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Sema3D and Sema7A Have Distinct Expression Patterns in Chick Embryonic Development

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Abstract

By RT-PCR, we isolated a partial cDNA clone for the chick *Semaphorin7A* (*Sema7A*) gene. We further analyzed its expression patterns and compared them with those of the *Sema3D* gene, in chick embryonic development. *Sema3D* and *Sema7A* appeared to be expressed in distinct cell populations. In mesoderm-derived structures, *Sema7A* expression was detected in the newly formed somites, whereas *Sema3D* expression was found in the notochord. In ectoderm-derived tissues, *Sema3D* is expressed broadly in the surface ectoderm, lens and nasal placodes. *Sema3D* is also expressed in the developing nervous system including diencephalon, dorsal neural tube, optical and otic vesicles. In the limb bud, *Sema3D* expression was found throughout the ectoderm excluding the apical ectoderm ridge (AER), where *Sema7A* is concentrated. Although both genes appeared to be expressed in the migrating neural crest cells, *Sema3D* expression is limited to neural crest cells migrating out of the midbrain/hindbrain regions, while *Sema7A* expression is widespread in both cranial and trunk neural crest cells.

Keywords

expression patterns; ectoderm; limb bud; chick; embryogenesis

INTRODUCTION

Semaphorins comprise one of the largest families of axon guidance cues, sharing a conserved SEMA domain containing approximately 500 amino acids (Raper, 2000; Pasterkamp and Kolodkin, 2003). The central feature of the SEMA domain is a seven-blade β -propeller fold similar to the β -propeller repeats of integrin α subunits. Based on sequence similarity and structural features, semaphorin proteins are classified into eight subclasses: Class 1 and 2 semaphorins are found in invertebrate species and class V semaphorins are found in the genomes of certain DNA viruses. Class 3–7 semaphorins are expressed in vertebrates: secreted proteins in class 3, glycosylphosphatidylinositol (GPI)-anchored proteins in class 7, and transmembrane proteins in classes 4 to 6. Two receptor families, plexins and neuropilins, have been characterized to mediate semaphorin functions. Except for class 3 semaphorins, which require neuropilins as co-receptors, other semaphorins bind to plexins and activate downstream signaling pathways.

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Many axon guidance factors have recently been shown to play a role in the development of non-neural tissues, including Netrin, Slit, Ephrin, and Semaphorin families (Hinck, 2004). It has been suggested that Netrin and the DCC-related receptor neogenin help stabilize cell layers during organogenesis of the mammary gland (Srinivasan et al., 2003). Netrin also interacts with integrin to mediate migration of pancreatic epithelial cells at early stages of development (Yebra et al., 2003). Slit proteins are expressed in a wide range of tissues outside the central nervous system, including the urogenital system, limb and tooth primordia (Piper and Little, 2003). Slit/Robo signaling has been shown to be important for kidney development (Grieshammer et al., 2004). Slit also regulates migration of myoblast cells, mesodermal cells, and leukocyte chemotaxis in *Drosophila* and vertebrates (Wong et al., 2002). It has been shown that ephrins and Eph receptors are involved in confining migrating cells to appropriate pathways and preventing intermixing of the cells at the boundaries, such as segmental interfaces in the hindbrain and somites (Durbin et al., 1998; Xu et al., 1999; Poliakov et al., 2004), in angiogenesis (Wang et al., 1998; Gerety et al., 1999), and during neural crest cell migration (Krull et al., 1997; Wang and Anderson, 1997; Santiago and Erickson, 2002).

Similarly, semaphorins have been shown to play many important roles in embryonic development, in addition to their roles as axon guidance factors. Autocrine stimulation by class 3 semaphorins such as *Sema3A* and *Sema3F* modulates endothelial cell adhesive and migratory properties in angiogenesis (Serini et al., 2003). Signaling by *Sema3E* to plexin-D1 independent of neuropillin controls vascular patterning in the somites by limiting angiogenic sprouting (Gu et al., 2005). Class 3 semaphorins are essential for heart morphogenesis. Targeted mutation of *Sema3C*, *Sema3A*, or *Plexin D1* in mice exhibits severe defects in various cardiac structures (Behar et al., 1996; Feiner et al., 2001; Gitler et al., 2004). In addition, *Sema6D* has been shown to play dual roles in cardiac morphogenesis, promoting migration of cells from the conotruncal segment, and inhibiting migration of ventricular cells (Toyofuku et al., 2004a). Reverse signaling by *Sema6D* enhances the migration of myocardial cells from the compact zone into the trabeculae (Toyofuku et al., 2004b). Semaphorins also additionally play a role in cell–cell interaction in the immune system, cancer formation, and metastasis (Kruger et al., 2005).

Although the expression patterns of semaphorins have been well documented in the development of the nervous systems (Shepherd et al., 1996; Chilton and Guthrie, 2003), expression analysis of semaphorin genes during early embryonic development is relatively limited. Here, we report characterization of a partial cDNA clone of the chick *Sema7A* gene, and its expression patterns in early embryonic development, in comparison to those of the *Sema3D* gene. Interestingly, these two genes exhibit largely non-overlapping, in some areas even complementary, expression patterns. In mesoderm-derived tissues, *Sema3D* transcripts were detected in the notochord, while *Sema7A* transcripts were detected in the newly formed somites. In the neural tube, *Sema3D* is expressed in forebrain, diencephalon, and dorsal neural tube in the spinal cord region, whereas *Sema7A* has only limited expression in the hindbrain. *Sema3D* is also expressed broadly in surface ectoderm, and ectodermal pla-codes such as lens placode and nasal placode. In the ectoderm of the limb bud, *Sema7A* is expressed predominantly in the apical ectodermal ridge (AER) where *Sema3D* expression is excluded, exhibiting complementary patterns. In addition, *Sema3D* appears to be expressed in cranial neural crest cells, but not in the trunk region. In contrast, *Sema7A* gene is broadly expressed in the neural crest cells including both cranial and trunk neural crest cells. These dynamic expression patterns suggest that these semaphorins may play distinct roles in early embryonic development.

RESULTS

Characterization of Chick *Sema7A* Partial cDNA Clone

Using cDNAs prepared from chick embryonic day 6 (E6) retinal and heart tissues as templates, we performed polymerase chain reactions (PCR) with degenerate primers, designed based on the conserved sequences in the SEMA domain, WTTFM(L)KA and DPYCA(G)WD (see Experimental Procedures section). We sequenced approximately 60 clones and analyzed the sequence by nucleotide and translated blast analyses using the programs provided by National Center for Biotechnology Information (NCBI). Several clones were confirmed as identical to the previously reported chick *Sema3D* gene (Luo et al., 1995; Chilton and Guthrie, 2003; Watanabe et al., 2004; Jin et al., 2006). Another clone was found to be identical to the newly annotated *Sema7A* gene from the chick genomic sequence (accession number: XM_413687), except a stretch of 44 nucleotides (Fig. 1A). This resulted in 16-amino acid sequence divergence in the SEMA domain. To distinguish it from the annotated *Sema7A* gene in the database, we named our clone “Sema7A-2.”

To determine whether *Sema7A-2* is a splice variant of the *Sema7A* gene, we performed blast searches in the expression sequence tag (EST) databases. Two chicken EST clones derived independently from a reproductive tract cDNA library (accession number CD217836) and a brain and testis library (accession number CN218431), respectively, show identical sequences to that of the *Sema7A-2*, including the divergent 44-nucleotide sequence. However, the 44-nucleotide sequence in the annotated chick *Sema7A* gene did not find its match in the EST databases. In addition, the 16-amino acid sequence translated from the 44 nucleotides in the *Sema7A-2* clone appears to be highly homologous to the equivalent regions in the human, mouse, and bovine *Sema7A* genes (87.5% similarity). The 16-amino acid sequence in the annotated chick *Sema7A* gene, however, appears not conserved compared to that of the mammalian genes (12.5% similarity). These results suggest that the *Sema7A-2* clone is likely a partial cDNA clone of the bona fide *Sema7A* gene. We currently do not know whether the 44-nucleotide sequence in the annotated chick *Sema7A* is expressed as a splice variant.

Expression of *Sema3D* and *Sema7A* Transcripts in Early Chick Embryonic Development

Further analysis with the bl2seq program provided by NCBI showed that *Sema3D* and *Sema7A-2* clones are divergent, with only 45% similarity in amino acid sequence, and no significant conservations in nucleotide sequence. In addition, blast searches against the entire NCBI databases confirmed that the nucleotide sequences of the *Sema3D* and *Sema7A-2* clones do not share significant identity with other genes, indicating that these cDNA clones can be used as templates for synthesizing specific probes for the chick *Sema3D* and *Sema7A* genes.

We carried out in situ hybridization experiments by using digoxigenin (DIG)-labeled RNA probes synthesized by using the *Sema3D* and *Sema7A-2* as templates. As the sequence of the *Sema7A-2* is identical to the annotated *Sema7A* gene in 640 out of total 684 nucleotides, in situ hybridization by this probe should detect mRNA transcripts of both the *Sema7A-2* and *Sema7A* genes, if they are expressed. We thus refer to the in situ hybridization patterns by this probe as the expression patterns of the *Sema7A* gene in the rest of this report. Multiple in situ hybridization experiments and negative controls with DIG-probes corresponding to the sense strand sequences were performed to ensure reproducibility and specificity.

The *Sema3D* mRNA expression was detected as early as Hamburger-Hamilton (HH) stage 7 in the notochord (Hamburger and Hamilton, 1992), and high levels of expression in the notochord persist to HH12 (Fig. 2A-C, F-H). A moderate level of expression was detected in the neural tube at HH12, and in the forebrain, diencephalons, and dorsal neural tube in the trunk region (Fig. 2C-H). Also at HH12, *Sema3D* expression was observed in optical and otic

vesicles (Fig. 2C-E), and in migrating neural crest cells in midbrain and hindbrain regions (Fig. 2D,F,G).

The expression of *Sema7A* was not detected before HH11–12. At HH12, *Sema7A* transcripts were found to be expressed in a restricted domain in the hindbrain (Fig. 3A,B), which on section, appear to be in the neuroepithelium in the medial portion of the neural tube (Fig. 3C). In addition, a group of cells in the dorsal midline are also positive for *Sema7A* (Fig. 3B). These cells are localized at the dorsal-most portion of the neural tube, corresponding to the premigratory neural crest cells in the hindbrain similar to the *Slug* gene (Del Barrio and Nieto, 2004) (Fig. 3C,E). In mesoderm-derived tissues, some expression was detected in the first 3–4 somites from the caudal side, but not in any of the rostral somites, suggesting that *Sema7A* is expressed only in the newly formed somites (Fig. 3A,D).

Expression of *Sema3D* and *Sema7A* at HH Stage 17–18

At HH17–18, the expression of *Sema3D* transcripts was visible widely in surface ectoderm and ectoderm-derived structures such as the lens placode and nasal placode (Fig. 4). *Sema3D* is additionally expressed in nascent limb bud and migrating neural crest cells. Interestingly, the expression in limb bud appears to be throughout the ectoderm except the apical ectodermal ridge (AER) region (Fig. 4A). Among the neural crest cells, *Sema3D* expression appears to be only in the cranial but not in the trunk neural crest (Fig. 4A,B, and E). Some *Sema3D*-positive cells were observed inside the heart in the outflow tract region. A high level of expression was also observed in the dorsal part of the neural tube, especially in the hindbrain and spinal cord regions (Fig. 4C-E).

At the same stages, *Sema7A* expression was detected widely in the neural crest cells, migrating from various levels of the neural tube, including mid-brain, hindbrain, and the trunk regions (Fig. 5). On cross-section, *Sema7A*-positive cells were found in dermomyotome, ventrolaterally migrating neural crest cells, but not in surface ectoderm. Unlike *Sema3D*, no visible in situ hybridization signal of *Sema7A* probe is present in the neuroepithelium in the neural tube (Fig. 5D).

Expression of *Sema3D* and *Sema7A* at HH Stage 22 (E3.5)

At E3.5 (HH22), *Sema3D* transcript expression persists broadly in surface ectoderm including most of the cranial facial and trunk regions (Fig. 6). In both the forelimb and hind limb, high levels of *Sema3D* transcripts were found in the surface ectoderm (Fig. 6B,E), but absent in the AER, similar to the patterns at HH17–18 (Fig. 4A). In the central nervous system, expression of *Sema3D* transcripts appears to be exclusively in the diencephalon (Fig. 6A,C). Similar to earlier stages, positive signals were also detected in the outflow tract region of the heart (Fig. 6A).

At E3.5, *Sema7A* transcript expression was observed in a different subset of ectoderm-derived tissues. The expression was still visible in the neural crest cells migrating via a dorsal-lateral route, as well as those via a ventral-medial route (Fig. 7C). *Sema7A* transcripts are not expressed in the neuroepithelium inside the neural tube. Except for the surface ectoderm next to the dorsal-most section of the neural tube in the hindbrain, there is no expression in the surface ectoderm elsewhere (Fig. 7B). The expression of *Sema7A* in the limb bud is complementary to that of *Sema3D* in that it is highly concentrated in AER, but not in the other regions of the limb bud.

DISCUSSION

In this study, we report isolation of a partial cDNA clone of the chick *Sema7A* gene, and expression analysis of *Sema7A* and *Sema3D* genes in early chick embryonic development. Interestingly, transcripts of these two semaphorin genes are mostly expressed in the areas with

active morphogenesis and cell migration. In addition, the expression patterns of *Sema7A* and *Sema3D* transcripts appear largely non-overlapping, even complementary in some areas such as the limb bud.

Except for a stretch of 44 nucleotides, *Sema7A-2* sequence is identical to the annotated chick *Sema7A* (640 out of total 684 nucleotides). However, the divergent 44-nucleotide sequence in *Sema7A-2* is identical to the sequences from two independent chicken *Sema7A* EST clones, and the translated amino acid sequence of *Sema7A-2* is highly homologous to those in the mammalian *Sema7A* proteins, suggesting that the *Sema7A-2* clone is the partial cDNA clone of the bona fide *Sema7A* gene. Because we failed to find any matching EST clone, we currently do not know whether the annotated chick *Sema7A* gene in the database is expressed and represents a splice variant of the gene.

Although initially characterized as axon guidance molecules (Raper, 2000; Pasterkamp and Kolodkin, 2003), semaphorins have been shown to play a role in cell migration, tissue morphogenesis, and remodeling. The best examples may be their roles in heart development. Targeted mutation of *Sema3C* in mice exhibit severe cardiac defects consisting of interruption of the aortic arch and improper septation of the cardiac outflow tract (Feiner et al., 2001). *Sema3A*^{-/-} mutant mice have postnatal hypertrophy of the right ventricle and ventricular septal defect (Behar et al., 1996). Deletion of one of the plexin receptors, plexinD1, results in cardiovascular defects involving the outflow tract of the heart and derivatives of the aortic arch arteries (Gitler et al., 2004). In addition, *Sema6D* has been shown to play dual roles in cardiac morphogenesis, promoting migration of cells from the conotruncal segment and inhibiting migration of ventricular cells (Toyofuku et al., 2004a). Reverse signaling by *Sema6D* enhances the migration of myocardial cells from the compact zone into the trabeculae (Toyofuku et al., 2004b). More recently, *Sema3D*, *Sema3F*, and *Sema5A* are shown to be expressed in cardiac cushions, an area characterized by active epithelial-mesenchymal transition (EMT) and remodeling (Jin et al., 2006). *Sema3D* is additionally expressed in the motile cells at the tips of ventricular trabeculae. These results suggest that semaphorins are expressed in the areas with active morphogenesis and possibly play a role in cell migration and embryogenesis.

Sema3F has been shown to be expressed in the posterior-half of the somites and repels trunk neural crest from migrating in the posterior half of the somites (Gammill et al., 2006) and *Sema3A* is required for correct migration of neural crest cells and patterning of the sympathetic nervous system (Eickholt et al., 1999; Kawasaki et al., 2002). Repulsion from the *Sema3* molecules also contributes to the guidance of migrating cranial neural crest cells (Osborne et al., 2005; Yu and Moens, 2005). *Sema3C*/plexin A2 interaction has been shown to be crucial for the migration and entry of the neural crest cells into the heart (Brown et al., 2001; Feiner et al., 2001). We detected some signals of *Sema3D* transcripts in the dorsal-most portion of the neural tube, corresponding to the premigratory neural crest cells, as well as in migrating cranial neural crest. This is similar to the previous finding that *Sema3D* is expressed in the motile cells at the tips of trabeculae in the ventricular chamber of the heart (Jin et al., 2006). As *Sema3D* encodes a secreted protein, its role in motile cells is currently unclear. It has been demonstrated that *SemaA3* controls vascular morphogenesis by inhibiting integrin function in an autocrine manner (Serini et al., 2003). Disruption of *SemaA3* signaling stimulates integrin-mediated adhesion and migration to extracellular matrices.

In early embryonic development, *Sema7A* is expressed in specific domains in the neural tube, limb bud, and somites. However, *Sema7A* is widely expressed in the migrating neural crest cells. Unlike the class 3 semaphorin genes, *Sema7A* encodes a membrane-anchored protein. In contrast to most semaphorin proteins, which act as negative factors for axonal growth and guidance, *Sema7A* enhances central and peripheral axon growth and is required for the proper formation of axonal tract during embryonic development (Pasterkamp et al., 2003). In addition,

the positive effect of *Sema7A* on axonal growth requires integrin receptor and MAPK signaling pathways. Moreover, *Sema7A* has been shown to be a potent stimulator of monocytes. *Sema7A* is expressed in the monocytes and acts by releasing through proteolysis in an autocrine fashion (Holmes et al., 2002). It awaits further study to uncover how these semaphorin genes are involved in cell migration and morphogenesis during early embryonic development.

EXPERIMENTAL PROCEDURES

Cloning of Partial cDNAs of the Chick Semaphorin Genes

Total RNAs were isolated from chick E6 heart and retinal tissues using Trizol (Invitrogen, La Jolla, CA). First-strand cDNA was synthesized using oligo (dT)₁₂₋₁₈ primer and Superscript II reverse transcriptase (Invitrogen). Subsequent PCR reactions were carried out with degenerate forward and reverse primers designed based on the conserved amino acid sequences in the SEMA domain, WTTFM(L)KA and DPYCA(G)WD, respectively. An EcoRI restriction site was added to the forward primer, and a XhoI site was added to the reverse primer for cloning into the pBluescript vector (Stratagene, La Jolla, CA). The sequences of the forward and reverse primers are: 5'-GGAATTCTGGACXACXTTYHTXAARGC (X=A+G+C+T, Y=C+T, H=C+T+A, R=A+G, S=G+C) and 5'-GCTCGAGTCCCAXSCRCARTAXGGRTC, respectively. The expected PCR product (700–750 bp) was cut out from the gel and subcloned into the pBluescript vector. The clones were analyzed by microsequencing and blast searches using programs of nucleotide-nucleotide BLAST and translated query vs. protein database provided by the National Center for Biotechnology Information (NCBI). The databases searched include non-redundant nucleotide sequence databases, EST databases, and non-redundant peptide sequence databases (NCBI). In addition, sequence similarity among the two genes was analyzed pairwise by using the *bl2seq* programs (*blastn* and *tblastx*) (NCBI). The sequence of *Sema7A-2* has been deposited in the GenBank (accession number DQ525915).

In Situ Hybridization

Standard specific pathogen-free white Leghorn chick embryos from closed flocks were provided fertilized by Charles River Laboratories (North Franklin, CT). Eggs were incubated inside a moisturized 38°C incubator and staged according to Hamburger and Hamilton (1992).

The sense and anti-sense RNA probes for *Sema3D* and *Sema7A* were synthesized by in vitro transcription with T3 or T7 RNA polymerases using digoxigenin-labeled UTPs (Roche). The procedure of whole mount in situ hybridization was carried out essentially as previously described (Jin et al., 2003; Zhang et al., 2004). Chick embryos of HH5, HH7–10, HH12 (~E1.5), HH17–18 (E2.5), and HH22 (E3.5) were dissected and fixed in 4% paraformaldehyde at 4°C for 12–24 hr. The embryos were treated with Proteinase K (Boehringer Mannheim, Indianapolis, IN) with increasingly higher concentrations for the older embryos, at 1 µg/ml for embryos younger than HH10, 5 µg/ml for HH12–17, and 10 µg/ml for embryos older than HH17. Hybridization was carried out by incubation with probes (~1 µg/ml) in the hybridization buffer (50% Formamide, 5×SSC, pH 4.5, 50 µg/ml yeast RNA, 1% SDS, 50 µg/ml heparin) overnight at 70°C. After hybridization, three washes were carried out for 30 min at 70°C with buffer I (50% formamide, 5×SSC, pH 4.5, 1% SDS) followed by three washes at 65°C with buffer III (50% formamide, 2×SSC, pH 4.5).

After completion of whole mount in situ hybridization, some of the embryos were sectioned. The embryos were postfixed in 4% paraformaldehyde and rinsed with PBS. The embryos were incubated in 15% sucrose in PBS with gentle rocking at 4°C for 24 hr. The embryos were then embedded in 15% sucrose, 7.5% gelatin in PBS, and sectioned on a cryostat (Leica, Deerfield, IL). The tissue sections were collected on Superfrost Plus slides (Fisher Scientific, Pittsburgh,

PA) and mounted. Results of whole mount in situ hybridization were photographed with a digital camera coupled to a dissection microscope (Nikon). The results of section in situ hybridization were observed on an upright microscope (Nikon TE 400) and photographed with a digital SPOT camera.

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REFERENCES

- Behar O, Golden JA, Mashimo H, Schoen FJ, Fishman MC. Semaphorin III is needed for normal patterning and growth of nerves, bones and heart. *Nature* 1996;383:525–528. [PubMed: 8849723]
- Brown CB, Feiner L, Lu MM, Li J, Ma X, Webber AL, Jia L, Raper JA, Epstein JA. PlexinA2 and semaphorin signaling during cardiac neural crest development. *Development* 2001;128:3071–3080. [PubMed: 11688557]
- Chilton JK, Guthrie S. Cranial expression of class 3 secreted semaphorins and their neuropilin receptors. *Dev Dyn* 2003;228:726–733. [PubMed: 14648849]
- Del Barrio MG, Nieto MA. Relative expression of Slug, RhoB, and HNK-1 in the cranial neural crest of the early chicken embryo. *Dev Dyn* 2004;229:136–139. [PubMed: 14699585]
- Durbin L, Brennan C, Shiomi K, Cooke J, Barrios A, Shanmugalingam S, Guthrie B, Lindberg R, Holder N. Eph signaling is required for segmentation and differentiation of the somites. *Genes Dev* 1998;12:3096–3109. [PubMed: 9765210]
- Eickholt BJ, Mackenzie SL, Graham A, Walsh FS, Doherty P. Evidence for collapsin-1 functioning in the control of neural crest migration in both trunk and hindbrain regions. *Development* 1999;126:2181–2189. [PubMed: 10207143]
- Feiner L, Webber AL, Brown CB, Lu MM, Jia L, Feinstein P, Mombaerts P, Epstein JA, Raper JA. Targeted disruption of semaphorin 3C leads to persistent truncus arteriosus and aortic arch interruption. *Development* 2001;128:3061–3070. [PubMed: 11688556]
- Gamill LS, Gonzalez C, Gu C, Bronner-Fraser M. Guidance of trunk neural crest migration requires neuropilin 2/semaphorin 3F signaling. *Development* 2006;133:99–106. [PubMed: 16319111]
- Gerety SS, Wang HU, Chen ZF, Anderson DJ. Symmetrical mutant phenotypes of the receptor EphB4 and its specific transmembrane ligand ephrin-B2 in cardiovascular development. *Mol Cell* 1999;4:403–414. [PubMed: 10518221]
- Gitler AD, Lu MM, Epstein JA. PlexinD1 and semaphorin signaling are required in endothelial cells for cardiovascular development. *Dev Cell* 2004;7:107–116. [PubMed: 15239958]
- Grieshammer U, Le M, Plump AS, Wang F, Tessier-Lavigne M, Martin GR. SLIT2-mediated ROBO2 signaling restricts kidney induction to a single site. *Dev Cell* 2004;6:709–717. [PubMed: 15130495]
- Gu C, Yoshida Y, Livet J, Reimert DV, Mann F, Merte J, Henderson CE, Jessell TM, Kolodkin AL, Ginty DD. Semaphorin 3E and plexin-D1 control vascular pattern independently of neuropilins. *Science* 2005;307:265–268. [PubMed: 15550623]
- Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. *Dev Dyn* 1992;195:231–272. [PubMed: 1304821]1951 (see comments)
- Hinck L. The versatile roles of “axon guidance” cues in tissue morphogenesis. *Dev Cell* 2004;7:783–793. [PubMed: 15572123]
- Holmes S, Downs AM, Fosberry A, Hayes PD, Michalovich D, Murdoch P, Moores K, Fox J, Deen K, Pettman G, Wattam T, Lewis C. Sema7A is a potent monocyte stimulator. *Scand J Immunol* 2002;56:270–275. [PubMed: 12193228]
- Jin Z, Zhang J, Klar A, Chedotal A, Rao Y, Cepko CL, Bao ZZ. Irx4 regulation of Slit1 expression contributes to the definition of early axonal paths inside the retina. *Development* 2003;130:1037–1048. [PubMed: 12571096]
- Jin Z, Chau MD, Bao ZZ. Sema3D, Sema3F, and Sema5A are expressed in overlapping and distinct patterns in chick embryonic heart. *Dev Dyn* 2006;235:163–169. [PubMed: 16261621]

- Kawasaki T, Bekku Y, Suto F, Kitsukawa T, Taniguchi M, Nagatsu I, Nagatsu T, Itoh K, Yagi T, Fujisawa H. Requirement of neuropilin 1-mediated Semaphorin 3A signals in patterning of the sympathetic nervous system. *Development* 2002;129:671–680. [PubMed: 11830568]
- Kruger RP, Aurandt J, Guan KL. Semaphorins command cells to move. *Nat Rev Mol Cell Biol* 2005;6:789–800. [PubMed: 16314868]
- Krull CE, Lansford R, Gale NW, Collazo A, Marcelle C, Yancopoulos GD, Fraser SE, Bronner-Fraser M. Interactions of Eph-related receptors and ligands confer rostrocaudal pattern to trunk neural crest migration. *Curr Biol* 1997;7:571–580. [PubMed: 9259560]
- Luo Y, Shepherd I, Li J, Renzi MJ, Chang S, Raper JA. A family of molecules related to collapsin in the embryonic chick nervous system. *Neuron* 1995;14:1131–1140. [PubMed: 7605628]
- Osborne NJ, Begbie J, Chilton JK, Schmidt H, Eickholt BJ. Semaphorin/neuropilin signaling influences the positioning of migratory neural crest cells within the hindbrain region of the chick. *Dev Dyn* 2005;232:939–949. [PubMed: 15729704]
- Pasterkamp RJ, Kolodkin AL. Semaphorin junction: making tracks toward neural connectivity. *Curr Opin Neurobiol* 2003;13:79–89. [PubMed: 12593985]
- Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL. Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. *Nature* 2003;424:398–405. [PubMed: 12879062]
- Piper M, Little M. Movement through Slits: cellular migration via the Slit family. *Bioessays* 2003;25:32–38. [PubMed: 12508280]
- Poliakov A, Cotrina M, Wilkinson DG. Diverse roles of eph receptors and ephrins in the regulation of cell migration and tissue assembly. *Dev Cell* 2004;7:465–480. [PubMed: 15469835]
- Raper JA. Semaphorins and their receptors in vertebrates and invertebrates. *Curr Opin Neurobiol* 2000;10:88–94. [PubMed: 10679438]
- Santiago A, Erickson CA. Ephrin-B ligands play a dual role in the control of neural crest cell migration. *Development* 2002;129:3621–3632. [PubMed: 12117812]
- Serini G, Valdembrì D, Zanivan S, Morterra G, Burkhardt C, Caccavari F, Zammataro L, Primo L, Tamagnone L, Logan M, Tessier-Lavigne M, Taniguchi M, Puschel AW, Bussolino F. Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. *Nature* 2003;424:391–397. [PubMed: 12879061]
- Shepherd I, Luo Y, Raper JA, Chang S. The distribution of collapsin-1 mRNA in the developing chick nervous system. *Dev Biol* 1996;173:185–199. [PubMed: 8575620]
- Srinivasan K, Strickland P, Valdes A, Shin GC, Hinck L. Netrin-1/neogenin interaction stabilizes multipotent progenitor cap cells during mammary gland morphogenesis. *Dev Cell* 2003;4:371–382. [PubMed: 12636918]
- Toyofuku T, Zhang H, Kumanogoh A, Takegahara N, Suto F, Kamei J, Aoki K, Yabuki M, Hori M, Fujisawa H, Kikutani H. Dual roles of Semaphorin 6D in cardiac morphogenesis through region-specific association of its receptor, Plexin-A1, with off-track and vascular endothelial growth factor receptor type 2. *Genes Dev* 2004a;18:435–447. [PubMed: 14977921]
- Toyofuku T, Zhang H, Kumanogoh A, Takegahara N, Yabuki M, Harada K, Hori M, Kikutani H. Guidance of myocardial patterning in cardiac development by Semaphorin 6D reverse signalling. *Nat Cell Biol* 2004b;6:1204–1211. [PubMed: 15543137]
- Wang HU, Anderson DJ. Eph family transmembrane ligands can mediate repulsive guidance of trunk neural crest migration and motor axon outgrowth. *Neuron* 1997;18:383–396. [PubMed: 9115733]
- Wang HU, Chen ZF, Anderson DJ. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell* 1998;93:741–753. [PubMed: 9630219]
- Watanabe Y, Toyoda R, Nakamura H. Navigation of trochlear motor axons along the midbrain-hindbrain boundary by neuropilin 2. *Development* 2004;131:681–692. [PubMed: 14729576]
- Wong K, Park HT, Wu JY, Rao Y. Slit proteins: molecular guidance cues for cells ranging from neurons to leukocytes. *Curr Opin Genet Dev* 2002;12:583–591. [PubMed: 12200164]
- Xu Q, Mellitzer G, Robinson V, Wilkinson DG. In vivo cell sorting in complementary segmental domains mediated by Eph receptors and ephrins. *Nature* 1999;399:267–271. [PubMed: 10353250]
- Yebra M, Montgomery AM, Diaferia GR, Kaido T, Silletti S, Perez B, Just ML, Hildbrand S, Hurford R, Florkiewicz E, Tessier-Lavigne M, Cirulli V. Recognition of the neural chemoattractant Netrin-1

by integrins alpha6beta4 and alpha3beta1 regulates epithelial cell adhesion and migration. *Dev Cell* 2003;5:695–707. [PubMed: 14602071]

Yu HH, Moens CB. Semaphorin signaling guides cranial neural crest cell migration in zebrafish. *Dev Biol* 2005;280:373–385. [PubMed: 15882579]

Zhang J, Jin Z, Bao ZZ. Disruption of gradient expression of Zic3 resulted in aberrant projection of the retinal ganglion axons to the optic disc. *Development* 2004;131:1553–1562. [PubMed: 14985256]

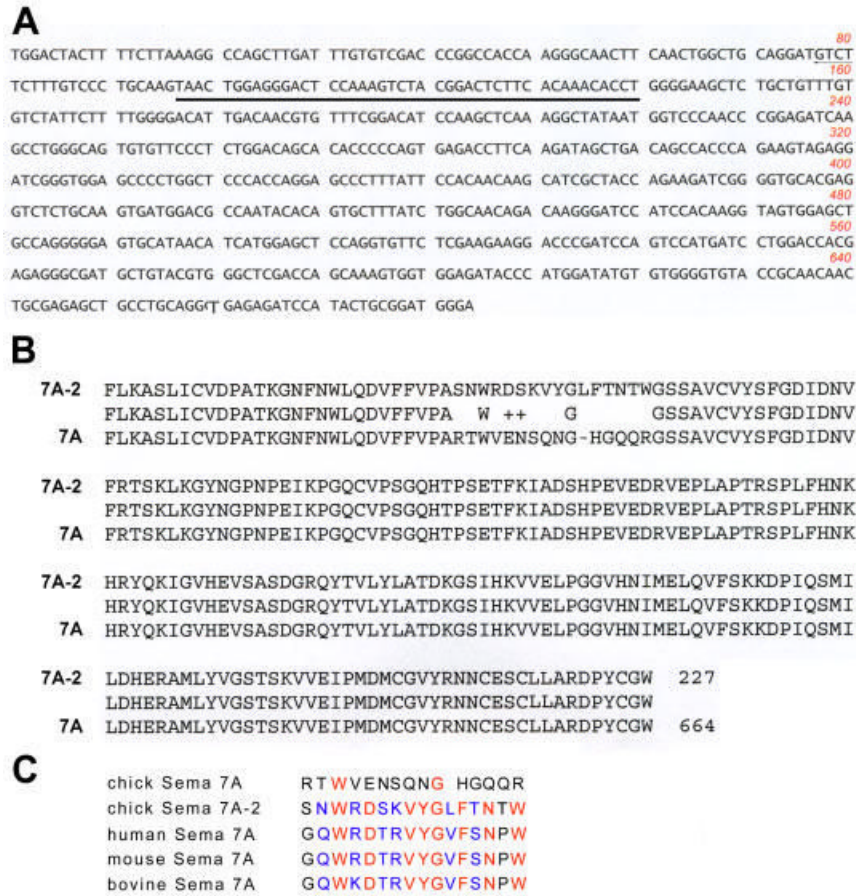


Fig. 1. Sequence analysis of the *Sema7A-2* clone. **A:** Nucleotide sequence of *Sema7A-2*. The 44-nucleotides that are different from the annotated *Sema7A* gene are underlined. **B:** Comparison of amino acid sequence translated from the *Sema7A-2* sequence with that of the annotated *Sema7A* gene. Note that the amino acid sequence is identical except the 16-amino acid stretch. **C:** Comparison of the 16-amino acid sequences encoded by human, mouse, bovine, and chick *Sema7A* genes with that by *Sema7A-2*. Note that the 16-amino acid sequence of the chick *Sema7A-2* is more homologous to the mammalian *Sema7A* proteins than the annotated chick *Sema7A*.

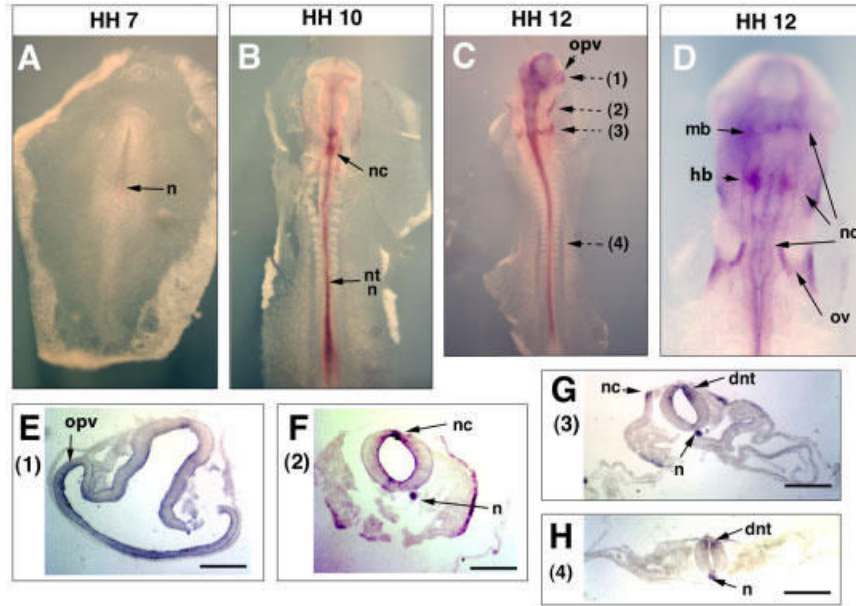


Fig. 2. Expression of *Sema3D* in early chick embryos (HH7 to HH12). Dorsal sides of embryos are shown for whole mount in situ hybridization results. For sections, the dorsal sides of the embryos are oriented upward. Whole mount in situ hybridization results are shown for embryos of (A) HH7, (B) HH10, (C) HH12 at a low magnification, and (D) HH12 at a higher magnification. After whole mount in situ hybridization, the embryos were sectioned. The planes of sections were shown with broken arrows and the numbers in parentheses next to the arrows indicate the figures showing the section results. E–H: Results from four section planes (1–4) of the HH12 embryo are shown after in situ hybridization with the *Sema3D* probe. n, notochord; nc, neural crest; nt, neural tube; mb, midbrain; hb, hindbrain; ov, otic vesicle; opv, optic vesicle; dnt, dorsal neural tube. Scale bars = 100 μm.

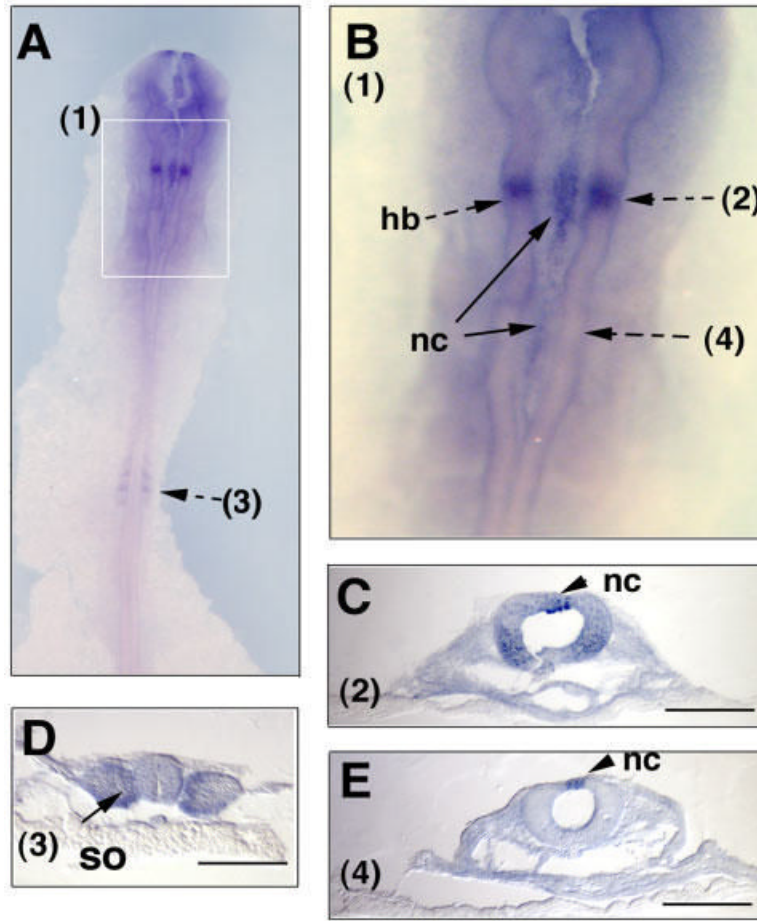


Fig. 3. Expression of *Sema7A* transcripts in early chick embryos at HH12. HH12 chick embryos were hybridized with the *Sema7A*-2 RNA probe, shown at the dorsal side of the embryo (A) and in the hindbrain region (B). The boxed area (1) in A is shown at a higher magnification in B. Note its expression in a restricted domain in hindbrain region and in some premigratory neural crest cells at the dorsal midline of the neural tube. The embryos were sectioned after whole mount in situ hybridization at the indicated section planes (2)–(4). nc, neural crest, hb, hindbrain, so, somite. Scale bars = 100 μm.

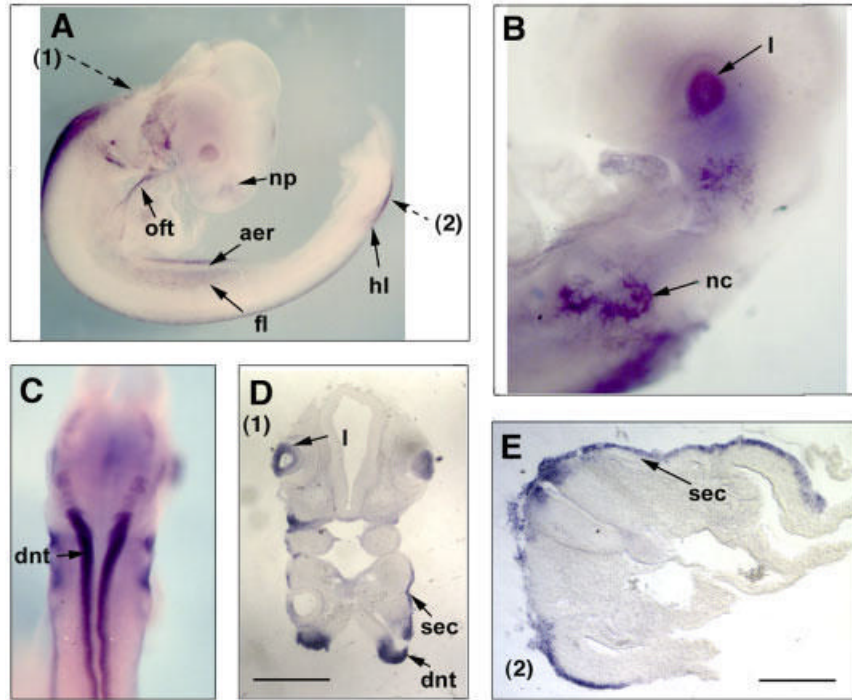


Fig. 4. Expression of *Sema3D* transcripts at HH stage 17. Lateral views (**A**, **B**) and a rear view (**C**) of the embryos are shown after whole mount in situ hybridization. The embryos were sectioned and the planes of sections are indicated by broken arrows. The numbers in parentheses next to the broken arrows indicate the figure numbers showing the section results. Note that the purple staining in the lens vesicles appears inside of the cells (**D**), suggesting that this is a signal of in situ hybridization, not trapping. aer, apical ectoderm ridge; np, nasal placode; fl, forelimb; hl, hindlimb; l, lens; nc, neural crest; oft, outflow tract; dnt, dorsal neural tube; sec, surface ectoderm. Scale bar in **D** = 250 μ m, in **E** = 100 μ m.

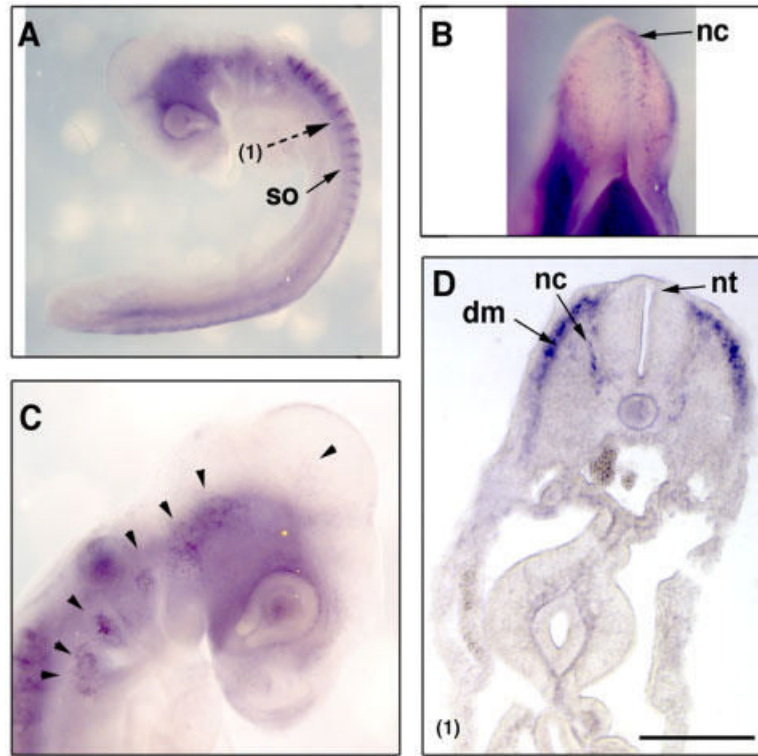


Fig. 5. Expression of *Sema7A* transcripts in HH17 chick embryos. Lateral views of the embryos (**A**, **C**) and a dorsal view of the midbrain/hindbrain regions (**B**) are shown. The section plane is indicated by a broken arrow and the section result is shown in **D**. Note the expression of *Sema7A* in migrating neural crest cells in cranial facial region (arrowheads in **C**) and in trunk region (**D**). so, somites; nc, neural crest; dm, dermomyotome; nt, neural tube. Scale bar = 100 μ m.

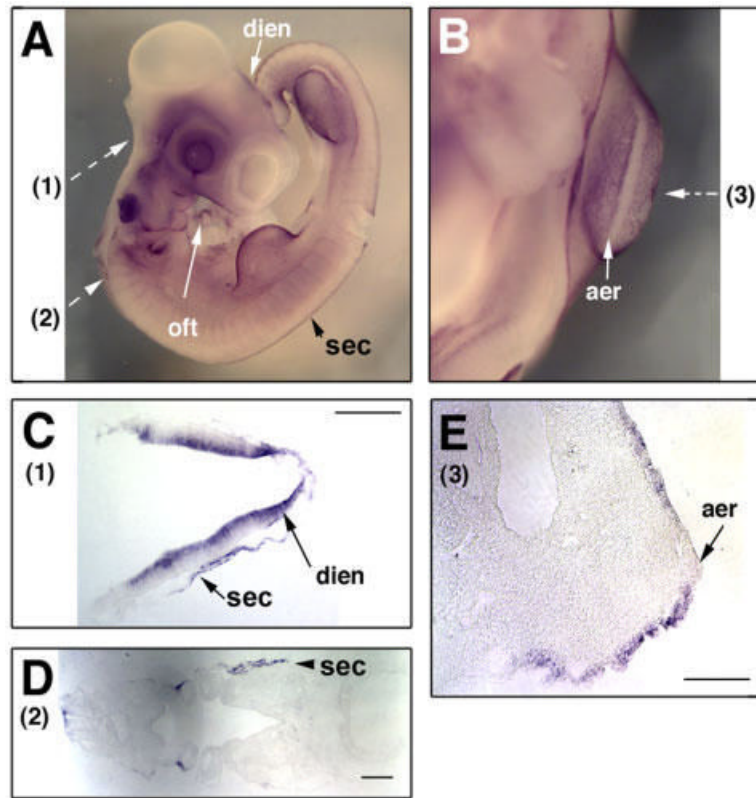


Fig. 6. Expression of *Sema3D* transcripts in E3.5 chick embryos. A lateral view (**A**) and a frontal view in the forelimb bud area (**B**) are shown. Section planes are indicated by broken arrows and the results of the sections are shown in **C–E**. Note the staining in the otic vesicle in (**A**) is due to trapping. Dien, diencephalon; oft, outflow tract; aer, apical ectoderm ridge; sec, surface ectoderm. Scale bars = 100 μ m.

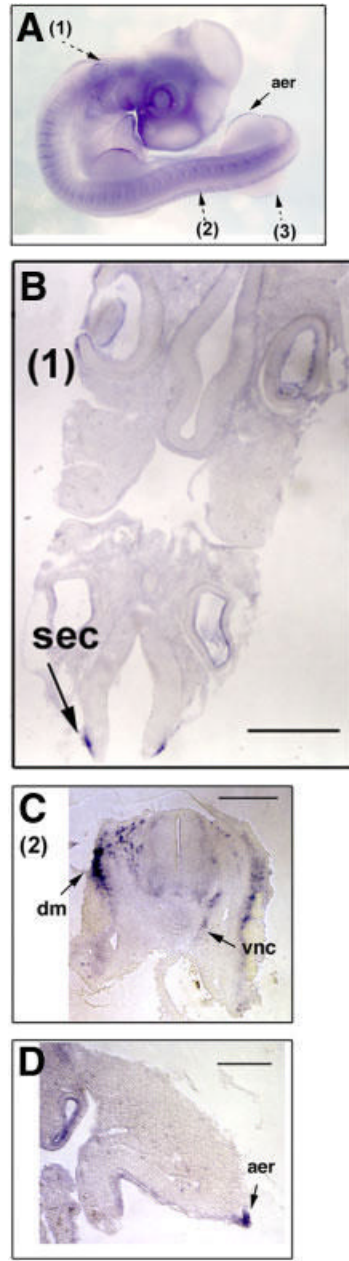


Fig. 7. Expression of *Sema7A* transcripts in E3.5 chick embryos. **A:** In situ hybridizations were performed on whole mount E3.5 chick embryos. Section planes are shown with broken arrows and the results of section after in situ hybridization are shown in **B–D** (1–3). aer, apical ectodermal ridge; sec, surface ectoderm; dm, dermomyotome; nc, dorsal-laterally migrated neural crest cells; vnc, ventral-medially migrated neural crest cells. Scale bars = 100 μ m.