Epigenetics as a first exit problem

E. Aurell\textsuperscript{1,2,3} and K. Sneppen\textsuperscript{3}

\textsuperscript{1}SICS, Box 1263, SE-164 29 Kista, Sweden
\textsuperscript{2}SANS/NADA/KTH, SE-100 44 Stockholm, Sweden
\textsuperscript{3}NORDITA, Blegdamsvej 17, DK-2100 Copenhagen, Denmark

(February 1, 2008)

Pacs numbers: 87.87.16-b

We develop a framework to discuss stability of epigenetic states as first exit problems in dynamical systems with noise. We consider in particular the stability of the lysogenic state of the $\lambda$ prophage, which is known to exhibit exceptionally large stability. The formalism defines a quantitative measure of robustness of inherited states.

Epigenetics is concerned with inherited states in living systems, which are not encoded as genes, but as the (inherited) patterns of expressions of genes. Modulation of gene expression, or functional genomics, underlies a wide number of biological phenomena, e.g. efficient use of nutrients available to an organism at a particular time. A familiar example of inherited patterns of gene expression is cell differentiation in multicellular organisms, which, if once established, can be propagated for long times. The stability of epigenetic states is important, as it simultaneously enables an organism to maintain a favorable state, and to keep the ability to change that state in a coordinated manner, if external conditions so dictate.

Some of the simplest examples of two-state systems in biology are found among bacteriophages, DNA viruses growing on bacterial hosts. When in the state of being stably integrated into the genome of the host, known as lysogeny, one set of viral genes is expressed. When on the other hand the virus is performing other tasks, such as directing translation of viral proteins leading to lysis, killing the bacterial host, other sets of genes are expressed. The classical example is the lysogenic state of phage $\lambda$ in *Escherichia coli* \textsuperscript{1}. Upon infection of an *E. coli* cell, either $\lambda$ enters a path leading to lysis, or it enters lysogeny, which can then be passively replicated for very long times. Indeed, the wild-type rate of spontaneous loss of $\lambda$ lysogeny is only about $10^{-5}$ per cell and generation \textsuperscript{2}, a life-time of the order of five years. Moreover, this number is but a consequence of random activation of another part of the genetic system, the SOS response involving RecA, and the intrinsic loss rate has in several independent experiments been found to be less than $10^{-7}$ per cell and generation \textsuperscript{3}, possibly as low as $2 \cdot 10^{-9}$ \textsuperscript{4}. The rate of mutations in the part of the lambda genome involved in lysogeny is between $10^{-6}$ and $10^{-7}$ per generation \textsuperscript{5}. Epigenetics is therefore in this system more stable than the genome itself.

One may recall that E. Schrödinger in “What is life?” \textsuperscript{6} starts by imagining that the stability of genetic inheritance stems from dynamic equilibria involving macroscopic numbers of molecules. On the basis of the then recent experimental data on the dependence of mutation rate on radiation, this hypothesis is discarded in favor of a molecular model of genetic memory, fore-shadowing the DNA-RNA machinery. Epigenetics, in particular $\lambda$ lysogeny as presented below, gives an example where Schrödinger’s first idea is essentially correct. The number of molecules needed to achieve stability, over biologically relevant time-scales, is however surprisingly small, only in the order of hundreds, and thus rather in the mesoscopic than in the macroscopic range. Such a number however nevertheless gives fluctuations in gene expression \textsuperscript{7}, and accordingly but a finite stability of many states.

A stable state can be likened to a control switch that is on. For $\lambda$ the analogy is quite direct \textsuperscript{8}: lysogeny is maintained by protein molecules and $\lambda$ DNA around an operator $O_R$, which consists of three binding sites $O_{R1}$, $O_{R2}$ and $O_{R3}$, overlapping with two promotor sites $P_{RM}$ and $P_R$, see Fig. \textsuperscript{9}. At the binding sites either one of two regulatory proteins CI and Cro can bind. These proteins are produced from the corresponding genes $cI$ and cro, which abut $O_R$, and which are regulated by CI and Cro. Hence transcription of cro starts at $P_R$, which partly overlaps $O_{R1}$ and $O_{R2}$, while transcription of $cI$ starts at $P_{RM}$, which partly overlaps $O_{R2}$ and $O_{R3}$. The affinity to the two promoters of RNA polymerase, the enzyme which catalyses the production of mRNA transcripts from DNA, depends on the pattern of Cro and CI bound to the operator sites. The rates of production of the two proteins are therefore functions of the concentrations of the proteins themselves, and balance decay and dilution in a stable stationary state with 200-300 CI and, on the average, few Cro per bacterium \textsuperscript{10}. This is a logical switch, because if CI concentration becomes sufficiently low, the increased activation of cro increases Cro concentration and decreases $cI$ activation, so that lysogeny is ended and lysis follows.
FIG. 1. Right operator complex, $O_R$, consisting of the three operators $O_{R1}$, $O_{R2}$ and $O_{R3}$. $cI$ is transcribed when $O_{R3}$ is free and $O_{R2}$ is occupied by CI. $cro$ is transcribed when both $O_{R2}$ and $O_{R1}$ is free. CI dimers bind cooperatively to $O_{R1}$ and $O_{R2}$.

The simplest mathematical model which embodies Fig. 1 is a set of coupled equations for the time rate of change of numbers of $CI$ and $Cro$ in a cell [11]:

\[
\begin{align*}
\dot{N}_{CI} &= \phi_{CI}(N_{CI}, N_{Cro}) - \frac{N_{CI}}{\tau_{CI}} \\
\dot{N}_{Cro} &= \phi_{Cro}(N_{CI}, N_{Cro}) - \frac{N_{Cro}}{\tau_{Cro}}
\end{align*}
\]

(1)

where the net production rates are

\[
\begin{align*}
\phi_{CI} &= f_{CI}(N_{CI}, N_{Cro}) - \frac{N_{CI}}{\tau_{CI}} \\
\phi_{Cro} &= f_{Cro}(N_{CI}, N_{Cro}) - \frac{N_{Cro}}{\tau_{Cro}}
\end{align*}
\]

(2)

The production terms $f_{CI}$ and $f_{Cro}$ are functions of CI and Cro concentrations. With no Cro in the system, the curve of $f_{CI}$ vs. CI concentration has been experimentally measured [11]. As reviewed in [6], these measurements are consistent with the best available data on protein-DNA affinities [12–14], dimerization constants [15], initiation rates of transcriptions of the genes, and the efficiency of translation of the mRNA transcripts into protein molecules. The decay constant $\tau_{CI}$ is proportional to the bacterial life-time, since CI molecules are not actively degraded in lysogeny, while $\tau_{Cro}$ is about 30 % smaller [16]. We remark that there is considerably more experimental uncertainty in the binding of Cro, both to other Cro and to DNA, than the binding of CI, see e.g. [17]. As a minimal model of the switch, we take $\tau_{CI}$ and $\tau_{Cro}$ from data, and deduce $f_{CI}$ and $f_{Cro}$ at non-zero concentrations of both CI and Cro with a standard set of assumed values of all binding constants, as done in [6]. Such a model is conveniently visualized by the phase space plot in Fig. 2.

If system (1) is in lysogeny, i.e. in the stable equilib-rum $S$, it will stay there indefinitely. The system leaves lysogeny when external perturbations push CI concentration to the left of the separatrix. In vivo, as sketched above, the important perturbation is RecA–mediated self-cleavage of CI, as a by-product of the SOS DNA repair mechanism when host DNA is damaged. The functional purpose of the switch, for the virus, is hence to sense if the host is in danger, and, if so, jump ship.

If the numbers of CI and Cro were macroscopically large, then (1) would be an entirely accurate description of the dynamics. The numbers are however only in the range of hundreds. The actual production process is influenced by many chance events, such as the time it takes for a CI or a Cro in solution to find a free operator site, or the time it takes a RNA polymerase molecule to find and attach itself to an available promotor. If in a time interval $\Delta t$ the expected number of molecules produced is $f_{CI} \Delta t$, then the number produced in an actual realization has scatter $\sqrt{f_{CI} \Delta t}$. As a minimal model of the switch with finite-$N$ noise, we therefore consider the following system of two coupled stochastic differential equations, with two independent standard Wiener noise sources $(d\omega^1_t, d\omega^2_t)$:

\[
\begin{align*}
\dot{N}_{CI} &= \phi_{CI}(N_{CI}, N_{Cro}) - \frac{N_{CI}}{\tau_{CI}} + g_{CI} d\omega^1_t \\
\dot{N}_{Cro} &= \phi_{Cro}(N_{CI}, N_{Cro}) - \frac{N_{Cro}}{\tau_{Cro}} + g_{Cro} d\omega^2_t
\end{align*}
\]

(3)

We assume that there is an equal amount of finite-$N$ noise in decay as in production, and the two noise amplitudes are hence

\[
\begin{align*}
g_{CI} &= \sqrt{f_{CI} + \frac{N_{CI}}{\tau_{CI}}} \\
g_{Cro} &= \sqrt{f_{Cro} + \frac{N_{Cro}}{\tau_{Cro}}}
\end{align*}
\]

(4)

The problem of escape from a stable equilibrium point like $S$ under a dynamics like (3) is a first-exit problem in the theory of stochastic processes. As such is it solved in Wentzel-Freidlin theory [18–19]. A special case of Wentzel-Freidlin theory is well-known from chemical physics, namely if the noise amplitudes $(g_{CI}, g_{Cro})$ are constant and the drift field is a potential field. If so, the problem of escape from $S$ is Kramers’ classical problem of thermal escape from a potential well [20–21]. The more general Wentzel-Freidlin problem has both similarities and differences to Kramers’ problem, as we will now explain.
The phase space plot of the dynamical system (1), and the optimal exit path in the stochastic dynamical system (3).

The lysogenic state is identified with a stable equilibrium $S$ at $(N_{CI}, N_{Cro}) \approx (225, 1)$. The basin of attraction of this equilibrium is delimited by a separatrix (basin boundary), which passes through the unstable equilibrium point $A$ at $(N_{CI}, N_{Cro}) \approx (56, 101)$. The separatrix crosses the CI-axis at $N_{CI} \approx 33$. Also indicated is the most probable exit path (full line with arrow) from $S$ to $A$. Insert shows a blow-up around unstable equilibrium $A$. Note that the most probable exit path goes into $A$ at a different angle compared to the trajectories of (1) going out of $A$. Parameter values are as in [6].

Proceeding heuristically, we note that the probability of a given realization of the noise in time $[0,T]$ is

$$
\text{Prob}(\{\omega_{CI}^{t}, \omega_{Cro}^{t}\}_{0}^{T}) 
\propto \exp \left( -\frac{1}{2} \int_{0}^{T} \left( \frac{\dot{N}_{CI} - \phi_{CI}}{\Gamma_{CI}} + \frac{\dot{N}_{Cro} - \phi_{Cro}}{\Gamma_{Cro}} \right)^{2} dt \right) \tag{5}
$$

where we have introduced the diagonal elements of the diffusion matrix, $\Gamma_{CI} = g_{CI}^{2}$ and $\Gamma_{Cro} = g_{Cro}^{2}$.

Of all the realizations that move the system from $S$ to $A$, the most probable is the one that minimizes the action functional

$$
\mathcal{A} = \frac{1}{2} \int_{0}^{T} \left( \frac{(\dot{N}_{CI} - \phi_{CI})^{2}}{\Gamma_{CI}} + \frac{(\dot{N}_{Cro} - \phi_{Cro})^{2}}{\Gamma_{Cro}} \right) dt \tag{6}
$$

where the initial position is $S$, the final position $A$, and the minimization is taken over all paths that go from $S$ to $A$ in time $T$. If $\mathcal{A}^{\text{min}} \gg 1$ it can be proved (see [19]) that the most probable exit point from the basin of attraction of $S$ is indeed $A$, and the rate of exit is to leading order

$$
\text{Rate}(\text{exit}) \propto \exp \left( -\mathcal{A}^{\text{min}} \right) \tag{7}
$$

The asymptotic formula (7) also contains a prefactor of dimension one over time $[13,19]$, which in the case of a potential field reduces to the prefactor in Kramers’ formula [20]. In our case the prefactor is of order once per bacterial generation. The optimal exit path obeys the appropriate Euler-Lagrange equations, being the extremal of variations of $\mathcal{A}$. Since the Lagrangian in (6) is not explicitly time-dependent, the Hamiltonian

$$
\mathcal{H} = \frac{1}{2} \left( \Gamma_{CI} p_{CI}^{2} + \Gamma_{Cro} p_{Cro}^{2} \right) + p_{CI} \phi_{CI} + p_{Cro} \phi_{Cro} \tag{8}
$$

is conserved along the path. The momenta $(p_{CI}, p_{Cro})$ are conjugate to the generalized coordinates $(N_{CI}, N_{Cro})$, and the Euler-Lagrange equations are equivalent to Hamilton’s equations $\dot{N} = \frac{\partial \mathcal{H}}{\partial p}$ and $\dot{p} = -\frac{\partial \mathcal{H}}{\partial N}$. We note that in this auxiliary classical mechanical system, the diffusion constants play the role of space-dependent elements of an inverse mass matrix, while the drift field is somewhat similar to a magnetic potential. We also note that the energy of the optimal exit path, the value of $\mathcal{H}$ along that path, must be non-negative, since the drift field vanishes at the two end points. On the other hand, we have in general $\partial \mathcal{H}/\partial T = -E$, where $E$ is the energy and $T$ is the transit time. It hence follows that...
the optimal exit path is a zero-energy path from $S$ to $A$ under the Hamiltonian in (8).

Fig. 2 shows the optimal fluctuation path. In contrast to thermal escape from a potential well, where the optimal path is always opposite in direction and equal in size to the drift field, there is no obvious simple prescription to directly compute the path from $S$ to $A$ in Fig. 2. The Hamiltonian analogy however suggest the following numerical procedure, using the relaxation method of computing solutions to 2-point boundary problems in an ODE [22,23].

We first find a natural parameter in the system, typically one of the binding constants, and vary that to get close to the bifurcation where the stable and unstable equilibria ($S$ and $A$) coalesce. The diffusion parameters $\Gamma_{CI}$ and $\Gamma_{Cro}$ are then practically constant in a neighbourhood around both points, while the drift field is small. We can then compute a path between the two points at high energy (equivalently, at a small transit time $T$), starting from a trial solution, which is a straight path at constant speed. In other words, $\dot{N}$ is taken constant along the trial path, and $\vec{p} = \frac{\partial L}{\partial \dot{N}}$ is given by $(\Gamma^{-1}) \cdot \dot{N} - \dot{\phi}$. The energy is then lowered incrementally, and the optimal path at each energy found by relaxation, using the previous solution as the trial solution at the new energy. A zero-energy path can thus be found close to the bifurcation, and, by changing back the parameter in small steps, again using relaxation to find each new path, a zero-energy path can be found at the original parameter value. The zero-energy motion in the intermediate neighbourhoods of the two points always needs to be taken care of by a local calculation, as explained in [19].

![Stable and unstable equilibria](image1.png) ![Change of Wentzel–Freidlin action](image2.png)

**FIG. 3.** Systemic changes due to changes in affinity of Cro to operator site OR3. The standard value is $-15.5$ kcal/mol. Stronger binding energies are investigated for use in numerical procedure (see main text), and to explore robustness of lysogeny to parameter changes. Other parameters are as in [3]. a) Location of stable equilibrium ($S$) and unstable equilibrium ($A$) as affinity is varied. The two equilibria are born in a bifurcation at affinity $-17.78$ kcal/mol and $(N_{CI}, N_{Cro}) \approx (176, 3.73)$. At increasing value of binding energy (weaker binding), the equilibria move apart, as indicated by the arrows. Insert shows the $N_{CI}$ value of the lysogenic state (stable equilibrium), as function of the affinity, in kcal/mol. b) Wentzel-Freidlin action as function of affinity. The escape rate from lysogeny is exponential in the action, with a prefactor of the order once per bacterial generation. At the standard value (-15.5 kcal/mol), the predicted rate from the model is hence about once in $10^{13}$ generations. We note that the escape rate depends very sensitively on parameters. A change of affinity by 1 kcal/mol to $-16.5$ kcal/mol gives an action of about 2.5, and an expected lifetime of the lysogenic state of only about ten generations.

There is an emerging consensus in molecular biology and biological physics that chemical networks in living cells have to be robust [24–26]. That is, they have not only to work under some conditions, but should work under a wide variety of conditions, possibly even under change or replacement of parts of the network, see e.g. recent mathematical modelling of cell signaling [27], and of a genetic control network for cell differentiation in *Drosophila* [28]. For the λ phage, robustness of lysogeny has been experimentally established for several large modifications of the OR complex [3]. The present work allows us to quantify robustness of epigenetic states. A state only exists at all if deterministic equations like (1) have a stable equilibrium with the corresponding properties. This state is stable for long times, even if the number of molecules involved is small, if the action $A$ in
and (2) is much larger than unity. The state is finally robust, if under a typical change of a changeable parameter \( \mu \), the state still exists and is stable. This means that \( \Delta \mu \cdot \frac{dA}{d\mu} \) must be significantly less than \( A \), where the typical change \( \Delta \mu \) could be the change in binding energy upon a single point mutation, of order 1 kcal/mol.

In Figs. 3a,b) we examine lysogenic stability as function of one parameter, the binding of Cro to \( O_R^3 \). If we first disregard the noise, we see that a change of affinity by 2.25 kcal/mol brings the stable and unstable equilibria together, such that the lysogenic state disappears altogether. We also observe a sensitive dependence of the position of the unstable equilibrium, while the number of CI in the stable equilibrium (lysogenic state) only changes by 30%. The lysogenic state therefore looks qualitatively similar over this range of parameters. These are features of the model embodied by equations (1) only. If we then bring in our model of the noise, equation (3), we see that the action \( A \) changes from more than 30 to less than 3 when affinity changes by 1 kcal/mol, the approximate change of binding energy under a single point mutation. Such a change hence suffices to destabilize the switch over biologically relevant time-scales, and the model is therefore not robust to such changes, in contradiction to (1). This implies the presence of some additional mechanism, in order for robustness to prevail. We stress that this lack of robustness is an inherent property of the model, true for all variations of parameters that have been put forward to quantitatively describe these generally accepted mechanisms of control, including the recent report that Cro may in fact bind cooperatively to \( O_R \) [17].

In conclusion, we have examined the general problem of escape from a stable equilibrium in more than one dimension, and demonstrated how this determines the stability of states of genetic networks. In contrast to Kramers’ escape from a potential well, the stability of inherited states in such networks is not a mathematically and computationally trivial problem. The most likely exit path does not go along a steepest decent of a potential – there is no potential. Instead, such a path can be described as a zero-energy trajectory between two equilibria in an auxiliary classical mechanical system. Finding it involves similar numerical problems as e.g. computing heteroclinic orbits in celestial mechanics. The overall lesson of this study is that an examination of equilibria and their bifurcations with changing parameter values allow us to quantify both the stability and the robustness of particular states of a genetic control system.

ACKNOWLEDGEMENTS

We thank Prof. G. Dahlquist for suggesting the numerical procedure used to find the minimum of the action, and B. Altshuler, S. Brown, P. Kraulis, P. Muratore-Ginanneschi and B. Öbrink for discussions and valuable comments. E.A. thanks the Swedish Natural Research Council for support under grant M-AA/FU/MA 01778-333. K.S. thanks the ITP (U.C. Santa Barbara) for hospitality, and consequently the National Science Foundation for financial support under grant No. PHY99-07949.

[1] A. D. Johnson, A. R. Poteete, G. Lauer, R. T. Sauer, G. K. Ackers, & M. Ptashne, Nature 294, 217-223 (1981).
[2] M. A. Shea, & G. K. Ackers J. Mol. Biol. 181, 211-230 (1985).
[3] M. Ptashne, A Genetic Switch; Phage \( \lambda \) and Higher Organisms, Blackwell Scientific Publications & Cell Press (1992).
[4] D. V. Rozanov, R. D’Ari & S. P. Sineokiy, J. Bacteriol. 180, 6306-6315 (1998).
[5] J. Little, D. P. Shepley & D. W. Wert, EMBO J. 18, 4299-4307 (1999).
[6] E. Aurell, S. Brown, J. Johansson & K. Sneppen (2000) Biophysical J. [submitted, cond-mat/0010286].
[7] J. W. Little, private communication (2000).
[8] E. Schrödinger, “What is Life” (1944).
[9] A. Arkin, J. Ross & H. H. McAdams Genetics 149, 1633-1648 (1998).
[10] J. Reinitz and J. R. Vaisnys, J. Theoretical Biol. 145, 295-318 (1990).
[11] D. K. Hawley, & W. R. McClure, J. Mol. Biol. 157, 493-525 (1982).
[12] J. G Kim, Y. Takeda, B. W. Matthews & W. F. Anderson, J. Mol. B 196, 149-158 (1987).
[13] S. K. Koblan & G. K. Ackers, Biochemistry 31, 57-65 (1992).
[14] Y. Takeda, P. D. Ross, & C. P. Mudd, Proc. Natl. Acad. Sci. USA 89, 8180-8184 (1992).
[15] S. K. Koblan & G. K. Ackers, Biochemistry 30, 7817-7821 (1991).
[16] A. A. Pakula, V. B. Young & R. T. Sauer, Proc. Natl. Acad. Sci. USA 83, 8829-8833 (1986).
[17] P. J. Darling, J. M. Holt & G. K. Ackers, J. Mol. Biol. 302, 625-38 (2000).
[18] M. Freidlin, & A. Wentzell, Random perturbations of Dynamical Systems, Springer-Verlag. New York/Berlin (1984).
[19] R. S. Maier & D. S. Stein, SIAM J. Appl. Math. 57, 752-790 (1997).
[20] H. A. Kramer, Physica 7, 284-304 (1940).
[21] P. Hänggi, P. Talkner, & M. Borkovec, Rev. Mod. Phys. 62, 251-341 (1990).
[22] W. Press, B. Flannery, S. Teukolsky & W. Vetterling, 
Numerical Recipes, Cambridge University Press (1988).
[23] P. P. Eggleton, Mon. Not. R. Astron. Soc. 151, 351 (1971).
[24] M. A. Savageau, Nature 229, 855-857 (1971).
[25] U. Alon, M. G. Surette, N. Barkai & S. Leibler, Nature 397, 168-171 (1999).
[26] W. Bialek, (2000), [cond-mat/0005235].
[27] U. Bhalla & R. Iyengar, Science 283, 381-387 (1999).
[28] G. von Dassow, E. Meir, E. Munro & G. Odell, Nature 406, 188-192 (2000).