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Left–right asymmetry is formed in individual cells by intrinsic cell chirality

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ABSTRACT

Many animals show left–right (LR) asymmetric morphology. The mechanisms of LR asymmetric development are evolutionarily divergent, and they remain elusive in invertebrates. Various organs in *Drosophila melanogaster* show stereotypic LR asymmetry, including the embryonic gut. The *Drosophila* embryonic hindgut twists 90° left-handedly, thereby generating directional LR asymmetry. We recently revealed that the hindgut epithelial cell is chiral in shape and other properties; this is termed planar cell chirality (PCC). We previously showed by computer modeling that PCC is sufficient to induce the hindgut rotation. In addition, both the PCC and the direction of hindgut twisting are reversed in *Myosin31DF* (*Myo31DF*) mutants. *Myo31DF* encodes *Drosophila* MyosinID, an actin-based motor protein, whose molecular functions in LR asymmetric development are largely unknown. Here, to understand how PCC directs the asymmetric cell-shape, we analyzed PCC in genetic mosaics composed of cells homozygous for mutant *Myo31DF*, some of which also overexpressed wild-type *Myo31DF*. Wild-type cell-shape chirality only formed in the *Myo31DF*-overexpressing cells, suggesting that cell-shape chirality was established in each cell and reflects intrinsic PCC. A computer model recapitulating the development of this genetic mosaic suggested that mechanical interactions between cells are required for the cell-shape behavior seen *in vivo*. Our mosaic analysis also suggested that during hindgut rotation *in vivo*, wild-type *Myo31DF* suppresses the elongation of cell boundaries, supporting the idea that cell-shape chirality is an intrinsic property determined in each cell. However, the amount and distribution of F-actin and Myosin II, which are known to help generate the contraction force on cell boundaries, did not show differences between *Myo31DF* mutant cells and wild-type cells, suggesting that the static amount and distribution of these proteins are not involved in the suppression of cell-boundary elongation. Taken together, our results suggest that cell-shape chirality is intrinsically formed in each cell, and that mechanical force from intercellular interactions contributes to its formation and/or maintenance.

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1. Introduction

Left–right (LR) asymmetry in body structures is a crucial aspect of development in many animals (Levin, 2005; Vandenberg and Levin, 2013; Okumura et al., 2008; Shiratori and Hamada, 2006). Several distinct mechanisms have been revealed that contribute to the formation of the LR axis in various species (Nakamura and Hamada, 2012; Palmer, 2004), including the LR-asymmetric flow of extra embryonic fluid (nodal flow), LR ion flux asymmetry in blastomeres, and LR-asymmetric cell migration. Some mechanisms may function simultaneously within a species (Yost, 2003; Adams et al., 2006; Gros et al., 2009). In some vertebrate species, including mice and fish, nodal flow created by the clockwise rotation of monocilia in the node initiates the formation of an LR axis in the embryo, and subsequently induces the LR-asymmetric expression of a conserved set of genes called the nodal cassette (Nonaka et al., 1998; Krebs et al., 2003; Essner et al., 2005; Larkins et al., 2012). However, cilia are not found in the node of the pig embryo, suggesting some diversity in the mechanism of LR axis formation (Gros et al., 2009). Furthermore, in the chick LR-asymmetrical cell movement around Hensen's node, which is equivalent to the mouse node, is observed as a first cue of LR asymmetric development (Gros et al., 2009). Monocilia do not exist in Hensen's node (Gros et al., 2009). In the frog, LR-asymmetric ion gradients precede nodal cassette expression, and the LR-asymmetric distribution of H^+/K^+ ATPase mRNA is observed as early as the 2–4-cell stage (Levin et al., 2002). It was also shown that an H^+/K^+ ATPase-dependent LR-difference in membrane voltage potential is important for LR patterning (Levin et al., 2002). In addition, LR asymmetry of H^+-V-ATPase-dependent H^+ flux at the 4–8-cell stage is also required for LR patterning in the chick, frog, and zebrafish (Adams et al., 2006). The mechanisms of LR-asymmetric development are more elusive in invertebrates, although LR asymmetry in blastomere shape at very early cleavage and in blastomere cytoskeletal organization is known to be involved in LR axis formation in snails and nematodes (Shibazaki et al., 2004; Kuroda et al., 2009; Meshcheriakov, 1976; Pohl and Bao, 2010). Thus, to ascertain all the mechanisms at work in LR-asymmetric development in animals, it is important to explore how these mechanisms function in various invertebrate species (Okumura et al., 2008).

In this respect, *Drosophila* is a useful model for studying the genetic and molecular mechanisms that form the LR axis and subsequent LR-asymmetric development (Okumura et al., 2008; Kuroda et al., 2012; Nakamura et al., 2013). Several organs in *Drosophila*, including the gut, testis, male genitalia, and brain, show stereotypical LR asymmetry (Hozumi et al., 2006; Spéder et al., 2006; Pascual et al., 2004). Among these organs, the gut of the developing embryo is the first structure to show LR asymmetry (Hayashi and Murakami, 2001; Hozumi et al., 2006); the foregut, midgut, and hindgut all show clear, genetically determined LR-asymmetric morphology (Hozumi et al., 2006). The embryonic hindgut has a simpler structure than the foregut or midgut, making it easier to analyze. The embryonic hindgut is composed of a single-layer epithelial tube covered by visceral muscle cells (Lengyel and Iwaki, 2002). Early in development, the embryonic hindgut is LR-symmetric and curves ventrally, but as it develops it undergoes a 90° left-handed rotation (Fig. 1A), causing it to curve toward the right (Fig. 1B) (Hayashi and Murakami, 2001; Taniguchi et al., 2011). This process occurs without cell division or apoptosis (Iwaki et al., 2001; Wells et al., 2013). Eliminating the overlying visceral muscle has no effect on the LR asymmetry of the embryonic hindgut (Hozumi et al., 2008), indicating that the hindgut epithelial tube is sufficient to induce this left-handed rotation without the assistance of the overlying visceral muscles (Hozumi et al., 2008; Vandenberg and Levin, 2013).

We recently reported that epithelial cells of the embryonic hindgut show planar cell chirality (PCC), a form of planar polarity that reflects the LR asymmetry of the cells on the plane of their cell layer (Taniguchi et al., 2011). PCC is observed in LR asymmetry in the shape, centrosome position, and *Drosophila* E-cadherin (DE-cad) distribution in individual cells (Taniguchi et al., 2011). In our previous study, a computer model suggested that cell-shape chirality is formed through mechanical force, possibly controlled by the LR-asymmetric distribution of DE-Cad (Taniguchi et al., 2011). This computer model also suggested that the cell-shape chirality can induce epithelial-tube rotation (Taniguchi et al., 2011). Interestingly, cell chirality is seen in various cultured mammalian cells, suggesting that cell chirality is a conserved property of cells (Wan et al., 2011; Xu et al., 2007; Chen et al., 2012). However, the mechanism of cell chirality formation is largely unexplored.

In *Drosophila* loss-of-function *Myo31DF* mutants, the direction of PCC in the embryonic hindgut epithelium and the LR asymmetry of various organs are reversed from those in wild-type *Drosophila* (Taniguchi et al., 2011) (Fig. 1C). Thus, it was proposed that these reversed states of PCC and organ laterality in *Myo31DF* mutants are the default state of LR asymmetry in *Drosophila* (Hozumi et al., 2006). According to this hypothesis, *Myo31DF* switches the mirror-image (sinistral) PCC found in *Myo31DF* mutants to wild-type (dextral) PCC (Taniguchi et al., 2011). *Myo31DF* encodes a type I unconventional Myosin, Myosin ID (Hozumi et al., 2006; Spéder et al., 2006). To understand how *Myo31DF* directs PCC formation, it is important to address whether the process by which *Myo31DF* determines cell-shape chirality is cell-autonomous. The answer to this question will help indicate whether PCC is individually formed in each cell or reflects a LR polarity established at the tissue level.

We previously generated a genetic mosaic hindgut epithelium of homozygous mutant-*Myo31DF* cells, some of which overexpressed wild-type *Myo31DF*; that study showed that the LR-asymmetric distribution of DE-Cad is controlled cell-autonomously by the *Myo31DF* gene (Taniguchi et al., 2011). However, whether or not wild-type *Myo31DF* directs chiral cell-shape cell-autonomously has not been clarified. This is a difficult issue, because predicting how mechanical forces will interact among hindgut epithelial cells, which show both dextral and sinistral PCC, during the formation of cell-shape chirality is not easy. It is challenging to analyze the

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