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Review

The balancing roles of mechanical forces during left-right patterning and asymmetric morphogenesis

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ABSTRACT

Left-right patterning and asymmetric morphogenesis arise from a complex set of molecular and cellular interactions that are particularly dynamic and associated with mechanical forces. How do mechanical forces translate into tissular asymmetries? Are these forces asymmetrical *de novo*, or do they build up from pre-existing asymmetries? Advances in developmental genetics, live imaging and cell biology have recently shed light on the origins of mechanical forces generated at the cell scale and their implication in asymmetric patterning and morphogenesis is now emerging. Here we ask when and how, molecular asymmetries and mechanical forces contribute to left-right patterning and organ asymmetries.

Contents

1. Introduction

Mechanical forces are ubiquitous and can modulate the developmental program of plants and animals (Mammoto and Ingber, 2010; Mirabet et al., 2011). Mechanical forces are influent in many steps of embryonic development, from gastrulation to organogenesis (Hamada, 2015; Heisenberg and Bellaiche, 2013; Mammoto and Ingber, 2010). Gastrulation (Behrndt et al., 2012; Farge, 2003; Hiramatsu et al., 2013; Maitre et al., 2012), kidney morphogenesis (Kramer-Zucker et al., 2005), inner ear and otolith formation (Colantonio et al., 2009; Wu et al., 2011), neuron migration (Sawamoto et al., 2006), cardiovascular development (Boselli et al., 2015; Freund et al., 2012; Peralta et al., 2013), haematopoiesis (Pardanaud and Eichmann, 2009), and left-right symmetry breaking (Nonaka et al., 1998) are all mediated by mechanical stresses and force mediated signaling (Zhang and Labouesse, 2012). Prominent mechanical forces-related diseases include cancer (Fernandez-Sanchez et

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al., 2015; Fernandez-Sanchez et al., 2010), ciliopathies (Hildebrandt et al., 2011) and cardiovascular diseases such as atherosclerosis (Hahn and Schwartz, 2009).

Despite the long recognition of the importance of mechanical forces in development, an understanding of how mechanical forces impact development has, until recently, remained elusive. Technological advances in recent years have allowed researchers to study the effects of physical forces on cell behaviors at unprecedented resolution (Ladoux et al., 2016; Lecuit et al., 2011). The results of these studies have led to a paradigm, where in its most extreme form, holds the idea that physical forces, independently of gene expression, can affect tissue development and growth by directly modulating cell behavior (Savin et al., 2011). Mechanical forces have also been shown to act as a key component in the coordination of cell behaviors at the tissue scale, in particular during tissue folding (Striedter et al., 2015). As a consequence, it is now clear that mechanical forces constitute an essential element in multiple aspects of the morphogenetic program (LeGoff and Lecuit, 2016; Zhang et al., 2010).

Forces can be sensed at the molecular and cellular scale through mechanosensitive proteins (Vogel and Sheetz, 2006). A major focus of research is now to define the molecules and signaling pathways associated with mechanotransduction and work from many different fields has now shown that pressure-sensitive membrane proteins, cytoskeletal elements, and extracellular matrix (ECM) components can participate in the interchange between mechanical forces and biochemical signals at the cellular scale (Mammoto et al., 2012; Vogel and Sheetz, 2006). Although much has been done in the study of biomechanical signaling at the cellular scale, the effects of forces at a tissue scale level have emerged only recently (Grill, 2011; Lecuit et al., 2011; Mammoto and Ingber, 2010). The field strongly benefits from concepts and formulation developed by physicists, which promoted the identification and quantification of the relevant forces through unifyed approaches (Grill, 2011). Recent advances in cell biology and live imaging are now allowing researchers to directly assess the distribution of tissue forces, thus helping them to have a better view of how mechanical forces can impact development (Sugimura et al., 2016). This, combined with the discoveries of novel mechanosensitive proteins and pathways, are consistently changing our view of how mechanical forces can impact development.

Left-right patterning and asymmetric morphogenesis is one of the most fascinating aspects of developmental biology. Both the symmetry and asymmetries of the body plan require a number of processes that need to be carefully controlled through a genetic program (Capdevila et al., 2000; Hamada et al., 2002; Pourquie, 2011). Being asymmetric certainly constitutes an advantage in the process of organ packing and positioning in a restrained space. Accordingly, most of our internal organs are asymmetrically positioned within the body cavity. Recent studies in the field of the left-right signaling and asymmetric tissue morphogenesis are now clarifying and reinforcing the interest in the field of mechanical forces and morphogenesis. Examples of tissue asymmetry can be seen in heart tube loop, brain folding, airway branching (Yashiro et al., 2007) and gut looping (Savin et al., 2011). Here, we review the molecular and sub-cellular basis of mechanical and biochemical pathways activated during left-right patterning and asymmetric morphogenesis. Throughout the review, we discuss the potential mechanosensors involved and the mechanical forces generated at cellular and tissue scale.

2. Left-right symmetry breaking mediated by cilia mediated flow forces

Fluid motion is usually mediated by motile cilia in the body. Motile cilia are organelles that protrude from nearly all vertebrate cells with typical lengths between 3 and 10 μm in growing tissues (Avasthi and Marshall, 2012; Ishikawa and Marshall, 2014; Keeling et al., 2016; Vincensini et al., 2011). In vertebrates, cilia are commonly thought to function as chemical and/or mechanical sensors. Motile cilia move fluids, and in doing so they participate in controlling several key developmental processes, such as chemical gradient formation, biomineralization or tubulogenesis (Cartwright et al., 2009). Left-right (LR) specification in vertebrates occurs in the left-right organizer (LRO), which is defined by a group of specialized cells located within the presomitic mesoderm. The cells delineating the LRO are ciliated and contain motile cilia that generate a slow-moving flow (the nodal flow) involved in the initial step of symmetry breaking (Nonaka et al., 1998) (Fig. 1A). Additionally, an intercellular amplification of the asymmetric signals occurs through genetic feedback mechanism near and around the LRO (Nakamura et al., 2006). The prominent models explaining symmetry breaking within the LRO suggests either an asymmetric chemical gradient (Okada et al., 2005), or that the LRO cells can mechanically sense flow due to a particular type of sensory cilia located in the periphery of the LRO, dictates the asymmetry (McGrath et al., 2003; Tabin and Vogan, 2003). While it is possible that these two mechanisms work together, a number of elements are still lacking for our complete understanding of the process (Pennekamp et al., 2015). Importantly, symmetry breakage occurs even in mutant mice with only two motile cilia (Shinohara et al., 2012). Experimental data using a mutant of the Notch signaling pathway and simulations of fluid flow dynamics in the zebrafish LRO revealed a threshold of approximately 30 motile cilia to get a proper LR symmetry breakage (Sampaio et al., 2014). This suggests that the flow detection apparatus is extremely efficient. When considering the flow velocities generated within the LRO of fish, mice and xenopus (Blum et al., 2009; Blum et al., 2014; Schweickert et al., 2007; Supatto and Vermot, 2011), it appears that they are much lower when compared to other organs - for example, they are 3 to 10 times lower than the hemodynamics generated in the vascular network even at its earliest embryonic stages (Anton et al., 2013; Cartwright et al., 2009; Goetz et al., 2014; Hove et al., 2003; Supatto and Vermot, 2011).

The mechanosensory hypothesis has been favored by the discovery that Trpp2 (PKD2 or polycystic kidney disease protein 2) is key for LR patterning (Field et al., 2011; Kamura et al., 2011; McGrath et al., 2003; Pennekamp et al., 2002; Schottenfeld et al., 2007; Yuan et al., 2015). Trrp2 is a potent mechanosensory protein (Patel et al., 2010; Sharif-Naeini et al., 2010) both in kidney and vasculature (Goetz et al., 2014; Nauli et al., 2003; Nauli et al., 2008) that acts in combination with Pkd1 at the cell membrane. In zebrafish, Trpp2 is necessary for the genesis of asymmetric calcium release around the LRO, which is initiated within cilia (Yuan et al., 2015). Mutant protein of Trpp2 that cannot bind to the membrane cannot rescue Trpp2 loss of function in the LRO and lead to LR symmetry defects (Yoshiba et al., 2012). Trpp2 belongs to the big family of transient receptor potential proteins (TRP) that contain a number of mechanosensitive channels. Yet, Trpp2 is not a 'canonical' stretch sensitive channel and its biology is extremely complex and cell type specific (Giamarchi et al., 2006): it is part of a multiprotein complex involved in transducing Ca^{2+}-dependent information. It localizes to primary cilia of renal epithelial cells, where it seems involved in mechanosensitive transduction signals (Nauli et al., 2003; Pazour et al., 2002; Yoder et al., 2002), but it has been observed at the cell membrane and in the ER. Trpp2 has been shown to inhibit the response of stretch activated cation channels in smooth muscle cells, suggesting that it can modulate mechanotransduction without being a mechanosensor itself (Sharif-Naeini et al., 2009). Recently, the group of David Clapham showed that intraciliary calcium increase is not observed in the mouse LRO in response to flow forces, suggesting that the primary function of TRP channels, including Trpp2, is not to modulate intraciliary calcium in response to cilia bending, and, as a consequence, do not act as mechanosensor in this context (Delling et al., 2016). In that aspect, it is worth mentioning that Pkd2 mutants do not present apparent defects in intracellular calcium levels in the node (Yoshiba et al., 2012). Importantly, Trpp2 frequently acts in combination with other mechanosensitive proteins such as Trpv4 (Du et al., 2014; Heckel et al., 2015; White et al., 2016), Pkd1 (Hanaoka et al.,