

Is(are) true concerning embryonic stem cells:

- A. They are derived from the pre-implantation embryo
- B. They can differentiate towards cardiomyocytes
- C. They are found in the tissues of fetal mice and humans
- D. They are totipotent

Which is (are) the true statement(s) concerning adult stem cells ?

- A. They are easy to maintain in a self-renewing state in vitro
- B. They are multipotent or unipotent, but never pluripotent
- C. They are typically more abundant than terminally differentiated cells
- D. They are typically used to make induced pluripotent stem cells

(Stem) Cell Plasticity

Prof. David Suter
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What you will hear about today

1. Potency and Plasticity
2. The Waddington landscape and epigenetics
3. What defines/determines the identity of a cell ? What are the barriers to changes in cell identity ?
4. Crossing cell identities
5. Plasticity of adult stem cells

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1. Potency and Plasticity

2. The Waddington landscape and epigenetics

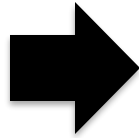
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4. Crossing cell identities

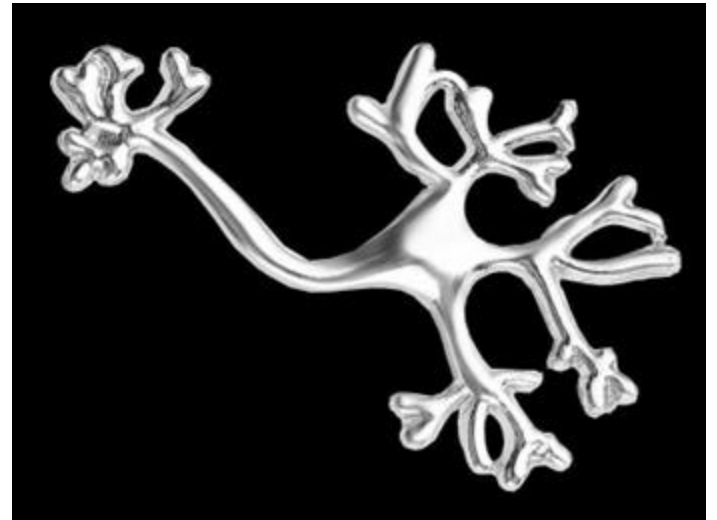
5. Plasticity of adult stem cells

What is plasticity ?

High plasticity



Low plasticity

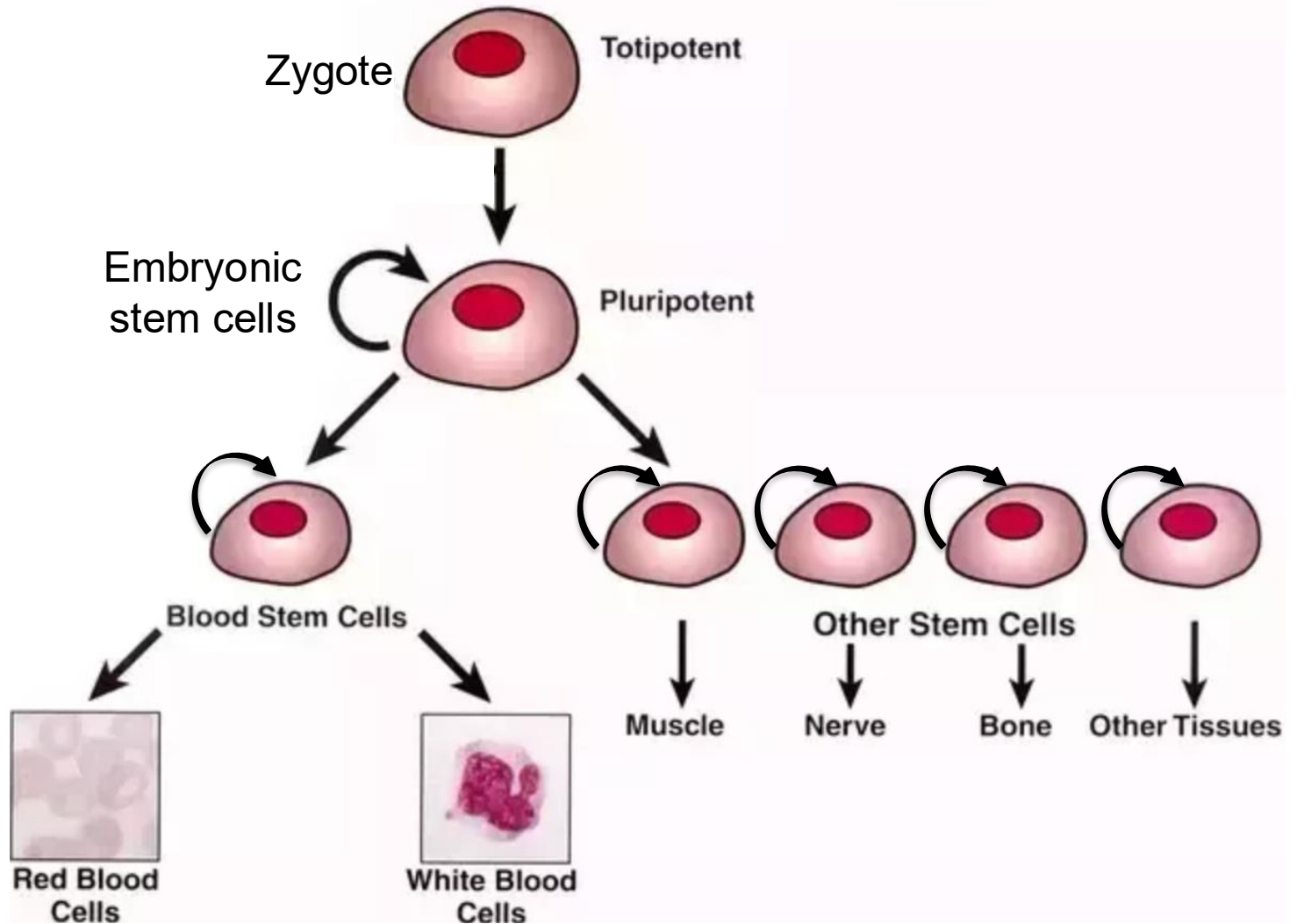


Potency and Plasticity

- Potency refers to the **number of different identities cells can acquire**
- Plasticity refers to how easy it is **to induce a change in cell identity**

Plasticity and potency

Plasticity

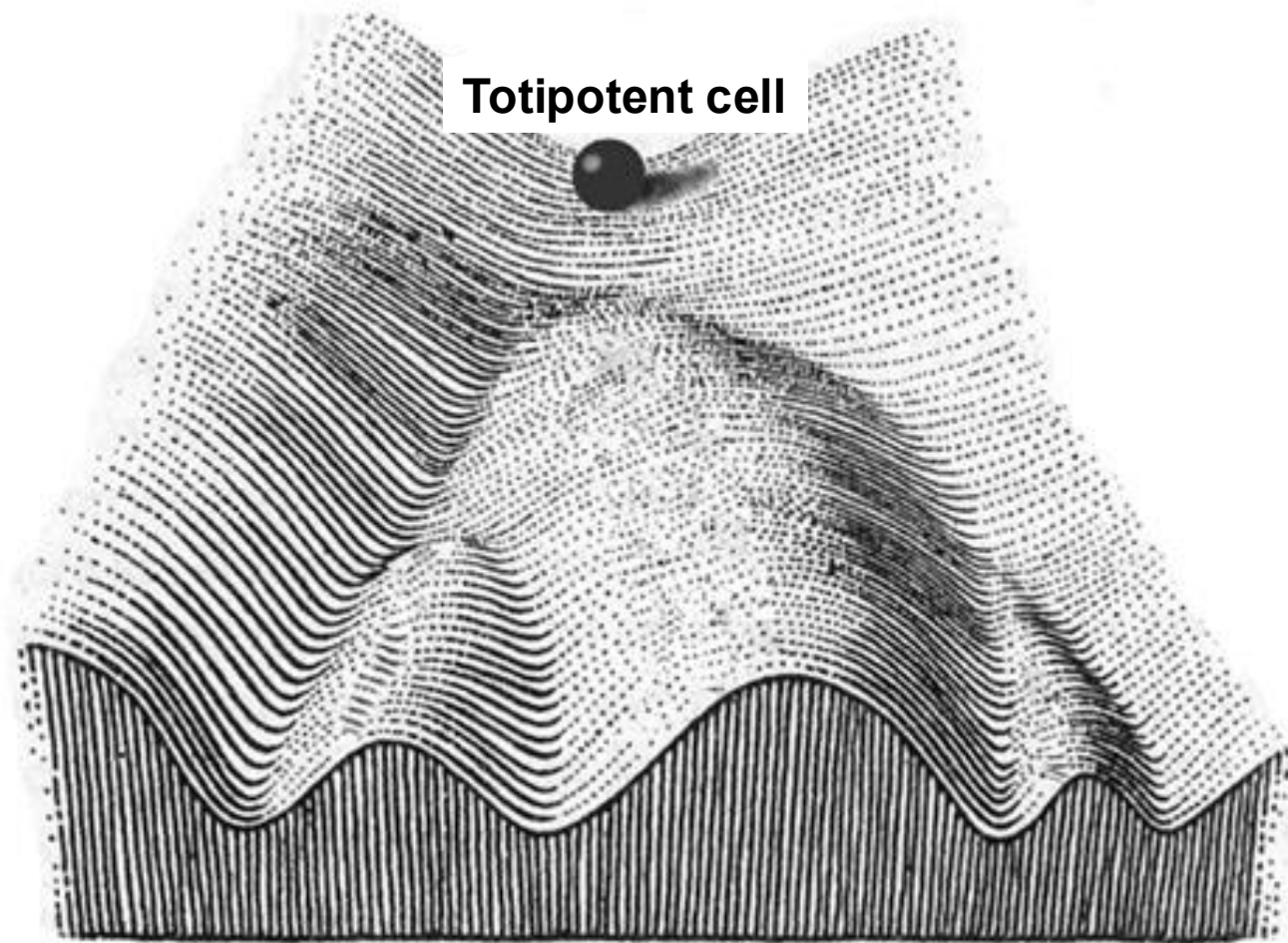


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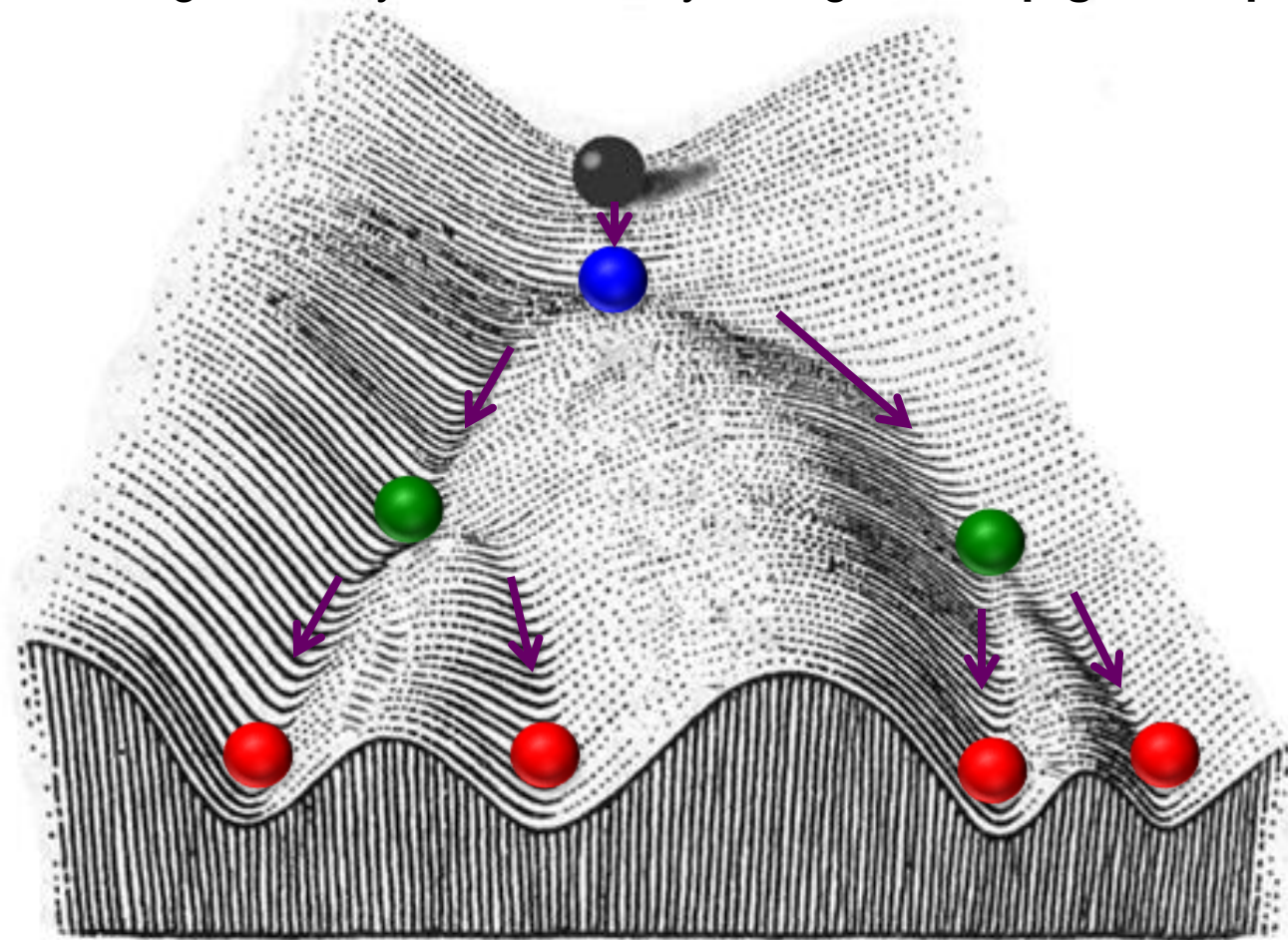
Waddington's landscape

In 1957, Conrad Waddington proposed an **epigenetic landscape** to represent the process of **cellular decision-making** during development.



Waddington's landscape

- A totipotent cell (the zygote) becomes restricted to **pluripotent stem cells**, **adult stem cells** and **terminally differentiated cells**
- Although cells are genetically identical, they change their **epigenetic properties**

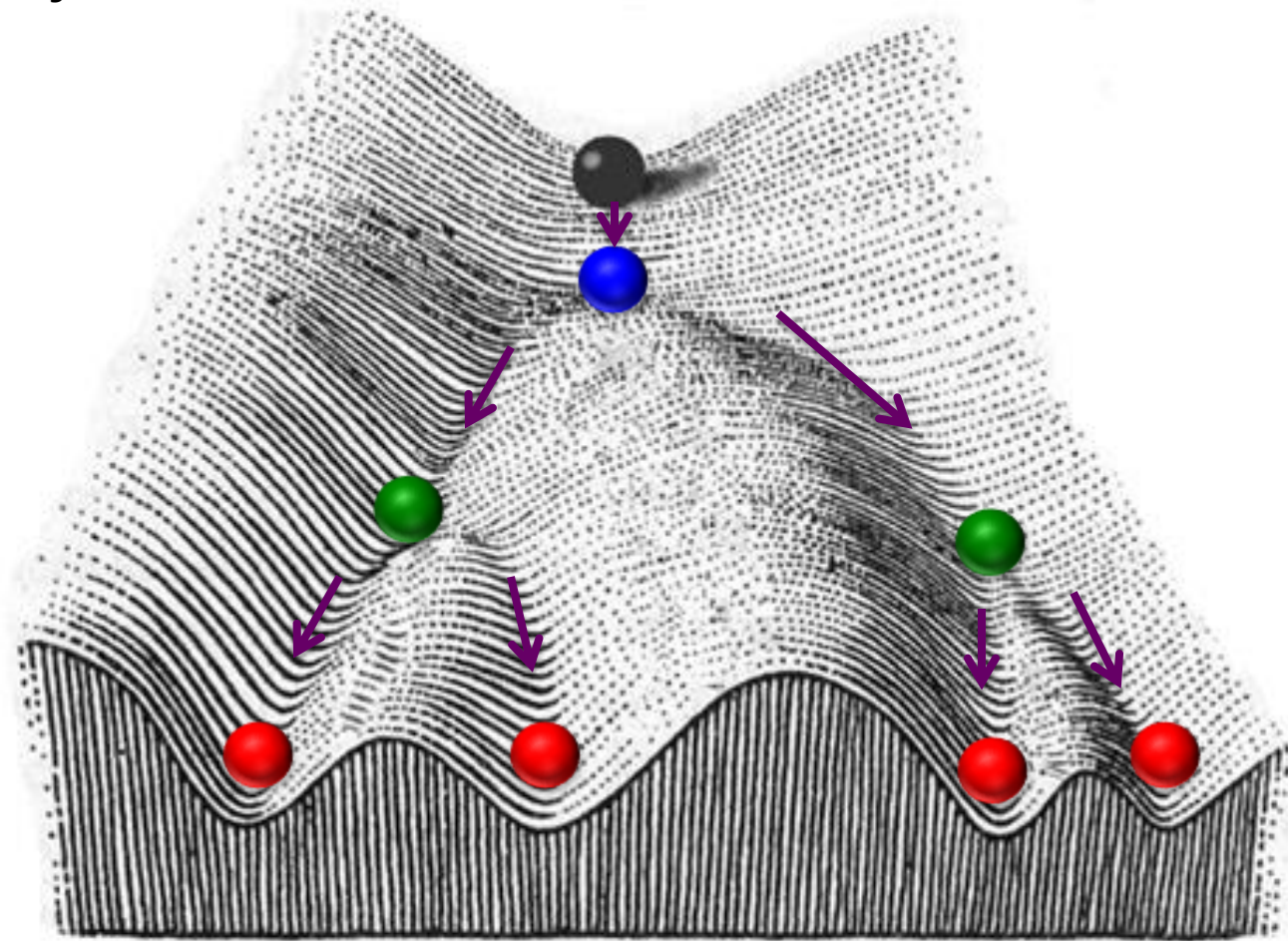


Epigenetic properties refer to **non-genetic mechanisms** conferring **stable modifications of cell identity**

Waddington's landscape

Two key elements in Waddington's landscape:

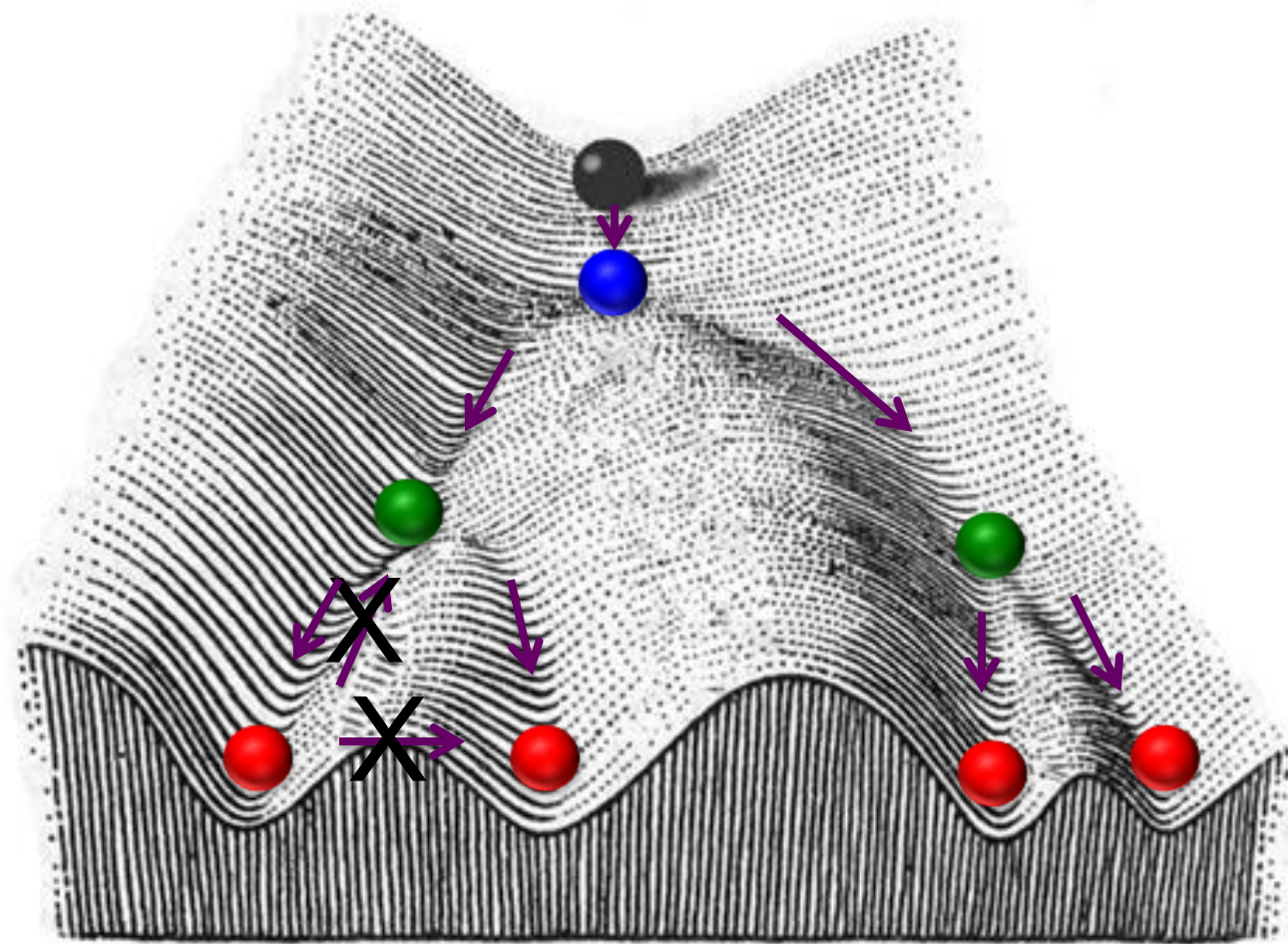
1. **Directionality** of cell fate



Waddington's landscape

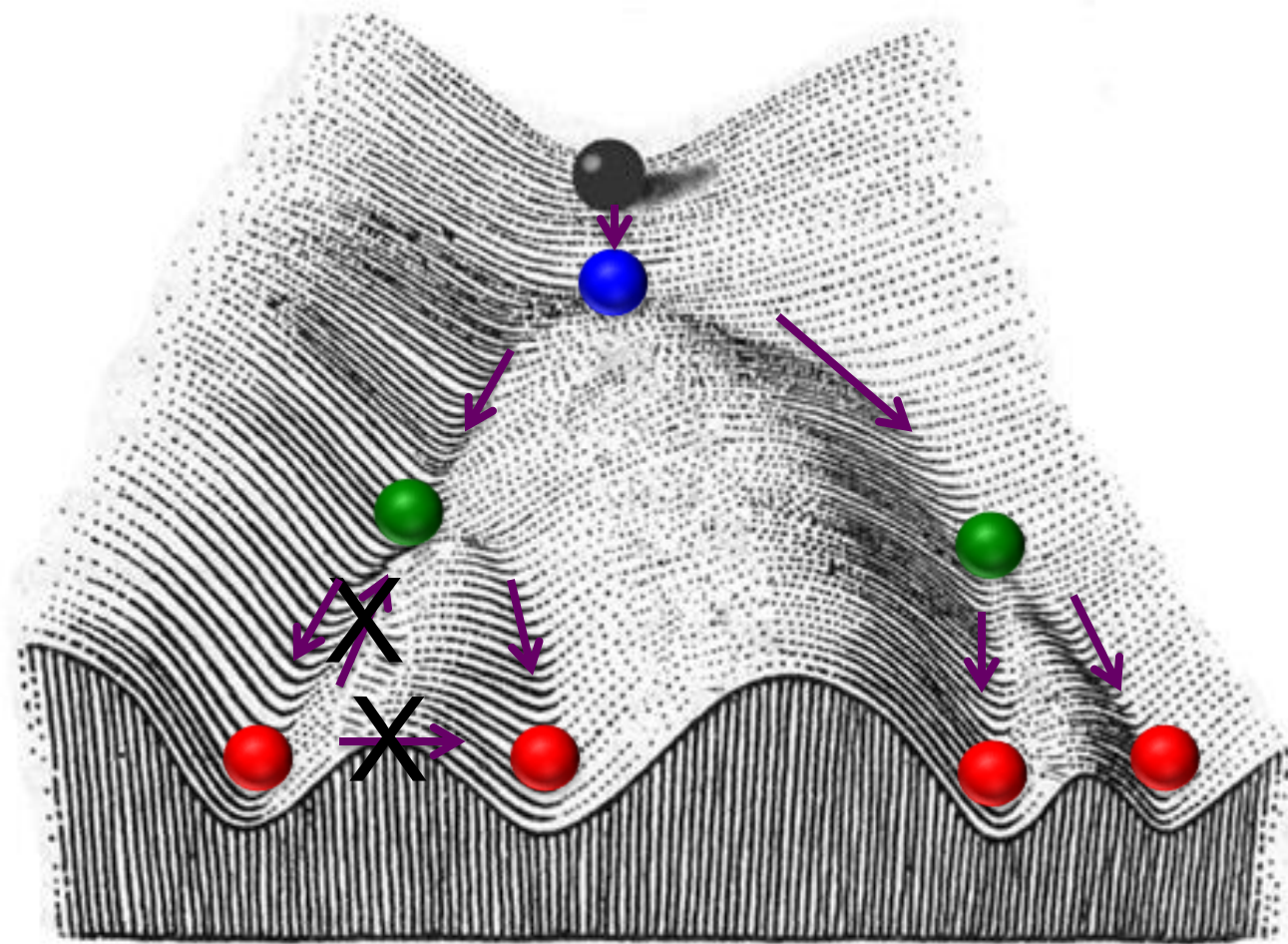
Two key elements in Waddington's landscape:

2. **Locking** of cell fate



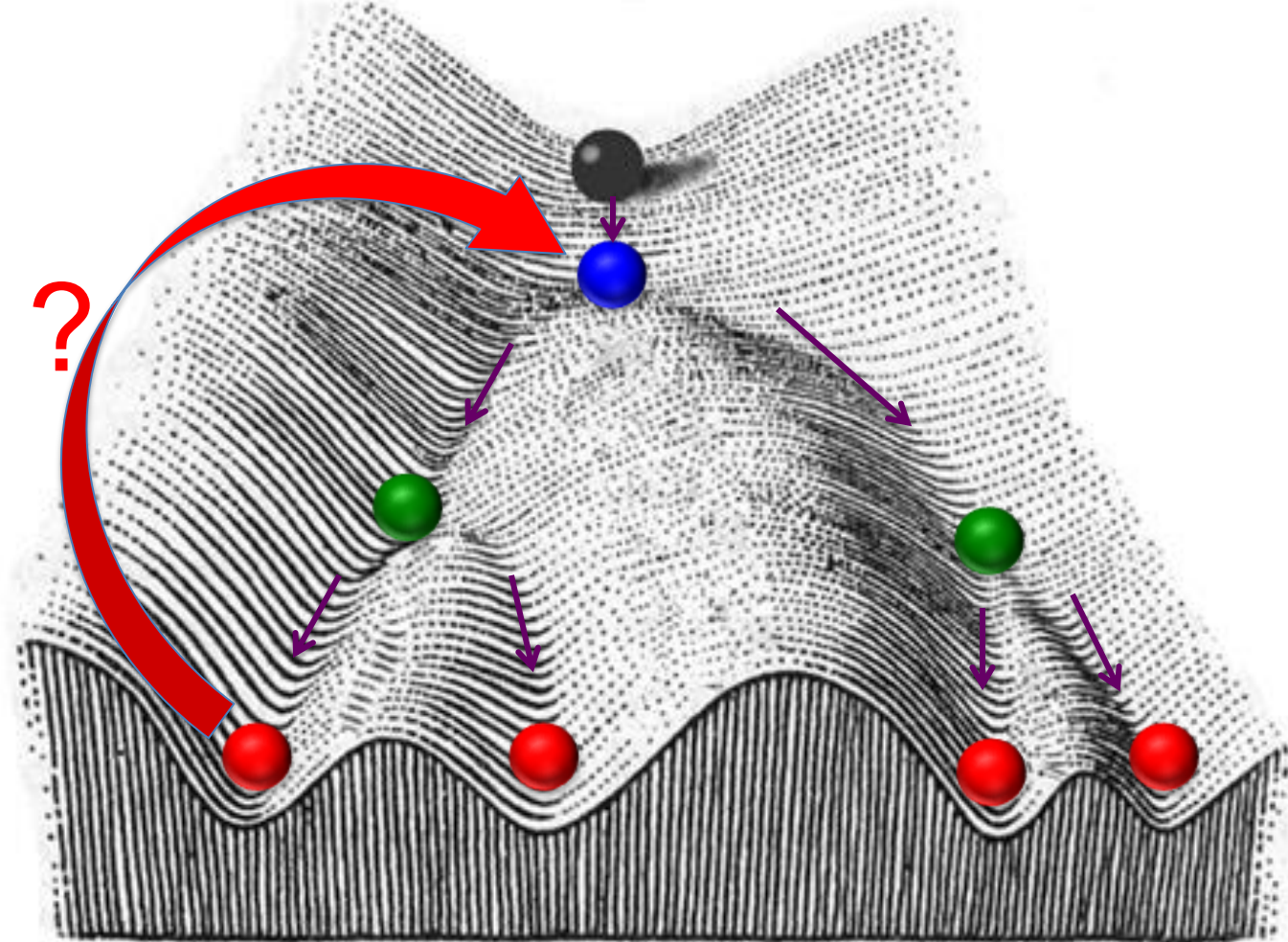
Key questions

- What are the molecular mechanisms allowing **directionality** of cell fate and **locking** in a certain state ?



Key questions

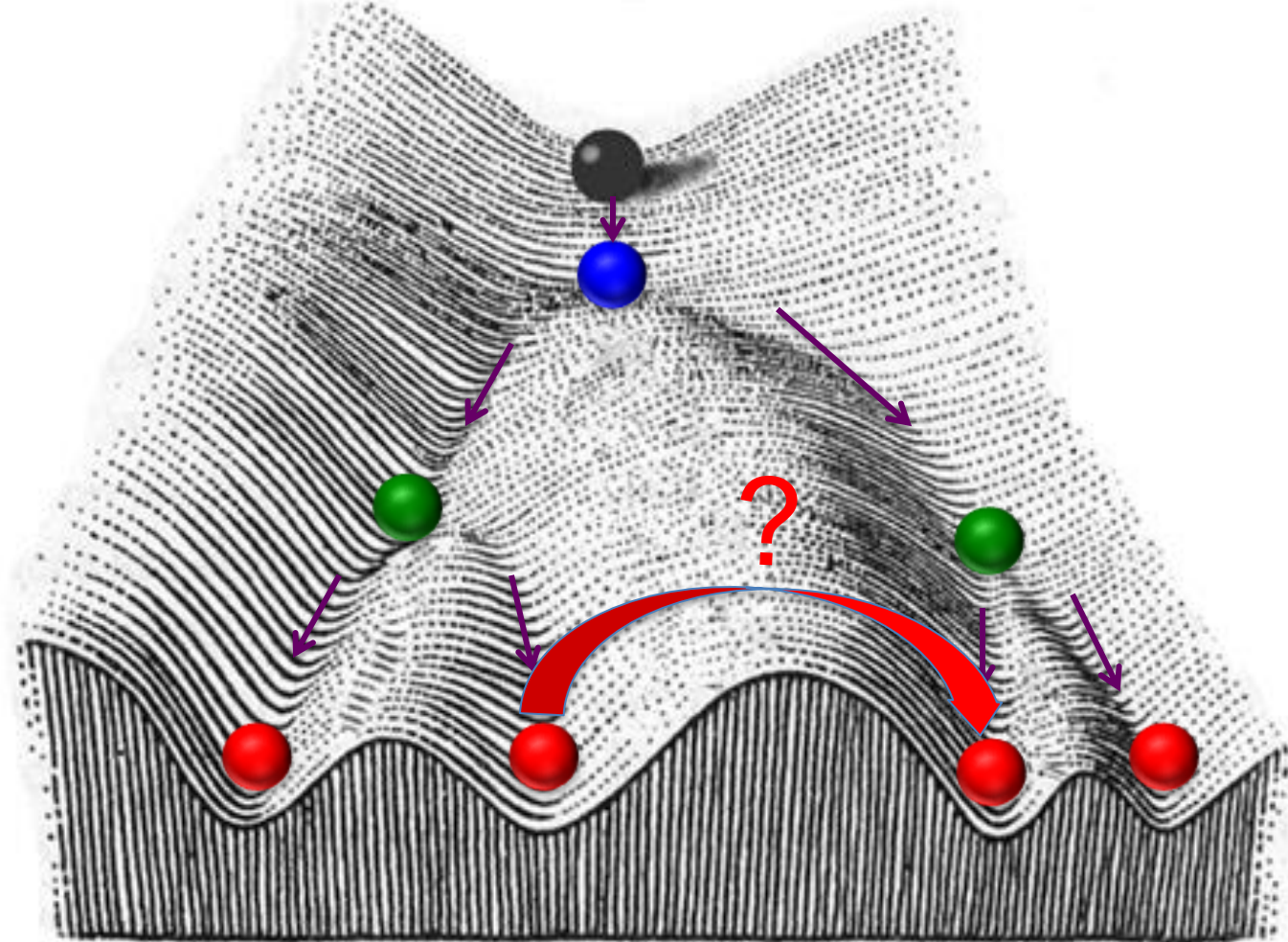
- How absolute are these restrictions in cell fate ?



Reprogramming to pluripotency

Key questions

- How absolute are these restrictions in cell fate ?



Transdifferentiation

What you will hear about today

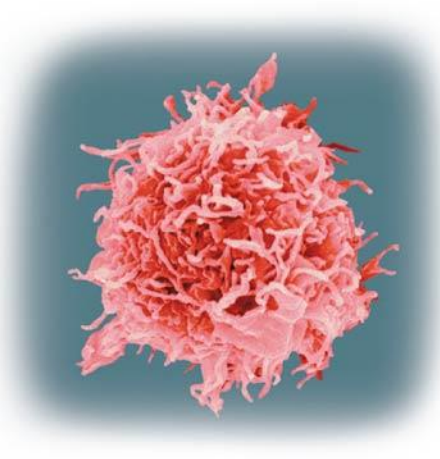
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What defines the identity of a cell ?



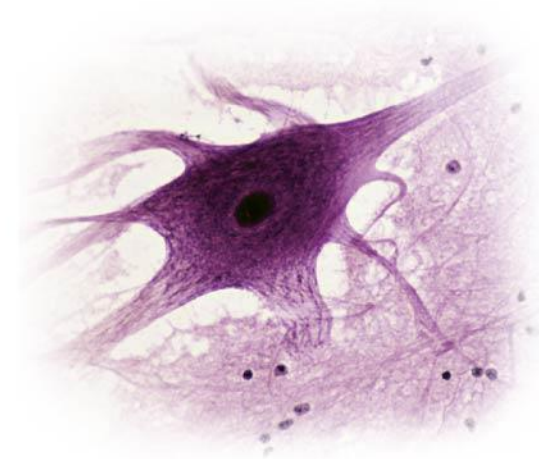
Colorized TEM

β -cell of the pancreas



Colorized SEM

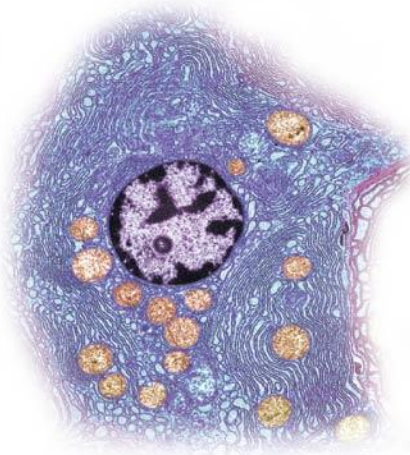
B lymphocyte



Colorized TEM

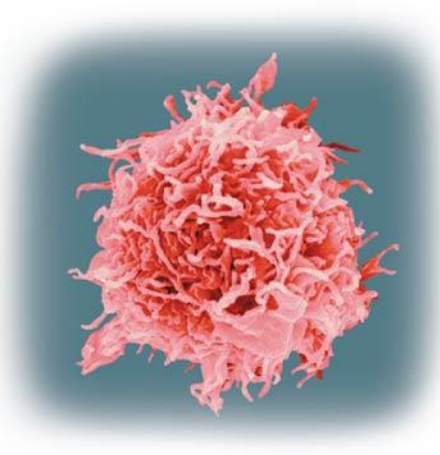
Neuron

What defines the identity of a cell ?



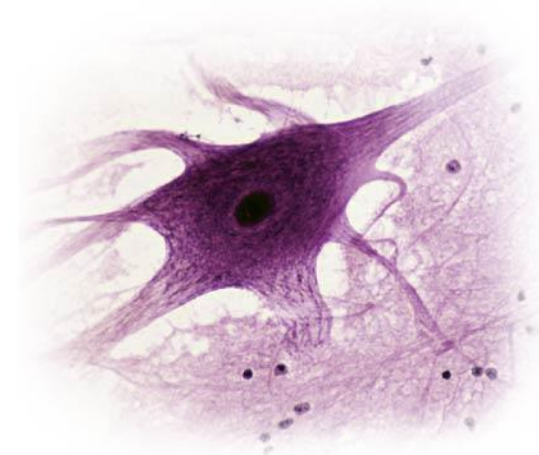
Colorized TEM

β -cell of the pancreas



Colorized SEM

B lymphocyte



Colorized TEM

Neuron

Gene for a glycolysis enzyme



Antibody gene



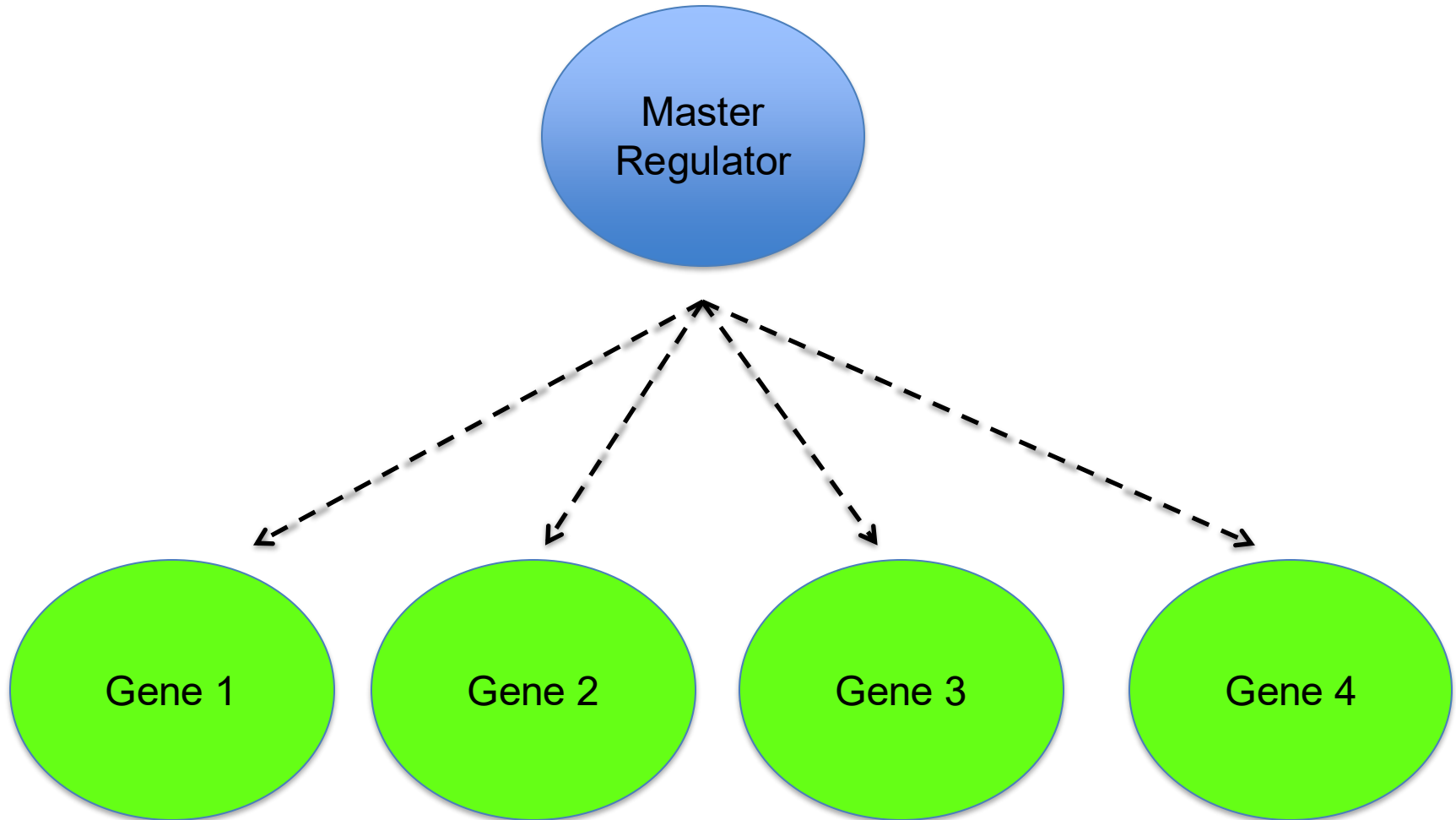
Insulin gene



Hemoglobin gene

What determines the identity of a cell ?

→ Are there Master regulators of cell identity ?



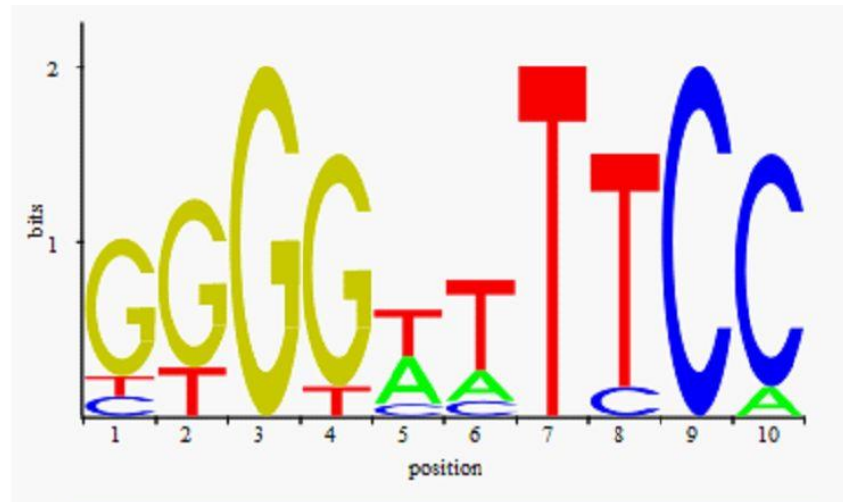
What properties should master regulators have ?

- Control positively or negatively the expression of other genes
- Specific for particular genes

Transcription factors

Eukaryotic transcription factors

- Transcription factors recognize specific short consensus DNA sequences of 6-15 DNA base pairs defined by a weight matrix. Example: NF- κ B



POSITIONAL FREQUENCY MATRIX

A	0	0	0	0	4	2	0	0	0	1
C	1	0	0	0	1	1	0	1	9	8
G	7	7	9	8	0	0	0	0	0	0
T	1	2	0	1	4	6	9	8	0	0

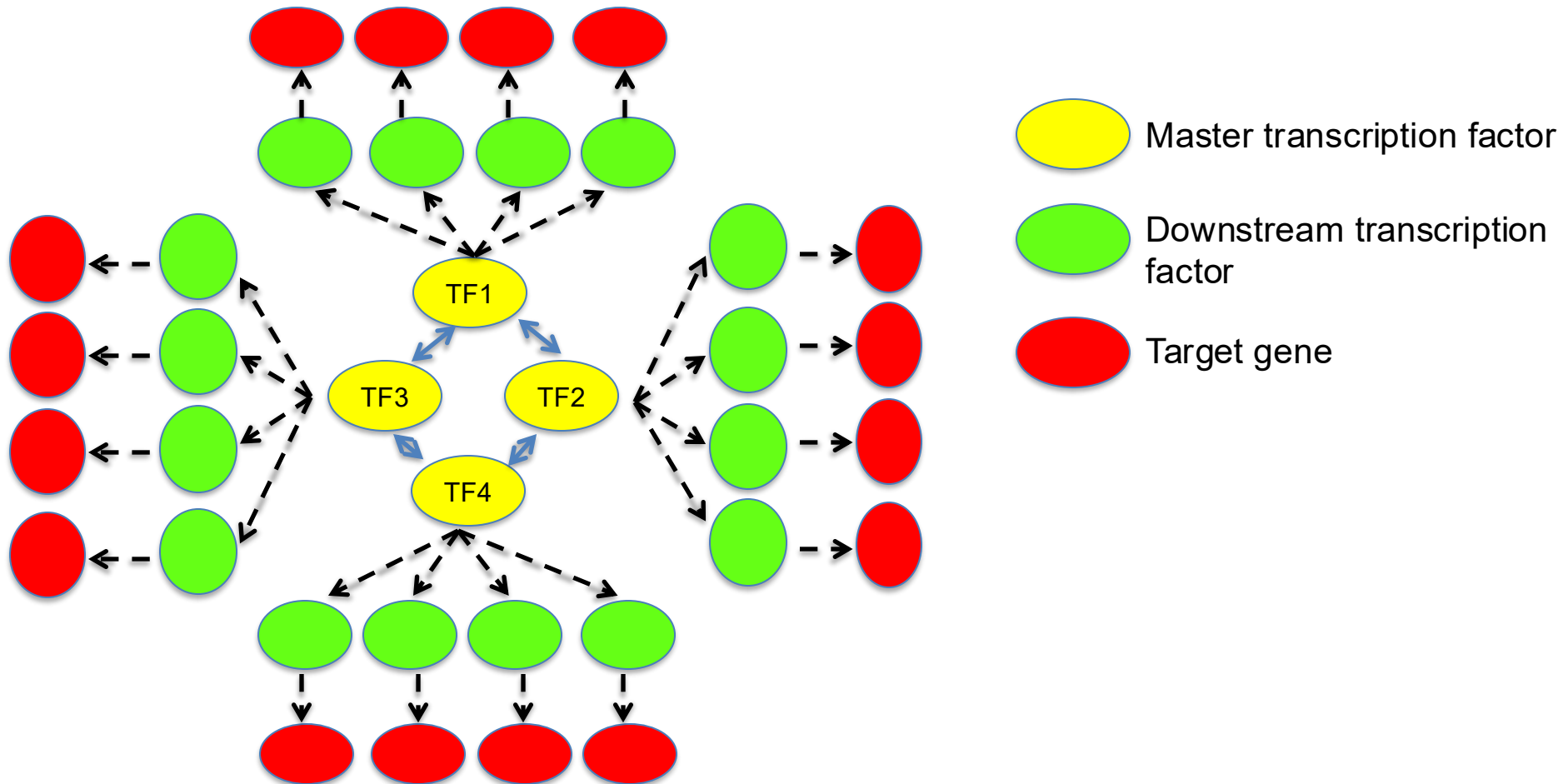
Eukaryotic transcription factors

- Interact with other proteins (co-activators, co-repressors) or other transcription factors to form heterodimers
- Control histone modifications → **control chromatin state** by recruiting histone modifiers
- There are > 1500 transcription factors encoded in the human genome
- **We don't know the function of hundreds of them**

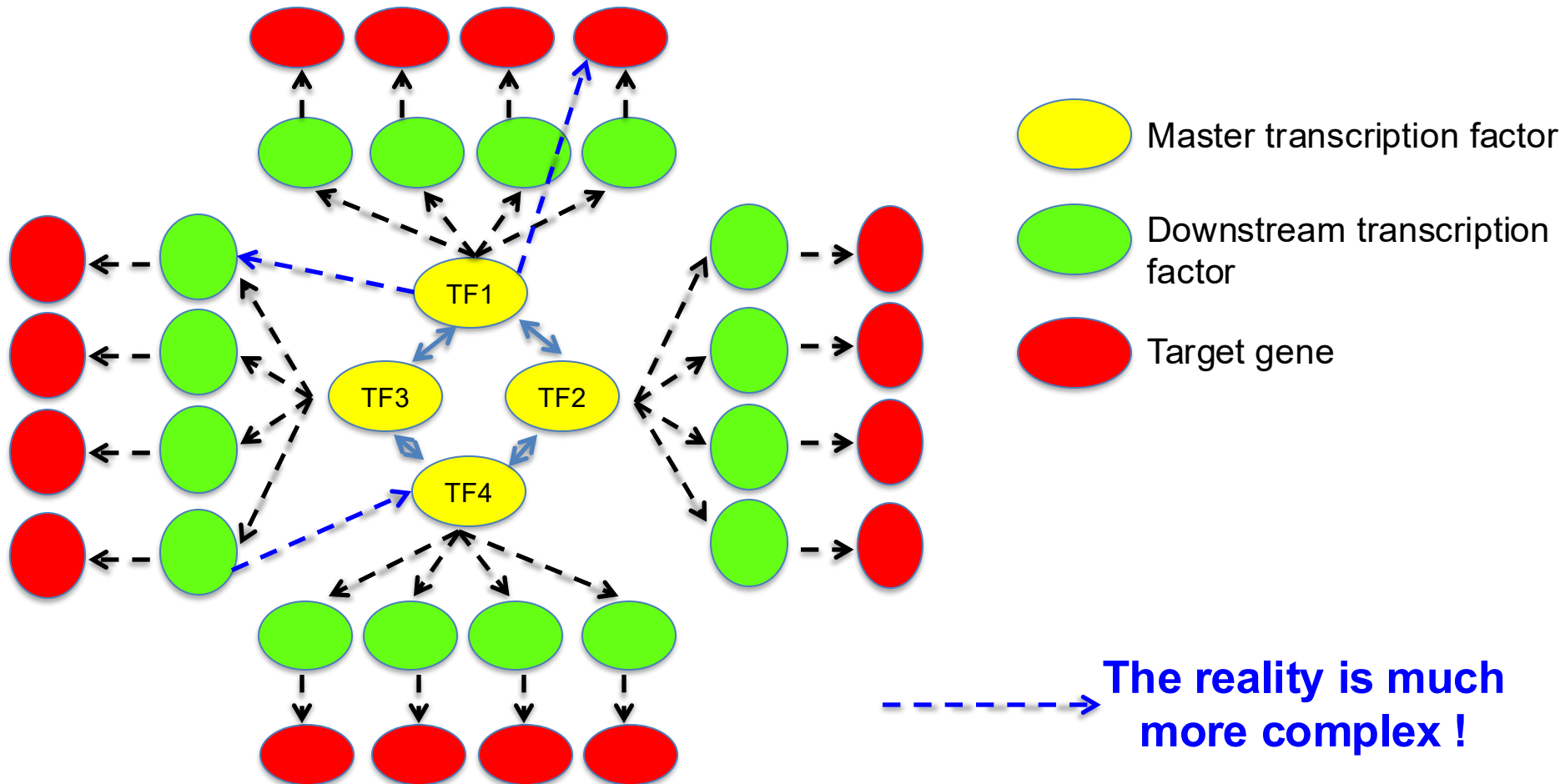
Eukaryotic transcription factors: key concepts

- The key feature of transcription factors is their **sequence specificity**
- They are the masters that will:
1. Turn genes on and off directly
 2. Recruit histone modifying enzymes to modify chromatin

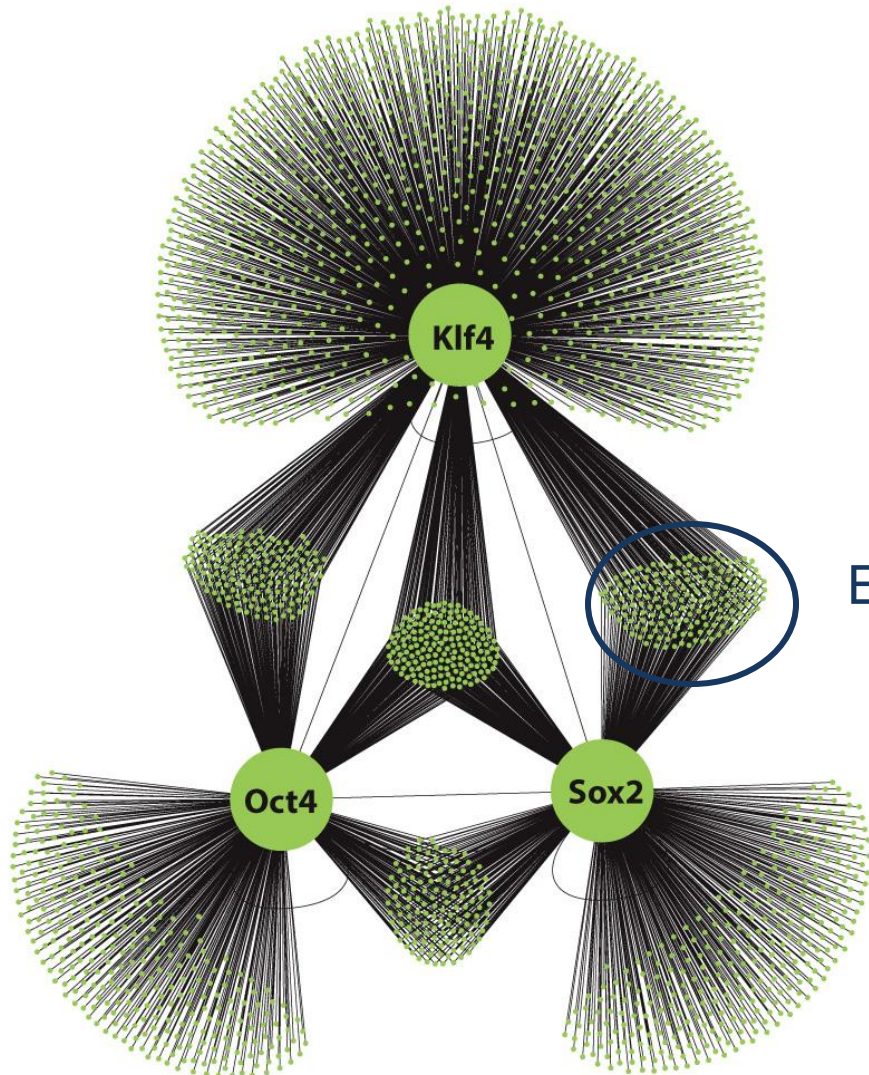
Transcription factors are organized in regulatory networks



Transcription factors are organized in regulatory networks

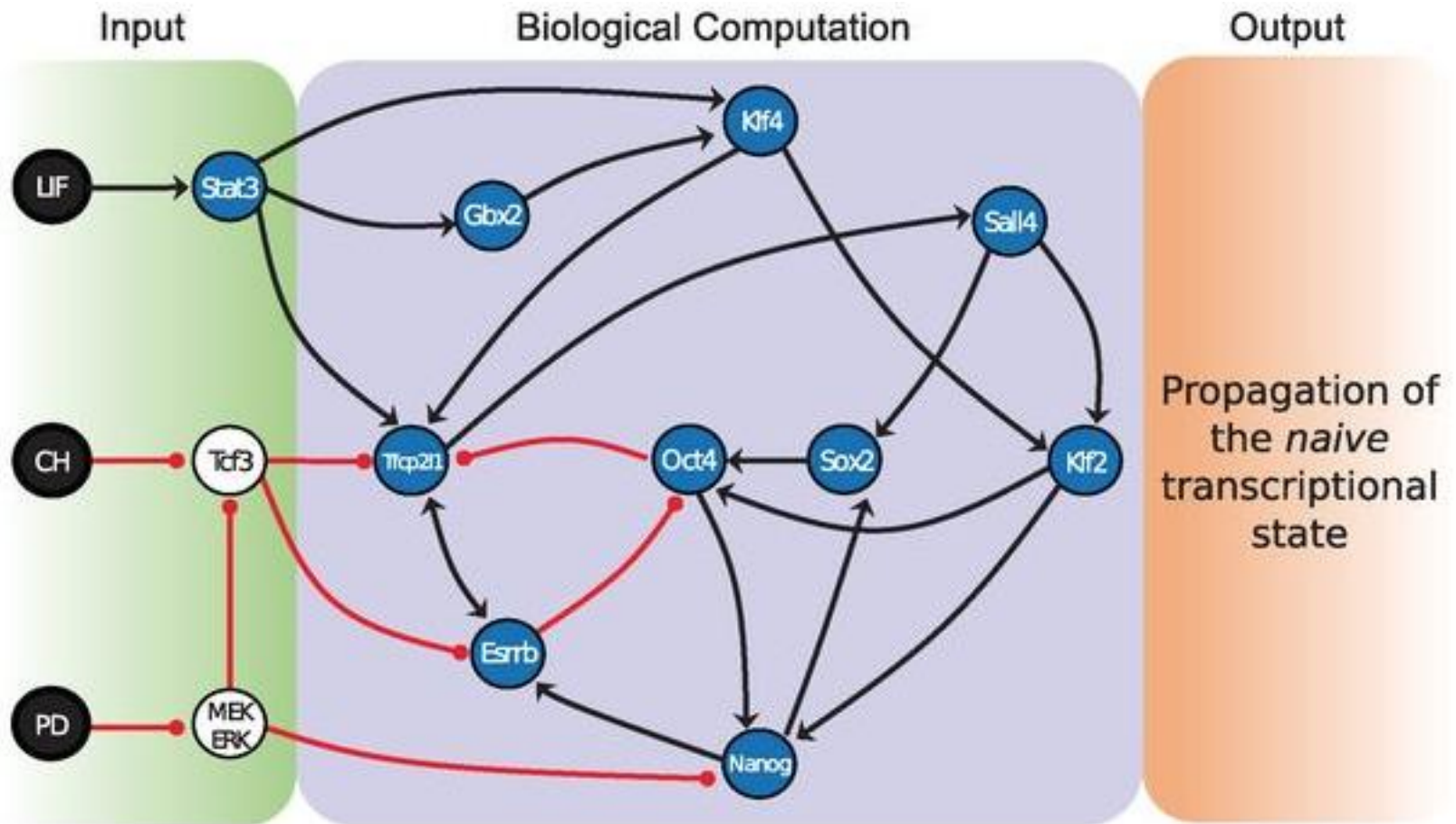


Three members of the pluripotency network and their targets



Each dot of this cloud is a gene bound by both Klf4 *and* Sox2

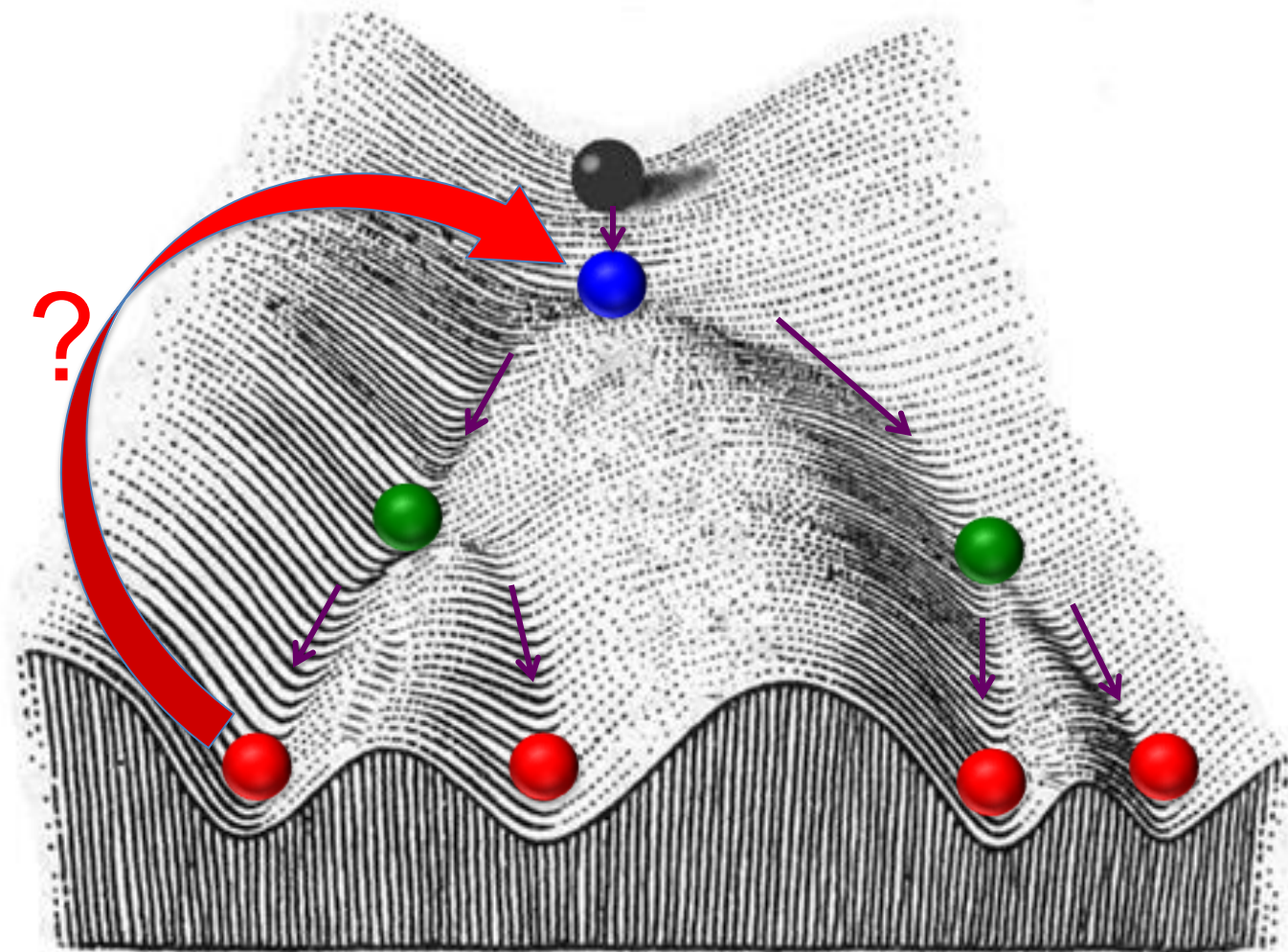
A minimal network of pluripotency



What you will hear about today

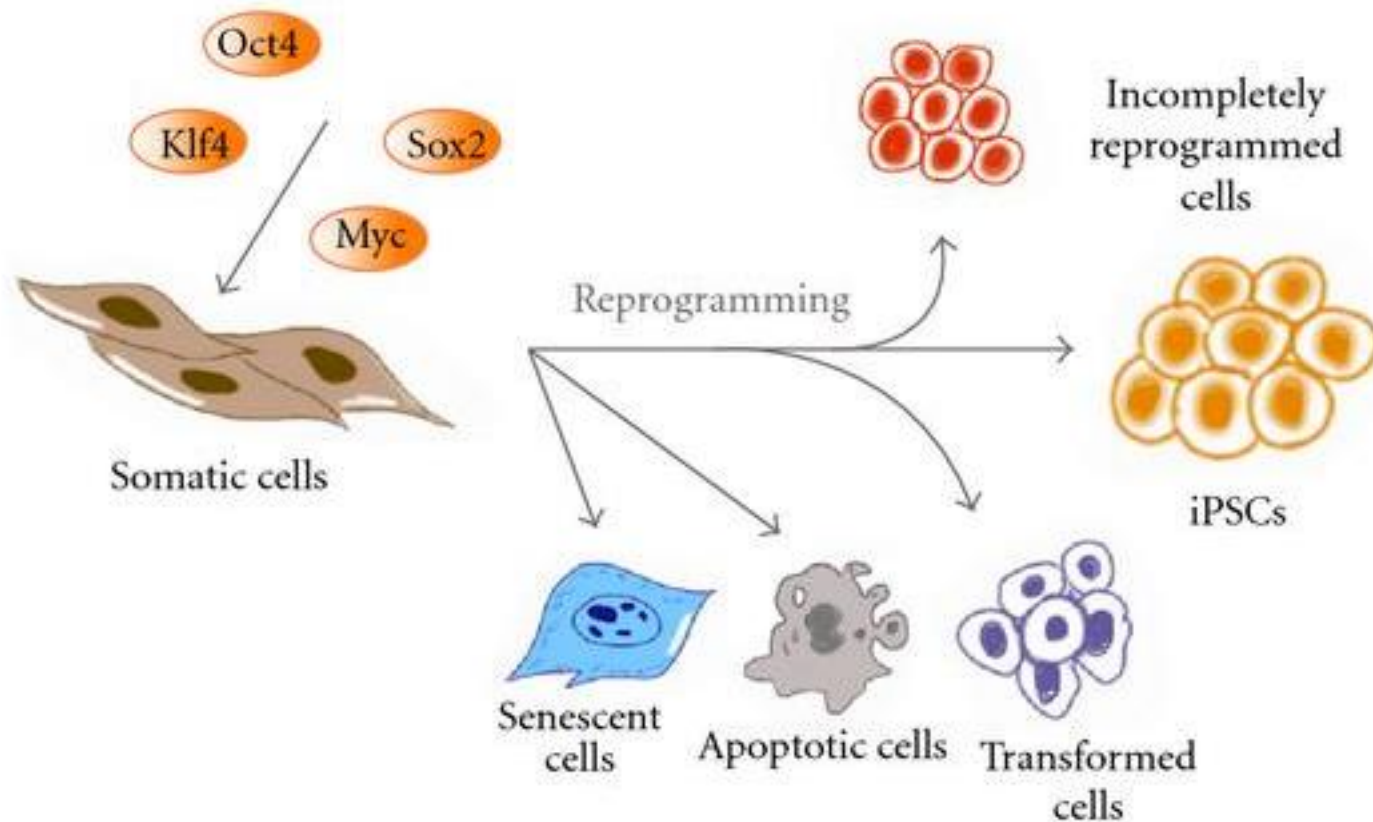
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Can transcription factors change the identity of a cell ?

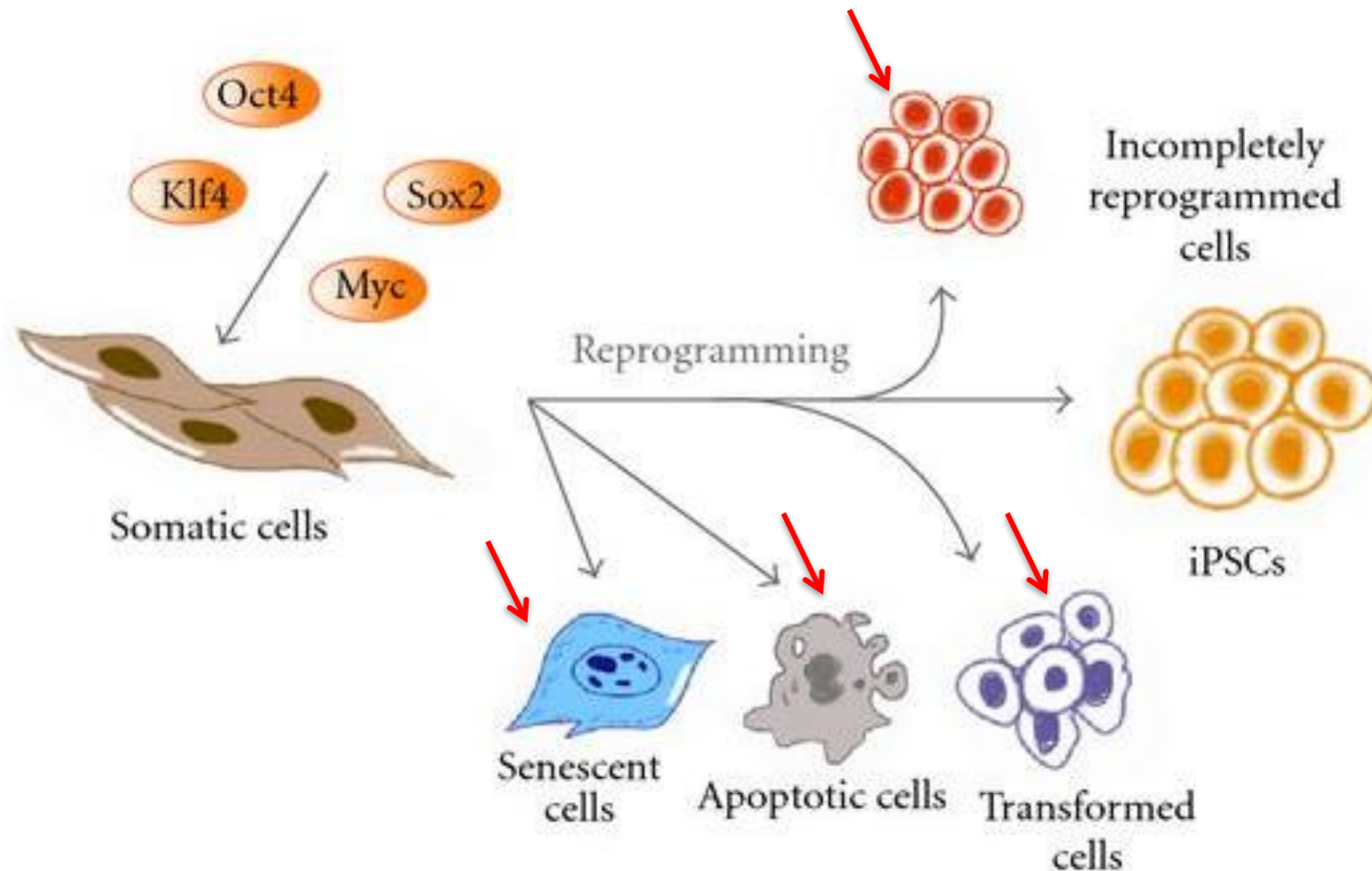


Reprogramming to pluripotency

Transcription factors mediate cellular reprogramming



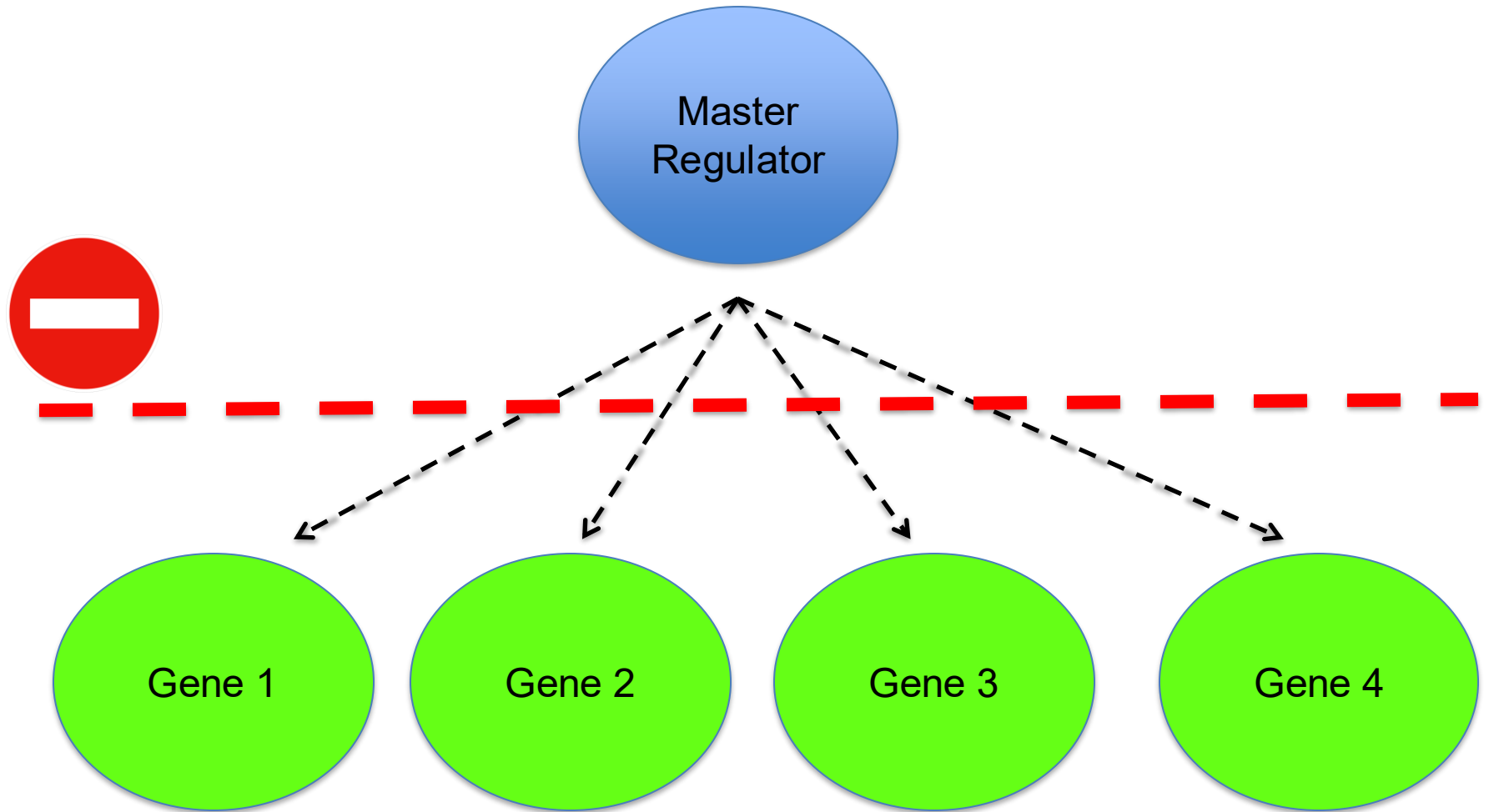
Transcription factors mediate cellular reprogramming



<http://www.hindawi.com/journals/isrn/2012/685852/fig1/>

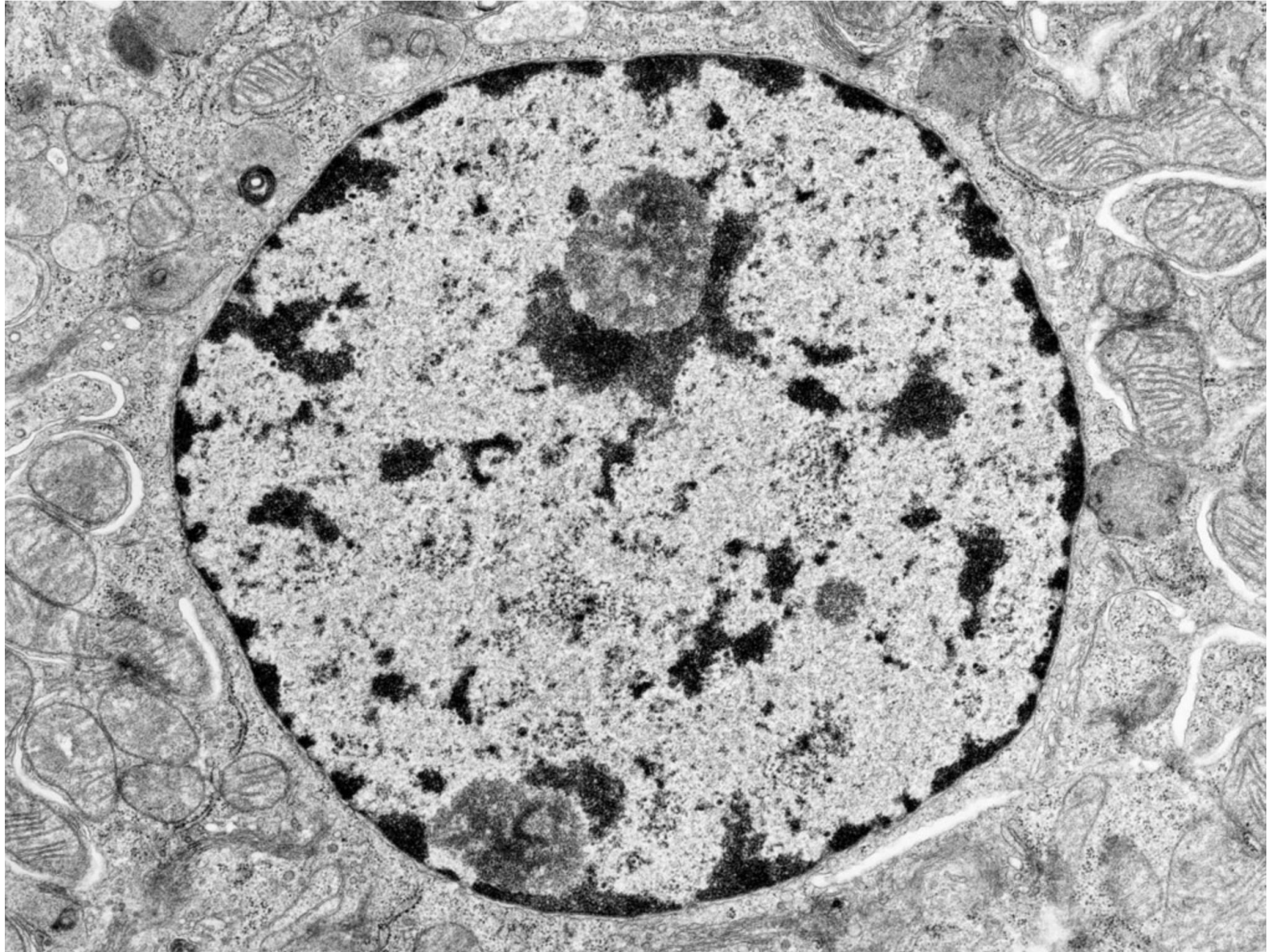
Reprogramming is very inefficient → barrier to overcome

Why is there a barrier ?

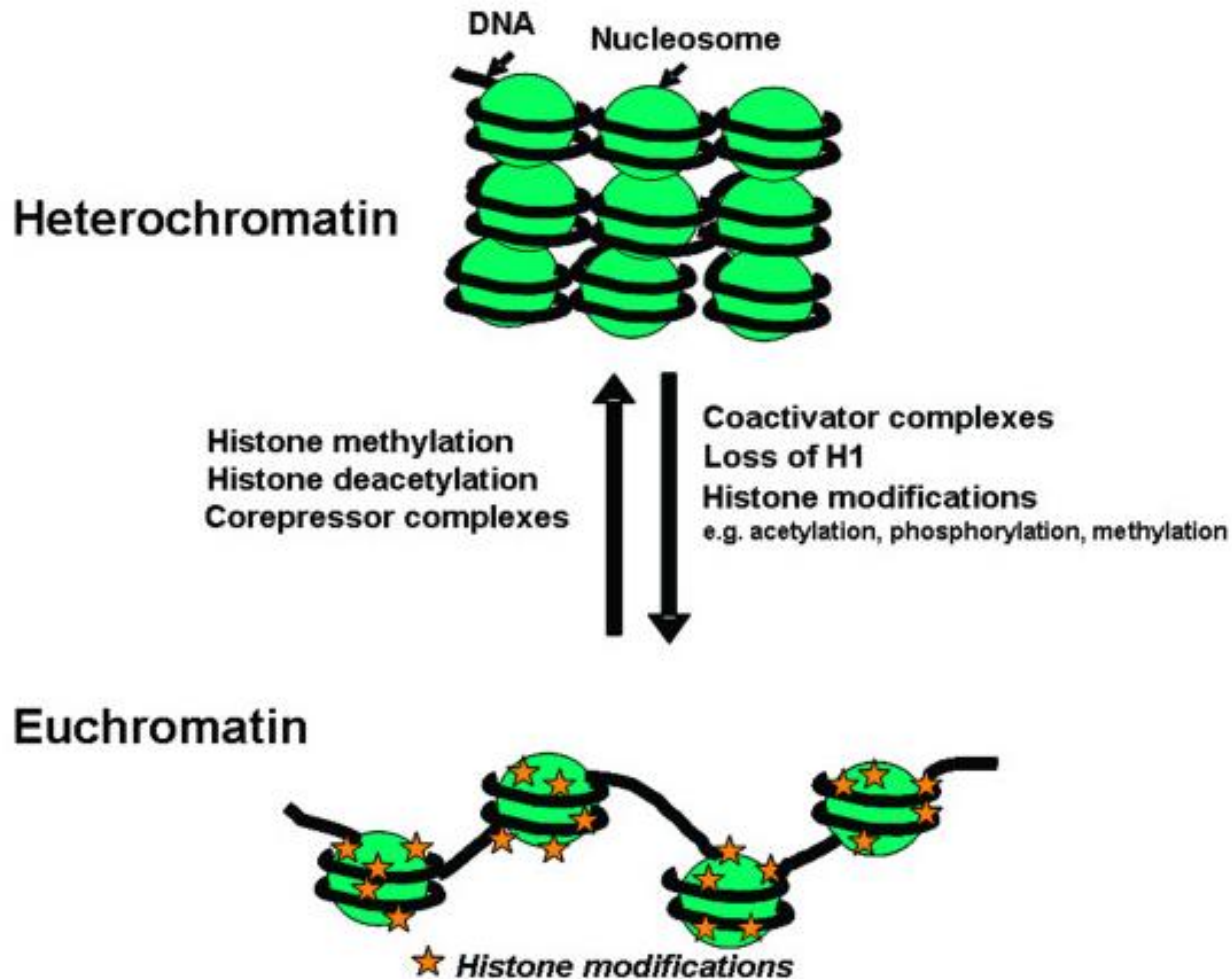


→ There must be something that makes the action of transcription factors difficult

Chromatin organization

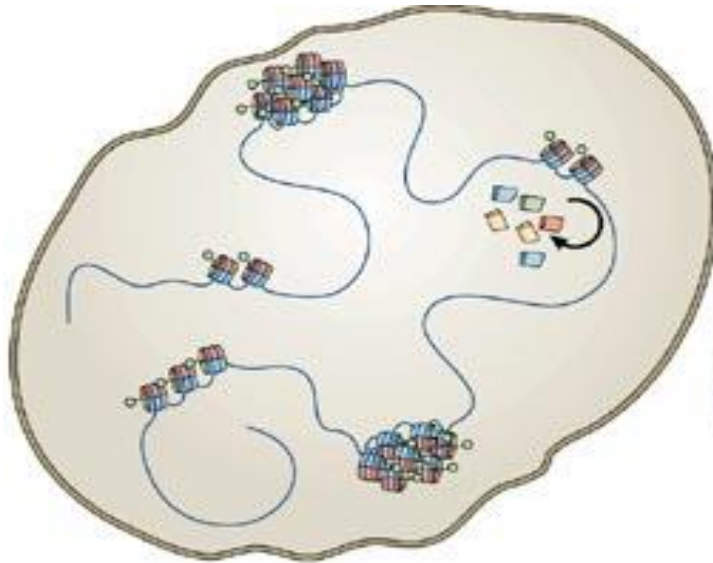


Chromatin organization

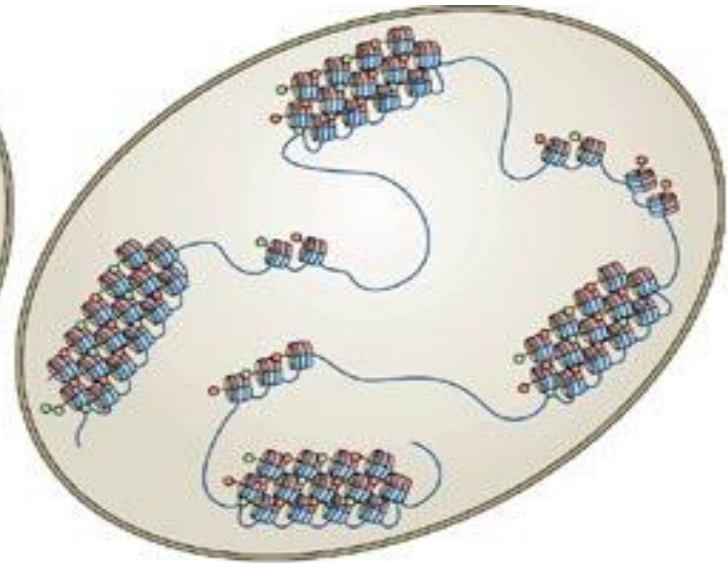


→ If target genes are embedded into heterochromatin, they are difficult to access for transcription factors

Chromatin organisation in pluripotent stem cells



Pluripotent cells



Differentiated cells

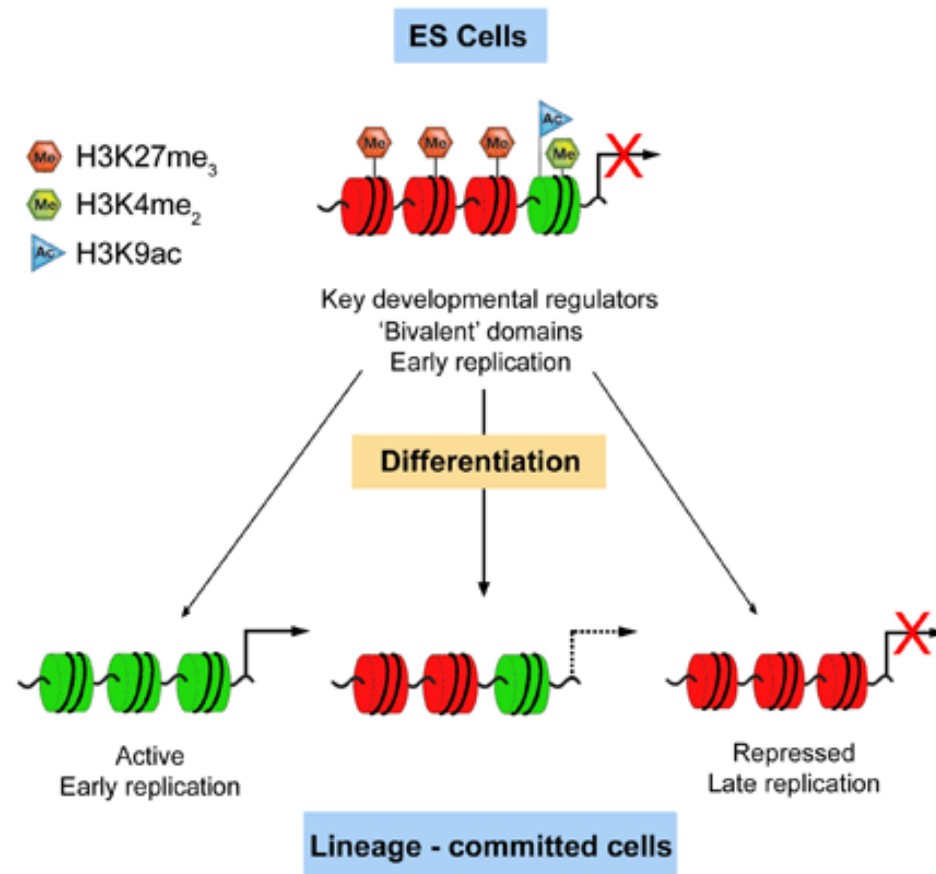
Modified from Meshorer and Mistelli, Nat Rev Mol Cell Biol 2006

Pluripotent cells:

- High proportion of euchromatin
- High amounts of chromatin remodelers (remove nucleosomes)

Bivalent chromatin domains in pluripotent stem cells

Pluripotent stem cells are characterized by bivalent chromatin domains carrying both active and inactive histone marks at developmental genes



Increased plasticity by modifiers of chromatin state

- Histone deacetylase inhibitors increase reprogramming efficiency
- Suppression of Mbd3 (member of a complex involved in histone deacetylation and inhibition of nucleosome remodeling) increases reprogramming efficiency

Chromatin state and plasticity

Key concepts

- The chromatin state is a major determinant of plasticity
- Chromatin state may be the major determinant of the barrier to overcome upon reprogramming or transdifferentiation

Chromatin modifications vs epigenetics

Epigenetics: by definition heritable

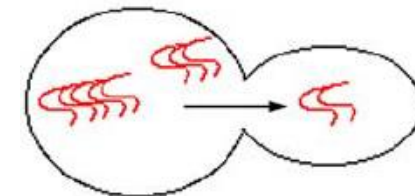
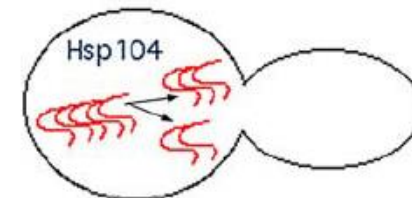
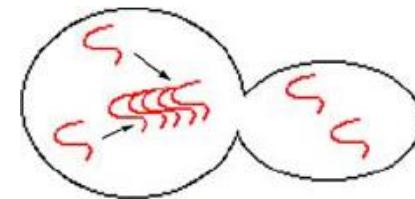
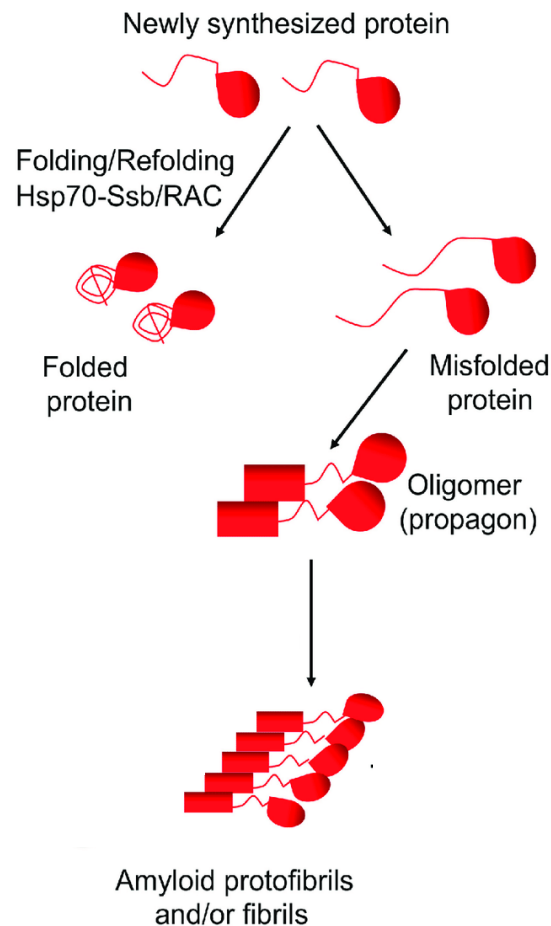
Not all histone modifications are heritable !

- **Activating histone marks: non-heritable**
- **Repressive marks: heritable**
- **DNA methylation: heritable (maintenance methylase active during S-phase)**

Chromatin modifications vs epigenetics

Epigenetics is not only mediated by chromatin

Example: prions

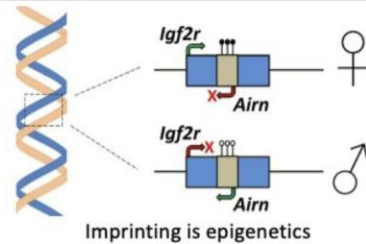


Epigenetic confusion

THE EPIGENETICS ALIGNMENT CHART

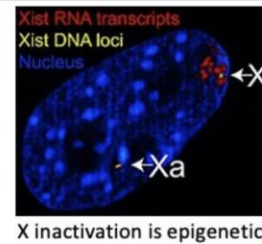
TIMESCALE PURIST

(Epigenetics must persist across generations of organisms)



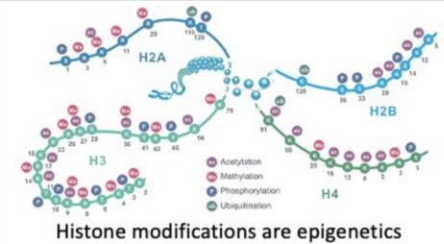
TIMESCALE NEUTRAL

(Epigenetics must persist across cell division)



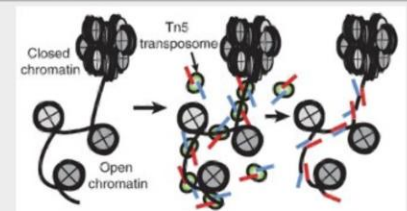
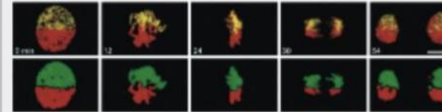
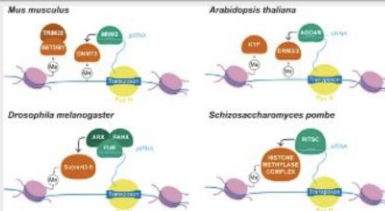
TIMESCALE REBEL

(Epigenetics can persist for any amount of time)



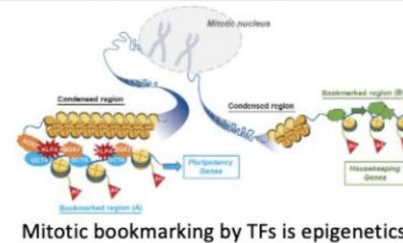
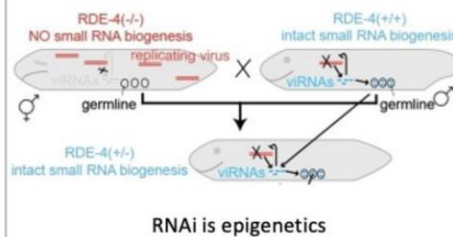
MECHANISM PURIST

(Epigenetics must be chemical modifications on top of DNA)



MECHANISM NEUTRAL

(Epigenetics must be functional changes to the genome)



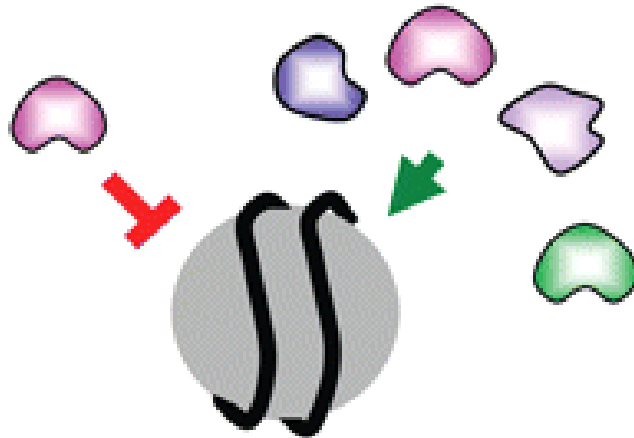
The functional relevance of many histone modifications is unclear

- Various histone marks correlate well with promoter/enhancer activity
- But: some of these marks are in fact dispensable
- Challenge in identifying role of marks versus other functions of the complexes putting these marks in place

How do transcription factors controlling cell identity interact with chromatin ?

Transcription factor interactions with nucleosomes

Most transcription factors

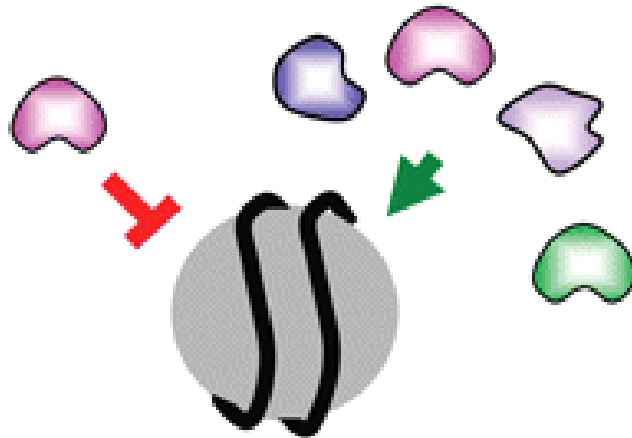


1- cooperativity allows nucleosome/chromatin binding

2 - simultaneous binding with other factors

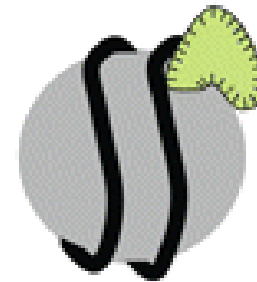
Transcription factor interactions with nucleosomes

Most transcription factors



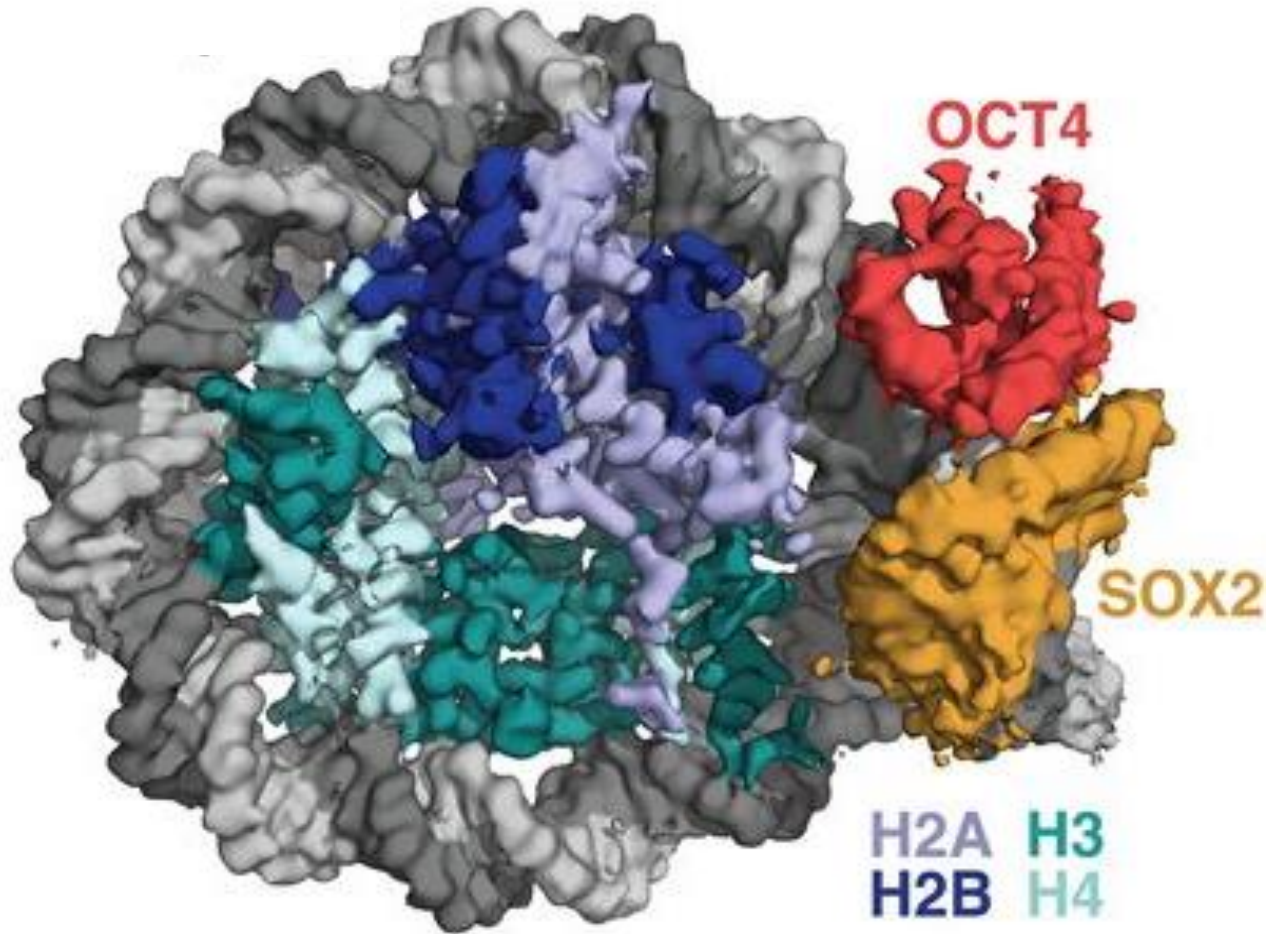
- 1- cooperativity allows nucleosome/chromatin binding
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Pioneer transcription factors

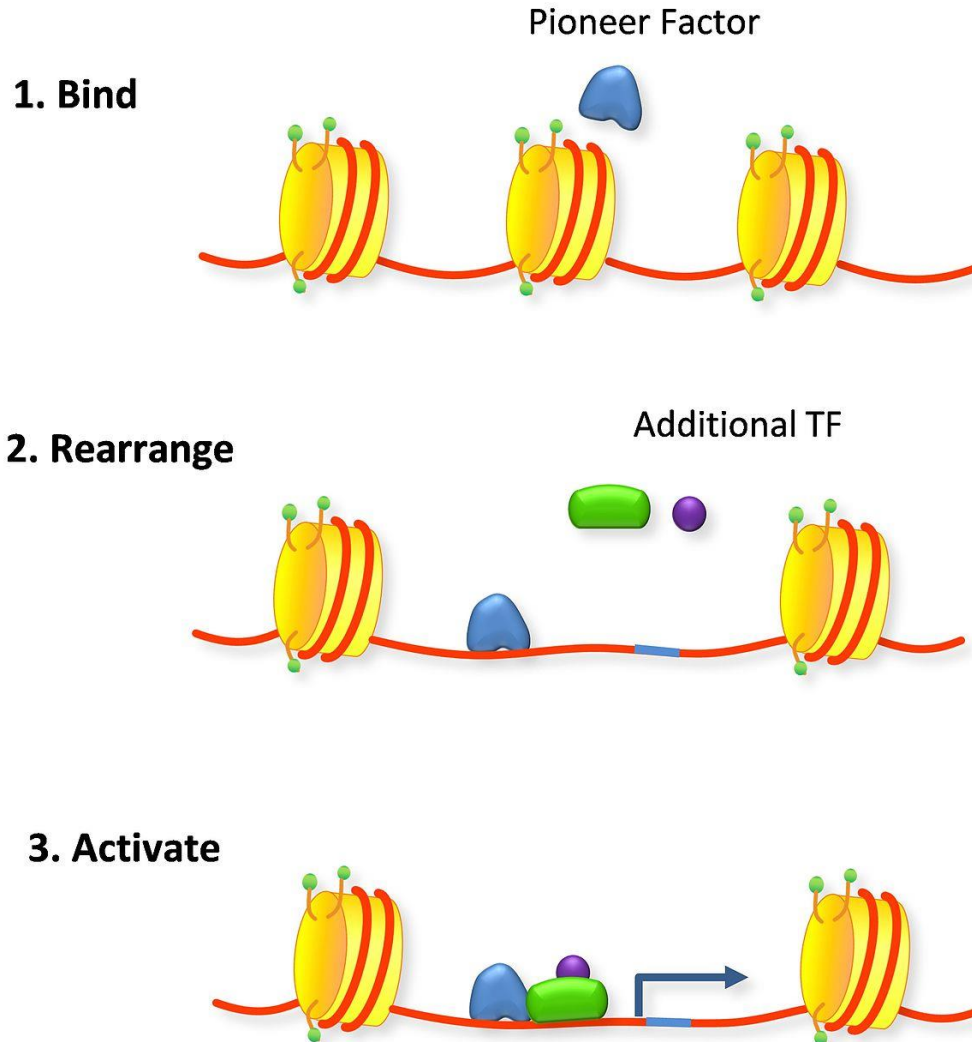


- 1 - independent nucleosome/chromatin binding
- 2 - precedes other factors binding

OCT4 and SOX2 pioneer TFs bound to the nucleosomes



Pioneer transcription factors

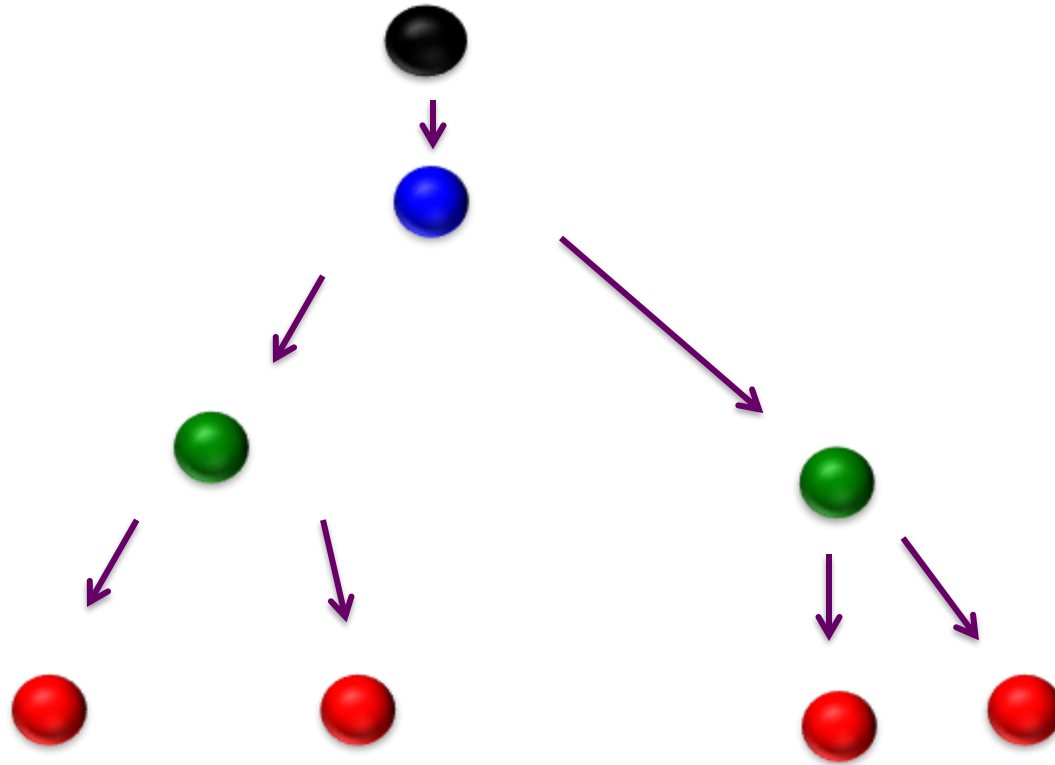


Pioneer transcription factors and plasticity

- Are able to bind nucleosomal DNA
- Are often key regulators of cell fate choices: FoxA1 (liver), Gata1 (erythroid lineage)
- Reprogramming: Oct4, Sox2, Klf4 and Myc → Oct4, Sox2 and Klf4 are pioneer factors

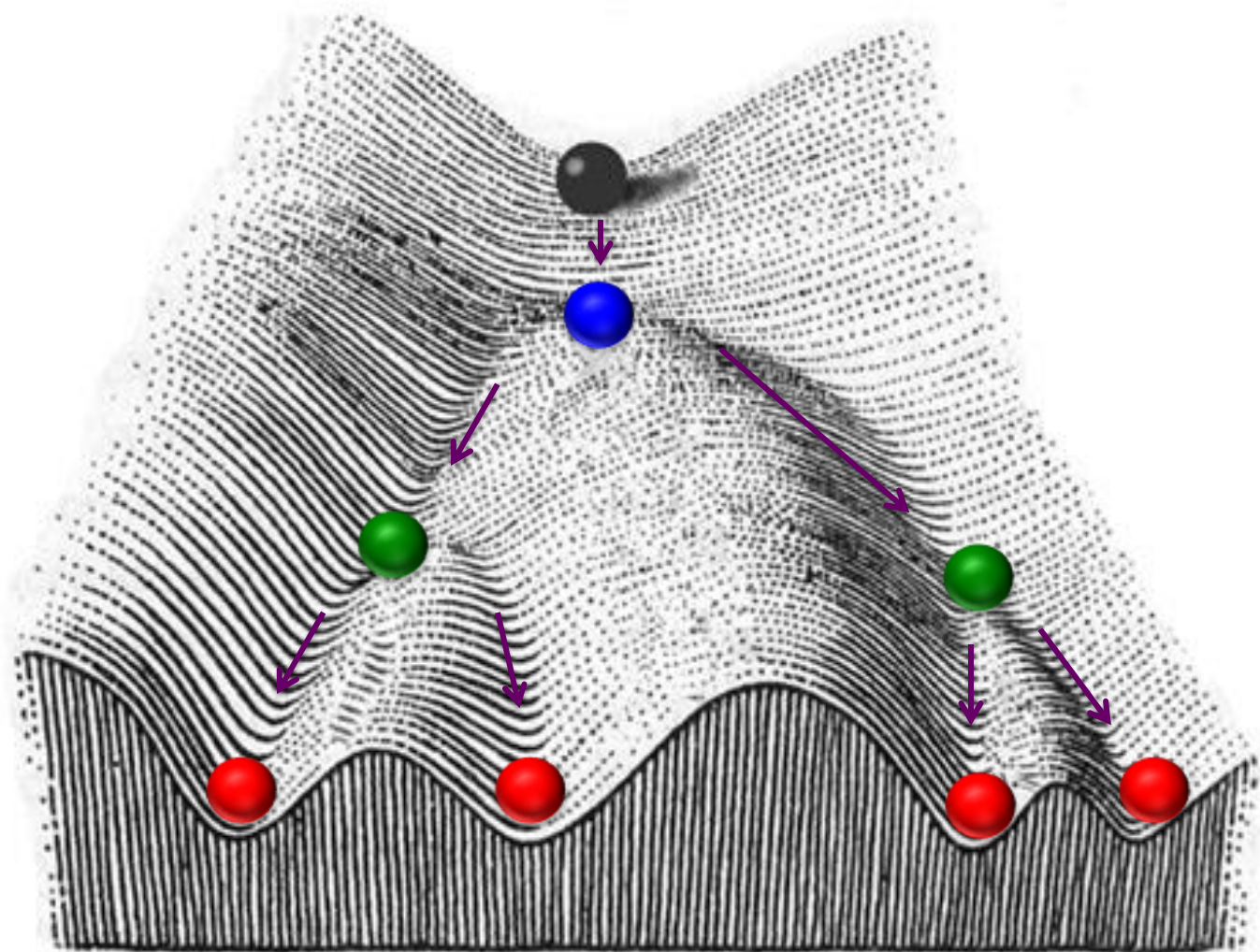
A working model: role of **transcription factors**

→ Define the identity of a cell and the direction of the transitions to a different cell type

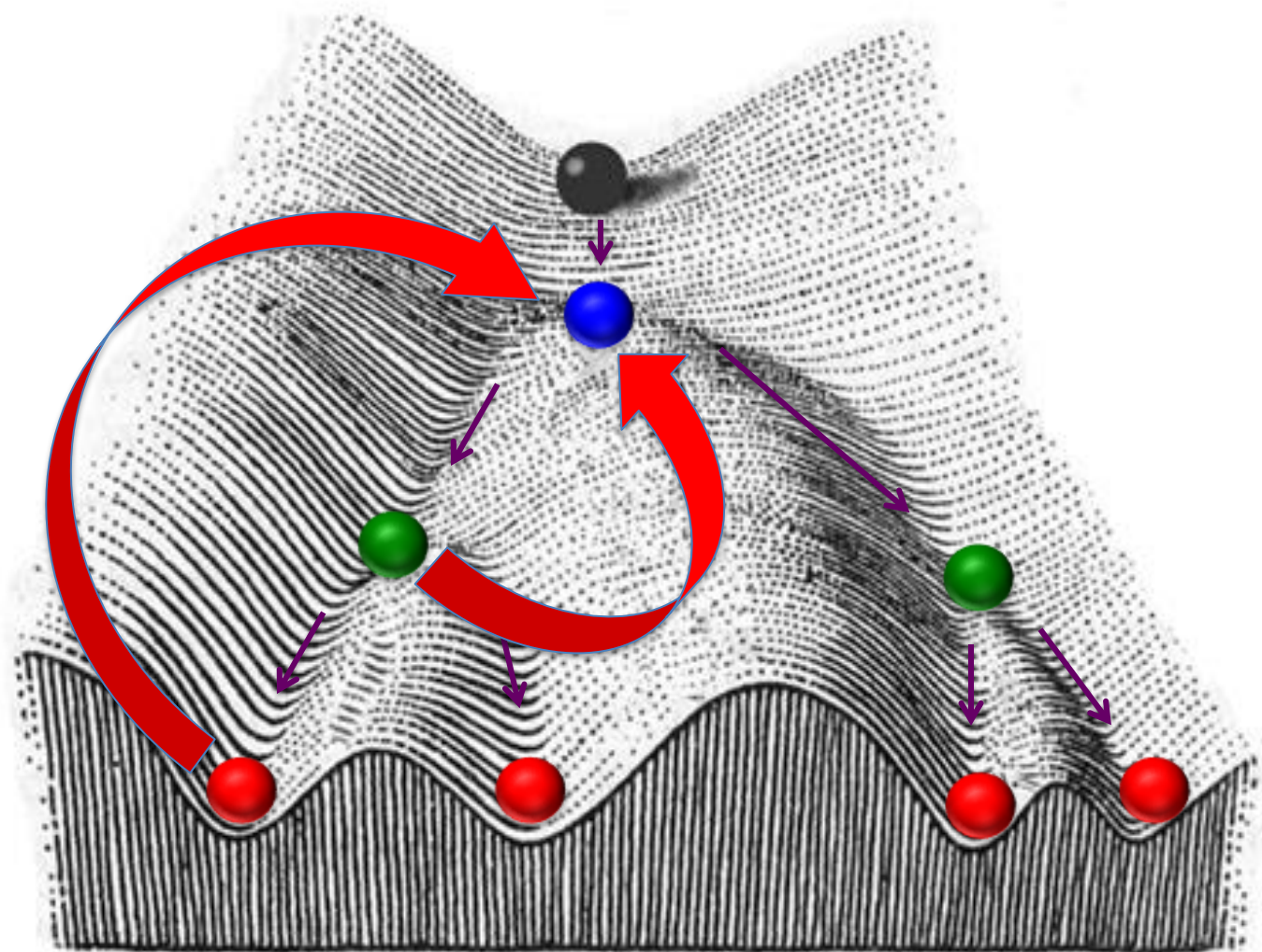


A working model: role of **chromatin state**

→ Defines the height of the hills and the valleys of the Waddington landscape

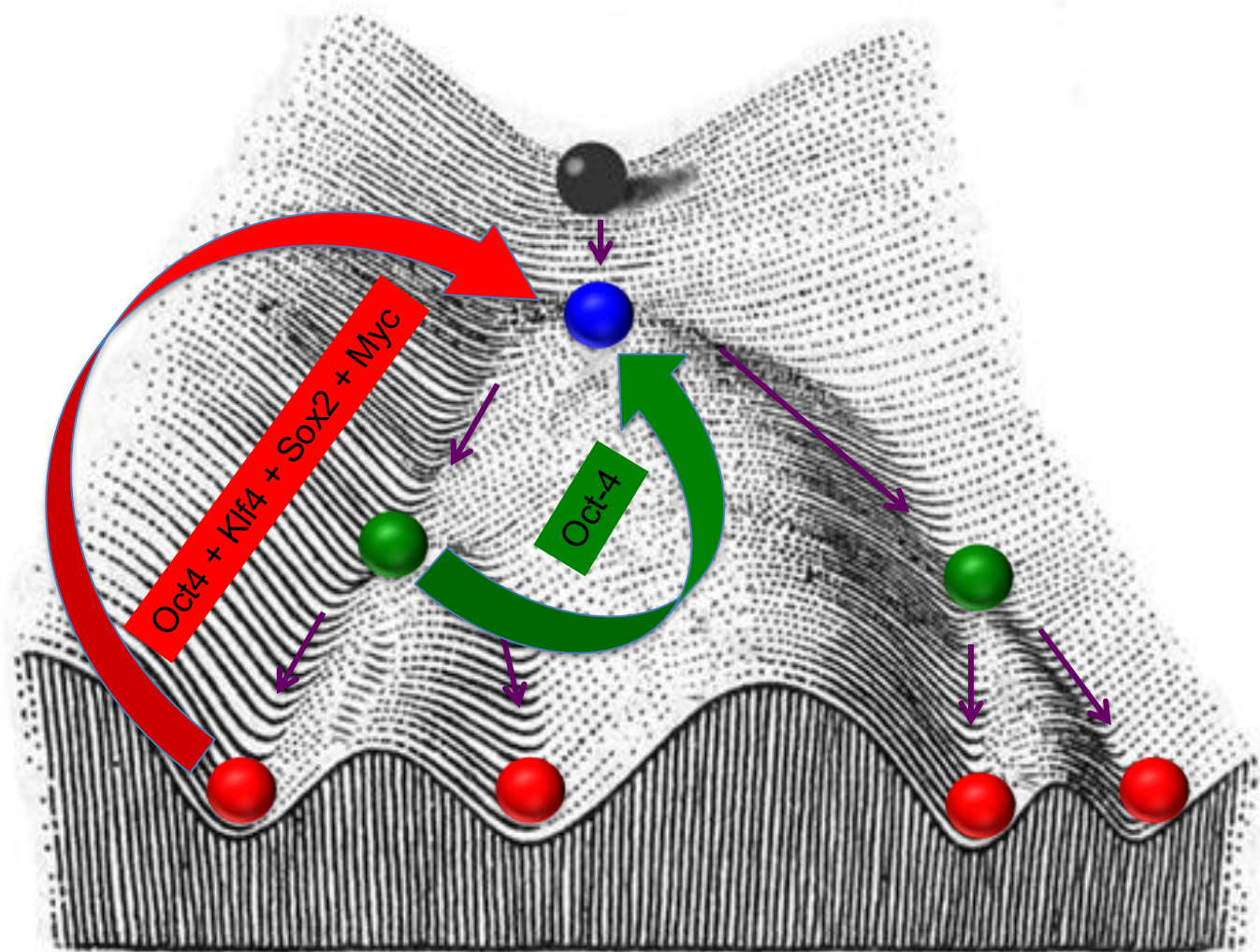


Is it easier to go from adult stem cell state to pluripotency ?



Reprogramming of adult neural stem cells vs fibroblasts

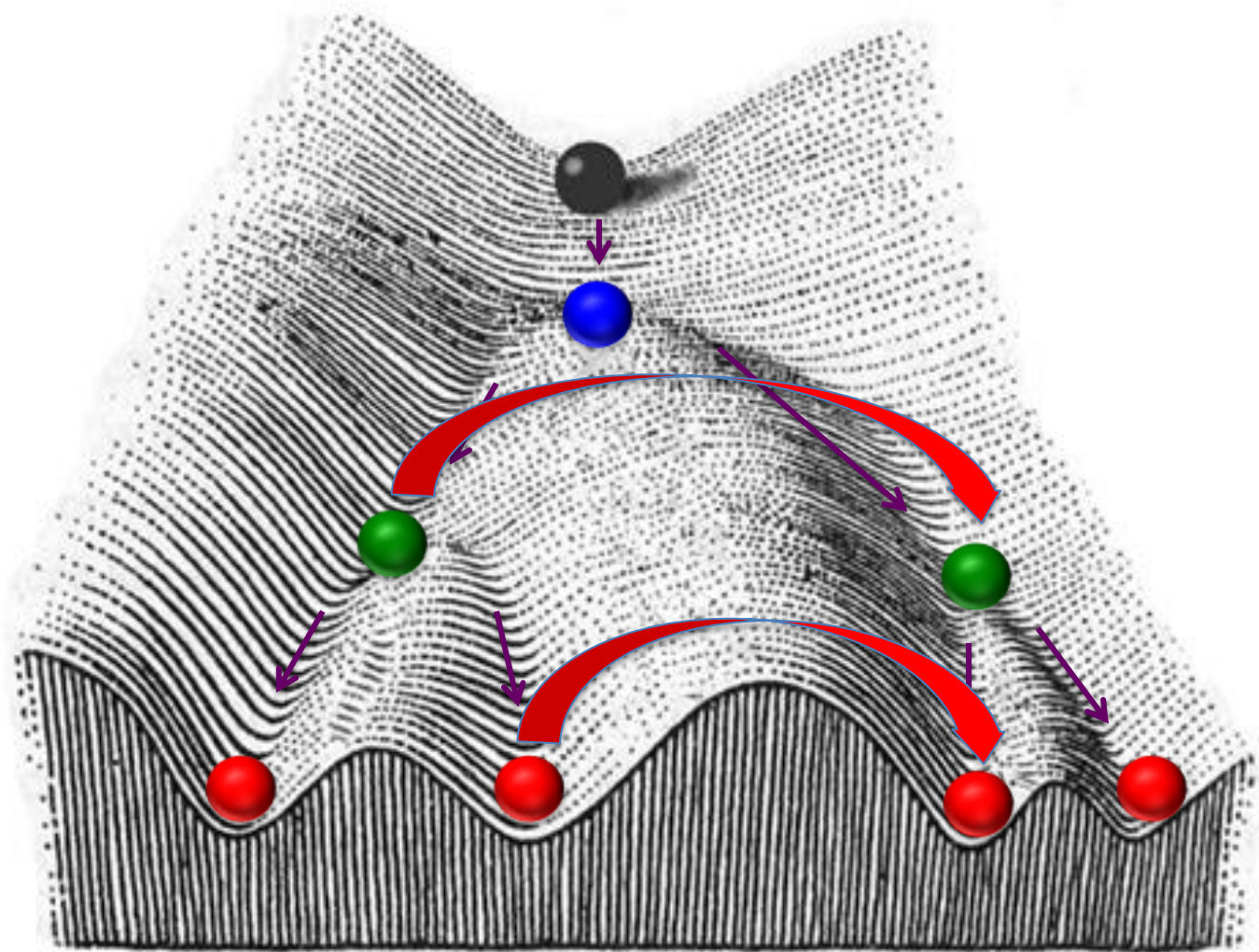
→ Oct-4 alone is sufficient to reprogram neural stem cells to pluripotency



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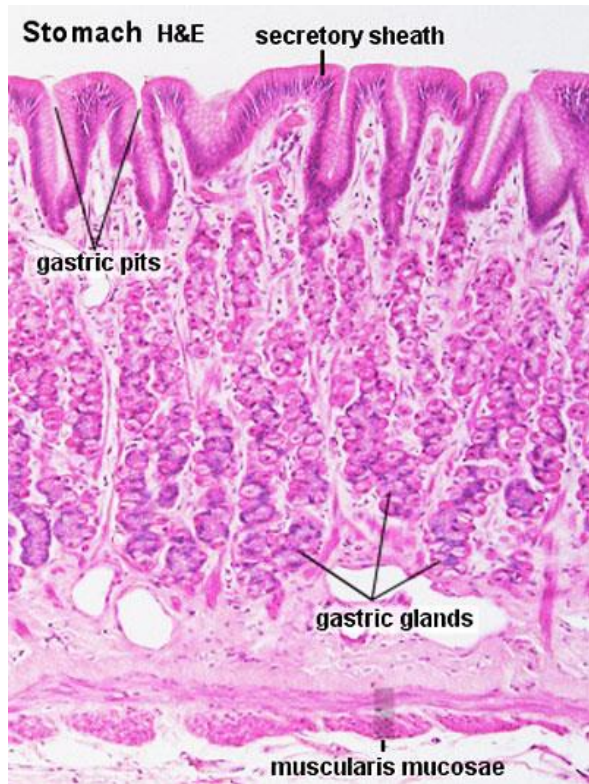
Metaplasia = switch from one cell type to another



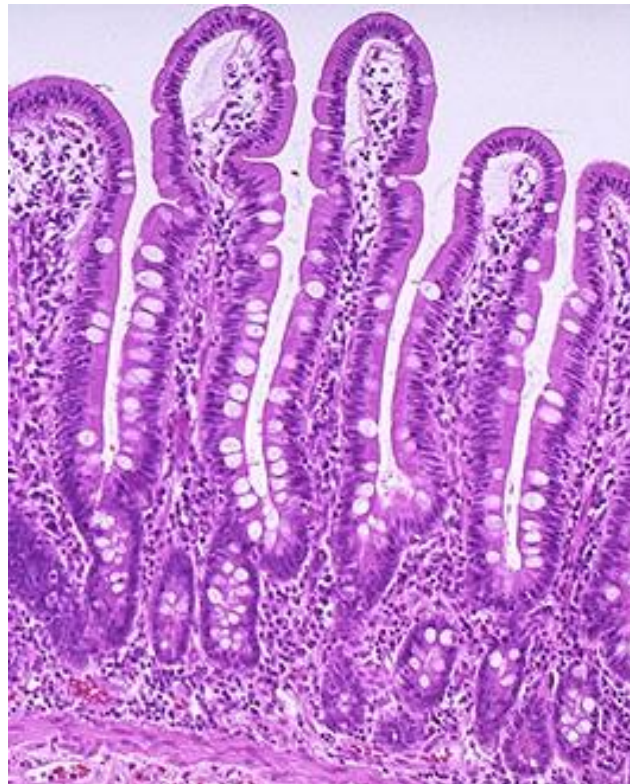
Example: intestinal metaplasia

- During chronic inflammation of the stomach, gastric cells transform into intestinal cells
- This is mediated by an increase in the expression of the transcription factor **Cdx2**

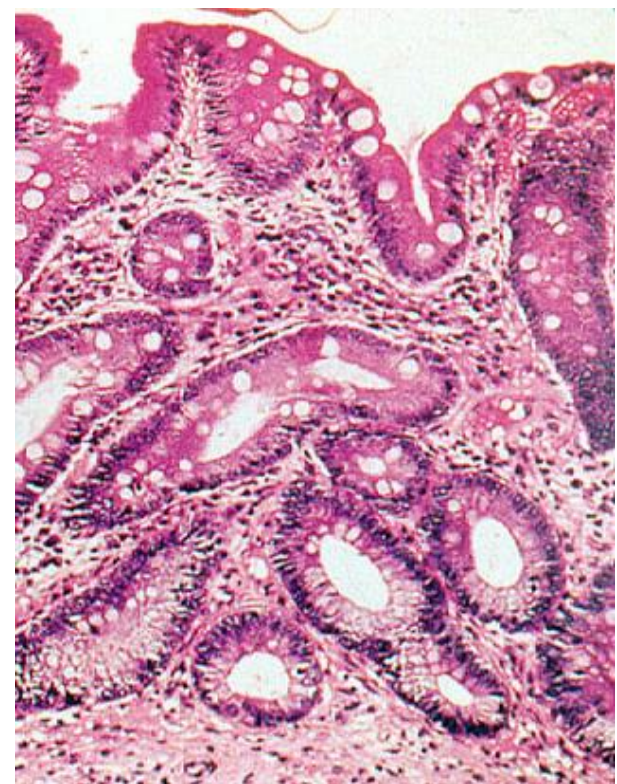
Normal stomach



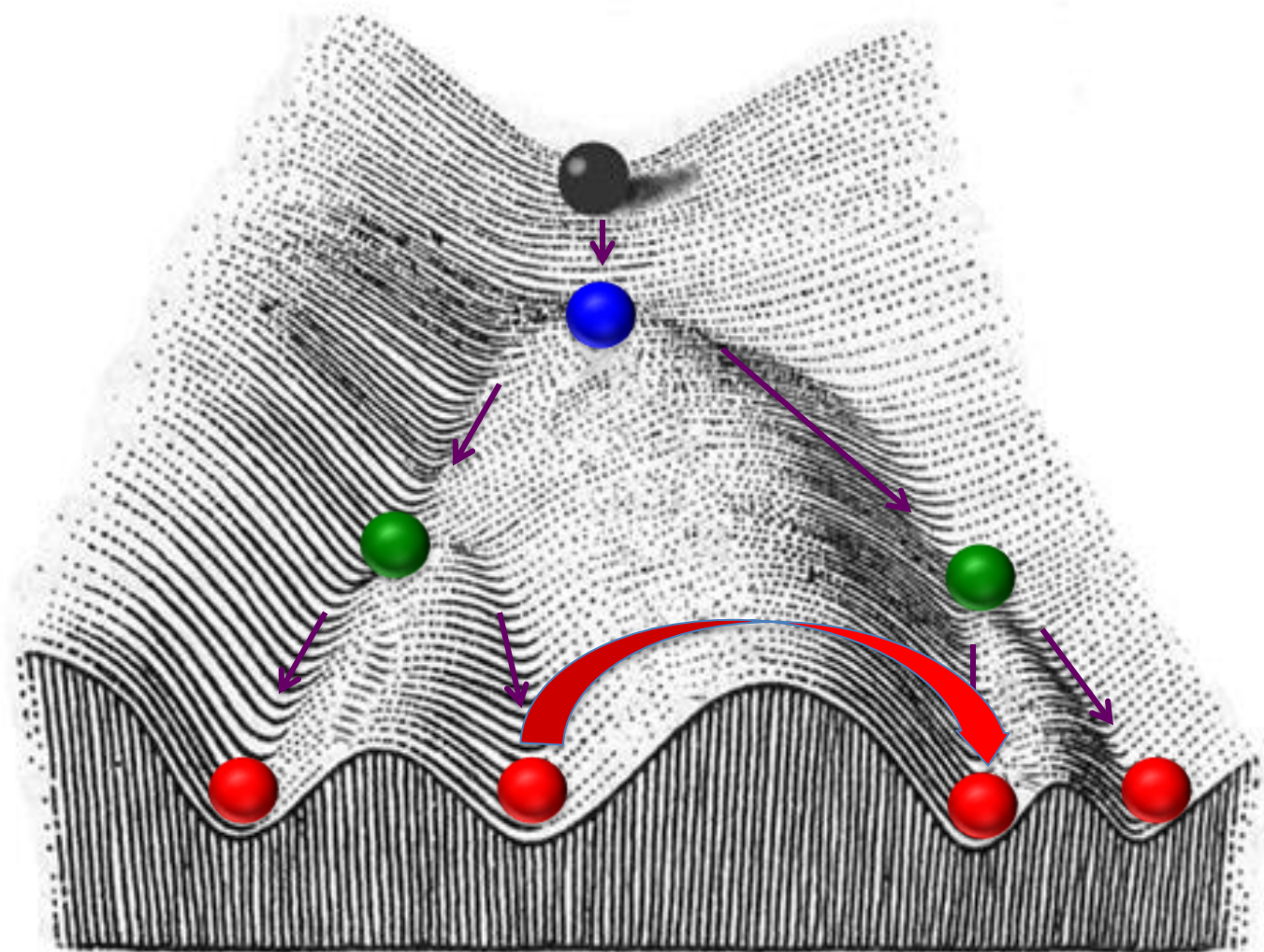
Normal intestine



Intestinal metaplasia



Transdifferentiation = switch from one differentiated cell type to another



Transdifferentiation or direct reprogramming

- Artificial process
- Most often, involves conversion of fibroblasts into various cell types
- Achieved by overexpression of master transcription factors

Example: induced neurons

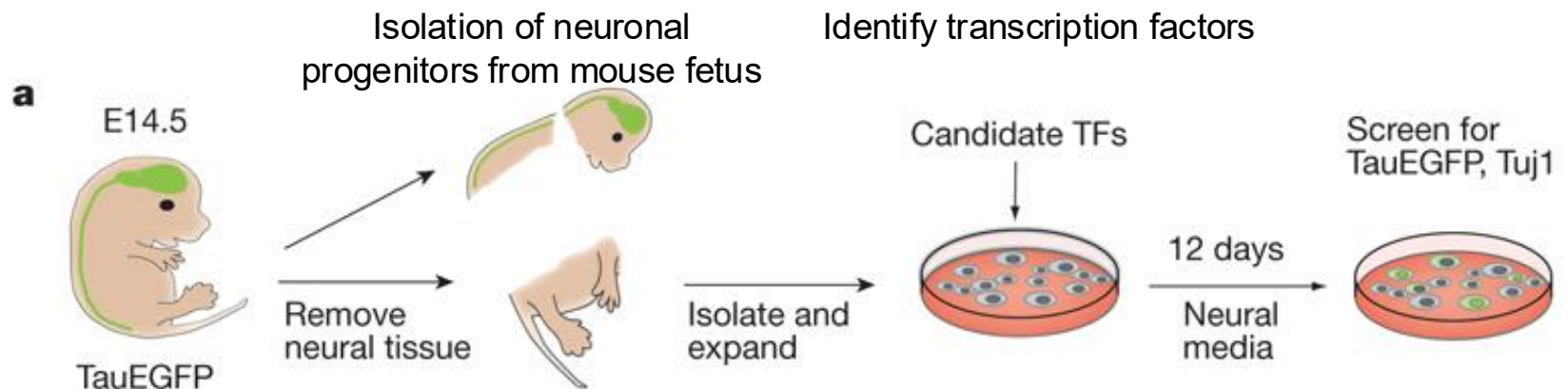
How to define a set of transcription factors allowing direct reprogramming ?

→ Isolate neuronal progenitors from mouse fetal tissue

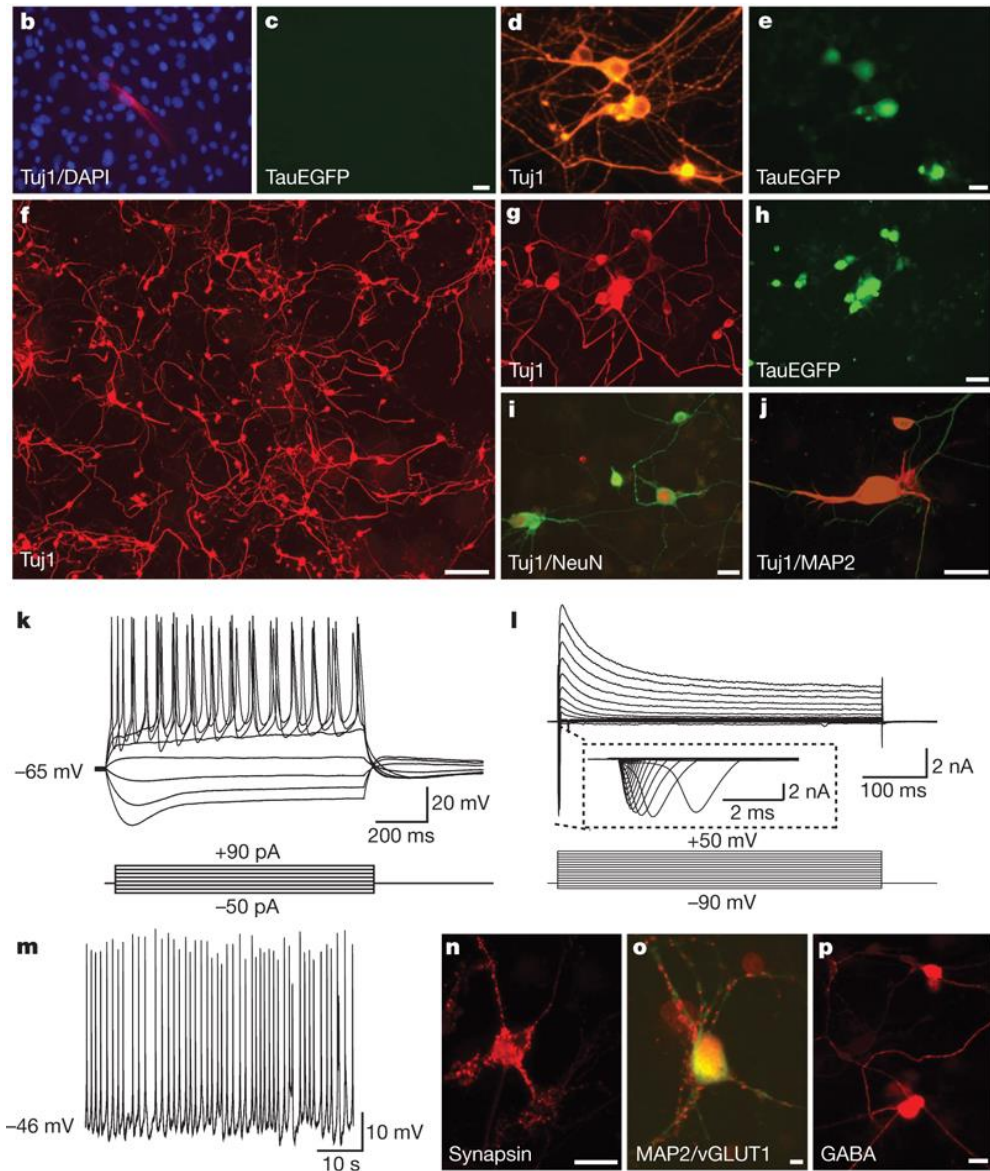
→ Identify 19 candidate transcription factors

→ Express them together in fibroblasts using viral vectors  **Neurons**

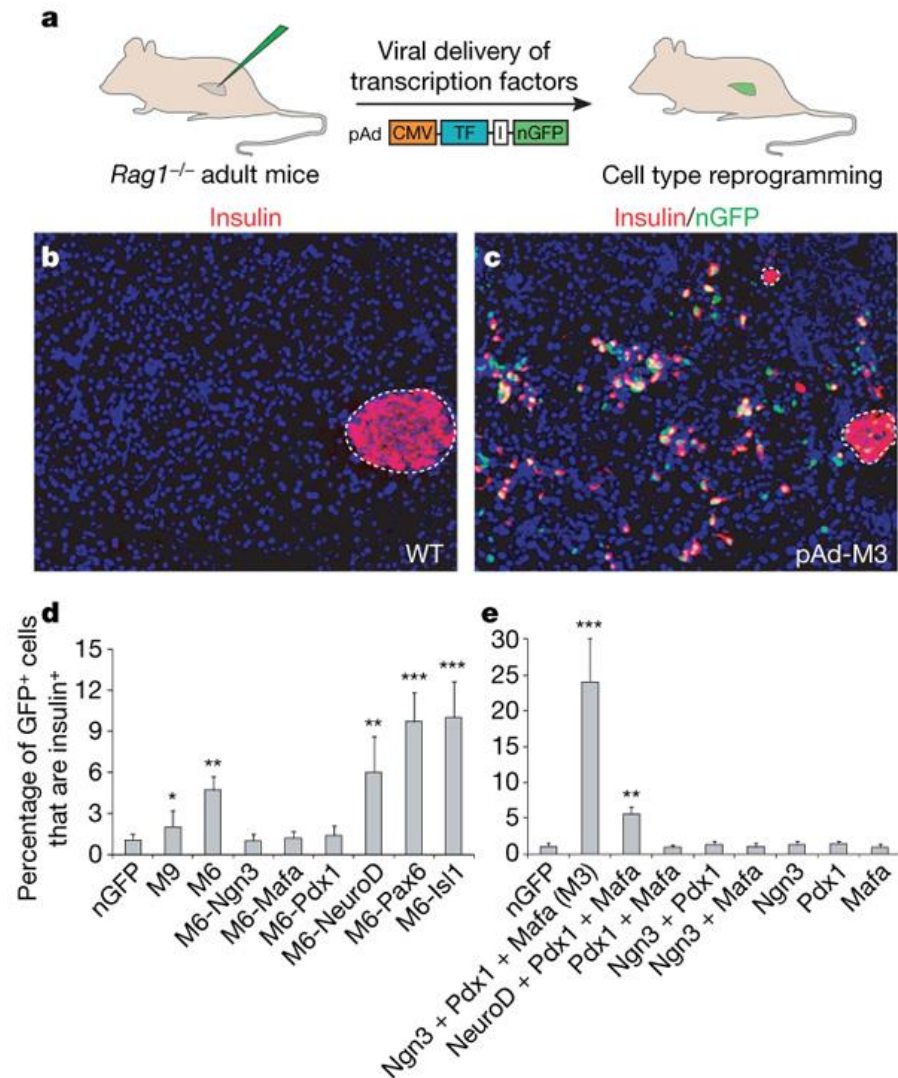
→ Test different subgroups of factors to identify the ones that allow reprogramming



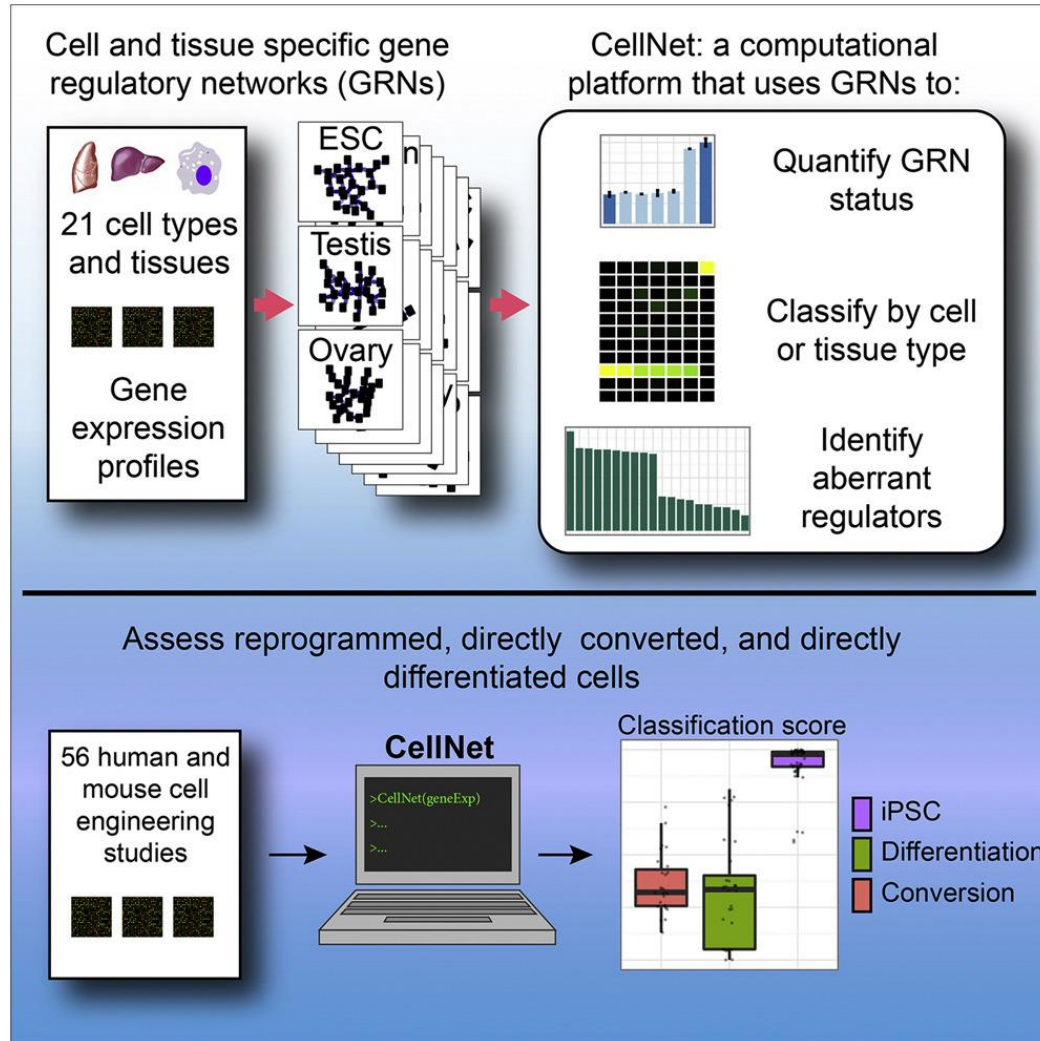
Brn2, Myt1l, Zic1, Olig2 and Ascl1 induced rapid conversion of fibroblasts to neurons



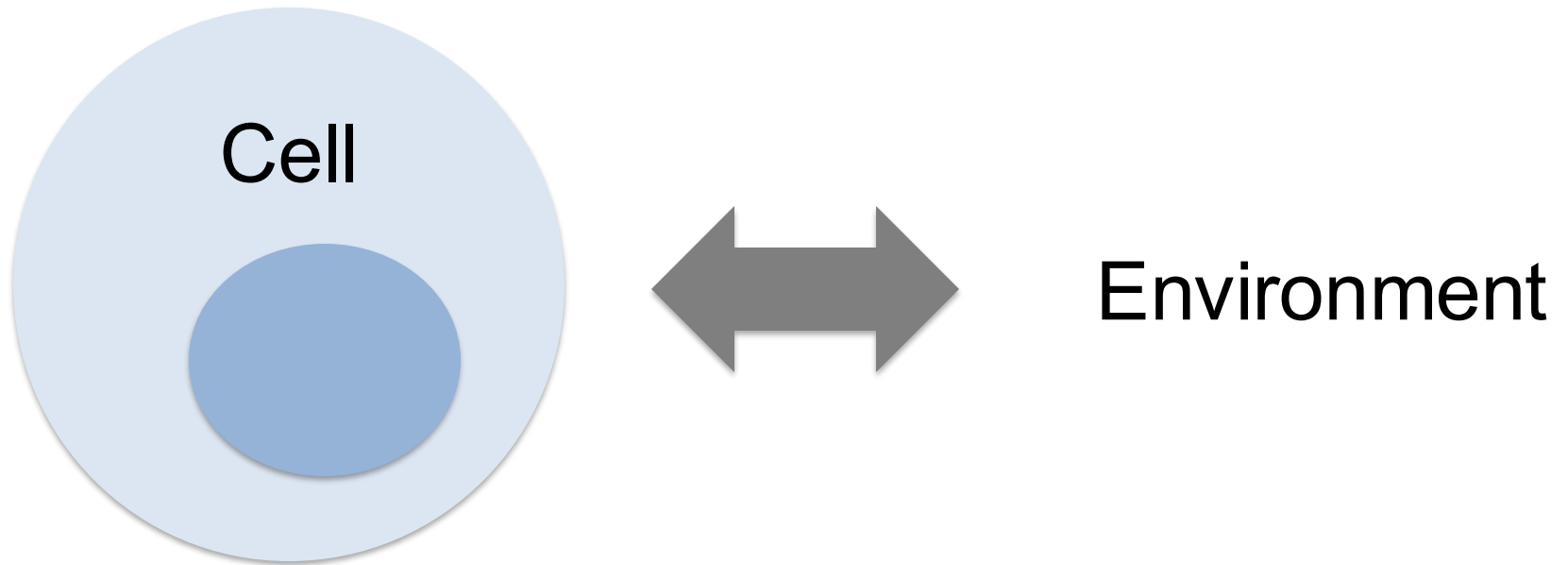
In vivo reprogramming



CellNet: computational prediction of master regulators

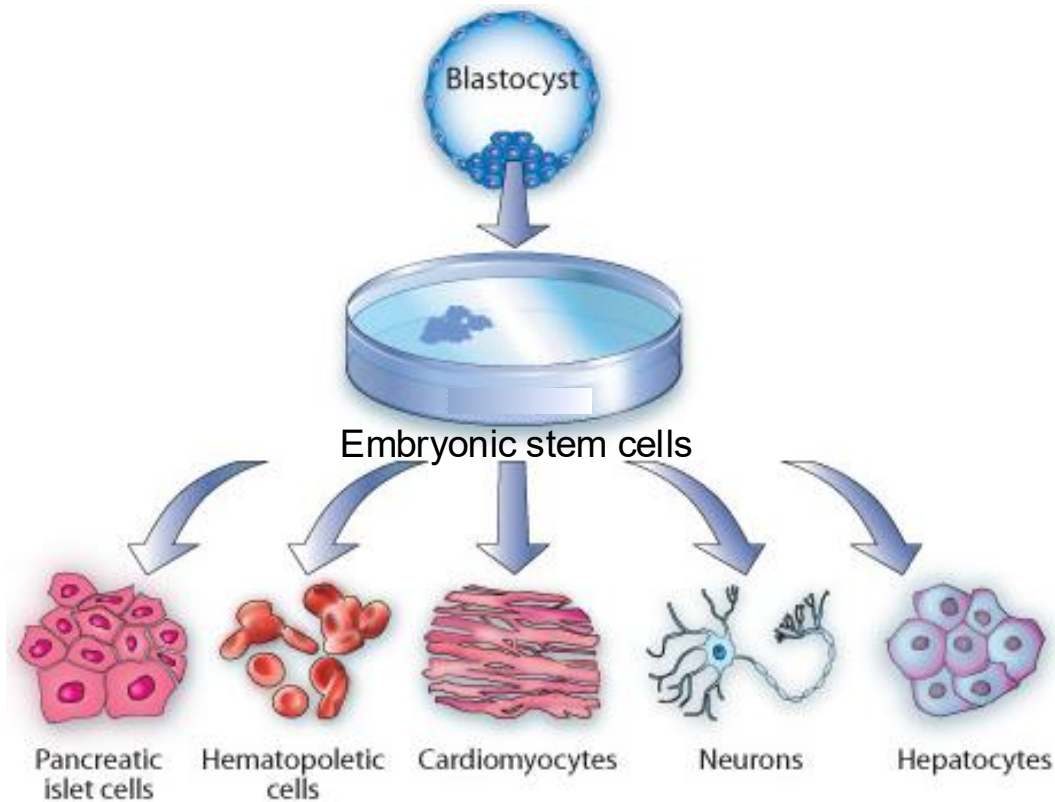


What is the role of the environment in instructing cell fate ?



The environment can instruct cell fate

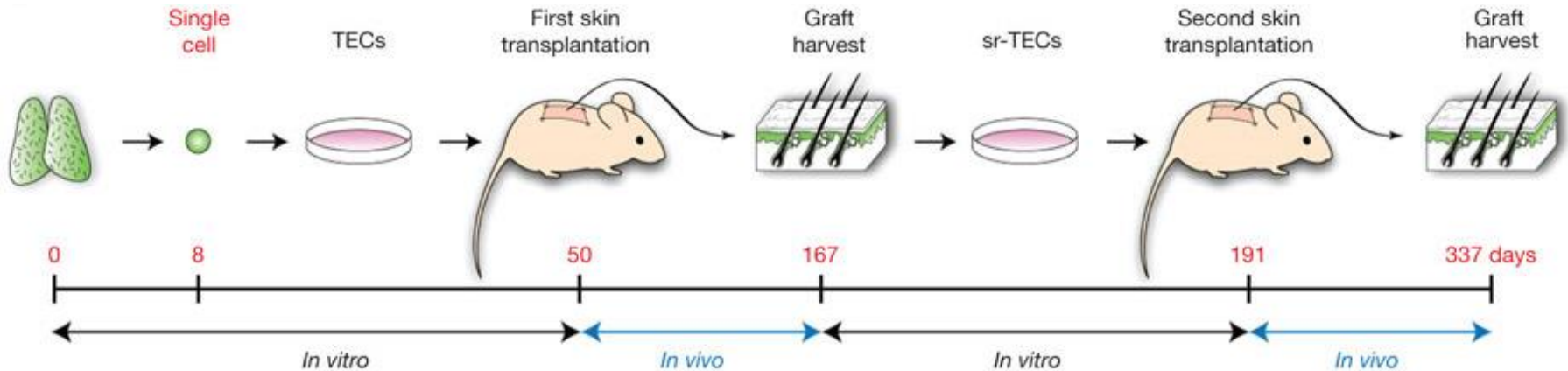
→ Embryonic stem (ES) cells will differentiate into virtually any cell type of the body by changing their culture conditions



→ **ES cells have a high degree of plasticity.** Terminally differentiated cells will generally not change their fate by simple changes of culture conditions.

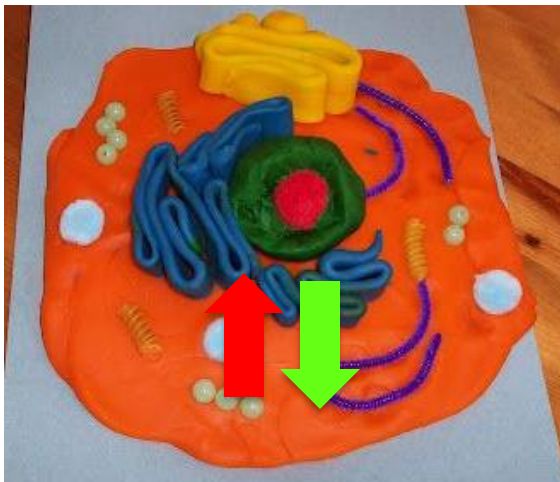
Microenvironmental transdifferentiation

- Isolation of thymic epithelial cells
- Grow them in culture
- Transplant into skin
- Transdifferentiation into skin



Cells with high plasticity

Environment



Modulation of the expression of master transcription factors and downstream genes



Change in cell fate

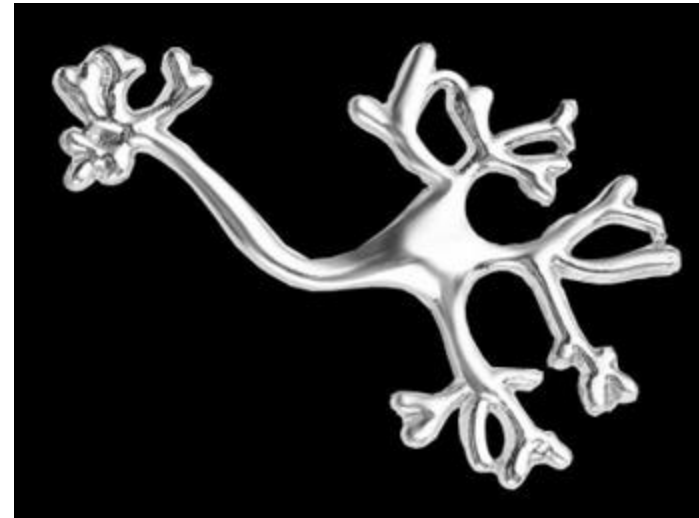


Cells with low plasticity

Environment



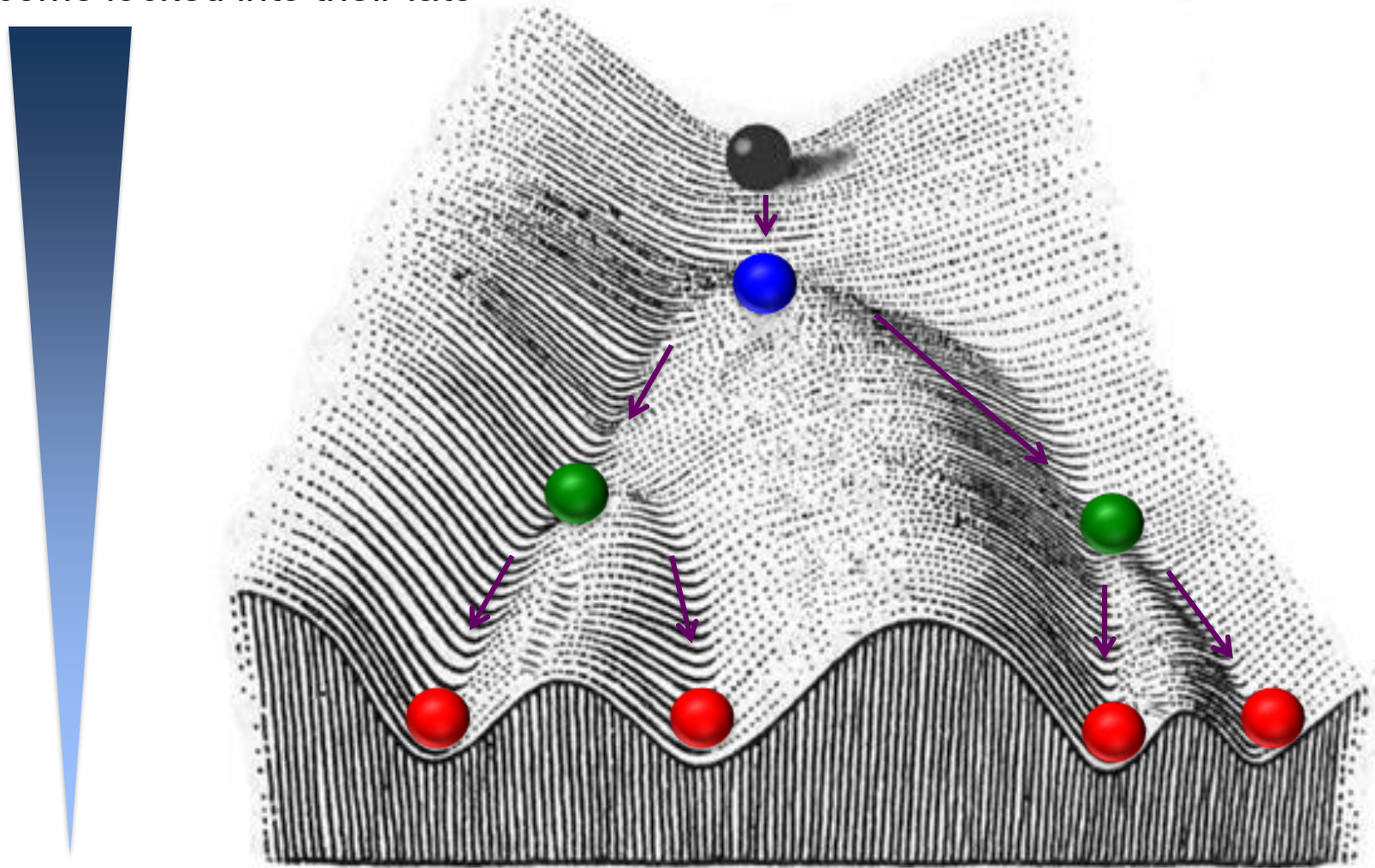
No expression changes of master transcription factors and downstream genes



Role of the environment in establishing cell identities

- Changes in the biochemical and physical environment mediate changes in cell identity
- Cells gradually lose their potential to respond to the environment as they become locked into their fate

Response to the environment

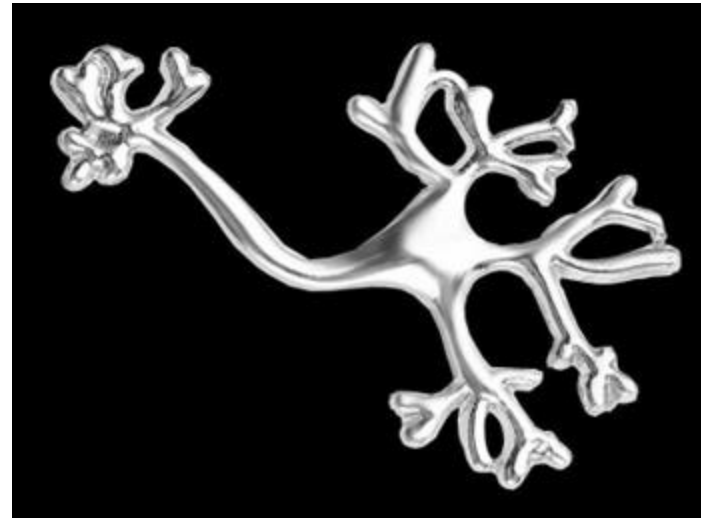


Overcoming low plasticity with master transcription factors

Forced expression of master transcription factors



Change in cell fate



Potential for regenerative medicine

1. Potential of reprogramming/transdifferentiation:

Cells can be directly derived from the patient and reprogrammed into the desired cell type

Good:

- No problem of immune compatibility
- Virtually no ethical issue as compared to ES cells

Bad:

- Most reprogramming/transdifferentiation protocols: involve the introduction of transgenes
- Potentially oncogenic if insertion disrupts tumor suppressor or activates an oncogene
- Reprogramming is not always complete

Potential for regenerative medicine

2. Potential of microenvironmental transdifferentiation:

Cells could be directly derived from the patient and re-implanted in another organ

Good:

- No problem of immune compatibility and no introduction of transgene

Bad:

- Limited number of situations in which this seems to work so far

What you will hear about today

1. Potency and Plasticity
2. The Waddington landscape and epigenetics
3. What defines/determines the identity of a cell ? What are the barriers to changes in cell identity ?
4. Crossing cell identities
- 5. Plasticity of adult stem cells**

Plasticity of adult stem cells

- Stem cells are by definition plastic, but different types of adult stem cells differ by their level of plasticity
- Plasticity can also become more restricted during development or aging

Plasticity of adult stem cells

1. Some stem cells keep a high level of plasticity from embryogenesis to adult life

→ Hematopoietic stem cells

→ Epidermal stem cells

→ Intestinal stem cells

Typical for rapidly regenerating tissues

Plasticity of adult stem cells

2. Other stem cells lose some of their plasticity over time

→ Neural stem cells:

- Embryonic development: allow to generate hundreds of different neuronal cell types.
- Adults: very limited capacity to regenerate neurons.

Summary

- Plasticity can be defined as the potential of a cell to change its identity
- Transcription factors and chromatin state play a key role in plasticity and changes in cell fates
- Cells with low plasticity can still change their fate upon artificial expression of transcription factors
- The environment can reprogram plastic cells to alternative fates
- Adult stem cells retain various degrees of plasticity

Next lecture 26.09
Neural Stem Cells