Sensitivity of proneural genes to lateral inhibition affects the pattern of primary neurons in *Xenopus* embryos

Ajay Chitnis* and Chris Kintner†

The Salk Institute for Biological Studies, PO Box 85800, San Diego, CA 92186-5800, USA

*Present address: Massachusetts General Hospital and Harvard Medical School, Cardiovascular Research Center, 149 13th St., Charlestown, MA 02129, USA

†Author for correspondence (e-mail: kintner@sc2.salk.edu)

**SUMMARY**

We have compared the roles of XASH-3 and NeuroD, two basic helix-loop-helix transcription factors, in the formation of primary neurons in early *Xenopus* embryos. When ectopically expressed in *xenopus* embryos, XASH-3 and NeuroD induce ectopic primary neurons in very different spatial patterns. We show that the pattern of primary neurons induced by XASH-3 and NeuroD can be accounted for by a difference in their sensitivity to inhibitory interactions mediated by the neurogenic genes, X-Delta-1 and X-Notch-1. Both NeuroD and XASH-3 promote the expression of the inhibitory ligand, X-Delta-1. However, XASH-3 appears to be sensitive to the inhibitory effects of X-Delta-1 while NeuroD is much less so. Consequently only a subset of cells that ectopically express XASH-3 eventually form neurons, giving a scattered pattern, while the ectopic expression of NeuroD leads to a relatively dense pattern of ectopic neurons. We propose that differences in the sensitivity of XASH-3 and NeuroD to lateral inhibition play an important role during their respective roles in neuronal determination and differentiation.

Key words: neurogenic genes, proneural genes, primary neurons, XASH-3, NeuroD

**INTRODUCTION**

MASH-1, XASH-1, and XASH-3 are basic helix-loop-helix (bHLH) transcription factors that were identified on the basis of sequence similarity to the *Drosophila* proneural genes belonging to the *achaete-scute* Complex (Mammalian or *Xenopus*, Achaete Scute Homolog; Johnson et al., 1990; Ferreiro et al., 1993; Zimmerman et al., 1993; Turner and Weintraub, 1994). In *Drosophila* the expression of proneural genes defines ‘proneural clusters’ in the neuroectoderm within which cells have the potential to adopt a neural fate (reviewed by Skeath and Carroll, 1994). Similarly, the vertebrate ASH genes also appear to promote differentiated neural fates, as evidenced by the loss of specific neurons in MASH-1 mutant mice (Guillemot et al., 1993) and by the induction of neuronal differentiation in neutralized *Xenopus* animal caps when XASH-3 is ectopically expressed (Ferreiro et al., 1994). More recently, Lee and colleagues identified a second type of neural bHLH transcription factor, called NeuroD (Lee et al., 1995). In both *Xenopus* and mouse embryos, the *ASH* genes appear to be expressed earlier than NeuroD during neurogenesis, suggesting that they play different roles in promoting neurogenesis (Lee et al., 1995). That these genes have different roles is also supported by ectopic expression experiments in *Xenopus* embryos. While the ectopic expression of NeuroD leads to precocious and a relatively dense pattern of ectopic neurons, XASH-3’s ability to promote a neuronal fate appears to be more restricted (Lee et al., 1995). This difference led us to ask whether these bHLH transcription factors interact with a second class of genes that appears to regulate neurogenesis: the vertebrate homologues of the *Drosophila* neurogenic genes.

In *Drosophila*, the neurogenic genes regulate the activity of the proneural genes in the *achaete-scute* complex via a process called lateral inhibition. Lateral inhibition is a form of local cell-cell interaction that acts within a proneural cluster to limit the number of cells that gives rise to neuroblasts (reviewed by Muskavitch, 1994). During this interaction, nascent neuroblasts express a membrane bound ligand, Delta, which binds and activates Notch on neighboring cells. Activation of Notch in a cell is thought to reduce the activity of the proneural genes, perhaps by activating genes in the Enhancer of split E(spl) complex which encode inhibitory bHLH transcription factors (Jennings et al., 1995). Inhibition of proneural gene activity via Notch not only inhibits cells from adopting a neural fate, but may also reduce their expression of Delta (Haenlin et al., 1994; Kunisch et al., 1994). In theory these interactions amplify small differences between cells in their potential to adopt a neural fate, ultimately restricting proneural activity to a single cell in each proneural cluster (Heitzler and Simpson, 1991; Ghysen et al., 1993; Heitzler et al., 1996). We therefore asked whether similar dynamic interactions might occur between XASH-3 or NeuroD, and the vertebrate homologs of the neurogenic genes, and whether such interactions might contribute to the role of these genes in regulating neurogenesis.

In *Xenopus* embryos, an early phase of neurogenesis gives rise to a simple pattern of neurons that can be identified using...
a neural-specific, type II beta-tubulin gene, called N-tubulin, whose expression marks neurons (Oschwald et al., 1991; Chitnis et al., 1995). Cells expressing N-tubulin (NECs) first arise in Xenopus embryos in three longitudinal domains, (medial, intermediate and lateral) on either side of the dorsal midline of the posterior neural plate (Fig. 1A). This pattern of NECs raises two questions: what determines the size and position of each ‘proneural domain’ where NECs form in the neural plate, and what determines the density of NECs that form within each domain? Recently, we have shown that the density of primary neuron formation appears to be controlled by the Xenopus homologs of Notch and Delta, called X-Notch-1 and X-Delta-1. Increased X-Delta-1 activity results in a loss of NECs, while blocking X-Delta-1 activity results in an increased number of NECs. Importantly, in embryos where X-Delta-1 is blocked, more NECs form by increasing the density of NECs within each domain; the position and size of each domain remains largely unperturbed. These observations suggested that the activity of the Xenopus neurogenic genes limits the number of neurons that form within each ‘proneural domain’ of the neural plate, and that other factors determine where in the neural plate the proneural domains are located.

Here we have examined the potential role of bHLH transcription factors like XASH-3 and NeuroD in defining proneural domains, and in determining the pattern of NECs that differentiate within these domains. We show that ectopic expression of XASH-3 can increase the size of the domains within the early neural plate within which NECs can be generated, however, because it promotes the expression of X-Delta-1, and its effects are sensitive to inhibition by neurogenic genes, only a subset of cells in these domains become NECs. In contrast, we show that NeuroD is relatively insensitive to inhibition by the neurogenic genes and this, at least in part, accounts for why its ectopic expression leads to a relatively dense pattern of ectopic NECs. These results are consistent with a role of bHLH transcription factors like XASH-3 and NeuroD in neuronal determination and differentiation.

RESULTS

The effects of ectopic NeuroD and XASH-3 expression on primary neurogenesis

We compared the effects of XASH-3 and NeuroD on the pattern of NECs, by injecting a mixture of synthetic XASH-3 or NeuroD RNA along with lacZ transcripts into one blastomere of a two- or four-cell stage embryo (Chitnis et al., 1995). Each blastomere contributes to one side of the developing embryo: the injected side is marked by β-galactosidase expression which can be detected by X-gal staining; the other side of the embryo serves as an uninjected control. Injected embryos were left to develop overnight, fixed at the neural plate stage, and analyzed by X-gal staining and for N-tubulin expression using whole-mount in situ hybridization (Chitnis et al., 1995).

As shown previously, ectopic expression of NeuroD in the neural plate leads to a widespread, relatively dense pattern of NECs in the posterior neural plate (Fig. 1F) (Lee et al., 1995). In contrast, ectopic expression of XASH-3 has a variable effect on the pattern of NECs, depending on the dose of XASH-3 RNA injected. At relatively high doses of RNA (2 pg/embryo), ectopic expression of XASH-3 eliminates or dramatically reduces the number of NECs in the neural plate (Fig. 1A, 10/10 embryos). At lower doses (0.25 pg/embryo), XASH-3 promotes the formation of ectopic NECs, but these, in contrast to those produced by NeuroD, arise as isolated cells interspersed amongst cells that do not express the tubulin marker (Fig. 1B versus Fig. 1F, 24/33 embryos). Thus while NeuroD promotes a relatively dense pattern of NECs, XASH-3 suppresses the formation of NECs, or produces ectopic NECs in a ‘salt-and-pepper’ pattern in the neural plate.

XASH-3 activates the expression of X-Delta-1

To examine why the spatial pattern of primary neurons that forms in response to NeuroD and XASH-3 is so different, we asked whether this difference could be due to the effects of lateral inhibition. Previously we showed that the Xenopus homologues of Delta and Notch, X-Delta-1 and X-Notch-1, appear to act by limiting the number of NECs that form within each of the three proneural domains on either side of the neural plate in Xenopus embryos (Chitnis et al., 1995). Ectopic expression of X-Delta-1 reduces or blocks the formation of primary neurons in Xenopus as predicted if it encodes a ligand that inhibits neurogenesis. Moreover, in Drosophila, the expression of Delta is thought to be promoted by proneural gene activity (Glysen et al., 1993; Haenlin et al., 1994; Kunisch et al., 1994). Thus, we hypothesized that if the ectopic expression of XASH-3 similarly promotes the expression of X-Delta-1 and if the proneural activity of
XASH-3 is sensitive to the inhibitory effect of the neurogenic genes, this could potentially account for the pattern of NECs seen in XASH-3 injected embryos. This hypothesis would predict that when XASH-3 RNA is injected at relatively low levels, XASH-3 would activate lateral inhibition by inducing X-\(\Delta\)-\(\delta\)-1 expression, thus allowing only a subset of cells in the XASH-3 expression domain to differentiate as neurons. However, when XASH-3 RNA is injected at relatively high levels, XASH-3 might induce a sustained expression of X-\(\Delta\)-\(\delta\)-1 that actually reduces the number of NECs or delays their differentiation.

To test for an interaction between XASH-3 and X-\(\Delta\)-\(\delta\)-1, we first examined the expression of X-\(\Delta\)-\(\delta\)-1 in embryos injected with XASH-3 mRNA (Fig. 1C). As above, two-cell embryos were injected in one cell with a mixture of synthetic XASH-3 and lacZ transcripts. Injected embryos were left to develop overnight, fixed at the neural plate stage, and analyzed for X-\(\Delta\)-\(\delta\)-1 expression by X-gal staining and whole-mount in situ hybridization. In 25/25 cases, we saw a striking induction of X-\(\Delta\)-\(\delta\)-1 expression on the injected side (Fig. 1C); this was not seen in control embryos injected with lacZ transcripts alone (9/9 cases). We conclude that XASH-3 is indeed an activator of X-\(\Delta\)-\(\delta\)-1.

In a parallel set of experiments we showed that XASH-3 induces the expression of X-\(\Delta\)-\(\delta\)-1 in animal cap assays. To generate neural tissue in isolated animal caps, we injected two-cell embryos with transcripts for the neural inducer Noggin (Lamb et al., 1993), either alone or along with transcripts for XASH-3. Animal caps isolated from these embryos were cultured to the equivalent of stage 14 (open neural plate) and assayed for the expression of X-\(\Delta\)-\(\delta\)-1 and X-\(\Delta\)-\(\delta\)-1 ICD by RNase protection. The results show that the expression of X-\(\Delta\)-\(\delta\)-1, and to a much lesser extent X-\(\Delta\)-\(\delta\)-1 ICD, is induced by XASH-3 in neural tissue formed in vitro (Fig. 2A).

The inhibitory effects of XASH-3 are blocked by an antimorphic form of X-\(\Delta\)-\(\delta\)-1

If XASH-3’s ability to block, or delay the appearance of NECs is due to its ability to activate X-\(\Delta\)-\(\delta\)-1, then we should be able to reverse this effect by concomitantly inhibiting the activity of X-\(\Delta\)-\(\delta\)-1. To block X-\(\Delta\)-\(\delta\)-1 activity, we used an antimorphic form of X-\(\Delta\)-\(\delta\)-1, called X-\(\Delta\)-\(\delta\)-1 Stu, which was engineered to lack an intracellular domain, and which we found in previous experiments to act as a dominant-negative mutant when expressed in embryos by RNA injection (Chitnis et al., 1995). Thus, embryos were injected on one side at the 2-4 cell stage with either XASH-3, with X-\(\Delta\)-\(\delta\)-1 Stu, or with a combination of both RNAs, along with lacZ RNA as a tracer (Fig. 1). Injected embryos were again processed at the neural plate stage for X-gal staining and \(N\)-tubulin expression using whole-mount in situ hybridization. As described above, many of the embryos injected with just XASH-3 RNA showed a reduction in the number of NECs (Fig. 1A; Table 1). As shown previously, embryos injected with just X-\(\Delta\)-\(\delta\)-1 Stu RNA showed an increase in the number of NECs, but the increase was apparent as a higher density of NECs that formed within the three proneural domains; the overall size and position of these domains remained for the most part unchanged (Fig. 1E; Table 1). In contrast, the pattern of NECs in embryos injected with a combination of XASH-3 and X-\(\Delta\)-\(\delta\)-1 Stu RNAs differed...
from those above in two respects (Fig. 1D). First, embryos injected with XASH-3 and X-Delta-1 RNAs showed an increase in the number of NECs relatively to those injected with just XASH-3 RNA (Fig. 1D versus Fig. 1A). We conclude from this result that the suppression of NECs by XASH-3 is indeed due to the ability of XASH-3 to activate X-Delta-1. Secondly, embryos injected with both XASH-3 and X-Delta-1 RNAs showed expanded domains in the posterior neural plate where NECs formed, often to the point where the distinction between the medial and intermediate, or intermediate and lateral, domains became blurred (Fig. 1D versus Fig. 1E). We conclude from this result that the size of domain where NECs can form is significantly increased by the ectopic expression of XASH-3, suggesting that XASH-3 has the potential to define a proneural domain.

The proneural activity of NeuroD is not blocked by activated Notch

The interaction between XASH-3 and X-Delta-1 revealed by the results described above also suggests that the salt-and-pepper pattern of ectopic NECs produced when XASH-3 is injected at lower concentrations (see Fig. 1B), is a consequence of lateral inhibition. Moreover, contrasting the pattern of NECs obtained with XASH-3 to the relatively dense pattern of NECs obtained with NeuroD suggests that the proneural activity of XASH-3 is more sensitive to lateral inhibition than is NeuroD’s. To examine this further, we asked whether the ability of XASH-3 and NeuroD to promote the formation of NECs can be blocked by constitutively active forms of Notch. In C. elegans, Drosophila and vertebrates, the Notch intracellular domain, or forms of Notch lacking an extracellular domain, act as constitutively activated molecules (Coffman et al., 1993; Roehl and Kimble, 1993; Struhl et al., 1993; Kopan et al., 1994). The intracellular domain of X-Notch-1 (ICD), for example, will block the formation of NECs when ectopically expressed in Xenopus embryos (Chitnis et al., 1995). Thus, two-cell or four-cell stage embryos were injected with either NeuroD RNA, with ICD RNA or with a combination of the two RNAs, along with lacZ as a tracer, and analyzed as in the experiments described above. The ectopic expression of NeuroD causes a dense pattern of NECs (Fig. 1F, 38/38 embryos), while that of ICD blocks the formation of NECs (Fig. 1I, 38/39 embryos). Embryos injected with both ICD and NeuroD RNA had the same pattern of NECs in the posterior

### Table 1. Effect of injection of embryos at the two-cell stage with synthetic RNAs

<table>
<thead>
<tr>
<th>Effect</th>
<th>Expt</th>
<th>XASH-3 (%)</th>
<th>XASH-3 + X-Delta-1 (%)</th>
<th>X-Delta-1 (%)</th>
<th>β-galactosidase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction or elimination of NECs</td>
<td>1</td>
<td>18/30 (60%)</td>
<td>4/50 (8)</td>
<td>0/18 (0)</td>
<td>7/40 (18)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>21/39 (54%)</td>
<td>6/40 (15)</td>
<td>0/35 (0)</td>
<td>1/42 (2)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>27/40 (68%)</td>
<td>11/51 (22)</td>
<td>3/31 (10)</td>
<td>0/34 (0)</td>
</tr>
<tr>
<td>Wider proneural domain</td>
<td>1</td>
<td>18/30 (60%)</td>
<td>42/50 (84)</td>
<td>3/18 (17)</td>
<td>4/40 (10)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18/39 (46%)</td>
<td>25/40 (62)</td>
<td>3/35 (9)</td>
<td>1/42 (2)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>21/40 (53%)</td>
<td>42/51 (82)</td>
<td>4/31 (13)</td>
<td>0/35 (0)</td>
</tr>
<tr>
<td>Higher density of NECs</td>
<td>1</td>
<td>2/30 (7)</td>
<td>14/28 (28)</td>
<td>11/18 (61)</td>
<td>0/40 (0)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0/39 (0)</td>
<td>9/40 (23)</td>
<td>27/35 (77)</td>
<td>0/42 (0)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2/40 (5)</td>
<td>30/51 (59)</td>
<td>25/31 (80)</td>
<td>0/35 (0)</td>
</tr>
</tbody>
</table>

Embryos injected at the two-cell stage with different synthetic RNAs were processed at the neural plate stage using X-gal and N-tubulin staining as described in Materials and Methods. In three separate experiments, embryos were scored for a (1) decrease or elimination of NECs, (2) increase in the domain size of NECs, or (3) increase in the density of NECs on the injected side. The number of embryos scored for each of the above phenotypes is expressed as a fraction of the total embryos examined in each experiment. The pattern of NECs for a given embryo might differ in two respects, for example, by showing a higher density as well as a wider domain of NECs. Thus, addition of the percentages for a given group of embryos can exceed 100%.
neural plate as those injected with just NeuroD RNA alone (Fig. 1G vs. Fig. 1F, 65/74 embryos), indicating that NeuroD is relatively resistant to the inhibitory effects of ICD. For comparison, embryos were also injected with a combination of XASH-3/X-Delta-1\textsuperscript{Stu} RNA, with ICD RNA, or with a combination of XASH-3/X-Delta-1\textsuperscript{Stu} and ICD RNA. In contrast to the results obtained with NeuroD, the ectopic NECs that normally appear in embryos injected with both XASH-3 and X-Delta-1\textsuperscript{Stu} RNAs (Fig. 1D, 8/10 embryos) were blocked by ICD (Fig. 1H, 20/21 embryos). Thus, these results demonstrate a differential sensitivity of NeuroD and XASH-3 to activated Notch.

In theory, NeuroD could also lead to a dense pattern of NECs because it does not promote the expression of the inhibitory ligand X-Delta-1. This, however, does not appear to be the case because both NeuroD and XASH-3 induce the expression of X-Delta-1 to similar levels in Noggin neutralized caps (Fig. 2). Furthermore, the ability of both NeuroD and XASH-3 to activate the expression of X-Delta-1 can be blocked by co-expressing ICD (Fig. 2). However, in the same experiment, NeuroD promotes the differentiation of neurons in Noggin neutralized caps as marked by N-tubulin expression, and this is not blocked by ICD (Fig. 2B). These in vitro results provide additional evidence that NeuroD can promote neuronal differentiation in the presence of activated Notch.

**DISCUSSION**

A set of interactions between the proneural genes in the achaete-scute Complex and Delta has been proposed in *Drosophila* to explain the selecting out of a single neuroblast from between eight to 20 cells in a proneural cluster (Heitzler and Simpson, 1991; Ghysen et al., 1993; Campos-Ortega, 1994). Our results suggest that a similar set of interactions between homologous genes in *Xenopus* are involved in creating the salt-and-pepper pattern of NECs in proneural domains within the neural plate. XASH-3 promotes a neuronal fate, and at the same time promotes the expression of the inhibitory ligand X-Delta-1. This activates X-Notch-1 on neighboring cells, thus reducing the activity of XASH-3, and decreasing the expression of X-Delta-1 in these cells. By amplifying small differences between neighboring cells, these interactions can then account for the salt-and-pepper pattern of ectopic NECs that arises from a relatively uniform expression of ectopic XASH-3 (Fig. 1B; see also Hinz et al., 1994). Thus, these results demonstrate how the dynamic regulation of transcription factor function through cell-cell interactions can lead to the emergence of the fine grain pattern of neuronal differentiation in the vertebrate nervous system.

The effects of ectopic XASH-3 expression are consistent with its behaviour as a proneural gene, however, its expression does not define the proneural domains where primary neurons are generated; it is expressed in a region that corresponds to the sulcus limitans later in development (Zimmerman et al., 1993; Turner and Weintraub, 1994). Consequently, ectopic expression of XASH-3 could lead to the generation of ectopic neurons by mimicking or promoting the activity of as yet undefined bHLH proneural transcription factors which are normally expressed in proneural domains. Indeed, genes within the achaete-scute Complex in *Drosophila* have been shown to have the ability to cross-regulate each other's expression (Martinez and Modolell, 1991; Skeath and Carroll, 1991). Therefore, ectopic XASH-3 expression might have proneural activity by driving the expression of related, neural bHLH transcription factors. Furthermore, bHLH transcription factors operate as heterodimers and indeed, are subjected to negative control when they form inactive dimers with HLH proteins that lack basic DNA binding domains (Garrell and Modolell, 1990; Van Doren et al., 1991; Cabrera et al., 1994). Hence ectopic XASH-3 expression could also promote the activity of other proneural genes by binding up negative regulatory molecules that normally inhibit their function. Regardless of how XASH-3 gives proneural activity in these experiments, it is clear that this activity is more sensitive to the inhibitory influence of the neurogenic genes. As a consequence of this sensitivity, only a subset of cells in the domains where XASH-3 is ectopically expressed become neurons.

How does the activity of the neurogenic genes repress the proneural activity of XASH-3 in these experiments? Since activated Notch inhibits the proneural activity of XASH-3 in embryos where its expression is driven by RNA injections there are two possible modes by which the inhibitory action could be mediated. The first is that XASH-3 is posttranscriptionally modified by the activity of the neurogenic genes. The second is that the neurogenic genes act on downstream targets of XASH-3. If XASH-3 and NeuroD activate different downstream targets to promote neurogenesis and if these targets are differentially sensitive to inhibition by neurogenic genes it would account for why NeuroD is much less sensitive to Notch repression (Fig. 3). This would be the case, for example, if XASH-3 promoted neurogenesis by activating other downstream bHLH transcription factors like NeuroD. The activity of the neurogenic genes may inhibit the expression of this class of bHLH transcription factors but may not inhibit their ability to promote the expression of neuron-specific genes. Thus, in this model the different response of XASH-3 and NeuroD to lateral inhibition reflects a differential ability of these two transcription factors to activate certain downstream target genes.

**Fig. 3.** Model for the interactions between proneural and neurogenic genes during primary neurogenesis in *Xenopus*. Diagrams in A and B represent potential interactions between proneural and neurogenic genes within cells of the neural plate where primary neurons are generated. In A, proneural genes expression promotes neuronal differentiation, but also promotes the expression of X-Delta-1. The expression of X-Delta-1 activates Notch in neighboring cells as shown in B, which blocks the ability of proneural genes to activate Delta in these cells. These interactions produce a negative feedback loop which allows the cell in A to express NeuroD, at which point it undergoes neuronal differentiation. Conversely, the cell in B cannot turn on NeuroD because of Notch activation, and thus fails to differentiate.
and a differential susceptibility of these target genes to repression by Notch. A test of this model depends on identifying the target genes activated by both NeuroD and XASH-3 when they promote neurogenesis.

Ectopic expression of NeuroD leads to ectopic neurons both in the neural plate and epidermises, while XASH-3’s effects are restricted to the neural plate (data not shown). These observations suggest that XASH-3 and NeuroD differ not only in their sensitivity to the inhibitory effect of the neurogenic genes but they also appear to differ in their sensitivity to inhibitory factors that restrict neurogenesis to the neural plate. The ability of NeuroD to generate ectopic neurons is itself limited to the ectoderm (Lee et al., 1995) hence the domains where XASH-3 and NeuroD are effective at generating ectopic neurons under different conditions reveal different levels of inhibitory control that restrict neurogenesis to specific domains in the embryo.

Lee and colleagues showed previously that the onset of NeuroD expression in Xenopus embryos correlates with the appearance of primary neurons as marked by N-tubulin expression (Lee et al., 1995). In contrast, the onset of XASH-3 expression occurs even earlier, and correlates to some extent with the time at which X-Delta-1 is expressed in domains of the neural plate where primary neurogenesis takes place (Zimmerman et al., 1993; Turner and Weintraub, 1994; Chitnis et al., 1995). Based on these and other observations, Lee and colleagues proposed that the XASH-3 and NeuroD have separate functions during neuronal determination and differentiation, respectively (Lee et al., 1995). In this view, determination is a state promoted by the expression of XASH-3 where cells in the neurogenic epithelium have the potential to adopt a neuronal fate, but where the ability to do so can be influenced by inhibitory factors. Determined cells, however, then progress to a state promoted by the expression of NeuroD where they initiate the irreversible process of neuronal differentiation. The roles of XASH-3 and NeuroD in neuronal determination and differentiation are similar to the roles that have been previously assigned to the bHLH transcription factors MyoD and Myf-5 in muscle determination, and to myogenin in muscle differentiation (Weintraub, 1993). Our results support the idea that the XASH-3 and NeuroD have different roles during determination and differentiation, respectively, and that one critical difference between these two transcription factors is their sensitivity to the effects of lateral inhibition, as mediated by Notch and Delta. Thus, one critical factor in controlling whether cells progress from determination to differentiation may be the differential effects of Notch activation on the proneural activity of XASH-3 and NeuroD. One reason why the expression of this second class of bHLH factors may be necessary is that the selection of cells by proneural genes like XASH-3 may be inherently unstable. Coupling the selection process, promoted by XASH-3, with a mechanism that stabilizes the differentiation of the selected cells, may be one way of reliably establishing a particular pattern of differentiation, as other cells in the environment continue to participate in inhibitory cell-cell interactions that determine future cell fate decisions.

The authors wish to thank Drs Anne Bang and Nancy Papalopoulou for comments on the manuscript. We are indebted to members of the laboratory for helpful discussion and to Dr Jackie Lee for providing the NeuroD expression clone. This work was supported by a NIH grant to C. K.

REFERENCES


(Accepted 1 April 1996)