Development 127, 3091-3100 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
DEV2562

Role of *frizzled 7* in the regulation of convergent extension movements during gastrulation in *Xenopus laevis*

Alexandre Djiane, Jean-François Riou, Muriel Umbhauer, Jean-Claude Boucaut and De-Li Shi*

Groupe de Biologie Expérimentale, Laboratoire de Biologie Moléculaire et Cellulaire du Développement, CNRS UMR 7622, Université Pierre et Marie Curie, 9 quai Saint-Bernard, 75005 Paris, France
*Author for correspondence (e-mail: dshi@ccr.jussieu.fr)

Accepted 25 April; published on WWW 22 June 2000

SUMMARY

Wnt signalling plays a crucial role in the control of morphogenetic movements. We describe the expression and functional analyses of *frizzled 7* (*Xfz7*) during gastrulation in *Xenopus*. Low levels of *Xfz7* transcripts are expressed maternally during cleavage stages; its zygotic expression strongly increases at the beginning of gastrulation and is predominantly localized to the presumptive neuroectoderm and deep cells of the involuting mesoderm. Overexpression of Xfz7 in the dorsal equatorial region affects the movements of convergent extension and delays mesodermal involution. It alters the correct localization, but not the expression, of mesodermal and neural markers. These effects can be rescued by extra-Xfz7, which is a secreted form of the receptor that also weakly inhibits convergent extension when overexpressed. This suggests that the wild-type and truncated receptors have opposing effects when coexpressed and that overexpression of Xfz7 causes an increased signalling activity. Consistent with this, Xfz7 biochemically and functionally interacts with Xwnt11. In addition, Dishevelled, but not β-catenin, synergizes with Xfz7 to affect convergent extension. Furthermore, overexpression of Xfz7 and Xwnt11 also affects convergent extension in activin-treated animal caps, and this can be efficiently reversed by coexpression of $Cdc42^{T17N}$, a dominant negative mutant of the small GTPase Cdc42 known as a key regulator of actin cytoskeleton. Conversely, $Cdc42^{G12V}$, a constitutively active mutant, rescues the effects of extra-Xfz7 on convergent extension in a dose-dependent manner. That both gain-of-function and loss-of-function of both *frizzled* and *dishevelled* produce the same phenotype has been well described in *Drosophila* tissue polarity. Therefore, our results suggest an endogenous role of Xfz7 in the regulation of convergent extension during gastrulation.

Key words: Frizzled, Wnt, Cdc42, Dishevelled, *Xenopus*, Gastrulation, Convergent extension, Morphogenetic movement

INTRODUCTION

Gastrulation in *Xenopus* involves a complex set of morphogenetic movements. The main engine producing the driving force for gastrulation is thought to be convergent extension which results from mediolateral intercalation of deep cells in the dorsal marginal zone (DMZ), located between the external epithelial layer and the Brachet's cleft. Cells deep to the Brachet's cleft migrate on the blastocoel roof and contribute to the prechordal mesoderm. Cells undergoing convergent extension will form axial and paraxial mesoderms, as well as neural tissue. Cell intercalation causes the asymmetric movements of the DMZ cells towards the midline (Keller et al., 1992). As a result, the region located just above the dorsal blastoporal lip moves inside the embryo to differentiate into mesoderm, while the region closer to the animal pole remains external to form neural tissue. Involuted cells continue mediolateral intercalation allowing the elongation of axial and paraxial mesoderms along the anteroposterior axis, and blastopore closure. Mediolateral intercalation results from coordinated regulation of the protrusive motility behaviour of the DMZ cells (Shih and Keller, 1992; Wacker et al., 1998). These processes are not established prior to gastrulation but reflect organizing events at gastrula stages (Domingo and Keller, 1995).

While the cellular basis of convergent extension is well documented, molecular mechanisms regulating this process remain poorly understood. Several lines of evidence have suggested that Wnt signalling may play a role in the control of cell behaviour during gastrulation. Wnts are a family of secreted proteins that are involved in intercellular signalling and pattern formation during development (for reviews see Moon, 1993; Cadigan and Nusse, 1997; Wodarz and Nusse, 1998). Functional analyses in *Xenopus* have suggested the presence of functionally distinct Wnts with distinct signalling pathways (reviewed by Moon et al., 1997; Miller et al., 1999). Overexpression in ventral-vegetal cells of the first group of Xwnts (Xwnt1, Xwnt3A, Xwnt8 and Xwnt8b) before gastrulation induces a complete secondary axis (reviewed by Wodarz and Nusse, 1998). These Xwnts activate the canonical Wnt/β-catenin signalling pathway and the transcription of target genes *siamois* and *Xnr3* (Carnac et al., 1996; Brannon

et al., 1997; McKendry et al., 1997; Fan et al., 1998). In contrast, overexpression of the second group of Xwnts (Xwnt4, Xwnt5A and Xwnt11) in embryos affects morphogenetic movements (Moon et al., 1993; Du et al., 1995; Ungar et al., 1995). These effects can be correlated with their ability to stimulate intracellular calcium release in a G-protein-dependent fashion (Slusarski et al., 1997a). Dishevelled (Dsh), a component of the Wnt pathway, was shown to play a role in morphogenetic movements (Sokol, 1996). However, little is known about the implication of other components of the Wnt pathway in these processes.

The effects elicited by the two groups of Xwnts are likely mediated by functional distinct frizzled receptors (Slusarski et al., 1997b; Sheldahl et al., 1999; Miller et al., 1999). The frizzled proteins constitute a large family of seven transmembrane receptors with at least nine members in mammals (Wang et al., 1996); they have a conserved extracellular cysteine-rich domain (CRD) and a divergent C-terminal cytoplasmic region. Some frizzled proteins can both recruit Wnts at the cell surface and activate a Wnt pathway (Bhanot et al., 1996; Yang-Snyder et al., 1996). The prototypic *Drosophila frizzled* is a tissue-polarity gene whose activity is crucial for the establishment of tissue polarity (Adler, 1992). It has been shown that Dsh and the Rho family proteins mediate frizzled signalling that is required for polarized cytoskeletal reorganisation (Eaton et al., 1996; Strutt et al., 1997; Boutros et al., 1998; Axelrod et al., 1998).

Several *Xenopus* homologues of the *frizzled* gene family have been identified recently (Shi et al., 1998; Deardorff et al., 1998; Itoh et al., 1998; Wheeler and Hoppler, 1999). We previously identified *Xfz3*, which is expressed in the neural folds and may play a role in the morphogenesis of the central nervous system (Shi et al., 1998). *Xfz8* is expressed in the migrating mesoderm and has potential functions in dorsoanterior development (Deardorff et al., 1998; Itoh et al., 1998). Overexpression of either a secreted form of Xfz8 or a naturally occuring Wnt antagonist was shown to inhibit convergent extension (Deardorff et al., 1998; Xu et al., 1998), indicating that signalling by frizzled receptors plays a role in morphogenetic movements of gastrulation.

In a further attempt to provide information on the role of Wnt receptors in the regulation of cell movements during gastrulation, we report here functional analyses of *Xfz7*. Analysis by in situ hybridization on sectioned embryos indicates that *Xfz7* transcripts are predominantly localized to the neuroectoderm and to deep cells of the involuting mesoderm. These cells undergo active mediolateral intercalation during convergent extension. Overexpression of Xfz7 affects convergent extension that is rescued by extra-Xfz7, a secreted form of the receptor. Furthermore, we show that Xfz7 biochemically and functionally interacts with Xwnt11. Based on these gain-of-function phenotypes, we analysed downstream components of Xfz7 signalling in convergent extension. Our analyses show that Xfz7 synergizes with Dsh, but not with β-catenin, in this process. Interestingly, a dominant negative form of the Rho family GTPase Cdc42 rescues the effect of Xfz7 and Xwnt11 in convergent extension, while a constitutively active mutant rescues the effects of extra-Xfz7. These results imply that cytoskeletal modifications may be potential targets of Xfz7 signalling. Therefore, *Xfz7* is expressed at the right time and place to play a role in the regulation of morphogenetic movements during gastrulation, and it might act through a mechanism similar to planar polarity signalling in *Drosophila*. Xwnt11, Dsh and cytoskeletal modifications may take part in Xfz7 signalling.

MATERIALS AND METHODS

Cloning of *Xfz7* cDNA

A partial *Xfz7* cDNA was initially obtained using a degenerate PCR procedure as previously described (Shi et al., 1998). To obtain a complete cDNA, specific primers (forward: 5′-CGGCCGATCATC-TCCTGTTC-3′; reverse, 5′-ATGGAACTGACCATGCCGAAG-3′) were used in PCR amplification to identify a positive clone in pools of a plasmid library made from LiCl-dorsalized gastrula mRNAs (Lemaire et al., 1995). After successive dilution of positive pools, a full-length cDNA clone (EMBL database accession number AJ243323) was obtained and sequenced.

Plasmid constructs

The *Xfz7* coding sequence was cloned into pCS2+ vector (Turner and Weintraub, 1994; Rupp et al., 1994). To obtain *pCS2-extra-Xfz7*, cDNA encoding the extracellular region of Xfz7 was amplified by PCR and cloned in the pCS2+ vector. The *pCS2-extra-Xfz7FLAG* was generated by introducing the FLAG epitope C-terminal to the extra-Xfz7. The *Xdsh* plasmid (Sokol, 1996) was provided by S. Sokol. The *pSP64T-β-catenin-GFP* plasmid was from P. Lemaire and H. Yasuo. The $Cdc42^{T17N}$ and $Cdc42^{G12V}$ cDNAs (Drechsel et al., 1996) were provided by A. Hall and D. Drechsel and cloned either in the pSP64T (Krieg and Melton, 1984) or the pCS2+ vector. *Xwnt11myc* was obtained by PCR amplification of *Xwnt11* coding sequence (Ku and Melton, 1993) and cloned into the pCS2-MT vector between *Bam*HI and *Cla*I sites in-frame with the six myc epitopes.

Microinjections of embryos and β-galactosidase staining

Capped mRNAs were made by in vitro transcription and injections of embryos were carried out in 0.1× MBS containing 3% Ficoll-400. After injections, embryos were kept in this solution for 3 hours and then cultured in 0.1× MBS until they reached appropriate stages (Nieuwkoop and Faber, 1967). β-galactosidase (β-gal) staining was carried out as described (Vize et al., 1991).

RNAse protection and RT-PCR

Extraction of RNA and RNAse protection assay were performed as previously described (Shi et al., 1998). For RT-PCR, RNA samples were treated with RNAse-free DNAse I (Boehringer Mannheim) and were reverse-transcribed using 200 units *SuperScript* (Life Technologies). PCR primers for *goosecoid* (*gsc*), *Xbra, Xwnt8, chordin, ornithine decarboxylase* (*ODC)* were as described (Lemaire and Gurdon, 1994; Bouwmeester et al., 1996).

In situ hybridization and immunostaining

Whole-mount in situ hybridization was performed according to standard protocol (Harland, 1991). In situ hybridization on sections was carried out essentially as described by Lemaire and Gurdon (1994), except that sections of 15 μm thickness were made from fixed embryos embedded in polyethylene glycol-400 distearate. Whole-mount immunocytochemistry using the muscle-specific 12/101 (Kintner and Brockes, 1984) and the notochord-specific MZ15 (Smith and Watt, 1985) monoclonal antibodies was performed as described (Dent et al., 1989).

Immunoprecipitation and western blotting

Interaction between Xwnt11 and Xfz7 was performed as described (Lin et al., 1997), except that the two proteins were expressed in embryos injected with 500 pg *Xwnt11myc* and *extra-Xfz7FLAG*

mRNAs at 2-cell stage. Five early gastrulae were extracted in 500 μl extraction buffer (100 mM NaCl, 5 mM EDTA, 0.5% NP-40, 10 mM Tris-HCl, pH 7.5, 2 mM PMSF, 25 μM leupeptin and 0.2 units/ml aprotinin). Cell lysates were incubated with 10 μl anti-FLAG M2 affinity gel (Sigma) for 1 hour at room temperature. After several washes with extraction buffer, the beads were subjected to 10% SDS-PAGE. Following electrophoresis, proteins were transferred to nitrocellulose sheets (Amersham). The membranes were incubated with 9E10 monoclonal antibody (Santa Cruz Biotechnology) followed by peroxidase-conjugated secondary antibody (Jackson ImmunoResearch Laboratories). Bound secondary antibodies were visualized using ECL reagents (Amersham).

Explant elongation assays

Synthetic mRNAs were injected at 4-cell stage near the animal pole region into all four blastomeres. Animal caps were dissected at stage 8 and incubated for 1 hour in 1× MBS containing 10 U/ml recombinant *Xenopus* activin A (provided by Dr J. C. Smith) and 0.5 mg/ml BSA. The stage of embryos and the duration of activin-treatment were rigorously controlled for each experiment. Explants were then cultured in 1× MBS until appropriate stages.

RESULTS

Expression of *Xfz7* in neuroectoderm and in deep cells of the involuting mesoderm

Xfz7 protein contains 549 amino acids and includes all characteristic features expected of members of frizzled family. It includes a putative signal peptide and an extracellular CRD with ten invariant cysteines, followed by seven putative transmembrane domains and a short C-terminal cytoplasmic domain of 24 amino acids (not shown). While this work was in progress, Wheeler and Hoppler (1999) reported the isolation of a cDNA which is very similar to the sequence described here. These two cDNAs probably results from polymorphic alleles of the same gene, due to the tetraploidy of the *Xenopus laevis* genome. However, a detailed temporal and spatial expression pattern of Xfz7 during gastrulation is still lacking.

Analysis by RNAse protection of the temporal expression of *Xfz7* indicated that it was expressed maternally at low levels during cleavage stages. Zygotic expression strongly increased at the beginning of gastrulation and remained constant at gastrula stages (Fig. 1). There was a decline in *Xfz7* mRNA levels from late neurula stage onwards.

We then performed in situ hybridization in whole embryos and on sections to analyse precisely the expression of *Xfz7* during gastrulation. The latter was used because it alleviates probe penetration problems associated with the whole-mount procedures (Lemaire and Gurdon, 1994). These analyses revealed that *Xfz7* was predominantly expressed in the dorsal region of gastrulae. At stage 10.5, *Xfz7* was detected both in the dorsal blastoporal lip and in the presumptive neuroectoderm. The superficial layer of the DMZ expressed low levels of *Xfz7*, while deep layers strongly expressed the transcripts (Fig. 2A,E,F). At this stage, *Xfz7* transcripts were also detected in mesendodermal cells deep to the Brachet's cleft (Fig. 2E,F). During gastrulation, *Xfz7* transcripts remained strongly expressed in the neuroectoderm and in deep cells of the DMZ, with lower levels of expression in the superficial layer (Fig. 2B,G,H). Consistent with the preferential dorsal expression in normal gastrula, lithium-dorsalized early gastrula showed uniform expression of *Xfz7* in the entire

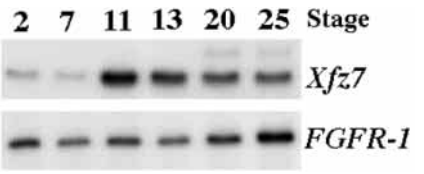

Fig. 1. Temporal expression of *Xfz7*. RNAse protection assay. *Xfz7* is maternally expressed with low levels during cleavage stages. Strong increase in the expression starts at the beginning of gastrulation. FGFR-1 probe was used as a loading control.

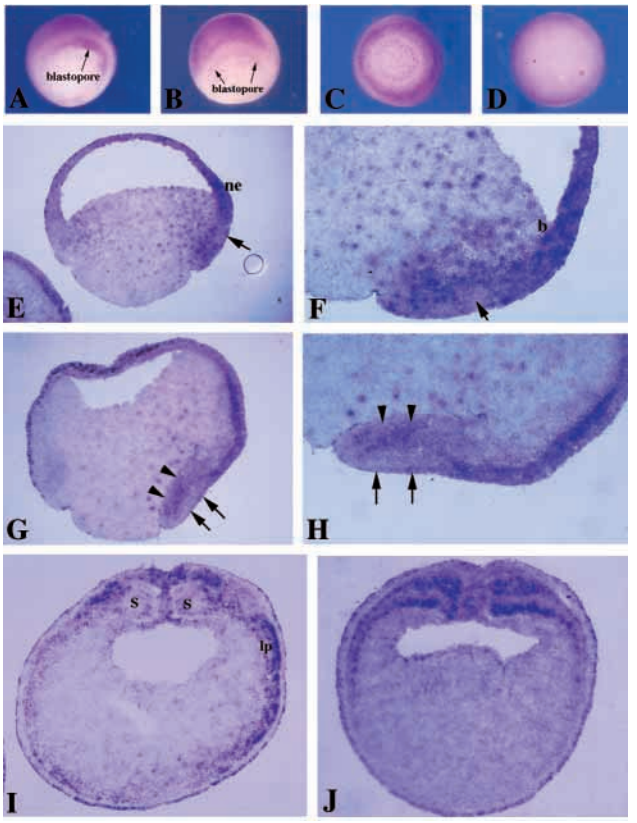

Fig. 2. Spatial expression of *Xfz7*. In situ hybridization on whole embryos (A-D) and on sections (E-J). (A) Dorsovegetal view of a stage 10 gastrula. *Xfz7* is strongly expressed in the blastoporal lip and in the presumptive neuroectoderm, with a gap of lower expression levels between these two regions. (B) Dorsovegetal view of a stage 11 gastrula. The presumptive neuroectoderm is strongly stained. (C) Lithium-dorsalized early gastrula shows expression of *Xfz7* in the entire marginal zone. (D) U.v.-ventralized early gastrula shows absence of *Xfz7* expression. (E) Sagittal section of a stage 10.5 gastrula. (F) Higher magnification of E. *Xfz7* transcripts are strongly expressed in the presumptive neuroectoderm (ne) and in deep cells of the dorsal mesoderm, located between the Brachet's cleft (b) and the superficial layers (arrow). (G) Sagittal section of a stage 11 gastrula. (H) Higher magnification of G. Strong expression of *Xfz7* is present in the presumptive neuroectoderm and in deep layers of dorsal mesoderm (arrowheads). Lower levels of expression are present in the superficial layers (arrows). (I,J) Transverse section from a stage 18 neurula. Anteriorly (I), *Xfz7* is expressed in the neural plate and in the lateral plate mesoderm (lp), but not in the somites (s). Posteriorly (J), it is expressed in the unsegmented somitic mesoderm.

marginal zone (Fig. 2C), while u.v.-ventralized early gastrula expressed lower levels of *Xfz7* transcripts (Fig. 2D). *Xfz7* expression pattern differs from that of *Xfz8* which was detected in the migrating mesendoderm deep to the Brachet's cleft but not in the involuting mesoderm (Deardorff et al., 1998; Itoh et al., 1998). Throughout neurula stages, *Xfz7* transcripts were most strongly expressed in the anterior neural plate (Fig. 2I). Anteriorly, *Xfz7* was not expressed in the somites but in the lateral plate mesoderm (Fig. 2I). Posteriorly, strong expression was found in the unsegmented somitic mesoderm (Fig. 2J). At later stages, *Xfz7* transcripts were predominantly expressed in the anterior central nervous system, pronephros and heart primordium (data not shown; Wheeler and Hoppler, 1999). These data both confirm and extend previous observations made by whole-mount in situ hybridization.

Overexpression of Xfz7 affects convergent extension

The function of Xfz7 during early development was analysed by overexpression in different regions of the embryo. Injections of various amounts of *Xfz7* mRNA into ventral-vegetal blastomeres at 4-cell stage did not induce a secondary axis. Accordingly, overexpression of Xfz7 alone in animal caps did not induce the expression of the canonical Wnt/β-catenin target gene *siamois* (data not shown). However, we found that injection of 400 pg *Xfz7* mRNA in the dorsal equatorial region at 4-cell stage resulted in embryos with gastrulation defects in 79% of cases (Table 1). At early gastrula stages (stages 10-10.5), dorsal blastoporal lip formed at the same time in *Xfz7*-injected embryos and in control *lacZ*-injected embryos (not shown). However, at mid-gastrula stages (stages 11-11.5), *Xfz7*-injected embryos showed a large-sized blastopore and yolk plug (compare Fig. 3A and B). By early neurula stage (stage 14), *Xfz7*-injected embryos showed delayed mesoderm involution and an open blastopore. They did not form trunk neural plate and exhibited externally visible endoderm on the dorsal side (Fig. 3C,D). As development proceeds, *Xfz7*-injected embryos had severe dorsal developmental defects including a shortening of the anteroposterior axis and microcephaly (Fig. 3E,F). Consistent with these phenotypes, immunostaining by muscle-specific 12/101 and notochord-specific MZ15 monoclonal antibodies revealed that *Xfz7*-injected embryos exhibit shortened axial and paraxial mesoderms (Fig. 3G-J).

The effect of Xfz7 overexpression on cell behaviours during gastrulation movements was further analysed using *lacZ* as a cell lineage tracer. In stage 11 mid-gastrulae injected with 400 pg *lacZ* mRNA alone, β-gal-stained cells were essentially located at the dorsal midline as a narrow strip that converges towards the blastoporal lip (Fig. 4A), indicating the asymmetric cell movements of the DMZ. In contrast, coinjection with 400 pg *Xfz7* mRNA resulted in β-gal-stained cells that were distributed uniformly on the dorsal region (Fig. 4B). This difference was much more pronounced by early neurula stage at which time β-gal-stained cells in *lacZ*-injected embryos contribute to the neural plate along the anteroposterior axis (Fig. 4C), whereas they occupy a large zone in the dorsal region of embryos coinjected with *Xfz7* (Fig. 4D). Accordingly, convergent extension movements and the elongation of the body axis were impaired. Sagittal sections from *lacZ*-injected embryos indicated that β-gal-stained cells were distributed in the entire notochord and the prechordal mesoderm (Fig. 4E). However, in coinjected embryos, β-gal-stained cells did not involute, a small archenteron cavity was formed by a limited involution of uninjected cells. Hence, the extent of dorsal involution was similar to that of uninjected ventral side (Fig. 4F). These in vivo analyses indicate that overexpression of Xfz7 affects convergent extension during gastrulation.

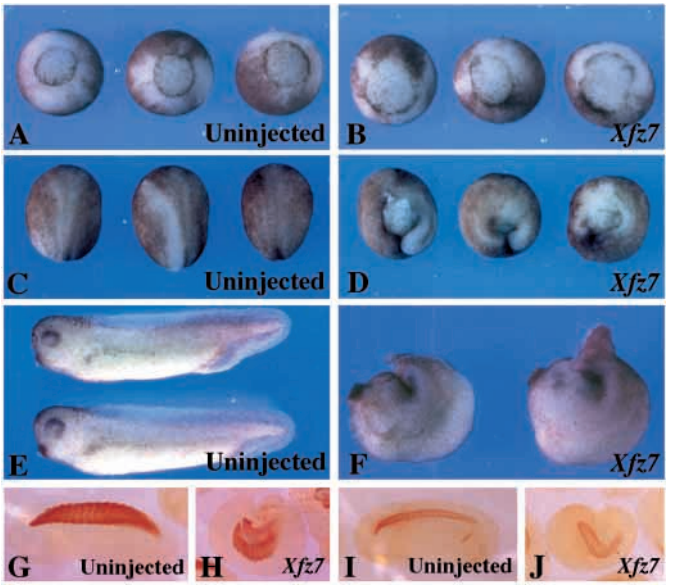

Fig. 3. Overexpression of Xfz7 affects gastrulation movements. 4-cell-stage embryos were injected at the dorsal equatorial region with 400 pg *Xfz7* mRNA and allowed to develop to different stages. (A) Control stage 11.5 embryos form a reduced circular blastopore. (B) *Xfz7*-injected embryos at stage 11.5 equivalent show a large circular blastopore. (C) Control stage 17 embryos have a closed blastopore. (D) *Xfz7*-injected embryos at stage 17 equivalent exhibit an open blastopore and externally visible endoderm. (E) Control stage 35 embryos. (F) *Xfz7*-injected embryos at stage 35 equivalent show dorsal development defects and shortened anteroposterior axis. (G-J) Immunostaining of muscle (G,H) and notochord (I,J) in stage 25 uninjected (G,I) and *Xfz7*-injected (H,J) embryos.

Table 1. Overexpression of Xfz7 on blastopore closure and early development

	Blastopore closure		Late phenotypes			
RNA-injected (pg)	Normal	Open blastopore	Normal	Bent axis	Others	*n*
lacZ (400)	90	10	90	6	4	150
Xfz7 (400)	21	79	18	76	6	198

Embryos were injected at 4-cell stage in the dorsal equatorial region. Blastopore closure was scored at stage 14 and late phenotypes were scored at stage 35. Examples of embryos with an open blastopore and a bent axis are as shown in Fig. 3D and F, respectively. Other phenotypes include anterior deficiency and absence of embryonic axes. Results were expressed as percentages except *n*, which refers to the total numbers of embryos scored.

Rescue of the Xfz7 phenotype by extra-Xfz7

In order to examine whether the effect of Xfz7 overexpression results from a gain-of-function or a loss-of-function by the titration of an endogenous ligand, we compared in whole embryos the effects of Xfz7 and of extra-Xfz7, which is a secreted form of Xfz7 retaining only the extracellular CRD and is not capable of transducing an intracellular signal. Injection of 400 pg *extra-Xfz7* mRNA in the dorsal equatorial region did not have a significant effect on gastrulation movements (not shown). When higher amounts of *extra-Xfz7* mRNA (1000 pg) were injected, 29% of injected embryos (*n*=127) exhibited an open blastopore at the end of gastrulation, while the rest (71%) gastrulated normally (see Table 2). That extra-Xfz7 inhibits gastrulation movements at higher doses is consistent with its dominant negative action to antagonize the activity of the wild-type receptor and/or of an endogenous ligand. Because of the weak activity of extra-Xfz7, it was not practical to rescue its effect by the wild-type receptor. We therefore tried to rescue the Xfz7 phenotype by extra-Xfz7. Different amounts of *Xfz7* mRNAs were injected in the dorsal equatorial region at 4-cell stage either alone or coinjected with 1000 pg *extra-Xfz7* mRNA. Blastopore closure was scored when control uninjected embryos reached stage 14 (early neurula). We obtained a significant rescue of blastopore closure when 1000 pg *extra-Xfz7* mRNA was coinjected with 200 pg *Xfz7* mRNA. Typically, injection of 200 pg *Xfz7* mRNA resulted in early neurulae with an open blastopore in 64% of cases (*n*=68). This frequency was reduced to 31% (*n*=54) by coinjecting with 1000 pg *extra-Xfz7* mRNA (Table 2). This result indicates that

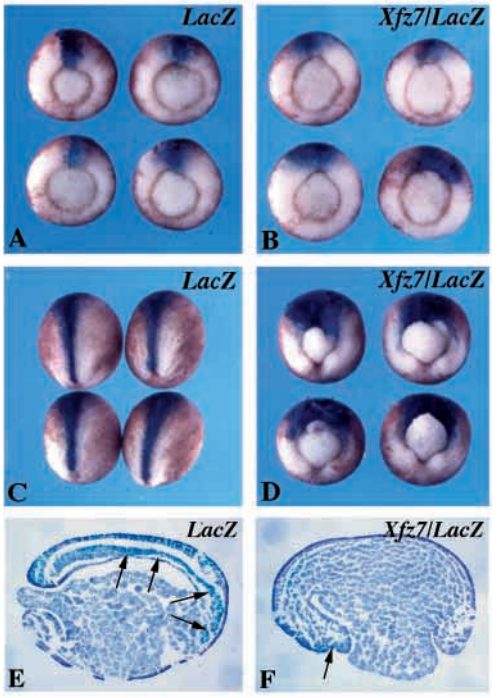

Fig. 4. Lineage tracer analysis of convergent extension after overexpression of Xfz7. Embryos at 4-cell stage were injected at the dorsal equatorial region with *lacZ* mRNA (400 pg) either alone or mixed with *Xfz7* mRNA (400 pg). (A) Control *lacZ*-injected embryos (stage 11). β-gal-stained cells are concentrated along the dorsal midline region. (B) *Xfz7*-injected embryos (stage 11) show absence of convergent extension by β-gal-stained cells. (C) *lacZ*-injected stage 15 embryos. β-gal-stained cells are concentrated along the medial region of the neural plate. (D) *Xfz7*-injected embryos at stage 15 equivalent lack convergent extension and the formation of neural plate. (E) Medial sagittal section from an embryo in C. β-gal-stained cells are distributed in the neural plate, the notochord and the prechordal mesoderm (arrows). (F) Medial sagittal section from an embryo in D, dorsal side is on the left. β-gal-stained cells do not involute (arrow). Notice the similar extent of involution on dorsal and ventral sides.

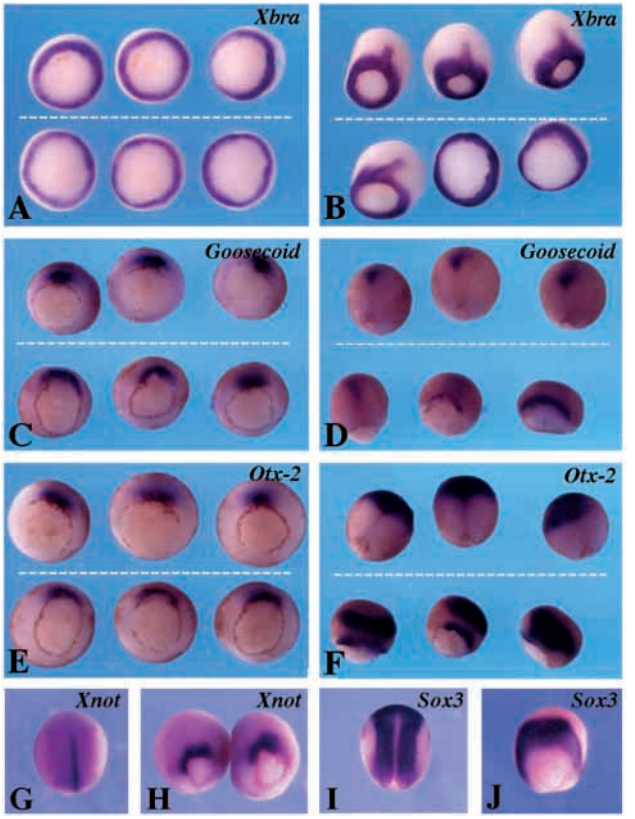

Fig. 5. Overexpression of Xfz7 affects the localization of mesodermal and neural markers. 4-cell-stage embryos were injected at the dorsal equatorial region with 400 pg *Xfz7* mRNA. (A) Expression of *Xbra* at stage 10. No significant difference can be noticed between control uninjected (top) and *Xfz7*-injected (bottom) embryos. (B) Expression of *Xbra* in stage 12 control embryos (top) is localised around the blastopore and in the notochordal mesoderm. In *Xfz7*-injected embryos (bottom), *Xbra* is absent or reduced in the notochordal mesoderm. (C) The pattern of *gsc* expression in stage 10.5 control embryos (top) reflects convergent extension of dorsal mesoderm toward the blastopore. *Xfz7*-injected embryos (bottom) do not display such a pattern. (D) At stage 13, *gsc* is located anteriorly in control embryos (top) whereas it remains near the blastopore and extends laterally in *Xfz7*-injected embryos (bottom). (E) Expression of *otx-2* in control (top) and in *Xfz7*-injected (bottom) embryos at stage 10.5. A difference can be noticed as in C. (F) At stage 13, control embryos (top) have only the anterior expression of *otx-2*; *Xfz7*-injected embryos (bottom) show *otx-2* expression around the dorsal blastoporal lip and in the dorsal ectoderm. (G) *Xnot* is expressed in the notochord in a stage 14 uninjected embryo. (H) Expression of *Xnot* in the dorsal blastoporal lip in *Xfz7*-injected embryos at stage 14 equivalent. (I) Expression of *Sox3* is present in the neural plate except in the midline in a stage 14 uninjected embryo. (J) Pattern of *Sox3* expression in a *Xfz7*-injected embryo at stage 14 equivalent.

Table 2. Rescue of blastopore closure by extra-Xfz7*

RNA-injected (pg)	Normal	Open blastopore	*n*
Uninjected	100		105
Extra-Xfz7 (1000)	71	29	127
Xfz7 (200)	36	64	68
Xfz7/*Extra-Xfz7* (200/1000)	69	31	54

*Injection of embryos and score of phenotypes were as described in Table 1.

Xfz7 and extra-Xfz7 exert opposing effects when coexpressed. Therefore, it is unlikely that Xfz7 exerts its effect by the titration of an endogenous ligand. This implies that overexpressed Xfz7 proteins interact with an endogenous ligand to activate a signalling pathway.

Xfz7 affects the localization, but not the expression, of mesodermal and neural markers

We then analysed the expression of *Xbra*, *gsc*, *otx-2*, *Xnot* and *Sox-3* in *Xfz7*-injected embryos to see if overexpression of Xfz7 directly modifies cell behaviour. *Xbra* is expressed in the entire marginal zone of the early gastrula. During gastrulation, it is expressed around the blastopore and in the developing notochord (Smith et al., 1991; Fig. 5A,B, top). *Gsc* and *otx-2* are expressed in the mesendodermal cells that will contribute to the prechordal mesoderm at the end of gastrulation (Cho et al., 1991; Pannese et al., 1995; Blitz and Cho, 1995; Fig. 5C-F, top). *Xnot* is first expressed in the Spemann organizer and then localized to the notochord by the end of gastrulation (von Dassow et al., 1993; Fig. 5G). *Sox-3* is restricted to the entire neural folds during neurulation (Penzel et al., 1997; Fig. 5I).

The distribution of *Xbra*, *gsc* and *otx-2* mRNAs at stage 10.5 was identical in *Xfz7*-injected and in control embryos (Fig. 5A,C,E). This shows that Xfz7 overexpression had no detectable effect on mesoderm specification prior to gastrulation. At later stages, the expression of *Xbra* in most *Xfz-7*-injected embryos was only detected around the blastopore. In embryos where mesoderm involution partially took place, some *Xbra* expression was detected in the notochord (Fig. 5B, bottom left). Expression of *gsc* and *otx-2* in *Xfz7*-injected late gastrulae was generally observed at the level of the dorsal blastoporal lip; it extended laterally instead of anteriorly (Fig. 5D,F, bottom). When mesoderm involution was less affected, anterior *gsc* expression was sometimes detected (Fig. 5D, bottom left). Interestingly, *Xfz7*-injected embryos exhibited two domains of *otx-2* expression at late gastrula stage. One was located near the dorsal blastoporal lip while the second appeared in the dorsal neuroectoderm. The latter is likely induced by planar neural induction in the absence of vertical signals from the underlying mesoderm (Blitz and Cho, 1995). Analysis of *Xnot* expression pattern clearly indicated that dorsal mesoderm failed to involute and notochord was not elongated in *Xfz7*-injected early neurula (Fig. 5G,H). Furthermore, analysis of *Sox3* expression revealed that trunk and posterior neural plates were not correctly formed in *Xfz7*-injected early neurula (Fig. 5I,J). These results suggest that signals transduced by Xfz7 directly modify the behaviour of DMZ cells participating in convergent extension movements.

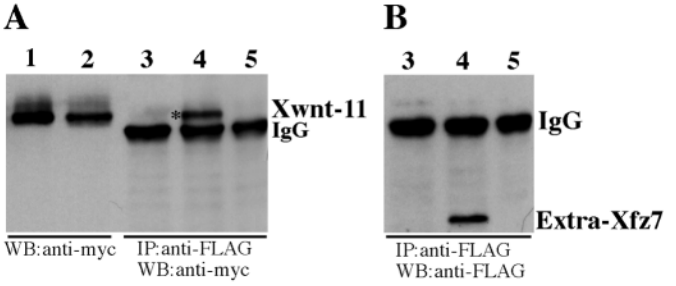

Fig. 6. Biochemical interaction between Xwnt11 and extra-Xfz7. (A) Proteins were extracted from embryos injected with 500 pg *Xwnt11myc* mRNA either alone (lanes 1, 3) or coinjected with 500 pg *extra-Xfz7FLAG* mRNA (lanes 2, 4), or from uninjected embryos (lane 5). Samples were immunoprecipitated (IP) using anti-FLAG M2 affinity gel (lanes 3, 4) followed by western blotting (WB) using 9E10 anti-myc antibody (lanes 1-5). Xwnt11myc (asterisk; lane 4) coimmunoprecipitates with extra-Xfz7FLAG. (B) Lanes 3-5 were probed with anti-FLAG antibody to show the presence of extra-Xfz7FLAG.

Interaction between Xwnt11 and Xfz7

Overexpression of Xwnt11 also affects morphogenetic movements (Du et al., 1995). In addition, *Xwnt11* (Ku and Melton, 1993; Tada and Smith, 2000) and *Xfz7* (Fig. 2) exhibit significant overlapping expression in the marginal zone of early gastrula. To see if Xwnt11 acts in the same pathway as Xfz7, we first examined the effect of Xwnt11 overexpression in whole embryos. Injection of 200 pg of the mRNA in the dorsal equatorial region produced the same phenotype as Xfz7 in 91% (*n*=79) of cases (Table 3). Furthermore, we found that Xwnt11 synergizes with Xfz7 to inhibit gastrulation movements. Injection of 100 pg *Xfz7* mRNA or 40 pg *Xwnt11* mRNA resulted in embryos with an open blastopore at the end of gastrulation in 28% (*n*=113) and 26% (*n*=54) of cases, respectively. Coinjection of *Xfz7* and *Xwnt11* mRNAs strongly increased the frequency (79%, *n*=46). Interestingly, extra-Xfz7 rescued the effect of Xwnt11 on blastopore closure in a dose-dependent manner (Table 3). Consistent with this functional interaction, we found that myc-tagged Xwnt11 coimmunoprecipitates with extra-Xfz7 (Fig. 6) and that the wild-type Xfz7 recruits Xwnt11 at the plasma membrane (data not shown). These results suggest that Xfz7 and Xwnt11 may act in the same pathway in convergent extension.

Dsh, but not β-catenin, synergizes with Xfz7

Genetic epistasis experiments in *Drosophila* suggest that Dsh

Table 3. Interactions between *Xfz7*, *Xwnt11* and *dsh**

RNA-injected (pg)	Normal	Open blastopore	*n*
Uninjected	98	2	198
Xwnt11 (200)	9	91	79
Xwnt11/*extra-Xfz7* (200/100)	26	74	61
Xwnt11/*extra-Xfz7* (200/500)	56	44	38
Xwnt11 (40)	74	26	54
Xfz7 (100)	72	28	113
Xfz7/*Xwnt11* (100/40)	21	79	46
dsh (200)	79	21	73
Xfz7/*dsh* (100/200)	18	82	76
β-catenin (100)	83	17	55
Xfz7/*β-catenin* (100/100)	72	28	57

*Injection of embryos and score of phenotypes were as described in Table 1.

and the Rho GTPase family proteins are required for frizzled signalling in tissue polarity (Krasnow et al., 1995; Eaton et al., 1996; Strutt et al., 1997; Boutros et al., 1998; Axelrod et al., 1998). We therefore examined whether a similar signalling cascade might be activated downstream of Xfz7 in convergent extension. As above, *Xfz7* mRNA (100 pg) was injected either alone or coinjected with *dsh* (200 pg) or *β-catenin* (100 pg) mRNAs. As shown in Table 3, overexpression of Dsh resulted in embryos with an open blastopore in 21% (n=73) of cases. Coinjection of Dsh with Xfz7 increased the frequency to 82% (n=76). By contrast, coexpression of β-catenin and Xfz7 did not have a significant effect on blastopore closure (Table 3). Consistent with this result, overexpression of Xwnt8, which activates the canonical Wnt/β-catenin pathway, had no effect on blastopore closure (not shown). These observations suggest that Xfz7 affects convergent extension by activating a distinct pathway via Dsh.

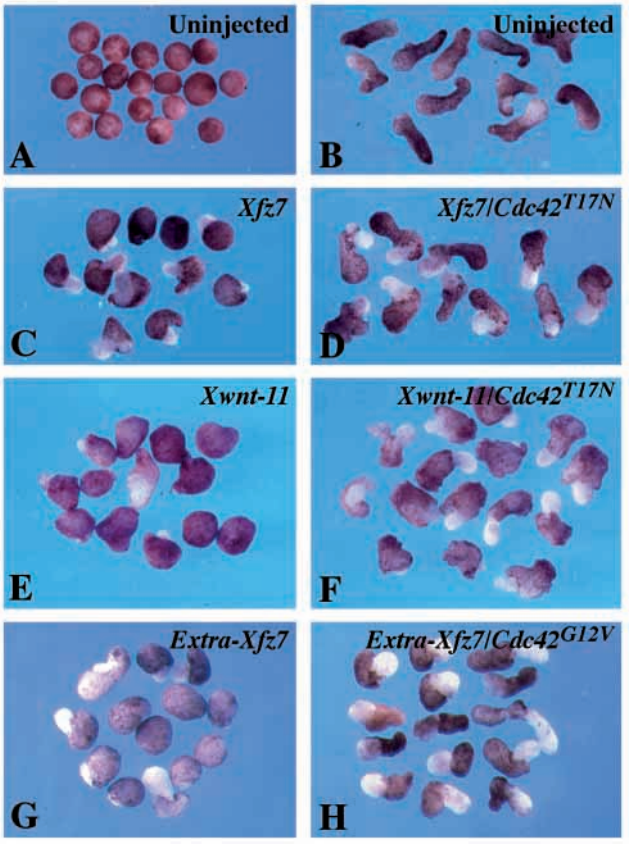

Fig. 7. Rescue of explant elongation by Cdc42 mutants. Animal caps were dissected at stage 8 and treated with activin. They were cultured to stage 17 equivalent. (A) Uninjected animal caps without activin treatment. (B) Uninjected animal caps treated with activin show extensive elongation. (C) Animal caps injected with 400 pg *Xfz7* mRNA and treated with activin remain rounded. (D) Coinjection of $Cdc42^{T17N}$ mRNA (500 pg) with *Xfz7* mRNA efficiently rescues the extent of explant elongation. (E) Animal caps injected with 100 pg *Xwnt11* mRNA and treated with activin. (F) Rescue of Xwnt11-blocked explant elongation by coinjection of 500 pg $Cdc42^{T17N}$ mRNA. (G) Animal caps injected with 2000 pg *extra-Xfz7* mRNA and treated with activin. (H) Coinjection of 200 pg $Cdc42^{G12V}$ DNA with *extra-Xfz7* mRNA rescues explant elongation.

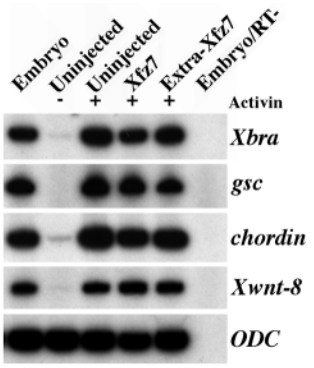

Fig. 8. RT-PCR analysis of the expression of dorsoventral mesodermal markers in stage 10.5 animal cap explants expressing Xfz7 or extra-Xfz7 and treated with activin. There is no significant difference in the expression of *Xbra*, *gsc*, *chordin* and *Xwnt8* between uninjected and injected explants treated with activin. RT–, whole embryo control sample without reverse transcriptase. *ODC* is a loading control.

A dominant negative Cdc42 mutant rescues the effects of Xfz7 and Xwnt11

To further characterize the signalling mechanism of Xfz7 in convergent extension, we screened the ability of different mutant forms of the Rho family of small GTPases (RhoA, Cdc42 and Rac) to rescue the effect of Xfz7 and Xwnt11 in activin-treated animal caps. Convergent extension movements also take place in activin-treated animal caps (Symes and Smith, 1987). In response to activin, uninjected animal caps showed extensive elongation (Fig. 7A,B), whereas explants injected with 400 pg *Xfz7* mRNA remained rounded (Fig. 7C). We found that injection of 500 pg mRNA encoding $Cdc42^{T17N}$, a dominant negative Cdc42 mutant (Drechsel et al., 1996), had no significant effect on explant elongation in untreated and activin-treated (10 U/ml) animal caps (not shown). Interestingly, coexpression of $Cdc42^{T17N}$ with Xfz7 efficiently and reproducibly rescued explant elongation (Fig. 7C,D). This rescue seems to be specific for $Cdc42^{T17N}$ because mutant forms of RhoA and Rac did not rescue elongation of activin-treated explants expressing Xfz7 (data not shown). Furthermore, $Cdc42^{T17N}$ also efficiently rescued the inhibitory effect of Xwnt11 overexpression on convergent extension (Fig. 7E,F). Overexpression of $Cdc42^{G12V}$, a constitutively active Cdc42 mutant, affects cell division at cleavage stages (Drechsel et al., 1996; data not shown). To see if it may rescue the effect of extra-Xfz7 on convergent extension, we used *pCS2-*$Cdc42^{G12V}$ DNA which contains $Cdc42^{G12V}$ cDNA under the control of the cytomegalovirus promoter. Injection of higher amounts of *extra-Xfz7* mRNA (2000 pg) inhibited activin-induced elongation of animal caps (Fig. 7G), this could be rescued by $Cdc42^{G12V}$ in a dose-dependent manner. An optimal rescue was obtained by coinjection of 200 pg $Cdc42^{G12V}$ DNA (Fig. 7H). As in whole embryos, overexpression of Xfz7 and extra-Xfz7 does not alter early mesoderm specification in activin-treated animal caps. RT-PCR analysis of the expression of early dorsoventral mesodermal markers showed that *gsc*, *chordin*, *Xbra* and *Xwnt8* were expressed at similar levels in activin-treated injected and uninjected explants (Fig. 8). Therefore, our results

suggest that Cdc42 may be an effector of Xfz7 signalling in convergent extension movements.

DISCUSSION

Expression of *Xfz7* in the involuting mesoderm during gastrulation

Xfz7 is a maternal mRNA expressed at low levels during cleavage stages, its zygotic expression sharply increases at the beginning of gastrulation. Maternal *Xfz7* may play a role in early dorsoventral mesoderm patterning since depletion of maternal *Xfz7* mRNA by antisense oligonucleotide results in embryos with dorsoanterior deficiency (Sumanas et al., 2000). In this study, in situ hybridization on sectioned gastrulae, which alleviates the probe penetration problems found in the whole-mount procedure (Lemaire and Gurdon, 1994), allowed us to detect high expression levels of *Xfz7* in the neuroectoderm and in deep cells of the DMZ, while lower levels were detected in the superficial layer. At the early gastrula stage, *Xfz7* was also expressed in the dorsal endodermal cells. This expression pattern correlates well with tissues undergoing active morphogenetic movements, in particular, mediolateral intercalation of the involuting mesoderm and active distortion movements of the endodermal mass (Keller et al., 1992; Winklbauer and Schürfeld, 1999). Previous works have shown that *Xfz8* is expressed in the migrating mesendodermal cells that lie deep to the Brachet's cleft and may be involved in the initial event of convergent extension (Deardorff et al., 1998). Therefore, the distinct expression pattern between *Xfz7* and *Xfz8* during gastrulation raises the possibility that they may regulate morphogenetic movements in distinct cell populations.

Xfz7 in the regulation of convergent extension

Overexpression of Xfz7 in the dorsal mesoderm affects convergent extension and delays mesodermal involution. The effects of Xfz7 on convergent extension are not due to changes on mesodermal fates and can be rescued by a secreted form of the receptor (see below). Overexpression of Xfz7 affects the correct localization of *Xbra*, *gsc*, *otx-2*, *Xnot* and *Sox-3* as a consequence of altered cell movements. In addition, it also affects activin-induced explant elongation but does not change the expression levels of dorsal and ventral mesodermal markers. In *Xenopus*, convergent extension involves active cell intercalation and the asymmetric movements of DMZ cells towards the midline (Keller et al., 1992). After involution, mesodermal cells continue to intercalate mediolaterally, allowing the elongation of axial mesoderm and the closing of blastopore. Our cell lineage and in situ hybridization analyses revealed that overexpression of Xfz7 prevented the asymmetric movements. In particular, we showed that the notochord marker *Xnot* was located at the blastoporal lip in *Xfz7*-injected early neurula instead of being extended along the entire notochord (see Fig. 5G,H). This further argues that overexpression of Xfz7 affects the asymmetric movements required for the elongation of notochordal precursor cells.

The effects of Xfz7 on convergent extension are consistent with its expression in the DMZ throughout gastrulation. Previous studies have shown that overexpressing an inhibitory form of Xfz8 affects morphogenetic movements (Deardorff et al., 1998). However, analysis using the dominant negative approach in the case of frizzled receptors may not fully address the specificity of a particular receptor involved in these processes. For example, overexpression of truncated forms of *Drosophila* frizzled 2 (DFz2N) and frizzled (FzN) indifferently interferes with wing margin development (Wg signalling) and with ommatidial polarity during eye development (frizzled signalling). The full-length frizzled 2, but not the full-length frizzled, rescues the effect of DFz2N and FzN on wing margin development. This observation suggests that DFz2N and FzN act in a dominant negative manner but they lose some aspects of signalling specificity (Zhang and Carthew, 1998). In support of this conclusion, overexpression of FrzA, a naturally occuring Wnt antagonist, also affects convergent extension during gastrulation, although FrzA mRNAs are not detected before neurulation (Xu et al., 1998). Since secreted Xfz8 and FrzA are not able to transduce a signal, it is possible that they interfere with an endogenous ligand involved in convergent extension.

The involvement of frizzled in the establishment of embryonic cell asymmetry has been well described in *Drosophila* (Adler, 1992) and *C. elegans* (Sawa et al., 1996; Rocheleau et al., 1997; Thorpe et al., 1997). A better described example is the prototypic *Drosophila frizzled* gene which is required for the establishment of tissue polarity. Both gain-of-function and loss-of-function mutations generate tissue-polarity phenotypes (Krasnow and Adler, 1994). Therefore, we could postulate that the regulation of cell polarity in vertebrates involves similar mechanisms as found in *Drosophila*. The effects resulting from Xfz7 gain-of-function may indeed reflect an endogenous function of this receptor in asymmetric movements during convergent extension (see below).

Functional and biochemical interactions between Xfz7 and Xwnt11

Overexpression of extra-Xfz7, a secreted form of Xfz7, also affects convergent extension, albeit to a less extent than the wild-type receptor. That extra-Xfz7 inhibits convergent extension at relatively higher doses is consistent with its dominant negative action to block the activity of an endogenous ligand. As an example, dominant negative Xwnt11 (see below) affects convergent extension at much higher doses than wild-type Xwnt11 (Du et al., 1995; Tada and Smith, 2000). The effect of Xfz7 could be reversed by extra-Xfz7, indicating that they have opposing effects when coexpressed. This result suggests that Xfz7 was not titrating an endogenous ligand. In contrast, it most likely interacts with a ligand and activates a signalling pathway. What may be the endogenous ligand interacting with Xfz7? A likely candidate would be Xwnt11. Several lines of evidence support this hypothesis. Firstly, *Xwnt11* (Ku and Melton, 1993; Tada and Smith, 2000) exhibits an overlapping expression pattern with *Xfz7* (this study) at early gastrula stage. Secondly, it synergizes with Xfz7 to affect morphogenetic movements (see Table 3). Thirdly, extra-Xfz7 coimmunoprecipitates with Xwnt11 (Fig. 6) and rescues its effect on gastrulation movements (Table 3). We also found that Xfz7 causes a significant increase in the association of myc-tagged Xwnt11 with the plasma membrane (data not shown). Finally, we show that, as Xfz7, the inhibitory effect of Xwnt11 on convergent extension in activin-treated animal caps could be rescued by $Cdc42^{T17N}$ (see Fig. 8). These strongly

suggest that Xwnt11 and Xfz7 act in the same pathway in convergent extension movements.

Recently, it has been reported that overexpression of a specific dominant negative Xwnt11 mutant blocks convergent extension (Tada and Smith, 2000). If Xwnt11/Xfz7 signalling is required for convergent extension, why overexpression of both wild-type and truncated forms of these proteins similarly blocks this process? By analogy with the *Drosophila* tissue-polarity gene *frizzled* which generates the same tissue-polarity phenotype following gain-of-function and loss-of-function (Krasnow and Adler, 1994), it is possible that cell polarity is sensitive to Xwnt11/Xfz7 signalling. Thus, either increasing or decreasing the activity would perturb the asymmetric cell movements of the DMZ. This conclusion is further supported by analyses in zebrafish embryos. It has been shown previously that overexpression of Wnt4, a Wnt5A/Wnt11 class of Wnts, inhibits cell movements (Ungar et al., 1995). Genetic analysis shows that *silberblick*$^{-/-}$ embryos, which carry a null mutation in the *Wnt11* gene, exhibit impaired convergent extension movements (J. C. Smith; personal communication). Taken together, these results strongly argue that the Xfz7 gain-of-function reflects a physiological role in convergent extension.

Cytoskeletal modifications may be potential targets of Xfz7 signalling

We demonstrated that Xfz7 activates a distinct pathway via Dsh in convergent extension. A similar situation was described in *Drosophila* where overexpression of Dsh phenocopies Frizzled gain-of-function (Krasnow et al., 1995). Our result is consistent with the observation that Dsh is required for convergent extension (Sokol, 1996). Dsh is a multifunctional protein involved in Wnt/β-catenin and planar polarity signallings (reviewed by Boutros and Mlodzik, 1999). In *Drosophila* planar polarity signalling, Dsh activates c-Jun N-terminal kinase (JNK) through the RhoA small GTPase (Strutt et al., 1997). The Rho family proteins are key regulators of signal transduction pathways that mediate distinct actin cytoskeleton changes required for cell migration (reviewed by Hall, 1998). Our analysis revealed that the effect of Xfz7 and Xwnt11 on activin-induced explant elongation was efficiently rescued by Cdc42^{T17N}, a dominant negative Cdc42 mutant (Drechsel et al., 1996). Conversely, the effect of extra-Xfz7 was rescued by Cdc42^{G12V}, a constitutively active mutant (Drechsel et al., 1996). This raises the possibility that Cdc42 may be an effector of Xwnt11/Xfz7/Dsh signalling. In this regard, it is worth noting that the same Cdc42 mutant as used in this study inhibits Dsh-induced JNK activation in NIH-3T3 cells (Moriguchi et al., 1999). A recent study also suggests that overexpression of Xfz7 blocks convergent extension and decreases cadherin-mediated cell adhesion (Medina et al., 2000). Therefore, the effects of Xfz7 overexpression on convergent extension movement may probably result from an increased JNK activity and/or an altered motility behaviour of individual cells. Interestingly, it was demonstrated that the Rho family proteins are required for polarized cytoskeletal reorganisation in planar polarity signalling in *Drosophila* (Eaton et al., 1996), and that signalling mediated by the frizzled receptor polarizes cell divisions in sense organ precursors (Gho and Schweisguth, 1998). We postulate that endogenous Xfz7 activity might be important for the regulation of a cytoskeletal system involved in cell polarisation during convergent extension. Higher activity might be functionally equivalent to no activity. Therefore, a similar mechanism regulating cytoskeletal modifications may be conserved from *Drosophila* to vertebrates and it will be of interest to examine whether JNK pathway is implicated in convergent extension.

The authors would like to thank P. Lemaire, H. Yasuo, S. Sokol, R. T. Moon, H. Grunz, R. Penzel, A. Hall, D. Drechsel and J. C. Smith for providing the reagents used in this study, J. C. Smith for communicating results prior to publication, C. Carron and L. Neves for reading the manuscript, A. Pascal and A. Bourdelas for excellent technical assistance and P. Nguyen for illustrations. This work was supported by grants from CNRS, AFM, ARC and MENRT.

REFERENCES

Adler, P. N. (1992). The genetic control of tissue polarity in *Drosophila*. *BioEssays* **14**, 735-741.

Axelrod, J. D., Miller, J. R., Shulman, J. M., Moon, R. T. and Perrimon, N. (1998). Differential recruitment of dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways. *Genes Dev*. **12**, 2610-2622.

Bhanot, P., Brink, M., Samos, C. H., Hsieh, J. C., Wang, Y. S., Macke, J. P., Andrew, D., Nathans, J. and Nusse, R. (1996). A new member of the *frizzled* family from *Drosophila* functions as a Wingless receptor. *Nature* **382**, 225-230.

Blitz, I. L. and Cho, K. W. Y. (1995). Anterior neuroectoderm is progressively induced during gastrulation: the role of the *Xenopus* homeobox gene *orthodenticle*. *Development* **121**, 993-1004.

Bouwmeester, T., Kim, S. H., Sasai, Y., Lu, B. and De Robertis, E. M. (1996). Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. *Nature* **382**, 595-601.

Boutros, M., Paricio, N., Strutt, D. I. and Mlodzik, M. (1998). Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and *wingless* signaling. *Cell* **94**, 109-118.

Boutros, M. and Mlodzik, M. (1999). Dishevelled: at the crossroads of divergent intracellular signaling pathways. *Mech. Dev*. **83**, 27-37.

Brannon, M., Gomperts, M., Sumoy, L., Moon, R. T. and Kimelman, D. (1997). A beta-catenin/XTcf-3 complex binds to the siamois promoter to regulate dorsal axis specification in *Xenopus*. *Genes Dev.* **11**, 2359-2370.

Cadigan, K. M. and Nusse, R. (1997). Wnt signalling: a common theme in animal development. *Genes Dev*. **11**, 3286-3305.

Carnac, G., Kodjabachian, L., Gurdon, J. B. and Lemaire, P. (1996). The homeobox gene *Siamois* is a target of the wnt dorsalization pathway and triggers organizer activity in the absence of mesoderm. *Development* **122**, 3055-3065.

Cho, K. W. Y., Morita, E. A., Wright, C. V. E. and De Robertis, E. M. (1991). Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene *goosecoid*. *Cell* **67**, 1111-1120.

Deardorff, M. A., Tan, C. G., Conrad, L. J. and Klein, P. S. (1998). Frizzled-8 is expressed in the Spemann organizer and plays a role in early morphogenesis. *Development* **125**, 2687-2700.

Dent, J. A., Polson, A. G. and Klymkowsky, M. W. (1989). A whole-mount immunocytochemical analysis of the expression of the intermediate filament protein vimentin in *Xenopus*. *Development* **105**, 61-74.

Domingo, C. and Keller, R. (1995). Induction of notochord cell intercalation behavior and differentiation by progressive signals in the gastrula of *Xenopus laevis*. *Development* **121**, 3311-3321.

Drechsel, D. N., Hyman, A. A., Hall, A. and Glotzer, M. (1996). A requirement for RhoA and Cdc42 during cytokinesis in *Xenopus* embryos. *Curr. Biol*. **7**, 12-23.

Du, S. J., Purcell, S. M., Christian, J. L., McGrew, L. L. and Moon, R. T. (1995). Identification of distinct classes and functional domains of wnts through expression of wild-type and chimeric proteins in *Xenopus* embryos. *Mol. Cell Biol*. **15**, 2625-2634.

Eaton, S., Wepf, R. and Simons, K. (1996). Roles for Rac1 and Cdc42 in planar polarization and hair outgrowth in the wing of *Drosophila*. *J. Cell Biol*. **135**, 1277-1289.

Fan, M. J., Grüning, W., Walz, G. and Sokol, S. Y. (1998). Wnt signaling and transcriptional control of *Siamois* in *Xenopus* embryos. *Proc. Natl. Acad. Sci. USA* **95**, 5626-5631.

Gho, M. and Schweisguth, F. (1998). Frizzled signalling controls orientation

of asymmetric sense organ precursor cell divisions in *Drosophila*. *Nature* **293**, 178-181.

Hall, A. (1998). Rho GTPases and the actin cytoskeleton. *Science* **279**, 509-514.

Harland, R. M. (1991). In situ hybridization: an improved whole mount method for *Xenopus* embryos. In *Methods in Cell Biology* (ed. B. K. Kay and H. B. Peng), vol. **36**, pp. 685-695. San Diego: Academic Press.

Itoh, K., Jacob, J. and Sokol, S. Y. (1998). A role for Frizzled 8 in dorsal development. *Mech. Dev.* **74**, 145-157.

Keller, R., Shih, J. and Domingo, C. (1992). The patterning and functioning of protrusing activity during convergence and extension of the *Xenopus* organizer. *Development***1992 Supplement**, 81-91.

Kintner, C. R. and Brockes, J. P. (1984). Monoclonal antibodies identify blastemal cells derived from dedifferentiated muscle in newt limb regeneration. *Nature* **308**, 67-69.

Krasnow, R. E. and Adler, P. N. (1994). A single *frizzled* protein has a dual function in tissue polarity. *Development* **120**, 1883-1893.

Krasnow, R. E., Wong, L. L. and Adler, P. N. (1995). *dishevelled* is a component of the *frizzled* signaling pathway in *Drosophila*. *Development*. **121**, 4095-4102.

Krieg, P. A. and Melton, D. A. (1984). Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs. *Nucl. Acids Res*. **12**, 7057-7070.

Ku, M. and Melton, D. A. (1993). *Xwnt-11*: A maternally expressed *Xenopus wnt* gene. *Development* **119**, 1161-1173.

Lemaire, P. and Gurdon, J. B. (1994). A role for cytoplasmic determinants in mesoderm patterning: cell-autonomous activation of *goosecoid* and *Xwnt-8* along the dorsoventral axis of early *Xenopus* embryos. *Development* **120**, 1191-1199.

Lemaire, P., Garrett, N. and Gurdon, J. B. (1995). Expression cloning of *Siamois*, a *Xenopus* homeobox gene expressed in dorsal-vegetal cells of blastula and able to induce a complete secondary axis. *Cell* **81**, 85-94.

Lin, K. M., Wang, S. W., Julius, M. A., Kitajewski, J., Moos, Jr. M. and Luyten, F. P. (1997). The cysteine-rich frizzled domain of Frzb-1 is required and sufficient for modulation of Wnt signaling. *Proc. Natl. Acad. Sci. USA* **94**, 11196-11200.

McKendry, R., Hsu, S. C., Harland, R. M. and Grosschedl, R. (1997). LEF1/TCF proteins mediate wnt-inducible transcription from the *Xenopus* nodal-related 3 promoter. *Dev. Biol*. **192**, 420-431.

Medina, A., Reintsch, W. and Steinbeisser, H. (2000). *Xenopus frizzled 7* can act in canonical and non-canonical Wnt signaling pathways: implications in early patterning and morphogenesis. *Mech. Dev.* **92**, 227-237.

Miller, J. R., Hocking, A. M., Brown, J. D. and Moon, R. T. (1999). Mechanism and function of signal transduction by the Wnt/β-catenin and Wnt/Ca^{2+} pathways. *Oncogene* **18**, 7860-7872.

Moon, R. T. (1993). In pursuit of the functions of the *Wnt* family of developmental regulators: insights from *Xenopus laevis*. *BioEssays* **15**, 91-97.

Moon, R. T., Campbell, R. M., Christian, J. L., McGrew, L. L., Shih, J. and Fraser, S. (1993). *Xwnt-5A*: a maternal *Wnt* that affects morphogenetic movements after overexpression in embryos of *Xenopus laevis*. *Development* **119**, 97-111.

Moon, R. T., Brown, J. D. and Torres M. (1997). WNTs modulate cell fate and behavior during vertebrate development. *Trends Genet.* **13**, 157-162.

Moriguchi, T., Kawachi, K., Kamakura, S., Masuyama, N., Yamanaka, H., Matsumoto, K., Kikachi, A. and Nishida, E. (1999) Distinct domains of mouse dishevelled are responsible for the c-Jun N-terminal Kinase/Stress-activated protein kinase activation and the axis formation in vertebrates. *J. Biol. Chem*. **274**, 30957-30962.

Nieuwkoop, P. D. and Faber, J. (1967). *Normal Table of Xenopus laevis (Daudin)*. Amsterdam: North Holland.

Pannese, M., Polo, C., Andreazzoli, M., Vignali, R., Kablar, B., Barsacchi, G. and Boncinelli, E. (1995). The *Xenopus* homologue of Otx-2 is a maternal homeobox gene that demarcates and specifies anterior body regions. *Development* **121**, 707-720.

Penzel, R., Oschwald, R., Chen, Y. L., Tacke, L. and Grunz, H. (1997). Characterization and early embryonic expression of a neural specific transcription factor xSox3 in *Xenopus laevis*. *Int. J. Dev. Biol*. **41**, 667-678.

Rocheleau. C. E., Downs, W. D., Lin, R. Y., Wittmann, C., Bei, Y. X., Cha, Y. H., Ali, M., Priess, J. R. and Mello, C. C. (1997). Wnt signaling and an APC-related gene specify endoderm in early C. elegans embryos. *Cell* **90**, 707-716.

Rupp, R. A. W., Snider, L. and Weintraub, H. (1994). *Xenopus* embryos regulate the nuclear localization of XMyoD. *Genes Dev*. **8**, 1311-1323.

Sawa, H., Lobel, L. and Horvitz, H. R. (1996). The *Caenorhabditis elegans* gene *lin-17*, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the *Drosophila* Frizzled protein. *Genes Dev.* **10**, 2189-2197.

Sheldahl, L. C., Park, M., Malbon, C. C. and Moon, R. T. (1999). Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. *Curr. Biol*. **9**, 695-698.

Shi, D. L., Goisset, C. and Boucaut, J. C. (1998). Expression of *Xfz3*, a *Xenopus frizzled* family member, is restricted to the early nervous system. *Mech. Dev.* **70**, 35-47.

Shih, J. and Keller, R. (1992). Cell motility driving mediolateral intercalation in explants of *Xenopus laevis*. *Development* **116**, 901-914.

Slusarski, D. C., Yang-Snyder, J., Busa, W. B. and Moon, R. T. (1997a). Modulation of embryonic intracellular Ca^{2+} signaling by *Wnt-5A*. *Dev. Biol*. **182**, 114-120.

Slusarski, D. C., Corces, V. G. and Moon, R. T. (1997b). Interaction of Wnt and a Frizzled homologue triggers G-protein-linked phosphatidylinositol signalling. *Nature* **390**, 410-413.

Smith, J. C. and Watt, F. M. (1985). Biochemical specificity of *Xenopus* notochord. *Differentiation* **59**, 109-115.

Smith, J. C., Price, B. M. J., Green, J. B. A., Weigel, D. and Herrmann, B. G. (1991). Expression of a Xenopus homolog of *Brachyury (T)* is an immediate-early response to mesoderm induction. *Cell* **67**, 79-87.

Sokol, S. Y. (1996). Analysis of Dishevelled signalling pathways during *Xenopus* development. *Curr. Biol.* **6**, 1456-1467.

Strutt, D. I., Weber, U. and Mlodzik, M. (1997). The role of RhoA in tissue polarity and Frizzled signalling. *Nature* **387**, 292-295.

Sumanas, S., Strege, P., Heasman, J. and Ekker, S. C. (2000). The putative Wnt receptor *Xenopus* frizzled-7 functions upstream of β-catenin in vertebrate dorsoventral mesoderm patterning. *Development* **127**, 1981-1990.

Symes, K. and Smith, J. C. (1987). Gastrulation movements provide an early marker of mesoderm induction in *Xenopus laevis*. *Development* **101**, 339-349.

Tada, M. and Smith, J. C. (2000). *Xwnt-11*, a target of *Xenopus* Brachyury, regulates gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. *Development* **127**, 2227-2238.

Thorpe, C. J., Schlesinger, A., Clayton, J. and Bowerman, B. (1997). Wnt signaling polarizes an early C. elegans blastomere to distinguish endoderm from mesoderm. *Cell* **90**, 695-705.

Turner, D. L. and Weintraub, H. (1994). Expression of achaete-scute homolog 3 in *Xenopus* embryos converts ectodermal cells to neural fate. *Genes Dev*. **8**, 1434-1447.

Ungar, A. R., Kelly, G. M. and Moon, R. T. (1995). *Wnt4* affects morphogenesis when misexpressed in the zabrafish embryo. *Mech. Dev*. **52**, 153-164.

von Dassow, G., Schmidt, J. E. and Kimelman, D. (1993). Induction of the *Xenopus* organizer: expression and regulation of *Xnot*, a novel FGF and activin-regulated homeo box gene. *Genes Dev*. **7**, 355-366.

Vize, P. D., Hemmatie-Brivanlou, A., Harland, R. M. and Melton, D. A. (1991). Assays for gene function in developing *Xenopus* embryos. In *Methods in Cell Biology* (ed. B. K. Kay and H. B. Peng), vol. **36**, pp. 367-387. San Diego: Academic Press.

Wacker, S., Brodbeck, A., Lemaire, P., Niehrs, C. and Winklbauer, R. (1998). Patterns and control of cell motility in the *Xenopus* gastrula. *Development* **125**, 1931-1942.

Wang, Y. S., Macke, J. P., Abella, B. S., Andreasson, K., Worley, P., Gilbert, D. J., Copeland, N. G., Jenkins, N. A. and Nathans, J. (1996). A large family of putative transmembrane receptors homologous to the product of the *Drosophila* tissue polarity gene *frizzled*. *J. Biol. Chem.* **271**, 4468-4476.

Wheeler, G. N. and Hoppler, S. (1999). Two novel *Xenopus* frizzled genes expressed in developing heart and brain. *Mech. Dev*. **86**, 203-207.

Winklbauer, R. and Schürfeld, M. (1999). Vegetal rotation, a new gastrulation movement involved in the internalization of the mesoderm and endoderm in *Xenopus*. *Development* **126**, 3703-3713.

Wodarz, A. and Nusse, R. (1998). Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* **14**, 59-88.

Xu, Q. H., D'Amore, P. A. and Sokol, S. Y. (1998). Functional and biochemical interactions of Wnts with FrzA, a secreted Wnt antagonist. *Development* **125**, 4767-4776

Yang-Snyder, J., Miller, J. R., Brown, J. D., Lai, C. J. and Moon, R. T. (1996). A *frizzled* homolog functions in a vertebrate wnt signaling pathway. *Curr. Biol.* **6**, 1302-1306.

Zhang, J. J. and Carthew, R. W. (1998). Interactions between Wingless and Dfz2 during *Drosophila* wing development. *Development* **125**, 3075-3085.