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Timing Is Everything: Direct Measurement of Retinol Production in Cones and Rods

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"In the last few years there has accumulated a considerable amount of new and highly precise data describing various visual functions." – Selig Hecht, 1937.

Vision research is one of the few subject areas in biology with a vigorous and active modern focus and a rich history of relevant quantitative literature. Now that it is no longer necessary for me to navigate the musty stacks of 80-yr-old Welch Hall to find the classic vision papers, many of which, for example, are available online in the *Journal of General Physiology*, one of my duties as a responsible commentator—to put the new work of Ala-Laurila and colleagues (see Ala-Laurila et al. on p. 153 of this issue) into its proper long-term context and perspective—becomes a bit easier.

That rod and cone ciliary photoreceptor cells are different was first recognized in the 19th century (Schultze, 1866). In fact, Schultze, armed with the physiological data obtained by Aubert and Helmholtz, proposed the so-called Duplicity Theory, which stated that the vertebrate retina is not one sensory organ, but two. Despite advances in the molecular genetics of vision and the biochemistry and molecular biology of phototransduction, a complete mechanistic understanding of rod and cone physiology has remained elusive. However, recent progress on a number of fronts suggests that we will, sooner rather than later, know how rod and cone photoreceptor systems partner to form a unified visual organ that functions more or less seamlessly over as much as the 10 orders of magnitude of photon flux that we might encounter in daily life (for review see Burns and Arshavsky, 2005).

Vertebrate visual pigments are not reversible photochromic sensors like invertebrate pigments. Invertebrate pigments can be switched on and off by different colors of light. However, photon capture by vertebrate visual pigments causes essentially irreversible photochemical isomerization of the covalently bound 11-cis-retinylidene chromophore. The new all-trans chromophore instantaneously generated by light acts akin to a pharmacological agonist and allows the 7-transmembrane receptor in which it resides to activate a heterotrimeric G protein as the first step in the phototransduction cascade. Receptor activity is modulated by phosphorylation, arrestin binding, and release of all-trans retinal from its binding pocket. But how does 11-cis retinal, the essential chromophore of most visual pigments, get back to where it belongs? And for that matter how is 11-cis retinal produced, and where?

The retinoid cycle, or the visual cycle, refers to the conversion of all-trans retinal to 11-cis retinal in the eye. The "regeneration" of opsin apo-protein with 11-cis retinal completes the cycle. There appear to be two separate and distinct cycles, one for rods residing in the retinal pigment epithelium and one for cones in the retinal Müller cells (Mata et al., 2002), although some controversy exists concerning the enzymology (Gollapalli and Rando, 2003; Mata et al., 2005). But in both photoreceptor classes, the first step of the cycle is the reduction of all-trans retinal by retinol dehydrogenase in the presence of NADPH, which occurs in the photoreceptor cells themselves.

The rates of recovery, or resensitization, after photobleaching vary dramatically between rods and cones. Cones recover much more rapidly than rods. The rate of recovery could be limited by the rate of binding of 11-cis retinal supplied by the retinoid cycle to opsin apoprotein or by the release rate of all-trans retinal. Interestingly, free opsins themselves can activate the visual cascade, and the basal activity of cone opsins are important in light adaptation, as is the basal activity of rod opsins in determining sensitivity; the ability of opsin to activate G proteins must be suppressed by the potent inverse agonist activity of 11-cis retinal for rod cells to achieve single photon sensitivity.

Ala-Laurila and colleagues performed microspectrophotometry and fluorescence imaging studies on isolated salamander photoreceptors. They were particularly interested in the rates of release of all-trans retinal from bleached pigment and its enzymatic reduction to all-trans retinol (vitamin A1). They took advantage of the fact that retinol is fluorescent and retinal is not (although it should be noted that amphibian photoreceptors contain a mixture of vitamins A1 and A2, dehydroretinol, and that the fluorescence quantum yield of vitamin A1 is nearly 40-fold higher than that of vitamin A2).

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Fluorescence imaging was used to measure the time course of the post-bleach appearance of retinol. Microspectrophotometery at multiple wavelengths and under two polarization conditions was used to quantitate the post-bleach photoproduct decay kinetics. This is no sleight of hand methodology, especially because it was applied to multiple individual photoreceptor cell types isolated from the salamander retina: red-sensitive rods containing the rod pigment RH1, green-sensitive rods containing the cone-type pigment SWS2, blue-sensitive cones containing the cone pigment SWS2, and red-sensitive cones containing the cone pigments M/LWS. Unfortunately, blue-sensitive cones, which contain the same pigment as the green-sensitive rods, could not be studied in detail for technical reasons.

The hypothesis underlying the experiments was that differences in rates of recovery among rods and cones might be related to the kinetics of photoproduct decay and the clearance of all-trans retinal by reduction to retinol. The results show that the rate of post-bleach retinol production can be determined either by the pigment's intrinsic photoproduct decay rate, which produces all-trans retinal, or by the retinol dehydrogenase reaction rate depending on the cell type. In rod outer segments after 90% bleach, retinol production occurs in a wave-like fashion from the base to the tip, with highest activity in the base. This observation, unique to rods, is most likely due to limits on the endogenous local NADPH concentration. The appearance of all-trans retinal, however, which should depend only on the distribution of bleached pigment, is uniform across the cell in both rods and cones. Interestingly, all-trans retinal appears to be completely converted to all-trans retinol in the photoreceptors. Virtually no all-trans retinal remains, which argues against the existence of secondary retinal binding sites on rhodopsin (Heck et al., 2003; Schädel et al., 2003).

The rate of dark adaptation is much faster in cones than rods and, as expected according to the hypothesis that photoproduct decay is rate limiting, all-trans retinal was released more rapidly by cone pigments compared with rod pigments. For example, the decay rate of red-sensitive cones was determined to be ~70 times greater than that of the red-sensitive rods; the green-sensitive rods were intermediate.

In summary, the work of Ala-Laurila et al. (2006) provides a mechanistic basis for understanding why the cone visual cycle spins much more rapidly than the rod cycle, at least with respect to the contributions of the photoreceptor cells themselves. Photoproduct decay to produce all-trans retinal, the reductive conversion of retinal to retinol, and the clearance of retinol all occur more rapidly in cones than in rods.

Hecht certainly could not have imagined the biochemical complexity underlying the physiology of a seemingly straightforward light reaction involving "visual purple," as the visual pigments were collectively then known. Although his goal was to describe the key elements of visual physiology by simple equations, he conceded, "reactions are certainly more involved than I have supposed" (Hecht, 1937). But with the proliferation of new experimental approaches and technologies, including the ability to interrogate single isolated rods and cones with more flexibility and precision (Kefalov et al., 2005) and the use of genetically engineered experimental animals (Wenzel et al., 2005; Nikonov et al., 2006), at least there is now substantial optimism that the scientific great grandchildren (or even grandchildren) of Hecht will witness a complete understanding of the mechanistic basis of the remarkable physiology of visual perception.

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