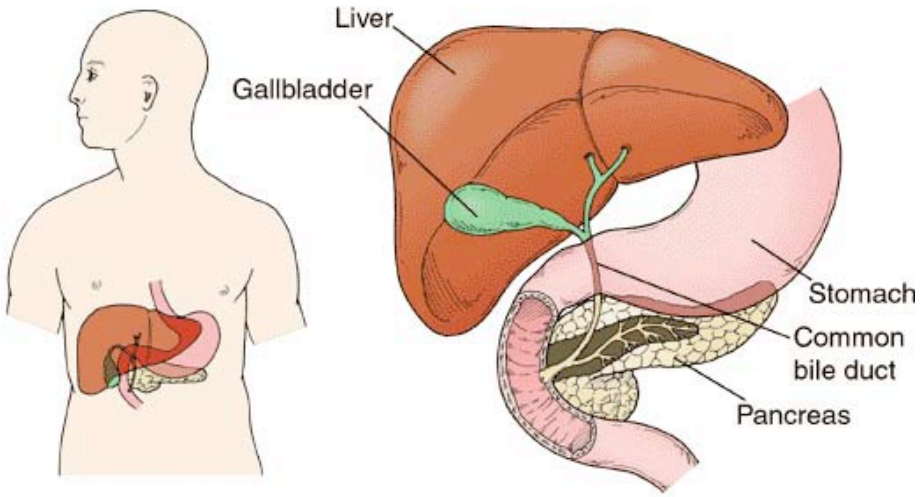
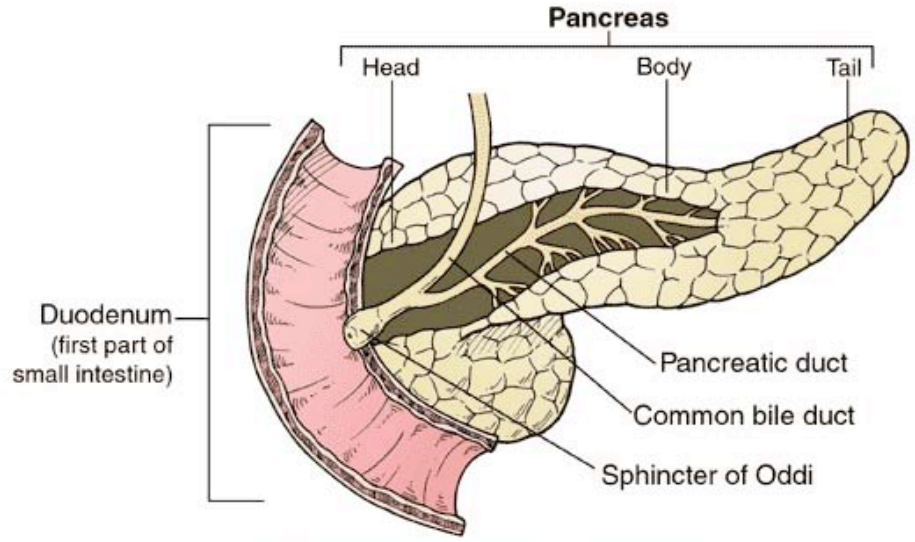
Pancreas and Liver Development

Human Development

Lori Sussel, PhD
Department of Genetics and Development
lgs2@columbia.edu

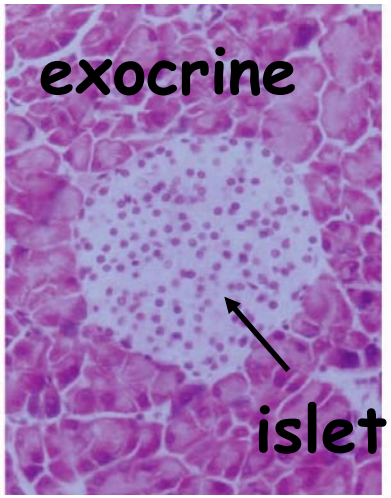
Location of the pancreas and liver



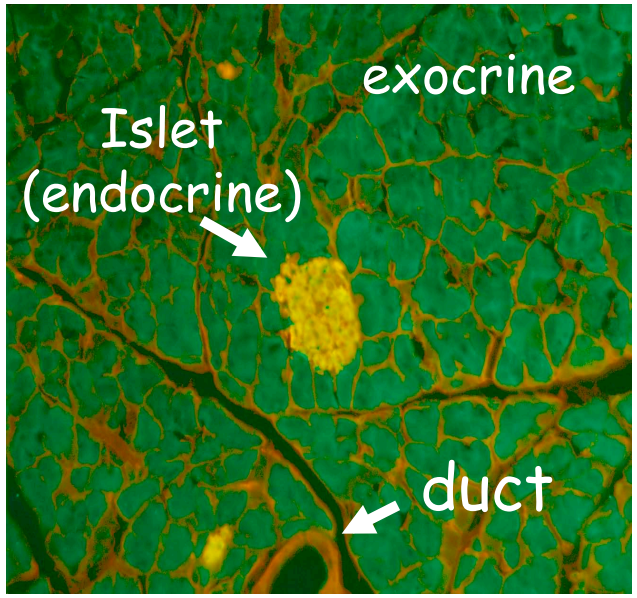


Pancreas

- gland responsible for energy homeostasis
- development has been major focus of research over the past 15 years

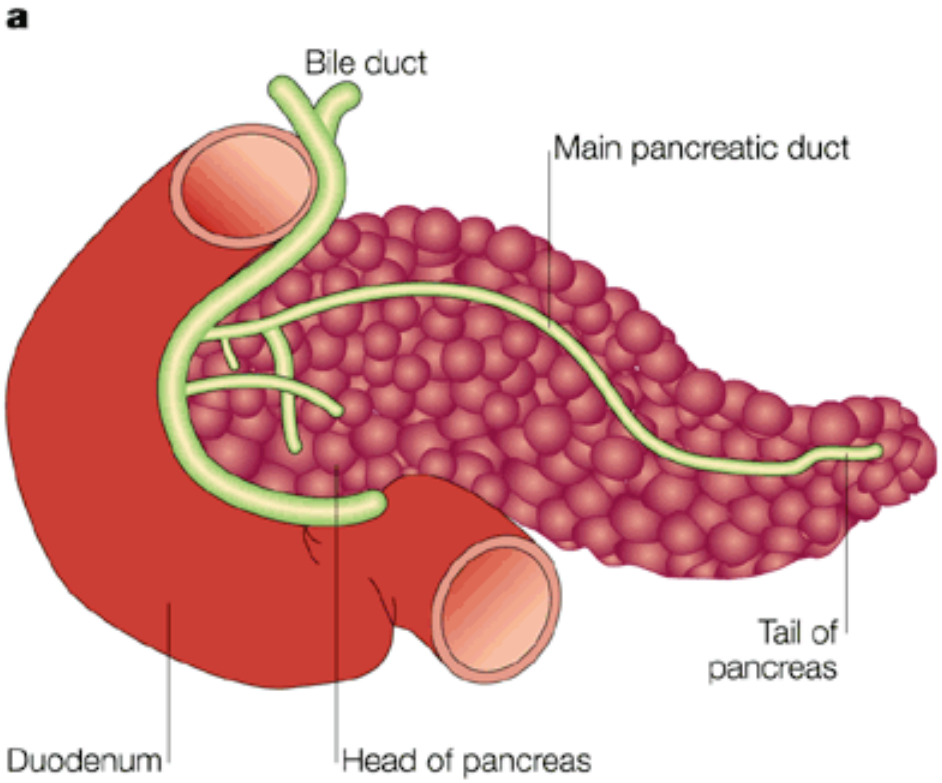


H&E

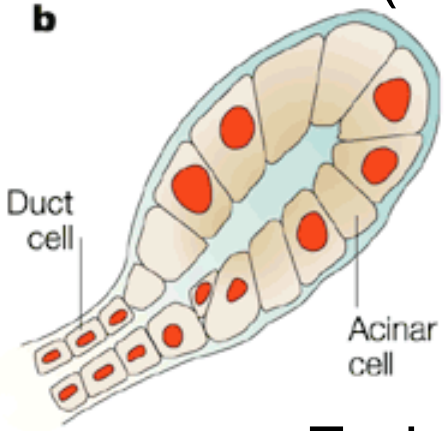


insulin, amylase

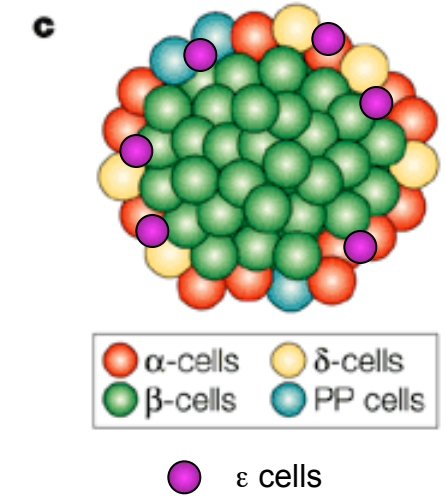
Pancreatic Cell Types



Exocrine (85%)



Endocrine (10%)
(Islets)



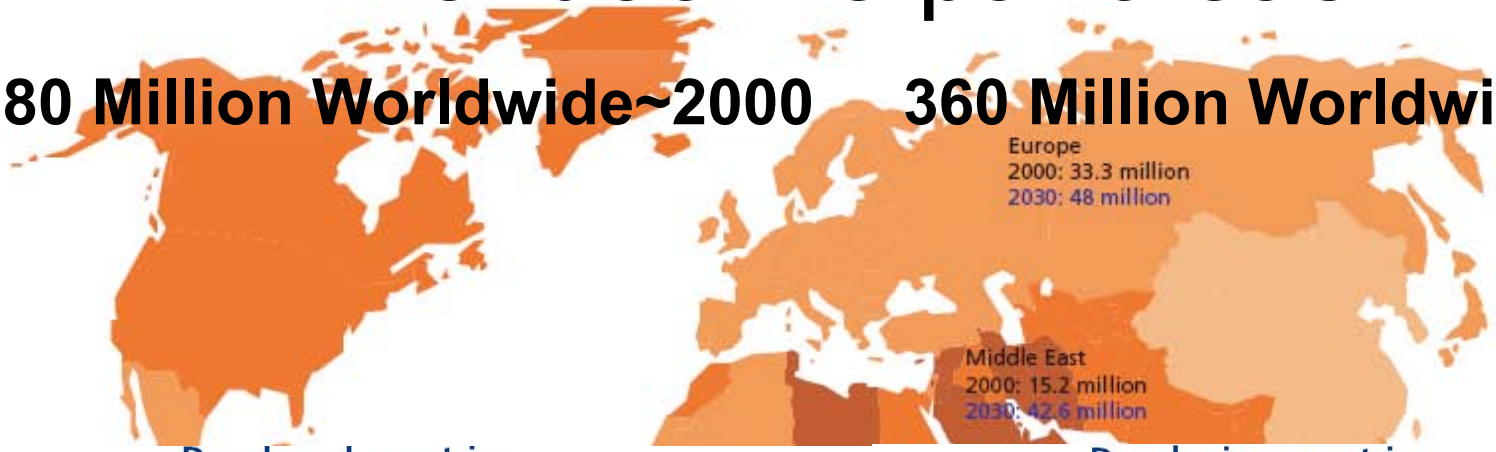
Edlund, 2002

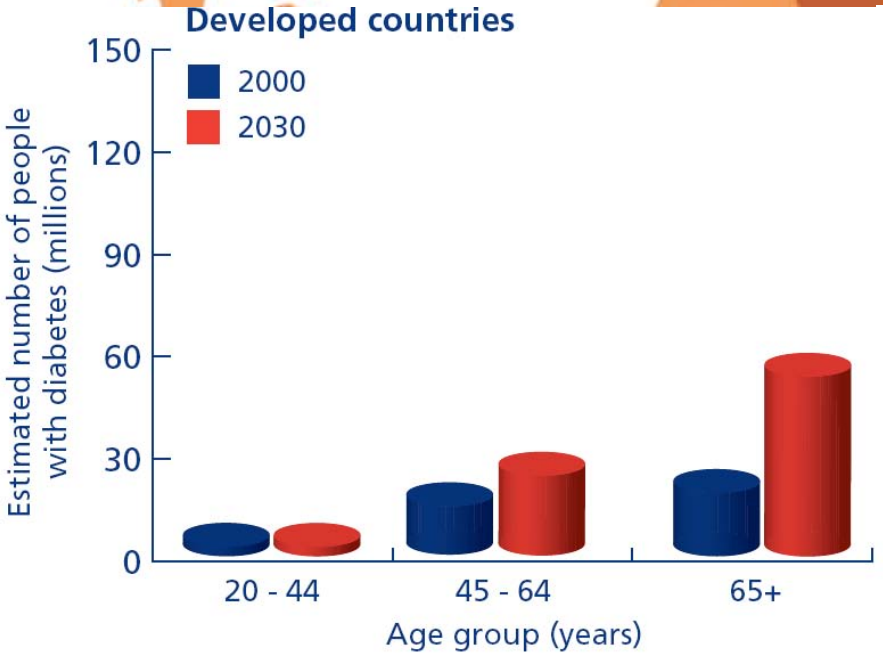
Cancer: disease of the exocrine pancreatic ducts

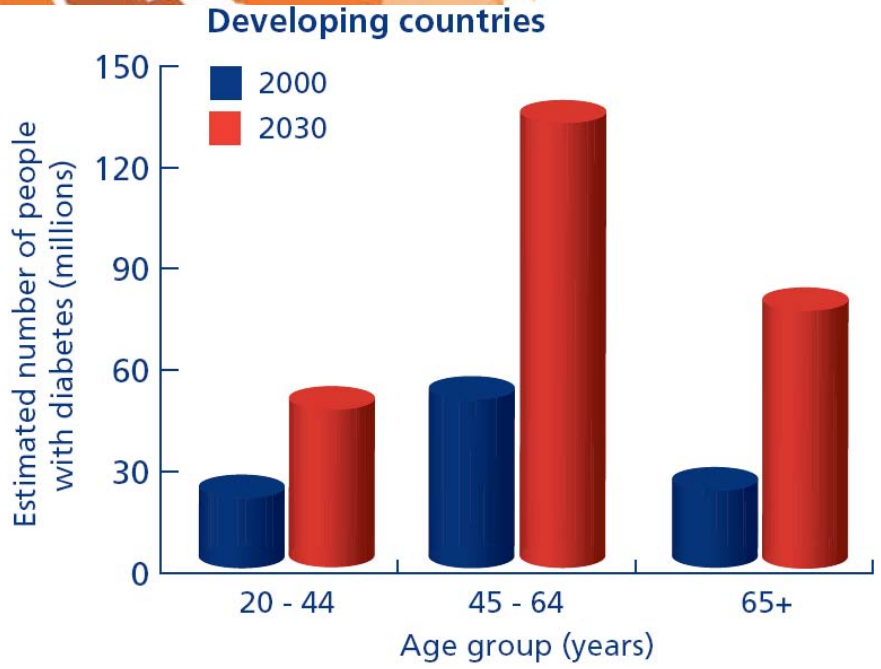
- 95 percent of pancreatic cancers start in the exocrine ductal cells
- diagnosed in ~ 30,000 people in the US each year
- 4th leading cause of cancer-related deaths
- often no symptoms early on; difficult to diagnose in its beginning stages; most pancreatic cancers have spread beyond gland by diagnosis
- high mortality rate
- pancreatic tumors have the poorest responses to treatment among all the major cancers

Diabetes: Disease of the endocrine pancreas

180 Million Worldwide~2000 **360 Million Worldwide~2030**



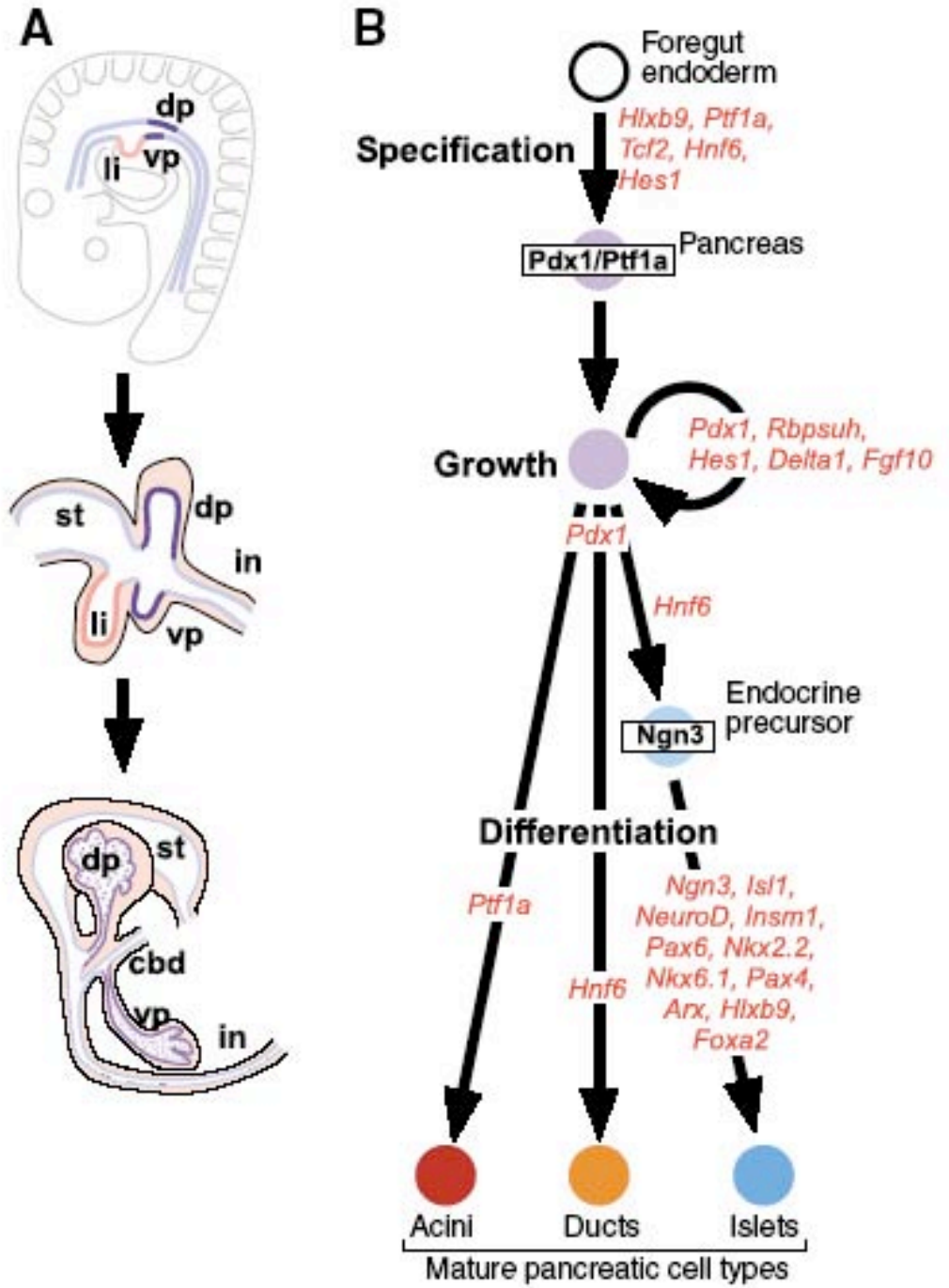




Organogenesis of the Pancreas

- arises from foregut endoderm
- initially forms as two separate and distinct rudiments which fuse to form a single organ containing all cell types
- mammals, birds, reptiles, amphibians and zebrafish have a pancreas with similar histology and mode of development
- organogenesis depends on complex interactions between epithelium and mesenchyme

Overview of pancreas development



Murtaugh, 2007

Pdx1

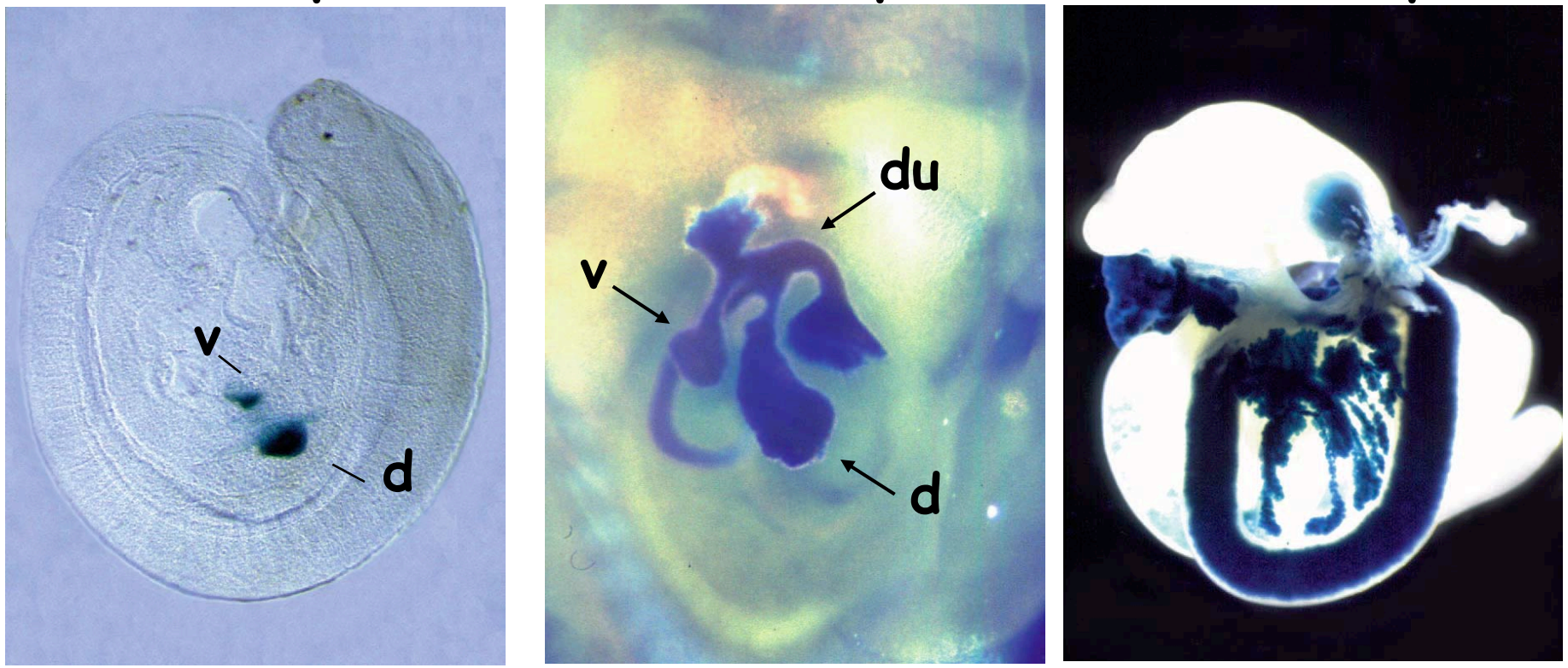
Definitive pancreas marker

Exocrine tissue = acinar cells

Endocrine tissue = islet cells

Pancreas development

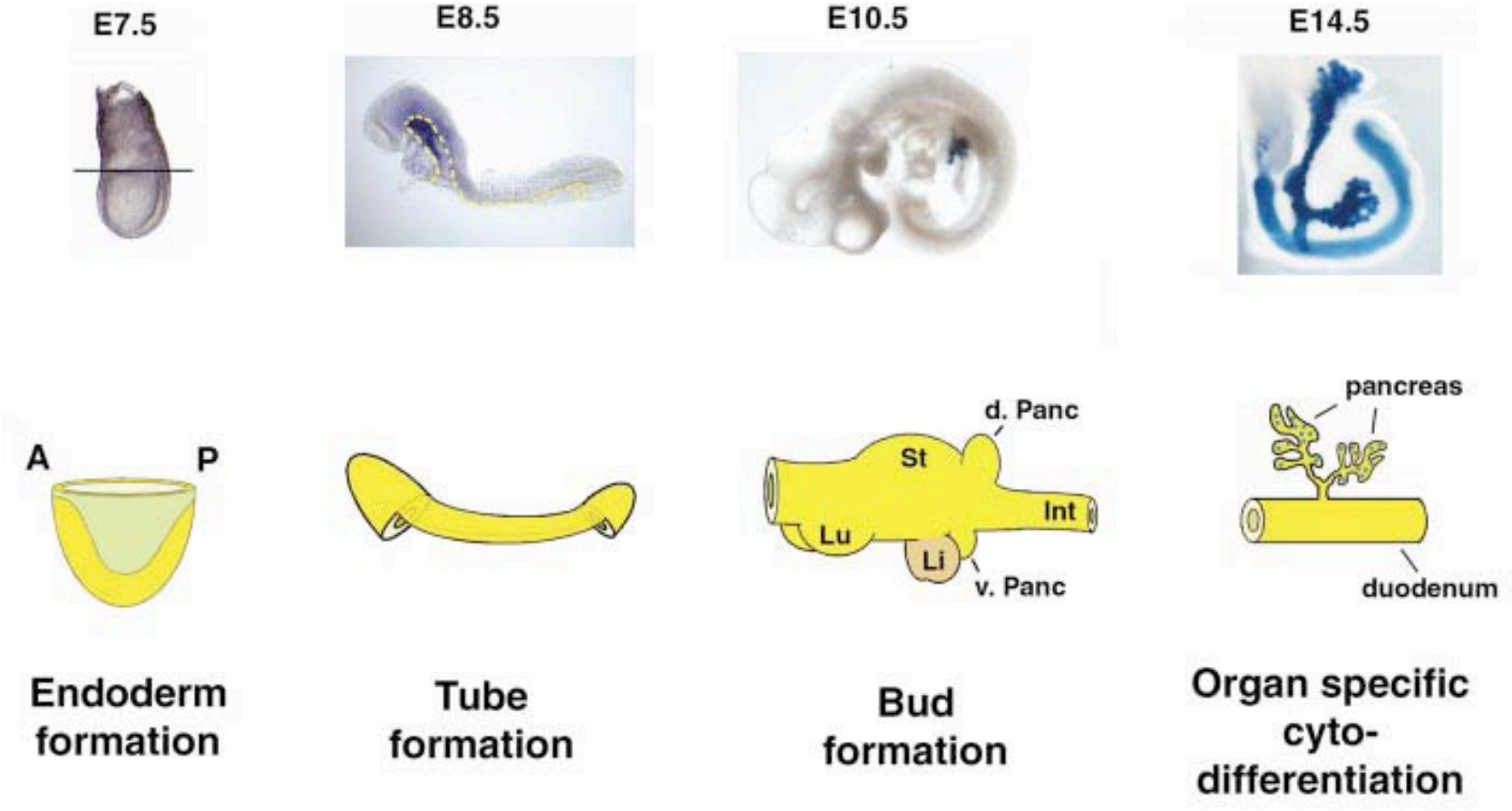
9.5 dpc **11.5 dpc** **16.5 dpc**



Pdx1:LacZ

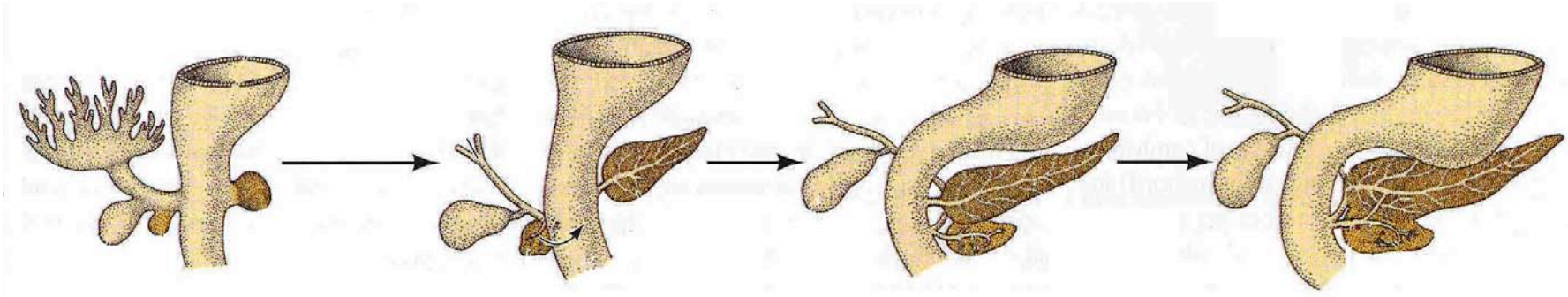
Offield et al., 1996

Pancreas development



Wells and Melton, 1999

Stages of pancreas development



9.5 dpc	10.5 - 14.5	14.5-16.5	16.5 - 18.5
26 dpc	30-60 dpc	12-20 wpc	25-29 wpc
Panc. bud evagination	endocrine differentiation	exocrine & endocrine differentiation	islet formation

Pancreas Looping

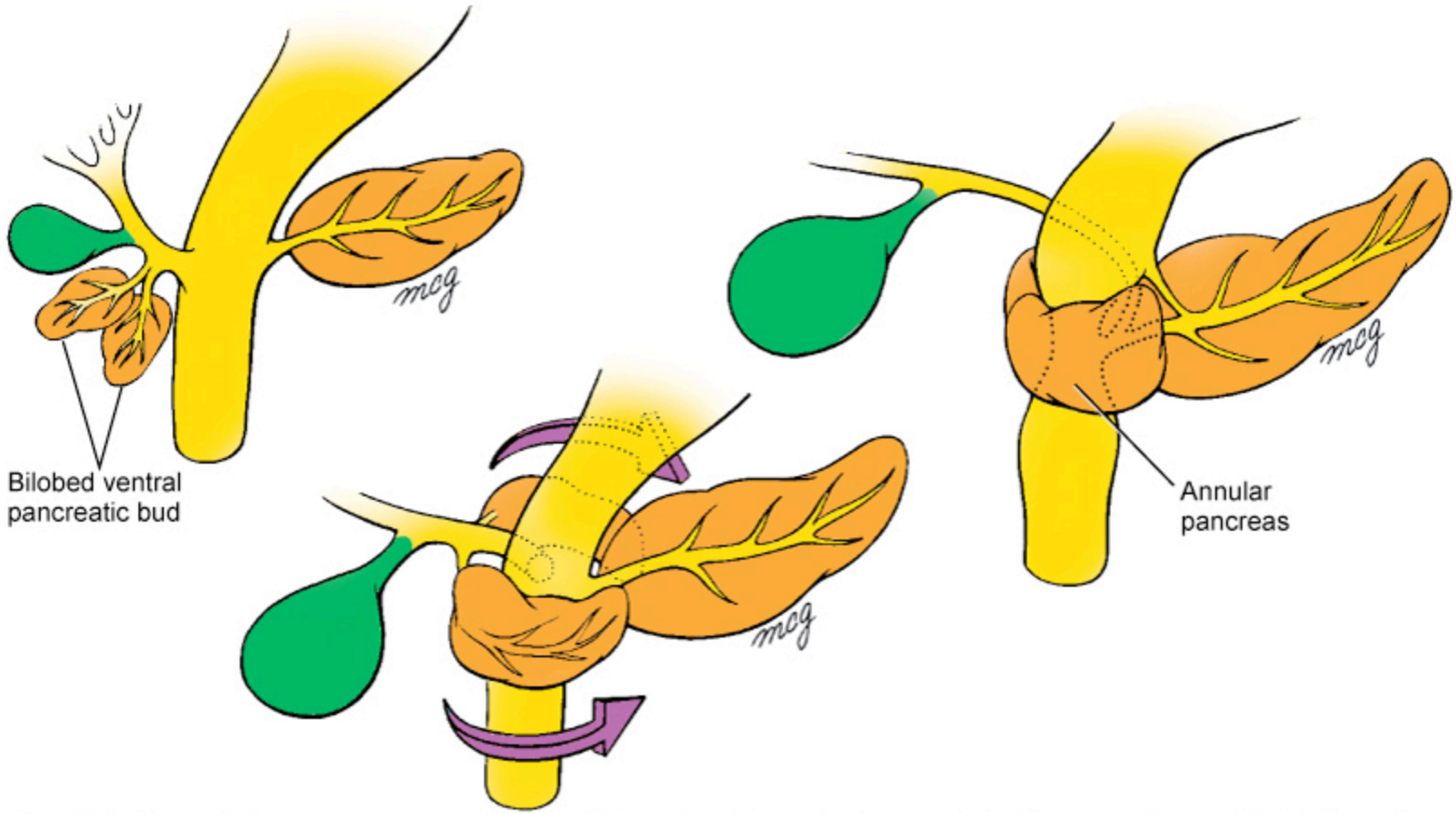
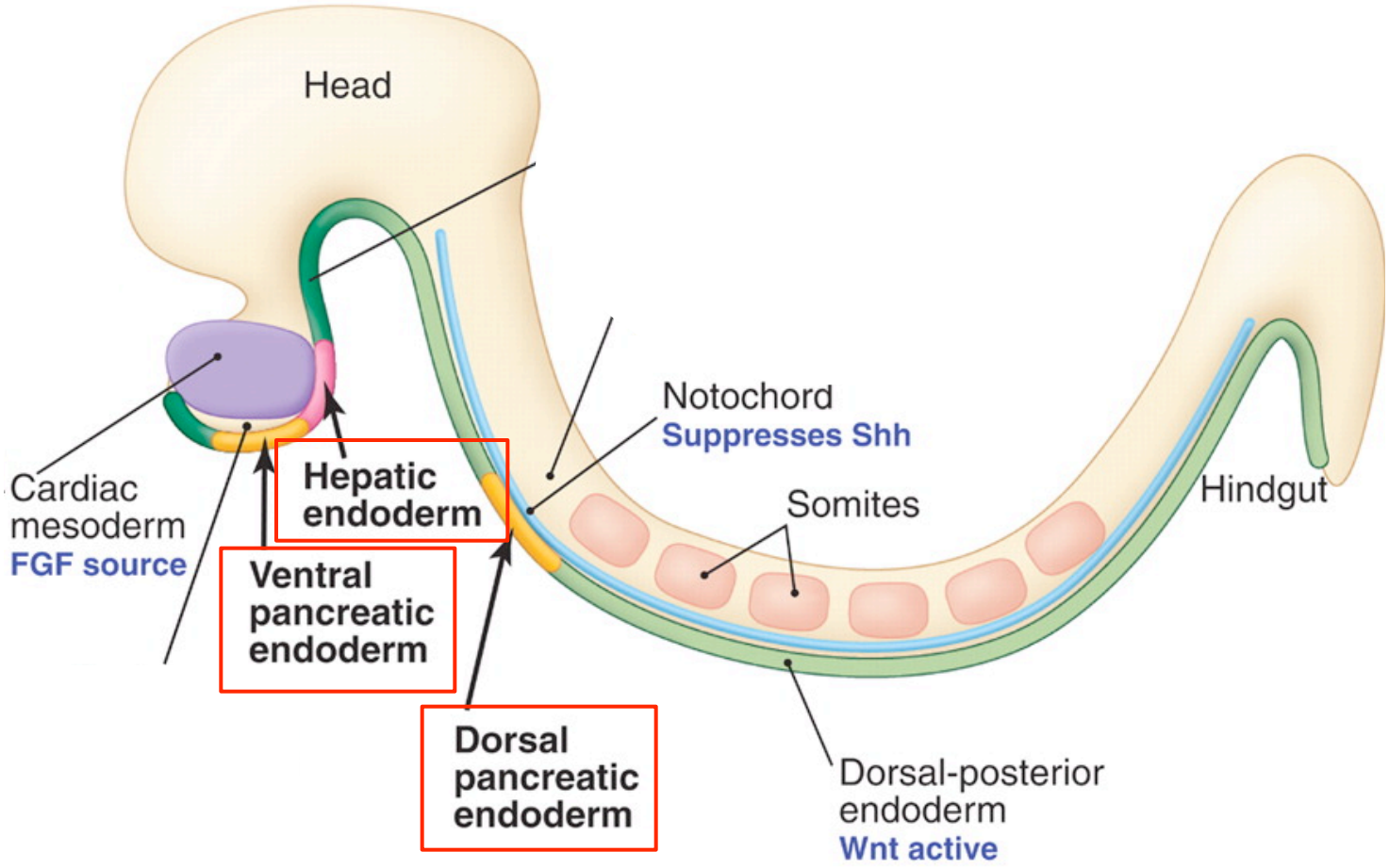


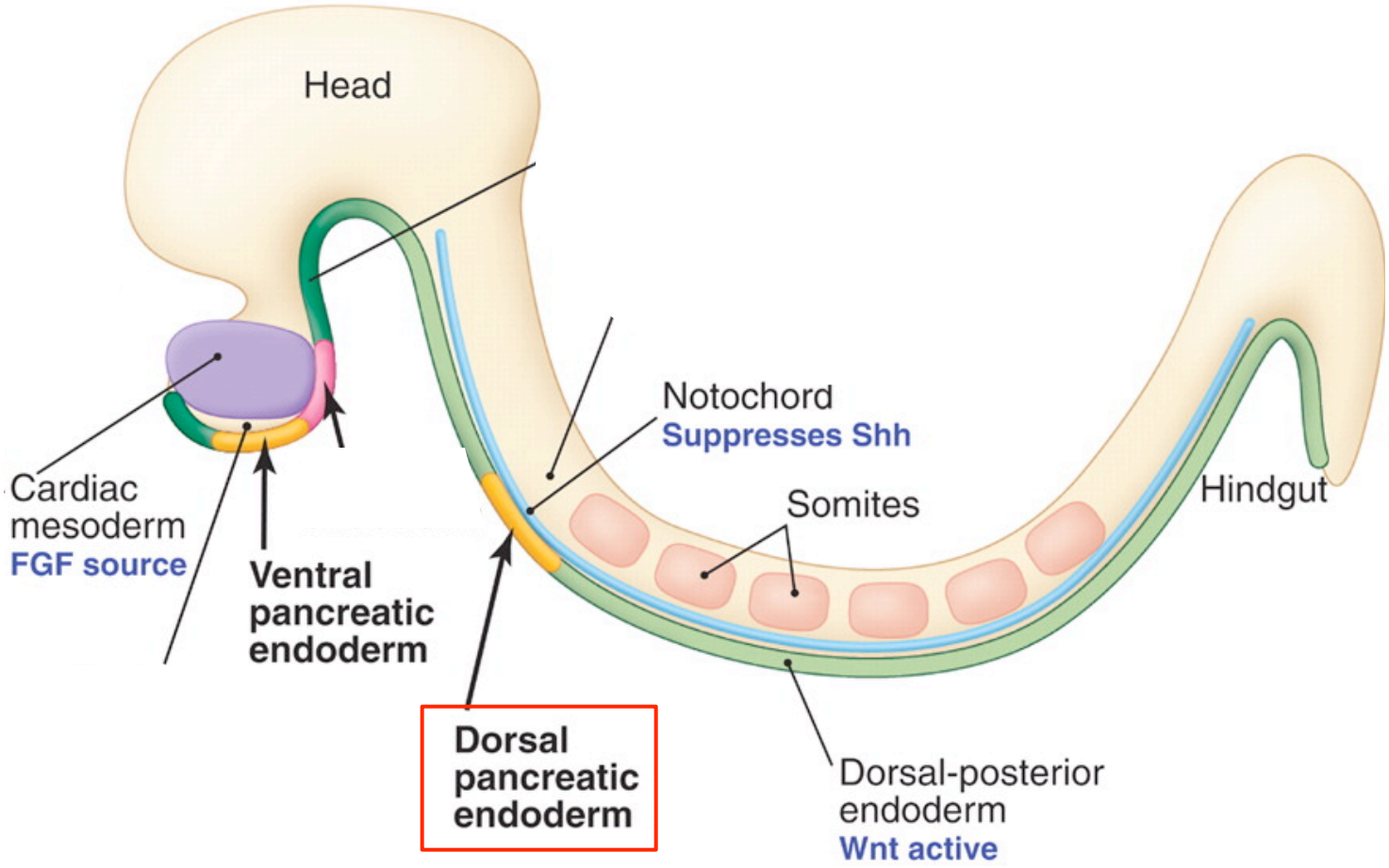
Fig. 14-12. The ventral pancreas may consist of two lobes. If the lobes migrate around the duodenum in opposite directions to fuse with the dorsal pancreatic bud, an annular pancreas is formed.

Early patterning of the endoderm



K. S. Zaret et al., Science 322, 1490 -1494 (2008)

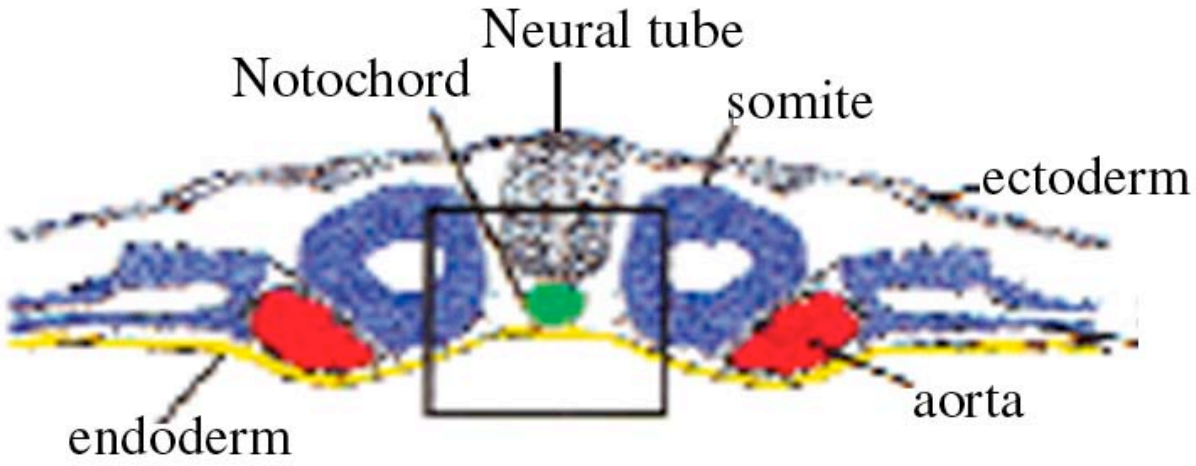
Early patterning of the endoderm

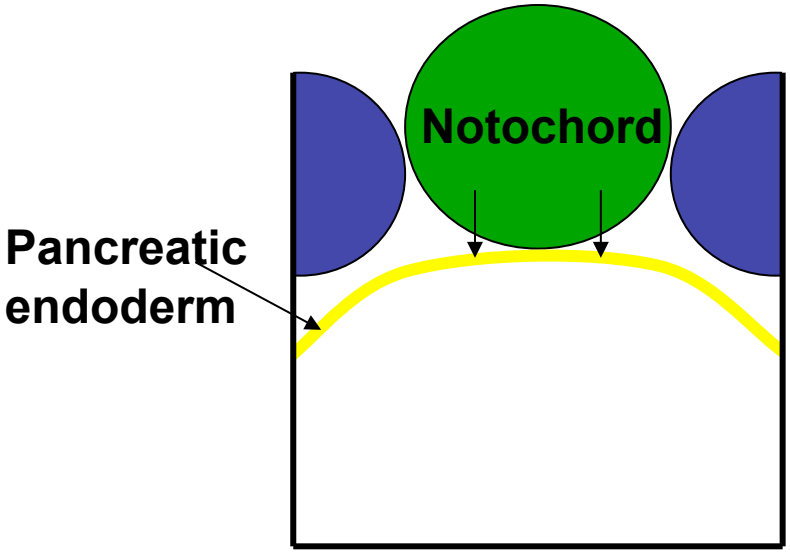


K. S. Zaret et al., Science 322, 1490 -1494 (2008)

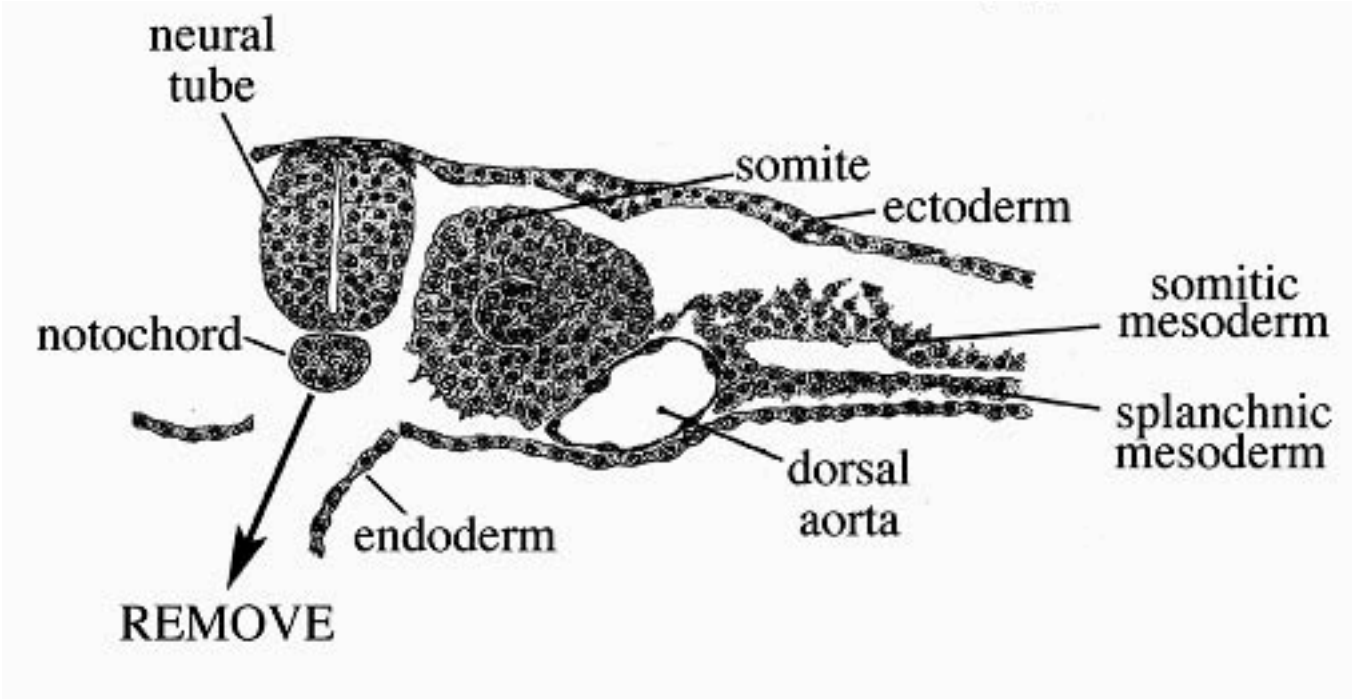
At e8.0, dorsal pancreatic endoderm is induced by the notochord

E8.5-9.0

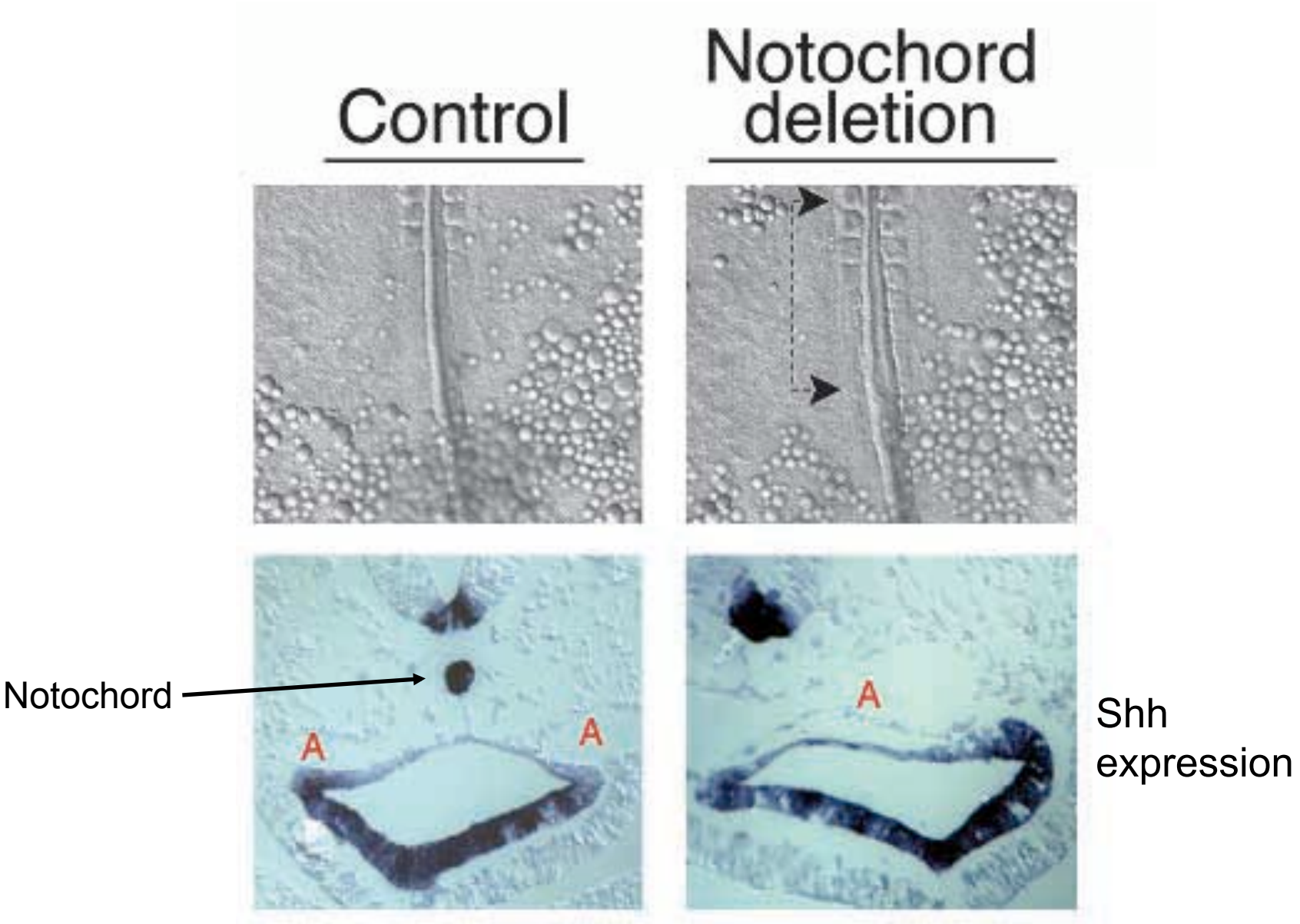




Experiment:
Remove the notochord and see what happens to the pancreas



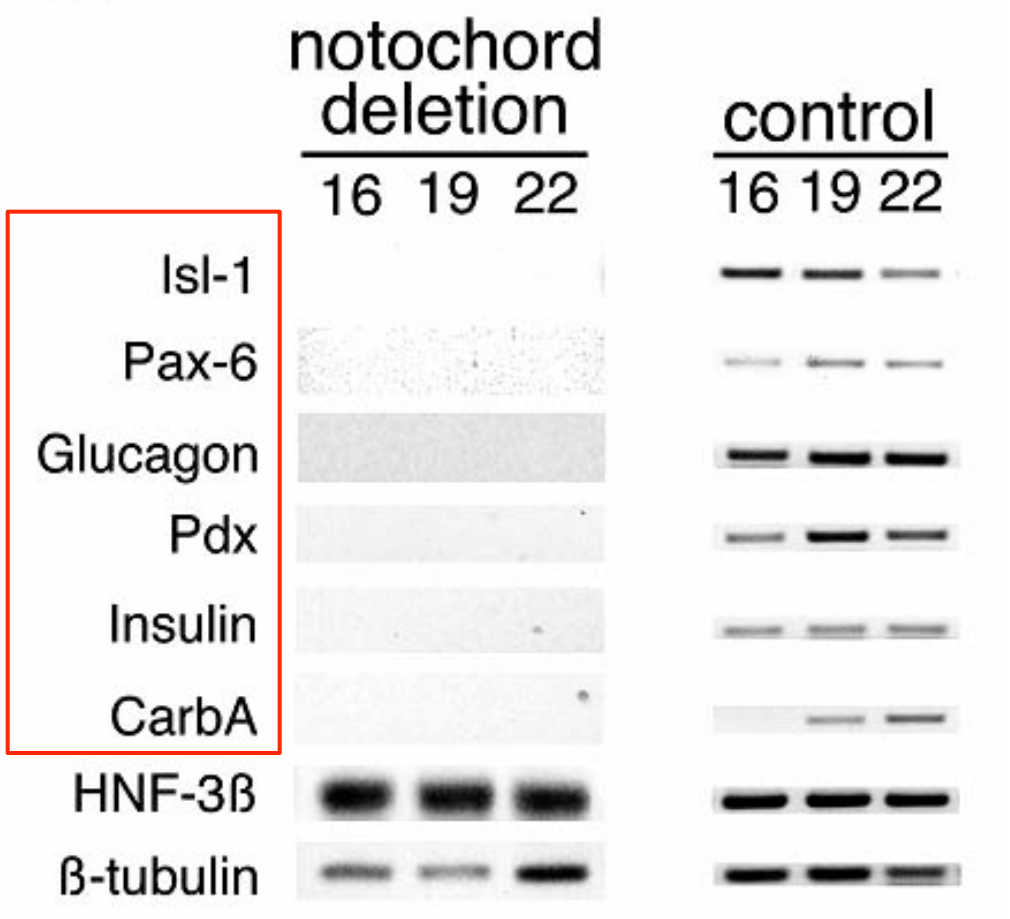
Kim, Hebrok and Melton, 1997-1999



Kim, Hebrok and Melton, 1997-1999

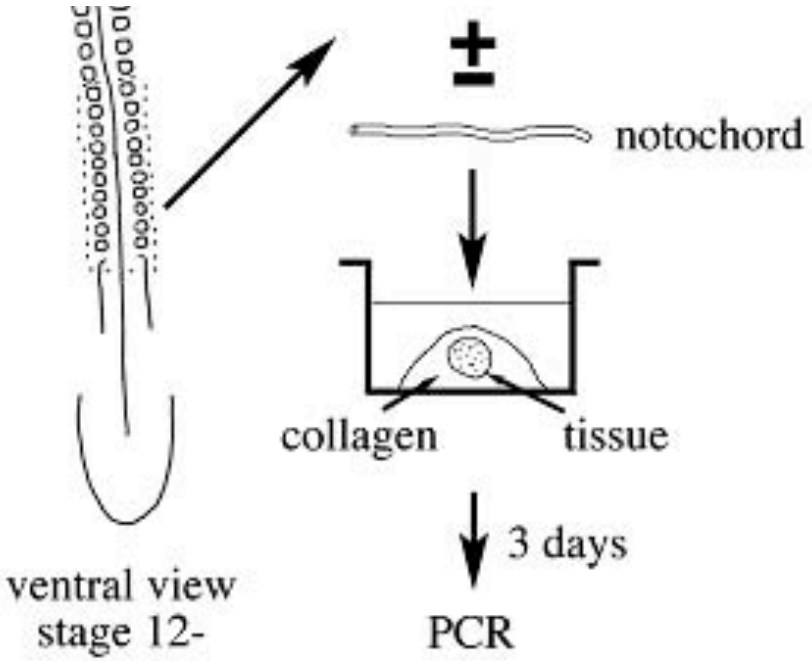
Notochord is necessary to specify pancreas

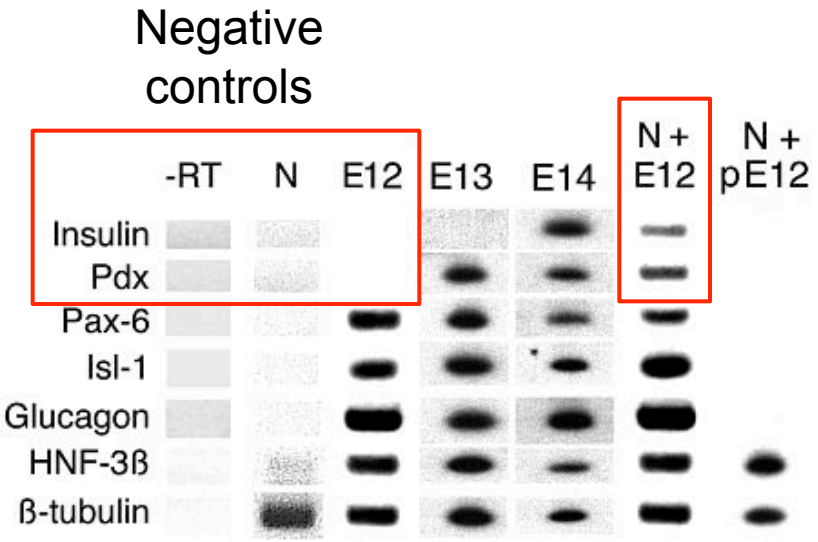
Pancreas markers



Kim, Hebrok and Melton, 1997-1999

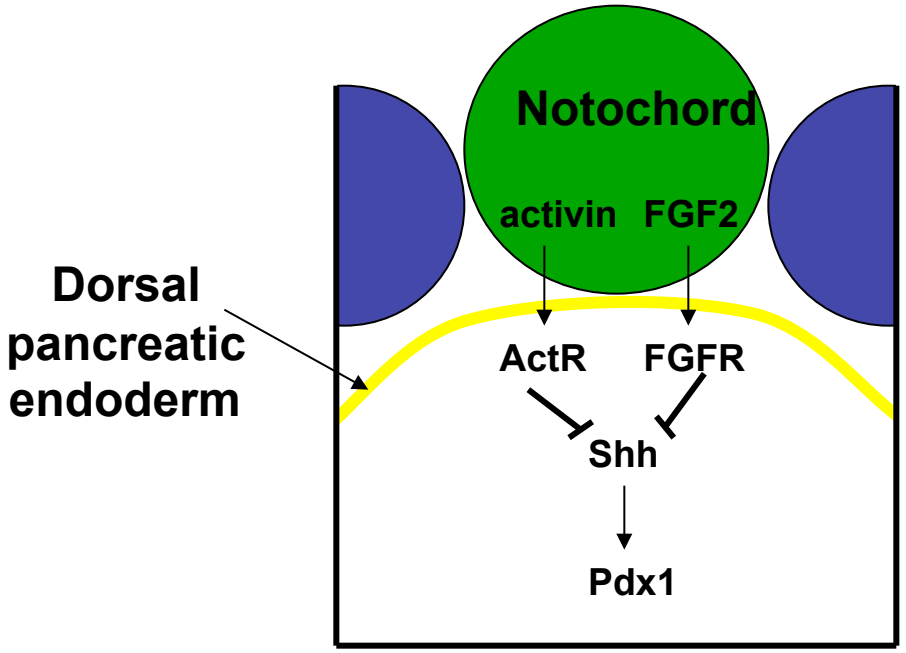
Notochord is sufficient to specify dorsal pancreas



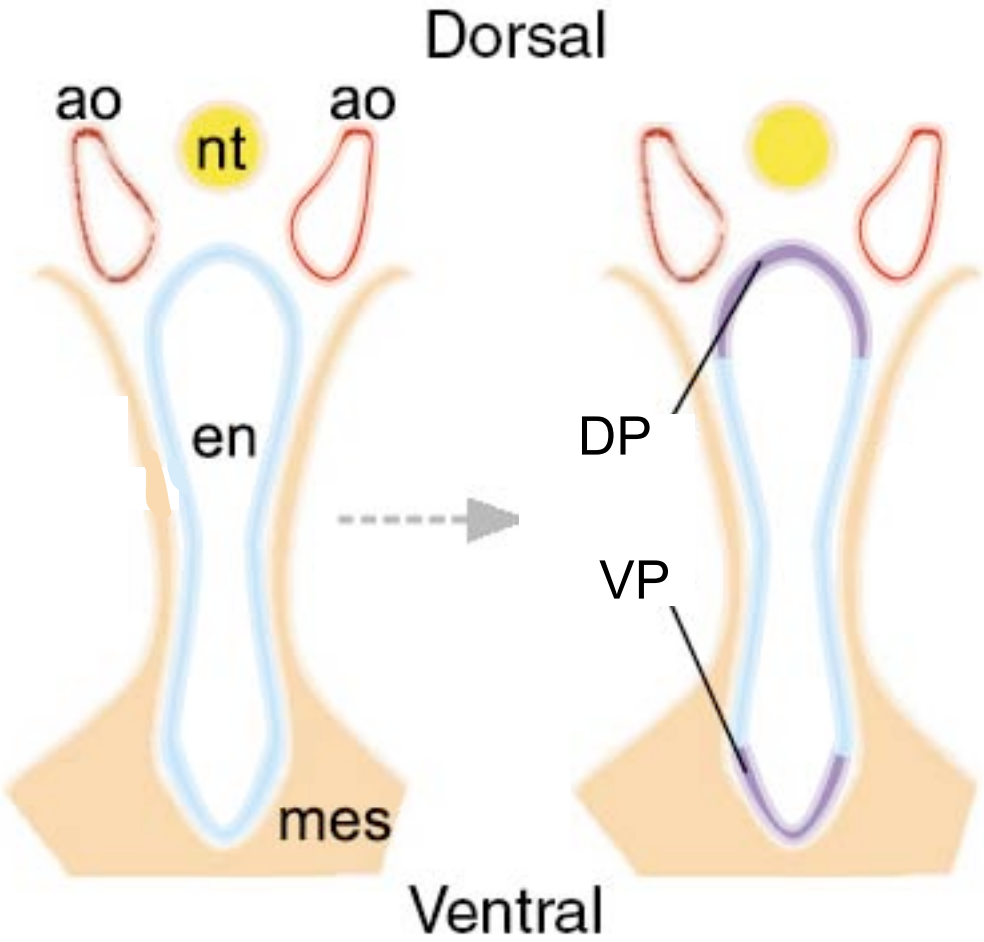


Kim, Hebrok and Melton, 1997-1999

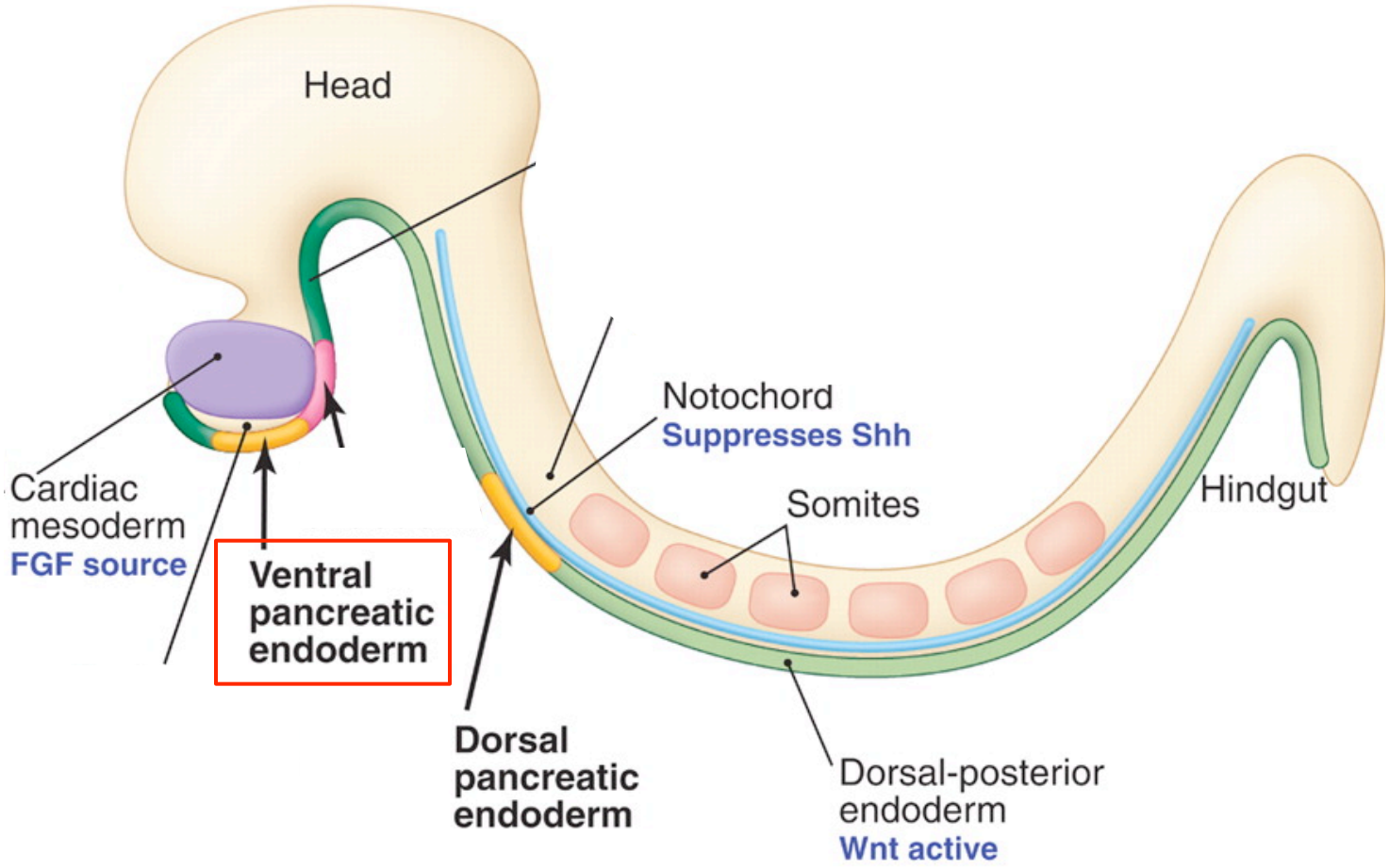
Signaling pathway directed by notochord



What patterns the ventral pancreas?

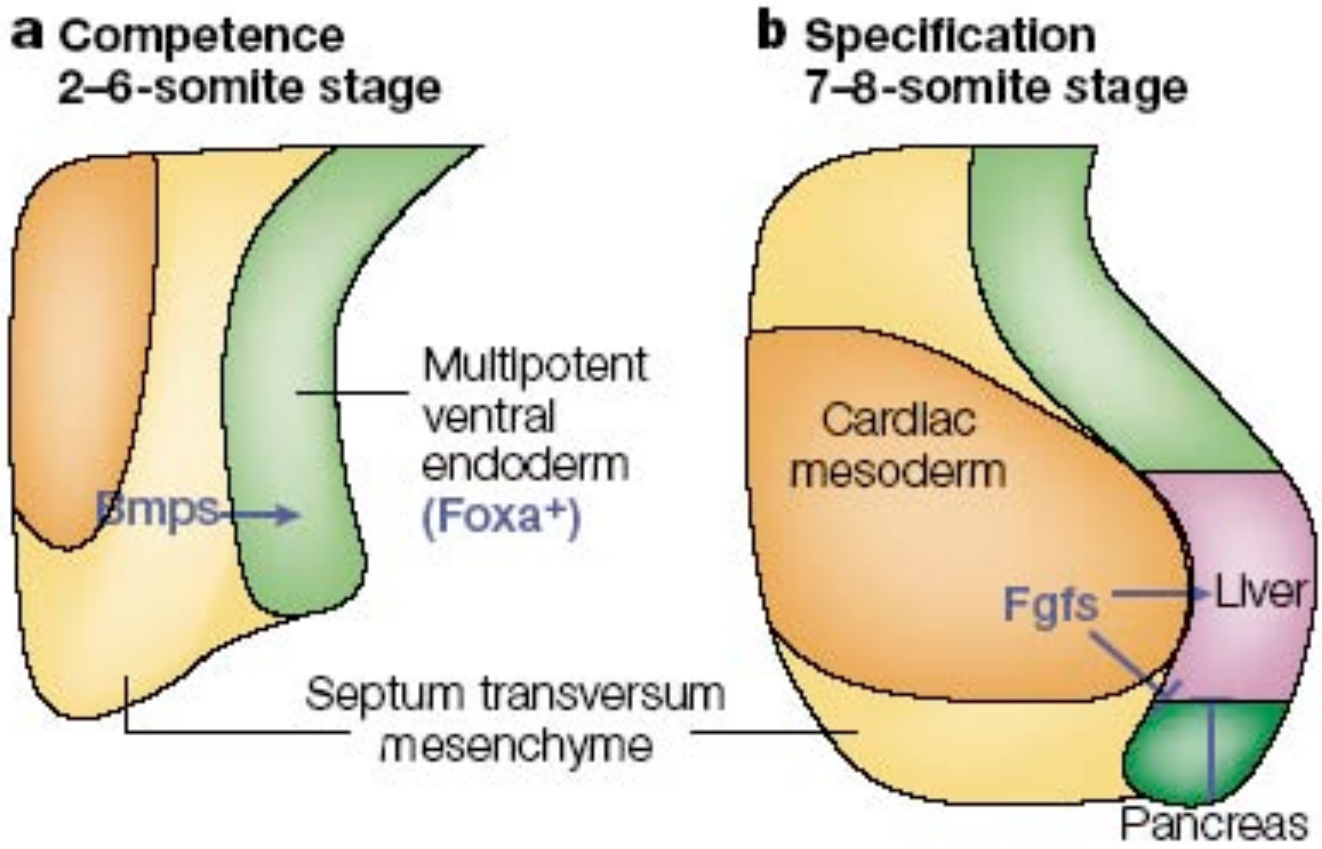


Early patterning of the endoderm



K. S. Zaret et al., Science 322, 1490 -1494 (2008)

Specification of ventral pancreas linked to liver specification

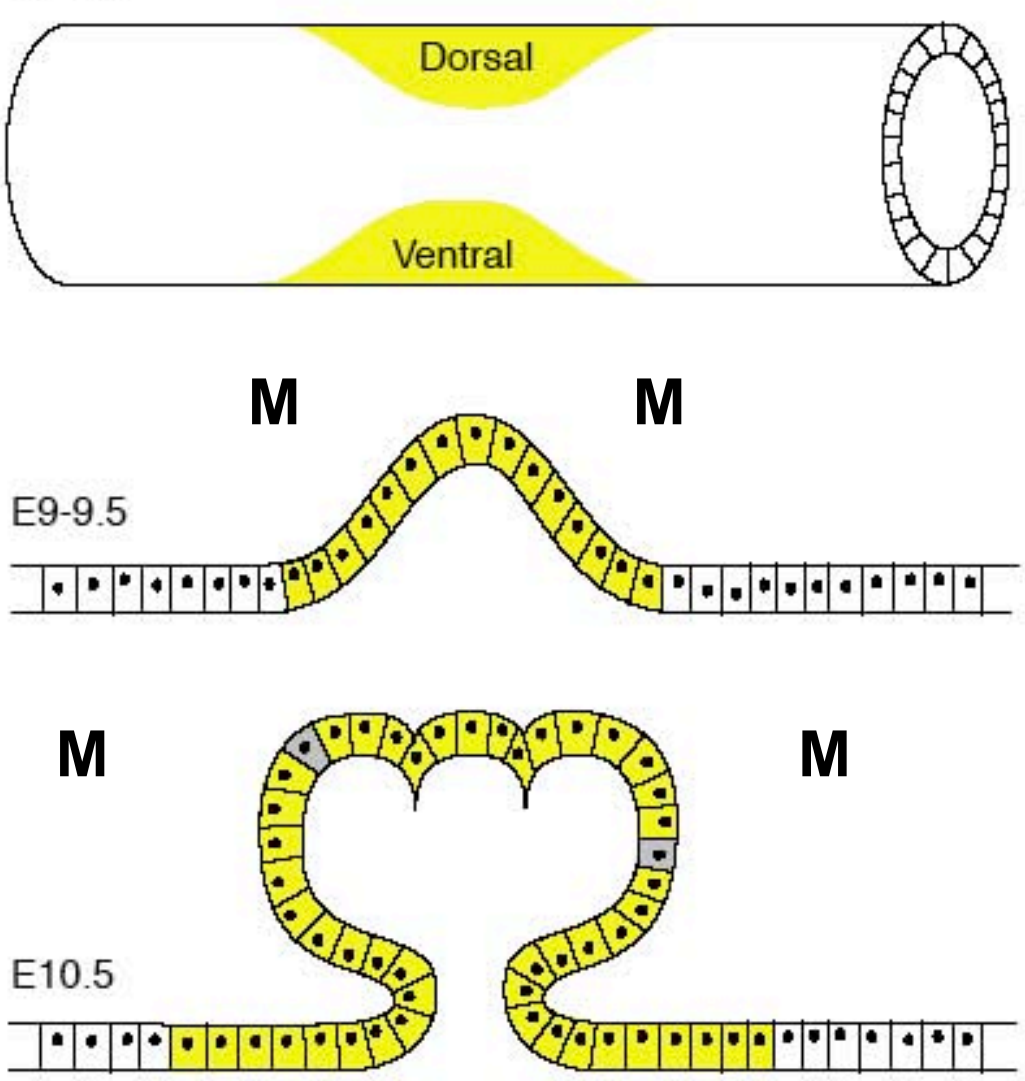


Zaret, 2002

Ventral pancreas induction

- Does not receive signals from notochord or dorsal aorta
- Develops next to cardiac mesoderm
- FGF and BMP signals from cardiac mesoderm required for liver induction and restriction of ventral pancreas domain (Zaret)
- Shh is activated (opposite from dorsal)

Pancreatic Mesenchyme

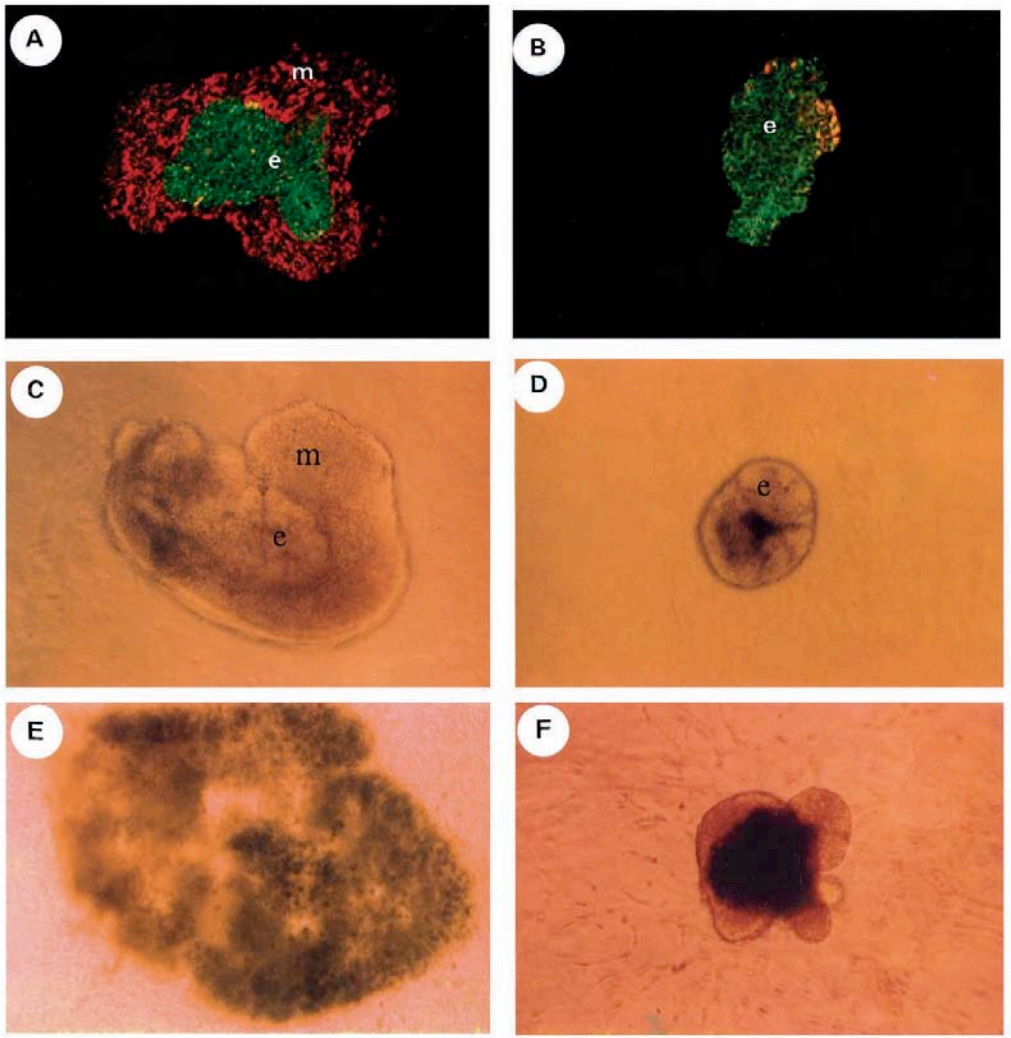


Kim and MacDonald, 2002

Pancreatic Mesenchyme

- Mesoderm accumulates around pancreatic epithelial buds
- Mesenchyme is necessary for cytodifferentiation and morphogenesis (Golosow and Grobstein, 1962)
- Signaling is permissive
  - FGF10
  - Notch
  - TGFβ family
  - Wnts
- Activation of pancreas transcriptional program

Mesenchymal signals are necessary for pancreatic growth and differentiation



G. Gittes

Mesenchymal signals: Time and space dependent

- Early experiments suggested endocrine was default lineage
  - Early mesenchyme favors endocrine development
  - Late mesenchyme favors exocrine
- Contact dependent signaling
  - proexocrine factor(s): cell-contact mediated
  - proendocrine factor(s): diffusible

Signaling pathways:
what molecules are involved?

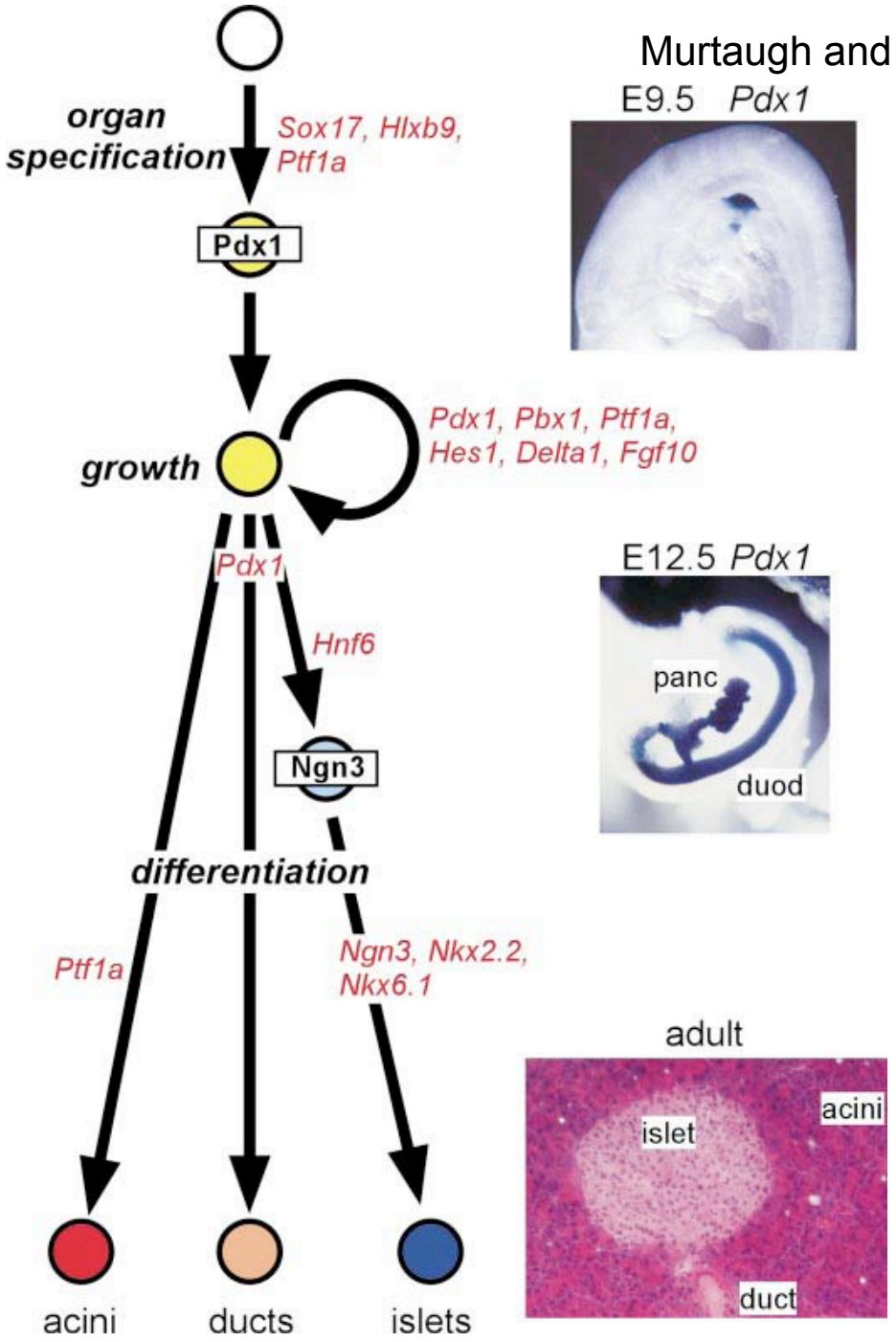
Murtaugh and Melton, 2003

Fgf
Activin

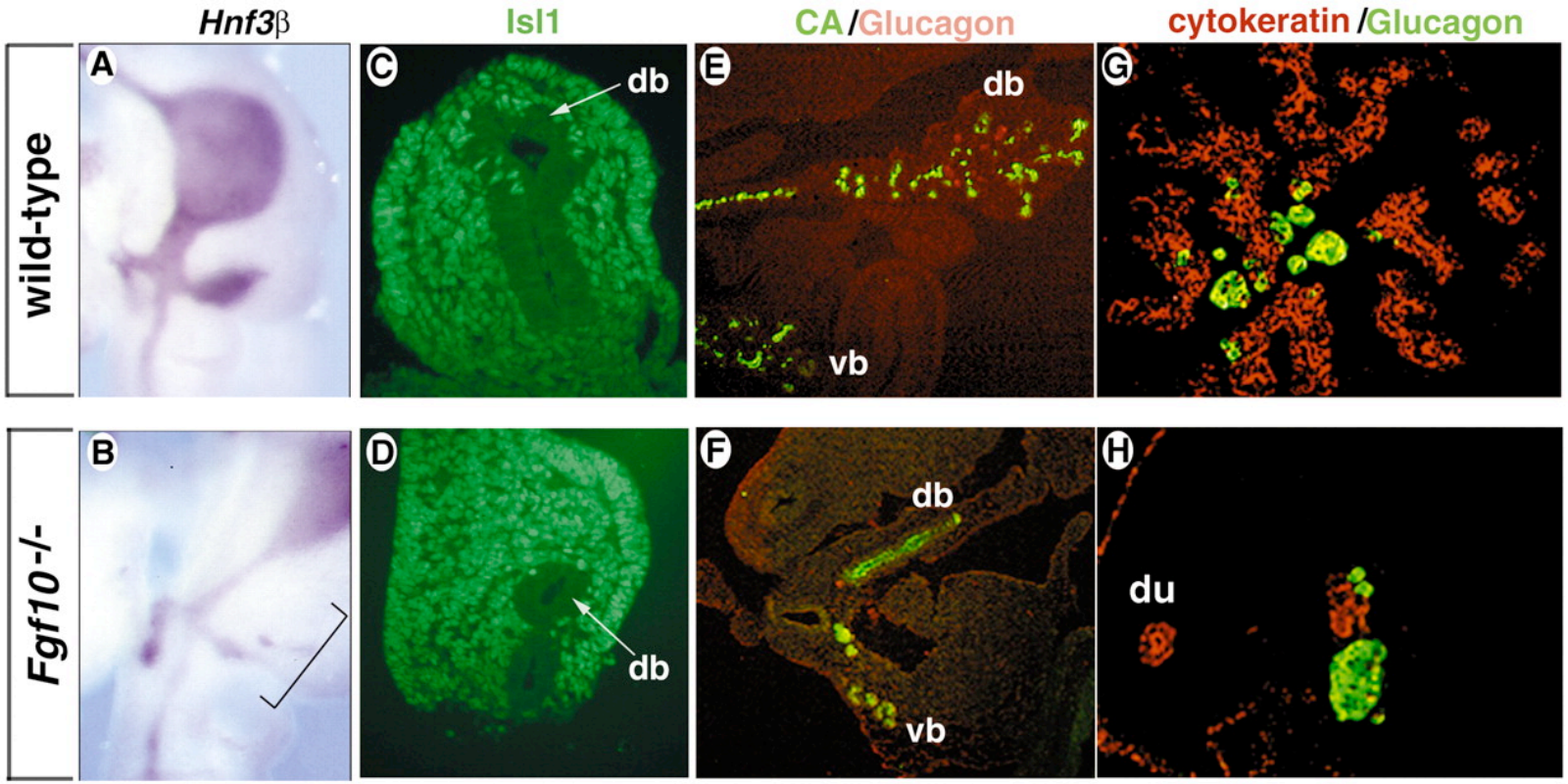
Fgf
Notch

Notch

?????
Shh



The size of the pancreatic epithelium in Fgf10-/- embryos is greatly reduced



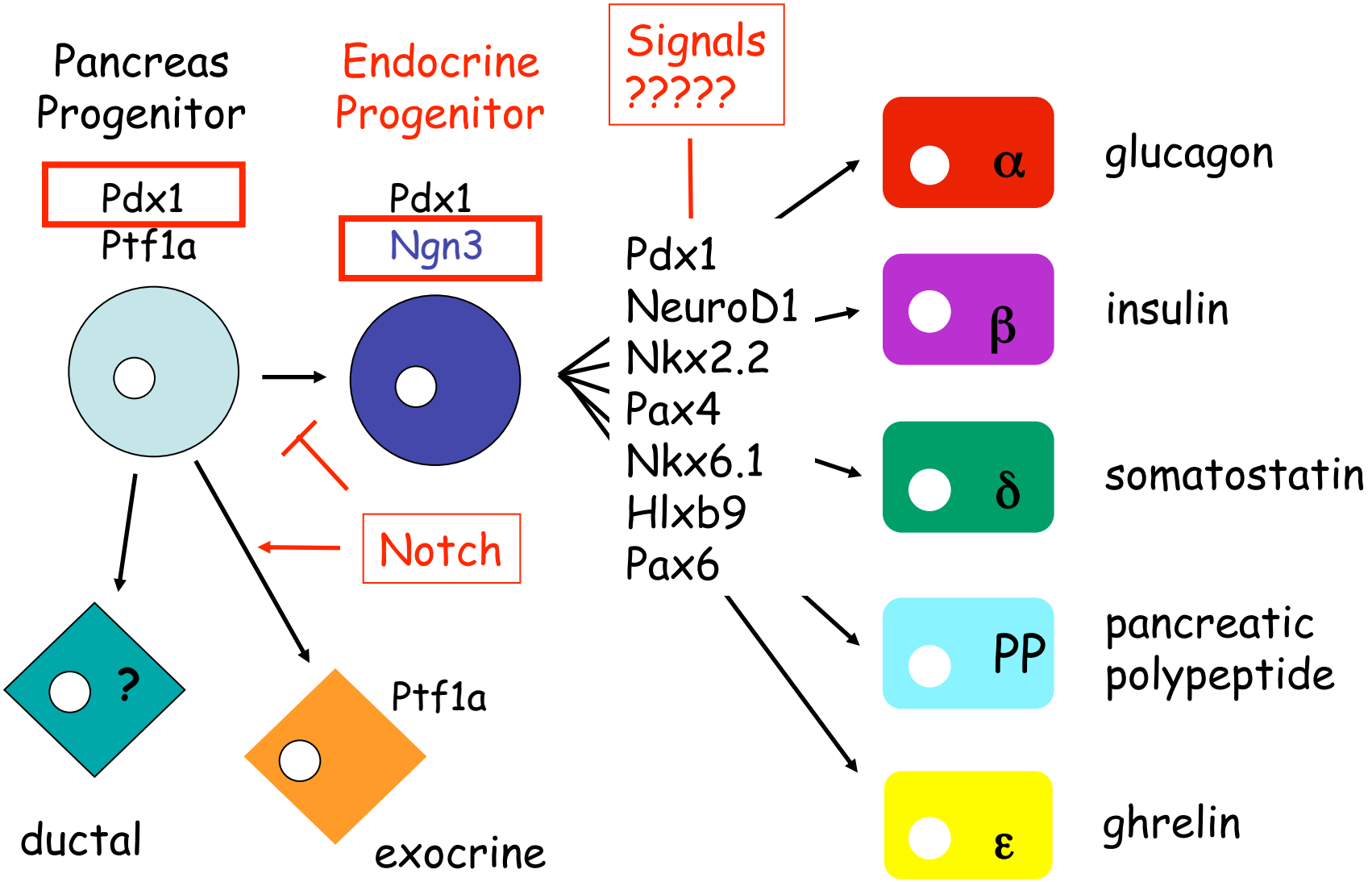
Bhushan, A. et al. Development 2001;128:5109-5117

Transcriptional control of pancreatic differentiation

Signaling events culminate in activation of transcriptional program

Transcription factor studies highlight several new and traditional mouse manipulation techniques

Pancreatic cell type specification

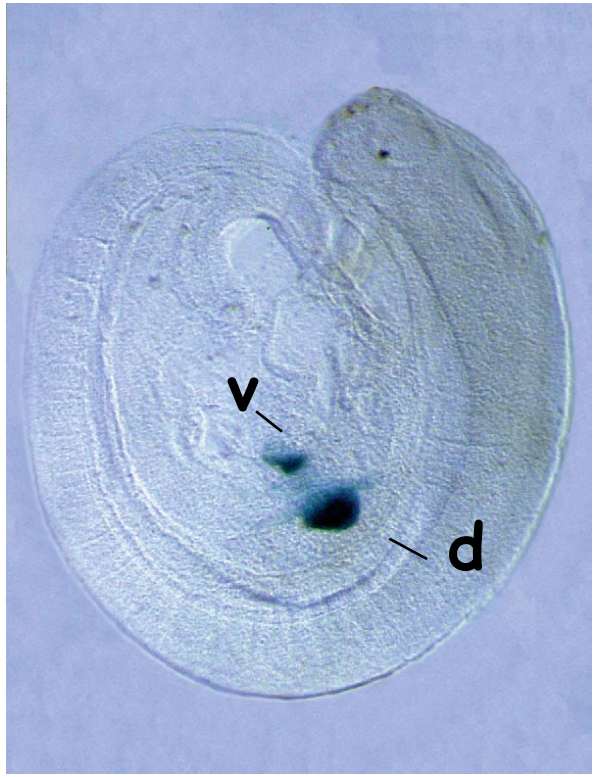


Pdx1
Pancreatic Duodenal Homeobox 1

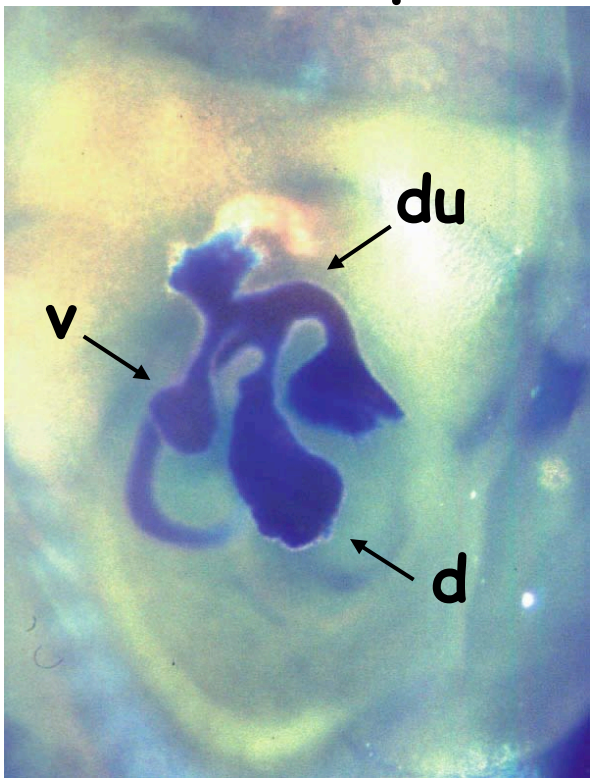
- Also known as IPF1, STF1, IDX1
- Expression identifies region of pancreas specification prior to visible morphological changes
- Earliest and one of the most specific genes expressed in pancreatic primordia
- Functions at several time points during pancreas development

Pdx1 expression

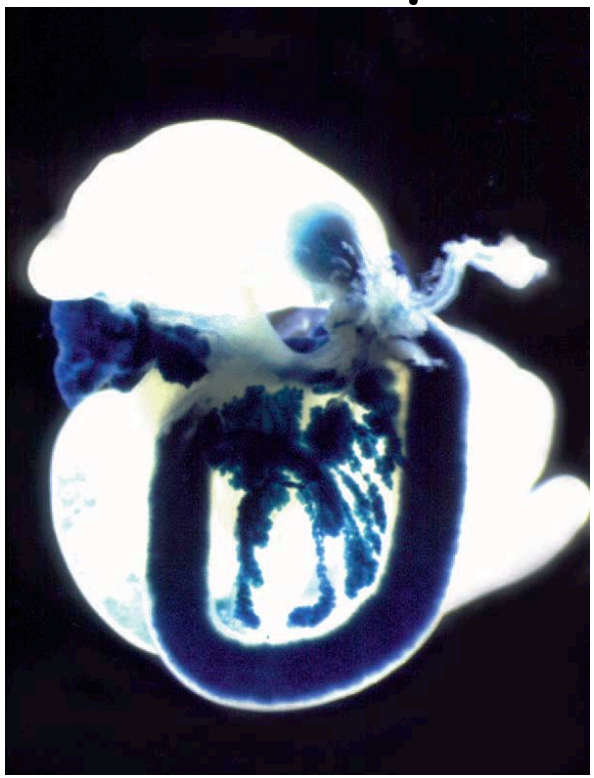
9.5 dpc



11.5 dpc

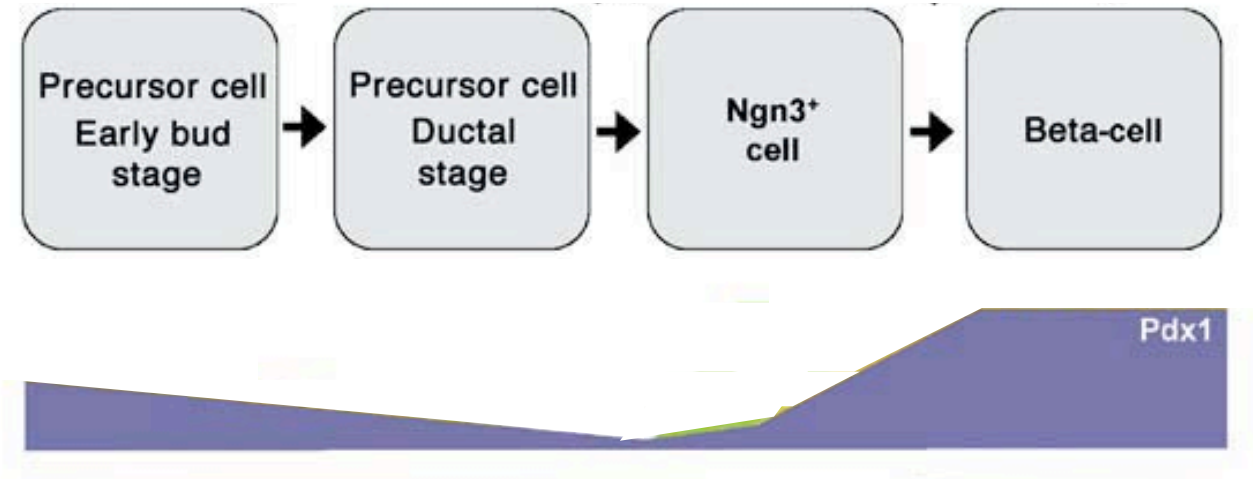


16.5 dpc



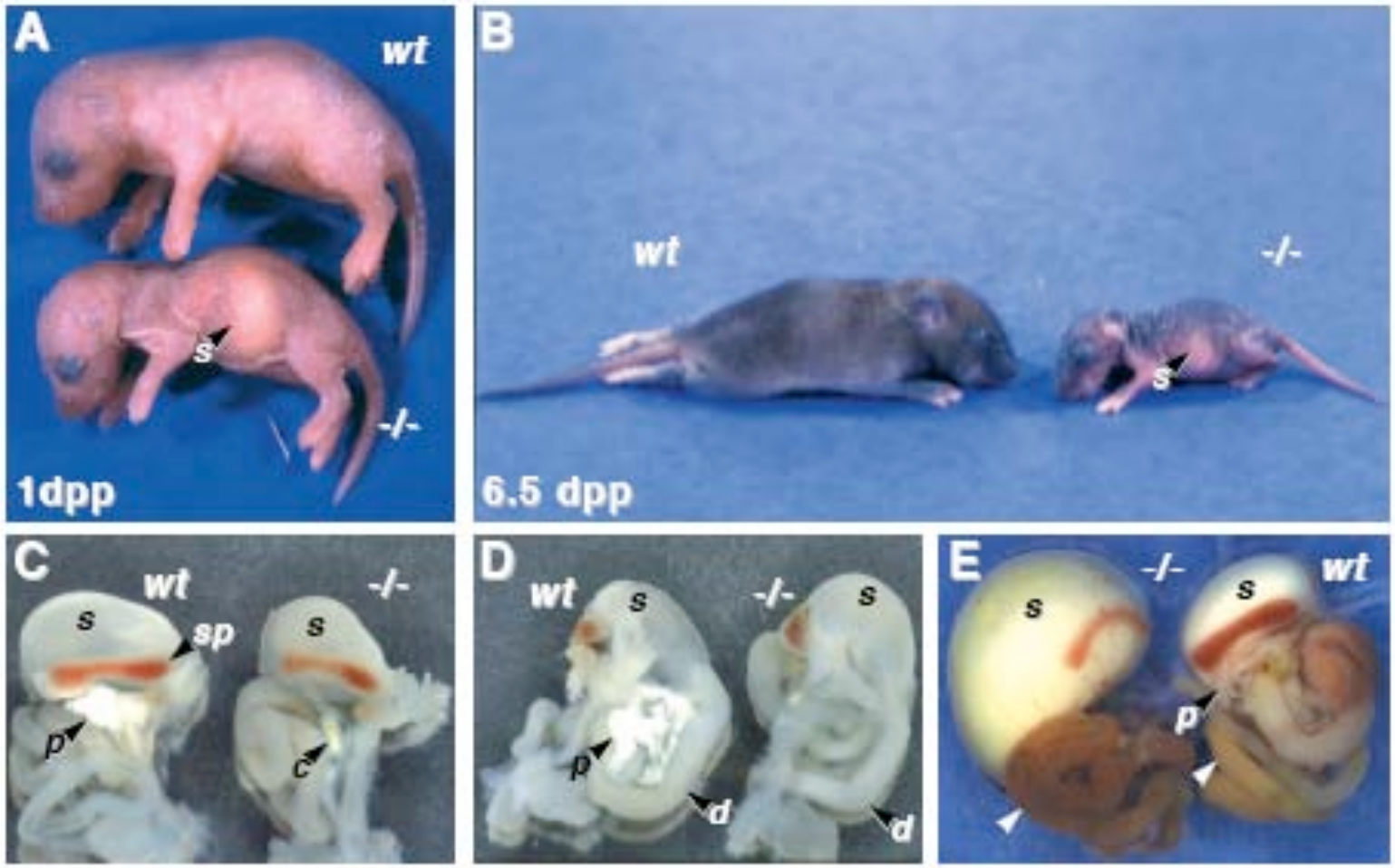
Offield et al., 1996

Pdx1 expression



- Throughout early pancreatic epithelium
- Pancreas progenitors
- β and δ cells (high levels)
- Exocrine cells (low levels)

Pdx1 null causes pancreatic agenesis

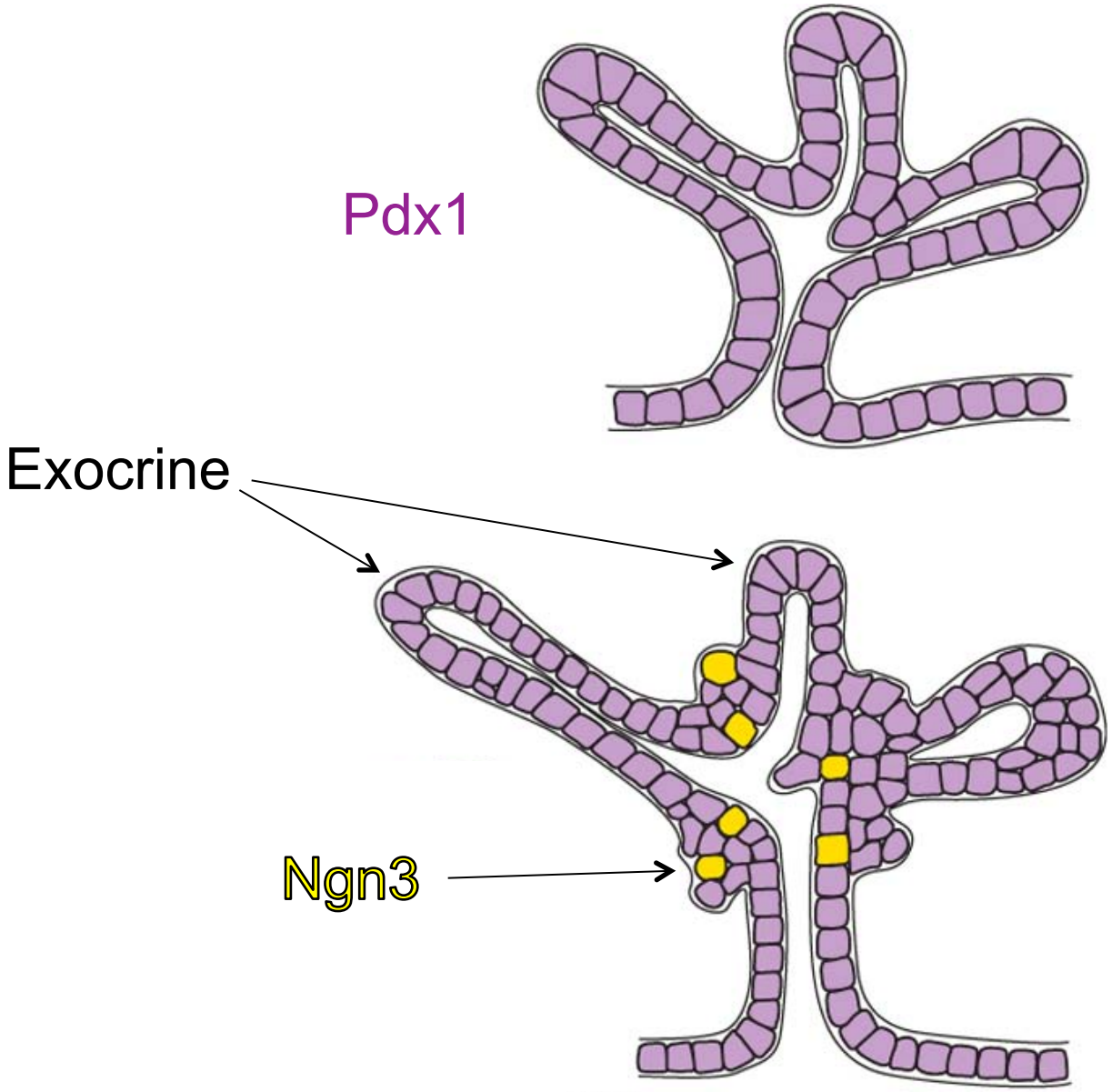


Offield et al., 1996

Pdx1 mutations in humans

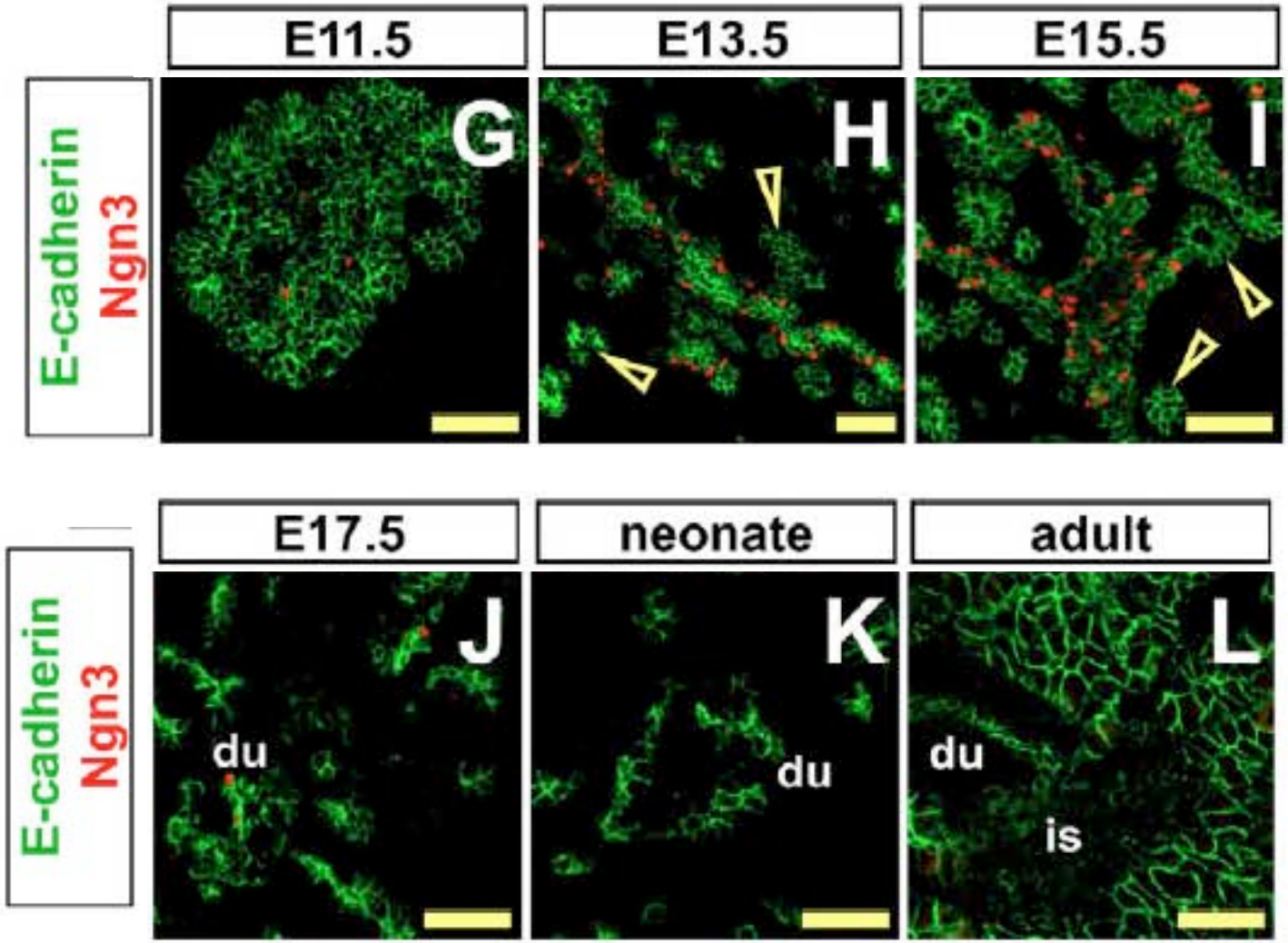
- Loss of function mutations cause apancreatic phenotype and perinatal lethality (failure to thrive infants)

- Reduced function mutations: MODY4
  - MODY = Maturity onset diabetes of the young

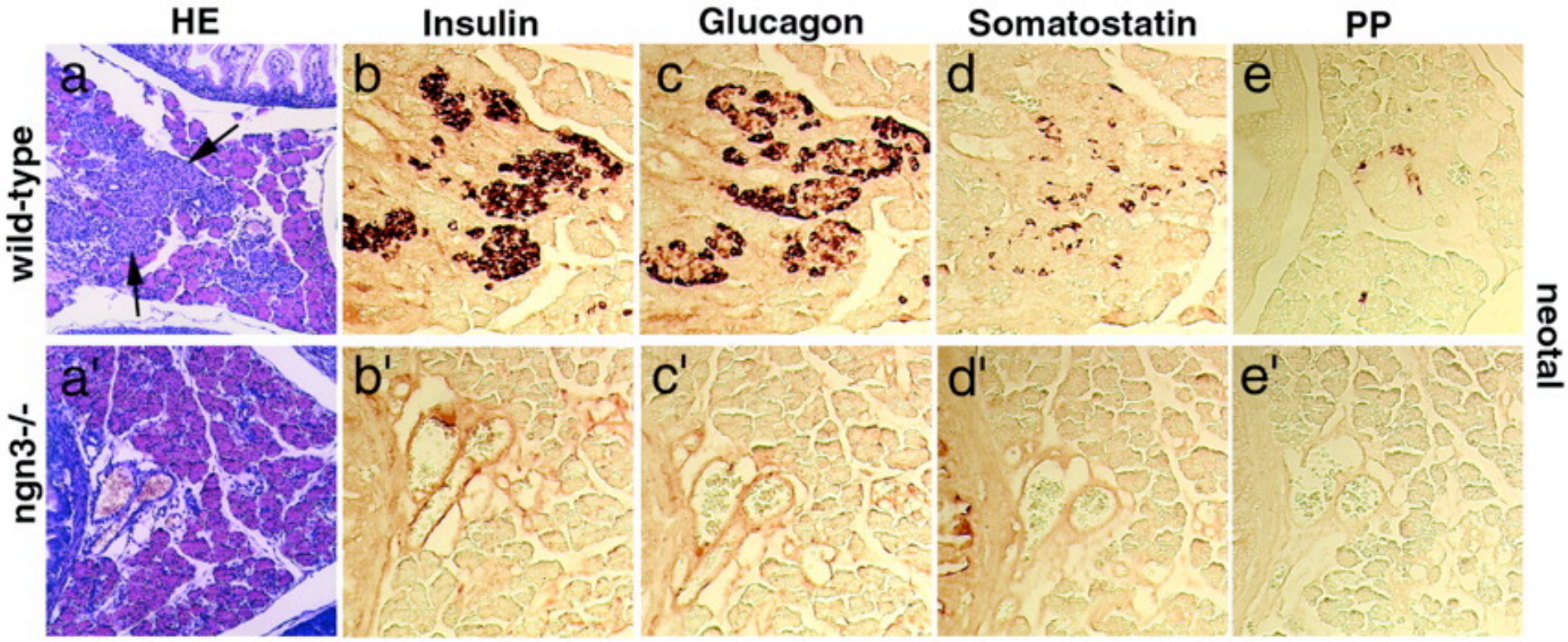


Edition.

Ngn3 is expressed in endocrine progenitors



Ngn3 null: all islet lineages are lost

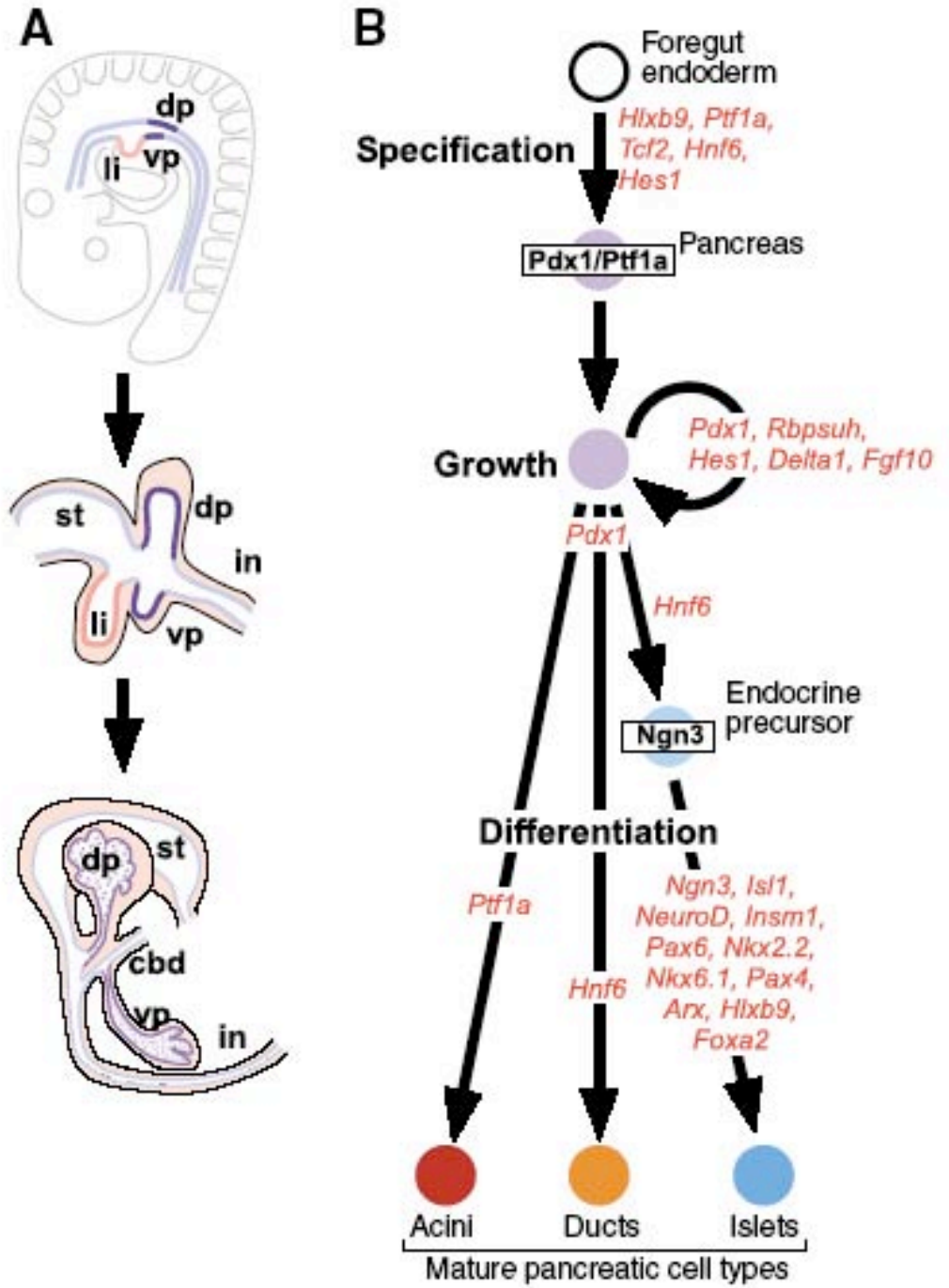


Gradwohl, Gérard et al. (2000) Proc. Natl. Acad. Sci. USA 97, 1607-1611

Ngn3 summary

- Ngn3 is expressed in the endocrine progenitor cells
- Ngn3 cells can give rise to all the islet cell populations
- The islet progenitor cells are differentially competent over time to give rise to the different islet cell types
- Reactivated during pancreas regeneration

Summary of pancreas development

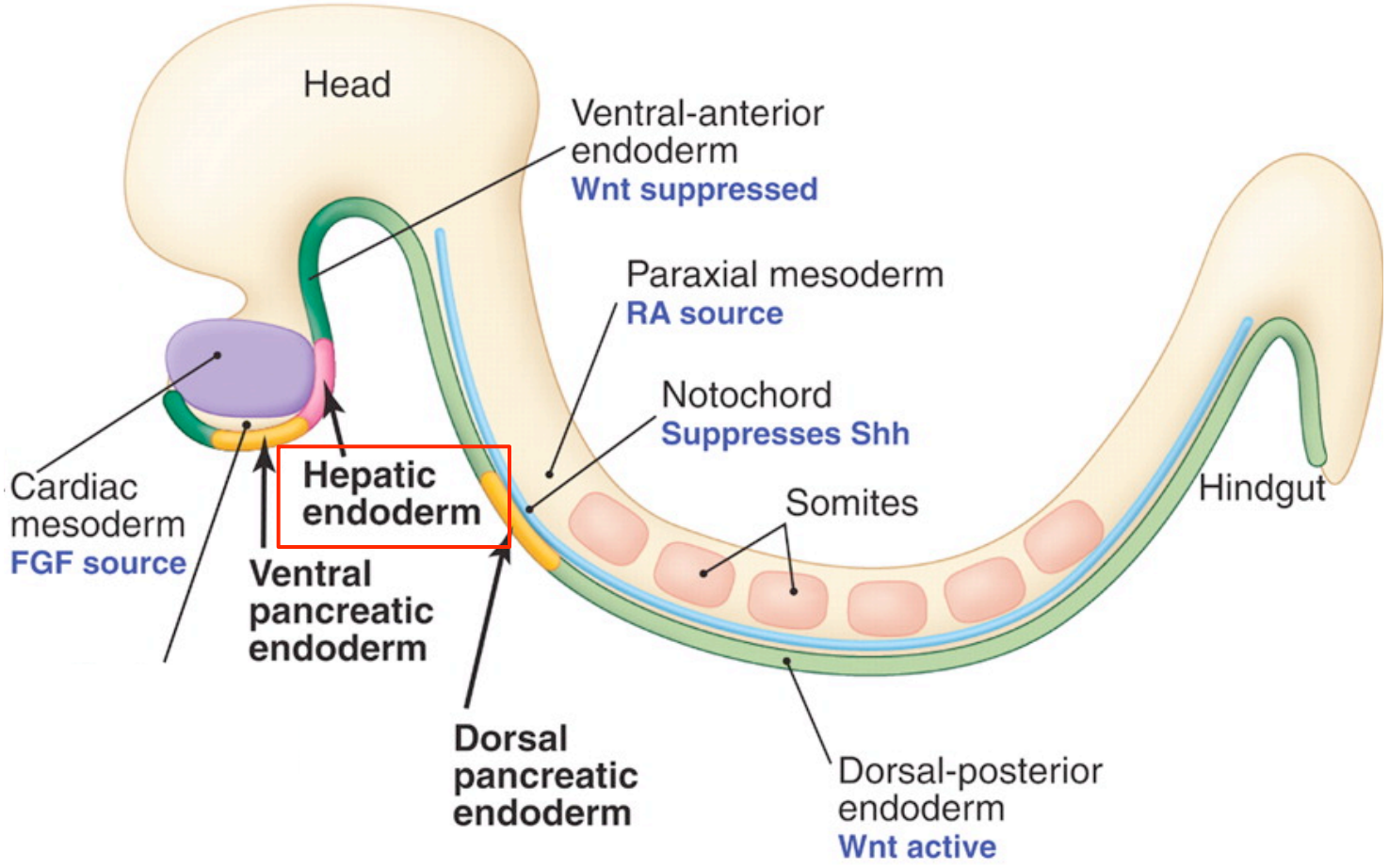


Murtaugh, 2007

Liver

- Largest internal organ in the body
- Two major lobes
- Hepatocytes (60-80% of liver cells) carry out main functions of the liver
- Many functions including fat breakdown, filtration, vitamin storage, glucose regulation, cholesterol production
- Genetic liver diseases, hepatitis, cirrhosis

Liver derived next to the v. pancreas



K. S. Zaret et al., Science 322, 1490 -1494 (2008)

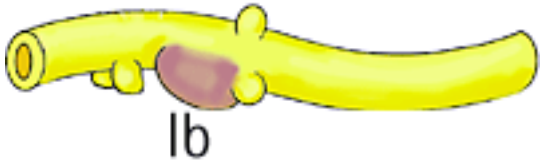
Liver organogenesis

- Derived from endoderm layer as a single rudiment
- Requires a series of inductive signals from at least 3 different mesodermal cell types
- Begins forming at e8.5 when hepatic epithelium thickens, delaminates and invades surrounding mesenchyme to form the liver bud
- Endothelial cells critical for liver development and differentiation
- Continued epithelial-mesenchymal interactions stimulate cell proliferation and morphogenesis as the organ grows
- High regenerative capacity (replication of existing cell types)

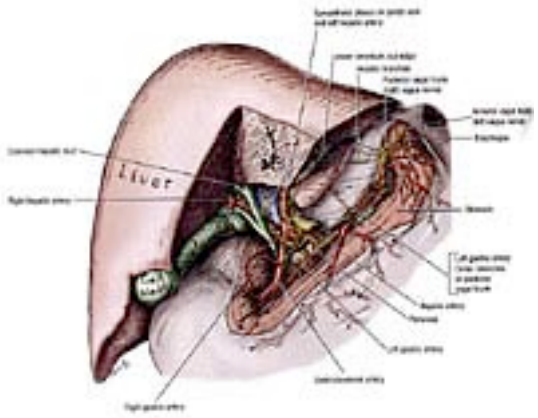
Sequential stages of liver development

Endoderm patterning and hepatic competence (gastrulation)

Liver induction and specification (4-8 somite)

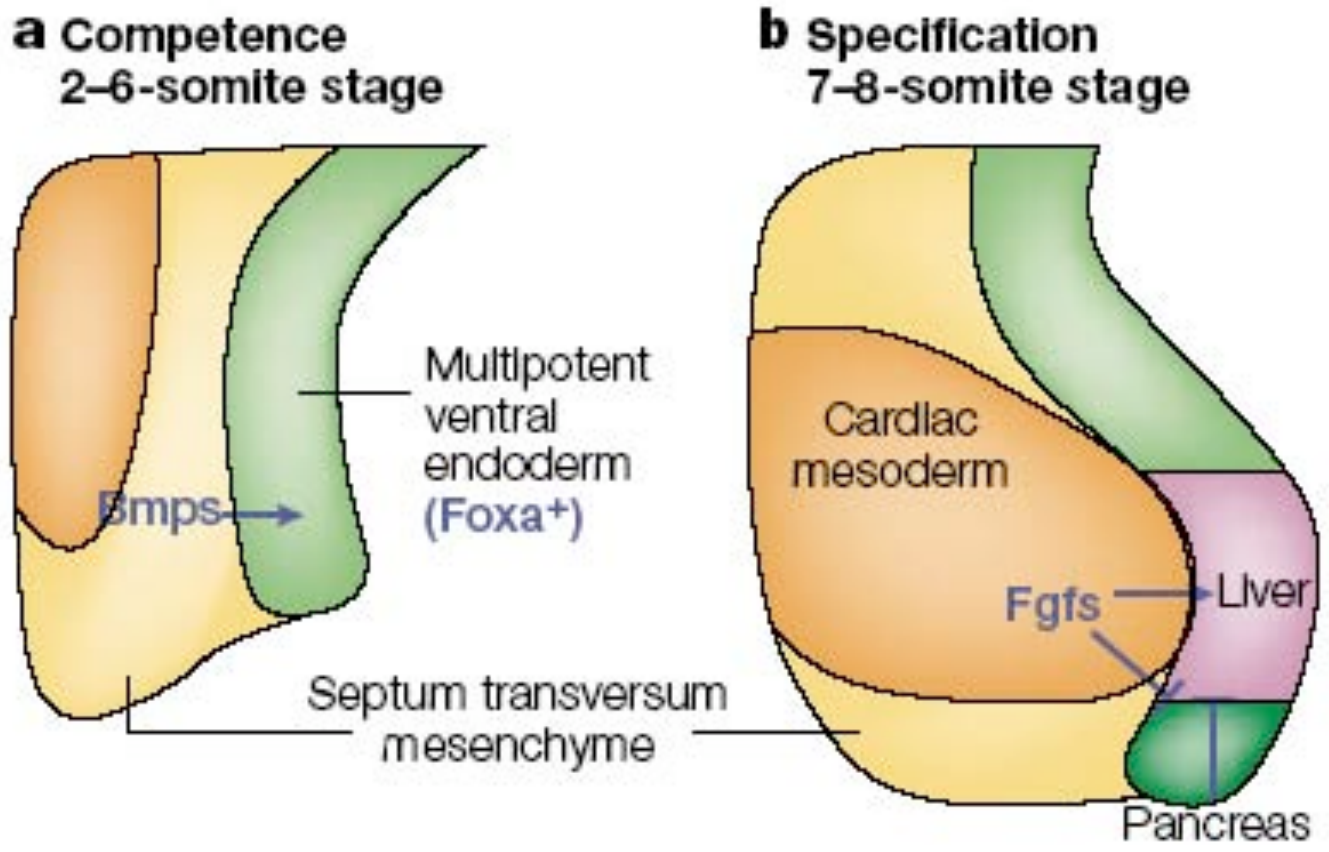


Liver bud proliferation morphogenesis

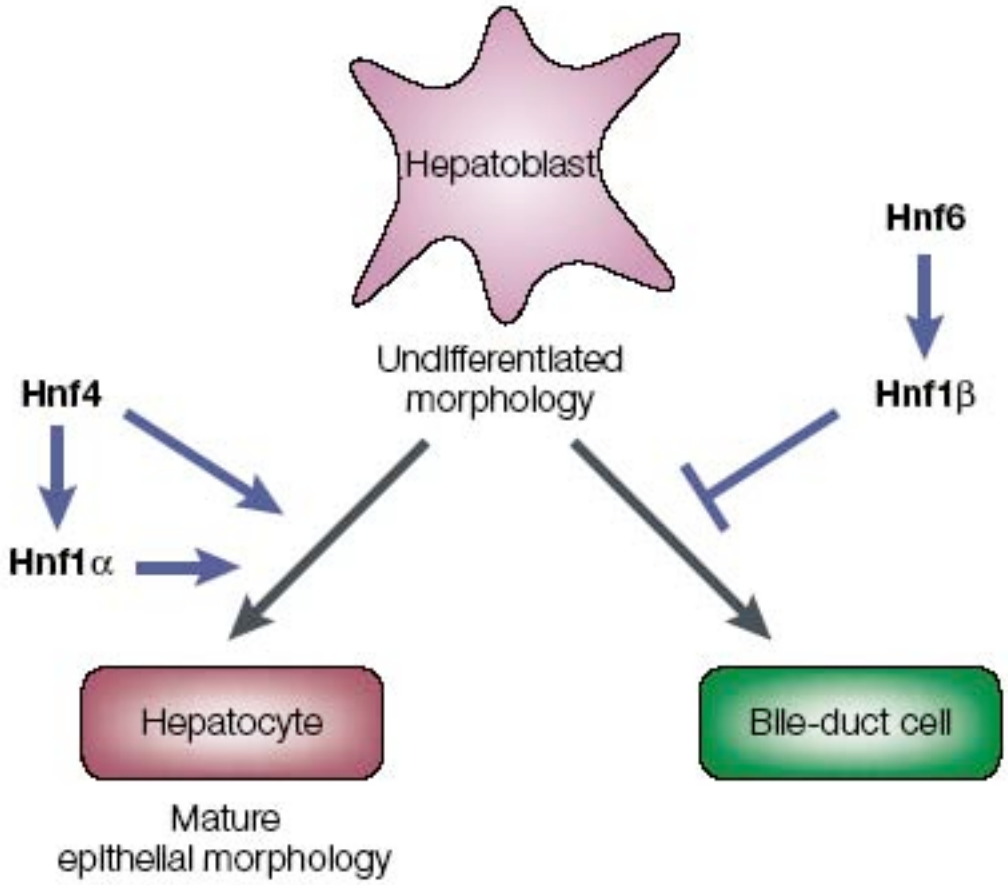


Hepatic differentiation

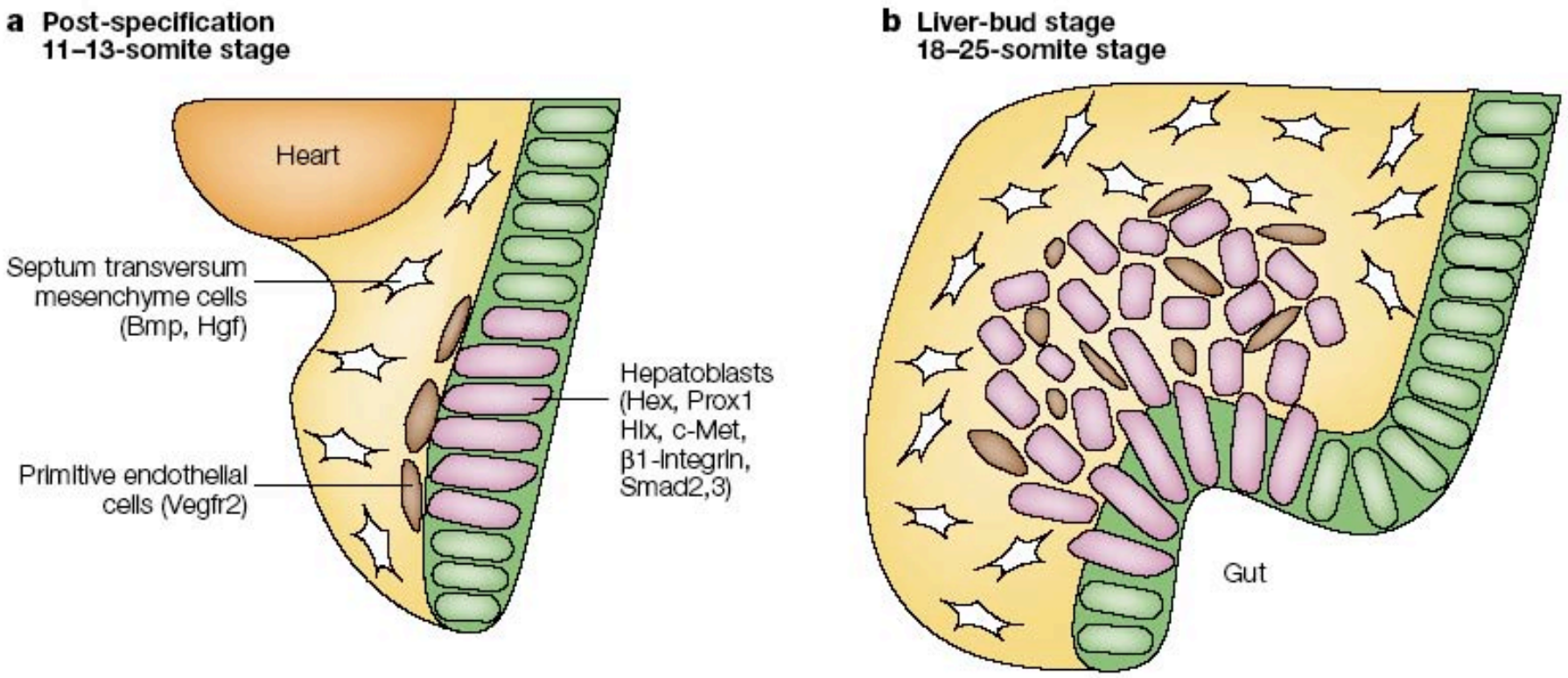
Establishment of competence and specification



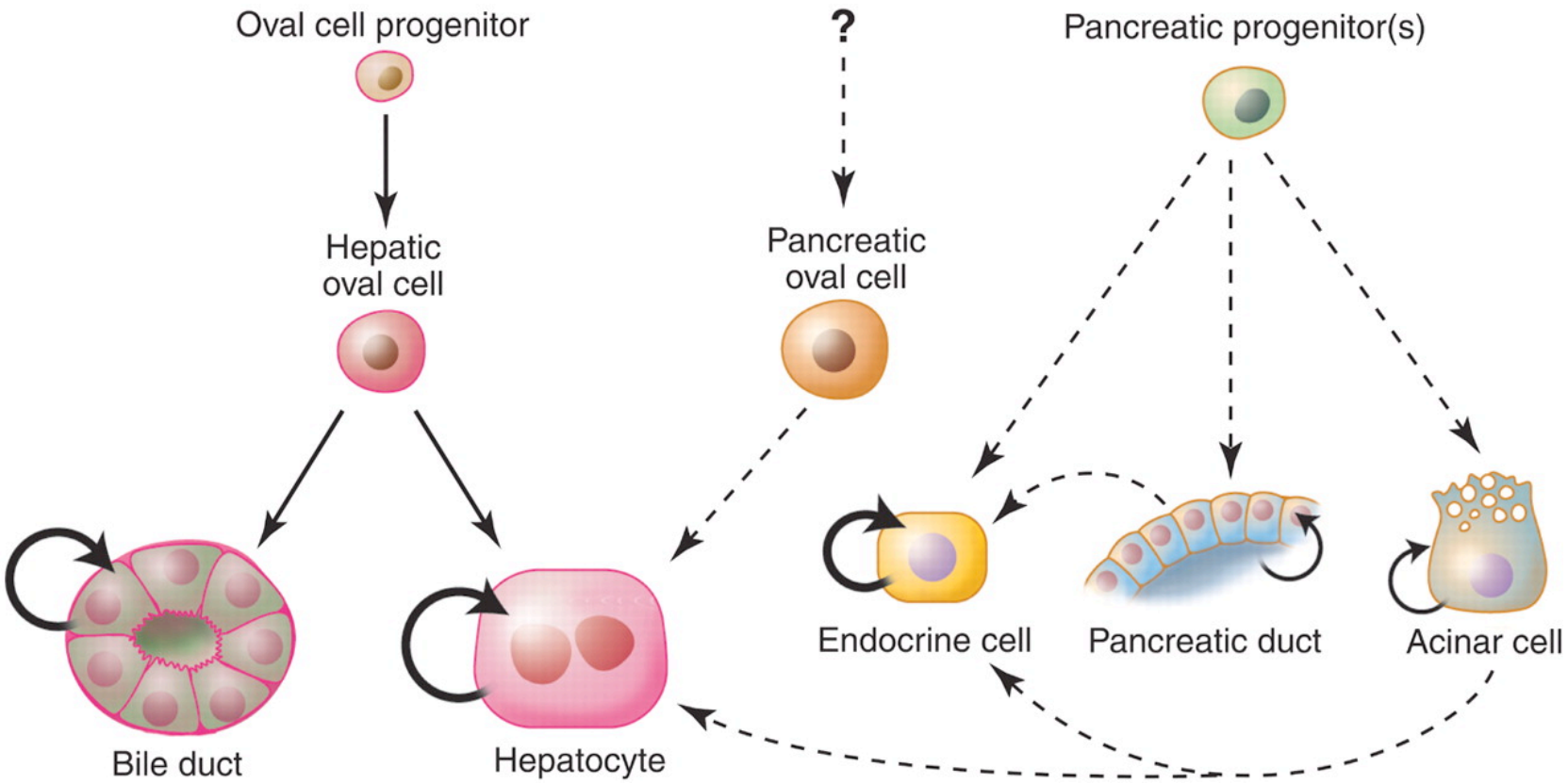
Cell type differentiation



Bud formation



Progenitor cells in liver and pancreas



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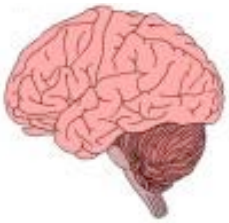
K. S. Zaret et al., Science 322, 1490 -1494 (2008)

Science
AAAS

Stem cells --> Islet cells

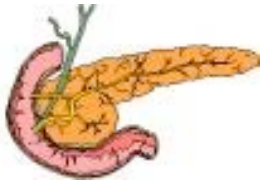


brain

or

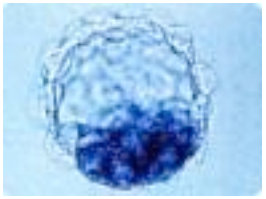
liver

?



pancreas

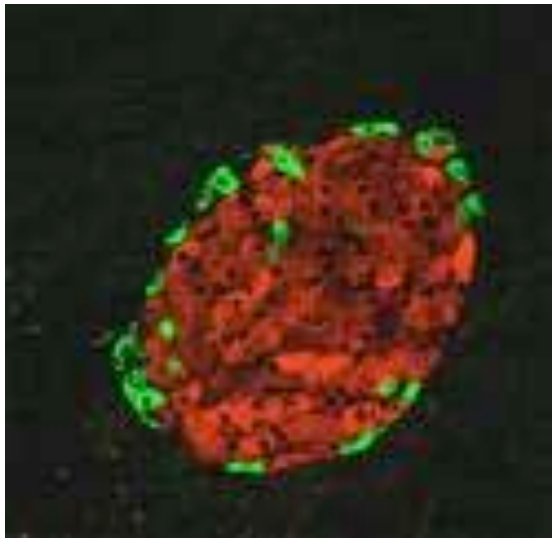
?



ES cells

iPS cells

?



ARTICLES

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nature biotechnology

Production of pancreatic hormone–expressing endocrine cells from human embryonic stem cells

Kevin A D'Amour, Anne G Bang, Susan Eliazer, Olivia G Kelly, Alan D Agulnick, Nora G Smart, Mark A Moorman, Evert Kroon, Melissa K Carpenter & Emmanuel E Baetge

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Stage 1		Stage 2	Stage 3	Stage 4	Stage 5
Definitive endoderm		Primitive gut tube	Posterior foregut	Pancreatic endoderm and endocrine precursor	Hormone expressing endocrine cell
Activin + Wnt	Activin	FGF10 + CYC	RA + CYC + FGF10	+/- DAPT; Ex4	+/- Ex4; IGF1; HGF
RPMI + 0% FBS	RPMI + 0.2% FBS	RPMI + 2% FBS	DMEM + 1% B27		CMRL + 1% B27