Cell Cycle Vignettes

Analysis of Cell Cycle Dynamics by Bifurcation Theory

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Bifurcation theory provides a classification of the expected ways in which the number and/or stability of invariant solutions ('attractors' and 'repellors') of nonlinear ordinary differential equations may change as parameter values are changed. The most common qualitative changes are 'saddle-node' bifurcations, 'Hopf' bifurcations, and 'SNIPER' bifurcations. At a saddle-node bifurcation, a pair of steady states, usually a stable node and an unstable saddle point, coalesce and disappear. At a Hopf bifurcation, a stable focus changes to an unstable focus and makes way for a small amplitude periodic solution ('limit cycle'). At a SNIPER bifurcation, the coalescence of a saddle point and a stable node creates an infinite-period limit cycle solution. These bifurcations have clear physiological correlates in the regulation of DNA replication, mitosis and cell division. Saddle-node bifurcations are related to checkpoints in the cell cycle: the establishment and removal of checkpoints correspond to the creation and annihilation of stable steady states at saddle-node bifurcations. The repetitive nature of the cell cycle (G1-S-G2-M-G1-etc.) is related to limit cycle solutions of the underlying kinetic equations: the ability to oscillate spontaneously arises at either a Hopf or a SNIPER bifurcation.

Historical background

Since the days of Isaac Newton, ordinary differential equations (ODEs) have been used throughout the physical and life sciences to describe the temporal development of dynamical systems: from the solar system, to the clock radio, to the regulation of DNA replication and cell division. Initially the focus was on ODEs that could be solved exactly in terms of 'elementary' functions of high-school algebra and trigonometry or 'special' functions of mathematical physics. But in the 1890's Poincare (1899) introduced the 'qualitative' theory of dynamical systems, i.e., systems of n nonlinear ODEs

$$\frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \mathbf{f}(\mathbf{x};\mathbf{p}), \quad \mathbf{x}(t) = \{x_1,...,x_n\} = \text{variables}, \quad \mathbf{p} = \{p_1,...,p_m\} = \text{parameters} \qquad [1]$$

Poincare proposed to interpret these equations as a vector field in n-dimensional state space, $\mathbf{x}$, and to characterize this vector field by its invariant solutions, which can be either attractors or repellors. The crucial question for Poincare was not "what is the exact solution of the ODE?" but "how do the qualitative features of the attractors and repellors depend on the values of the parameters?" This latter question is the subject of bifurcation theory (Odell 1980; Strogatz 1994), which was developed in the mid-20th

Century by Andronov's school of Russian physicists and engineers for $n = 2$ (Andronov et al. 1966), and later by a host of mathematicians for the general case (Kuznetsov 2004).

A one-parameter bifurcation diagram begins with a plot of the steady state value of a chosen dynamical variable, x_i, as a function of a chosen parameter, p_j, the 'bifurcation parameter'. In Fig. 1A, we plot a typical bifurcation diagram for a bistable system. Between the two thresholds, $\theta_{\text{inact}} < p_j < \theta_{\text{act}}$, the system can persist in either of two stable steady states (x_i small or x_i large). Precisely at the thresholds, $p_j = \theta_{\text{inact}}$ and $p_j = \theta_{\text{act}}$, the dynamical system undergoes a bifurcation from one type of behavior (a single stable steady state) to a qualitatively different type of behavior (bistability). This type of bifurcation is called a 'saddle-node' or 'fold'. In Fig. 1B, we illustrate a 'Hopf' bifurcation, in which a stable steady state loses stability and gives rise to stable limit-cycle oscillations. The limit cycles are born with small amplitude and grow in size as the parameter value pulls away from the bifurcation point. We shall meet some other types of bifurcations shortly.

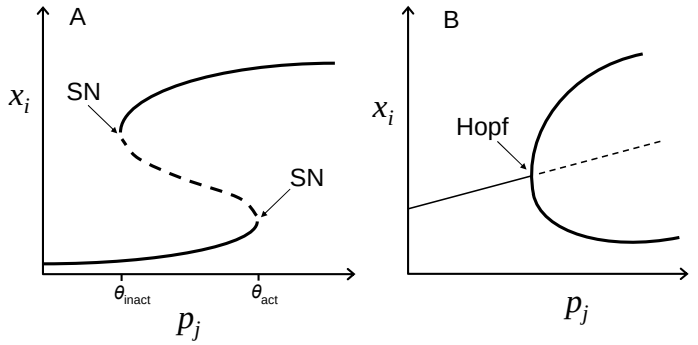

Figure 1. One-parameter bifurcation diagrams. (A) Saddle-node bifurcation. Solid line: stable steady state; dashed line: unstable steady state. (B) Hopf bifurcation. Thin solid line: stable steady state; thin dashed line: unstable steady state; thick solid line: maximum and minimum values attained by a stable limit cycle oscillation.

Of special interest to systems biologists are the musings of Rene Thom (1989) on 'structural stability and morphogenesis'. Thom was highly regarded among mathematicians for his study of gradient dynamical systems,

$$\frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \nabla U(x_1,...,x_n) \qquad [2]$$

where $U(\mathbf{x})$ is a scalar function of the variables (think of it as the 'potential energy' of the system) and $\nabla = \left(\partial/\partial x_1,...,\partial/\partial x_n\right)$ is the gradient operator. The steady state solutions of Eq. [2] are places where $\nabla U = 0$, i.e. 'singularities' of the potential function. Thom's great contribution was to classify the topologically distinct types of singularities of potential functions in n dimensions. Next Thom took the unusual step—unusual for a famous mathematician—to speculate that the bifurcations he had characterized might underlie the 'unfolding' of a fertilized egg into a larva. Following Waddington's hypothesis that embryonic development is the evolution of a dynamical system on an

'energy landscape', Thom pointed out that his complete classification of the qualitative changes of behavior that could be observed under this type of gradient dynamic must provide the key to understanding morphogenetic transitions.

Thom's ideas were bitterly opposed by both theoretical and experimental biologists of his generation, and (perhaps) for good reasons. First of all, morphogenesis is governed, we know, by the interactions of genes and proteins (i.e., a biochemical interaction network), which is *not* a gradient dynamical system. Hence, the bifurcations of relevance to molecular cell biologists are not the singularities of potential functions (Thom's case) but rather the generic bifurcations of nonlinear ODEs (the case of Andronov et al.). But Thom's more fundamental idea (stripped of its unfortunate alliance to Waddington's energy landscape)—that qualitative changes in cell physiology should be correlated with qualitative changes in the attractors and repellors of a vector field (a system of nonlinear ODEs)—is absolutely correct. It is the basis of the application of bifurcation theory to problems in molecular cell biology.

One-parameter bifurcation diagrams and signal-response curves

The connection between bifurcation theory and cell physiology is the signal-response curve. In a typical experiment, a molecular cell biologist might challenge cells with increasing amounts of an extracellular signal molecule and measure whether certain downstream genes are expressed or not. And a typical result is that, for low signal levels there is no expression, but for signal levels above a certain threshold there is strong expression of the gene (Fig. 2). In this circumstance, it is natural to ask what happens if the signal level is steadily decreased in cells that are expressing protein R? Do they turn off at the same signal strength where they turned on? Or at a much lower signal strength? Or not at all?

In the first case, the signal-response curve is perfectly smooth and reversible; there are no qualitative changes in the behavior of the control system as the signal varies up and down. In the second case, there is a region of bistability between the two thresholds, and the behavior of the control system is qualitatively different over three ranges of signal strength: for $S < \theta_{inact}$ there is a single stable steady state with R small; for $\theta_{inact} < S < \theta_{act}$ the control system can persist in either of two attractors (R small or R large) that are separated by an unstable steady state; and for $S > \theta_{act}$ there is a single stable steady state with R large. This is exactly the case of a one-parameter bifurcation diagram with saddle-node bifurcations bounding a zone of bistability (Fig. 1A).

It is possible that $\theta_{inact} < 0$, in which case the signal, $S = [S]$ (a positive number), cannot be made small enough to flip the switch off. In this case, the control system is said to be a one-way switch. By increasing S, the switch can be turned on, but it can't be turned off by decreasing S.

There are many convincing examples of toggle switches in molecular and cell biology generally (Tyson et al. 2003) and in cell cycle regulation particularly (see the vignette on 'Bistability and Oscillations').

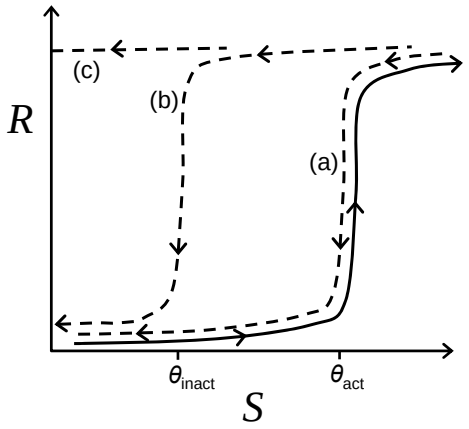

Figure 2. Signal-response curve. The experimentalist can vary the signal strength S (say, the concentration of an extracellular ligand) and observe the response R (say, the expression level of a gene induced by S). As S is slowly increased, the expression of R turns on abruptly; a typical threshold-type response. What happens as S is slowly decreased? There are three possibilities. (a) The gene expression turns on and off at the same threshold signal strength: the signal-response curve is smooth and reversible; e.g., a Hill function. (b) The threshold for gene inactivation (θ_{inact}) is lower than the threshold for gene activation (θ_{act}): the signal-response curve has a region of bistability and functions like a toggle switch. (c) The gene cannot be inactivated by lowering the signal strength even to zero: the control system functions as a one-way switch.

In another common physiological situation, a cellular process begins to oscillate when a stimulating signal gets large enough (Goldbeter 1996). In this case, the signal-response curve exhibits a Hopf bifurcation, as in Fig. 1B.

These examples suggest that the signal-response curves often measured by cell physiologists are none other than one-parameter bifurcation diagrams in the parlance of applied mathematicians. If we may associate abrupt, qualitative changes in signal-response characteristics of living cells with bifurcations in vector fields of nonlinear dynamical systems, then it is natural to ask how many different types of generic bifurcations are exhibited by dynamical systems and what do they look like? Are there hundreds of different types of bifurcations to match the seemingly boundless variety of cellular behaviors? Or are all the peculiarities of cellular signal processing simply variations on a few common themes?

The answer is the latter. In addition to the saddle-node and Hopf bifurcations illustrated in Fig. 1, there are only a few other common, generic, one-parameter bifurcations: subcritical Hopf, cyclic fold, saddle-loop and SNIPER bifurcations (Fig. 3 and Table 1).

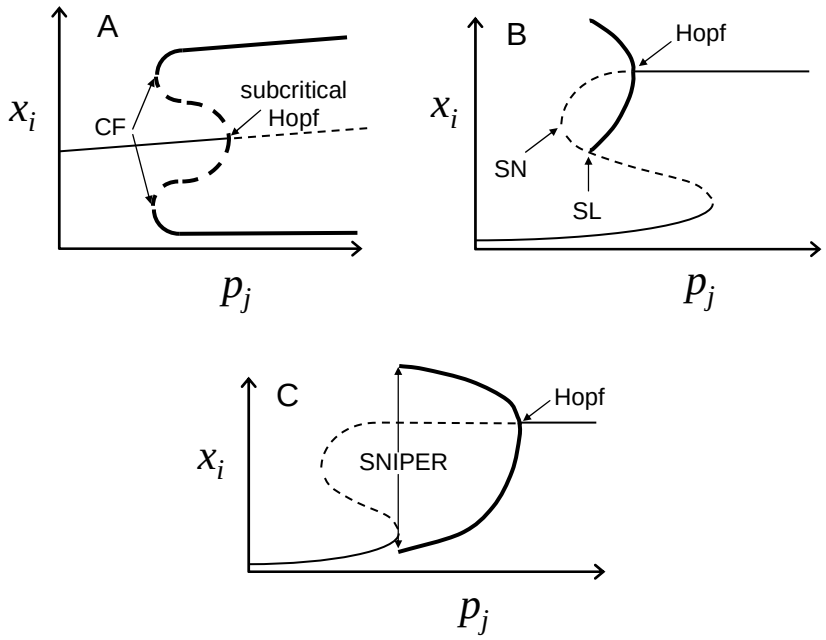

Figure 3. The other common types of bifurcation points. (A) Subcritical Hopf bifurcation and cyclic fold (CF) bifurcation. (B) Saddle-loop (SL) bifurcation. (C) Saddle-node infinite-period (SNIPER) bifurcation.

Of this we can be certain: Cell physiology is governed by underlying regulatory networks that consist of biochemical reactions among genes, RNAs and proteins. These networks are dynamical systems; their dynamics are governed by nonlinear ODEs (biochemical kinetic equations). The solutions of these equations determine the time-dependent behavior of the cell, and the nature of these solutions are determined by the attractors and repellors of the dynamical vector field in state space. Qualitative changes in the behavior of cells must be reflections of qualitative changes in the nature of these attractors and repellors, i.e., on the generic bifurcations of nonlinear vector fields. Hence, the six types of bifurcations we have introduced must be the basic building blocks of all cellular signal-response curves. It is this connection between signal-response curves of living cells and one-parameter bifurcation diagrams of dynamical systems that is the heritage of Rene Thom's proposal.

An important caveat to this interpretation of bifurcation theory is the fact that single cells are very small, with limited numbers of molecules (10s, 100s, 1000s) of each of the interacting species. Hence, continuous ODEs are only a first approximation to the dynamics of intracellular molecular control systems. The effects of stochastic variations of small numbers of molecules can have significant effects on the qualitative features of dynamical systems. Stochastic effects must be given due consideration, but subsequent to a thorough study of the system by bifurcation theory.

Relation to cell cycle regulation

Bifurcation theory has been used to study the molecular basis of cell cycle regulation (e.g., Borisuk and Tyson 1998, Tyson et al. 2003, Czikasz-Nagy et al. 2006). The basic idea behind these papers is that a eukaryotic cell progresses through the DNA replication-division cycle by a series of transitions (G1/S, G2/M, M/G1) that correspond to bifurcations of the underlying molecular control system. Before each transition, the cell is arrested in a stable steady state of the dynamical system that corresponds to a particular physiological state: G1-arrest, G2-arrest or metaphase-arrest. To pass to the next stage of the cell cycle, the stable arrested state must be lifted, either by annihilation (at a SN or SNIPER bifurcation) or by losing stability (at a Hopf bifurcation). To prevent a transition, e.g., if there is some damage to the DNA or some problem in aligning chromosomes on the metaphase plate, then a 'checkpoint' mechanism stabilizes the arrested state by moving the bifurcation point to some higher value of the progression signal(s). For example, a schematic diagram of the fission yeast cell cycle is provided in Fig. 4.

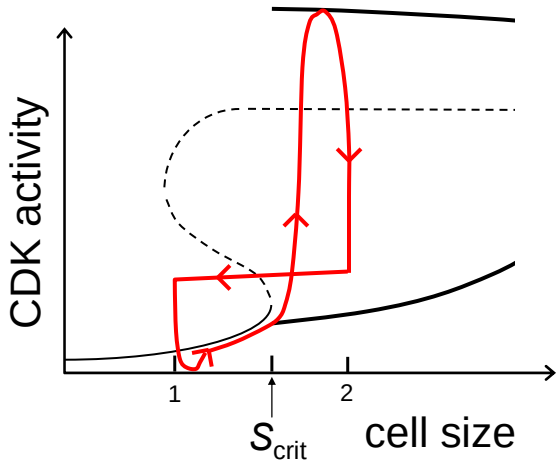

Figure 4. A schematic diagram of the fission yeast cell cycle. The bifurcation diagram in Fig. 3C is interpreted here as a signal-response curve relating cyclin-dependent kinase (CDK) activity to cell growth. CDK is a protein kinase that governs progression through the cell cycle. In fission yeast, low CDK activity corresponds to a G1/S/G2 state and high activity to mitosis. Cell size can be thought of as a bifurcation parameter: cell size increases slowly as the cell grows, and the CDK control adapts quickly to an attractor of the vector field at the current size of the cell. The red curve is a 'cell cycle trajectory'. At the size of a newborn cell (size = 1), the only attractor is a stable steady state of low CDK activity. After a brief G1 period, the cell replicates its DNA and then pauses in G2 phase until it grows large enough to surpass the SNIPER bifurcation. The bifurcation point is the 'critical size' for the G2/M transition in fission yeast. The dynamical system is attracted to a large amplitude limit cycle, which carries the cell into mitosis (CDK increasing). The cell exits mitosis when CDK activity is destroyed, and this is the signal for the cell to divide. Cell size is abruptly halved, and the newborn cells (each of size = 1) are attracted to the stable G1/S/G2 steady state. Notice that the cell cycle time (the time required to progress around the red loop) is identical to the mass-doubling time (the time necessary to grow from birth size =1 to division size = 2).

During early embryogenesis, from fertilization to the mid-blastula transition, mitotic cycles proceed rapidly and synchronously, without checkpoint controls. In this case, the DNA replication-division cycles seem to be driven by spontaneous limit cycle oscillations. For more details, see the vignette on 'Bistability and Oscillations'.

Table 1. Generic bifurcations of dynamical systems

Name	**Characteristics**	**Cell cycle correlate**
Saddle-node	Creation and annihilation of pairs of steady states	Irreversible transitions; checkpoints
Hopf, supercritical	Birth of stable limit cycles of small amplitude and finite frequency	Spontaneous MPF oscillations in embryos
Hopf, subcritical	Birth of unstable limit cycles of small amplitude and finite frequency	Subcrit Hopf and cyclic fold bif'ns occur in pairs and may correlate with embryonic MPF oscill'ns
Cyclic fold	Creation and annihilation of pairs of limit cycles	
Saddle-loop	Annihilation of a limit cycle by a homoclinic orbit at a saddle point; finite amplitude and small frequency	SL and SNIPER bif'ns are closely related; they are involved in irrev transitions in the yeast cell cycle (budding yeast and fission yeast)
SNIPER* or SNIC* (synonomous)	Annihilation of a limit cycle by a homoclinic orbit at a saddle-node; finite amplitude and small frequency	

SNIPER = saddle-node infinite-period; SNIC = saddle-node invariant-circle

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