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Oxidative stress defense and repair systems of the ocular lens

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1. ABSTRACT

It is well accepted that reactive oxygen species (ROS) play a critical role in many biological processes including disease and longevity. Oxidation of proteins has been linked to many disease states and even the aging process itself. This was first proposed as "The free radical theory of aging" in 1956 by Denham Harman which suggests that free radicals causes cumulative and irreversible damage to macromolecules, loss of cellular function and cell death over time directly impacting health and lifespan. Cellular damage from ROS exposure has been termed oxidative stress, which is an imbalance between cellular ROS production and the ability of the cell to regulate ROS levels and repair damage caused by ROS. This review focuses on the role of oxidative stress in the eye lens as a model for understanding the role of oxidative stress systems in age-related human disease.

2. THE HUMAN EYE LENS

The lens is among the most accessible part of the body exposed to environmental insult and its transparent function in transmitting light into visual information has led to the evolution of distinctive protein and antioxidant systems to defend against environmental damage. Light of various wavelengths enters the eye, passes through the cornea to the aqueous humor and to the lens where it is focused. Focused light then passes through the vitreous humor before striking the retina. Both the cornea and the lens filter and focus light coming into the eye. It is primarily the lens that filters damaging ultra violet (UV) light so that light hitting the retina is almost exclusively composed of visible wavelengths (1). This UV filtering role of the lens is known to cause damage to lens proteins and may contribute to the development of cataract (2) (3) (4). To achieve the necessary refractive index for the lens to transmit light information, the protein concentration of the

lens must be very high. The center of a human lens contains a protein concentration as high as 450 mg/ml (5). The lens is an avascular, encapsulated, transparent tissue with a center of terminally differentiated fiber cells that are organelle free and incapable of protein turnover (4). The lens epithelium, which covers the anterior surface of the organ, contains the majority of transporters and metabolic enzymes in the lens (6) (7) and is responsible for the growth and development of the entire lens (8). Lens epithelial cells near the lens equator differentiate to form the fiber cells. This constant addition of fiber cells requires the lens to grow throughout life (4). This area of lens differentiation is called the superficial lens cortex. Mitochondrial respiration, carried out in the lens epithelium and superficial cortical fibers, accounts for 90% of the oxygen consumed by the lens, although other oxygen consumers are found in the area beyond these mitochondria containing cells (9). Oxygen enters the lens tissue via diffusion from the surrounding aqueous and vitreous humors but its consumption of oxygen keeps the partial pressure of oxygen in the lens fibers lower than at the surface of the lens (9). In this way, potentially detrimental effects of oxygen on lens fiber cell proteins are minimized. This is important for the maintenance of lens homeostasis since the fiber cells are devoid of organelles and contain the majority of lens crystallin proteins required for light refraction (10). Crystallins in the lens, described later in more detail, are synthesized during gestation, thus the lens is truly unique in that the cells and proteins contained in the lens fibers must remain functional for the life of the individual (4). Protein aggregation in the fibers as a result of oxidation, other protein modification and/or proteolysis results in loss of lens transparency and cataract formation (11) (12) (13) (14). In patients treated with hyperbaric oxygen (higher than normal oxygen tension), a high proportion go on to develop cataracts (15). Treatment of guinea pigs with hyperbaric oxygen induced cross linking of guinea pig lens nuclear crystallins into large disulfide-bonded aggregates capable of scattering light (16). Collectively these properties of the lens make it uniquely susceptible to oxidative stress and consequently the lens has evolved a multitude of systems to combat ROS damage.

2.1. Sources of lens ROS

Reactive oxygen species (ROS) are small readily diffusible molecules that contain unpaired electrons and include the hydroxyl radical (•OH), the superoxide anion ($O_2^{\cdot -}$), hydroperoxyl radicals ($HO\bullet_2$) and peroxynitrite ($OONO^-$). Hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) contain their full complement of electrons but exist in an unstable or reactive state.

ROS form in cells as a consequence of both endogenous and exogenous oxidative stress initiators including altered cellular respiration, viral infection, immune activity, disease states, U.V.-light exposure, radiation exposure, smoke, metals and drugs. Importantly, a fundamental source of endogenous ROS is produced in the mitochondria via the electron transport chain where inefficient electron coupling leads to the formation of $O_2^{\cdot -}$ (17) (18). It has been estimated that as much as 2% of oxygen is converted to $O_2^{\cdot -}$ under normal respiratory conditions (17) (19) (20). $O_2^{\cdot -}$ is converted by the superoxide dismutases (detailed later) to form H_2O_2, H_2O_2 in the presence of metal ions in the Fenton reaction can form the highly reactive and damaging •OH radical (21). Interaction of $O_2^{\cdot -}$ and nitric oxide (NO), a well characterized signaling molecule, gives rise to $OONO^-$, while $^1O_2$ may arise from absorption of photochemical energy by O_2 (22). These many propagation reactions demonstrate that production of $O_2^{\cdot -}$ can give rise to multiple damaging ROS species and thus the lens requires specialist antioxidant and enzyme systems to prevent damage to lens proteins exposed to ROS sources. Mitochondria produce less ATP and more ROS upon aging in mammalian tissues (22). This leads to further mitochondrial damage and more ROS production. This gradual progression towards $O_2^{\cdot -}$ production coupled with decreased antioxidant activity and levels in aging tissue is believed to contribute to a number of age-related degenerative disorders including age-related cataract. Evidence that mitochondrial ROS may play a role in lens protein oxidation and cataract formation comes from the fact that some inherited mitochondrial diseases are associated with an elevated risk of cataract (23), although the mechanisms underlying this phenomenon have not fully been elucidated. Several studies have provided evidence that loss of lens repair systems results in loss of mitochondrial combined with increased ROS levels in lens cells (24).

2.2. Defense systems in the lens

ROS causes oxidative modifications to proteins resulting in loss or gain of function and protein aggregation which results in light scatter and cataract formation. Oxidative stress defense mechanisms operating to defend or protect the lens against ROS range from simple ROS scavenger systems to more advanced enzyme protective systems, free metal binding proteins, and actual repair proteins that can restore normal function to oxidatively damaged proteins. The lens is also home to an interesting family of chaperone proteins that act to prevent aggregation of oxidized proteins within the cell. Failures in antioxidant defense/detoxifying systems to protect proteins against oxidative stress-induced damage or subsequent failure of the protein repair systems to restore protein activity by reversing oxidative stress-induced damage requires the action of protein salvaging systems such as the proteasome (25) which degrade damaged proteins. Importantly, many of these antioxidant and repair systems also depend on the availability of specific reducing systems which may critically limit and regulate the function of these systems in the lens. The many layered systems of lens antioxidant defense and repair under normal circumstances function efficiently to prevent damage to lens proteins and prevent cataract formation. Oxidative stress has been implicated in a number of ocular diseases including age-related macular degeneration (AMD) (26) (27), cataract (12) (13) (14) (28), Uveitis (29), corneal inflammation (30), glaucoma (31) (32) and Keratitis (33). Understanding the roles and mechanisms that underlie the activities of these lens oxidative defense systems provides insight into the

understanding of these and other oxidative stress-associated diseases.

3. GENERAL ROS SCAVENGERS OF THE EYE LENS

General ROS scavengers can be roughly described as those molecules that non-enzymatically eliminate ROS by acting as chain breakers to directly prevent the propagation of ROS formation through direct binding and detoxification of ROS and/or ROS generators. In the lens, the primary reducing system or ROS scavenger is glutathione (GSH). Other important and well studied ROS scavengers in the lens are ascorbate (vitamin c) and vitamin E, while the cartotenoids, which are supplied soley in the diet, have been the subject of multiple supplementation studies to determine their ability to prevent or delay AMD and cataract.

3.1. Glutathione

GSH is the primary scavenger of ROS in the lens and the predominant reducing system in other ocular tissues, including the retina and cornea where it protects against chemical and oxidative stress-induced damage (34). GSH is a dual function peptide that also participates in regulation of DNA and protein synthesis, cell-cycle control, signal transduction and proteolysis. It is also involved in multiple metabolic pathways and the immune response (35). GSH exists in unusually high levels in the lens. GSH functions to maintain protein thiol groups in their reduced form and therefore maintaining normal protein function (34). GSH is maintained in its reduced form by the enzyme glutathione reductase. Under normal cellular conditions in the lens epithelium (36) (37) GSH is almost entirely found in its reduced state (38) (39) with barely detectable levels of the oxidized form of GSH (GSSG). GSH is also found at high levels in the lens fiber cells where it is likely transported from the epithelium to the fiber cells through lens connexins. GSH is completely broken down and resynthesized in the rabbit lens about every 48 h (40). The epithelium of the lens also contains an active glutathione redox cycle. This pathway involves glutathione reductase (GSH-Rx), NADPH and the hexose monophosphate shunt (HMPS) which all function to reduce GSSG back to active GSH (41) (42) (43). Cultured human lens cells challenged with H_2O_2 show a linear increase in HMPS activity while rabbit lenses subjected to t-butyl hydroperoxide (tBHP) stress show near complete oxidation of GSH to GSSG, but this ratio is reversed after recovery in complete medium (44) possibly as a consequence of increased shunt and glutathione redox cycle activity. Treatment of cultured lens epithelial cells with 0.1mM H_2O_2 for up to 180 min showed depletion of the GSH pool over the first 60 min followed by full recovery (45), illustrating that lens cells react quickly to reduced GSH levels.

Levels of reduced GSH have been shown to decrease in the human lens with increased aging (36) (46) (47) and upon cataract formation (37). GSH was also observed to decrease in normal guinea pig lenses with aging, while slight decreases in guinea pig lens GSH were found following hyperbaric oxygen treatment (48). Decreased concentrations of GSH or a shift from GSH to GSSG in the lens is believed to increase the rate of post translational modifications of crystallins (49), and to perpetuate damage to key proteins containing –SH groups including Na/K-ATPase, cytoskeletal proteins and proteins associated with membrane permeability (44).

3.2. Ascorbate

Ascorbate (vitamin C) is found at high levels in the lens and is believed to be capable of consuming oxygen in the lens (50). In the presence of redox available metal ions, ascorbate is oxidized to dehydroascorbate and H_2O_2 and in the process O_2 is consumed (9). Dehydroascorbate is subsequently reduced back to ascorbate with reduced GSH and/or NADPH (51). Ascorbate is transported from the plasma across the blood-aqueous barrier by the ciliary body into the aqueous humor where it is believed to supply the rest of the eye (52). In addition to its oxidative role in H_2O_2 production, ascorbate can also act as a strong reductant and scavenger of ROS, particularly $O_2\cdot^-$. Ascorbate decreases membrane damage in diabetic rats (53), and photoperoxidation of lens membranes (54). It also prevents riboflavin mediated light-induced damage to cation pumps in the lens (55) (56) and it plays a role in lens development and maintenance of transparency during development (57). Ascorbate has been shown to protect the rat lens against selenite-induced cataract (58). Incubation of mouse lenses with high levels of ascorbate also protected against ROS-mediated decreases in membrane transport, ATP production and decreased GSH levels (59). It is known that measured levels of ascorbate decline with age, possibly contributing to cataract formation. In fact, the ratio of ascorbate to dehydroascorbate decreased in cataractous lenses compared to clear lenses (60) (61) (62) (63), suggesting a requirement for ascorbate in lens homeostasis and prevention of cataract.

These data provide evidence for a protective role for ascorbate in the lens, however, oxidized ascorbate – dehydroascorbate is likely damaging to the lens, suggesting that ascorbate can also contribute to lens oxidation and cataract formation, for instance, dehydroascorbate causes precipitation and cross-linking of bovine lens crystallins *in vitro* (64). The interaction of ascorbate with light in the presence of metal ions, as described above, leads to the formation of H_2O_2 and subsequent interaction of H_2O_2 with metal ions, particularly Iron (Fe) that could produce the damaging species •OH. In this way ascorbate can actually become a prooxidant and lead to protein damage via both H_2O_2 and Fenton production of •OH. The addition of both Fe and ascorbate to cultured lens epithelial cells led to a significant increase in ferritin synthesis (the intracellular iron storage protein) (65) and increased Fe loading into ferritin (66), therefore protecting against oxidative damage. A number of studies (reviewed by Chui & Taylor, 2007 (67)) indicate that consuming elevated levels of antioxidants such as ascorbate, carotenoids, and tocopherol is associated with delayed development of various forms of cataract. Indeed the Blue Mountains Eye Study found that participants with the highest quintile of total intake of vitamin C had a reduced risk of incident nuclear cataract (68). Even more effective was an above-median intake of

combined antioxidants (vitamins C and E, β-carotene, and zinc) which was also associated with a reduced risk of incident nuclear cataract (68).

3.3. Vitamin E

Vitamin E, the most important fat soluble antioxidant, is proposed to be an important chain breaking antioxidant for prevention of lipid peroxidation. Vitamin E is actually an umbrella term for a group of compounds called tocopherols and tocotrienols. α-tocopherol, the most active naturally occurring form, contains a chromane ring which reacts with organic peroxyl radicals and accounts for its antioxidant activity. It protects tissue lipids from free radical attack (69). There is evidence to suggest that vitamin c acts to recycle the α-tocopheryl radical back to α-tocopherol, therefore implying a synergistic function between these two antioxidants (51). Vitamin E supplementation has been shown to protect rat lenses against radiation-induced cataract (70). The delaying or preventive effect of vitamin E on cataractogenesis has also been studied in *in vivo* sugar cataract models, for example galactose cataracts. Two studies showed that vitamin E supplementation showed no protective effect on galactosemic cataract in rat lenses (71) (72) but two other studies showed that direct topical instillation of vitamin E liposomes into eyes increased rat lens vitamin E and helped protect against galactosemic cataract (73) (74). Just as the animal models for vitamin E supplementation and cataractogenesis produce mixed results so do the many human trials examining vitamin E supplementation and/or dietary/plasma levels and their association with cataract. Levels of lutein-zeaxanthin, retinoid, or α-tocopherol showed no differences between normal and cataractous human lenses (75) but in regular users of multivitamin supplements, the risk of nuclear opacification was reduced by one third. In regular users of vitamin E supplements and persons with higher plasma levels of vitamin E, the risk was reduced by approximately half (76). A low plasma vitamin E level was associated with a 3.7-fold excess risk of the progression of early cortical lens opacities compared with the highest quartile in hypercholesterolemic eastern Finnish men (77). In the Beaver Dam Eye Study, persons in with higher total serum tocopherol had a lower risk of cataract than those with a lower serum tocopherol (78) and lens opacities were associated with lower levels of riboflavin, vitamin E, Fe, and protein nutritional status (79). By contrast, McNeil *et al* (2004) found that vitamin E supplemented for 4 years at a dose of 500 IU daily did not reduce the risk of nuclear, cortical or posterior subcapsular (80).

3.4. Carotenoids

The best studied carotenoids in the eye are lutein and zeaxanthin. Carotenoids are made up of more than 600 structural variants that are all lipophilic pigments. They are synthesized in plants, fungi, bacteria and algae and provided to mammalians only in their diet, particularly in egg yolk and dark leafy green vegetables such as spinach or kale. Some carotenoids, including β-carotene, can protect against photo-oxidative damage by scavenging $^1O_2$ and peroxyl radicals and can interact synergistically with other antioxidants (81). They may lower the risk for several degenerative disorders, cardiovascular and ophthalmological diseases, and various types of cancer (82). In the eye, lutein and zeaxanthin have been shown to filter high-energy wavelengths of blue visible light, primarily in the macula of the retina and to act as antioxidants that protect against the formation of ROS (1).

A study by Gale *et al.*, (2001) showed that the risk of nuclear cataract was lowest in people with the highest plasma concentrations of α- or β-carotene while the risk of cortical cataract was lowest in people with the highest plasma concentrations of lycopene. In addition the risk of posterior subcapsular cataract was lowest in those with higher concentrations of lutein but high plasma concentrations of vitamin C, vitamin E, or zeaxanthin and β-cryptoxanthin were not associated with decreased risk (83). In the Blue Mountain Eye Study, as mentioned above, an increased intake of vitamins C and E, β-carotene, and zinc was associated with a reduced risk of nuclear cataract but not with cortical or posterior subcapsular cataract (68). The Beaver Dam Eye Study, also mentioned above, found that patients with the highest quintile of lutein intake were half as likely to develop nuclear cataract as those in the lowest quintile of intake (84). In the Pathologies Oculaires Liées à l'Age (POLA) Study, the highest quintile of plasma zeaxanthin was significantly associated with reduced risk of nuclear or other cataract, among other carotenoids, only β-carotene showed a significant negative association with nuclear cataract (85). In the Carotenoids in the Age Related Eye Disease Study (CAREDs), women in the group with high dietary levels of lutein and zeaxanthin had a 23% lower prevalence of nuclear cataract compared with those with low levels (86). Despite the conclusions of these studies, an analysis of a number of supplementation studies involving carotenoids using the FDA evidence review system, found no evidence to support the fact that lutein or zeaxanthin can protect against cataract formation (87).

4. ANTIOXIDANT ENZYMES OF THE EYE LENS

4.1. The superoxide dismutases

The antioxidant enzymes of the eye catalytically remove ROS, in some cases generating other reactive or unstable species but generally resulting in a less toxic environment. Mitochondrial superoxide is dismutated to H_2O_2 and O_2 by the superoxide dismutases. Manganese superoxide dismutase (MnSOD, SOD 2) is present in the mitochondrial matrix while copper zinc superoxide dismutase (CuZnSOD, SOD 1) in localized to the intra-membrane space of the mitochondria and in the cytosol (18). In the absence of metal ions and thus the Fenton reaction, H_2O_2 produced in this reaction can then be detoxified by specialized enzymes including glutathione peroxidase (GSH-Px), the peroxiredoxins (Prxs) and catalase, described below.

A recent study examined total SOD (TSOD), MnSOD and CuZnSOD activities in lens epithelial cells derived from different types of cataract in patients having phacoemulsification cataract surgery. The highest level of all TSOD, MnSOD and CuZnSOD activity was in patients 50 years or younger with the activity gradually declining

with age. The level of TSOD activity increased in cortical cataract and the individual levels of CuZn-SOD and Mn-SOD activities in lens epithelial cells were also higher in cortical cataracts (88). MnSOD has been shown to protect human lens epithelial cells against oxidative stress using up- and down regulation of the enzyme in lens epithelial cells (89). The intrinsic apoptotic pathway was also significantly increased in MnSOD down-regulated lens epithelial cells compared to those with up-regulated MnSOD when exposed to superoxide (90).

CuZnSOD, which is not mitochondrial specific and makes up 90% of total SOD (91), was shown to prevent H_2O_2-induced oxidative damage when over-expressed in whole rat lenses (92). Treatment of human lens epithelial cells with a bolus of 17 β-estradiol, known to protect lens cells against oxidative stress by preserving the mitochondria, resulted in a significant rapid increase in the activity of MnSOD with no effect on levels of mRNA or protein (93).

4.2. Hydrogen peroxide detoxifiers

4.2.1. Catalase

Human Catalase is normally found localized in the peroxisomes, where it functions to decompose H_2O_2 to H_2O. One study investigated the effect of over expressing catalase in both the nucleus and mitochondria of mice, two areas normally devoid of catalase activity. They found that oxidative damage was reduced, H_2O_2 production and H_2O_2-induced aconitase (an enzyme involved in the TCA cycle) inactivation were attenuated, and importantly that cataract development, cardiac pathology, and the development of mitochondrial deletions were reduced (94). Resveratrol (a polyphenol antioxidant found in plants) was found to protect human lens epithelial cells against H_2O_2-induced oxidative stress by increasing catalase, SOD-1, and heme oxygenase (HO-1) expression (95). In rat lens epithelial explants, increased levels of GSH and catalase suppressed changes typically associated with TGFβ-induced transdifferentiation including wrinkling of the lens capsule, cell-surface blebbing, apoptotic cell loss, induction of αSMA, and loss of Pax6 expression (96). However, lenses from mice deficient in catalase did not show any increased susceptibility to oxidative stress generated by photochemical reaction (97), suggesting that catalase is not the most important enzyme involved in decomposing H_2O_2.

4.2.2. The peroxiredoxins

Given its peroxisomal location and the evidence outlined above it is unlikely that catalase is the major H_2O_2 detoxification enzyme in the lens. One likely H_2O_2 detoxification system in the lens is the Prxs, which are peroxide scavengers that possess redox active cysteines and use the thioredoxin system as an electron donor to detoxify H_2O_2, $OONO^-$ and a wide range of hydroperoxides (99). Six mammalians Prxs are known, with Prx 1, 2 and 6 found in the cytoplasm, Prx 3 in the mitochondria, Prx 4 in the endoplasmic reticulum, and Prx 5 found in various compartments in the cell including the peroxisomes and mitochondria (98) (99) (100). Since the mitochondria is the major source of H_2O_2 formation, Prx3 is well studied. Significant levels of Prx 3 mRNA and protein have been detected in human lens epithelial and fiber cells and it has been shown that Prx 3 is inducible by H_2O_2 in human lens epithelial cells but not by tBHP or heat shock (100).

4.2.3. Glutathione peroxidase

Another potentially important H_2O_2 detoxification enzyme is GSH-Px which also reduces H_2O_2 to H_2O or alkyl peroxides to alcohols at the expense of reduced GSH (34). In the lenses of H_2O_2-treated mice with deficient levels of GSH-Px, DNA strand breaks in the mice with elevated GSH-Px were 40% of those with normal GSH-Px activity (101), while in the GSH-Px knockout mouse lens DNA damage was 5 fold that of GSH-Px rich transgenic mice (101).

5. FREE METAL DETOXIFIERS OF THE EYE LENS

5.1. Metallothioneins

Fenton-type reactions, where H_2O_2 reacts with free metal ions i.e. Iron (Fe^{2+}) or copper (Cu^{2+}) to produce the •OH radical, are a major source of oxidative stress initiated by transition metals (102) and are thought to be involved in the formation of cataract (4) (103) (104). Detoxification of these metals by metallothioneins in combination with careful control of free iron levels by ferritin is central to prevent ROS production. Human exposures to toxic metals such as Fe, Cu, cadmium (Cd), lead, aluminum, mercury and others, may arise from fossil fuel emissions, industrial waste, cigarette smoke, and air pollution (105). These metals have been associated with increased risk of cataract formation (106) and it has been proposed that detoxification of metals may play a major role in prevention of cataract. In support of this theory, increased Cd levels (107) and increased Cu levels (108) have been reported in cataract compared to clear lenses. Another study that compared corticonuclear and mature cataracts found higher Fe levels in the mature cataract (109) suggesting a role for these metals in cataract formation.

Metallothioneins (MTs) serve to detoxify free metals, there are 16 known isoforms of MTs in humans, grouped into four classes: I, II, III, and IV. The human lens expresses MT classes I and II. Only one isoform, MTIIa, is specific for the lens epithelium, whereas the MTI isoforms are expressed at lower levels in both the lens epithelium and lens fibers (110). In addition, MTIIa exhibits increased expression in age-related cataract compared with clear human lenses (111) suggesting a possible role for MTIIa in lens protection. Lens MTs (Ig, If, Ih, Ie, and IIa) were differentially induced by specific metals in human lens epithelial cells, specifically by Cd^{2+} and Zn^{2+}, but not Cu^{2+} (112). Similar responses of the MTIIa gene were detected in identically treated primary human lens epithelial cells. Cd^{2+} and Zn^{2+} induced MTIIa to five times higher levels than MTIg (112). Overexpression of MTIIa in lens epithelial cells has been shown to protect against Cd as well as TBHP-induced oxidative stress (113). The same study also shows that MTIIa may play a role in regulating expression of other important antioxidant genes, HO-1, thioredoxin reductase and MnSOD, antioxidant molecules that could further enhance protection against oxidative

stress in lens cells, potentially delaying onset of cataract (113).

5.2. Ferritin

Fe is transported in the plasma using transferrin, the Fe transport protein, while ferritin is the intracellular protein responsible for iron binding and storage. Ferritin which is found throughout the lens (114) (115), is a multimeric iron-storage protein consisting of 24 subunits of two types: heavy (H) and light (L). The ratio of these two chains is tissue specific and controls iron storage and availability (116). Fe levels in the eye lens have been reported to be between 0.18 and 9.6 μg/g wet weight (117). A number of studies have found increased Fe levels in cataractous lenses (109) (114) with redox active Fe (not bound to ferritin) also found at higher levels in cataractous compared to control lenses (103) (104). Low levels of Fe have been found in both the aqueous and vitreous humors (118). Levels are kept low by the blood ocular barrier preventing transferrin entrance into the eye (117). Inflammation which causes a breakdown of this barrier results in large increases in iron concentration in both the aqueous and vitreous humors (119). The lens tightly controls Fe levels and during inflammation the lens accumulates Fe by taking it up from increased levels in the aqueous and vitreous humors in both the transferrin and non-transferrin-bound forms. The lens Fe concentration returns to control levels following resolution of the inflammatory episode (120). Why the lens would act as a sink for excess Fe is not known and is the subject of much research.

In a recent study using knockdown of ferritin heavy chains in human lens epithelial cells, increased iron availability resulted in increased cystine uptake and GSH concentration and decreased nuclear translocation of hypoxia-inducible factor 1-α (a transcription factor that regulates vascular endothelial growth factor (VEGF) expression) and VEGF accumulation in the cell-conditioned medium (121).

6. PROTEIN REPAIR SYSTEMS OF THE EYE LENS

6.1. Methionine sulfoxide reductases

While all amino acids are susceptible to oxidation, methionine and cysteine residues are among the most vulnerable due to the presence of a sulfur containing side chain that is sensitive to attack from ROS such as H_2O_2, •OH, hypochlorous acid, chloramines and $OONO^-$ (122) (123). In the eye lens protein methionine sulfoxide (PMSO), the oxidized form of methionine, levels increase upon aging (124) and in human cataractous lenses 60%-70% of total lens protein is found as PMSO (125). ROS mediated oxidation of methionine results in an asymmetric sulfur center and thus PMSO exists as two epimers, the S- and R-epimers. Methionine oxidation in proteins can result in altered conformation, activity, sub-cellular localization patterns and aggregation states which are associated with loss of cellular functions, apoptosis and cell death (126). Methionine oxidation is, however, reversible via a thioredoxin dependent reaction in which PMSO is converted to reduced methionine. This reaction is catalyzed by a family of enzymes called the methionine sulfoxide reductases (Msrs). The Msr family consists of MsrA (found in the cytosol and mitochondria) and three MsrBs; MsrB1 (localized in the cytosol and nucleus), MsrB2 (localized in the mitochondria) and MsrB3 (localized in the endoplasmic reticulum and mitochondria) that act on the S- and R-epimers respectively (127). The oxidized thioredoxin produced during the reduction of PMSO is subsequently reduced by thioredoxin reductase (TrxR) in an NADPH-dependent reaction.

It has been shown that in bovine lenses 40% of Msr activity is due to MsrB while the remaining is MsrA (128). MsrA has been shown to play an important role in protection of lens cells against oxidative damage and it has been shown to be required for the maintenance of lens transparency *in vivo (*24) (129) (130) (131). Gene silencing of MsrA decreases the resistance of lens epithelial cells to H_2O_2-induced oxidative stress resulting in increased mitochondrial ROS levels in human lens cells (129) and loss of lens cell mitochondrial function (24). Similarly gene silencing of MsrB1, B2 and B3 results in decreased resistance to tBHP-induced oxidative stress and increased cell death in human lens epithelial cells (129). Deletion of the MsrA gene in mice leads to oxidative stress-induced cataract (130). By contrast, over-expression of MsrA in human lens cells protects against oxidative stress and preserves mitochondrial function (129). Recently, both cytochrome c (cyt c) (130) and α-crystallin/sHSP (131) have been identified as key targets of MsrA function in the lens. Both proteins are critical for lens function. Cyt c is essential for mitochondrial electron transfer and is a key initiator of apoptosis in mammalian cells (132). α-crystallin/sHSP is a molecular chaperone that is essential for the maintenance of lens transparency whose deletion has been shown to result in cataract formation (133) (134) (135) (136) and is discussed in more detail below.

6.2. Thioltransferases

Thioltransferase, known interchangeably as glutaredoxin, is a GSH-dependent cytosolic protein and a member of the thiol-disulfide oxidoreductase enzyme family, containing a conserved CXXC active site (137). Two isoenzymes of thioltransferase are known: cytosolic TTase-1 (Grx1) and mitochondrial TTase-2 (Grx2) (137). The TTases use reduced GSH in their reaction to reduce protein thiols (s-thiolation) preventing disulfide bond formation and protein aggregation (137). TTase activity was found to be higher in the lens epithelial layer than in the rest of the lens (138).

In cultured lens epithelial cells TTase 1 levels and activity were shown to be upregulated by treatment with H_2O_2. Depletion of GSH and subsequent treatment with H_2O_2 also increased levels of TTase in human lens epithelial cells (139). Porcine lenses cultured in H_2O_2 over a 24 hr period showed similar trends for TTase, with slow transient increases in TTase activity, mRNA transcript and protein levels in response to low level of H_2O_2 and a more rapid response to higher levels of H_2O_2 (140). In a separate study, treatment of cultured lens epithelial cells with 0.1mM H_2O_2 over 3 h inactivated the key glycolytic

enzyme glyceraldehyde-3-phopshate dehydrogenase (G-3PD) by more than 50% over the first 15 min, this returned to 80% of normal activity by 180 min. Interestingly, the inactivated cellular G-3PD in the cell extract could be partially reactivated by human recombinant TTase but not GSH (141). The absence of TTase 1 was also shown to increase lens cell susceptibility to UV-B radiation (UVR-B), light scattering was increased in TTase$^{-/-}$ mouse lenses compared to wild type mouse lens after treatment with UVR-B (142). TTase-2 has been shown to protect against disruption of the mitochondrial transmembrane potential in lens epithelial cells during oxidative stress conditions (143) and more recently TTase-2 was shown to protect complex I of the electron transport chain and to prevent H_2O_2-induced apoptosis in lens epithelial cells (144).

7. REDUCING SYSTEMS OF THE EYE LENS

7.1. Thioredoxin and thioredoxin reductase

This section could easily contain GSH which we have grouped under ROS scavengers. It could also contain a section on NADPH, which is required for Msr repair of PMSO, thioredoxin repair of disulfide bonds and the recycling of GSH but NADPH is mentioned specifically in many other sections in this review. Thioredoxin (Trx) is the major disulfide reductase responsible for maintaining proteins in a reduced state within the cell. It is a small, cysteine rich protein possessing a dithiol/disulfide active site (CGPC) that acts to reduce protein disulfides. Its action results in an internal disulfide on the thioredoxin protein itself and this must be reduced by TrxR in an NADPH dependent reaction in order to recycle Trx. Trx serves as an electron donor, via the thiol/disulfide exchange reaction, for a number of enzymes including Msrs, ribonucleotide reductases, as well the peroxiredoxins (145). Reduction of disulfide bonds is thought to be an important regulatory step where Trx could control the redox state of critical SH groups involved in structure and catalytic function in proteins.

There are two nuclear encoded isoforms of Trx, Trx 1 found in the cytosol, and Trx 2 which is localized solely to the mitochondria. Trx 1 not only is an important part of the antioxidant defensive against oxidative stress but it also plays a role in transcription, growth control, and immune function (145). Early embryonic lethality results from knockout of either Trx 1 (146) or Trx 2 (147) and it has also been shown in mice that a haploinsufficiency (a single functional copy of a gene, where insufficient product is made) of Trx 2 results in the reduction of ATP production and increased ROS production (148). Early work on mouse lenses revealed that *in vivo* photochemical oxidative stress to Emory mice resulted in a fivefold upregulation of the Trx 1 gene in the lens at 3 weeks and a fourfold increase of the lens Trx 1 protein but that Trx 2 was unchanged (149). In cultured lens epithelial cells, Trx 1 is up regulated at both transcript and protein levels in response to H_2O_2 treatment (150). Interestingly, human lens epithelial cells treated with exogenous human recombinant Trx 1 showed a simultaneous increase in mRNA expressions of mitochondrial MnSOD, TTase 1, TTase 2, and thioredoxin peroxidase IV (Prx3) (151).

Recently a study on twenty three normal human lenses of 19-77 yrs which were grouped into 2nd, 3rd, 5th, 6th and 7th decades, indicated that Trx 1 activity decreased with age. Activity of Trx 1 in the 7th decade appeared to be 30% of the activity found in lenses from the 2nd decade, a significant drop in activity. Interestingly the corresponding protein levels of Trx 1 were unaffected and remained steady throughout the 7 decades in the lenses studied (152).

7.2. Thioredoxin like proteins

Recently a thioredoxin-like protein (TXNL) containing the conserved CXXC reducing motif was discovered in the retina of mice and termed rod derived cone viability factor (RdCVF) (153). This protein known as TXNL6 in humans is a product of the NXNL1 gene. The discovery of a novel CXXC containing protein led to the hypothesis that other reducing systems could function in the eye in addition to or as an alternative to the better characterized reducing systems such as Trx. TXNL6 is part of a small family of thioredoxin-like proteins which consists of TXNL1, TXNL2, TXNL4 and TXNL5. TXNLs share the CXXC active site of Trx 1 and/or the thioredoxin fold which consists of a four stranded β sheets surrounding three α helices (154). Little is known about the disulphide reducing action of many of the TXNLs but recent work has shown that TXNL6 is present in both the lens epithelium and lens fiber cells, that it is inducible by oxidative stress and that it can serve as a reducing agent for MsrA in the repair of essential lens proteins (155). TXNL1 is known to be a redox sensor and part of the 26 s proteasome subunit (156) (157). TXNL2 is also known as glutaredoxin 3 and may catalyze the reduction of glutathione mixed disulfides (158). Little is known about the oxidoreductase role of TXNL4 if any but it is known to be essential for the G2/M transition of the cell cycle (159). TXNL5 is known to have redox potential similar to that of Trx 1 (160) and it is known that it can itself be reduced by TxrR and that it inhibits TNF-α induced NFκB activation to a greater extent than Trx 1 (161).

8. CHAPERONE PROTEINS OF THE EYE LENS

8.1. α -crystallin

Heat shock proteins (Hsps) are highly conserved proteins that are induced in response to various physiological and environmental stressors (162), interestingly HSPs form essential partnerships with the proteasome and lysosomes in protein degradation processes (162). The molecular chaperones expressed in lens epithelial cells include αA-crystallin, αB-crystallin, Hsp25/27, Hsp40, Hsc70, Hsp70 and TCP-1 (163) (164) (165) (166). In the lens the predominant Hsp is α-crystallin. α-crystallin is made up of two small HSPs (sHSPs); αA- and αB-crystallin, α-crystallin makes up approximately 40-50% of total lens protein and is crucial for maintaining lens transparency.

In addition to its structural and refractive role in the lens, α-crystallin is a molecular chaperone (133) (135) that functions to prevent protein aggregation (134), and formation of high molecular weight aggregates in the lens. α-crystallin has also been implicated in apoptotic control

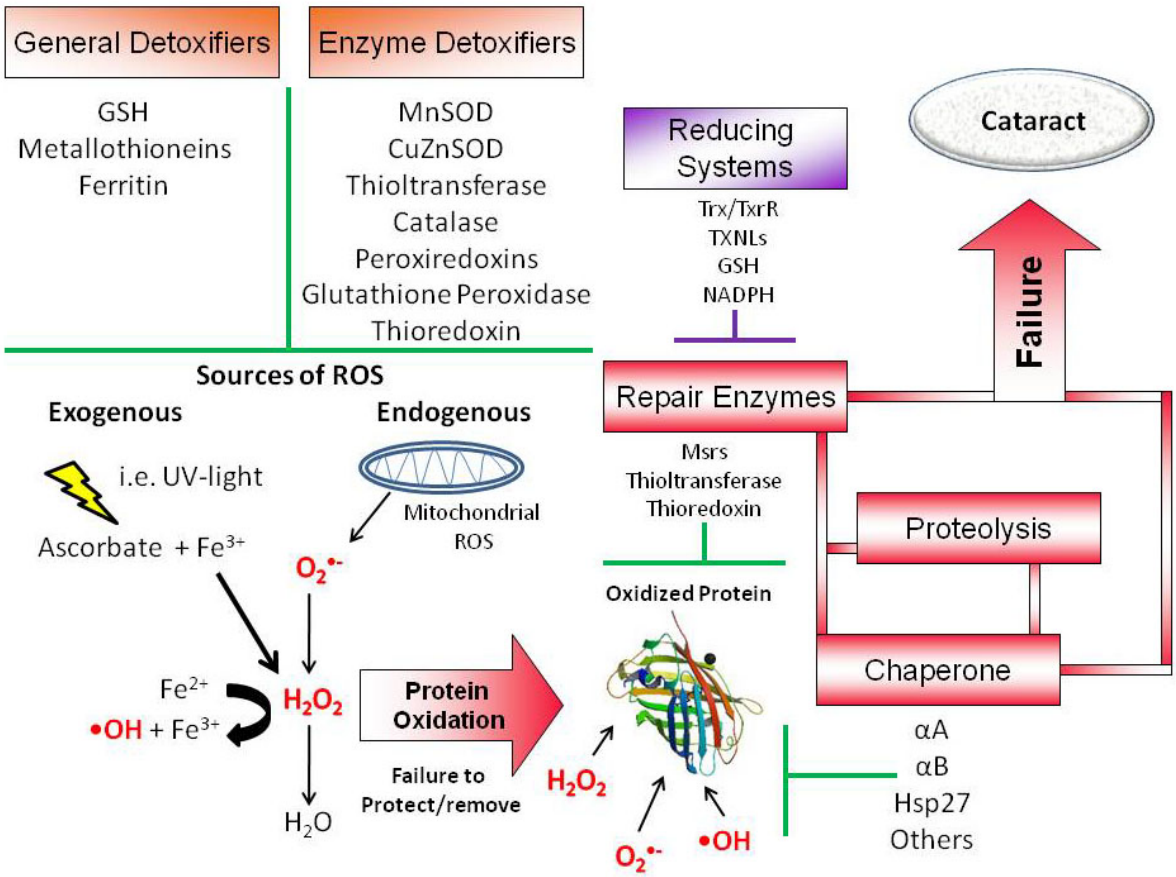

Figure 1. ROS production arises from endogenous (altered cellular respiration, peroxisomes, lipooxygenases, immune activity, disease states and cytochrome P450) or exogenous sources (U.V.-light exposure, ionizing radiation, viral infection, smoke, metals and drugs), in this Figure we give one example of each for clarity. The frontline primary defense against ROS are the general and enzymatic detoxifiers such as GSH, MnSOD, CuZnSOD, Catalase, the peroxiredoxins, glutathione peroxidase, thioredoxins and thioltransferases. Failure of the primary defenses may result in oxidative damage to important lens cellular proteins, activating specific repair enzymes, heat shock proteins/molecular chaperones or protein proteolysis processes aided by specific reducing systems. Failure of protein repair systems, chaperones or proteolysis, systems that work in concert to prevent accumulation of damaged proteins, leads to protein aggregation, increased light scattering and ultimately cataract formation.

and cell survival (8). In its native state in the lens, α crystallin consists of two subunits called αA- and αB-crystallin that consist of 173 and 175 amino acids respectively. αA- and αB-crystallin share as much as 57% homology and exist in the lens in a 3:1 ratio (167). Deletion of αA-crystallin in mice, results in smaller lenses compared to wild type, and the development of opacification that spreads with age (136). Mutation of αA-crystallin (R116C), which causes loss of chaperone function, leads to cataract in humans (168). Similarly a mutation of αB-crystallin (R120G) that causes cataract and desmin-related myopathy in humans, also leads to loss of chaperone function (169). Importantly, the chaperone function of α-crystallin is also lost upon oxidative conditions (131) (170) (171). Specific oxidation of bovine α-crystallin methionines to PMSO resulted in loss of chaperone function *in vitro* (131) and αA-crystallin methionines are found as PMSO in rat hereditary cataracts (172) and in MsrA knockout mice (131). Oxidation by Fenton-type reaction of rat recombinant αA- and αB-crystallin was shown to result in higher molecular weight proteins that lacked chaperone function (173). These results suggest that oxidation of α-crystallin results in loss of chaperone function and that loss of chaperone function plays a role in cataract formation. αA-crystallin and αB-crystallin as well as HSP27 can be induced by specific metals in SRA01/04 human lens epithelial cells. Cd^{2+} and Cu^{2+}, but not Zn^{2+}, induced αB-crystallin and HSP27 while αA-crystallin was induced by Cu^{2+} only (174).

9. SUMMARY

It is clear that the lens has evolved a multitude of systems to defend against oxidative stress damage which collectively are required to maintain the transparent function of the lens and prevent cataract formation. The evidence presented here suggests that lens oxidative protective systems and oxidative repair systems are linked through their requirements for common reducing systems and that their actions to repair and or maintain common lens proteins and each other are concerted and mutually dependent. These systems and their functions are summarized in Figure 1. As discussed in this review multiple exogenous (UV-light, drugs, metals, and cigarette smoke) and endogenous (altered mitochondrial respiration, respiratory burst of

phagocytes, viral infection) sources of oxidative stress result in the formation of multiple forms of ROS such as $O_2^{\cdot -}$, H_2O_2, and •OH. Two types of antioxidant systems (general and enzymatic detoxifiers) normally detoxify these ROS species to maintain lens homeostasis. These include GSH, MnSOD, CuZnSOD, catalase, the peroxiredoxins and glutathioine peroxidase. Simultaneously, metallothioneins and ferritin function to limit access to free metals. Failure of these antioxidant systems leads to oxidation of proteins, loss of protein function and protein aggregation. Specific repair systems including the Msrs and thioltransferase as well as thioredoxin can repair proteins restoring their normal function. Chaperone proteins such as αA-crystallin, αB-crystallin and Hsp27 can remove toxic protein aggregates. Failure of these antioxidant defense and repair systems results in irreversible protein aggregation, loss of lens cell function and ultimately cataract formation.

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Abbreviations: ROS: reactive oxygen species; UV: Ultra violet; •OH: hydroxyl radical; $O_2\cdot^-$: the superoxide anion; $HO\bullet_2$: hydroperoxyl radicals; $OONO^-$: peroxynitrite; H_2O_2: hydrogen peroxide; $^1O_2$: singlet oxygen; NO: nitric oxide; AMD: age related macular degeneration; GSH: glutathione; GSSG: oxidized glutathione; GSH-Rx: glutathione reductase; HMPs: hexose monophosphate shunt; tBHP: t-butyl hydroperoxide; Fe: Iron; MnSOD: manganese superoxide dismutase; CuZnSOD: copper-zinc superoxide dismutase; TSOD: total superoxide dismutase; HO-1: heme oxygenase-1; Cu: copper; Cd: cadium; MTs: metallothioneins; VEGF: vascular endothelial growth factor; PMSO protein methionine sulfoxide; Msrs methionine sulfoxide reductases; thioredoxin reductase (TxrR); Cyt c: cytochrome c; TTase: thioltransferase; G-3PD: glyceraldehyde-3-phopshate dehydrogenase; Trx: thioredoxin; TXNL: thioredoxin-like protein; RdCVF Rod derived cone viability factor; Hsps: heat shock proteins.

Key Words: Oxidative Stress, Reactive Oxygen Species, Antioxidant Defense, Antioxidant Enzymes, Chaperone Proteins, Protein Repair, Lens, Age-Related Cataract, Protein Oxidation, Protein Aggregation, Review

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