ELSEVIER

Gene Expression Patterns 5 (2005) 647–653

GEP

www.elsevier.com/locate/modgep

Developmental expression of sema3G, a novel zebrafish semaphorin

Craig B. Stevens[a], Mary C. Halloran[a,b,*]

[a]*Department of Zoology, University of Wisconsin, 1117 West Johnson St., Madison, WI 53706, USA*
[b]*Department of Anatomy, University of Wisconsin, 1117 West Johnson St., Madison, WI 53706, USA*

Received 5 January 2005; received in revised form 27 February 2005; accepted 28 February 2005
Available online 11 April 2005

Abstract

The semaphorins are a large, evolutionarily conserved family of signaling molecules with broad functions during development. The class 3 semaphorins are a subclass of secreted semaphorins found in vertebrates. There have been six class 3 semaphorins identified to date (*sema3A* to *sema3F*) and some have been shown to function in axon guidance and cardiovascular development. However, the functions of many class 3 semaphorins and their potential interactions in vivo are still not well understood. As a step toward understanding the actions of all class 3 semaphorins in vivo, we have cloned and analyzed the developmental expression pattern of a novel zebrafish class 3 semaphorin, *sema3G*. *sema3G* is expressed in a dynamic pattern throughout the first 3 days of development. It is expressed in the adaxial cells of the somite during somitogenesis. In the brain, *sema3G* is expressed in cell clusters in the midbrain and diencephalon, and is expressed in the telencephalon in close proximity to the olfactory epithelium. *sema3G* also is expressed in the pharyngeal arches, the pectoral fin bud, and the developing pronephros. These results provide a basis for studying how expression of multiple semaphorins could be essential for aspects of early development.

Keywords: Semaphorin; Zebrafish; Secreted; Class 3; sema3G; Sema3A; Sema3D; Adaxial; Somite; Pronephros; Fin bud; Pharyngeal arches; Axon; Olfactory; Midbrain; Telencephalon; Eye; Optic nerve; Diencephalon; Sema; Plexin; Neuropilin; Immunoglobulin

1. Results and discussion

Semaphorins make up a large gene family divided into seven classes. The class 3 semaphorins are secreted proteins originally identified as repulsive axon guidance signals, however, their expression patterns and other functional data suggest they have multiple roles during vertebrate development (Raper, 2000; Deutsch, 2004; Fujisawa, 2004). Six class 3 semaphorins have been identified in mammals (*sema3A* to *3F*), and 5 have been isolated in chicken (*sema3A* and *sema3C to 3F*) (Luo et al., 1993; Luo et al., 1995; Puschel et al., 1995; Feiner et al., 1997; Christensen et al., 1998; Eckhardt and Meyerhans, 1998). Class 3 semaphorin genes are expressed in distinct patterns in the nervous system as well as several other tissues, including the somites, limbs, and sensory organs (Luo et al., 1995; Puschel et al., 1995; Shepherd et al., 1996; Bagnard et al., 1998; Roos et al., 1999; Yee et al., 1999; Williams-Hogarth et al., 2000). Disruption of individual class 3 semaphorins in vivo leads to defects in axon pathfinding and cardiovascular development (Behar et al., 1996; Taniguchi et al., 1997; Feiner et al., 2001; Sahay et al., 2003; Serini et al., 2003; Shoji et al., 2003; Liu et al., 2004; Torres-Vazquez et al., 2004; Wolman et al., 2004). However, for several class 3 semaphorins, there still is no in vivo functional information. In addition, some class 3 semaphorins can antagonize the action of other class 3 semaphorins in vitro, suggesting the class 3 semaphorins may interact with each other during development in vivo (Takahashi et al., 1998). In order to fully understand the functional roles of the class 3 semaphorins and their potential interactions in vivo, it is necessary to identify the complete complement of class 3 semaphorins in vertebrates and determine their detailed expression patterns during development.

The zebrafish is an ideal model system to study semaphorin functions and the role of coordinated class 3 semaphorin activities. However, in zebrafish complete sequences of only three class 3 semaphorins have been identified to date (Halloran et al., 1999; Roos et al., 1999; Yee et al., 1999). Here we report the cloning and expression

* Corresponding author. Tel.: +1 608 263 7875; fax: +1 608 262 9083.
E-mail address: mchalloran@wisc.edu (M.C. Halloran).

1567-133X/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.modgep.2005.02.009

pattern of a novel zebrafish class 3 semaphorin, *sema3G*. *sema3G* is specifically and dynamically expressed in both neural and non-neural tissues during zebrafish development. In addition, while the *sema3G* expression pattern is unique, it shares some overlap with the expression of other class 3 semaphorins. These results will assist in determining the roles played by class 3 semaphorins during development.

1.1. Cloning of sema3G

A partial sequence of this gene (formerly named *semaZ8*) was previously reported (Halloran et al., 1998). To obtain the complete sequence, we performed 5′ rapid amplification of cDNA ends (RACE) followed by nested PCR on total mRNA isolated from zebrafish embryos at 19 h post fertilization (hpf). The resulting product was sequenced and found to contain an open reading frame encoding a protein 748 amino acids in length. Analysis of this protein sequence using the Conserved Domain Database (Marchler-Bauer et al., 2003) showed that it encodes a semaphorin domain, an immunoglobulin domain, and a highly basic carboxyl terminus (data not shown), the characteristic domains of the class 3 secreted semaphorins (Luo et al., 1995; Puschel et al., 1995). In addition, *sema3G* contains 17 of 18 highly conserved cysteine residues shared with other class 3 semaphorins (Luo et al., 1995; Puschel et al., 1995).

Sequence analysis suggests that *sema3G* is a novel class 3 semaphorin and not an orthologue to previously identified semaphorins. A clustalW alignment revealed that Sema3G has no outstanding amino acid identity to any of the known class 3 semaphorins in zebrafish, human, mouse, chicken, or *Xenopus* (Fig. 1, and data not shown). Rather, Sema3G has approximately the same percentage amino acid identity (about 40%) with each of the class 3 semaphorin genes in mouse, chicken, *Xenopus*, and zebrafish (Table 1). In contrast, other zebrafish semaphorins share significantly higher amino acid identity to their orthologues in other species. For example, zebrafish Sema3A1 and Sema3A2 have average identities of 64 and 75%, respectively, to Sema3A orthologues in other species, but only 40 and 45% average identities to all other class 3 homologues (Yee et al., 1999). In addition the mouse class 3 semaphorins, like the zebrafish, also have high identities (78–97%) to their orthologues in other species and low identities (41–46%) to all other class 3 semaphorins.

We examined the intron/exon structure of *sema3G* and compared it to each of the mouse class 3 semaphorin genes (obtained from the Ensembl genome database, www.ensembl.org) to determine if *sema3G* could be assigned as an orthologue to one of the known class 3 genes. However, the class 3 semaphorin genes in mouse, with the exception of *sema3B*, share a common intron/exon structure of 17 exons distributed across the coding sequence. *sema3B* differs slightly, with part of the coding region divided into additional smaller-sized exons (data not shown). *sema3G* shares the same overall intron/exon structure as mouse

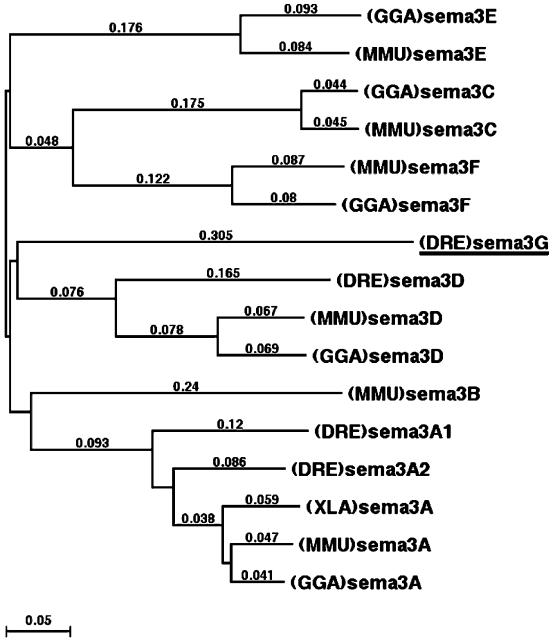

Fig. 1. Phylogenetic tree comparing Sema3G to other representatives of class 3 semaphorins. Sema3G is significantly distant from the other groupings of class 3 semaphorins, suggesting it represents a novel class 3 semaphorin. The phylogenetic tree was calculated using nearest neighbor joining with the proportion of distances estimated, and gaps ignored. Species abbreviations: (GGA) *Gallus gallus*; (DRE) *Danio rerio*; (MMU) *Mus musculus*; (XLA) *Xenopus laevis*. See experimental procedures for GenBank accession numbers.

sema3A and *3C to 3F*. Therefore, this type of analysis does not distinguish between different class 3 semaphorins. Based on the sequence analysis described above, we conclude that this is a novel class 3 semaphorin and thus assign it the name *sema3G* (Semaphorin Nomenclature Committee, 1999).

We conducted BLAST searches of other vertebrate genomes using the Ensembl genome database, but failed to identify any putative class 3 semaphorins with significant identity to *sema3G*. *sema3G* has been mapped previously to linkage group 8 of the zebrafish genome, a region that has been found to be syntenic with part of human chromosome 1 (Postlethwait et al., 2000; Talbot and Rauch, 2003).

Table 1
Percentage amino acid identity of Sema3G in comparison to other class 3 secreted semaphorins

	Mouse	Chicken	Xenopus	Zebrafish
Sema3A	42%	41%	40%	37/41%[a]
Sema3B	36%			
Sema3C	38%	38%		
Sema3D	41%	42%		40%
Sema3E	38%	37%		
Sema3F	36%	38%		

Sema3G does not have an outstanding similarity to any single representative, suggesting that *sema3G* represents a novel zebrafish semaphorin gene.

[a] Comparison to zebrafish Sema3A1 and Sema3A2, respectively.