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Molecular and cellular mechanisms of signaling in Retina: Lessons from the developmental regulation of neuronal circuit formation.

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Even so, in embryonic days newly formed retina represent a smooth assembly of yet unspecified cells (Fig. 1a), shortly after birth retina is rapidly progressing into highly organized structure of the nervous system with main neuronal sub-layers (Fig. 1b). In the mature state these layers are nearly completely separated from each other. Functional significance of this organization - is to establish a remarkable flow of visual information into brain. Signals are passing straight from the photoreceptors (PR), positioned in the outer part of the retina, via the bipolar cells (BP) in the middle of the retina to the retinal ganglion cells (RGC), positioned in the inner part. The geometrical proportion between these cell types PR:BP:GCL in adult mammalian retina can be almost 10:5:1 representing strong pyramidal hierarchy directed toward RGC.
Remarkably, before this pyramidal composition start to be functional, two other cell types will emerge and divide a mix of mitotic and post-mitotic cells (Fig. 1a). These "check points" layers of retina consist of horizontal cells (HC), that almost precisely divide sum of neurons into two parts, separating now the photoreceptor layer from the bipolar cells (BP). Almost at the same time amacrine cells (AC) start to control interface between the BP cells and the main input RGCs. These two layers are less investigated and probably the most interesting for the control signaling and information flow. A key aspect of the most intriguing features in spatial regulation of cell-cell contacts and signaling is its adjustability first in development, second – in the adaptation to light.

Recently we have shown that newly formed HC and AC layers express high level of Drebrin – Developmental Regulated Brain Protein, (MS in preparation). We showed direct binding of Cx43 to drebrin that was analysed in details in previous work. Here we perform in vivo and in vitro analyses for the role of drebrin, that expressed in two splice isoformes E and A - Adult and Embryonic). Our idea was that drebrin may be required for connexin signaling during fast rearrangements of cell-cell contacts and upon establishment of retinal layer specificity. Both HC and AC are also known to express array of neuronal connexins, Cx36, Cx45, Cx57. The functional link between Drebrin and Connexins remained obscur. Gap junctions appear to be involved in restricts tracer coupling between neighboring HC in newly formed layer of retina and between AC but not yet in ganglion-ganglion cell coupling. However, tracer coupling between HC and amacrine cells at the earliest ages is yet to be defined, probably to the rearrangements of cell-cell contacts during developmental migration. At this time (E15-20), combinations of glutamate antagonists or GABA-A antagonist does not influence cell cell-coupling. The direct roles of connexins in HC and AC layers is still remained to be analysed in greater details. One idea is that gap junction coupling between HCs and ACs (possibly the cholinergic amacrine cells) is a transient early phase of transmission employed before the chemical synapses.

Interestingly, completely mature electrical transmission and generation of retinal waves in mammals can proceed via gap junction before synaptic. Connexins are known to be permeable to cAMP. This second messenger can modulate intracellular levels of cAMP in a cluster of connected cells and have dramatic effects on the propagation of Ca^{2+} waves. Thus, gap junctions play a very important role in the cell-layer definition before chemical synapses have started to act.

To better understand the role of early Drebrin expression we combined in utero electroporation of drebrin RNAi in embryo brain with in vitro cellular approaches and expressed Drebrin together with

neuronal connexins in neuronal and non-neuronal cells. Our in vitro reconstruction experiments revealed stunning role of Drebrin in stabilizing neuronal connexins at the cell-cell interface. To control the obtained data we also applied RNAi of Drebrin and showed that in the absence of drebrin the neuronal connexins were unable to be delivered to the plasma membrane and to form contacts with neighboring cells. Single cell assay of RNAi using high resolution Live Cell Microscopy show how in the absence of drebrin Cx36 was unable to be delivered to the plasma membrane and to establish cell-cell. Instead, unsupported by submembrane cytoskeletal scaffold Cx36 was co-localized with lysosomal markers and revealed degradation bands when analyzed in WB and Cx36 specific antibodies.

The obtained results confirm ability of highly morphogenic protein drebrin to stabilize and spatially regulate rearrangements of cell-cell contacts thus quickly providing the adjustability of intercellular signaling. This unexpected finding not only adds to the developmental complexity in regulation of signaling but also shows how cross-talks between cytoskeleton and cell-cell connectivity may influence complex processes as signaling and transmission of visual signal in retina and brain. Cx36 in known to be the most permeable to the cAMP and broadly expressed in inhibitory GABA-ergic interneurons. Thus drebrin-induced rearrangements of cell-cell contacts may regulate not only excitatory pathways of retinal PR but also provide complex inhibitory regulation of ganglion cells. The question still remained to answer, how the expression of drebrin itself is regulated in development and under local conditions.

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