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REVIEW

The twists and turns of left-right asymmetric gut morphogenesis

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ABSTRACT

Many organs develop left-right asymmetric shapes and positions that are crucial for normal function. Indeed, anomalous laterality is associated with multiple severe birth defects. Although the events that initially orient the left-right body axis are beginning to be understood, the mechanisms that shape the asymmetries of individual organs remain less clear. Here, we summarize new evidence challenging century-old ideas about the development of stomach and intestine laterality. We compare classical and contemporary models of asymmetric gut morphogenesis and highlight key unanswered questions for future investigation.

KEY WORDS: Gut tube, Intestine, Laterality, Left-right asymmetry, Morphogenesis, Stomach

Introduction

Left-right (LR) asymmetry is a fundamental characteristic of vertebrate anatomy. In humans, this is evident in the left- or right-sided positioning of multiple organs in the thoracic and abdominal cavities (Fig. 1). Moreover, individual organs exhibit morphological laterality independent of their location, as evident in the unequally sized left and right lobes of the liver, or the disparate lobation patterns of the left versus right lungs. In many cases, these asymmetries are evolutionarily conserved and necessary for physiological function, indicating that the formation of LR asymmetry is an essential phase of vertebrate organogenesis. Indeed, abnormal LR axis formation – a condition known as heterotaxy – is associated with some of the most common and severe structural birth defects, including complex congenital heart defects (CHDs), intestinal malrotation, extrahepatic biliary atresia, asplenia/polysplenia and other anomalies (Bartram et al., 2005; Chinya et al., 2019; Desgrange et al., 2018; Gabriel and Lo, 2020; Kothari, 2014; Ticho et al., 2000). Therefore, understanding the mechanisms that shape individual organ lateralities is not only necessary for illuminating the morphogenesis of numerous organs but could also be crucial for explaining the etiology of a wide variety of birth defects.

Decades of studies have revealed the developmental processes that initially orient the LR body axis in vertebrate embryos (reviewed by Almirantis, 1995; Blum et al., 2014; Brueckner et al., 1991; Capdevila et al., 2000; Grimes and Burdine, 2017; Levin, 2005; Norris, 2012). In brief, a crucial phase of the symmetry-breaking process that occurs in many species involves cilia that are found within specialized LR organizer (LRO) regions (Dasgupta and Amack, 2016; Schweickert et al., 2017). The action of these cilia generates an asymmetrical fluid flow that establishes local asymmetries in gene expression (Cartwright et al., 2004; Okada et al., 2005; Schweickert et al., 2017; Smith et al., 2008), ultimately leading to the expression of Nodal, a transforming growth factor β superfamily ligand, in the left lateral plate mesoderm (LPM). Nodal then activates expression of the transcription factor Pitx2 in the left LPM and, ultimately, on the left side of developing organs, where it is required for the proper morphogenesis of various anatomical asymmetries (Burn and Hill, 2009; Campione et al., 1999; Davis et al., 2017; Liu et al., 2001; Mahadevan et al., 2014; Muller et al., 2003; Plageman et al., 2011; Ryan et al., 1998; Welsh et al., 2013; Womble et al., 2018). In some species, cilia are not involved and the early symmetry-breaking events remain unclear (Gros et al., 2009; Hamada and Tam, 2020; Kajikawa et al., 2020); nonetheless, the process culminates in LR asymmetrical gene expression in the LPM.

Although we know that asymmetrical Nodal/Pitx2 expression is required to confer 'leftness' to tissues left of the midline, what 'leftness' (or 'rightness') means, in terms of the morphogenesis of each individual organ, has been unclear and is only just beginning to be defined. Progress in understanding this last crucial phase of organogenesis has been slower than progress in elucidating the initial symmetry-breaking events. This is partly because, for most asymmetrical organs, the putative differences between the morphogenesis of their left and right sides is understudied or entirely unknown. Furthermore, what we think we know about the most basic developmental events that generate asymmetry in each organ is dominated by hypothetical descriptions proffered over one century ago by classical human embryologists, despite such ideas being supported by little quantitative or experimental data, or even being refuted.

In this Review, our goal is to reconcile historical models with contemporary studies that have provided new insights into asymmetric morphogenesis of the digestive organs, focusing on the stomach and intestine, but also touching on other gut-derived/associated organs such as the liver, pancreas, spleen and lung. We focus on tissue level changes, rather than molecular details, and aim to re-contextualize common dogma, highlight unanswered questions regarding the mechanisms that generate organ-specific LR asymmetries, and illuminate fruitful paths for future study.

Stomach curvature

The earliest LR asymmetrical morphology to appear during the development of the vertebrate digestive tract is the curvature of the stomach. This organ originates as a straight segment of the foregut that gradually acquires a leftward curved, J-shaped morphology with a convex (outward bending) curvature on the left side of the body and a concave (inward curling) curvature on the right (Fig. 1). The prevailing classical view of stomach development asserts that this curved shape arises as a result of repositioning the original dorsal wall of the stomach to the left side of the body via active 'rotation' of the primitive organ on its own longitudinal axis. In this model, the dorsal wall will become the convex side of the stomach (classically termed the 'greater' curvature), while the ventral wall, which is simultaneously rotated to the right side, becomes the concave side (or 'lesser' curvature; Fig. 2A).

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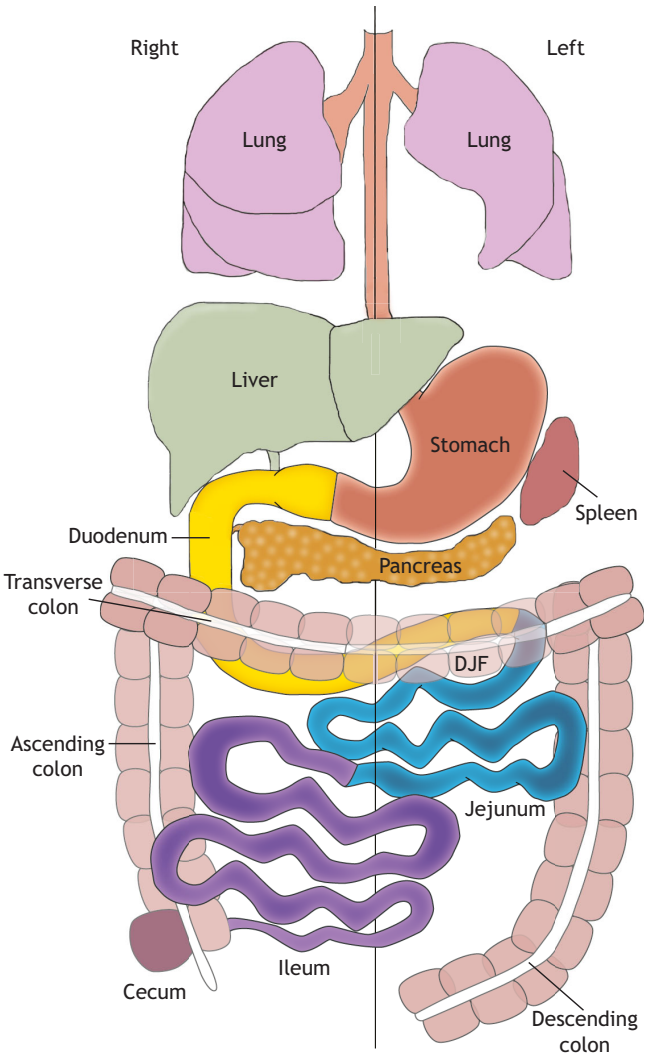

Fig. 1. The left-right asymmetry of gut derived organs. A cartoon of human anatomy shows the positions and morphologies of left-right asymmetrical organs derived from the embryonic primitive gut tube and/or gut-related tissues. Ventral view; the vertical line divides anatomical left and right sides. The stomach, small intestine [duodenum, duodenojejunal flexure (DJF), jejunum and ileum] and large intestine (ascending colon, cecum, transverse colon and descending colon) are segments of the primitive gut tube itself. The lungs, liver and pancreas bud off from the primitive gut tube, while the spleen originates from the splanchnic mesoderm surrounding the gut tube.

This classical 'rotation' model arose in the late 19th and early 20th century, based largely on two observations made by human embryologists. The first observation was that the dorsal mesogastrium, which anchors the stomach to the dorsal body wall, is ultimately found attached on the left side of the organ after curvature (Fig. 2A). Interestingly, the right side of the dorsal mesogastrium is known to undergo vacuolization and breakdown as the structure lengthens to form the greater omentum (Fig. 2A; Liebermann-Meffert, 2000). This remodeling process was presumed to enable the mobility of the attached stomach during its rotation, although the morphogenetic and/or biomechanical mechanisms capable of driving such large-scale organ movement remained unexplained. The second observation was that the left and right vagus nerves arise along the left and right side of the stomach early in development, but are later branched along the ventral and dorsal faces of the curved organ, respectively (Larsen, 2014). Together, these two observations led to the conclusion that, in order to give rise to the observed repositioning of associated mesogastria and nerves during curvature formation, the stomach must rotate around its dorsoventral axis (Fig. 2A).

Interestingly, an alternative model of stomach curvature morphogenesis was put forward by embryologists in the mid-20th century. This alternative view posited that the asymmetrical shape of the organ forms independently of its rotation. In this model, the development of curvature is attributed to predominant growth of the left side of the stomach over the right, such that the original left wall expands to form the convex side (Macarulla-Sanz et al., 1996; Miete, 1960). The concave side, by contrast, is derived from the original right side. This 'LR asymmetrical growth' model (Fig. 2B) originated from skepticism about the practicability of whole-organ rotation, as well as observed differences in epithelial architecture on the left versus right sides of the human stomach during curvature (Dankmejer and Miette, 1961).

Perhaps owing to the simplicity of the classical rotation model, the LR asymmetrical growth model is less prevalent in the literature and often overlooked in medical textbooks. This is likely because the non-rotation-based mechanism is thought to be at odds with the apparent rotation of the mesogastria and vagus nerves. However, proponents of the asymmetrical growth hypothesis argued that the connection of the dorsal mesogastrium to the dorsal midline of the stomach is not an immutable tether but a dynamic linkage that shifts as the stomach grows. They suggested that breakdown of the right side of the dorsal mesogastrium allows it to be 'pulled' to the left with expansion of the left stomach wall (Kanagasuntheram, 1957). In this model, the final position of the dorsal mesogastrium on the left side of the curved stomach is not indicative of a rotation event, but merely the result of the original midline attachment point shifting to the left as the organ grows preferentially leftward (Fig. 2B). But what about the left and right vagus nerves becoming located on the ventral/anterior and dorsal/posterior surfaces of the curved stomach? Non-rotation proponents point out that the left and right vagus nerves also assume 'rotated', i.e. ventral and dorsal, positions in the esophagus (Kanagasuntheram, 1957), a region of the gut that does not curve or rotate (Borghi et al., 2002). Therefore, the vagus nerves may pathfind by mechanisms independent of organ orientation per se, such that their final positions are potentially irrelevant to the argument of stomach curvature.

It should be noted that both the rotation and non-rotation (i.e. LR asymmetrical growth) models were proposed prior to the advent of modern developmental biology and were based largely on qualitative, retrospective observations of a series of preserved human embryos. However, several groups have recently addressed the origin of stomach curvature using quantitative methodologies and *in vivo* experiments in animal models. Interestingly, these studies largely support the non-rotation, LR asymmetrical growth hypothesis. For example, highly quantitative, 3D morphometric analyses of human embryonic stomachs revealed predominant growth of the left gastric wall over the right, finding little evidence for rotation around the dorsoventral axis that is proposed by the classical rotation model; only a gradual deflection of the stomach caudally and to the left was observed, thus reflecting differential growth (Kaigai et al., 2014; Nebot-Cegarra et al., 1999). More recently, animal model studies have directly addressed the cellular and molecular basis for stomach laterality (Davis et al., 2017). These revealed that, in both mouse and frog embryos, differences in the morphogenesis of the left versus right sides of the stomach facilitate preferential thinning and expansion of the left stomach wall, consistent with predominant 'growth' of the left side of the organ

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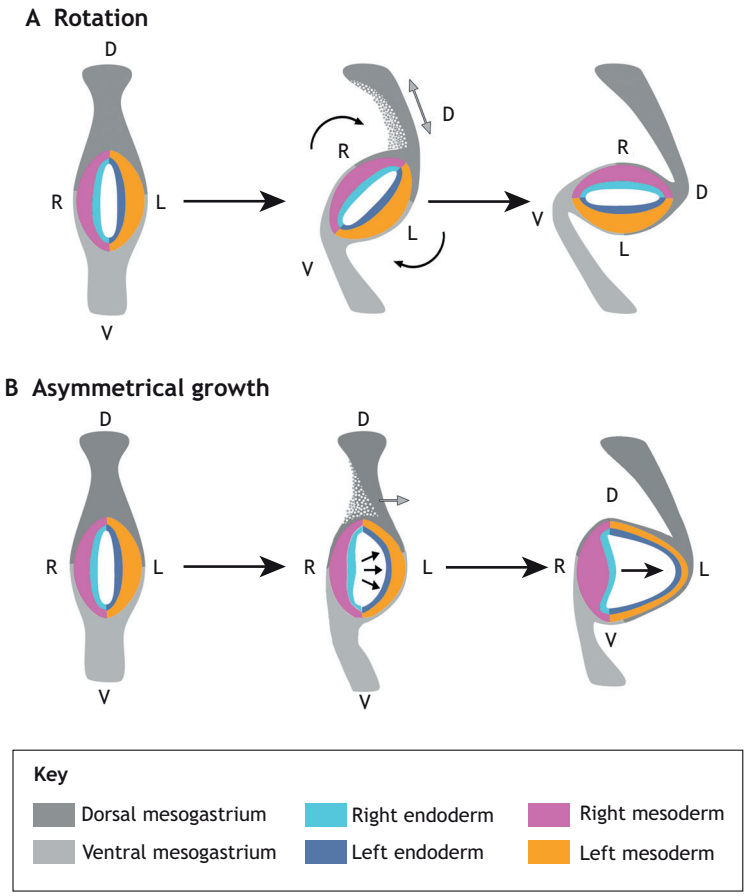

Fig. 2. Classical and contemporized models of stomach curvature. Cartoons depicting the successive stages of stomach development based on classical ('rotation'; A) and contemporary ('asymmetrical growth'; B) models. The stomach region of the primitive gut tube is shown in transverse cross-section and color coded to highlight the component tissue layers, including the dorsal mesogastrium (dark gray), ventral mesogastrium (light gray), right endoderm (light blue), left endoderm (dark blue), right mesoderm (pink) and left mesoderm (orange). The original dorsal (D), ventral (V), left (L) and right (R) faces of the organ are indicated to show their changing locations during stomach curvature. (A) In the classical 'rotation' model, the stomach is thought to rotate such that the original dorsal surface becomes the left side and the original ventral surface becomes the right side. The original right side of the dorsal mesogastrium then breaks down (as indicted by stippled gray shading) as it lengthens (gray arrow), but it remains connected to the original dorsal midline of the rotating stomach. (B) In the contemporary 'asymmetrical growth' model, rotation does not occur. Instead, the left wall of the embryonic stomach undergoes differential thinning and expansion. Breakdown of the original right side of the dorsal mesogastrium (stippled gray shading) enables the connection point of the dorsal mesogastrium with the stomach to shift to the left (gray arrow) as the left wall expands outward (black arrows).

(Fig. 2B). Surprisingly, this expansion was found to be independent of LR asymmetries in cell numbers; instead, it appears to be driven by asymmetrical cell rearrangements, facilitated by precocious polarization and radial intercalary rearrangement of the endoderm cells that differentiate into the gastric epithelium in the left stomach wall (Davis et al., 2017; see Fig. 4B). This early morphogenetic difference is thought to leave the primitive stomach tube with unequal left and right side dimensions, manifested as whole organ-scale curvature. In both species, the changes in LR asymmetric cell polarization and rearrangement were found to be dependent on earlier LR patterning events, including cilia function (in mouse), Nodal activity (in frog), and left side-specific activity of Pitx2c (in frog) (Davis et al., 2017). Thus, accumulating evidence suggests that, despite the widespread propagation of the rotation theory, the early curvature of the stomach is likely generated by asymmetrical growth of its contralateral walls.

Intestine morphogenesis

Caudal/posterior to the curved stomach, discrete segments of the small and large intestine also become positioned to the left or right of the midline. In humans, for example, the descending duodenum is on the right, the duodenojejunal flexure is on the left, the ascending colon and cecum are on the right, and the descending colon is on the left (Fig. 1). These asymmetries are derived from the development of the embryonic midgut, which forms a prominent 'primary' loop during gut morphogenesis (Fig. 3A). The cranial limb of this loop is composed of the future duodenum, jejunum and proximal ileum, while the caudal limb will become the distal ileum, cecum and colon [the cranial and caudal limbs were traditionally designated based on their relative position with respect to the superior mesenteric artery (SMA); Fig. 3A].

Historically, the process of establishing small and large intestinal laterality was deemed to occur via 'rotation'. This was because the final anatomical positions of the various midgut derivatives and the twisted appearance of the attached mesentery suggested that the cranial and caudal limbs of the primary loop must rotate around each other in a counter-clockwise (CCW) direction during development (Blechschmidt and Kircheiss, 1973; Gasser, 1975; Kim et al., 2003; Mall, 1898; Snyder and Chaffin, 1954). In this classical view (the 'Rotation' model, Fig. 3B), the entire midgut loop, as a complete

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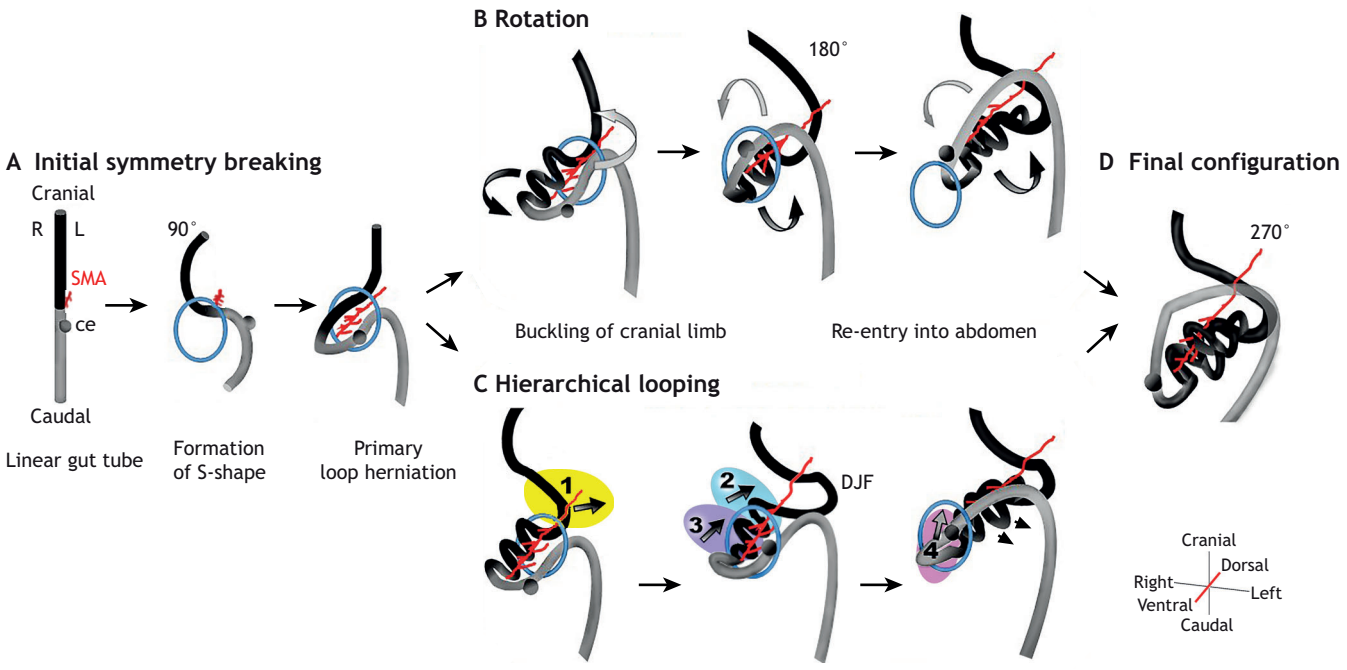

Fig. 3. Classical and contemporary models of asymmetric intestine morphogenesis. The midgut is divided into cranial (black) and caudal (gray) segments, defined by their position with respect to the superior mesenteric artery (SMA, red). The cecum (ce) is depicted as a small spherical protrusion from the caudal limb. (A) The initial symmetry of the gut tube is broken by the formation of an 'S-shape'. The contiguous cranial and caudal segments then form a primary loop that undergoes herniation beyond the boundary of the body wall (blue ring) into the umbilical coelom ('primary loop herniation'). Successive stages according to classical (B, 'rotation') or contemporary (C, 'hierarchical looping') models are depicted. In both models, the cranial limb begins to elongate extensively, buckling to form smaller loops depicted as sine-wave undulations. (B) The rotation model assumes that the initial S-shape represents the first 90° of a full 270° counter-clockwise (CCW) rotation process around the SMA; the entire primary loop then undergoes 180° of additional CCW rotation during and/or after its re-entry into the abdominal space. (C) In the hierarchical looping model, four secondary loops locally and sequentially orchestrate anatomical asymmetry. The first loop (1, yellow) forms in the intra-abdominal region of the cranial limb and becomes the duodenojejunal flexure (DJF). Subsequently, the second (distal jejunum, 2, blue) and third (proximal ileum, 3, purple) secondary loops form in the umbilical region of the cranial limb. These loops re-enter the body cavity first, and then shift leftward, likely guided by their connection with the left-sided DJF. The fourth secondary loop (distal ileum and cecum, 4, pink) then forms in the caudal limb and re-enters last, sliding into the remaining space on the right. (D) Both models result in the same asymmetrical intestine configuration. Cranial-caudal, dorsal-ventral and left-right axes are indicated.

entity, is thought to undergo a series of twisting turns, ultimately completing a 270° course of rotation to place the various segments of the intestine and colon in their final left- or right-sided locations. The first 90° rotation occurs prior to formation of the primary loop itself, as the cranial end of the primitive midgut bends to the right and the caudal end to the left, forming an 'S'-shape when viewed ventrally (Fig. 3A; Snyder and Chaffin, 1954). In amniotes, rapid lengthening of the midgut tube compared with the embryonic body cavity then leads to extension of the partially rotated primary loop into the umbilical space (known as the physiological umbilical hernia; see Fig. 3A); later, the gut must re-enter the abdominal cavity. According to the rotation model, an additional 180° CCW rotation occurs while the midgut is inside the umbilicus (Kim et al., 2003) and/or as it undergoes re-entry (Frazer and Robbins, 1915; Snyder and Chaffin, 1954); the cranial limb shifts caudally and then to the left, and the caudal limb moves cranially and then to the right (Fig. 3B). In this classical view, the cranial and caudal limbs of the primary loop cross over each other as they rotate, twisting around the SMA at the root of the mesentery, to place the segments of the mature duodenum, jejunum, ileum, cecum and colon in their final left- or right-sided positions (Fig. 3D; Kim et al., 2003; Mall, 1898; Snyder and Chaffin, 1954). In humans with congenital intestinal defects, the abnormal relative locations of these segments were interpreted to be the result of the midgut failing to properly complete the rotation process, leading to such anatomical configurations being termed intestinal 'malrotations' (Andrew, 1961; Deitch and Engel, 1980; Dott, 1923; Freitas and Ventura, 1980; Glowniak, 1988; Price and Kane, 1955; Schwalbe, 1906; Torres and Ziegler, 1993; Valioulis et al., 1997).

Like stomach rotation, the concept of midgut rotation distills the convoluted topological transformations of intestine morphogenesis into a straightforward succession of rotating turns; this likely underlies the persistence of the century-old rotation model in modern literature and textbooks. However, over the years, several authors have questioned the assumption that intestine laterality is derived from such a user-friendly operation, suggesting that the rotation model is oversimplified and misleading. Below, we discuss classical and recent observations that challenge 'simple' rotation as the mechanism that generates intestine laterality, and outline an alternative interpretation of the process as a hierarchical series of spatiotemporally coordinated looping and lengthening events that occur in discrete regions of the midgut (Fig. 3C).

As mentioned above, a key symmetry-breaking event in midgut morphogenesis is the formation of the initial S-shape in which the cranial portion of the primitive midgut curves to the right, while the caudal region curves to the left (Fig. 3A). Although the classical rotation model interprets this configuration as the initial 90° turn in an active 270° rotation process (Snyder and Chaffin, 1954), recent

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3D imaging analyses of human embryos (Hikspoors et al., 2018; Soffers et al., 2015) suggest that the contour of the early midgut loop merely follows the helical shape of the body axis, which is created by the normal 'turning' of the mammalian embryo – a process that happens to coincide with the early stages of gut development. Moreover, it has been noted that the formation of the S-shape coincides with the normal caudalward descent of the curved stomach, which would logically be expected to shift the cranial end/limb of the midgut (which is contiguous with the right-sided, pyloric end of the stomach) caudally and rightward (Hikspoors et al., 2018; Soffers et al., 2015). Thus, although the first break in midgut symmetry may be described (geometrically) as a 90° rotation of the primitive midgut, this configuration alone is not necessarily evidence of an active organ-scale rotation process, as the S-shape could arise as a passive response to the pre-existing helical asymmetry of the body axis and/or the influence of apposed organs or attached tissues (discussed later).

After the establishment of the S-shape, continued lengthening of the midgut causes it to form the primary loop, the apex of which protrudes into the umbilicus, with cranial and caudal limbs still in their partially 'rotated' right- and left-sided positions (Fig. 3A). While herniated, the cranial limb then forms smaller loops along its length, resembling 'sine wave' undulations, likely a result of the rapidly lengthening gut tube buckling under the mechanical strain of its attachment to the mesentery (Fig. 3B; Savin et al., 2011); these smaller loops have recently been termed 'tertiary loops' (discussed in detail later). In contrast, the caudal limb does not undergo such convolutions, allowing it to be visually distinguished from the cranial limb. Multiple authors have used this morphological distinction to observe that, while herniated, the segments of the cranial and caudal limbs leading to (and contained within) the umbilicus do not alter their positions or twist around each other but remain on the right and left sides of the SMA, respectively. Thus, in contrast to depictions in classical rotation models, the midgut loop does not actually rotate prior to re-entry (compare Fig. 3B with C; Frazer and Robbins, 1915; Hikspoors et al., 2018; Soffers et al., 2015). Moreover, both classical and modern studies have noted that the cecum – a morphological landmark at the apex of the midgut loop, often used to track rotation – moves only 'passively' while herniated, i.e. it adopts different orientations as the mass of intestinal loops forms in the cranial limb but nonetheless remains to the left of the midline until re-entry (Fig. 3C; Frazer and Robbins, 1915; Metzger et al., 2011; Ueda et al., 2016). Consequently, the position of the cecum, although often depicted as adopting successively more CCW orientations during intestinal rotation (as in Fig. 3B; Sivakumar et al., 2018), is likely not an accurate reflection of gut *situs*. Indeed, in several species (e.g. rat and chick), the tip of the midgut loop even appears to (at least initially) rotate clockwise (Metzger et al., 2011; Southwell, 2006).

While the umbilical region of the cranial limb is forming its mass of smaller loops, the segment of this limb still inside the abdominal cavity, which includes the duodenum and proximal jejunum, forms only a single large loop (labelled as '1' in Fig. 3C). This intra-abdominal loop (i.e. as opposed to the herniated, intra-umbilical loops) originates on the right side but elongates extensively, crossing the midline caudal to the SMA, ultimately becoming the left-sided duodenojejunal flexure (DJF; see Figs 1 and 3C; Kluth et al., 1995; Kluth et al., 2003; Metzger et al., 2011). The final asymmetrical position of the DJF shifts the cranial end of the midgut leftward, thereby influencing the motility and orientation of the contiguous jejunal and ileal loops (Long et al., 1996). Indeed, striking correlations between intestinal malrotation/volvulus and abnormal orientations of the DJF in humans suggest proper morphogenesis of this unique intra-abdominal loop is essential for normal intestine laterality (Long et al., 1996). Although classical embryologists concluded that the DJF must be brought into its critical left-sided position by the coordinated rotation of the entire midgut, it actually achieves its asymmetric location early in gut development, well before the hypothetical rotation and re-entry of the herniated segments of the primary loop (Bardeen, 1914; Kluth et al., 2003). Thus, at least one essential intestinal laterality must form independently of the hypothetical rotation process (see also 'Hierarchical looping' model below).

Proponents of the rotation model suggested that growth of the caudal limb during and/or after re-entry into the body cavity must provide the force to propel rotational movement of the primary loop (Frazer and Robbins, 1915). In this view, the caudal limb would push the cranial limb leftward as it extends further rightward, each limb presumably re-orienting the other in a yin-yang duality (Fig. 3B). Yet multiple studies (Bardeen, 1914; Mall, 1898; Ueda et al., 2016) have shown that the caudal limb does not grow rapidly or extensively enough to impel the hypothesized rotation. Moreover, although the mechanisms that underlie re-entry remain poorly understood, it has been known since the early 20th century (Frazer and Robbins, 1915) that the herniated intestine does not return to the body cavity all at once. Instead, the mass of jejunal and ileal coils (derived from the cranial limb) returns to the body cavity first and moves to the left while the cecum and colon (derived from the caudal limb) are still herniated (Fig. 3C; Frazer and Robbins, 1915). Thus, the derivatives of the cranial limb assume their respective LR positions independently of the growth trajectory of the caudal limb.

The discrepancies discussed above, especially the spatially compartmentalized growth of the intra-abdominal (i.e. the DJF) versus herniated segments of the cranial limb, and the temporally independent re-entry of the cranial versus caudal limbs, suggest that coordinated rotation of the entire midgut during development is an implausible mechanism for generating digestive laterality. Without an actively orchestrated rotation process how, then, is the final LR positioning of the various derivatives of the midgut accomplished? Interestingly, recent 3D reconstructions of discrete stages of human intestine development (Soffers et al., 2015) have led to the rediscovery and further characterization of a second generation of four invariant 'secondary' loops that form along the length of the midgut. These anatomical features, first recognized over one century ago (Mall, 1898), are demarcated by areas of shorter mesentery between them, an embryological artifact that remains identifiable even in the adult intestine (Mall, 1898). These four stereotypical loops form in a predictable, hierarchical order along the cranial-caudal axis of the midgut, corresponding to the order of their re-entry into the body cavity (Fig. 3C; Soffers et al., 2015).

The identification of these loops led to the proposition of the 'Hierarchical looping' model (Fig. 3C; Soffers et al., 2015). In this model, the first secondary loop forms within the intra-abdominal region of the cranial limb (labelled '1' in Fig. 3C; Soffers et al., 2015). As discussed above, this first secondary loop becomes the DJF, elongating extensively to assume a left-sided position in the body prior to re-entry of the herniated loops. The second and third secondary loops (labelled '2' and '3' in Fig. 3C) form in the intra-umbilical segments of the cranial limb, within the distal jejunum and proximal ileum, respectively. During re-entry, the coils of these second and third secondary loops pass sequentially under the SMA, spreading to the left, likely guided by their connection with the left-sided DJF. The fourth secondary loop (labelled '4' in Fig. 3C) forms last, in the caudal limb, between the tip of the primary loop and the

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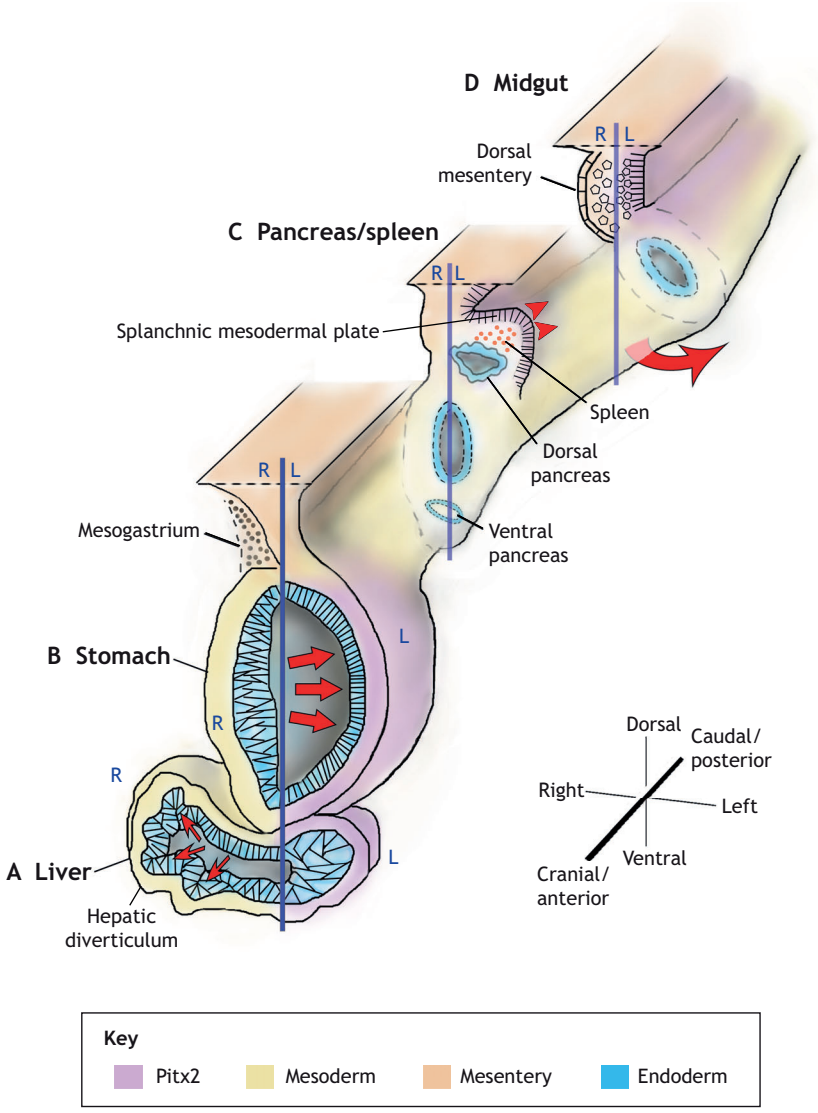

Fig. 4. Divergent mechanisms of asymmetric morphogenesis. Multiple asymmetric morphogenesis events are depicted in a generic vertebrate embryonic gut tube (left anterior view). Cellular and molecular asymmetries arise in foregut and midgut tissues derived directly from the lateral plate mesoderm (LPM), i.e. in the outer layer of the gut tube (yellow) or in the mesenteries (orange) that suspend the tube from the body wall. Asymmetries also develop in underlying endoderm (blue) tissues in direct apposition to LPM-derived mesoderm. In each case, *Pitx2* (purple) is locally expressed on the left side; however, the exact tissue layers and cellular events involved vary for each organ and/or region. (A) *Pitx2* is expressed in the mesoderm surrounding the left side of the hepatic diverticulum and suppresses changes in cell shape and rearrangement in the underlying left endodermal epithelium, enabling the left side to remain condensed while the right side expands (thin arrows). These early asymmetries are associated with later differences in the size and morphology of the left and right liver lobes. (B) *Pitx2* is also expressed in the mesoderm and/or endoderm layers of the left stomach wall (depending on species), and is required for left endoderm cells to polarize and rearrange into a thinner expanded layer. This asymmetry causes the left wall to bulge outward (thick arrows), ultimately driving leftward curvature of the entire stomach tube. Although LR asymmetric morphogenesis events occur in the endoderm layer of both the stomach and liver, the resultant tissue-level changes on each side are very different, e.g. the left stomach wall thins and expands, while the left side of the hepatic diverticulum remains condensed. (C) Posterior to the stomach, *Pitx2* is expressed in the outer epithelial layer on the left side of the dorsal mesentery, in the splanchnic mesodermal plate (SMP), and is required to retain its columnar morphology. The left SMP consequently undergoes outgrowth (arrowheads) as the underlying left mesenchyme proliferates to form the spleen, and the dorsal pancreatic endoderm is recruited leftward. (D) In the early midgut dorsal mesentery, the asymmetrical expression of *Pitx2* regulates the emergence of LR asymmetries in cell shape, polarity and ECM remodeling that cause the right side of the mesentery to expand while the left side condenses. As a consequence, the mesentery and midgut tube tilt leftward (curved arrow); the relationship of this early tilt to the formation of the initial midgut S-shape and later intestinal laterality is unresolved. Interestingly, in multiple species, *Pitx2* is also expressed asymmetrically in the mesoderm layer of the gut tube itself; however, its role in asymmetric gut morphogenesis is unknown. Not all mesentery-derived tissues undergo the same tissue-level shape changes, e.g. the left SMP exhibits outgrowth while the left midgut mesentery condenses. Organs and regions depicted are not to scale; the relative cranial-caudal positioning of each organ has been adjusted for ease of illustration on the same schematic, and the asymmetries shown do not necessarily exist at the same developmental stage or in all vertebrates.

cecum, within the distal ileum (Soffers et al., 2015). This fourth loop is thought to be the last to re-enter the body cavity, filling the remaining available space on the right (Metzger et al., 2011; Soffers et al., 2015). Following re-entry, the distal ileum and cecum then slide dorso-caudally (rather than rotate) into their final position in the right lower abdomen (Fig. 3D).

In this contemporized view, individual segments of the midgut assume their left- or right-sided anatomical positions according to

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the order of looping and/or re-entry, the space available in the abdominal cavity, and/or the relative orientation and forces provided by neighboring intra-abdominal segments of the gut tube. Thus, according to the hierarchical looping model, the final orientation of the intestine segments, flexures and twisted mesentery merely creates the illusion that the gut rotates 270° around the SMA; the end result is the same but the developmental process generating the final laterality is distinctly different. Although more challenging to comprehend than a coordinated rotation process, hierarchical looping has the advantage of being entirely compatible with the spatially compartmentalized and temporally distinct development of the cranial versus caudal and intra-abdominal versus intra-umbilical segments of the primary midgut loop discussed above. This re-interpretation may have important implications for future studies of the morphogenetic mechanisms that underlie normal and abnormal intestinal laterality (discussed in detail by Soffers et al., 2015).

The asymmetric morphogenesis of other organs

It has been hypothesized that, during vertebrate evolution, the primitive gut tube is the first structure to acquire LR asymmetry (i.e. as opposed to the heart; Blum et al., 2014), allowing longer, compartmentalized digestive tracts to be consistently packaged within the body cavity. However, LR asymmetries are not only formed by segments of the gut tube itself; many other lateralities in digestive and non-digestive organs originate from gut tube-derived or -associated structures (Fig. 1). Below, we touch briefly on a few examples for which recent studies have begun to shed light on the morphogenesis of anatomical LR asymmetries (Fig. 4).

Liver

The liver diverticulum buds off from the ventral surface of the primitive gut tube at the foregut-midgut boundary, posterior to the stomach. Notwithstanding some variation in accessory lobes between species, the right side of the liver usually becomes larger than the left side (Abdel-Misih and Bloomston, 2010). The classical explanation for this asymmetry is that the displacement of the cardiac sinus venosus to the right during heart looping enables greater blood flow (i.e. a growth advantage) to the right side of the embryonic liver (Grover and Moore, 1988). However, even prior to vascularization (i.e. shortly after the initial budding of the liver diverticulum from the primitive gut tube), the majority of the volume of the organ was found to lie on the right, suggesting the early bud is already inherently LR asymmetric (Grover and Moore, 1988; Heisler, 1907). Indeed, recent lineage tracing investigations identified the presence of left- and right-side clones in the embryonic liver, suggesting that cells contributing to each lobe become segregated early, and that the left and right halves of the organ develop largely independently (Weiss et al., 2016).

Consistent with this idea, a recent study in *Xenopus* showed that, as the early liver diverticulum buds off from the gut tube, hepatic endoderm cells on the right side become more apically constricted, with a greater length-to-width ratio, whereas left-sided cells appear rounder and more compact, indicating that distinct left- and right-sided morphogenetic programs are distinguished very early in liver morphogenesis (Fig. 4A; Womble et al., 2018). In this study, asymmetric expression of *pitx2c* in the mesoderm surrounding the left side of the early hepatic diverticulum (Fig. 4A) was found to be necessary and sufficient to elicit left-sided epithelial morphogenesis and lobe formation in the underlying endoderm. The nature of the molecular events mediating this tissue interaction, and the relationship between early asymmetries in epithelial architecture and later differences in the gross size and shape of the left versus right lobes, remain to be determined. *Pitx2c* is also expressed in the left side of the septum transversum mesenchyme surrounding the budding liver in mammalian embryos (Shiratori et al., 2006), but whether similar asymmetries in the cellular morphogenesis of the hepatic endoderm exist in other vertebrates, and how such differences might affect the later morphogenesis of the mammalian liver, have not been investigated.

In zebrafish, the liver bud does not exhibit (obvious) asymmetrical lobation but the organ does become positioned asymmetrically, albeit on the left (not right) side of the gut tube, as driven by asymmetrical leftward migration of early hepatoblasts from the hepatic endoderm. This process is dependent on epithelial-mesenchymal interactions mediated via ephrin B1/EphB3b signaling (Cayuso et al., 2016). Although hepatic expression of ephrin B1 has also been reported in other species (Costa et al., 2003; Fletcher et al., 1994), it is unknown if this pathway is involved in asymmetrical liver lobation or anatomical positioning in other vertebrates.

Pancreas and spleen

Both the pancreas and the spleen arise at the foregut-midgut boundary (Brendolan et al., 2007; Jennings et al., 2013) and eventually become situated on the left side of the body near the convex side of the stomach (Fig. 1). These tissues arise within the dorsal mesentery (DM), an embryonic structure derived from the splanchnic layer of the LPM that connects the primitive gut tube to the body wall (Fig. 4C). A thickened columnar epithelium known as the splanchnic mesoderm plate (SMP; Green, 1967; Hecksher-Sørensen et al., 2004) covers the DM from the posterior stomach to the anterior duodenum. Although the SMP is initially bilaterally symmetrical, the SMP on the right recedes while the left SMP, which expresses *Pitx2*, maintains its columnar morphology, undergoes rapid proliferation and bulges outward to the left of the gut tube. The leftward outgrowth of the SMP and underlying splenopancreatic mesenchyme forms a dorsal, left-sided protrusion posterior to the stomach that contains both the spleen anlage and the dorsal pancreas bud (Fig. 4C). In embryos with no or abnormal SMP development, the spleen is absent and the pancreas adopts abnormal orientations (Hecksher-Sørensen et al., 2004). Moreover, in embryos with a reversed LR axis (i.e. *inv/inv* mice; Watanabe et al., 2003), the SMP bulges towards the right side, confirming its asymmetry is determined by global LR patterning cues (Hecksher-Sørensen et al., 2004).

It should be noted that, although the asymmetrical influence of the SMP may compel the dorsal pancreas to grow to the left of the midline, the morphogenesis of the mature pancreas involves the fusion of both dorsal and ventral pancreas rudiments. Although the fusion process itself remains virtually unstudied, it is often attributed to the rotation of the gut tube (Gittes, 2009; Jennings et al., 2013; Pan and Brissova, 2014), with most textbooks illustrating the ventral pancreas bud(s) moving rightward around to the dorsal side of a rotating duodenum to meet the dorsal bud (Larsen, 2014). However, the means by which such a translocation might occur are unknown.

Lungs

In many vertebrates, the left lung exhibits fewer lobes than its right counterpart (Matthew et al., 2009). For example, in humans, the left lung has two lobes whereas the right lung is trilobed (Fig. 1; Larsen, 2014). In individuals with aberrant LR asymmetry, the laterality of lung lobation may be reversed or even eliminated (e.g. both lungs develop a bilobed left lung or a trilobed right lung morphology; Aylsworth, 2001; Casey, 1998; Lin et al., 2014; Yim et al., 2018). Early in foregut development, the left and right lung buds emerge

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from the foregut just anterior to the stomach and undergo distinct branching events; the bifurcating patterns are reversed in embryos with reversed LR asymmetry, suggesting that left and right lung morphologies result from early initiation of disparate side-specific morphogenetic programs (Metzger et al., 2008). The left side program is likely specified by *Pitx2c*, which is expressed only in the left lung in mice, as loss of *Pitx2c* leads to both lungs developing right lung morphology (Cardoso and Lü, 2006; Hogan, 1999; Liu et al., 2001). However, the downstream cellular events and molecular targets of Pitx2c in the lung remain unknown. Interestingly, a recent investigation suggested that, in snakes, the left lung often develops as a shortened or even vestigial organ (van Soldt et al., 2015). This LR asymmetry is hypothesized to be achieved by the left lung bud undergoing slowed or arrested growth compared to the right (van Soldt et al., 2015). It remains to be seen whether such mechanisms contribute to the Pitx2-mediated asymmetries in lung morphology observed in other vertebrates.

Similarities and differences between asymmetric organs

Although our knowledge of asymmetric gut morphogenesis is still limited, some commonalities between organs may be noted. For example, in every organ, the cell populations involved originate from the splanchnic layer of the LPM and/or from endoderm immediately juxtaposed to splanchnic mesoderm-derived tissues (Fig. 4). Thus, the executors of asymmetric organ morphogenesis are directly derived from, or influenced by, the LPM, i.e. the tissue in which LR asymmetric gene expression patterns are broadly established earlier in development (although the exact, organ-specific fates of *Nodal/Pitx2*-expressing cell populations from the LPM have not been explicitly delineated for most species). Another common theme is that the emergence of laterality occurs early in organogenesis and often involves LR asymmetries in the architecture of mesoderm- and/or endoderm-derived epithelial tissues within or associated with each organ. Finally, these early tissue asymmetries are all directly or indirectly dependent on late-stage, organ-specific (left-sided) expression of *Pitx2* within the developing gut tube and/or its associated mesenteries (Fig. 4).

Despite these similarities, there are many differences in the morphogenetic programs that drive the formation of asymmetric morphology in each discrete region of the gut tube (Fig. 4). For example, it is obvious that there is no universal tissue-level shape change that signifies ‘leftness’ or ‘rightness’. The left stomach wall thins and expands while the left side of the liver bud remains compact. Likewise, while tissue expansion characterizes the left side of the dorsal mesentery-derived SMP, tissue condensation contracts the left side of the midgut dorsal mesentery (see below). Furthermore, while the endoderm exhibits cellular-level asymmetries in the developing stomach and liver, no such lateralities have been identified in this tissue layer in the early midgut (Davis et al., 2008). The underlying cellular mechanisms at play in each tissue also vary widely, from LR differences in proliferation to asymmetries in cell shape, polarity, rearrangement and/or ECM remodeling (Davis et al., 2008, 2017; Hecksher-Sørensen et al., 2004; Kurpios et al., 2008; Sivakumar et al., 2018; Welsh et al., 2013; Womble et al., 2018; Fig. 4). Finally, at the molecular level, there appears to be little consensus regarding the effectors and networks that function in parallel with or downstream of Pitx2, although only a small number of genes are currently known to be associated with asymmetric organ morphogenesis (Table 1). Further elucidation of the relevant tissue-, cellular- and molecular-level asymmetries operating in both the left and right sides of multiple organs is necessary to determine whether this apparent divergence might in fact be underlain by common themes.

Unanswered questions in the field

With new insights into the development of stomach and intestine laterality, the field is now poised to address key unanswered questions in the context of contemporized models of asymmetrical gut morphogenesis.

Table 1. Molecules expressed asymmetrically in the gut tube and associated tissues

Gene	Expression	References
Transcription factors		
Pitx2	Left stomach, left gut tube, left DM, left SMP, left liver	Davis et al. (2008), Hecksher-Sørensen et al. (2004), Liu et al. (2001), Logan et al. (1998), Sivakumar et al.(2018), Welsh et al. (2013), Womble et al. (2018)
Isl1	Left stomach, left DM	Davis et al. (2008), Yuan and Schoenwolf, (2000)
Barx1	Left SMP, left stomach	Hecksher-Sørensen et al. (2004), Kim et al. (2007), Kim et al. (2005)
Tbx18	Right DM	Davis et al. (2008)
Nkx3-2 (*Bapx1*)	Left SMP	Hecksher-Sørensen et al. (2004)
Capsulin (*Tcf21*; *Pod1*)	Left SMP	Hecksher-Sørensen et al. (2004), Lu et al. (2000)
Hox11	Left SMP	Dear et al. (1995), Hecksher-Sørensen et al. (2004), Roberts et al. (1994)
Nkx2-5	Left SMP	Burn et al. (2008)
Wt1	Left SMP	Hecksher-Sørensen et al. (2004), Herzer et al. (1999)
Signaling molecules		
Daam2	Left DM	Welsh et al. (2013)
Fzd8	Left DM	Welsh et al. (2013)
Fzd4	Left DM	Welsh et al. (2013)
Gpc3	Left DM	Welsh et al. (2013)
Prickle1	Right DM	Welsh et al. (2013)
Sfrp1	Right DM	Welsh et al. (2013)
Sfrp2	Right DM	Welsh et al. (2013)
Growth factors		
Cxcl12	Left DM	Sivakumar et al. (2018)
Fgf10	Left SMP	Hecksher-Sørensen et al. (2004)
Cell-adhesion proteins		
N-cadherin (*Cdh2*)	Left DM	Kurpios et al. (2008)
Tsg6 (*Tnfaip6*)	Right DM	Sivakumar et al. (2018)

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What factors contribute to stomach curvature?

Although the early curvature of the stomach is initially generated by asymmetrical expansion of its contralateral walls, the curved organ does rotate to varying degrees in different directions as it grows larger and descends into the abdominal cavity (Kaigai et al., 2014), and other factors contribute to its final size, shape and anatomical orientation. For example, Wnt/PCP signaling contributes to the lengthening of the anterior stomach via oriented cell division (Matsuyama et al., 2009). However, the relationship between this process and the radial intercalation of endoderm cells (Davis et al., 2017) is unknown. Likewise, the mesoderm layer of the developing stomach also exhibits LR asymmetries in thickness and tissue architecture (Davis et al., 2017), but the cellular morphogenetic events that underlie these LR differences, and their influence on the nascent gastric musculature or vagus innervation remain to be elucidated. There is some evidence for interaction between the mesoderm and endoderm tissue layers (Davis et al., 2017) and, although Pitx2 targets are likely involved, the molecules that mediate this interplay have not been identified. Whether instructive morphogenetic changes also occur on the right side of the stomach, and whether there is coordination and crosstalk between the contralateral sides, are intriguing unresolved issues with important implications for the development of other asymmetric organs. Finally, it will be interesting to ascertain whether and/or how tissues outside the stomach tube itself (e.g. the splanchnic mesoderm-derived, asymmetrically remodeling mesogastrium) influence the process of curvature, and how the various cellular- and tissue-level asymmetries in the developing stomach influence, and are influenced by, the dynamic mechanical properties of the entire organ or neighboring regions of the gut tube. Indeed, it is intriguing that the stomach lies in the midst of a concentrated zone of asymmetric morphogenesis at the foregut-midgut boundary that also gives rise to several other asymmetric structures, including the lungs, pancreas, spleen and liver (Fig. 4). Thus, defining the spatiotemporal and mechanistic inter-relationships between the morphogenesis of the stomach walls, mesogastrium, lungs, SMP and hepatic diverticulum has the potential to yield tremendous insight into how multiple organogenesis events are coordinated to generate consistent anatomical complexity. In the context of experimental models of abnormal LR asymmetry (e.g. Hummel and Chapman, 1959; Ryan et al., 1998; Sempou and Khokha, 2019; Yokoyama et al., 1993), such studies may help explain the convoluted, seemingly random anatomical patterns and combinations of laterality-related organ defects seen in humans (Casey, 1998; Shiraishi and Ichikawa, 2012; Sutherland and Ware, 2009).

How does the midgut ‘S-shape’ arise?

The formation of the initial S-shape of the midgut places the cranial limb of the prospective primary loop on the right and the caudal limb on the left, making it arguably the most important symmetry-breaking event in intestine morphogenesis. However, the mechanisms that underlie the development of this key morphological feature remain unclear. As discussed above, it is hypothesized that the formation of the S-shape may be a passive response to the constraints of a helical body axis and/or the descent of more cranial organs. However, the midgut also forms an initial S-shape in non-amniote vertebrates that do not have helical body axes, e.g. *Xenopus* (Muller et al., 2003), suggesting other factors must contribute to the initial break in midgut symmetry, at least in lower vertebrates. Moreover, active symmetry-breaking events linked to LR patterning pathways may influence the formation of this crucial asymmetry in amniote species (discussed below).

Despite the enigmatic expression of *Pitx2* on the left side of the primitive midgut (Fig. 4D; Logan et al., 1998; Piedra et al., 1998), cellular- or tissue-level asymmetries have yet to be found in the early midgut tube itself (Kurpios et al., 2008). However, prior to the formation of the S-shape, striking differences in cell/tissue morphology, extracellular matrix (ECM) composition and gene expression exist between the left and right sides of the DM that suspends the midgut, i.e. posterior and/or caudal to the SMP (see Fig. 4D and Table 1; Davis et al., 2008; Kurpios et al., 2008; Welsh et al., 2013). Tissue expansion on the right side of the DM, followed by Pitx2-dependent compression on the left side (Sivakumar et al., 2018), creates an asymmetrical architecture that is essential for properly lateralized gut vasculature (Mahadevan et al., 2014; Sivakumar et al., 2018). In addition, because the asymmetrical morphogenesis of this region distorts the shape of the mesenteric stalk itself, tilting the attached gut tube to the left, it has been hypothesized that asymmetrical remodeling of the DM is also the first step in establishing the intestine laterality that is observed later (Davis et al., 2008). However, it is not obvious exactly how the early DM asymmetry might affect the later morphogenesis of the intestine, as the tilting occurs early, during closure of the open midgut into a tube and prior to primary loop formation (Davis et al., 2008; Kurpios et al., 2008; Sivakumar et al., 2018; Southwell, 2006; Welsh et al., 2013). It has been hypothesized that the early DM tilt could somehow influence the initial S-shape of the midgut (Davis et al., 2008), although the cranial region of the ‘S’ curves rightward, in the opposite direction to the mesentery-mediated leftward tilt. It is also possible that the leftward tilt of the DM somehow influences the laterality of a mechanical buckling event in the early midgut, resulting in the S-shape (Davis et al., 2008), although this remains to be demonstrated. Experiments that perturb DM tilting in the chick embryo do result in abnormal gut laterality, as revealed by reversals in the curvature of the stomach (Davis et al., 2008). Unfortunately, however, the experimentally manipulated embryos do not survive to the point of midgut looping (Davis et al., 2008), and the intestine of the chicken does not develop the same ‘rotated’ LR asymmetries as the mammalian tract (Southwell, 2006), so the relationship between DM tilting and specific intestine lateralities may only be inferred in the chick model. However, in mice, knockout of an ECM-modifying gene normally restricted to the right side of the cranial DM, *Tsg6*, was recently found to cause striking intestinal malrotation and volvulus phenotypes (Sivakumar et al., 2018). Investigating the formation of the initial S-shape, DJF or later secondary loops in this model has great potential to illuminate our understanding of the development of mammalian intestinal laterality and the underlying pathogenesis of human malrotation and volvulus.

How do secondary loops arise and what roles do they play?

The hierarchical looping model re-interprets the complex events of intestine development, providing a plausible alternative to rotation-based theories of asymmetric morphogenesis, but fundamental unanswered questions still exist. For example, what genetic patterning events or morphogenetic gradients underlie the stereotypical placement of discrete growth zones at defined positions along the cranio-caudal, dorsal-ventral or left-right axis of the gut tube? Recently, LR differences in tissue thickness and cellular orientation suggestive of asymmetrical cell rearrangement were reported to exist in the inner versus outer curvatures of the DJF, i.e. within the first secondary loop (Onouchi et al., 2013, 2015, 2016). Although the inner and outer curvatures of the DJF are derived from the original left and right sides of the primitive

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duodenum, respectively, the relationship of the observed cellular asymmetries to LR axis formation, DM tilting or the expression of LR genes such as *Pitx2* is unknown. It will be interesting to determine whether similar cellular asymmetries accompany the formation of other secondary loops. Furthermore, determining whether and/or how the formation of secondary loops is altered in models with LR asymmetry defects (such as *situs inversus* or heterotaxy) versus isolated intestinal malrotation (e.g. the *Tsg6* mouse) could reveal whether and/or how LR patterning affects specific features of intestine laterality, and/or whether secondary looping is executed independently of LR patterning per se. Another fascinating area to explore is the conservation of secondary loops between species. The existence of species-specific patterns of secondary loops could underlie the wide variety of vertebrate intestinal morphologies that are difficult to explain by the rotation of a single primary loop, e.g. the large hairpin bends observed in the horse colon or the multi-layered spirals seen in ruminants (Singh, 2017). Finally, the biomechanical factors that control the hierarchical formation of the secondary loops or provide the force for their sequential retraction and placement within the body cavity, also remain to be identified.

How do mechanical forces influence asymmetric morphogenesis?

Regardless of whether rotation, differential growth and/or hierarchical looping generates the various twists and turns of vertebrate digestive anatomy, physical and mechanical forces must underlie the massive topological changes that occur. It is important to note that the entire process of intestine morphogenesis coincides with, and indeed is driven by, proper gut tube elongation. Therefore, the biomechanical mechanisms impelling and supporting the lengthening of the gut tube are likely to be integral to the development of proper laterality. Underscoring this point is a recent review of cases of congenital short bowel (from 1969 to present), which revealed that malrotation occurs in 98.4% of individuals with shortened gut tubes (Negri et al., 2020). Moreover, intestinal malrotation has been observed in multiple contexts in which short guts were induced by experimental perturbations of signaling pathways involved in gut elongation (Pitera et al., 2001; Lipscomb et al., 2006; Yamada et al., 2010).

The link between gut elongation and laterality is also supported by computational modelling indicating that gut lengthening is mechanically coupled with looping. As mentioned above, disproportionate lengthening of the gut tube versus the attached DM is thought to create a growth strain that drives compressive buckling of the tube, resulting in the formation of predictable ‘sine wave’ undulations (Fig. 3B; Savin et al., 2011). Soffers et al. (2015) named these buckling events 'tertiary' loops; they exist within the secondary loops discussed above, but are thought neither to arise in precise locations along the gut tube (Soffers et al., 2015) nor to represent LR asymmetries in and of themselves. Nonetheless, their existence could influence the formation, re-entry and/or retraction of the secondary loops, thus altering final laterality. Although the role of the attached DM in secondary loop formation is unknown, the shorter mesentery observed between secondary loops (Mall, 1898; Soffers et al., 2015) may serve to define and/or mechanically isolate these key segments.

As the number and curvature of gut loops is dependent not only on the rate of gut lengthening, but also on the radius of the gut and the length/thickness of the mesentery (Nerurkar et al., 2017), any perturbation that alters the geometric and/or physical properties of different segments of the gut tube and/or the attached DM might influence the mechanics of looping and ‘rotation’. For example, defects in the radial patterning and diameter of the gut tube itself may affect its mechanical properties and ‘loopability’; indeed, intestinal narrowing (stenosis and/or atresia) has been associated with malrotation in humans (Adams and Stanton, 2014; Chinya et al., 2019; Ishii et al., 2020; Morikawa et al., 2009). Interestingly, these narrowing defects are often attributed to ‘vascular accidents’ that inhibit blood flow during gut development (Adams and Stanton, 2014; Ishii et al., 2020; Martin and Shaw-Smith, 2010). Thus, the DM, which is crucial for development of the gut vasculature (Sivakumar et al., 2018), may play a key role in asymmetric intestine morphogenesis simply by supporting proper gut tube growth and/or elongation. Further questions arise: e.g. how does perturbing the early leftward tilting of the DM affect its dimensions and mechanical properties, and how does abnormal DM tilting affect the vascularization, length and/or mechanical properties of associated loops of intestine? Pursuing such integrative lines of inquiry could begin to define the relative contributions of early versus late, cell biological versus physiological, and molecular versus mechanical mechanisms in the complex multi-phasic process that sculpts the final configuration of the vertebrate gut tube.

Conclusions

The overall breadth and variety of vertebrate organ lateralities suggests that variation in the asymmetric morphogenesis of gut-related tissues may have been a rich source of novel functional morphology during evolution. Although we are just beginning to understand what makes individual organs develop as LR asymmetric entities, some trends are emerging. First, rotational translocations of large segments of the gut tube are likely not the primary events that shape the anatomical lateralities of the stomach or intestine, despite the dogmatic illustrations found throughout the literature and textbooks. Second, many of the events that shape anatomical asymmetries involve tissues directly derived from, or immediately influenced by, the splanchnic layer of the LPM. Third, within these tissues, a wide variety of asymmetric cellular morphogenetic processes, dependent on localized, tissue-specific, left-sided expression of Pitx2, are deployed early in organogenesis. Unraveling how all these levels and layers of LR asymmetric morphogenesis have been integrated into the development of the vertebrate gut tube could provide profound insight into not only the etiology of laterality-related birth defects, but also the fundamental nature of organogenesis itself.

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Competing interests

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References

Abdel-Misih, S. R. Z. and Bloomston, M. (2010). Liver anatomy. *Surg. Clin. North Am.* **90**, 643-653. doi:10.1016/j.suc.2010.04.017

Adams, S. D. and Stanton, M. P. (2014). Malrotation and intestinal atresias. *Early Hum. Dev.* **90**, 921-925. doi:10.1016/j.earlhumdev.2014.09.017

Almirantis, Y. (1995). Left-right asymmetry in vertebrates. *BioEssays* **17**, 79-83. doi:10.1002/bies.950170114

Andrew, A. (1961). A simple teaching model illustrating the rotation of the gut. *J. Med. Educ.* **36**, 892-898.

Aylsworth, A. S. (2001). Clinical aspects of defects in the determination of laterality. *Am. J. Med. Genet.* **101**, 345-355. doi:10.1002/ajmg.1219

Bardeen, C. R. (1914). The critical period in the development of the intestines. *Am. J. Anat.* **16**, 427-445. doi:10.1002/aja.1000160403
Bartram, U., Wirbelauer, J. and Speer, C. P. (2005). Heterotaxy syndrome – asplenia and polysplenia as indicators of visceral malposition and complex congenital heart disease. *Biol. Neonate* **88**, 278-290. doi:10.1159/000087625
Blechschmidt, E. and Kircheiss, W. (1973). *Die pränatalen Organsysteme des Menschen: untersucht unter funktionellen Gesichtspunkten*. Hippokrates-Verlag.
Blum, M., Feistel, K., Thumberger, T. and Schweickert, A. (2014). The evolution and conservation of left-right patterning mechanisms. *Development* **141**, 1603-1613. doi:10.1242/dev.100560
Borghi, F., Gattolin, A., Bogliatto, F., Garavoglia, M. and Levi, A. C. (2002). Relationships between gastric development and anatomic bases of radical surgery for cancer. *World J. Surg.* **26**, 1139-1144. doi:10.1007/s00268-002-6346-0
Brendolan, A., Rosado, M. M., Carsetti, R., Selleri, L. and Dear, T. N. (2007). Development and function of the mammalian spleen. *BioEssays* **29**, 166-177. doi:10.1002/bies.20528
Brueckner, M., McGrath, J., D'Eustachio, P. and Horwich, A. L. (1991). Establishment of left-right asymmetry in vertebrates: genetically distinct steps are involved. *Ciba Found. Symp.* **162**, 202-212. doi:10.1002/9780470514160.ch12
Burn, S. F. and Hill, R. E. (2009). Left-right asymmetry in gut development: what happens next? *BioEssays* **31**, 1026-1037. doi:10.1002/bies.200900056
Burn, S. F., Boot, M. J., de Angelis, C., Doohan, R., Arques, C. G., Torres, M. and Hill, R. E. (2008). The dynamics of spleen morphogenesis. *Dev. Biol.* **318**, 303-311. doi:10.1016/j.ydbio.2008.03.031
Campione, M., Steinbeisser, H., Schweickert, A., Deissler, K., van Bebber, F., Lowe, L. A., Nowotschin, S., Viebahn, C., Haffter, P., Kuehn, M. R. et al. (1999). The homeobox gene Pitx2: mediator of asymmetric left-right signaling in vertebrate heart and gut looping. *Development* **126**, 1225-1234.
Capdevila, J., Vogan, K. J., Tabin, C. J. and Izpisúa Belmonte, J. C. (2000). Mechanisms of left-right determination in vertebrates. *Cell* **101**, 9-21. doi:10.1016/S0092-8674(00)80619-4
Cardoso, W. V. and Lü, J. (2006). Regulation of early lung morphogenesis: questions, facts and controversies. *Development* **133**, 1611-1624. doi:10.1242/dev.02310
Cartwright, J. H. E., Piro, O. and Tuval, I. (2004). Fluid-dynamical basis of the embryonic development of left–right asymmetry in vertebrates. *Proc. Natl. Acad. Sci. USA* **101**, 7234-7239. doi:10.1073/pnas.0402001101
Casey, B. (1998). Two rights make a wrong: human left–right malformations. *Hum. Mol. Genet.* **7**, 1565. doi:10.1093/hmg/7.10.1565
Cayuso, J., Dzementsei, A., Fischer, J. C., Karemore, G., Caviglia, S., Bartholdson, J., Wright, G. J. and Ober, E. A. (2016). EphrinB1/EphB3b coordinate bidirectional epithelial-mesenchymal interactions controlling liver morphogenesis and laterality. *Dev. Cell* **39**, 316-328. doi:10.1016/j.devcel.2016.10.009
Chinya, A., Naranje, K. and Mandelia, A. (2019). Situs inversus abdominalis, polysplenia, complex jejunal atresia and malrotation in a neonate: a rare association. *Int. J. Surg. Case Rep.* **56**, 93-95. doi:10.1016/j.ijscr.2019.02.016
Costa, R. M. B., Mason, J., Lee, M., Amaya, E. and Zorn, A. M. (2003). Novel gene expression domains reveal early patterning of the Xenopus endoderm. *Gene Expr. Patterns* **3**, 509-519. doi:10.1016/S1567-133X(03)00086-3
Dankmejer, J. and Miette, M. (1961). Le rôle de l'épithélium dans le développement précoce de l'estomac chez l'homme. *CR Assoc Anat* **47**, 256-260.
Dasgupta, A. and Amack, J. D. (2016). Cilia in vertebrate left-right patterning. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **371**, 20150410. doi:10.1098/rstb.2015.0410
Davis, N. M., Kurpios, N. A., Sun, X., Gros, J., Martin, J. F. and Tabin, C. J. (2008). The chirality of gut rotation derives from left-right asymmetric changes in the architecture of the dorsal mesentery. *Dev. Cell* **15**, 134-145. doi:10.1016/j.devcel.2008.05.001
Davis, A., Amin, N. M., Johnson, C., Bagley, K., Troy Ghashghaei, H. and Nascone-Yoder, N. (2017). Stomach curvature is generated by left-right asymmetric gut morphogenesis. *Development* **144**, 1477-1483. doi:10.1242/dev.143701
Dear, T. N., Colledge, W. H., Carlton, M. B., Lavenir, I., Larson, T., Smith, A. J., Warren, A. J., Evans, M. J., Sofroniew, M. V. and Rabbitts, T. H. (1995). The Hox11 gene is essential for cell survival during spleen development. *Development* **121**, 2909-2915.
Deitch, E. A. and Engel, J. M. (1980). Anomalies of gut rotation mimicking appendicitis in the adult. *Am. Surg.* **46**, 226-229.
Desgrange, A., Le Garrec, J.-F. and Meilhac, S. M. (2018). Left-right asymmetry in heart development and disease: forming the right loop. *Development* **145**, dev162776. doi:10.1242/dev.162776
Dott, N. M. (1923). Anomalies of intestinal rotation: their embryology and surgical aspects: with report of five cases. *Br. J. Surg.* **11**, 251-286. doi:10.1002/bjs.1800114207
Fletcher, F. A., Carpenter, M. K., Shilling, H., Baum, P., Ziegler, S. F., Gimpel, S., Hollingsworth, T., Vanden Bos, T., James, L. and Hjerrild, K. (1994). LERK-2, a binding protein for the receptor-tyrosine kinase ELK, is evolutionarily conserved and expressed in a developmentally regulated pattern. *Oncogene* **9**, 3241-3247.
Frazer, J. E. and Robbins, R. H. (1915). On the Factors concerned in causing Rotation of the Intestine in Man. *J. Anat. Physiol.* **50**, 75-110.
Freitas, J. E. and Ventura, V. (1980). Complete failure of gut rotation: detection by hepatobiliary imaging. *Clin. Nucl. Med.* **5**, 370-371. doi:10.1097/00003072-198008000-00009
Gabriel, G. C. and Lo, C. W. (2020). Left-right patterning in congenital heart disease beyond heterotaxy. *Am. J. Med. Genet. C Semin. Med. Genet.* **184**, 90-96. doi:10.1002/ajmg.c.31768
Gasser, R. F. (1975). *Atlas of Human Embryos*. Medical Department, Harper & Row.
Gittes, G. K. (2009). Developmental biology of the pancreas: a comprehensive review. *Dev. Biol.* **326**, 4-35. doi:10.1016/j.ydbio.2008.10.024
Glowniak, J. V. (1988). Intestinal malrotation diagnosed by cholescintigraphy. *Clin. Nucl. Med.* **13**, 835-836. doi:10.1097/00003072-198811000-00017
Green, M. C. (1967). A defect of the splanchnic mesoderm caused by the mutant gene dominant hemimelia in the mouse. *Dev. Biol.* **15**, 62-89. doi:10.1016/0012-1606(67)90006-1
Grimes, D. T. and Burdine, R. D. (2017). Left-right patterning: breaking symmetry to asymmetric morphogenesis. *Trends Genet.* **33**, 616-628. doi:10.1016/j.tig.2017.06.004
Gros, J., Feistel, K., Viebahn, C., Blum, M. and Tabin, C. J. (2009). Cell movements at Hensen's node establish left/right asymmetric gene expression in the chick. *Science* **324**, 941-944. doi:10.1126/science.1172478
Grover, M. H. and Moore, G. W. (1988). Growth and asymmetry of the human liver during the embryonic period. *Pediatr. Pathol.* **8**, 17-24. doi:10.3109/15513818809022276
Hamada, H. and Tam, P. (2020). Diversity of left-right symmetry breaking strategy in animals. *F1000Res.* **9**, 123. doi:10.12688/f1000research.21670.1
Hecksher-Sørensen, J., Watson, R. P., Lettice, L. A., Serup, P., Eley, L., De Angelis, C., Ahlgren, U. and Hill, R. E. (2004). The splanchnic mesodermal plate directs spleen and pancreatic laterality, and is regulated by Bapx1/Nkx3.2. *Development* **131**, 4665-4675. doi:10.1242/dev.01364
Heisler, J. C. (1907). *A Text-Book of Embryology for Students of Medicine*. W. B. Saunders
Herzer, U., Crocoll, A., Barton, D., Howells, N. and Englert, C. (1999). The Wilms tumor suppressor gene wt1 is required for development of the spleen. *Curr. Biol.* **9**, 837-840. doi:10.1016/S0960-9822(99)80369-8
Hikspoors, J. P. J. M., Kruepunga, N., Mommen, G. M. C., Peeters, J.-M. P. W. U., Hülsman, C. J. M., Eleonore Köhler, S. and Lamers, W. H. (2018). The development of the dorsal mesentery in human embryos and fetuses. *Semin. Cell Dev. Biol.* **92**, 18-26. doi:10.1016/j.semcdb.2018.08.009
Hogan, B. L. (1999). Morphogenesis. *Cell* **96**, 225-233. doi:10.1016/S0092-8674(00)80562-0
Hummel, K. P. and Chapman, D. B. (1959). Visceral inversion and associated anomalies in the mouse. *J. Hered.* **50**, 9-13. doi:10.1093/oxfordjournals.jhered.a106870
Ishii, D., Miyagi, H., Hirasawa, M. and Miyamoto, K. (2020). Congenital multiple colonic atresias with intestinal malrotation: a case report. *Surg Case Rep* **6**, 60. doi:10.1186/s40792-020-00822-z
Jennings, R. E., Berry, A. A., Kirkwood-Wilson, R., Roberts, N. A., Hearn, T., Salisbury, R. J., Blaylock, J., Piper Hanley, K. and Hanley, N. A. (2013). Development of the human pancreas from foregut to endocrine commitment. *Diabetes* **62**, 3514-3522. doi:10.2337/db12-1479
Kaigai, N., Nako, A., Yamada, S., Uwabe, C., Kose, K. and Takakuwa, T. (2014). Morphogenesis and three-dimensional movement of the stomach during the human embryonic period. *Anat. Rec.* **297**, 791-797. doi:10.1002/ar.22833
Kajikawa, E., Horo, U., Ide, T., Mizuno, K., Minegishi, K., Hara, Y., Ikawa, Y., Nishimura, H., Uchikawa, M., Kiyonari, H. et al. (2020). Nodal paralogues underlie distinct mechanisms for visceral left-right asymmetry in reptiles and mammals. *Nat Ecol Evol* **4**, 261-269. doi:10.1038/s41559-019-1072-2
Kanagasuntheram, R. (1957). Development of the human lesser sac. *J. Anat.* **91**, 188-206.
Kim, W. K., Kim, H., Ahn, D. H., Kim, M. H. and Park, H. W. (2003). Timetable for intestinal rotation in staged human embryos and fetuses. *Birth Defects Res. A Clin. Mol. Teratol.* **67**, 941-945. doi:10.1002/bdra.10094
Kim, B.-M., Buchner, G., Miletich, I., Sharpe, P. T. Shivdasani, R. A. (2005). The stomach mesenchymal transcription factor Barx1 specifies gastric epithelial identity through inhibition of transient Wnt signaling. *Dev. Cell* **8**, 611-622. doi:10.1016/j.devcel.2005.01.015. PMID: 15809042.
Kim, B.-M., Miletich, I., Mao, J., McMahon, A. P., Sharpe, P. A. and Shivdasani, R. A. (2007). Independent functions and mechanisms for homeobox gene Barx1 in patterning mouse stomach and spleen. *Development* **134**, 3603-3613. doi:10.1242/dev.009308
Kluth, D., Kaestner, M., Tibboel, D. and Lambrecht, W. (1995). Rotation of the gut: fact or fantasy? *J. Pediatr. Surg.* **30**, 448-453. doi:10.1016/0022-3468(95)90053-5
Kluth, D., Jaeschke-Melli, S. and Fiegel, H. (2003). The embryology of gut rotation. *Semin. Pediatr. Surg.* **12**, 275-279. doi:10.1053/j.sempedsurg.2003.08.009
Kothari, S. S. (2014). Non-cardiac issues in patients with heterotaxy syndrome. *Ann. Pediatr. Cardiol.* **7**, 187-192. doi:10.4103/0974-2069.140834
Kurpios, N. A., Ibañes, M., Davis, N. M., Lui, W., Katz, T., Martin, J. F., Izpisúa Belmonte, J. C. and Tabin, C. J. (2008). The direction of gut looping is

established by changes in the extracellular matrix and in cell:cell adhesion. *Proc. Natl. Acad. Sci. USA* **105**, 8499-8506. doi:10.1073/pnas.0803578105
Larsen, W. J. (2014). *Human Embryology Fifth Edition*. Churchill Livingstone Inc.
Levin, M. (2005). Left–right asymmetry in embryonic development: a comprehensive review. *Mech. Dev.* **122**, 3-25. doi:10.1016/j.mod.2004.08.006
Liebermann-Meffert, D. (2000). The greater omentum. Anatomy, embryology, and surgical applications. *Surg. Clin. North Am.* **80**, 275-293. doi:10.1016/S0039-6109(05)70406-0
Lin, A. E., Krikov, S., Riehle-Colarusso, T., Frías, J. L., Belmont, J., Anderka, M., Geva, T., Getz, K. D., Botto, L. D. and Study, N. B. D. P. (2014). Laterality defects in the national birth defects prevention study (1998–2007): birth prevalence and descriptive epidemiology. *Am. J. Med. Genet. A* **164**, 2581-2591. doi:10.1002/ajmg.a.36695
Lipscomb, K., Schmitt, C., Sablyak, A., Yoder, J. A. and Nascone-Yoder, N. (2006). Role for retinoid signaling in left-right asymmetric digestive organ morphogenesis. *Dev. Dyn.* **235**, 2266-2275. doi:10.1002/dvdy.20879
Liu, C., Liu, W., Lu, M. F., Brown, N. A. and Martin, J. F. (2001). Regulation of left-right asymmetry by thresholds of Pitx2c activity. *Development* **128**, 2039-2048.
Logan, M., Pagán-Westphal, S. M., Smith, D. M., Paganessi, L. and Tabin, C. J. (1998). The transcription factor Pitx2 mediates situs-specific morphogenesis in response to left-right asymmetric signals. *Cell* **94**, 307-317. doi:10.1016/S0092-8674(00)81474-9
Long, F. R., Kramer, S. S., Markowitz, R. I. and Taylor, G. E. (1996). Radiographic patterns of intestinal malrotation in children. *Radiographics* **16**, 547-556; discussion 556–60. doi:10.1148/radiographics.16.3.8897623
Lu, J., Chang, P., Richardson, J. A., Gan, L., Weiler, H. and Olson, E. N. (2000). The basic helix-loop-helix transcription factor capsulin controls spleen organogenesis. *Proc. Natl. Acad. Sci. USA* **97**, 9525-9530. doi:10.1073/pnas.97.17.9525
Macarulla-Sanz, E., Nebot-Cegarra, J. and Reina-de la Torre, F. (1996). Computer-assisted stereological analysis of gastric volume during the human embryonic period. *J. Anat.* **188**, 395-401.
Mahadevan, A., Welsh, I. C., Sivakumar, A., Gludish, D. W., Shilvock, A. R., Noden, D. M., Huss, D., Lansford, R. and Kurpios, N. A. (2014). The left-right Pitx2 pathway drives organ-specific arterial and lymphatic development in the intestine. *Dev. Cell* **31**, 690-706. doi:10.1016/j.devcel.2014.11.002
Mall, F. P. (1898). Development of the human intestine and its position in the adult. *Bull. Johns Hopkins Hosp.* **9**, 197-208.
Martin, V. and Shaw-Smith, C. (2010). Review of genetic factors in intestinal malrotation. *Pediatr. Surg. Int.* **26**, 769-781. doi:10.1007/s00383-010-2622-5
Matsuyama, M., Aizawa, S. and Shimono, A. (2009). Sfrp controls apicobasal polarity and oriented cell division in developing gut epithelium. *PLoS Genet.* **5**, e1000427. doi:10.1371/journal.pgen.1000427
Matthew, W. and Wanda, H. and **Colin Rousseaux ed** (2009). *Fundamentals of Toxicologic Pathology*. Academic Press.
Metzger, R. J., Klein, O. D., Martin, G. R. and Krasnow, M. A. (2008). The branching programme of mouse lung development. *Nature* **453**, 745-750. doi:10.1038/nature07005
Metzger, R., Metzger, U., Fiegel, H. C. and Kluth, D. (2011). Embryology of the midgut. *Semin. Pediatr. Surg.* **20**, 145-151. doi:10.1053/j.sempedsurg.2011.03.005
Miete, M. (1960). Enkele aspecten van de embryonale ontwikkeling van de menselijke maag. *Ned Tijdschr Geneeskd* **104**, 1216.
Morikawa, N., Kuroda, T., Honna, T., Kitano, Y., Tanaka, H., Takayasu, H., Fijino, A., Kawashima, N., Tanemura, H., Muto, M. et al. (2009). A novel association of duodenal atresia, malrotation, segmental dilatation of the colon, and anorectal malformation. *Pediatr. Surg. Int.* **25**, 1003-1005. doi:10.1007/s00383-009-2459-y
Muller, J. K., Prather, D. R. and Nascone-Yoder, N. M. (2003). Left-right asymmetric morphogenesis in the Xenopus digestive system. *Dev. Dyn.* **228**, 672-682. doi:10.1002/dvdy.10415
Nebot-Cegarra, J., Maraculla-Sanz, E. and Reina-De La Torre, F. (1999). Factors involved in the “rotation” of the human embryonic stomach around its longitudinal axis: computer-assisted morphometric analysis. *J. Anat.* **194**, 61-69. doi:10.1046/j.1469-7580.1999.19410061.x
Negri, E., Coletta, R. and Morabito, A. (2020). Congenital short bowel syndrome: systematic review of a rare condition. *J. Pediatr. Surg.* **55**, 1809-1814. doi:10.1016/j.jpedsurg.2020.03.009
Nerurkar, N. L., Mahadevan, L. and Tabin, C. J. (2017). BMP signaling controls buckling forces to modulate looping morphogenesis of the gut. *Proc. Natl. Acad. Sci. USA* **114**, 2277-2282. doi:10.1073/pnas.1700307114
Norris, D. P. (2012). Cilia, calcium and the basis of left-right asymmetry. *BMC Biol.* **10**, 102. doi:10.1186/1741-7007-10-102
Okada, Y., Takeda, S., Tanaka, Y., Belmonte, J.-C. I. and Hirokawa, N. (2005). Mechanism of nodal flow: a conserved symmetry breaking event in left-right axis determination. *Cell* **121**, 633-644. doi:10.1016/j.cell.2005.04.008
Onouchi, S., Ichii, O., Otsuka, S., Hashimoto, Y. and Kon, Y. (2013). Analysis of duodenojejunal flexure formation in mice: implications for understanding the genetic basis for gastrointestinal morphology in mammals. *J. Anat.* **223**, 385-398. doi:10.1111/joa.12093
Onouchi, S., Ichii, O., Otsuka-Kanazawa, S. and Kon, Y. (2015). Asymmetric morphology of the cells comprising the inner and outer bending sides of the murine duodenojejunal flexure. *Cell Tissue Res.* **360**, 273-285. doi:10.1007/s00441-014-2091-6
Onouchi, S., Ichii, O., Nakamura, T., Elewa, Y. H. A. and Kon, Y. (2016). Spatiotemporal distribution of extracellular matrix changes during mouse duodenojejunal flexure formation. *Cell Tissue Res.* **365**, 367-379. doi:10.1007/s00441-016-2390-1
Pan, F. C. and Brissova, M. (2014). Pancreas development in humans. *Curr. Opin. Endocrinol. Diabetes Obes.* **21**, 77-82. doi:10.1097/MED.0000000000000047
Piedra, M. E., Icardo, J. M., Albajar, M., Rodriguez-Rey, J. C. and Ros, M. A. (1998). *Pitx2* Participates in the late phase of the pathway controlling left-right asymmetry. *Cell* **94**, 319-324. doi:10.1016/S0092-8674(00)81475-0
Pitera, J. E., Smith, V. V., Woolf, A. S. and Milla, P. J. (2001). Embryonic gut anomalies in a mouse model of retinoic Acid-induced caudal regression syndrome: delayed gut looping, rudimentary cecum, and anorectal anomalies. *Am. J. Pathol.* **159**, 2321-2329. doi:10.1016/S0002-9440(10)63082-9
Plageman, T. F., Jr., Zacharias, A. L., Gage, P. J. and Lang, R. A. (2011). Shroom3 and a Pitx2-N-cadherin pathway function cooperatively to generate asymmetric cell shape changes during gut morphogenesis. *Dev. Biol.* **357**, 227-234. doi:10.1016/j.ydbio.2011.06.027
Price, E. A. and Kane, G. (1955). Non-rotation of gut; with report of case of left-sided appendiceal abscess. *S. Afr. Med. J.* **29**, 655-658.
Roberts, C. W. M., Shutter, J. R. and Korsmeyer, S. J. (1994). Hox11 controls the genesis of the spleen. *Nature* **368**, 747-749. doi:10.1038/368747a0
Ryan, A. K., Blumberg, B., Rodriguez-Esteban, C., Yonei-Tamura, S., Tamura, K., Tsukui, T., de la Peña, J., Sabbagh, W., Greenwald, J., Choe, S. et al. (1998). Pitx2 determines left–right asymmetry of internal organs in vertebrates. *Nature* **394**, 545-551. doi:10.1038/29004
Savin, T., Kurpios, N. A., Shyer, A. E., Florescu, P., Liang, H., Mahadevan, L. and Tabin, C. J. (2011). On the growth and form of the gut. *Nature* **476**, 57-62. doi:10.1038/nature10277
Schwalbe, E. (1906). *Die Morphologie der Missbildungen des Menschen und der Tiere*. Fischer.
Schweickert, A., Ott, T., Kurz, S., Tingler, M., Maerker, M., Fuhl, F. and Blum, M. (2017). Vertebrate left-right asymmetry: what can nodal cascade gene expression patterns tell us? *J Cardiovasc Dev Dis* **5**, 1. doi:10.3390/jcdd5010001
Sempou, E. and Khokha, M. K. (2019). Genes and mechanisms of heterotaxy: patients drive the search. *Curr. Opin. Genet. Dev.* **56**, 34-40. doi:10.1016/j.gde.2019.05.003
Shiraishi, I. and Ichikawa, H. (2012). Human heterotaxy syndrome. *Circ. J.* **76**, 2066-2075. doi:10.1253/circj.CJ-12-0957
Shiratori, H., Yashiro, K., Shen, M. M. and Hamada, H. (2006). Conserved regulation and role of Pitx2 in situs-specific morphogenesis of visceral organs. *Development* **133**, 3015-3025. doi:10.1242/dev.02470
Singh, B. (2017). *Dyce, Sack, and Wensing’s Textbook of Veterinary Anatomy*. Elsevier.
Sivakumar, A., Mahadevan, A., Lauer, M. E., Narvaez, R. J., Ramesh, S., Demler, C. M., Souchet, N. R., Hascall, V. C., Midura, R. J., Garantziotis, S. et al. (2018). Midgut laterality is driven by hyaluronan on the right. *Dev. Cell* **46**, 533-551.e5. doi:10.1016/j.devcel.2018.08.002
Smith, D. J., Blake, J. R. and Gaffney, E. A. (2008). Fluid mechanics of nodal flow due to embryonic primary cilia. *J. R. Soc. Interface* **5**, 567-573. doi:10.1098/rsif.2007.1306
Snyder, W. H., Jr. and Chaffin, L. (1954). Embryology and pathology of the intestinal tract: presentation of 40 cases of malrotation. *Ann. Surg.* **140**, 368-379. doi:10.1097/00000658-195409000-00013
Soffers, J. H. M., Hikspoors, J. P. J. M., Mekonen, H. K., Koehler, S. E. and Lamers, W. H. (2015). The growth pattern of the human intestine and its mesentery. *BMC Dev. Biol.* **15**, 31. doi:10.1186/s12861-015-0081-x
Southwell, B. R. (2006). Staging of intestinal development in the chick embryo. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* **288**, 909-920. doi:10.1002/ar.a.20349
Sutherland, M. J. and Ware, S. M. (2009). Disorders of left–right asymmetry: heterotaxy and situs inversus. In *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, pp. 307-317. Wiley Online Library.
Ticho, B. S., Goldstein, A. M. and Van Praagh, R. (2000). Extracardiac anomalies in the heterotaxy syndromes with focus on anomalies of midline-associated structures. *Am. J. Cardiol.* **85**, 729-734. doi:10.1016/S0002-9149(99)00849-8
Torres, A. M. and Ziegler, M. M. (1993). Malrotation of the intestine. *World J. Surg.* **17**, 326-331. doi:10.1007/BF01658699
Ueda, Y., Yamada, S., Uwabe, C., Kose, K. and Takakuwa, T. (2016). Intestinal rotation and physiological umbilical herniation during the embryonic period. *Anat. Rec.* **299**, 197-206. doi:10.1002/ar.23296
Valioulis, I., Anagnostopoulos, D. and Sfougaris, D. (1997). Reversed midgut rotation in a neonate: case report with a brief review of the literature. *J. Pediatr. Surg.* **32**, 643-645. doi:10.1016/S0022-3468(97)90730-2
van Soldt, B. J., Metscher, B. D., Poelmann, R. E., Vervust, B., Vonk, F. J., Müller, G. B. and Richardson, M. K. (2015). Heterochrony and early left-right asymmetry in the development of the cardiorespiratory system of snakes. *PLoS ONE* **10**, e116416. doi:10.1371/journal.pone.0116416

Watanabe, D., Saijoh, Y., Nonaka, S., Sasaki, G., Ikawa, Y., Yokoyama, T. and Hamada, H. (2003). The left-right determinant Inversin is a component of node monocilia and other 9+0 cilia. *Development* **130**, 1725-1734. doi:10.1242/dev.00407

Weiss, M. C., Le Garrec, J.-F., Coqueran, S., Strick-Marchand, H. and Buckingham, M. (2016). Progressive developmental restriction, acquisition of left-right identity and cell growth behavior during lobe formation in mouse liver development. *Development* **143**, 1149-1159. doi:10.1242/dev.132886

Welsh, I. C., Thomsen, M., Gludish, D. W., Alfonso-Parra, C., Bai, Y., Martin, J. F. and Kurpios, N. A. (2013). Integration of left-right Pitx2 transcription and Wnt signaling drives asymmetric gut morphogenesis via Daam2. *Dev. Cell* **26**, 629-644. doi:10.1016/j.devcel.2013.07.019

Womble, M., Amin, N. M. and Nascone-Yoder, N. (2018). The left-right asymmetry of liver lobation is generated by Pitx2c-mediated asymmetries in the hepatic diverticulum. *Dev. Biol.* **439**, 80-91. doi:10.1016/j.ydbio.2018.04.021

Yamada, M., Udagawa, J., Matsumoto, A., Hashimoto, R., Hatta, T., Nishita, M., Minami, Y. and Otani, H. (2010). Ror2 is required for midgut elongation during mouse development. *Dev. Dyn.* **239**, 941-953. doi:10.1002/dvdy.22212

Yim, D., Nagata, H., Lam Christopher, Z., Grosse-Wortmann, L., Seed, M., Jaeggi, E. and Yoo, S.-J. (2018). Disharmonious Patterns of Heterotaxy and Isomerism. *Circ. Cardiovasc. Imaging* **11**, e006917. doi:10.1161/CIRCIMAGING.117.006917

Yokoyama, T., Copeland, N. G., Jenkins, N. A., Montgomery, C. A., Elder, F. F. and Overbeek, P. A. (1993). Reversal of left-right asymmetry: a situs inversus mutation. *Science* **260**, 679-682. doi:10.1126/science.8480178

Yuan, S. and Schoenwolf, G. C. (2000). Islet-1 marks the early heart rudiments and is asymmetrically expressed during early rotation of the foregut in the chick embryo. *Anat. Rec.* **260**, 204-207. doi:10.1002/1097-0185(20001001)260:2<204::AID-AR90>3.0.CO;2-5