KING'S College LONDON

INSTITUTE OF PSYCHIATRY,
PSYCHOLOGY & NEUROSCIENCE

Module:
Biological Foundations of Mental Health

Week 2:
Building blocks of the brain

Prof Jack Price

Topic 3:

Exploring mental health using stem cells

Part 1 of 3

Part 1

Topic list

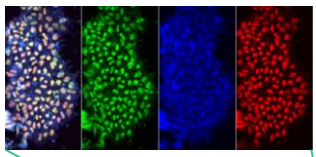
This week, we will be looking at the following topics:

- Topic 1: Neuron-glial interactions and mental health
- Topic 2: From embryonic neural progenitor cells (NPCs) to adult hippocampal neurogenesis (AHN)
- ***Topic 3: Exploring mental health using stem cells***

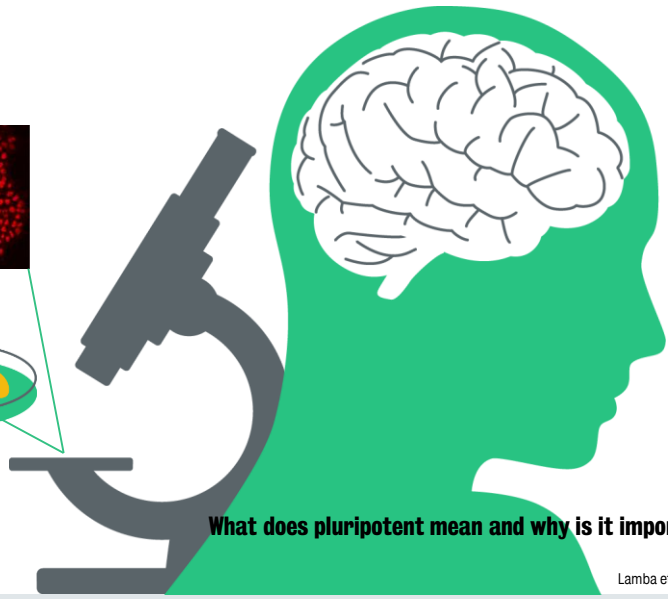
Click **Next** to continue

What are iPSCs?

iPSCs
Induced **Pluripotent** Stem Cells



iPSCs grown in tissue culture



What does pluripotent mean and why is it important?

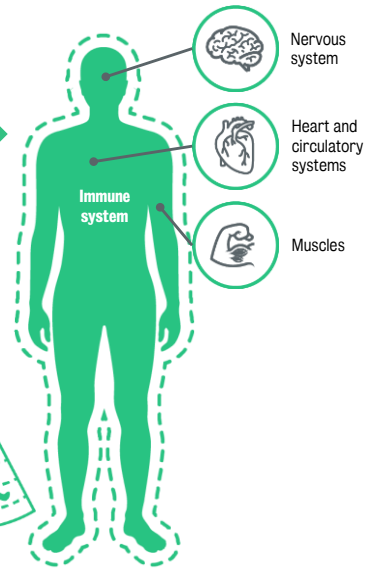
Lamba et al. (2010)

Understanding pluripotentiality

Pluripotentiality

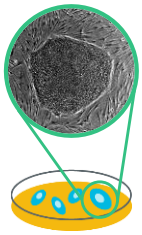
Foetal and adult bodies

Pluripotent cells



This inner cell mass cells are incredibly **ephemeral**.

The property of pluripotency only lasts a few days and never reappears during the entire life cycle.



ES (embryonic stem) cells

The pluripotent property in these cells is **permanent**.

Similarly to the inner cell mass cells, these can give rise to all the different cell types that make up the body.

Pluripotency: John Gurdon

What's the biological basis for pluripotency?

The experiment that gave us the first clue about this question was done by John Gurdon.

(Sir John Bertrand Gurdon, British biologist)

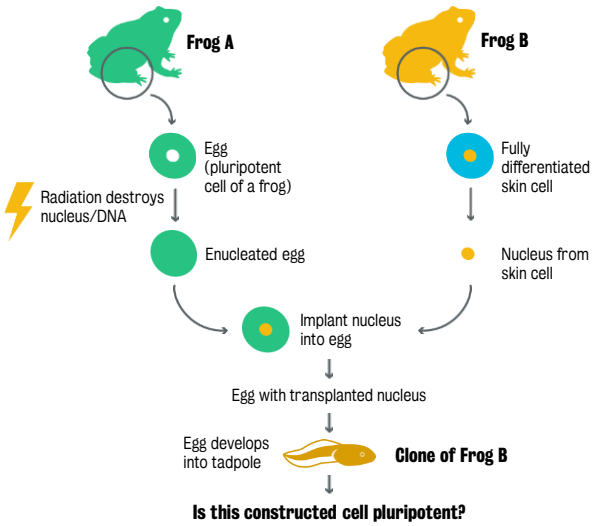
Conclusions:

The nucleus, even though it was from a fully differentiated cell, had all the information required to generate an entire organism.

There must be factors in the enucleated egg that, once combined, tell the nucleus that it has to start behaving as a pluripotent cell.

This informs us of the existence of factors in the cytoplasm of pluripotent cells that dictate pluripotency.

Experiment showing that pluripotency resides in the cell cytoplasm



Pluripotency: Shinya Yamanaka

The key finding from Gurdon's experiment was the **existence of factors in the cytoplasm of pluripotent cells that capture the essence of pluripotency**.

But what are those factors?

Work published in 2006 (and in 2007) by Shinya Yamanaka helped to provide understanding to this question.

(Dr Shinya Yamanaka, Japanese Nobel Prize-winning Researcher)

Yamanaka suggested that the factors must be **gene products** themselves or, in order words, **proteins**.

He proposed a list of 24 factors that seemed to be associated with pluripotency.

24 pluripotency factors

Oct3/4	Dnmt3l
Sox2	Ecat8
Klf4	Gdf3
c-Myc	Sox15
Ecat1	Fthl17
Dppa2	Sall4
Dppa3	Rex1
Dppa4	Utf1
Dppa5	Tcl1
Fbx15	β-catenin
Nanog	Stat3
ERas	Grb2

Key questions:

How could Yamanaka show that these 24 factors do include the ones that give rise to pluripotency?

How could he differentiate between the really important factors and the less important ones?

Discovering iPS cells (1)

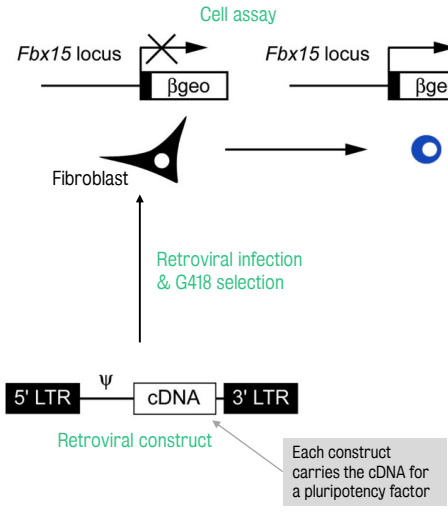
Step 1:
Have an assay: a way to recognize if he'd produced pluripotency in cells that weren't pluripotent.

Method:
Use of skin fibroblasts.

Challenge:
To make the skin fibroblasts become pluripotent and, if they did, to recognise that this has happened.

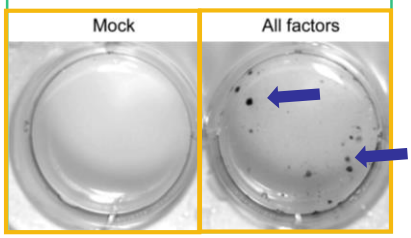
Conclusions:
Among those 24 factors are the ones that are capable of inducing pluripotency in otherwise non-pluripotent fibroblasts.

Assay for pluripotency



Fbx15:
Pluripotent cells always seem to have this gene active

Transfection of fibroblasts with the 24 pluripotency factors



Takahashi & Yamanaka (2006)

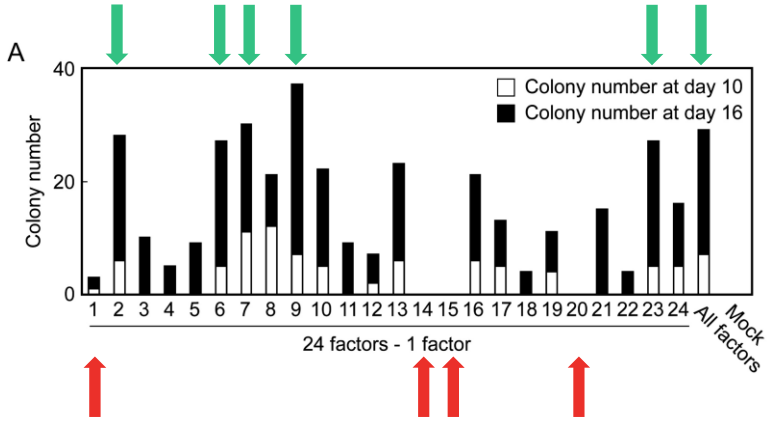
Discovering iPS cells (2)

Step 2:
In order to work out which of the 24 factors are essential to induce pluripotency, the author repeated the experiment several times, each time leaving a factor out.

Green arrows:
Factors not needed to induce pluripotency.

Red arrows:
Factors needed to induce pluripotency.

Conclusion:
It is very likely that the factors highlighted by the red arrows are necessary to induce pluripotency.



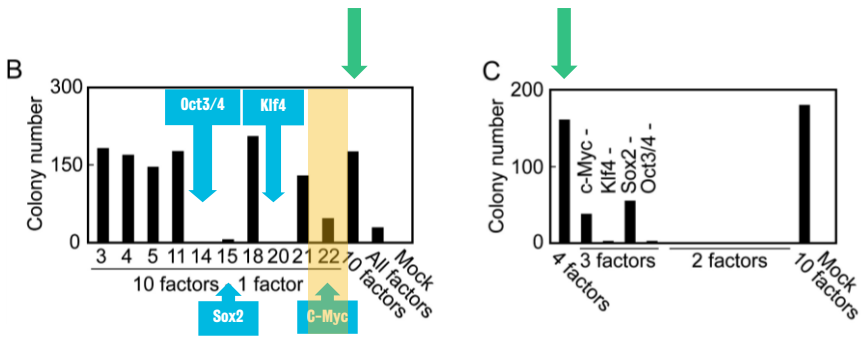
Takahashi & Yamanaka (2006)

Discovering iPS cells (3)

Step 3:
After narrowing the 24 factors down to 10, the author repeated the experiment with only the 10 remaining factors, sequentially leaving one out at a time.

Yamanaka factors:
Oct3/4, Sox2, Klf4, c-Myc

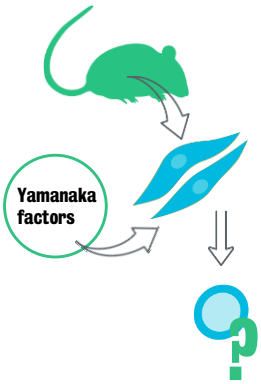
Conclusion:
- all of the 4 remaining factors are needed to generate pluripotent cells
- these 4 factors are sufficient on their own



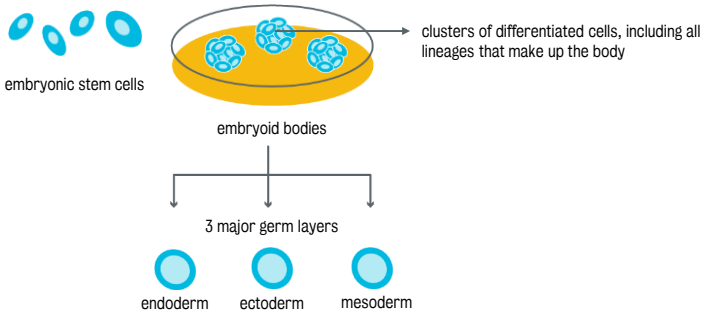
Takahashi & Yamanaka (2006)

Proving pluripotency (1)

Step 4:
To generate embryoid bodies.



Challenge:
At this stage, it was only a hypothesis that the blue cells truly were pluripotent.



Takahashi & Yamanaka (2006)

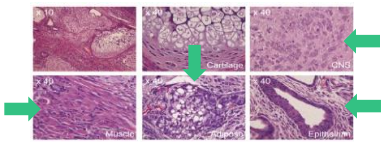
Proving pluripotency (2)

Step 5:
Take the fibroblasts that had been transduced with the 4 factors, grow them as embryoid bodies and show that each of the 3 germ layers were represented within the embryoid bodies.

Findings:
Histological sections of mice confirmed that the green cells had contributed to the creation of all the different body tissues.

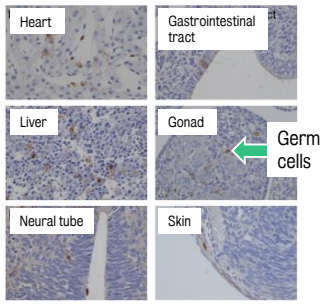
Conclusion:
The fibroblasts that were injected into the blastocyst contributed to the germ line in such a significant way, that it made it possible to produce mice that were entirely derived from these.

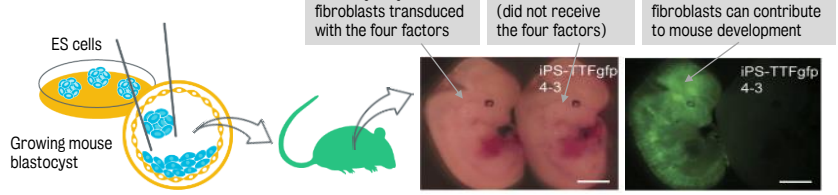
Histological sections through embryoid bodies



Mesoderm derivatives:
- muscles and smooth muscle
- cartilage
- epithelial tissue
- brain tissue
- adipose tissue

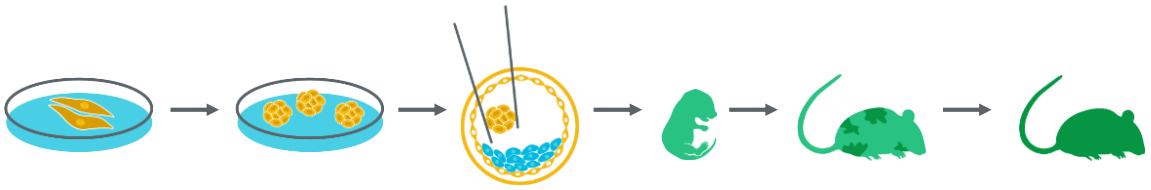
Histological sections through mouse tissue





Takahashi & Yamanaka (2006)

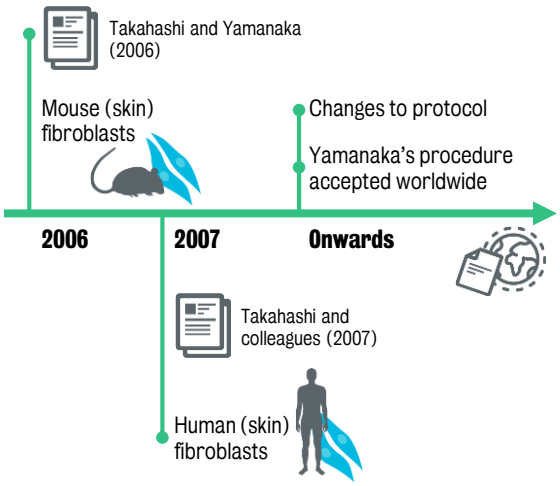
Proving pluripotency (3)

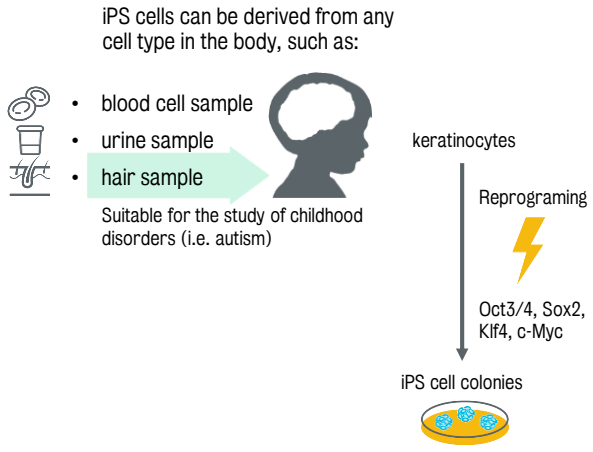


With his experiments, **Yamanaka found the biological basis for pluripotency**.

Takahashi & Yamanaka (2006)

Generation of iPSC lines and the study of childhood disorders





Takahashi & Yamanaka (2006); Takahashi et al. (2007)

End of part 1