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Review

Structural mechanisms of plexin signaling

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ABSTRACT

Signaling through plexin, the major cell surface receptor for semaphorin, plays critical roles in regulating processes such as neuronal axon guidance, angiogenesis and immune response. Plexin is normally kept inactive in the absence of semaphorin. Upon binding of semaphorin to the extracellular region, plexin is activated and transduces signal to the inside of the cell through its cytoplasmic region. The GTPase Activating Protein (GAP) domain in the plexin cytoplasmic region mediates the major intracellular signaling pathway. The substrate specificity and regulation mechanisms of the GAP domain have only been revealed recently. Many intracellular proteins serve as either upstream regulators or downstream transducers by directly interacting with plexin. The mechanisms of action for some of these proteins also start to emerge from recent studies. We review here these advances in the mechanistic understanding of plexin intracellular signaling from a structural perspective.

Contents

1. Introduction

Plexins are the major cell surface receptors for the axon guidance proteins semaphorins (Tamagnone et al., 1999; Winberg et al., 1998). The over 20 semaphorins are divided according to sequence conservation into eight classes (Kolodkin et al., 1993; Semaphorin Nomenclature Committee, 1999). Some semaphorins are secreted proteins, while others are cell surface attached through a transmembrane region or a glycosylphosphatidylinositol-linker (Semaphorin Nomenclature Committee, 1999). Invertebrates have two plexins (Plexins A and B), whereas the nine vertebrate plexins are organized into four classes (A, B, C and D) (Tamagnone et al., 1999). Some class A plexins require the co-receptor neuropilin (neuropilin 1 or 2) to form holo-receptors for secreted class III semaphorins (Takahashi et al., 1999).

Signals through semaphorin/plexin play essential roles in many aspects of the development of the nervous system, including axon guidance, fasciculation, branching and synapse formation

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(reviewed in Kruger et al., 2005; Tran et al., 2007). Some plexin family members are expressed in adult tissues, playing roles in controlling tissue homeostasis and regeneration after injury to the nervous system (Shim et al., 2012). While in general the semaphorin/plexin signal is repulsive in axon guidance, it can be attractive under some circumstances (reviewed in Kruger et al., 2005; Tran et al., 2007). In addition to their roles in the nervous system, plexins are involved in regulating angiogenesis and cardiovascular development (reviewed in Gu and Giraudo, 2013; Sakurai et al., 2012). Other functions of plexins include regulation of immunity and bone homeostasis (reviewed in Kang and Kumanogoh, 2013; Takamatsu and Kumanogoh, 2012). Genetic knockout of plexins or semaphorins are often embryonically lethal, causing severe defects in the development of the nervous and cardiovascular systems (reviewed in Worzfeld and Offermanns, 2014). Malfunction of the plexin pathway has been implicated in human diseases, including neurological disorder and cancer (reviewed in Gu and Giraudo, 2013; Sakurai et al., 2012; Tamagnone, 2012; Worzfeld and Offermanns, 2014).

In the past few years, the understanding of the mechanisms governing the regulation and signaling of plexin has grown tremendously (reviewed in Jones, 2015). This progress is owned largely to insights from structural studies of both the extracellular and intracellular regions of several plexin family members in various states. The N-terminal Sema domain in the plexin extracellular region binds semaphorin, on it's own or together with the extracellular region of the neuropilin co-receptor (Janssen et al., 2012, 2010; Liu et al., 2010; Nogi et al., 2010). The cytoplasmic region of neuropilin is short (~30 residues) and appears non-essential for semaphorin signaling (Nakamura et al., 1998). The activating signal initiated by semaphorin binding propagates through the multiple membrane proximal domains and the transmembrane helix to the cytoplasmic region of plexin. The cytoplasmic region is responsible for triggering intracellular signaling cascades, which ultimately lead to a variety of cellular responses that underlie the biological functions of plexin. Therefore, the cytoplasmic region of plexin and proteins associated with it have been subjected to extensive investigations to elucidate the signaling mechanisms of plexin. These aspects of plexin signaling will be the focus of this review.

2. Overall architecture and signaling mechanisms of the plexin cytoplasmic domain

The ~600-residue cytoplasmic regions of the plexin family members are highly conserved and share a common architecture. It was discovered over a decade ago that this region contains two segments (C1 and C2) that show sequence similarity to GTPase Activating Proteins (GAPs) for Ras (Fig. 1A) (Hu et al., 2001; Rohm et al., 2000). The two segments are interrupted by an insertion region. Despite this interruption, structural studies have shown that the two GAP-homology segments fold together into one intact GAP domain, which indeed structurally resembles RasGAPs such as p120GAP and neurofibromin (Fig. 1B) (He et al., 2009; Tong et al., 2009). There are two conserved arginine residues in the plexin GAP domain, one in each of the two segments, corresponding to the two catalytically essential arginine residues in RasGAPs (Fig. 1). Mutating these arginine residues abolishes plexin activity both in vitro and in vivo, demonstrating the essential role of the GAP domain in plexin function (Rohm et al., 2000; Worzfeld et al., 2014).

The identification of the GAP domain in plexins was of particular significance for establishing the signaling mechanisms underlying plexin function. Small GTPases such as Ras and Rac1 are master regulators of many fundamental cellular processes such as proliferation and cytoskeletal dynamics. They act as molecular switches, cycling between the GDP-bound inactive and GTP-bound active states. GAPs turn off GTPases by accelerating hydrolysis of the bound GTP to GDP, whereas guanine nucleotide exchange factors (GEFs) activate them by promoting the exchange of GDP for GTP. The presence of the GAP domain makes it possible for plexins to directly control the activity of small GTPases, consistent with their roles in regulating cell morphology and migration. To date, plexins remain unique as the only group of cell surface receptors known to contain a GAP domain.

The ~200-residue insertion segment between the two GAP homology regions forms an independent domain that packs against one side of the GAP domain (Fig. 1B) (He et al., 2009; Tong et al., 2007, 2009). This domain interacts with Rho family small GTPases such as Rac1, RND1 and RhoD, and is therefore referred to as the RhoGTPase Binding Domain (RBD) (Driessens et al., 2001; Hu et al., 2001; Oinuma et al., 2003; Rohm et al., 2000; Tong et al., 2007; Turner et al., 2004; Vikis et al., 2002, 2000; Zanata et al., 2002). The RBD/RhoGTPase interaction plays a regulatory role in plexin signaling. In addition to the GAP domain and the RBD, the crystal structures also revealed a juxtamembrane segment at the N-terminus of the plexin cytoplasmic region (Fig. 1), which is an essential regulatory element (He et al., 2009; Wang et al., 2013).

Over 20 proteins have been reported to interact with the cytoplasmic region of plexins and contribute to signaling. Some plexin family members contain unique protein-interaction sites that mediate member-specific signaling pathways. For example, Class B plexins (PlexinB1, B2 and B3) specifically interact with two related GEFs, PDZ−RhoGEF and LARG (leukemia-associated RhoGEF) (Fig. 1B). The interaction is mediated by a conserved C-terminal "VTDL" motif in class B plexins and the PDZ (PSD-95, Dlg-1 and ZO-1) domains in PDZ−RhoGEF and LARG (Aurandt et al., 2002; Driessens et al., 2002; Hirotani et al., 2002; Oinuma et al., 2003; Perrot et al., 2002; Swiercz et al., 2002). PDZ−RhoGEF and LARG recruited by Class B plexins activate RhoA, mediating an important branch of the signaling pathway (Worzfeld et al., 2014). Class A plexins interact with two other highly related GEFs FARP1 (FERM, RhoGEF, and pleckstrin homology domain protein 1) and FARP2 (Fig. 1B) (Toyofuku et al., 2005; Zhuang et al., 2009). FARP1 and FARP2 contribute to plexin-mediated dendrite growth and axonal repulsion, respectively. FARP2 has also been implicated in osteoprotection by Semaphorin3A/PlexinA1 (Hayashi et al., 2012). FARPs are normally autoinhibited by adopting a closed conformation that blocks the GEF active site (He et al., 2013). The mechanism by which plexin binds FARPs and regulates their activity is not clear at present.

For comprehensive discussions on the interactions between plexins and their intracellular binding partners, please refer to recent review articles (Gay et al., 2011; Hota and Buck, 2012). Here we will focus on the GAP domain and the RBD of plexins, because they constitute the core signaling and regulatory components, and their mechanisms of action are better understood in light of recent structural studies.

3. Substrate specificity of the plexin GAP domain

Since the discovery of the GAP domain in plexin over a decade ago, many studies have been devoted to identifying its small GTPase substrate and experimentally demonstrating its catalytic activity (reviewed in Kruger et al., 2005). The two studies that originally identified the GAP domain did not report GAP activity to any small GTPase (Hu et al., 2001; Rohm et al., 2000). Later, a study showed that PlexinB1 is active specifically to the Ras homolog R-Ras but not Ras (Oinuma et al., 2004). This study further showed that plexin signaling is critically dependent on inactivation of R-Ras. The GAP activity, assessed by the levels of GTP-bound R-Ras in cells