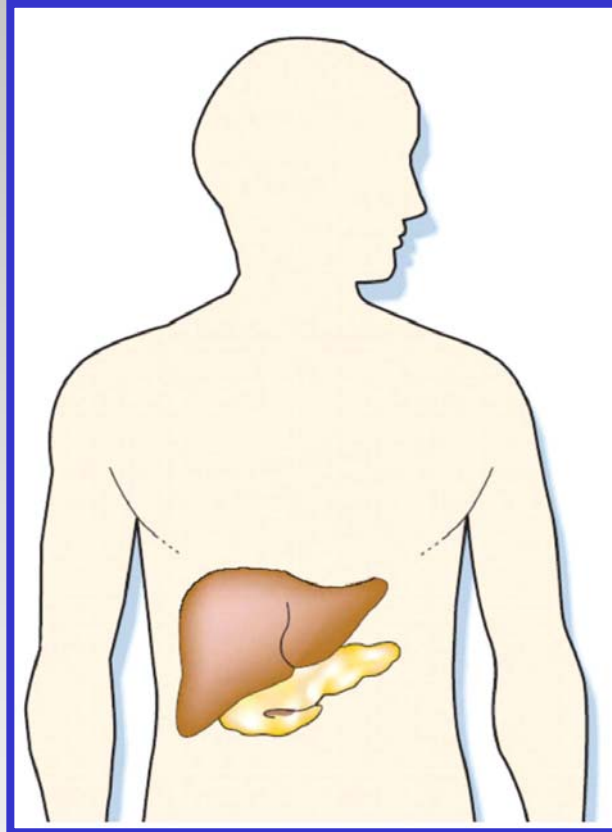


Science to Stem Cell Therapeutics



Ken Zaret, Ph.D.

Fox Chase Cancer Center, Philadelphia, PA

Needs and Sources of Cells

- acute liver failure
- genetic liver disease

- type I diabetes

1. **Cadaveric tissue** - *limited*

2. **Duct-associated progenitors**

- *activated after damage to hepatocytes or beta cells*

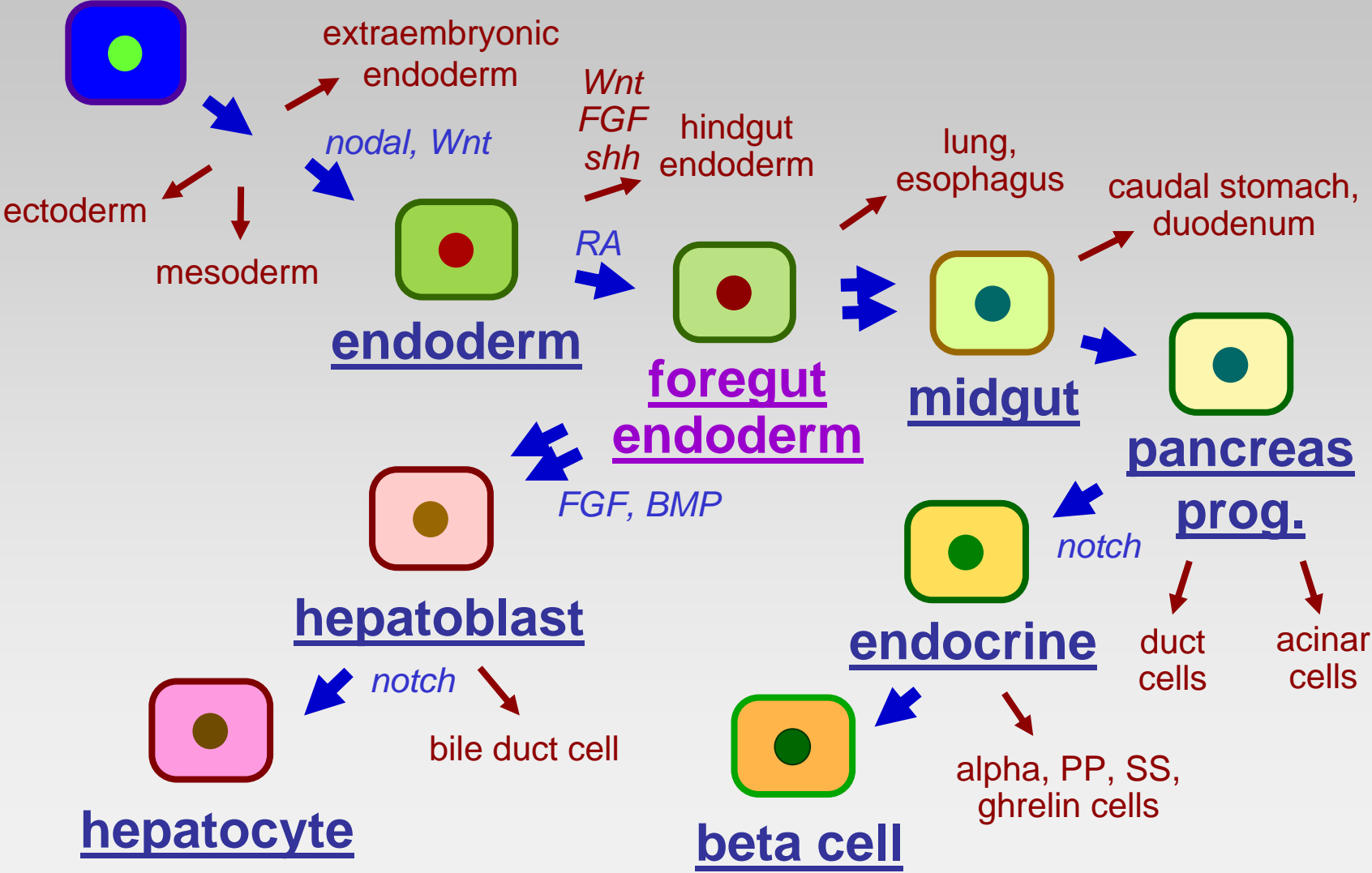
3. **Fetal liver or pancreatic cells** - *limited*

4. **Trans-differentiation from other cell types**

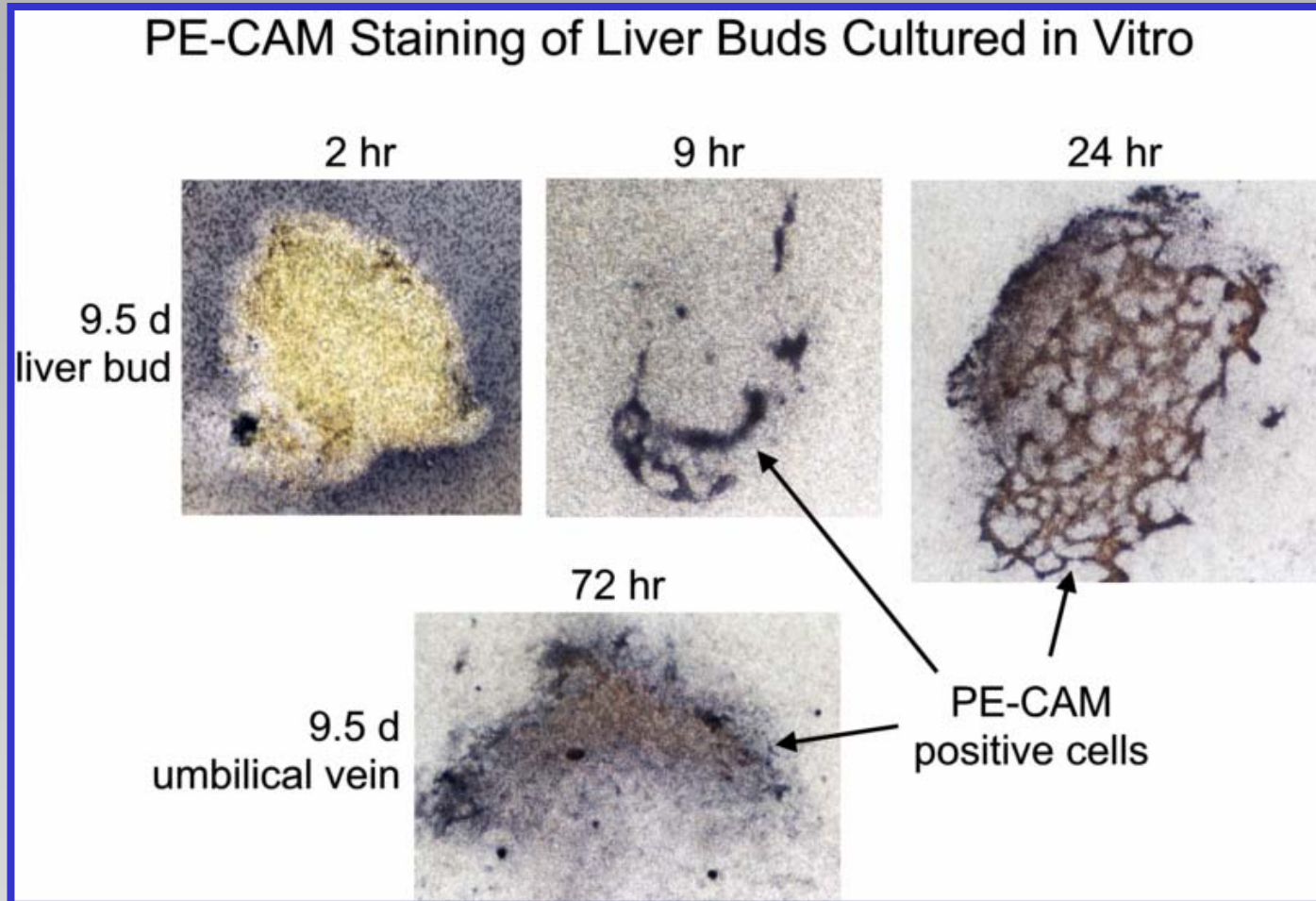
5. **ES cells or induced pluripotent cells**

Developmental Programming

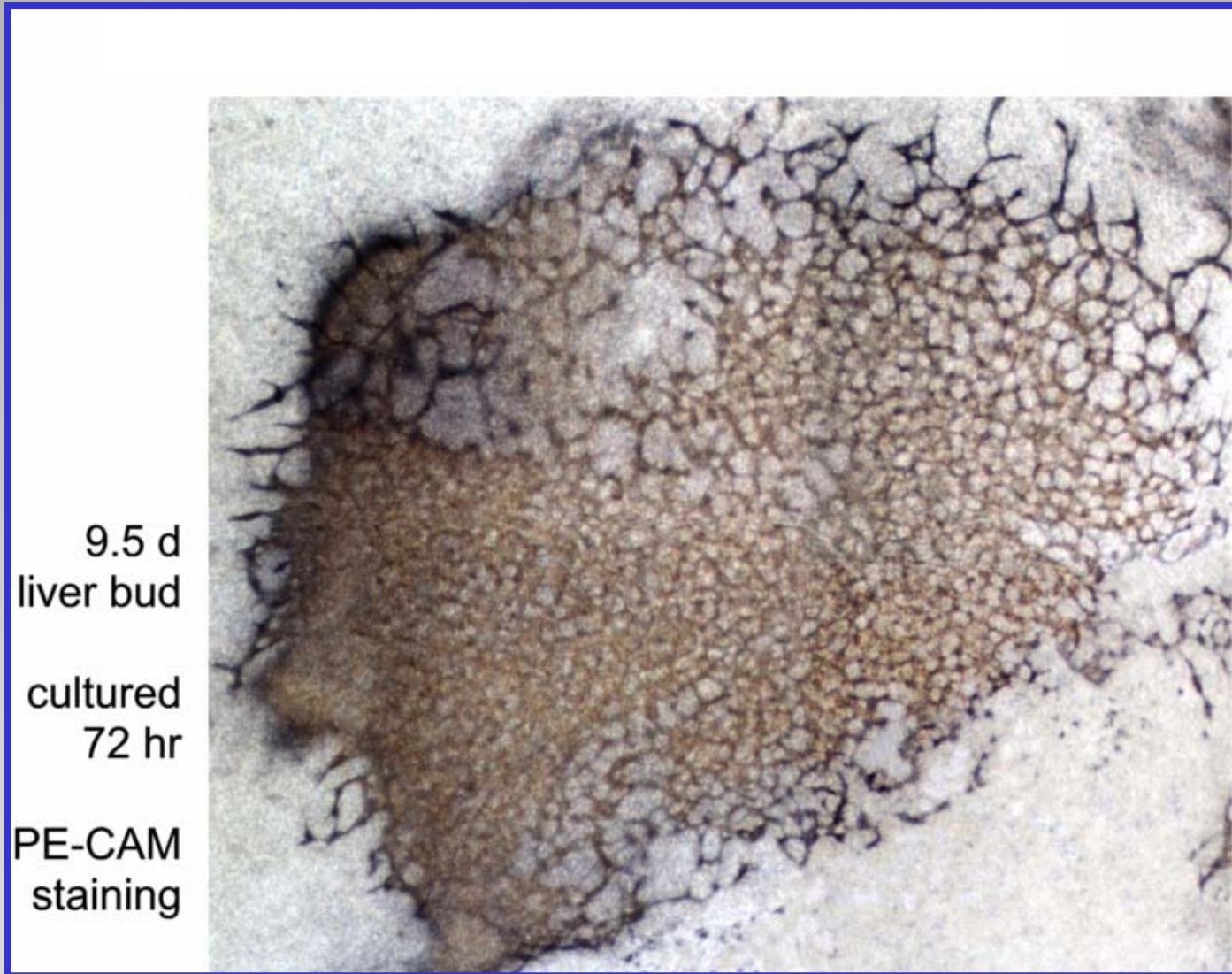
blastomere (ES, iPS)



Reconstituting Organogenesis in Vitro



Reconstituting Organogenesis in Vitro



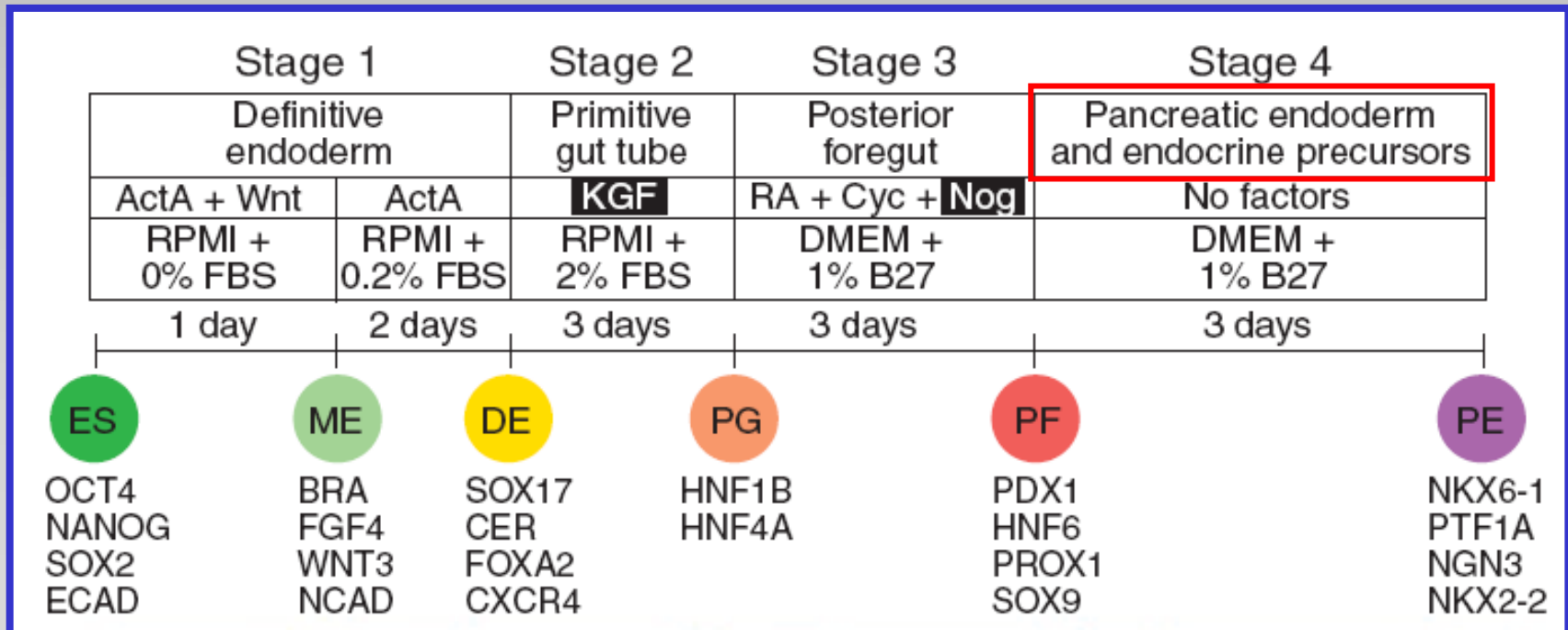
Pancreatic Development From ES Cells

Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*

Evert Kroon, Laura A Martinson, Kuniko Kadoya, Anne G Bang, Olivia G Kelly, Susan Eliazar, Holly Young, Mike Richardson, Nora G Smart, Justine Cunningham, Alan D Agulnick, Kevin A D'Amour, Melissa K Carpenter, Emmanuel E Baetge

NATURE BIOTECHNOLOGY VOLUME 26 NUMBER 4 APRIL 2008

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Liver Development From ES Cells

BMP-4 is required for hepatic specification of mouse embryonic stem cell-derived definitive endoderm

Valerie Gouon-Evans¹, Lise Boussemart¹, Paul Gadue¹, Dirk Nierhoff^{2,3}, Christoph I Koehler², Atsushi Kubo⁴, David A Shafritz² & Gordon Keller¹

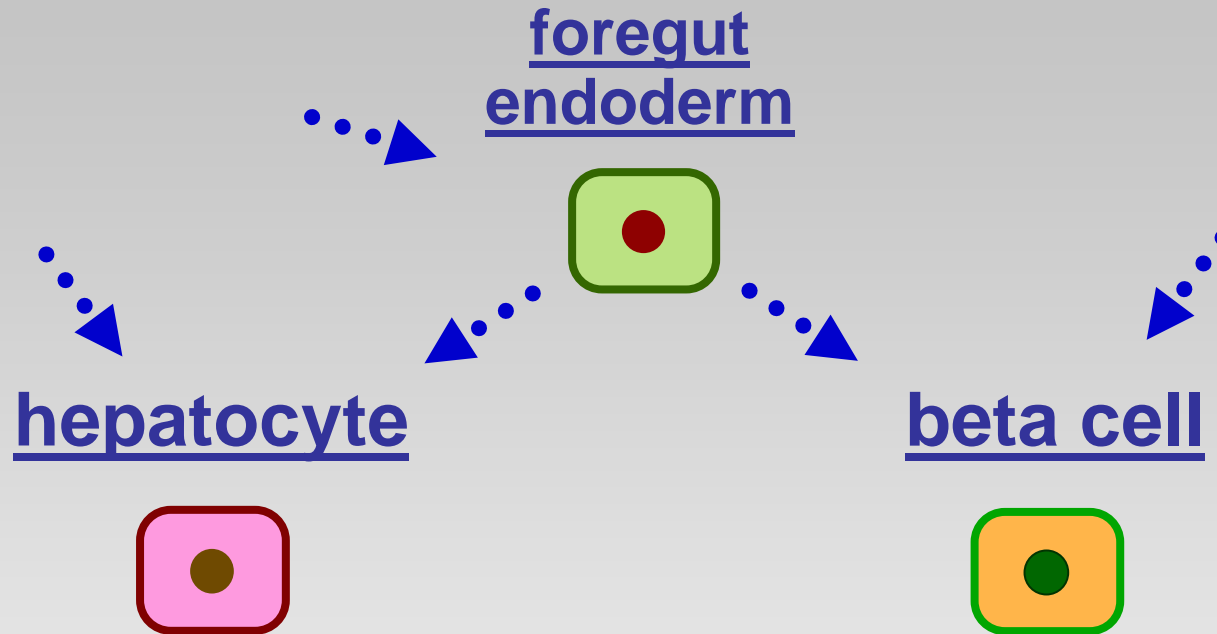
VOLUME 24 NUMBER 11 NOVEMBER 2006 **NATURE BIOTECHNOLOGY**

Distinct mesodermal signals, including BMPs from the septum transversum mesenchyme, are required in combination for hepatogenesis from the endoderm

Jennifer M. Rossi,^{1,2} N. Ray Dunn,³ Brigid L.M. Hogan,³ and Kenneth S. Zaret^{2,4}

¹Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, Rhode Island 02912, USA; ²Cell and Developmental Biology Program, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA;

Goals for Stem Cell Differentiation

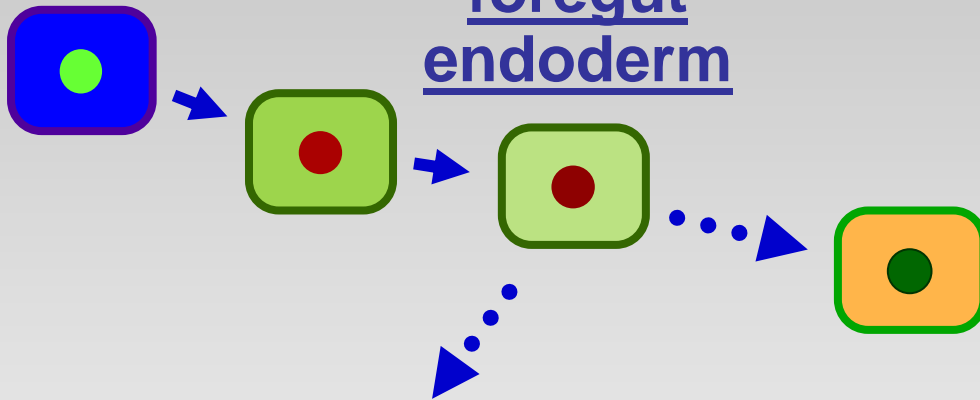


- secrete serum proteins
- secrete bile
- metabolize nutrients and toxicants (P450s)

- glucose-stimulated insulin secretion (GSIS)

Problem of Incomplete Programming Despite Complete Potential

hES cells



D'Amour et al. 2006:

- insulin expressing,
adherent to dish,
not glucose responsive

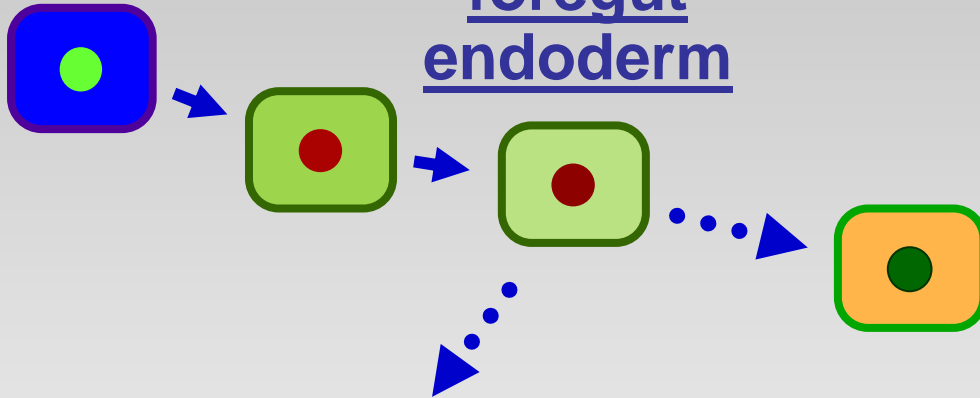
Kroon et al. 2008:

- insulin nonexpressing,
generated glucose-responsive
cells long after transplant

•••▶ occasional
teratomas

Utility of Generating Tissue Architecture

hES cells



Kroon et al. 2008:

- insulin nonexpressing,
generated glucose-responsive
cells long after transplant

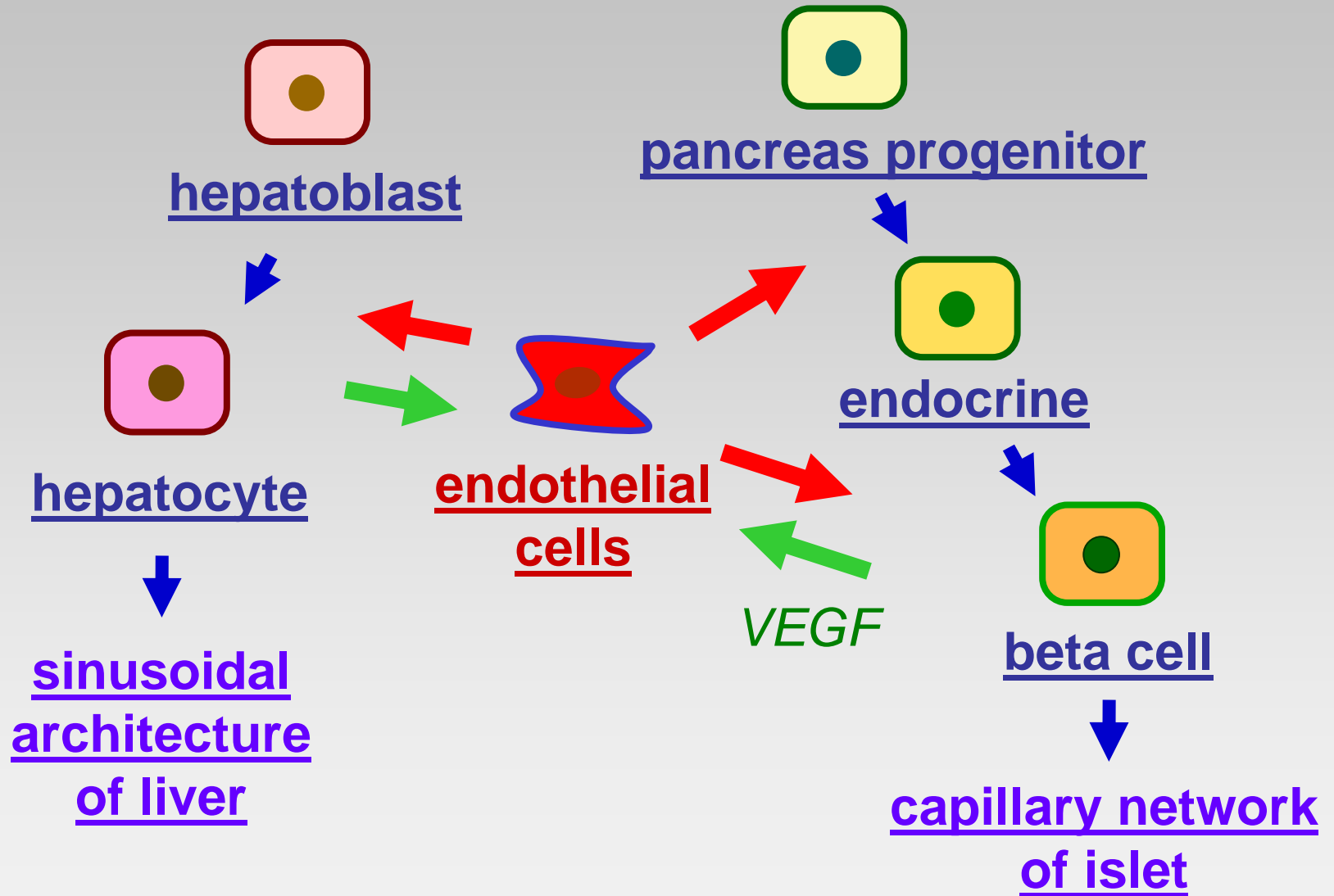
D'Amour et al. 2006:

- insulin expressing,
adherent to dish,
not glucose responsive

Jiang et al. 2007:

- insulin expressing,
glucose responsive
in nonadherent clusters

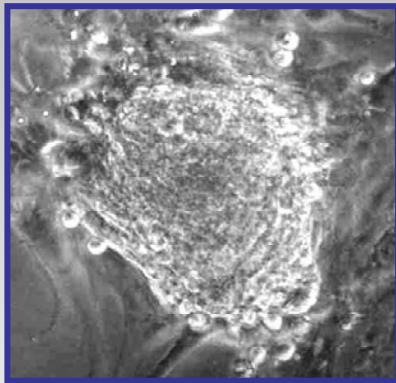
Principle of Co-Differentiation



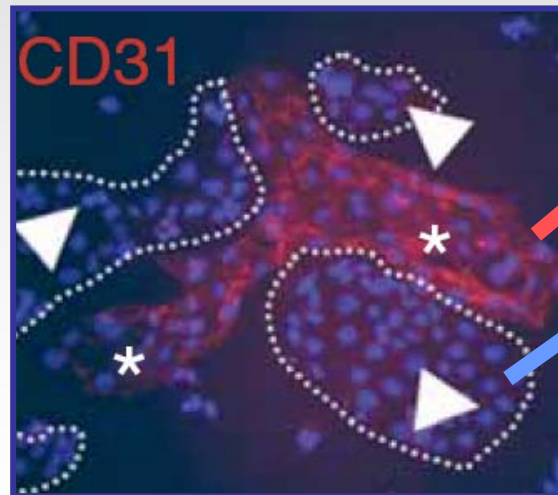
Co-Differentiation in ES Cell Cultures

Gouon-Evans/Keller (2006) *Nat. Biotech.*

mES cells



select for endoderm
and liver markers

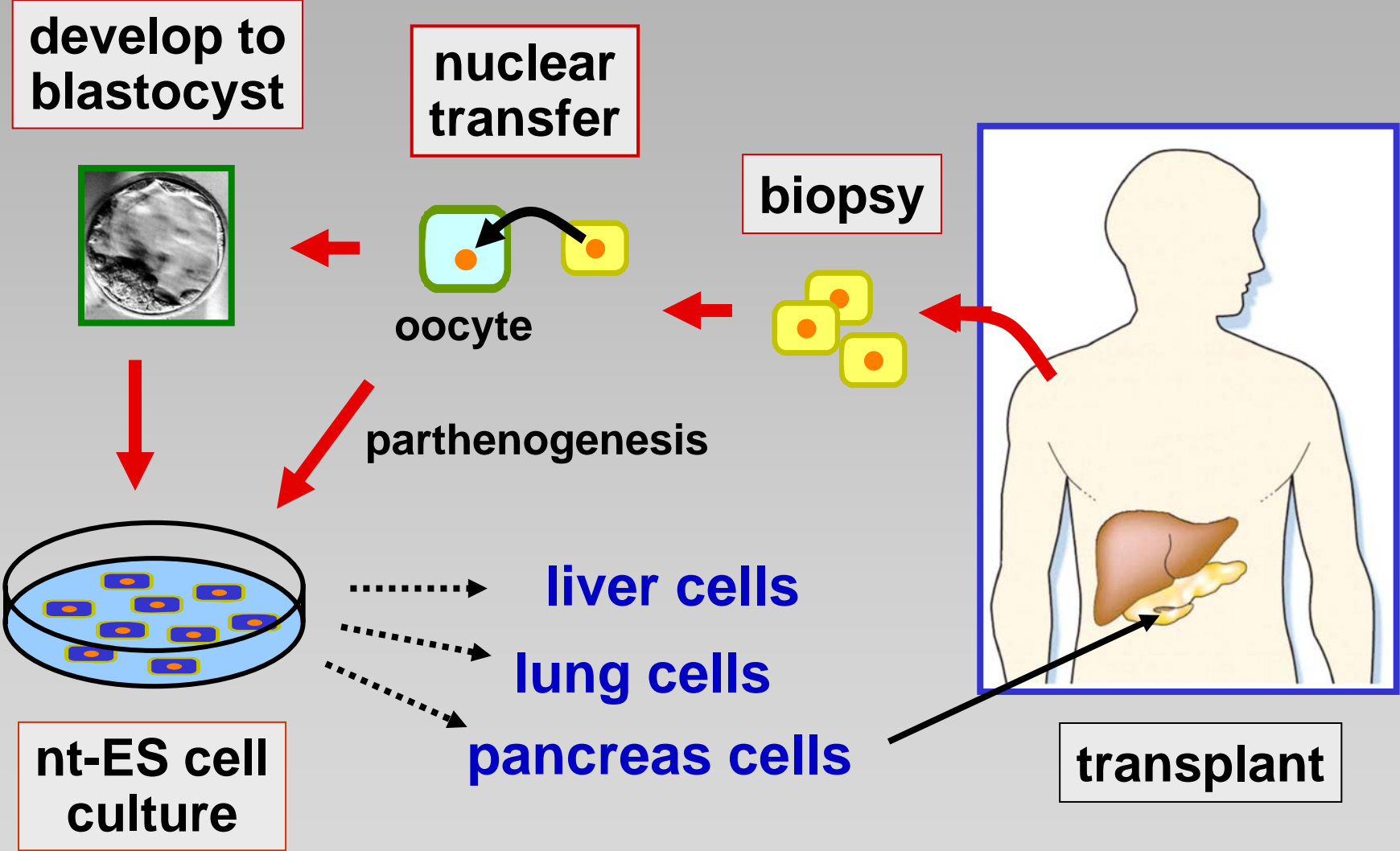


endothelial

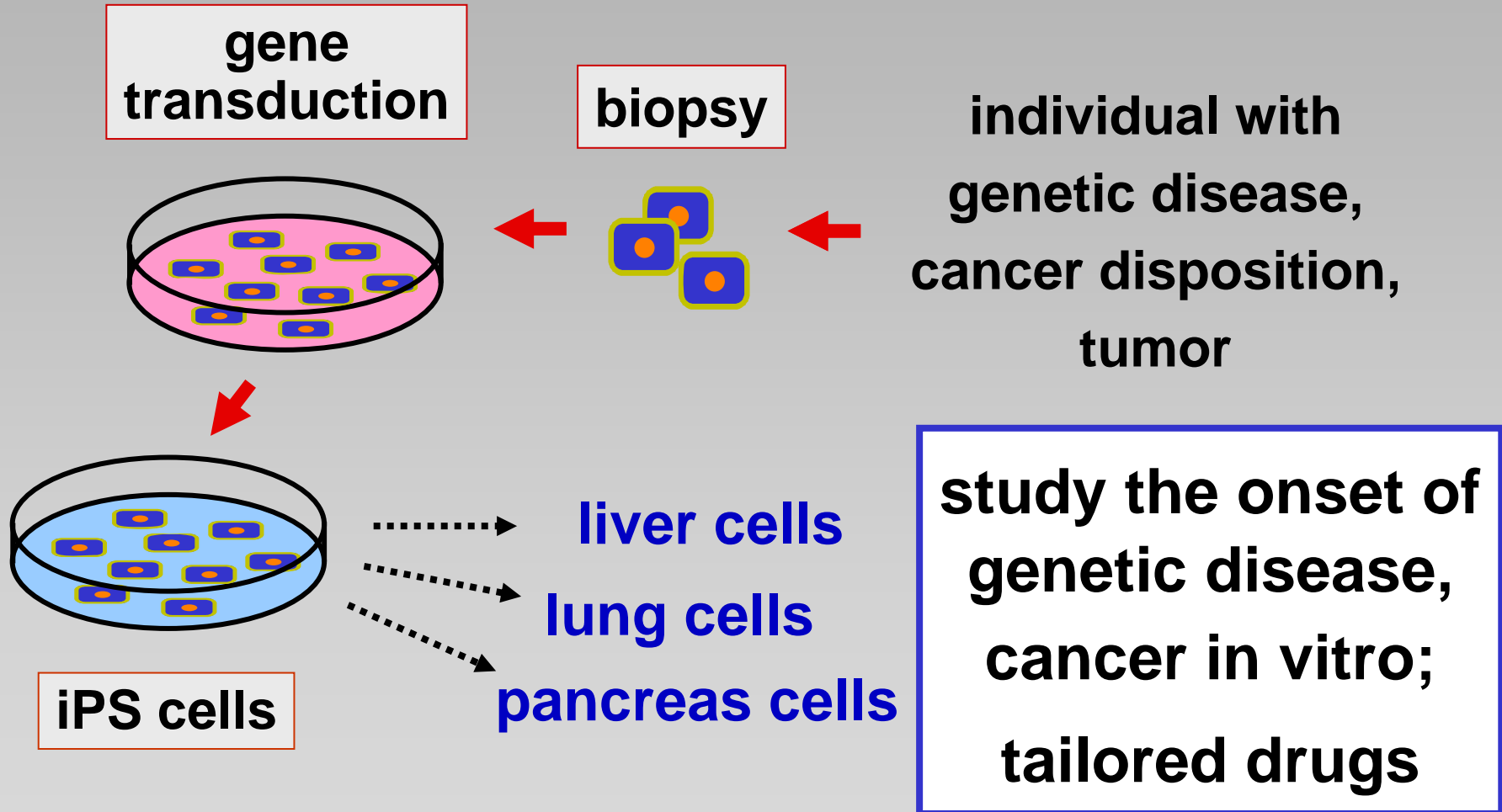
hepatoblasts

afp+, *alb1+*

Patient-Specific Cell Therapy



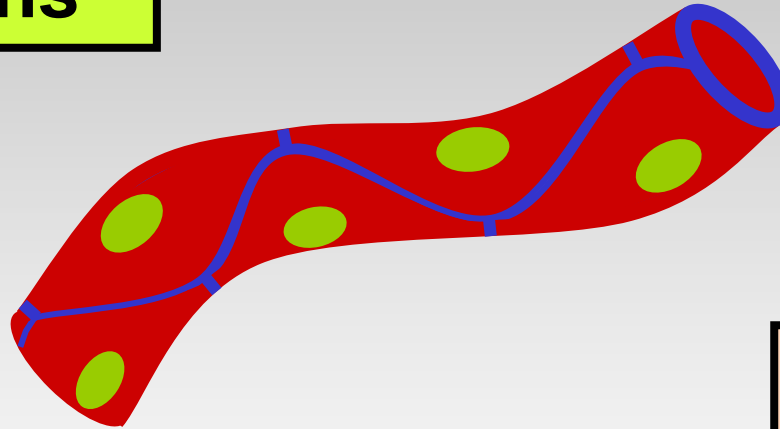
Disease-Specific Cells for Research



Special Roles of the Vasculature

endocrine
organs

oncogenesis

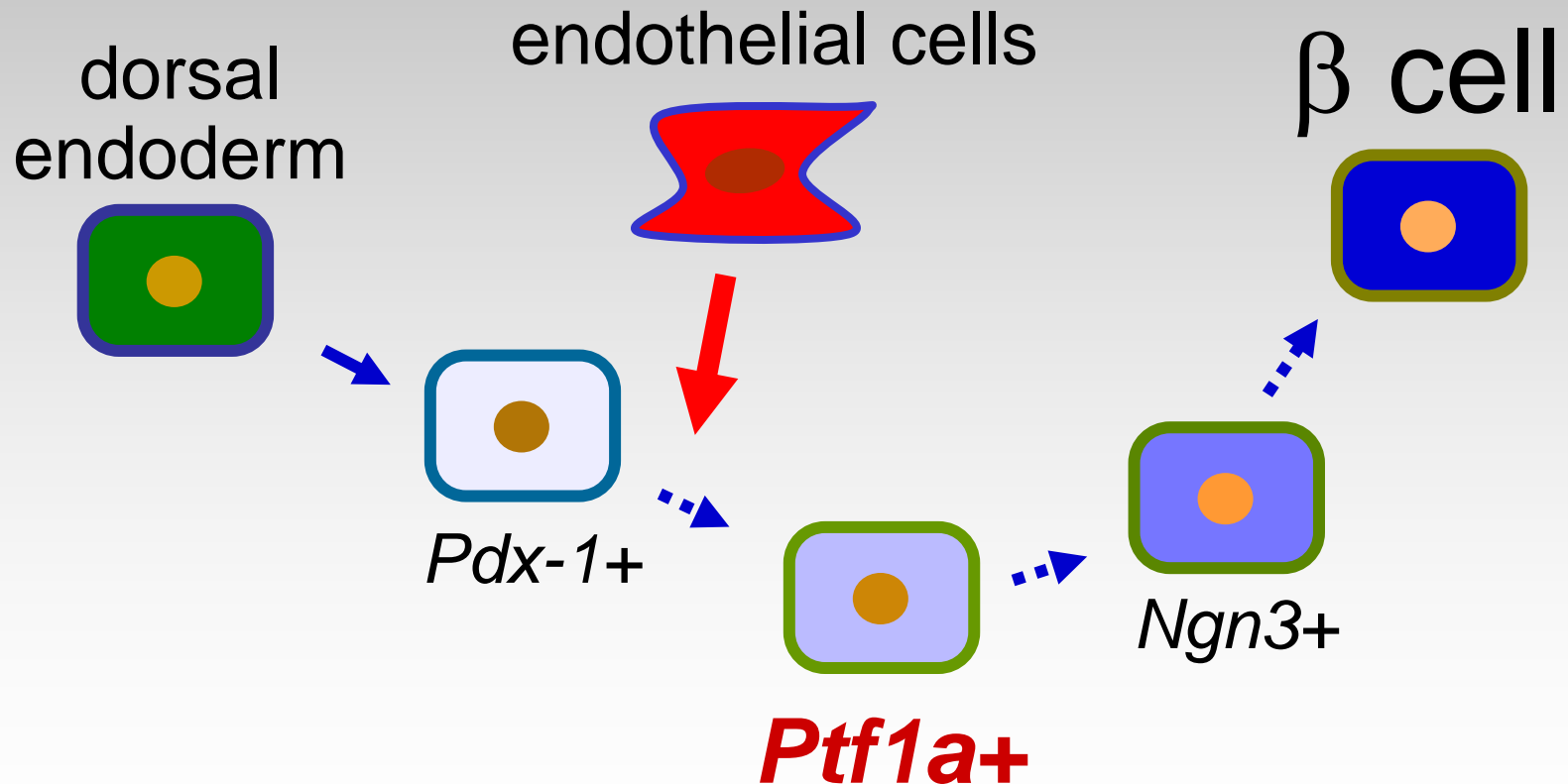


neural, renal
development

response
to tissue
damage

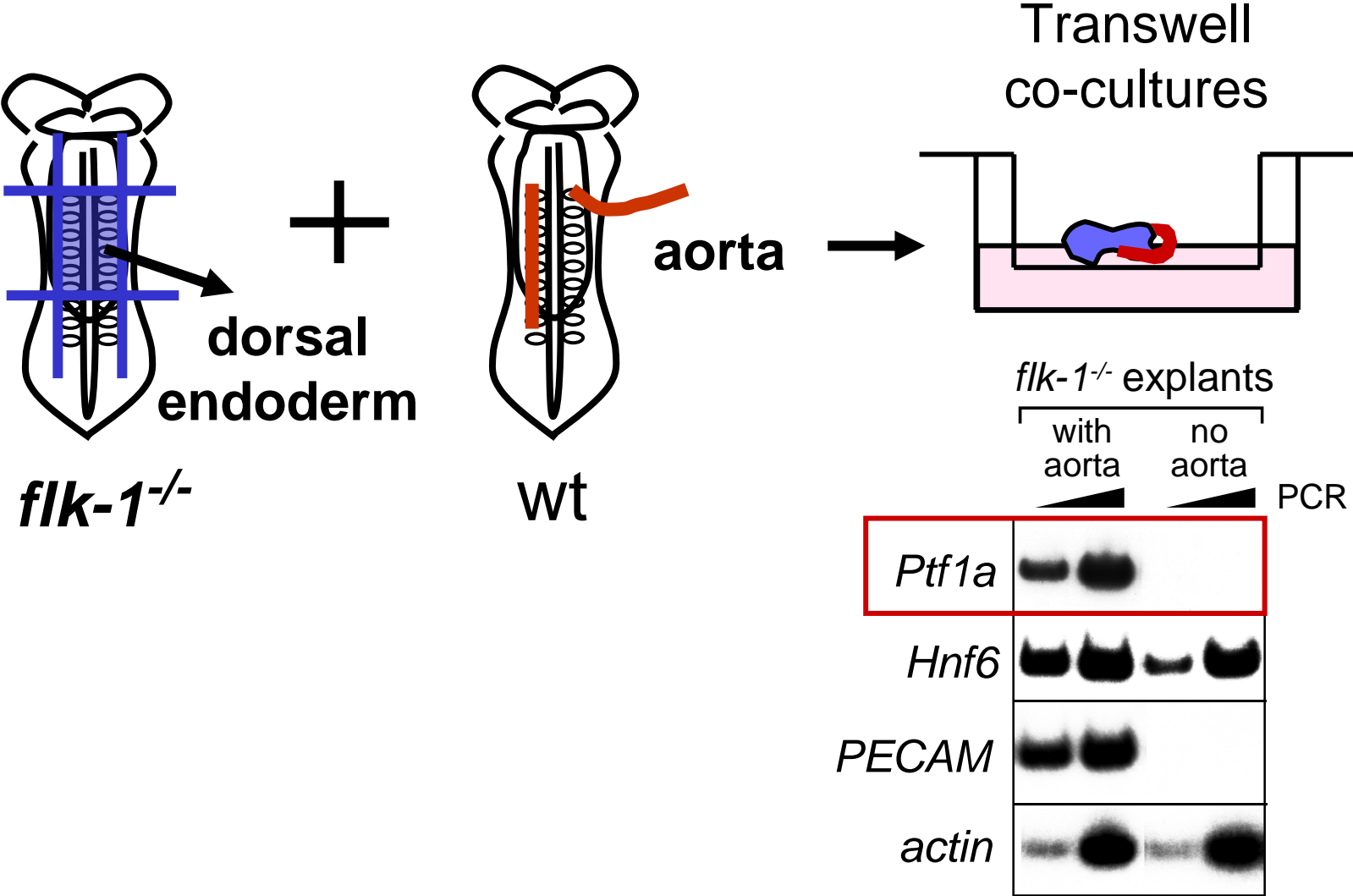
Blood Vessel Cells Needed for Early Pancreatic Development

Yoshitomi & Zaret (2004) *Development*



Co-Culture of *flk-1*^{-/-} Dorsal Endoderm & Aorta

Yoshitomi & Zaret (2004) *Development*



Endothelial Cell Lines for Co-Culture Experiments

	eEND.2	bEND.3	HUVEC	HUAEC
species	mouse	mouse	human	human
tissue	cavernous hemangioma	brain endothelioma	umbilical vein	umbilical artery
antigen expression	von Willebrand factor	ICAM-1 VCAM-1	factor VIII	factor VIII

Control cell lines: 3T3 cells, 293T cells

Endothelial Cell Lines Induce Ptf1a

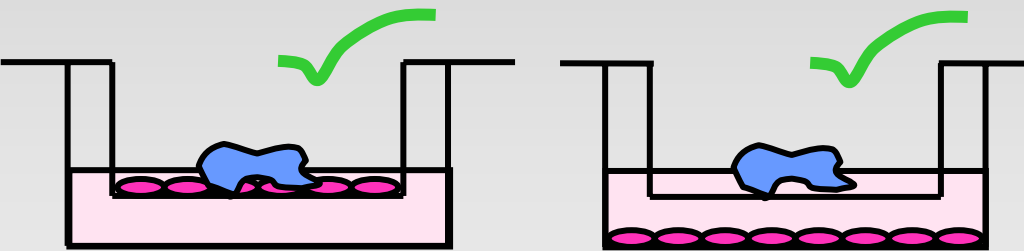
Ptf1a induction assay:

flk-1^{-/-} dorsal endoderm



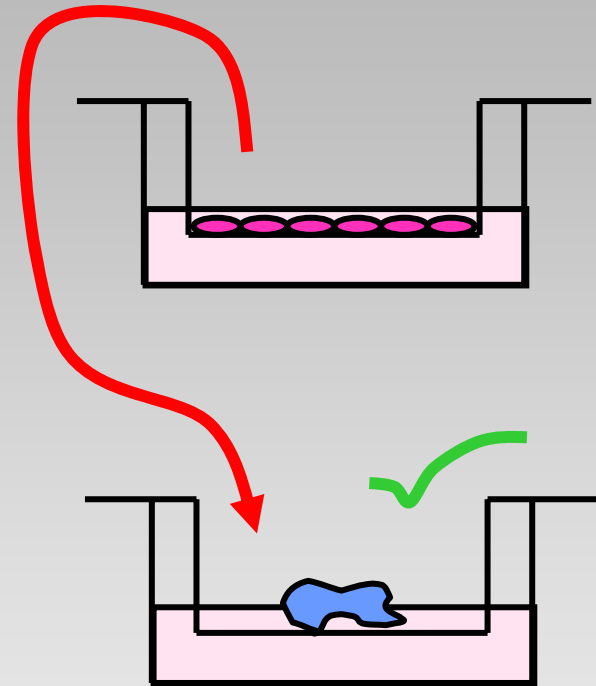
+

endothelial cell lines



contact +

contact -

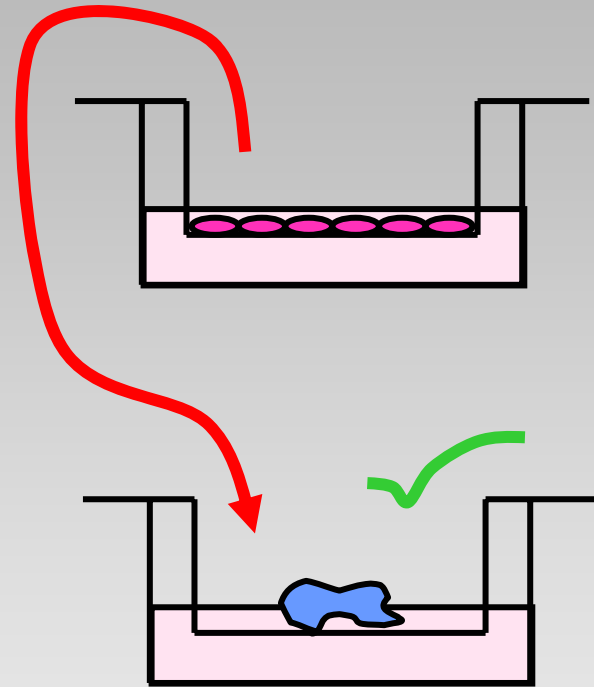


conditioned medium

Search for Endothelial Factors that Induce Ptf1a

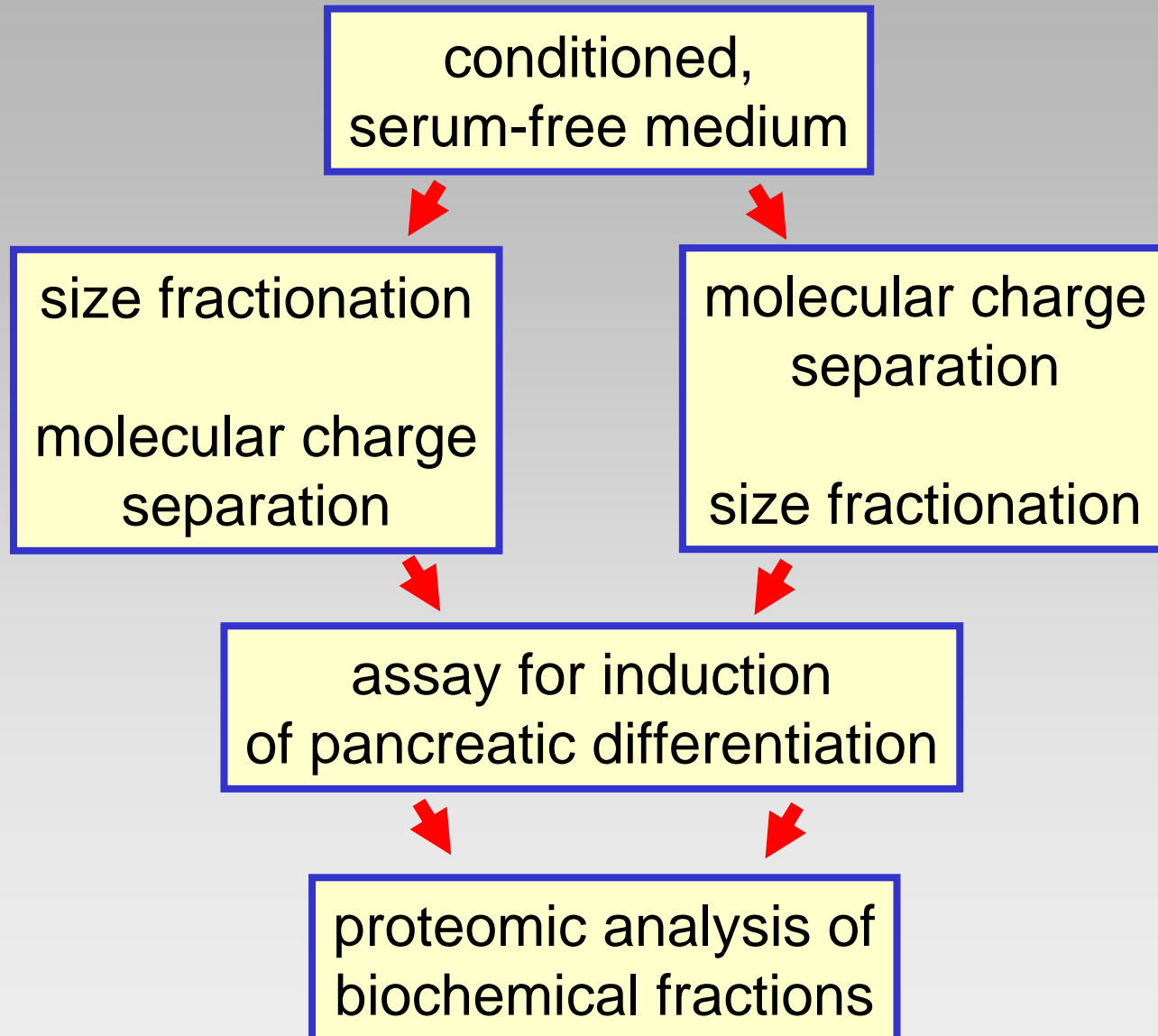
Experimental Stages:

- 1 - optimum medium for each cell line
- 2 - common medium
- 3 - minimal DMEM for 2 days:
 - clarify,
 - 0.2 μm filter
 - store -80°C



***stage 3: conditioned,
serum-free medium***

Biochemical Fractionation of Secreted Proteins



Licensing and Collaborations with Industry

secreted proteins
from endothelial cells

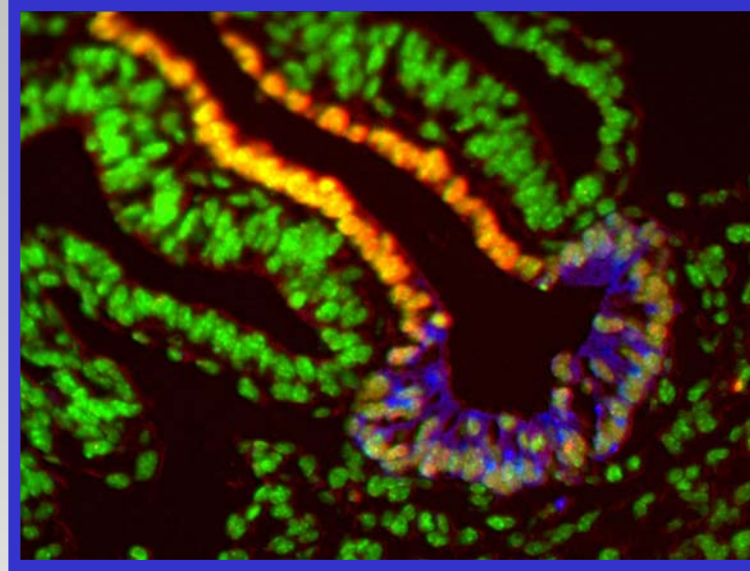
secreted proteins
from fibroblast/stromal cells

biochemical fractions stored

protein I.D.'s in each fraction known

use fractions in functional screens
in the pharmaceutical/biotech setting

Acknowledgements



Deborah Freedman, Takashi Sekiya, Ewa Wandzioch
Jason Watts, Nandita Mullapudi, David Metzger
Chenran Xu, Andrea Wecker, Angela Hines

**FCCC; NIH-NIGMS, NIDDK (BCBC), NCI
W.W. Smith; Mathers Found., Searle**