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The long and the short of SAD-1 kinase

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The Ser/Thr SAD kinases are evolutionarily conserved, critical regulators of neural development. Exciting findings in recent years have significantly advanced our understanding of the mechanism through which SAD kinases regulate neural development. Mammalian SAD-A and SAD-B, activated by a master kinase LKB1, regulate microtubule dynamics and polarize neurons. In *C. elegans*, the *sad-1* gene encodes two isoforms, namely the long and the short, which exhibit overlapping and yet distinct functions in neuronal polarity and synaptic organization. Surprisingly, our most recent findings in *C. elegans* revealed a SAD-1-independent LKB1 activity in neuronal polarity. We also found that the long SAD-1 isoform directly interacts with a STRADα pseudokinase, STRD-1, to regulate neuronal polarity and synaptic organization. We elaborate here a working model of SAD-1 in which the two isoforms dimer/oligomerize to form a functional complex, and STRD-1 clusters and localizes the SAD-1 complex to synapses. While the mechanistic difference between the vertebrate and invertebrate SAD kinases may be puzzling, a recent discovery of the functionally distinct SAD-B isoforms predicts that the difference likely arises from our incomplete understanding of the SAD kinase mechanism and may eventually be reconciled as the revelation continues.

Key words: SAD kinases, SAD-1, *C. elegans*, neural development, neuronal polarity, synapse, isoform

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The transformation of a nascent amorphous cell to a mature polarized neuron requires a concerted interplay of various factors. These include extracellular morphogens and growth factors providing cues; intracellular messengers relaying the information; and cytoskeletal networks effecting neurite extension and directional transport of axon- or dendrite-specific organelles.[1-5]

SAD Kinases Regulate Neural Development through Distinct Mechanisms

C. elegans SAD-1 kinase (Synapses of the Amphid Defective) was the first regulator of neuronal polarity identified in vivo.[6] It restricts the localization of synaptic vesicle proteins to axons and also organizes them at synapses.[6,7] Its mammalian counterparts, SAD-A and SAD-B, together also play essential roles in neuronal polarization.[8,9] SAD-A and SAD-B are activated by LKB1,[9] a tumour suppressor[10,11] and master kinase[12] implicated in various processes including cell cycle regulation[13] and cell polarity.[14,15] LKB1 functions in a complex with a STRAD pseudokinase that stabilizes,[16] activates[12] and properly localizes the kinase.[16,17] Activated SAD kinases phosphorylate a microtubule-associated protein, Tau, and modulate microtubule dynamics during neuronal polarization.[9] SAD-B also regulates neurotransmitter release.[18] SAD kinases therefore are conserved regulators of neural development and functions.

Notwithstanding their functional conservation, our recent study revealed an unexpected difference in the mechanism between the vertebrate and invertebrate SAD kinases.[19] Unlike the linear mammalian pathway of the LKB1-STRAD complex functioning through SAD kinases, in *C. elegans*, the sole LKB1 ortholog, PAR-4,[14,20] displayed SAD-1-independent activities in neuronal polarity. Instead, PAR-4 regulates neuronal polarity by activating

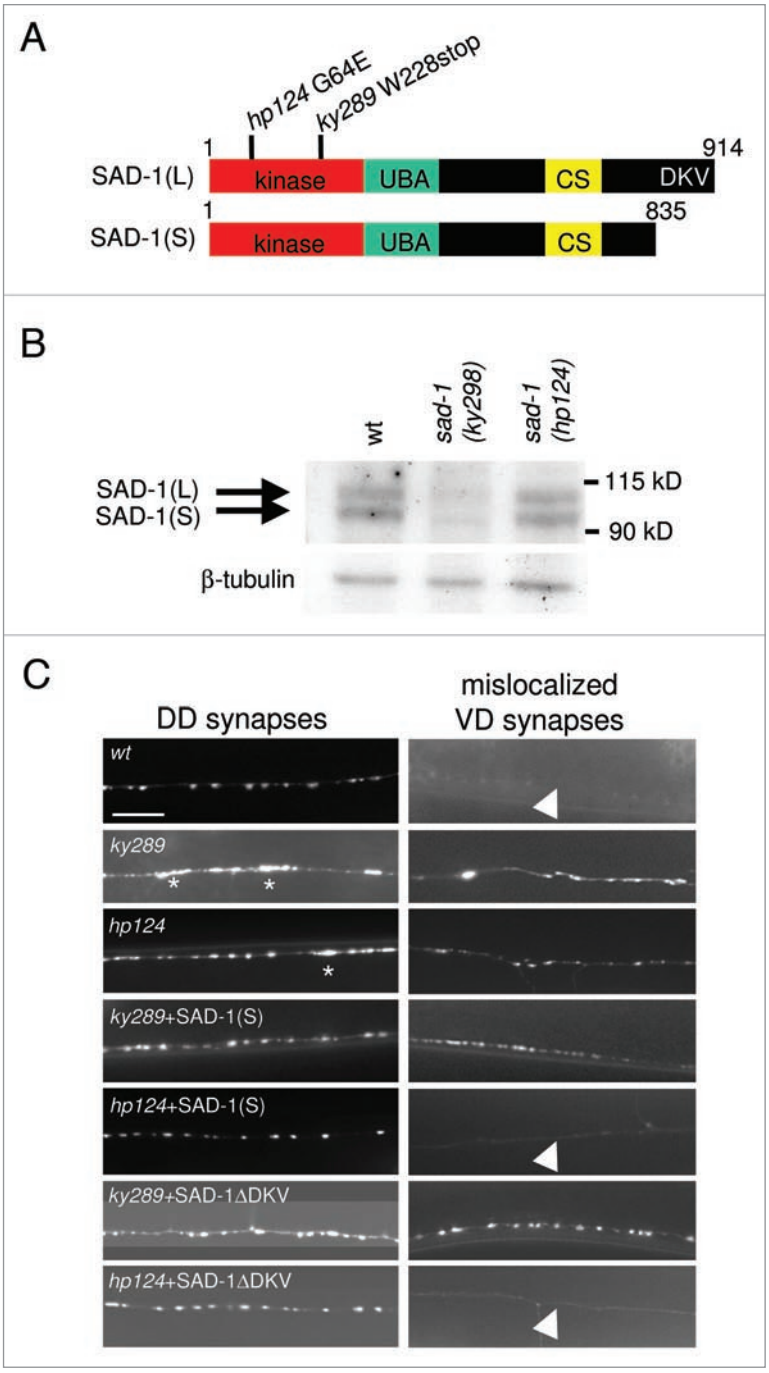

Figure 1. SAD-1 dimer/oligomerizes. (A) Schematic representations of SAD-1(L) and SAD-1(S) protein structures. Each isoform comprises a kinase domain, ubiquitin-associated (UBA) domain, and a unique C-terminal sequence (CS) conserved amongst SAD kinases. In addition, SAD-1(L) has a PDZ domain-binding consensus sequence at the C-terminus (DKV) to which NAB-1 binds. The molecular lesions of two *sad-1* mutants, *ky289* and *hp124*, are shown. The *ky289* mutation causes an early stop codon whereas *hp124* changes a conserved glycine residue in the kinase domain to glutamic acid. (B) Biochemical characterization of *sad-1* mutants. SAD-1 protein levels were examined in wild-type, *ky289*, and *hp124* animals by immunoblotting using anti-SAD-1 (top) and anti-β-tubulin for loading control (bottom). While no SAD-1 was detected in *ky289* null mutants, both isoforms were detected in *hp124* mutants. (C) Differential neuronal phenotype rescues by SAD-1(S) or SAD-1ΔDKV in *ky289* and *hp124* mutants. Neuronal phenotypes of the GABAergic neurons along the dorsal nerve cord (DNC) were examined using a pre-synaptic vesicle marker, SNB-1::GFP. For synaptic organization, SNB-1::GFP signals in the axons of the DD class GABAergic neurons were examined (left). In wild-type animals, SNB-1::GFP exhibited uniform shape, size, and spacing. Both alleles of *sad-1* displayed uneven and diffuse SNB-1::GFP morphology (asterisks) which was fully rescued by either SAD-1(S) or SAD-1ΔDKV. For neuronal polarity, SNB-1::GFP signals mis-localizing to the dendrites of the VD class GABAergic neurons were examined (right). In wild-type animals, no pre-synaptic SNB-1::GFP was mis-localized (arrowhead). In both alleles of *sad-1*, ectopic SNB-1::GFP signals were observed. This defect was rescued only when SAD-1(S) or SAD-1ΔDKV was expressed in SAD-1(KD)-producing *hp124* mutants and not in the protein-null *ky289* mutants. Scale bar, 5 μm.

another kinase, PAR-1.[19,21] SAD-1, on the other hand, directly associates with and functions exclusively through a *C. elegans* ortholog of STRADα, STRD-1. These findings also challenge the common notion that STRADα functions exclusively through LKB1. Do these findings simply denote evolutionary divergence in the function of LKB1 and SAD kinases? Or do they also reflect the complexity in the regulation of, and interplay between, these signaling components in vivo? These questions warrant further commentary presented here and investigations to follow.

SAD Isoforms Perform Distinct Functions through Different Partners

Not all SAD kinases are created equal. Previously, we reported the identification of two SAD-1 isoforms[7] (**Fig. 1A**). The long isoform (SAD-1(L)) rescued both neuronal polarity and synaptic organization defects[7] of *sad-1*(*ky289*) protein-null mutants (**Fig. 1B**). In contrast, the short isoform (SAD-1(S)), truncated at the C-terminus, restored synaptic organization but not neuronal polarity.[7] We showed that SAD-1(S) failed to interact with NAB-1, the sole *C. elegans* ortholog of an F-actin binding scaffold protein, Neurabin, which regulates neuronal polarity through its interaction with the C-terminus of SAD-1(L)[7] (**Fig. 1A**). The SAD-1 isoforms therefore serve overlapping and yet distinct functions through different effectors.

The presence of multiple isoforms of SAD kinases is not unique to *C. elegans*.

Recently, isoforms of mouse SAD-B have also been reported, and one isoform was implicated in centrosome duplication during cell cycle progression.[22] This newly-identified role of the SAD-B isoform has an interesting connection to the neural functions of SAD kinases, revealed in a previous study which implicated a role of centrosome localization in determining the axonal fate.[23] Consistently, in both neuronal polarization and cell cycle regulation, SAD kinases regulate microtubule dynamics through Tau or tubulin, respectively. It is then not inconceivable that different isoforms of SAD kinases regulate neural development in parallel, via distinct mechanisms and effectors, in different cellular contexts. In view of this, the STRD-1-dependent activity of SAD-1 in *C. elegans*[19] and the LKB1-dependent activation of SAD-A and SAD-B in mammals[9] may simply represent our limited understanding of all aspects of the mechanism governing SAD activities.

SAD-1 Dimer/Oligomerizes

In our recent work, we reported an additional difference between the two SAD-1 isoforms in their organization along the axon.[19] When fluorescently-tagged and expressed separately, SAD-1(L) organized into tight clusters along the axon whereas SAD-1(S) appeared more diffuse.[19] Co-expressed SAD-1(L) and SAD-1(S) resembled the tight clustering pattern of SAD-1(L),[19] implying that the two isoforms might interact.

Indeed, our new in vivo and in vitro data strongly support this possibility. SAD-1(S) or SAD-1(L) lacking the C-terminus (SAD-1ΔDKV) cannot interact with NAB-1 and fails to rescue the neuronal polarity defect of *sad-1*(*ky289*) protein-null mutants.[7] However, when expressed in *sad-1*(*hp124*) loss-of-function mutants, which produce a kinase-dead but otherwise intact SAD-1 (SAD-1(KD)) protein (**Fig. 1B**), both SAD-1(S) and SAD-1ΔDKV fully restored both neuronal polarity and synaptic organization (**Fig. 1C**). As neither SAD-1(KD) nor SAD-1(S)/SAD-1ΔDKV alone can rescue the neuronal polarity defect,[7] a plausible explanation for this observation is that a fully functional complex comprised of the SAD-1(KD) long isoform and SAD-1(S) or SAD-1ΔDKV was formed.

We further confirmed that SAD-1 proteins interact with each other using the yeast-two-hybrid system in which SAD-1(S) and SAD-1(L) exhibited robust interactions through their ubiquitin-associated (UBA) domain (**Fig. 1A**; data not shown). The UBA domain has been shown to interact with kinase domains,[24] suggesting that the protein-protein interaction within a SAD-1 complex may be mediated by the UBA domain of one SAD-1 molecule and the kinase domain of another. Together, these findings are consistent with our co-expression data in which SAD-1(S) assumed the tight clustering pattern of SAD-1(L).[19]

SAD-1 Clusters at and Localizes to Synapses through SAD-1(L) and STRD-1 Interaction

The two SAD-1 isoforms also differed in their interaction with STRD-1.[19] The *strd-1* gene shares the same genetic pathway with *sad-1* to regulate neuronal polarity and synaptic organization, and STRD-1 physically interacts with SAD-1(L).[19] In *strd-1* loss-of-function mutants, co-expressed SAD-1(L) and SAD-1(S) failed to cluster along the axon, suggesting that STRD-1 regulates the sub-cellular organization and localization of the SAD-1(L)/SAD-1(S) complex.[19] On the other hand, when expressed separately, only SAD-1(L) displayed abnormal clustering and localization in *strd-1* mutants whereas SAD-1(S) remained unaffected.[19] Taken together, these data suggest that the clustering and localization of the SAD-1(L)/SAD-1(S) complex at synapses are mediated by STRD-1 through its direct interaction with SAD-1(L).

A Working Model for SAD-1

In view of the presented data, we propose the following model (**Fig. 2**). Along the axon, SAD-1(L) interacts with other SAD-1 through its UBA domain. The transformation of the diffuse SAD-1(S) localization pattern to tight clusters resembling that of SAD-1(L) when the two isoforms are co-expressed suggests that SAD-1(S) preferentially interacts with SAD-1(L) over another SAD-1(S) (**Fig. 2A and B**). The SAD-1(L)/SAD-1(S) complex interacts with STRD-1 via SAD-1(L), and this association promotes the clustering and localization of the SAD-1 complex at synapses. The complex subsequently interacts with NAB-1 to establish neuronal polarity.

We have confirmed that both isoforms are indeed co-expressed in the same neurons (data not shown). What, then, is the physiological role of SAD-1(S) when SAD-1(L) alone can fully rescue the neural defects of *sad-1* complete loss-of-function mutants? The simplest explanation is that there may be additional, distinct functions for SAD-1(S) yet to be discovered. Here, we propose an alternative scenario in which the expression of the SAD-1 isoforms is regulated by a developmental switch during neural development.

Previously, we demonstrated that the establishment of neuronal polarity and synaptic organization has distinct temporal requirements for SAD-1 kinase activity.[25] While SAD-1 activity is strictly required during a narrow window of time to establish neuronal polarity, synaptic organization could be established or even corrected at flexible developmental time points.[25] Furthermore, whereas establishing neuronal polarity depends strictly on SAD-1(L), either isoform suffices for synaptic organization, suggesting that neuronal polarization is a much more tightly-controlled process. We thus speculate that a temporal switch for SAD-1(S) expression is activated once neuronal polarity is established and when SAD-1(L) is no longer necessary (**Fig. 2C and D**). In polarized neurons, newly-synthesized SAD-1(S) associates with the existing SAD-1(L) and STRD-1 to perform 'surveillance' functions, correcting any abnormal synaptic organization.

Implications of the SAD-1 Working Model on Other SAD Kinases

Clear species differences exist between the vertebrate and invertebrate SAD kinases. For instance, while the two SAD kinases function redundantly in mammals,[8] only a single kinase suffices in *C. elegans*.[6] Also, the SAD-1—NAB-1 interaction may not be conserved given the poor sequence

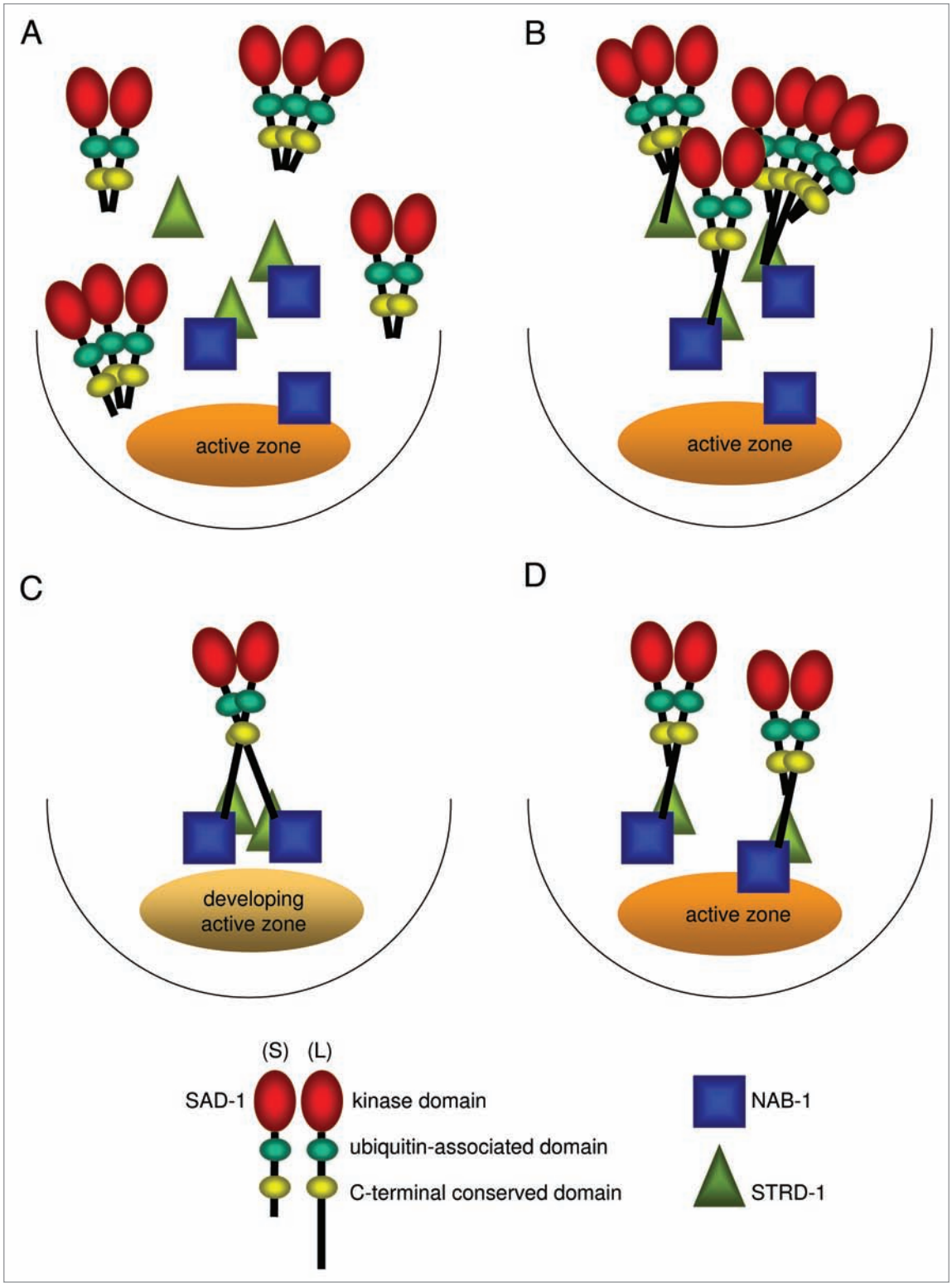

Figure 2. SAD-1 working model. (A and B) Formation of the SAD-1 complex. Expressed alone, SAD-1(S) fails to interact and cluster with STRD-1 or NAB-1 (A). When co-expressed, SAD-1(L) recruits SAD-1(S), and the SAD-1 complex binds STRD-1 and NAB-1 through the C-terminus of the long isoform (B). The interaction with STRD-1 is crucial for clustering and localizing the SAD-1 complex along the axon. The localization of STRD-1 and NAB-1 is unaffected by SAD-1 as evidenced in our previous studies. (C and D) Developmental regulation of the isoform expression. During neuronal polarization, SAD-1(L) is predominantly expressed (C). Once polarity is established, SAD-1(S) is expressed and dimer/oligomerizes with SAD-1(L) to perform a 'surveillance' role in synaptic organization (D).

similarities in the C-terminus amongst SAD kinases.[6]

However, SAD kinases may in fact share much in common as demonstrated above. As SAD-1—SAD-1 interaction is mediated through the conserved UBA domain, SAD kinases may also dimer/oligomerize in mammals. In future studies, it will be important to test and refine our model across species to better understand the complex mechanism of SAD kinases in the regulation of neural development.

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