A quest for redefining stem cell induction strategies:

How to deal with ethical objections and patenting problems

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Workshop
Native and Induced Embryonic Stem Cell Standardization
Florence, March 19-21, 2012

What we mostly read about the ethical aspects of **iPS cell** use:

„Direct reprogramming through the ectopic expression of defined transcription factors… represents a simple way to obtain pluripotent stem-cell lines from almost any somatic tissue and mammalian species. The use of such cells also **circumvents the ethical issues associated with human cells**.“

F. González et al.: Methods for making induced pluripotent stem cells: reprogramming à la carte. *Nature Reviews / Genetics* 12: 231 (2011)

On contrast, but only more rarely found in the literature:

„The **use of iPSCs and tetraploid complementation for human reproductive cloning** would raise profound **ethical objections**. Professional standards and laws that ban human reproductive cloning by somatic cell nuclear transfer **should be revised** to also **forbid** it by other methods, such as iPSCs via tetraploid complementation.“

B. Lo et al.: Cloning Mice and Men: Prohibiting the Use of iPS Cells for Human Reproductive Cloning. *Cell Stem Cell* 6: 16 (2010)

And already before:

„**iPS cells:** There would be severe ethical problems associated with **using tetraploid complementation technology in humans**, even without the intention of implanting the resulting artificially created embryos into a uterus (see, for example, H.-W. Denker *Reprod. Biomed. Online* **19**, suppl. 1, 34–37; 2009). The issues are similar to those that have arisen over embryonic stem cells and include aspects of **patentability**.“

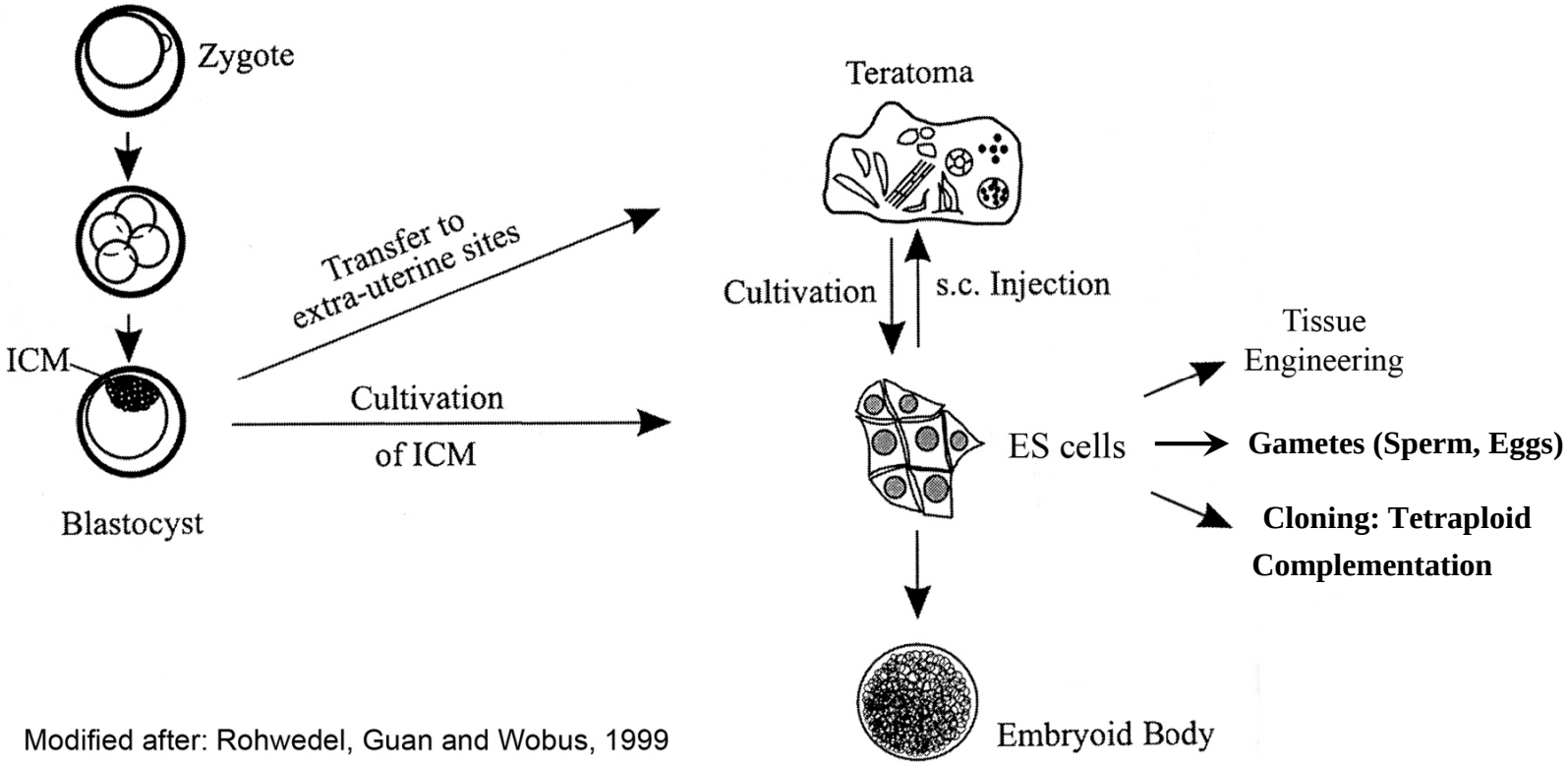
H.-W. Denker: Ethical concerns over use of new cloning technique in humans. *Nature* 461: 341 (2009)

iPS cells: Ethical Problems Solved?

1. Definition, Derivation

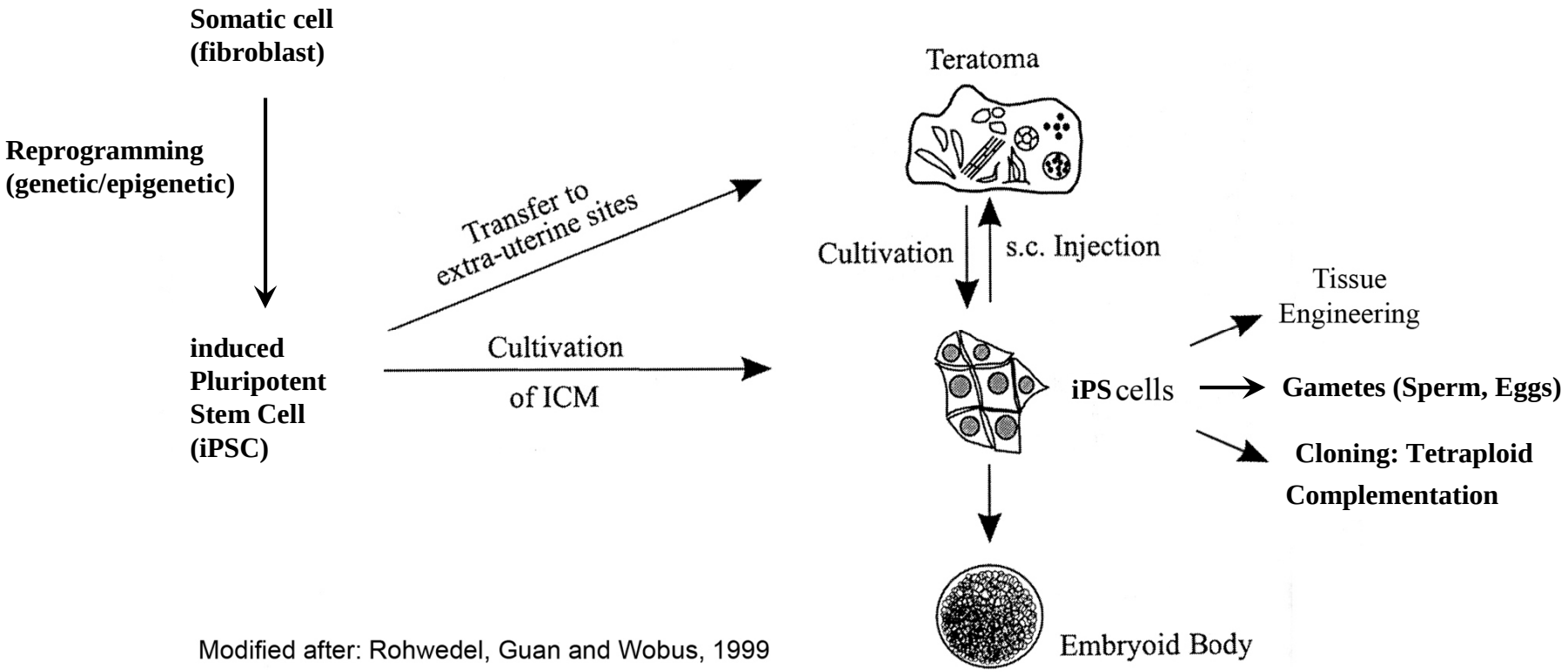
2. Pattern Formation Potential:
    - Autonomous: Embryoid Bodies
    - Aided: Tetraploid Complementation (TC)
3. Patenting
4. Alternative Approaches

ESCs: Derivation and Properties



Modified after: Rohwedel, Guan and Wobus, 1999

iPSCs: Derivation and Properties



iPS cells: Ethical Problems Solved?

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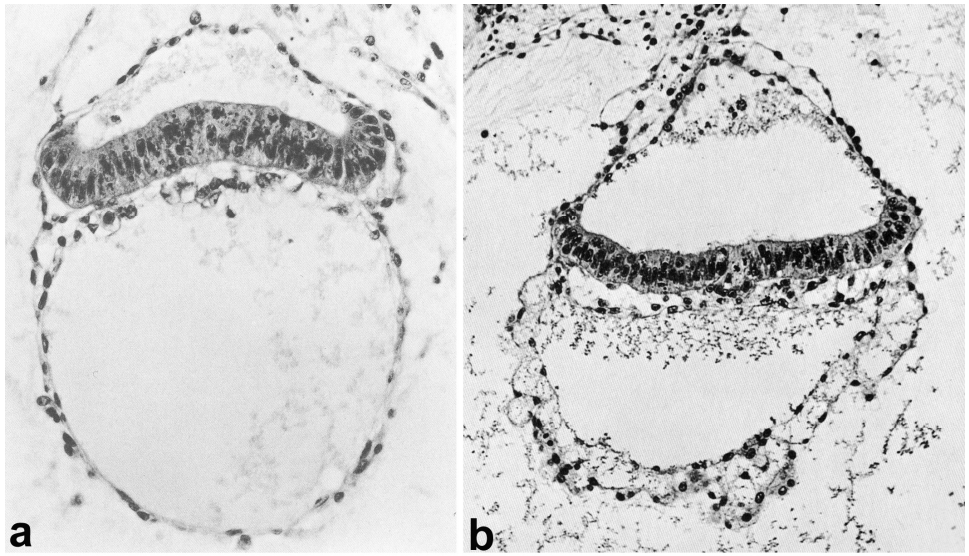
Human and Monkey „Embryoid Bodies“

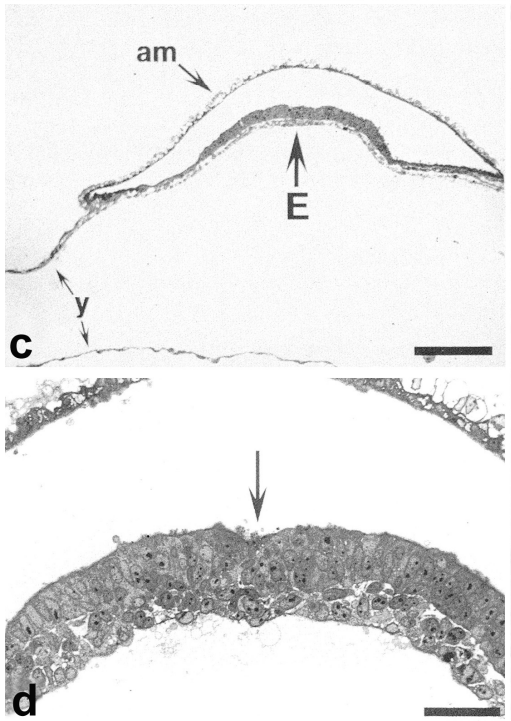
a, b:

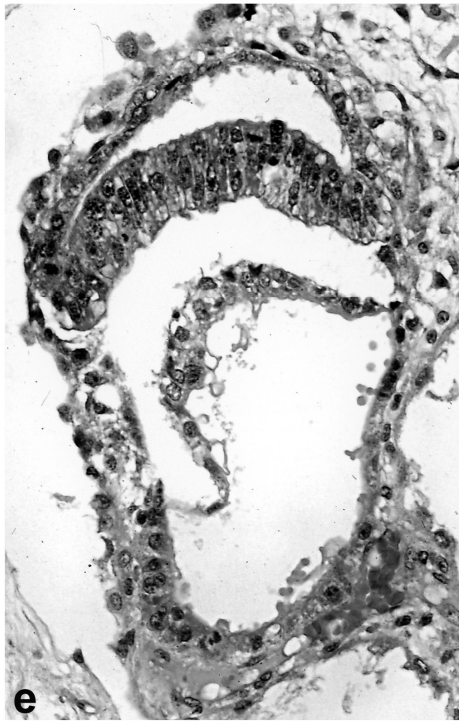
Human embryos in vivo (Carnegie Collection)



c, d:

„Embryoid body“: Marmoset Monkey ES cell colony in vitro (J. Thomson et al.: Biol. Reprod. 55, 254-259, 1996)





e:

„Embryoid body“ from a **human** terato-carcinoma in vivo (I. Damjanov/ P. Andrews)

Denker, H.-W.: *Naturwissenschaften* 91: 1-21 (2004)

The most important event in your life is not birth, marriage, or death but gastrulation.

(Lewis Wolpert)

Pattern Formation Potential

Literature on Autonomous Early Embryonic Pattern Formation Potential of ESCs in „Embryoid Bodies“ (Gastrulation, Self-Organization, Basic Body Plan):

- **Thomson, J.A.** et al.: Pluripotent cell lines derived from Common Marmoset (Callithrix jacchus) blastocysts. *Biol. Reprod.* 55: 254-259 (1996)
- **Behr, R.** et al.: Epithelial–mesenchymal transition in colonies of Rhesus Monkey embryonic stem cells: A model for processes involved in gastrulation. *Stem Cells* 23:805–816 (2005)
- **ten Berge, D.** et al.: Wnt signaling mediates self-organization and axis formation in embryoid bodies. *Cell Stem Cell* 3: 508-518 (2008)
- **Fuchs, C.** et al.: Self-organization phenomena in embryonic stem cell-derived embryoid bodies. *Cells Tissues Organs* (publ. online-first August 19, 2011)

What can „embryoid bodies“ teach us?

- Pluripotent stem cells possess **gastrulation** potential and can show impressive early embryonic pattern formation (**self-organization**) potential *in vitro*.
- These processes are central elements of **basic body plan** formation and **individuation** during embryogenesis.
- „Embryoid bodies“ formed *in vitro*, however, rarely reach the high degree of order of a harmonious basic body plan.

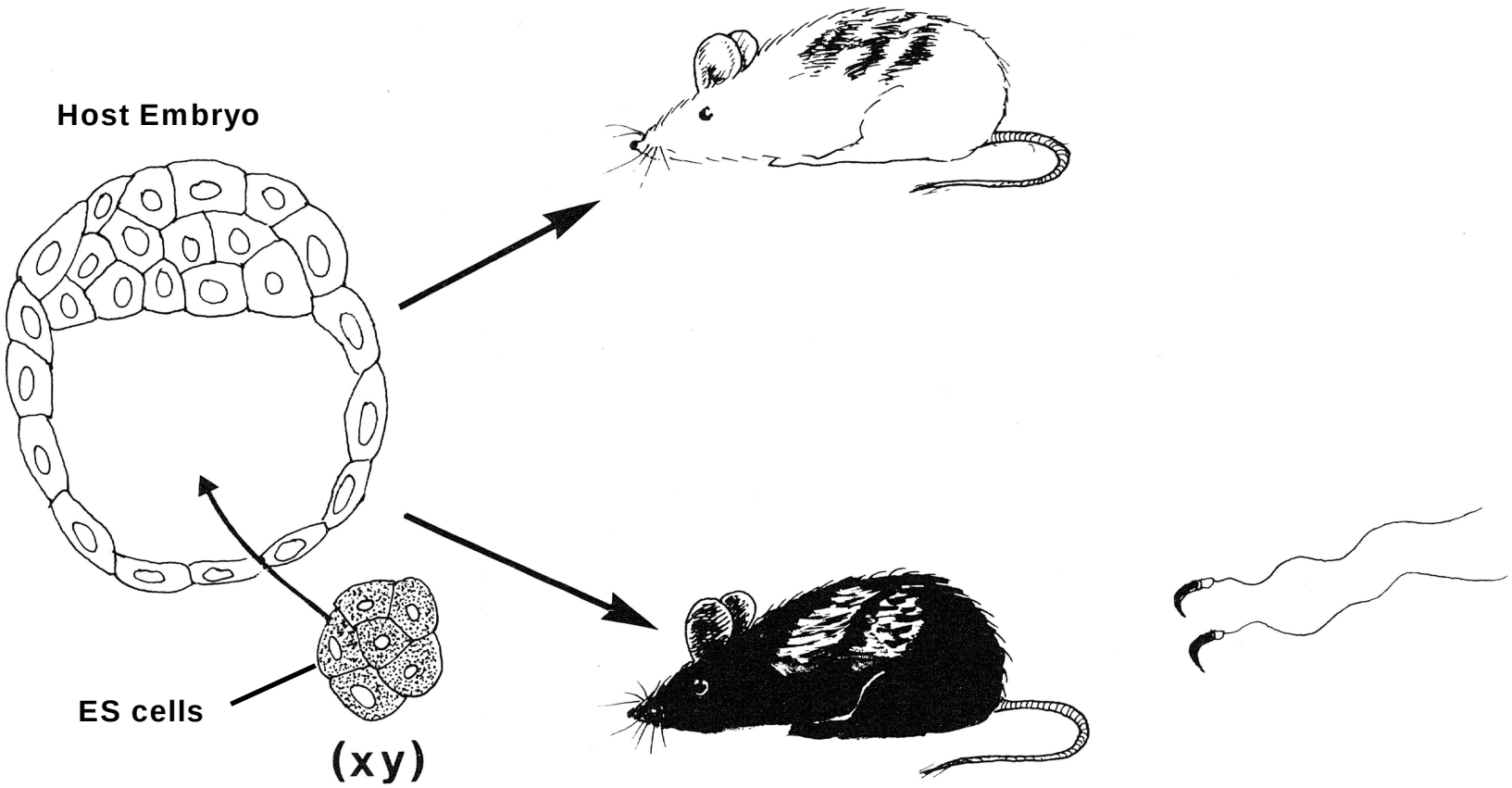
iPS cells: Ethical Problems Solved?

1. Definition, Derivation
2. **Pattern formation Potential:**
    - Autonomous: Embryoid Bodies
    - **Aided: Tetraploid Complementation (TC)**
3. Patenting
4. Alternative Approaches

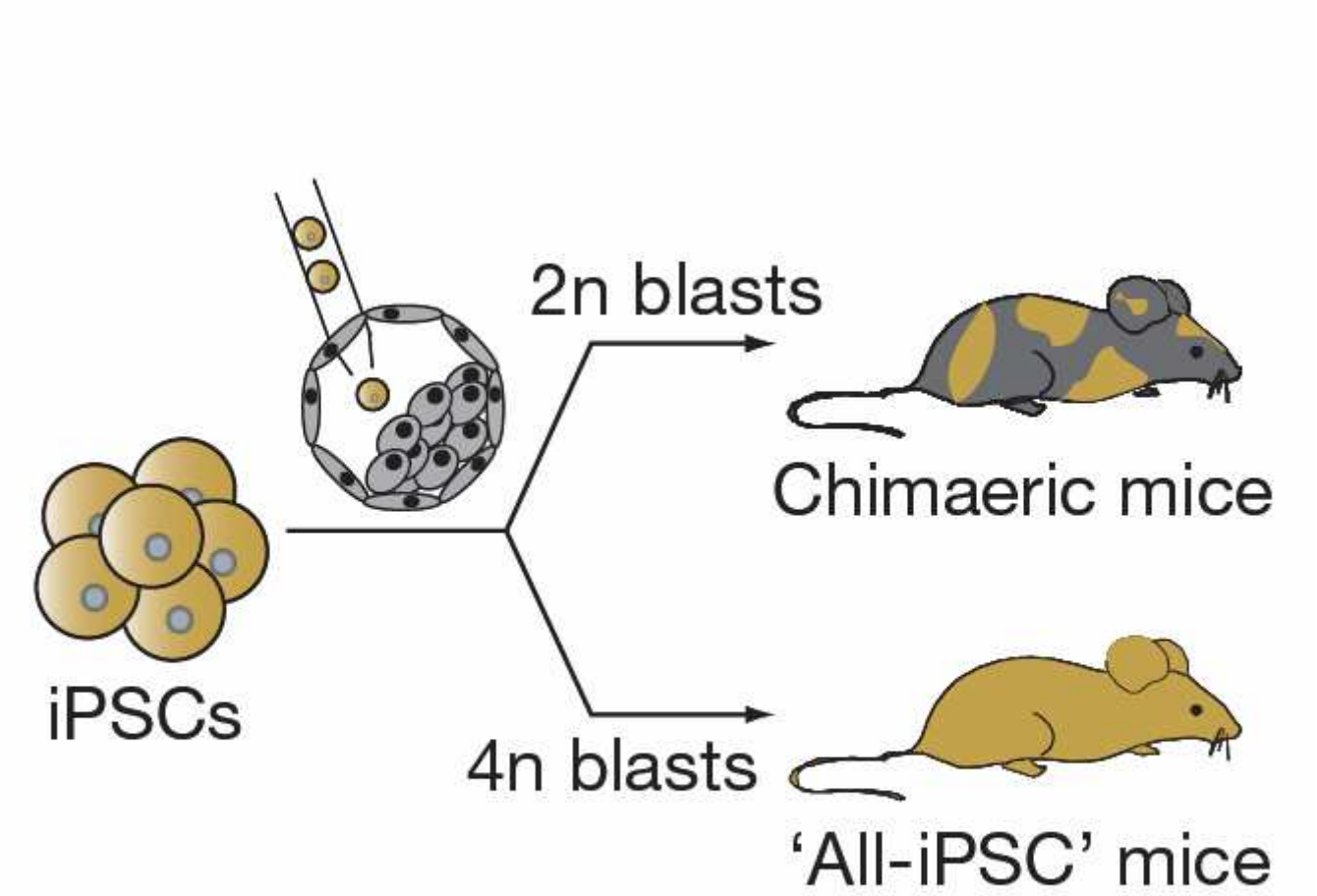
TC offers a method for **cloning viable individuals from pluripotent stem cells (ESCs, but also iPSCs)**.

This is a topic for **ethics, legislation and patenting regulations** that is just beginning to be recognized.

Chimera Formation and Tetraploid Complementation



Tetraploid Complementation:
If the normal host embryo is replaced by tetraploid blastomeres, the resulting mouse consists exclusively of ES cell derivatives (Nagy et al.: Proc. Natl. Acad. Sci. USA 90, 8424-8428, 1993)



Stadtfeld, M. et al.: Nature 465: 175-81 (2010) Fig. 2

Direct cloning of viable mice from iPSCs

- **Boland, M. J.** et al.: Adult mice generated from induced pluripotent stem cells. *Nature* 461: 91 (2009)
- **Kang, L.** et al.: iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. *Cell Stem Cell* 5: 135 (2009)
- **Zhao, X.-y.** et al.: iPS cells produce viable mice through tetraploid complementation. *Nature* **461**: 86 (2009)
- **Zhao, X.-y.** et al.: Production of mice using iPS cells and tetraploid complementation. *Nature Protocols* 5: 963 (2010)

Why might anyone intend to use TC with **human** iPSCs?

- **Reproductive Cloning**

  Worldwide consensus NOT to permit Reproductive Cloning. Will it hold?

- Research Cloning

Reproductive Cloning

The consensus may not last: (Re-)Construction of human embryos from ESCs for reproductive purposes has indeed already been proposed:

Devolder, K.; Ward, C.M.: Rescuing human embryonic stem cell research: The possibility of embryo reconstruction after stem cell derivation. *Metaphilosophy* 38: 245 (2007)

Why might anyone intend to use TC with **human** iPSCs?

- Reproductive Cloning

  Worldwide consensus NOT to permit reproductive cloning. Will it hold?

- **Research Cloning**

  (without intending to transfer the artificially created embryo to a uterus)

  Recent literature on its use in the mouse seems to suggest application with human cells

Why do Authors Argue for **Research Cloning** using TC?

- Individual iPS cell lines are observed to differ with respect to:
  - -- differentiation capacities
  - -- gene expression patterns
  - -- epigenetic marks

- This appears to argue for:
  - -- quality control
  - -- optimization of derivation protocols

TC is advertised as the most rigorous pluripotency test („gold standard“) for iPS cells in the mouse

- „We therefore consider the tetraploid complementation as the state-of-the-art technique to assess the pluripotency of a given cell line.“

  Wu, G. et al.: Generation of Healthy Mice from Gene-Corrected Disease-Specific Induced Pluripotent Stem Cells. *PLoS Biol* 9(7): e1001099 (2011)

- „This study underscores the intrinsic qualitative differences between iPS cells generated by different methods and highlights the need to rigorously characterize iPS cells beyond *in vitro* studies.“

  Han, J. et al.: Tbx3 improves the germ-line competency of induced pluripotent stem cells. *Nature* 463: 1096 (2010)

Publications underscoring the need for testing iPS cells due to epigenetic peculiarities

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

Matthias Stadtfeld[1,2,3]*, Effie Apostolou[1,2,3]*, Hidenori Akutsu[4], Atsushi Fukuda[5], Patricia Follett[1], Sridaran Natesan[6], Tomohiro Kono[5], Toshi Shioda[2] & Konrad Hochedlinger[1,2,3]

Induced pluripotent stem cells (iPSCs) have been generated by enforced expression of defined sets of transcription factors in somatic cells. It remains controversial whether iPSCs are molecularly and functionally equivalent to blastocyst-derived embryonic stem (ES) cells. By comparing genetically identical mouse ES cells and iPSCs, we show here that their overall messenger RNA and microRNA expression patterns are indistinguishable with the exception of a few transcripts encoded within the imprinted *Dlk1–Dio3* gene cluster on chromosome 12qF1, which were aberrantly silenced in most of the iPSC clones. Consistent with a developmental role of the *Dlk1–Dio3* gene cluster, these iPSC clones contributed poorly to chimaeras and failed to support the development of entirely iPSC-derived animals ('all-iPSC mice'). In contrast, iPSC clones with normal expression of the *Dlk1–Dio3* cluster contributed to high-grade chimaeras and generated viable all-iPSC mice. Notably, treatment of an iPSC clone that had silenced *Dlk1–Dio3* with a histone deacetylase inhibitor reactivated the locus and rescued its ability to support full-term development of all-iPSC mice. Thus, the expression state of a single imprinted gene cluster seems to distinguish most murine iPSCs from ES cells and allows for the prospective identification of iPSC clones that have the full development potential of ES cells.

Nature 465: 175-81 (2010)

Other publications documenting **epigenetic peculiarities and epigenetic memory** of iPSCs

- **Liu, L.** et al.: Activation of the imprinted Dlk1-Dio3 region correlates with pluripotency levels of mouse stem cells. *J. Biol. Chem.* 285: 19483 (2010)
- **Kim, K.** et al.: Epigenetic memory in induced pluripotent stem cells. *Nature* 467: 285 (2010)
- **Bar-Nur, O.** et al.: Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet Beta cells. *Cell Stem Cell* 9: 17 (2011)
- **Lister, R.** et al.: Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature* 471: 68 (2011)

…and even chromosomal aberrations and gene deletions:

- **Mayshar, Y.** et al.: Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. *Cell Stem Cell* 7: 521 (2010)
- **Laurent, L. C.** et al.: Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. *Cell Stem Cell* 8: 106 (2011)

Use of iPSCs for disease modelling

„**Genetic manipulation of iPS cells in combination with tetraploid embryo aggregation** provides a practical and rapid approach to evaluate the efficacy of gene correction of human diseases in mouse models.“

Wu, G. et al.: Generation of Healthy Mice from Gene-Corrected Disease-Specific Induced Pluripotent Stem Cells. *PLoS Biol* 9(7): e1001099 (2011)

→ **How could that be translated to human therapy without testing human iPSCs?**
TC with human cells? Animal-human chimeras?
Same questions apply to iPSC use in tissue engineering!

Would it be ethically acceptable to use cloning by TC with human iPSCs for quality/safety testing purposes *in vitro*, i.e. without transferring embryos to a uterus („Research Cloning“)?

- Legal problems (Germany: Embryo Protection Law, ESchG): Embryo destruction!
- Informed consent of cell donors

Even without transferring the products of TC („artificial“/test embryos) to a uterus such a procedure would re-create the problem of embryo destruction which the original idea of iPSC technology intends to eliminate.

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4. Alternative Approaches

Published online 18 October 2011 | Nature | doi:10.1038/news.2011.597

News

European court bans patents based on embryonic stem cells

Final decision could stifle investment in developing therapies.

Ewen Callaway

TC Capability and Patenting

Any „fully pluripotent“ stem cell (possessing TC capability) cannot be considered patentable.

→ **My prediction: European regulations will finally take this into consideration after iPS cell patenting has become a topic.**

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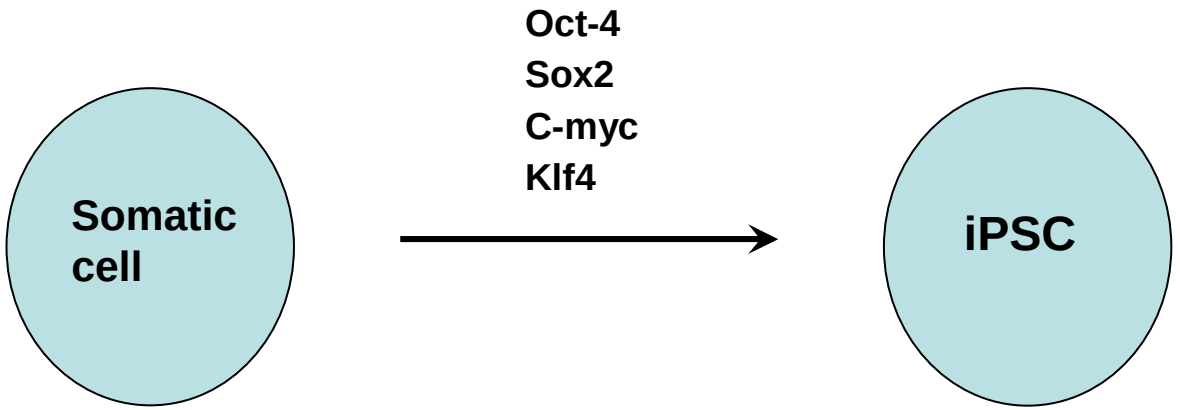
The Traditional Way of Pluripotency Induction: The 4 Yamanaka Factors



Methodology:

DNA-based (integrative / non-integrative)

RNA-based

Protein-based

Recent Review:

González et al.: *Nature Reviews*

12: 231 (2011)

Induction of Pluripotency Using the 4 Yamanaka Factors

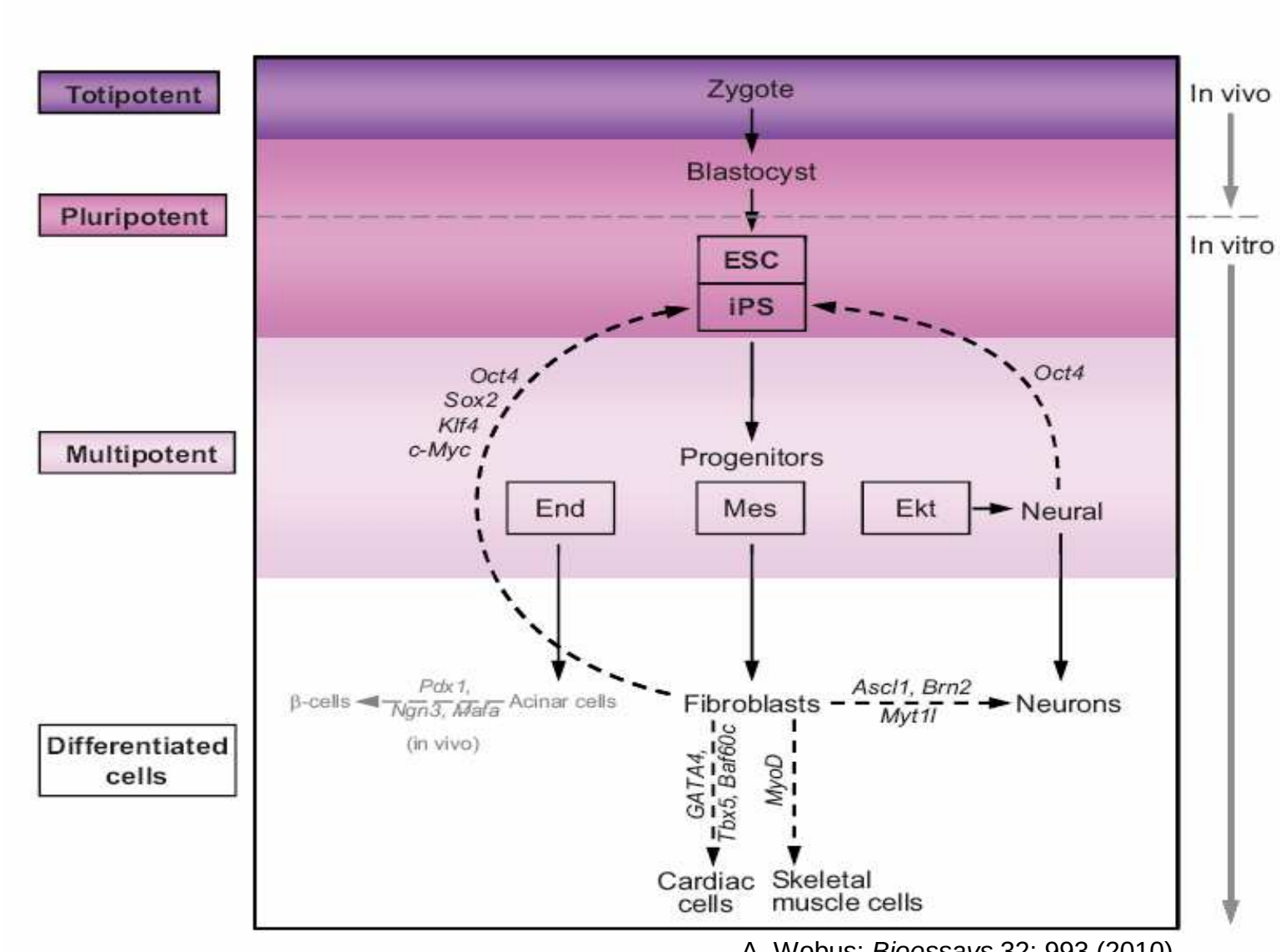
→ If **pluripotency** is the endpoint, the ethical problem just discussed is NOT eliminated,

- neither by the **methodology** chosen for **induction**
- nor by the **cell type of origin** (e.g. neuronal stem cells endogenously expressing some of the Yamanaka factors).

The Alternative: Bypassing Pluripotency

Direct conversion of somatic cells into stem or progenitor cells that lack self-organization and TC capability, i.e. remain at lower degrees of potentiality

4. Alternative Approaches



A. Wobus: *Bioessays* 32: 993 (2010)

The Alternative: Bypassing Pluripotency

Direct conversion of somatic cells into stem or progenitor cells that lack self-organization and TC capability, i.e. remain at lower degrees of potentiality:

- No Yamanaka factors, but directly target genes regulating lineage specification.

OR:

- Do use (some of the) Yamanaka factors but suppress self-organization processes genetically or epigenetically during stem cell derivation (e.g. culturing conditions).

  (Caveat: Pluripotency may be transitory and remain undetected during derivation, and may be regained later).

Recent Literature on Bypassing Pluripotency

- **Ieda, M.** et al.: Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors. *Cell* 142: 375 **(2010)**
- **Szabo, E.** et al.: Direct conversion of human fibroblasts to multilineage blood progenitors. *Nature* 468: 521 **(2010)**
- **Vierbuchen, T.** et al.: Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* 463: 1035 **(2010)**
- **Caiazzo, M.** et al.: Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature* 476: 224 **(2011)**
- **Pfisterer, U.** et al.: Direct conversion of human fibroblasts to dopaminergic neurons. *PNAS* 108: 10343 **(2011)**
- **Son, E. Y.** et al.: Conversion of Mouse and Human Fibroblasts into Functional Spinal Motor Neurons. *Cell Stem Cell* 9: 205 **(2011)**
- **Qiang, L.** et al.: Directed conversion of Alzheimer's disease patient skin fibroblasts into functional neurons. *Cell* 146: 359 **(2011)**
- **Yoo, A. S.** et al.: MicroRNA-mediated conversion of human fibroblasts to neurons. *Nature* 476: 228 **(2011)**

Conclusion

Recently proposed alternative strategies how to create stem and progenitor cells with **restricted** developmental potential (lack of TC capability) promise to avoid the ethical and patenting problems posed by the potentiality of pluripotent stem cells (ESCs and iPSCs).

Take-Home Message

- It is not the cell source chosen for stem cell derivation but the **potentiality** of the produced stem cells that is the most challenging theme for future stem cell research and legislation.
- Be prudent: **Avoid pluripotency**, do NOT consider it the ultimate goal of your research!
- Choose the emerging **alternative** stem cell derivation strategies **bypassing pluripotency** and creating stem cells with **restricted** potentiality!

Thanks

Experimental Stem Cell Studies (Denker Lab)

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Birgit Maranca-Hüwel
Dorothee Schünke
Michael Thie

Ethics

Thomas Heinemann (Bonn)
Ludger Honnefelder (Bonn)
Søren Holm (Manchester)

Further Reading:

http://www.uni-due.de/denker/

My primary ethical concern raised by the pluripotency of stem cells

My primary ethical concern here is that after the advent and worldwide use of pluripotent stem cell technology (and with the technology of TC at hand) we may not be able to maintain **control over our individual genetic / epigenetic uniqueness**:

-- in case of ES cells: the genetic / epigenetic uniqueness of our **offspring**;

-- in case of iPS cells: **our own** genetic / epigenetic uniqueness.