**Molecular Medicine**

**Vangl2 Acts via RhoA Signaling to Regulate Polarized Cell Movements During Development of the Proximal Outflow Tract**

Helen M. Phillips, Jennifer N. Murdoch, Bill Chaudhry, Andrew J. Copp, Deborah J. Henderson

Abstract—Loop-tail (Lp) mice develop double outlet right ventricle and aortic arch defects, and the defects are caused by mutations in the Vangl2 gene. Vangl2 mRNA is found in the outflow tract myocardium, including the myocardializing cells that migrate into the outflow tract cushions. Analysis of muscularization of the outflow tract septum showed that this process is compromised in Lp/Lp fetuses. Vangl2 is a component of the noncanonical Wnt, planar cell polarity (PCP) pathway that signals via RhoA. We therefore looked for evidence of polarization in myocardializing cells. In wild-type fetuses, myocardializing cells extend lamellipodia and filopodia into the cushion tissue and reorganize their actin cytoskeleton from a cortical form to stress fibers; behaviors that are characteristic of polarized cells. In contrast, Lp/Lp littermates do not extend lamellipodia or filopodia into the cushion tissue, and their actin remains in a cortical form, suggesting that polarized cell migration of myocardializing cells is inhibited in Lp/Lp. Several other components of the PCP pathway are also localized in the outflow tract myocardium. In wild-type fetuses, the myocardializing cells coexpress RhoA and one of its downstream mediators, ROCK1. RhoA expression is disrupted in Lp/Lp, and is lost from the myocardial-cushion tissue interface, including the presumptive myocardializing cells. These data suggest that Vangl2 is required for the polarization and movement of myocardializing cells into the outflow tract cushions, and that RhoA and ROCK1 are downstream mediators of the PCP signaling pathway in the developing outflow tract. (Circ Res. 2005;96:292-299.)

Key Words: congenital heart defects ■ genes ■ mouse mutant ■ development ■ cardiac muscle

Loop-tail (Lp) is a naturally occurring mouse mutant, which develops severe cardiovascular defects in association with abnormalities of midline development. Lp homozygotes exhibit double outlet right ventricle with perimembranous ventricular septal defects, coarctation of the aorta, and retention of the right aortic arch. Although abnormalities of neural crest cell migration and colonization of the outflow tract are frequently associated with outflow tract and aortic arch abnormalities, neural crest defects have never been identified in Lp/Lp embryos.

The Lp gene, Vangl2 (also known as strabismus, Lpp1, or Ltap), has been shown to encode a protein homologous to Drosophila strabismus/van gogh (Vang). Both mammalian and Drosophila Vang proteins contain four putative transmembrane domains and a PDZ-binding domain, consistent with conserved functional properties. In Drosophila, Vang is a key component of a frizzled/disheveled pathway that is essential for regulating planar cell polarity and, in some tissues, cell fate. Although the activating ligand has not been identified in Drosophila, the pathway is highly conserved in vertebrates where it appears to be activated by Wnt binding to the frizzled receptor. This pathway differs, however, from canonical Wnt signaling via β-catenin, as it is β-catenin–independent. Vangl2 functions, therefore, in a noncanonical Wnt, or planar cell polarity, pathway (see reviews). Studies in Xenopus, zebrafish, and mice suggest that Vangl2/strabismus regulates convergent extension movements in vertebrate embryos. These convergent extension movements occur at gastrulation and involve polarized cell movements that drive organized patterns of cell intercalation, resulting in the narrowing and lengthening of the embryo (see reviews). Whereas planar cell polarity occurs within the plane of an epithelium in Drosophila, converging tissues in vertebrates, which include presumptive notochord, neural tube, and somites, consist of a layer of epithelium overlying several layers of mesenchymal cells. Mediolateral intercalation of cells is driven by polarized protrusive behavior involving directed lamellipodia. These lamellipodia exert traction on adjacent cells, thereby pulling the cells between one another. Conservation of this pathway suggests that Vangl2 may play roles in control of cell polarity, cell adhesion, and cell movements, and indeed, Lp mutants have a shortened body axis, possibly indicating a defect in convergent extension movements.
Although convergent extension-type cell movements have not been described in the developing cardiovascular system, the muscularization of the proximal outflow tract cushions shares some common elements. In both birds and mammals, the initially mesenchymal outflow tract cushions gradually become myocardial, starting at around the time of outflow tract septation. This muscularization of the septum is suggested to occur, at least in the early stages, by the migration/ingrowth of existing cardiomyocytes into the flanking cushion mesenchyme. This process has been termed “myocardialization” (see review11). In this study, we have tested the hypothesis that muscularization of the outflow tract cushions involves polarized cell movements that are dependent on Vangl2 signaling. We show that the myocardializing cells display characteristics of motile polarized cells. Furthermore, we show that these characteristics are absent in Lp mutants, resulting in a failure to muscularize the proximal outflow tract cushions. This is associated with disrupted planar cell polarity pathway signaling.

Materials and Methods

Embryonic Preparation and Analysis

The LPT/Le inbred strain, which carries the Lp mutation, was originally obtained from the Jackson Laboratory (Bar Harbor, Maine). Mice were bred and genotyped as described previously12 to generate litters containing Lp/Lp, Lp/+, and +/+ embryos. All animals were maintained and killed according to the requirements of the Animals (Scientific Procedures) Act 1986 of the UK Government. Embryos were dissected from the uterus and processed for immunohistochemistry and in situ hybridization.

Immunohistochemistry

Each experiment was repeated a minimum of three times on an average of six embryos per genotype. Immunoperoxidase staining of paraffin-embedded tissues was performed as previously described.12 The α-smooth muscle actin antibody (clone 1A4) and the skeletal slow myosin antibody (clone NOQ7.5.4D) were obtained from Sigma Chemical Co. The MF20 antibody, which recognizes myosin, was obtained from the Developmental Studies Hybridoma Bank, University of Iowa, and was used interchangeably with the skeletal slow myosin antibody. The ROCK-1 (sc-6055) and RhoA (sc-418) antibodies were obtained from Santa Cruz Biotechnology, and for this analysis, embryos were fixed in 1% acetic acid, 95% ethanol overnight, then processed for wax embedding. Antigen retrieval (microwaving in 0.01 mol/L citrate buffer) was required for ROCK1 staining. For immunofluorescence staining, tissues were cryosectioned.14 Fluorescently labeled secondary antibodies were visualized using laser confocal microscopy. In each case, negative controls were performed in the absence of primary antibody, and no staining was obtained. Phalloidin staining was performed as described previously.14 Overlays of RhoA and ROCK1 immunohistochemistry were created using Adobe Photoshop 4.0.

cDNA Probes and In Situ Hybridization

The Vangl2 probe was designed against the 3′ untranslated region of the gene (Genbank accession AY035370, nucleotides 1716 to 2200). Primers were designed against the published sequences of Dvl2 (XM_12646), Wnt11 (MMWNT), and ROCK1 (NM_009071), and the resulting fragments were cloned into pGEM-T easy (Promega) and sequenced to confirm identity. The Wnt5a probe was a gift from Dr Andrew McMahon from the Roche Institute of Molecular Biology, Nutley, NJ. Slide in situ hybridization using digoxigeninated labeled riboprobes was performed as described previously.3

Results

Vangl2 Is Expressed in the Outflow Tract Myocardium

We have previously shown that Lp mice, which carry mutations in the Vangl2 gene2,3, develop outflow tract defects that manifest principally as double outlet right ventricle, commonly associated with parallel arterial trunks4 (Figure 1A through 1F). Vangl2 is first expressed in the outflow tract myocardium. This is maintained at E13.5, colocalizing with MF20 staining in cardiomyocytes. L, This is maintained at E14.5 (arrows) in the cardiomyocytes at E12.5 (arrows). J and K, Vangl2 expression is maintained at E13.5, colocalizing with MF20 staining in cardiomyocytes. L, This is maintained at E14.5 (arrows) although the expression is downregulated in the ventricular myocardium. ao indicates aorta; lv, left ventricle; pos, proximal outlet septum; pt, pulmonary trunk; rv, right ventricle.
myocardium at embryonic day (E) 9.5 although at this stage the levels are low (data not shown). By E11.5, however, \( Vangl2 \) is expressed abundantly, with a punctate pattern, in the outflow tract myocardium (Figure 1G and 1H) and can be seen in the cardiomyocytes extending into the proximal outlet septum as the myocardialization process begins. This pattern is maintained at E12.5 through E14.5, closely mirroring myosin staining of cardiomyocytes, after which it is down-regulated (Figure 11 through 1L and data not shown). Myosin immunohistochemistry was also performed on sections that had been previously hybridized with a \( Vangl2 \) riboprobe. This confirmed that \( Vangl2 \) mRNA is found in myosin-expressing cells (data not shown). \( Vangl2 \) mRNA is not found in the cells of the mesenchymal cushion tissue or the endocardium at any stage of development.

### Abnormal Muscularization of the Proximal Outlet Septum in \( Lp/Lp \)

At E10.5 through E11.5, the general morphology of the outflow tract, and the cellular organization of the epithelial outflow tract myocardium specifically, is indistinguishable between +/-, \( Lp/+ \), and their \( Lp/Lp \) littermates (Figure 2A and 2B). By E12.5, the myocardial cells closest to the cushion mesenchyme have lost their epithelial contacts in +/-, \( Lp/+ \), and \( Lp/Lp \) fetuses, and are extending into the endocardial cushion tissue (Figure 2C and 2D). There are no apparent differences in the numbers of cardiomyocytes extending into the proximal outlet septum in +/-, \( Lp/+ \), and \( Lp/Lp \) fetuses, although, in the \( Lp/Lp \) fetuses, the myocardial cells do not appear to extend as far into the septum as in their littermates.

At E13.5, many myosin-positive cardiomyocytes can be seen extending into the septum in +/- and \( Lp/+ \) fetuses (arrows in Figure 2E). Furthermore, the region of the proximal outlet septum that remains nonmuscularized, and therefore mesenchymal, has reduced in size in comparison to a day earlier in gestation (compare the relative breadth of the septum in Figure 2E with 2C). By this stage, there is an obvious reduction in the number of cardiomyocytes extending into the septum in \( Lp/Lp \) fetuses, and the boundary of the myocardial wall with the mesenchymal septum does not follow the smooth curve that is evident in the \( Lp/+ \) fetuses. Furthermore, the region of the proximal outlet septum that remains nonmuscularized appears larger in the \( Lp/Lp \) fetuses than in the +/- and \( Lp/+ \) littermates (compare Figure 2G with 2E). At the same stage, and shown in adjacent sections, \( \alpha \)-smooth muscle actin (\( \alpha \)SMA) is strongly expressed in myosin-negative cells within the mesenchymal septum of +/- and \( Lp/+ \) fetuses (compare Figure 2F with 2E), which are in contact with the myocardial wall. Although \( \alpha \)SMA is expressed in a number of different cell types in the outflow tract at different stages of development, these myosin-negative, \( \alpha \)SMA-positive cells that are adjacent to the myocardial wall have been suggested to be mesenchymal cells that are differentiating into muscle under the influence of cardiomyocytes penetrating the septum from the myocardial wall of the outflow tract.15–17 Although the cells in the myocardial wall in \( Lp/Lp \) strongly express \( \alpha \)SMA, few myosin-negative cells express \( \alpha \)SMA (compare Figure 2H with 2G), suggesting that there may be a reduction in recruitment of mesenchymal cushion cells into the cardiomyocyte lineage in \( Lp/Lp \) fetuses.

As muscularization of the proximal outlet septum is completed, at E15.5, the outlet septum of \( Lp/+ \) fetuses is a thick wedge of muscular tissue separating the aorta from the right ventricle (arrow in Figure 2I). In contrast, the outlet septum in the \( Lp/Lp \) fetuses is malpositioned and remains unmuscularized.
Figure 3. Myocardializing cells show characteristics of motile polarized cells. A and B, Fluorescent MF20 staining, of Lp/+ fetuses at E12.5, shows that the cardiomyocytes in the outflow tract wall extend lamellipodia and filopodia into the flanking cushion tissue. In Lp/Lp littermates, the cardiomyocytes remain rounded and do not extend into the flanking cushion tissue. C and D, Phalloidin (Phal) staining of actin filaments reveals that in Lp/+, the cardiomyocytes extending into the cushion tissue have lost the cortical distribution seen within the vessel wall (arrowheads in C), and instead show stress fibers (arrows in C). In Lp/Lp littermates, the cardiomyocytes flanking the cushion tissue retain a cortical actin distribution (arrows in D).

Myocardializing Cells Display Characteristics of Polarized Cells That Are Disrupted in Lp

Because Vangl2 is thought to function in the vertebrate counterpart of the Drosophila planar cell polarity pathway, we investigated whether there is evidence of polarized cell movements in the developing outflow tract. In vertebrates, directional protrusive behavior is the key feature of polarized cell movements.7 This involves the formation of unipolar or bipolar lamellipodial extensions and the reorganization of the actin cytoskeleton from a cortical to filamentous form, seen as the appearance of stress fibers.

In wild-type embryos, at E12.5, the myocardial cells of the outflow tract wall lose their epithelial contacts and appear to penetrate into the adjacent cushion tissue (as shown by van den Hoff and coauthors11,15–17). Fluorescent labeling of these cells with MF20 shows that the cells at the leading edge of the myocardium, flanking the endocardial cushion tissue, have an elongated appearance, and lamellipodia and filopodia can be seen extending into the unstained cushion mesenchyme (Figure 3A). Staining similar outflow tract sections with rhodamine-conjugated phalloidin reveals that the cardiomyocytes in the outflow tract wall show cortical actin fibers (arrowheads in Figure 3C), whereas the cells extending into the septum have reorganized their actin cytoskeleton such that the bulk of the filamentous actin is seen as stress fibers running parallel to the long axis of the cells (arrows in Figure 3C). In Lp/Lp, the cardiomyocytes in the outflow tract wall have also deep epithelialized and thus have lost their smooth boundary with the endocardial cushion tissue. In contrast to their littermates, however, no lamellipodia or filopodia can be seen extending into the endocardial cushion tissue, and the cells have retained the rounded morphology that is characteristic of the cells in the myocardial wall of the outflow tract (arrows in Figure 3B). Furthermore, phalloidin staining reveals that the majority of the cells in the leading edge maintain a cortical filamentous actin distribution, rather than displaying stress fibers (arrows in Figure 3D). These data show that during the early stages of muscularization of the proximal outflow tract cushions, when the myocardial cells in the outflow tract wall move into the septum, they display characteristics of polarized cells, and significantly, these are disturbed in Lp/Lp fetuses where a key planar cell polarity gene is mutated.

Components of the Noncanonical Wnt Pathway Are Expressed During Remodeling of the Proximal Outflow Tract

We looked at the expression patterns of other planar cell polarity genes during muscularization of the proximal outflow septum to establish whether the components are in place for pathway signaling. The expression patterns of Wnt5a and Wnt11, which are thought to be the specific Wnt ligands that activate the noncanonical pathway, Dvl1, Dvl2, and Dvl3 whose protein products are predicted to bind Vangl2, and ROCK1 and ROCK2, which potentially act downstream in the pathway, were analyzed in outflow tract sections from E11.5 to E15.5.

Wnt5a is expressed in the outflow tract endocardial cushions at E11.5 (Figure 4B) but is downregulated within the developing septum by E12.5, although there is low level expression in the cardiomyocytes penetrating the septum at E12.5 and E13.5 (data not shown). In contrast, Wnt11 is expressed in a similar pattern to Vangl2 in the myocardium of the outflow tract from E9.5 to E15.5, and in the cardiomyocytes extending into the septum during muscularization (Figure 4C and data not shown). Of the Disheveled (Dvl) genes, only Dvl2 is expressed in the myocardium of the outflow tract and in the cardiomyocytes extending into the septum (Figure 4E), with Dvl1 and Dvl3 found at very low levels in the developing outflow tract (data not shown). ROCK1 and ROCK2 transcripts are, like Wnt11, expressed in the myocardium of the outflow tract from E9.5 to E15.5 (Figure 4F and data not shown) in a strikingly similar pattern to Vangl2, and thus were considered to be good candidates for acting downstream of Vangl2 in the outflow tract myocardium. None of these genes, including Vangl2 itself, were misexpressed at the mRNA level in Lp/Lp embryos (Figure 4D and data not shown), suggesting that the absence of functional Vangl2 protein does not affect the transcription of these genes.
Pathway activation by Wnt results in activation of RhoA, which in turn activates downstream signaling cascades, culminating in remodeling. Interestingly, and as might be predicted from the activity during the myocardialization step of outflow tract septation, the initially mesenchymal endocardial cushions are transformed into the mature muscular subpulmonary infundibulum, which receives the outlet of the right ventricle and supports the pulmonary trunk, and in doing so confines the aorta to the left ventricle. The precise cellular events that underlie this process are becoming clearer, and it seems that an early step requires cardiomyocyte polarization of the myocardializing cells is inhibited in Lp/Lp, but these cells are not extending into the septum to the same extent as in their Lp/+ littermates (Figure 5H and arrows in 5M), as has also been seen with other markers. Overlaying sections stained for RhoA and ROCK1 in Lp/Lp embryos (Figure 5L through 5N) showed that the RhoA and ROCK1 expression domains are nonoverlapping and the two proteins do not colocalize at the myocardial-cushion tissue interface. These data therefore show that in wild-type embryos RhoA and ROCK1 expression domains overlap, but that in Lp, where polarized myocardial cells are absent, RhoA expression is lost.

**Discussion**

During outflow tract septation, the initially mesenchymal endocardial cushions are transformed into the mature muscular subpulmonary infundibulum, which receives the outlet of the right ventricle and supports the pulmonary trunk, and in doing so confines the aorta to the left ventricle. The precise cellular events that underlie this process are becoming clearer, and it seems that an early step requires cardiomyocytes within the outflow tract wall to respond to an inductive signal by loss of epithelial contacts and protrusion into the mesenchymal cushion tissue. These migrating muscle cells then induce flanking mesenchymal cushion cells to differentiate into cardiomyocytes, and these, in turn, induce differentiation of further cushion cells, until the entire septum is muscularized (see review11). The data presented in this study suggest that the establishment and/or maintenance of cellular polarity is essential for the muscularization process to proceed normally.

Polarized cell movements are a common feature of embryonic development in both vertebrates and invertebrates. Our data indicates that the myocardializing cells of the outflow tract exhibit characteristics of polarized cells, including directional protrusive behavior and reorganization of the cortical actin cytoskeleton. Importantly, we show that the polarization of the myocardializing cells is inhibited in Lp mutants, which fail to muscularize their proximal outlet septum as a key phenotypic feature. This suggests that the protein encoded by the Vangl2 gene, which is disrupted in Lp mice and is known to act in the noncanonical Wnt (planar cell polarity) pathway, is essential for normal polarized cell activity during the myocardialization step of outflow tract remodeling. Interestingly, and as might be predicted from the

**Figure 4.** Expression pattern of components of the planar cell polarity pathway in wild-type embryos at E11.5. A and D, Vangl2 is expressed in the outflow tract myocardium in Lp/+ and Lp/Lp littermates. B, Wnt5a is expressed in the endocardial cushions at E11.5 (arrows) and not in the outflow tract myocardium. C, E, and F, Wnt11, Dvl2, and ROCK1 are expressed in the outflow tract myocardium. G, Noncanonical Wnt signaling. Vangl2 is a component of a multiprotein complex at the cell membrane. Pathway activation by Wnt results in activation of RhoA, which in turn activates downstream signaling cascades, culminating in affects on polarized cell movements. pos indicates proximal outlet septum; rv, right ventricle; Dgo, diego; Dvl, Disheveled; Fmi, flamingo, Fz, frizzled; Pk, prickle.

**RhoA Is Lost From the Myocardial-Cushion Tissue Interface in Lp**

Once we had established that components of the noncanonical Wnt pathway are expressed in the outflow tract myocardium, and that polarity of myocardializing cells is disturbed in Lp, we set out to determine the specific pathway that acts downstream of Vangl2 to regulate polarized cell movements. Vangl2 is thought to be part of a multiprotein complex at the cell membrane, which activates RhoA. RhoA, in turn, activates the Rho kinases, which then phosphorylate downstream components of the pathway, resulting in reorganization of the actin cytoskeleton and cell movement (Figure 4G).

RhoA protein is found in the mesenchymal cushion cells adjacent to the outflow tract myocardium in Lp/+ embryos at early E12.5 and, by E13.5, is abundant at the boundary between the two cell types (Figure 5A and 5C). JNK, which is also thought to act downstream of noncanonical Wnt signaling in vertebrates, could not be detected in the outflow tract region, either in the myocardium or the endocardial cushions (data not shown), and so was ruled out as mediating signaling downstream of Vangl2 in the outflow tract. ROCK1 protein, in contrast, is found throughout the myocardium in Lp/+ embryos at E12.5, but by E13.5, it is most abundant in the myocardializing cells that penetrate the proximal outlet septum (Figure 5E and 5G and arrows in 5I). To colocalize RhoA and ROCK1, we tried a number of fixation and immunohistochemical protocols, but were unable to visualize the two antigens on the same section. However, by overlaying adjacent sections, we were able to show that the RhoA and ROCK1 expression domains overlap, and that the two proteins appear to be coexpressed at the myocardial-cushion tissue interface (Figure 5I through 5K).

In Lp/Lp littermates, at both E12.5 and E13.5, RhoA is abundantly expressed in some regions of the cushion tissue, but minimal staining is found in other regions (asterisks in Figure 5B and 5D). ROCK1 protein can be seen in some of the myocardial cells interdigitating with the septum in Lp/Lp, but these cells are not extending into the septum to the same extent as in their Lp/+ littermates (Figure 5H and arrows in 5M), as has also been seen with other markers. Overlaying sections stained for RhoA and ROCK1 in Lp/Lp embryos (Figure 5L through 5N) showed that the RhoA and ROCK1 expression domains are nonoverlapping and the two proteins do not colocalize at the myocardial-cushion tissue interface. These data therefore show that in wild-type embryos RhoA and ROCK1 expression domains overlap, but that in Lp, where polarized myocardial cells are absent, RhoA expression is lost.
model proposed by van den Hoff and colleagues,11 when the numbers of myocardial cells extending into the septum are reduced, fewer mesenchymal cushion cells are induced to differentiate into cardiomycocytes, as shown by their expression of α-smooth muscle actin.17 Thus, by a combination of failure of extension of the myocardial cells into the septum, and insufficient induction of mesenchyme cells into the cardiomycocyte lineage, the Lp/Lp septum remains unmuscularized. Whether this defect in muscleization of the proximal outlet septum has a direct affect on alignment of the great vessels with the ventricular chambers remains to be established.

Noncanonical Wnt signaling has been shown to be involved in a number of morphological processes in vertebrate species, although it has not, until now, been described in cardiovascular development. Mice carrying null mutations in the Dvl2 gene, which we have shown to be expressed in the outflow tract myocardium and is a key molecule in both canonical and noncanonical Wnt signaling, have similar outflow tract defects to those seen in Lp, including double outlet right ventricle and ventricular septal defects.18 The outflow tract defects in Dvl2 null mutants have been linked, via the canonical Wnt pathway, to abnormalities in neural crest development in the outflow tract, although there is no evidence for either defects in neural crest cell migration into the outflow tract or disruption of the canonical Wnt pathway in Lp (our unpublished data). Interestingly, the severe neural tube defects (craniorachischisis) in double mutants of Dvl1 and Dvl2, which appear to be identical to the neural tube defect found in Lp, have been ascribed to abnormalities in planar cell polarity pathway signaling.18 Although it is possible that Dvl2 plays different roles in different tissues, our observation that Dvl2 is expressed in the outflow tract myocardium, raises the possibility that it may also be acting in the planar cell polarity pathway in this tissue, and this may be compounding abnormalities in neural crest development in the outflow tract in Dvl2 null mutants. Mouse embryos lacking the flamingo homologue, Celsr1,19 which also plays a key role in PCP signaling,6 have outflow tract defects similar to those found in Lp (our unpublished data), further supporting the notion that noncanonical Wnt signaling is essential for normal outflow tract development.

Reviewing data from vertebrate and invertebrate species, it appears that Vang/strabismus is a component of a membrane-associated multiprotein complex which includes frizzled, flamingo, disheveled, prickle, and diego homologues (see reviews6,7). The formin-homology protein, Daam1, binds directly to both Disheveled and RhoA, which is a critical regulator of the actin cytoskeleton and also plays important roles in cell migration (see review20). The binding of Wnt to the frizzled receptor thus initiates this pathway, activates RhoA, and culminates in reorganization of the actin skeleton, facilitating polarized cell movements.7 In this article, we show that Vangl2, and several other key components of the PCP pathway, are expressed in the outflow tract. Both Wnt5a and Wnt11 are found in the outflow tract at E11.5, just before myocardialization begins; Wnt5a is found in the endocardial cushion tissue, whereas Wnt11 is found in the myocardium. Wnt5a is therefore ideally localized to induce polarized cell migration from the myocardium into the cushion tissue, and
markers. Activation of ROCK1 requires its recruitment to the interface, although its expression domain does not overlap with ROCK1. In these regions, although ROCK1 is expressed in the endocardial cushion tissue, at its interface with the myocardium, overlapping with one if its key effectors, RhoA, is also expressed in the myocardium. RhoA is expressed in the endocardial cushion tissue, but is also coexpressed with ROCK1 in the myocardializing cells. Myocardializing cells recruit flanking mesenchymal cushion cells into the myocardial lineage, which in turn recruit the next layer of cushion cells to become cardiomyocytes. This results in complete muscularization of the septum. In Lp/Lp embryos, where Vangl2 signaling is disrupted, the myocardial cells flanking the septum do not extend into the tissue and reduced numbers of cushion cells are recruited to muscle. The developing septum in Lp/Lp retains a mesenchymal composition.

Figure 6. Diagram illustrating our model for the role of Vangl2 in muscularization of the proximal outlet septum. In the normal embryo, Vangl2 is expressed in the outflow tract myocardium, including the cells that penetrate the cushion tissue in the process of myocardialization. These myocardializing cells extend lamellipodia and filopodia into the flanking mesenchymal cushion tissue. Other components of the planar cell polarity pathway, including ROCK1, are also expressed in the myocardium. RhoA is expressed in the endocardial cushion tissue, but is also coexpressed with ROCK1 in the myocardializing cells. Myocardializing cells recruit flanking mesenchymal cushion cells into the myocardial lineage, which in turn recruit the next layer of cushion cells to become cardiomyocytes. This results in complete muscularization of the septum. In Lp/Lp embryos, where Vangl2 signaling is disrupted, the myocardial cells flanking the septum do not extend into the tissue and reduced numbers of cushion cells are recruited to muscle. The developing septum in Lp/Lp remains in an inactive form. This failure to activate ROCK1 would block downstream propagation of the signal, and thus inhibit reorganization of the actin cytoskeleton and polarized cell movements. We are currently designing strategies and reagents to study the activation state of RhoA, ROCK1, and downstream components of the pathway in the myocardializing cells.

RhoA and the Rho kinases have recently been shown to play crucial roles in cardiac development and inhibition of either of these factors prevents fusion of the bilateral heart primordia and results in disturbed trabeculation and chamber demarcation, depending on developmental stage. These latter defects are associated with effects on the cell cycle, resulting in a decrease in cell proliferation, rather than obvious effects on the cytoskeleton. In contrast, the defects in heart tube fusion appear to result from abnormalities in migration of the bilateral heart primordia, and thus may involve similar polarity-related migratory processes to those described here. RhoA/ROCK signaling thus appears to play an important role in myocardial cell migration, both at the earliest stages of heart tube formation, and at the remodeling stages of cardiac development, when the heart is septating. In summary, we have described the mechanism by which myocardializing cells extend into the developing outlet septum and have identified a pivotal role for the planar cell polarity signaling pathway in this muscularization process.

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References


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