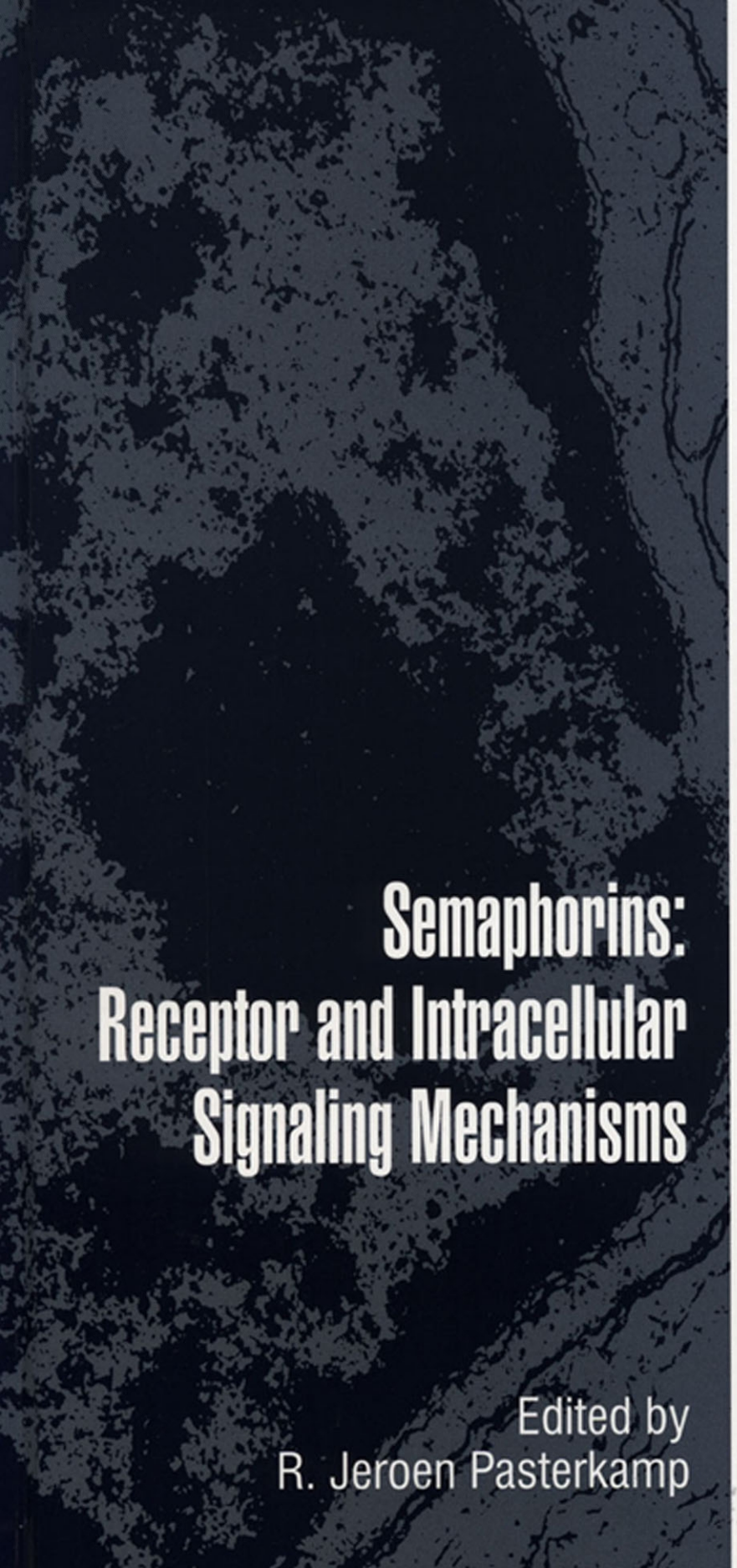

ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Volume 600

Semaphorins: Receptor and Intracellular Signaling Mechanisms

Edited by
R. Jeroen Pasterkamp

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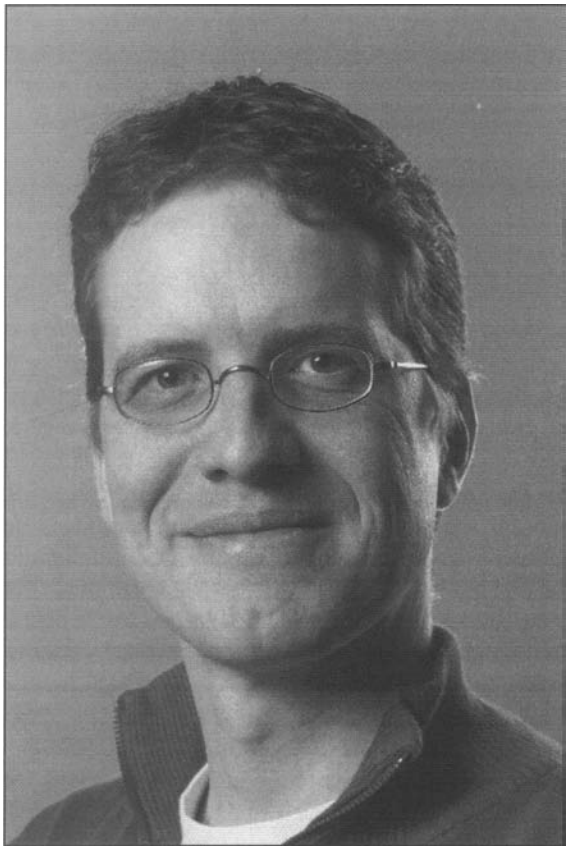

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R. JEROEN PASTERKAMP is an Assistant Professor at the Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands. The focus of his laboratory is directed towards understanding the intracellular signaling events involved in the formation of neuronal connections. His research team concentrates on the developing mouse embryo using an integrated approach involving molecular biology, cell biology, in vivo functional proteomics, and mouse genetics. He received his Ph.D. from the Netherlands Institute for Neurosciences (Amsterdam, The Netherlands) and did his Postdoctoral at the Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore.

PREFACE

Since the identification of the first two semaphorins in the early 1990s, Sema-1a (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

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. elegens 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.