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DSCAM: an endogenous promoter drives expression in the developing CNS and neural crest

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Abstract

The development of central nervous system (CNS) neuronal networks involves processes including neuroblast migration, axonal pathfinding, and synaptogenesis. To evaluate the role of the axonal guidance molecule DSCAM in CNS connectivity, we generated a *lacZ* reporter construct, Pr1.8-βgal, containing a 1.8 kb fragment of the human *DSCAM* promoter region, and analyzed its expression in four E12.5 transgenic mouse embryos. We found that Pr1.8-βgal drives *lacZ* expression in the choroid plexus and roof of the fourth ventricle, the floor plate of the fourth ventricle, pons and medulla oblongata, and the eye, limb buds, and dorsal root ganglion. This recapitulates a subset of *DSCAM* expression as demonstrated by in situ hybridization, supporting this 1.8 kb fragment as a component of the endogenous *DSCAM* promoter. The Pr1.8-βgal expression pattern supports a role for DSCAM in CNS development, providing an endogenous promoter to investigate the contribution of DSCAM to Down syndrome neural defects.

The neuronal networks of the central nervous system (CNS) are formed through the development of distinct neuronal populations, which migrate to their final positions, extend axons to connect with their synaptic targets, and form dendrites to receive afferent input on the soma and dendritic spines [1–3]. In approaches to defining the perturbations of these neural systems that underlie genetically determined cognitive deficits, Down syndrome (DS) provides an important model with which to link individual genes and pathways to morphogenetic processes and cognitive functions.

Down syndrome cell adhesion molecule (DSCAM) is a chromosome 21 axonal guidance molecule expressed by the developing neurons of the central and peripheral nervous systems (CNS and PNS) [4] and defines a novel subclass within the immunoglobulin superfamily of cell adhesion molecules (Ig-CAMs) [4]. *DSCAM* is particularly highly expressed in higher-order cortical structures [5,6], and we have proposed that its overexpression may contribute to pre- and postnatal defects in neuronal morphogenesis, and thus to the cognitive defects of DS.

In order to investigate the contributions of chromosome 21 genes to the pathogenesis of DS, it is important to generate models that overexpress individual genes in subsets of their endogenous patterns. The large size of the *DSCAM* gene, which spans more than 840 kb [7], presents challenges for the generation of models utilizing the endogenous promoter. However, many of the well-studied genes whose promoters have been used to drive expression in other mouse models, such as the neuron-specific enolase (NSE) promoter [8], are expressed after the initial development of CNS and PNS connections, and after the onset of *DSCAM* expression.

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To define the promoter elements and control regions that direct *DSCAM* expression in individual neural tissues during specific morphogenetic events and processes, we have generated a series of reporter constructs with subsets of the human *DSCAM* promoter driving the expression of a *lacZ* reporter gene. We now report that one of these constructs, Pr1.8-βgal, recapitulates a subset of the *DSCAM* expression pattern previously demonstrated by tissue in situ hybridization.

Methods

Generation of the transgenic construct Pr1.8-βgal. The Pr1.8-βgal transgene was constructed as follows. A 3 kb *Xho*I–*Hin*dIII fragment of genomic DNA surrounding the first exon of human *DSCAM* was subcloned into the *Xho*I and *Hin*dIII sites of pBluescript. This subclone was digested at the *Ngo*MIV site 33 bp upstream of the translational start site (ATG, see Fig. 1), the ends were blunted using T4 DNA Polymerase (Promega), and the DNA was redigested with *Xho*I, yielding a 1.8 kb genomic fragment up to, but not including, the translational start site of human DSCAM. This fragment was subcloned into the reporter vector pβgal-enhancer (Clontech), which had been prepared by digesting the vector DNA with *Hin*dIII, blunting the ends using T4 DNA Polymerase (Promega), and redigesting with *Hin*dIII. The resulting construct, Pr1.8-βgal, contains 1.8 kb of the putative promoter region of human *DSCAM* driving the expression of the *lacZ* gene.

Cell culture and transfection assays. The Pr1.8-βgal construct was tested for promoter activity in three different cell lines from different tissue types: COS-7, a kidney SV40-transformed fibroblastic cell line from African green monkey (ATCC); NIH/3T3, a contact-inhibited cell line established from mouse embryo cultures ([9], ATCC); and MEQC, a quail embryonic cardiac mesenchyme cell line [10]. Cells were cultured in Dulbecco's modified Eagle's medium high glucose (Gibco-BRL) containing 1 mM sodium pyruvate and the following sera: 10% fetal calf serum (COS-7), 10% calf serum (NIH/3T3) or 10% fetal calf serum and 2% chick serum (MEQC). All cell lines were cultured at 37 °C with 5% CO_2.

Transfection conditions for each cell line were optimized using the control vector pβgal-control (Clontech) and the transfection reagents Superfect (Qiagen), Effectene (Qiagen), and Lipofectamine (Gibco-BRL) according to manufacturers' instructions. Briefly, $4–5 \times 10^4$ cells were seeded per well of a 24-well plate 24 h prior to transfection. The following day, master mixes designed to give final DNA concentrations of 100, 200, or 400 ng per well in combination with low, medium or high concentrations of the transfection reagent were preincubated according to manufacturers' instructions in serum-free (Lipofectamine, Superfect) or serum-containing (Effectene) media. The cells were washed twice with 1×PBS and the transfection complexes were added to the cells. In the case of cells transfected with Superfect, the transfection complexes were removed after 2–4 h and replaced with normal growth medium. Cells were assayed for βgal activity 24 and 48 h post-transfection using the βgal Staining Kit (Invitrogen) according to manufacturers' instructions. The transfection conditions resulting in the highest percentage of transfected cells (cells showing *lacZ* expression) and the lowest levels of cytotoxicity were used in all subsequent experiments.

Generation and analysis of Pr1.8-βgal transgenic mouse embryos. The vector backbone of the Pr1.8-βgal construct was removed by digestion with *Xho*I and *Sal*I, and the resulting 6.6 kb DNA fragment was isolated by gel electrophoresis and purified using the QIAquick Gel Extraction Kit (Qiagen). The purified DNA fragment was introduced into fertilized mouse oocytes by pronuclear injection and the oocytes were transferred into the oviducts of pseudopregnant dams. Embryos were harvested at E12.5, fixed, and analyzed for the expression of the *lacZ* transgene by staining with X-gal as described previously [11]. Due to problems at the injection facility, the construct was not successfully reinjected and no further timepoints were available for analysis.

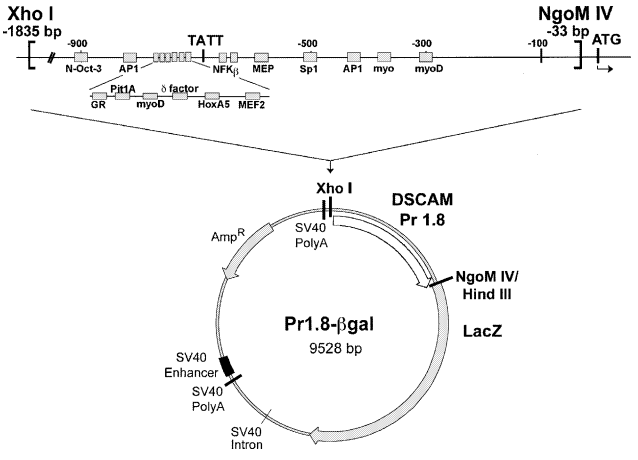

Fig. 1. Construction of the Pr1.8-βgal transgene. A 1.8 kb *Xho*I–*Ngo*MIV fragment of human genomic DNA spanning from positions −1835 to −33 with respect to the DSCAM translational start site (ATG) was subcloned into the *Xho*I and *Hin*dIII sites of the reporter vector pβgal-enhancer (Clontech) (see Methods for details). Potential transcription factor binding sites within this 1.8 kb region are represented by grey boxes and the positions of the *Xho*I and *Ngo*MIV restriction sites, the translational start site (ATG) and TATT sequence are indicated. Within the transgenic construct, the 1.8 kb promoter fragment is denoted by a white arrow, the open reading frames of the *lacZ* and ampicillin resistance genes are denoted by grey arrows, and SV40 elements are denoted by black boxes or lines. GR, glucocorticoid receptor; myo, myogenin.

Results

A 1.8 kb human DSCAM promoter fragment drives expression of a lacZ transgene in mouse embryos

To identify functional elements of the endogenous DSCAM promoter for use in directing transgene expression in the mouse, we subcloned a fragment containing 1.8 kb of human genomic DNA immediately upstream of the DSCAM translational start site into the reporter vector pβgal-enhancer (Clontech) (see Fig. 1). The resulting transgenic construct, Pr1.8-βgal, was first tested for promoter activity in cell lines derived from several different tissue types, including COS-7 (ATCC), NIH/3T3 (ATCC), and a quail embryonic cardiac mesenchyme (MEQC) cell line [10] (see Methods). Transfection conditions were initially optimized for each cell line using the pβgal-control vector (Clontech) and a variety of transfection reagents, including Effectene (Qiagen), Superfect (Qiagen), and Lipofectamine (Gibco-BRL) (see Methods). Having

determined the optimal transfection conditions for each cell line, transfections were then performed using the Pr1.8-βgal transgenic construct. The 1.8 kb promoter fragment showed definite promoter activity in approximately 5–10% of cells transfected for each cell line, as compared to 50–60% of cells transfected with the pβgal-control vector (data not shown). The Pr1.8-βgal construct was then assayed in transgenic mouse embryos at E12.5 (see Methods).

Whole-mount staining of transgenic embryos revealed consistent patterns of Pr1.8-βgal expression in four embryos (Fig. 2 and data not shown), with strongest expression in regions of the developing brain including expression in the fourth ventricle, midbrain, and hindbrain, which was conserved in all four embryos (Figs. 2b, d, e and data not shown). The transgene is also expressed in the dorsal root ganglia in embryo #1 (Figs. 2a and b), and in the eye and limb buds in embryo #2 (Fig. 2d). The embryos were then sectioned in order to further analyze the distribution of the transgene.

Transgene expression in the developing brain

The Pr1.8-βgal transgene has a very restricted distribution in the developing mouse brain at E12.5 (Figs. 2–4). This includes strong expression in the roof of the fourth ventricle, where expression is localized to choroid plexus epithelium and midline roof plate ependyma (Figs. 3b and 4a). The transgene is also expressed focally in the paramedian ependyma of the floor of the metencephalon (Figs. 3b and 4b), but only in a few ependymal cells opposite the site of the motor neurons at the

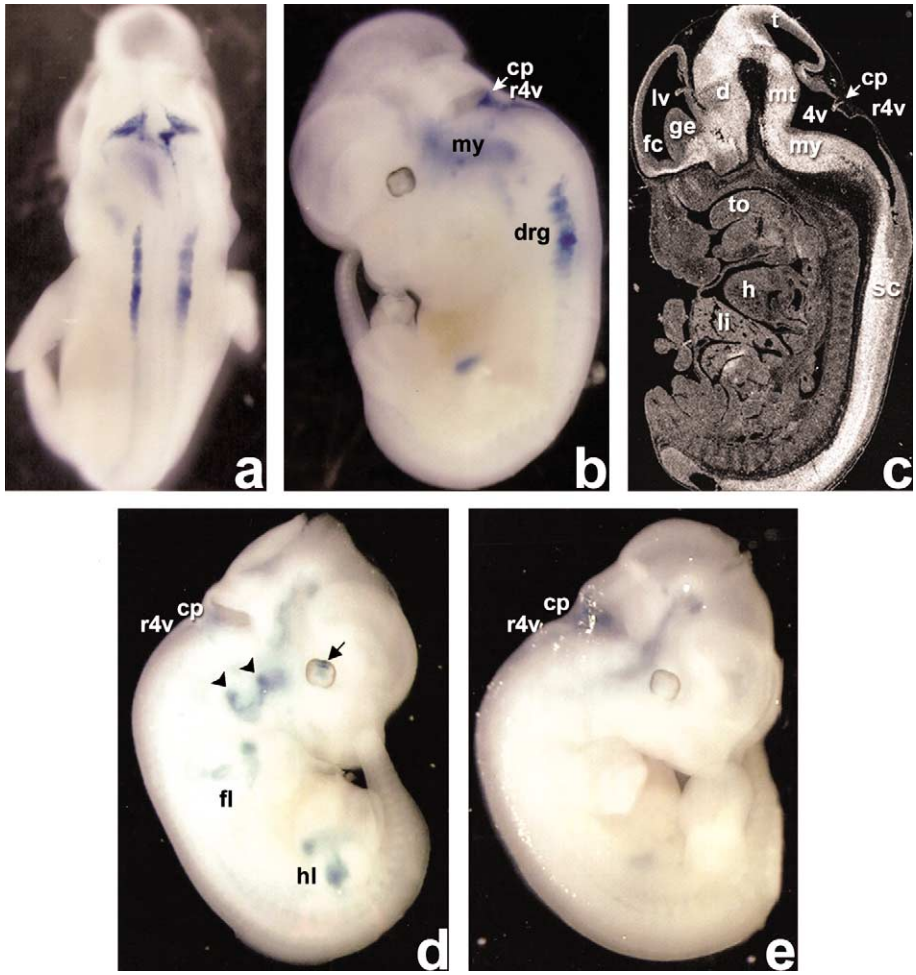

Fig. 2. Pr1.8-βgal transgenic mouse embryos following whole-mount staining with X-gal. The blue color indicates transgene expression. (a) Dorsal and (b) lateral views of embryo #1 at E12.5. (c) Sagittal section of a normal mouse embryo at E12.5 following tissue in situ hybridization with a mouse *DSCAM* probe [6]. (d) Lateral view of embryo #2 at E12.5. The expression indicated by the arrowheads may reflect transgene expression in facial and cranial nerve ganglia (see Figs. 3 and 4), but may also correspond to migrating neural crest. (e) Lateral view of embryo #3 at E12.5. Cp, choroid plexus; r4v, roof of fourth ventricle; my, myelencephalon; drg, dorsal root ganglia; fc, frontal cortex; lv, lateral ventricle; ge, ganglionic eminence; d, diencephalon; t, tectum; mt, metencephalon; 4v, fourth ventricle; to, tongue; h, heart; li, liver; sc, spinal cord; fl, forelimb; hl; hindlimb. The arrow in (d) indicates transgene expression in the eye.

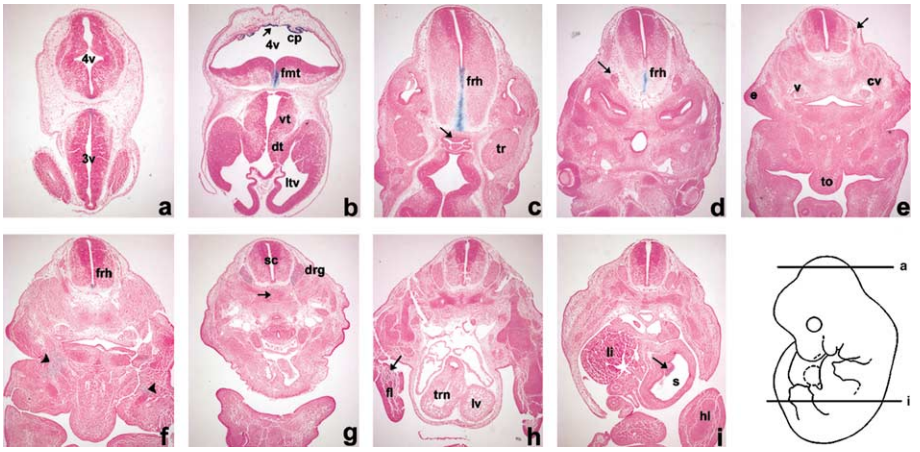

Fig. 3. Transverse sections through Pr1.8-βgal transgenic mouse embryos at E12.5 following whole-mount staining with X-gal. Sections were cut from rostral (a) to caudal (i) between the boundaries indicated in cartoon and reveal Pr1.8-βgal expression in the following regions: (a) the roof of the hindbrain; (b) the roof of the fourth ventricle (arrow), the choroid plexus (cp), and the floor of the metencephalon (fmt); (c) the floor of the rhombencephalon (frh), trigeminal ganglion (tr), and infundibulum (arrow); (d) the floor of the rhombencephalon (frh) and the superior ganglion of vagus–cranial accessory ganglion complex (arrow); (e) the inferior ganglion of the vagal nerve (v) and the spinal accessory nerve (arrow); (f) the pattern of expression indicated by arrowheads may correspond to migrating neural crest; (g) the dorsal root ganglia (drg) and notochord (arrow); (h) the forelimb (fl); and (i) the stomach (s). Abbreviations: 4v, fourth ventricle; 3v, third ventricle; vt, ventral thalamus; dt, dorsal thalamus; ltv, lateral ventricle; e, primordium of pinna of ear; cv, anterior cardinal vein; to, tongue; sc, spinal cord; trn, truncus; lv, left ventricle; hl, hindlimb; li, liver.

junction of the floor plate with the paramedian ependyma, and not in the more lateral ependyma. This pattern was conserved in all four embryos (data not shown). Lower levels of transgene expression can be detected in the ependyma of the third ventricle, but no expression is detected in the diencephalon (Fig. 3b). More caudally, Pr1.8-βgal is strongly expressed in the floor plate ependyma of the rhombencephalon (pons and medulla oblongata) (Figs. 3c,d and 4c,d) but is not expressed in the floor plate ependyma of the spinal cord (Figs. 3g–i). Again, this pattern was conserved in all four embryos (data not shown). Finally, coarsely granular and rod-shaped structures are visualized in the notochord (Fig. 4k), but no expression is detected in the surrounding sclerotome or prechordal mesoderm at the level of the forebrain.

Transgene expression in cranial nerve ganglia and dorsal root ganglion

Pr1.8-βgal shows conserved expression in several facial and cranial nerve ganglia, including subsets of cells in the trigeminal ganglion (Figs. 3c and 4f), the superior ganglion of vagus–cranial accessory ganglion complex (Figs. 3d and 4h) and the inferior ganglion of the vagal nerve (Figs. 3e and 4i), and in cells of the infundibulum (Figs. 3c and 4e). A more diffuse expression pattern is seen caudally (Fig. 3f), which may correspond to migrating neural crest. In the dorsal root ganglia, the transgene is expressed in specific primary sensory neurons, mostly in the ventral part (Figs. 3g and 4j), but is not expressed in the spinal cord at the same levels. Lastly, expression is seen in the stomach epithelium

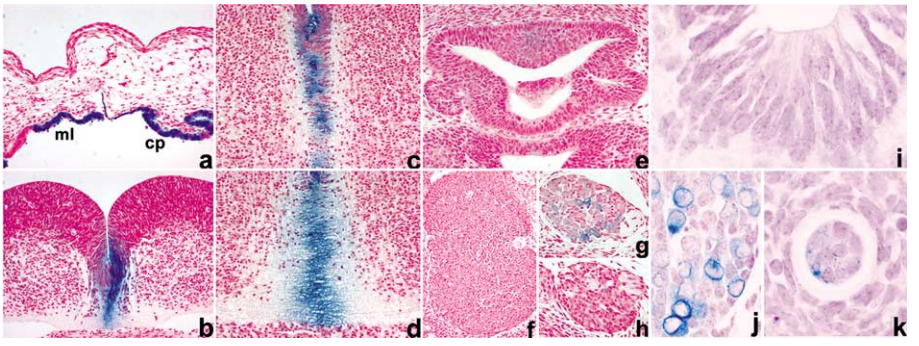

Fig. 4. Details of Pr1.8-βgal expression in: (a) the midline ependyma (ml) of the roof of the fourth ventricle and the epithelium of the choroid plexus (cp); (b) the floor plate ependyma of the metencephalon; (c) and (d) the floor plate ependyma of the rhombencephalon; (e) cells of the infundibulum; (f) cells of the trigeminal ganglion; (g) the inferior ganglion of the vagal nerve; (h) the superior ganglion of vagus–cranial accessory ganglion complex; (i) the floorplate; (j) specific primary sensory neurons of the dorsal root ganglion; and (k) the notochord (adjacent to the floorplate shown in (i)).

(Fig. 3h) which may correspond to neuroendocrine precursors.

Discussion

In this report, we describe a 1.8 kb upstream region with human DSCAM promoter activity which drives expression in subsets of CNS and other tissues. The *DSCAM* gene encodes a putative axonal guidance molecule expressed in neurons of the developing cortex, cerebellum, and spinal cord [6], and we have proposed that its overexpression may affect axonal outgrowth and/or the development of neural networks, thus contributing to cognitive defects [6]. The expression pattern of the Pr1.8-βgal transgene recapitulates a subset of the endogenous *DSCAM* expression pattern determined by tissue in situ hybridization (TISH) [4,6], supporting a role for this 1.8 kb region in endogenous control of *DSCAM* expression. Further, the pattern of transgene expression is consistent with a role for DSCAM in mediating axonal and possibly also neuroblast migrations, and may provide clues as to the roles of DSCAM in the morphogenetic processes underlying cognitive development.

Pr1.8-βgal expression pattern recapitulates a subset of DSCAM expression

The patterns of Pr1.8-βgal transgene expression shown here at E12.5 represent a subset of tissues which we have previously shown to express the *DSCAM* mRNA [4,6]. It is unlikely that these expression patterns result from insertional effects, given that the point of insertion for transgenes is random whereas the patterns of expression seen here are conserved in several different embryos. In the developing brain, the conserved pattern of transgene expression in the fourth ventricle, midbrain, and hindbrain (Figs. 2b and 3b–d) is similar to that of *DSCAM* in the developing metencephalon and myelencephalon (Fig. 2c, [6]). However, the transgene is not expressed in the diencephalon or frontal cortex (Fig. 3b) which also expresses *DSCAM* at this time (Fig. 2c, [6]). The expression of Pr1.8-βgal also corresponds to that of *DSCAM* in the choroid plexus of the fourth ventricle (Figs. 2b and c), but not in the choroid plexi of the third or lateral ventricles [4,6]. The expression of Pr1.8-βgal in the developing eye (Fig. 2d) is consistent with *DSCAM* expression in the ganglion cell layer of the retina at E16.5 [4], and the patterns of Pr1.8-βgal expression in cranial ganglia such as the vagal and trigeminal ganglia (Figs. 4f–h) and in the limb buds (Fig. 2d) also reflect patterns of *DSCAM* expression observed by TISH [4,12]. The transgene is also expressed in specific primary sensory neurons in the ventral regions of the dorsal root ganglia (DRG) (Figs. 3g and 4j), which is consistent with *DSCAM* expression in the DRG at E12.5 [4]. However, no transgene expression is detected in the spinal cord at the same levels (Fig. 3g), which is of interest as we have shown by TISH that *DSCAM* is strongly expressed in both the DRG and the ventral spinal cord at this time [4,6]. Lastly, the expression of Pr1.8-βgal in the DRG, which are of neural crest origin, as well as in a pattern which may correspond to neuroendocrine precursors (Fig. 3h) and to neural crest migration into the branchial arches (Figs. 2d and 3f), is consistent with the expression of *DSCAM* in neural crest derivatives we have previously demonstrated using TISH [4].

Taken together, these results suggest that the 1.8 kb region described here constitutes part of the endogenous *DSCAM* promoter, containing elements driving expression in the metencephalon, myelencephalon, cranial and facial nerve ganglia, limb buds, dorsal root ganglia and other neural crest derivatives, and that the promoter elements driving DSCAM expression in the diencephalon, forebrain, and spinal cord lie outside of this region. Several of the transcription factor binding sites predicted within the 1.8 kb region (Fig. 1) support the proposed roles for *DSCAM* in both CNS and heart [13] development, although the lack of Pr1.8-βgal expression in the heart (Figs. 2 and 3) shows clearly that other promoter elements are required to drive *DSCAM* expression in the heart.

Pr1.8-βgal expression in the floor plate, choroid plexus, and notochord

The Pr1.8-βgal transgene shows strong expression in the choroid plexus epithelium and midline roof plate ependyma of the fourth ventricle, as well as in the floor plate ependyma of the metencephalon and myelencephalon (Figs. 4a–d). This pattern is strongly reminiscent of the expression patterns of S-100β, a secreted neurotrophic factor, and of vimentin, an intermediate filament cytoskeletal protein, in the human fetus at 10–14 weeks gestation [14,15]. The expression of Pr1.8-βgal in these ependymal cells is of interest given the roles they are thought to play in axonal and neuronal migrations: the basal processes of floor plate ependyma are thought to guide the trajectories of axons of the developing long tracts of the brainstem [14,15]; and at least in the mouse, the choroid plexus is thought to repel migrating cortical neurons and ganglionic eminence neurons [16]. We have previously suggested that DSCAM functions as a guidance receptor in both axonal and neuronal migrations, as do related immunoglobulin superfamily members such as DCC [17,18], and the identification of DSCAM promoter activity in these cells, particularly in their basal processes, during this time in development may support this hypothesis.

Lastly, the ependymal cells of the floor plate are induced by *Sonic hedgehog* (*Shh*), which is secreted by the underlying notochord, and potentiated by retinoic acid [14,15], both of which are molecules known to function in the specification of ventral cell fates [2,3]. DSCAM is expressed specifically in the ventral regions of the developing spinal cord [6] and we have previously suggested that it may mediate some of the downstream effects of *Shh* and retinoic acid [6]. The identification of DSCAM promoter activity in cells of the notochord (Fig. 4) may support a role for DSCAM in these pathways, and may also support our hypothesis that DSCAM is not directly controlled by the homeodomain transcription factor "code" specifying the fates of the distinct neuronal subpopulations within the ventral spinal cord [19,20].

In summary, we have identified a 1.8 kb fragment of the putative promoter region of the human *DSCAM* gene which drives expression in subsets of CNS and other tissues. The pattern of transgene expression revealed in mouse embryos supports a role for DSCAM in the processes underlying the development of neural networks. Understanding this role will be critical in defining how perturbations of these neural networks give rise to genetically determined cognitive deficits. Moreover, the use of this and other promoter elements in specific overexpressing models may elucidate the contribution of DSCAM overexpression to DS defects in neuronal morphogenesis, and thus to the cognitive defects of DS.

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