CHAPTER 2

Netrins and Their Receptors

Simon W. Moore, Marc Tessier-Lavigne and Timothy E. Kennedy*

Abstract

Netrins are a family of proteins that direct cell and axon migration during development. Three secreted netrins (netrin-1, -3 and -4) have been identified in mammals, in addition to two GPI-anchored membrane proteins, netrin-G1 and G2. Orthologues of netrin-1 play a highly conserved role as guidance cues at the midline of the developing CNS of vertebrates and some bilaterally symmetric invertebrates. In vertebrates, floor plate cells at the ventral midline of the embryonic neural tube secrete netrin-1, generating a circumferential gradient of netrin protein in the neuroepithelium. This protein gradient is bifunctional, attracting some axons to the midline and repelling others. Receptors for the secreted netrins include DCC (deleted in colorectal cancer) and the UNC5 homologues: UNC5A, B, C and D in mammals. DCC mediates chemoattraction, while repulsion requires an UNC5 homologue and, in some cases, DCC. The netrin-G proteins bind NGLs (netrin G ligands), single pass transmembrane proteins unrelated to either DCC or the UNC5 homologues. Netrin function is not limited to the developing CNS midline. Various netrins direct cell and axon migration throughout the embryonic CNS, and in some cases continue to be expressed in the mature nervous system. Furthermore, although initially identified for their ability to guide axons, functional roles for netrins have now been identified outside the nervous system where they influence tissue morphogenesis by directing cell migration and regulating cell-cell and cell-matrix adhesion.

Introduction

The discovery of netrins can be traced back to insights provided by Santiago Ramón y Cajal at the end of the 19th century, when he proposed that axons may be guided by diffusible cues.[1] Upon observing, in fixed sections, the projections of spinal commissural neuron axons towards the ventral midline of the embryonic spinal cord, he hypothesized that floor plate cells at the midline secreted a diffusible cue that established a chemotropic gradient in the neuroepithelium (Fig. 1A). Direct evidence of chemotropic axon guidance began to accumulate in the 1980s through single cell turning assays and coculture of explanted embryonic neural tissue.[2] Notably, explants of embryonic rat spinal floor plate, when cultured at a distance from explants of dorsal spinal cord, evoked commissural axon outgrowth (Fig. 1D),[3] and an ectopic floor plate cocultured alongside an embryonic spinal cord attracted commissural axons, deflecting them away from their normal dorsal-ventral trajectory (Fig. 1E).[4] These findings provided strong evidence for the existence of a chemotropic axon guidance factor(s) secreted by the floor plate.

In parallel, studies in the nematode *Caenorhabditis elegans* identified genes required for circumferential axon guidance.[5,6] One of the genes identified, *unc-6*, encoded a secreted

*Corresponding Author: Timothy E. Kennedy—Centre for Neuronal Survival, Montreal Neurological Institute, McGill University, 3801 University Avenue, Montreal, Quebec, H3A 2B4, Canada. Email: timothy.kennedy@mcgill.ca

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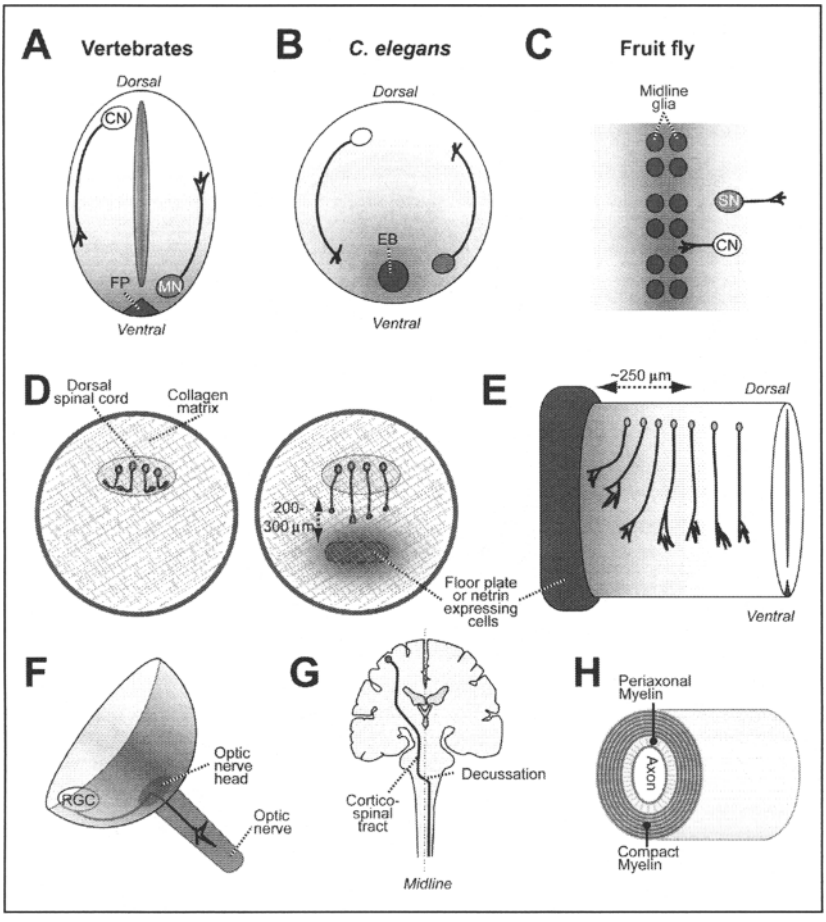


Figure 1. Netrins are important midline axon guidance cues: A) Netrin-1 secreted by the floor plate (FP) attracts commissural neuron (CN) axons and repels motoneuron (MN) axons from the ventral midline. B) During early neural development in *C. elegans*, axons are guided towards and away from a row of epidermoblasts (EB) expressing the netrin homologue UNC-6 at the ventral midline. C) Netrin-A and -B emanating from midline glia guides commissural (CN) axons to and segmental nerve (SN) axons away from the *D. melanogaster* midline. D) Embryonic spinal commissural axon outgrowth assay: An explant of dorsal embryonic rat spinal cord containing the commissural neuron cell bodies is embedded in a collagen matrix. In the absence of a source of netrin-1, such as the floor plate, the extending axons remain within the explant. In the presence of netrin-1, the axons emerge from the explant and grow into the collagen. E) Embryonic spinal commissural axon turning assay: A segment of embryonic rat spinal cord is embedded into a collagen matrix and an explant of the floor plate is grafted onto one end. Neurons within ~250 µm of the ectopic floor plate turn away from their normal dorsal to ventral trajectory and grow toward the grafted floor plate. F) Netrin-1, expressed at the optic nerve head, is required for retinal ganglion cell (RGC) axons to exit from the retina into optic nerve. G) Netrin and its receptors DCC and UNC5C are required for the decussation of the corticospinal tract at the spinal medulla boundary. H) In the mature mammalian CNS, netrin-1 is localized to periaxonal myelin suggesting a role regulating interactions between axonal and oligodendroglial membranes. Panels A, D, E and H have been reprinted from Current Opinions in Neurobiology 16:529-534 with permission from Elsevier, ©2006.[124]

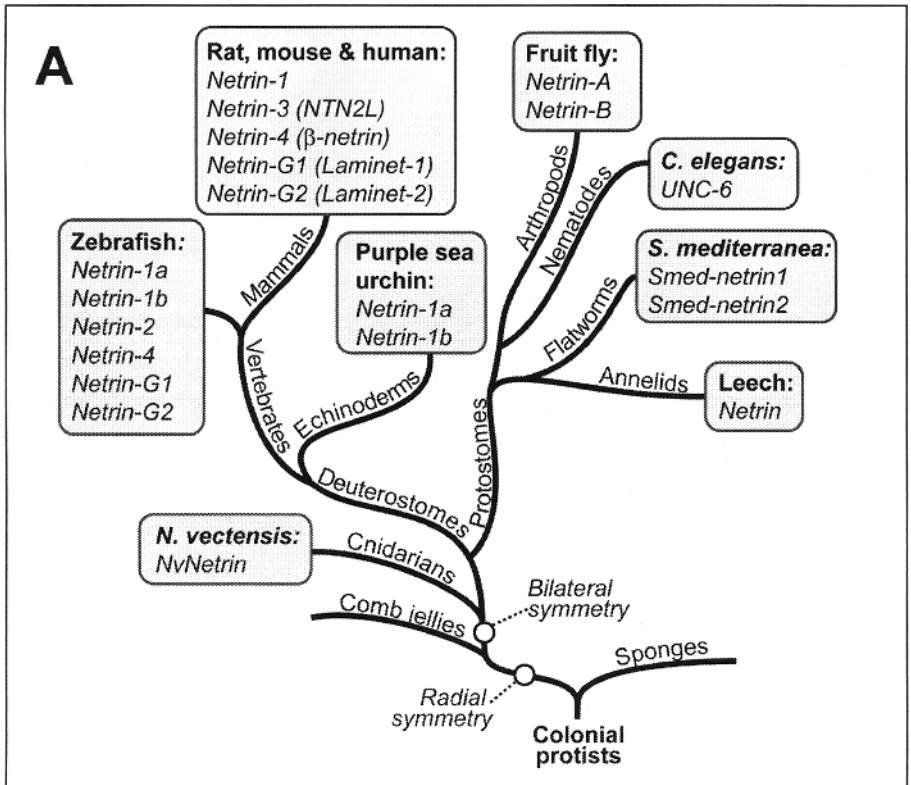
protein with sequence homology to laminins.[7] In 1994, using commissural axon outgrowth from explants of embryonic rat dorsal spinal cord as a functional assay, two proteins were purified from homogenates of embryonic chick brain and discovered to be homologous to UNC-6.[8] They were named netrin-1 and netrin-2 based on the Sanskrit word 'netr' meaning 'one who guides'. Netrin-1 is expressed by floor plate cells[9] and forms a gradient in the spinal neuroepithelium as commissural axons extend to the floor plate.[10] Engineering an aggregate of cells to express either netrin-1 or netrin-2, mimicked the commissural axon guidance activity of the floor plate (Fig. 1D-E).[9] Identification of the mouse ortholog of netrin-1, and generation of netrin-1 mutant mice, demonstrated that netrin-1 is essential for appropriate spinal commissural axon extension in the embryonic spinal cord.[11] In parallel, *C. elegans unc-6* was shown to be expressed at the ventral midline.[12] and to function as a long-range midline attractant guidance cue.[13] Furthermore, two netrins, Netrin-A and Netrin-B, were implicated in midline attraction in *Drosophila,*[14,15] although in this case netrin mediated attraction is apparently only essential at short-range close to the midline.[16] Thus, a century after chemotropic mechanisms were proposed to direct axon guidance, netrins were identified as diffusible chemotropic cues that guide spinal commissural axon extension, with homologues implicated in long- and short-range guidance in worms and flies. Netrins are now known to function not only as attractants, but also as repellents, and to be essential for the development of numerous axonal tracts.

Netrin Structure

Netrins are highly conserved in the course of animal evolution. Illustrating this, a netrin homologue has recently been identified in the sea anemone *Nematostella vectensis*, an organism thought to exhibit some of the earliest hallmarks of bilateral symmetry (Fig. 2A).[17] Vertebrate species express the secreted netrins, netrins 1-4, and two related GPI-anchored membrane proteins, netrin-G1 and -G2 (Fig. 2A). All netrins are composed of approximately 600 amino acids, and have a molecular mass of approximately 70 kilodaltons. They share two characteristic amino terminal domains, V and VI, that are homologous to domains V and VI found at the amino terminal ends of laminins (Fig. 3A). Laminins are large secreted heterotrimers made up of α, β, and γ subunits.[18] Domains V and VI of netrin-4 and netrin-Gs are most similar to β subunits of laminins, while those of netrins 1-3 are more similar to the γ subunits (Fig. 3C).[19]

Netrins 1, 3, 4, G1 and G2 are expressed in mammals, including rats, mice and humans, whereas orthologues of netrin-2 have thus far only been identified in chicken[8] and zebrafish.[20] The amino acid sequences of netrins 1-3 are highly similar (Fig. 3C) and, consistent with this, cellular sources of any of these proteins mimic the chemoattractant function of the floor plate.[8,9,21] The sequences of netrin-4 and netrin-Gs are substantially divergent, notably exhibiting a higher degree of homology to laminins than to netrins 1-3 (Fig. 3C).[22-25] Orthologues of netrin-4 or the netrin-Gs have thus far only been found in vertebrates, while orthologues of netrins 1-3 have been identified in distantly related animals, including the nematode worm *C. elegans,*[7] the flatworm *Schmidea mediterranea,*[26] the fruit fly *Drosophila melanogaster,*[14,15] the leech *Hirudo medicinalis*[27] and the sea anemone *Nematostella vectensis* (Fig. 2A).[17]

In laminins, domain VI, approximately 300 amino acids in length, is capable of binding heparin, cell surface receptors and ECM proteins[28,29] and is required for calcium-dependent multimerization between laminin molecules.[30] Mutational studies carried out in *C. elegans* indicate that domain VI of netrin is critical for both axon attraction and repulsion.[31] The motif SXDXGXS/TW is present in domain VI of all netrins and mutation of these residues in the *C. elegans* netrin UNC-6 disrupts guidance functions.[19,31] Interestingly, only the β subunits of laminin contain this motif. This is noteworthy because, as described above, netrins 1 through 3 are most homologous to the γ chain. Domain VI of netrins 1-3 also contains two cysteine residues not present in other netrins or laminins. One of these cysteines replaces a tryptophan that is strictly conserved among laminin subunits.[19] Domain V of netrins contains three tandem arrays of cysteine-rich epidermal growth factor (EGF) repeats named V-1, V-2 and V-3, and is approximately 150 amino acids in size.[7] Mutation of domain V-3 in the *C. elegans* netrin



B

	DCC	UNC5	NGL
Mammals	Neogenin DCC	UNC5A *(UNC5h1)* UNC5B *(UNC5h2)* UNC5C *(UNC5h3, RCM)* UNC5D *(UNC5h4)*	NGL-1 NGL-2
Chicken	Neogenin cDCC	UNC5h2 cUNC5h3	
Zebrafish	zDCC zNeo	UNC5a UNC5b	
X. laevis	xDCC	xUNC5	
Fruit fly	Frazzled	UNC5	
C. elegans	UNC-40	UNC-5	
S. mediterranea	Smed-netR		

Figure 2. Netrins and their receptors in various organisms: A) Evolutionary tree diagram highlighting the presence of netrin homologues in a wide variety of bilaterally symmetrical organisms. B) Netrin 1-3 receptors (DCC and UNC5) and the netrin-G receptors (NGL) in various organisms.

UNC-6 disrupts attractant mechanisms, whereas repulsion is lost following mutation of either V-2 or V-3 domains.[12,31]

Netrins 1-4 contain a conserved carboxyl terminal domain, domain C (Fig. 3A), that has a predicted α-helical secondary structure and is homologous to domains found in the complement C3, 4 and 5 protein family (CC3, 4 and 5), secreted frizzled-related proteins (sFRP), type

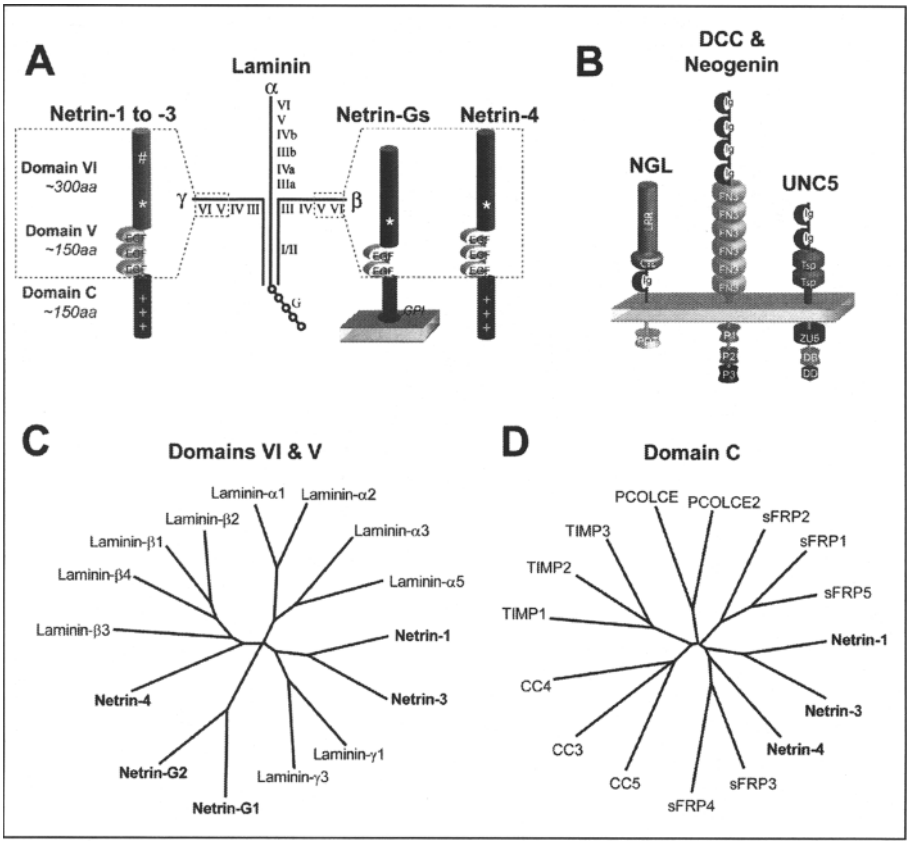


Figure 3. Netrin and netrin receptor structure: A) All netrins contain amino terminal domains V and VI related to corresponding amino terminal domains of laminins. Domain V is composed of cysteine-rich epidermal growth factor (EGF) repeats. Domain C in secreted netrins contains many positively charged, basic residues. B) DCC and UNC5 are receptors for netrin-1 to -3. NGL1 and NGL2 are receptors for netrin-G_1 and -G_2, respectively. C) Tree illustrating a phylogenetic relationship based on sequence of the VI and V domains in human netrins and laminins. D) Phylogenetic tree based on human protein sequences related to the C domain of netrin-1 (see text for details). Panels A and B have been reprinted with permission from 'A830: Netrins' in the Encyclopedia of Life Sciences by John Wiley & Sons, Ltd.

I C-proteinase enhancer proteins (PCOLCEs) and tissue inhibitors of metalloproteinases (TIMPs) (Fig. 3D). Deletion of domain C from UNC-6 netrin in *C. elegans* does not appear to disrupt axon guidance, although increased axon branching has been detected.[32] Most netrin-1 protein in the vertebrate CNS is not freely soluble, but bound to cell surfaces or extracellular matrix.[33,34] A notable feature of the netrin C domain is that it contains many basic amino acids. It has been hypothesized that these may bind to negatively charged sugars associated with proteoglycans on cell surfaces, such as heparin sulfate proteoglycans and chondroitin sulfate proteoglycans.[8,35,36] Presentation of netrins closely associated with cell surfaces may be a common mode of action in the netrin family. Although the C domain is not conserved in the netrin-Gs, a C terminal GPI-link anchors them to cell surfaces.

Functional Roles for Netrins during Nervous System Development

During embryogenesis in *C. elegans* and *D. melanogaster*, secretion of the netrin UNC-6 and netrins A/B respectively, are essential for orienting cell and axon migration with respect to the ventral midline of the developing nervous system (Fig. 1B,C).[6,7,15,37,38] Similarly, netrin-1 expressed by the floor plate in mouse plays an essential role directing axon extension relative to the ventral midline of the embryonic spinal cord. Netrin-1 deficiency in mouse also disrupts the formation of major axon projections to the midline in brain, including the corpus callosum and hippocampal commissure,[11] indicating that numerous axon tracts require netrin-1 to cross from one side of the CNS to the other. Acting as a repellent, netrin-1 directs axon extension by subsets of motoneurons, including: trochlear motoneurons,[39] cranial motoneurons[40] and spinal accessory motoneurons.[41]

Away from the midline, netrin-1 expression at the optic nerve head is required for the axons of retinal ganglion cells to exit the retina and enter the optic nerve (Fig. 1F).[42] Netrin-1 is also implicated in the guidance of dopaminergic axons within the ventral midbrain,[43] in the thalamocortical projection,[44] as well as in the formation of axon projections within the hippocampus.[45]

In contrast to netrin-1, the function of other netrin family members in vertebrates is relatively poorly understood. Netrin-3 can mimic the ability of netrin-1 to attract spinal commissural axons and repel trochlear motor neuron axons in vitro,[21] however, netrin-3 expression in the spinal cord begins after the initial commissural axons have pioneered the path to the floor plate. Netrin-3 is, however, expressed in dorsal root ganglia in the developing PNS, and by mesodermal cells that may influence axon guidance to peripheral targets.[46] Netrin-4 is widely expressed in the developing nervous system, including in the olfactory bulb, retina, dorsal root ganglia, as well as by cerebellar granule, hippocampal, and cortical neurons.[22] In the developing spinal cord, a relatively low level of netrin-4 is expressed adjacent to floor plate cells; however, like netrin-3, this begins after the first commissural axons have crossed the midline. Both netrin-G1 and -G2 are expressed primarily by neurons, with very limited expression outside the nervous system.[25,47] Netrin-G1 is expressed in the dorsal thalamus, olfactory bulb and inferior colliculus, while netrin-G2 is expressed in the cerebral cortex. *Netrin-G1* gene mutations in humans produce symptoms similar to Rett syndrome,[48] characterized by normal early development followed by loss of purposeful use of the hands, distinctive hand movements, slowed brain and head growth, gait abnormalities, seizures, and mental retardation. Netrin G1-deficient mice have no obvious abnormalities in gross anatomy and neural circuitry, but exhibit altered synaptic responses and defects in sensorimotor gating behavior.[49] These findings led to the suggestion that the major role for netrin-G proteins may be in the maturation, refinement, and maintenance of synapses, rather than axonal outgrowth and guidance. Consistent with this, the netrin-G receptor NGL-2 influences the formation of glutamatergic synapses through an interaction with the post-synaptic scaffold protein PSD-95.[50]

Netrin Signal Transduction

The signal transduction mechanisms regulated by netrins are currently the subject of intense scrutiny. The majority of the studies carried out have focused on the role of netrin-1 as a chemoattractant axon guidance cue and comparatively little is known regarding signal transduction by other netrins. The following provides an overview of signal transduction events implicated in the response to netrin-1, for a detailed (for a detailed review see ref. 51,52).

Netrin receptors in vertebrates include DCC (deleted in colorectal cancer), the DCC paralogue neogenin, and four UNC5 proteins, UNC5A-D (Fig. 2B). Although DCC, neogenin, and the UNC5 proteins all bind netrin-1, the majority of studies of netrin signaling have focused on DCC. Attractant responses to netrin-1 require DCC. In contrast, repellent responses require expression of an UNC5 protein, with coexpression of DCC in some cases. Interestingly, neogenin also interacts with a GPI-linked protein called Repulsive Guidance Molecule.[53]

Netrin-1 Mediated Chemoattraction

Unc-40 encodes the *C. elegans* orthologue of DCC.[6,54] *C. elegans unc-40* mutants predominantly exhibit defects in ventrally-directed migration of cells and axons, in contrast to *unc-6 (netrin)* mutants in which migrations both toward and away from the ventral midline are disrupted. Consistent with the *unc-40* mutant phenotype in the nematode, application of DCC function blocking antibodies to explants of embryonic mouse spinal cord blocked netrin-1 induced commissural axon outgrowth.[55] Furthermore, *dcc* gene knockout produced a phenotype very similar to that generated by loss of netrin-1 function, including loss of the spinal ventral commissure, corpus callosum and hippocampal commissure.[56]

The extracellular domain of DCC is composed of six fibronectin type 3 (FN3) repeats and four immunoglobulin (Ig) repeats (Fig. 3B). The DCC FN3 domains are implicated as netrin-1 binding sites, but exactly which FN3 domain binds netrin-1 remains controversial.[57-59] The DCC intracellular domain has no known intrinsic catalytic activity, but contains several putative protein binding and phosphorylation sites. Based on particularly strong identity between DCC family members, three regions of the intracellular domain of DCC, termed domains, P1, P2 and P3, have been identified (Figs. 3B and 4A).[60] The P1 domain is a highly conserved 17 amino acid motif, the P2 domain is rich in proline residues, containing four PXXP putative SH3 domain-binding motifs (Fig. 4A), and the P3 domain contains several highly conserved possible phosphorylation sites.

The ability of a cue to attract axon growth is thought to reflect its capacity to regulate membrane protrusions made by the growth cone. Rho GTPases are a family of intracellular proteins that coordinate cytoskeletal organization and adhesive interactions.[61] In particular, the activation of the Rho GTPases Rac and Cdc42 has been shown to be essential for attractant responses to a number of guidance cues,[62,63] including netrin-1.[64,65] The exact sequence of events linking DCC to Rho GTPase activation, and their downstream effectors, remains unclear. Multimerization of the DCC P3 domain following binding to netrin-1 is implicated as an initial event in mediating chemoattraction.[66,67] The DCC intracellular domain associates with the adaptor protein Nck1,[68] the tyrosine kinases Fak[69] and Fyn,[70] the serine/threonine kinase Pak,[64] as well as the actin binding proteins Ena/Vasp[71] and N-WASP.[64] In addition to Rac and Cdc42 activation, application of netrin-1 leads to production of phosphoinositides by recruitment of phosphatidylinositol transfer protein-α,[72] activation of phosphatidylinositol-3 kinase,[73] and the breakdown of phosphoinositides by phospholipase C into IP3 and diacylglycerol (DAG).[73] IP3 promotes intracellular calcium release from intracellular stores and DAG activates protein kinase C.[74] Supporting a role for IP3 production in netrin-1 mediated chemoattraction, elevating intracellular calcium is required for turning to netrin-1.[75] Notably, such calcium increases can contribute to Rac and Cdc42 activation.[76] Figure 4C presents a speculative model of how these events may contribute to netrin-1 mediated axonal chemoattraction.

Netrin-1 Mediated Chemorepulsion

UNC5 netrin receptors were first implicated as mediators of repellent responses to the netrin UNC-6 from studies in *C. elegans*.[6,77] *Unc-5* mutants exhibit defects in dorsally-directed migrations, away from the ventral midline source of UNC-6 netrin, and misexpression of *unc-5* by neurons caused their axons to be redirected along a dorsal trajectory.[37] As in *C. elegans*, a single UNC5 family member has been identified in *D. melanogaster*.[38] Four have been found in mammals: UNC5A, B, C and D (Fig. 2B).[78-81] UNC5s are composed of two extracellular Ig domains, that bind netrin, and two extracellular Tsp (thrombospondin) domains (Fig. 3B).[58] The UNC5 intracellular domain is made up of three conserved domains: a ZU5 domain, a DCC-binding (DB) domain and a death domain (DD, Fig. 3B). The function of the ZU5 domain is unknown, however it is homologous to a sequence in the scaffolding protein Zona Occludens-1 found at tight junctions.[82]

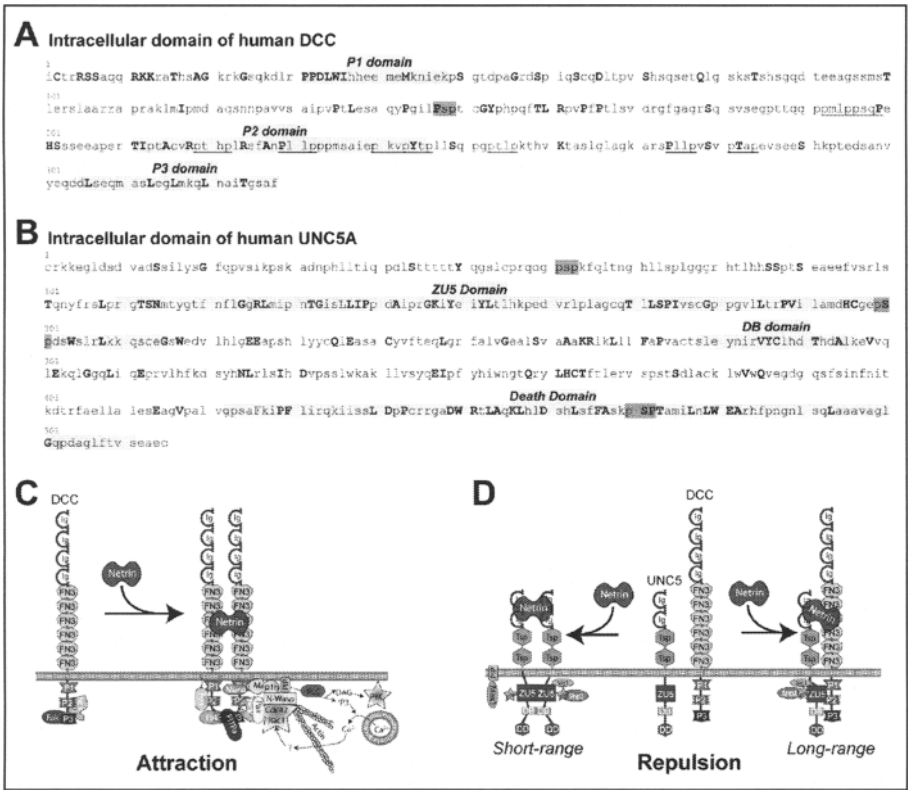


Figure 4. Model of netrin signal transduction: Amino acid sequences of the intracellular domains of human DCC (A) and UNC5A (B). Amino acids conserved between C. elegans, X. laevis and humans are in bold capital letters. Assigned domains are lightly shaded, while WW class IV motifs (PSP) are more darkly shaded. Core SH3 PXXP motifs are underlined. Panel C and D summarize signaling events involved in attractive and repellent responses, respectively (see text for details). Panels A and B have been reprinted with permission from 'A830: Netrins' in the Encyclopedia of Life Sciences by John Wiley & Sons, Ltd.

Studies in worms, flies and vertebrates suggest that long-range repulsion to netrin requires the cooperation of UNC5 and DCC, but that UNC5 without DCC is sufficient for short-range repulsion.[38,66] Although the reason for this difference is not clear, it may be the case that DCC and UNC5 together form a more sensitive netrin receptor complex that is able to respond to lower concentrations of protein found at a greater distance from a source of netrin secretion. At long-range, direct association between the cytoplasmic domains of UNC5 and DCC appears to be essential.[66,83] While mediating short-range responses to netrin independently of DCC, genetic studies in *C. elegans* have stressed the importance of the region between UNC5 cytoplasmic ZU5 and DD domains.[84] Several proteins have been proposed to interact with UNC5 family members in mediating a repellent response, including: the tyrosine kinase Src1, the tyrosine phosphatase Shp2,[85] the F-actin anti-capping protein Mena,[86] the structural protein ankryn, and the adaptor protein Max1.[87] Repellent responses to netrin-1 are thought to involve tyrosine phosphorylation of UNC5's intracellular domains at multiple sites.[85] Figure 4D outlines a speculative model of the intracellular events occurring during short and long-range repulsion.

Regulating the Response to Netrin-1

Growth cones respond rapidly to local guidance cues and exhibit substantial autonomy from the neuronal cell body. Growth cones react to netrin along a continuum that ranges from repulsion to unresponsiveness to attraction. The mechanisms that control this shift in netrin responsiveness are just beginning to be understood.

Many of the factors shown to regulate the response of growth cones to netrin can be correlated with changes in the expression of either UNC5 or DCC. At the transcriptional level, mis-expressing the homeobox transcription factor even-skipped in *D. melanogaster* resulted in disruption of *unc5* expression and motoneuron axon guidance defects.[88] Local protein synthesis within the growth cone is required for chemoattraction of cultured *X. laevis* neurons to netrin.[89] The newly synthesized proteins have been suggested to influence either the recovery of growth cones from desensitization, or netrin signal transduction directly.[90] Conversely, DCC function is negatively regulated by proteolysis, including both extracellular metalloproteinase implicated in shedding of the DCC ectodomain,[91] and ubiquitination of the DCC intracellular domain through an interaction with Siah-1, a RING domain containing protein that promotes DCC degradation via the ubiquitin-proteasome pathway.[92,93] In mammals, the intracellular domains of UNC5 proteins are substrates for caspases.[94]

Intracellular concentrations of cyclic nucleotides are key regulators of growth cone responsiveness to several guidance cues (see Chapter 10 for further discussion). Manipulating the intracellular concentration of cAMP, thereby activating protein kinase A (PKA), regulates the response of growth cones to netrin-1. Initial experiments demonstrated that axons of cultured *X. laevis* spinal neurons attracted to a pipette puffing netrin-1, were instead repelled when PKA was inhibited.[95] These studies led to the proposal that PKA can control the direction of growth cone turning by regulating intracellular signal transduction pathways downstream of netrin-1. PKA activation has been shown to selectively recruit DCC from an intracellular vesicular pool to the plasma membrane of commissural neuron growth cones, and the increased levels of DCC potentiate the outgrowth and turning response of these neurons to netrin-1.[96,97] Interestingly, activation of protein kinase C (PKC) induces endocytosis of UNC5 homologues resulting in cultured cerebellar granule cell neurons switching from chemorepellent to chemoattractant responses to netrin-1.[98] These findings suggest that extracellular factors that regulate PKA and PKC will influence axon outgrowth by determining which receptors are presented by the growth cone.

Other Potential Netrin Receptors

Other receptors, in addition to DCC and UNC5 proteins, have been suggested for netrins 1-3. The G-protein coupled adenosine receptor, A2B, was reported to bind netrin-1 and cooperate with DCC in spinal commissural axon guidance.[99] However, subsequent studies provided evidence that A2B is neither expressed by these neurons nor required for commissural axon guidance in response to netrin-1.[67] The $\alpha 6\beta 4$ and $\alpha 3\beta 1$ integrins bind netrin-1 and these interactions have been implicated in the development of the pancreas.[100] Given the homology of the N-terminus of netrin-1 to laminins, it might be predicted that netrins would bind integrins through N-terminal domains; but surprisingly $\alpha 6\beta 3$ and $\alpha 3\beta 1$ integrins interact with a highly charged sequence of basic amino acids at the C-terminus of netrin-1 that is not homologous to laminins. While these findings raise the exciting possibility that integrins may function as netrin receptors in other contexts, the significance of netrin-integrin interactions in vivo remains to be demonstrated. In contrast to the secreted netrins, netrin-Gs bind transmembrane proteins called the netrin-G ligands (NGL) (Fig. 3B) and netrin-Gs do not appear to interact with DCC, neogenin, or the UNC5 proteins.[24,101]

Netrin in the Adult Nervous System

Netrins and netrin receptors are expressed in the adult vertebrate nervous system.[9,21,22,24,25,33,102-108] Netrin-1 is expressed by many types of neurons and by myelinating

glia: oligodendrocytes in the CNS[33] and Schwann cells in the PNS.[105,107] Subcellular fractionation of CNS white matter indicated that netrin-1 is enriched in periaxonal myelin membranes (Fig. 1H),[33] suggesting that it may normally mediate interactions between axonal and oligodendrocyte membranes. Expression by mature myelinating oligodendrocytes raises the possibility that netrin-1 may influence axon regeneration. Notably, netrin-1, DCC and UNC5s influence the development of the corticospinal tract, which transmits information controlling voluntary limb movements, suggesting that netrin-1 might play an important role following spinal cord injury (Fig. 1G).[109,110] During maturation of the mammalian spinal cord, DCC expression is downregulated, while UNC5 homologue expression increases,[108] indicating that UNC5 repellent signaling may be the dominant response to netrin in the adult spinal cord.

An examination of the consequences of spinal cord injury in the adult rat found that levels of netrin-1 mRNA and protein were substantially reduced at the site of injury itself, and this decreased expression persisted for at least 7 months.[111] Netrin-1 was not associated with the glial scar, but netrin-1 was expressed in an apparently normal distribution by neurons and oligodendrocytes adjacent to the lesion. The expression of DCC and UNC5 proteins was also reduced after injury. Although DCC expression remained low, UNC5 expression recovered and subsets of neurites adjacent to the lesion exhibited elevated UNC5 immunoreactivity. These findings are consistent with earlier studies carried out in the optic nerve, indicating that both DCC and UNC5B continue to be expressed by retinal ganglion cells following axotomy, albeit at reduced levels, as their axons attempt to extend along either the injured optic nerve itself or into a growth permissive peripheral nerve graft.[106,107] While a role for netrin-1 in axon regeneration remains to be demonstrated directly, these findings suggest a role for netrin-1 as a component of CNS myelin that inhibits axon regeneration by neurons expressing UNC5 following injury.

Although the functional significance of netrin-1 expression in the adult CNS remains unknown, an intriguing hypothesis is that netrins may contribute to maintaining appropriate connections in the intact CNS by restraining inappropriate axonal sprouting. A consequence of this may be that netrins subsequently inhibit the reestablishment of connections following injury. In line with this hypothesis, studies carried out in lamprey, a primitive vertebrate with the ability to recover significant function following spinal cord transaction,[112] indicate a correlation between UNC5 expression and poor axonal regeneration following lesion.[113] Importantly, it may be possible to reverse such an inhibitory role for netrin in the adult mammalian CNS by manipulating cAMP levels within regenerating axons. As described above, increasing cAMP converts netrin-mediated repulsion to attraction, and encouraging findings indicate that increasing the concentration of cAMP in neurons promotes axon regeneration in the mature CNS following injury.[114,115]

Conclusion and Perspectives

Since their discovery a little over a decade ago, significant insight has been gained into netrin function. Extending axons have been found to be directed by netrins in multiple contexts. Netrins also direct the migration of numerous cell types during development, including: inferior olivary,[116] basilar pontine[117] and LHRH neurons,[118] as well as, striatal neuronal precursors,[119] cerebellar granule cells,[120] spinal accessory neurons[41] and oligodendrocyte precursor cells.[121,122] An exciting new avenue of research has identified roles for netrins in the morphogenesis of a variety of tissues.[123,124] Netrins are now implicated in the development of the lung,[125,126] mammary gland[127] and vascular networks.[128-131] Although aspects of this work is in its initial stages, the studies described here identify roles for netrins in axon guidance, cell migration, tissue morphogenesis, and the maintanance of appropriate cell-cell interactions, supporting the conclusion that netrins influence development in a broad range of biological contexts.

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