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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.

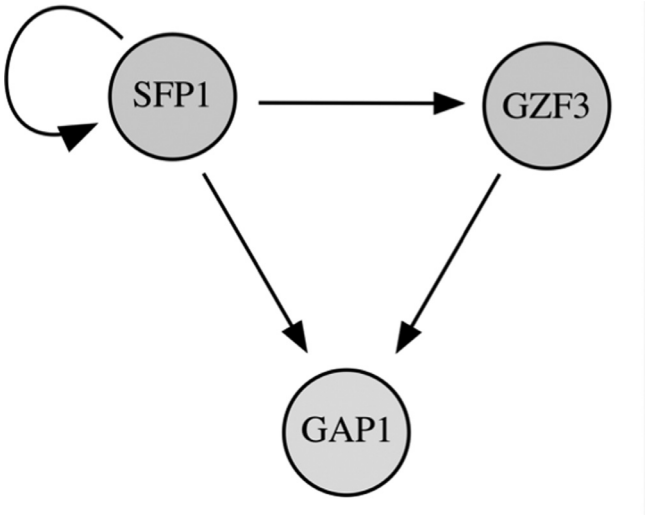

Fig. 1. A feed-forward loop motif of yeast *Saccharomyces cerevisiae* involving genes SFP1, GZF3 and GAP1. SFP1 is a self-regulated motif embedded in the larger GRN motif. Arrows indicate coupling between two genes, but the information about the type of coupling (activation or repression) is not available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

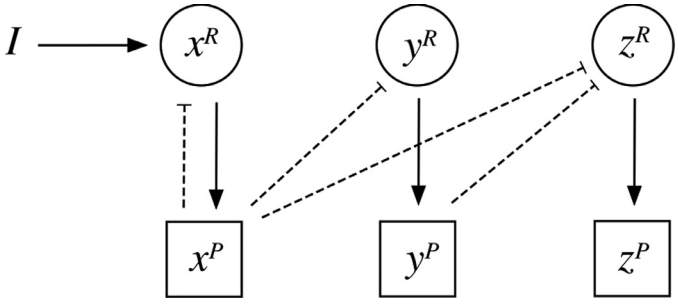

Fig. 2. A 3-gene 6-node feed-forward loop GRN motif. All arrows are different but for simplicity this is not made explicit in the figure. Circles stands for mRNA variables and squares for protein variables. Solid lines indicate positive coupling and dashed lines indicate negative or positive coupling (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.