Organised by the ESHRE Special Interest Group "Stem Cells" in collaboration with the Spanish Stem Cell Bank (Centre de Medicina Regenerativa de Barcelona, Centro de Investigacion Principe Felipe - Valencia and Centro de Investigaciones Biomédicas - Granada)

Basic course on "Update on pluripotent stem cells (hESC and iPS)
Hands on course on "Derivation and culture of pluripotent stem cells"

ESHRE Campus 2010
Valencia Spain, 8-12 November 2010

Cell reprogramming (induced pluripotent stem cells, iPS)

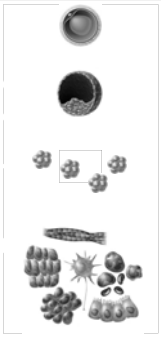
Angel Raya
ICREA Research Professor
Institute for Bioengineering of Catalonia
Barcelona, Spain

November 8, 2010

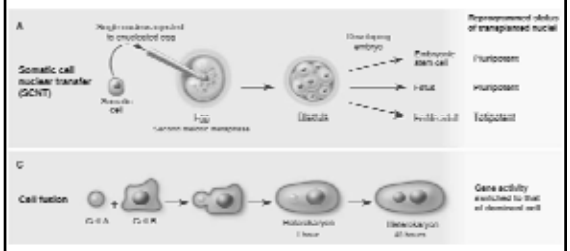
The progressive loss of potency

DEVELOPMENT

POTENCY

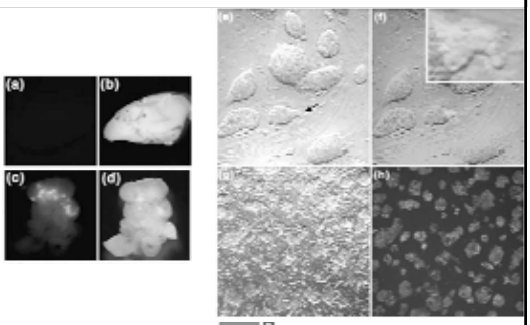


Reprogramming to pluripotency



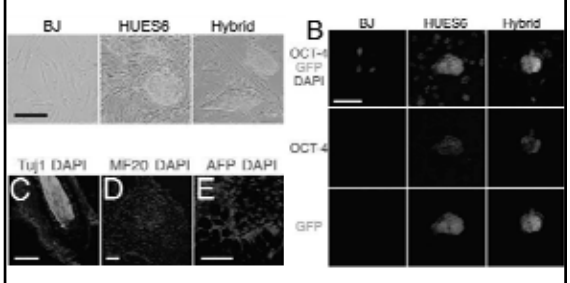
Gurdon & Melton, *Science*, 2008

Reprogramming through cell fusion



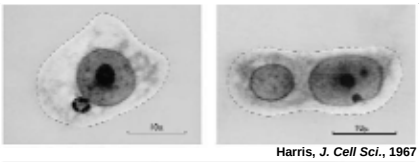
Tada et al., *Curr. Biol.*, 2001

Reprogramming through cell fusion

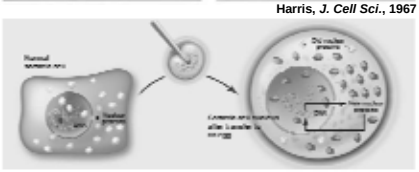


Cowan et al., *Science*, 2005

Reprogramming through cell fusion



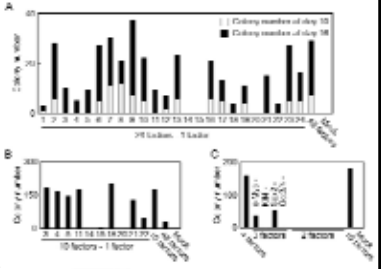
Harris, *J. Cell Sci.*, 1967



Gurdon & Melton, *Science*, 2008

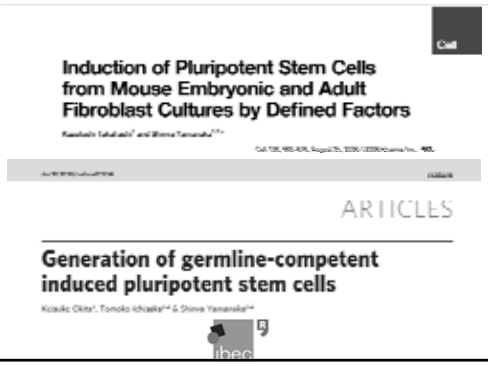
Induced reprogramming of mouse cells

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



Takahashi & Yamanaka, *Cell*, 2006

Induced reprogramming of mouse cells

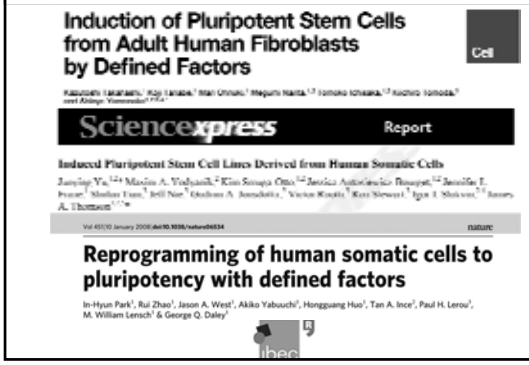


Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

ARTICLES

Generation of germline-competent induced pluripotent stem cells

Induced reprogramming of human cells



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

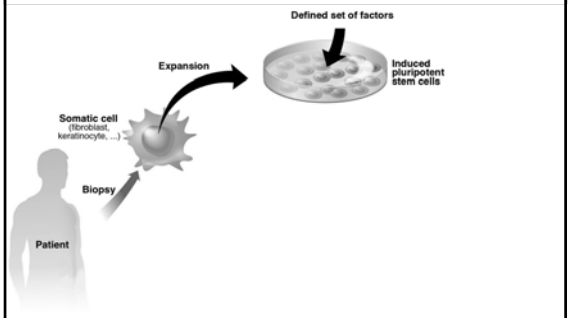
Cell

Sciencexpress Report

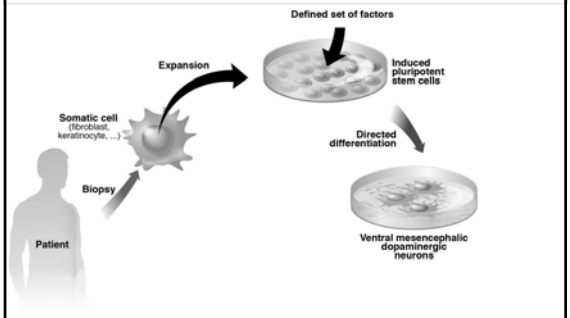
Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Reprogramming of human somatic cells to pluripotency with defined factors

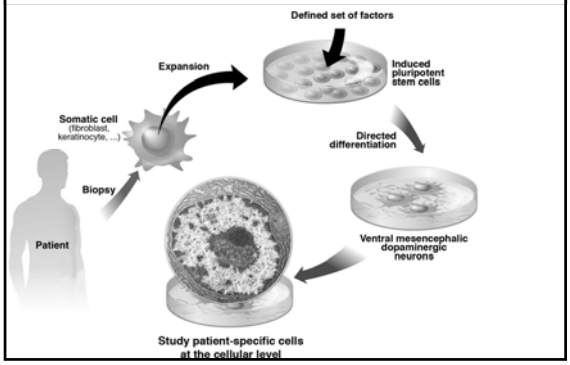
Modeling human disease with iPS cells



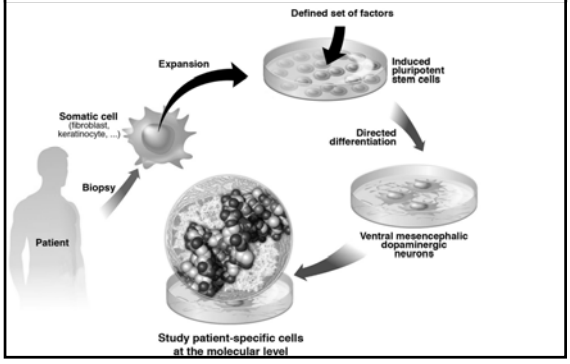
Modeling human disease with iPS cells



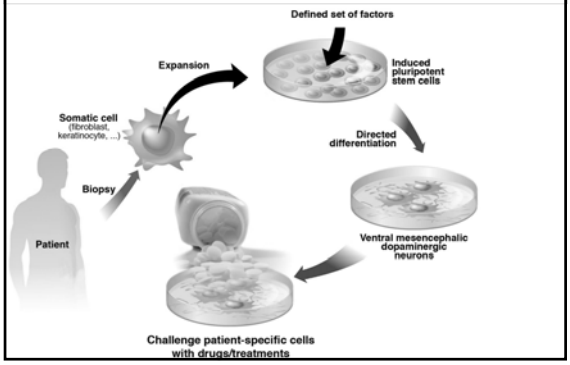
Modeling human disease with iPS cells



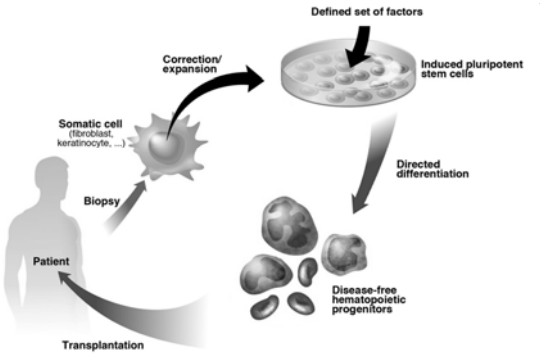
Modeling human disease with iPS cells



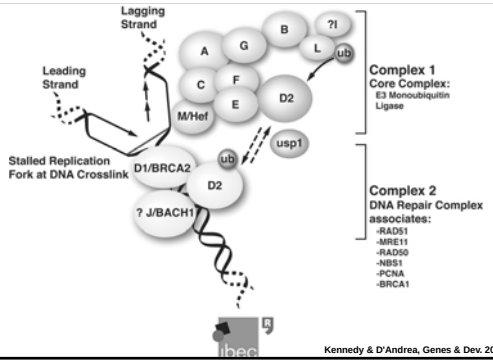
Modeling human disease with iPS cells



Treating human disease with iPS cells



The FA/BRCA2 pathway



Kennedy & D'Andrea, Genes & Dev. 2005

Generation of FA patient-specific iPS cells

Juan Bueren
CIEMAT

	Promoters	Transgene		ID	Source	Passages
RV	viral LTR	FANCA FANCA –EGFP FANCA-Neo	for FANCA	FA5 FA90 FA153 FA404	Skin Skin Skin Skin	High High High Fresh
LV	internal promoters: SFFV, CMV, PGK, VAV	FANCA FANCA- Wpre FANCA – EGFP				
RV	viral LTR	FANCD2 FANCD2 –EGFP FANCD2-Neo	for FANCD2	FA430 FA431	Skin Skin	Mid Low
LV	internal promoters: VAV	FANCD2- Wpre				

Raya et al.,Nature 2009

Generation of FA patient-specific iPS cells

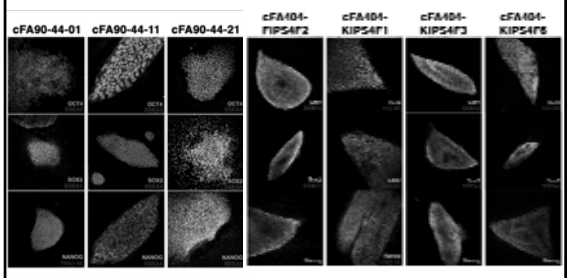
Patient ID	FA group	Somatic cell	Attempts	iPS-like colonies	Lines generated	Lines characterized: Markers	In vitro	Terat.
FA5	A	Fibr.	5	0	0	NA	NA	NA
FA5	A	cFibr.	5	0	0	NA	NA	NA
FA90	A	Fibr.	3	0	0	NA	NA	NA
FA90	A	cFibr.	3	~37	10 cFA90-44-X	5	5	3
FA153	A	Fibr.	5	0	0	NA	NA	NA
FA153	A	cFibr.	5	0	0	NA	NA	NA
FA404	A	Fibr.	3	0	0	NA	NA	NA
FA404	A	cFibr.	3	~30	2 cFA404-FiPS4FX	2	2	2
FA404	A	Kerat.	3	0	0	NA	NA	NA
FA404	A	cKerat	3	~30	3 cFA404-KiPS4FX	3	3	3
FA430	D2	Fibr.	6	0	0	NA	NA	NA
FA430	D2	cFibr.	6	0	0	NA	NA	NA
FA431	D2	Fibr.	3	~10	2*	2*	NA	NA
FA431	D2	cFibr.	3	~10	2 cFA431-44-X	2	2	NT

Raya et al.,Nature 2009

Criteria for defining *bona fide* iPS cells

- Self-renewal
  - > 20 passages
  - Karyotypic stability
- Pluripotency
  - Expression of pluripotency-associated markers
  - *In vitro* differentiation
  - Teratoma formation
- Molecular
  - DNA fingerprinting
  - Integration of reprogramming transgenes
  - Silencing of reprogramming transgenes
  - Reprogramming of gene expression profile
  - Reprogramming of DNA methylation profile

Generation of FA patient-specific iPS cells



Raya et al.,Nature 2009

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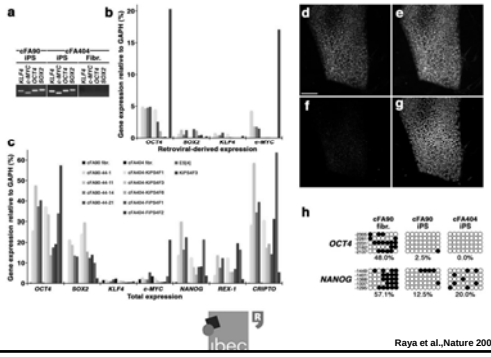

Raya et al.,Nature 2009

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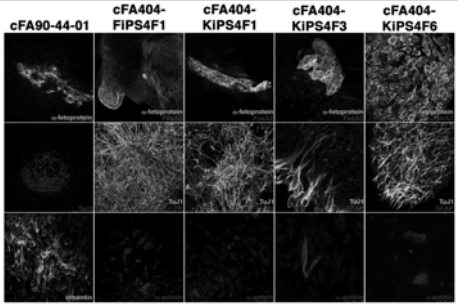
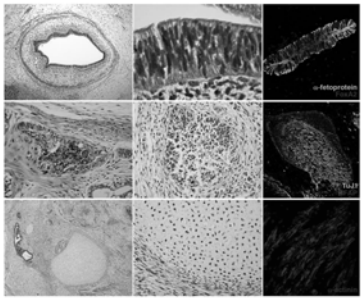


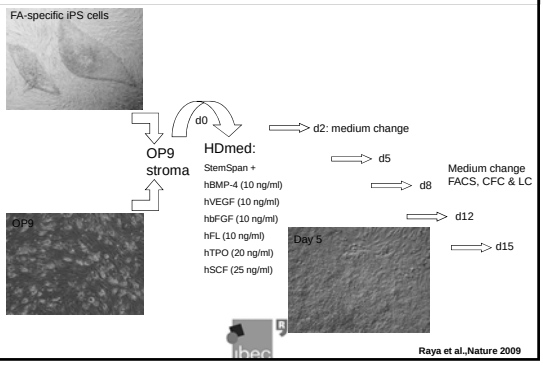
Raya et al.,Nature 2009

Generation of FA patient-specific iPS cells



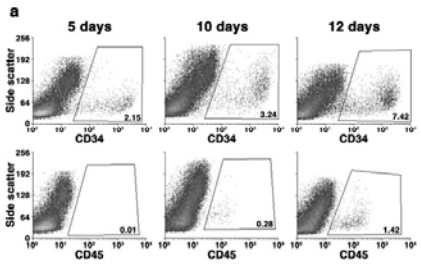
Raya et al.,Nature 2009

Hematopoietic differentiation of FA iPS cells



Raya et al.,Nature 2009

FA iPS-derived hematopoietic progenitor cells



Raya et al.,Nature 2009

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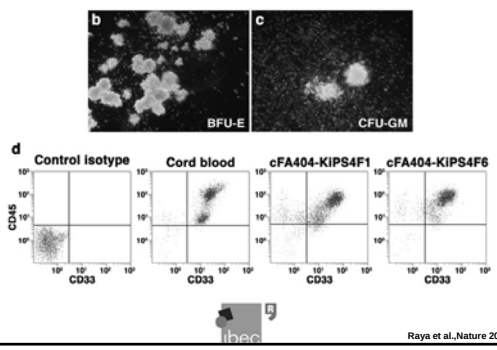
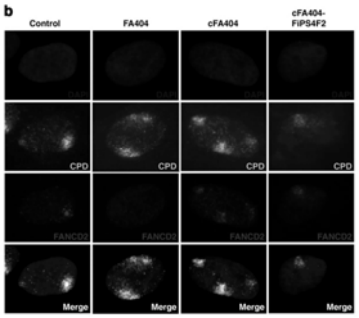


Raya et al.,Nature 2009

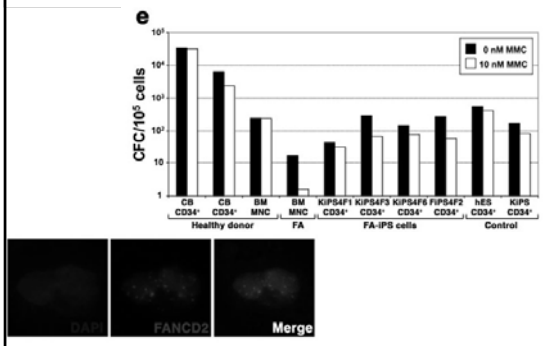
FA iPS-derived cells are disease free

Jordi Surrallés
UAB



Raya et al.,Nature 2009

Disease-free hematopoietic progenitors



Raya et al.,Nature 2009

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FA153	A	Fibr.	5	0	0	NA	NA	NA
FA153	A	cFibr.	5	0	0	NA	NA	NA
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Raya et al.,Nature 2009

FA pathway is critical for iPS cell proliferation

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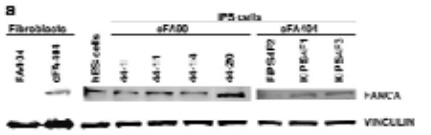
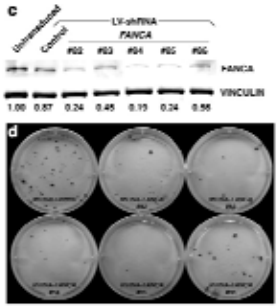


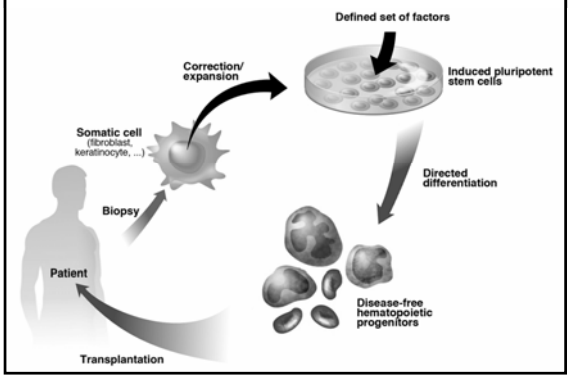
Raya et al.,Nature 2009

FA pathway is critical for iPS cell proliferation



Raya et al.,Nature 2009

Proof-of-concept for iPS-based cell therapy



Shortcomings of iPS cell therapy

Derivation of human iPS cells
- **Retroviral integrations**
- **Clinical-grade lines**

Control of cell proliferation
- **Selective killing**
- **Understanding self-renewal**

Specific differentiation protocols
- **Directed differentiation protocols**
- **Clinical-grade protocols**

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Ignasi Rodríguez-Pizà
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CIEMAT

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UAB

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