XIF3, a *Xenopus* peripherin gene, requires an inductive signal for enhanced expression in anterior neural tissue

C. R. SHARPE, A. PLUCK and J. B. GURDON

*Cancer Research Campaign Molecular Embryology Research Group, Department of Zoology, University of Cambridge, UK*

**Summary**

A full-length cDNA clone for the *Xenopus* intermediate filament gene XIF3 has been isolated. It is very similar in sequence to the rat intermediate filament cDNA clone 73 that is thought to encode the neuronal intermediate filament protein 'peripherin'. By analysing dissected embryos, we show that XIF3 is expressed predominantly in anterior and dorsal structures and most strongly in the brain of the tailbud (stage 26) embryo. *In situ* hybridization shows XIF3 transcripts to be localized in neural tissue and especially in regions that most probably correspond to the motor neurones of the neural tube and to some cranial nerve ganglia.

New XIF3 transcripts are first found at the start of gastrulation at a low level throughout the ectoderm and are not localized to the presumptive neurectoderm. Expression subsequently increases by about 10-fold in neural tissue, and requires an interaction of the mesoderm with overlying ectoderm. Because new transcripts are found predominantly in neural tissue of the head, this response can be used as a marker of anterior neural induction.

**Key words:** *Xenopus*, neural induction, intermediate filaments, peripherin.

**Introduction**

The generation of neural tissue in Amphibia involves an interaction between mesodermal and ectodermal cell layers as they move into close proximity during gastrulation (Spemann, 1938; Gurdon, 1987; Saxen, 1989). As a result, a part of the ectoderm is directed away from an epidermal pathway of differentiation to one forming neural tissue. In order to understand these interactions, we have attempted to isolate genes whose expression can be used to recognise the induced neurectoderm at an early stage of its development. In this way, we can avoid assays for neural induction that rely on morphological or histological techniques, processes that require subjective analysis at relatively late stages of development, sometimes days after the induction has taken place.

One marker gene that we find particularly useful is XIF6, which encodes a *Xenopus* intermediate filament, the neurofilament-M (NF-M) (Sharpe, 1988). The neurofilament proteins in general have been detected throughout the central nervous system from stage 33 (Godsave et al. 1986), and NF-M in particular, is widely distributed in neural tissue at the swimming tadpole stage (Szarø and Gainer, 1989). We have been able to use this gene as a general neural marker at the tailbud stage (stage 26) and transcripts can be detected as early as the late neurula stage. Expression of a second marker, the homeobox-containing gene XlHbox-6, is restricted to the posterior nervous system and can therefore be used as a regional marker (Sharpe et al. 1987). In order to investigate the regional basis of neural induction that leads to anterior-posterior differentiation, we have looked for other genes that are expressed in distinct regions of the nervous system.

During the screening that led to the isolation of the *Xenopus* NF-M gene, two other intermediate filament (IF) genes were found (Sharpe, 1988). One of these, XIF1, was found by sequence comparison to be a *Xenopus* vimentin gene, expressed in neural and mesodermal cell types. The other, XIF3, was closely related to the vimentin gene, but by limited sequence analysis could not be designated as the *Xenopus* form of either vimentin, desmin or GFAP which together form the type III IF subfamily. At the tailbud stage of development, expression of XIF3 is confined predominantly to the anterior portion of the embryo, a pattern of expression not seen for either XIF1 or XIF6 (NF-M) genes.

In this paper we extend these findings by presenting a cDNA clone comprising the entire coding sequence for XIF3. The predicted translation product is most similar to the protein encoded by the rat clone 73 (Leonard et al. 1988), an IF protein also known as peripherin (reviewed by Greene, 1989). We also show that the activation of XIF3 transcription in the embryo is dependent on two processes, only the second of which involves neural induction.

XIF3 expression can be used as a marker for the developing anterior nervous system and, when used in
conjunction with our previously identified markers of neural induction, XIF6 and XIIBox-6, it will enable us to analyse the formation of a regionally differentiated nervous system during embryonic neural induction.

Materials and methods

(1) Screening the cDNA libraries

_Xenopus_ cDNA libraries prepared from neurulae (Kintner and Melton, 1987) and tailbud embryos (provided by D. Cleveland and M. Lopata) by the method of Gubler and Hoffman (1983) were screened at high stringency (Benton and Davis, 1977) with a fragment of the XIF3 gene containing the 222 bp exon encoding the carboxy-terminus of the central helical rod domain of the predicted protein (Sharpe, 1988). Fragments from full-length clones were subcloned into M13 mp18 vectors (Messing, 1983) and sequenced by the dideoxy protocol (Sanger et al., 1977; Henikoff, 1984).

(2) Analysis of embryonic expression

Embryos were prepared, grown and dejellied as described by Gurdon (1977), and staged according to Nieuwkoo and Faber (1967). Neural conjugates were constructed as described previously (Sharpe et al., 1987). RNase protection assays were performed according to Krieg and Melton (1987) using one or two embryo equivalents of total RNA per assay prepared as described by Mohun et al. (1984). The XIF3 probe was made by transcribing HindIII-cut pBSXIF3 (Sharpe, 1988) with T7 RNA polymerase. The 5S probe was synthesised by transcribing the plasmid pSP 5S (Sharpe et al., 1987) with SP6 RNA polymerase after linearizing the template with PvuII.

(3) In situ hybridization

Antisense RNA probes were synthesized by _in vitro_ transcription in the presence of [35S]UTP (Krieg and Melton, 1987). The XIF3 probe comprised the transcripts of the HindIII-linearised pBSXIF3 transcribed from the T7 promoter and of the plasmid pBSXIF3-HS, a 184 bp HindIII-Sau3A fragment cloned into the vector Bluescribe (Stratagene), linearized with HindIII and transcribed with T7 polymerase. The method used was that of Kintner and Melton, (1987) and Hopwood et al. (1989).

Results

(1) The nucleotide sequence of XIF3 and its relationship to other intermediate filament genes

We have determined the complete coding sequence of XIF3 from two cDNA clones, XIF3.1 and XIF3.2. The longest clone (XIF3.1) extended from the 3' poly(A) tail for 2137 residues. It is likely that XIF3.1 lacks some nucleotides from the 5' untranslated region of the full-length message. XIF3.2 is missing the first 254 residues at the 5' end compared to XIF3.1. Only one further difference was found between the clones; in the carboxy terminal domain of the protein, there is a conservative change of an alanine to a valine residue due to a single point mutation at position 1254. This may represent a naturally occurring polymorphism or an artefact introduced during cloning. In all other respects, the sequences of clone XIF3.1 and clone XIF3.2 are identical. The sequence in Fig. 1 represents the clone XIF3.1; the single observed change in XIF3.2 is indicated.

From the cDNA clones, the predicted messenger RNA length of XIF3 is approximately 2.2 kb, in good agreement with the size determined by RNA blotting (Sharpe, 1988) and confirms the greater length of the XIF3 transcript compared to that of XIF1 (Vimentin, 1.8 kb). Conceptual translation of the open reading frame (shown in Fig. 2) beginning with the first ATG codon at position 17 generates a protein of 456 amino acid residues compared with 458 and 463 amino acids for two forms of the related protein, _Xenopus_ vimentin (Herrmann et al. 1989a). XIF3 was identified as encoding a type III IF (the classification of the IF proteins has been reviewed in Steinert and Roop, 1988) by limited DNA sequence analysis of one exon of the genomic clone covering 70 amino acid residues of the sequence (Sharpe, 1988) and the classification is confirmed by the full-length cDNA sequence.

All IF proteins have a similar three-domain structure, namely a non-helical head and tail separated by a central helical rod-shaped domain. The head domain of XIF3 is particularly rich in serine residues (40 %, 25/63) (shown in bold italic in Fig. 2) compared to other type III IF proteins, the exception being clone 73 (30 %, 21/62), an IF cDNA isolated from rat phaeochromocytoma (PC12) cells. For clone 73, it has been suggested that the serine residues may be involved in the phosphorylation of the protein (Leonard et al. 1988). The central helical rod domain contains distinctive regions of periodic heptad repeats and near its carboxy end is the conserved amino acid sequence TYRKLLEGEE, both features common to all intermediate filament proteins (Steinert and Roop, 1988). The non-helical tail domain contains a high proportion of the amino acid residues serine, threonine and proline.

Table 1 summarizes the amino acid sequence identities between _Xenopus_ intermediate filament proteins and their rodent homologues. Percentage identities within the individual head, rod and tail domains are calculated for three pairs of proteins; XIF3 with rat clone 73, _Xenopus_ vimentin (Herrmann et al. 1989a) with hamster vimentin (Quax et al. 1983), and _Xenopus_ desmin (Herrmann et al. 1989b) with hamster desmin.

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<td>% Amino acid identity</td>
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1 Figures are expressed as percentages either across the whole protein (overall), or across the individual domains as defined by Steinert and Roop (1988).

clonel 73 is the rat form of peripherin (Leonard et al. 1988).

Xen I is the _Xenopus_ form of vimentin (Herrmann et al. 1989a).

vim is hamster vimentin (Quax et al. 1983).

Xdes is the _Xenopus_ form of desmin (Herrmann et al. 1989b).

des is the hamster form of desmin (Quax et al. 1985).
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Fig. 1. The complete nucleotide sequence of Xenopus XIF3 cDNA clone pXIF3.1. The 5' EcoRI site, (position 1–6), is bold and the first downstream ATG, the probable translational start site, is underlined at position 17. The boundaries to the head, rod and tail domains of the protein are indicated. The intermediate filament consensus sequence towards the end of the rod domain is boxed and the termination codon marked as an asterisk. The poly(A) addition sequence is double underlined. The variant nucleotide at position 1254 and the altered amino acid residue found in pXIF3.2 are shown below and above the line, respectively. This and the following sequence data will appear in the EMBL/GENBANK/DDBJ Nucleotide Sequence. Databases under the accession number X16570.
Fig. 2. A comparison of the amino acid sequences of clone 73, the rat peripherin cDNA clone (Leonard et al. 1988), and XIF3. Conserved residues are indicated by two dots, conservative changes by one dot. The abundant serine residues in the head domain are shown in bold italic. Regions of the central helical rod: coils 1a, 1b and 2, that show the regions of extensive heptad repeats of hydrophobic residues distinctive of intermediate filament proteins, are boxed. A stop codon is represented by an asterisk. Gaps have been introduced to give better alignment.
(Quax et al. 1985). From these data, it can be seen that XIF3 is, over its full length, less similar to its putative rodent homologue, clone 73, than are either Xenopus vimentin or desmin to their rodent counterparts. This is particularly the case when the tail regions, thought to be important in conferring the different functions on the IF protein, are compared (Steinert et al. 1985). However, XIF3 is more similar to clone 73 than to any other IF protein. As XIF3 is in some respects homologous of clone 73 and as clone 73 is expressed only in neural tissue, we have investigated the embryonic expression of XIF3 as a candidate neural marker during Xenopus development.

(2) The location of XIF3 expression in the tailbud embryo
We have previously shown that XIF3 is expressed predominantly in the head of the tailbud (stage 26) embryo (Sharpe, 1988). We now extend this observation; protection assays of RNA from stage 26 embryos show that XIF3 expression outside the head region is predominantly in the dorsal tissues of the embryo (Fig. 3A). This general pattern of expression remains essentially unchanged from the tailbud to the free swimming tadpole stages (unpublished observation). The XIF3 protection assay gives a doublet protected band of around 222 bases. This could represent either the protection of two very closely related molecules such as alternatively spliced transcripts of the kind seen in the rodent homologue, peripherin (Landon et al. 1989), or an artefact of the RNase reaction.

We have used RNase protection analysis of dissected embryos and in situ hybridization to determine more accurately the location of embryonic XIF3 expression. First, a more detailed dissection of the head was performed. Heads were divided into four parts, namely ectoderm (including sucker), brain, eyes and a remnant composed of mesoderm and ectoderm. The level of XIF3 transcripts in each fragment was then compared to the level in an equal number of intact heads and whole embryos at the same developmental stage. The total level of RNA was found by determining the amount of 5S RNA in a fraction of each sample. The advantages of this approach are that it allows a quantitative comparison of the number of transcripts at any stage, and that even low levels of transcripts can be detected. The result, shown in Fig. 3B, is that the majority of XIF3 transcripts in the head region are located in the brain. It is possible that the low levels found in the other fractions may represent errors of dissection.

Second, in order to resolve more finely the spatial distribution of XIF3 transcripts, antisense RNA probes against the XIF3 message were used for in situ hybridization to sections of Xenopus embryos at different stages of development. Fig. 4B shows a longitudinal (parasagittal) section of a stage 26 embryo with hybridization to XIF3 message in the neural tube. There is no hybridization to the extreme anterior neural tube (forebrain) in Fig. 4B. The same results are found in the adjacent sections (not shown). Most notably, there is no detectable XIF3 signal along the posterior quarter of the axis confirming the generally anterior nature of XIF3 expression seen in the simple dissections.

The oblique longitudinal section shown in Fig. 4C shows a heavily labelled region extending from the level of the hindbrain forward to the most anterior part of the embryo and then turning ventrally towards the sucker. It is likely that this represents hybridization to XIF3 transcripts in a cranial nerve. The results from the longitudinal sections do not explain the preponderance of XIF3 transcripts found in the head by protection assays. This phenomenon is probably due to high levels of XIF3 transcripts in regions not covered by the longitudinal section in Fig. 4B, and represents transcripts from regions seen in the transverse sections of stage 18, 22 and 32 embryos shown in Figs 5, 6 and 7. The most anterior sections show no hybridization to the neural tube. As early as stage 18 there is intense labelling outside the neural tube (Fig. 5B) in regions that most likely correspond to the migrating neural crest. By stage 22 (Fig. 6B), these spots are located in the positions expected for the cranial ganglia, tissues that are, in part, derived from the neural crest (Sadaghiani and Thiebaud, 1987). The patterns of labelling of the cranial ganglia are more apparent in the stage 32 transverse sections shown in Figs 7B, C, and D. These tissues can be seen in the histologically stained section (Fig. 7H) where they appear as dense clusters of cells. No labelling was seen outside the neural tube in more posterior regions.

There is a second contribution to the observed high levels of XIF3 transcripts in the head. Within the neural tube itself it is apparent, even at stage 18, that there are regions of more intense labelling (Fig. 5C). These are located in two patches ventrolaterally on either side of the neural tube in the anterior part of the embryo and from their location might represent regions of motor neurones. Roberts and Clarke (1982) showed by horseradish peroxidase labelling that, in Xenopus embryos, the cell bodies of motor neurones were located in a single patch, ventrolaterally on either side of the neural tube. By stage 22, it is possible to distinguish only one intensely labelled patch on either side of the neural tube (Fig. 6C) consistent with the location of the motor neurones. In the stage 32 embryo, the patches are most strongly labelled in the posterior hindbrain (Fig. 7D). It is likely that these would not be seen in the longitudinal sections if they bisected the centre of the neural tube. In sections posterior to the hindbrain (Figs 5D and E, 6D and E, 7E–G) labelling is weaker and more diffuse throughout the neural tube. In conclusion, the observed high level of XIF3 transcripts in the head, shown by RNase protection assays, is due to the contribution of XIF3 transcripts from some of the cranial ganglia and the motor neurones of the hindbrain.

The use of both RNase protection assays on dissected tissue and in situ hybridization to sections therefore provides complementary information. The protection assays give quantitative data on the distribution of XIF3 transcripts along the anterior posterior axis whilst in situ...
hybridization gives localisation data at a resolution that is not possible by dissection.

(3) Tissue interactions in the developing embryo are required for elevated XIF3 expression

During neural induction, part of the ectoderm is diverted from a pathway of differentiation that leads to the formation of epidermis by an interaction with the migrating mesoderm during gastrulation (Spemann, 1938; Gurdon, 1987). This event has been shown to result in the expression of specific genes that can be used as markers of neural differentiation (Jacobson and Rutishauser, 1987; Kintner and Melton, 1987; Sharpe et

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**Fig. 3.** RNase protection assays using the pBSXIF3 probe to detect XIF3 transcripts in dissected regions of the stage 26 (tailbud) embryo. Panel A shows the division of an embryo into head, dorsal and ventral regions and confirms that XIF3 is found predominantly in the head with a smaller amount in the dorsal region. SM, HinfI-digested pBR322 size markers; Pr, undigested probe. Panel B shows the result of dissecting five tailbud stage heads into fractions containing ectoderm (including sucker), neural tissue (brain), eyes (including the optic nerve) and remnant material (including mesoderm and endoderm). An aliquot of the RNA from each sample was diluted 100-fold and assayed for the presence of 5S transcripts by RNase protection assay to quantitate recoveries (lower panel). The strong signal for XIF3 in the brain sample is not due to a proportionately increased recovery of RNA from this tissue.
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Fig. 4. Figs 4, 5, 6 and 7 show in situ hybridizations of sections of Xenopus laevis embryos using a XIF3 specific antisense RNA probe derived from pBSXIF3 and pBSXIF3-HS. (A) Diagram of a stage 25 embryo (Nieuwkoop and Faber, 1967). (B) An autoradiograph (dark-field) of a longitudinal (parasagittal) section through a stage 25 embryo hybridised to the XIF3 probe. The signal (arrowed) is present in the hindbrain region and extends caudally along the neural tube for approximately three quarters the length of the embryo. (C) An oblique longitudinal section through a stage 25 embryo with hybridization to the neural tube in the hindbrain region and in a structure projecting anteriorly then ventrally that could be one of the cranial nerves (large arrow). (D) Dorsal view of the anterior end of a stage 25 embryo showing the plane of the oblique section presented in panel C (Adapted from Nieuwkoop and Faber, 1967).
Fig. 5. (A) A stage 18 embryo showing the planes of section (b–e) of the dark-field autoradiographs shown in panels B–E. (B) An anterior section showing the earliest hybridization to XIF3 in the neural tube (small arrow). The large arrow shows intense labelling of cells external to the neural tube that are probably of neural crest origin. (C) A section through the hindbrain region. Diffuse labelling in the neural tube is shown by the small arrow. Two ventrolaterally located patches of intense hybridization, on one side of the neural tube, are shown by the shadowed arrows. (D and E) Progressively more caudal sections with diffuse labelling in the neural tube (small arrows), but no intense labelling either within or external to the neural tube.
Fig. 6. (A) A stage 22 embryo showing the planes of section (b–e) of panels B–E. (Nieuwkoop and Faber, 1967) (B–E) Dark-field autoradiographs of equivalent regions to those depicted in Fig. 5. In panel C the diffuse labelling in the hindbrain region of the neural tube is heavier, and only one region of intense labelling is apparent, probably corresponding to the motor neurone region.

second model in which it is first expressed, at a low level, equally throughout the ectoderm.

Within the neural tissue XIF3 transcripts are localized predominantly in the anterior regions. We have asked whether the localization of XIF3 transcription occurs at the same time as the activation of the XIF3 gene by neural induction, or whether these are two independent events. Embryos were therefore taken at three stages, late gastrula, late neurula and tailbud and then divided into prospective neural plate (later anterior–dorsal structures), prospective spinal cord (later posterior–dorsal structures) and the rest of the embryo.
Fig. 7. (A) A stage 32 embryo showing the planes of section (b–g) of panels B–G (Nieuwkoop and Faber, 1967). (B–G) Dark-field autoradiographs showing regions of XIF3 expression. Large arrows depict regions of intense labelling outside the neural tube that are probably due to the accumulation of XIF3 transcripts in cranial nerves and ganglia. The shadowed arrows show the intensely labelled regions that probably correspond to the motor neurones of the neural tube. Small arrows depict the diffuse labelling in the neural tube. (H) Haematoxylin–eosin-stained transverse section from a separate embryo at a level along the anterior–posterior axis equivalent to panel c. The dense bundles of cells outside the neural tube that label strongly with XIF3 in panel c are indicated by the black arrows. nt, neural tube; ov, otic vesicle; no, notocord; ph, pharynx; h, heart.
Fig. 8. (A) RNase protection assay showing the first step of XIF3 gene activation during development. Animal cap dissections were either taken at stage 8 or 10 for immediate analysis, or grown as explants to the tailbud stage (stage 26) then assayed for the presence of XIF3 transcripts. (B) RNase protection assay showing the second step of XIF3 gene activation. Neural conjugates and explants of stage 10 ectoderm and stage 11 mesoderm were grown to the tailbud stage and assayed along with an equivalent number of whole embryos at the same stage. SM; HinfI digested pBR322 size markers; Pr, undigested probe.

Fig. 9. RNase protection assays showing that XIF3 expression is equally distributed throughout the ectoderm prior to neural induction. (A) The dissected regions of early gastrula ectoderm. Segments b and d contain regions of ectoderm that during normal development would form neural tissue. (B) XIF3 protection assay of RNA from ectoderm explants grown on to stage 26. To control for the recovery of RNA from each sample the levels of 5S RNA were also determined from aliquots of the same RNA.
Fig. 10. Graph showing the abundance of XIF3 transcripts at late gastrula (stage 13), late neurula (stage 19) and tailbud (stage 30) embryos when divided into anterior-dorsal, posterior-dorsal and the rest of the embryo and frozen directly for analysis compared to whole embryos at stage 30.

Each region was frozen as soon as it was isolated and assayed by RNase protection for expression of XIF3, the levels being quantified by densitometry. One of two results might be expected. If localization occurs at the same time as neural induction then the anterior-dorsal region should always be the major site of XIF3 transcript accumulation. On the other hand, XIF3 gene activation by neural induction might initially result in an equal level of the transcripts in both the anterior-dorsal and the posterior-dorsal regions with localization occurring later, independently of neural induction. In this case, the preponderance of XIF3 transcripts in the head would occur subsequent to neural induction and would become apparent only at the tailbud stage. From Fig. 10 it can be seen that at the late-gastrula stage XIF3 expression is detectable at a low level in all the dissected samples and that as development progresses the elevated level of XIF3 expression is always the greatest in the anterior-dorsal region. These results agree with the proposed model in which neural induction and the regionally restricted pattern of XIF3 expression occur concurrently.

(4) XIF3 transcripts are more abundant in anteriorized embryos

Early cleavage stage embryos can be forced to develop an altered body plan that consists of an increased proportion of dorsal and anterior structures by subjecting the embryos to LiCl (Kao et al. 1986). The severity of the phenotype depends on the length of exposure to LiCl and has been graded from type 5, the normal embryo, to type 10, the most severely dorsalised and anteriorised phenotype (Kao and Elinson, 1988).

It is predicted that transcripts from an anterior neural marker gene would accumulate to increased levels in LiCl-treated embryos. Similarly, the amount of transcripts from a posterior neural marker gene might be expected to decrease in these embryos. As shown in Fig. 11 this is indeed the case. There is a large increase in the level of XIF3 transcripts in type 9 embryos and this is found to be a cumulative effect, dependent on the
severity of the LiCl phenotype. At the same time there is the expected decrease in the level of transcripts from the posterior neural marker gene XIHbox-6.

LiCl-treated embryos may have approximately twice the amount of neural tissue compared to normal embryos (Breckenridge and Warner, 1987). It is therefore unlikely that the increase in XIF3 transcripts is due entirely to increases in the total amount of neural tissue. Furthermore, in our experiments, the level of transcripts from the general neural marker gene, XIF6 (NF-M), remains essentially constant across the range of phenotypes. This again suggests that there is no marked increase in the total amount of neural tissue in LiCl-treated embryos.

These results suggest that the increased level of XIF3 transcripts is due to an increase in the amount of anterior neural tissue in LiCl-treated embryos. This supports the contention that an elevated level of XIF3 transcripts can be regarded as a marker for anterior neural tissue. In these experiments XIF3 clearly differs from the general neural marker XIF6 (NF-M).

Discussion

In this paper, we describe the complete coding sequence of XIF3, a new type III intermediate filament cDNA isolated from *Xenopus* embryos, and the interactions that lead to its high level of expression in the developing anterior nervous system.

XIF3 has an unusual pattern of expression during embryogenesis for a prospective neural marker. In the embryo, it is first transcribed at a low level in cells of the ectodermal lineage (Sharpe, 1988) beginning at gastrulation. It is then elevated to a high level of expression in a subset of cells that, following neural induction, form the anterior nervous system. This contrasts with markers such as XIF6 and XIHbox6 which are expressed only after neural induction has occurred. It is also possible to detect transcripts for another neural marker, N-CAM, in the fertilized egg, but these probably originate from a maternal store and so do not represent gene activation in the embryo prior to neural induction (Kintner and Melton, 1987). A similar two-step pattern of IF gene activation has previously been described, on a very different time scale, for the 63×10³ $M_r$ keratins during *Xenopus* metamorphosis, (Mathisen and Miller, 1988). These genes are activated at a low level in the epidermis at late stages of development (stage 48–52) and are then induced to a high level of expression before metamorphosis under the influence of thyroid hormone T₃.

There is growing evidence to suggest that the gastrula ectoderm is not a homogeneous tissue at the start of gastrulation: Dorsal and ventral regions are not equally responsive to the neural inductive stimulus (Sharpe et al. 1987) and they express the epidermal marker ep-i-1 asymmetrically (London et al. 1988; Savage and Philips, 1989). One possibility is that the difference in ability of the gastrula ectoderm to respond to neural induction might also be indicated by different patterns of gene expression at this stage. XIF3, which is initially expressed in gastrula ectoderm and ultimately in neural tissue, might represent an example of regional gene expression in the gastrula embryo. That is, the low level of XIF3 transcripts might have been found in that part of the ectoderm that in normal development is destined to become neural tissue. However, this is not the case, since XIF3 transcripts are found equally in dorsal and ventral ectoderm removed from early gastrulae, and throughout the ectoderm of the late gastrula embryo.

We consider that the elevated levels of XIF3 transcripts can be used as a marker of anterior neural tissue for two reasons. First, the expression of the gene has been shown to be predominantly in anterior neural tissue compared to the distribution of transcripts from the neurofilament gene, XIF6, which are more widely distributed at the tailbud stage (Sharpe, 1988). Second, XIF3 transcripts accumulate to high levels in embryos with anterior structures which are enlarged due to the effects of LiCl. Again this is not the case for the general neural marker XIF6. Because of these results we do not think that the level of XIF3 transcripts simply reflects the greater amount of neural tissue at the anterior end of the tailbud embryo.

There are certain reservations to this conclusion. XIF3 is expressed predominantly in the hindbrain region, so it is conceivable that there may be other markers that are expressed in more anterior regions. Furthermore, XIF3 is expressed at a very low level in uninduced ectoderm and at a level detectable by *in situ* hybridization along much of the spinal cord. This may complicate the analysis of future conjugate experiments designed to look at regionalization within the central nervous system if intermediate levels of transcripts are seen. This may be less of a problem if these analyses are combined with others using markers such as the general neural marker XIF6 and the posterior neural marker XIHbox 6.

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References


