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Original Article

The expression of *XIF3* in undifferentiated anterior neuroectoderm, but not in primary neurons, is induced by the neuralizing agent noggin

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ABSTRACT The gene *XIF3* encodes a neural-specific type-III intermediate filament protein whose expression in the embryo precedes that of the neurofilaments by several hours. We now show, by *in situ* hybridization, that it is expressed at the neurula stage in primary neurons and, to a lesser extent, in undifferentiated anterior neuroectoderm. At the swimming tadpole stage, strong expression is restricted to the midbrain-hindbrain boundary, even-numbered rhombomeres of the hindbrain and the Vth and VIIth cranial ganglia. *XIF3* gene expression can be induced in ectodermal cells (animal caps) derived from blastula when grown to the neurula stage in the presence of the neuralizing agent noggin. In agreement with the proposed ability of noggin to neuralize, but not to promote neuronal differentiation, we find that the pattern of noggin-inducible *XIF3* expression in animal caps is consistent with expression in undifferentiated anterior neuroectoderm but not in primary neurons.

KEY WORDS: *Xenopus, intermediate filament, XIF3, primary neurons, noggin*

Introduction

The dynamic organization of the cell is maintained by a cytoskeleton consisting of microtubules, actin filaments and intermediate filaments (IFs). All the IF proteins share a conserved central alpha-helical rod domain that is important for protein dimerization and aggregation into higher order structures culminating in 8-10 nm filaments. The intermediate filaments have been subdivided into a number of classes according to sequence similarity (Steinert and Roop, 1988). Included in the type III IFs are vimentin, glial fibrillary acidic protein (GFAP) and desmin. Vimentin is expressed in neural cells at an early stage of development and widely within cells of mesenchymal origin where it has been attributed a range of functions (Evans, 1998). In contrast, the expression of GFAP in glial cells and desmin in muscle is normally restricted to a single cell type. The expression of the three type IV IFs is also restricted, in this case to neurons and consequently the type IV IFs are known collectively as the neurofilaments though it is clear that neurons often express other classes of IF proteins in addition to the neurofilaments.

The gene *XIF3* encodes a type III intermediate filament protein found predominantly in neural tissue (Sharpe *et al.*, 1989). It is closely related in sequence to mouse peripherin, a gene which is expressed widely in the peripheral nervous system and induced in PC12 cells in response to nerve growth factor (NGF) promoted neuronal differentiation (reviewed in Greene, 1989). In *Xenopus*, *XIF3* mRNA is first found at a low level in animal cap cells and then accumulates rapidly in the neurectoderm. *XIF3* therefore represents a gene encoding a type III intermediate filament protein whose expression becomes neural specific. *XIF3* gene expression in the neurula embryo precedes that of the type IV neurofilaments which in *Xenopus* are expressed first at the early tailbud stage (Sharpe, 1988).

During *Xenopus* development, the dorsal part of the animal cap becomes the neuroectoderm which will form the neural tube. Probably as an adaptation to a free swimming larval lifestyle, a small number of neurons rapidly differentiate to control the earliest movements of the newly hatched larva (Roberts and Clarke, 1982). These are the primary neurons, defined by their large size, precocious commitment to a neuronal fate and early axonal extension (Lamborghini, 1980; Hartenstein, 1989; Hartenstein, 1993). Within the neuroectoderm the primary neurons arise in restricted domains, marked at an early stage by the expression of *neurogenin*, a homolog of the fly proneural genes (Ma *et al.*, 1996). Within these regions, some cells are selected to become primary neurons by lateral inhibition, a process mediated by Delta-Notch signaling (Chitnis *et al.*, 1995). Selected cells then begin to differentiate and by the mid-neurula stage express a neuron-specific type-II β-

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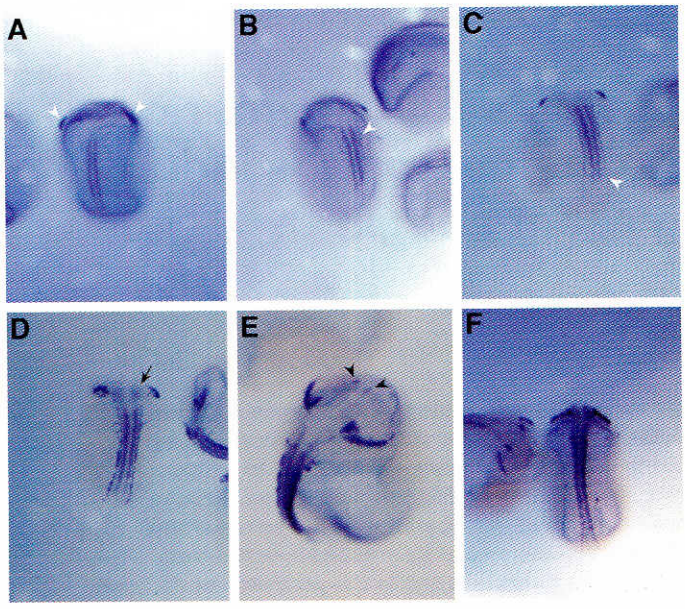


Fig. 1. The pattern of expression of the *XIF3* gene during the neurula stages. **(A)** *At stage 15, XIF3 transcripts are found in a line either side of the midline and probably correspond to the future primary motor neurons. Bilateral patches adjacent to the anterior neural plate represent expression in the placodal component of the trigeminal ganglia (arrowheads).* **(B)** *By stage 15+, XIF3 expression is also detectable in a stripe either side of the midline corresponding to the primary interneurons (arrowhead).* **(C)** *By stage 16, punctate XIF3 staining is apparent in the primary sensory neuron (Rohan-Beard cell) stripe (arrowhead). In addition there is diffuse XIF3 staining in neuroectoderm between the motor neuron and interneuron stripes and to the same anterior limit as these stripes.* **(D)** *At stage 17 there is additional diffuse bilateral staining in the prospective midbrain (arrow), though this expression is transient.* **(E)** *By stage 18, punctate staining is seen in two patches adjacent to the anterior boundary of the neuroectoderm corresponding to neurons in the nasal placodes (arrowed).* **(F)** *At stage 20, the neural tube is almost completely folded and the primary neurons are strongly stained. Expression in the trigeminal ganglia extends around the eye.*

tubulin (NST) (Chitnis *et al.*, 1995). The first axons sprout at the early tailbud stage, little more than one day after fertilization (Jacobson and Huang, 1985).

Under experimental conditions the whole of the animal cap can be made to form neural tissue (Grunz and Tacke, 1989), but this ability is repressed in the embryo by the extracellular protein BMP-4 (Wilson and Hemmati-Brivanlou, 1995). Consequently neural tissue forms when BMP-4 activity is itself compromised in dorsal ectoderm through the activity of proteins such as noggin and chordin that bind BMP-4 (Holley *et al.*, 1996; Piccolo *et al.*, 1996). It has been observed that isolated animal cap ectoderm cultured in the presence of noggin develops into neuroectoderm and expresses high levels of XIF3, however, these cells do not express NST and fail to differentiate into neurons (Lamb *et al.*, 1993). Consequently, the observation that XIF3 is expressed predominantly in primary neurons, yet is noggin inducible, at first seems incongruent.

In this paper we show that *XIF3* transcripts are found in two separate cell types at the neurula stage. The first are the rapidly differentiating primary neurons of the caudal neurectoderm, the trigeminal ganglion and the olfactory placode whilst the second cell type consists of a region of undifferentiated anterior neurectoderm. The pattern of *XIF3* expression in noggin treated animal caps reflects elevated transcript levels in undifferentiated anterior neuroectoderm and therefore is consistent with the ability of noggin to neuralize but not to promote neuronal differentiation (Lamb *et al.*, 1993).

Results

The distribution of XIF3 transcripts

RNAse protection assays have previously shown that *XIF3* transcripts are predominantly in the anterior third of the embryo at the tailbud stage (Sharpe *et al.*, 1989). We now describe the pattern of *XIF3* expression during development using whole-mount *in situ* hybridization with an antisense *XIF3* RNA probe.

Quantitative RNase protection assays detect a low level (approximately 10^5 transcripts) of *XIF3* in the egg and throughout the early stages of development (Sharpe *et al.*, 1989). Transcription of *XIF3* in the embryo begins around the start of gastrulation and transcripts accumulate to a plateau of approximately 10^6 per embryo at the late neurula stage (Sharpe *et al.*, 1989). We were unable to detect the low maternal level by *in situ* hybridization and first detected *XIF3* by this method in the mid-neurula embryo. At this stage expression is confined to two longitudinal stripes in the neuroectoderm adjacent to the midline and bilateral patches within the epidermis at the boundary of the neuroectoderm (Fig. 1A). The

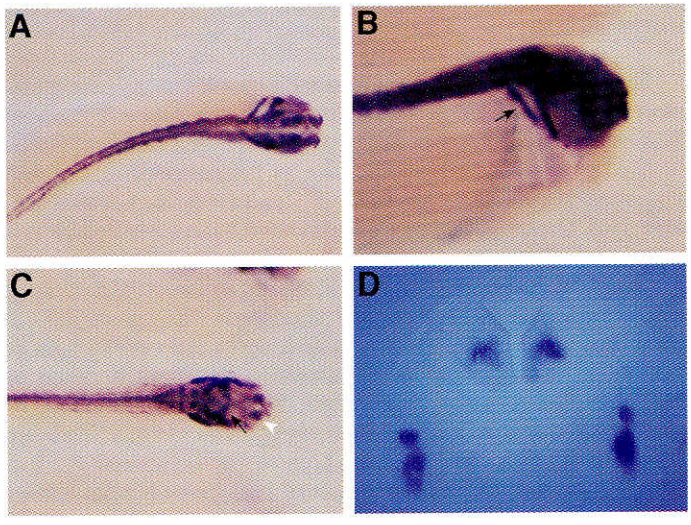


Fig. 2. The pattern of XIF3 expression in the later embryo. **(A)** *In the late tailbud (stage 28) expression in the VIIth cranial nerve is now apparent and within the anterior neural tube expression of XIF3 resolves into patches of high and low expression along the A-P axis.* **(B)** *Lateral view showing expression in the Vth and (arrowed) VIIth cranial ganglia and in the eye.* **(C)** *By stage 36/37 expression in the caudal primary neurons has almost completely disappeared, though staining in the cranial ganglia remains strong. Within the CNS, XIF3 is now clearly expressed in distinct domains. There is a domain in the forebrain that lies beneath the nasal pits (arrowhead) and another at the midbrain-hindbrain boundary (arrow). Within the hindbrain expression is mainly confined to rhombomeres 2,4 and 6.* **(D)** *A transverse section through a stage 36/37 embryo shows that XIF3 expression within the even rhombomeres is confined to a ventro-lateral domain.*

stripes probably correspond to expression in primary motor neurons, whilst the patches are likely to be cells within the neural placodes that contribute to the trigeminal (or Vth cranial) ganglia (Chitnis, *et al.*, 1995). In both cases the staining is punctate as this reflects the selection through lateral inhibition of some cells in these areas to differentiate as neurons (Chitnis *et al.*, 1995).

As development progresses, punctate *XIF3* staining is seen in additional longitudinal stripes corresponding to expression in the primary interneurons and then the primary sensory neurons (Rohan-Beard cells) (Fig. 1B,C). Towards the end of neurulation (stage 17-18), transcripts are first detected in the neuroectoderm in a pattern that is diffuse, affecting all cells in a particular area, rather than the punctate staining associated with the primary neuron stripes. This type of *XIF3* expression is seen in the presumptive midbrain either side of, but not including, the midline and in the neuroectoderm at the anterior end of the primary neuron stripes (Fig. 1D).

A short time later (st. 18-19) two small patches of punctate *XIF3* staining appear at the anterior end of the embryo which probably correspond to the prospective olfactory neurons of the nasal placodes (Fig. 1E). In the tailbud embryo (stage 28), *XIF3* staining in the neural tube has resolved into separate domains (Fig. 2A). Staining in the trigeminal ganglion remains intense whilst XIF3 expression in the VIIth ganglion is just detectable. In addition there is weak staining in the eye.

After hatching (stage 35) expression in primary neurons along the spinal cord is strongly reduced. Within the head there are bands of strong staining corresponding to the midbrain-hindbrain boundary and hindbrain rhombomeres 2, 4 and 6 (Fig. 2C), though not all cells within these rhombomeres are affected (Fig. 2D). Staining in cranial ganglia V and VII remains strong and there is weak staining in the nasal pits and the adjacent forebrain region. Transverse sections through the eye show that staining is restricted to the ciliary marginal zone (data not shown).

XIF3 is expressed in primary neurons in the tailbud embryo

The pattern of *XIF3* staining in the neurula embryo suggests the gene is expressed in primary neurons. To confirm this assumption we have identified *XIF3* transcripts by *in situ* hybridization and co-stained with the anti-HNK-1 monoclonal antibody. In *Xenopus* embryos at the tailbud stage, anti-HNK-1 (clone VC1.1) recognizes a range of neural cells including, Rohan-Beard cells (primary sensory neurons), cells of the trigeminal ganglion and, weakly, the primary motorneurons (Nordlander, 1989). We have also examined comparable embryos using an antisense neuron-specific type-II β-tubulin (*NST*) probe (as a known marker of primary neurons) (Chitnis *et al.*, 1995) and then co-stained with the same antibody. Sections through embryos show an intimate association between *XIF3* and HNK-1 staining (Fig. 3A,C) and a similar close correlation is seen for *NST* and HNK-1 (Fig. 3B,D). These results strongly indicate that *XIF3*, like *NST*, is expressed in primary neurons at the early tailbud stage.

Induction of XIF3 by noggin

The expression of noggin in animal caps diverts these cells from an epidermal to a *neural* fate (Lamb *et al.*, 1993). It is important to note though, that noggin alone is insufficient to propel cells along a pathway of *neuronal* differentiation in which cells extend axons and express markers such as *NST*. It has previously been shown however, that noggin induces isolated animal cap explants to express elevated levels of *XIF3* (Lamb *et al.*, 1993). Given the pattern of *XIF3* expression we have described above there are two possible explanations; first, the elevated level of *XIF3* will be found in precursor cells that have undergone selection through lateral inhibition but which, in the absence of factors other than noggin, are unable to complete differentiation as primary neurons, or second, noggin induced *XIF3* expression will not be associated with primary neurons but instead will be found in the equivalent of undifferentiated anterior neurectoderm. From the observations on whole embryos we know that in the former case the staining pattern will be characteristically punctate whereas in the latter the staining will be evenly distributed across areas consisting of many cells.

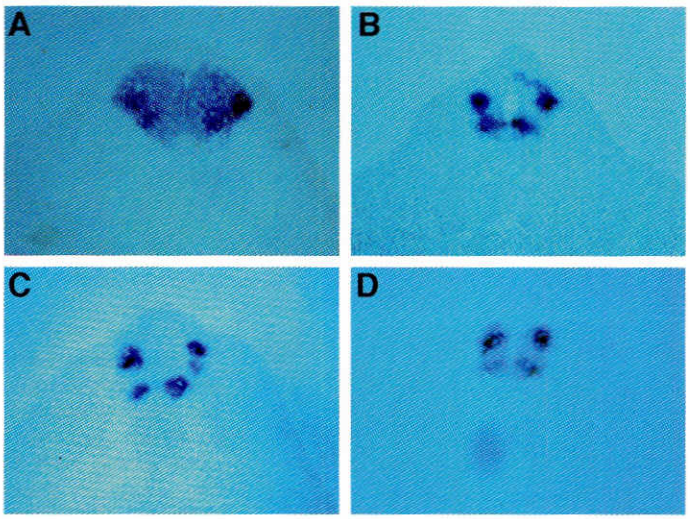


Fig. 3. Co-expression of *XIF3* and neural specific tubulin with antibody markers for primary neurons. (A-D) *Transverse sections of stage 22 embryos at two positions along the A-P axis:* **(A** *and* **C)** *embryos at stage 22 stained by in situ hybridization with an DIG-labeled antisense RNA probe to XIF3 (blue) and then by whole-mount antibody staining with anti-HNK-1 (brown).* **(B** *and* **D)** *Sections stained by in situ hybridization with a neural specific tubulin (NST) specific antisense probe (blue) and then by whole-mount antibody staining with anti-HNK-1 (brown). At this stage the antibody stains a structure within the cell that is probably the Golgi apparatus. Note the co-localization of the two stains in the primary neurons. In addition XIF3 staining is seen in undifferentiated neuroectoderm at more rostral levels (panel A).*

Embryos at the two-cell stage were injected with synthetic *noggin* mRNA, animal caps removed (at stage 9) and cultured to the equivalent of the late neurula stage. The induction of *XIF3* in *noggin* injected animal caps was monitored by Northern blots which also confirmed the lack of *NST* expression (data not shown). Similarly, *NST* transcripts were not detected by *in situ* hybridization in *noggin* injected animal caps (Fig. 4A and B). In contrast, animal caps taken from embryos receiving more than 125 pg of *noggin* mRNA expressed *XIF3* in large, diffuse patches (Fig. 4C and D) whilst transcripts were not detected in uninjected animal caps (Fig. 4E). Culturing noggin injected animal caps with a small amount of dorsal mesoderm resulted in a combination of diffuse and punctate *XIF3* staining (Fig. 4F) showing that the two patterns are clearly distinguishable.

Above we correlated the expression of *XIF3* to the expression of the epitope recognized by the anti-HNK-1 antibody in primary

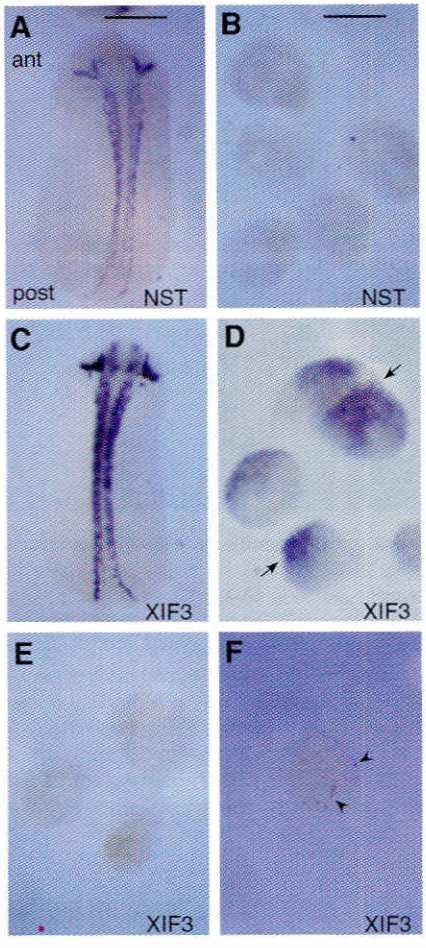


Fig. 4. Noggin induces a pattern of expression of XIF3 in animal caps that is similar to that found in anterior neuroectoderm but not in primary neurons. (A) *Stage 22 embryo stained by in situ hybridization with a NST specific antisense RNA probe. The anterior of the embryo (ant) is at the top.* **(B)** *Noggin injected animal caps also stained with the NST specific probe at the equivalent stage to (A) lack NST expression.* **(C)** *Stage 22 embryo stained by in situ hybridization with a XIF3 specific antisense RNA probe.* **(D)** *Noggin injected animal caps at the same stage stained with the XIF3 specific probe. Arrows indicate large patches of evenly distributed XIF3 staining. However the staining does not cover the entire animal cap.* **(E)** *Control uninjected animal caps assayed at stage 22 are negative for XIF3 expression.* **(F)** *Explant of animal cap and a small piece of dorsal mesoderm that induces the formation of primary neurons and at stage 22 results in a punctate pattern of XIF3 expression. Bar in A, 0.5 mm; in B, 0.25 mm.*

neurons. Neither noggin-injected nor uninjected animal caps react with the anti-HNK-1 antibody (Fig. 5A-C) at the early tailbud stage. However, *noggin*-injected (Fig. 5E) but not uninjected (Fig. 5F) animal caps reacted with the monoclonal antibody 6F11 which recognizes the neural marker, NCAM at the tailbud stage. Sections through 6F11 stained animal caps did not show an altered morphology in the injected explant (Fig. 5 G-I) though stained cells appeared more elongated than cells in uninjected controls. It has previously been shown that noggin induces animal caps to form neural tissue without forming mesoderm (Lamb *et al.*, 1995) and this is also the case in our experiments as neither *noggin* -injected nor uninjected animal caps react with the antibody 12/101 which marks somitic mesoderm (Fig. 5 J-L) (Kintner and Brockes, 1984). Together these results suggest that *noggin* induces *XIF3* in animal caps in a pattern that looks like that found in undifferentiated anterior neuroectoderm rather than in primary neurons as they differentiate.

Discussion

Expression of XIF3 during the formation of the nervous system

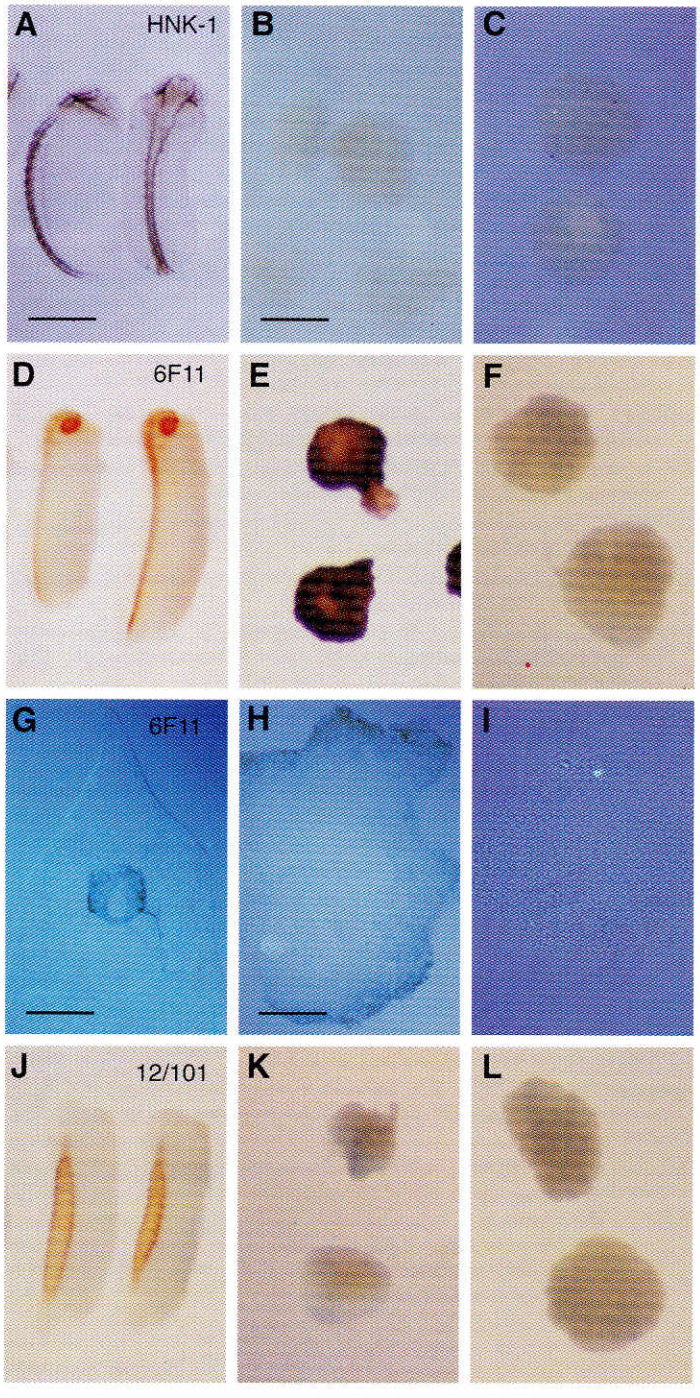
In this report we extend previous studies of *XIF3*, a gene encoding a neural-specific type III intermediate filament protein (Sharpe, 1988; Sharpe *et al.*, 1989). Using *in situ* hybridization we have shown that there are two distinct domains of *XIF3* expression in the neurula stage neurectoderm. The first is within the cells that contribute to the columns of primary neurons, and therefore appears punctate, whilst the second, at a lower level, is in all cells within a restricted part of the anterior neuroectoderm and therefore gives rise to an even, diffuse staining pattern.

At later stages *XIF3* expression is generally similar to that reported for tanabin, itself an intermediate filament protein though distinct in sequence from XIF3 (Hemmati-Brivanlou *et al.*, 1992). Both are expressed strongly in cranial ganglia and the even-numbered rhombomeres of the hindbrain. However, the patterns of *XIF3* and *tanabin* expression also show notable differences. For example, transverse sections at the tailbud stage show that Rohan-Beard cells, which do not express *tanabin* (Hemmati-Brivanlou *et al.*, 1992), clearly express *XIF3* (Fig. 3).

It has been suggested previously that cells entering a pathway of neural development are subject to sequential patterns of IF expression (Bennett, 1987) and we can now add *XIF3* to an overlapping sequence of IF gene expression in *Xenopus* neural tissue. Following neural induction, the pre-neural cells probably first express vimentin, as they do in chick embryos (Tapscott *et al.*, 1981), although the expression of nestin (Lendahl *et al.*, 1990), which is found in the pre-neural cells of higher vertebrates, has yet to be examined in *Xenopus*. From the mid-neurula stage the post-mitotic primary neurons express neural-specific IFs such as XIF3 and tanabin (Hemmati-Brivanlou *et al.*, 1992). Several hours later at the beginning of the tailbud stage the primary neurons complete differentiation and begin to extend axons (Jacobson and Huang, 1985) and this stage marks the first expression of NF-M a type IV neurofilament (Sharpe, 1988). Subsequently neurons may express NF-L and NF-H, or, in *Xenopus*, alternative IFs encoded by the genes *XNIF* (Charnas *et al.*, 1992), which is thought to be the alpha-internexin equivalent (Fleigner *et al.*, 1994) and *Xfiltin* (Zhao and Szaro, 1997).

XIF3 is induced in animal cap cells by noggin

XIF3 is strongly expressed in animal caps in response to *noggin* (Lamb *et al.*, 1993). This was initially surprising since *XIF3*, like *NST*, is expressed predominantly in primary neurons at the neurula stage, yet noggin treatment does not induce NST nor result in the formation of differentiated neurons in animal caps (Lamb *et al.*, 1993). We have resolved this issue by showing that *XIF3*, but not *NST* expression is found in undifferentiated anterior neurectoderm. Staining in these regions appears diffuse as many adjacent cells express *XIF3*. In contrast staining in primary neuron domains is punctate as only those cells selected by lateral inhibition will differentiate and express *XIF3*. Animal caps injected with *noggin* mRNA stain evenly for *XIF3* in large patches of cells rather than in a punctate pattern suggesting that noggin results in the formation of undifferentiated anterior neurectoderm. These results indicate that noggin is sufficient for XIF3 expression in anterior neuroectoderm whereas additional, as yet unknown, factors are required for expression in primary neurons. The cell surface protein NCAM is expressed throughout most of the neurectoderm, and is also detected in animal caps following noggin injection lending further support to the suggestion that XIF3 is expressed in undifferentiated neuroectoderm in response to noggin. Interestingly, whereas NCAM appears to be expressed throughout noggin injected animal



caps, XIF3 expression by *in situ* hybridization in noggin animal caps was usually restricted to a part of the animal cap (compare Figs. 4D and 5E). In the whole embryo, NCAM is expressed throughout the neuroectoderm whilst XIF3 in undifferentiated neuroectoderm is confined to an anterior domain, and this differential pattern of expression may be recapitulated in the *noggin* injected animal caps.

In conclusion we have shown that *XIF3* is expressed at an early stage in sets of rapidly differentiating primary neurons in the *Xenopus* embryo and also in a restricted domain of undifferentiated anterior neurectoderm. An analysis of the *XIF3* promoter may well identify elements that control the expression of the *XIF3* gene in each these two domains.

Materials and Methods

Maintenance of embryos

Xenopus embryos were dejellied in 2% cysteine-HCl (pH 8.0) and grown as previously described in hypotonic 0.1xMBS (Gurdon, 1977). Animal caps were removed in isotonic 1xMBS with forceps and needles at stage 9 (stages according to Nieuwkoop and Faber 1994) and grown as pairs in 1xMBS on agarose coated dishes.

In situ hybridization and whole-mount immunocytochemistry

In situ hybridization was performed according to Harland (1991) with the modifications of Baker *et al.*, (1995). At the appropriate stage embryos were fixed for 2 h in formaldehyde-based MEMFA and stored at -20°C in methanol. Following *in situ* hybridization with either a XIF3 antisense probe derived from the XIF3 EcoRI fragment cloned into Bluescript linearized with BamH1 and synthesized with SP6 or an NST antisense probe generated with T3 from a BamHI linearized template, embryos were refixed in MEMFA and photographed as cleared specimens in Murrays Clear using Kodak 160T slide film.

Embryos for whole-mount immunocytochemistry were fixed in MEMFA. Anti-HNK-1 (Sigma) was used as a primary antibody at 1 in 500, 6F11(XAN3) which recognizes an epitope on NCAM was used at a dilution of 1:1 (Sakaguchi *et al.*, 1989) and 12/101 which recognizes somites (Kintner and Brockes, 1984) was used at 1 in 200. Each was followed with an HRP-conjugated secondary antibody and developed with the Pierce DAB staining kit according to the manufacturers instructions. Embryos were dehydrated in methanol, transferred to PEDS wax as described previously (Sharpe and Goldstone, 1997) and cut into 14 μm sections on a rotary microtome.

Noggin injections and animal cap assays

Synthetic noggin mRNA was injected at no more than 0.1 mg/ml in an injection volume of 10 nl. For the induction of XIF3, embryos in 1xMBS 2.5% Ficoll were injected with 125 pg of noggin mRNA into the animal pole of both cells at the two cell stage and grown in 1xMBS to the early blastula stage

Fig. 5. Further analysis of noggin injected animal caps. (A) *Stage 24 embryos stained with the anti-HNK-1 monoclonal antibody which marks primary neurons. Anterior is at the top of the panel.* **(B)** *At the same early neurula stage, noggin injected animal caps are negative for the anti-HNK-1 epitope.* **(C)** *Uninjected animal caps are also negative with the anti-HNK-1 antibody.* **(D)** *Whole embryos at the late tailbud stage stained with the monoclonal antibody 6F11 that recognizes an NCAM antigen and marks neural tissue.* **(E)** *Noggin injected animal caps react positively with 6F11 across most if not all of the animal cap showing that noggin injection results in the animal cap acquiring a neural fate.* **(F)** *Uninjected animal caps are negative with the 6F11 antibody.* **(G,H,I)** *Sections through the samples shown in D,E and F respectively. 6F11 is seen throughout the injected animal cap explant but is strongest in the superficial cells, though this may be due to difficulties in antibody penetration in the whole-mount sample. There is little difference in the morphology of the animal caps in noggin injected (H) and uninjected (I) animal caps.* **(J)** *Late tailbud embryos stained with the somite marker 12/101.* **(K)** *Noggin injected animal caps do not express the antigen recognized by 12/101 confirming that noggin can neuralize animal caps without inducing the formation of mesoderm.* **(L)** *Uninjected animal caps do not express the 12/101 antigen. Bars in A and for the other whole embryos, 1.3 mm; in B and the other intact animal caps, 0.25 mm; in G, 0.33 mm and in H, 0.06 mm.*

and then in 0.1xMBS to the late blastula stage. Animal caps were removed and cultured as described above then fixed when control embryos were at the late neurula, early tailbud stage.

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References

BAKER, C.V.H., TORPEY, N.B., SHARPE, C.R., HEASMAN, J. and WYLIE, C.C. (1995). A *Xenopus c-kit* related receptor tyrosine kinase expressed in migrating stem cells of the lateral line system. *Mech. Dev. 50:* 217-228.

BENNETT, G.S. (1987). Changes in intermediate filament composition during neurogenesis. In *Current Topic in Developmental Biology* 21 151-183, Ed. R.K. Hunt, Academic Press, Orlando, USA.

CHARNAS, L.R., SZARO, B.G. and GAINER, H. (1992). Identification and developmntal expression of a low molecular weight neuronal intermediate filament protein expressed in *Xenopus laevis. J. Neurosci 12:* 3010-3024.

CHITNIS, A., HENRIQUE, D., LEWIS, J., ISH-HOROWICZ, D. and KINTNER, C.R. (1995). Primary neurogenesis in *Xenopus* embryos regulated by a homologue of the *Drosophila* neurogenic gene Delta. *Nature 375:* 761-766.

EVANS, R.M. (1998). Vimentin: the conundrum of the intermediate filament gene family. *Bioessays 20:* 79-86.

FLEIGNER, K.H., KAPLAN, M.P. WOOD, T.L., PINTAR, J.E. and LIEM, R.K.H. (1994) Expression of the gene for the neuronal intermediate filament protein alpha *internexin coincides* with the onset of neuronal differentiaition in the developing rat nervous system. *J. Comp. Neurol. 342:* 161-173.

GREENE, L.A. (1989). A new neuronal intermediate filament protein. *Trends Neurosci. 12:* 228-230.

GRUNZ, H. and TACKE T. (1989). Neural differentiation of *Xenopus laevis* ectoderm takes place after disaggregation and delayed reaggregation without inducer. *Cell Differ. Dev. 28:* 211-218.

GURDON, J.B. (1977). Methods for nuclear transplantation in Amphibia. *Methods Cell Biol. 16:* 125-139.

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

HARTENSTEIN, V. (1989) Early neurogenesis in *Xenopus*: The spatio-temporal pattern of proliferation and cell lineages in the embryonic spinal cord. *Neuron 3:* 399-411.

HARTENSTEIN, V. (1993). Early pattern of neuronal differentiation in the embryonic brainstem and spinal cord. *J. Comp. Neurol. 328:* 213-231.

HEMMATI-BRIVANLOU, A., MANN, R.W. and HARLAND, R.M. (1992). A protein expressed in the growth cones of embryonic vertebrate neurons defines a new class of intermediate filament protein. *Neuron 9:* 417-428.

HOLLEY, S., NEUL, J., ATTISANO, L., WRANA, J., SASAI, Y., O'CONNOR, M., DEROBERTIS, E. and FERGUSON, E. (1996). The *Xenopus* dorsalising factor noggin ventralises *Drosophila* embryos by preventing DPP from activating its receptor. *Cell 86:* 607-617.

JACOBSON, M. and HUANG, S. (1985). Neurite outgrowth traced by means of horseradish peroxidase inherited from neuronal ancestral cells in frog embryos. *Dev. Biol. 110:* 102-113.

KINTNER, C.R. and BROCKES, J.P. (1984). Monoclonal antibodies identify blastemal cells derived from dedifferentiating muscle in newt limb regeneration. *Nature 308:* 67-69.

LAMB T.M., KNECHT A.K., SMITH W.C., STACHEL S.E., ECONOMIDES A.N., STAHL N., YANCOPOLOUS, G.D. and HARLAND, R.M. (1993). Neural induction by the secreted polypeptide noggin. *Science 262:* 713-718.

LAMBORGHINI, J.R., (1980). Rohan-Beard cells and other large neurons in *Xenopus* embryos originate during gastrulation. *J. Comp. Neurol. 189:* 323-333.

LENDAHL, U., ZIMMERMAN, L.B. and MCKAY, R.D.G. (1990). CNS stem cells express a new class of intermediate filament protein. *Cell 60:* 585-595.

MA, Q., KINTNER, C. and ANDERSON, D.J. (1996). Identification of *neurogenin* a vertebrate neuronal determination gene. *Cell 87:* 43-52.

NIEUWKOOP, P.D. and FABER, J. (1994). *Normal Table of Xenopus laevis* (Daudin). 2nd edition. North Holland Publishing Co. Amsterdam.

NORDLANDER, R.H. (1989). HNK-1 marks earliest axonal outgrowth in *Xenopus. Dev. Brain Res.* 50: 147-153.

PICCOLO, S., SASAI, Y., LU, B. and DEROBERTIS, E. (1996). Dorsoventral patterning in *Xenopus*: inhibition of ventral signals by direct binding of chordin to BMP4. *Cell 86:* 589-598.

ROBERTS, A. and CLARK J.D.W. (1982). The neuroanatomy of an amphibian embryo spinal cord. *Philos. Trans. R. Soc. Lond. [Biol.] 296:* 195-212.

SAKAGUCHI, D.S., MOELLER, J.F., CLARK, R., COFFMAN, C.R., GALLENSON, N. and HARRIS, W.A. (1989). Growth cone interactions with a glial cell line from embryonic retina. *Dev. Biol. 134:* 158-174.

SHARPE, C.R. (1988). Developmental expression of a neurofilament M and two vimentin-like genes in *Xenopus laevis. Development 103:* 269-277.

SHARPE, C.R. and GOLDSTONE, K. (1997). Retinoid receptors promote primary neurogenesis in *Xenopus. Development 124:* 515-523.

SHARPE, C.R. PLUCK A. and GURDON, J.B. (1989). XIF3 a *Xenopus* peripherin gene, requires an inductive signal for enhanced expression in anterior neural tissue. *Development 107:* 701-714.

STEINERT, P.M. and ROOP, D.R. (1988). Molecular and cellular biology of intermediate filaments. *Annu. Rev. Biochem. 57:* 593-626.

TAPSCOTT, S.J., BENNETT, G.S., TOYAMA, Y., KLEINBART, F.A. and HOLTZER, H. (1981). Intermediate filament proteins in the developing chick spinal cord. *Dev. Biol. 86:* 40-54.

WILSON, P.A. and HEMMATI-BRIVANLOU, A. (1995). Induction of epidermis and inhibition of neural fate by BMP-4. *Nature 376:* 331-333.

ZHAO, Y. and SZARO, B.G. (1997). Xefiltin, a new low molecular weight neuronal intermediate filament protein of *Xenopus laevis* shares sequence features with goldfish gefiltin and mammalian alpha internexin and differs in expression from XNIF and NF-L. *J. Comp. Neurol. 377:* 351-364.

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