Cell. Mol. Life Sci. 62 (2005) 2599–2616
1420-682X/05/222599-18
DOI 10.1007/s00018-005-5191-3

Cellular and Molecular Life Sciences

Review

Netrin-1: when a neuronal guidance cue turns out to be a regulator of tumorigenesis

P. Mehlen* **and C. Furne**

Apoptosis, Cancer and Development Laboratory- Equipe labellisée 'La Ligue', CNRS FRE2870, Centre Léon Bérard, 69008 Lyon (France), Fax: + 33.4.78782887, e-mail: mehlen@lyon.fnclcc.fr

Received 8 May 2005; received after revision 6 July 2005; accepted 22 July 2005
Online First 15 September 2005

Abstract. Netrin-1 has been shown to play a crucial role in neuronal navigation during nervous system development mainly through its interaction with its receptors DCC and UNC5H. However, initially the *DCC* (deleted in colorectal cancer) gene was proposed as a putative tumor suppressor gene. It was then difficult to reconcile the two activities of DCC until the observation that DCC belongs to an emerging family of receptors named dependence receptors. Such receptors share the property of inducing apoptosis in the absence of ligand, hence creating a cellular state of dependence on the ligand. Thus, netrin-1 may not only be a chemotropic factor for neurons but also a survival factor. We will review here the identification of netrin-1 and its receptors, the signaling pathways initiated in the presence or absence of netrin-1. We will suggest some possible roles of netrin-1 in nervous system development, neovascularisation, adhesion and tumorigenesis.

Key words. Netrin; axon guidance; apoptosis; dependence receptor; cancer; angiogenesis; DCC; UNC5H.

Netrin-1 identification and netrin family

Tessier-Lavigne and collaborators discovered the first netrins in vertebrates by using a functional assay designed to identify proteins that could promote commissural axon outgrowth [1]. Commissural neurons located in the dorsal part of the developing spinal cord were known to first extend axons toward the ventral part of the spinal cord through a mechanism dependent on a ventral midline structure named the floor plate [1, 2]. The elegant assay developed by Tessier-Lavigne and collaborators was based on the hypothesis that spinal commissural axons are attracted by diffusible cue(s) secreted by the floor plate [2–4]. Searching for such diffusible cue(s) that can promote commissural axon outgrowth ex vivo in a collagen matrix, they identified a protein that they named netrin-1, from the Sanskrit term for one who guides [1, 5]. *Netrin-1* is expressed by floor plate cells as commissural axons extend toward the ventral midline [5]. The definitive evidence for a major role of netrin-1 in mediating commissural axon development was further obtained by studying the defect of commissural neuron projections in mice lacking netrin-1 function [6]. In netrin-1 null mice, the corpus callosum, hippocampal commissure, anterior commissure and ventral spinal commissure were abnormal, indicating that netrin-1 is required for the development of multiple commissural projections [6]. Netrin-1 guides not only commissural neurons but many other neurons, such as retinal axons [7]. On a functional basis, netrin-1 not only induces axon outgrowth but also directs the orientation of growth cones, as was elegantly shown by Holt's and Poo's teams using dissociated *Xenopus* neurons [8, 9]. Although initial studies have focused on the ability of netrin-1 to attract extending axons, netrin-1 has also been shown to act as a repellent for other

* Corresponding author.

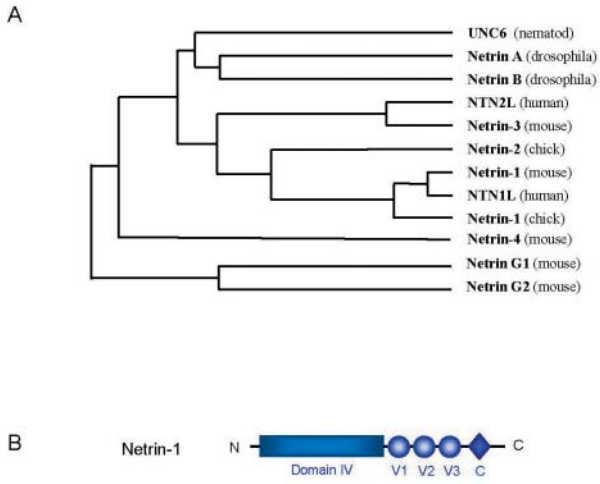

Figure 1. Netrin-1 within netrins. (A) Representation of the different netrins identified so far. (B) Schematic representation of netrin-1, a laminin-related molecule.

axons [10]. Together, these findings led to the proposal that a gradient of netrin-1 protein emanating from the floor plate orients the growth of multiple populations of axons as they extend circumferentially toward or away from the ventral midline of the embryonic central nervous system, even though evidence for this gradient has not been obtained.

Interestingly, netrin-1 belongs to a wide family of conserved proteins. Several members of the *netrin* gene family have been identified in mammals: *netrin-1*, *netrin-3*, *netrin-G1*, *netrin-G2* and *netrin-4*, also called *β-netrin* [1, 6, 11–13] (see fig.1). Orthologs of these netrin family members have been identified in the human genome, and the human ortholog of netrin-1 is also named NTN1L. All encode ~60–80 kDa proteins (fig.1) composed of three domains (V, VI and C) and an amino terminal signal peptide characteristic of secreted proteins. Domains V and VI of netrins are homologous to domains V and VI of laminins, while the netrin C domain shares some sequence similarity with domains present in the complement and tissue inhibitors of metalloprotease (TIMP) protein families [1, 14]. Netrins have been conserved among species; the *Caenorhabditis elegans* netrin – i.e. UNC6 [15] – was actually the first member of the netrin family identified using a genetic screen for defects in neural development by examining mutants with uncoordinated (unc) phenotypes [15, 16]. Loss of UNC6 function produces defects in the trajectories of axons that normally extend circumferentially toward or away from the ventral midline of the developing nematode. Similarly, loss of netrin function causes anomalies in commissure formation in the *Drosophila melanogaster* nervous system [17]. Thus, netrin function in axon guidance is conserved through evolution.

Netrins are not only involved in axon guidance but also play a central role in the migration of neurons, glial oligodendrocyte precursors and mesodermal cells during embryogenesis [18–26].

Netrin-1 receptors

DCC, a netrin-1 receptor and a putative tumor suppressor

Candidate netrin receptors were first identified in *C. elegans* based on the similarity of *unc-5*, *unc-40* and *netrin/unc-6* mutant phenotypes [16]. Mutation of *unc-5* causes defects in ventral to dorsal migration, away from *unc-6*-expressing cells, mutation of *unc-40* produces defects in dorsal to ventral migration, toward *unc-6* expressing cells, and mutation of *unc-6* causes defects in both trajectories. *unc-40* and *unc-5* genes encode type I transmembrane receptors, and both are expressed by neurons as they extend axons [27, 28]. Interestingly, an ortholog of *unc-40* was found in mammals [29] and was proposed a few years before netrin-1 discovery as a putative type I transmembrane receptor whose gene is deleted through allelic loss in a majority of colorectal cancers; it was consequently named deleted in colorectal cancer (*DCC*) [30].

Indeed, based on early studies of tumor suppressor genes such as the retinoblastoma gene [31] and pioneer work by Knudson [32], allelic loss [also known as loss of heterozygosity (LOH)] has been implicated as an important mechanism of tumor suppressor gene inactivation. In the late 1980s, efforts of Vogelstein and colleagues to identify chromosomal regions most likely to harbor tumor suppressor genes in colorectal cancer revealed that chromosome regions 5q, 17p and 18q were commonly affected by LOH [33, 34]. While LOH of chromosome 5q and 17p respectively reflects the inactivation of an adenomatous polyposis coli (APC) allele and of a p53 allele [35, 36], the finding that about 70% of primary colorectal cancers had LOH of chromosome 18q suggested that the inactivation of one or more novel genes on 18q might play a significant role in colorectal cancer [30]. The identification of somatic mutations nearby an anonymous DNA marker from the 18q21 region in two primary colorectal cancers ultimately led to the identification of the *DCC* gene [30]. Subsequent studies have suggested that *DCC* has 29 or more exons and spans about 1.3 million Mb [37, 38].

The *DCC* gene encodes different protein products as a result of alternative splicing [39], though all known isoforms appear to be type I transmembrane glycoproteins of 175–190 kDa with a single membrane spanning domain. The sequences present in the large extracellular domain – i.e., about 1100 amino acids – bear strong similarity to those found in neural cell adhesion molecule

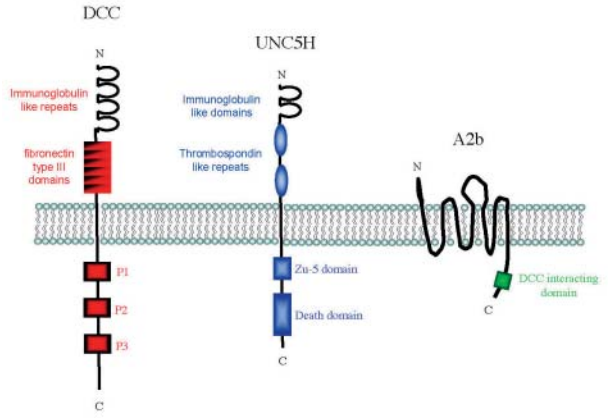

Figure 2. Netrin-1 receptors. Schematic representation of netrin-1 receptors DCC, UNC5H and A_{2B}. The extracellular domain of DCC family members is composed of four immunoglobulin-like domains and six fibronectin type III domains, followed by a transmembrane domain and an intracellular domain composed of three conserved domains named P1, P2 and P3. UNC5H receptors contain two extracellular immunoglobulin-like domains, two thrombospondin type 1 domains, followed by a transmembrane domain and an intracellular domain. The intracellular domain of UNC5H homologues contains a ZU5 domain and death domain. A_{2B} is a seven transmembrane receptor that interacts with DCC in the C-terminal intracellular domain.

(NCAM) protein family members, and include four immunoglobulin-like domains and six fibronectin type III-like motifs (fig. 2). The DCC cytoplasmic domain of 325 amino acids shows little similarity to proteins with well-established functions. However, within the cytoplasmic domain, three regions named P1, P2 and P3 appear more specifically conserved among orthologs of DCC and are then proposed to play functional roles in DCC activity [40] (fig. 2). DCC is known to have a homolog in mammals, named neogenin, but little is known of this protein [41]. While neogenin was initially proposed to be a netrin-1 receptor, more recent studies have demonstrated that RGM (repulsive guidance molecule) is probably a better candidate for being ligand of neogenin [42, 43]. Well-conserved DCC orthologs have been identified in *C. elegans* (UNC40), *Drosophila* (Frazzled) and *Xenopus* [16, 28, 44, 45]. In humans, a low level of *DCC* expression has been detected in many developing and adult tissues, with the highest level in the brain [29, 30, 39, 41].

As noted above, LOH of chromosome 18q that includes the *DCC* gene – i.e. 90% of all 18q LOH – has been found in a large fraction of colorectal cancers [30, 34, 46]. Interestingly, in most reports, allelic losses of 18q are infrequent in early stage tumors (e.g. small adenomas), but are common in primary colorectal carcinomas and in nearly 100% of hepatic metastases arising from colorectal primaries, implying that chromosome 18q LOH may contribute more to the progression than to the initiation of colorectal cancer. Most studies have linked chromosome 18q LOH in colorectal cancers to a reduction in *DCC* expression [47–51]. LOH of chromosome 18q and/or decreased *DCC* expression have also been seen in various other cancers, including gastric [52–54], prostate [55–57], endometrial [58–60], ovarian [61, 62], esophageal [63, 64], breast [65, 66], testicular [67, 68], and glial [69, 70] cancers, as well as neuroblastoma [71, 72] and hematologic malignancies [73–75]. Most of studies have associated chromosome 18q LOH and loss of *DCC* expression with poor prognosis in colorectal cancer patients [76–80] and with decreased responsiveness to chemotherapeutic agents, such as 5-fluorouracil-based adjuvant [81].

Some evidence that *DCC* inactivation may in fact be associated with tumorigenic growth properties in colon and other cancers has been obtained. For example, the introduction of an intact copy of chromosome 18 into a colorectal cancer cell line lacking endogenous *DCC* expression has yielded detectable levels of *DCC* transcripts and resulted in the suppression of growth in soft agar and tumorigenicity in nude mice [82]. In addition, the ectopic expression of *DCC* in a tumorigenic keratinocyte cell line lacking endogenous *DCC* expression has been shown to suppress tumorigenic cell growth in nude mice [83]. Interestingly, in this study, it was observed that tumorigenic reversion was associated with loss of *DCC* expression and loss/rearrangement of the transfected *DCC* expression vector [83]. Several more recent studies also indicate that the restoration of *DCC* expression can suppress tumorigenic growth properties in vitro or in nude mice [84, 85]. However, the rarity of point mutations identified in *DCC* coding sequences, the presence of other known/candidate tumor suppressor genes on chromosome 18q [47, 86, 87] and the lack of a tumor predisposition phenotype in mice heterozygous for *DCC* inactivating mutations [88] have raised questions about DCC's candidacy as a tumor suppressor. Even though, as discussed elsewhere [89], none of this evidence is sufficient to exclude the tumor suppressor function of DCC, the absence of (i) any demonstration that the loss of DCC expression represents a selective advantage for tumor cell development in vivo and (ii) any proposed molecular mechanism that might link DCC to its in vitro tumor suppressor activity has insidiously decreased the interest of DCC in cancer. On the other hand, the observation that DCC null mice die a few hours after birth and show defects in the developing nervous system similar to those of netrin-1 mutant mice has led to considering DCC mainly as a receptor or as part of a receptor complex for netrin-1 and, as such, involved in neuronal guidance [29]. Numerous studies using either a DCC blocking antibody, transfection experiments or DCC null mice have further demonstrated that netrin-1 requires the presence of DCC to mediate both axon outgrowth and orientation [29, 90, 91].

UNC5H, a netrin-1 receptor family

DCC is, however, not the only known netrin-1 receptor. Indeed, as shown in *C. elegans*, the *unc*-5 gene also encodes a putative UNC6/netrin receptor [16, 27]. UNC5 and its vertebrate homologs UNC5H are type I transmembrane proteins composed of two extracellular immunoglobulin (Ig)-like domains, two thrombospondin type I domains, and an intracellular sequence that contains a ZU5 domain and a death domain (fig. 2) [92]. Four UNC5 homologs have been found in mammals UNC5H1, UNC5H2, UNC5H3 and UNC5H4 (also called UNC5A, B, C and D in humans) and have been grouped under the term UNC5H [92–94]. UNC5H1–H3 have been shown to bind netrin-1 in cell culture systems, suggesting that UNC5H molecules function as netrin receptors [92, 95]. The role of UNC5 protein in axon guidance in *C. elegans* has been clearly demonstrated since the trajectories of axons extending away from a source of UNC6/netrin are disrupted in *C. elegans unc-5* mutants [16]. In *D. melanogaster*, *unc-5* is expressed by a subset of motoneurons whose axons exit the central nervous system without crossing the midline and then avoid netrin-expressing muscles in the periphery [96]. Furthermore, in both *C. elegans* and *D. melanogaster*, the ectopic expression of *unc-5* in neurons that either normally do not respond to netrin or are attracted toward a source of netrin, causes their axons to be repelled by netrin in vivo [96, 97]. These findings are consistent with UNC5 mediating a repellent response to netrin. Moreover, an analysis of the *unc5H3* mouse mutant (rostral cerebellar malformation, rcm, [95, 98]) indicates that UNC5H3 is required for the migration of granule cell and Purkinje cell precursors during cerebellar development and for the guidance of corticospinal tract axons [95, 99]. Moreover, Hong et al. have proposed, using ectopic expression of rat UNC5H in dissociated *Xenopus* commissural neurons, that UNC5H, through its ability to interact with DCC in the presence of netrin-1, mediates axon repulsion [40]. However, because UNC5H1 and UNC5H2 inactivation in mice cannot be used to study the role of UNC5H1-2 in axon/neuronal guidance, since homozygous embryos die too early at respectively E9.5 and E8.5 ([100] and M. Tessier-Lavigne, personal communication), and because the phenotype of unc5H3 mutant mice is not clearly associated with a guidance/migration defect, it is fair to say that the possible involvement of mammalian UNC5H in neuronal guidance in vivo is unclear.

Other putative netrin-1 receptors

More recently, another type of receptor for netrin-1 has been identified by our group. While performing a search for intracellular partners of DCC by a two-hybrid screen, we observed that DCC actually interacted with the last intracellular domain of the G-protein-coupled-receptor A_{2B} (fig. 2) [101]. A_{2B} belongs to the family of adenosine receptors that comprises four members, A_1, A_{2A}, A_{2B} and A_3 [102]. A_{2B} was initially presented as a receptor for adenosine but its weak affinity for adenosine raised the question of the physiological role of this receptor [103]. We then described how A_{2B}/DCC interaction occurrs mainly in the presence of netrin-1, and we actually provided evidence that netrin-1 is able to bind directly to A_{2B} with a dissociation constant of 22 nM independent of the presence of DCC [101]. Moreover, we demonstrated that this binding activates A_{2B}, leading to cyclic AMP (cAMP) production (see also below). In an attempt to elucidate the role of this interaction in netrin-1 function, we proposed, using ex vivo explant assays, that A_{2B} was involved in netrin-1-mediated rat commissural axon outgrowth [101]. However, the in vivo significance of the interaction between A_{2B} and netrin-1 was challenged by Tessier-Lavigne and collaborators. They proposed that inhibiting A_{2B} has no effect, or actually has a potentialization effect on netrin-1-induced axon outgrowth in both *Xenopus* and rat commissural axons [90]. Along these lines, no clear ortholog of A_{2B} was found in *C. elegans*, while the other components of the netrin-signaling complex (UNC6, UNC40, UNC5) were evolutionarily conserved. However, more recently, a third report suggested that the same A_{2B} inhibitors modulate netrin-1 responsiveness of *Xenopus* retinal axons [104]. Interestingly, Holt and colleagues have reported that netrin-1-mediated axon attraction is first dependent on A_{2B}, while retinal growth cones are young, in the sense of their pathfinding. When the same axons age, they show decreased A_{2B} expression and a loss of A_{2B}-dependent netrin-1-mediated axon orientation [104]. Moreover, it is worth noting that in their report challenging the role of A_{2B} in axon guidance, Tessier-Lavigne and collaborators studied A_{2B} in an experimental setting leading to rapid A_{2B} desensitization – i.e., this purified netrin-1 used to monitor axon outgrowth triggers loss of A_{2B} plasma membrane localization and consequently fails to transducing cAMP production [V. Corset et al., unpublished]. All told, it is fair to say that if it is reasonable to consider A_{2B} as a netrin receptor, its role in netrin-1 mediated axon guidance is still uncertain.

A recent report also suggested that netrin-1 interacts with integrin $\alpha6\beta4$ and $\alpha3\beta1$ [105]. This interaction relies on the fact that (i) blocking antibodies to DCC and neogenin failed to block netrin-1 binding to fetal pancreatic epithelial cells while blocking $\alpha6\beta4$ inhibited netrin-1 recruitment to these cells and (ii) integrins $\alpha6\beta4$ and $\alpha3\beta1$ from fetal pancreatic epithelial cell lysate were retained by a netrin-1-peptide affinity column. This report proposed that this interaction is important for the migration of pancreatic epithelial cells, netrin-1 then appearing more like an adhesive substrate.

Several concerns, however, do emerge from this study, in particular on how other netrin-1 receptors such as UNC5H or A_{2B} are expressed in the analyzed cells and whether integrins such as $\alpha6\beta4$ and $\alpha3\beta1$, probably present in many other cells, might rather act as part of the netrin-1 receptor complex than as direct netrin-1 receptors.

Netrin-1 receptors DCC and UNC5H are dependence receptors

The view of DCC as a putative tumor suppressor in numerous types of malignancies is in strong contrast to its role in mediating axon guidance during nervous system development. Such dual functions were hypothesized to be a trait of the so-called dependence receptors. Dependence receptors create cellular states of dependence on their respective ligands by either inducing apoptosis when unoccupied by the ligand, or inhibiting apoptosis in the presence of the ligand [106, 107]. We then demonstrated that, in the absence of netrin-1, the forced expression of DCC in various cell lines devoid of DCC expression led to apoptosis induction while the presence of netrin-1 was sufficient to block this cell death process [108]. Further studies confirmed that DCC induces apoptosis when expressed in settings in which netrin-1 is not expressed [109, 110] while netrin-1 blocks this effect [110, 111]. Therefore, DCC is functionally related to other dependence receptors such as $p75^{NTR}$, the common neurotrophin receptor, the androgen receptor, Ptc, RET, $\alpha v\beta3$ integrin and neogenin [43, 112–115].

Interestingly, DCC is not the only netrin-1 receptor to display the trait of inducing apoptosis in the absence of netrin-1. Indeed, the observation that the intracellular sequence of UNC5H receptors contains a death domain, very often associated with cell death regulation, led us to investigate whether UNC5H receptors have a similar pro-apoptotic activity. We demonstrated that the forced expression of UNC5H1, UNC5H2 and UNC5H3 led to apoptosis induction, a phenomenon that was blocked by the presence of netrin-1 [116]. Similar results were reported by other groups [117, 118]. It is then interesting to note that, while reporting on the chemorepulsive activity of ectopically expressed UNC5H in *Xenopus* spinal neurons, Tessier-Lavigne and colleagues used a death domain-deleted mutant of UNC5H to observe guidance-related effects because expressing wild-type UNC5H killed commissural neurons [40]. Hence, netrin-1 receptors DCC and UNC5H are dependence receptors that can trigger two completely opposite signals depending on the presence of the ligand netrin-1. Thus, netrin-1 may be both a chemotropic molecule for axonal guidance and a survival factor [116].

Netrin-1 receptor induced signaling

Death signaling observed in the absence of netrin-1

The specific molecular mechanisms by which DCC exerts its pro-apoptotic effects when netrin is absent have not been fully clarified. However, a first hint is that DCC pro-apoptotic activity requires the activation of cysteine aspartate proteases named caspases [108]. When unbound to its ligand, DCC is cleaved roughly in the middle of its intracellular domain (aspartic acid residue 1290) by an unknown active caspase [108]. While DCC is cleaved in vitro by caspase 3, it may be cleaved in vivo by another caspase or another protease. The current model suggests that the cleavage of DCC allows the release of DCC's inhibitory C-terminal domain and the exposure of a domain located upstream to the caspase cleavage site. This upstream DCC domain, which is sufficient for cell death induction and further caspase activation, is named ADD for addiction/dependence domain [108]. How ADD drives cell death is still under investigation. Chen and collaborators have described an Akt-like molecule, named DIP13 for DCC interacting protein 13, that specifically interacts with ADD [111]. These authors demonstrated that DIP13 is required for DCC-induced cell death [111]. It is not known how DIP13 mediates apoptosis. Interestingly, however, we proposed that the ADD of DCC also interacts with the initiator caspase-9 in the absence of netrin-1, while netrin-1 presence is associated with a reduction of DCC/caspase-9 interaction [110]. Moreover, we demonstrated that DCC-induced cell death requires caspase-9. However, it does not require the two frequently described pathways for apoptosis – the death receptor pathway implicating caspase-8 recruitment to the DISC (death-inducing signaling complex) and the mitochondria-dependent pathway dependent on apoptosome formation involving cytochrome c, Apaf-1 and caspase-9 [119, 110]. Moreover, DCC was also shown to interact with caspase-3, but contrary to caspase-9, this interaction occurs in the presence of ligand only and in the DCC C-terminal region, the one released after DCC cleavage. Hence, a speculative model would be that in the absence of netrin-1, DCC, via interactions with caspase-9, promotes direct caspase-3 activation and subsequent caspase-dependent cell death [110] (fig. 3). While the interaction of caspase-3 with DCC is direct, the interaction of DCC with caspase-9 is probably indirect and may require an adaptor protein, possibly DIP13.

The molecular mechanisms that lead UNC5H proteins to initiate cell death in the absence of netrin-1 are beginning to be documented (fig. 4). Interestingly, similarly to DCC, UNC5H1, UNC5H2 and UNC5H3 are all cleaved by caspase. Moreover, as for the other dependence receptors, DCC, AR and RET, this caspase cleavage of UNC5H2 and UNC5H3 is required for cell death induction [116, 117]. However, a slight difference exists between DCC

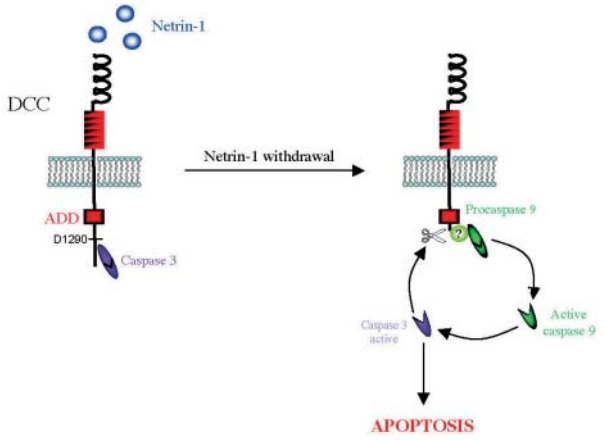

Figure 3. Putative mechanism for DCC-mediated apoptosis. In the presence of netrin-1, DCC interacts with caspase-3 in its C-terminal domain. When netrin-1 is withdrawn, DCC is cleaved by active caspase. This preliminary cleavage leads to the recruitment of caspase-9, probably through an adaptor protein. This interaction allows caspase-9 activation and caspase-3 cleavage, leading to (i) more DCC cleavage and (ii) caspase-3 dependent cell death.

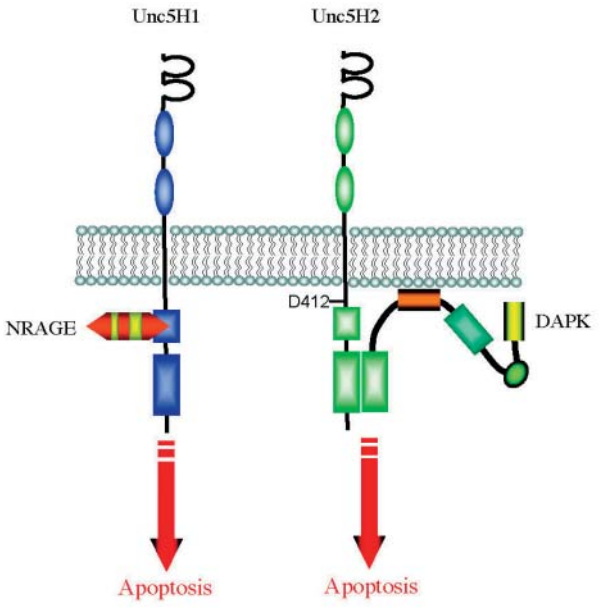

Figure 4. Putative mechanism for UNC5H-mediated apoptosis. UNC5H receptors display in their intracellular region a death domain and a ZU-5 domain. Through interaction with the ZU-5 domain of UNC5H1, NRAGE mediates UNC5H1-induced cell death. Through interaction with the death domain of UNC5H2, DAPK is catalytically activated and triggers UNC5H2-induced cell death.

and UNC5H2, as the domain required for cell death induction is located after the caspase cleavage site [116]. Interestingly, two domains of UNC5H receptors seem to be important for UNC5H pro-apoptotic activity, – i. e. the ZU-5 domain and the death domain. Indeed, UNC5H1, but not so much UNC5H2 or UNC5H3, appears to interact with the adaptor protein NRAGE via the UNC5H1 ZU-5 domain, and UNC5H1-induced cell death has been shown to be dependent on the ZU domain of UNC5H1 and on the presence of NRAGE [118]. Conversely, the deletion of the death domain in both UNC5H1 and UNC5H3 has been shown to be sufficient to abrogate UNC5H-induced cell death. In the case of UNC5H2, but not of UNC5H1 and UNC5H3, it has recently been shown that the death domain interacts with a protein serine threonine kinase named DAPK (death-associated protein kinase). DAPK was initially described as a mediator of apoptosis via its serine-threonine kinase activity [120, 121]. The death domains of UNC5H2 and DAPK share 49.4% homology, and it has been shown that UNC5H2 not only interacts with DAPK but also induces the catalytic activity of DAPK thanks to the cooperation of their death domains – i. e. the deletion of UNC5H2 death domain renders UNC5H2 unable to (i) induce apoptosis or (ii) trigger the catalytic activity of DAPK [122]. Thus, even though UNC5H receptors are close homologs, they do not appear to be functionally identical.

In any case, the observation that UNC5H1, UNC5H2, UNC5H3 and DCC are all cleaved by caspases suggests the existence of a conserved mechanism, implying that these receptors, when unengaged by netrin-1, are recognized and cleaved by active caspase(s). How then can the first cleavage take place? The model requires a first cleavage that would occur in settings in which the classic dogma describes caspases as inactive proenzymes. How can a receptor that requires caspase cleavage to produce a pro-apoptotic molecule participate in apoptosis induction, rather than simply serve a passive role in the process? One possibility is that the process may be initiated by a non-caspase protease, then propagated via caspase cleavage. Only a few cleavage events induced by a non-caspase protease would then be sufficient to initiate the cell death pathway by locally activating enough caspase to generate a caspase amplification loop via these receptors. Alternatively, the now old dogma suggesting that caspases are completely inactive in non-apoptotic cells and are only activated massively upon pro-apoptotic stimuli might just be wrong. Recent findings have shown that caspase zymogens display some protease activity [123–125], whereas cells express endogenous caspase inhibitors, such as inhibitor of apoptosis proteins (IAPs), that prevent the propagation of active caspases [125]. Similarly, local caspase activation without cell death induction has been documented [126, 127]. Cell death induction could therefore result from caspase amplification rather than from caspase initiation, and this would support the importance of the cellular control of caspase activation/inhibition in cell fate determination: cell death induction would be the result of a move from low/local

caspase activation – i.e. that may have a positive input in the cell, like cell differentiation – to high/distributed caspase activation. The balance between low/local and high/distributed caspase activation would therefore be likely modulated by endogenous caspase inhibitors such as IAPs, and by endogenous caspase amplifiers such as dependence receptors. However, it is fair to say that a large amount of work is needed to pinpoint the precise intracellular molecular mechanisms at work when netrin-1 unbinds from DCC or UNC5H receptors.

Positive signaling observed in the presence of netrin-1

While insights into how the absence of netrin-1 triggers the death of DCC/UNC5H-expressing cells have emerged, knowledge about the molecular mechanisms that lead to axon outgrowth and orientation is still very incomplete (fig. 5). The first hint of netrin-1-induced signaling was reported by Poo and collaborators. They demonstrated that cytosolic cAMP and calcium levels were crucial for determining the nature of the chemotropic activity of netrin-1. Hence, modulating cAMP levels by cAMP agonists or antagonists modifies the turning behavior of *Xenopus* commissural neurons – i.e. low cAMP levels are associated with chemorepulsion by netrin-1, while high cAMP is associated with chemoattraction [8]. However, the observation did not prove that netrin-1 enhances or down-regulates cAMP levels and calcium release. Holt and colleagues have demonstrated that the presence of netrin-1 induces cAMP increase in the growth cones of *Xenopus* retinal axons [128], and Poo and colleagues have demonstrated a transient release of calcium [129]. The netrin-1-induced increase of cAMP is probably not mediated by DCC since attempts to demonstrate signaling leading to cAMP production through DCC have been unsuccessful [V. Corset and P. Mehlen, unpublished]. In contrast, Corset et al. have proposed that netrin-1-induced cAMP production is mediated by A_{2B} at least in transfection experiments [101]. This mechanism sounds reasonable, since A_{2B} is known to be coupled to Gs protein and to transduce cAMP production upon adenosine binding [103]. This observation is also supported by the fact that in retinal neurons, the loss of netrin-1-induced cAMP production is associated with the down-regulation of A_{2B} that is observed when retinal axons age [104]. Regarding the increase of calcium, two reports have recently demonstrated the involvement of the transient receptor potential channels (TRPCs) and more specifically of TRPC1 [130, 131]. Poo and colleagues and Ming and colleagues have indeed shown that TRPC1 is required for netrin-1-induced axon turning and netrin-1-induced calcium influx. Yet, whether this(ese) receptor(s) is(are) activated in response to DCC or A_{2B} or alternative netrin-1 receptors is unknown. Moreover, this requirement is probably downstream in netrin-1 signaling because it

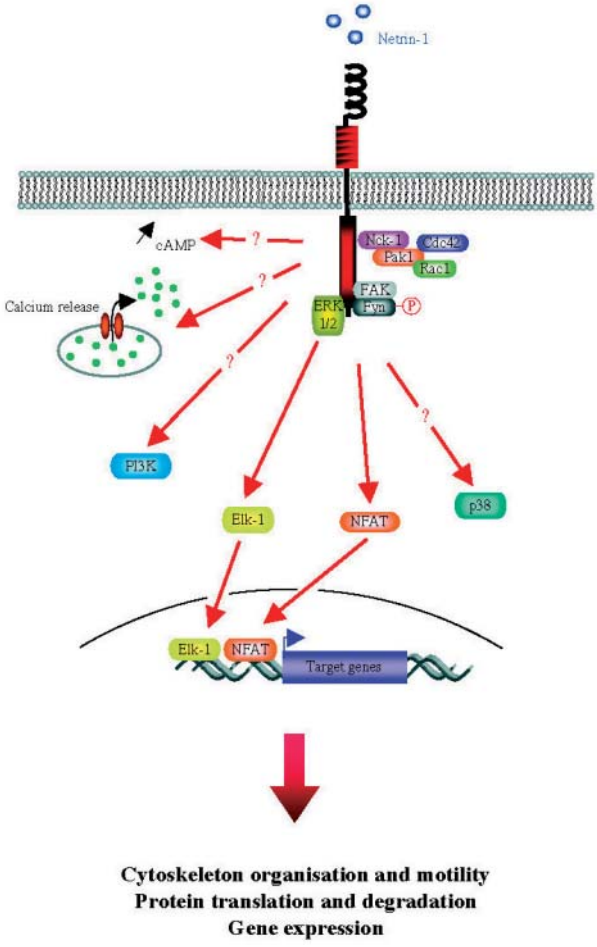

Figure 5. Schematic representation of the known signaling pathways induced by DCC, the DCC/netrin-1 pair or netrin-1. In the presence of netrin-1, DCC has been shown to activate Rac-1, the ERK-1/2 dependent pathway, to interact with FAK and to phosphorylate Fyn. Netrin-1 has been also shown to induce cAMP increase, calcium release, PI3K and p38 activation, but the nature of the receptor(s) involved in these signaling pathways is unknown. These signalings may then affect the cytoskeleton, protein translation and gene expression via Elk-1 and NF-AT.

appears also to be true for other axon guidance cues, such as BNDF or Sema3A.

Similarly, phosphatidylinositol-3 (PI3) kinase activation was observed in *Xenopus* retinal axons upon netrin-1 treatment [132], and PI3 kinase inhibitors block netrin-1-mediated axon guidance [132, 133]. However, it is not known what receptor(s) is(are) involved in this activation. In any case, this PI3 kinase activation was shown to be important to modulate protein degradation and protein translation within the growth cone, two mechanisms that appear of key importance for axon guidance [132].

In a search for direct signaling of DCC upon ligation with netrin-1, Forcet et al. and Ming et al. [91, 134] proposed that netrin-1 may activate a specific MAPK pathway. Indeed, DCC has been shown to specifically activate

ERK-1/2 MAPK but not JNK MAPK or p38 MAPK in response to netrin-1. This activation is dependent on the direct binding of ERK-1/2 to DCC, suggesting that the intracellular domain of DCC acts as a scaffold for MAPK activation [91]. However, it is not known whether DCC directly triggers MAPK activation through ERK binding or whether this ERK binding to DCC represents a mechanism for amplifying ERK activation previously initiated through another DCC-independent signaling cascade. In any case, this netrin-1/DCC-mediated ERK activation has been shown to be required for netrin-1-induced axon outgrowth and orientation [91], even though the targets of ERK-1/2 involved in axon guidance are not known. Recently, Holt and colleagues confirmed that netrin-1 stimulates ERK-1/2 activation, but they also reported that, in *Xenopus* retinal neurons, netrin-1 induces p38 activation [127]. While, as reported in Forcet et al., p38 inhibitor fails to modulate the outgrowth and turning of commissural axons, in *Xenopus* retinal neurons a similar p38 inhibitor blocks netrin-1-induced axon attraction and growth cone collapse. This interesting contrast suggests that netrin-1-induced signaling may be cell specific. It is also possible that, in retinal neurons, another netrin-1 receptor is involved, since it has not been shown whether DCC is involved in p38 activation [127]. Interestingly, Campbell and Holt have proposed that this p38 activation leads to caspase-3 cleavage – and probably activation – within the growth cone of *Xenopus* retinal axon. Conversely to what has been proposed by other groups in various cell types [108, 111, 117], this caspase activation occurs in the presence rather than in the absence of netrin-1. They have also proposed that caspase-3 inhibition converts netrin-1-mediated attraction to repulsion. These data imply that local caspase activation within growth cones can be transient – without cell death induction – and act positively on axon guidance. How could these results be related to the death induction and caspase activation mediated by DCC or UNC5H observed in the absence of netrin-1? The two events are actually probably independent, because the transient caspase activation observed in the presence of netrin-1 is not specific for netrin-1 but is also noted with various axonal guidance cues, such as semaphorin3A or LPA [127]. In any case further work will be required to elucidate the nature and role of this transient caspase activation that seems to occur very rapidly (less than 5 min after netrin-1 addition) and is downstream of p38 activation. Such activation would then require direct action of p38, or one of its cytoplasmic targets, in caspase-3 cleavage and activation.

Another type of netrin-1 signaling was also recently proposed. DCC has been shown to trigger the activation of small GTPases, such as Rac-1 and cdc42 upon netrin-1 binding [135, 136]. Interestingly, Rac-1 and cdc42 activation has been widely shown to modulate cytoskeleton organization and influence motility [137]. Specifically, it was recently shown that, in rat commissural neurons, netrin-1 induces the recruitment of cdc42, Rac-1 together with Pak-1 and N-WAS into a DCC complex [138]. The activation of small GTPases would then represent a very tempting signal for the cytoskeletal changes that are known to occur during the process of axon guidance. Along this line, Bargmann, Tessier-Lavigne and colleagues have proposed, through a genetic approach in *C. elegans,* that ced-10, which encodes a Rac-like protein, acts downstream of the UNC40/DCC signaling cascade [139]. Lamarche and colleagues have proposed that DCC-mediated Rac-1 activation occurs through the binding of DCC to Nck-1, an adaptor protein [140].

More recently, three groups simultaneously reported that netrin-1 signaling might involve recruitment of the protein kinase FAK, classically implicated in integrin-mediated signaling [141–143]. Because DCC lacks a kinase catalytic domain, it is tempting to consider FAK/DCC as a receptor complex allowing kinase-dependent signaling. It has also been shown that not only FAK but also Fyn, a Src family kinase, can be found in a DCC intracellular complex and that netrin-1 induces DCC-dependent FAK, Fyn and DCC phosphorylation [141, 142]. This DCC phosphorylation appears to depend on FAK and Fyn and is important for netrin-1-induced axon outgrowth and turning [141, 142, 144]. Several issues are not yet understood: (i) netrin-1 does not seem to modulate either FAK or Fyn binding to DCC complex, suggesting that other parameters/DCC interactors trigger netrin-1-dependent FAK phosphorylation; (ii) the main phosphorylation site Y1420 (or Y1418 depending on the report) is not conserved in *C. elegans* UNC40, suggesting that this netrin-1/DCC signaling is a relatively late event in evolution [144]; (iii) whether Fyn, a Src-related molecule, or Src itself is required remains to be clarified [141,144]

The signaling pathways described above are assumed to initiate downstream effectors. Considering the role of DCC in neuronal guidance, the effectors can be multiple: molecules involved in growth cone motility and molecules involved in controlling protein/genome expression. Even though yet not demonstrated, the known activation of small GTPases and MAPKs in response to netrin-1 probably affects effectors involved in cytoskeleton rearrangements, as both proteins are known to display cytoskeleton targets [91, 135]. Along these lines, it has been shown that Ena/VASP, a protein regulating the assembly and geometry of actin networks, is required for netrin-1-mediated axon guidance and is downstream of DCC [145]. Similarly, MAP1B, a specific microtubule-associated protein implicated in the crosstalk between microtubules and actin filaments, has been shown to be phosphorylated in response to netrin-1 in a GSK-3-dependent mechanism and to be required for netrin-1 effects on neuronal migration and axonal guidance [146]. However, the receptor involved has not been

investigated. Other targets of these signaling pathways may be translational regulators. Indeed, it has recently been proposed that neo protein translation is stimulated by netrin-1 and is required for netrin-1-mediating attraction of *Xenopus* retinal growth cones in vitro [132]. Key translation initiation factors like eIF4E and its regulatory protein eIF4E-BP1 are known to be phosphorylated by an ERK-1/2-dependent pathway [147], thus constituting a potential mechanism linking netrin-1 to novel protein synthesis for axon growth and guidance. Other targets may be transcription factors. It has been reported that Elk-1, a target of ERK-1/2, is activated in response to netrin-1, yet this has been observed in immortalized cells and still has to be demonstrated in vivo [91]. A stronger case was made with NFAT signaling. Indeed it has been shown that the netrin-1-induced DCC-dependent activation of NFAT transcription factors and netrin-1-mediated axon outgrowth require this activation [148]. However, it is still unclear what genes are targeted by these transcription factors that may be important for netrin-1 functions.

Very few, if any, intracellular signals are known to be induced through UNC5H receptors. The main observation regarding a putative role of UNC5H in netrin-1 signaling is the fact that, in the presence of netrin-1, the intracellular domain of UNC5H interacts with DCC and somehow down-regulates the signaling induced by DCC, hence converting axon attraction – when DCC is expressed alone – to axon repulsion – when DCC is expressed in the presence of UNC5H [40]. However, the view of UNC5H receptors as co-receptors and regulators of DCC does not completely fit with the observation that, in various systems, UNC5H receptors are either expressed without DCC or expressed at a higher level than DCC. Moreover, the recent analysis of the phenotype of mice bearing a mutation of DCC deleting its P3 domain shown to interact in vitro with UNC5H failed to match the proposed model, at least regarding corticospinal tract axon guidance [99]. Hence, it is tempting to speculate that UNC5H receptors initiate important signaling independent of DCC. Interestingly, it has been reported that UNC5H is submitted to the phosphorylation of its intracellular domain, a phosphorylation that is dependent on the presence of netrin-1 [149]. Moreover, cytoplasmic phosphorylated tyrosine 482 has been shown to be crucial for UNC5 function in *C. elegans* [150]. A recent report also suggested that UNC5H2 interacts with a Giα2 protein [151]. However, further work will have to analyze the intracellular signals that can be initiated via UNC5H upon netrin-1 binding.

It has become clear over the last few years that an important concept in neuronal guidance and in netrin-1 function in general is the exact interpretation of netrin-1 information, as netrin-1 appears as a survival factor, a chemoattractant and also a chemorepellent. Some cell-specific mechanisms then regulate the nature of the receptor complex expressed, depending on the desired effect of netrin-1. Along these lines, Tessier-Lavigne and colleagues have reported that DCC can interact with UNC5H receptors [40] and with Robo receptors [152]. The interaction of UNC5H with DCC is assumed to mediate the chemorepulsive activity of netrin-1 (see above). In contrast, the interaction of the intracellular domain of Robo with the intracellular domain of DCC that occurs in the presence of the Robo ligand Slit is supposed to silence the DCC effect. The DCC/Robo interaction is hypothesized to down-regulate DCC activity once commissural axons have crossed the midline, leading to a loss of responsiveness of these axons to netrin-1. However, it is not yet known what effect Robo interaction exerts on the various signaling pathways described above.

Another mechanism that regulates receptor presentation/activation appears to be their presence in lipid rafts. Accumulating evidence suggests that lipid rafts are dynamic, tightly packed and ordered membrane microdomains enriched in sphingolipids and cholesterol [153, 154]. This liquid ordered phase favors the dynamic assembly of different lipid-anchored proteins as well as transmembrane proteins [155]. Recent studies have shown that rafts play an important role in cell signaling, in particular through the organization of surface receptors, signaling enzymes and adaptor molecules into complexes at specific sites in the membrane [155]. Recently, it was shown that DCC is in part localized in lipid rafts [156, 157]. This localization is due to the palmitoylation of DCC [157] and is poorly dependent on netrin-1 presence [156, 157]. Importantly, the inhibition of DCC raft localization abolishes netrin-1-induced DCC signaling – i. e.MAPK pathway – and the disruption of raft integrity inhibits netrin-1 induced axon outgrowth [157] and turning [156]. Thus, it is tempting to speculate that a change of DCC in the lipid rafts could be a mechanism that regulates the netrin-1 response. How other netrin-1 receptors also associate with lipid rafts remains to be shown. Similarly, another mechanism that may regulate netrin-1 response is the surface expression of netrin-1 receptor. Hinck and colleagues have demonstrated that the presence of UNC5H1 at the cell membrane is regulated through the interaction of UNC5H1 with PICK1 (protein interacting with C kinase-1) and with protein kinase C (PKC). In particular, PKC activation has been shown to inhibit the UNC5H1-dependent netrin-1 response because UNC5H1 is no longer located at the plasma membrane [158].

When a neuronal cue turns out to be a survival factor in vivo

Role of DCC/UNC5H-induced death in axonal/neuronal migration

The role of the positive signaling pathways initiated upon netrin-1 binding to its receptors is easy to grasp.

They all participate in a way in the chemotropic activity of netrin-1 guidance, attraction, or repulsion of axons or neurons. However, the observation that four of the five known netrin-1 receptors induce cell death when expressed in settings in which netrin-1 is not available also suggests that the ability of netrin-1 to block cell death represents an important mechanism for guiding neurons. Thus, netrin-1-induced guidance may be the result of two mechanisms: (i) a positive mechanism that guides axon or neurons toward a gradient of ligand and (ii) a negative mechanism that kills neurons or axons that would grow out of the specific region of netrin-1 expression. This second mechanism would then determine the required migration path by regulating the fate of cells migrating out of this path (fig. 6). The positive mechanism has clearly been demonstrated by (i) the various ex vivo assays showing that experimentally generated netrin-1 gradient attracts or repels axons through a DCC-dependent mechanism, and (ii) by the phenotype of DCC and netrin-1 mutant mice in which commissural axons do not correctly extend [1, 5, 6, 88]. The negative mechanism needs support other than in vitro analysis showing neuronal cell death in the absence of netrin-1. Initial support has been provided by the analysis of inferior olivary neurons in netrin-1 null mutants [18, 19]. Indeed, inferior olivary neurons that originate from post-mitotic neurons expressing DCC, UNC5H or both receptors are known to migrate ventrally through a netrin-1 dependent process. In netrin-1 mutant mice, these neurons do not fail to migrate but actually die during the early stage of migration [116]. Interestingly, in DCC mutant mice, the inferior olive is also missing, but a similar cell death analysis shows a much reduced cell death induction [E. Bloch-Gallego, personal communication], suggesting that in this case the absence of inferior olive is related to the loss of migration due to the absence of positive signal. Along the same lines, Jiang et al. recently reported that neural crest cells that migrate and colonize the developing bowel and pancreas are also dependent on the DCC/netrin-1 pair for both migratory behavior – i.e. attraction mediated by a positive signal of bound DCC – and survival – i.e. inhibition of DCC-mediated cell death by netrin-1 presence [159]. Indeed, these authors report that netrin-1 mediates the survival of neural crest cell probably by inhibiting DCC-induced cell death [159]. One could, however, argue that, if this dual model fits well with chemoattraction, the model of cell death induction in settings with low netrin-1 concentration appears more difficult to apprehend in a context of chemorepulsion. A possible explanation is that chemorepulsion necessarily occurs in a context in which axonal/neuronal growth is still promoted by the repulsive clue, otherwise the axon/neuron would stop. Thus, chemorepulsion should represent a displacement toward a lower concentration of cues such as netrin-1 rather than a displacement toward no netrin-1. Moreover, while achieving its migration, the repulsed axon/neuron would necessarily find another cue that can promote its growth and probably its survival. In this context, repulsion toward a decreased concentration of netrin-1 is completely distinct from the death-promoting activity observed in the absence of netrin-1. However, even though this dual model of death/guidance is tempting, more definite evidence has to be provided to support the hypothesis conclusively.

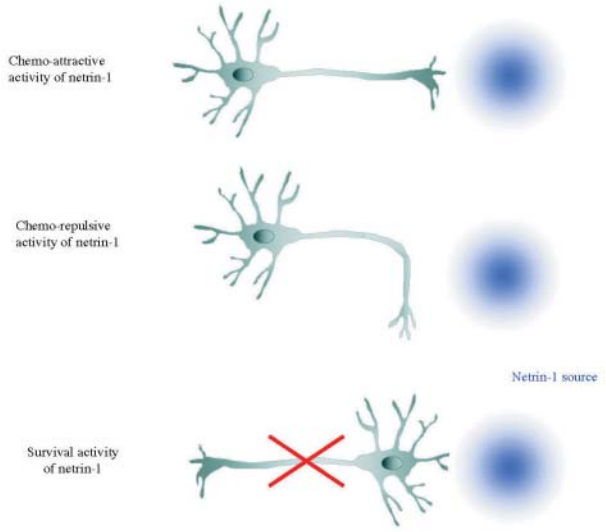

Figure 6. Putative model for axonal/neuronal guidance. Axonal/neuronal guidance may be the result of two opposite signaling pathways: (i) a positive signaling pathway leading to the well-demonstrated chemotropic activity of netrin-1 that attracts or repulses neurons/axons and (ii) the control of neuron/axon fate by the induction of apoptosis in neurons/axons that would grow or extend out of the region of netrin-1 availability.

Role of DCC/UNC5H-induced death in the control of tumorigenesis

Although the role of DCC in mediating netrin-1 effects has clearly been demonstrated during nervous system development, DCC cellular function outside the nervous system remains unknown. As noted above, *DCC* transcripts and protein have been detected in various adult normal tissues outside the nervous system [30, 160, 161]. Consistent with a possible role for DCC in transmitting netrin signals in tissues outside the nervous system, netrin-1 is also expressed in various adult tissues [162]. Northern blot studies indicate that netrin-1 transcripts may in fact be considerably more abundant in adult heart, small intestine and colon tissues than in the adult brain [162]. Within adult intestinal tissue, netrin-1 expression appears to be greatest at the base of crypts [89]. Given the absence of an obvious role for DCC and netrin-1 in the development of the intestine [88], what might be the

significance of DCC and netrin-1 expression in the intestine? The tight regulation of epithelium proliferation and differentiation in the intestine is known to be crucial for normal organ function and likely for inhibition of tumorigenesis [163]. Presumably intestinal stem cells reside near the base of the crypt, and the bulk of proliferating cells are located in the lower third of the crypt. As the cells migrate toward the villus tip, they become more differentiated. Upon reaching the tip, the cells are shed into the intestinal lumen, where they presumably undergo apoptosis. Perhaps the DCC/netrin-1 interaction may play a role in the regulation of cell proliferation and differentiation. Consistent with this idea, in certain cell lines forced DCC expression has been shown to induce G2/M cell cycle arrest [109], and in other cells ectopic DCC expression has been linked to a marked loss of proliferation [84]. Unfortunately, these studies have not explored the role of netrin-1 in DCC effects on cell cycle progression or proliferation. Moreover, while an initial study indicated that DCC might play a role in goblet cell differentiation [160], the possible role of DCC in differentiation along the goblet cell lineage has not been confirmed [84, 164]. Additionally, the possible role of DCC in differentiation does not fit neatly with data obtained in various cell lines, showing that, upon netrin-1 binding, DCC activates the ERK-1/2 MAPK pathway [91], while ERK-1/2 activation has been suggested to inhibit intestinal epithelium differentiation [165]. However, since small GTPases have been involved in DCC signaling, it is of some interest to consider the possible role of netrin-1-mediated DCC-dependent activation of small GTPases in the intestine. Indeed, transgenic mice expressing a constitutive form of Rac-1 in their intestines showed a precocious differentiation of the epithelium with accompanying alterations in apical actin [166]. Hence, DCC-mediated Rac-1 activation may be important for epithelium differentiation.

However, small GTPase, ERK-1/2 and FAK activation by DCC is unlikely to account for the putative DCC tumor suppressor activity, since small GTPase, MAPK and FAK/Src signaling have been shown to be activated in cancer and/or to participate directly in promoting tumor development [167, 168]. An alternative hypothesis for DCC/netrin-1 function in the intestine that may fit with DCC tumor suppressor activity is the possibility that DCC and netrin-1 have a role in cell survival regulation. The pro-apoptotic activity of DCC in the absence of netrin ligand would be consistent with a role for the DCC/netrin-1 pair in the homeostatic regulation of the intestinal epithelium and possibly as well in the suppression of tumorigenesis. Following the dependence receptor mechanism described above, we can hypothesize that the DCC/netrin-1 pair, and possibly UNC5H/netrin-1, may indeed limit tumor development by inducing the apoptosis of cells that acquire transforming capacities (fig. 7). Any tumor cell submitted to an inappropriate environment would have unbound dependence receptors, which would trigger the pro-apoptotic activity of DCC and/or UNC5H, and ultimately lead to cell death and subsequent tumor regression. This is particularly the case when highly proliferative cells grow in regions with low netrin-1 concentration or migrate to other tissues because of metastatic spread. In cancer, the deletion of genes coding for DCC and UNC5H would induce the loss of the pro-apoptotic signal, thus providing a selective advantage for tumor escape.

Accordingly, as described above, the DCC gene is known to be deleted in many tumors. Interestingly, a strongly

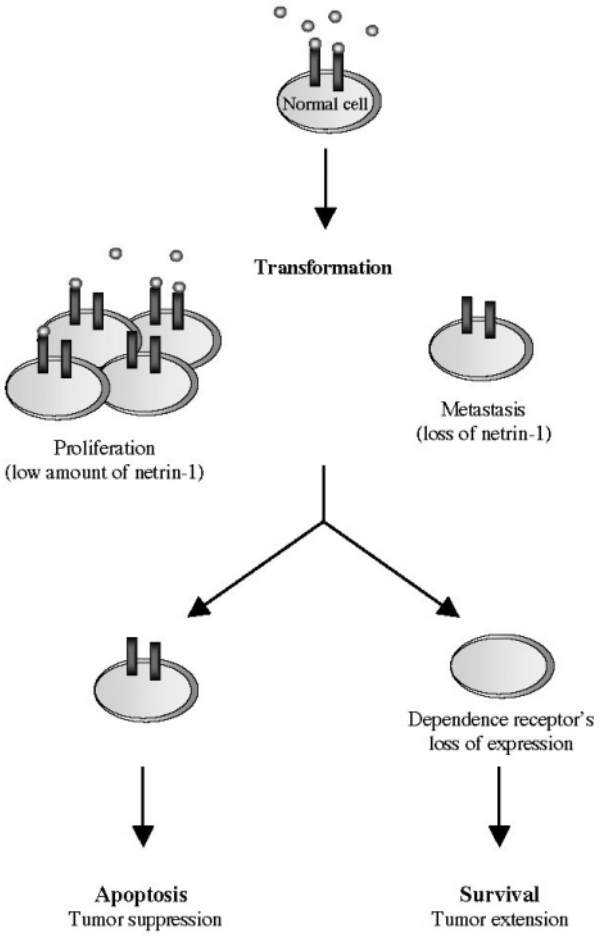

Figure 7. DCC and UNC5H receptors may function as tumor suppressors. In a normal context a cell expresses DCC/UNC5 in the setting of netrin-1 presence. On acquiring a transformed phenotype, this cell migrates toward other tissues (metastasis) or proliferates. In both cases, netrin-1 concentration decreases in regard to DCC/UNC5H expression, either because netrin-1 is not expressed in other tissues or because the number of transformed cells expressing DCC/UNC5H has increased while netrin-1 concentration remains unchanged. This turns DCC/UNC5H into a death inducer, hence limiting tumor extension. Such a mechanism cannot occur if the cell has acquired the selective advantage of losing/reducing DCC/UNC5H expression.

reduced expression of UNC5H has also been reported in over 90% of colorectal cancers and many other tumors. In colorectal cancers, this reduction has been mostly associated with UNC5H1 and UNC5H3 [94]. Although allelic losses have also been observed, the loss of UNC5H expression might rather be consecutive to epigenetic mechanisms such as promoter methylation [94]. Moreover, UNC5H2 has been shown to be a direct transcriptional target of the p53 tumor suppressor gene. Of major interest, p53 pro-apoptotic activity has been shown to be dependent on the expression of UNC5H2 and can be antagonized by netrin-1 [117]. Moreover, several in vitro experiments have shown that, similarly to DCC, UNC5H expression compromises the two hallmarks of cell transformation: anchorage-independent growth and ability to invade through a reconstituted basement membrane (Matrigel) [89, 94].

Interestingly, in normal colorectal tissues in which the loss of DCC and/or UNC5H in tumors is very common, netrin-1, DCC and UNC5H have a very interesting expression pattern [89]. In these tissues, netrin-1 is preferentially expressed at the base of the intestinal crypt [89], whereas DCC is distributed throughout the villi [169]. This is consistent with the classic view that highly proliferative cells from the base of the intestinal crypt differentiate while moving to the distal part of the villi/crypt and eventually detach and die or die and detach. Proliferating crypt cells that express DCC in a netrin-1-rich environment would be protected from cell death, whereas epithelial cells that have stopped proliferating to differentiate and move toward the villus tip would progressively be placed into a netrin-1-deprived environment, inducing apoptotic cell death. Along these lines, overexpressing netrin-1 throughout the intestinal epithelium contributes to reduce apoptosis by approximately 50% [89]. One may speculate that the apparent proximal-to-distal netrin-1 gradient may function as a regulatory system that limits the lifespan of cells that undergo (i) multiple proliferative steps within the crypt and (ii) repeated mechanical and chemical insults originating from the intestinal lumen, two conditions that may increase the risk of cell damage and resultant aberrant behavior. The cell death induced by the absence of superficial netrin-1 expression, together with the mechanical detachment of cells in the lumen, may thus be key factors for limiting the initiation of malignant transformation.

To evaluate the role of these DCC and UNC5H receptors in tumorigenesis, and to avoid the common bias of using the gene knockout method in which both the positive – netrin-1-dependent signals – and negative – apoptosis in the absence of netrin-1 – pathways are inactivated, we recently developed an alternative strategy. Mice forced to overexpress netrin-1 in the intestinal epithelium were used to prevent receptor-induced cell death. As seen above, the targeted overexpression of netrin-1 throughout the digestive tract produces approximately 50% cell death inhibition in the intestinal epithelium [89]. In agreement with the model proposed for the regulation of cell life span by netrin-1-controlled DCC-induced cell death, this inhibition is associated with the formation of numerous focal hyperplasias (compared with control mice) and adenomas [89]. Thus, cell death inhibition by netrin-1 is associated with increased initiation of colorectal tumorigenesis. Because DCC loss is very often considered a late event in human colorectal tumors, netrin-1-overexpressing mice were backcrossed with a mouse model in which colorectal tumorigenesis is initiated by a mutation in *adenomatous polyposis coli (APC)* [170]. APC mutated mice usually develop low-grade adenomas, but the tumor that developed in APC/netrin-1 mice progressed toward high grade adenoma and adenocarcinoma [89]. The above results demonstrate that blocking the cell death by netrin-1 overexpression can both initiate and stimulate colorectal tumorigenesis [89], thus confirming the tumorigenic role of dependence receptors expressed in the digestive tract.

Several key issues remain to be resolved. First, is the receptor involved DCC or UNC5H1-3? Interestingly, UNC5H and DCC proteins have distinct localizations in intestinal villi – UNC5H2 and DCC appear to be present along the entire villus, whereas UNC5H3 is found only in the intestinal crypt [89]. Together with the fact that the overexpression of netrin-1 in the mouse model described above is affected by two time window effects – one during early tumorigenesis, as exemplified by the adenomas and focal hyperplasias observed in the mice, and a later effect accompanied by the formation of adenoma carcinomas in APC/netrin-1 mice. This would be in favor of a regulatory role of these dependence receptors at different phases of colorectal tumorigenesis. The second issue concerns netrin-1 expression in human colorectal tumors. Indeed, the prediction from the model proposed would be that a similar selective advantage is conferred when tumor cells lose the expression of a receptor – as is the case for DCC and/or UNC5H – or gain netrin-1 expression. A preliminary set of data has shown that netrin-1 overexpression is only rarely associated with human colorectal cancer, i. e. 7% of tested tumors [89], suggesting that the gain of netrin-1 or the loss of the receptor does not confer similar selective advantages. Further work is required to analyze whether this effect is restricted to colorectal tumors or if it can be extrapolated to other types of cancer.

Other roles for netrin-1 and its receptors

In recent years, not only did netrin-1 move from a neuronal navigation cue to a survival molecule that may control tumorigenesis, but recent reports also proposed that netrin-1 may play alternative roles. The first one is a role

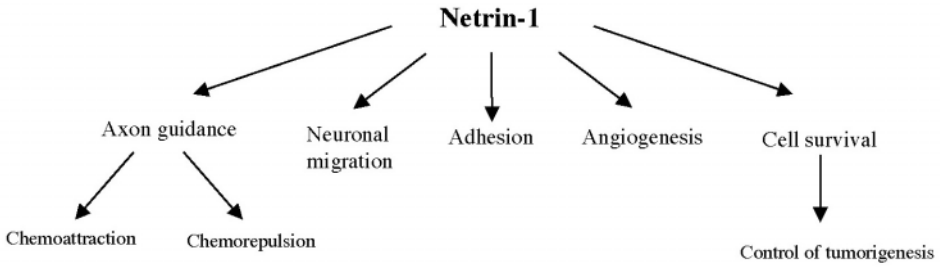

Figure 8. Netrin-1, a multi-functional molecule. Netrin is a multi-functional molecule that mediates axon chemoattraction, chemorepulsion, neuronal migration, angiogenesis, adhesion and consequent morphogenesis, and cell survival leading to the control of tumorigenesis.

as a cell adhesion molecule, the other one being a role in angiogenesis. Even though these roles are still poorly defined and remain controversial, they may turn out to be important facets of netrin-1.

Netrin-1 as an adhesion molecule involved in morphogenesis

Although the roles of netrin-1 as a neuronal guidance cue and as a survival factor are now largely documented, it is interesting to note that netrin-1, even though considered as a diffusible molecule, resembles laminin. Netrin-1 may then be hypothesized not only as a guidance cue that is expressed in gradient but as a matrix protein important for adhesion. Along this line, Hinck and colleagues have proposed that netrin-1 is important for mammary gland morphogenesis [171]. Indeed, netrin-1 is expressed in prelumenal cells; while analyzing the mammary gland development of netrin-1 mutants, it was observed that netrin-1 is required to stabilize multipotent progenitor cap cell layers. Similarly, netrins have been shown to be implicated in lung branching morphogenesis [172]. Moreover, Yebra and colleagues have proposed that netrin-1 mediates pancreatic epithelial cell adhesion. Interestingly, the puzzling aspect of these studies is the nature of the receptor involved in netrin-1 activity: it has been proposed that neogenin is the netrin-1 receptor involved in netrin-1 effects on mammary glands, but (i) whether netrin-1 is actually a functional ligand for neogenin is still a matter of controversy [29, 42, 43], and (ii) neogenin mutant mice have failed to show any obvious phenotype. Similarly, the nature of the receptor involved in lung branching is not known while integrins such as putative netrin-1 receptors (see above) may be implicated in pancreatic epithelial cell adhesion.

Netrin-1 as an angiogenic factor

Very recently, netrin-1 and its receptors were investigated in the context of angiogenesis. Indeed, it is a classic observation that molecules initially discovered in neuronal guidance also play a role in the morphogenesis of the vascular system [173]. Two recent papers have proposed a role for the netrin-1 receptor UNC5H2 and for netrin-1 in angiogenesis [100, 174]. In the first study, Lu and colleagues investigated the phenotype of UNC5H2 mutant mice and observed that homozygous mutant embryos display an abnormal vascular system phenotype with aberrant extension of endothelial tip cell filopodia, excessive vessel branching and abnormal navigation. Interestingly, the effect is probably independent of netrin-1, at least in mammals, as the inactivation of netrin-1 did not show a similar vascular phenotype. Whether other netrins or other unknown ligands are important for this angiogenic effect remains to be shown. Park and colleagues have used in vitro/ex vivo culture assays to show that netrin-1 triggers an angiogenic effect on endothelial cells. However, contrasting with the study of Eichmann and colleagues, the effect appears not to be mediated by DCC or UNC5H receptors. Another contradiction will have to be examined further: in the study by Eichmann and colleagues, UNC5H2 appears to play an inhibitory role for angiogenesis – i.e. mutants show excessive extension of endothelial tip cells or excessive vessel branching – while in the report by Park et al. netrin-1 appears to stimulate angiogenesis. In any case, these interesting studies support the role of netrins or netrin-1 receptors in angiogenesis. Whether this effect is related to a guidance effect or to a survival effect needs to be evaluated.

Concluding remarks

Initially described as an axon guidance cue, netrin-1 appears today as a fantastic molecule with multiple functions, from attracting to repulsing axons, from serving as a matrix molecule involved in tissue morphogenesis to regulating cell survival and tumorigenesis. Significant effort has been made in the last decade to clarify the role of netrin-1 in controlling the migration of neurons and the growth/orientation of axons during development. However, almost nothing is known about netrin-1 in adult. For sure netrin-1 and netrin family members are expressed in adults and particularly in adult brain: is this expression related to plasticity, survival/maintenance or to a completely different yet unknown function of netrin-1? We have shown that netrin-1 is also expressed in adult gut and serves there as a survival signal that limits

tumor formation. The next challenges are to understand whether, owing to its multiples facets, netrin-1 is a plausible target for therapeutic strategies against cancer and possibly other diseases.

Acknowledgments. We wish to thank A. Chédotal for critical reading and comments on the manuscript and D. Reynaud for text correction. C. F. is supported by a fellowship from the Ministère de la Recherche et de la Technologie. The work performed in Mehlen's laboratory is supported by the Ligue Contre le Cancer, the Centre National de la Recherche Scientifique, the Ministère de la Recherche et de la Technologie (ACI), the Association pour la Recherche contre le Cancer (ARC), the Fondation de la Recherche Médicale (FRM) and the National Institutes of Health (NIH).

1 Serafini T., Kennedy T. E., Galko M. J., Mirzayan C., Jessell T. M. and Tessier-Lavigne M. (1994) The netrins define a family of axon outgrowth-promoting proteins homologous to C. elegans UNC-6. Cell **78** (3): 409–424
2 Tessier-Lavigne M., Placzek M., Lumsden A. G., Dodd J., Jessell T. M. (1988) Chemotropic guidance of developing axons in the mammalian central nervous system. Nature **336** (6201): 775–778
3 Ramon y Cajal S. (1909) Histologie du System Nerveux de l'Homme et des Vertebres, Consejo Superior de investigaciones Cientificas, Madrid
4 Placzek M., Tessier-Lavigne M., Jessell T. and Dodd J. (1990) Orientation of commissural axons in vitro in response to a floor plate-derived chemoattractant. Development **110** (1): 19–30
5 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** (3): 425–435
6 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** (6): 1001–1014
7 Deiner M. S., Kennedy T. E., Fazeli A., Serafini T., Tessier-Lavigne M. and Sretavan D. W. (1997) Netrin-1 and DCC mediate axon guidance locally at the optic disc: loss of function leads to optic nerve hypoplasia. Neuron **19** (3): 575–589
8 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** (6): 1225–1235
9 de la Torre J. R., Hopker V. H., Ming G. L., Poo M. M., Tessier-Lavigne M., Hemmati-Brivanlou A. et al. (1997) Turning of retinal growth cones in a netrin-1 gradient mediated by the netrin receptor DCC. Neuron **19** (6): 1211–1224
10 Colamarino S. A. and Tessier-Lavigne M. (1995) The axonal chemoattractant netrin-1 is also a chemorepellent for trochlear motor axons. Cell **81** (4): 621–629
11 Wang H, Copeland NG, Gilbert DJ, Jenkins NA, Tessier-Lavigne M. (1999) Netrin-3, a mouse homolog of human NTN2L, is highly expressed in sensory ganglia and shows differential binding to netrin receptors. J. Neurosci. **19** (12): 4938–4947
12 Koch M., Murrell J. R., Hunter D. D., Olson P. F., Jin W., Keene D. R. et al. (2000) A novel member of the netrin family, beta-netrin, shares homology with the beta chain of laminin: identification, expression, and functional characterization. J. Cell Biol. **151** (2): 221–234
13 Nakashiba T., Nishimura S., Ikeda T. and Itohara S. (2002) Complementary expression and neurite outgrowth activity of netrin-G subfamily members. Mech. Dev. **111** (1–2): 47–60
14 Banyai L. and Patthy L. (1999) The NTR module: domains of netrins, secreted frizzled related proteins, and type I procollagen C-proteinase enhancer protein are homologous with tissue inhibitors of metalloproteases. Protein Sci. **8** (8): 1636–1642
15 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** (5): 873–881
16 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 **4** (1): 61–85
17 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** (2): 203–215
18 Yee K. T., Simon H. H., Tessier-Lavigne M. and O'Leary D. M. (1999) Extension of long leading processes and neuronal migration in the mammalian brain directed by the chemoattractant netrin-1. Neuron **24** (3): 607–622
19 Bloch-Gallego E., Ezan F., Tessier-Lavigne M. and Sotelo C. (1999) Floor plate and netrin-1 are involved in the migration and survival of inferior olivary neurons. J. Neurosci. **19** (11): 4407–4420
20 Alcantara S., Ruiz M., De Castro F., Soriano E. and Sotelo C. (2000) Netrin 1 acts as an attractive or as a repulsive cue for distinct migrating neurons during the development of the cerebellar system. Development **127** (7): 1359–1372
21 Su M., Merz D. C., Killeen M. T., Zhou Y., Zheng H., Kramer J. M. et al. (2000) Regulation of the UNC-5 netrin receptor initiates the first reorientation of migrating distal tip cells in Caenorhabditis elegans. Development **127** (3): 585–594
22 Sugimoto Y., Taniguchi M., Yagi T., Akagi Y., Nojyo Y. and Tamamaki N. (2001) Guidance of glial precursor cell migration by secreted cues in the developing optic nerve. Development **128** (17): 3321–3330
23 Hamasaki T, Goto S, Nishikawa S, Ushio Y. (2001) A role of netrin-1 in the formation of the subcortical structure striatum: repulsive action on the migration of late-born striatal neurons. J. Neurosci. **21** (12): 4272–4280
24 Lim Y. S. and Wadsworth W. G. (2002) Identification of domains of netrin UNC-6 that mediate attractive and repulsive guidance and responses from cells and growth cones. J. Neurosci. **22** (16): 7080–7087
25 Spassky N., de Castro F., Le Bras B., Heydon K., Queraud-Le-Saux F., Bloch-Gallego E. et al. (2002) Directional guidance of oligodendroglial migration by class 3 semaphorins and netrin-1. J. Neurosci. **22** (14): 5992–6004
26 Tsai H.H., Tessier-Lavigne M. and Miller R. H. (2003) Netrin 1 mediates spinal cord oligodendrocyte precursor dispersal. Development **130** (10): 2095–2105
27 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** (2): 289–299
28 Chan S. S., 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** (2): 187–195
29 Keino-Masu K., Masu M., Hinck L., Leonardo E. D., Chan S. S., Culotti J. G. et al. (1996) Deleted in Colorectal Cancer (DCC) encodes a netrin receptor. Cell **87** (2): 175–185
30 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** (4938): 49–56
31 Cavenee W. K., Dryja T. P., Phillips R. A., Benedict W. F., Godbout R., Gallie B. L. et al. (1983) Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature **305** (5937): 779–784
32 Knudson A. G. Jr (1985) Hereditary cancer, oncogenes and antioncogenes. Cancer Res **45** (4): 1437–1443
33 Vogelstein B., Fearon E. R., Hamilton S. R., Kern S. E., Preisinger A. C., Leppert M. et al. (1988) Genetic alterations dur-

ing colorectal-tumor development. N. Engl. J. Med. **319** (9): 525–532
34 Vogelstein B., Fearon E. R., Kern S. E., Hamilton S. R., Preisinger A. C., Nakamura Y. et al. (1989) Allelotype of colorectal carcinomas. Science **244** (4901): 207–211
35 Fearon E. R. and Vogelstein B. (1990) A genetic model for colorectal tumorigenesis. Cell **61** (5): 759–767
36 Kinzler K. W. and Vogelstein B. (1996) Lessons from hereditary colorectal cancer. Cell **87** (2): 159–170
37 Cho K. R., Oliner J. D., Simons J. W., Hedrick L., Fearon E. R., Preisinger A. C. et al. (1994) The DCC gene: structural analysis and mutations in colorectal carcinomas. Genomics **19** (3): 525–531
38 Ekstrand B. C., Mansfield T. A., Bigner S. H. and Fearon E. R. (1995) DCC expression is altered by multiple mechanisms in brain tumours. Oncogene **11** (11): 2393–2402
39 Reale M. A., Hu G., Zafar A. I., Getzenberg R. H., Levine S. M. and Fearon E. R. (1994) Expression and alternative splicing of the deleted in colorectal cancer (DCC) gene in normal and malignant tissues. Cancer Res. **54** (16): 4493–4501
40 Hong K., Hinck L., Nishiyama M., Poo M. M., Tessier-Lavigne M. and Stein E. (1999) A ligand-gated association between cytoplasmic domains of UNC5 and DCC family receptors converts netrin-induced growth cone attraction to repulsion. Cell **97** (7): 927–941
41 Meyerhardt J. A., Look A. T., Bigner S. H. and Fearon E. R. (1997) Identification and characterization of neogenin, a DCC-related gene. Oncogene **14** (10): 1129–1136
42 Rajagopalan S., Deitinghoff L., Davis D., Conrad S., Skutella T., Chedotal A. et al. (2004) Neogenin mediates the action of repulsive guidance molecule. Nat. Cell Biol. **6** (8): 756–762
43 Matsunaga E., Tauszig-Delamasure S., Monnier P. P., Mueller B. K., Strittmatter S. M., Mehlen P. et al. (2004) RGM and its receptor neogenin regulate neuronal survival. Nat. Cell Biol. **6** (8): 749–755
44 Pierceall W. E., Reale M. A., Candia A. F., Wright C. V., 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** (2): 654–665
45 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** (2): 197–204
46 Watanabe T., Wu T. T., Catalano P. J., Ueki T., Satriano R., Haller D. G. et al. (2001) Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N. Engl. J. Med. **344** (16): 1196–1206
47 Thiagalingam S., Lengauer C., Leach F. S., Schutte M., Hahn S. A., Overhauser J. et al. (1996) Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers. Nat. Genet. **13** (3): 343–346
48 Itoh F., Hinoda Y., Ohe M., Ohe Y., Ban T., Endo T. et al. (1993) Decreased expression of DCC mRNA in human colorectal cancers. Int. J. Cancer **53** (2): 260–263
49 Goi T., Yamaguchi A., Nakagawara G., Urano T., Shiku H. and Furukawa K. (1998) Reduced expression of deleted colorectal carcinoma (DCC) protein in established colon cancers. Br. J. Cancer **77** (3): 466–471
50 Schmitt C. A., Thaler K. R., Wittig B. M., Kaulen H., Meyer zum Buschenfelde K. H. and Dippold W. G. (1998) Detection of the DCC gene product in normal and malignant colorectal tissues and its relation to a codon 201 mutation. Br. J. Cancer **77** (4): 588–594
51 Tarafa G., Villanueva A., Farre L., Rodriguez J., Musulen E., Reyes G. et al. (2000) DCC and SMAD4 alterations in human colorectal and pancreatic tumor dissemination. Oncogene **19** (4): 546–555
52 Uchino S., Tsuda H., Noguchi M., Yokota J., Terada M., Saito T. et al. (1992) Frequent loss of heterozygosity at the DCC locus in gastric cancer. Cancer Res. **52** (11): 3099–3102
53 Fang D. C., Jass J. R. and Wang D. X. (1998) Loss of heterozygosity and loss of expression of the DCC gene in gastric cancer. J. Clin. Pathol. **51** (8): 593–596
54 Yoshida Y., Itoh F., Endo T., Hinoda Y. and Imai K. (1998) Decreased DCC mRNA expression in human gastric cancers is clinicopathologically significant. Int. J. Cancer **79** (6): 634–639
55 Brewster S. F., Gingell J. C., Browne S. and Brown K. W. (1994) Loss of heterozygosity on chromosome 18q is associated with muscle-invasive transitional cell carcinoma of the bladder. Br. J. Cancer **70** (4): 697–700
56 Latil A., Baron J. C., Cussenot O., Fournier G., Soussi T., Boccon-Gibod L. et al. (1994) Genetic alterations in localized prostate cancer: identification of a common region of deletion on chromosome arm 18q. Genes Chromosomes Cancer **11** (2): 119–125
57 Latil A., Chene L., Cochant-Priollet B., Mangin P., Fournier G., Berthon P. et al. (2003) Quantification of expression of netrins, slits and their receptors in human prostate tumors. Int. J. Cancer **103** (3): 306–315
58 Imamura T., Arima T., Kato H., Miyamoto S., Sasazuki T. and Wake N. (1992) Chromosomal deletions and K-ras gene mutations in human endometrial carcinomas. Int. J. Cancer **51** (1): 47–52
59 Gima T., Kato H., Honda T., Imamura T., Sasazuki T. and Wake N. (1994) DCC gene alteration in human endometrial carcinomas. Int. J. Cancer **57** (4): 480–485
60 Saegusa M., Hashimura M., Hara A. and Okayasu I. (1999) Loss of expression of the gene deleted in colon carcinoma (DCC) is closely related to histologic differentiation and lymph node metastasis in endometrial carcinoma. Cancer **85** (2): 453–464
61 Enomoto T., Fujita M., Cheng C., Nakashima R., Ozaki M., Inoue M. et al. (1995) Loss of expression and loss of heterozygosity in the DCC gene in neoplasms of the human female reproductive tract. Br. J. Cancer **71** (3): 462–467.
62 Saegusa M, Machida D, Okayasu I. (2000) Loss of DCC gene expression during ovarian tumorigenesis: relation to tumour differentiation and progression. Br. J. Cancer **82** (3): 571–578
63 Huang Y., Boynton R. F., Blount P. L., Silverstein R. J., Yin J., Tong Y. et al. (1992) Loss of heterozygosity involves multiple tumor suppressor genes in human esophageal cancers. Cancer Res. **52** (23): 6525–6530
64 Dolan K., Garde J., Gosney J., Sissons M., Wright T., Kingsnorth A. N. et al. (1998) Allelotype analysis of oesophageal adenocarcinoma: loss of heterozygosity occurs at multiple sites. Br. J. Cancer **78** (7): 950–957
65 Devilee P., van Vliet M., Kuipers-Dijkshoorn N., Pearson P. L. and Cornelisse C. J. (1991) Somatic genetic changes on chromosome 18 in breast carcinomas: is the DCC gene involved? Oncogene **6** (2): 311–315
66 Thompson A. M., Morris R. G., Wallace M., Wyllie A. H., Steel C. M. and Carter D. C. (1993) Allele loss from 5q21 (APC/MCC) and 18q21 (DCC) and DCC mRNA expression in breast cancer. Br. J. Cancer **68** (1): 64–68
67 Murty V. V., Li R. G., Houldsworth J., Bronson D. L., Reuter V. E., Bosl G. J. et al. (1994) Frequent allelic deletions and loss of expression characterize the DCC gene in male germ cell tumors. Oncogene **9** (11): 3227–3231
68 Strohmeyer D., Langenhof S., Ackermann R., Hartmann M., Strohmeyer T. and Schmidt B. (1997) Analysis of the DCC tumor suppressor gene in testicular germ cell tumors: mutations and loss of expression. J. Urol. **157** (5): 1973–1976
69 Scheck A. C. and Coons S. W. (1993) Expression of the tumor suppressor gene DCC in human gliomas. Cancer Res. **53** (23): 5605–5609
70 Reyes-Mugica M., Rieger-Christ K., Ohgaki H., Ekstrand B. C., Helie M., Kleinman G. et al. (1997) Loss of DCC expression and glioma progression. Cancer Res. **57** (3): 382–386

71 Kong X. T., Choi S. H., Inoue A., Takita J., Yokota J., Hanada R. et al. (1997) Alterations of the tumour suppressor gene DCC in neuroblastoma. Eur. J. Cancer **33** (12): 1962–1965
72 Reyes-Mugica M., Lin P., Yokota J. and Reale M. A. (1998) Status of deleted in colorectal cancer gene expression correlates with neuroblastoma metastasis. Lab. Invest. **78** (6): 669–675
73 Porfiri E., Secker-Walker L. M., Hoffbrand A. V. and Hancock J. F. (1993) DCC tumor suppressor gene is inactivated in hematologic malignancies showing monosomy 18. Blood **81** (10): 2696–2701
74 Miyake K., Inokuchi K., Dan K. and Nomura T. (1993) Alterations in the deleted in colorectal carcinoma gene in human primary leukemia. Blood **82** (3): 927–930
75 Miyake K., Inokuchi K. and Nomura T. (1994) Expression of the DCC gene in human hematological malignancies. Leuk. Lymphoma **16** (1–2): 13–18
76 Jen J., Kim H., Piantadosi S., Liu Z. F., Levitt R. C., Sistonen P. et al. (1994) Allelic loss of chromosome 18q and prognosis in colorectal cancer. N. Engl. J. Med **331** (4): 213–221
77 Lanza G., Matteuzzi M., Gafa R., Orvieto E., Maestri I., Santini A. et al. (1998) Chromosome 18q allelic loss and prognosis in stage II and III colon cancer. Int. J. Cancer **79** (4): 390–395
78 Sun X. F., Rutten S., Zhang H. and Nordenskjold B. (1999) Expression of the deleted in colorectal cancer gene is related to prognosis in DNA diploid and low proliferative colorectal adenocarcinoma. J. Clin. Oncol. **17** (6): 1745–1750
79 Saito M., Yamaguchi A., Goi T., Tsuchiyama T., Nakagawara G., Urano T. et al. (1999) Expression of DCC protein in colorectal tumors and its relationship to tumor progression and metastasis. Oncology **56** (2): 134–141
80 Saw R. P., Koorey D., Painter D., Gallagher P. J. and Solomon M. J. (2002) p53, DCC and thymidylate synthase as predictors of survival after resection of hepatic metastases from colorectal cancer. Br. J. Surg. **89** (11): 1409–1415
81 Barratt P. L., Seymour M. T., Stenning S. P., Georgiades I., Walker C., Birbeck K. et al. (2002) DNA markers predicting benefit from adjuvant fluorouracil in patients with colon cancer: a molecular study. Lancet **360** (9343): 1381–1391
82 Tanaka K., Oshimura M., Kikuchi R., Seki M., Hayashi T. and Miyaki M. (1991) Suppression of tumorigenicity in human colon carcinoma cells by introduction of normal chromosome 5 or 18. Nature **349** (6307): 340–342
83 Klingelhutz A. J., Smith P. P., Garrett L. R. and McDougall J. K. (1993) Alteration of the DCC tumor-suppressor gene in tumorigenic HPV-18 immortalized human keratinocytes transformed by nitrosomethylurea. Oncogene **8** (1): 95–99
84 Velcich A., Corner G., Palumbo L. and Augenlicht L. (1999) Altered phenotype of HT29 colonic adenocarcinoma cells following expression of the DCC gene. Oncogene **18** (16): 2599–2606
85 Kato H., Zhou Y., Asanoma K., Kondo H., Yoshikawa Y., Watanabe K. et al. (2000) Suppressed tumorigenicity of human endometrial cancer cells by the restored expression of the DCC gene. Br. J. Cancer **82** (2): 459–466
86 Riggins G. J., Thiagalingam S., Rozenblum E., Weinstein C. L., Kern S. E., Hamilton S. R. et al. (1996) Mad-related genes in the human. Nat. Genet. **13** (3): 347–349
87 Takaku K., Oshima M., Miyoshi H., Matsui M., Seldin M. F. and Taketo M. M. (1998) Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes. Cell **92** (5): 645–656
88 Fazeli A., Dickinson S. L., Hermiston M. L., Tighe R. V., Steen R. G., Small C. G. et al. (1997) Phenotype of mice lacking functional Deleted in colorectal cancer (Dcc) gene. Nature **386** (6627): 796–804
89 Mazelin L., Bernet A., Bonod-Bidaud C., Pays L., Arnaud S., Gespach C. et al. (2004) Netrin-1 controls colorectal tumorigenesis by regulating apoptosis. Nature **431** (7004): 80–84
90 Stein E., Zou Y., Poo M. and Tessier-Lavigne M. (2001) Binding of DCC by netrin-1 to mediate axon guidance independent of adenosine A2B receptor activation. Science **291** (5510): 1976–1982
91 Forcet C., Stein E., Pays L., Corset V., Llambi F., Tessier-Lavigne M. et al. (2002) Netrin-1-mediated axon outgrowth requires deleted in colorectal cancer-dependent MAPK activation. Nature **417** (6887): 443–447
92 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 **386** (6627): 833–838
93 Engelkamp D. (2002) Cloning of three mouse Unc5 genes and their expression patterns at mid-gestation. Mech. Dev. **118** (1–2): 191–197
94 Thiebault K., Mazelin L., Pays L., Llambi F., Joly M. O., Saurin J. C. et al. (2003) The netrin-1 receptors UNC5H are putative tumor suppressors controlling cell death commitment. Proc. Natl. Acad. Sci. USA **100** (7): 4173–4178
95 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 **386** (6627): 838–842
96 Keleman K. and Dickson B. J. (2001) Short- and long-range repulsion by the Drosophila Unc5 netrin receptor. Neuron **32** (4): 605–617
97 Hamelin M., Zhou Y., Su M. W., Scott I. M. and Culotti J. G. (1993) Expression of the UNC-5 guidance receptor in the touch neurons of C. elegans steers their axons dorsally. Nature **364** (6435): 327–330
98 Przyborski S. A., Knowles B. B. and Ackerman S. L. (1998) Embryonic phenotype of Unc5h3 mutant mice suggests chemorepulsion during the formation of the rostral cerebellar boundary. Development **125** (1): 41–50
99 Finger J. H., Bronson R. T., Harris B., Johnson K., Przyborski S. A. and Ackerman S. L. (2002) The netrin 1 receptors Unc5h3 and Dcc are necessary at multiple choice points for the guidance of corticospinal tract axons. J. Neurosci. **22** (23): 10346–10356
100 Lu X., Le Noble F., Yuan L., Jiang Q., De Lafarge B., Sugiyama D. et al. (2004) The netrin receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system. Nature **432** (7014): 179–186
101 Corset V., Nguyen-Ba-Charvet K. T., Forcet C., Moyse E., Chedotal A. and Mehlen P. (2000) Netrin-1-mediated axon outgrowth and cAMP production requires interaction with adenosine A2b receptor. Nature **407** (6805): 747–750
102 Fredholm B. B., AP I. J., Jacobson K. A., Klotz K. N. and Linden J. (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. **53** (4): 527–552
103 Feoktistov I. and Biaggioni I. (1997) Adenosine A2B receptors. Pharmacol. Rev. **49** (4): 381–402
104 Shewan D., Dwivedy A., Anderson R. and Holt C. E. (2002) Age-related changes underlie switch in netrin-1 responsiveness as growth cones advance along visual pathway. Nat. Neurosci. **5** (10): 955–962
105 Yebra M., Montgomery A. M., Diaferia G. R., Kaido T., Silletti S., Perez B. et al. (2003) Recognition of the neural chemoattractant Netrin-1 by integrins alpha6beta4 and alpha3beta1 regulates epithelial cell adhesion and migration. Dev. Cell **5** (5): 695–707
106 Mehlen P. and Bredesen D. E. (2004) The dependence receptor hypothesis. Apoptosis **9**: 37–49
107 Mehlen P. and Thibert C. (2004) Dependence receptors: between life and death. Cell. Mol. Life Sci. **61** (15): 1854–1866
108 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 mechanism requiring receptor proteolysis. Nature **395** (6704): 801–804
109 Chen Y. Q., Hsieh J. T., Yao F., Fang B., Pong R. C., Cipriano S. C. et al. (1999) Induction of apoptosis and G2/M cell cycle arrest by DCC. Oncogene **18** (17): 2747–2754.

110 Forcet C., Ye X., Granger L., Corset V., Shin H., Bredesen D. E. et al. (2001) Thedependence receptor DCC (deleted in colorectal cancer) defines an alternative mechanism for caspase activation. Proc. Natl. Acad. Sci. USA **98** (6): 3416–3421

111 Liu J., Yao F., Wu R., Morgan M., Thorburn A., Finley R. L. Jr et al. (2002) Mediation of the DCC apoptotic signal by DIP13 alpha. J. Biol. Chem. **277** (29): 26281–26285

112 Rabizadeh S., Oh J., Zhong L. T., Yang J., Bitler C. M., Butcher L. L. et al. (1993) Induction of apoptosis by the low-affinity NGF receptor. Science **261** (5119): 345–348

113 Ellerby L. M., Hackam A. S., Propp S. S., Ellerby H. M., Rabizadeh S., Cashman N. R. et al. (1999) Kennedy's disease: caspase cleavage of the androgen receptor is a crucial event in cytotoxicity. J. Neurochem. **72** (1): 185–195

114 Bordeaux M. C., Forcet C., Granger L., Corset V., Bidaud C., Billaud M. et al. (2000) The RET proto-oncogene induces apoptosis: a novel mechanism for Hirschsprung disease. EMBO J. **19** (15): 4056–4063

115 Stupack D. G., Puente X. S., Boutsaboualoy S., Storgard C. M. and Cheresh D. A. (2001) Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. J. Cell. Biol. **155** (3): 459–470

116 Llambi F., Causeret F., Bloch-Gallego E. and Mehlen P. (2001) Netrin-1 acts as a survival factor via its receptors UNC5H and DCC. EMBO J. **20** (11): 2715–2722

117 Tanikawa C., Matsuda K., Fukuda S., Nakamura Y. and Arakawa H. (2003) p53RDL1 regulates p53-dependent apoptosis. Nat. Cell Biol. **5** (3): 216–223

118 Williams M. E., Strickland P., Watanabe K. and Hinck L. (2003) UNC5H1 induces apoptosis via its juxtamembrane domain through an interaction with NRAGE. J. Biol. Chem. **278** (19): 17483–17490

119 Zimmermann K. C. and Green D. R. (2001) How cells die: apoptosis pathways. J. Allergy Clin. Immunol. **108** (4 Suppl.): S99–103

120 Deiss L. P., Feinstein E., Berissi H., Cohen O. and Kimchi A. (1995) Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death. Genes Dev. **9** (1): 15–30

121 Cohen O., Inbal B., Kissil J. L., Raveh T., Berissi H., Spivak-Kroizaman T. et al. (1999) DAP-kinase participates in TNF-alpha- and Fas-induced apoptosis and its function requires the death domain. J. Cell Biol. **146** (1): 141–148

122 Llambi F., Laurenco F. C., Gozuacik D., Guix C., Pays L., Del Rio G. et al. (2005) The dependence receptor UNC5H2 mediates apoptosis through DAP-kinase. EMBO J. **24:** 1192–1201

123 Salvesen G. S. and Dixit V. M. (1997) Caspases: intracellular signaling by proteolysis. Cell **91** (4): 443–446

124 Yang X., Chang H. Y. and Baltimore D. (1998) Autoproteolytic activation of pro-caspases by oligomerization. Mol. Cell **1** (2): 319–325

125 Salvesen G. S. and Duckett C. S. (2002) IAP proteins: blocking the road to death's door. Nat. Rev. Mol. Cell. Biol. **3** (6): 401–410

126 Fernando P., Kelly J. F., Balazsi K., Slack R. S. and Megeney L. A. (2002) Caspase 3 activity is required for skeletal muscle differentiation. Proc. Natl. Acad. Sci. USA **99** (17): 11025–11030

127 Campbell D. S. and Holt C. E. (2003) Apoptotic Pathway and MAPKs Differentially Regulate Chemotropic Responses of Retinal Growth Cones. Neuron **37** (6): 939–952

128 Hopker V. H., Shewan D., Tessier-Lavigne M., Poo M. and Holt C. (1999) Growth-cone attraction to netrin-1 is converted to repulsion by laminin-1. Nature **401** (6748): 69–73

129 Hong K., Nishiyama M., Henley J., Tessier-Lavigne M. and Poo M. (2000) Calcium signalling in the guidance of nerve growth by netrin-1. Nature **403** (6765): 93–98

130 Wang G. X. and Poo M. M. (2005) Requirement of TRPC channels in netrin-1-induced chemotropic turning of nerve growth cones. Nature **434** (7035): 898–904

131 Shim S., Goh E. L., Ge S., Sailor K., Yuan J. P., Roderick H. L. et al. (2005) XTRPC1-dependent chemotropic guidance of neuronal growth cones. Nat. Neurosci. **8** (6): 730–735

132 Campbell D. S. and Holt C. E. (2001) Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. Neuron **32** (6): 1013–1026

133 Ming G., Song H., Berninger B., Inagaki N., Tessier-Lavigne M. and Poo M. (1999) Phospholipase C-gamma and phosphoinositide 3-kinase mediate cytoplasmic signaling in nerve growth cone guidance. Neuron **23** (1): 139–148

134 Ming G. L., Wong S. T., Henley J., Yuan X. B., Song H. J., Spitzer N. C. et al. (2002) Adaptation in the chemotactic guidance of nerve growth cones. Nature **417** (6887): 411–418

135 Li X., Saint-Cyr-Proulx E., Aktories K. and Lamarche-Vane N. (2002) Rac1 and Cdc42 but not RhoA or Rho kinase activities are required for neurite outgrowth induced by the Netrin-1 receptor DCC (deleted in colorectal cancer) in N1E-115 neuroblastoma cells. J. Biol. Chem. **277** (17): 15207–15214

136 Shekarabi M. and Kennedy T. E. (2002) The netrin-1 receptor DCC promotes filopodia formation and cell spreading by activating Cdc42 and Rac1. Mol. Cell. Neurosci. **19** (1): 1–17

137 Etienne-Manneville S. and Hall A. (2002) Rho GTPases in cell biology. Nature **420** (6916): 629–635

138 Shekarabi M., Moore S. W., Tritsch N. X., Morris S. J., Bouchard J. F. and Kennedy T. E. (2005) Deleted in colorectal cancer binding netrin-1 mediates cell substrate adhesion and recruits Cdc42, Rac1, Pak1, and N-WASP into an intracellular signaling complex that promotes growth cone expansion. J. Neurosci. **25** (12): 3132–3141

139 Gitai Z., Yu T. W., Lundquist E. A., Tessier-Lavigne M. and Bargmann C. I. (2003) The Netrin Receptor UNC-40/DCC Stimulates Axon Attraction and Outgrowth through Enabled and, in Parallel, Rac and UNC-115/AbLIM. Neuron **37** (1): 53–65

140 Li X., Meriane M., Triki I., Shekarabi M., Kennedy T. E., Larose L. et al. (2002) The adaptor protein Nck-1 couples the netrin-1 receptor DCC (deleted in colorectal cancer) to the activation of the small GTPase Rac1 through an atypical mechanism. J. Biol. Chem. **277** (40): 37788–37797

141 Li W., Lee J., Vikis H. G., Lee S. H., Liu G., Aurandt J. et al. (2004) Activation of FAK and Src are receptor-proximal events required for netrin signaling. Nat. Neurosci. **7** (11): 1213–1221

142 Liu G., Beggs H., Jurgensen C., Park H. T., Tang H., Gorski J. et al. (2004) Netrin requires focal adhesion kinase and Src family kinases for axon outgrowth and attraction. Nat. Neurosci. **7** (11): 1222–1232

143 Ren X. R., Ming G. L., Xie Y., Hong Y., Sun D. M., Zhao Z. Q. et al. (2004) Focal adhesion kinase in netrin-1 signaling. Nat. Neurosci. **7** (11): 1204–1212

144 Meriane M., Tcherkezian J., Webber C. A., Danek E. I., Triki I., McFarlane S. et al. (2004) Phosphorylation of DCC by Fyn mediates Netrin-1 signaling in growth cone guidance. J. Cell. Biol. **167** (4): 687–698

145 Lebrand C., Dent E. W., Strasser G. A., Lanier L. M., Krause M., Svitkina T. M. et al. (2004) Critical role of Ena/VASP proteins for filopodia formation in neurons and in function downstream of netrin-1. Neuron **42** (1): 37–49

146 Del Rio J. A., Gonzalez-Billault C., Urena J. M., Jimenez E. M., Barallobre M. J., Pascual M. et al. (2004) MAP1B is required for Netrin 1 signaling in neuronal migration and axonal guidance. Curr. Biol. **14** (10): 840–850

147 Herbert T. P., Tee A. R. and Proud C. G. (2002) The extracellular signal-regulated kinase pathway regulates the phosphorylation of 4E-BP1 at multiple sites. J. Biol. Chem. **277** (13): 11591–11596

148 Graef I. A., Wang F., Charron F., Chen L., Neilson J., Tessier-Lavigne M. et al. (2003) Neurotrophins and netrins require calcineurin/NFAT signaling to stimulate outgrowth of embryonic axons. Cell **113** (5): 657–670

149 Tong J., Killeen M., Steven R., Binns K. L., Culotti J. and Pawson T. (2001) Netrin stimulates tyrosine phosphorylation of the UNC-5 family of netrin receptors and induces Shp2 binding to the RCM cytodomain. J. Biol. Chem. **276** (44): 40917–40925
150 Killeen M., Tong J., Krizus A., Steven R., Scott I., Pawson T. et al. (2002) UNC-5 function requires phosphorylation of cytoplasmic tyrosine 482, but its UNC-40-independent functions also require a region between the ZU-5 and death domains. Dev. Biol. **251** (2): 348–366
151 Komatsuzaki K., Dalvin S. and Kinane T. B. (2002) Modulation of G(ialpha(2)) signaling by the axonal guidance molecule UNC5H2. Biochem. Biophys. Res. Commun. **297** (4): 898–905
152 Stein E. and Tessier-Lavigne M. (2001) Hierarchical organization of guidance receptors: silencing of netrin attraction by slit through a Robo/DCC receptor complex. Science **291** (5510): 1928–1938
153 Pike L. J. (2003) Lipid rafts: Heterogeneity on the high seas. Biochem. J. **378** (Pt. 2): 281–291
154 Hueber A. O. (2003) Role of membrane microdomain rafts in TNFR-mediated signal transduction. Cell Death Differ. **10** (1): 7–9
155 Simons K. and Toomre D. (2000) Lipid rafts and signal transduction. Nat. Rev. Mol. Cell. Biol. **1** (1): 31–39
156 Guirland C., Suzuki S., Kojima M., Lu B. and Zheng J. Q. (2004) Lipid rafts mediate chemotropic guidance of nerve growth cones. Neuron **42** (1): 51–62
157 Herincs Z., Corset V., Cahuzac N., Furne C., Castellani V., Hueber A. O. et al. (2005) DCC association with lipid rafts is required for netrin-1-mediated axon guidance. J. Cell Sci. **118** (Pt 8): 1687–1692
158 Williams M. E., Wu S. C., McKenna W. L. and Hinck L. (2003) Surface expression of the netrin receptor UNC5H1 is regulated through a protein kinase C-interacting protein/protein kinase-dependent mechanism. J. Neurosci. **23** (36): 11279–11288
159 Jiang Y., Liu M. I. and Gershon M. D. (2003) Netrins and DCC in the guidance of migrating neural Crest-Derived Cells in the developing bowel and pancreas. Dev. Biol. **258:** 364–384
160 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** (10): 1174–1183
161 Mehlen P. and Fearon E. R. (2004) Role of the dependence receptor DCC in colorectal cancer pathogenesis. J. Clin. Oncol. **22** (16): 3420–3428
162 Meyerhardt J. A., Caca K., Eckstrand B. C., Hu G., Lengauer C., Banavali S. et al. (1999) Netrin-1: interaction with deleted in colorectal cancer (DCC) and alterations in brain tumors and neuroblastomas. Cell Growth Differ. **10** (1): 35–42
163 Potten C. S. (1992) The significance of spontaneous and induced apoptosis in the gastrointestinal tract of mice. Cancer Metastasis Rev. **11** (2): 179–195
164 Fabre M., Martin M., Ulloa F. and Real F. X. (1999) In vitro analysis of the role of DCC in mucus-secreting intestinal differentiation. Int. J. Cancer **81** (5): 799–807
165 Taupin D. and Podolsky D. K. (1999) Mitogen-activated protein kinase activation regulates intestinal epithelial differentiation. Gastroenterology **116** (5): 1072–1080
166 Stappenbeck T. S. and Gordon J. I. (2001) Extranuclear sequestration of phospho-Jun N-terminal kinase and distorted villi produced by activated Rac1 in the intestinal epithelium of chimeric mice. Development **128** (13): 2603–2614
167 Johnson G. L. and Lapadat R. (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK and p38 protein kinases. Science **298** (5600): 1911–1912
168 Jaffe A. B. and Hall A. (2002) Rho GTPases in transformation and metastasis. Adv. Cancer Res. **84**: 57–80
169 Hsu Y. H., Shaw C. K. and Chuong C. M. (2001) Immunohistochemical localization of deleted-in-colon-cancer (DCC) protein in human epithelial, neural and neuro-endocrine tissues in paraffin sections with antigen retrieval. Kaohsiung J. Med. Sci. **17** (7): 351–357
170 Fodde R., Edelmann W., Yang K., van Leeuwen C., Carlson C., Renault B. et al. (1994) A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors. Proc. Natl. Acad. Sci. USA **91** (19): 8969–8973
171 Srinivasan K., Strickland P., Valdes A., Shin G. C. and Hinck L. (2003) Netrin-1/neogenin interaction stabilizes multipotent progenitor cap cells during mammary gland morphogenesis. Dev. Cell **4** (3): 371–382
172 Liu Y., Stein E., Oliver T., Li Y., Brunken W. J., Koch M. et al. (2004) Novel role for Netrins in regulating epithelial behavior during lung branching morphogenesis. Curr. Biol. **14** (10): 897–905
173 Autiero M., De Smet F., Claes F. and Carmeliet P. (2005) Role of neural guidance signals in blood vessel navigation. Cardiovasc. Res. **65** (3): 629–638
174 Park K. W., Crouse D., Lee M., Karnik S. K., Sorensen L. K., Murphy K. J. et al. (2004) The axonal attractant Netrin-1 is an angiogenic factor. Proc. Natl. Acad. Sci. USA **101** (46): 16210–16215