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Developmental localization of adhesion and scaffolding proteins at the cone synapse

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ABSTRACT

The cone synapse is a complex signaling hub composed of the cone photoreceptor terminal and the dendrites of bipolar and horizontal cells converging around multiple ribbon synapses. Factors that promote organization of this structure are largely unexplored. In this study we characterize the localization of adhesion and scaffolding proteins that are localized to the cone synapse, including alpha-n-catenin, beta-catenin, gamma-protocadherin, cadherin-8, MAGI2 and CASK. We describe the localization of these proteins during development of the mouse retina and in the adult macaque retina and find that these proteins are concentrated at the cone synapse. The localization of these proteins was then characterized at the cellular and subcellular levels. Alpha-n-catenin, gamma-protocadherin and cadherin-8 were concentrated in the dendrites of bipolar cells that project to the cone synapse but were not detected or stained very dimly in the dendrites of cells projecting to rod synapses. This study adds to our knowledge of cone synapse development by characterizing the developmental localization of these factors and identifies these factors as candidates for functional analysis of cone synapse formation.

Development of the nervous system requires the integration of a large number of cell types into functional neural circuits. The differential adhesion hypothesis posits that expression of cell adhesion molecules that promote adhesion and repulsion underlies much of this connectivity. The cone synapse of the retina, the focus of this study, is the site of the first synapses in the photopic visual pathway. The cone synapse is a complex synapse between a single cone and the dendrites of multiple horizontal and bipolar cells, organized around a presynaptic ribbon, with the invaginating dendrites of ON bipolar cells and horizontal cells, referred to as a triad, and the dendrites of OFF bipolar cells making flat contacts at the base of each triad (Boycott and Hopkins, 1991; Hopkins and Boycott, 1992). Each cone synapse contains multiple triads; for example, in the primate retina each cone pedicle is estimated to contain approximately 30 triads (Ahnelt and Kolb, 1994). A further level of complexity is added in that a given bipolar or horizontal cell will sample multiple such synapses but will rarely contact the same cone twice (Reese, 2011).

Much work has successfully focused on understanding the organization and structure of the presynaptic ribbon. For example, bassoon is required for anchoring and maintenance of the presynaptic ribbon apposed to bipolar and horizontal cell neurites (Dick et al., 2003; Spiwoks-Becker et al., 2013). Additional studies have identified factors required for organization of the more simple rod synapse. MAGI and sidekick proteins were shown to promote development of the rod synapse and NGL-2 was shown to direct horizontal cell axons to the rod synapse (Soto et al., 2013; Yamagata and Sanes, 2010). Less work was devoted to understanding how the various types of bipolar cells and horizontal cells organize themselves into the cone synapse. In this study, the expression of adhesion and scaffolding proteins at the cone synapse was assayed. The developmental, cell type specific and sub-cellular localization of six such proteins is described. This work identifies these factors as candidates for further functions studies.

1. Results

The retina is composed of three cellular layers, the retinal ganglion cell layer, the inner nuclear layer and the outer nuclear layer, and two synaptic layers, the outer plexiform layer and the inner plexiform layer (Fig. 1). To help address how the specificity of synaptic contacts is generated during development, a library of antibodies to adhesion and scaffolding proteins was screened to identify proteins that are localized preferentially or specifically to the mouse cone synapse. Of these, six were chosen for further investigation because of strong staining localized to the cone synapse, limited data

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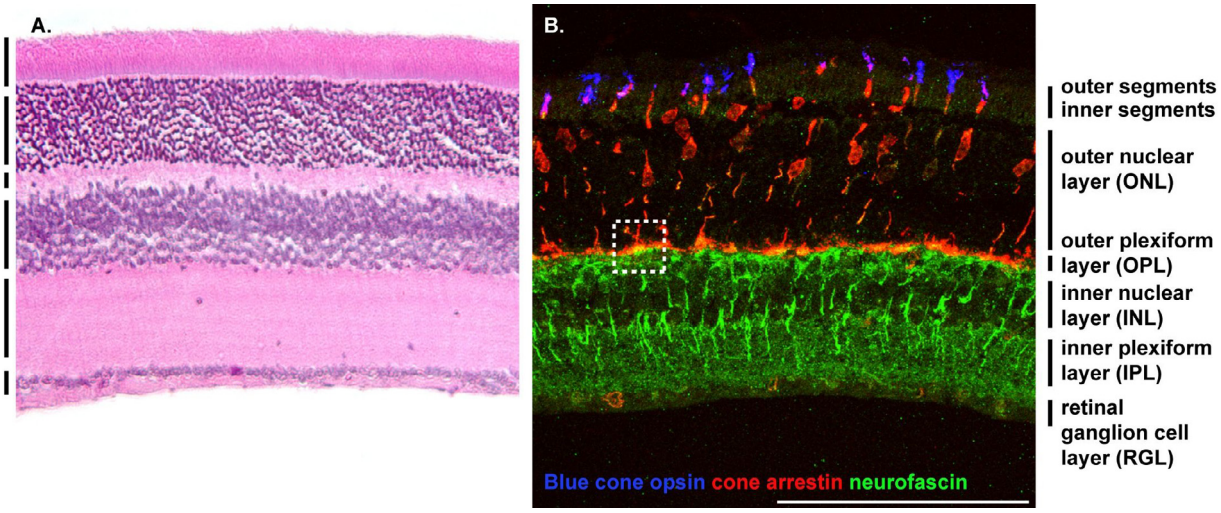

Fig. 1. Overview of the mouse retina. Sections of mouse retina stained with H&E or with antibodies to cone arrestin, blue cone opin and neurofascin. The retina is organized in three cellular layers and two synaptic layers. The outer nuclear layer (ONL) contains the cell bodies of rods and cones. The inner nuclear layer (INL) contains the soma of bipolar cells, horizontal cells, amacrine cells and Müller glia. The retinal ganglion cell layer (RGL) contains the soma of retinal ganglion cells and amacrine cells. The outer plexiform layer (OPL) contains the synapses between photoreceptors and bipolar and horizontal cells, while the inner plexiform layer (IPL) contains the synapses of bipolar cells, amacrine cells and retinal ganglion cells. The focus of this study is on synapses localized in the outer plexiform layer (dashed box). The scale bar in (B) is equivalent to 106.5 μm.

about their localization in the retina and as representatives of classes of adhesion and scaffolding molecules. These included α-n-catenin and β-catenin, two scaffolding molecules that connect cadherins to the cytoskeleton, CASK, a PDZ protein, MAGI2, a PDZ scaffolding protein involved in organization of retinal circuits in the outer plexiform layer and two adhesion molecules: cadherin-8 and γ-protocadherin.

1.1. Adhesion and scaffolding proteins at the cone synapse

These proteins were all concentrated around the cone synapse, as visualized with antibodies to PSD95 and PNA (Fig. 2). The localization of each of these proteins was also assayed with respect to the cone synapse in whole retina. α-N-catenin was localized on the postsynaptic face of the cone synapse and was nested within PNA staining, which labels the dendritic tips of ON bipolar cells (Fig. 3A) (Koike et al., 2010). γ-Protocadherin was also concentrated on the postsynaptic face of the cone synapse. γ-Protocadherin was observed to both overlap with PNA staining and in between PNA positive processes (Fig. 3B). Cadherin-8 was observed along the cell body and dendrites of cells matching the morphology of bipolar cells. Cadherin-8 was also observed to both overlap with PNA staining and between PNA positive processes and in some sections dim staining was observed near the outer nuclear layer (Fig. 3C). β-catenin was localized around and within the cone synapse but did not overlap with PNA (Fig. 3D). Magi-2 was concentrated on the postsynaptic face of the cone pedicle, but was also observed within the rod spherule-containing portion of the outer plexiform layer (Fig. 3E). CASK overlapped with PNA staining at the cone pedicle and a single puncta of immunoreactivity was observed in each rod spherule (Fig. 3F).

1.2. Developmental dynamics of protein localization

Each of these markers was assayed during development of the mouse cone synapse, at postnatal days 6, 8, 10 and 12, in the macaque retina, and in combination with markers that label Müller glia, horizontal cells and multiple types of bipolar cells.

α-N-catenin was widely localized in the developing retina but became concentrated apposed to the presynaptic marker PSD95 at the cone synapse by postnatal day 10, consistent with a postsynaptic localization (Fig. 4A–E). α-N-catenin was also concentrated apposed to PSD95 staining in sections of macaque retina, as well as around cells in the macaque inner and outer plexiform layers (Fig. 4F). α-N-catenin was observed around horizontal cells and overlapped with markers of bipolar cell subtypes (Fig. 4G–I and Tables 1 and 2).

Limited γ-protocadherin staining was observed in the developing outer plexiform layer until post natal day 8, after which it was concentrated apposed to PSD95 staining at the cone synapse (Fig. 5A–E). A similar staining pattern was observed in mouse and macaque retina (Fig. 5F). Overlap between calbindin, a marker of horizontal cells, was not observed opposite of the cone synapse, but was observed in the portion of the outer plexiform layer facing the outer nuclear layer (Fig. 5G). Likewise overlap between GS (glutamine synthetase), a marker of Müller glia, and γ-protocadherin was not observed at the cone synapse but was observed in processes in between cone synapses (Fig. 5H). Expression of γ-protocadherin in both of these cell types was previously described (Lefebvre et al., 2008). Overlap was also detected between γ-protocadherin and all assayed bipolar cell types (Fig. 5I and Tables 1 and 2).

Cadherin-8 was first observed at postnatal day 10 and was observed dimly in cell bodies and apposed to PSD95 staining at the cone synapse (Fig. 6A–E). Cadherin-8 staining was not observed at the macaque cone pedicle (Fig. 6F). Cadherin-8 did not overlap with calbindin or GS, a marker of Müller glia but was observed in a limited subset of bipolar cells consistent with it being expressed by type 2 OFF bipolar cells and some ON bipolar cells (Fig. 6G–I, Fig. 8 and Tables 1 and 2). All type 2 bipolar cells colocalized with cadherin-8 and the majority (81.8%) of cadherin-8 positive cells expressed Syt2 (Fig. 6I).

β-catenin was widely distributed in the mouse retina, as previously described (Fu et al., 2006). β-catenin became concentrated at the cone synapse as this structure developed, around P10 (Fig. 7A–E). A similar pattern of distribution was observed in mouse and in