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Homeostasis in a feed forward loop gene regulatory motif

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ABSTRACT

The internal state of a cell is affected by inputs from the extra-cellular environment such as external temperature. If some output, such as the concentration of a target protein, remains approximately constant as inputs vary, the system exhibits homeostasis. Special sub-networks called motifs are unusually common in gene regulatory networks (GRNs), suggesting that they may have a significant biological function. Potentially, one such function is homeostasis.

In support of this hypothesis, we show that the feed-forward loop GRN produces homeostasis. Here the inputs are subsumed into a single parameter that affects only the first node in the motif, and the output is the concentration of a target protein. The analysis uses the notion of infinitesimal homeostasis, which occurs when the input-output map has a critical point (zero derivative). In model equations such points can be located using implicit differentiation. If the second derivative of the input-output map also vanishes, the critical point is a *chair*: the output rises roughly linearly, then flattens out (the homeostasis region or *plateau*), and then starts to rise again. Chair points are a common cause of homeostasis. In more complicated equations or networks, numerical exploration would have to augment analysis. Thus, in terms of finding chairs, this paper presents a proof of concept.

We apply this method to a standard family of differential equations modeling the feed-forward loop GRN, and deduce that chair points occur. This function determines the production of a particular mRNA and the resulting chair points are found analytically. The same method can potentially be used to find homeostasis regions in other GRNs. In the discussion and conclusion section, we also discuss why homeostasis in the motif may persist even when the rest of the network is taken into account.

1. Introduction

Homeostasis occurs in a biological or chemical system when some output variable remains approximately constant as an input parameter varies over some range. The notion of homeostasis is often associated with regulating global physiological parameters like temperature, hormone levels, or concentrations of molecules in the bloodstream in complex multicellular organisms. However, it also can be applied to unicellular organisms, where the issue is how some internal cell state of interest (the copy number of an mRNA transcript or a protein expression level, for example) responds to changes in the intra-cellular or extra-cellular environment (such as changes in the expression level of an upstream transcription factor or environmental factors, such as temperature).

1.1. Infinitesimal homeostasis

The biological notion of homeostasis can be defined in the context of systems of differential equations as follows. Assume that the system depends on an input parameter $I \in \mathbb{R}$

$$\dot{X} = F(X, I), \tag{1.1}$$

where $X \in \mathbb{R}^n$ represents internal variables such as chemical concentrations. Assume also that (1.1) has a stable equilibrium at $X = X_0$ when $I = I_0$; thus,

$$F(X_0, I_0) = 0.$$

The implicit function theorem coupled with stability implies that there is a stable equilibrium $X(I)$ of (1.1) near X_0 for each I near I_0; that is,

$$F(X(I), I) \equiv 0.$$

Homeostasis means that a certain quantity Z that depends on the family of equilibria $X(I) = (x_1(I), \ldots, x_n(I))$ is approximately constant as I varies. Often $Z(I) = x_j(I)$ for some coordinate j. We call

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$Z(I)$ the *input-output map*. In general, it is not easy to find regions of homeostasis.

Recently, Golubitsky and Stewart (2017) introduced the notion of *infinitesimal homeostasis* to enable the use of implicit differentiation to find regions of homeostasis. Specifically, the idea is to search for parameter values I_0 where

$$Z_I(I_0) = 0, \tag{1.2}$$

where the subscript I indicates partial differentiation with respect to I.

We make three remarks about (1.2). First, although this a local condition, in many biological examples it is likely to lead to approximate constancy on a wide range of I values. Second, it is usually easier to use implicit differentiation to find points of infinitesimal homeostasis than it is to find intervals over which $Z(I)$ is approximately constant. Finally, the existence of infinitesimal homeostasis requires that the model equations are nonlinear, which is always the case in biological models. These issues are discussed in detail in Reed et al. (2017) for a number of biochemical network motifs. Similiar ideas (using a slightly different terminology) have been discussed in Tang and McMillen (2016).

Nijhout et al. (2014) observe that homeostasis often appears in three parts: first the output increases roughly linearly as a function of the input, then it remains approximately constant, and then it increases again. They call this type of homeostasis a *chair*, and the first and third parts *escape from homeostasis*. An *infinitesimal chair point* (Golubitsky and Stewart, 2017) is a point I_0 where $Z_I(I_0) = Z_{II}(I_0) = 0$ and $Z_{III}(I_0) \neq 0$. Golubitsky and Stewart (2018a) also give a mathematical justification for why infinitesimal chairs are important when considering homeostasis. Specifically, chair points are the simplest singularities that occur in a system that evolves towards homeostasis. Mathematically, chair points are *codimension 1* singularities. That is, they can occur robustly when, as one parameter is varied, the system goes from a region of non-homeostasis to a region of homeostasis.

1.2. Gene regulatory networks

In this paper we use singularity theory to find infinitesimal chairs in differential equation models for a specific type of *gene regulatory network* (GRN), the *feed-forward loop* motif (FFL). Our results both suggest an explanation for the ubiquity of feed-forward loop motifs among those GRN that are expected to display homeostasis and provide a proof of concept for finding homeostasis in other network motifs. Fig. 1 gives an example of an FFL motif in yeast.

Each node in a GRN represents two related variables: the concentration of mRNA, and the concentration of the associated protein. In order to account for the internal structure, we follow (Zak et al., 2005) and adopt a more refined way of drawing the network diagram (see Fig. 2) that makes the two-variable structure of the nodes explicit:

(1) Each node corresponds to a scalar variable, which can be an mRNA concentration indicated by superscript R and represented by a circle or a protein concentration indicated by superscript P and represented by a square.
(2) A protein node (square) receives an input from exactly one mRNA node (circle), and the effect of this coupling is always positive and represented by a solid arrow (mRNA-protein coupling). The equation of an mRNA node depends only on the state variable for that node and the state variable of the tail cell of the solid input arrows to that node.
(3) An mRNA node (circle) receives inputs only from protein nodes (square). It can receive as many inputs as necessary, represented by dashed arrows (gene-gene coupling). The effect of each dashed arrow into an mRNA node depends only on the equation of that node. It can be repression (binding affinity decreases when concentration increases) or activation (binding affinity increases when concentration increases), depending on the form of the input function at that node.

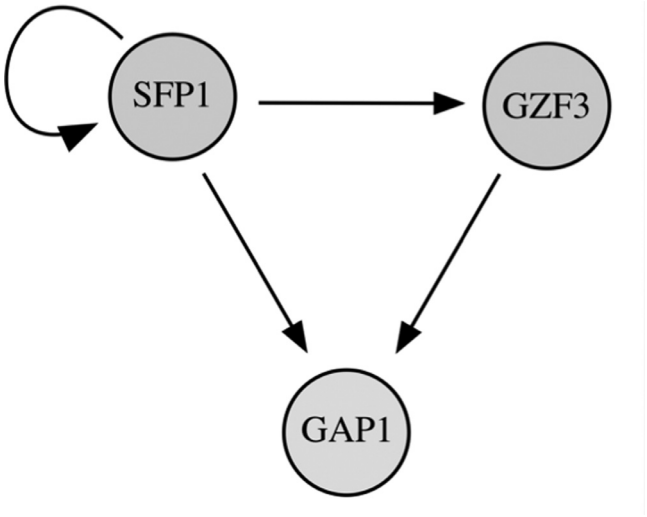

Fig. 1. A feed-forward loop motif of yeast *Saccharomyces cerevisiae* involving genes SFP1, GZF3 and GAP1. SFP1 is a self-regulated motif embedded in the larger GRN motif. Arrows indicate coupling between two genes, but the information about the type of coupling (activation or repression) is not available (Cipollina et al., 2008; Hu et al., 2007). Adapted from GDB (Cherry et al., 2012).

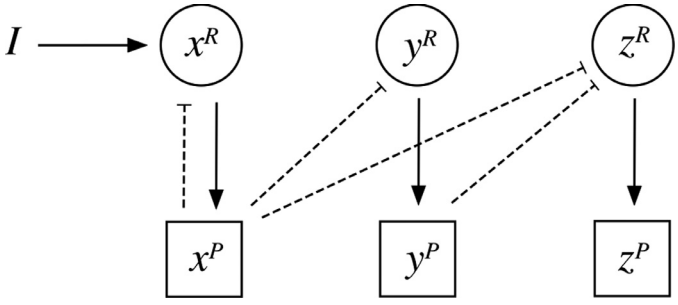

Fig. 2. A 3-gene 6-node feed-forward loop GRN motif. All arrows are different but for simplicity this is not made explicit in the figure. Circles stands for mRNA variables and squares for protein variables. Solid lines indicate positive coupling and dashed lines indicate negative or positive coupling (depending on the form of the equations at that node). The general form of the system of differential equations associated with this diagram is given in (1.3). It is shown in Theorem 1.1 that homeostasis occurs in z^P.

Fig. 2 shows a diagram representing a general 3-gene 6-node feed-forward loop, which includes the feed-forward loop shown in Fig. 1 as a particular case (the solid lines of Fig. 1 correspond to the dashed lines of Fig. 2). The equations corresponding to the GRN motif in Fig. 2 have the form:

$$\begin{aligned}
\dot{x^R} &= f^R(x^R, x^P) + I \\
\dot{x^P} &= f^P(x^R, x^P) \\
\dot{y^R} &= g^R(x^P, y^R) \\
\dot{y^P} &= g^P(y^R, y^P) \\
\dot{z^R} &= h^R(x^P, y^P, z^R) \\
\dot{z^P} &= h^P(z^R, z^P)
\end{aligned} \tag{1.3}$$

The input parameter I represents the action of all other upstream transcription factors that affect the x gene and do not come from the y and z genes.

1.3. Homeostasis in a specific model

In Section 2 Proposition 2.1 we derive general conditions for the occurrence of infinitesimal homeostasis points and chair points in (1.3). These conditions are suitable for numerical solution; for an example, see Fig. 5 and the discussion at the end of Section 4. By choosing specific functions, analytic solutions can be derived in special cases, such as:

$$\begin{aligned} f^R(x^R,x^P) &= -\delta x^R + \mathcal{S}(x^P) + I \\ f^P(x^R,x^P) &= -\alpha x^P + x^R \\ g^R(x^P,y^R) &= -\delta y^R + \mathcal{S}(x^P) \\ g^P(y^R,y^P) &= -\alpha y^P + y^R \\ h^R(x^P,y^P,z^R) &= -\delta z^R + \mathcal{T}(x^P + y^P) \\ h^P(z^R,z^P) &= -\alpha z^P + z^R \end{aligned} \tag{1.4}$$

where $\mathcal{S}$ and $\mathcal{T}$ are positive decreasing sigmoid functions. This form is discussed in Section 3.

Theorem 1.1. *For each $I > -1$ the standard model (1.4) has a unique equilibrium which is stable and has all coordinates positive. The equilibrium has an infinitesimal chair point at I_0 if and only if x^P is a solution of the system*

$$\begin{aligned} \mathcal{S}(x^P) &= \sigma x^P - I \\ \mathcal{S}'(x^P) &= -\sigma \\ \mathcal{S}''(x^P) &= 0 \\ \mathcal{S}'''(x^P) &\neq 0 \end{aligned} \tag{1.5}$$

where $\sigma = \alpha\delta$.

The proof proceeds in several stages in Sections 2 and 3. A specific example using this theorem is given in Section 4.

Remark 1.2. The third equation in (1.5) implies that infinitesimal chairs occur in the simplified standard model (1.4) only at points of inflection of the sigmoid function $\mathcal{S}$.

1.4. Remarks from singularity theory

The universal unfoldings of chair points determined by (1.5) are also present in (1.4). It follows that the chair points are structurally stable and hence occur in a larger family of models than those in (1.4). The determinacy conditions for a chair point ($z_I = z_{II} = 0$) also provide a numerical method for finding chairs when analytic methods fail. We return to these points in Section 4.

1.5. Structure of the paper

The feed-forward loop GRN that we study is discussed in Section 2. Specifically, Proposition 2.1 gives conditions for the occurrence of a chair in the output variable z^P for the general Eq. (1.3). Here we discuss the use of implicit differentiation to find points of infinitesimal homeostasis and infinitesimal chairs. Section 3 introduces the standard model (3.2) that we use. Theorem 1.1, which applies to a simplified version of the standard model, is also proved in this section. Couplings in GRNs are often done through sigmoid functions, and Section 4 completes the calculations for a specific choice of sigmoid functions. The calculations for other choices would be similar. We also show that these added assumptions can be relaxed if the calculations for finding homeostasis points are performed numerically. The paper ends with Section 5 on Discussions and Conclusions.

2. Feed-forward loop GRN motif

Consider the GRN motif consisting of three genes shown in Fig. 2, where solid lines represent mRNA-protein coupling and dashed lines represent gene-gene coupling. Since experimental information about the type of coupling is unavailable we are assuming repression regulation for all mRNA nodes in the GRN motif. As we shall see, this assumption implies that the system has a unique stable equilibrium for all admissible parameter values. It has been experimentally observed that repression regulation (more specifically, self-regulation by repression) provide stability, thereby limiting the range over which the concentrations of network components fluctuate (Becksei and Serrano, 2000).

Our goal is to find regions of homeostasis in the steady-state protein concentration z^P as a function of the input parameter I. The steady state equations associated to (1.3) are

$$\begin{aligned} f^R(x^R,x^P) + I &= 0 \\ f^P(x^R,x^P) &= 0 \\ g^R(x^P,y^R) &= 0 \\ g^P(y^R,y^P) &= 0 \\ h^R(x^P,y^P,z^R) &= 0 \\ h^P(z^R,z^P) &= 0 \end{aligned} \tag{2.1}$$

The Jacobian of (1.3) is

$$J = \begin{bmatrix} f^R_{x^R} & f^R_{x^P} & 0 & 0 & 0 & 0 \\ f^P_{x^R} & f^P_{x^P} & 0 & 0 & 0 & 0 \\ 0 & g^R_{x^P} & g^R_{y^R} & 0 & 0 & 0 \\ 0 & 0 & g^P_{y^R} & g^P_{y^P} & 0 & 0 \\ 0 & h^R_{x^P} & 0 & h^R_{y^P} & h^R_{z^R} & 0 \\ 0 & 0 & 0 & 0 & h^P_{z^R} & h^P_{z^P} \end{bmatrix} \tag{2.2}$$

Subscripts in (2.2) and in the following indicate partial derivatives. The block-triangular form implies that an equilibrium of (1.3) is stable if and only if

$$h^P_{z^P} < 0 \quad h^R_{z^R} < 0 \quad g^P_{y^P} < 0 \quad g^R_{y^R} < 0 \quad \mathrm{tr}(K) < 0 \quad \det(K) > 0 \tag{2.3}$$

where

$$K = \begin{bmatrix} f^R_{x^R} & f^R_{x^P} \\ f^P_{x^R} & f^P_{x^P} \end{bmatrix}$$

By the implicit function theorem, if an equilibrium Y_0 at I_0 is stable then there exists a unique family of stable equilibria near $Y(I)$ for I near I_0.

Proposition 2.1. *A stable equilibrium $Y_0 = (x^R, x^P, y^R, y^P, z^R, z^P)$ at I_0 satisfies $z^P_I(I_0) = 0$ if and only if*

$$\rho(I_0) \equiv g^R_{y^R} g^P_{y^P} h^R_{x^P} + h^R_{y^P} g^P_{y^R} g^R_{x^P} = 0. \tag{2.4}$$

An infinitesimal chair point for output z^P occurs if in addition

$$\frac{d\rho}{dI}(I_0) = 0 \qquad \text{and} \qquad \frac{d^2\rho}{dI^2}(I_0) \neq 0. \tag{2.5}$$

Proof. Eq. (2.1) define x, y, z implicitly as functions of I. Implicit differentiation of (2.1) with respect to I yields

$$\begin{aligned} f^R_{x^R} x^R_I + f^R_{x^P} x^P_I &= -1 \\ f^P_{x^R} x^R_I + f^P_{x^P} x^P_I &= 0 \\ g^R_{y^R} y^R_I + g^R_{x^P} x^P_I &= 0 \\ g^P_{y^R} y^R_I + g^P_{y^P} y^P_I &= 0 \\ h^R_{z^R} z^R_I + h^R_{x^P} x^P_I + h^R_{y^P} y^P_I &= 0 \\ h^P_{z^R} z^R_I + h^P_{z^P} z^P_I &= 0 \end{aligned} \tag{2.6}$$

The first two equations in (2.6) can be solved for x_I^R and x_I^P. We assume $f^P_{x^R} \neq 0$ so that $x_I^P \neq 0$. Next we work backwards. By the last equation in (2.6), $z_I^P = 0$ implies $z_I^R = 0$, so

$$h^R_{x^P} x_I^P + h^R_{y^P} y_I^P = 0$$

or

$$y_I^P = -\frac{h^R_{x^P}}{h^R_{y^P}}\, x_I^P.$$

Substitute in the fourth equation in (2.6) to get

$$h^R_{y^P} g^P_{y^R} y_I^R - g^P_{y^P} h^R_{x^P} x_I^P = 0.$$

Eliminating y_I^R from the third equation,

$$-(g^R_{y^R} g^P_{y^P} h^R_{x^P} + h^R_{y^P} g^P_{y^R} g^R_{x^P}) x_I^P = 0$$

Since $x_I^P \neq 0$, (2.4) is satisfied. We have shown that infinitesimal homeostasis occurs when (2.4) is satisfied.

The equation $\rho = 0$ for infinitesimal homeostasis has a chair if ρ has a double zero; that is, $\rho_I = 0$. The nondegeneracy condition states that the equation does not have a triple zero; that is, $\rho_{II} \neq 0$. This completes the proof of (2.5) and of the proposition. □

3. Homeostasis in a standard model for the feed-forward loop

It is common in deterministic modeling of transcription and translation to assume that the equations are linear with constant coefficients, called *rate constants*, except for x^P and t_J in the first equation (Kaern et al., 2005). Hence we arrive at the following *standard model*:

$$\begin{aligned} \dot{x}^R &= -\delta^x x^R + \gamma^x \tilde{f}(x^P, t_J) + I \\ \dot{x}^P &= -\alpha^x x^P + \beta^x x^R \end{aligned} \tag{3.1}$$

Here the function $\tilde{f}$ is an *input function* with range [0, 1] that governs transitions between the active and repressed states of the promoter by converting protein concentrations x^P and t_J into binding affinity. The parameter δ^x is the degradation rate of the mRNA, α^x is the degradation rate of the protein, γ^x is rate of mRNA synthesis, and β^x is the rate of protein synthesis. All of these quantities are constants. We employ this unorthodox notation because the superscript shows which variables the constants are associated with. This is useful for (3.2) below. The parameter I acts on the production rate of mRNA variable, which implies that

$$\sup_{(x^P, t_J)} \{\gamma^x \tilde{f}(x^P, t_J)\} + I = \gamma^x + I \geqslant 0.$$

Next we show that the standard model (3.1) for the three gene network in Fig. 2 has a family of stable equilibria, one for each admissible value of the input parameter I. The general standard model for this GRN motif is:

$$\begin{aligned} f^R(x^R, x^P) &= -\delta^x x^R + \gamma^x \tilde{f}(x^P) + I \\ f^P(x^R, x^P) &= -\alpha^x x^P + \beta^x x^R \\ g^R(x^P, y^R) &= -\delta^y y^R + \gamma^y \tilde{g}(x^P) \\ g^P(y^R, y^P) &= -\alpha^y y^P + \beta^y y^R \\ h^R(x^P, y^P, z^R) &= -\delta^z z^R + \gamma^z \tilde{h}(x^P, y^P) \\ h^P(z^R, z^P) &= -\alpha^z z^P + \beta^z z^R \end{aligned} \tag{3.2}$$

where δ^x, δ^y, δ^z, α^x, α^y, $\alpha^z\gamma^x$, γ^y, γ^z, β^x, β^y, β^z are positive constants, and $\tilde{f}$, $\tilde{g}$, $\tilde{h}$ are *input functions*. More precisely, $\tilde{f}$ and $\tilde{g}$ are non-negative and strictly decreasing (repression coupling) sigmoid functions and $\tilde{h}$ is a two-dimensional generalization of a sigmoid function of repression type (Alon, 2007).

In order to apply Proposition 2.1 to find an explicit infinitesimal chair point of (3.2), we introduce several simplifying assumptions:

- The mRNA degradation constants have the same value for all genes.
- The protein degradation constants have the same value for all genes.
- The protein synthesis constants are the same for all genes.
- Introducing dimensionless parameters and variables, we may assume that the synthesis constants are normalized to 1.
- The input functions $\tilde{f}$ and $\tilde{g}$ are equal and $\tilde{h}$ is a scalar function of $x^P + y^P$. This means that the concentrations x^P and y^P act independently and additively on gene z.

These assumptions lead to the simplified model (1.3) given in the Introduction:

Proof of Theorem 1.1. Since the maximum value of $\mathcal{S}$ is $\mathcal{S}(0) = 1$, we have $I + 1 \geqslant 0$. We solve for the zeros of (3.2) as follows:

$$\begin{aligned} x^R &= \alpha x^P \\ y^R &= \alpha y^P \\ z^R &= \alpha z^P \\ y^P &= \tfrac{1}{\sigma}\mathcal{S}(x^P) \\ z^P &= \tfrac{1}{\sigma}\mathcal{T}(x^P + y^P) \\ \mathcal{S}(x^P) &= \sigma x^P - I \end{aligned} \tag{3.3}$$

For all admissible values of I the last equation in (3.3) has a unique solution $x^P > 0$, since the left hand side is strictly decreasing in x^P and always positive and the right hand side is strictly increasing in x^P. Hence the first equation in (1.5) is satisfied.

Given x^P the fourth equation in (3.3) yields y^P; the second equation yields y^R; the first equation yields x^R; the fifth equation yields z^P; and the third equation yields z^R. Thus the equilibrium exists and is unique. In addition, $y^P > 0$ implies that all other components of the equilibrium are positive for the model Eq. (1.4).

Moreover, by (2.2), the eigenvalues of the Jacobian at this equilibrium are $-\alpha, -\delta$ and the eigenvalues of the 2×2 matrix

$$K = \begin{bmatrix} -\delta & \mathcal{S}' \\ 1 & -\alpha \end{bmatrix}$$

Since $\mathrm{tr}(K) < 0$ and $\det(K) > 0$, the eigenvalues of M both have negative real part and the equilibrium is stable.

Next we use the specific form of (1.4) to calculate infinitesimal homeostasis points by solving (2.4). We calculate

$$\begin{aligned} g^R_{y^R} g^P_{y^P} h^R_{x^P} + h^R_{y^P} g^P_{y^R} g^R_{x^P} &= [-\delta][-\alpha][\mathcal{T}'(x^P + y^P)] \\ &\quad + [\mathcal{T}'(x^P + y^P)][1][\mathcal{S}'(x^P)] \\ &= \mathcal{T}'(x^P + y^P)(\sigma + \mathcal{S}'(x^P)) \\ &= 0. \end{aligned}$$

Since $\mathcal{T}' < 0$ the second equation in (1.5) is satisfied.

When two infinitesimal homeostasis points come together as a parameter is varied, they do so at a value of I where $\rho(I) = \rho_I(I) = 0$. Therefore infinitesimal chair points occur when $\rho_I = 0$, and they are nondegenerate when $\rho_{II} \neq 0$. Differentiating

$$\rho(I) = \mathcal{T}'(x^P(I) + y^P(I))(\sigma + \mathcal{S}'(x^P(I)))$$

twice with respect to I, and evaluating at I_0, yields the third and fourth equations in (1.5). This completes the derivation of (1.5). □

4. Explicit examples

We now specialize the model further, making it possible to obtain an explicit value of I_0 at which the infinitesimal chair point occurs. To do so, we assume that $\mathcal{S}$ is a normalized Hill function of repression type (Santillán, 2008), that is

$$\mathcal{S}(x) = \frac{1}{1 + x^n}.$$

The exponent n, called the *Hill coefficient*, measures the steepness of the input function. Typically, input functions are moderately steep with n between 1 and 3, see (Alon, 2007). To obtain a function with an inflection point, which requires $n > 1$, we choose

$$\mathcal{S}(x) = \frac{1}{1+x^2}. \tag{4.1}$$

Now

$$\mathcal{S}'(x) = -\frac{2x}{(1+x^2)^2}$$
$$\mathcal{S}''(x) = -2\frac{1-3x^2}{(1+x^2)^3}$$
$$\mathcal{S}'''(x) = 24x\frac{1-x^2}{(1+x^2)^4}$$

Note that $\mathcal{S}''(x) = 0$ implies $x_0^P = \frac{1}{\sqrt{3}}$ since we are interested only in positive roots of $\mathcal{S}''$. Therefore

$$\mathcal{S}(x_0^P) = \frac{3}{4}$$
$$\mathcal{S}'(x_0^P) = -\frac{3\sqrt{3}}{8}$$
$$\mathcal{S}'''(x_0^P) > 0$$

Moreover,

$$\sigma_0 = -\mathcal{S}'(x_0^P) = \frac{3\sqrt{3}}{8}. \tag{4.2}$$

A critical point of I occurs when

$$I_0 = \sigma_0 x_0^P - \mathcal{S}(x_0^P) = \frac{3\sqrt{3}}{8}x_0^P - \frac{3}{4} = -\frac{3}{8},$$

which is greater than -1. Finally, the *set point* (the value about which homeostasis is approximately constant) is

$$z^P(I_0) = \frac{1}{\sigma_0}\mathcal{T}(x_0^P + y_0^P) = \frac{1}{\sigma_0}\mathcal{T}\left((1+\sigma_0)x_0^P - I_0\right)$$
$$= \frac{8}{3\sqrt{3}}\mathcal{T}\left(\frac{1}{\sqrt{3}} + \frac{3}{4}\right)$$

Therefore the set point is determined by the choice of the function $\mathcal{T}$.

We now consider the case where the synthesis constants γ and β are not normalized to 1. The parameter σ becomes $\sigma = \frac{\alpha\delta}{\beta\gamma}$. The equation for x^P is $\mathcal{S}(x^P) = \sigma x^P - \frac{I}{\gamma}$, the value of the input parameter I at which the infinitesimal chair point occurs is $I_0 = \frac{3}{8}(1-2\gamma)$ (since $\gamma > 0$ we always have $I_0 + \gamma \geqslant 0$) and the set point value is

$$z^P(I_0) = \frac{8}{3\sqrt{3}}\mathcal{T}\left(\frac{1}{\sqrt{3}} + \frac{3}{4}\gamma\right)$$

Nevertheless, the corresponding rate constants for the three different genes remain equal, and so do the two Hill functions.

4.1. Homeostasis as an FFL network phenomenon

In this model x^P and y^P are never infinitesimally homeostatic with respect to variation of I (using (2.3) and (2.6)) — but, as we see, z^P can be. This suggests that the network topology of the feed-forward loop is important in generating homeostasis. We emphasize this point by graphing $x^P(I)$, $y^P(I)$, $z^P(I)$ in Fig. 3.

First, $x^P(I)$ is obtained by solving $\mathcal{S}(x) - \sigma_0 x + I = 0$ for x as function of I with σ_0 given by (4.2). Then

$$y^P(I) = \frac{1}{\sigma_0}\mathcal{S}(x^P(I))$$
$$z^P(I) = \frac{1}{\sigma_0}\mathcal{S}(x^P(I) + y^P(I)). \tag{4.3}$$

For $\mathcal{S}$ given by (4.1) the equation $\mathcal{S}(x) - \sigma_0 x + I = 0$ is a cubic equation with exactly one real root for each I. Hence $x^P(I)$ is the unique real root x of

$$\frac{3\sqrt{3}}{8}x^3 + Ix^2 + \frac{3\sqrt{3}}{8}x - I = 0. \tag{4.4}$$

4.2. Implications of singularity theory

Now we can appeal to singularity theory as explained in Golubitsky and Stewart (2017). Without loss of generality, we assume that $I_0 = 0$, which can always be arranged by translating the coordinate I. Because the input-output function $z(I)$ is 1-dimensional we consider singularity types near 0 of a single-variable function. Such singularities are determined by the first non-vanishing derivative $\frac{d^k z}{dI^k}(0)$. Informally, the *codimension* of a singularity is the number of conditions on derivatives that determine it. This is also the minimum number of extra variables required to specify all small perturbations of the singularity, up to suitable changes of coordinates. These perturbations can be organized into a family of maps called the *universal unfolding*, which has that number of extra variables. The *normal form*, which is obtained when the extra variables are set to zero, is the 'simplest' expression for the function near 0, up to suitable changes of coordinates. Typically it is a polynomial.

Using standard results of elementary catastrophe theory (see Golubitsky and Stewart, 2017 for details), it can be shown that the normal form of the input-output function for simple homeostasis is $z(I) = \pm I^2$ and no unfolding parameter is required (codimension 0). The normal form of the input-output function for an infinitesimal chair is $z(I) = \pm I^3$, and its universal unfolding is $\tilde{z}_\sigma(I) = \pm I^3 + \sigma I$ (codimension 1). Fig. 4 shows a numerical plot of z^P against I for three values of σ: one at the infinitesimal chair point I_0 and one on either side. The graphs have the same qualitative shape as the functions $-I^3 + aI$, $-I^3$, and $-I^3 - aI$ in the universal unfolding, for some $a > 0$. See Golubitsky and Stewart (2017).

The universality property implies that any small perturbation of the input-output map induced by a small perturbation of the equations is given, up to a change of coordinates, by a member of the universal unfolding. In particular, taking the constant rates of the three genes to be slightly different from each other is such a small perturbation of the input-output map.

4.3. Comments on numerics

The specific analysis of system (1.5) in the proof of Theorem 1.1 is valid only for (a reasonable class of) specialized equations. However, this analysis paves the way for a more extensive numerical exploration of the more general system (3.2) or even the yet more general system (1.3). We make two points. First, the analysis given here shows how to find chair points numerically. For example, see Fig. 5 where a chair point is found in the system (3.2) using numerics carried out for unequal δ's based on the analytic methods given here. Second, although numerical methods can be useful for finding chair points somewhat automatically, one still has to specify the model system. Thus, we leave a full-scale numerical exploration for this feed-forward loop motif (and other possible systems of interest) for future work.

5. Discussions and conclusions

5.1. Summary of results

In this paper we studied a differential equation model (1.3), and in more detail the special case (1.4), for a gene regulatory network motif called a feed-forward loop. This motif appears to be

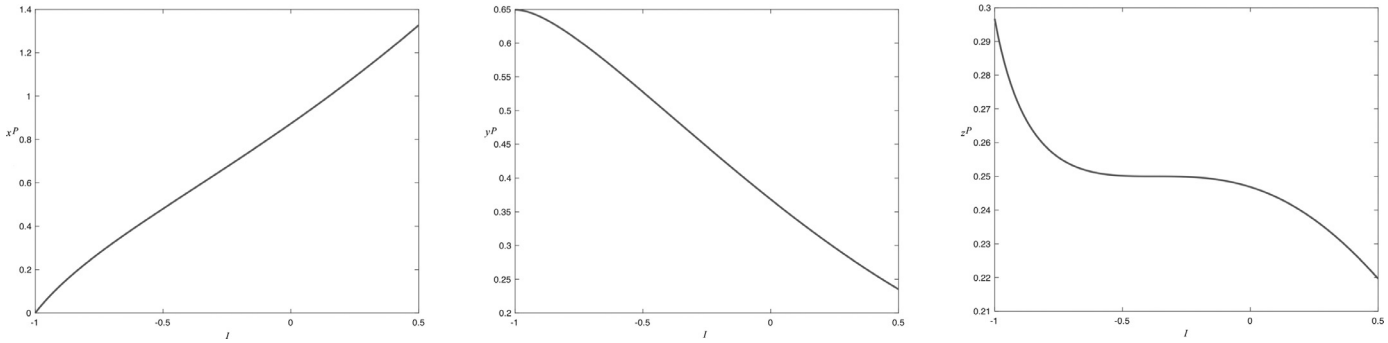

Fig. 3. Stable equilibrium in the feed-forward loop GRN motif (1.4) near z^P-homeostasis. The horizontal coordinate on the three graphs is I and includes the infinitesimal homeostasis point $I_0 = -\frac{3}{8} = -0.375$. The vertical coordinates are, respectively, (left) $x^P(I)$, (center) $y^P(I)$, (right) $z^P(I)$. See (4.3) and (4.4). Neither x^P nor y^P exhibits homeostasis, whereas z^P does.

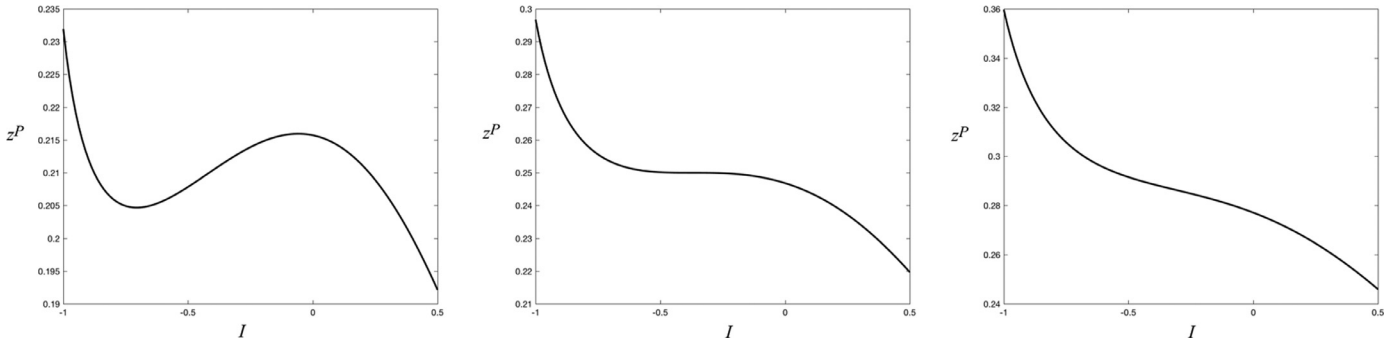

Fig. 4. Chair singularity and universal unfolding of the feedforward loop GRN motif (1.4). The horizontal coordinate is I and includes the infinitesimal homeostasis point $I_0 = -\frac{3}{8} = -0.375$. The vertical coordinate is $z^P(I, \sigma)$ with (left) $\sigma = \sigma_0 - 0.1 \approx 0.55$, (center) $\sigma = \sigma_0 = \frac{3}{8}\sqrt{3} \approx 0.65$, (right) $\sigma = \sigma_0 + 0.1 \approx 0.75$. Here, we obtain $x^P(I, \sigma)$ by solving $S(x) - \sigma x + I = 0$ for x as function of I and σ, and use (4.3) to obtain $y^P(I, \sigma)$ and $z^P(I, \sigma)$. Consequently, σ is a universal unfolding parameter of this chair singularity (Golubitsky and Stewart, 2017).

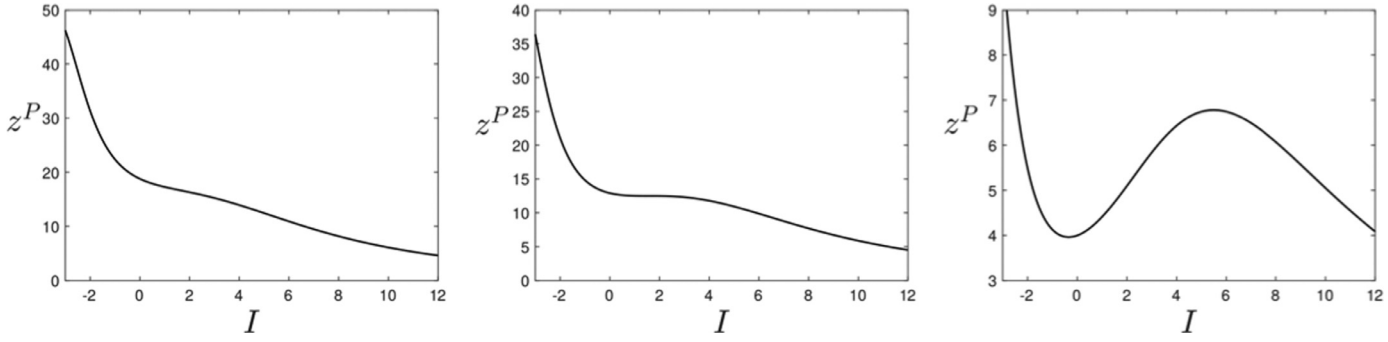

Fig. 5. Using numerical exploration starting at $\delta^x = 10$, $\delta^y = 1$, $\delta^z = 0.1$, $\alpha^x = 0.4$, $\alpha^y = 0.3$, $\alpha^z = 0.2$, a chair singularity (3.2) was found by varying δ^y. The chair point occurs at $I = 1.5593$ and $\delta^y = 2.1651$. As δ^y decreases from 3 to 2.1651 to 1, the input-output graph (I, z^P) changes from decreasing to chair to a minimum and a maximum. This is the behavior one expects from the structurally stable universal unfolding of a chair point. We thank Yangyang Wang for using XPP and Matlab to perform these calculations.

highly prevalent in single-cell organisms such as yeast. Here we proved the existence of homeostasis in the output protein concentration with respect to an input parameter I that represents the collective influence of the other parts of the regulatory network. Using infinitesimal homeostasis, we have shown that the feed-forward loop can display a strong form of expression level homeostasis, called *chair homeostasis* (Golubitsky and Stewart, 2017; Nijhout et al., 2014). Moreover, it is clear that these techniques, usually in combination with numerical methods, can be used to search for infinitesimal chairs in other gene regulatory networks.

Homeostatic mechanisms in nature not only produce a steady-state output that remains constant under variation of the input, but this output even remains constant when parameters (such as enzyme affinity or maximum activation rate) in the regulated system change slightly. This notion of *robust homeostasis* has been considered in the systems biology literature, more specifically to *biological circuit design* (Ang and McMillen, 2013; Ma et al., 2009; Sontag, 2010). Tang and McMillen (2016) attempt to formalize the notion of robust homeostasis in order to obtain an algorithm to design networks supporting robust homeostasis. However, being based on the theory of singularities (Golubitsky and Stewart, 2017), the notion of chair homeostasis is, in a natural way and without the need of any additional assumptions, a form of robust homeostasis. In fact, the stability of universal unfoldings of elementary catastrophes ensures that chair homeostasis is robust in a very strict sense.

5.2. Discussion of other issues

5.2.1. Embedding of motifs in large networks

Small sub-network patterns that appear with high frequency in complex large networks, called network motifs, have played an in-

creasingly important role in biology and systems biology. However, a deep and natural question is less frequently addressed. Since motifs in biological networks do not exist in isolation, but are embedded in much larger networks, we may ask under what conditions the imbedded motif operates in the same way as the isolated motif. The notion of homeostasis provides the simplest context where this question might be addressed rigorously.

A partial answer can be given provided suitable modeling assumptions are valid. Suppose that the remainder of the network inputs *only* to the first node (here x^R). Then whatever the dynamics of the whole network may be, its effect on the motif is the same as varying I dynamically. It can be proved (Golubitsky and Stewart, 2018b) that if I varies slowly, and equilibrium states in the motif are sufficiently strongly attracting, then the output remains homeostatic for the full network as long as I remains within the homeostasis region of the motif (or perhaps a slightly smaller region). In short: Under these conditions, homeostasis in the motif implies homeostasis in the full network, for the same output variable. Similar remarks apply in the presence of stochastic noise, provided fluctuations are sufficiently small. Additional inputs to the motif also do not destroy homeostasis if they are sufficiently weak, but this issue is more delicate.

5.2.2. Multiple inputs

An important issue is: how do other parts of the regulatory network influences a motif? A partial answer was given above, when the other parts of the network affects only one node of the motif. That is, we have considered motifs that generate homeostasis in one output variable as a function of one input variable. But one might be interested also in homeostasis as a function of several input parameters affecting different nodes of the GRN motif. See Golubitsky and Stewart (2018a). For example, in the case of the FFL motif, one could consider a second input parameter I_2 affecting the y^R-node in Fig. 2.

5.2.3. Experimental findings of homeostasis

Finally: Can one experimentally observe expression level homeostasis in some gene? In fact, there are some experiments reported in the literature that could be interpreted as direct observation of expression level homeostasis in a housekeeping gene. Recall that a *housekeeping gene* is a constitutive gene that is required to maintain basic cellular function, and is expressed under normal and pathological conditions. It has been observed that some housekeeping genes are transcribed at a 'relatively constant rate' in most non-pathological situations (Kozera and Rapacz, 2013), and these genes are a candidate for homeostasis.

Cankorur-Cetinkaya et al. (2012) seek to identify certain housekeeping genes in yeast that are used as *reference genes* to measure the response to glucose or ammonium limitations. The idea is that a reference gene will not change its expression level when the amount of glucose or ammonium is varied, whereas the target genes will change their expression levels. Thus the authors are trying to show that there is homeostasis in the reference gene with respect to variation in the target gene. Moreover, most of these candidate reference genes reported in Cankorur-Cetinkaya et al. (2012) appear as the last gene in a feed-forward loop (Cherry et al., 2012).

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References

Alon, U., 2007. An Introduction to Systems Biology: Design Principles of Biological Circuits. Chapman & Hall/CRC.

Ang, J., McMillen, D.R., 2013. Physical constraints on biological integral control design for homeostasis and sensory adaptation. Biophys. J. 104, 505–515.

Becksei, A., Serrano, L., 2000. Engineering stability in gene networks by autoregulation. Nature 405, 590–593.

Cankorur-Cetinkaya, A., Dereli, E., Eraslan, S., Karabekmez, E., Dikicioglu, D., Kirdar, B., 2012. A novel strategy for selection and validation of reference genes in dynamic multidimensional experimental design in yeast. PLoS ONE 7 (6), e38351.

Cherry, J.M., Hong, E.L., Amundsen, C., Balakrishnan, R., Binkley, G., Chan, E.T., Christie, K.R., Costanzo, M.C., Dwight, S.S., Engel, S.R., Fisk, D.G., Hirschman, J.E., Hitz, B.C., Karra, K., Krieger, C.J., Miyasato, S.R., Nash, R.S., Park, J., Skrzypek, M.S., Simison, M., Weng, S., Wong, E.D., 2012. Saccharomyces genome database: the genomics resource of budding yeast. Nuc. Acids Res. (Database issue) 40. D700–5. http://www.yeastgenome.org.

Cipollina, C., van den Brink, J., Daran-Lapujade, P., Pronk, J.T., Porro, D., de Winde, J.H., 2008. Saccharomyces cerevisiae SFP1: at the crossroads of central metabolism and ribosome biogenesis. Microbiology 154 (6), 1686–1699.

Golubitsky, M., Stewart, I., 2018a. Homeostasis with multiple inputs. http://people.mbi.ohio-state.edu/golubitsky.4/reprintweb-0.5/output/papers/homeo_multiple_10_10_17.pdf.

Golubitsky, M., Stewart, I., 2017. Homeostasis, singularities and networks. J. Math. Biol. 74, 387–407.

Golubitsky, M., Stewart, I., 2018b. Motifs and homeostasis in networks. In preparation.

Hu, Z., Killion, P.J., Iyer, V.R., 2007. Genetic reconstruction of a functional transcriptional regulatory network. Nat Genet. 39 (5), 683–687.

Kaern, M., Elston, T.C., Blake, W.J., Collins, J.J., 2005. Stochasticity in gene expression: from theories to phenotypes. Nat. Genet. Rev. 6. 451–62.

Kozera, B., Rapacz, M., 2013. Reference genes in real-time PCR. J. Appl. Genet. 54 (4), 391–406.

Ma, W., Trusina, A., El-Samad, H., Lim, W.A., Tang, C., 2009. Defining network topologies that can achieve biochemical adaptation. Cell 138 (4), 760–773.

Nijhout, H.F., Best, J., Reed, M., 2014. Escape from homeostasis. Math. Biosci. 257, 104–110.

Reed, M., Best, J., Golubitsky, M., Stewart, I., Nijhout, H.F., 2017. Analysis of homeostatic mechanisms in biochemical networks. Bull. Math. Biol. 79 (11), 2534–2557. doi:10.1007/s11538-017-0340-z.

Santillán, M., 2008. On the use of the hill functions in mathematical models of gene regulatory networks. Math. Model. Nat. Phenom. 3 (2), 85–97.

Sontag, E., 2010. Remarks on feedforward circuits, adaptation, and pulse memory. IET Syst. Biol. 4, 39–51.

Tang, Z.F., McMillen, D.R., 2016. Design principles for the analysis and construction of robustly homeostatic biological networks. J. Theor. Biol. 408, 274–289.

Zak, D.E., Vadigepalli, R., Gonye III, G.E., Doyle, F.J., Schwaber, J.S., Ogunnaike, B.A., 2005. Unconventional systems analysis problems in molecular biology: a case study in gene regulatory network modeling. Comp. Chem. Eng. 29, 547–563.