Available online at www.sciencedirect.com
ScienceDirect
Developmental Biology 301 (2007) 404–416
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Xenopus fibrillin regulates directed convergence and extension

Paul Skoglund *, Ray Keller

Department of Biology-Gilmer Hall Rm. 241, University of Virginia, Charlottesville, VA 22903, USA

Received for publication 12 May 2006; revised 5 September 2006; accepted 5 September 2006
Available online 9 September 2006

Abstract

Fibrillin-based human diseases such as Marfan syndrome and congenital contractural arachnodactyly implicate fibrillins in the function and homeostasis of multiple adult tissues. Fibrillins are also expressed in embryos, but no early developmental role has been described for these proteins. We use three independent methods to reveal a role for *Xenopus* fibrillin (XF) at gastrulation. First, expressing truncated forms of XF in the embryo leads to failure of gastrulation concomitant with a dominant-negative effect on native fibrillin fibril assembly. Expressing truncated XF also inhibits normal progression of the patterned, polarized cell motility that drives convergence and extension at gastrulation and perturbs directed extension in cultured explants of dorsal mesoderm. Second, injection of a synthetic peptide encoding a cell-binding domain of XF into midgastrula embryos causes acute failure of gastrulation associated with defective fibrillin fibril assembly. These injections also reveal a critical role for this peptide in the fibril assembly process. Third, morpholino-mediated knockdown of translation of XF in the embryo also perturbs normal gastrulation and directed extension. Together, these data show that native *Xenopus* fibrillin is essential for the process of directed convergent extension in presumptive notochord at gastrulation.

Keywords: *Xenopus*; Gastrulation; Fibrillin; Morphogenesis; Notochord

Introduction

Vertebrate gastrulation is a combination of two intertwined processes organized by Spemann's Organizer: morphogenetic tissue movements driven by patterned cell motility that remodel the embryo and the concomitant induction of specific cell fates, and these spatially and temporally regulated cellular behaviors are integrated over the entire presumptive notochordal/somitic region, or dorsal involuting marginal zone (DIMZ), to drive directed extension of this tissue along the anterior–posterior axis and ultimately the generation of a mature notochord flanked by segmented somites (Shih and Keller, 1992a,b; Keller et al., 2000). The DIMZ is the "mechanical organizer" because these deformations drive gastrulation in the whole embryo, and cultured explants of DIMZ tissue (Keller explants) can autonomously undergo morphological extension to generate measurable force while anisotropically stiffening in the direction of extension (Moore et al., 1995). Regulation of cellular motility in the DIMZ is critical for this morphogenesis to occur; however, the locally acting molecular mechanisms that modulate these behaviors to generate anisotropic tissue level forces remain poorly defined (Shih and Keller, 1992b; Keller et al., 2000).

Blastopore closure, most of involution at gastrulation, and elongation of the embryo during normal gastrulation are brought about by the posterior progression of expression of specific cell behaviors collectively called mediolateral intercalation behavior (MIB) (Keller et al., 2000). DIMZ cells of the early gastrula are isodiametric and exhibit randomly directed protrusive activity but change their behavior during notochordal morphogenesis. These cells adopt both an elongated shape perpendicular to the axis of extension and exhibit mediolaterally oriented bipolar protrusive activity (Shih and Keller, 1992a). Expression of MIB is induced in presumptive notochordal cells in an anterior-to-posterior and lateral-to-medial progression driving cell intercalation and leading to convergence (narrowing) and extension (lengthening) of notochord (convergent extension or CE) (Shih and Keller, 1992b). During this time, morphologically distinct structures composed of extracellular

* Corresponding author. Fax: +1 434 982 5626.
E-mail address: ps5d@virginia.edu (P. Skoglund).

0012-1606/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2006.09.005

matrix (ECM) become apparent at the bilateral presumptive notochordal–somitic boundaries. These boundaries elongate from anterior-to-posterior concomitant with notochordal development and are essential for the "boundary capture" mechanism of cell intercalation. An intercalating cell remains bipolar until it contacts the boundary, at which time it locally ceases the invasive intercalating type of protrusive activity at the boundary-contacting end. Instead, it spreads in the plane of the boundary and is stabilized or "captured" there, transiting to a monopolar protrusive mode while at the same time elongating the boundary (Shih and Keller, 1992b).

The first ECM component of the developing notochord–somite boundary is *Xenopus* fibrillin (XF) (Skoglund et al., 2006). Chick, mouse and human fibrillins are also found around notochords (Gallagher et al., 1993; Wunsch et al., 1994; Zhang et al., 1995; Rongish et al., 1998; Quondamatteo et al., 2002; Czirok et al., 2004). Human fibrillins-1, -2 and -3 (Fbns1–3) comprise a family of ECM glycoproteins that form filamentous microfibrils that both supply biomechanical support in the body and regulate signaling events. These proteins interact with cells through both integrin and proteoglycan receptors as well as ligands that include other ECM molecules and growth factors (reviewed in Robinson and Godfrey, 2000). Mutations in Fbn 1 and 2 are responsible for the human diseases Marfan syndrome (MFS) and congenital contractural arachnodactyly (CCA) and MFS patients have variable life expectancy that correlates with the severity of the allele inherited; some alleles exhibit a mild phenotype with near-normal lifespan whereas others are more severe, ranging to neonatal mortality (Dietz et al., 1994).

Here we examine the function of XF in the frog embryo by employing three distinct interdictions of fibrillin fibril assembly, all of which block normal progression of gastrulation in a manner consistent with a defect in axial morphogenesis. These data strongly implicate native XF fibrils at the notochord–somite boundary in the normal process of convergent extension of dorsal mesoderm during amphibian gastrulation and additionally reveal a role for a conserved cell-binding domain in XF in fibril assembly.

Results

Expression of a truncation mutant of Xenopus fibrillin (XF) blocks gastrulation when expressed on the dorsal but not ventral sides of the gastrula

Targeted injection of synthetic mRNA encoding two amino-terminal truncated forms of XF, deletion-XF (delXF) and twice-deleted XF (TdXF), was performed into either the presumptive dorsal or ventral marginal zone of developing *Xenopus* embryos (Fig. 1A). These experiments were motivated by the observation that truncation alleles of human Fbn1 exert dominant-negative effects on fibrillin deposition into matrix (see Schrijver et al., 2002). When expressed on the dorsal side of the gastrula, where native XF is expressed (Skoglund et al., 2006), normal progression of gastrulation is blocked (Figs. 1B–G). In injected embryos the blastopore fails to close (Figs. 1C–G), leading to failure of normal internalization of mesoderm and lack of archenteron elongation. This failure is apparent first at late midgastulae stage (St. 11), when dorsally injected embryos are retarded in closing their blastopores with respect to control injected animals (Figs. 1G, H). Failure of morphogenesis in injected animals is not due to a change in dorsal mesodermal cell fate because notochord is still specified in injected animals (Fig. 1F). Notochordal tissue is found in a ring around the open blastopore and appears similar to classic "ring embryos" generated by mechanically disrupting dorsal, axial tissue (Schectman, 1942). In contrast, expression of deltaXF or TdXF on the ventral side of the blastopore leads to background levels of gastrulation defects, similar to expression of bovine pre-prolactin on the dorsal or ventral side (Fig. 1B).

Gastrulation blocking activity localizes to carboxy-terminal unique sequences

To address the molecular mechanism by which expressing amino-terminal deleted forms of XF on the dorsal side of the embryo perturbs gastrulation, we constructed two carboxy-terminal truncations of TdXF (Fig. 1A). One of these, TdXF-Protease (TdXF-P), is truncated at a putative furin protease processing site where fibrillins are normally cleaved by proteases during maturation (arrow in Fig. 1A). This truncation acts like the parent construct (TdXF) and blocks normal progression of gastrulation (Fig. 1B). In contrast, further deletion of carboxy-terminal unique sequences produces TdXF-Repeats (TdXF-R), which does not exhibit appreciable gastrulation blocking activity and thus implicates the unique sequences deleted from this protein in the molecular mechanism that perturbs gastrulation (Fig. 1B).

Explants of DIMZ-expressing deltaXF extend abnormally

Because the phenotype of deltaXF/TdXF-injected embryos and the localization of XF expression in the dorsal mesoderm are both consistent with involvement of XF in DIMZ convergent extension, we examined the effect of expressing deltaXF in DIMZ explants (Keller explants) that converge and extend autonomously. Keller sandwiches made from pairs of dorsal marginal zones of injected embryos do not extend properly. Notochords of approximately normal length and volume form in explants derived from injected animals, but these notochords are twisted and curled, and the severity of this phenotype depends on the dose of deltaXF RNA injected (Figs. 2A–D). These results suggest that deltaXF injections perturb the biomechanical mechanisms normally responsible for directed extension of this tissue.

To further explore this effect, we examined open face explants (derived from single embryos) unilaterally expressing deltaXF. These explants were assayed for bending, either towards or away from the injected side as determined by co-expression of green fluorescent protein (GFP). Sixty-nine percent (11 of 16) of deltaXF-injected explants bent towards the injected side (Fig. 2E/F), and none bent away, suggesting that extension is retarded in deltaXF-expressing regions. In