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. Invulated 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 Wnts (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 morphogenic 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 Wnts 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 occurring 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 Wnt11. 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 Wnt11 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'-CGGCGGATCCATC-TCCGTGTC-3'; reverse, 5'-ATGGAATCTGACATGCGCGAAG-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 Weinstein, 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 Cdc42T17N and Cdc42G12V 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 BamHI and ClaI 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 disperse. 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 dorsal region of the involuting 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. 2F). 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 un.injected 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.

<table>
<thead>
<tr>
<th>RNA-injected (pg)</th>
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<th>Late phenotypes</th>
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<td></td>
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<tr>
<td>lacZ (400)</td>
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<tr>
<td>Xfz7 (400)</td>
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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.
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). 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 Xfz7-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.

**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

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

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

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

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*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.

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 Cdc42T17N, 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 Cdc42T17N with Xfz7 efficiently and reproducibly rescued explant elongation (Fig. 7C,D). This rescue seems to be specific for Cdc42T17N because mutant forms of RhoA and Rac did not rescue elongation of activin-treated explants expressing Xfz7 (data not shown). Furthermore, Cdc42T17N also efficiently rescued the inhibitory effect of Xwnt11 overexpression on convergent extension (Fig. 7E,F). Overexpression of Cdc42G12V, 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-Cdc42G12V 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 Cdc42G12V in a dose-dependent manner. An optimal rescue was obtained by coinjection of 200 pg Cdc42G12V 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...
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, Knot 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 Knot 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 occurring 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 coinmunoprecipitates 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 Cdc42T17N (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/b-catenin and planar polarity signalling (reviewed by Bourtos 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 Cdc42T17N, a dominant negative Cdc42 mutant (Drechsel et al., 1996). Conversely, the effect of extra-Xfz7 was rescued by Cdc42G12V, 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.

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