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SYD-1 promotes multiple developmental steps leading to neuronal connectivity

Yan Xu¹ and Christopher C. Quinn^{1,*}

¹Department of Biological Sciences, University of Wisconsin-Milwaukee; Milwaukee, WI, 53201, U.S.A.

Abstract

The establishment of neuronal connectivity requires precise orchestration of multiple developmental steps, including axon specification, axon guidance, selection of synaptic target sites, and development of synaptic specializations. Although these are separate developmental steps, evidence indicates that some of the signaling molecules that regulate these steps are shared. In this review, we focus on SYD-1, a RhoGAP-like protein that has been implicated in each step of axonal development. We discuss interactions between SYD-1, UNC-40(DCC) and RhoGTPases and highlight both similarities and differences in how SYD-1 functions to regulate the different steps of axonal development. These observations reveal an example of how a signaling protein can be repurposed across sequential developmental steps.

Introduction

Axon specification, navigation and synaptogenesis are key steps in neuronal development. Failures in any of these steps can underlie developmental disorders of the nervous system such as epilepsy, dyslexia, autism and schizophrenia [1-3]. In fact, many of the genes that have been implicated in the development of axonal connections have also been linked to these disorders. Therefore, fundamental knowledge of the genetic pathways that control the development of axonal connections may lead to future advances in understanding, diagnosing and treating neurodevelopmental disorders. Axonal connections are established through a series of developmental steps. Following birth, the neuronal cell body migrates to its final location and begins to extend processes. One of these processes is specified as the axon and undergoes long-range migration to its target region. Upon arriving at its target region, the axon must innervate the correct layer and begin the process of synaptogenesis. To understand how the multiple steps of axonal development are controlled and coordinated, it is important to learn about the function of single signaling proteins that participate in multiple developmental steps.

Here, we discuss research implicating the synapse defective protein-1 SYD-1 (mSYD1A, dSyd-1) in multiple steps of axonal development including axonal specification, axon navigation, target site selection and synaptogenesis. SYD-1 is a phylogenetically conserved

*To whom correspondence should be addressed at: Department Biological Sciences, 3209 N. Maryland Avenue, Milwaukee, WI 53211, quinncc@uwm.edu, Phone: (414)229-2602, Fax: (414)229-3926.

protein that has been studied in *C. elegans*, *Drosophila* and mice (Figure 1). A long form of SYD-1 that contains a PDZ domain, a C2 domain and a Rho GAP-like domain is expressed in *C. elegans* and *Drosophila*. The PDZ domain of SYD-1 is essential for its role in both synaptogenesis and axonal specification [4,5]. The Rho GAP-like domain of SYD-1 can bind to activated MIG-2 (Rac) GTPase and is required for SYD-1 function in axon guidance [4,6-8]. In *C. elegans* and mice, a short form of SYD-1 is also expressed, which is missing the PDZ domain. In mice, the PDZ domain is replaced by an N-terminal Intrinsically Disordered (ID) domain that can mediate interactions with multiple binding partners [7]. In this review, we discuss the different roles of SYD-1 in each step of axonal development and consider how this signaling protein can be repurposed across multiple developmental steps. We consider data from *C. elegans*, *Drosophila* and mice to arrive at an understanding of the similarities and differences in the function of SYD-1 in these different developmental steps.

SYD-1 promotes acquisition of neuronal polarity

Neurons are polarized, such that a single process functions as an axon to transmit signals and other processes function as dendrites to receive signals. This polarization depends on the segregation of specialized presynaptic components to axons and specialized postsynaptic components to dendrites. The polarity of neurons is determined early in neuronal development when a single process is specified as an axon, with other processes becoming dendrites. In *C. elegans* *syd-1* mutants, presynaptic specializations are located in both axons and dendrites, indicating that SYD-1 is required for the establishment of neuronal polarity [4]. The mechanism that allows SYD-1 to specify axonal identity is unknown. However, Rac GTPases have been extensively implicated in the establishment of neuronal polarity [9]. Moreover, as discussed below, SYD-1 can regulate Rac GTPase activity within other contexts [6,8]. Therefore, SYD-1 is likely to promote axonal specification by regulating RacGTPase activity.

SYD-1 functions with UNC-40 (DCC) and SAX-3 (Robo) to promote axon guidance

Following the specification of axonal identity, the axon must grow and migrate towards its synaptic targets. The growing axon is guided by extracellular guidance cues, such as UNC-6 (netrin) and SLT-1 (slit) that activate the UNC-40 (DCC) and SAX-3 (Robo) receptors, respectively. Activation of these receptors is thought to recruit intracellular signaling components, leading to directional responses that cause the axon to grow towards or away from the source of guidance cue. The signaling pathways that mediate these directional responses are not well understood, and are an area of active investigation.

The UNC-40 and SAX-3 guidance receptors can function in parallel to each other to mediate axon guidance in *C. elegans*. However, evidence indicates that UNC-40 and SAX-3 can also function in combination with each other, through a mechanism that is likely to involve direct binding between these receptors [10-14,8]. Recent research has suggested that SYD-1C, a short form of SYD-1 that lacks the PDZ domain, can mediate this combinatorial signaling between UNC-40 and SAX-3 [8]. SYD-1C binds to the cytoplasmic domains of both UNC-40 and SAX-3 and genetic evidence indicates that SYD-1C mediates signaling that requires both UNC-40 and SAX-3. The RhoGAP-like domain of SYD-1C binds to the activated MIG-2 (Rac) GTPase and can negatively regulate its activity to promote axon

guidance. The role of MIG-2 in promoting axon guidance involves the regulation of the WAVE complex, which can promote the f-actin nucleating activity of the ARP2/3 complex [15]. Thus, SYD-1 may promote axon guidance by negatively regulating actin polymerization in the growth cone.

One unresolved question involves the relationship between SYD-1C and the MIG-2 GTPase. Although SYD-1C contains a RhoGAP-like domain, a GAP activity has not yet been detected for SYD-1 and key residues in the SYD-1 GAP-like domain are not conserved, suggesting that SYD-1 may not possess GAP activity on its own [16,17,8]. One possibility is that SYD-1 may bind to activated MIG-2 and recruit a second protein that does have GAP activity. Alternatively, it remains possible that SYD-1 could have its own GAP activity.

SYD-1 promotes selection of synaptic target sites

Once axons reach their target regions, they must extend to the correct layer and elaborate presynaptic boutons. For example, in *Drosophila*, the R7 photoreceptor neurons project axons into the medulla, which has ten layers M1-M10. Once the axons arrive in the medulla, they travel past layers M1-M5 and form synaptic connections within layer M6 [18]. In *Drosophila Dsyd-1* mutants, the R7 axons initially reach the M6 layer, but later retract and terminate in layers M3-M5 [6]. In addition to this early defect, *Dsyd-1* mutants also exhibit a late defect, where axons that do reach the M6 layer, extend processes that reach beyond the M6 layer. Genetic evidence suggests that the primary function for *Dsyd-1* in R7 photoreceptor axon targeting is to promote Trio activity. Trio is a Guanine Nucleotide Exchange Factor (GEF), which can guide photoreceptor axons by activating the Rac GTPases [19-21]. These GTPases bind directly to the P21 activated Kinase (Pak) and cause a conformational shift that promotes its kinase activity. The kinase activity of Pak is thought to regulate actin polymerization in the growth cone of the photoreceptor axons, thereby influencing target site selection.

SYD-1 promotes synaptic maturation and transynaptic signaling

Following the initial selection of synaptic sites, presynaptic structures mature through the recruitment and assembly of specialized presynaptic proteins. For example, in *C. elegans* HSN axons, the SYG-1 transmembrane protein localizes to the nascent presynaptic site and interacts with the WAVE complex, leading to the formation of an F-actin network [22,23]. Next, the adaptor protein NAB-1 (neurabin) associates with the F-actin network and recruits key scaffolding components including SYD-1, SYD-2 and ELKS [24,25]. These scaffolding components are thought to recruit additional specialized presynaptic proteins, leading to maturation of the pre synaptic compartment [25,5].

SYD-1 can also promote presynaptic maturation in the AIY neuron of *C. elegans*. However, in this neuron, SYD-1 accumulation is instructed by UNC-40, rather than SYG-1 [26,27]. In response to UNC-6 (Netrin), UNC-40 localizes to presynaptic regions in the AIY neuron and recruits SYD-1 and SYD-2, which function upstream of MIG-10 (lamellipodin), a protein that can promote actin polymerization through an interaction with ABI-1 and the WAVE complex [28-34]. In addition, a second pathway can function in parallel to SYD-1 and SYD-2 to promote presynaptic maturation in the AIY axon. This second pathway includes

UNC-40, the CED-5 Guanine nucleotide exchange factor (GEF), the CED-10 GTPase and MIG-10. In this pathway, CED-5 activates the CED-10 GTPase, which functions upstream of MIG-10. Thus, UNC-40 may cause localized F-actin polymerization in the AIY presynaptic regions in a manner similar to SYG-1 at the HSN presynaptic regions.

In the *Drosophila* neuromuscular junction, the homologs of the active zone proteins SYD-1, SYD-2 and ELKS (Dsyd-1, Liprin- α and Bruchpilot, respectively) are also involved in presynaptic maturation [35]. In addition, Dsyd-1 functions with the transsynaptic Neurexin-Neurologin complex to coordinate presynaptic and postsynaptic maturation [36]. Dsyd-1 accumulates early in the nascent presynaptic compartment and its PDZ domain binds to Neurexin. Neurexin interacts with Neurologin, a protein that is located in the postsynaptic compartment. The interaction between Neurexin and Neurologin stabilizes the nascent Dsyd-1/Liprin- α complexes, promoting maturation of both the pre and postsynaptic complexes.

The mammalian homolog of SYD-1, mSYD1A, is required for presynaptic assembly and transsynaptic signaling in cultured mouse neurons [7]. Unlike its invertebrate homologs, mSYD1A has an active GAP domain that is specific for the RhoA GTPase. However, the mSYD1A GAP domain is not required for promoting presynaptic assembly, but rather may participate in transsynaptic signaling to promote postsynaptic development. Interestingly, mSYD1A has an N-terminal Intrinsically Disordered Domain (ID domain) that is necessary and sufficient to promote presynaptic assembly. The ID domain can physically interact with Liprin- α (SYD-2) and Munc-18, key active zone components required for the development and function of synapses.

Differing roles of SYD-1 in the regulation of Rac GTPases

One intriguing aspect of SYD-1 function is that in different developmental steps it can have different effects on the activity of the Rac GTPases. For example, during axon guidance SYD-1 can negatively regulate Rac. However, during synaptic target site selection, Dsyd-1 can positively regulate Rac (Figure 2a) We speculate that these opposite roles of SYD-1 could be enabled by different binding partners at each of these developmental steps (Figure 2b-c). For example, during axon guidance, SYD-1 can negatively regulate Rac [8]. Although the molecular mechanism accounting for this negative regulation is unknown, we hypothesize that SYD-1 can negatively regulate Rac by recruiting it to a complex that also includes GAP proteins (Figure 2b). Consistent with this idea, SYD-1 can bind to both activated Rac and the cytoplasmic tail of SAX-3 [8]. Based on work in other organisms [37-40], we speculate that SAX-3 may interact with GAP proteins. Therefore, the interaction between SYD-1 and SAX-3 could expose activated Rac to negative regulation by GAP proteins.

In contrast to its role during axon guidance, Dsyd-1 is thought to positively regulate Rac by promoting the GEF activity of Trio during synaptic target site selection [6]. Whereas the molecular mechanism accounting for this positive regulation of Trio is unknown, we hypothesize that Dsyd-1 could activate Trio by recruiting Rac to a complex that includes Trio. For example, The LAR receptor protein tyrosine phosphatase binds to Trio and to a complex of proteins that includes Liprin- α , Bruchpilot (BRP) and Dsyd-1 (Figure 2C).

Thus, Dsyd-1 could facilitate Trio activity by bringing Trio and its substrate, Rac, into a complex.

Actin polymerization may be a common downstream target of UNC-40 and SYD-1 in both axon guidance and synaptogenesis

Localized accumulation of f-actin is a key feature of both axon guidance and synaptogenesis. For example, asymmetric accumulation of f-actin is thought to provide a directional force that promotes growth cone turning in response to extracellular guidance cues [41-46]. Likewise, f-actin also accumulates at the nascent synapse and is thought to promote the recruitment of proteins that promote synaptogenesis [22,24,47]. The mechanisms that regulate the accumulation of f-actin in axon guidance and synaptogenesis are not well understood, but are likely to involve both UNC-40 and SYD-1. In axon guidance, genetic and biochemical studies have identified interactions between UNC-40 and SYD-1 with Rac, a regulator of actin polymerization [48-50,8]. In synaptogenesis, genetic studies have indicated that UNC-40 and SYD-1 function upstream of MIG-10 [27,51], a regulator of actin polymerization. In both synaptogenesis and axon guidance, the mechanism for f-actin accumulation is thought to involve localization of UNC-40 to the site of actin accumulation. For example, in axon guidance UNC-40 localizes to the leading edge of the HSN neuron, closest to the source of attractive guidance cue [28]. In synaptogenesis, UNC-40 localizes to the site of synapse formation [27,51]. Imaging studies have revealed a strong association between UNC-40 localization and sites of f-actin accumulation, suggesting that the localized accumulation of UNC-40 is likely to promote localized accumulation of f-actin [52]. Thus, localized accumulation of UNC-40 is thought to drive the localized accumulation of f-actin, which promotes both axon guidance and synaptogenesis.

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Figure 1. SYD-1 is a phylogenetically conserved protein that includes PDZ, C2 and RhoGAP-like domains

The SYD-1 family of proteins have been studied in *Drosophila* (Dsyd-1), *C. elegans* (SYD-1B and SYD-1C), and mouse (mSYD1A). *Drosophila* Dsyd-1 and *C. elegans* SYD-1B include PDZ, C2 and RhoGAP-like domains. However, these proteins are missing residues that are thought to be important for RhoGAP activity and RhoGAP activity has not yet been observed from these proteins. Mice express mSYD1A, a short form of SYD-1 that is missing the PDZ domain, but includes an N-terminal Intrinsically Disordered Domain (IDD). This IDD domain can bind to multiple partners that are involved in synaptic development and function. Unlike its homologs in *Drosophila* and *C. elegans*, mouse SYD1A possesses a GAP activity for RhoA. *C. elegans* also expresses a short form of SYD-1, SYD-1C that does not include the PDZ domain. Although SYD-1C is predicted to contain an N-terminal IDD domain, this domain has not yet been experimentally verified and is not conserved relative to the IDD domain of mouse mSYD1A.

Figure 2. Model for positive and negative regulation of Rac by SYD-1

(a) Relationships between SYD-1 and Rac as inferred from genetic interactions in axon guidance and synaptic targeting. (b-c) Hypothetical models of molecular interactions that can explain the relationships between SYD-1 and Rac in axon guidance and synaptic targeting. (b) In axon guidance, SYD-1 can negatively regulate Rac (MIG-2) [8]. We hypothesize that SYD-1 may recruit activated Rac to the UNC-40/SAX-3 receptor complex, exposing it to GAP activity. This hypothesis is suggested by the fact that SYD-1 can bind to both the cytoplasmic tail of SAX-3 and to activated Rac [8]. Moreover, the *Drosophila* homolog of SAX-3 can bind to srGAP and CrossGAP [37-40]. Thus, SYD-1 could recruit Rac to the UNC-40/SAX-3 complex, which may contain GAP proteins, thus leading to the negative regulation of Rac. (c) During synaptic target site selection, Dsyd-1 can promote Trio activity, which would cause activation of Rac [6]. We hypothesize that Syd-1 can promote Trio activity by recruiting its substrate, Rac, into a complex with Trio. This hypothesis is supported by the fact that the LAR receptor protein tyrosine phosphatase can bind to Trio and to a complex that includes Liprin- α , Bruchpilot (BRP), and Dsyd-1.