An Age-dependent Feedback Control Model for Calcium and Reactive Oxygen Species in Yeast Cells

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Abstract
Calcium and reactive oxygen species (ROS) interact with each other and play an important role in cell signaling networks. Based on the existing mathematical models, we develop an age-dependent feedback control model to simulate the interaction. The model consists of three subsystems: cytosolic calcium dynamics, ROS generation from the respiratory chain in mitochondria, and mitochondrial energy metabolism. In the model, we hypothesized that ROS induces calcium release from the yeast endoplasmic reticulum, Golgi apparatus, and vacuoles, and that ROS damages calmodulin and calcineurin by oxidizing them. The dependence of calcium uptake by Vcx1p on ATP is incorporated into the model. The model can approximately reproduce the log phase calcium dynamics. The simulated interaction between the cytosolic calcium and mitochondrial ROS shows that an increase in calcium results in a decrease in ROS initially (in log phase), but the increase-decrease relation is changed to an increase-increase relation when the cell is getting old. This could accord with the experimental observation that calcium diminishes ROS from complexes I and III of the respiratory chain under normal conditions, but enhances ROS when the complex formations are inhibited. The model predicts that the subsystem of the calcium regulators Pmc1p, Pmr1p, and Vex1p is stable, controllable, and observable. These structural properties of the dynamical system could mathematically confirm that cells have evolved delicate feedback control mechanisms to maintain their calcium homeostasis.

1 Introduction
Calcium and reactive oxygen species (ROS) interact with each other and play an important role in cell signaling networks, as demonstrated in Fig[1] Mitochondrial Ca\(^{2+}\) activates allosterically pyruvate dehydrogenase, isocitrate dehydrogenase, and \(\alpha\)-ketoglutarate dehydrogenase (McCormack et al, 1993), and stimulates the ATP synthase (Das et al, 1990), \(\alpha\)-glycerophosphate dehydrogenase (Wernette et al, 1981), and the adenine nucleotide translocase (ANT) (Mildaziene et al, 1995). Under physiological conditions, mitochondrial Ca\(^{2+}\) upregulates the mitochondrial oxidative phosphorylation pathway and results in faster respiratory chain activity and higher ATP output (for review, see Brookes et al, 2004).

ROS can damage cellular components such as proteins, lipids, and DNA. A rise of ROS may cause mutations in mitochondrial DNA or loss of heterozygosity in chromosomal DNA and lead to cell death. ROS is required for cell proliferation, but can also induce apoptosis (Beckman et al, 1998). Although ROS clearly possess the capacity to behave in a random and destructive fashion, growing evidence highlights a

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Figure 1: Regulatory system of intracellular calcium and reactive oxygen species (ROS) homeostasis in a budding yeast cell. Ca\(^{2+}\) ions are pumped into a cell by a unknown Ca\(^{2+}\) pump X\(_1\) in the normal conditions and by Mid1p activated by amiodarone. A rise of cytosolic Ca\(^{2+}\) level triggers a cascade of activations of calmodulin, calcineurin, and Crz1p, leading to the activation of transcription of genes PMC1 and PMR1, and inhibition of VCX1. Then Pmc1p pumps Ca\(^{2+}\) into the vacuole, Pmr1p pumps Ca\(^{2+}\) into the Golgi apparatus, Pmr1p and Cod1p pumps Ca\(^{2+}\) into the endoplasmic reticulum (ER), and uniporter transports Ca\(^{2+}\) into mitochondria. We assume that Vcx1p can be activated directly by Ca\(^{2+}\) with a delay and it pumps Ca\(^{2+}\) into the vacuole. Ca\(^{2+}\) ions can leave the vacuole through Yvc1p. Ca\(^{2+}\) ions in the Golgi apparatus can be transported into the vacuole, the ER, or out of the cell by vesicles and Ca\(^{2+}\) ions in the ER can be transported into the Golgi apparatus by vesicles. Ca\(^{2+}\) ions in the ER and the Golgi apparatus can be released to the cytosol under the stimulation of IP3. Ca\(^{2+}\) ions in mitochondria are transported by the antiporter to the cytosol. We hypothesized that ROS induces calcium release from the yeast vacuoles, Golgi apparatus, and endoplasmic reticulum and that ROS damages calmodulin and calcineurin by oxidizing them. H\(^+\) ions are pumped by Vma1p from the cytosol into the vacuole, by Vcx1p from the vacuole into the cytosol, and by Pma1p from the cytosol to the outside of the cell. H\(^+\) ions in mitochondria are ejected by the respiratory chain driven by the energy released from oxidation of NADP, which are produced from the tricarboxylic acid (TCA) cycle (or Krebs cycle). H\(^+\) ions in the cytosol flow back to mitochondria through F\(_{1}\)F\(_{0}\)-ATPase to power the ATP synthesis. Superoxide \(O_2^-\) produced by the respiratory chain is transported into the cytosol through the inner membrane anion channel.

specific role in redox cell signaling and suggests that in many instances the production of ROS is tightly regulated and its downstream targets are exquisitely specific (for review, see Brookes et al, 2004 and Finkel, 2003).

ROS as a marker for cell senescence are generated by mitochondria and several other intracellular
sources. The respiratory chain of mitochondria is the main source. Other sources include a wide range of extramitochondrial enzymes (Gordeeva et al, 2003), such as NADPH-oxidase and myeloperoxidase, and the endoplasmic reticulum, where the superoxide is generated by a leakage of electrons from NADPH-cytochrome-P450 reductase (Gordeeva et al, 2003). In addition, elevated ROS was caused by elevated redox potentials including elevated GSSG levels and NADP⁺ levels (Monteiro et al., 2004).

ROS generation has been shown to be modulated by calcium. A rise of calcium can increase ROS. On the other hand, an elevated ROS may result in an increase in calcium (Gordeeva et al, 2003). In mitochondria, it appears that calcium diminishes ROS from complexes I and III under normal conditions, but enhances ROS when the complex formations are inhibited (Brookes et al, 2004). Deletion of yeast cytosolic thioredoxin peroxidase I greatly decreases the reduced glutathione GSH / the oxidized glutathione GSSG ratio in mitochondria upon calcium treatment (Monteiro et al., 2004). A low ratio of GSH/GSSG indicates a high oxidative potential within a cell. In yeast cells, Dawes and co-workers observed a switch of anti-ROS system from log phase (young) to stationary phase (Agarwal et al., 2005). These previous observations suggest the contribution of cytosolic calcium homeostasis to organelle functions and overall cellular redox homeostasis.

It appears that Ca²⁺ is a global positive effector of cell function. Malfunction of the calcium homeostasis may cause intracellular senescence and aging (for review see Foster, 2007; Murchison and Griffith, 2007; Tang et al, 2008b). Indeed, the calcium hypothesis of brain aging is widely accepted (Thibault et al., 2007). The transition from a robust control to malfunciton of calcium homeostasis may signal or be the cause of aging.

The cytosolic calcium concentration is the net results of pump proteins (Mid1p, Cch1p, Yvc1p, etc) that increase the concentration and pump proteins (Pmc1p, Pmr1p, Vcx1p, etc) that decrease the concentration. The functions of these two categories of proteins are coordinated by calmodulin and calcineurin. Yeast cells uptake calcium from the environment via Mid1p, Cch1p, and possibly other unidentified transporters (Courchesne and Ozturk, 2003). The rise of cytosolic calcium activates calmodulin which in turn activates the serine/threonine phosphatase calcineurin. The activated calcineurin de-phosphorylates Crz1p and suppresses the activity of Vcx1p. Activated Crz1p enters the nucleus and up-regulates the expression of PMR1 and PMC1 (see Cyert 2001). Pmr1p pumps calcium ions into the organelle Golgi and possibly endoplasmic reticulum (ER). The calcium in ER and Golgi will be secreted along with the canonical secretory pathways. Pmc1p pumps calcium ions into vacuole, an organelle that stores excess ions and nutrients. While most calcium ions inside vacuoles form polyphosphate salts and are not re-usable, a small fraction of calcium ions can be pumped to the cytosol by Yvc1p. Yvc1p channels calcium to the cytosol and also contributes to the rise of cytosolic calcium concentration (Dennis and Cyert, 2002). In this intricate system, damage of one protein such as Pmc1p or Vcx1p may not affect cell’s ability to adjust to small variations of calcium burst. On the contrary, a decline of the whole system will ruin the robustness.

Based on the intracellular calcium model developed by the authors (Tang and Liu, 2008a), mitochondrial calcium models developed by Cortassa et al (2003) and Magnus et al (1997, 1998), and the mitochondrial energy metabolism model developed by Cortassa et al (2003), we further established an age-dependent feedback control model to simulate aging calcium and ROS dynamics and their interaction. The model consists of three subsystems: cytosolic calcium dynamics, ROS generation from the respiratory chain in mitochondria, and mitochondrial energy metabolism. In smooth muscle, superoxide radical O₂⁻ has been shown to inhibit both Ca²⁺-ATPase activity and Ca²⁺ uptake into the sarcoplasmic reticulum (SR) while stimulating inositol 1,4,5-trisphosphate-induced Ca²⁺ release (Suzuki et al, 1991, 1992). Favero et al (1995) reported that hydrogen peroxide H₂O₂ stimulates the Ca²⁺ release channel from skeletal muscle sarcoplasmic reticulum. Thus, in our model, we hypothesized that ROS induces calcium release from the yeast endoplasmic reticulum, Golgi apparatus, and vacuoles although there is no such a report about yeast cells. We also hypothesized that ROS damages calmodulin and calcineurin by oxidizing them. The dependence of calcium uptake by Vcx1p on ATP is incorporated into the model.

The model can approximately reproduce the log phase calcium dynamics. The simulated interaction
between the cytosolic calcium and mitochondrial ROS shows that an increase in calcium results in a decrease in ROS initially (in log phase), but the increase-decrease relation is changed to an increase-increase relation when the cell is getting old. This could accord with the experimental observation that calcium diminishes ROS from complexes I and III of the respiratory chain under normal conditions, but enhances ROS when the complex formations are inhibited. Such inhibition could come from a disproportion of lipid component or other alterations in the membrane during the aging process. The model predicts that the subsystem of the calcium regulators Pmc1p, Pmr1p, and Vex1p is stable, controllable, and observable. These structural properties of the dynamical system could mathematically confirm that cells have evolved delicate feedback control mechanisms to maintain their calcium homeostasis.

2 Results

2.1 The model contains two new features

Our model presented in the next section, Feedback control model, is built on the intracellular calcium model developed by the authors (Tang and Liu, 2008a), mitochondrial calcium models developed by Cortassa et el (2003) and Magnus et al (1997, 1998), and the mitochondrial energy metabolism model developed by Cortassa et el (2003). Two new features are added to these existing models.

In smooth muscle, superoxide radical $O_2^-$ has been shown to inhibit both Ca$^{2+}$-ATPase activity and Ca$^{2+}$ uptake into the sarcoplasmic reticulum (SR) while stimulating inositol 1,4,5-trisphosphate-induced Ca$^{2+}$ release (Suzuki et al, 1991, 1992). Favero et al (1995) reported that hydrogen peroxide H$_2$O$_2$ stimulates the Ca$^{2+}$ release channel from skeletal muscle sarcoplasmic reticulum. Thus we hypothesized that ROS induces calcium release from the yeast endoplasmic reticulum, Golgi apparatus, and vacuoles although there is no such a report about yeast cells. The release rate is determined by fitting the experimental data of Favero et al (1995) into the Michaelis-Menton function

$$ r_{ROS}(H_2O_2) = \frac{V_{H2O2,max}[H_2O_2]}{K_{H2O2,M} + [H_2O_2]}, $$

with $V_{H2O2,max} = 7.28$ (nmol/mg/min) and $K_{H2O2,M} = 11.28$ (mM), as shown in Fig 2. The unit, nmol/mg/min, is converted into min$^{-1}$ by multiplying the factor of 0.2/0.685. This leads to changes in the equations (22), (26), (27), and (28) in the next section.
We also hypothesized that ROS damages calmodulin and calcineurin by oxidizing them:

\[
3\text{Ca}^{2+} + \text{calmodulin} \xrightarrow{k_1} \text{CaM,} \quad \text{CaM + calcineurin} \xrightarrow{k_2} \text{CaN,} \\
\text{calmodulin + ROS} \xrightarrow{K_{ROS}} \text{ROS - calmodulin,} \quad \text{CaM + ROS} \xrightarrow{K_{ROS}} \text{ROS - CaM,} \\
\text{calcineurin + ROS} \xrightarrow{K_{ROS}} \text{ROS - calcineurin,} \quad \text{CaN + ROS} \xrightarrow{K_{ROS}} \text{ROS - CaN,}
\]

where CaM denotes the Ca\(^{2+}\)-bound calmodulin and CaN denotes the CaM-bound calcineurin. The ROS-calmodulin and other ROS-damaged molecules are dead in function. They will likely be removed by the proteolysis systems such as autophagy of the cell. Using the law of mass action, we can write down the differential equations for these reactions as follows:

\[
\frac{d[\text{calm}]}{dt} = -k_1[\text{Ca}^{2+}]^3[\text{calm}] + k_{-1}[\text{CaM}] - K_{ROS}[\text{calm}][\text{ROS}], \quad (2) \\
\frac{d[\text{CaM}]}{dt} = k_1[\text{Ca}^{2+}]^3[\text{calm}] - k_{-1}[\text{CaM}] - K_{ROS}[\text{CaM}][\text{ROS}] - k_2[\text{CaM}][\text{calc}] + k_{-2}[\text{CaN}], \quad (3) \\
\frac{d[\text{calc}]}{dt} = -k_2[\text{CaM}][\text{calc}] + k_{-2}[\text{CaN}] - K_{ROS}[\text{calc}][\text{ROS}], \quad (4) \\
\frac{d[\text{CaN}]}{dt} = k_2[\text{CaM}][\text{calc}] - k_{-2}[\text{CaN}] - K_{ROS}[\text{CaN}][\text{ROS}], \quad (5)
\]

Adding the equations (2), (3), and (5) together gives

\[
\frac{d}{dt}(\text{[calm]} + \text{[CaM]} + \text{[CaN]}) = -K_{ROS}[\text{ROS}](\text{[calm]} + \text{[CaM]} + \text{[CaN]}),
\]

which implies

\[
\text{[calm]}(t) + \text{[CaM]}(t) + \text{[CaN]}(t) = ([\text{CaM}_0] + [\text{CaN}_0](0)) \exp \left( -K_{ROS} \int_0^t [\text{ROS}](s) ds \right),
\]

where \([\text{CaM}_0] = [\text{calm}](0) + [\text{CaM}](0)\) denotes the total initial concentration of Ca\(^{2+}\)-free and Ca\(^{2+}\)-bound calmodulin. This results in the equation (23) in the next section. Adding the equations (4) and (5) together gives

\[
\frac{d}{dt}(\text{[calc]} + \text{[CaN]}) = -K_{ROS}[\text{ROS}](\text{[calc]} + \text{[CaN]}),
\]

which implies

\[
\text{[calc]}(t) + \text{[CaN]}(t) = [\text{CaN}_0] \exp \left( -K_{ROS} \int_0^t [\text{ROS}](s) ds \right),
\]

where \([\text{CaN}_0] = [\text{calc}](0) + [\text{CaN}](0)\) denotes the total initial concentration of CaM-free and CaM-bound calcineurin. This leads to the equation (24) in the next section.

Another new feature is that the dependence of calcium uptake by Vcx1p on ATP is incorporated into the model. Ohsumi et al (1983) showed that calcium uptake by the antiporter Vcx1p is driven by an
Figure 3: The dependence of calcium uptake by Vcx1p on ATP. The calcium uptake rate with respect to ATP is determined by fitting the data of Ohsumi et al. (1983) into the following polynomial
\[ r_{Ca}(\text{ATP}) = c_7 \text{ATP}^7 + c_6 \text{ATP}^6 + c_5 \text{ATP}^5 + c_4 \text{ATP}^4 + c_3 \text{ATP}^3 + c_2 \text{ATP}^2 + c_1 \text{ATP} + c_0, \]
with
\[ c_7 = -1.306693554068647 \times 10^4, \]
\[ c_6 = -1.001717287517461 \times 10^4, \]
\[ c_5 = 9.327964163212047 \times 10^4, \]
\[ c_4 = -1.293683912191992 \times 10^5, \]
\[ c_3 = 7.97624058714334 \times 10^4, \]
\[ c_2 = -2.460051295828870 \times 10^4, \]
\[ c_1 = 3.602510489478877 \times 10^3, \]
\[ c_0 = -30.478458046941080. \]

as shown in Fig 3. The unit, nmol/mg/min, is converted into min\(^{-1}\) by multiplying the factor of 2. This feature can be seen from the equations (22) and (26) in the next section.

2.2 The model can approximately reproduce the log phase calcium dynamics

Figs 4 and 5 indicate that the model can approximately reproduce the experimental results of log phase calcium dynamics. In reproducing the result (Fig 4) obtained by Förster and Kane (2000), the environmental calcium is set to 20 \(\mu\)M during the initial 10 seconds and then is suddenly changed to 50000 \(\mu\)M, following the experiment of Förster and Kane (2000). All parameters and initial conditions are listed in Tables 1 and 2. To simulate the case where calcineurin is inhibited, the rate constants \(k_2\) and \(k_{-2}\) are set to 0. In reproducing the result (Fig 5) obtained by Ohsumi and Auraku (1983), the environmental calcium is set to 300 \(\mu\)M. In both cases, the simulated calcium dynamics agrees approximately with the experimental data in a general tendency, although they do not match perfectly.
Figure 4: Numerical reproduction of calcium homeostasis in log phase. In reproducing calcium homeostasis in log phase observed by Förster and Kane (2000), the environmental calcium is set to 20 µM during the initial 10 seconds and then is suddenly changed to 50000 µM in accordance with the experiment. All parameters and initial conditions are listed in Tables 1 and 2. To simulate the case where calcineurin is inhibited, the rate constants $k_2$ and $k_{-2}$ are set to 0. The system (22)-(49) is solved numerically by using MATLAB. The simulated calcium shocks (black lines) agree approximately with the experimental data of Förster and Kane (red dotted lines), although they do not match perfectly.

Figure 5: Numerical reproduction of vacuolar calcium uptake. In reproducing the result of vacuolar calcium uptake obtained by Ohsumi and Anraku (1983), the environmental calcium is set to 300 µM. All parameters and initial conditions are listed in Tables 1 and 2. The system (22)-(49) is solved numerically by using MATLAB. The simulated calcium uptake agrees approximately with the experimental data in a general tendency. Left: reproduction of Fig.1a of Ohsumi and Anraku (1983); right: simulation.

2.3 Lifespan can be predicted by the calcium level simulated by the model

The cytosolic calcium level in yeast cells is maintained in a narrow range of 50 - 200 nM (Aiello et al., 2002; Dunn et al., 1994; Miseta et al., 1999a). A higher calcium level could result in cell death. Using the model, we simulated calcium levels during aging. Fig.6 shows that the calcium level gradually increases with time and exceeds 0.2 µM around 4000 minutes. If the cell dies at the calcium level of 0.2 µM, then the lifespan of the cell is 4000/120 =33 generations, which is close to the experimental average lifespan of 29 generation (Tang and Liu, 2008a).
Figure 6: Prediction of aging calcium dynamics. In producing this aging calcium dynamics, the environmental calcium is set to 300 \( \mu \text{M} \). All parameters and initial conditions are listed in Tables 1 and 2. The calcium level gradually increases with time and exceeds 0.2 \( \mu \text{M} \) around 4000 minutes. The cytosolic calcium level in yeast cells is maintained in a narrow range of 50 - 200 nM (Aiello et al., 2002; Dunn et al., 1994; Miseta et al., 1999a). If the cell dies at the calcium level of 0.2 \( \mu \text{M} \), then the lifespan of the cell is 4000/120 = 33 generations, which is close to the experimental average lifespan of 29 generation (Tang and Liu, 2008a).

2.4 The model can simulate the interaction between calcium and ROS

ROS generation has been shown to be modulated by calcium. A rise of calcium can increase ROS. On the other hand, an elevated ROS may result in an increase in calcium (Gordeeva et al., 2003). In mitochondria, it appears that calcium diminishes ROS from complexes I and III of the electron transport chain under normal conditions, but enhances ROS when the complex formations are inhibited (Brookes et al., 2004). We used the model to simulate this interaction. The phase plot of Fig. shows that an increase in calcium results in a decrease in ROS initially (in log phase), but the increase-decrease relation is changed to an increase-increase relation when the cell is getting old. An explanation about the increase-increase relation is a “two-hit” hypothesis: in addition to calcium increase, the function of the complexes of the electron transport chain is declining as the cell is getting old. Such declination may have an effect similar to the inhibition of the complexes.
Figure 7: Prediction of the interaction between calcium and ROS. Green triangle: initial time; red dot: final time. The phase plot shows that an increase in calcium results in a decrease in ROS initially (in log phase), but the increase-decrease relation is changed to an increase-increase relation when the cell is getting old.
2.5 The model predicts that the subsystem of the calcium regulators Pmc1p, Pmr1p, and Vex1p is stable, controllable, and observable

Following control analysis (Liu and Tang, 2008), we analyzed the stability, controllability, and observability of the subsystem of the calcium regulators Pmc1p, Pmr1p, and Vex1p:

\[
\frac{d[Ca^{2+}]_c}{dt} = f_1([Ca^{2+}]_c) \frac{V_{ex}[Ca^{2+}]_{ex}}{K_{ex} + [Ca^{2+}]_{ex}} - h(t)\theta \left( \frac{1}{[CaN]} \right) \frac{V_{pmc}[Ca^{2+}]_c}{K_{pmc} + [Ca^{2+}]_c} \\
- f_2([CaN])f_3([Ca^{2+}]_d) \frac{V_{vex}[Ca^{2+}]_c}{K_{vex} + [Ca^{2+}]_c} + f_4(Ca)_c \frac{V_{vex}[Ca^{2+}]_v}{K_{vex} + [Ca^{2+}]_v} \\
- h(t)\theta \left( \frac{1}{[CaN]} \right) \frac{V_{pmr}[Ca^{2+}]_c}{K_{pmr} + [Ca^{2+}]_c} - g(t)V_{cod}[Ca^{2+}]_c \\
- h(t)\theta \left( \frac{1}{[CaN]} \right) \frac{V_{erpmr}[Ca^{2+}]_c}{K_{erpmr} + [Ca^{2+}]_c},
\]

\[
\frac{d[Ca^{2+}]_v}{dt} = h(t)\theta \left( \frac{1}{[CaN]} \right) \frac{V_{pmc}[Ca^{2+}]_c}{K_{pmc} + [Ca^{2+}]_c} + f_2([CaN])f_3([Ca^{2+}]_d) \frac{V_{vex}[Ca^{2+}]_c}{K_{vex} + [Ca^{2+}]_c} \\
- k_6[Ca^{2+}]_v - f_4([Ca^{2+}]_c) \frac{V_{vex}[Ca^{2+}]_v}{K_{vex} + [Ca^{2+}]_v} + k_7k_9f_5([Ca^{2+}]_g)[Ca^{2+}]_g,
\]

\[
\frac{d[Ca^{2+}]_g}{dt} = h(t)\theta \left( \frac{1}{[CaN]} \right) \frac{V_{pmr}[Ca^{2+}]_c}{K_{pmr} + [Ca^{2+}]_c} + k_6f_6([Ca^{2+}]_c)[Ca^{2+}]_c \\
- k_9f_5([Ca^{2+}]_g)[Ca^{2+}]_g,
\]

\[
\frac{d[Ca^{2+}]_r}{dt} = h(t)\theta \left( \frac{1}{[CaN]} \right) \frac{V_{erpmr}[Ca^{2+}]_c}{K_{erpmr} + [Ca^{2+}]_c} + \frac{V_{cod}[Ca^{2+}]_c}{K_{cod} + [Ca^{2+}]_c} \\
- k_8f_6([Ca^{2+}]_g)[Ca^{2+}]_r + k_9k_{10}f_5([Ca^{2+}]_g)[Ca^{2+}]_g,
\]

\[
\frac{d[Ca^{2+}]_d}{dt} = t_d([Ca^{2+}]_c - [Ca^{2+}]_d),
\]

\[
\frac{d[CaM]}{dt} = k_1[Ca^{2+}]_c^3 (([CaM]_0 + CaN(0) - [CaM] - [CaN])) \\
- k_{-1}[CaM] - k_2[CaM] ([CaN]_0 - [CaN]) + k_{-2}[CaN],
\]

\[
\frac{d[CaN]}{dt} = k_2[CaM] ([CaN]_0 - [CaN]) - k_{-2}[CaN],
\]

\[
\frac{dh}{dt} = k_3\phi \left( \frac{1}{[CaN]} \right) (1 - h) - k_4 \left[ 1 - \phi \left( \frac{1}{[CaN]} \right) \right] h.
\]

Detailed explanations about the model and various functions are presented in the next section. From the point of view of control theory, the equations (7)-(11) are state equations of a plant, and the equations (12)-(14) constitute a controller. Thus, variables h and [CaN] in the equations (7)-(11) are control inputs (manipulated variables) that control the calcium pumps Pmc1p, Pmr1p, and Vex1p. In order to use control theory to analyze the problem, we rewrite the equations (13)-(14) as follows

\[
\frac{d[CaN]}{dt} = -k_{-2}[CaN] + u_1,
\]

\[
\frac{dh}{dt} = -k_4h + u_2,
\]

where

\[
u_1 = k_2[CaM] ([CaN]_0 - [CaN]),
\]

\[
u_2 = k_3\phi \left( \frac{1}{[CaN]} \right) (1 - h) + k_4\phi h \left( \frac{1}{[CaN]} \right).
\]
are feedback controllers. Then the equations (7)-(11) and (15)-(16) constitute a standard nonlinear open-loop control system with the following output equation

\[ y = [Ca^{2+}]_c. \] (19)

Since the steady state equations of the control system cannot be solved explicitly, we use MATLAB to solve it numerically to obtain the following equilibrium:

\[
\begin{align*}
[Ca^{2+}]_c &= 0.083, \\
[CaN] &= 1.54, \\
\eta &= 0.85, \\
[Ca^{2+}]_{ir} &= 877, \\
[Ca^{2+}]_g &= 363, \\
[Ca^{2+}]_{er} &= 12, \\
[Ca^{2+}]_d &= 0.083.
\end{align*}
\]

Using the Maple software to linearize the system (7)-(11) and (15)-(16) at the equilibrium, we obtain the following Jacobian

\[
A = 
\begin{bmatrix}
-8219.8 & 2.7159 \times 10^{-9} & 0 & 0 & -3.2428 & -3284.7 & -959.12 \\
2885.6 & -0.5 & 7.0488 & 0 & 3.2428 & 990.34 & 289.21 \\
3733.5 & 0 & -23.496 & 70.791 & 0 & 2262 & 660.47 \\
83.336 & 0 & 2.3496 & -70.791 & 0 & 32.315 & 9.4354 \\
1 & 0 & 0 & 0 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -5 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -0.1
\end{bmatrix}
\]

The input matrix is given by

\[
B = 
\begin{bmatrix}
0 & 0 \\
0 & 0 \\
0 & 0 \\
0 & 0 \\
1 & 0 \\
0 & 1
\end{bmatrix}
\]

and the output matrix is \( C = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \). This leads to a standard linear control system

\[
\frac{dx}{dt} = Ax + Bu, \quad \text{(20)}
\]

\[
y = Cx. \quad \text{(21)}
\]

The eigenvalues of the Jacobian \( A \) are all negative: -8219.8, -74.079, -20.208, -1.0004, -0.5, -5, -0.1. Thus the linear control system (20) is exponentially stable and then the original nonlinear control system (7)-(11) and (15)-(16) is locally exponentially stable (Khalil, 2002, Corollary 5.1).

The system (20) is controllable if for any initial state \( x_0 \) and any desired state \( x_f \), there exists a control \( u \) such that \( x(T) = x_f \) for some \( T > 0 \). The system (20)-(21) is observable if any initial state can be uniquely determined by the output \( y(t) \) (cytosolic calcium) over \((0, T)\) for some \( T > 0 \).

To check the controllability of (20), it suffices to examine the rank of the Kalman controllability matrix (Morris, 2001, Ogata, 2002)

\[
\mathcal{C} = [B \ AB \ \cdots \ A^6B].
\]
Using the MATLAB control system toolbox, we found that the matrix $C$ has a rank of 7 and then the system (20) is controllable (Morris, 2001). In the same way, we found that Kalman observability matrix

$$\mathcal{O} = [C^T \mid A^T C^T \mid \cdots \mid (A^T)^6 C^T]$$

has a rank of 7 and then the system (20)–(21) is observable (Morris, 2001).

It is well known that if a control system is controllable and observable, we can design (locally stabilizable) linear output feedback controllers, such as observer-based feedback controllers, to regulate the calcium to its equilibrium. This could confirm that cells have developed the smart feedback controllers (17) and (18) to maintain their calcium homeostasis. In control engineering, it is usually difficult to design globally-stabilizable nonlinear output feedback controllers. Thus such a cell-developed output controller could have potential applications in control engineering.

3 Feedback control model

Based on the intracellular calcium model developed by the authors (Tang and Liu, 2008a), mitochondrial calcium models developed by Cortassa et al (2003) and Magnus et al (1997, 1998), and the mitochondrial energy metabolism model developed by Cortassa et al (2003), we establish the following feedback control
\[
\begin{align*}
\frac{d[Ca^{2+}]_c}{dt} &= V_{pmx,Ca} + V_{vcc,Ca} + V_{gxx,Ca} + V_{ex,Ca} + f_m(V_{NaCa} - V_{uni}) + [r_{Ca}[H_2O_2]] + r_{Ca}([O_2^-])[(Ca^{2+})_v + [Ca^{2+}]_g + [Ca^{2+}]_er] - V_{pmr,Ca} - V_{vcc,Ca} - V_{pmr,Ca} - V_{erpmr,Ca} - V_{cord,Ca}, \\
\frac{d[CaM]}{dt} &= V_{CaM} - k_{-1}[CaM] - V_{CaN} + k_{-2}[CaN] - K_{ROS}[CaM][([O_2^-] + [H_2O_2]), \\
\frac{d[CaN]}{dt} &= V_{CaN} - k_{-2}[CaN] - K_{ROS}[CaN][([O_2^-] + [H_2O_2]), \\
\frac{d[Ca^{2+}]_v}{dt} &= V_{pmr,Ca} + V_{vcc,Ca} - k_6[Ca^{2+}]_v - V_{gxx,Ca} + k_7k_9f_5([Ca^{2+}]_g)[Ca^{2+}]_g - V_{gxx,Ca} - [r_{Ca}([H_2O_2]) + r_{Ca}([O_2^-])][Ca^{2+}]_v, \\
\frac{d[Ca^{2+}]_g}{dt} &= V_{pmr,Ca} + k_8f_6([Ca^{2+}]_er)[Ca^{2+}]_er - k_9f_5([Ca^{2+}]_g)[Ca^{2+}]_g - V_{gxx,Ca} - [r_{Ca}([H_2O_2]) + r_{Ca}([O_2^-])][Ca^{2+}]_g, \\
\frac{d[Ca^{2+}]_er}{dt} &= V_{erpmr,Ca} + V_{cord,Ca} - k_8f_6([Ca^{2+}]_er)[Ca^{2+}]_er + k_9k_10f_5([Ca^{2+}]_g)[Ca^{2+}]_g - [r_{Ca}([H_2O_2]) + r_{Ca}([O_2^-])][Ca^{2+}]_er, \\
\frac{d[Ca^{2+}]_d}{dt} &= t_d([Ca^{2+}]_c - [Ca^{2+}]_d), \\
\frac{d[C]}{dt} &= p(i - [M]), \\
\frac{d[M]}{dt} &= p \left( k_{11}[C] + k_{12}[C][M]^2 - \frac{k_{13}[M]}{[M] + 1} \right), \\
\frac{d[IP3]}{dt} &= \frac{k_{14}[M]}{k_{15} + [M]k_{16} + [Ca^{2+}]_c} - k_{17}[IP3], \\
\frac{d[ADP]_m}{dt} &= V_{ANT} - V_{ATPase} - V_{SL}, \\
\frac{d[\Delta \Psi]_m}{dt} &= V_{He} + V_{He,F} - V_{Hta} - V_{ANT} - V_{Htak} - V_{NaCa} - 2V_{uni}, \\
\frac{d[Ca^{2+}]_m}{dt} &= f_m(V_{uni} - V_{NaCa}),
\end{align*}
\]
\[
\frac{d[O_2^-]}{dt}_m = r_{ROS\text{increase}}(t) \times shunt \times V_{O_2} - V_{ROS}^{Tr},
\]
(36)
\[
\frac{d[O_2^-]}{dt}_c = V_{ROS}^{Tr} - V_{SOD},
\]
(37)
\[
\frac{d[H_2O_2]}{dt} = V_{SOD} - V_{CAT} - V_{GPX},
\]
(38)
\[
\frac{d[GS]}{dt} = V_{GR} - V_{GPX},
\]
(39)
\[
\frac{d[ISOC]}{dt} = V_{Aco} - V_{IDH},
\]
(40)
\[
\frac{d[C]}{dt} = V_{Aco} - V_{IDH},
\]
(41)
\[
\frac{d[SCoA]}{dt} = V_{KGDH} - V_{SL},
\]
(42)
\[
\frac{d[Suc]}{dt} = V_{SL} - V_{SDH},
\]
(43)
\[
\frac{d[FUM]}{dt} = V_{SDH} - V_{FH},
\]
(44)
\[
\frac{d[MAL]}{dt} = V_{FH} - V_{MDH},
\]
(45)
\[
\frac{d[OAA]}{dt} = V_{MDH} - V_{CS} - V_{AAT},
\]
(46)
\[
\frac{d[ASP]}{dt} = V_{AAT} - V_{C\_ASP},
\]
(47)
\[
\frac{d[NADH]}{dt} = V_{IDH} + V_{KGDH} + V_{MDH} - V_{O_2},
\]
(48)
\[
\frac{d[ATP]}{dt}_c = -V_{ANT} - V_{gly} + V_{hyd}.
\]
(49)

For the detailed derivation of the equations (22)-(32), we refer to the work by Cui et al (2006), and Tang and Liu (2008a). For the other equations, we refer to the work by Cortassa et al (2003, 2004) and Magnus et al (1997, 1998).

The state variables are described as follows:

1. \([Ca^{2+}]_c\): the concentration of cytosolic calcium (µM);
2. \([CaM]\): the concentration of Ca^{2+}-bound calmodulin (µM);
3. \([CaN]\): the concentration of CaM-bound calcineurin (µM);
4. \([CaM_0]\): the total concentration of Ca^{2+}-free and Ca^{2+}-bound calmodulin (µM);
5. \([CaN_0]\): the total concentration of CaM-free and CaM-bound calcineurin (µM);
6. \(h\): the total nuclear fraction of Crz1p;
7. \([Ca^{2+}]_v\): the concentration of calcium in the vacuole (µM);
8. \([Ca^{2+}]_g\): the concentration of calcium in the Golgi apparatus (µM);
9. \([Ca^{2+}]_{er}\): the concentration of calcium in ER (µM);
10. \([Ca^{2+}]_d\): delayed cytosolic calcium signal (µM);
11. $[C]$: the cyclin (dimensionless);
12. $[M]$: the maturation promoting factor (dimensionless),
13. $[IP3]$: the concentration of IP3 (µM);
14. $[ADP]_m$: the concentration of mitochondrial ATP (mM);
15. $[\Delta \Psi]_m$: the electrical potential difference across the inner mitochondrial membrane $\Psi_i - \Psi_m$ (V), where $\Psi_i$ denotes the voltage of the outside of the inner mitochondrial membrane and $\Psi_m$ is the voltage of the matrix side;
16. $[Ca^{2+}]_m$: the concentration of mitochondrial calcium (µM);
17. $[O_2^-]_m$: the concentration of mitochondrial superoxide (mM);
18. $[O_2^-]_c$: the concentration of intracellular superoxide (mM);
19. $[H_2O_2]$: the concentration of intracellular hydrogen peroxide (mM);
20. $[GSH]$: the concentration of intracellular glutathione (mM);
21. $[ISOC]$: the concentration of isocitrate (mM);
22. $[\alpha KG]$: the concentration of $\alpha$-ketoglutarate (mM);
23. $[SCoA]$: the concentration of succinyl CoA (mM);
24. $[Suc]$: the concentration of succinate (mM);
25. $[FUM]$: the concentration of fumarate (mM);
26. $[MAL]$: the concentration of malate (mM);
27. $[OAA]$: the concentration of oxalacetate (mM);
28. $[ASP]$: the concentration of aspartate (mM);
29. $[NADH]$: the concentration of NADH in mitochondrial matrix (mM);
30. $[ATP]_c$: the concentration of intracellular ATP (mM);

In what follows, $g(t)$ is the experimental survival curve of wild type yeast (BY4742) cells to describe the aging process of proteins (see Fig. 2 of Tang and Liu (2008a)). Reaction velocities are described as follows:

1. The velocity of calcium transport through a unknown channel X on the plasma membrane:

$$V_{pmx, Ca} = f_1([Ca]_c) \frac{V_{ex}[Ca^{2+}]_{ex}}{K_{ex} + [Ca^{2+}]_{ex}},$$

where $[Ca^{2+}]_{ex}$ is the environmental calcium.

2. The velocity of calcium transport through Pmc1p:

$$V_{pmc, Ca} = h(t)\theta \left( \frac{1}{[CaN]} \right) \frac{g(t)V_{pmc}[Ca^{2+}]_c}{K_{pmc} + [Ca^{2+}]_c},$$
3. The velocity of calcium transport through Vcx1p:

$$V_{v cx,Ca} = r_{Ca}(\frac{[ATP]_c}{[CaN]})(\frac{[Ca^{2+}]}{d}) \frac{g(t)V_{v cx}[Ca^{2+}]}{K_{v cx} + [Ca^{2+}]}.$$ 

4. The velocity of calcium transport through Yvc1p:

$$V_{y vc,Ca} = f_4(Ca)_c \frac{g(t)V_{y vc,Ca}^2}{K_{y vc} + [Ca^{2+}]}.$$ 

5. The velocity of calcium transport through Pmr1p:

$$V_{p mr,Ca} = h(t) \theta \left( \frac{1}{[CaN]} \right) \frac{g(t)V_{p mr}[Ca^{2+}]}{K_{p mr} + [Ca^{2+}]}.$$ 

6. The velocity of calcium transport through Pmr1p on ER:

$$V_{e r p mr,Ca} = h(t) \theta \left( \frac{1}{[CaN]} \right) \frac{g(t)V_{e r p mr,Ca}^2}{K_{e r p mr} + [Ca^{2+}]}.$$ 

7. The velocity of calcium transport through Cord1p:

$$V_{c o d,Ca} = \frac{g(t)V_{c o d}[Ca^{2+}]}{K_{c o d} + [Ca^{2+}]}.$$ 

8. The velocity of calcium transport through a unknown channel X on Golgi stimulated by IP3:

$$V_{g x,Ca} = \frac{[IP3]g(t)V_{g x}[Ca^{2+}]}{K_{g x} + [Ca^{2+}]}.$$ 

9. The velocity of calcium transport through a unknown channel X on the vacuole stimulated by IP3:

$$V_{v x,Ca} = \frac{[IP3]g(t)V_{v x}[Ca^{2+}]}{K_{v x} + [Ca^{2+}]}.$$ 

10. The velocity of calcium binding to calmodulin:

$$V_{CaM} = k_1[Ca^{2+}]_c \left( [CaM_0] + [CaN(0)] \right) \exp \left( -K_{ROS} \int_0^t ([O_2^-]_c + [H_2O_2]_c)ds \right)$$

$$- [CaM] - [CaN].$$

11. The velocity of binding of calcium-bound calmodulin to calcineurin:

$$V_{CaN} = k_2[CaM] \left( [CaN_0] \exp \left( -K_{ROS} \int_0^t ([O_2^-]_c + [H_2O_2]_c)ds \right) - [CaN] \right).$$

12. The velocity of proton $H^+$ transport across inner mitochondrial membrane driven by NADH:

$$V_{H e} = 6a r_{res} \sqrt{\frac{NADH}{NAD}} - (a + b) \exp \left( \frac{6gF\Delta \mu_{H}}{RT} \right) \left[ \frac{a}{1 + a r_{res} \sqrt{\frac{NADH}{NAD}}} \right] \left[ \frac{b}{1 + b r_{res} \sqrt{\frac{NADH}{NAD}}} \right] \exp \left( \frac{6gF\Delta \mu_{H}}{RT} \right) + \left[ \frac{2}{1 + 2 r_{res} \sqrt{\frac{NADH}{NAD}}} \right] \left[ \frac{3}{1 + 3 r_{res} \sqrt{\frac{NADH}{NAD}}} \right] \exp \left( \frac{6gF\Delta \mu_{H}}{RT} \right) \left( \frac{6gF\Delta \mu_{H}}{RT} \right) \left( \frac{6gF\Delta \mu_{H}}{RT} \right) \left( \frac{6gF\Delta \mu_{H}}{RT} \right) \left( \frac{6gF\Delta \mu_{H}}{RT} \right) \left( \frac{6gF\Delta \mu_{H}}{RT} \right) \left( \frac{6gF\Delta \mu_{H}}{RT} \right).$$
13. The velocity of proton H\(^+\) transport across inner mitochondrial membrane driven by FADH\(_2\):
\[
V_{He,F} = 6 \rho_{res} \left( \frac{r_a K_{res,F}}{1 + r_1 K_{res,F}} \right) \exp \left( \frac{4gF \Delta \mu_H}{RT} \right) = \left( \frac{r_a K_{res,F}}{1 + r_1 K_{res,F}} \right) \exp \left( \frac{4gF \Delta \mu_H}{RT} \right).
\]

14. The velocity of ATP synthesis by the F\(_1\)F\(_0\)-ATPase:
\[
V_{ATPase} = -g(t)\rho_{F1} \left[ \frac{10^2 p_a + p_c \exp \left( \frac{3F \Delta \psi_R}{RT} \right) - K_{F1} [ATP]_m [ADP]_m F} {1 + p_1 K_{F1} [ATP]_m [ADP]_m F} \right] \exp \left( \frac{3F \Delta \psi_R}{RT} \right) + \left( \frac{p_2 + p_3 K_{F1} [ATP]_m [ADP]_m F} {1 + p K_{uni} [ATP]_m [ADP]_m F} \right) \exp \left( \frac{3F \Delta \mu_H}{RT} \right).
\]

15. The velocity of H\(^+\) uptake by mitochondria via F\(_1\)F\(_0\)-ATPase:
\[
V_{Hu} = -3 \rho_{F1} \left[ 10^2 p_a + p_c \exp \left( \frac{3F \Delta \psi_R}{RT} \right) - K_{F1} [ATP]_m [ADP]_m F \right] \exp \left( \frac{3F \Delta \psi_R}{RT} \right) + \left( \frac{p_2 + p_3 K_{F1} [ATP]_m [ADP]_m F} {1 + p K_{uni} [ATP]_m [ADP]_m F} \right) \exp \left( \frac{3F \Delta \mu_H}{RT} \right).
\]

16. The velocity of ATP and ADP translocation across inner mitochondrial membrane via the adenine nucleotide translocator (ANT):
\[
V_{ANT} = V_{max,ANT} \left( \frac{1 - \frac{[ATP^3-]}{[ATP^4-]}} {1 + \frac{[ADP^3-]}{[ADP^4-]}} \exp \left( \frac{-bF \Delta \psi} {RT} \right) \right) \left( \frac{1 + \frac{[ATP^4-]}{[ATP^3-]} \exp \left( \frac{-bF \Delta \psi} {RT} \right)} {1 + \frac{[ATP^4-]}{[ATP^3-]} \exp \left( \frac{-bF \Delta \psi} {RT} \right)} \right).
\]

17. The velocity of H\(^+\) leak across the inner mitochondrial membrane:
\[
V_{Hleak} = gh \Delta \mu_H.
\]

18. The velocity of calcium transport through the calcium uniporter:
\[
V_{uni} = g(t) \left[ \frac{[Ca^{2+}]}{K_{trans}} \left( 1 + \frac{[Ca^{2+}]}{K_{trans}} \right)^4 \frac{1 + \frac{[Ca^{2+}]}{K_{trans}}}{1 + \frac{[Ca^{2+}]}{K_{trans}}} \exp \left( \frac{-2F(\Delta \psi - \Delta \psi_o)} {RT} \right) \right] \left( 1 + \frac{[Ca^{2+}]}{K_{trans}} \right)^n \left( 1 + \frac{[Ca^{2+}]}{K_{trans}} \right)^m.
\]

19. The velocity of calcium transport out of mitochondria through the Na\(^+\)/Ca\(^{2+}\) antiporter:
\[
V_{NaCa} = g(t) \left[ \frac{[Ca^{2+}]}{K_{trans}} \exp \left( \frac{bF(\Delta \psi - \Delta \psi_o)} {RT} \right) \right] \left( 1 + \frac{[Na^+]_c}{[Ca^{2+}]} \right)^n \left( 1 + \frac{[Na^+]_c}{[Ca^{2+}]} \right)^m.
\]

20. The velocity of oxygen consumption in the respiratory chain of mitochondria:
\[
V_{O_2} = 0.5 \rho_{res} \left[ r_a + r_c \exp \left( \frac{6gF \Delta \mu_H}{RT} \right) \right] \exp \left( \frac{6gF \Delta \mu_H}{RT} \right).
\]

21. The velocity of conversion of the intracellular superoxide [O\(_2^-\)]\(_c\) into hydrogen peroxide H\(_2\)O\(_2\) by superoxide dismutase (SOD):
\[
V_{SOD} = \frac{2k_{SOD1}k_{SOD5} (k_{SOD1} + k_{SOD3}) \left( 1 + \frac{[O_2^-]_c}{[O_2^-]_c} \right) \exp \left( \frac{6gF \Delta \mu_H}{RT} \right)} {k_{SOD5} (2k_{SOD1} + k_{SOD3}) \left( 1 + \frac{[O_2^-]_c}{[O_2^-]_c} \right)}.
\]
22. The velocity of conversion of the intracellular hydrogen peroxide $H_2O_2$ into water by catalase (CAT):

$$V_{\text{CAT}} = 2k_{\text{CAT}} E_{\text{CAT}}^T [H_2O_2] \exp(-f_r [H_2O_2]).$$

23. The velocity of reduction of $H_2O_2$ by glutathione peroxidase (GPX):

$$V_{\text{GPX}} = \frac{E_{\text{GPX}}^T[H_2O_2][GSH]}{\Phi_1[GSH] + \Phi_2[H_2O_2]}.$$

24. The velocity of reduction of oxidized glutathione (GSSH) by glutathione reductase (GR):

$$V_{\text{GR}} = \frac{k_{\text{RRI}} E_{\text{GR}}^T [GSSG] [NADPH]}{[GSSG][NADPH] + K_{\text{MSSG}}^G [NADPH] + K_{\text{MADPH}}^N [GSSG] + K_{\text{MSSG}}^G K_{\text{MADPH}}^N}.$$

25. The velocity of $[O_2^-]_m$ transport out of the mitochondrial matrix through the inner membrane anion channel:

$$V_{\text{ROS}}^T = \frac{j_{\text{IMAC}}}{\Delta \Psi_m} \left( \Delta \Psi_m + \frac{RT}{F} \ln \left( \frac{[O_2^-]_m}{[O_2^-]/_i} \right) \right).$$

26. The velocity of conversion of oxaloacetic acid (OAA) and acetyl CoA (AcCoA) to citrate (CIT) by citrate synthase (CS):

$$V_{\text{CS}} = \frac{k_{\text{Sac}}^E E_{\text{CS}}}{1 + \frac{K_{\text{Mssac}}^A}{[\text{AcCoA}]} + \frac{K_{\text{Maa}}^A}{[\text{OAA}]} + \frac{K_{\text{Mssac}}^A K_{\text{Maa}}^A}{[\text{AcCoA}][\text{OAA}]}}. $$

27. The velocity of conversion of citrate to isocitrate by aconitase:

$$V_{\text{ACO}} = k_{\text{faco}}^C \left( \frac{[\text{CIT}]}{K_{\text{Eaco}}^C} - \frac{[\text{ISOC}]}{K_{\text{Eaco}}^C} \right).$$

28. The velocity of conversion of isocitrate to α-ketoglutarate by isocitrate dehydrogenase (IDH):

$$V_{\text{IDH}} = \frac{k_{\text{isdh}}^E E_{\text{IDH}}}{1 + \frac{[H^+]_{m}}{K_{\text{H}}^T} + \frac{K_{\text{NAD}}^N}{[\text{NAD}]} \left( 1 + \frac{[\text{NADH}]}{K_{\text{r},\text{NADH}}} \right) + \frac{\left( K_{\text{isoc}}^T \right)^m}{1 + \frac{K_{\text{NAD}}^N}{[\text{NAD}]} \left( 1 + \frac{[\text{NADH}]}{K_{\text{r},\text{NADH}}} \right)}}.$$

29. The velocity of conversion of α-ketoglutarate to succinyl CoA by α-ketoglutarate dehydrogenase (KGDH):

$$V_{\text{KGDH}} = \frac{k_{\text{kghd}}^E E_{\text{KGDH}}}{1 + \frac{\left( K_{\text{kghd}}^T \right)^m}{1 + \frac{K_{\text{NAD}}^N}{[\text{NAD}]} \left( 1 + \frac{[\text{NADH}]}{K_{\text{r},\text{NADH}}} \right)}}.$$

30. The velocity of conversion of succinyl CoA into succinate by Succinyl CoA lyase (SL):

$$V_{\text{SL}} = k_{\text{fsl}}^E \left( [\text{SCoA}][\text{ADP}]_m - \frac{[\text{Suc}][\text{ATP}]_m [\text{CoA}]}{K_{\text{sl}}^E} \right).$$

31. The velocity of conversion of succinate to fumarate by Succinate dehydrogenase (SDH):

$$V_{\text{SDH}} = \frac{k_{\text{fsdh}}^E E_{\text{SDH}}}{1 + \frac{K_{\text{fsdh}}^T}{[\text{Suc}]_m} \left( 1 + \frac{[\text{OAA}]}{K_{\text{r},\text{adh}}^T} \right) \left( 1 + \frac{[\text{FUM}]}{K_{\text{r},\text{adh}}^T} \right)}.
32. The velocity of conversion of fumarate to malate by Fumarase (FH):

\[ V_{FH} = k_{FH}^F \left( [FUM] - \frac{[MAL]}{K_{E}^{FH}} \right). \]

33. The velocity of conversion of malate to oxaloacetate (OAA) by the enzyme malate dehydrogenase (MDH):

\[ V_{MDH} = \frac{k_{cat}^{MDH} E_{P}^{MDH} f_{h,a} f_{h,i}}{1 + \frac{[MAL]}{K_{i}^{MDH}} \left( 1 + \frac{[OAA]}{K_{i}^{MDH}} \right) \left( \frac{K_{NAD}^{MDH}}{[NAD]} + K_{NAD}^{MDH} \right)} \]

34. The velocity of conversion between Oxaloacetate and \( \alpha \)-ketoglutarate of the TCA cycle and the amino acids aspartate (ASP) and glutamate (GLU) by aspartate aminotransferases (AAT):

\[ V_{AAT} = k_{j}^{AAT} \left( [OAA][GLU] - \frac{[\alpha KG][ASP]}{K_{E}^{AAT}} \right). \]

35. The velocity of consumption of the aspartate:

\[ V_{C-ASP} = k_{c-ASP} [ASP]. \]

Numerous functions used in the above velocities are described as follows. The function

\[ f_1([Ca]_c) = \frac{1}{1 + a_1 \exp \left[ a_2([Ca]_c - [Ca]_c) \right]} \]  

(50)

control the Ca\(^{2+}\) entering the cell from the environment through the unknown pump X. The function \( \phi \) is given by

\[ \phi(x) = \frac{1}{1 + L_0 \left( \frac{(\lambda x)^N + 1}{(x-1)^N + 1} \right)}, \]  

(51)

where \( L_0 \) is a basic equilibrium constant, \( \lambda \) is an increment factor, and \( N \) is an integer. Within the nuclear fraction, the fully dephosphorylated state is transcriptionally active and is given by

\[ h_0(t) = h(t) \theta \left( \frac{1}{[CaN]} \right), \]  

(52)

where

\[ \theta(x) = \frac{1 + L_0}{x^{N+1} - 1} + L_0 \frac{(\lambda x)^N + 1}{(x-1)^N + 1}. \]  

(53)

The function

\[ g(t) = \frac{1}{b_1 + b_2 \exp(b_3 t)}, \]  

(54)

is an aging factor of various proteins such as calmodulin, calcineurin, Pmc1p, Vcx1p, and Yvc1p. For feedback control laws

\[ f_2([CaN]) = \frac{1}{1 + k_5[CaN]}, \]  

(55)

\[ f_3([Ca]_c) = \frac{1}{1 + a_3 \exp \left[ a_4([Ca]_c - [Ca]_c) \right]}, \]  

(56)

\[ f_4([Ca]_c) = \frac{1}{1 + a_5 \exp \left[ a_6([Ca]_c - [Ca]_c) \right]}, \]  

(57)

\[ f_5([Ca]_g) = \frac{1}{1 + a_7 \exp \left[ a_8([Ca]_g - [Ca]_g) \right]}, \]  

(58)

19
\( f_2 \) represents the negative regulation of calcineurin on \( Vcx1p \). \( f_3 \) is a feedback control describing how \( Vcx1p \) is activated by \( Ca^{2+} \) directly. \( f_4 \) is a feedback control describing how \( Ca^{2+} \) are transported back to the cytosol through \( Yvc1p \) in response to a low cytosolic \( Ca^{2+} \) concentration. \( f_5 \) is a feedback control describing how \( Ca^{2+} \) are transported out of the Golgi by vesicles in response to a high \( Ca^{2+} \) concentration in the Golgi. The function

\[
f_6([Ca]_{er}) = \frac{1}{1 + a_9 \exp\left[a_{10}([Ca]_{er} - [Ca]_{er})\right]}
\]  

(59)

is a feedback control that maintains the calcium homeostasis in the ER. The electrochemical gradient, or proton motive force \( \Delta \mu_H \), is given by

\[
\Delta \mu_H = [\Delta \Psi]_m + \frac{RT}{F} \Delta pH.
\]  

(60)

Mitochondrial \( NAD^+ \) is assumed to be conserved as follows

\[
[NAD] = C_{PN} - [NADH]
\]

with \( C_{PN} \) as the total concentration of pyrimidine nucleotides. Mitochondrial ATP, \([ATP]_m\), is assumed to be conserved as follows

\[
[ATP]_m = C_m - [ADP]_m
\]

with \( C_m \) as the total concentration of adenine nucleotides and \([ADP]_m\). Here are other relations:

\[
[ATP]^-_c = 0.05[ATP]_c,
\]

\[
[ADP]^+_-c = 0.45[ADP]_c,
\]

\[
[ATP]^+_m = 0.05[ATP]_m,
\]

\[
[ADP]^-_-m = 0.45 \cdot 0.8[ADP]_m,
\]

\[
f_{h,a} = \frac{1}{1 + [H^+]_{K_{h1}} + [H^+]^2_{K_{h1}K_{h2}}} + k_{offset},
\]

\[
f_{h,i} = \left(\frac{1}{1 + [H^+]_{K_{h1}} + [H^+]^2_{K_{h1}K_{h2}}}\right)^2,
\]

\[
[ADP]^-_c = [totalATP]_c - [ATP]_c,
\]

\[
R_{PDH} = \frac{1}{1 + u_2(1 + u_1(1 + C_{am}/K_{Ca,PDH})^{-2})},
\]

\[
AcCoA = \frac{2R_{PDH}\beta_{max}(1 + \beta_1[Glc])[Glc][ATP]_c}{1 + \beta_3[ATP]_c + (1 + \beta_4[ATP]_c\beta_5[Glc] + (1 + \beta_6[ATP]_c)\beta_7[Glc]^2},
\]

\[
r_{ROSincrease}(t) = b_1 + b_2 \exp(b_3t),
\]

\[
r_{ROS}(x) = \frac{V_{H2O2,\max}x}{K_{H2O2,\max} + x}.
\]

Numerous parameters and their values in the model are listed in Table 1. Initial conditions used in our computations are listed in Table 2 and all other initial conditions not listed are zero.

To simulate calcium, ROS, and ATP responses to glucose inputs (Glc), we used the experimental data of the exogenous glucose input obtained by Korach-André et al (2004). The data was extended periodically, as shown in Fig 8. Using MATLAB, we solved numerically the system (22)–(49). Fig 8 shows that the calcium and ROS stay at their equilibrium levels for about 3000 minutes and then increase with time while ATP stays in its homeostasis ranges all the time.
Figure 8: Exogenous glucose input. The experimental data of the exogenous glucose input obtained by Korach-Andrée et al (2004) was extended periodically.

4 Discussion

We established an age-dependent feedback control model to simulate aging calcium and ROS dynamics and their interaction by integrating the existing calcium models, ROS model, and the mitochondrial energy metabolism model. The model approximately reproduced the log phase calcium dynamics. The simulated interaction between the cytosolic calcium and mitochondrial ROS showed that an increase in calcium results in a decrease in ROS initially (in log phase), but the increase-decrease relation was changed to an increase-increase relation when the cell is getting old. The model predicted that the subsystem of the calcium regulators Pmc1p, Pmr1p, and Vex1p is stable, controllable, and observable. These structural properties of the dynamical system could mathematically confirm that cells have evolved delicate feedback control mechanisms to maintain their calcium homeostasis.

Although high levels of ROS are toxic to cells, moderate levels of ROS may be beneficial. In this paper, we only focused on ROS production by mitochondria. Jazwinski and co-workers identified that mitochondrial dysfunction triggers a retrograde response, which is beneficial for lifespan extension (Kirchman et al., 1999; Jazwinski 2005). Moreover, moderate ROS stimulation enhances autophagy, a vacuolar process that degrades damaged proteins and organelles (Scherz-Shouval and Elazar 2007). We recently found that inability to degrade the ROS-damaged materials inside vacuoles shortens the lifespan (Tang et al., 2008c). On the other hand, over-activation of autophagy usually leads to autophagic cell death (Chen et al., 2007). Including the retrograde response and the vacuole-dependent removal of ROS-damaged materials in our future studies should provide a model about the fine-tuning of ROS.

Mathematical models for the ions H\(^+\), Na\(^+\), and K\(^+\) have been established by Kapela et al (2008) and Pokhilko et al (2006). Our model may be enhanced by including these models. Since the functions of calmodulin and calcineurin depend on pH in cytosol, the integration of the dynamics of ions H\(^+\) into our model is important.

In our recent work (Tang and Liu, 2008a), a sensitivity analysis showed that the subsystem of calcium regulators Pmc1p, Pmr1p, and Vcx1p is robust with respect to perturbations of some important parameters, such as the proportional feedback control gains $V_{ex}, V_{pmc}, V_{xcx},$ and $V_{pmr}$. In another work (Liu and Tang, 2008), a sensitivity analysis showed that the simulated glucose regulation by insulin is robust with respect to feedback control gains. Thus we speculate that cells may have developed the robustness during their evolution and it can be expected that a similar robustness result can be obtained for the augmented system through a sensitivity analysis. In control engineering, the feedback gain robustness is
required in the feedback control designs.

An interesting control problem is to design observer-based output feedback controllers for the subsystem of the calcium regulators. Since we have shown that the subsystem is controllable and observable, such a output feedback controller can be designed. It is interesting to compare the human-designed controllers with the cell-developed controllers and then to apply the cell-developed controller to problems in control engineering.

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Table 1: Values of Parameters of the Model (22)-(49)

| Parameter | Value       | Description                                                                 |
|-----------|-------------|-----------------------------------------------------------------------------|
| $V_{ex}$  | 2500 (µM/min) | Rate constant of Channel X                                                  |
| $V_{pmc}$ | 40000 (µM/min) | Rate constant of Pmc1p                                                       |
| $V_{vcx}$ | 70000 (µM/min) | Rate constant of Vcx1p                                                       |
| $V_{yvc}$ | 10 (µM/min)    | Rate constant of Yvc1p                                                       |
| $V_{pmr}$ | 7000 (µM/min)  | Rate constant of Pmr1p on Golgi                                              |
| $V_{gx}$  | 10 (µM/min)    | Rate constant of a unknown IP3-stimulated calcium pump on Golgi             |
| $V_{erpmr}$ | 100 (µM/min) | Rate constant of Pmr1p on ER                                                |
| $V_{cod}$ | 10 (µM/min)    | Rate constant of Cod1p                                                       |
| $V_{vx}$  | 100 (µM/min)   | Rate constant of a unknown IP3-stimulated calcium pump on vacuole          |
| $K_{ex}$  | 500 (µM)       | Michaelis-Menten constant of Channel X (Cui et al, 2006)                    |
| $K_{pmc}$ | 2.3 (µM)       | Michaelis-Menten constant of Pmc1p (Cui et al, 2006)                        |
| $K_{vcx}$ | 100 (µM)       | Michaelis-Menten constant of Vcx1p (Cui et al, 2006)                        |
| $K_{yvc}$ | 0.2 (µM)       | Michaelis-Menten constant of Yvc1p                                           |
| $K_{pmr}$ | 100 (µM)       | Michaelis-Menten constant of a unknown IP3-stimulated calcium pump on vacuole |
| $K_{gx}$  | 50 (µM)        | Michaelis-Menten constant of a unknown IP3-stimulated calcium pump on Golgi |
| $K_{erpmr}$ | 0.1 (µM) | Michaelis-Menten constant of Pmr1p on ER                                    |
| $K_{cod}$ | 0.1 (µM)       | Michaelis-Menten constant of Cod1p                                          |
| $\left[Ca^2+\right]_c$ | 0.06 (µM) | Steady state of Ca$^{2+}$ in the cytosol (Aiello et al, 2002)               |
| $\left[Ca^2+\right]_g$ | 300 (µM) | Steady state of Ca$^{2+}$ in Golgi (Pinton et al, 1998)                     |
| $\left[Ca^2+\right]_{er}$ | 10 (µM) | Steady state of Ca$^{2+}$ in ER (Aiello et al, 2002)                        |
| $[CuM_0]$ | 25 (µM)        | The total concentration of calmodulin (Cui et al, 2006)                     |
| $[CuN_0]$ | 25 (µM)        | The total concentration of calcineurin (Cui et al, 2006)                    |
| $N$       | 13            | The number of relevant regulatory phosphorylation sites (Cui et al, 2006)    |
| $L_0$     | $10^{-N/2}$    | The basic equilibrium constant (Cui et al, 2006)                            |
| $\lambda$ | 5             | The increment factor (Cui et al, 2006)                                      |
| $t_d$     | 1 (1/min)      | Calcium signal delay rate                                                   |
| Parameter | Value                  | Description                                           |
|-----------|------------------------|-------------------------------------------------------|
| $a_1$     | 0.1                    | The feedback control constant                         |
| $a_2$     | 15 (1/µM)              | The feedback control constant                         |
| $a_3$     | 1                      | The feedback control constant                         |
| $a_4$     | 50 (1/µM)              | The feedback control constant                         |
| $a_5$     | 100                    | The feedback control constant                         |
| $a_6$     | 100 (1/µM)             | The feedback control constant                         |
| $a_7$     | 50                     | The feedback control constant                         |
| $a_8$     | 0.05 (1/µM)            | The feedback control constant                         |
| $a_9$     | 50                     | The feedback control constant                         |
| $a_{10}$  | 1 (1/µM)               | The feedback control constant                         |
| $k_1$     | 500 (1/(µM)^3 min)     | The forward rate constant (Cui et al, 2006)           |
| $k_2$     | 5 (1/(µM min))         | The forward rate constant (Cui et al, 2006)           |
| $k_3$     | 0.4 (1/min)            | The nuclear import rate constant (Cui et al, 2006)   |
| $k_4$     | 0.1 (1/min)            | The nuclear export rate constant (Cui et al, 2006)   |
| $k_5$     | 10 (1/µM)              | The feedback control constant (Cui et al, 2006)       |
| $k_6$     | 0.5 (1/min)            | The feedback control constant                         |
| $k_7$     | 0.3                    | The feedback control constant                         |
| $k_8$     | 40 (1/min)             | The feedback control constant                         |
| $k_9$     | 5.5 (1/min)            | The feedback control constant                         |
| $k_{10}$  | 0.1                    | The feedback control constant                         |
| $k_{11}$  | 3.5                    | Cell cycle constant (Norel et al, 1991)               |
| $k_{12}$  | 1                      | Cell cycle constant (Norel et al, 1991)               |
| $k_{13}$  | 10                     | Cell cycle constant (Norel et al, 1991)               |
| $k_{14}$  | 500000 (µM/min)        | Rate constant in cell cycle                           |
| $k_{15}$  | 100                    | Cell cycle constant                                   |
| $k_{16}$  | 100                    | Cell cycle constant                                   |
| $k_{17}$  | 0.3 (1/min)            | Degradation Rate of IP3                               |
| $k_{-1}$  | 100 (1/min)            | The backward rate constant (Cui et al, 2006)          |
| $k_{-2}$  | 5 (1/min)              | The backward rate constant (Cui et al, 2006)          |
| $p$       | 0.01                   | Cell cycle scaling                                    |
| $i$       | 1.2                    | Cyclin input (Norel et al, 1991)                      |
| $b_1$     | 0.92                   | The aging constant                                    |
| $b_2$     | 0.08                   | The aging constant                                    |
| $b_3$     | 0.00115 (1/min)        | The aging rate constant                               |
Table 1 continued (descriptions of the rest of parameters are referred to Cortassa et al (2003, 2004))

| Parameter | Value |
|-----------|-------|
| $V_{H2O2,max}$ | 2.1255 (/min) |
| $K_{H2O2,M}$ | 11.28 (mM) |
| $C_m$ | 15 (mM) |
| $[totalATP]_c$ | 2 (mM) |
| $C_{PN}$ | 10 (mM) |
| $minute$ | 60 (s) |
| $r_a$ | minute × $6.394 \times 10^{-10}$ (/min) |
| $r_b$ | minute × $1.762 \times 10^{-13}$ (/min) |
| $r_1$ | $2.077 \times 10^{-18}$ |
| $r_2$ | $1.728 \times 10^{-9}$ |
| $r_3$ | $1.059 \times 10^{-26}$ |
| $\rho_{res}$ | 0.0006 (mM) |
| $\rho_{res,F}$ | 0.0045 (mM) |
| $K_{res}$ | $1.35 \times 10^{18}$ |
| $K_{res,F}$ | $5.765 \times 10^{13}$ |
| $\Delta \Psi_B$ | 0.05 (V) |
| $g$ | 0.85 |
| $FADH_2$ | 1.24 (mM) |
| $FAD$ | 0.01 (mM) |
| $p_a$ | minute × $1.656 \times 10^{-5}$ (/min) |
| $p_b$ | minute × $3.373 \times 10^{-7}$ (/min) |
| $p_{c1}$ | minute × $9.651 \times 10^{-14}$ (/min) |
| $p_{c2}$ | minute × $4.585 \times 10^{-19}$ (/min) |
| $p_1$ | $1.346 \times 10^{-8}$ |
| $p_2$ | $7.379 \times 10^{-7}$ |
| $p_3$ | $6.65 \times 10^{-15}$ |
| $\rho_{F1}$ | 0.525 (mM) |
| $K_{F1}$ | $1.71 \times 10^{6}$ |
| $R$ | 8.315 (V C/mol/K) |
| $T$ | 310.6 (K) |
| $F$ | 96480 (C/mol) |
| $P_i$ | 20 (mM) |
| $V_{max,ANT}$ | 0.05 (mM) |
| $f_p$ | 0.5 |
| $g_H$ | 0.01 (mM/s/V) |
| $\Delta pH$ | -0.6 (pH units) |
| $C_{mito}$ | 1.812 (MM/V) |
| $V_{max,uni}$ | minute × 0.625 (muM/min) |
| $\Delta \Psi^0$ | 0.091 (Volts) |
Table 1 continued

| Parameter | Value |
|-----------|-------|
| $K_{act}$ | $3.8 \times 10^{-4}$ (mM) |
| $K_{trans}$ | 0.019 (mM) |
| $L$ | 110 |
| $n_a$ | 2.8 |
| $V_{max,NaCa}$ | minute $\times$ 0.005 (mM/min) |
| $b$ | 0.5 |
| $Na_i$ | 10 (mM) |
| $K_{Na}$ | 9.4 (mM) |
| $K_{Ca}$ | $3.75 \times 10^{-4}$ (mM) |
| $n$ | 3 |
| $f_m$ | 0.0003 |
| $r_{c1}$ | minute $\times$ 2.656 $\times 10^{-19}$ (/min) |
| $r_{c2}$ | minute $\times$ 8.632 $\times 10^{-27}$ (/min) |
| $G_L$ | 0.0782 (mM/s/V) |
| $G_{max}$ | 7.82 (mM/s/V) |
| $a$ | $10^{-3}$ |
| $b_{ROS}$ | $10^4$ |
| $\kappa$ | 70 (/V) |
| $\Delta \Psi_m$ | 0.004 (V) |
| $K_{cc}$ | 0.01 (mM) |
| $k_{1SOD}$ | minute $\times$ 2.4 $\times 10^6$ (/mM/min) |
| $k_{3SOD}$ | minute $\times$ 4.8 $\times 10^4$ (/mM/min) |
| $k_{5SOD}$ | minute $\times$ 0.5 (/min) |
| $E_{SOD_T}$ | 1 $\times 10^{-3}$ (mM) |
| $K_i^{H_2O_2}$ | 0.5 (mM) |
| $k_{1CAT}$ | minute $\times$ 1.7 $\times 10^4$ (/mM/min) |
| $E_{CAT_T}$ | 0.001 (mM) |
| $f_r$ | 50 |
| $E_{GPX_T}$ | 0.00141 (mM) |
| $P_{HI_1}$ | 2.5/minute (mM min) |
| $P_{HI_2}$ | 0.5/minute (mM min) |
| $K_{GSSG}^{GSH}$ | 1.94 (mM) |
| $K_N^{ADPH}$ | 38.7 (mM) |
| $k_{1GR}$ | minute $\times$ 0.0308 (/min) |
| $E_{GR_T}$ | 1.27 $\times 10^{-3}$ (mM) |
| $G_T$ | 1.5 (mM) |
| $shunt$ | 0.05 |
| $j$ | 0.12 |
Table 1 continued

| Parameter          | Value                                           |
|--------------------|-------------------------------------------------|
| $K_{ROS}$          | 3550 (/min/mM)                                  |
| $NADPH$            | 50 (mM)                                         |
| $k_{cat}^{CS}$     | minute×3.2 (/min)                               |
| $E_{CS}$           | 0.4 (mM)                                        |
| $K_{NADCoA}^{M}$   | $1.26 \times 10^{-2}$ (mM)                     |
| $K_{OAA}^{M}$      | $6.4 \times 10^{-4}$ (mM)                      |
| $C_{Kint}$         | 1.0 (mM)                                        |
| $k_{ACO}^{T}$      | minute×12.5 (/min)                              |
| $K_{ACO}^{E}$      | 2.22                                            |
| $k_{IDH,at}$       | minute×9 (/min)                                 |
| $E_{IDH}^{T}$      | 0.109 (mM)                                      |
| $K_{NADH}^{i}$     | 0.19 (mM)                                       |
| $K_{P,ADP}^{a}$    | $6.2 \times 10^{-2}$ (mM)                      |
| $[H^+]$            | $2.5 \times 10^{-5}$ (mM)                      |
| $k_{h,1}$          | $8.1 \times 10^{-5}$ (mM)                      |
| $k_{h,2}$          | $5.98 \times 10^{-5}$ (mM)                     |
| $K_{ISOC}^{M}$     | 1.52 (mM)                                       |
| $K_{a}^{Ca}$       | 0.00141 (mM)                                    |
| $k_{KDGDH}^{cat}$  | minute×2.5 (/min)                               |
| $E_{KDGDH}^{T}$    | 0.5 (mM)                                        |
| $K_{\text{alphaKG}}^{M}$ | 1.94 (mM)                           |
| $K_{NAD}^{M}$      | 38.7 (mM)                                       |
| $K_{Mg}^{M}$       | 0.0308 (mM)                                     |
| $K_{Ca}^{D}$       | $1.27 \times 10^{-3}$ (mM)                     |
| $n_{aKG}$          | 1.2                                             |
| $n_i$              | 1.7                                             |
| $Mg$               | 0.4 (mM)                                        |
| $k_{SL}^{SL}$      | minute×0.127 (/mM/min)                          |
| $K_{E}^{SL}$       | 3.115                                           |
| $CoA$              | 0.02 (mM)                                       |
| $k_{SDH}^{cat}$    | minute×1.0 (/min)                               |
| $E_{SDH}^{T}$      | 0.5 (mM)                                        |
| $K_{Suc}^{M}$      | $3 \times 10^{-2}$ (mM)                        |
| $K_{FUM}^{M}$      | 1.3 (mM)                                        |
| $K_{OAA}^{i,sdh}$  | 0.15 (mM)                                       |
| $k_{FHH}^{FH}$     | minute×0.83 (/min)                              |
| $K_{E}^{FH}$       | 1                                              |
Table 1 continued

| Parameter | Value |
|-----------|-------|
| $k_{h1}$  | $1.13 \times 10^{-3}$ (mM) |
| $k_{h2}$  | 26.7 (mM) |
| $k_{h3}$  | $6.68 \times 10^{-9}$ (mM) |
| $k_{h4}$  | $5.62 \times 10^{-6}$ (mM) |
| $k_{\text{offset}}$ | $3.99 \times 10^{-2}$ |
| $k_{MDH}^{\text{T}}$ | minute $\times$ 27.75 (/min) |
| $E_T^{MDH}$ | 0.154 (mM) |
| $K_{M_{MAL}}$ | 1.493 (mM) |
| $K_{D_{AA}}$ | $3.1 \times 10^{-3}$ (mM) |
| $k_{f_{AAT}}$ | minute $\times$ 0.644 (/min) |
| $K_{E}^{AAT}$ | 6.6 |
| $k_{G_{ASP}}$ | minute $\times$ 0.01 (/min) |
| Glc | 6 (mM) |
| $u_1$ | 15 |
| $u_2$ | 1.1 |
| $K_{PDH_{Ca}}$ | 0.05 (µM) |
| $\beta_{\text{max}}$ | $126 \times 0.125$ (/min/mM) |
| $\beta_1$ | 1.66 (/mM) |
| $\beta_3$ | 4 (/mM) |
| $\beta_4$ | 2.83 (/mM) |
| $\beta_5$ | 1.3 (/mM) |
| $\beta_6$ | 2.66 (/mM) |
| $\beta_7$ | 0.16 (/mM) |

Table 2: Initial Conditions for the Model (22)-(49)

| Parameter | Value | References |
|-----------|-------|------------|
| $[Ca]_{c}(0)$ | 0.08 (µM) | Norel et al, 1991 |
| $[CaN](0)$ | $10^{-18}$ (µM) | Norel et al, 1991 |
| $C](0)$ | 0.8 | Norel et al, 1991 |
| $[M](0)$ | 0.4 | Norel et al, 1991 |
| $[ADP]_m(0)$ | 10 (mM) | |
| $[\Delta\Psi]_{m}(0)$ | 0.1 (V) | |
| $[O_{2}^-]_{m}(0)$ | $2 \times 10^{-13}$ (mM) | |
| $[O_{2}^-]_{c}(0)$ | $1 \times 10^{-13}$ (mM) | |
| $[GSH](0)$ | 0.2 (mM) | |
| $[ADP]_{c}(0)$ | 1 (mM) | |