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Mémoire présenté par Benjamin Pfeuty

Nonlinear Dynamics of Cellular Decision Making

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Abstract

The multiscale, heterogeneous and functional attributes of biochemical networks raise great challenges for mathematical modeling approaches, related for instance to problems of identifiability, tractability or reducibility which are quite specific to biology. However, many fundamental cellular functions, such as proliferation, death, differentiation, clock or signaling, are prone to rely on the common – multistable and oscillatory – behaviors, which offers gateways to decipher complex biological processes with low-dimensional dynamical models. An objective of my research over the last decade has been to explore the dynamical and structural features of protein networks which regulate the decisions of cells made during their division and differentiation processes.

Whereas the simplest bistable decision switch can be implemented by a strong enough positive feedback in protein networks, we found that, in general, cellular decision making is prone to involve much more sophisticated regulatory architecture and nonlinear dynamics, which can be summarized in three main results. First, the well-designed combinations of several positive feedback, negative feedback and feedforward loops enable to produce transition trajectories featured with tunable speed and reversibility as well as robust sequentiality, as observed for diverse cellular decisions. Second, intracellular oscillatory dynamics can be exploited by creating tunable windows of opportunities for customized cell decision making among many fate alternatives. Importantly also, high-codimension bifurcations provide the possibility to unfold well-distinct cellular decision properties to fit with a specific environmental or social context.

Overall, these results support the notion that the low-dimensional topological and dynamical features of regulatory networks can already endow cell with a vast repertoire of adaptive skills.

Chapter 1

Cell decision making: An overview

> "Living systems are cognitive systems, and living as a process is a process of cognition. This statement is valid for all organisms, with and without a nervous system"
>
> H. Maturana and F. Varela

1.1 The biological basis of decision making

Understanding the biological and psychological mechanisms that guide human decisions has always been a key topic in neurosciences [Schall01, Gold07] and social sciences [Tversky81]. By conventional definition, ***decision making is the cognitive process of choosing between alternative courses of action, based on informations and uncertainties, beliefs and goals, values and risks***. This broad definition applies not only to humans, but more generally to any living organisms equipped with some cognitive capacity, including prokariotic and eukariotic cells [Perkins08, Balazsi11, BenJacob14]. This first section introduces the biological basis for decision making as a specific and fundamental mode of interaction between a living organism and its environment.

1.1.1 Decision making as an adaptive strategy

In order to understand the biological roots of decision making, it is helpful to envision the *living process* as arising from the interplay between ***metabolic and adaptive processes*** defining two embedded levels of living system organization (Fig. 1.1):

- ***From metabolism to adaptation***

From a thermodynamic viewpoint, living systems are open and non-equilibrium systems that maintain their internal organization by importing and dissipating free energy from their surrounding area and by producing and exporting high-entropy energy into it [Schrodinger44]. Accordingly, their internal organization fundamentally consists in a ***metabolic/catalytic network of (bio)chemical reactions*** that consume energy-rich molecules and photons to maintain or regenerate such organization in face to various endogenous and exogenous sources of disorganization (thermal fluctuations or radiative/chemical/mechanical perturbations). Another critical feature of such a network is its encapsulation and circular relationship within a physical

boundary: the metabolic network produces and regenerates the semi-permeable lipidic membrane that, in turn, encloses an internal space and filters the fluxes that are required to perpetuate the metabolic network and process [Varela74, Adamala13].

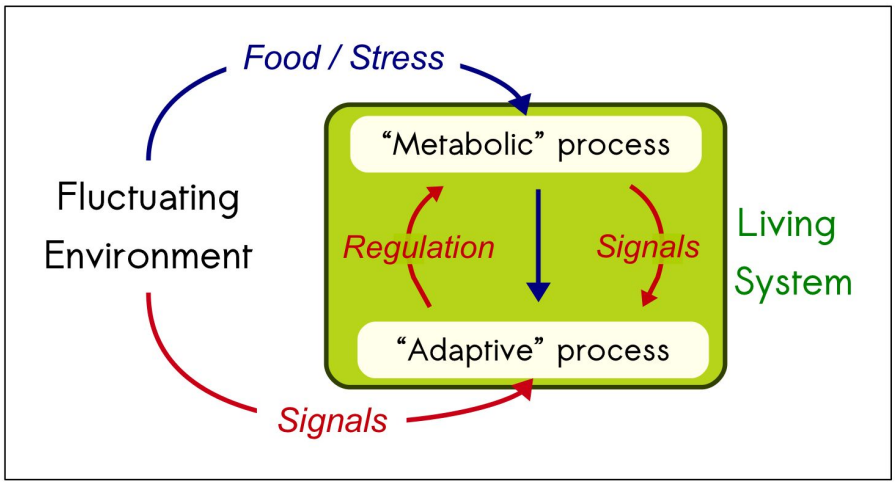


Figure 1.1: **Life's interplay between metabolic and adaptive processes.** *Living organisms are traversed by flows of energy and of information. Blue: the metabolic process consists in importing and exploiting free energy (i.e., food) from the environment and in minimizing and exporting entropy production (i.e., stress), in order to sustain the living low-entropy organizational state. Red: the adaptive process consists in tracking and interpreting various extracellular and intracellular signals from the environment in order for the organism to best adapt its whole organizational state to spatiotemporal fluctuations of this environment.*

However, non-equilibrium dissipative structures, even when compartmentalized, remain strongly dependent on specific boundary conditions and are therefore vulnerable to their temporal variations that commonly occur on the highly dynamic earth environment. In other words, the perpetuation of dependent and vulnerable living dissipative structures over billions years and their expansion all over the earth have been made possible by developing powerful *"adaptive capacity to keep pace with environmental conditions that change over short and long periods"* [Brooks11]. The adaptive strategy of living systems would thus be based on their capacity to develop a spatiotemporal mode of interactions with their surroundings. Shortly, two main adaptive mechanisms can be distinguished [Kussell05, Bleuven16]:

(*i*) Variation/selection mechanisms operate at the level of populations and involve the interplay between stochastic diversification processes and selective amplification processes (through differential survival and proliferation rates). The diversification processes can be more or less heritable and reversible depending on it acts at genetic level (adaptive evolution) or epigenetic/phenotypic levels (bet-hedging).

(*ii*) Signaling/regulatory mechanisms consist in the interplay between sensing, memory and response processes, which enables a living organism to adjust its organizational state to best adapt to environmental changes. This strategy is likely to confer *cognitive abilities* in exploiting the stream of past and ongoing environmental perturbations to infer the best adaptive internal responses, but also to infer the most probable environmental states in future time and in other places in order to predict and anticipate.

Whether one or another strategy prevails depends on the characteristics of environmental changes and the respective metabolic cost of sensing and proliferating [Kussell05]. However, these two strategies are also somehow mixed as the stochastic diversification process can be regulated [Maxwell17] and the regulatory process is stochastic to some extent [Maheshri07, Raj08] and can diversify [Voordeckers15]. As we shall see next, the decision-making process is a particular mode of regulatory adaptive mechanisms.

- ***Adaptive decisions in rugged fitness landscapes***

  "Cells and organisms sense, compute and make decisions [...] to invest their limited resources to their maximal benefit." — [Tkačik16]

A fundamental mode of biological adaptive response to changeable environment is to produce discrete outputs in response to gradual changes of internal or external milieu, which is referred to as a decision-making process. The requirement for such a discrete response process presumably stems from the nonlinear nature of the adaptive problems to which living systems are confronted. According to the framework of optimality theory, living organisms cope with adaptive problems by looking for an optimal trade-off between various costs, constraints, criteria and objectives, thus trying to maximize their fitness [Parker90]. Multi-criteria/combinatorial optimization problems are likely to display multiple local optima, drawing a rugged fitness landscape [Kauffman87, Visser14], but a single global optimum. Changing the setting of the problem would usually shift both the coordinates and the fitness values of the local optima, which can eventually lead to a ***discrete change of the global optimum solution*** (Fig. 1.2).

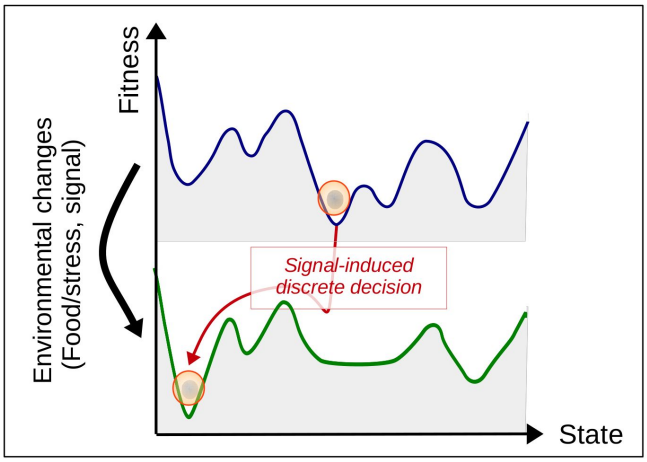


Figure 1.2: **Decision making as an adaptive strategy.** *Discrete cell-state switching toward a fitter local optimum of a rugged fitness landscape, upon environmental changes.*

The decision-making process may thus be viewed as an attempt to track such discrete change of the global optimum, or, at least, to switch to a neighboring better-fitted valley. For instance, a living organism threatened by a predator can elicit several possible adapted behaviors, such as as to fight, to escape, to hide or to request help, while mixed response such as hide-and-fight are generally suboptimal. Which decision is the most appropriate and, then, should be selected among many others, depends on multiple environmental factors as well as on the previous history and actual condition of the organism. In brief, ***the decision process relies on the detection and interpretation of diverse external and internal signals, and discreteness is a constitutive feature of decision making***.

1.1.2 Cellular decisions regulated by protein networks

- ***A broad spectrum of cellular decisions***

All aspects of the life of an organism potentially involve decision-making steps, hence giving rise to a great diversity of cellular decision-making behaviors (Fig. 1.3A):

(*i*) Reproduction decisions contribute to proliferation and diversification processes. Examples: DNA replication, mitosis, meiosis, fusion, fission, polyploidization, DNA uptake [Morgan07].

(*ii*) Metabolic decisions participate to the adaptation to temporal fluctuations of resources and stress. Examples: metabolic transitions toward the utilization of alternative resources (diauxic shift, glucose/galactose switch) or to a low activity state of dormancy (or quiescence) [Stanley15, Rittershaus16].

(*iii*) Motility decisions are involved in the adaptation of spatial fluctuations of resources and stress in order to explore more favorable environment. Examples: phototaxis, chemotaxis, aerotaxis, swimming, swarming, gliding, floating, walking [Jarrell08].

(*iv*) Social decisions are associated to the division of labor in a population of communicating cells. For instance, distinct pools of cells in colony or multicellular organisms may specialize in diverse and complementary tasks such as stress protection, nutrient incorporation, spatial patterning or repopulation [Celiker13, West16].

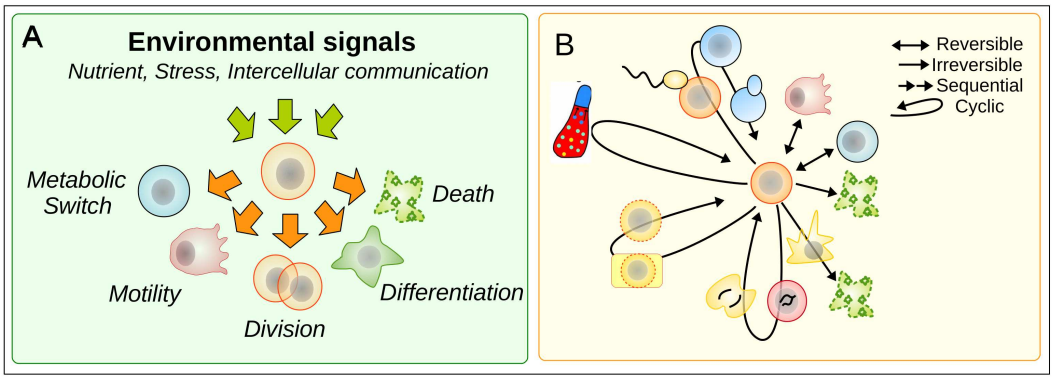


Figure 1.3: **A broad spectrum of cellular decisions.** *(A) Variety of signal-induced decisions. (B) A diversity of developmental cycles involving sequential, cyclic, reversible or irreversible decision making.*

Upon spatiotemporal fluctuations of their environment, especially when resources are running out or when stress factors are accumulating, unicellular organisms can deploy complex patterns of decisions selected among a vast repertoire, giving rise to elaborate ***developmental cycles*** (Fig. 1.3B), for instance in Cyanobacteria [Meeks02], Caulobacter [Kirkpatrick12], Bacillus [Kuchina11], Saccharomyces [Herskowitz88] and Dictyostelium [Li11]. In the course of evolution, some unicellular eukaryotes have undergone a transition into truly multicellular organisms [Miller10]. Since then, multicellular eukaryotic organisms have evolved an impressive degree of morphological innovation involving a huge number of cells and diversity of cell types (i.e., $100 - 1000$ in animals) spatially organized into tissues and organs. The developmental cycle of multicellular organisms usually involves a spatially- and temporally-regulated developmental process during which a single zygotic cell - originating from sporic or gametic meiosis

- undergoes innumerable rounds of division and differentiation decisions before giving rise to a mature multicellular organism. Proliferation and differentiation events continue to play a crucial role in fully developed tissues in replenishing wounded area or replacing cells that are routinely eliminated due to aging [Pellettieri07, Visvader16]. In short, ***cellular decisions such as resting, dividing, differentiating or dying must be carefully controlled, both spatially and temporally, to support the development and homeostasis of viable multicellular organisms***. Inappropriate decisions may lead, notably, to developmental defects, tumorigenesis or premature aging.

- ***Protein regulatory networks***

In animals, the sensorimotor and cognitive behaviors are orchestrated by a specific organ – the brain – and by a specific type of cells – the neurons –. The complexity of the organization and operations of neural circuits in the brain provides huge potentialities for integrating a multiplicity of cues from the external world, the internal body and long-term/working memories as well as for elaborating a diversity of routine or creative decision responses.

In unicellular organisms, decision-making processes are implemented at the level of so-called ***protein regulatory networks*** (Fig. 1.4A). Proteins are biological macromolecules consisting of amino acid chains. The life cycle of a protein essentially involves its synthesis ($DNA \longrightarrow mRNA \longrightarrow P$), some reversible or irreversible modifications ($P \longrightarrow P^*$) and its degradation ($P^{(*)} \longrightarrow \oslash$). Within a protein network, two proteins can interact, directly or indirectly, in various ways depending on which step of the protein cycle is regulated:

(i) Transcriptional/posttranscriptional regulation of protein synthesis is respectively mediated (i) by transcription factors that control the rate of transcription by promoting (as activator), or preventing (as repressor) the recruitment of RNA polymerase to specific genes and (ii) by mRNA-binding proteins that control the rates of mRNA degradation and translation.

(ii) Posttranslational regulation of protein activity is typically mediated by the enzymes that may induce diverse post-translational modifications (PTM) of protein structure/state: the most common PTM is ***phosphorylation*** which involves addition (resp., removal) of a functional phosphate group linked to a particular protein residue by protein kinases (resp., phosphatases); ***multimerization*** with other proteins is another ubiquitous mechanism used to modulate the state and activity of a protein. Such modifications can directly alter not only the functional activity of a protein, but also its localization by promoting its translocation into different subcellular structures.

(iii) Posttranslational regulation of protein stability/degradation is mediated through the interplay between PTMs, for instance when some phosphorylation events promote the ubiquitination level of a protein that, later on, will be degraded by the ubiquitin proteasome system.

The fact that a given protein species can both regulate and be regulated by many protein species and through many modes of regulation gives rise to ***large and intricate*** protein networks. However, the specificity of these regulations with respect to their mechanisms, targets and effects give also rise to ***organized and heterogeneous*** protein networks. One great challenge of modeling investigations of these large and organized protein networks is to find suitable levels of description and analysis as we will discuss in the next section.

1.2 Dynamical modeling of cellular decisions

1.2.1 Differential equation models

- ***General formalism***

In eukaryotic cells, the protein number for a given species (defined by a specific amino acid sequence and encoded by a specific gene promoter) is highly variable depending on its function and the context. On average, there is typically $\sim 10^{4-6}$ copies of a protein species translated from a pool of $\sim 10^{2-4}$ copies of mRNA and one or two copy of gene [Milo16]. Despite the low number of gene copy, the high rate of binding and unbinding events ($\sim 10^3 s^{-1}$) on the promoter compared with the mRNA transcription and degradation rates ($< s^{-1}$) enables a temporal averaging of promoter occupancy and activation. These quantitative considerations justify ***the use of rate equations to describe the dynamics of protein concentration over slow enough timescales***. Each step of the protein life cycle consists in fact several complex biochemical reaction steps involving for instance multiple cofactors, multiple binding sites, polymerization, multimerization, DNA conformation, protein folding etc... ***It is therefore convenient to describe protein network models using effective reactions*** to describe the synthesis, degradation or post-translational modification processes using implicitely linearization, averaging or timescale arguments. In any case, effective reaction rates are typically monotonous, linear or nonlinear, functions of substrates and regulator concentrations, involving for instance ***cooperative or saturation mechanisms***. Although modeling studies take into account such nonlinearities in diverse manners (Michaelis-Menten for degradation rates, sigmoidal or steep Hill functions for transcripton rates), a general scheme with mass-action kinetics is the following:

$$\oslash \xrightarrow{f_k(x_k)} x_i \xrightarrow{f_l(x_l)} \oslash \tag{1.1}$$

$$x_j \xrightarrow{f_m(x_m)} x_i \xrightarrow{f_n(x_n)} x_j \tag{1.2}$$

can be translated into the following differential equation for the time evolution of the species i, at a concentration x_i:

$$\frac{dx_i}{dt} = f_k(x_k, \mathbf{p}_k) - f_l(x_l, \mathbf{p}_l)\, x_i + f_m(x_m, \mathbf{p}_m)\, x_j - f_n(x_n, \mathbf{p}_n)\, x_i \tag{1.3}$$

where $f(x, \mathbf{p})$ generally consists in linear, quadratic polynomial or Hill functions. Furthermore, some extracellular or intracellular concentration species (damage, nutrient, intercellular communications) may reflect the state of the external environment to which the cell should adapt, and must therefore be treated as ***signaling inputs*** ($\mathbf{s}(t)$) to which the protein regulatory network should respond. Such distinctions between signal inputs, intracellular variables, kinetic parameters and reaction rates lead to the following smooth nonlinear ODE system:

$$\frac{d\mathbf{x}}{dt} = \mathbf{F}(\mathbf{x}(t), \mathbf{p}, \mathbf{s}(t))\ \mathbf{x} \in \mathbb{R}^{n1}\,,\ \mathbf{p} \in \mathbb{R}^{n2}\,,\ \mathbf{s} \in \mathbb{R}^{n3} \tag{1.4}$$

whose behavior can be studied and classified in various spaces: the state space, the parameter space, the signal space, and diverse mapping between those spaces (Fig. 1.4B).

- ***Design and reduction of regulatory network models***

In textbooks, the modeling of protein networks typically follows a stepwise task sequence with (i) the identification of proteins and their interactions, (ii) the formalization of the corresponding ODE system Eq.1.4, (iii) the estimation of parameter constants, (iv) the dynamical analysis of the model. It is however worth to highlight now the main difficulty encountered in this

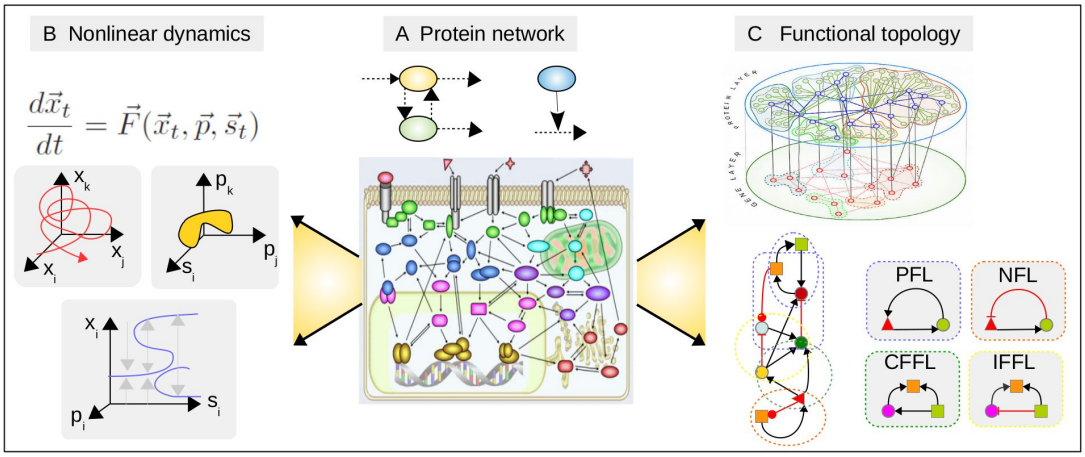


Figure 1.4: **Topology and dynamics of protein networks.** *(A) A set of multimodal interactions between diverse biomolecular species produces a complex and dense protein network characterized by (B) nonlinear dynamical properties (e.g., in state space, parameter space and signal space) and (C) topological properties (e.g., graphs, loops). Upper panel extracted from [Srivastava14].*

modeling process. The above mentioned large-scale and multi-scale features of protein regulatory networks can lead to dynamical systems Eq.1.4 characterized with ***high-dimensional state space and parameter space***. First, the nonlinear nature of these multiscale networks make their theoretical and computational analysis challenging. But more importantly, experimental studies do not bring enough informations regarding the regulatory scheme, the values of kinetic parameters or the dynamics of variables, to reliably determine the detailed structure and accurate parametrization of such high-dimensional models. In other words, such incomplete biochemical and biological knowledge may typically lead to problems of non-identifiability, for which the same set of data can be equally explained by several model structure or parametrizations. To solve these problems, many implicit or explicit methods must be used to reduce the dimensionality of the model [Radulescu12,Snowden17], ***so as to match with the dimension of experimental data and to facilitate its analysis***:

(i) Network design: Network design is an essential, difficult and controversial part of the modeling work, which can be discussed along three main considerations. Because the tasks realized by cells are usually segregated in time and space, the regulatory network typically shows a modular structure [Hartwell99, Papin04]. Furthermore, a broad class of functional behaviors such as transitions, oscillations, perfect adaptation, signal processing can be performed by small circuits [Tyson03, Alon07, Brandman08]. Accordingly, ***a first step to design a network model of reasonable dimensionality is to isolate a task-specific subnetwork or module***, keeping in mind that such subnetwork must be somehow coupled to others to ensure coordination between diverse tasks. Because task-specific network can remain large, ***a second step consists in restricting to a core set of species*** whose significant role is supported by experimental studies (knockdown, overexpression) and by their position within the network (hub, feedback), which amounts to delete the irrelevant parts of a network. ***Another related network design step consists in gathering/lumping several species or regulations together***, for instance by describing a family or class of species (e.g., CKI, Rb, Dll) as a single effective species or by describing a signaling cascade (e.g., MAPK, PI3K) as a single effective regulation. These simplification steps are generally performed on the basis of prior experimental knowledge and working hypothesis about which species/regulations are important or not for the data

or phenomena of interest. However, these could in principle be derived from diverse – inference, decomposition, truncation, projection, pruning, lumping or coarse-graining – techniques (reviewed in [Radulescu12, Snowden17]) when applied to a well-defined and parameterized network.

(*ii*) Timescale separation: The dynamical systems above (Eq. 1.4) typically displays a broad range of timescales associated with the different regulatory mechanisms (of the order of seconds for phosphorylation cycles, of minutes to hours for protein turnover rate and of hours to days for epigenetic changes of chromatin), but also related to the nonlinear dynamics of the system (oscillatory periods, fast/slow modes). Timescale separation is the most common argument to reduce the dimensionality of the system, where for instance the slowest variables can be assumed to be constant while the fastest variables can be assumed to quickly equilibrate. In fact, diverse methods are available ranging from the common quasi-steady state approximation or averaging techniques to more elaborate manifold-based techniques (e.g., computation of and projection onto slow invariant manifolds) [Radulescu12].

(*iii*) Nondimensionalisation: Nondimensionalization of concentration and time is a simple procedure to reduce the number of variables and of parameters. Because concentrations are positive but bounded (due to crowding effect), such rescaling can enable to define and restrict the relevant state-space as an hypercube.

(*iv*) Parametric analysis: Regardless whether models are more or less reduced, many parameters are either effective, difficult to estimate experimentally or highly dependent on the context or cell type, meaning that a critical part of model setting is to estimate the biologically- and functionally-relevant domains of parameters. This difficulty of parametric incompleteness can be circumvented using diverse methods of parametric analysis such as optimization methods (e.g., local or global, heuristic or iterative), sensitivity analysis (e.g., identification of stiff versus sloppy parameters) or parameter space exploration (e.g., bifurcation and phase diagrams).

(*v*) Discreteness and randomness: Modeling frameworks other than ODE can be more appropriate to account for the influence of the discrete and stochastic nature of certain regulatory events on intracellular dynamics and cell behaviours. For instance, the sigmoid binary-like response of transcriptional rate as a function of promoter occupancy has inspired logic-based boolean models, which are suited for discrete-state analysis of large gene regulatory networks [Albert14, Dunn14]. As well, the overall effects of various sources of stochasticity (molecular noise, low copy number, chromatin switching, transcriptional bursting etc...) must be treated with statistical physic tools, such as the chemical master/Fokker-Planck equations or stochastic simulations [Maheshri07, Gillespie13, Tsimring14], though it can be still possible to keep using the ODE framework by introducing Langevin noise terms [Maheshri07, Gillespie13].

In the models defined and analyzed in this thesis, both rather detailed ($n_1, n_2 > 10$) and coarse-grained/reduced ($n_1, n_2 < 10$) network models have been studied, depending on whether the model aims to study specific biological process and regulatory mechanisms or to extract some network design principles involved for a class of biological behavior. In any case, the elaboration of network models was carefully based on literature and experimental evidences, and exploited in diverse manner and rigorness the techniques presented above (see for instance [Pfeuty18] and its Fig. S8).

1.2.2 Network topology

Like many other networks (neuronal, social, ecological, communication), a protein regulatory network can be viewed as a network of nodes connected by directed edges and characterized by a specific topology (Fig. 1.4C). The topology of a large network can be analyzed with respect to the statistical features of their edge distribution (degree distribution, clustering coefficient, assortativity, hierarchy, reciprocity...) and, then, be shown to belong or relate to a particular class or subclass of network topology (random, scale-free, small world, democratic, bow-tie...). These large-scale topological features of protein networks [Barabasi04, Friedlander15, Mengistu16] provide key insights on evolutionary forces and constraints that shape the network structure.

However, ***the adaptive dynamics of protein regulatory networks tends to rather depend on the operations and combination of small-scale topological features called motifs***, each of which can already underlie sophisticated dynamics and functional tasks [Tyson03, Prill05, Alon07]. A primary classification of these functional motifs can be first made by depicting a protein regulatory network as a graph with directed and signed edges characterized with a particular Jacobian $J_{ij} = \mathcal{S}\left(\left[\frac{\partial F_i}{\partial x_j}(\mathbf{x})\right]\right)$. Feedforward loops (FFL) are defined by two or more paths that connect the same source and target nodes, and they can be coherent (CFFL) or incoherent (IFFL) depending on whether the overall effect along these paths have same or opposite signs. Motifs with feedforward loops only can already trigger diverse complex dynamical behaviors such as signal filtering, non-monotonic transient dynamics and non-monotonic steady-state outputs [Kaplan08, Goentoro09, Ma09]. Feedback loops are defined by a cycle path that connect the same source/target node, and they can be positive (PFL) or negative (NFL) depending on the sign of the overall effect along this path. In contrast with FFL only, feedback loops have a critical impact on the Jacobian determinant, and thus on the fixed point stability. For instance, a NFL is a necessary condition for an Hopf bifurcation giving rise to oscillations while PFL is a necessary conditions for multistationarity [Thomas81]. From a biological viewpoint, feedbacks play critical roles in the adaptive and developmental abilities of living organisms [Lewis08]:

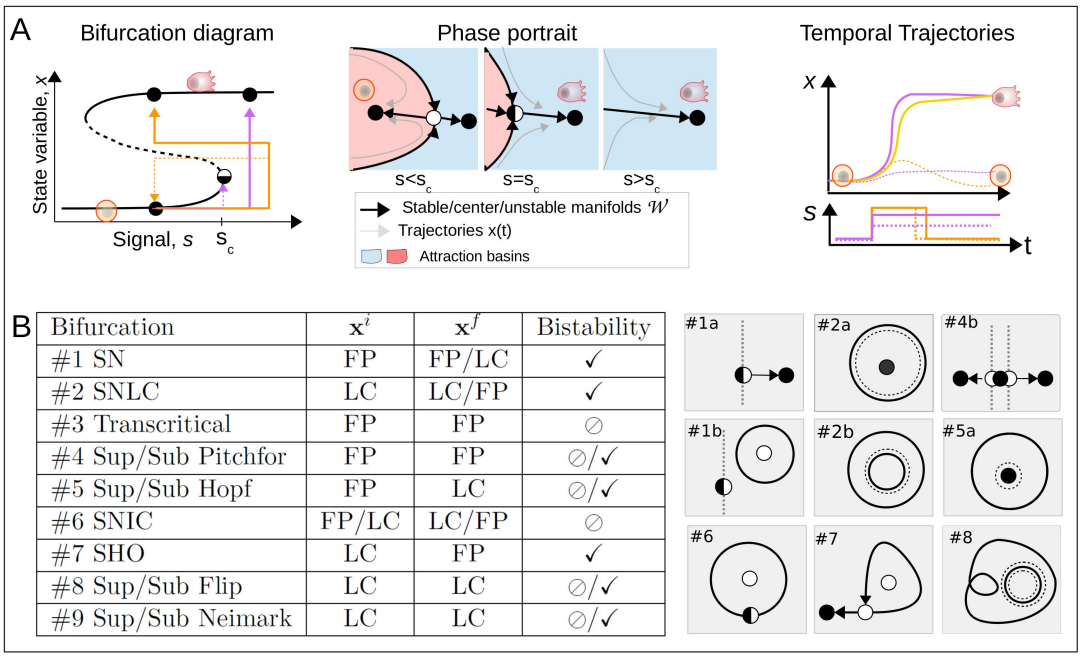
> *"Positive feedback can give a system a flip/flop choice between alternative steady states; it can endow a system with enduring memory of its exposure to past signals; it can generate inhomogeneity in a system that starts out spatially uniform. Negative feedback can smooth out irregularities; it can enable a system to respond to a signal more rapidly; operating with a delay, it can give rise to temporal oscillations. These behaviors are not immediately obvious intuitively, but mathematics allows us to predict and compute them."*

In fact, protein networks often comprise a large number of such loops intermeshed in various ways. Such interplay between a multiplicity of feedback loops is likely to give rise to a broad repertoire of sophisticated dynamical behaviors, while a given dynamical behavior can be implemented by a diversity of loop topologies. This combinatorial potential have motivated the investigation of ***the design principles of protein networks*** [Lim13] involved for instance in signaling cascades [Kholodenko06], oscillatory circuits [Novak08, Jolley12], adaptative responses [Ma09, Ferrell16], fold-change detection [Adler17] and, of course, switching behaviours [Brandman05, Kim07, Guantes08, Shah11, Kim12, Oyarzun15, Cardelli17].

1.2.3 Nonlinear dynamics

The main proposal of our dynamical modeling approach is that ***important properties of cellular decision making can be primarily explained at the level of the nonlinear dynamics of the protein regulatory networks***, which can be investigated through computational analysis of Eq. 1.4. Decision making can be viewed as a dynamical process that converts

a continuous stream of signal inputs into discrete behavioral outputs. From a dynamical system perspective, this process is often described as a discrete transition from a destabilized pre-decision attractor state toward a well-distinct post-decision attractor states, eventually among several possible attractor states. This kind of behavior is likely to occur in the presence of a ***bifurcation or structural instability*** corresponding to changes in the qualitative (or topological) structure of the phase portrait of the dynamical network system, where the phase portrait of a dynamical system is a flow-invariant partitioning of the state space into a set of particular orbits (See dynamical system glossary of Appendix C) that offer skeletal information about the topology of the flow [Wiggins90]. The phase portrait and its transformation upon changes of signals and parameters are likely to provide important informations about decision-making properties by depicting attractors, attraction basins, basin boundaries, bifurcation events and transition trajectories. This is illustrated for the simplest decision-making switch (Fig. 1.5A) associated with a signal-induced destabilization of a fixed point through a saddle-node bifurcation point (at $s = s_c$) toward another stable fixed point that is stable for $s < s_c$, which implies bistability and possibly hysteresis.



B

Bifurcation	$\mathbf{x}^i$	$\mathbf{x}^f$	Bistability
#1 SN	FP	FP/LC	✓
#2 SNLC	LC	LC/FP	✓
#3 Transcritical	FP	FP	⊘
#4 Sup/Sub Pitchfor	FP	FP	⊘/✓
#5 Sup/Sub Hopf	FP	LC	⊘/✓
#6 SNIC	FP/LC	LC/FP	⊘
#7 SHO	LC	FP	✓
#8 Sup/Sub Flip	LC	LC	⊘/✓
#9 Sup/Sub Neimark	LC	LC	⊘/✓

Figure 1.5: **Bifurcation mechanisms for discrete decisions.** *(A) The basic decision switch can be described by a signal-induced destabilization of a (fixed-point) attractor when crossing a (saddle-node) bifurcation point toward another (fixed-point) attractor, during which the phase portrait structured by several invariant manifolds displays qualitative changes. Accordingly, decision making is sensitive to inducing signal intensity and duration. (B) Repertoire of bifurcation scenarios underlying a transition between two states, which are restricted to fixed points (FP) and limit cycles of period-1/2 ($LC^{1/2}$). Sup: supercritical. Sub: subcritical. Bistability: coexistence of two stable states* $\mathbf{x}^i$ *and* $\mathbf{x}^f$ *for* $s = s_c - \epsilon$.

- ***The diversity of bifurcation scenarios***

Although the simplest decision switch can be described by a one-dimensional bistable system (Fig. 1.5A), refined and elaborate decision-making behaviours may involve a diversity of decision attractors (fixed point, one or multiple frequency oscillations, perhaps chaotic attractors) and

of bifurcation types, some of which are listed and depicted in Fig. 1.5B. In fact, the way how temporal variations in bifurcation parameters and stochasticity (e.g., Langevin noise) control the transition dynamics tends to depend on the type of underlying bifurcation [Wiggins90, Berglund06]. It is also noteworthy that codimension-1 bifurcation scenarios can intersect in the parameter space into higher codimension bifurcation scenarios (e.g., Bautin, cusp, pitchfork, Bogdanov-Takens, SNHO, SNSHO; some are depicted in Fig. B.1). This thesis also explores the conjecture that distinct types of bifurcation can underlie different decision properties, and high-codimension bifurcations can underlie flexible decision properties.

1.2.4 Thesis outline

This thesis investigates differential equation models of protein networks that regulate important cellular decisions related with proliferation and differentiation processes. The main objective is to decipher the relationship between the structural and dynamical properties of these network in order to shed light on key decision-making properties and strategies of living cells. This thesis will be organized into chapters that will address these questions regarding four important decision-making properties that are typically involved for cell division and differentiation decisions:

- **Chapter 2**: Decision speed
  How do cells control the timing of their decisions?
- **Chapter 3**: Decision reversibility
  How do cells control the (ir)reversility of their decisions?
- **Chapter 4**: Decision gating
  How do cells seize or create decision-making opportunities?
- **Chapter 5**: Decision paths
  How do cells control the sequentiality of their decision process?

Chapter 2

Decision speed

"Do you have the patience to wait until your mud settles and the water is clear?"

Lao Tzu

Speed-accuracy trade-off is an essential feature of biological decision-making processes in general, and of division initiation decision in particular (Section 2.1). This Chapter presents several studies of the G1-phase progression and G1/S transition in the mammalian cell-cycle, which reveals a complex organization of the regulatory network (Section 2.2.1 and [Pfeuty08]) that specifically allows for a tunable control of the timing of G1/S transition (Section 2.2.2 and [Pfeuty12b, Pfeuty12c]). These studies highlight network and dynamic mechanisms that enables a fine-tuned control of decision speed, regardless to some extent of the decision-making impulse (Section 2.3).

2.1 Introduction

2.1.1 Insights from neuroscience: speed-accuracy trade-off

In experimental psychology, the measures of reaction times and error rates in decision tasks involving varying temporal constraints have established a fundamental principle in decision theory known as the speed-accuracy trade-off [Wickelgren77, Bogacz10, Heitz12]: in such a trade-off, decision makers implicitly compare the relative advantage between reacting quickly and well-preparing and weighting their decision, in order to decide when to initiate the irreversible step of decision making. On the one hand, urgent decisions with tight deadlines require quick reaction times and commitment. On the other hand, error-free decisions in ambiguous situations and in the absence of stringent deadlines require prior accumulation of evidences and complex information processing before commitment. Despite the diversity and multiplicity of sensory and motor modalities involved in decision tasks, the speed-accuracy trade-off seems to be captured by rather simple computational models (called integrated accumulators or bounded integration) whose parameters can be nevertheless highly flexible and adjustable depending on the attentional effort, the motivation or the environmental context [Salinas14, Standage14, Drugowitsch15].

2.1.2 Speed and checkpoints for cell proliferation

The efficiency of cellular decision-making processes is dependent on various contingencies related to readily available informations, temporal constraints or metabolic costs, which should also entail a trade-off between speed and accuracy. The temporal gap between signal exposure and irreversible commitment can be highly variable and may significantly increase in certain situations. Among the many cellular functions that are based on discrete decisional processes, the issue of whether to divide or not is probably the most archaic and ubiquitous decision to which cells are confronted. The proliferation cycle of growth and division has indeed evolved as one of the most fundamental strategy involved in the perpetuation and expansion of life. It basically relies on the temporal coordination of diverse catabolic, anabolic and morphological processes including protein synthesis, mitochondrial fusion, DNA replication, chromatin segregation and cytokinesis. The accurate and successful orchestration of these events requires favorable external and internal conditions (related to nutrient import, metabolic state, cell integrity, stress events...) which are carefully evaluated through the integration and computation of diverse signals [Morgan07]. During the decision-making process of division initiation, the speed-accuracy trade-off manifests itself through the competing requirements for:

(i) a quick enough division initiation to sustain high proliferation rate and to maximize the fitness of the population.

(ii) a careful enough division initiation to avoid partial or complete failures and maximize the chances of giving birth to healthy daughter cells. This stringency of this requirement is demonstrated by the occurrence of so-called ***cell-cycle checkpoints*** at the transition between different cell-cycle phases, such as, for instance, the growth-dependent checkpoints [Hartwell74, Pardee74] or the DNA-damage checkpoint [Bartek01].

Expectedly, the optimal trade-off between the two requirements would be determined by the respective costs of cell division failures and division delays, an thus, would vary depending on environmental context (e.g., poor versus rich nutrient conditions), species attributes (e.g., prokaryotes versus eukaryotes) or cellular lifestyle (e.g., unicellular versus multicellular). As a matter of fact, the duration of the cell-division cycle of mammalian cells, especially their G1 phase, exhibits an extreme variability, notably over the course of development [Takahashi99, Lange10, Roccio13, Dong18] (Fig. 2.1A).

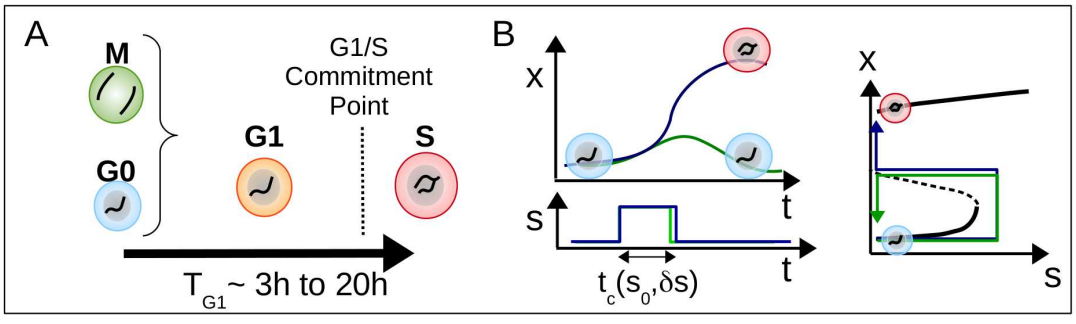


Figure 2.1: **Decision speed during the G1-phase of the cell cycle.** *(A) The decision time T_{G1} – the time between G0 exit (or M-phase exit) and S-phase initiation – ranges in mammalian stem cells between* 3hr *to* 30hr *depending on various factors. (B) Decision speed relates to the time t_c between the onset of the inducing signals (e.g., growth mitogenic factors) and the irreversible crossing of a saddle separatrix (see Eqs 2.1 to 2.5).*

2.1.3 How to regulate transition speed?

The control of the decision speed associated for the initiation of cell division can be addressed from a dynamical perspective as follows: let assume that a step signal (Eq. 2.1) makes a bistable protein network transit from the steady state $\mathbf{x}^0$ (e.g., G0) toward the stable steady state $\mathbf{x}^1$ state (e.g., S phase) (Eq. 2.2). The decision speed will mostly be related to the time t_c (Eq. 2.3) between the onset of the step signal and the crossing of a saddle separatrix beyond which decision becomes irreversible despite the removal of decision-inducing signals (Eq. 2.4) (Fig. 2.1B). Within this scheme, the control of decision speed can be investigated by determining how this critical time t_c depends on signal properties ($\mathbf{s}_0$, $\delta\mathbf{s}$) and network properties ($\mathbf{p}$) (Eq. 2.5).

Step signal:	$\mathbf{s}(t) = \mathbf{s}_0 + \delta\mathbf{s}\,\mathcal{H}(t)$	(2.1)
Bistability:	$\phi^\infty(\mathbf{x}^{0,sad,1}, \mathbf{s}_0) = \mathbf{x}^{0,sad,1}$	(2.2)
Decision time:	$t_c = \{t : \phi^t(\mathbf{x}^0, \mathbf{s}_0) \in \mathcal{W}^s(\mathbf{x}^{sad})\}$	(2.3)
Saddle separatrix:	$\mathcal{W}^s(\mathbf{x}^{sad}) = \{\mathbf{x} : \phi^\infty(\mathbf{x}, \mathbf{s}_0) = \mathbf{x}^{sad}\}$	(2.4)
Speed control?	$t_c = f(\mathbf{s}_0, \delta\mathbf{s}, \mathbf{p})$	(2.5)

2.2 Results: Temporal control of G1-phase decisions

2.2.1 Flexible G1-phase transit through intricate feedback loops

See [Pfeuty08] for more details

- ***The multiplicity of positive feedback loops***

As emphasized earlier, the growth-dependent checkpoint that occurs in late G1 phase before S-phase entry and initiation of DNA replication is the first and most important cell decision-making step of cell division. Beyond this G1/S checkpoint, a sudden deprivation of growth or mitogenic factors will not prevent the progression through the S phase, which suggests the occurrence of a saddle instability (like in Fig. 2.1B) induced by strong positive feedback mechanisms. This irreversible transition can be explained by the structure of the G1-phase regulatory network which comprises several positive feedback loops involving cyclin/Cdks complexes, notably cyclin D/Cdk4,6 (cycD) and cyclin E/Cdk2 (cycE), and cell-cycle inhibitors, notably Rb proteins and Cdk-inhibitors (CKI) (Fig. 2.2A):

$$
\begin{aligned}
&\text{PFL1}: \quad E2F \to E2F \\
&\text{PFL2}: \quad E2F \to cycE \multimap Rb \multimap E2F \\
&\text{PFL3}: \quad cycE \multimap CKI \multimap cycE \\
&\text{PFL4}: \quad cycE \multimap Rb \dashv cycE
\end{aligned}
$$

Although cooperation between these PFLs is sufficient to explain the irreversible transition from G1 to S phase [Novak04, Gerard09], the coupling between growth and division or other regulatory refinement may need to be taken into account in order to explain the timing of the transition. In unicellular organisms, models of the cell-division cycle usually assume that the rate

of cyclin synthesis is cell size-dependent so that G1-phase duration, T_{G1}, essentially depends on the time required to reach a critical cell size [Csikasz-Nagy06, Pfeuty07]. Cells from multicellular organisms display a more flexible and reciprocal coupling between their growth and division processes [David06, Ginzberg15] as senescent or differentiated cells can grow very large without dividing while some stem cells and cancer cells divide at very small sizes. Such a reciprocal coupling between growth and division is also expected to enable mammalian cells to finely tune their G1-phase duration in a context-dependent manner.

In order to confirm this hypothesis, we have proposed a model of the mammalian G1-phase regulatory network [Pfeuty08] that notably includes many reciprocal interactions between ribosomes and cell-cycle regulators (Fig. 2.2A), which thus implement many additional feedback loops:

$$\begin{aligned}
\text{PFL4}: &\quad Rb \dashv Rib \rightarrow cycD \multimap Rb \\
\text{PFL5/6}: &\quad cycE \rightarrow Rib \rightarrow cycD, E \\
\text{NFL1}: &\quad cycE \rightarrow Rib \rightarrow CKI \multimap cycE \\
\text{NFL2}: &\quad p53 \dashv Rib \rightarrow p53 \\
\text{NFL3}: &\quad cycD \multimap CKI \rightarrow cycD
\end{aligned}$$

Among the many positive feedback loops, PFL4 is especially interesting as it operates upstream independently on cyclin E. Although the early-G1 PFLs should lead to the activation of late-G1 PFLs (because cycD activates cycE synthesis via Rb phosphorylation and derepression), basal or stress-induced activation of the p53-CKI pathway can inhibit late G1 progression and S phase entry without significant interference with early G1-phase progression and G0 exit.

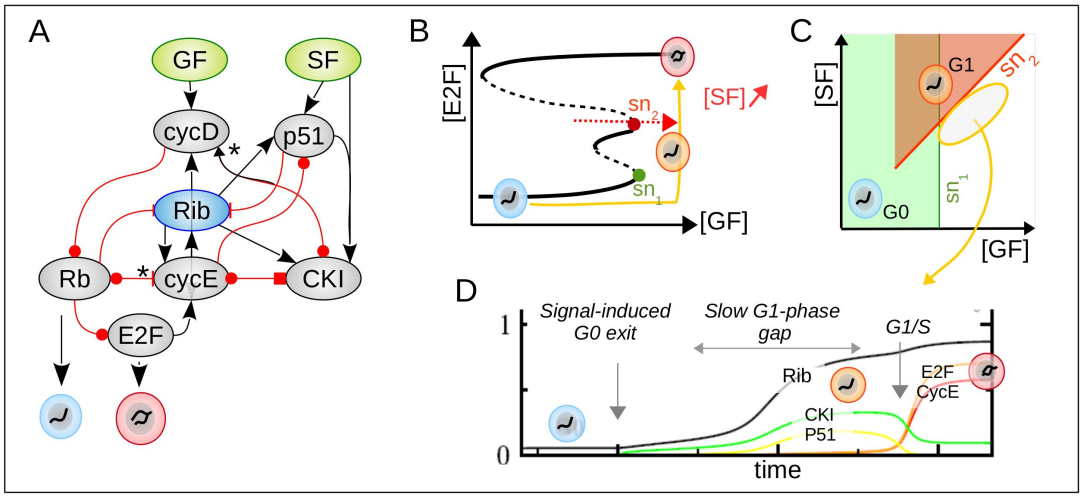


Figure 2.2: **G1-phase arrest before G1/S transition.** *(A) Mammalian G1-phase regulatory network including the interactions between cell-cycle regulators and ribosomes (* are disrupted in section 2.2.3 and Fig. 2.3). (B) Schematic bifurcation diagram showing that increasing $[SF]$ stabilize a G1-phase arrested state between the G0 state and the S-phase state, by shiftin $[GF]_{sn_2}$ threshold. (C) Schematic phase diagram showing the separable control of two SN bifurcation for G0 exit $[GF]_{sn_1}$ and for G1/S transition $[GF]_{sn_2}([SF])$. (D) Very slow G1 phase progression with transient upregulation of CKI and P51 close to the SN_2 bifurcation. All panels are extracted or adapted from [Pfeuty08].*

Accordingly, increasing the level of stress factors $[SF]$ ***can stabilize an intermediate G1-phase arrested state*** (Figs. 2.2B,C) without compromising G0 exit (i.e., $[GF]_{sn1}$ at

which G0-state branch is destabilized) as demonstrated by ***the existence of a tristability region*** (Figs. 2.2C). Moreover, $[SF]$ levels that are not high enough to stabilize this intermediate G1-phase arrested state can nevertheless significantly delay the irreversible transition to the S phase state, which correlates with a transient increase of P53 and CKI during early G1 phase (Figs. 2.2D), consistently with experiments [Loewer10]. Such a non-monotonic response of cell-cycle inhibitors following a step of $[SF]$ manifests the presence of NFL and/or IFFL mechanisms such as NFL1 and NFL2. A further question is to whether the observed slow-down of G1-phase progression requires particular bifurcation and network mechanisms.

2.2.2 Flexible decision speed through saddle-node ghosts

See [Pfeuty12b, Pfeuty12c] for more details

- ***G1/S decision speed depends on network-driven transition trajectories***

A simplified G1-phase regulatory network without Ribosomal and P51 proteins [Pfeuty12b] is studied to clarify the dynamical mechanism by which an intermediate G1-phase arrest state contributes to a flexible decision speed [Pfeuty12b]. The $[SF]$-dependent control of G1-phase speed/duration was found to tightly depend on the network organization (Fig. 2.3A). Indeed, disrupting specific seemingly-minor regulations ($Rb \dashv cycE$ and $CKI \rightarrow cycD$) qualitative alter the manner how G1-phase duration depends on $[SF]$ and how much (ir)reversible is the $[SF]$-dependent G1-phase arrest (Fig. 2.3A). In the *wild-type* model (left), the G1-phase progression speed smoothly decreases with increasing levels of $[SF]$ when $[SF] \to [SF]_{sn}$ as $1/T_{G1} \propto ([SF]_{sn} - [SF])^{1/2}$, whereas the G1-phase arrest is fully reversed when $[SF]$ decreases below $[SF]_c$. Following the two network alterations (among others), the G1-phase progression speed sharply decreases with increasing levels of $[SF]$ when $[SF] \to [SF]_c$ as $1/T_{G1} \propto -\log([SF]_c - [SF])^{-1}$, whereas the G1-phase arrest is reversed only when $[SF]$ decreases below $[SF]_{sn} < [SF]_c$. The difference between these two behaviours relates to whether the saddle-node bifurcation associated with the appearance of the G1-phase arrested state occurs on the G1-phase trajectory ($\phi^{\infty}(\mathbf{x}^{G0}) = \mathbf{x}^{sn}$) or not. In the latter case, G1-phase arrest would arise when the G1-phase trajectories meet the saddle fixed point ($\phi^{\infty}(\mathbf{x}^{G0}) = \mathbf{x}^{sad}$) (Fig. 2.3B). Given these differences, tunable transition speed relies on smooth slowing down of the flow speed in a state-space region (called a bottleneck region) where a saddle-node is close to appear under a parametric perturbation (called a saddle-node ghost or remnant).

Because the network model was simplified and not specific to a particular stress context, we do not really expect or predict that disrupting one of these two regulations will induce qualitative changes of G1-phase progression. However, this result demonstates that the qualitative properties of G1-phase progression can drastically change by removing a subset of feedback or feedforward motifs in the network, thus highlighting the functional importance of such regulatory sophistications.

- ***Relations between bifurcation scenarios and decision properties***

Like in the above models, the dynamics of cell-cycle transition dynamics between two phases (G0/G1, G1/S, G2/M etc...) can be studied by analyzing the multistable switching behavior of a network model restricted to the cell-cycle module and phases of interest. However, most cell-cycle models describe the whole cell-division cycle as a limit cycle oscillator [Novak04, Csikasz-Nagy06, Gerard09]. The above described relationship between decision-making speed properties and bifurcation mechanisms can be recapitulated more generically for limit cycle scenarios (Fig. 2.4 and [Pfeuty12c]):

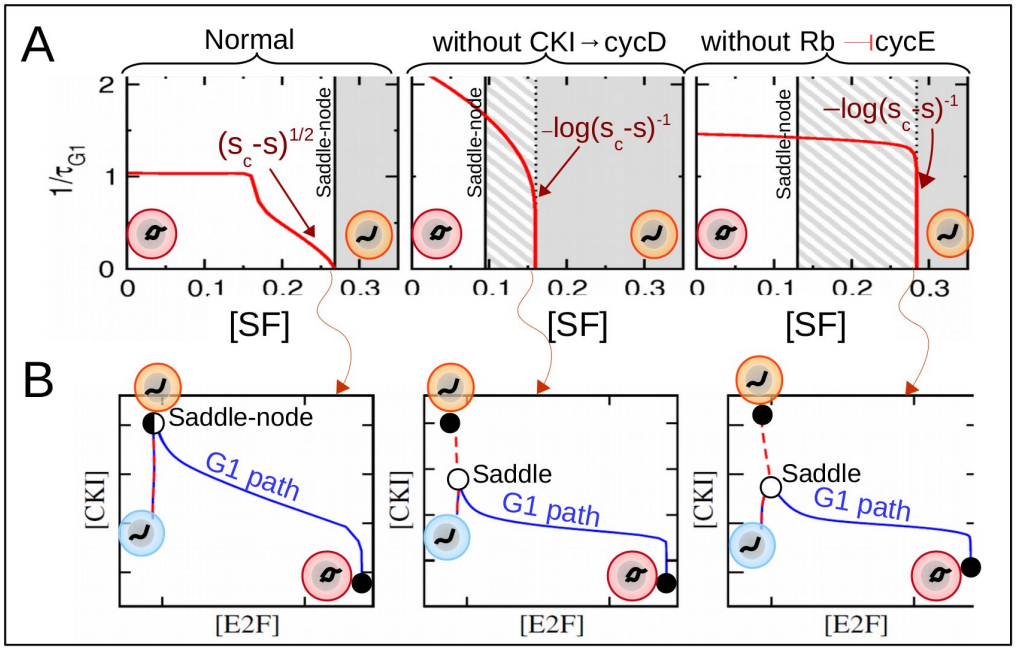


Figure 2.3: **Two distinct classes of G1-phase arrest depending on network features.** *Alterations of the G1-phase regulatory network can qualitatively change (A) the controllability of G1-phase duration ($T_{G1} = f([SF])$) from tunable to non-tunable and (B) the corresponding slow G1-phase trajectories from a saddle-node to a saddle colneigbhorhood. Panels are extracted or adapted from [Pfeuty12b].*

(*i*) **Tunable control of decision speed** typically occurs in the vicinity of a saddle node on invariant circle (SNIC) bifurcation (Figs. 2.4, left). This bifurcation scenario entails a fully reversible cell-cycle arrest.

(*ii*) **Non-tunable (and quick) decision speed** typically occurs in the vicinity of a saddle-homoclinic bifurcation (SHO) (Figs. 2.4, right). This scenario is associated to an irreversible, to varying extent, cell-cycle arrest.

(*iii*) **Tunable decision speed and tunable irreversibility** can nevertheless co-occur in the vicinity of higher codimension bifurcations such as a saddle node bifurcation on a saddle homoclinic orbit (SNSHO) (Fig. 2.4, middle). This scenario has been evidenced in a model of cell-cycle arrest and differentiation decisions in neural stem cells [Pfeuty15a], and is more consistent with the observation of distinct, reversible and irreversible, cell-cycle arrest in response to varying level of type of stress [Toettcher09, Purvis12].

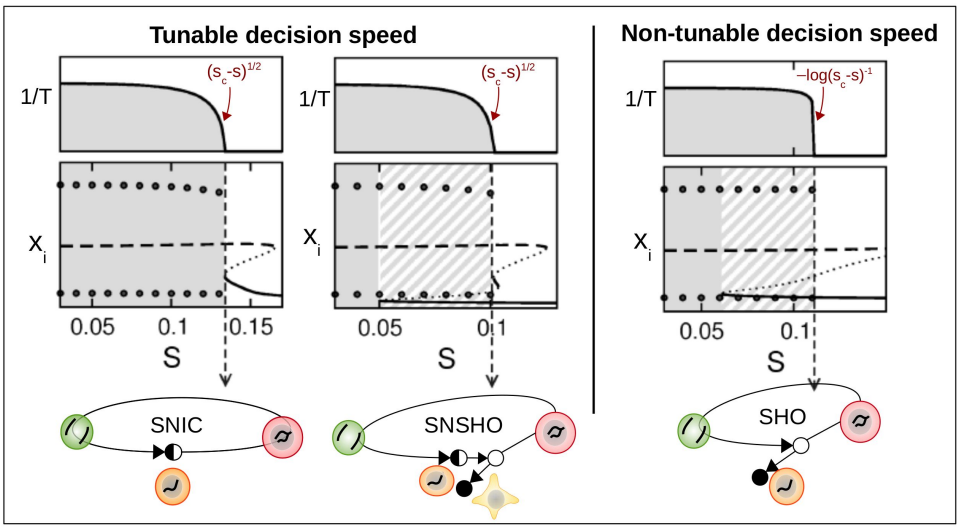


Figure 2.4: **Limit cycle bifurcation scenarios associated with tunable or non-tunable decision speed.** *Saddle-node in Left-to-right panels represent three bifurcation routes associated with local bifurcation of a limit cycle giving rise to tunable or non-tunable decision making. Upper panels are extracted from [Pfeuty12c]*

2.3 Conclusion

In a basic bistable decision-making switch generated by a single PFL, the transition speed from $\mathbf{x}^0$ to $\mathbf{x}^1$ is related to the degree of instability of the initial state measured by the flow speed at $|\mathbf{F}(\mathbf{x}^0, \mathbf{s})| > 0$. However, speed-accuracy trade-off suggests that the decision-making speed should also depend on other context-dependent requirements such as the accumulation of evidences or temporal deadlines. Our studies of division initiation decision (G1/S transition) have revealed that *part of the complex feedback and feedfoward organization of the network* contribute to a generic dynamical mechanism enabling for a flexible control of decision speed:

> *Cellular decisions featured with a temporally-flexible preparation stage before irreversible commitment may build upon "a saddle-node ghost mechanism"*.

In the multistable G1-phase regulatory networks that are studied in [Pfeuty08, Pfeuty12b], we showed that the condition for the decision-making trajectory to traverse this ghost region depends on the feedback-feedforward organization of the network, in a manner that is not yet really understood but that would require to depict more carefully the phase portraits and stability properties of invariant sets. The relationship between decision-making speed tunability and the properties of transition trajectories near some singularity at $s = s_c$ is summarized by (Appendix C or SM of [Pfeuty12b]):

Trajectory through	SN ghost	Near saddle
Speed $\Sigma = 1/t_c$	$\propto (s - s_c)^{1/2}$	$\propto -\log(s - s_c)^{-1}$
Tunability $(\partial_s \Sigma)^{-1}$	$\propto \Sigma$	$\propto \Sigma^{-2} e^{-B/\Sigma}$
$\Sigma \gg \Sigma^{-2} e^{-B/\Sigma}$ for Σ small enough		

The existence of a temporally-flexible preparation stage before irreversible commitment can also be viewed as a reflection stage that would ultimately lead to make or not the decision (to divide). This notion have been corroborated by a body of evidences

showing the existence of a reversible and tunable G1-phase arrest well distinct to G0 arrest: (i) upon wound or normal regeneration, adult quiescent stem cells first switch (eventually back and forth) into a metabolically-activated early G1 state [Rodgers14, Visvader16], before to eventually divide asymmetrically and give rise to transit-amplifying progenitors; (ii) upon moderate stress, cultured cells can enter into a fully reversible G1-phase arrest state before to divide and proliferate again [Toettcher09, Purvis12]. More generally, it is tempting to propose that a reflection stage is all the more necessary during such G1 phase where many decision outcomes other than to divide are possible (e.g., wait, repair, differentiate, senesce or die [Wainwright01, David06]).

Conversely, the speed-accuracy trade-off should also favor the ability to make *fast and unchecked* decisions when it is needed. For instance, some stem cells, especially embryonic stem cells and some neural stem cells, display a very short G1-phase length and weak variations in their cyclin level, which suggests that they are lacking checkpoints [White05, Ballabeni11, Roccio13]. Noteworthily also, transmission and repartition of maternal factors (P21, Arf) critically determine whether daughter cells will born in a G0-like state (with hyperphosphorylated Rb) or a G1-like state (with hypophosphorylated Rb), thereby contributing to both checked and unchecked G1-phase progression [Moser18]. It is therefore conceivable that the cell cycle dynamics can be switched between a low-amplitude limit cycle to make a quick division decision or large-amplitude and speed-tunable limit cycle to make careful division decision among diverse alternatives.

Time-lapse imaging using cell-cycle reporter probes (e.g., FUCCI) can provide accurate measurements of the duration of cell-cycle phase, especially G1-phase [Roccio13, Kafri13]. Coupling such measurements with manipulations of (i) the cell environment (e.g., using microfluidic techniques [Albrecht10]) or/and (ii) the regulatory structure (e.g., using genome engineering techniques) would be nevertheless required to assess the nonlinear dynamical mechanism highlighted in this chapter.

Chapter 3

Decision reversibility

"There is nothing wrong with (mind) change, if it is in the right direction"

Winston Churchill

A key question related to decision making is whether and to which extent a decision should be maintained upon removal of the decision-inducing signal (Section 3.1). We have shown that a well-design combination of positive and negative feedback generates a bistability featured with flexible hysteretic behaviors (Section 3.2.1 and [Pfeuty09]). Such feedback and dynamical mechanism is illustrated in the case of tissue-level control of cell fate decisions (Section 3.2.2 and [Pfeuty16]). These studies highlight network and dynamic mechanisms which allow for a fine-tuned control of decision-making reversibility, regardless to some extent of decision-making initiation (Section 3.3).

3.1 Introduction

3.1.1 Insights from neuroscience: changes of mind or firm commitments

A well-known aspect of human decision making is whether one can easily change its mind and reconsider its decision or one is bound to its decision for a long time [Fleming09, Resulaj09, Alabatankis11]. On the one hand, decisions must be kept for long time enough to bring a true benefit, which requires that decision attractors be both insensitive to and robust against various sources of perturbations and signals. On the other hand, decisions need also to be reevaluated in the light of the ongoing stream of new external or internal cues. These two conflicting requirements suggest the existence of a trade-off between robustness and flexibility, which would depend on the relative adaptive advantages of keeping its commitment or of changing its minds throughout the decision process.

This fundamental trade-off is illustrated by the artificial perceptual phenomena of binocular rivalry where perception alternates at a certain periodicity between the different images presented to each eye [Kovacs96]. This phenomena is explained in neural network models that incorporate a mutual inhibition process (i.e., positive feedback loop) and a slow self-inhibition process (i.e., negative feedback loops) [Theodoni11]: the positive feedback loop triggers switching transitions between the two perception attractors, and the negative feedback loop destabilizes these perceptual states after some time.

3.1.2 Reversible cellular decisions with negative feedbacks

The properties of reversibility and of negative feedback are also observed for many cell decisions. The most archetypal case corresponds to intrinsically transient decisions such as the extensively-studied case of bacterial competence where pieces of DNA are transiently up-taken from the environment [Suel06]. These studies reveal how both positive and negative feedbacks cooperate within a simple genetic circuit to produce an excitable dynamics that underlies transient cellular differentiation. While some cell decisions like bacterial competence are reversed after some time unrelatedly to some extent to environmental changes, many others are reversed upon new environmental signals of specific nature and intensity. For instance, the cell decision to switch its metabolism between the use of different nutrient (e.g., carbon) sources depends on both the intracellular state of the metabolism and the environmental state of nutrient availability, which involves regulated hysteretic behavior [Acar05, Oyarzun15]. Such hysteresis is expected to satisfy a trade-off between *memory* (to avoid spurious switching upon small fluctuations of signaling cues), and *flexibility* (to quickly switch to another resource when one starts lacking [Nguyen15, Wang15]). Interestingly, this trade-off seems to involve particular regulatory designs [Acar05, Oyarzun15].

3.1.3 How to regulate hysteretic behavior?

The issue related to the reversibility of decision making can be formulated in terms of hysteretic behavior in a multistable system. Let assume that a protein network implements a decision switch driven by a given signal s, from a steady state $\mathbf{x}^0$ (stable for $s < s_{c0}$) to another steady state $\mathbf{x}^1$ (stable for $s > s_{c1}$) (Eqs 3.1). In this case, reversibility would be governed by the way how the hysteresis size δs_c (Eq. 3.2) and other decision properties (e.g., attractor coordinates and distances) depend on the network architecture in general, and the positive and negative feedback architecture in particular (Eq. 3.3):

Bistable switch	$\phi(\mathbf{x}^0, s < s_{c0}) = \mathbf{x}^0 \cup \phi(\mathbf{x}^1, s > s_{c1}) = \mathbf{x}^1$	(3.1)
Hysteresis size:	$\delta s_c = s_{c0} - s_{c1}$	(3.2)
Hysteresis control?	$\{\delta s_c, \mathbf{x}^\alpha(s_{c\alpha})\} = f(\mathbf{p})$	(3.3)

3.2 Results: Negative-feedback control of decision reversibility

3.2.1 Transition from bistability to oscillation

See [Pfeuty09] for more details

- ***Cross-shaped phase diagram***

The bifurcation behavior of bistable systems with a single positive feedback loop can be captured by the normal form: $x' = s + \beta x - x^3$. The phase diagram as a function of s and β typically shows a codimension-2 cusp singularity for $s = \beta = 0$ at the intersection of the two saddle-node bifurcation lines corresponding to a collision between two stable and one unstable equilibria. In this system, hysteresis is observed by varying s back and forth while its extent is controlled by $\beta > 0$. Importantly, the size of the hysteretic jump smoothly increase with β as

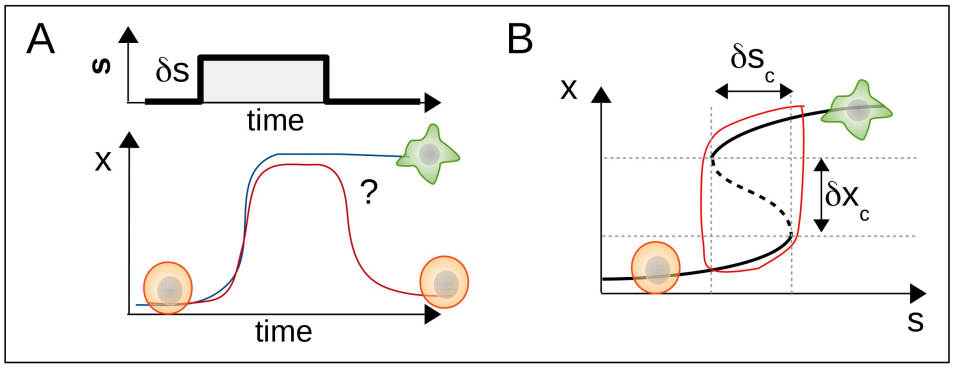


Figure 3.1: **Decision reversibility.** *(A) Reversible versus robust decisions would typically differ in whether decision is retained after signal removal or not. (B) In the case of decision making based on a bistable switch, this property relates to the notion of hysteresis defined by some signal range* $\delta\mathbf{s}$ *and attractor distance* $\delta\mathbf{x}$.

$\delta x = x^{sn_1} - x^{sn_2} \propto \beta^{1/2}$. How does the presence of negative feedback modify these hysteretic behaviors? This has been analyzed in details using a two-dimensional dynamical model of chemical reaction [Boissonade80]. The system displays ***a cross-shaped phase diagram*** characterized with intersecting domains of bistability (two stable fixed points) and oscillation (one stable limit cycle). The phase diagram notably exhibits a particular codimension-2 singularity where the bistable regime disappears through two simultaneous local subcritical Hopf bifurcations.

Actually, many positive-feedback protein networks that regulate cellular decisions turn out to involve negative feedback loops [Kim07, Pfeuty09], which questions the possible roles of those negative feedbacks in decision-making properties.

- ***Tunable hysteresis***

The significance of the transition from bistability to oscillations for decision making has been investigated using a generic protein regulatory network where two positive-feedback modules are coupled through a negative feedback loop [Pfeuty09] (Fig. 3.2A):

$$\begin{aligned} \text{PFL1}: &\quad X \to X \\ \text{PFL2}: &\quad Y \to Y \\ \text{NFL1}: &\quad X \to Y \dashv X \end{aligned}$$

For some parameter range, the model displays a cross-shaped diagram (Fig. 3.2B) where, in contrast to the results of [Boissonade80], the bistability regime disappears through two saddle-node bifurcation on a limit cycle (Fig. 3.2C). In both the Hopf or saddle-node cases, the fact that two local bifurcations co-occur at separate state-space coordinates allows for discrete and highly reversible decisions as well as the modulation of the hysteretic properties without significant changes of decision states themselves (Fig. 3.2C,D). This sharply contrasts with the cusp bifurcation scenario described above. As a result, decision making can be more flexibly tuned by using two appropriate bifurcation parameters (signals) that operate a separate control of the initiation of decision making and of its reversibility/exit (shift of the two saddle-node coordinates in Fig. 3.2D). Moreover, a negative feedback can also impact on the transition speed, probabilities and trajectories (Figs. 4, 5 and 6 of [Pfeuty09]). However, only a small and specific domain in the space of network topologies and parameters displays this particular bifurcation and phase diagram. It remains thus debatable whether such a dynamical mechanism is really used to control hysteresis and, afterwards, the reversibility of cellular decisions.

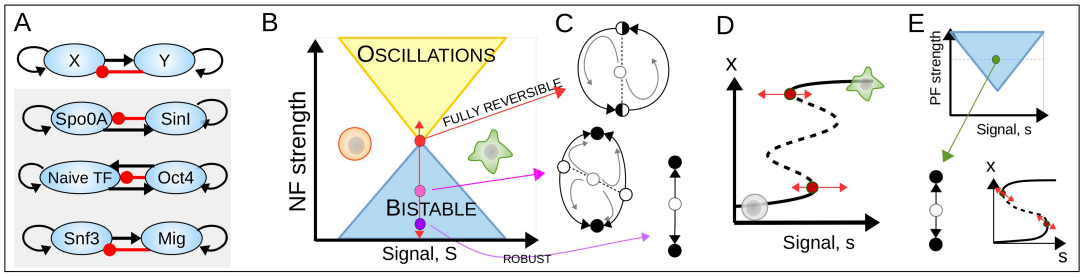


Figure 3.2: **Flexible control of hysteresis and decision reversibility.** *(A) A generic class of protein network that combines two PFLs and a NFL. (B) Cross-shaped phase diagram depending on NFL strength that display bistable and oscillatory regimes intersecting at a codimension-2 bifurcation point. (C) Typical phase portraits associated with reversible or robust bistability on an invariant circle. (D) Typical bifurcation diagram showin how hysteresis size can be controlled by two "unconnected" saddle-node bifurcation allows for a separate control of hysteresis and discreteness (red arrows). (E) Typical phase diagram, phase portrait and bifurcation diagram when hysteresis size is controlled by the PFL only. All panels are adapted from [Pfeuty09].*

3.2.2 Differentiation decisions with tissue-level negative feedbacks

See [Pfeuty16] for more details

A modeling study of binary differentiation during development further illustrates the biological significance of tuning the strength of a negative feedback loop for modulating the reversibility of decision making [Pfeuty16]. At the tissue level, differentiation decisions are the by-product of intracellular dynamics and intercellular signaling, which has motivated several models investigating how (diffusive) coupling between cell dynamical systems can lead to the symmetry-breaking and clustering behaviors [Kaneko94, Koseska10]. The present model considers a particular type of intracellular dynamics characterized with signal-induced bistability like in previous minimal models of binary differentiation decisions [Huang07, Wang11] and a particular type of intercellular coupling known to trigger divergent fate decision mediates a so-called ***lateral inhibition*** between neighboring cells (say A and B) [Lewis08, Bessonnard14, Matsuda15]:

$$Notch_A \rightarrow Lfng/Hes_A \dashv Dll_A \rightarrow Notch_B \tag{3.1}$$

$$Fgf2/Erk_A \dashv Nanog_A \rightarrow Fgf4_A \rightarrow Fgf2/Erk_B \tag{3.2}$$

These intracellular and intercellular mechanisms (Fig. 3.3A) supplemented by the inherent stochasticity of the intracellular dynamics, can be recapitulated into a simple stochastic dynamical system model [Pfeuty16]:

$$\begin{aligned} \frac{dx_i}{dt} &= s + \rho\, x_i - x_i^3 - \gamma m + \sqrt{2D}\,\zeta_i(t) \\ \tau_m \frac{dm}{dt} &= \frac{1}{N}\sum_{i=1,N} x_i - m \end{aligned}$$

which, in the homogeneous and noiseless limit, display a cross-shaped phase diagram similar to [Boissonade80]. This model belongs to ***the class of globally-coupled bistable systems*** [Desai78] where the coupling is inhibitory and delayed with a timescale τ_m. In these equations, ρ represents the strength of intracellular PFLs while γ represents the strength of *global* intercellular NFL. This model is well-suited to study how the interplay between positive and negative feedbacks and between oscillatory and transition dynamics contribute to an efficient collective

symmetry-breaking process (Fig. 3.3B). Indeed, a population of stem cells must split in two populations of differentiated cell where the relative size of each subpopulation is developmentally controlled and may differ (i.e., proportion regulation and homeostasis [Mizuguchi95]).

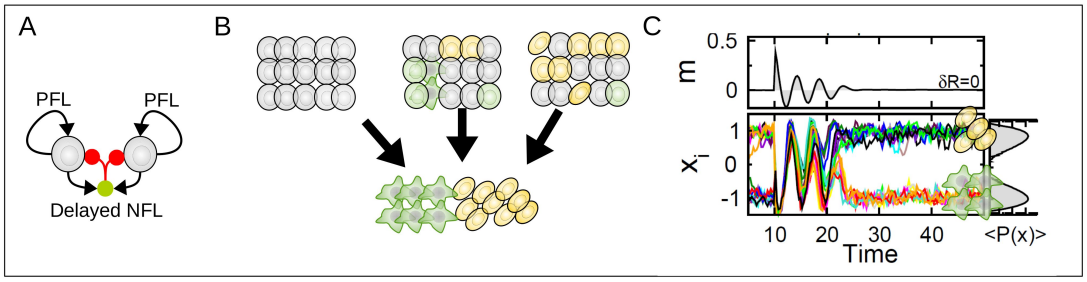


Figure 3.3: **From tunable to robust differentiation decisions in cell populations.** *(A) During development, binary differentiation decisions often involve the interplay of intracellular PFLs and intercellular NFL (e.g., Notch-mediated lateral inhibition). (B) This interplay helps a population in a given initial state to rapidly reach a steady state with two populations of differentiated cells. (C) Such relaxation dynamics is characterized with a preliminary phase where cells make a reversible switching between the two states (due to mean-field fluctuations of $m(t)$) followed by a phase where cells acquire a differentiated state in a robust and quasi-irreversible manner (as $m(t)$ has reached steady-state).*

Consistently with a previous study [Huber03], this type of system can produce a stable oscillatory state driven by the delayed negative feedback and a stable symmetry-broken state with a bimodal distribution $\tilde{\mathcal{P}}(x)$ (with $\tilde{m} = \int \tilde{\mathcal{P}}(x)$) for which the negative feedback is neutralized. The symmetry-broken state is always stable and coexists with a stable collective oscillation for large enough γ and τ_m. ***The most efficient (i.e., noise-robust and quick) relaxation dynamics toward a symmetry-broken state is shown to arise through a state-dependent control of hysteresis***:

(i) First, when (if) the initial population state is far away from the target stationary state ($m(t) - \tilde{m}$ large enough), individual cells switch very easily, back-and-forth, between the two states, due to noise or oscillatory mechanisms.

(ii) Such noisy oscillatory and reversible switching behaviors enable individual cells to quickly redistribute in the two cell-type attractors in the right proportions as $m(t) \to \tilde{m}$.

(iii) Finally, when such symmetry-broken stationary state is approached ($m(t) \sim \tilde{m}$), individual cells start behaving in the robust bistable regime and they are therefore trapped or frozen into one of their two cell-type attractors, thereby precluding the possibility from *changing their mind* and differentiating again.

At the tissue level, the cooperation between negative feedback and noise facilitates an efficient relaxation of any initial conditions (Fig. 3.3B) toward the symmetry-broken steady state with a given proportion of each cell types, in which noise quickly damps the possible *spurious* oscillations and negative feedback avoids the *spurious* metastability and subsequent logarithmically-slow relaxation (see Fig. 2 of [Pfeuty16]). In comparison to the schematic intracellular network model of [Pfeuty09] and Fig. 3.2A, the negative feedback loop is mediated by an intercellular mechanism and its strength depends on a slow population variable, which makes possible a context-dependent shift from an oscillatory or noise-sensitive bistable system associated with flexible cell fate priming to an highly-robust bistable system associated with irreversible cell fate specification.

This model shares many similitudes with other models investigating the binary differentiation process of starved dictyostellium cells [Mizuguchi95, Rafols13] and of embryonic stem cells [Bessonnard14, DeMot16], where in both case robust symmetry-breaking relies on the interplay between intracellular multistability and lateral inhibition mediated by Dif1 and Fgf2 respectively.

3.3 Conclusion

The regulatory network mechanisms enabling robust and irreversible switching behavior have generally been addressed by investigating how an ultrasensitive switch-like response can be turned into an hysteretic or irreversible switch-like response, for instance by combining positive feedback loops or adding cooperativity [Ferrell01, Brandman05, Shah11, Kim12, Hsu16]. This chapter proposes that *well-designed combinations of several PFLs and a strong enough NFL* can implement a simple mechanism to also tune hysteretic properties:

> *Cellular decisions featured with a tunable (ir)reversibility may build upon "regulated hysteresis associated with bistability on an invariant circle".*

The possibility to control separately the signal-dependent thresholds (saddle-node) for decision initiation and exit without altering the decision states themselves sharply differ from the control of hysteresis by positive feedback only, especially when approaching the cusp bifurcation. It is to note that this mechanism entails that state-space trajectories are well-distinct for the back and forth transition, which has also been shown to be the natural consequence of a *curl flux in the vector field* [Wang11, Li14], which increases with the presence of negative feedbacks.

The control of hysteretic behavior and decision reversibility can be exploited for various cellular decision-making context ranging from differentiation process and metabolic adaptation. Developmental differentiation processes often involve ***an early stage of lineage priming*** characterized with versatile transitions between precursor states [Chattwood13, Betizeau13], before to commit terminal differentiation in a robust manner. Conversely, fully differentiated cells can sometimes dedifferentiate or transdifferentiate upon injury [**?**, Merrell16]. Similar trade-off applies for metabolic decisions which need be robust in order to prevent cells from a costly, back-and-forth switching between two equally-available nutrient sources, but must also be made flexible in order to motivate cells to seek other nutrient supplies when those in current use starts running out [Nguyen15, Wang15]. Our proposal that intracellular or intercellular negative feedback can contribute to such plasticity is well-supported by the common presence of NFLs in metabolic pathways [Kaniak04, Acar05] and differentiation pathways (such as through lateral/intercellular inhibition [Rafols13, Matsuda15, DeMot16]).

Chapter 4

Decision gating

"Luck is what happens when preparation meets opportunity"

Seneca

Living cells display various types of endogenous dynamics - e.g., circadian, cell-cycle or metabolic oscillations - which are likely to influence and bias decision outcomes in response to inducing signals, which we call decision gating (Section 4.1). The intracellular oscillations are shown to allow a tunable control of cell decision outcomes, both in the context neural stem cell differentiation (Section 4.2.1 and [Pfeuty15a, Pfeuty15b]) and from a more general viewpoint (Section 4.2.2 and [Pfeuty14]). These studies highlight network and dynamic mechanisms that allow for a fine-tuned control of decision outcomes through the interplay of oscillatory and signaling dynamics (Section 4.3).

4.1 Introduction

4.1.1 Insights from neuroscience: attentional gating

Magicians know very well that attention is a critical cognitive process that shapes our perceptual decision making, and can therefore bias them as perceptual illusions [Macknik08]. In most situations however, ***attention and the related processes of expectation, anticipation and preparedness aim to improve further decision-making processes*** [Summerfield14, Battistoni17]. Interestingly, focused attention is a highly dynamic process where, for instance, enhanced brain rhythms at various frequencies participate to the performance and accuracy of signal-processing and decision tasks [Schroeder09, Fiebelkorn14, Petro15, Nobre18]. Even without any attentional focus, the resting brain settles in fact in a highly dynamic spontaneous activities [Berkes11, Tozzi16], which may influence further signal-induced decision making [Hesselmann08]. Last but not least, it has been proposed that a main function of spontaneous or evoked brain oscillations would be to control the flow of signals and informations, notably through gating and multiplexing mechanisms [Akam14].

4.1.2 Cellular decisions gated by intracellular oscillations

A living cell often exhibits endogenous dynamical behaviors related to the ongoing functions of signaling, metabolism and proliferation. Such intracellular dynamics may influence the manner how transient extracellular perturbations/signals are converted into decision outputs. In unicellular organisms, the decision to divide or not is typically gated, in a more or less stringent manner, by their circadian clock [Moulager10] or by their metabolic clock [Papagiannakis17]. In multicellular organisms as well, the cellular decision to differentiate into a particular subtype has been shown to depend on the phase of the cell-division cycle [Pauklin13] or of the circadian clock [Brown14]. More surprising is the involvment of oscillatory pathways driven by intercellular signaling in regulating cell fate decision outputs in animal stem cells [Imayoshi14, Isomura14], but also in bacteria [Schultz13, BenJacob14] where it was proposed that *"each oscillation opens a short interval with high transition probability turning oscillation into opportunities*. In these diverse situations, it is natural to hypothesize that intracellular oscillations can *in principle* modulate the cellular sensitivity to decision-inducing signals or the cellular competence to make a decisions, questioning the network and dynamical mechanisms involved in this gating process.

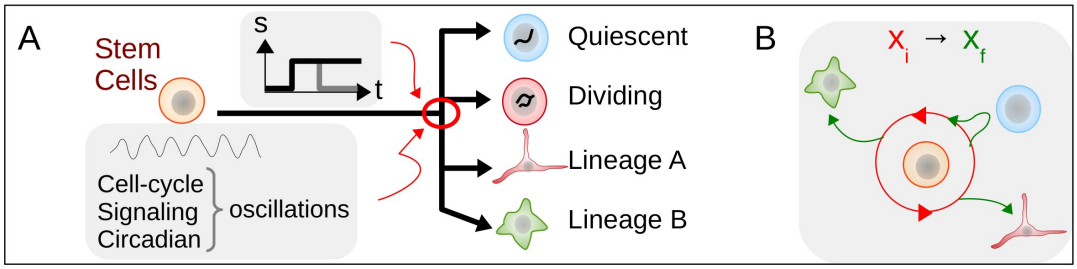


Figure 4.1: **Cell decisions gated by intracellular oscillations.** *(A) Examples of intracellular oscillations that can influence stem cell fate decisions. (B) Decision gating as a state-dependent - reversible or irreversible- transition from basal oscillations to many fate alternatives.*

4.1.3 How to gate transitions with oscillations?

In the following, decision gating is investigated in the case where a signal input (say scalar to make it simple) (Eq. 4.1) can make a cell eventually switch from a basal oscillatory state γ^0 stable for $s < s_{c0}$ and of period T^0 (e.g., proliferating stem cells) to a stable state $\mathbf{x}^j$ eventually among many alternatives (Eq. 4.3) (e.g., non-proliferating differentiated/quiescent/senescent states). The question that arises is whether and how decision outcomes would depend on the interplay of signal properties and oscillations properties.

Step/Rectangular signal	$s(t) = s_0 + \delta s \mathcal{H}(t)/\Pi(t, T_S)$	(4.1)
Pre-decision oscillations:	$\phi^\infty(\mathbf{x} \in \gamma^0, s < s_{c0}) \in \gamma^0$	(4.2)
Post-decision states:	$\phi^\infty(\mathbf{x}^{j=1,N}, s > s_{cj}) = \mathbf{x}^{j=1,N}$	(4.3)
Gate control?	$\phi^\infty(\mathbf{x} \in \gamma^0, \mathbf{s}(t)) = f(\mathbf{x}, T_0, s_0, \delta s, T_S, \mathbf{p})$	(4.4)

4.2 Results: Oscillatory control of differentiation decisions

4.2.1 Fate decisions of neural stem cells

See [Pfeuty15a, Pfeuty15b] for more details

- ***Regulatory network for neural stem cell fate decisions***

The embryonic and postnatal development of the central nervous system entails the differentiation of neural stem cells (NSCs) into a multiplicity of cell types (neural, glial) and subtypes (e.g., pyramidal or GABAergic neurons) [Greig13]. Before reaching a decision on their fate, neural stem cells actively divide and are subjected to highly dynamic intercellular signaling. This begs the question of the role that the fast oscillations in the Notch-Hes1 pathway and the slow oscillation of cell-division-cycle pathways play together in the cell-fate decision of neural stem cells.

Like for cell-cycle pathways and other signaling pathways [Isomura14], the Notch-Hes1 pathways also combines PFL and NFL in a manner that can give rise to oscillations (of a period about 3hr) as well as two steady states associated with either high or low levels of Notch and Hes1 ([Goodfellow14] and Fig. S2 of [Pfeuty15a]):

$$\begin{aligned}
&\text{NFL1}: \quad Hes1 \dashv Hes1 \\
&\text{PFL1}: \quad Hes1 \dashv miR9 \dashv Hes1 \\
&\text{PFL2}: \quad Hes1_A \dashv Dll_A \overset{*}{\to} Hes1_B \dashv Dll_B \overset{*}{\to} Hes1_A
\end{aligned}$$

Interestingly, there is a significant crosstalk between Notch-Hes1 signaling pathways, G1-phase regulatory pathways, especially toward the regulation of the neural differentiation factor Ngn2 (Fig. 4.2A):

$$\begin{aligned}
&\text{PFL3}: \quad Ngn2_A \to Dll_A \overset{*}{\to} Hes1_B \dashv Ngn2_A \to Dll_B \overset{*}{\to} Hes1_A \dashv Ngn2_A \\
&\text{PFL4}: \quad Ngn2 \dashv cycD, E \multimap Ngn2 \\
&\text{IFFL}: \quad Hes1 \overset{\dashv}{\multimap} Ngn2 \cup Hes1 \dashv cycD, E \multimap Ngn2 \\
&\text{CFFL}: \quad S_{DIF} \dashv Hes1 \overset{\dashv}{\multimap} Ngn2 \cup S_{DIF} \to Ngn2
\end{aligned}$$

- ***From oscillatory to transition dynamics***

Numerical simulations and bifurcation analysis of the overall NSC regulatory network show that the crosstalk between signaling, cell-cycle and differentiation pathways generates a subtle and sophisticated fate decision dynamics. First, under the simplifying assumption of static $[Hes1]$ levels as a control parameter, various combinations of $[Hes1]$ levels and $[S_{Dif}]$ levels (e.g., differentiation factors such as Fgf10, Wnt, Shh) can generate diverse cellular *phenotypes* ranging from fast and slow G1-phase progression to reversible (G0) or irreversible (late G1) cell-cycle arrest depending on the occurrence of SNIC or SHO bifurcations (Fig. 4.2B and Fig. 3 of [Pfeuty15a]). This diverse phenotypes have been characterized experimentally including the result that intermediate Hes1 levels promote cell-division cycles while high or low Hes1 levels promote well-distinct cell-cycle arrest. With respect to decision gating, ***the distinct properties and localization of these two limit-cycle bifurcations entail that a same*** $[S_{Dif}]$ ***signal can lead to different G0 or G1 cell-cycle arrest outcomes depending on the cell-cycle state and the Hes1 levels of the cell when signal is received.***

This picture is further complicated when we consider Hes1 dynamics driven by the Delta-Notch *lateral inhibitory* coupling between cells. We found that Hes1 oscillations is likely to

contribute to an highly dynamical proliferation state that make coupled cells eventually desynchronize each other thereby generating dynamic cell-to-cell heterogeneity (Fig. 4.2C,D). Such desynchronization and heterogeneity is further found to promote divergent fate decisions in response to transient $[S_{Dif}]$, where the cell starting first to differentiate into neurons (High $[Ngn2]$ and $[Dll]$) inhibit the differentiation of the coupled cell (through lateral inhibition) which either shift to a quiescent G0 state or proliferate again (Fig. 4.2C,D). In sharp contrast, two synchronized cells, hence having the same sensitivity to differentiation factors $[S_{Dif}]$ and the same competence to differentiate, may inhbibit and neutralize each other from differentiating when receiving similar $[S_{Dif}]$ inputs (Fig. 4.2D and Fig. S3 of [Pfeuty15a]).

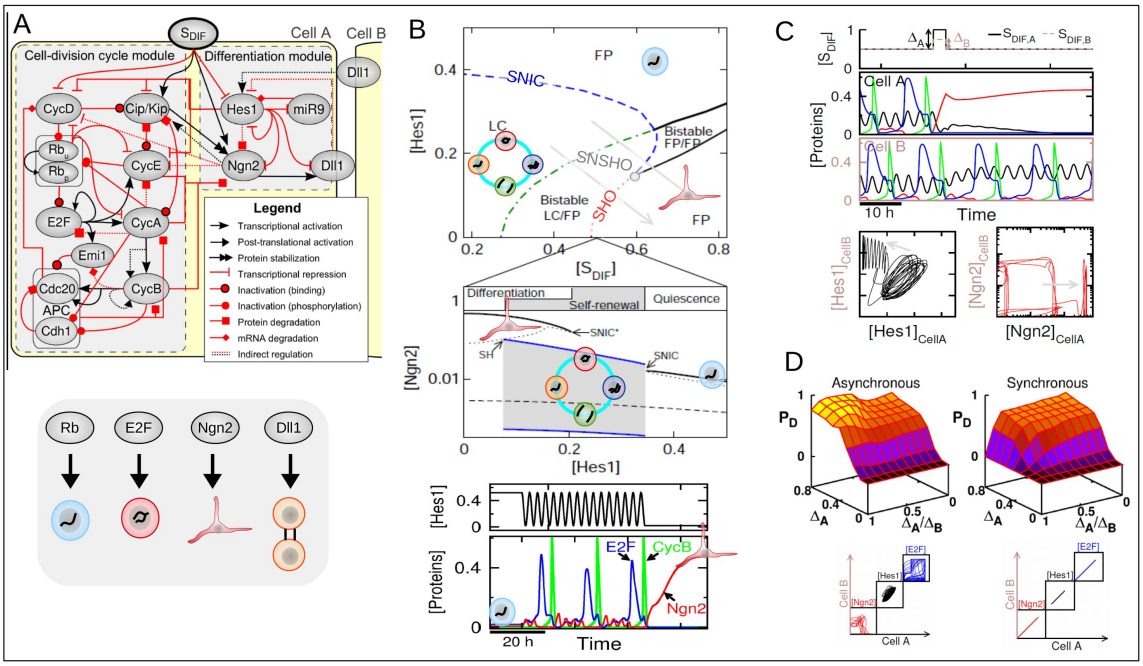


Figure 4.2: **Fate decision dynamics of neural stem cells.** *(A) Neural differentiation depends on several signaling pathways, cell-cycle and differentiation pathways. (B) Two limit-cycle bifurcation scenarios towards a reversible quiescent G0 state and a irreversible differentiation state. Up panel: phase diagram where $[Hes1]$ is taken as a bifurcation parameter. Middle panel: Bifurcation diagram as function of Hes1. Down panel: Examples of Hes1 driven transitions between cell-division cycle and two well-distinct cell-cycle arrested state. (C) Time course of signal and concentration associated with a asymmetric fate decision in a two coupled-cell model where Delta-Notch coupling produces Hes1 oscillations. (D) Probability P_D that one of the coupled cells differentiate is much higher when coupled cells are desynchronized (especially when the cells receive the same differentiation signal). All panels are extracted from [Pfeuty15a]*

To summarize, cell-division-cycle and Hes1 signaling oscillations play cooperative roles in tuning decision probabilities through opportunity windows and dynamic heterogeneity:

(i) Opportunity windows: a pulse of $[S_{Dif}]$ (if not too long and high) must occur during the G1-phase in order to promote differentiation. In consequence, the lengthening of G1 phase increases the chance that a signal occurring in G1 would induce neural differentiation in line with experimental observations [Lange10].

(ii) Dynamic heterogeneity: a pulse of $[S_{Dif}]$ must coincide with a low Notch activity in order to induce differentiation, such that occurrence of asynchronous and heterogeneous Hes1

and cell-cycle dynamics between cells enables then to reach divergent fate decisions in response to the same $[S_{Dif}]$ signal.

4.2.2 Oscillatory phase-dependent decisions

In the context of stem cell differentiation decisions, we have identified ***several mechanisms by which oscillations can drive flexible decision-making*** (schematically represented in Fig. 4.3 in the case of asymmetric and binary fate decisions of neural stem cells [Pfeuty15b]):

- The signal-induced switch between a limit cycle and another attractor can depend on the phase φ at which the signal is received, which leads to ***phase-dependent relationship between signal inputs and fate decision outputs*** ($\phi^{\infty}(\gamma, \mathbf{s}(t)) = f(\varphi, \mathbf{p}_s)$). In comparison, a perturbed steady state can only have a single decision output for a given signal input ($\phi^{\infty}(\gamma, \mathbf{s}(t)) = f(\mathbf{p}_s)$). This is somehow similar to the concept of phase response curve (PRC) describing the relation between signal input and phase change ouput.

- Such phase-dependent input-output relationship can be ***modulated by signal-dependent changes of limit cycle trajectories, period and bifurcation type***. In the case of PRCs, their shape is indeed known to tightly depend on the nonlinear dynamics of oscillators (e.g., [Pfeuty03, Pfeuty11]).

- Finally, the phase itself can be ***modulated by previous perturbations or ongoing (de)synchronization processes*** between intracellular oscillations (e.g., cell cycle and Hes1) and between neighboring cells (e.g., through Delta-Notch coupling). In particular, desynchronized oscillations provides a tunable source of cell-to-cell heterogeneity that can be used for symmetry-breaking without requiring any source of stochastic fluctuations [Matsuda15, DeMot16, Pfeuty16].

In contrast with the PRC properties of neuronal oscillations or circadian clocks, these phase-dependent decision properties cannot be studied in the limit of small perturbations. However, some investigations can nevertheless be done on low-dimensional models to study how the probability to make a given decision depend on signaling and oscillatory properties [Pfeuty14].

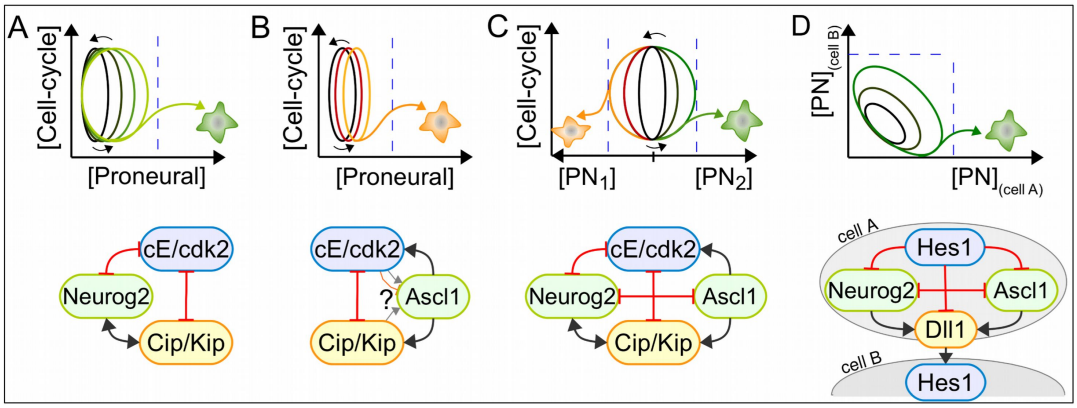


Figure 4.3: **Schematic illustration of NSC fate decisions based on phase/shape/synchronization of cell-cycle and signalling oscillations.** *Extracted from [Pfeuty15b].*

4.3 Conclusion

Unlike a steady-state attractor, a limit-cycle attractor can retain some memory or encode some information through its phase, period and state-space coordinates. Context-dependent regulation of the amplitude, frequency, shape and synchronization of cellular oscillations therefore provide a substrate for temporal coding and functional pleiotropy, especially in neurons [Akam14] but in any other cells as well [Behar10, Levine13]. In line with this idea, our study of neural stem cell fate decisions suggests that:

> *Cellular decisions featured with tunable opportunity windows may build upon "oscillatory dynamics whose phase, trajectory, period, synchronization and bifurcation properties" modulate signal sensitivity and decision outcomes.*

If a limit cycle is the simplest attractor that can perform temporal discrimination between signaling inputs, other high-dimensional attractors and dynamics (e.g., line attractors, limit torus or itinerancy) could as well confer this type of flexible signal processing.

Intracellular oscillations are well-suited to underlie various cellular functions such as circadian clocks, developmental cycles or spatial periodic stripes [Kruse05, Tiana07]. Their proposed role in driving cellular differentiation and symmetry-breaking processes is much less transparent but quite appealing [Kaneko94, Kaneko97, Schultz13, Isomura14]. Our work suggests that the oscillatory drive of cellular decisions provide an additional lever of control that is particularly suitable in context where many decision outcomes are possibles and should be distributed over time and space. This is typically the case during the development of the central nervous where the well-scheduled production and layering of many subtypes of neuronal and glial cells [Greig13] clearly rely on the dynamic versatility of neural stem and progenitor cells [Betizeau13, Imayoshi14].

Testing the existence of oscillatory gating mechanisms should only to measure the probability for a given cellular transition (e.g., death, differentiation) to occur as a function of signal properties and the phase of the intracellular (cell-cycle, circadian or signaling) oscillations, using for instance time-lapse imaging techniques [Roccio13, Kafri13]. Quantifying how such gating, if exists, has been exploited for a collective and temporal control of developmental processes is much more difficult to assess. To address how oscillatory gating may influence spatial patterning (stripes, clusters, salt-and-pepper), a possibility could be the design and study of multicellular synthetic systems endowed with oscillatory, multistable and synchronization properties [Ullner07, Koseska10, Matsuda15, PerezCarrasco18].

Chapter 5

Decision paths

"The path is the goal"

Siddhārtha Gautama

Many complex biological processes can be described as a coordinated sequence of transition events where the path is somehow more important than the initial and the final state (Section 5.1). The study of two sequential cellular decision processes – oocyte meiotic maturation and pluripotent stem cell transitions – reveals that a bistable transition path can be both very complicated and very robust to diverse perturbations, which rely on a complex feedback/feedforward architecture of their regulatory network (Section 5.2). These studies highlight network and dynamic mechanisms allowing a fine-tuned and robust control of decision paths, regardless to some extent of the starting point and the final destination (Section 5.3).

5.1 Introduction

5.1.1 Insights from neuroscience: transient neural and cognitive dynamics

When one learns to play tennis, the teacher usually stresses the importance of your posture and gesture to reach your goal, which is to send the ball at the desired place and pace. This idea of an important role for transient dynamics have been proposed as an alternative paradigm to understand information-processing and decision making [Rabinovich08a, Rabinotich08b]:

> *"Fixed-point attractor dynamics express no useful dynamics; only the state the network settles into, given by its initial conditions, matters, not the path taken to reach that state. An alternative theoretical framework may explain some forms of neural network dynamics that are consistent with experiments, in which transient dynamics are resistant to noise and small variations in initial conditions such that the succession of states visited by the system is stable."* –

Transient neural dynamics has been proposed to serve various purposes during decision making, such as the generation of complex sensorimotor tasks [Rokni12], the dynamic coding of sensorial representations such as odors [Mazor05] or working memory processes [Spaak17, Nachstedt17].

5.1.2 Sequential decision-making during developmental processes

In the context of cellular decisions, the critical importance of the notion of trajectories/paths is illustrated by the Waddington's epigenetic landscape [Waddington42] that depicts the developmental control of cell-fate decisions as branching and diverging paths from the zigotic cell to fully-differentiated cells (e.g., neuron, erythrocyte, fibroblast, myocyte) (Fig. 5.1A). In parallel to cell proliferation required to increase the number of cells, this branching process generally corresponds to the binary cell-fate decision process through which a given stem or progenitor cell type differentiates into two possible cell subtypes, whereby, on the whole, it gives rise to a diversity of lineage paths. Alternatively, in vitro culturing and manipulation of cells also enable to induce reprogramming paths which can be used to trigger transdifferentiation or induced pluripotency [Takahashi15]. These developmental and reprogramming paths raise the issue as to (i) what are the dynamical and biochemical mechanisms that allow for a stringent and directional control of these assorted cell-lineage paths in such a way as to avoid developmental defects related to spurious cellular states (e.g., cancer cells) or spurious tissue states (e.g., hypertrophy, hypotrophy), and (ii) how to exploit these mechanisms to reprogram cell fates and states for medical purposes.

The occurrence of diversified, yet robust, cellular decision paths is also a salient feature of much more basic developmental cycles. Many species ranging from yeast, amoeba, plants or animals can display diverse developmental cycles in relation with various patterns of ploidy (number of sets of chromosomes) and of growth-division coordination (Fig. 5.1B). Besides the usual cell-division cycle which include the successive G1, S, G2, M and cytokinesis phases, some cells alternatively can (i) duplicate their genomic DNA without undergoing mitosis (endocycling) or cytokinesis (endomitosis); (ii) reduce their chromosome number by decreasing ploidy (meiosis); (iii) undergo nuclear division in the absence of growth or cell division (syncytial cycles), or (iv) fuse to create polyploid cells (mating or fertilization). The fact that these cell-cycle variants share some events though they differ in their sequential patterning provides a valuable framework to study cellullar decision paths.

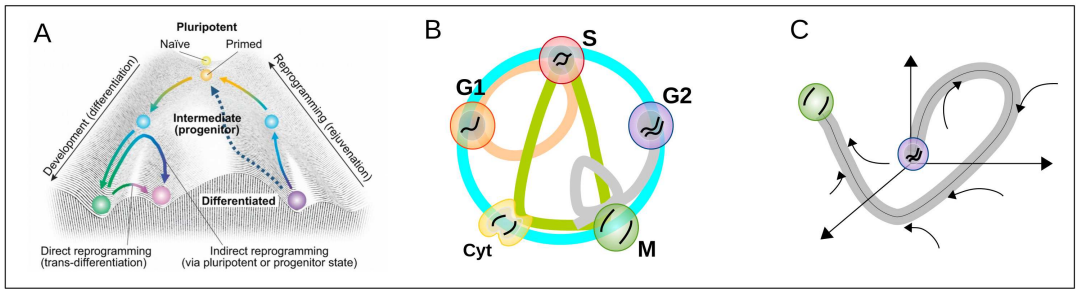


Figure 5.1: **Developmental paths.** *(A) Diversity of developmental or reprogramming paths associated with stepwise cell-fate transitions between cellular subtypes including totipotency, pluripotency, multipotency, bipotency and fully diffentiated cells (extracted from [Takahashi15]). (B) Diversity of developmental paths associated with cell-cycle variants (blue: mitotic cycle; Orange: endocycle; grey: meiotic maturation; green: syncytial cycle). (C) A schematic state-space representation of robust and complex transient dynamics of a cell-fate decision switch (Eqs 5.1-3).*

5.1.3 How to shape transition paths?

Decision-making paths can be investigated in the case where, upon application of a rectangular (or step) signal $\mathbf{s}(t)$ (Eq. 5.1), a bistable regulatory network (Eq. 5.2) initially setting in a stable

state $\mathbf{x}^0$ is destabilized and follows a particular temporal and state-space trajectories (Eq. 5.2 and Fig. 5.1C) toward a final stable state $\mathbf{x}^1$. Path control would require such trajectory to be implemented at the level of network organization and, at the same time, to be robust against many sources of structural, dynamic and environmental variability (Eq. 5.3):

Rectangular signal	$\mathbf{s}(t) = \mathbf{s}_0 + \delta\mathbf{s}\,\Pi(t, T_s)$	(5.1)
Bistability	$\phi(\mathbf{x}^{0/1}, \mathbf{s}_0) = \mathbf{x}^{0/1}$	(53.2)
Decision Path	$\mathbf{x}(t) = \phi^{0<t<T}(\mathbf{x}^0, \mathbf{s}_0 + \delta s) \cup \phi^{t>T}(\phi^T(\mathbf{x}^0, \mathbf{s}_0 + \delta s), \mathbf{s}_0)$	(5.2)
Path control?	$\mathbf{x}(t) = f(\mathbf{x}^0, T, \delta\mathbf{s}, \mathbf{p})$	(5.3)

5.2 Results: Sequential control of developmental decisions

5.2.1 Meiotic transitions during oocyte maturation

See [Pfeuty12a] for more details

The cell-division cycle events associated with oocyte maturation follows a well-elaborated decision-making pattern that occurs only in oocyte though it is critical for the life cycle of vertebrate organisms (Fig. 5.1C and 5.2A)): upon exposure to progesterone ($s = [Pg]$), G2-arrested immature oocytes accomplish a firt meiotic division followed by a second one that remains unfinished and ends with an arrest in metaphase II, which is reflected by a non-monotonic timecourse of MPF activity (Fig. 5.2A). How can a sequential decision-making pattern be elicited be a single signal input and, at the same time, highly robust to a broad range of perturbation?

To address this issue, we have elaborated a model of the maturation regulatory network (including 12 variables and 50 parameters) which couples two pathways that usually operate independently (Fig. 5.2B that is a simplified representation of Fig 2 of [Pfeuty12a]). First, the mitotic regulatory network driven by cyclinB-Cdks (called here maturation promoting factor MPF) involves a set of feedback loops that generate an excitable (and oscillatory) behavior underlying M-phase initiation and completion:

$$\begin{aligned} &\text{PFL1}: \quad MPF \dashv\!\bullet\, Myt \dashv\!\bullet\, MPF \\ &\text{PFL2}: \quad MPF \rightarrow cdc25 \rightarrow MPF \\ &\text{NFL1}: \quad MPF \overset{\rightarrow}{\rightarrow} APC \dashv\!\blacksquare\, MPF \end{aligned} \tag{5.1}$$

Second, the MAPK pathway, involved in processing a variety of extracellular signals and in initiating a diversity of cellular responses, typically includes a positive feedback loop which contributes to induce bistable transitions, here:

$$\text{PFL3}: \quad Mos \rightarrow Erk \rightarrow Mos$$

During the oocyte maturation, these oscillatory and bistable modules are tightly coupled at several levels, implementing additional positive feedback loops and negative feedback loops

whose respective roles remained unclear:

$$\begin{aligned}
\text{PFL4}: &\quad MPF \to Mos \multimap Myt \multimap MPF \\
\text{PFL5}: &\quad MPF \to Mos \to Erk \to cdc25 \to MPF \\
\text{PFL6}: &\quad MPF \to Mos \to Erk \to Rsk \multimap Myt \multimap MPF \\
\text{PFL7}: &\quad MPF \to Mos \to Erk \to Rsk \to Emi2 \multimap APC \multimap MPF \\
\text{NFL2}: &\quad MPF \multimap Emi2 \multimap APC \multimap MPF
\end{aligned}$$

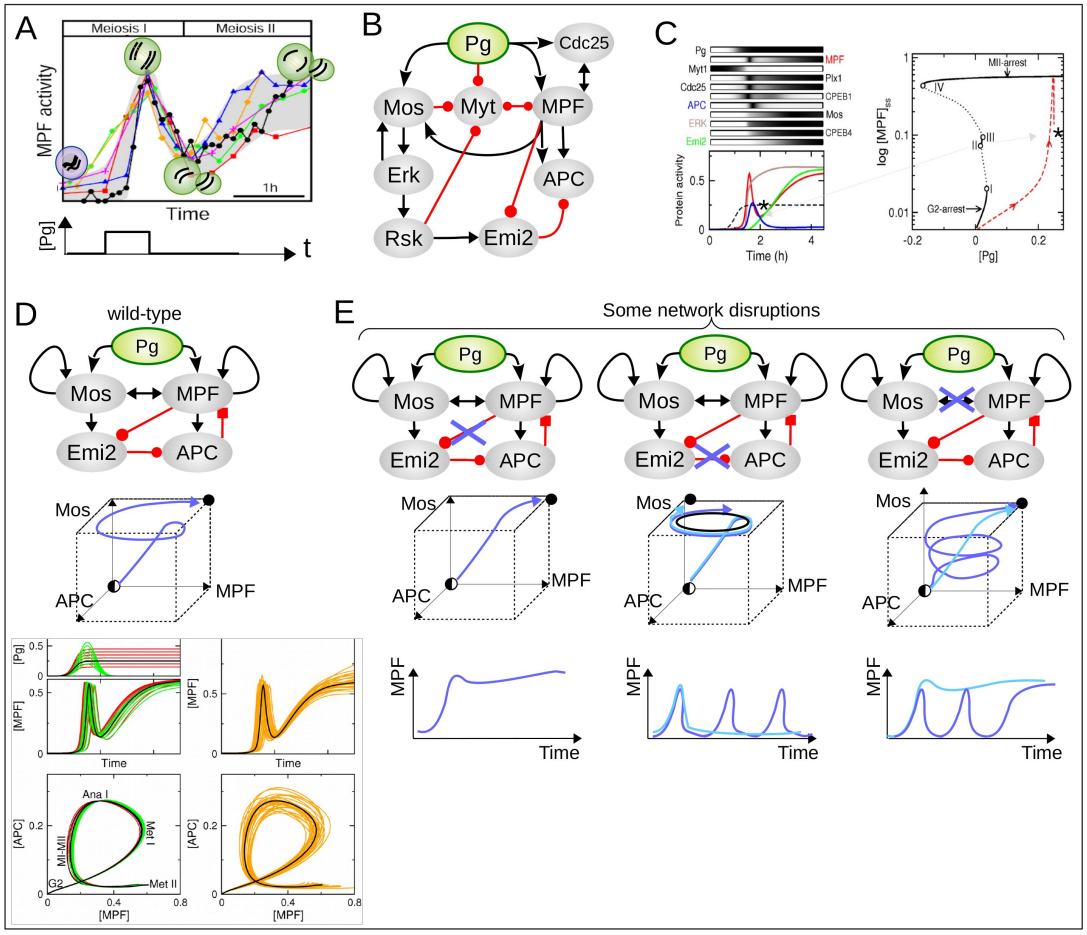


Figure 5.2: **State-space dynamics and feedback logic of meiotic transitions.** *(A) Time courses of MPF associated with meitoic maturation consisting in G2/M transition, first meiosis, meiotic transition and Mitotic arrest. (B) Simplified representation of the maturation regulatory network model. (C) Simulated temporal concentration trajectories associated the stepwise maturation process and corresponding bifurcation diagram showing multistability (bistability and a small region of tristability). (D-E) Up, middle and bottom panels respectively display the network logic, state-space trajectories and temporal trajectories. (D) The wild-type network display concentration trajectories that are consistent with experimental observations (A) and that are robust to variations in signal levels (left) and parameter values (right). (E) The networks where specific link between MAPK and MPF modules are disrupted typically show spurious dynamical trajectories and, thus, oocyte maturation failure, which can be due to both spurious attractors (middle) or spurious transient dynamics (left and right). All panels are extracted, adapted or inspired from [Pfeuty12a].*

While the MAPK and MFP modules respectively display a bistable behavior and an excitable/oscillatory behavior, their coupling through PF4-7 and NFL2 generates a specific transition dynamics similar to those observed during oocyte meiotic maturation (compare MPF trajectory in Fig. 5.2C and Fig. 5.2A). This complex transition dynamics is characterized with :

(*i*) A high-dimensional and non-monotonous trajectory between the destabilized G2-arrest steady state (through a SN bifurcation and the final metaphase-II-arrested steady state (Fig. 5.2C).

(*ii*) The state-space and temporal trajectories are shown to be very robust against moderate variations of initial conditions (not shown), signaling levels and parameter values (bottom left panels of Fig. 5.2D), though the existence of another attractor that is not reached but that bottlenecks the trajectory through the meiotic transition (Fig. 5.2C).

(*iii*) This particular trajectory requires the multimodal coupling between the MAPK and MFP modules, as the disruption of one mode of coupling can lead to spurious transition trajectories and final states (Fig. 5.2E). Note that one type of failure occurs because the decision-making trajectory reaches and stops at the intermediate MI-MII steady-state attractors whose role was rather to shape the transition trajectory. Some of these defects have been observed experimentally upon ablation or overexpression of specific proteins (see Table 1 of [Pfeuty12a]).

Overall, these findings suggest that complex sequential decision making can be built upon the well-designed coupling between modules and their dynamics, in order ***to perform both temporal coordination and segregation of several decision steps***.

5.2.2 Pluripotent transitions during embryonic development

See [Pfeuty18] for more details

Pluripotency is an early stage of vertebrate development during which (pluripotent) stem cells progress from a naive to a primed state before differentiating into lineage-restricted multipotent stem cells (Fig. 5.3A) such as neuroectoderm, mesoderm and endoderm lineages. This cell fate progression from a naive to a primed state occurs in parallel with discrete changes in the epigenetic and transcriptional programs as well as with tissue-specific changes in extracellular signaling and spatial patterning. Such a well-scheduled set of coordinated decisions is thus thought to be of critical importance to unfold, in space and time, multilineage diffentiation [Smith17].

Strikingly yet, this simple pattern of sequential fate decisions is regulated by a quite complex protein network (Fig. 5.3B) which combines a dense set of PFLs and a small set of NFLs and IFFLs essentially mediated by Oct4:

$$
\begin{aligned}
\text{PFL1/8}: &\quad Oct \leftrightarrow Nanog \leftrightarrow Klf \leftrightarrow Esrrb \leftrightarrow Nanog \\
\text{PFL9/12}: &\quad Esrrb \leftrightarrow Oct \leftrightarrow Klf \\
\text{NFL1}: &\quad Oct \to Tcf3 \dashv Esrrb \to Oct4 \\
\text{NLF2}: &\quad Oct \to Erk \multimap Klf4 \to Oct4 \\
\text{IFFL1}: &\quad Oct \to Tcf3 \dashv Esrrb \cup Oct4 \to Esrrb \\
\text{IFFL2}: &\quad Oct \to Erk \multimap Klf4 \cup Oct4 \to Klf4 \\
\text{CFFL}: &\quad LIF \multimap Gsk3/Tcf3 \dashv Esrrb \cup LIF \to Stat3 \to Klf4 \to Essrb \\
\text{CFFL}: &\quad Stat3 \to Myc \multimap Erk \multimap Klf4 \cup Stat3 \to Klf4
\end{aligned}
$$

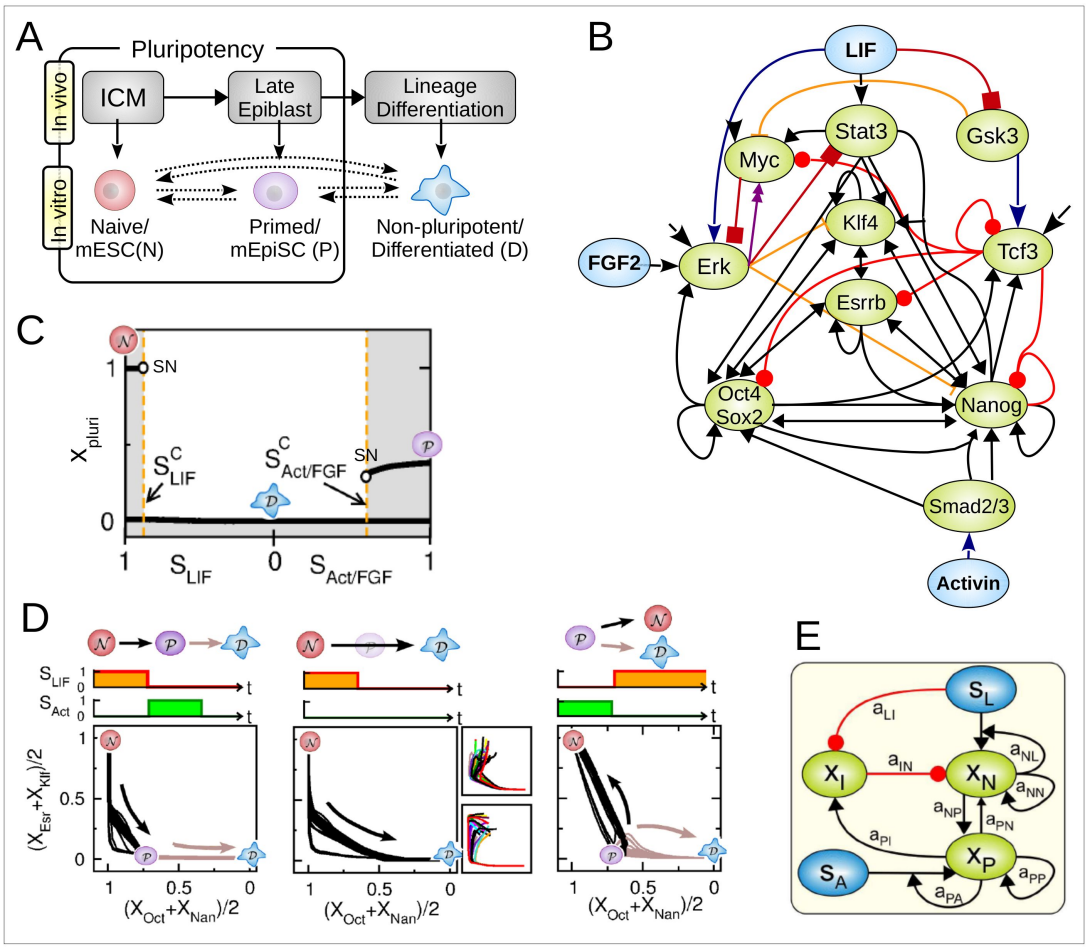


Figure 5.3: **State-space dynamics and feedback logic of pluripotent stem cell transitions.** *(A) The forward transition between naive and primed pluripotent states follows a specific logic in vivo and can be controlled in vitro. (B) Pluripotency regulatory network model from which a set of parameter-optimized models is obtained by fitting experimental data (Fig 2 and 3 of [Pfeuty18]). (C) The overall set of optimized models share similar signal-dependent multistability between the naive, primed and non-pluripotent states. (D) The overall set of optimized models share similar state-transition trajectories featured with canalization (through the primed state) and directionality (from naive to differentiated).. (E) A coarse-grained modular model that retains the multistable and canalization properties of the detailed mode (see Fig 7 of [Pfeuty18]). All panels are extracted from [Pfeuty18].*

Identifiability, sensitivity and perturbation analysis of the complex network structure shows that the existence of a core architecture that implement two important properties of decision-making dynamics:

(i) Signal-dependent multistability between naive pluripotency, primed pluripotency and non-pluripotent states (Fig. 5.3C), despite the intrication of PFLs suggesting that some network features contribute to a segregation between naive and primed states.

(ii) Canalization where the destabilization of the naive pluripotent state fosters a state-space trajectory that is canalized through the primed pluripotent state in a manner that is robust (Fig. 5.3D) to variability in initial conditions (two small panels), in signaling pattern

(compare left to middle panel) in parameters (the different lines represent models with different optimized parameter set), before to exit pluripotency.

Network perturbation approach and modular analysis allows to derive a simplified coarse-grained model that summarizes the core regulatory features of the detailed network including PFLs, NFL and FFLs (Fig. 5.3E):

$$\begin{aligned}
\text{PFLs}: &\quad X_N \leftrightarrow X_N \leftrightarrow X_P \leftrightarrow X_P \\
\text{NFL}: &\quad X_P \rightarrow X_I \dashv X_N \rightarrow X_P \\
\text{IFFL}: &\quad X_P \rightarrow X_I \dashv X_N \cup X_P \rightarrow X_N \\
\text{CFFL}: &\quad S_L \rightarrow X_N \cup S_L \dashv X_i \dashv X_N
\end{aligned}$$

This low-dimensional ($n_1 = 3$) dynamical system model can indeed reproduce accurately the multistable and transitional properties of the original detailed ($n_1 = 10$) model (compare Fig. 6 of [Pfeuty18] with Fig. 5.3, which highlights the core design principles that underlie the multistable and canalization properties. Similarly to the meiotic maturation network, the feedback and feedforward coupling between PFL modules seems important for the temporal coordination and segregation of intermediate metastable states and decision steps.

5.3 Conclusion

For the two biological case studies of oocyte meiotic maturation and pluripotent stem cell transitions, the sequential decision dynamics is shown (i) to be captured by *a core network organization where several PFLs are combined in a complicated manner* and (ii) to correspond to state-space trajectories that are very robust against diverse sources of variability, which suggests that:

> *Cellular decisions featured with a complex and robust sequence of intermediate steps may build upon "the shaping of a slow invariant attracting manifold".*

The Invariance property would ensure that such manifold is a *particular solution trajectory* that organizes and partitions the phase portrait and that can be shaped like other solutions (such as a limit cycle). ***The slowness and attraction properties*** would ensure that transverse perturbations and neighboring trajectories are *quickly* relaxing and converging to this particular trajectory, ***which bears some analogy with the Waddington's concept of canalization*** [Waddington42]. In the cases studied in this chapter, the slow manifold of interest is likely to be one-dimensional as it is the *remnant of the heteroclinic connection between the initial saddle instability and the final stable steady state* (i.e., $\mathcal{W}^U(\mathbf{x}^{sad}) \cup \mathcal{W}^S(\mathbf{x}^f)$). The conjecture above needs to be supported by more careful analysis of the phase portraits and the stability properties of invariant manifolds.

We also have to understand how the shape, the stability and the flow properties of such slow attracting manifold depend on the network structure, though we hypothesize that PFLs critically contribute to generate saddle-node ghosts and transverse stability, while negative feedback and feedforward loops shape and drive trajectories between and through those ghosts with a certain speed (similarly to NFL-PFL limit cycles where NFL sets the angular speed while PFL contributes to robust amplitude and local slowing down of oscillations [Novak08, Tsai08]). This hypothesis also entails that the slowness of the manifold emerges from the feedback-feedforward architecture rather than the existence of *slow* variables and regulatory mechanisms, related for instance to slow epigenetic changes. It is to note that a slow manifold mechanism has been characterized in modeling studies of spatial patterning during embryonic development [Manu09, Tufcea15, Verd17] and that a related concept of ***dominant kinetic path*** have been developed for stochastic protein networks [Wang11, Li13].

Noteworthily also, the decision-making process with a flexible preparation phase (discussed in Chapter 2) can be also viewed as a sequential decision process –first preparing to make a decision and then making the decision–. In fact, the bifurcation diagrams also share similitude with the possible existence of tristability (compare Fig. 2.2B, Fig. 5.3C and Fig. 2 of [Pfeuty12a]) where the intermediate stable states may not be reachable during the decision switching behavior, but can still shape transition trajectories through *bottleneck state-space region*.

Last but not least, the development of genome-scale single-cell measurement techniques have allowed the possibility to characterize developmental trajectories bringing to the fore the notion of continuum [Morgani17, Smith17], or continuum of microstates [Hormoz16, Jang17], which remains to be characterized from dynamical system perspective.

Chapter 6

Perspectives

"There is no (living) organism without teleology; there is no teleology without inwardness; and life can be known only by life"

Hans Jonas

6.1 A brief summary

In this thesis, I have discussed how diverse cell decision properties - speed, reversibility, gating and paths - can be controlled and tuned at the levels of nonlinear dynamics and loop motifs of intracellular biochemical networks. The fact that complex network and dynamical mechanisms can produce subtle decision properties was quite expected. Less expected was the critical importance of coupled PFL-NFL-FFL mechanisms, oscillatory behaviors and bifurcation types in producing diverse and tunable decision-making properties (Fig. 6.1).

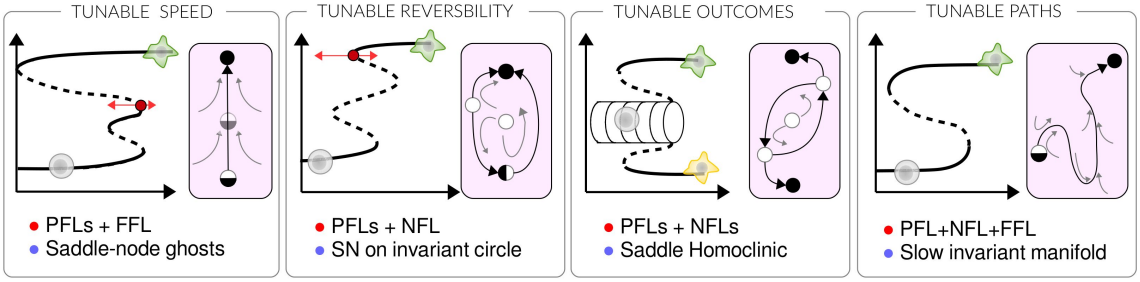


Figure 6.1: **Nonlinear dynamics for fine-tuning diverse cell decision properties.**

Notably, while PFLs shape the attractor landscape featured with multistability that define alternatives and instabilities that trigger transitions, the role of NFLs and FFLs is rather to shape and drive the transient and transition dynamics, whereby they contribute to a *fine-tuned control* of the speed, reversibility, opportunities, sequentiality of decision making. In parallel, oscillatory dynamics, slow manifold and related bifurcation scenarios illustrate the nonlinear dynamics that can implement such fine-tuned control of decision making. This is in line with ***the proposal that controllability is a crucial feature of adaptive regulatory process*** [BarYam09, Muller11, Zanudo17]. However, this thesis has barely scratched the surface of some important topics regarding the manner how cellular decision making can be shaped by evolutionary trade-offs, engineering tools or stochastic processes.

6.2 Other dimensions of cell decision making

6.2.1 Optimality in cellular decision-making

- ***Evolutionary trade-offs***

The organization of intracellular networks has evolved to track and keep memory of slow and fast changes in the cell environment [Brooks11, Voordeckers15]. Regulatory networks and regulated phenotypes have co-evolved to satisfy trade-offs between a variety of costs and benefits, which can typically be studied within the framework of multi-criteria optimization problems [Poelwijk11, Shoval12, Hart15]. Cost-benefit trade-offs reflect the constraints acting at different levels of biological organization such that, at some point, fitness improvement in one phenotypic trait (objective) is achievable at the expense of others. This trade-off defines a high-dimensional Pareto optimality front in the objective space (related to the network dynamics) corresponding to an optimal set of regulatory network designs and parameters (Fig. 6.2A). Therefore, slow evolutionary and context-dependent adaptation of network dynamics and structure are therefore prone to occur on this sub-manifold. In determining Pareto fronts with respect to cell decision making [Giagkiozis14], the main difficulty is to identify and quantify the relevant constraints, costs and benefits, which are inherent to any decision tasks such as:

(*i*) Energetic costs corresponding to the unavoidable dissipation or consumption of free energy required for information processing [Mehta12, Parrondo15] or for the maintenance of network organization [Mengistu16]. However, such thermodynamic and metabolic costs remain exceedingly difficult to quantify despite several attempts [Lan12, Li14, Milo16].

(*ii*) Adaptive benefits provided to cells when they take the most appropriate decision among many other possible ones. This is again very challenging to quantify especially because fitness is mostly effective at the level of cell populations. While some insights into adaptive benefits can be obtained by measuring time-averaged and population-averaged growth rate in a controlled environment [Perfeito11,Mitchell15], it is much harder to assess the extent to which specific cell decision-making properties contribute to improving the development and homeostasis of multicellular organisms. A more reasonable strategy in this case would be to focus on one particular decision ability such as speed, accuracy, distribution, information-processing (e.g., [Kobayashi10, Siggia13]) or one multicellular feature (see (iii)).

(*iii*) Social constraints that shape cellular decision making toward both the development and adaptation of the multicellular organisms. Intercellular interactions provide additional layers of negative and positive feedbacks, which contribute to various important developmental processes such as cell-fate diversification, spatial patterning, temporal schedules, size control, wound repair, ageing and others [Freeman00, Lewis08]. Although these processes involve the crosstalk of specific and complicated pathways regulating diverse cellular functions such as intercellular communication, metabolism, proliferation, differentiation, shape or motility, some principles can be nevertheless captured by simple models where colonies or tissues are described as a spatially-continuous [Vakulenko09, Kondo10] or spatially-discrete [Kaneko94, Mizuguchi95, Koseska10, Ares12] models of cells, where both intracellular and intercellular dynamics are idealized.

- ***Engineering approaches of cell decisions***

This notion that network architecture has evolved to meet some optimal trade-off between several objectives can be exploited ***to engineer and manipulate the configuration of such network architecture*** toward scientific, therapeutic or industrial purposes. Synthetic biology

provides indeed techniques to *explore network spaces* which have already been applied to investigate various cell decision-making problems, for instance to reveal the critical role of noise on bacterial fate decisions [Cagatay09], to clarify the role of intercellular coupling in binary cell-fate decisions [Matsuda15], to question the interplay between dynamic signaling and decisions outcomes [Gordley16], to force cells to remain in an undecided saddle state [Lugagne17], to reprogramm cell fate [DelVecchio16] or to engineer a kill switch [Chan16].

6.2.2 Statistical aspects of cellular decisions

Thermal fluctuations or other sources of randomness impact on a broad spectrum of intracellular processes (conformation, binding, polymerization, transport etc...) as illustrated by the highly stochastic gene expression dynamics [Maheshri07,Raj08], while such noises have many functional implications for the adaptive, developmental and evolutionary behaviors of living organisms [Eldar10, Tsimring14]. In the context of decision making, the significance of intracellular sources of noise and variability can be addressed essentially in terms of the statistical and information-theory features of decision dynamics (Fig. 6.2B).

- ***Dynamical and cell-to-cell variability***

In this thesis, decision-making processes were essentially described in terms of state-space attractors and trajectories, while their robustness was analyzed in terms of local stability or sensitivities. In presence of intrinsic and extrinsic sources of noise, more suitable framework and tools stemming from statistical physics (master equation, Fokker-Planck equation, stochastic differential equation, stochastic simulations) describe decisions in terms of probability distributions and transitions rates and is definitively necessary to explain and quantify cell-to-cell variability of gene/protein expression in isogenic populations [Simon18].

In addition, the advent and recent use of single-cell genome-wide techniques, such as single-cell transcriptome sequencing (scRNA-seq) [Tang09], profiles, follows and compares the state of many (isogenic) cells in their high-dimension ($\sim 10^4$) gene expression space. The application and combination of cutting-edge statistical techniques (feature selection, dimensionality reduction, clustering...) to these high-dimensional data can thus shed new lights to cell decision making especially during development [Trapnell14,Kester18,Kumar17,Griffiths18] (and reference therein), for instance to discriminate and map cellular states, to quantify gene expression variability over time and between cells, to characterize developmental trajectories and bifurcation events, to reconstruct lineage structure or to infer regulatory network architecture and models.

As well-illustrated in the case of binary fate decision of stem cells, these diverse statistical approaches are equally-important to determine how the interplay of stochasticity and multistability leads to particular statistical properties of fate decisions [Wang11, DeMot16] or how stochastic and multistable properties can be inferred from statistical analysis of single-cell measurements during fate decisions [Marco14, Semrau17].

- ***Information processing***

Besides its influence on gene expression dynamics and cell-to-cell heterogeneity, the biological significance of stochasticity could also be addressed from a *cognitive* viewpoint. Indeed, a living cell (or organism) must make decisions under various sources of *uncertainties* due to the inherent unpredictability of environmental changes and the incompleteness of sensory and stored informations. Cell decision making can thus be viewed as a probabilistic process in which a cell infers the most probable actual and future states of its environment, from a finite and partial amount of available informations. Diverse information-theoretical approaches and measures have been proposed to investigate how cellular signaling pathways encode and decode informations over time and how those events impose constraints on decision accuracy and speed [Kobayashi10,Siggia13, Bowsher14].

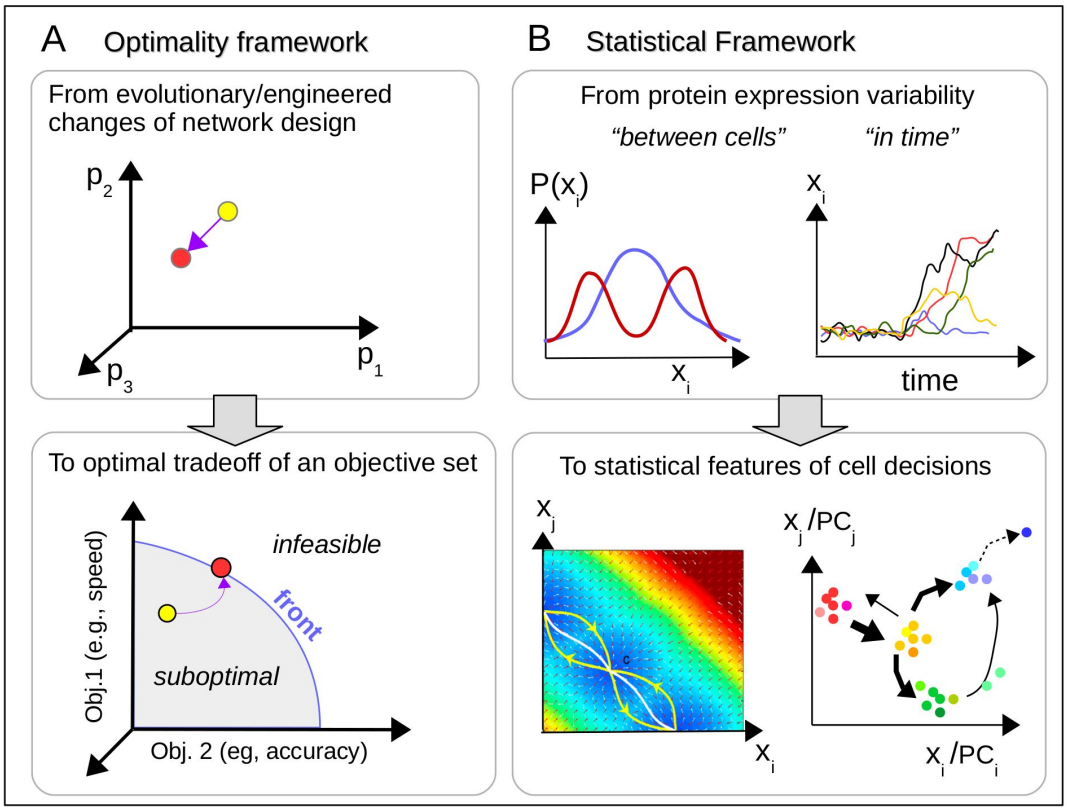


Figure 6.2: **Optimality and statistical approaches of decision-making.** *(A) How do evolutionary/engineering changes of network design translate into an more or less optimal trade-off between natural or desired objectives for decision-making tasks? (B) How do the many sources of protein expression variability translates into statistical properties of decision making (transition paths and rates..)? The bottom left panel is extracted from [Wang11].*

6.3 On-going project: cell decisions between life and death

The ongoing research projects are primarily dedicated to the topic of stress-induced cell death decision-making and are parts of a collaborative and interdisciplinary program which combines theory, modeling and experiments [1].

6.3.1 Design principles of stress-response networks

- ***Adaptive homeostasis through negative feedback***

Living cells are continually exposed to a diversity of stress conditions, be they nutritional, chemical, oxidative, thermal, mechanical or others, which produce intracellular damages that threat the homeostasis and survival of cells. To cope with this threat, cells also evolved adaptive mechanisms through which stress and damage signals are processed via a regulatory network that elicits a repair and defense response aiming at preserving cellular homeostasis and survival. This network is implemented by a core negative feedback loop, in which stresses – such as oxidative stress (OxS), heat shock (HS), carbon starvation (CS), ionizing radiation (IR) or

[1] Theory: Q. Thommen (MCF), D. Labavic (PostDoc), M. Ladjimi (Thesis); Experiments: E. Courtade (MCF), F. Anquez (MCF), M. Guibert (PostDoc)

osmotic shock (OsS)– produce cell damages or imbalances – production of reactive species (H_2O_2) or of misfolded proteins (MFP), ATP depletion (AMP), DNA double-strand break (DSB) or turgor pressure changes (DP) –, which leads, directly or indirectly, to the activation or synthesis of regulatory and repair proteins (Fig. 6.3A):

Stress	Damage		Regulator		Repair		Damage
OxS:	H_2O_2	$\overset{*}{\rightarrow}$	G6P	$\rightarrow$	NADPH	$\dashv$	H_2O_2
HS:	MFP	$\overset{*}{\rightarrow}$	HSF	$\rightarrow$	HSP	$\dashv$	MFP
CS:	AMP	$\rightarrow$	AMPK	$\overset{*}{\rightarrow}$	ATP	$\dashv$	AMP
IR:	DSB	$\rightarrow$	ATM	$\overset{*}{\rightarrow}$	DDR	$\dashv$	DSB
OsS:	ΔP	$\rightarrow$	HOG	$\overset{*}{\rightarrow}$	Glyc	$\dashv$	ΔP

where $*$ indicates an indirect and effective regulation mediated by several regulatory species and mechanisms.

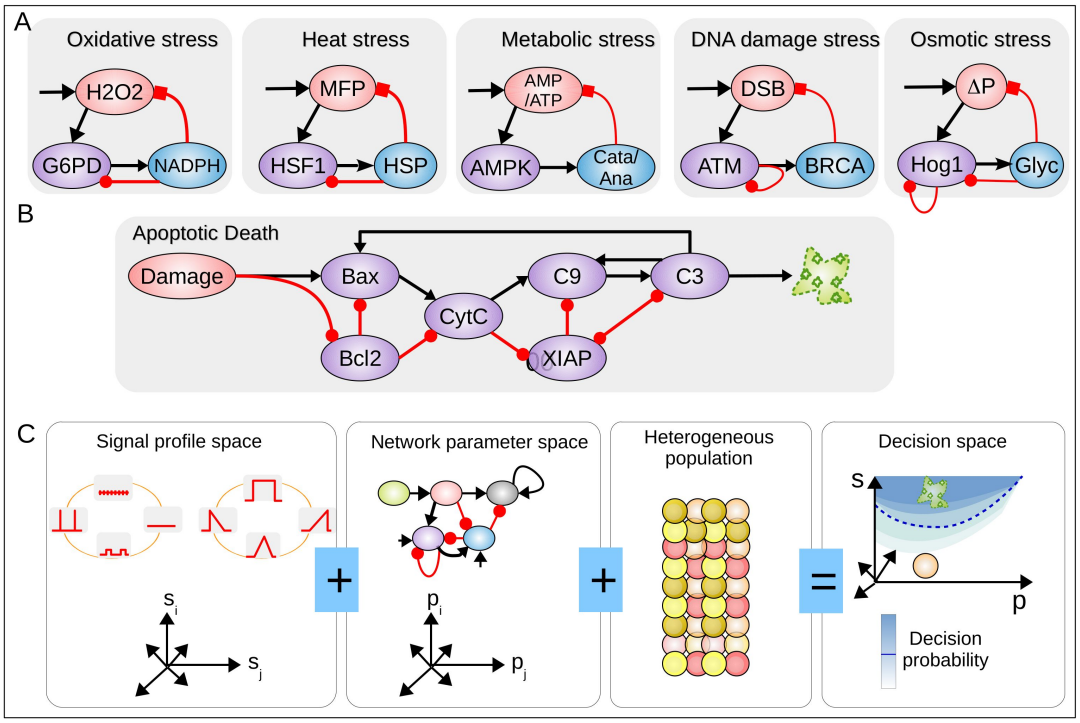


Figure 6.3: **Interplay of negative and positive feedbacks in stress-response networks.** *(A) Schematic representation of the negative feedback network involved in the adaptative response to oxidative, heat, carbon starvation, DNA-damage and osmotic stresses. (B) Schematic representation of the positive feedback network involved in the death response to damage. (C) Study of the relationship between stress profile, network structure, non-genetic heterogeneity and decision properties.*

- ***Cell death through positive feedback***

From an individualistic viewpoint, stress-induced cell death in unicellular organisms can merely be seen as a failure of the cell to survive or adapt to a too high level of damages. By

contrast, in cell colonies or multicellular organisms, individual cell death can be seen as an expression of a social behavior as death may occur and even be actively induced in the absence of intracellular damages, for the benefit of the whole colony/organism, a phenomenom that is called *programmed cell death* [Ameisen02]. In these cell societies, heterogeneous levels of stress exposition or sensitivity among diverse cell types may justify the strategy to make or let die part of the population, and eventually replacing it through division and differentiation processes, whether it occurs during bacterial bet-hedging process [Veening08] or tissue regeneration [Pellettieri07, Visvader16]. In the course of metazoan evolution, cell death has become an increasingly prominent and well-regulated process used for instance for promoting the interdependency between cells, for avoiding the survival of misrepaired and misbehaving cells, for improving cellular debris clearance or for shaping body plans and organs. Cells are thus equipped with death regulatory pathways that are sensitive to various extracellular and intracellular signals and that can elicit cell death through a variety of enzymatic and morphological processes, including apoptosis but also regulated necrosis, necropoptosis, autophagy, mitotic catastrophe or senescence [Kroemer09]. The irreversible nature of the cell-death process typically relies on the presence of strong positive feedback loops such as those operating during apoptosis [Legewie06] (Fig. 6.3B):

$$
\begin{aligned}
PFL1: &\quad Cytc_c \rightarrow Cytc_{m\rightarrow c}\\
PFL2: &\quad C3 \dashv XIAP \dashv C3\\
PFL3: &\quad C3 \dashv XIAP \dashv C9 \rightarrow C3\\
PFL4: &\quad C3 \rightarrow C9 \rightarrow C3\\
PFL5: &\quad C3 \rightarrow Bax, Bid \rightarrow [CytC]_{release} \rightarrow C9 \rightarrow C3
\end{aligned}
$$

These positive feedback loops contribute to amplification mechanisms when some threshold of caspase activity is reached, which triggers a set of irreversible events starting first with the mitochondrial changes and culminating in the activation of effector caspases and cell bebbling.

6.3.2 Stress time profiles and fractional killing

The forward coupling between an adaptive negative feedback process and an irreversible positive feedback process raises a number of issues about life-death decisions. On the one hand, adaptive negative feedback do not only lower steady-state damages, but can also induce an accelerated response [Rosenfeld02], noise-filtering properties [Singh09, Guantes10] or a transient overshoot [Ma09, Ray10, Karin16]. On the other hand, death positive feedback induces an irreversible commitment [Legewie06], with nevertheless a highly variable kinetics and outcomes [Spencer09, Roux15, Paek16]. Their coupling may therefore shape or produce decision behaviors sensitive to signal dynamics and stochasticity in sophisticated and cell-specific manner (Fig. 6.3C). All these considerations are investigated in several related projects:

(i) Stress time profile: How does the temporal profile of stress signals influence life-death fate decisions? This issue can be explored by, first, looking for the relation between the characteristics of signal inputs (e.g., duration, intensity, frequency, shape index, asymmetry index...) and those of the decision-making output (e.g., death type, probabilities and kinetics), and then, by determining how this relation depends on the characteristics of the regulatory network (e.g., regulatory timescale, feedback structure). Such a systematic approach would be valuable to identify relevant evolutionary and adaptive trade-offs related to the natural environment, resource allocation and social constraints and, *in fine*, to design therapeutic protocols that would selectively optimize or minimize cell death. This

issue is actually addressed from a dose-response viewpoint focusing on hyperthermia dose responses [2] and on general dose-rate effects [3].

(*ii*) Fractional killing: How do various sources of stochasticity and heterogeneity influence the variability of cell death fate decisions ? Fractional killing is a process whereby varying level of stress is prone to induce ***only a fraction of cell to die*** (and survive). Whereas many theoretical and experimental works have investigated how fractional killing may depend on stochastic damage production [Loewer13], stochastic gene expression dynamics [Gaudet12, Bertaux14] and on the negative feedback adaptive dynamics [Roux15, Paek16], it remains to understand their respective interplay. Indeed, the effect of diverse sources of stochasticity on cell fate decision properties (i.e., death probabilities and death time) should depend, in various ways, on the nonlinear dynamics of stress-response regulatory networks (i.e., phase-portrait characteristics).

Related to these theoretical issues, decades of dose-response studies have provided systematic statistical measurements of cellular survival response in response to various types, combination and time profile of stress, thereby providing an extensive dataset to fuel or test models. In our team, experimental setups are readily available and will be further refined to monitor heat and oxidative stress inputs and to evaluate cell death outputs (time, type and kinetics), so as to generate multi-dimensional and multi-parametric dose response curves that will allow us to address the subtle relationship between stress time profile, regulatory network design and cell-fate decision properties.

6.4 Epistemic considerations

6.4.1 From biochemical mechanisms to dynamical principles

While experiments aim at interacting with natural phenomena to gain knowledge, models use such knowledge to construct an effective (able to generalize and predict) conceptualization of these phenomena. However, it seems intrinsically difficult to reconcile the search for unifying theoretical principles and the diversity and complexity of life's biochemical mechanisms. Modeling approaches in life sciences are therefore pulled between a given theoretical framework (e.g., dynamical system theory, statistical theory, information theory or network theory) and the complex multiscale biological reality. Such gap is continously widening with the development of measurement techniques that lead to an accumulation of quantitative structural and dynamical informations over a broad range of spatial and temporal scales, though sophisticated methods to read out such informations are being developed. To bridge this gap, a modeling strategy that has been used in our framework is to build models with varying levels of resolution ranging from (i) reduced or coarse-grained models keeping a minimal set of properties in order to match qualitative and general behaviors to (ii) detailed or fine-grained models incorporating an extensive set of properties in order to match quantitative and specific behaviors. To illustrate this idea, the electrical activity of neurons has been described using a wide spectrum of models ranging from low-dimensional phase, rate or pulse models to high-dimensional conductance-based and compartmentalized models, in which each description level is useful to explain a certain class of behaviors and is consistent with that of all the others (e.g., [Pfeuty03]).

A more ambitious strategy is to use methods for model reduction that are suited for multiscale phenomena [Gorban06, Radulescu12, Snowden17, Transtrum16], which *"alleviate the issue of complexity by seeking to eliminate those portions of a biochemical reaction network that have*

[2] Ladjimi et al, A dynamical framework for refining thermal dose models and capturing dose-time profile effects, in preparation

[3] Labavic et al, Inferring cellular adaptive properties from dose-rate response curves, in preparation

little or no effect upon the outcomes of interest, hence yielding simplified systems that retain an accurate predictive capacity and a strong explanatory power". As mentioned in the introduction, the diversity of biomolecule types, of regulatory mechanisms and of timescales involved in biochemical reactions leads to dynamical systems of high dimensionality, nonlinearity and stiffness, which makes model reduction both necessary and challenging. However, the existence of a few stiff parameters combinations in systems biology models [Gutenkunst07] or the mapping of high-dimensional single-cell data to low-dimensional space and manifolds [Trapnell14] support the relevance and usefulness of low-dimensional effective models. A broad spectrum of model reduction methods are now available and will certainly help in the quest of structural and dynamical principles.

6.4.2 The nonlinear dynamics of life

The working hypothesis underlying our dynamical modeling framework is that *"important cell decision-making properties primarily emerge from the organization and the transformation of the phase portrait of the protein network dynamics"*. This view of biological processes builds upon the long-standing idea that living systems are specified by some dynamical and structural features that are to some extent independent of their (bio)physical and (bio)chemical substrate.

Indeed, far-from-equilibrium dissipative systems of diverse nature can display complex spatiotemporal behaviors ranging from symmetry-breaking and patterning to turbulence and chaos. The presence of additional layers of organization and nonlinearities in living dissipative systems is likely to refine, but also restrict, their dynamical behavior in a life-specific mode that still needs to be clarified. A few centuries ago, Kant already intuited the self-organized and self-directed nature of living beings [Kant1790, Weber02]:

> *"Living systems display a unity that is related to their essence as globally functioning, actively integrated, cohesive and purposive entities. Living systems are in other words organised essentially in view of certain purposes. They are natural purposes - both a cause and effect of itself - in which nothing whatsoever is the result of chance."*

Since, many philosophers, biologists or physicists have developed concepts and theories to refine this idea in the light of new scientific knowledge around two poles of nonlinear processes:

(*i*) The organizational circularity between the metabolism and its membrane boundary [Varela74] or its repair system [Rosen91], between the thermodynamic work and its constraints [Kauffman00], between an ergodic system and its markov blanket [Friston13]...

(*ii*) The adaptability to changing environments in its various dynamic aspects such as homeostasis [Ashby62], evolvability [Kirschner06], anticipation [Rosen85], free energy minimization [Friston12]...

How do *self-production over space* and *self-preservation over time* translate into universal laws and principles of biology? The answer probably requires the development of a self-consistent theoretical framework that encompasses nonlinear dynamical system theory, network theory, statistical theory, information theory and biosemiotics theory. Decision making, as an intuitive, ubiquitous, dynamical, cognitive behavior, seems well-suited to the pursuit of this goal.

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Appendix A

Glossary and nomenclature

Acronyms or abbreviations	
MPF	Maturation Promoting Factor (i.e., p34cdc2-cycB)
MFP	Mis-Folded Proteins
ROS	Reactive Oxygen Species (e.g., H_2O_2, O_2^-)
DSB	DNA strand breaks
CKI	Cyclin-dependent Kinase Inhibitor (e.g., p21, p27, P57, Ink4)
Dll	Delta ligand
Ngn	Neurogenin
GF	Growth/mitogenic factors (e.g., Insulin, nutrients, cytokines)
SF	Stress factors (e.g., ROS, MFP, DSB)
DF	Differentiation factors (e.g., Notch, Fgf, BMP)
NSC	Neural Stem Cells
Network links	
$\rightarrow$	Transcriptional/translational upregulation
$\rightarrow$	Post-translational activation
$\dashv$	Transcriptional/translational downregulation
$-\bullet$	Post-translation inactivation
$-\blacksquare$	Upregulation of protein degradation
$\overset{*}{\rightarrow}$	Intercellular interaction (here $\rightarrow$).
Network motifs	
PFL	Positive Feedback Loop
NFL	Negative Feedback Loop
CFFL	Coherent Feed-Forward Loop
IFFL	Incoherent Feed-Forward Loop

Table A.1: **Protein networks**.

.

Acronyms	
ODE	Ordinary Differential Equations
PRC	Phase Response Curve
FP	Fixed Point
LC	Limit Cycle
SN	Saddle Node
SNIC	Saddle Node on Invariant Circle
SNLC	Saddle Node of Limit Cycle
SHO	Saddle Homoclinic Orbit (or loop)
SNHO	Saddle-Node Homoclinic Orbit (or loop)
SNSHO	Saddle-Node on a Saddle Homoclinic Orbit (or loop)
Mathematical notations and symbols	
$\mathbf{x}$ vs x_i	vector vs scalar
$\mathbf{x}(t)$	Set of biomolecule (mRNAs, proteins) concentrations
$\mathbf{s}(t)$	Set of signaling molecules concentrations
$\mathbf{p}$	Set of kinetic reaction rates
$\mathbf{F}(\mathbf{x}, \mathbf{p}, \mathbf{s})$	Smooth vector field produced by set of selected biochemical reactions
$\phi^t(\mathbf{x})$	Flow/evolution operator associated with $\mathbf{F}$: $\phi^t(\mathbf{x}_0) = \{\mathbf{x}(t), \mathbf{x}(0) = \mathbf{x}_0\}$
$\mathbf{x}^\alpha$	Steady state α solution of $\phi^t(\mathbf{x}^\alpha) = \mathbf{x}^\alpha$
γ^α	T-periodic limit cycle orbit where $\phi^T(\mathbf{x} \subset \gamma^\alpha) = \mathbf{x}$
φ	Phase of a limit cycle
$\mathcal{H}(t)$	Heaviside step
$\Pi(t, \Delta t)$	Rectangular function
$\mathcal{W}$	Invariant manifold $\phi^t(\mathcal{M}) = \mathcal{M} \, \forall \, t$
$\mathcal{W}^{s/u/c}(u)$	Stable/unstable/center manifold of a limit set u
$\mathcal{P}(x_i, t)$	Probability distribution of variable x_i at time t
Phase portraits/Bifurcation diagrams	
●/○/◐	Stable/Unstable/Saddle-node fixed point
—/- - -	Stable/Unstable steady-state branch

Table A.2: **Dynamical system modeling**.

Glossary for nonlinear dynamical systems

Invariant manifold - Flow-invariant set of points that are continuously and smoothly parameterizable geometric objects. Important ones are closed limit sets and (un)stable/center manifolds of limit sets.

ω/α **limit sets** - Asymptotic orbits for $t \to \pm\infty$ that can be fixed, periodic, quasi-periodic or aperiodic orbits depending on their geometry.

Attractors/repellor - ω/α limit sets of *all* their neighboring points.

Saddle limit set - Both ω and α limit set of two subsets of neighboring points.

Stable/unstable/center manifolds of a limit set ($\mathcal{W}^{s,u,c}(.)$) - Invariant manifolds that converges to it for $t \to \pm\infty$ and are tangent to their stable/unstable/center eigenspaces (i.e., $\Re(\lambda_i) < 0, > 0, = 0$).

Weak/strong manifolds - Submanifolds of $\mathcal{W}^{s,u}$ associated with the eigenspace corresponding to the eigenvalue of lesser/higher magnitude when well-separated.

Slow manifold - In slow-fast systems, an invariant or locally-invariant manifold that is normally hyperbolic (the expansion or contraction in the transversal direction is much larger than in the tangential direction). A slow manifold can be locally attracting, repelling or of saddle-type, and is often the center manifold of a non-elliptic fixed point ($\lambda_i = 0$).

Attraction basin - the stable manifold of an attractor delimited by boundaries (generally defined by the stable manifold of saddle limit set).

Homoclinic/heteroclinic manifolds - Intersections of stable and unstable manifolds of the same/different limit set(s).

Bifurcation - Occurrence of *sudden, local or global, qualitative/topological* change in the dynamical system behavior upon a small smooth parametric change, which can be described as a collision between invariant sets.

Codimension of bifurcation - the number of parameters which must be varied for the bifurcation to occur.

1D bifurcation - Family of bifurcation of 1D flow (fold[1], cusp[2], swallowtail[3], butterfly[4], pitchfork$^{1/\infty}$, transcritical$^{1/\infty}$).

SN-type bifurcation - Local bifurcation associated with the collision and disappearance of two equilibria, and can occur for instance on a one-dimensional invariant manifold (e.g., invariant circle for SNIC or a homoclinic orbit for SNSHO).

Homoclinic-type bifurcation - Global bifurcation associated with the collision of a limit cycle with an equilibria (e.g., saddle for SHO, saddle-node for SNHO).

Appendix B

Bifurcation unfolding

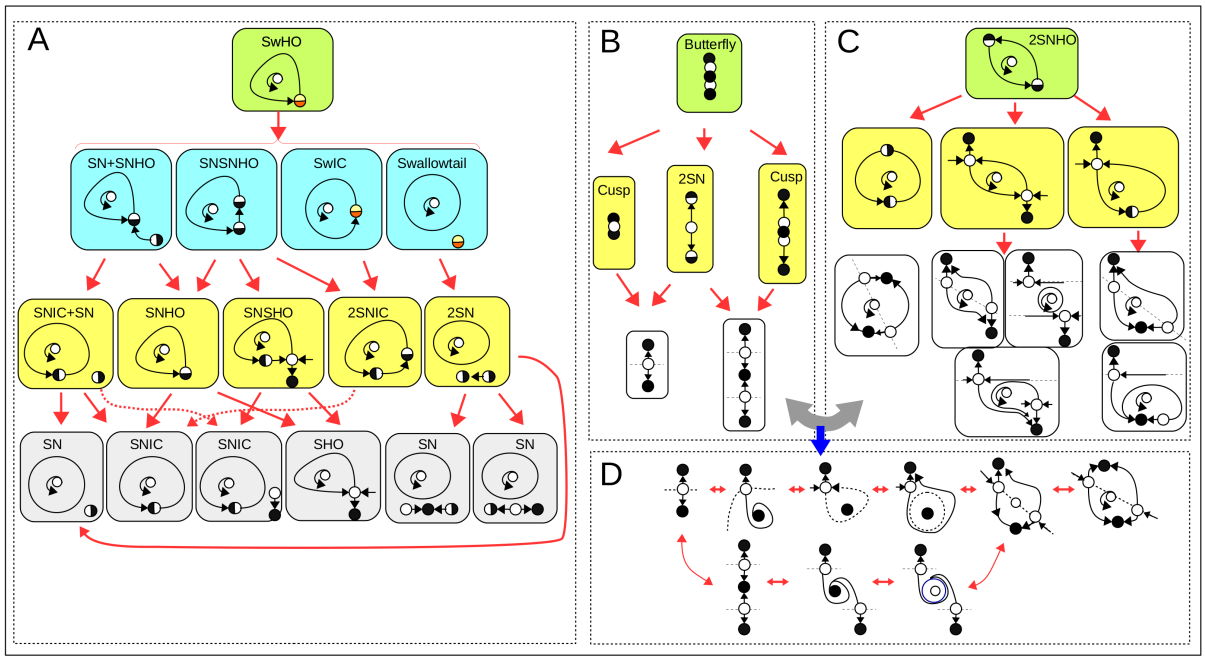

Figure B.1: **Unfolding of high codimension bifurcations**. White: Non-singular; Grey/Yellow/Blue/Green: codimension-1/2/3/4 singularities. (A) Bifurcation scenarios of limit cycles possibly relevant for cell-cycle arrest and exit. (B,C) Typical bifurcation scenarios and phase portraits for competing/binary decisions with PFLs only (B) or with both PFLs and NFLs (C). (D) Stepwise changes of phase portraits from (B) to (C).

Appendix C

Some maths

Tunable speed through saddle-node ghost

The topological normal form of a saddle-node bifurcation reads $x' = \epsilon + x^2$. For ϵ small and positive, integrating this equation :

$$\int_0^{t'} dt = \int_{x_0}^{x(t')} (x^2 + \epsilon)^{-1} \, dx \tag{C.1}$$

which allows to compute the time to go from x_0 to $x(t')$:

$$t' = 1/\sqrt{\epsilon} \, \left[\arctan\left(x(t')/\epsilon\right) - \arctan\left(x_0/\epsilon\right)\right] \to 1/\sqrt{\epsilon} \tag{C.2}$$

by taking the limit $\epsilon \to 0^+$. For ϵ small, enough most of this time is spent within the saddle-node ghost ($x_{sn}(\epsilon = 0) = 0$) (i.e., just before to make the decision). Defining speed as $\Sigma = 1/t'$, the speed tunability (by s) can be defined as the inverse derivative:

$$\left(\frac{d\Sigma}{d\epsilon}\right)^{-1} = \sqrt{\epsilon} = \Sigma^{-1} \tag{C.3}$$

Non-tunable speed near a saddle

Let consider a system that contains a saddle fixed point and a stable and unstable manifold ($\mathcal{W}^{s/u}(\mathbf{x}^{sad})$) associated to this saddle point, where the linearized dynamics along the single unstable direction reads $x' = \lambda_u x$. For some critical signal parameter $\mathbf{s}_c$, the initial state (e.g., a destabilized steady state) of the system belongs to the ($(n_1 - 1)$-dimensional) stable manifold and will converge to such saddle fixed point:

$$\mathbf{x}^0 \in \mathcal{W}^s(\mathbf{x}^{sad}) \tag{C.4}$$

However, a small parametric perturbation induces a shift between $\mathbf{x}^0$ and $\mathcal{W}^s(\mathbf{x}^{sad})$ that further translates into a shift ϵ between the $\mathbf{x}(t)$ and $\mathcal{W}^s(\mathbf{x}^{sad})$ at the entry of an hypercube Δ around the saddle wherein trajectories evolve according to the linearized flow. The time spent in this hypercube reads:

$$t' = 1/\lambda_u \ln\left(\Delta/\epsilon\right) \to -(\lambda_u)^{-1} \ln \epsilon \tag{C.5}$$

by taking the limit $\epsilon \ll \Delta$. Again, defining $\Sigma = 1/t'$, the inverse derivative reads:

$$\left(\frac{d\Sigma}{d\epsilon}\right)^{-1} = \lambda_u^{-1} \, \epsilon (\ln \epsilon)^2 = \lambda_u \, e^{-\lambda_u/\Sigma} \, \Sigma^2 \tag{C.6}$$

It remains to determine how ϵ scales with the parametric perturbations (i.e., $[SF]$ in the model studied in Chapter 2).

Appendix D

CV

Cheminement

- 2000-2001: Master de Science Cognitives. Laboratoire Neurosciences Cognitives et Imagerie Cérébrale. Hopital la Pitié Salpetrière.
- 2001-2004: PhD Neurosciences. Laboratory of Neurophysics and Physiology of Motor System. Université Descartes.
- 2005: Post-Doctoral fellow. Interdisciplinary Center for Neural Computation. Hebrew University of Jerusalem.
- 2006-2009: Post-Doctoral JSPS fellow. Laboratory of Complex Systems and Non Linear Sciences. Tokyo University.
- 2009-2011: Post-Doctoral ANR fellow. Institut de Recherche Interdisciplinaire / Laboratoire de Physique des Lasers, Atomes and Molécules. Université Lille I.
- 2011-2018: Chargé de recherche CNRS. Laboratoire de Physique des Lasers, Atomes and Molécules. Université de Lille

Liste de publications

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