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REVIEW ARTICLE

Cellular diversity in the developing nervous system: a temporal view from *Drosophila*

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SUMMARY

This article considers the evidence for temporal transitions in CNS neural precursor cell gene expression during development. In *Drosophila*, five prospective competence states have so far been identified, characterized by the successive expression of Hb→Kr→Pdm→Cas→Gh in many, but not all, neuroblasts. In each temporal window of transcription factor expression, the neuroblast generates sublineages whose temporal identity is determined by the competence state of the neuroblast at the time of birth of the sublineage. Although similar regulatory programs have not yet been identified in mammals, candidate regulatory genes have been identified. Further investigation of the genetic programs that guide both invertebrate and vertebrate neural precursor cell lineage development will ultimately lead to an understanding of the molecular events that control neuronal diversity.

Key words: Drosophila, Nervous system, Neural precursor cell

INTRODUCTION

During nervous system development, individual neural precursor cells (NPCs) generate multiple uniquely fated neural subtypes. Although the regulatory networks that provide spatial identity to NPCs have been investigated, little is know about the genetic circuitry that regulates the generation of different cellular types within individual NPC lineages. The molecular mechanisms that underlie the choreographed appearance of these uniquely fated cells are a subject of great interest. Recent studies on vertebrate NPC lineages suggest that many or perhaps all NPCs pass through successive developmental competence states during the generation of their neural offspring (Desai and McConnell, 2000; Qian et al., 2000; Harris, 2001; Livesey and Cepko, 2001). Likewise, individual NPCs of the *Drosophila* CNS, termed neuroblasts (NBs), have been shown to undergo temporally ordered changes in cell-identity gene expression programs (Kambadur et al., 1998; Brody and Odenwald, 2000; Isshiki et al., 2001; Novotny et al., 2002). Collectively, these recent studies suggest that once NPCs initiate lineage development, they become temporally restricted in the types of neural cells that they can generate. Deciphering the regulatory inputs that dictate these orchestrated decisions is central to our understanding of the molecular events that control neural development.

The fundamental issues posed by the ability of NPCs to generate multiple neural subtypes are the same for both vertebrates and invertebrates: what are the developmental prerequisites affecting the potential of the NPC to generate uniquely fated progeny? Is the birth order of different neural subtypes fixed and to what extent is the NPC responsive to external cues? What are the intrinsic regulatory circuits that control the birth order of the uniquely fated cells, and are the regulatory mechanisms conserved? Understanding the cellular and molecular aspects of these temporally sensitive events has far-reaching consequences for both developmental neurobiology and for the potential efficacy of therapeutic stem cell transplantation. This article examines what is currently known about the temporal transitions in NPC gene expression during *Drosophila* CNS development and the relationship of these transitions to the changing developmental competence of NBs during lineage formation. The invertebrate model system will be compared with observations made with vertebrates, first analyzing the developmental prerequisites for initiating neural lineage development, then examining the evidence for temporally ordered transitions in NB gene expression, and finally addressing cellular mechanisms for assuring the inheritance by neural progeny of cell fate decisions made in the NB.

DEVELOPMENTAL PREREQUISITES FOR INITIATING NEURAL LINEAGE DEVELOPMENT

Interrelated studies that address the timing and location of NB identity decisions are examined in this article. This information is important in assessing whether the mechanisms that control NPC commitment are conserved between invertebrates and

vertebrates. Collectively, the experiments from *Drosophila* reveal that NB positional identity precedes the development of lineages, but whether this is true in vertebrate model systems remains unclear.

During *Drosophila* gastrulation, subsets of neuroectoderm cells are singled out to become NBs by a complex set of integrated regulatory cascades that employ both extrinsic signaling molecules and intrinsic genetic programs (Campos-Ortega, 1995; Skeath, 1999). During this process, or shortly thereafter, these cells physically enlarge, more than doubling their cell diameters, and alter their cell-cell adhesion properties with respect to their neighboring ectodermal cells. Coincident with these morphological changes, NBs exit the neuroectoderm, via a process known as delamination, and move inwards to reside in a subectodermal proliferative zone (PZ). Morphological studies have shown that the NBs exit the neuroectoderm in staggered waves (Hartenstein and Campos-Ortega, 1984), with early NBs entering the PZ at stage 7 of development and late NBs delaminating from the neuroectoderm at stage 10 (3.5 and 5 hours respectively). Shortly after their arrival to the PZ, NBs initiate a series of asymmetrical self-renewing cell divisions, with each mitotic event generating a smaller ganglion mother cell (GMC). GMCs then divide once to yield either neurons or glia (reviewed by Fuerstenberg et al., 1998).

Transplantation experiments

Both homo- and heterotypic cell transplantation studies performed before neural lineage development reveal that NB positional identity is established before their entry into the PZ. At the beginning of gastrulation, along the anterior-to-posterior (AP) axis of the neuroectodermal region, NBs are irreversibly committed with regard to their segmental identities. For example, at this developmental stage, cells transplanted from the thoracic into the abdominal neuroectoderm retain their thoracic identities and vice versa (Prokop and Technau, 1994; Prokop et al., 1998). Along the dorsal-to-ventral (DV) axis (often referred to as the lateral-to-medial axis), cell fates at ventral sites (adjacent to the ventral midline) of the neuroectoderm are also firmly committed at stage 7 (which is slightly more than 3 hours of zygotic development). This commitment is evidenced by observations that ventral cells retain their fate upon transplantation to more dorsal sites (Udolph et al., 1995). However, during this stage, neuroectodermal cells at more dorsal sites are still able to change their fate when exposed to ectopic ventral positions (Udolph et al., 1998).

Heterochronic transplantation experiments have shown that cell fate plasticity also exists over time. The developmental potential of early delaminating NBs (stage 7) versus the potential of those that initiate lineage development late (stage 10) has been assessed (Berger et al., 2001). NBs remain competent to interpret extrinsic signals properly and can adjust their temporal fates in both directions, i.e. from late to early and from early to late NB identities. This study also revealed that late delaminating NBs do not require a cell division cycle to segregate from the neuroectoderm nor do they depend on a previous division to undergo lineage development. Additionally, these transplantation studies indicated that surrounding tissue can influence proliferation of individual neuroectodermal progenitors.

Mutational and genetic analyses

Both loss- and gain-of-function studies have identified two *Drosophila* regulatory networks that encompass the proneural and neurogenic genes that are required to establish NB fates (reviewed by Campos-Ortega, 1995; Arendt and Nübler-Jung, 1999). Additional regulatory networks, which function both in the AP and DV axes, determine the positional identity of each NB and consequently the fate of neurons and glia generated by the NB (reviewed by Bhat, 1999; Skeath, 1999). Specifically, segment polarity genes determine positional identity in the AP axis of the ventral cord and homeobox genes determine positional identity in the DV axis. As each NB generates different repertoires of neurons, and/or glia (Bossing et al., 1996; Schmidt et al., 1997; Schmid et al., 1999), it is likely that the combinatorial effects of these two gene systems function to regulate a variety of other gene networks to specify uniquely the full range of cell types generated by each NB. It is clear that many NB identity genes initiate expression in the neuroectoderm and are still expressed in NBs during lineage development (Bhat, 1999; Skeath, 1999). It would seem that their continued expression is required to maintain NB identity throughout lineage development.

Commitment of the vertebrate NPC

Regulatory networks similar to those found in *Drosophila* function in vertebrates to control positional identity (reviewed by Arendt and Nübler-Jung, 1999; Jessell, 2000). Gene expression studies suggest that the process of establishing regional identity within NPCs occurs before lineage development (reviewed by Temple, 2001). In addition, mutational analysis of homeobox genes also shows that NPC positional identity within the murine cortex is established before lineage development. For example, mice that bear only one functional copy of the *Otx2* homeobox gene show dramatic brain malformations, implicating *Otx2* in the early specification of positional identity in regions of the forebrain, midbrain and rostral hindbrain (Acampora et al., 1997). Altered cell fate patterning in *Otx1;Otx2* double mutants is detectable as early as 8.5 days of development before the onset of widespread lineage development.

Extrinsic cues, as evidenced by in vivo transplantation studies and in vitro cell culture experiments, play an important role in establishing specific cell fates within vertebrate NPC lineages (reviewed by Doe et al., 1998; Anderson, 2001). Transplantation studies in zebrafish, using cells from the hindbrain, indicate a continued plasticity in cell fate (Schilling et al., 2001). However, responsiveness to non-cell autonomous signaling decreases with increasing age of transplanted cells. These and other studies indicate that the extrinsic factors act to promote the choice of one fate at the expense of others, rather than by selectively supporting the survival or proliferation of lineage-committed progenitors. For example, BMP2/4 promotes neuronal differentiation of cortical ventricular zone precursors (Li et al., 1998). The sequential actions of two different BMP receptors appear to control this temporal switch (Panchision et al., 2001). By contrast, BMPs inhibit neuronal differentiation of adult subventricular zone (SVZ) precursors, owing to their promotion of astrocyte differentiation (Lim et al., 2000). In the vertebrate retina, even postmitotic cells can be respecified by experimental manipulations. Specifically, CNTF treatment shifts rod

photoreceptor cells to a bipolar cell fate (Ezzeddine et al., 1997). Embryonic retinal progenitor cells, when cultured in the presence of excess postnatal retinal cells (Belliveau and Cepko, 1999), can be respecified to produce rod cells, but are not able to generate cell fates restricted to postnatal ages (cone cells). Thus, although extrinsic cues can alter the fate of the neural subtypes produced by progenitor cells, they are limited by the cell fate repertoire of the progenitor pool. It is not yet clear whether the apparent plasticity seen in transplantation and culture studies is due to different subsets of NPCs, or whether individual, spatially restricted NPCs can indeed undergo fate changes. If precursor cells are heterogeneous with regard to their developmental commitment, the apparent plasticity may be the result of distinct lineages 'selected' in different environments.

Arguing against extended periods of developmental plasticity in vertebrates, other studies suggest that cell fate identity in terms of regional identity might occur relatively early, prior to lineage development. For example, mouse mid-hindbrain progenitors from embryonic day 13.5 are already incapable of adopting a forebrain phenotype after grafting into the telencephalon (Olsson et al., 1997). Additional evidence for early commitment of spatial identity comes from the study of the regulation of the intermediate filament gene, nestin (Yaworsky and Kappen, 1999). Nestin is an early marker for most, but not all, stem cells in the mammalian central nervous system (Dahlstrand et al., 1995). Distinct CNS progenitor-specific enhancers have been identified that regulate expression of this gene in different regions of the developing CNS. This study implies spatial regulation of transcriptional repertoires in NPCs. Rather than continued plasticity, there appears to be a distinct order in the production of neural subtypes (Qian et al., 2000). It is therefore likely that many mammalian NPCs are spatially restricted in their ability to generate specific cell types. Only through the development of better tools designed to identify and mark both invertebrate and vertebrate NPCs (Anderson, 2001) will the extent, cellular basis and timing of cell commitment be understood.

TEMPORALLY ORDERED TRANSITIONS IN NB GENE EXPRESSION DURING LINEAGE DEVELOPMENT

Cell lineage tracing studies in the developing fly CNS have revealed that many of the first-born, oldest NB offspring are positioned deepest in the developing ganglia, while the last-born, younger cells occupy more superficial positions (Bossing et al., 1996). Development in the vertebrate model systems differs. For example, the vertebrate NPCs maintain an internal position in the developing cortex, with their first born, oldest sublineages positioned adjacent to the NPCs, while subsequent sublineages migrate through the early born layers, occupying more superficial strata (reviewed by McConnell, 1989). Whereas in vertebrates the neural subtypes are arrayed in morphological strata, in *Drosophila* little morphological evidence exists for a similar layering. Nevertheless, when viewed with molecular markers (discussed below), sequentially born NB sublineages establish basal to apical layered gene expression domains in all CNS ganglia.

Molecular evidence for the temporal diversification of *Drosophila* NB progeny comes from studies of the zinc-finger transcription factor Castor (Cas; also known as Ming) (Cui and Doe, 1992; Mellerick et al., 1992). Although Cas is expressed in most if not all NBs during mid to late sublineage development, onset of expression is delayed in early delaminating NBs. Additional evidence for temporally defined windows of NB gene expression comes from an analysis of Cas transcriptional regulatory targets. In vitro DNA-binding studies have revealed that the DNA-binding specificity of Cas is similar if not identical to that of another structurally different zinc-finger protein known as Hunchback (Hb) (Stanojevic et al., 1989; Treisman and Desplan, 1989; Kambadur et al., 1998). Previous work examining Hb transcriptional targets identified two genes encoding the functionally redundant POU domain transcription factors, *pdm-1* and *pdm-2* (Johnson and Hirsh, 1990; Billin et al., 1991; Dick et al., 1991), referred to here as *pdm*. In the cellular blastoderm, Hb is a repressor of *pdm* expression (Lloyd and Sakonju, 1991; Cockerill et al., 1993). It was reasoned that Hb might target *pdm* during neurogenesis, and as Cas binds to Hb consensus binding sites, perhaps Cas too regulates *pdm* during neurogenesis. Subsequent studies have shown that Hb and Cas act early and late, respectively, to restrict *pdm* expression to a subset of neural cells that maintain an intermediate position sandwiched between early-born neural progeny expressing Hb and late-born neural progeny expressing Cas (Kambadur et al., 1998). Additional lineage marking studies and in vitro culture studies have revealed that these layered expression domains are formed by transitions in NB gene expression, and that the GMC and neural progeny maintain expression of the transcription factor active in the NB during each temporal window (Brody and Odenwald, 2000). Taken together, these studies indicate that many NBs undergo sequential changes in their gene expression profiles during lineage development.

Subsequent to the initial description of this network (Kambadur et al., 1998), two additional temporal gene expression windows have been identified. They are characterized by expression of *Kruppel* (*Kr*), a zinc-finger transcription factor (Schuh et al., 1986), and *Grainyhead* (*Grh*), a bHLH transcription factor (Bray et al., 1989). The *Kr* NB expression window is between the Hb and Pdm windows (Isshiki et al., 2001), and *Grh* is expressed after *cas* (Brody and Odenwald, 2000). Fig. 1 illustrates the temporal progression of these transcription factors in the NB and the generation of sublineages that occupy each temporal window. Although many NBs, in all CNS ganglia, transition through each of the gene expression windows, some NBs express only a subset of the temporal factors. One example of a NB that exits the network early is NB7-3. NB7-3 expresses Hb→Kr→Pdm but not Cas or Grh (Isshiki et al., 2001; Novotny et al., 2002). An example of a late delaminating NB, NB6-1, expresses only Cas (Cui and Doe, 1992; Mellerick et al., 1992) and not the early sublineage determinants.

In vertebrates there is now both cellular (conserved birth order of progeny) and molecular evidence for ordered transitions in gene expression within NPCs (reviewed by Harris, 2001; Livesey and Cepko, 2001). Temporally ordered changes in gene expression has been observed during murine NPC lineage development. For example, the NPC late sublineage gene, termed *Svet1* has been shown to follow the

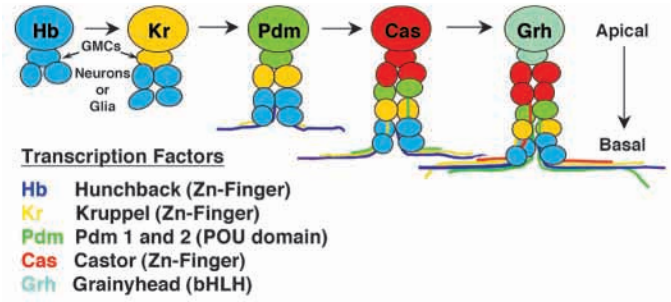

Fig. 1. Sequential transitions in neuroblast gene expression generate layered sublineage expression domains. During each temporal gene expression window, asymmetric NB divisions give rise to GMCs that are marked by the continued presence of the temporal factor that is expressed in the NB during its birth. These transcription factors are also detected in nascent postmitotic neurons and glia. Cells that express Hb are positioned on the inner basal surface of the developing ganglion, and are pushed deeper into the developing neuromere upon the birth of subsequent lineages. As a consequence of these transitions in NB gene expression during lineage development, layered transcription factor expression domains are formed throughout the developing CNS. The temporal factors may act as competence factors, determining the ability of the NB to generate progeny with distinct differentiative states.

expression of Otx1 in neural precursors (Tarabykin et al., 2001). *Svet1* expression marks the subventricular NPCs fated to give rise to later born cortical neural subpopulations that reside in the cortical layers II, III and IV, while Otx1 expression marks the ventricular NPCs, which give rise to earlier born progeny that reside in even deeper cortical layers. In addition, the staggered expression of Otx1 and Otx2 define layered expression domains in the cortex and cerebellum (Frantz et al., 1994). Transient expression patterns in retinal NPCs have also been observed (Perron et al., 1998). Although homologs of the *Drosophila* temporal transcription factors have been identified in non-insect species, including mammals, their roles in temporal development of the nervous system has not yet been investigated. Expression of *hb* homologs is detected in the embryonic CNS of the leech (Savage and Shankland, 1996; Iwasa et al., 2000) and *C. elegans* (Fay et al., 1999). In mammals, Hb-related genes of the Ikaros family are expressed in the developing CNS (Honma et al., 1999). A mammalian Pdm homolog, SCIP/Oct6, is expressed in specific cortical layers of the brain (Frantz et al., 1994) and a mammalian Cas cognate exists but has not been characterized (GenBank Accession Number, BAA91089).

DOES THE TEMPORAL NB NETWORK ESTABLISH NEURAL SUBTYPE IDENTITY?

Clonal analyses of NB lineages reveal that both early- and late-born descendents are fated to be either motoneurons, interneurons or glia (Bossing et al., 1996; Schmidt et al., 1997; Schmid et al., 1999). For example, during the Hb, Kr or Cas expression windows, NBs can generate all three cell types, indicating that that the temporal transcription factor network does not directly regulate cell-type identity (Isshiki et al., 2001). Because these sublineage determinants impart temporal and not subtype identity, it is necessary to distinguish between two types of sublineage determinants.

Mutational analyses of the temporal network genes show that they are required for proper sublineage development (Kambadur et al., 1998; Isshiki et al., 2001; Novotny et al., 2002). The temporal factors, specifically Hb and Cas, play essential roles during neurogenesis in all CNS ganglia, as evidenced by their mutant phenotypes. The number of Pdm-positive cells is increased in *hb*-null mutants, suggesting loss of *hb* causes a switch in lineage production and an augmentation of the number of Pdm-expressing cells. Similarly, loss of *cas* function results in an expansion of the Pdm expression domain (Kambadur et al., 1998). Thus, in many instances, absence of a temporal factor results in a failure to generate a particular GMC cell fate found in that temporal window. In the NB7-4 lineage, *hb* mutants lack the first-born glial fate but show no change in the number of later-born glia (Isshiki et al., 2001). Also, loss of *hb* function in the NB 7-3 lineage results in the loss of first-born neurons without affecting later lineages (Novotny et al., 2002). In addition, loss of *Kr* function can result in the absence of the neural cells that normally express that transcription factor, and in some lineages (7-1 or 7-3) loss of *Kr* results in the presence of necrotic neurons, supporting the idea that loss of a temporal factor can result in cell death (Isshiki et al., 2001). Thus, loss of a temporal factor results in an alteration of the neural identities in the layer in which that factor is usually expressed and may result in an increase in cells in an adjacent layer that express the adjacent temporal factor (Kambadur et al., 1998; Isshiki et al., 2001). However, recent work indicates that the cells within the sublineage that misexpress a temporal factor do not undergo a complete switch in temporal identity. For example, analysis of the NB 7-3 lineage reveals that loss of *hb* does not result in early neurons adopting fates found in later sublineages (Novotny et al., 2002).

Gain-of-function experiments reveal that these transcription factors can alter NB gene expression programs in adjacent temporal domains. For example, ectopic expression of Hb and Kr has shown that when these factors are expressed outside their temporal window, they redirect the fate of later sublineages to an earlier fate. When NBs ectopically express Hb outside of the normal temporal window of Hb expression, they generate progeny that express markers and morphology characteristic of early-born neurons (Isshiki et al., 2001; Novotony et al., 2002). Although Hb is necessary for early sublineage identity, loss of *hb* does not affect later-born sublineages, as evidenced by the near wild-type expression of *cas* in *hb* mutants. Continuous Hb expression can transform many or all progeny towards an early fate. Similarly, ectopic expression of Kr during NB lineage production results in more cells that maintain fates similar to those normally found in the Kr expression domain (Isshiki et al., 2001). Likewise, the targeted misexpression of Cas during early sublineage development reduces the number of Pdm-expressing cells (Kambadur et al., 1998).

These temporal transcription factors can be considered ‘competence factors’ in that they regulate the ability of NBs to give rise to different uniquely fated sublineages; each sublineage is marked by the continued expression of each of these factors. It is clear from the studies cited above that these transcription factors regulate the temporal identities (early

versus late) of the NB and its progeny and not cell-type specific identity (i.e. neural versus glia). For example, loss of *hb* function affects both neural and glia cell fates (Isshiki et al., 2001). The lack of both neurons and glia in *hb* mutants suggests that cell-type identity may be generated in the context of temporal identity. Temporal identity seems to be working neither upstream nor downstream, but rather in conjunction with the program of cellular differentiation unique to each NB. For example, recent examination of the generation of glia by the NB1-1 abdominal NB indicates that individual GMCs, generated after the first-born GMC, give rise to both a single neural and a single glial progeny (Udolph et al., 2001).

DO THE TEMPORAL TRANSCRIPTION FACTORS CONTINUE TO FUNCTION IN NB OFFSPRING?

Studies have shown that the temporal transcription factors purdure in postmitotic cells generated during lineage development. The stable inheritance of the transcription factors in neurons and glia suggests that these factors may be of importance in establishing or maintaining postmitotic cell identities. Although the temporal factors are maintained in the GMC, they are absent in the next temporal state of the NB. It is noteworthy that while *cas* mRNA and protein are expressed in NBs, only its encoded protein is detected in GMCs and their progeny (Kambadur et al., 1998). Therefore it is important to ask whether the temporal transcription factors and their mRNAs are asymmetrically distributed between the NB and its GMC progeny and how this asymmetrical distribution is achieved.

One mechanism for assuring the transfer of cell-fate determinants from the NPC to its progeny is asymmetric cell division. There are striking parallels between the mechanisms by which fruit flies and nematodes assure the uneven distribution of cell fate determinants between the progeny of an asymmetrically dividing precursor cell, particularly with reference to the conservation of the role of PAR proteins in this process (reviewed by Lu et al., 2000; Knoblich, 2001). It is likely that these mechanisms are also conserved in vertebrates. Divisions of NPCs in retinal development are asymmetrical (Livesey and Cepko, 2001; Zhong et al., 2000). The asymmetrical transfer of the daughter cell fate determinant Numb (reviewed by Jan and Jan, 1998) from neural precursors to their progeny is clearly conserved when comparing the fly with mammals. Asymmetrical division of the cortical precursors (Zhong et al., 1996) and retinal precursors (Cayouette et al., 2001) is accompanied by the asymmetrical localization of the mammalian Numb protein. However, there is no direct evidence that the partitioning of temporal factors into the GMC in *Drosophila* is carried out by known determinants of asymmetry. It is also possible that the transition to a new state is accompanied by active destruction of the temporal factor and its message in the NB as the NB transits from one temporal state to the next.

Onset of expression of temporal factors need not occur first in NBs, nor are their effects likely to be felt solely in NBs, GMCs or neurons. One exception to the onset of temporal factors in NBs occurs in the NB4-2 lineage. The *pdm* genes are known to specify first GMC identity in the progeny of NB4-2 (Bhat et al., 1995; Yeo et al., 1995), but *pdm* expression is detected only in the GMC and not in its NB. The lack of Hb/Pdm-1 overlap in GMCs or in their progeny suggests Hb may dynamically regulate *pdm* expression by first silencing early *pdm* NB expression and then, in absentia, permitting *pdm* reactivation in the GMC.

Another example of temporal regulation of gene expression during lineage development, involving onset in GMCs, is the expression of *klumpfuss* (*klu*) (Yang et al., 1997). Klu protein is first expressed in a subset of NBs during stage 10. Each of these NBs is born during the initial wave of NB delamination (late stage 8), but onset of Klu expression occurs after these NBs have generated their first GMCs. Klu is also activated in GMCs. Specifically, Klu is first activated in the second GMC generated during the divisions of the NB4-2. In *klu* mutants, the identity of this GMC is transformed into a first-born fate. In addition, driving Klu expression in the first-born GMC of this lineage changes its fate to that of the second-born GMC. Thus, the absence or presence of Klu determines the identity of both the first and second GMCs generated from the NB. These results suggest that in contrast to the almost global functions of Hb, Pdm, Cas and possibly of Grh within the temporal cascade, it is likely that the function of other components of the temporal cascade and/or their regulatory targets may be restricted to specific lineages.

Evidence from a study of the adult CNS of *Drosophila* points to regional differences between inner and outer neural processing centers that may have their origin from different sublineages during embryonic development. Thoracic neuromeres of adult insects are partitioned into discrete dorsal (inner) and ventral (outer) domains that are the processing centers for different sensory modalities (Pfluger et al., 1988; Murphey et al., 1989; Murphey et al., 1999). We suggest that the functioning of the temporal network during the embryonic period could be realized through the differentiation of neuronal subtypes that assume different functions in the adult CNS. Ultimately, only through an understanding of the cellular specializations of early- and late-born neurons will the function of the temporal network be understood in terms of cell function.

Evidence exists for both transcriptional regulation of the ordered birth of mammalian neural subtypes, and for the downstream consequences of such a program. Examination of the spatial patterns of 15 genes involved in early and late phases of *Xenopus* retinal development suggests a spatial ordering of gene expression that predicts a genetic hierarchy governing vertebrate retinogenesis (Perron et al., 1998). In mammals, there is now evidence that NPCs provide regulatory inputs to their progeny, being differentially transmitted to one of the two progeny of an asymmetrical division (Livesey and Cepko, 2001). For example, transcription of genes required for exit of retinal precursors from the cell cycle (Dyer and Cepko, 2001) and for the function of differentiated postmitotic retinal ganglion neurons occurs before M phase in progenitors (Waid and McLoon, 1995). This suggests that the retinal ganglion cell fate may be determined before mitosis. In addition, progenitor cells lose their responsiveness to external cues as they enter M phase of the cell cycle, prior to undergoing differentiation (Bellveau and Cepko, 1999). These observations indicate that the decision to assume a particular fate might be made by the progenitor cell and actuated only at the next level of development.

HOW ARE THE TEMPORAL TRANSITIONS IN GENE EXPRESSION REGULATED?

Several observations relating to the changes in NB gene expression must be taken into account when considering a possible mechanism for these transitions. For this discussion, an existing temporal window will be referred to as state #1 and the succeeding gene expression window will be referred to as state #2. Thus, the transition from state 1 to state 2 is accompanied by the downregulation of a temporal factor expressed in state 1 and by the upregulation of the succeeding factor characteristic of state 2.

Feedback and feedforward regulation

Studies on the regulation of these transitions have thus far shown that the transcription factors that are expressed in state 1 activate factors in state 2 and repress factors in previous states and states subsequent to state 2 (Kambadur et al., 1998; Isshiki et al., 2001) (Fig. 2). Thus, temporal factors are responsible for upregulation of the next state, but also insulate the genetic programs from regulators of adjacent temporal windows. Evidence points to the interdependence of Hb, Kr, Pdm and Cas in promoting or repressing the expression of one another. For example, *Kr* function is required for *pdm* expression and *pdm* function is required for proper *cas* expression. In addition, both Hb and Cas repress *pdm* expression. Altogether, the current work suggests that these transcription factors may be thought of as developmental 'progression factors' with reference to their role in promoting the sequential changes in NB gene expression.

It has been suggested that each gene functions to activate the next gene in the pathway and represses the 'next plus one' gene (Isshiki et al., 2001). This model of regulation by consecutive inputs has great heuristic value, and elements of it are likely to be correct. Nevertheless, in some instances only subtle alterations in downstream gene expression profiles are observed. For example, in *hb*-null mutants the temporal and spatial activation dynamics of *cas* expression appear to be similar to those of wild type (Kambadur et al., 1998; Isshiki et al., 2001). It is possible that additional inputs, either transcription factors or other signaling pathways participate in controlling the temporal network. The consecutive input model is testable only when all of the regulatory components acting on each of the promoters of network genes are understood in detail.

Cell cycle regulation of the temporal network

Evidence for the importance of cell cycle progression comes from studies on cell cycle-arrested NBs (Cui and Doe, 1995; Isshiki et al., 2001). When NBs are arrested in their cell cycle at the start of lineage development, they fail to undergo the temporal transitions in gene expression. Another study (Weigmann and Lehner, 1995) highlighting the importance of cell cycle progression rather than developmental time has demonstrated that, when the NB cell cycle is temporarily arrested, the NB fails to skip ahead to express a late sublineage marker; rather, it proceeds with normal lineage development when released from the block. In addition, cell cycle progression has been shown to be necessary for sublineage specific gene activation during mammalian lymphocyte differentiation (Bird et al., 1998). Taken together, all the available data suggest that a developmental switch tied to the cell cycle guarantees both that the NB progresses to the next competence state at the same time that it perpetuates the existing competence state in its GMC progeny via asymmetrical distribution of key regulators. Indeed, decay of an existing set of factors associated with a temporal state, or removal of that factor from the NB, may be controlled by cell cycle regulatory factors. In addition, transfer of the temporal progression factors from the NB to the GMC is likely to be integrated with cell cycle regulatory networks. However, no evidence has yet been provided for an involvement of asymmetry determinants such as Inscuteable, Partner of Inscuteable and Bazooka in the sub-cellular distribution of Hb, Kr, Pdm, Cas or Grh.

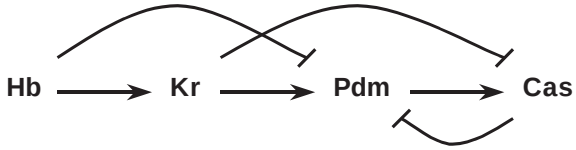

Fig. 2. Crossregulation of the temporal transcription factors. Both loss- and gain-of-function studies have demonstrated cross-regulatory interactions between the temporal transcription factors. These interactions include both activation (arrows) and repression of transcription (T-bars). As detailed in the text, these regulatory interactions can both stabilize the current state and promote the next state, thus ensuring the sequential progression of temporal states during lineage development.

We favor a transcription factor 'reshuffling' model triggered by chromatin condensation/remodeling during mitosis to explain the transitions in NB gene expression. Condensation of chromatin during mitosis could trigger the reshuffling of transcription factor occupancy on cis-regulatory sites controlling NB temporal genes. During state 1, the concentration of factors that dictate state 2 could increase relative to those that regulate state 1. The changes in the relative concentrations between state 1 and 2 factors could be brought about by differences between transcriptional activities and/or differences in the stability of their encoded proteins. The stable transcription factor-DNA complexes that dictate state 1 gene expression would require chromatin remodeling to initiate their release. Several studies have indicated that transcription factors are removed from DNA during the process of chromatin condensation accompanying mitosis (Segil et al., 1991; Martinez-Balbas et al., 1995; Kellum et al., 1995; Platero et al., 1998; Mullen et al., 2001; Gottesfeld and Forbes, 1997). After cytokinesis, state 2 factors would then out-compete state 1 factors for promoter occupancy in the NB, thus leading to a switch in gene expression programs. Although there is no direct evidence that demonstrates competition between temporal factors for promoter occupancy, examination of the cis-regulatory modules controlling one of the temporal factors, Pdm-1 (Kambadur et al., 1998; Berman et al., 2001), reveals that multiple Hb/Cas binding sites overlap or are immediately adjacent to Kr-binding sites. This may indicate competition between transcription factors for a common set of cis-regulatory modules. In instances where state 1 is not confined to a single mitotic cycle, the buildup of state 2 factors relative to state 1 regulators would be the rate-limiting step. The stable inheritance of state 1 gene expression programs in the state 1

GMC(s) could be established by cell-type-specific chromatin remodeling [as suggested by Isshiki et al. (Isshiki et al., 2001)]. [For chromatin remodeling reviews, see Farkas et al. (Farkas et al., 2000) and Müller and Leutz (Müller and Leutz, 2001).]

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