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A Hybrid Model of a Genetic Regulatory Network in Mammalian Sclera

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Myopia in human and animals is caused by the axial elongation of the eye and is closely linked to the thinning of the sclera which supports the eye tissue. This thinning has been correlated with the overproduction of matrix metalloproteinase (MMP-2), an enzyme that degrades the collagen structure of the sclera. In this short paper, we propose a descriptive model of a regulatory network with hysteresis, which seems necessary for creating oscillatory behavior in the hybrid model between MMP-2, MT1-MMP and TIMP-2. Numerical results provide insight on the type of equilibria present in the system.

1 Introduction

This short paper presents a descriptive model of a genetic regulatory network in the mammalian sclera using the formalism of hybrid dynamical systems. This model is deduced from experimental observations of enzyme interactions that govern the remodeling of the collagen tissue in the sclera. A number of research publications indicate that myopia is closely related to an unbalanced remodeling in sclera [12, 22]. Myopia is an optical condition in which the eye grows abnormally in the axial direction, causing images to form in front of the retina compared to on the retina, as it normally occurs [9, 12, 13, 18]. The excessive length of the eye is driven by the remodeling of the scleral extra cellular matrix (ECM) (e.g., loss of Type I collagen, COL1A1), leading the progressive thinning of this tissue [1, 11, 12]. Scleral remodeling is regulated by a large number of growth factors, membrane receptors, proteases, and protease inhibitors, which work in concert to optimize the dynamic synthesis and degradation of COL1A1[3, 11, 27]. One of the most studied actors in sclera remodeling is the Type II matrix metalloproteinase (MMP-2), because of its role in the degradation of COL1A1 [6, 12, 16]. MMP-2 is regulated by the Type II tissue inhibitor of the matrix metalloproteinases (TIMP-2), and when the two enzymes are properly balanced, the sclera develops normally. MMP-2 regulation by TIMP-2 shows a particular mechanism in which TIMP-2 not only inhibits the proteolytic activity of MMP-2, but is also necessary for the production of this metalloproteinase in its active form [12, 24, 26]. Such a mechanism is very important for the balance between COL1A1 production and degradation in sclera, and hence, should play a key role in a model of a genetic regulatory network in this tissue.

The remainder of the paper is organized as follows. Section 2 introduces the mechanisms governing the regulatory network of interest and proposes a hybrid system model. Section 3 presents results from simulations of the proposed model, which, for a particular set of parameters, identify both isolated equilibria and limit cycles. Final remarks and a discussion of the current efforts appear in Section 4.

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2 Modeling

We develop a model of a regulatory network in mammalian sclera from the following experimental observations. Sufficient high levels of MMP-2 protein cause the expression of TIMP-2 [12, 21] (considering expression as the result of transcription, translation and activation of the protein latent form). When the concentration of TIMP-2 exceeds a minimum threshold, this protein indirectly modulates the increment of MMP-2: TIMP-2 triggers the expression of active membrane-type I matrix metalloproteinase (MT1-MMP) [12, 21, 24], which is necessary for the activation of latent MMP-2 [12, 24, 26]. When the concentration of TIMP-2 protein is sufficiently high, TIMP-2 inhibits the proteolytic activity of MMP-2 and MT1-MMP [12, 19, 21, 24, 26]. As we mentioned above, MT1-MMP triggers the activation of latent MMP-2 when sufficiently high [5, 15]; therefore, by blocking MT1-MMP, TIMP-2 is also inhibiting the activation of latent MMP-2 [12, 24, 26]. In fact, [12, 26] argue that the increased TIMP-2 mRNA and protein levels are significant as TIMP-2 is not only a protein inhibitor of both the active and latent form of MMP-2 but also paradoxically essential for the MT1-MMP dependent activation of MMP-2. The genetic network capturing these mechanisms is depicted in Figure 1.

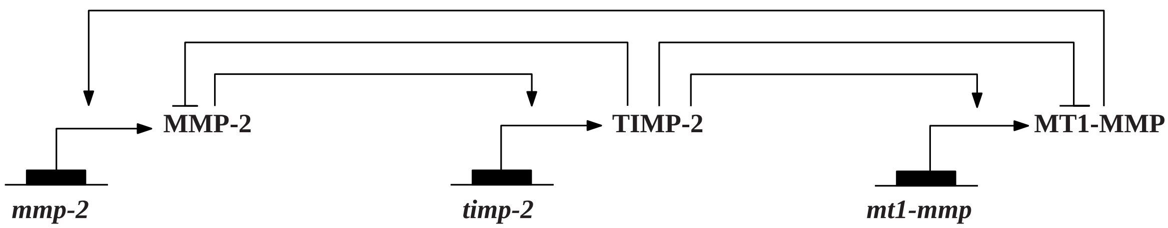


Figure 1: *Proposed genetic regulatory network for sclera. Lowercase names refer to genes, uppercase names refer to proteins. Lines ending in arrows represent expression triggers and lines ending in flatheads refer to inhibition triggers.*

The mechanisms described above can be encoded in a piecewise-linear differential equation following the modeling technique in [6, 14]. However, the resulting model of the genetic network in sclera would not incorporate hysteresis, which is a key player in genetic regulatory networks [2, 7, 10, 20]. To incorporate hysteresis, we follow the approach in [23] and propose a hybrid system model in the framework of [4]. To this end, we define the state of the hybrid system as

$$z = [x_1, x_2, x_3, q_1, q_2, q_3, q_4]^\top \tag{1}$$

where $z \in Z := \mathbb{R}^3_{\geq 0} \times \{0,1\}^4$. The continuous states x_1, x_2, x_3 represent the protein concentrations, where x_1 represents the protein concentration of TIMP-2, x_2 the concentration of MT1-MMP, and x_3 the concentration of MMP-2. Positive constants $\gamma_1, \gamma_2, \gamma_3$ define the decay rates and k_1, k_2, k_3 define the growth rates, respectively, for each of the concentrations. The discrete states (logic variables) q_1, q_2, q_3, q_4 define the boolean value (1 or 0) of the hysteresis functions associated with each of the thresholds $\theta_1, \theta_2, \theta_3, \theta_4$ and the hysteresis half-width constants h_1, h_2, h_3, h_4 associated with each of the thresholds, respectively.

Threshold	Definition
θ_1	TIMP-2 level for MT1-MMP expression
θ_2	MT1-MMP level for MMP-2 expression
θ_3	TIMP-2 level for MT1-MMP/MMP-2 inhibition
θ_4	MMP-2 level for TIMP-2 expression

Table 1: Definition of protein thresholds in the genetic regulatory network for sclera.

Following the definitions in Table 1, the thresholds and hysteresis half-width constants are used to determine when, for current values of the protein concentrations and of the logic variables, changes of the logic variables should occur. For instance, according to the mechanisms described above, if $q_4 = 0$ and x_3 is small, then x_1 should decay according to its decay rate γ_1. However, if $q_4 = 0$ and x_3 becomes large (i.e., the concentration of MMP-2 is large) then q_4 should change to 1 and x_1 should be expressed according to its own growth rate k_1. The continuous evolution of x_3 can be captured mathematically by the differential equation

$$\dot{x}_1 = k_1 q_4 - \gamma_1 x_1$$

while the discrete change of q_4 can be captured by the difference equation

$$q_4^+ = 1 - q_4 \qquad \text{when} \qquad q_4 = 0 \text{ and } x_3 \geq \theta_4 + h_4, \qquad \text{or} \quad q_4 = 1 \text{ and } x_3 \leq \theta_4 - h_4$$

In this way, the *flow map* of the hybrid system defining the continuous dynamics of z is given by

$$F(z) := \begin{bmatrix} k_1 q_4 - \gamma_1 x_1 \\ k_2 q_1 (1 - q_3) - \gamma_2 x_2 \\ k_3 q_2 (1 - q_3) - \gamma_3 x_3 \\ 0_{4\times 1} \end{bmatrix} \tag{2}$$

Changes of the variables occur when z is in the *jump set*, which is conveniently written as

$$D := \bigcup_{i=1}^{4} D_i$$

where

$$\begin{aligned} D_1 &:= \{z : q_1 = 1, x_1 \leq \theta_1 - h_1\} \cup \{z : q_1 = 0, x_1 \geq \theta_1 + h_1\} \\ D_2 &:= \{z : q_2 = 1, x_2 \leq \theta_2 - h_2\} \cup \{z : q_2 = 0, x_2 \geq \theta_2 + h_2\} \\ D_3 &:= \{z : q_3 = 1, x_1 \leq \theta_3 - h_3\} \cup \{z : q_3 = 0, x_1 \geq \theta_3 + h_3\} \\ D_4 &:= \{z : q_4 = 1, x_3 \leq \theta_4 - h_4\} \cup \{z : q_4 = 0, x_3 \geq \theta_4 + h_4\} \end{aligned}$$

The right-hand side of the difference equation discretely updating the logic variables is given by the *jump map*

$$G(z) = \begin{cases} g_1(z) & z \in D_1 \setminus (D_2 \cup D_3 \cup D_4) \\ g_2(z) & z \in D_2 \setminus (D_1 \cup D_3 \cup D_4) \\ g_3(z) & z \in D_3 \setminus (D_1 \cup D_2 \cup D_4) \\ g_4(z) & z \in D_4 \setminus (D_1 \cup D_2 \cup D_3) \\ \hat{g}(z) & z \in D_1 \cap D_2 \cap D_3 \cap D_4 \end{cases} \tag{3}$$

where

$$g_1(z) = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ 1 - q_1 \\ q_2 \\ q_3 \\ q_4 \end{bmatrix}, \quad g_2(z) = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ q_1 \\ 1 - q_2 \\ q_3 \\ q_4 \end{bmatrix}, \quad g_3(z) = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ q_1 \\ q_2 \\ 1 - q_3 \\ q_4 \end{bmatrix}, \quad g_4(z) = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ q_1 \\ q_2 \\ q_3 \\ 1 - q_4 \end{bmatrix}$$

and $\hat{g}(z) = \{g_1(z), g_2(z), g_3(z), g_4(z)\}$. Note that x_1 and its associated logic variables q_1 and q_3 are the only "inputs" to the dynamics of x_2, which suggests that $\theta_1 + h_1 < \theta_3 - h_3$ should hold for x_2 to ever grow. Moreover, x_1 and its associated logic variable q_3 are "inputs" to the dynamics of x_3, while x_3 and q_4 are "inputs" to the dynamics of x_1, in what resembles to a feedback interconnection.

With the definitions mentioned before, a hybrid system $\mathscr{H} = (F, C, G, D)$ in the framework of [4] capturing the mechanism in the genetic network of sclera with hysteresis is given as

$$\mathscr{H}: \quad z \in Z \quad \begin{cases} \dot{z} = F(z) & z \in C := \overline{Z \setminus D} \\ z^+ \in G(z) & z \in D \end{cases} \tag{4}$$

3 Simulation Results

We simulate the hybrid model of the scleral genetic network within a Matlab/Simulink toolbox [20]. Unless otherwise stated, the growth rate k_i and decay rates γ_i, $i = 1, 2, 3$ for the three proteins are identically set to 1 and the hysteresis h_i, $i = 1, 2, 3, 4$ are set to 0.01.

3.1 Isolated Equilibrium Points

Figure 2(a) and Figure 2(b) present simulation results in which the hybrid system evolves to the equilibrium point at $x^* = (0, 0, 0)$. Under these initial conditions and protein thresholds, the concentration of TIMP-2 (x_1) is not sufficiently high to permit continued expression of the MT1-MMP (x_2) and MMP-2 (x_3) genes. The protein concentration associated with the MMP-2 gene continues to grow, but when the MT1-MMP gene is inhibited, MMP-2 will become inhibited with time.

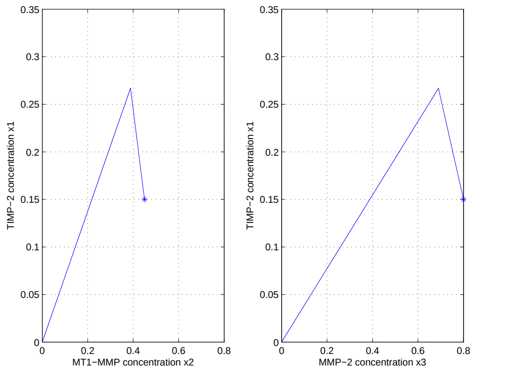
Figure 2(c) and Figure 2(d) show that the solution of the hybrid system goes toward the equilibrium point at $x^* = \left(\frac{k_1}{\gamma_1}, \frac{k_2}{\gamma_2}, \frac{k_3}{\gamma_3}\right)$. With the given initial conditions and parameters, the concentration of TIMP-2 (x_1) is not high enough to inhibit the expression of the MT1-MMP (x_2) and MMP-2 (x_3) genes. This situation can be a cause of high myopia [12, 17].

3.2 Limit cycles

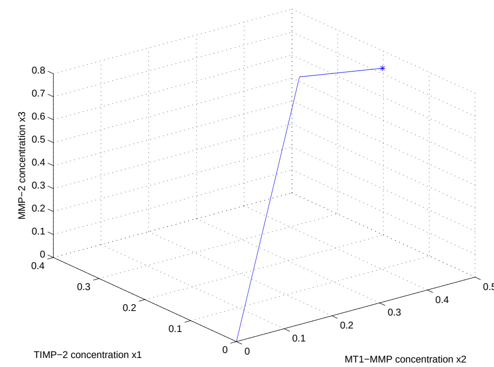
Figure 3(a) and Figure 3(b) illustrates the oscillatory behavior in the hybrid system when the concentration of TIMP-2 exceeds θ_1 and the concentration of MMP-2 exceeds θ_4 recurrently. In this scenario, the discrete state behavior stabilizes to a periodic orbit. It is apparent that the TIMP-2 protein as modeled here has a stabilizing effect on the other two protein concentrations when it is at a sufficiently high level. In this scenario, the sclera develops normally. To illustrate that such normal development of the sclera is only possible when hysteresis is present, the previous simulation is repeated for half-width hysteresis constants equal to zero. Figure 3(c) and Figure 3(d) show the corresponding system response. The solution to the hybrid system converges to an isolated equilibrium point.

4 Conclusion

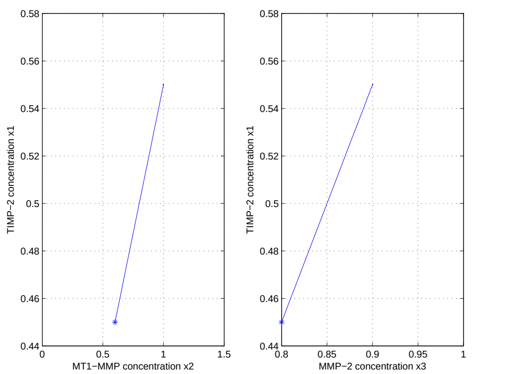
A mathematical model of a regulatory network with hysteresis to describe the mechanisms in the mammalian sclera was introduced. The model captures the interaction between MMP-2, MT1-MMP, and TIMP-2. Numerical results indicate that the system can have both isolated equilibria and limit cycles in the 3-dimensional space of protein concentrations. For the arbitrarily chosen parameters, numerical results seem to suggest that hysteresis is needed for normal development of sclera. Current efforts include characterizing the type of equilibria in terms of the values of the systems constants using the hybrid



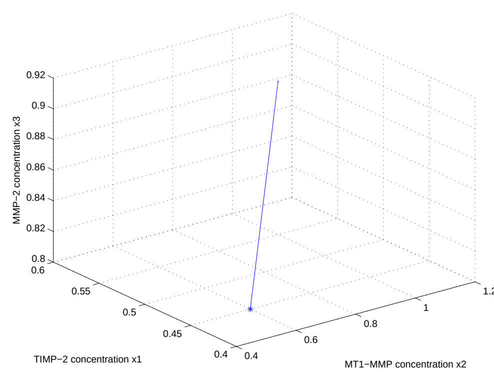
(a) $\theta_1 = 0.4, \theta_2 = 0.5, \theta_3 = 0.6, \theta_4 = 0.7, x_1(0) = 0.15, x_2(0) = 0.45, x_3(0) = 0.8, q_1(0) = 1, q_2(0) = 1, q_3(0) = 0, q_4(0) = 1$, $*$ is the initial point.



(b) $\theta_1 = 0.4, \theta_2 = 0.5, \theta_3 = 0.6, \theta_4 = 0.7, x_1(0) = 0.15, x_2(0) = 0.45, x_3(0) = 0.8, q_1(0) = 1, q_2(0) = 1, q_3(0) = 0, q_4(0) = 1$, $*$ is the initial point.



(c) $k_1 = 0.55, k_3 = 0.9, \theta_1 = 0.4, \theta_2 = 0.5, \theta_3 = 0.6, \theta_4 = 0.7, x_1(0) = 0.45, x_2(0) = 0.6, x_3(0) = 0.8, q_1(0) = 1, q_2(0) = 1, q_3(0) = 0, q_4(0) = 1$, $*$ is the initial point.



(d) $k_1 = 0.55, k_3 = 0.9, \theta_1 = 0.4, \theta_2 = 0.5, \theta_3 = 0.6, \theta_4 = 0.7, x_1(0) = 0.45, x_2(0) = 0.6, x_3(0) = 0.8, q_1(0) = 1, q_2(0) = 1, q_3(0) = 0, q_4(0) = 1$, $*$ is the initial point.

Figure 2: Solutions to $\mathscr{H}$ for different parameters and initial conditions. For the chosen values, solutions converge to isolated equilibrium points.

systems techniques employed in [23] and the design of in-vivo experiments to identify the parameters of the genetic model.

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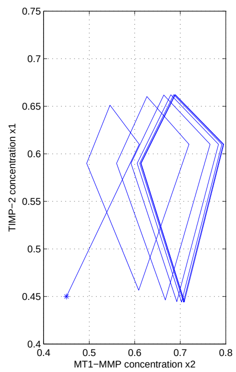
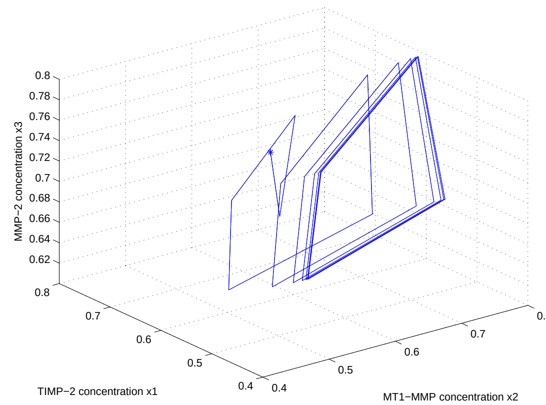
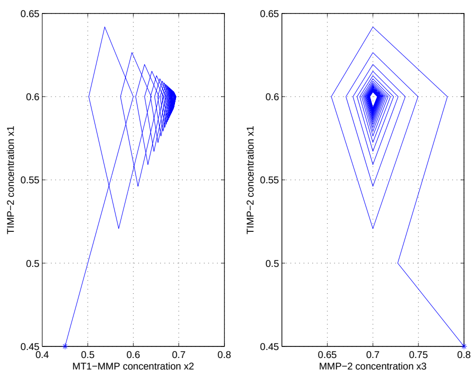


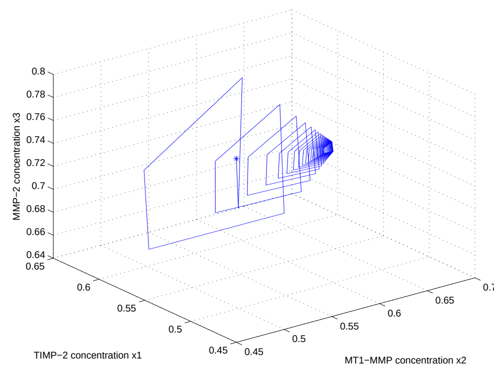
(a) $h_i = 0.01$ for each $i = 1,2,3,4$, $*$ is the initial point.



(b) $h_i = 0.01$ for each $i = 1,2,3,4$, $*$ is the initial point.



(c) $h_i = 0$ for each $i = 1,2,3,4$, $*$ is the initial point.



(d) $h_i = 0$ for each $i = 1,2,3,4$, $*$ is the initial point.

Figure 3: Solutions to $\mathscr{H}$ for $\theta_1 = 0.4, \theta_2 = 0.5, \theta_3 = 0.6, \theta_4 = 0.7, x_1(0) = 0.45, x_2(0) = 0.45, x_3(0) = 0.8, q_1(0) = 1, q_2(0) = 1, q_3(0) = 0, q_4(0) = 1$ with nonzero and zero h_i for each $i = 1,2,3,4$.

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