ENDODERM AND EPITHEL STEM CELLS

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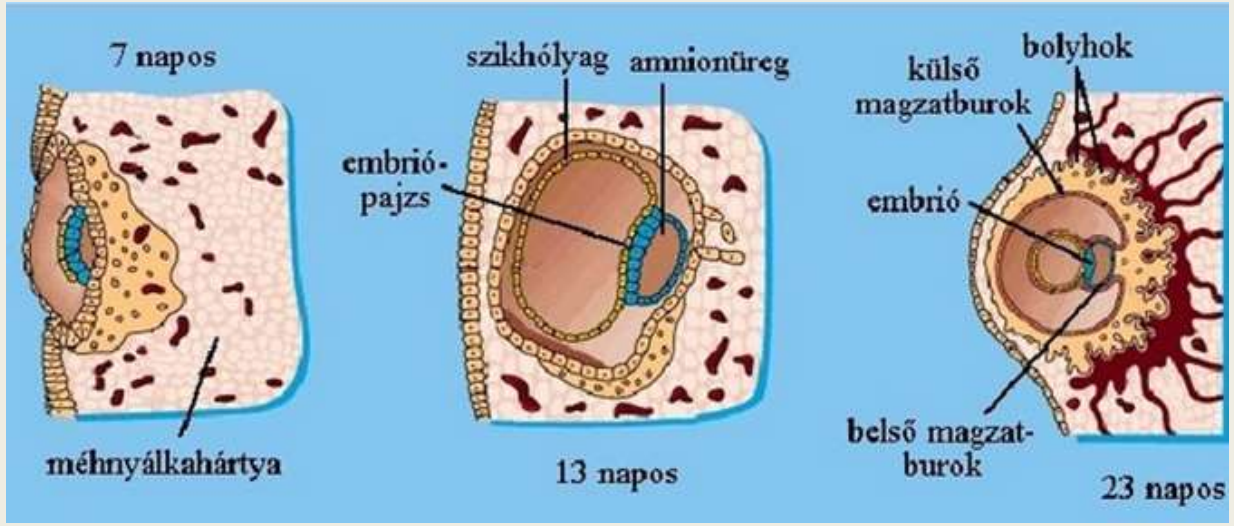
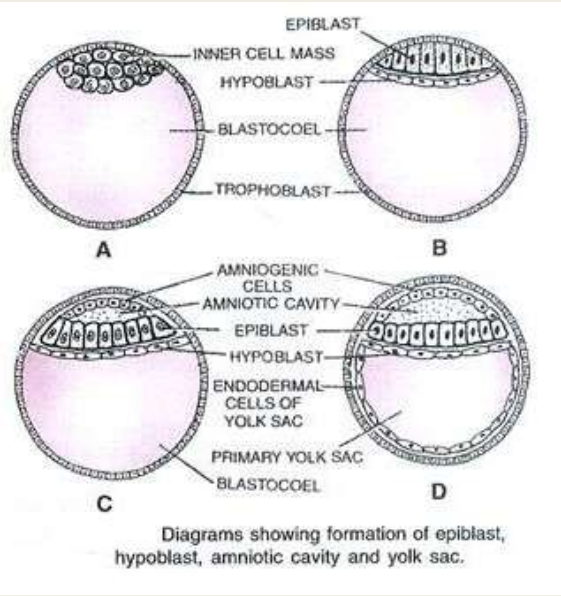
Primer tissues

Let’s start embryogenesis!





Diagrams showing formation of epiblast, hypoblast, amniotic cavity and yolk sac.

Formation of Embryonic Disc:

We have seen that early blastocyst consists of inner cell mass and trophoblast. The inner cell mass contains cells called stem cells which have the potency to give rise to all tissues and organs. The cells of the inner cell mass differentiate into two layers around 8 days after fertilization, a hypoblast and epiblast.

The hypoblast (primitive endoderm) is a layer of columnar cells and epiblast (primitive ectoderm) is a layer of cuboidal cells. The cells of the hypoblast and epiblast together form a two layered embryonic disc.

Formation of Amniotic Cavity:

A space appears between epiblast and trophoblast, called amniotic cavity filled with amniotic fluid. The roof of this cavity is formed by amniogenic cells derived from the trophoblast, while its floor is formed by the epiblast.

- After the blastocyst stadium, the embryo consists two germ layers, and it consists essentially of two epithelial cell layers: the dorsal, single-layer epithelium is the epiblast layer, and the ventrally located single-layer epithelial-like hypoblast cells. So, the earliest type of embryonic tissue is the epithelium.
- With gastrulation, another important type of tissue in the early embryo is the mesenchym.
- The three germ layers embryo (germinal disc) consists of the epiblast / ectoderm, under that the connective tissue cells of the mesoderm, and at the third layer is the monolayer of the endoderm.
- For a long time, the embryo has these two types of relatively undifferentiated tissues: the epithelium and the earliest connective tissue..

"It is not birth, marriage, or death, but gastrulation, which is truly the most important time in your life." Lewis Wolpert (1986)

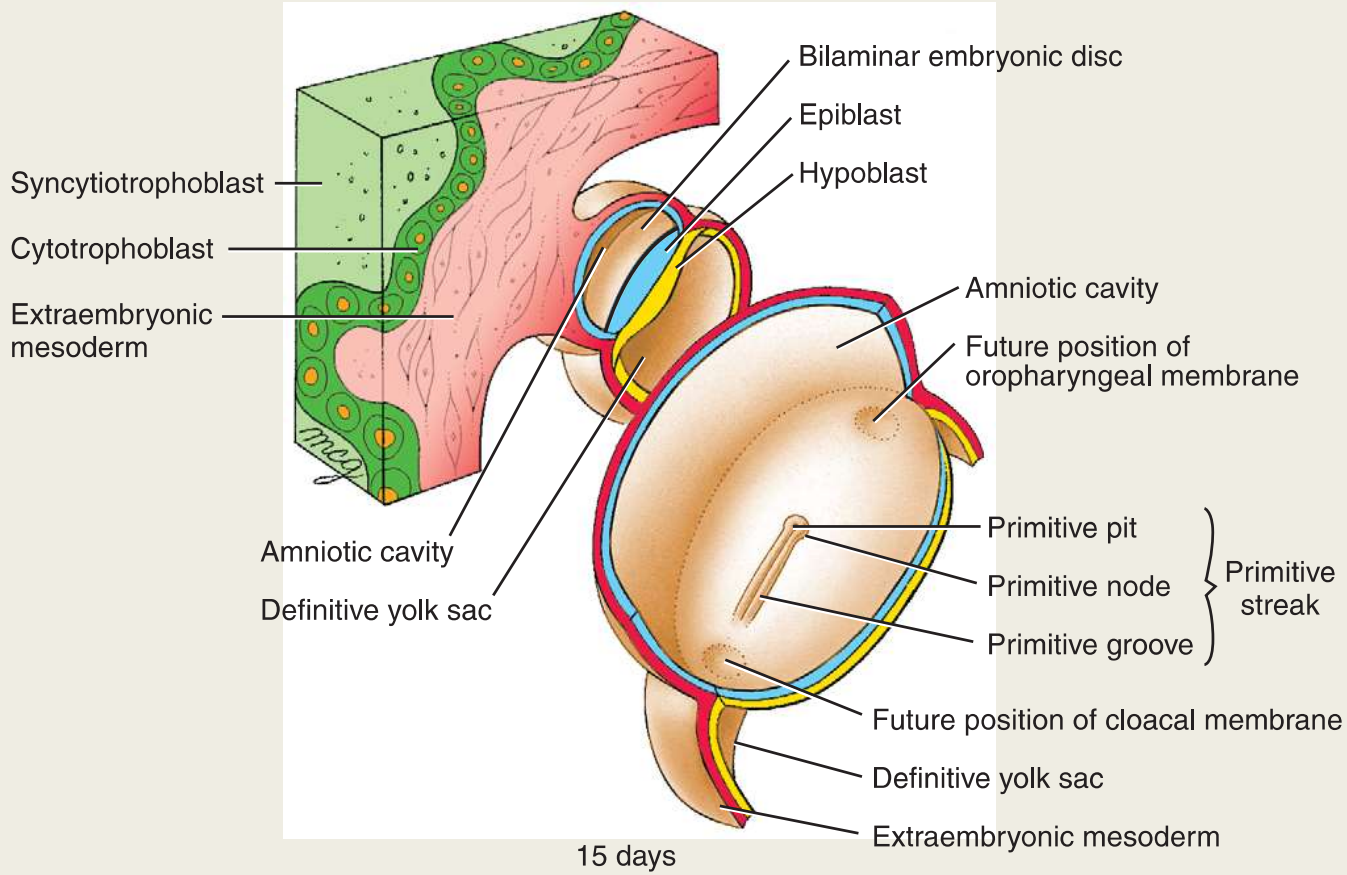
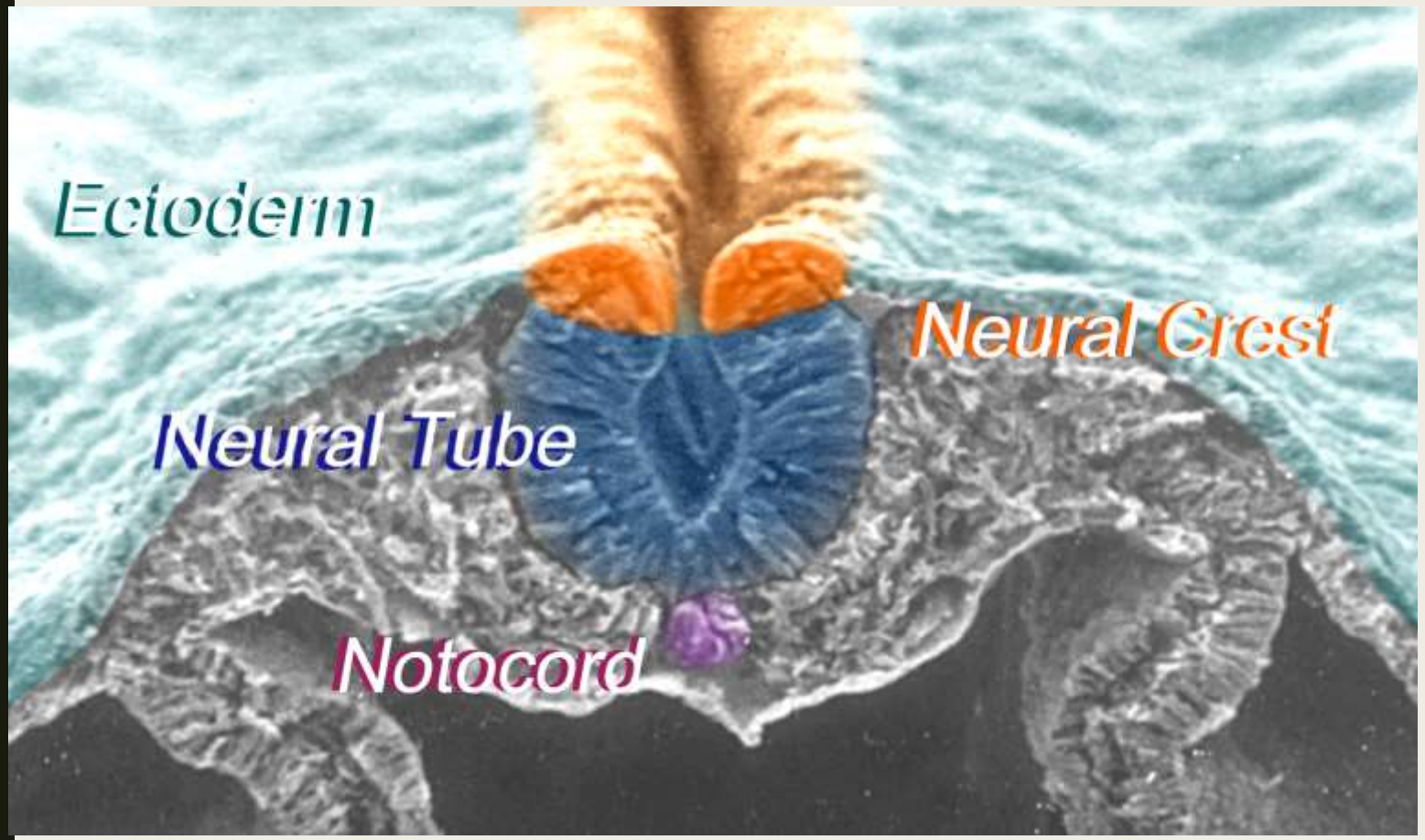
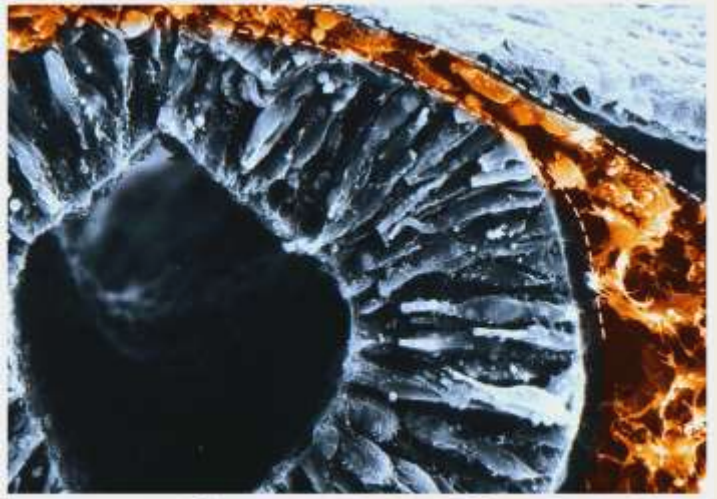
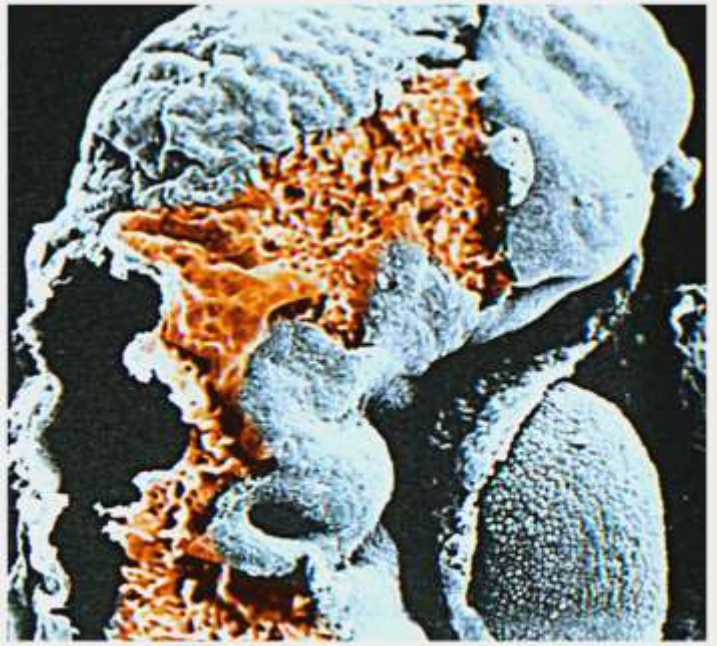


Figure 3-1. View of dorsal surface of bilaminar embryonic disc through sectioned amnion and yolk sac. Inset at upper left shows relation of the embryo to the wall of the chorionic cavity. The primitive streak, now 1 day old, occupies 50% of the length of the embryonic disc. The future positions of oropharyngeal and cloacal membranes are indicated.


Ectoderm
Neural Crest
Neural Tube
Notocord





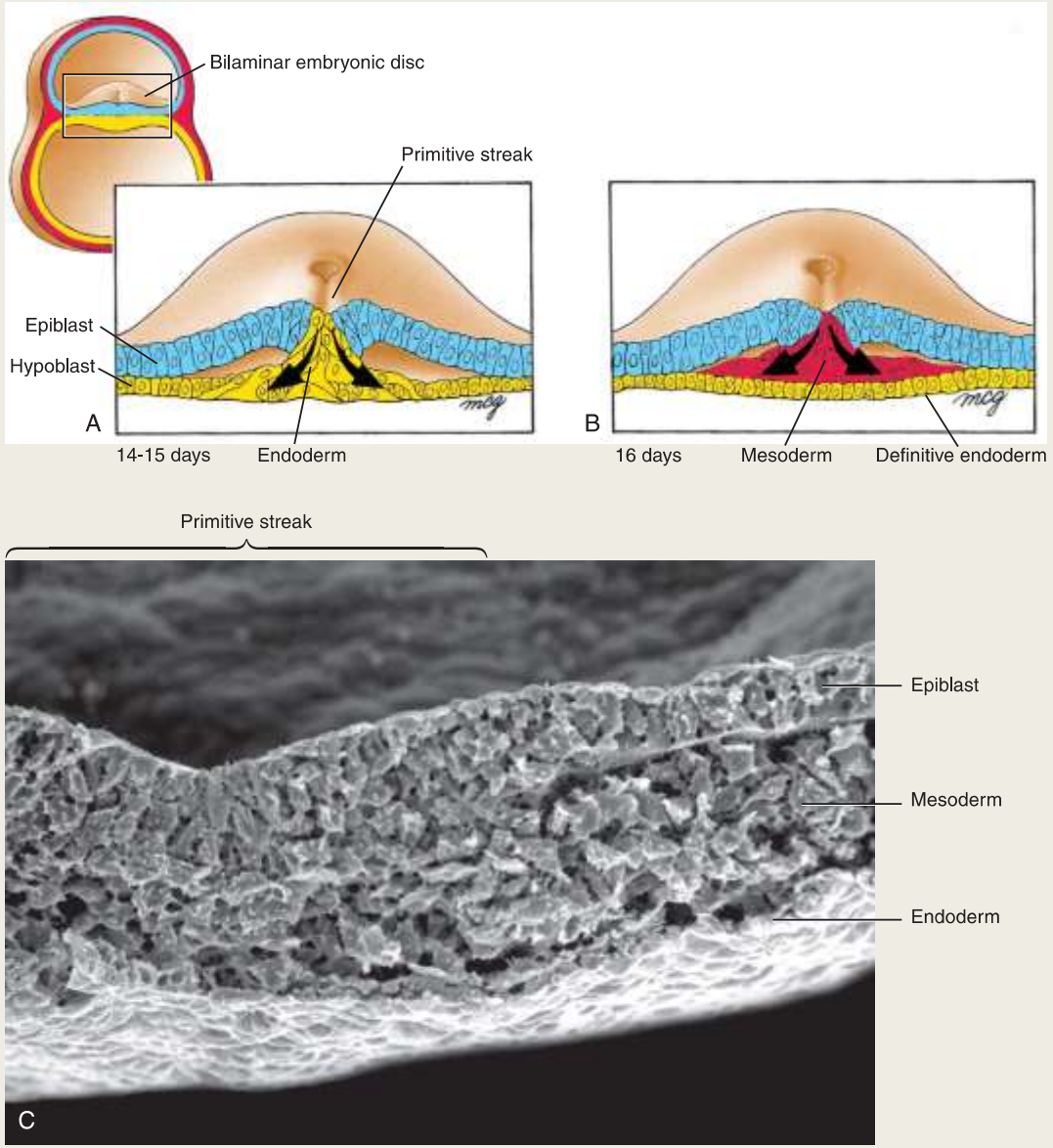


Figure 3-6. Embryonic discs sectioned through the region of primitive streak, showing ingression of epiblast cells during gastrulation. *A*, On days 14 and 15, ingressing epiblast cells displace hypoblast and form definitive endoderm. *B*, Epiblast that ingresses on day 16 migrates between endoderm and epiblast layers to form intraembryonic mesoderm. *C*, Scanning electron micrograph of a cross section through the chick primitive streak.

Primary tissues

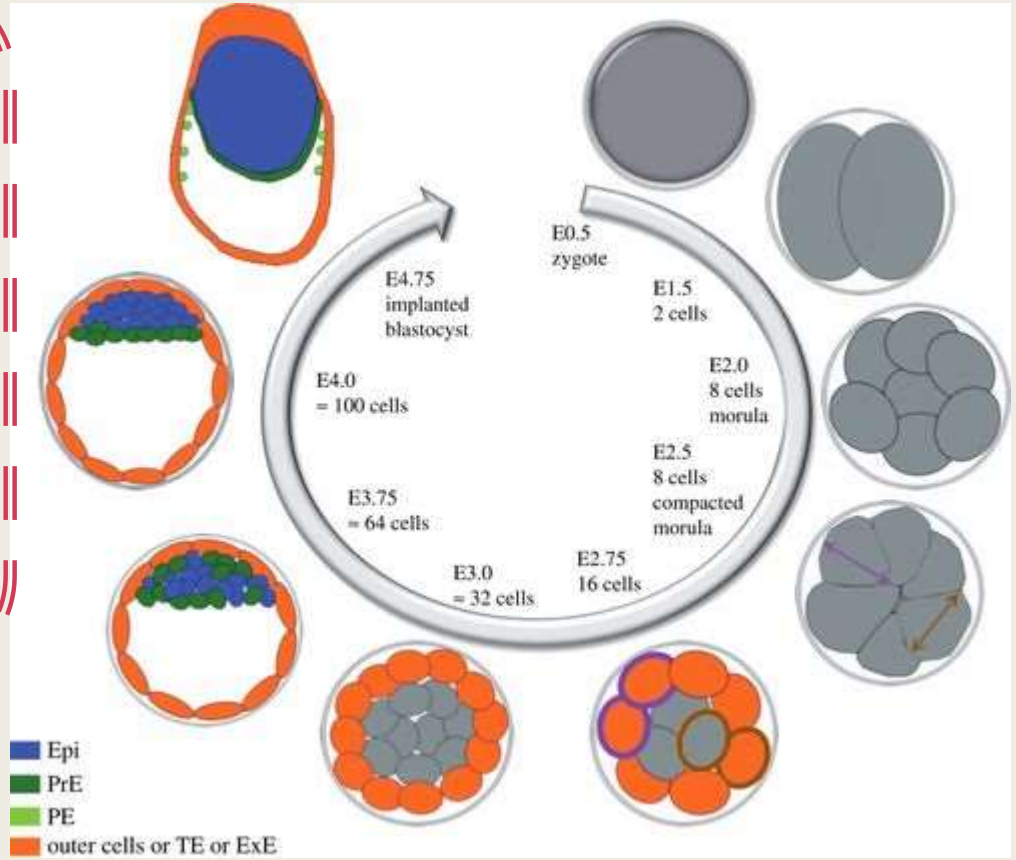
- The earliest connective tissue of the embryo is the mesenchym. (up to the end of the embryonic age, up to the end of 2th month) These cells are embedded in ECM, actively migrate and can differentiate in a wide spectrum. (pluripotency). These mesenchymal cells can differentiate not only to connective tissues, but also bone, cartilage, adipose tissue, muscle tissue.
- The term mesenchymal refers primarily to the shape and differentiation property (see above), not to from which germinal layer is derived: mesenchymal is usually a derivative of mesoderm but not always! Mesenchymal cells are also derived from the neural crest.
- mesoderm differentiates not only to mesenchym but also epithelum, e.g. the epithelium of the kidneys.
- Ergo: the mesenchym and the mesoderm are not synonymous!

Primary tissues develop to each other

- The first type of tissue in the embryo is therefore the epithelium.
- During the gastrulation cells migrate from this tissue the mesenchymal cells
- Thus, during the gastrulation, epithelial cells lose their epithelial properties (closely related cells: with cell-cell connections, no ECM, no migrating phenotype) and become mobile, individual ECM-producing cells.
- This process is the **epithelio-mesenchymal transformation** (**EMT)**.
- EMT is not uncommon in early embryos: not only the gastrulation is an EMT, but also the outflow of neural crest cells from the ectoderm (see neurulation) or from somites (epithelial somite ball), sclerotom cells, myotom cells, dermato cells.
- Other times, the reverse of this process is observed: stellate, migrating cells become epithelial cells: **mesenchymal-epithelial transformation, MET**. We will see this at the kidney develops.

The core molecular regulatory network for primitive endoderm versus epiblast cell specification

The early ICM progenitors co-express Nanog and Gata6 before acquiring distinct identities cell by cell.



Epi and PrE cells specify within the ICM in an apparent random salt and pepper pattern.

https://royalsocietypublishing.org/doi/full/10.1098/rstb.2013.0537

expression of Nanog and Gata6 depends on FGF4 expression

the Erk pathway inhibits PrE specification while promoting Epi identity, visualized by Nanog expression in all ICM cells

FGF4 administration induces PrE cells, at the expense of the Epi cells. The ICM precursor has a binary fate choice, which is dependent on low or high Erk activity leading either to an Epi or to a PrE identity, respectively.

Grabarek *et al.* showed that plasticity is lost in all ICM cells only after the late blastocyst stage.

the expression of Nanog and Gata6 in an exclusive manner is not sufficient to lock the cell identity

Nanog requirements for epiblast specification

Several groups have analysed *Nanog*$^{-/-}$ embryos [24–27], and showed that the first role of Nanog is to specify Epi.

Indeed, in *Nanog*$^{-/-}$ embryos, all ICM cells express Gata6. This also confirms that Nanog represses Gata6 expression *in vivo*.

The ICM marker Oct4 and the trophectoderm marker Cdx2 are correctly expressed in these mutants, demonstrating that cell specification between ICM and trophectoderm occurs properly.

Fgf4 was shown to be expressed specifically in Epi precursor cells of wild-type embryos

Fluorescent *in situ* hybridization analyses showed that this specific Epi expression disappears in *Nanog* mutants, strongly suggesting that *Fgf4* expression is induced by Nanog

This indicates that in *Fgf4*$^{-/-}$ embryos, the decay of Gata6 expression is not directly due to the absence of FGF4, but is rather the consequence of Nanog high expression that inhibits that of Gata6.

While Gata6 induction of expression is not impaired before the 8-cell stage in the absence of FGF4, it is inhibited when FGFR and Mek activities are blocked at an early time point (before compaction) in a *Nanog* mutant context

another RTK ligand is active early on to induce Gata6 expression, even if *Fgf4* is expressed at these early stages. Thus, another RTK must be active to induce Gata6 expression.

there are two consecutive phases of Gata6 expression, first induced through an unknown RTK activation and then maintained independently of the direct RTK/FGF4 signalling.

Gata6 requirements for primitive endoderm specification

Gata6 mutant ES cells are unable to differentiate into PrE, even in the presence of retinoic acid that normally drives them towards a PrE identity

Gata6 is necessary to specify PrE cells and is an important component of the binary Epi/PrE cell fate decision as all ICM cells express the Epi markers Nanog and Sox2 in *Gata6*$^{-/-}$ embryos

However, Gata6 early expression does not depend on the presence of FGF4 as was shown with *Fgf4* and *Nanog* mutant analyses

Other factors implicated in epiblast/primitive endoderm specification

Despite Oct4 impact on ES cell pluripotency, *Oct4* mutant embryos produce Epi and PrE cells in correct proportions

This suggests that Oct4 is dispensable for Epi versus PrE specification.

The RTK pathway does not impact ICM cell specification in human embryos, whereas salt and pepper expression of Nanog and Gata6 is present

Bmp4 is indeed specifically expressed in Epi precursors at E3.5 and plays a role in extraembryonic endoderm lineage differentiation, possibly as early as PrE formation, in embryoid bodies .

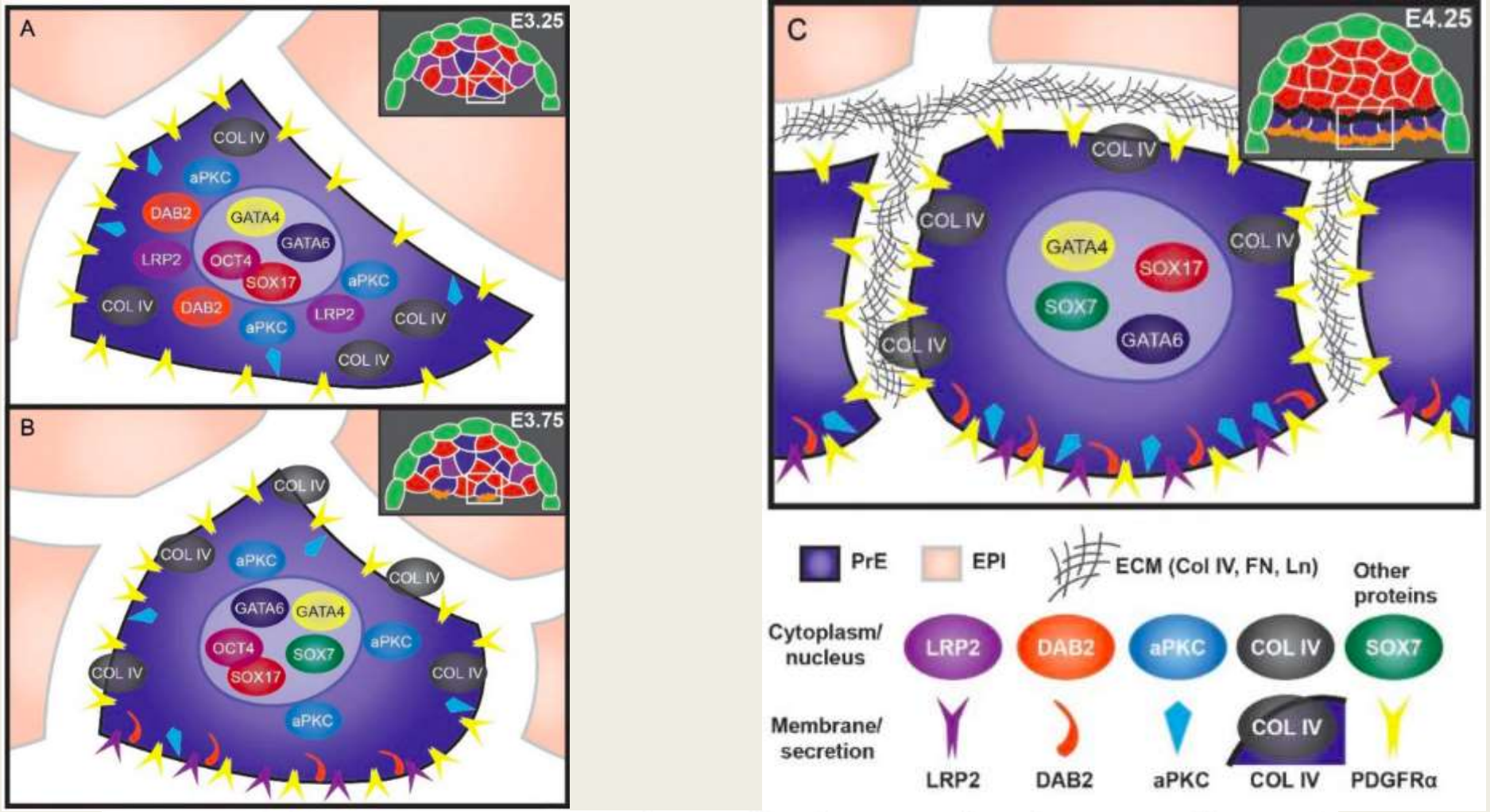
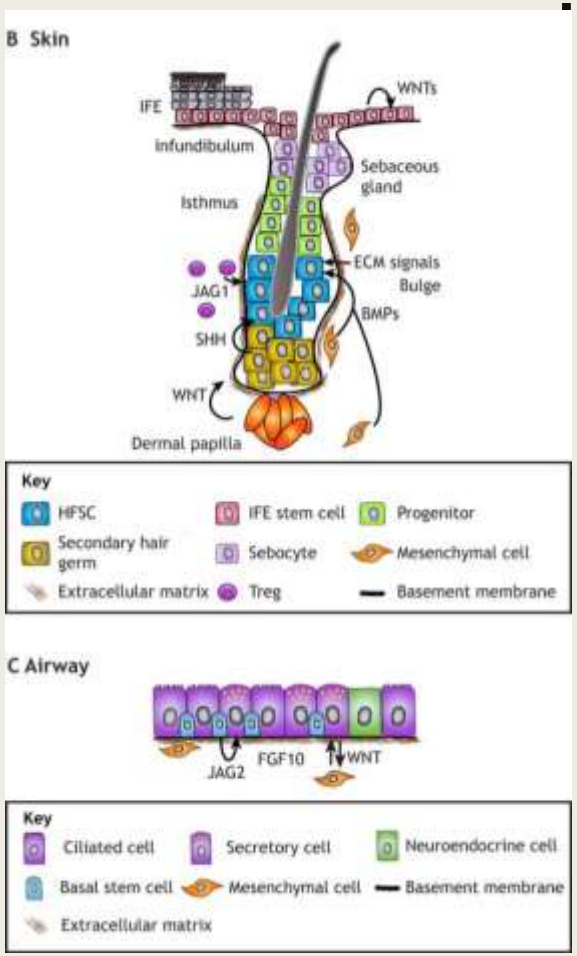
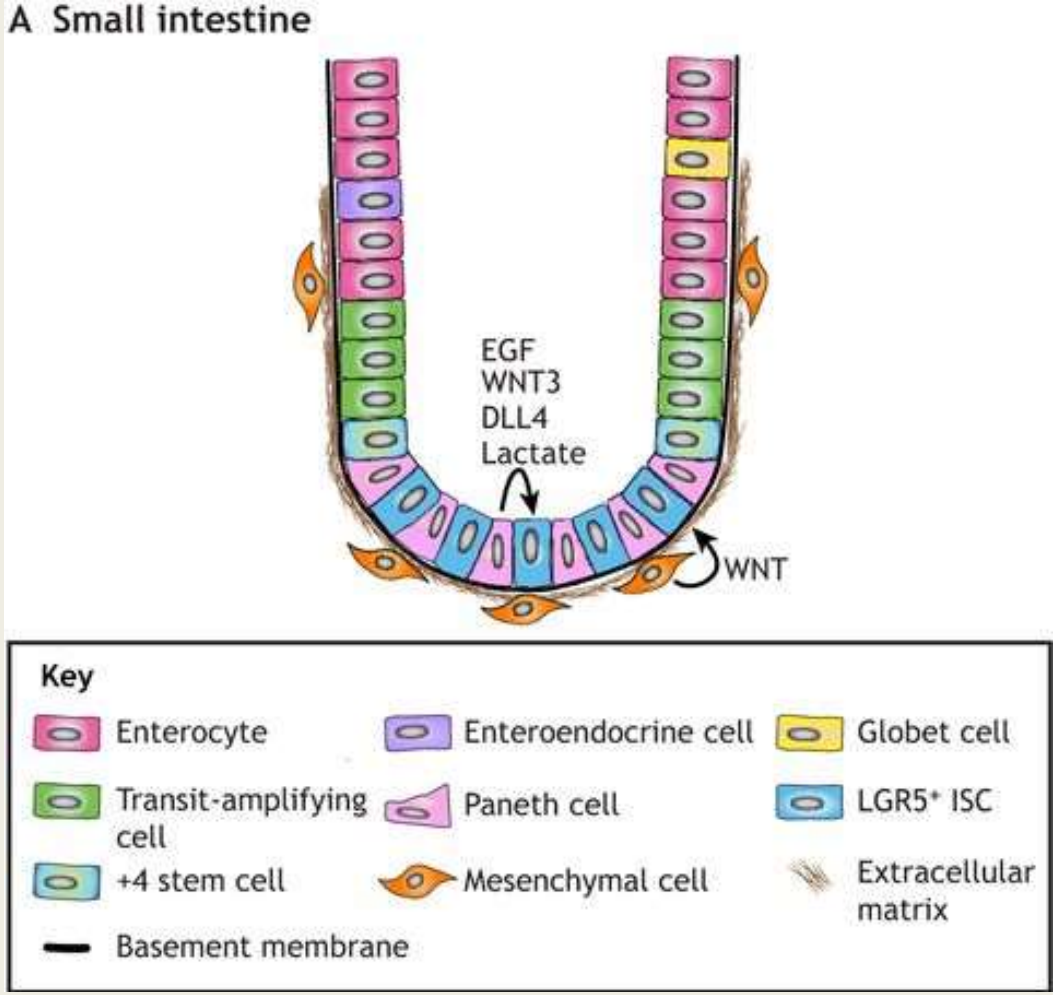


Fig. 3 (A) Before E3.75, some PrE cells are already situated at the ICM surface and express specific markers but are not polarized yet. (B) Around E3.75, PrE cells that have emerged to the surface start to be polarized, evidenced by DAB2 and LRP2 localized in the future apical membrane that faces the blastocoel cavity. SOX7 is now expressed within the nucleus of these cells. It is not clear if COLIV is already secreted by then or at the next step. (C) At E4.25, sorting is completed as all PrE cells have arisen at the surface, and start to form a structured epithelium with a basolateral extracellular matrix (ECM), and an apical pole labeled by LRP2, DAB2, and now aPKC.

Stem cells drive dynamic tissue turnover in epithelia





these stem cells migrate out of their niche, they differentiate either into the absorptive or secretory lineages and finally into one of four differentiated cell types: enterocytes, mucin-secreting goblet cells, peptide hormone-secreting neuroendocrine cells and microbicide-secreting Paneth cells

the intestinal epithelium is one of the fastest self-rene tissues and completely regenerates within 3-5 days

It has been shown that co-culturing LGR5$^+$ ISCs with a Paneth cell-enriched population or adding exogenous WNT3A, enhances the efficiency of LGR5$^+$ ISCs in forming differentiated intestinal organoids *in vitro*

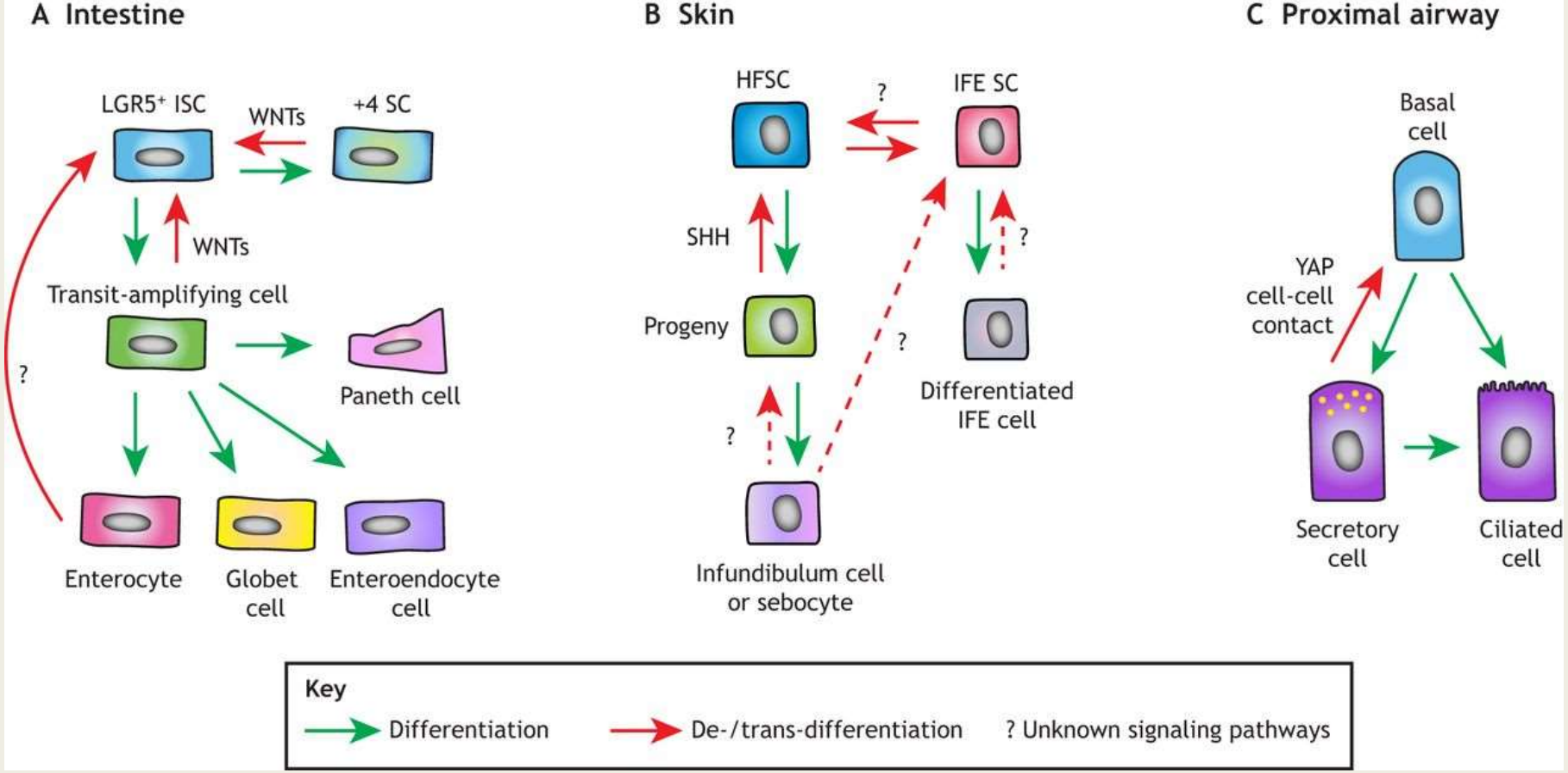
Paneth cells play a role in immunity and host-defense, but also secrete important signaling molecules such as WNT3, EGF and Notch ligand DLL4, suggesting that they might signal to ISCs. In line with this, it has been shown that co-culturing LGR5$^+$ ISCs with a Paneth cell-enriched population or adding exogenous WNT3A, enhances the efficiency of LGR5$^+$ ISCs in forming differentiated intestinal organoids *in vitro*

depletion of Paneth cells *in vivo* using three different genetic mouse models leads to reduced stem cell numbers indicating that daughter cells of LGR5$^+$ ISCs provide essential niche signals for these stem cells.

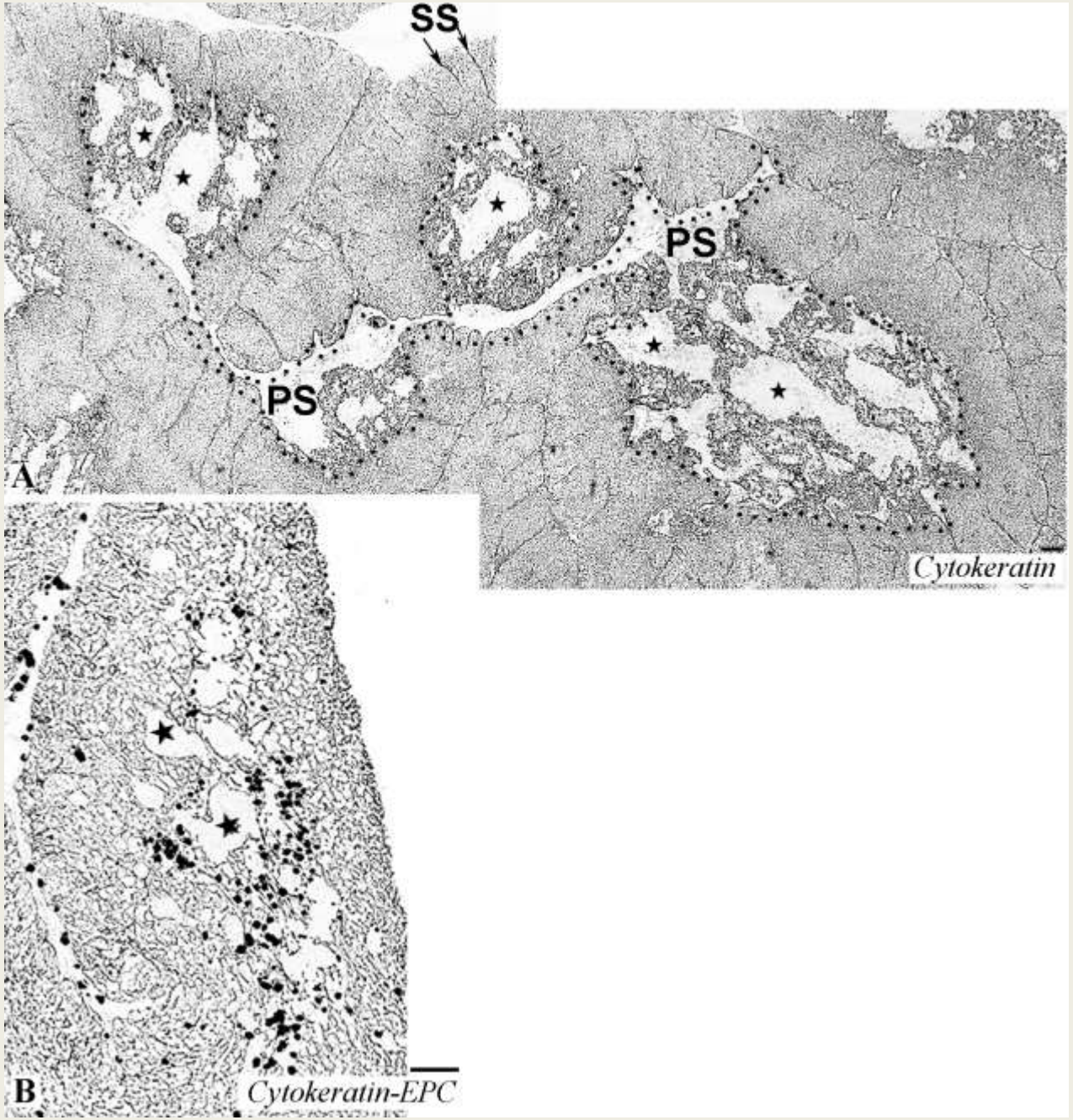
Deletion of epithelial *Wnt3*, although necessary for organoid cultures, has no effect on stem cell function in adult mice, as stromal secretion of WNTs could fully support intestinal homeostasis

Interestingly, WNT alone is not sufficient to promote LGR5$^+$ ISC self-renewal, but additional signals from R-spondins are required. WNT stabilizes R-spondin receptor expression (LGR4, LGR5, LGR6), enabling R-spondin to drive stem cell expansion

Collectively this indicates that Paneth cells are a dispensable source of WNT *in vivo*, and thus the outcome of Paneth cell depletion might be dependent on the approach used and its indirect impact on the WNT signaling status of the niche



Niche-controled differentiation trajectories and plasticity in epithelia. (A) In the intestine, progenitors and also enterocytes can dedifferentiate to LGR5$^+$ intestinal stem cells (ISCs) through WNT-mediated niche signals. (B) In the skin, the immediate progeny of hair follicle stem cells (HFSCs) as well as more distant populations located in the interfollicular epidermis (IFE), infundibulum and sebaceous glands can repopulate the bulge stem cell niche upon HFSC loss. The precise signals that control this plasticity are unclear but *in vitro* studies implicate sonic hedgehog (SHH) signaling in this process. In response to wounding, HFSCs are able to migrate into the IFE to regenerate the epidermis and, vice versa, IFE stem cells can generate hair follicles upon transplantation. Although experimental evidence for many dedifferentiation events is compelling (solid arrows) for others it is preliminary (broken arrows). (C) In the lung proximal airway epithelium, secretory cells can dedifferentiate into basal cells through signals that involve direct cell-cell



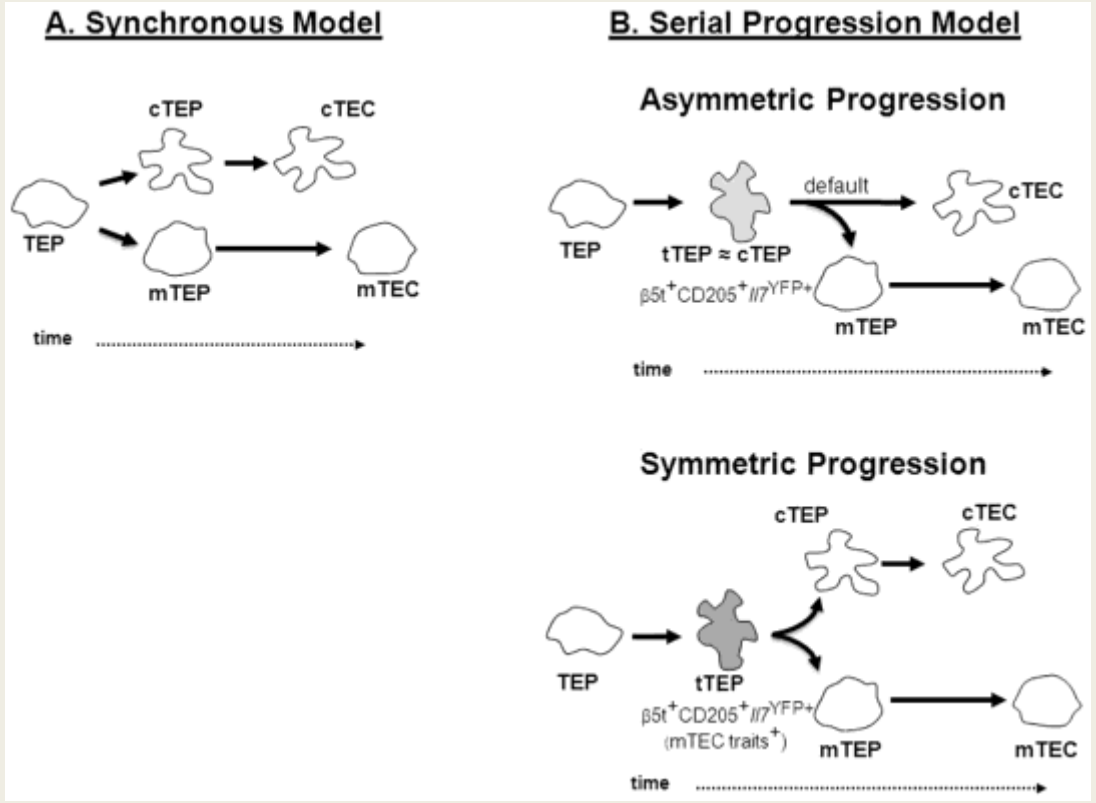
The cytokeratin intermedier filaments (KIFs) are specific for the thymic epithelial cells, which have endodermal origin.

There are 2 groups of KIF:

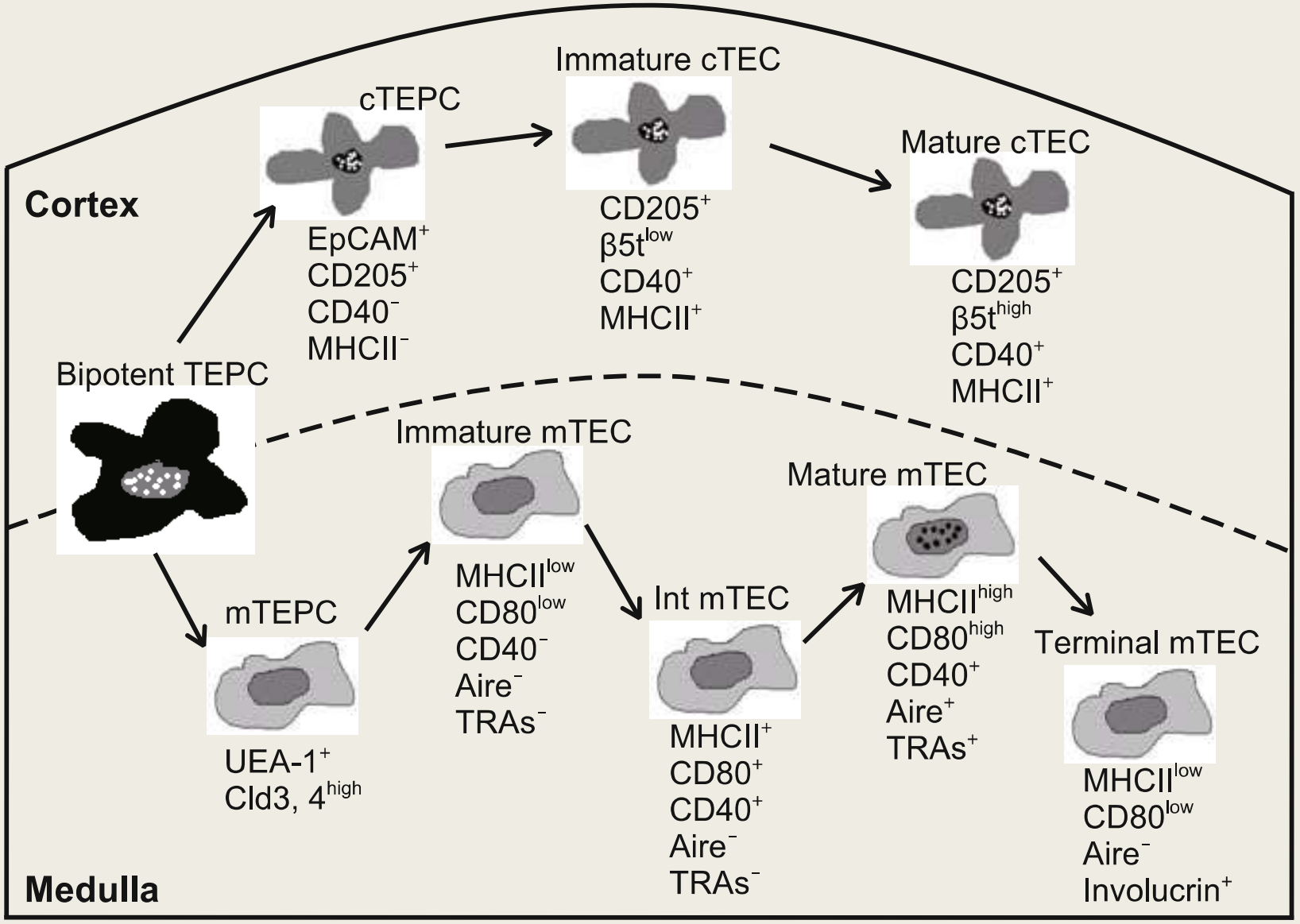
Ist class: K9-K23 and

IInd class: K1-K8 and K71-K80) (Odaka et al. 2013).

The appearance of KIFs can be a characteristic indicator of a particular cell line.



Models of thymic epithelial cell development. (A) In the “synchronous” model, uncommitted bipotent TEC progenitors (TEPs) diverge simultaneously to lineage-restricted cortical (cTEPs) and medullary (mTEPs) progenitors, which then progress into mature cTECs and mTECs.
(B) In the “serial progression”model, TEPs transverse through a “transitional TEC progenitor” stage (tTEP) that expresses phenotypic and molecular traits associated with cTECs prior to the commitment into a cTEC or mTEC fate. In the asymmetric scenario (top), tTEPs are more closely linked, at the phenotypic and molecular levels, with cTEPs and have the potential to generate both mTEC progenitors and mature cTECs, with the cortical lineage being the “default” pathway. In the symmetric scenario (bottom), tTEPs express both cTEC and as-yet-unidentified mTEC traits prior to lineage specification. (Alves et al. 2014)


Cortex
cTEPC
EpCAM+
CD205+
CD40−
MHCII−
Immature cTEC
CD205+
β5tlow
CD40+
MHCII+
Mature cTEC
CD205+
β5thigh
CD40+
MHCII+
Bipotent TEPC
Immature mTEC
MHCIIlow
CD80low
CD40−
Aire−
TRAs−
Mature mTEC
MHCIIhigh
CD80high
CD40+
Aire+
TRAs+
mTEPC
UEA-1+
Cld3, 4high
Int mTEC
MHCII+
CD80+
CD40+
Aire−
TRAs−
Terminal mTEC
MHCIIlow
CD80low
Aire−
Involucrin+
Medulla

Epidermal stem cells

- The proliferation capacity of epithelial cells, although very high
- There is a lot of evidence that proper signals from the cell's immediate environment are needed to maintain the stem cell property.

- Adherence of basal epithelial cells to the underlying membrane appears to be essential in maintaining the epidermal stem cell population.

- Membrane-forming proteins regulate the maintenance of epidermal stem cell populations via mitogen-activated protein kinase (MAPK) binding to cellular beta1 integrin receptors (Zhu et al., 1999).

- Beta1 integrin also plays an essential role in the normal formation of epithelial tissue and hair follicles

- Knockout of beta1 integrin in epithelial cells in mice results in severe disorders between the hair follicle and the epithelium (Brakebusch et al., 2000; Raghavan et al., 2000).

- In cells that are thought to have unrestricted reproductive capacity (stem cells, embryonic cells, immortalized and tumor cells), the telomerase enzyme prevents shortening of chromosomal ends.

- In mature healthy organisms, telomerase activity was detected in bone marrow, umbilical cord and peripheral blood cells (bone marrow stem cells), and in basal cells of the epithelium in addition to gametes (Härle-Bachor-Boukamp,1996).