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# Functional dissection of SYG-1 and SYG-2, cell adhesion molecules required for selective synaptogenesis in *C. elegans*

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ABSTRACT

Cell adhesion molecules of the Immunoglobulin superfamily (IgCAMs) play diverse functions during neural development. Previously, we have identified SYG-1/Neph1 and SYG-2/Nephrin, IgCAMs necessary for synaptic specificity in *Caenorhabditis elegans*. Here, we conduct an *in vivo* structure-function analysis of SYG-1 and SYG-2 to identify domains of SYG-1 and SYG-2 necessary for heterophilic binding as well as synaptic specificity. We find the first Ig domain of SYG-1 and the first 5 Ig domains of SYG-2 are necessary and sufficient for their binding *in vivo*, as well as for synapse formation. We also find the SYG-2 cytoplasmic domain is required for SYG-2 subcellular trafficking, while the intracellular region of SYG-1 is required for synaptic function at earlier developmental stages, but is dispensable for later stages. This study defines the domain requirements for SYG-1/SYG-2 heterophilic binding and suggests that unknown SYG-1 extracellular interactors may play a role in SYG-1-mediated synaptic specificity.



## Introduction

The formation of neural circuits requires the coordination of multiple developmental events such as cell migration, axon and dendrite outgrowth and guidance, followed by target recognition and synapse assembly. Although it is has been documented that synaptic connections in the brain are precise and stereotyped, relatively little is known about the molecular mechanisms by which neurons select their correct synaptic partners to initiate synaptic assembly while rejecting other contacting cells in the same target field. It has been proposed that cell adhesion molecules found on pre-and postsynaptic cells may be used to mediate cell recognition and initiate synaptogenesis.

Recently, a number of cell adhesion molecules of the Immunoglobulin domain family (IgCAMs) have been implicated in synapse formation. SynCAMs, homophilic IgCAMs, have been shown to promote synaptogenesis *in vitro* (Biederer et al., 2002). Sidekicks, Ig domain containing proteins, have been implicated in the laminar choices within the inner plexiform layer of the vertebrate retina (Yamagata et al., 2002). Recently, another well recognized IgCAM, UNC-40/DCC, was shown to be enriched at presynaptic sites and to be essential for normal synaptogenesis in the AIY neuron of *Caenorhabditis elegans* (Colon-Ramos et al., 2007). In addition, we have previously shown that heterophilic interactions between SYG-1 and SYG-2, a pair of IgSF proteins, are required for specification of synapses in the HSNL neuron of *C. elegans* (Shen and Bargmann, 2003; Shen et al., 2004).

SYG-1, SYG-2, and their homologs have been demonstrated to be cell adhesion molecules that play diverse roles during development. In *Drosophila*, there are two SYG-1 homologs, Irregular chiasm C-roughest (IrreC-Rst) and Kin of IrreC/DumbFounded (Kirre or Duf), and two SYG-2 homologs, Sticks and Stones (Sns) and Hibris. IrreC-Rst and Hibris are required for proper patterning of the *Drosophila* eye (Ramos et al., 1993). During ommatidial development, heterophilic interaction between IrreC-Rst, expressed on the interommantidial precursor cells (IPCs) and Hibris, expressed on the primary pigment cells, is necessary for proper IPC cell sorting and remodeling of adhesive contacts, which then leads to apoptotic death of surplus IPCs (Bao and Cagan, 2005; Carthew, 2007). Additionally, heterophilic interactions between IrreC-Rst and Sns, as well as between Kirre and SNS have been shown to be important for *Drosophila* myoblast fusion. IrreC-Rst and Kirre are expressed on muscle founder cells while SNS and Hibris are expressed on fusion competent myoblasts. IrreC-Rst and Kirre act redundantly to bind SNS, while Hibris is thought modify SNS activity (Chen et al., 2007; Dworak and Sink, 2002). While weak homophilic interactions of IrreC-Rst and Kirre have been shown in cell culture, heterophilic interactions between SNS and IrreC-Rst as well as between SNS and Kirre are thought to be most important for myoblast fusion. Additionally, a zebrafish Kirre-like molecule has been shown to be required for myoblast fusion, suggesting that this pathway may be conserved in vertebrates (Srinivas et al., 2007).

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In vertebrates, Neph1/Kirrel1 and Nephrin, orthologs of SYG-1 and SYG-2 respectively, play essential roles in kidney development. There are three homologs of SYG-1 in vertebrates: Neph1/Kirrel1, Neph2/Kirrel3, and Neph3/Kirrel2, and a single homolog of SYG-2, nephrin (for simplicity, SYG-1 homologs will be called neph1, neph2 and neph3 in this paper). Neph1 and Nephrin have been implicated in glomerular slit diaphragm formation, the permeable membrane which allows for filtration of solutes in the kidney. In either humans with inherited mutations or mice with targeted deletions, loss of either Neph1 or Nephrin function leads to failure of glomerular slit membrane formation and lethal proteineuria (Donoviel et al., 2001; Kestila et al., 1998). In cell culture experiments it has been shown that both Neph1 and Nephrin exhibit homotypic as well as heterotypic interactions, but which of these interactions are of functional importance is unclear (Gerke et al., 2003; Khoshnoodi et al., 2003; Liu et al., 2003). In addition, Neph1 and Neph2 have been shown to be expressed at synaptic sites in the brain, and Neph1 and Neph2 physically associate with CASK, a synaptic scaffolding protein, suggesting that neph proteins may play a role in synapse formation in the vertebrate CNS (Gerke et al., 2006). In addition, Neph2 and

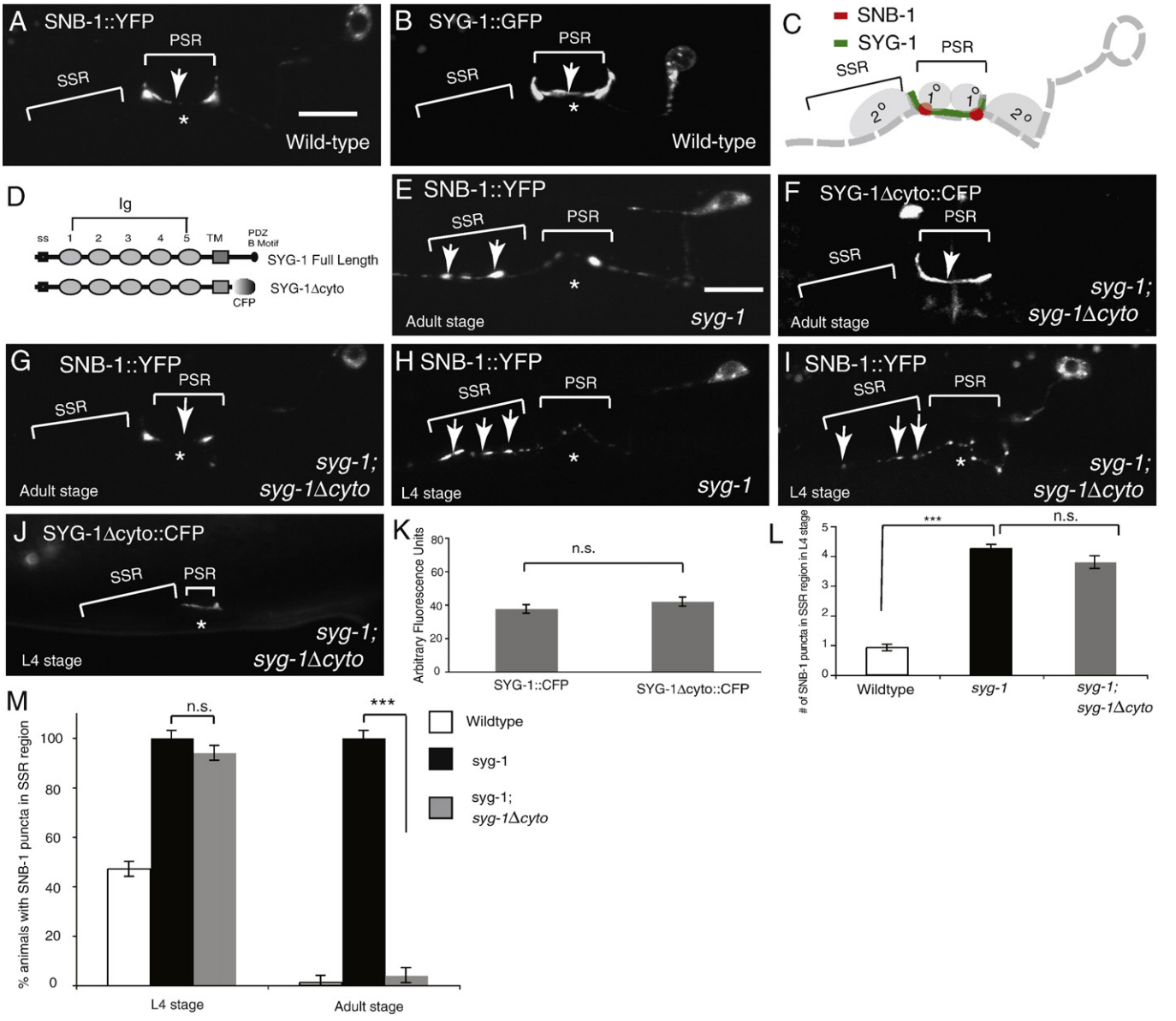


**Fig. 1.** The SYG-1 Extracellular domain is sufficient to rescue the *syg-1* phenotype in adults. (A) Representative wild-type animals expressing the synaptic vesicle marker SNB-1::YFP in HSNL. Asterisk marks position of the vulva. Note that SNB-1 expression (arrow) is concentrated around the vulva at the primary synaptic region (PSR) (bracket). Secondary synaptic region (SSR) is defined as region anterior to the vulva (bracket) Scale bar is 10 um. (B) Wild-type animal expressing SYG-1::GFP in HSNL. Note that SYG-1 is localized to the PSR (arrow). (C) Schematic of synaptobrevin and SYG-1 localization in HSNL. 1° and 2° vulval epithelial cells are shown in light grey. SYG-1 is localized by SYG-2 expressed in 1° epithelial cells. (D) Schematic of domain structure of SYG-1 and the SYG-1Δcyto construct. SYG-1 contains a signal sequence, 5 Ig domains, a transmembrane domain and a PDZ binding motif. SYG-1Δcyto is SYG-1 trunctated after the transmembrane domain and replaced with CFP. (E) Representative *syg-1* adult expressing SNB-1::YFP. Ectopic SNB-1 clusters are present anteriorly at the secondary synaptic region (SSR)(arrows). (F) Localization of SYG-1Δcyto::CFP construct. SYG-1Δcyto::CFP localizes to the vulva in an identical fashion as the full-length SYG-1 protein (arrow). (G) *syg-1* adult expressing the SYG-1Δcyto construct in HSNL. SNB-1 is no longer at the SSR but is localized at the vulva in the PSR, similar to the wild-type animals. (H) *syg-1* animal expressing SNB-1::YFP in the L4 stage. Ectopic SNB-1 clusters are found anteriorly in the SSR(arrows). (I) *syg-1* animal in the L4 stage expressing the SYG-1Δcyto construct in HSNL. Ectopic SNB-1 clusters are present anteriorly in the SSR at this stage (arrows). (J) *syg-1* animal expressing SYG-1Δcyto::CFP construct in L4 stage. SYG-1Δcyto::CFP is localized to the PSR in L4 stage (bracket). (K) Comparison of average intensity of signal in the PSR between SYG-1Δcyto::CFP and SYG-1::CFP in L4 stage. No statistically significant difference is observed. (L) Quantification of number of SSR puncta between wild-type, *syg-1*, and *syg-1* animals expressing the SYG-1Δcyto::CFP construct in the L4 stage. Error bars, standard error. $n>50$. $^{***}p<0.001$, student's t-test. (M) Quantification of the adult and L4 stage phenotypes. Error bars, standard error of proportion. $n>50$. $^{***}p<0.001$, Chi-squared test.