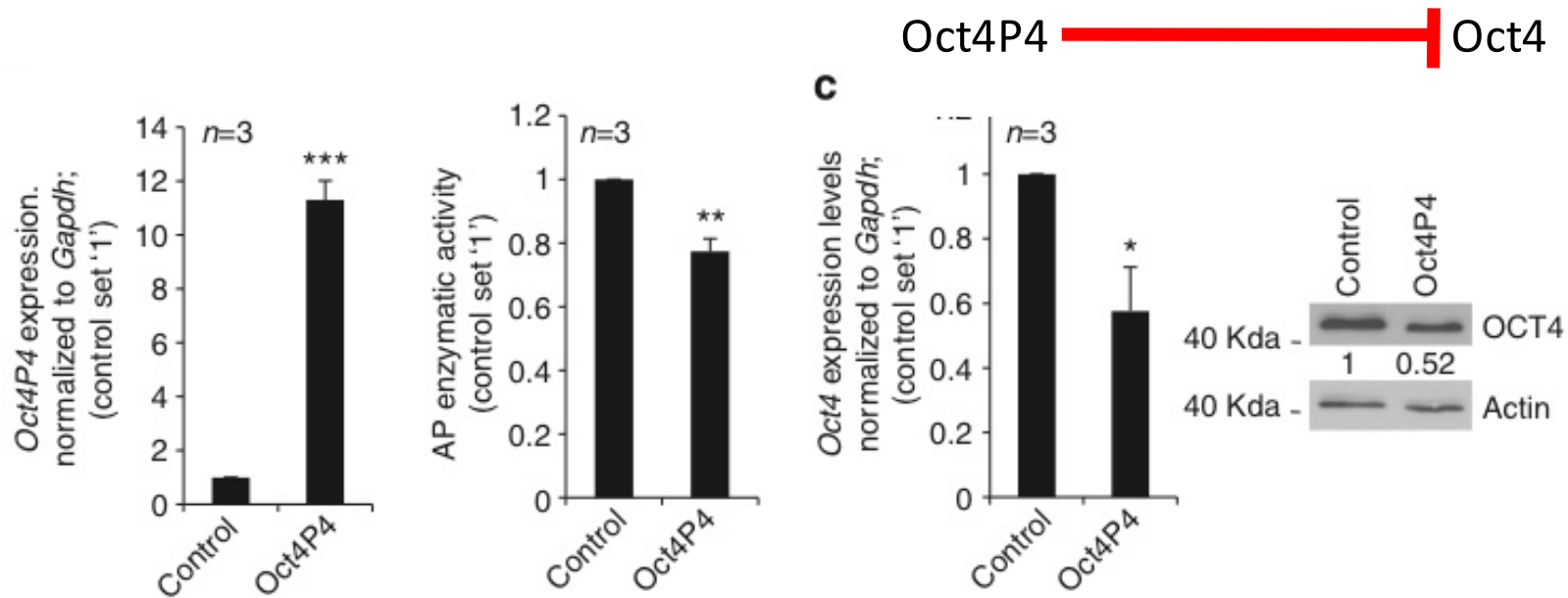
Evolution of  
pseudogenes  
to fine-tune the  
expression  
of ancestral genes

# Cytoplasmatic OCT4P1 acts as Oct4/Rb1 ceRNA



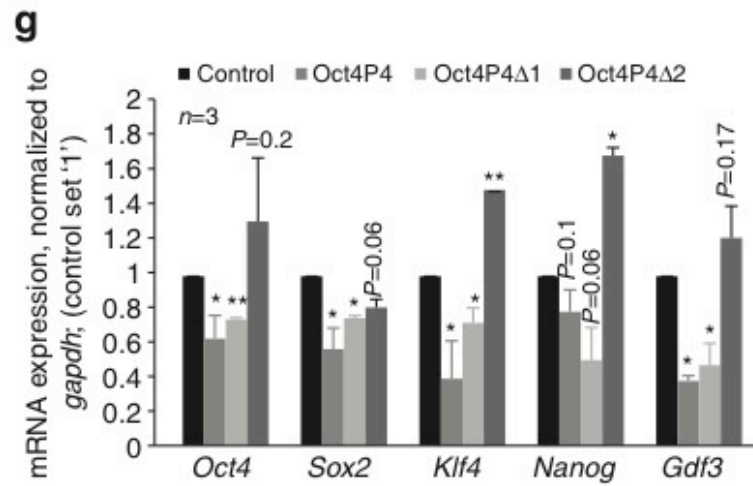
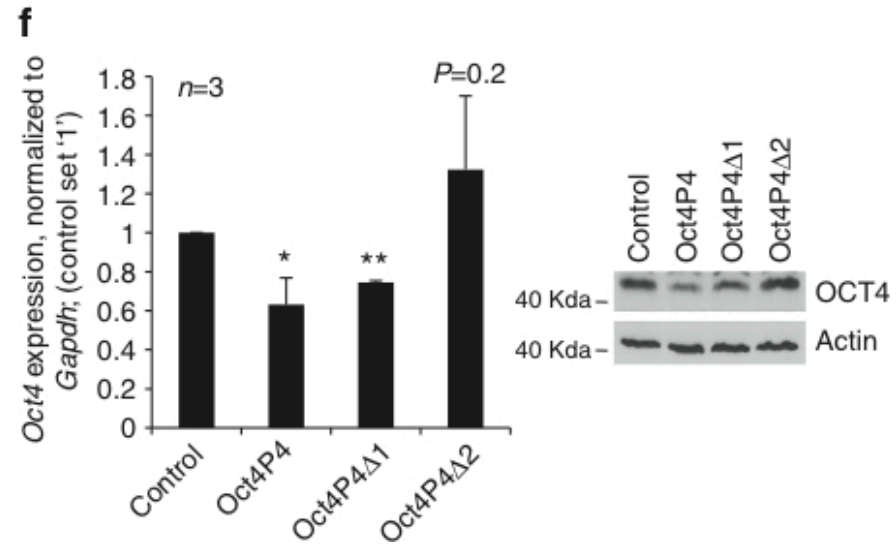
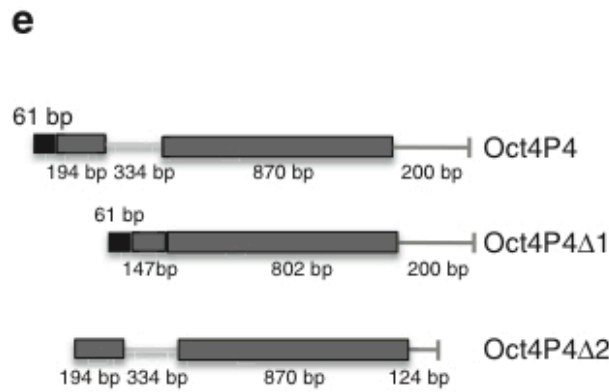
**OCT4P1 promotes self-renewal by sponging miRNAs that target Oct4**

# Nuclear OCT4P4 promotes mESC differentiation

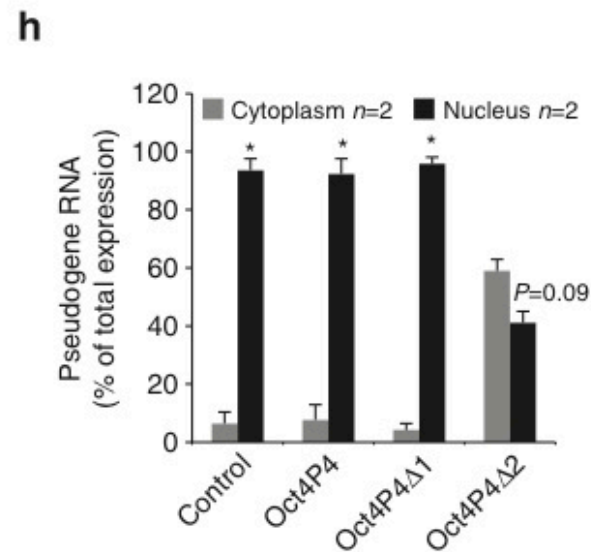




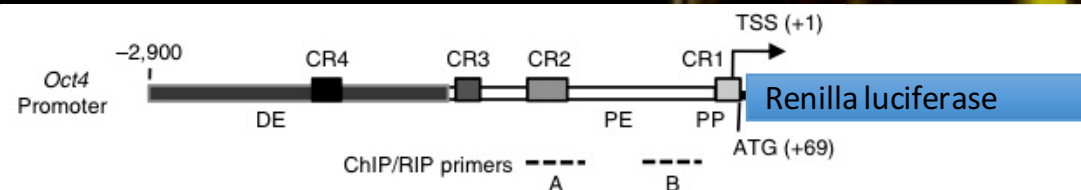
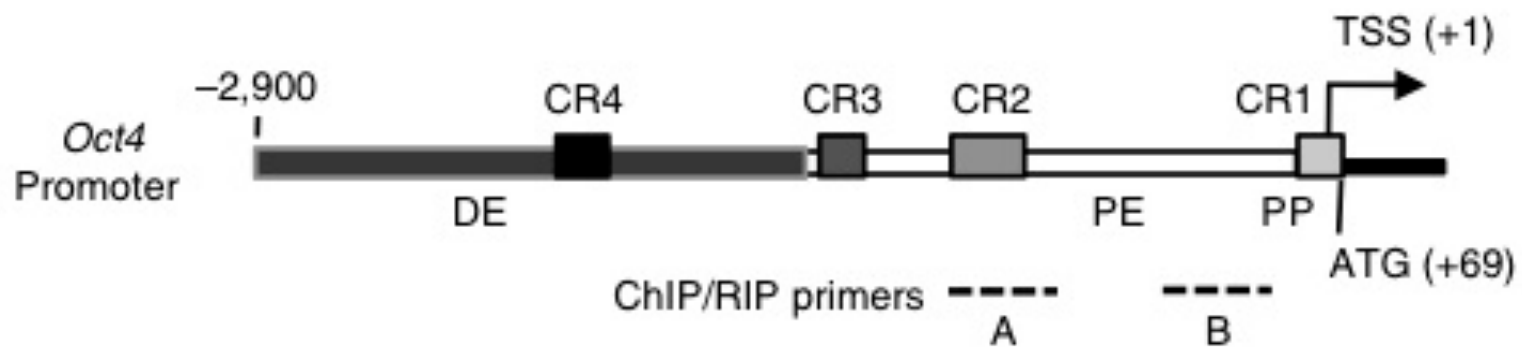
# 5' and 3' UTR homology domains are required to repress self-renewal marker genes



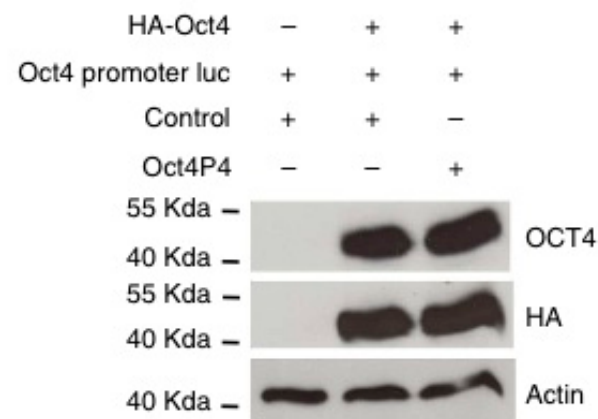
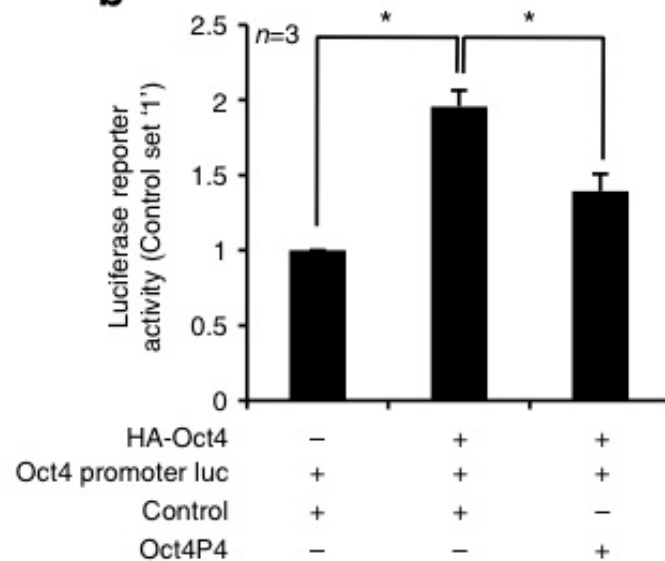
Self-renewal genes



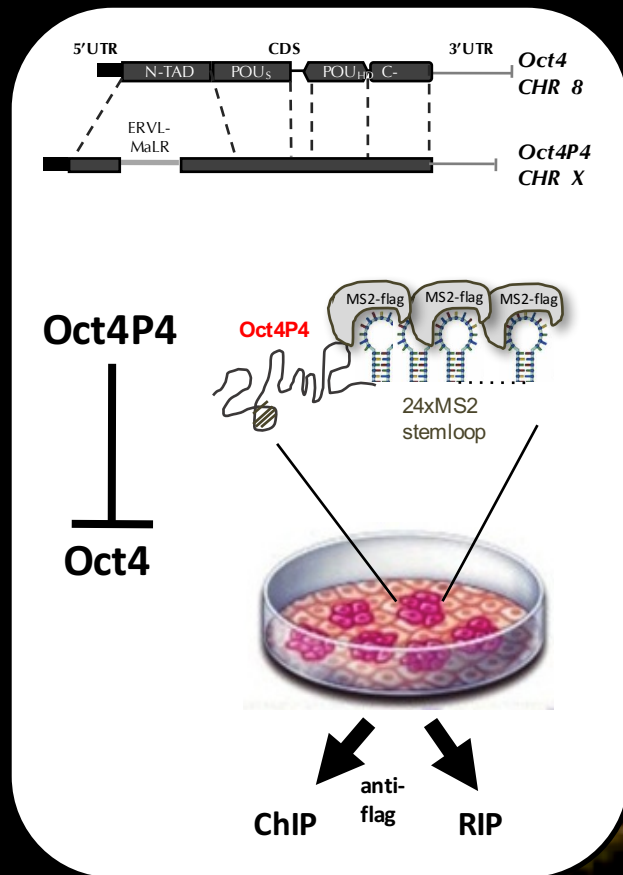
# Oct4P4 interferes with the ancestral Oct4 promoter



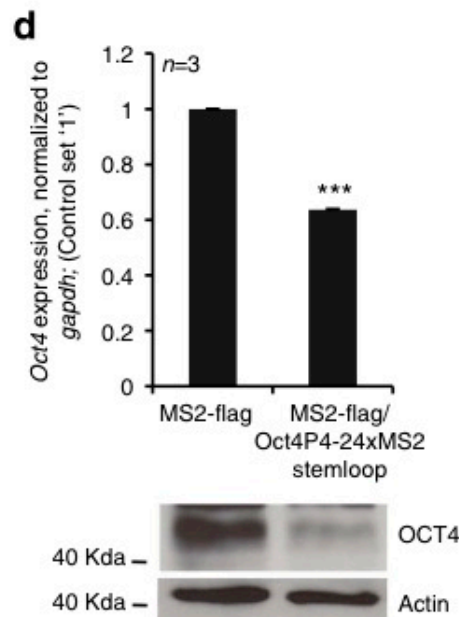
**b**



# A model system to study Oct4P4 lncRNA localization

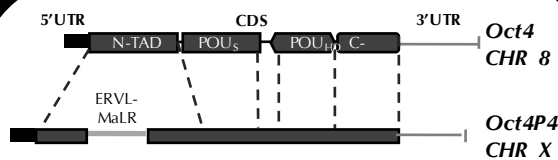
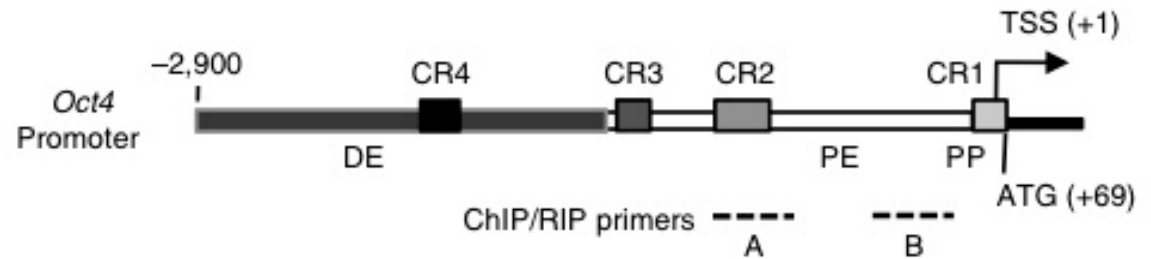


## RNA immunoprecipitation anti-flag then RT-PCR for Oct4P4 lncRNA

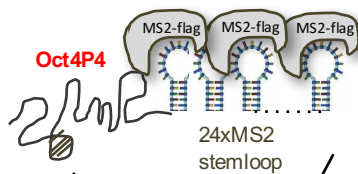


MS2 stem loop tagged Oct4P4 co-expressed with flag-MS2

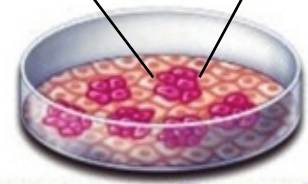
# A model system to study Oct4P4 lncRNA localization



Oct4P4

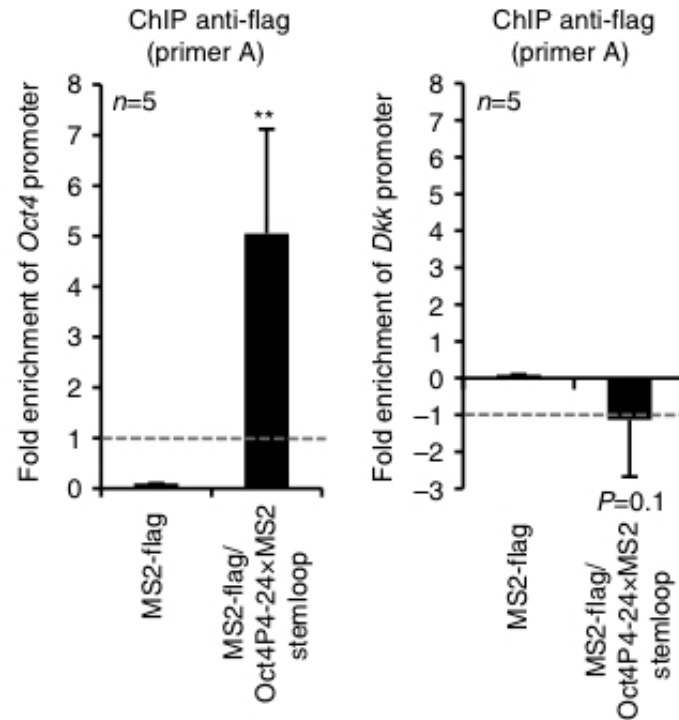


Oct4



CHIP anti-flag RIP

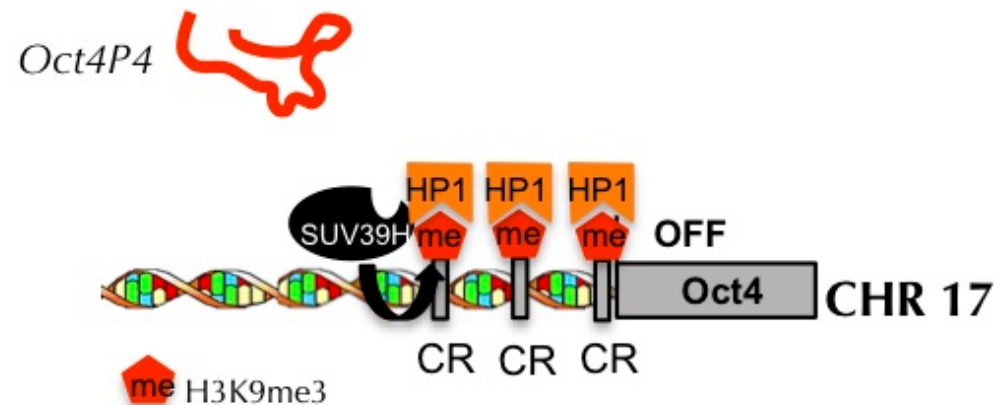
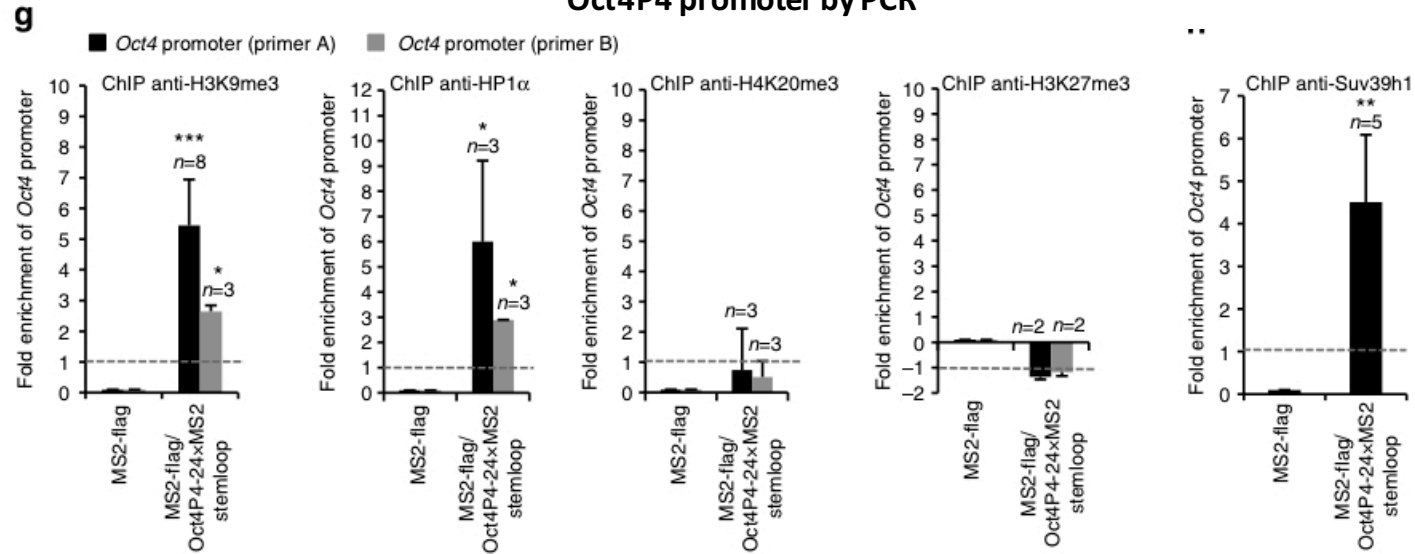
ChIP using anti-flag then use the immuno-precipitate to detect the Oct4P promoter by PCR



Oct4P4-MS2 lncRNA localizes to Oct4 promoter

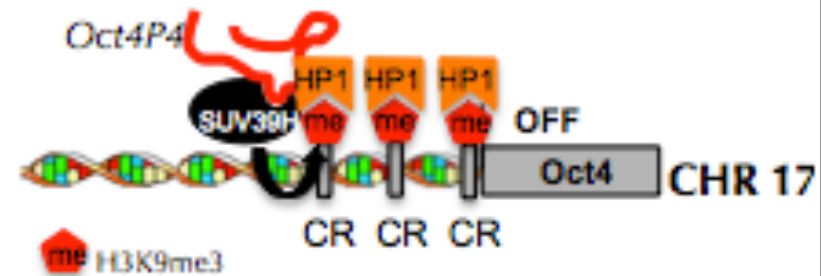
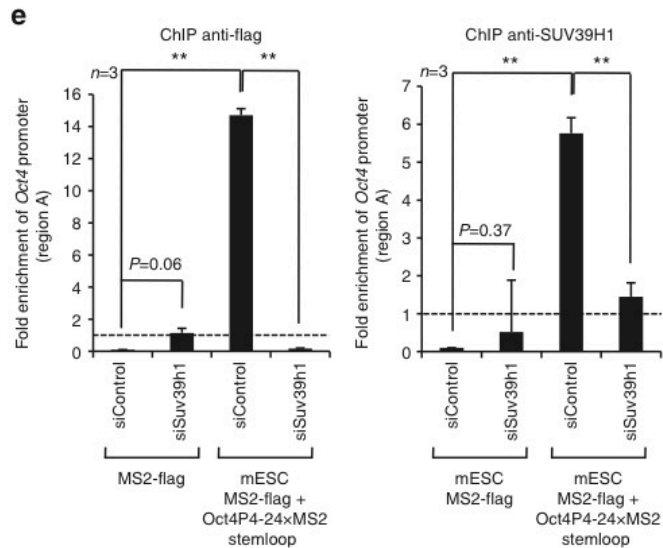
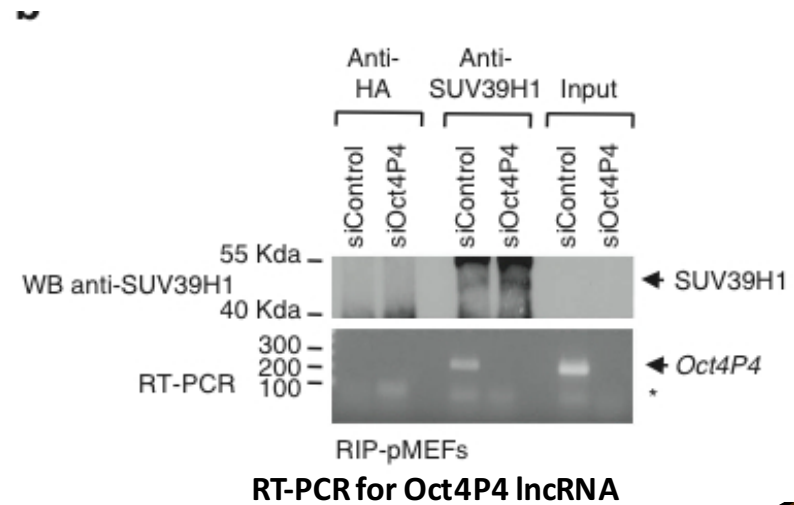
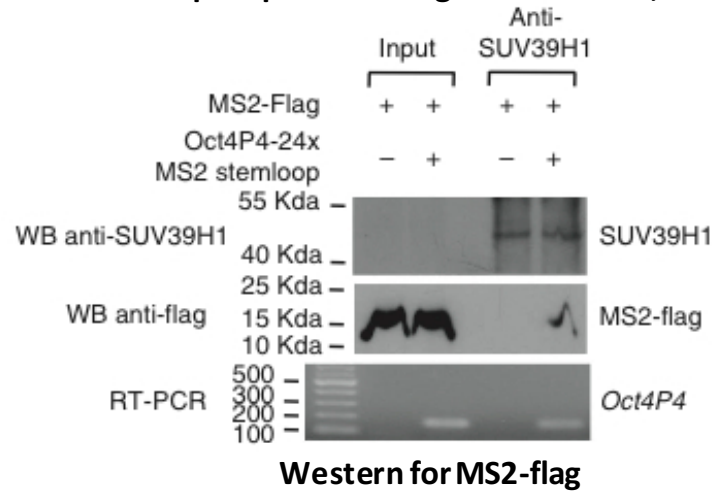
# Oct4P4-MS2 directs Suv39h1 to Oct4 promoter

ChIP using specific antibodies then use the immuno-precipitate to detect the Oct4P4 promoter by PCR



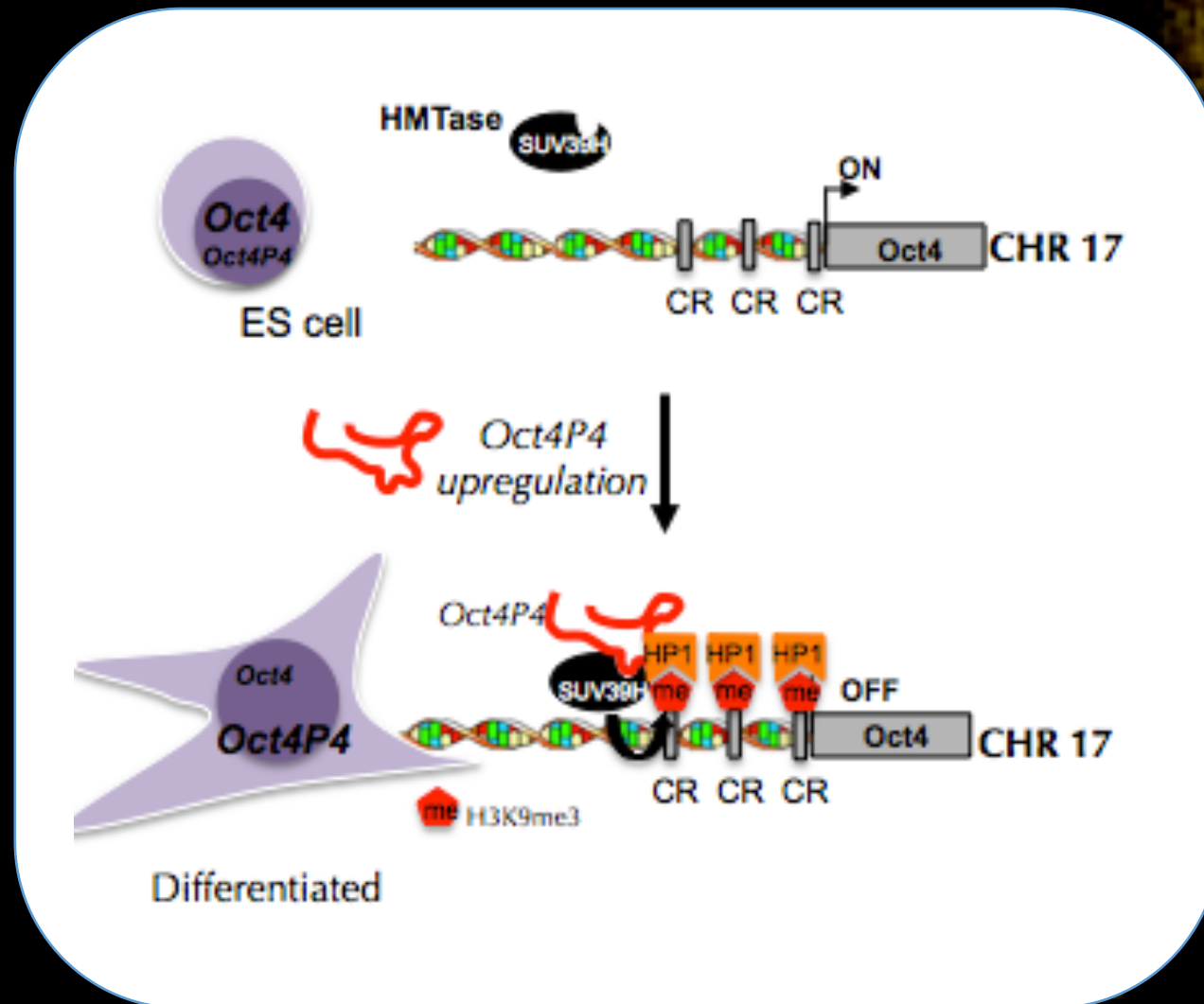
# Oct4P4-MS2 directly interacts with Suv39h1

RNA immunoprecipitation using anti-SUV39h1;



ChIP using specific antibodies then use the immuno-precipitate to detect the Oct4P4 promoter by PCR

# Oct4P4-MS2 recruits Suv39h1 To direct silencing of the Oct4 promoter

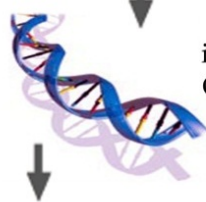


**REVERSIBILITY???**

## INDUCING PLURIPOTENCY IN ADULT CELLS



Adult cells



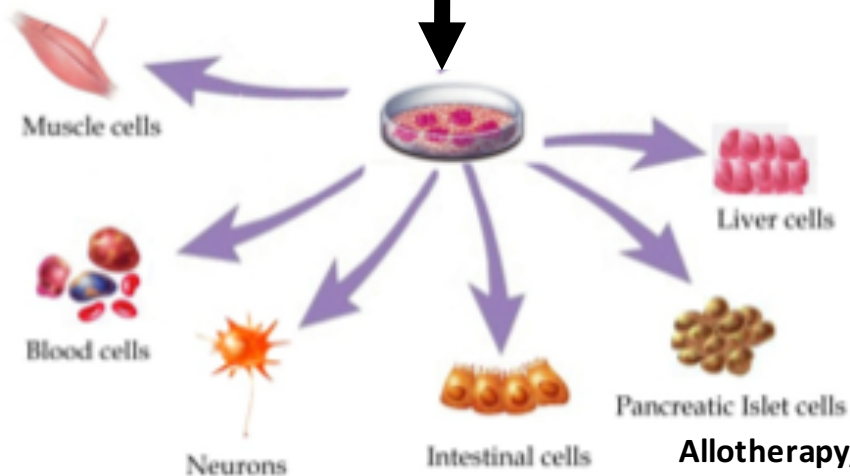
Genes inserted to induce reprogramming  
Oct4, Klf4, Sox2, c-Myc

Reprogram into  
ES like-cells



**iPS cells**

=induced pluripotent cells



DIFFERENTIATED

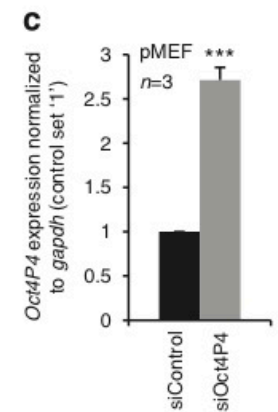
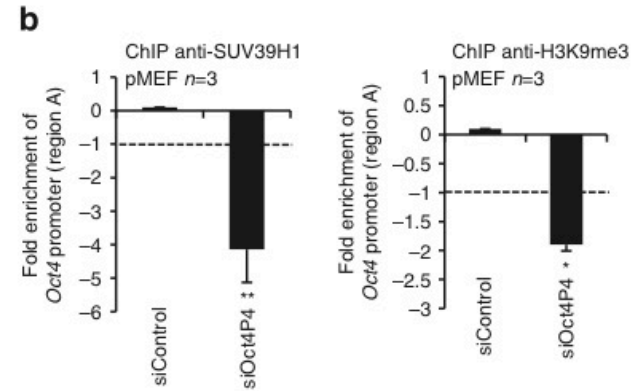
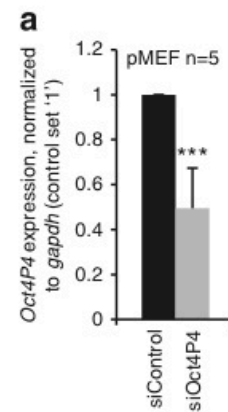
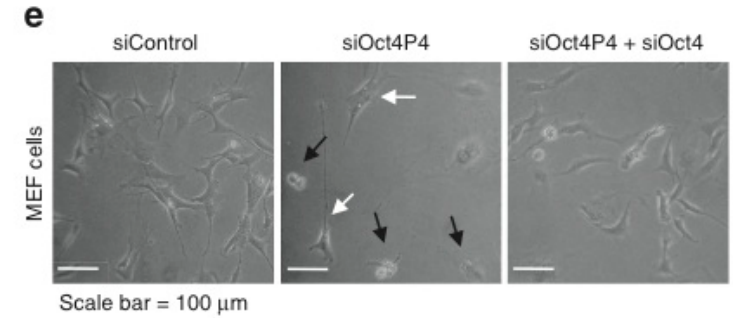
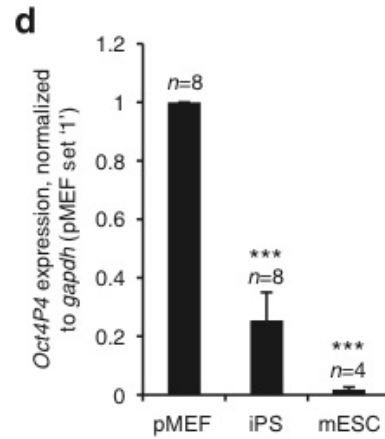
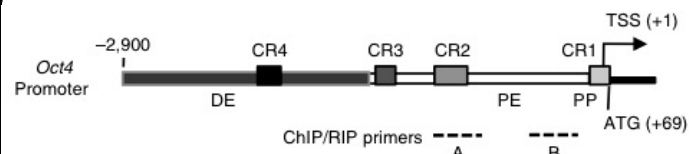


UNDIFFERENTIATED  
(SELF-RENEWAL)

INDUCE CELL  
LINE SPECIFIC  
DIFFERENTIATION

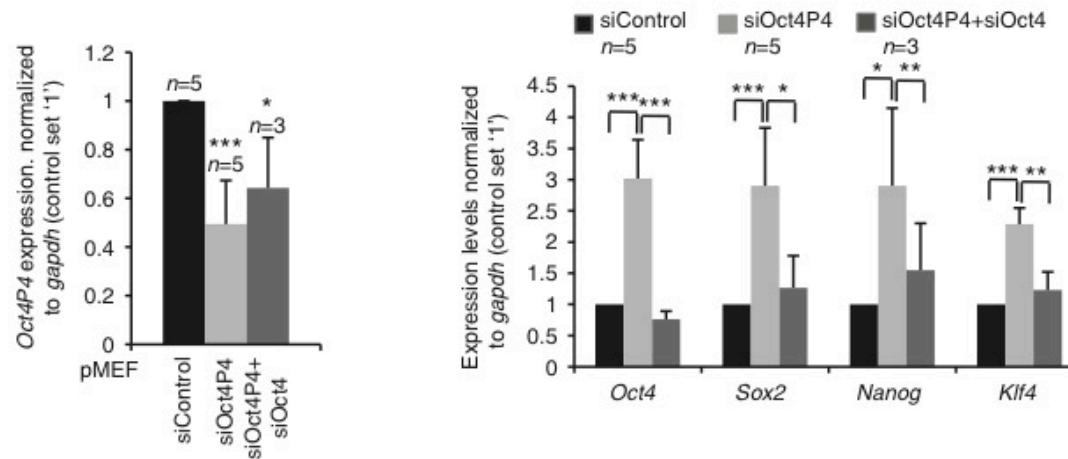


# Oct4P4 depletion in pMEFs causes the re-acquisition of self-renewal features

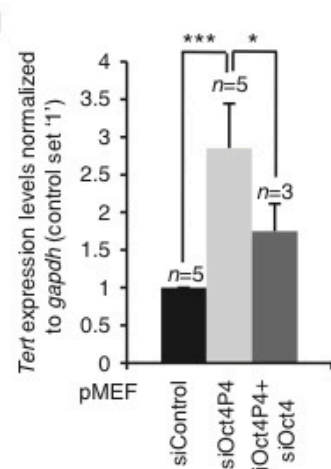


# Oct4P4 depletion in pMEFs causes the re-acquisition of self-renewal features

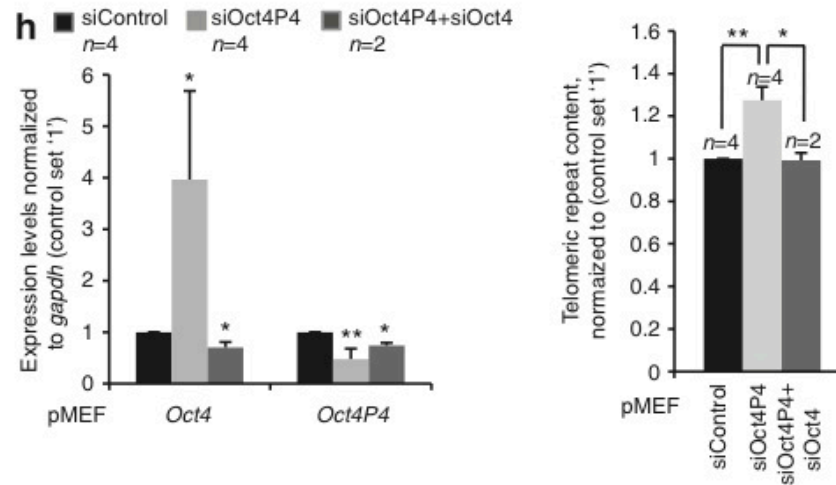
**f**



**g**

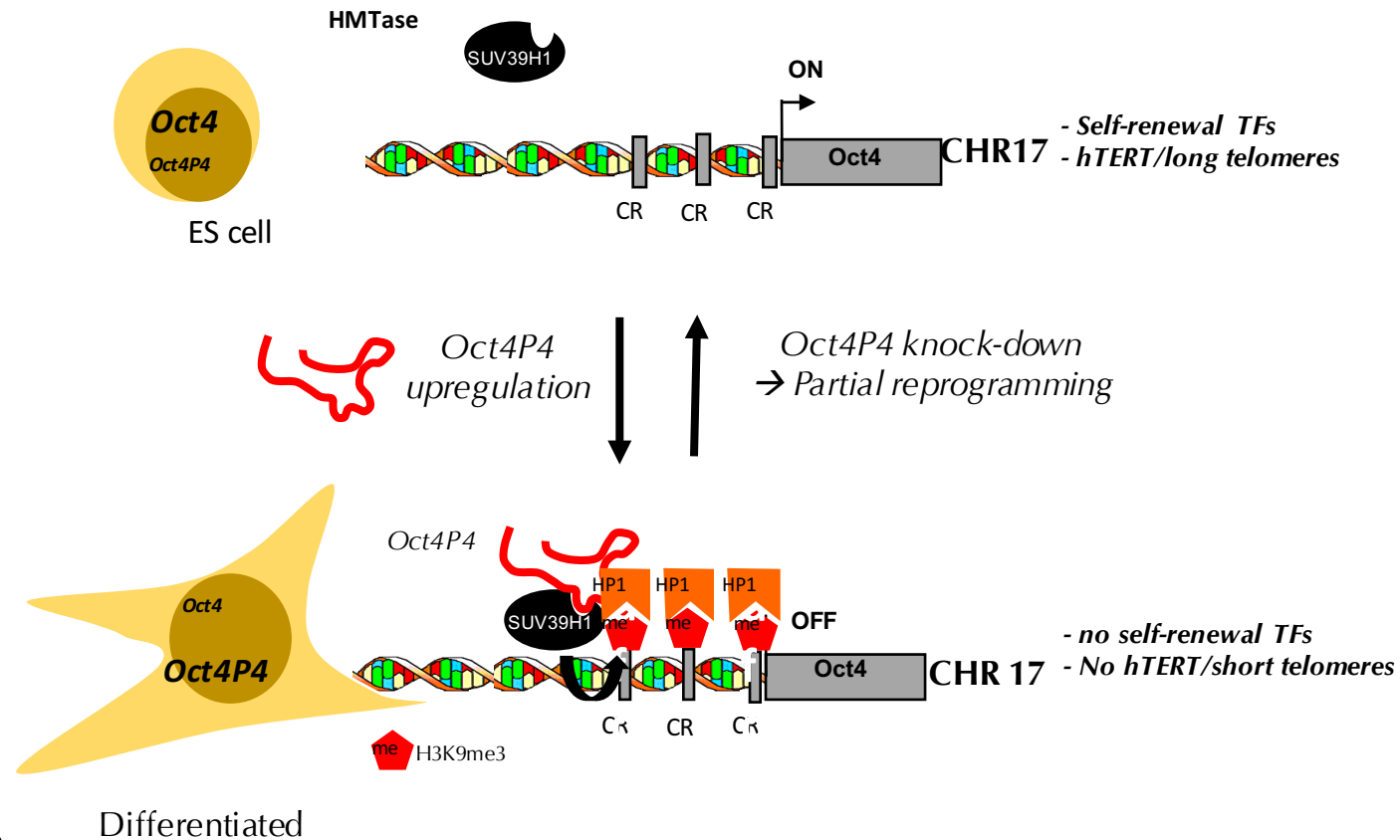


**h**



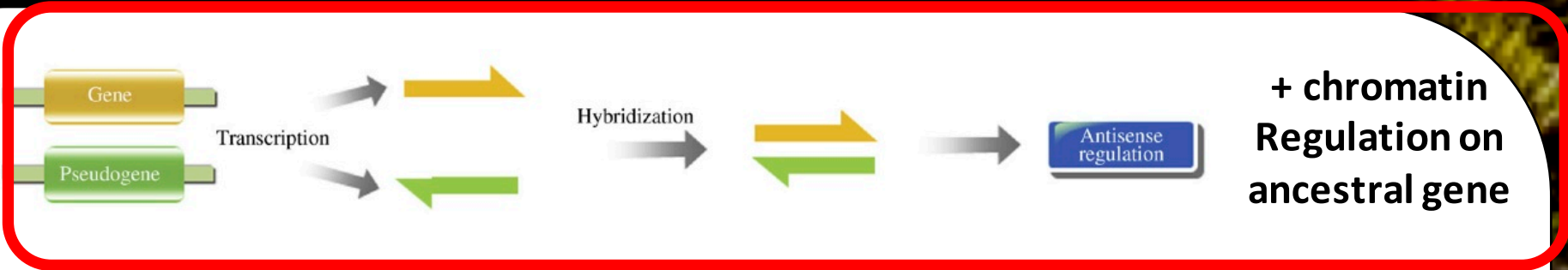
# Pseudogenes control the epigenetic status of ancestral genes

## *Oct4* pseudogene lncRNA silences ancestral gene

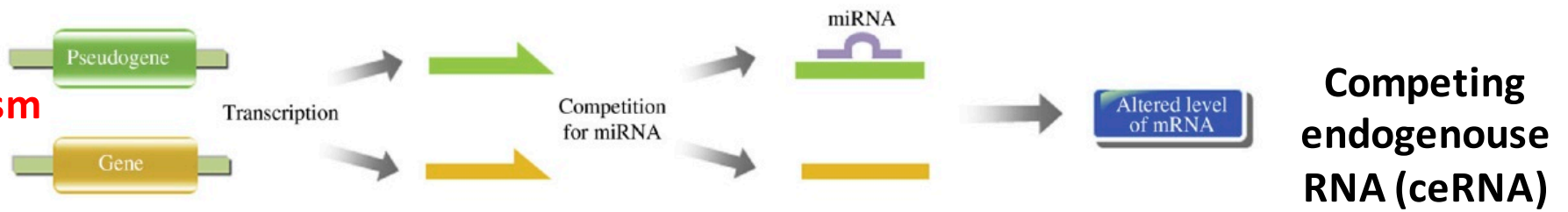


# Pseudogenes are powerful regulators of gene expression

**A**  
**Nuclear**



**B**  
**Cytoplasm**



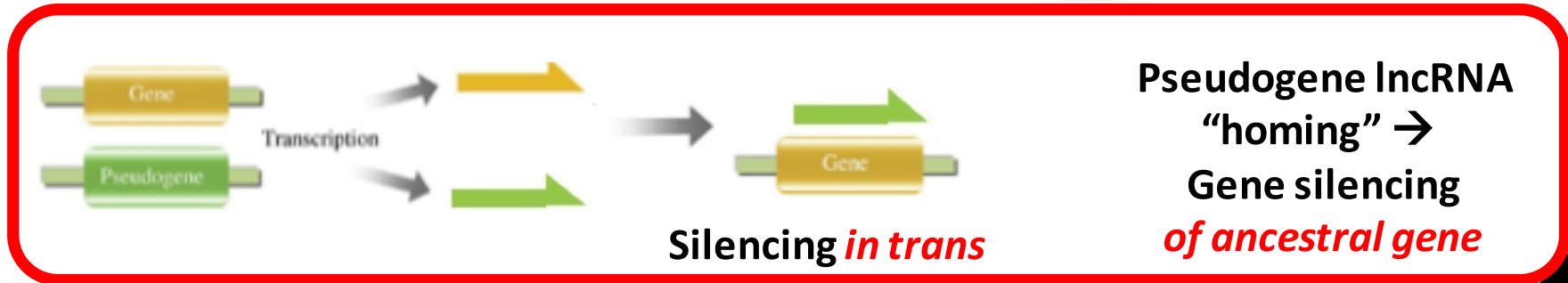
**C**  
**Cytoplasm**



**D**  
**Cytoplasm**



**Nuclear**



# WHAT WOULD YOU DO NEXT TO UNDERSTAND OCT4P4 FUNCTION?

*Oct4 pseudogene lncRNA silences ancestral gene*

