CMLS, Cell. Mol. Life Sci. 56 (1999) 62–68
1420-682X/99/020062-07 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Netrins and netrin receptors

F. J. Livesey

Department of Genetics and Howard Hughes Medical Institute, Harvard Medical School, 200 Longwood Ave., Boston (Massachusetts 02115, USA), Fax + 1 617 432 7595, e-mail: livesey@rascal.med.harvard.edu

Received 5 February 1999; received after revision 18 March 1999; accepted 25 March 1999

Abstract. The formation of precise connections between neurons and their targets during development is dependent on extracellular guidance cues that allow growing axons to navigate to their targets. One family of such guidance molecules, conserved across all species examined, is that of the netrin/UNC-6 proteins. Netrins act to both attract and repel the growing axons of a broad range of neuronal cell types during development and are also involved in controling neuronal cell migration. These actions are mediated by specific receptor complexes containing either the colorectal cancer (DCC) or neogenin protein, in the case of the attractive receptor, or UNC-5-related proteins, in the case of the repellent receptor. Recent work has identified a key role for intracellular cyclic nucleotide levels in regulating the nature of the response of the growing axon to netrins as either attractive or repulsive. Netrin-DCC signaling has also been shown to regulate cell death in epithelial cells in vitro, raising the interesting possibility that netrins may also regulate cell death in the developing nervous system.

Key words. Axon guidance; cell migration; netrin; DCC; neogenin; *unc-6*; *unc-5*; *unc-40*.

Introduction

Axon guidance in development of the nervous system

A fundamental concept in developmental neurobiology is that of the steering of growing axons by specific extracellular signals, or axon guidance molecules. This hypothesis was first articulated by Ramon y Cajal around the turn of the century, based on his observations of developing neurons in fixed tissue sections and experiments on peripheral nerve regeneration [1]. From these studies, he suggested the term 'growth cone' to describe the highly pleomorphic leading edge of the growing axon and also postulated the existence of diffusible substances that guide growing axons [1]. In contrast with the suggestion of guidance of growing axons by diffusible factors, Roger Sperry coined the term 'chemoaffinity hypothesis' in the 1960s to encapsulate his theory that there existed molecular markers that mapped groups of neurons to one another during development [2]. This hypothesis was based on the results of Sperry's own experiments on the regeneration of axons from the retina to the optic tectum. The molecular basis of such topographic maps was uncovered during the 1980s in a long series of experiments by Bonhoeffer and colleagues to model in vitro Sperry's topographic map between the retina and the optic tectum [3]. As part of this work, those workers found evidence for a factor produced by the posterior part of the tectum which repelled ingrowing axons, a protein they later found to be a ligand for the Eph family of receptor tyrosine kinases [4].

However, the concept of active chemoattraction of growing axons remained decidedly theoretical until the late 1970s, when Gundersen and Barrett demonstrated turning of growth cones towards a point source of nerve growth factor (NGF) [5]. Although that finding was thought to be of doubtful physiological significance, it was the first demonstration of guidance of growing axons by secreted or environmental factors. A second key experiment was that of Lumsden and Davies in the

early 1980s, which demonstrated directed, specific growth of axons towards their normal targets in vitro, using explants of tissues placed in a gel of collagen [6]. The development of this system was critical for future experiments on axon guidance and, as described below, the isolation of the first vertebrate chemoattractive factor.

During the 1980s there were several other in vitro demonstrations of axon guidance and also of the concept of chemorepulsion, that is the exclusion of axon growth from particular areas by secreted factors. In the case of repulsive factors, Keynes and colleagues demonstrated that growing motor and sensory neuron axons were excluded from a part of the chick embryo (the posterior sclerotome) by a diffusible repellent molecule [7, 8], whose identity remains to be discovered. Around the same time, Raper and colleagues isolated a protein from chick brain that caused the collapse of the leading edge of the growing axon, the growth cone, in tissue culture, which they termed collapsin [9, 10]. In what was to become a repeated finding in studies of axon guidance molecules, collapsin turned out to be a vertebrate protein closely related to the protein product of a *Drosophila* gene required for normal development of the peripheral nervous system, initially named *fasciclin IV* [11, 12]. As more vertebrate and invertebrate homologues of both of these genes were discovered, it became clear that they were members of a very diverse, evolutionarily conserved family of axon guidance molecules which was renamed the semaphorin family [12].

Netrin—the floor plate-derived axon guidance molecule

In addition to the seminal Lumsden and Davies experiment, the second demonstration of chemoattraction in the developing vertebrate nervous system during this period was that of attraction of the growing axons of a group of neurons in the spinal cord towards the structure which defines the ventral midline of the developing spinal cord, the floor plate. In this work, Marc Tessier-Lavigne and his colleagues used the collagen gel culture method to demonstrate the existence of a factor secreted by the floor plate that specifically attracted the growth of commissural axons towards the floor plate [13, 14]. In a now classical series of experiments, Tessier-Lavigne and colleagues followed up those initial observations and used the assay of commissural axon outgrowth from explants of dorsal neural tube in collagen gels *in vitro* to biochemically isolate the factor responsible [15, 16]. They isolated two proteins of approximately 80 kDa molecular weight and cloned their corresponding complementary DNAs (cDNAs), naming the genes *netrin-1* and *netrin-2*, from the Sanskrit term for 'one who guides' [15]. The two genes encode proteins that are 78% identical to one another.

An interesting twist in the isolation of the 'floor plate factor' was that netrins proved to be vertebrate homologues of the *Caenorhabditis elegans* protein UNC-6, a laminin-related molecule involved in axon guidance and cell migration [15–18]. In the worm, mutations in the *unc-6* gene disrupt both axon guidance and mesodermal cell migration [17], and studies on its expression during development suggest that, in addition to regulating dorsoventral axon guidance and mesoblast migration, UNC-6 is expressed by pioneer neurons to guide later-following axons to their targets [18].

Following the isolation of the vertebrate netrins and the recognition of their homology with UNC-6, homologues were identified in a variety of other species, including two *Drosophila* homologues (netrin A and netrin B) and a single netrin in zebrafish [19–22]. Netrin proteins are remarkably conserved across species, comprised in all cases of an N-terminus signal peptide, followed by two domains homologous to domains V and VI of the B1 chain of laminin, with the domain VI-homologous region containing the predicted three EGF-repeats, and a C-terminal basic region that is the most variable part of the protein between species [16].

Netrin receptors—DCC/UNC-40 and UNC-5 homologues

The same genetic screen in *C. elegans* that identified *unc-6* as a gene which functioned in dorsoventral axon guidance also identified two other genes acting in that genetic pathway, *unc-5* and *unc-40*, mutations in which also affected dorsoventral axon guidance [23, 24]. Specifically, mutations in *unc-5* result in disruption of dorsal axon and cell migrations, whereas ventral and longitudinal axon growth and cell migrations are unaffected. In contrast, mutations in *unc-40* result in defects in both dorsal and ventral axon guidance and cell migration [23, 24].

Both of these genes encode UNC-6/netrin receptors. The *unc-40* gene encodes a transmembrane protein of the immunoglobulin superfamily that is closely related to the protein product of the human *dcc*, or deleted in colorectal cancer, gene [25]. The *dcc* gene was identified by Vogelstein and colleagues as a gene lying within a region of chromosome 18q commonly deleted in human metastatic colorectal cancer, as the name would suggest [26, 27]. This gene was found to be expressed at highest levels in the human central nervous system and is expressed normally in the gastrointestinal tract [28]. Homologues of this gene have been identified in mouse [29], chick [30] and *Xenopus* [31], and expression studies have shown that the gene is expressed in the nervous system of all these species and is also expressed in the basal cell layer of many epithelia, such as gut, skin, lung

and bladder [30]. The DCC protein is a transmembrane protein containing four immunoglobulin domains (Ig domains), six type III fibronectin repeats (FnIII repeats) and a cytoplasmic tail with no homologies to known proteins. In addition, one other closely related protein, neogenin, has been identified in the developing chick nervous system [32].

Based on the cloning of *unc-40* and its identification as a putative receptor [25], the possible role of DCC as a netrin receptor was investigated. The mammalian DCC protein was shown to function as part of a UNC-6/netrin receptor, in that DCC-blocking antibodies inhibit netrin-stimulated outgrowth of commissural neuron axons in vitro [33]. The neogenin protein has also been shown to bind netrin-1 in vitro [33], suggesting that it, too, is a component of a netrin receptor. The *Drosophila* homologue of *DCC*, *frazzled*, was proposed to encode a netrin receptor based on the similarity of the *frazzled* and *netrin* mutant phenotypes [34]. Interestingly, DCC has also been shown to bind heparin and heparan sulphate, components of the extracellular matrix and also found on the cell surface, through its fifth fibronectin type III repeat [35]. In addition, blockade of this interaction with a monoclonal antibody that does not block netrin-DCC interactions disrupts netrin-stimulated axonal outgrowth [33], suggesting that netrin-DCC signaling is dependent on a number of molecular interactions [35]. This is reminiscent of fibroblast growth factor (FGF) receptor activation, where full activation of the receptor by FGF is dependent on heparan sulphate [35].

The *C. elegans unc-5* gene encodes a 919-amino acid transmembrane protein, the extracellular portion of which contains two immunoglobulin and two thrombospondin type 1 domains, placing it in the immunoglobulin superfamily and immediately suggesting that it functions as a cell adhesion molecule or receptor [36]. On the basis of the effects of mutations in this gene on dorsal axon guidance, it was proposed that this protein acts as a 'repellent' UNC-6/netrin receptor, that is that it mediates axon growth away from a source of UNC-6/netrin protein [36]. Three vertebrate homologues of this gene have been cloned and shown to bind netrin-1 in vitro, again supporting the proposal that UNC-5 and its homologues encode components of netrin receptors [37, 38].

Actions of netrins in vitro and in vivo

One of the clearest functions of the netrins, and their defining feature, is their demarcation of the midline for a variety of growing axons in vertebrates and invertebrates [9]. Thus, axons that normally cross the midline in the developing spinal cord in vertebrates or nerve cord in invertebrates do not do so in the absence of netrin function in mouse [39], *C. elegans* [17, 23, 24] and *Drosophila* [19, 20]. Netrin also acts to guide axon growth towards the midline at levels of the nervous system rostral to the spinal cord, such that axons that leave the developing cerebellum and cross the floor plate in vivo are attracted by floor plate explants in vitro, an effect again reproduced by aggregates of netrin-expressing cells [40]. A number of other midline-defining effects of netrins were uncovered by the described phenotype of a *netrin-1* hypomorphic mutant mouse generated by insertional mutagenesis, including the absence of a number of forebrain commissures [39].

In addition, however, netrins have a barrier function at the midline, repelling certain classes of growing axons. For example, a floor plate factor was shown to repel trochlear motor neuron axons away from the midline in vitro and this effect can be mimicked by netrin [41], which also repels cranial motor axon growth in vitro [42]. However, the floor plate was also shown to repel other classes of axons in a netrin-independent fashion [40]. Analysis of the *netrin-1* mutant revealed that, although commissural axon guidance was markedly abnormal in these animals, trochlear motor axon growth was normal, and the placement of trochlear neuron cell bodies was slightly abnormal [39]. In addition, the floor plate from *netrin-1* mutant animals retains the ability to repel trochlear neuron axons in vitro, although the same floor plate lost its ability to attract commissural axons [39]. Of note, a further surprising finding was that *netrin-1* mutant floor plates also retain the ability to cause commissural axon turning in an in vitro assay, even though the outgrowth-promoting aspect of netrin function was lost. These findings highlight the fact that the floor plate produces several factors affecting axon guidance in the developing spinal cord in addition to netrin which remain to be defined.

In addition to its effects at the midline, netrins have been implicated in axon guidance in a range of other parts of the nervous systems of vertebrates and invertebrates. The best-studied example to date is the role of *Drosophila* netrin in development of the peripheral nervous system. Both of the *Drosophila* netrin genes are expressed in discrete muscle groups in the periphery, in addition to their expression by midline glia, considered to be the fruitfly equivalent of floor plate cells [19, 20]. Misexpression of either netrin in muscle groups leads to misrouting of motor axons to inappropriate muscles, implicating netrins in motor axon guidance in the periphery. Mutations in *frazzled*, the *Drosophila* UNC-40 homologue, result in abnormal projection of motor neurons that normally innervate netrin-expressing muscles [34], whereas ectopic expression of netrins in muscles leads to rerouting of frazzled-expressing motor neurons to innervate those muscles. It is not clear

whether a similar mechanism operates in vertebrates, although DCC is expressed by developing motor neurons [22, 33] and netrin in some muscle groups in the periphery, including the extraocular muscles [22]. However, no effects of mutations in either DCC or netrin-1 on motor axon guidance in vertebrates have been reported [39, 43].

Within the developing nervous system, netrin and netrin receptor expression have been documented in a diverse group of structures, suggesting a role in axon guidance in those structures [15, 22, 44]. One particular example was that of retinal ganglion expression of DCC [22, 33] and netrin expression in the developing optic nerve [15, 22], leading to the suggestion that netrin serves to guide retinal ganglion cell axons to the optic nerve head [22, 33]. This was proven to be the case by analysis of both DCC and netrin mutant mice [45]. Netrins have also been shown to guide axons leaving the developing cerebral cortex by netrin produced by neighbouring tissues [46].

Given the clear role of netrin/UNC-6 in controlling cell migration in *C. elegans*, the possible role of netrin in regulating cell migration during vertebrate development was of obvious interest [15]. The distribution of netrin and DCC-expressing cells in the developing cerebellum and midbrain suggested an involvement of netrin signaling in controlling granule cell migration in the developing cerebellum and the development of midbrain dopaminergic neurons [22]. The *rcm* mutant mouse, in which a UNC-5 homologue is disrupted, has both midbrain and cerebellar defects attributed to aberrant cell migration [37]. In addition, aberrant migration of pontine neurons in the hindbrain has been observed in both *netrin-1* and DCC mutant mice [39, 43].

Finally, a question that has received little attention to date is the function of netrin signaling in nonneuronal tissues. Netrin and DCC are expressed in a range of epithelial cell types, including the gastrointestinal tract and skin [15, 30]. Given the original description of DCC as a putative tumour suppressor gene and the recent demonstration of a role for DCC in regulating apoptosis (described below), there is clearly much to be learned about possible netrin functions in controlling cell migration, differentiation or apoptosis.

Signal transduction by netrin receptors

Little is currently known of the signal transduction pathways downstream of netrin receptors. As stated above, the cytoplasmic tails of DCC/UNC-40 and UNC-5 have no obvious homologies to known proteins, with the exception of a short region of homology within the cytoplasmic tail of UNC-5 to a unique region in the carboxy terminus of zona occludens-1, a protein involved in the formation of intercellular junctions [38]. The elucidation of the signal transduction pathways downstream of both receptors is an obvious target for the application of the yeast two-hybrid system. Using this approach, Fearon and colleagues used the cytoplasmic tail of DCC in the yeast two-hybrid system to isolate a human homologue of the *Drosophila seven-in-absentia (sina)* gene that binds to the DCC receptor in vivo [47]. The normal function of *sina* in *Drosophila* development is not clear, although it functions in a pathway controlling R7 photoreceptor differentiation [48]. However, one function of *sina* is to regulate ubiquitination and degradation of its target proteins and thereby regulate signaling by those proteins [48]. Given the recent demonstration of caspase-dependent cleavage of the cytoplasmic tail of DCC leading to cell death [49] (discussed below), it is tempting to speculate that the vertebrate homologues of *sina* may act to regulate such an apoptosis-promoting pathway.

Recent experiments by Poo and colleagues have demonstrated the critical importance of levels of cyclic nucleotides within the growth cone for regulating the nature of the growth cone's response to a given axon guidance molecule [50–52]. In an elegant series of experiments, they found that treatments that increased the levels of cAMP or cGMP within the growth cone had the effect of converting repulsive cues into attractive ones, whereas decreasing cyclic nucleotide levels converted attractive cues into repulsive signals. In addition, this effect is specific for certain cyclic nucleotide-axon guidance molecule pairs: for example, increasing cGMP levels in a variety of neurons that are normally repelled by semaphorin III (sema III) results in the growth cones of those neurons being attracted towards a source of sema III [52]. Conversely, neurons that are normally attracted towards netrin-1 are repelled by this molecule when intracellular levels of cAMP are reduced [51].

However, these experiments do not provide any easy answers about what the functional consequences of altering cyclic nucleotide levels are within the growth cone. As is stated by the authors, cyclic nucleotides act as second messengers for a large number of cell surface receptors, and the regulation of growth cone responses by cyclic nucleotide levels suggests a possible mechanism whereby the growth cone can integrate other extracellular signals to respond to the same cue in different ways under different circumstances [52]. The best example of such growth cone behaviour is the phenomenon described whereby growth cones that grow towards the floor plate in response to netrin become completely unresponsive to netrin having crossed the floor plate [53]. As suggested by Song and colleagues, it is possible that the mechanism underlying this phenomenon involves regulation of cyclic nucleotide levels. Although cyclic nucleotides may be in-

volved in the signal transduction pathways themselves, there are a number of ways in which cyclic nucleotide levels may affect the coupling of netrin receptor activation to cytoskeletal reorganization indirectly. For example, some of the small G proteins (GTPases) that are regulated by cAMP, such as Rho, and that modulate growth cone activity [54] may be the point of action of changes in cyclic nucleotide levels.

A genetic approach to finding key components of a netrin signaling network has been taken recently in the Culotti laboratory [55, 56]. This mutant screen made use of the phenotype observed of axon repulsion from sources of UNC-6 when *unc-5* is ectopically expressed in a group of sensory neurons that do not normally express that receptor. Eight different genes which suppress the *unc-5* misexpression phenotype were discovered, some of which were already known to be involved in netrin signaling, such as *unc-6* and *unc-40*, and other known genes not previously known to have a role in the netrin pathway, such as *unc-44*. Four novel genes were identified, one of which has been characterized to date as a transforming growth factor-β (TGF-β) family member, *unc-129* [56]. In *C. elegans* this factor appears to directly guide axons during development, although it may act to induce production of an axon guidance molecule from neighbouring cells [56]. The finding of this and the other genes from this screen also raises the possibility of the existence of a similar genetic pathway in vertebrates, particularly given the degree of conservation of the netrin signaling pathway between all species.

A final intriguing feature of DCC receptor signaling, discussed in more detail below, is the presence of a caspase-sensitive site within the cytoplasmic tail, cleavage at which leads to cell death [49]. The function of this domain in normal development is not known, but it may be involved in regulated neuronal cell death during development.

Future findings for the netrins and their receptors

Although identified from relatively simple beginnings, the netrin story is far from complete. The cloning of netrin opened up a very rich field of enquiry in developmental neurobiology and a number of unexpected findings. The use of netrins in different parts of the nervous system to guide a variety of neuronal types was not an obvious finding for a family of proteins of such conserved function in the nerve cord/neural tube in all species examined.

With respect to axon guidance, the cell biology of netrin signaling is far from clear. Although the receptors have been cloned, the composition of the receptors is not known. Most importantly, little is known of the signal transduction pathways downstream of the receptors that couple receptor activation to cytoskeletal changes in the growth cone, other than the recent findings on the importance of cyclic nucleotide levels in regulating those pathways. Although it makes elucidating the pathways more arduous, it is exciting that the intracellular faces of all of the known netrin receptors have no obvious domains of homology to known signaling molecules, suggesting that a novel, fundamental pathway involved in regulating cell motility remains to be uncovered. As discussed above, the recent genetic studies in *C. elegans* may already have uncovered several key components of such a pathway [55, 56].

Finally, the recent finding of a role for netrin-DCC signaling in regulating cell death in epithelial cells in vitro adds a possible novel function for netrins [49]. In a convincing series of experiments, it was shown that the unoccupied DCC receptor constitutively activates a cell death pathway when expressed in a human embryonic kidney cell line (293T cells) and that this pathway was inhibited when the receptor was occupied by netrin. This work is important both for the function of *DCC* as a tumour suppressor gene and its functions in the developing and adult nervous system. As discussed above, *DCC* was implicated as a tumour suppressor gene on the basis that the *DCC* gene lies in a region of chromosome 18q that is frequently deleted in a number of tumours, but most commonly colorectal carcinoma, and that this deletion is associated with metastasis [27]. A possible mechanism for the tumour- or metastasis-suppressing function of DCC is implicit in its role as a dependence receptor, that is that survival of the DCC-expressing cells is dependent on continued presence of the ligand—in the event of metastasis to a region without netrin, for example, such cells would die, whereas cells with deletions in both *DCC* genes would be viable.

The possibility that DCC signaling may be involved in regulating cell death during development is intriguing, given that DCC is expressed on both growing axons and migrating neurons during development [22, 33]. One well-described feature of developing nervous systems is the careful pruning of incorrect axonal projections. It would be remarkable if DCC had a built-in editing function during development to aid in the elimination of cells which migrated to incorrect regions, or of eliminating neurons whose axons make extreme routing errors, in addition to being a key cell surface molecule for guiding those axons and cells.

1 Cajal S. R. y (1991) Cajal's Degeneration and Regeneration of the Nervous System, May R. M. (ed.), Oxford University Press, Oxford
2 Sperry R. G. (1963) Chemoaffinity in the orderly growth of nerve fiber patterns and connections. Proc. Natl. Acad. Sci. USA **50:** 703–710
3 Walter J., Kern-Veits B., Huf J., Stolze B. and Bonhoeffer F. (1987) Recognition of position-specific properties of tectal cell

membranes by retinal axons in vitro. Development **101:** 685–696
4 Drescher U., Kremoser C., Handwerker C., Loschinger J., Noda M. and Bonhoeffer F. (1995) In vitro guidance of retinal ganglion cell axons by RAGS, a 25 kDa tectal protein related to ligands for Eph receptor tyrosine kinases. Cell **82:** 359–370
5 Gundersen R. W. and Barrett J. N. (1979) Neuronal chemotaxis: chick dorsal-root axons turn toward high concentrations of nerve growth factor. Science **206:** 1079–1080
6 Lumsden A. G. S. and Davies A. D. (1983) Earliest sensory fibres are guided to peripheral targets by attractants other than nerve growth factor. Nature **306:** 786–788
7 Rickmann M., Fawcett J. W. and Keynes R. J. (1985) The migration of neural crest cells and the growth of motor axons through the rostral half of the chick somite. J. Embryol. Exp. Morphol. **90:** 437–455
8 Davies J. A., Cook G. M., Stern C. D. and Keynes R. J. (1990) Isolation from chick somites of a glycoprotein fraction that causes collapse of dorsal root ganglion growth cones. Neuron **4:** 11–20
9 Raper J. A. and Kapfhammer J. P. (1990) The enrichment of a neuronal growth cone collapsing activity from embryonic chick brain. Neuron **4:** 21–29
10 Luo Y., Raible D. and Raper J. A. (1993) Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones. Cell **75:** 217–227
11 Luo Y., Shepherd I., Li J., Renzi M. J., Chang S. and Raper J. A. (1995) A family of molecules related to collapsin in the embryonic chick nervous system. Neuron **14:** 1131–1140
12 Goodman C. S. (1996) Mechanisms and molecules that control growth cone guidance. Ann. Rev. Neurosci. **19:** 341–377
13 Tessier-Lavigne M., Placzek M., Lumsden A., Dodd J. and Jessell T. (1988) Chemotropic guidance of developing axons in the mammalial central nervous system. Nature **336:** 775–778
14 Placzek M., Tessier-Lavigne M., Jessell T. and Dodd J. (1990) Orientation of commissural axons in vitro to a floor-plate derived chemoattractant. Development **110:** 19–30
15 Kennedy T. E., Serafini T., de la Torre J. R. and Tessier-Lavigne M. (1994) Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. Cell **78:** 425–435
16 Serafini T., Kennedy T. E., Galko M. J., Mirzayan C., Jessell T. M. and Tessier-Lavigne T. M. (1994) The netrins define a family of axon outgrowth-promoting proteins homologous to *C. elegans* UNC-6. Cell **78:** 409–424
17 Ishii N., Wadsworth W. G., Stern B. D., Culotti J. G. and Hedgecock E. M. (1992) UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in *C. elegans*. Neuron **9:** 873–881
18 Wadsworth W. G., Bhatt H. and Hedgecock E. M. (1996) Neuroglia and pioneer neurons express UNC-6 to provide global and local netrin cues for guiding migrations in *C. elegans*. Neuron **16:** 35–46
19 Harris R., Sabatelli L. M. and Seeger M. A. (1996) Guidance cues at the *Drosophila* CNS midline: identification and characterization of two *Drosophila* netrin/UNC-6 homologs. Neuron **17:** 217–228
20 Mitchell K. J., Doyle J. L., Serafini T., Kennedy T. E., Tessier-Lavigne M., Goodman C. S. et al. (1996) Genetic analysis of netrin genes in *Drosophila*: netrins guide CNS commissural axons and peripheral motor axons. Neuron **17:** 203–215
21 Lauderdale J. D., Davis N. M. and Kuwada J. Y. (1997) Axon tracts correlate with netrin-1a expression in the zebrafish embryo. Mol. Cell. Neurosci. **9:** 293–313
22 Livesey F. J. and Hunt S. P. (1997) Netrin and netrin receptor expression in the embryonic mammalian nervous system suggests roles in retinal, striatal, nigral, and cerebellar development. Mol. Cell. Neurosci. **8:** 417–429
23 Hedgecock E. M., Culotti J. G., Hall D. H. and Stern B. D. (1987) Genetics of cell and axon migrations in *Caenorhabditis elegans*. Development **100:** 365–382
24 Hedgecock E. M., Culotti J. G. and Hall D. H. (1990) The unc-5, unc-6 and unc-40 genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in *C. elegans*. Neuron **2:** 61–85
25 Chan S. S.-Y., Zheng H., Su M.-W., Wilk R., Killeen M. T., Hedgecock E. M. et al. (1996) UNC-40, a *C. elegans* homolog of DCC (deleted in colorectal cancer), is required in motile cells responding to UNC-6 netrin cues. Cell **87:** 187–195
26 Fearon E. R., Cho K. R., Nigro J. M., Kern S. E., Simons J. W., Ruppert J. M. et al. (1990) Identification of a chromosome 18q gene that is altered in colorectal cancers. Science **247:** 49–56
27 Cho K. R. and Fearon E. R. (1995) DCC: linking tumor suppressor genes and altered cell surface interactions in cancer? Curr. Opin. Genet. Dev. **5:** 72–78
28 Hedrick L., Cho K. R., Fearon E. R., Wu T.-C., Kinzler K. W. and Vogelstein B. (1994) The DCC gene product in cellular differentiation and colorectal tumorigenesis. Genes Dev. **8:** 1174–1183
29 Cooper H. M., Armes P., Britto J., Gad J. and Wilks A. F. (1995) Cloning of the mouse homologue of the deleted in colorectal cancer gene (mDCC) and its expression in the developing mouse embryo. Oncogene **11:** 2243–2254
30 Chuong C.-M., Jiang T.-X., Yin E. and Widelitz R. B. (1994) cDCC (chicken homologue to a gene deleted in colorectal carcinoma) is an epithelial adhesion molecule expressed in the basal cells and involved in epithelial-mesenchymal interaction. Dev. Biol. **164:** 383–397
31 Pierceall W. E., Reale M. A., Candia A. F., Wright C. V. E., Cho K. R. and Fearon E. R. (1994) Expression of a homologue of the deleted in colorectal cancer (DCC) gene in the nervous system of developing *Xenopus* embryos. Dev. Biol. **166:** 654–665
32 Vielmetter J., Kayyem J. F., Roman J. M. and Dreyer W. J. (1994) Neogenin, an avian cell surface protein expressed during terminal neuronal differentiation, is closely related to the human tumor suppressor molecule deleted in colorectal cancer. J. Cell Biol. **127:** 2009–2020
33 Keino-Masu K., Masu M., Hinck L., Leonardo E. D., Chan S. S.-Y., Culotti J. G. et al. (1996) Deleted in colorectal cancer (DCC) encodes a netrin receptor. Cell **87:** 175–185
34 Kolodziej P. A., Timpe L. C., Mitchell K. J., Fried S. R., Goodman C. S., Jan L. Y. et al. (1996) frazzled encodes a Drosophila member of the DCC immunoglobulin subfamily and is required for CNS and motor axon guidance. Cell **87:** 197–204
35 Bennett K. L., Bradshaw J., Youngman T., Rodgers J., Greenfield B., Aruffo A. et al. (1997) Deleted in colorectal carcinoma (DCC) binds heparin via its fifth fibronectin type III domain. J. Biol. Chem. **272:** 26940–26946
36 Leung-Hagesteijn C., Spence A. M., Stern B. D., Zhou Y., Su M.-W., Hedgecock E. M. et al. (1992) UNC-5, a transmembrane protein with immunoglobulin and thrombospondin type 1 domains, guides cell and pioneer axon migrations in *C. elegans*. Cell **71:** 289–299
37 Ackerman S. L., Kozak L. P., Przyborski S. A., Rund L. A., Boyer B. B. and Knowles B. B. (1997) The mouse rostral cerebellar malformation gene encodes an UNC-5-like protein. Nature **388:** 838–842
38 Leonardo E. D., Hinck L., Masu M., Keino-Masu K., Ackerman S. L. and Tessier-Lavigne M. (1997) Vertebrate homologues of *C. elegans* UNC-5 are candidate netrin receptors. Nature **388:** 833–838
39 Serafini T., Colamarino S. A., Leonardo E. D., Wang H., Beddington R., Skarnes W. C. et al. (1996) Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. Cell **87:** 1001–1014
40 Shirasaki R., Mirzayan C., Tessier-Lavigne M. and Murakami F. (1996) Guidance of circumferentially growing axons by netrin-dependent and -independent floor plate chemotropism in the vertebrate brain. Neuron **17:** 1079–1088
41 Colamarino S. A. and Tessier-Lavigne M. (1995) The axonal chemoattractant netrin-1 is also a chemorepellent for trochlear motor axons. Cell **81:** 621–629

42 Guthrie S. and Pini A. (1995) Chemorepulsion of developing motor axons by the floor plate. Neuron **14:** 1117–1130
43 Fazeli A., Dickinson S. L., Hermiston M. L., Tighe R. V., Steen R. G. and Small C. G. (1997) Phenotype of mice lacking functional deleted in colorectal cancer (dcc) gene. Nature **386:** 796–804
44 MacLennan A. J., McLaurin D. L., Marks L., Vinson E. N., Pfeifer M., Szulc S. V. et al. (1997) Immunohistochemical localization of netrin-1 in the embryonic chick nervous system. J. Neurosci. **17:** 5466–5479
45 Deiner M. S., Kennedy T. E., Fazeli A., Serafini T., Tessier-Lavigne M. and Sretavan D. (1997) Netrin-1 and DCC mediate axon guidance locally at the optic disc: loss of function leads to optic nerve hypoplasia. Neuron **19:** 575–589
46 Metin C., Deleglise D., Serafini T., Kennedy T. E. and Tessier-Lavigne M. (1997) A role for netrin-1 in the guidance of cortical efferents. Development **124:** 5063–5074
47 Hu. G., Zhang S., Vidal M., LaBaer J., Xu T. and Fearon E. R. (1997) Mammalian homologs of seven in absentia regulate DCC via the ubiquitin-proteasome pathway. Genes Dev. **11:** 2701–2714
48 Dickson B. J. (1998) Photoreceptor development: breaking down the barriers. Curr. Biol. **8:** R90–R92
49 Mehlen P., Rabizadeh S., Snipas S. J., Assa-Munt N., Salvesen G. S. and Bredesen D. E. (1998) The DCC gene product induces apoptosis by a mechanims requiring receptor proteolysis. Nature **395:** 801–804
50 Song H. J., Ming G. L. and Poo M. M. (1997) cAMP-induced switching in turning direction of nerve growth cones. Nature **388:** 275–2799
51 Ming G. L., Song H. J., Berninger B., Holt C. E., Tessier-Lavigne M. and Poo M. M. (1997) cAMP-dependent growth cone guidance by netrin-1. Neuron **19:** 1225–1235
52 Song H. J., Ming G. L., He Z., Lehmann M., McKerracher L., Tessier-Lavigne M. et al. (1998) Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides. Science **281:** 1515–1518
53 Shirasaki R., Katsumata R. and Murakami F. (1998) Change in chemoattractant responsiveness of developing axons at an intermediate target. Science **279:** 105–107
54 Gallo G. and Letourneau P. C. (1998) Axon guidance: GTPases help axons reach their targets. Curr. Biol. **8:** R80–R82
55 Colavita A. and Culotti J. G. (1998) Suppressors of ectopic UNC-5 growth cone steering identify eight genes involved in axon guidance in *Caenorhabditis elegans*. Dev. Biol. **194:** 72–85
56 Colavita A., Krishna S., Zheng H., Padgett R. W. and Culotti J. G. (1998) Pioneer axon guidance by UNC-129, a *C. elegans* TGF-β. Science **281:** 706–709