# Rod Bipolar Cell Networks in a Retinal Pathoconnectome

Rebecca L. Pfeiffer[1], James R. Anderson[1], Daniel P. Emrich[1], Jeebika Dahal[1], Crystal L. Sigulinsky[1], Jia-Hui Yang[1], Kevin D. Rapp[1], Carl B. Watt[1], Jessica C. Garcia[1], Mineo Kondo[2], Hiroko Terasaki[3], Robert E. Marc[1,4], Bryan W. Jones[1]

*1. Department of Ophthalmology, Moran Eye Center, University of Utah, Salt Lake City UT, USA*

*2. Mei University, Tsu, Mie Japan*

*3. Kyoto University, Kyoto, Japan*

*4. Signature Immunologics, Torrey, UT, USA*

**Purpose:** Ultrastructural connectomics has allowed for precise identification of neural network topologies in retina, exposing synaptic connectivity associated with specific pathways involved in neural retinal processing. In pathological degenerate retina such as retinitis pigmentosa (RP), retinal remodeling emerges as a phenomenon through a series of negative plasticity events originating from neural deafferentation initiated by photoreceptor degeneration. Early stages of remodeling include glial changes, GluR receptor alterations (reprogramming), and rewiring of retinal networks. The connectivities initiated by these processes are currently unknown. To address this problem, we have created an ultrastructural pathoconnnectome of early retinal remodeling in a rabbit model of retinitis pigmentosa, Retinal Pathoconnectome 1 (RPC1).

**Approach**: The tissue for RPC1 was obtained from a 10-month-old transgenic P347L rabbit model of autosomal dominant RP. Our control connectome is RC1, which was generated from a 13-month-old Dutch-Belted rabbit. Tissue was fixed in mixed aldehydes, osmicated, dehydrated, embedded in epon resin, and sectioned at 70nm. Serial sections were placed on grids, stained, and imaged using a JEOL JEM-1400 TEM using SerialEM software. Every 30th section was reserved for computational molecular phenotyping (CMP), and probed for small molecules: glutamate, glutamine, glycine, GABA, taurine, glutathione; or TEM compatible proteins GFAP and GS. The pathoconnectome volume is explored and annotated using the Viking software suite.

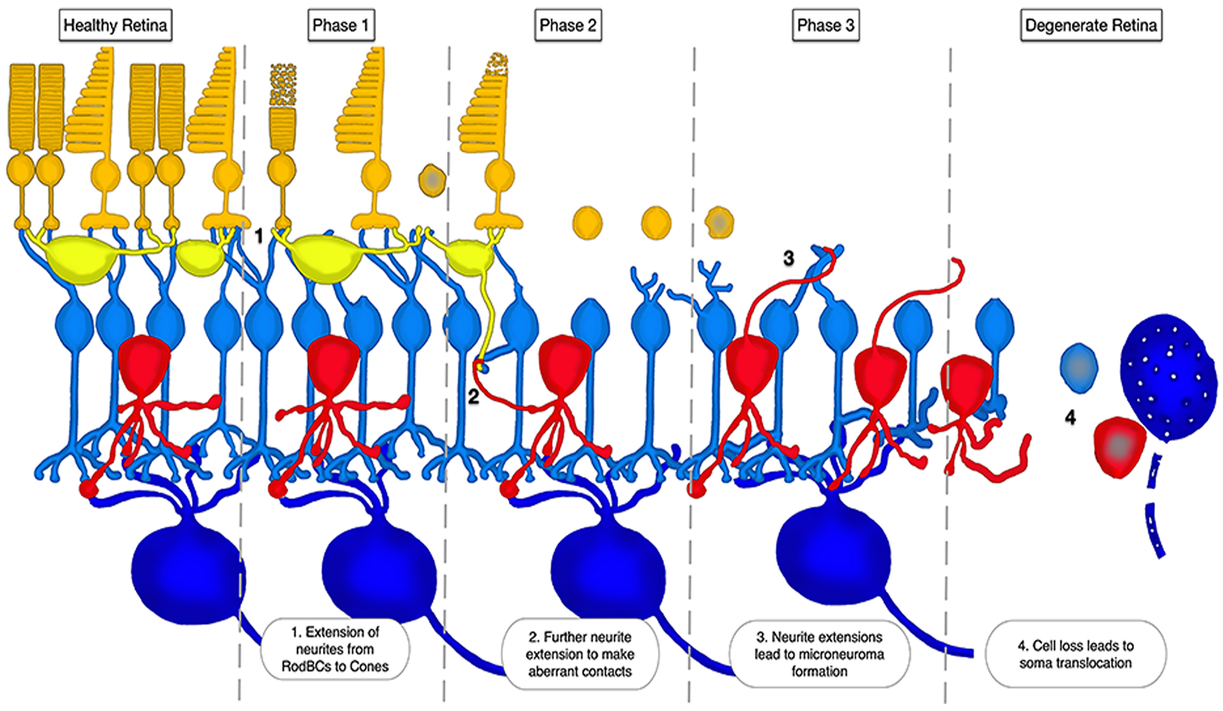


**Figure 1: Rewiring in Retinal Remodeling:** Following photoreceptor degeneration, retinal networks enter a period of plasticity collectively termed remodeling. A key component of remodeling is rewiring, in which gross chages have been described, but the local network impacts are still being explored. During rewiring, retinal neurons extend neurites in an aberrant fashion within the neural retina. Phase 1 is characterized by rod degeneration. During this time, it has previously described that rod photoreceptors sprout neurites that extend beyond the OPL into the IPL, occasionally reaching the GCL. In phase 2, cone photoreceptors degenerate and more pronounced neurite sprouting occurs from all neuronal types of the retina. In phase 3, the neurites coalesce into microneuromas. Microneuromas are collections of neuronal aberrant processes from all cell classes of retinal neurons and create new areas of IPL, exhibiting ultrastructural findings consistent with active synapses, internal to the microneuromas, although the partners involved are currently unknown.

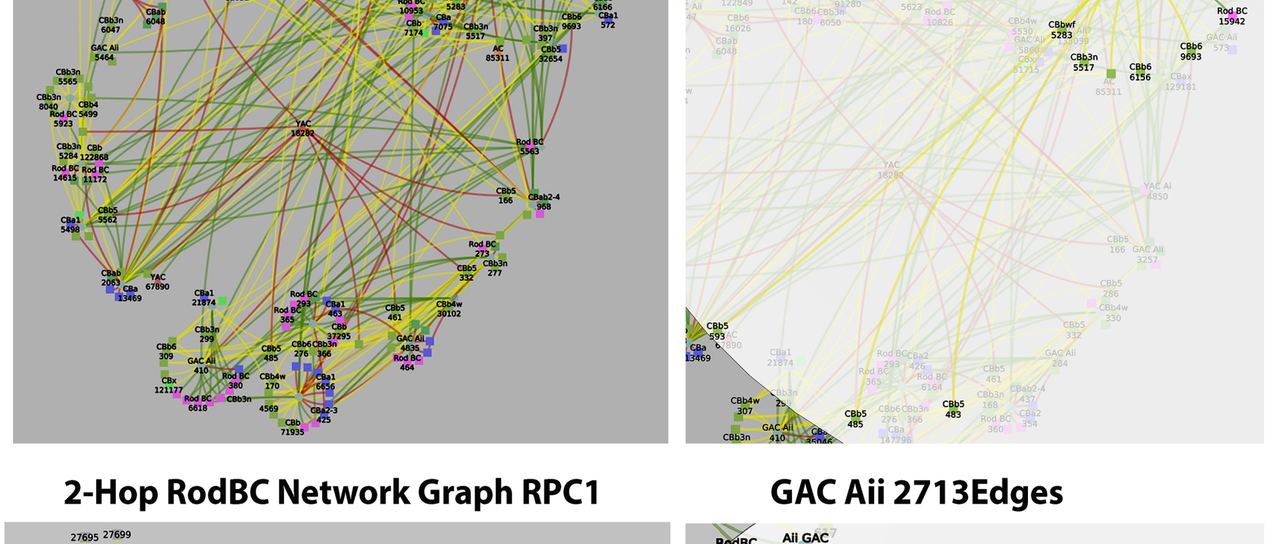


**Figure 2: Retinal Pathoconnectome 1 (RPC1)** (A) Description of the RPC1 volume. (B) Overlay of a top down view of the 3D renderings of all confirmed 16 rod bipolar cells (RodBCs) and cone-contacting rod bipolar cells (XRodBCs) in the RPC1 volume on a representative TEM section from RPC1. (C) Vertical view of the 3D reconstruction of RodBC and XRodBCs in RPC1.




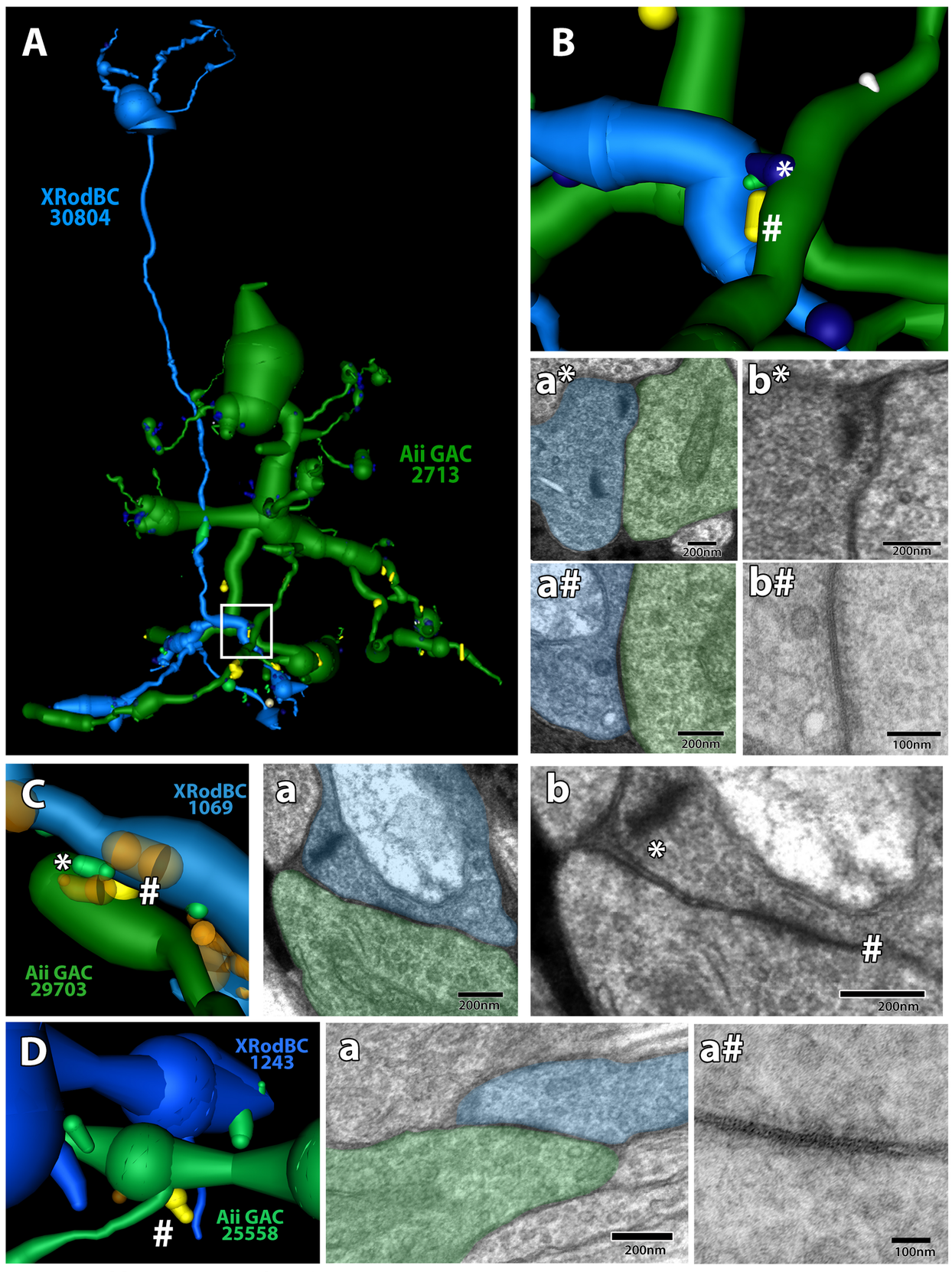


**Figure 3: Abberant synaptology in RPC1.** (**A**) 3D rendering of XRodBC 30804 (blue) and Aii GAC 2713 (green). (**B**) Higher magnification of region indicated by box in A. * indicates a glutamatergic ribbon and # indicates a gap junction between the XRodBC and Aii GAC (a*) Pseudo-colored TEM image of the ribbon indicated in B. (b*) Higher magnification of ribbon indicated in B. (a#) Pseudo-colored TEM image of gap junction indicated in B. (b#) Recaptured TEM image at 25k (.436nm/px) tilted to -5 degrees. (**C**) 3D rendering of gap junction and ribbon annotations between XRodBC 1069(blue) and Aii GAC 29703 (dark green). (a) Pseudo-colored TEM image of ribbon and gap junction in C. (b) Higher magnification TEM image of ribbon (*) and gap junction (#) in C. (**d**) 3D rendering of gap junction annotation between XRodBC 1243(blue) and Aii GAC 25558 (dark green). (a) Pseudo-colored TEM image of gap junction in d. (a#) Recaptured TEM image at .436nm/px tilted to -10 degrees.

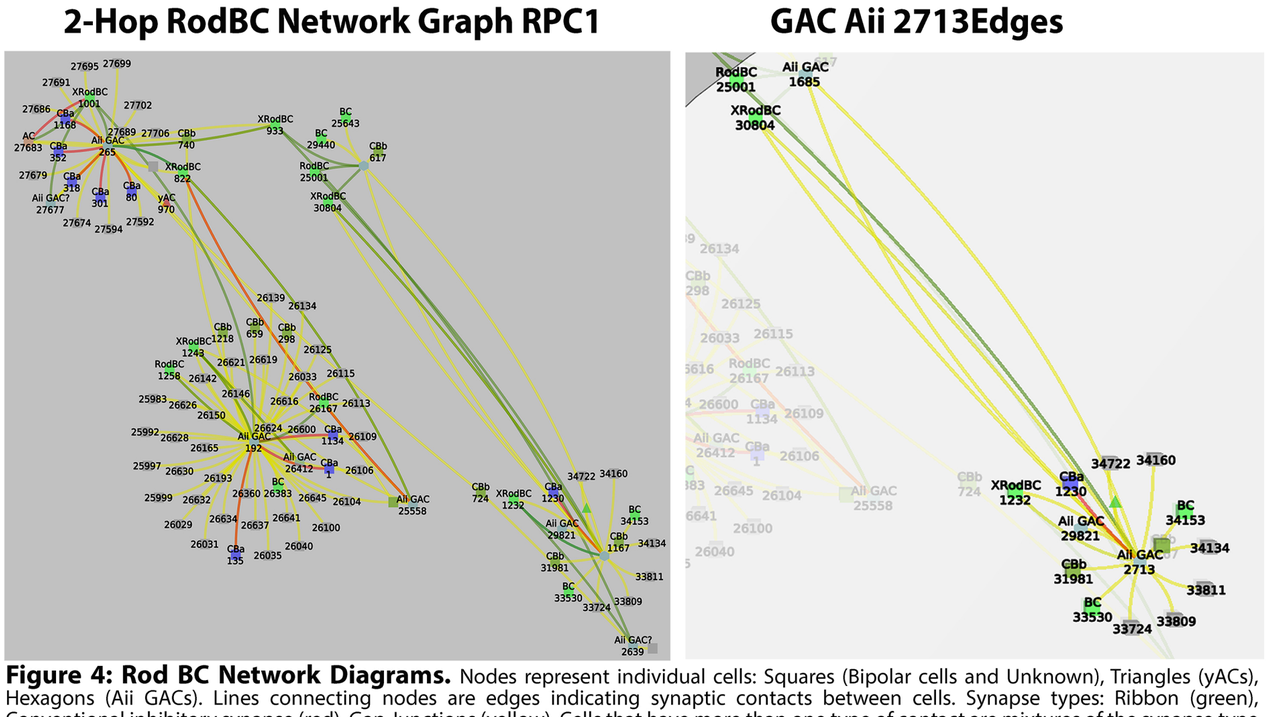


**Figure 4: Rod BC Network Diagrams.** Nodes represent individual cells: Squares (Bipolar cells and Unknown), Triangles (yACs), Hexagons (Aii GACs). Lines connecting nodes are edges indicating synaptic contacts between cells. Synapse types: Ribbon (green), Conventional inhibitory synapse (red), Gap Junctions (yellow). Cells that have more than one type of contact are mixtures of the synapse type colors. The RC1 network (above) illustrates normal synaptic connectivy of the rod pathway in the retina. The RPC1network (below) highlights the variation in synaptic connectivity observed in the degenerate retina.

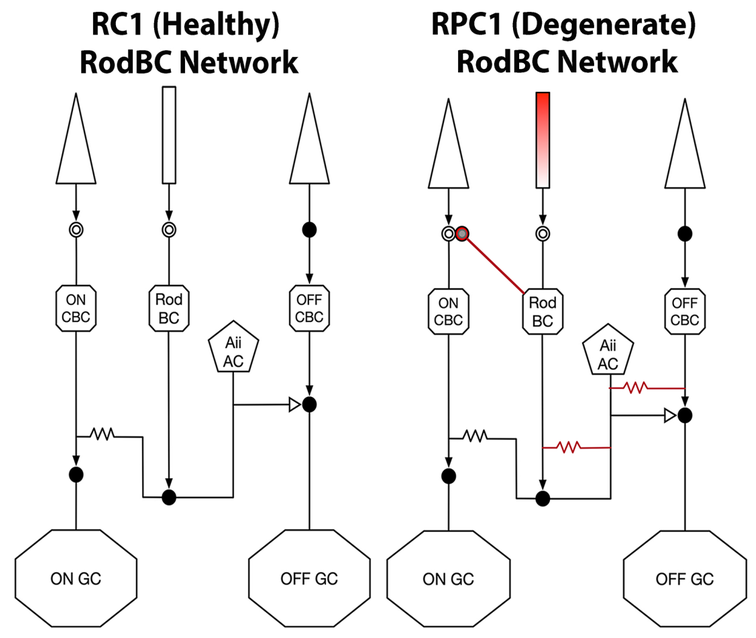


**Figure 5: Network alterations from rod photoreceptor stress and degeneration.** Healthy circuit diagram (left) illustrates the normal conectivity of the scotopic (low-light) pathway. Degenerate RodBC network (right) highlights the changes (red) in the network that occur as a consequence of rod degeneration.

**Funding and Disclosures:**

R01 Grant EY015128(BWJ), R01 Grant EY02576(BWJ), T32 Grant EY024234 (RLP), P30 Grant EY014800(Core), Undergraduate Research Opportunities Program at the University of Utah (JCG), Unrestricted Research Grant from Research to Prevent Blindness (Moran Eye Center)

REM is a Prinicipal of Signature Imunologics