**XASH genes promote neurogenesis in *Xenopus* embryos**

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**SUMMARY**

Neural development in *Drosophila* is promoted by a family of basic helix-loop-helix (bHLH) transcription factors encoded within the Achaete Scute-Complex (AS-C). XASH-3, a *Xenopus* homolog of the *Drosophila* AS-C genes, is expressed during neural induction within a portion of the dorsal ectoderm that gives rise to the neural plate and tube. Here, we show that XASH-3, when expressed with the promiscuous binding partner XE12, specifically activates the expression of neural genes in naive ectoderm, suggesting that XASH-3 promotes neural development. Moreover, XASH-3/XE12 RNA injections into embryos lead to hyper trophy of the neural tube. Interestingly, XASH-3 misexpression does not lead to the formation of ectopic neural tissue in ventral regions, suggesting that the domain of XASH proneural function is restricted in the embryo. In contrast to the neural inducer *noggin*, which permanently activates the NCAM gene, the activation of neural genes by XASH-3/XE12 is not stable in naive ectoderm, yet XASH-3/XE12 powerfully and stably activates NCAM, Neurofilament and type III β-tubulin gene expression in noggin-treated ectoderm. These results show that the XASH-3 promotes neural development, and suggest that its activity depends on additional factors which are induced in ectoderm by factors such as *noggin*.

Key words: XASH, basic helix-loop-helix transcription factor, neurogenesis, *Xenopus*

**INTRODUCTION**

The AS-C genes in flies comprise a set of four genes that are ascribed proneural functions, in that their activation augments neural determination (Alonso and Cabrera, 1988; Ghysen and Dambly, 1988; Campuzano and Modollel, 1992). They code for transcription factors of the basic-helix-loop-helix family (bHLH; Murre et al., 1989a), and are believed to promote neural differentiation in a manner similar to the proposed role of the myogenic bHLH proteins, MyoD, myf-5 and myogenin, in muscle development (Weintraub et al., 1991a). Flies that lack all of the AS-C genes have severe neural hypoplasia both in the central and peripheral nervous system, while ectopic expression of the AS-C genes can lead to additional neural differentiation (reviewed by Campos-Ortega and Jan, 1991; Campos-Ortega, 1993).

The *Xenopus* genes, XASH-1 (Ferreiro et al., 1993) and XASH-3 (Zimmerman et al., 1993) were cloned based on their sequence homology with the *Drosophila* AS-C genes. MASH-1, a mammalian AS-C homolog (Johnson et al., 1990), is required for the development of the peripheral autonomic and olfactory nervous system in mice (Guillemot et al., 1993). XASH-1, named because of its homology to MASH-1, is first expressed zygotically in the anterior neuropilethium of the embryo during folding of the neural tube in a pattern similar to but not identical with MASH-1 (Ferreiro et al., 1993). XASH-3, however, can be detected much earlier, at stage 11.5, in an area fated to become part of the neural plate, including presumptive brain and spinal areas (Zimmerman et al., 1993). This makes XASH-3 one of the earliest expressed neural-specific transcription factors in the *Xenopus* embryo. Since induction of the neural plate by the organizer begins at the onset of gastrulation (Kintner, 1992; Slack and Tannahill, 1992) just an hour and a half earlier, XASH-3 could act early during neural induction to promote neural development.

To determine whether XASH-3 promotes neural development in *Xenopus* embryos, we investigated whether XASH-3 can activate neural genes in ectoderm by injecting *Xenopus* embryos with XASH-3 RNA, along with RNA encoding the promiscuous bHLH heterodimer partner XE12. Ectodermal caps isolated from these embryos were found to express neural genes but not a mesoderm-specific gene. This activation of neural genes, however, appeared to be transient, and was not maintained in culture. The proneural activity of the XASH genes was reinforced by the finding that the expression of either XASH-1/XE12 or XASH-3/XE12 in embryos results in a stable enlargement of the neural tube. Ectopic XASH expression, however, did not cause the formation of neural tissue in ventral regions of the embryo. These results suggested that XASH-mediated activation of neural genes normally works in the context of a neural inducing signal and cannot substitute for this signal. In agreement with this idea, we found that...
**MATERIAL AND METHODS**

**Whole embryo analysis**

cDNAs for XASH-1 and XASH-3 were subcloned into sp64T and transcribed into capped mRNA in vitro using SP6 polymerase (Kreig and Melton, 1987). One blastomere of 2-cell or 4-cell stage embryos were injected with 5 nl of mRNA solution. All injection solutions contained 80 ng β-gal mRNA (for doses of other RNAs see text and figure legends). In those cases where XASH RNA and XE12 RNA (Rashbass et al., 1992) were injected together, they were mixed and added to the β-gal mRNA. Embryos were fixed in 4% paraformaldehyde, 15-36 hours after the injections, all were stained for β-gal activity with X-gal, and some were stained for NCAM or Xtwist message using a whole-mount protocol (Harland, 1991). The whole-mount embryos were cleared in benzyl-benzoate (Harland, 1991). The whole-mount protocol (Harland, 1991) was used for the RNase protection assay of the expression of the cultured caps or from 3 control embryos and divided into a 90% portion, used for the RNase protection assay of the expression of muscle-specific actin. Probes are described in detail by Dixon and Kintner (1989) and Lamb et al. (1993). Approximately equivalent amounts of animal cap RNA were assayed in each case as monitored by the levels of EF1α transcription or the cross-hybridization with cytoskeletal actin.

**Animal cap studies**

Both blastomers of 2-cell stage embryos were injected with 5 nl of mRNA solution including the types and amounts described below. Noggin mRNA (Lamb et al., 1993) was generated from a PCR product corresponding to the noggin cDNA that was subcloned into sp64T. Six animal caps per condition were dissected out and cultured for 24 hours (or until undissected siblings reached NF stage 24/25) on 1% agarose dishes in 0.5x MMR plus antibiotics. RNA was isolated from the cultured caps or from 3 control embryos and divided into a 90% portion, used for the RNase protection assay of the expression of NF, type III β-tubulin, NCAM and EF-1A, and a 10% portion, used for the RNase protection assay of the expression of muscle-specific actin. Probes are described in detail by Dixon and Kintner (1989) and Lamb et al. (1993). Approximately equivalent amounts of animal cap RNA were assayed in each case as monitored by the levels of EF1α transcripts or the cross-hybridization with cytoskeletal actin.

**RESULTS**

**The effect of XASH-3 on the expression of neural-specific genes in uninduced animal caps**

Animal cap tissue isolated from the late blastula normally differentiates into epidermis but can be induced to become mesodermal or neural tissue if combined with a source of appropriate inducing signals (Kintner and Melton, 1987; Sharpe et al., 1987; Dixon and Kintner, 1989; Green and Smith, 1991). In order to determine whether XASH-3 promotes neural development in ectoderm in the absence of neural inducing signals, animal caps were isolated from embryos injected at the one or two cell stage with XASH-3, cultured overnight, and then assayed for the expression of several neural-specific genes by an RNAase protection assay. Animal caps from embryos injected with just XASH-3 alone (Fig. 1, lane d) expressed only very small amounts of the neural-specific transcript NCAM. Since XASH-3, like other bHLH transcription factors, is likely to act as a heterodimer with the promiscuous bHLH partner E12, the vertebrate homolog of da (which may be in limiting quantity in the embryo; Lassar et al., 1989; Murre et al., 1989; Rashbass et al., 1992), XASH-3 RNA was injected along with low amounts of XE12 (Rashbass et al., 1992). Caps isolated from these embryos expressed NCAM transcripts as well as neuronal-specific transcripts for Neurofilament (NF) similar to those present in stage-matched embryos, even though the caps were cultured without any neural inducers (Fig. 1, top lanes e and i). Since ectoderm from embryos injected with just XE12 or XASH-3 alone failed to express significant levels of NCAM RNA (Fig. 1, top lanes c and d), XASH-3 and XE12 appear to act synergistically to induce neural gene expression.

Ectoderm from XASH-3/XE12-injected embryos was also examined the expression of the dorsal mesodermal marker, XASH-3/XE12 was a much more efficient activator of neuronal differentiation in noggin induced ectoderm. These results support the notion that the XASH genes are proneural in Xenopus, and that XASH-3 proneural activity in the early neuroectoderm is enhanced by neural induction.

![Fig. 1. The specificity of expression of neural and muscle transcripts in animal caps from embryos injected with different bHLH encoding mRNAs. Top: 125 pg (high) but not 25 pg (low) of XE12 mRNA induces NCAM and neurofilament expression (lanes b and c). 833 pg of XASH-3 mRNA alone induces only slight NCAM expression, but when it is combined with 25 pg of XE12 mRNA, there is a dramatic increase in NCAM and NF expression (lanes d and e). Note that the level of neural transcripts in the XASH-3/XE12-injected condition are close to the levels in whole un.injected embryos (lane i). 833 pg of XMyoD mRNA induces both NF and NCAM expression, but when this is combined with 25 pg of XE12, the levels of NCAM and NF expression are not increased (lanes f and g). Bottom: 125 pg of XE12 mRNA induces some expression of muscle-specific actin (lane b), but neither XE12 at 25 pg or XASH-3 at 833 pg alone, or in combination, induce muscle actin expression (lanes c-e). However, XMyoD at 833 pg does activate muscle actin expression and this is dramatically enhanced by combination with 25 pg of XE12 mRNA (lanes f and g). These results were replicated twice in independent experiments. The asterisk shows cytoskeletal actin mRNA, which is recognized by the cardiac actin probe and acts as a loading control. These lanes were cut and rearranged from a single exposure of one experiment.](image)
RNA (125 pg instead of 25 pg) also expressed in naive ectoderm by the injection of XASH-3/XE12 when the caps are assayed 1 day after the injection (lane a). If the caps are cultured for a second or third day, the level of these neural genes is dramatically reduced (lane d). Control caps (lanes b and e) show no neural gene expression on day 1 or 2. This experiment was repeated at least three times, and although there was some variation in the extent of the decrease in NCAM and NF expression XASH-3/XE12 induced caps, the decrease was always dramatic by 3 days.

Cardiac Actin or Muscle Specific Actin (MSA; Hopwood and Gurdon, 1990). In contrast to the clear induction of neural genes, there was no induced expression of MSA (Fig. 1, lane c), suggesting that the effect of XASH-3 on neural development is direct in the sense that the neural induction it is not mediated through the primary induction of dorsal mesoderm in these caps.

Specificity of MyoD/XE12 for myogenesis and XASH-3/XE12 for neurogenesis

In order to assess further the specificity of XASH-3, we also examined the effects of MMyoD (Hopwood et al., 1989), a bHLH transcription factor that was previously shown to induce the expression of muscle-specific genes (Weintraub et al., 1991b), and MSA expression in animal cap assays (Hopwood and Gurdon, 1990). Surprisingly, injection of MMyoD RNA alone induced low levels of NCAM and NF as well as MSA RNA expression (Fig. 1, lane f). Reducing the amounts MMyoD RNA injected into embryos, simultaneously reduced the levels of MSA and of NF and NCAM RNA. Thus, we could not find a dose of MMyoD RNA that induced MSA but not neural gene expression. Previous work with MMyoD had shown that coexpression with XE12 results in formation of heterodimers, and an enhancement in MMyoD’s ability to activate muscle-specific genes (Rashbass et al., 1992). Thus, we injected embryos with MMyoD plus XE12, at a concentration of XE12 that alone did not induce any of the marker genes. We saw a specific enhancement of MSA expression, and no enhancement of either NCAM or NF expression (Fig. 3, lane g). This result is similar to the above result with XASH-3, which when combined with XE12, also greatly enhanced the expression of the the neural-specific markers, but still did not cause any induction of the muscle-specific marker (Fig. 3, lanes d and e). This indicates that, in the presence of XE12, XASH-3 is a specific activator of neural gene expression while MMyoD is primarily an activator of muscle gene expression.

It is not clear why MMyoD RNA injected alone activates low levels of NCAM and NF RNA expression. We noted, however, that animal caps isolated from embryos injected with five times the dose of XE12 RNA (125 pg instead of 25 pg) also expressed RNAs for neural-specific genes as well as MSA (Fig. 3, lane b). Misexpression of bHLH proteins may have indirect effects because of their propensity to heterodimerize indiscriminately, and to dilute out potential inhibitors such as id (Benezza et al., 1990) or activators such as E12. (Parkhurst et al., 1990; Jarman et al., 1993). Since XASH-3 binds the mammalian muscle creatine kinase (MCK) E-box in vitro and transactivates the MCK/CAT reporter gene in transfected 10T1/2 cells (Zimmerman et al., 1993), it is perhaps surprising that XASH-3 activates neural gene expression but not muscle gene expression in vivo. This suggests that XASH-3 is not a promiscuous in vivo, and that it may show this particular specificity in the context of unidentified factors that exist in cells of the developing embryo.

Activation of neural gene expression in animal caps by XASH-3 is unstable

Activation of neural gene expression by XASH-3 suggests that XASH-3 promotes neural differentiation. Similar studies using injection of XMyoD RNA, however, have shown that XMyoD can activate muscle gene expression in animal cap assays without causing the differentiation of ectoderm cells into muscle (Hopwood and Gurdon, 1990). In the case of XMyoD, muscle gene expression can be detected in ectoderm after 1 day in culture, but the expression is then lost, presumably following the loss of the misexpressed XMyoD transcripts and protein. Similarly, ectoderm from embryos injected with XASH-3/XE12, after 1 day in culture, expressed large amounts of NCAM and NF RNA, but after 2-3 days in culture, the levels of these RNAs dropped, and in some experiments disappeared altogether (Fig. 2). These results indicate that while XASH-3 can activate neural gene expression in naive ectoderm, XASH-3 is probably not acting as a switch, causing cells that express it transiently to become neural. In this sense, the injection of XASH-3/XE12 RNA does not mimic the normal events of neural induction. This result also agrees with our whole embryo misexpression studies (see below), and indicates that XASH-3 probably does not activate neural genes by inducing a neural inducer, as all neural inducers so far studied stably activate neural development.

The effect of XASH-3 in animals caps is very sensitive to concentration. Relatively large amounts of XASH-3/XE12 RNA are needed to turn on neural gene expression, yet when the XASH-3 RNA has been diluted out even 2- to 3-fold, there is little or no activation (data not shown). The sharp dose response curve is consistent with the idea that XASH-3 acts by activating its own expression as has been proposed for the myogenic bHLH transcription factors (Weintraub et al., 1991a). XASH-3 injection, however, does not appear to turn on XASH-1 expression in isolated ectoderm (data not shown) suggesting that cross-regulation between the XASH genes may not be a factor in this dosage sensitivity.

XASH genes in embryos lead to neural hypertrophy in dorsal regions of the embryo

To explore the role of XASH genes in neural development in vivo, we examined the phenotypes of embryos that had been exposed to ectopic XASH-1 and XASH-3 by RNA injection. When embryos were injected with the same levels of XASH-
RNA that led to the activation of neural genes in the animal cap assay, they failed to gastrulate normally and thus could not be analyzed. Therefore, we examined embryos that had been injected with a ten-fold dilution of \textit{XASH-3} RNA. At this dose, \textit{XASH-3/XE12} did not have any effect on neural gene expression in the animal cap assay (see below). However, when these embryos were allowed to develop until neural plate or neural tube stages, they showed extra neural tissue when they were examined both histologically and for the expression of the neural-specific gene \textit{NCAM}. (Fig. 3A-C). Histological analysis reveals that the neural plate of younger embryos and the neural tube of older ones expands dramatically on the injected side, spreading out laterally (Figs 3C, 4). No expansion of the notochord was observed (Fig. 3 C), nor was there enhanced expression of the notochord-specific gene \textit{Xlim-1} (Taira et al., 1992) (data not shown). Similar, though less dramatic, results were obtained with \textit{XASH-3/XE12} injections (Figs 3D, 4). This, combined with the evidence from animal caps above, confirms that the expansion of the neural tube is not a secondary consequence of the \textit{XASH} genes leading to extra inducing tissue on the injected side. In addition, the expansion of the neural tube is not simply the result of injecting the \textit{XE12} molecule. We also looked at embryos in which \textit{XE12} was injected alone at twice the dose of the \textit{XASH-3/XE12} experiments and in no case did we see an expansion of the neural tube (Fig. 4). Thus, \textit{XASH} RNA can perturb the amount of neural tissue that forms in embryos at doses where it has no effects on isolated animal cap tissue.

Importantly, the enlargement of neural tissue following the injection of low amounts of \textit{XASH-3/XE12} RNA was autonomous and restricted. Neural hypertrophy was only in the side of the embryo where the ectopic \textit{XASH-3/XE12} was expressed, and only in the region of the neural plate/tube. If, for instance, \textit{XASH-3/XE12} was expressed in the posterior part of the embryo, then the neural plate would expand only in the region of ectopic expression. When \textit{XASH-1/XE12} or \textit{XASH-3/XE12} was misexpressed in ventral regions of the embryo, this did not lead to an extra neural tissue, suggesting that the proneural function of \textit{XASH} is restricted to the dorsal ectoderm, near its normal zone of expression.

Since the lateral expansion of the neural tube could be at the expense of tissue around the neural plate, we looked at the ectodermal expression of \textit{Xtwist}, a cephalic neural crest marker, expressed at the lateral borders of the anterior neural plate (Hopwood et al., 1989). Consistent with this idea, embryos misexpressing \textit{XASH-3/XE12} showed reduced \textit{Xtwist} expression on the injected side (Figs 3B, 4). Similar results on the expansion of the neural tube at the expense of lateral ectoderm in \textit{XASH-3} injected embryos have been found by Turner and Weintraub (1994).
**XASH-3 promotes neuronal differentiation in noggin-treated animal caps**

The result described above indicates that XASH-3 can promote neuronal development but only in regions of the embryo where neural induction normally takes place. To test this further, we examined the effects of XASH-3 expression in ectoderm that had been induced to form neural tissue by the neural inducer noggin (Lamb et al., 1993). Noggin-treated ectoderm expresses large amounts of NCAM RNA as well as the anterior CNS homeodomain transcript OtxA (Lamb et al., 1993). In contrast to XASH-3-expressing ectoderm, however, noggin-induced ectoderm does not appear to undergo neuronal differentiation as marked by the expression of neuronal genes such as type III neural-specific tubulin (Lamb et al., 1993). Thus, we injected XASH-3/E12 RNA along with noggin RNA into animal caps and assayed for type III tubulin expression. The results of this analysis show that noggin-injected ectoderm does not express type III tubulin (Fig. 5 lane 2), as shown previously (Lamb et al., 1993). However, when these caps are co-injected with XASH-3/E12, they express type III tubulin at levels near those of control embryos (Fig. 5 lanes 4 and 5). Importantly, the levels of XASH-3 required for the induction of neural tubulin in noggin-induced ectoderm are at least 10-fold less than the levels required to induce tubulin in naive ectoderm, and similar to the levels required to cause neural expansion in whole embryos. When this amount of XASH-3/E12 RNA is injected without noggin, there is no induction of either NCAM or tubulin (Fig. 5 lane 3). In addition, the effects of XASH-3/E12 expression on tubulin expression in noggin-induced caps appears to be stable for up to 2 days in culture (data not shown). Thus, these results support the idea that XASH-3 is more effective in promoting neuronal development in the context of noggin-induced ectoderm than it is in uninduced ectoderm.

**DISCUSSION**

The principle finding of this study is that XASH-3 acts as a proneural gene. In embryos, XASH-3/E12 results in an increase in the size of the neural tube, perhaps by increasing the lateral extent of the neural plate. In the animal cap assay, XASH-3 when combined with XE12 induces neural but not muscle gene expression. Thus, these assays provide strong support for the conclusion that XASH-3 is a proneural gene. Our results with misexpression corroborate the proneural role of the vertebrate AS-C homologs that has been suggested by the knock out of MASH-1 in the mouse (Guillemat et al., 1993).

In overexpression studies with bHLH proteins, the question of specificity must arise. Because bHLH genes heterodimerize, they may have non-specific effects when overexpressed. The classic example of this is the effect of hairy on sex determination in flies (Parkhurst et al., 1990). Thus, in the context of the whole organism, where a myriad of bHLH proteins may be operating in a dosage sensitive way, the overexpression of a single member of this class can create a phenotype which may be less informative about the overexpressed gene than it is about its partner. We found that either XmixoD and XE12, when expressed alone in animal caps at high enough levels, lead to the expression of both neural and muscle-specific markers. Why either turns on neural genes in the animal cap assay is unclear. Perhaps they have less target specificity as homodimers, perhaps they are diluted out or compete with an endoge-
nous inhibitory protein (Benezra et al., 1990; Ellis et al., 1990; Garrell and Modolell, 1990; Ruezinsky et al., 1991), or perhaps they form activating heterodimers with the low levels of maternal XASH-1 (data not shown). The problem of specificity is overcome to some extent in the animal cap studies where a direct comparison of several different bHLH members can be made on exactly the same tissue. Thus, when XASH-3 or XM\textit{y}od were combined with low doses XE12 (at doses where XE12 did not by itself activate neural or muscle genes), we found that XASH-3/XE12 heterodimers strongly induce neural genes without inducing muscle genes, while XM\textit{y}od/XE12 heterodimers preferentially activate myogenic genes over neural genes. The heterodimers thus show exquisite tissue specificity that presumably reflects their normal site of action.

Another major finding to emerge from these studies is that the ability of XASH-3 to act in naive ectoderm appears to be compromised relative to its ability to act in the embryo or in noggin-induced ectoderm. Thus, while XASH-3 will turn on neural genes in naive ectoderm, this occurs only when relatively large amounts of XASH-3 RNA are injected and in most experiments, the effects on neural gene expression is only transient. In contrast, 10-fold less XASH-3 RNA is required to expand the neural tube in embryos or to activate neuronal gene expression in noggin-induced ectoderm, and in both cases, the effects appear to be irreversible. These observations strongly suggest that the normal expression of XASH-3 is not sufficient to allow ectoderm to take on the neural fate. Neural induction, therefore, cannot be viewed simply as the activation of XASH-3 expression.

One of the important unresolved questions raised by these studies, concerns where XASH-3 normally acts during the early stages of neural development. In this respect, it is interesting to compare the effects obtained by the expression of XASH-3 in ectoderm with those obtained by treating ectoderm with the neural inducer, noggin. While ectoderm treated with noggin appears to differentiate into neural tissue as measured by the expression of large amounts NCAM RNA, it does not appear to undergo neuronal differentiation as measured by the expression of genes such as NF or tubulin (Lamb et al., 1993). In contrast, ectoderm injected with large amounts of XASH-3/XE12 RNA goes on to express large amounts of both NF and tubulin RNA relative to the amounts of the neural marker NCAM. Moreover, tubulin expression can be activated in noggin-treated caps even by relatively low levels of XASH-3/XE12 RNA. Thus, one model based on these observations is that XASH-3 acts downstream of neural induction to promote neuronal differentiation. In this model, the primary role of the proneural XASH genes is not to establish the neuroepithelium of the neural plate and tube, but rather to act within this region of the embryo to promote the neuronal fate.

In \textit{Drosophila}, \textit{Notch} mutant embryos make neural tissue at the expense of ventral epidermis, suggesting that the cells of the ventral neurogenic region may flip between these two states (Artavanis-Tsakonas and Simpson, 1991). Yet in mutants for \textit{AS-C}, although there is a severe neural deficiency, there is no noticeable extra epidermal tissue (Wieschaus et al., 1984; Jimenez and Campos-Ortega, 1990). In \textit{AS-C}/\textit{Notch} double mutants, the neural hypertrophic phenotype is repressed, yet these embryos still cannot make ventral epidermis (Martinez Arias, 1993). These results suggest that \textit{AS-C} function is not a simply a switch between the epidermal and neural state, and show that the lack of \textit{AS-C} function does not lead cells to take on an epidermal fate. While we have no data on the loss of XASH function, we note that loss of \textit{MASH-1} in the mouse leads to a loss of the olfactory neurons, but not to the neuroepithelium from which these neurons arise. From these data, we suggest that the loss of XASH-3 may not cause cells in the neural plate to take on an epidermal fate, but rather to differentiate into an alternative fate within the neural tube such as, for example, into ependymal cells.

Our studies are directly comparable to overexpression studies in flies, using a heat shock promoter to induce the temporary expression of \textit{scute} throughout the developing fly deficient for the endogenous \textit{achaete} and \textit{scute} gene function (Rodriguez et al., 1990). One finding from such studies concerns the redundancy of the \textit{AS-C} genes, such that heat shock expression of one of them can substitute for the expression of another. Thus, sensory bristles that are lost in \textit{achaete} mutants are regained in these flies where \textit{scute} or \textit{asense} are ubiquitously expressed through heat shock (Rodriguez et al., 1990; Dominguez and Campuzano, 1993). Like the \textit{AS-C} genes, the two XASH genes are expressed in distinct but overlapping spatiotemporal domains, yet either one has the potential, when overexpressed, to cause neural hypertrophy, suggesting that they also may have functional redundancy.

A critical comparison between our results and those in the fly concerns the finding that the \textit{AS-C} genes can only function in restricted regions of neurogenic potential, so that even when there is ubiquitous heat-shock induced expression of one of these genes, sensory organs still tend to appear only at their appropriate sites on the fly (Rodriguez et al., 1990; Dominguez and Campuzano, 1993). The spacing between sensory bristles is regulated in part by the inhibitor of \textit{AS-C} function, \textit{emc} (Ellis et al., 1990; Garrell and Modolell, 1990). As a bHLH protein lacking a basic domain, \textit{emc} has an anti-neural function: it inactivates \textit{AS-C} proteins by forming ‘dead’ heterodimers with them that are incapable of binding DNA (Ellis et al., 1990; Garrell and Modolell, 1990). In \textit{emc} mutants, heat shock induced \textit{AS-C} activity leads to ectopic bristles, suggesting that the spatial restriction of \textit{AS-C} proneural function in these flies is in part controlled by the endogenous pattern of \textit{emc} expression (Cubas and Modolell, 1992). We have previously shown that \textit{emc} similarly inhibits the XASH-1 DNA binding in vitro (Ferreiro et al., 1993). Similarly, in the blastoderm there appears to negative regulation of the \textit{AS-C} genes by \textit{twist} and \textit{snail} in ventral region during establishment of the mesoderm (Kosman et al., 1991), and in dorsal regions through the action of \textit{dpp} during the establishment of the dorsal epidermis (Ferguson and Anderson, 1992). Thus, the dorsal restriction we see in the proneural function of overexpressed XASH, in \textit{Xenopus} embryos could in part be due to the expression of inhibitory factors in ventral regions of the embryo. Neural induction may therefore not only turn on XASH-3 expression, but also eliminate negative regulators thereby providing a domain in which the proneural properties of XASH-3 are effective. We cannot, of course, rule out the possibility that there may be spatially restricted proneural factors co-expressed with XASH-3 in the neural plate and tube that help to turn on neural genes.

In sum, these studies suggest that XASH-3 plays a role in amphibian embryos in promoting neural development during the progression of ectoderm into neural tissue. It will be of
interest to determine what factors are normally responsible for activating XASH gene expression within the neural plate, and whether other genes, such as the vertebrate homologs for the Drosophila neurogenic genes or the antineural genes, regulate the activity of the XASH genes in the neural plate and tube.

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