Control of neurogenesis – lessons from frogs, fish and flies

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Two types of genes activated by neural inducers have been identified, those that lead to the activation of proneural genes and those that limit the activity of these genes to specific domains in the neural plate. The analysis of these genes has begun to fill gaps in our understanding of events that lead from neural induction to the generation of neurons within three longitudinal columns in the *Xenopus* and zebrafish neural plate.

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Abbreviations

BMP	bone morphogenetic protein
EGF	epidermal growth factor
FGF	fibroblast growth factor
oep	*one-eyed pinhead*
sbn	*somitabun*
snh	*snailhouse*
swr	*swirl*

Introduction

The analysis of early neurogenesis in *Xenopus* and zebrafish embryos has revealed the remarkable conservation between vertebrates and invertebrates of molecular mechanisms by which cells in the ectoderm adopt a neural fate (reviewed in [1]) and by which cells within the neuroectoderm are selected to become neurons (reviewed in [2]). Neural inducers such as chordin, follistatin and noggin are functional antagonists of bone morphogenetic proteins (BMPs), such as BMP2 and BMP4. BMP signaling blocks the ectoderm's ability to adopt a neural fate, and an important role of neural inducers is to define an area of the ectoderm in which the anti-neural activity of the BMPs is antagonized. Neural inducers are expressed in the dorsal organizer and influence fate in the ectoderm by planar signaling or by vertical signaling when axial mesoderm expressing these genes comes to lie under the ectoderm. In *Drosophila*, *short gastrulation* (*sog*), a *chordin* orthologue, blocks the activity of *Decapentaplegic* (*Dpp*), a BMP4 homologue, and plays a similar role in defining the domain of the ectoderm that will become the neuroectoderm.

Similarities are also seen in the mechanisms whereby neuroectodermal cells are selected to become neurons. Early neurons in *Xenopus* and zebrafish embryos are distributed in a simple pattern: a subset of cells in three bilateral longitudinal domains are selected to become neurons in the neural plate. As in *Drosophila*, expression of a basic helix-loop-helix (bHLH) transcription factor, neurogenin (Xngnr1) [3], appears to define domains in the *Xenopus* neural plate where cells have the potential to form early neurons. Within these domains, lateral inhibition mediated by the neurogenic genes *Notch* and *Delta* limits the activity of neurogenin to a subset of cells that are permitted to become neurons [2,4].

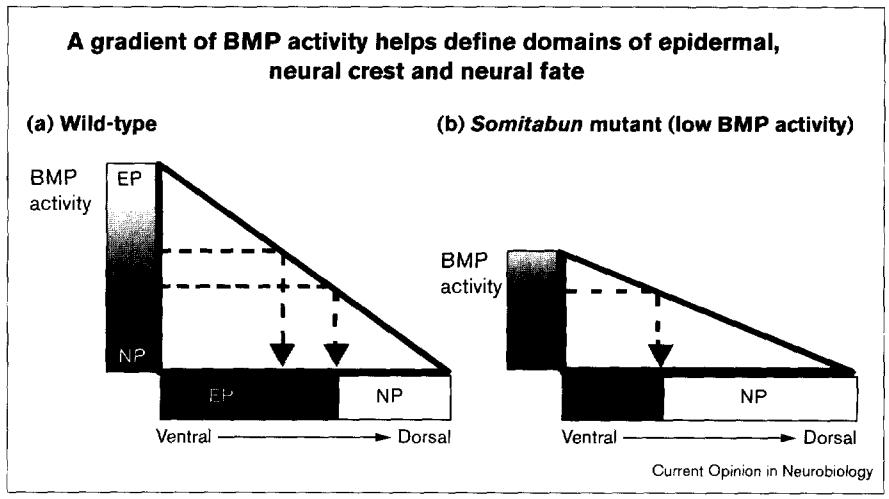
This review will focus on papers describing recent insights from the *Xenopus* and zebrafish model systems that emphasize the potential role of a gradient of BMP activity in determining dorsoventral fate in the ectoderm, and that have recently identified molecules, downstream of neural inducers, that influence neurogenesis by modulating the activity of neural and proneural genes. The review concludes with the discussion of recent work that shows how neuroblasts are generated in three bilateral longitudinal domains in the *Drosophila* neuroectoderm. These studies point to mechanisms that may potentially be conserved and important for understanding how neurons in the *Xenopus* and zebrafish neural plate are generated in three longitudinal columns.

Role of BMP activity in determining dorsoventral fates in the ectoderm

To assess the role of BMP activity in determining dorsoventral fates in the ectoderm, the effect of different doses of neural inducers, BMPs, or mediators of BMP signaling on the fate of ectodermal cells has been examined [5•,6••,7•,8••,9•,10•,11]. These studies show that the ectoderm responds to BMP activation in a dose-dependent manner, with neural fate being associated with the lowest BMP activity and epidermal fate with the highest. Recent analysis of zebrafish mutants provides further evidence for the role of BMP activity in defining dorsoventral fate in the ectoderm. Ventralized *chordino* mutants have a mutation in the zebrafish homologue of *chordin* and are associated with a smaller neural plate [12–16]. The ventralized phenotype seen in *chordino* mutants is dependent on the activity of *swirl*, a zebrafish *BMP2b* homologue, which is consistent with *chordino* working by suppressing the activity of this gene [16,17•]. A mutation in *swirl*, on the other hand, is associated with a severely dorsalized phenotype in which the neural plate is expanded at the cost of more lateral and ventral derivatives, the neural crest and epidermis.

The effects on early neural crest in a series of progressively more dorsalized zebrafish mutants, *snailhouse* (*snh*), *somitabun* (*sbn*) and *swirl* (*swr*), provide an important insight into the potential role of a gradient of BMP activity in determining dorsoventral fates in the ectoderm [18••]. Neural crest is thought to be determined as a consequence of local interactions at the boundary of the neural plate and epidermal cells [19,20]. If this is the case, the location of the crest should shift ventrally in mutants in which the neural plate is expanded, corresponding to the altered location of the

Figure 1



A gradient of BMP activity determining dorsoventral fates in the ectoderm accounts for the expansion of the neural crest domain seen in *sbn* mutants. If high (white), intermediate (grey) and low (black) levels of BMP activity determine epidermal (EP), neural crest (NC) and neural plate (NP) fates, respectively, along the dorsoventral axis of the ectoderm, then a change in the shape of the gradient due to lower BMP activation could lead to a level of BMP activation in the ventral ectoderm that corresponds to the threshold for neural crest determination rather than epidermis. The change in the shape of the gradient would alter the size of the neural plate and neural crest domains. Dashed horizontal lines indicate the window of BMP activity required for neural crest determination, and dashed vertical lines indicate the location along the dorsoventral axis of corresponding levels of BMP activation.

boundary between neural and epidermal cells; however, no change is expected in the size of the neural crest domain. The dorsalized phenotype of *sbn* mutants, however, is characterized by an expanded neural plate, loss of epidermis and an expanded neural crest domain [18••]. The local interactions model does not provide a simple explanation for the expanded neural crest domain in *sbn* mutants. If, on the other hand, a gradient of BMP activity is responsible for generating epidermis, neural crest and neural plate at high, intermediate and low levels, respectively, it would be easier to account for this phenotype, as suggested below [18••]. Lowered BMP activity in *sbn* mutants changes the profile of BMP activity along the dorsoventral axis of the ectoderm. As a consequence of this change BMP activity in the ventral ectoderm could correspond to the requirement for neural crest rather than epidermal cells, accounting for the expansion of neural crest at the cost of epidermis (Figure 1).

A role for a BMP activity gradient in determining neural crest fate is also supported by studies in *Xenopus*, which show that neural crest is induced with intermediate levels of BMP activation in the ectoderm [6••,7•]. Whether or not BMP activation plays an early role in determining neural crest fate in the ectoderm, however, remains controversial, and recent studies in chick embryos specifically argue against it [21•]. Differences in the time at which neural crest fate is determined in different organisms may be one factor that contributes to differences in the interpretation of the role of BMPs in neural crest formation in chick versus *Xenopus* and zebrafish. An early role for a BMP gradient in determining neural crest fate does not rule out a role for local interactions later in development. In any event, BMP signaling alone is not sufficient to produce neural crest in the ectoderm, other signaling pathways such as the Wnts and fibroblast growth factors (FGFs) are also necessary [22,23]. How the gradient of BMP activity is established and maintained is not yet completely clear, and the roles of diffusible antagonists, FGF and cell movement in this process are being determined [5•,8••,10•].

Molecules linking neural inducers to activation of proneural genes

BMP signaling promotes epidermal fate in the ventral ectoderm by activating at least three classes of genes, including ventral-specific homeobox genes (e.g. *PV.1* and *Xvent1*), *GATA1* and *Msx1* [24•]. Suppression of BMP signaling, on the other hand, by neural inducers leads to the activation of a number of recently discovered genes that promote neural fate in the dorsal ectoderm (see Table 1). Differential screens designed to identify genes upregulated by *chordin* or *noggin* in the *Xenopus* ectoderm have led to identification of genes in the *Sox* and *Zic* families [24•,25,26•]. *Sox* genes encode *Sry*-related transcription factors that contain a high mobility group (HMG) domain that binds to DNA in a sequence-specific manner; recently identified members include *SoxD* and *Sox2*. *Zic*-related genes, on the other hand, are homologues of *Drosophila odd paired* and recently identified members include *Zic-r1* [26•], *Zic3* [27], *Zic1* [28], *Zic2* [29••] and *opl* [25,30]. Mediators of neural induction have also been identified in expression screens aimed at isolating mRNAs that lead to an expansion of neural tissue when ectopically expressed. This has led to the identification of a novel bifunctional gene, *Geminin*, that both controls cell cycle and is an important mediator of neural fate [31•].

Amongst the recently identified neuralizing genes, *SoxD* is one of the earliest to be expressed [32••]. It is initially widely expressed in the prospective ectoderm at the late blastula stage, and its expression is then restricted to the dorsal ectoderm by midgastrulation. Ectopic expression of *SoxD* in animal caps promotes the expression of genes required for neural and neuronal differentiation. Initial results suggest that *SoxD*'s early expression may help account for the 'default' [33] ability of ectoderm to adopt

Table1

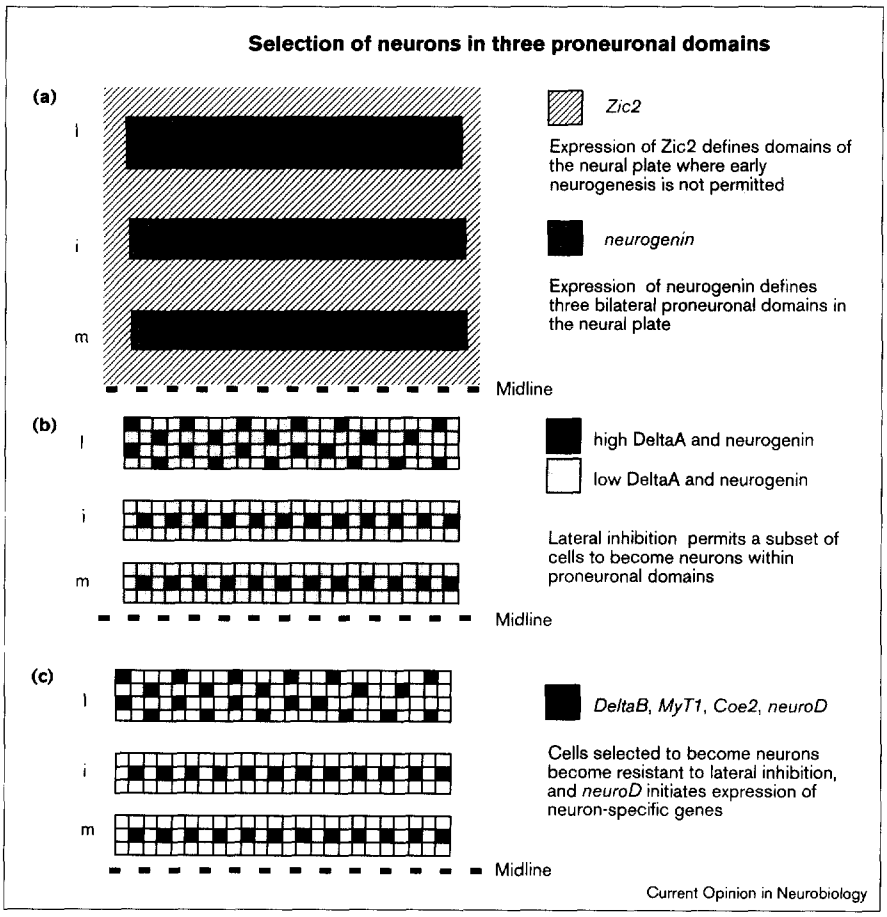
Candidate genes linking neural induction to neurogenesis.

Gene	Regulation in ectoderm	Expression in ectoderm		Effects of ectopic expression on				
		Early gastrula	Late gastrula	Epidermis	Neural crest	Neural	Proneural genes	Neurons
SoxD	Positive by Chd Negative by BMP	Pan ectodermal	Neuroectoderm	Decrease	Increase	Increase anterior	Increase Xngnr-1	Increase N-tubulin
Zic-r1	Positive by Chd Negative by BMP	Dorsal	Lateral anterior neuroectoderm (later in the lateral neural plate)	Decrease	Increase	Increase anterior	Increase Xngnr-1	Increase N-tubulin
Zic3	Positive by Chd Negative by BMP	Dorsal	Lateral anterior neuroectoderm (later in the lateral neural plate)	Decrease	Increase	Increase anterior	Increase XASH-3	Increase neuroD
Sox2	Positive by Chd Negative by BMP	Dorsal	Neuroectoderm	Decrease (+ bFGF)	Increase (+ bFGF)	Increase (+ bFGF) posterior	Increase Xngnr-1 (+ bFGF)	Increase N-tubulin (+ bFGF)
opl	Positive by noggin	Dorsal	Lateral anterior neuroectoderm (later in the lateral neural plate)	Decrease	Increase	Increase dorsal	Not known	Not known
geminin	Positive by Chd and noggin	Dorsal (maternal expression in the animal hemisphere)	Neuroectoderm	Decrease	Decrease (high dose) Increase (low dose)	Increase posterior	Increase Xngnr-1	Increase N-tubulin
Xiro1/Xiro2	Positive by noggin + RA Positive by Gli proteins	Not detected	Lateral anterior neuroectoderm (later in the lateral neural plate)	Decrease	Decrease	Increase	More XASH-3 More Xngnr-1	Variable increase ATH-3
Xiro3	Positive by noggin + FGF Negative by Xngnr-1	Not detected	Between prospective medial and intermediate proneural domains	Decrease	Decrease	Increase	More XASH-3 Less Xngnr-1	Decrease N-tubulin

Adapted from [24•]. bFGF, basic FGF; Chd, chordin; RA, retinoic acid.

Figure 2

A series of inhibitory interactions restrict neurogenesis to a subset of cells within three proneuronal domains in the neuroectoderm. **(a)** *Zic2* limits the expression of neurogenin to three domains, medial (m), intermediate (i) and lateral (l), in the neural plate. **(b)** Neurogenin drives the expression of the inhibitory ligand DeltaA, which activates Notch in neighboring cells and reduces the activity of neurogenin in those cells. As a consequence of these interactions, a subset of cells are selected that maintain high levels of *neurogenin* and *DeltaA*, while neighboring cells are inhibited from doing the same. **(c)** Cells selected to maintain high levels of *neurogenin* begin to express another *Delta* homologue, *DeltaB*. They also express *MyT1* and *Coe2*, which makes them resistant to lateral inhibition. Eventually, these cells express NeuroD, which controls the expression of genes responsible for the differentiation of neurons.



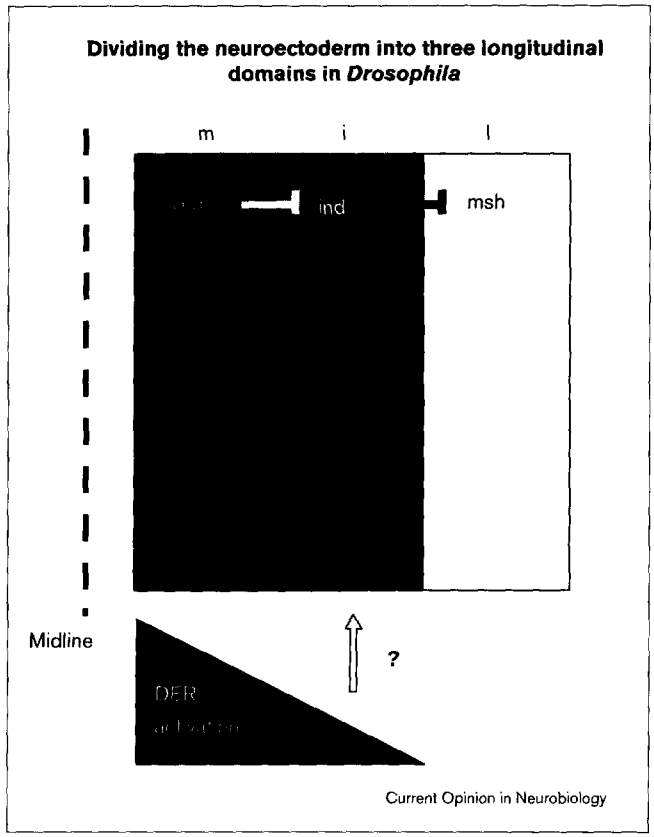
a neural fate. Like *SoxD*, *Zic-r1* and *Zic3* initiate neural and neuronal differentiation when they are ectopically expressed; however, unlike *SoxD*, which remains widely expressed in the CNS, their expression is eventually restricted to the dorsal nervous system. *Sox2*, another *Sry*-related gene, also has a paneural expression but differs from *SoxD*, *Zic-r1* and *Zic3* in its ability to have a neuralizing effect on its own [26•]. *Sox2* requires additional factors to reveal this potential, and its role is thought to be in changing the competence of ectoderm, allowing it to respond to neuralizing factors such as FGF. Like *Sox2*, *opl* does not have a neuralizing effect on its own, but it sensitizes the animal cap ectoderm to the neural inducer *noggin* and alters the anteroposterior nature of neural tissue induced by it [25]. Like *noggin*, *SoxD*, *Zic3* and *Zic-r1* generate neural tissue with an anterior character, whereas *geminin* and *Sox2* (with *Fgf*) generate neural tissue with caudal characteristics [26•,27,28,31•]. Many of the *Zic* genes activated by neural inducers also induce neural crest markers when expressed ectopically, which is consistent with their expression later in the dorsal neural tube [25,26•,27,28,29••,30].

Genes in the Iroquois complex control proneural gene expression in *Drosophila* [34]. The discovery of vertebrate iroquois homologues has led to the discovery of another class of genes that promote expression of proneural genes in vertebrates [35,36•,37•]. The *Xenopus* homologue, *Xiro3* does not lead, however, to the expression of the proneural gene *neurogenin*, which is involved in neuronal determination. Rather, it suppress the expression of this gene and promotes the expression of another proneural gene homologue, *XASH-3*. Like the *Xiro* homologs, *XASH-3* also suppresses differentiation of neurons and causes an expansion of the neural plate. The ability of *XASH-3* to suppress primary neurogenesis is attributable, at least in part, to the activation of neurogenic genes [38]. *Xiro-3*, however, continues to suppress differentiation of neurons when neurogenic genes are suppressed by a dominant-negative form of Delta, suggesting that the effects on primary neurogenesis may be mediated by another mechanism [37•]. While the physiological role of *XASH-3* remains a little unclear, recent work has re-emphasized the role this type of proneural gene may play in determining neural fate [7•,39]. Morgan and Sargent [7•] suggest that *XASH-3* helps define the part of the neuroectoderm that will form neural plate rather than neural crest.

Zic2 limits neurogenesis to longitudinal domains in the neural plate

Genes such as *Zic3*, *Zic-r1* and *SoxD* are widely expressed in the prospective neural plate and are capable of promoting

Figure 3



A series of inhibitory interactions divide the neuroectoderm in *Drosophila* into three longitudinal compartments in which three homeobox genes, *vnd, ind* and *msh* are expressed. *ind* inhibits *msh* from being expressed in the intermediate compartment, whereas *vnd* inhibits *ind* from being expressed in the ventral compartment. This limits their expression to three distinct domains of the neural plate where they play an essential role in determining the fate of neuroblasts in three longitudinal domains. *Drosophila* EGF receptor (DER) activation plays an essential role in determining the fate of neuroblasts in the intermediate domain. This could potentially be attributable to DER activation driving *ind* expression.

the expression of the proneural gene *neurogenin*, so why is *neurogenin* expression restricted to three bilateral longitudinal domains in the neural plate? One answer to this question comes from the discovery of another member of the *Zic* family, *Zic2* [29••]. *Zic2* inhibits formation of neurons and is expressed in stripes that are complementary to longitudinal domains in which early neurons are generated. This zinc-finger transcription factor contains mono-amino-acid stretches characteristic of repressor domains, and it is thought to repress the function of other more widely expressed members of the *Zic* and *Gli* superfamilies, limiting their function to specific domains of the neural plate where *neurogenin* is expressed (Figure 2). Replacement of the repressor domain in *Zic2* with an activator domain makes it promote formation of neurons, similar to other members of this family. Like other *Zic* genes, however, *Zic2* promotes differentiation of the neural crest [29••].

The role of proneural and neurogenic genes

Functional analysis of zebrafish homologues of *neurogenin* and *Delta* provides more evidence for the role of neurogenic genes in limiting *neurogenin* function to a subset of cells within 'proneuronal' domains [40–42,43•,44••]. Ectopic expression of *neurogenin* in zebrafish embryos leads to formation of ectopic neurons in a salt-and-pepper pattern, primarily in the neuroectoderm. This suggests that lateral inhibition limits the number of *neurogenin*-expressing cells that are permitted to become neurons and that additional patterning mechanisms limit neurogenin's activity to the dorsal ectoderm. Dynamic changes in the expression of zebrafish *DeltaA* are consistent with the role of this inhibitory ligand in selecting cells that become neurons by lateral inhibition [43•,44••]. *DeltaA* is initially expressed widely in all cells in the proneuronal domains but is later expressed at a particularly high level in a subset of these cells that begin to express neuronal markers and another *Delta* homologue, *DeltaB*. The sequentially restricted pattern of expression of multiple *Delta* homologues in zebrafish suggests that neurogenic genes may be involved in restricting neural fate in a series of fate determination events that eventually lead to the formation of neurons. The neurogenic phenotype of the zebrafish *mind bomb* mutant supports the role of these genes in selecting cells that become neurons within proneuronal domains [45,46]. *MyT1*, whose expression makes cells resistant to the effects of lateral inhibition, facilitates stable adoption of a neuronal fate in cells selected to become neurons [47]. Recently, it has been shown that this is also facilitated by another class of transcription factors in the Col/Olf-1/EBF family (Xcoe2 and Zcoe2) [48,49]. Finally, cells selected to become neurons begin to express neuroD, a bHLH transcription factor, which initiates expression of genes important for differentiation of neurons (Figure 2) [42,50].

Making three stripes – more hints from *Drosophila?*

Ectopic expression of *neurogenin-1* in zebrafish shows that although *neurogenin* gives cells the potential to adopt a neuronal fate, the type of neurons generated is determined independently by dorsoventral patterning mechanisms [40]. Mechanisms that generate the three proneuronal domains are still poorly understood in zebrafish and *Xenopus*. It is interesting that neuroblasts produced in the early waves of neurogenesis in the *Drosophila* neuroectoderm are also produced in three longitudinal columns. Analysis of three homeobox genes, *vnd* (*ventral neural defective*), *ind* (*intermediate neuroblast defective*) and *msh* (*muscle segment homeobox*), shows how a cascade of inhibitory interactions divides the neuroectoderm into three domains: *vnd* represses *ind* in the ventral column, and *ind* represses *msh* in the intermediate column, limiting their expression to three distinct, medial, intermediate and lateral domains in which early neuroblasts are generated [51••,52••] (see Figure 3). The identification of vertebrate homologues of these homeobox genes, *NK2.2* (*vnd*) [53], *Gsh1* (*ind*) [54,55], and *Msx1* and *Msx3* (*msh*) [56], which are expressed in corresponding domains of the vertebrate

neural plate, points to potential similarities in the patterning mechanisms that define the proneuronal domains in vertebrates. In *Drosophila*, epidermal growth factor (EGF) signaling has also been shown to play an important role in dorsoventral patterning of the neuroectoderm longitudinal domains [57–59] and potentially influences the expression of *ind* in the intermediate domain. In vertebrates, midline hedgehog signals rather than EGF play an important role in dorsoventral patterning [60]. The recent cloning of *one-eyed pinhead* (*oep*) in zebrafish, which encodes an EGF-related molecule, however, points to the potential importance of EGF-related signals in vertebrates as well [61••].

Conclusions

BMP signaling divides the ectoderm into three dorsoventral domains in which the neural plate, neural crest and epidermis form. Neural inducers suppress BMP signaling and lead to the expression a number of transcription factors, including *Sox* and *Zic* genes, that promote expression of genes required for neural and neuronal differentiation in the dorsal ectoderm. *Zic2* helps limit primary neurogenesis to three bilateral proneuronal domains in the neural plate where *neurogenin* is expressed. Finally, neurogenic genes limit the number of cells that become neurons within these domains. A challenge that remains for the future is to understand the interactions between genes activated by neural inducers and to characterize the mechanisms that generate the three bilateral proneuronal domains in the neural plate. Analysis of early neurogenesis in the neural plate of zebrafish and *Xenopus* embryos through the discovery of new genes and mutants will continue to provide important insights into the fundamental patterning mechanisms that operate in the developing nervous system. Research in past decade has emphasized the discovery of similarities in molecular mechanisms that operate in diverse animal systems. It is important, however, to recognize and understand the differences in developmental processes in diverse developmental model systems so as to understand how differences in timing, size of tissue, and gene duplication have contributed to diversity in neurogenesis.

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