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The cadherin domains and the kinesin-binding intracellular domain of CASY-1/calsyntenin function in a redundant manner for learning

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Abstract

Taste avoidance learning in *Caenorhabditis elegans* is regulated by the calsyntenin/alcadein homolog CASY-1, which transports the insulin receptor DAF-2c to the synaptic region. This transport involves binding of the CASY-1 intracellular domain to the kinesin-1 (KIF5) complex. However, a previous study showed that the intracellular domain of CASY-1 is dispensable for learning. To investigate how CASY-1 functions, we performed functional domain mapping of CASY-1. Both the cadherin domains of CASY-1 and its binding to kinesin-1 are individually dispensable, while simultaneous loss of both abolished the CASY-1 function, suggesting that CASY-1 enables robust intracellular transport through physical interactions with multiple proteins.

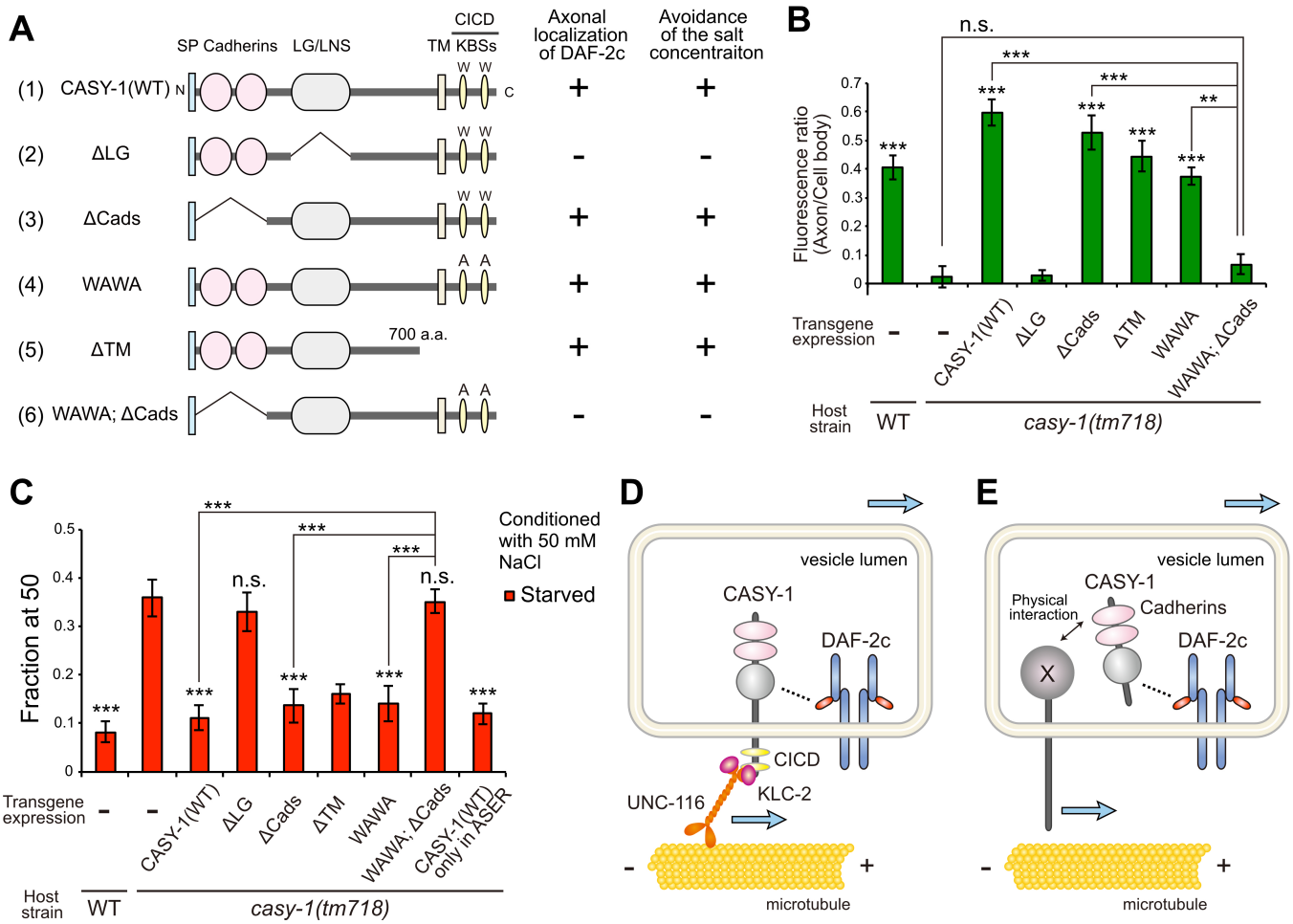

Figure 1. The interaction between CASY-1 and kinesin-1 is involved in the synaptic DAF-2c localization:

(**A**) Schematic depiction and functions of the mutated variants of CASY-1. SP, signal peptide; Cadherins, Cadherin domains; LG/LNS, LG/LNS-like domain; TM, transmembrane region; KBSs, kinesin binding segments; CICD, CASY-1 intracellular C-terminal domain. (**B**) Axonal localization of DAF-2c::Venus was quantified in ASER of wild type and *casy-1* mutants transformed with the mutated variants of *casy-1* that are shown in (A). $n \geq 9$ animals. Difference from *casy-1(tm718)* animals

carrying only the transformation marker (−) unless otherwise indicated. ***$p < 0.001$, **$p < 0.01$ (ANOVA with Tukey's post-test). n.s., not significant. (**C**) The salt concentration preference assay was performed on wild type and *casy-1* mutants transformed with the mutant constructs of *casy-1* that are shown in (A). $n = 21$. Difference from *casy-1(tm718)* animals carrying only the transformation marker (−) unless otherwise indicated; ***$p < 0.001$ (ANOVA with Tukey's post-test). n.s., not significant. (B and C) The *casy-1* promoter was used to express transgenes unless otherwise noted. The *gcy-5* promoter was used to express the *casy-1* transgene only in ASER in (C). (**D** and **E**) A possible model for the mechanisms by which CASY-1 regulates the axonal DAF-2c localization. CASY-1 interacts with the kinesin-1 complex through the intracellular domain (D) and with an unknown axonal protein(s) through the cadherin domains (E), to be transported to the axon together with DAF-2c.

Description

The nematode *Caenorhabditis elegans* can memorize external salt concentrations and modify the preference for them according to past experiences; they are attracted to the past salt concentration where they had been fed, whereas they avoid it if they were starved (Kunitomo et al., 2013; Ohno et al., 2014). We found that the avoidance of the salt concentrations associated with starvation was impaired in mutants of *casy-1* (Ikeda et al., 2008; Ohno et al., 2014). CASY-1 is the sole *C. elegans* ortholog of calsyntenins/alcadeins, a family of transmembrane proteins highly expressed in neurons. CASY-1 has the extracellular cadherin and LG/LNS domains and the intracellular kinesin-1 binding segments (KBSs) (Ikeda et al., 2008, Fig. 1A). It has been suggested that CASY-1 functions in intracellular trafficking and cell adhesion through physical interactions with BAM-2/neurexin-related (Kim & Emmons, 2017), UNC-104/kinesin-3 (KIF1) (Thapliyal et al., 2018), and KLC-2/kinesin-1 (KIF5) light chain (Ohno et al., 2014).

In a previous study (Ohno et al., 2014), we reported that CASY-1 induces starvation-associated learning by acting as an adaptor linking the insulin receptor isoform DAF-2c and the kinesin-1 complex to transport DAF-2c to the synaptic region, based on the following results: (1) CASY-1 physically interacts with KLC-2 and DAF-2c, (2) mutation or knockdown of *unc-116*/kinesin-1 heavy chain or *klc-2* inhibits the synaptic DAF-2c localization, and (3) MAPK-dependent KLC-2 S452 phosphorylation prevents physical interaction between CASY-1 and KLC-2 and this phosphorylation also prevents the synaptic DAF-2c localization. However, another study (Ikeda et al., 2008) found that the expression of the extracellular domain of CASY-1, which lacks intracellular KBSs, is sufficient to restore the learning defects of *casy-1* mutants.

To understand how CASY-1 regulates the DAF-2c transport and learning, we examined the functions of mutated variants of CASY-1 (Fig. 1A) by transgenic rescue experiments, in which the constructs were expressed under the *casy-1* promoter in the *casy-1(tm718)* deletion mutant strain. Consistent with the previous study (Ikeda et al., 2008), deletion of the central LG/LNS domain (ΔLG) abolished the functionality of CASY-1, whereas that of the cadherin domains (ΔCads) did not, in both the regulation of DAF-2c localization and the starvation-associated learning (Fig. 1A(2)(3), Fig. 1B, and Fig. 1C). Interestingly, replacement of the tryptophans of both kinesin-binding motifs in KBSs by alanines (WAWA), which completely disrupts the binding of the intracellular domain of CASY-1 with KLC-2 (Ohno et al., 2014), did not affect the functions of CASY-1 (Fig. 1A(4), Fig. 1B, and Fig. 1C). Even the extracellular domain alone (ΔTM), which is assumed to be released from the cell surface, was functional (Fig. 1A(5), Fig. 1B, and Fig. 1C). By contrast, the construct that harbors both the replacement of the tryptophans by alanines and the deletion of cadherin domains (WAWA; ΔCads) failed to rescue the *casy-1* mutant phenotypes (Fig. 1A(6), Fig. 1B, and Fig. 1C), suggesting that the cadherin domains and the kinesin binding intracellular domain function in a redundant fashion. These results may be explained by assuming that CASY-1 mediates the transport of DAF-2c via two interactions, the interaction of the intracellular domain with kinesin-1 (Fig. 1D) and the interaction of the extracellular cadherin domains with an unknown axonal protein(s), which is itself transported probably by interaction with motor proteins (Fig. 1E). Since the point mutations in kinesin-1 binding motifs of CASY-1 abolished the functions of the cadherin domains-deleted construct (Fig. 1A(6)) and the expression of a gain-of-function form of KLC-2 increased the axonal DAF-2c localization in a CASY-1-dependent fashion (Ohno et al., 2014), the association of CASY-1 with kinesin-1 appears to have a significant contribution to the axonal transport of DAF-2c.

Methods

Salt concentration preference assay was performed as described (Ohno et al., 2014). Young adult worms were exposed to 50 mM NaCl without food for 4 h followed by a behavioral test in which five to nine worms were transferred to an agar assay plate (56 mm [w] x 38 mm [d] plastic dish poured with 4 mL of 2% agar, 5 mM potassium phosphate [pH 6.0], 1 mM $CaCl_2$, 1 mM $MgSO_4$) with a gradient of NaCl, covering a concentration range of approximately 20 mM to 80 mM. Worms that moved to the ~50 mM (the central half area of an assay plate) area were classified as “50”.

Axonal localization of DAF-2c::venus was evaluated as described (Ohno et al., 2014). Fluorescence was observed with a Leica HCX PL APO 63×/1.30 objective on a Leica TCS-SP5 confocal microscope. Images were analyzed with the software attached to the confocal microscope (LAS AF ver 2.2).

Statistic analyses were performed with a statistic package (Prism v.5, GraphPad software).

Reagents

JN1505	*peEx1505*[P*myo-3*::*venus*].
JN1527	*casy-1*(*tm718*) *II*; *peEx1527*[P*myo-3*::*venus*].
JN1528	*casy-1*(*tm718*) *II*; *peEx1528*[P*casy-1*::*casy-1*; P*myo-3*::*venus*].
JN1529	*casy-1*(*tm718*) *II*; *peEx1529*[P*casy-1*::*casy-1*(ΔLG); P*myo-3*::*venus*].
JN1530	*casy-1*(*tm718*) *II*; *peEx1530*[P*casy-1*::*casy-1*(ΔCads); P*myo-3*::*venus*].
JN1531	*casy-1*(*tm718*) *II*; *peEx1531*[P*casy-1*::*casy-1*(ΔTM); P*myo-3*::*venus*].
JN1532	*casy-1*(*tm718*) *II*; *peEx1532*[P*casy-1*::*casy-1*(*WAWA*); P*myo-3*::*venus*].
JN1533	*casy-1*(*tm718*) *II*; *peEx1533*[P*casy-1*::*casy-1*(*WAWA;* ΔCads); P*myo-3*::*venus*].
JN1538	*casy-1*(*tm718*) *II*; *peEx1538*[P*gcy-5*::*casy-1*; P*myo-3*::*venus*].
JN1560	*casy-1*(*tm718*) *II*; *peIs1524*[P*gcy-5*::*daf-2c*::*Venus*; P*unc-122*::*mCherry*]; *peEx1560*[P*casy-1*::*casy-1*; P*gcy-5*::*mCherry*; P*lin-44*::*gfp*].
JN1561	*casy-1*(*tm718*) *II*; *peIs1524*[P*gcy-5*::*daf-2c*::*Venus*; P*unc-122*::*mCherry*]; *peEx1561*[P*casy-1*::*casy-1*(ΔLG); P*gcy-5*::*mCherry*; P*lin-44*::*gfp*].
JN1562	*casy-1*(*tm718*) *II*; *peIs1524*[P*gcy-5*::*daf-2c*::*Venus*; P*unc-122*::*mCherry*]; *peEx1562*[P*casy-1*::*casy-1*(ΔCads); P*gcy-5*::*mCherry*; P*lin-44*::*gfp*].
JN1563	*casy-1*(*tm718*) *II*; *peIs1524*[P*gcy-5*::*daf-2c*::*Venus*; P*unc-122*::*mCherry*]; *peEx1563*[P*casy-1*::*casy-1*(ΔTM); P*gcy-5*::*mCherry*; P*lin-44*::*gfp*].
JN1564	*casy-1*(*tm718*) *II*; *peIs1524*[P*gcy-5*::*daf-2c*::*Venus*; P*unc-122*::*mCherry*]; *peEx1564*[P*casy-1*::*casy-1*(*WAWA*); P*gcy-5*::*mCherry*; P*lin-44*::*gfp*].
JN1565	*casy-1*(*tm718*) *II*; *peIs1524*[P*gcy-5*::*daf-2c*::*Venus*; P*unc-122*::*mCherry*]; *peEx1565*[P*casy-1*::*casy-1*(*WAWA;* ΔCads); P*gcy-5*::*mCherry*; P*lin-44*::*gfp*].

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