Coupling architecture of the Aii/ON cone bipolar cell network in the degenerate retina

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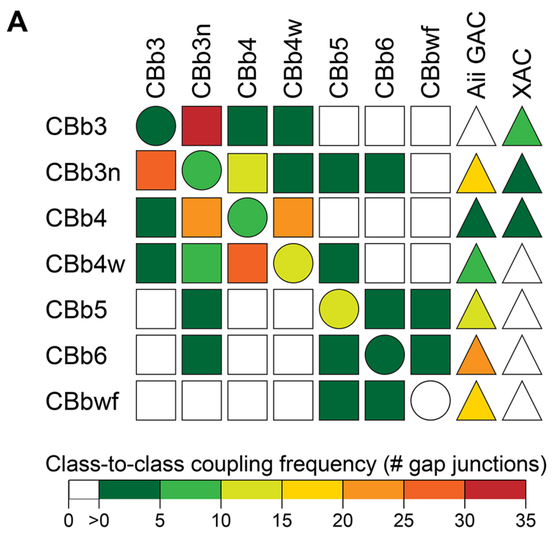
Purpose: Retinal network hyperactivity within degenerative retinal networks is a component of the disease process with implications for therapeutic interventions for blinding diseases that depend upon the surviving retinal network. Connexin36-containing gap junctions centered on the Aii amacrine cell network appear to mediate the aberrant signaling observed in mouse models of retinal degeneration. However, it remains unclear whether this hyperactivity reflects changes in the underlying circuitry or dysfunction/dysregulation of the normative circuitry. Mapping retinal circuitry in the ultrastructural rabbit Retinal Connectome, RC1, has revealed Aii network topologies explicitly involving gap junctions. In addition to canonical Aii-to-Aii and Aii-to-ON cone bipolar cell (CBC) coupling, we describe pervasive in- and cross-class coupling motifs among ON CBCs that extend and dramatically expand the coupled Aii network topologies. Since virtually every gap junction in the inner plexiform layer contains Connexin36, these circuits likely participate in the aberrant signaling of degenerate retinas. This study examines these Aii and ON CBC coupling motifs in Retinal PathoConnectome 1 (RPC1), an ultrastructural pathoconnectome of a rabbit model of retinitis pigmentosa.

Approach: RPC1 is a 2nm/pixel resolution volume of retina from a 10 month old, transgenic P347L rabbit model of autosomal dominant retinitis pigmentosa in early phase 1 retinal remodeling, a time point where cone and rod photoreceptors are still present, albeit going through cell stress. RPC1 spans the vitreous to basal outer nuclear layer and was built by automated transmission electron microscopy and computational assembly. ON CBCs, Aii amacrine cells, and their coupling partners were annotated using the Viking application and explored with 3D rendering and graph visualization of connectivity. Gap junctions were validated by 0.25 nm resolution recapture with goniometric tilt when necessary. Motifs were compared to those discovered in RC1. RC1 is a 2 nm resolution, 0.25 mm diameter volume of a light-adapted adult female Dutch Belted rabbit retina spanning the ganglion cell through inner nuclear layers.



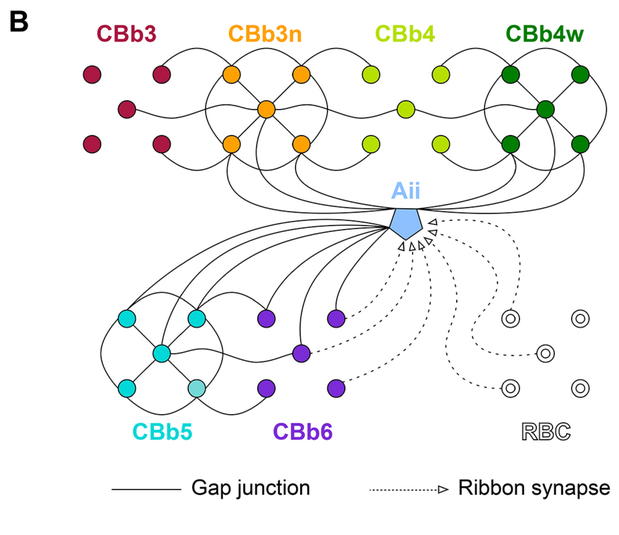
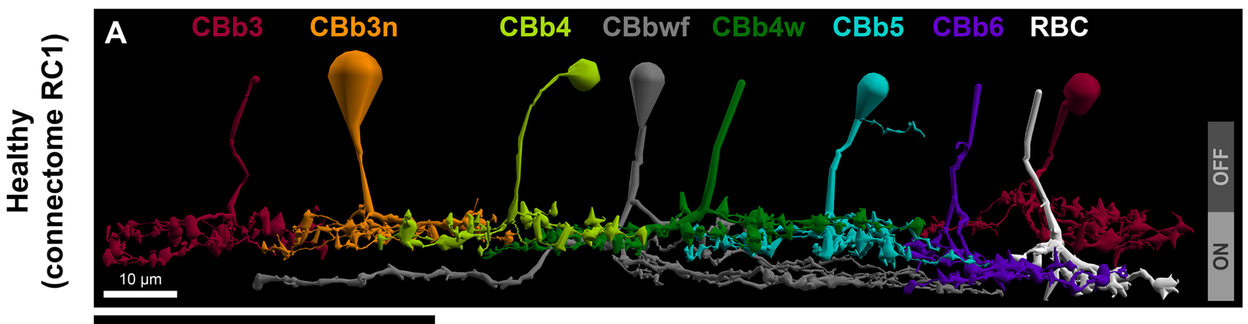


Figure 1. Summary of ON CBC coupling profiles and the Aii/ON CBC network for a healthy rabbit retina derived from connectome RC1. (A) Coupling matrix depicting the per cell average coupling frequency for a given class. Values are encoded by a linear heat map scale where dark red represents the highest coupling frequency and dark green represents the lowest frequency, while absence of coupling is encoded by empty white icons. Icons denote the type of class pairing: circles (in-class homocellular coupling), squares (cross-class homocellular coupling), and triangles (heterocellular coupling). The graph illustrates that each ON CBC class exhibits a unique combination of coupled partner classes and frequencies. (B) Expanded circuitry of the Aii/ON CBC network due to ON CBC coupling.



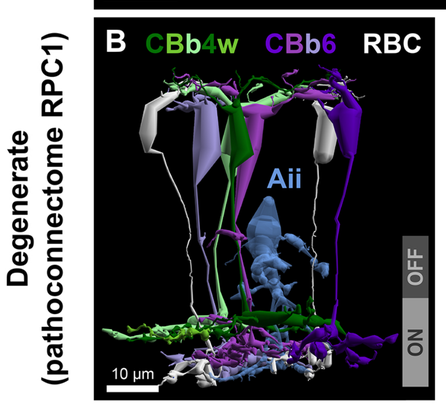
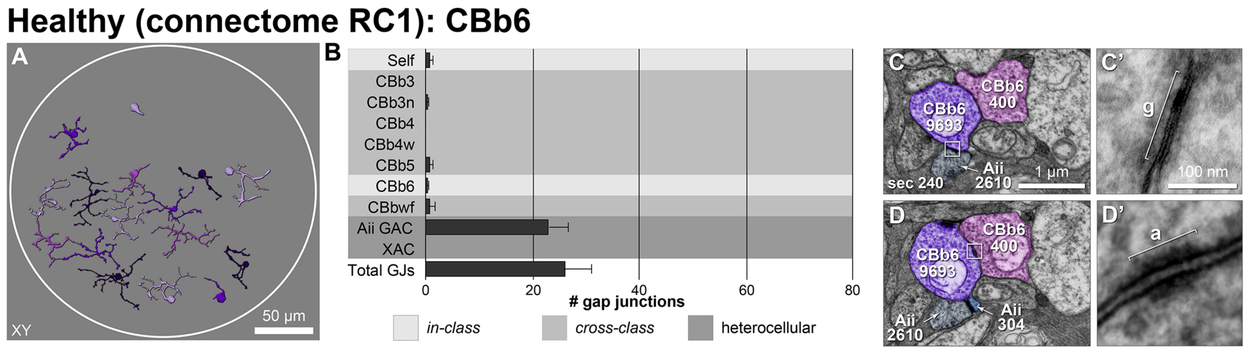
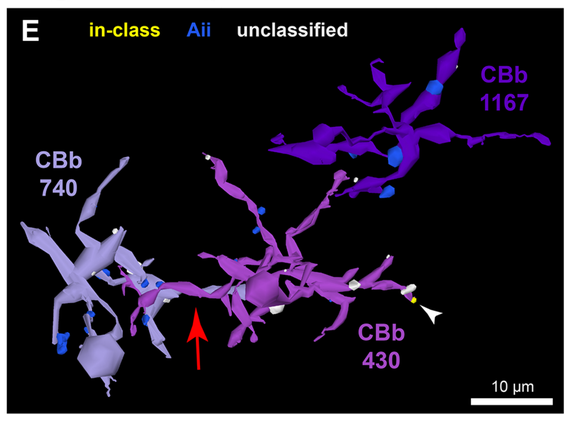


Figure 2. Stratification of ON CBCs in the healthy and degenerate retinas. (A) The seven identified classes of ON CBCs in rabbit retina derived from connectome RC1. Shallow classes exhibit extensive costratification while deeper classes are more isolated. The coupling profiles among ON CBCs reflect this, but stratification alone is insufficient to define ON CBC coupling specificty. (B) Members of the three classes of identified bipolar cells in RPC1 thus far. Aii cells help identify the ON/OFF sublamina. RBC terminals stratify the deepest with members of the CBb6 class (shades of purple) arborizing just above. The CBb5 class should be positioned in the "gap" between the CBb6 arbors and those of the shallow CBb4w class (shades of green).

Healthy (connectome RC1): CBb6



Degenerate (pathoconnectome RPC1): Candidate CBb6

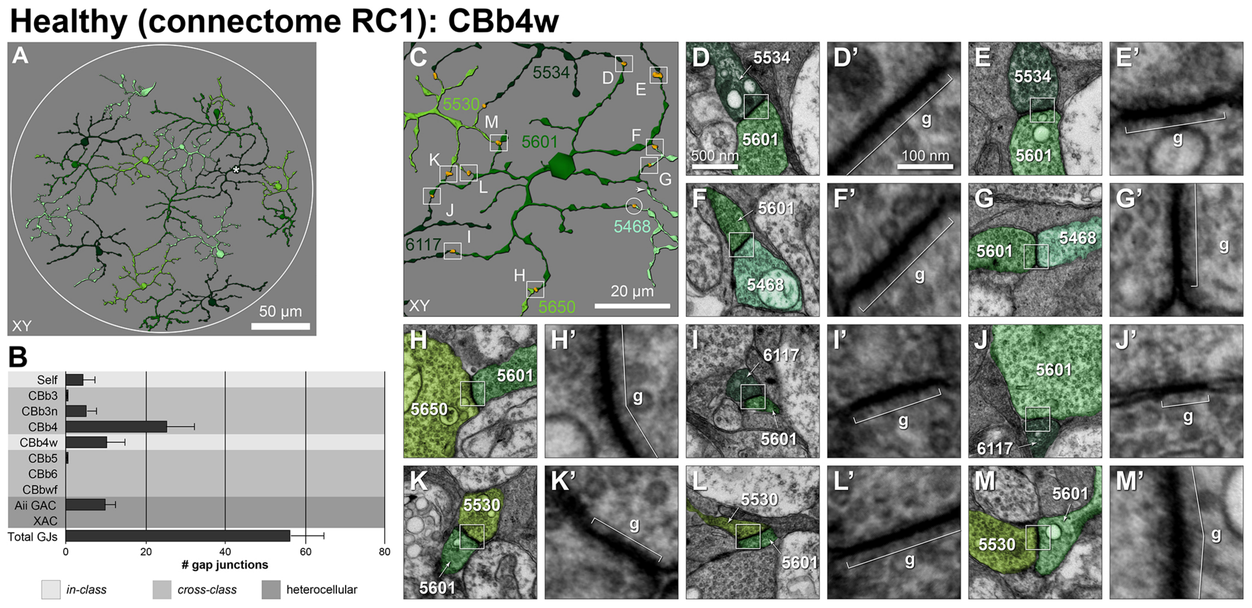


F

	Contacts			Candidate Class
	Aii	CBb (tiled)	CBb (overlap)	
CBb 740	10 (g) ; 1(r)	0	0	CBb6
CBb 430	5 (g)	1 (g)	0	CBb6
CBb 1167	4 (g)	0	0	CBb

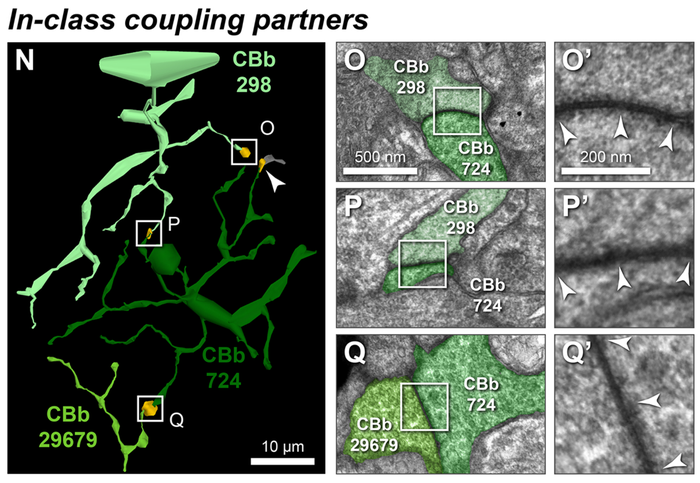
Figure 3. Identification of candidate CBb6 cells in pathoconnectome RPC1 reveals altered morphological relationships but largely intact coupling motifs in the degenerate retina. Evaluation of CBb6 cells in healthy retina (connectome RC1) reveals that they roughly tile the retinal plane with some gaps, but never overlap (A). They exhibit the simplest coupling profile, almost exclusively coupling with Aii cells (B). CBb6 cells share membrane contact with in-class neighbors, but never couple (D), depsite the localization of gap junction proteins at immediately adjacent sites of Aii coupling (C). (E) Candidate CBb6 cells in pathoconnectome RPC1, showing the classification of their gap junctions according to partner identification: Aii (blue), in-class (yellow), and unclassified (white). Note that the arbors of CBb6 cells 740 and 430 overlap, violating tiling. However, CBb6 cells in RPC1 retain their deep stratification, robust Aii coupling, limited coupling with other ON CBCs and occasional ribbon input to Aii cells (F). The gap junction mediating coupling with another ON CBC, may actually reflect cross-class coupling rather than in-class, but the cell extended beyond the volume and could not be classified. Since it has tip-to-tip topology, it is currently clssified as an in-class gap junction. Abbreviations: a, adherens junction; g, gap junction.

Healthy (connectome RC1): CBb4w



Degenerate (pathoconnectome RPC1): Candidate CBb4w

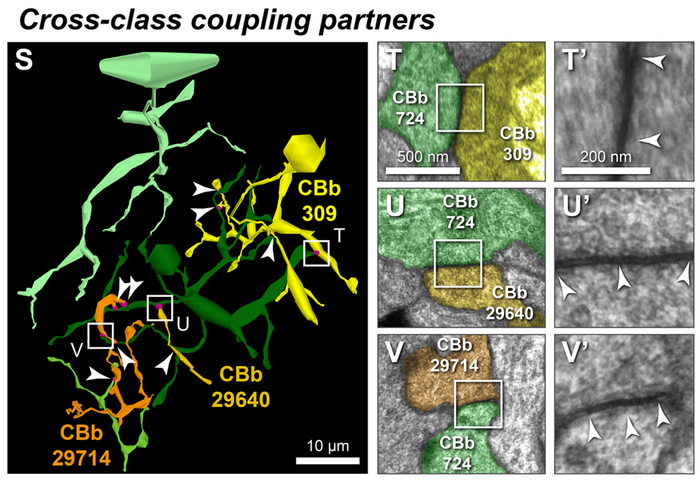
In-class coupling partners



R

	Contacts			Candidate Class
	Aii	CBb (tiled)	CBb (overlap)	
CBb 298	3 (g)	1 (g)	0	CBb4w
CBb 724	7 (g)	3 (g)	10 (g)	CBb4w
CBb 29679	2 (g)	1 (g)	1 (g)	CBb4w

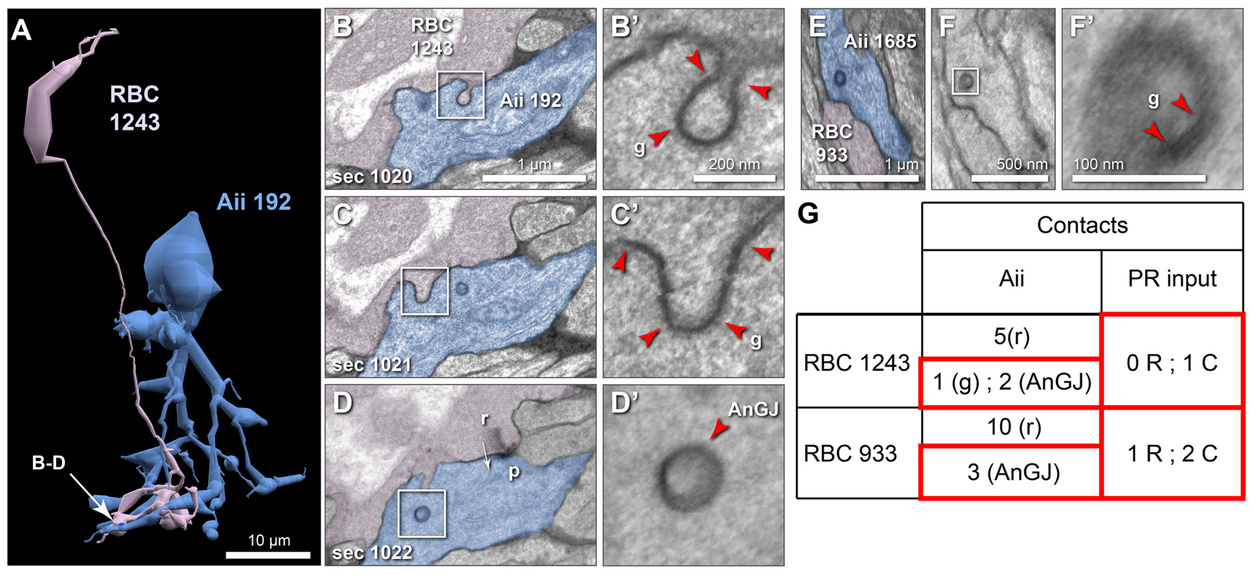
Cross-class coupling partners



W

	Contacts			Candidate Class
	Aii	CBb (tiled)	CBb (overlap)	
CBb 309	0	1 (g)	4 (g)	**CBb4**/3
CBb 29640	0	1 (g)	2 (g)	**CBb4**/3
CBb 29714	0	0	5 (g)	**CBb4**/3
CBb /	++	+	+++	CBb3n

Figure 4. Identification of candidate CBb4w cells in pathoconnectome RPC1 reveals altered coupling motifs in the degenerate retina. Evaluation of CBb4w cells in healthy retina (connectome RC1) reveals that they nearly perfectly tile the retinal plane (A), forming in-class homocellular gap junctions (yellow dots, C) with their immediate neighbors in tip-to-tip or tip-to-edge topology (C-M). They also couple with a number of other ON CBC classes with specific frequencies (B). (N-O) Candidate CBb4w cells were identified in pathoconnectome RPC1 due to their robust tiling and presence of tip-to-tip gap junctions. (R) Each heavily couples with Aii cells and other ON CBCs. (S-V) Many of these ON CBC partners violate tiling (S), indicative of cross-class coupling (pink dots, S). (W) These partners lack coupling with Aii cells, suggestive of the CBb3 or CBb4 classes. CBb4 is a common cross-class motif, but CBb3 is not in the helathy retina (B). Candidate CBb3n cells, recognizable by their frequent Aii coupling, have not been identified as CBb4w coupling partners in RPC1 (W), suggesting this motif may be lost in the degenerate retina. Boxed regions in (C,N,S) are shown with TEM. Boxed regions in TEM are shown in enlarged views. Arrowheads indicate location of in-class (yellow) or cross-class (magenta) gap junctions (in C,N,S). Gap junctions are indicated with bars or arrowheads. Abbreviations: g, gap junction.



G

	Contacts	
	Aii	PR input
RBC 1243	5(r)	0 R ; 1 C
	1 (g) ; 2 (AnGJ)	
RBC 933	10 (r)	1 R ; 2 C
	3 (AnGJ)	

Figure 5. RBCs form rare aberrant gap junctions with Aii GACs. (A) RBC 1243 forms normal and aberrant connections with Aii 192 at a single contact site. (B-D) Serial TEM sections illustrating the presence of a gap junction (C) initiation of annular gap junction formation (B) and a fully internalized annular gap junction (D). RBC 1243 maintains appropriate ribbon synapses nearby (D). (E) Second example of an annular gap junction associated with contact between RBC 933 and Aii 1685 revealing previous existence of an gap junction between these cells. (F) Recapture with goniometric tilt reveals characteristic pentalaminar structure. (G) Summary of RBC contacts. Both cells receive cone photoreceptor input indicative of dendritic remodeling. Abbreviations: AnGJ, annular gao junction; C, cone; g, gap junction; p, post-synaptic density; PR, photoreceptor; r, ribbon; R, rod.

Conclusions: RPC1 shows degeneration of rod outer segments, Müller cell hypertrophy and neuronal sprouting, characteristic of early stage retinal degeneration and phase 1 remodeling, when retinal hyperactivity and its reliance on gap junctional coupling has likely already initiated and human patients would still have some vision. All major coupling motifs (Aii-to-Aii, Aii-to-ON CBC, and ON CBC-to-ON CBC) were observed. Preliminary examinations indicate that several ON CBC classes retained their class-specific coupling profiles, accepting and rejecting specific combinations of Aii and ON CBC class partnerships. However, recent findings reveal aberrant partnerships in the coupled network, including both loss of prominent motifs and acquisition of novel ones. Thus, clear aberrant morphological and synaptic changes have been identified in RPC1, including changes in the coupling specificity and gap junction distributions of both Aii amacrine cells and ON CBCs (Figure 6). This suggests that the Aii/ON CBC circuit topology is already altered during early phase 1 remodeling, with substantial implications for therapeutic interventions in human subjects. The full coupling network is actively being examined and progress has begun on RPC2, a second pathoconnectome for examining later, phase 2 remodeling in this same model.

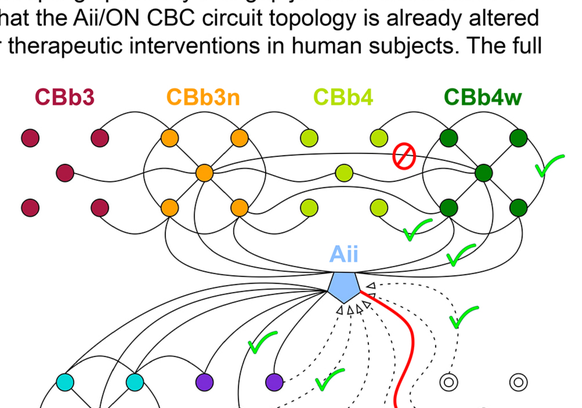


Figure 6. Summary of changes in the Aii/ON CBC network in degenerate retina. RBCs, which normally never form gap junctions, show evidence of aberrant coupling (red line) with Aii cells, immediately adjacent sites of canonical ribbon synapses. No evidence of coupling between CBb4w cells and their canonical coupling partner, the CBb3n class, was observed, suggesting this motif may be lost in the degenerate retina. Presence of a single tip-to-tip gap junction with a CBb6 cell may indicate aberrant coupling among members of this class in the degernate retina. However, many coupling motifs were retained at these early stages of degeneration (green check marks).

Disclosures: Marc, RE, Signature Immunologics, Inc.

Support: This work was supported by the National Eye Institute: NIH grants R01 EY02576, R01 EY015128, and P30 EY014800 Vision Core Grant (REM); T32 EY024234 Vision Training Grant (REM and CLS); the Calvin and JeNeal Hatch Presidential Endowed Chair (REM); an unrestricted grant from Research to Prevent Blindness to the Moran Eye Center; and a Research to Prevent Blindness Career Development Award (BWJ). Funding for the JEOL JEM-1400 was generously provided by the late Martha Ann Healy, a friend of the Moran Eye Center.