# From Dolly to disease models in a dish




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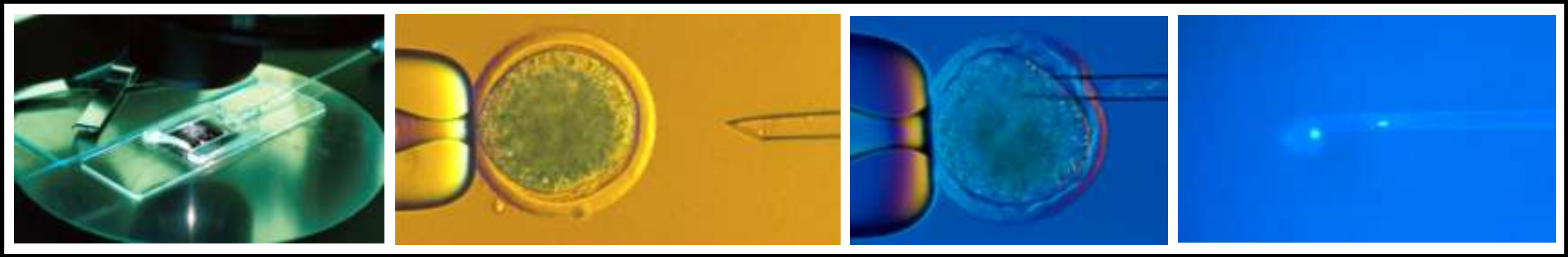
# Cloning, stem cells and regenerative medicine

- Understanding the control of development
- Biomedical products
- Animal models of human diseases
- Patient specific cell lines
- Animal stem cell lines



# Fundamental research questions

How does the single cell of an embryo form all of the tissues of an adult?

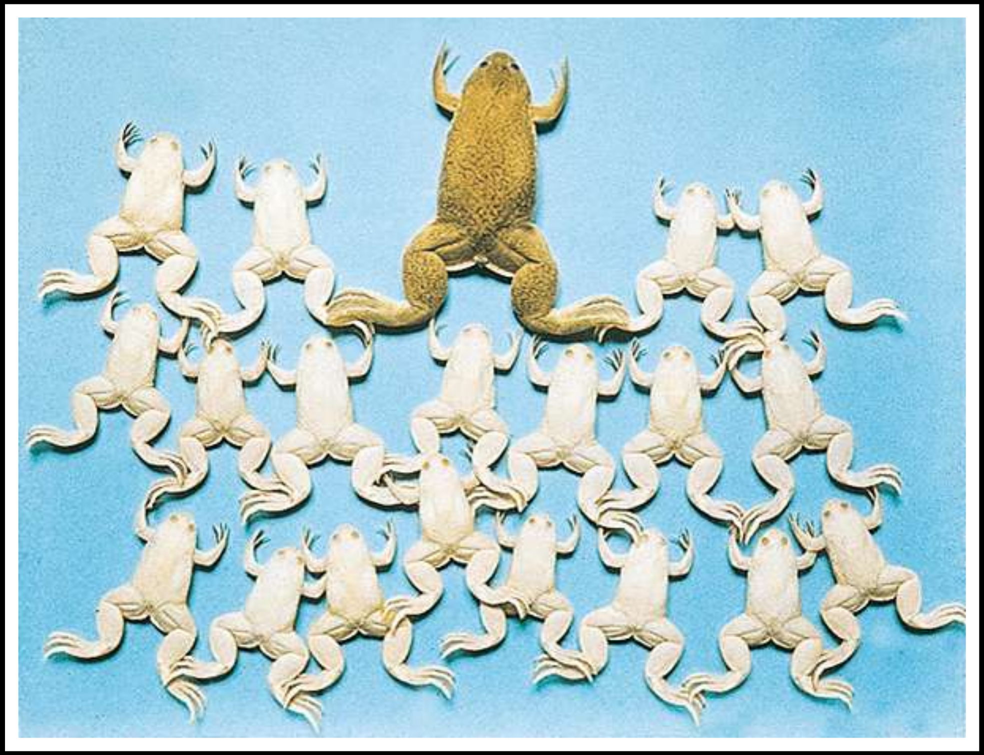
- Is genetic information lost as development takes place?

# 1952 - 1962 Nuclear transfer in frogs

**Briggs and King 1952; Gurdon 1962**

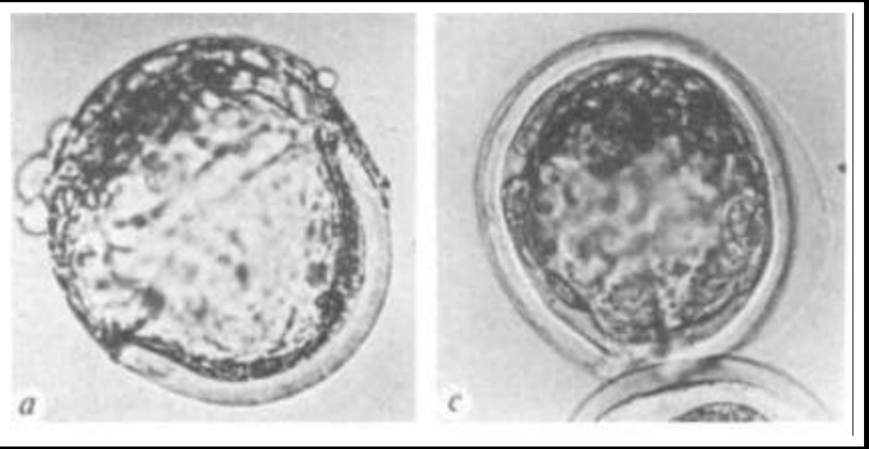
- Donor nucleus from cells of albino embryos
- Egg from dark green frog
- Many albino frogs

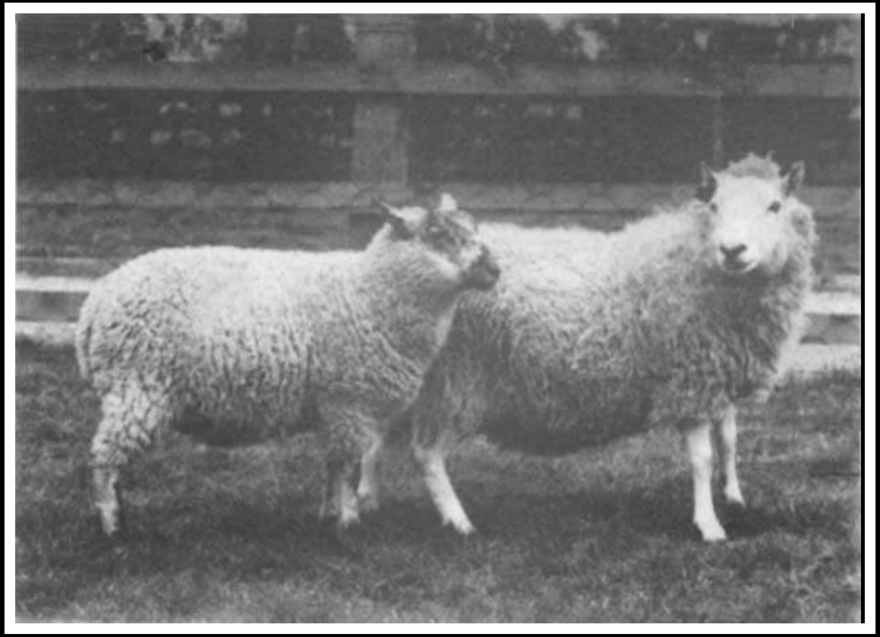
- Efficiency drops with nuclei from later stages
- There have been no adult clones of adult frogs



# 1986 First nuclear transfer in mammals

**Willadsen Nature 1986**



- **Two embryos derived by nuclear transfer**
- **Cloned cross-bred lamb (on the left)**
- **Clones only from early embryos**

# 1997 First clone of an adult animal

- Better co-ordination of donor and recipient cells
- Donor cell from mammary gland of Finn Dorset ewe early in 3rd trimester pregnancy
- Egg without nucleus from Blackface ewe
- Surrogate mother Blackface ewe
- Death age 6 after virally induced lung cancer

# 1996 – 2006 10 years of somatic cell cloning

SHEEP 1996

CATTLE 1998

MICE 1998

GOATS 1999

PIGS 2000

GAUR 2000

MOUFLON 2001

CAT 2002

RABBIT 2002

BANTENG 2003

RAT 2003

MULE 2003

DEER 2003

HORSE 2003

DOG 2005

FERRET 2006

# Fundamental research questions

**How does the single cell of an embryo form all of the tissues of an adult?**

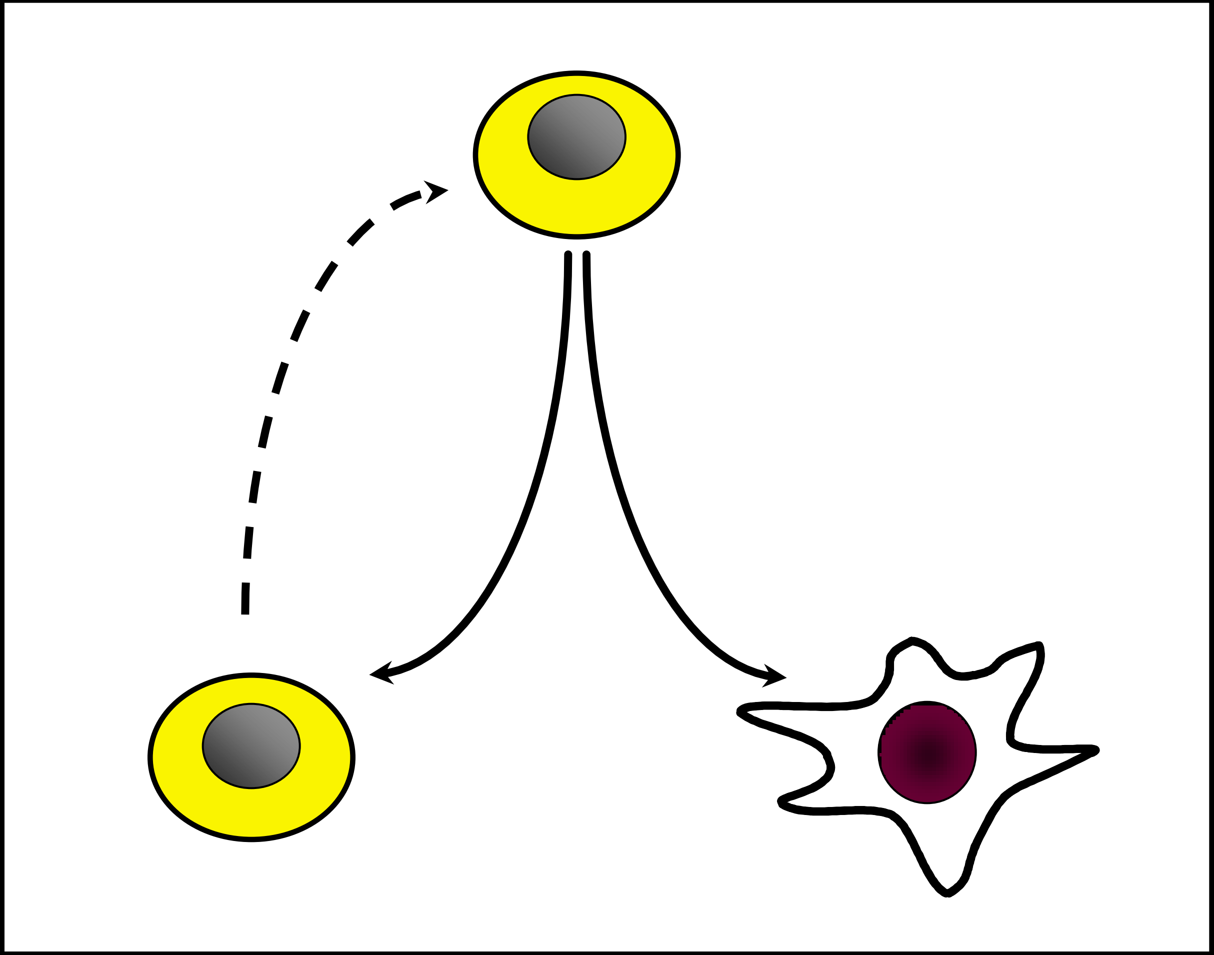
- **Is genetic information lost as development takes place?**

**Apparently not**

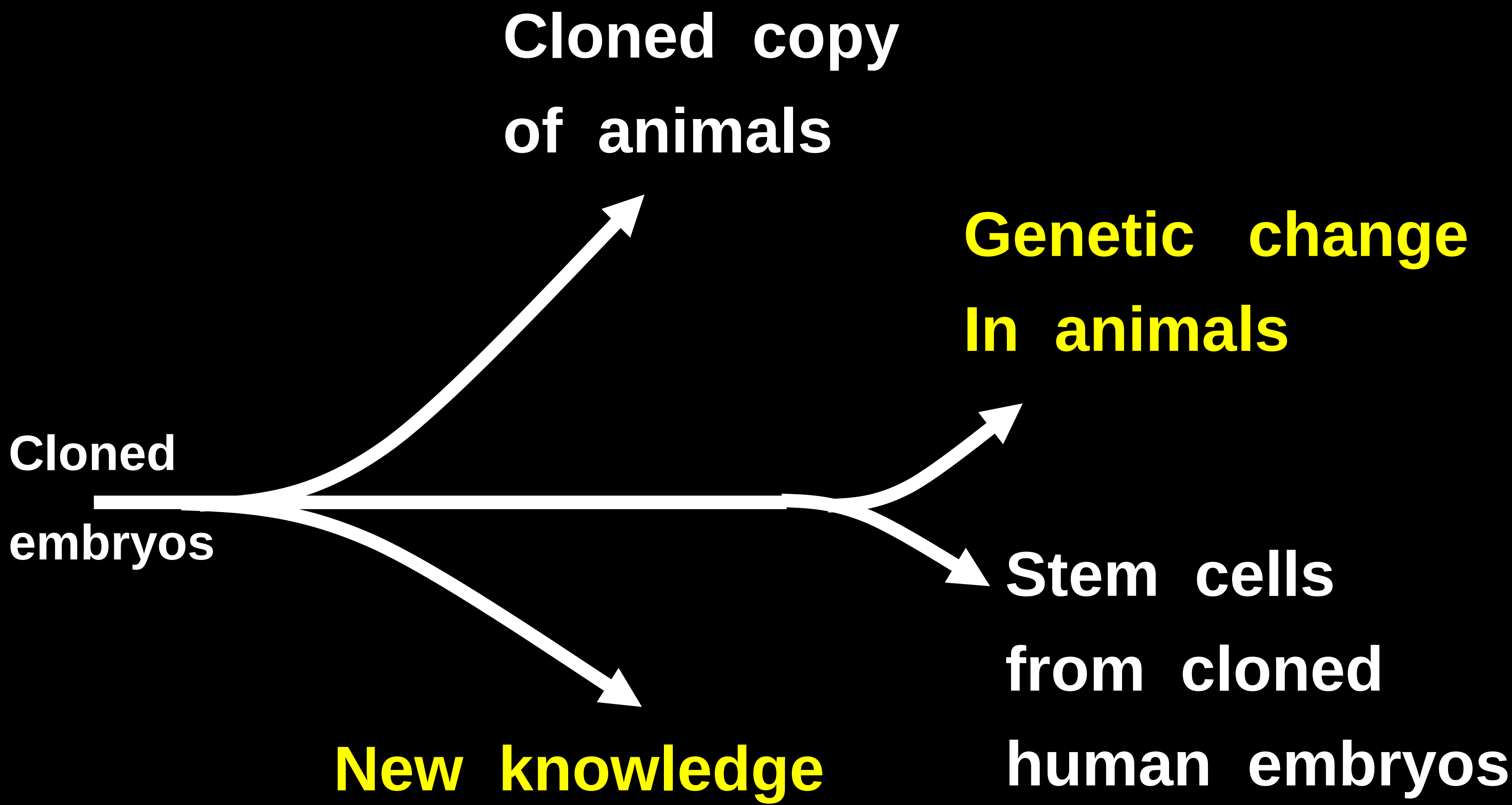
- **Can cell function be modified?**

- **Yes, but inefficiently**

# Biology of stem cells



# Opportunity through cloning



# Genetic modification of animals

- Xenotransplantation
- Production of human proteins
- Animal Disease Models

## Production of human antibodies from cattle

**Hematech : Jim Robl, Phillipe Collas**

- Created artificial chromosome
  - Carrying human heavy and light chain locii
- Introduced into fetal fibroblasts
- Cloned to make embryos - transferred to recipients
- Calves born - expressed human genes
- Deleted bovine immunoglobulin genes and PrP
- Antibodies produced against anthrax

# Disease models

## Pig model of cystic fibrosis

### Rogers et al 2008 Science

Disruption of both CFTR alleles

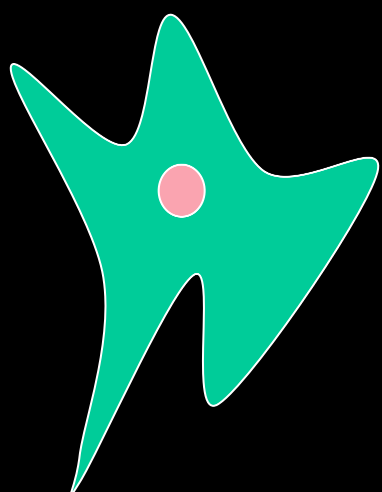
Defective chloride transport.
Developed meconium ileus,
Exocrine pancreatic destruction
Focal biliary cirrhosis,

Abnormalities seen in newborn patients with CF.

# Reprogramming
## by expression of defined genes – iPS cells

Shinya Yamanaka

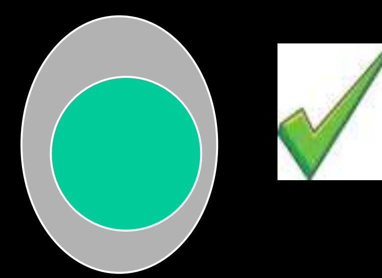
4 factors can induce pluripotency



Somatic cell
fibroblast

4 factors

Oct4
Sox2
Klf4
c-Myc



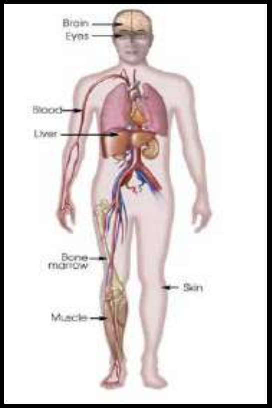
Induced pluripotent cell

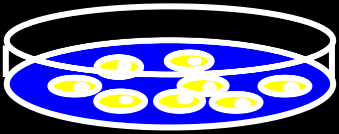
# Induced pluripotent stem cells

iPS cells

Selected genetics

Long life in culture
Form all tissues ?

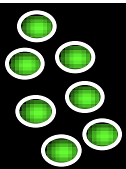


Direct reprogramming

Blood

ß cells

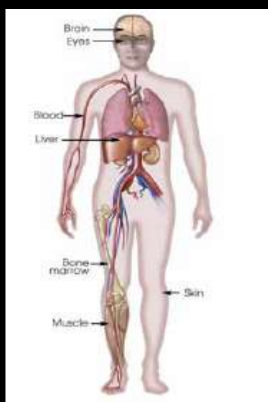


Heart

Nerve

iPS cells also
form teratomas

# Directed fate conversion




Skin

Blood

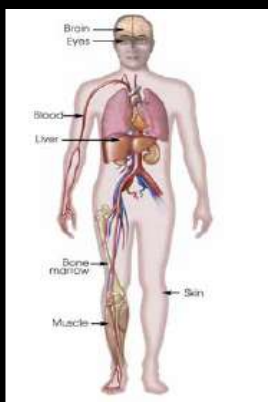
Heart

Nerve

Selected genetics

Form all tissues ?

# Directed fate conversion




Skin

Blood

Heart

Nerve

Progenitors or terminally differentiated cells ?

Progenitors do not form teratomas

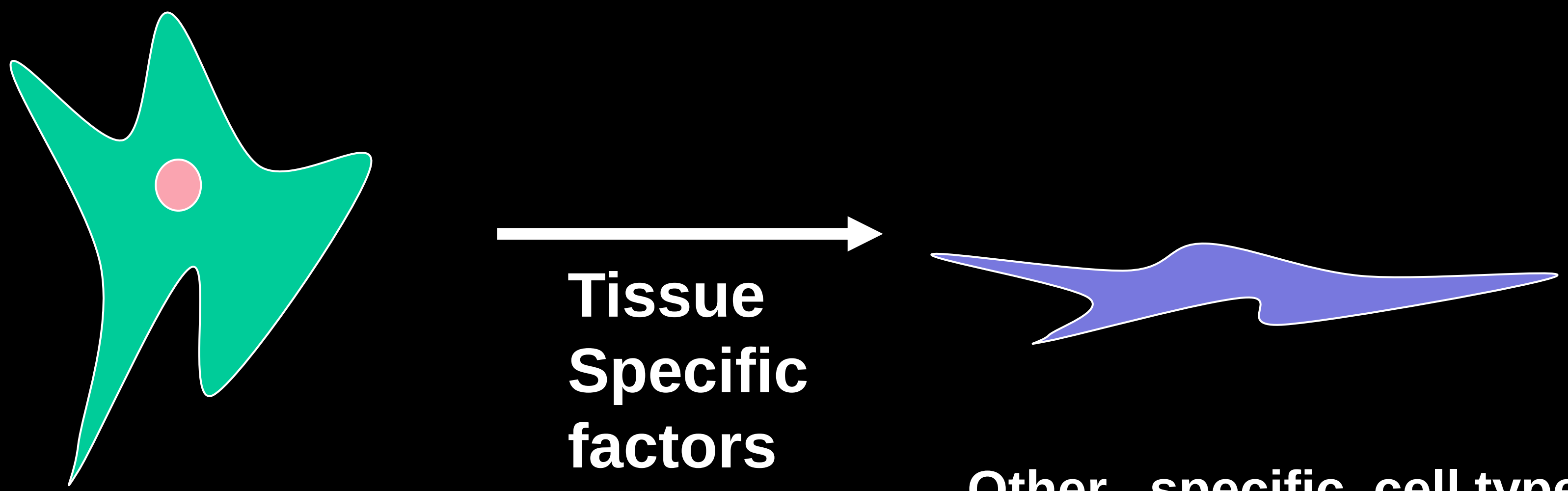
But might integrate inappropriately

Selected genetics

Form all tissues ?

# What are the limits to reprogramming ?

**Changes can be made from one tissue to another**



# Expectations for the future

- Fate change of cells will be routine

- Procedures will be simplified
- Accuracy of change improved

- New sources of cells offer a great deal
  - Used for research
  - Library established for cell therapy
  - Opportunity for gene therapy ??

**Beginning of a new era**

# Potential uses of stem cells

**Stem cell derivatives used to replace cells that have died or no longer function normally**

# Can stem cells identify new drugs for specific diseases ?

- Many inherited diseases have no treatment
- Produce cells identical to patient
- Can study cause of disease

# Can stem cells identify new drugs for specific diseases ?

- Many inherited diseases have no treatment
- Produce cells identical to patient
- Can study cause of disease

- Use these cells to look for new drugs
- These then tested in animals before use

**Mechanisms in sporadic and inherited cases will sometimes be the same**

# Modelling familial dysautonomia

**Lee et al, Nature Sept 2009**

- **Rare but fatal disease caused by death of specific nerves,**

- **Point mutation in a gene (IKBKAP8)**
- **Involved in function of other genes**

- **Tissue-specific defect ?**
- **Varying reduction in level of normal IKAP protein**

# Familial dysautonomia

Cells derived from

patients and controls

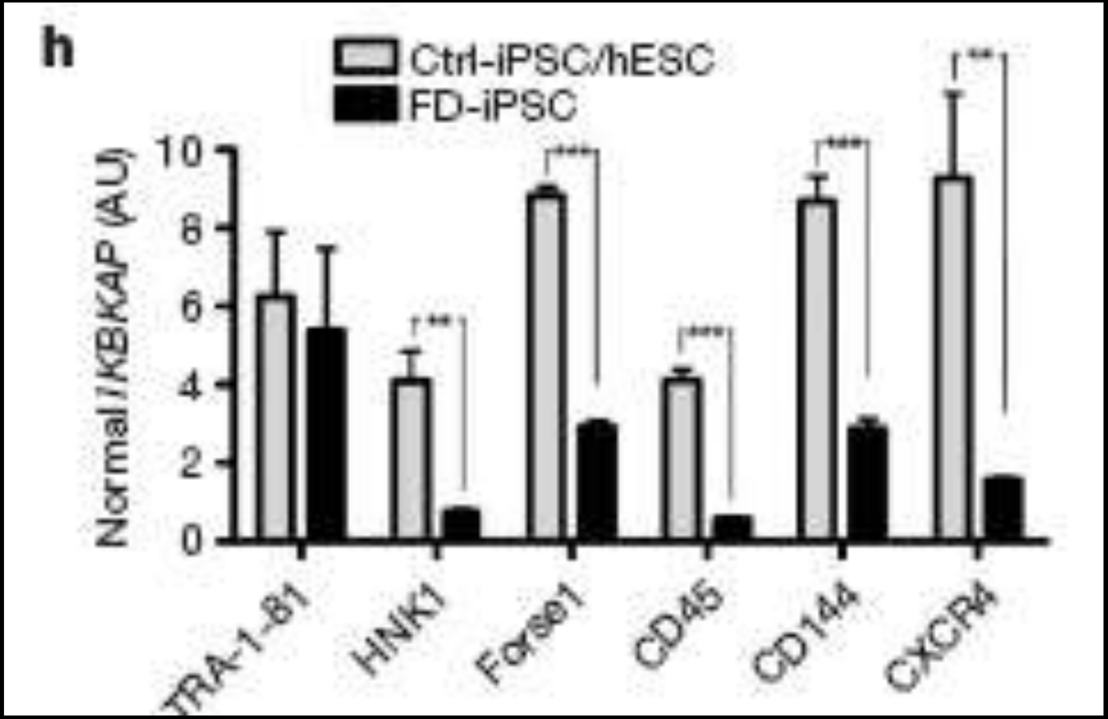
Hnk1 Neural crest

Forse1 Neural rosette

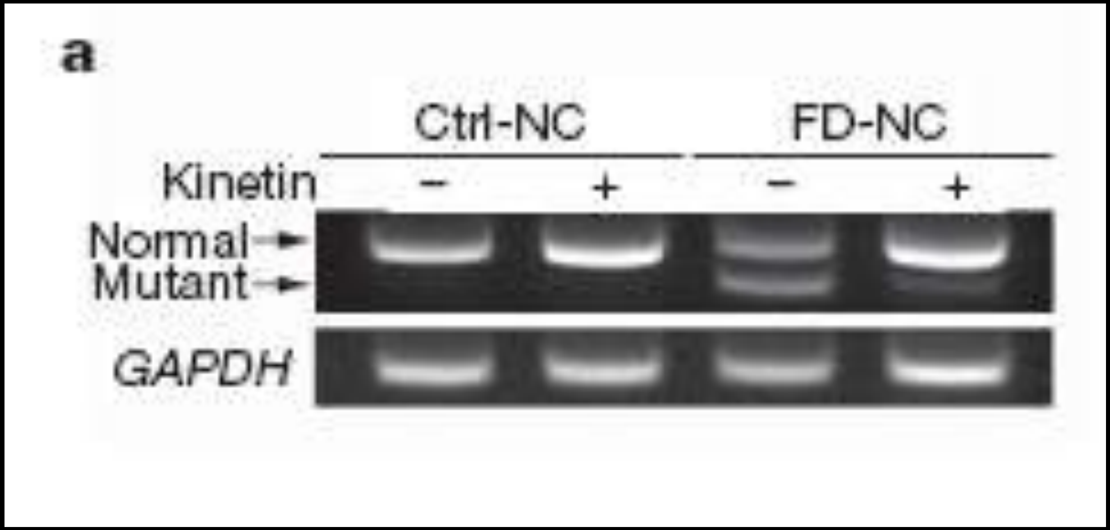
CD45 HSC

CD144 Endothelial cells

CXCR4 Endoderm



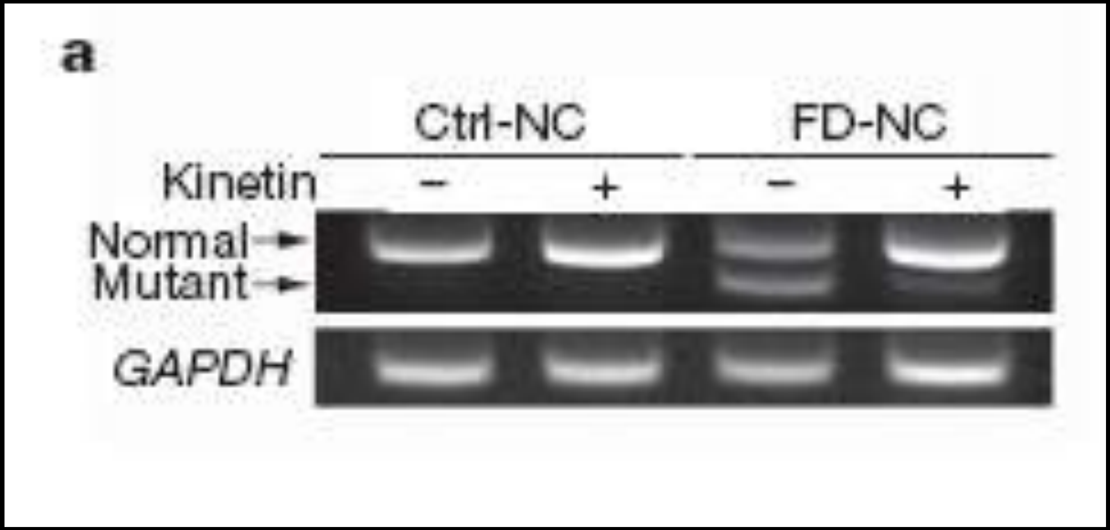
# Kinetin as a candidate therapeutic
**Lee et al 2009**




**Inclusion of kinetin reduced the production of shorter transcript in neural crest cells**

# Kinetin as a candidate therapeutic

**Lee et al 2009**




**Inclusion of kinetin reduced the production of shorter transcript in neural crest cells**

- Differences in gene function mimiced in iPS cell derivatives
- Tissue specificity of the effect unexpected
- First candidate drug identified
- May provide effective test system

# Value of iPS cells in research

- iPS cells offer important advantages
  - No need to know causative mutation
  - Can assess effect of other genetic variation
  - Readily obtained

- Inherited cases will sometimes inform sporadic cases

- Motor neuron disease, Parkinson's , cancers
- Psychiatric diseases, inherited learning difficulty


**There are a very large number of inherited diseases**

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