Semaphorins: Green Light for Redox Signaling?

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During neuronal development, extending axons are guided to their targets by a combination of repelling and attracting cues (*1*). These include members of the semaphorin family of ligands and their receptors, plexins and neuropilins (*2*, *3*). Semaphorins were initially identified for their ability to induce axon steering and collapse of the growth cone in vitro, a process that requires local rearrangement of the actin cytoskeleton (*4*, *5*). Although in the past decade we have learned a great deal about the biology of semaphorins and their receptors, the exact mechanisms through which they signal and their downstream effectors remain largely unknown. Terman and colleagues report the identification of a family of plexin-interacting cytoplasmic proteins with an unusual domain organization that suggests a role for redox regulation in axon guidance (*6*).

Semaphorins represent a large family of proteins (more than 30 members, subdivided into seven classes), which are highly conserved in both invertebrates and vertebrates. An additional class is found in some nonneurotropic DNA viruses. Based on their biochemical properties, semaphorins can also be classified as secreted, transmembrane, or glycophosphatidylinositol (GPI)-anchored proteins (*7*).

Semaphorins were originally identified as chemorepellents. However, it is now becoming clear that semaphorins serve more complex biological functions than just chemorepulsion and that individual members have different biological specificities (*2*). For example, Sema3A can attract cortical apical dendrites (*8*), whereas Sema3C behaves as a chemorepellent for sympathetic neurons and a chemoattractant for cortical neurons (*9*). Functions outside the nervous system have also been described: Sema4D (CD100) modulates the function of B and T lymphocytes (*10*, *11*) and secreted semaphorins encoded by the genome of some DNA viruses appear to play a role in allowing viral escape from the host immune system. Semaphorins may also play a role in controlling invasive growth (*12*, *13*) and in the development of the cardiovascular system (*2*).

Two families of semaphorin receptors have been identified: plexins and neuropilins. Plexins comprise nine proteins divided into four classes (A through D). They are transmembrane proteins, and their cytoplasmic tails are required for signal transduction (*14*). Transmembrane and GPI-anchored semaphorins signal through direct binding to plexins, whereas neuropilins serve as coreceptors for the interaction between plexins and secreted semaphorins. The most remarkable consequence of semaphorin signaling is the local rearrangement of the cytoskeleton. How plexins signal to the cytoskeleton remains, however, unknown. Plexins lack any detectable enzymatic activity and likely function by recruiting effector proteins in response to ligand-induced conformational changes. Strong evidence implicates small guanosine triphosphatases (GTPases) of the Rho and Rac families as downstream effectors (*3*). Both GTPases regulate the cytoskeleton in neurons, and genetic studies in *Drosophila* have revealed that Rho activation is required for semaphorin signaling [reviewed in (*15*)]. Furthermore, members of the B class of plexins bind PDZ-Rho GEF, a guanine exchange factor for RhoA (*16*), and inhibit Rac signaling. Although plexins are phosphorylated on tyrosine residues, the responsible kinase(s) have not yet been identified, nor it is known whether phosphorylation is required for growth cone collapse. A putative receptor tyrosine kinase protein named OffTrack (OTK) interacts with PlexA, and genetic evidence suggests that it could participate in Sema1A-PlexA signaling (*17*). Finally, members of a family of putative intracellular signal transducers, called CRMPs (collapsin response mediator proteins), which are expressed exclusively in the developing nervous system, have been implicated in semaphorin-induced growth cone collapse (*18*).

Complexity is increased by the fact that different semaphorin-plexin combinations signal through different effectors, and it is likely that the same semaphorin can activate different intracellular pathways, depending on the cellular context, as suggested, for example, by the bifunctional activity of Sema3C.

The paper from Kolodkin's group (*6*) adds new players to the scene and suggests a previously unknown signaling mechanism. In a yeast two-hybrid screen for interacting partners of PlexA, the authors have identified a large cytosolic protein called MICAL [the vertebrate homolog had been previously cloned by Suzuki *et al*. as a partner of CasL, a protein that localizes at focal adhesions and stress fibers (*19*)]. In an elegant series of genetic and biochemical experiments, Terman *et al*. provide evidence that MICAL interacts with semaphorin and PlexA and is required for normal development of *Drosophila* motor neurons (*6*).

MICAL is a member of a family of large cytosolic proteins conserved in organisms ranging from flies to mammals. In addition to a number of protein-protein interaction domains (a coiled-coil motif, a calponin homology domain, a LIM domain, and a proline-rich region), MICALs are characterized by a highly conserved NH_2-terminal flavoprotein monoxygenase domain of about 500 amino acids. Flavoprotein monoxygensase are oxidoreductases that use the flavin adenin dinucleotide (FAD) as a cofactor to insert one atom of molecular oxygen into their substrates (*20*). In some contexts, these enzymes can act as oxidases and generate reactive oxygen species (ROS). Indeed, the purified NH_2-terminal domain of MICAL binds FAD, and this binding was required for semaphorin signaling (*6*). Although Terman *et al.* did not formally prove that MICAL functions as a monoxygenase, they showed that pharmacological inhibitors of flavoprotein monoxygenases suppress semaphorin signaling in an in vitro growth cone repulsion assay. Although further work is needed to clarify the role of MICALs in semaphorin signaling, these experiments strongly suggest that redox modifications play an important role in axon guidance, which may have important implications for human diseases.

ROS have been regarded as toxic molecules because of their great reactivity and ability to damage cellular macromolecules, such as DNA, lipids, and proteins. During the past decade, however, we have begun to recognize that controlled production of ROS and regulated redox modifications of transcription factors or enzymes are an essential part of signal transduction pathways (*21*, *22*).

Among free radicals, nitric oxide (NO) was the first to be

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recognized as a second messenger (*23*). It is generated by nitric oxide synthases (NOSs), which oxidize L-arginine to produce L-citrulline and NO. NO binds and activates guanylate cyclase, thereby inducing intracellular elevation of guanosine 3′,5′ monophosphate (cGMP) and activation of a number of targets, such as cGMP-dependent protein kinase, cGMP-gated ion channels, and cGMP-phosphodiesterases. NO can induce a number of biological responses, including the relaxation of smooth muscle cells, inhibition of platelet aggregation, and regulation of the immune system [reviewed in (*24-26*)].

Alternatively, ROS can directly oxidize specific amino acid residues of target proteins and modulate their function. For example, after treatment with epidermal growth factor, platelet-derived growth factor (*27*), or nerve growth factor (*28*), cells produce a burst of ROS that oxidizes a highly reactive cysteine residue in the catalytic site of a number of protein tyrosine phosphatases, resulting in their inactivation and thus allowing a transient peak of tyrosine kinase activity (*29*). The fact that ROS production plays a role in signaling by growth factors was confirmed by the observation that pretreatment with antioxidants or overexpression of a ROS-scavenging enzyme blocks tyrosine phosphorylation and growth factor signaling (*27*, *28*).

In addition, ROS have been implicated in the mechanisms involved in the release of mitochondrial proteins during p53-dependent apoptosis. The tumor suppressor p53 induces transcriptional activation of redox-related genes and stimulates a sustained rise in ROS levels, possibly involved in opening of the mitochondrial pore and activation of the mitochondrial permeability transition (*30*). Indeed, antioxidant treatment prevents apoptosis. The idea that signal transduction pathways can finely tune ROS levels is confirmed by studies of the adaptor protein p66Shc. Mouse embryo fibroblasts derived from p66Shc null mice have significantly reduced levels of intracellular ROS and increased resistance to oxidative stress-induced apoptosis (*31-33*).

Variations of intracellular ROS might also affect gene-specific transcription. In prokaryotes, the transcription factor OxyR is a stress-responsive transcription factor whose affinity for target promoters depends on the specific redox modification of a single cysteine residue (*34*, *35*). Changes in the cell's redox status are reflected by changes in the ratio of reduced cofactors to oxidized cofactors, such as nicotinamide adeninine dinucleotide (NADH), nicotinamide adenine dinucleotide diphosphate (NADPH), and flavin adenine dinucleotide (FADH). In eukaryotes, upon binding to NADH, the corepressor CtBP undergoes a conformational change that results in increased affinity for a number of binding partners and transcriptional repression of several target genes (*36*). CtBP, therefore, might function as a redox-sensitive transcriptional regulator. The AP-1 transcription is another well-known example of a redox-regulated transcription factor. AP-1 activity requires that two critical cysteine residues in its DNA binding domain be in a reduced form. Various types of oxidative stress activate the nuclear redox factor Ref-1, which in turn reduces AP-1, hence triggering its ability to activate gene-specific transcription (*37*, *38*). A similar mechanism has been demonstrated for nuclear factor-κB (NF-κB) (*21*).

As for any relevant discovery, new questions always exceed resolved issues: Does MICAL act by modifying the global or local intracellular concentration of ROS? Does it modify other proteins? Is it a general effector of all semaphorin-plexin signaling, or does it play a role only in a subset of them? How do MICAL activity and localization change in response to ligand-induced plexin activation?

Actin and small GTPases of the Rho and Rac families (or their respective GEFs) are good candidate substrates for the redox effect of MICAL. The collapse of the growth cone induced by semaphorins requires localized depolymerization of actin, and actin's oxidation leads to disassembly of actin filaments and reduces the ability of actin monomers to form polymers. MICAL had been previously been identified as an interactor with vimentin and CasL, both of which are involved in cytoskeleton regulation (*19*). Rho and Rac family members are important downstream effectors of semaphorins, and there is also a precedent for redox regulation of a small GTPase (*39*).

One can speculate that MICALs, through their many protein-protein interaction domains, may act as platforms to recruit downstream effectors to the site of plexin activation. The activity of these effectors could then be selectively modulated by redox modifications of key amino acid residues that could either be the direct effect of MICAL's monoxygenase activity or be the indirect consequence of a local increase in ROS (Fig. 1).

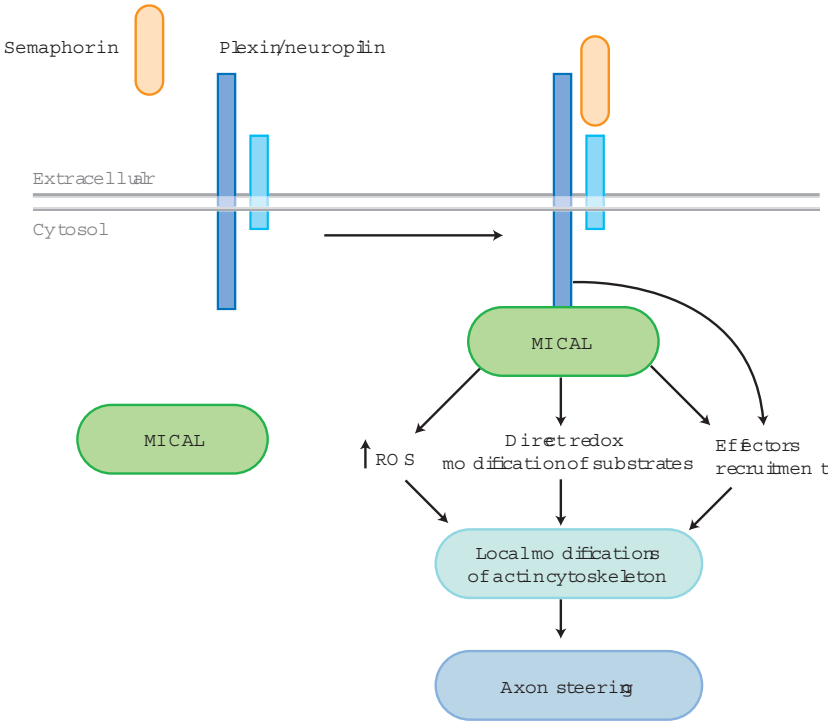

Fig. 1. How MICALs could signal plexin-mediated responses. A schematic representation of how members of the MICAL family of putative flavoprotein monoxygenases could signal to the cytoskeleton is shown. MICALs could serve (i) as a platform to recruit effector proteins; (ii) to increase the local concentration of ROS, whereby ROS would act as a second messenger; and (iii) to introduce specific redox modifications into downstream effectors through direct enzymatic oxidation.

In addition to the relevance for our understanding of neuronal development, Terman's paper has important implications for human diseases. At sites of spinal cord traumatic lesions, there are increased intracellular concentrations of ROS and other oxidants, which result in inhibition of axonal growth. In principle, interfering with the redox signaling by MICALs through the use of flavoprotein monoxygenase inhibitors could facilitate axonal regeneration by preventing growth cone collapse.

Semaphorin-plexin signaling also occurs outside the nervous system. Of particular interest is its role in immune regulation and cancer invasion and metastasis. If MICALs prove to be important in these contexts as well, they could become relevant targets for novel immunomodulating and anticancer drugs.

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