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Review

Cellular and molecular mechanisms of intestinal elongation in mammals: the long and short of it

Sara Cervantes[1,2]

[1]Diabetes and Obesity Research Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer IDIBAPS-Hospital Clínic, Barcelona, Spain and [2]Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERDEM), Spain

Summary. The gastrointestinal tract carries out essential functions for the organism, including the digestion and absorption of nutrients. The cells lining the lumen of the gut tube derive from the endoderm, one of the three germ layers formed during gastrulation. The length of the intestinal tract determines its digestive and absorptive capacity, and so the intestine expands several times the length of the whole body to ensure an adequate absorptive area to meet nutritional demands. However, the endoderm starts out as a small sheet of cells spanning less than the whole length of the head-fold embryo. In order to achieve its final shape and size, the cells in the endoderm undergo extensive growth and profound morphogenetic changes, which are governed by embryonic signaling pathways and transcription factors. This review, based on mouse development, summarizes our current knowledge of the cellular and molecular mechanisms underlying the morphogenetic changes that participate in shaping the mature intestinal tract in vertebrates.

Key words: Intestinal elongation, Gut, Morphogenesis, Wnt/PCP, Convergence extension

Introduction

The intestinal tract is essential for ingestion, digestion, nutrient absorption, endocrine function, metabolic homeostasis and immunity. The gastrointestinal tube is compartmentalized into the esophagus, stomach, small intestines and large intestines, which carry out unique roles along the rostrocaudal axis of the gut. In addition, each of these regions contains a plethora of cellular types originating from the three embryonic germ layers (ectoderm, mesoderm and endoderm), which are organized to form distinct concentric tissues that perform specialized functions. Cells derived from the endoderm occupy the innermost layer of the gastrointestinal tract, while cells originated from the mesoderm give rise to the surrounding muscular cell layers in the submucosa and muscularis propria. These tissues are populated by a dense network of blood vessels and ectoderm-derived enteric neurons. The endoderm at the level of the intestines gives rise to four cell lineages: 1) Enterocytes, which line the epithelium and perform absorptive functions; 2) Goblet cells, also known as mucus-secreting cells; 3) Enteroendocrine cells, which secrete hormones; and 4) Paneth cells, which carry out defensive functions against microbial damage (Heath, 2010; Noah et al., 2012).

In vertebrates, the endoderm-derived cells lining the intestines organize in pit-shaped structures, the crypts of Lieberkühn, which expand into the subjacent layer and contain, among others, stem cells that will give rise to all four intestinal cell lineages to maintain the homeostasis of the constantly self-renewing intestinal epithelium. This epithelium at the level of the small intestine is further compartmentalized into villi, epithelial projections that exponentially increase the absorptive surface of the intestines. Moreover, the tremendous length of the intestine additionally contributes to increase its digestive and absorptive area. At the end of gestation, the gastrointestinal tract extends several times the length of the body, despite starting out in mammals as a flat sheet of cells located at the outside of the cup-shaped embryo after gastrulation. Hence, the endoderm needs to undergo extensive growth and morphogenetic changes to give rise to a long, complex and convoluted gut tube at the end of gestation. Crypt-villus

,Offprint requests to: Sara Cervantes PhD, Diabetes and Obesity Research Laboratory, IDIBAPS, Esther Koplowitz Center (CEK), 5th floor, Rosselló 149-153, 08036 Barcelona, Spain. e-mail: scervant@clinic.ub.es

morphogenesis and differentiation of the specialized intestinal cell types has been the focus of a number of reports and excellent reviews (Rubin, 2007; Zorn and Wells, 2009; Noah et al., 2012). This review instead focuses on a poorly unexplored area, the cellular and molecular mechanisms that govern the morphogenetic changes in the shape and size of the intestine.

Formation of the gut tube dramatically differs between non-amniotes (zebrafish and frogs) and amniotes (humans, mice and chicks). While zebrafish and frogs develop an occluded rod-like structure that will elongate and form a lumen by apical polarization, humans, mice and chicks form a tubular gut from a sheet of endodermal cells (Chalmers and Slack, 2000; Grapin-Botton, 2005; Ng et al., 2005; Lewis and Tam, 2006; Zorn and Wells, 2009; Heath, 2010). For the purpose of this review, I will take mouse development as an example to summarize the mechanisms involved in intestinal formation and elongation in amniotes. The different aspects of intestinal elongation conveyed are divided into two main sections that cover the cellular (section 1) and molecular (section 2) aspects of intestinal elongation, which are subsequently divided into early (up to formation and closure of the gut tube at E9.5-10.0) and late (those occurring during mid-gestation, starting at E10.5) events.

Cellular mechanisms of gut elongation in mammals

The three embryonic germ layers, namely ectoderm, mesoderm, and endoderm, that will give rise to all the tissues and organs in the animal body are generated during gastrulation. In this section, I will cover the cell movements and morphogenetic events occurring upon completion of this process. Excellent reviews covering different aspects of endoderm formation during the initial stages of embryo development can be found elsewhere (Grapin-Botton and Constam, 2007; Tam et al., 2007; Zorn and Wells, 2007).

Early stages in the formation and elongation of the primitive gut (before E10.5)

The endoderm, which will give rise to the intestinal tract and associated organs, is formed as cells from the epiblast that ingress through the primitive streak move backwards towards the anterior region of the embryo while displacing the visceral endoderm (reviewed in (Lewis and Tam, 2006)). As a result of these cellular movements, the definitive endoderm emerges at its earliest stage as a layer of squamous epithelium that covers the entire embryo on its ventral side (Fig. 1). The first elongating event of the endoderm starts in head-fold stage embryos (E8.0) and continues until the embryos develop to ~14-somite stage (E9.5), when the closure of the gut tube is almost complete. During these stages (E8.0-E9.5), the mediolateral axis of the layer of definitive endoderm narrows while the anterior-posterior axis extends (Garcia-Garcia et al., 2008). As a result of this process, known as convergence and extension, the endoderm reshapes into a thin, long structure. Recent studies indicate that, at the cellular level, gut elongation is possibly driven by the intercalation of lateral cells towards the midline (mediolateral intercalation) and exclude the contribution of cell proliferation, which minimally accounts for the lengthening of the endoderm at this stage (Tremblay and Zaret, 2005; Garcia-Garcia et al., 2008).

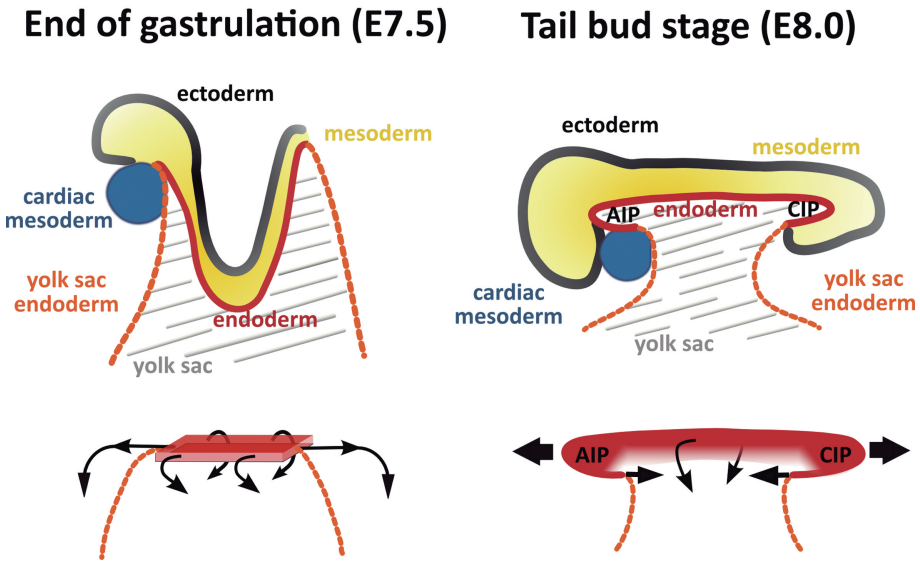

Fig. 1. Diagram depicting the changes in the shape of the endodermal layer (red) during gut tubulogenesis at the indicated embryonic stages in mouse.

Besides elongation, the endoderm undergoes profound changes in shape, resulting in the formation of a tube from a tissue that started out as a sheet of cells. Gut tubulogenesis and lengthening occur simultaneously, suggesting that the same cellular events can participate in one process or the other. Gut tube morphogenesis begins when the epithelial sheet of endoderm folds over at the anterior and posterior ends, forming the anterior and caudal intestinal pockets or portals. The initial pockets grow deeper as midline cells from the anterior and posterior edges of the endoderm move caudally and rostrally, respectively, while cells on the lateral endoderm are displaced towards its ventral side, to eventually form the Anterior and Caudal Intestinal Portals (AIP and CIP, respectively) (Fig. 1). Both openings move towards each other while the lateral endoderm walls fold towards the ventral midline in a process that entails extensive cell rearrangements, including migration (Tremblay and Zaret, 2005; Tam et al., 2007; Franklin et al., 2008). During the course of portal and gut tube formation, the anterior and posterior ends of the ventrally opened gut remain connected to the yolk sac (Kaufman and Bard, 1999; Sulik, 2003) (Figs. 1, 2). By 20-22 somites, the leading edge of the AIP and CIP meet in the midgut region, while the embryo has completed turning and has thus adopted a fetal position (Kaufman and Bard, 1999; Lewis and Tam, 2006) (Fig. 2). The process of gut tube closure is intimately associated with the embryo turning, so mutant embryos that fail to properly undergo body rotation develop with an open gut tube (Lewis and Tam, 2006). As a consequence of body rotation, the endoderm relocates to its eventual position in the inside of the embryo, while still connected to the yolk sac by the vitelline duct or yolk stalk (Figs. 1, 2). This duct originated from the opening of the endoderm into the cavity of the yolk sac, which progressively narrowed as the AIP and CIP come closer (Kaufman and Bard, 1999; Sulik, 2003) (Fig. 1). Complete closure of the vitelline duct in a timely manner is also important to ensure proper gut morphogenesis. In fact, failure to close the midgut before the second wave of gut elongation commences at E10.5 results in the formation of a secondary axis in the gut tube (Cervantes et al., 2009). Thus, formation of the primitive gut tube involves cell migration and convergence and extension movements that need to be coordinated in order to reach the appropriate shape of the organ.

Initial endoderm and body axis elongation occur simultaneously and, more importantly, proportionately between E8.0-E9.5 In this context, expansion of the body axis involves coordinated growth of all three germ layers, and therefore defects in one germ layer may have effects in the expansion of the others. In fact, defects of mesodermal origin bear consequences for intestinal formation, as seen in mutant embryos displaying severe posterior truncations that primarily affect paraxial mesoderm (Takada et al., 1994; Galceran et al., 1999; Yamaguchi et al., 1999; Pinson et al., 2000). While these studies indicate that growth of the mesoderm

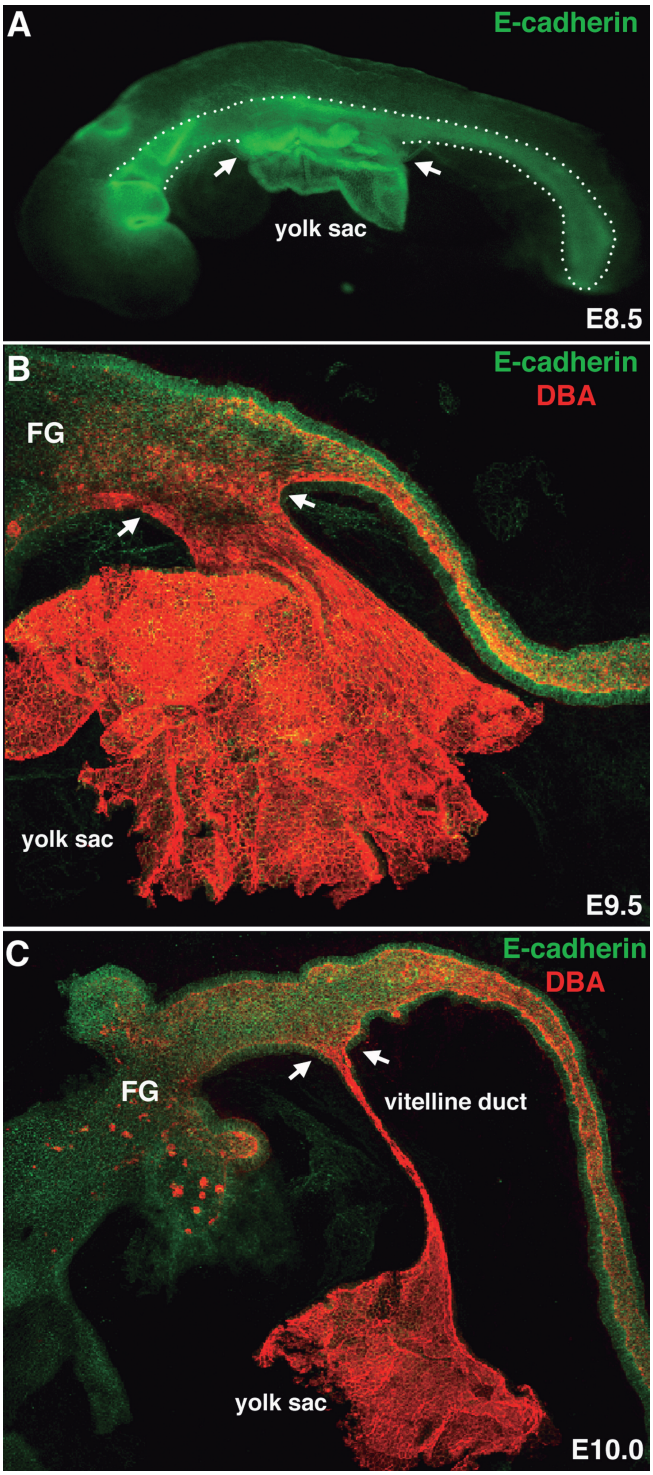

Fig. 2. The opening connecting the gut endoderm and the yolk sac progressively narrows and closes by E10.0. Whole mount immunofluorescence stainings using an antibody against E-cadherin (green), and the lectin Dolichos Bifluorus Agglutinin (red), which bind to cell-surface glycoproteins. Arrows indicate the opening of the yolk sac. **A.** By E8.5, about one-third of the gut tube, outlined with a broken line, opens into the yolk sac. **B and C.** The opening progressively diminishes as the gut tube narrows and elongates until it converts into an occluded rod-like structure by E10.0, before the second wave of intestinal elongation starts. FG; foregut.

accompanies endoderm formation, anterior endoderm fate map studies rule out a direct connection between mesodermal and endodermal cell movements, since endoderm and adjacent mesoderm can end up at different locations (Tremblay and Zaret, 2005). Nevertheless, the endoderm is no exception and, like many other organs, the gut does not develop properly without an interaction between the endoderm and the

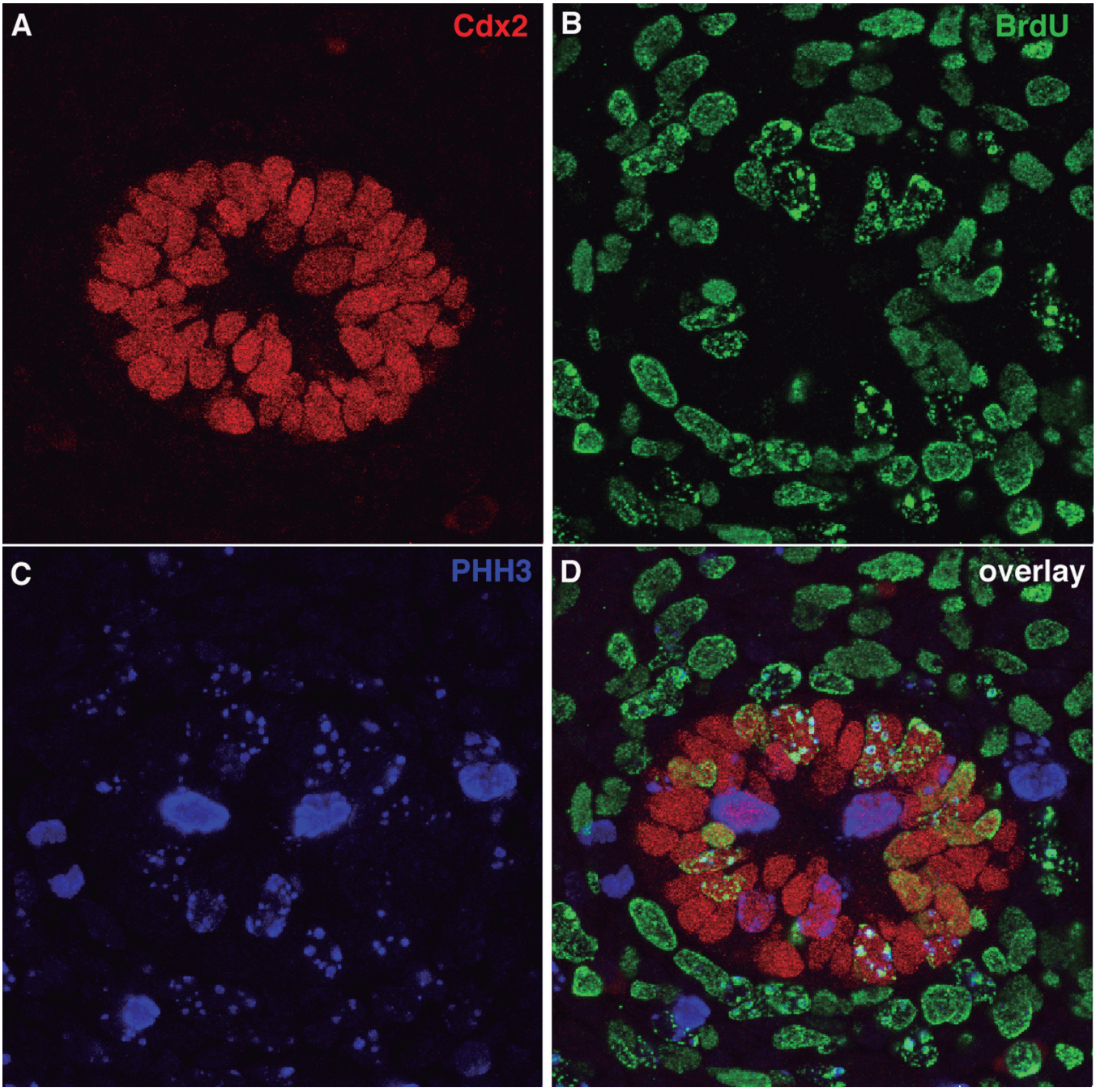

Fig. 3. Extensive cell proliferation in the endoderm and mesenchyme of the gut at E10.5. **A.** Immunofluoresence staining on sections to mark the cells in the endoderm of the gut, marked with the intestinal marker Cdx2 (red). **B.** BrdU was injected into pregnant females 8 hours before dissection to mark the proliferating cells, which have incorporated BrdU into the DNA during the S phase. Brdu is detected here with an anti-BrdU antibody (green). **C.** Phospho Histone H3 (PHH3), detected with an anti-PHH3 antibody (blue), is found in cells at the late G2 and M phases of the cell cycle, thus marking dividing cells. **D.** Image overlay reveals the presence of proliferating cells in both the endoderm (Cdx2 positive) and mesenchyme.

mesoderm, both at early and later stages (see below) (Grapin-Botton and Melton, 2000; Roberts, 2000).

Intestinal elongation starting at mid-gestation (E10.5 and onwards)

At the end of gestation, the intestine, which corresponds to the midgut and hindgut region in the embryo, is an elongated and convoluted tube. Thus, the primitive gut tube, which only extends to the length of the embryo by E10.5, undergoes extensive elongation and coiling to attain its mature shape and size. In fact, during the second wave of intestinal elongation, the gut tube expands disproportionately to total body length. The extensive proliferation of the cells in the monolayered epithelium of the endoderm underlies the exponential growth of the gut (Fig. 3). Cell divisions in the gut epithelium during the initial stages of gut elongation (E11.5) require a transient movement of the cell nuclei towards the luminal side of the epithelium, followed by their re-intercalation in the epithelial sheet (Cervantes et al., 2009). Whether dividing cells at this stage of intestinal morphogenesis remain connected to the basal side of the epithelium and undergo interkinetic nuclear movements, as they do at later stages of intestinal morphogenesis, remains to be investigated (Grosse et al., 2011). Such a burst of cell proliferation in the endodermal layer needs to be coupled with proliferation of the surrounding mesenchyme (Fig. 2), which provides, in addition to patterning information, growth cues to the epithelium. In fact, the mesenchyme surrounding the endoderm is essential to driving and sustaining proper endodermal growth, as demonstrated in mouse mutants in which the mesenchyme does not expand appropriately (Geske et al., 2008; Mao et al. 2010; Kim et al., 2011). Nevertheless, the extensive proliferation of the gut endoderm results in the formation of a loop in the midgut region that expands rapidly on the ventral side of the embryo towards the umbilical cord. By E11.5, the midgut loop adopts a hairpin shape, which will progressively enlarge and coil. Due to space restrictions within the abdominal cavity, which at this stage is mainly occupied by the growing liver, the gut grows outside of the embryo until E15.5, forming the physiological umbilical hernia (Kaufman and Bard, 1999; Bates and Deutsch, 2003; Sulik, 2003). Recent studies based on mathematical modeling show that gut looping is driven by the relative growth rates of the epithelium of the endoderm and the anchoring dorsal mesentery, underscoring the importance of context and mechanical tissue interactions in shaping the mouse intestines (Savin et al., 2011).

Molecular mechanisms of gut elongation

Components of the main embryonic signaling pathways, as well as key transcription factors, have been shown to be required in at least one of the multiple steps involved in intestinal development, including elongation, morphogenesis and differentiation of the different intestinal cell types. The following sections cover the signaling molecules and transcription factors described to contribute to intestinal elongation, either during early stages of endoderm and primitive gut tube formation (section 2.1), or later during mid-gestation (section 2.2).

Molecular control of early gut formation and elongation (before E10.5)

A considerable number of Wnt signaling components belonging to its canonical Wnt/ß-catenin or non-canonical branches take part in intestinal elongation. Mice deficient in the Wnt/ß-catenin transcription factors Tcf4 and Tcf1 (*Tcf4*$^{-/-}$;*Tcf1*$^{-/-}$), which are downstream components of canonical signaling, fail to form caudal structures. In these double mutant embryos, the caudal endoderm, including the hindgut and the CIP, appears severely disrupted, while the paraxial mesoderm is

Table 1. Glossary terms used in this review.

Term	Definition
Gastrulation	Process involving migratory and cell rearrangement events that lead to the formation of the three germ layers: ectoderm, mesoderm and endoderm.
Neurulation	Process leading to the formation of the neural tube from the ectodermal cells in the neural plate.
Paraxial mesoderm	Portion of the mesoderm formed lateral to the neural tube on both sides.
Visceral endoderm (VE)	Outer layer of cells covering the embryo before gastrulation (egg-cylinder stage).
Definitive endoderm (DE)	Germ layer formed during gastrulation that later gives rise to the epithelial lining of the respiratory and intestinal organs.
Convergence and extension	Process by which a given tissue rearranges to narrow along one axis (mediolateral) and elongate along a perpendicular axis (anterioposterior).
Planar Cell Polarity (PCP)	Polarization of a field of cells within the plane of an epithelial layer. It represents a level of polarization distinct from apicobasal polarity.
Wnt/ß-catenin	Refers to the arm of the Wnt signaling pathway involving transcriptional events triggered by the stabilization of the cytoplasmic pool of ß-catenin.
Wnt/Planar Cell Polarity (PCP)	Refers to the arm of the Wnt signaling pathway independent of ß-catenin that controls planar cell polarity.

appropriately specified. The primary affected process in these mutant embryos is the specification of the caudal endoderm that will give rise to the hindgut, which is the last to ingress through the primitive streak during gastrulation, rather than its elongation (Gregorieff et al., 2004). In agreement with this endodermal phenotype arising from perturbed Wnt/ß-catenin signaling, it has recently been shown that the pathway is active in the posterior young definitive endoderm (E7.5-E8.5) (Sherwood et al., 2011).

The importance of the non-canonical branch of Wnt signaling and, more specifically, Wnt/Planar Cell Polarity (Wnt/PCP) in body axis elongation during gastrulation and neurulation, was first uncovered in Xenopus and zebrafish (reviewed in Wallingford et al., 2002). These morphogenetic processes entail convergence and extension (narrowing along the mediolateral axis while extending in the anterioposterior), a coordinated cellular process that requires planar cell polarity, which is defined as the orientation of cells within the plane of a tissue. In mice, similarly to frogs and zebrafish, a conserved Wnt/PCP pathway is required to drive body axis elongation and neural tube closure (Wang and Nathans, 2007). Mouse embryos deficient in non-canonical Wnt components, including the ligand Wnt5a (*Wnt5a*$^{-/-}$) (Yamaguchi et al., 1999), the receptors Fz3 and Fz6 (*Fzd3*$^{-/-}$;*Fzd6*$^{-/-}$) (Wang et al., 2006b) and type I Sfrps (*Sfrp1*$^{-/-}$;*2*$^{-/-}$;*5*$^{+/-}$) (Satoh et al., 2006, 2008), or other Wnt/PCP membrane and intracellular components [i.e. Vangl2 (*Vangl2*$^{Lp/Lp}$) (Torban et al., 2004), Disheveled1 and 2 (*Dvl1*$^{-/-}$;*Dvl2*$^{-/-}$) (Wang et al., 2005, 2006a), Dact1 (*Dact1*$^{-/-}$) (Suriben et al., 2009; Wen et al., 2010), Celsr1 (*Celsr1*$^{Scy/Scy}$ or *Celsr1*$^{Crsh/Crsh}$) (Curtin et al., 2003) and PTK7 (*PTK7*$^{-/-}$) (Lu et al., 2004)], display a shortened body axis and, in some cases, an opened neural tube, in addition to defects in hair orientation and cochlear formation (reviewed in (Karner et al., 2006) and (Wang and Nathans, 2007)). Furthermore, mice deficient in the receptor tyrosine kinase *Ror2*, a component of an alternative non-canonical Wnt/Ror2 signaling pathway, display a shortened body axis (DeChiara et al., 2000; Oishi et al., 2003). Thus, mouse Wnt/PCP and non-canonical Wnt mutants display PCP phenotypes similar to those caused by defects in convergence and extension in frogs and zebrafish. Indeed, studies in *Vangl2*$^{Lp/Lp}$ and *Dvl1*$^{-/-}$;*Dvl2*$^{-/-}$ mouse embryos, in which Wnt/PCP is disrupted, showed that, consistent with what has been reported in frogs and zebrafish, Wnt/PCP is also required for convergence and extension of the notochord and neural tube. Loss of Wnt/PCP components generated wider and shorter mutant embryos as compared to control littermates, measured by calculating the progression of length to width ratios (LWR) during body axis elongation (Wang et al., 2006a; Ybot-Gonzalez et al., 2007). Furthermore, direct analysis of convergence and extension by cell tracing experiments in *Vangl2*$^{Lp/Lp}$ embryos revealed that Wnt/PCP is necessary for midline extension in both axial mesoderm and neural plate (Ybot-Gonzalez et al., 2007). The study of Wnt/PCP phenotypes in mice has mostly been restricted to tissues that display clear planar cell polarity (orientation within the tissue plane), such as hair bundle orientation in inner ear sensory cells, and hair follicle orientation in the skin, as well as morphogenetic processes, such as neural tube and eyelid closure and, as mentioned above, notochord extension. Even though defects in the elongation of the endoderm were not specifically investigated in most of the Wnt/PCP mutant mice, a cumulative body of evidence suggests that Wnt/PCP is involved in gut elongation. Thus, the fact that i) body axis extension includes the three germ layers, ii) the body and the endoderm grow proportionally in head-fold stage embryos (E8.0 to E9.5), and iii) Wnt/PCP regulates body axis extension by convergence and extension in mice, indicates that a Wnt/PCP signaling module also participates in primitive gut elongation. Indeed, analysis of *Wnt5a*$^{-/-}$, *Vang2*$^{Lp/Lp}$ and *Dvl1*$^{-/-}$;*Dvl2*$^{-/-}$ primitive gut tube revealed a shorter and wider intestine, a defect that is consistent with perturbed convergence and extension in these mutants (Cervantes and Hebrok, unpublished observations). Furthermore, loss of *Wnt5a* results in a delay or failure to complete gut tube closure in the midgut by the time it starts to massively elongate, thus causing the formation of a duplicated gut tube (Cervantes et al., 2009). Similarly to the observed phenotypes in *Wnt5a*$^{-/-}$ and *Vangl2*$^{Lp/Lp}$, *Dact1*$^{-/-}$ mutant mice display defects in the elongation of the paraxial mesoderm and endoderm and fail to form a hindgut (Suriben et al., 2009; Wen et al., 2010). The Wnt/PCP defects observed in these mice arise from a perturbed modulation of Vangl2 (Suriben et al., 2009) or Dvl2 (Wen et al., 2010) by Dact1, an intracellular phosphoprotein involved in the regulation of Wnt signaling.

Forward genetic screens have led to the identification of other proteins required for body axis extension. The study of chato mutants revealed that Zfp568, a KRAB domain zinc-finger protein, which possibly functions as a transcriptional regulator, is required for convergence and extension of endodermal cells (Garcia-Garcia et al., 2008). Lulu mutants, deficient in the FERM-domain gene erythrocyte protein band 4.1-like 5 (Epb4.115), display defects in the epithelial to mesenchymal transition occurring in cells that ingress through the primitive streak during gastrulation (Lee et al., 2007). Interestingly, the fact that chato functions independently of non-canonical Wnt signaling (Garcia-Garcia et al., 2008) indicates that, although Wnt/PCP has traditionally been regarded as the main regulator of convergence and extension and body axis elongation, multiple signaling pathways are likely to be necessary to drive body axis and gut elongation. In this sense, our understanding of this process in the mouse is far from being complete and needs to be addressed in order to define the Wnt/PCP signaling module and other signaling pathways that control the early stages of endoderm elongation.

Molecular control of exponential gut growth during mid-gestation (E10.5 and onwards)

The signaling molecules and pathways controlling the wave of intestinal proliferation and elongation during mid-gestation in mice have not been characterized. The large majority of reports focus on villi morphogenesis and cell differentiation, and only three recent studies reveal some aspects of the molecular mechanisms required for the proper formation of intestinal shape. In addition to its role during early stages of body axis elongation, which has been described in the previous section, some components of non-canonical Wnt signaling are again instrumental later in midgut elongation during mid-gestation. Loss of the non-canonical Wnt5a ligand, which is expressed in the mesenchyme surrounding the midgut and hindgut, results in a profound reduction in intestinal elongation during mid-gestation, which adds up to the already shortened and wider primitive gut tube (described in section 2.1). As mentioned above (section 1.2), at the epithelial level Wnt5a is necessary to maintain cell proliferation in the epithelium and proper epithelial organization at the earliest stages of midgut elongation (E10.5-E11.5). Specifically, Wnt5a ensures proper nuclear re-intercalation after cell divisions to maintain the epithelial layer of the gut (Cervantes et al., 2009). At later stages (E13.5), Wnt5a has been shown to maintain apico-basal polarity, including proper apical localization of the Wnt/PCP components Dvl2 and Fz3 in the endoderm (Matsuyama et al., 2009). Whether this loss of apico-basal polarity also explains the defective cell divisions observed at earlier stages in Wnt5a mutant embryos remains to be investigated. At these later stages, loss of type I Sfrps in *Sfrp1*$^{-/-}$; *2*$^{-/-}$; *5*$^{+/-}$ compound mutants impairs intestinal growth and promotes the formation of epithelial cell clumps, similar to those observed in *Wnt5a*$^{-/-}$ intestinal epithelium (Cervantes et al., 2009; Matsuyama et al., 2009). Conversely, loss of Vangl2, which is normally expressed in the epithelium of the endoderm, does not impair midgut elongation at E10.5, indicating that the Wnt/PCP pathway is unlikely to be the main driver of intestinal elongation (Cervantes et al., 2009). In contrast, mouse embryos deficient in Ror2, which is expressed in both the epithelium and mesenchyme of the midgut, also display a shortened gut, although not as much as that of *Wnt5a*$^{-/-}$ embryos. In addition, non-proliferative cell clumps in the epithelium of the endoderm suggest that Ror2 mediates, in part, Wnt5a-driven intestinal elongation (Yamada et al., 2010). Studies in lung and limb development revealed that Wnt5a functions through a Ror2/Vangl2 signaling module to curve canonical Wnt/ß-catenin signaling (Mikels et al., 2009; Gao et al., 2011). However, this pathway is not active in the elongating midgut (E10.5-E16.5) (Kim et al., 2007; Cervantes et al., 2009), thus, the signaling outcome downstream of Wnt5a and Ror2 signaling during intestinal elongation remains to be uncovered.

While the studies presented above involve mesenchyme-to-epithelium signaling to maintain the epithelial layer, a number of reports show that the expansion and maintenance of the mesenchymal cell layer is crucial to support gut expansion during mid-gestation. Simultaneous ablation of Sonic Hedgehog (*Shh*) and Indian Hedgehog (*Ihh*) (Mao et al., 2010), or inactivation of *Fgf9* (Geske et al., 2008), which signal from the epithelium to the mesenchyme, resulted in a dramatic reduction in intestinal length. These studies further demonstrate that these factors, secreted by the endoderm, act as mitogenic input in the surrounding mesenchyme by acting on their corresponding receptors: Smoothened (Shh and Ihh) and Fgfr1 and Fgfr2 (Fgf9). However, the requirements for these factors are different in the cellular subtypes residing in the mesenchyme. Both factors are necessary for the proliferation of mesenchymal cells, but, while *Fgf9*$^{-/-}$ embryos displayed normal proliferation and differentiation of the muscularis propria, containing smooth muscle cells, mice deficient in Hedgehog signals are devoid of this cell layer (Geske et al., 2008; Mao et al., 2010). Conversely, excess Hedgehog signaling in the gut mesenchyme driven by the expression of a constitutively active form of Hh receptor (SmoM2), results in the expansion of both the mesenchymal layer and the smooth muscle cells (Mao et al. 2010). In addition to Hh and Fgf signaling, Notch signaling is also active in the developing intestinal mesenchyme (and epithelium). Blocking Notch signaling by ablation of its main signal transducer RBP-J in the gut mesenchyme leads to a reduction in intestinal length. Moreover, constitutive activation of Notch signaling mediated by the overexpression of its active intracellular domain (NICD) causes a more severe reduction in intestinal length (Kim et al., 2011). In both cases, the layer of subepithelial fibroblasts surrounding the gut endoderm fails to expand. Despite the similarities in the observed phenotypes generated by loss and gain of Notch signaling, the cellular mechanisms underlying these defects are not the same: while loss of Notch signaling results in reduced proliferation, overactivation of the pathway results in cell death. Nevertheless, these observations argue that Notch signaling is tightly regulated in the mesenchyme, and, in fact, Hedgehog signals curve Notch activity in the surrounding subepithelial fibroblasts (Kim et al., 2011). Besides signaling pathways, the homeobox-containing transcription factor Hlx also plays a role in the mesenchyme that is crucial to drive intestinal expansion. *Hlx* is expressed in the intestinal mesenchyme, and its ablation does not impact formation of the mesenchymal cell layers. However, expansion of the intestines is dramatically perturbed in *Hlx*$^{-/-}$, suggesting that Hlx controls a mesenchyme-to-epithelium signal that is required for intestinal expansion (Hentsch et al., 1996).

Relevance to human disease and concluding remarks

In humans, the major expansion of the intestinal tract occurs during the last 15 weeks of gestation, when

there is an acceleration of its growth rate to ensure an adequate intestinal length to meet postnatal nutritional demands (Weaver et al., 1991). Insufficient intestinal length results in intestinal failure, which is defined as a reduction of the functional gut mass below that needed to sustain proper weight growth and maintenance in children and adults, respectively. One subtype of intestinal failure is short bowel syndrome (SBS), which can be defined as a residual small bowel length of less than 25% predicted by gestational age, mostly due to resection. The growing number of surviving preterm newborns suffering from necrotizing enterocolitis (NEC) and thus subject to bowel resection has resulted in an increase in the incidence of neonatal SBS (Wales et al., 2005; Barclay et al., 2010; Gutierrez et al., 2011). Neonatal SBS is associated with high mortality and morbidity due to surgical complications or infections resulting from parenteral nutrition, regardless of the etiology of SBS (Wales et al., 2005).

Short bowel syndrome management involves nutritional, surgical and pharmacologic interventions to achieve full enteral nutrition. Growth factors, specifically Growth Hormone, have been used in mice and humans to stimulate gut adaptation, yielding contradictory and short-term results (Barclay et al., 2010; Wales et al., 2010). In this context, a better knowledge of the signaling pathways and molecules that provide growth cues to promote intestinal elongation during development could be very useful to envision alternative treatment options for this condition. On one hand, the identification of novel therapeutic targets may help in designing specific therapies to promote intestinal adaptation after bowel resection. On the other hand, identifying the molecules that drive intestinal elongation *in utero* may contribute to defining the etiology of congenital SBS and better understanding this disease. However, we are only now beginning to understand the molecular and cellular mechanisms that orchestrate intestinal morphogenesis. Our knowledge of how different signaling events, summarized in this review, act in a coordinated fashion to promote appropriate intestinal length is far from being complete, and more studies are needed to achieve a comprehensive understanding of intestinal growth.

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