INTRODUCTION
A fundamental question in vertebrate development concerns the formation of the major body axes. In *Xenopus*, the dorsal-ventral axis is characterized by muscle, notochord and neural tube (dorsal tissues), mesenchyme and blood (ventral tissues). Before fertilization, the radially symmetric egg bears no indication of where the future dorsal-ventral axis will be. Shortly after fertilization, a cytoplasmic rearrangement occurs that is crucial for the establishment of dorsal axial structures (reviewed in Gerhart et al., 1989). Treatments that prevent the cytoplasmic rearrangement, such as UV irradiation of the vegetal pole, also prevent the formation of the dorsal-most tissues. Thus, UV-ventralized embryos do not develop a complete dorsal-ventral nor anterior-posterior axis and remain radially symmetric.

Not all the molecules responsible for the inductions necessary to develop a complete dorsal-ventral axis have yet been identified. Not only is mesoderm induced in the marginal zone of the embryo, but it is also patterned such that dorsal-type mesoderm is formed on one side of the embryo, and ventral-type mesoderm on the other side. It is not clear if induction and patterning of mesoderm involve different molecular mechanisms or if the same molecules can induce and pattern mesoderm simultaneously (Ruiz i Altaba and Melton, 1989; Green and Smith, 1990). In addition, experiments using animal caps induced with either activin or FGF indicate that the type of induction obtained can be modified by the presence of other secreted molecules. For example, FGF inductions can be modified by Xwnt-8 and activin inductions can be modified by Xwnt-8 or BMP-4 (Christian et al., 1992; Dale et al., 1992; Jones et al., 1992; Sokol and Melton, 1992).

Recent experiments highlight the potential involvement of several different types of secreted molecules in axis formation: activin, which is a member of the transforming growth factor ß (TGF-β) family, FGF, noggin and wnts (see reviews Melton, 1991; Jessell and Melton, 1992; Kimelman et al., 1992; McMahon, 1992; Nusse and Varmus, 1992; Sive, 1993). The roles of activin and FGF in the early embryo have been tested directly by injecting synthetic mRNA for truncated, or dominant negative activin and FGF receptors. Injection of a dominant negative activin receptor into an early embryo can block the formation of mesoderm and dorsal axial structures (Hemmati-Brivanlou and Melton, 1992). Injection with a dominant negative FGF receptor results in decreased muscle actin expression and posterior defects (Amaya et al., 1991). These latter injected embryos still have neural tissue, some muscle and notochord, indicating that not all mesoderm is eliminated. Similar ‘dominant negative’ experiments have not yet been tried for noggin and wnts, because the receptors for these ligands are unknown. However, injection experiments with synthetic mRNA indicate that noggin and wnts can cause the formation of complete axes (McMahon and Moon, 1989; Smith and Harland, 1991; Sokol et al., 1991; Chakrabati et al., 1992; Smith and Harland, 1992).

The *wnt* gene family encodes glycosylated, cysteine-rich proteins that are involved in the regulation of cell growth and differentiation. One member of this family, *Xwnt-11*, has been shown to be important for dorsal-ventral axis formation in *Xenopus* embryos.

**Xwnt-11: a maternally expressed *Xenopus* wnt gene**

Min Ku* and Douglas A. Melton

Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Ave., Cambridge, MA 02138, USA

*Author for correspondence

SUMMARY

We have isolated and characterized a novel *Xenopus wnt* gene, *Xwnt-11*, whose expression pattern and overexpression phenotype suggest that it may be important for dorsal-ventral axis formation. *Xwnt-11* mRNA is present during oogenesis and embryonic development through swimming tadpole stages. *Xwnt-11* mRNA is ubiquitous in early oocytes and is localized during mid-oogenesis. By late oocyte stages, *Xwnt-11* mRNA is localized to the vegetal cortex, with some mRNA in the vegetal cytoplasm. After egg maturation, *Xwnt-11* mRNA is released from the vegetal cortex and is found in the vegetal cytoplasm. This early pattern of *Xwnt-11* mRNA localization is similar to another vegetally localized maternal mRNA, Vg1 (D. A. Melton (1987) *Nature* 328, 80-82). In the late blastula, *Xwnt-11* mRNA is found at high levels in the dorsal marginal zone. As gastrulation proceeds, *Xwnt-11* mRNA appears in the lateral and ventral marginal zone and, during tadpole stages, it is found in the somites and first branchial arch. Injection of *Xwnt-11* mRNA into UV-ventralized embryos can substantially rescue the UV defect by inducing the formation of dorsal tissues. The rescued embryos develop somitic muscle and neural tube; however, they lack notochord and anterior head structures.

Key words: axis formation, mesoderm induction, *Xenopus* embryogenesis, *Xwnt* genes, localized mRNAs
secreted proteins that are probably involved in short-range cell-cell communication (reviewed in Nusse and Varmus, 1992). The name ‘wnt’ is an amalgam of wingless and int, two of the first known members of this gene family. Mouse wnt-1 (int-1) was first discovered in mammary gland tumors as a locus that is transcriptionally activated upon integration of mouse mammary tumor virus DNA (Nusse et al., 1984). The Drosophila segment polarity gene wingless is a homolog of the mouse wnt-1 gene (Rijsewijk et al., 1987).

Wnt proteins have hydrophobic N-terminal signal peptides, one or more sites for N-linked glycosylation and most wnt proteins range in size from 350-380 amino acids, although recently new Drosophila wnt genes have been identified whose protein products are predicted to be more than 100×10^3 M_r (Eisenberg et al., 1992; Russell et al., 1992). Wnt proteins also have up to 24 conserved cysteine residues in nearly parallel positions. Overexpression of wnt-1 in tissue culture experiments indicates that most of the protein is secreted but is bound by the extracellular matrix instead of freely diffusing into the conditioned medium (Papkoff et al., 1987; Bradley and Brown, 1990; Papkoff and Schryver, 1990). Antibody staining in Drosophila also shows that wg protein is found within a few cell diameters of wg-expressing cells (van den Heuvel et al., 1989).

There is evidence that wnt genes can play a crucial role in cell fate determination. Transgenic mice that do not express wnt-1 protein lack extensive regions of the cerebellum and midbrain (McMahon and Bradley, 1990; Thomas and Capecchi, 1990; Thomas et al., 1991; McMahon et al., 1992). In Drosophila, wingless protein is important for embryonic segmentation, for Malpighian tubule formation in the larva, and for proper leg and wing formation in the adult (Lane, 1967; Sharma, 1973; Nusslein-Volhard and Wieschaus, 1980; Baker, 1988; Skaer and Martinez Arias, 1992; Struhl and Basler, 1993). Drosophila genetics has yielded clues about other genes involved in the wg pathway, such as armadillo and zeste-white 3, but a wnt receptor has not yet been identified (Peifer et al., 1991; Siegfried et al., 1992). Detailed biochemical characterization of wnt proteins and the wnt signal transduction pathway has been hampered by the inability to express soluble, biologically active forms of wnt proteins (Papkoff et al., 1987; Papkoff, 1989; Bradley and Brown, 1990; Papkoff and Schryver, 1990). It is known, however, that injection of synthetic wnt mRNA into Xenopus embryos can increase gap junction permeability (Olson et al., 1991; Olson and Moon, 1992).

Interest in wnt genes and Xenopus development stems from experiments in which the injection of mouse wnt-1 mRNA into the ventral side of a normal Xenopus embryo caused the formation of a complete second axis (McMahon and Moon, 1989). A related member of this family called Xwnt-8 is also a potent inducer of the dorsal axis and it can completely rescue UV-ventralized embryos (Sokol et al., 1991; Smith and Harland, 1991). These experiments, while dramatic, raise new questions because the expression pattern of these genes is inconsistent with their possible action during early axis formation. Xwnt-8 is not expressed until late blastula or early gastrula, which is several hours after mesoderm has been induced. Moreover, Xwnt-8 mRNA is found on the ventral side of the embryo (Christian et al., 1991), exactly opposite to the expected localization if its function is to induce dorsal axial development. Xwnt-1 is not expressed until the neurula stage, and therefore cannot be involved in the establishment of the axis (Noordermeer et al., 1989). Recently another gene, Xwnt-3A, has been shown to cause axial duplication upon injection of its mRNA, but it is also not normally expressed until the neurula stage, well after mesoderm induction and patterning have taken place (Wolda et al., 1993). There is no information yet about the distribution of Xwnt proteins, and the possibility remains that Xwnt proteins could be deposited in growing oocytes, as has been suggested for activin (Asashima et al., 1991; Dohrmann et al., 1993).

A simple explanation for the potency of injected Xwnt-1 and Xwnt-8 mRNA is that they are able to cross react with a wnt receptor, whose normal function is to transmit the signal encoded by a maternally expressed wnt gene. We have therefore undertaken to clone maternally expressed wnt genes, in order to test the hypothesis that a wnt molecule is involved in dorsal-ventral axis formation.

We report here the identification of Xwnt-11, a new member of the wnt gene family. Xwnt-11 mRNA is localized vegetally during oogenesis; by late oocyte stage V-VI, it is associated with the vegetal cortex, as well as in patches in the vegetal cytoplasm. The unfertilized egg and early cleavage stage embryos still contain vegetally localized Xwnt-11 mRNA, but it is now no longer cortical, and is instead diffuse throughout the vegetal cytoplasm. After zygotic transcription starts at the midblastula transition (MBT), Xwnt-11 mRNA is present in the marginal zone, first on the dorsal side and then laterally and ventrally as gastrulation progresses. During early tadpole stages, Xwnt-11 is also expressed in somitic muscle and in the first branchial arch. The injection of synthetic Xwnt-11 mRNA can significantly rescue UV-ventralized embryos by inducing the formation of somitic muscle and neural tube. However, rescued embryos do not develop notochord or anterior head structures. As far as we know, this is the first example of a maternally expressed Xwnt gene with a localized mRNA, and for which there is evidence from injection experiments that it could be important for dorsal-ventral axis formation.

MATERIALS AND METHODS

PCR

Degenerate oligonucleotide primers were synthesized that correspond to conserved peptide sequences among most mouse and Xenopus wnt genes. The sense-strand primer included BamHI and NdeI sites at the 5′ end, encoding the peptide sequence CKCHG. The sequence is:

5′ GGGGATCCATATG(TC)AA(AG)(TG)(TC)CA(CT)GG 3′

The antisense-strand primer included EcoRI and XbaI sites, encoding the peptide sequence FHWCC. The sequence is:

5′ GGGATCTCTAGA(CA)(CA)(ACCA)(AG)(TG)(AG)AA 3′

PCR amplification was performed on 0.5 µg of DNA from a Xenopus oocyte cDNA library. The 25 µl reactions consisted of 50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.1% Triton X-100, 1.5 mM MgCl_2, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 1.0 µg of
each primer and 1.5 U of Taq DNA Polymerase. The reactions were cycled 30 times with the cycle profile consisting of 1 minute each at 93°C, 60°C, and 72°C. 1 µl of this amplified product was then cycled again for a further 30 cycles. The reaction was then extracted with phenol-chloroform, ethanol precipitated and resuspended in TE. The DNA was then run out on a 1% agarose gel; the expected PCR fragment of approximately 400 bp was excised and purified. The purified DNA was then digested with BamHI and EcoRI, and was subcloned into the vector pGEM-7Zf(+). The PCR fragments were then sequenced by the dideoxy termination method for sequencing (Sanger et al., 1977) using Sequenase (US Biochemical Company).

**Isolation and sequencing of Xwnt-11 cDNA**

A Agt10 Xenopus oocyte cDNA library was screened using random primed DNA probes made from the BamHI-EcoRI Xwnt-11 PCR fragment. Hybridization was done overnight at 42°C in 50% formamide, 5x SSC, 0.1% SDS and 100 µg/ml denatured salmon sperm DNA. Approximately 8×10⁶ plaques were screened and 16 positive plaques were identified on duplicate filters. Five of the initial positive plaques were purified, and four different insert sizes were found, ranging from approximately 1 to 2 kb. The inserts from four different lambda clones were subcloned into pGEM-7Zf(+). Partial sequence confirmed that all four clones contained Xwnt-11 sequence. The complete sequence on both strands of the longest insert was determined by the dideoxy termination method, using specific oligos as sequencing primers. Dideoxy sequencing was also done on Xwnt-11 templates prepared by exonuclease III digestion (Promega).

**Northern blots**

Total RNA was extracted from staged whole oocytes and embryos, or from dissected pieces, as described in Krieg and Melton, 1987. Oocytes and embryos were staged according to Dumont (1971) and Nieuwkoop and Faber (1967). RNA was run on a 1% agarose gel containing formaldehyde, blotted onto nylon membranes and hybridized with an antisense RNA probe in 50% formamide, 5x SSPE, 5% SDS, and 100 µg/ml denatured salmon sperm DNA. The entire EcoRI fragment of Xwnt-11 cDNA was used as a probe. Hybridization was done overnight at 60°C and the blots were washed the next day in 0.1x SSPE, 0.1% SDS at 65-75°C two times for 1 hour each.

**UV irradiation**

Eggs were dejellied, then irradiated with short-wave UV light for 75 seconds at 30 minutes postfertilization. The eggs were irradiated by placing them on a quartz slide, which was directly on top of a hand-held 254 nm short-wave UV lamp (UVP, Inc., Model UVG-11). Embryos were assigned a value on the dorsoanterior index (DAI) (Kao and Elinson, 1988). A normal embryo has a DAI value of 5, and the extreme UV phenotype is assigned a value of 0. These values are opposite to those used for the index of axial deficiency (IAD) (Scharf and Gerhart 1983). The DAI grades are:

0. No somites present; trace of tail mesenchyme occasionally seen
1. No otic vesicle(s) present; somites present in trunk or portions thereof
2. No visible retinal pigment; otic vesicles or single vesicle still visible
3. Eyes fused or cyclopic, but at least some retinal pigment visible
4. Reduced forehead; eyes smaller than normal and sometimes joined
5. Normal in all externally visible respects

The average DAI value was calculated by summing individual grades and dividing by the total number of embryos. Embryos were scored at control stages 26-28 (Nieuwkoop and Faber, 1967).

**Whole-mount in situ hybridization and immunohistochemistry**

Whole-mount in situ hybridization was performed as described previously (Hemmati-Brivanlou et al., 1990; Harland, 1991). Whole-mount immunohistochemistry was carried out as described previously (Hemmati-Brivanlou and Harland, 1989). The muscle-specific monoclonal antibody (mAb) 12/101 (Kintner and Brockes, 1984) was used at a 1:500 dilution. The notochord-specific mAb Tor 70 (Kushner, 1984; Bolce et al., 1992) was used at a 1:1000 dilution. The goat anti-mouse secondary antibody (Jackson Laboratories) was used at a 1:200 dilution.

**In situ hybridization**

In situ hybridization was performed with 35S-labelled probes as described in O’Keefe et al. (1991).

**In vitro transcription and microinjection**

To generate sense Xwnt-11 transcripts, the EcoRI fragment of Xwnt-11 was cut out of pGEM-7Zf(+) and ligated into a blunt-ended BglII site in pSP64T (Krieg and Melton, 1984). SP6 RNA Polymerase was used for in vitro transcription. Fertilized eggs were placed in 3% Ficoll/0.5x MMR for injection. Embryos were injected at the 4- to 8-cell stage into one blastomere subequatorially, with 10 nl of Xwnt-11 mRNA (60 pg/nl).

**Histology**

Embryos were fixed in Bouin’s, dehydrated and embedded in Paraplast. Sections were cut at 10 µm thickness and were stained with Giemsa.

**RESULTS**

**Cloning and characterization of Xwnt-11**

Maternally expressed Xenopus wnt genes were cloned using a PCR amplification strategy. Degenerate oligonucleotides were made to amino acid sequences conserved among several known mouse and Xenopus wnt genes (see Materials and Methods). PCR amplification was performed using an oocyte cDNA library as template. PCR products of the expected size were subcloned and sequenced to verify that the PCR products were wnt-related. Three different sequences were found, one of which corresponds to the previously described Xwnt-5A (Christian et al., 1991) and two of which appear to be novel Xwnt sequences. Experiments reported here concern one of the two novel Xwnt sequences, Xwnt-11. The nomenclature of Xenopus wnt genes follows that of the mouse genes. Therefore, the number eleven indicates that the sequence is not similar to that of any known mouse wnt genes.

The PCR fragment of Xwnt-11 was used as a probe to screen an oocyte cDNA library. The longest clone recovered contains a 2.1 kb insert that includes all of the coding region as well as 360 bp of 5′ untranslated sequences, and 782 bp of 3′ untranslated sequences. A poly(A) tail was not included in this sequence. The open reading frame contains 353 amino acids, including a hydrophobic signal sequence at the 5′ end and all 24 of the cysteine residues that are conserved among most of the other known mouse wnt genes (Fig. 1A,B). Comparison of the Xwnt-11 amino acid sequence with other Xwnt genes for which complete coding
The localization of Xwnt-11 transcripts in the embryo was investigated by northern blot analysis, in situ hybridization to sectioned tissue and whole-mount in situ hybridization. The Xwnt-11 probe hybridizes to an approximately 2.5 kb transcript that is present from oocyte to stage 28 tadpole (Fig. 2). Isolation of RNA from dissected animal and vegetal blastomeres indicates that Xwnt-11 mRNA is localized to the vegetal hemisphere at the 8-cell stage (Fig. 2). At this stage, there is no difference between dorsal and ventral levels of Xwnt-11 RNA (data not shown).

In situ hybridization to sectioned tissue was done on oocytes, 8-cell and early gastrula embryos. Xwnt-11 mRNA is present in the youngest oocytes (stage I-II) and is distributed ubiquitously in the cytoplasm (Fig. 3A). During the middle stages of oogenesis, at a time when yolk platelets start to become asymmetrically distributed in the animal-vegetal axis, Xwnt-11 mRNA becomes localized to one pole of the oocyte (Fig. 3B). In the oldest oocytes (stage V-VI), Xwnt-11 mRNA shows two patterns of localization in the vegetal hemisphere; it is tightly associated with the vegetal cortex and there is also some mRNA in the vegetal hemisphere at the 8-cell stage (Fig. 2). At this stage, there is no difference between dorsal and ventral levels of Xwnt-11 RNA (data not shown).
Xwnt-11: a maternally expressed Xenopus wnt gene

Cytoplasm. The animal-vegetal axis of late stage oocytes can be clearly discerned by the location of the germinal vesicle in the animal hemisphere, and by the large yolk platelets in the vegetal hemisphere. In the 8-cell-stage embryo, Xwnt-11 mRNA is vegetally localized, confirming the result from northern blot analysis, but the mRNA is no longer confined to the cortex and is instead found throughout the vegetal cytoplasm (Fig. 3D).

Whole-mount in situ hybridization was done on stages from egg to tadpole. For reasons as yet unclear, we were unable to detect a signal above background in early embryos (egg to stage 8) with antisense probes to either Xwnt-11 or a relatively abundant mRNA, Vg1. However, whole-mount in situ hybridization was successful from late blastula to tadpole stages. At late blastula (stage 9), Xwnt-11 mRNA is visible as a crescent in the marginal zone on one side of the embryo (Fig. 4A). We think that this staining is on the dorsal side of the embryo because in slightly older embryos at stage 10, when the blastopore lip that marks the dorsal midline is just barely perceptible, the highest levels of Xwnt-11 mRNA are seen on the dorsal side above the lip. The whole-mount in situ hybridization technique does not seem to detect transcripts in yolky vegetal cells (Smith and Harland, 1992); therefore it is possible that Xwnt-11 mRNA is present at this stage more vegetally than is apparent from Fig. 4A. Staining is present initially in both the superficial and deep layer of marginal zone cells. By mid gastrulation, only cells that have not involuted, including superficial epithelial and inner cells, express Xwnt-11 (Fig. 5). During early gastrulation (stage 10.5), staining persists at highest levels on the dorsal side, but it starts to appear laterally and ventrally (Fig. 4B). As gastrulation proceeds (stage 12.5), Xwnt-11 mRNA is expressed in a ring around the closing blastopore, still at highest levels dorsally and only in the non-involuted cells (Fig. 4C). In late neurula, Xwnt-11 is expressed both dorsal to, and ventral to, the closed blastopore (Fig. 4D). By early tailbud (stage 22-23), most of the Xwnt-11 expression around the blastopore has faded and the predominant localization is in the somites around the somite nuclei and in the first branchial arch (Fig. 4E). The first branchial arch will later give rise to head mesenchymal structures. Due to the vertical alignment of the somitic nuclei, Xwnt-11 staining appears as vertical stripes along the dorsal axis (Fig. 4E,F). Hybridization with a control sense Xwnt-11 RNA probe did not show any of these staining patterns (data not shown).

Xwnt-11 mRNA injection into UV-ventralized embryos

Injection of in vitro transcribed mRNA into Xenopus embryos is a good assay to test for axis-inducing ability. Injections can be made into either a ventral blastomere of a normal embryo to test for second axis induction, or into an axially deficient UV-irradiated embryo to test for axis rescue. We found that Xwnt-11 mRNA will induce partial axis formation in both cases, but the experiments reported here all involve injection into UV-ventralized embryos.
because the percentage of embryos exhibiting a phenotype is greater than for normal embryos injected on the ventral side. The reason for this difference is unclear.

The injection of Xwnt-11 mRNA into UV-ventralized embryos can significantly rescue the formation of dorsal axial structures. On a DAI scale of 0 to 5, where 0 is the most severe UV defect and 5 is completely normal, Xwnt-11-injected embryos can be rescued to an average DAI of 1.8 (Table 1). Although numerically this may not appear to be a significant rescue, there is actually a dramatic difference since the DAI scale is not ‘linear’ in terms of morphological features. The most severe UV-ventralized embryos are radially symmetric and do not develop any features of a dorsal-ventral axis. UV embryos injected with Xwnt-11 mRNA develop a well-formed trunk, with somitic muscle and a neural tube. Brain vesicles and otic vesicles are sometimes present. The comparison between UV-ventralized and Xwnt-11-injected UV embryos is clearly visible both by histology (Fig. 6) and by whole-mount immunohistochemistry with tissue-specific antibodies (Fig. 7; Table 2). The structures that are missing from these rescued embryos are the most anterior head structures such as forebrain, eyes and cement gland. Histological sections also show that the notochord is absent from Xwnt-11-injected UV embryos. When these embryos are stained at stage 26-28 with the notochord-specific antibody Tor 70 (Bolce et al., 1992), only a few scattered positive staining cells can be detected. It is possible that the notochord formed normally and then degenerated, but we see no Tor 70 staining at earlier stages (20-22) in Xwnt-11-injected UV embryos, whereas control embryos already express the Tor 70 antigen (data not shown). The muscle found in these embryos is abundant. Unlike normal embryos where the somites are arranged on each side of the notochord (Fig. 6E), Xwnt-11-injected embryos have somites that have fused across the midline (Fig. 6C).

In order to achieve this rescued phenotype, the most effective dose of RNA was 300-600 pg, and the experiments reported here all used 600 pg. This is a much greater amount than that reported for other Xwnt injection experiments (10 pg or less for Xwnt-8 and Xwnt-3A), but is less than that reported for wnt-1 (1-2 ng) and for wg (high con-
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A 10 pg injection of Xwnt-11 mRNA is not effective in rescuing the UV defect. Increasing the amount of RNA injected from 600 pg to 2 ng does not change the phenotype observed in rescued embryos, but does decrease the frequency of survivors. Differences in the stability of injected RNA, or in efficiency of translation may explain why more Xwnt-11 mRNA is needed to obtain a phenotype compared with Xwnt-8 mRNA injection. The Xwnt-11 mRNA used for injection contains both 5′ and 3′ untranslated sequences. Eliminating the flanking untranslated sequences (in case they decrease the efficiency of translation) does not affect the observed phenotype.

Despite the lack of a normal notochord, these embryos have been induced to develop a spinal cord; however, it is not perfectly formed. Normal spinal cords have two bundles of axon tracts, or ‘marginal zones’, located on either side of the ventral midline. The spinal cord in Xwnt-11-injected UV embryos has only one large marginal zone located in the ventral midline (Fig. 6C).

Are Xwnt-11’s effects the result of inducing new mesoderm tissue, or are they the result of changing the

Fig. 4. Whole-mount in situ hybridization with a Xwnt-11 probe on embryos of different stages. (A) Late blastula (stage 9), vegetal view, dorsal is up. Staining is present in a broad arc in the marginal zone of the embryo on the dorsal side. This arc is much broader than the organizer. (B) Early gastrula (stage 10.5), vegetal view, dorsal is up. The marginal zone staining has extended laterally and ventrally. Highest levels of expression are on the dorsal side in an area roughly corresponding to the organizer (60°around the dorsal mid-line). The lateral and ventral marginal zone staining does not extend down to the blastopore lip. (C) Late gastrula (stage 12.5), vegetal view, dorsal is up. A ring of staining around the closing blastopore is present. The few dorsal-most staining cells extending out along the dorsal midline probably include presumptive posterior neural plate and archenteron roof cells. (D) Late neurula (neural folds have closed), view from posterior-ventral side, dorsal is up. The blastopore is almost completely closed and is visible only as a small slit. Xwnt-11 expressing cells are present both dorsal and ventral to the blastopore. (E) Early tailbud (stage 22-23), anterior to the right, dorsal is up. The predominant staining is around somite nuclei, which are aligned vertically in each somite, and in the first branchial arch. (F) Tadpole (stage 28), anterior to the right, dorsal is up. Xwnt-11 staining is present around the somite nuclei, which are aligned vertically in each somite, and in the first branchial arch. Scale bars all represent 500 µm.
dorsal-ventral pattern of pre-existing mesoderm tissue? When Xwnt-11 mRNA is injected into the animal hemisphere of 1-cell embryos and animal caps are subsequently cut several hours later at the blastula stage, these animal caps are not induced to become mesoderm. Morphologically, they do not elongate but remain rounded up like control uninjected animal caps, and Northern blots indicate that muscle actin is not induced (data not shown). These results suggest that Xwnt-11 is not a mesoderm inducer, but is a molecule capable of dorsalizing pre-existing mesoderm. This interpretation is similar to conclusions drawn from previous experiments where it appears that Xwnt-8 can modify the type of mesoderm induced by other growth factors such as FGF or activin (Christian et al., 1992; Sokol and Melton, 1992).

DISCUSSION

We set out to clone new Xenopus wnt genes to test our hypothesis that there exists a maternally expressed wnt gene whose function in normal development is in the establishment of dorsal tissues (muscle, notochord and neural tube). The hypothesis stems from an interpretation of previous experiments where injection of wnt-1 or Xwnt-8 mRNA into Xenopus embryos can cause a duplication of the existing axis, or cause the formation of an axis in an axially deficient UV-irradiated embryo (McMahon and Moon, 1989; Smith and Harland, 1991; Sokol et al., 1991). One interpretation of these experiments is that the proteins from injected wnt-1 or Xwnt-8 mRNA act by cross-reacting with an endogenous receptor whose normal function is to transmit a Xwnt signal. Injected wnt-1 and Xwnt-8 could therefore be ‘mimicking’ a maternally expressed wnt gene. This interpretation would also predict that injection into Xenopus embryos of the synthetic mRNA for this maternal wnt gene could induce axis formation.

Xwnt-11 has some, but not all, of the properties predicted for such a maternal wnt gene. Xwnt-11 is expressed maternally from the earliest stages of oogenesis, as well as later during embryogenesis and tadpole stages. During oogenesis, Xwnt-11 mRNA is first uniformly distributed (stage I-II), then becomes gradually localized to one pole (stage III-IV) and is tightly localized to the vegetal cortex with some small patches in the vegetal cytoplasm by stage V-VI. In the mature, unfertilized egg, Xwnt-11 mRNA is still vegetally localized but it is no longer tightly bound to the cortex. This pattern persists during early cleavage stages, as Xwnt-11 mRNA is localized to the vegetal cytoplasm at the 8-cell stage.

The early localization pattern of Xwnt-11 is very similar to that of another maternal transcript, Vg1 (Rebagliati et al., 1985; Melton, 1987). Both transcripts are localized to the vegetal cortex during oogenesis, although one difference between the two is that Xwnt-11 mRNA is also present to some extent in the vegetal cytoplasm of stage V-VI oocytes. Xwnt-11 and Vg1 mRNA appear to be released from the vegetal cortex after oocyte maturation and before fertilization. The Vg1 mRNA localization signal has been shown to reside in the 3′ UTR (Mowry and Melton, 1992). Sequence comparisons of the Xwnt-11 and Vg1 untranslated sequences do not show any obvious homology, although this does not rule out the possibility that the same RNA localization machinery is used in both cases.

During late blastula and early gastrula stages, Xwnt-11

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total number*</th>
<th>Average DAI</th>
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<tbody>
<tr>
<td>UV</td>
<td>324</td>
<td>0.8</td>
</tr>
<tr>
<td>UV+X11</td>
<td>87</td>
<td>1.8</td>
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<tr>
<td>UV+X8</td>
<td>78</td>
<td>5.3</td>
</tr>
<tr>
<td>Normal</td>
<td>166</td>
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*These numbers are from five separate experiments, except for UV+X8 which is from two experiments.
mRNA is present in the marginal zone, with highest levels of expression on the dorsal side. At this stage, both surface and deep cells express Xwnt-11. As gastrulation progresses, Xwnt-11 expression expands laterally and ventrally in the marginal zone to form a ring around the closing blastopore. By mid-gastrulation, only cells that have not yet involuted express Xwnt-11. In early tadpole stages, Xwnt-11 mRNA is present in each somite around the somite nuclei and in the first branchial arch. Different patterns of Xwnt-11 expression throughout early development probably reflect different

Fig. 6. Histological sections of UV-ventralized eggs injected with Xwnt-11 mRNA. UV-ventralized embryos, UV embryos injected with Xwnt-11 mRNA and normal embryos were fixed at stage 26-28, sectioned and stained to determine what structures had formed. (A) UV-ventralized embryo, cross section. (B) UV embryos injected with Xwnt-11 mRNA. Mid-trunk cross section, dorsal is up. (C) Higher magnification of a UV embryo injected with Xwnt-11 mRNA. The neural tube (nt) consists of a dark- and a pale-stained region. There is no lumen in this section, but other sections sometimes do show a lumen. The pale-stained region is the ‘marginal zone (mz)’, where axon tracts are located. There is one large marginal zone in the ventral midline (compare with Fig. 6E). There is also one large mass of muscle (m) that extends across the midline. (D) Normal embryo. Mid-trunk cross-section, dorsal is up. (E) Higher magnification view of a normal embryo. Neural tube (nt) and notochord (n) are visible. One of the two files of somitic muscle on each side of the notochord is marked (m). Note the two lateral pale-stained ‘marginal zones’ (mz) separated by ventral floor plate cells. The scale bars in A, B and D) represent 100 µm and in D and E represent 50 µm.
functions. In *Drosophila*, it is well documented that the wg signal is used several times for proper embryonic, larval and adult development (reviewed in Nusse and Varmus, 1992; Peifer and Bejsovec, 1992).

The expression pattern of *Xwnt-11* can be contrasted with that of *Xwnt-8*. *Xwnt-8* is first detectable after zygotic transcription commences at the midblastula transition (MBT) (Christian et al., 1991). *Xwnt-8* expression increases rapidly

### Table 2. The presence of muscle, notochord and neural tube in UV irradiated and injected embryos as determined by tissue specific antibody staining and histology†

<table>
<thead>
<tr>
<th></th>
<th>12101 mAb (muscle)</th>
<th>Tor70 mAb (notochord)</th>
<th>Histology (neural tube)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>−</td>
<td>Total</td>
</tr>
<tr>
<td>UV</td>
<td>5*</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>UV+Xwnt-11</td>
<td>24</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

†The 12101 monoclonal antibody (Kintner and Brookes, 1984) was used to assess the presence of muscle. The Tor70 monoclonal antibody (Bolce et al., 1992) was used to score for the presence of notochord. These results were also confirmed by examining histological sections. The presence of neural tubes was scored by looking at serial sections. Again, in all of the UV plus Xwnt-11 embryos that contained neural tubes, none of them had notochords (n=25).

*In these 5 embryos that contained muscle, the amount of muscle is much less than in normal or injected embryos.

**This number includes 12 out of the total 22 that have a few scattered positively staining cells (see Fig. 7B). In no case did we observe an organized notochord tube.

**Fig. 7.** Whole-mount immunohistochemistry of *Xwnt-11*-injected embryos. UV-ventralized embryos (A,D), UV embryos injected with *Xwnt-11* mRNA (B,E) and normal embryos (C,F) were fixed at stage 26-28, and whole-mount immunohistochemistry was done with tissue-specific antibodies. UV-ventralized embryos have neither notochord nor muscle. UV embryos injected with *Xwnt-11* mRNA have somitic muscle but no notochord. Normal control embryos have both muscle and notochord. (A-C) Incubated with the notochord-specific monoclonal antibody (mAb) Tor 70 (Kushner, 1984; Bolce et al., 1992). (D-F) Incubated with the muscle-specific monoclonal (mAb) antibody 12101 (Kintner and Brookes, 1984). (A) UV-ventralized embryo plus Tor 70 mAb. There are no Tor 70-positive cells. (B) UV embryo injected with *Xwnt-11* mRNA plus Tor 70 mAb. There are a few scattered Tor 70-positive cells but no organized notochord is present. (C) Normal embryo plus Tor 70 mAb. An extensive notochord is present along the dorsal axis. (D) UV-ventralized embryo plus 12101 mAb. There are no 12101-positive cells. (E) UV embryo injected with *Xwnt-11* mRNA plus 12101 mAb. Extensive somitic muscle is present along the dorsal axis. (F) Normal embryo plus 12101 mAb. Somitic muscle is present. Scale bars all represent 100 µm.
the cortical rotation remain a mystery, it has long been
(Malacinski et al., 1981; Youn and Malacinski, 1981; Clarke
mRNA is evenly distributed in the dorsal-ventral
et al., 1991).

The axis; it appears first in a broad arc in the dorsal marginal
zone, and subsequently in the lateral and ventral marginal
zone. This expression pattern is consistent with a role in pat-
terning dorsal mesoderm, as there is thought to be a signal
behaves similarly to Xwnt-8, is noggin (Smith and Harland,
1992). Noggin was isolated in a screen for molecules that
could completely rescue UV-ventralized embryos. Unlike
Xwnt-11, noggin can completely rescue UV embryos to a
normal DAI of 5, including notochord and anterior head
structures such as eyes, forebrain and cement gland (Smith
and Harland, 1992). However, noggin mRNA injection by
itself does not induce mesoderm in animal caps (Smith
and Harland, 1992). Noggin protein applied to ventral marginal
zone explants from normal embryos can ‘dorsalize’ pre-
existing ventral mesoderm towards more dorsal fates (Smith
et al., 1993). Therefore, noggin mRNA injection into UV
embryos is probably also ‘dorsalizing’ pre-existing ventral
mesoderm in UV embryos, rather than directly inducing
dorsal mesoderm. Noggin mRNA is present maternally and
is uniformly distributed in the dorsal-ventral and animal-
vegetal axes. Zygotically expressed noggin mRNA is
localized to presumptive dorsal mesoderm cells (Smith

Xwnt-11 and noggin are two maternally expressed genes
that, to different extents, can cause dorsal axis formation in
UV-ventralized embryos. There is evidence from expression
data and from the UV rescue assay that both molecules
might be important in the establishment of the dorsal-ventral
axis. Although Xwnt-11 cannot completely rescue UV-ven-
tralized embryos, a more conclusive demonstration of its
function might come from antisense knockout experiments
and antibody studies of Xwnt-11 protein distribution. There
is also nothing known yet about the Xwnt-11 receptor or its
signal transduction pathway. Further studies to address these
issues should help clarify the role of Xwnt-11 in normal
development and begin to elucidate its relationship with
other cell-signalling molecules.

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was previously called Xwnt-9; we wish to acknowledge Arend
Sidow, who suggested that this gene should instead be called
Xwnt-11 to avoid confusion with the previously known hagfish
and thresher shark wnt-9 sequences. This work was supported by
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dominant negative mutant of the FGF receptor disrupts mesoderm
wingless signaling pathway in both embryonic and adult pattern formation. Development 111, 1029-1043.


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