CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2013-2014 :

“Reprogrammations développementales, induites et pathologiques ”

Cours I

Reprogrammation de l’identité cellulaire – introduction historique

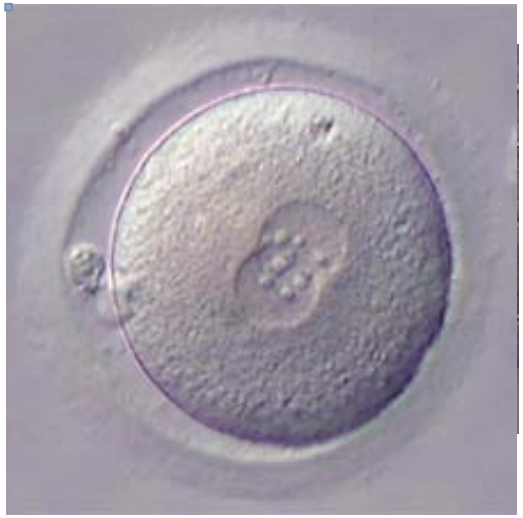
10 mars 2014

Seminaire:
Sir John Gurdon,
le vendredi 14 mars à 17h30

COLLÈGE DE FRANCE 1530

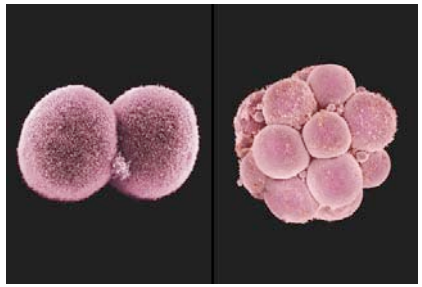
Omnis cellula e cellula

(Virchow, 1855)

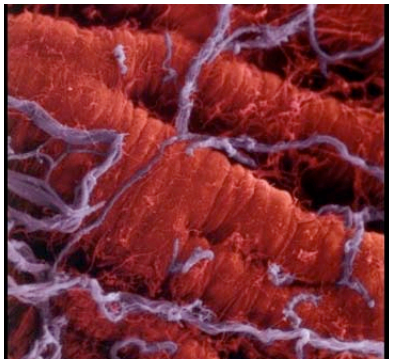


"*And thus the wonderful truth became manifest that a single cell may contain within its microscopic compass the sum total of the heritage of the species*".

EB Wilson, 1900



Eye



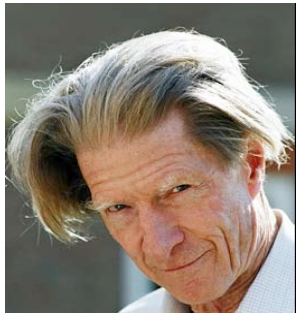
Heart and nerves

Macrophage

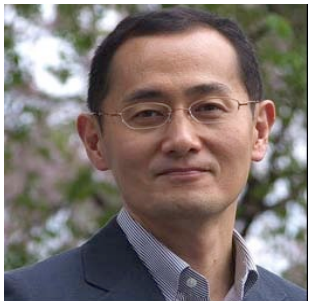
Lennart Nilsson ©

Epigenesis: establishing organized diversity from a single cell

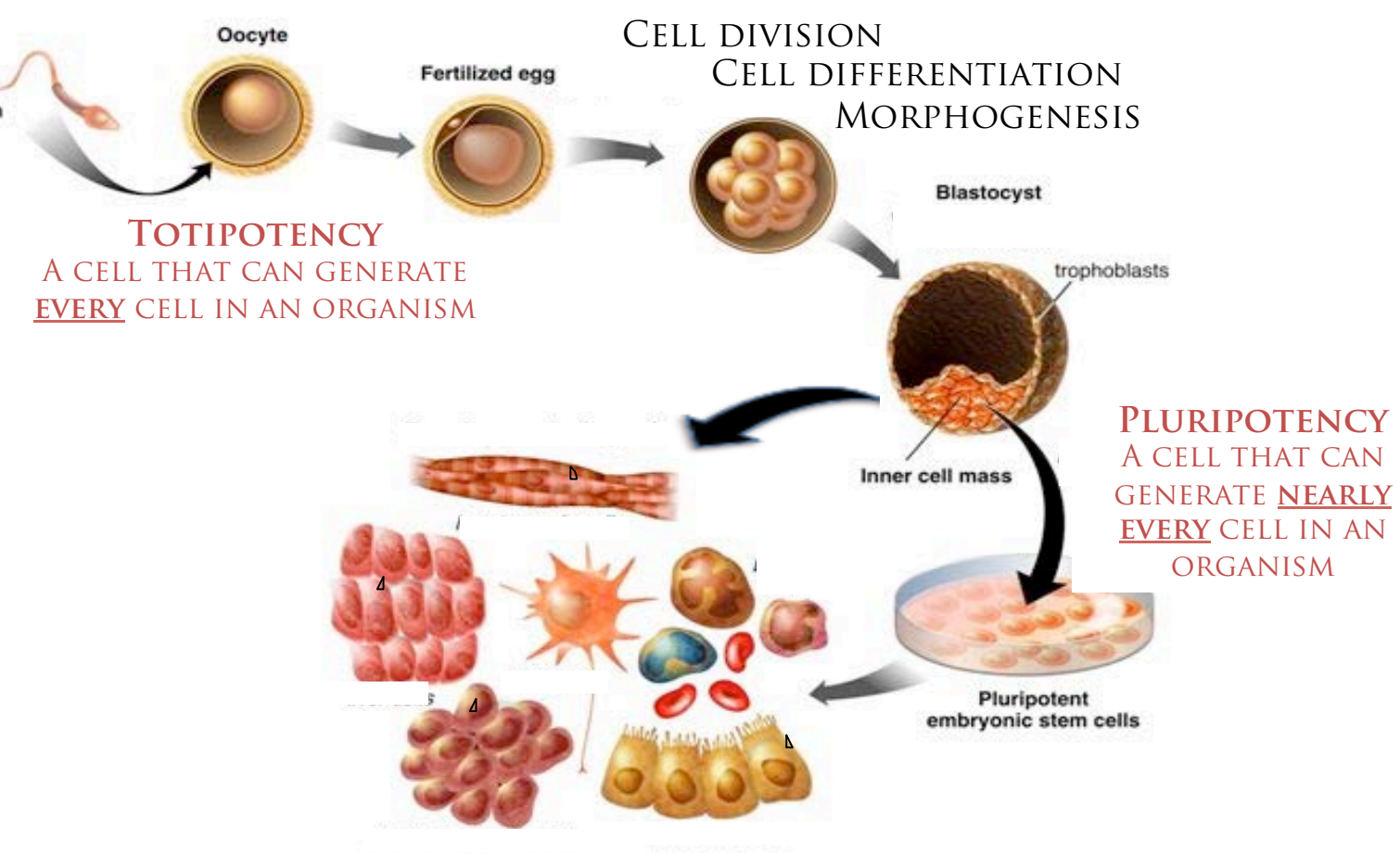
2012 Nobel Prize for Physiology and Medicine



Sir John Gurdon



Yamanaka Sensei



TOTIPOTENCY
A CELL THAT CAN GENERATE **EVERY** CELL IN AN ORGANISM

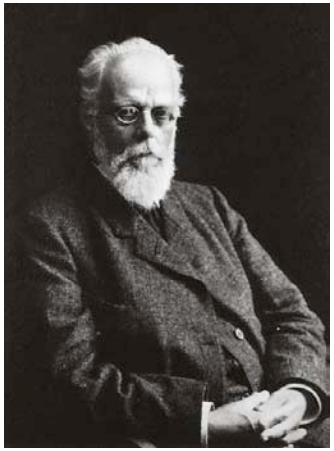
PLURIPOTENCY
A CELL THAT CAN GENERATE **NEARLY EVERY** CELL IN AN ORGANISM

Decades of research on cell fate changes during development led to the view that, in vivo, differentiated cells are irreversibly committed to their fate.

Can a cell's fate be reversed? Can it forget its state? Lose its identity?

Does a differentiated cell have the capacity to form all cells of an organism or is this solely the business of the germ line?

The unidirectionality of development



August Weismann
(1834–1914)
Evolutionary biologist

Wilhelm Roux
(1850–1924)
Zoologist
Experimental embryologist

The "Weismann barrier":

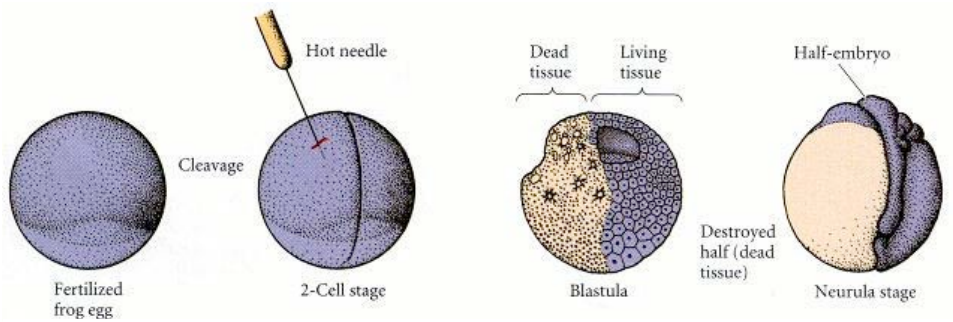
Genetic information *cannot* pass from soma to germ plasm and on to the next generation.
=> Acquired characteristics *cannot* be inherited (contrary to Jean Baptiste Lamarck)

"Germ Plasm Theory":

Inheritance only takes place via germ cells (gametes)
Development is a unidirectional process
The differentiated state of specialised cells, like skin or liver, is fixed irreversibly…

"Mosaic hypothesis":

Supported by **Wilhelm Roux's** cell ablation experiments killing one cell of a 2-cell frog embryo leads to half an embryo

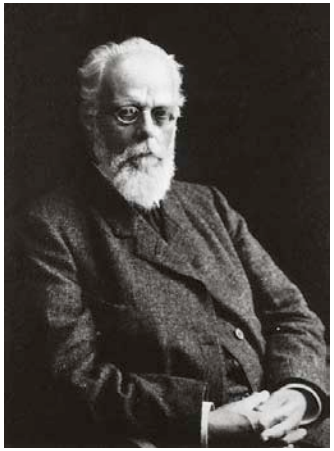


From: The Developmental Mechanics of Cell Specification
Developmental Biology, Gilbert SF.

Each cell plays its own unique part in the entire design
and cannot play any other part

Conclusions of Weismann and Roux experiments led to the prevailing view that cellular **differentiation proceeds with progressive selective "loss" of genetic material** not relevant to specific function, resulting in **genetic mosaicism.**
Only the germ cells are set aside and preserved from this….

The unidirectionality of development



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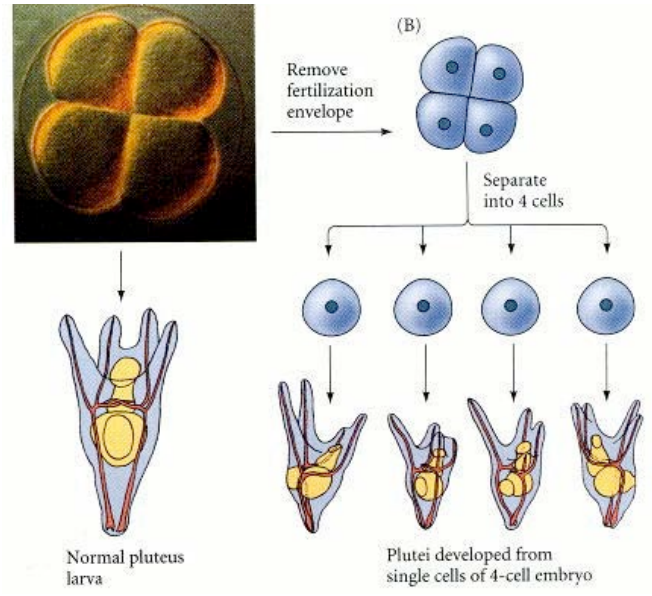
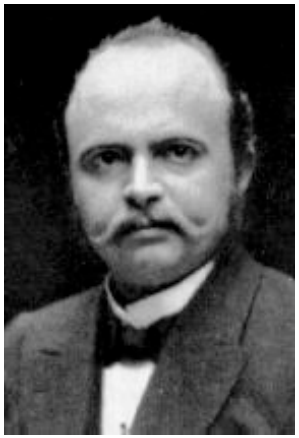
Totipotency of early blastomeres :

Any cell of an early sea urchin embryos has the ability to become an embryo.
Each cell still possesses all determinants.

Artificial twinning (not "cloning")



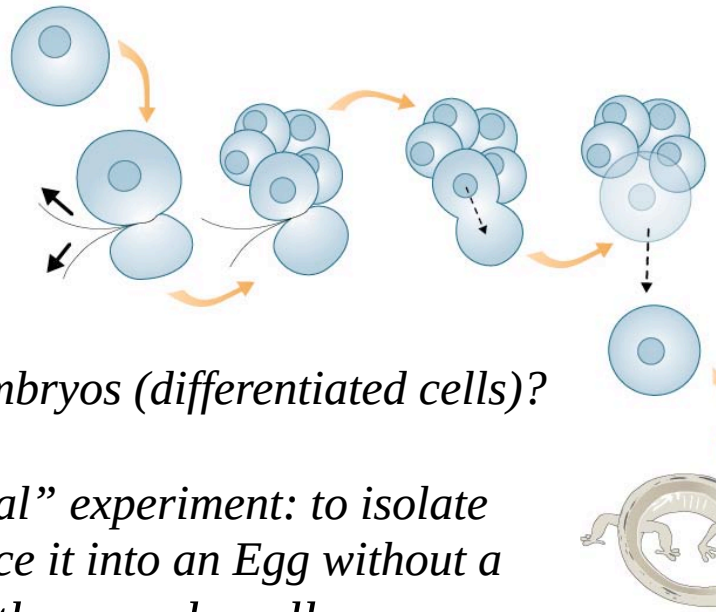


Hans Driesch
(1867-1941)
Experimental embryologist

Spemann and Mangold demonstration of totipotency at 8-cell stage

Hans Spemann*
(1869–1941)

- Experiments on Salamander embryos to determine a cells developmental potential (range of structures to which it can give rise)
- Embryonic fates are affected by distribution of determinants and the pattern of cleavage
- The first two blastomeres of the frog embryo are **totipotent** (can develop into all the possible cell types)
- **Single cells of an 8-cell embryo are also totipotent**



First example of nuclear transfer: Nucleus from an early embryonic cell directs the complete growth of a salamander, effectively substituting for the nucleus in a fertilized egg!

Spemann, H. (1928). Die Entwicklung seitlicher und dorso-ventraler Keimhälften bei verzögerter Kernversorgung. *Ztschr. f. Wiss. Zool*. 132, 105–134

Could this work with later stage embryos (differentiated cells)?

*Spemann proposed a "fantastical" experiment: to isolate nucleus of a morula and introduce it into an Egg without a nucleus…ie to **CLONE** the morula cell…*

whereby one cell or tissue directs the development of another, neighboring, cell or tissue, via biochemical signals that lead to cellular differentiation in the nervous system and other embryonic organs.

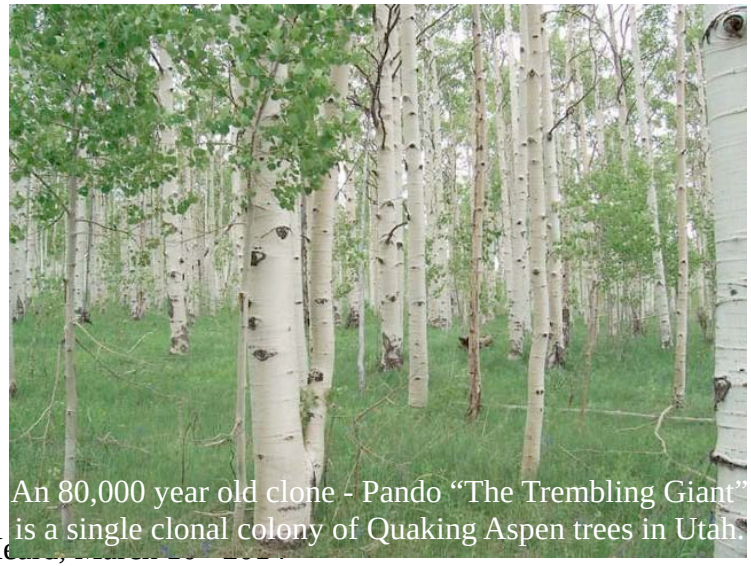
The term clone is derived from the Ancient Greek word κλών (klōn, “twig”): the process whereby a new plant can be created from a twig.

There are many clones in nature: vegetative (asexual) reproduction (“apomixis” in plants) - results in clonal populations of genetically identical individuals

Some aphids and many trees, shrubs, vines - parts of a plant may become detached by fragmentation and grow on to become separate clonal individuals….

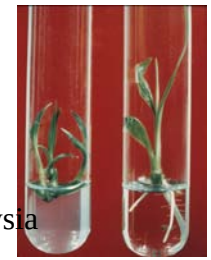
Some European cultivars of grapes represent clones that have been propagated for over two millennia

Natural clones



An 80,000 year old clone - Pando “The Trembling Giant” is a single clonal colony of Quaking Aspen trees in Utah.

Artificially generated clones



Cloning oil palm trees in Malaysia (Courtesy R. Martienssen)

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Testing the Weissman Roux hypothesis: The developmental potential of a differentiated cell nucleus

Weissman Roux hypothesis:

Nuclei of differentiated cells lose their ability to generate a new organism.

Spemann:

If all genes are retained and the process of differentiation is reversible, a somatic nucleus would maintain the potential to form a new organism when transplanted into the egg.

Robert Briggs (1911-1983)

Thomas J. King (1921-2000)

*TRANSPLANTATION OF LIVING NUCLEI FROM BLASTULA CELLS INTO ENUCLEATED FROGS' EGGS**

By Robert Briggs and Thomas J. King

Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia, Pennsylvania

Communicated by C. W. Metz, March 15, 1952

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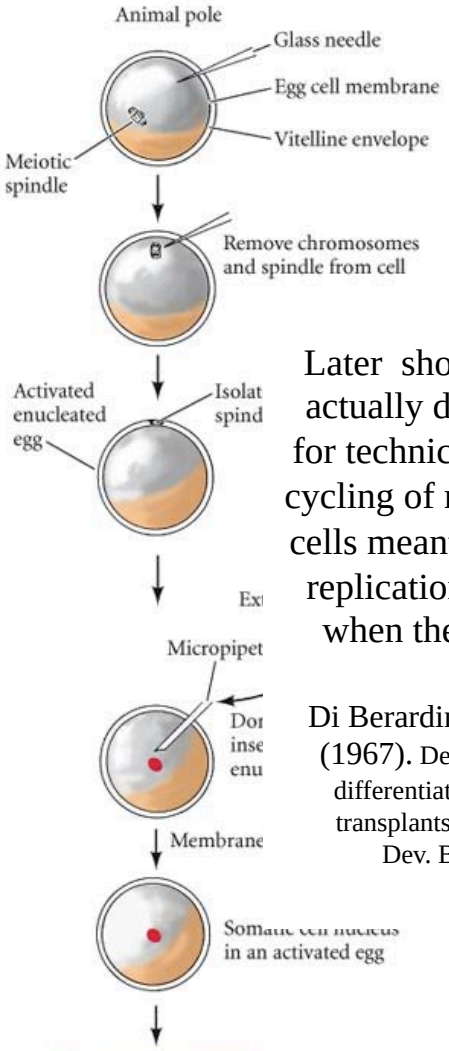
Briggs and King:
Nuclear Transfer experiments in the frog, Rania pipiens



Later showed that this was actually due to genetic loss for technical reasons (slower cycling of more differentiated cells meant that chromosome replication was incomplete when the egg divided…)

Di Berardino M. A., King T. J. (1967). Development and cellular differentiation of neural nuclear-transplants of known karyotype. Dev. Biol. 15, 102-128.

"Freddy" derived using the te DiBerardino and N. Hoffner Orr. (photograph courtesy of M. DiBe

Conclusions of Briggs and King papers (1952, 1956)

1. Nuclear transfer (NT) into enucleated eggs was a viable cloning technique!
2. The nucleus directs cell growth and, ultimately, an organism's development.
3. Embryonic cells early in development are better for cloning than cells at later stages.
4. Loss of developmental potential was heritable (following serial transfer NTs)

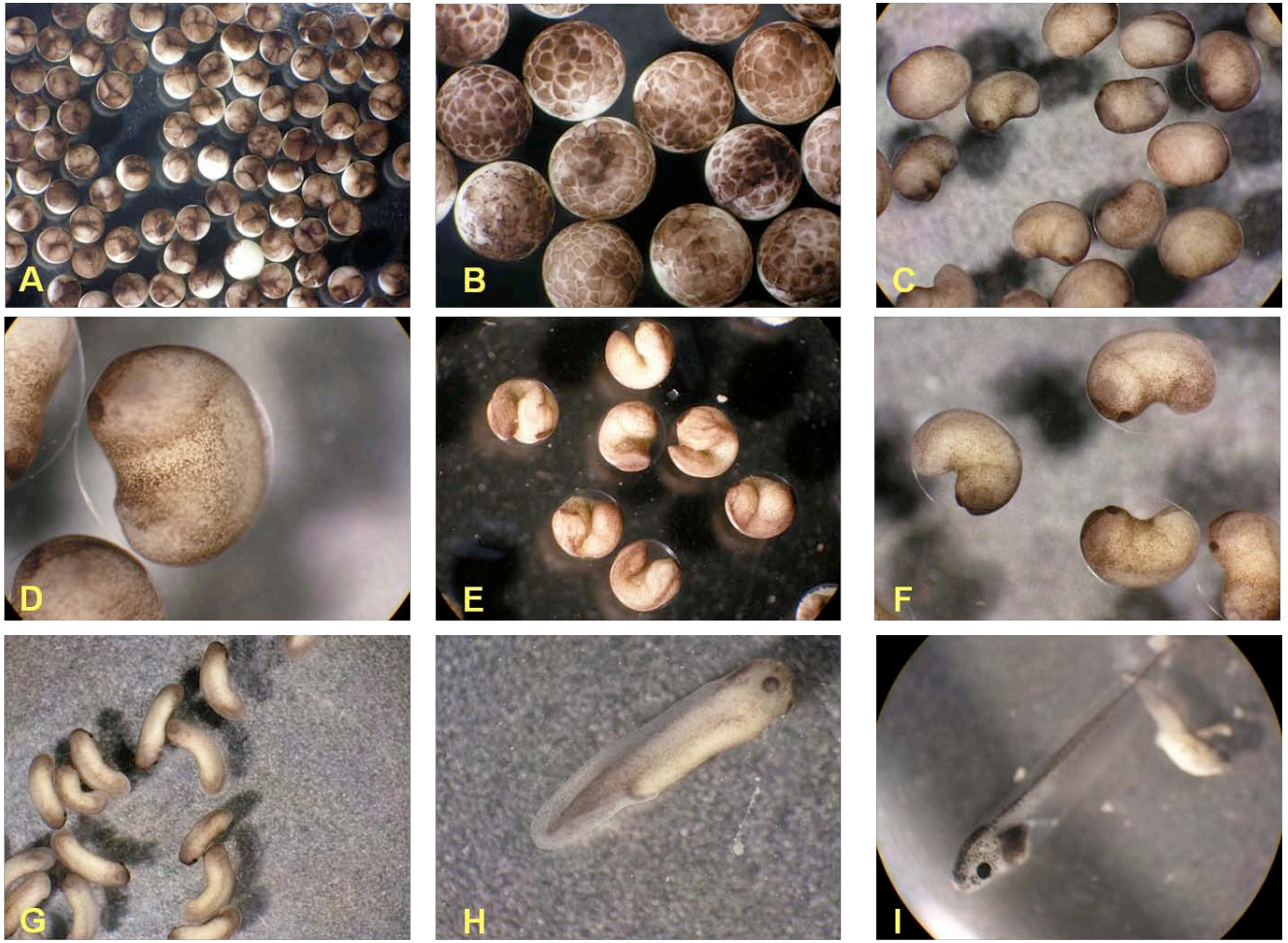
Loss of developmental potential of differentiated nuclei could still be due to genetic loss?
(consistent with Weismann-Roux?)

⇒ The question as to whether the genome itself changes during development, or whether it is the way genes are expressed that is responsible for differentiation remained unanswered….

Gurdon:
Nuclear Transfer experiments in the frog, Xenopus laevis

Is the genome irreversibly altered as cells become more specialized during development?

John B. Gurdon



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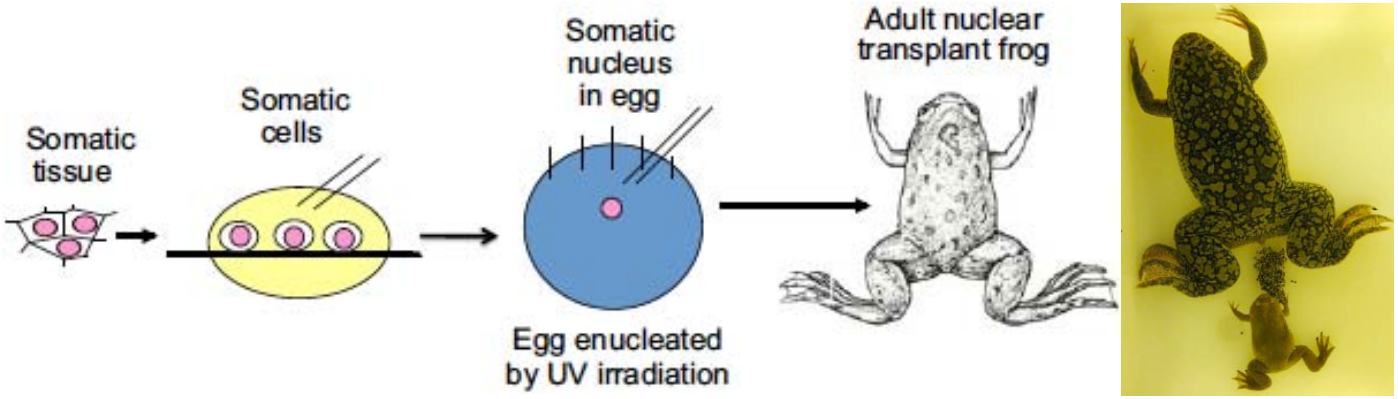
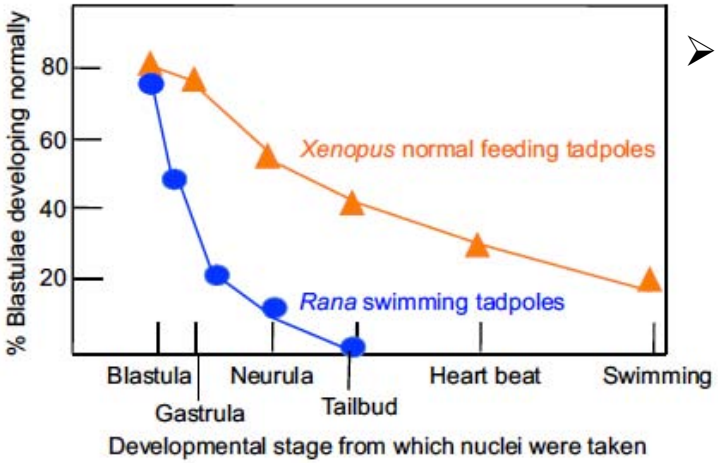
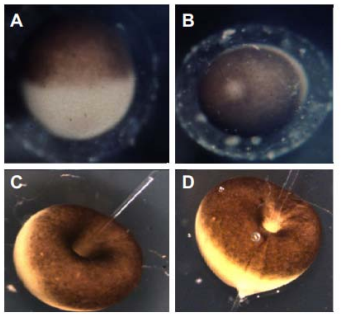




- Easier model system, *Xenopus laevis*
- Improved technique (UV to remove egg nucleus)
- Markers of donor nuclei

(Fischberg et al, 1958; Brown and Gurdon, 1964)



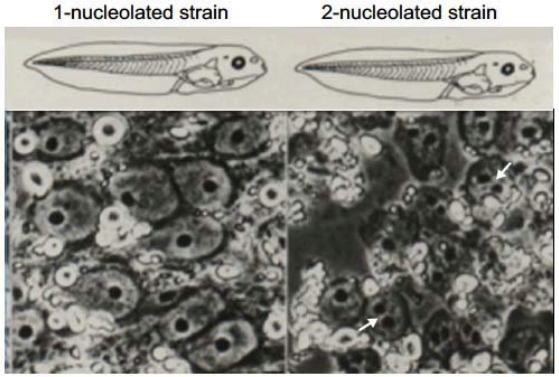


Fig. 5. A nucleolar genetic marker for *Xenopus laevis*. Heterozygotes

Gurdon:
Nuclear Transfer experiments in the frog, Xenopus laevis

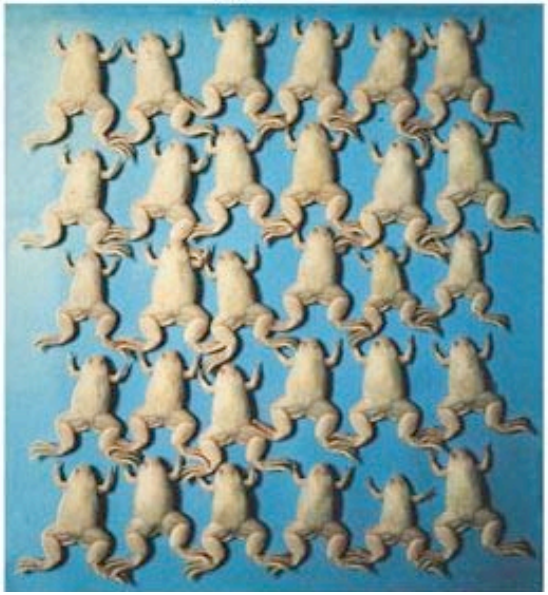
Is the genome irreversibly altered as cells become more specialized during development?

John B. Gurdon

African clawed toad, *Xenopus laevis*, (now a classic amphibian model).

Wild-type donor of enucleated eggs

Albino parents of nucleus donor



The development resulting from the transplantation of nuclei from differentiated and embryonic cells of Xenopus laevis

Donor stage (Nieuwkoop & Faber, 1956)	*Total transfers*	*No cleavage*	*Total transfers resulting in cleavage*	*Development resulting from transplanted nuclei*								
				Abortive cleavage	*Partial cleavage*	*Complete blastulae*	*Arrested blastulae*	*Abnormal gastrulae*	*Abnormal post-neurulae*	*Stunted tadpoles*	*Died as swimming tadpoles*	*Normal feeding tadpoles*
Intestinal epithelium cell nuclei (stage 46–48)	726	347	379	175	156	48	18	8	5	6	1	10
	100%	48%	52%	24%	21·5%	6·5%	—	—	—	—	—	1·5%
Blastula or gastrula endoderm nuclei (stage 8–12)	279	66	213	8	32	173	4	17	19	27	6	100
	100%	24%	76%	3%	11%	62%	—	—	—	—	—	36%

Gurdon, J. B. (1962). The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J. Embryol. Exp. Morphol.* **10**, 622-640.

Gurdon:
Nuclear Transfer experiments in the frog, Xenopus laevis

Is the genome irreversibly altered as cells become more specialized during development?

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Generated live frogs (though with low efficiency):
- from transplanted neurula stage endoderm nuclei
- from differentiated intestinal nuclei of tadpoles (1.5%)
⇒ resulted in **fertile adult frogs** after nuclear transfer

The nuclei of differentiated cells retain their totipotency
(can generate all cell types, including germ line)

Gurdon, J. B. (1960). The developmental capacity of nuclei taken from differentiating endoderm cells of Xenopus laevis. *J. Embryol. Exp. Morphol*. **8**, 505-526.
Gurdon, J. B. (1962). The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J. Embryol. Exp. Morphol.* **10**, 622-640.
Gurdon, J. B. and Uehlinger, V. (1966). 'Fertile' intestine nuclei. *Nature* **210**, 1240-1241.

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Nuclear Transfer experiments in the frog, Xenopus laevis

Is the genome irreversibly altered as cells become more specialized during development?

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Gurdon's Conclusions:

1. Cell differentiation did not involve permanent changes to the genome

⇒ Genetic equivalence of somatic and embryonic cell nuclei

First proof that cell differentiation depends on changes in the expression rather than the content of the genome

2. Remarkable reprogramming capacity of the egg cytoplasm

3. **Lack of fertile clones from adult nuclei and the many abnormal embryos - probably due to failures in the correct reprogramming of the nuclei by the cytoplasm of the egg**

⇒ **Incomplete chromosome replication**?

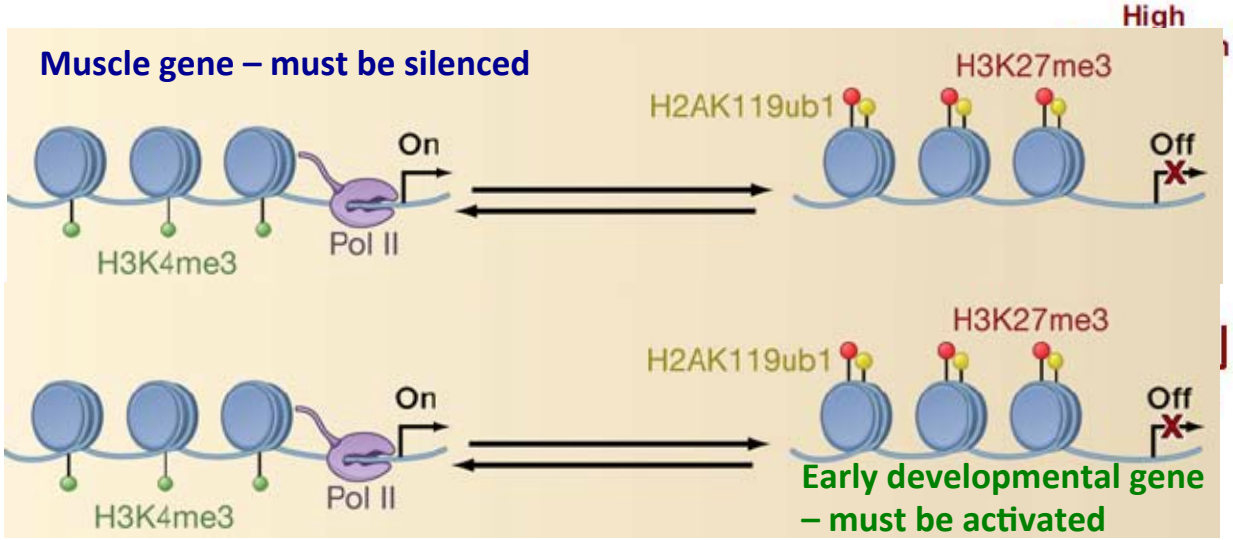
and **Epigenetic resistance**?

Epigenetic Memory as a cause of inefficient NT?

Types of "epigenetic memory" that could interfere with efficient reprogramming:

- Repressed state of developmental genes in differentiated nucleus?
- Active state of specialised genes characteristic of the differentiated nucleus?

Inappropriate expression (memory of the active state) of muscle genes from a muscle cell donor nucleus in about half of the NT embryos



Memory of the *active* state in cells that normally do not express muscle genes

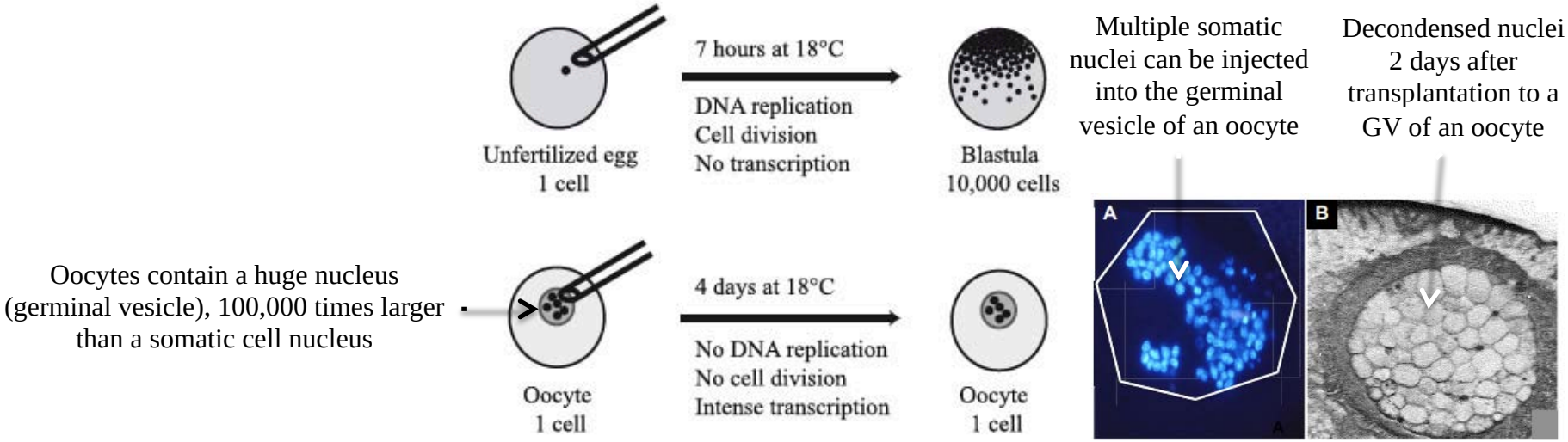
What could the nature of this "active" state memory be?
How efficiently is the silent state of developmental genes erased?

Sir John Gurdon, Seminar on March 14th, 5.30pm

The inefficiency of NT could be due to incompatibility of the quiescent state of the donor nucleus and the egg's rapid cell division

"We think **rapid cell division** and **DNA replication** enforced on an amphibian transplanted nucleus by an activated egg has **a high probability of introducing replication defects**, as is seen in Rana pipiens (Di Berardino and King, 1967), thereby greatly reducing the chance of obtaining entirely normal development from the nucleus of an adult cell." Gurdon, 2013, *Development* 140, 2449-2456

To avoid these problems, Gurdon went on to use growing oocytes (no DNA replication and no cell division), rather than unfertilized eggs, for his investigation of <u>reprogramming mechanisms</u> and the factors underlying <u>epigenetic memory</u>



Sir John Gurdon, Seminar on March 14th, 5.30pm

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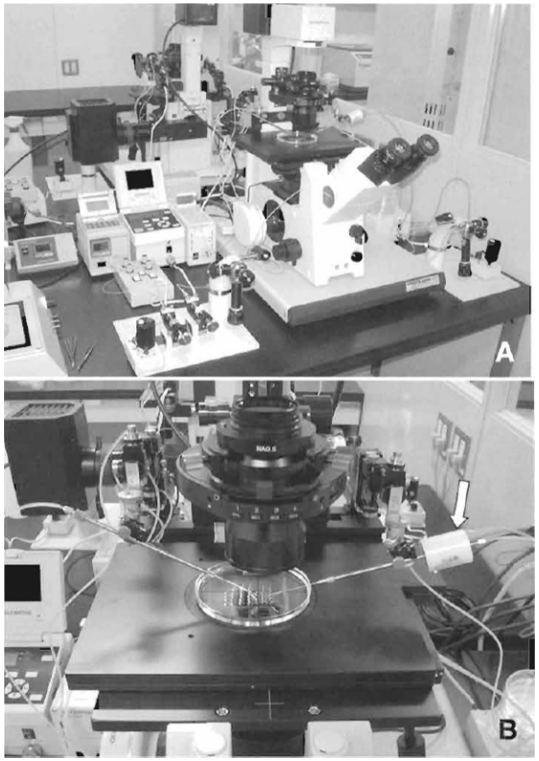
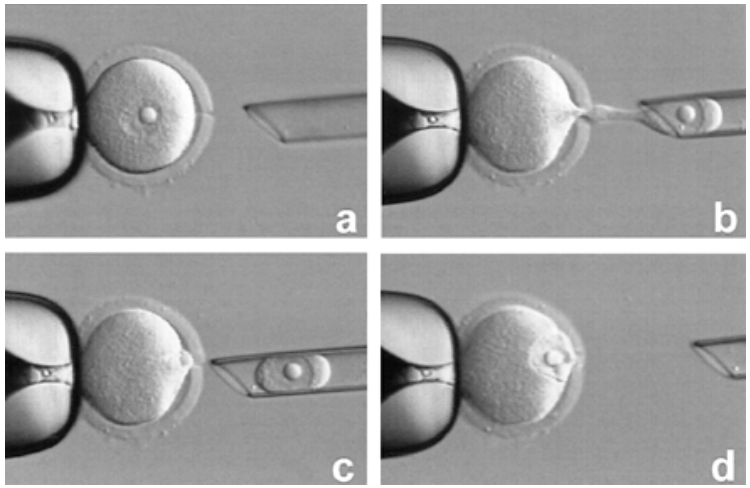
Nuclear Transfer and Cloning in Mammals

Why did it take almost 30 years before successful NT was achieved in mammals?

- Mammalian egg cells are much smaller than those of frogs or salamanders
  ⇒ much harder to manipulate: required micromanipulation techniques
  (Graham, 1969; Barensdat, 1970, Lin 1971)
- Different mammals have different characteristics in terms of accessibility, timing, growth
  - Efficient embryo transfer techniques had to be developed





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Nuclear Transfer and Cloning in Mammals

Discovery of imprinting in mouse embryos

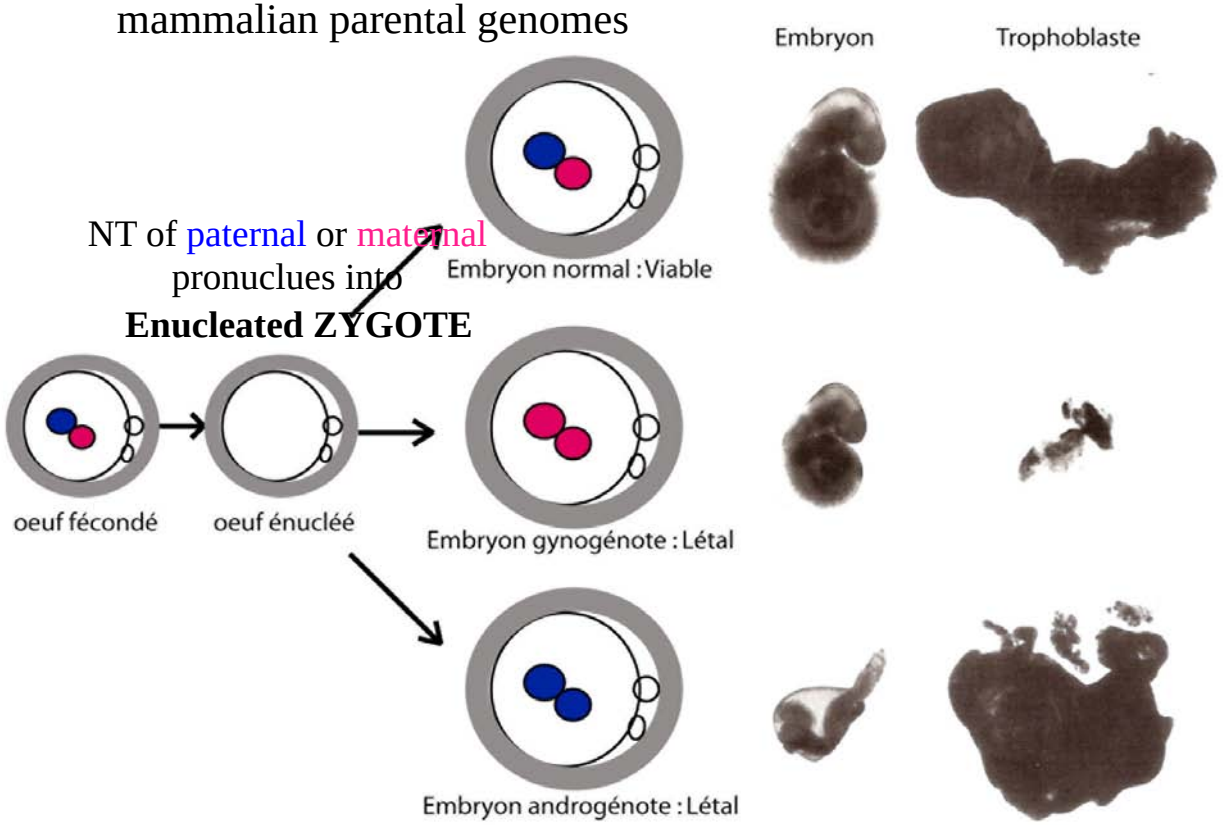
Transplant paternal or maternal pronuclei into enucleated fertilised egg (zygote)

Azim Surani Davor Solter

However, this SCNT approach was unsuccessful for production of mammalian clones…

Nuclear transplantation experiments in mice by Azim Surani and Davor Solter :

- Two male or two female pronuclei are incompatible with normal development
- Formal demonstration of the functional non-equivalence of mammalian parental genomes



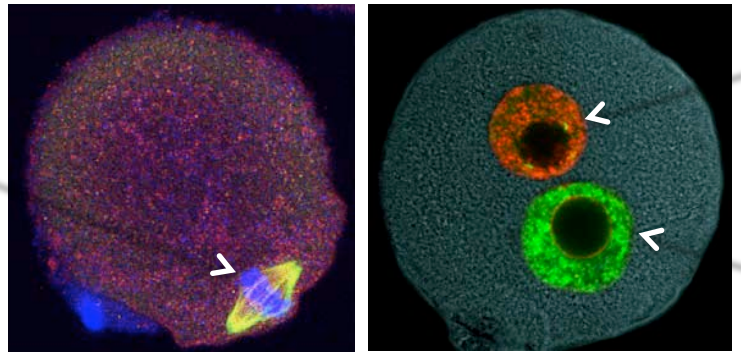
BARTON, S. C , SURANI, M. A. AND NORRI
and maternal genomes in mouse development. *N*
SURANI, M. A., BARTON, S. C. AND NORRI
reconstituted mouse eggs suggests imprinting of
gametogenesis. *Nature* 308, 548-550.
MCGRATH, J. AND SOLTER, D. (1984). Comp
requires both the maternal and paternal genomes

Nuclear Transfer and Cloning in Mammals

Why did it take almost 30 years before successful NT was achieved in mammals?

<u>Recipient cell used for enucleation:</u>
Initially, enucleated zygotes were used, not unfertilized eggs!
Zygote nuclei are in *interphase*
Oocytes are in *metaphase* (no nuclear envelope)

Metaphase chromosomes in MII Oocyte



Maternal interphase pronucleus

Paternal interphase pronucleus

Nuclear factors required for reprogramming may be retained in nucleus?

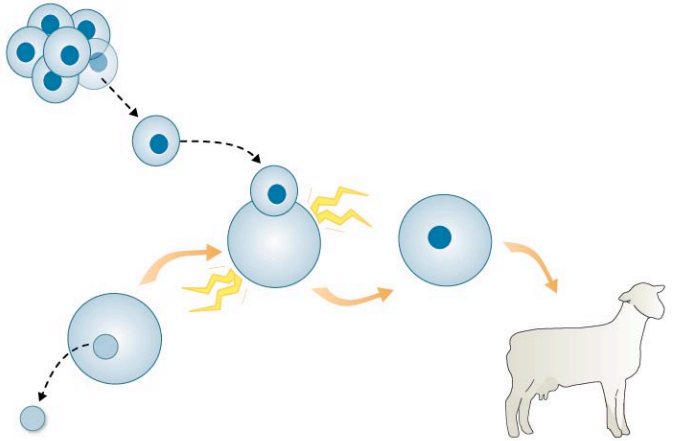
Nuclear Transfer and Cloning in Mammals

From rabbits, to sheep, to cows, mice and beyond….

1975 - Bromhall transferred a nucleus from a rabbit embryo cell into an enucleated rabbit egg cell and produced a morula after a couple of days.

1978 –Louise Brown, the first baby conceived via in-vitro fertilization, is born - successful embryo transfer techniques have become available.

1985 – Willadsen separated one cell from an 8-cell lamb embryo and used a sm electrical shock to fuse it to an enucleated egg cell – after a few days he transplanted this into a surrogate ewe and 3 lambs were born.

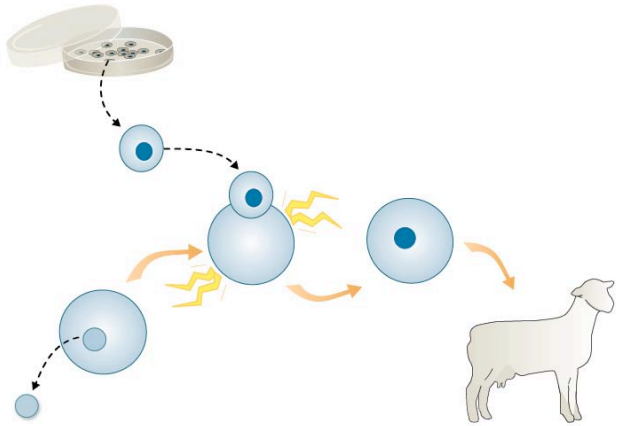


1987 - Prather and Eyestone produced two cloned calves: “Fusion” and “Copy”

1996 - Wilmut and Campbell transferred nuclei from cultured cells into enucleated sheep egg cells. Two lambs born “Megan” and “Morag”.

1996 - Wilmut and Campbell created a lamb “Dolly” by transferring the nucleus from an adult sheep's udder cell into an enucleated egg. Of 277 attempts, only one produced an embryo that was carried to term in a surrogate mother.

⇒ **First ever mammal cloned from an adult somatic cell….**



Dolly: the first mammalian clone

Viable offspring derived from fetal and adult mammalian cells

I. Wilmut, A. E. Schnieke*, J. McWhir, A. J. Kind*
& K. H. S. Campbell

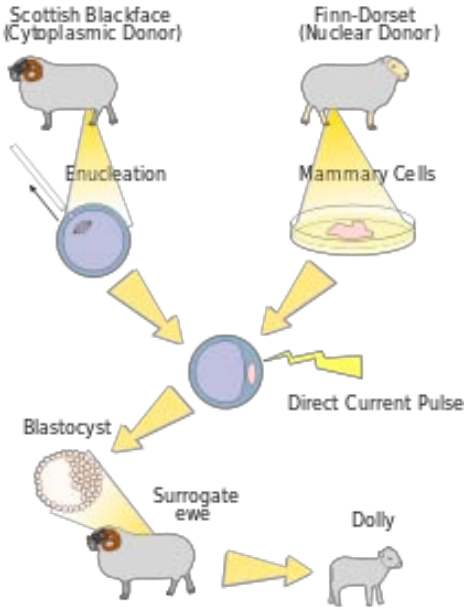
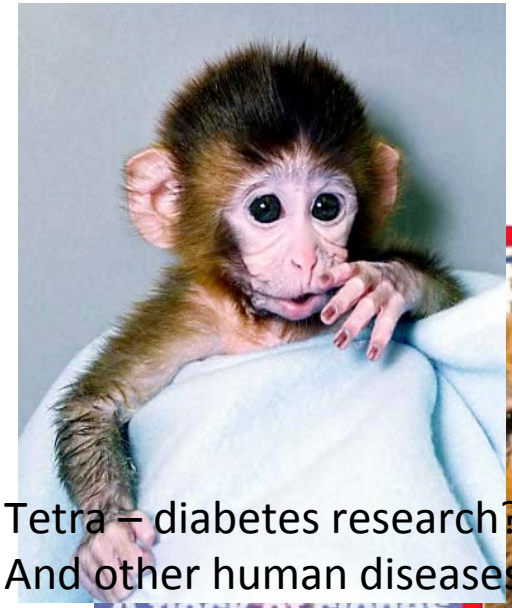


Table 1 Development of embryos reconstructed with three different cell types

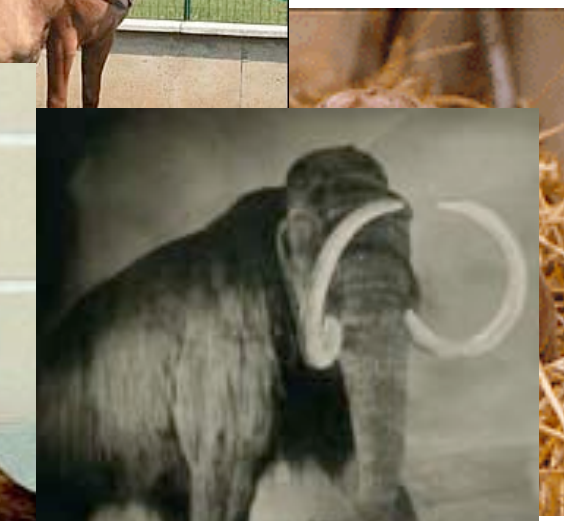
Cell type	No. of fused couplets (%)*	No. recovered from oviduct (%)	No. cultured	No. of morula/ blastocyst (%)	No. of morula or blastocysts transferred†	No. of pregnancies/ no. of recipients (%)	No. of live lambs (%)‡
Mammary epithelium	277 (63.8)[a]	247 (89.2)	-	29 (11.7)[a]	29	1/13 (7.7)	1 (3.4%)
Fetal fibroblast	172 (84.7)[b]	124 (86.7)	- 24	34 (27.4)[b] 13 (54.2)[b]	34 6	4/10 (40.0) 1/6 (16.6)	2 (5.9%) 1 (16.6%)§
Embryo-derived	385 (82.8)[b]	231 (85.3)	- 92	90 (39.0)[b] 36 (39.0)[b]	72 15	14/27 (51.8) 1/5 (20.0)	4 (5.6%) 0

* As assessed 1 h after fusion by examination on a dissecting microscope. Superscripts a or b within a column indicate a significant difference between donor cell types in the efficiency of fusion ($P < 0.001$) or the proportion of embryos that developed to morula or blastocyst ($P < 0.001$).
† It was not practicable to transfer all morulae/blastocysts.
‡ As a proportion of morulae or blastocysts transferred. Not all recipients were perfectly synchronized.
§ This lamb died within a few minutes of birth.

Bringing in the Clones…



Tetra – diabetes research?
And other human diseases



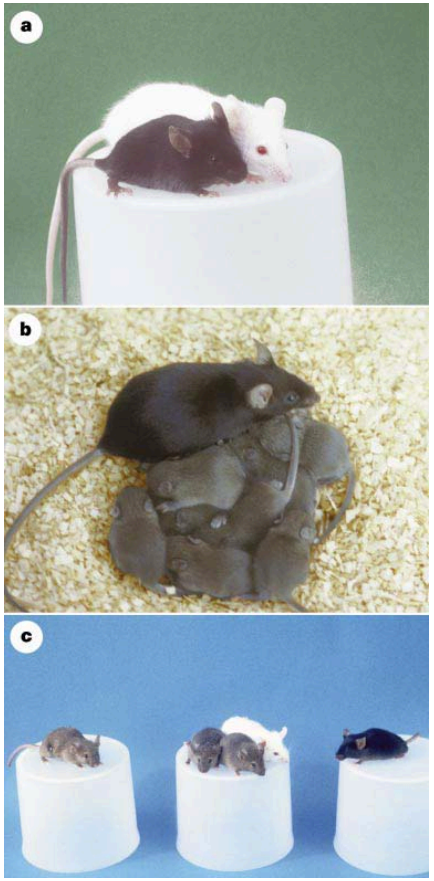
Cloning pet animals…

To clone????

On the brink of extinction

Mules are sterile – unless cloned

Cumulina: the first mouse clone



First cloned mouse (Cumulina) from a cumulus cell, and she herself produced progeny (Wakayama et al., 1998)

Teruhiko Wakayama

Transferred nuclei were reprogrammed to totipotency i.e. ability to form not only all of the cells of the adult organism (as is the case for pluripotency) but also extraembryonic tissues including the trophectoderm of the placenta.

Efficiency was low, however.

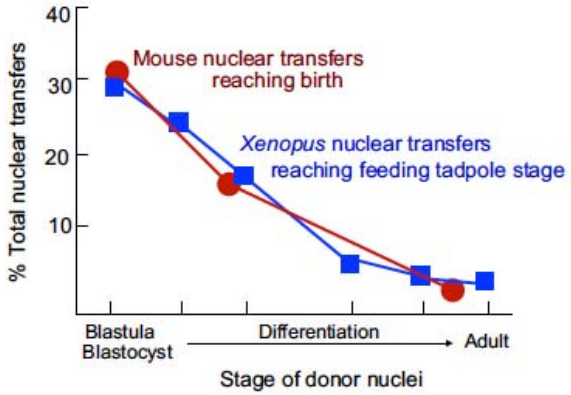


Fig. 8. Survival of nuclear transplant embryos in *Xenopus* and mouse. *Xenopus* data taken from Gurdon (Gurdon, 1962) and mouse data from Wakayama et al. (Wakayama et al., 1998).

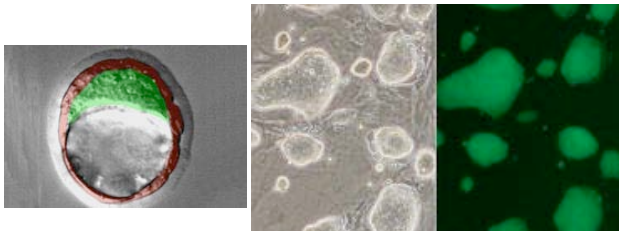
COLLÈGE DE FRANCE 1530

Mammalian Embryonic Stem cells

• 1950s-60s – Teratocarcinomas (Stevens and Little 1956) can give rise to embryonic carconoma (EC) cell lines that are pluripotent – can form all 3 germ layers (Finch and Ephrussi, 1967; Kleismith and Pierce, 1964) & contribute to the soma once transferred into normal embryos (Brinster, 1974).

• 1981– Derivation of embryonic stem (ES) cells from mouse blastocysts (Evans and Kaufman1981; Martin, 1981)

• 1992 - and embryonic germ (EG) cells from primordial germ cells (Matsui et al, 1992; Resnick et al, 1992)



Mario R. Capecchi, Sir Martin J. Evans, Oliver Smithies
(Nobel Prize, 2007)

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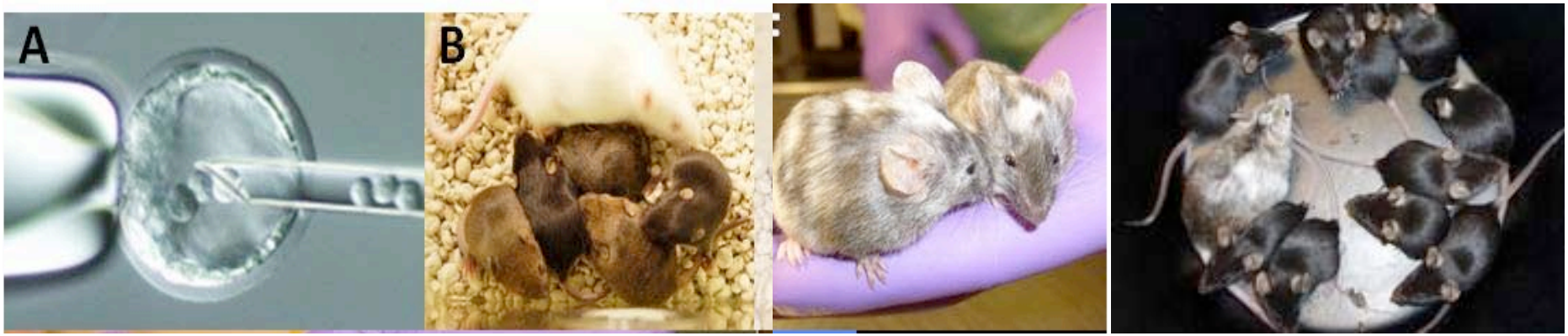
• 1998 – Derivation of human ES cells (Thomson et al, 1998)



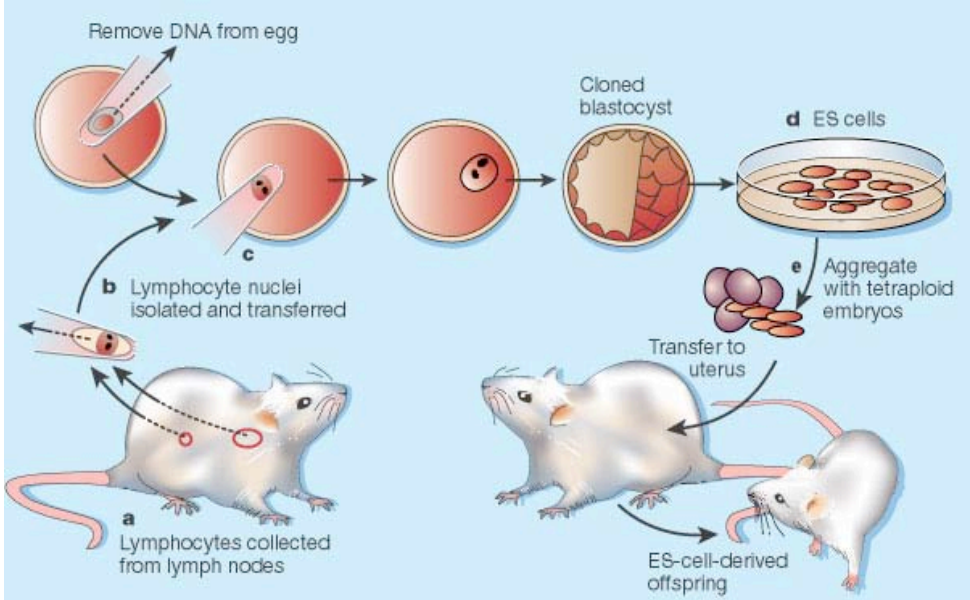
- Remain undifferentiated and immortal in culture: SELF RENEWAL
- Form chimeras, differentiate into ALL 3 germ layers and produce germ cells when reintroduced into blastocvsts: PLURIPOTENT

PROVIDE REMARKABLE TOOLS FOR GENETIC MANIPULATION
UNDERSTANDING OF PLURIPOTENCY
AND A MORE EFFICIENT MEANS OF CLONING IN MICE

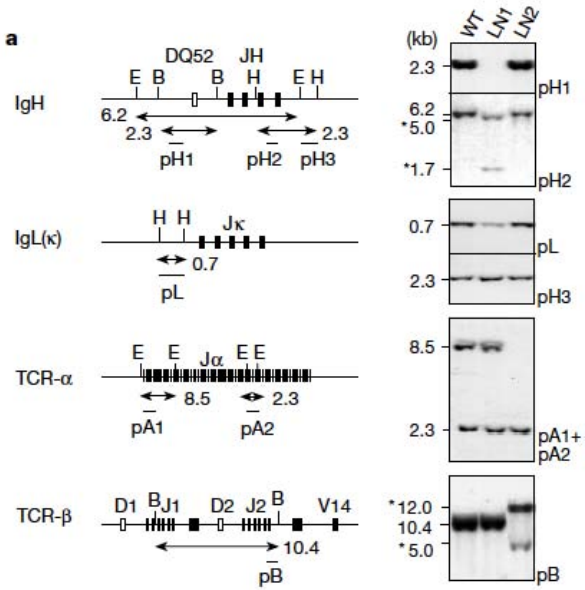
Monoclonal mice derived by SCNT: ultimate proof that fully differentiated cells can be used for successful cloning

Rudolph Jaenisch set out to prove that fully differentiated mature cells are indeed capable of creating ALL cell types in a mouse (rather than a rare stem or progenitor cell)

But, could NOT derive mice by **direct transfer** of blastocysts cloned from **mature lymphocytes** into recipient mothers .
Used an ES cell intermediate provided more efficient reprogramming



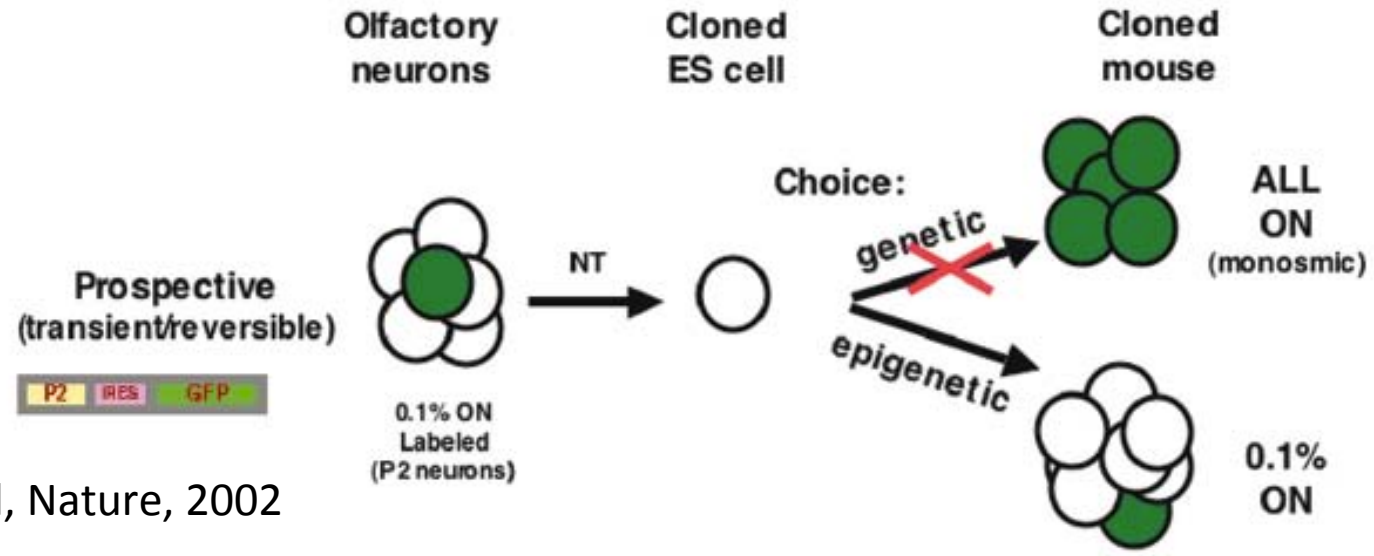
Hochedlinger and Jaenisch, 2002, Nature 415: 1035-1038



Mature T and B cells are rare examples of cells in which the genome sequence is altered as they mature. Using the genome of a T cell or B cell for cloning by nuclear transfer, the genomic rearrangement should be detected in all the cells of the clones

Mice cloned by SCNT from post-mitotic neurons

Jaenisch went on to perform similar experiments with Olfactory Neurons:
These are also mature cells but no genetic change in theory
Only single olfactory receptor genes our of many chosen to be expressed during development: purely epigenetic?



Eggan et al, Nature, 2002

The genome of a **post-mitotic, terminally differentiated neuron can re-enter the cell cycle and be reprogrammed to a state of totipotency after nuclear transfer**. Moreover, the pattern of odorant receptor gene expression and the organization of odorant receptor genes in cloned mice was indistinguishable from wild-type animals, indicating that irreversible changes to the DNA of olfactory neurons do not accompany receptor gene choice.

Lessons from Cloning ?

$$$ to clone a pet cat: was it worth it?

Genetic Savings & Clone,
Provided commercial gene banking and cloning services to pet owners
(closed down in 2006)

Rainbow

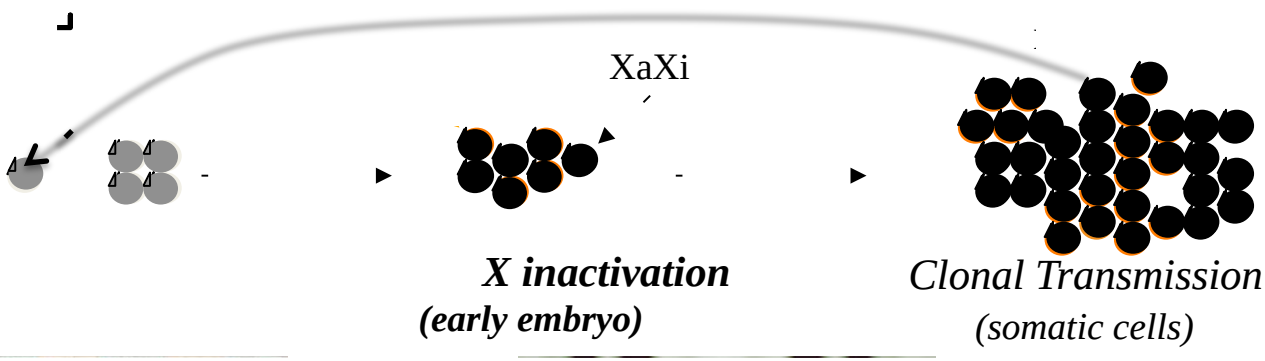
SCNT

CC (Carbon Copy)
b. Dec 22, 2001

No “rainbow” (orange and black) fur color for carbon copy!

Why are they not identical?

Clones are not identical…



- Even though two clones are genetically identical, they may not look or act the same way! Experimentally produced clones may show even more variation (inefficient reprogramming?)

In the case of Carbon Copy:
the somatic nucleus from Rainbow may not have been fully reprogrammed… as one X appears to be silent in all cells of Cc (the allele for orange fur) unlike her donor where either X is active (orange and black fur)!

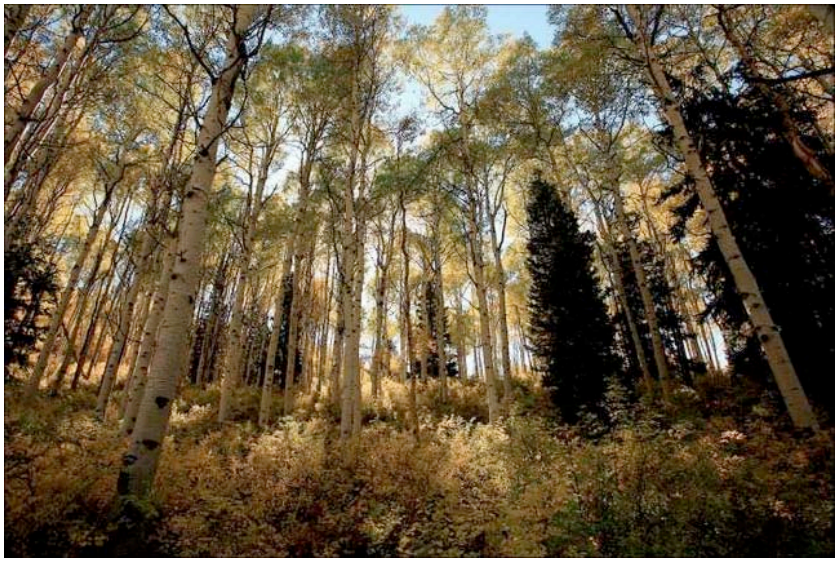
Cloning results in reduced fitness

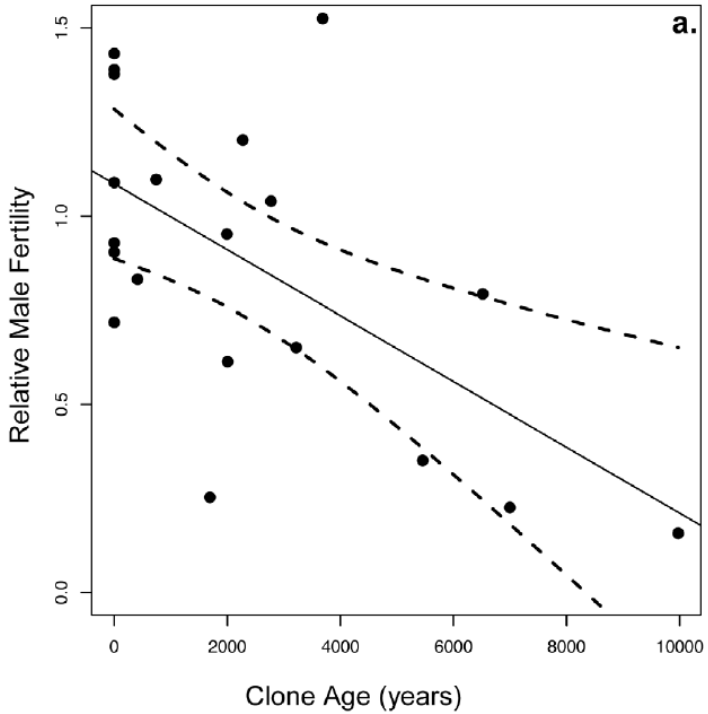
OPEN ACCESS Freely available online PLOS BIOLOGY

Aging in a Long-Lived Clonal Tree

Dilara Ally[1,2]*, Kermit Ritland[3], Sarah P. Otto[2]

1 Department of Biological Sciences, University of Idaho, Moscow, Idaho, United States of America, 2 Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada, 3 Department of Forest Sciences, University of British Columbia, Vancouver, British Columbia, Canada

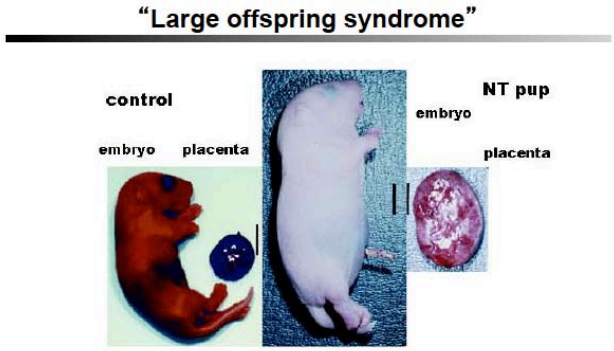




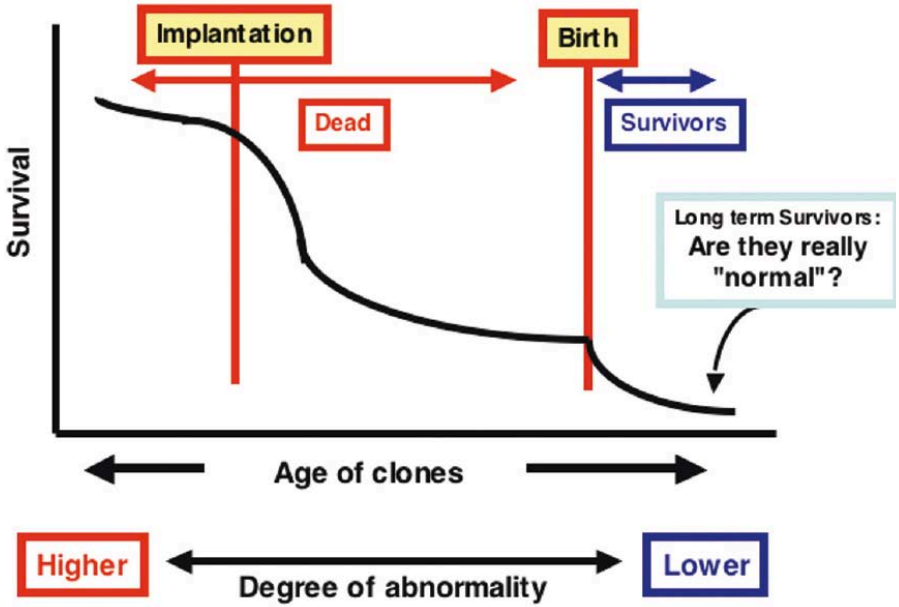
Disadvantages to cloning: mutations, or genetic errors, that gradually and steadily build up in the genetic material of the plants' cells. The longer an aspen depends on cloning to survive, the worse it is at sexual reproduction

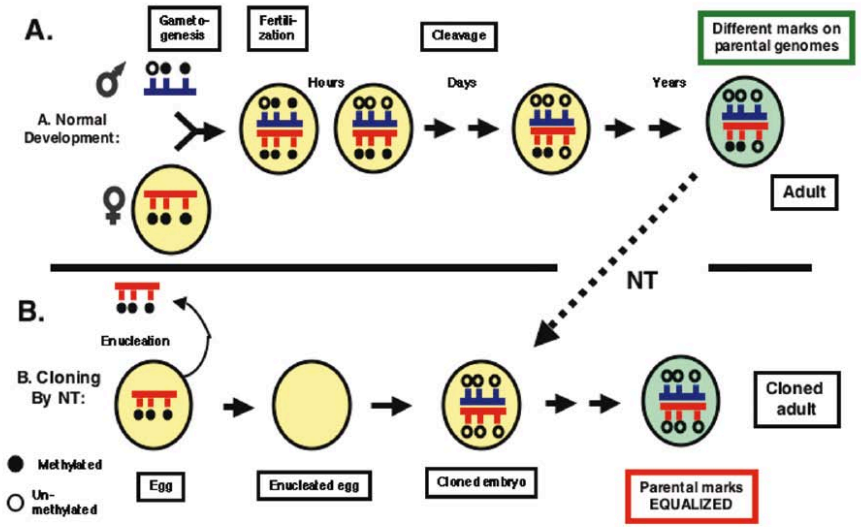
Cloning mammals is inefficient

- 277 oocytes for Dolly
- 613 oocytes => 5 mouse pups
- 1852 oocytes => 6 rabbits
- 72 oocytes => 5 pigs
- 496 oocytes => 24 cattle
- 188 oocytes => 1 kitten



Are the offspring of cloned animals normal?





COLLÈGE DE FRANCE 1530

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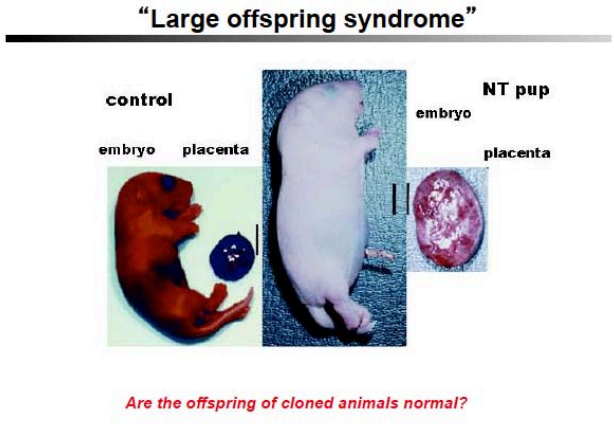
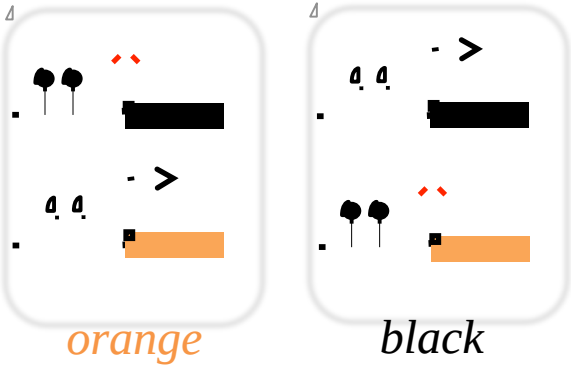
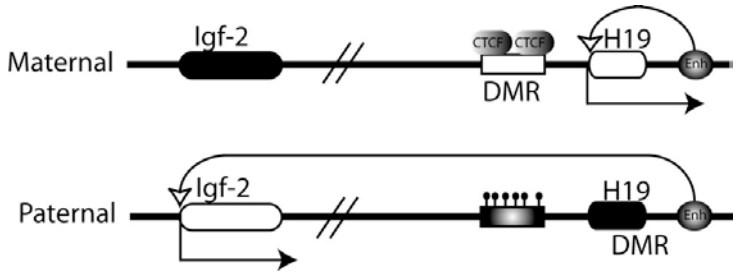


X inactivation



Imprinting



Epigenetic processes can be perturbed:

- Aberrant methylation at imprinted loci particularly in extraembryonic tissues
- Aberrant X-inactivation patterns

Cloning can result in pathologies

Table 1 **Summary of the pathologies described in cloned fetuses and offspring**

Species	Type of embryo manipulation	Embryo loss	Gestation length	Placental abnormalities	Fetal size	Respiratory/cardiovascular dysfunction	Organ dysplasia	Perinatal mortality	Post-natal development
Cow	Nuclear transfer	High	Prolonged	Common	Increases	Common	Common	Raised	Altered
	Other	High	Prolonged	Common	Increases	Occasional	Occasional	Raised	Altered
Sheep	Nuclear transfer	High	Prolonged	Common	Increases	Common	Common	Raised	Altered
	Other	High	Prolonged	Common	Increases	-	Occasional	Raised	Altered
Goat	Nuclear transfer	High	Prolonged	None observed	Normal	No	No	Normal	Normal
	Other	High	Normal	None observed	Normal	No	No	Normal	Normal
Pig	Nuclear transfer	High	Prolonged	None observed	Reductions	Occasional	Occasional	Raised	Altered
	Other	High	Normal	None observed	Reductions	No	No	Raised	Normal
Mouse	Nuclear transfer	High	Caesareans	Common	Altered	Common	-	Raised	Altered
	Other	High	-	-	Reduced	-	-	-	Altered

From : Wilmut, N. Beaujean, P. A. de Sousa, A. Dinnyes, T. J. King, L. A. Paterson, D. N. Wells‡ & L. E. Young (2002) Somatic cell nuclear transfer, Nature, 419.

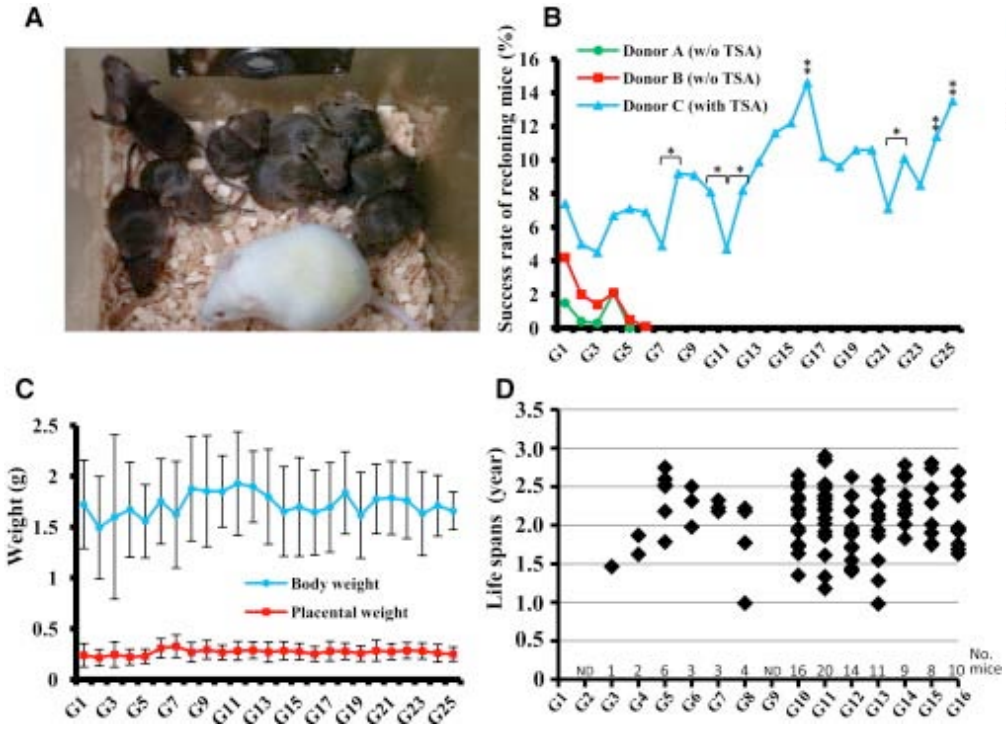
- Clones only from some donor cell types (eg cumulus cells for mice)?
- Developmental and physiological abnormalities in placentas?
- although not inherited (Eggan and Jaenisch 2002)
- => due to **failure to reprogram the epigenome** rather than to genetic abnormalities?
- Imprinting and X-inactivation errors in cloned embryos?
- Passage through ES cells can improve efficiency (corrects some epigenetic abnormalities?)
- Premature ageing ? Shorter telomeres? Dolly: short telomeres; Cloned cows:longer telomeres telomeres…=> *likely to depend on species,and on balance between telomere shortening and elongation*

Improved technique and epigenetic drug treatment reduces abnormalities and results in normal life span?

Successful Serial Recloning in the Mouse over Multiple Generations

Sayaka Wakayama,[1] Takashi Kohda,[2] Haruko Obokata,[1,3] Mikiko Tokoro,[1,4] Chong Li,[1,5] Yukari Terashita,[1,6] Eiji Mizutani,[1,7] Van Thuan Nguyen,[1,8] Satoshi Kishigami,[1,9] Fumitoshi Ishino,[2] and Teruhiko Wakayama[1,7,*]

Wakayam et al, Cell Stem Cell, 2013



Telomere length is reset during early mammalian embryogenesis

Sonja Schaetzlein*, Andrea Lucas-Hahn†, Erika Lemme†, Wilfried A. Kues†, Martina Dorsch‡, Michael P. M
Heiner Niemann†§, and K. Lenhard Rudolph*§

Schaetzlein et al, PNAS, 2004

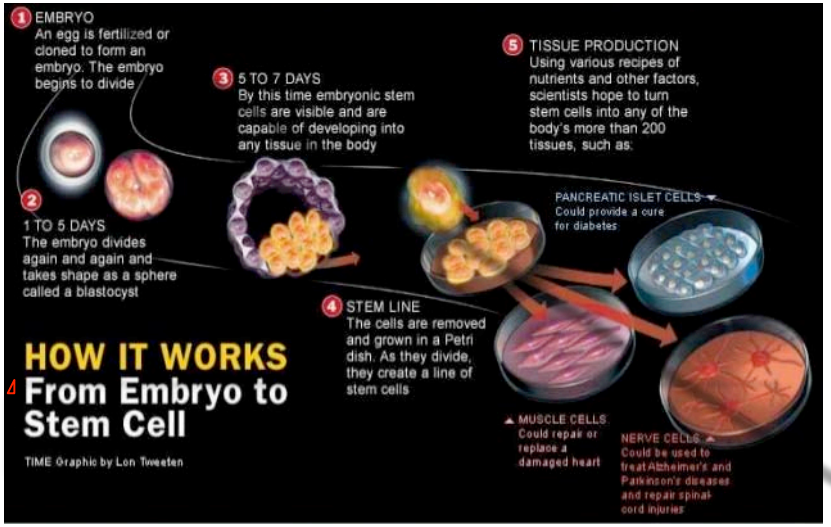
RNAi-mediated knockdown of *Xist* can rescue the impaired postimplantation development of cloned mouse embryos

Shogo Matoba[a,1], Kimiko Inoue[a,b,1,2], Takashi Kohda[c,1], Michihiko Sugimoto[a,1], Eiji Mizutani[a], Narumi Ogonuki[a], Toshinobu Nakamura[d,3], Kuniya Abe[a], Toru Nakano[d], Fumitoshi Ishino[c,2], and Atsuo Ogura[a,b,e,2]

Matoba et al, PNAS, 2011

Using TSA to treat cells, generation of healthy mouse clones that live a normal lifespan and can be sequentially cloned indefinitely (> 25 generations).

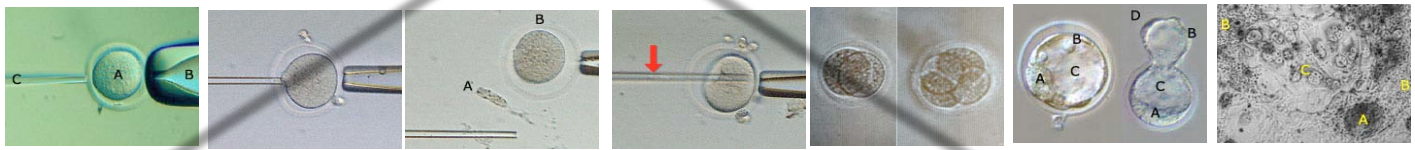
Therapeutic Cloning?

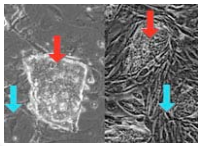


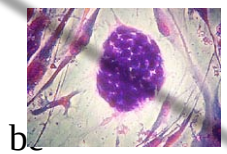
Derivation of human ES cells by Jamie Thomson in 1998 and more recent work by Noggle et al 2011 showing that it is, in principle, possible to reprogram human somatic cells up to the blastocyst stage at least…

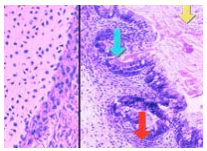
Led to the hope that patient specific pluripotent ESCs could be obtained by SCNT of for eg a skin cell nucleus reprogrammed in a human egg, which could then be differentiated to the cell type that was defective in the patient: "**Therapeutic Cloning**"
For organ transplant replacement, skin grafts, treatment of degenerative diseases (eg Parkinson's) , spinal cord repair or leukemia

Therapeutic cloning was shown to work in animals, but raised **serious ethical issues in humans….**









Proposed to avoid using human embryos – but use activated human eggs instead (parthenogenotes)??

- Parthenogenesis: an egg that activates spontaneously on its own. This is relatively common in women. Eggs activate and often form cysts or benign tumors in the ovary.
- activated eggs begin to divide, form embryos at early stages, blastocysts with stem cells
- Use for therapeutic purposes? Eg young woman with Type 1 diabetes, could donate her eggs, to be activated artificially in the laboratory without being fertilized. Develop to the blastocyst stage in vitro,derive embryonic stem cells and use them to treat Type 1 diabetes?

http://www.pbs.org/wgbh/nova/sciencenow/3209/04-clon-nf.html

Therapeutic Cloning has Serious Ethical Issues

Great **hope** and **hype**, but also great **fear**:

Ethical Issues:

- Moral values, legal issues and religious considerations
- Manipulation of human germ cells (eggs)
- Impact of on women (extensive hormonal treatments, repeated surgery) to gather enough eggs (could use other species cows/pigs – but raises other issues!)
- Destruction of embryo
- Killing of life (cf debate on whether en embryo is a human being prior to implantation)

Practical limitations:

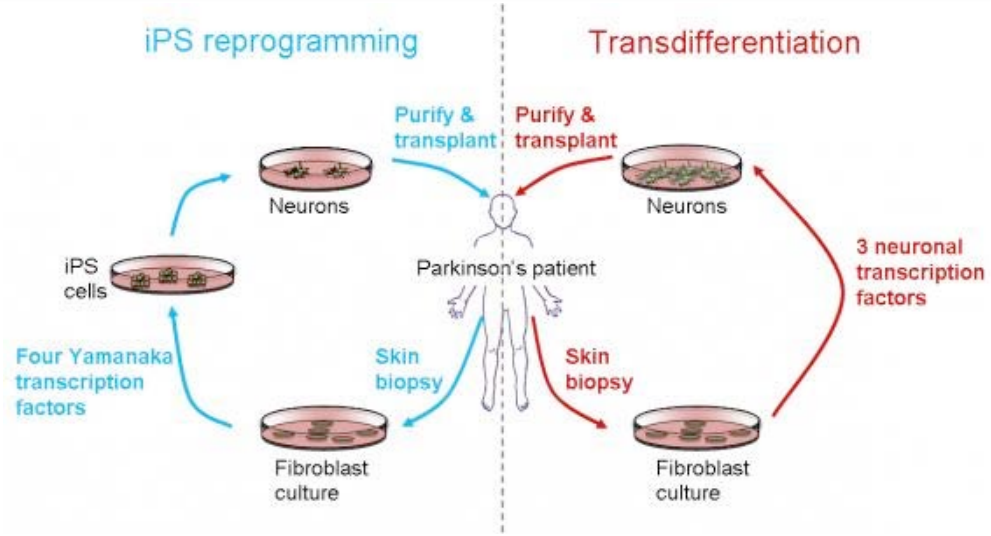
- Sufficient numbers of human eggs could never be obtained
- Some immune rejection may occur:
- Mitochondrial DNA only comes from the egg – not the donor

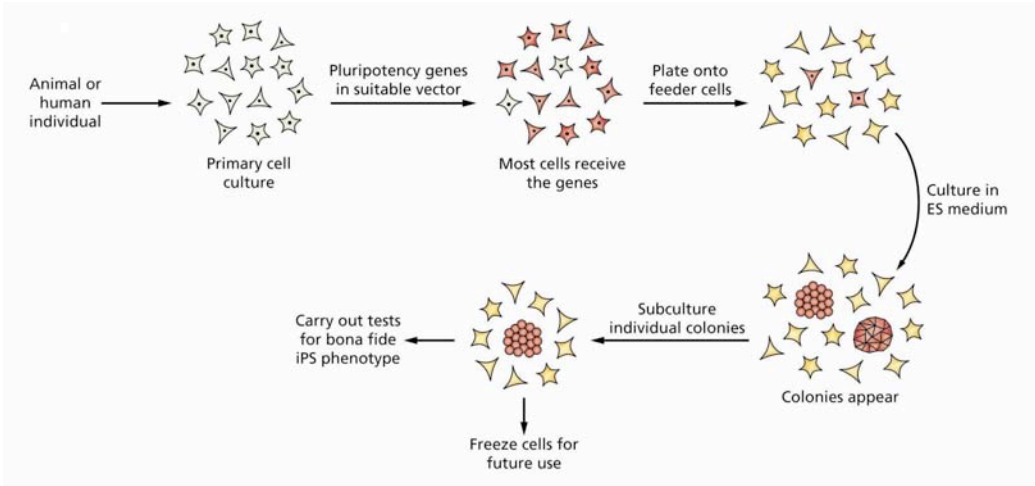
Therapeutic Cloning has Serious Ethical Issues

The therapeutic hopes raised by NT, but the realisation that this avenue should NOT be explored, led to increased intensive efforts to develop alternatives – and to understand how the egg accomplishes the resetting of the somatic genome to a pluripotent state.

In this way the need to use human eggs in the process would be circumvented…

Several alternatives to Therapeutic Cloning may now be possible

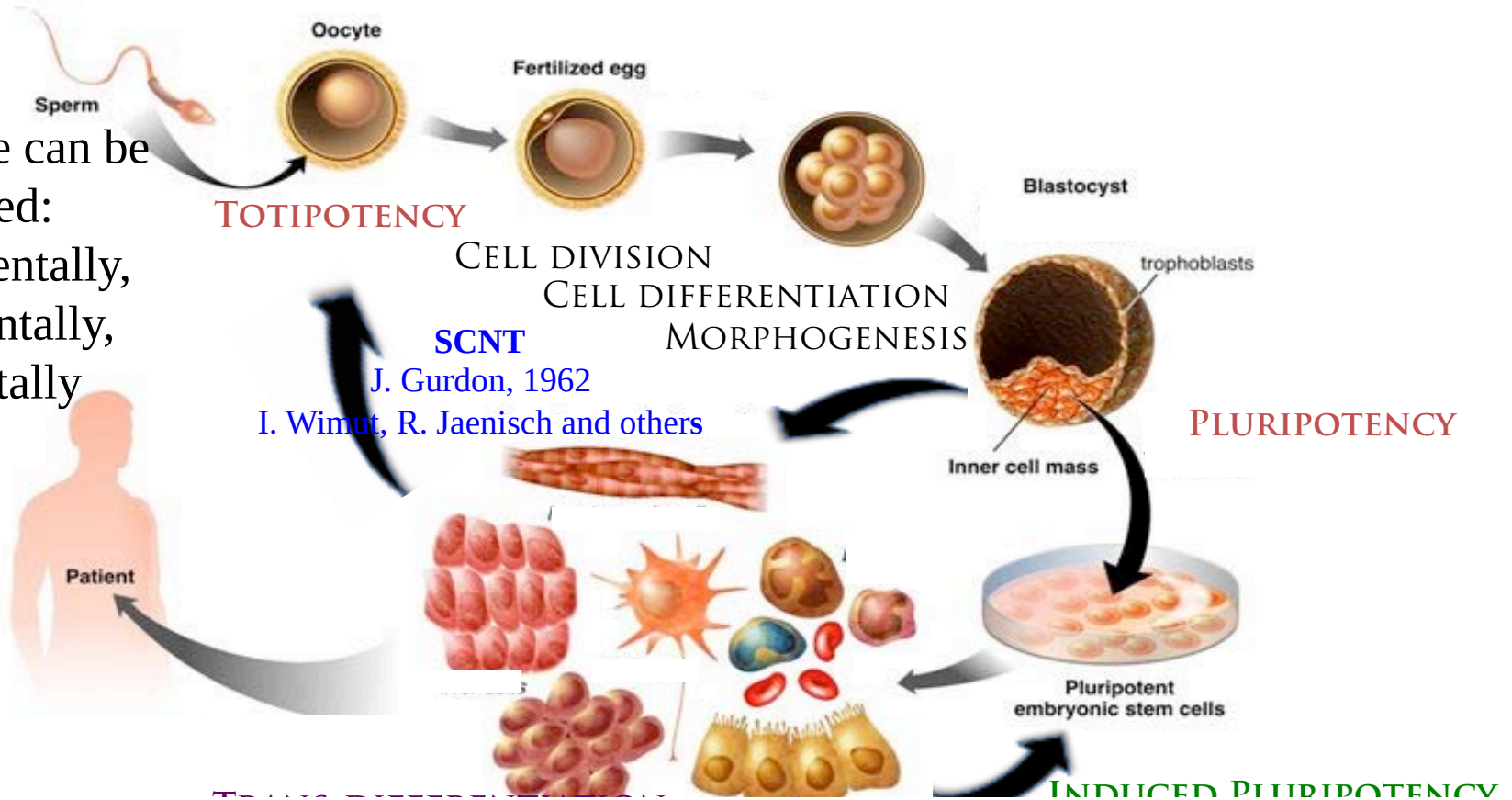




Cours III et V

All cells have the capacity to form a whole organism – through differential gene expression

A cell's fate can be reversed: developmentally, experimentally, accidentally

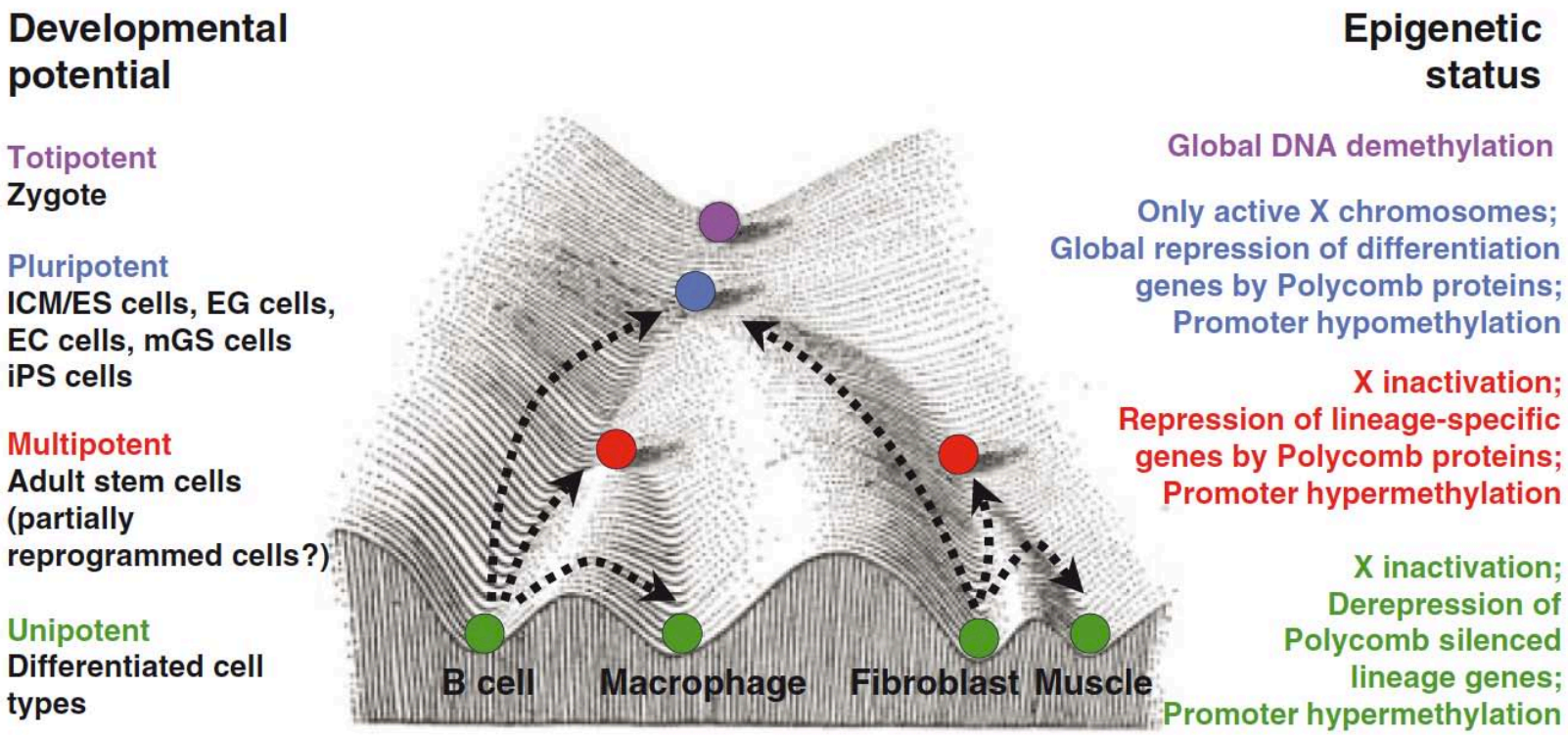


Differentiated cells are NOT irreversibly committed to their fate
but can be REPROGRAMMED and/or REPURPOSED

⇒ Developmental restrictions imposed on the genome during differentiation must be due to reversible epigenetic modifications rather than to permanent genetic changes

⇒ Epigenetic changes allow the **maintenance of cell identity** but can be overriden by TFs, as well as by active and passive loss

Reprogramming:
How to climb back up the Waddington landscape….



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