

Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 28

**Embryonic stem cells
and**

Transcription factor-mediated epigenetic reprogramming

Regulation of gene expression during embryonic development

Lecture 24	Gene Regulation during Drosophila Development
Lecture 25	Signal transduction pathways involved in Development
Lecture 26	Homeotic genes
Lecture 27	Epigenetic regulation of gene expression during development

Lecture 28	Embryonic stem cells and transcription Factor-mediated Epigenetic Reprogramming
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Normal development depends on a precise sequence of changes in the configuration of the chromatin, which are primarily related to the acetylation and methylation status of histones and the methylation of genomic DNA.

These epigenetic modifications control the precise tissue-specific expression of genes.

The correct pattern of cytosine methylation in CpG dinucleotides is required for normal mammalian development.

During mammalian development, the paternal DNA is actively and rapidly demethylated after fertilization, while the maternal DNA undergoes passive demethylation.

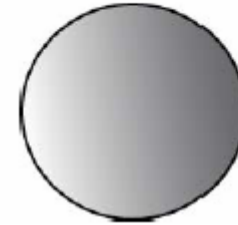
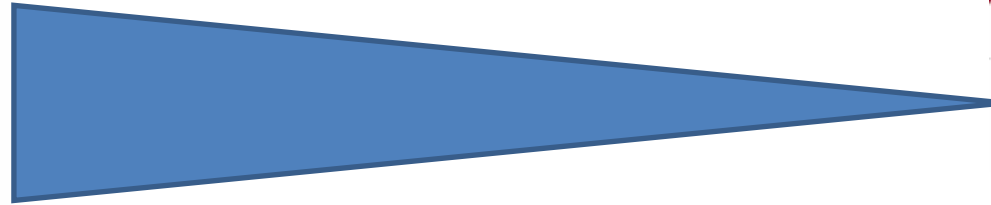
The embryonic DNA is remethylated between the two-cell and the blastocyst stages in waves, which correlates with the onset of transcription of the zygotic genes.

These mechanisms ensure that critical steps during early development, such as the timing of first cell division, compaction, blastocyst formation, expansion and hatching are regulated by a well-orchestrated expression of genes.

pluripotent cell

DEVELOPMENT POTENTIAL

unipotent cell

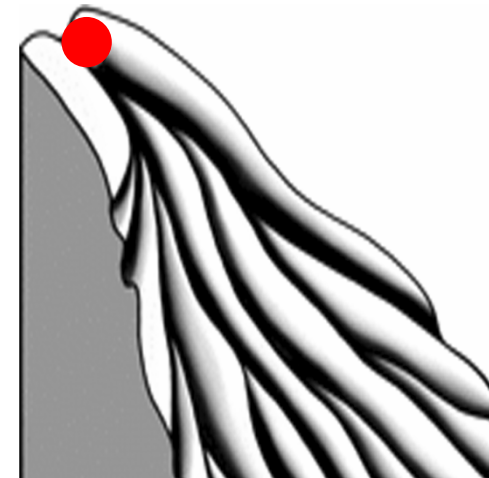


A long-held dogma in developmental biology is that mammalian somatic cell differentiation is irreversible

The process of differentiation is comparable to a ball rolling down a hill with valleys.

When the ball is on top of the hill, it can roll down through any valley below, i.e., a totipotent cell can differentiate into any tissue of the body.

However, once the ball reaches the bottom of the hill, it cannot move to another valley or back to the top of the hill. i.e., once a cell is terminally differentiated, it can no longer transdifferentiate into another cell type or become totipotent again.



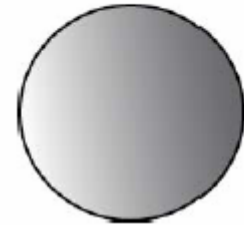
pluripotent cell



REPROGRAMMING



unipotent cell



In 1952, Robert Briggs and Thomas King, working on a frog, *Rana pipiens*, transplanted nuclei from blastula into enucleated eggs, which then developed into normal embryos.

The cells in blastula are still in undifferentiated stage.

Briggs, R. & King, T. J. Transplantation of living nuclei from blastula cells into enucleated frogs' eggs.

Proc. Natl Acad. Sci. USA **38**, 455–463 (1952)

Can you produce a normal embryo from a nucleus taken from a differentiated cell?

In the late 1950s and early 1960s, Gurdon transplanted intestinal epithelium-cell nuclei from *Xenopus* tadpoles into enucleated frog eggs and produced 10 normal tadpoles: Molly and her fellow clones.

This demonstrated that nuclei of differentiated cells retain their totipotency.

The genome remains intact during differentiation and that the epigenetic changes to the somatic-cell nucleus are reversible.

Gurdon, J. B. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles.

J. Embryol. Exp. Morphol. **34**, 93–112 (1962)

Dolly

(5 July 1996 – 14 February 2003)

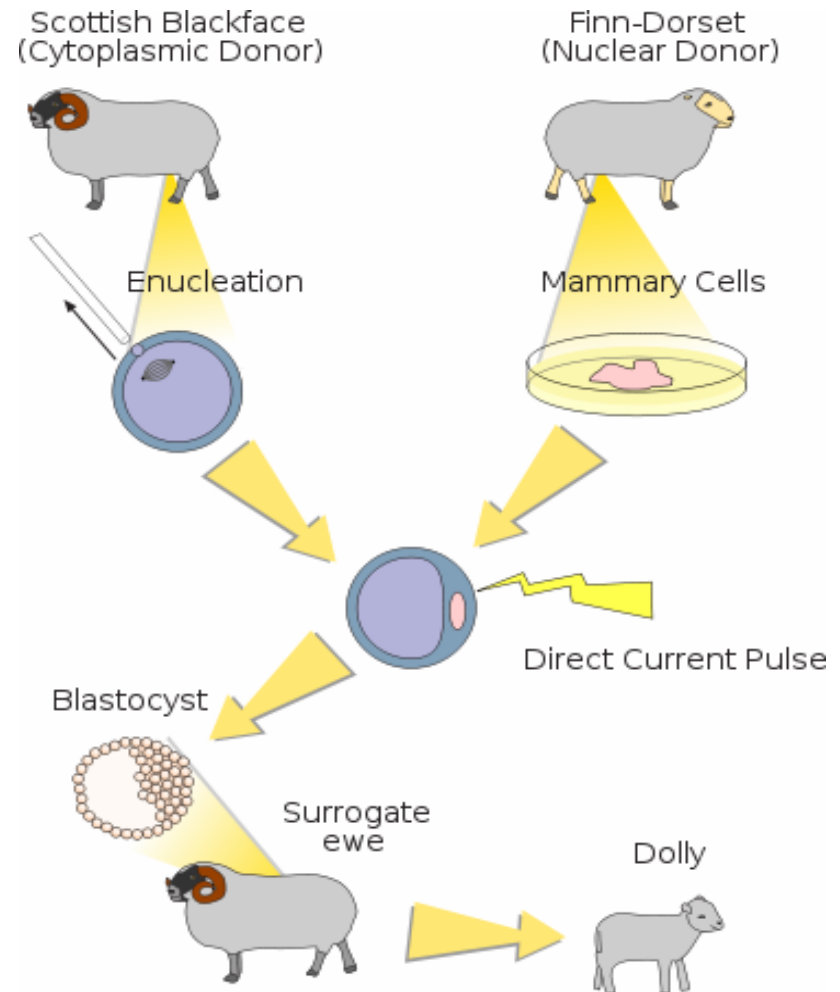
Dolly was a female domestic sheep who was the first mammal to be cloned from an adult somatic cell (mammary gland), using the process of somatic cell nuclear transfer.

She was cloned by **Ian Wilmut, Keith Campbell and colleagues** at the Roslin Institute near Edinburgh in Scotland.

Dolly was born on 5 July 1996 and she lived until the age of six

Wilmut, I. et al. Viable offspring derived from fetal and adult mammalian cells.

Nature **385**, 810–813 (1997)



Subsequently, somatic cloning was demonstrated in other species, such as the cow, mouse, goat, pig, cat, and rabbit

Gurdon, J.B., and Byrne, J.A. (2003). The first half-century of nuclear transplantation. *Proc. Natl. Acad. Sci. USA* 100, 8048–8052.

Jaenisch and his colleagues generated mice from B lymphocytes that had undergone immunoglobulin rearrangement

Hochedlinger, K., and Jaenisch, R. (2002). Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature* 415, 1035–1038.

A major concern in these experiments involving **somatic cell nuclear transfer (SCNT)** experiments is the low CLONING EFFICIENCY. At present <1% of nuclear-transfer embryos develop to adulthood.

SCNT was a hit-and-miss procedure

However, the efforts of Briggs, King, Gurdon and Wilmut are very significant since it demonstrated that **cell differentiation is not irreversible and differentiated nuclei can be reprogrammed to achieve totipotency.**

These findings had enormous implications in the fields of stem-cell biology and epigenetics.

Somatic cell nuclear transfer (SCNT)

The expression profile of a differentiated cell is abolished and the new embryo-specific expression profile is established to drive embryonic and foetal development.

This involves abolishing the expression of ~8000–10000 genes of the somatic cell program and the initiation of the embryonic program with ~10000 genes.

Pre-zygotic reprogramming includes the erasure of somatic cell epigenetic modifications and is followed by a post-zygotic establishment of embryonic modifications.

Other post-zygotic reprogrammings involve X-chromosome inactivation and adjustment of telomere lengths.

The story of STEM CELLS

What Are Stem Cells?

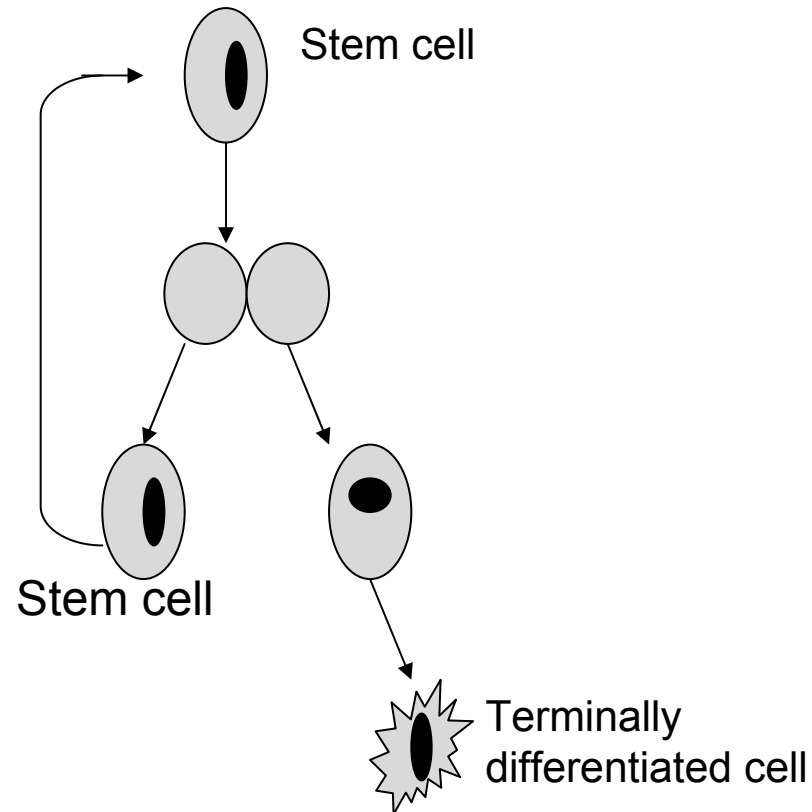
Stem cells are undifferentiated cells that can reproduce themselves indefinitely and under appropriate conditions develop into a wide variety of differentiated cells with specialized functions.

Two important characteristics of stem cells:

-Self-renewal

- Pluripotency

A stem cell is a clonal, self-renewing entity that is multipotent and thus can generate several differentiated cell types.



Types of Stem Cells

Embryonic stem cells (ES cells)

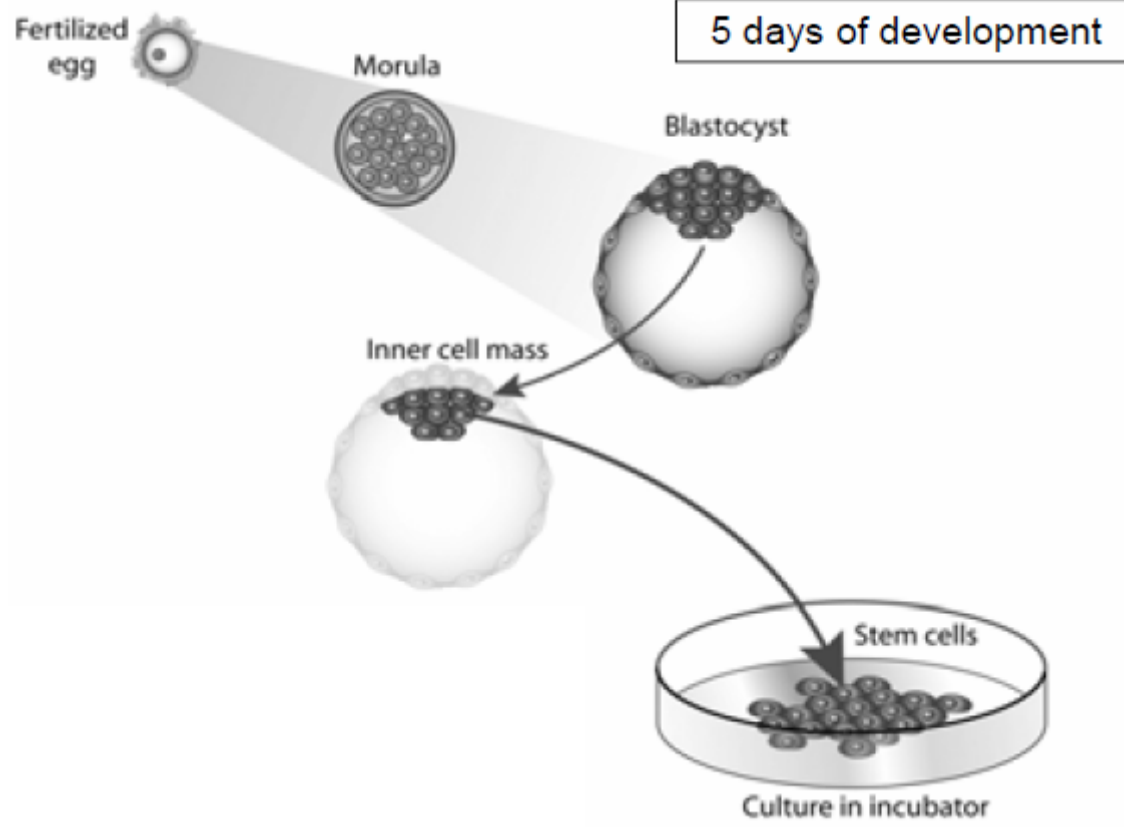
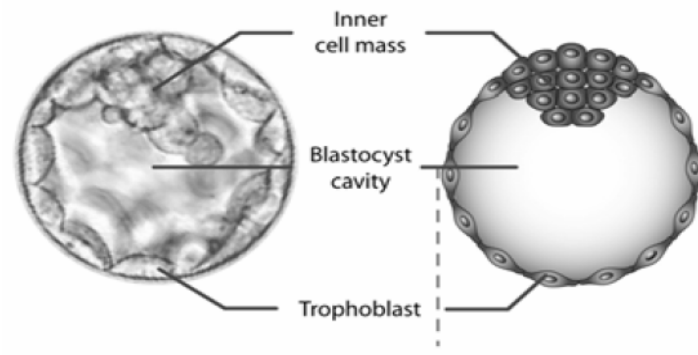
They come from a 4 to 7-day-old embryo (**blastocyst**) . They have the ability to form virtually any type of cell found in the human body.

Adult stem cells

These are more specialized than embryonic stem cells. They are found in the majority of tissues and organs in our body and generate the mature cell types within that tissue or organ. They have a restricted ability to produce different cell types and to self-renew.

Adult stem cells are present in tissues where cell turnover is high.
Ex. Skin, Digestive tract, Bone marrow etc.

What is a blastocyst?



The concept of stem cells dates back to 1960s when researchers were trying to do bone marrow transplants as well as study a group of cells known as teratocarcinomas, which are tumors derived from an embryo.

These teratocarcinoma cells could be grown in cell culture indefinitely and could be induced to differentiate into specific cell types. These were named as **embryonic carcinoma (EC)** cells.

EC cells became very useful tools to understand early embryonic development. However, they harbored genetic mutations and exhibited abnormal karyotypes.

It therefore became necessary to isolate pluripotent embryonic stem (ES) cells directly from the inner cell mass of developing embryo.



Sir Martin J. Evans

The Nobel Prize in Physiology or Medicine 2007 was awarded jointly to Mario R. Capecchi, Sir Martin J. Evans and Oliver Smithies *"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"*.

Leroy Stevens at the Jackson Laboratory found that a specific strain of mice (129Sv strain) has a high propensity to develop testicular tumours and such tumours contain totipotent cells.

He showed that their cells could develop into embryoid bodies, i.e. aggregates of embryonic cells. When transplanted, such aggregates could induce solid tumours with many different cell types. A few years later, it was shown that such tumours were derived from undifferentiated **embryonal carcinoma (EC)** cells

Stevens LC, Little, C. C. Spontaneous testicular teratomas in an inbred strain of mice. Proc Natl Acad Sci (USA). 1954;40:1080-1087.

Stevens LC. Embryonic potency of embryoid bodies derived from a transplantable testicular teratoma of the mouse. Dev Biol. 1960;2:285-297.

Martin Evans injected these totipotent EC cells into blastocysts and reimplanted them into foster mice and generated offspring that was chimeric, with contributions from EC cells in nearly every tissue.

However, these chimeric mice carrying EC derived cells developed multiple tumours and could not contribute to the germ line due to karyotypic abnormalities.

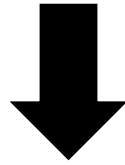
With the use of monoclonal antibodies, he characterized cell surface macromolecules of EC cells and their normal counterparts, thus identifying molecular markers of early differentiation.

The results suggested that normal cells with a similar phenotype as EC cells could be found and used for experiments. In 1980, Evans teamed up with the embryologist Matt Kaufman to combine cell culture and embryo manipulation.

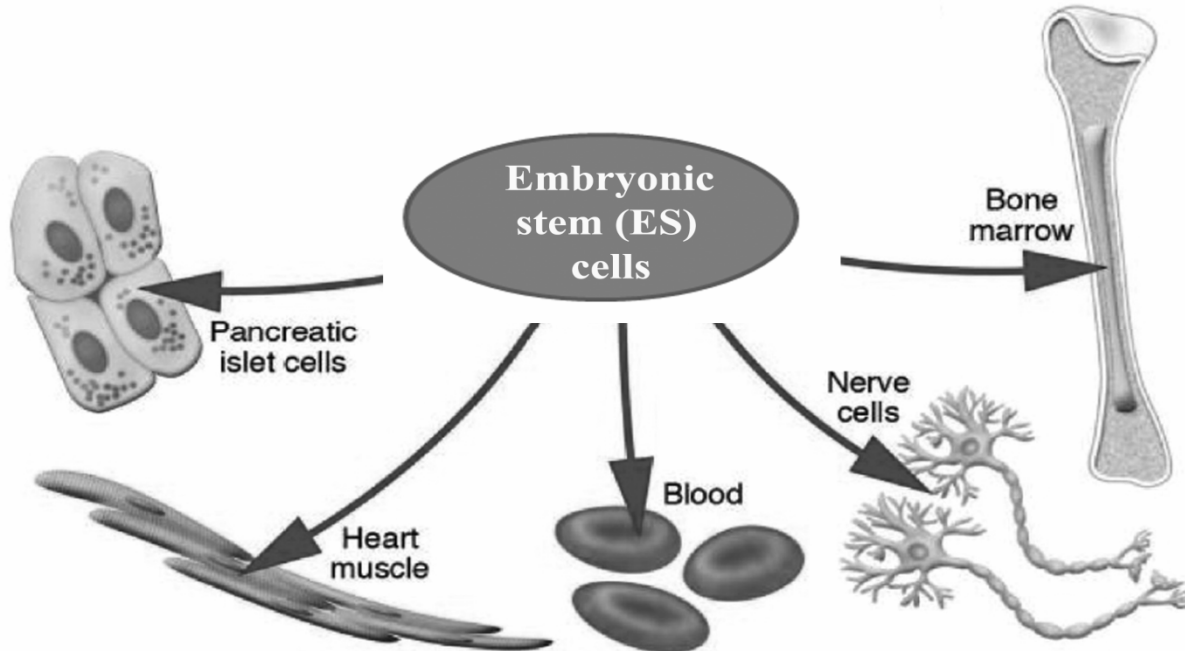
He started culturing cells from blastocysts and started to get EC-like cells which formed teratomas in vivo and differentiated in vitro.

These cells were named **embryonic stem cells (ES cells)**

The importance of ES cells



Culture these pluripotent stem cells *in vitro* as Embryonic stem (ES) cells and induce them to differentiate into specific cell types



Thus, ES cells have tremendous potential both as research tools to understand the mechanism by which transcription factors induce cell differentiation during development as well as in regenerative medicine.

**How to culture ES cells without losing
their totipotency?**

In the year 1981, the isolation of ES cells from mouse embryo was first described in 1981 by Martin Evans and Matthew Kaufman.

Evans M, Kaufman M (1981) Establishment in culture of pluripotent cells from mouse embryos. *Nature* **292**: 154–156.

Martin G (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA* **78**: 7634–7638.

In the year 1998, researchers, lead by James Thomson at the University of Wisconsin at Madison, first developed a technique to isolate and grow human embryonic stem cells in cell culture

Thomson J, Itskovitz-Eldor J, Shapiro S, Waknitz M, Swiergiel J, Marshall V, Jones J (1998) Embryonic stem cell lines derived from human blastocysts. *Science* **282**: 1145–1147.

Culturing embryonic stem cells derived from the inner cell mass of the early embryo.

Following isolation from the embryo, these cells are cultured on plates containing feeder cells which are fibroblasts that are rendered mitotically inactive by treatment with cells mitomycin-c. Cells cultured in this fashion form teratomas and embryoid bodies and can be induced to differentiate *in vitro* indicating that these cells are pluripotent.

Once it became possible to culture ES cells without loss of pluripotency, it became possible to identify the factors that are essential for retention of their pluripotency.

For ex., it was discovered that the feeder cells provide a factor known as the **leukemic inhibitory factor (LIF)** and serum provides **bone morphogenetic proteins (BMPs)** that are necessary to prevent ES cells from differentiating. These factors are extremely important for the efficiency of deriving ES cells.

Deriving mouse ES cells and culturing them without loss of pluripotency is a major challenge.

Deriving human ES cell and culturing them in a pluripotent state has been an even more daunting task.

Molecular basis of pluripotency

1. Extracellular environments:

mouse embryonic fibroblast feeder layer to maintain ES pluripotency– effects of LIF (leukemia inhibiting factor), also known as differentiation inhibiting activity; LIF expressed in trophectoderm and LIF receptor in inner cell mass (ICM), a paracrine interaction

2. Signaling cascades to the stem cell nucleus:

gp130 receptor, Jak/STAT for self-renewal or ERK for differentiation

3. The master regulators for pluripotency

**By expressing specific transcription factors,
one can alter the cell lineage or cell fate**

Ectopic expression of transcription factors can reprogram cells into another lineage:

Gata1 convert lymphoid/myeloid cells into Mega/Erythroid cells

CAN YOU INDUCE PLURIPOTENCY IN A DIFFERENTIATED ADULT CELL?

IN OTHER WORDS,

CAN WE DERIVE

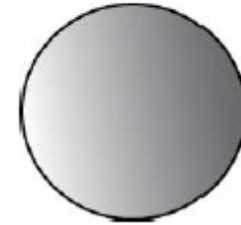
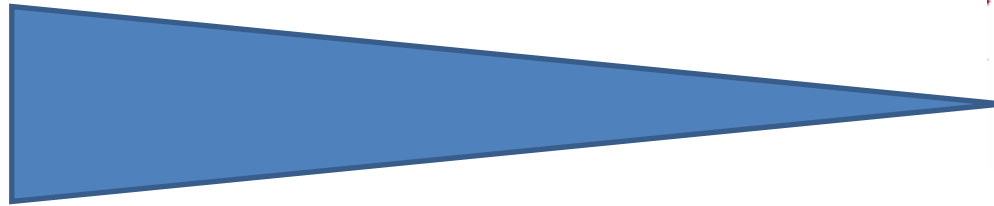
INDUCED PLURIPOTENT STEM CELLS OR iPS CELLS BY

EXPRESSING SPECIFIC TRANSCRIPTION FACTORS?

pluripotent cell

DEVELOPMENT POTENTIAL

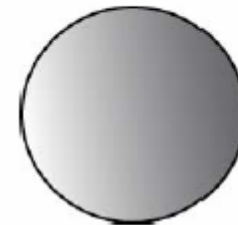
unipotent cell



pluripotent cell

REPROGRAMMING

unipotent cell



Can we reprogram cells from an adult tissue into the equivalent of human pluripotent embryonic stem cells without using an actual embryo.

Several proteins were shown to play roles in reprogramming in frog oocytes, and their identities may well give clues to the overall requirements for reprogramming in other species as well. These include ISWI, which is involved in protein exchange between the transferred nucleus and the oocyte cytoplasm and Brg1, which is required for the activation of **Oct-3/4, a transcription factor specifically expressed in undifferentiated cells.**

Both ISWI and Brg1 are chromatin remodeling ATPases, thus indicating the crucial role of chromatin remodeling in nuclear reprogramming. In addition, the germ cell proteins FRGY2a and FRGY2b reversibly disassemble somatic nucleoli in egg cytoplasm whereas the egg protein nucleophosmin may be involved in chromatin decondensation.

Nanog

A homeobox transcription factor specifically expressed in early mouse embryos and ES cells was identified as one of the key players required for maintaining pluripotency of ES cells.

Overexpression of Nanog in mouse ES cells enabled them to undergo self-renewal in the absence of leukemia inhibitory factor (LIF) .

Similarly, overexpression of Nanog in human ES cells enabled growth without feeder cells.

Nanog null embryos show disorganization of the extraembryonic tissues at E5.5, with no discernible epiblast or primitive ectoderm.

ES cells lacking Nanog can be derived, but they tend to differentiate spontaneously into extraembryonic endoderm lineages even in the presence of LIF.

Even heterozygous Nanog mutant ES cells were unstable and susceptible to spontaneous differentiation.

RNAi-mediated knockdown of Nanog led to differentiation in both mouse and human ES cells.

These data indicated that Nanog plays a crucial role in the induction and maintenance of pluripotency of ES cells..



**SHINYA
YAMANAKA**

After four years of experimentation, Shinya Yamanaka discovered 24 separate factors which when injected into fibroblasts under appropriate culture conditions, created pluripotent cells identical to embryonic stem cells

- Each factor alone could not do the job; a specific combination was required
- The specific combination was observed in FOUR separate genes

**GENERATION OF IPS CELLS BY THE EXPRESISON OF
A DEFINED SET OF TRANSCRIPTION FACTORS**

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi¹ and Shinya Yamanaka^{1,2,*}

Cell. 2006 Aug 25;126(4):663-76.



SHINYA YAMANAKA

24 candidate factors

Ecat1, Dpp5(Esg1), Fbx015, Nanog, ERas, Dnmt3l, Ecat8, Gdf3, Sox15, Dppa4, Dppa2, Fthl17, Sall4, Oct4, Sox2, Rex1, Utf1, Tcl1, Dppa3, Klf4, b-cat, cMyc, Stat3, Grb2

Overexpression of FOUR transcription factors (Oct-3/4, Sox2, c-Myc, KLF4) in mouse fibroblasts transforms them into ES-like cells

**Yamanaka factors
Oct4, Sox2, Klf4, and c-Myc**

HOW ABOUT DERIVING HUMAN iPS cells?

Human induced pluripotent stem cells

In November 2007, two independent research groups announced the creation of human iPS cells:

James Thomson at University of Wisconsin–Madison

Shinya Yamanaka and colleagues at Kyoto University, Japan

Using retroviral vectors, Yamanaka expressed **Oct3/4**, **Sox2**, **Klf4**, and **c-Myc** in human fibroblasts and transformed them into pluripotent stem cells

Cell. 2007 Nov 30;131(5):861-872

Thomson and colleagues used lentiviral vectors to express **OCT4**, **SOX2**, **NANOG**, and a different gene **LIN28** for the generation of human iPS cells.

Science. 2007 Dec 21;318(5858):1917-20



Dr. Shinya Yamanaka



Dr. James Thomson

The Yamanaka factors are transcription factors / master regulators

Yamanaka factors Oct4, Sox2, Klf4, and c-Myc

Oct4:

- POU-domain containing transcription factor.
- Oocytes, fertilized embryo, ICM, epiblast, ES cells, and germ cells.
- Crucial for the maintenance of pluripotency; homodimerizes and heterodimerizes with other co-factors such as Sox2 to regulate the ES cell state; deletion in ES cells results in loss of pluripotency and embryonic lethality (blastocyst stage)

Sox2:

- SRY-related HMG-box DNA-binding protein
- Oocytes, ICM, epiblast, germ cells, multipotent cells of extra-embryonic ectoderm, cells of neural lineage, brachial arches, and gut endoderm.
- Regulates the pluripotent state; deletion in ES cells results in loss of pluripotency; deletion in mouse results in embryonic lethality (E6.5) because of failure to maintain epiblast

Yamanaka factors
Oct4, Sox2, Klf4, and c-Myc

KIF4

- Member of the Kruppel-like factor family of transcription
- Enriched in the gut, skin, and ES cells; also expressed in cells of the blood such as B cells and monocytes
- Tumor suppressor or oncogene that functions in regulating cell differentiation, cell growth, and cell cycle; deletion in the mouse leads to death postnatally because of skin barrier deficiencies; mice also have an intestinal and hematopoietic Phenotype.

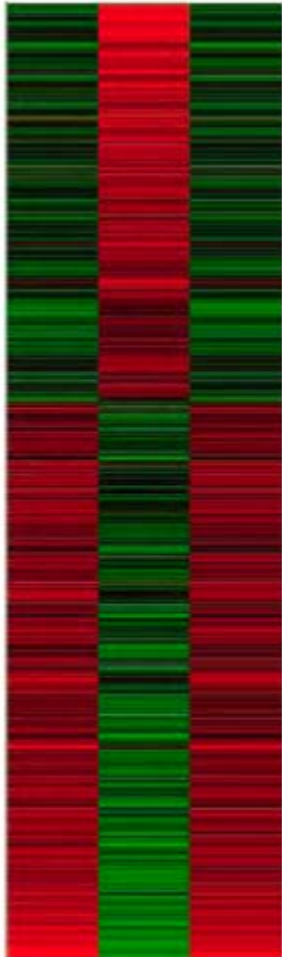
C-Myc

- Basic helix-loop-helix transcription factor
- Begins at the morula stage and to varying degrees in proliferating cells throughout development; multiple tissues including the heart, liver, intestine, spleen, kidney, lung, and mammary gland
- Involved in cell cycle progression, apoptosis, and cellular transformation; loss of function in mouse leads to embryonic lethality (E10.5) because of defects in growth/cardiac and neural development; c-Myc null ES cells have impaired tumor progression

Oct 4 and Nanog are essential for maintenance of pluripotent state of ES cells and are expressed only in pluripotent cells

Yamanaka and others have derived iPS cells from a variety of tissue types (liver, stomach, brain) and turned them into skin, muscle, gut, cartilage, and nerve cells

ES MEF iPS



Analysis of differentially expressed genes between ES cells and MEFs

Transcriptional profiles are indistinguishable

Global epigenetic reprogramming in iPS cells

Approach

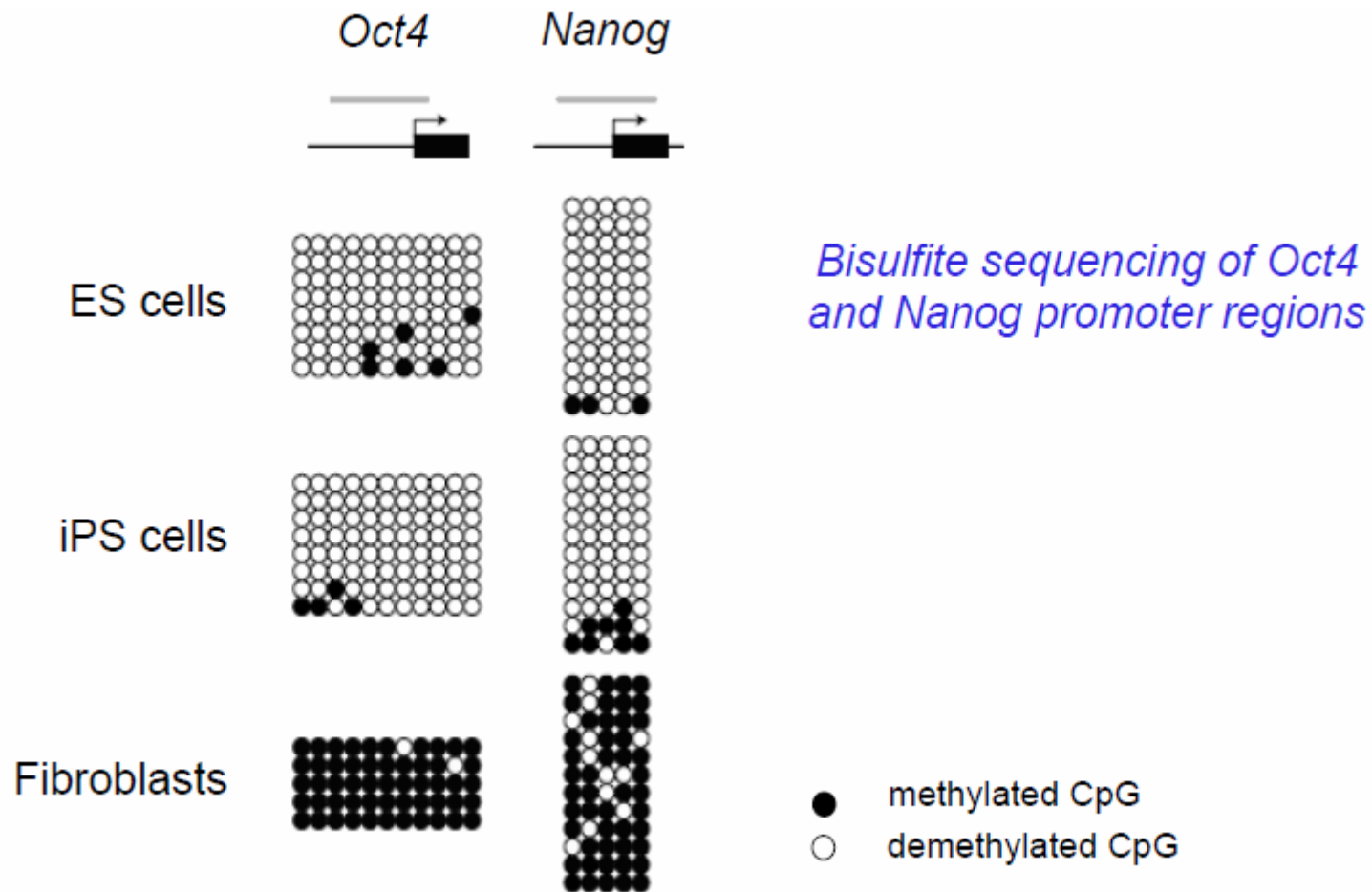
Genome-wide ChIP-chip analysis of K4/K27 trimethylation (16,500 promoters)

Findings

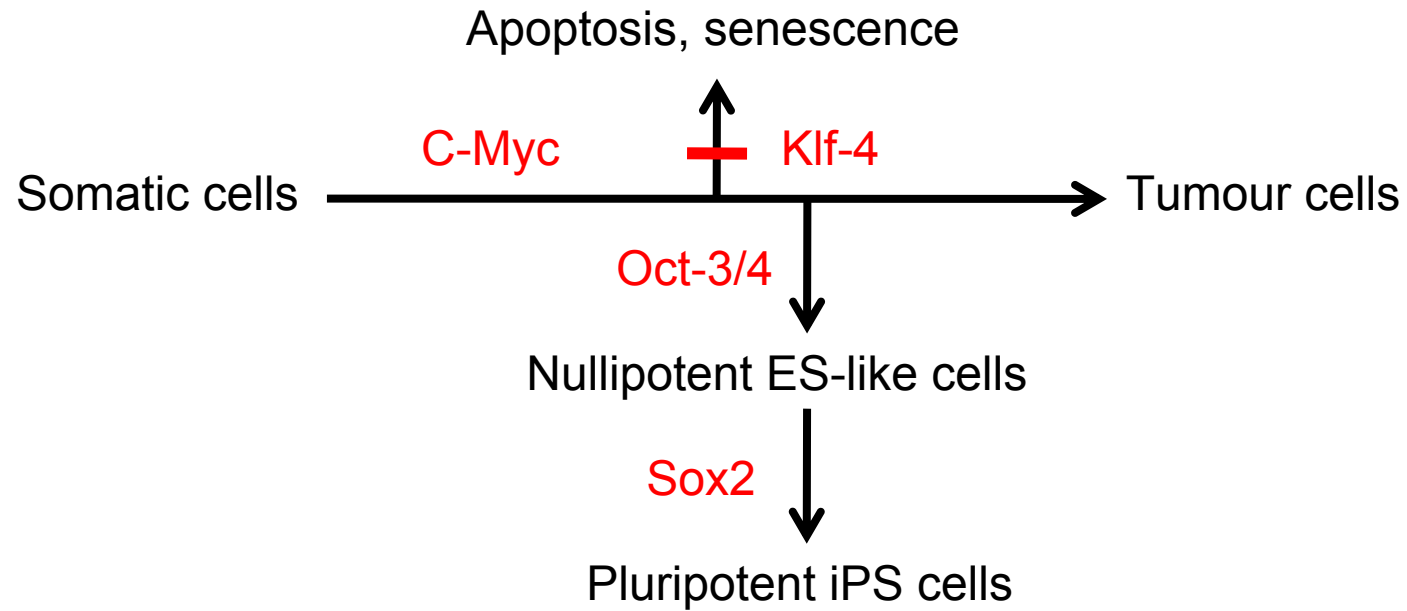
iPS and ES cells are indistinguishable

Reprogramming mainly associated with changes in repressive methylation (K27)

Gene-specific epigenetic reprogramming?



How do the Four Factors Induce Pluripotent Stem Cells?



The criteria to define a bona fide iPS cell

- Non viable staining : Positive for alkaline phosphatase staining and pluripotency factors including NANOG.
- Viable staining for cell surface antigen expression: Tra-1-81(+), Tra-1-60(+), SSEA3(+) SSEA4(+)
- Expressing endogenous pluripotency associated genes, including OCT4, SOX2, NANOG, REX1, FGF4, ESG1, DPPA2/4, hTERT, DNMT3B, etc.
- The ability to differentiate into all three germ lineages in embryoid bodies and form teratoma in immunodeficient animals.
- The erasure of imprinting (DNA methylation) at pluripotency gene promoters, including Oct4, Sox2, Nanog etc.

iPS cells generation in patient fibroblasts

- Parkinson's disease (Wernig and Jaenisch, 2008, Maehr and Melton PNAS 2009).
- Amyopathic Lateral Sclerosis, (Dimos and Eggan Science 2008)
- Type I diabetes (Maehr and Melton PNAS 2009)
- ADA-SCID, SBDS, Gaucher disease, Duchenne and Becker Muscular dystrophin, Parkinson's disease, Huntington disease, JDM, Down syndrome, Lesch-Nyhan syndrome. (Park and Daley Cell 2008).

iPS cells generation from other cell types

- Blood cells (Loh and Daley 2009). B-cells (Hanna and Jaenisch Cell 2008)
- Blood stem cells (Emiinli and Hochedlinger Nat Genet 2009)
- Pancreatic β -cells (Stadtfeld and Hochedlinger Cell Stem Cell 2008)
- Hepatic and gastric endoderm (Aoi and Yamanaka Science 2008)
- Neural stem cells (Kim and Scholar, Nature 2008)

Summary

- Reprogramming patient cells into a pluripotent state provides the best matching and the most abundant source for tissue regeneration.
- Among all different methods, the most achievable is direct reprogramming, by introducing pluripotency associated transcription factors into primary tissue culture.
- Direct reprogramming generates induced pluripotent stem cells that are functionally and phenotypically identical to embryonic stem cells.
- “Safer” protocol in generating less tumorigenic induced pluripotent stem cells are rapidly evolving.
- More works are needed in order to employ iPS cells for tissue replacement therapeutics.

The viral transfection systems used to insert the genes at random locations in the host's genome created concern for potential therapeutic applications of these iPSCs, because the created cells might be prone to form tumors.

It was therefore demonstrated by Konrad Hochedlinger and his colleague at Harvard University that expressing the four genes using adenoviral vectors in adult skin and liver cells can lead to the generation of mouse iPS cells that are identical to embryonic stem cells.

In fact it was later shown that reprogramming adult differentiated cells into an iPS cells can be achieved by simply transfecting expression plasmids encoding the requisite four genes although the efficiency is drastically reduced.

More recently, teams led by Keisuke Kaji, of the University of Edinburgh, and Andras Nagy, of the University of Toronto, reported in May 2009 that human iPS cells can be generated by direct delivery of proteins, thus eliminating the need for viruses or genetic modification.

A group of Sheng Ding in La Jolla, California, has also shown that mouse iPS cells can be generated by repeated treatment of the cells with certain proteins channeled into the cells via poly-arginine anchors.

The expression of pluripotency induction genes can also be increased by treating somatic cells with FGF2 under low oxygen conditions.

Cell Stem Cell

Brief Report

Generation of Induced Pluripotent Stem Cells Using Recombinant Proteins

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iPS cell research – major breakthroughs

First demonstration of reprogramming;
Oct3/4, Sox2, c-Myc and Klf4

Takahashi and Yamanaka Cell 126:663

Findings confirmed; improved selection
method; chimera formation with germline
competence

Okita et al. Nature 448: 313; Wernig et al.
Nature 448: 260; Maherali et al. Cell Stem
Cell 1:55

Drug selection not required; unmodified
somatic donor cells

Meissner et al. Nature Biotechnol. 25: 1177;
Blelloch et al. Cell Stem Cell 1: 245

c-Myc is not required to direct reprogramming
of somatic cells

Nakagawa et al. Nature Biotechnol. 26:101;
Wernig et al. Cell Stem Cell 2:1

First demonstrations of direct reprogramming
of human somatic cells; use of alternate
factors Nanog and Lin28 (no c-Myc or Klf4)

Yu et al. Science 318:1917; Takahashi et al.
Cell 131: 861

First model therapeutic application

Hanna et al. Science 318: 1920

Developments toward “safer” generation of iPS cells

Reduced number of transcription factor use:

- No myc: Nakagawa and Yamanaka, Nat Biotechnol 2008, Wernig and Jaenisch, Cell Stem Cell 2009
- No Sox2: by adding GSK-3 inhibitor, Zhou and Ding, Stem cell 2009, in neural stem cell, Kim and Scholer Nature 2008
- No Klf4/myc, by addition of Valproic acid : Huangfu and Melton, Nat Biotech 2008
- No Myc and Sox2, by addition of BIX01294 and PD0325901 (Zhou and Ding, Cell Stem Cell 2008).
- Klf4 only by adding Kenpaullone (Lyssiotis and Jaenisch, PNAS 2009)

Developments toward “safer” generation of iPS cells

Specific pathways:

- TGFb inhibitor replace Sox2 and cMyc and induce Nanog (Maherali and Hochedlinger, Curr Biol 2009, Ichida and Eggan 2009)
- p53 inhibition augments iPS efficiency (Hong and Yamanaka, Nature 2009, Utikal and Hochedlinger Nature 2009, Marion and Blastco Nature 2009, Li and Serrano Nature 2009, Kawamura and Belmonte 2009)
- Hypoxia – Yoshida and Yamanaka Cell Stem Cell 2009
- WNT signaling stimulates reprogramming efficiency (Marsonm, Jaenisch Cell Stem Cell 2008)

Developments toward “safer” generation of iPS cells

Better vectors:

- Drug Inducible vectors: Wernig and Jaenisch, Nat Biotechnol 2008, Hockemeyer and Jaenisch, Cell Stem Cell 2008
- Non-integrating vectors : adenovirus in hepatocyte (Stadtfeld and Hochedlinger Science 2008)
- Self-inactivating vectors: Piggy Bac (Yusa and Bradley, Nat Methods 2009)
- multi-cistronic vectors: single lentiviral cassette (Carey and Jaenisch, PNAS 2009, Sommer and Mostoslavsky, Stem Cell 2009)
- Vector free (episome Yu and Thomson, Science 2009; direct transfection Okita and Yamanaka Science 2008)
- Direct protein induction: poly arginine modification of recombinant protein (Zhou and Ding, Cell Stem Cell 2009),

PUBLICATIONS FROM YAMANAKA LAB ON iPS CELLS

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Turning Back the Cellular Clock: A Farewell to Embryonic Stem Cells?

Shinya Yamanaka discovered how to revert adult cells to an embryonic state. These induced pluripotent stem cells might soon supplant their embryonic cousins in therapeutic promise

The two landmark papers that describe production of human iPS cells

Cell. 2007 Nov 30;131(5):861-72.

Induction of pluripotent stem cells from adult human fibroblasts by defined factors.

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S.

(Yamanaka factors: Oct4, Sox2, Klf4, and c-Myc – transcription factors)

Science. 2007 Dec 21;318(5858):1917-20.

Induced pluripotent stem cell lines derived from human somatic cells.

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J,

Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA.

(Oct4, Sox2, Nanog, Lin28)

The generation of iPSCs suggests that a specific combination of defined factors, rather than a single factor, could epigenetically alter the global gene expression of a cell

Neurogenin 3, in combination with Pdx1 and Mafa, can efficiently reprogram pancreatic exocrine cells into functional beta cells in vivo

Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J., and Melton, D.A. (2008). In vivo.

Reprogramming of adult pancreatic exocrine cells to beta-cells.
Nature 455, 627–632.

A combination of three factors, Ascl1, Brn2, and Myt1l, converts dermal fibroblasts to functional neurons

Vierbuchen, T., Ostermeier, A., Pang, Z.P., Kokubu, Y., Sudhof, T.C., and Wernig, M. (2010).

Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* 463, 1035–1041.

Direct reprogramming of dermal fibroblasts into cardiomyocytes

A combination of three developmental transcription factors (i.e., Gata4, Mef2c, and Tbx5) rapidly and efficiently reprogrammed postnatal cardiac or dermal fibroblasts directly into differentiated cardiomyocyte-like cells.

Masaki Ieda et al., Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors.

Cell 142, 375–386, August 6, 2010

Masaki Ieda et al., Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors.

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The three reprogramming factors, Gata4, Mef2c, and Tbx5, are core transcription factors during early heart development. They interact with one another, coactivate cardiac gene expression (e.g., Nppa, Gja5 [Cx40], and Myh6), and promote cardiomyocyte differentiation. Gata4 is considered a “pioneer” factor and might open chromatin structure in cardiac loci thus allowing binding of Mef2c and Tbx5 to their specific target sites and leading to full activation of the cardiac program.