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Morphogenetic movements driving neural tube closure in *Xenopus* require myosin IIB

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ABSTRACT

Vertebrate neural tube formation involves two distinct morphogenetic events — convergent extension (CE) driven by mediolateral cell intercalation, and bending of the neural plate driven largely by cellular apical constriction. However, the cellular and molecular biomechanics of these processes are not understood. Here, using tissue-targeting techniques, we show that the myosin IIB motor protein complex is essential for both these processes, as well as for conferring resistance to deformation to the neural plate tissue. We show that myosin IIB is required for actin-cytoskeletal organization in both superficial and deep layers of the *Xenopus* neural plate. In the superficial layer, myosin IIB is needed for apical actin accumulation, which underlies constriction of the neuroepithelial cells, and that ultimately drive neural plate bending, whereas in the deep neural cells myosin IIB organizes a cortical actin cytoskeleton, which we describe for the first time, and that is necessary for both normal neural cell cortical tension and shape and for autonomous CE of the neural tissue. We also show that myosin IIB is required for resistance to deformation ("stiffness") in the neural plate, indicating that the cytoskeleton-organizing roles of this protein translate in regulation of the biomechanical properties of the neural plate at the tissue-level.

Introduction

The vertebrate neural tube forms from an initially flat, short and broad neural plate which then undergoes dramatic morphogenetic events that not only bend and roll it into a tube, but also greatly narrow and lengthen it along its antero-posterior axis (Jacobson and Gordon, 1976; Schoenwolf and Smith, 1990). This narrowing and lengthening of the neural tissue, commonly referred to as convergent extension (CE), is important for neural tube closure, as it brings the neural folds toward the midline, where they will meet and fuse (Wallingford and Harland, 2002). In a urodele (tailed) amphibian, *Taricha torosus*, neural CE is largely dependent on CE of the underlying dorsal mesoderm (Jacobson and Gordon, 1976). The anuran (tailless) amphibian *Xenopus laevis*, however, shows a form of neural CE that is mechanically independent of the underlying mesoderm (Elul et al., 1997; Keller and Danilchik, 1988; Keller et al., 1992a,b). In sandwich explants, this mode of neural CE is induced and maintained by planar organizer signals without continued vertical signaling from the underlying mesoderm (Keller et al., 1992b). In isolated deep neural plate explants, it occurs by mediolateral cell intercalation of the deep mesenchymal cells of the double layered *Xenopus* neural plate, using a bipolar protrusive activity like that seen in the intercalating presumptive mesodermal cells (Elul et al., 1997). In contrast, with normal, continued vertical interactions with the underlying mesoderm, this bipolar mode is replaced with a monopolar, medially directed protrusive activity that is more efficient in producing neural cell intercalation and CE (Elul and Keller, 2000; Ezin et al., 2003). This medially-directed protrusive activity is dependent on unknown signals emanating from the midline tissues of notochord and the overlying notoplate (Ezin et al., 2003, 2006). CE of the neural plate, like that of the dorsal mesoderm, is dependent on the vertebrate non-canonical Wnt/planar cell polarity (PCP) pathway (Wallingford and Harland, 2001, 2002). However, the mechanism by which the neural cells are polarized, how they produce the forces that drive their intercalation, and the relative contribution of the autonomous neural CE movements to the forces that shape the neural plate and roll it into a tube remain unresolved.

Medial movement of the neural folds involves bending of the neural plate. This process is driven largely by shape changes in neuroepithelial cells, which undergo apical constriction and assume a wedge or bottle-cell morphology, thus causing the neural sheet to bend (Burnside and Jacobson, 1968; Schoenwolf and Smith, 1990). The actin-binding protein Shroom is a key regulator of apical constriction during *Xenopus* anterior neural tube closure (Haigo et al., 2003), and in cultured MDCK cells, Shroom causes apical constriction by regulating the apical positioning of a contractile actomyosin network (Hildebrand, 2005).

Myosin II is a motor protein that binds to actin filaments and moves along them by hydrolyzing ATP, and also serves as an actin cross-linker (Laevsky and Knecht, 2003; Xu et al., 2001). Vertebrates have at least two genes encoding two different non-muscle myosin II

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heavy chain proteins — MHC-A and MHC-B (Katsuragawa et al., 1989; Kawamoto and Adelstein, 1991; Kelley et al., 1995). A third isoform, MHC-C, has been identified in mouse (Golomb et al., 2004). MHC-A is the predominant isoform expressed in *Xenopus* early embryonic cells (Kelley et al., 1996), but MHC-B is up-regulated in dorsal tissues that undergo CE (Bhatia-Dey et al., 1998) and myosin IIB is necessary for CE