Cx46 gap junctions provide a pathway for the delivery of glutathione to the lens nucleus

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Glutathione Synthesis, Diffusion and Utilization

The model calculations here follow the data presented in the main paper. Glutathione (GSH) is assumed to be primarily synthesized in peripheral cells. It is membrane impermeant but permeates lens gap junction channels, so it diffuses away from the site of synthesis into central fiber cells. As it moves into the lens it is consumed through its detox action and GSSG is the product. GSSG is membrane impermeant but does not permeate lens gap junctions. These ideas are summarized in ***Fig A1***.

The rate constant K_3 (***Fig A1***) is for some unknown pathway that results in the breakdown or removal of *GSSG*. If such a pathway were not present, there would be no diffusion gradient for *GSH* since utilization and reformation would be equal and opposite. Viz, at steady state

$$\Delta j_{GSH} = -K_1 GSH + K_2 GSSG$$
$$0 = K_1 GSH - (K_2 + K_3) GSSG$$

This implies: $$GSSG = \frac{K_1}{K_2+K_3} GSH$$

$$\Delta j_{GSH} = -K\ GSH$$

Where $$K = K_1 - \frac{K_1 K_2}{K_2+K_3} \quad (\text{sec}^{-1})$$

So, if K_3 were zero, Δj_{GSH} would be zero and there would be no gradient. In spherical coordinates, Δj_{GSH} is given by the left hand side of the differential equation below, whereas the net rate of consumption is K:

$$\frac{1}{r^2}\frac{d}{dr}(r^2 J_{GSH}) = -K\ GSH$$

J_{GSH} = diffusion + conduction + advection (moles/(cm^{2}sec));

$$J_{GSH} = -D\left(\frac{dGSH}{dr} - GSH\,\frac{F}{RT}\frac{d\psi_i}{dr}\right) - GSH\ \Lambda_i \frac{dp_i}{dr} \qquad (1)$$

D (cm^2/sec) is the diffusion coefficient for GSH, ψ_i (volts) is the intracellular voltage, Λ_i (cm^2/sec/mmHg) is the intracellular hydraulic conductivity, and p_i (mmHg) is the intracellular hydrostatic pressure.

In a layer of superficial fiber cells GSH is synthesized at a rate that is probably regulated through feedback to maintain a constant concentration GSH(a). We will therefore assume the boundary condition at the surface is the experimentally measured value of GSH:

$$GSH(a) \approx 20\ mM \qquad (2)$$

The second boundary condition at the lens center is:

$$\frac{dGSH(0)}{dr} = 0 \qquad (3)$$

Wang et al. (2009) showed the intracellular voltage is well described by:

$$\psi_i(r) = \psi_i(a) + \Delta\psi(1 - r^2/a^2) \quad (4)$$

Gao et al. (2011) showed intracellular pressure is well described by:

$$p_i(r) = \Delta p(1 - r^2/a^2) \quad (5)$$

Based on experiments in these papers: $\Delta\psi$ = 10 mV, Δp = 335 mmHg, and using the result in this paper that *GSH* partitions into gap junction channels 12-times less than sodium, $\Lambda_i = 2\text{x}10^{-11}$ (cm^2/s)/mmHg, and $D = 0.3\text{x}10^{-7}$ cm^2/s.

Define:

$$\lambda = \sqrt{D/K} \quad (6)$$
$$R = r/\lambda \quad (7)$$
$$A = a/\lambda \quad (8)$$
$$G(r) = GSH(r)/GSH(a) \quad (9)$$
$$\phi(r) = \psi(r)/(RT/F) \quad (10)$$

Inserting Eqs 4 & 5 into Eq 1 yields:

$$\frac{1}{R^2}\frac{d}{dR}R^2\frac{dG(R)}{dR} - \varepsilon\frac{1}{R^2}\frac{d}{dR}R^3G(R) = G(R) \quad (11)$$

$$G(A) = 1 \quad (12)$$

A small parameter ε emerges from Eq 1. The parameter ε represents the difference between the force of conduction where voltage is pulling GSH toward the lens center and advection where water flow is pushing GSH toward the lens surface. These forces are individually small in comparison to diffusion, but they are even less important because they tend to cancel each other.

$$\varepsilon = \frac{2(\Delta\phi - \Lambda_i\Delta p/D)}{(a/\lambda)^2} \quad (13)$$

Conduction and advection are small components (order ε) of the flux when a large diffusion gradient is present. Normalized glutathione concentration can be expanded in a perturbation series in ε. Viz.

$$G(R) = G^{(0)}(R) + \varepsilon G^{(1)}(R) + \ldots\ldots \quad (14)$$

Eq 14 is inserted into Eqs 11 & 12, then terms of like power in ε are collected to generate a series of problems, each of which can be analytically solved.

The $G^{(0)}$ Problem (Diffusion and Consumption).

$$\frac{1}{R^2}\frac{d}{dR}R^2\frac{dG^{(0)}(R)}{dR} = G^{(0)}(R) \quad (15)$$

$$G^{(0)}(A) = 1 \quad (16)$$

The solution is given by

$$G^{(0)}(R) = \frac{\mathrm{A}sinhR}{Rsinh\mathrm{A}} \tag{17}$$

The $G^{(1)}$ Problem (The Correction for Conduction and Advection).

$$\frac{1}{R^2}\frac{d}{dR}R^2\frac{dG^{(1)}(R)}{dR} - \frac{1}{R^2}\frac{d}{dR}R^3G^{(0)}(R) = G^{(1)}(R) \tag{18}$$

$$G^{(1)}(\mathrm{A}) = 0 \tag{19}$$

The solution that obeys Eqs 18 & 19 is given by

$$G^{(1)}(R) = \left(\frac{3AcoshR+ARsinhR}{4sinh\mathrm{A}}\right) - \left(\frac{3AcoshA+A^2sinhA}{4sinh\mathrm{A}}\right)\frac{\mathrm{A}sinh\mathrm{R}}{R\ sinh\ \mathrm{A}} \tag{20}$$

The solution to within order ε^2 is given by:

$$G(R) = G^{(0)}(R) + \varepsilon G^{(1)}(R) \tag{21}$$

Dimensional Results

If we ignore the order ε solution, the concentration of GSH is given by

$$\frac{GSH(r)}{GSH(a)} = \frac{a\ sinh\ r/\lambda}{r\ sinh\ a/\lambda} \tag{22}$$

If a graph of Eq 22 (***Fig 7A***) is compared to the data from Lim et al. (2007) (see ***Fig 7C,*** *plot in blue*), the best fit of the model to the data approximately occurs when

$$\lambda = 0.012 \text{ cm} \tag{23}$$

Eq 13 gives

$$\varepsilon = 0.002 \tag{24}$$

And, from Eq 6, the rate of GSH utilization is

$$K = 2\text{x}10^{-4}\ \text{sec}^{-1} \tag{25}$$

$$\text{K}^{-1} = 1.4 \text{ hours}$$

The rate of consumption must be quite large to create the observed peripheral gradient shown in Lim et al., (2007) **(see *Fig 7C)***. But such a large rate of consumption in the central fiber cells will drive the concentration to zero, whereas data show the concentration does not go to zero. One possible reason for the difference is that the rate of consumption is not uniform, but instead declines to near zero in the central fibers. Then, the concentration should not go to zero. Another possibility is that there is some small residual *de novo* synthesis or uptake of GSH in all fiber cells. To examine these two possibilities, the models below were constructed.

Consider first the idea of little to no GSH consumption in central fiber cells. In a real lens, a

decline in GSH consumption would be a continuous function of distance into the lens. However, a continuous decline does not lead to equations that can be analytically solved. To test the idea of no consumption in central fibers, we assumed the rate of consumption had two values, *K* (Eq 25) in fibers located less than 500 μm from the surface and zero in fiber located more than 500 μm from the surface. Thus the discontinuity in consumption is assumed to occur at:

$$R = B = \frac{b}{\lambda} \quad where \quad b = 0.11 \text{ cm} \tag{26}$$

The differential equations are:

$$\frac{1}{R^2}\frac{d}{dR}R^2\frac{dG^{(0)}(R)}{dR} = \begin{cases} 0 & 0 \le R \le B \\ G^{(0)}(R) & B \le R \le A \end{cases} \tag{27}$$

The boundary conditions are the same as before. The matching of the solutions at R = B is done by invoking continuity in both GSH concentration and concentration gradient. The solutions are:

$$\frac{GSH(r)}{GSH(a)} = \begin{cases} k_0(0)\frac{b\sinh r/\lambda}{r\sinh b/\lambda} & 0 \le r \le b \\ k_1(0)\frac{b\sinh r/\lambda}{r\sinh b/\lambda} + k_2(0)\frac{b\cosh r/\lambda}{r\cosh b/\lambda} & b \le r \le a \end{cases} \tag{28}$$

The constants $k_0(0)$, $k_1(0)$ and $k_2(0)$ are defined below in Eqs 34-36 with S = 0.

The second idea was that GSH synthesis persists in the fiber cells, but at a greatly diminished rate from synthesis in surface cells. The rate of synthesis is:

$$s \quad \left(\frac{moles}{cm^3 sec}\right) \qquad S = \frac{\lambda^2 s}{D\ GSH(a)} \tag{29}$$

The differential equation is:

$$\frac{1}{R^2}\frac{d}{dR}R^2\frac{dG^{(0)}(R)}{dR} = G^{(0)}(R) - S \tag{30}$$

The solution to Eq 30 is:

$$\frac{GSH(r)}{GSH(a)} = S + (1 - S)\frac{a\ sinh\ r/\lambda}{r\ sinh\ a/\lambda} \tag{31}$$

These two different assumptions produce virtually identical results, with either predicting a pedestal in GSH concentration in the central fibers (see ***Fig 7B***). However the data from Lim et al., adapted in ***Fig 7C,*** show a negative slope in the GSH concentration in central fibers. Neither of these assumptions is capable of producing a negative slope. However, if both assumptions are simultaneously invoked: there is no consumption in central fibers but there is some synthesis, these assumptions lead to GSH accumulation and a negative slope (***Fig 7C,*** *plot in red****)***.

The differential equations describing both assumptions simultaneously are

$$\frac{1}{R^2}\frac{d}{dR}R^2\frac{dG^{(0)}(R)}{dR} = \begin{cases} -S & 0 \le R \le B \\ G^{(0)}(R) - S & B \le R \le A \end{cases} \tag{32}$$

The solutions are:

$$\frac{GSH(r)}{GSH(a)} = \begin{cases} (1-S)k_0(S) + S + S\frac{(b/\lambda)^2}{6}(1-r^2/b^2) & 0 \le r \le b \\ S + (1-S)\left(k_1(S)\frac{b\sinh r/\lambda}{r\sinh b/\lambda} + k_2(S)\frac{b\cosh r/\lambda}{r\cosh b/\lambda}\right) & b \le r \le a \end{cases} \quad (33)$$

The constants in Eqs 28 and 33 are the same except for their dependence on S = 0.007. Viz

$$k_0(S) = \frac{a}{\lambda}\frac{1+\frac{S\ (b/\lambda)^2}{1-S\ 3a/\lambda}\sinh(a-b)/\lambda}{(b/\lambda)\cosh(\mathrm{a-b})/\lambda + \sinh(a/b)/\lambda} \quad (34)$$

$$k_1(S) = \frac{a}{b}\frac{\sinh b/\lambda\cosh b/\lambda - (b/\lambda)sinh^2 b/\lambda - \frac{S\ (b/\lambda)^3}{1-S\ 3a/\lambda}\sinh b/\lambda\cosh a/\lambda}{(b/\lambda)\cosh(\mathrm{a-b})/\lambda + \sinh(a-b)/\lambda} \quad (35)$$

$$k_2(S) = -\frac{a}{b}\frac{\sinh b/\lambda\cosh b/\lambda - (b/\lambda)cosh^2 b/\lambda - \frac{S\ (b/\lambda)^3}{1-S\ 3a/\lambda}\cosh b/\lambda\sinh a/\lambda}{(b/\lambda)\cosh(\mathrm{a-b})/\lambda + \sinh(a-b)/\lambda} \quad (36)$$

In Eq 28 the constants are given by Eqs 34-36 with $S = 0$.

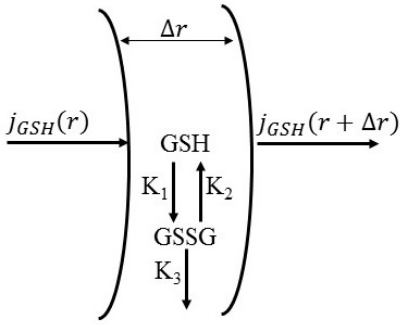

Figure A1. GSH diffusion, consumption and resynthesis from GSSG. The sketch depicts a fiber cell at a distance r cm from the lens center. Only the essential details of the reaction are shown. In actuality, two oxidized GSH molecules combine to form GSSG, which is enzymatically cleaved to form two unoxidized GSH molecules. These intermediate steps are not shown.