Enhancement of the stability of genetic switches by overlapping upstream regulatory domains

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We study genetic switches formed from pairs of mutually repressing operons. The switch stability is characterised by a well defined lifetime which grows sub-exponentially with the number of copies of the most-expressed transcription factor, in the regime accessible by our numerical simulations. The stability can be markedly enhanced by a suitable choice of overlap between the upstream regulatory domains. Our results suggest that robustness against biochemical noise can provide a selection pressure that drives operons, that regulate each other, together in the course of evolution.

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Biochemical networks are the analog computers of life. They allow living cells, to detect, transmit, and amplify environmental signals, as well as integrate different signals in order to recognize patterns in, say, the food supply. Indeed, biochemical networks can perform a variety of computational tasks analogous to electronic circuits. However, their design principles are markedly different. In a biochemical network, computations are performed by molecules that chemically and physically interact with each other. These interactions are stochastic in nature. This becomes particularly important when the concentrations are low. In gene regulatory networks, this is generally the case: not only the DNA, but also the proteins that regulate gene expression are often present in very small numbers, which can be as low as ten, or even fewer. Hence, one would expect that gene regulatory networks, in contrast to electronic circuits, are highly stochastic and error prone \cite{1, 2, 3, 4, 5}. An important question, therefore, is how the ability to resist biochemical noise constrains the design of the network \cite{2, 3}.

In prokaryotes, the expression of operons—groups of contiguous genes that are transcribed into single mRNA molecules—is regulated by the binding of transcription factors (TFs) to upstream regulatory domains on the DNA. A spatial arrangement in which two operons are transcribed in diverging directions allows the upstream regulatory domains to interfere with each other. This affords additional regulatory control. In particular, biochemical noise in the expression of operons can become correlated or anticorrelated. Just as the existence of operons provides for correlated gene expression, interference between the regulatory domains of two diverging operons allows for correlated or anticorrelated expression of operons. Here, we show that this can have a dramatic influence on the stability of gene regulatory networks.

Recently, we have performed a statistical analysis of the spatial distribution of operons on the genome of \textit{Escherichia coli} \cite{6}. The analysis identified a large number of motifs in which the operator regions of two operons overlap and interfere \cite{6}. Among them are well-known examples such as the lysA-lysR and the araBAD-araC operon pairs \cite{7}. But perhaps the best-known and arguably the most studied example of such a motif is provided by the $\lambda$-phage switch, which consists of two adjacent operons that mutually repress each other \cite{8}. Here, we study a minimalist model of such a switch, as shown in Fig. 1. In particular, we compare the stability of an ‘exclusive’ (XOR) switch, for which the simultaneous binding of the repressive TFs for both operons is inhibited, to that of a general switch. We find that the exclusive switch is much more stable than the general switch. The basis reason is that one cannot simultaneously turn off both operons in the exclusive switch. This demonstrates the potential importance of such motifs in making gene regulatory networks robust against biochemical noise. Although these constructions seem peculiar to prokaryotes, evidence for correlated and anticorrelated gene expression has also been reported for eukaryotes \cite{9, 10}. 

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1}
\caption{(a) In a prokaryote, a so-called toggle switch can be formed from a pair of operons that mutually repress each other. (b) If these operons are transcribed in diverging orientations, the upstream regulatory domains can interfere with each other.}
\end{figure}
TABLE I: Distinct possibilities for the subsets of allowed genome states for our switch model.

| case / genome states | O   | OAₙ | OBₘ | OAₙBₘ |
|----------------------|-----|-----|-----|-------|
| general              | ✓   | ✓   | ✓   | ✓     |
| exclusive            | ✓   | ✓   | ✓   | ×     |
| partially co-operative| ✓  | ✓   | ×   | ✓     |
| totally co-operative | ✓  | ×   | ×   | ✓     |

The starting point of our analysis is a set of chemical reactions that constitute the switches shown in Fig. 1. As chemical species, we introduce a pair of TFs which can exist as monomers, A and B, or multimers, Aₙ and Bₘ. The state of the genome is represented by O, OAₙ, etc., depending on the binding of the TF multimers. Adopting a condensed notation in which ‘|’ indicates alternative sets of reactants and ‘→’ indicates that the reactants are not destroyed by the reaction, the set of chemical reactions are

\[
\begin{align*}
  nA &\rightleftharpoons Aₙ, \\
  mB &\rightleftharpoons Bₘ, \\
  O + Aₙ &\rightleftharpoons OAₙ, \\
  O + Bₘ &\rightleftharpoons OBₘ, \\
  OAₙ + Bₘ &\rightleftharpoons OAₙBₘ, \\
  OAₙ + OBₘ + Aₙ &\rightleftharpoons OAₙBₘ, \\
  O | OAₙ &\rightleftharpoons A, \\
  O | OBₘ &\rightleftharpoons B, \\
  A | B &\rightarrow \emptyset.
\end{align*}
\]

These reactions account for, respectively, the formation of multimers, the binding of TF multimers to the genome (Eqs. (1b) and (1c)), the expression of TF monomers, and the degradation of TF monomers. Repression of gene expression is implicit in Eqs. (1d), thus A is expressed if and only if Bₘ is not bound, etc. Reaction rates are as indicated, and we define equilibrium constants for multimerisation, \(K_d = k_t/k_b\), and binding to the genome, \(K_b = k_{on}/k_{off}\).

Whilst detailed and biologically faithful models can be constructed as has been done for the λ-phage switch \([11, 12]\), the above model is intentionally ‘as simple as possible’. We believe such an approach is as important as detailed biological modelling in elucidating the basic physics behind switches. Thus, for example, we have condensed the details of transcription and translation into a single reaction step in Eqs. (1f), governed by rate coefficients \(k_A\) and \(k_B\). On the other hand, as Cherry and Adler have shown \([13]\), the TF binding isotherms must satisfy certain criteria in order to make a working switch. In the present model this is effected by introducing cooperativity through the binding of TF multimers rather than monomers.

In our model the genome is in one of four states \(\{O, OAₙ, OBₘ, OAₙBₘ\}\). We now include the effect of interference between the upstream regulatory domains by disallowing some of these states (this is in the spirit of simplicity, strictly speaking the effect is to modify the probabilities of the states). Since the empty genome is always a possibility and both Aₙ and Bₘ should be allowed to bind otherwise they would not be TFs, it turns out that there are only five possibilities, two of which are related by symmetry. The four distinct cases are shown in Table I and are implemented by excluding some of the reactions in Eqs. (1b) and (1c). For example, the exclusive switch is obtained by discarding the reactions in Eqs. (1b) thereby removing the state OAₙBₘ.

We first use mean-field theory to analyse the behaviour of Eqs. (1). Switching behaviour corresponds to the appearance of two distinct stable states in the space of TF molecule numbers. Previously, general switches were studied in detail by Cherry and Adler \([13]\), and a specific example of the exclusive switch was studied by Kepler and Elston \([14]\). We extend the analysis of Cherry and Adler to determine where switching behaviour can occur, for all the cases in Table I. Firstly, for \(n = m = 1\), no switching behaviour can be found for any case. This confirms that some form of co-operative binding is required. For the totally co-operative switch though, switching behaviour cannot be found for any values of \(n\) and \(m\). For the remaining cases, we have analysed in detail the situation for \(n = m = 2\) where both TFs bind as dimers.

Fig. 2 shows the regions in the \((\mu_A/k_A, \mu_B/k_B)\) plane where switching behaviour is found. Clearly, switching behaviour is more extensive for the exclusive switch compared to the general switch, and is strongly suppressed for the partially co-operative switch. Thus we conclude that, at least in mean field theory, the structure of the switch has a strong influence on the extent of switching behaviour.

To go beyond mean field theory, we have simulated the reactions in Eqs. (1) using Gillespie’s kinetic Monte-
Carlo scheme which generates sample trajectories appropriate to the chemical master equation \[12\]. We focus on dimerising \((n = m = 2)\) general and exclusive switches, and on the symmetry line \(\mu_A = \mu_B = \mu\) and \(\mu_A = \mu_B = \mu\). We will use the expression rate \(k \approx 0.1–1\,\text{s}^{-1}\) \[12\] as a unit of \((\text{inverse})\) time and the degradation rate \(\mu\) as the main control parameter. The choice of the rate constants is biologically motivated, in particular we expect expression to be a slow step and the binding equilibrium to be biased in favour of bound states \[12\]. For a baseline set we use \(k_b/V = k_\text{on} = 5k_{\text{off}} = 5k\) \((K_d/V = 1\) and \(K_b/V = 5\)), where \(V \approx 2\,\mu\text{m}^3\) is the cell volume \[12\]; we assume one copy of the genome is present.

We monitor the total numbers of the TFs, \(N_A\) and \(N_B\), including those in dimers and those bound to the genome. If the system is behaving as a switch then we typically see that one of the TFs is strongly repressed compared to the other one. A switching event occurs when the roles of the two TFs flip spontaneously, as shown in Fig. 4.

We can obtain more insight into the switching behaviour by sampling the probability distribution \(P(N_A, N_B)\) for states in the \((N_A, N_B)\) plane, as shown in Fig. 4. Switching behaviour appears as a double maximum in probability in this representation, and the transition state is seen to lie at low numbers of both TFs. Three points are worthy of note. First of all, it is seen that the positions of the two stable steady states do not depend much on the architecture of the switch. This is not surprising, because if one species dominates, both switches will behave similarly. What is perhaps more surprising, is that the pathways for switching are different. The transition paths of the exclusive switch cross the transition state surface at higher values of \(N_A = N_B\), as compared to the general switch. The reason is that in the general switch both genes can be repressed simultaneously, while in the exclusive switch only one gene can be turned off at the time. More importantly, however, the barrier for flipping the switch is higher for the exclusive switch than for the general switch, as can be seen in the insets in Fig. 4. This is because for a switch to flip, two events have to happen. First of all, the system has to wait for a rare fluctuation by which the concentration of the dominant species decreases; this allows for the synthesis of the other component. Subsequently, the latter component has to bind to its operator site in order to toggle the switch. In the general switch, the latter event is more probable, because the minor component can bind to its site as soon as it is synthesized, while in the exclusive switch the dominant species first has to dissociate from the DNA. This is the main reason why the exclusive switch is more stable than the general switch.

We have also characterised the switching dynamics, by constructing the cumulative distribution function \(F(\Delta t)\) for the time intervals \(\Delta t\) between zero crossings of the order parameter \(N_A – N_B\). About 50% of \(F\) arises from noise on a time scale \(\Delta t \sim k^{-1}\) as the system jitters around the transition state, but for \(\Delta t \gg k^{-1}\), and provided we are well into the switching regime, we invariably see Poisson statistics with \(F \rightarrow 1 – \exp[-\Delta t/\tau]\) (see Fig. 4b). This firstly confirms that the switch states have a well defined lifetime \(\tau\), and secondly allows us to extract an accurate estimate of the value of \(\tau\).

Bialek has suggested that the switch lifetime may grow exponentially with the number of molecules involved in switching between states \[10\]. Motivated by this, we monitor the mean number \(\langle N \rangle\) of the most-expressed TF,
defined to be the time average of $\max(N_A, N_B)$. We can also calculate $\overline{N}$ from mean field theory, and we find good agreement between this and the value measured in the simulations, as $\mu$ varies.

Qualitative support for Bialek’s conjecture comes from Fig. 3. It shows that $\tau$ grows very rapidly with $N$, which is the basic reason why extremely stable switches can be built with at most a few hundred expressed proteins. In contrast to Bialek’s conjecture, however, $\tau(N)$ appears to be sub-exponential in $N$. Interestingly, we can fit $\tau(N)$ to a form that is suggested by an analysis of a related problem, namely that of switching between broken-symmetry phases in a driven diffusive model. This suggests that the ultimate scaling is $\tau \sim N^\alpha \exp[bN]$, where $\alpha$ and $b$ are constants. Note that this corresponds to Bialek’s conjecture, but with a logarithmic correction in $N$.

More importantly, however, Fig. 3 clearly demonstrates that the switch construction does indeed have a marked influence on the stability of the switch. It shows that the lifetime of the exclusive switch grows much more rapidly with mean copy number than that of the general switch. Our simulations cover $10 \lesssim N \lesssim 30$, but if we extrapolate our results to $N \approx 100$, then $k\tau \approx 10^4 - 10^6$ for the general switch but $k\tau \approx 10^8 - 10^{10}$ for the exclusive switch. In the latter case, this corresponds to lifetimes measured in tens of years. Such extremely long lifetimes have been reported for λ-phage.

In summary, a genetic switch is intrinsically stochastic, because of the molecular character of its components. However, our simulations demonstrate that the stability of a genetic switch can be strongly enhanced by spatially arranging the operons such that competing regulatory molecules mutually exclude each other at the operator regions. Such a spatial arrangement can be achieved if the two operons lie next to each other on the DNA and are transcribed in diverging directions – a network motif that has been identified by our statistical analysis of the gene regulatory network of E. coli. Hence, our simulations suggest that robustness against biochemical noise can provide a selection pressure that drives pairs of operons, that either regulate each other or are controlled by a common transcription factor, towards each other in the course of evolution.

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FIG. 5: Switch lifetime as a function of mean number of most-expressed TF, for the general (solid line) and exclusive (dashed line) cases. The exclusive switch becomes orders of magnitude more stable than the general switch at high numbers of the expressed TF.

[1] H. H. McAdams and A. Arkin, Proc. Natl. Acad. Sci. 94, 814 (1997).
[2] M. B. Elowitz and S. Leibler, Nature 403, 335 (2000).
[3] N. Barkai and S. Leibler, Nature 403, 268 (2000).
[4] E. M. Ozbudak, M. Thattai, I. Kurtser, A. D. Grossman, and A. van Oudenaarden, Nat. Genet. 31, 69 (2002).
[5] M. B. Elowitz, A. J. Levine, E. D. Siggia, and P. S. Swain, Science 297, 1183 (2002).
[6] P. R. ten Wolde and P. B. Warren, q-bio/0310029
[7] R. Wagner, Transcription regulation in prokaryotes (OUP, Oxford, 2000).
[8] M. Ptashne, A genetic switch: phage lambda and higher organisms, 2nd edn (Blackwell, Oxford, 1992).
[9] P. J. Willy, R. Kobayashi, and J. T. Kadonaga, Science 290, 982 (2000).
[10] Z. Szallasi, TRENDS Pharmacol. Sci. 22, 110 (2001).
[11] A. Arkin, J. Ross, and H. H. McAdams, Genetics 149, 1633 (1998).
[12] E. Aurell, S. Brown, J. Johanson, and K. Sneppen, Phys. Rev. E 65, 051914 (2002).
[13] J. L. Cherry and F. R. Adler, J. theor. Biol. 203, 117 (2000).
[14] T. B. Kepler and T. C. Elston, Biophys. J. 81, 3116 (2001).
[15] D. T. Gillespie, J. Phys. Chem. 81, 2340 (1977).
[16] W. Bialek, in Advances in Neural Information Processing 13, eds T. K. Leen, T. G. Dietterich and V. Tresp (MIT Press, Cambridge, 2001); see also cond-mat/0005235
[17] M. R. Evans, D. P. Foster, C. Godrèche, and D. Mukamel, Phys. Rev. Lett. 74, 208 (1995).
[18] C. Godrèche, J. M. Luck, M. R. Evans, D. Mukamel, S. Sandow, and E. R. Speer, J. Phys. A: Math. Gen. 28, 6039 (1995).
[19] We have also examined the effect of varying parameters away from the baseline set, such as increasing the number of TFs generated per expression reaction in order to mimic the synthesis of multiple copies of TFs from a single mRNA transcript. The associated changes in the noise of gene expression affect the switch stability, but the main conclusion that the exclusive switch is much more stable than the general switch remains unchanged.