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**Semaphorins:
Receptor and Intracellular
Signaling Mechanisms**

Edited by
R. Jeroen Pasterkamp

Semaphorins: Receptor and Intracellular Signaling Mechanisms

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Edited by R. Jeroen Pasterkamp

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Edited by

R. Jeroen Pasterkamp

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of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands*

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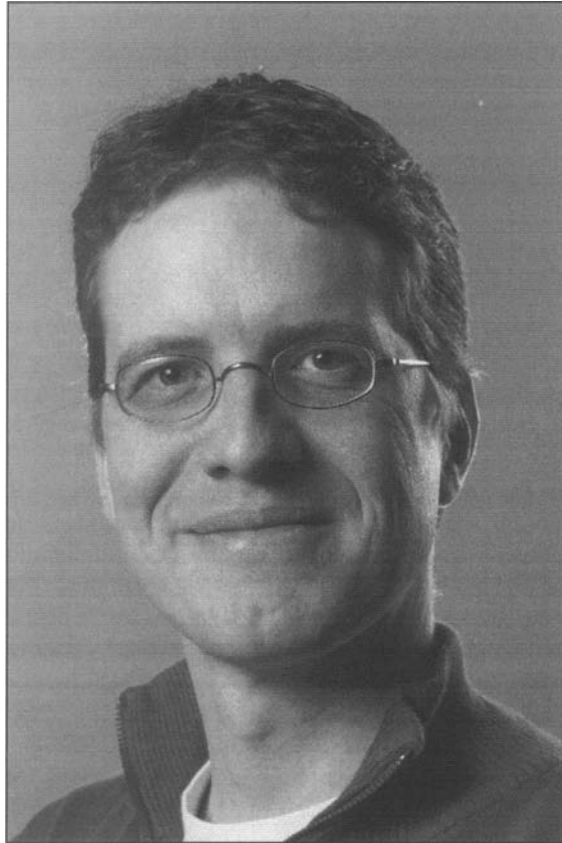
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PREFACE

Since the identification of the first two semaphorins in the early 1990s, Sema1a (Fasciclin IV) and Sema3A (collapsin), more than 25 semaphorin genes have been described. Although originally identified as repulsive guidance signals for extending axons, these secreted and membrane-associated glycoproteins are extremely pleiotropic, and many serve diverse roles unrelated to axon guidance. These include multiple distinct roles within a given biological system or tissue, including axon guidance, cell migration and neuronal apoptosis in the nervous system, and also parallel functions in seemingly disparate systems, such as cell migration in the nervous, cardiovascular and immune systems. Our knowledge of the cellular actions of semaphorin family members has advanced significantly over the past several years, and the receptors and intracellular signaling mechanisms that underlie semaphorin function are being unveiled at a rapid pace.

Although plexins are the predominant family of semaphorin receptors, multiple (co-)receptor proteins function in several semaphorin signaling events. A unifying principle that defines the function of high-affinity semaphorin receptors characterized to date is their multimeric character. Unrelated receptor proteins with distinct functions (e.g., ligand-binding, signal-transducing, modulatory) are assembled into large holoreceptor complexes to detect and respond to semaphorin proteins present in the extracellular space. There is a growing appreciation that the composition of a semaphorin receptor complex not only determines ligand specificity and sensitivity but also dictates the functional outcome of a ligand-receptor interaction. For example, a semaphorin receptor complex may trigger attractive or repulsive cell migration events in response to the same semaphorin ligand depending on the presence or absence of certain co-receptor proteins.

Recent semaphorin research is characterized by an impressive effort to decipher the intracellular signal transduction networks downstream of semaphorins and their receptors. An ever-increasing number of cytosolic signaling molecules is being implicated in semaphorin signaling, and common principles begin to emerge that underlie the molecular basis of semaphorin function. Activation of small GTPases, and phosphorylation of both receptors and intracellular effector proteins, are crucial for semaphorin-mediated effects. In addition, recent evidence suggests the involvement of less well-known modulatory mechanisms, such as redox signaling and local protein synthesis.

The chapters included in this book are intended to provide a representative survey of recent progress on research devoted to semaphorins with an emphasis on receptor and intracellular signaling mechanisms. The first four chapters address several of the key families of cytosolic signaling cues implicated in semaphorin signaling (including CRMPs, small GTPases, protein kinases, and MICALs). The following three chapters cover the intracellular and extracellular factors that modulate semaphorin signaling (various cytosolic cues, Ig superfamily cell adhesion molecules, and proteoglycans). Finally, the last four chapters review recent progress in our understanding of how semaphorin signaling pathways may contribute to the development and disease of specific biological systems.

It is likely that work on semaphorin function such as outlined in this book will continue to advance our understanding of key regulator influences on cellular morphology, and that these studies will serve as a model for the complex and elaborate cellular and molecular functions of all major families of guidance cues.

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CONTENTS

PREFACE VI

**1. THE CRMP FAMILY OF PROTEINS AND THEIR ROLE
IN SEMA3A SIGNALING 1**

Eric F. Schmidt and Stephen M. Strittmatter

Properties and Expression of CRMPs 1
Evidence For CRMPs in Sema3A Signaling 2
The Regulation of the Cytoskeleton by CRMPs 5
CRMP, Endocytosis and Other Signaling Events 7
CRMP and Disease 8
Concluding Remarks 9

2. GTPASES IN SEMAPHORIN SIGNALING 12

Andreas W. Püschel

Summary 12
Introduction 12
The Function of GTPases for Semaphorin Signaling 13
Interaction of GTPases with Plexins 14
GTPases and Signaling by Plexin-A1 16
GTPases and Signaling by Plexin-B1 19
Invertebrate Semaphorins 20
Open Questions 20

3. INTRACELLULAR KINASES IN SEMAPHORIN SIGNALING 24

Aminul Ahmed and Britta J. Eickholt

Introduction 24
**Semaphorins Regulate Actin Filament Dynamics by Controlling
Cofilin Activity 25**
Modulation of Semaphorin Responses by Neurotrophins 27
Regulation of PI3K Signaling by Semaphorins 28
**Synergistic Control of CRMP-2 Phosphorylation by CDK-5
and GSK3 Mediates Sema3A Function 29**
Cdk5 and Sema3A-Mediated Increases in Axonal Transport 30

MAPK Signaling and the Control of Sema3A Induced Translation of Axonal mRNA	31
Semaphorin Signaling Leading to Selective Cell Death Responses	32
Concluding Remarks	32
4. MICAL FLAVOPROTEIN MONOOXYGENASES: STRUCTURE, FUNCTION AND ROLE IN SEMAPHORIN SIGNALING	38
Sharon M. Kolk and R. Jeroen Pasterkamp	
Introduction	38
The MICAL Family	39
MICALs in Semaphorin Signaling	41
A MICAL Connection to the Cytoskeleton?	43
MICALs: Redox Regulators of Axon Guidance Events?	46
Concluding Remarks	47
5. SIGNALING OF SECRETED SEMAPHORINS IN GROWTH CONE STEERING	52
Sangwoo Shim and Guo-li Ming	
Introduction	52
In Vitro Neuronal Growth Cone Steering Assays	52
Receptor Complex in Mediating Growth Cone Turning Responses to Class 3 Semaphorins	53
Intracellular Mediators for Class 3 Semaphorin-Induced Growth Cone Turning	55
Modulation of Growth Cone Turning Responses to Class 3 Semaphorins	57
Summary	57
6. MODULATION OF SEMAPHORIN SIGNALING BY IG SUPERFAMILY CELL ADHESION MOLECULES	61
Ahmad Bechara, Julien Falk, Frédéric Moret and Valérie Castellani	
Summary	61
Introduction	61
Soluble Forms of IgSFCAMs Convert Repulsive Responses to Class III Semaphorins into Attraction	62
Transmembrane Forms of IgCAMs Are Components of Class III Semaphorin Receptors	64
Molecular Interactions Underlying the Modulation of Semaphorin Signaling by Soluble IgSFCAMs	64
Receptor Internalization and Modulation of Sema3A Signaling	65
Biological Contexts for Regulation of Semaphorin Signals by IgSFCAMS	66
Conclusions	68

**7. PROTEOGLYCANS AS MODULATORS OF AXON GUIDANCE
CUE FUNCTION 73**

Joris de Wit and Joost Verhaagen

Introduction 73
Role of Heparan Sulfate Proteoglycans in Axon Guidance 74
Role of Chondroitin Sulfate Proteoglycans in Axon Guidance 80
Proteoglycans and Guidance Molecules in the Regeneration of Adult Neurons 82
Concluding Remarks 84

**8. SEMAPHORIN SIGNALS IN CELL ADHESION AND CELL
MIGRATION: FUNCTIONAL ROLE AND MOLECULAR
MECHANISMS 90**

Andrea Casazza, Pietro Fazzari and Luca Tamagnone

Introduction 90
**Functional Role of Semaphorins in Cell Adhesion
and Cell Migration 91**
**Signaling Molecules Mediating Semaphorin Function
in Cell Adhesion and Migration 98**
Some Open Questions 102
Conclusions 103

**9. SEMAPHORIN SIGNALING DURING CARDIAC
DEVELOPMENT 109**

Toshihiko Toyofuku and Hitoshi Kikutani

Cardiac Morphogenesis: An Overview 109
Sema6D-Plexin-A1 Axis in Cardiac Morphogenesis 111
Semaphorin Signaling in Vascular Connections to the Heart 112
Signals of Sema3A in Endothelial Cell Migration 115
Summary and Perspectives 115

**10. SEMAPHORIN SIGNALING IN VASCULAR AND TUMOR
BIOLOGY 118**

Gera Neufeld, Tali Lange, Asya Varshavsky and Ofra Kessler

**Receptors Belonging to the Neuropilin and Plexin Families and Their
Semaphorin Ligands 118**
The Role of the Neuropilins in VEGF Signaling 123
**The Role of Class-3 Semaphorins in the Control of Angiogenesis
and Tumor Progression 124**
**Cell Surface Attached Semaphorins as Modulators of Angiogenesis
and Tumor Progression 125**
Semaphorins as Direct Regulators of Tumor Cell Behavior 127
Conclusions 127

11. SEMAPHORIN SIGNALING IN THE IMMUNE SYSTEM	132
Vincent Potiron, Patrick Nasarre, Joëlle Roche, Cynthia Healy and Laurence Bounsell	
Introduction	132
CD100/SEMA4D	133
SEMA4A	137
Viral Semaphorins and SEMA7A / CD108	139
Other Immune Semaphorins and Related Proteins	141
Semaphorins in Lymphoid Disorders	142
Conclusion	143
Abbreviations	144
INDEX	145

CHAPTER 1

The CRMP Family of Proteins and Their Role in Sema3A Signaling

Eric F. Schmidt and Stephen M. Strittmatter*

Abstract

The CRMP proteins were originally identified as mediators of Sema3A signaling and neuronal differentiation. Much has been learned about the mechanism by which CRMPs regulate cellular responses to Sema3A. In this review, the evidence for CRMP as a component of the Sema3A signaling cascade and the modulation of CRMP by plexin and phosphorylation are considered. In addition, current knowledge of the function of CRMP in a variety of cellular processes, including regulation of the cytoskeleton and endocytosis, is discussed in relationship to the mechanisms of axonal growth cone Sema3A response.

The secreted protein Sema3A (collapsin-1) was the first identified vertebrate semaphorin. Sema3A acts primarily as a repulsive axon guidance cue, and can cause a dramatic collapse of the growth cone lamellipodium. This process results from the redistribution of the F-actin cytoskeleton^{1,2} and endocytosis of the growth cone cell membrane.²⁻⁴ Neuropilin-1 (NP1) and members of the class A plexins (PlexA) form a Sema3A receptor complex, with NP1 serving as a high-affinity ligand binding partner, and PlexA transducing the signal into the cell via its large intracellular domain. Although the effect of Sema3A on growth cones was first described nearly 15 years ago, the intracellular signaling pathways that lead to the cellular effects have only recently begun to be understood. Monomeric G-proteins, various kinases, the redox protein, MICAL, and protein turnover have all been implicated in PlexA transduction. In addition, the collapsin-response-mediator protein (CRMP) family of cytosolic phosphoproteins plays a crucial role in Sema3A/NP1/PlexA signal transduction. Current knowledge regarding CRMP functions are reviewed here.

Properties and Expression of CRMPs

A number of CRMP genes were identified independently in different species around the same time, and were named according to their method of discovery. CRMPs are also known as turned on after division (TOAD-64),⁵ dihydropyrimidinase related protein (DRP),⁶ unc33 like protein (Ulip),⁷ and TUC (TOAD64/Ulip/CRMP).⁸ Five vertebrate CRMP genes (CRMP1-5) have been identified, while the *Drosophila* genome appears to encode for only a single CRMP. CRMP1-4 share ~75% protein sequence identity with each other, however CRMP5 (also referred to as CRAM) is only 50-51% homologous. CRMPs share a high sequence homology with the *C. elegans unc-33* gene,^{5,7,9,10} although two other nematode genes, CeCRMP1 and 2, have been classified in the CRMP family.¹¹ In addition, mammalian CRMP1, 2, and 4 appear to undergo alternative splicing.^{12,13} CRMP isoforms strongly interact with

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each other and exist as heterotetramers when purified from brain.¹⁴ Specificity exists for the hetero-oligomerization in that different isoforms have varying affinities for each other.¹⁴ Information obtained from the examination of the crystal structure of CRMP1 homotetramers reveals that this specificity is likely due to differential polar and hydrophobic residues between isoforms at the two oligomerization interfaces.¹⁵ CRMP1-4 genes share a high sequence homology (60%) with the liver dihydropyrimidinase (DHPase) and structural similarity with members of the metal-dependent amidohydrolases, both of which form stable tetramers. However, none of the CRMP isoforms demonstrate any enzymatic activity, likely due to the fact that they lack crucial His residues which coordinate binding of a metal atom at the active site of amidohydrolase enzymes.^{6,14,15}

CRMPs were discovered to be one of the first proteins expressed in newly born neurons in the developing brain,⁵ and CRMP2 expression has been shown to be induced by factors that promote neuronal differentiation such as noggin, chordin, GDNF, and FGF.¹⁶⁻¹⁸ Not surprisingly, CRMPs are most highly expressed during the neurogenic period of brain development and expression peaks during the period of axon growth.¹⁹ In addition, CRMP1, 2 and 5 are expressed in immature interneurons in the adult olfactory bulb,²⁰ a site of ongoing neurogenesis in adulthood.²¹ The expression of CRMPs is restricted primarily to the nervous system, however some isoforms show a differential pattern of expression in various nervous system structures.^{19,22} CRMP2, and to some extent CRMP3, are expressed in mature neurons at low levels. These expression patterns, when taken together with the fact that CRMPs form heterotetramers, imply that oligomers consisting of different combinations of monomeric isoforms may have different functional effects in various cell types.

The significant sequence similarity of CRMPs with the worm *unc-33* gene implies a role for CRMP in axon growth and morphology, since *unc-33* mutants display severe axonal abnormalities.^{23,24} Also, overexpression of CRMP2 induces ectopic axon formation in cultured hippocampal cells.²⁵ Although CRMP is a cytosolic protein, a significant fraction has been shown to be tightly associated with the cell membrane.^{5,26} This membrane-associated pool of CRMP is enriched at the leading edge of the growth cone lamellipodium and filopodia, further supporting a role in axon outgrowth and guidance.⁵ All CRMP isoforms continue to be expressed through the period of axon pathfinding and synaptogenesis,¹⁹ and CRMP expression is induced in sprouting fibers after injury in both the central and peripheral nervous system.^{5,27,28}

Evidence For CRMPs in *Sema3A* Signaling

The first evidence for the involvement of CRMPs in *Sema3A* signaling came from the identification of chick CRMP-62 (CRMP2) in an expression cloning screen looking for *Sema3A* signaling components.¹⁰ Injection of CRMP-62 (CRMP2) RNA rendered *Xenopus* oocytes electrophysiologically responsive to *Sema3A* (collapsin-1). Further, treatment of DRG neurons with antibodies developed against an N-terminal region (a.a. 30-48) of CRMP-62 blocked the ability of *Sema3A* to collapse their growth cones.

Sema3A signaling can be reconstituted in a nonneuronal heterologous system.^{29,30} COS7 cells overexpressing PlexA1 and NP1 contract when *Sema3A* is applied to the media. This effect is easily quantified using alkaline phosphatase (AP)-tagged ligand and measuring the surface area of the cells with bound AP. AP-*Sema3F* treatment fails to cause contraction of PlexA1/NP1-expressing cells, demonstrating the specificity of the assay. COS7 cells overexpressing CRMPs in addition to the PlexA1/NP1 receptor components undergo contraction at a much more rapid rate than cells in which the receptors are expressed alone.¹⁵ Therefore, CRMP proteins are able to facilitate *Sema3A*-mediated morphological changes in nonneuronal cells, further suggesting that they play a role in the signaling pathway (Fig. 1).

In addition, CRMP1-4 are all able to form a complex with the *Sema3A* receptor PlexA1 in transfected nonneuronal cells.¹⁵ Although the presence of NP1 attenuates this interaction, stimulation with *Sema3A* reestablishes the complex. This is a specific effect since treatment with *Sema3F* is unable to promote PlexA1-CRMP interactions in the presence of NP1.