OSCILLATORY DYNAMICS IN A DOUBLE ACTIVATOR FEEDBACK MOTIF

Srinandan Dasmahapatra

Faculty of Physical and Applied Sciences,
University of Southampton, Southampton SO17 1BJ, UK
sd@ecs.soton.ac.uk

ABSTRACT

Biological oscillators, specially those that constitute the circadian clock, have been extensively modelled as coupled feedback loops of positive and negative elements in gene regulatory circuits. The presence of a negative feedback loop is a necessary condition for the onset of oscillations, and different mechanistic implementations have been modelled, such as transcriptional repression (such as CRY/PER down-regulating activation by CLOCK:BMAL1 in the mammalian clock) or by enhancing proteolysis. It is also a characteristic feature of transcriptional control in clock systems that conserved *cis*-regulatory sequences (such as E- and D-boxes) are competitively bound by transctiption factors. Using this feature, we propose a new duplicated autoregulated motif where competition for the same promoters by differentially activating transctiption factors drives oscillations.

1. INTRODUCTION

The dynamics of small gene regulatory networks has been an active area of investigation, gaining importance as putative modules of core functional phenotypes in organisms as well as design elements in synthetic biology. For instance, some of the identified over-represented network motifs [1] such as feed-forward networks have been the subject of much theoretical and experimental investigation; feedback loops of positive and negative regulatory elements have been investigated as models of switching behaviour [2] and oscillating phenotypes, such as in NF-κB signalling or in circadian rhythms [3]. The role of negative regulators in the toggle switch is most commonly exemplified by repressive Cro-CI binding to a common set of operator sites of bacteriophage λ in the context of the lysis/lysogeny switch[4]. This is typically modelled as a pair of mutually repressive genes. Negative feedback has also been identified as the key ingredient in oscillatory dynamics – and various models have explored how this is brought about by direct repression at the *cis*-regulatory level or by proteolysis[5]. In the case of circadian rhythms, the conservation of this oscillatory behaviour across phyla has led to detailed investigation of homologies between known systems, pointing to several conserved genomic binding sites. For instance, the mammalian circadian system contains *cis*-regulatory regions called the E/E'-box, the RRE and the D-box which form the loci of *trans* action of proteins within the clock system as well as in enhancer regions of clock-controlled peripheral systems [6]. Thus a number of transcription factors bind to common consensus elements in the enhancers of the clock genes, to which the positive and negative regulators of transcription associate, with some binding to the DNA and others interacting via protein-protein interactions to the DNA-bound proteins.

The knowledge of positive or negative regulation is inferred from expression levels following knockout experiments and (sometimes) substantiated by the detection of protein-bound *cis*-regulatory sites upstream of transcription start sites. However, the mechanisms of transcription initiation and elongation are complex and not known in detail for many systems. In particular, there are several steps related to the recruitment of co-activators and co-repressors and core transcritional machinery including the Mediator complex and RNA Pol II whic involve the affinity between multi-unit proteins[7]. In particular, [8] has shown that the inferred CLOCK/BMAL1 activity from such mutant studies can have a dual role – it can act as an activator for some genes (such as *Per1, Dbp*) and as a repressor for others (such as *Cry1*). This dual regulatory aspect has been observed in many other systems. For instance, it has been shown [9] that repression in *Drosophila* is achived by activators competing for the same genomic binding sites. We take this as central to our study.

The model introduced in this paper mimics the repression by the action of two activators which have differential affinity for the transcriptional machinery. While this effect had been noted in [10] and in the non-monotonic dependence of the Shea-Ackers[11] functional forms for the gene regulatory function [12] its functional significance has not been addressed before. In particular, we demonstrate that this activator mediated repression can account for the negative arm in bringing about oscillations in gene networks. To motivate the result intuitively, we argue that if two activators that produce transcripts with different rates, the weaker transcription activator can potentially impair the ability of the stronger one to generate more transcript. The effects are most acute if there is steric repulsion between the two activators. The effectiveness of steric repulsion in altering the stability of network dynamics builds on the enhanced (bi-)stability properties of the model for the toggle switch in phage lambda [13].

In this paper we propose a simple two-activator motif that can be used to generate bistability and oscillations. Here we focus on the oscillatory dynamics. We note that the result and its biological interpretation in terms of competitive dynamics at the promoter appears in contradiction to [14] which reports on the promoter-competition driven ablation of oscillations in the standard 2-gene activator-repressor oscillator. We proceed by first building the gene regulatory function of a double activator from thermodynamic arguments following [11, 15]. We then "wire up" the circuit composed of double positive feedback loops (in the spirit of [16] where an activator and repressor double feedback motif[17] was engineered) and demonstrate that the coupled effect of slow degradation of one component with the competitive recruitment of the core transcription machinery is sufficient to generate oscillations via a Hopf bifurcation.

2. THERMODYNAMIC MODEL OF GENE REGULATION

The thermodynamic models of gene regulation [11, 15] assume that protein-DNA (un-)binding transitions occur at a much faster time-scale than the processes of transcript elongation and protein synthesis, thus presenting only an average, or net probability of occurence of poised (Pol II bound) promoter to the latter processes. As noted in section 1, transcription factors bound to the enhancer recruit RNA polymerase via the Mediator complex [7], and the thermodynamic formalism can easily accommodate different binding free energies of protein-DNA and protein-protein interactions in a uniform manner. In this paper, we summarize the composite effects of transcription factor-Mediator and Mediator-RNA polymerase binding by energy terms $\epsilon_{A_i p}$ for each activator A_i. This is a vastly simplified model for gene regulation, as it ignores a wide variiety of activating mechanisms including the contribution of nucleosome binding to DNAand energetic effects due to histone modifications. We introduce Boltzmann factors for all possible configurations for binding of activators $A_{1,2}$ and a proxy for the Mediator-Pol II complex P to calculate the partition function $Z_{tot}(P, A_1, A_2)$. A subset of these configurations are poised for transcription *i.e.*, those with RNA polymerase or Pol II bound to the promoter. The rate of mRNA synthesis is taken to be proportional to the probability of the Pol II bound promoter, which is taken to be the ratio of the Boltzmann factors for the favourable configurations with promoter-specific bound Pol II to $Z_{tot}(P, A_1, A_2)$.

The binding energies for non-specific (site-specific) binding are denoted $\varepsilon^0(\varepsilon^s)$, with appropriate subscripts which identify the binding of activators or the polymerases to the DNA. For the case where k polymerases, l activators of type 1 and m activators of type 2 bind to the *cis*-regulatory region of the DNA their energy contribution is

$$\begin{aligned} E^s(k,l,m) \equiv\ & k(\varepsilon^s_{pd} + l\varepsilon_{A_1 p} + m\varepsilon_{A_2 p} + \\ & l\varepsilon^s_{A_1 d} + m\varepsilon^s_{A_2 d} + lm\varepsilon_{A_1 A_2} + \\ & lm\varepsilon_{A_1 A_2 p}), \end{aligned} \quad (1)$$

where the subscripts indicate the protein-protein binding energies as well, including $\varepsilon_{A_1 A_2 p}$ which captures the net energy of recruitment of transcriptional machinery due to the combined action of the activators. In this paper, we set that to infinity to indicate steric repulsion. If the proteins bind to non-cognate sites, the binding energy is

$$E^0(k,l,m) := k\varepsilon^0_{pd} + l\varepsilon^0_{A_1 d} + m\varepsilon^0_{A_2 d}. \quad (2)$$

For P the number of Pol II molecules, $A_{1,2}$ the number of transcription activators of each type, we introduce

$$\zeta(P, A_1, A_2) = \binom{N}{P, A_1, A_2} e^{-\beta E^0(P, A_1, A_2)}, \quad (3)$$

where the right hand side includes in the exponent $\beta = 1/(k_B T)$, and the trinomial coefficient contains the number of binding sites in the genome N. The partition function for 0 or 1 molecules of type $P, A_{1,2}$ bound to the relevant DNA sites is

$$Z_{tot}(P, A_1, A_2) = \sum \zeta(P-k, A_1-l, A_2-m) e^{-\beta E^s(k,l,m)}, \quad (4)$$

with $(k,l,m) \in \{0,1\}^3$. We use the Stirling approximation and the definitions

$$\begin{aligned} \alpha_i = \frac{A_i}{N} e^{-\beta(\varepsilon^s_{A_i d} - \varepsilon^0_{A_i d})}, \quad r_i = e^{-\beta\varepsilon_{A_i p}}, \quad i = 1,2 \\ \rho = \frac{P}{N} e^{-\beta(\varepsilon^s_{pd} - \varepsilon^0_{pd})},\ r_{12} = e^{-\beta\varepsilon_{A_1 A_2 p}},\ \omega_{12} = e^{-\beta\varepsilon_{A_1 A_2}} \end{aligned} \quad (5)$$

to simplify Z_{tot} which enables us to compute the probability of occupancy of the promoter by RNA Pol II:

$$\sum_{(l,m)\in\{0,1\}^2} \frac{\zeta(P-1, A_1-l, A_2-m)}{Z_{tot}(P, A_1, A_2)} e^{-\beta E^s(1,l,m)} \quad (6)$$

and express the transcription rate as proportional to

$$\frac{\rho\,(1 + r_1\alpha_1 + r_2\alpha_2)}{(1 + \alpha_1 + \alpha_2) + \rho\,(1 + r_1\alpha_1 + r_2\alpha_2)}. \quad (7)$$

For this paper, we assume complete exclusion of simultaneous occupation of the promoter and/or recruitment of Pol II by the activators, so we set $r_{12} = 0 = \omega_{12}$.

3. INSTABILITY IN DUAL ACTIVATOR CIRCUIT

The rate of transcription is commonly taken to be proportional to the probability of finding a promoter poised for transcript elongtion. If we assume both activators $A_{1,2}$ are functional only as dimers, and they activate each other and themselves (see Figure 1). In the circuit design of [17, 16] contained a dimeric activator and a tetrameric repressor. Introducing the variables x and y for the scaled monomer concentrations of the activators A_1 and A_2, and the definitions $\mathbf{x} = (x, y)$, $\mathbf{c} = (c_x, c_y)$, matrix $\mathbf{\Delta} = \mathrm{diag}(\Delta_1, \Delta_2)$ and

$$\varphi(\mathbf{x}, \mathbf{r}) = \frac{\rho(1 + r_1 x^2 + r_2 y^2)}{(1+\rho) + (1+\rho r_1)x^2 + (1+\rho r_2)y^2}, \quad (8)$$

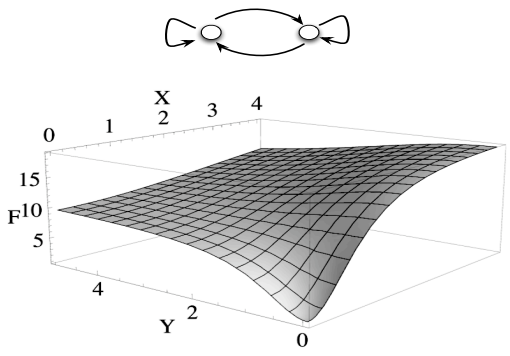

Figure 1. The dual activator regulatory motif and the fold change of transcriptional efficiency relative to the basal rate. Note that y behaves as an activator for low values of x and as a repressor when x is abundant. This is so because $r_1 > r_2$.

we arrive at the the pair of equations describing the system dynamics:

$$\dot{\mathbf{x}} = \mathbf{c}\varphi(\mathbf{x}, \mathbf{r}) - \mathbf{\Delta} \cdot \mathbf{x}. \tag{9}$$

In the above, we have made the assumption that the protein-DNA dissociation constants (K_D) are the same for both dimers, and as in (7), that the binding of the activators to the Pol II proxy is independent of genomic background, *i.e.* that there are no other co-factors that influence those rates. These assumptions underlie our definition of the scaled variables $x, y = [A_{1,2}]/\sqrt{K_{dim}K_D}$ where K_{dim} is the dimer dissociation constant for either activator (assumed equal for simplicity). The decay rates of the proteins are $\Delta_{1,2}$ while $c_i = [d_i]\mu_i\pi_i/(\delta_i\sqrt{K_{dim}K_D})$ which amalgamates the transcription (μ_i), translation (π_i) and mRNA decay rates (δ_i) as well as the copy number of the gene promoter $[d_i]$ by eliminating the fast reactions.

A couple of remarks are in order. This approximation relies on a separation of time-scales for DNA binding and dimerization processes from the slower transcription and translation events. However, the equations (9) are "sped up" with respect to the full system which includes the detailed steps of each of the processes. For the purpose of this paper it is sufficient to work with this simplified model (9) as we have checked that the principal result, that of the onset of oscillations, is valid for a similar range of parameter values for the full system. The period of the oscillations is higher in (9) than in the more detailed model. Further, we have checked that the oscillatory behaviour reported below is also present in a full stochastic simulation using the Gillespie algorithm provided one sets the "volume factor" which translates between numbers and concentrations to be high enough (numbers of order 50 or above). This is also the case for the circadian clock models studied in [18].

The fixed points of (9) satisfy $(x^*/y^*) = (c_x\Delta_2/c_y\Delta_1)$ which are obtained from the solutions of the cubic equation $\varphi_x(x, y^*(x^*)) = 0$. The cubic equation has the form $\sum_{i=1}^{4}(-1)^i a_i x^{i-1}$ where $a_i > 0$; thus, by Descartes' rule of signs, there are either 3 or 1 real positive solutions.

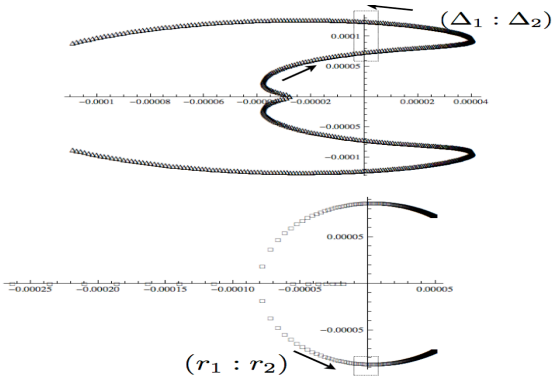

Figure 2. Hopf bifurcation: the complex plane (real part on the abscissa, imaginary part on the ordinate) of eigenvalues of the Jacobian when the ratios of protein degradation (top) and the ratios of Pol II recruitment by activators (bottom) are changed. In the top figure, the ratio $r_1/r_2 = 40$, while in the bottom figure, $\Delta_1/\Delta_2 = 10$. The values for $c_x/c_y = 4$ and the basal activation rate is taken to be 10^{-3} of the activation rate r_2. A similar pair of bifurcations to the $\Delta_1 : \Delta_2$ control parameter occurs for the $c_x : c_y$ ratio (figure not shown).

In this paper, we focus on the regime of parameter space where oscillations occur via a Hopf bifurcation, which is local to a particular fixed point. The analysis of stability reduces to finding the eigenvalues of a (2×2) Jacobian matrix $J_{ij} - \delta_{ij}\Delta_i$, where $J_{ij} = (\partial(\varphi, \varphi)/\partial(x, y))_{ij}$ evaluated at a fixed point.

For a (2×2) Jacobian, a Hopf bifurcation occurs when the trace vanishes ($\Delta_1 + \Delta_2 = J_{11} + J_{22}$) while the determinant is positive. In Figure 2 we plot the eigenvalues on the complex plane and show that the system becomes unstable when the eigenvalues intersect the imaginary axis in a continuous fashion.

4. DIFFERENT ACTIVATION STRENGTHS DRIVES OSCILLATONS

The Figure 2 shows that as the parameters change, the steady state becomes unstable as the system undergoes a Hopf bifurcation. As a consequence limit cycle oscillations occur as shown in the figure 3 which displays the trajectory drawn against the vectorfield background around the intersection of the x- and y-nullclines. It is known that in genetic oscillators composed of activators and repressors, the times scales of activation and repression have to be well-separated, and this is usually captured by the ratios of the decay times of the two species. The Hopf bifurcation as a function of changing the $\Delta_1 : \Delta_2$ parameter bears testimony to this. What we also find is that for two activators, the ration of activation strengths can also be a parameter driving such a Hopf bifurcation, using the definitions in (5). To get a sense of scale, for the set of parameters used in Figure 3, the differences of the binding free energies is around $3k_BT$.

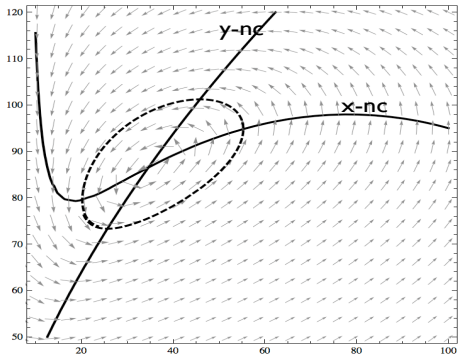

Figure 3. The x- and y- nullclines of the dynamical system and a limit cycle trajectory shown as a dotted line. The parameters used to plot the curves are ($r_0 = 0.001$, $(r_1/r_2) = 24$, $(c_x/c_y) = 4$, $(\Delta_1/\Delta_2) = 10$.

5. DISCUSSION

Mechanisms by which observational patterns are generated enables one to solicit corresponding interventions. In the study of biochemical oscillations, the role of repressors to generate negative feedback has almost always been assumed. The claims in the literature [14] made about the nature of promoter occupancy and competition are made on the basis of such an assumption. Assuming monotonicity of the contributions of transcription factors can be a rather restrictive assumption as well, as it precludes the search for mechanisms such as the one proposed here. We close with a speculation about the origin of such a motif in evolution. Circadian clocks are autonomous oscillators that are entrained by the physical signatures of the earth's rotation. It is likely that a transition from an externally driven responsive system to an autonomous mechanism must have occurred at least once in evolution. Gene duplication is considered to be a major source of evolutionary novelty. The duplicated nature of the transcription control in (9) suggests a putative origin of such a motif in gene duplication and diversification processes[19] operating on an autoactivating gene.

6. REFERENCES

[1] U. Alon, "Network motifs: theory and experimental approaches," *Nat Rev Gen*, vol. 8, pp. 450–461, Jun 2007.

[2] A. Arkin, *et al*, "Stochastic kinetic analysis of developmental pathway bifurcation in phage λ-infected *Escherichia coli* cells," *Genetics*, vol. 149, pp. 1633–1648, 1998.

[3] B. Novak and J. J. Tyson, " Design principles of biochemical oscillators," *Nat Rev Mol Cell Biol*, vol. 9, pp. 981–991, 12 2008.

[4] M. Ptashne, *A Genetic Switch: Phage Lambda Revisited*, CSHL Press, 3rd ed., 2004.

[5] R. Guantes and J. F. Poyatos, "Dynamical principles of two-component genetic oscillators," *PLoS Comput Biol*, vol. 2, no. 3, pp. e30, 03 2006.

[6] H. Ukai and H. R. Ueda, "Systems biology of mammalian circadian clocks," *Ann Rev Physiol*, vol. 72, pp. 579–603, 2010.

[7] R. D. Kornberg, "Mediator and the mechanism of transcriptional activation," *Trends in Biochem Sci*, vol. 30, pp. 235–239, 2005.

[8] R. V. Kondratov, *et al*, "Dual role of the CLOCK/BMAL1 circadian complex in transcriptional regulation," *The FASEB Journal*, 2006.

[9] K. Senger, *et al*, "Gene repression by coactivator repulsion," *Mol Cell*, vol. 6, no. 4, pp. 931–937, Oct 2000.

[10] V. H. Pakka, *Dynamics of molecular fluctuations in gene regulatory networks.*, Ph.D. thesis, University of Southampton, UK, 2009.

[11] G. K. Ackers, *et al*, "Quantitative model for gene regulation by lambda phage repressor," *PNAS*, vol. 79, no. 4, pp. 1129–1133, 1982.

[12] T. Gedeon, *et al*, "When activators repress and repressors activate: A qualitative analysis of the shea–ackers model," *Bull Math Bio*, vol. 70, pp. 1660–1683, 2008.

[13] P. B. Warren and P. R. ten Wolde, "Enhancement of the stability of genetic switches by overlapping upstream regulatory domains," *Phys. Rev. Lett.*, vol. 92, no. 12, pp. 128101, Mar 2004.

[14] A. Munteanu, *et al*, "Avoiding transcription factor competition at promoter level increases the chances of obtaining oscillation," *BMC Sys Bio*, vol. 4, no. 1, pp. 66, 2010.

[15] L. Bintu, *et al*, "Transcriptional regulation by the numbers: models," *Curr Op Gen and Dev*, vol. 15, no. 2, pp. 116 – 124, 2005.

[16] J. Stricker, *et al*, "A fast, robust and tunable synthetic gene oscillator," *Nature*, vol. 456, pp. 516–519, 2008.

[17] J. Hasty,*et al*, "Synthetic gene network for entraining and amplifying cellular oscillations," *Phys. Rev. Lett.*, vol. 88, no. 14, pp. 148101, Mar 2002.

[18] D. Gonze, *et al*, "Robustness of circadian rhythms with respect to molecular noise," *PNAS*, vol. 99, no. 2, pp. 673–678, 2002.

[19] S. A. Teichmann and M. M. Babu, "Gene regulatory network growth by duplication," *Nat Gen*, vol. 36, pp. 492–6, 2004.