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Pintallavis, a gene expressed in the organizer and midline cells of frog embryos: involvement in the development of the neural axis

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Summary

We have identified a novel frog gene, *Pintallavis* (the Catalan for lipstick), that is related to the fly *fork head* and rat HNF-3 genes. *Pintallavis* is expressed in the organizer region of gastrula embryos as a direct zygotic response to dorsal mesodermal induction. Subsequently, *Pintallavis* is expressed in axial midline cells of all three germ layers. In axial mesoderm expression is graded with highest levels posteriorly. Midline neural plate cells that give rise to the floor plate transiently express *Pintallavis*, apparently in response to induction by the notochord. Overexpression of *Pintallavis* perturbs the development of the neural axis, suppressing the differentiation of anterior and dorsal neural cell types but causing an expansion of the posterior neural tube. Our results suggest that *Pintallavis* functions in the induction and patterning of the neural axis.

Key words: axial mesoderm, midline, neural induction, organizer, *Pintallavis*, *Xenopus laevis*.

Introduction

The development of the basic body plan of vertebrate embryos depends on inductive interactions and morphogenetic movements that take place during gastrulation (Gerhart and Keller, 1986; Hamburger, 1988). The process of gastrulation results in the formation of two midline mesodermal structures, the prechordal plate and the notochord, and in the induction of the neural plate. Subsequently, the notochord induces the overlying neural ectoderm to differentiate into the floor plate, the ventral midline of the neural tube. The patterning properties of these midline cell groups are, in large part, responsible for the regional differentiation of the nervous system along the antero-posterior (A-P) axis, leading to the formation of the brain and spinal cord.

The first insight into the events that control the formation of the neural axis of the amphibian embryo derived from the transplantation assays of Spemann and Mangold (1924). In these experiments, transplantation of the dorsal lip of early gastrulae to the ventral region of a sibling embryo resulted in the formation of a twinned embryo with a second neural axis. The cells of the dorsal lip, termed the organizer, changed the fate of host ventral cells, resulting in the formation of axial mesoderm and induced neural tissue. In contrast, the midline structures of the induced second axis derived primarily from the descendents of the grafted dorsal lip cells (Spemann, 1938; Smith and Slack, 1983).

In normal *Xenopus* embryos, the dorsal lip contains mesodermal cells that involute at the blastopore during gastrulation and extend along the A-P axis giving rise primarily to the prechordal plate and notochord. Ectodermal cells adjacent to the dorsal lip, a region known as the dorsal noninvoluting marginal zone (NIMZ, Keller and Danilchik, 1988) remain in the ectoderm but also extend along the midline, to form the notoplate, which is coextensive with the underlying notochord (Jacobson, 1981; Keller et al., 1985; Keller and Danilchik, 1988). In addition, the superficial cells of the dorsal lip give rise to the midline endodermal cells that line the archenteron roof. Thus, cells in and adjacent to the dorsal lip form midline structures in each of the three germ layers of the embryo after gastrulation.

The ability of the dorsal lip to induce neural tissue is maintained by its axial mesodermal derivatives. The notochord, in particular, is a potent inducer of neural tissue in competent ectoderm. These and other experiments have led to the idea that the involuted axial mesoderm is responsible for induction of the neural tube (Mangold, 1933; Holtfreter, 1933; Nieuwkoop et al., 1952; Sharpe and Gurdon, 1990). However, signals from the dorsal lip that spread through the plane of the ectoderm are also involved in neural induction and A-P patterning (Spemann, 1938; Savage and Phillips, 1989; Dixon and Kintner, 1989; Ruiz i Altaba, 1990, 1992). Moreover, recent studies suggest that the notoplate may be a source of planar signals involved in the formation of the neural tube and that these cooperate with vertical signals from the head mesoderm and notochord to induce the forebrain and ventral neuronal types in the hindbrain and spinal cord (Ruiz i Altaba, 1992).

The induction of ventral neurons by the notochord appears to involve the floor plate, a group of cells with notochord-like properties located along the ventral midline of the embryonic neural tube with the exception of the forebrain (Kingsbury, 1930; Placzek et al., 1990, 1991; Yamada et al., 1991; Hatta et al., 1991). The differentiation of the floor plate from cells at the midline of the neural plate is induced by contact with the underlying notochord (van Straaten et al., 1988; Placzek, et al., 1990; Clarke et al., 1991). Thus, in *Xenopus*, the floor plate is likely to be derived from the notoplate in response to signals from the notochord. The induction and patterning of the *Xenopus* nervous system may therefore require signals from midline mesodermal cell groups: the dorsal lip, the notochord and the prechordal mesoderm, and from midline neural cell groups: the notoplate and the floor plate. The different inductive properties of these midline cell groups could reflect distinct signals or the action of the same signal on cells with different response properties.

An understanding of the molecular events involved in the induction and patterning of the nervous system requires the identification of genes that control the properties of these midline structures. In addition to the conserved signalling properties of the notochord and floor plate, both structures are unsegmented but are surrounded by segmented tissues. The notochord is adjacent to the somites and the floor plate is adjacent to the rhombomeres of the hindbrain (Vaage, 1969; Lumsden and Keynes, 1989). The division of tissues into segmented and unsegmented structures could reflect a fundamental and evolutionarily conserved decision. In flies, the *fork head* gene has been suggested to be involved in repressing thoracic segmentation in the head (Jürgens and Weigel, 1988). *Fork head* encodes a nuclear protein (Weigel et al., 1989) that contains a sequence very similar to that of the DNA-binding domain of the HNF-3 liver transcription factors (Weigel and Jäckle, 1990; Lai et al., 1990, 1991). The conservation in sequence between *fork head* and the HNF-3 genes enabled us to search for related genes expressed in midline cells of *Xenopus* embryos that could be involved in establishing their common properties, the lack of segmentation and their inductive abilities.

We describe here the isolation and characterization of a *Xenopus* gene, *Pintallavis*, that is related to the rat HNF-3 and *Drosophila fork head* genes. *Pintallavis* is expressed first in the dorsal lip as a direct response to dorsal mesodermal induction. After the onset of gastrulation, *Pintallavis* is transiently expressed at high levels in the notochord and at low levels in the prechordal mesoderm. *Pintallavis* is also expressed at the midline of the neural plate although it is absent from the notoplate in exogastrula embryos, suggesting that its expression is induced by the notochord. Injection of synthetic *Pintallavis* RNA into developing embryos results in axial defects. These include a disruption in the extension of the embryo and a suppression of anterior neural structures. In contrast, in posterior regions there is an overproduction of neural tissue and an absence of certain dorsal neurons. The restricted expression of *Pintallavis* in midline cells and the phenotype obtained after its overexpression suggest that this gene is involved in the development of the neural axis.

In recent studies, the cloning of XFKHI, a closely related *Xenopus* gene, has been reported (Dirksen and Jamrich, 1992). The distributions of *Pintallavis* and XFKHI are similar but not identical, suggesting that they may represent the two copies of the same gene in the tetraploid *Xenopus* genome.

Materials and methods

Embryological manipulations

Xenopus laevis embryos were obtained by inducing female pigmented or albino frogs to lay eggs with human chorionic gonadotropin which were fertilized in vitro with testis homogenates. Embryos were freed from their jelly coats by treatment with 3% cysteine pH 7.6 in tap water and reared in 0.1×MMR (Newport and Kirschner, 1982). Embryos were staged according to Nieuwkoop and Faber (1967).

Treatment of embryos before first cleavage with ultraviolet light was performed for 30 seconds, 1 minute and 1.5 minutes by irradiating the vegetal poles approximately 40 minutes after fertilization. Treatment of early blastula stage embryos (stage 6-7) with Li^+ was performed by incubating embryos in 0.25 M LiCl in tap water for 8-10 minutes. A fraction of treated embryos was allowed to develop to the tadpole stage for the assessment of the degree of axial defects (Kao and Elinson, 1988).

Animal pole explants were cut from late blastula stage embryos (stage 7-8) and incubated in 0.5×MMR. Both activin and basic fibroblast growth factor were supplied as transformed COS cell supernatants and were a kind gift from C. Hume (Columbia University). Treatment of animal caps with all-*trans* retinoic acid (RA; Sigma) was performed by diluting the stock of 5×10^{-3}M in dimethylsulfoxide with 0.1×MMR to 10^{-8}M. RA-treated embryos were protected from light. Cycloheximide (5 μM; Sigma) was applied to whole embryos 1 hour before cutting the animal caps, which were also incubated in the drug.

Complete exogastrulae were obtained by incubation of embryos from which the vitelline membrane had been removed in 1.3×MMR. All explants were kept in agarose dishes in the presence of antibiotics.

Embryo injections

Injection of synthetic RNA was performed by injecting 1-10 nl of RNA solution in water with a pressure driven injector. Embryos were kept in 1×MMR, 3% Ficoll before, during and 1 hour following the injection. Rhodamine/lysine/dextran (M_r 10,000; Molecular Probes) at 50 mg/ml was mixed with the RNA solution at a ratio of ~1:10 immediately before injection.

RNA isolation, cDNA synthesis and PCR

RNA was isolated from homogenized embryos by proteinase K digestion followed by precipitations with ethanol and LiCl. Dissection of embryo parts for RNA isolation was performed by fixing the embryos in acid alcohol (95% ethanol, 5% glacial acetic acid) for 5-20 minutes before dissection.

First-strand cDNA was prepared by priming the RNA with random primers in the presence of Superscript reverse transcriptase (BRL). The polymerase chain reaction (PCR) was performed with degenerate primers on cDNA samples with a 40°C annealing step in the presence of AmpliTaq DNA polymerase (Perkin Elmer-Cetus). Oligonucleotides flanked by *Eco*RI or *Bam*HI restriction sites with inosines in completely degenerate positions were directed against the conserved sequences ITMAIQ and MFENGC of the HNF-3 (Lai et al., 1991) and *fork head* (Weigel et al., 1989) genes. PCR reactions were also performed with

primers directed to the internal sequence QNQQRWQ. The presence of an amplified band was dependent on the addition of reverse transcriptase to the cDNA reaction ruling out contamination by genomic DNA. The PCR products were digested with restriction enzymes, purified in low melting point agarose gels and ligated to a phage M13 vector for sequencing with sequenase (USB) in the presence of ^{35}S-labelled dATP. The sequence of 20 independent clones showed only a few third-base changes and one conservative amino acid substitution. These variations are likely to reflect the polymorphisms of single genes in the teraploid *Xenopus* genome.

Library screens and subclones

Screening of a neurula stage cDNA library in lambda gt10 (Kintner and Melton, 1987) was performed at high stringency with ^{32}P-labelled random-primed M13 PCR inserts on phage lifts transferred to Hybond (DuPont) filters. Phage DNA was cross-linked to filters by UV light.

The insert of the longest cDNA isolated in the library screens did not have internal *Eco*RI sites. The ~1.8 kb cDNA insert was then subcloned into the *Eco*RI site of pBSKS (Stratagene) to yield pF5.

RNase protections and in vitro RNA synthesis

RNase protection assays and the EF-I alpha probe were as described (Ruiz i Altaba and Melton, 1989a). For the *Pintallavis* probe, a *Pst*I deletion subclone of pF5, pF5Pstdelta, was digested with *Xmn*I and transcribed with phage T7 RNA poymerase. Ten and one embryo or animal cap equivalents were used for the *Pintallavis* and the EF-I alpha probes, respectively. RNase digestion was carried out at room temperature for 1 hour. The undigested products were analysed in denaturing sequencing gels.

Synthetic, capped RNA was made by transcription of the full-length *Pintallavis* cDNA insert at 30ºC, cloned by blunt-end ligation into the filled *Bgl*II site of pSP64T, with SP6 RNA polymerase (Krieg and Melton, 1984). pSP64T (Melton, 1985) provides 5′ and 3′ untranslated sequences and a poly(A)-poly(C) tail that enhance the translation and the stability of injected messages. In vitro translation of *Pintallavis* RNA in reticulocyte lysates (Promega) was performed with 0.5 μg of RNA per reaction in the presence of ^{35}S-labelled methionine using bromo mosaic virus RNA as a control. The products were analysed by electrophoresis in 10% polyacrylamide gels.

For control studies, a frame-shift mutation was introduced into the *Pintallavis* sequence by digestion with *Bgl*II (nucleotide 507), filled-in with Klenow polymerase and religated, yielding pF5(B). The translation of this RNA is predicted to terminate after an additional 29 amino acids that are not present in *Pintallavis*, thus truncating the putative DNA-binding domain.

Whole-mount in situ hybridization

Whole-mount in situ hybridization was performed with digoxigenin-labelled probes according to Harland (1991; see also Hemmati-Brivanlou et al., 1990). The full-length cDNA insert was used as template to generate an antisense RNA probe from pF5 after digestion with *Hin*dIII and transcription with T7 RNA polymerase in the presence of dig-11 UTP. Hybridization of embryos with the sense probe gave no signal. Hybridization with an anti-sense probe derived from the 3′ untranslated region gave the same results as the full-length probe. In situ hybridization with *Xenopus Brachyury* was performed by transcribing the full insert of pXbra6 with T7 RNA polymerase, kindly provided by J. Smith (Smith et al., 1991). Transcription reactions were traced by adding a small amount of ^{32}P-labelled UTP. The RNA probes were not hydrolysed and used at a concentration of 1 μg/ml. The hybridization signal was detected by a secondary antibody coupled to alkaline phosphatase, used at 1:2000, followed by the phosphatase reaction with BCIP and X-phosphate (Boehringer Mannheim). Reaction times varied from 1 to 8 hours.

Whole-mount antibody labelling and histology

Whole-mount antibody labelling was performed essentially as described by Dent et al. (1989), using MEMFA as fixative (Patel et al., 1989; Hemmati-Brivanlou and Harland, 1989). Monoclonal antibodies (mAbs) Xen1 (Ruiz i Altaba, 1992) and HNK-1 (Abo and Balch, 1981) were used as culture supernatants diluted 1:1. mAb 12/101 (Kintner and Brockes, 1984) was used as ascites fluid diluted 1:500. Secondary antibodies (Fab fragments) coupled to horseradish peroxidase (Boehringer Mannheim) were used at 1:100. Antibody binding was detected by the peroxidase reaction in the presence of H_2O_2 and diaminobenzidine. Labelled embryos were cleared in benzyl alcohol-benzyl benzoate. Histological sections (12.5 μm) were obtained by sectioning paraplast-embedded embryos. The sections were subsequently dewaxed in xylene and mounted in Permount.

Results

Isolation and structural features of Pintallavis

The *Drosophila fork head* and the rat HNF-3 genes are very similar in a region encoding their putative DNA-binding domains (Weigel and Jäckle, 1990; Lai et al., 1991). This permitted the design of degenerate oligonucleotide primers with which to screen for related *Xenopus* genes using the polymerase chain reaction. First-strand cDNA was prepared from a combined RNA sample isolated from the dorsal lip of early gastrula (stage 10) embryos and from the midline of mid-gastrula (stage 12-12½) embryos. The primers amplified a ~240 bp fragment the sequence of which is highly similar to that of the conserved domain in the HNF-3 and *fork head* genes (Fig. 1A, B).

To isolate a full-length cDNA clone, a mid-neurula (stage 17) library (Kintner and Melton, 1987) was screened with the random-primed PCR product. Three recombinant lamda clones were isolated which contained unique *Eco*RI inserts of 1.6 to 1.8 kb in length. The longest cDNA clone was sequenced, revealing an open reading frame that encodes a protein of 399 amino acids that is similar to the HNF-3 and *fork head* proteins (Fig. 1A, B). The position of the first methionine in *Pintallavis* was assigned by sequence similarity with the NH_2-terminal region of the HNF-3 proteins (Fig. 1B). A high degree of similarity in the putative DNA-binding domains was observed among all five proteins (positions 107 and 222 of *Pintallavis*; Fig. 1B). Although this region exhibits over 90% identity in all five proteins, *Pintallavis* is not significantly more closely related to any individual protein of this family. The region of *Pintallavis* that is NH_2-terminal to the conserved putative DNA-binding domain has a very high methionine content as reported for the HNF-3 and *fork head* proteins (Weigel et al., 1989; Lai et al., 1991). Two other regions of similarity between HNF-3 and *fork head* are also found in *Pintallavis*, a domain between positions 316 and 341 containing the HPFSI motif, and a small domain from position 386 to the COOH terminus of the protein (Fig. 1A, B).

Pintallavis exhibits 47% overall identity with HNF-3 gamma, 46% identity with HNF-3 alpha, 45% identity with

A

PINTALLAVIS

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                                                                    75
GAATTCCGTTAACTCTTTCTAGAACTGGAAGGTCTTCTTGTTGGAGAAACCATACCTCCGCTGTGGACTCCAGAACATG CTA AAT AGA GTC AAA
                                                                      Met Leu Asn Arg Val Lys
                                                                   150
CTG GAA ATT AAG GAT CCT ATG GAC TGG AAT ACC ATG TAC CAA GAG AAT GAG ATG TAT TCT GGA ATA CAC AAC ATG
Leu Glu Ile Lys Asp Pro Met Asp Trp Asn Thr Met Tyr Gln Glu Asn Glu Met Tyr Ser Gly Ile His Asn Met
                                                                   225
ACC AAT GTC TTG CCA AGC AAT TCA TTC TTA CCA AAT GAT GTT TCC ACA GTA ACA ACA AGC ATG CCT TAT ATG AGT
Thr Asn Val Leu Pro Ser Asn Ser Phe Leu Pro Asn Asp Val Ser Thr Val Thr Thr Ser Met Pro Tyr Met Ser
                                                                   300
AAT GGC TTA CCA GGG CCT GTT ACC AGT ATT CAG GGA AAC ATA GGC TCT CTT GGT TCC ATG CCT CAA GGA ATG GTG
Asn Gly Leu Pro Gly Pro Val Thr Ser Ile Gln Gly Asn Ile Gly Ser Leu Gly Ser Met Pro Gln Gly Met Val
                                                                   375
GGA TCC CTT GCT CCT CCA CCT TCC ACT GCT GCT TAT CCA TTG GGC TAT TGC CAA GGG GAG TCT GAA TTT CAG AGA
Gly Ser Leu Ala Pro Pro Pro Ser Thr Ala Ala Tyr Pro Leu Gly Tyr Cys Gln Gly Glu Ser Glu Phe Gln Arg
                                                                   450
GAT CCT CGT ACC TAT CGT AGG AAT TAC TCT CAT GCT AAG CCC CCA TAC TCT TAC ATC TCC CTT ATT ACC ATG GCA
Asp Pro Arg Thr Tyr Arg Arg Asn Tyr Ser His Ala Lys Pro Pro Tyr Ser Tyr Ile Ser Leu Ile Thr Met Ala
                                                                   525
ATA CAG CAG GCA CCC AAC AAG ATG ATG ACA CTA AAT GAG ATC TAC CAG TGG ATC ATA GAC CTT TTC CCT TAC TAC
Ile Gln Gln Ala Pro Asn Lys Met Met Thr Leu Asn Glu Ile Tyr Gln Trp Ile Ile Asp Leu Phe Pro Tyr Tyr
                                                                   600
AGG CAG AAC CAG CAA AGA TGG CAG AAC TCT ATA CGC CAT TCA CTA TCC TTT AAT GAC TGT TTT GTT AAA GTT CCA
Arg Gln Asn Gln Gln Arg Trp Gln Asn Ser Ile Arg His Ser Leu Ser Phe Asn Asp Cys Phe Val Lys Val Pro
                                                                   675
CGT TCT CCA GAA AAG CCA GGT AAA GGC TCC TAT TGG ACT CTG CAC CCC GAA TCT GGC AAT ATG TTT GAG AAT GGT
Arg Ser Pro Glu Lys Pro Gly Lys Gly Ser Tyr Trp Thr Leu His Pro Glu Ser Gly Asn Met Phe Glu Asn Gly
                                                                   750
TGT TAC TTA AGA AGG CAG AAG AGA TTC AAA TGT GAA AGG AGC AAG TCT GGA GAA GGA GAG AAA AAA GTA AAC AAA
Cys Tyr Leu Arg Arg Gln Lys Arg Phe Lys Cys Glu Arg Ser Lys Ser Gly Glu Gly Glu Lys Lys Val Asn Lys
                                                                   825
CCA GGG GAG GAG ACT GGA GGA AAC TTA AAG GAG AAT CCA CTT GGC TAT GAT GAC TGT TCC TCT TCA AGA TCC CCA
Pro Gly Glu Glu Thr Gly Gly Asn Leu Lys Glu Asn Pro Leu Gly Tyr Asp Asp Cys Ser Ser Ser Arg Ser Pro
                                                                   900
CAA GCT GCA GTT AAT GAT GGA GGT AGG GAC TCC ACT GGA TCA AGT ATA CAT CAG GCT TGT GGA AGT TCT CCA GTT
Gln Ala Ala Val Asn Asp Gly Gly Arg Asp Ser Thr Gly Ser Ser Ile His Gln Ala Cys Gly Ser Ser Pro Val
                                                                   975
GGC CTT AGC CCT ACA TCT GAA CAA GCA GGA ACA GCA TCT CAA CTC ATG TAT CCA TTA GGT CTC TCT AAT GAC GGC
Gly Leu Ser Pro Thr Ser Glu Gln Ala Gly Thr Ala Ser Gln Leu Met Tyr Pro Leu Gly Leu Ser Asn Asp Gly
                                                                  1050
TAT CTT GGC CTA GTT GGA GAA GAT GTC CAT TTG AAG CAT GAT CCT TTC TCT GGG AGA CAT CCA TTT TCT ATT ACA
Tyr Leu Gly Leu Val Gly Glu Asp Val His Leu Lys His Asp Pro Phe Ser Gly Arg His Pro Phe Ser Ile Thr
                                                                  1125
CAA CTA ATG TCT TCT GAA CAG GAC CAA ACA TAC GCA AAT AAA ATG GAA ATG TGT CCA ACC ACA GAC CAT CTT GTC
Gln Leu Met Ser Ser Glu Gln Asp Gln Thr Tyr Ala Asn Lys Met Glu Met Cys Pro Thr Thr Asp His Leu Val
                                                                  1200
CAC TAC TCT AAC TAC AGC TCT GAC TAT CAC AAC ATG GCC TCA AAA AAT GGA CTA GAC ATG CAG ACA TCC TCA TCC
His Tyr Ser Asn Tyr Ser Ser Asp Tyr His Asn Met Ala Ser Lys Asn Gly Leu Asp Met Gln Thr Ser Ser Ser
                                                                  1275
ACT GAT AAT GGA TAT TAT GCC AAC ATG TAC TCC AGA CCT ATC CTC AGC TCC CTT TAAACATTTGTTTCTGTTGTGCTTTAC
Thr Asp Asn Gly Tyr Tyr Ala Asn Met Tyr Ser Arg Pro Ile Leu Ser Ser Leu ***
                                                            1350
AGACATTTCATATTCAAAGTAAATGCCTTACTAATCAAAGCAGCTGTATTACCTACAAGTAGGTTCTTAAAAACCCAATCGTTTTTGAGACGCAGTGAT
                                1425
TATGATTCTAAACCAGTACTTAGAATATCACTCAGTAAGAGAAAAGATTAAAAATCAGTATTTATTTATAATGCTTGATTGTCTGACCTAAAGGCTTTA
                                                                                                  1575
CCTACCTTGACTTAAAAAAACATTCATATAATATTAAGGAAATCATTATATGAACTTTATTCTGTATATGTTGTATAGGCAAGATGACCATACTGTTAA
                                                          1650
ATGCATCACATTTAGCAAAATTATCCTTTACTTTCTAAAACAGTCAAAAAGTAATATTGCTATTATGTATTTTGGGTTAATATACTATACAAGAAGCAG
                                1725
ATTTTGCTATGTTTAAATATGTATATATTGTTTCATGTTTTCTCTATGATATTAAAGTTGAATAGCATTTAAAGGTGTCAGTTCTGAATGTTTTATTTC
 1800                                                                                             1875
TATGTGTTTCACATGAAACCTGTTTTTTTTTTCTTATGTCTTTGCTATTCTTGATATTAATATATAAACTTGTTCCGGAATTC
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Fig. 1. *Nucleotide and amino acid sequence of* Pintallavis. (A) Nucleotide sequence of a *Pintallavis* cDNA. The translation of the encoded open reading frame is provided. The termination codon is indicated by three stars. The sequence is flanked by *Eco*RI sites that may be derived from cloning linkers. This sequence has been entered into the EMBL and GenBank data bases under the accesion number X65171.(B) Alignement of protein sequences showing the conserved regions of identity (overlined). Dots refer to gaps included to allow maximal matching of the five sequences. Positions with two or more conserved methionines in the NH_2-terminal region are also denoted by overlining. The location of the conserved sequences used to design degenerate oligonucleotides for PCR are denoted by arrows. The HNF-3 sequences are from Lai et al. (1990, 1991) and E. Lai, personal communication. The *fork head* sequence is from Weigel et al. (1989).

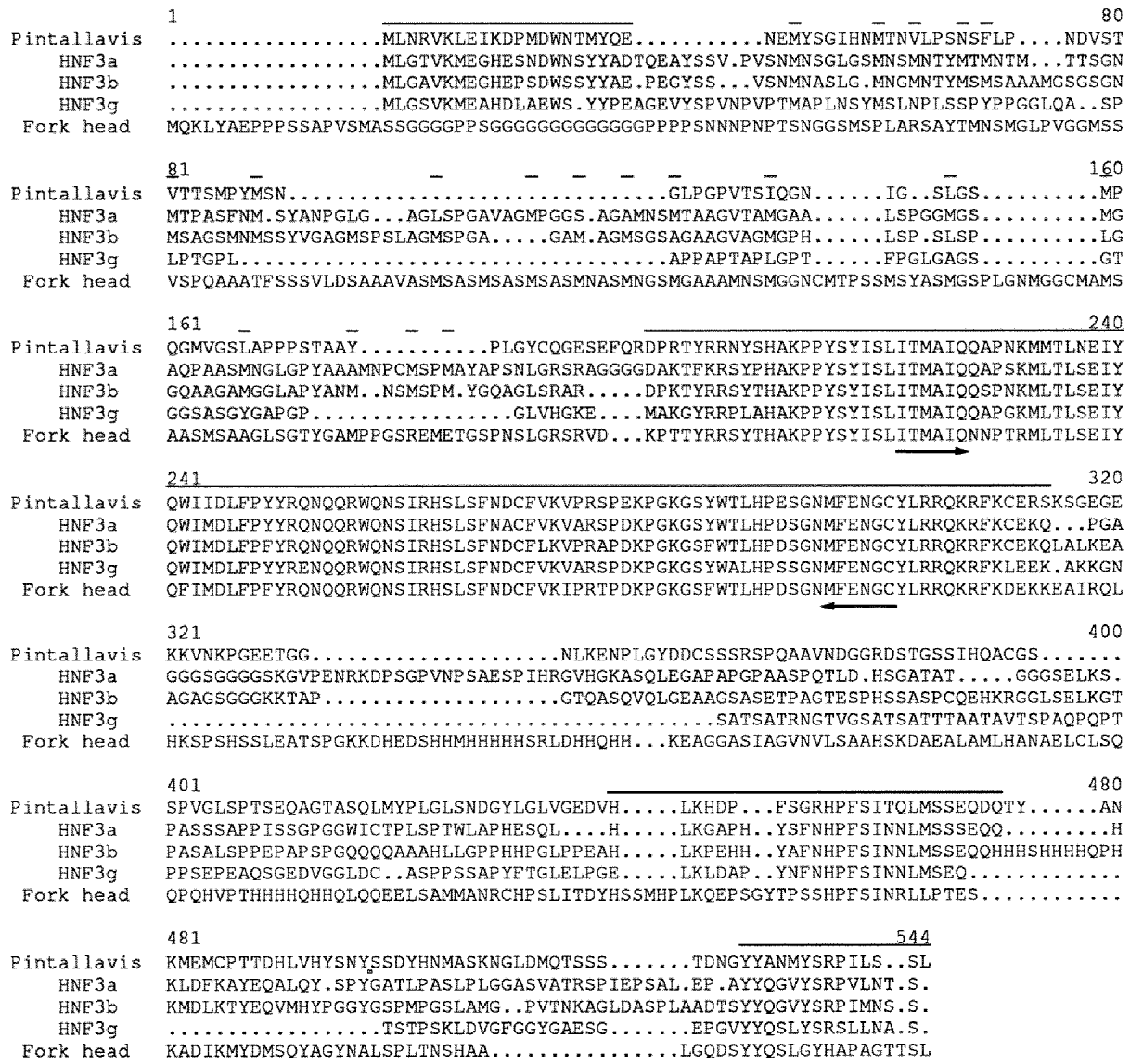
HNF-3 beta, and 42% identity with *fork head*. The NH_2-terminal region of *Pintallavis* is similar to that of the HNF-3 genes; however, the rat genes are more closely related (~60%) to each other than to the frog gene. *Pintallavis* is therefore unlikely to be the *Xenopus* homologue of the rat HNF-3 genes. The conservation of several distinct domains between *Pintallavis*, the three HNF-3 genes and *fork head*, and the distinct patterns of expression of the vertebrate genes (see below) suggests that *Pintallavis* is a new member of this gene family.

Transient embryonic expression of Pintallavis

The period over which *Pintallavis* is expressed during embryonic development was examined by RNase protection. RNA was isolated from embryos at different embryonic stages and the expression of *Pintallavis* compared with that of EF-1 alpha, a gene expressed at similar levels in all cells of the *Xenopus* embryo (Krieg et al., 1989). The expression of *Pintallavis* is first detected in the late blastula (stage 9) embryo and its expression peaks in mid-gastrula (stage 12) embryos (Fig. 2A). *Pintallavis* expression declines at later stages and is barely detectable in the late tailbud-early tadpole stage (stage ~26). Thus, the transcription of the *Pintallavis* gene is zygotic and transient.

To determine the time of onset of *Pintallavis* expression more precisely, RNA samples were prepared at 1-hour intervals from batches of timed embryos from the same frog starting at the early blastula stage (stage 6) and analysed by RNase protection (Fig. 2B). There is a large increase in abundance of EF-1 alpha transcripts in the 1-hour interval between stages ~8 and ~9. This marks the mid-blastula transition, the time at which zygotic transcription commences

B



(Newport and Kirschner, 1982; Krieg et al., 1989). *Pintallavis* expression appears first at stage ~8 and its levels increase markedly by stage ~9. These result show that *Pintallavis* is transcribed at the midblastula transition.

Expression of Pintallavis *in the dorsal lip and midline cells*

To determine the regional pattern of expression of *Pintallavis* RNA we performed in situ hybridization analyses by whole-mount labelling of albino embryos using RNA probes (Harland, 1991). Expression of *Pintallavis* RNA was first detected in the marginal zone of late blastulae (stage 8-8½) in an arc of ~30º (not shown). The level of expression of *Pintallavis* RNA then increases and by stage ~9½, before the appearance of the dorsal lip, *Pintallavis* expression occupies about a 60º arc in the marginal zone (Fig. 3A). Labelling of pigmented embryos shows that the early expression of *Pintallavis* is confined to the dorsal, less pigmented, side (not shown). By stage 10 when the dorsal lip is first apparent there is a coincidence of the organizer and the expression of *Pintallavis* RNA (Fig. 3B).

After the onset of gastrulation (stage 10⁺-11) *Pintallavis* RNA is present at high levels in cells undergoing convergent-extension movements at the midline, and at lower abundance in more lateral regions (Fig. 3C). By stage 11½ a few weakly labelled cells were detected all around the blastopore (not shown). At stage 12 *Pintallavis* RNA is expressed in the dorsal midline (Fig. 3D, E) including the prechordal region. Expression of *Pintallavis* RNA in the axial mesoderm is graded with the highest levels detectable in posterior regions (Fig. 3D, F, 4G). At this stage *Pintallavis* RNA is expressed at very low levels in the paraxial mesoderm that gives rise to the somites (Fig. 3D and not shown). Expression of *Pintallavis* decreases at later stages of development and by the mid-late neurula stage (stage 18-20) there is very low expression in the midline (Fig. 3F). By the late tailbud-early tadpole stage (stage ~28)

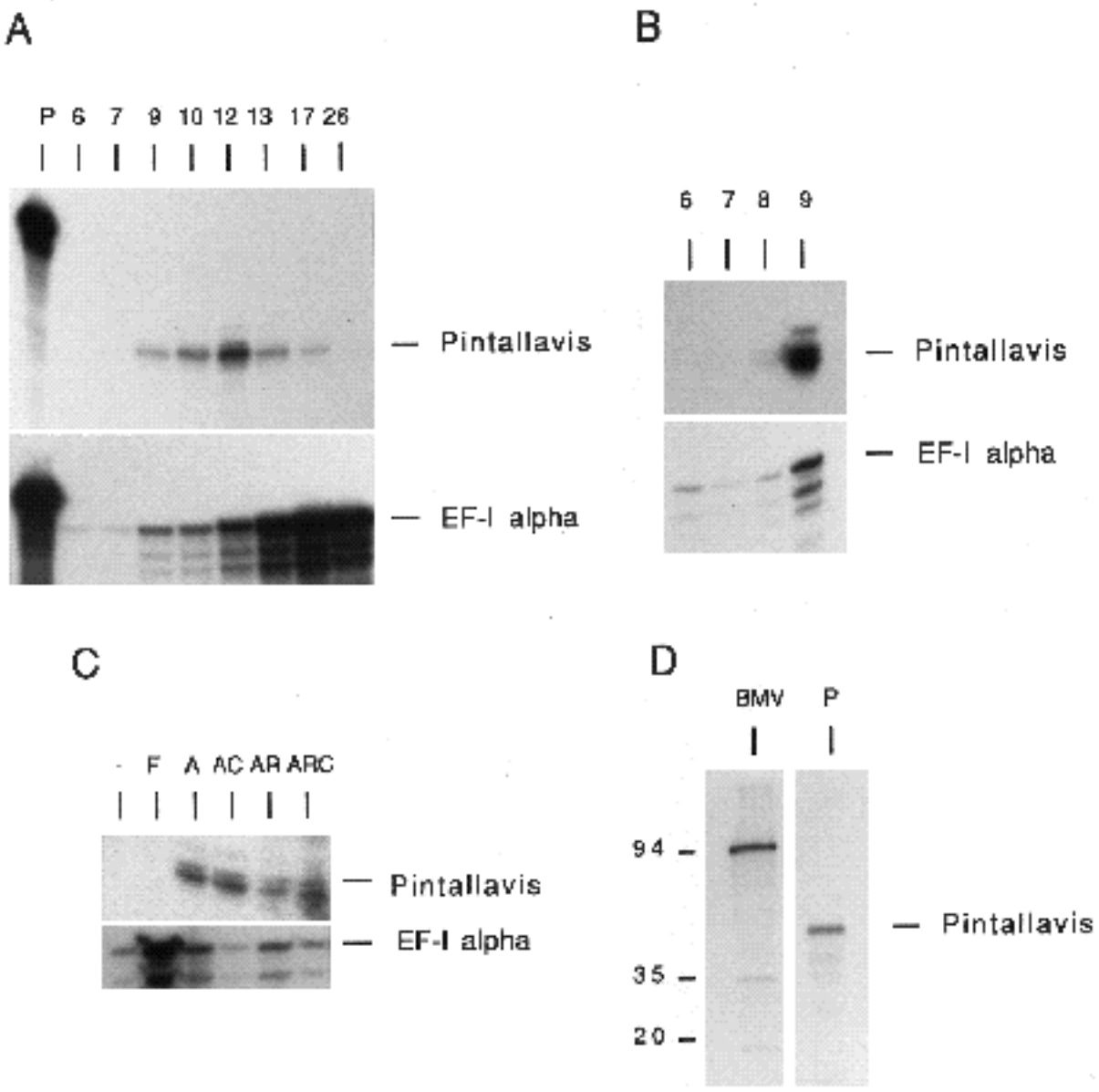


Fig. 2. *Temporal expression, induction and translation of* Pintallavis *RNA.* (A) Temporal expression of *Pintallavis* RNA during embryonic development assayed by RNase protection. P, probe. Numbers refer to embryonic stages. 6-7, early blastula; 9, late blastula; 10, early gastrula; 12, midgastrula; 13, late gastrula-early neurula; 17, neurula; 26, late tailbud. The profile of EF-I alpha shows low maternal levels of expression which start to increase at the late blastula stage (stage 9). (B) *Pintallavis* is first expressed at the midblastula transition as assayed by RNase protection. Numbers refer to embryonic stages. The midblastula transition occurs at stage 8. Note that EF-I alpha transcripts increase rapidly after the midblastula transition. (C) *Pintallavis* RNA is induced in animal caps by activin (A) but it is not detected in untreated animal caps (−) or caps induced by bFGF (F). RA treatment (10^{-8}M) of activin-induced animal caps (AR) decreases the level of *Pintallavis* expression. Cycloheximide treatment of activin-induced animal caps (AC) does not affect *Pintallavis* expression, while simultaneous treatment of activin-induced caps with cycloheximide and RA (ARC) prevents the repression of *Pintallavis* RNA levels observed after RA treatment. Note that cycloheximide treatment results in consistently lower levels of EF-I alpha transcripts. All samples were assayed at stage 10 with the exception of animal caps treated with FGF, which were assayed for *Pintallavis* expression at stage 20. At stage 10, FGF-treated caps do not express *Pintallavis* RNA (not shown). In A-C the RNase-protection products detected with the *Pintallavis* RNA probe include a major and one or two minor bands. All these bands are specific for the *Pintallavis* probe with the weaker bands probably representing alternate digestion products proper of the conditions used or allelic variations of the gene. (D) Translation of synthetic *Pintallavis* RNA (P) in reticulocyte lysates results in the production of a protein of ~40-45×10^3 M_r. As control, the translation products of Bromo Mosaic virus RNA (BMV) are shown.

Pintallavis RNA is expressed solely in the tailbud and at the ventral midline of the newly formed posterior spinal cord (Fig. 3G). Very low levels of *Pintallavis* RNA were also detected in the tailbud of older tadpoles (stage 36; not shown).

Sections of whole-mount labelled embryos at stage 12½

confirmed that *Pintallavis* RNA is present at high levels in the notochord (Fig. 3I) with lower levels in the prechordal plate mesoderm (Fig. 3J). These sections also revealed that *Pintallavis* is expressed in the midline of the neural plate overlying the notochord but not above the prechordal mesoderm (Fig. 3H-J). Moreover, transverse sections show that the expression of *Pintallavis* RNA in the midline of the neural plate appears to be restricted to deep layer cells (not shown). *Pintallavis* RNA is also expressed in the midline endodermal cells lining the archenteron (Fig. 3H, I). Thus, *Pintallavis* RNA is expressed by midline cells of the three germ layers.

Pintallavis *expression in the dorsal lip requires dorsalizing signals*

The presence of *Pintallavis* RNA in the dorsal lip suggests that the expression of this gene requires signals that confer dorsal axial properties. To examine this possibility we determined the expression of *Pintallavis* in embryos in which the development of axial structures had been modified. Treatment of blastula stage embryos with high doses of Li^+ results in the acquisition of a dorsal character by all mesodermal cells (Kao and Elinson, 1986; Kao et al., 1988). Inversely, irradiation of fertilized eggs before first cleavage with high levels of UV light results in the acquisition of an exclusively ventral character by all mesodermal cells (Scharf and Gerhart, 1983; Cooke and Smith, 1987). To determine whether *Pintallavis* expression is modified in accordance with the dorso-ventral character of the mesoderm, embryos were treated with high doses of Li^+ or irradiated with UV light. In Li^+-treated embryos, the normal region of expression of *Pintallavis* at stage 9-10 in the dorsal lip (Fig. 4A) expands to include the entire marginal zone (Fig. 4B). Inversely, *Pintallavis* expression is completely absent in UV-treated embryos (Fig. 4C). These results suggest that *Pintallavis* expression in vivo requires signals that confer dorsal character to the mesoderm.

The early expression of *Pintallavis* is more restricted than that of the *Xenopus Brachyury* gene (*Xbra*; Smith et al., 1991). However, we observed that the expression of *Xbra* RNA in the marginal zone at stage 9-10 is also graded, with high levels in the dorsal lip region and low levels ventrally (Fig. 4D). Li^+ treatment abolished this graded expression resulting in a high levels of Xbra in the entire marginal zone (Fig. 4E). In contrast, UV irradiation resulted in a low level of expression of Xbra RNA throughout the entire marginal zone (Fig. 4F).

The presence of *Pintallavis* in the dorsal lip together with the alteration of its expression pattern by Li^+ and UV suggests that it is regulated by factors that induce dorsal mesodermal cell types (Smith, 1987; Smith et al., 1990; Thomsen et al., 1990). To test this possibility, *Pintallavis* expression was assayed in animal cap explants treated with activin at concentrations that induce dorsal mesoderm or with basic fibroblast growth factor (bFGF) at concentrations that induce posterior paraxial and ventral mesoderm. No expression of *Pintallavis* was detected in untreated animal caps or in caps treated with FGF as determined at stages 10 and 20 by in situ hybridization (not shown) and RNase protection assays (Fig. 2C, lane F). As a positive control, FGF was found to induce Xbra expression (not shown; Smith et al., 1991). By contrast, exposure of animal caps to activin resulted in a significant induction of *Pintallavis* mRNA (Fig. 2C, lane A). This result is consistent with the ability of activin but not FGF-treated caps to behave as an organizer (Ruiz i Altaba and Melton, 1989c; Cooke, 1989) and to induce notochord tissue (Smith, 1987; Slack et al., 1988; Kimmelman and Kirschner, 1988).

In situ hybridization analyses revealed that in activin-treated animal caps expression of *Pintallavis*, unlike that of *Brachyury*, was usually restricted to a small region of the induced cap (not shown). This provides further evidence for the heterogeneity of animal cap cells that can be revealed by mesoderm-inducing factors (Ruiz i Altaba and Jessell, 1991a; Sokol and Melton, 1991).

The rapid expression of *Pintallavis* at the mid-blastula transition raised the possibility that its expression in the dorsal lip is a direct response to induction. To test this possibility animal caps were treated with activin in the presence of cycloheximide, to prevent protein synthesis (Cascio and Gurdon, 1987; Rosa, 1989). Treatment with cycloheximide did not change the levels of *Pintallavis* RNA induced by activin at stage 10 (Fig. 2C, lane AC). This suggests that the transcription of *Pintallavis* is a direct response to dorsal mesoderm-inducing factors.

Treatment of pre-gastrula *Xenopus* embryos with retinoic acid (RA) results in a dose-dependent truncation of axial structures in an anterior-to-posterior progression (Durston et al., 1989; Sive et al., 1990; Ruiz i Altaba and Jessell, 1991a). This effect is mediated, in part, by inhibiting the development of dorsal mesoderm (Ruiz i Altaba and Jessell, 1991a). Moreover, in animal caps treated with high concentrations of activin, RA modifies the character of the induced mesoderm from anterior-dorsal to posterior-ventral in a concentration-dependent manner (Ruiz i Altaba and Jessell, 1991a). The restricted expression of *Pintallavis* in dorsal-axial structures raised the possibility that its expression may be sensitive to RA. Exposure of activin-induced animal caps to RA resulted in a decrease in the level of *Pintallavis* mRNA at stage 10 (Fig. 2C, lane AR). This effect appears to require protein synthesis, since exposure of RA and activin-treated caps to cycloheximide blocked the ability of RA to repress *Pintallavis* RNA (Fig. 2C, lane ARC).

Pintallavis *is not expressed in exogastrula ectoderm*

In *Xenopus*, cells that are located at the midline of the neural plate have been termed the notoplate. The notoplate is derived from cells of the dorsal NIMZ, a cell group adjacent to the dorsal lip mesoderm before gastrulation (Jacobson, 1981; Keller and Danilchik, 1988). The notoplate is formed by the convergent and extension movements of NIMZ cells during gastrulation. The neural expression of *Pintallavis* in midline neural plate cells could therefore result from its earlier expression in cells in the dorsal NIMZ, the notoplate precursors, or may require inductive signals from the notochord that are transmitted during gastrulation. However, the small size of the dorsal NIMZ makes it difficult to determine whether these cells express *Pintallavis* prior to gastrulation.

To determine whether *Pintallavis* is expressed by notoplate cells in the absence of underlying notochord, we

examined the expression of *Pintallavis* in complete exogastrulae. In exogastrulae, the mesoderm does not involute under the ectoderm but the extension movements of the notochord and notoplate still occur, resulting in the elongation but lack of apposition of these two midline structures (Ruiz i Altaba, 1992). In complete exogastrulae examined at stage 12-12½, *Pintallavis* was present in the region that contains endoderm and mesoderm, where its expression was detected in the notochord and prechordal mesoderm (Fig. 4G-I). Within the mesoderm, *Pintallavis* expression exhibited a posterior-to-anterior gradient (Fig. 4G). In contrast, *Pintallavis* RNA was not detected in the ectodermal region of the exogastrulae, despite the presence of notoplate cells, as defined by their convergent extension movements and of extensive neural tissue (Fig. 4I; Ruiz i Altaba, 1992). Similarly, *Xbra* expression was absent from the ectoderm of exogastrulae (Fig. 4J). These results show that *Pintallavis* is not expressed in the notoplate of exogastrulae. Expression of *Pintallavis* by cells at the midline of the neural plate in normal embryos may therefore be dependent on inductive or maintenance signals from the underlying notochord.

Fig. 3. *Localization of* Pintallavis *RNA in developing embryos by whole-mount in situ hybridization*. (A) Ventral view of a late blastula stage embryo (stage 9) showing the expression of *Pintallavis* RNA in a >60º arc. (B) Ventral view of an early gastrula stage embryo (stage 10) showing that the *Pintallavis* expression marks the site of appearance of the dorsal lip. (C) *Pintallavis* expression is detected in cells undergoing convergent extension movements at stage 10^{+}-11, after the onset of gastrulation. Note the spread of *Pintallavis* RNA into regions lateral to the dorsal midline, which bisects the growing dorsal lip. As with other RNAs, the hybridization signal of *Pintallavis* at these early stages is nuclear. (D) At stage ~12, *Pintallavis* RNA is detected in the dorsal midline of the entire embryo, including the anterior region (upper right corner). Note the low levels of expression in paraxial tissues. The blastopore marks the posterior pole. (E) High magnification photograph of the posterior midline of a late gastrula stage embryo (stage 12½-13) viewed from the dorsal side. (F) *Pintallavis* expression in the midline rapidly decreases by the late neurula stage (stage 17-18). Note the graded posterior-to-anterior expression of *Pintallavis* as seen in this dorso-lateral view. The posterior of the embryo is at the lower right of the picture. The weak labelling represents non-specific background in the archenteron. (G) Lateral view of a late tailbud-early tadpole stage embryo (stage 28) showing expression of *Pintallavis* exclusively in the tailbud and in the newly formed floor plate of the neural tube (arrowhead). Anterior is to the right. (H) Cross-section of a late gastrula-early neurula (stage 13) embryo labelled in whole-mount showing the expression of *Pintallavis* in the midline of the three germ layers. Dorsal side is at the top. (I) Mid-sagittal section of a stage 12½ embryo showing expression of *Pintallavis* in the posterior mesoderm (notochord), the overlying midline cells of the neural plate and the endodermal cells of the archenteron roof. Note the weak expression of *Pintallavis* in the head mesoderm. (Anterior is to the right). (J) High-magnification photograph of a sagittal section of a stage 12½ embryo showing expression of *Pintallavis* in the prechordal plate mesoderm (arrowheads) but not in the overlying neural plate. Scale bars: A, B, D, F, G=200 μm; C, H-J=100 μm; E=50 μm.

Injection of synthetic Pintallavis *RNA perturbs axial development*

To begin to examine the function of *Pintallavis* we injected synthetic RNA into fertilized eggs. The injected *Pintallavis* RNA is functional as its translation in vitro (see Materials and methods) yielded a protein of 40-45×10^3 M_r (Fig. 2D), in agreement with its predicted size (Fig. 1A).

Pintallavis RNA was first injected into the equatorial region of a single cell of two-cell stage embryos. Rhodamine/lysine/dextran was routinely coinjected as a lineage tracer to identify the injected side later in development. Since the first cleavage plane defines the axis of bilateral symmetry in most embryos (Klein, 1987), the phenotypes observed in the injected and uninjected sides were compared. We first describe the general features of the phenotypes observed and then analyse mesodermal and neural tissues in more detail.

The injected halves of tadpole-stage embryos displayed a markedly shortened axis, a decrease in or absence of anterior neural structures and a widening of the neural folds of the future hindbrain and spinal cord (Fig. 5A-C; Table 1). In neurula embryos, the head folds and the eye primordia were usually absent from the injected side (Fig. 5B). The cement gland, an anterior ectodermal structure, was also markedly reduced in size (Fig. 5A, B). At tadpole stages, embryos lacked an eye (Fig. 5D) and exhibited a deformed hindbrain with malformed melanocytes (not shown).

The defects in axial development observed after injection of *Pintallavis* RNA were not observed in control embryos or in embryos injected with RNA encoding a frame-shift mutant, that is predicted to generate a mutated protein that lacks virtually the entire putative DNA-binding domain (Table 1A), or antisense *Pintallavis* (not shown). Similarly, injection of RNA encoding the rat *Isl-1* homeobox gene (Thor et al., 1991) did not cause these defects (not shown). Moreover, injection of *Wnt* RNAs produced hyperdorsalized and twinned embryos (not shown, see McMahon and Moon, 1989; Smith and Harland, 1991; Sokol et al., 1991), phenotypes that are distinct from those obtained with *Pintallavis* RNA. Distinct phenotypes have also been obtained by injection of RNA encoding the homeobox gene *Xhox3* (Ruiz i Altaba and Melton, 1989b; Ruiz i Altaba et al., 1991) or the helix-loop-helix gene *MyoD* (Hopwood and Gurdon, 1990). Thus, it appears that the phenotype obtained after *Pintallavis* injection is specific.

We only occasionally (<5% of injected embryos, see Table 1) observed secondary axes after injection of *Pintallavis* RNA (not shown). This suggests that the action of *Pintallavis* differs from that of *Goosecoid*, another gene expressed transiently in the dorsal lip (Blumberg et al., 1991) since injection of *Goosecoid* RNA into the ventral region of early *Xenopus* embryos results in secondary axes (Cho et al., 1991).

Pintallavis *affects axial development when injected into blastomeres that give rise to mesodermal and neural tissues*

To determine the cell types in which *Pintallavis* overexpression can induce axial defects, we injected *Pintallavis* RNA into single blastomeres of 32-cell embryos, the fates of which are known (Table 1C; Dale and Slack, 1987a,b; Moody, 1987). Injection of *Pintallavis* RNA into blas-

tomeres that give rise to axial (C1) and paraxial (C2/C3) mesodermal structures resulted in embryos with axial defects (Table 1B). Injection of *Pintallavis* RNA into blastomeres that give rise to the dorsal equatorial region (C1) which forms the organizer and the axial mesoderm resulted in a high incidence of defective axes. In contrast, there was no obvious effect when *Pintallavis* RNA was injected into blastomeres that give rise predominantly to ventral mesoderm (C4; Table 1B, C).

Axial defects were also observed after injection of *Pintallavis* into blastomeres that normally give rise to neural tissue (A1/B1, B2; Table IB). The occurence of axial defects after injection both into blastomeres that give rise to mesodermal or neural cells suggests that *Pintallavis* overexpression affects the development of the neural axis indirectly by actions on mesoderm and directly by actions on ectoderm.

Table1. *Summary of results obtained after injection of* Pintallavis *RNA into one blastomere at the 2-cell stage (A) or into a single blastomere at the 32-cell stage (B)*

A. *Injection of* Pintallavis *RNA into 1 blastomere at the 2-cell stage*

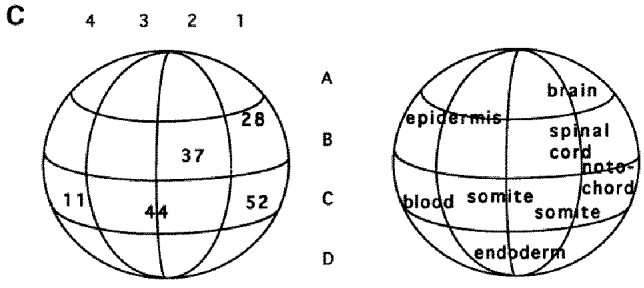
Sample (ng)	*n*	Defective axis	Normal axis head defects	Normal	% affected
Pintallavis					
0.5	182	125	5	52	69
1	147	106	3	38	72
2	147	102	2	43	69
5	75	67	0	8	89
Frame shift					
0.5	159	19	2	138	12
Uninjected	322	19	4	299	6

B. *Injection of* Pintallavis *RNA into 1 blastomere at the 32-cell stage*

Sample	*n*	Defective axis	Normal	% affected
500 pg				
A1/B1	68	19	49	28
B2	35	13	22	37
C1	208	108	100	52
C2/C3	386	170	216	44
C4	225	24	201	11
Uninjected	289	19	270	6

All embryos were scored for phenotype externally. Numbers in the sample column of A refer to the amount of the injected *Pintallavis* or control RNAs. In B, embryos were injected with 500 pg of *Pintallavis* RNA into different blastomeres. *n*, total number of embryos; Defective axis, number of embryos displaying specific axial defects (see text); Normal axis head defects, number of embryos showing head defects with normal axes (see text); Normal, number of normal embryos; % affected, percentage of embryos displaying specific axial defects; uninjected refers to control embryos. Embryos defective in gastrulation were not included in the sample.

C. *Schematic map of the 32-cell stage embryo and injection results*



This diagram shows the position of the different blastomeres and the incidence of axial defects following injections as described in (B) (left) and their normal fates according to Dale and Slack (1987a) (right). In this maps the animal pole is at the top and the dorsal side to the right.

Injection of Pintallavis *RNA does not transform paraxial into axial mesoderm*

The extension of the embryo along the A-P axis is thought to be dependent on forces generated by the paraxial mesoderm (Wilson et al., 1989). The shortened axis observed in injected embryos (Fig. 5C) could therefore be due to a change in fate of paraxial mesodermal cells. However, injected embryos had segmented somites and the diameter of the notochord appeared to be only slightly larger than in control embryos (Fig. 6; and not shown). In addition, the formation of muscle in activin-induced animal caps as detected by the expression of the 12/101 antigen (Kintner and Brockes, 1984) was similar in caps derived from *Pintallavis*-injected (*n*=45) and control embryos (not shown). These observations provide evidence that *Pintallavis* does not transform the paraxial somitic mesoderm into notochord. Thus, overexpression of *Pintallavis*, unlike *Xhox3* (Ruiz i Altaba and Melton, 1989a), results in a defective axis with apparently normal axial mesoderm. The defects that occur after injection of *Pintallavis* RNA are partly similar to those observed after treatment of early neurula-stage embryos with RA (Ruiz i Altaba and Jessell, 1991b).

Together, these results suggest that *Pintallavis* expression does not produce an obvious change in the fate of mesodermal cells even though it appears to alter their extension properties.

Pintallavis overexpression affects neural differentiation

Embryos injected at the 2-cell stage with *Pintallavis* RNA exhibited defects in anterior neural structures. At the neurula stage, the anterior neural folds were absent (Fig. 5B), and by the tadpole stage, anterior neural structures, including the eye were missing (Fig. 5D, 6C). Labelling of affected (*n*=10) and control embryos with a neural-specific marker, mAb Xen1 (Ruiz i Altaba, 1992) showed that forebrain structures are reduced in size (not shown). In contrast to the decrease in size of forebrain structures, there was an increase in the amount of posterior neural tissue (Fig. 6D, E, G, H), which sometimes formed multiple tubular structures (Fig. 6D, E).

This expansion of posterior neural tissue together with the ability of *Pintallavis* RNA to cause axial defects when injected into blastomeres that give rise to neural ectoderm (Table 1B, C) raised the possibility that *Pintallavis* causes neural differentiation when expressed in ectodermal cells. To test this possibility, explanted animal caps derived from embryos injected with *Pintallavis* RNA into the animal pole of one blastomere at the 2-cell stage were assayed for the

presence of neural tissue. Animal caps from injected embryos did not differentiate neural tissue as assessed by the lack of Xen1 immunoreactivity (*n*=68; not shown). Thus, the overproduction of neural tissue observed in injected embryos is unlikely to result from *Pintallavis* conferring neural properties to recipient ectodermal cells.

In chick embryos, floor plate grafts placed next to the dorsal neural tube induce ectopic ventral neurons while repressing dorsal neuronal differentiation (Yamada et al., 1991; Placzek et al., 1991). The normal expression of *Pintallavis* by midline neural plate cells, which later become the floor plate, raises the possibility that expression of *Pintallavis* in dorsal regions affects adjacent neural tissue in a manner analogous to that of floor plate grafts. Since floor plate and notochord grafts repress dorsal neuronal differentiation (Yamada et al., 1991), we examined whether ectopic *Pintallavis* expression affects the differentiation of dorsal neurons in the spinal cord. At the early tailbud stage (stage ~24-26) Rohon-Beard sensory neurons (Hughes, 1957) can be identified in the dorsal spinal cord by their morphology and by expression of the HNK-1 epitope (Figs 5E, 6F; Nordlander, 1989). Embryos injected into one side with *Pintallavis* RNA displayed a unilateral reduction or complete loss of Rohon-Beard neurons (*n*=10; Figs 5E, F, 6F-H). In addition, in injected embryos there was a decrease in the number of melanocytes and dorsal fin mesenchymal cells which derive from the neural crest (not shown).

Further experiments are required to determine whether *Pintallavis* expression mimicks a floor plate graft in inducing ectopic ventral neurons in adjacent neural ectoderm.

These results provide evidence that *Pintallavis* overexpression represses the differentiation of anterior neural structures but increases the amount of neural tissue in the posterior neural tube. In addition, there appears to be a suppression of neuronal differentiation in the dorsal spinal cord.

Discussion

The frog gene *Pintallavis* is related to the rat *HNF-3* and fly *fork head* genes. *Pintallavis* is transiently expressed by midline cells in all three germ layers of gastrula and neurula embryos. The phenotype detected after overexpression provides no evidence that *Pintallavis* is involved in repressing segmentation in midline structures but suggests a function in the development of the neural axis.

Pintallavis *is expressed in the organizer*

Pintallavis is expressed in the dorsal mesoderm soon after the mid-blastula transition. Like *Brachyury* (Smith et al., 1991), *Goosecoid* (Cho et al., 1991) and *Xlim1* (Taira et al., 1992), three other genes expressed in the organizer, *Pintallavis* transcription is a direct response to mesodermal induction and requires dorsalizing signals. The coincident expression of *Pintallavis*, *Brachyury*, *Goosecoid* and *Xlim1* in the dorsal lip raises the possibility that these genes interact to determine the different cell types that derive from the organizer and to control their patterning along the A-P axis. Moreover, the differing lateral extents of the graded expression of *Pintallavis* and *Brachyury* observed in mesodermal cells adjacent to the organizer may be important in patterning the mesoderm. Indeed, studies of the mouse *Brachyury* gene suggest that different levels of its function contribute to mesodermal patterning along the A-P axis (Yanagisawa, 1990; Hermann, 1991).

At later stages of development, the tail bud has organizer-like properties, producing patterned mesodermal and neural tissues (Spofford, 1948). *Pintallavis*, like *Xhox3* (Ruiz i Altaba and Melton, 1989a; Ruiz i Altaba et al., 1991) and *Brachyury* (not shown), is expressed in this region, consistent with the combined function of these genes in the induction and patterning of axial tissues.

Pintallavis *is expressed in the midline*

After the onset of gastrulation *Pintallavis* is expressed in axial mesodermal cells of the prechordal plate and notochord, and in the endoderm of the archenteron roof. Expression of *Pintallavis* in midline mesodermal cells is graded with lowest levels in the prechordal plate and highest levels in the posterior notochord. *Pintallavis* is also expressed by cells at the midline of the neural plate that gives rise to the floor plate. A comparison of *Pintallavis* expression in normal and exogastrula embryos suggests that this gene is not expressed in notoplate cells in the absence of inducing signals from the notochord. Thus, *Pintallavis* is expressed in midline mesodermal structures derived from the organizer and may be rapidly induced in the overlying neural plate. The expression of *Pintallavis* in both the notochord and the midline cells of the neural plate may reflect the common properties of these two midline axial structures (Jessell et al., 1988; Wagner et al., 1990; Jessell and Dodd, 1992). It remains unclear whether the notochord induces the expression of *Pintallavis* in endodermal cells of the archenteron roof.

The expression patterns of *Pintallavis* and XFKH1 (Dirksen and Jamrich, 1992) are similar but differ in two aspects. First, Pintallavis is expressed before stage 9 in a restricted region (~30º of arc) of the dorsal marginal zone, whereas XFKH1 is transcribed at similar stages over a much wider area (>90º) and only later becomes restricted. By stage 10, the onset of gastrulation, the expression of both genes is coincident with the site of dorsal lip formation. Second, at the late neurula stage, Pintallavis expression decreases rapidly and anterior regions exhibit little or no Pintallavis mRNA. In contrast, XFKH1 mRNA is still evident in midline cells that will form the floor plate of the hindbrain.

The identity of mesodermal cells is not affected by Pintallavis *overexpression*

Injection of *Pintallavis* RNA into cells that give rise to axial and paraxial mesoderm results in embryos that display pronounced axial defects. However, there are no overt defects in the posterior mesoderm. Expression of high levels of *Pintallavis* RNA in presumptive somitic mesodermal cells may disturb their normal movements without affecting their specification. Elevated levels of *Pintallavis* RNA could also cause anterior mesodermal cells to acquire a more posterior character. In this case, the embryo might elongate less, since posterior axial cells appear to undergo less pronounced extension movements than their anterior counterparts (Symes and Smith, 1987; Keller and Danilchik, 1988). In

either case, it appears that expression of *Pintallavis* in presumptive somitic mesoderm affects only some properties of the recipient cells without altering their overall determination. This is similar to the result observed after ectopic expression of myoD in *Xenopus*, where non-muscle cells acquire some muscle properties; for example, the expression of the alpha actin gene, in the absence of myogenesis (Hopwood and Gurdon, 1990).

Overexpression of Pintallavis *affects neural development*

Pintallavis overexpression has distinct neural phenotypes. First, the development of anterior neural structures, such as the eye, is repressed. Second, there is an overproduction of posterior neural tissue. Third there is a repression of dorsal neuronal differentiation. The effects of overexpression of *Pintallavis* on the differentiation of anterior neural structures could be due to an increase in the low level of *Pintallavis* RNA that is normally present in the prechordal plate. As a consequence, these cells may acquire a more posterior character eliminating their ability to induce forebrain structures.

The increase in posterior neural tissue observed after injection of *Pintallavis* RNA could reflect an enhanced neural-inducing activity of paraxial mesoderm. In *Xenopus*, neural-inducing activity is concentrated in the notochord, where *Pintallavis* expression is high, but is also present at much lower levels in the paraxial mesoderm (Jones and Woodland, 1989), where *Pintallavis* expression is very low. Indeed, preliminary experiments suggest that presumptive somitic mesoderm isolated from embryos injected with *Pintallavis* RNA has a greater ability to induce neural tissue in recombinates with animal cap ectoderm than equivalent regions of mesoderm from control embryos (not shown).

The neural phenotype observed after *Pintallavis* overexpression could also be related to its expression at the midline of the neural plate, which later gives rise to the floor plate. Since the floor plate is a ventral neural structure present in the hindbrain and spinal cord but absent from the forebrain, the neural phenotypes observed after injection of *Pintallavis* RNA could result from the partial acquisition of floor plate properties by neural cells. The repression of the differentiation of anterior neural cells could result from the acquisition of floor plate properties in the anterior neural tissue. Similarly, repression of the differentiation of dorsal neurons could reflect a dorsal expansion of floor plate properties (Yamada et al., 1991).

Pintallavis *and neural induction*

The pattern of expression of *Pintallavis* RNA in midline cells with inductive properties, together with the neural phenotypes that result from its injection suggest an involvement of *Pintallavis* in neural induction.

Pintallavis alone, however, does not appear to be able to direct neural differentiation, since animal cap ectoderm isolated from injected embryos does not develop into neural tissue. Moreover, *Pintallavis* is not expressed by all neural plate cells which might be expected if its expression were required in ectodermal cells that become neural. Indeed, the lateral extent of the neural plate is thought to be regulated by the propagation, from cell to cell, of a midline-derived neuralizing signal combined with a gradual loss of competence of the responding ectoderm (Leussink, 1970; Nieuwkoop and Albers, 1990; Servetnick and Grainger, 1991). In addition, *Pintallavis* expression in exogastrula embryos is not detected in the notoplate, a cell group implicated in neural induction (Ruiz i Altaba, 1992).

Neural induction may depend on the combined action of *Pintallavis* and other mesodermal genes, such as *Brachyury*. Such genes, expressed in the dorsal lip, could initiate the production of a neural-inducing signal. The spread of this signal, from the dorsal lip to adjacent ectoderm, and its subsequent propagation through the ectoderm (Leussink, 1970) may, however, be independent of the mesodermal genes responsible for the initiation of the signal. Thus, cells of the notoplate may acquire neural-inducing properties (Ruiz i Altaba, 1992) in the absence of *Pintallavis* expression.

The graded expression of *Pintallavis* along the A-P axis of the mesodermal midline could contribute to the ability of the notochord to induce a floor plate in the overlying neural ectoderm. The expression of *Pintallavis* by midline neural plate cells, the floor plate precursors, may then be involved in specifying the floor plate and its signalling properties.

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References

Abo, T. and Balch, C. M. (1981). A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J. Immunol*. **127**, 1024-1029.

Blumberg, B., Wright, C. V. E., De Robertis, E. M. and Cho, K. W. Y. (1991). Organizer-specific homeobox genes in *Xenopus laevis* embryos. *Science* **253**, 194-196.

Cascio, S. and Gurdon, J. B. (1987). The initiation of new gene transcription during *Xenopus* gastrulation requires immediately preceding protein synthesis. *Development* **100**, 297-305.

Cho, K. W. Y., Blumberg, B., Steinbeisser, H. and De Robertis, E. M. (1991). Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene *Goosecoid*. *Cell* **67**, 1111-1120.

Clarke, J. D. N., Holder, N., Soffe, S. R. and Storm-Mathisen, J. (1991). Neuroanatomical and functional analysis of neural tube formation in notochordless *Xenopus* embryos: laterality of the spinal cord is lost. *Development* **112**, 499-516.

Cooke, J. and Smith, J. C. (1987). The mid-blastula cell-cycle transition and the character of mesoderm in UV-induced nonaxial *Xenopus* development. *Development* **99**, 197-210.

Dale, L. and Slack, J. M. W. (1987a). Fate map for the 32-cell stage of *Xenopus laevis. Development* **99**, 527-551.

Dale, L. and Slack, J. M. W. (1987b). Regional specification within the mesoderm of early embryos of *Xenopus laevis. Development* **100**, 279-295.

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

Dirksen, M. L. and Jamrich, M. (1992). A novel, activin-inducible, blastopore lip-specific gene of *Xenopus laevis* contains a *fork head* DNA-binding domain. *Genes Dev.* **6**, 599-608.

Dixon, J. and Kintner, C. R. (1989). Cellular contacts required for neural induction in *Xenopus* embryos: evidence for two signals. *Development* **106**, 749-757.

Durston, A. J., Timmermans, J. P. M., Hage, W. J., Hendricks, H. F. J., de Vries, N. J., Heideveld, M. and Nieuwkoop, P. D. (1989). Retinoic acid causes an anteroposterior transformation of the developing central nervous system. *Nature* **340**, 140-144.

Gerhart, J. and Keller, R. (1986). Region-specific cell activities in amphibian gastrulation. *Ann. Rev. Cell Biol.* **2**, 201-229.

Hamburger, V. (1988). *The Heritage of Experimental Embryology. Hans Spemann and the Organizer.* Oxford: Oxford University Press.

Harland, R. M. (1991). In situ hybridization: an improved whole mount method for *Xenopus* embryos. *Meth. Enzymol.* **36**, 675-685.

Hatta, K., Kimmel, C. B., Ho, R. K. and Walker, C. (1991). The cyclops mutation blocks specification of the floor plate of the zebrafish central nervous system. *Nature* **350**, 339-341.

Hemmati-Brivanlou, A., Frank, D., Bolce, M. E., Brown, B. D., Sive, H. L. and Harland, R. M. (1990). Localization of specific mRNAs in *Xenopus* embryos by whole-mount *in situ* hybridization. *Development* **110**, 325-330.

Hemmati-Brivanlou, A. and Harland, R. M. (1989). Expression of an *engrailed*-related protein is induced in the anterior ectoderm of early *Xenopus* embryos. *Development* **106**, 611-617.

Hermann, B. G. (1991). Expression pattern of the *Brachyury* gene in whole-mount T^{wis}/T^{wis} mutant embryos. *Development* **113**, 913-917.

Holtfreter, J. (1933). Die totale exogastrulation, eine Selbstablosung des Ektoderms von Entomesoderm. Entwicklung und funktionelles Verhalten nervenloser Organe. *Wilhelm Roux Arch. EnwMech. Org.* **129**, 669-793.

Hopwood, N. and Gurdon, J. B. (1990). Activation of muscle genes without myogenesis by ectopic expression of MyoD in frog embryo cells. *Nature* **347**, 197-200.

Hughes, A. F. (1957). The development of the primary sensory neurons in Xenopus laevis (Daudin). *J. Anat. Lond.* **91**, 323-338.

Jacobson, A. G. (1981). Morphogenesis of the neural plate and tube. In *Morphogenesis and Pattern Formation* (ed. Connelly T.G. et al.) pp. 233-263. Raven Press: New York.

Jessell, T. M., Bovolenta, P., Placzek, M., Tessier-Lavigne, M. and Dodd, J. (1988). Polarity and patterning in the neural tube: the orgin and role of the floor plate. *Ciba Found. Symp.* **144**, 255-280.

Jessell, T. M. and Dodd, J. (1992). Floor plate-derived signals and the control of neural cell pattern in vertebrates. *Harvey Lecture Series.* (in press).

Jones, E. A. and Woodland, H. R. (1989). Spatial aspects of neural induction in *Xenopus laevis. Development* **107**, 785-791.

Jürgens, G. and Weigel, D. (1988). Terminal versus segmental development in the Drosophila embryo: the role of the homeotic gene *fork head. Roux's Arch. Devl. Biol.* **197**, 345-354.

Kao, K. R. and Elinson, R. P. (1988). The entire mesodermal mantle behaves as Spemann's organizer in dorso-anterior enhanced *Xenopus laevis* embryos. *Devl. Biol.* **127**, 64-77.

Kao, K. R., Masui, Y. and Elinson, R. P. (1986) Lithium-induced respecification of pattern in *Xenopus laevis* embryos. *Nature* **322**, 371-373.

Keller, R. and Danilchik, M. (1988). Regional expression, pattern and timing of convergence and extension during gastrulation of *Xenopus laevis. Development* **103**, 193-209.

Keller, R., Danilchik, M., Gimlich, R. and Shih, J. (1985). Convergent extension and cell intercalation during gastrulation of *Xenopus laevis*. In *Molecular Determinants of Animal Form.* (ed. G.M. Edelman). pp. 111-141. Alan Liss: New York.

Kimelman, D. and Kirschner, M. (1987). Synergistic induction of mesoderm by FGF and TGFβ and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. *Cell* **61**, 859-877.

Kingsbury, B. F. (1930). The developmental significance of the floor-plate of the brain and spinal cord. *J. Comp. Neurol.* **50**, 177-207.

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

Kintner, C. R. and Melton, D. A. (1987). Expression of the *Xenopus* N-CAM RNA in ectoderm is an early response to neural induction. *Development* **99**, 311-325.

Klein, S. L. (1987). The first cleavage furrow demarcates the dorsal-ventral axis in *Xenopus* embryos. *Devl. Biol.* **120**, 299-304.

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

Krieg, P. A., Varnum, S. M., Wormington, M. and Melton, D. A. (1989). The mRNA encoding elongation factor 1 alpha (EF-1 alpha) is a major transcript at the midblastula transition in *Xenopus. Devl. Biol.* **133**, 93-100.

Lai, E., Prezioso, V. R., Smith, E., Litvin, O., Costa, R. H. and Darnell, J. E. (1990). HNF-3A, a hepatocyte-enriched transcription factor of novel structure is regulated transcriptionally. *Genes Dev.* **4**,1427-1436.

Lai, E., Prezioso, V. R., Tao, W., Chen, W. S. and Darnell, J. E. (1991). Hepatocyte nuclear factor 3a belongs to a gene family in mammals that is homologous to the Drosophila homeotic gene *fork head*. *Genes Dev*. **5**, 416-427.

Leussink, J. A. (1970). The spatial distribution of inductive capacities in the neural plate and archeteron roof of urodeles. *Neth. J. Zool.* **20**, 1-79.

Lumsden, A. and Keynes, R. (1989). Segmental patterns of neuronal development in the chick hindbrain. *Nature* **337**, 424-428.

McMahon, A. P. and Moon, R. T. (1989). Ectopic expression of the proto-oncogene int-1 in *Xenopus* embryos leads to duplication of the embryonic axis. *Cell* **58**, 1075-1084.

Mangold, O. (1933). Uber die Induktionsfahigkeit der verschiedenen Bezirke der Neurula von Urodelen. *Naturwissenshaften* **21**, 761-766.

Melton, D. A. (1985). Injected anti-sense RNAs specifically block messenger RNA translation in vivo. *Proc. Nat. Acad. Sci. USA* **82**, 144-148.

Moody, S. A. (1987). Fates of the blastomeres of the 32 cell-stage *Xenopus* embryo. *Devl. Biol.* **122**, 300-319.

Newport, J. and Kirschner, M. (1982). A major developmental transition in early *Xenopus* embryos: II. Control of the onset of transcription. *Cell* **30**, 687-696.

Nieuwkoop, P. D. and Albers, B. (1990). The role of competence in the craniocaudal segregation of the central nervous system. *Dev. Growth Differ*. **32**, 23-31.

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

Nieuwkoop, P. D., Boterenbrood, E. C., Kremer, A., Bloesma, F. F. S. N., Hoessels, E. L. M. J., Meyer, G. and Verheyen, F. J. (1952). Activation and organization of the central nervous system in amphibians. *J. Exp. Zool.* **120**, 1-108.

Nordlander, R. H. (1989). HNK-1 marks earliest axonal growth in *Xenopus*. *Devl. Brain Res.* **50**, 147-153.

Patel, N. H., Martin-Blanco, E., Coleman, K. G., Poole, S. J., Ellis, M. C., Kornberg, T. B. and Goodman, C. S. (1989). Expression of engrailed proteins in arthropods, annelids and chordates. *Cell* **58**, 955-968.

Placzek, M., Tessier-Lavigne, M., Yamada, T., Jessell, T. and Dodd, J. (1990). Mesodermal control of neural cell identity: floor plate induction by the notochord. *Science* **250**, 985-988.

Placzek, M., Yamada, T., Tessier-Lavigne, M., Jessell, T. M. and Dodd, J. (1991). Control of dorsoventral pattern in vertebrate neural development: induction and polarizing properties of the floor plate. *DevelopmentSupplement* **2**, 105-122.

Rosa, F. M. (1989). Mix 1, a homeobox mRNA inducible by mesoderm inducers, is expressed mostly in the presumptive endodermal cells of *Xenopus* embryos. *Cell* **57**, 965-974.

Ruiz i Altaba, A. (1990). Neural expression of the *Xenopus* homeobox gene Xhox3: evidence for a patterning neural signal that spreads through the ectoderm. *Development* **108**, 595-604.

Ruiz i Altaba, A. (1992). Planar and vertical signals in the induction and patterning of the *Xenopus* nervous system. *Development*, in press.

Ruiz i Altaba, A., Choi, T. and Melton, D. A. (1991). Expression of the *Xhox* 3 homeobox protein in *Xenopus* Embryos: Blocking its early function suggests the requirement of *Xhox* 3 for normal posterior development. *Dev. Growth Differ.* **33**, 651-669.

Ruiz i Altaba, A. and Jessell, T. M. (1991a). Retinoic acid modifies mesodermal patterning in early *Xenopus* embryos. *Genes Dev.* **5**, 175-187.

Ruiz i Altaba, A. and Jessell, T. M. (1991b). Retinoic acid modifies the pattern of cell differentiation in the central nervous system of neurula stage *Xenopus* embryos. *Development* **112**, 945-958.

Ruiz i Altaba, A. and Melton, D. A. (1989a). Bimodal and graded expression of the *Xenopus* homeobox gene *Xhox3* during embryonic development. *Development* **106**, 173-183.

Ruiz i Altaba, A. and Melton, D. A. (1989b). Involvement of the *Xenopus* homeobox gene *Xhox3* in pattern formation along the anterior-posterior axis. *Cell* **57**, 317-326.

Ruiz i Altaba, A. and Melton, D. A. (1989c). Interaction between peptide

growth factors and homeobox genes in the establishment of anterior-posterior polarity in frog embryos. *Nature* **341**, 33-38.

Savage, R. and Phillips, C. R. (1989). Signals from the dorsal blastopore lip during gastrulation bring the ectoderm toward a non-epidermal pathway of differentiation. *Devl. Biol.* **133**, 157-168.

Scharf, S. R. and Gerhart, J. C. (1983). Axis determination in eggs of *Xenopus laevis*: a critical period before first cleavage, identified by common effects of cold, pressure and ultraviolet irradiation. *Devl. Biol*. **99**, 75-87.

Servetnick, M. and Grainger, R. (1991). Homeogenetic neural induction in *Xenopus. Devl. Biol*. **147**, 73-82.

Sharpe, C. and Gurdon, J. B. (1990). The induction of anterior and posterior neural genes in *Xenopus laevis. Development* **109**, 765-774.

Sive, H. L., Draper, B. W., Harland, R. M. and Weintraub, H. (1990). Identification of a retinoic acid sensitive period during primary axis formation in *Xenopus laevis. Genes Dev.* **4**, 932-942.

Slack, J. M. W., Darlington, B. G., Heath, J. K. and Godsave, S. F. (1988). Mesoderm induction in early *Xenopus* embryos by heparin binding growth factors. *Nature* **326**, 197-200.

Smith, J. C. (1987). A mesoderm-inducing factor is produced by a *Xenopus* cell line. *Development* **99**, 3-14.

Smith, J. C. and Slack, J. M. W. (1983) Dorsalization and neural induction: properties of the organizer in *Xenopus laevis. J. Embryol. Exp. Morph.* **78**, 299-317.

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

Smith, J. C., Price, B. M. J., Van Nimmen, K. and Huylebroeck, D. (1990). Identification of a potent *Xenopus* mesoderm-inducing factor as a homologue of activin A. *Nature* **345**, 729-731.

Smith, W. C. and Harland, R. M. (1991). Injected Xwnt-8 RNA acts early in *Xenopus* embryos to promote formation of a vegetal dorsalizing center. *Cell* **67**, 735-765.

Sokol, S., Christian, J. L., Moon, R. T. and Melton, D. A. (1991). Injected Wnt RNA induces a complete body axis in *Xenopus* embryos. *Cell* **67**, 741-752.

Sokol, S. and Melton, D. A. (1991). Preexistent pattern in *Xenopus* animal pole cells revealed by induction with activin. *Nature* **361**, 409-411.

Spemann, H. (1938). *Embryonic Development and Induction.* New Haven: Yale University Press.

Spemann, H. and Mangold, H. (1924). Uber Induktion von Embryonalanlangen durch Implantation artfremder Organisatoren. *Wilhelm Roux Arch. EntwMech. Org*. **100**, 599-638.

Spofford, W. R. (1948). Observations on the posterior part of the neural plate in *Ambystoma.*II. The inductive effect of the intact posterior part of the chorda-mesodermal axis on competent prospective ectoderm. *J. Exp. Zool.* **107**, 123-164.

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

Taira, M., Jamrich, M., Good, P. J. and Dawid, I. B. (1992). The LIM domain-containing homeobox gene *Xlim1* is expressed specifically in the organizer region of *Xenopus* gastrula embryos. *Genes Dev.* **6**, 356-366.

Thomsen, G., Woolf, T., Whitman, M., Sokol, S., Vaughan, J., Vale, W. and Melton, D. A. (1990). Activins are expressed early in *Xenopus* embryogenesis and can induce axial mesoderm and anterior structures. *Cell* **63**, 485-493.

Thor, S., Ericson, J., Brännström, T. and Edlund, T. (1991). The homeodomain LIM protein Isl-1 is expressed in subsets of neurons and endocrine cells in the adult rat. *Neuron* **7**, 881-889.

Vaage, S. (1969). The segmentation of the primitive neural tube in chick embryos (*Gallus domesticus*) *Advan. Anat. Embryol. Cell. Biol.* **41**, 1-88.

van Straaten, H. W. M., Hekking, J. W. M., Wiertz-Hoessels, E. L., Thors, F. and Drukker, J. (1988). Effect of the notochord on the differentiation of a floor plate area in the neural tube of the chick embryo. *Anat. Embryol.* **177**, 317-324.

Wagner, M., Thaller, C., Jessell, T. M. and Eichele, G. (1990). Polarizing activity and retinoid synthesis in the floor plate of the neural tube. *Nature* **345**, 819-822.

Weigel, D. and Jäckle, H. (1990). The *fork head* domain: a novel DNA binding motif of eukaryotic transcription factors? *Cell* **63**, 455-456.

Weigel, D., Jürgens, G., Küttner, F., Seifert, E. and Jäckle, H. (1989). The homeotic gene *fork head* encodes a nuclear protein and is expressed in the terminal regions of the Drosophila embryo. *Cell* **57**, 645-658.

Wilson, P., Oster, G. and Keller, R. (1989). Cell rearrangement and segmentation in *Xenopus*: direct observation of cultured explants. *Development* **105,** 155-166.

Yamada, T., Placzek, M., Tanaka, H., Dodd, J. and Jessell, T. M. (1991). Control of cell pattern in the developing nervous system: Polarizing activity of the floor plate and notochord. *Cell* **64**, 635-647.

Yanagisawa, K. O. (1990). Does the T gene determine the anteroposterior axis of the mouse embryo? *Japan J. Genet.* **65**, 287-297.

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Fig. 4. *Localization of* Pintallavis *(A-C, G-I) and* Brachyury *(D-F, J) RNAs in normal, Li^+- and UV-treated embryos and exogastrulae.* (A) Ventral view of a normal stage 9^+ embryo showing the expression of *Pintallavis* in the dorsal marginal zone. (B) Ventral view of a stage 9-10 Li^+-treated embryo showing the expression of *Pintallavis* in the entire marginal zone. (C) Ventral view of a stage 9-10 UV-treated embryo showing the absence of *Pintallavis* expression. (D) Ventral view of a normal stage 9-10 embryo showing the graded expression of *Brachyury* in the marginal zone. Dorsal to the right. (E) Ventral view of a stage 9-10 Li^+-treated embryo showing a high level of *Brachyury* expression in the entire marginal zone. (F) Ventral view of a stage 9-10 UV-treated embryo showing a low level of *Brachyury* expression in the entire marginal zone. (G) *Pintallavis* expression in complete exogastrula at stage 12-12½ is detected only in the mesoderm where it shows a posterior-to-anterior gradient. (Posterior mesoderm is to the top). The arrowhead indicates the junction between ectoderm and mesoderm. (H) Longer development of the alkaline phosphatase reaction shows that the expression of *Pintallavis* RNA in exogastrula at stage 12-12½ is high in the notochord and low in the prechordal plate (arrowhead). The outline of the unlabelled ectoderm is indicated. (I) High magnification photograph of the exogastrula in (H) showing the absence of *Pintallavis* expression in the ectoderm. The groove detected at the midline of the ectoderm denotes the notoplate (arrowheads). (J) Expression of *Brachyury* RNA in exogastrula at stage 12-12½ showing its expression in all mesodermal cells in the posterior mesoderm, equivalent to the region around the blastopore in normal embryos, and at lower levels in the notochord. The outline of the unlabelled ectoderm is indicated. Scale bars: A-F, H=100 μm; G, I=50 μm; J=80 μm.

Fig. 5. *External phenotype of embryos injected at the 2-cell stage with* Pintallavis *RNA.* (A) Antero-dorsal view of a late neurula (stage ~18) embryo injected into its left side. Note the decreased anterior extent of the neural folds (arrowhead) and the asymmetric position of the small cement gland, which is mostly absent from the injected side. (B) Anterior view of late neurula-early tailbud stage (stage ~20-22) injected (left) and control (right) embryos. The injected embryo was injected into the left side. Note the diminution in the elevation of the neural fold of the injected side (arrowhead) compared to that on the uninjected side and in the control embryo. The cement gland and the head folds in the injected embryo are found largely in the uninjected side. Compare the position of these axial structures in relation to the axis of bilateral symmetry in the injected and control embryos. (C) Dorsal view of early tadpole (stage ~30-32) control (top) and injected (bottom) embryos. Note the shorter length and wider axis of the injected embryo. (D) Lateral view of tadpole (stage ~36) control (top) and injected (bottom) embryos showing the abnormal head structures, in particular, the lack of an eye. (E) Dorsal view of a tailbud stage (stage ~24) embryo injected with *Pintallavis* RNA into the right side after labelling in whole-mount with mAb HNK-1. The red labelling is the DAB-peroxidase reaction product in Rohon-Beard sensory neurons (arrowhead). Note the reduced number of these neurons in the injected (right) side. (F) High magnification photograph of the embryo in (E) showing the position, arrangement and axonal projections of Rohon-Beard neurons on the uninjected (left) and injected (right) sides. Scale bars: A, E=200 μm; B=400 μm; C=100 μm; D=150 μm; F= 50 μm.

Fig. 6. *Internal phenotype of embryos injected with* Pintallavis *RNA.* (A) Lateral view of an early tadpole (stage ~30) embryo labelled in whole mount with mAb Xen1 showing the neural-specific expression of this antigen. (B) Lateral view of a tadpole (stage ~34) labelled in whole mount with mAb 12/101 showing the somitic mesoderm. (C) Cross-section through the head of an embryo (stage ~36) injected with *Pintallavis* RNA into the left side and labelled with mAb Xen1 prior to sectioning. Note the lack of the left eye (e) and the inclusion of the notochord (not) in the neural tissue (n) that is deformed in the left side. Inclusion of the notochord in the anterior neural tube was commonly observed in injected embryos. The uneven labelling is due to the fact that this and other (D) embryos were processed as whole-mounts before sectioning. (D) Cross-section through the trunk of an embryo (stage ~34) injected with *Pintallavis* RNA into the right side labelled with mAb 12/101 prior to sectioning. The embryo displays normal somites (s) and notochord. The neural tube (n) on the injected side is enlarged. (E) Cross-section through the trunk of an embryo (stage ~30) injected with *Pintallavis* RNA into the right side. Note that the enlarged neural tube (n) in the injected side has formed a small secondary tubular structure (arrowhead). (F) Cross-section through the trunk of a normal embryo (stage ~34) labelled with mAb HNK-1 showing the dorsal position of Rohon-Beard neurons (arrowheads) within the spinal cord. The axons of Rohon-Beard neurons are detected under the skin (right-hand arrowhead). (G, H) Cross-sections through the trunk of embryos injected with *Pintallavis* RNA into the right side after labelling with mAb HNK-1. Note the absence of HNK-1-labelled Rohon-Beard neurons from the enlarged injected sides (arrows). The expanded area of somitic mesoderm on the injected side is probably a result of the inability of these cells to extend vertically. All sections show representative phenotypes. Scale bars: A, B= 200 μm; C-H=20 μm.