# Neurogenesis genes: mechanisms of proneural activity

In vertebrates and insects, the prospective ectoderm is subdivided into a neurogenic and a non-neurogenic portion by the antagonistic activity of homologous secreted molecules *decapentaplegic/BMP-4* and *short gastrulation/chordin* [1]. The neurogenic ectoderm, composed initially of proliferative cells, give rise to neural progenitor cells, most of these being multipotential. In order to acquire their specificity, these cells go through a complex and tightly regulated set of events. Parallel studies of neural lineages in vertebrates and *Drosophila* indicate that, despite their differences, proneural genes (genes that control neurogenesis) in these organisms have similar structure and function. [1-3, 5]

Following the efforts to elucidate the genes and mechanisms associated with neurogenesis, several proneural and regulatory genes were identified in vertebrates in *Drosophila* , and their modes of action studied. These efforts provided evidence that in the initiation of neurogenesis and specification of neuron identity a small number of ‘ proneural genes’, which encode transcription factors of the basic-helix-loop-helix (bHLH) class, are a key element. Research in this field begun in the 1970s, when a set of genes involved in regulating the early steps of *Drosophila* neurogenesis was identified. Molecular analysis led to the isolation of the four sequence similar genes of this set ( *achaete* ( *ac* ), *scute* ( *sc* ), *lethal of scute* ( *lsc* ) and *asense* ( *ase* )). Another proneural gene belonging to a different bHLH family, *atonal* ( *ato* ), was later isolated. Two *ato* -related genes, *amos* ( *absent MD neurons and olfactory sensilla* ) and *cato* ( *cousin of atonal* ), were more recently found, defining a second family of proneural genes. By now, many

genes related to *asc* and *ato* were identified in vertebrates through loss-of-function (LoF) and gain-of-function (GoF) studies [1-3].

Table 1 – Genes and proteins involved in vertebrate neurogenesis. Current studies suggest that there are more key elements in neurogenesis still unknown. For futher information relating to these molecules see references[1-5].

| Family | Examples | Type of study | Function |
|---|---|---|---|
| *asc* | *Cash1* (chicken) | LoF | Neurogenesis in the ventral telencephalon and the olfactory sensory epithelium; commitment of multipotent progenitors to a neuronal lineage and in the inhibition of a glial fate; |
| | *Cash4* (chicken) | | |
| | *Mash1* (mouse) | | |
| | *Mash2* (mammals) | | |
| | *Xash1* (*Xenopu* | | |

| | | | |
|---|---|---|---|
| | *s* ) | | in conjunction with *Ngn* genes, is involved in the generation of all progenitors of the cerebral cortex |
| | *Xash3* ( *Xenopus* ) | | |
| | *Zash1* (Zebrafish) | | |
| *ato* related | *Math1* (mouse) | LoF/ GoF | Development of certain neuronal lineages in the CNS: cerebellar granule cells, hair cells in the inner ear, D1 interneurons and other non-neural cell types; specification of interneuron identity; specification of |
| | *Math5* (mouse) | LoF | |
| | *ath5* (mouse, Zebrafish) | GoF | |
| | *Xath5* ( *Xenopus* ) | | |
| | *Cath5* (Chicken) | | |

| | | | |
|---|---|---|---|
| | | | retinal ganglion cells fate in multipotent retinal progenitors |
| | *Xath1* (Xenopus) | | shown to induce ectopic neuronal differentiation in non-neural ectoderm |
| | | | ectopic expression in the chick neural tube leads to precocious differentiation of neuroepithelial cells |
| *Neurogenin* | *Ngn1, Ngn2* | LoF/ | Neurogenesis of cranial |

| | | |
|---|---|---|
| | (mouse) GoF | sensory ganglia; neurogenesis of spinal sensory ganglia and ventral spinal cord neurons; activation of Notch signaling; commitment of multipotent progenitors to a neuronal lineage and the inhibition of a glial fate; promote neuronal differentiation through activation of the differentiation |

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|---|---|---|---|
| | | | genes *NeuroD* and *ath3* ; generation of all cranial and spinal sensory progenitors; cell cycle withdrawal |
| *NeuroD* | *Math3/ NeuroM NeuroD* | LoF | Neuronal vs. glial cell-fate decision in defined CNS regions; proliferation, differentiation and survival of granule cells in the cerebellum and hippocampus |
| *Olig* | Olig2 | | DNA-binding repressors of proneural gene |

| | | |
|---|---|---|
| | | transcription |
| Proneural gene inhibitors | Id<br>Hes/ Her/Esr | Passive repressors of proneural gene activity (interference with heterodimerization of bHLH proteins, a process essential to DNA binding) |

Mechanisms of proneural activity

*Notch signaling*

A critical step in neurogenesis is the commitment of progenitor cells to their respective fates. One of the mechanisms that control this phase is called Notch signaling, also known as “ lateral inhibition”. A set of transcription factors (encoded by patterning genes) and extracellular signaling molecules act together to control the spatiotemporal pattern of the expression of proneural proteins and proteins that antagonize their function. The progenitor cells were these antagonizing proteins are expressed at lower levels are the ones that will suffer further differentiation by action of

proneural genes. In addiction to this fact, the expression proneural proteins in these cells inhibit the expression of the same proteins in neighboring cells through the activation of the Notch/Delta signaling pathway – preventing them to differentiate.

The expression of Delta, a cell-surface protein, is promoted by bHLH proneural genes. The interaction of Delta with Notch, a cell-surface receptor, activates the expression of a transcription factor, which in turn activates the expression of Hes/Her/Esr in vertebrates — that directly downregulate proneural gene expression. This leads to a further decrease in Delta expression in neighboring cells – a reinforcement of cell-cell signaling, that decreases neural potential in all but one cell of a proneural cluster [6]. It is worth noting that LoF mutations in the neurogenic genes lead to a neuronal commitment of all or most cells of the proneural cluster [1, 5].

*Positive-feedback loops.*

In order to increase and/or maintain the levels of proneural gene expression in the selected neural progenitors, positive-feedback mechanisms are required. The vertebrate HLH proteins Xcoe2 and Hes6 are induced by proneural genes and act as upregulators of proneural gene expression (e. g. Hes6 interferes at a post-transcriptional level with the inhibitory activity of the bHLH factor Hes1 on *Mash1* transcription and function). Another protein, Myt1, is also induced by proneural genes, and in turn confers insensitivity to lateral inhibition to the selected progenitors. In vertebrates, autoregulation has been demonstrated for the gene *Math1* , but it does not seem to have a role in the regulation of *Mash1* or *Ngn* genes [3].

*Cascades of neuronal-differentiation genes.*

The activation of neuronal-differentiation mechanisms is done by a set of bHLH regulatory genes, structurally related to proneural genes. Proneural genes, of which the expression is transient, control the activation of these regulatory genes, in a cascade of events that form the basis of sequential cell determination and differentiation.

The existence of bHLH genes that can induce neuronal differentiation when ectopically expressed, but are expressed later than proneural genes and are under their transcriptional control, has been confirmed in both vertebrates and *Drosophila* [3].

Several publications report examples that illustrate that *NeuroD* -family genes in vertebrates are activated and act downstream of proneural genes in a similar fashion to *ase* and *cato* in *Drosophila* neurogenesis. Furthermore, is has been shown that certain neuronal-differentiation genes can be cross-activated, and do not show the capability to activate proneural genes, reinforcing the idea that neurogenesis is a complex cascade process where a multitude of genes act sequentially in order to produce mature cells. Recent studies indicate that some proneural genes, such as *Mash1* , *Ngn1* and *ato* , have a dual capacity to promote the selection of progenitor cells and to interfere in differentiation steps, leading to the inference that both intrinsic molecular properties and temporal expression patterns are responsible for the distinct developmental roles of proneural and differentiation bHLH genes [1, 3, 4].

*Inhibition of glial fates.*

Like in other neurogenesis aspects, neurons and glia are generated from common multipotent progenitors in a temporally coordinated fashion. Adding to their important (and better characterized) role in neurogenesis, some vertebrate proneural genes have recently been shown to inhibit glial fates, namely *Mash1* , *Math3* and *Ngn2* that were studied in mutant mice [3] .

*Cell-cycle regulation.*

Cell-cycle withdrawal and differentiation in the nervous system are two closely related events. It is apparent that cell-division arrest occurs has a way to " immunize" already specified progenitor cells against extrinsic fate-determining cues. Once again, proneural genes, this time interacting with cyclin-dependent kinase (Cdk) inhibitors, have a determinant role in this event (e. g. overexpression of *Ngn2* in the chick neural tube leads to premature neuronal differentiation in neuroepithelial cells and cell-cycle exit) [3, 4].

Neuronal-subtype specification

The structural diversity of proneural genes on vertebrates and *Drosophila* , and the fact that some families of proneural genes are preferentially expressed in certain progenitor domains specific for the production of particular types of neurons indicate that they are key element in the specification of neuronal-subtype characteristics. Linkage between proneural genes and neuronal fate has been observed for *Math1* , *Ngn1* and *Mash1* , in mice LoF studies. The role of this last gene in the specification of noradrenergic neurons is illustrative of the importance of proneural genes for neuronal sub-type specification. In cells that have a noradrenergic

phenotype *Mash1* acts in combination with a determinant of the noradrenergic phenotype, the homeodomain protein Phox2b, to induce the expression of the homeobox gene *Phox2a* and of the noradrenaline-synthesizing enzyme dopamine ï ¢-hydroxylase (DBH). Other experiments showed that *Mash1* is determinant in the specification of other neuronal subtypes. The fact that *Mash1* is involved in this phase of the maturation of different types of cells of the nervous system is a proof that it works in conjuction with regionally expressed factors that modify its specificity. Interestingly, it was found that other bHLH non-proneural genes ( *NeuroD* and *Math3* ) are involved in neuronal-subtype specification, with a *modus operandi* similar to that of proneural genes [1, 3].

Recent studies also indicate that secondary neurogenesis is regulated by other factors, such as hormones, transmitters, growth factors and environmental cues [7].

Bibliography

[1] Salzberg, A., Bellen, H. J. (1996) *Invertebrate versus vertebrate neurogenesis: variations on the same theme?* ; Dev. Genet. 18(1), 1-10

[2] Laufer, E., Marigo, V. (1994) *Evolution in developmental biology: Of morphology and molecules* ; Trends Genet. 10, 261-263.

[3] Bertrand, N., Castro, D. S., Guillemot, F. (2002) *Proneural genes and the specification of neural cell types* ; Nat. Rev. Neurosci. 3, 517–530

[4] Finlay, B. L., Hersman, M. N., Darlington, R. B. (1998) *Patterns of vertebrate neurogenesis and the paths of vertebrate evolution* ; Brain Behav Evol52(4-5), 232-242

[5] Chan, Y. M., Jan, Y. N. (1999) *Conservation of neurogenic genes and mechanisms* ; Curr. Opin. Neurobiol. 9, 582-588

[6] Lodish, H. *et al* . (2000) *Molecular Cell Biology* . 4th ed. New York: W. H. Freeman & Co.

[7] Cayre, M. (2002) *The common properties of neurogenesis in the adult brain: from invertebrates to vertebrates* ; Comp Biochem Physiol B Biochem Mol Biol. 132(1), 1-15