# FGF3 in the floor plate directs notochord convergent extension in the *Ciona* tadpole

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Convergent extension (CE) is the narrowing and lengthening of an embryonic field along a defined axis. It underlies a variety of complex morphogenetic movements, such as mesoderm elongation and neural tube closure in vertebrate embryos. Convergent extension relies on the same intracellular molecular machinery that directs planar cell polarity (PCP) in epithelial tissues, including non-canonical Wnt signaling components. However, it is not known what signals coordinate CE movements across cell fields. In the simple chordate *Ciona intestinalis*, the notochord plate consists of just 40 cells, which undergo mediolateral convergence (intercalation) to form a single cell row. Here we present evidence that a localized source of FGF3 in the developing nerve cord directs notochord intercalation through non-MAPK signaling. A dominant-negative form of the *Ciona* FGF receptor suppresses the formation of polarized actin-rich protrusions in notochord cells, resulting in defective notochord intercalation. Inhibition of *Ciona FGF3* activity results in similar defects, even though it is expressed in an adjacent tissue: the floor plate of the nerve cord. In *Xenopus* mesoderm explants, inhibiting FGF signaling perturbs CE and disrupts membrane localization of Dishevelled (Dsh), a key regulator of PCP and CE. We propose that FGF signaling coordinates CE movements by regulating PCP pathway components such as Dsh.

**KEY WORDS: *Ciona*, FGF, Ascidian, Convergent extension, Notochord, Planar cell polarity**

## INTRODUCTION

In vertebrates and ascidians, the formation of the primary embryonic body axis depends on the lengthening of the axial mesoderm by convergent extension (CE), a process in which cells intercalate medially along the anteroposterior (AP) axis (Keller, 2002; Wallingford et al., 2002). Members of the planar cell polarity (PCP) pathway, including Dishevelled (Dsh), Prickle and Van Gogh/ Strabismus, are recruited to specific regions on the cell membrane upon activation of the non-canonical Wnt pathway (Lawrence et al., 2004; Jones and Chen, 2007; Seifert and Mlodzik, 2007) and transduce the Wnt/PCP signal into changes in actin dynamics and cellular motility. Disrupting these membrane protein complexes causes severe defects in tail extension and neural tube closure (Wallingford, 2006), suggesting a central role for these genes in chordate embryogenesis.

Tissue-level CE typically begins with coordinated polarization and movement of individual cells. During *Xenopus* mesoderm formation, cells across the dorsal marginal zone (DMZ) form actin protrusions and lamellopodia preferentially along the mediolateral (ML) axis, events that are controlled by Dsh and other PCP genes (Wallingford et al., 2000). However, most of the known PCP components are intracellular molecules that function cell-autonomously to regulate cytoskeletal rearrangements. The putative extracellular signals that might establish the initial polarization of a field of cells have yet to be identified (Barrow, 2006; Casal et al., 2006; Lawrence et al., 2007).

Here, we present evidence for a non-autonomous mechanism for establishing ML polarity in the *Ciona* notochord. A localized source of FGF3 in the ventral midline of the neural tube signals to the underlying notochord plate to direct ML intercalation. Hence, FGF signaling could serve as the instructive signal for coordinating cell behavior over long distances and establishing the direction of CE in chordates.

## MATERIALS AND METHODS

### *Ciona*
Adult *Ciona intestinalis* were obtained and maintained as previously described (Corbo et al., 1997).

### In situ hybridization and immunohistochemistry
In situ hybridization, diphosphorylated (dp) Erk staining and double antibody/in situ hybridization were performed according to Davidson et al. (Davidson et al., 2006). Phalloidin staining and lamellopodia counting were performed according to Munro et al. (Munro et al., 2002a).

### Morpholinos, PCR and enhancer constructs
*C. intestinalis FGF3* (*Ci-FGF3*) splice donor (5′-CCGATGTTTGA-CTTACTTTGCGGCG-3′) and splice acceptor (5′-CAATCTCA-GCTGTGAAAATAGAAAT-3′) morpholinos were co-injected with Brachyury::GFP (10 ng/μl) at a total concentration of 1.5 mM. qRT-PCR was performed with total RNA extracted from ~15 embryos using SYBR chemistry on an ABI 3700 real time PCR system with the following primers: Ci-FGF3 (5′-ATAACAAGTCGCCGCAAACT-3′, 3′-TTGCTGTGTC-GGTTCATAGC-5′) and Cytoplasmic actin 7 (internal control, 5′-CTC-CATCATGAAGTGCGATGTT-3′, 3′-CATTCTGTCGGCGATTCCA-5′). A nerve cord-specific enhancer for the *FGF3* homolog in *Ciona savignyi* (*Cs-FGF3*) was cloned by fusing the first intron (~6.2 kb) to a 5 kb genomic DNA fragment from the 5′ flanking region. The nerve cord enhancer 00124 (5′-TATATATACTGTTGTGCCAG-3′, 3′-CATCTTGGTTAAAACTGA-TTC-5′), notochord enhancer Noto1 (5′-GGCTTGGTCAGTTGAATC-3′, 3′-CGTAAACAACTTCATAATTTTG-5′), muscle enhancer MyoD (5′-GGCTTACGCATCTCGAGCGAACC-3′, 3′-CTCTTGAGAGATACA-CGTCATCG-5′) and endodermal strand enhancer 00794 (5′-CAT-TCTGCGCTGCTGTTG-3′, 3′-CGGTTTTGCTTTCACAACTTT-5′) were cloned using the indicated primers.

### Time-lapse movies
For the time-lapse movie examining notochord intercalation, the dorsal anterior quadrants from wild-type and mutant embryos were isolated at the mid-gastrula stage and recorded over a period of 5 hours every 15 seconds.

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For notochord cell protrusive activity, electroporated embryos were developed to the mid-neurula stage, dissociated in $Ca^{2+}/Mg^{2+}$-free medium with trypsin (Christiaen et al., 2008) and recovered for 15 minutes before mounting on a poly-L-lysine-coated slide. Movies were taken every 6 seconds over a period of 15 minutes.

### *Xenopus* explants
*Xenopus* embryos were obtained, reared and injected as described (Sive et al., 2000). Embryos were injected in the animal hemisphere at the 2-cell stage for isolation of animal caps and equatorially into two dorsal blastomeres at the 4-cell stage for isolation of DMZ. Capped mRNAs were synthesized using the mMessage Machine Kit (Ambion) and injected as follows: Xdsh-GFP (Rothbacher et al., 2000) 120 pg; PKC-δ-YFP (Kinoshita et al., 2003) 100 pg; membrane RFP 200 pg. Animal caps (stage 9) were removed and cultured in 3/4× NAM (Sive et al., 2000) for 30 minutes before fixation and immunohistochemistry. The DMZ above the blastopore lip was excised from stage 10+ embryos in 1× Steinberg's medium. DMZs were allowed to heal until whole-embryo siblings reached stage 10.5, then transferred to 1× Steinberg's medium containing 120 μM SU5402 or DMSO and cultured at 15°C until stage 12. Embryos and explants were fixed in MEMFA (Sive et al., 2000), dehydrated in methanol, then rehydrated and processed for in situ hybridization or bleached in 1% hydrogen peroxide solution prior to immunohistochemistry. The following antibodies were used: chicken anti-GFP 1:500 (Abcam); rabbit anti-RFP 1:500 (Molecular Probes); Tor70 (notochord, mouse IgM) (Bolce et al., 1992); and 12/101 (muscle, mouse IgG) (Kintner and Brockes, 1984).

## RESULTS AND DISCUSSION
### Non-MAPK FGFR signaling is required for notochord convergent extension
In tadpoles of *Ciona intestinalis*, the notochord is composed of a single row of 40 cells that forms through ML intercalation, a common mode of CE (Miyamoto and Crowther, 1985). To identify potential regulators of notochord intercalation, we searched for genes that are specifically expressed in the notochord plate and found that the *Ciona FGFR* gene is strongly expressed in the notochord before and after intercalation (Fig. 1A,B). This expression is distinct from the role of FGF signaling in notochord induction at the 32-cell stage of embryogenesis (Kim et al., 2000; Kim and Nishida, 2001), which depends on MAPK signaling through maternal FGFR and the transcriptional activation of *Ciona Brachyury* (*Ci-Bra*). By contrast, the later zygotic expression of *FGFR* in the notochord plate and definitive notochord was not associated with MAPK activity (Fig. 1G,H). Moreover, inhibition of MAPK activation with the pharmacological MEK inhibitor U0126 did not affect notochord intercalation when applied after the the notochord fate is specified (Fig. 1I).

FGFR signaling acts through three major downstream pathways: Ras/MAPK, PLCγ/$Ca^{2+}$ and PI3K/Akt (Bottcher and Niehrs, 2005). To determine whether an alternative FGF signaling pathway might be essential for notochord development, a dominant-negative form of the *Ciona* FGFR (dnFGFR) (Davidson et al., 2006) was specifically expressed in the notochord lineage after the late gastrula stage using the *Ci-Noto1* enhancer (Takahashi et al., 1999). The transgene caused defects in notochord intercalation (Fig. 1C-F) but did not affect notochord cell fate specification (see Fig. S2 in the supplementary material). Many of the defective notochord cells failed to undergo appropriate shape changes (Fig. 1E′) and rearrangement. The resulting embryos had shorter and wider tails owing to the failure in CE (Fig. 1, compare F with D).

To better visualize notochord morphogenesis, we performed time-lapse microscopy on dorsal-anterior notochord/neural tube explants (DA explants) (Munro and Odell, 2002b) isolated from wild-type and *dnFGFR Ciona* embryos. These DA explants preserve the normal spatial relationship between notochord and neural plate. In wild-type DA explants, notochord cells polarize, form dynamic membrane protrusions and intercalate along a distinct axis to produce an elongated structure (see Movie 1 in the supplementary material). By contrast, in DA explants from *dnFGFR* mutant embryos, notochord cells had diminished membrane protrusions and failed to intercalate, resulting in a wide notochord plate resembling that seen in wild-type embryos before CE (see Movie 2 in the supplementary material).

In *Drosophila* wing bristle development, *Frizzled* mutants exhibit both cell-autonomous and non-cell-autonomous PCP defects (Klein and Mlodzik, 2005). The *Ciona FGFR* seems to function strictly in a cell-autonomous manner. In mosaically transformed embryos, only those notochord cells that express the *dnFGFR* transgene displayed a rounded shape and failed to intercalate (Fig. 1E′). By contrast, neighboring wild-type notochord cells lacking the transgene underwent normal intercalation to form a single row of cells. Together, these data suggest that FGF signaling – independent of the MAPK pathway – is essential for notochord intercalation in the *Ciona* tadpole.

### FGFR signaling controls polarized actin organization in notochord cells
Lamellopodia-like protrusions form at the ML edges of notochord precursor cells within the *Ciona* notochord plate (Munro and Odell, 2002a; Jiang et al., 2005) and are presumed to be the driving force for directional intercalation. To determine whether the *dnFGFR* transgene interferes with the formation of polarized lamellopodia, phalloidin staining and confocal microscopy were used to reconstruct the three-dimensional organization of actin protrusions in the notochord (Fig. 1J). In wild-type embryos at the late neurula stage, an average of ~0.42 actin protrusions per internal edge was observed in the notochord plate, comparable with previous results from another ascidian species, *Boltenia villosa* (Munro and Odell, 2002a). Most of these protrusions were positioned within 45° of the ML axis as compared with the AP axis in the notochord plate, which is consistent with the ML direction of intercalation. When the *dnFGFR* transgene was expressed in the notochord, there was a ~50% reduction in the number of actin protrusions at internal notochord plate cell boundaries (Fig. 1J). Moreover, the remaining protrusions displayed a randomized orientation instead of the ~2:1 ML:AP ratio seen in wild-type notochord. This combined reduction in actin protrusions and polarity might underlie the defective notochord intercalation in *dnFGFR* embryos.

FGF signaling is not only necessary for the formation of oriented actin protrusions in the notochord, but is also sufficient to induce ectopic cell protrusive activity. Dissociated wild-type notochord cells form random protrusions at low frequency in culture (Jiang et al., 2005) (see Movie 3 in the supplementary material). Incubating notochord cells with recombinant human basic FGF (bFGF) protein causes the cells to form multiple and larger protrusions at much higher frequency (see Movie 4 in the supplementary material), whereas cells expressing the *dnFGFR* transgene failed to respond to the ectopic FGF signal (see Fig. S5 and Movie 5 in the supplementary material). Thus, FGF signaling might regulate *Ciona* notochord intercalation by inducing locally oriented, actin-based protrusions.

### *Ciona FGF3* is expressed in the floor plate above the converging notochord
The preceding results suggest an instructive role for FGF signaling in notochord intercalation. To identify the putative ligand, we examined the expression patterns of all six FGF genes in the *Ciona* genome.
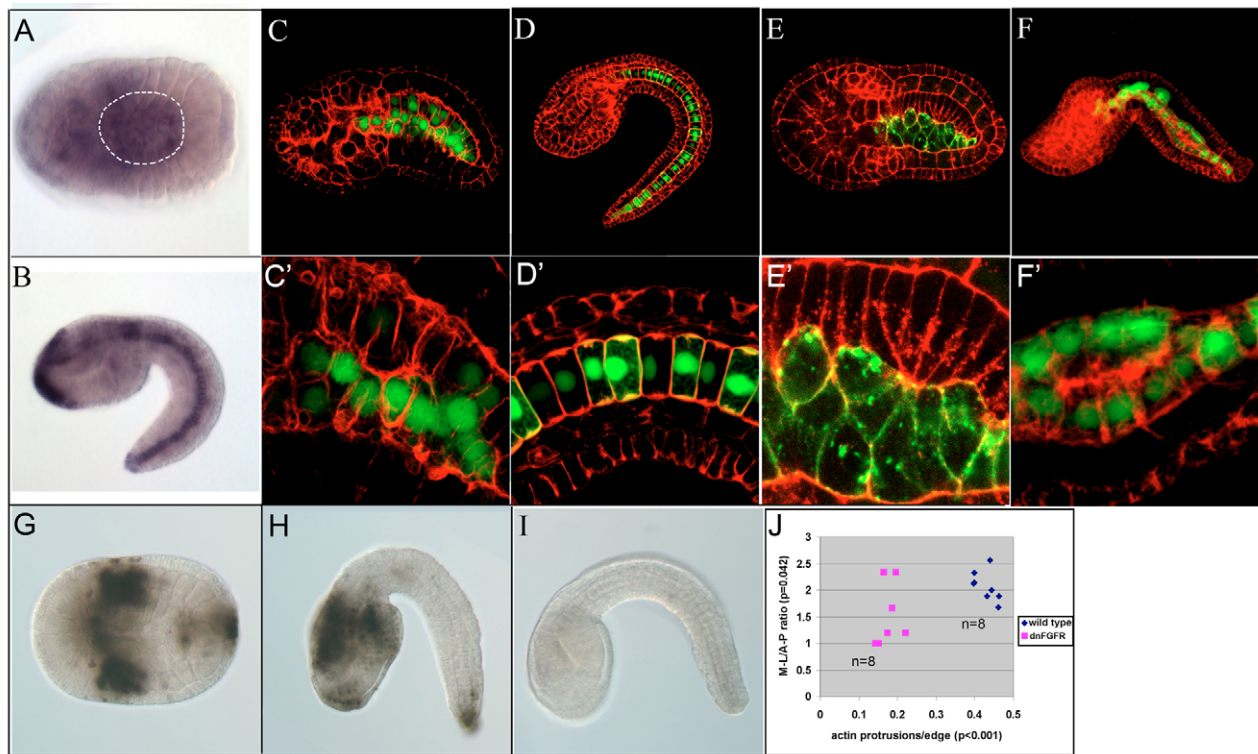
**Fig. 1. *FGFR* is required for mediolateral convergence of the *Ciona* notochord.** (**A**,**B**) *Ci-FGFR* is strongly expressed in the developing notochord plate (A, neurula stage, white circle) and in notochord after intercalation (B, tailbud stage). (**C-D′**) Wild-type notochord cells (Bra::GFP, green; phalloidin, red) undergo progressive ML intercalation (C,C′, early tailbud) to form a single cell row (D,D′, late tailbud). (**E,E′**) Noto1::dnFGFR-Venus-expressing (green) notochord cells display cell-autonomous defects in intercalation at early tailbud stage. (**F,F′**) Noto1::dnFGFR+Bra::GFP (green) embryos display strong intercalation defects at late tailbud stage. (53/75 embryos displayed CE defects.) Images in C′-F′ are magnifications from C-F, respectively. (**G**,**H**) dpErk staining of wild-type embryos at neurula (G) and late tailbud (H) stages shows absence of MAPK activation in the notochord. (**I**) Treatment of embryos with U0126 from late neurula stage on eliminates MAPK activation but does not affect notochord intercalation (103/103 embryos). (**J**) Distribution of the number of actin protrusions per internal notochord edge versus the ratio of ML- to AP-oriented protrusions. Note the difference between wild-type (blue) and *dnFGFR* (red) notochord indices (eight embryos each). *P*-values are indicated for each axis.

Only one of these, *Ci-FGF3/7/10/22* (*Ci-FGF3*), is expressed in close proximity to the developing notochord (Imai et al., 2002). The *Ciona* dorsal nerve cord consists of four rows of ependymal cells in the tail (Crowther and Whittaker, 1992). *Ci-FGF3* is expressed in the ventral-most row of cells, which constitutes the rudimentary floor plate of the nerve cord in tailbud stage embryos (Fig. 2A) and is located immediately above the midline of the notochord.

To determine the detailed expression pattern of *Ci-FGF3*, we characterized *FGF3* regulatory DNA sequences. Because the *Ci-FGF3* gene is located at the end of a contig in the *C. intestinalis* genome assembly, we used another ascidian species, *Ciona savignyi*. Previous studies have shown that orthologous enhancers work equally well in both species (Bertrand et al., 2003; Davidson et al., 2005). Indeed, an 11-kb *Cs-FGF3* regulatory DNA fully recapitulated the normal *FGF3* expression pattern in *C. intestinalis* when attached to a *lacZ* reporter gene, including staining in a single row of cells comprising the floor plate at tailbud stages (Fig. 2, compare C with A). At earlier stages, when the notochord plate starts to undergo intercalation, the *Cs-FGF3* regulatory DNA drove reporter expression in the two rows of floor plate progenitors, which are in direct contact with the midline of the notochord plate (Fig. 2D,E). Later in development, the floor plate progenitors intercalate at the midline during neural closure to form the floor plate (Fig. 2F,G). Thus, it appears that *Ci-FGF3* is expressed at the right time and place to serve as a signal for notochord intercalation.

## *Ci-FGF3* is required for actin polarization and notochord convergent extension

To address the function of *Ci-FGF3* in *Ciona* notochord development, we designed two antisense morpholino oligos (MOs) targeting the splice donor/acceptor sites flanking the first intron of *Ci-FGF3*. qRT-PCR assays indicated that injection of both MOs caused a substantial reduction in the steady-state levels of *Ci-FGF3* transcripts (see Fig. S3 in the supplementary material). *Ci-FGF3* morphant embryos displayed a variety of CE defects, including noticeably shortened tails (Fig. 3A-C). Confocal imaging revealed several classes of mutant phenotypes with varying severity. In the most extreme cases, the mutant notochord consisted of two or three rows of cells instead of one, resulting in an extremely reduced tail that was about the same length as the trunk region (Fig. 3A′-C′). To determine whether *Ci-FGF3* regulates actin organization in the notochord, we performed phalloidin staining and confocal reconstruction of actin structures in *Ci-FGF3* morphants. As seen for the *dnFGFR* transgene, the *FGF3* MOs caused both a reduction in the number of actin protrusions and a randomization of protrusion orientation (Fig. 3E). The *FGF3* morphant phenotype was stronger than that of *dnFGFR* mutants because the dominant-negative form of the receptor is not fully penetrant in the notochord.

*Ci-FGF3* is expressed in a spatially localized pattern that corresponds to the ML polarity of actin protrusions. To determine whether *FGF3* provides a positional cue triggering CE, we asked
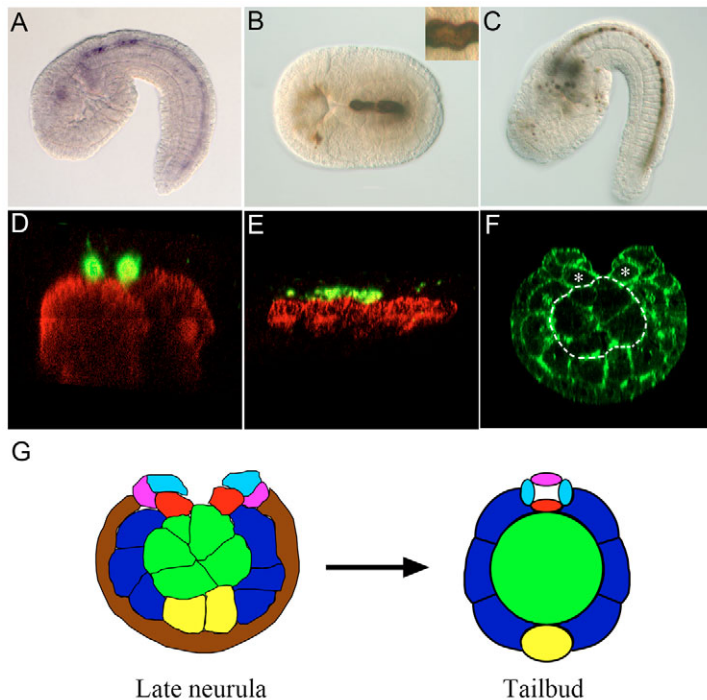
**Fig. 2. *Ci-FGF3* is expressed in the ventral midline of the nerve cord during notochord CE.** (**A**) *Ci-FGF3* in situ showing expression in the floor plate at mid-tailbud stage. (**B,C**) *Cs-FGF3* enhancer::*lacZ* in situ in late neurula (B; inset, high magnification) and mid-tailbud (C) embryos recapitulates endogenous *Ci-FGF3* expression. (**D,E**) Cross-sectional (D) and lateral (E) views of late neurula embryos double labeled for *Cs-FGF3* enhancer (green, *lacZ* in situ) and Bra::GFP (red, GFP antibody). (**F**) Cross-sectional view of late neurula embryo stained with phalloidin (green). The notochord plate (dashed line) and floor plate precursors (asterisks) are indicated. (**G**) Cross-sectional illustration of the organization of major *Ciona* tissues: ventral nerve cord (red), lateral nerve cord (light blue), dorsal nerve cord (purple), notochord (green), tail muscle (dark blue), endoderm (yellow) and epidermis (brown).

whether misexpressing *FGF3* in neighboring tissues could affect notochord intercalation. We used four different tissue-specific enhancers to direct *Ci-FGF3* expression in the nerve cord, notochord, tail muscles and endodermal strand. Such misexpression resulted in varying degrees of intercalation defects in the notochord (Fig. 3F-I′). The most severe defects were obtained upon

misexpression of *Ci-FGF3* in the notochord (Fig. 3F,F′), which emphasizes the need for the instructive cue to be expressed in a neighboring tissue. Relatively mild defects were obtained when *Ci-FGF3* was misexpressed in the tail muscles (Fig. 3H,H′), which is consistent with the possibility that the muscles serve as a secondary source of intercalary signals, as seen in other ascidians (Munro and
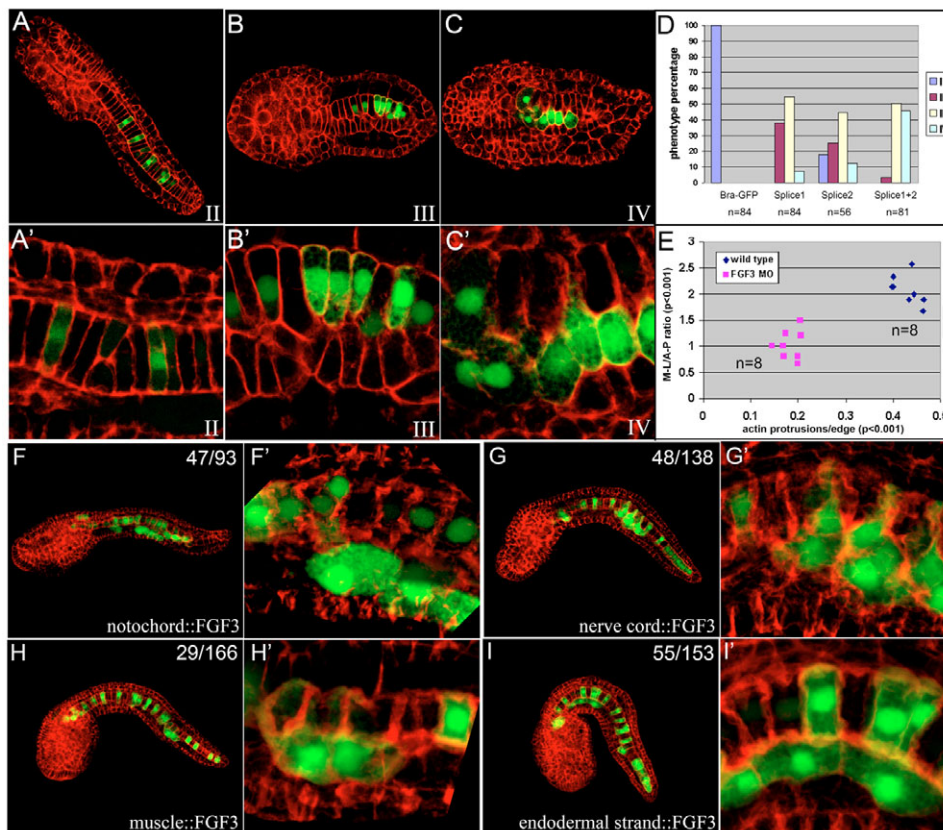


**Fig. 3. *Ci-FGF3* is required for notochord CE.** (**A-C′**) Phenotypic series of embryos co-injected with *Ci-FGF3* splice morpholinos (MOs) and Bra::GFP (green) and counterstained with phalloidin (red). The notochord displays progressively stronger intercalation defects ranging from an occasional two cells per row (A,A′, category II), two-cell row (B,B′, category III) and more than two cells per row (C,C′, category IV). (**D**) Summary of MO phenotypes. *n*, number of embryos examined. (**E**) Distribution of actin protrusion abundance and ML/AP ratio in wild-type and MO embryos. The MO embryos display a more severe reduction in protrusions and loss of ML polarity than *dnFGFR* notochord (see Fig. 1J). (**F-I′**) Misexpression of *Ci-FGF3* disrupts notochord intercalation non-cell-autonomously. Notochord (F,F′), ventral and dorsal nerve cord (G,G′), muscle (H,H′) and endodermal strand (I,I′) enhancer-driven *Ci-FGF3* causes varying degrees of intercalation defects (number of defective embryos/total embryos) in the notochord. All embryos are co-electroporated with Bra::GFP (green) and counterstained with phalloidin (red). Images in A′-I′ are magnifications from A-I, respectively.
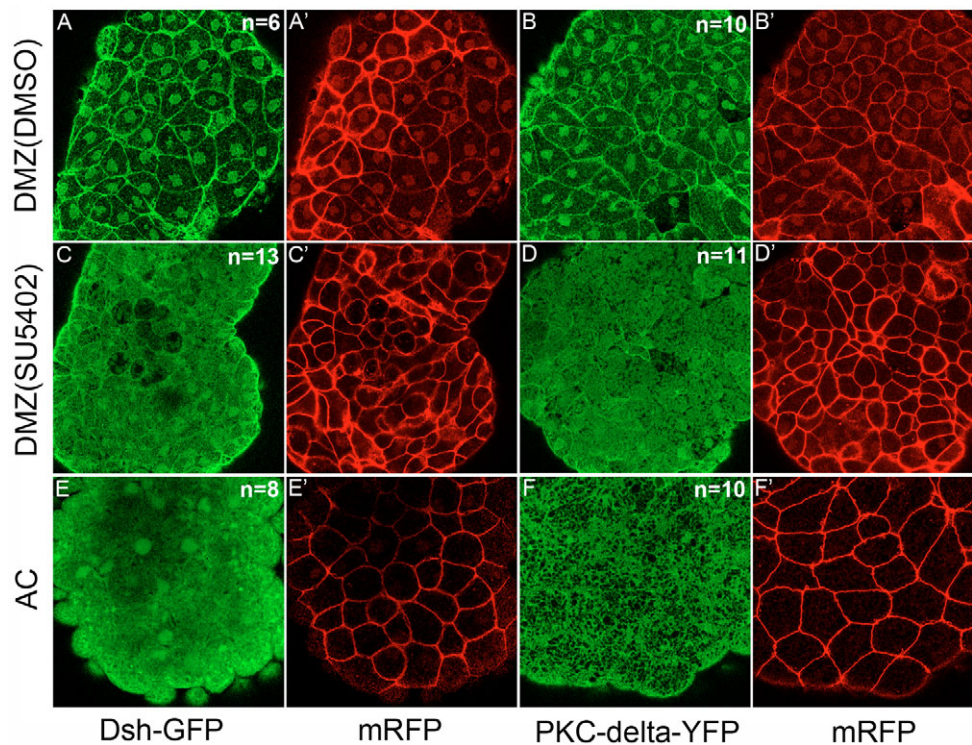
**Fig. 4. FGF signaling is required for membrane localization of the PCP pathway proteins Dsh and PKC-δ in *Xenopus* dorsal mesoderm.** Dsh-GFP and PKC-δ-YFP proteins are membrane-localized in wild-type *Xenopus* dorsal marginal zone (DMZ) (**A-B'**) and cytoplasmically localized in the animal cap (AC) (**E-F'**). Treatment with the FGFR inhibitor SU5402 between stages 10.5 and 12 causes Dsh and PKC-δ to delocalize from the membrane in DMZ (**C-D'**); compare with DMSO-treated controls (**A-B'**). All embryos are co-injected with membrane RFP (mRFP, red) to label the cell membrane.

Odell, 2002b). Finally, overexpression of *Ci-FGF3* in the nerve cord resulted in severe intercalary defects (Fig. 3G,G′), suggesting that both the levels and location of the *Ci-FGF3* signal are important for proper notochord intercalation. Defects in notochord intercalary behavior did not result from changes in notochord fates (see Fig. S4 in the supplementary material).

### FGFR signaling is required for membrane localization of Dishevelled and PKC-δ in *Xenopus*

One of the hallmarks of PCP and CE is the translocation of Dsh to the plasma membrane, which signals to regulators of actin dynamics to establish cell polarity (Habas et al., 2003). We asked whether FGF signaling is required to promote membrane localization of the Dsh protein. This is hard to address in *Ciona* owing to the lack of Ci-Dsh antibodies and the variable penetrance of the *dnFGFR* transgene in the *Ciona* notochord. However, FGF signaling has also been implicated in CE of the mesoderm in *Xenopus laevis* (Sivak et al., 2005). Therefore, we used *Xenopus* DMZ explants to assess the relationship between FGF signaling and the subcellular localization of a *Xenopus* Dsh (Xdsh)-GFP fusion protein. As a control, we also assayed the localization of PKC-δ-YFP, which is a known downstream target of the FGF pathway that becomes membrane-localized upon FGFR activation (Kinoshita et al., 2003; Sivak et al., 2005). Because FGF signaling is required for specification of the mesoderm (including notochord) in *Xenopus* (see Fig. S6 in the supplementary material), we manipulated FGF signaling by treatment with the FGFR pharmacological inhibitor SU5402 after mesoderm induction (from stage 10.5 onwards). This treatment results in short embryos owing to defects in CE (see Fig. S7 in the supplementary material).

In wild-type *Xenopus* DMZ explants undergoing CE, Xdsh-GFP and PKC-δ-YFP were localized primarily to the cell membrane (Fig. 4A,B). Upon SU5402 treatment, Xdsh-GFP was excluded from the membrane and was instead distributed throughout the cytoplasm (Fig. 4C), suggesting that FGFR signaling is required for Xdsh

membrane localization. PKC-δ-YFP was exclusively localized to the cell membrane in wild-type DMZ explants, whereas the majority of the protein was present in the cytoplasm after treatment with SU5402 (Fig. 4D). As expected, DMZ explants did not elongate after drug treatment. Together, these data suggest that FGF signaling is required for Dsh membrane localization in the *Xenopus* dorsal mesoderm during CE. Since Dsh is also required for CE in *Ciona* (Keys et al., 2002), similar mechanisms are likely to operate in *Ciona* notochord intercalation.

Directional movement of individual cells in embryogenesis can be achieved via diverse mechanisms, including chemotaxis, differential protrusive activity and differential adhesion. A key feature of CE movements is the coordination of uniform asymmetric cell behavior across large fields of cells. We have presented evidence that during *Ciona* notochord formation, FGF3 is released from the developing floor plate of the neural tube to coordinate ML polarity and CE movements of the notochord plate. This is consistent with the previous observation that ascidian DA explants can form elongated notochord rudiments in culture, whereas isolated notochord precursors alone do not (Munro and Odell, 2002b). Our results show that the instructive signal emanating from the neural plate to direct notochord CE in *Ciona* is a member of the FGF signaling pathway that works through the non-MAPK pathway downstream of FGFR. One candidate is the NRH receptor that functions downstream of FGFR to promote DMZ protrusive activity (Chung et al., 2005), although *Ciona* lacks a clear NRH homolog.

FGF signaling might play similar roles in coordinating complex morphogenetic processes in vertebrate development. As in *Ciona*, an early phase of MAPK-mediated FGF signaling is required to induce mesoderm fate in the frog embryo through the activation of genes such as *Brachyury*. After mesoderm specification, non-MAPK FGF signaling has been implicated in axial elongation in the *Xenopus* neurula (Sivak et al., 2005), but the details of this mechanism remain unclear. The complex and often overlapping expression patterns of 23 FGFs and four FGFRs in the early

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*Xenopus* embryo (S.P., unpublished) prevent the unambiguous identification of a ligand-receptor relationship underlying CE, in contrast to *Ciona*. However, it is possible that a similar tissue-tissue interaction is involved in establishing the polarity of *Xenopus* DMZ cells.

Cells undergoing CE need to transduce the extrinsic polarity cues (such as FGF) into coordinated (planar) cell behavior (Lawrence et al., 2002; Lawrence et al., 2004). It is likely that FGF signaling achieves this effect through interacting with the non-canonical Wnt/PCP pathway. It has been shown previously that FGFR is required for membrane localization of PKC-δ, which physically interacts with Dsh (Kinoshita et al., 2003; Sivak et al., 2005). The *Ciona* genome encodes ten Wnts, but none of these is expressed in the notochord, raising the possibility they might play permissive roles in the notochord, as seen in other systems. We propose that a localized FGF signal released by a developing tissue functions as an extracellular positional cue to directionally activate the intracellular PCP pathway in cells of an adjacent tissue (see Fig. S1 in the supplementary material). This coordination in cell polarity is the first step towards the orchestrated cell movements that underlie CE.

**Supplementary material**
Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/136/1/23/DC1

**References**
**Barrow, J. R.** (2006). Wnt/PCP signaling: a veritable polar star in establishing patterns of polarity in embryonic tissues. *Semin. Cell Dev. Biol.* **17**, 185-193.
**Bertrand, V., Hudson, C., Caillol, D., Popovici, C. and Lemaire, P.** (2003). Neural tissue in ascidian embryos is induced by FGF9/16/20, acting via a combination of maternal GATA and Ets transcription factors. *Cell* **115**, 615-627.
**Bolce, M. E., Hemmati-Brivanlou, A., Kushner, P. D. and Harland, R. M.** (1992). Ventral ectoderm of Xenopus forms neural tissue, including hindbrain, in response to activin. *Development* **115**, 681-688.
**Bottcher, R. T. and Niehrs, C.** (2005). Fibroblast growth factor signaling during early vertebrate development. *Endocr. Rev.* **26**, 63-77.
**Casal, J., Lawrence, P. A. and Struhl, G.** (2006). Two separate molecular systems, Dachsous/Fat and Starry night/Frizzled, act independently to confer planar cell polarity. *Development* **133**, 4561-4572.
**Christiaen, L., Davidson, B., Kawashima, T., Powell, W., Nolla, H., Vranizan, K. and Levine, M.** (2008). The transcription/migration interface in heart precursors of Ciona intestinalis. *Science* **320**, 1349-1352.
**Chung, H. A., Hyodo-Miura, J., Nagamune, T. and Ueno, N.** (2005). FGF signal regulates gastrulation cell movements and morphology through its target NRH. *Dev. Biol.* **282**, 95-110.
**Corbo, J. C., Levine, M. and Zeller, R. W.** (1997). Characterization of a notochord-specific enhancer from the Brachyury promoter region of the ascidian, Ciona intestinalis. *Development* **124**, 589-602.
**Crowther, R. J. and Whittaker, J. R.** (1992). Structure of the caudal neural tube in an ascidian larva: vestiges of its possible evolutionary origin from a ciliated band. *J. Neurobiol.* **23**, 280-292.
**Davidson, B., Shi, W. and Levine, M.** (2005). Uncoupling heart cell specification and migration in the simple chordate Ciona intestinalis. *Development* **132**, 4811-4818.
**Davidson, B., Shi, W., Beh, J., Christiaen, L. and Levine, M.** (2006). FGF signaling delineates the cardiac progenitor field in the simple chordate, Ciona intestinalis. *Genes Dev.* **20**, 2728-2738.
**Habas, R., Dawid, I. B. and He, X.** (2003). Coactivation of Rac and Rho by Wnt/Frizzled signaling is required for vertebrate gastrulation. *Genes Dev.* **17**, 295-309.
**Imai, K. S., Satoh, N. and Satou, Y.** (2002). Region specific gene expressions in the central nervous system of the ascidian embryo. *Gene Expr. Patterns* **2**, 319-321.
**Jiang, D., Munro, E. M. and Smith, W. C.** (2005). Ascidian prickle regulates both mediolateral and anterior-posterior cell polarity of notochord cells. *Curr. Biol.* **15**, 79-85.
**Jones, C. and Chen, P.** (2007). Planar cell polarity signaling in vertebrates. *BioEssays* **29**, 120-132.
**Keller, R.** (2002). Shaping the vertebrate body plan by polarized embryonic cell movements. *Science* **298**, 1950-1954.
**Keys, D. N., Levine, M., Harland, R. M. and Wallingford, J. B.** (2002). Control of intercalation is cell-autonomous in the notochord of Ciona intestinalis. *Dev. Biol.* **246**, 329-340.
**Kim, G. J. and Nishida, H.** (2001). Role of the FGF and MEK signaling pathway in the ascidian embryo. *Dev. Growth Differ.* **43**, 521-533.
**Kim, G. J., Yamada, A. and Nishida, H.** (2000). An FGF signal from endoderm and localized factors in the posterior-vegetal egg cytoplasm pattern the mesodermal tissues in the ascidian embryo. *Development* **127**, 2853-2862.
**Kinoshita, N., Iioka, H., Miyakoshi, A. and Ueno, N.** (2003). PKC delta is essential for Dishevelled function in a noncanonical Wnt pathway that regulates Xenopus convergent extension movements. *Genes Dev.* **17**, 1663-1676.
**Kintner, C. R. and Brockes, J. P.** (1984). Monoclonal antibodies identify blastemal cells derived from dedifferentiating limb regeneration. *Nature* **308**, 67-69.
**Klein, T. J. and Mlodzik, M.** (2005). Planar cell polarization: an emerging model points in the right direction. *Annu. Rev. Cell Dev. Biol.* **21**, 155-176.
**Lawrence, P. A., Casal, J. and Struhl, G.** (2002). Towards a model of the organisation of planar polarity and pattern in the Drosophila abdomen. *Development* **129**, 2749-2760.
**Lawrence, P. A., Casal, J. and Struhl, G.** (2004). Cell interactions and planar polarity in the abdominal epidermis of Drosophila. *Development* **131**, 4651-4664.
**Lawrence, P. A., Struhl, G. and Casal, J.** (2007). Planar cell polarity: one or two pathways? *Nat. Rev. Genet.* **8**, 555-563.
**Miyamoto, D. M. and Crowther, R. J.** (1985). Formation of the notochord in living ascidian embryos. *J. Embryol. Exp. Morphol.* **86**, 1-17.
**Munro, E. M. and Odell, G. M.** (2002a). Polarized basolateral cell motility underlies invagination and convergent extension of the ascidian notochord. *Development* **129**, 13-24.
**Munro, E. M. and Odell, G.** (2002b). Morphogenetic pattern formation during ascidian notochord formation is regulative and highly robust. *Development* **129**, 1-12.
**Rothbacher, U., Laurent, M. N., Deardorff, M. A., Klein, P. S., Cho, K. W. and Fraser, S. E.** (2000). Dishevelled phosphorylation, subcellular localization and multimerization regulate its role in early embryogenesis. *EMBO J.* **19**, 1010-1022.
**Seifert, J. R. and Mlodzik, M.** (2007). Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. *Nat. Rev. Genet.* **8**, 126-138.
**Sivak, J. M., Petersen, L. F. and Amaya, E.** (2005). FGF signal interpretation is directed by Sprouty and Spred proteins during mesoderm formation. *Dev. Cell* **8**, 689-701.
**Sive, H., Grainger, R. and Harland, R.** (2000). *Early Development of Xenopus Laevis: A Laboratory Manual*. New York, NY: CSHL Press.
**Takahashi, H., Hotta, K., Erives, A., Di Gregorio, A., Zeller, R. W., Levine, M. and Satoh, N.** (1999). Brachyury downstream notochord differentiation in the ascidian embryo. *Genes Dev.* **13**, 1519-1523.
**Wallingford, J. B.** (2006). Planar cell polarity, ciliogenesis and neural tube defects. *Hum. Mol. Genet.* **15 Spec No. 2**, R227-R234.
**Wallingford, J. B., Rowning, B. A., Vogeli, K. M., Rothbacher, U., Fraser, S. E. and Harland, R. M.** (2000). Dishevelled controls cell polarity during Xenopus gastrulation. *Nature* **405**, 81-85.
**Wallingford, J. B., Fraser, S. E. and Harland, R. M.** (2002). Convergent extension: the molecular control of polarized cell movement during embryonic development. *Dev. Cell* **2**, 695-706.