Complex Patterns and Simple Architects: Molecular Guidance Cues for Developing Axonal Pathways in the Telencephalon

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1 Introduction

The aim of this review is to discuss the current research on the spatio-temporal distribution and function of the four major classes of axonal guidance cues (netrins, semaphorins, slits, and ephrins) and their receptors in the developing mammalian telencephalon. In the first part, we briefly describe guidance molecules and their receptors. In the second part, we review their overlapping distribution in the specific architectonic zones of the cerebral wall during the embryonic and early postnatal period. In the third part, we describe complementary and/or overlapping functions of these molecules in the development of all major classes of telencephalic axon pathways: subcortical (thalamic and extrathalamic) afferent systems, corticofugal (projection) systems, and cortico-cortical (commissural and ipsilateral) fiber systems. To conclude, we discuss several general themes which emerge from the current research, and point out that most axonal guidance cues have other developmental roles as well, including possible involvement in synaptic plasticity in the adult brain.

2 The Four Major Classes of Axonal Guidance Cues Are Netrins, Semaphorins, Slits, and Ephrins

Growth cones of developing axon pathways successfully navigate through the complex environment of the fetal telencephalon to their intermediate and final targets, by responding to a variety of substrate-bound or diffusible molecular signals (Tessier-Lavigne and Goodman 1996; Mueller 1999). These molecular guidance cues on cell surfaces and

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in the extracellular matrix (ECM) are expressed in stereotypical patterns and either positively or negatively regulate the direction and speed of axon growth and the degree of axon fasciculation (Letourneau et al. 1994; Goodman 1996; Mueller 1999). The molecules anchored to the cell membrane or localized to the ECM surrounding the cell that secretes them serve as local guidance cues and influence the behavior of growth cones via contact-mediated mechanisms. Soluble molecules secreted by the intermediate or final targets of growing axons serve as long-range guidance cues, and growth cones advance up or down a concentration gradient of a particular guidance cue (Goodman 1996; Mueller 1999).

Axonal guidance cues represent a complex set of signaling molecules: cell surface receptors and adhesion molecules, nondiffusible ECM molecules and short-range ECM-bound guidance cues; and diffusible long-range attractants or repellents (Tessier-Lavigne and Goodman 1996; Chisholm and Tessier-Lavigne 1999). Many of the molecular guidance cues and their receptors are evolutionarily conserved, displaying similarities in the structure and functional roles in organisms as different as a nematode (*C. elegans*) or a fruitfly (*Drosophila*) and mammals, including humans (Chisholm and Tessier-Lavigne 1999). The four major families of recently identified axonal guidance molecules include the netrins, semaphorins, slits, and ephrins (Culotti and Kolodkin 1996; Flanagan and Vanderhaeghen 1998; Chisholm and Tessier-Lavigne 1999; Mueller 1999; Raper 2000; Mellitzer et al. 2000).

2.1 Netrins Usually Function as Chemoattractants and Bind to Deleted in Colorectal Cancer and Neogenin

The netrins, a small family of secreted laminin-related proteins, were the first diffusible chemoattractant molecules identified in the vertebrate central nervous system (Kennedy et al. 1994; Serafini et al. 1994). Receptors for netrins are Deleted in Colorectal Cancer (DCC) and the neogenin (Mueller 1999; Chisholm and Tessier-Lavigne 1999). The netrins are expressed along the ventral midline of the CNS in both invertebrates and vertebrates, and they attract commissural axons to the midline (Colamarino and Tessier-Lavigne 1995; Culotti and Kolodkin 1996). Mutations in mouse netrins result in defects in commissure formation in both the spinal cord and the forebrain (Serafini et al. 1996). In the spinal cord, Netrin-1-responsive commissural axons express cell adhesion molecule TAG-1 as they approach the midline, and cell adhesion molecule L1 after they cross it (Dodd et al. 1988). However, in the

forebrain, commissural axons retain the expression of both the TAG-1 and the L1 after they cross the midline (Fujimori et al. 2000). Netrins are also chemoattractants for cerebellofugal axons in the hindbrain (Shirasaki et al. 1995). However, netrins can function as chemorepellents for certain classes of axons – for example, axons of the trochlear nerve are repelled away from the midline by netrin (Varela-Echevarria et al. 1997).

2.2
Semaphorins Act as Chemorepellents for Most and Chemoattractants for Some Axons and Bind to Neuropilins, Plexins, and L1

The semaphorins act as chemorepellents for most axons and as chemoattractants for some growing axons (Mark et al. 1997; Raper 2000). The semaphorins are a large family of transmembrane and secreted molecules, currently classified into eight groups (Chisholm and Tessier-Lavigne 1999; Semaphorin Nomenclature Committee 1999; Nakamura et al. 2000). Classes 3–7 are vertebrate semaphorins; class 3 semaphorins are secreted proteins, classes 4–6 are transmembrane proteins, and class 7 are GPI-anchored proteins (Nakamura et al. 2000). The semaphorins function is mediated by receptor complexes composed of neuropilins and plexins (Tamagnone and Comoglio 2000). It has been suggested that neuropilins are ligand-binding subunits and plexins are signaling subunits of class 3 semaphorin holoreceptor complexes (Raper 2000; Liu and Strittmatter 2001). In addition, it has been recently found that the cell adhesion molecule L1 is an essential component of the Sema3A receptor complex (Castellani et al. 2000). Recent studies suggest that Neuropilin-1 is the major receptor for the most studied Sema3A (He and Tessier-Lavigne 1997; Kolodkin et al. 1997), whereas Neuropilin-2 is the major receptor for Sema3F in the nervous system (Chen et al. 2000; Giger et al. 2000). Mutant Sema3A or Neuropilin-1 knock-out mice display very similar and striking defects in peripheral nerve projections (Behar et al. 1996; Kitsukawa et al. 1997; Taniguchi et al. 1997; Catalano et al. 1998), but many major CNS axon projections develop normally (Catalano et al. 1998). In contrast to Neuropilin-1 mutant mice, which die during the second half of gestation (Kitsukawa et al. 1997), many Neuropilin-2 mutant mice are viable into adulthood and thus suitable for the detailed analysis of axon guidance defects (Chen et al. 2000; Giger et al. 2000).

Sema3A acts as a chemorepellent for a number of axonal classes in vitro and in vivo: most rat cranial motor axons (Varela-Echavarria et al. 1997), cerebellar mossy fibres (Rabacchi et al. 1999), and neurites

growing from pontine, cortical, and thalamic explants in vitro (Bagnard et al. 1998).

2.3 Slits Are Midline Repellents That Bind to Robo Receptors

Slits are secreted proteins involved in axon guidance and neuronal migration (Chisholm and Tessier-Lavigne 1999; Mueller 1999). Their receptor is the transmembrane protein Robo (Kidd et al. 1998a, b). Three *slit* genes (*slit1*, *slit2*, *slit3*) and three robo genes (*robo1*, *robo2*, *rig-1*) have been identified in mammals (Holmes et al. 1998; Itoh et al. 1998; Brose et al. 1999; Yuan et al. 1999). In *Drosophila*, Slits are repellent proteins made by midline glial cells (Kidd et al. 1998a, b, 1999), and play an important role in preventing commissural axons that have crossed the midline from recrossing the midline (Battye et al. 1999; Kidd et al. 1999). Commissural axons express low levels of Robo receptors on their surfaces prior to crossing the midline, due to the action of a negative regulator, the Commissureless protein (Kidd et al. 1998a, b). Thus, axons are able to cross the midline a single time. After crossing, however, the expression of Robo is dramatically upregulated, axons become highly responsive to the Slit repellent, and can no longer recross (Kidd et al. 1998a, b).

In vertebrates, commissural axons in the spinal cord are also responsive to the repellent activity created by floor plate cells; however, this activity is dependent on both Slits and Semaphorins (Zou et al. 2000). Slits are also expressed at the midline of the brain stem and the prosencephalon. For example, in the mouse, Slit proteins repel retinal axons in vivo, and cooperate to establish a corridor through which the axons are channeled and the optic chiasm forms in the ventral diencephalon (Erskine et al. 2000; Ringstedt et al. 2000; Plump et al. 2002). The role of Slits in the telencephalon is described below.

2.4 Ephrins and Eph Receptor Tyrosine Kinases Are Involved in Bidirectional Signaling

Ephrins are cell surface-associated ligands for Eph receptor tyrosine kinases implicated in repulsive axon guidance, cell migration, topographic mapping and angiogenesis (Flanagan and Vanderhaeghen 1998; Mellitzer et al. 2000; Knöll and Drescher 2002). In vertebrates, they comprise two major subclasses: EphA receptors and EphrinA ligands, and EphB receptors and EphrinB ligands (Mellitzer et al. 2000). Ephrin-

A1–A5 ligands are tethered to the cell surface by a GPI-anchor and bind EphA1–A8 receptors, whereas Ephrin-B1–B3 ligands are transmembrane proteins which posses a cytoplasmic portion and bind to EphB1–B6 and EphA4 receptors (Flanagan and Vanderhaeghen 1998; O'Leary and Wilkinson 1999; Klein 2001). In the Ephrin-Eph receptor signaling system, both classes of molecules function as receptors and as ligands. This phenomenon is described as bi-directional signaling and involves the mutual activation of particular signaling pathways in both of the interacting cells (Knöll and Drescher 2002). Whereas most receptor tyrosine kinases have soluble ligands, Ephrins must be membrane-bound in order to function properly (Knöll and Drescher 2002). The roles of Ephrins and Eph receptors in the developing telencephalon are described below.

3 Axonal Guidance Cues Display a Characteristic Spatio-Temporal Pattern of Expression in Embryonic and Fetal Zones of the Telencephalon

Fetal development of the telencephalon is characterized by continuous transformations and reorganization of the fetal cerebral wall which consists of transient, cytoarchitectonically defined compartments, the fetal zones (Rakic 1982; Kostovic and Rakic 1990; Kostovic et al. 1995, 2002; Kostovic and Judaš 2002). The cellular and fiber content of these zones is permanently changing; thus, fetal neuronal circuitry elements (afferent fibers, synapses, postsynaptic neurons) display transient patterns of areal, laminar, and modular organization (Kostovic et al. 1995). The fetal zones also display specific spatio-temporal patterns of expression of the ECM components and axonal guidance cues (Letourneau et al. 1994; Pearlman and Sheppard 1996; Kostovic et al. 2002). At least in the developing human brain, differences in the relative abundance and nature of ECM molecules enable the selective visualization of fetal zones (Kostovic et al. 2002). The transient subplate zone is a key compartment for transient fetal neuronal circuitry and competitive cellular interactions within the subplate zone are crucial for the areal specification of the cerebral cortex and the formation of cortical connectivity (Kostovic and Rakic 1990; Allendoerfer and Shatz 1994; Kostovic and Judaš 1995).