SYSTEMS BIOLOGY AND DEVIATION CURVATURE TENSOR

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Abstract. In this article, we study the robustness of biological systems by means of the eigenstructure of the deviation curvature tensor. This is the differential geometric theory of the variational equations for deviation of whole trajectories to nearby ones. We apply this theory to the Van der Pohl equations and some biological models, and examine the relationship between the linear stability of steady-states and the stability of transient states. The main application is the G_1-model for the cell cycle, where Jacobi stability reveals the robustness and fragility of the cell arrest states and suggests the existence of more subtle checkpoints.

1. Introduction

Recent genome sequencing projects and further functional analysis of complete gene sets provided in the last few years a huge mass of molecular information for a wide range of model organisms. The large number of structural components, interactions and control processes between them make the internal mechanism of the cell extremely difficult to deal with only by empirical considerations. It is also almost impossible to formulate any general hypothesis or framework that are consistent with all these data. To overcome this situation, technology based on computer science with firm mathematical background is strongly required.

The difficulty of mathematical modeling in biology comes from the differences between the notions of *"what to look for"* used in biology and mathematics. Biology usually classifies the components of the biological system (eg. genes, mRNA's, proteins) and the interactions between them by experiments and empirical observations without paying much attention to the physical-mathematical principles that lie underneath. Because of this, in general, biological models lack information essential for simulation analysis and do not have a mathematical background (eg. statistics or theory of differential equations).

On the other hand, mathematical models include information essential for simulation analysis, but the interaction between the components of the biological systems are less visualized and less easy to understand and interpret.

We consider that both approaches are not enough by themselves to understand the complex life process. Biologists often claim that they do not need complicated mathematics to analyze their systems and that traditional empirical methods are enough. However, identifying all genes and proteins in a living cell is like listing all the components of a computer: wires, chips, transistors,

condensers, display, mouse, etc. This will provide only a sort of catalog of the computer. Even knowing how these components are inter-connected, such that the computer will work, won't reveal the sophisticated underlying mathematics and engineering. Biology may provide the components of the cell (genes, proteins, mitochondrion, etc.), as well as some of the interactions between them (regulatory gene network, metabolic pathways, etc). But, it cannot answer questions such as: how are signals encoded, how is noise fluctuation connected with adaptability, how do cells act when a malfunction occurs, or what are the design principles and circuit patterns of the cell.

In order to understand these topics a combined *biology-mathematics* approach, called also **systems biology**, [Kitano 2002], is needed and the bridge between these two fields should be *computer science*.

Once we have an understanding of the system structure, we can approach the system dynamics. There are a lot of methods of doing this in mathematics and engineering and the preference for one or another depends on the availability of biological data incorporated in the model. In the present paper, we will use steady-states analysis and KCC-theory to do this.

Cell simulation is a very important topic because the experiments *in vitro* are quite time- and money- consuming. By simulating the behavior of the cell within the computer one can save a lot of time and money in drug discovery research ([Kitano 2002]).

To conduct a system-level analysis, some mathematical tools have to be used. There have been many attempts to use mathematics in Life Science, but much of it is not of relevance to real biological modeling. There are yet interesting methods, from both the biological and mathematical points of view, for example the algebraic approach to sequence analysis and the Central Dogma of molecular biology ([Carbone–Gromov 2001]), and also modeling metabolic pathways and chemical reactions by the use of differential equations ([Murray 1993]), etc. We are also going to show in this paper that differential geometric methods, namely KCC-theory, or Jacobi stability, can be a useful tool in the study of the properties of metabolic pathways, [Antonelli et al. 1993].

One of the most important properties of biological systems is their *robustness* and their *fragility*. By *robustness* we mean both the relative insensitivity to alteration of their internal parameters and the ability to adapt to changes in their environment. In very robust systems, even damage to their internal topological structure produces only minor alterations in their behavior. Such properties of *robustness* are achieved through feedback, modularity, redundancy and structural stability. However, the robustness of biological systems does not come without a cost, and this is their *fragility*. All complex systems that are very robust against common or known perturbations can often be very sensitive to new perturbations. We point out that the robustness of a system is not always an advantage for the organism as a whole. For instance, cancer cells are extremely robust for their own growth and survival against various perturbations. They proliferate by the cell division cycle mechanism eliminating the communication with the environment, becoming in this way insensitive to

external perturbations. However, in spite of this kind of *robustness*, it may still be possible to eliminate the cancer cells by exploiting their *fragility*.

Biological processes, especially protein production, can be described by first order differential equations ([Murray 1993]). By elimination of some variables or using the conservation of enzymes, we can transform the initial system of first order differential equations into a topologically conjugate second order system of differential equations (SODE) with fewer equations. In the general case this is not always possible, but the differential equations concerning us are based on the law of mass action or Michaelis–Menten formalism and this kind of transformation is always possible ([Murray 1993]).

In this paper we propose a method of studying the *robustness* and *fragility* of a biological system (genetic network, pathway) by means of the deviation curvature tensor of a semispray.

This Jacobi stability analysis is complementary to the linear stability of Step 4 above. The differential geometric theory of the variational equations for the deviation of whole trajectories to nearby ones allows us to estimate the admissible perturbation around the steady-states of a differential equations system. Here, by *admissible* we mean perturbations that do not change the stability ranges of the system (see also [Antonelli et al. 1993], [Antonelli et al. 1998], [Lackey, B. 1999], [Antonelli et al. 2002], [Antonelli–Bradbury 1996] for related discussions and applications).

The aims of the present paper are as follows,

(1) To define the robustness of a biological system as the Jacobi stability of nearby trajectories. Therefore, we propose the following "algorithm" for studying the robustness:
   - **Step 1.** The key variables of the biological phenomenon are identified together with the their interactions (feedbacks, loops).
   - **Step 2.** Differential equations describing the temporal evolution of the system are constructed (by the law of mass action or Michaelis-Menten formalism, see [Murray 1993]).
   - **Step 3.** The steady-states of these differential equations are determined.
   - **Step 4.** The stability of the steady-states is studied (linear stability, bifurcation analysis, existence of limit cycles, etc).
   - **Step 5.** The Jacobi stability of nearby trajectories is studied. The theoretical predictions of the model for some specific range of the parameters are compared with the experimental data or theoretical biological facts. If the model's predictions do not agree with the existing biological knowledge, then the model has to be modified accordingly.

(2) To study the relationships between the linear (Liapunov) stability of steady-states of a first order system of differential equations (FODE) and the Jacobi stability of the deviation curvature tensor of the attached SODE. This is done by evaluating the sign of the real part of

the eigenvalues of the *deviation curvature (Second KCC-invariant tensor* P_i^j). Generally, linear stability and Jacobi stability do not lead to the same stability ranges. These analysis methods are complementary, but distinct from one another. Jacobi stability leads to another kind of bifurcation type analysis, which is nevertheless a Liapunov analysis.

(3) We will illustrate our method with several examples: Van der Pohl equations, the Tyson model ([Tyson 1991]) for frog eggs cell cycle and the G_1-phase of the cell division cycle (for details see [Tecarro et al. 2003]). We point out that similar to the usual bifurcation analysis, Jacobi critical points can be seen biologically as checkpoints in the cell cycle mechanism, yet different from the ones indicated by the Hopf bifurcation analysis.

2. Biological Setting

2.1. **Central Dogma of Molecular Biology.** Living biological systems are very complex but, at the same time, they are highly ordered in a remarkably efficient way. Such systems store the information and the means necessary for cellular reproduction, organization, control, etc. in each generation in the *genome*, the total collection of genetic material in the chromosomes of an organism. It is known that a large number of cell types are capable of protein production, self-regulation and control. The regulatory mechanism in cellular physiology starts with the synthesis of mRNA copied from a gene. This process is called *transcription*. From these mRNAs, a *protein* (an enzyme) is generated according to the genetic code carried by mRNA. This second step is called *translation*. This above described transcription and translation form together *The Central Dogma of Molecular Biology*, or Crick's dogma.

2.2. **Pathways.** Identification of metabolic pathways is a major challenge in Life Science.

A cell is a chemical mechanism in constant interaction with the environment. The processing of chemicals, needed for the cell to stay alive and replicate itself, is organized into *metabolic pathways*. One can think of it as a directed graph $\mathcal{M}$ where the vertices are marked by various chemical compounds, and where edges represent chemical transformations ([Carbone–Gromov 2001], http://www.genome.ad.jp/kegg/kegg2.html).

2.3. **Reaction Kinetics.** Biochemical reactions are continuously present in all living cells and lie in certain biochemical pathways. These pathways can be inferred from the literature or directly from experimental data.

Our point here is that always when working with biochemical reactions it is highly probable that the number of equations can be reduced mainly for the following two reasons:

1. The differential equations describing the variation in time of concentrations of reactants are not all independent.

2. The enzymes are working as catalysts in biochemical reactions, this means that their concentration is conserved.

In general, reaction kinetics of n reactions result in an n-th order system of first order ODE

$$\frac{du_i}{dt} = f_i(u_1, u_2, ..., u_n), \quad i \in \{1, 2, ..., n\}, \tag{2.1}$$

where $u_1, u_2, \ldots, u_n$ are the concentrations of the substrates, and the functions $f_1, f_2, \ldots, f_n$ encode the biochemical reactions between them.

We are only concerned with non-negative solutions since $u(t)$ is a vector of concentrations. The classical methods of stability analysis are applicable, as we shall see.

The examples presented so far are quite simple and they are meant to illustrate the biochemical mechanism encoded by the Central Dogma. However, even the simplest biological organisms are much more complicated than these examples.

2.4. **The cell division cycle.** Somatic cells reproduce by duplicating their contents and then dividing in two. This cell division cycle is the fundamental means by which all somatic cell types are duplicated in an individual. The cell cycle has four phases: synthesis (S), mitosis (M), and some gap phases (G_1), (G_2).

During the cell division cycle the cell accomplishes four tasks:

(1) cell growth (during the G_1-phase)
(2) replicate its DNA (during the S-phase)
(3) segregate the chromosomes (during the G_2-phase)
(4) cellular division (during the M-phase)

The standard cell cycle (for adult cells) is generally quite long in mammals, its length depending on the type of cell (for brain cells, a very long period, and for the liver, a shorter period). Cells in G_1, if they have not yet committed themselves to DNA replication, can pause in their progress through the cell cycle and enter a specialized *resting state*, often called G_0 where they can remain for days, weeks or even years before resuming proliferation (see [Alberts et al. 1994] for details).

It is also important that S and M phases alternate in time. For example, if a haploid cell attempts two mitotic nuclear divisions in a row, without a complete S phase, the resulting daughter cell will only inherit incomplete genomes and die.

Proper progression through the cell cycle is monitored at some *checkpoints* where the cell verifies whether it is prepared to enter the next stage of the cell division cycle.

If problems arise at these checkpoints, they block the exit of the cell from the previous stage.

3. Stability

3.1. **Linear stability of ODE's.** Given the differential equation $\dot{x}(t) = f(x)$, let $\varphi^t(x_0)$ be the solution $x(t)$ with the initial condition x_0 at $t = 0$, i.e.

$$\varphi^0(x_0) = x_0, \qquad \frac{d}{dt}\varphi^t(x_0) = f(\varphi^t(x_0)), \tag{3.1}$$

at all t for which the solution is defined. It is customary to write $\varphi(t, x_0)$ for $\varphi^t(x_0)$. The function $\varphi^t(x_0)$ is called the *flow* of the differential equation, and the function f defining the differential equation is called the *vector field which generates the flow.*

The *phase-portrait* of a two-dimensional differential equation $\dot{x} = f(x)$ is a drawing of the solution curves with the direction of increasing time indicated. In an abstract sense, the phase portrait is the drawing of all solutions, but in practice it only includes the representative trajectories. The *phase space* is the domain of all x's considered. For a standard analysis see for eg. [Murray 1993].

REMARK.

Having Liapunov stability ([Robinson 1995])means geometrically that the trajectories are "converging". In other words, the nonlinear flow near the fixed point sink is an exponential contraction if the linear flow is one. Indeed, let p be a fixed point for the equation $\dot{x} = f(x)$, with $x \in I\!R^n$, and let us assume that all the eigenvalues λ for $(Df)_{|p}$ have negative real parts; so there exists a constant $a > 0$ such that $Re(\lambda) < -a < 0$ for all λ. Then, for any norm $||\cdot||$ on $I\!R^n$ there exists a neighborhood $U \in I\!R^n$ of p and a constant $c \geq 1$ such that for any initial condition $x \in U$, the solution is defined for all $t \geq 0$ and satisfies $||\varphi^t(x) - p|| \leq c \cdot e^{-ta}||x - p||$, for all $t > 0$.

3.2. **KCC-theory and Jacobi stability.** Let us recall first some basics ([Antonelli 2000], [Antonelli et al. 1993][1], [Antonelli, Bucataru 2003]). Let $(x^1, \ldots, x^n) = (x)$,

$$\left(\frac{dx^1}{dt}, \frac{dx^2}{dt}, \ldots, \frac{dx^n}{dt}\right) = \left(\frac{dx}{dt}\right) = \dot{x} \tag{3.2}$$

and t be $2n + 1$ coordinates of an open connected subset Ω of the Euclidean $(2n+1)$-dimensional space $I\!R^n \times I\!R^n \times I\!R^1$. And let us consider a second order differential equation (SODE) of the form

$$\frac{d^2x^i}{dt^2} + g^i(x, \dot{x}, t) = 0, \ i \in \{1, 2, \ldots, n\}, \tag{3.3}$$

where each function $g^i(x, \dot{x}, t)$ is C^∞ in a neighborhood of some initial conditions $((x)_0, (\dot{x})_0, t_0)$ in Ω.

In order to find the basic differential invariants of the system (3.3) under the non-singular coordinate transformations

$$\begin{aligned} &\bar{x}^i = f^i(x^1, \ldots, x^n), \ i \in \{1, 2, \ldots, n\}, \\ &\bar{t} = t, \end{aligned} \tag{3.4}$$

[1]The term "KCC-theory" was coined for the first time by P.L. Antonelli in [Antonelli et al. 1993].

we define the KCC-covariant differential of a contravariant vector field $\xi^i(x)$ on the open subset Ω by

$$\frac{D\xi^i}{dt} = \frac{d\xi^i}{dt} + \frac{1}{2}\, g^i_{;r}\xi^r, \tag{3.5}$$

where the semicolon ";" indicates partial differentiation with respect to $\dot{x}$. The idea of this approach belongs to Kosambi [Kosambi 1933], E. Cartan [Cartan 1933] (who corrected Kosambi's work), and S.S. Chern (for the most general version) [Chern 1939]. The Einstein summation convention will be used throughout.

Using (3.5), the system (3.3) becomes

$$\frac{D\dot{x}^i}{dt} = \frac{1}{2}\, g^i_{;r}\dot{x}^r - g^i = \varepsilon^i, \tag{3.6}$$

where ε^i defined here is a contravariant vector field on Ω and is called the *first* KCC-*invariant*. It is interpreted as an external force [Antonelli 2000].

The functions $g^i = g^i(x, \dot{x}, t)$ are 2-homogeneous in $\dot{x}$ if and only if $\varepsilon^i = 0$. In other words, $\varepsilon^i = 0$ is a necessary and sufficient condition for a semispray to be a spray. It is obvious that for the geodesic spray of a Riemannian or Finsler manifold, the first invariant vanishes.

Let us vary the trajectories $x^i(t)$ of (3.3) into nearby ones according to

$$\bar{x}^i(t) = x^i(t) + \eta\, \xi^i(t), \tag{3.7}$$

where η denotes a parameter with $|\eta|$ small and where $\xi^i(t)$ are the components of some contravariant vector field defined along the path $x(t)$. Since $\bar{x}$ and x are solutions of (3.3), it is not difficult to see that $\eta\ddot{\xi} + (\bar{g} - g) = 0$, where $\bar{g} - g := g(t, x + \eta\xi, \dot{x} + \eta\dot{\xi}) - g(t, x, \dot{x})$. View $\bar{g} - g$ as a function of η and apply the mean value theorem. This enables us to cancel off the η which multiplies $\ddot{\xi}$. Finally, take the limit $\eta \to 0$ to obtain the variational equations

$$\frac{d^2\xi^i}{dt^2} + g^i{}_{;r}\, \frac{d\xi^r}{dt} + g^i{}_{,r}\xi^r = 0, \tag{3.8}$$

where the comma "," indicates partial differentiation with respect to x^r.

Using now the KCC-covariant differential (3.5), one obtains (3.8) in the covariant form

$$\frac{D^2\xi^i}{dt^2} = P^i_r\xi^r, \tag{3.9}$$

where

$$P^i_j = -g^i{}_{,j} - \frac{1}{2}g^r g^i{}_{;r;j} + \frac{1}{2}\dot{x}^r g^i{}_{,r;j} + \frac{1}{4}g^i{}_{;r}g^r{}_{;j} + \frac{1}{2}\frac{\partial g^i{}_{;j}}{\partial t} \tag{3.10}$$

is called the *second* KCC-*invariant* of the system (3.3), or *deviation curvature tensor*.

Note that (3.9) is the *Jacobi field equation* whenever system (3.3) are the geodesic equations in either Finsler or Riemannian geometry. The notion of Jacobi stability for SODE's is thus a generalization of that for the geodesics of a Riemannian or Finsler manifold. This justifies the usage of the term *Jacobi stability* for KCC-Theory.

We are interested in the "*focusing tendency*" of trajectories of (3.3) in a vecinity of a point $x^i(t_0)$ of it. For simplicity we will consider $t_0 = 0$.

Let us consider the trajectories $x^i = x^i(t)$ of (3.3) as curves in the Euclidean space $(I\!R^n, < \cdot, \cdot >)$, where $< \cdot, \cdot >$ is the canonical inner product of $I\!R^n$, and let us impose to the deviation vector ξ the initial conditions

$$\xi(0) = O, \qquad \dot{\xi}(0) = W \neq O,$$

where $O \in I\!R^n$ is the null vector.

We consider now an "adapted" inner product $<< \cdot, \cdot >>$ to the deviation tensor ξ by

$$<< X, Y >>:= \frac{1}{< W, W >} \cdot < X, Y >$$

for any vectors $X, Y \in I\!R^n$. Obviously, $||W||^2 :=<< W, W >>= 1$.

Then, we can imagine the "*focusing tendency*" of trajectories around 0 as:

- trajectories are **bunching together** if $||\xi(t)|| < t^2$, $t \approx 0^+$,
- trajectories are **dispersing** if $||\xi(t)|| > t^2$, $t \approx 0^+$

(see the pioneers work [Auslander 1955], [Rund 1959], [Laugwitz, D. 1965], and [Bao et al. 2000] for a modern explanation treatment).

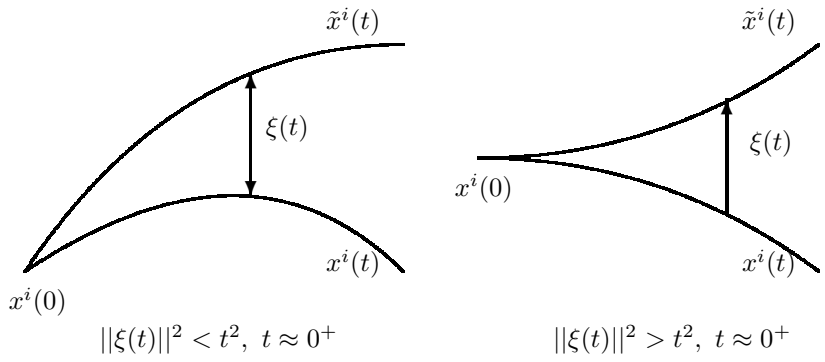


Figure 1. Behavior of trajectories near zero.

It follows:

Lemma 3.1

Let us consider the SODE (3.3) and its variation (3.7) with the tensor $\xi(t)$ satisfying the initial conditions:

$$\xi(0) = O, \qquad \dot{\xi}(0) = W \neq O,$$

where $O \in I\!R^n$ is the null vector.

Then, for $t \approx 0^+$, the trajectories of (3.3) are

- *bunching together iff the real part of the eigenvalues of $P^i_j(0)$ are strict negative,*
- *dispersing iff the real part of the eigenvalues of $P^i_j(0)$ are strict positive.*

Proof.

This proof follows the one in [Bao et al. 2000] for the Finslerian geodesics. Starting with the "Jacobi equations" (3.9), one calculates

$$\begin{aligned}
\xi^{(1)}: &= \Big(\frac{D\xi^i}{dt}\Big)e_i,\\
\xi^{(2)}: &= \Big(\frac{D^2\xi^i}{dt^2}\Big)e_i = (P^i_r\xi^r)e_i,\\
\xi^{(3)}: &= \Big(\frac{D^3\xi^i}{dt^3}\Big)e_i = \Big(\frac{DP^i_r}{dt}\xi^r + P^i_r\frac{D\xi^r}{dt}\Big)e_i\\
\xi^{(4)}: &= \Big(\frac{D^4\xi^i}{dt^4}\Big)e_i,
\end{aligned}$$

where $e_i,\ i\in\{1,2,...,n\}$ is an orthonormal basis of $I\!R^n$ with respect to the inner product $<<\cdot,\cdot>>$.

And evaluating these in $t=0$, one gets

$$\begin{aligned}
\xi(0) &= 0,\ \xi^{(1)}(0) = W,\\
\xi^{(2)}(0) &= 0,\ \xi^{(3)}(0) = (P^i_r(0)W^r)e_i.
\end{aligned}$$

If denoting $f(t) :=<<\xi(t),\xi(t)>>$, then

$$\begin{aligned}
f(0) &= 0\\
f^{(1)}(0) &= 0\\
f^{(2)}(0) &= 2<<W,W>>= 2\\
f^{(3)}(0) &= 0\\
f^{(4)}(0) &= 8<<P^i_r(0)W^re_i,W>>.
\end{aligned}$$

Then, locally, for $t\approx 0^+$, one has

$$\begin{aligned}
f(t) &= f(0)+\frac{1}{1!}f^{(1)}(0)t+\frac{1}{2!}f^{(2)}(0)t^2+\frac{1}{3!}f^{(3)}(0)t^3+\frac{1}{4!}f^{(4)}(0)t^4+O(5)\\
&= <<W,W>>t^2+\frac{1}{3}<<P^i_rW^re_i,W>>t^4+O(t^5)\\
&= t^2+\frac{1}{3}(P^i_rW^rW_i)t^4+O(t^5) = t^2+\frac{1}{3}Kt^4+O(t^5),
\end{aligned}$$

where $W_j := \delta_{ij}W^i$, and $K := P^i_rW^rW_i$.

It is clear that

- if $K<0$, then $f(t)<t^2$, i.e. trajectories are bunching together,
- if if $K>0$, then $f(t)>t^2$, i.e. trajectories are dispersing.

And this is equivalent with the fact that the matrix $P^j_i(0)$ has to have only eigenvalues with strictly negative, and positive real parts, respectively.

□

Now we can introduce the notion of Jacobi stability as follows. This kind of stability refers to the "focusing tendency" (in a small vecinity of t_0) of trajectories of (3.3) with respect to a variation (3.7) that satisfy the condition

$$||x^i(t_0)-\tilde{x}^i(t_0)|| = 0,\ ||\dot{x}^i(t_0)-\dot{\tilde{x}}^i(t_0)|| \neq 0.$$

Definition.
The trajectories of (3.3) are called Jacobi stable at $(x(t_0), \dot{x}(t_0))$ *if and only if the real parts of the eigenvalues of the deviation tensor* $P_i^j|_{t_0}$ *are strict negative, and Jacobi unstable, otherwise.*

Remark. The third, fourth and fifth KCC-invariants are:

$$R_{jk}^i = \frac{1}{3}(P_{j;k}^i - P_{k;j}^i), \quad B_j{}^i{}_{k\ell} = R_{jk;\ell}^i, \quad D_j{}^i{}_{k\ell} = g_{;j;k;\ell}^i. \tag{3.11}$$

A basic result of the KCC-theory is the following

Proposition 3.2 ([Antonelli 2000]) *Two* SODE's *of form* (3.3) *on* Ω *can be locally transformed, relative to* (3.4)*, one into other, if and only if their five* KCC-*invariants* ε^i, P_j^i, R_{jk}^i, $B_j{}^i{}_{k\ell}$, $D_j{}^i{}_{k\ell}$ *are equivalent tensors. In particular, there are local coordinates* $(\bar{x})$ *for which* $g^i(\bar{x}, \dot{\bar{x}}, t) = 0$ *if and only if all five* KCC-*tensors vanish.*

Remark. It would be interesting to correlate Linear stability with Jacobi stability. In other words, to compare the signs of the eigenvalues of the Jacobian matrix J at a fixed point with the signs of the eigenvalues of the deviation curvature tensor P_i^j evaluated at the same point. Even though this should be possible in the general case, we give here only a discussion for the 2-dimensional case.

Let us consider the following system of ODE:

$$\frac{du}{dt} = f(u, v) \qquad \frac{dv}{dt} = g(u, v) \tag{3.12}$$

such that the point $(0, 0)$ is a fixed point, i.e. $f(0, 0) = g(0, 0) = 0$. In general, even though the fixed point is (u_0, v_0), by the change of variables $\bar{u} = u - u_0$, $\bar{v} = v - v_0$, one can always obtain $(0, 0)$ as a fixed point. We denote by J the Jacobian matrix of (3.12), i.e.

$$J(u, v) = \begin{pmatrix} f_u & f_v \\ g_u & g_v \end{pmatrix} \tag{3.13}$$

where the subscripts indicate partial derivatives with respect to u and v.

The oscillation conditions of (3.12) are $trA > 0$ and $detA > 0$, where A is the Jacobian J evaluated at the fixed point $(0, 0)$, i.e. $J|_{(0,0)} = A$. Using the characteristic equation and some elementary algebra, we see that only the following *periodic behavior of* (3.12) *are possible*:

(1) The point $(0, 0)$ is an unstable spiral, i.e.

$$\Delta < 0, \qquad S > 0.$$

(2) The point $(0, 0)$ is an unstable node, i.e.

$$\Delta > 0, \qquad S > 0, \qquad P > 0,$$

where $\Delta = (f_u - g_v)^2 + 4 f_v g_u = (trA)^2 - 4\, detA$, $S = f_u + g_v = trA$, and $P = f_u g_v - f_v g_u = detA$ are the discriminant of the characteristic equation, the sum and product of the eigenvalues, respectively.

By elimination of one of the variables, we can transform (3.12) into a SODE of the form (3.3) and compute the deviation curvature. It does not matter which of the two variables we eliminate, because the resulting differential equations are topologically conjugate ([Robinson 1995]).

Let us relabel v as x, and $g(u,v)$ as y, and let us assume that $g_u|_{(0,0)} \neq 0$. The variable to be eliminated, u in this case, should be chosen such that this condition holds. Since $(u,v) = (0,0)$ is a fixed point, the theorem of implicit functions implies that the equation $g(u,x) - y = 0$ has a solution $u = u(x,y)$ in the vicinity of $(x,y) = (0,0)$. Now, $\ddot{x} = \dot{g} = g_u\, f + g_v\, y$. Hence we obtain an autonomous one-dimensional case of the SODE (3.3), namely $\ddot{x}^1 + g^1 = 0$, where

$$g^1(x,y) = -g_u(u(x,y),x)\, f(u(x,y),x) - g_v(u(x,y),x)\, y. \tag{3.14}$$

Evaluating now the curvature tensor P_1^1 from (3.10) at the fixed point $(0,0)$, we obtain after some computation:

$$4\, P_1^1|_{(0,0)} = -4 g^1_{,1}|_{(0,0)} + (g^1_{;1})^2|_{(0,0)} = \Delta := (trA)^2 - 4\, detA. \tag{3.15}$$

It can be checked that the second equality would not change if one had used the first equation of (3.12) to eliminate the variable v instead. Now, straightforward calculations give the following.

Theorem 3.3 *Let us consider the ODE (3.12) with the fixed point p=(0,0) such that* $g_u|_{(0,0)} \neq 0$.

Then, the Jacobian J estimated at p has complex eigenvalues with positive real parts if and only if p is a Jacobi stable point.

Corollary 3.4

(i) If p is an unstable spiral , then it is Jacobi stable. Conversely, if $trA > 0$ *and p is Jacobi stable, then it is an unstable spiral.*

(ii) If p is an unstable node, then it is Jacobi unstable. Conversely, if we have $trA > 0$, $detA > 0$, *and p is Jacobi unstable, then it is an unstable node.*

4. Worked Examples

4.1. **Van der Pohl equations.** Van der Pohl equations form a classical example of an oscillatory system. This system also exhibits a limit cycle behavior, but in this case, there are regions in the parameter space where the periodic trajectories are Jacobi stable in their entirety. In other words, for these regions, the *periodic solutions of the Van der Pohl equations are robust.*

Even though it is possible to imagine a set of chemical reactions (i.e. a metabolic pathway) that leads to the Van der Pohl equations, we choose to start directly with the system of differential equations, which reads:

$$\begin{aligned} \frac{du}{dt} &= v := f(u,v) \\ \frac{dv}{dt} &= a(1-u^2)v - u := g(u,v) \end{aligned} \tag{4.1}$$

where $a \in \mathbb{R}$.

4.1.1. **Linear stability.** The unique fixed point is $O(u_0 = 0, v_0 = 0)$, and the Jacobian matrix is

$$J = \begin{pmatrix} 0 & 1 \\ -2auv - 1 & a(1-u^2) \end{pmatrix}. \tag{4.2}$$

It follows that

$$A = J|_{(0,0)} = \begin{pmatrix} 0 & 1 \\ -1 & a \end{pmatrix} \tag{4.3}$$

with the characteristic equation $\lambda^2 - a\lambda + 1 = 0$. We have $trA = a$ and $detA = 1$. The eigenvalues of the system are

$$\begin{aligned} \lambda_1 &= \frac{1}{2}a + \frac{1}{2}\sqrt{a^2-4} \\ \lambda_2 &= \frac{1}{2}a - \frac{1}{2}\sqrt{a^2-4}. \end{aligned} \tag{4.4}$$

The condition for oscillations, $trA > 0$ and $detA > 0$, are equivalent to $a > 0$. On the other hand, the sum and product of the eigenvalues being $S = a$ and $P = 1$, respectively, from the oscillation condition we obtain $S > 0$, $P > 0$.

Under these conditions, we recall that

- if $\Delta > 0$, then the point S is an *unstable node*, and
- if $\Delta < 0$, then the point S is an *unstable spiral*,

where $\Delta = (trA)^2 - 4\,detA$.

Since $\Delta = a^2 - 4$, it follows that:

- if $a \in (2, \infty)$ then the fixed point $O(0,0)$ is an *unstable node*, and
- if $a \in (0, 2)$ then the fixed point $O(0,0)$ is an *unstable spiral*.

The following result is known ([Murray 1993]):

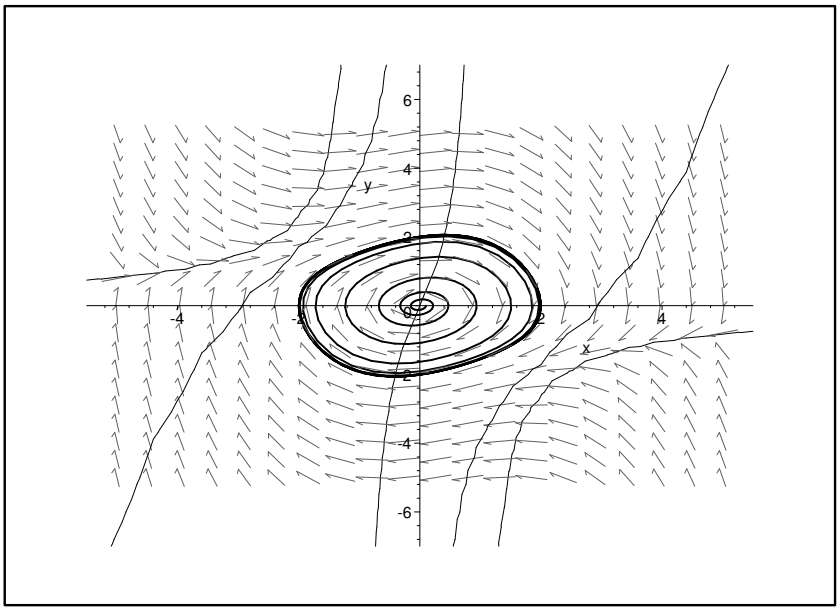
Proposition 4.1 *The solution of the system (4.1) is periodic for any $a \in (0,2)$. Moreover, here the system has a limit cycle behavior.*

4.1.2. **Jacobi stability.** From Collorary 3.4 it follows that:

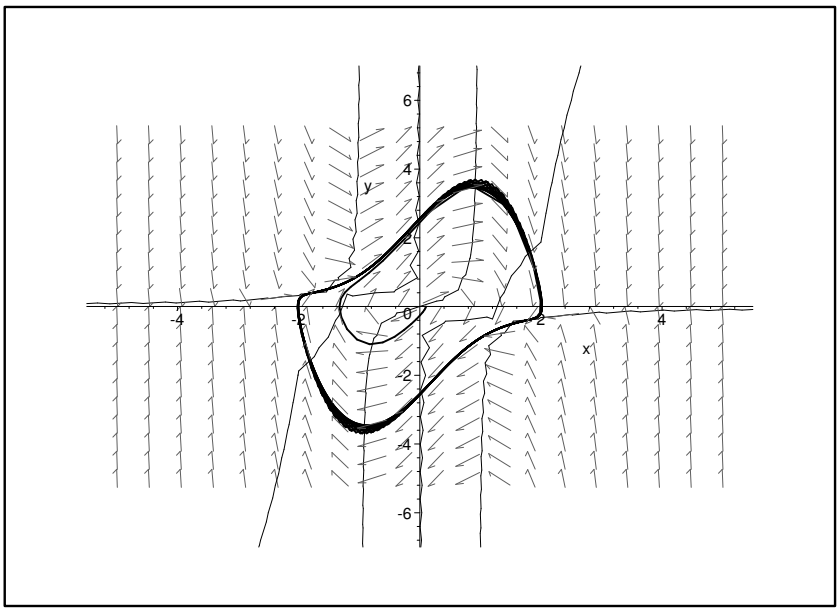
Proposition 4.2 *The fixed point O(0,0) is in the Jacobi stability region if and only if $a \in (0,2)$.*

This can also be seen by a direct calculation of the deviation curvature:

$$P_1^1 = -axy - 1 + \frac{1}{4}a^2(1-x^2)^2. \tag{4.5}$$



(a) For small values of a, the limit cycle lies in the interior of the Jacobi stability region.



(b) For large a, the limit cycle does not lie inside the Jacobi stability region.

Figure 2. The limit cycle of the Van der Pohl equations.

Numerical simulations show that for small values of the parameter $a \in (0, 2)$, for example $a \in (0, 0.4)$, the limit cycle lies in the interior of the Jacobi stability region (see Figure 2 (a)). For this range of parameter, the system exhibits a *robust periodic behavior*. However, for large values, near 2, the limit cycle is no longer contained in the Jacobi stability region (Figure 2 (b)).

In other words, there is a "Jacobi bifurcation" value for a generic solution near 0.4, but we haven't yet for the moment an analytical method to find its value.

4.2. **Cell division cycle for frog embryos.** Let us consider now a mathematical model proposed by Tyson ([Tyson 1991]) for frog eggs. This model was studied in detail in [Antonelli et al. 2002], and we only recall it here. However, the interpretation of Jacobi stability as *robustness* and *fragility* is new.

The periodic trajectories of this biological system are not Jacobi stable in their entirety; therefore, they are not robust.

The mitotic cycles in both embryonic and somatic cells appear to be controlled by the action of an enzyme, the maturation promoting factor (MPF), that peaks abruptly at metaphase (M). The enzyme MPF is a heterodimer composed of two other proteins: *cyclin* and a protein kinase (*cdc*2).

It is known that cyclin accumulation and destruction controls the activation and inactivation of MPF. In other words, the cyclin has to build up to a threshold concentration to activate MPF, and the destruction of cyclin is coupled to the inactivation of MPF and the exit from mitosis.

Since this is a very simplified model for the cell cycle, it is difficult to obtain a cancerous cell model from it.

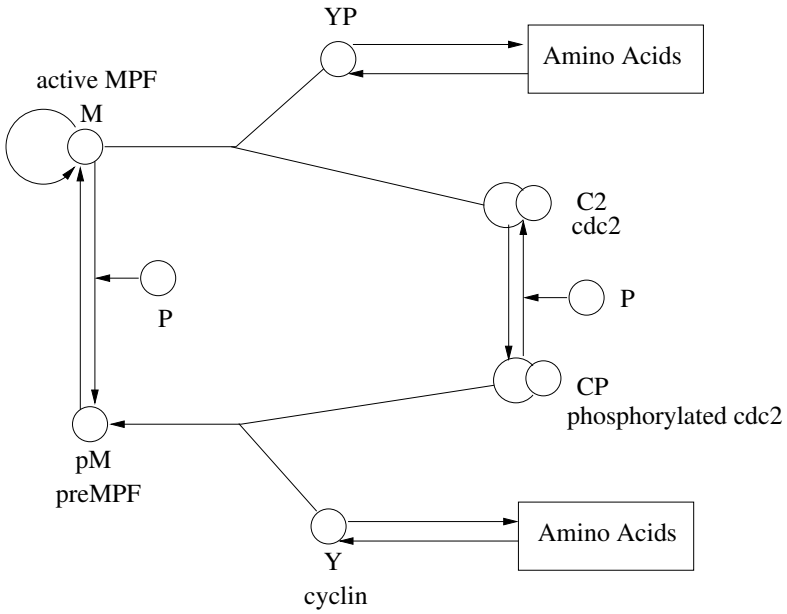


Figure 3. The cell cycle regulatory pathway.

The interplay between *cyclin* and *cdc*2 in generating MPF is understood in detail ([Tyson 1991]).

Newly synthesized cyclin combines with preexisting *cdc*2 to form an inactive MPF complex. The complex is then activated, via autocatalysis, by dephosphorylation at a specific tyrosine residue of *cdc*2. Active MPF is known to stimulate a number of processes essential for nuclear and cell division. At the transition from metaphase to anaphase, the MPF complex dissociates, and the cyclin is rapidly degraded. Then the cycle repeats itself.

The following system of differential equations models what we described:

$$\begin{aligned}
\frac{dC_2}{dt} &= k_6 \cdot M - k_8 \cdot P \cdot C_2 + k_9 \cdot CP \\
\frac{dCP}{dt} &= -k_3 \cdot CP \cdot Y + k_8 \cdot P \cdot C_2 - k_9 \cdot CP \\
\frac{dpM}{dt} &= k_3 \cdot CP \cdot Y - pM \cdot F(M) + k_5 \cdot P \cdot M \\
\frac{dM}{dt} &= pM \cdot F(M) - k_5 \cdot P \cdot M - k_6 \cdot M \\
\frac{dY}{dt} &= k_1 \cdot CT - k_2 \cdot Y - k_3 \cdot CP \cdot Y \\
\frac{dYP}{dt} &= k_6 \cdot M - k_7 \cdot YP,
\end{aligned} \tag{4.6}$$

where the activation by MPF is given by the function $F(M) = k + k_4 M/CT^2$ and CT is the total *cdc*2. We have here six time-dependent variables C_2, CP, pM, M, Y, YP, and 10 parameters.

After some simplifications, the cell cycle regulatory pathway can be expressed by the following system of differential equations:

$$\begin{aligned}
\frac{du}{dt} &= (v-u)(k + k_4 u^2) - k_6 u \\
\frac{dv}{dt} &= k_1 - k_6 u,
\end{aligned} \tag{4.7}$$

where u and v are the relative concentration of active MPF and total cyclin minus degraded cyclin relative to total *cdc*2, respectively, ([Tyson 1991]). The parameter ranges are as follows:

Rate	*Type*	*Value*	*Meaning*
k	constant	0.018 min^{-1}	*cdc*2 dephosphorylation
k_1	constant	0.015 min^{-1}	*cyclin* synthesis
k_4	adjustable	10–1000 min^{-1}	autocatalythic activation of MPF
k_6	adjustable	0.1–10 min^{-1}	breakdown *cdc*2-*cyclin* complex

Table 1. Parameter values for the frog embryos model.

Using the phase-plane analysis, Tyson ([Tyson 1991]) has shown that, depending on the values of k_4 and k_6, the cell cycle regulatory system exhibits three different *modes of control*. For small values of k_6, the system displays a steady-state of high MPF activity, which can be associated with the metaphase arrest of unfertilized eggs (A). For moderate values of k_6, the system executes autonomous oscillations modeling rapid cell cycling in early embryos (B). For large values of k_6, the system is attracted to an excitable steady-state of low MPF activity, which corresponds to interphase arrest of resting somatic cells or to growth-controlled bursts of MPF activity in proliferating somatic cells (C).

The system of ODEs (4.6) can be rewritten in the form (4.7) with the parameter values $k = 0.018$, $k_1 = 0.015$. For k_4 we choose the value 180 when studying the behavior of (4.7) with respect to the values of $k_6 \in (0.1, 10)$.

4.2.1. **Linear stability analysis.** From (4.7) it follows that the only fixed point is $S(u_0, v_0)$, where

$$u_0 = \frac{k_1}{k_6}, \quad v_0 = \frac{k_1}{k_6^2}\frac{(k+k_4)k_6 + k_1^2 k_4}{k_1 k_4 + k k_6}. \tag{4.8}$$

The Jacobian of the system (4.7) is

$$J := \begin{pmatrix} -k - k_4u^2 + 2(v-u)k_4u - k_6 & k + k_4u^2 \\ -k_6 & 0 \end{pmatrix} \tag{4.9}$$

and

$$\begin{aligned} trJ &= -k - k_4u^2 + 2(v-u)k_4u - k_6, \\ detJ &= (k + k_4u^2)k_6 \end{aligned} \tag{4.10}$$

Calculating Δ, the discriminant of the characteristic equation of (4.7), and the trace and determinant of $A = J|_{(u_0,v_0)}$ for the parameter values in Table 1 ($k_4 = 180$), the linear stability of the fixed point $S(u_0, v_0)$ can be given by the following table.

k_6	0.1		0.19		0.36		1.46		1.9	
trA	–		–	0	+	0	–		–	
$detA$	+		+		+		+		+	
Δ	+	0	–		–		–	0	+	
Linear stability	stable			unstable (limit cycle)			stable			

Table 2. Linear stability analysis of the Tyson model.

In other words, the stability of the solution changes accordingly to the values of k_6 passing through two bifurcation points $k_6^{(1)} = 0.36$ and $k_6^{(2)} = 1.46$, respectively. This means that the system (4.7) describes three distinct modes of the cell developmental cycle:

- *mode A*: $k_6 \in [0.1, 0.36)$: linear stability
- *mode B*: $k_6 \in [0.36, 1.46)$: linear instability (limit cycle)
- *mode C*: $k_6 \in [1.46, 10)$: linear stability.

Tyson pointed out that these three modes correspond to the unfertilized egg, early embryos, and somatic cell proliferation, respectively ([Tyson 1991]).

4.2.2. **Jacobi stability analysis.** We have ([Antonelli et al. 2002])

Proposition 4.3
(i) If the system (4.7) is in mode B, i.e. $k_6 \in [0.36, 1.46)$*, then the fixed point S is in the Jacobi stability region.*
(ii) In the linear stability regions A and C, Jacobi stability changes as in Table 3.

k_6	0.1	0.19	0.36	1.46	1.9
Linear stability	S	S	uS (l.c.)	S	S
Jacobi stability	uS	S	S	S	uS

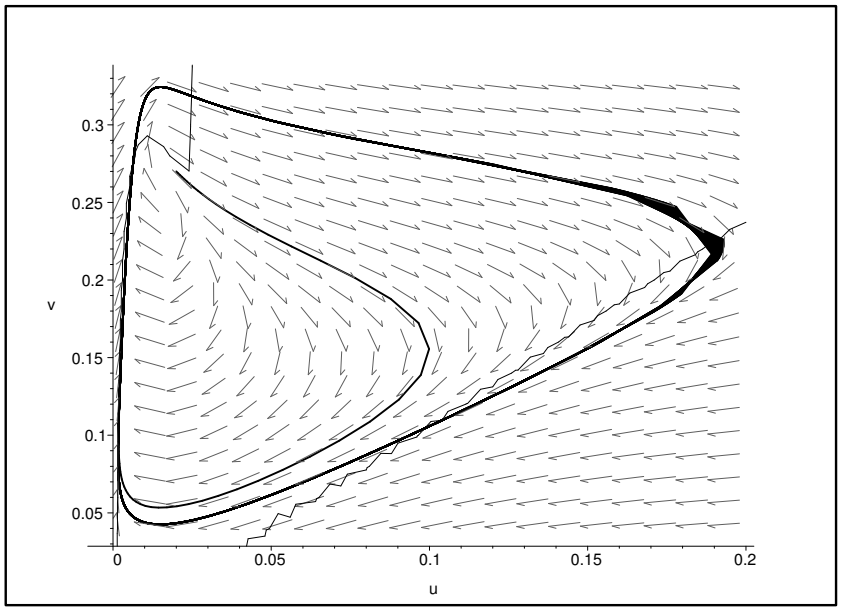
Table 3. Linear stability vs. Jacobi stability of the fixed point S for the Tyson model (here, S = stable, uS = unstable, l.c. = limit cycle).

The Jacobi stability analysis of the steady-state solutions is important because they are the measurable phenotype in the cell.

The whole limit cycle is not included in the Jacobi stability region, but only a vicinity of the fixed point S is (see Figure 4). In other words, *the periodic trajectories are robust only in the interior of this vicinity and fragile outside it.*

Let us explain briefly the biological interpretation of the Jacobi stability analysis on this model. See [Antonelli et al. 2002] for detailed explanations.

(1) In *mode A* (corresponding to the unfertilized eggs), the biological system is fragile for most values of the parameter k_6, becoming robust only close to the boundary with mode B. This means that if the breakdown of the active *cdc2*-cyclin active complex is less than a threshold (in this case $k_6 = 0.19$), then when disturbed enough from the basin of attraction, the unfertilized egg dies away.
(2) In *mode B* (the early embryos), the system exhibits bursts of growing controlled by the MPF activity indicated by the existence of a limit cycle). In this case the system is robust, being very unlikely to lose its normal development.
(3) Finally, in *mode C* (the adult cell), the system exhibits again a fragility when far from the boundary with B. In other words, if the rate of breakdown of the *cdc2*-cyclin active complex increases over a threshold (in our case $k_6 = 1.9$) due, say, to abnormal chemical levels, then the cell dies away.



The limit cycle is not Jacobi stable in its entirety.

Figure 4. Jacobi stability for the Tyson model.

4.3. **G_1-phase of the cell division cycle.** In this subsection we are going to study a model for the G_1-phase of the cell division cycle in mammals, as proposed by Tecarro, Obeyesekere, Achmuty in [Tecarro et al. 2003].

We will study the reduced version of their model, the model for a cancerous cell. This model is important for the insights it brings to the mechanism of proliferation of cancerous cells, and for applications to drug design.

The Jacobi stability analysis adds new information (on the robustness and fragility of the system) to the classical linear stability analysis. It reveals the existence of more subtle checkpoints in the cell division cycle, and clarifies the robust cell arrest states.

Even though the typical cell division cycle is divided into four distinct phases: G_1, S, G_2, M, as explained in 2.6, this model mimics the cell cycle divided into two distinct phases only: the G_1-phase (the gap between the end of mitosis and beginning of synthesis), and the rest of the cell cycle thought of as a phase, i.e. a S-G_2-M-phase. In other words, for simplicity, the distinction among the phases S, G_2 and M is ignored.

The regulatory pathway is discussed in detail in [Tecarro et al. 2003], so we briefly overview it here only.

The main actors in this model are:

- a protein called cyclin E: *cycE*,
- a cyclin-dependent kinase: *cdk2*,
- the retinoblastoma protein: *Rb*,

- the maturation promoting factor: *MPF*.

The cell division cycle starts when *cycE* combines with *cdk2* to form the active complex *cycE/cdk2*, and a high concentration of *Rb* is present in the cell. A necessary condition for the $G_1 \mapsto S$ checkpoint to allow synthesis initiation is the increase of *cycE/cdk2* concentration above a threshold close to its peak, and maximum phosphorylation of *Rb* protein.

For this simplified model, the condition for the progression through the rest of the cell cycle S-G_2-M is the increase of hypo-phosphorylated *Rb*, decrease of concentration of the complex *cycE/cdk2*, and the increase of the MPF concentration. The check of these conditions is performed at the only two checkpoints present in this model: $G_1 \mapsto S$, and $M \mapsto G_1$,

A flow chart representing this molecular mechanism can be found in [Tecarro et al. 2003]. Its corresponding ODE's are:

$$\begin{aligned}
\frac{dM}{dt} &= a_M \cdot E + f(R_T - R) + g \cdot M^2 \cdot E - d_M \cdot M \\
\frac{dE}{dt} &= \frac{a_E}{h + R} - d_E \cdot M \cdot E \\
\frac{dR}{dt} &= \frac{p_1(R_T - R)M}{q_1 + (R_T - R) + M} - \frac{p_2 R \cdot E}{q + R + E},
\end{aligned}$$

where E is the concentration of *cycE/cdk2*, M of MPF, and R of the unphosphorylated *Rb*. The rest of the letters are reaction rate constants (see [Tecarro et al. 2003] for details).

4.3.1. **A cancerous cell model.** The $G_1 \mapsto S$ checkpoint verifies if the DNA has suffered some damages, the cell is ready for synthesis, etc.

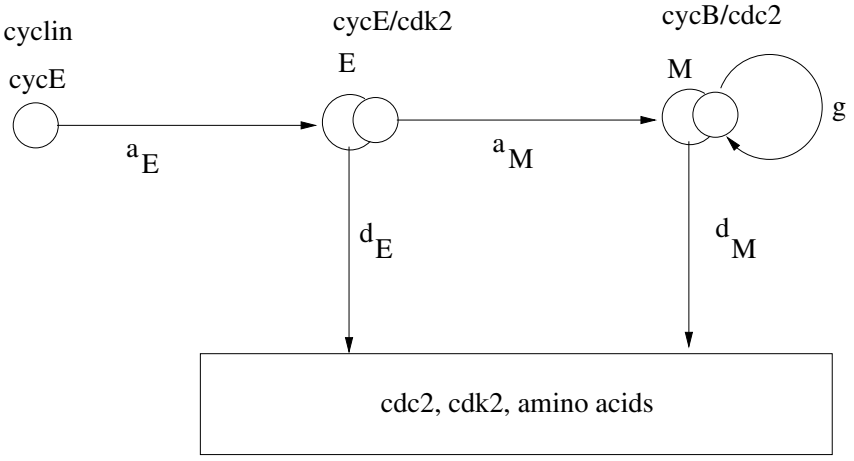


Figure 5. Regulatory pathway of the cell division cycle for the G_1-model in mammals.

The real molecular mechanism of this checkpoint is quite complex, but in the present model, its task is accomplished by the action of active (i.e. phosphorylated) protein *Rb*.

In order to model a cancerous cell, it is plausible to assume that, for example due to point mutations in the genome, *Rb* has lost its tumor suppressing functions, so the cell cannot be impeded from growing and dividing endlessly. The flow chart of this molecular mechanism can be represented like in Figure 5.

The corresponding ODE's are

$$\begin{aligned} \frac{dM}{dt} &= a_M \cdot E + g \cdot M^2 \cdot E - d_M \cdot M \\ \frac{dE}{dt} &= a_E - d_E \cdot M \cdot E. \end{aligned} \tag{4.11}$$

For the sake of simplicity we will write the system as

$$\begin{aligned} \frac{du}{dt} &= a_M v + g u^2 v - d_M u = f(u,v) \\ \frac{dv}{dt} &= a_E - d_E u v = g(u,v), \end{aligned} \tag{4.12}$$

where we have put $u := M$, $v := E$, letting the rest of the parameters as they are in order to not completely lose the biological meaning (see Table 4).

4.3.2. **Linear stability analysis.** From (4.12), it follows that the system

$$f(u,v) = 0, \quad g(u,v) = 0$$

has solutions if and only if the condition

$$a_E < \frac{d_E d_M}{g} \tag{4.13}$$

holds. If it doesn't, then the nullclines $f = 0$ and $g = 0$ do not intersect and therefore there are no steady-states.

Condition (4.13) states that in order to have steady-states, the synthesis rate of *cycE*/*cdk*2 has to be less than the ratio of the product of degradation constants to the autocatalysis ratio of M. This condition makes biological sense and mainly characterizes the arrest of the cell in the G_1-phase ([Alberts et al. 1994]).

Assuming from now on the condition (4.13), the steady-states of (4.12) are

$$u_0 = \sqrt{\frac{a_E a_M}{d_E d_M - g a_E}}, \quad v_0 = \frac{a_E}{d_E}\sqrt{\frac{d_E d_M - g a_E}{a_E a_M}}, \tag{4.14}$$

and the Jacobian matrix evaluated at the fixed point $S(u_0, v_0)$ is given by

$$A = J\big|_{(u_0,v_0)} = \begin{pmatrix} 2guv - d_M & a_M + gu^2 \\ -d_E v & -d_E u \end{pmatrix}\Big|_{(u_0,v_0)} = \begin{pmatrix} 2g\dfrac{a_E}{d_E} - d_M & a_M + gu_0^2 \\ -d_E v_0 & -d_E u_0. \end{pmatrix}. \tag{4.15}$$

In order to obtain the oscillation conditions, we calculate

$$\begin{aligned} trA &= 2g\frac{a_E}{d_E} - d_M - d_E u_0 \\ detA &= -d_E[u_0(g\frac{a_E}{d_E} - d_M) - v_0 a_M] \\ \Delta &= (trA)^2 - 4\, detA \\ &= (2g\frac{a_E}{d_E} - d_M)^2 + d_E u_0^2 - 2u_0 d_M d_E - 4v_0 a_M d_E \end{aligned} \tag{4.16}$$

From condition (4.13) it follows that $detA > 0$. In other words, the oscillation condition is $trA > 0$ (with $\Delta < 0$ for having an unstable spiral). Unlike the general estimation of $trA > 0$ in [Tecarro et al. 2003], we pursue a numerical study, using the parameter values in Table 4 below. Here, c and t are arbitrary units for concentration and time.

Rate	*Type*	*Value*	*Meaning*
a_E	adjustable	0-0.17 c^2t^{-1}	$cycE/cdk2$ production
a_M	constant	0.18 t^{-1}	MPF production
g	constant	0.067 $c^{-2}t^{-1}$	MPF self-activation
d_E	constant	0.03 $c^{-1}t^{-1}$	$cycE/cdk2$ degradation
d_M	constant	0.38 t^{-1}	MPF degradation

Table 4. The cancerous cell model's parameter values [Tecarro et al. 2003].

Since the progression through cell cycle is triggered by E, it is natural to let its production rate a_E vary in order to depict the behavior of the model.

With the values in Table 4, one finds that the linear stability of (4.12) is given by (as before: S = stable, uS = unstable, l.c. = limit cycle)

a_E	0		0.0355		0.097		0.167		0.168		0.17
tr A	–		–	0	+	0	–		–		
det A	+		+		+		+		+		
Δ	+	0	–		–		–	0	+		
Linear stability	S			uS (l. c.)			S				

Table 5. Linear stability of the G_1-model for cancerous cells.

Proposition 4.4 [Tecarro et al. 2003] *For the parameter values in Table 4, and* $a_E \in (0.097, 0.167)$ *the fixed point* $S(u_0, v_0)$ *is an unstable spiral. Moreover, the values* $a_E^1 = 0.097$, $a_E^2 = 0.167$ *are Hopf bifurcation points and the system exhibits a limit cycle behavior on this interval.*

4.3.3. **Jacobi stability.** Let us put $y = \dfrac{du}{dt}$ and $x = u$. By the procedure presented in §3.2, from the system (4.12) we obtain the "Jacobi equation"

$$\frac{d^2x}{dt^2} + g^1(x, y) = 0,$$

where

$$g^1(x,y) = -\frac{2g}{a_M + gx^2}y^2 + \frac{gd_Mx^2 - a_Md_Ex - d_Ma_M}{a_M + gx^2}y$$

$$-[(ga_E - d_Ed_M)x^2 + a_Ea_M].$$

Next, using (3.10) we obtain

$$P_1^1(u,v) = \frac{\Lambda(u)}{2(a_M + gu^2)}v + \frac{\Omega(u)}{4(a_M + gu^2)^2},$$

where we have put

$$\Lambda(u) = -g^2d_Eu^4 - 2a_Mgd_Eu^2 + 4ga_Md_Mu - a_M^2d_E$$

$$\Omega(u) = g^2d_E^2u^6 + g(2a_Md_E^2 + gd_M^2)u^4 - 4gd_Ed_Ma_Mu^3 +$$

$$+a_M(a_Md_E^2 - 10gd_M^2)u^2 - 4a_M^2d_Ed_Mu + d_M^2a_M^2.$$

Evaluating the tensor $P_1^1(u,v)$ at the parameter values in Table 4 and $a_E = 0.1$, we find that the Jacobi stability region is like that in Figure 6.

Numerical simulations shows that the limit cycle barely fails to completely stay within the Jacobi stability region.

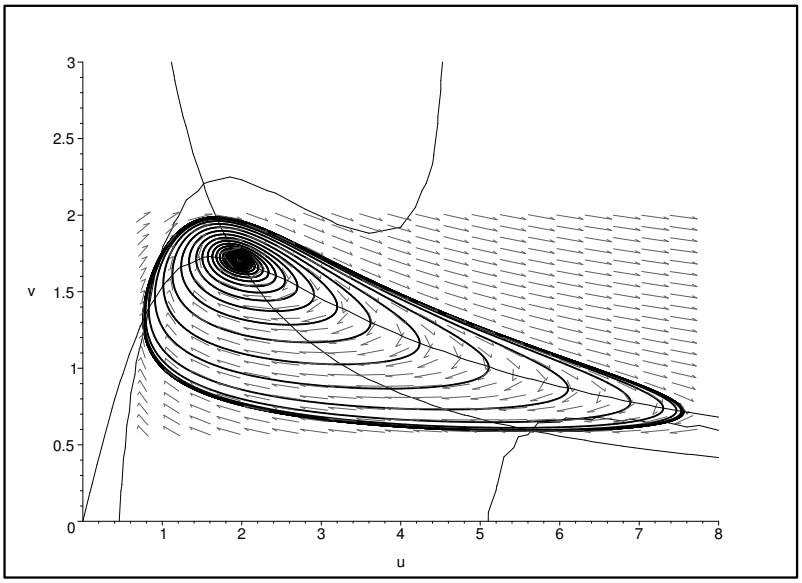


Figure 6. Jacobi stability of the cancerous cell model. The limit cycle barely fails to enter the stability region.

From Corollary 3.4 and the above discussion, we conclude the following.

Proposition 4.5 *For the parameter values in the Table 4,*

(1) *if $a_E \in (0.097, 0.167)$, then the fixed point $S(u_0, v_0)$ of the system (4.12) belongs to the Jacobi stability region;*
(2) *the Jacobi stability of the system (4.172 changes as in Table 6.*

a_E	0	0.035	0.097	0.167	0.168	0.17
Lin. stab.	S	S	uS (l.c.)	S	S	
Jacobi stab.	uS	S	S	S	uS	
Bio. mean'g	frg arr	rob arr	rob osc	rob arr	frg arr	

Table 6. Jacobi stability of the cancerous cell model, with familiar abbreviations. Also, frg = fragile, rob = robust, arr = arrest, osc = oscillations.

4.3.4. **Biological interpretation.** We discuss in the following the biological meaning of the Jacobi stability analysis for the cancerous cell model (4.11). Many of the results are true for the normal cell model also. This shows that the deletion of the *Rb* protein does not obstruct the cell cycle regulation ([Tecarro et al. 2003]).

The bifurcation with respect to the production rate a_E of $cycE/cdk2$ gives an insight on the mechanism of cancerous cell proliferation.

The linear stability analysis shows that, for low concentrations of E, in our case $a_E \in (0, 0.097)$, the cell exhibits stable steady-state. This corresponds to the G_1-phase arrest state present in both normal and cancerous cells (see [Alberts et al. 1994]). A stable state is biologically interpretable as a cell arrest state ([Tecarro et al. 2003]).

For intermediate values of a_E, i.e. $a_E \in (0.097, 0.167)$, the biological system exhibits oscillatory behavior (limit cycle). In other words, the cyclin concentration periodically oscillates driving the cell through synthesis and mitosis phases.

For large values of a_E, in our case $a_E \in (0.167, 0.17)$, the cell enters again a steady-state that can be associated with another cell arrest state. The two Hopf bifurcation points $a_E^1 = 0.097$, and $a_E^2 = 0.167$ represent the two checkpoints existent in this model of the cell division cycle.

If we study the Jacobi stability of this biological system, more information can be added to the above picture. Firstly, we find out that there are two "Jacobi bifurcation" points $a_E^{J1} = 0.035$, and $a_E^{J2} = 0.168$ where the Jacobi stability of the steady solution changes. If we interprete this kind of stability as indicating the robustness and fragility of a system, from Table 6 we have:

(1) **for $a_E \in (0, 0.035)$ the cell is in a "fragile arrest"**. This means that at a very small concentration of $cycE/cdk2$ the cell rests at the begining of G_1-phase, but small perturbations can easily drive it away

from this stable state (for example increase of level of some proteins, or failure of the checkpoint $M \mapsto G_1$).

(2) **for $a_E \in (0.0355, 0.097)$ the cell is in a "robust arrest"**. We associate this state of the cell with the usual G_1-phase. There is biological evidence ([Alberts et al. 1994]) that this arrest state is robust because here the cell grows and prepares for its DNA synthesis.

(3) **for $a_E \in (0.097, 0.167)$ the cell enters "robust oscillations"**. This state corrsponds to the M-G_2-S-phase where the increase of the concentration of $cycE/cdk2$ complex indicates an increase of the concentration of MPF which propells the cell through the rest of cell cycle, provided that the $G_1 \mapsto S$ checkpoint is cleared. In the model of a cancerous cell, because of the lack of Rb, the checkpoint $G_1 \mapsto S$ is not functional anymore, so cells with abnormal growth or damaged DNA sequence cannot be stopped from entering synthesis and mitosis.

(4) **for $a_E \in (0.167, 0.168)$ the cell is in a "robust arrest", and for $a_E \in (0.168, 0.17)$ in a "fragile arrest"**. It is difficult to find biological evidence in the literature for these states. This may be because the concentrations varies in a thin interval making their experimental authentification very difficult, or they may be some artifacts of these models.

We conclude that Jacobi stability analysis adds a degree of accuracy to the classical linear stability analysis by studying the robustness of a biological system. In the case of cancerous cells, the cell arrest is very important. We consider that the "robust arrest" region is of main interest for applications. The picture we just painted could not have been obtained solely from linear stability analysis.

5. Conclusions

Various mathematical theories and methods can be used for the study and simulation of biological systems: combinatorial algebra, linear algebra, differential equations, differential geometry, etc. However, *in the case of systems biology, differential equations and differential geometry should be preferred.*

Studying the properties of a biological system by an associated set of differential equations is not a new topic ([Kitano 2002]). However, linear stability analysis of differential equations involves linearizations via the Jacobian of a non-linear system.

On the other hand, KCC-analysis is one based on the study of Liapunov stability of whole trajectories in a region. Therefore, in this case, the perturbations represent trajectories close to the reference trajectory. The results of such method, even when derived at a particular point, yields information about the behavior of trajectories (solutions to the non-linear system) in a neighborhood, or open region surrounding that point. To be more specific, evaluating the eigenstructure of the deviation curvature tensor P^i_j (of the SODE obtained

from the original equations) at the steady-states of the system gives us information about the behavior of trajectories, or transient (i.e. non-steady) states, in an open region of these steady-states (see also [Antonelli et al. 1993]).

We have shown using concrete examples that it is natural to interpret Jacobi stability as the *robustness* of a (biological) system. Although the information about the stability of a solution is true only in an open region around the steady-state, this kind of information can shed new light on the problem.

Acknowledgments. I would like to thank P.L. Antonelli and D. Bao for their advice and guidance in writing this paper.

References

[Alberts et al. 1994] Alberts, B., Bray, D., Lewis, J., Rolf, M., Roberts, K., Watson, J.D., *Molecular Biology of the Cell, Third Edition*, Garland Publ. Inc., 1994.

[Antonelli et al. 1993] Antonelli, P.L., Ingarden, R., Matsumoto, M., *The theory of sprays and Finsler spaces with applications in physics and biology,* Kluwer Acad. Publ., FTPH vol. 58, 1993.

[Antonelli–Bradbury 1996] Antonelli, P.L., Bradbury, R.H., *Volterra–Hamilton models in the Ecology and Evolution of Colonial organisms*, World Scientific, 1996.

[Antonelli et al. 1998] Antonelli, P.L., Auger, P. and Bradbury, R.H., *Corals and starfish waves on the Grear Barrier Reef:Analytical trophodynamics and 2-patch aggregation methods*, Mathl. Comput. Modeling, **27** (1998), 121–135

[Antonelli 2000] Antonelli, P.L., *Equivalence problem for Systems of second-order ordinary differential equations,* Encyclopedia of Math., Kluwer Acad. Publ., 2000.

[Antonelli et al. 2001] Antonelli, P.L., Maculan, N., Portugal, R., Rutz, S.F., and Sabau, V.S., *Transient-states analysis of a 2-species dynamical ecological model via KCC-theory*, Nonlinear Studies **8** (2001), 283–296.

[Antonelli et al. 2002] Antonelli, P.L., Rutz, S., Sabau, V.S., *A transient-state analysis of Tyson's model for the Cell division cycle by means of KCC-theory*, Open Systems & Information Dynamics **9**(3) (2002), 223–238.

[Antonelli, Bucataru 2003] Antonelli, P. L., Bucataru, I., *Second Order Differential Equations*(in "Handbook of Finsler Geometry", Edited by P. L. Antonelli), Kluwer Acad. Publ., 2003, 123–132.

[Auslander 1955] Auslander, L., *On curvature in Finsler geometry*, Trans. Amer. Math. Soc.,**79**,(1955), 378–388.

[Bao et al. 2000] Bao, D., Chern, S.S., Shen, Z., *An introduction to Riemann–Finsler geometry*, Springer, GTM 200, 2000.

[Carbone–Gromov 2001] Carbone, A., Gromov, M., *Mathematical slices of Molecular Biology*, Gazette des Mathématiciens, Numérce spécial, Société Mathématique de France **88** (2001), 11–80.

[Cartan 1933] Cartan, E., *Observations sur le mémoir précédent*, Math. Zeitschrift **37** (1933), 619–622.

[Chern 1939] Chern, S.S., *Sur la géometrie d'un système d'equations differentialles du second ordre*, Bull. Sci. Math. **63** (1939), 206–212.

[Kitano 2002] Kitano, H., *Systems Biology: A Brief Overview*, Science **259** (2002), 1662–1664.

[Kosambi 1933] Kosambi, D.D., *Parallelism and path-space*, Math. Zeitschrift **37** (1933), 608–618.

[Lackey, B. 1999] Lackey, B., *A model of trophodynamics*, Nonlinear Analysis, **35** (1999), 37–57.

[Laugwitz, D. 1965] Laugwitz, D., *Differential and Riemannian Geometry*, Academic Press, New York, 1965.

[Murray 1993] Murray, J.D., *Mathematical Biology*, Springer, Biomathematical Texts, vol. 19, 1993.

[Robinson 1995] Robinson, C., *Dynamical Systems: Stability, Symbolic Dynamics and Chaos*, CRC Press, 1995.

[Rund 1959] Rund, H., *The Differential Geometry of Finsler Spaces*, Springer–Verlag, 1959.

[Tecarro et al. 2003] Tecarro, E.S., Obeyesekere, M.N., Achmuty, G., *Mathematical analysis of a 3-variable cell cycle model*, Nonlinear Analysis **4** (2003), 87–107.

[Tyson 1991] Tyson, J., *Modeling the cell division cycle: cdc2 and cyclin interactions*, Proc. Natl. Acad. Sci. USA, Cell Biology **88** (1991), 7328–7332.

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