Direct reprogramming

Jana Bartoňková
Lukáš Bálek

Direct reprogramming
= transdiferenciace

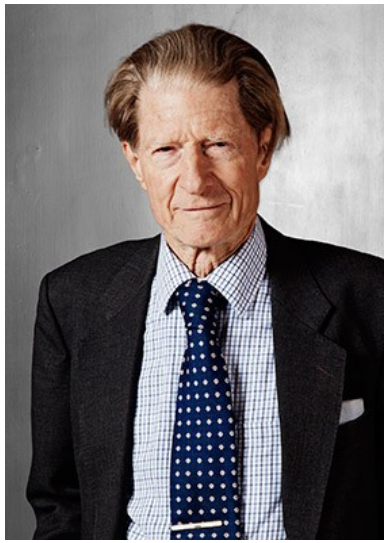
The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cells of Feeding Tadpoles

by J. B. GURDON[1]

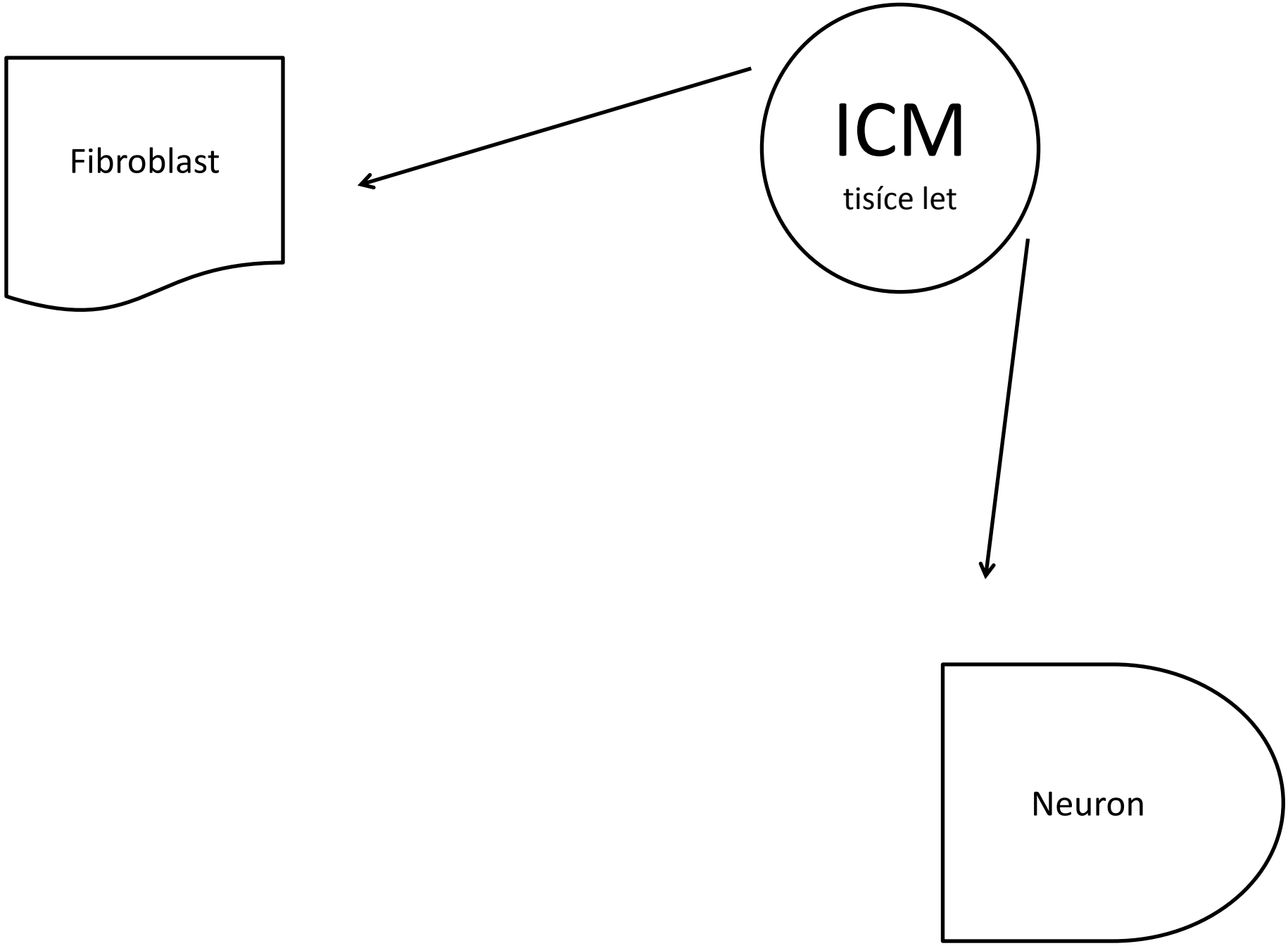
From the Embryology Laboratory, Department of Zoology, Oxford

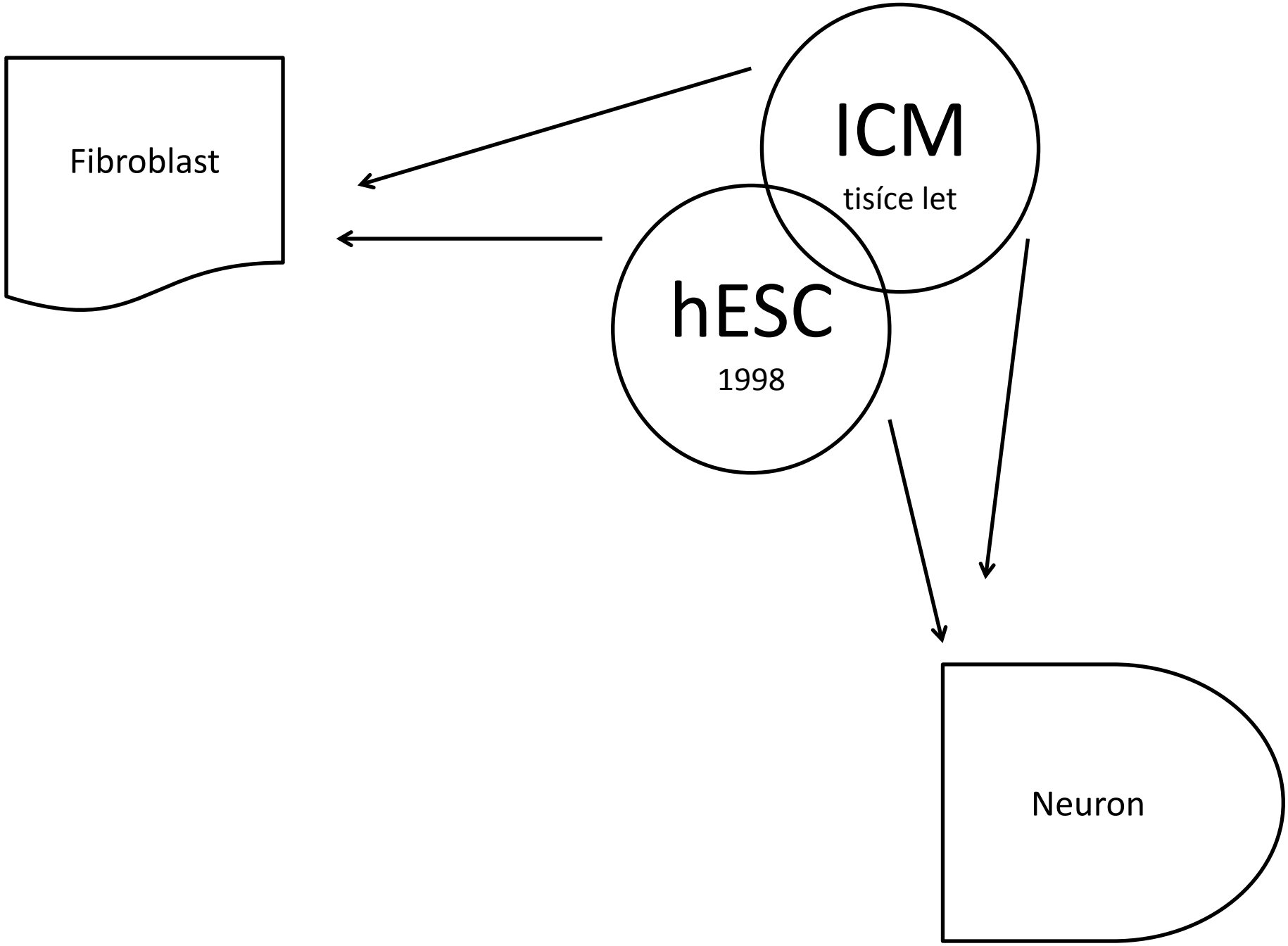
WITH ONE PLATE

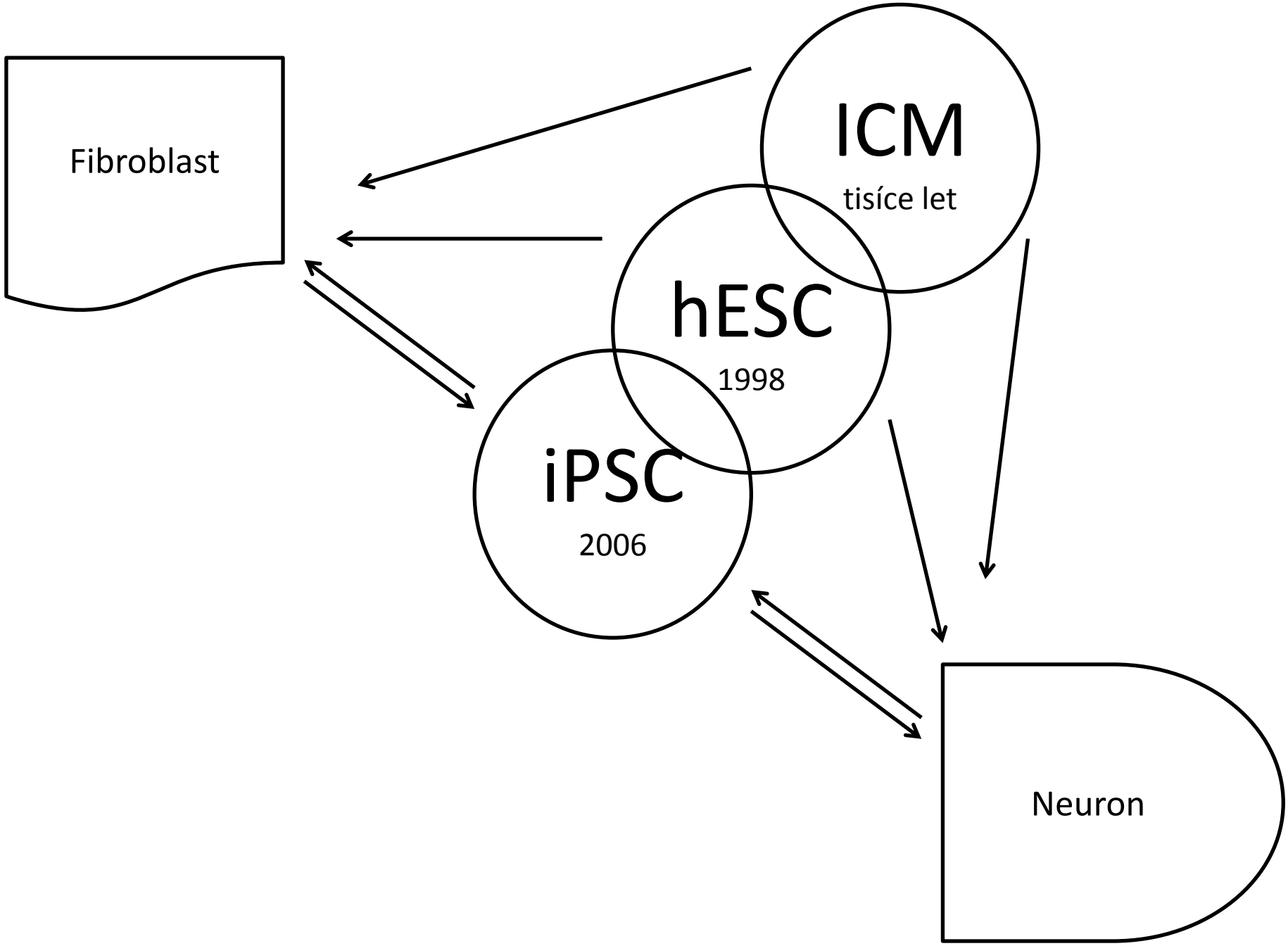
[J. Embryol. exp. Morph., Vol. 10, Part 4, pp. 622–40 December 1962]

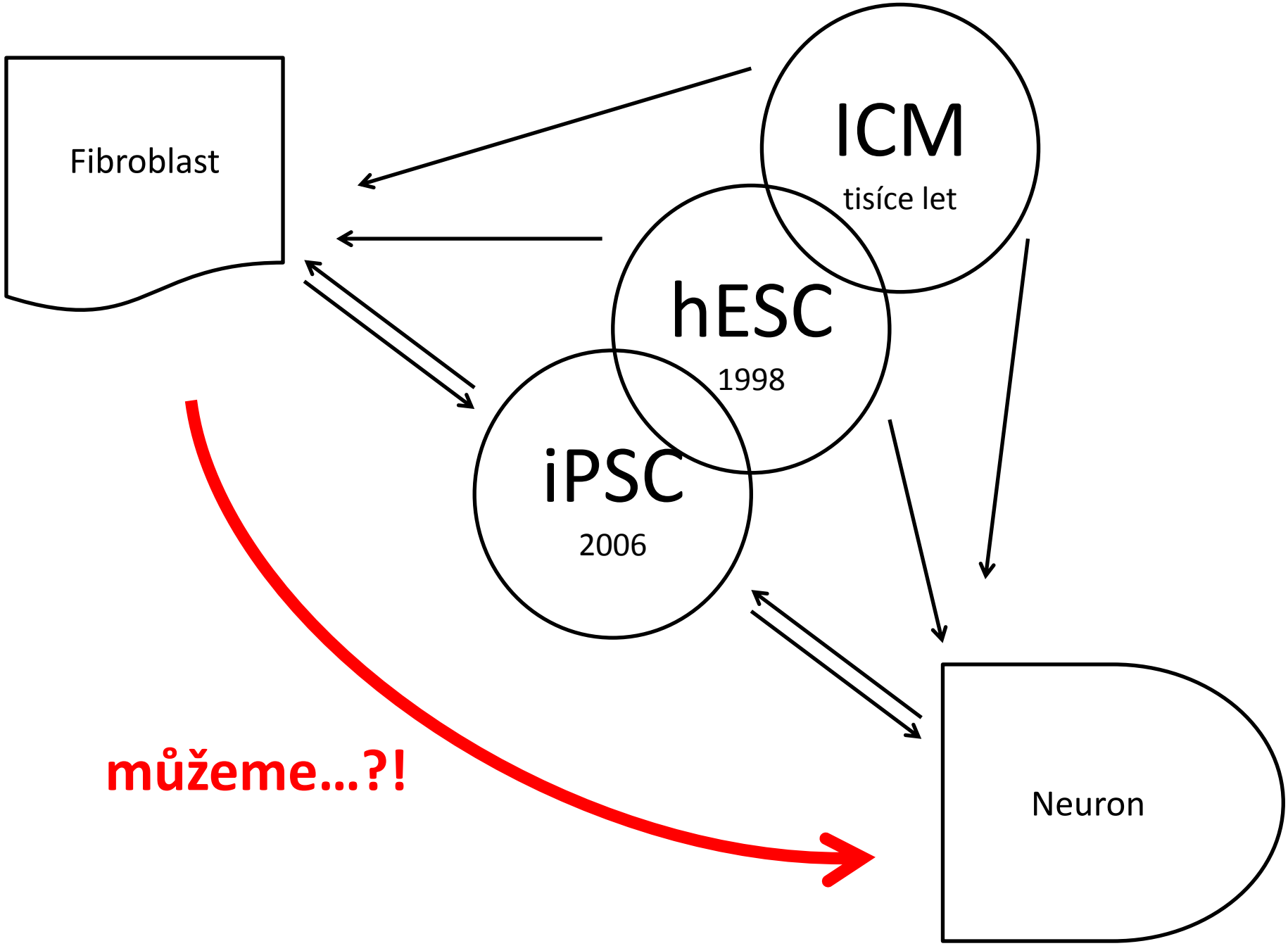


Nobelova cena 2012 (S. Yamanaka)


Fibroblast
ICM
tisíce let
Neuron


Fibroblast
ICM
tisíce let
hESC
1998
Neuron


Fibroblast
ICM
tisíce let
hESC
1998
iPSC
2006
Neuron


Fibroblast
ICM
tisíce let
hESC
1998
iPSC
2006
Neuron
můžeme…?!

náhrada/oprava buněk, tkání a orgánů

model nemoci na buněčné úrovni

Medicína

IN VITRO

iPSC

↓

Diferencovaná buňka

↓

Implantace

IN VITRO

Diferencovaná buňka

↓

Transdiferencovaná buňka *in vitro*

↓

Implantace

IN SITU

Transdiferenciace *in vivo*

Jak na to?

- transkripční faktory
- epigenetické regulátory
- miRNA
- malé chemické molekuly

Jak na to?

transkripční faktory

- hodiny po transfekci

- stabilní i po odstranění stimulu

Jak na to?

<u>epigenetické regulátory</u>

- Dnmt1 (Dhawan et al., 2011)

- Baf60c (Takeuchi and Bruneau, 2009)

- polycomb repressor complex 2 (PRC2) (Patel et al., 2012)

Jak na to?

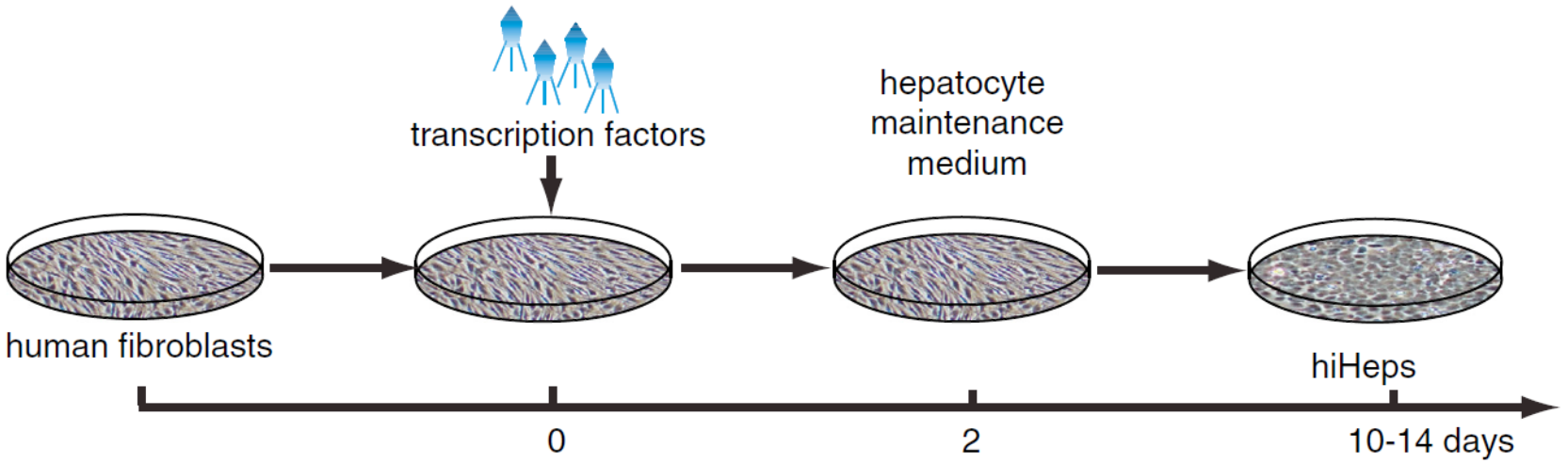
<u>miRNA</u>

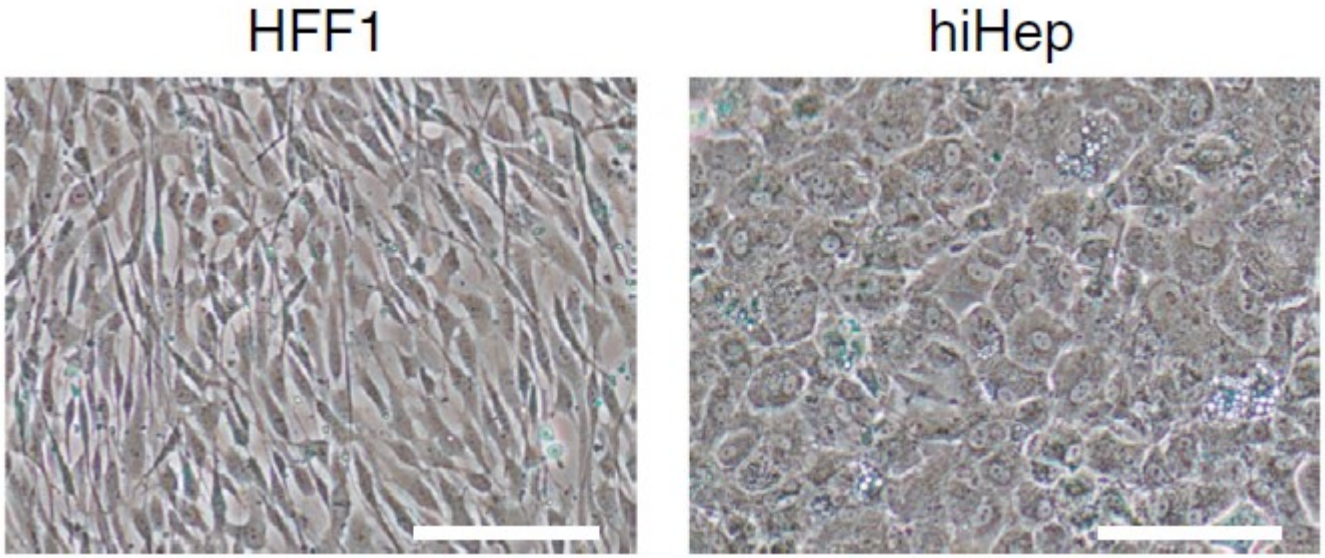
- miR-9/9 and miR-124 (Yoo et al., 2011)
- není tak efektivní jako TF

Jak na to?

malé chemické molekuly

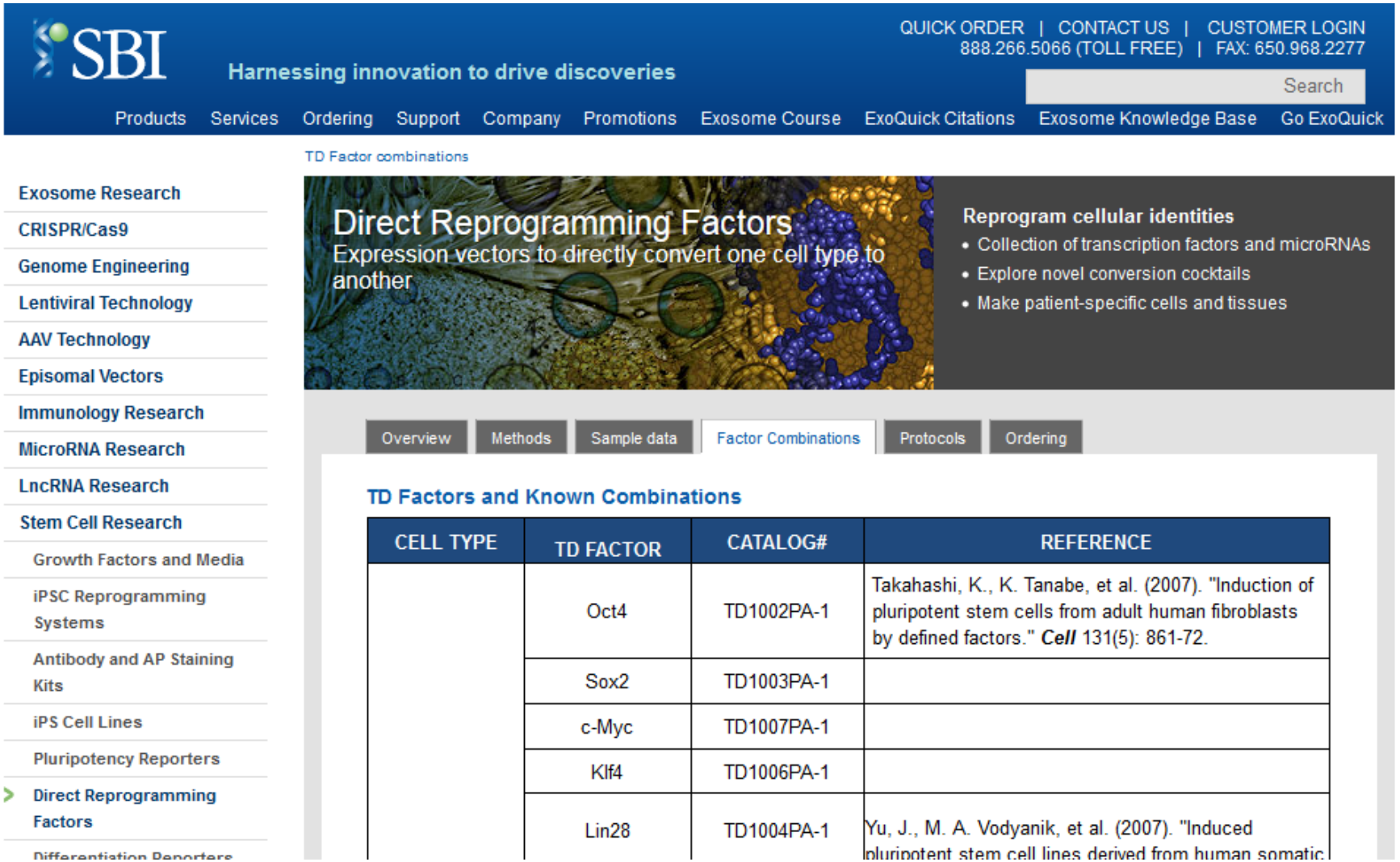
- především u iPSC
- předmět výzkumu





HFF = human fetal limb fibroblast

Huang P., Zhang L., Gao Y. et al. (2014). Cell Stem Cell 14, 370–384



www.systembio.com

IN VITRO

Kardiomyocyty	Tbx5, Mef2c, Gata-4, Mesp1	Ieda, M., J. D. Fu, et al. (2010). "Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors." Cell 142(3): 375-86.
	Oct4, Sox2, Klf4, C-Myc	Efe, J. A., S. Hilcove, et al. (2011). "Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy." Nat Cell Biol 13(3): 215-22.
Pankreas	Ngn3, Pdx1, MafA, VP16	Zhou, Q., J. Brown, et al. (2008). "In vivo reprogramming of adult pancreatic exocrine cells to beta-cells." Nature 455(7213): 627-32.
Krevni progenitory	Oct4, Gata1, Gata2, Gata3, Gata-4	Szabo, E., S. Rampalli, et al. "Direct conversion of **human** fibroblasts to multilineage blood progenitors." Nature 468(7323): 521-6
Osteoblasty	Mir-2861	Ivey, K. N. and D. Srivastava (2011). MicroRNAs as regulators of differentiation and cell fate decisions." Cell Stem Cell 7(1): 36-41.

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Neurony

Brn2, Ascl1, Mytl1, Zic1	Vierbuchen, T., et al. (2010). "Direct conversion of fibroblasts to functional neurons by defined factors." Nature 463(7284): 1035-41.
Brn2, Ascl1, Mytl1, NeuroD1	Pang, Z. P., et al. (2011). "Induction of **human** neuronal cells by defined transcription factors." Nature 476(7359): 220-3.
Mir-9, Mir-124, Ascl1, Mytl1	Yoo, A. S., et al. (2011). "MicroRNA-mediated conversion of **human** fibroblasts to neurons." Nature 476(7359): 228-31.
Ascl1, Brn2, Mytl1, Lmx1a, FoxA2	Caiazzo, M.et al. (2011). "Direct generation of functional dopaminergic neurons from mouse and human fibroblasts." Nature 476(7359): 224-7.
Mytl1, Brn2, Mir-124	Ambasudhan, R., M. Talantova, et al. (2011). "Direct Reprogramming of Adult **Human** Fibroblasts to Functional Neurons under Defined Conditions." Cell Stem Cell 9(2): 113-8.
Oct4, Sox2, Klf4, C-Myc	Kim, J., J. A. Efe, et al. (2011). "Direct reprogramming of mouse fibroblasts to neural progenitors." Proc Natl Acad Sci. 108(19): 7838-43.

IN VITRO

Dopaminergní neurony	Ascl1, Brn2, Mytl1, Foxa2, Lmx1a	Pfisterer, U., A. Kirkeby, et al. (2011). "Direct conversion of **human** fibroblasts to dopaminergic neurons." Proc Natl Acad Sci.108(25):10343-8.
Motorické neurony	Lhx3, Ascl1, Brn2, Mytl1, Ngn2, Hb9, Isl1, NeuroD1	Son, E. Y., J. K. Ichida, et al. (2011). "Conversion of Mouse and Human Fibroblasts into Functional Spinal Motor Neurons." Cell Stem Cell.
Hepatocyty	Gata-4, HNF1-alpha, Foxa3	Huang, P., Z. He, et al. (2011). "Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors." Nature 475(7356): 386-9.
	HNF4-alpha, Foxa1, Foxa2, Foxa3	Sekiya, S. and A. Suzuki (2011). "Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors." Nature 475(7356): 390-3.
	Foxa3, HNF1A, HNF4A	Huang P., Zhang L., Gao Y. et al.(2014). Direct Reprogramming of **Human** Fibroblasts to Functional and Expandable Hepatocytes. Cell Stem Cell 14, 370–384

IN SITU

2008 D. Melton: exokrinní buňky slinivky břišní > beta buňky (Ngn3, Pdx1 a Mafa)

2012 D. Srivastava: fibroblasty z jizvy srdce po infaktu > bijící kardiomyocyty (Gata4, Mef2c and Tbx5)

2014 Guo: gliové buňky > neurony (NeuroD1)

2015 Weider: satelitní gliové buňky > oligodendrocyte-like buňky (Sox10)

Překážky ve využití

- udržení/expanze buněk
- nízká efektivita
- bezpečnost použití


YEAR 1 XXX
REPROGRAMMING CELLS FOR DUMMIES