Transcriptional control of glial cell development in *Drosophila*

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Abstract

Neurons and glia are generated from multipotent neural progenitors. In *Drosophila*, the transcriptional regulation of glial vs. neuronal fates is controlled by the expression of the transcription factor encoded by the *glial cells missing* gene (*gcm*) in multiple neural lineages. The *cis*-regulatory control of *gcm* transcription serves as a nodal point to translate a complex array of spatially and temporally regulated transcription factors in distinct neural lineages into glial-specific expression. Gcm acts synergistically with several downstream transcription factors to initiate and maintain glial-specific gene expression. The identification of a large set of glial-specific genes through the application of computational and whole genome tools provides the opportunity to analyze the transcriptional regulation of glial cell development at the genomic level in a relatively simple genetic model system.

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Introduction

One of the great challenges of biology is to understand how cell diversity is generated during development. This challenge is especially daunting when one considers such a complex organ as the nervous system. A functional nervous system requires the correct specification and precise organization of a large number of neural cell types. Neural cells can be classified into two distinct groups, neurons and glia. Neurons play the leading role in processing and transmitting information, while glia play the supporting role, nourishing and insulating neurons. One general rule that has emerged from lineage analysis of neurogenesis is that both neurons and glia are generated from multipotent neural progenitors or neural stem cells (e.g., Bossing et al., 1996; Davis and Temple, 1994; Frank and Sanes, 1991; Schmid et al., 1999; Schmidt et al., 1997; Stemple and Anderson, 1992; Turner and Cepko, 1987; Udolph et al., 1993; Williams and Price, 1995). While much effort has been made to identify neural progenitors and the mechanisms controlling their fates, the mechanisms that control whether neural progenitor cells will adopt glial vs. neuronal cell fates are only beginning to be understood.

In vertebrates, neural stem cells respond to multiple cell-intrinsic, cell-extrinsic, and temporal cues that induce glial cell fates, yet no single developmental pathway leading to gliogenesis has been discovered (Anderson, 2001; Götz, 2003). Even so, much recent progress has been made in understanding the transcriptional control of glial cell fates in vertebrates, and three classes of transcription factors have emerged to be among the major determinants of the neuronal vs. glial cell fate decision as well as glial subtype specification: the basic helix-loop-helix (bHLH) transcription factors homologous to the *Drosophila* proneural genes (e.g., Nieto et al., 2001; Sun et al., 2001a,b; Tomita et al., 2000; Zhou and Anderson, 2002; reviewed in Ross et al., 2002; Rowitch et al., 2002; Vetter, 2001), members of the Sox family of high-mobility-group (HMG) factors (e.g., Kim et al., 2003; Stolt et al., 2003), and regionally expressed homeodomain factors (Sun et al., 2001a,b; Zhou et al., 2001). Current models present a complex mechanism for glial cell specification, whereby these transcription factors control the progressive restriction or loss of nonglial
(or neuronal) potentials of neural progenitors, rather than the promotion of glial cell fate in the neuron-glia decision; and, a combinatorial code of bHLH factors and regionally restricted factors determines whether progenitors will produce neuronal or glial subtypes. By contrast, the fruit fly Drosophila melanogaster has a seemingly simpler mechanism controlling gliogenesis. A single gene, glial cells missing (gcm, also known as glide), is the primary regulator of glial cell determination. gcm encodes a novel transcription factor that is transiently expressed in nearly all embryonic glia, except for the midline glia (Akiyama et al., 1996; Hosoya et al., 1995; Jones et al., 1995; Schreiber et al., 1997; Vincent et al., 1996). gcm loss-of-function mutant embryos lack nearly all glial cells, and presumptive glial cells are transformed into neurons (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). Conversely, in gain-of-function conditions when gcm is ectopically expressed, presumptive neurons are transformed into glia (Hosoya et al., 1995; Jones et al., 1995). Thus, within the nervous system, gcm acts as a binary genetic switch, with Gcm-positive cells becoming glia and Gcm-negative cells becoming neurons (Fig. 1).

Because gcm is transiently expressed, it is likely that it controls only the initiation of glial cell differentiation. Genes downstream of gcm must accomplish the differentiation and maintenance of glial cell fate. To date, several transcription factors have been described that are regulated by gcm and are required to propagate the glial cell fate decision. In addition, through the use of traditional genetic screens, and a variety of molecular genetic and genomic techniques, including expression screens, cDNA microarrays, and new computational and bioinformatic methods, a growing number of glial-specific genes have been identified, and many are potential transcriptional targets of gcm and its downstream factors (e.g., Egger et al., 2002; Freeman et al., 2003). The intent of this review is to highlight recent research on the transcriptional control of glial cell development in Drosophila, focusing on current questions that are being addressed in the field and future directions it may take.

Drosophila has proven to be an excellent model system for dissecting mechanisms of neural development and lends itself well to a systematic study of glial cell differentiation. In addition to its sophisticated classical and molecular genetic tools, much is known about the lineages, patterns, and identities of glia and neurons in the developing CNS and PNS. Glial cells and their progenitors are arranged in stereotypic patterns repeated in each segment of the embryo, and are easily identified by position, and by a large array of markers. Nearly all embryonic glial lineages have been completely described from progenitor to fully differentiated glia (Bossing et al., 1996; Ito et al., 1995; Schmid et al., 1999; Schmidt et al., 1997).

Drosophila has also long been at the forefront of studies of transcriptional regulation, and is currently one of the most tractable models for animal transcriptional control (reviewed in Biggin and Tjian, 2001). Many fundamental concepts such as cis-regulatory DNA elements acting over long distances, and the regulation of development by hierarchical cascades of transcription factors originated in research on Drosophila. Whether or not homologous mechanisms are controlling glial cell differentiation in vertebrates, the study of mechanisms regulating glial cell differentiation in Drosophila should prove to be of valuable interest in the general understanding of transcriptional regulation of cell fate specification. Some of the specific questions being addressed by researchers in the field are the following. How do single neural progenitors sequentially generate different cell types that include both neurons and glia? How is glial cell differentiation activated in these progenitors? How do combinations of transcription factors initiate and maintain glial cell fates? How are glial-specific genes coordinately controlled to carry out glial cell differentiation, and how are glial subtypes differentiated? Can new genomic and bioinformatic tools be used to decipher these phenomena?

Specification of neural progenitors and the glial cell fate decision

In Drosophila, glia can be split into two distinct groups, midline glia and lateral glia. In this review, we focus on the transcriptional regulation of lateral glial cell differentiation. Lateral glia comprise the majority of glial cells and are defined by their dependence on the transcription factor Gcm. In the embryo, lateral glia are derived from neural
progenitors that originate in the ventral neurogenic ectoderm and the peripheral ectoderm lateral to the ventral midline (Bossing et al., 1996; Campos-Ortega and Hartenstein, 1997; Goodman and Doe, 1993; Jan and Jan, 1993; Jones, 2001; Schmid et al., 1999; Schmidt et al., 1997). In the PNS, progenitors called sensory organ precursors (SOPs) delaminate from the ectoderm and undergo a series of cell divisions that generate specific types of neurons, glia, and other support cells. In the CNS, neuro progenitors delaminate from the ectoderm and cycle through a series of asymmetric divisions, producing a secondary precursor called a ganglion mother cell (GMC) with each event. Each GMC then passes through a single division to yield differentiated neurons and/or glia. One population of CNS progenitors, neuroblasts (NBs), gives rise to only neurons, whereas glial producing progenitors come in two forms—glioblasts (GBs), which give rise to only glial cells, and neuroglioblasts (NGBs), which produce mixed glial/neuronal lineages. In addition, NGBs can be further subdivided into at least two types (Udolph et al., 2001). Type 1 NGBs produce a glioblast and a neuroblast after the first division, and type 2 NGBs generate a series of GMCs that divide once to yield either two sibling neurons or a neuron/glia sibling pair. For simplicity, CNS progenitors are often collectively called NBs. NBs and SOPs generate unique, reproducible, stereotypic patterns of neuronal and glial progeny.

The transcriptional apparatus controlling the initial formation of NBs and SOPs has been well studied (reviewed in Artavanis-Tsakonas et al., 1995; Campos-Ortega, 1995; Gibert and Simpson, 2003). The balance of proneural and neurogenic gene activity regulates the initial selection of neuroblasts and SOPs from the ectoderm. Proneural genes are bHLH transcription factors that are expressed in clusters of neuroblasts and SOPs from the ectoderm. Proneural genes such as Prospero and Numb, and are subject to signaling from the Notch pathway, which differentiates sibling cell fates through the activity of the Suppressor of Hairless [Su(H)] transcription factor (Doe et al., 1991; Hirata et al., 1995; Knoblich et al., 1995; Rhyu et al., 1994; Udolph et al., 2001; Uemura et al., 1989; Umesono et al., 2002; Vaessin et al., 1991). Notch signaling has been shown to influence gcm transcription and glial cell differentiation in binary cell fate decisions, with the general rule that it promotes gliogenesis in the case of neuronal/glial sibling pairs, but has the opposite effect on secondary precursor/sibling pairs (Udolph et al., 2001; Umesono et al., 2002; Van De Bor and Giangrande, 2001).

To summarize, a plethora of transcription factors are expressed in complex temporal and spatial manners to specify unique neural lineages during development. How all these factors come together to produce different patterns of glial vs. neuronal progeny is poorly understood. The resulting readout of combinations of temporal and spatial cues somehow leads to the activation of gcm and the initiation of glial cell differentiation in precise and stereotypic patterns in the nervous system.

**Transcriptional regulation of gcm controls gliogenesis**

The expression of gcm mRNA and protein closely profiles the initiation of gliogenesis in the various neural lineages that generate glia. gcm is both necessary and sufficient for glial cell development. These observations lead to the conclusion that the primary event controlling gliogenesis is the transcriptional regulation of gcm. Thus, the cis-regulatory control of gcm transcription serves as a nodal point to translate the complex array of transcription factors found in distinct neural lineages into glial-specific expression. Understanding the transcriptional regulation of gcm may provide a model for understanding how diverse transcriptional inputs are integrated in different neural lineages to give rise to stereotypic patterns of glial vs. neuronal progeny.

Deciphering the transcriptional regulation of any given gene in multicellular animals is not a straightforward process. Most of what we know about the structure, organization, and action of cis-regulatory DNAs has been derived through the painstaking dissection of DNA sequences proximal to gene transcription units, testing each DNA for its ability to influence the transcription of reporter genes
or effect genetic rescue in transgenic animals, and identifying and characterizing trans-acting factors. Because this process is laborious, surprisingly few cis-regulatory DNAs have been examined in any detail (Davidson, 2001; Marks-tein and Levine, 2002). However, common features of animal regulatory sequences have become apparent (Armone and Davidson, 1997; Howard and Davidson, 2004; Levine and Tjian, 2003). Most regulatory DNAs are modular in nature. Cis-regulatory elements or modules are typically several hundred base pairs to 1 kilobase (kb) in length, are located within several kb (roughly 10 kb in flies and up to 100 kb in mammals) of the exons or within the introns of the genes they control, and contain clustered binding sites for multiple transcriptional activators and repressors. Modules often work independently of one another to direct composite patterns of cell-specific gene expression when linked within common cis-regulatory regions.

Thus, one model for gcm transcription is that its regulatory DNA is modular, where each module integrates a different combination of inputs to activate gcm in different lineages. At one extreme, it is possible that each glial lineage and glial subtype has separate regulatory modules with unique modes of regulation. Alternatively, the gcm locus may have a limited number of regulatory elements that respond to signals present in multiple developmental environments. Two recent studies set out to dissect the cis-regulatory structure of the gcm locus (Jones et al., 2004; Ragone et al., 2003) (Fig. 2). The results indicate that gcm transcription is controlled by a combination of tissue-specific and lineage-specific modular elements, but not by glial subtype-specific elements, nor by elements that control expression in progenitors that undergo a specific mode of division (e.g., GB vs. type 1 NGB vs. type 2 NGB).

The first study used genomic DNA from the gcm locus to rescue the gcm loss-of-function phenotype in transgenic embryos (Ragone et al., 2003). Four genomic DNA rescue constructs were tested, each containing the gcm coding sequences and 1.7 kb of 3′DNA, but each differing by the length of 5′DNA by 2, 4, 6, and 9 kb, respectively. The 4-kb 5′ transgene was able to rescue glial cell differentiation in most CNS glial lineages, indicating that it contains most the information to direct glial-specific transcription; however, the ability to rescue was not fully penetrant, and required additional upstream DNA to provide “quantitative” elements that boost expression. Full rescue in nearly all CNS glial lineages was only achieved with 9 kb of 5′DNA. A comparison of the 4 kb 5′ rescue construct to a shorter 2 kb 5′ rescue construct showed that specific information for different GB and NGB lineages resides on different segments of DNA; the 2 kb upstream construct was able to rescue only three of nine identified thoracic and abdominal glial producing lineages, indicating that it contains information for three specific lineages, but lacks information for six others. In addition, the 2 kb 5′ transgene showed precocious expression of gcm in three non-glia-producing NB lineages, causing them to generate ectopic glial cells instead of neurons. These results indicate that there is also cis-regulatory information for other neural lineages in the 2 kb 5′ transgene that must normally be repressed by 5′DNA sequences contained in the longer constructs. Thus, some lineage-specific expression is achieved by both specific activation and repression.

The second study presented a systematic dissection of cis-regulatory DNA elements of gcm using lacZ reporter activity in transgenic embryos, testing the activity of ~35 kilobases of DNA from the gcm locus dissected into 18 fragments (Jones et al., 2004). In agreement with the previous study, most glial-specific cis-regulatory activity mapped to the first 9 kb of DNA 5′to the gcm transcription unit; additional glial-specific activity also was found in a small region of 3′DNA. Further dissection of these regions revealed that glial-promoting cis-regulatory activity could be divided into at least three components (1) a general neural component, (2) a lineage-specific component, and (3) an autoregulatory component that is dependent on gcm activity.

Close to the gcm promoter, a 1-kb region promotes broad, but weak, pan-neural expression. More distal to the promoter is a 3-kb segment that promotes expression in most CNS GBs and NGBs. Adding this 3-kb segment to the

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Fig. 2. Cis-regulatory DNA elements of gcm. Black bar represents approximately 20 kb of genomic DNA at the gcm locus; orange ovals represent Gcm binding sites (determined in vitro). Transcribed genes are represented as rectangles above the bar, arrows represent direction of transcription; gcm exons are shown with shaded part (red) representing coding sequence. DNA regions promoting specific activities are shown as colored bars below the map. 1-1A, 2-2T, and 1-3 are CNS neuroglioblasts lineages. Data is derived from Jones et al. (2004) and Ragone et al. (2003) as described in the text.
proximal pan-neural promoting region caused a loss of neuronal expression. These data support a model where gcm expression in the CNS requires both lineage-specific activation and general neuronal repression.

Most striking was the discovery of a small DNA element in the 3′ region that promotes expression in glia derived from a single glial progenitor, the abdominal glioblast GB 6-4A. Interestingly, expression was absent in the analogous precursor, NGB 6-4T, found in the thoracic segments; NGB 6-4T undergoes a slightly different pattern of divisions, generating both neurons and glia. It has long been recognized that the homeotic selector genes of the Hox complex are responsible for the segmental differences in neural cell lineage specification along the anterior posterior axis (e.g., Prokop et al., 1998). By crossing reporter lines into homeotic gene mutant backgrounds, it was shown that expression driven by the GB 6-4A element in the abdomen is dependent on the homeotic selector gene abdominal-A, demonstrating that homeotic genes may control segmental differences in glial lineages by directly regulating gcm transcription.

The third component contributing to gcm glial expression is autoregulation. Previous genetic data had implicated a positive autoregulatory feedback mechanism controlling gcm transcription (Miller et al., 1998). The cis-regulatory analysis confirms these observations. A DNA segment that drives gcm-dependent glial cell expression is located further distal (−7.4 to −4.4 kb) to the gcm promoter (Jones et al., 2004), and the sequence contains two high-affinity Gcm protein binding sites (GBS) and as many as three or more lower-affinity GBSs (Ragone et al., 2003). Autoregulation provides the “quantitative” component that boosts gcm transcription, as high levels of gcm transcription are required for triggering gliogenesis (Freeman and Doe, 2001; Ragone et al., 2003).

Taken together, these two studies give a hint that gcm may be regulated by multiple cis-regulatory modules controlling lineage-specific transcription (summarized in Fig. 2). The existence of a cis-regulatory element for a single neural precursor, GB 6-4A, indicates that at least one neural precursor lineage has a unique mode of regulation. The genetic rescue data suggest the existence of additional lineage-specific elements in the 5′ region. In principle, a fine dissection of the 3-kb 5′ region that promotes most CNS glial cell expression will possibly reveal additional neural precursor specific elements. How any set of transcription factors control these lineage-specific modules remains to be explored.

Transcriptional control of glial differentiation downstream of gcm

Gcm is thought to initiate gliogenesis through the transcriptional activation of glial-specific target genes. However, glia are not the only cells that require gcm during development. gcm, together with its closely related homolog gcm2, is also required for the differentiation of the plasmatocyte/macrophage lineage of blood cells, or hemocytes (Alfonso and Jones, 2002; Bernardoni et al., 1997; Lebestky et al., 2000). gcm2 is closely linked to gcm on the chromosome, but is dispensable for glial cell differentiation. Before gcm is activated in glial progenitors, both gcm and gcm2 are expressed in hemocyte precursors derived from proephalic mesoderm, where they act to trigger the maturation of hemocytes into macrophages. Embryos that are double mutants for gcm and gcm2 show abnormal macrophage development, and expression of gcm alone is sufficient to induce macrophage development within hemocyte lineages. Thus, gcm has the ability to promote either glial cell differentiation or hemocyte differentiation in different developmental contexts. How some gcm target genes are activated in glial cells but not in hemocytes is not understood. This behavior suggests that additional factors act in combination with Gcm to promote glial differentiation. The identity of these cofactors is not known. However, one may guess that these glial-promoting cofactors may be common to the transcriptional apparatus controlling both neuronal and glial differentiation, as in the absence of gcm glia are transformed into neurons.

Gcm protein binds with high affinity to the octameric consensus DNA sequence AT(G/A)CGGG(T/C) (Akiyama et al., 1996; Schreiber et al., 1997, 1998). DNA binding activity maps to the highly conserved 153 amino N-terminal DNA binding domain, the Gcm domain, that is shared with its vertebrate homologs. The C-terminal part of the protein acts as a potent transactivator. Reporter gene activation by Gcm in transfected cells is dependent on the presence of Gcm binding sites (Miller et al., 1998; Schreiber et al., 1997). Taken together, Gcm has all the characteristics of a sequence-specific DNA-binding transcriptional activator.

Variations of the Gcm-binding site (GBS) are found repeated in the putative regulatory regions of a number of glial-specific genes that are dependent on gcm expression (Akiyama et al., 1996; Freeman et al., 2003; Granderath et al., 2000; Schreiber et al., 1997). These potential target genes include the glial-specific transcription factors encoded by the repo, pointed, and tramtrack genes. The repo gene encodes a homeomain transcription factor that is expressed in all lateral glial cells (Campbell et al., 1994; Halter et al., 1995; Xiong et al., 1994), repo expression is gcm-dependent. Transient expression of gcm is followed by maintained expression of repo. Eleven sequences that match or have one mismatch from the consensus GBS are located within the first 4 kb upstream of the repo transcription unit (Akiyama et al., 1996). Mutations in repo do not affect initial glial determination, but do affect their differentiation and the expression of late glial cell markers. Defects associated with repo mutations include a reduction in the number of glial cells, disrupted fasciculation of axons, and
increased neuronal cell death. Thus, repo appears to control important aspects of terminal glial differentiation.

Also dependent on gcm expression are the P1 form of the ETS domain transcription factor encoded by the pointed (ptd) gene (Klaes et al., 1994; Klämbt and Goodman, 1991), and the P69 form of the BTB-zinc-finger transcription factor encoded by the tramtrack gene (ttk) (Giesen et al., 1997). Like repo, mutations in these genes do not prevent the initiation of glial cell development, but have terminal differentiation defects. pointedP1 promotes different aspects of glial cell differentiation, and is required for the expression of several glial markers. In contrast, ttk acts to repress neuronal differentiation. In ttk mutants, glial cells ectopically express neuronal antigens. In addition, Ttk69 inhibits the expression of the pan-neural bHLH genes asense and deadpan, which promote the neuronal potential of neural progenitors (Badenhorst, 2001). These data support a model whereby gcm promotes glial cell characteristics by initiating the glial-specific transcriptional activators repo and pointed, while simultaneously repressing neuronal characteristics by activating the transcriptional repressor ttk (Giesen et al., 1997). Additional data suggests that repo may also cooperate with ttk to suppress neuronal fates, thereby reinforcing the glial cell fate choice (Yuasa et al., 2003) (Fig. 3A).

The cis-regulatory network downstream of gcm is not strictly linear. In the few examples that exist, gcm and its downstream regulators repo, pointed, and ttk appear to act cooperatively at the cis-regulatory level to initiate and maintain the expression of glial-specific genes. Most instructive have been studies of cis-regulatory elements controlling the expression of the loco gene, which encodes a family member of the Regulators of G-Protein Signaling (RGS) proteins expressed in lateral glia (Granderath et al., 1999, 2000; Yuasa et al., 2003). A 1.9-kb cis-regulatory DNA element of loco can direct glial-specific expression of a reporter gene in vivo (Granderath et al., 2000). Scattered in the DNA sequence of this element are three GBSs and an ETS binding site (the consensus site for PointedP1 protein). Specific mutation of at least two of the GBSs caused a complete loss of expression, demonstrating the requirement for Gcm for the initiation of expression and cooperativity of multiple binding sites. Mutation of the ETS binding site caused a premature decay of reporter expression, suggesting that PointedP1 is required for the maintenance of loco expression. Ectopic expression of either gcm or pointed drives weak expression of the loco reporter; however, co-expression of gcm and pointed induces robust loco reporter expression, demonstrating a synergistic interaction of the two transcription factors on the cis-regulatory element.

Similar studies show that repo and pointed also cooperate to regulate loco expression (Yuasa et al., 2003). Like pointed, repo is required for the maintenance of loco expression, and ectopic expression of repo and pointed together induces stronger ectopic expression of loco than either gene can alone. Here, at least for one gene, a picture is emerging in which Gcm initiates the expression of glial-specific genes (along with unknown co-factors), and simultaneously activates downstream transcription factors that cooperate on the same promoters with Gcm to activate expression (Fig. 3B). As Gcm is expressed transiently, glial-specific expression is maintained by its downstream transcription factors after Gcm disappears. While it remains to be seen, it would not be surprising if many glial-specific genes, if not most, are regulated similarly.

Fig. 3. Transcriptional regulatory networks controlling gliogenesis in Drosophila. (A) Summary of gcm pathway. gcm transcription is regulated by multiple inputs in different neural lineages. gcm initiates glial cell development by the simultaneous activation of glial differentiation and repression of neuronal differentiation. Additional neuronal factors (X) may be required to activate glial fate. Glial differentiation is promoted by the factors repo, pointed (pnt), and others. Neuronal differentiation is blocked by tramtrack (ttk) through the repression of neuronal factors such as asense (ase) and deadpan (dpt). repo may be required as a co-factor for neuronal repression (see text for additional detail). (B) Circuit diagram for the transcriptional regulation of the glial-specific gene loco. gcm cooperates with downstream factors repo and pnt to initiate and maintain loco expression. gcm autoregulates to boost its own expression. Dashed lines represent hypothetical autofeedback loops regulating repo and pnt. Transient expression of gcm activates the circuit; loco expression is maintained by repo and pnt.
Genome-wide analysis of glial-specific gene transcription

Analysis of individual cis-regulatory DNAs in transgenic promoter constructs, and the characterization of trans-acting factors, their specific binding sites and mode of action, have provided valuable information about the structure of cis-regulatory modules and transcriptional regulatory networks controlling the differentiation of cell lineages. However, this approach is still too laborious to allow more than a few examples to be examined in any detail. The availability of whole-genome sequences now offers different approaches to the study of cell-specific transcriptional regulation. One current and future goal in the post genome era is to identify and characterize cis-regulatory modules and networks among coordinately regulated genes through the application of computational, bioinformatic, and other whole-genome tools.

The transcriptional regulation of glial cell differentiation in Drosophila is proving to be an excellent model system for the application of these tools. Several recent studies have taken advantage some of the striking features of Gcm to identify Gcm target genes through whole genome analyses (Egger et al., 2002; Freeman et al., 2003). These features include the strong neuron/glial transformation of loss-of-function and gain-of-function gcm phenotypes, and the characteristic Gcm binding site that is found repeated in the regulatory regions of Gcm target genes. In one study, Freeman et al. (2003) used a combination of genomic approaches—computational DNA-binding site search algorithms, cDNA microarrays, and database searches—and identified 45-Gcm-regulated glial genes, and 5-Gcm-regulated macrophage genes. Twenty of these Gcm-regulated glial genes and the macrophage genes are likely to be direct targets of Gcm because of the presence of multiple Gcm-binding sites in flanking genomic DNA.

An analysis of the expression profiles of this glial-specific gene collection has already revealed some important insights into their transcriptional regulation (Freeman et al., 2003). Glial-specific genes can be clustered into groups that share distinct spatial or temporal patterns. Temporal profiles include genes expressed throughout gliogenesis and genes that are first expressed early, midway, or late in gliogenesis; spatial profiles include either all glia or characteristic subsets of glia. Each spatial and temporal group of Gcm target genes showed a different response to ectopic Gcm expression. Genes expressed in all CNS glia were induced throughout the entire CNS by ectopic Gcm expression; genes expressed in subsets of glia were only induced ectopically in restricted spatial domains; and, genes expressed late in development, were induced ectopically only late in development. These data suggest that most Gcm target genes require both Gcm and a spatially or temporally restricted co-factor to be activated, contributing to the generation of glial subtype diversity, and adjusting the time of gene expression for glial function.

The identification of a large number of Gcm-regulated genes provides the opportunity to explore the transcriptional regulation of glial cell differentiation at the genomic level. By comparing cis-regulatory regions of co-expressed glial-specific transcripts, it may be possible to identify shared DNA motifs that identify shared transcription factors required for their expression. Do glial-specific Gcm target genes share DNA motifs that are distinct from macrophage-specific target genes? Are there distinct motifs shared by spatially or temporally regulated genes? Can the trans-acting factors that interact with motifs be identified? Is it possible to decipher a cis-regulatory code for glial specific expression? With the combination of new computational tools, and verification by classical transgenic analysis, many of these questions will possibly answered in the coming years and should prove to be a valuable contribution to our emerging understanding of cell-specific transcriptional regulation.

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References


