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Spatial and temporal specification of neural fates by transcription factor codes

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The vertebrate central nervous system contains a great diversity of neurons and glial cells, which are generated in the embryonic neural tube at specific times and positions. Several classes of transcription factors have been shown to control various steps in the differentiation of progenitor cells in the neural tube and to determine the identity of the cells produced. Recent evidence indicates that combinations of transcription factors of the homeodomain and basic helix-loop-helix families establish molecular codes that determine both where and when the different kinds of neurons and glial cells are generated.

Introduction

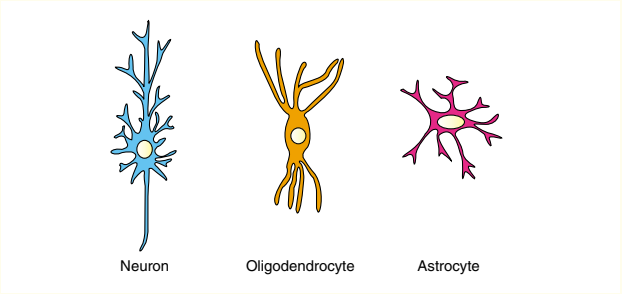
A multitude of neurons of different types, as well as oligodendrocytes and astrocytes (see Box 1), are generated as the vertebrate central nervous system develops. These different neural cells are generated at defined times and positions by multipotent progenitors located in the walls of the embryonic neural tube. Progenitors located in the ventral neural tube at spinal cord level first produce motor neurons, which innervate skeletal muscles and later produce oligodendrocytes (Fig. 1). The first cells produced by progenitors at more dorsal or ventral positions in the spinal cord are interneurons of different classes. The generation of a particular class of neuron or glial cell from a multipotent progenitor is a complex process that can be subdivided into a series of sequential steps (Fig. 2A,B). First, progenitor cells acquire unique positional identities through a process of spatial patterning of the neural primordium. Thus, progenitors in the ventral spinal cord that produce motor neurons and oligodendrocytes acquire a distinct identity from that of progenitors in the dorsal spinal cord or brain. Multipotent progenitors then produce daughter progenitor cells that are restricted to produce only one of the primary neural cell types – neurons, oligodendrocytes or astrocytes – in a step called cell type selection or commitment. Committed neuronal progenitors also become specified to produce neurons of a particular kind, e.g. a particular class of motor neuron or interneuron, a step called subtype specification that is conceptually distinct from, but mechanistically tightly linked to, the step of cell type commitment, as we will see. Neuronal progenitors then stop dividing, migrate out of the progenitor zones they occupy (see Fig. 1B) and towards more differentiated areas of the neural tube. There they initiate a programme of terminal differentiation. Oligodendrocytes, astrocytes and some types of neurons begin to migrate and differentiate while still dividing.

The fact that particular classes of neurons and glial cells are produced only at particular locations in the embryonic neural tube suggests that the mechanisms that govern spatial patterning and the acquisition of diverse cell fates are linked. Moreover, neurons and glial cells are produced in a defined order (first neurons, then oligodendrocytes, then astrocytes), and different classes of neurons originating from the same progenitors are also produced in a particular sequence, suggesting that the mechanisms controlling the specification of cell fates and the timing of progenitor division arrest and differentiation are also coordinated (Temple, 2001).

Great efforts have been made in the last 15 years to elucidate the genetic programmes underlying the generation of cell diversity in the nervous system, and transcription factors have been shown to play a central role in this process. Initially, different transcription factor families were thought to control particular steps in the differentiation of progenitor cells into neurons or glia, but their function turned out later to be broader and more complex. Thus, homeodomain proteins (HD) such as paired box 6 (Pax6) and orthodenticle homolog 2 (Otx2), were first shown to pattern the neural primordium, whereas basic helix-loop-helix (bHLH) proteins such as achaete-scute complex homolog-like 1 (Ascl1; also known as Mash1) and neurogenin 2 (Neurog2; also known as Ngn2), were initially shown to promote the cell cycle arrest and neuronal differentiation of progenitors. Additional functions of these transcription factor families have emerged more recently, in particular in the generation of a diverse array of neurons and glia. Recent evidence moreover suggests that generation of cell diversity in the nervous system involves extensive interactions between transcription factors of the HD and bHLH families.

The purpose of this review is to provide an update on how the differentiation of neural progenitor cells into different classes of neurons and glia is coordinately regulated by different families of

Box 1. Neurons and glial cells



The vertebrate central nervous system comprises three primary cell types, including neurons and two types of glial cells. Neurons are electrically excitable cells that process and transmit information via the release of neurotransmitters at synapses. Different subtypes of neurons can be distinguished by the morphology of their cell body and dendritic tree, the type of cells they connect with via their axon, the type of neurotransmitter used, etc. The two main types of glial cells are oligodendrocytes and astrocytes. Oligodendrocytes form the myelin sheaths that wrap and insulate axons and they promote the saltatory conduction of electric signals. Astrocytes contribute to the structural integrity of the brain, provide metabolic support to neurons, maintain water and ionic balance and modulate synaptic transmission.

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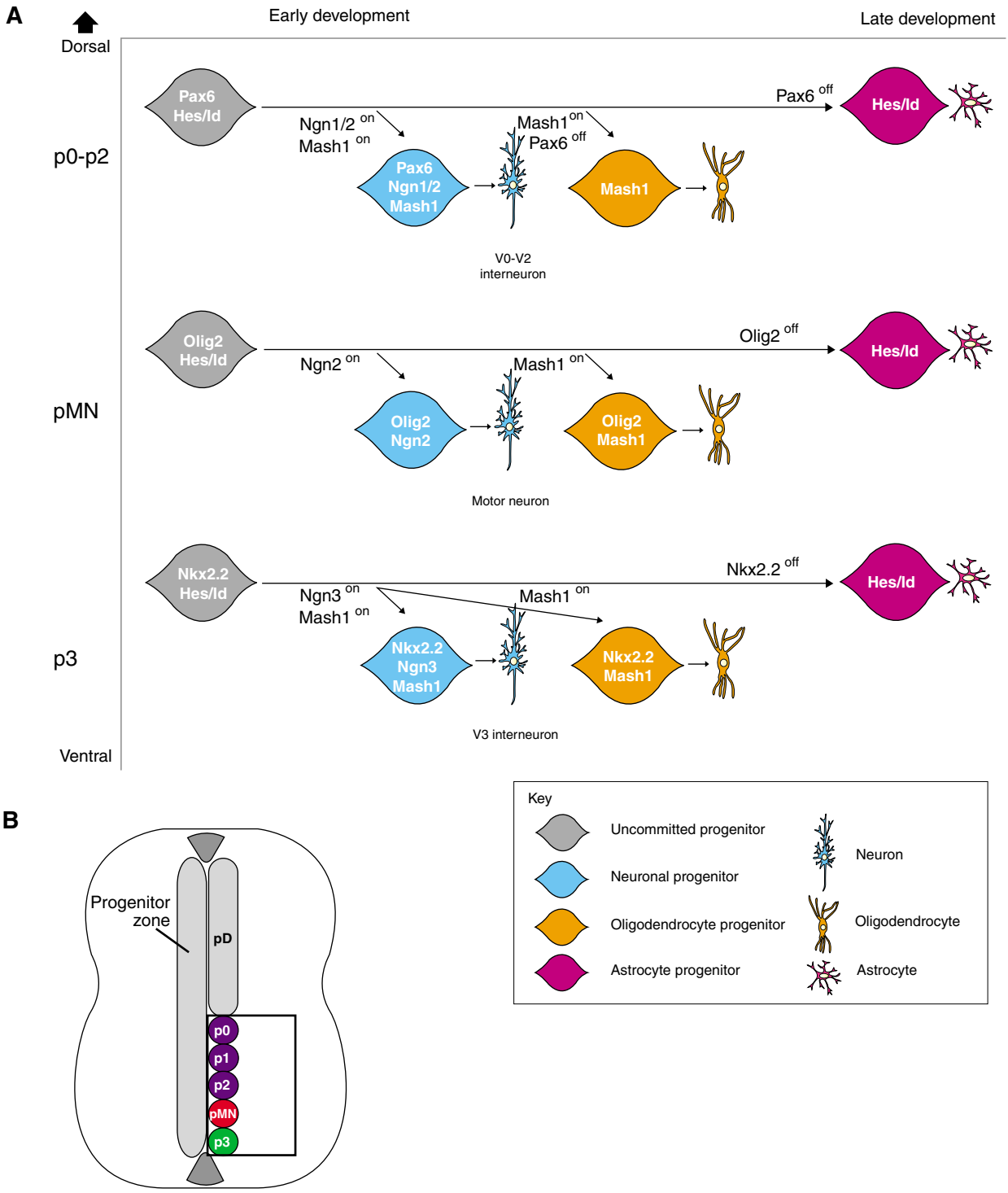

Fig. 1. Sequential generation of different classes of neurons and glia in different domains of the ventral spinal cord. (**A**) Progenitor domains in the ventral part of the mouse embryonic spinal cord. The vertical axis represents the dorsoventral axis of the spinal cord, the horizontal axis represents developmental time. (**B**) A cross-section of a mouse embryonic spinal cord (dorsal, top), indicating the position of the progenitor domains shown in A. Progenitor domains shown in A and B are: p0-p2, which generate sequentially V0-V2 interneurons, oligodendrocytes and astrocytes; pMN, which generates sequentially motor neurons (MNs), oligodendrocytes and astrocytes; p3, which generates V3 interneurons, oligodendrocytes and astrocytes. In the ventral spinal cord, oligodendrocyte progenitors (orange) are generated from the pMN and p3 domains and also from the p0 and p1 domains (Fogarty et al., 2005). Patterning proteins (see Box 2), including the homeodomain (HD) proteins Pax6 and Nkx2.2, and the basic helix-loop-helix (bHLH) protein Olig2, which establish the progenitor domains, are initially coexpressed with the inhibitory HLH proteins Id and Hes in uncommitted progenitor cells (grey). The induction of the proneural proteins Ngn2 and Mash1 in progenitors promotes neurogenesis (blue), whereas the induction of Mash1, the maintenance of Olig2 and Nkx2.2 and the downregulation of Pax6 promote oligodendrogenesis (orange), and the downregulation of patterning proteins and the maintenance of inhibitory HLH proteins promote astrogenesis (pink). pD, progenitor domain for dorsal neurons. See text and Sugimori et al. (Sugimori et al., 2007) for further details.

Box 2. Different classes of transcription factors specify neural cell fates

The differentiation of neural stem cells into specific classes of neurons and glia involves different categories of transcription factors that act at different stages and have different functions.

Patterning proteins: these transcription factors act early in neural development by subdividing the neural primordium into distinct domains and by providing progenitor cells in these domains with distinct positional identities. The homeodomain (HD) factors of the Pax, Nkx and Irx families and the basic helix-loop-helix (bHLH) protein Olig2 provide positional identity along the dorsoventral axis of the neural tube. Patterning proteins that provide positional identity along the anteroposterior axis include HD proteins of the Otx, Gbx, En and Hox families.

Progenitor proteins: these are fate determinants that are expressed in dividing neural progenitors, including patterning proteins and other factors expressed later in progenitor cells. They control various aspects of a cell's fate, such as its neurotransmission type or axon path and include LIM-HD proteins such as Lhx3.

Proneural proteins: these transcription factors of the bHLH family initiate programmes of neurogenesis in neural progenitors. Their expression leads to neuronal commitment, cell cycle exit and differentiation, and to Notch signalling activation in adjacent progenitors. The main mouse proneural proteins are Mash1 (Ascl1), neurogenin (Ngn; Neurog) 1-3 and Math1 (Atoh1).

Neuronal differentiation bHLH proteins: these include factors such as NeuroM (Neurod4) and NeuroD (Neurod1) that are induced by proneural proteins in postmitotic cells and contribute to the neuronal differentiation programme.

Neuronal HD proteins: these include proteins such as Hb9 (Mnx1), Mbh1 (Barhl2) and Brn3 (Pou4f1) that are only expressed in neural cells as they become postmitotic. They contribute to the subtype-specific differentiation programmes that are activated in postmitotic neuronal precursors.

Inhibitory HLH proteins: the HLH Id proteins and the bHLH Hes proteins have anti-neurogenic and anti-oligodendrogenic activity and act by inhibiting proneural bHLH proteins (Id and Hes proteins) and Olig2 (Id proteins) and by repressing proneural gene expression (Hes proteins).

transcription factors (see Box 2). The first part of the review discusses the role of progenitor proteins in regulating both the positional identity of progenitors and their fate. The second part discusses the role of another group of transcription factors, the proneural proteins, in regulating both the fate of progenitors and their differentiation. Finally, in the third part, I discuss the evidence that progenitor proteins and proneural proteins interact to specify cell fates, and that interactions between transcription factors of the HD and bHLH families, in particular, play a major role in determining where and when different classes of neurons and glia are produced during development. The review mostly focuses on mouse and chick, in which most of the information on the role of transcription factors in specification of neural cell fates has been obtained.

Coupling spatial patterning and fate specification

Soon after neural induction, neural cells acquire distinct characteristics and different fates depending on their positions along the anteroposterior (AP, or rostrocaudal, RC) and dorsoventral (DV) axes of the neural tube. This reflects the expression of different combinations of transcription factors that confer their positional identities on progenitors. These transcription factors also promote the generation of different cell types at different positions, and thus link the early step of neural tube patterning with the subsequent specification of different cell fates.

A number of progenitor proteins have been shown to subdivide the neural tube into distinct DV domains, in which neural cells have distinct identities and fates (Jessell, 2000). Among the best-studied are the HD proteins Pax6 and Nk2 homeobox 2 (Nkx2.2; also known as Nkx2-2) and the bHLH protein oligodendrocyte transcription factor 2 (Olig2). Nkx2.2 establishes the ventral-most progenitor cell domain in the spinal cord (called p3), which generates both neurons of the V3 interneuron class and oligodendrocytes (see Fig. 1). Olig2 is required for the generation of the pMN progenitor domain, which is situated just dorsal to p3 and generates, sequentially, neurons of the motor neuron class and oligodendrocytes, whereas Pax6 is involved in the establishment of the progenitor domains dorsal to pMN (called p0-p2) that produce different classes of interneurons followed by oligodendrocytes and astrocytes (Fig. 1).

Besides the establishment of progenitor cell domains, these patterning proteins (Box 2) also play a later role in the selection of the cell types produced by these progenitors. Hence, Pax6 expression in mouse spinal cord or forebrain progenitors induces the formation of neurons, whereas loss of *Pax6* results in reduced neurogenesis and in precocious formation of oligodendrocyte and astrocyte precursors (Hack et al., 2005; Heins et al., 2002; Sugimori et al., 2007). Loss of *Olig2* in the mouse spinal cord results in the absence of both motor neurons and oligodendrocytes, and overexpression experiments in chick embryos and in progenitor cultures have shown that Olig2 can promote either neurogenesis or oligodendrogenesis, depending on the developmental stage and the expression of other factors by progenitors (Mizuguchi et al., 2001; Sugimori et al., 2007; Sun, T. et al., 2001; Zhou and Anderson, 2002) (Fig. 1 and see below). Thus, patterning proteins are also involved in the generation of progeny from multipotent progenitor cells that have a restricted neuronal, astroglial or oligodendroglial fate.

Different classes of neurons are produced by progenitors in each domain of the neural primordium, and the choice of neuronal subtype often also involves patterning proteins. In the ventral spinal cord, Olig2 is involved in the specification of motor neuron identity, as shown by Olig2 misexpression in the chick neural tube, which results in the generation of motor neurons at ectopic positions (Mizuguchi et al., 2001; Novitch et al., 2001). Another striking example of a patterning transcription factor that controls sequential steps in neural development is Otx2, a HD protein that plays a central role in the initial specification of the anterior neural primordium and in its subsequent regionalisation into forebrain and midbrain territories (Martinez-Barbera et al., 2001). Otx2 also controls the identity of diverse neuronal populations generated much later in these anterior structures (Nishida et al., 2003; Puelles et al., 2006; Vernay et al., 2005).

How can the same transcription factors control so many different steps in neural development? This is one of the main questions that developmental neurobiologists are now facing, and there is, so far, no satisfactory answer. Many patterning proteins act by repressing the expression of other transcription factors, thereby restricting developmental programmes to particular progenitor populations (Lee et al., 2004; Muhr et al., 2001). Hence, the cross-repression of *Nkx2.2* and *Olig2* contributes to the establishment of distinct interneuron and motor neuron domains in the ventral spinal cord (Briscoe et al., 2000; Novitch et al., 2001), or the cross-repression of *Otx2* and another patterning gene, gastrulation brain homeobox 2 (*Gbx2*), establishes the boundary between the midbrain and hindbrain territories (Liu and Joyner, 2001). Patterning genes then activate region-specific differentiation programmes, involving the

expression of a large number of transcription factors that control particular aspects of a cell's identity, including other progenitor proteins, proneural bHLH proteins and neuronal HD proteins (see Boxes 1 and 2). Hence, Olig2 controls the motor neuron fate through the inhibition of *Nkx2.2* and through the activation of transcription factors that are involved in motor neuron specification, including the progenitor protein LIM homeobox protein 3 (Lim3; also known as Lhx3), the proneural factor Ngn2 and the neuronal HD protein motor neuron and pancreas homeobox 1 (Mnx1; also known as Hb9) (Lee et al., 2005; Novitch et al., 2001). Otx2 also regulates both proneural proteins, such as atonal homolog 1 (Atoh1 or Math1) in the midbrain and Mash1 in the thalamus, and neuronal HD proteins, such as Lim1 in the thalamus and cone-rod homeobox (Crx) in the retina (Nishida et al., 2003; Puelles et al., 2006; Vernay et al., 2005).

The functions of patterning proteins thus vary in different parts of the nervous system and at different times. A better understanding of these functions will require the systematic characterisation of their transcriptional targets, and the elucidation of the mechanisms that determine the stage- and cell type-specific expression of these targets.

Coupling fate specification and differentiation

Once a progenitor cell has acquired a particular neuronal or glial identity, the next step in the development of the cell lineage involves the arrest of cell divisions (in the case of most neurons but not glial cells) and the initiation of a programme of terminal differentiation (Fig. 2A). Different classes of transcription factors coordinately regulate cell fate specification and differentiation in neuronal and glial lineages.

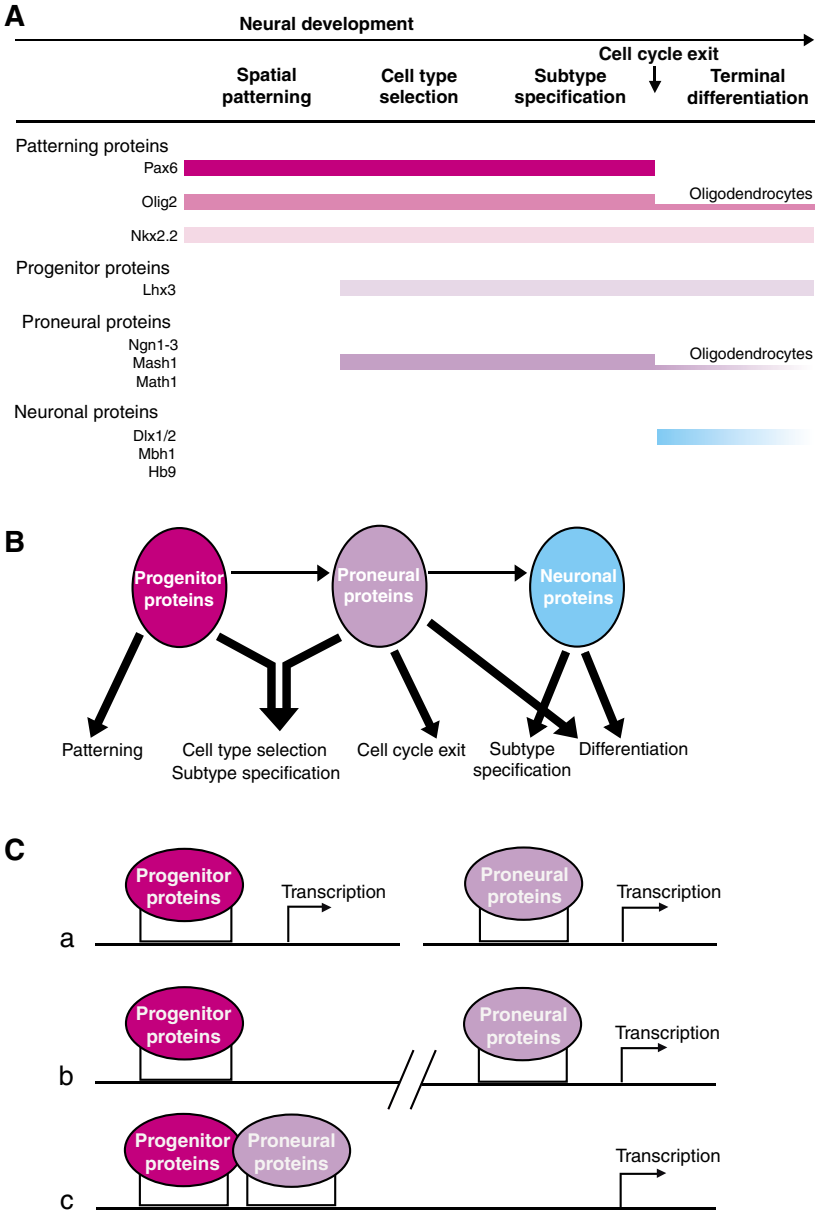

Fig. 2. Distinct and overlapping functions of homeodomain proteins and bHLH proteins in neural development. (**A**) Different families of transcription factors are expressed during sequential phases of neural development. Patterning proteins are expressed early in neural development. Pax6 is then downregulated when progenitor cells become postmitotic. Olig2 expression is maintained in oligodendrocyte progenitors but is downregulated in postmitotic neurons (thinner bar), while Nkx2.2 expression is maintained in both neurons and oligodendrocyte progenitors. Progenitor proteins, such as Lhx3, are induced in mitotic progenitors after the onset of patterning protein expression and remain expressed in postmitotic neurons. Proneural protein expression is induced in subsets of progenitor cells after spatial patterning. Progenitors that express proneural proteins undergo cell type selection and initiate neuronal subtype specification, rapidly followed by cell cycle exit. Proneural protein expression is then switched off in most newborn neurons. Mash1 expression is maintained transiently in oligodendrocyte progenitors (represented by a thinner bar). Neuronal protein expression is induced in progenitor cells following their cell cycle exit. (**B**) The differentiation of multipotent progenitor cells into specific classes of postmitotic neurons and glia involves transcriptional cascades in which patterning proteins induce proneural proteins, which in turn induce, often directly, neuronal homeodomain proteins (thin arrows). These factors regulate different phases of neural development (thick arrows; see text). Subtype specification is initiated in dividing progenitors coordinately by progenitor proteins and proneural proteins and further promoted by neuronal proteins after cell cycle exit. (**C**) The molecular mechanisms that underlie the synergistic activity of patterning/progenitor proteins and proneural proteins are largely unknown and could include: (a) indirect interactions through regulation of distinct target genes (e.g. Olig2 and Ngn2) (Mizuguchi et al., 2001; Novitch et al., 2001); (b) binding to distinct sites in the promoter of a common target gene and synergistically activating target gene transcription [e.g. Isl1, Lhx3, Ngn2 and NeuroM (Neurod4)] (Lee and Pfaff, 2003); (c) Cooperative binding of the progenitor protein and the proneural protein to adjacent sites in the promoter of a common target [e.g. Mash1 and Brn2 (also known as Pou3f2)] (Castro et al., 2006). White boxes represent transcription factor binding sites.

Neurogenesis: proneural factors integrate spatial and temporal cues

Transcription factors of the bHLH family play a central role in the differentiation of neural progenitors into neurons. The expression of proneural bHLH proteins (see Box 2), which in the mouse include Mash1, Ngn1-3 and Math1, is both necessary and sufficient to promote the generation of differentiated neurons from undifferentiated progenitor cells (reviewed by Bertrand et al., 2002; Ross et al., 2003). Proneural proteins control the commitment of multipotent progenitors to a neuronal fate (Nieto et al., 2001; Sun, Y. et al., 2001; Tomita et al., 2000), but also influence the particular neuronal subtypes produced in a region-specific manner (reviewed by Bertrand et al., 2002; Brunet and Ghysen, 1999). Thus, the expression of Mash1 or Ngn2 in forebrain progenitor cells promotes the generation of GABAergic and glutamatergic neurons, respectively (Berninger et al., 2007; Parras et al., 2002), whereas expression of Ngn2 in spinal cord progenitors is required for motor neuron identity (Lee and Pfaff, 2003; Mizuguchi et al., 2001; Novitch et al., 2001; Scardigli et al., 2003). Proneural genes also control later aspects of the neurogenic process, including the arrest of progenitor divisions (Farah et al., 2000; Mizuguchi et al., 2001; Nakada et al., 2004), as well as the subsequent migration of newborn neurons out of the progenitor zone of the neural tube and their terminal differentiation (Berninger et al., 2007; Hand et al., 2005; Nakada et al., 2004; Seibt et al., 2003).

This central role of proneural proteins in neurogenesis is likely to reflect the regulation of numerous genes that control the different steps in this process (Fig. 3). In order to promote neurogenesis, proneural proteins must first inhibit the expression of the SoxB1 genes (*Sox1*, *Sox2* and *Sox3*), which promote the self-renewal and multipotency of neural progenitors, and also block their activity through the activation of an antagonistic Sox gene, *Sox21* (Bylund et al., 2003; Sandberg et al., 2005). The exact mechanism by which proneural proteins commit progenitors to a neuronal fate is not known. However, by analogy with other developmental systems in which mechanisms of cell fate specification have been analysed in depth, such as specification of the endomesoderm in sea urchin embryos or DV patterning in *Drosophila* embryos (Levine and Davidson, 2005), this step is likely to involve the activation of numerous downstream transcription factors, the expression of which is stabilised through the formation of a regulatory network, which in turn promotes the differentiation of committed neuronal progenitors. Support for this model comes from studies in the developing retina, cerebral cortex and spinal cord, where Ngn2 regulates the expression of multiple genes that encode transcription factors of the bHLH, T-box and Sox families, which have been implicated in neuronal differentiation (Bergsland et al., 2006; Kanekar et al., 1997; Matter-Sadzinski et al., 2005; Schuurmans et al., 2004) (Fig. 3). The neuronal commitment of multipotent progenitors brought about by proneural proteins also involves the inhibition of astrocyte differentiation by distinct mechanisms, including the sequestration of a gliogenic transcriptional complex away from glial promoters, and inhibition of the expression of components of the gliogenic JAK-STAT signalling pathway (He et al., 2005; Sun, Y. et al., 2001).

The specification of neuronal identities by proneural proteins involves the regulation of neuronal HD proteins (Box 2, Fig. 1 and Fig. 2B). These factors have diverse roles in the specification of neuronal identities, and their mutation result in a range of phenotypes. A mutation in even skipped homeotic gene 1 (*Evx1*)

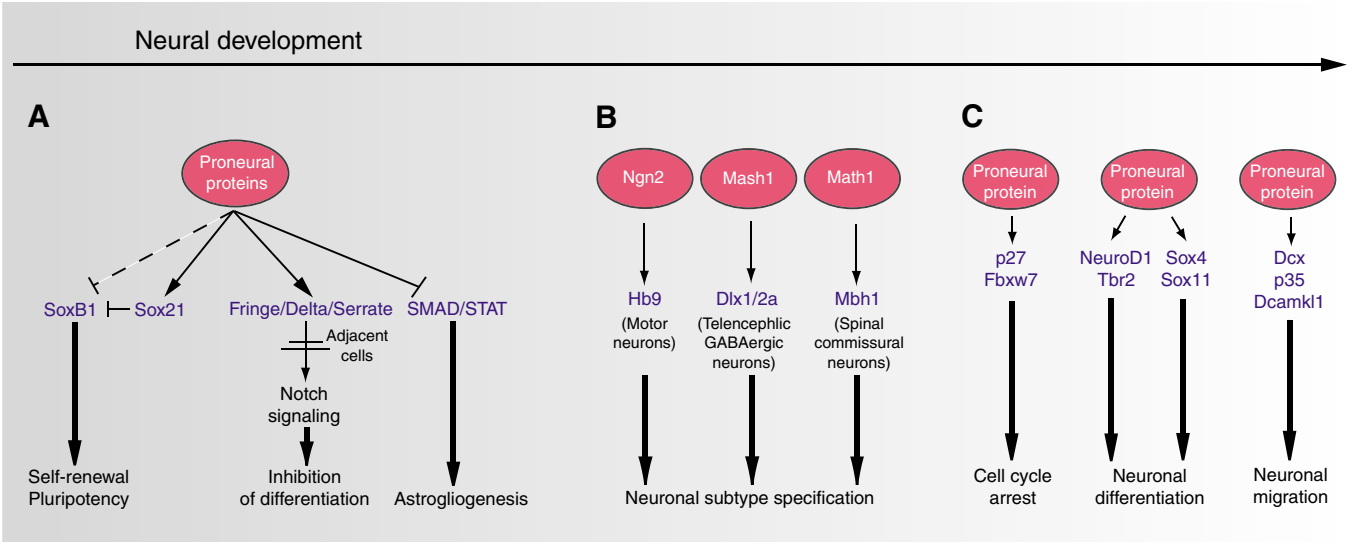

Fig. 3. Proneural proteins control multiple cellular processes and activate multiple target genes during neurogenesis. Proneural proteins control many aspects of neurogenesis and some of their targets have been identified. (**A**) Proneural proteins suppress the neural stem cell programme by interfering with the activity of SoxB1 genes (see text); they select neuronal progenitors by directly activating Notch ligands and suppress astrogenesis by interfering with SMAD and STAT signalling (see text). (**B**) Different proneural proteins specify different neuronal subtype identities by directly activating HD protein-encoding genes, such as *Hb9* (Lee and Pfaff, 2003), *Dlx1/2* (Poitras et al., 2007) and *Mbh1* (also known as Barhl2) (Saba et al., 2005). (**C**) Proneural proteins also induce the expression of transcription factors that promote neuronal differentiation, including bHLH proteins, T-box proteins and Sox proteins (see text). In addition to regulating transcription factors involved in cell fate specification, proneural proteins also regulate genes that control later steps in the neurogenic programme, such as cell cycle arrest, neuronal differentiation and migration (Farah et al., 2000; Castro et al., 2006; Ge et al., 2006). Some of these genes (e.g. *Fbxw7* and doublecortin-like kinase) are regulated cooperatively by the proneural protein Mash1 and the POU HD proteins Brn1 (also known as Pou3f3) and Brn2 (Castro et al., 2006). Tbr2 (Eomes); Dcamkl1 (Dclk1).

leads to a complete switch in identity of V0 interneurons into V1 interneurons (Moran-Rivard et al., 2001), whereas mutations in distal-less homeobox 1 and 2 (*Dlx1* and *Dlx2*), two genes directly induced by Mash1, results in a block in the differentiation of striatal neurons (Anderson et al., 1997; Yun et al., 2002; Poitras et al., 2007). Mice mutant for *Hb9*, a direct target of Ngn2, present a more subtle axon pathfinding defect in motor neurons (Arber et al., 1999; Lee and Pfaff, 2001; Thaler et al., 1999). Importantly, the same proneural proteins specify different types of neurons in different regions of the nervous system. For example, Ngn2 promotes the generation of motor neurons in the ventral spinal cord and that of cortical pyramidal neurons in the dorsal telencephalon (Parras et al., 2002; Lee and Pfaff, 2003; Schuurmans et al., 2004). This reflects the regulation by proneural proteins of different sets of target genes in different regions, possibly owing to the differential expression of interacting transcription factors (see Lee and Pfaff, 2003).

Proneural proteins also influence the fate of progenitor cells indirectly by determining the timing of their last division. Different classes of neurons are produced at different times in all regions of the developing nervous system. This is in part owing to temporal changes in the composition of the signalling environment that directs the fate of progenitor cells (Cepko et al., 1996; Edlund and Jessell, 1999; McConnell, 1995; Ohnuma and Harris, 2003; Sockanathan and Jessell, 1998). By controlling the timing of cell cycle exit, proneural proteins determine the nature of the inductive signals that progenitors are exposed to at the time their fate is fixed, i.e. during their final division. This role in controlling the birth date of neurons reflects two particular properties of proneural proteins. First, these factors have a unique role in promoting cell cycle exit, an activity that is not shared by progenitor proteins such as Olig2 or Pax6. Second, proneural proteins are only transiently expressed by neural progenitors around the time of their final division (e.g. Britz et al., 2006; Miyata et al., 2004), in contrast to patterning proteins, which are expressed more uniformly by progenitor cells (Fig. 2A). Whereas the spatial pattern of proneural gene expression is likely to be controlled by patterning proteins (e.g. Scardigli et al., 2003; Zhou and Anderson, 2002) (see Fig. 2B) and by cross-repression between proneural genes (Fode et al., 2000; Gowan et al., 2001), their timing of expression is controlled by extrinsic signalling pathways that regulate the differentiation of progenitor cells, either positively [e.g. Wnt signalling (Hirabayashi et al., 2004)] or negatively [e.g. Notch signalling (Kageyama et al., 2005)]. Thus, proneural factors integrate spatial and temporal cues received from patterning proteins and from neurogenic and anti-neurogenic signals, respectively. They convert this information into neuronal subtype-specific differentiation programmes by activating neuronal HD determinants and by selecting the timing of progenitor cell division arrest.

Gliogenesis: choosing between astroglial and oligodendroglial fates

The sequential generation of neurons and glia is a general feature of the developing nervous system in vertebrates. This neuron-to-glia switch is controlled by multiple mechanisms, which include extrinsic signals, transcription factors and modifications of histones and DNA (reviewed by Rowitch, 2004; Guillemot, 2007; Miller and Gauthier, 2007). At the transcriptional level, a key step in the switch of neural progenitors to gliogenesis is the induction of Sox9 and NFIA, two proteins that promote both astroglial and oligodendroglial fates. These factors also inhibit neurogenesis and thus contribute to coordinating the onset of gliogenesis with the arrest of neurogenesis. The gliogenic function of the HMG-box transcription factor Sox9 was revealed by analyzing *Sox9*-null mice, which produce fewer oligodendrocyte precursors and astrocytes and show a transient increase in motor neuron numbers in the ventral spinal cord (Stolt et al., 2003). Sox9 is subsequently expressed in oligodendrocyte precursors, along with the related proteins Sox8 and Sox10, and the three factors play redundant functions in oligodendrocyte differentiation (Wegner and Stolt, 2005). The gliogenic activity of the CCAAT box element-binding transcription factor NFIA has been demonstrated by silencing the *Nfia* gene in the chick spinal cord, which prevents the generation of both astrocyte and oligodendrocyte precursors and leads to premature neuronal differentiation. Conversely, misexpressing *Nfia* in the chick spinal cord leads to precocious expression of glial markers in the ventricular zone and to the precocious and excessive emigration of astrocyte precursors. NFIA and NFIB are subsequently required for terminal astrocyte differentiation (Deneen et al., 2006). Sox9 and NFIA therefore have a common role in the specification of both oligodendroglial and astroglial progenitors, but the two factors later have divergent functions in the differentiation of oligodendrocytes and astrocytes, respectively. The nature of the mechanisms that underlie the activity of these two gliogenic factors, and whether they act independently or in the same pathway, are not known.

The choice between the alternative astroglial and oligodendroglial fates is controlled by different transcription factors (reviewed by Rowitch, 2004). The progenitor proteins Olig2 and Nkx2.2, which specify progenitor identities and neuronal fates in the ventral spinal cord (see above), later promote oligodendrogenesis and inhibit astrogenesis in the same progenitor domains. Olig2 is required to generate oligodendrocyte precursors and to inhibit ectopic astrocyte production in the mouse spinal cord, whereas coexpression of Olig2 and Nkx2.2 in the chick spinal cord is sufficient to induce oligodendrocyte precursors at ectopic locations (Lu et al., 2002; Zhou and Anderson, 2002; Zhou et al., 2001). The proneural protein Mash1, an essential regulator of neurogenesis in many parts of the nervous system, is also involved in the specification of a subset of oligodendrocyte precursors in the spinal cord and forebrain (Parras et al., 2004; Parras et al., 2007; Sugimori et al., 2007). There is therefore a striking convergence between the transcriptional programme that controls oligodendrogenesis and those regulating neurogenesis in the ventral neural tube. Olig2, Nkx2.2 and Mash1 remain expressed in oligodendrocyte precursors and control their differentiation into myelinated oligodendrocytes, suggesting that the same transcriptional mechanisms underlie cell type commitment and terminal differentiation in the oligodendroglial lineage (Liu et al., 2007; Qi et al., 2001) (M. Nakafuku, personal communication).

The bHLH protein SCL (also known as Tal1), which is expressed in a restricted domain of the ventral spinal cord, has the opposite role of promoting astrogenesis by inducing astrocyte precursor-specific genes, and inhibiting oligodendrogenesis via repression of *Olig2* (Muroyama et al., 2005). Other transcription factors known to promote astrocyte development act downstream of gliogenic signalling pathways, such as STATs, SMADs and RBP-Jk (also known as Rbpj), which are activated by the cytokine/JAK, BMP and Notch pathways, respectively (reviewed by Guillemot, 2007; Miller and Gauthier, 2007). However, these factors have been shown to regulate the expression of late astrocytic differentiation markers, such as glial fibrillary acid protein (Gfap), and it is unclear whether they also act in the specification of the astrocytic fate, perhaps in combination with other factors, such as NFIA, or only in terminal astrocyte differentiation (Miller and Gauthier, 2007).

It is clear from the foregoing discussion that many transcription factors involved in cell fate specification in the nervous system promote multiple fates, suggesting that transcription factors work in a combinatorial manner to instruct progenitor cells to generate a particular class of neurons or glia at a given time and location. In the next section, I discuss recent evidence that transcription factors indeed act in combinations, in particular for the specification of the three primary neural cell types in the spinal cord, and for the specification of the different neuronal subtypes of the retina.

Transcription factor codes specifying cell fates

Specifying primary neural fates

The idea that different combinations of patterning proteins and proneural proteins determine cell fates in the spinal cord was first put forward by David Anderson and colleagues to explain their finding that *Olig2* mutant mice lack both oligodendrocytes and motor neurons (Zhou and Anderson, 2002). They proposed a model whereby a simple combinatorial code composed of Olig and Ngn proteins determines the production of neurons, oligodendrocytes or astrocytes by multipotent progenitor cells in the ventral spinal cord. During the neurogenic period, the combination of Olig2 and Ngn2 in the pMN progenitor domain would specify the motor neuron fate, whereas expression of Ngn2 alone in the p2 domain would promote an interneuron fate. Following the downregulation of Ngn2 expression at the onset of the gliogenic period, expression of Olig2 alone in the pMN domain would lead to the generation of oligodendrocytes, whereas the absence of either Olig2 or Ngn2 in p2 would result in the production of astrocytes (Zhou and Anderson, 2002) (see also Novitch et al., 2001; Mizuguchi et al., 2001). This model was recently extended and updated by Sugimori et al. (Sugimori et al., 2007) to include other patterning and proneural proteins as well as inhibitory HLH factors (see Box 2) that are expressed in the ventral spinal cord. To directly test the hypothesis that patterning, proneural and inhibitory HLH proteins act combinatorially, the authors systematically expressed different gene pairs in spinal cord-derived stem cell cultures and then assessed the cell types produced (Sugimori et al., 2007). These experiments showed that the patterning factors Pax6, Nkx2.2 and Olig2 influence the fate of progenitors by modulating the activity of the proneural neurogenin and Mash1 proteins and of the inhibitory HLH proteins Hes and Id (Fig. 4). For example, whereas expression of Mash1 alone in stem cells generated clones that contained both neurons and oligodendrocytes, the coexpression of Mash1 and Pax6 generated clones that contained only neurons, whereas coexpression of Mash1 and Olig2 generated clones that contained only oligodendrocytes. Also, whereas expression of Id1 or Hes1 alone in neural stem cell cultures promoted astrogenesis, coexpression of these proteins with any of the patterning proteins tested maintained progenitors in an undifferentiated state instead. These results obtained in culture were corroborated by a detailed analysis of the expression of patterning and HLH proteins in the developing rat ventral spinal cord (Sugimori et al., 2007). For example, the beginning and end of the neurogenic period in the Nkx2.2^{+} and Olig2^{+} progenitor domains is paralleled by the onset and termination of neurogenin protein expression, whereas the generation of oligodendrocyte precursors in the pMN domain coincides with the onset of Mash1 expression, and the beginning of astrogenesis is marked by the termination of patterning protein expression and the maintenance of Id and Hes expression in progenitors (Fig. 1).

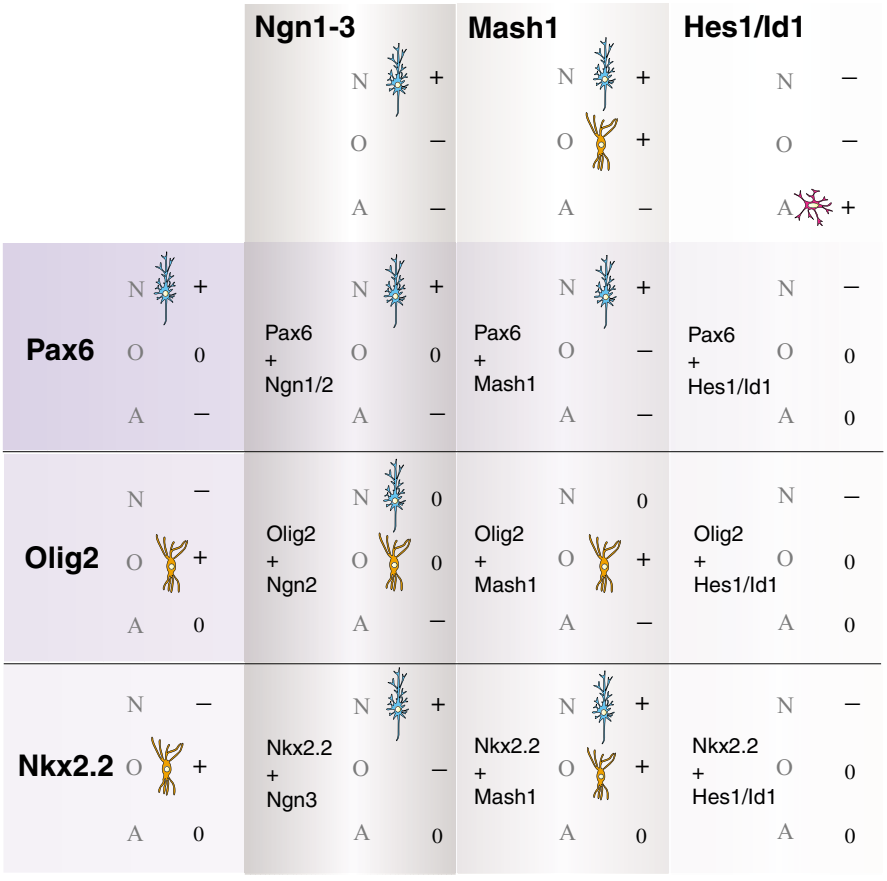

Fig. 4. The combinatorial activity of patterning proteins and proneural proteins promotes neural cell type commitment. The combinatorial activities of patterning proteins (vertical axis) and of proneural proteins and inhibitory HLH proteins (horizontal axis) in the commitment of progenitor cells to neuronal (N), oligodendroglial (O) or astroglial (A) fates are shown as entries on a matrix. These results were obtained by overexpressing combinations of factors in neural stem cell population cultures derived from rat embryonic spinal cord. The effect of particular transcription factor combinations on the generation of the primary neural cell types is represented by '+' for significant induction, '–' for significant repression and '0' for no activity. See text and Sugimori et al. (Sugimori et al., 2007) for details.

The study by Sugimori et al. (Sugimori et al., 2007) thus provides evidence that cooperation between patterning, proneural and inhibitory HLH proteins establishes a molecular code that determines both the spatial and the temporal patterns of neurogenesis and gliogenesis. Proneural proteins determine the timing of the neuron-to-glia switch, as shown by the analysis of various mutant mice (Sugimori et al., 2007), as well as the rate of cell cycle exit and differentiation (see above), and thus control the numbers of neurons and glial cells produced by each progenitor domain. Patterning proteins also contribute to the timing of the generation of the different neuronal and glial populations, and their final size, by attenuating the neurogenic activity of Ngn2, by modulating the neurogenic and oligodendrogenic activities of Mash1, and by suppressing the astrogenic activity of the Hes and Id proteins (Sugimori et al., 2007). Moreover, patterning proteins determine the spatial patterns of neurogenesis and gliogenesis, by establishing distinct profiles of proneural gene expression in different progenitor domains (Scardigli et al., 2003; Zhou and Anderson, 2002) and by modulating the neurogenic or gliogenic activity of proneural proteins in each domain (Sugimori et al., 2007). Other factors are likely to be involved as well, such as the Dlx1 and Dlx2 proteins, which promote neurogenesis and inhibit oligodendrogenesis via repression of Olig2, in progenitors of the mouse forebrain (Petryniac et al., 2007).

Specifying neuronal subtypes

Synergies between progenitor proteins, mostly of the HD family, and bHLH proteins have also been implicated in the specification of neuronal subtype identities in different model systems, particularly in the mouse and *Xenopus* retina (see Hatakeyama and Kageyama, 2004; Wang and Harris, 2005). Multiple bHLH proteins are expressed in dividing retinal progenitor cells and some of them are maintained in particular retinal neuron populations. Thus, both Mash1 and another bHLH protein, Math3 (also known as Neurod4), are expressed transiently by differentiating bipolar cells, implicating these factors in the specification of this neuronal subtype. There is indeed a reduction in the number of bipolar cells in *Mash1* single-mutant mouse embryos and an almost complete loss in *Mash1*; *Math3* double-mutant embryos with a compensatory increase in the number of Müller glial cells (Tomita et al., 2000). However, misexpression of Mash1 or Math3 in retinal progenitors results in the production of photoreceptors at the expense of Müller glial cells, suggesting that these bHLH proteins are not sufficient to specify bipolar cells (Hatakeyama et al., 2001). The HD protein Chx10 (also known as Vsx2) is also expressed in bipolar cells, and a mutation in mouse *Chx10* results in a complete loss of these neurons (Hatakeyama and Kageyama, 2004). However, misexpression of Chx10 produces Müller glial cells or undifferentiated cells in the inner nuclear layer of the retina, indicating again that Chx10 expression is not sufficient to promote the bipolar cell identity. By contrast, misexpression of Chx10 together with Mash1 or Math3 promotes the generation of bipolar cells (Hatakeyama et al., 2001). Thus, the bHLH proteins Mash1 and Math3 alone can promote neurogenesis, but they must interact with the HD protein Chx10 to specify the particular bipolar subtype. Reciprocally, Chx10 alone provides retinal cells with a laminar (inner nuclear layer) identity, but it must act with Mash1 or Math3 to specify a particular neuronal subtype. Loss-of-function and misexpression studies in mouse with other transcription factors expressed by subsets of retinal neurons lead Ryoichiro Kageyama and colleagues to propose a generalisation of this model in which a transcription factor code that involves combinations of bHLH and HD proteins controls the specification of the different classes of retinal cells (Hatakeyama and Kageyama, 2004) (Fig. 5). In this model, HD proteins determine the laminar position of retinal cells, whereas bHLH proteins determine their time of birth, which is tightly correlated with the identity of the cells produced (Cepko et al., 1996). bHLH proteins may thus contribute to cell fate specification by determining the precise timing of cell cycle exit of retinal progenitors (Ohnuma and Harris, 2003), as well as by directly activating the expression of cell fate determinants, as discussed above in the context of the spinal cord.

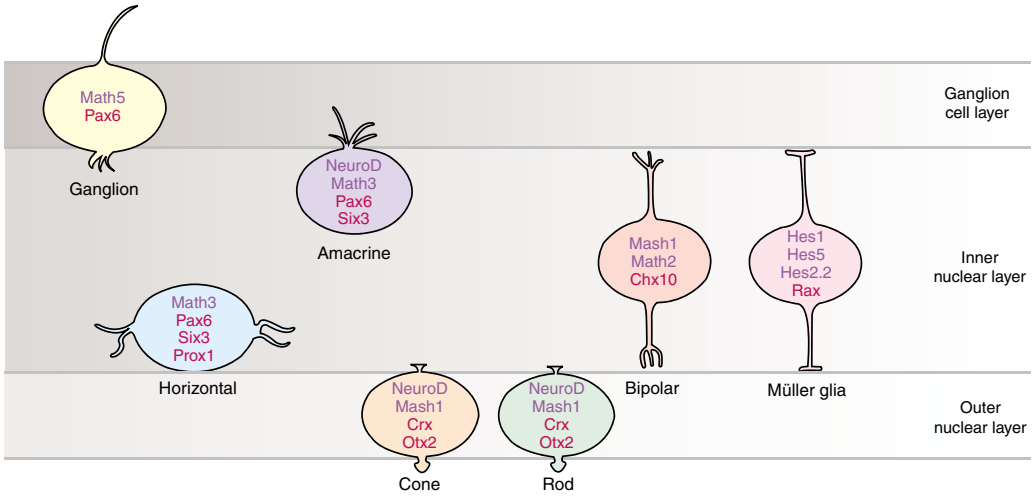

Fig. 5. Model of neuronal subtype specification in the retina by combinations of HD and bHLH proteins. Different combinations of bHLH proteins (purple) and HD proteins (red) are expressed by the different classes of neurons and glia in the retina. From mouse mutant analyses and from coexpression of HD proteins and bHLH proteins in mouse retinal explants, Hatakeyama and Kageyama have proposed that the combinatorial activity of different HD and bHLH proteins determines the fate of retinal cells. See text and Hatakeyama and Kageyama (Hatakeyama and Kageyama, 2004) for details. NeuroD (Neurod1).

Conclusion

This review has focused on a few well-studied examples, and one must ask the question of how general is the role of progenitor protein-proneural protein interactions in neural cell fate specification. The fact that such interactions have been implicated in the selection of the primary neural fates and in the specification of neuronal phenotypes in regions as diverse as the spinal cord, the retina and the forebrain, suggests that this is a fundamental mechanism that operates throughout the nervous system, although this remains to be established.

The mechanisms that underlie the cooperative activity of HD and bHLH proteins in the different systems discussed above remain largely unknown (Fig. 2C). An attractive model is that these factors cooperate to regulate common target genes that are themselves involved in fate specification. In one of the few examples that have been well documented, the cooperation of Ngn2 with the LIM-HD proteins Lhx3 and Isl1 to specify motor neurons involves the synergistic activation by these factors of the *Hb9* gene (Lee and Pfaff, 2003). There are other examples in the nervous system and in other tissues of bHLH proteins and HD proteins that regulate gene expression through cooperative DNA binding or synergistic transcriptional activation, involving in some cases physical interactions between members of these two families (Ohneda et al., 2000; Poulin et al., 2000; Sun et al., 2003; Berkes et al., 2004; Castro et al., 2006). Similar mechanisms might operate for the specification of neural cell fates. Current efforts to characterise the molecular pathways controlled by transcription factors in the developing nervous system will eventually elucidate the mechanisms that underlie their combinatorial activities, including the synergistic regulation of common target genes and perhaps also less direct mechanisms.

Ten years after David Anderson and Yuh Nung Jan discussed the respective contributions of bHLH proteins and HD proteins in the determination of the neuronal phenotype (Anderson and Jan, 1997), one can appreciate how much has been learned in the interval about the functions of individual transcription factors, but also how much remains to be learnt about how they perform these functions.

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