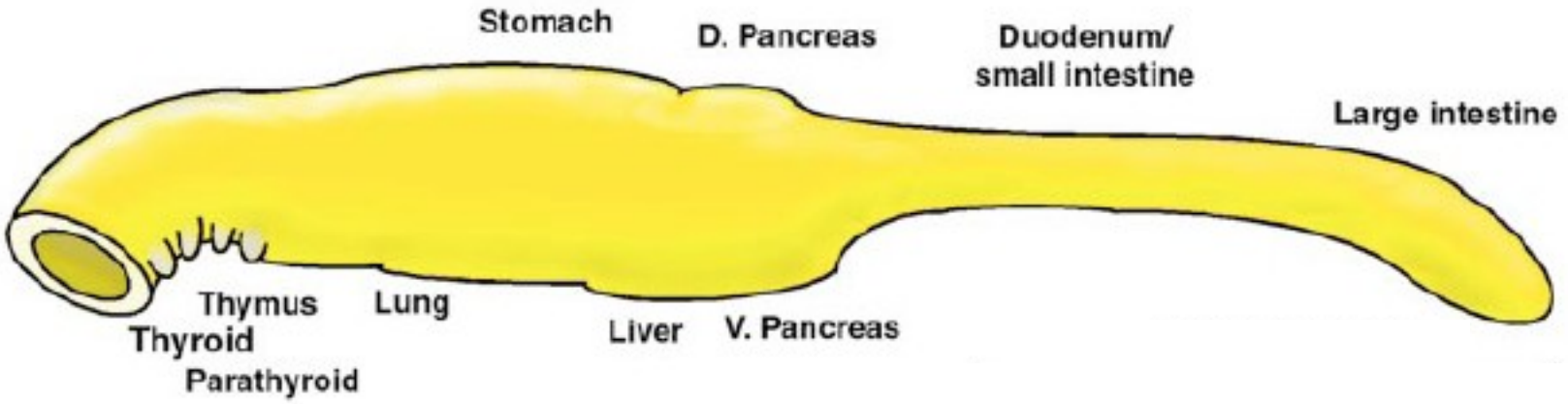
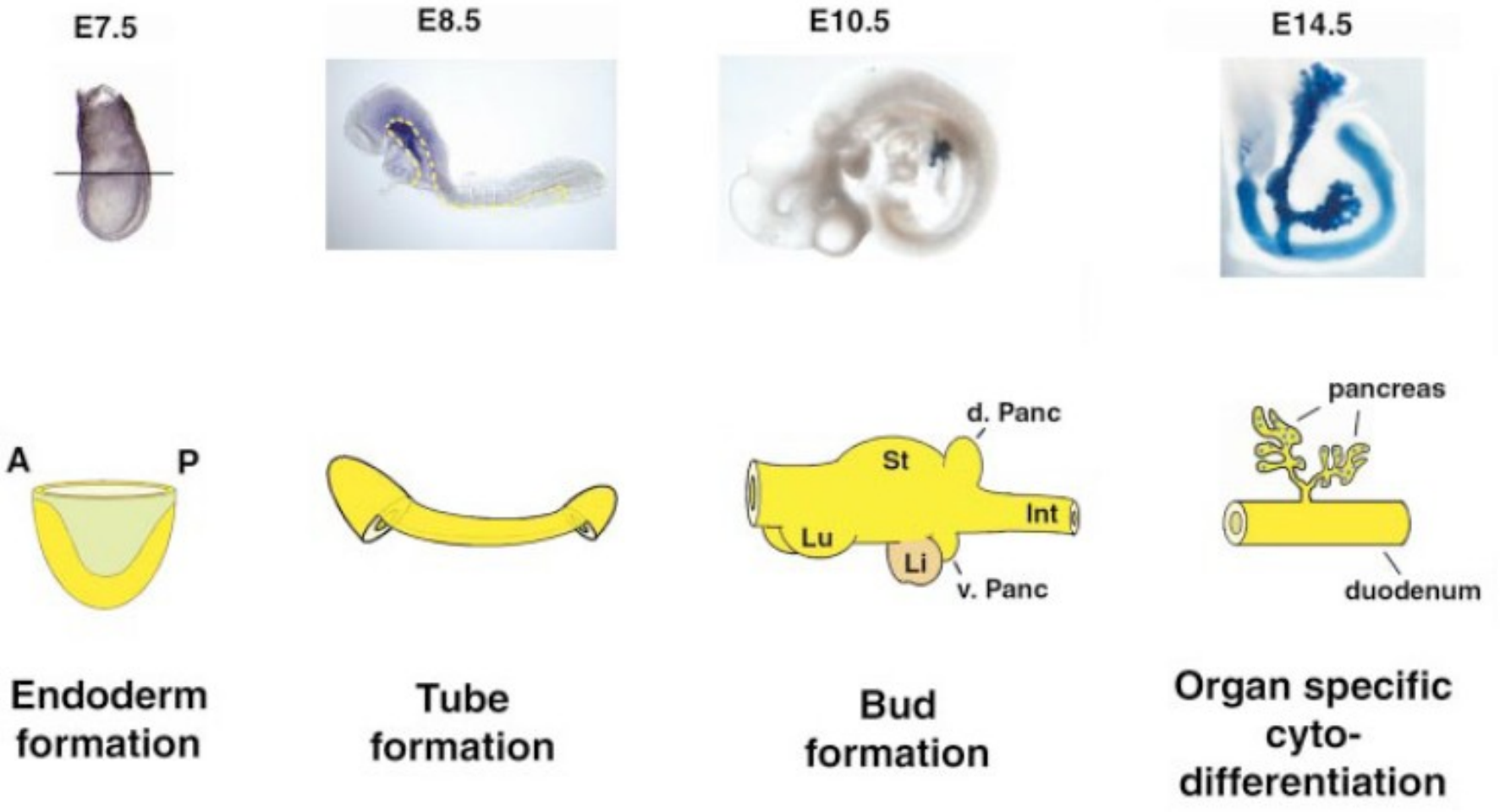
DEVELOPMENT OF ENDODERMAL ORGANS



Fáze vývoje endodermálních orgánů (Časování u myši)



1. DETERMINATION OF THE ENDODERM:

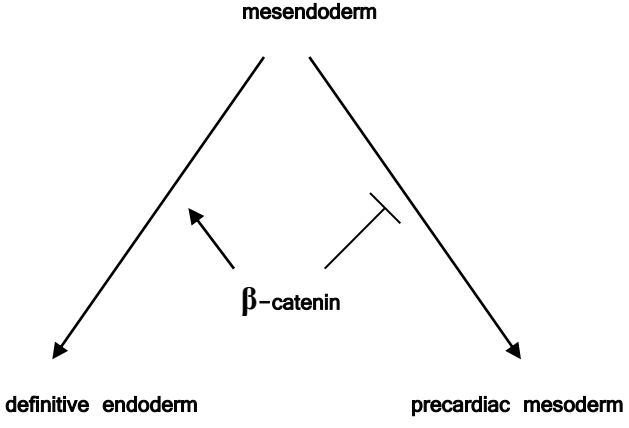
- při základní specifikaci zárodečných listů - klíčová role maternálních faktorů

- navzdory odlišné gastrulaci je molekulárně velmi konzervovaná

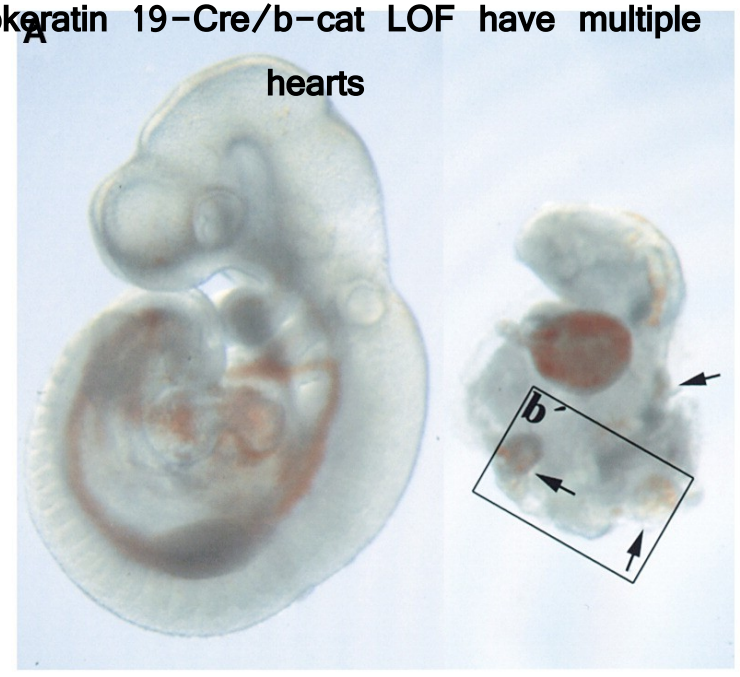
- klíčové transkripční faktory: VegT, **Sox17**, **Mix1**, **Mixer** and **GATA1-4**.

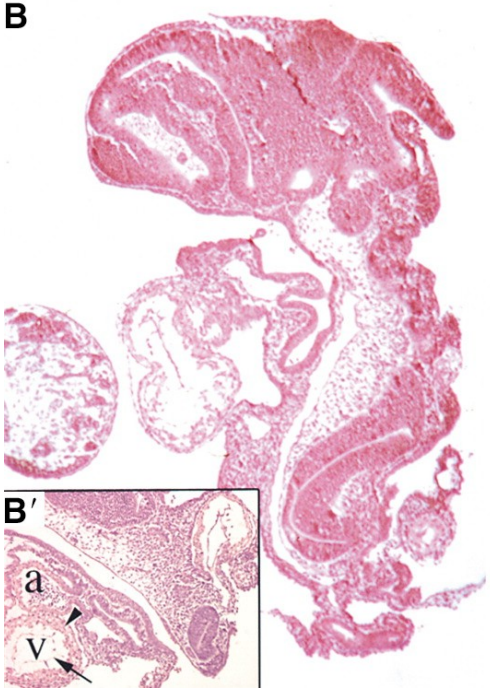
-animals with **nodal loss-of-function** can not form the **definitive endoderm**.

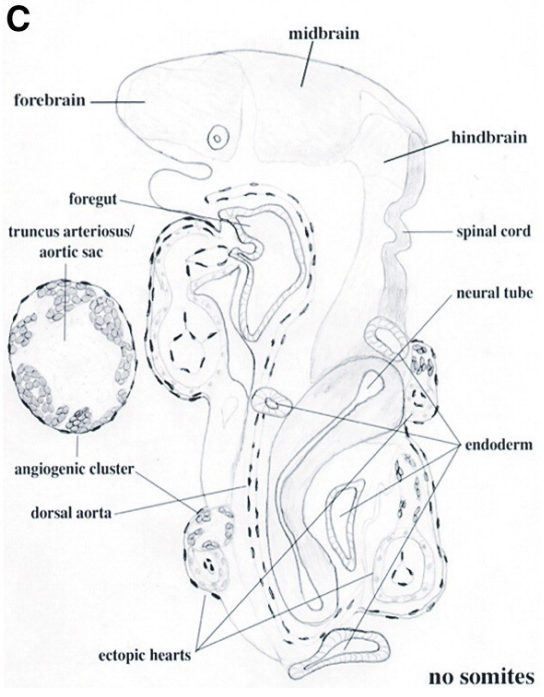
Role of beta-catenin in definitive endoderm: blocks specification of heart forming mesoderm



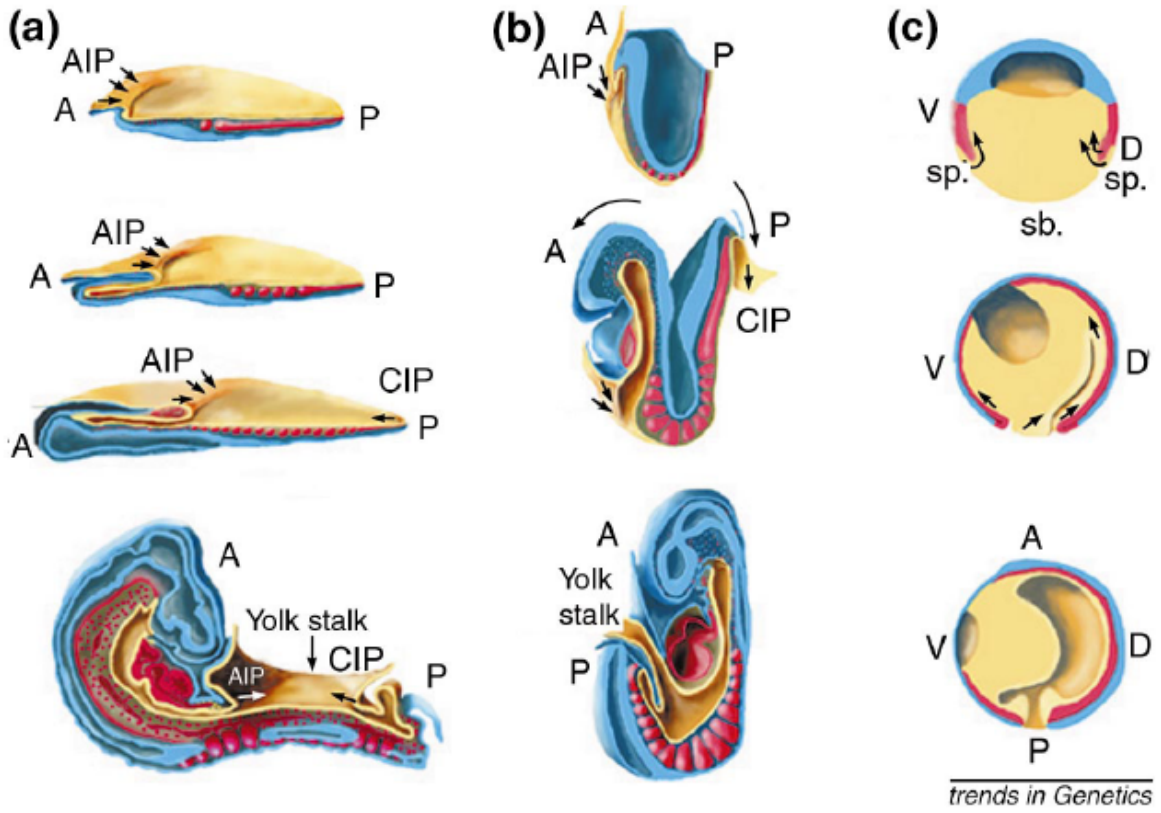
Cytokeratin 19-Cre/b-cat LOF have multiple hearts



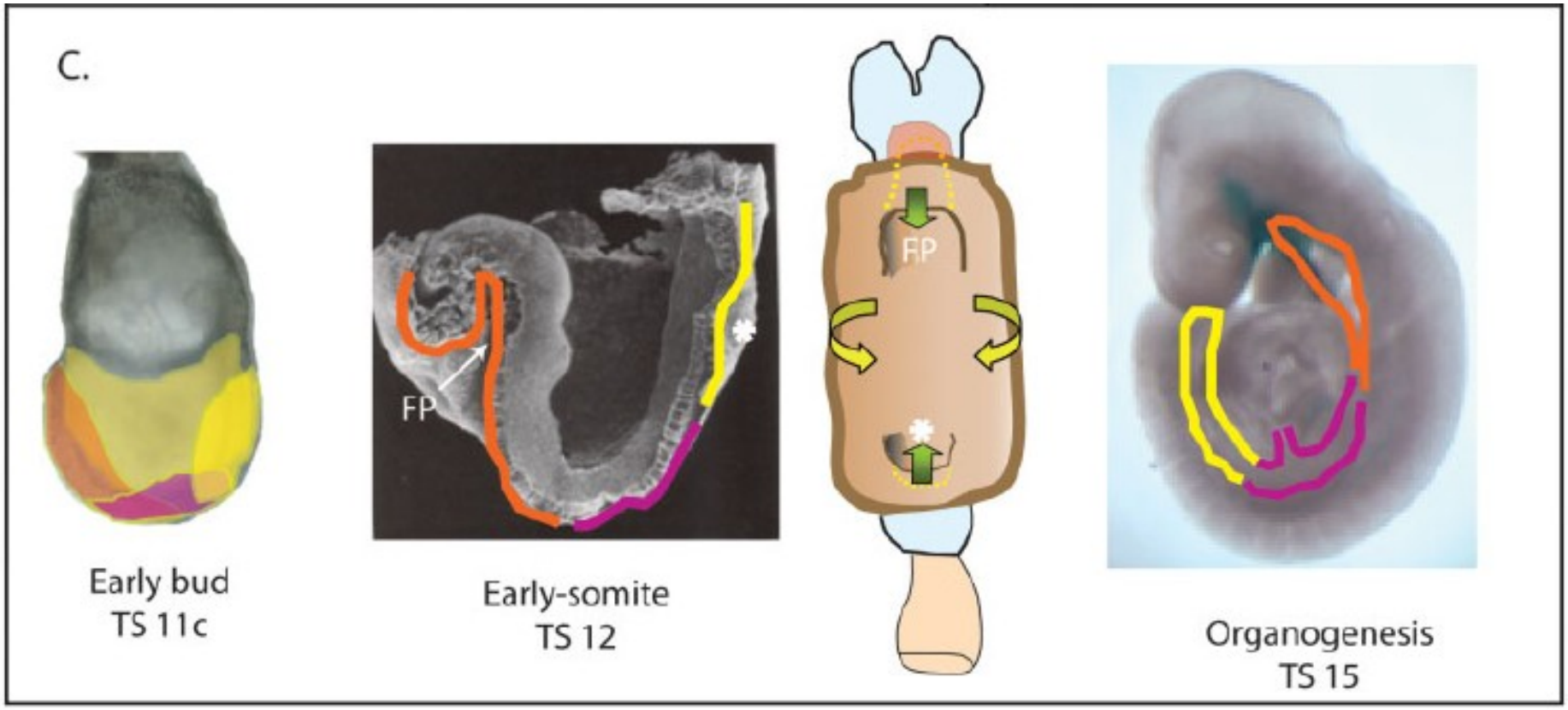




2. Formování endodermální trubice

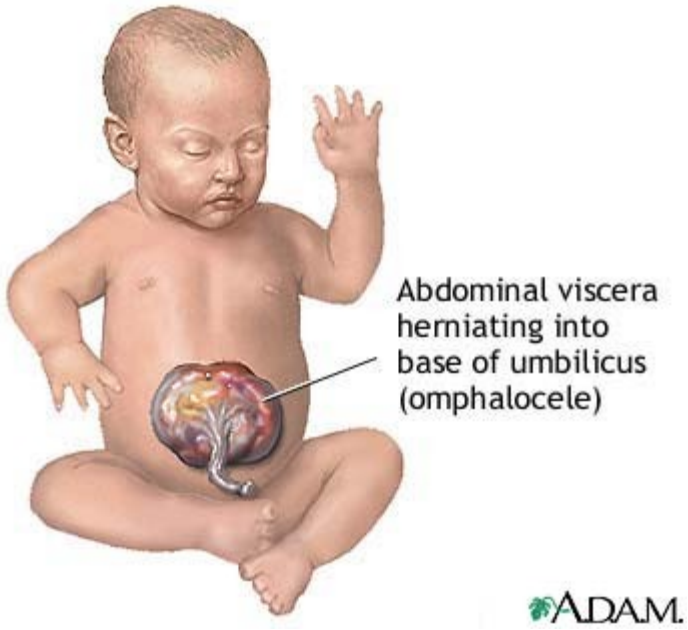


Endodermal, mesodermal and ectodermal layers are respectively shown in yellow, red and blue. (a) A series of chicken embryos at 1-, 5-, 15- and 46-somite-stage (E3.5). (b) 5-, 11- and 20-somite-stage mouse embryos (E8.0, 8.5 and 9.0). (c) Gastrulating *Xenopus* embryos at stages 10, 11 and 13. In chicken and mouse embryos, the anterior intestinal portal (AIP), a crescent-shaped fold, appears in the endoderm at the level of the tip of the neural tube and moves posteriorly as indicated by arrows. A similar fold, called the caudal intestinal portal (CIP), later arises at the very posterior end of the embryo and moves anteriorly. The two folds meet at the yolk stalk, which connects the embryo to the yolk sac. In the mouse, convergence of the AIP and CIP is facilitated by the turning of the embryo (big arrows). The latest stages of mouse and chicken development show that the mouth is open anteriorly whereas anal/urogenital plates are not yet open. In *Xenopus*, the endoderm that gives rise to the ventral part of the gut tube comes from the subblastopore region (sb), whereas the endoderm that gives rise to the dorsal part ingresses from the suprablastopore zone (sp) on the dorsal side. Deep endoderm cells of the gastrula will contribute to the foregut. Abbreviations: A, anterior; P, posterior; D, dorsal; V, ventral.

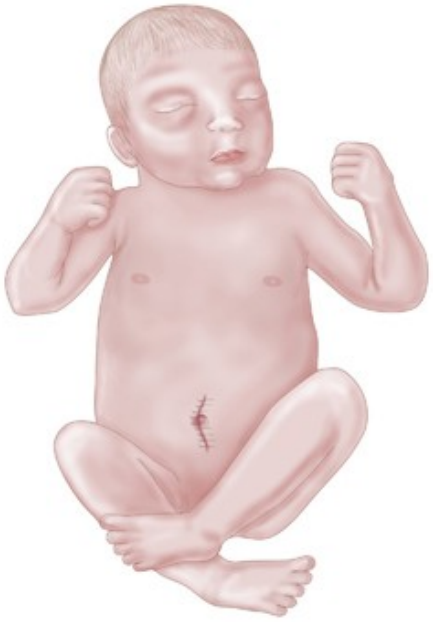


expressed in the definitive endoderm (arrow) and anterior visceral endoderm (AVE, asterisk). C: Formation of the gut tube showing the location of the endodermal precursors of the foregut (orange), midgut (pink), and hindgut (yellow) at the early-bud, early-somite, and forelimb-bud stages. At the early somite stage, the foregut pocket (FP) is extending deeply into the anterior region of the embryo and closing, but the hindgut pocket (asterisk) is just beginning to form. The embryonic gut is shown schematically in a ventral view to illustrate the morphogenetic movement (arrows) associated with the closure of the foregut and hindgut pockets and the ventral folding of the lateral body wall in the formation of the embryonic gut. At the organogenesis stage, the foregut pocket, hindgut pocket, and the lateral endodermal walls have met at the yolk stalk, thus closing the gut tube.

Omphalocele （omfalokéla）



MoŽnost chirurgické nápravy



3. Anteroposteriorní specifikace

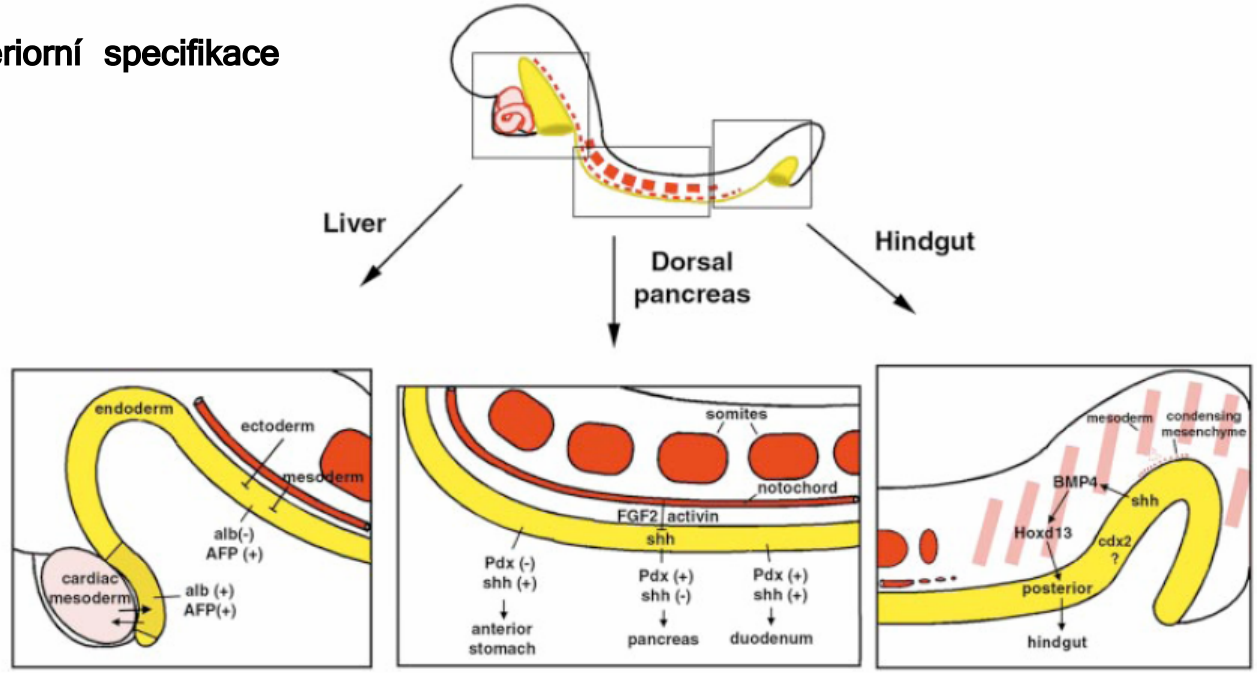
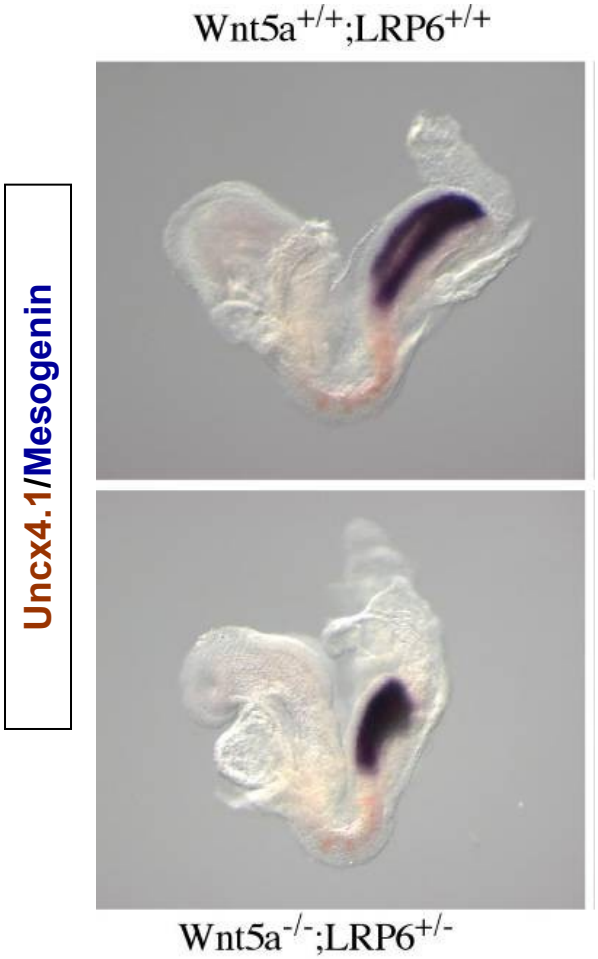


Figure 5 Signals that establish pattern in the forming gut tube. The *top panel* represents an embryo that has a notochord, somites, a foregut and a hindgut, but not a fully formed gut tube (early somite stage chick/mouse embryo). Endoderm, yellow; notochord and somites, red; and cardiac mesoderm and posterior mesoderm, pink. The boxed regions are shown schematically enlarged below. The *lower left panel* is a schematic of early hepatogenesis (liver formation) in mouse. As the anterior endoderm of the embryo folds over to form the foregut pocket, cardiac mesoderm begins to condense next to the ventral foregut endoderm. Signals arising from the cardiac mesenchyme act positively on adjacent endoderm and result in expression of the liver marker albumin. Negative signals from dorsal mesoderm and/or ectoderm act concurrently to repress albumin expression outside of the ventral foregut (liver) domain. Interestingly, ventral foregut endoderm is necessary for cardiac induction as well (arrow from endoderm to cardiac mesoderm). The *lower middle panel* depicts signals involved in pancreas formation in chick. Prepancreatic endoderm (endoderm fated to become pancreatic) responds to permissive factors from the adjacent notochord by expressing pancreatic markers. Deletion of the notochord results in loss of pancreas formation. Specifically, FGF2 and activin secreted by the notochord act to repress expression of *shh* in pancreatic endoderm, which results in pancreas marker expression. The *lower right panel* illustrates signals that pattern the hindgut in chick. *shh* expression in hindgut endoderm induces *bmp4* expression in adjacent mesoderm, which induces posterior *Hoxd13* expression in mesoderm, thus posteriorizing adjacent endoderm. At this time mesoderm is condensing into mesenchyme around the gut, which acts later to further pattern the gut tube.

Opakování: Wnt/β-cateninová dráha determinuje zadní části těla.



Klíčové morfogenetické události při specifikaci endodermální trubice.

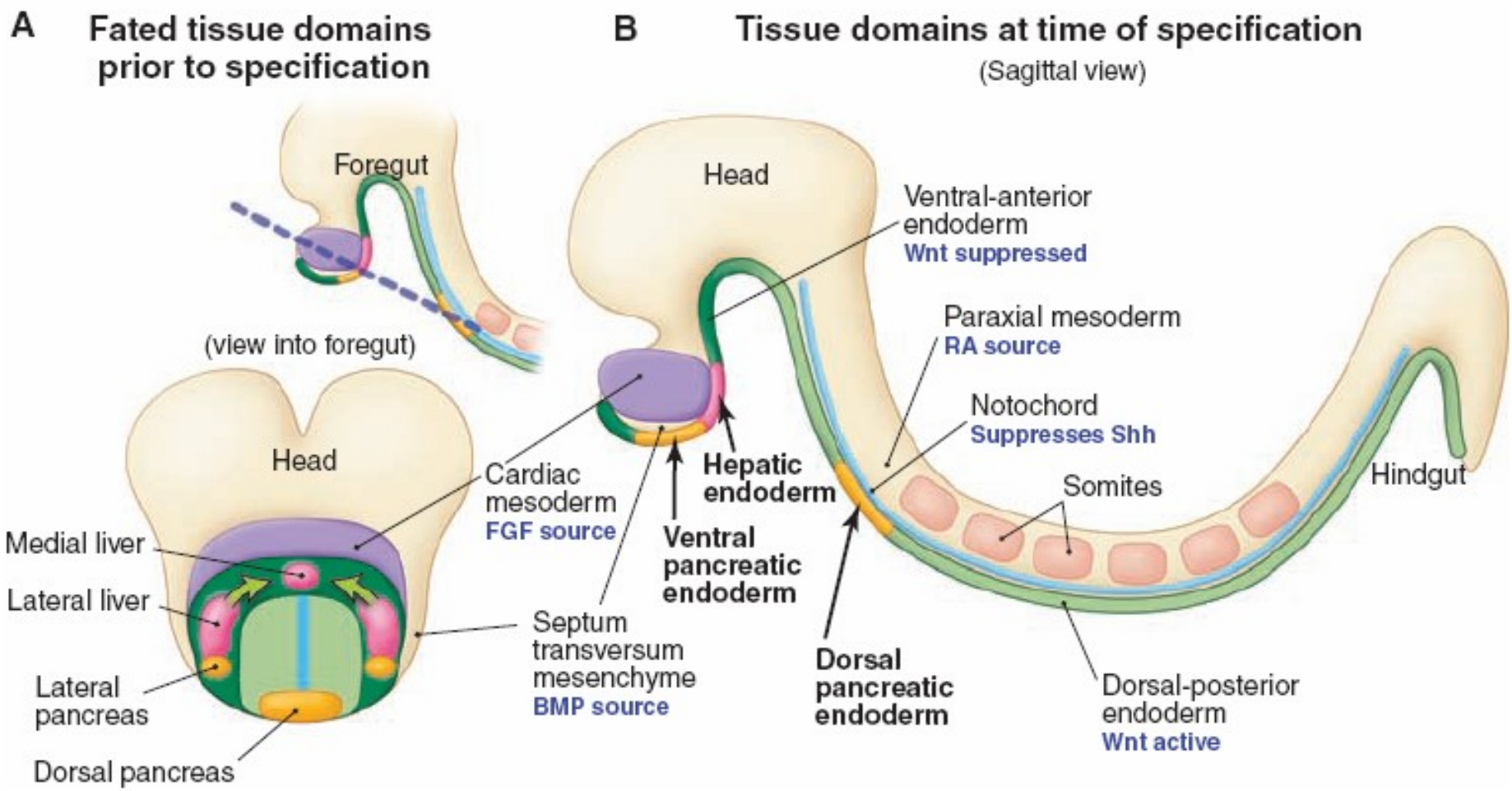
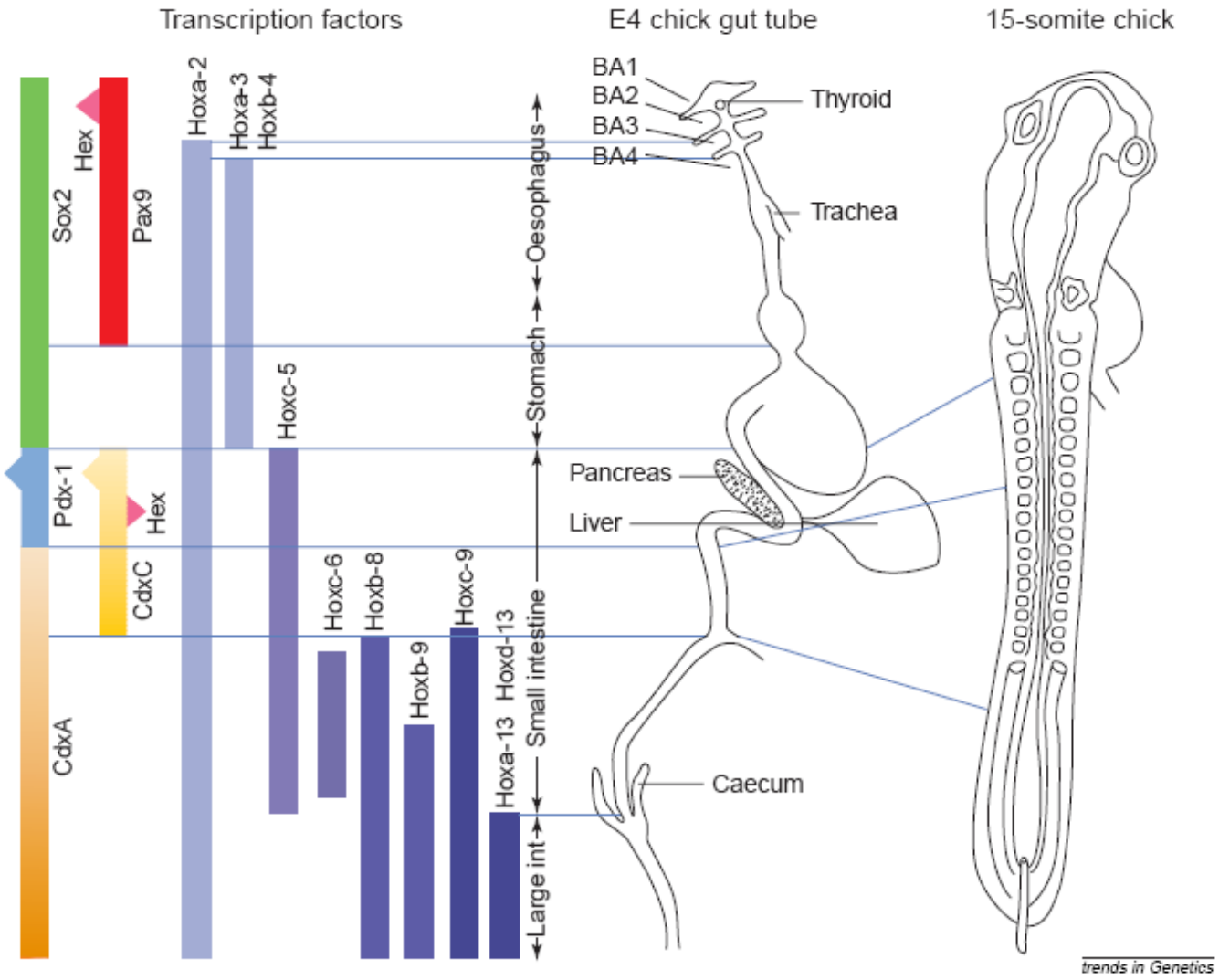


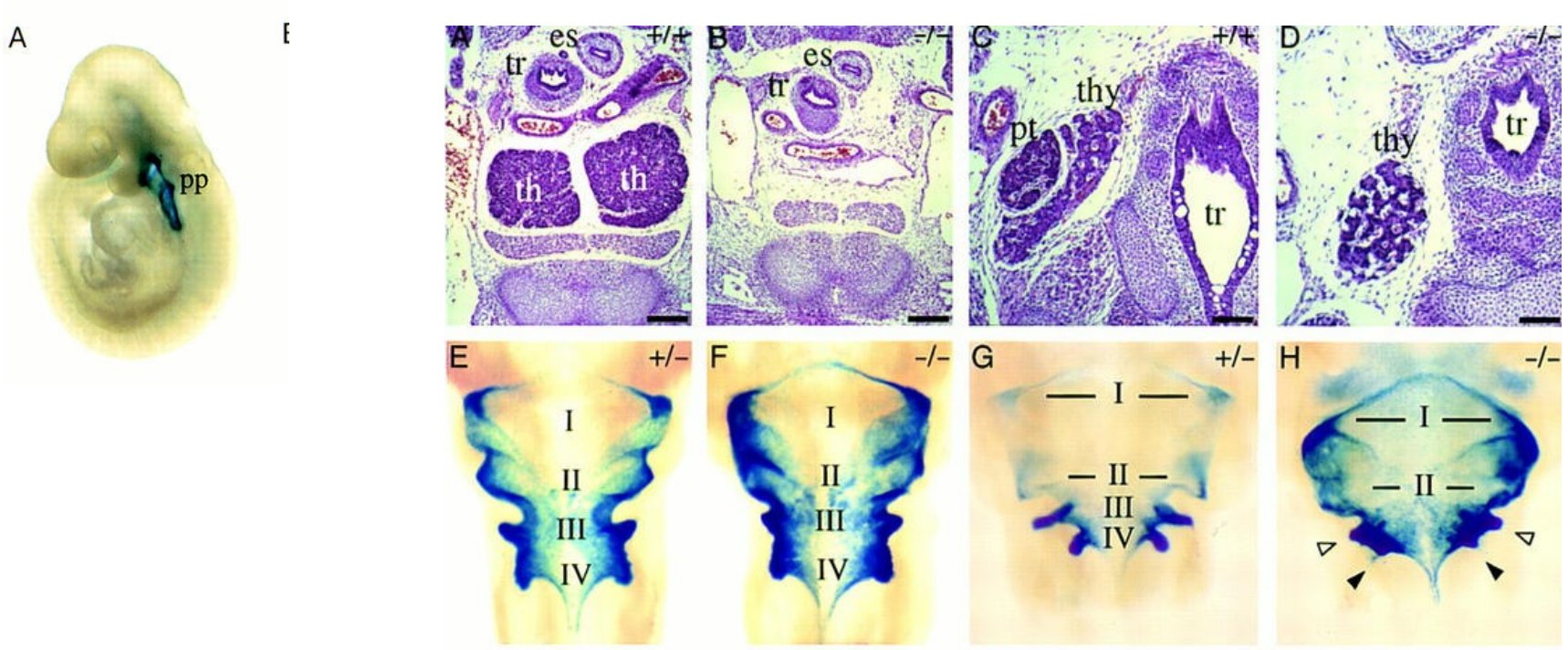
FIGURE 2. Regional expression of transcription factors in endoderm



The transcription factors shown on the left, mostly homeobox genes, are mapped to specific regions of the endoderm, as shown on the right. Although we have mapped their expression in the chicken embryo, their homologs are also expressed in a regional manner in the mouse. These genes are not only regionally expressed in already shaped organs (as shown in the E4 chicken gut tube), but also in the endodermal sheet prior to organ formation (as shown at 15-somite stage), with stable expression domains that can be used as markers of presumptive regions. The top left pink triangle shows *Hex* expression in the thyroid. The bottom left triangle refers to pancreas bud and the bottom right triangle to liver bud. BA1–4 represent branchial arches 1–4. Compiled mostly from unpublished *in situ* hybridizations on adjacent sections performed in our laboratory as well as from Ref. 71.

PŘÍKLADY KLÍČOVÝCH TRANSKRIPČNÍCH FAKTORŮ:

Pax9 that is critical for development of thymus and parathyroid



Absence of thymus, parathyroid glands, and ultimobranchial bodies in *Pax9*-deficient mice. (*A–D*) H/E-stained transverse sections of the neck region at E14.5. (*A*) Two thymic lobes (th) are present in the wild-type embryo. (*B*) At the same level, *Pax9*-deficient embryos are completely devoid of the thymus. Thymic rudiments were also not found in other regions of the neck and upper trunk (data not shown). (*C*) The parathyroid glands (pt) are attached to the thyroid gland (thy) in wild-type embryos and are absent in homozygous *Pax9lacZ* mutant embryos (*D*). (*E–J,M,N*) Ventral view of cleared whole-mount X-gal stainings of the pharyngeal pouches and their derivatives in *Pax9lacZ* mutant embryos. (*E*) In heterozygous *Pax9lacZ* mutant embryos at E10.0, *Pax9lacZ* is expressed in all four pharyngeal pouches (I–IV). (*F*) At the same stage, the third and fourth pharyngeal pouches are also present in homozygous *Pax9lacZ* mutant embryos. (

PŘÍKLADY KLÍČOVÝCH TRANSKRIPČNÍCH FAKTORŮ:

Pax9 that is critical for development of thymus and parathyroid

Nkx2.1 is essential for for thyroid and lungs

Pdx1 that is a critical regulator of pancreas development

FoxA2 which loss-of function leads to lack of development of fore- and midgut.

4. Bud formation – "pučení endodermálních orgánů"

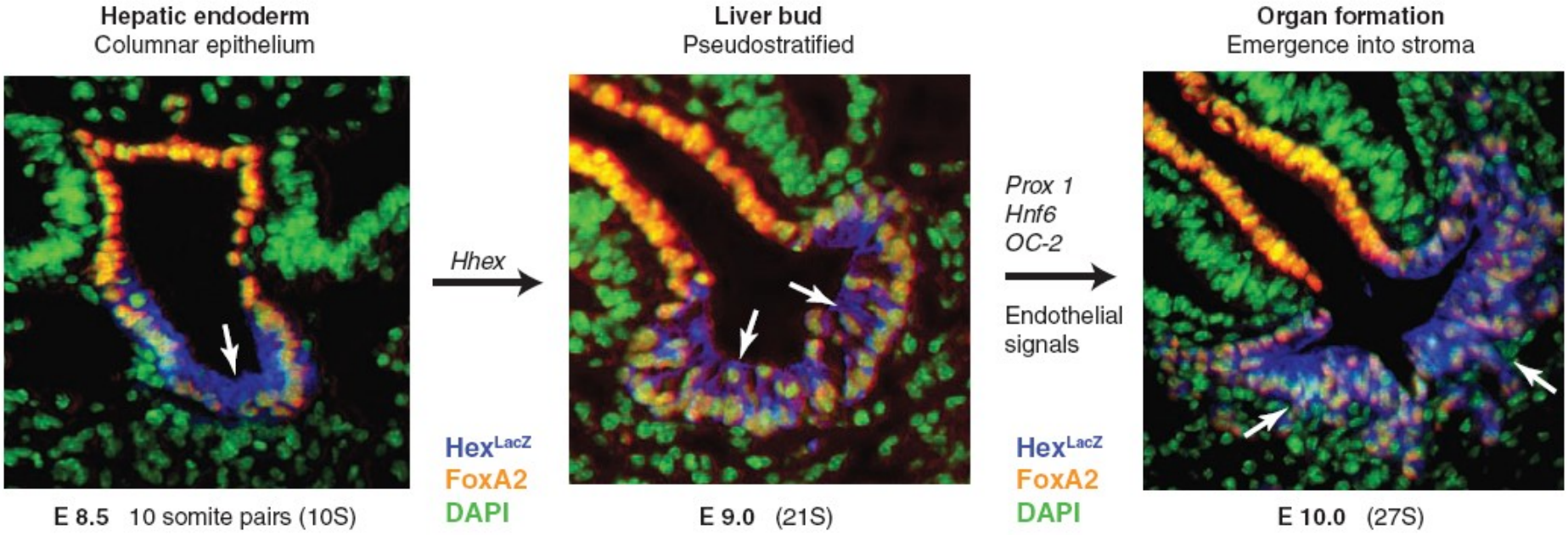
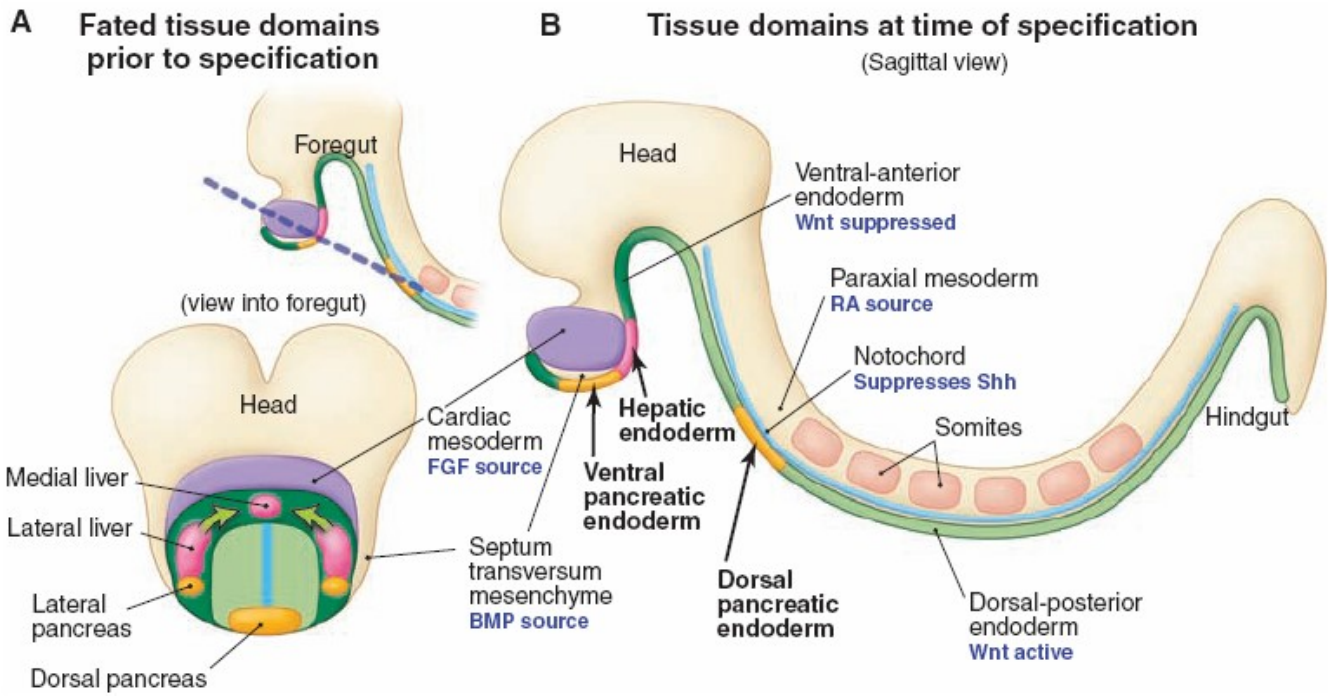
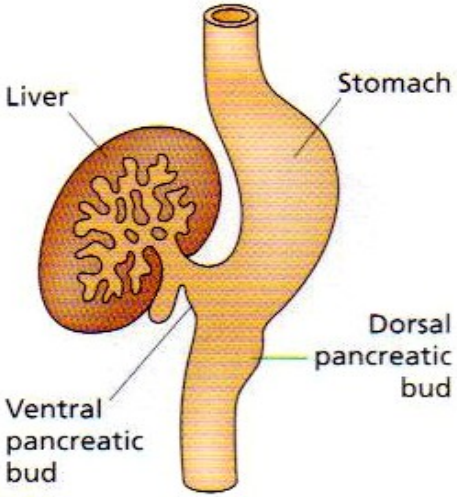


Fig. 2. Stages of liver bud organogenesis. Hepatoblasts are stained blue (Hex^{LacZ+}), cells with orange nuclei are gut endoderm ($FoxA2^{+}$), and all nuclei were stained green by 4′,6′-diamidino-2-phenylindole. White arrows point to the hepatic cells. Genes and signals that promote each transition are indicated. Similar morphogenetic stages occur during pancreas bud organogenesis. Images are adapted from (*36*). S, pair somite stage; E, embryonic day.

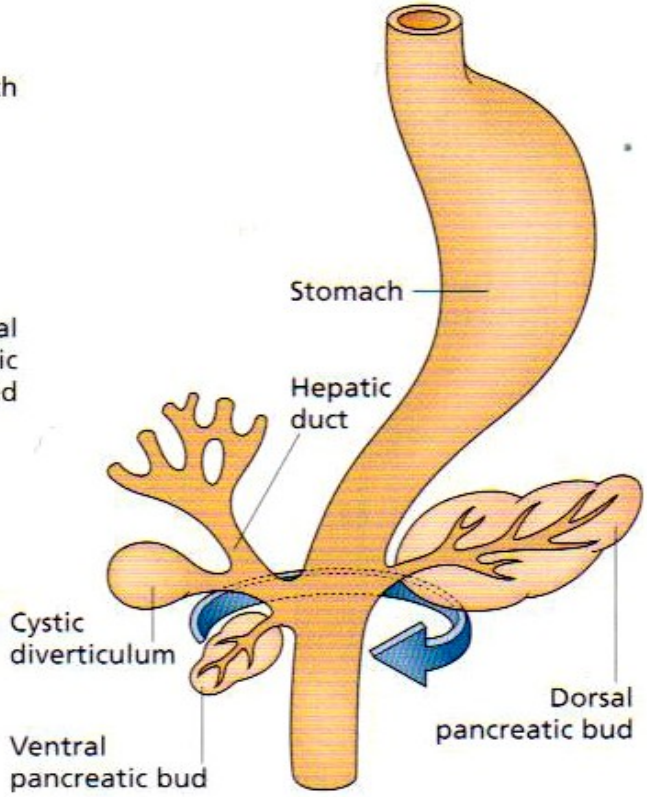
Příklad

INDUCTION OF PANCREATIC BUDS:
- both dorsal and ventral buds give rise to the same range of pancreatic cells
- dorsal bud appears where notochord contacts the gut roof. It is the region of suppression of the otherwise ubiquitous expression of **Shh** and **Indian hedgehog** (**Ihh**). The effect of notochord can be mimicked by administration of **Activin** or **FGF**
- ventral pancreas is formed from the adjacent region of the foregut floor to the liver only in the **absence** of FGF, that functions in maintaining the Shh, It thus appears that dorsal bud is induced by FGF whereas the ventral bud develops because of an absence of FGF, although the common factor is suppression of Shh expression in the endoderm. Once both buds are formed, their continued outgrowth and differentiation depends on close proximity of the pancreatic mesenchyme, that executes a **permissive effect** on bud outgrowth. A signal that carries this function appears to be **FGF10**.

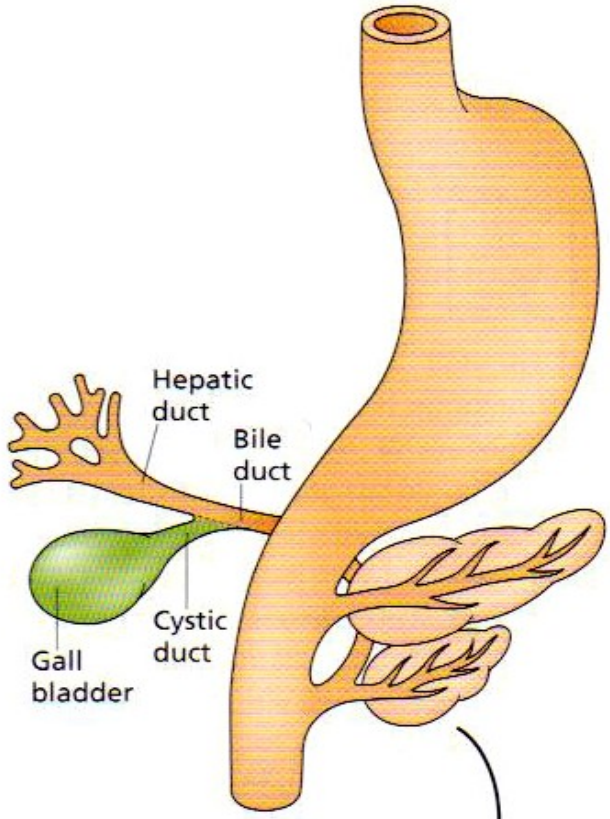




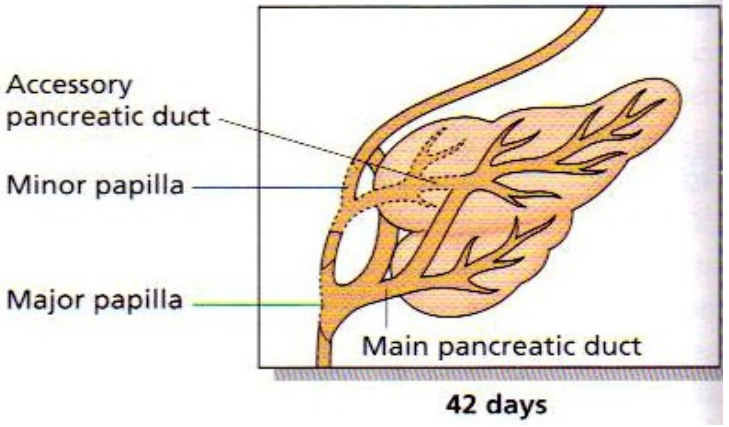
30 days



32 days



35 days



42 days

5. Diferenciace endodermálních progenitorů

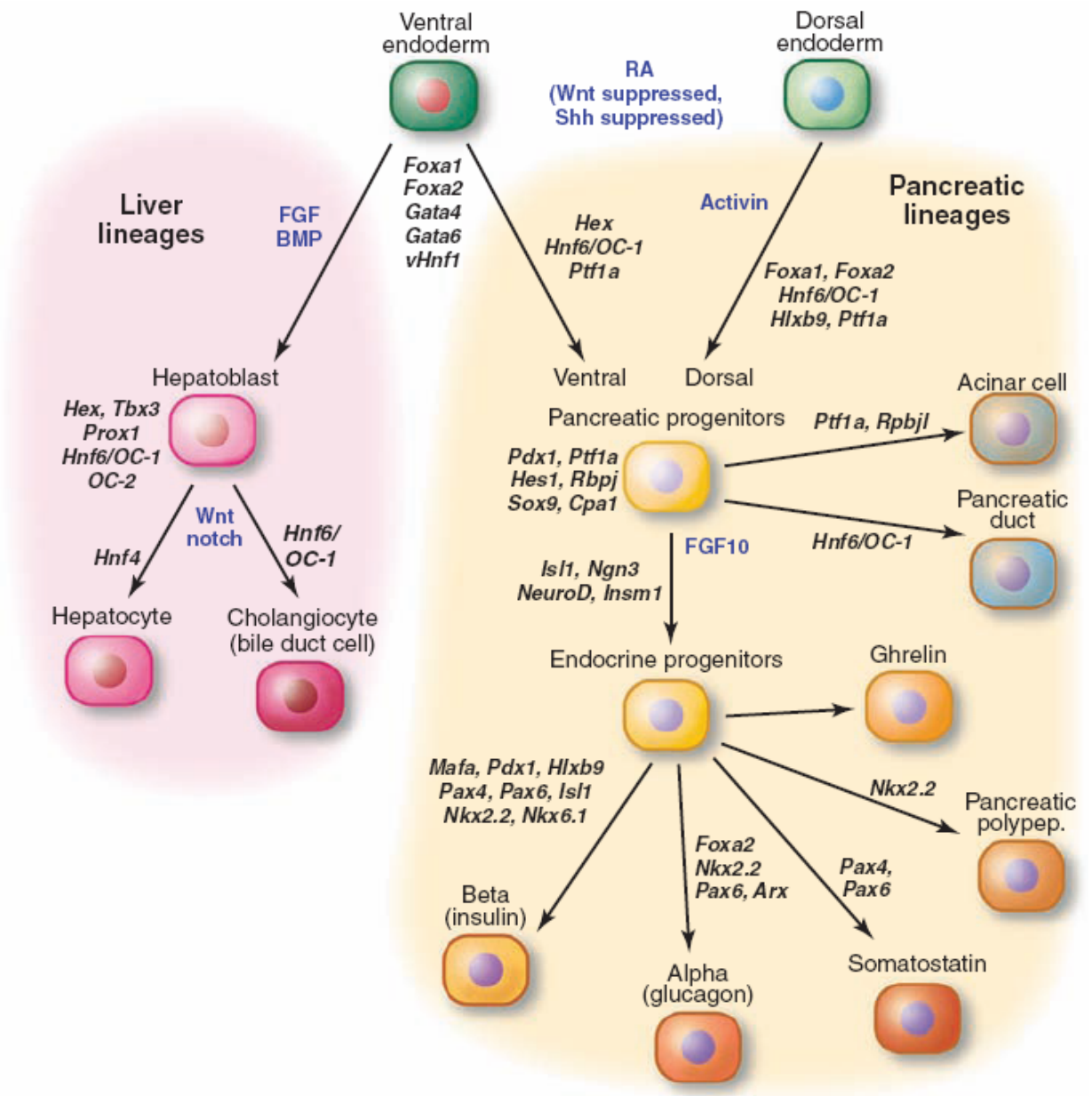


Fig. 3. Regulatory factors controlling cell type lineages within the liver and pancreas. Transcription factor genes are shown in bold; their functions have been reviewed in the text and elsewhere (*23*, *55–57*), except for *vHnf1* in hepatic development (*81*). *Pdx1* initially marks duodenum and caudal stomach progenitors (not shown) as well as the pancreatic domains (*28*).

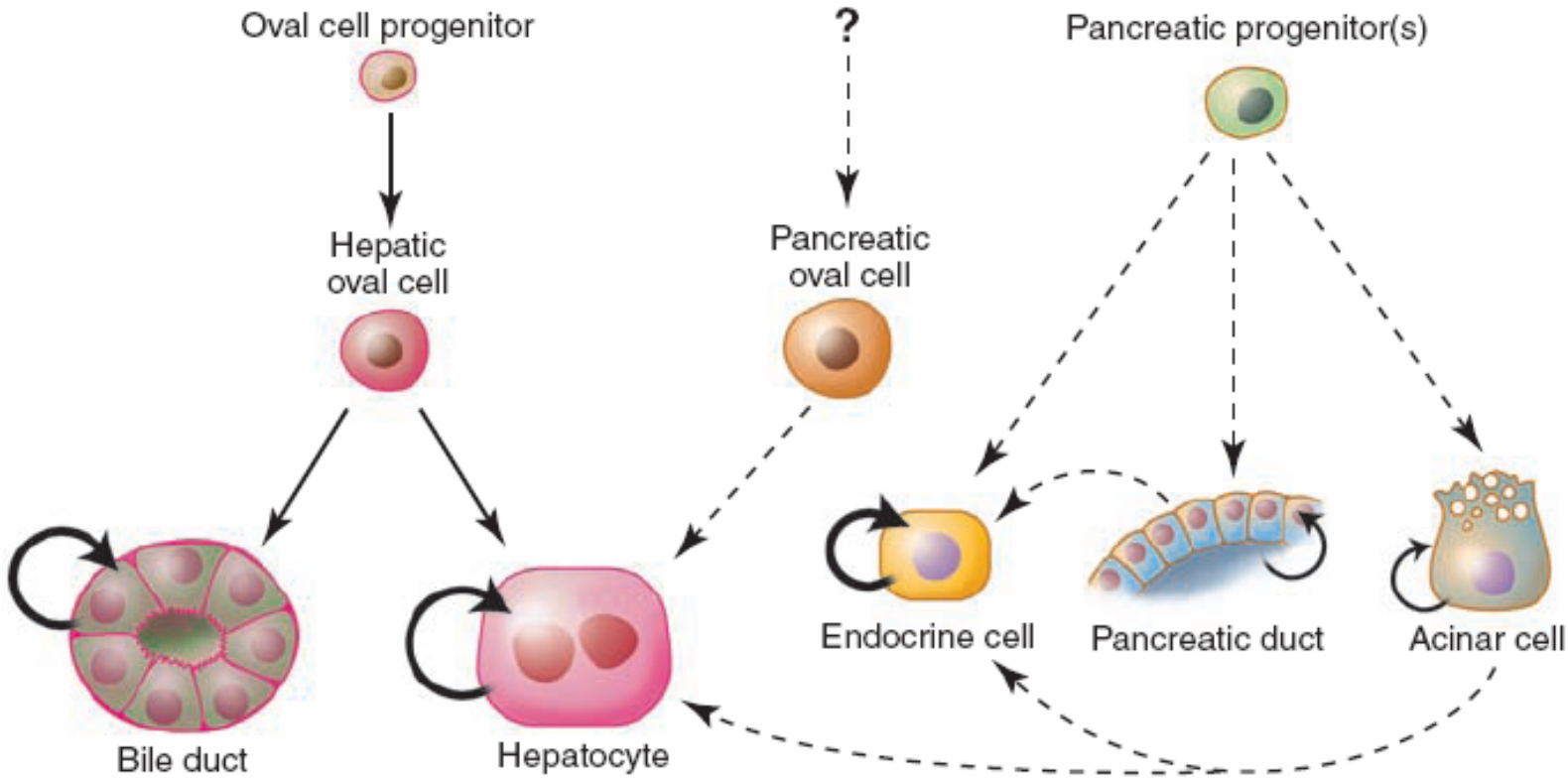


Fig. 4. Progenitor lineage relationships in adult liver and pancreas. The thickness of the arrows indicates the dominant mode of regeneration. Dashed lines delineate rare or hypothetical cell-fate transitions that occur only under specific experimental conditions.

Praktické důvody pro detailní poznání vývoje endodermu:

- příprava hepatocytů pro toxikologické studie - doposud se hepatocyty nedají kultivovat in vitro!
- cell replacement therapy - zejména pro cukrovku

Cell replacement therapy for diabetes

Some slides provided by: I. Kubíková, J. Mašek

Diabetes mellitus

- metabolická porucha – nerovnovážné hospodaření s cukry hyperglykémie
- hlavní mechanismus - působení vzájemně protichůdných hormonů ovlivňující hodnotu glykemie
- katabolické hormony - glukagon, katecholamin, tyroxin a somatostatin
- jeden anabolický hormon inzulin

Typy diabetes

ČR 800 000/svět 215 000 000

rok 2030 cca 350 milionů diabetiků

7-10% typ I/80-90% typ II

typ 1 – (IDDM)

- chronické onemocnění způsobené autoimunitní reakcí T-lymfocytů - destrukce β(B) buněk nedostatečná produkce inzulinu

typ 2 – rezistence k inzulinu (NIDDM) především blokování receptorů na inzulin– staří, obézní jedinci

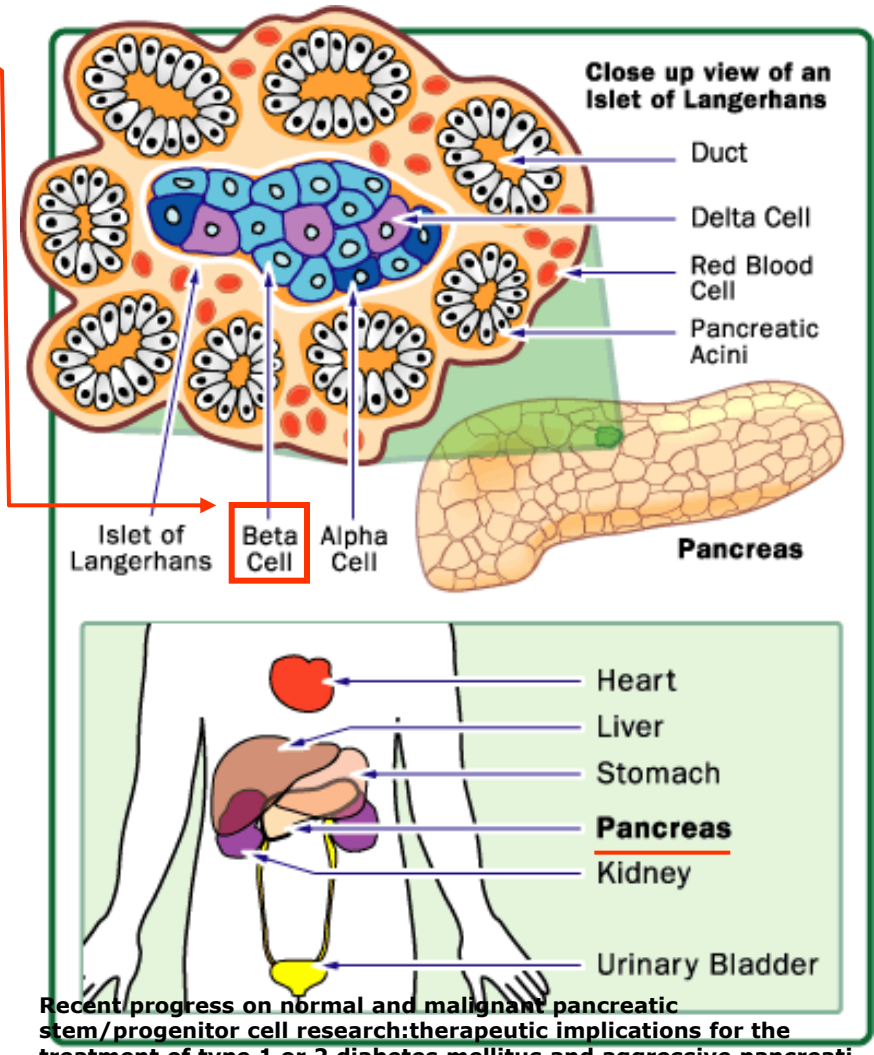
Fyziologická tvorba inzulinu - β(B) buňky

produkce inzulinu –β buňky lokalizované v Langerhansových ostrůvcích pankreatu

hlodavci – organizace b. v ostrůvku - krev protéká v pořadí β b. - α b. - δ b., dochází u nich k replikaci β b. v adultním org. - u člověka ne

ostrůvky - 100 000 - 2,5 mil. na pankreas, 50-300um - každý ostrůvek několik tisíc b.

různé typy b. - β buněk 70%, glukagon sekretující α b., somatostatin sekretující δ b. a PP (pankreatický polypeptid) sekretující b.



Recent progress on normal and malignant pancreatic stem/progenitor cell research:therapeutic implications for the treatment of type 1 or 2 diabetes mellitus and aggressive pancreaticcancerM Mimeault and S K Batra *Gut* 2008;57;1456-1468

1921 příprava čistého inzulinu

typ 1 – injikace inzulinu brání projevům choroby – pumpa, pero, injekční stříkačka

typ 2 – kombinace cvičení, diety, dodávání inzulinu, některých nepřímých léčiv; střevní bypass - normalizuje hladiny glukózy v krvi u 80-100% obézních pacientů

Transplantační terapie

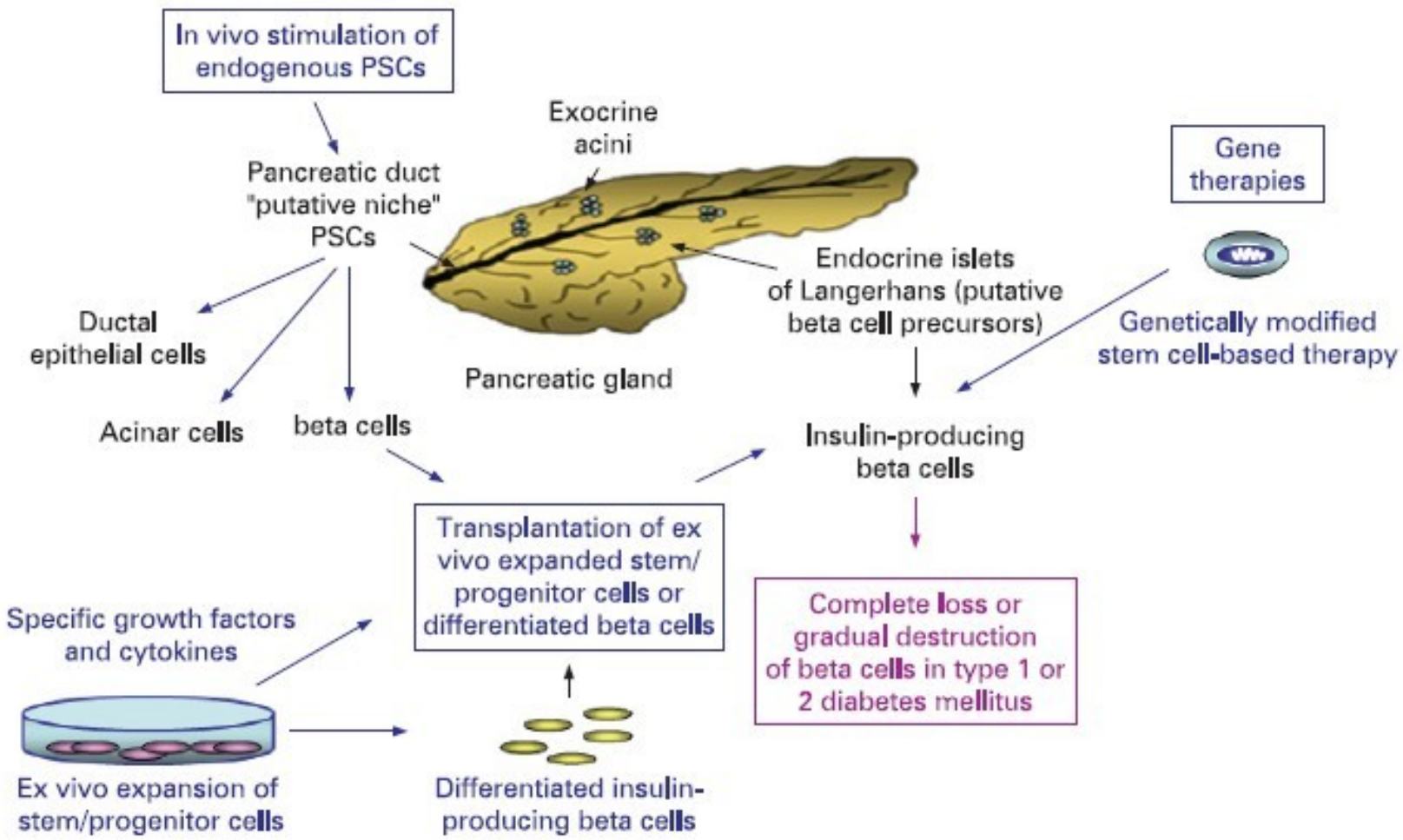
transplantace celé slinivky nebo Langerhansových ostr. x transplantace β buněk

Tzv. Edmonton protocol

po 5 letech 85 % z pacientů opět na nižších dávkách inzulinu

časté odvržení štěpu

Typy buněčných terapií



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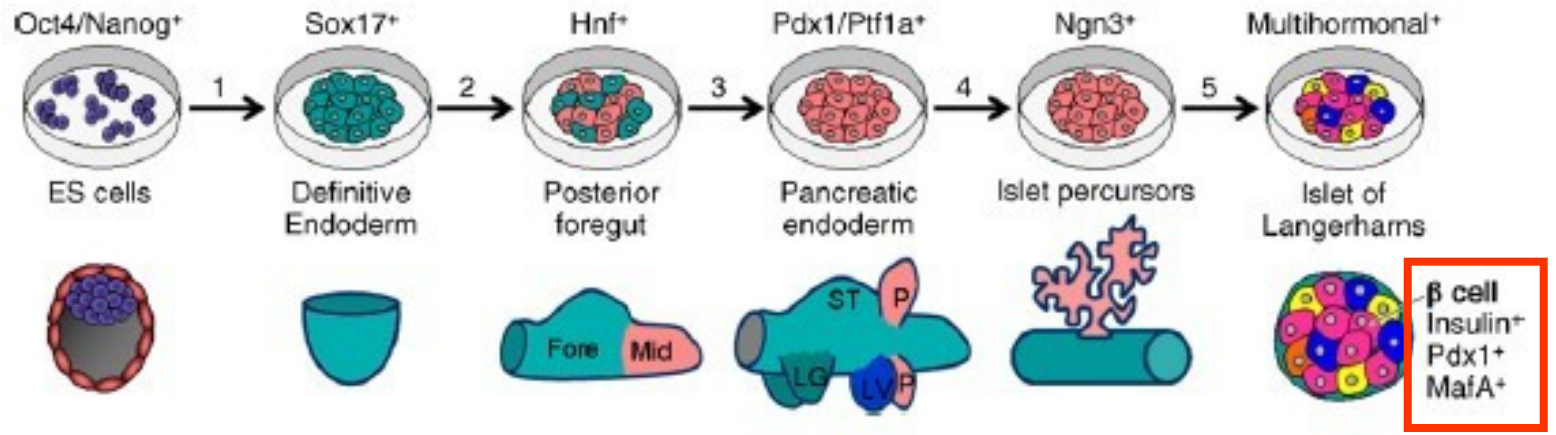
Diferenciace z ESC/iPSC

K. Docherty et al. / Seminars in Cell & Developmental Biology 18 (2007) 827–838



Artifactual Insulin Release From Differentiated Embryonic Stem Cells

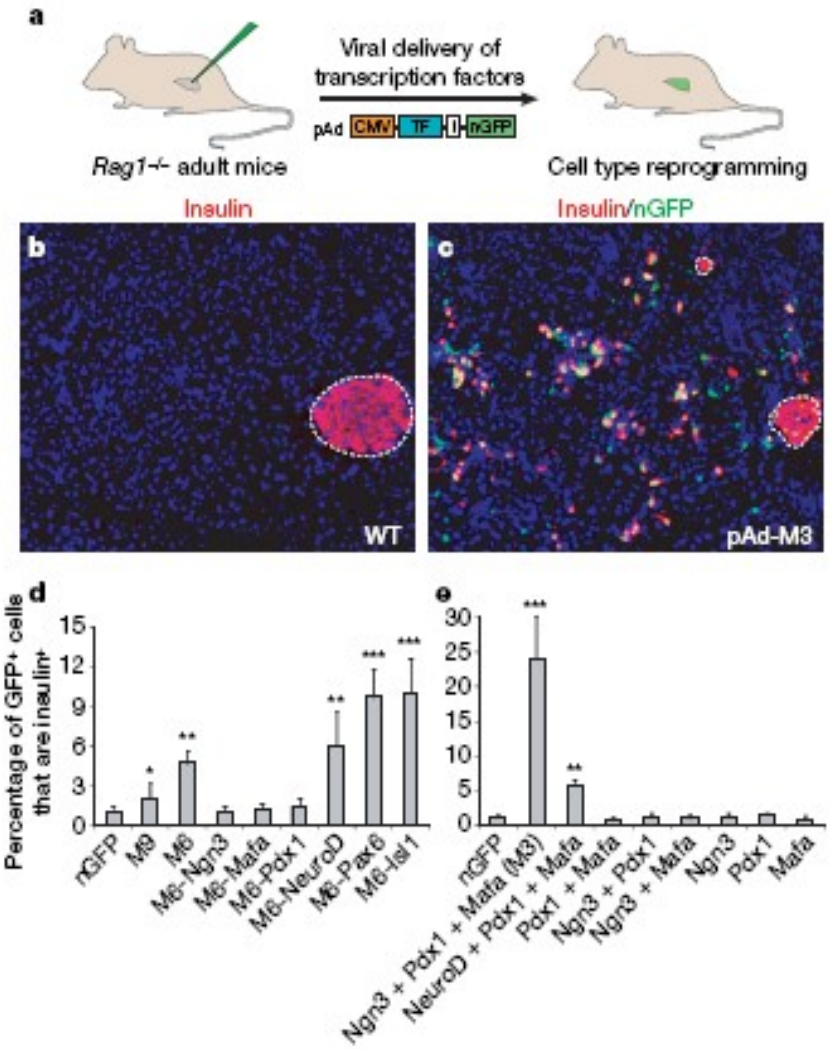
Mattias Hansson,[1] Anna Tonning,[2] Ulrik Frandsen,[1] Andreas Petri,[1,3] Jayaraj Rajagopal,[4] Mikael C.O. Englund,[5] R. Scott Heller,[1] Joakim Håkansson,[2] Jan Fleckner,[3] Helen Nilsson Sköld,[2] Douglas Melton,[4] Henrik Semb,[2] and Palle Serup[1] DIABETES, VOL. 53, OCTOBER 2004

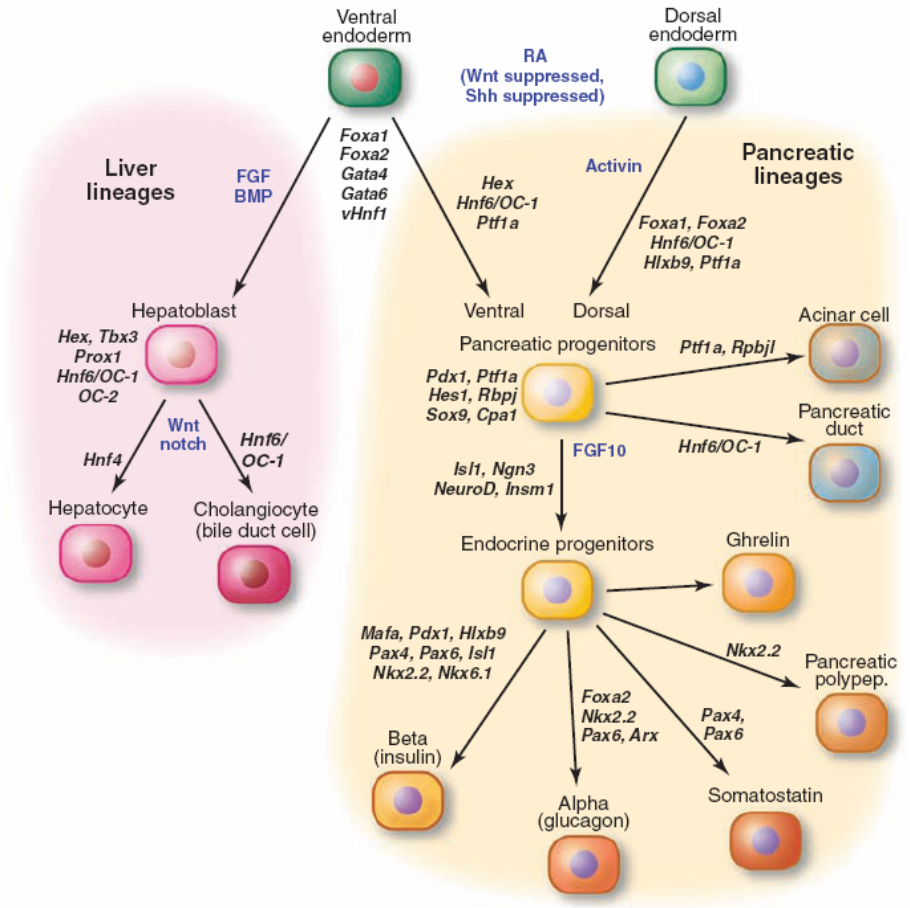
Vol 455|2 October 2008|doi:10.1038/nature07314 nature

ARTICLES

In vivo reprogramming of adult pancreatic exocrine cells to β-cells

Qiao Zhou[1], Juliana Brown[2], Andrew Kanarek[1], Jayaraj Rajagopal[1] & Douglas A. Melton[1]





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Neurogenin3 Is Sufficient for Transdetermination of Hepatic Progenitor Cells into Neo-Islets In Vivo but Not Transdifferentiation of Hepatocytes

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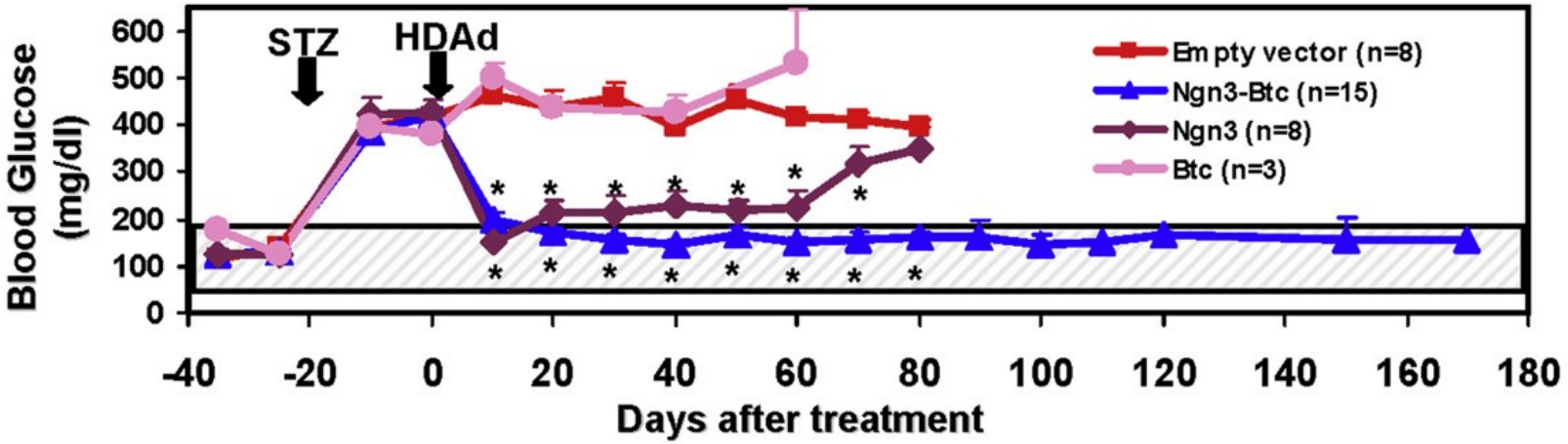
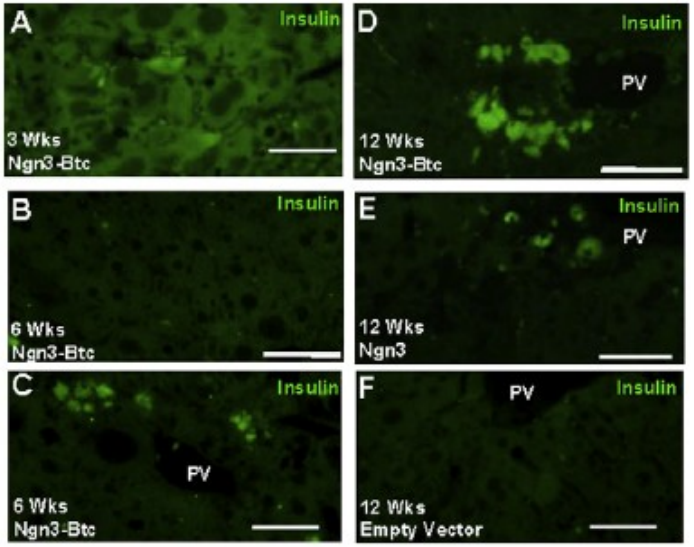
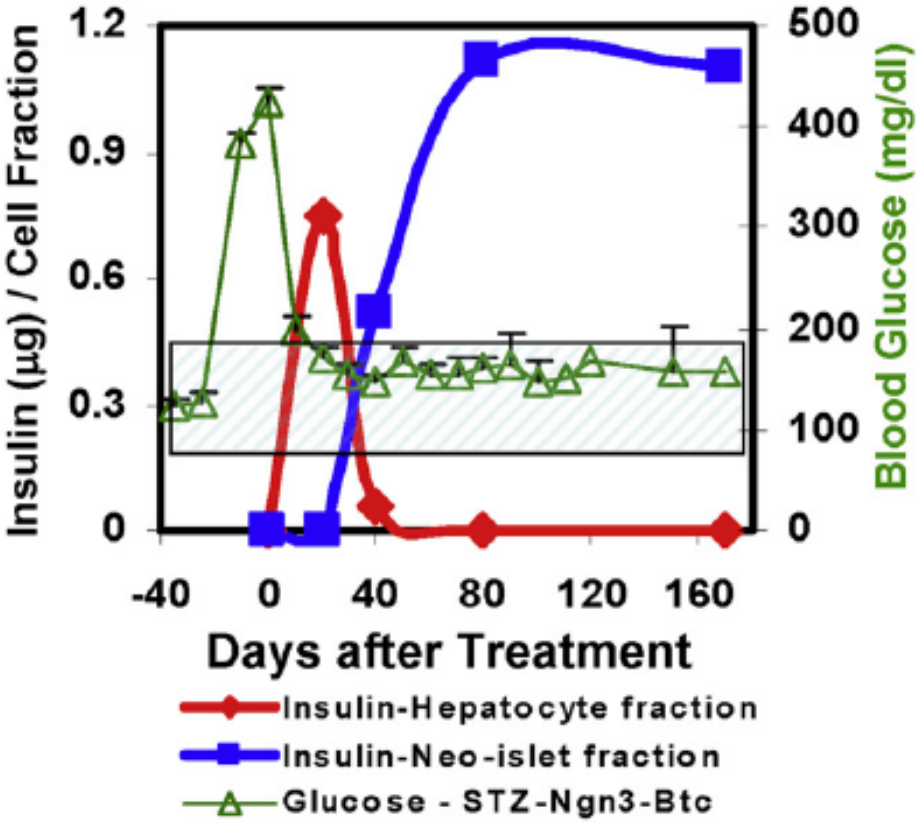


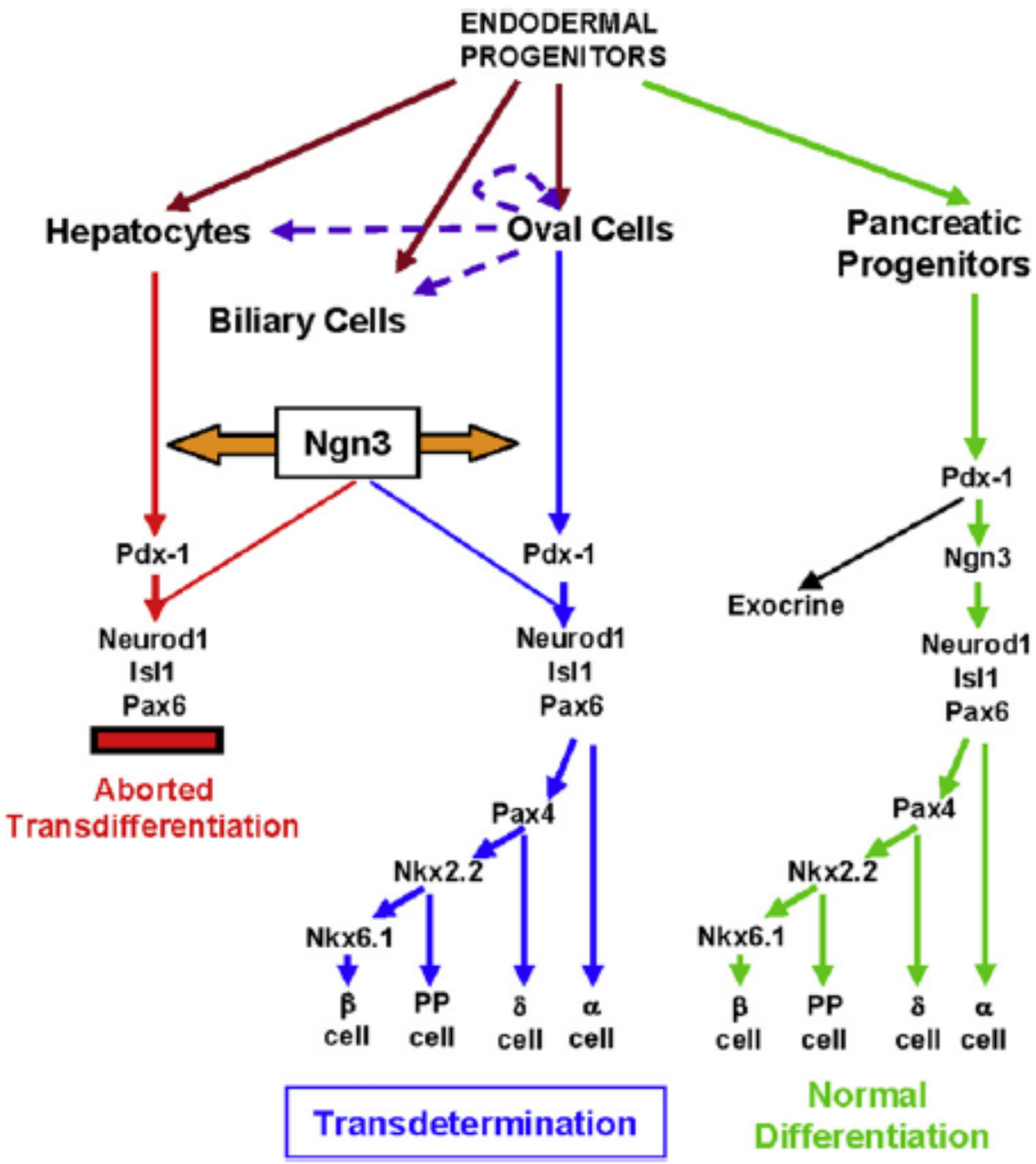
Btc – betacellulin

(A–F) Representative sections of STZ-diabetic mouse liver stained for insulin (green) by immunofluorescence (IF).





(C) Estimated insulin content of the parenchymal hepatocyte and the neo-islet fractions (left *y* axis) and the blood glucose of the Ngn3-Btc-treated group (right *y* axis) and normal range (hatched area) (from Figure 1A; all values are mean ± SEM). Note the concordance of the estimated insulin content (upper panel) with the insulin immunostaining predominantly in the parenchymal hepatocytes at 3 weeks and in the periportal neo-islets at 12 weeks (lower panels).



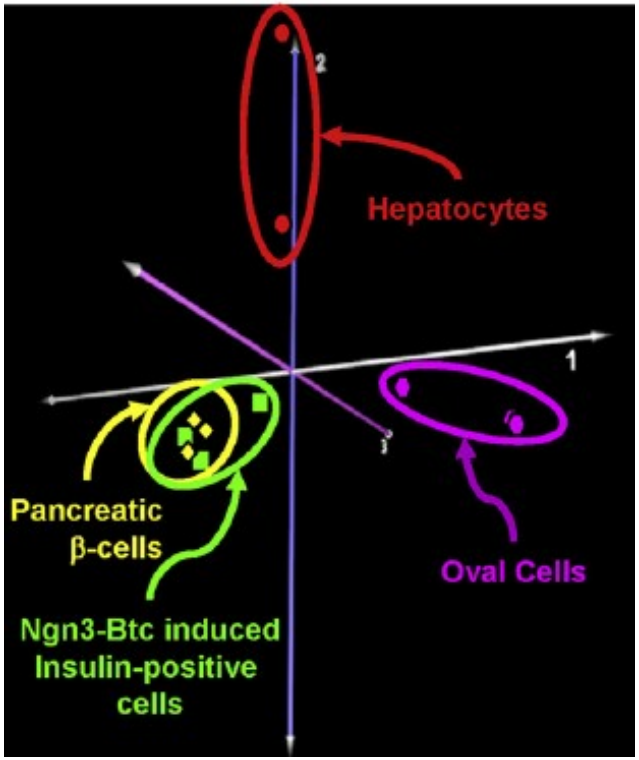


Figure 8. Model for Ngn3-Induced Transdetermination of Hepatic Oval Cells

Ngn3 induces transdetermination in oval cells (in blue) and an aborted transdifferentiation in Ngn3-transduced parenchymal hepatocytes (in red). The normal islet differentiation cascade is also shown (in green). Normally, oval cells give rise to hepatocytes and biliary cells (interrupted lines). The origin of hepatocytes and biliary cells from the common endodermal progenitors is also shown (in brown).

Praktické důvody pro detailní poznání vývoje endodermu:

- příprava hepatocytů pro toxikologické studie - doposud se hepatocyty nedají kultivovat in vitro!
- cell replacement therapy - zejména pro cukrovku
- pochopení vzniku a progrese nádorů (zejména tlustého střeva, plic, jater a slinivky břišní) - dohromady zodpovědné za více než 60 % úmrtí na nádorová onemocnění

Figure 1.1: The 20 most common causes of death from cancer, UK, 2006