Identification and Characterization of *fullback*, a Novel Posteriorly-Expressed *Xenopus* Gene

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Abstract:

We have identified *fullback*, a *Xenopus laevis* gene which shows highest similarity to the low affinity nerve growth factor receptor *p75*. Extracellular portions of the protein are conserved between fullback and p75, although regions of p75 which have been implicated in its role in apoptosis are not especially conserved in fullback. *fullback* is expressed in the posterior of the embryo from gastrulation onwards and is found dorsally during tailbud stages. This pattern of expression apparently does not coincide with the incidence of apoptosis in the early *Xenopus* embryo and therefore indicates that fullback may have a different function during embryogenesis.

Results:

The low affinity nerve growth factor receptor p75 is a member of the "death receptor" family of transmembrane proteins, most of which have some role in apoptosis (Liepinsh, et al, 1997). This receptor sends a pro-apoptotic signal leading to neuronal death unless it binds a neurotrophic ligand such as NGF which causes it to send an anti-apoptotic signal instead (reviewed in Bredesen and Rabizadeh, 1997). Using a yeast invertase screen to find secreted and transmembrane proteins which may play a role in patterning the early embryo, we have identified *fullback*, a *Xenopus* gene with highest similarity to p75 (Jacobs et al, 1997). Here we report a sequence comparison of fullback to p75 in other vertebrates and a spatial and temporal analysis of *fullback* expression in *Xenopus*.

fullback encodes a 427 amino acid protein with a signal sequence peptide at the amino terminus (von Heijne, 1986) and a single putative transmembrane domain. While the p75 genes from human, rat and chicken share between 75-85%

identity with each other, fullback is only approximately 40% similar to any of them and is therefore unclear whether fullback is a *Xenopus* homolog of p75 (Fig 1). The death receptor family of proteins is characterized by their cysteine-rich extracellular domains (Liepinsh et al, 1997). A protein sequence alignment of fullback with the three p75 proteins illustrates that all 25 of the extracellular cysteines in p75 are also conserved in this region of fullback (Fig 1, boxed). This implies that fullback may have extracellular structural similarity to p75. However, the "death domain", a six-helix portion of the intracellular domain present in most death receptor family members which is thought to be essential for apoptotic signaling (underlined, Fig 1) (Tartaglia, et al, 1993) is not well conserved in fullback, suggesting that this protein may have an entirely different, non-apoptotic function in the embryo.

Northern analysis of *fullback* expression (Fig 2), shows a full length transcript of approximately 3kb. Weak maternal expression can be detected by RT-PCR (data not shown), but is not apparent by Northern blot. Zygotic expression of *fullback* begins at midgastrula stages, peaks during neurulation and then declines by swimming tadpole stages.

In situ hybridization analysis was performed in order to determine the location of *fullback* transcripts within the embryo (Fig 3). During gastrula and neurula stages, *fullback* expression is posteriorly localized. At early gastrula (Fig 3A, panel a) weak expression is found in a wide rim around the blastopore. At midgastrula (panel b)*fullback* expression remains around the blastopore and increases in intensity. A cross section through a halved embryo of this stage (panel f) shows that *fullback* is expressed in the superficial tissue of the blastopore lip but not in the involuted mesoderm. At neurulation (panel c)*fullback* reaches its highest levels of expression and is found in a ring around the closed blastopore in the

posterior of the embryo, both in the ectoderm and in the underlying mesoderm (panel g). At this stage, dorsal expression begins to extend out from the posterior of the embryo in a small streak along the midline (arrow, panel c). Cross sections of neurula embryos suggest that this staining does not coincide with the notochord but may correspond to migrating neural crest. At late neurula (panel d) expression persists in the posterior of the embryo and continues to extend dorsally into regions near the developing somites. A comparative *in situ* done on halved embryos with *fullback* and *m-actin* probes clearly shows non-overlapping regions of expression, implying that this dorsal *fullback* expression is not myotomal (panels h and j). At tailbud stages *fullback* is maintained in the posterior and the dorsal expression expands ventrally (panel e). A transverse cut through these embryos reveals that the dorsal staining overlaps with *muscle actin* staining in the somite, implying that at this stage *fullback* RNA is present in the myotome (panels i and k).

In order to assess whether *fullback* plays a role in modulating apoptosis, we compared its expression with TUNEL assays which profile the temporal and spatial occurrence of apoptosis within the *Xenopus* embryo (Hensey and Gautier, 1998). Using this assay, these authors found that apoptosis begins at midgastrula stages in a variable pattern including every region of the embryo except the cells immediately surrounding the blastopore. Later, TUNEL staining coincides with regions of neurogenesis in the ectoderm during neurula and tailbud stages. These regions of the embryo do not correspond with those expressing *fullback*, implying that *fullback* is not essential for apoptotic signaling. These data, together with the significant intracellular sequence divergence between p75, suggest that the function of fullback is different from that of p75.

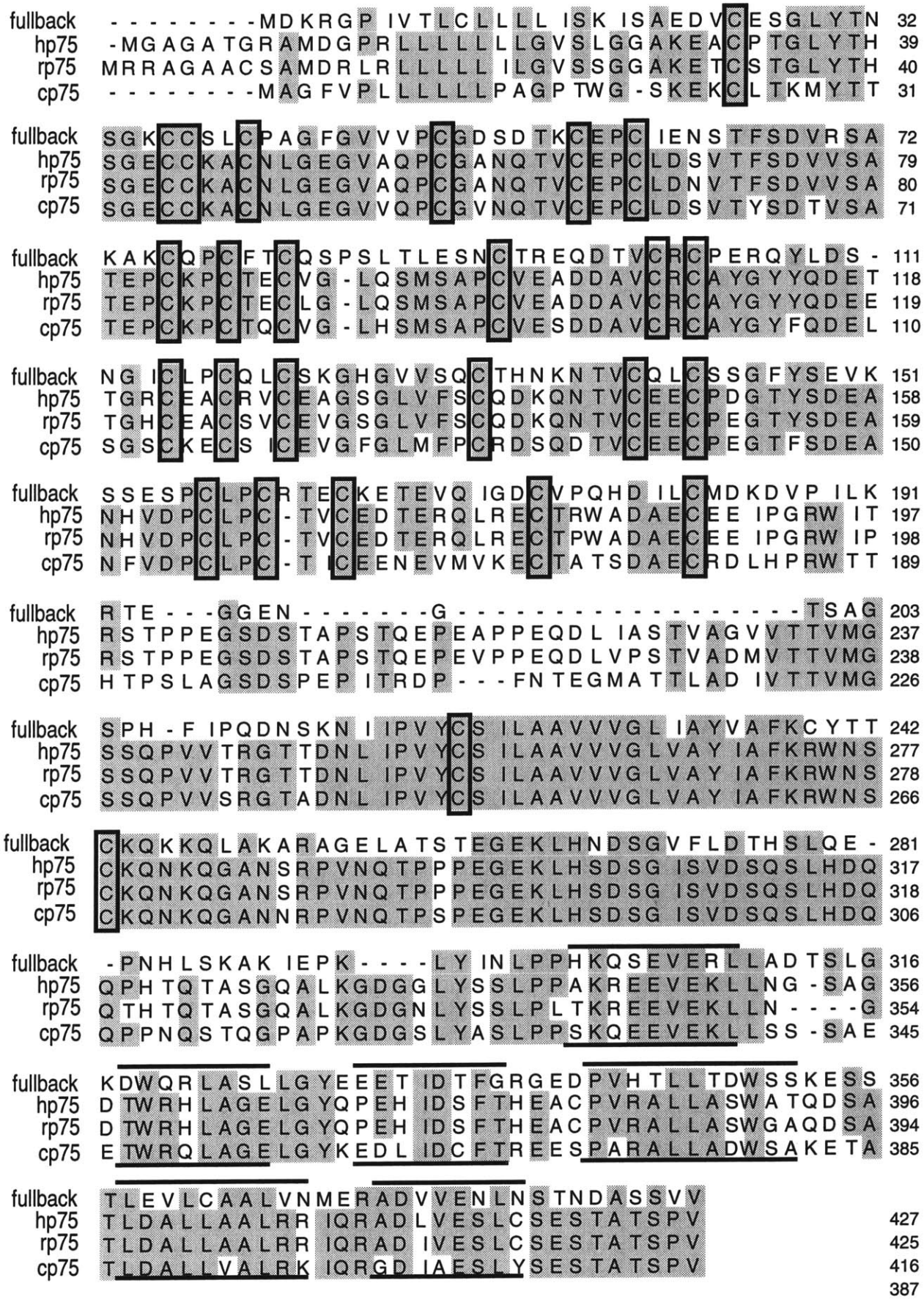
Methods:

The full length clone of *fullback* was isolated from a random-primed cDNA library made from ventral mesoderm and ectoderm from mid-gastrula Xenopus embryos (stage 11.5). Embryos were staged according to Nieuwkoop and Faber, 1967. Two oligos were used to form an *EcoR* I adapter: BIS3, 5'-AATTCCCATAGCAACAAACAGTA-3', unphosphorylated and BIS4, 5'-TACTGTTTGTTGCTATGGG-3', phosphorylated.

The random-primed cDNA library was subjected to a yeast selection as described previously (Jacobs et al., 1997). The Genbank accession number of *fullback* is AF131890.

in situ hybridization was performed as described in Kuo, et al., 1998. Most in situ panels show albino embryos, although some show wild type embryos which have been bleached in 2% hydrogen peroxide for 45 minutes.

Northern blot analysis was performed as described in Sagerström et al., 1997.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hp75 -MGAGATGRAMDGPRLLLLLLLGVSLGGAKEACPTGLYTH 39
rp75 MRRAGAACSAMDRLRLLLLL ILGVSSGGAKETCSTGLYTH 40
cp75 -------MAGFVPLLLLLLPAGPTWG-SKEKCLTKMYTT 31
fullback SGKCCSLCPAGFGVVVPCGDSDTKCEPC IENSTFSDVRSA 72
hp75 SGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSA 79
rp75 SGECCKACNLGEGVAQPCGANQTVCEPCLDNVTFSDVVSA 80
cp75 SGECCKACNLGEGVVQPCGVNQTVCEPCLDSVTYSDTVSA 71
fullback KAKCQPCFTCQSPSLTLESNCTREQDTVCRCPERQYLDS- 111
hp75 TEPCKPCTECVG-LQSMSAPCVEADDAVCRCAYGYYQDET 118
rp75 TEPCKPCTECLG-LQSMSAPCVEADDAVCRCAYGYYQDEE 119
cp75 TEPCKPCTQCVG-LHSMSAPCVESDDAVCRCAYGYFQDEL 110
fullback NG ICLPCQLCSKGHGVVSQCTHNKNTVCQLCSSGFYSEVK 151
hp75 TGRCEACRVCEAGSGLVFSCQDKQNTVCEECPDGTYSDEA 158
rp75 TGHCEACSVCEVGSGLVFSCQDKQNTVCEECPEGTYSDEA 159
cp75 SGSCKECS ICEVGFGLMFPCRDSQDTVCEECPEGTFSDEA 150
fullback SSESPCLPCRTECKETEVQ IGDCVPQHD ILCMDKDVP ILK 191
hp75 NHVDPCLPC-TVCEDTERQLRECTRWADAECEE IPGRW IT 197
rp75 NHVDPCLPC-TVCEDTERQLRECTPWADAECEE IPGRW IP 198
cp75 NFVDPCLPC-T ICEENEVMVKECTATSDAECRDLHPRWTT 189
fullback RTE---GGEN-------G--------------------TSAG 203
hp75 RSTPPEGSDSTAPSTQEPEAPPEQDL IASTVAGVVTTVMG 237
rp75 RSTPPEGSDSTAPSTQEPEVPPEQDLVPSTVADMVTTVMG 238
cp75 HTPSLAGSDSPEP ITRDP---FNTEGMATTLAD IVTTVMG 226
fullback SPH-F IPQDNSKN I IPVYCS ILAAVVVGL IAYVAFKCYTT 242
hp75 SSQPVVTRGTTDNL IPVYCS ILAAVVVGLVAY IAFKRWNS 277
rp75 SSQPVVTRGTTDNL IPVYCS ILAAVVVGLVAY IAFKRWNS 278
cp75 SSQPVVSRGTADNL IPVYCS ILAAVVVGLVAY IAFKRWNS 266
fullback CKQKKQLAKARAGELATSTEGEKLHNDSGVFLDTHSLQE- 281
hp75 CKQNKQGANSRPVNQTPPPEGEKLHSDSG ISVDSQSLHDQ 317
rp75 CKQNKQGANSRPVNQTPPPEGEKLHSDSG ISVDSQSLHDQ 318
cp75 CKQNKQGANNRPVNQTPSPEGEKLHSDSG ISVDSQSLHDQ 306
fullback -PNHLSKAK IEPK----LY INLPPHKQSEVERLLADTSLG 316
hp75 QPHTQTASGQALKGDGGLYSSLPPAKREEVEKLLNG-SAG 356
rp75 QTHTQTASGQALKGDGNLYSSLPLTKREEVEKLLN----G 354
cp75 QPPNQSTQGPAPKGDGSLYASLPPSKQEEVEKLLSS-SAE 345
fullback KDWQRLASLLGYEEETIDTFGRGEDPVHTLLTDWSSKESS 356
hp75 DTWRHLAGELGYQPEHIDSFTHEACPVRALLASWATQDSA 396
rp75 DTWRHLAGELGYQPEHIDSFTHEACPVRALLASWGAQDSA 394
cp75 ETWRQLAGELGYKEDLIDCFTREESPARALLADWSAKETA 385
fullback TLEVLCAALVNMERADVVENLNSTNDASSVV
hp75 TLDALLAALRRIQRADLVESLCSESTATSPV 427
rp75 TLDALLAALRRIQRADIVESLCSESTATSPV 425
cp75 TLDALLVALRKIQRGDIAESLYSESTATSPV 416
387

Figure 1: Protein Sequence Alignment of fullback with p75 from Other Vertebrates

The p75 proteins shown are from human (hp75), rat (rp75) and chicken (cp75). Identical amino acids are shaded. Numbers to the right of the sequences indicate the numerical positions of the amino acids. Boxes denote cysteines conserved among all four proteins. The six-helix death domain region is underlined and overlined.

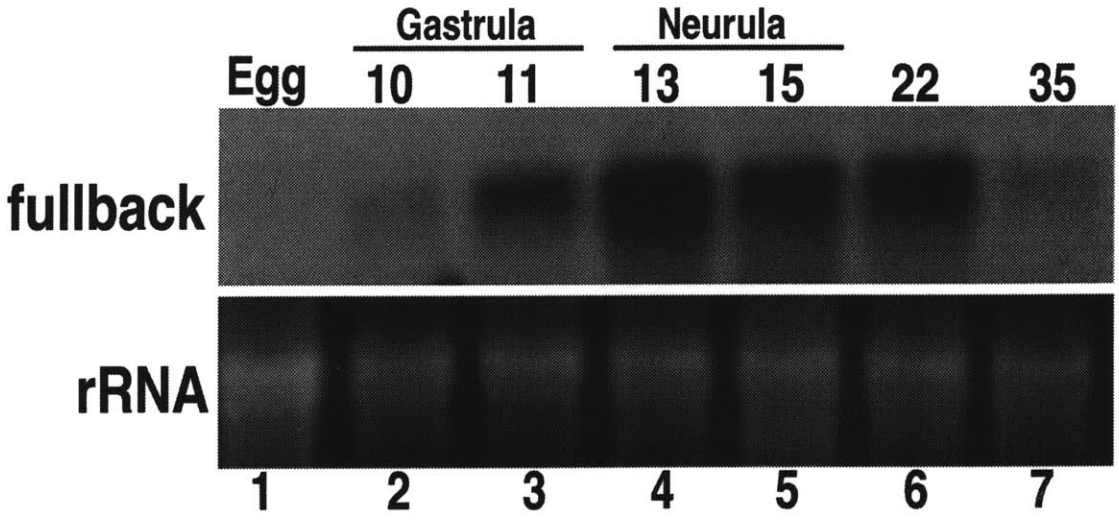
Gastrula
Neurula
Egg
10
11
13
15
22
35
fullback
rRNA
1
2
3
4
5
6
7

Figure 2: Northern Analysis of *fullback* mRNA

fullback RNA (upper panel). Lane 1 contains unfertilized egg RNA. Lane 2 contains RNA from early gastrula, stage 10. Lane 3 contains RNA from midgastrula, stage 11. Lane 4 contains RNA from early neurula, stage 13. Lane 5 contains RNA from midneurula, stage 15. Lane 6 contains RNA from early tailbud, stage 22. Lane 7 contains RNA from swimming tadpole, stage 35. 18s Ribosomal RNA is shown in the lower panel as a loading control.

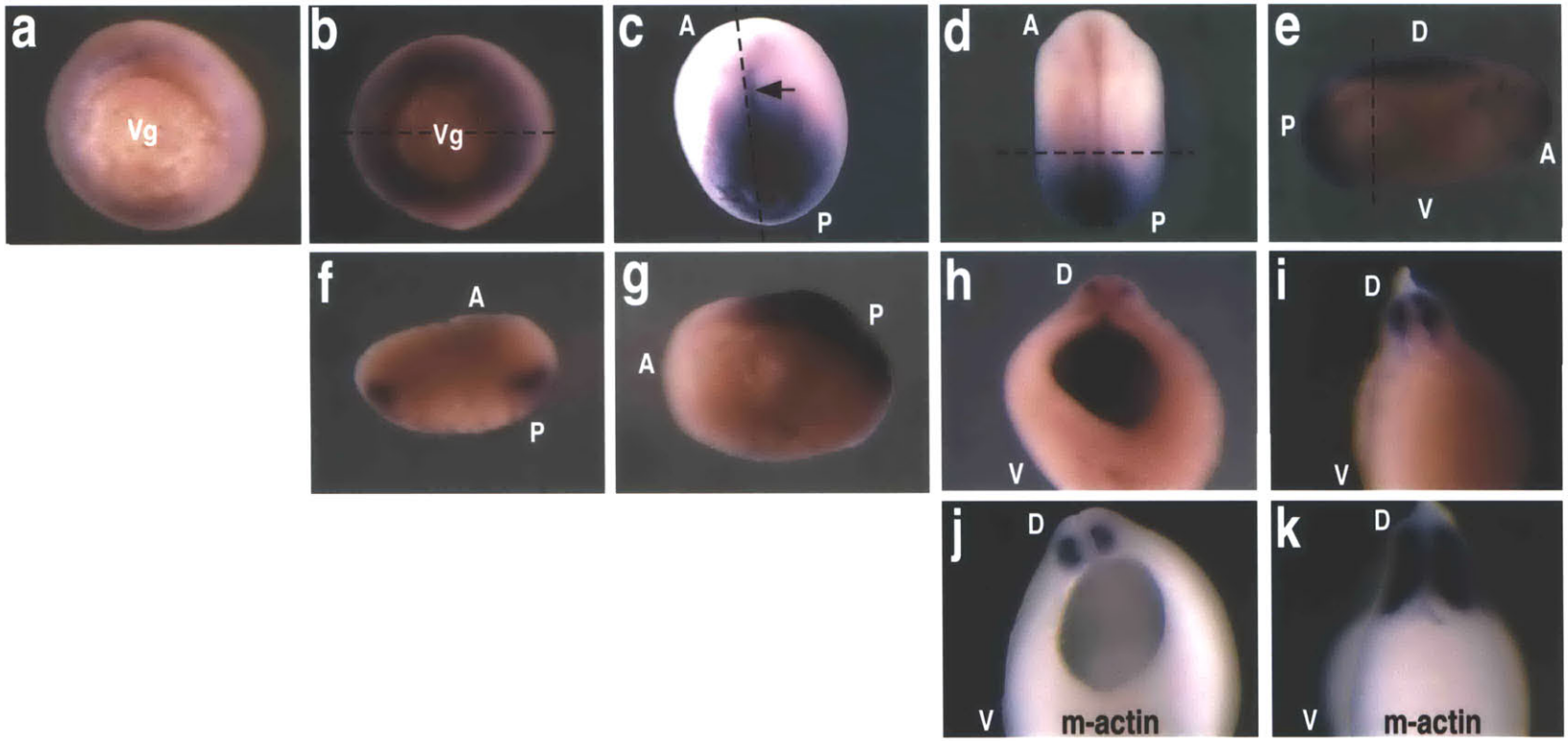
a
Vg
b
Vg
c
A
P
d
A
P
e
D
P
A
V
f
A
P
g
P
A
h
D
V
i
D
V
j
D
V
m-actin
k
D
V
m-actin

Figure 3: *In Situ* Hybridization Analysis of *fullback* RNA Expression

In situ hybridization was performed on whole (panels a-e) or halved (panels f-k) *Xenopus* embryos. Dashed lines in panels b-e denote the plane in which each embryo was cut in half.

a. Vegetal view of an early gastrula (stage 10.5). b. Posterior view of an embryo at mid-gastrula (stage 11.5). c. Dorsal view of an early neurula (stage 13). Arrow indicates the weakly perceptible stripe of staining at the dorsal midline. d. Dorsal view of a late neurula (stage 18). e. Lateral view of a tailbud (stage 25). f. Interior view of a halved embryo at mid-gastrula (stage 11.5). g. Interior view of an early neurula (stage 13) which has been parasagittally cut in half. h. Interior view of a late neurula halved transversely. The dorsal expression of *fullback* is shown. i. Interior view of a late tailbud (stage 25) embryo cut transversely in half.

Panels j and k: Comparative *in situ* hybridizations were performed using a probe for *muscle actin*, which is expressed in the myotome of the *Xenopus* somite. Panel j shows a late neurula embryo (stage 18) cut in half transversely. Panel k shows a tailbud stage embryo (stage 25).

In all panels, Vg= vegetal, A= anterior, P= posterior, D= dorsal, V= ventral.

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