— **Review** —

Role of Glutathione S-Transferases in Lens under Oxidative Stress

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The polymorphic expression of glutathione S-transferase (GST) in animal lenses has been demonstrated by purification with affinity chromatography, immunochemical analysis with Western-blotting and amino acid sequencing of the *N*-terminal regions. Three major classes of GST (class α, γ or π) have been identified in human, bovine, pig, rabbit, rat and guinea pig. They show a typical GSH-conjugation reaction with various chemicals, including organic peroxides. These GSTs possess unique properties against oxidative stress, that is, α-class GST exhibits a GSH-peroxidase-like activity, μ-class GST shows a potent resistance to naphthoquinone and H_2O_2, and π-class GST shows a high sensitivity to a variety of oxidants. Because the oxidative stress is believed to be a major factor in the development of cataracts, these unique expression patterns of GST in animal lenses reveal an important factor in the formation and development of oxidant-induced cataracts, including naphthalene cataract and senile cataract. The most typical example has been presented that effect of curucumin on the prevention of cataract through the induction of GST. This review summarizes the evidence for the involvement of GST in cataractogenesis, which may be seen to support it as an important risk.

Key words —— cataract, naphthalene, glutathione S-transferase, polymorphism, oxidative stress

INTRODUCTION

Cataracts are the leading cause of blindness worldwide and accounts for visual loss in more than 25% of the cases in the U.S. in people over 65.[1)] With the increasing of the elderly population, senile cataracts is developing into a serious health hazard in Japan. "Cataract" refers to the opacification of the lens and cataract formation is one of the irreversible processes for which modern medical science has no effective, available treatment except surgery. Drugs with prophylactic or ameliorative effects for the condition are sorely lacking.

The epidemiological relationship between smoking and cataracts has been well studied[2–5)] and there is evidently a dose-response relationship between the cumulative effects of smoking and the risk of cataract development. It is possible that this exposure to excess smoke causes increasing harm to the lens, either by direct entry of the combustion and condensation products of tobacco into the eyes, or by continually raising the temperature of the lens.

The mechanisms of cataract development are complex. Smoking is just one of the many established or putative risk factors for cataracts, which also include advanced age, trauma, persistent intraocular inflammation, ultraviolet radiation, ionizing radiation, diabetes mellitus, hypoparathyroidism, prolonged corticosteroid administration, aromatic hydrocarbon intake and high body mass index.[1–6)]

In cataracts associated with smoking, naphthalene is reported to be a risk factor.[2–6)] Under naphthalene exposure, it has been suggested that cataract formation follows a similar process to the one seen in the development of senile cataracts,[7)] suggesting that studies of the mechanisms of naphthalene-cataracts could help lead to a prevention of cataracts in-

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duced by oxidative stress or aging. This review summarizes the important factors related to cataract development, some of which afford themselves as targets for the development of therapeutic agents.

I. Importance of Redox Regulation in Lens

Glutathione (GSH) is the most important reductant in the living materials. As is the case in other tissues, the high content of glutathione in the lens is believed to protect the thiols in structural proteins and enzymes and thus allow for proper biological function.[8–11)]

Aging lenses or lenses under oxidative stress exhibit an extensively consumed GSH pool. The lens depends on a balanced redox state to maintain transparency. The endogenous high concentration of glutathione plays an important role in the defense against both exogenous and endogenous reactive oxygen species produced from a variety of sources, and maintains proteins in the lens in a reduced state. The second line of defense for the health of the lens is its intrinsic antioxidant-enzymes for protecting or restoring the lens functions/activities of proteins/enzymes.

Reactive oxygen species (ROS) have long been considered to be toxic, harmful by-products of living in an aerobic environment. Both the superoxide anion (O_2^-) and hydroxyl radical (•OH) are extremely unstable and short lived. On the other hand, hydrogen peroxide (H_2O_2) is freely diffusible and relatively long lived.[11)] ROS can be generated endogenously by several different enzymatic systems or exogenously from the environment. The endogenous sources include mitochondria, peroxisomes, lipooxygenases, NADPH oxidase, cytochrome P450 and inflammatory cytokines. Exogenous sources include ultraviolet light, ionizing radiation, chemotherapeutics, and environmental toxins.[8–12)] Of the exogenous sources, polycyclic aromatic hydrocarbons are an important risk factor for the cataract formation through lipid peroxidation generated from the redox-imbalance in the cell, namely, oxidative stress. Additionally, ROS also directly damage the antioxidant-system, indicating that such damage leads to a lens weakened in certain aspects of the innate protection against oxidative stress.

II. Human Glutathione S-Transferases

Human glutathione S-transferase (GST) is a family of dimeric enzymes classified into 8 groups, α-class GST (GSTA, alpha), μ-class GST (GSTM, mu), π-class GST (GSTP, pi), θ-class GST (GSTT, theta), ζ-class GST (GSTZ, zeta), ω-class GST (GSTO, omega), κ-class GST (GSTK, kappa) and microsomal-class GST (mGST, microsomal), with additional subclasses in each class.[3–17)] Among these GSTs, the majority (GSTA, GSTM, GSTP, GSTT, GSTZ and GSTO) are located in the cytosolic fraction and GSTK is located in the mitochondrial fraction. Based on the amino acid sequences, cytosolic GSTs within α class exhibit a greater than 40% identity and those between classes share an identity of less than 25%. As presented in Table 1, Hayes *et al*. described a typical classification scheme of homodimeric GSTs with 24 subclasses and unique substrate specifities.[14)] Additionally, the chromosomal distribution of GST genes also shown in Table 1. Judging from these results, various GSTs in human may be understood to have unique functions and transcriptional regulation. Furthermore, we have reported the amino acid sequences of *N*-terminal of GSTs which were purified from animal lenses and identified them with class-specific antiserum on Western-blotting, as shown in Table 2.

Numerous reports have been presented that GSTs can catalyze the conjugation reaction of various chemicals with GSH. Including GSH-conjugation activity,[13–17)] GSTs are a typical multifunctional enzyme which plays a role in binding to hydrophobic compounds (ligandin),[18)] as a thioltransferase-like redox regulator,[19)] Se-independent GSH-peroxidase[20)] and steroid isomerase.[21)] The unique properties of the major class of GST are described in the following.

1. α Class Glutathione S-Transferase

A large number of the homodimeric and heterodimeric GSTA thus far reported can be generated from these subunits.[14–17)] The most characteristic function of GSTA distinct from the other classes is a Se-independent GSH-peroxidase activity. It has been reported that the majority of enzymatic activity in the Se-deficient rat is dependent on the GSTA activity in the aspect of GSH-peroxidase-activity.[22)] Under Se-deficient conditions, it is estimated that Se-dependent GSH-peroxidase loses its enzymatic activity. Under oxidative conditions, cellular apoptosis is caused mainly by the lipid peroxides of membrane lipids. Compared with Se-dependent GSH-peroxidase, GSTA, exhibits a potent GSH-peroxidase activity toward organic peroxides presented by lipid peroxides. With regard to the activity toward hydrogen peroxide, the Se-dependent GSH-peroxidase shows a more potent enzymatic activity

Table 1. Substrate Preferences of Human Glutathione Transferases

Family	Class, enzyme	Substrates or reaction
Cytosolic	Alpha, A1-1	5-ADD, BCDE, BPDE, Busulfan, Chlorambucil, DBADE, DBPDE, BPhDE, N-a-PhIP
	Alpha, A2-2	CuOOH, DBPDE, 7-chloro-4-nitrobenz-2-oxa-1,3-diazole
	Alpha, A3-3	Δ^5-ADD, Δ^5-pregnene-3,20-dione, DBPDE
	Alpha, A4-4	COMC-6, ethacrynic acid, 4-hydroxynonenal, 4-hydroxydecenal
	Alpha, A5-5	not done
Cytosolic	Mu, M1-1	*t*-PBO, BPDE, CDE, DBADE, trans-stilbene oxide, styrene-7,8-oxide
	Mu, M2-2	COMC-6, DCNB, aminochrome, dopa O-quninoe, PGH_2 to PGE_2
	Mu, M3-3	BCNU, PGH_2 to PGE_2
	Mu, M4-4	CDNB
	Mu, M5-5	low for CDNB
Cytosolic	Pi, P1-1	acrolein, base propenals, BPDE, CDE, Chlorambucil, COMC-6, EA, Thiotepa
Cytosolic	Sigma, S1-1	PGH_2 to PGD_2
Cytosolic	Theta, T1-1	BCNU, butadiene epoxide, CH_2Cl_2, EPNP, ethylene oxide
	Theta, T2-2	CuOOH, menaphthyl sulfate
Cytosolic	Zeta, Z1-1	dichloroacetate, fluoroacetate, 2-chloropropionate, malelyacetoacetate
Cytosolic	Omega, O1-1	monomethylarsonic acid, dehydroascorbic acid
	Omega, O2-2	dehydroascorbic acid
Mitochondrial	Kappa, K1-1	CDNB, CuOOH, (S)-15-hydroperoxy-5,8,11,13-eicosatetraenoic acid
MAPEG	group I, MGST2	CDNB, LTA_4 to LTC_4, (S)-5-hydroperoxy-8,11,14-*cis*-6-*trans*-eicosatetraenoic acid
	group I, FLAP	nonenzymatic binding of arachidonic acid
	group I, LTC4S	LTA_4 to LTC_4
MAPEG	group II, MGST3	CDNB, LTA_4 to LTC_4, (S)-5-hydroperoxy-8,11,14-*cis*-6-*trans*-eicosatetraenoic acid
MAPEG	group IV, MGST1	CDNB, CuOOH, hexachlorobuta-1,3-diene
	group IV, PGES1	PGH_2 to PGE_2

This table summarizes two tables of the paper which Hayers, J. D. *et al.*[14] Abbreviations: MAPEG, microsomal GST; 5-ADD, Δ^5-androstene-3,17-dione; BCDE, benzo[*g*]chrysene diol epoxide; BPDE, benzo[*a*]pyrene diol epoxide; DBADE, ; DBPDE, dibenz[*a*,*h*]anthracene diol epoxide; BPhDE, benzo[*c*]phenanthrene diol epoxide; N-a-PhIP, N-acetoxy-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; CuOOH, cumeme hydroperoxide; COMC-6, crotonyloxymethyl-2-cyclohexenone; *t*-PBO, tert-butylhydroperoxide; CDE, chrysene diol epoxide; CDNB, 1-chloro-2,4-dinitro-benzene; BCNU, 1,3-bis(2-chloroethyl)-crotonyloxymethyl-2-cyclohexenone; EPNP, 1,2-epoxy-3-(p-nitrophenoxyl)propane.

Table 2. Different Expression of Glutathione S-Transferases in Mammalian Lenses

Animal	No. GST	Class	References
Human	2	α, π	32
Porcine	1	π	32
Dog	2	α, π	37
Bovine	2	μ	32
Rabbit	2	μ, π	33
Rat	2	μ, π	36
Guinea pig	2	μ	44

The classes of lens GSTs were analyzed with class-specific antiserum and Western-blotting.

than GSTA. Considering these results, GSTA may be the dominant enzyme in the reduction of organic peroxides under conditions such as Se-deficiency.

In addition, a unique GST (GST5.8) that may belong to the GSTA class exhibited an extremely high activity in the aspect of GSH-conjugation activity toward 4-hydroxy nonenal, which is presumed to be a final product of lipid peroxidation under oxidative stress.[23] However, judging from the properties presented in recent papers GST5.8 may be a distinct enzyme from the other class α GTSs (GSTA1, GSTA2, GSTA3 and GSTA4) reported to date.

Curcumin (a reductant of turmeric) treatment caused a significant induction of the GST isozyme rat GST8-8 (rGST8-8) (a family of GSTA) in lens epithelium. Because rGST8-8 utilizes 4-hydroxy nonenal as the preferred substrate, it has been suggest that the protective effect of curcumin against cataracts may be mediated through the induction of this GST isozyme, suggesting that curcumin may be an effective protective agent against the cataractogenesis induced by lipid peroxidation.[24,25]

2. μ-Class Glutathione S-Transferase

GSTMs display a GSH-conjugation activity

similar to that of the other class GSTs. The thioltransferase-like (TTase-like) activity is the most characteristic property of GSTM.[19] The first report of this activity was presented in a paper on bovine lens GSTM by Raghavachari, N. *et al*.[26] TTase, a small enzyme of < 10 kDa, can catalyze the thiol/disulfide exchange reaction between small molecular weight thiols and proteins, and plays an important role in the redox regulation of protein thiols in living cells.[27–30] We have previously reported that TTase displays a strong sensitivity against oxidants, including disulfides. However, GSTMs show a potent resistance to oxidants and prooxidants, including H_2O_2, organic peroxides and naphthoquinone. These results suggest that GSTM can play a role as the dominant reductant in the restoration of protein thiols from the oxidized form under oxidative conditions. This TTase-like activity is apparently generated despite the fact that GSTM doesn't have the TTase motif (Cys-x-x-Cys) which is widely conserved in the characterisitic enzymes having a thiol/disulfide exchange reaction such as TTase (glutaredoxin, Grx), thioredoxin (Trx) and protein disulfide isomerase (PDI).[29]

GSTM1-1 may be involved in the p38 signaling pathway to protect cells from apoptosis through a dissociation from apoptosis signal-regulating kinase 1 (Ask1) in the presence of heat shock proteins under oxidative stress.[31] This result has made it evident that overexpression of GSTM1-1 inhibits the induction of p38 in the cells.

3. π-Class Glutathione S-Transferase

Among the classes of GST studied, GSTP was found to possess a strong sensitivity to oxidants.[29,32–38] A typical GSTP which exists in human placenta shows an efficient inactivation under oxidative conditions (oxidative stress) induced with oxidants (disulfides, H_2O_2, superoxide anion) and a prooxidant (naphthoquinone). However, the resulting inactivated GSTP is effectively restored to its activity by treatment with thiols including GSH, cysteamine and dithiothreitol, but not other chemical reductants such as butylated hydroxytoluene, α-tocopherol, ascorbate, uric acid, mannitol, tyrosine, tryptophan, histidine, quercitrin or bilirubin. Furthermore, this restoration is accelerated in the presence of TTase with a relatively low concentration of GSH, suggesting that GSTP is inactivated by the formation of mixed disulfide bonds between small disulfides and protein thiols and the inactivated GSTP is restored to its activity through the reduction of mixed disulfides.[34] It is supposed that GSTP escapes complete inactivation by shunting temporarily through the formation of reversibly mixed disulfides.

On the other hand, the GSTP gene has been effectively induced by treatment with polycyclic aromatic hydrocarbons, inorganic arsenics and estrogen.[39–41] This induction of GSTP can lead to cancer or tumor cell multidrug resistance, but certain reductants, such as curcumin, are able to inhibit this induction of this GSTP gene such that the cancer cells are induced to enter appoptosis. Furthermore, GSTP directly inhibited c-Jun N-terminal kinase (JNK) so as to prevent cells from oxidative-damage triggered apoptosis in liver, embryo fibroblasts and PC12 cells. This indicates GST has the critical function of serving as an endogenous negative regulatory switch for these same regulatory kinase pathways by binding to and inhibiting JNK activity. Mechanistically, JNK activity has been shown to be stimulated upon dissociation of the GST : JNK complex[42,43] and GSH peptidomimetics can interfere with protein-protein interactions and lead to JNK activation. NO directly effects the expression of multidrug-resistance protein 1, suggesting an indirect effect on the elimination of prooxidants from lens cells because the excretion ratio of GSH-conjugate is altered. These results indicated that GSTP may be involved in the cell damage through its large induction.

4. Polymorphism of Glutathione S-Transferases

Recently, certain results on the polymorphic expression of GST in animal lenses have been reported.[32,33,35–37,44] GSTs were isolated and purified with similar purification procedures using Sephacryl S-100 gelfiltration, S-hexylglutathione Agarose and Q-Sepharose column chromatographies. The multiple forms of GST obtained from dog, rabbit and guinea pig lenses were isolated by Q-Sepharose anion chromatography. The amino acid sequences of GST *N*-terminal regions were determined with a Shimadzu PS-Q peptide sequencer. Only certain α-class GST amino acid sequence were undetectable because of the blocked *N*-terminal, as being reported in othe studies of GST α. The *N*-terminal amino acid sequences support the polymorphic expression of GST in animal lenses (Fig. 1).

To investigate the involvement of GST in the oxidant-induced cataract formation in lenses, the resistance of GST to oxidants and/or prooxidants such as naphthoquinone, H_2O_2 and the superoxide anion from the xanthine-xanthine oxidase reaction

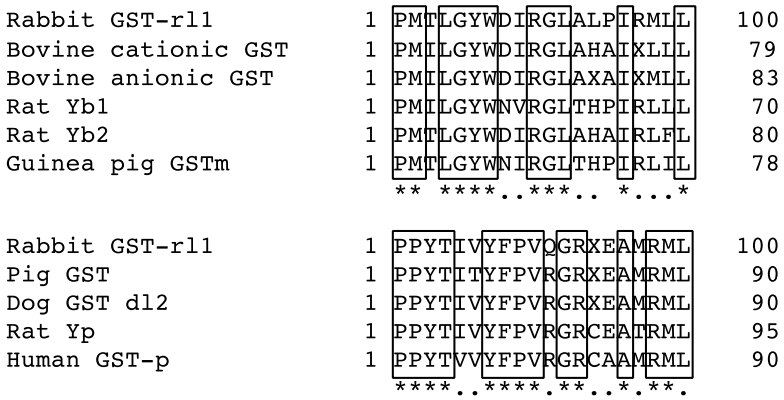

Fig. 1. *N*-Terminal Amino Acid Sequences of Glutathione S-Transferase in Class μ and π
The *N*-terminal amino acid sequences of GSTs have been summarized in our recent papers.[33,35–37]

Table 3. Sensitivities of Glutathione S-Transferase Class μ and π against Oxidants

Oxidant	Porcine π	Human π	Bovine μ	Guinea pig μ
None	100.0	100.0	100.0	100.0
5 μM Naphthoquinone	37.0	14.8	103.5	99.7
Xanthine-xanthine oxidase	42.0	10.7	99.6	100.1
5 mM H_2O_2	47.6	13.1	102.2	98.0

The sensitivities of glutathione S-transferase against oxidants were determined under the concentrations of oxidants described in the table. The enzymes were purified from animal lenses.[27,32,33,35,38]

products (Table 3). The results of inactivation studies suggested the different sensitivities of these classes of GST towards oxidants or prooxidants, namely, the π-class GST, is very sensitive, but the μ-class GST is resistant. Considering the GSH-peroxidase activity of α-class GST together with the results of these inactivation studies and immunochemical analyses, the sensitivity of the lens against oxidants such as nathoquinone or peroxides may be dependent on the type of classes present. Additionally, further evidence of epidemiological investigation will be necessary to clarify the relationship between the class dependent-expression pattern of GST in lenses and cataract development.

III. Naphthalene Metabolism

Naphthalene is a typical polycyclic aromatic hydrocarbon that is toxic to humans if ingested, inhaled, or absorbed by the skin. Its most frequent use is as a raw material in the industrial production of the phthalic anhydride used to manufacture polyvinyl chloride piping, vanity table tops, and boat hulas, as well as in the manufacturing of synthetic resins, solvents, and lubricants. The major commercial products made from naphthalene include mothballs and toilet deodorant blocks. Furthermore, naphthalene is present in tar and tobacco smoke, *etc*. Animals fed naphthalene quickly develop cataracts. It is well-known that naphthalene is metabolized *via* a detoxification pathway through monooxygenation with phase I enzymes and a cojugation reaction with phase II enzymes, and further metabolized through the mercaputuric pathway (Fig. 2). Naphthoquinone, an intermediate of naphthalene metabolism, plays a potential prooxidant role through its redox cycle. The resulted oxidants produced from naphthquinone such as reactive oxygen species or radicals oxidize membrane lipids and/or proteins. The cytotoxicity of naphthoquinones has been attributed to intracellular ROS generation through one-electron-reductase-mediated redox cycling and to arylation of cellular nucleophiles, suggesting that aldose reductase plays a role in the redox cycling.[45,46] Xu, G. T. *et al*. have reported that naphthalene dihydrodiol is found in the aqueous humor and lens of naphthalene-fed rats. It is proposed in their study that the naphthalene dihydrodiol produced in the liver reaches the aqueous humor and penetrates the lens where it is fur-

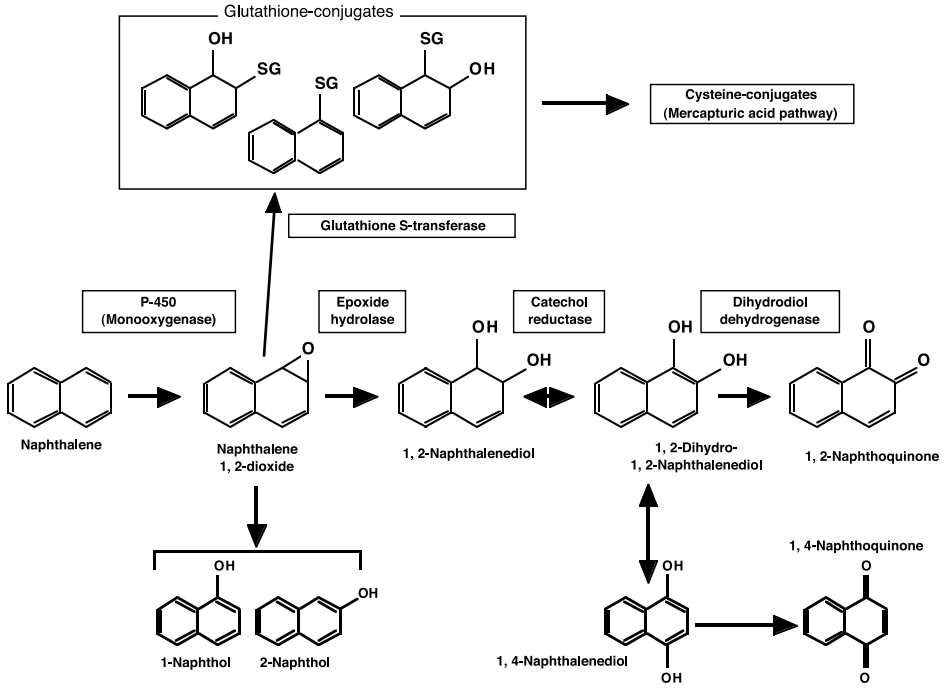

Fig. 2. Metabolism of Naphthalene and Involvement of Glutathione S-Transferase

ther metabolized to its ultimate form the toxic species, naphthoquinone.[47] Because naphthoquinone is highly reactive and quickly forms covalent bonds with various cellular thiols such as glutathaione, cysteine, and protein thiols.[48] The formation of NQ in the lens is considered to be the basic mechanism for the formation of naphthalene-induced cataracts. The lens protein modification by naphtoquinone is also considered to serve as a model for the lens protein modifications, such as disulfide bond formation, which are induced by various oxidative insults. Similar protein modifications such as those mediated by xanthurenic acid have been reported in senile cataracts.[49] This naphthalene-induced cataract model has been widely utilized for human senile cataracts.

CONCLUSION

As has been widely described and is reiterated above, the cataract is one of the major risk factors for blindness in the world today. Numerous pieces of evidence have been presented on how oxidative stress may be a major cause of cataractogenesis, including those produced from hyperglycemia in diabetes, ionizing irradiation, UV-irradiation, aging, aromatic hydrocarbon-metabolism and so forth.[1] In the metabolic pathway of aromatic hydrocarbon, the metabolic intermediates appear regardless of whether lens, liver or other organs produce the reactive oxygen species, radicals, peroxides and other oxidants/prooxidants. During aging, the lens loses its antixodant potencies such as may be seen with the decrease of glutathione or the expression levels of anti-oxidant enzyme.[2,49] Among the anti-oxidant enzymes, GST is an important enzyme, as are superoxide dismutase, catalase and glutathione peroxidase.[14] Papers has suggested a role for the alteration of GST expression in the resistance of the lens to oxidative cataracts in murine animals.[50,51] Furthermore, GSTM exhibits a dethiolate activity such as that of thioltransferase, suggesting that GSTM plays an important role in the cleavage of the mixed disulfides produced under oxidative stress.[19,26] These results suggest that GSTs play a role as an important anti-oxidant enzyme under oxidative stress. Indeed, epidemiological studies have been presented the implication of GST M1 having an important role in cataractogenesis.[2,52] Taking these results into consideration together with the effects of curcumin on the prevention against naphthalene-cataract and the induction of GST,[24,53] the level of GST expression is suggested to be an important risk factor for cataract development, such as seen in both naphthalene- and senile cataracts, a typical model of oxidant-cataract.

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