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Mechanical control of notochord morphogenesis by extra-embryonic tissues in mouse embryos

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ABSTRACT

Mammalian embryos develop in coordination with extraembryonic tissues, which support embryonic development by implanting embryos into the uterus, supplying nutrition, providing a confined niche, and also providing patterning signals to embryos. Here, we show that in mouse embryos, the expansion of the amniotic cavity (AC), which is formed between embryonic and extraembryonic tissues, provides the mechanical forces required for a type of morphogenetic movement of the notochord known as convergent extension (CE) in which the cells converge to the midline and the tissue elongates along the antero-posterior (AP) axis. The notochord is stretched along the AP axis, and the expansion of the AC is required for CE. Both mathematical modeling and physical simulation showed that a rectangular morphology of the early notochord caused the application of anisotropic force along the AP axis to the notochord through the isotropic expansion of the AC. AC expansion acts upstream of planar cell polarity (PCP) signaling, which regulates CE movement. Our results highlight the importance of extraembryonic tissues as a source of the forces that control the morphogenesis of embryos.

1. Introduction

Mammalian embryos develop inside of the liquid-filled cavities that are formed by extraembryonic tissues (Fig. 1A). The development of embryos is coordinated with extraembryonic tissue formation, which support embryonic development by implanting embryos into the uterus (Cha et al., 2012), supplying nutrition (Bielinska et al., 1999), providing a confined

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niche (Schmidt, 1992), and also providing patterning signals (Arnold and Robertson, 2009; Tam and Loebel, 2007) to embryos. Growing evidence suggests the importance of mechanical signals in the control of cellular responses and embryonic development (Halder et al., 2012; Hiramatsu et al., 2013; Orr et al., 2006; Vogel and Sheetz, 2009). Because embryos are connected with extraembryonic tissues, the forces generated by the embryonic/extraembryonic tissues also likely control the development of these tissues. To understand the mechanical roles of extraembryonic tissues on mouse embryonic development, we used mouse embryos and focused on the evolutionarily conserved morphogenesis of the notochord, specifically, convergent extension (CE) movement of the notochord (Keller, 2002; Keller et al., 1989; Sausedo and Schoenwolf, 1993, 1994; Wallingford et al., 2002; Wood and Thorogood, 1994) in which the cells converge to the midline and the tissue elongates along the antero-posterior (AP) axis. Studies in *Xenopus* showed that CE of the notochord relies on cell rearrangement (Keller, 2002; Wallingford et al., 2002). Wnt/Frizzled/planar cell polarity (PCP) signaling is required for the polarization of cells (Keller, 2002; Myers et al., 2002; Wallingford et al., 2002), and the AP patterning systems acting upstream or in parallel determine the direction of tissue elongation (Ninomiya et al., 2004). PCP signaling also plays important roles in CE of the mouse notochord (Wang et al., 2006; Ybot-Gonzalez et al., 2007; Yen et al., 2009), but cellular behaviors during CE in the mouse notochord has not been studied in detail.

In this study, we performed time-lapse imaging of cellular behaviors during CE in the mouse notochord, and found that the cellular behaviors are different from those of *Xenopus* notochord. We also revealed that, in mouse embryos, the force generated by expansion of the amniotic cavity (AC) plays critical roles in CE of the notochord.

2. Results

2.1. *Mouse notochord is stretched along the AP axis*

To understand the cellular basis of notochord morphogenesis, we performed time-lapse imaging of the notochord during CE movements. To monitor the cell movements, we used *Foxa2*$^{nEGFP–CreERT2}$ embryos, which express enhanced green fluorescent protein (EGFP) in the nuclei of the notochord and the endoderm (Imuta et al., 2013), and time-lapse movies were obtained between the early head fold (EHF) (Downs and Davies, 1993) and approximately 5-somite stages. A dissected EHF stage embryo was fixed in a modified culture dish, which was placed on the inverted confocal microscope, as to visualize the posterior notochord region (Yamanaka et al., 2007) (Fig. 1B), and were cultured with imaging for approximately 10 h. At this stage, the notochord is a one-cell-thick sheet continuous with the endoderm and is exposed to the visceral yolk sac cavity (Fig. 1A).

During this period, the notochord undergoes CE morphogenesis (Sausedo and Schoenwolf, 1994), and our live-imaging analyses revealed dynamic cell rearrangements during the CE movements (Fig. 1C–F, Movie S1). Unexpectedly, the notochord cell nuclei were initially oriented toward the left–right (LR) axis, but gradually reoriented toward the AP axis (Fig. 2A–F, Movie S2). Such reorientation of the notochord cells has not been previously reported in *Xenopus* notochord studies (Keller, 2002; Keller et al., 1989; Wallingford et al., 2002). The orientation of the cell division axis was also biased toward the AP and the LR axes in the notochord and endoderm, respectively (Fig. 2G, Movie S3). Such cellular behavior gave us an impression that the notochord is stretched along the AP axis during CE, and that the cells are rearranged and reoriented to support the rapid extension of this tissue.

To examine whether the notochord is stretched along the AP axis, we ablated a notochord cell by using laser-pulse irradiation and examined the movement of the cells surrounding the ablated cell (Fig. 2H, Movie S4). Because ablation of a cell releases the tension from the surrounding cells, the released cells move toward the direction of the tension that the cells are receiving. Wound healing slowly occurred following the rapid relaxation behavior. Therefore, the distance of cell movement immediately after ablation mainly reflects the effects of relaxation behaviors and, thus, correlates with the strength of the tension. Upon ablation of a notochord cell, the cells located anterior (A) or posterior (P) to the ablated cell showed movements that were two to three times larger than those of the cells located at the left (L) or right (R) positions (Fig. 2H, and J–L). Ablation of a nearby endoderm cell caused smaller movements of the surrounding cells; the cells at the LR positions had a tendency to show larger movements (Fig. 2I, M and N). These results are consistent with the hypothesis that the notochord cells receive strong anisotropic tension along the AP axis, and that the neighboring endoderm cells experience weaker tension, which is slightly biased toward the LR axis.

2.2. *AC expansion is required for proper notochord morphogenesis*

Notochord extension progresses with the growth of the embryonic and extraembryonic tissues, including the expansion of the AC (Fig. 1A). We hypothesized that the expansion of the AC could be a source of the force required for CE. To test this hypothesis, we inserted an open-tip fine glass capillary into the amniotic cavity by piercing through the visceral yolk sac and the amnion (Fig. 3A), which suppressed the accumulation of the inner pressure and the expansion of the AC (Fig. 3A, B, E and N). The capillary remained *in situ* throughout the culture period, and another capillary was inserted after 4 h to overcome clogging of the first capillary. In pierced embryos, the elongation of the body axis and CE of the notochord was reduced, and the notochord cells remained oriented toward the LR axis at the end of *in vitro* culture (Fig. 3C, D, and F–I). The oriented division of the notochord cells along the AP axis was also reduced (Fig. 3J). Despite the morphological changes in the pierced embryos, the expression of *Foxa2* and *T* genes in the notochord (Ang and Rossant, 1994; Herrmann, 1991; Sasaki and Hogan, 1993), which was monitored by the knock-in of *H2B-EGFP* into the respective loci (Imuta et al., 2013), were comparable between the pierced and control embryos (Fig. 3K and L). Furthermore, the rate of somite formation at this stage is faster than later stages (about one pair in every hour) (Tam, 1981), and this fast rate was not