Dorsalizing and neuralizing properties of Xdsh, a maternally expressed Xenopus homolog of dishevelled

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SUMMARY

Signaling factors of the Wnt proto-oncogene family are implicated in dorsal axis formation during vertebrate development, but the molecular mechanism of this process is not known. Studies in Drosophila have indicated that the dishevelled gene product is required for wingless (Wnt1 homolog) signal transduction. We demonstrate that injection of mRNA encoding a Xenopus homolog of dishevelled (Xdsh) into prospective ventral mesodermal cells triggers a complete dorsal axis formation in Xenopus embryos. Lineage tracing experiments show that cells derived from the injected blastomere contribute to anterior and dorsal structures of the induced axis. In contrast to its effect on mesoderm, overexpression of Xdsh mRNA in prospective ectodermal cells triggers anterior neural tissue differentiation. These studies suggest that Wnt signal transduction pathway is conserved between Drosophila and vertebrates and point to a role for maternal Xdsh product in dorsal axis formation and in neural induction.

Key words: Xenopus, Wnt, dishevelled, dorsal axis formation, neuralizing activity

INTRODUCTION

An amphibian egg is laid with a clear animal-vegetal polarity, but its dorsoventral axis is not specified. Dorsoventral differences are specified quite early in Xenopus development as a result of a cortico-cytoplasmic rotation that occurs soon after fertilization. During this microtubule-mediated displacement of internal egg cytoplasm relative to the cell cortex (Gerhart et al., 1989; Elinson and Rowning, 1988), dorsal cytoplasm acquires an ability to trigger dorsal development upon microinjection into a ventral blastomere (Fujisue et al., 1993; Holowacz and Elinson, 1993). Two models may be proposed to explain dorsoventral patterning of mesoderm. According to the ‘permissive’ model of dorsoventral patterning, the axis-inducing activity is mediated by maternally encoded factor(s), called dorsal determinants or modifiers, which cause a local change in marginal zone cell competence to mesoderm-inducing signals produced by vegetal pole cells (Sokol et al., 1993; Holowacz and Elinson, 1993). Two models may be proposed to explain dorsoventral patterning of mesoderm. According to the ‘permissive’ model of dorsoventral patterning, the axis-inducing activity is mediated by maternally encoded factor(s), called dorsal determinants or modifiers, which cause a local change in marginal zone cell competence to mesoderm-inducing signals produced by vegetal pole cells (Sokol and Melton, 1991; Moon and Christian, 1992). As a result of this change, not only mesoderm is induced, but it becomes polarized (or regionalized) into future dorsal (notochord, muscle) and ventral (mesenchyme, kidney, blood) tissues. According to the ‘instructive’ model, multiple inducers or different levels of a single inducer directly specify formation of mesoderm with different dorsal or ventral character (Nieuwkoop, 1973; Dale and Slack, 1987b).

Whereas soluble peptide growth factors from the TGFβ and FGF families are thought to play a role in mesoderm induction (see Smith, 1993; Dawid, 1991, for reviews), two other classes of secreted polypeptides, Wnts, related to the int-1 (Wnt1) proto-oncogene product (Sokol et al., 1991; Smith and Harland, 1991), and noggin (Smith and Harland, 1992) have been shown to affect dorsoventral patterning of embryonic mesoderm. Although low levels of noggin mRNA are detected maternally (Smith and Harland, 1992), it is mainly expressed in the Spemann organizer region after the midblastula transition and the onset of zygotic transcription (Newport and Kirschner, 1982). Noggin has been shown to possess both neuralizing and dorsalizing activities (Smith et al., 1993; Lamb et al., 1993), suggesting that it mediates some of the Spemann organizer activities.

Different members of the Wnt family are expressed in specific regions of the embryos of various species and have been implicated in Drosophila segmentation, murine central nervous system development and in MMTV-induced mammary gland carcinogenesis (see Dickinson and McMahon, 1992; Nüssle and Varmus, 1992, for reviews). Several Wnt products have been shown to induce dorsal axis formation in Xenopus embryos (Moon et al., 1993; Klein and Melton, 1994). Since these Wnts are not expressed at the right time to perform this function during normal development (Moon et al., 1993), they were proposed to mimic yet unknown Wnt product(s) and to affect the same signal transduction pathway that operates in the embryo. Interestingly, a maternal Wnt, Xwnt11, has been identified which is capable of induction of a partial dorsal axis (Ku and Melton, 1993).
While the molecular mechanism by which a Wnt signal is transmitted in embryonic cells is unknown, genetic studies in Drosophila have implicated products of several segment polarity genes: wingless (wg, a homolog of Wnt1 gene), dishevelled (dsh) and armadillo (arm) in this pathway. Indeed, the phenotypes of these segment polarity mutants are almost identical as judged by similar cuticular defects, lack of segmental furrows, fusion of tracheal pits and a characteristic pattern of cell death (Perrimon, 1994). In cells responding to wg signaling, all three genes are required for the correct expression of the target gene engrailed (Perrimon, 1994). The dsh product is required for the stimulatory effect of wg on arm protein (Riggleman et al., 1990; Noordermeer et al., 1994), which is a homolog of mammalian plakoglobin and β-catenin (McCrea et al., 1991). In addition to these gene products, zeste-white 3, a homolog of mammalian glycogen synthase kinase (Woodgett, 1990), has been shown to suppress the wg effect on arm and to function in the same pathway downstream of dsh (Siegfried et al., 1992, 1994).

The phenotype of flies overexpressing wg is completely suppressed in dsh mutants (Noordermeer et al., 1994; Siegfried et al., 1994), indicating that the dsh product is required for wg to function. Clonal analysis of dsh mutations during either embryonic or imaginal development has shown that cells with a mutant dsh product display the mutant phenotype and cannot be rescued by the dsh product supplied by neighboring wild-type cells (Klingensmith et al., 1994; Theisen et al., 1994). Thus, dsh functions cell autonomously and is likely to participate in the reception or interpretation of the wg signal by the responding cells. Taken together, these observations suggest that the dsh protein is a critical regulatory element of the Wnt signal transduction pathway.

To test for the possible involvement of dishevelled in dorsoventral patterning in vertebrate embryos, we isolated a Xenopus homolog of dsh (Xdsh). The results of this study, which evaluates the role of Xdsh in Xenopus development, suggest that dorsal body axis is determined through the Wnt signaling pathway and that this pathway is evolutionarily conserved between Drosophila and vertebrates.

**MATERIALS AND METHODS**

**Eggs and embryos**

Eggs were obtained by injecting Xenopus laevis females with 800 units of human chorionic gonadotropin. Fertilization and embryo culture were done in 0.1x MMR (1x MMR =100 mM NaCl, 2 mM KCl, 1 mM MgSO4, 2 mM CaCl2, 5 mM Hepes (pH 7.6), 0.1 mM EDTA) as described (Newport and Kirschner, 1982). Staging was according to Nieuwkoop and Faber (1967). Ultraviolet light irradiation was performed similar to Scharf and Gerhart (1980). Embryos in a plastic chamber with a Saran Wrap bottom were placed on the surface of a UV lamp (UVG-11, 254 nm) and were irradiated for 1 minute within 30-35 minutes after fertilization. The optimal time of exposure to UV light was determined in preliminary experiments. Only experiments on embryos with an average dorsoanterior index (DAI) of less than 1 (Kao and Elinson, 1988) were taken into account.

**Cloning, in vitro transcription and microinjection of embryos with RNA**

The 0.7 kb XhoI-PstI fragment of the Drosophila dishevelled cDNA (Klingensmith et al., 1994) was labeled by random hexanucleotide priming (Sambrook et al., 1989). The labeled fragment was used to probe a Xenopus oocyte λgt10 cDNA library (Rebagliati et al., 1985).
Properties of Xenopus dishevelled under low stringency conditions as described (Sambrook et al., 1989). A 3.3 kb insert, containing full length Xdsh cDNA, was subcloned into the EcoRI site of the pBluescript-SK vector (Stratagene), and both DNA strands were sequenced. Alignment of the deduced Xdsh amino acid sequence with the sequences of the Drosophila and mouse dsh proteins was carried out using the PILEUP program of the Computer Genetics Group (Madison, WI).

To overexpress the Xdsh product in embryos, the 3.3 kb Xdsh cDNA fragment was subcloned into the EcoRI site of the pSP64R1 vector, a modified version of pSP64T (Vize et al., 1991), which contains several convenient cloning sites and allows in vitro synthesis of efficiently translated mRNAs. A control out-of-frame ∆Xdsh construct was made by digesting the plasmid containing Xdsh cDNA with ApaI, followed by filling-in protruding ends with Klenow.

Fig. 2. Xdsh transcripts are present maternally and are equally distributed in different regions of the early blastula. Total RNA isolated from embryos at different developmental stages or from embryonic explants was analyzed by Northern blotting with specific antisense RNA probes. (A) Expression of Xdsh during embryogenesis: E, fertilized eggs; B, stage 7 blastulae; G, stage 11 gastrulae; N, stage 15 neurulae and T, tailbud embryos. (B) Spatial distribution of the Xdsh transcripts in stage 7 blastulae. Explants were isolated from A, animal; M, marginal; V, vegetal; D, dorsal; V’, ventral regions; T, RNA prepared from whole embryos. Two embryo equivalents of total RNA were loaded per each lane. FN, Xwnt8 and Vg1-specific probes were used as controls. Fibronectin RNA (FN) is a control for loading. Xwnt8 transcripts are known to appear only after the midblastula transition (Christian et al., 1991). Vg1 RNA is a vegetally localized maternal mRNA (Rebagliati et al., 1985).

Fig. 3. The effect of Xdsh mRNA depends on the site of injection. A single prospective dorsal (A) or ventral (B,D) vegetal blastomere of cleavage-stage embryos (8-16 cells) was injected with 0.4 ng of Xdsh mRNA. Phenotypes of the injected neurulae (A,B) and of tadpoles at stages 40-42 (C,D) are presented. Axis duplications are clearly visible in embryos injected with Xdsh RNA in a ventral blastomere (B,D). Embryos, injected ventrally with 0.4 ng of a control ∆Xdsh mRNA (C), are indistinguishable from normal tadpoles or from the tadpoles, injected dorsally with Xdsh mRNA (as in A). Note that three embryos in D have completely duplicated body axes including most anterior and posterior structures, whereas in one embryo both dorsal axes oppose each other and posterior development is inhibited.
enzyme and re-ligating the construct. A plasmid encoding β-galactosidase was a gift of R. Harland.

Capped synthetic RNAs were generated as described (Krieg and Melton, 1984) by in vitro transcription of different plasmids using SP6 RNA polymerase. Embryos, incubated in 3% Ficoll, 0.5x MMR were injected with 10 nl of RNA solution in distilled water at 8- to 16-cell stage into a single blastomere. After 1-2 hours of incubation, the medium was changed to 0.1x MMR with 50 μg/ml of gentamicin for long-term culture. Death rate for the injected embryos was usually below 5%. The prospective dorsal and ventral sides were determined by pigmentation differences in the early embryo (Nieuwkoop and Faber, 1967). Prospective ventral blastomeres are more heavily pigmented than their dorsal counterparts. The accuracy of this determination was tested in each experiment by allowing a group of control embryos to develop to determine whether the pigmentation differences correctly predicted the position of dorsal blastopore lip. The usual error of such determinations was 5-7%.

**Lineage tracing and histology**

Cleavage-stage embryos (8- to 32-cell stages) were injected with 10 nl of a solution containing 0.2-0.4 ng of Xdsh mRNA and 0.2 ng of β-gal RNA in water. After two days of culturing in 0.1x MMR, embryos were fixed for an additional 2 hours with 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 10 mM EDTA, 0.5% SDS and 200 μg/ml proteinase K, and incubated for 1 hour at 37°C. Homogenates were extracted twice with phenol/chloroform (1:1) and once with chloroform, and RNA was precipitated by ethanol.

**RESULTS**

**Identification of a Xenopus homolog of dishevelled**

To isolate a Xenopus homolog of dsh, a 0.7 kb Psfl-XhoI fragment of the Drosophila dsh cDNA (Klingensmith et al., 1994) was used to probe a Xenopus oocyte cDNA Agt10 library at low stringency conditions. This screen resulted in isolation of a phage containing a 2.5 kb insert, strongly hybridizing to the Drosophila dsh probe. Partial sequencing of the clone revealed significant similarity of its primary structure with the sequence of the deduced Drosophila dsh protein.

The 5'-terminal 0.6 kb fragment of the cloned partial length cDNA was used to rescreen the same cDNA library. As a result of this screen, a 3.3 kb Xdsh cDNA was isolated. The cDNA has been sequenced revealing an open reading frame of 2208 amino acids. The predicted overall amino acid sequence of the Xdsh protein is 46% identical to the sequence of the Drosophila dsh protein (Klingensmith et al., 1994; Theisen et al., 1994) and 60% identical to the product of the recently isolated mouse dishevelled cDNA (Sussman et al., 1994). The structural elements of Xdsh include two proline-rich stretches and the discs large homology region (DHR), a motif found in the product of the recently isolated mouse discs large and, in several proteins, associated with cytoskeleton and with tight junctions (Bryant et al., 1993; Anderson et al., 1993) (Fig. 1).

The first 20 amino acids of the deduced N terminus of the...
Xdsh protein, starting with the first available methionine, are virtually identical (with two conservative amino acid changes) to the N terminus of mouse dishevelled product (Sussman et al., 1994), suggesting that the first AUG codon is the true translation start. The A--AUGG sequence surrounding the AUG codon is a good match to Kozak consensus sequences for translation initiation. According to Northern analysis, the endogenous Xdsh mRNA is approximately 3.5 kb. Together, these data indicate that the cloned 3.3 kb Xdsh cDNA is likely to encode the full-length Xdsh protein.

**Xdsh RNA is a ubiquitous maternal message**

If Xdsh protein is necessary for Wnt signal transduction, it should be expressed in the blastomeres that can respond to Wnts and at the time when cells are competent to respond. Injected Xwnt8 mRNA has an effect as early as at the 32- to 64-cell stage (Olson et al., 1991), and it seems to affect any ventral or lateral blastomere within the marginal zone (Sokol et al., 1991). Thus, Xdsh is expected to be present maternally.

Northern analysis was used to study the expression pattern of Xdsh mRNA during Xenopus embryonic development (Fig. 2). A single mRNA species (approximately 3.5 kb) was detected throughout different developmental stages, being most abundant in eggs.

To determine which regions of the embryo express Xdsh mRNA, blastulae (stage 7-7.5) were dissected manually into animal, marginal and vegetal or into dorsal and ventral parts. It is fairly easy to distinguish different embryonic regions based on the difference in pigmentation (Nieuwkoop and Faber, 1967). A probe specific to the vegetally localized Vg1 RNA was used to control dissections along the animal-vegetal axis, while dissections along the dorsal-ventral axis were controlled by culturing five explants until the gastrula stages (see Materials and Methods). Total RNA from the dissected pieces was analyzed on Northern blots with different antisense RNA probes. While a control probe detected Vg1 mRNA mainly in the vegetal explants, Xdsh mRNA seems equally distributed in both animal-vegetal and dorsal-ventral directions (Fig. 2B).

**Microinjection of Xdsh mRNA leads to induction of a complete dorsal axis**

To determine whether the Xdsh product is sufficient to mimic the ability of Wnt1 mRNA to cause duplication of the body axis (McMahon and Moon, 1989), the full length Xdsh CDNA was subcloned into the pSP64R1 vector. In vitro synthesized Xdsh mRNA was microinjected into single ventral blastomeres of 8- to 16-cell Xenopus embryos. In several independent experiments, microinjection of Xdsh mRNA leads to induction of a complete dorsal axis.

**Table 2. Injection of Xdsh mRNA induces dorsal axes in UV-treated embryos**

<table>
<thead>
<tr>
<th>RNA injected</th>
<th>Total number of injected embryos</th>
<th>Ventralized phenotype</th>
<th>Partial rescue of dorsal axis</th>
<th>Complete rescue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xdsh</td>
<td>57</td>
<td>6 (11%)</td>
<td>23 (40%)</td>
<td>28 (49%)</td>
</tr>
<tr>
<td>β-gal</td>
<td>53</td>
<td>45 (90%)</td>
<td>7 (13%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Embryos were treated with UV light and injected at the 4- to 8-cell stage into a single blastomere with 0.4 ng of mRNA and left to develop to the equivalent of stages 40-45. Embryonic phenotypes were scored morphologically into three categories according to the DAI scale of Kao and Elinson (1988): ventralized phenotype (DAI 0-1), partial rescue (DAI 2-3) and complete rescue (DAI 4-5). Data from two independent experiments are presented.
experiments, Xdsh mRNA triggered the formation of a secondary dorsal axis (Fig. 3B,D) similar to the effects of Wnt1 or Xwnt8 mRNAs (Sokol et al., 1991). Xdsh mRNA injections into dorsal blastomeres at the same stage did not alter normal development (Fig. 3A). Embryos injected with a control Xdsh mRNA containing a short deletion that disrupts the open reading frame, developed normally (Fig. 3C), suggesting that the intact Xdsh protein is necessary for the observed effect. The induced secondary axes frequently (in more than half of the injected embryos) contained a full set of dorsal structures including the most anterior structures (eyes and cement glands) (Table 1). Histological examination of the injected embryos revealed two properly organized axes with notochords, neural tubes and somites (Fig. 4). Consistent with our morphological observations, Xdsh mRNA injections activated the expression of goosecoid mRNA, a dorsal region-specific marker (Cho et al., 1991), and reduced the level of Xwnt8 mRNA, a ventrolateral marker (Christian et al., 1991), in stage 10.5 gastrulae (data not shown).

The effect of Xdsh mRNA was dose dependent: 0.5-1 ng of the mRNA was the optimal dose, 50-100 pg induced partial secondary axes, and injection of more than 2 ng resulted in a shortened tail and spinal cord with the head being almost normal in size. Thus, phenotypes of embryos injected with the high dose (2-4 ng) of Xdsh mRNA were somewhat different from the radially symmetric embryos, dorsalized by the high doses of Xwnt8 mRNA (Christian et al., 1991) or by lithium chloride (Kao and Elinson, 1988). This result may be related to the inability of the Xdsh mRNA and protein to spread from one cell to another, in contrast to diffusion of lithium chloride or transmission of Wnt proteins.

These observations show that overexpression of Xdsh mRNA alone is sufficient to trigger dorsal axis formation, and that Xdsh may, thus, transduce an endogenous signal responsible for determination of dorsal mesoderm.

**Injection of Xdsh mRNA rescues embryos ventralized by UV light**

Although ventral injections of Xdsh mRNA suggest that its effect does not depend on the dorsally located endogenous Spemann organizer, these observations do not exclude the possibility that the organizer synergizes with the injected mRNA. Since embryos treated with ultraviolet light (UV) are deficient in the Spemann organizer activity and fail to develop dorsal and anterior structures (Gerhart et al., 1989), they represent a useful system to study potential dorsal determinants. Several gene products including noggin, a chimeric BMP-Vg1 protein (Thomsen and Melton, 1993; Dale et al., 1993) and certain Wnt products (Moon et al., 1993) are known to restore normal development in embryos ventralized by UV light.

To test whether Xdsh is able to trigger dorsal development in the absence of the Spemann organizer, embryos were irradiated with UV light after fertilization and injected with in
Properties of *Xenopus dishevelled* vitro synthesized Xdsh mRNA in a single vegetal blastomere of the 8- to 16-cell-stage embryos. While UV-treated embryos injected with water or with an unrelated RNA (β-gal RNA) did not have visible dorsal structures, in embryos that received Xdsh mRNA, the dorsal axis was rescued (Fig. 5; Table 2). In four independent experiments, we consistently observed a complete rescue of axial development of the ventralized embryos from dorsoanterior index (DAI) of less than 1 to DAI 5 (Kao and Elinson, 1988).

Together, these findings demonstrate that the Xdsh mRNA
Cells overexpressing Xdsh mRNA directly contribute to the most anterior and dorsal axial structures

To determine which tissues in the induced axes are formed by the progeny of blastomeres, injected with Xdsh mRNA, lineage tracing was carried out by coinjecting Xdsh mRNA and β-gal mRNAs into a single ventrovegetal blastomere of 8- to 16-cell embryos (Dale and Slack, 1987a). When the injected embryos reached tadpole stages (stage 40-42), they were fixed and stained for β-gal activity. In embryos injected with β-gal RNA alone (total number 28), staining was found in ventrolateral tissues (Fig. 6A), consistent with the normal fate of the injected cells (Moody, 1987; Dale and Slack, 1987a). In contrast, all embryos injected with β-gal and Xdsh mRNAs (32 out of 32) were stained in dorsal and anterior tissues, e. g. in notochord, head and branchial mesenchyme and in pharyngeal endoderm (Fig. 6A). Only one out of two axes in each embryo was stained. These findings suggest that Xdsh mRNA functions cell autonomously: cells that received Xdsh mRNA change their ventral fate and, instead, may form an ectopic organizing center.

Staining of anterior and dorsal structures was also observed in the rescue experiments, where β-gal RNA was coinjected with Xdsh RNA into embryos ventralized by UV irradiation (Fig. 6B,C). These results are similar to what was observed in studies with Xwnt8 RNA injections (Sokol et al., 1991; Smith and Harland, 1991), in which the majority of injected blastomeres formed a Spemann organizer and only a small percentage of them contributed exclusively to endoderm, thus, mimicking the vegetal organizing center (Gimlich and Gerhart, 1984). Interestingly, there was a correlation between the degree of rescue and the fate of the injected cells. While in completely rescued embryos (0.4 ng Xdsh RNA per embryo; n=37), the injected cells were found exclusively in the head mesenchyme/pharyngeal endoderm region (Fig. 6B), in partially rescued embryos (0.05 ng of Xdsh RNA per embryo; n=28), cells injected with Xdsh mRNA populated mostly notochord and anterior mesoderm (Fig. 6C).

To extend lineage tracing analysis to the 32-cell-stage embryos, Xdsh mRNA (0.5 ng) was injected into C4 or D4 blastomere (according to nomenclature of Dale and Slack, 1987a). Progeny of D4 ventral blastomere injected with the same dose of Xdsh mRNA contributed to head mesoderm and anterior endoderm (Fig. 6E), while injected C4-derived cells were found mainly in the axial mesoderm (Fig. 6F). Thus, fates of injected cells depend on the site of injection, which is consistent with the idea that Xdsh dorsalizes prospective ventral mesoderm creating a new Spemann organizer on the ventral side. Solely endodermal staining was not observed in any of the injected embryos (n=35), arguing that the effects of Xdsh mRNA on the organizer formation are cell autonomous. Although Xdsh mRNA clearly causes changes in cell fate and, therefore, affects cell behavior during gastrulation, we cannot exclude the possibility that Xdsh directly influences cell migration, as was proposed for goosecoid (Niehrs et al., 1993).

Neuralizing activity of Xdsh in presumptive ectodermal cells

Xdsh mRNA may influence dorsal axis formation either by inducing mesoderm de novo (similar to members of TGFβ and FGF families) or by altering polarity of mesodermal cells, similar to the competence modifiers, such as some Wnts and noggin.

To discriminate between these two possibilities, differentiation of animal pole cells overexpressing Xdsh RNA was studied. At later stages, cultured animal caps formed prominent cement glands which are normally induced during neural induction (data not shown). Subsequent analysis revealed activation of XA-1, an anterior ectodermal marker (Sive et al., 1989), and Otx2, a forebrain-specific marker (E. Boncinelli, personal communication, also called OtxA, Lamb et al., 1993), but not muscle-specific actin transcripts (Mohun et al., 1984) (Fig. 7A). Whereas NCAM, a pan-neural marker (Kintner and Melton, 1987) is only marginally visible in Fig. 7, it was well induced in other experiments (data not shown). Northern analysis of mesoderm-specific gene expression at the midgastrula stage failed to detect significant amounts of mRNAs for Xbra, Xwnt8 and goosecoid, early mesoderm-specific markers (Fig. 7B; Smith, 1993). These findings suggest that Xdsh mRNA can induce neural tissue formation directly, in the absence of mesoderm.

Taken together, these observations indicate that the Xdsh properties are very similar to the effects of noggin, a factor possessing both dorsalizing and neuralizing activities (Smith et al.,
antisense oligonucleotides were reported to affect phatase). Thus, it is conceivable that DHR is involved in Maekawa et al., 1994). Moreover, some of these proteins have junctions and/or with the cytoskeleton (Anderson et al., 1993; Smith, 1993; Klein and Melton, 1994) directly induce dorsal mesoderm in animal caps. Dorsal modifiers, in contrast, such as several Wnts and noggin, do not induce mesoderm on their own, but synergize with the endogenous mesoderm inducers and change the character of mesoderm from ventral to dorsal (Christian et al., 1992; Sokol and Melton, 1992). Dorsal modifiers trigger mesoderm formation in the animal caps only when animal caps are isolated from the embryo after stage 9 (Sokol, 1993; Lamb et al., 1993). Xdsh does not induce mesoderm in blastula animal caps, but expression of muscle-specific actin is activated if animal caps injected with Xdsh are isolated at stage 10 (data not shown). Thus, axis-inducing activity of Xdsh is very similar to the modifying effects of Wnts or noggin. It is important to point out, that Xdsh transcripts are mostly maternal, whereas noggin expression peaks after mid-blastula transition in the Spemann organizer, and the known Wnts that are capable of inducing dorsal axis (Moon et al., 1993) are also expressed zygotically. Studies of interactions between different dorsal modifiers may lead to identification of molecular pathways of vertebrate dorsal axis determination.

Neuralization of ectodermal cells overexpressing Xdsh mRNA indicates that Xdsh may have a second important function connected with neural tissue formation. Dorsalizing and neuralizing activities of Xdsh are similar to those of noggin (Lamb et al., 1993). Experiments are in progress to establish potential connections between Xdsh and other neuralizing factors, including noggin, follistatin (Hemmatti-Brivanlou et al., 1994), vertebrate hedgehog (Roelink et al., 1994; Echelard et al., 1993) and protein kinase C (Otte et al., 1988).

The requirement for wingless in Drosophila segmentation, in the imaginal discs and at the wing margin (Perrimon, 1994) suggests that both Wnt and dsh homologs may play multiple roles in vertebrate morphogenesis as well. Since several Wnts have been implicated in CNS development (Dickinson and McMahon, 1992; Nusse and Varmus, 1992), the neuralizing activity of Xdsh is consistent with Xdsh playing a role in the transmission of Wnt signals during CNS patterning. Alternatively, the maternal Xdsh protein could be directly activated by the cortical rotation on the prospective dorsal side of the embryo and may participate in modification of the cell responses both to mesoderm induction and to neural induction. Experiments aimed at inactivation of the Xdsh function should clarify its role in Wnt signal transduction and in embryogenesis.

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Properties of Xenopus dishevelled


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