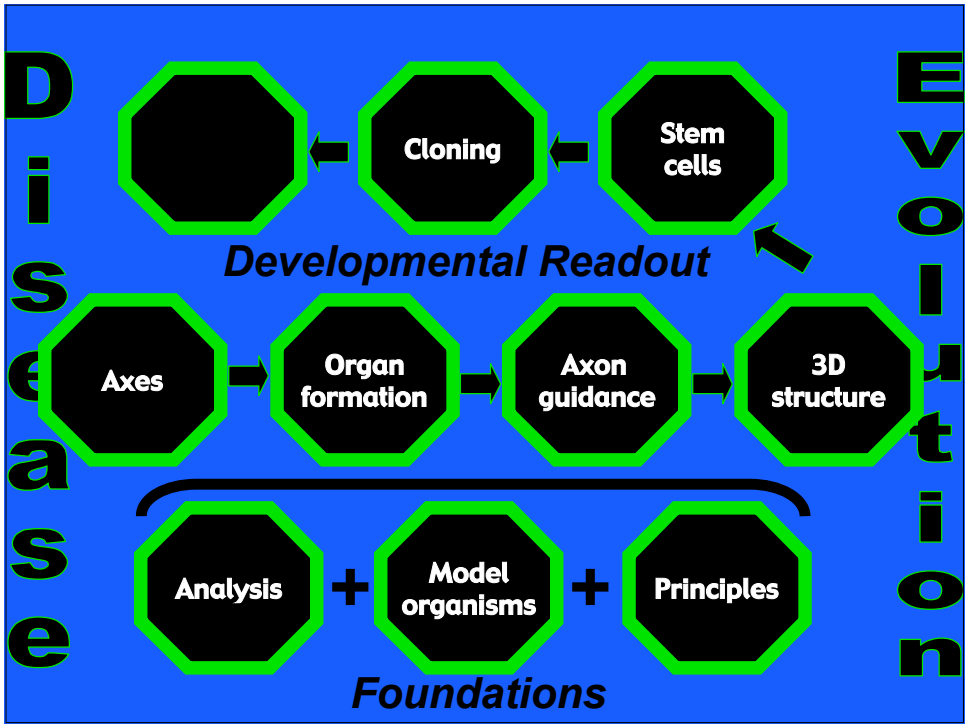
7.72
12.4.06

Cloning and Epigenetics



1

Cloning:

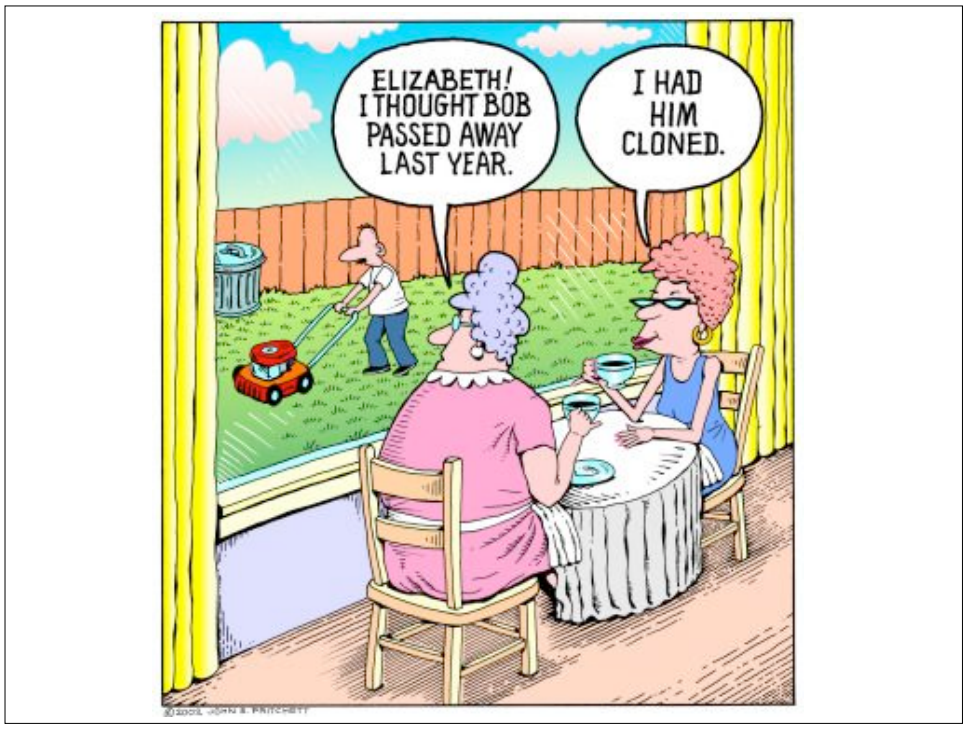
Making an identical copy

2

Epigenetics:

Transcriptional control that does not directly involve DNA base sequence, and can be reversible without mutation

1. Reproductive vs therapeutic cloning



Artisan, 1978

3

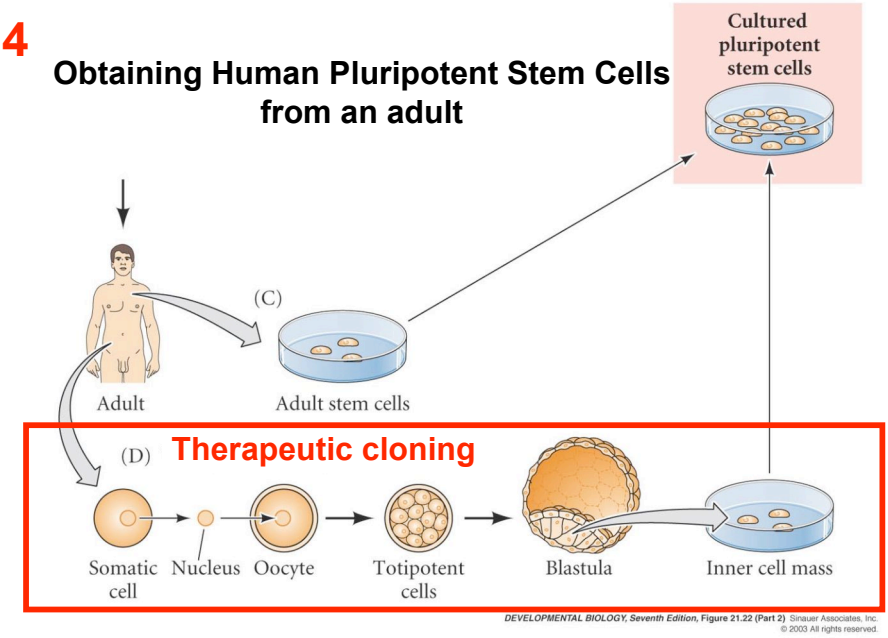
Why clone?

Reproductive
replacement baby
infertility
spare parts
gene therapy
useful producer animal

Therapeutic
autologous stem cells
gene therapy

4

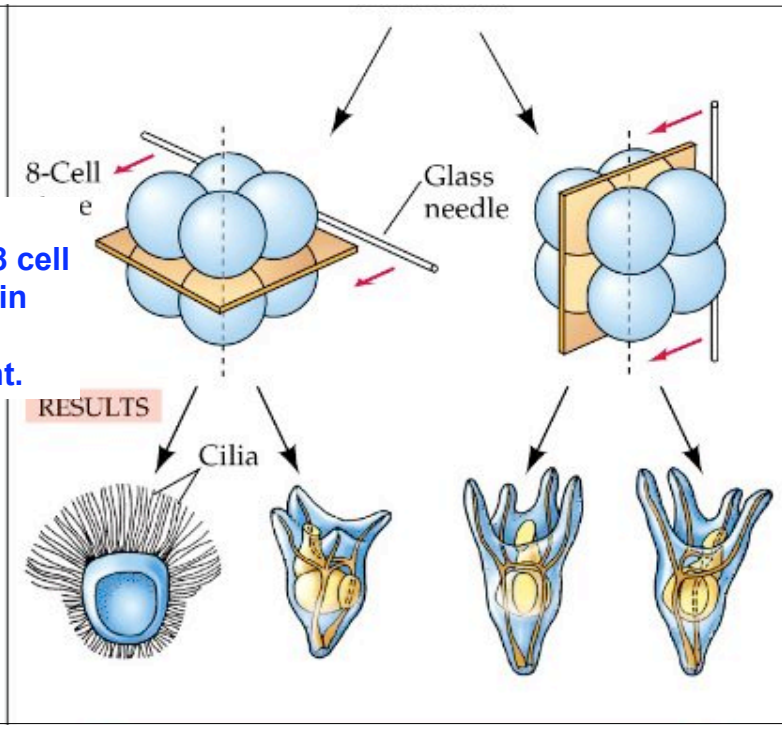
Obtaining Human Pluripotent Stem Cells from an adult



DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 21.22 (Part 2) Sinauer Associates, Inc.

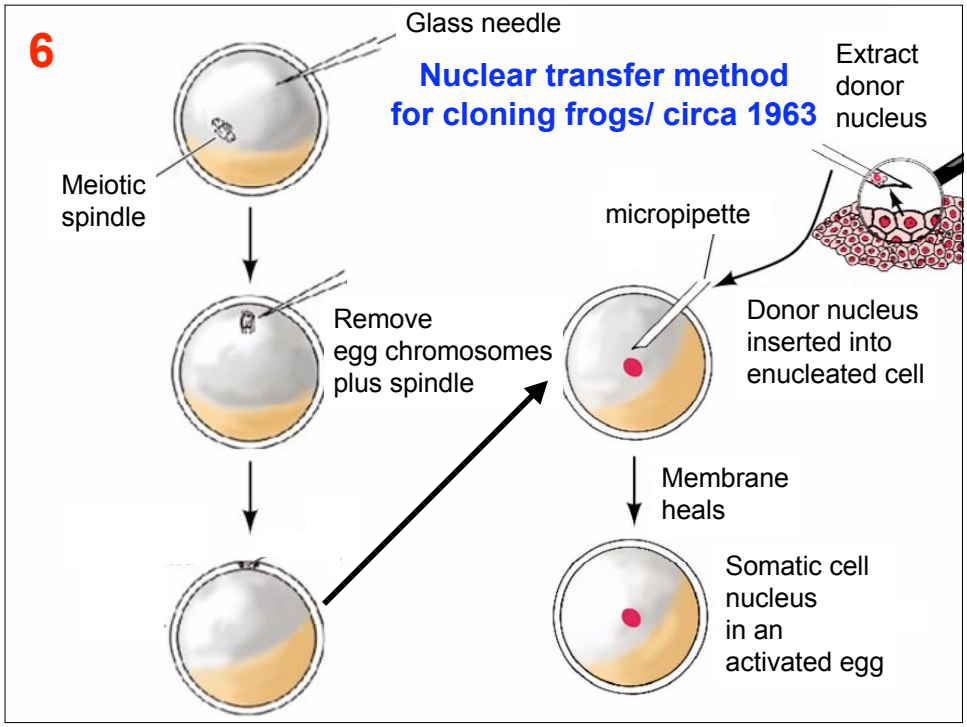
2. History: the question of cellular potency

5

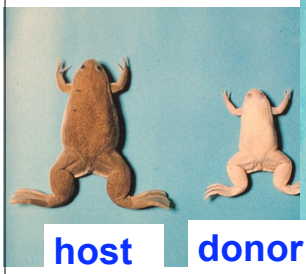


Cells in 8 cell sea urchin are not totipotent.

3. Testing the potency of nuclei



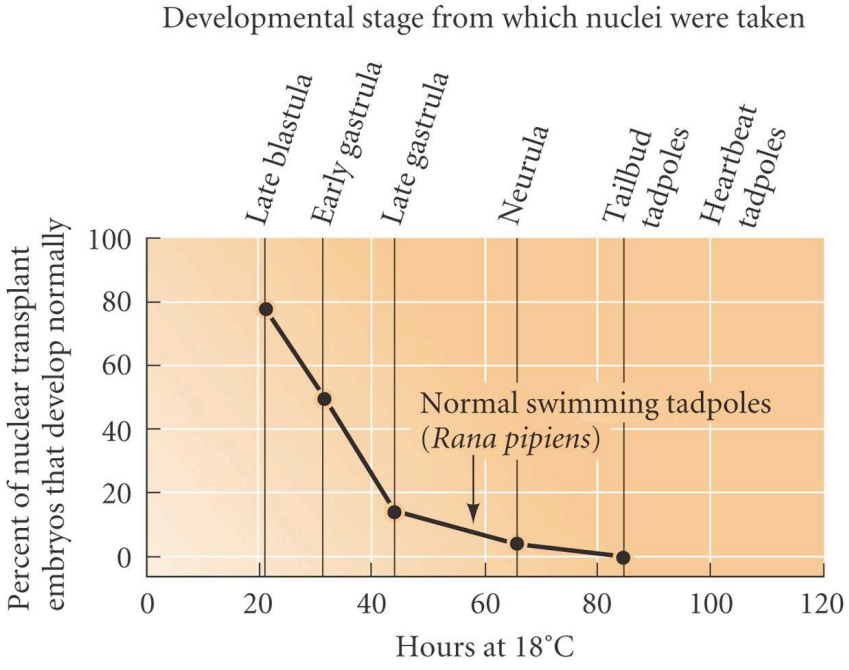
7



host donor

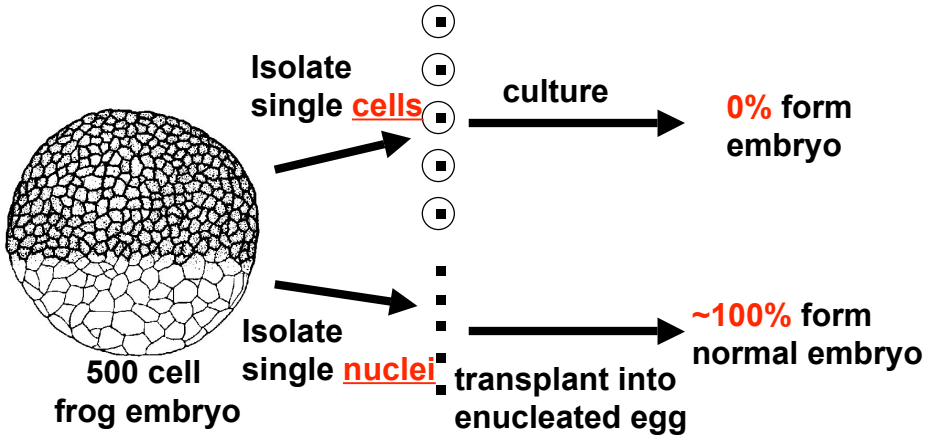
cloned frogs

8



Decrease in potency of nuclei with age of donor

9



Cellular versus nuclear potency

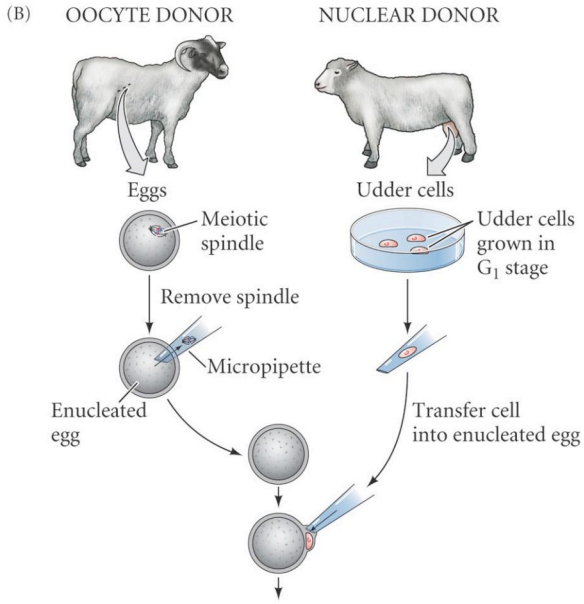
4. SCNT Methodology

10

Dolly:
first mammal cloned by nuclear transfer 1997

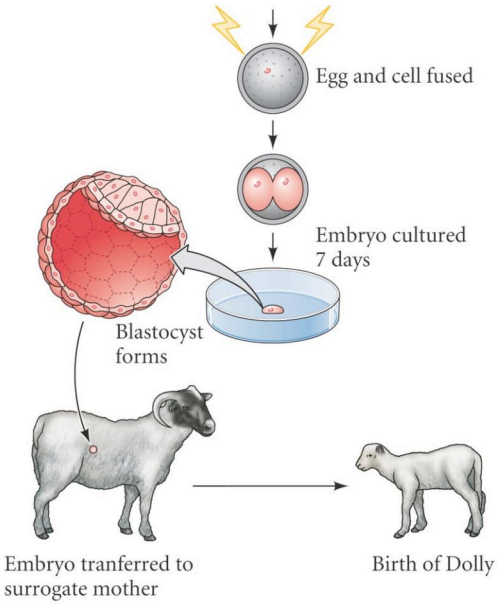
© Roddy Field, the Roslin Institute

11a Cloning mammals using adult somatic nuclei



DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 4.8 (Part 2) Sinauer Associates, Inc.

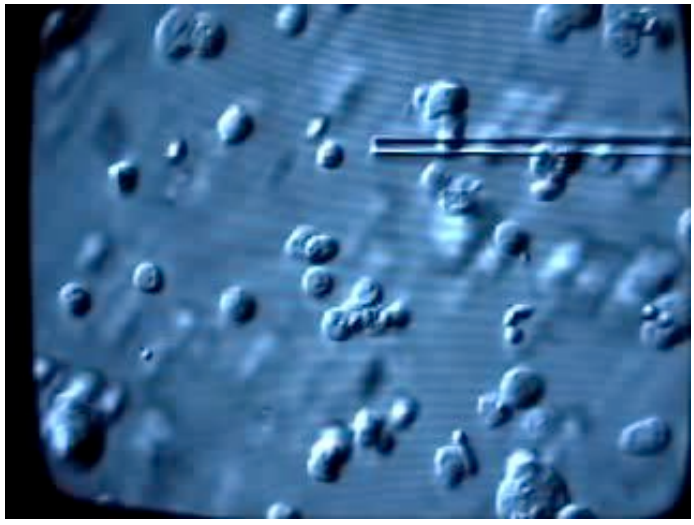
11b



Cloning mammals using adult somatic nuclei

12

13



Isolation of somatic cell nuclei
Kevin Eggan and Rudolf Jaenisch, Whitehead Institute

14

Removal of egg chromosomes (metaphase plate)
Kevin Eggan and Rudolf Jaenisch, Whitehead Institute

15

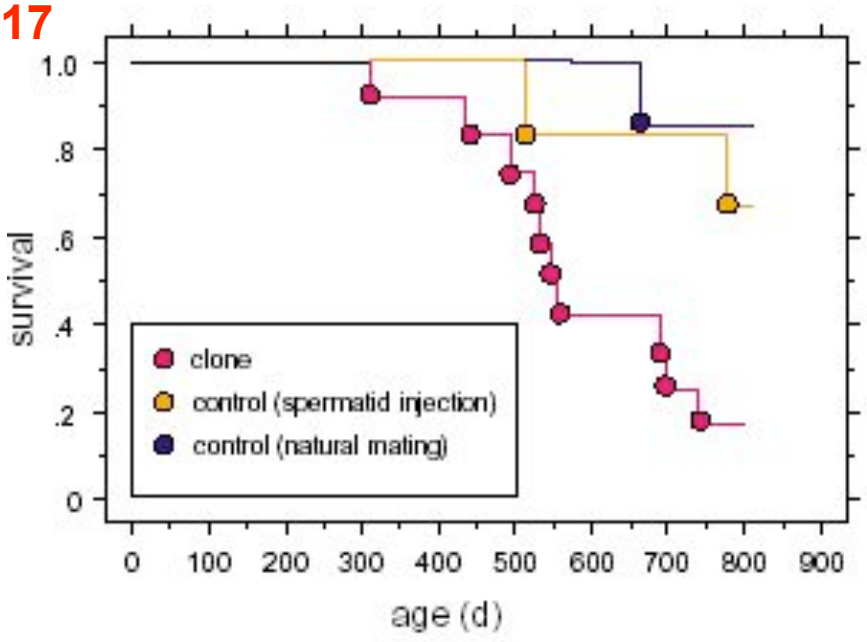
Injection of somatic cell nucleus into enucleated egg
Kevin Eggan and Rudolf Jaenisch, Whitehead Institute

5. Problems with animals derived from nuclear transfer

16 "Large Offspring Syndrome" In Cloned Mice

normal cloned

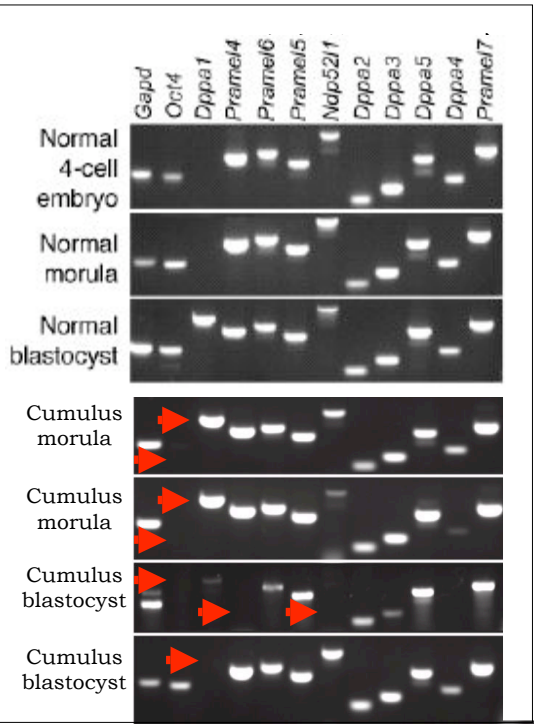
17



Early death of mice cloned from somatic cells

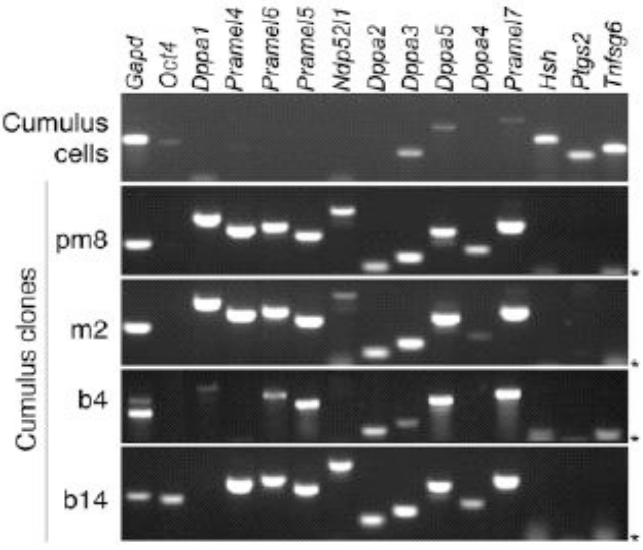
18

Embryos derived from cumulus cells do not show normal early gene expression patterns



Bortvin et al, 2003

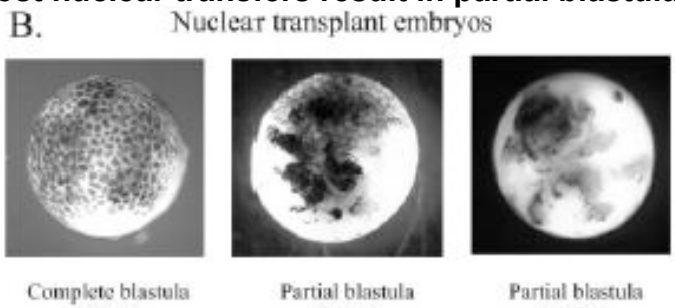
19



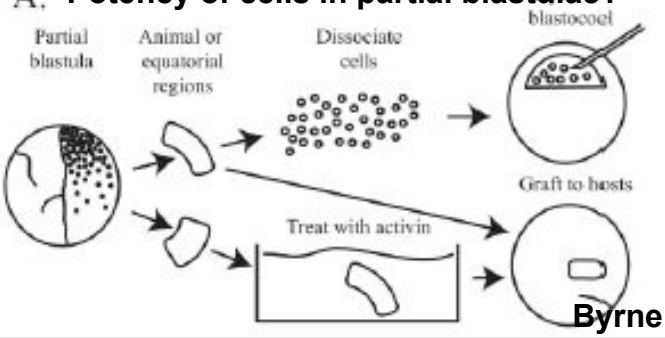
Individual cloned embryos are different

Bortvin et al, 2003

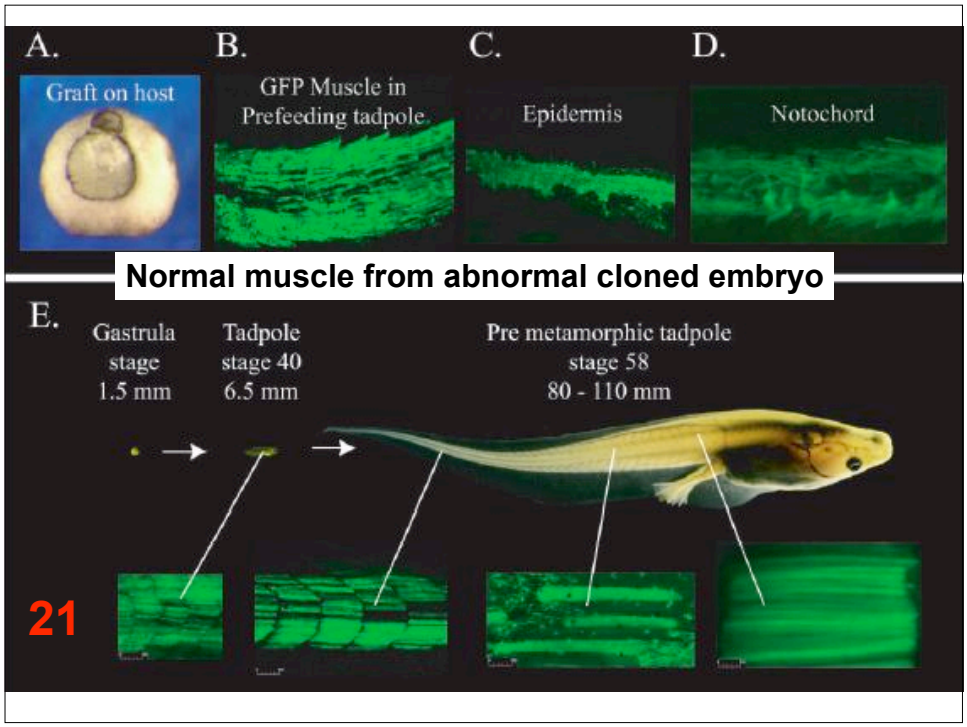
20 **Most nuclear transfers result in partial blastulae**



A. **Potency of cells in partial blastulae?**



Byrne et al, 2003



Normal muscle from abnormal cloned embryo

21

6. Epigenetic control of gene expression

22

The kitten "CC" is a non-identical clone produced using somatic nuclear transfer from "Rainbow" (B)

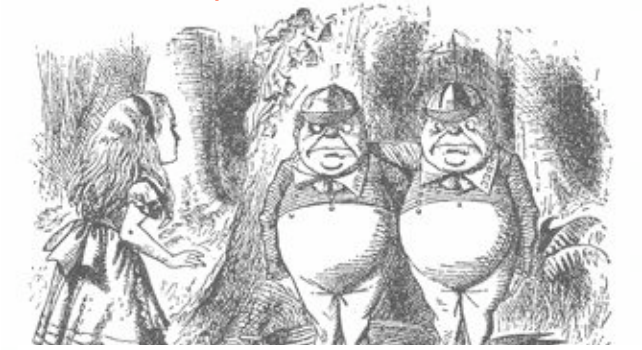
(A) 

(B) 

Genes in A and B are same, gene expression is not.

23

How identical are identical twins?
Concordance (= do both twins share a trait?)



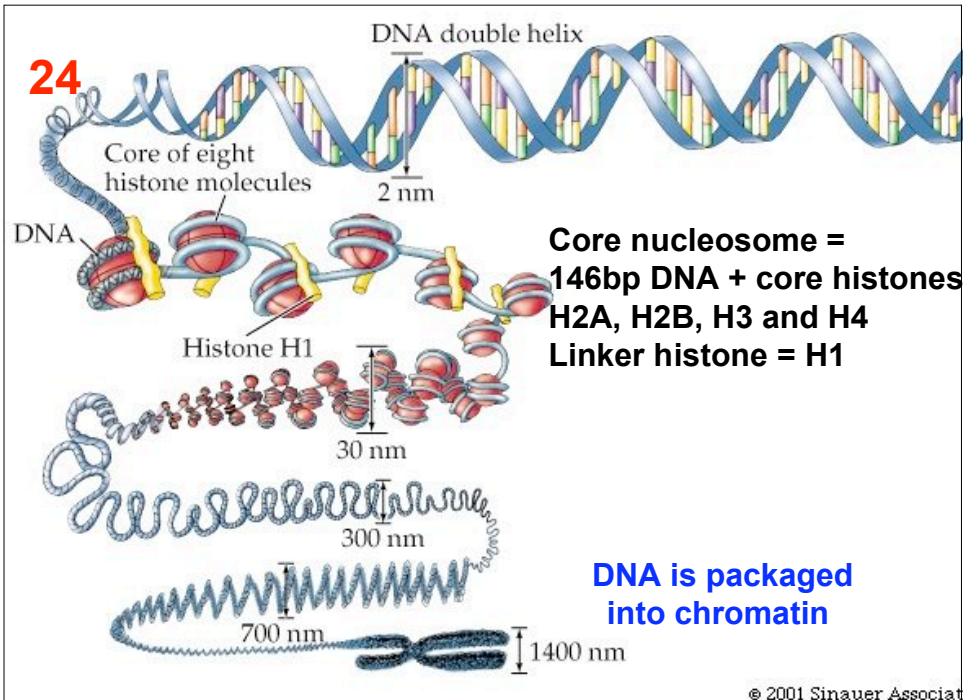
scleroderma 98% identical, 10% fraternal

Due to DNA base sequence

asthma 54% identical, 24% fraternal

DNA base sequence + something else = Epigenetics

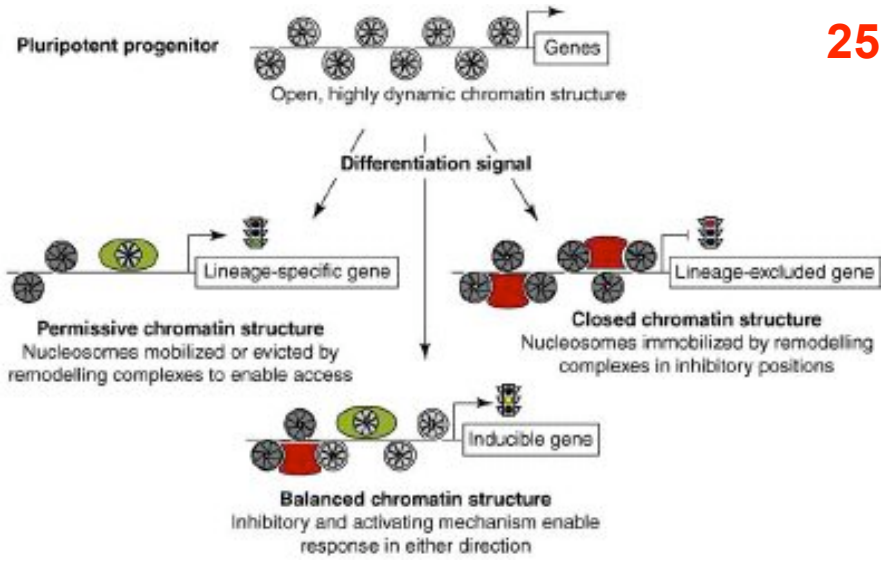
24



Core nucleosome =
146bp DNA + core histones
H2A, H2B, H3 and H4
Linker histone = H1

DNA is packaged into chromatin

25



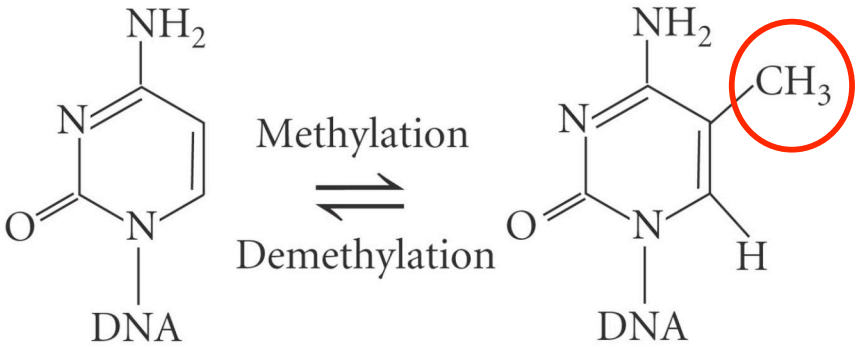
Determination, differentiation and chromatin

Kaeser and Emerson, 2006

DNA Methylation

26

(A)



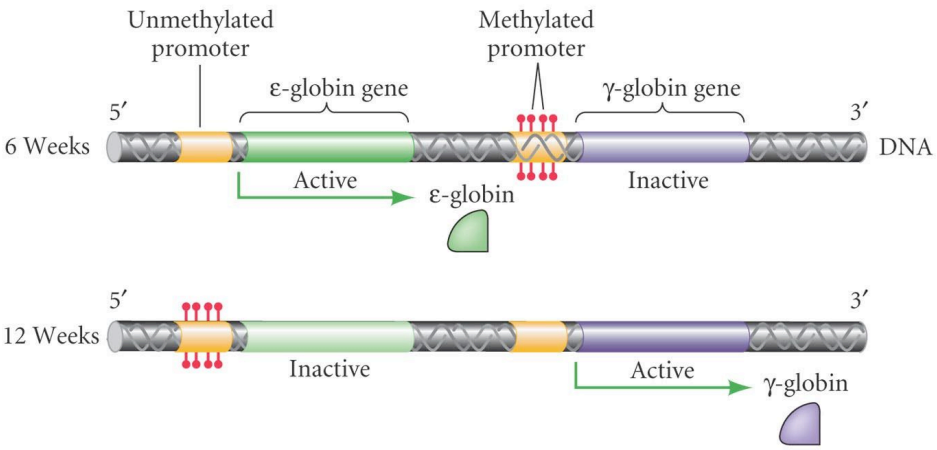
Cytosine **5-Methylcytosine**

In DNA, cytosine can be replaced by 5-methylcytosine: this does not change the base sequence of the DNA

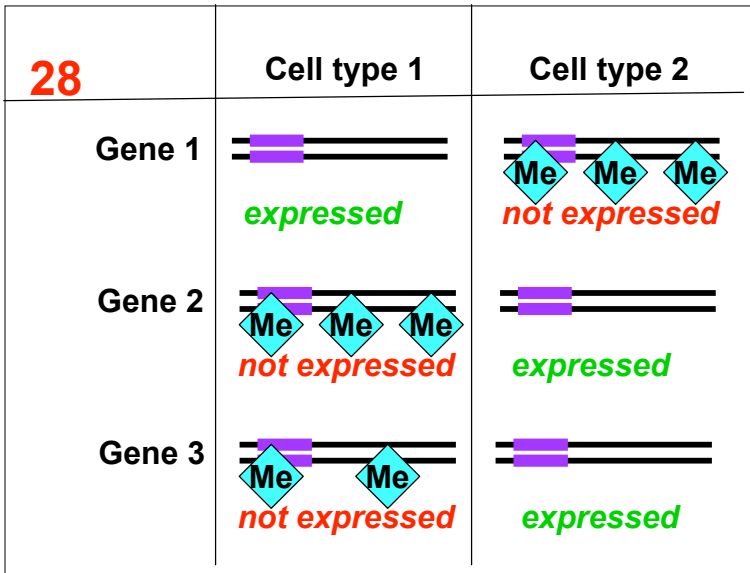
27

(B)

Methylation of Globin Genes represses their expression



28

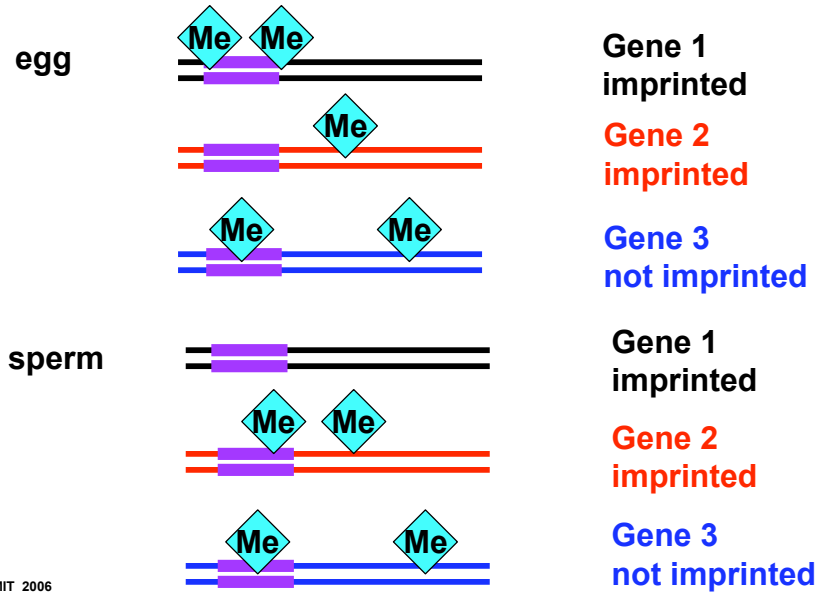


Cell type-specific methylation patterns
help regulate which genes are expressed

H.Sive MIT 2006

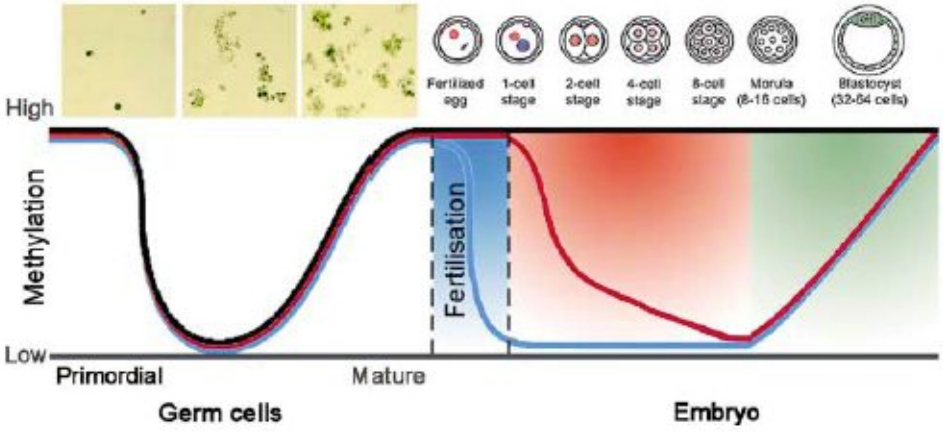
29

Another epigenetic control
paternal or maternal-specific methylation = imprinting



H.Sive MIT 2006

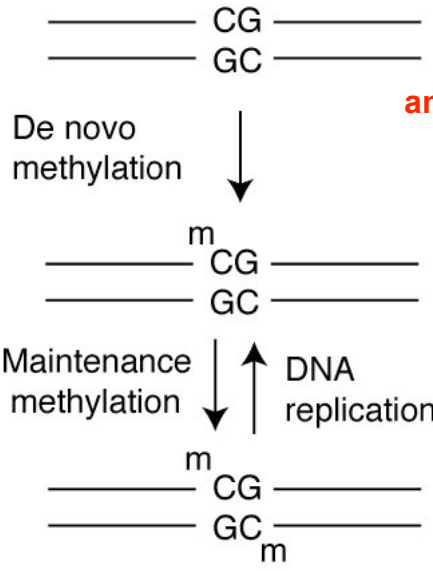
30 **Global changes in paternal and maternal methylation during mouse development**



Red: maternal
Blue: paternal

Santos and Dean, 2004

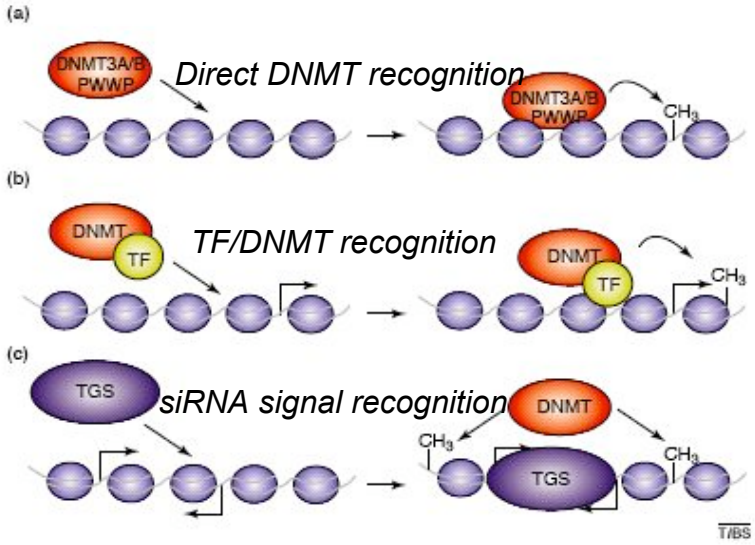
31



Germ cells and embryonic cells establish methylation patterns

Somatic cells maintain cell type-specific methylation patterns

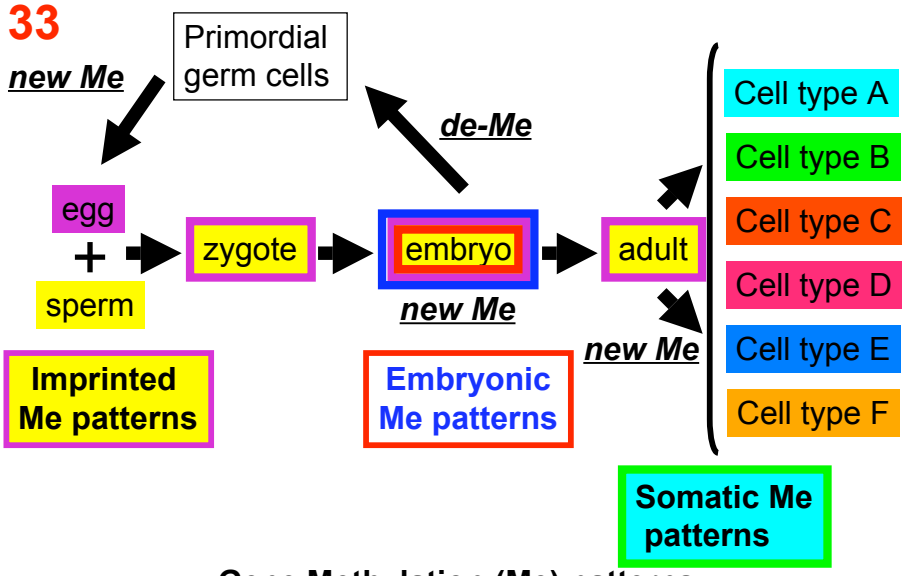
32



Targetting de novo DNA methylation via
DNA sequence recognition

Klose and Bird, 2006

33

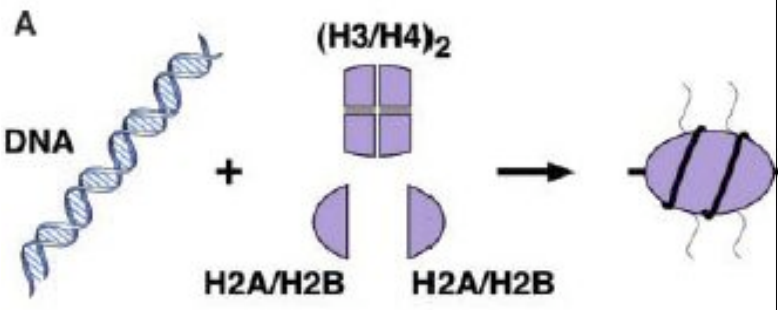


Gene Methylation (Me) patterns
(and therefore gene activity) change during development

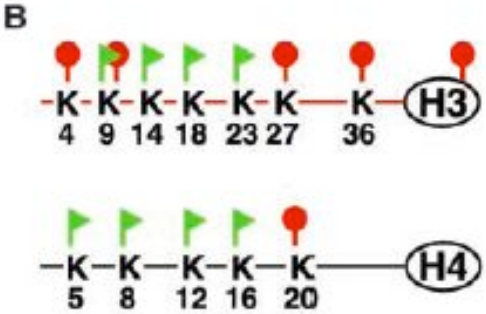
H.Sive MIT 2006

Histone modification and DNA methylation connection

34

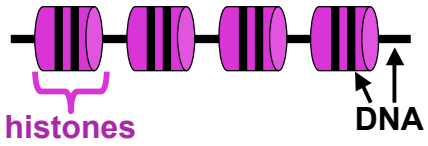


"Histone code". Sites of histone H3, H4 acetylation and methylation



Grewal and Mozaed, 2003

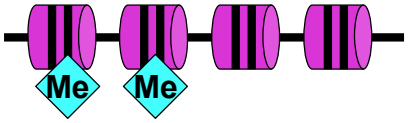
dsDNA is shown as one line



35

In chromatin
DNA
is wound around
histone proteins

Histones must be
removed or modified
to activate transcription

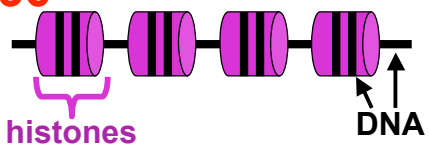


Methylation of cytosine
prevents histone
removal/modification and so
represses transcription

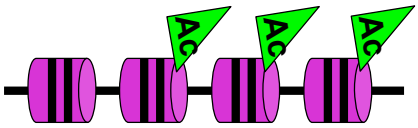
Histone removal, and therefore transcription, is inhibited by DNA methylation

H.Sive MIT 2006

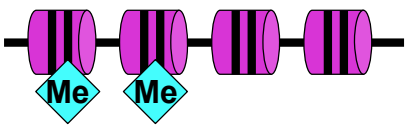
36



In chromatin
DNA
is wound around
histone proteins



Addition of acetyl groups
to histone proteins
unwinds chromatin to
activate transcription

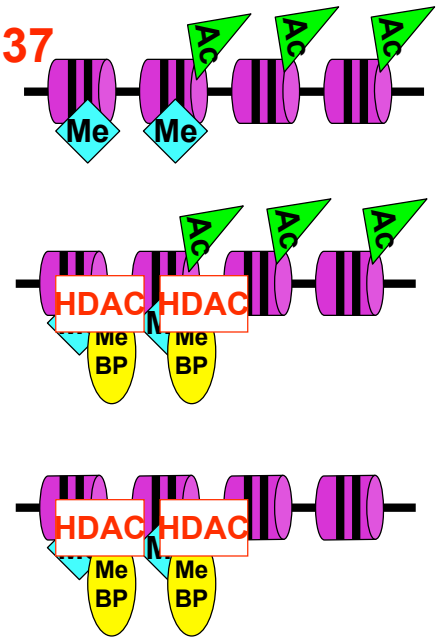


Methylation of cytosine
represses transcription

Transcription requires acetylated histones and is inhibited by DNA methylation

H.Sive MIT 2006

37



DNA Methylation inhibits transcription

because…

MeDNA binds specific proteins

which bind Histone Deacetylase (HDAC)

which… de-acetylates histones are therefore inhibits transcription

DNA methylation directs histone de-acetylation

H.Sive MIT 2006

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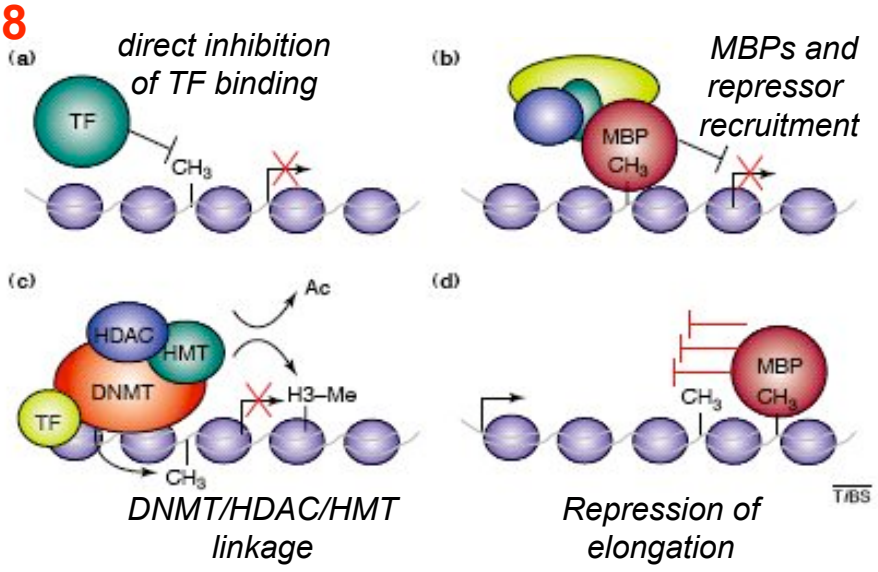


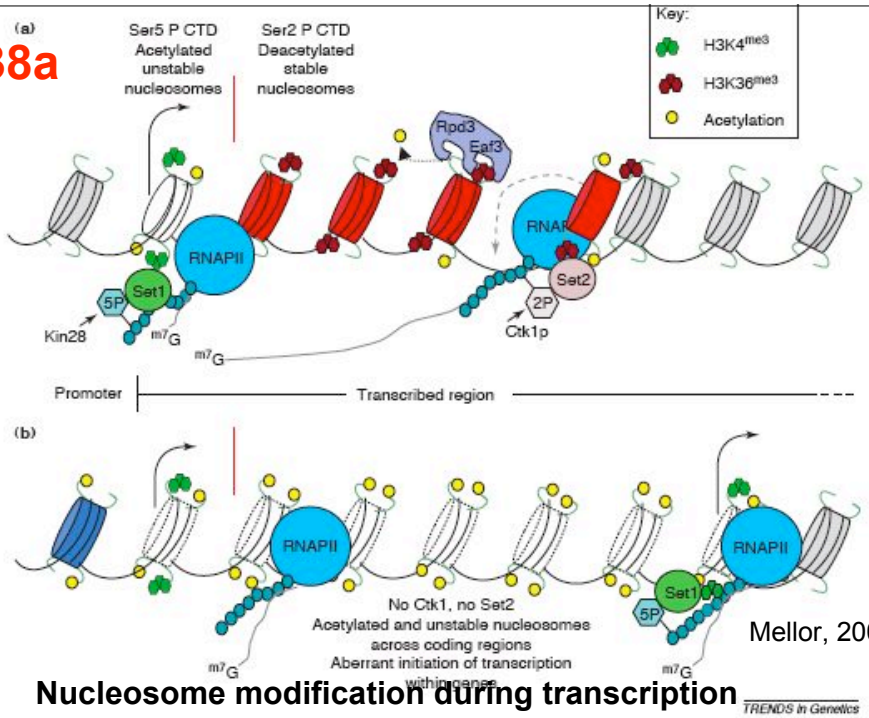
DNMT/HDAC/HMT linkage

Repression of elongation

Multiple mechanisms of DNA-methylation mediated repression

Klose and Bird, 2006

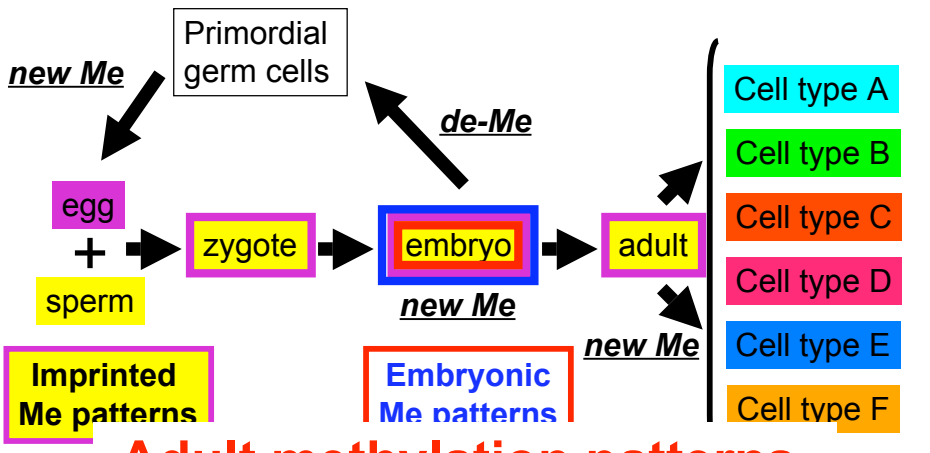
38a



Mellor, 2006

Nucleosome modification during transcription

Rephrasing:
why cloned embryos are abnormal



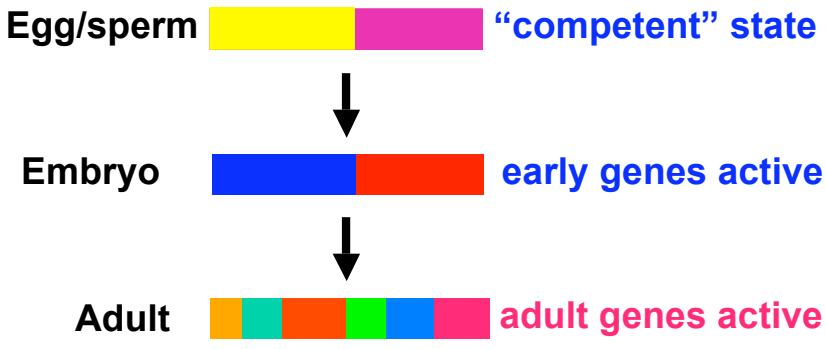
Adult methylation patterns do not normally revert to embryonic patterns

H.Sive MIT 2006

40

Methylation patterns (chromatin structure) and correct gene expression

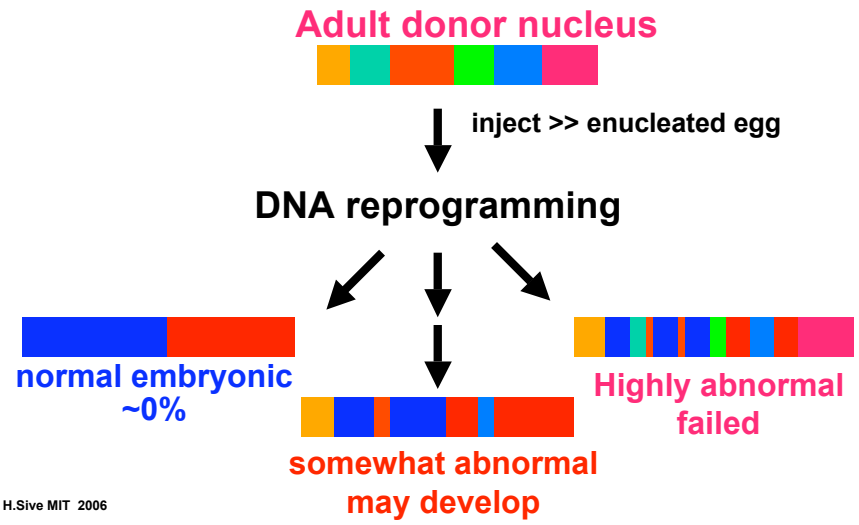
Normal development



H.Sive MIT 2005

41 Methylation patterns (chromatin) and competence for correct gene expression

After somatic nuclear transfer



H.Sive MIT 2006

7. How to reprogram nuclei?

42

How to reprogram adult nucleus to an embryonic state?

adult donor nucleus

inject >> enucleated egg >> ICM

Sequential nuclear transfer

partially reprogrammed nucleus >> cell line

inject >> enucleated egg

normal embryonic pattern
>> pluripotent ES cells,
normal embryo

H.Sive MIT 2006

43

Table 1. First nuclear transfers using determined and differentiated cells

Species	Donor tissue	Donor stage	% of total nuclear transfers reaching muscular response stage	Ref.
Xenopus laevis	Endoderm	Muscular response stages 23–26	17–24	3
X. laevis	Endoderm	Heart beat stage 36	9–13	3
X. laevis	Muscle	Muscular response stage 26	2	42
X. laevis	Intestinal epithelium	Early feeding tadpole stage 47	1–3	4

Table 2. The combination of first and serial nuclear transfer results

Species	Donor tissue	Donor stage	% of total nuclear transfers reaching muscular response stage	Ref.
X. laevis	Intestinal epithelium	Early feeding tadpole stage 47	20	4
X. laevis	Skin outgrowth	Adult	11–12	14
X. laevis	Kidney, lung, heart	Adult	13	47

Serial nuclear transfer increases developmental potential

44

How to reprogram adult nucleus to an embryonic state?

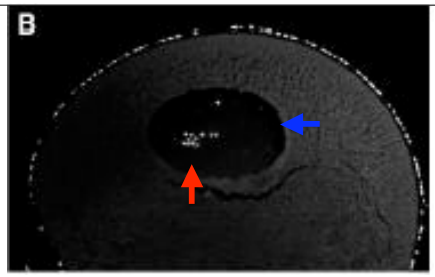
adult donor nucleus/cell

Incubate in “reprogramming factors”

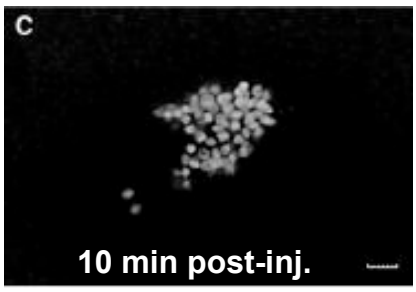
normal
embryonic nucleus/cell

H.Sive MIT 2006

45

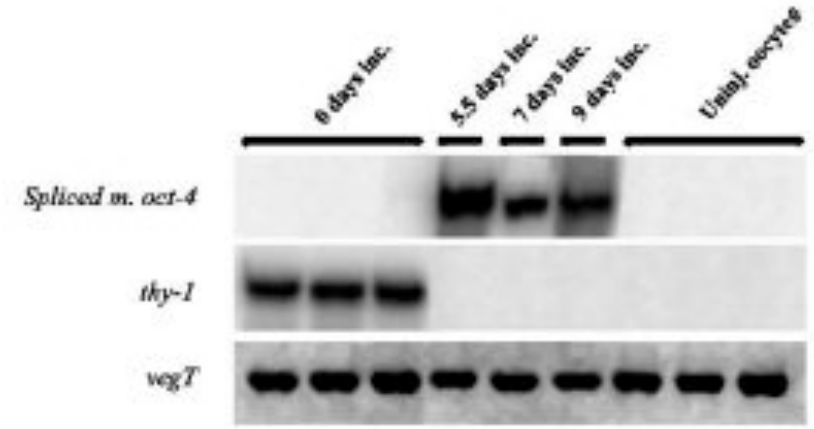


Oocyte nucleus
+ mouse nuclei
Low mag.



Injection of mouse thymocyte nuclei into frog oocyte nucleus
Byrne et al, 2003

46

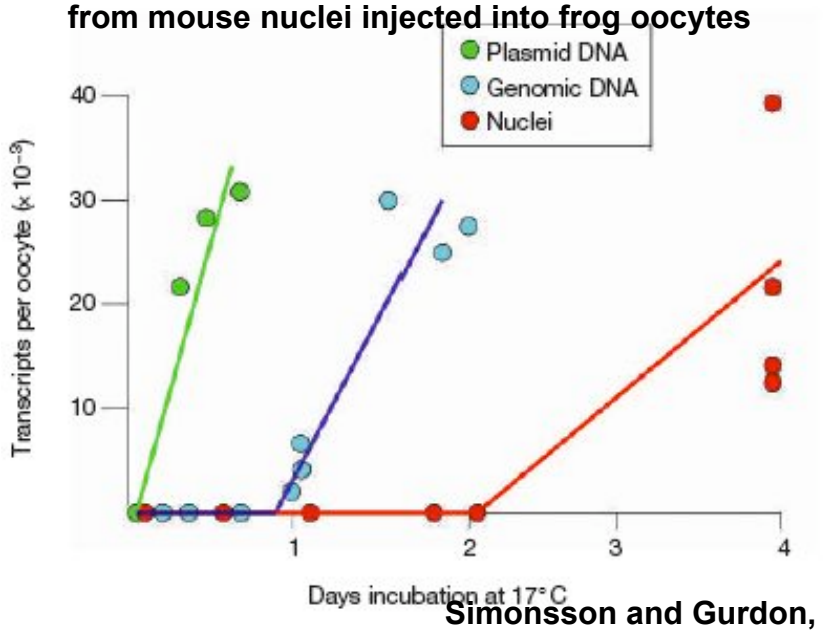


Activation of *oct4* expression in mouse thymocyte nuclei injected into frog oocyte nuclei

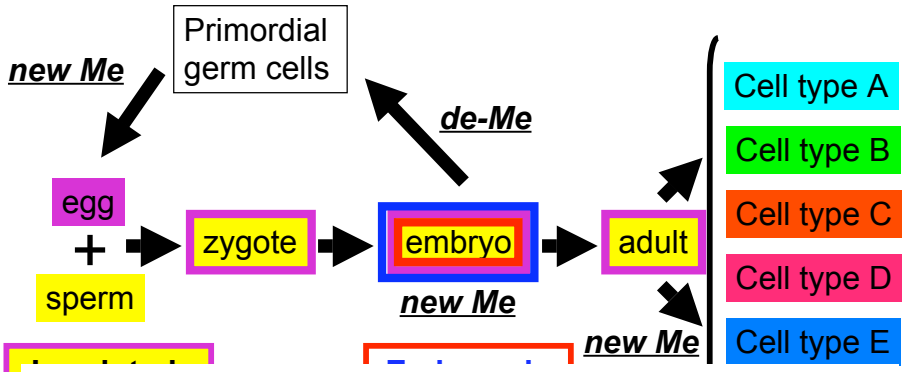
Byrne et al, 2003

47

Deproteinization and demethylation decreases delay in activation of *oct4* expression from mouse nuclei injected into frog oocytes



Simonsson and Gurdon, 2004

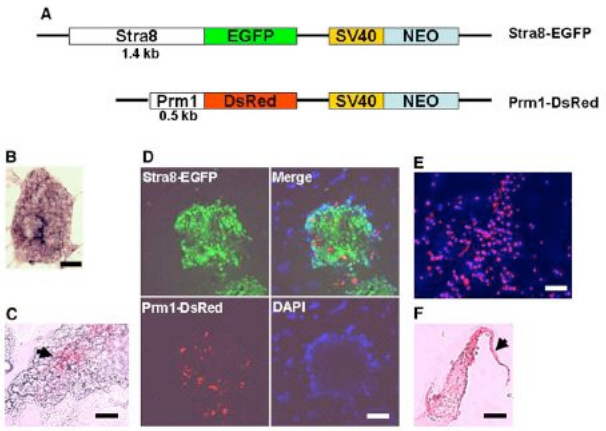


ISSUE: different maternal and paternal Me patterns needed for normal development, so may always need egg + sperm.

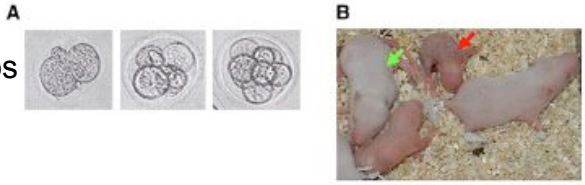
H.Sive MIT 2006

48

Selection for sperm markers



Embryos/pups



Functional sperm derived from ES-derived cells

Nayernia et al, 2006

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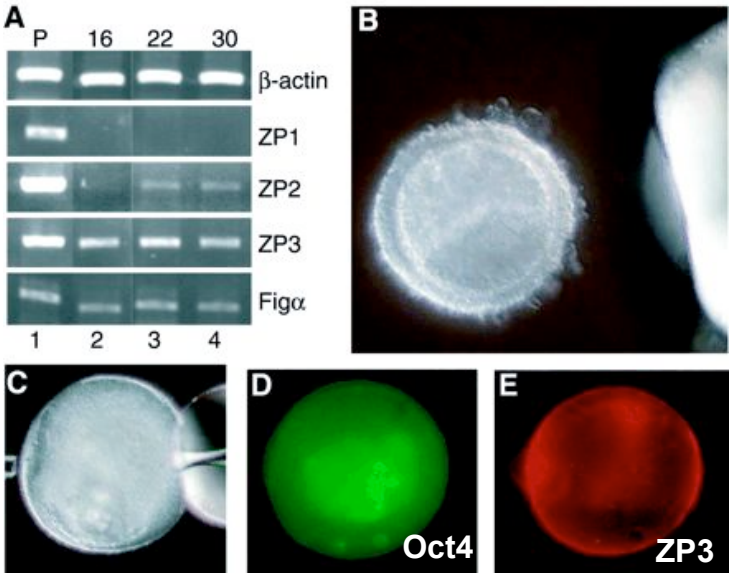

Creating germ cells from ES cells (maybe)

Schöler et al 2003

8. Current and future issues

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Issues

Ethics

Use of human embryos

Source of eggs/ surrogate mothers

Fate of abnormal babies

Methods

How to clone primates?

ES cells >> eggs

How to reprogram adult nuclei

Gene therapy