Induced Pluripotent Stem Cells

Stem Cell Models for Environmental Health
The National Academies, Keck Center
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M. William Lensch, Ph.D.
Division of Hematology/Oncology
Children's Hospital Boston/HHMI
Department of Pediatrics
Harvard Medical School
Harvard Stem Cell Institute
Outline

- **Induced pluripotent stem cells (iPS)**
  - History, methods, limitations
- **In vitro disease models**
  - Down syndrome and malignancy
  - Dyskeratosis congenita and telomeres
  - Fanconi anemia, Fragile-X, and the limits of various model systems
- **The road ahead**
  - Research today, needs, questions
August Weismann (1834 – 1914)

**Hypothesis:**
Daughter cells differ as each inherits a fraction of the entire set of genes.

How does development work?

“Qualitative Divisions”
Mechanisms of lineage commitment

Hans Spemann
(1869 – 1941)

1920’s
Mechanisms of lineage commitment

Figure 3·12. Spemann’s constriction experiment.
Hypothesis: Daughter cells do not differ genetically.

Epigenetic mechanism
Chromatin remodeling during development
DNA methylation and histone acetylation

Nuclear Transfer (NT)

Fig. 1. Robert Briggs (A; 1911–1983) and Thomas J. King (B; 1921–2000).

TABLE 1

<table>
<thead>
<tr>
<th>Cleavage of Enucleated Eggs Injected with Blastula Cell Nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of eggs injected</td>
</tr>
<tr>
<td>Cleavage</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Abortive</td>
</tr>
<tr>
<td>Cleaved</td>
</tr>
<tr>
<td>Blastulæ</td>
</tr>
<tr>
<td>Partial</td>
</tr>
<tr>
<td>&lt;1/2</td>
</tr>
<tr>
<td>&gt;1/2</td>
</tr>
<tr>
<td>Complete</td>
</tr>
</tbody>
</table>

~32%

1952

John Gurdon

Vol. 38, 1952

ZOLOGY: BRIGGS AND KING

TRANSPPLANTATION 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
"Direct" cellular reprogramming

What makes cell types different from one another?

The expression of a single gene (MyoD) is able to impose a myogenic phenotype in fibroblasts
Viable offspring derived from fetal and adult mammalian cells


Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, UK
* PPL Therapeutics, Roslin, Midlothian EH25 9PP, UK

# oocytes used
# blastocysts
1/29/277
0.36%

“Dolly”
first mammal produced via NT from an adult cell (mammary epith.)

1997
Survey of ~17,500 intrauterine transfers from 6 species (sheep, cow, goat, pig, mouse, and monkey)

~0.8% efficiency overall

High birth weight, Pulmonary problems, Cardiovascular abnormalities, Immune system abnormalities/infection, Kidney and/or liver abnormalities, Placental abnormalities, Joint malformations or other gross deformities
Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi and Shinya Yamanaka

Generation of germline-competent induced pluripotent stem cells

Keisuke Okita, Tomoko Ichisaka & Shinya Yamanaka

Virally-delivered: Oct3/4, Sox2, c-Myc, Klf4
Selection: (Fbx15) (Oct4) (Nanog)
Refinement of Reprogramming

Taken from: Yamanaka, Cell Stem Cell, 2007
Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,1,2*, Maxim A. Vodyanik,2 Kim Smuga-Otto,1,2 Jessica Antosiewicz-Bourget,1,2 Jennifer L. Frane,3 Shulan Tian,3 Jeff Nie,3 Gudrun A. Jonsdottir,3 Victor Ruotti,3 Ron Stewart,3 Igor I. Slukvin,2,4 James A. Thomson1,2,5*

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,1 Koji Tanabe,1 Mari Ohnuki,1 Megumi Narita,1,2 Tomoko Ichisaka,1,2 Kiichiro Tomoda,3 and Shinya Yamanaka1,2,3,4,*

Reprogramming of human somatic cells to pluripotency with defined factors

In-Hyun Park1, Rui Zhao1, Jason A. West1, Akiko Yabuuchi,1 Hongguang Huo1, Tan A. Ince1, Paul H. Lerou1, M. William Lensch1 & George Q. Daley1

all appeared in late 2007
Disease-specific induced pluripotent stem cells permit modeling and screening with human cells.
Incremental advances in iPS


**Table 1. Small Molecules and Other Factors Used to Enhance the Reprogramming Process**

<table>
<thead>
<tr>
<th>Molecule/Factor</th>
<th>Target/Mode of Action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valproic Acid</td>
<td>histone deacetylase inhibitor</td>
<td>Huangfu et al., 2008a, 2008b</td>
</tr>
<tr>
<td>5-azacytidine; shRNA against Dnmt1</td>
<td>DNA demethylating agent</td>
<td>Mikkelsen et al., 2008</td>
</tr>
<tr>
<td>BIX01294</td>
<td>histone methyltransferase inhibitor</td>
<td>Shi et al., 2008b</td>
</tr>
<tr>
<td>BayK8644</td>
<td>L-type calcium channel agonist</td>
<td>Shi et al., 2008a</td>
</tr>
<tr>
<td>Wnt3a</td>
<td>cell signaling molecule; transcriptional activation of multiple downstream targets, including c-Myc</td>
<td>Marson et al., 2008</td>
</tr>
<tr>
<td>siRNA against p53 and Utf1 cDNA</td>
<td>tumor suppressor; ESC-specific cofactor, respectively</td>
<td>Zhao et al., 2008</td>
</tr>
</tbody>
</table>

O, Oct4; S, Sox2; M, c-Myc; K, Klf4; Dnmt1, DNA methyltransferase 1.
Table 1
Mouse and human IPS cells have been generated in a variety of ways

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Reprogramming factors</th>
<th>Method of delivery</th>
<th>Timeline (wk)</th>
<th>Efficiency</th>
<th>Genomic integration</th>
<th>Genomic integration removed</th>
<th>Retrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEFs</td>
<td>O,S,K,M</td>
<td>Retroviral vectors</td>
<td>2–3</td>
<td>0.01%–0.050%</td>
<td>Yes</td>
<td>No</td>
<td>10,12–15</td>
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<tr>
<td>MEFs</td>
<td>O,S,K</td>
<td>Retroviral vectors</td>
<td>3–4</td>
<td>0.001%–0.01%</td>
<td>Yes</td>
<td>No</td>
<td>56,65,98</td>
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<tr>
<td>Hepatocytes and stomach</td>
<td>O,S,K,M or O,S,K</td>
<td>Retroviral vectors</td>
<td>2–3</td>
<td>0.5%–8%</td>
<td>Yes</td>
<td>No</td>
<td>46</td>
</tr>
<tr>
<td>Neural stem cells</td>
<td>O,K or O,M or O</td>
<td>Retroviral vectors</td>
<td>1–4</td>
<td>0.1%–5%</td>
<td>Yes</td>
<td>No</td>
<td>48,99</td>
</tr>
<tr>
<td>D lymphocytes</td>
<td>O,S,K,M</td>
<td>Adenoviral vectors</td>
<td>4–5</td>
<td>0.0001%–0.001%</td>
<td>No</td>
<td>–</td>
<td>29</td>
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<tr>
<td>MEFs</td>
<td>O,S,K,M</td>
<td>Adenoviral vectors</td>
<td>2–3</td>
<td>0.01%–0.1%</td>
<td>No</td>
<td>–</td>
<td>34</td>
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<tr>
<td>MEFs</td>
<td>O,S,K,M</td>
<td>Lentiviral vectors</td>
<td>4–5</td>
<td>0.002%–0.008%</td>
<td>No</td>
<td>–</td>
<td>31</td>
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<tr>
<td>MEFs</td>
<td>O,K + Bix and BayK</td>
<td>Retroviral vectors</td>
<td>2–3</td>
<td>0.007%–0.02%</td>
<td>Yes</td>
<td>No</td>
<td>60</td>
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<tr>
<td>MEFs</td>
<td>O,S,K,M</td>
<td>Plasmid nucleofection</td>
<td>2–3</td>
<td>0.002%</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>MEFs, TTFs</td>
<td>O,K + RepSox or</td>
<td>Retroviral vectors</td>
<td>2–3</td>
<td>0.01%–1%</td>
<td>Yes</td>
<td>No</td>
<td>61</td>
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<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Skin fibroblasts</td>
<td>O,S,K,M or O,S,K,M,T,S,SV or O,S,K,M,N or O,S,K</td>
<td>Retroviral vectors</td>
<td>2–5</td>
<td>0.001%–1%</td>
<td>Yes</td>
<td>No</td>
<td>2,16,18,19</td>
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<tr>
<td>Keratinocytes</td>
<td>O,S,K,M or O,S,K</td>
<td>Retroviral vectors</td>
<td>1–2</td>
<td>0.001%–0.01%</td>
<td>Yes</td>
<td>No</td>
<td>49</td>
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<td>Peripheral blood cells</td>
<td>O,S,K,M</td>
<td>Retroviral vectors</td>
<td>2</td>
<td>0.01%–0.02%</td>
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<td>No</td>
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<tr>
<td>Skin fibroblasts</td>
<td>O,S,K,M or O,S,K,M,N</td>
<td>Lentiviral vectors</td>
<td>3–4</td>
<td>0.002%</td>
<td>Yes</td>
<td>No</td>
<td>101</td>
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<tr>
<td>keratinocytes</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Skin fibroblasts</td>
<td>O,S,N,L or O,S,N,L,M,K,K,M</td>
<td>Lentiviral vectors</td>
<td>2–3</td>
<td>0.01%–1%</td>
<td>Yes</td>
<td>No</td>
<td>17,20,33</td>
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<tr>
<td>Skin fibroblasts</td>
<td>O,S,K,M or O,S,K</td>
<td>Lentiviral vectors</td>
<td>3–5</td>
<td>NR</td>
<td>Yes</td>
<td>Partial</td>
<td>36</td>
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<tr>
<td>Embryonic fibroblasts</td>
<td>O,S,K,M</td>
<td>piggyBac transposon</td>
<td>2–4</td>
<td>NR</td>
<td>Yes</td>
<td>No</td>
<td>67</td>
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<tr>
<td>Embryonic fibroblasts</td>
<td>O,S,K,M</td>
<td>piggyBac transposon</td>
<td>2–4</td>
<td>0.006%–0.01%</td>
<td>Yes</td>
<td>No</td>
<td>66</td>
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<tr>
<td>Skin fibroblasts</td>
<td>O,S,N,L,M,K,S,SV</td>
<td>Episomal vectors</td>
<td>NR</td>
<td>0.003%–0.006%</td>
<td>No</td>
<td>–</td>
<td>33</td>
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<tr>
<td>Adipose stem cells</td>
<td>O,S,K,M</td>
<td>Lentiviral vectors</td>
<td>2–3</td>
<td>0.2%</td>
<td>Yes</td>
<td>No</td>
<td>51</td>
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<tr>
<td>Skin fibroblasts</td>
<td>O,S,K,M</td>
<td>Reombinant proteins</td>
<td>8</td>
<td>0.001%</td>
<td>No</td>
<td>–</td>
<td>32</td>
</tr>
</tbody>
</table>

*a*Deox-inducible systems, *a*Viral LTR is not excised, *a*Proteins were delivered in the form of whole cell extracts. O, Oct4; S, Sox2; K, Klf4; M, c-Myc; N, Nanog; L, Lin28; SV, SV40 LT; T, hTert; MEF, mouse embryonic fibroblast; TTF, tail tip fibroblast; NR, not reported; –, not relevant.
Outline

• Induced pluripotent stem cells (iPS)
  – History, methods, limitations

• *In vitro* disease models
  – Down syndrome and malignancy
  – Dyskeratosis congenita and telomeres
  – Fanconi anemia, Fragile-X, and the limits of various model systems

• The road ahead
  – Research today, needs, questions
• Transient myeloproliferative disease (TMPD) in the first few neonatal months
• 20% of TMD patient relapse and progress to DS-AMKL
• Incidence of solid tumors in DS ~ 10% of age-matched individuals
  • What is the “protective” effect of trisomy 21?
  • Anti-angiogenesis?

Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1

Kwan-Hyuck Baek¹, Alexander Zaslavsky⁴, Ryan C. Lynch¹⁺, Carmeila Britt¹, Yoshiaki Okada², Richard J. Siarey³, M. William Lensch⁵, In-Hyun Park⁶, Sam S. Yoon⁵, Takashi Minami⁶, Julie R. Korenberg⁷, Judah Folkman¹, George Q. Daley¹, William C. Aird², Zygmunt Galdzicki⁸ & Sandra Ryeom¹
Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients

Suneet Agarwal¹, Yuin-Han Loh¹, Erin M. McLoughlin¹, Junjiu Huang²,³, In-Hyun Park¹, Justine D. Miller¹, Hongguang Huo¹, Maja Okuka⁶, Rosana Maria dos Reis², Sabine Loewer¹, Huck-Hui Ng⁴, David L. Keefe⁵, Frederick D. Goldman⁵, Aloysius J. Klingelhofer⁶, Lin Liu²,⁷ & George Q. Daley¹,⁸

2010

Calado and Young, Blood, 2008
• DC-iPS telomere erosion
• \textit{TERT} doesn’t help
• However, continued culture leads to increase in telomere length
TERC is transcriptionally upregulated, perhaps by OCT4
Fanconi anemia (FA)

- rare, autosomal recess. \((FA-B = X)\)
- >13 complementation groups
- sensitivity to X-linking agents
  - \((DEB and MMC exposure)\)
- 2/3 cases dysmorphic
- short stature, microcephaly
- radial-ray abnormalities
- \textit{café-au lait} spots
- renal, cardiac, central nervous system, reproductive anomalies
- aplastic anemia, pancytopenia
- bone marrow failure
- hypersens. to inhib. cytokines
- cancer predisposition

Marrow failure → leukemic evolution in FA

physiological levels of cytokines = apoptosis
(de-facto selective pressure)

1. physiological levels of cytokines = apoptosis
(de-facto selective pressure)

2. aplastic anemia

3. marrow failure

4. clonal outgrowth

modified from: Lensch et al., Leukemia 1999.
Fanconi cells cannot make iPSC

- Could not reprogram FA cells
- Antecedent gene correction permitted iPSC generation from FA fibroblasts
- iPSC may not facilitate all studies
Lentiviral-based RNAi (shRNAs) for FA gene K/D

Knockdown of Fanconi anemia genes in human embryonic stem cells reveals early developmental defects in the hematopoietic lineage

Asmin Tulpule, 1-3 M. William Lensch, 1,3,4 Justine D. Miller, 1,3 Karyn Austin, 1 Alan D’Andrea, 5 Thorsten M. Schlaeger, 1,3 Akiko Shimamura, 1,6 and George Q. Daley 1-4,7

- Two hESC lines, H9 (Thomson, 1998) and BG01 (Mitalipova, 2003)
- Chose 3 oligos for FANCA and FANCD2 (FANCAi and FANCD2i)
- Controls: Vector only & Luciferase RNAi vector (LUCi)
FAi-hESC fail to respond to genotoxic stress

All data at 24 hrs

<table>
<thead>
<tr>
<th></th>
<th>LUCi</th>
<th>FANCAi</th>
<th>LUCi</th>
<th>FANCAi</th>
</tr>
</thead>
<tbody>
<tr>
<td>FANCD2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L/S</td>
<td>0.1</td>
<td>0</td>
<td>0.3</td>
<td>0.06</td>
</tr>
<tr>
<td>untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mM HU</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Graph showing FANCD2 nuclear foci (%) at different MMC concentrations.

Images: DAPI, GFP, FANCD2, Merge.
FA gene correction rescues hematopoietic deficits

- **A**
  - FANCD2i + hFANCD2
  - FANCD2i + empty vector

- **B**
  - 2mM HU 24 hrs
  - FANCD2-L
  - FANCD2-S
  - FANCAi + hFANCA
  - FANCAi + empty vector

- **C**
  - Relative % CD45+ (FANCD2i + hFANCD2 vs FANCD2i + empty vector)
  - ***

- **D**
  - Relative % CD45+ (FANCAi + hFANCA vs FANCAi + empty vector)
  - *

- **E**
  - Relative CFU (FANCD2i + hFANCD2 vs FANCD2i + empty vector)
  - ***

- **F**
  - Relative CFU (FANCAi + hFANCA vs FANCAi + empty vector)
  - *

**pMMP-puro, pMMP-puro hFANCA, and pMMP-puro hFANCD2**

selection – 2x (1 μg/ml puro) for 72 hours

D20 EBs into H4434
Hox gene expression is down in FAi-hESCs

- HOX genes with decreased expression
  - FANCD2i
    - PROX1
    - VAX1
    - SIX1
    - LHX1
    - HOXB9
    - HHEX
  - FANCAi
    - CDX1
    - HOXD3
    - TLX1
    - PAX3
    - DLX2
    - HOXB1
    - HOXD1
    - BARX1

D20 EBs
> Δ1.5x
Marrow failure → leukemic evolution in FA

1. Physiological levels of cytokines = apoptosis
   (de-facto selective pressure)

2. Aplastic anemia

3. Marrow failure

4. Clonal outgrowth

Modified from: Lensch et al., Leukemia 1999.
Marrow failure → leukemic evolution in FA

- physiological levels of cytokines = apoptosis (de-facto selective pressure)
- aplastic anemia
- marrow failure
- clonal outgrowth

modified from: Lensch et al., Leukemia 1999.
Fragile X Syndrome → silencing of FMR1 gene
Modeled by FX-ES cells from PGD embryos

CGG tandem repeat expansion, 5’ UTR of FMR1 gene
**Brief Report**

**Differential Modeling of Fragile X Syndrome by Human Embryonic Stem Cells and Induced Pluripotent Stem Cells**

Achia Urbach,¹,²,⁴ Ori Bar-Nur,³,⁴ George Q. Daley,¹,²,⁴ and Nissim Benvenisty³,⁴

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Expression of FMR1 gene in normal fibroblasts, iPS, and ES cells
Outline

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  – History, methods, limitations

• *In vitro* disease models
  – Down syndrome and malignancy
  – Dyskeratosis congenita and telomeres
  – Fanconi anemia, Fragile-X, and the limits of various model systems

• The road ahead
  – Research today, needs, questions
iPS cells and ES cells are not the same

* Gene expression in iPS cells becomes more ES-like over time
* Differences remain - what will these differences mean for research?
Identification of differentially-methylated regions using a genome-wide approach
Epigenetics and development

Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells

Matthias Stadtfeld1,2,3*, Effie Apostolou1,2,3*, Hidenori Akutsu4, Atsushi Fukuda5, Patricia Follett1, Sridaran Natesan6, Tomohiro Kono3, Toshi Shioda2 & Konrad Hochedlinger1,2,3

- A single cluster of imprinted genes was different between mouse ES and iPS
- Treatment with histone-deacetylase inhibit. (VPA) gives chimerism (4n as well)
What makes ES and iPS different?

- Differences may relate to many things
  - viral insertions, mutations, selection vs. morphology
- The greatest cause is likely epigenetic
- After cellular reprogramming
  - some tissue-specific genes remain ”ON”
  - some pluripotency genes remain ”OFF”
- “Confused” states of gene expression result

What are consequences of these differences?

How can iPS cells be improved?
Summary

- Road to iPS cells was long and interesting
  - Road ahead will undoubtedly be the same
- iPS cells are not amenable to all studies
  - Epigenetic conditions, others… need ESC and NT
- Field continues to refine iPSC methodologies
  - Improved efficiency, safety, and sources
- Mechanistic understanding of reprogramming
  - Collateral insights into cellular lineage specification
- Current thrust dedicated to study of epigenetics
  - Also sheds light on human diseases
- iPSC and ESC already tremendously valuable
  - Disease models, screening platforms, basic science
Many thanks…

- Susan Fisher, Amanda Cline, Marilee Shelton-Davenport, Fran Sharples
- George Daley and the Daley Laboratory
- Leukemia & Lymphoma Society, Harvard Stem Cell Institute, HHMI, RoFAR, NIH, DoD, Children’s Hospital Boston, Manton Center for Orphan Disease Research
- The patients and their families… the true experts

http://daley.med.harvard.edu