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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

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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.

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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).

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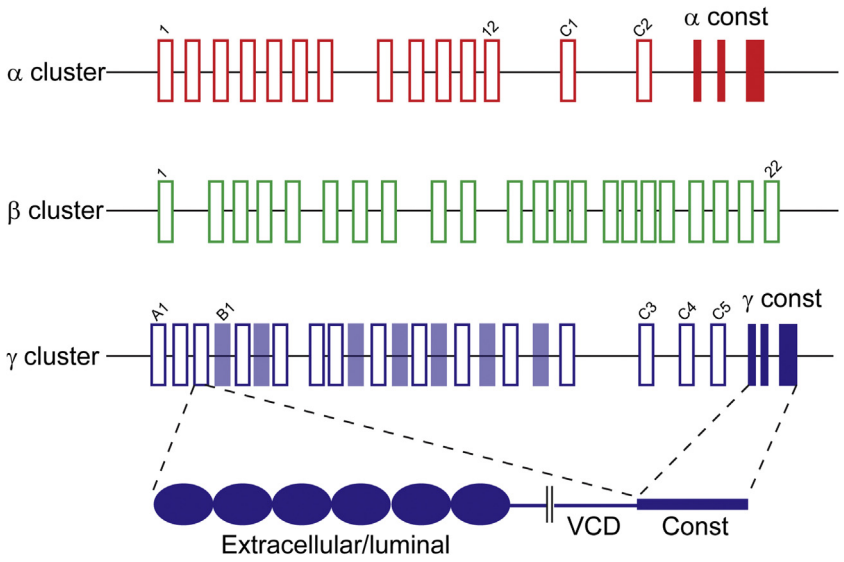

Fig. 1. Organization of the Pcdh gene cluster. The α, β and γ subclusters are arranged in tandem. Single exons (white or light blue bars; 14 in α, 22 in β and 22 in γ) encode the majority of each Pcdh molecule. There are "C-type" (C1-C5) exons in α and γ that encode molecules with less similarity to 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-