Seminars in Cell & Developmental Biology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Clustered protocadherin trafficking

Greg R. Phillips[a,b,*], Nicole LaMassa[a], Yan Mei Nie[a]

[a] Department of Biology, Center for Developmental Neuroscience, College of Staten Island, City University of New York, United States
[b] Program in Neuroscience, The Graduate Center, City University of New York, United States

ARTICLE INFO

Article history:
Received 13 March 2017
Received in revised form 26 April 2017
Accepted 3 May 2017
Available online xxx

Keywords:
Cell adhesion
Self-avoidance
Endosome
Synapse

ABSTRACT

The cluster of almost 60 protocadherin genes, divided into the α, β and γ subgroups, is a hallmark of vertebrate nervous system evolution. These clustered protocadherins (Pcdhs) are of interest for several reasons, one being the arrangement of the genes, which allows epigenetic regulation at the cluster and single-cell identity. Another reason is the still ambiguous effect of Pcdhs on cell–cell interaction. Unlike the case for classical cadherins, which typically mediate strong cell adhesion and formation of adherens junctions, it has been challenging to ascertain exactly how Pcdhs affect interacting cells. In some instances, Pcdhs appear to promote the association of membranes, while in other cases the Pcdhs are anti-adhesive and cause avoidance of interacting membranes. It is clear that Pcdh extracellular domains bind homophillically in an antiparallel conformation, typical of adhesive interactions. How can molecules that would seemingly bind cells together be able to promote the avoidance of membranes? It is possible that Pcdh trafficking will eventually provide insights into the role of these molecules at the cell surface. We have found that endogenous and expressed Pcdhs are generally less efficient at targeting to cell junctions and synapses than are classical cadherins. Instead, Pcdhs are prominently sequestered in the endolysosome system or other intracellular compartments. What role this trafficking plays in the unique mode of cell–cell interaction is a current topic of investigation. It is tempting to speculate that modulation of endocytosis and endolysosomal trafficking may be a part of the mechanism by which Pcdhs convert from adhesive to avoidance molecules.

Contents

1. Introduction

The discovery of Pcdhs [1,2] and the Pcdh gene cluster [3,4] were exciting breakthroughs to investigators working in the field of cell adhesion and recognition at the synapse. The properties of the cluster suggested the potential for an adhesive code, differentially expressed among neurons, that might form a basis for synaptic specification, supplementing and/or refining other adhesion/recognition systems in play at the synapse. The regulation of individual genes in the cluster by methylation supports the notion that neurons acquire the code in an epigenetic fashion [5–8]. The clustered arrangement features alternative splicing of single exons, encoding each Pcdh, to constant exons that encode an identical segment appended to the cytoplasmic domains of Pcdh-αs and -γs (Fig. 1). The combinatorial association of multiple Pcdhs into "adhesive units" has been calculated [9,10] to provide sufficient

* Corresponding author at: Department of Biology, College of Staten Island, City University of New York. 2800 Victory Blvd. Staten Island, NY 10314, United States.
E-mail address: Greg.Phillips@csi.cuny.edu (G.R. Phillips).

http://dx.doi.org/10.1016/j.semcdb.2017.05.001
1084-9521/

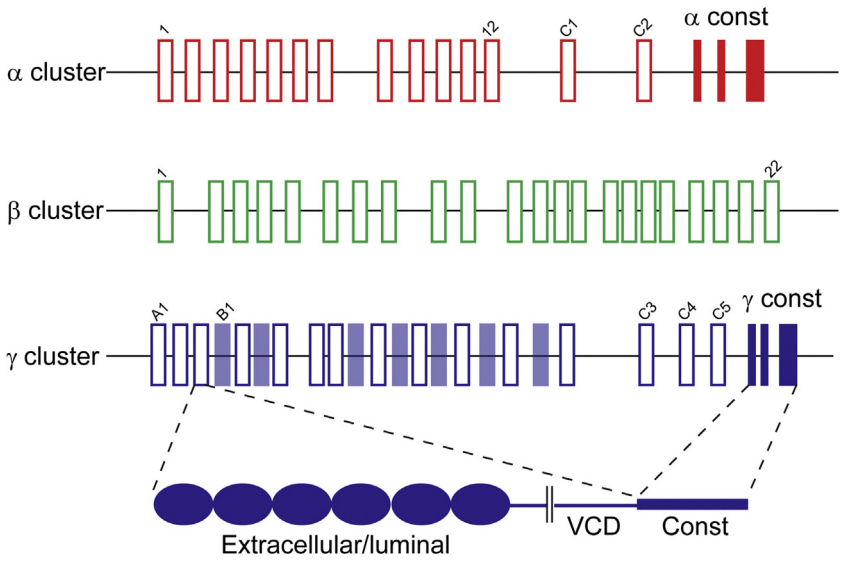

Fig. 1. Organization of the Pcdh gene cluster. The α, β and γ subclusters are arranged in tandem. Single exons (white or light blue bars; 14 in α, 22 in β and 22 in γ) encode the majority of each Pcdh molecule. There are "C-type" (C1-C5) exons in α and γ that encode molecules with less similarity to the other exons within their cluster. There are 2 additional subclasses of Pcdh-γs (γAs and γBs; white and light blue bars, respectively). At the end of the α and γ clusters are 3 constant exons that are spliced at the mRNA level to the individual Pcdh exons from the cluster. Thus the Pcdh-αs and -γs have their own identical cytoplasmic moiety appended to the carboxy terminus. Shown below is how one Pcdh-γ (γA3) is spliced to the constant exons. The relevant domains include the extracellular or luminal domain which contains the adhesive cadherin repeats, the portion of the cytoplasmic domain encoded by the individual or "variable" Pcdh exons (variable cytoplasmic domain, VCD) and the constant domain.
Pcdh nomenclature is from [3,4].

complexity necessary for at least a partial, if not primary role, in synaptogenic recognition and single cell identity. Thus, to understand how these molecules operate at the cellular level promises to fundamentally change how we view neural development and wiring and could shed new light on certain neurodevelopmental disorders.

2. Are Pcdhs adhesive?

One of the original criteria for assessing the activity of a putative adhesion molecule is to transfect the coding cDNA into cell lines that normally lack adhesion [11]. Early on, it was noted that one Pcdh-γ, Pcdh-γC3, exhibited weak adhesion in transfected cells [1] as compared to a classical cadherin. However, it was then noted that adhesion of this Pcdh increased dramatically when the cytoplasmic domain was replaced with that of a classical cadherin. This was the first indication that Pcdh cytoplasmic domains might negatively regulate adhesion. The K562 cell line was later found to be optimal for studying the adhesive interactions promoted by Pcdhs [12] and other groups have used these cells to work out the homophilic specificity of Pcdh interactions [9,10,13]. In these cells, Pcdh overexpression is able to promote significant cell aggregation while in others, the effect is less pronounced. In parallel, the binding interactions of Pcdh extracellular domains were characterized extensively by x-ray crystallography [10,13–15]. It is now very clear that Pcdhs exhibit homophilic binding via their cadherin repeats. However, this cell–cell binding activity has been difficult to reconcile with the behavior of the molecules in cells and neurons. In some cases, such as neuron-astrocyte interactions [16], as well as synaptic interactions within clonal neuron populations [8], Pcdhs seem to promote cell–cell interaction. On the other hand, at least for the case of dendrite self-avoidance, [17,18] Pcdhs induce the opposite of what is normally considered to be "cell adhesion". How is it possible that molecules which contain extracellular domains that should bind apposing membranes can actually cause the detachment and avoidance of the same interacting membranes, particularly when other stronger adhesive proteins are certain to co-exist on these membranes? The same question has been posed for another class of self-avoidance molecules, the Dscams in *Drosophilia* [19–21] but has only begun to be addressed at the cell biological level. It is possible that elaboration of the differences in the intracellular trafficking between Pcdhs and classical cadherins will provide insights into this question.

3. Pcdh localization at membrane contact points in neurons and other cells

An important criterion for evaluating the activity of putative cell adhesion molecules is their localization or recruitment to sites of membrane contact between cells. Classical cadherins, for example, typically line up precisely at cell junctions and are recruited to the synaptic cleft [22,23]. An antibody to the Pcdh-γ constant domain has been effective at localizing Pcdh-γs at the light and electron microscopic levels in hippocamapal neurons in culture and in vivo [24–26]. Early in development in vitro, as dendrites are beginning to extend, these neurons exhibit fine dendro-dendritic protrusions that span the space between same-cell dendrites. Very similar structures are seen in developing starburst amacrine cells in which Pcdh-γ dependent self-avoidance has been studied [17]. In hippocampal neurons, many of the points of contact between these "dendritic bridges" and the main dendrite exhibited a strong punctum of Pcdh-γ immunoreactivity [25] (Fig. 2). In contrast, N-cadherin immunoreactivity was more generalized along the dendrites at this stage and lacked the enrichment at dendritic bridge contacts [25]. Transfected Pcdhs can mimic the distribution of endogenous Pcdh-γs at dendritic bridges [25] (Fig. 2). The exact function of these bridges is currently unknown but it is possible that the bridges relate to proper dendrite self-avoidance or patterning. Similar bridging structures, albeit abnormally persistent, have been observed in Drosophilia Dscam mutants that lack the Dscam cytoplasmic domain and that also exhibit defective dendrite self-avoidance in vivo [19]. Interestingly, abnormal dendritic bridges were also revealed by the CLARITY method to be present in brains of autism patients [27].

Astrocytes express abundant Pcdh-γs [16] and most likely other clustered Pcdhs. It has been shown through targeted dele-

Please cite this article in press as: G.R. Phillips, et al., Clustered protocadherin trafficking, Semin Cell Dev Biol (2017), http://dx.doi.org/10.1016/j.semcdb.2017.05.001

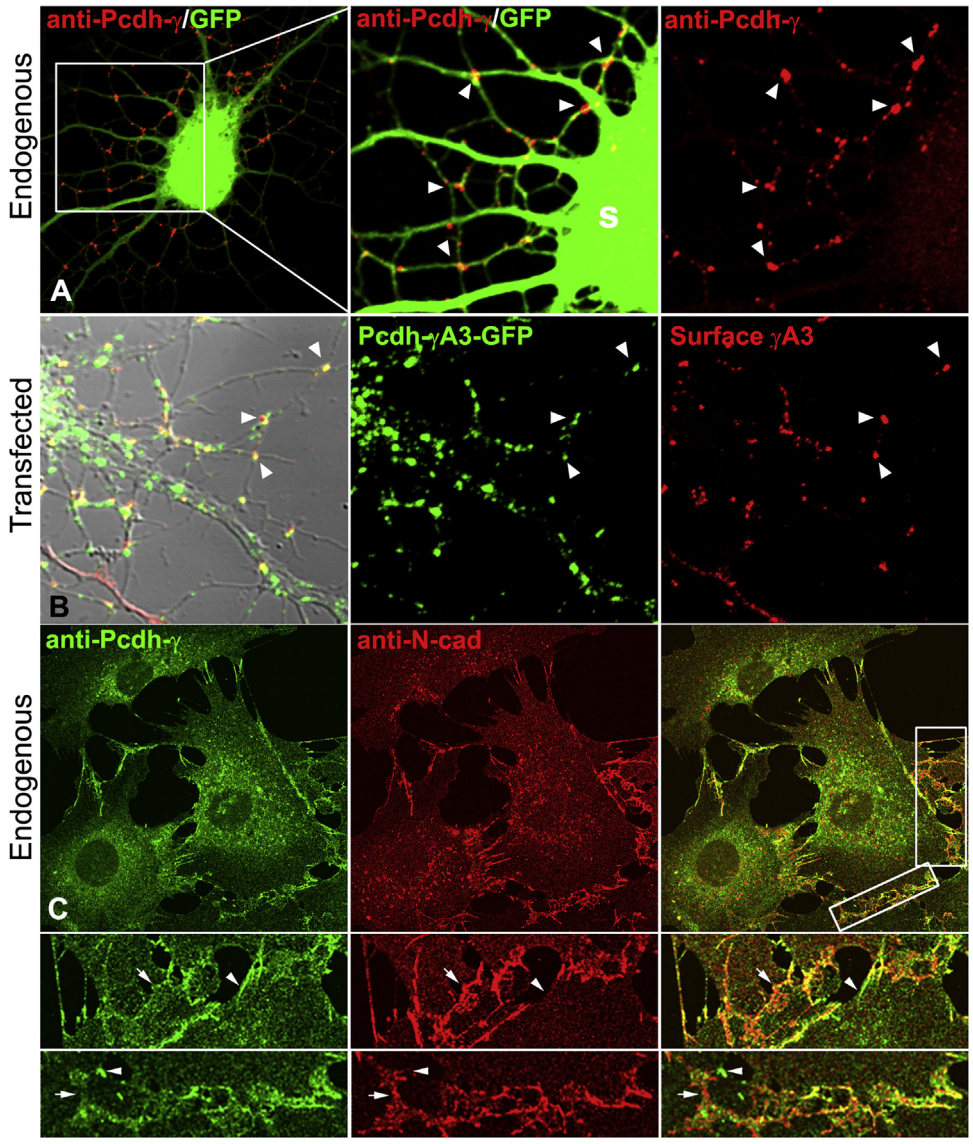

Fig. 2. Accumulation of Pcdh-γs at contact points in neurons and astrocytes. (A) During early development of hippocampal neurons in vitro, same cell dendrites make contact through dendritic bridges. Immunolocalization of Pcdh-γs using an affinity purified antibody to the constant domain shows that Pcdh-γs are precisely located at the contact point between a bridge and the main dendrite (arrowheads). (B) Transfected Pcdh-γA3-GFP mimics the distribution of native Pcdhs at bridge contacts in neurons. Surface expression of Pcdh-γA3 was detected by immunostaining with an extracellular antibody without permeabilization. Surface expression was mainly at bridge contacts (arrowheads). Pcdh-γA3 was enriched in organelles in the main dendrite, unlabeled with the surface antibody. (C) Astrocytes in culture also express abundant Pcdh-γs. Immunostaining with the constant domain antibody shows that the molecules can accumulate at cell–cell interfaces together with N-cadherin. Subtle differences in distribution of Pcdh-γs and N-cadherin were found at these interfaces (arrows and arrowheads, boxed regions).
A and B adapted from [24]. C, unpublished data.

tion or modification of the Pcdh-γ repertoire in astrocytes and neurons that homophilic astrocyte/neuron interactions can modulate dendrite arborziation and synaptogenesis [16,28]. The results from these studies are consistent with an adhesive role for the molecules in that a lack of astrocyte/neuron Pcdh interaction caused stunted dendrites and reduced synaptic density. Consistently, Pcdh-γ immunolocalization in astrocytes shows that the molecules accumulate quite prominently at cell–cell interfaces together with N-cadherin with subtle differences in the fine distribution of the two cadherins within cell contacts (Fig. 2). At these contacts, whether or not Pcdhs biochemically associate with classical cadherins or other adhesive molecules is still unresolved. One proteomic study of Pcdh-γ immunoisolated complexes identified the presence of classical cadherins in these complexes [29] but the functionality of the association remains to be studied.

The discovery of the Pcdh gene cluster initially generated great interest because of the potential for a long hypothesized a "synaptic adhesive code" [30] that would, at least in part, specify the pattern of synaptic connectivity between neurons. Some of the earliest studies on Pcdh localization were therefore focused on their distribution relative to the synapse. This was done both in vivo and in cultured neurons. At the light level, immunostaining experiments using antibodies to the GFP moiety appended to all Pcdh-γs in a knock-in mouse noted that Pcdhs could overlap with synaptic markers but that there was a substantial non-synaptic signal [31]. In dissociated cells, anti-Pcdh-γ constant domain antibodies also detected some synaptic overlap of Pcdh puncta but noted that the non-synaptic signal was non-diffuse, punctate, and located in a likely interior region of the dendrite [24].

Please cite this article in press as: G.R. Phillips, et al., Clustered protocadherin trafficking, Semin Cell Dev Biol (2017), http://dx.doi.org/10.1016/j.semcdb.2017.05.001

4. Pcdh localization in synaptic intracellular compartments

Postembedding immunogold electron microscopy has been highly effective for characterizing the unusual relationship of Pcdh-γs to the synapse. The localization of Pcdh-γs at the synapse was compared to that of N-cadherin, a known synaptic cleft molecule. These experiments have found, in agreement with light level studies, that Pcdh-γs were more enriched in axonal and spinous intracellular compartments versus the synaptic cleft, while, in contrast, N-cadherin was primarily located at the cleft [24,26]. The exact type of synaptic compartment that harbors Pcdh-γs is a topic of current investigation. One possibility, based on results from cultured cell experiments (see below), is that Pcdh-γs, and possibly other Pcdhs, are located in a type of recycling endosome compartment. Such compartments have been studied in dendrites and spines and are critical for the trafficking of glutamate [32] and other receptors [33] to the postsynaptic membrane in response to activity. When located in the dendrite shaft, some of these compartments are thought to serve as a local storage compartment for multiple spines [34]. It will be important to determine whether or not the Pcdhs are located in dendritic recycling endosomes and whether the molecules play a role in synapse development and function via these compartments. Axon bouton trafficking compartments and endosomes are less well characterized than those in dendrites and spines [35] but organelles distinct from synaptic vesicles appear to harbor Pcdh-γs in boutons [24].

Regardless of the exact type and function of Pcdh trafficking organelles in neurons, the results so far support the hypothesis that Pcdhs and classical cadherins mediate fundamentally distinct and possibly opposing cellular processes related to cell–cell interaction. One of the most outstanding questions might be whether or not Pcdhs play an "avoidance" role at the synapse, discouraging autapses and other inappropriate synapses, similar to their role at same-cell dendrites, or an adhesive role as originally envisioned, as well as how the unique Pcdh trafficking might fit into one or another role. New studies indicate that synapses between daughter clone neurons from the same precursor might be promoted by matching Pcdh interactions [8]. On the other hand, Pcdh-γs might play a role in synaptic pruning. Pcdh-γ inactivation in starburst amacrine cells causes exuberant synaptogenesis with increases in autapse formation in these cells [18] suggesting an anti-adhesive role at these synapses. Increased synapses upon Pcdh-γ disruption could also be due to an inhibitory interaction of Pcdh-γ ectodomains with the synaptogenic adhesion molecule neuroligin-1 [36] although Pcdh-γ adhesive binding is not involved in this interaction. Perhaps Pcdhs, stored within recycling organelles, can insert into nascent synapses from these compartments and modify the synaptic junction between neurons with matching Pcdh repertoires as had been proposed previously [37]. Storage in recycling organelles might ensure rapid delivery upon synaptic reorganization or plasticity as well as local retrieval and sorting. Depending on cell type, Pcdh linkages may then promote or destabilize the synapse.

5. Using cultured cells to study the mechanism of Pcdh trafficking

Cell culture experiments have been essential to unravel the function and dynamics of classical cadherins as well as other adhesion molecules. Cultured cells have also been used successfully to study Pcdh homophilic interactions. It may be fruitful to use cell cultures to examine the dynamics of Pcdhs at cell–cell contacts and their intracellular trafficking. In contrast to classical cadherins and as well as other adhesion molecules, Pcdhs induce weaker, yet detectable, cellular aggregation when transfected into cultured cells. One of the reasons for this weak adhesion may be that transfected Pcdhs are largely retained in intracellular compartments [25,38–40] much like native Pcdhs in synaptic compartments. Thus, understanding of Pcdh trafficking may shed light on the unique modes of cell–cell interaction mediated by these molecules. For Pcdh-γs, it has been possible to study trafficking of GFP fusions given the fact that appending GFP to the carboxy-terminus of all Pcdh-γs in knock-in mice does not interfere with function in vivo [17,31,41,42]. For Pcdh-γA3, the GFP fused full-length molecule accumulated strikingly within intracellular compartments in HEK293 cells and primary neurons. Inaccessibility to an antibody generated against the extracellular domain of Pcdh-γA3 was used to confirm the intracellular localization of most puncta in neurons [25,38,39]. Furthermore, this pattern was very similar of the punctate immunostaining pattern for Pcdh-γs in primary neurons [24,25].

Classical cadherin cytoplasmic interactions normally have a stabilizing effect on junctions by ultimately linking to the actin-based cytoskeleton. The role of Pcdh cytoplasmic interactions are less clear but trafficking appears to be significantly affected by the cytoplasmic domains. Upon removal of the cytoplasmic domain, transfected Pcdhs typically exhibit much greater surface expression where the extracellular domains could then engage in cell–cell binding. Cytoplasmic deletion of Pcdh-γA3 caused a dramatic accumulation at cell–cell junctions in HEK293 cells and at dendro-dendritic contact points in primary neurons [25]. Other groups found that cytoplasmic deletion of several Pcdhs induced greater cellular aggregation in the K562 assay [9,15] and early on it was shown that replacement of the Pcdh-γC3 cytoplasmic domain with a classical cadherin cytoplasmic domain greatly enhanced cell aggregation [1]. Thus, the cytoplasmic domains of most Pcdhs studied so far have a very clear negative influence on adhesive activity in most assays and this appears to be most likely due to the presence of cytoplasmic intracellular trafficking and retention signals.

Endocytosis [43] and intracellular retention could be a property that might help to explain the unique mode(s) of cell–cell interaction mediated by Pcdhs. Characterization of the trafficking mechanisms would then help to shed new light on the roles of the Pcdhs in neural development. Studies have therefore been undertaken to characterize the organelles that harbor Pcdhs and to pinpoint the signals present on the cytoplasmic domain that promote intracellular trafficking. A simple yet effective cellular system that has been used by others to characterize intracellular trafficking and recycling of various neural transmembrane receptors has been HEK293 cells [44]. Trafficking mechanisms in these cells can be similar to neurons at least for some molecules [33]. Using HEK293 cells, it was found that two transfected Pcdh-γs (-γA3 and -γB2) localized to organelles that were positive for the late endosome marker LAMP2 and/or for the autophagy marker LC3 [38,39]. Transfection experiments from other groups also revealed abundant punctate intracellular accumulation of Pcdhs in K562 cells [10]. Interestingly, a Pcdh-α (α1) completely avoided these compartments when transfected into cells as did Pcdh-γC4 [38]. Others have also noted the distinct trafficking of Pcdh-αs and -γC4 in transfection experiments [10]. Most importantly, Pcdh-γA3 and -γB2 were targeted very differently than the classical cadherin, N-cadherin, which could never co-cluster with LC3 and was minimally trafficked in LAMP2 positive organelles [38,39]. Cytoplasmic deletion of Pcdh-γA3, including the constant domain and most of the unique part of the cytoplasmic domain (variable cytoplasmic domain, VCD, see Fig. 1), which caused an increase in surface delivery, completely eliminated the trafficking to LAMP2 and LC3 positive organelles with prominent display at the interfaces between transfected cells [38,39]. Deletion of the constant domain only also promoted greater contact site localization [25] but the molecule could still cluster with LAMP2 and LC3 [38,39]. Of note, the constant domain contains polybasic residues together with a serine residue that can be

Please cite this article in press as: G.R. Phillips, et al., Clustered protocadherin trafficking, Semin Cell Dev Biol (2017), http://dx.doi.org/10.1016/j.semcdb.2017.05.001

phosphorylated [45]. This motif was shown to bind to phospholipids in a regulated manner and disruption of this motif caused deficiencies in Pcdh-γ function as it relates to dendrite arborization. Whether these residues of the constant domain play a role in Pcdh trafficking is currently under investigation.

There are a variety of endosomally related organelles that can be positive for LAMP2 and/or LC3. Ultrastructural techniques are necessary to provide additional information about the specific compartment type. Correlative light and electron microscopy (CLEM) has been a highly effective way to study trafficking of GFP fusions in endosomal or autophagy related compartments [46,47]. By CLEM, both Pcdh-γA3-GFP and -γB2-GFP positive areas within cells corresponded to massive accumulations of ~250 nm multivesicular bodies that were absent in control cells [38,39] (Fig. 3). These resembled late or recycling endosomes in that they were also associated with tubular projections [48,49]. Deletion of the cytoplasmic domain including the VCD and constant portions completely eliminated trafficking to these organelles [38]. When found inside the cell, the cytoplasmic deletion corresponded to whorled ER membranes instead of multivesicular bodies. Thus it is clear that a major function of the cytoplasmic domain for at least some Pcdhs is to direct the molecule to the endolysosome system.

The role of Pcdhs in multivesicular bodies is currently being investigated. One possibility is that Pcdhs could be secreted in the form of exosomes which originate from the fusion of the late endosome/multivesicular body with the plasma membrane. There is some evidence that Pcdhs can be detected in exosomes but further investigation is required [50–52]. On the other hand, tubulation of endosomes is a way to partition cargo from one subdomain of the endosome to another [49] for recycling back to the cell surface and the secretory pathway. There are accessory factors that participate in this partitioning [53]. The tubules that emanate from Pcdh-γ positive endosomes are strongly reminiscent of tubules involved in recycling of cargo from sorting endosomal compartments allowing the recycled molecules to avoid degradation. In particular, studies of beta-2 adrenergic receptor (β2AR) trafficking could provide a template to study how Pcdhs are trafficked and the significance of this trafficking [44]. Like Pcdhs, the trafficking of the β2AR has been characterized extensively in transfected HEK293 cells and in neurons [33,44]. Super-resolution microscopy has shown that β2AR becomes partitioned to tubules connected to endosomes and this partitioning is dependent on the retromer complex that mediates endosome to Golgi/plasma membrane recycling. Interestingly, β2AR and retromer are also markers for dendritic recycling endosome domains that provide a local supply of membrane proteins to dendritic microdomains. In preliminary studies, Pcdh-γA3 and the β2AR can colocalize in HEK293 cells (Fig. 3), particularly within internal compartments, suggesting the possibility that the two molecules share similar trafficking mechanisms.

Cytoplasmic interactions for classical cadherins are essential for function and have been studied for decades. We know relatively little about Pcdh cytoplasmic function despite the identification of some binding interactions [40,45,54,55]. It seems reasonable to propose that the sequences controlling Pcdh endolysosomal trafficking will likewise be important to characterize if we are to understand the distinctive mode of cell–cell interaction that Pcdhs promote. The clear differences between the full-length molecule and the cytoplasmic deletion, in terms of trafficking, provided a simple assay to map the cytoplasmic sequences that control how Pcdh-γA3, and other Pcdhs, traffic in the endolysosome system. Progressive cytoplasmic truncations were tested for their ability to colocalize with LAMP2 and LC3 in transfected cells. Surprisingly, it was found that the constant domain was not necessary for the molecule to colocalize with endolysosomal markers [39]. Further Pcdh-γA3 truncations into the VCD were evaluated for trafficking and it was found that deletion up until 163 amino acids from the carboxy-terminus of Pcdh-γA3 preserved colocalization with LAMP2 and LC3 but that deletion after 190 amino acids completely eliminated this colocalization. Elimination of endolysosomal trafficking correlated with more localization at cell–cell interfaces in an adhesive conformation.

This defined an amino acid segment within the VCD of Pcdh-γA3 critical for trafficking, almost identical for all Pcdh-γA molecules [39] (Fig. 4). Inspection of Pcdhs from the three subclusters starting at approximately 30 amino acids downstream from the transmembrane domain showed that there is indeed similarity amongst most clustered Pcdhs in the VCD trafficking region [40]. A highly conserved valine residue (valine 753 in Pcdh-γA3) can be used as an anchor to align the VCDs from other Pcdhs. Downstream from this residue is a cluster of conserved basic residues next to serines and threonines followed by a cluster of hydrophobic and aromatic amino acids. Just upstream of the conserved valine is another segment that contains a more divergent region still with common serines, tyrosines and glutamine residues. Detailed studies to examine the role of each of these residues in regulating Pcdh trafficking are now being conducted.

Earlier studies on Pcdh surface delivery noted that surface expression of a Pcdh-α was enhanced when coexpressed with a Pcdh-γ in HEK293 cells. The functional interaction in this study was mapped to the extracellular domain [56]. This finding has been confirmed and expanded on in structural studies which showed a *cis* interaction between the sixth cadherin repeats of various Pcdhs that can enhance surface delivery in cotransfected cells [10]. Exactly how extracellular *cis* interaction influences Pcdh surface delivery remains to be determined. However, it was also noted in the early studies that a cytoplasmic interaction between Pcdhs-α and -γ also exists that could also influence surface delivery [56]. This cytoplasmic binding activity was not mapped to a particular domain or set of residues in these Pcdhs. The possibility that the VCD trafficking motif mediates, at least part, of this cytoplasmic interaction was tested by co-immunoprecipitation experiments. It was found that both extracellular and cytoplasmic deletion Pcdh-γA3 constructs were equally effective at co-immunoprecipitating full length Pcdh-γA3 and γB2 in cotransfected cells confirming the extensive *cis* interactions that occur within the γ family between the same Pcdh as well as different Pcdhs. A surprising finding was that co-precipitation of full length Pcdh-γs was just as efficient when the interacting construct contained only the transmembrane and VCD region, lacking the constant and extracellular domains. Mapping of the VCD region that mediates cytoplasmic interaction found that approximately the same residues important for trafficking of Pcdh-γA3 were responsible for mediating cytoplasmic interaction of Pcdh-γA3 with itself and with -γB2. Further analysis showed that the Pcdh-γA3 VCD can interact with Pcdh-αs and -βs. We have termed the VCD region within Pcdh-γA3 and the similar segment from other Pcdhs, the "VCD motif" [40] (Fig. 4).

6. Pcdh endocytosis

In contrast to the characterization of Pcdh endolysosomal trafficking, much less is known about the behavior of the molecules when found at the cell surface. How do Pcdhs arrive at the endolysosome system and what does this trafficking have to do with the function of Pcdhs at the cell surface? A model for clustered Pcdh function may come from studies of the non-clustered protocadherin, Pcdh8, which can, in some cases, correlate with an apparent deregulation of adhesion. In hippocampal neurons, Pcdh8/Arcadlin/PAPC, initially identified as a molecule upregulated at the mRNA level in response to activity, was found to induce the removal of N-cadherin from synapses via endocytosis [57]. Another study found that Pcdh8 can induce N-cadherin

Please cite this article in press as: G.R. Phillips, et al., Clustered protocadherin trafficking, Semin Cell Dev Biol (2017), http://dx.doi.org/10.1016/j.semcdb.2017.05.001

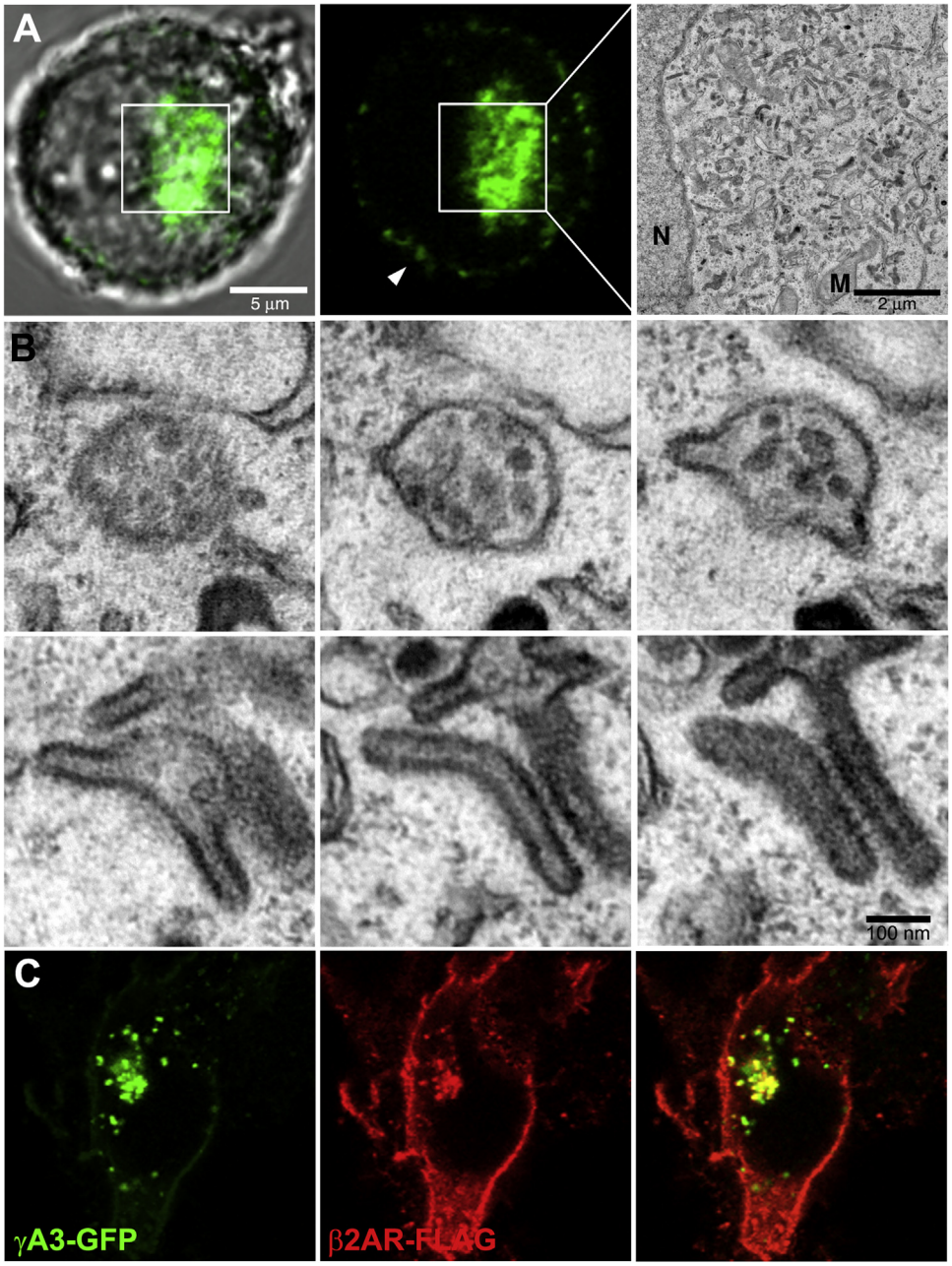

Fig. 3. Pcdh-γA3 positive trafficking organelles in HEK 293 cells. (A) CLEM of transfected cells. A massive accumulation of trafficking organelles is seen in the region of GFP fluorescence. (B) Serial section images through a single organelle observed in Pcdh-γA3 transfected cells. These organelles have characteristics of multivesicular bodies/late endosomes as well as tubules that are commonly found on recycling endosomes. (C) β2AR has been shown to exhibit prominent trafficking in recycling endosomes [33,44] and can colocalize with Pcdh-γA3 in transfected cells.

A from [35], B and C unpublished.

internalization during somitogenesis [58]. Whether this property of triggering endocytosis is unique to Pcdh8 or is also found in other non-clustered Pcdhs is unknown. Other non-clustered Pcdhs can deregulate or modify adhesion by pathways that may not involve endocytosis or the endolysosome system. For example, the autism associated Pcdh10 [59] interacts with ubiquitinated PSD-95 which is then subjected to degradation by the proteosome and which results in elimination of synapses [60]. Pcdh19, a non-clustered Pcdh linked to a female restricted epilepsy and mental retardation [61,62], can bind to N-cadherin and alter the homophilic specificity of adhesion [63].

Some data indicates that clustered Pcdhs themselves may also be prominent targets for endocytosis when displayed at the surface which could in part explain their low levels of surface expression, weak cell aggregation activity and accumulation in the endolysosome system. Pcdh-α and -γ families can be cleaved by γ-secretase, after extracellular cleavage, to generate a soluble cytoplasmic carboxy-terminal fragment (CTF) [12,64]. In some cases, the CTF can be translocated to the nucleus. It was shown that cleavage of a Pcdh-α and accumulation of the CTF was prevented by blocking endocytosis [43]. Other studies showed by live imaging of full length Pcdh-γA3 in hippocampal neurons that vesicles containing this molecule can be dynamically trafficked to and from synapses [25]. These mobile Pcdh vesicles were eliminated upon deletion of the cytoplasmic domain.

Please cite this article in press as: G.R. Phillips, et al., Clustered protocadherin trafficking, Semin Cell Dev Biol (2017), http://dx.doi.org/10.1016/j.semcdb.2017.05.001

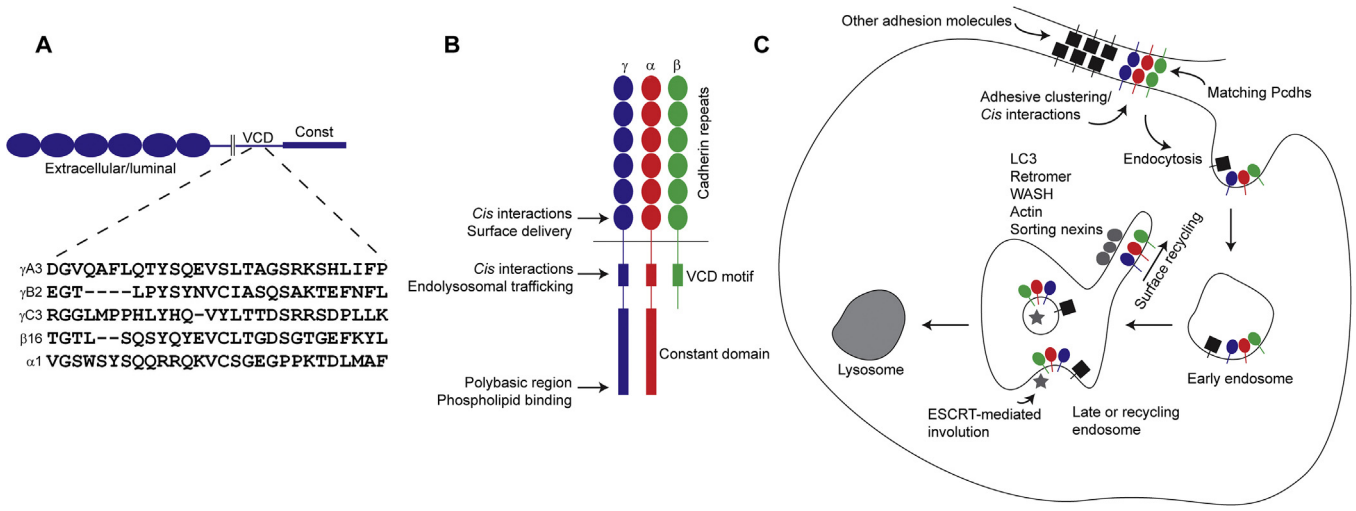

Fig. 4. Model for Pcdh trafficking with respect to function. (A) Segment of the VCD found to be critical for trafficking in the endolysosome system and for cytoplasmic *cis* interactions (VCD motif) [39,40]. Segment was initially mapped in Pcdh-γA3 and similarities are found in other Pcdhs. (B) Identified Pcdh domains with a role in trafficking. Extracellular cadherin repeats 1–4 are involved in adhesion while the cadherin repeat 6 mediates *cis* interaction that can promote surface delivery [10,13]. On the cytoplasmic side, the VCD motif can participate in *cis* interactions and this segment is also important for endolysosomal trafficking of the molecule. At the carboxy terminus of the constant domains for Pcdh-γs and -αs is a polybasic region that, for Pcdh-γs, can interact with phospholipids with effects on signaling [45]. It is possible that the polybasic region can also influence Pcdh trafficking. (C) A model for how trafficking might alter adhesion. Adhesion between membranes expressing matching Pcdhs will cluster Pcdhs and other adhesion molecules at the junction. Under certain conditions, Pcdh adhesive clustering and/or *cis* interaction might trigger membrane curvature and endocytosis [43] which could also cause the internalization of other adhesion molecules. Trafficking of the internalized complex would be directed to the late or recycling endosome via trafficking signals in the Pcdh VCD motif. Here, Pcdhs might be involuted into interior vesicles of the multivesicular body due to the association with endosomal sorting complexes required for transport (ESCRT complex) [43]. These interior vesicles, containing both Pcdhs and other adhesion molecules, could be destined for degradation in the lysosome or possibly secreted as exosomes. This could essentially be a mechanism by which Pcdhs downregulate adhesion after surface membrane recognition. Based on the ultrastructure of Pcdh containing organelles, Pcdhs may be sorted into tubules that emanate from the endosome and which could indicate recycling to the cell surface. Potential intracellular markers that are being used to study whether Pcdhs are recycled to the surface are indicated. These include LC3, which plays a role in tubulation, as well as the retromer, WASH (WASP and SCAR homologue) and sorting nexin complexes, involved in sorting cargo to endosomal subdomains. Actin can also play a role in sorting cargo in endosomes.

7. A model for assembly and trafficking of the Pcdh complex

Some combination of Pcdh-αs, βs and -γs are thought to generate a heteromeric complex that is the basis for an "adhesive unit", with an enormous number of combinations and specificity [9,10]. How might this complex be assembled? Most Pcdh-γs, as well as -βs (unpublished observations), target efficiently to the endolysosome system in transfected cells. In contrast, Pcdh-αs appear to have a completely different trafficking pattern. Targeting to the various compartments appears to be affected by different combinations of Pcdhs. Earlier studies had shown that a Pcdh-α was directed to a cytoplasmic compartment when overexpressed and that co-transfection with a Pcdh-γ could increase the trafficking of Pcdh-α to the cell surface [56]. CLEM on transfected cells confirmed the ER-like localization Pcdh-α1 and the striking differences in trafficking between Pcdh-γs and Pcdh-αs [38]. It has been suggested that Pcdh-α/γ *cis*-binding in the ER may be a factor in the export of the Pcdh-α/β/γ complex to the surface, ensuring that the entire Pcdh complex is properly assembled prior to surface display [56]. Extracellular Pcdh-α/γ *cis*-interaction was found to be important for promotion of Pcdh-α surface localization and a cytoplasmic interaction between the two also participated in trafficking. It remains to be determined if interaction between Pcdh-αs and -γs at the VCD motif can also influence Pcdh-α trafficking from the ER. It is notable that the Pcdh-α VCD motif contains a group of three basic residues [40] that are similar to other segments which may function as an ER retention motif, and that can be masked upon complex formation [65].

How might the distinct trafficking exhibited by Pcdhs relate to their role at cell surfaces? We know that adhesive activity for the Pcdh extracellular domains can be detected in cell aggregation assays and also that the adhesive interfaces for several Pcdhs have been described by x-ray crystallography. In addition, replacement or deletion of the cytoplasmic domains from some Pcdhs can have a positive effect on adhesion. Therefore, the cytoplasmic domains of at least some Pcdhs have a clear negative effect on adhesive activity and their role appears to be intracellular retention. This situation is very unlike the classical cadherins where the cytoplasmic domain has additional stabilizing activity towards adhesion. Therefore, what is the mode(s) of action for Pcdhs in cell–cell interaction and how might this relate to self-avoidance, which is by definition a process that must involve detachment? It is possible that endolysosomal trafficking, which is distinct for the clustered Pcdhs, may be part of the mechanism behind the self-avoidance activity for these molecules. In contrast to avoidance mechanisms operative during axon outgrowth, which involve the turning of growth cones, self-avoidance and tiling of dendrites appears to involve a "sorting" process. For starburst amacrine cells, same cell dendrites appear to be connected by dendritic bridges that eventually become sorted out during postnatal development [17]. These dendrites are certain to express strong adhesion molecules from both the cadherin and immunoglobulin superfamily, among others. Adhesion through pro-adhesive molecules on same-cell dendrites must be deactivated or downregulated for same-cell dendrites to detach and tile. It could be that adhesion between same-cell dendrites is downregulated by endocytosis triggered by the extensive matching Pcdh contacts that must exist on same-cell membranes (Fig. 4C), with subsequent trafficking of resultant organelles to the endolysosome system directed via the trafficking motifs in Pcdh VCDs or elsewhere. Pro-adhesive molecules might then be subjected to degradation in lysosomes while Pcdhs might be recycled back to the surface as suggested by the tubulated morphology of Pcdh-associated endosomes. Endocytosis of Pcdhs may be tightly regulated such that the molecules can be converted from adhe-

Please cite this article in press as: G.R. Phillips, et al., Clustered protocadherin trafficking, Semin Cell Dev Biol (2017), http://dx.doi.org/10.1016/j.semcdb.2017.05.001

sive to anti-adhesive by activating endocytic and endolysosomal trafficking signals on Pcdh cytoplasmic domains.

Acknowledgements

Work in the GRP lab has been funded by grants from the NIH (R21NS048367, R01NS051238 and R03MH110980), the Irma T. Hirschl Charitable Trust, the PSC-CUNY, and a Provost's Research Award from the College of Staten Island.

References

[1] H. Obata, N. Sago, J.M. Mori, M.F. Rochelle, M. Seldin, S. St John, S.T. Suzuki, Protocadherin Pcdh2 shows properties similar to, but distinct from, those of classical cadherins, J. Cell Sci. 108 (Pt 12) (1995) 3765–3773.
[2] N. Kohmura, K. Senzaki, S. Hamada, N. Kai, R. Yasuda, M. Watanabe, H. Ishii, M. Yasuda, M. Mishina, T. Yagi, Diversity revealed by a novel family of cadherins expressed in neurons at a synaptic complex, Neuron 20 (6) (1998) 1137–1151.
[3] Q. Wu, T. Maniatis, A striking organization of a large family of human neural cadherin-like cell adhesion genes, Cell 97 (6) (1999) 779–790.
[4] Q. Wu, T. Zhang, J.F. Cheng, Y. Kim, J. Grimwood, J. Schmutz, M. Dickson, J.P. Noonan, M.Q. Zhang, R.M. Myers, T. Maniatis, Comparative DNA sequence analysis of mouse and human protocadherin gene clusters, Genome Res. 11 (3) (2001) 389–404.
[5] B. Tasic, C.E. Nabholz, K.K. Baldwin, Y. Kim, E.H. Rueckert, S.A. Ribich, P. Cramer, Q. Wu, R. Axel, T. Maniatis, Promoter choice determines splice site selection in protocadherin alpha and gamma pre-mRNA splicing, Mol. Cell 10 (1) (2002) 21–33.
[6] M. Kawaguchi, T. Toyama, R. Kaneko, T. Hirayama, Y. Kawamura, T. Yagi, Relationship between DNA methylation states and transcription of individual isoforms encoded by the protocadherin-alpha gene cluster, J. Biol. Chem. 283 (18) (2008) 12064–12075.
[7] S. Toyoda, M. Kawaguchi, T. Kobayashi, E. Tarusawa, T. Toyama, M. Okano, M. Oda, H. Nakauchi, Y. Yoshimura, M. Sanbo, M. Hirabayashi, T. Hirayama, T. Hirabayashi, T. Yagi, Developmental epigenetic modification regulates stochastic expression of clustered protocadherin genes, generating single neuron diversity, Neuron 82 (1) (2014) 94–108.
[8] E. Tarusawa, M. Sanbo, A. Okayama, T. Miyashita, T. Kitsukawa, T. Hirayama, T. Hirabayashi, S. Hasegawa, R. Kaneko, S. Toyoda, T. Kobayashi, M. Kato-Itoh, H. Nakauchi, M. Hirabayashi, T. Yagi, Y. Yoshimura, Establishment of high reciprocal connectivity between clonal cortical neurons is regulated by the Dnmt3b DNA methyltransferase and clustered protocadherins, BMC Biol. 14 (1) (2016) 103.
[9] D. Schreiner, J.A. Weiner, Combinatorial homophilic interaction between gamma-protocadherin multimers greatly expands the molecular diversity of cell adhesion, Proc. Natl. Acad. Sci. U. S. A. 107 (33) (2016) 14893–14898.
[10] C.A. Thu, W.V. Chen, R. Rubinstein, M. Chevee, H.N. Wolcott, K.O. Felsovalyi, J.C. Tapia, L. Shapiro, B. Honig, T. Maniatis, Single-cell identity generated by combinatorial homophilic interactions between alpha, beta, and gamma protocadherins, Cell 158 (5) (2014) 1045–1059.
[11] A. Nose, A. Nagafuchi, M. Takeichi, Expressed recombinant cadherins mediate cell sorting in model systems, Cell 54 (7) (1988) 993–1001.
[12] K. Reiss, T. Maretzky, I.G. Haas, M. Schulte, A. Ludwig, M. Frank, P. Saftig, Regulated ADAM10-dependent ectodomain shedding of gamma-protocadherin C3 modulates cell–cell adhesion, J. Biol. Chem. 281 (31) (2006) 21735–21744.
[13] R. Rubinstein, C.A. Thu, K.M. Goodman, H.N. Wolcott, F. Bahna, S. Mannepalli, G. Ahlsen, M. Chevee, A. Halim, H. Clausen, T. Maniatis, L. Shapiro, B. Honig, Molecular logic of neuronal self-recognition through protocadherin domain interactions, Cell 163 (3) (2015) 629–642.
[14] K.M. Goodman, R. Rubinstein, C.A. Thu, F. Bahna, S. Mannepalli, G. Ahlsen, C. Rittenhouse, T. Maniatis, B. Honig, L. Shapiro, Structural basis of diverse homophilic recognition by clustered alpha- and beta-protocadherins, Neuron 90 (4) (2016) 709–723.
[15] K.M. Goodman, R. Rubinstein, C.A. Thu, S. Mannepalli, F. Bahna, G. Ahlsen, C. Rittenhouse, T. Maniatis, B. Honig, L. Shapiro, gamma-Protocadherin structural diversity and functional implications, Elife 5 (2016).
[16] A.M. Garrett, J.A. Weiner, Control of CNS synapse development by {gamma}-protocadherin-mediated astrocyte-neuron contact, J. Neurosci. 29 (38) (2009) 11723–11731.
[17] J.L. Lefebvre, D. Kostadinov, W.V. Chen, T. Maniatis, J.R. Sanes, Protocadherins mediate dendritic self-avoidance in the mammalian nervous system, Nature 488 (7412) (2012) 517–521.
[18] D. Kostadinov, J.R. Sanes, Protocadherin-dependent dendritic self-avoidance regulates neural connectivity and circuit function, Elife 4 (2015).
[19] B.J. Matthews, M.E. Kim, J.J. Flanagan, D. Hattori, J.C. Clemens, S.L. Zipursky, W.B. Grueber, Dendrite self-avoidance is controlled by Dscam, Cell 129 (3) (2007) 593–604.
[20] W.B. Grueber, A. Sagasti, Self-avoidance and tiling: mechanisms of dendrite and axon spacing, Cold Spring Harb. Perspect. Biol. 2 (9) (2010) a001750.
[21] S.L. Zipursky, W.B. Grueber, The molecular basis of self-avoidance, Annu. Rev. Neurosci. 36 (2013) 547–568.
[22] A.M. Fannon, D.R. Colman, A model for central synaptic junctional complex formation based on the differential adhesive specificities of the cadherins, Neuron 17 (3) (1996) 423–434.
[23] N. Uchida, Y. Honjo, K.R. Johnson, M.J. Wheelock, M. Takeichi, The catenin/cadherin adhesion system is localized in synaptic junctions bordering transmitter release zones, J. Cell Biol. 135 (3) (1996) 767–779.
[24] G.R. Phillips, H. Tanaka, M. Frank, A. Elste, L. Fidler, D.L. Benson, D.R. Colman, Gamma-protocadherins are targeted to subsets of synapses and intracellular organelles in neurons, J. Neurosci. 23 (12) (2003) 5096–5104.
[25] M. Fernandez-Monreal, S. Kang, G.R. Phillips, Gamma-protocadherin homophilic interaction and intracellular trafficking is controlled by the cytoplasmic domain in neurons, Mol. Cell. Neurosci. 40 (3) (2009) 344–353.
[26] M. Fernandez-Monreal, T. Oung, H.H. Hanson, R. O'Leary, W.G. Janssen, G. Dolios, R. Wang, G.R. Phillips, Gamma-protocadherins are enriched and transported in specialized vesicles associated with the secretory pathway in neurons, Eur. J. Neurosci. 32 (6) (2010) 921–931.
[27] K. Chung, J. Wallace, S.Y. Kim, S. Kalyanasundaram, A.S. Andalman, T.J. Davidson, J.J. Mirzabekov, K.A. Zalocusky, J. Mattis, A.K. Denisin, S. Pak, H. Bernstein, C. Ramakrishnan, L. Grosenick, V. Gradinaru, K. Deisseroth, Structural and molecular interrogation of intact biological systems, Nature 497 (7449) (2013) 332–337.
[28] M.J. Molumby, A.B. Keeler, J.A. Weiner, Homophilic protocadherin cell–cell interactions promote dendrite complexity, Cell Rep. 15 (5) (2016) 1037–1050.
[29] M.H. Han, C. Lin, S. Meng, X. Wang, Proteomics analysis reveals overlapping functions of clustered protocadherins, Mol. Cell. Proteomics 9 (1) (2010) 71–83.
[30] L. Shapiro, D.R. Colman, The diversity of cadherins and implications for a synaptic adhesive code in the CNS, Neuron 23 (3) (1999) 427–430.
[31] X. Wang, J.A. Weiner, S. Levi, A.M. Craig, A. Bradley, J.R. Sanes, Gamma protocadherins are required for survival of spinal interneurons, Neuron 36 (5) (2002) 843–854.
[32] M. Park, E.C. Penick, J.G. Edwards, J.A. Kauer, M.D. Ehlers, Recycling endosomes supply AMPA receptors for LTP, Science 305 (5692) (2004) 1972–1975.
[33] R.W. Choy, M. Park, P. Temkin, B.E. Herring, A. Marley, R.A. Nicoll, M. von Zastrow, Retromer mediates a discrete route of local membrane delivery to dendrites, Neuron 82 (1) (2014) 55–62.
[34] J.R. Cooney, J.L. Hurlburt, D.K. Selig, K.M. Harris, J.C. Fiala, Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane, J. Neurosci. 22 (6) (2002) 2215–2224.
[35] K.M. Harris, R.J. Weinberg, Ultrastructure of synapses in the mammalian brain, Cold Spring Harb. Perspect Biol. 4 (5) (2012).
[36] M.J. Molumby, R.M. Anderson, D.J. Newbold, N.K. Koblesky, A.M. Garrett, D. Schreiner, J.J. Radley, J.A. Weiner, Gamma-protocadherins interact with neuroligin-1 and negatively regulate dendritic spine morphogenesis, Cell Rep. 18 (11) (2017) 2702–2714.
[37] J.D. Jontes, G.R. Phillips, Selective stabilization and synaptic specificity: a new cell-biological model, Trends Neurosci. 29 (4) (2006) 186–191.
[38] H.H. Hanson, S. Kang, M. Fernandez-Monreal, T. Oung, M. Yildirim, R. Lee, K. Suyama, R.B. Hazan, G.R. Phillips, LC3-dependent intracellular membrane tubules induced by gamma-protocadherins A3 and B2: A role for intraluminal interactions, J. Biol. Chem. 285 (2010) 20982–20992.
[39] R. O'Leary, J. Reilly, H.H. Hanson, S. Kang, N. Lou, G.R. Phillips, A variable cytoplasmic domain segment is necessary for gamma-protocadherin trafficking and tubulation in the endosome/lysosome pathway, Mol. Biol. Cell 22 (22) (2011) 4362–4372.
[40] A. Shonubi, C. Roman, G.R. Phillips, The clustered protocadherin endolysosomal trafficking motif mediates cytoplasmic association, BMC Cell Biol. 16 (1) (2015) 28.
[41] J.A. Weiner, X. Wang, J.C. Tapia, J.R. Sanes, Gamma protocadherins are required for synaptic development in the spinal cord, Proc. Natl. Acad. Sci. U. S. A. 102 (1) (2005) 8–14.
[42] J.L. Lefebvre, Y. Zhang, M. Meister, X. Wang, J.R. Sanes, {gamma}-Protocadherins regulate neuronal survival but are dispensable for circuit formation in retina, Development 135 (24) (2008) 4141–4151.
[43] S.M. Buchanan, S.S. Schalm, T. Maniatis, Proteolytic processing of protocadherin proteins requires endocytosis, Proc. Natl. Acad. Sci. U. S. A. 107 (41) (2010) 17774–17779.
[44] M.A. Puthenveedu, B. Lauffer, P. Temkin, R. Vistein, P. Carlton, K. Thorn, J. Taunton, O.D. Weiner, R.G. Parton, M. von Zastrow, Sequence-dependent sorting of recycling proteins by actin-stabilized endosomal microdomains, Cell 143 (5) (2010) 761–773.
[45] A.B. Keeler, D. Schreiner, J.A. Weiner, Protein kinase C phosphorylation of a gamma-protocadherin C-terminal lipid binding domain regulates focal adhesion kinase inhibition and dendrite arborization, J. Biol. Chem. 290 (34) (2015) 20674–20686.
[46] M. Razi, S.A. Tooze, Correlative light and electron microscopy, Methods Enzymol. 452 (2009) 261–275.
[47] H.H. Hanson, J.E. Reilly, R. Lee, W.G. Janssen, G.R. Phillips, Streamlined embedding of cell monolayers on gridded live imaging dishes for correlative light and electron microscopy, Microsc. Microanal. 20 (2010) 1–8.
[48] H.J. Geuze, J.W. Slot, G.J. Strous, H.F. Lodish, A.L. Schwartz, Intracellular site of asialoglycoprotein receptor-ligand uncoupling: double-label immunoelectron microscopy during receptor-mediated endocytosis, Cell 32 (1) (1983) 277–287.

Please cite this article in press as: G.R. Phillips, et al., Clustered protocadherin trafficking, Semin Cell Dev Biol (2017), http://dx.doi.org/10.1016/j.semcdb.2017.05.001

[49] J.R. Goldenring, Recycling endosomes, Curr. Opin. Cell Biol. 35 (2015) 117–122.
[50] M. Gonzalez-Begne, B. Lu, X. Han, F.K. Hagen, A.R. Hand, J.E. Melvin, J.R. Yates, Proteomic analysis of human parotid gland exosomes by multidimensional protein identification technology (MudPIT), J. Proteome Res. 8 (3) (2009) 1304–1314.
[51] K. Carayon, K. Chaoui, E. Ronzier, I. Lazar, J. Bertrand-Michel, V. Roques, S. Balor, F. Terce, A. Lopez, L. Salome, E. Joly, Proteolipidic composition of exosomes changes during reticulocyte maturation, J. Biol. Chem. 286 (39) (2011) 34426–34439.
[52] T.A. Reinhardt, R.E. Sacco, B.J. Nonnecke, J.D. Lippolis, Bovine milk proteome: quantitative changes in normal milk exosomes, milk fat globule membranes and whey proteomes resulting from Staphylococcus aureus mastitis, J. Proteomics 82 (2013) 141–154.
[53] M.N.J. Seaman, The retromer complex –endosomal protein recycling and beyond, J. Cell Sci. 125 (20) (2013) 4693–4702.
[54] J. Chen, Y. Lu, S. Meng, M.H. Han, C. Lin, X. Wang, Alpha- and gamma-protocadherins negatively regulate PYK2, J. Biol. Chem. 284 (5) (2009) 2880–2890.
[55] C. Lin, S. Meng, T. Zhu, X. Wang, PDCD10/CCM3 acts downstream of {gamma}-protocadherins to regulate neuronal survival, J. Biol. Chem. 285 (53) (2010) 41675–41685.
[56] Y. Murata, S. Hamada, H. Morishita, T. Mutoh, T. Yagi, Interaction with protocadherin-gamma regulates the cell surface expression of protocadherin-alpha, J. Biol. Chem. 279 (47) (2004) 49508–49516.
[57] S. Yasuda, H. Tanaka, H. Sugiura, K. Okamura, T. Sakaguchi, U. Tran, T. Takemiya, A. Mizoguchi, Y. Yagita, T. Sakurai, E.M. De Robertis, K. Yamagata, Activity-induced protocadherin arcadlin regulates dendritic spine number by triggering N-cadherin endocytosis via TAO2beta and p38 MAP kinases, Neuron 56 (3) (2007) 456–471.
[58] J. Chal, C. Guillot, O. Pourquie, PAPC couples the segmentation clock to somite morphogenesis by regulating N-cadherin dependent adhesion, Development 144 (4) (2017) 664–676.
[59] E.M. Morrow, S.Y. Yoo, S.W. Flavell, T.K. Kim, Y. Lin, R.S. Hill, N.M. Mukaddes, S. Balkhy, G. Gascon, A. Hashmi, S. Al-Saad, J. Ware, R.M. Joseph, R. Greenblatt, D. Gleason, J.A. Ertelt, K.A. Apse, A. Bodell, J.N. Partlow, B. Barry, H. Yao, K. Markianos, R.J. Ferland, M.E. Greenberg, C.A. Walsh, Identifying autism loci and genes by tracing recent shared ancestry, Science 321 (5886) (2008) 218–223.
[60] N.P. Tsai, J.R. Wilkerson, W. Guo, M.A. Maksimova, G.N. DeMartino, C.W. Cowan, K.M. Huber, Multiple autism-linked genes mediate synapse elimination via proteasomal degradation of a synaptic scaffold PSD-95, Cell 151 (7) (2012) 1581–1594.
[61] L.M. Dibbens, P.S. Tarpey, K. Hynes, M.A. Bayly, I.E. Scheffer, R. Smith, J. Bomar, E. Sutton, L. Vandeleur, C. Shoubridge, S. Edkins, S.J. Turner, C. Stevens, S. O'Meara, C. Tofts, S. Barthorpe, G. Buck, J. Cole, K. Halliday, D. Jones, R. Lee, M. Madison, T. Mironenko, J. Varian, S. West, S. Widaa, P. Wray, J. Teague, E. Dicks, A. Butler, A. Menzies, A. Jenkinson, R. Shepherd, J.F. Gusella, Z. Afawi, A. Mazarib, M.Y. Neufeld, S. Kivity, D. Lev, T. Lerman-Sagie, A.D. Korczyn, C.P. Derry, G.R. Sutherland, K. Friend, M. Shaw, M. Corbett, H.G. Kim, D.H. Geschwind, P. Thomas, E. Haan, S. Ryan, S. McKee, S.F. Berkovic, P.A. Futreal, M.R. Stratton, J.C. Mulley, J. Gecz, X-linked protocadherin 19 mutations cause female-limited epilepsy and cognitive impairment, Nat. Genet. 40 (6) (2008) 776–781.
[62] C. Depienne, D. Bouteiller, B. Keren, E. Cheuret, K. Poirier, O. Trouillard, B. Benyahia, C. Quelin, W. Carpentier, S. Julia, A. Afenjar, A. Gautier, F. Rivier, S. Meyer, P. Berquin, M. Helias, I. Py, S. Rivera, N. Bahi-Buisson, I. Gourfinkel-An, C. Cazeneuve, M. Ruberg, A. Brice, R. Nabbout, E. Leguern, Sporadic infantile epileptic encephalopathy caused by mutations in PCDH19 resembles Dravet syndrome but mainly affects females, PLoS Genet. 5 (2) (2009) e1000381.
[63] M.R. Emond, S. Biswas, C.J. Blevins, J.D. Jontes, A complex of Protocadherin-19 and N-cadherin mediates a novel mechanism of cell adhesion, J. Cell Biol. 195 (7) (2011) 1115–1121.
[64] I.G. Haas, M. Frank, N. Veron, R. Kemler, Presenilin-dependent processing and nuclear function of gamma-protocadherins, J. Biol. Chem. 280 (10) (2005) 9313–9319.
[65] K. Michelsen, H. Yuan, B. Schwappach, Hide and run. Arginine-based endoplasmic-reticulum-sorting motifs in the assembly of heteromultimeric membrane proteins, EMBO Rep. 6 (8) (2005) 717–722.

Please cite this article in press as: G.R. Phillips, et al., Clustered protocadherin trafficking, Semin Cell Dev Biol (2017), http://dx.doi.org/10.1016/j.semcdb.2017.05.001