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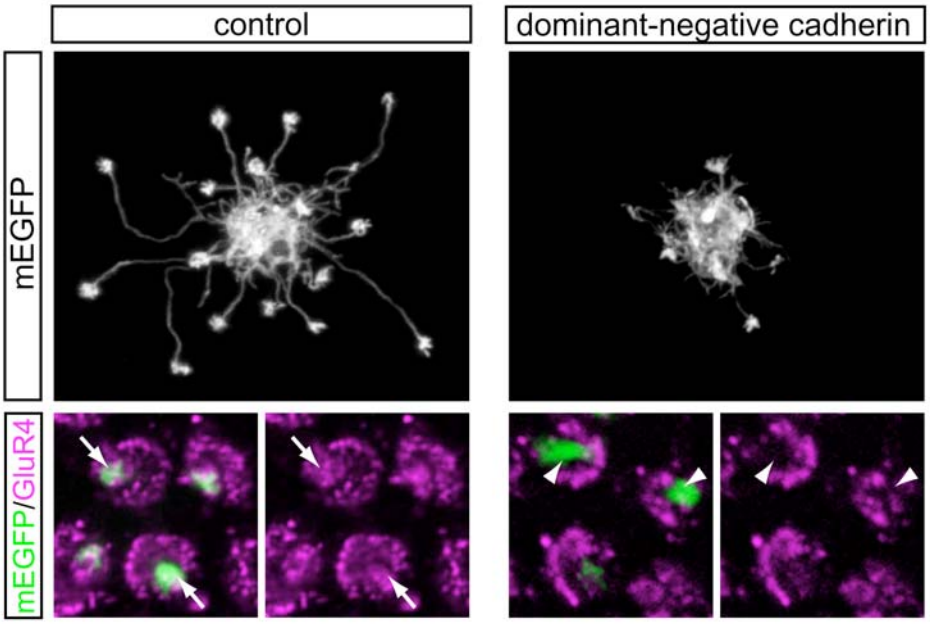
Keeping an eye on cadherin function in retinal synaptogenesis in vivo

October 31, 2006 – The role of cadherin cell-cell adhesion machinery in regulating the organization of the vertebrate synapse has been amply shown in cultured neurons, but has yet to be demonstrated directly in vivo. Invertebrate models, such as the fruit fly *Drosophila melanogaster* have provided evidence that cadherins and associated molecules regulate the numerous aspects of the structure and activity of the cellular processes known as dendrites, which extend from the cell body, or soma, to form synaptic connections and receive signals transmitted from other neurons. But the difficulty of performing and observing the effects of genetic manipulations in the intact vertebrate nervous system have prevented researchers from confirming what the in vitro data suggest – that cadherins play similar roles in vertebrate synapses.

Now, in an article published in the journal *Development*, Koji Tanabe and colleagues in the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi; Group Director) report the use of a new gene transfer system that enabled them to study the function of cadherins in horizontal cells in the embryonic retina in chicken. These cells, which receive input from photoreceptors and modulate intercellular feedback in the retinal network, form elaborate dendritic fields that enable them to make contacts with multiple surrounding neurons. Tanabe, who later moved to the RIKEN Frontier Research Program Nakagawa Research Initiative Unit (Shinichi Nakagawa; Unit Leader), found that in neurons in which cadherin function had been lost, the size of these fields was smaller and that the individual dendrites frequently failed to connect properly with photoreceptor cells.



Overexpression of dominant negative cadherin inhibits dendrite growth and disrupts the morphologies of dendritic terminals (Upper panels, TypeIII horizontal cells are shown). Cadherin blockage also perturbs synapse formation. Although the synapse marker GluR4 accumulates strongly on dendritic terminals of control cells (arrows), the accumulation is perturbed in dominant-negative cadherin expressing cells (arrowheads).

This success in observing cellular phenotypes in living tissue was made possible by their development of a novel transposon-based system of conditional transgenesis. To enable the visualization of horizontal cells in vivo, the group first used this

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method to introduce a combination of plasmids whose net effect was to allow the group to observe this specific subset of the retinal cellular population labeled by the membrane-targeted enhanced green fluorescent protein (mEGFP) at single-cell resolution. Looking at late-stage chick embryos, they discovered that they were able to classify these cells by shape. In this taxonomy, Type I horizontal cells possess axons and have small, bushy dendritic fields; Type II cells lack axons long, sinuous dendrites covering a wider field; while Type III, also axonless, exhibited what the authors describe as a “candelabrum” structure formed by single-branched dendrites.

The ability to observe horizontal cells as they developed in their natural habitat enabled the group to watch as the initially indistinguishable cells gradually transformed, extending dendrites that went on to form synaptic connections in patterns specific to their morphological type. Type I dendrites were found to project to both principal and accessory populations of double cone photoreceptors; dendrites from Type III cells, however, terminated only in accessory cones. The picture of Type II horizontal cells was less clear, as there are no molecular markers capable of distinguishing their targets at the resolution needed.

After verifying that horizontal cells express the cadherin family member N-cadherin in vivo, Tanabe used a plasmid vector to overexpress a dominant-negative version of this molecule together with mEGFP in horizontal cells, thereby blocking cadherin function. The cadherin-inhibited cells labeled by mEGFP diversified into the three horizontal cell morphologies, but in all three types, the dendritic fields were smaller than normal. Axon outgrowth, in contrast, was unaffected. Looking next at the level of individual dendrites, Tanabe found that although dendrites appeared to home in to their appropriate targets, the morphologies of the dendritic terminals were severely affected. Moreover, Tanabe showed that cadherin regulates synapse formation by observing that, even in less morphologically affected dendritic terminals, cadherin blockage perturbed the accumulation of the synaptic marker GluR4.

Given that the cadherin-negative horizontal cells showed defects in both the global organization of the dendritic and the local ability to form functional synapses, Tanabe et al. suggest that cadherins may be involved in two distinct stages of dendritic morphogenesis; first as the dendrites elongate, then again later as they form synaptic contacts. This represents an important in vivo demonstration of cell adhesion machinery in synapses, and a new stride forward toward a better understanding of how the neural network forms from assemblies of initially discrete cells.

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