**PERSPECTIVES**

**An Hypothesis to Account for the Renewal of Outer Segments in Rod and Cone Photoreceptor Cells: Renewal as a Surrogate Antioxidant**

It is undisputed that glutathione (GSH) is an important cellular antioxidant. Although it is commonly believed that GSH is present in all retinal cells, several publications show that GSH is not immunologically detectable in outer segments of rod and cone photoreceptor cells, but is present in inner retinal cells and the pigment epithelium. Using these intriguing and surprising findings as a starting point, an hypothesis is proposed that the renewal of outer segments serves as a surrogate antioxidant for GSH and that the exceptional vulnerability of photoreceptor cells to certain toxic chemicals is linked to the deficiency in GSH in outer segments as a reductant, a detoxificant, and as an enzymatic cofactor. It is suggested that this deficiency of GSH is not damaging to outer segments under normal conditions, because renewal serves to replace any damaged molecules before they increase to detrimental levels. However, when photoreceptors are stressed, the renewal of outer segments alone is not capable of overcoming the higher rates of oxidizing and detrimental chemical reactions, and the health of the entire photoreceptor cell is at risk. The hypothesis is supported by a consideration of the essential role of the NADPH-dependent retinol reductase, by the different localization within photoreceptor cells of two key metabolic enzymes that are sensitive to oxidation, NADPH-dependent glutathione reductase and glucose dehydrogenase and the sodium-potassium ATPase, and by a consideration of the effects of toxic chemicals that selectively damage photoreceptor cells. (Invest Ophthalmol Vis Sci. 2008; 49:3259–3261) DOI:10.1167/iovs.08-1785

It has been 40 years since Young published his classic paper on the renewal of photoreceptor cell outer segments, arguably one of the most important contributions in the field of the biology of photoreceptor cells. Although the general principles of renewal of outer segments in rod and cone photoreceptor cells are now known in relation to the assembly and shedding of discs, it is still a mystery why these organelles evolved a daily renewal (~10%/d) of their molecular and structural constituents. Conventional wisdom suggests that certain constituents of the outer segment (e.g., the polyunsaturated fatty acids) are particularly prone to oxidative damage due to exposure to photons in the presence of a near-arterial level of oxygen. If, in fact, daily oxidant stress on photoreceptor cells is considerable, then an obvious question is how is it that in most of us (and in most of the animals we study) photoreceptor cells survive quite well throughout many decades? The answer presumably resides in the outer segments' having a substantial capability to respond to ongoing oxidative stress. Any consideration of intracellular antioxidant defense systems begins with glutathione (GSH), ascorbic acid (AA), and vitamin E. GSH and AA are water soluble and function principally in the cytoplasm and mitochondria, whereas vitamin E is the principal free radical, chain-breaking antioxidant in membranes. What makes GSH, AA, and vitamin E special is that they interact in a series of coupled oxidation-reduction reactions. Most important, GSH is the “engine” that drives the coupled cascade, because it has the highest redox potential and thus is capable of reducing dehydroascorbic acid back to AA, which, in turn, reduces oxidized vitamin E. GSH is normally regenerated from the oxidized disulfide (GSSG) by a mechanism involving the NADPH-dependent glutathione reductase and glucose metabolism. In addition, of the three antioxidants, only GSH is capable of reducing S-S bonds in oxidatively modified proteins back to SH-bonds, thereby reversing the damage to the proteins.

Our laboratory made the first measurements of GSH in freshly excised rat retinas. Since these studies used whole retinas for the measurements of GSH, its cellular localization could not be determined. In the absence of such localization, it was assumed that all retinal cells contain GSH. It was thus rather surprising when immunocytochemical reports appeared in the literature showing weak to no GSH immunoreactivity in outer segments of rod and cone photoreceptors from rodent, primate and zebrafish retinas. In contrast, Müller cells and inner retinal neurons appear to contain substantial pools of this compound. There was also a hint from an earlier biochemical study that rat rod photoreceptors may not contain GSH (or AA), since the content of GSH (or AA), expressed as nanomoles per retina, was similar in normal and photoreceptor-less (RCS) rat retinas, a result consistent with the suggestion that the bulk of the GSH (and AA) is in the inner retina layers.

An important question is, how is it that under normal conditions photoreceptor outer segments are not compromised by this apparent deficit in antioxidant capacity? Is there an alternative mechanism? Could it be that Nature has evolved a “fail-safe” mechanism to ensure that the damage potential of molecular constituents prone to oxidation is minimized? By continuously renewing outer segments, damaged molecules have a minimal residence time in those organelles. Thus, I propose that the absence of GSH is not damaging to outer segments under normal diurnal conditions, because renewal of the outer segment serves to replace any damaged molecules, renewal serving as a “surrogate antioxidant.”

Let us consider two instructive examples of how it might act in this capacity. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) is a cytosolic protein whose active center contains an SH group that is sensitive to oxidation. G3PDH has a very high specific activity and makes up as much as 2% of the total protein in rod outer segments. Those features favor its activity being kept above that needed to sustain glycolysis in this organelle during its 10-day transit time along the outer segments. Alternatively, proteins that are susceptible to oxidation could be excluded from outer segments. An excellent example is the Na+-K+ ATPase. Autoradiography of 3H-Houabain binding in frog retinas and immunocytochemistry of the enzyme in rat rods show little to no enzyme in the outer segments. From the Eye Research Institute, Oakland University, Rochester, Michigan.

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segments, the site of the high-Na\(^+\) conductance (dark current), but enzyme activity is high in inner segments.

A second and even more likely explanation for the low-to-undetectable level of GSH in outer segments is that retinol dehydrogenase, like glutathione reductase, requires NADPH produced by the glucose-dependent hexose monophosphate shunt (HMP shunt).\(^{18-21}\) The reaction catalyzed by retinol dehydrogenase is the first step in the series of reactions that regenerate the visual pigments in rods and cones, a reaction of critical importance for re-establishing sensitivity to light. It may be that the primacy of the visual cycle dictated a need to eliminate competing pathways for NADPH in outer segments.

I offer this suggestion despite the findings of Hsu and Molday\(^{22}\) who reported that the rate of NADPH production from the HMP shunt in bovine outer segments is sufficient to support both the reduction of retinal to retinol and glutathione reduction. However, their argument was based on the stimulation of HMP shunt activity observed when NADP was added to isolated bovine rod outer segments (see Fig. 2 in their paper\(^{22}\)). Since intact plasma membranes are impermeable to NADP, this result means that the outer segments were probably leaky. In the presence of intact plasma membranes, compounds in the incubation media that do not penetrate membranes (e.g., NADP, ATP, and phosphorylated sugars) should not directly affect cellular metabolic activities (i.e., HMP activity) of the retina. Our results support this contention because the addition of phosphorylated compounds to media bathing isolated, intact rat retinas does not stimulate metabolic activity.\(^{25}\)

The idea that the retinol reductase and glutathione reductase are necessarily in competition for NADPH is supported by Kolesnikov et al.\(^{22}\) who have proposed that “the speed of retinol reduction is set by the availability of NADPH.” This also suggests that there is insufficient NADPH for the control of rhodopsin bleaching and for GSSG reduction.

Vertebrate photoreceptor cells may have reducing systems that do not depend on GSH. One such reducing system is thioredoxin and thioredoxin reductase,\(^{25,26}\) which has the capability of scavenging free radicals and maintaining SH groups. Because this redox system, like glutathione reductase, uses NADPH, my hypothesis predicts that this enzyme should not be present in outer segments, but it could supply reducing power in the mitochondria-rich inner segments. This is exactly what was reported for the immunocytochemical localization of thioredoxin and thioredoxin reductase in normal and tubby mouse retinas;\(^{27}\) neither was present in outer segments, but both were present in inner segments.

It is well known that vertebrate photoreceptors are uniquely sensitive to conditions that interfere with their enzymatic or metabolic activities. In addition to the case of inherited retinal degenerations, selective death of photoreceptor cells has been observed in response to the administration of certain chemical agents. Of the several agents known to produce photoreceptor cell death, without damaging the inner retinal layers, iodoacetate (IAA) is perhaps the best characterized.\(^{28-29}\) IAA is a potent inhibitor of G3PDH,\(^{30}\) and loss of photoreceptor function and viability is believed to result from depression of metabolic energy production to critical levels. Why is the detrimental action of IAA expressed only in photoreceptor cells? I feel the answer to this question is linked to the absence of GSH in these cells. It is well known that GSH is carboxymethylated by IAA, and the carboxymethylated product does not inhibit G3PDH.\(^{31,32}\) Thus, GSH serves to inactivate or detoxify IAA. Since outer segments of photoreceptor cells have an immunologically undetectable level of GSH,\(^{11,12}\) they are “sensitized” to low concentrations IAA. In contrast, inner retinal cells are protected against the same low concentrations of IAA, because these cells contain a high concentration of GSH. In addition to IAA, there are several chemical agents (i.e., N-methyl-N-nitrosourea\(^ {33-36} \) and ferrous ion),\(^ {37,38} \) that produce selective photoreceptor cell death. As in the case of IAA, I hypothesize that the absence of GSH sensitizes photoreceptor cells to their actions, whereas the presence of GSH in the inner retinal cells protects. For example, GSH both catalyzes the decomposition of N-methyl-N-nitrosourea and scavenges the methylating agent produced.\(^ {39} \) Reactive ferrous ion in the presence of hydrogen peroxide generates free radicals,\(^ {40-42} \) GSH scavenges free radicals and detoxifies hydrogen peroxide,\(^ {9} \) both of which protect against the detrimental reactions initiated by ferrous ion and oxygen.

In summary, it is proposed that renewal of outer segments in rods and cones substitutes for a deficiency in GSH, and overall antioxidant capacity in these organs and supports the health and vitality of these cells. However, in the face of an excessive oxidizing challenge or a chemical stress, the renewal of outer segments alone is no longer sufficient to overcome the higher rate of modification of proteins, fatty acids, and lipids. Hence, when outer segments are damaged, the health of the entire photoreceptor cell is at risk. In this regard, it is tempting to suggest that a slowing in the rate of renewal of outer segments in rod and cone photoreceptor cells could be a risk factor involved in the death of these cells in a variety of human diseases that are suggested to involve reactive oxygen species.\(^ {43-50} \) A deficiency in antioxidant capacity in photoreceptor cells may also provide an explanation for the enhanced susceptibility of RCS rat photoreceptor cells to light (relative to normal rats), since phagocytosis of rod outer segments by the pigment epithelium does not occur in these animals.\(^ {31,52} \)

References


