CHAPTER 4

MICAL Flavoprotein Monooxygenases: Structure, Function and Role in Semaphorin Signaling
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Abstract

MICALs (for Molecule Interacting with CasL) form a recently discovered family of evolutionarily conserved signal transduction proteins. They contain multiple well-conserved domains known for interactions with the cytoskeleton, cytoskeletal adaptor proteins, and other signaling proteins. In addition to their ability to bind other proteins, MICALs contain a large NADPH-dependent flavoprotein monooxygenase enzymatic domain. Although MICALs have already been implicated in a variety of cellular processes, their function during axonal pathfinding in the Drosophila neuromuscular system has been best characterized. During the establishment of neuromuscular connectivity in the fruit fly, MICAL binds the axon guidance receptor Plexin A and transduces semaphorin-1a-mediated repulsive axon guidance. Intriguingly, mutagenesis and pharmacological inhibitor studies suggest a role for MICAL flavoenzyme redox functions in semaphorin/plexin-mediated axonal pathfinding events. This review summarizes our current understanding of MICALs, with an emphasis on their role in semaphorin signaling.

Introduction

The formation of neuronal circuits during embryonic development relies upon the guidance of growing axons to their synaptic targets. To help neurons find these targets, axons are tipped with a highly motile sensory structure called the growth cone. Growth cones are instructed to follow predetermined trajectories by heterogeneously distributed guidance molecules in their extracellular environment. Binding of axon guidance molecules to receptor complexes on the growth cone surface initiates intracellular signaling events, which in turn modulate growth cone morphology and directional motility through local modifications of the neuronal cytoskeleton. Axon guidance molecules can act as attractants or repellents, either directing growth cones towards a specific structure or preventing them from entering inappropriate regions of the embryo. Furthermore, they exist as membrane-associated or soluble agents, acting at short ranges or at long distance, respectively.1-3

The semaphorins are among the largest of the conserved families of axon guidance molecules, affecting axon steering, zonal segregation of distinct axon populations, axon branching, axon fasciculation, neuron polarity, and synapse formation and function.4,5 In addition, semaphorins and their receptors participate in the regulation of various nonneuronal processes such as angiogenesis, organogenesis, tumorigenesis and immune cell function. Furthermore, they may contribute to the onset and/or progression of human (brain) disease.6-9 Semaphorin

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signaling during axon guidance is dependent on multimeric receptor complexes on the growth cone cell surface that contain plexin proteins as obligatory signal-transducing subunits. Semaphorins can either bind directly to plexins (Sema classes 1, 2, 4-7 and Sema3E) or may require specialized ligand-binding coreceptors, such as neuropilin proteins (Sema class 3), to achieve steering of neuronal processes. In addition to plexins and neuropilins, several structurally unrelated receptors have been identified, some of which function in the nervous system including receptor tyrosine kinases (Otk), Ig superfamily cell adhesion molecules (L1 and NrCAM), integrins, and CD72. In sharp contrast to the wealth of information on the biology of semaphorins and their (co)receptors, the cytosolic signaling pathways that mediate growth cone responses to semaphorins are only now beginning to be understood. An ever increasing number of signaling proteins are now implicated in linking semaphorin receptors to the neuronal cytoskeleton including members of the Rho family of small GTPases, collapsin response mediator proteins (CRMPs), and intracellular protein kinases. Here we focus on MICALs, a novel family of cytosolic signaling proteins implicated in mediating semaphorin signaling events in neurons. A short description of the recently established neuronal MICAL expression patterns precedes our overview of potential roles played by these multidomain proteins in repulsive semaphorin/plexin signaling. We then review potential roles for MICALs in the regulation of cytoskeletal dynamics and summarize recent insights into the structure and function of the MICAL flavoprotein monoxygenase domain.

The MICAL Family

A critical step in neuronal semaphorin signaling is the activation of specific signal transduction pathways by plexins. Several of the semaphorin signaling cues identified to date can associate directly with the cytoplasmic domain of plexin. The MICAL proteins (for Molecule Interacting with CasL) form a family of cytosolic plexin-interacting proteins that participate in repulsive semaphorin signaling in neurons. In invertebrate species such as Drosophila, a single MICAL protein has been identified (D-MICAL), while vertebrates have three MICAL genes (MICAL-1, MICAL-2 and MICAL-3). In addition, several MICAL-like genes (MICAL-L) exist which encode potentially MICAL-related proteins that lack the highly conserved NH2-terminal region present in 'full-length' MICALs. From sequence analysis it has been shown that MICALs contain multiple domains and motifs known to be important for interactions with the actin cytoskeleton and other proteins critical for signaling events to the cytoskeleton. In addition, MICALs uniquely combine their protein-binding properties with an NH2-terminal flavoprotein monoxygenase domain (Fig. 1A). MICALs are expressed in various tissues including lung, heart, thymus, and brain.

MICAL Expression

Thus far, MICAL expression patterns in invertebrate and vertebrate nervous systems have been studied in most detail. In the early embryonic fruit fly (stage 7-8), prominent D-MICAL labeling is found in the ventral neurogenic region. At later stages (stage 13 onward), D-MICAL transcripts are present within the developing Drosophila brain and ventral cord in most, if not all, central nervous system (CNS) neurons. In contrast, mRNA expression in peripheral nervous system (PNS) neurons is weak. In line with D-MICAL's role downstream of Plexin A (PlexA), D-MICAL and PlexA distribution patterns are highly similar. Immunohistochemistry for D-MICAL protein labels neuronal cell bodies, axons and growth cones.

Vertebrate members of the MICAL family are expressed throughout the developing and mature rat nervous system. In contrast to MICAL-1 and -3, the onset of MICAL-2 expression is delayed (late embryonic/postnatal) and MICAL-2 is absent from certain specific brain areas such as the hypothalamus and striatum. MICAL-2 expression patterns in the embryonic and postnatal nervous system support the idea that MICALs play roles in neural development, while their presence in adult neurons hints at a possible function in the control of adult (structural) plasticity. Furthermore, the overlap between MICAL, plexin and neuropilin distribution patterns supports a role for vertebrate MICALs in semaphorin signaling.