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Semaphorin/Neuropilin binding specificities are stable over 400 million years of evolution

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Abstract

Semaphorins are a large and important family of signaling molecules conserved in Bilateria. An important determinant of the biological function of their largest class, the secreted class 3 semaphorins, is the specificity of their binding to neuropilins, a key component of a larger holoreceptor complex. We compared these binding specificities in mice and zebrafish, whose most recent common ancestor was more than 400 million years in the past. We also compared the binding specificities of zebrafish class 3 semaphorins that were duplicated very early within the teleost lineage. We found a surprising conservation of neuropilin binding specificities when comparing both paralogous zebrafish semaphorin pairs and orthologous zebrafish and mouse semaphorin pairs. This finding was further supported by a remarkable conservation of binding specificities in cross-species pairings of semaphorins and neuropilins. Our results suggest that the qualitative specificities with which particular semaphorins bind to particular neuropilins has remained nearly invarient over approximately 400 million years of evolution.

Keywords

semaphorins; neuropilins; evolutionary conservation; axonal guidance

Introduction

Semaphorins (semas) are a family of secreted and transmembrane proteins that mediate signaling in a wide variety of biological processes including axon guidance [1], immune system regulation [2], angiogenesis [3, 4] synapse formation [5, 6, 7], cellular migration [8], , and cancer metastasis [9, 10, 11]. The semaphorin family has been categorized into 8 classes based upon their domain structure [12]. Classes 3–7 are expressed in vertebrates. Class 3 is the largest class in the family. Each of its members contain a signal sequence, an Ig domain, and a short basic domain. Chick Sema3A was the first identified vertebrate axonal guidance cue and the first discovered vertebrate member of the semaphorin family. Its original name, Collapsin, was inspired by its ability to induce the collapse of growth

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cones through the dramatic loss of fibrillar actin in growth cones [1, 13]. The majority of higher vertebrates, including the mouse and human, have 7 class 3 members designated sema3A through 3G [14].

For the most part, class 3 semaphorins signal through a receptor complex composed of a pair of neuropilins (nrps) complexed with a pair of A class Plexins [15, 16]. One exception is sema3E which can directly bind and activate the plexin D1 receptor, although it can also bind to, and its activity can be affected by, neuropilin1 [17, 18]. Although Plexins mediate semaphorin signal transduction, semaphorin to neuropilin binding plays a key role in determining the specificity and sensitivity of semaphorin action. Class 3 semaphorins bind to specific neuropilins and this specificity is a major contributor to the functional specificity of semaphorin signaling.

The objective of this study was to determine the degree to which the members of an important family of signaling molecules, the class 3 semaphorins, has retained their specific functional characteristics over a long evolutionary time scale. Tetropod and teleost lineages diverged from one another approximately 400 million years ago, so there has been ample time for specific identities of individual semaphorin orthologs to assume new functional characteristics. Shortly thereafter, a presumptive duplication at the base of the teleost lineage produced multiple class 3 semaphorin paralogs in zebrafish. It is possible that related semaphorins could assume very different properties over such a long time scale, especially in when comparing terrestrial and marine species that are very different. As a first step in assaying the functional properties of particular class 3 semaphorins, we examined the qualitative binding preferences of all class 3 mouse and teleost fish semaphorin orthologs to all mouse and teleost neuropilins, both within the same species and across species. We found that the binding preferences of zebrafish semaphorin paralogs, and of zebrafish and mouse orthologs, is remarkably well conserved. This suggests that any specialization of semaphorin function will be found to depend less on changes in binding specificities, and more on either changes in gene expression patterns, or in the molecular details of semaphorin activated signaling pathways.

Material sand methods

Sequence analysis and bioinformatics.

Reference sequences for the mouse and zebrafish classs semaphorins and neuropilins were obtained from NCBI using HomoloGene as an entry point (https://www.ncbi.nlm.nih.gov/homologene/). Genomic and protein alignments were performed using ClustalW2 at EMBL (https://www.ebi.ac.uk/Tools/msa/clustalw2/). Phylogenetic trees were generated from peptide sequences with EMBL Simple Phylogeny using nearest neighbor joining (https://www.ebi.ac.uk/seqdb/confluence/display/THD/Simple+Phylogeny). Genes surrounding semaphorins were identified manually using the Ensembl (Sanger) genome browser (https://useast.ensembl.org/index.html).

Plasmid construction and protein expression.

Total RNA was extracted using Trizol (ThermoFisher[TF] Cat. #15596018) from 3 day postfertilized ZV9 zebrafish embryos or new born WT mice. cDNA was generated using an oligo (dT) primer based kit (Invitrogen SuperScript III, TF Cat. #18080400). Target genes were amplified from cDNA using nested PCR primers and incorporated into the pAG-3AP vector to generate AP-semaphorin fusion proteins [19]. Neuropilins were generated in the same way and inserted into the pAG-CT vector that incorporated myc plus a 6xhis tag [20]. Plasmids were prepared for transfection using GeneJET maxiprep kit (TF Cat. #K0491). HEK-293T cells were seeded one day before transfection to ~70% −90% confluence. Plasmids encoding semaphorin3s were transfected into HEK-293T cells via calcium phosphate co-precipitation. The cells were transfected for 4–6 hrs and then washed with fresh medium. After an recovering overnight, the temperature were lowered to 30°C. AP-sema3 containing medium was collected 2 days posttransfection. Neuropilin encoding plasmids were transfected into COS-7 cells plated into a 24 well tissue culture dishes using lipofactamine 2000 (TF Cat. #11668019). The plasmid and lipofactamine were diluted in Opti-MEM (TF # 31985062) separately and the ratio of plasmid to lipofactamine was 1 μg:2.3–2.5 μL. After incubating the DNA and lipafectamine to form a complex for 20 minutes, the mixture was added into the 500 uL medium bathing COS-7 cells. Fresh medium replaced the transfection medium 6 hours later. Neuropilin transfected COS-7 cells were used the next day for semaphorin binding experiments [20].

Binding assay.

The relative concentrations of AP-sema3 in supernatants was determined by slot-blots reacted for AP [21]. Supernatants were diluted in PBS containing 10%FBS so that the same amount of each semaphorin was applied to neuropilin transfected COS-7 cells. COS-7 cells were incubated with sema3 for 1.5 hours at room temperature before fixing the COS-7 cells in 4% PFA with 10% sucrose in PBS for 20min at room temperature. After 3 hours heat inactivation at 65°C, the AP reaction was developed at 37°C for 5 hours with NBT/BCIP substrate solution (TF Cat# N6495 /34040) at 37°C. COS-7 cells that expressed neuropilin and bound AP-semaphorin turned purple.

Immunostaining.

Cos-7 cells following transfection were fixed in 4% PFA containing 10% sucrose for 20 min at room temperature. Cells were washed in1x PBS followed by an overnight incubation with 9-E10 anti-Myc primary antibody (1:500 dilution) (Sigma-Aldrich M4439) at 4c. Cells were gently washed with in 1x PBS, then incubated with AP conjugated second antibody (1:1000 dilution) (Jackson Immuno Research 715-055-150) for 1hr at RT. Cells were then washed in 1x PBS. The cells were then heat inactivated at 56c for 3hrs to kill endogenous AP. They were then developed at 37c with NBT/BCIP for 1–2hrs.

Results

Comparison of class 3 semaphorin sequences in zebrafish and mice.

Mouse and zebrafish class 3 semaphorin peptide sequences were compared to identify likely paralogous and orthologous semaphorins (Figure 1). Several patterns are immediately apparent. First, class 3 semaphorins can be divided into three clades containing semaphorins 3A,B,D; 3C,F; and 3E,G respectively. Second, the close relationship between zebrafish paralogs sema3aa/sema3ab, sema3fa/sema3fb, and sema3ga/sema3gb are readily apparent and, relatively speaking, appear to be the most recent divergent events in the reconstructed phylogenetic tree. This is consistent with the duplication of these genes after the divergence of the tetropod and teleost lineages. Third, clear orthologous groupings of mouse as compared to zebrafish semaphorins are apparent for sema3A/sema3aa/sema3ab, sema3B/sema3b, sema3C/sema3c, sema3D/sema3d, sema3E/sema3e, sema3F/sema3fa/sema3fb, and sema3G/sema3bl. zebrafish sema3ga, sema3gb, and sema3h genes do not have any likely orthologs in the mouse.

The clustering of class 3 semaphorins.

To further examine the interrelationships between class 3 semaphorins we compared the genes surrounding both semaphorin orthologs and paralogs in a variety of representative species including mouse, chick, pufferfish, zebrafish, and Coelocanth. Mouse and chick sema3D, 3A, 3E are clustered immediately adjacent to each other while sema3C is located nearby. Only 3 intervening genes separate the sema3DAE cluster from sema3C (Figure 2A). The sema3DAE cluster is flanked by Grm3 on one side and Pclo on the other. The zebrafish orthologs of these semaphorins: sema3d, sema3ab, and sema3e are similarly clustered together on zChr 18 and have the same genes on either flank of the cluster. Thus, the sema3DAE cluster has been maintained intact separately in the teleost and tetrapod lines. This is not the case for the zebrafish sema3c gene which no longer resides near the sema3DAE cluster, but is instead found on a separate chromosome (Z4 in figure 2). As it is in the mouse and chicken, sema3c is flanked on one side by cd36 and other genes related to those flanking mouse and chick sema3C. The sema3aa gene is also situated on zChr 4 but at a considerable distance form sema3c. All of these semaphorins and their flanking genes are similarly arranged in another teleost that is highly divergent from zebrafish, a pufferfish from the genus Tetraodon. The separation of sema3c from the sema3DEF cluster likely occurred early in the teleost lineage or even earlier. Coelacanth genomic data is still fragmentary and cannot be mapped to specific chromosomes. However, the immediate genes flanking the sema3DAE cluster and sema3c gene are remarkably similar to the arrangement in zebrafish, chick, and mouse.

The chromosomal arrangement of the remaining class 3 semaphorins is more complex. Chick sema3B, sema3F, and sema3G are all situated on chromosome 12 but are separated each from another by several genes (Figure 2B). Sema3B and 3F are similarly arranged on mouse chromosome 9, but sema3G resides separately on chromosome 14. The genes flanking each side of sema3B and sema3F are well conserved in mouse and chick. Both zebrafish and pufferfish genes orthologous to sema3B (sema3b) and sema3G (sema3bl) are immediately adjacent to one another. Interestingly, this is not the case in the Coelacanth,

where the arrangement of sema3b and sema3f resembles that in tetrapods, consistent with tetrapods diverging from the Coelacanth lineage after Coelacanths and teleosts diverged [22]. The orthologs of sema3F, and their paralogs, are separated from the sema3b/sema3G cluster in both teleosts. In the zebrafish, sema3fa and 3fb are on separate chromosomes, while in pufferfish they are at a substantial distance apart on the same chromosome. Overall, the presence of similar neighboring genes to each of the semaphorins in these species are consistent with the orthologous groupings suggested by peptide sequence similarities.

Mouse semaphorin to mouse neuropilin binding preferences.

One important determinant of class 3 semaphorin function is whether they bind to neuropilin 1, neuropilin 2, or both. We performed qualitative binding studies to examine the relative specificities of every combination of mouse or zebrafish class 3 semaphorin to mouse or zebrafish neuropilins. For baseline purposes, we tested mouse class 3 semaphorin binding to mouse neuropilins 1 and 2. Although this information is available in the literature, previous data were collected in disparate labs using varied methodologies. We wanted to ensure that our findings were comparable between and across species. Consistent with previous reports [16], we found (Table 1 and Figure S1) that sema3A and 3D bind nrp1; sema3F binds nrp2; and sema3C, 3E, and 3G bind both nrp1 and nrp2.

Zebrafish semaphorin to zebrafish neuropilin binding preferences.

We performed qualitative binding studies to examine the relative specificities of every combination of zebrafish class 3 semaphorin to zebrafish neuropilin. As in mouse, we observed three general categories of binding specificity (Table 1 and Figure S3). One category is comprised of those semaphorins that bind best to nrp1s as compared to nrp2s (sema3aa, sema3ab, sema3d). A second category bind nrp2s better than nrp1s (sema3b, sema3fa, sema3fb, sema3ga, and sema3gb). Finally, a third category bind both nrp1s and nrp2s (sema3bl, sema3c, sema3e, and sema3h). Both members of each paralogous pair (sema3aa/3ab; sema3fa/3fb; and sema3ga/3gb) had the same relative preferences for nrp1s as compared to nrp2s. We observed one notable difference between the mouse and zebrafish paralogs in their patterns of binding specificities. Mouse sema3b binds both mouse nrp1 and nrp2, while zebrafish sema3b binds to zebrafish nrp2a. With the exception of sema3B, the zebrafish and the mouse binding patterns are very similar.

Cross species semaphorin to neuropilin binding specificities.

To further test how well semaphorins and neuropilins have preserved their relative binding specificities over time, we tested the degree to which mouse semaphorins bind zebrafish neuropilins (Table 1 and Figure S2), and conversely, zebrafish semaphorins bind mouse neuropilins (Table 1 and Figure S4). For the most part, zebrafish and mouse orthologs bind mouse and zebrafish neuropilins with the same specificities. For example, mouse sema3A binds both zebrafish nrp1a and nrp1b (but not nrp2a or nrp2b); while zebrafish sema3aa and sema3ab bind mouse nrp1 (but not nrp2). Occasional exceptions were noted. The clearest example is the failure of zebrafish sema3e to bind either mouse nrp1 or nrp2, even though it binds to all zebrafish nrps; while mouse sema3e binds to all mouse and zebrafish nrps. When all the binding studies are viewed together in Table 1, blocks of nrp1 binding activity (top box), nrp2 binding activity (middle box), or both nrp1 and 2 binding activity (bottom box)

can be visualized for groups of orthologous and paralogous semaphorins. It is remarkable the extent to which mouse and zebrafish semaphorin and nrp orthologs appear almost functionally interchangeable.

Discussion

It has been proposed that a wholesale genomewide duplication occurred very early within the teleost lineage that promoted gene diversity, and potentially, helped stimulate the extraordinary radiation of divergent teleost species over the past 300 million years [23]. Although not all class 3 semaphorins have paralogs, presumably because some redundant genes were lost after accumulating deleterious mutations, there are three clear parologous pairs. We examined whether some of these ‘newly’ minted class 3 semaphorin genes have altered their functional properties over evolutionary time. We were also interested in the degree to which teleost and tetropod class 3 semaphorins may have altered their functional profiles over an even longer time. We found that both paralogous zebrafish semaphorins and orthologous mouse and zebrafish semaphorins have preserved surprisingly similar patterns of neuropilin binding specificities over a very long time scale.

Paralogous zebrafish class 3 semaphorin pairs are easily apparent by sequence similarity and are already recognized by current nomenclature. Pairs are generally denoted by an ‘a’ or ‘b’ appended to the ends of their names (eg. sema3aa and sema3ab). With a few important exceptions, orthogonal relationships between tetrapod and teleost species are also well reflected by the current established nomenclature. For example mouse sema3A is the mouse semaphorin most closely related to zebrafish sema3aa/sema3ab by sequence similarity. However, the current nomenclature is not a perfect guide to gene orthology. Mouse sema3G is not likely to be the ortholog of zebrafish sema3ga/sema3gb as zebrafish sema3bl is its closer relative. Zebrafish sema3ga/gb is more closely related to a clade of semas that include sema3C, sema3F, and sema3fa/fb than mouse sema3G.

The overall genomic organization of the class 3 semaphorins suggests that they may have existed as two gene clusters before the divergence of the teleost and tetropod lines. If one assumes that clustered genes represent a more highly ordered state than the same genes dispersed on separate chromosomes, then finding genes clustered together suggests that they more likely originated in a clustered configuration than that they clustered together over time. In this respect, the chicken genome may most accurately resemble the ancestral state of class 3 semaphorin gene organization with sema3D, sema3A, sema3E, and sema3C grouped together on Chromosome 5; and sema3B, sema3F, and sema3G grouped together on Chromosome 12. In zebrafish, this organization has been partially broken. Although sema3d, sema3ab, and sema3e remain clustered on Chromosome 18; sema3aa and sema3c are situated at a large distance from one another on Chromosome 4. Similarly, sema3B and the orthologue of mouse sema3G (semabl) are grouped together on zebrafish Chromosome 8; sema3fa and sema3fb are each situated individually on their own Chromosomes. Interestingly, sema3b and sema3f are grouped together in the Coelacanth in a very similar arrangement to that in the chick. If the chick genomic arrangement of class 3 semaphorins is most representative of the ancient common ancestor of both teleosts and tetrapods, and if the Coelacanth is closer to the root of the tetrapod lineage than the teleost

lineage, then the similar (re)arrangements of semaphorin genes in zebrafish and pufferfish likely represent early events in the teleost lineage, perhaps in part related to whole genome duplication.

Finally, a very interesting pattern emerges in examining the degree of relatedness between class 3 semaphorins as compared to their presumed ancestral configuration in two separate gene clusters. Intriguingly, sema3A and 3D are members of one clade, sema3C and 3F are members of another, and sema3E and 3G are members of a third. One interpretation of this pattern is that although highly diverged from one another now, the sema3A,C,E and the sema3B,F,G clusters ultimately originated from a single very ancient common gene cluster of three class 3 semaphorins. This early single cluster must then have divided into two separate clusters before the divergence of teleosts and tetropods.

We anticipated that teleost and tetropod orthologs might posses divergent neuropilin binding specificities acquired over time, and that even zebrafish paralogs could have diverged from one another in function. The peptide sequences of zebrafish paralogs sema3aa/ab are ~75% identical, of sema3fa/fb are 85% identical, and of sema3ga/gb are 78% identical. In comparison, the mouse and zebrafish orthologs range from ~60%−75% identical. Upon testing, we were astounded at the degree of conservation between the binding specificities of both zebrafish paralogs and mouse/fish orthologs. In every instance, zebrafish paralogs bound the same pattern of both zebrafish and mouse neuropilins. Even more surprisingly, the same was true for 5 of the mouse / zebrafish orthologs. Mouse sema3A, 3C, 3D, 3E, and 3F all bound the same mouse and zebrafish neuropilins as their othologous counterparts. There were only two exceptions. First, mouse sema3B binds both mouse nrps 1 and 2 as well as zebrafish nrp1a and nrp2a. In contrast, zebrafish sema3b only binds mouse nrp2 and zebrafish nrp2a. Thus, zebrafish sema3b has a narrower spectrum of nrp binding and is apparently more specialized in function than its mouse counterpart. Second, mouse sema3G binds to mouse and zebrafish nrp2s far better than to nrp1s. In contrast, its apparent zebrafish ortholog sema3bl binds all mouse and zebrafish nrps. In this instance the zebrafish ortholog has a broader binding spectrum than the mouse. It is interesting to note that mouse sema3 / zebrafish sema3b and mouse 3G / sema3bl orthologues have about 10% less peptide sequence identity when comparing the mouse and zebrafish versions than the five other orthologous pairs. This greater divergence in peptide identity may reflect the changed neuropilin binding profiles between the two species.

Altogether, we detected 2 instances of orthologs or paralogs with major differences in neuropilin binding specificities in 16 opportunities. Thus, the overall evolutionary story for class 3 semaphorins appears to be the conservation of binding functions rather than their divergence. Importantly, class 3 semaphorins generally bind a holoreceptor complex composed of neuropilins, plexins, and other components [24], so there my be other differences between the paralogs or orthologs that our simple qualitative neuropilin-specific binding studies could not detect. Even so, the conservation in function we observed is striking.

How could the functional properties of these genes remain stable for so long? With regards to the zebrafish semaphorin 3 paralogous pairs, the most likely explanation is the

subfunctionalization of paralog expression [23]. In this model, duplicate genes that are expressed in many different contexts under a variety of transcriptional control elements gradually specialize their expression profiles as control elements are differentially lost or changed over time (Force et al, 1999/Genetics). This would predict that the current zebrafish paralogs should have distinctive expression patterns when comparing one to another. Consistent with this idea, sema3aa and sema3ab have distinct expression patterns in the embryonic neural crest and neural tube, as do each parologous pair of sema3fa/fb, sema3ga/gb, nrp1a/nrp1b, and nrp2a/2b all [25]. If the origin of semaphorin class 3 paralogues in the fish is a genome-wide duplication near the origin of all teleost species, then one might expect there to be many additional semaphorin paralogs. Presumably many of these have been lost through nonfunctionalization as redundant genes accumulated deleterious mutations and became dispensable [23]. Finally, the high conservation of neuropilin binding specificities between mouse and zebrafish orthologues argues that at least this aspect of their function is nearly fixed, presumably through structural constraints imposed by their receptor complexes, as well as their possessing crucial roles in development that cannot be easily altered without compromising fitness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Sema	Semaphorin
Nrp	Neuropilin
WT	wild type
AP	alkaline phosphatase
6xhis tag	polyhistidine tag
BCIP	5-Bromo-4-chloro-3-indolyl phosphate
NBT	Nitro Blue Tetrazolium

References

[1]. Raible D, Raper J and Luo Y, “Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones.,” Cell, vol. 75, no. 2, p. 217–27. [PubMed: 8402908]

[2]. M. I D. H. e. Bougeret C, “Increased surface expression of a newly identified 150-kDa dimer early after human T lymphocyte activation,” J. Immunol, vol. 148, no. 2, p. 318–23, 1992. [PubMed: 1530858]

[3]. M. T-L Carmeliet P, “Common mechanisms of nerve and blood vessel wiring,” Nature, vol. 436, no. 7048, p. 193–200, . [PubMed: 16015319]

[4]. Gelfand MV, Hong S and Gu C, “Guidance from above: common cues direct distinct signaling outcomes in vascular and neural patterning.,” Trends in Cell Biology, vol. 19, no. 3, pp. 99–110, 2009. [PubMed: 19200729]

[5]. Pasterkamp RJ and Giger RJ, “Semaphorin function in neural plasticity and disease,” Current Opinion in Neurobiology, vol. 19, no. 3, pp. 263–274, 2009. [PubMed: 19541473]

[6]. Tran TS, Kolodkin AL and Bharadwaj R, “Semaphorin Regulation of Cellular Morphology,” Annual Review of Cell and Developmental Biology, vol. 23, no. 1, pp. 263–292, 2007.

[7]. Ruhrberg C and Schwarz Q, “In the beginning Generating neural crest cell diversity,” Cell Adhesion & Migration, vol. 4, no. 4, pp. 622–630, 2010. [PubMed: 20930541]

[8]. Bc J and Rj P, “Semaphorin signalling during development,” Development, vol. 141, no. 17, pp. 3292–3297, 2014. [PubMed: 25139851]

[9]. Pan H and Bachelder RE, “Autocrine Semaphorin3A stimulates eukaryotic initiation factor 4E-dependent RhoA translation in breast tumor cells.,” Experimental Cell Research, vol. 316, no. 17, pp. 2825–2832, 2010. [PubMed: 20655307]

[10]. Xiang R, Davalos AR, Hensel CH, Zhou X-J, Tse C and Naylor SL, “Semaphorin 3F Gene from Human 3p21.3 Suppresses Tumor Formation in Nude Mice,” Cancer Research, vol. 62, no. 9, pp. 2637–2643, 2002. [PubMed: 11980661]

[11]. Tomizawa Y, Sekido Y, Sekido Y, Kondo M, Gao B, Yokota J, Roche J, Drabkin HA, Lerman MI, Gazdar AF and Minna JD, “Inhibition of lung cancer cell growth and induction of apoptosis after reexpression of 3p21.3 candidate tumor suppressor gene SEMA3B,” Proceedings of the National Academy of Sciences of the United States of America, vol. 98, no. 24, pp. 13954–13959, 2001. [PubMed: 11717452]

[12] (. Committee N)), “Unified nomenclature for the semaphorins/collapsins,” Cell, vol. 97, no. 5, p. 551–2, . [PubMed: 10367884]

[13]. Fan J, Mansfield SG, Redmond T, Gordon-Weeks PR and Raper JA, “The organization of F-actin and microtubules in growth cones exposed to a brain-derived collapsing factor,” Journal of Cell Biology, vol. 121, no. 4, pp. 867–878, 1993. [PubMed: 8491778]

[14]. Koppel AM, Feiner L, Kobayashi H and Raper JA, “A 70 Amino Acid Region within the Semaphorin Domain Activates Specific Cellular Response of Semaphorin Family Members,” Neuron, vol. 19, no. 3, pp. 531–537, 1997. [PubMed: 9331346]

[15]. Janssen BJC, Malinauskas T, Weir GA, Cader MZ, Siebold C and Jones EY, “Neuropilins lock secreted semaphorins onto plexins in a ternary signaling complex.,” Nature Structural & Molecular Biology, vol. 19, no. 12, pp. 1293–1299, 2012.

[16]. Toledano S, Nir-Zvi I, Engelman R, Kessler O and Neufeld G, “Class-3 Semaphorins and Their Receptors: Potent Multifunctional Modulators of Tumor Progression,” International Journal of Molecular Sciences, vol. 20, no. 3, p. 556, 2019. [PubMed: 30696103]

[17]. Gu C, “Semaphorin 3E and plexin-D1 control vascular pattern independently of neuropilins,” Science, vol. 307, no. 5707, p. 265–8, 2005. [PubMed: 15550623]

[18]. Chauvet SCSMF, “Gating of Sema3E/PlexinD1 Signaling by Neuropilin-1 Switches Axonal Repulsion to Attraction during Brain Development,” Neuron, pp. 807–822, 2007. [PubMed: 18054858]

[19]. Kobayashi NR, Kobayashi NR, Fan D-P, Giehl KM, Bedard AM, Wiegand SJ, Tetzlaff W and Tetzlaff W, “BDNF and NT-4/5 Prevent Atrophy of Rat Rubrospinal Neurons after Cervical Axotomy, Stimulate GAP-43 and Tα1-Tubulin mRNA Expression, and Promote Axonal Regeneration,” The Journal of Neuroscience, vol. 17, no. 24, pp. 9583–9595, 1997. [PubMed: 9391013]

[20]. Kapfhammer JP, Xu H and Raper JA, “The detection and quantification of growth cone collapsing activities,” Nature Protocols, vol. 2, no. 8, pp. 2005–2011, 2007. [PubMed: 17703212]

[21]. Feiner L, Koppel AM, Kobayashi H and Raper JA, “Secreted Chick Semaphorins Bind Recombinant Neuropilin with Similar Affinities but Bind Different Subsets of Neurons In Situ,” Neuron, vol. 19, no. 3, pp. 539–545, 1997. [PubMed: 9331347]

[22]. Amaral DB and Schneider I, “Fins into limbs: Recent insights from sarcopterygian fish,” Genesis, vol. 56, no. 1, p. , 2018.

[23]. Glasauer SMK and Neuhauss SCF, “Whole-genome duplication in teleost fishes and its evolutionary consequences,” Molecular Genetics and Genomics, vol. 289, no. 6, pp. 1045–1060, 2014. [PubMed: 25092473]

[24]. Alto LT and Terman JR, “Semaphorins and their Signaling Mechanisms,” Methods of Molecular Biology, vol. 1493, no. , pp. 1–25, 2017.

[25]. Yu H-H and Moens CB, “Semaphorin signaling guides cranial neural crest cell migration in zebrafish.,” Developmental Biology, vol. 280, no. 2, pp. 373–385, 2005. [PubMed: 15882579]

Summary

- The last common ancestors of terrestrial vertebrates and teleost fishes are thought to have diverged approximately 400 million years ago. We examined the functional conservation of a key family of axonal guidance cues and signaling molecules, the class III semaphorins, during this time period.
- With only a few exceptions, othologous mouse and zebrafish semaphorins prefer to bind to the same (neuropilin) binding partners.
- Duplicated semaphorins, thought to have diverged near the beginning of the teleost lineage, all had nearly identical binding preferences.
- These findings suggest a very high conservation of functional identity within this important class of signaling molecules over an extremely long time period. They are consistent with a model of semaphorin specialization that is more dependent upon their acquiring unique developmental expression patterns than assuming new specialized biochemical properties.

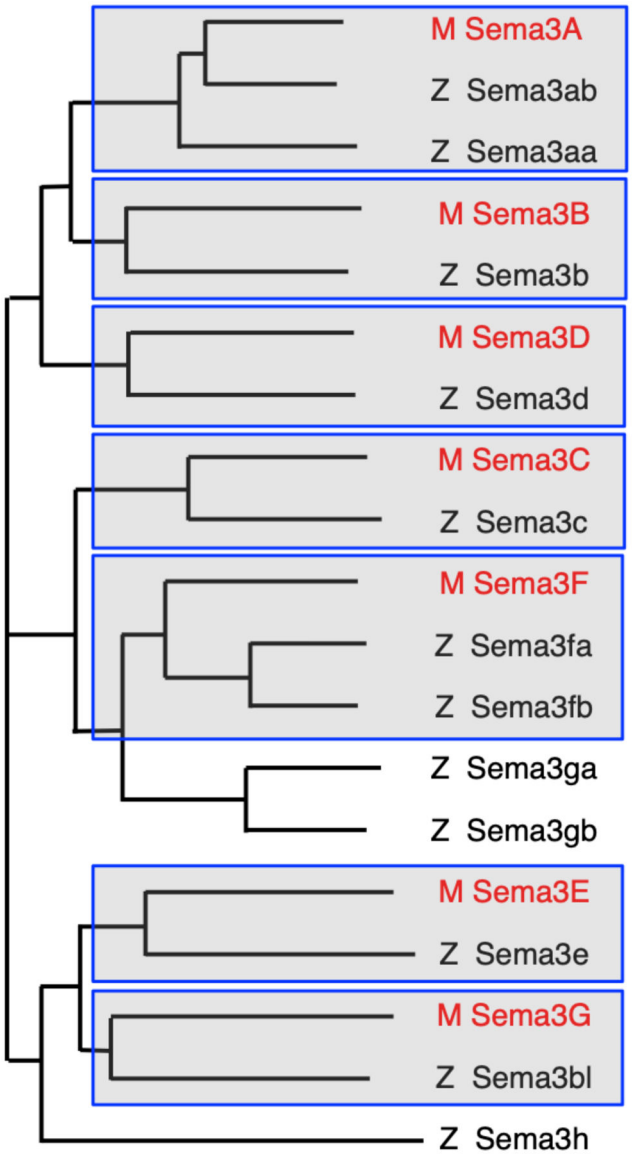

Figure 1. Nearest neighbor relationships between mouse and zebrafish semaphorin peptide sequences.

Zebrafish paralogs are grouped together with their mouse orthologues (gray boxes).

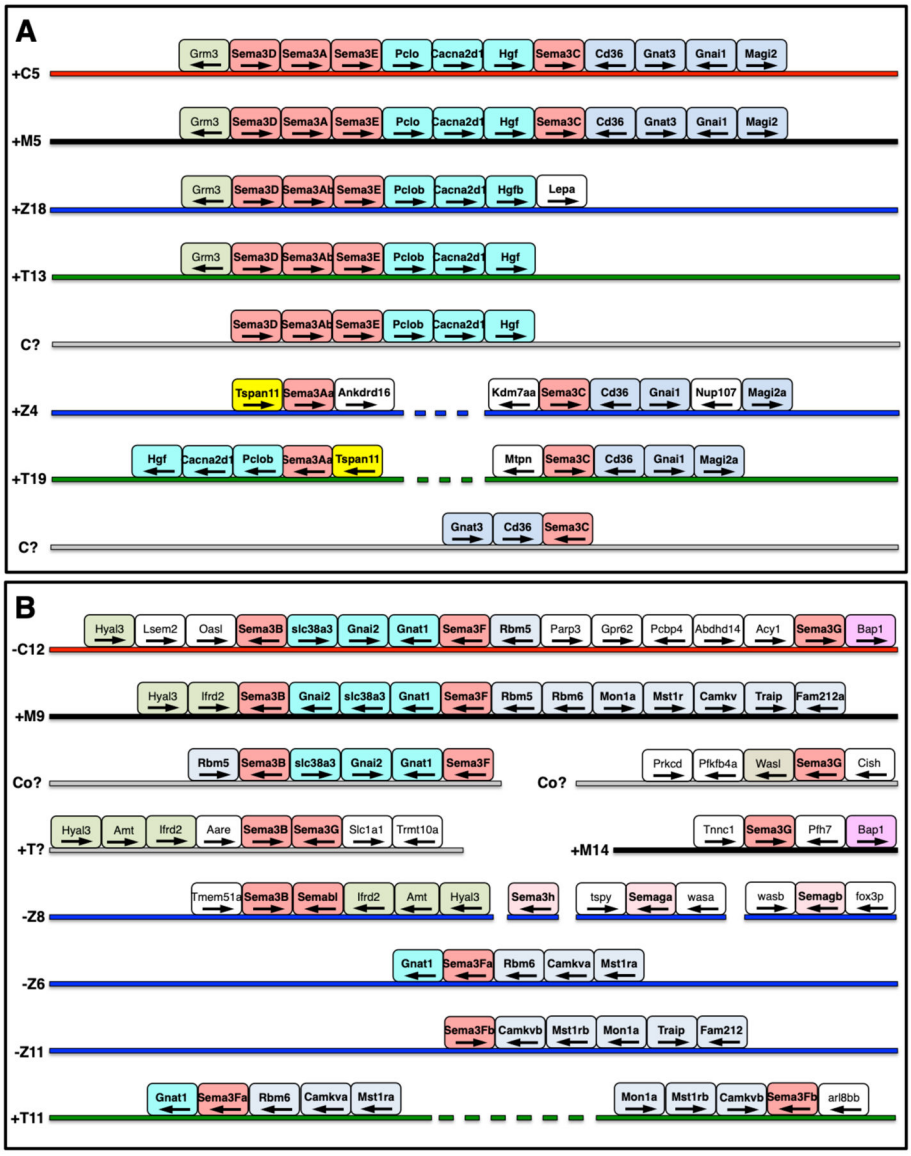

Figure 2. Clustering and arrangement of semaphorins in representative genomes.

The relative arrangements of semaphorin genes with their nearest neighbors in the chick, mouse, pufferfish, Ceolocanth, and zebrafish. Chromosome and strand orientation are indicated where known to the left. Gene orientation indicated by arrows. Large distances between genes on the same chromosomes are indicated by gaps. Not to scale.

Table 1.

A summary of zebrafish and mouse semaphorin binding specificities to zebrafish or mouse neuropilins.

Qualitatively stronger binding is indicated by the darker shaded cells, and weaker binding by the lightly shaded cells. M (red: mouse), Z (blue: zebrafish). The first boxed grouping binds Nrp1s but not Nrp2s, the second grouping binds Nrp2s and not Nrp1s, and the third grouping binds both Nrp1s and Nrp2s.

	M nrp1	Z nrp1a	Z nrp1b	M nrp2	Z nrp2a	Z nrp2b
M sema3A						
F sema3aa						
F sema3ab						
M sema3D						
Z sema3d						
M sema3F						
Z sema3fa						
Z sema3fb						
Z sema3ga						
Z sema3gb						
M sema3B						
Z sema3b						
M sema3C						
F sema3C						
M sema3E						
Z sema3e						
M sema3G						
Z sema3bl						
Z sema3h						