How retroactivity impacts the robustness of genetic networks

How Retroactivity Impacts the Robustness of Genetic Networks

Shaoshuai Mou Domitilla Del Vecchio

Abstract— **This paper studies how retroactivity impacts the robustness of gene transcription networks against parameter perturbations. By employing the linearization technique and the real stability radius, we provide comparisons of the robustness between gene transcription networks with retroactivity and ones without retroactivity. Both numerical and analytical results show that retroactivity tends to decrease such robustness. This finding in turn implies that modular genetic networks tend to be more robust against parameter perturbations.**

I. Introduction

Regulation of gene expression is a major component of the molecular activities in cells. The pathway from gene to protein includes many steps such as transcription, mRNA degradation, translation, and protein decay, all of which can in principle be regulated [1]. For most genes, the regulation of transcription is of primary importance since only transcriptional regulation can ensure that no unnecessary intermediates are synthesized. Regulation of transcription is usually controlled by the reversible binding of *transcription factors* (TF) to promoter sites of genes, which leads to complex networks of interactions between multiple genes and TFs. A collection of genes and interactions among them through regulation by TFs constitute a *gene transcription network* (GTN). Each node represents a transcriptional component, which takes several TFs as input and outputs a single TF. Each directed edge between two nodes from the *parent* node to the *child* node indicates that the output of the parent node regulates the transcription of the child node.

Similar to electrical networks, transmitting a signal to a downstream system affects the dynamics of the sending component arises in gene transcription networks. This effect is called *retroactivity* [2]. Because of retroactivity, the dynamics of a node in in GTNs changes dramatically when it regulates the transcription of a child node. It has been shown experimentally that such dramatic changes include response time [3], and the steady state input-output characteristics [4]. Thus, much attention has recently been given to obtain ordinary differential equation (ODE) models for genetic networks with retroactivity [5]–[7]. In particular, the authors of [7] have made progress in explicitly quantify the retroactivity for a large class of GTNs.

Robustness as a key system property of genetic networks has been investigated for a long time in both fields of control, and synthetic biology [8]–[14]. By *robustness* in this paper is meant the ability to maintain a certain property, such as stability or response time, in the face of parameter perturbations, which may result from genetic mutations [9], changes of interactions among genes [10], or physical changes in the environment [11]. Robustness enables gene regulatory networks to continue to function despite noisy expression of their constituent genes or even when facing substantial environment variation. Many factors have been found to contribute to robustness of genetic networks, such as integral feedback [12] and network structures [13]. Sufficient conditions in terms of LMIs have been given in [14] to check whether a gene transcription network has a globally asymptotically stable equilibrium by assuming all parameter perturbations are constrained in a polytope. Although the LMI feasibility test in [14] involves solving several optimization problems, the result has provided a sufficient condition to guarantee that a gene transcription network has no multiple equilibria or limit cycles when facing a specific type of parameter perturbations.

The aim of this paper is to study how retroactivity impacts the robustness of gene transcription networks against parameter perturbations. The dynamics of gene transcription networks with and without retroactivity will be modeled by two systems of ODEs. A robustness index called stability radius [15] will be introduced to compare the robustness of these two systems close to their equilibria. The system linearized at its equilibrium with larger real stability radius is said to be more robust in the sense that it maintains the stability of its equilibrium when facing larger parameter perturbations in the 2-norm sense. Both simulations and analytical results will be given for such comparisons, which indicate that retroactivity generally decreases GTNs' robustness against parameter perturbations. On the one hand, this finding suggests that natural systems, being inherently robust, may have evolved ways to attenuate retroactivity and thus enforce modularity [16]–[18]. On the other hand, our finding implies that methods to mitigate or avoid the retroactivity [19], [20] when building complex multi-module circuits may lead to more robust systems. This is another advantage of modularity in addition to already being crucial for bottom-up design approaches [21], [22].

This paper is organized as follows: Section II gives a set of ODEs that model the dynamics of GTNs with retroactivity. In Section III, robustness comparisons between GTNs without retroactivity and GTNs with retroactivity are provided. Simulations on a multi-module system, the event detector, are given in Section IV. We conclude in Section V and all the proofs are provided in Section VI.

This work is supported by the grant NSF-CCF-1058127. S. Mou and D. Del Vecchio are with the Department of Mechanical Engineering, Massachusetts Institute of Technology. Email: smou@mit.edu, ddv@mit.edu

II. Model and Problem Formulation

Consider an n-node gene transcription network as in Fig.1, in which each node represents a gene or transcriptional component, whose inputs are the output transcription factors from other nodes. Each directed edge from node i to j indicated by $i \to j$ in Fig.1 means that the output of node i regulates the transcription of node j. In short, gene transcription networks of interest here are composed of "nodes" representing genes and "directed edges" representing regulatory interactions among genes.

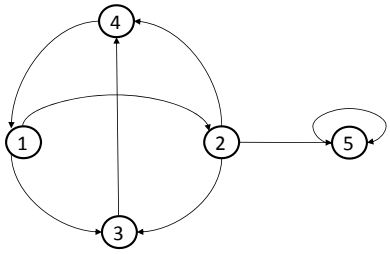

Fig. 1. A five-node gene transcription network

Let x_i denote output protein of node i and let x_i denote the concentration of x_i. For simplicity of notation we suppose each node has at most two parents. Then, the dynamics of a gene transcription network without considering retroactivity can be described by the following ODE :

$$\Sigma_1: \quad \dot{x} = f(x,u) \tag{1}$$

where $x = \begin{bmatrix} x_1 & x_2 & \cdots & x_n \end{bmatrix}' \in \mathbb{R}^n$, $u = \begin{bmatrix} u_1 & u_2 & \cdots & u_n \end{bmatrix}'$ with u_i representing external input to node i, and the ith element of $f(x,u)$ is

$$f_i(x,u) = u_i - \delta_i x_i + H_i(x) \tag{2}$$

with δ_i denoting the protein decay rate of x_i. The $h_i(x)$ in (2) is the Hill function which models the production rate of x_i with respect to its two parents x_p and x_q given by

$$H_i(x) = \eta_i \frac{\pi_i + \pi_{ip}\frac{x_p^{m_{ip}}}{k_{ip}} + \pi_{iq}\frac{x_q^{m_{iq}}}{k_{iq}} + \pi_{ipq}\frac{x_p^{m_{ip}}x_q^{m_{iq}}}{k_{ipq}}}{1 + \frac{x_p^{m_{ip}}}{k_{ip}} + \frac{x_q^{m_{iq}}}{k_{iq}} + \frac{x_p^{m_{ip}}x_q^{m_{iq}}}{k_{ipq}}} \tag{3}$$

where η_i denotes the total concentration of the promoters of i; the binding of a parent to the free promoter of a child node as an m-multimer forms a complex with dissociation constant denoted by k; π denotes the production rate from the corresponding complexes.

The hill function $H_i(x)$ in (3) is related to the number of x_i's parents. If node i has no parent, we let $\frac{1}{k_{ip}} = \frac{1}{k_{iq}} = \frac{1}{k_{ipq}} = 0$ in (3); if node i has a single parent node x_p, we let $\frac{1}{k_{iq}} = \frac{1}{k_{ipq}} = 0$ in (3). When node i has two or more parents, $H_i(x)$ is also affect by the type of their bindings to x_i, which usually include the following three types:

- *Competitive binding:* x_p and x_q bind exclusively to the promoters of their common child;
- *Independent binding:* x_p and x_q do not affect each other in their bindings to a common child. That is, even if a node's promoter is bound with one parent, it is still available to be bound with its other parents.
- *Cooperative binding:* x_p must be bound to its child's promoters before x_q can bind.

In the case of independent binding, $H_i(x)$ is as defined in (3); if node i's two parents' bindings are competitive, we have $\frac{1}{k_{ipq}} = 0$ in (3); if node i's two parents' bindings are cooperative with node x_q bound after x_p, one has $\frac{1}{k_{iq}} = 0$ in (3).

When retroactivity is considered, the dynamics of a gene transcription network modifies to [7]:

$$\Sigma_2: \quad \dot{x} = [I + R(x)]^{-1} f(x,u) \tag{4}$$

where $R(x) \in \mathbb{R}^{n\times n}$ is called the *retroactivity matrix*. Note that $R(x) = 0$ when retroactivity is not considered and (4) becomes (1). When retroactivity is considered, $R(x) \neq 0$ and (4) is significantly different from (1). Please refer to [7] for the derivation of $R(x)$ with respect to different types of bindings. Here, we will use that when all the bindings are independent, $R(x)$ is a diagonal matrix with the ith diagonal entry given by

$$r_{ii} = \sum_{j\in\mathcal{C}_i} \frac{m_{ji}^2 \eta_j \frac{x_i^{m_{ji}-1}}{k_{ji}}}{(1 + \frac{x_i^{m_{ji}}}{k_{ji}})^2} \tag{5}$$

where $\mathcal{C}_i$ denotes the set of node i's children. For other types of bindings, $R(x)$ is not a diagonal matrix. Refer to the Appendix for the expression of $R(x)$ when all bindings are competitive and the number of each node's parents is not limited to two.

Note that the retroactivity matrix $R(x)$ leads to significant changes in the dynamics of a gene transcription network. These changes have been studied on oscillators, toggle switches, and other motifs [7]. The aim of the rest of this paper is to explore how such changes resulting from retroactivity affect the robustness of a gene transcription network against parameter perturbations, namely we will compare the robustness of systems Σ_1 and system Σ_2.

III. Effect of Retroactivity on Robustness

In this section, we will compare the robustness of the system without retroactivity Σ_1 given in (1) to that of the system with retroactivity Σ_2 given in (4) against parameter perturbations. In order to do so, we focus on the behavior of these two systems around a common equilibrium x^*, where x^* is such that $f(x^*,u) = 0$ for a fixed u. Linearization of Σ_1 and Σ_2 about x^* leads to the following two linear systems under abusing the notations for simplicity:

$$\bar{\Sigma}_1: \quad \dot{x} = Ax$$

and

$$\bar{\Sigma}_2: \quad \dot{x} = (I+R)^{-1}Ax$$

where

$$A = \left(\frac{\partial f(x,u)}{\partial x}\right)_{x=x^*}, \quad R = R(x)_{x=x^*}$$

are constant matrices. To mathematically compare the robustness of $\bar{\Sigma}_1$ and $\bar{\Sigma}_2$ close to x^* against parameter perturbations, we introduce the following stability radius [15], [23].

A. Robustness Index: Stability Radius

Let $\Lambda(M)$ denote the spectrum of a square matrix $M \in \mathbb{K}^{n\times n}$, where $\mathbb{K} = \mathbb{C}$ or $\mathbb{R}$. Let $\mathbb{C}^-$ denote the open left-half complex plane and let $\mathbb{C}^+$ denote the closed right-half complex plane. Define the *stability radius* of M as

$$r_{\mathbb{K}}(M) \triangleq \inf\{|\Delta| : \Delta \in \mathbb{K}^{n\times n}, \Lambda(M+\Delta)\cap\mathbb{C}^+ \neq \emptyset\} \quad (6)$$

where $|\cdot|$ denotes the 2-norm. Then $r_{\mathbb{K}}(M)$ is the 2-norm of the smallest perturbation forcing $M+\Delta$ to be unstable. The stability radius defined in (6) is a natural measure of a system's ability to maintain stability of an equilibrium point under perturbations to elements of the system matrix. A system with larger stability radius is able to maintain its stability under larger perturbations to the system's matrix in the 2-norm sense. If $\Lambda(M)\cap\mathbb{C}^+ \neq \emptyset$, one has $r_{\mathbb{K}}(M) = 0$. In the following, we only consider the non-trivial case: $\Lambda(M)\cap\mathbb{C}^+ = \emptyset$, that is, M is a Hurwtiz stable matrix.

By the continuity of eigenvalues of a matrix with respect to its entries, the eigenvalue leaving $\mathbb{C}^-$ towards $\mathbb{C}^+$ must lie on $\partial\mathbb{C}^-$, which is the boundary of $\mathbb{C}^-$. Thus we can write

$$r_{\mathbb{K}}(M) = \inf_{s\in\partial\mathbb{C}^-}\left(\inf_{\Delta\in\mathbb{K}^{n\times n}}\{|\Delta| : \det(sI-M-\Delta)=0\}\right) \quad (7)$$

According to [15], one has the following relationship for the *complex stability radius* for $\Delta \in \mathbb{C}^{n\times n}$:

$$r_{\mathbb{C}}(M) = \inf_{s\in\partial\mathbb{C}^-}|(sI-M)^{-1}|^{-1} = ||M||_{H_\infty}^{-1} \quad (8)$$

where

$$||M||_{H_\infty} = \sup_{\omega\in\mathbb{R}}\sigma_1\left((j\omega - M)^{-1}\right) \quad (9)$$

with $\sigma_1(\cdot)$ the largest singular value of a matrix. This makes the computation of $r_{\mathbb{C}}(M)$ possible. By [23] one has that the real stability radius for $\Delta \in \mathbb{R}^{n\times n}$ is given by

$$r_{\mathbb{R}}(M) = \min_{\omega\in\mathbb{R}}\sup_{\gamma\in(0,1]}\sigma_{2n-1}\begin{bmatrix} M & \gamma\omega I \\ -\gamma^{-1}\omega I & M\end{bmatrix} \quad (10)$$

The real stability radius can be computed from (10), or by algorithms proposed in [24]. However, the computation of $r_{\mathbb{R}}(M)$ involves the minimization of unimodal functions [23], which is a challenging problem [25]. To avoid complex computations, we determine lower and upper bounds of $r_{\mathbb{R}}(M)$ which can be easily computed.

Lemma 1: Suppose $M, \Delta \in \mathbb{R}^{n\times n}$. Then

$$||M||_{H_\infty}^{-1} \leq r_{\mathbb{R}}(M) \leq \sigma_n(M) \quad (11)$$

where $\sigma_n(M)$ denotes the smallest singular value of M.

The bounds obtained in Lemma 1 are tight in the sense that they can be reached under certain conditions as indicated by the following lemma:

Lemma 2: If the H_∞ norm of M is achieved at $\omega = 0$, one has

$$\sigma_n(M)||M||_{H_\infty} = 1. \quad (12)$$

B. Robustness Comparison

For genetic networks in practice, parameter perturbations are usually real. If the real stability radius of $\bar{\Sigma}_1$ is larger than that of $\bar{\Sigma}_2$, we say Σ_1 is *more robust* than Σ_2 at their equilibrium. By definition of real stability radius, this means that for all real parameter perturbations with a certain upper bound in its 2-norm, the system Σ_1 is stable at x^* while Σ_2 in practice may become unstable and converge to a different equilibrium. This will lead the system Σ_2 to exhibit a different phenotype, which may be associated with malfunction.

In this subsection we will compare the robustness of the two linearized systems $\bar{\Sigma}_1$ without retroactivity and $\bar{\Sigma}_2$ with retroactivity under real perturbations to elements of their system matrices A and $(I+R)^{-1}A$, respectively, by comparing their real stability radiuses. Since the real stability radius of an unstable matrix is 0, we only consider the case when A and $(I+R)^{-1}A$ are both Hurwitz stable.

Based on Lemma 1, one can immediately conclude that Σ_1 is more robust than Σ_2 if

$$||A||_{H_\infty}^{-1} > \sigma_n((I+R)^{-1}A) \quad (13)$$

and Σ_2 is more robust than Σ_1 if

$$||(I+R)^{-1}A||_{H_\infty}^{-1} > \sigma_n(A) \quad (14)$$

These two inequalities (13) and (14) give sufficient conditions to determine whether retroactivity increases or decreases the robustness of the gene transcription network against parameter perturbations. To find out which of the conditions (13) and (14) holds in general, we first perform numerical experiments as follows.

Experiment 1: First, we randomly generate a Hurwitz stable matrix A and compute $||A||_{H_\infty}^{-1}$ and $\sigma_n(A)$. Second, we randomly generate an $n\times n$ square matrix R such that $(I+R)^{-1}A$ is also Hurwitz stable and compute $||(I+R)^{-1}A||_{H_\infty}^{-1}$ and $\sigma_n((I+R)^{-1}A)$. Third, we check conditions (13) and (14). We repeat the above three-step computation 10000 times and summarize our findings in Table I.

TABLE I

NUMBER OF EXPERIMENTS FOR WHICH (13) AND (14) HOLDS

n	(13) holds	(14) holds	No answer
1	10000	0	0
2	9603	4	393
3	9622	2	376
4	9594	0	406
5	9593	0	407
6	9590	0	410
7	9610	0	390
8	9585	0	415
9	9614	0	386
10	9569	0	431

Table I has the following implications:

- (13) holds, which means Σ_1 is more robust than Σ_2, in more than 95.5% of 10000 repeated computations.

This implies that retroactivity usually decreases a gene transcription network's robustness against parameter perturbations;

- Several extreme examples (less than 0.04%) have been found such that (14) holds, which means Σ_2 is more robust than Σ_1, and thus retroactivity increases the robustness against parameter perturbations. One artificial instance of this case will be illustrated in Example 1;
- There are a few cases (less than 4.5%) in which neither (13) nor (14) holds, from which we can not draw any conclusion about the robustness of Σ_1 and Σ_2. This is because the conditions given in (13) or (14) are only sufficient.

Example 1: Here we give an artificial example where retroactivity increases the robustness of a gene transcription network against parameter perturbations. Consider a three-node network in Fig. 2 in which node 1 regulates node 2 and 3, node 2 regulates 1 and 3.

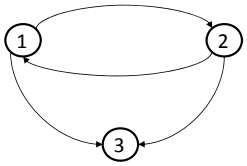

Fig. 2. A three-node gene transcription network

Suppose x_1 and x_2 bind competitively to x_3. Without losing any generality, we assume all $m_{ij} = 1$ and all $k_{ij} = 1$. The dynamics of node 1 and 2 for this example are

$$\begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \end{bmatrix} = [I + R(x_1, x_2)]^{-1} \begin{bmatrix} f_1(x_1, x_2, u_1) \\ f_2(x_1, x_2, u_2) \end{bmatrix} \quad (15)$$

where

$$f_1(x_1, x_2, u_1) = u_1 - \delta_1 x_1 + \eta_1 \frac{\pi_1 + \pi_{12} x_2}{1 + x_2}$$
$$f_2(x_1, x_2, u_2) = u_2 - \delta_1 x_2 + \eta_2 \frac{\pi_2 + \pi_{21} x_1}{1 + x_1}$$

with

$$R(x_1, x_2) = \begin{bmatrix} \frac{\eta_2}{(1+x_1)^2} + \frac{\eta_3(1+x_2)}{(1+x_1+x_2)^2} & -\frac{\eta_2 x_1}{(1+x_1+x_2)^2} \\ -\frac{\eta_1 x_2}{(1+x_1+x_2)^2} & \frac{\eta_1}{(1+x_2)^2} + \frac{\eta_3(1+x_1)}{(1+x_1+x_2)^2} \end{bmatrix}$$

Let $u_1 = -2.6631, u_2 = -6.1849$, $\eta_1 = 36.9, \eta_2 = 9.356, \eta_3 = 0.186$, $\pi_1 = 0.0014, \pi_{12} = 0.14, \pi_2 = 0.0009, \pi_{21} = 1.181$, $\delta_1 = 0.6994, \delta_2 = 0.3110$. One has the system matrix A and the retroactivity matrix R of the system (15) at $x_1 = 2.4, x_2 = 5.2$ are:

$$A = \begin{bmatrix} -0.6994 & 0.1330 \\ 0.9511 & -0.3110 \end{bmatrix}, \quad R = \begin{bmatrix} 0.8168 & -0.3036 \\ -2.5974 & 0.9685 \end{bmatrix}$$

which leads to

$$(I + R)^{-1} = \begin{bmatrix} 0.7061 & 0.1089 \\ 0.9317 & 0.6517 \end{bmatrix}$$

Then

$$||(I + R)^{-1} A||_{H_\infty}^{-1} = 0.0826, \quad \sigma_n(A) = 0.0743$$

It follows that the stability radius of $\bar{\Sigma}_2$ is larger than that of $\bar{\Sigma}_1$, which in turn implies that retroactivity increases the robustness. Note however that this example is an artificial example since negative values of u_1 and u_2 are not realizable in practice. Actually we have not found a set of physically realizable parameters such that Σ_2 is more robust than Σ_1 even for this simple three-node network.

When the bindings are competitive or cooperative, the retroactivity matrix R has no special structure, for which one can only check conditions (13) and (14) numerically in order to conclude whether retroactivity increases or decreases the robustness against parameter perturbations. Computations in Table I show that retroactivity generally decreases robustness for these cases. When it comes to independent bindings, the retroactivity matrix R is diagonal, which allows us to obtain further analytical results as follows.

C. Independent Bindings: R is diagonal

Suppose all the bindings are independent. Then R is a diagonal matrix with non-negative diagonal entries given in [7]. Let $\underline{r}$ and $\overline{r}$ denote the smallest and largest diagonal entry of R. Then

$$\sigma_n(I + R) = 1 + \underline{r}$$

We further suppose that each node has at least one children. Then $\underline{r} > 0$ and thus

$$\sigma_n(I + R) > 1$$

from which and (24) one has

$$||(I+R)^{-1} A||_{H_\infty}^{-1} \leq \sigma_n((I+R)^{-1} A) \leq \frac{\sigma_n(A)}{\sigma_n(I + R)} < \sigma_n(A)$$

which implies that the condition (14) can not be satisfied in the case of independent bindings. Numerical computations suggest that (13) holds in general, the proof of which is quite challenging. We first look at two extreme cases:

Case 1: Retroactivities corresponding to all TF/promoter bindings in a gene transcription network are *balanced* in the sense that $\frac{\underline{r}}{\overline{r}} \approx 1$. Let

$$\bar{R} = (I + R)^{-1} - \frac{1}{1 + \overline{r}} I$$

By (7), one has

$$\begin{aligned} & r_{\mathbb{R}}\left((I + R)^{-1} A\right) \quad (16) \\ = & \inf_{s \in \partial \mathbb{C}^-} \left(\inf_{\Delta \in \mathbb{R}^{n \times n}} \{ |\Delta| : \det(sI - \frac{1}{1 + \overline{r}} A - \Delta - \bar{R} A) = 0 \} \right) \\ = & \inf_{\bar{s} \in \partial \mathbb{C}^-} \left(\inf_{\bar{\Delta} \in \mathbb{R}^{n \times n}} \{ |\frac{\Delta}{1 + \overline{r}} - \bar{R} A| : \det\left(\bar{s} I - A - \bar{\Delta}\right)) = 0 \} \right) \\ \leq & \inf_{\bar{s} \in \partial \mathbb{C}^-} \left(\inf_{\bar{\Delta} \in \mathbb{R}^{n \times n}} \{ \frac{|\Delta|}{1 + \overline{r}} + |\bar{R}||A| : \det\left(\bar{s} I - A - \bar{\Delta}\right)) = 0 \} \right) \\ = & \frac{r_{\mathbb{R}}(A)}{1 + \overline{r}} + (\frac{1}{1 + \underline{r}} - \frac{1}{1 + \overline{r}}) \sigma_1(A) \quad (17) \end{aligned}$$

Since $\alpha(R) \approx 1$, then

$$1 - \frac{\underline{r}}{\overline{r}} < \frac{||A||_{H_\infty}^{-1}}{\sigma_1(A)}$$

It follows that

$$(\frac{1}{1+\underline{r}}-\frac{1}{1+\overline{r}})\sigma_1(A)<(1-\frac{1}{1+\overline{r}})||A||_{H_\infty}^{-1}$$

from which, $||A||_{H_\infty}^{-1}\leq r_{\mathbb{R}}(A)$ and (17), one has

$$r_{\mathbb{R}}\left((I+R)^{-1}A\right)<r_{\mathbb{R}}(A).$$

Then Σ_1 is more robust than Σ_2.

Case 2: There exists one TF/promoter binding which leads to extremely large retroactivity, or in other words, $\overline{r}\to\infty$. Note that

$$\lim_{\overline{r}\to\infty}\sigma_n\left((I+R)^{-1}A\right)=0$$

By the continuity of eigenvalues of a matrix with respect to its entries, there must exist a finite real number μ such that for all $\overline{r}\in[\mu,\infty)$, $\sigma_n\left((I+R)^{-1}A\right)<||A||_{H_\infty}^{-1}$. Then Σ_1 is more robust than Σ_2.

To include more cases in which Σ_1 is more robust than Σ_2, we introduce the following lemma.

Lemma 3: If

$$\sigma_n(I+R)>\sigma_n(A)||A||_{H_\infty} \tag{18}$$

one has that Σ_1 is more robust than Σ_2.

The proof of Lemma 3 is given in the Appendix. It is worth mentioning that the condition in Lemma 3 separates the retroactivity matrix R and the system matrix A. This enables us to conclude the followings:

Case 3: The retroactivity corresponding to each TF/promoter binding in a gene transcription network is sufficiently large. Since A is Hurwtitz stable, one has $||A||_{H_\infty}$ and $\sigma_n(A)$ are bounded. Then when $\underline{r}$ is large enough, one has (18).

Case 4: The H_∞ norm of the matrix A is achieved at $\omega=0$. By Lemma 2 on has $||A||_{H_\infty}\sigma_n(A)=1$. Note that $\sigma_n(I+R)=1+\underline{r}>1$. Then (18) holds, which implies Σ_1 is more robust than Σ_2. In practice this case suggests that Σ_1 has a "low-pass filter" behavior. Because of its benefit to ignore rapid variations and only respond to longer-lasting changes, this low-pass filtering capacity is a common feature of regulation of transcription, as suggested in *E. coli* theoretically [26], verified experimentally [27] and recently observed in eukaryotes [28]. This suggests that in practice Σ_1 is more robust than Σ_2.

As a summary of the above findings, we have the following theorem

Theorem 1: In the case of independent bindings, let $\overline{r}$ and $\underline{r}$ denote the largest and the smallest diagonal entry of R, respectively. Σ_1 is more robust than Σ_2 if any of the followings holds:

- retroactivities corresponding to all TF/promoter bindings in a gene transcription network are *balanced* in the sense that $\frac{\underline{r}}{\overline{r}}\approx 1$;
- there exists one TF/promoter binding which leads to extremely large retroactivity in the sense that $\overline{r}\approx\infty$;
- the retroactivity corresponding to each TF/promoter binding in a gene transcription network is sufficiently large in the sense that $\underline{r}$ is large;
- the H_∞ norm of the matrix A is achieved at $\omega=0$.

IV. Numerical Experiments on An Event Detector

In this section, we perform simulations on an event detector circuit and illustrate how retroactivity affects the robustness of such a multi-module system against parameter perturbations. The event detector consisting of six nodes is shown in Fig. 3, in which $i\to j$ and $i\dashv j$ represent that i is an activator and a repressor of j, respectively. The mechanism for the event detector to work is as follows: In the presence of a low input u, the cascade consisting of nodes $1,2,3$ propagates the signal to remove repression on the inverter 4, eventually resulting in a switch in the state of the toggle module, which leads to that the output of node 7 is changed and maintained to be low.

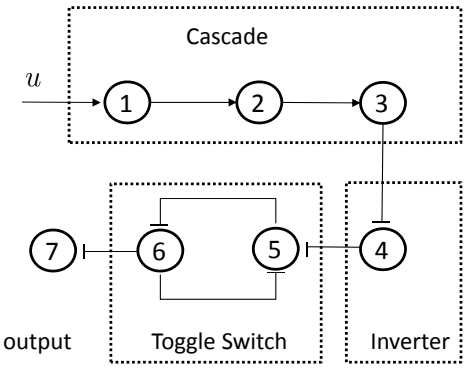

Fig. 3. An Event Detector

The dynamics of the event detector without considering retroactivity is

$$\Sigma_1:\quad \dot{x}=f(x,u) \tag{19}$$

where the ith element of $f(x,u)$ are:

$$\begin{aligned}
f_1(x,u) &= -\delta_1 x_1+u\\
f_2(x) &= -\delta_2 x_2+\eta_2\pi_{21}\frac{\frac{x_1}{k_{21}}}{1+\frac{x_1}{k_{21}}}\\
f_3(x) &= -\delta_3 x_3+\eta_3\pi_{32}\frac{\frac{x_2}{k_{32}}}{1+\frac{x_2}{k_{32}}}\\
f_4(x) &= -\delta_4 x_4+\eta_4\pi_4\frac{1}{1+\frac{x_3}{k_{43}}}\\
f_5(x) &= -\delta_5 x_5+\eta_5\pi_5\frac{1}{1+\frac{x_4}{k_{54}}+\frac{x_6^2}{k_{56}}}\\
f_6(x) &= -\delta_6 x_6+\eta_6\pi_6\frac{1}{1+\frac{x_5^2}{k_{65}}}\\
f_7(x) &= -\delta_7 x_7+\eta_7\pi_7\frac{1}{1+\frac{x_6^2}{k_{76}}}
\end{aligned}$$

Suppose 4 and 6 bind to 5 competitively. By considering retroactivity, one has

$$\Sigma_2: \quad \dot{x} = [I + R(x)]^{-1} f(x,u) \tag{20}$$

where $R(x) = [r_{ij}]_{7\times 7}$ only have the following non-zero entries:

$$r_{11} = \frac{\frac{\eta_2}{k_{21}}}{(1+\frac{x_1}{k_{21}})^2}, \quad r_{22} = \frac{\frac{\eta_3}{k_{32}}}{(1+\frac{x_2}{k_{32}})^2}, \quad r_{33} = \frac{\frac{\eta_4}{k_{43}}}{(1+\frac{x_3}{k_{43}})^2}$$

$$r_{44} = \frac{\frac{\eta_5}{k_{54}}(1+\frac{x_6^2}{k_{56}})}{(1+\frac{x_4}{k_{54}}+\frac{x_6^2}{k_{56}})^2}, \qquad r_{46} = -\frac{\frac{2\eta_5 x_4}{k_{54}}\frac{x_6}{k_{56}}(1+\frac{x_6^2}{k_{56}})}{(1+\frac{x_4}{k_{54}}+\frac{x_6^2}{k_{56}})^2}$$

and

$$r_{55} = \frac{\frac{4\eta_6 x_5}{k_{65}}}{(1+\frac{x_5^2}{k_{65}})^2}, \; r_{66} = \frac{\frac{4\eta_5 x_6}{k_{56}}(1+\frac{x_4^2}{k_{54}})}{(1+\frac{x_4}{k_{54}}+\frac{x_6^2}{k_{56}})^2} + \frac{\frac{4\eta_7 x_6}{k_{76}}}{(1+\frac{x_6^2}{k_{76}})^2}$$

Please refer to [7] and Lemma 4 in the Appendix for the derivation of the above equations (19) and (20).

Let x^* be such that $f(x^*,u) = 0$ with a fixed $u = 1$. Without losing generality, we suppose $\eta_i = 1$. By linearization of the system Σ_1 in (19) and the system Σ_2 in (20) about x^*, one has two linearized systems $\bar{\Sigma}_1$ and $\bar{\Sigma}_2$ with system matrices A and $(I+R)^{-1}A$, respectively, where

$$A = \left(\frac{\partial f(x,u)}{\partial x}\right)_{x=x^*}, \quad R = R(x)|_{x=x^*}.$$

To compare the robustness of Σ_1 and Σ_2, we perform the following numerical experiments. First we randomly choose each δ_i from the interval $[0.01, 0.1]$ hr^{-1}, each k_{ij} from the interval $[1, 10]$ nM or nM^2 and each π_{ij}, π_i from the interval $[1, 10]$hr^{-1}. All these chosen intervals are realizable in practical gene transcription networks [7]. Then one has two constant matrices A and $(I+R)^{-1}A$. Second, we check the conditions (13) and (14). Third, we repeat the above steps for 10000 times. Results are summarized in Table II.

TABLE II

NUMBER OF EXPERIMENTS FOR WHICH (13) AND (14) HOLDS

(13) holds	(14) holds	No answer	Total Experiments
8439	0	1561	10000

Table II suggests that the system Σ_1 without retroactivity is more robust than the system Σ_2 with retroactivity against parameter perturbations for more than 85% of the total 10000 cases. By considering the definition of stability radius, there exists certain parameter perturbations under which Σ_1 is stable at x^* while Σ_2 becomes unstable at x^* and may converge to a different equilibrium. Under these perturbations the output of toggle switch may not switch, which ultimately leads to no change of the output of node 7 from high to low and the event detector fails to work correctly.

V. CONCLUSIONS

In this paper we have employed the stability radius to determine the effect of retroactivity on networks' robustness. It has been shown both analytically and numerically that retroactivity usually decreases the robustness of gene transcription networks against parameter perturbations in the sense that the stability radius of the linearized system with retroactivity is smaller than that of the system without retroactivity. This, in turn, implies that modularity may lead to more robust biological systems in addition to its known evolutionary advantages.

VI. APPENDIX

Proof of Lemma 1: By the definition of the stability radius in (6), one immediately has $r_{\mathbb{C}}(M) \le r_{\mathbb{R}}(M)$, which together with (8) implies the following lower bound

$$r_{\mathbb{R}}(M) \ge ||M||_{H_\infty}^{-1} \tag{21}$$

On the other hand, (7) implies

$$\begin{aligned} r_{\mathbb{R}}(M) &\le \inf_{s=0}\left(\inf_{\Delta\in\mathbb{R}^{n\times n}}\{|\Delta| : \det(sI - M - \Delta) = 0\}\right) \\ &= \inf_{\Delta\in\mathbb{R}^{n\times n}}\{|\Delta| : \det(M+\Delta) = 0\} \\ &\le \inf_{\Delta\in\mathbb{R}^{n\times n}, \det\Delta = 0}\{|\Delta| : \det(M+\Delta) = 0\} \end{aligned}$$

which is equal to $\sigma_n(M)$ by the Schmidt-Mirsky Theorem [29]. Then one has the following upper bound

$$r_{\mathbb{R}}(M) \le \sigma_n(M) \tag{22}$$

We complete the proof. ■

Proof of Lemma 2: Since the H_∞ norm of M is achieved at ω,

$$||M||_{H_\infty} = \sigma_1\left((j\omega - M)^{-1}\right) = \sigma_n^{-1}(j\omega - M).$$

Then

$$||M||_{H_\infty}\sigma_n(M) = \sigma_n(M)\sigma_n^{-1}(j\omega - M)$$

which is equal to 1 at $\omega = 0$. We complete the proof. ■

Proof of Lemma 3: Let $\bar{q} = \frac{(I+R')q}{|(I+R')q|}$, where q is the unit vector such that $q'AA'q = \lambda_{\min}(AA')$. Then

$$\begin{aligned} &\sigma_n\left((I+R)^{-1}A\right) \qquad (23) \\ = &\min_{v\in\mathbb{R}^n, |v|=1}\sqrt{v'(I+R)^{-1}AA'(I+R')^{-1}v} \\ \le &\sqrt{\bar{q}'(I+R)^{-1}AA'(I+R')^{-1}\bar{q}} \\ = &\sqrt{\frac{qAA'q}{q'(I+R)(I+R')q}} \\ = &\sqrt{\frac{\lambda_{\min}(AA')}{q'(I+R)(I+R')q}} \\ \le &\frac{\sigma_n(A)}{\sigma_n(I+R)} \qquad (24) \end{aligned}$$

which and (18) imply

$$\sigma_n\left((I+R)^{-1}A\right) < ||A||_{H_\infty}^{-1}$$

Then by Lemma 1 one has $r_{\mathbb{R}}((I+R)^{-1}A) < r_{\mathbb{R}}(A)$. We complete the proof. ■

In the following, we will generalize the equations (1) and (4) in the case of competitive bindings by removing the assumption that each node has at most two parents.

Lemma 4: Suppose all the bindings are competitive. Let $\mathcal{N}_i$ and $\mathcal{C}_i$ denote the set of node i's parents and children, respectively. The system without considering retroactivity is

$$\Sigma_1: \quad \dot{x} = f(x) \tag{25}$$

where the ith element of $f(x)$ is

$$f_i(x) = u_i - \delta_i x_i + h_i(x) \tag{26}$$

with

$$h_i(x) = \eta_i \frac{\pi_i + \sum_{j\in\mathcal{N}_i} \pi_{ij} \frac{x_j^{m_{ij}}}{k_{ij}}}{1 + \sum_{j\in\mathcal{N}_i} \frac{x_j^{m_{ij}}}{k_{ij}}}.$$

By considering the retroactivity one has:

$$\Sigma_2: \quad \dot{x} = [I + R(x)]^{-1} f(x) \tag{27}$$

where $R(x) = [r_{ij}]_{n\times n}$ with

$$r_{ij} = \begin{cases} \sum_{q\in\mathcal{C}_i} \frac{\frac{\eta_q m_{qi}^2 \frac{x_i^{m_{qi}-1}}{k_{qi}} \left(1+\sum_{l\in\mathcal{N}_q/\{i\}} \frac{x_l^{m_{ql}}}{k_{ql}}\right)}{\left(1+\sum_{l\in\mathcal{N}_q} \frac{x_l^{m_{ql}}}{k_{ql}}\right)^2}, & i = j; \\ 0, & i \neq j \text{ and } \mathcal{C}_i \cap \mathcal{C}_j = \emptyset; \\ -\sum_{q\in\mathcal{C}_i\cap\mathcal{C}_j} \frac{\eta_q m_{qi} \frac{x_i^{m_{qi}}}{k_{qi}} m_{qj} \frac{x_j^{m_{qj}-1}}{k_{qj}}}{\left(1+\sum_{l\in\mathcal{N}_q} \frac{x_l^{m_{ql}}}{k_{ql}}\right)^2}, & \text{otherwise.} \end{cases} \tag{28}$$

Proof of Lemma 4: We assume that reactions in gene transcription networks can be divided into two time-separable steps: the reversible binding reactions, and protein production/decay process. The reactions in a gene transcription network include the followings

- Competitive binding reactions:

$$m_{ij}\mathrm{x}_j + \mathrm{c}_i \underset{k_{ij}^-}{\overset{k_{ij}^+}{\rightleftharpoons}} \mathrm{c}_{ij}, \quad j \in \mathcal{N}_i, \quad i \in \mathbf{n}$$

- Protein production/decay reactions:

$$\mathrm{c}_i \xrightarrow{\pi_i} \mathrm{x}_i, \quad \mathrm{c}_{ij} \xrightarrow{\pi_{ij}} \mathrm{x}_i, \quad j \in \mathcal{N}_i, \quad i \in \mathbf{n}$$

and

$$\mathrm{x}_i \underset{\zeta_i}{\overset{\delta_i}{\rightleftharpoons}} \emptyset, \quad i \in \mathbf{n}$$

where $\mathbf{n} = \{1, 2, ..., n\}$.

The ODEs for these reactions are

$$\dot{c}_{ij} = k_{ij}^+ x_j^{m_{ij}} c_i - k_{ij}^- c_{ij}, \quad j \in \mathcal{N}_i \tag{29}$$

and

$$\dot{x}_i = u_i - \delta_i x_i + \pi_i c_i + \sum_{j\in\mathcal{N}_i} \pi_{ij} c_{ij} - R_i(x) \tag{30}$$

where

$$R_i(x) = \sum_{q\in\mathcal{C}_i} m_{qi}(k_{qi}^+ x_i^{m_{qi}} c_q - k_{qi}^- c_{qi}). \tag{31}$$

Note that $R_i(x)$ results from that x_i is bind to its children and regulates their transcriptions. When retroactivity is not considered, $R_i(x) = 0$.

Note that the equation in (30) contains both protein production/decay reactions, which are very slow, and reversible bindings, which are very fast. To extract a pure slow dynamics, we let

$$w_i = x_i + s_i, \quad i \in \mathbf{n} \tag{32}$$

where

$$s_i = \sum_{q\in\mathcal{C}_i} m_{qi} c_{qi} \tag{33}$$

By (29), one has

$$\dot{s}_i = R_i(x)$$

It follows that

$$\dot{w}_i = u_i - \delta_i x_i + \pi_i c_i + \sum_{j\in\mathcal{N}_i} \pi_{ij} c_{ij} \quad i \in \mathbf{n} \tag{34}$$

which is much slower than the reversible binding process (29). By applying singular perturbation theory [30] to the system consisting of equations (29) and (34), one could set the left hand side of (29) to be 0, which leads to

$$c_{ij} = \frac{x_j^{m_{ij}}}{k_{ij}} c_i, \quad j \in \mathcal{N}_i, \quad i \in \mathbf{n} \tag{35}$$

Since the total concentration of the promoters of i is a constant η_i, then

$$\sum_{j\in\mathcal{N}_i} c_{ij} + c_i = \eta_i$$

which and (35) imply

$$c_i = \frac{\eta_i}{1 + \sum_{j\in\mathcal{N}_i} \frac{x_j^{m_{ij}}}{k_{ij}}}, \quad i \in \mathbf{n} \tag{36}$$

From (34), (35) and (36), one has

$$\dot{w}_i = f_i(x), \quad i \in \mathbf{n} \tag{37}$$

where f_i is as in (26). When retroactivity is not considered, one has $s_i(x) = 0$ and $R_i(x) = 0$ and then $w_i = x_i$. Thus when retroactivity is not considered, one has $\dot{x} = f(x)$ with the ith element equal to f_i in (26).

When retroactivity is considered, one needs to find the relation between $\dot{w}_i$ and $\dot{x}_i$ in order to get the expression of $\dot{x}$. Note that the total concentration of the promoters of q is a constant η_q, one has for each $q \in \mathcal{C}_i, \quad i \in \mathbf{n}$,

$$\sum_{l\in\mathcal{N}_q} c_{ql} + c_q = \eta_j$$

from which and (35), one has

$$c_q = \frac{\eta_q}{1 + \sum_{l \in \mathcal{N}_q} \frac{x_l^{m_{ql}}}{k_{ql}}}$$

Then

$$c_{qi} = \frac{\eta_q \frac{x_i^{m_{qi}}}{k_{qi}}}{1 + \sum_{l \in \mathcal{N}_q} \frac{x_l^{m_{ql}}}{k_{ql}}}, \quad q \in \mathcal{C}_i, \quad i \in \mathbf{n} \tag{38}$$

by which and (33) one has

$$s_i(x) = \sum_{q \in \mathcal{C}_i} \frac{\eta_q m_{qi} \frac{x_i^{m_{qi}}}{k_{qi}}}{1 + \sum_{l \in \mathcal{N}_q} \frac{x_l^{m_{ql}}}{k_{ql}}} \tag{39}$$

Let $w = \begin{bmatrix} w_1 & w_2 & \cdots & w_n \end{bmatrix}'$. From $w_i = x_i + s_i(x)$ and (39), one has

$$\dot{w} = (I + R)\dot{x}$$

where $R = [r_{ij}]_{n \times n}$ is such that

$$r_{ij} = \frac{\partial s_i(x)}{\partial x_j}.$$

Then one has r_{ij} is as defined in (28). We complete the proof. ■

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