Received: 24 March 2019 | Revised: 9 August 2019 | Accepted: 13 August 2019 | Uncorrected manuscript published: 16 August 2019

DOI: 10.1111/tra.12685

REVIEW

Traffic WILEY

Claudins in the brain: Unconventional functions in neurons

Vina Tikiyani[1] | Kavita Babu[1,2]

[1]Department of Biological Sciences, Indian Institute of Science Education and Research (IISER), Mohali, Punjab, India

[2]Centre for Neuroscience (CNS), Indian Institute of Science (IISc), Bangalore, India

Correspondence
Vina Tikiyani and Kavita Babu, Department of Biological Sciences, Indian Institute of Science Education and Research (IISER), Mohali, Punjab, India.
Email: vina.tikiyani@nih.gov (V. T.) and kavita.babu@babulab.org, kavitababu@iisc.ac.in (K. B.)

Present address
Vina Tikiyani, National Institute of Dental and Craniofacial Research (NIDCR), NIH, Bethesda, MD.

Funding information
DST-SERB, Grant/Award Number: EMR/2016/005332; IISc; IISER; Council of Scientific and Industrial Research, India

Peer Review
The peer review history for this article is available at https://publons.com/publon/10.1111/tra.12685/

Abstract

Bonafide claudin proteins are functional and structural components of tight junctions and are largely responsible for barrier formation across epithelial and endothelial membranes. However, current advances in the understanding of claudin biology have revealed their unexpected functions in the brain. Apart from maintaining blood-brain barriers in the brain, other functions of claudins in neurons and at synapses have been largely elusive and are just coming to light. In this review, we summarize the functions of claudins in the brain and their association in neuronal diseases. Further, we go on to cover some recent studies that show that claudins play signaling functions in neurons by regulating trafficking of postsynaptic receptors and controlling dendritic morphogenesis in the model organism *Caenorhabditis elegans*.

KEYWORDS

acetylcholine receptors, *C. elegans*, claudin, neuromuscular junction, synapse, tight junction

1 | STRUCTURAL SIMILARITY AT EPITHELIAL AND SYNAPTIC MEMBRANES

The epithelial tissue is held intact by the formation of tight junctions (TJ). A tight junction is a multiprotein complex whose primary function is to maintain paracellular permeability and barrier functions across epithelial cells.[1] The TJ complex consists of more than 40 different proteins and some of the functionally important proteins include transmembrane proteins, claudins, occludins and the cytoplasmic scaffolding proteins, ZO-1, 2 and 3.[2] These proteins are involved in regulating paracellular movement of water, solutes and immune cells across the plasma membrane via ion selectivity functions residing on the extracellular loops of claudin and occludin proteins.[3] Claudins also participate in intracellular signaling and gene expression functions by interacting with actin-binding proteins ZO-1, 2 and 3 through their post synaptic density protein (PSD95), drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1) (PDZ) binding motifs.[4]

Formation, function and plasticity at the synapse are mediated by various cell adhesion molecules (CAMs).[5-8] Apart from reports on the function of CAMs in maintaining cell-cell and cell-extracellular matrix contacts (reviewed in Reference 9), there are various modes by which CAMs could function at the synapse; they could function in target recognition such as SYG-1 and sidekicks,[10,11] formation and alignment of synaptic specializations such as SynCAMs, neurexins and neuroligins,[12,13] regulation of synaptic structure and function such as Cadherins and Syndecan (reviewed in Reference 14) as well as the regulation of activity-dependent synaptic plasticity such as RIG-3 which prevents synaptic potentiation by regulating Wnt signaling.[15,16]

Despite differential functions and mechanisms that operate at epithelial TJs and synapses, there is a striking similarity between proteins found at the TJ and the synapse. Proteomic and Bioinformatic analysis of epithelial TJs has revealed some unexpected clusters of synaptic molecules.[17] Moreover, studies validate the presence of various presynaptic (synaptotagmin VII, rabaptin-5, glutamate transporter EAAT1)

and postsynaptic molecules (GluR1, NMDA zeta subunit, mGlur1, mGlur5, piccolo, homer and GRIP) at epithelial tight junctions.[17] A neuronal protein, Peripheral myelin protein (PMP-22) which is largely present at myelinated Schwann cells of peripheral neurons is also detected at intercellular junctions in epithelia.[18] It appears that similar structural elements have evolved at these sites to build these two types of cellular junctions.[19] Not only have synaptic elements have been witnessed at the epithelial tight junction, but TJ proteins have also been identified at the synapses of chick ciliary ganglion.[20,21] Tight junctions have also been reported to be present at invertebrate synapses. For example, high-resolution electron micrographs from the neuropile region of the blowfly larvae, *Protophormia terraenovae* confirms the presence of TJ proteins in the neuropile.[22,23]

Two kinds of junctional structures are found at the synapse; synaptic junctions and puncta adherentia junctions.[24,25] The Puncta adherentia junctions are mechanical adhesion sites in the neurons, and they share many morphological and molecular features with the adherens junctions of the epithelium.

Very recently, two studies in *Caenorhabditis elegans* have shown that claudin-like proteins interact with actin/actin binding proteins at synapses/dendrites. First, In polymodal nociceptive for mechanosensation and thermosensation (PVD) neurons, higher order dendritic branching is maintained by the claudin HPO-30, which directly interacts with the Wave regulatory complex (WRC).[26] Second, we have shown that a claudin-like protein, HIC-1 interacts with an actin-binding protein, NAB-1/Neurabin through its PDZ binding motif in *C. elegans* cholinergic synapses.[27] These studies suggest that claudins function at dendrites and synapses similar to how they function at the epithelium.

An intriguing possibility is that neuronal synaptic adhesion molecules may share characteristics with epithelial tight junction structures. This could open new avenues on studies of adhesion molecules in neurons and the brain.

2 | CLAUDINS

Claudins are four transmembrane domain proteins with their N- and C-termini inside the cell and two extracellular loops that participate in making homo/heterotypic interactions with other claudins.[28] Based on sequence similarity, claudin superfamily proteins have been divided into two groups in humans; the classical claudins (1-10, 14, 15, 17, 19) and nonclassical claudins (11-13, 16, 18, 20-24).[29] Tetraspan claudin proteins have two extracellular loops; the first large extracellular loop is required for specific ion permeability and for making a paracellular barrier, while the second shorter extracellular loop is thought to be involved in the holding function between the apposing cell membranes and making homo/heterophilic interactions.[30] Further, most claudins possess a PDZ binding motif at their C-terminus tail by which they interact with PDZ domain-containing scaffolding proteins such as ZOs, PATJ and MUPP1, which in turn act as adaptors that links claudins to the actin cytoskeleton in epithelial cells (reviewed in Reference 31). The PDZ-binding motif, -K/R/H-X-Y-V, is present largely at −3,-2,-1, 0 positions at the C-terminal tail of the claudins. The -Y-V motif at the C-terminal positions −1 and 0 show high conservation in classical claudins in contrast to the large variations that are seen in nonclassical claudins (H/S/Y/D/E/R-V/L). It is thought that because of the conserved -Y-V motif in the classical claudins, they bind to the PDZ1 domain of ZO-1 in the epithelium (reviewed in Reference 29). The Claudin superfamily of proteins is conserved structurally but is highly divergent at the sequence level (reviewed in Reference 29). There are 24 gene families of claudins in mammals, which exhibit complex tissue-specific patterns of expression and function. Therefore, differential expression and function of claudins in mammals has been reported in many tissues including intestine, kidney, gall bladder, inner ear, retina, prostate glands, brain, etc. (reviewed in Reference 29). Apart from the function of claudins in regulating paracellular ion transport and membrane polarity at TJs, evidence indicates that they are also present at sites outside of the tight junction where they perform various non-canonical functions in cell signaling.[32] They regulate cell proliferation, differentiation and gene expression in different cell types such as differentiation of bone cells osteoclasts and osteoblasts (reviewed in Reference 33). An example of claudin function in signaling is shown by Claudin-1 that acts as a co-receptor for entry of the hepatitis C virus.[34]

Although, there are no typical TJ structures reported in invertebrates, the components required for making TJs such as claudins proteins are expressed widely in these organisms. A claudin-like molecule "sinuous" in *Drosophila* is required to maintain septate junctions. The septate junctions are considered to be equivalent to the vertebrate tight junctions in terms of maintaining barrier and paracellular permeability functions in the epithelium.[35] In *C. elegans*, 18 claudin and claudin-like molecules are thought to be present, including CLC-1, CLC-2, CLC-3 and CLC-4 that show structural similarity and, in some cases, functional similarity to their vertebrate counterparts. (Reviewed in References 36 and 37 and WormBase). Their functions involve regulating channel activity, intercellular signaling and maintaining cell morphology. CLC-1 is expressed in the epithelial cell junctions in the pharynx and regulates barrier function while CLC-2 is expressed in the seam cells of the hypodermis where it maintains hypodermal barrier functions.[38] Meanwhile, the functions of the remaining CLC proteins are as yet unknown. A putative claudin-like protein, VAB-9, is similar to the vertebrate BCMP1 (brain cell membrane protein 1) protein. VAB-9 localizes to epithelial cell contacts and has shown to interact with the cadherin-catenin complex during epidermal morphogenesis.[39] Taken together, the diverse expression and function of the claudin superfamily suggest that they could be involved in maintaining homeostasis in different tissue types and are likely to have other functions beyond their function at epithelial tight junctions.

3 | CLAUDINS IN THE NERVOUS SYSTEM

A growing body of evidence suggests functional roles for claudins in the brain. They have been shown to be essential components of the Blood-Brain Barrier (BBB) and changes in their expression and/or function has been shown to be associated with various brain disorders (reviewed in Reference 40). The BBB protects a delicate and intricate network of neurons from noxious blood-borne or surrounding stimuli.

Claudins-1,3,5,11,12,19, occludin, Zona occludens-1 (ZO-1) and tricellulin have been identified as key proteins in making these neural barriers.[41] An example of claudins involved in maintaining normal brain function comes from the fact that Claudin-5 positive leukocytes have been detected in the brain during neuroinflammation.[42] Claudin-5 also regulates tumor cell motility across the BBB, Indicating the involvement of Claudin-5 in brain metastasis.[43] Activation of matrix metalloproteinases (MMPs) opens the BBB by degrading tight junction proteins, including Claudins-5 and occludin.[44] Meanwhile, Claudin-11 regulates magnesium ion permeability across the myelin sheath membrane and is crucial for mice behavior and neurotransmitter release.[45]

More recently, studies on the functional roles of the claudin superfamily proteins in neurons are gaining traction through work in model organisms. Out of six claudin-like molecules found in *Drosophila melanogaster*, two have been reported to be essential to make septate junctions to allow for maintaining the blood-brain barrier integrity in the fly brain.[46] Claudin-5a in zebrafish is required for brain ventricle morphogenesis where it establishes neuroepithelial-ventricular barriers for maintaining the hydrostatic pressure within the ventricular cavity (Reference 47 and (reviewed in Reference 48). NSY-4, a Claudin-like protein in *C. elegans* is required for the stochastic activation of AWC olfactory neurons. The AWCs are a left/right bilateral pair of neurons. In each animal, one AWC neuron is activated or ON at any given time, while the other remains inactivated or OFF. NSY-4 co-ordinates lateral signaling between the AWC neurons, which allows them to take an ON or OFF state, respectively.[49]The above examples indicate that claudins play essential roles in the nervous system. However, how they might be performing their function in the brain or at the synapse and their mechanism of action is still poorly understood.

4 | SIGNALING FUNCTIONS OF CLAUDINS IN THE NERVOUS SYSTEM

Apart from making tight junction complexes, claudins also interact with various non-tight junction proteins including cell adhesion molecules, EpCam and tetraspanin, signaling proteins ephrin A, ephrin B and their receptors EphA and EphB.[50-53] These interactions of claudins with the actin cytoskeleton and with non-tight junction proteins suggest that claudins could have functions other than those involved in barrier formation. A few members of the claudin superfamily of proteins that play noncanonical functions in neurons are described here:

5 | HPO-30 REGULATES DENDRITIC MORPHOGENESIS AND LACHR LOCALIZATION

A claudin-like molecule, HPO-30 (Hypersensitive to Pore-forming toxin) is expressed in multiple neurons in *C. elegans* including PVD, FLP and cholinergic neurons. This protein is also expressed in the body-wall muscles of *C. elegans*.[54,55] Ever since its discovery as a dendritic stability molecule in PVD neurons,[26,55] various functions have been attributed to HPO-30 in the *C. elegans* nervous system.

Studies have shown that HPO-30 is required for lateral branching of *C. elegans* PVD and FLP neurons.[55] Dendrite morphogenesis is essential for nervous system assembly and circuit function. PVD is a multidendritic nociceptor neuron, which responds to harsh touch and cold temperature. The branching pattern of the PVD neuron involves multiple pathways and is used as a model for dendritic development. One pathway that allows for normal dendritic patterning involves DMA-1, a leucine-rich transmembrane receptor. DMA-1 interacts and forms a complex with the claudin, HPO-30 (illustrated in Figure 1A). Here, HPO-30 regulates the surface expression and trafficking of the DMA-1 receptor. Loss of *hpo-30* increases, whereas HPO-30 gain of function decreases the membrane surface expression of DMA-1 receptors in the PVD neuron.[56] The mobile fraction of DMA-1 receptors tagged to GFP also showed decreased in the *hpo-30* mutants, indicating a role for HPO-30 in DMA-1 receptor trafficking.[56] In proposing a model for the function of HPO-30 in the PVD neurons, Zou et al show that the intracellular domain of DMA-1 binds to the Rac GTP exchange factor, TIAM-1, while the intracellular domain of HPO-30 binds to the actin modulator, Wave Regulatory Complex (WRC). They go on to propose that the association between DMA-1 and HPO-30 recruits both TIAM-1 and WRC to the dendritic membranes. This signaling complex is required for the localization and dynamics of the actin cytoskeleton, which ultimately controls the highly branched dendritic arbors in PVD neurons (Figure 1A and Reference 26).

Acetylcholine receptors are responsible for various brain activities in both vertebrates and invertebrates. In *C. elegans*, there are two kinds of receptors that respond to the acetylcholine neurotransmitter; levamisole-sensitive acetylcholine receptors (LAChRs) and nicotine-sensitive acetylcholine receptors (nAChRs).[57] The LAChRs are the most extensively studied subtype of acetylcholine receptors in *C. elegans* and contribute to ~20% of synaptic transmission mediated by acetylcholine in worms. They respond to the anthelmintic drug, levamisole. They are nematode-specific and consist of five different subunits; UNC-38, UNC-29, UNC-63, LEV-8 and LEV-1.[58] It has been shown that HPO-30 localizes to neuromuscular junctions and shows genetic and physical interaction with levamisole-sensitive receptor (LAChR) subunits UNC-29 and UNC-38. Further, it regulates LAChR clustering through interacting with another cell adhesion molecule neuroligin (NLG-1) (Figure 1B and Reference 54).

LAChRs are targets of anthelmintic drugs, hence detailed studies on these receptors' and their regulation could help develop new strategies to prevent parasitic worm infections.

6 | STARGAZIN AND REGULATION OF α-AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLEPROPIONIC ACID (AMPA) RECEPTORS TRAFFICKING

Claudin superfamily member, Stargazin (STG-1) is highly conserved across the phyla. It acts as an obligate auxiliary subunit of transmembrane AMPA receptor regulatory proteins (TARPs) for AMPA receptors and is required for glutamatergic currents.[59,60] The Stargazin

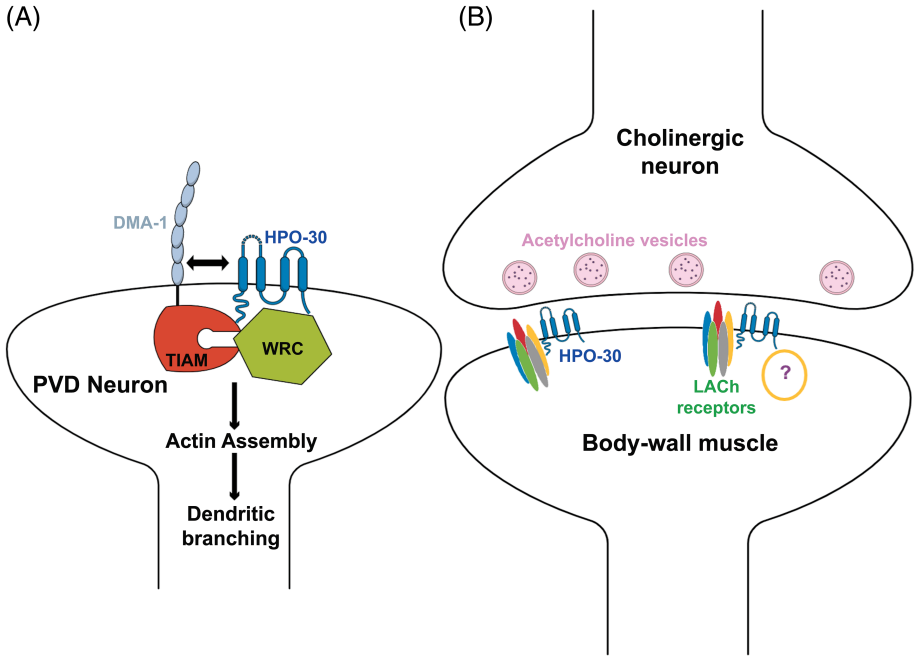

FIGURE 1 HPO-30 functions in dendrites and synapses. A, HPO-30 interaction with DMA-1 receptor regulates actin cytoskeleton and dentritic branching in *C. elegans* PVD neurons. This image is adapted with modifications from Reference 26. B, HPO-30 regulates LAChRs at the *C. elegans* neuromuscular junction. HPO-30 co-precipitates with the levamisole sensitive receptor subunits UNC-29 and UNC-38[54]

proteins are an extensively studied class of claudin-related molecules functioning at glutamate synapses.[61-63] Moreover, they are capable of forming adhesion contacts when expressed in L-fibroblasts.[64] Stargazin shares structural and functional features with claudin proteins. It contains four transmembrane domains with N- and C-termini inside the cell and two extracellular loops. The larger extracellular loop regulates the biophysical properties of AMPA receptors while the C-terminus is responsible for the trafficking of these receptors. Recent reports indicate that the C-terminus domain of TARP proteins play various unexpected and versatile functions in AMPA receptor regulation.[65] Being a positive allosteric regulator of AMPA receptors, it stabilizes the localization and surface expression of the receptors by facilitating the interaction of AMPA receptors with scaffolding proteins of the postsynaptic density, PSD95 (illustrated in Figure 2) Further, the exchange of AMPA receptor by lateral diffusion between extrasynaptic and synaptic sites is also dependent on the interaction between Stargazin and PSD95.[67] Stargazin also forms a ternary complex with adaptor proteins AP-2 and AP-3 and hence has an important role in NMDA-dependent LTD by regulating trafficking pathways of AMPA receptors.[68]

AMPA type glutamate receptors are responsible for fast excitatory transmission. They are one of the major contributors of synaptic plasticity, and defects in these receptors have been associated with neurodegenerative and neuropsychiatric disorders.[69] Over the past decade studies on AMPA receptor regulation has brought out the function of the Stargazin in maintaining these receptors. Since excessive AMPA activity and excitotoxicity is thought to be the cause of neuropsychiatric disorders like stroke and epilepsy, therefore molecules that regulate AMPA receptors, like Stargazin are now considered to be potential target for designing antagonists against AMPA receptors.[70]

7 | HIC-1 AND THE REGULATION OF ACR-16/A7 RECEPTOR TRAFFICKING

HIC-1 is a divergent claudin-like protein that has recently been characterized for its function at *C. elegans* cholinergic synapses.[27] HIC-1 is

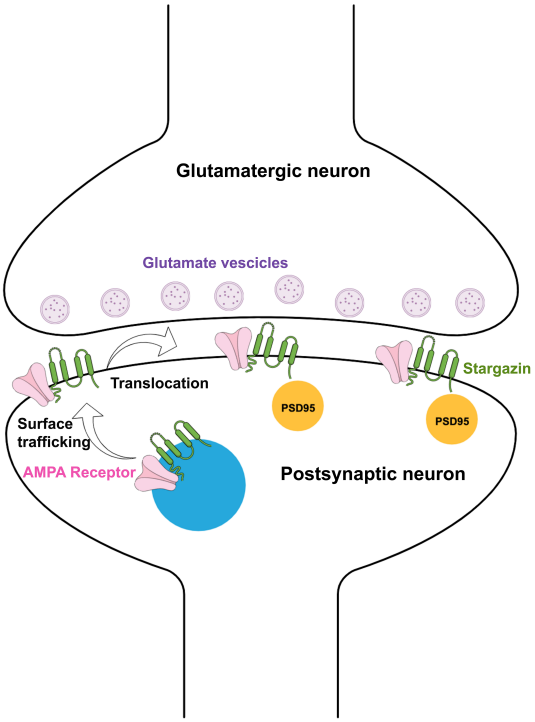

FIGURE 2 Stargazin regulates AMPA receptor trafficking at the synapse. Stargazin interacts with AMPA receptors in the intracellular compartment and regulates their trafficking and stability at the synapse. This image is adapted with modifications from Reference 66

expressed at the presynaptic terminus of cholinergic synapses. The claudin-like molecule, HIC-1 also possesses a -Y (Tyrosine) amino acid at its C-terminus similar to most of the classical claudins and hence could have a putative PDZ binding motif at its C-terminus.

At cholinergic synapses, HIC-1 has been shown to interact with an actin-binding protein, NAB-1 through its putative PDZ binding motif. Comparison of HIC-1 function at synapses with claudin functions in epithelial cells suggests that the C-terminal region of HIC-1 functions in a manner similar to conventional claudins in epithelial cells in terms of their interaction with actin-binding proteins and maintaining the integrity of the actin cytoskeleton.

Most of the excitatory currents at the *C. elegans* NMJs are mediated by nicotine-sensitive ACR-16/α7 (nAChR) receptors. The surface expression of these receptors is regulated through the Wnt signaling pathway.[15,71-74] Jensen et al have elegantly dissected out the Wnt signaling pathway, which regulates the translocation of ACR-16/α7 receptors at the *C. elegans* NMJs. Their study shows that ACR-16/α7 receptor translocation is mediated by members of the canonical Wnt signaling pathway.[75] Out of five Wnts LIN-44, EGL-20, CWN-1, CWN-2 and MOM-2 in *C. elegans*,[76] CWN-2[75] and LIN-44[73] are involved in this pathway. The Wnts, CWN-2 and LIN-44 secreted from the presynaptic neurons, bind and activate the postsynaptic Wnt receptor Lin-17/Frizzled. Lin-17/Frizzled then activates downstream signaling involving disheveled (DSH-1), which in turn regulates the trafficking of ACR-16/α7 onto the postsynaptic muscle membrane.[75] In *hic-1* mutants, increased levels of Wnt secretion have been seen which leads to increased trafficking of ACR-16/α7 at the muscle membrane.[27] HIC-1 appears to regulate the secretion of Wnt ligands CWN-2 and LIN-44 from *C. elegans* cholinergic neurons by binding to the actin-binding protein, NAB-1. HIC-1 and NAB-1 together regulate the integrity of the actin cytoskeleton. The actin cytoskeleton in turn, regulates the controlled release of Wnt vesicles, which ultimately controls ACR-16/α7 receptor trafficking at cholinergic synapses (Illustrated in Figure 3 and Reference 27).

Aberrant Wnt secretion is implicated in synaptic plasticity and cognitive deficiencies such as schizophrenia, bipolar disorders and Alzheimer's Disease.[77,78] An in-depth, comprehensive study of how Wnt secretion and acetylcholine receptors are regulated by a claudin-like molecule HIC-1 at the synapse could provide a better understanding of neuromuscular junction structure and function.

8 | CONCLUDING REMARKS

Claudin superfamily of protein play differential functions in various tissues (reviewed in Reference 29). Although they were initially characterized for their function in barrier formation across the epithelial tissue, they are also emerging as neuronal proteins according to recent studies. They play diverse roles in the maintenance of the synaptic and neuronal structure and function. In this review, we have summarized functions of some of the claudins HPO-30, Stargazin and HIC-1 in *C. elegans* nervous system where they are mainly involved in

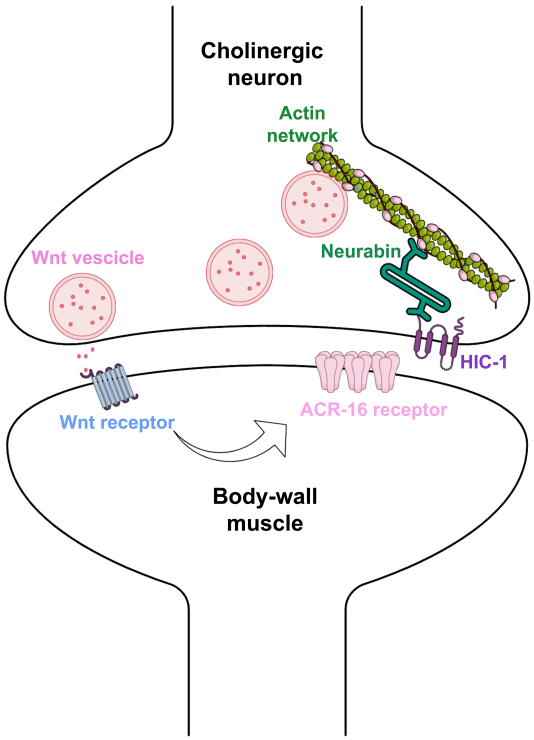

FIGURE 3 HIC-1 functions to maintain ACR-16/α7 receptors at the synapse. The Claudin-like protein HIC-1 regulates acetylcholine receptors (ACR-16/α7) at the cholinergic synapse. HIC-1 modulates the actin cytoskeleton through interacting with an actin binding protein, Neurabin (NAB-1). The actin cytoskeleton regulates the release of Wnt vesicles, which in turn regulate ACR-16/α7 receptor at the postsynaptic muscle membrane. This image is adapted from Reference 27

maintaining the surface expression and trafficking of various receptors.

As mentioned earlier, two kinds of acetylcholine receptors, LAChRs and nAChRs are functional at the *C. elegans* neuromuscular junctions. Although both subtypes of receptors are colocalized at the *C. elegans* neuromuscular junction,[79] distinct mechanisms are involved for their synaptic localization and clustering. On one hand the Levamisole-sensitive receptors (LAChRs) are maintained at the synapse by a mechanism involving the claudin, HPO-30, while on the other hand nicotine-sensitive acetylcholine receptors (ACR-16/α7) are regulated by the Wnt signaling pathway involving another claudin-like protein, HIC-1. Acetylcholine receptors are associated with various neuropsychiatric and neurodegenerative disorders such as Alzheimer's and Parkinson.[80-82] A study from our lab and other labs[26,54-56] indicate a striking dichotomy for HPO-30 in *C. elegans*. In the body-wall muscles, it is required for the stability of LAChRs while in PVD neurons HPO-30 regulates DMA-1 receptors and together with DMA-1, controls downstream assembly of F-actin, allowing for normal dendritic branching.

As mentioned earlier, Stargazin regulates AMPA receptor trafficking and localization at the glutamatergic synapses. AMPA receptors are the principle ionotropic glutamate receptors involved in the

mediation of fast excitatory neurotransmission in the mammalian brain and hence are a major component of synaptic plasticity,[83] learning and memory formation.[84] AMPA receptor levels are altered in many neurological diseases such as amyotrophic lateral sclerosis, epilepsy, ischemia and Alzheimer's Disease. Despite the wealth of knowledge gathered in the past decade about the deregulated levels of AMPA receptors in these diseases, only limited success is achieved in directly targeting AMPA receptors.[85] Therefore, researchers are now proposing that indirectly affecting AMPA by modulating AMPA regulatory proteins such as Stargazin could provide new therapeutic potentials to treat neuronal diseases associated with AMPA receptors.[85] A detailed, comprehensive study on how these different receptors are regulated through the claudin superfamily of protein could give more insight into how these receptors are regulated in the nervous system.

ACKNOWLEDGMENTS

V.T. was funded by the Council of Scientific and Industrial Research (CSIR) for a graduate fellowship. K.B. was funded by IISER Mohali and is currently funded by IISc, Bangalore and a DST-SERB grant (grant no. EMR/2016/005332).

ORCID

Kavita Babu https://orcid.org/0000-0002-7527-3666

REFERENCES

1. Anderson JM, Van Itallie CM. Physiology and function of the tight junction. *Cold Spring Harb Perspect Biol*. 2009;1(2):a002584.
2. Schneeberger EE, Lynch RD. The tight junction: a multifunctional complex. *Am J Physiol Cell Physiol*. 2004;286(6):C1213-C1228.
3. Anderson J, Van Itallie CM. Tight junctions and the molecular basis for regulation of paracellular permeability. *Am J Physiol*. 1995;269(4):G467-G475.
4. Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta*. 2008;1778(3):660-669.
5. Abbas L. Synapse formation: let's stick together. *Curr Biol*. 2003;13(1):R25-R27.
6. Tallafuss A, Constable JR, Washbourne P. Organization of central synapses by adhesion molecules. *Eur J Neurosci*. 2010;32(2):198-206.
7. Wu H, Xiong WC, Mei L. To build a synapse: signaling pathways in neuromuscular junction assembly. *Development*. 2010;137(7):1017-1033.
8. Yamagata M, Sanes JR, Weiner JA. Synaptic adhesion molecules. *Curr Opin Cell Biol*. 2003;15(5):621-632.
9. Albelda SM. Endothelial and epithelial cell adhesion molecules. *Am J Respir Cell Mol Biol*. 1991;4(3):195-203.
10. Shen K, Bargmann CI. The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in *C. elegans*. *Cell*. 2003;112(5):619-630.
11. Yamagata M, Weiner JA, Sanes JR. Sidekicks: synaptic adhesion molecules that promote lamina-specific connectivity in the retina. *Cell*. 2002;110(5):649-660.
12. Biederer T, Sara Y, Mozhayeva M, et al. SynCAM, a synaptic adhesion molecule that drives synapse assembly. *Science*. 2002;297(5586):1525-1531.
13. Hu Z, Hom S, Kudze T, et al. Neurexin and neuroligin mediate retrograde synaptic inhibition in *C. elegans*. *Science*. 2012;337(6097):980-984.
14. Couchman JR. Syndecans: proteoglycan regulators of cell-surface microdomains? *Nat Rev Mol Cell Biol*. 2003;4(12):926-937.
15. Babu K, Hu Z, Chien SC, Garriga G, Kaplan JM. The immunoglobulin super family protein RIG-3 prevents synaptic potentiation and regulates Wnt signaling. *Neuron*. 2011;71(1):103-116.
16. Pandey P. Bhardwaj, a., and Babu, K. the immunoglobulin superfamily protein, RIG-3, regulates WNT signaling by interacting with the non-conventional Wnt receptor, CAM-1 at the *C. elegans* neuromuscular junction. *Genetics*. 2017;206(3):1521-1534.
17. Tang VW. Proteomic and bioinformatic analysis of epithelial tight junction reveals an unexpected cluster of synaptic molecules. *Biol Direct*. 2006;1:37.
18. Notterpek L, Roux KJ, Amici SA, Yazdanpour A, Rahner C, Fletcher BS. Peripheral myelin protein 22 is a constituent of intercellular junctions in epithelia. *Proc Natl Acad Sci U S A*. 2001;98(25):14404-14409.
19. Tomita S, Nicoll RA, Bredt DS. PDZ protein interactions regulating glutamate receptor function and plasticity. *J Cell Biol*. 2001;153(5):F19-F24.
20. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. *Cell*. 2012;149(6):1192-1205.
21. Darin de Lorenzo AJ. Electron microscopy: tight junctions in synapses of the chick ciliary ganglion. *Science*. 1966;152(3718):76.
22. McDonald PW, Hardie SL, Jessen TN, Carvelli L, Matthies DS, Blakely RD. Vigorous motor activity in *Caenorhabditis elegans* requires efficient clearance of dopamine mediated by synaptic localization of the dopamine transporter DAT-1. *J Neurosci*. 2007;27(51):14216-14227.
23. Osborne MP. The fine structure of synapses and tight junctions in the central nervous system of the blowfly larva. *J Insect Physiol*. 1966;12(12):1503-1512.
24. Togashi H, Sakisaka T, Takai Y. Cell adhesion molecules in the central nervous system. *Cell Adh Migr*. 2009;3(1):29-35.
25. Mizoguchi A, Nakanishi H, Kimura K, et al. Nectin: an adhesion molecule involved in formation of synapses. *J Cell Biol*. 2002;156(3):555-565.
26. Zou W, Dong X, Broederdorf TR, et al. A dendritic guidance receptor complex brings together distinct Actin regulators to drive efficient F-Actin assembly and branching. *Dev Cell*. 2018;45(3):362-375. e363.
27. Tikiyani V, Li L, Sharma P, Liu H, Hu Z, Babu K. Wnt secretion is regulated by the tetraspan protein HIC-1 through its interaction with Neurabin/NAB-1. *Cell Rep*. 2018;25(7):1856-1871.e1856.
28. Suzuki H, Nishizawa T, Tani K, et al. Crystal structure of a claudin provides insight into the architecture of tight junctions. *Science*. 2014;344(6181):304-307.
29. Krause G, Winkler L, Mueller SL, Haseloff RF, Piontek J, Blasig IE. Structure and function of claudins. *Biochim Biophys Acta (BBA)—Biomembranes*. 2008;1778(3):631-645.
30. Lal-Nag M, Morin PJ. The claudins. *Genome Biol*. 2009;10(8):235.
31. Gunzel D, Yu AS. Claudins and the modulation of tight junction permeability. *Physiol Rev*. 2013;93(2):525-569.
32. Hagen SJ. Non-canonical functions of claudin proteins: beyond the regulation of cell-cell adhesions. *Tissue Barriers*. 2017;5(2):e1327839.
33. Alshbool FZ, Mohan S. Emerging multifunctional roles of Claudin tight junction proteins in bone. *Endocrinology*. 2014;155(7):2363-2376.
34. Evans MJ, von Hahn T, Tscherne DM, et al. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature*. 2007;446(7137):801-805.

35. Wu VM, Schulte J, Hirschi A, Tepass U, Beitel GJ. Sinuous is a drosophila claudin required for septate junction organization and epithelial tube size control. *J Cell Biol*. 2004;164(2):313-323.
36. Cox EA, Hardin J. Sticky worms: adhesion complexes in *C. elegans*. *J Cell Sci*. 2004;117(Pt 10):1885-1897.
37. Cox EA, Tuskey C, Hardin J. Cell adhesion receptors in *C. elegans*. *J Cell Sci*. 2004;117(Pt 10):1867-1870.
38. Asano A, Asano K, Sasaki H, Furuse M, Tsukita S. Claudins in *Caenorhabditis elegans*. *Curr Biol*. 2003;13(12):1042-1046.
39. Simske JS, Köppen M, Sims P, Hodgkin J, Yonkof A, Hardin J. The cell junction protein VAB-9 regulates adhesion and epidermal morphology in *C. elegans*. *Nat Cell Biol*. 2003;5:619-625.
40. Goncalves A, Ambrosio AF, Fernandes R. Regulation of claudins in blood-tissue barriers under physiological and pathological states. *Tissue Barriers*. 2013;1(3):e24782.
41. Reinhold AK, Rittner HL. Barrier function in the peripheral and central nervous system—a review. *Pflügers Archiv—Eur J Physiol*. 2016;469(1):123-134.
42. Paul D, Baena V, Ge S, et al. Appearance of claudin-5+ leukocytes in the central nervous system during neuroinflammation: a novel role for endothelial-derived extracellular vesicles. *J Neuroinflammation*. 2016;13(1):292.
43. Jia W, Lu R, Martin TA, Jiang WG. The role of claudin-5 in blood-brain barrier (BBB) and brain metastases (review). *Mol Med Rep*. 2014;9(3):779-785.
44. Yang Y, Rosenberg GA. MMP-mediated disruption of claudin-5 in the blood–brain barrier of rat brain after cerebral ischemia. *Methods Mol Biol*. 2011;762:333-345.
45. Maheras KJ, Peppi M, Ghoddoussi F, Galloway MP, Perrine SA, Gow A. Absence of claudin 11 in CNS myelin perturbs behavior and neurotransmitter levels in mice. *Sci Rep*. 2018;8(1):3798.
46. Stork T, Engelen D, Krudewig A, Silies M, Bainton RJ, Klämbt C. Organization and function of the blood–brain barrier in *Drosophila*. *J Neurosci*. 2008;28(3):587-597.
47. Zhang J, Liss M, Wolburg H, Blasig IE, Abdelilah-Seyfried S. Involvement of claudins in zebrafish brain ventricle morphogenesis. *Ann N Y Acad Sci*. 2012;1257(1):193-198.
48. Abdelilah-Seyfried S. Claudin-5a in developing zebrafish brain barriers: another brick in the wall. *Bioessays*. 2010;32(9):768-776.
49. Hsieh Y-W, Alqadah A, Chuang C-F. Asymmetric neural development in the *C. elegans* olfactory system. *Genesis*. 2014;52(6):544-554.
50. Kovalenko OV, Yang XH, Hemler ME. A novel cysteine cross-linking method reveals a direct association between claudin-1 and tetraspanin CD9. *Mol Cell Proteomics*. 2007;6(11):1855-1867.
51. Ladwein M, Pape U-F, Schmidt D-S, et al. The cell–cell adhesion molecule EpCAM interacts directly with the tight junction protein claudin-7. *Exp Cell Res*. 2005;309(2):345-357.
52. Rubinstein E. The complexity of tetraspanins. *Biochem Soc Trans*. 2011;39(2):501-505.
53. Tanaka M, Kamata R, Sakai R. EphA2 phosphorylates the cytoplasmic tail of Claudin-4 and mediates paracellular permeability. *J Biol Chem*. 2005;280:42375-42382.
54. Sharma P, Li L, Liu H, Tikiyani V, Hu Z, Babu K. The claudin-like protein HPO-30 is required to maintain LAChRs at the *C. elegans* neuromuscular junction. *J Neurosci*. 2018;38(32):7072-7087.
55. Smith CJ, O'Brien T, Chatzigeorgiou M, et al. Sensory neuron fates are distinguished by a transcriptional switch that regulates dendrite branch stabilization. *Neuron*. 2013;79(2):266-280.
56. Tang LT, Diaz-Balzac CA, Rahman M, et al. TIAM-1/GEF can shape somatosensory dendrites independently of its GEF activity by regulating F-actin localization. *Elife*. 2019;8:e38949.
57. Richmond JE, Jorgensen EM. One GABA and two acetylcholine receptors function at the *C. elegans* neuromuscular junction. *Nat Neurosci*. 1999;2(9):791-797.
58. Boulin T, Gielen M, Richmond JE, Williams DC, Paoletti P, Bessereau JL. Eight genes are required for functional reconstitution of the *Caenorhabditis elegans* levamisole-sensitive acetylcholine receptor. *Proc Natl Acad Sci U S A*. 2008;105(47):18590-18595.
59. Walker CS, Brockie PJ, Madsen DM, et al. Reconstitution of invertebrate glutamate receptor function depends on stargazin-like proteins. *Proc Natl Acad Sci U S A*. 2006;103(28):10781-10786.
60. Wang R, Walker CS, Brockie PJ, et al. TARP proteins have fundamental roles in the gating of glutamate receptors and the tuning of synaptic function. *Neuron*. 2008;59(6):997-1008.
61. Vandenberghe W, Nicoll RA, Bredt DS. Stargazin is an AMPA receptor auxiliary subunit. *Proc Natl Acad Sci U S A*. 2005;102(2):485-490.
62. Chen L, Chetkovich DM, Petralia RS, et al. Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature*. 2000;408:936-943.
63. Qiao X, Meng H. Nonchannel functions of the calcium channel γ subunit: insight from research on the stargazer mutant. *J Bioenerg Biomembr*. 2003;35(6):661-670.
64. Price MG, Davis CF, Deng F, DL B. The α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate receptor trafficking regulator "stargazin" is related to the claudin family of proteins by its ability to mediate cell-cell adhesion. *J Biol Chem*. 2005;280(20):19711-19720.
65. Sager C, Tapken D, Hollmann M. The C-terminal domains of TARPs: unexpectedly versatile domains. *Channels*. 2010;4(3):155-158.
66. Nakagawa T, Sheng M. A *Stargazer* foretells the way to the synapse. *Science*. 2000;290(5500):2270-2271.
67. Bats C, Groc L, Choquet D. The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. *Neuron*. 2007;53(5):719-734.
68. Matsuda S, Kakegawa W, Budisantoso T, Nomura T, Kohda K, Yuzaki M. Stargazin regulates AMPA receptor trafficking through adaptor protein complexes during long-term depression. *Nat Commun*. 2013;4:2759.
69. Palmer CL, Cotton L, Henley JM. The molecular pharmacology and cell biology of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. *Pharmacol Rev*. 2005;57(2):253-277.
70. Gill MB, Bredt DS. An emerging role for TARPs in neuropsychiatric disorders. *Neuropsychopharmacology*. 2010;36:362.
71. Jensen M, Hoerndli Frédéric J, Brockie Penelope J, et al. Wnt signaling regulates acetylcholine receptor translocation and synaptic plasticity in the adult nervous system. *Cell*. 2012;149(1):173-187.
72. Wang J, Ruan N-J, Qian L, Lei W-l, Chen F, Luo Z-G. Wnt/β-catenin signaling suppresses Rapsyn expression and inhibits acetylcholine receptor clustering at the neuromuscular junction. *J Biol Chem*. 2008;283(31):21668-21675.
73. Pandey P, Bhardwaj A, Babu K. Regulation of WNT signaling at the neuromuscular junction by the immunoglobulin superfamily protein RIG-3 in *Caenorhabditis elegans*. *Genetics*. 2017;206(3):1521-1534.
74. Barik A, Zhang B, Sohal GS, Xiong WC, Mei L. Crosstalk between Agrin and Wnt signaling pathways in development of vertebrate neuromuscular junction. *Dev Neurobiol*. 2014;74(8):828-838.
75. Jensen M, Brockie PJ, Maricq AV. Wnt signaling regulates experience-dependent synaptic plasticity in the adult nervous system. *Cell Cycle*. 2012;11(14):2585-2586.
76. Hilliard MA, Bargmann CI. Wnt signals and frizzled activity orient anterior-posterior axon outgrowth in *C. elegans*. *Dev Cell*. 2006;10(3):379-390.
77. Oliva CA, Vargas JY, Inestrosa NC. Wnts in adult brain: from synaptic plasticity to cognitive deficiencies. *Front Cell Neurosci*. 2013;7:224.
78. Hoseth EZ, Krull F, Dieset I, et al. Exploring the Wnt signaling pathway in schizophrenia and bipolar disorder. *Transl Psychiatry*. 2018;8(1):55.

79. Francis MM, Evans SP, Jensen M, et al. The Ror receptor tyrosine kinase CAM-1 is required for ACR-16-mediated synaptic transmission at the *C. elegans* neuromuscular junction. *Neuron*. 2005;46(4):581-594.
80. Ellis J, Villemagne VL, Nathan P, et al. Relationship between nicotinic receptors and cognitive function in early Alzheimer's disease: a 2-[18F] fluoro-A-85380 PET study. *Neurobiol Learn Mem*. 2008;90(2):404-412.
81. Rinne J, Myllykyla T, Lönnberg P, Marjamäki P. A postmortem study of brain nicotinic receptors in Parkinson's and Alzheimer's disease. *Brain Res*. 1991;547(1):155-158.
82. Counts SE, He B, Che S, et al. α7 nicotinic receptor up-regulation in cholinergic basal forebrain neurons in Alzheimer disease. *Arch Neurol*. 2007;64(12):1771-1776.
83. Fleming JJ, England PM. AMPA receptors and synaptic plasticity: a chemist's perspective. *Nat Chem Biol*. 2010;6:89-97.
84. Keifer J, Zheng Z. AMPA receptor trafficking and learning. *Eur J Neurosci*. 2010;32(2):269-277.
85. Chang PKY, Verbich D, McKinney RA. AMPA receptors as drug targets in neurological disease–advantages, caveats, and future outlook. *Eur J Neurosci*. 2012;35(12):1908-1916.

How to cite this article: Tikiyani V, Babu K. Claudins in the brain: Unconventional functions in neurons. *Traffic*. 2019;20:807–814. https://doi.org/10.1111/tra.12685