

Gene expression pattern

Cloning and expression of *Xenopus Prickle*, an orthologue of a *Drosophila* planar cell polarity gene

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Abstract

We have cloned *Xenopus* orthologues of the *Drosophila* planar cell polarity (PCP) gene *Prickle*. *Xenopus Prickle* (*XPk*) is expressed in tissues at the dorsal midline during gastrulation and early neurulation. *XPk* is later expressed in a segmental pattern in the presomitic mesoderm and then in recently formed somites. *XPk* is also expressed in the tailbud, pronephric duct, retina, and the otic vesicle. The complex expression pattern of *XPk* suggests that PCP signaling is used in a diverse array of developmental processes in vertebrate embryos. © 2002 Published by Elsevier Science Ireland Ltd.

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1. Results and discussion

The planar cell polarity (PCP) signaling cascade plays important roles in establishing epithelial planar polarity in invertebrates (Shulman et al., 1998). Recent experiments have revealed that a similar PCP pathway is critical for controlling cell polarity during convergent extension (Heisenberg et al., 2000; Tada and Smith, 2000; Wallingford et al., 2000) and neurulation (Kibar et al., 2001; Wallingford and Harland, 2001). The *Drosophila* Prickle protein is a critical player in the control of planar polarity (Adler et al., 2000; Gubb et al., 1999), and a *Prickle* gene is expressed in notochord cells during convergent extension in the primitive chordate *Ciona intestinalis* (Hotta et al., 2000). To further define the PCP signaling pathway in vertebrates we have cloned a *Xenopus* orthologue of *Drosophila* Prickle (Gubb et al., 1999) and examined its expression pattern during early development.

We obtained clones encoding two different proteins (97% identical) both with a high degree of identity to *Drosophila* Prickle at the amino acid level. We have named the genes *Xenopus Prickle-A* and *B* (*XPk-A* and *XPk-B*). *XPk* is almost equally similar to *Drosophila* Prickle, *Ciona Prickle-1* and *Drosophila Espinas*. *XPk* is roughly 85% identical to the

uncharacterized human protein BAB71198 and about 64% identical to human LIM-only protein 6 (LMO6) (Fig. 1A, B). Prickle proteins contain four conserved domains in the N-terminal portion of the protein (Gubb et al., 1999). First is the PET domain, conserved in Prickle, Espinas and Testin; the function of this domain is unknown. The PET domain is followed closely by three LIM domains. When only the PET and LIM domains are compared, *XPk* is 90% identical to BAB71198 and 67% identical to LMO6. Alignment of the PET and LIM domains of *XPk*, BAB71198, and LMO6 is shown in Fig. 1. *XPk* is less than 40% identical to human testin or LMO4 (Fig. 1B).

We examined the developmental expression profile of *XPk* by RT-PCR and by in situ hybridization. *XPk* is expressed maternally, and zygotic expression commences at about the onset of gastrulation (st. 10+) and steadily increases until tadpole stages (st. 30) (Fig. 1C). At the onset of blastopore lip formation (st. 10+), expression of *XPk* begins in the dorsal marginal zone (Fig. 2A). As gastrulation proceeds, expression expands to include the lateral and ventral marginal zones (Fig. 2B). The first few rows of cells above the blastopore lip are free of *XPk* expression (Fig. 2A, B), reminiscent of *Xenopus Brachyury* expression and consistent with the observation that *Ciona Prickle* is expressed downstream of *Ciona Brachyury* (Takahashi et al., 1999). The *XPk* expression pattern moves dorsally as gastrulation movements bring the marginal zone tissues to the dorsal side of the embryo (Fig. 2C). Cross-sections

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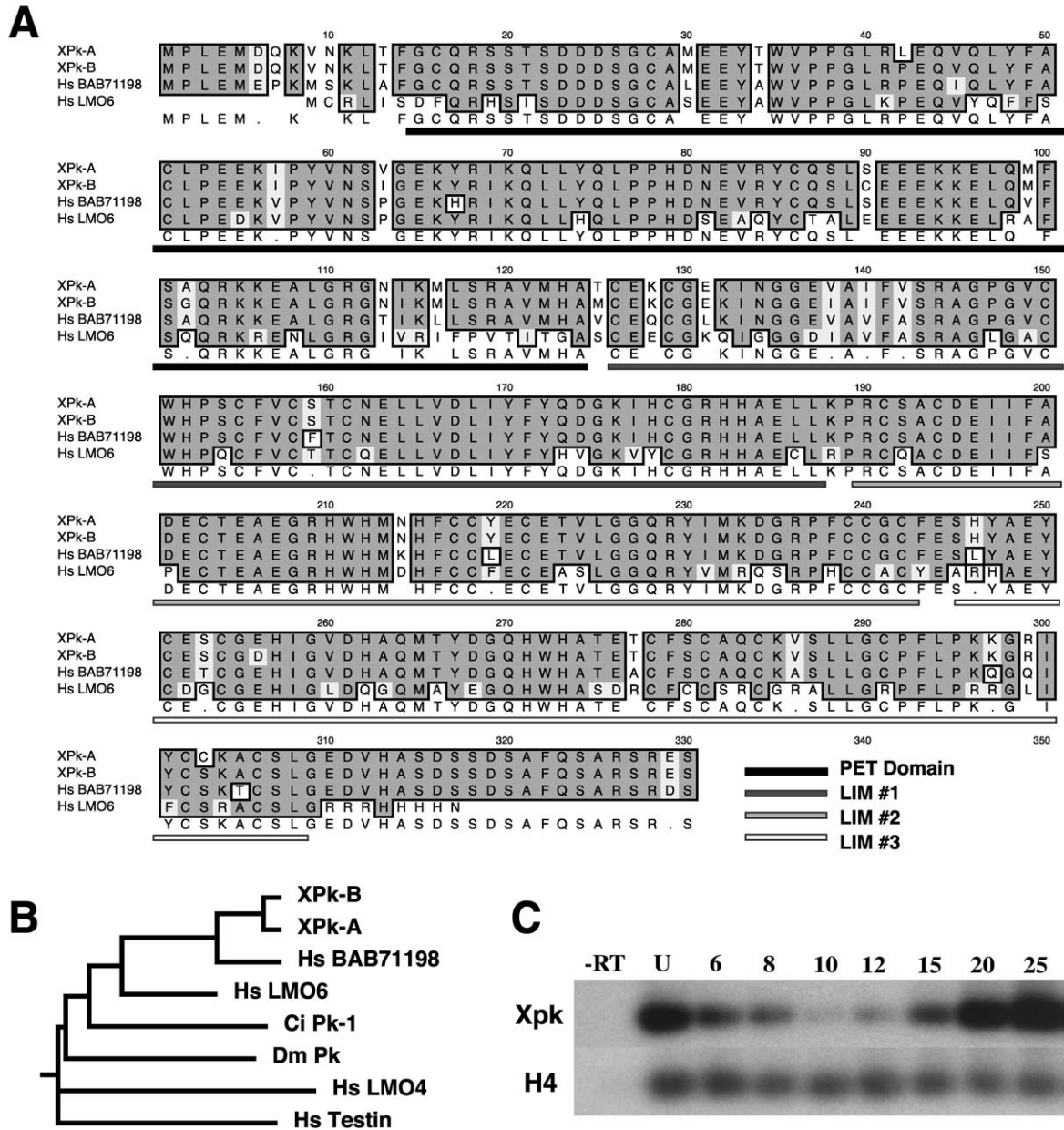


Fig. 1. Sequence of *Xpk*. (A) Alignment of PET and LIM domains of Xpk-A, Xpk-B, Hs BAB871198, and Hs LMO6. PET and LIM domains are indicated by shaded bars, key is at bottom right of panel. (B) Dendrogram of protein sequence relationships of Prickle proteins from *Xenopus*, human, *Ciona*, and *Drosophila*. Human LMO4 and Testin serve as outgroups. (C) RT-PCR of *Xpk* expression. Numbers indicate developmental stages; U = unfertilized egg; H4 = histone H4.

revealed expression in both involuting and non-involuting marginal zone (Fig. 2D). By the end of gastrulation, *Xpk* is expressed very strongly in the dorsal midline and more weakly in paraxial tissues (Fig. 2E). *Xpk* is excluded from anterior neural ectoderm, but is expressed in dorsal mesoderm and posterior neural ectoderm (Fig. 2D, F). At the end of gastrulation, *Xpk* expression begins to be downregulated in the mesoderm, but remains strong in posterior ectoderm through neurula stages (Fig. 2D, F; Fig. 3A, a'). *Xpk* is therefore expressed in tissues involved in convergent extension during gastrula and neurula stages (Keller et al., 2000),

consistent with the proposed role of PCP signaling in this process (Wallingford and Harland, 2001).

At stage 21, faint expression can be observed in the forming pronephric anlage (not shown). By stage 24, this expression domain resolves specifically to the anlage of the pronephric duct (Fig. 3B, C). Duct-specific staining is obvious by stage 28 (Fig. 3C, E). Expression is not seen in the pronephric tubules or glomus. Beginning at stage 24, expression can also be seen in the tailbud and later in the tail tip (Fig. 3B, C).

During tailbud stages, *Xpk* is expressed in structures at the

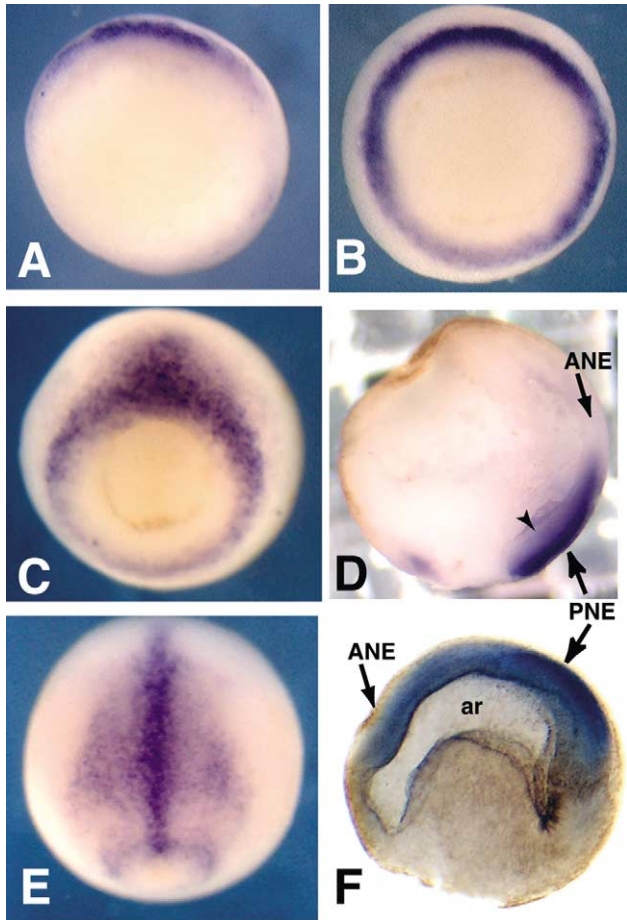


Fig. 2. Early expression of *Xpk*. (A–C) Vegetal view, dorsal at top. (A) st. 10 + . (B) st. 10.5. (C) st. 11.5. (D) Sagittal section of st. 11.5, dorsal to right; arrowhead indicates weakening expression in mesoderm, arrow indicates strong expression in posterior neural ectoderm (PNE); ANE = anterior neural ectoderm. (E) Dorsal view, anterior at top. st. 12. (F) Cleared embryo st. 13; sagittal view, anterior to left. ar = archenteron.

developing dorsal midline and in the forming somites. *Xpk* is expressed weakly in the notochord at more anterior levels (Fig. 3D, d1) and expression increases in more posterior notochord (Fig. 3, d2, d3). In the most posterior regions, *Xpk* is expressed in both notochord and the floorplate of the neural tube with weak expression sometimes observed in the roofplate (Fig. 3D, d3). A dynamic pattern is observed in forming somites. Expression is strongest in one or two recently formed somites (black arrowheads, Fig. 3B, C) and weaker in the presomitic mesoderm. These expression domains are parallel to the regions of notochord and somite which display defects in *loop-tail* mutant mice (Greene et al., 1998), which express a mutant form of the PCP gene *Strabismus* (Kibar et al., 2001).

At stage 30, a complex expression pattern is observed in the head. *Xpk* is strongly expressed in the lens and the otic vesicle (Fig. 3E, e'). Expression is also seen in the more anterior branchial pouches and the mandibular arch (Fig. 3E).

2. Materials and methods

Degenerate PCR and low-stringency screening were both used to obtain *Xpk* clones. Degenerate PCR (5'-GTGYT-GYGGMMGRCA YCAYGCN-3' and 5'-RTCDGTDGCR-TGCCARTGYTGN-3') of st. 10 *Xenopus* cDNA generated a 298 bp fragment, which was used to screen a st. 10 *Xenopus* cDNA library (ZAP cDNA synthesis kit; Stratagene) at high stringency, resulting in *Xpk-A* and *Xpk-B* clones. For low-stringency screening, a DNA probe was generated by random priming from the ~870 bp *AccI/StuI* fragment of *Ciona Pk-1* (gift of David Keys). An arrayed, st. 13 *Xenopus* cDNA library (RZPD, Germany) was hybridized to the probe overnight at 44°C. A 4.5 kb *Xpk-B* clone was obtained (RZPD clone DKFZp546K2053Q2).

RT-PCR was performed using the following primers: 5'-GCTTCTAATGTTGGACTGCC-3' and 5'-TCAGGAAT-GATCCGGCAAAC-3'. Products were loaded on 2% agarose gels, electrophoresed, transferred to nylon membrane, and the membranes were hybridized to the isotope-labeled fragment of *Xpk* and autoradiographed.

In situ hybridization was performed as described (Sive et al., 2000) using digoxigenin-labelled probes; BM-Purple was used for all staining.

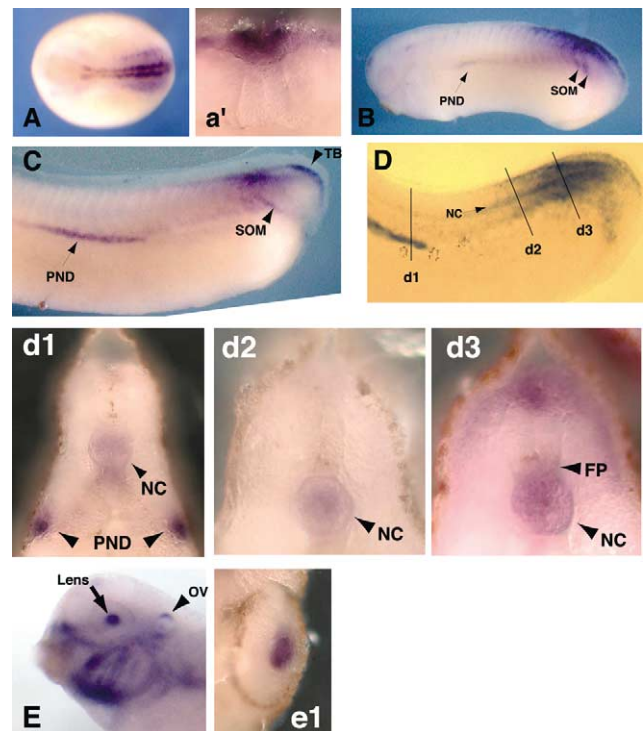


Fig. 3. Later expression of *Xpk*. (A) st. 17, dorsal view, anterior to left. (a') Transverse section through stage 17 embryo. (B) st. 26. (C–D) St. 30; sagittal view, anterior to left. (D) Cleared embryo. PND = pronephric duct; SOM = forming somites; NC = notochord; TB = tailbud; FP = floorplate (d1–d3). Transverse sections at levels indicated by lines in panel (D). (E) Head of stage 32 embryo. OV = otic vesicle. (e') Section through eye, st. 32.

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