# Connexin36 Forms Synapses Essential for Night Vision

**Rod signals reach ganglion cells via at least five pathways. A new study in this issue of *Neuron* shows that all pathways from rods to ON-type ganglion cells require connexin36. This is the first physiological demonstration in mammals that a well-defined neural circuit requires a gap junction as an obligatory transmission step.**

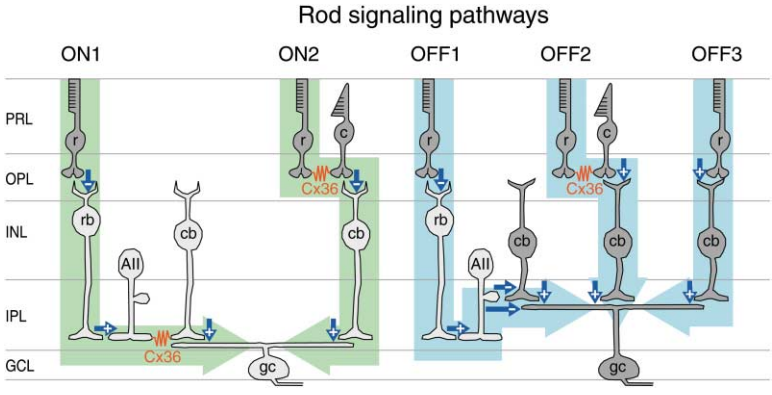
The visual system encodes light signals in parallel pathways at every stage of processing. At the first stage in the retina, photoreceptors divide the job of phototransduction between rods, specialized for night vision, and cones, specialized for day vision. Photoreceptors synapse onto relay interneurons (bipolar cells), which divide their task between an ON type specialized for signaling light increments and an OFF type specialized for signaling light decrements. Bipolar cells synapse onto the output neurons of the retina (ganglion cells), most of which collect inputs from one type of bipolar cell and so adopt an ON or OFF response themselves. Further distinguishing features of a ganglion cell are created by laterally connecting interneurons, horizontal cells, and amacrine cells (Sterling, 1998). Retinal cells communicate via chemical synapses, using the same neurotransmitters and receptors common to the brain and spinal cord. In addition, many mammalian retinal cells are electrically coupled to one another via gap junctions (Sterling, 1998). But do these retinal gap junctions serve as essential circuit elements, as has long been known to occur in invertebrates (Furshpan and Potter, 1957), or do they serve primarily as long-range synchronizing elements (Galarreta and Hestrin, 2001)?

In this issue, Deans, Volgyi, and colleagues investigated the retina of a mouse lacking connexin36 (Cx36). They found that all pathways from rods to ON ganglion cells require Cx36 gap junctions for signal transmission: without Cx36 gap junctions, rod-driven signals cannot get through (Deans et al., 2002).

Previous studies established five parallel pathways for rod vision (Figure; Sterling, 1998). In the dark, the rod releases glutamate continuously and hyperpolarizes the rod bipolar through a sign-inverting metabotropic synapse (mGluR6). In the "starlight" pathways (ON1, OFF1), single photons transiently hyperpolarize the rod, suppressing its glutamate release, which depolarizes the rod bipolar. The rod bipolar excites the AII amacrine, which makes a sign-conserving gap junction synapse onto an ON cone bipolar and a sign-inverting glycinergic synapse onto an OFF cone bipolar; ON and OFF cone bipolars excite corresponding ganglion cells. In the "twilight" pathways (ON2, OFF2), the rod transmits signals through the pedicle of a neighboring cone via a sign-conserving gap junction, and the cone in turn signals through ON and OFF cone bipolar cells to corresponding ganglion cells (Figure; Smith et al., 1986; DeVries and Baylor, 1995). In an additional pathway so far demonstrated only in rodents, the rod releases glutamate directly onto an OFF cone bipolar, which excites an OFF ganglion cell (OFF3; Hack et al., 1999; Soucy et al., 1998; Tsukamoto et al., 2001). Rod pathway wiring is complex: there are two kinds of sign-inverting synapses (i.e., mGluR6 and glycine receptors), and one pathway uses two sign inversions (OFF1).

Deans, Volgyi, and colleagues used the Cx36 Knockout (KO) mouse and a variety of methods to test the prediction that rod signaling to ON ganglion cells requires obligatory transmission through Cx36 synapses (Deans et al., 2002). To locate Cx36, the authors replaced the *Cx36* coding sequence with two histochemical reporters and found that several cell types express Cx36 including rods, some types of cone bipolar cells, and amacrine cells. Cones also likely express Cx36, but this could not be verified. Immunocytochemistry confirmed that the AII amacrine cell expresses Cx36. Furthermore, intracellular injection of Neurobiotin tracer, which passes through retinal gap junctions, showed that the Cx36 KO mouse does not have functional gap junctions either between AII amacrine cells and ON cone bipolars or between neighboring AII cells, which normally couple to one another (Sterling, 1998).

To confirm the anatomical observations, the authors recorded extracellularly from ON ganglion cells in vitro.




Five Signaling Pathways from a Rod to a Ganglion Cell

A rod connects to an ON ganglion cell via two pathways (ON1, ON2) and to an OFF ganglion cell via three pathways (OFF1–OFF3). Both pathways from the rod to ON ganglion cells depend on the connexin36 gap junction as an essential output synapse (Cx36; red). ON cells depolarize to light (lighter gray) whereas OFF cells depolarize to dark (darker gray). Arrows indicate chemical synapses that are sign conserving (+) or sign reversing (−). All cells release glutamate except the AII amacrine cell, which releases glycine. All chemical synapses and gap junctions depolarize the postsynaptic cell with two exceptions: thephotoreceptor hyperpolarizes the rb or ON cb via a metabotropic glutamate receptor (mGluR6) cascade and the AII hyperpolarizes the OFF cb and OFF gc via a glycine ionotropic receptor. Abbreviations: r, rod; c, cone; rb, rod bipolar cell; cb, cone bipolar cell; AII, AII amacrine cell; gc, ganglion cell; PRL, photoreceptor layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

The threshold light intensity for eliciting a cone-driven response in the wild-type (WT) ON ganglion cell was estimated in two ways, using 468 nm light: with the response to 10 Hz flicker, a frequency readily transmitted by the cones, but not by the more sluggish rods, and with the response to a 500 ms light pulse delivered when rods are strongly light adapted and in saturation. Both methods found the cone threshold to be an intensity that generates ~30 isomerized Rhodopsins (Rh*) $rod^{-1}$ $s^{-1}$. The authors then measured thresholds for a dim light pulse in WT and Cx36 KO ganglion cells. Most WT cells had a low threshold of $<0.5$ Rh* $rod^{-1}$ $s^{-1}$, far below cone threshold. However, all Cx36 KO cells had a much higher threshold of ~30 Rh* $rod^{-1}$ $s^{-1}$, matching the WT cone threshold. In short, Cx36 KO's ON ganglion cells do not receive rod-driven signals.

These physiological results establish that the Cx36 gap junction acts as an obligatory synapse in all pathways from rods to ON ganglion cells. This result is consistent with the prediction based on the two known pathways (ON1, ON2; Figure), but encompasses other possible pathways. Thus, if there is another undiscovered rod pathway to the ON ganglion cell, it too must use the Cx36 gap junction as an obligatory synapse. Such a strong conclusion about the role of Cx36 gap junctions in rod signal transmission is unlikely to have been reached with pharmacological manipulations as gap junction antagonists lack adequate specificity in their action. Reason to believe that ON1 and ON2 constitute the only pathways from rods to ON ganglion cells comes from previous pharmacological investigations using the mGluR6 agonist L-AP-4 (Slaughter and Miller, 1981). Application of L-AP-4 pins the rod bipolar and cone ON bipolars in the hyperpolarized dark state, making them unresponsive to the decrease in glutamate release that occurs when rods hyperpolarize in response to light. Evidence from many studies since 1981 using L-AP-4 dovetails nicely with the new evidence from the Cx36 KO data in supporting ON1 and ON2 as the only pathways from rods to ON ganglion cells.

An issue that remains unresolved is whether an ON ganglion cell receives signals from the starlight pathway (ON1) only under scotopic (rod-mediated) conditions and the twilight pathway (ON2) only under mesopic (rod- and cone-mediated) conditions (Smith et al., 1986). To resolve this, one needs a mouse where Cx36 gap junctions can be selectively inactivated, at either the AII→cone bipolar synapse, or the rod→cone synapse.

Two results from the wild-type mouse were unexpected based on established retinal circuitry (Figure; Deans et al., 2002). The first unexpected result was that certain ON ganglion cells ("high-sensitivity cells") appear to receive inputs only from rods. Such rod-only input seems puzzling, because rod signals can reach an ON ganglion cell only via an ON cone bipolar cell, which should also transmit cone signals (ON1, ON2; Figure). This paradox might be explained by an unusual feature of the mouse retina. Mouse cones coexpress both a middle-wavelength sensitive (M) pigment ($\lambda_{max}$ = 508 nm) and an ultraviolet sensitive (UV) pigment ($\lambda_{max}$ ~360 nm); coexpression varies across the retina such that cones in the most dorsal retina (ca. 20% of the total) express mostly M pigment, whereas cones in ventral retina (ca. 80% of the total) express mostly the UV pigment (Lyubarsky et al., 1999; Applebury et al., 2000). The 468 nm stimulus used in the study would be effective in stimulating the rod pigment (rhodopsin) and the M pigment, but more than 10,000-fold less effective for the UV pigment. Thus, depending on the level of coexpression of M-pigment in cones expressing the UV pigment, any ganglion cell recorded in ventral retina might appear to receive inputs only from rods. The second unexpected result was that certain ON ganglion cells ("low-sensitivity cells") receive inputs only from cones. The cone-only input seems puzzling, because all cones receive rod signals via the rod→cone gap junction (ON2, OFF2; Figure); thus, any cell that receives cone input should also receive rod input. However, the low-sensitivity cells were rare (14% of recordings) and so, perhaps rods were, in some way, insensitive in these recordings. The in vitro mouse retina is a delicate preparation, and the fact that rod signals were evident in ~86% of recordings is nonetheless impressive.

Cones are likely the original type of vertebrate photoreceptor, and so when rods evolved they had to "stitch" themselves into the existing cone pathways. Why would an important signaling pathway, such as the rod system, use gap junctions rather than conventional chemical synapses at so many critical junctures to stitch itself in? One idea is that rod pathways require modulation with the diurnal cycle: upregulation at night and downregulation during the day. One way to accomplish this diurnal switch is to release a neuromodulator in the retina that effectively modulates rod pathway synapses (Wang and Mangel, 1996). Perhaps such modulation works more efficiently when the target is a gap junction synapse rather than a subset of chemical synapses. A second idea is that the gap junction allows an inhibitory interneuron to simultaneously inhibit certain cells (via chemical synapses) and excite others (via gap junction synapses; Sterling, 1998). The AII amacrine cell uses this seemingly efficient strategy to transmit the rod's single photon signal to both ON and OFF pathways.

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