Coupling Architecture of the retinal Aii/ON cone bipolar cell network and alteration in degeneration

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

[1]Department of Ophthalmology, John A. Moran Eye Center, University of Utah, Salt Lake City, Utah, USA; [2]Mie University, Tsu, Japan; [3]Nagoya University, Nagoya-shi, Japan

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1. Background.

➤ Gap junctions in the Aii/ON cone bipolar (CB) network of the mammalian retina are essential for night vision.

➤ In retinal degeneration, the Aii/ON CB network is the epicenter of oscillatory behavior underlying retinal network hyperactivity that contributes to visual impairment.

  ➢ Oscillations require Cx36-containing gap junctions.

➤ Reports of additional gap junctions among ON CBs.

2. Aim.

Does this hyperactivity reflect changes in circuitry or dysfunction of normative circuitry?

1) Define the coupling architecture of the Aii/ON CB network in healthy adult retina using connectome RC1.

2) Evaluate changes in coupling motifs in RPC1, a pathoconnectome from a rabbit retinal degeneration model of retinitis pigmentosa.

Figure 1. Vertical pathways mediating day and night vision.

3. Normative coupling motifs in healthy adult rabbit retina (Connectome RC1).

RC1:
- 13-month old, female, Dutch Belted rabbit. Light adapted, mid-periphery. (Anderson et al., 2009, 2011a,b).
- 0.25 mm diameter volume built from 341 serial TEM sections (70-90 nm thick) at 2.18 nm/pixel resolution.
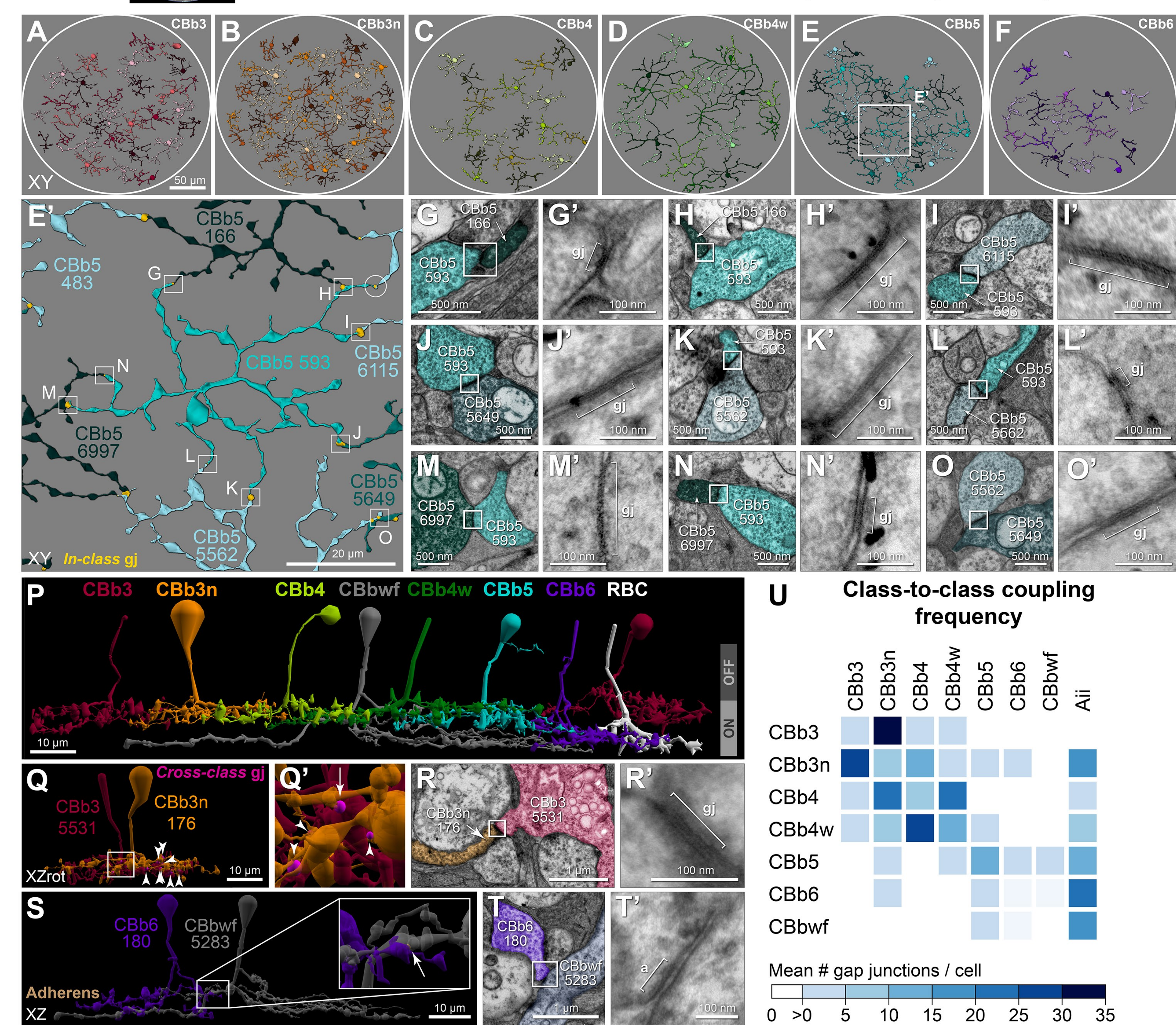

Figure 2. Connectomics-based reconstruction of ON CBs reveals pervasive, class-specific coupling motifs both within and across classes of ON CBs. (A-F) The 145 ON CBs contained within RC1 were classified into 7 distinct classes. CB axonal arbors within each class jigsaw together to cover the retina optimally, without overlap, a pattern known as *tiling*. CBbwf arbors exhibit overlap (n = 2 cells, not shown). Circles approximate RC1 volume boundaries. (E') Processes contacting neighbors of the same class often terminate in tip-to-tip or tip-to-shaft gap junctions (yellow dots, scaled by a factor of 6 for visibility), that mediate *in-class* homocellular coupling (except CBb6). (G-O) Gap junctions in E' confirmed by TEM recapture at 40,000X (0.27 nm/pixel) with goniometric tilt (G'-O'). (P) ON CB axons costratify to varying extents. Most costratifying classes participate in *cross-class* coupling (Q-R), but some class pairings do not, despite opportunities (S-T). Gap junctions mediating *cross-class* coupling (magenta, scaled by factor of 4 for visibility) and adherens junctions (tan) indicated by arrows and arrowheads in Q,Q',S. (R,T) Contacts confirmed by TEM recapture at 40,000X (0.27 nm/pixel) with goniometric tilt. (U) Summary of ON CB coupling profiles. Heat map reflects the mean number of gap junctions formed by an individual cell of each class with every other class of ON CB and Aii cells. Abbreviations: a, adherens junction; gj, gap junction.
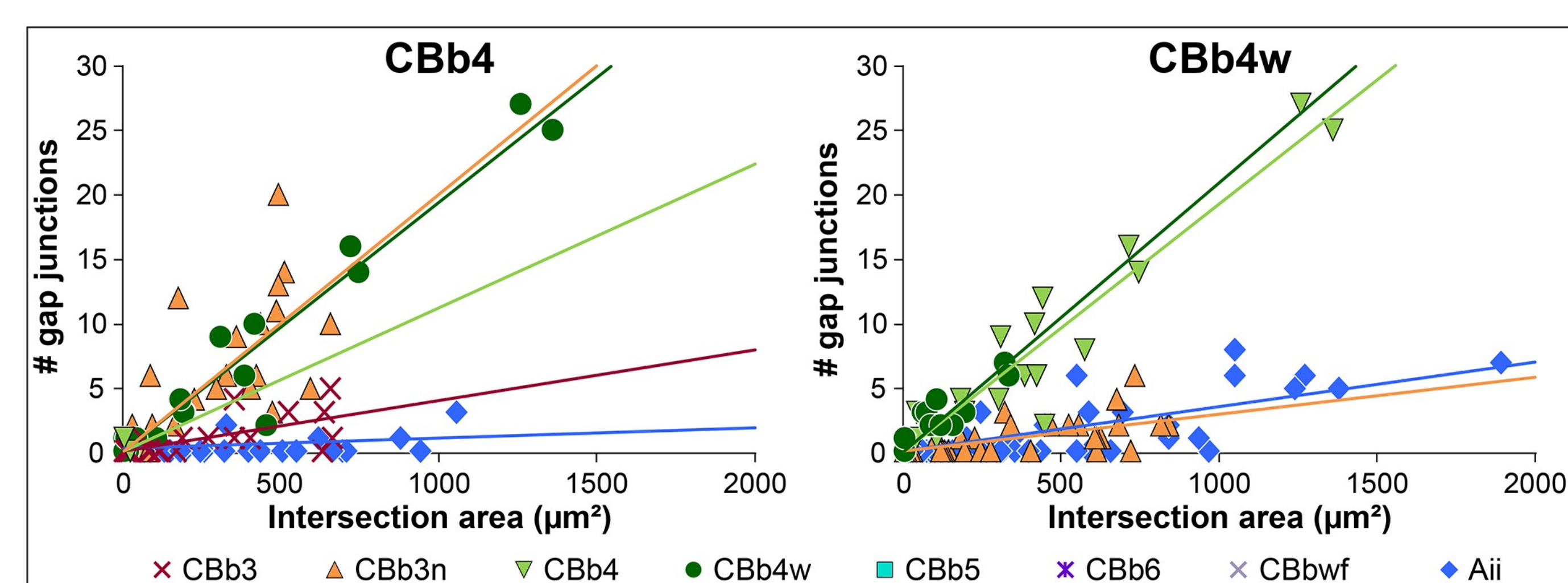
Class-to-class coupling frequency

Mean # gap junctions / cell: 0 >0 5 10 15 20 25 30 35

Figure 3. Geometry accounts for the variation in, but not specificity of, ON CB coupling. Graphs depict coupling frequency as a function of the degree of axonal field intersection between cell pairings for each class. E.g. CBb4 cells are 6 times as likely to couple with CBb4w cells as costratifying CBb4w cells to couple with CBb3n cells, despite having smaller and less branched arbors. CBb4 cells virtually lack coupling with Aii cells, while CBb4w cells lack coupling with CBb3 cells.
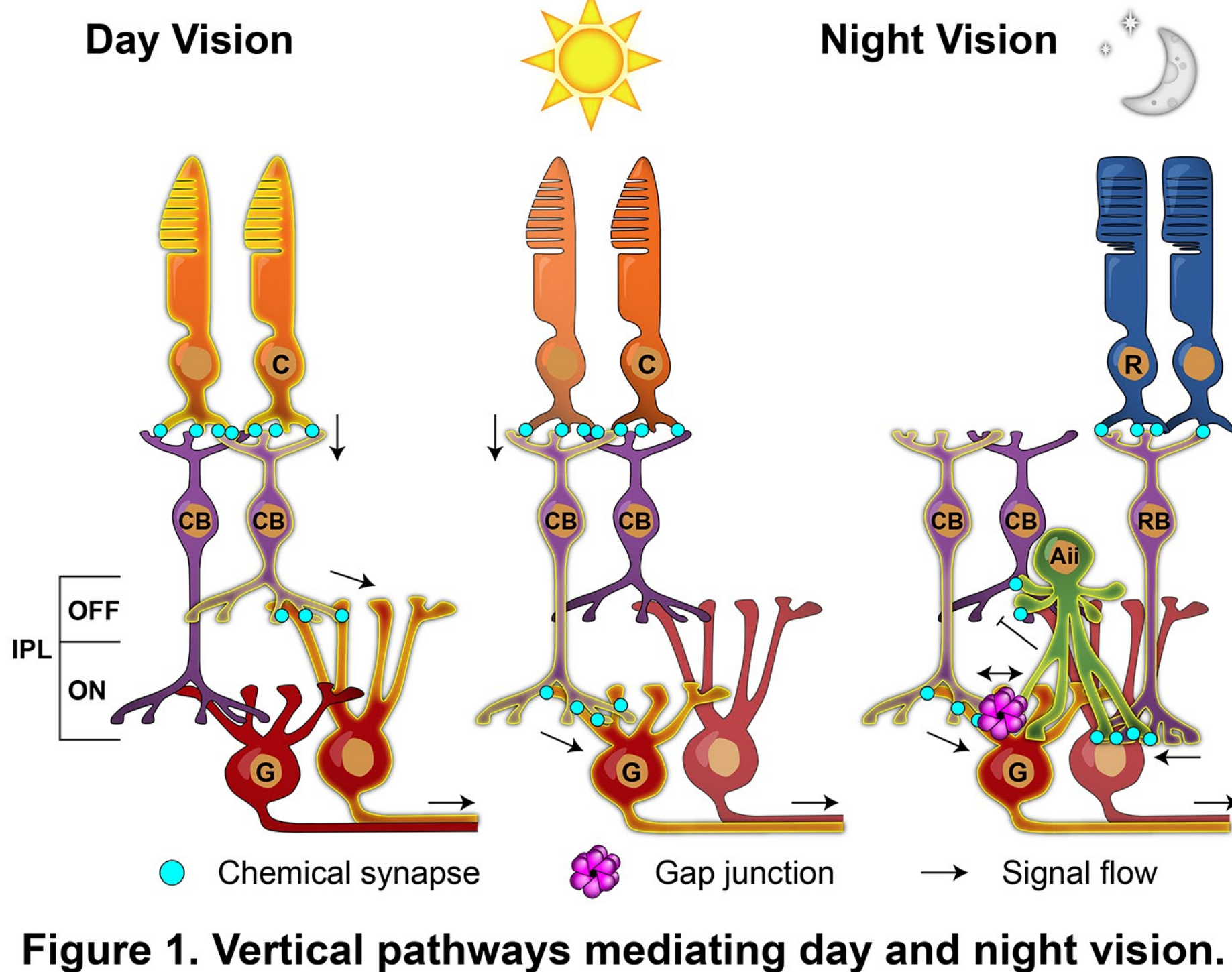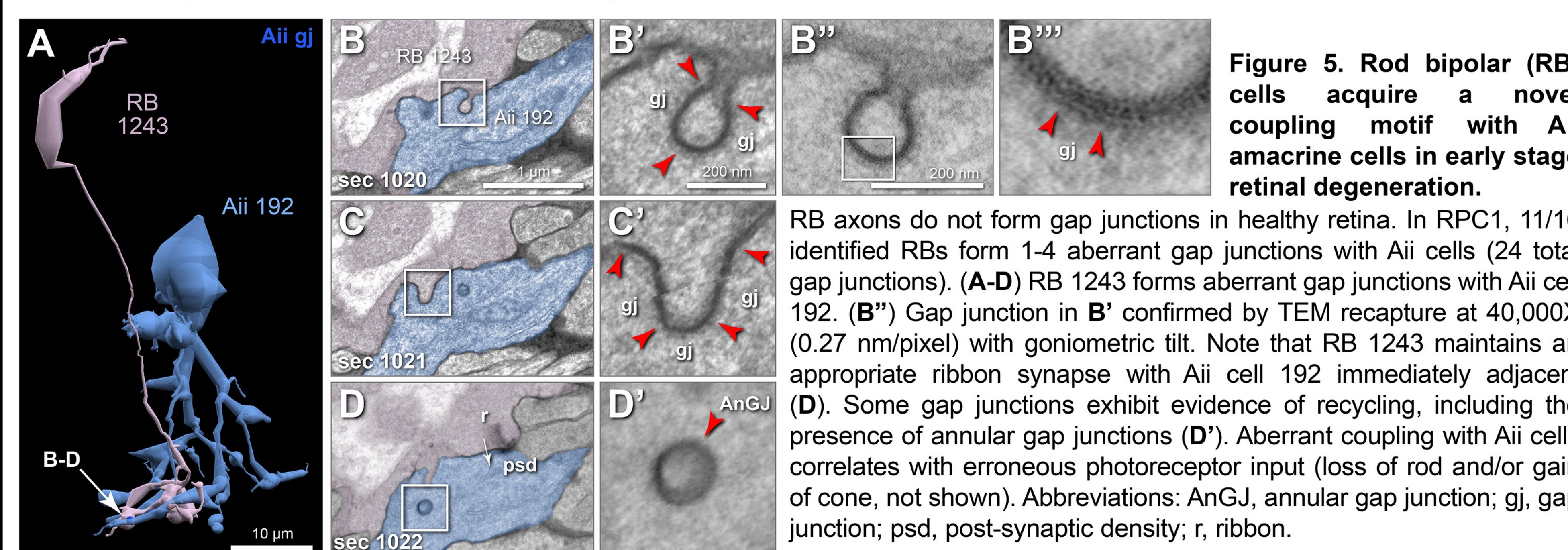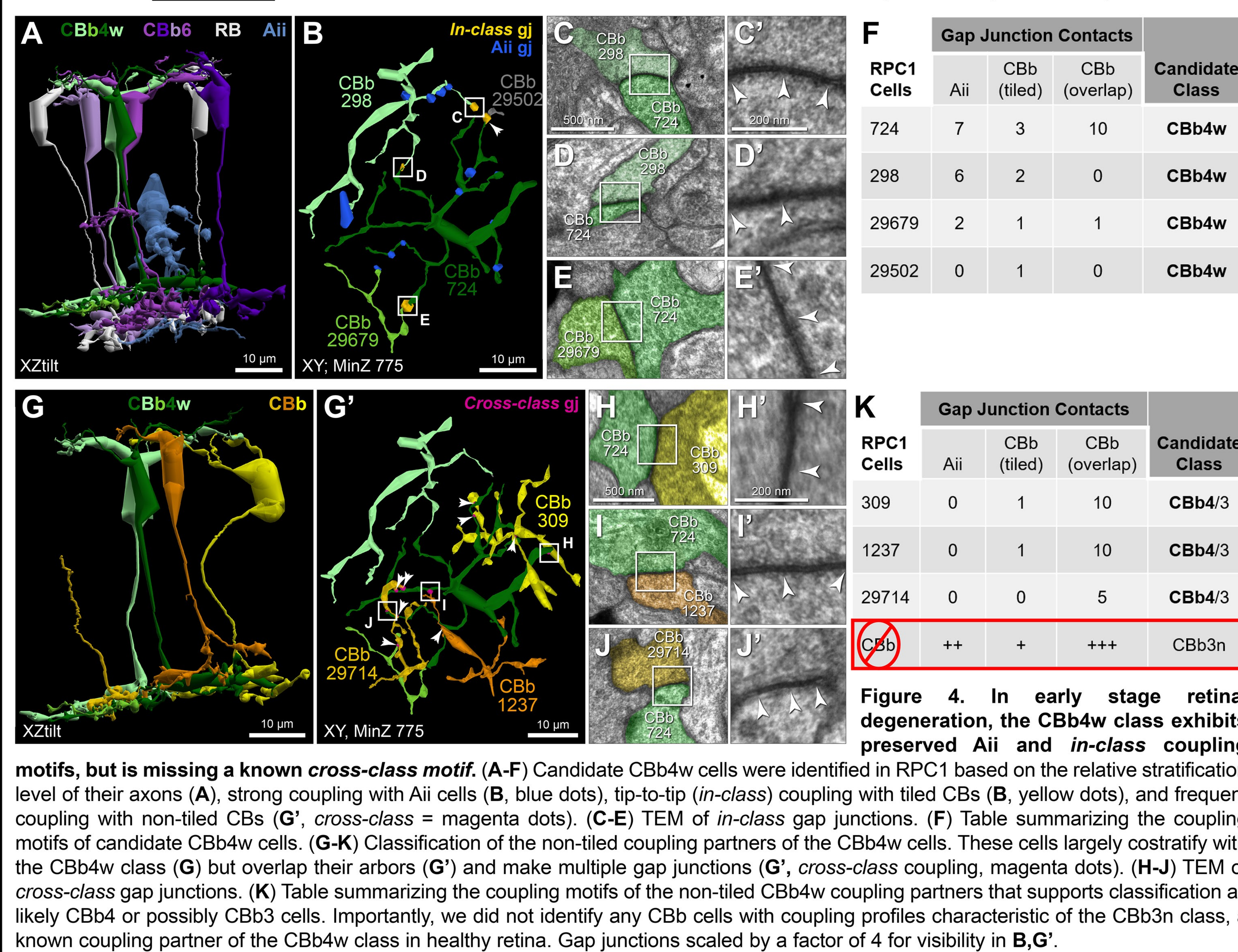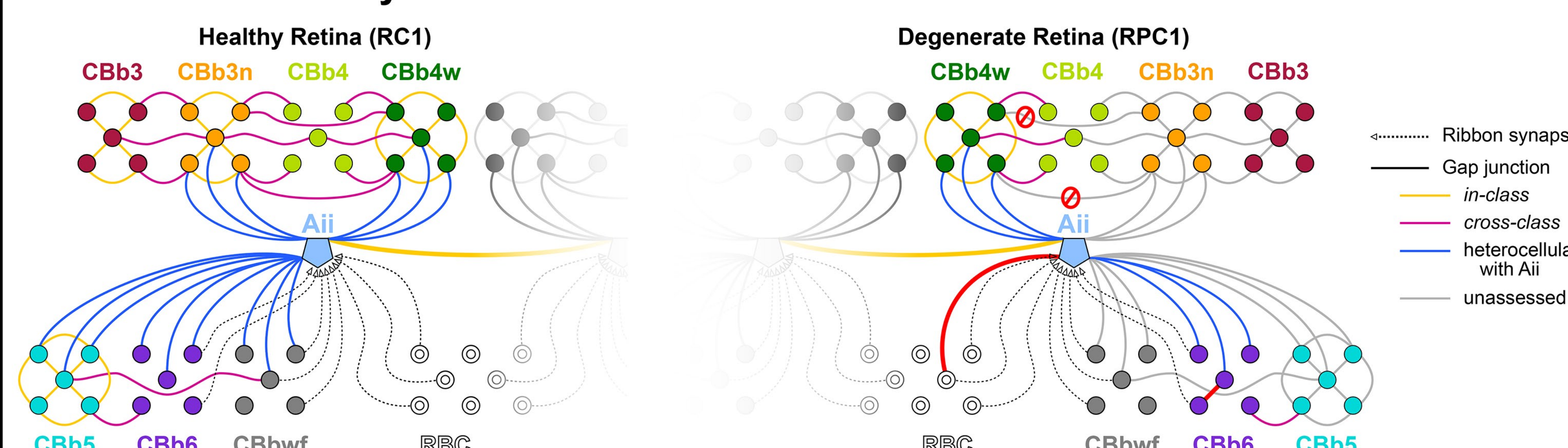
4. Altered coupling motifs in degenerate retina (Pathoconnectome RPC1).

RPC1:
- Transgenic Rhodopsin P347L rabbit model of autosomal dominant retinitis pigmentosa (Kondo et al., IOVS 2009).
  - 10-months old, male, New Zealand White background. Light adapted, para-visual streak.
  - Presenting with ~50% rod photoreceptor loss and early stage 1 retinal remodeling.
  - 0.07 mm diameter volume built from 946 serial TEM sections (70 nm thick) at 2.18 nm/pixel.

Table F:

RPC1 Cells	Gap Junction Contacts			Candidate Class
	Aii	CBb (tiled)	CBb (overlap)	
724	7	3	10	CBb4w
298	6	2	0	CBb4w
29679	2	1	1	CBb4w
29502	0	1	0	CBb4w

Table K:

RPC1 Cells	Gap Junction Contacts			Candidate Class
	Aii	CBb (tiled)	CBb (overlap)	
309	0	1	10	CBb4/3
1237	0	1	10	CBb4/3
29714	0	0	5	CBb4/3
CBb	++	+	+++	CBb3n

Figure 4. In early stage retinal degeneration, the CBb4w class exhibits preserved Aii and *in-class* coupling motifs, but is missing a known *cross-class* motif. (A-F) Candidate CBb4w cells were identified in RPC1 based on the relative stratification level of their axons (A), strong coupling with Aii cells (B, blue dots), tip-to-tip (*in-class*) coupling with tiled CBs (B, yellow dots), and frequent coupling with non-tiled CBs (G', *cross-class* = magenta dots). (C-E) TEM of *in-class* gap junctions. (F) Table summarizing the coupling motifs of candidate CBb4w cells. (G-K) Classification of the non-tiled coupling partners of the CBb4w cells. These cells largely costratify with the CBb4w class (G) but overlap their arbors (G') and make multiple gap junctions (G', *cross-class* coupling, magenta dots). (H-J) TEM of *cross-class* gap junctions. (K) Table summarizing the coupling motifs of the non-tiled CBb4w coupling partners that supports classification as likely CBb4 or possibly CBb3 cells. Importantly, we did not identify any CBb cells with coupling profiles characteristic of the CBb3n class, a known coupling partner of the CBb4w class in healthy retina. Gap junctions scaled by a factor of 4 for visibility in B,G'.

Figure 5. Rod bipolar (RB) cells acquire a novel coupling motif with Aii amacrine cells in early stage retinal degeneration. RB axons do not form gap junctions in healthy retina. In RPC1, 11/16 identified RBs form 1-4 aberrant gap junctions with Aii cells (24 total gap junctions). (A-D) RB 1243 forms aberrant gap junctions with Aii cell 192. (B'') Gap junction in B' confirmed by TEM recapture at 40,000X (0.27 nm/pixel) with goniometric tilt. Note that RB 1243 maintains an appropriate ribbon synapse with Aii cell 192 immediately adjacent (D). Some gap junctions exhibit evidence of recycling, including the presence of annular gap junctions (D'). Aberrant coupling with Aii cells correlates with erroneous photoreceptor input (loss of rod and/or gain of cone, not shown). Abbreviations: AnGJ, annular gap junction; gj, gap junction; psd, post-synaptic density; r, ribbon.

5. Network Summary & Conclusions

Healthy Retina (RC1) — Degenerate Retina (RPC1)

Legend: Ribbon synapse; Gap junction; in-class; cross-class; heterocellular with Aii; unassessed

➤ Gap Junctions formed by ON CBCs are prominent network components with specificity rivaling that of chemical synapses.

➤ Gap junctions provide a mechanism for extensive lateral signal transfer within and across parallel processing streams.

➤ Circuit topology is altered in retinal degeneration prior to complete loss of rods, with implications for therapeutic interventions.

6. Support & Disclosures