Simplified models for the mammalian circadian clock

Jean-Paul Comet[1], Gilles Bernot[1], Aparna Das[2],
Francine Diener[2], Camille Massot[1] and Amélie Cessieux[1]

[1] Laboratoire I3S, UMR 7271 UNS-CNRS, Université de Nice,
2000, route des Lucioles, B.P. 121, 06903 Sophia Antipolis CEDEX, France.
e-mail : {bernot,comet}@unice.fr

[2] Laboratoire J.A. Dieudonné, UMR CNRS 7351, Université de Nice,
Parc Valrose, 06108 Nice Cedex 02, France.
e-mail : {aparna,diener}@unice.fr

Abstract

Numerous biological mechanisms are synchronized by the circadian rhythm in species so diverse as mushrooms, drosophiles or mammals. Because of its ubiquity and of its implication in numerous cellular functions, we believe important to be able to extract the main "coarse-grain" mechanisms common to a majority of organisms. In this chapter we consider both differential equation models and discrete models and we deliberately choose to push the simplicity of the model as far as possible, focusing only on a few biological behaviours of interest. The hope is to get the essential abstract causalities that govern these behaviours.

1 Introduction

The growing interest for the circadian cycle in biology is motivated by several facts, including an important proportion of the population with staggered working timetable, the effect of jet lag, and, also importantly, the drug chronotherapy. Many of these studies involve careful modelling where the circadian cycle interacts with other subsystems such as the metabolic pathway influenced by a particular drug [1]. A good design of such complex models implies, among others, a proper global understanding of the main features of the circadian cycle behaviours. In this perspective the goal of this paper is to design models of the circadian cycle that focus on these main features whilst being sufficiently simple to offer a human comprehension of the global circadian system.

Mathematical models of biological systems are often classified into three main frameworks.

- Continuous models using differential equations constitute probably the most frequently used framework, see for example [2].

- With the growing interest for logical approaches, discrete models constitute a class of frameworks which focus on the causality relationships between events, this kind of models is well suited to take into account qualitative information [3, 4].

- Lastly the stochastic approaches form a sort of intermediate class of frameworks: the global dynamics of the model is divided into elementary steps to which are attributed some probabilities [5].

Among others, Leloup and Goldbeter [6] proposed a carefully detailed differential model of the circadian clock; Forger and Peskin [7] proposed a stochastic model. There does not yet exist any qualitative model of the mammalian circadian clock, which we incidentally propose in this article because discrete approaches push further simplicity. As a first step towards simplification we propose two successive differential models reflecting a two-step simplification of the Leloup-Goldbeter's model. We then jump directly to a discrete model in order to take the day-night alternation into account, this additional simplification being sufficiently intuitive to avoid addressing a stochastic modelling.

In section 2 we describe the main molecular mechanisms that constitute circadian cycle in the suprachiasmatic nucleus. Although partly governed by the light/dark alternation, it is known that this cycle is actually an endogenous cycle, governed by an internal mechanism in each cell and the goal of section 3 is to introduce two simplified EDP models and section 4 establishes on the smaller model a mathematical proof of the existence of sensible parameters that lead to a limit cycle in the dynamics. Section 5 introduces a supplementary simplification using the discrete Thomas' framework whereas introducing the light influence on the system. Lastly section 6 takes into consideration some delays and gives an obvious explanation of the main reasons for the robustness against the day length variations.

2 Molecular processes underlying the mammalian circadian clock

Mammalian circadian clocks regulate numerous biological functions (sleeping, locomotive activity, internal temperature, hormonal secretions and so on). In the absence of temporal reference point, the circadian rhythm shows a sustained oscillation both at the cellular and the organism levels, indicating that the body possesses an endogenous daily clock. Although each cell is equipped with an internal clock, there are external synchronizers, called Zeitgebers ("donors of time"), such as light, social rhythms, food or physical exercise that maintain a 24-hour oscillation.

In mammals there is an internal supervisor made of two neuronal groups situated at the base of the hypothalamus, that is called the *suprachiasmatic*

nucleus (SCN) [8]. All the cells that constitute the SCN exhibit a rhythmic circadian clock and they are strongly synchronized (apparently using some local signals). The cells in the peripherical tissues (liver, muscles...) also exhibit a rhythmic behaviour [9] but it seems that the SCN plays the role of main oscillator synchronising the peripheral oscillators. Injuries of the SCN entail a visible disappearance of the global rhythm, and a transplant restores it [10, 11, 12]. In the suprachiasmatic nucleus, the main Zeitgeber is light. This "pacemaker" receives signals from the retina *via* the retinohypothalamic tract which is independent from the vision mechanism [13]. According to [14] these signals are passed on by the SCN into the pineal gland which, through the night-secretion of melatonin, informs the whole body of the arrival of night.

At the molecular level in the SCN cells, the regulation of the circadian cycle is driven by the expression of the clock genes, generating a feedback loop detailed in Figure 1:

- During the day, the protein complex BMAL1/CLOCK induces the expression of clock genes by fixing their promoters. It stimulates the production of CRY1 and CRY2 (cryptochromes), as well as of PER1, PER2 and PER3, RORA and REV-ERBα. Proteins RORA activate bmal1 and cry1 while REV-ERBα inhibits them [15, 16]. Moreover, in the suprachiasmatic nucleus, the light activates the transcription of genes per by inducing the acetylation of their promoters, that allows the slackening of the chromatin and their transcription [17]. Proteins PER are then phosphorylated by CKIε (Casein Kinase I ε), see [18]. Then these proteins can follow three different pathways: they can be destroyed *via* ubiquitinization, or they can form complexes PER1-PER2-CKIε which accumulate in the cytoplasm, or they can also form complexes PER1-CRY1-CKIε and PER2-CRY2-CKIε which also accumulate in the cytoplasm[1].

- During the night, complexes PER1-PER2-CKIε and PER-CRY-CKIε are hyperphosphorylated by CKIε before entering the nucleus where they inhibit BMAL1/CLOCK [19]. This degradation probably originates from CKIε since it can lead to the degradation of complexes (PER or CRY)-PER-CKIε-BMAL1/CLOCK by phosphorylating BMAL1 [18]. Thus during the night there is no transcription anymore of the circadian genes, leading to a negative retro-control of PER and CRY on themselves.
  In the cytoplasm, PER-CRY-CKIε captures REV-ERBα. RORA can then activate the transcription of BMAL1 which will form a complex with CLOCK. Nevertheless a period of time is mandatory to neutralize

[1] Let us remark that the function of PER3 is not yet known.

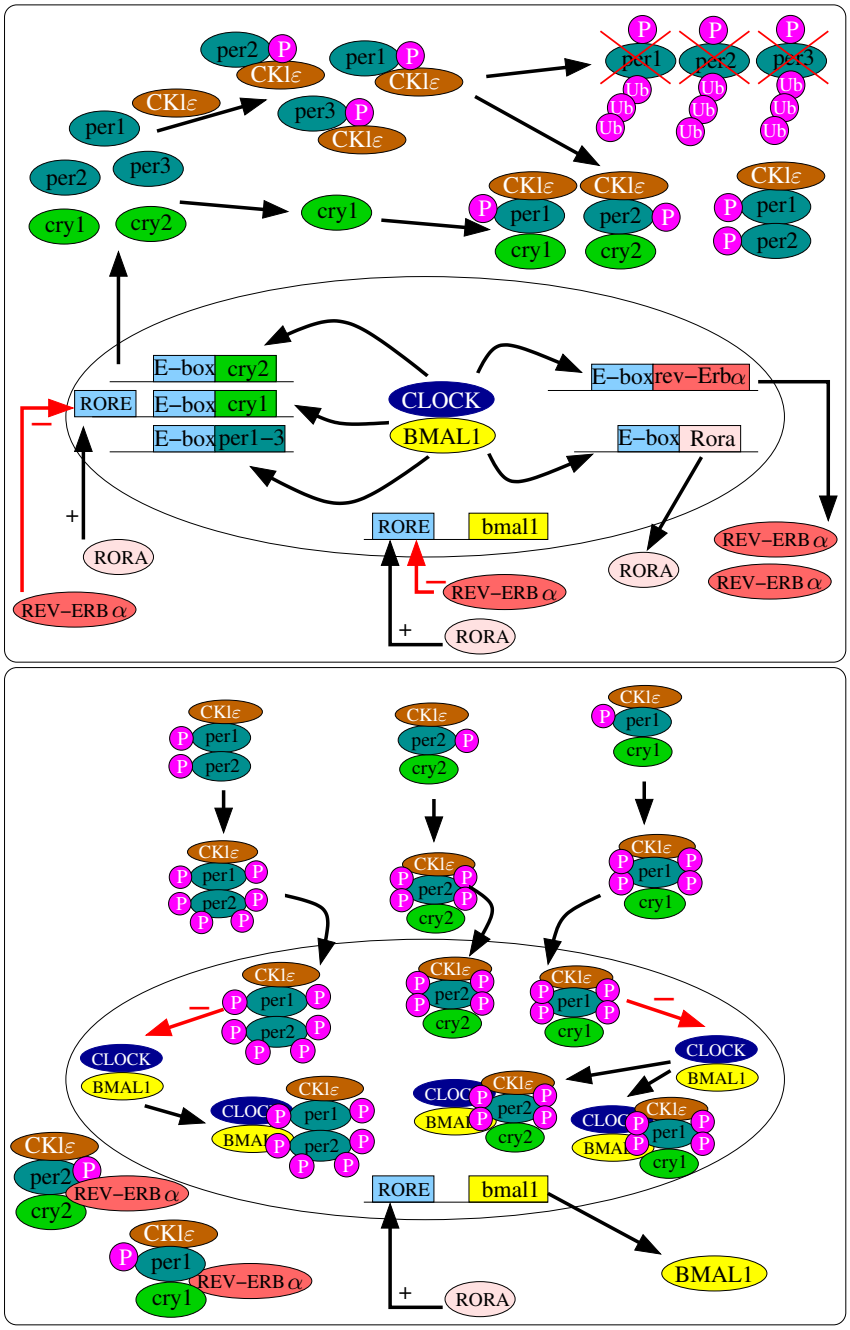

Figure 1: Molecular regulations of the mouse circadian clock during the day (top) and during the night (bottom).

all molecules REV-ERBα, this explains why the peak of transcription of BMAL1 takes place several hours before dawn. At dawn, proteins CRY and PER have all been destroyed by the degradation of complexes. REV-ERBα is consequently again active and inhibits bmal1 *via* RORE. The cycle can then restart [20, 21, 22].

3 Simplified EDP models for the mammalian circadian clock

In this section we propose a mathematical model of the circadian cycle obtained as a simplification of the model of Leloup and Goldbeter [6]. In the next section we will establish the existence of a limit cycle with a default period of 24 hours.

We will focus in this section on the behavior of the complex PER-CRY. During the day, the proteins PER and CRY are phosphorylated by CKIϵ and form complexes PER-CRY-CKIϵ which then accumulate in the cytoplasm. During the night, PER-CRY-CKIϵ captures REV-ERBα in the cytoplasm and will be hyperphosphorylated by CKIϵ before entering the nucleus where it inhibits its own transcription.

The continuous time model of Leloup and Goldbeter [6] contains 16 differential equations. The considered genes are per, cry, bmal1, clock and rev-erbα. They take into account the phosphorylation cycles, membrane exchanges between nucleus and cytosol, the translations of mRNA into protein, as well as the association of PER and CRY proteins to form the complex PER-CRY. The trajectories resulting from the equations can be cyclic with a sensible circadian period. Goldbeter and Leloup [6] also focus on the behavior of the concentrations over time under some light-dark alternation patterns: they consider the constant darkness case as well as the case "12 hours light – 12 hours dark." Their model is quite close to the biological reality, despite some simplifications. This model also correctly predicts several mutant behaviours of the circadian rhythm.

3.1 Model with 8 variables

In this subsection, we reduce the original model of Leloup and Goldbeter without losing too much information. This approach allows us to get a simpler model with 8 variables and 8 equations, leading to a better focus on the main involved mechanisms and on their importance within the overall schema.

We first decided to remove CLOCK protein because, although important to form a complex with BMAL1, its amount is known to be constant and is never a critical resource. Secondly, following Leloup and Goldbeter, we removed REV-ERBα from our EDP model. In fact the action of REV-ERBα on bmal1 is hidden in the negative feedback from the PER-CRY complex in the nucleus

to the PER and CRY in the cytosol, see Figure 2. Lastly, we have represented as a unique step both translation and traduction, leading to the removal of all mRNA variables.

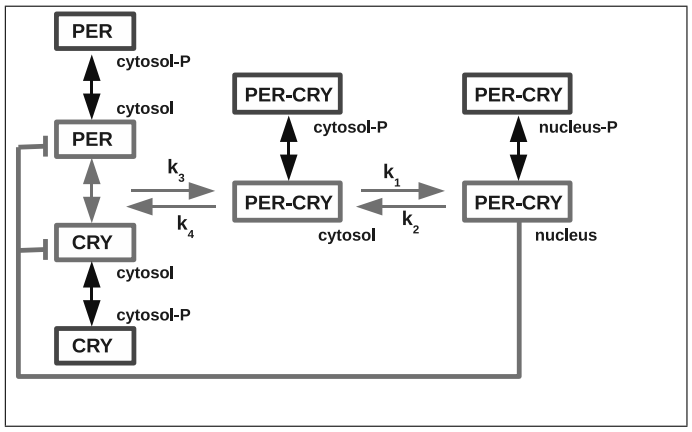

Figure 2: The 8 variable model contains a negative feedback loop (grey arrows) where (1) the PER-CRY complex of the nucleus inhibits the proteins PER and CRY, (2) PER and CRY form a complex in the cytosol and (3) this complex enters the nucleus. Black arrows represent reversible phosphorylations.

So, the model of Figure 2 is described by a set of 8 kinetic equations with 28 parameter values. We have chosen similar parameter values to those of Leloup and Goldbeter, with very few modifications.

$$\begin{aligned}
\frac{dP_C}{dt} &= \frac{K^n}{K^n + PC_N^n} v_1 - v_{1p} \frac{P_C}{k_p + P_C} + v_{2p} \frac{P_{CP}}{k_{dp} + P_{CP}} \\
&\quad -k_3 P_C C_C + k_4 PC_C - kd_1 P_C \qquad (1) \\
\frac{dC_C}{dt} &= \frac{K^n}{K^n + PC_N^n} v_2 - v_{1c} \frac{C_C}{k_p + C_C} + v_{2c} \frac{C_{CP}}{k_{dp} + C_{CP}} \\
&\quad -k_3 P_C C_C + k_4 PC_C - kd_2 C_C \qquad (2) \\
\frac{dPC_C}{dt} &= -v_{1pc} \frac{PC_C}{k_p + PC_C} + v_{2pc} \frac{PC_{CP}}{k_{dp} + PC_{CP}} \\
&\quad +k_3 P_C C_C - k_4 PC_C - k_1 PC_C + k_2 PC_N - kd_3 PC_C \qquad (3) \\
\frac{dPC_N}{dt} &= -v_{3pc} \frac{PC_N}{k_p + PC_N} + v_{4pc} \frac{PC_{NP}}{k_{dp} + PC_{NP}} \\
&\quad +k_1 PC_C - k_2 PC_N - kd_4 PC_N \qquad (4)
\end{aligned}$$

$$\frac{dP_{CP}}{dt} = v_{1p}\frac{P_C}{k_p + P_C} - v_{2p}\frac{P_{CP}}{k_{dp} + P_{CP}} - v_{dpc}\frac{P_{CP}}{k_d + P_{CP}} - k_{dn}P_{CP} \quad (5)$$

$$\frac{dC_{CP}}{dt} = v_{1c}\frac{C_C}{k_p + C_C} - v_{2c}\frac{C_{CP}}{k_{dp} + C_{CP}} - v_{dcc}\frac{C_{CP}}{k_d + C_{CP}} - k_{dn}C_{CP} \quad (6)$$

$$\frac{dPC_{CP}}{dt} = v_{1pc}\frac{PC_C}{k_p + PC_C} - v_{2pc}\frac{PC_{CP}}{k_{dp} + PC_{CP}} - v_{dpcc}\frac{PC_{CP}}{k_d + PC_{CP}} - k_{dn}PC_{CP} \quad (7)$$

$$\frac{dPC_{NP}}{dt} = v_{3pc}\frac{PC_N}{k_p + PC_N} - v_{4pc}\frac{PC_{NP}}{k_{dp} + PC_{NP}} - v_{dpcn}\frac{PC_{NP}}{k_d + PC_{NP}} - k_{dn}PC_{NP} \quad (8)$$

Variables P_C and C_C correspond to the amount of PER and CRY proteins respectively in the cytosol, and PC_C and PC_N are the amount of PER-CRY complex in the cytosol and in the nucleus respectively. The complexation of PER and CRY follows the rate k_3, with corresponding dissociation rate k_4. The use of Hill functions in equations (1) and (2) simulate the inhibition of PER-CRY (PC_N) on PER (P_C) and CRY (C_C) with n as degree of cooperativity.

3.2 Model with 4 variables

In order to mathematically prove the existence of a limit cycle, we again simplify the model of Figure 2: the model of figure 3 does not incorporate the phosphorylation of the proteins and, consequently, contains four variables instead of eight variables. The dynamics of cytosolic PER protein (P_C) and

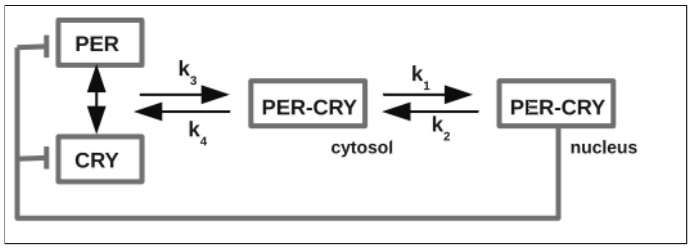

Figure 3: The 4 variable model in which the phosphorylation steps are omitted.

CRY protein (C_C) and cytosolic PER-CRY (PC_C) and nuclear PER-CRY (PC_N) is governed by the following system of four kinetic equations:

$$\frac{dP_C}{dt} = \frac{K^n}{K^n + PC_N^n} v_1 - k_3 P_C C_C + k_4 PC_C - kd_1 P_C \tag{9}$$

$$\frac{dC_C}{dt} = \frac{K^n}{K^n + PC_N^n} v_2 - k_3 P_C C_C + k_4 PC_C - kd_2 C_C \tag{10}$$

$$\frac{dPC_C}{dt} = k_3 P_C C_C - k_4 PC_C - k_1 PC_C + k_2 PC_N - kd_3 PC_C \tag{11}$$

$$\frac{dPC_N}{dt} = k_1 PC_C - k_2 PC_N - kd_4 PC_N \tag{12}$$

The next section is devoted to the investigation of the dynamics of the 4 variable model *via* numerical simulations and bifurcation diagram analysis. Most results were obtained using MATHEMATICA.

4 Analysing the 4-variable EDP model

Not surprisingly, the existence of the cycle depends on the degradation rate of the complex PER-CRY in the nucleus. Thus kd_4 is the control parameter. When all parameters are fixed except kd_4, we observe that a Hopf bifurcation takes place with disappearance of the limit cycle for kd_4 crossing some value. This implies that for a sufficiently small value of kd_4 there exists one limit cycle.

To study the stability of the equilibrium points we apply the Lyapunov's first (indirect) method [23]. If $kd_4 = 0.41$, the system (9-12) has one fixed point. The Jacobian matrix of the system (9-12) at the equilibrium point is

$$\begin{pmatrix} -0.277892 & -0.162354 & 0.06 & -11.1584 \\ -0.227892 & -0.212354 & 0.06 & -12.2743 \\ 0.227892 & 0.162354 & -0.19 & 0.06 \\ 0 & 0 & 0.08 & -0.16 - kd4 \end{pmatrix}$$

This matrix has 4 eigenvalues.

- Two of them are real eigenvalues with negative real part.
- The two others are complex conjugates and their common real parts:
  - are positive when the parameter kd_4 is less than the bifurcation value, which is close to 0.42 in our case (see the figure 4(a), 4(b) and 4(c))
  - and become negative when the parameter crosses this bifurcation value.

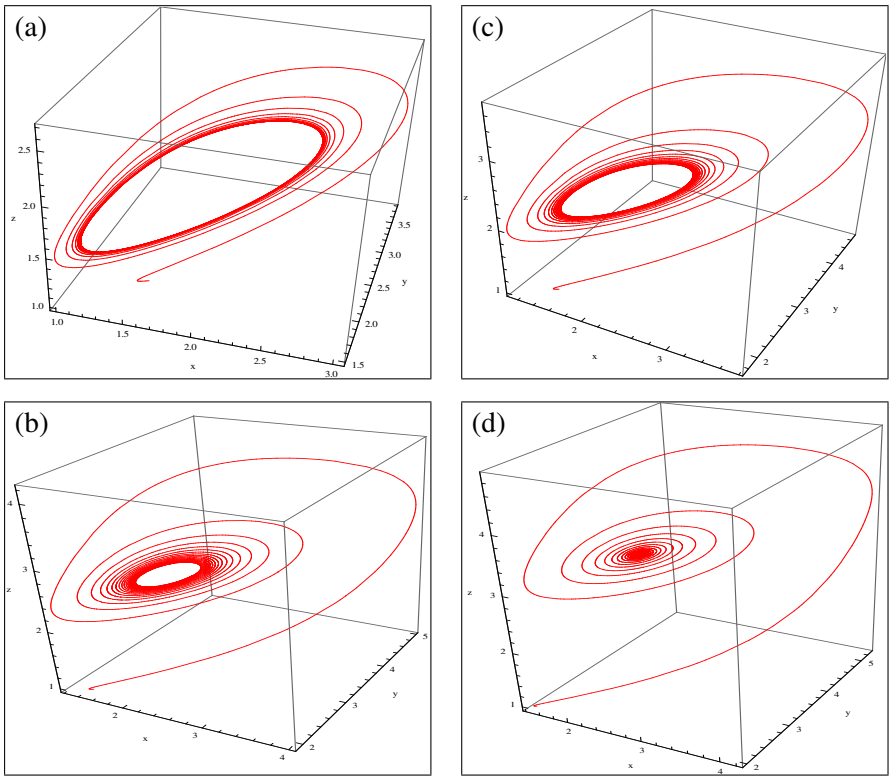

Figure 4: 3D projections of some phase trajectories. Common parameters are $K = 0.4$, $n = 15$, $v_1 = 2$, $v_2 = 2.2$, $k_1 = 0.08$, $k_2 = 0.06$, $k_3 = 0.08$, $k_4 = 0.06$, $kd_1 = 0.05$, $kd_2 = 0.05$ and $kd_3 = 0.05$, with $(1.5, 2, 1, 0.5)$ as initial condition. In (a), $kd_4 = 0.25$. In (b), $kd_4 = 0.35$. In (c), $kd_4 = 0.42$. In (d), $kd_4 = 0.55$. We observe a stable limit cycle for Figures (a), (b) and (c) only.

This computation can be made easily using the Routh-Hurwitz criterion [24] applied to the characteristic polynomial of the Jacobian matrix.

It is also possible to get the same bifurcation schema with kd_3 around 0.39, where kd_3 is the degradation rate of the complex PER-CRY in the cytosol.

Following Gérard, Gonze and Goldbeter in [25], we suspect that 3 variables are needed to observe the same bahaviour (this will be the object of a future research).

5 Simplified purely discrete model

The discrete modelling frameworks aim at providing a logical explanation of the observed behaviours from a qualitative point of view. For biological

networks, René Thomas [26] established a framework where the continuous phase space is partitioned into qualitative regions in such a way that, within a region, the action of a species on other species is qualitatively constant. Trajectories are then abstracted by transitions from a region to another one. René Thomas has defined this abstraction in such a way that it is consistent with the continuous framework [27].

5.1 Thomas' discrete modeling framework

To define a Thomas' model one has first to design an *interaction graph* whose nodes are most of the time the molecular species of the biological network under consideration, and whose edges are labelled with signs: "+" for activations and "–" for inhibitions. For example, similarly to model of Figure 3, the genes per and cry have a positive action on the complex PER-CRY in the cytosol, which in turn has a positive action on the complex PER-CRY in the nucleus, and lastly the former inhibits the genes per and cry, see Figure 5.

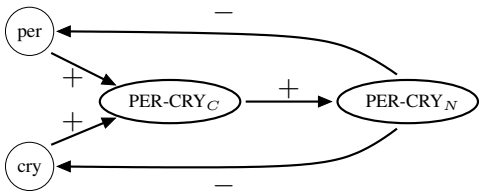

Figure 5: Thomas' model with 4 variables.

René Thomas has remarked that, when a gene x acts on a gene y, there is a threshold in the concentration space of x such that :

- if the concentration of x is below the threshold, x is unable to regulate y, and the regulation is inactive,
- if its concentration passes the threshold, x is able to regulate y and the regulation is active.

More generally, when x has an action on several targets $y_1, y_2 ... y_k$, there are k thresholds (one threshold associated each target). Then from a qualitative point of view, x can have $k+1$ different behaviours: either it does not regulate any target, either it regulates a unique target, either it regulates 2 targets, and so on. Thus, the concentration space of the variable x can be divided into $k+1$ intervals where k is the number of targets. These intervals are called *abstract levels* and are denoted by integers in $[0, k]$.

The qualitative dynamics of the network is then defined by the successive *states* of each variable, where a state is an interval number. Let us consider a variable y regulated by $x_1, x_2, ...x_n$. A qualitative state being given, there is a subset ω of $\{x_1, x_2, ...x_n\}$ such that $x_i \in \omega$ if and only if the qualitative state of x_i denotes an interval greater than the $x_i \rightarrow y$ threshold. According to René Thomas, the abstract level toward which y is attracted only depends on ω. Consequently, if y has n regulators there are 2^n different parameters for variable y, which we denote by $K_{y,\omega}$, one for each possible subset ω of regulators of y.

A model of a gene network is consequently defined by

- an interaction graph $G = (V, E)$ where edges are labelled with a sign and an integer (which is the number of the interval immediately after the threshold),
- the family of parameters $\{K_{y,\omega}\}$, y being any member of V, and ω being any subset of $G^-(y)$, the set of predecessors of y in the graph G.

A model of a gene network being given (in particular all parameter values are known), we associate a state graph:

- a state is an assignment s that associates to each variable of the graph an integer value representing a possible interval for this variable (see above),
- a transition $s \rightarrow s'$ from one state to another state is obtained by modifying only *one* variable assignment as follows:
  - the modified variable x must satisfy $s(x) \neq K_{x,\omega}$ where ω is the set of active regulators of x according to the state s,
  - if $s(x) < K_{x,\omega}$, then $s'(x) = s(x) + 1$ and $s'(y) = s(y)$ for all $y \neq x$,
  - if $s(x) > K_{x,\omega}$, then $s'(x) = s(x) - 1$ and $s'(y) = s(y)$ for all $y \neq x$.

Thus the dynamics of the model is *nondeterministic* and *asynchronous* because at a particular state, even if several variables are attracted towards a different value, only one variable can change simultaneously (because there is no reason that several variables reach their threshold exactly at the same time). Moreover variables never jump several thresholds as the discrete model is an abstraction of a continuous phenomenon.

5.2 Oversimplified circadian clock and introducing light influence

In first approximation the two negative cycles of Figure 5 are known to act roughly in the same time sustaining the circadian oscillation. Moreover the main role of per and cry is to produce the PER-CRY complex. it suggests that if we wish to simplify again, the next step is to amalgamate per and cry into an abstract "set of genes" that we denote G. Then we get a unique cycle with three nodes in which the only role of PER-CRY$_C$ is to "produce" PER-CRY$_N$. So we remove the node PER-CRY$_C$, leading to the model of Figure 6 (left) where PER-CRY$_N$ is abbreviated as PC.

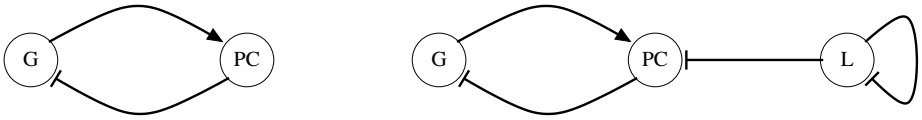

Figure 6: **(left)** A simplified model of the interactions in the mammalian circadian clock. **(right)** Adding the light variable in order to take into account the driving of the system by light/dark alternation.

Since light trains the rhythmicity of the mammalian circadian clock by inhibiting the transport of PER-CRY$_C$ into the nucleus, it has an inhibitor effect on complex PC in Figure 6 (right).

Moreover because of the natural alternance of day and night, the model has to be able to produce an oscillation of light. This is done by adding a fictitious auto-inhibition: light can be "on" or "off", when it is "on", it has an inhibitor effect on itself and light tends to go "off" and conversely.

5.3 Identifying the discrete parameters

The interaction graph being given, it remains many possible parameter values leading to a large number of possible models with different state graphs. Obviously, a parameterization is valid if the corresponding state graph is coherent with the biological knowledge about the dynamics of the system.

Let us first treat the parameters for the variable L (light). The fictitious feedback loop must generate oscillations, thus $K_{L,\{\}} = 1$ and $K_{L,\{L\}} = 0$ (otherwise no oscillation on L could be observed).

We also know that the circadian clock oscillates in constant darkness ($L = O$). So, the feedback loop $G \leftrightarrow PC$ generates oscillation and we must have $K_{G,\{\}} = 1$ and $K_{G,\{PC\}} = 0$ (otherwise no sustained oscillation on L could be observed).

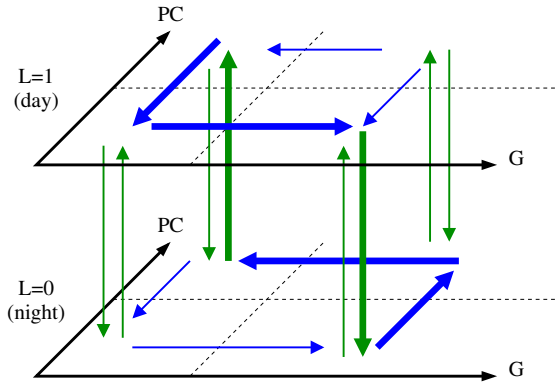

Figure 7: Qualitative dynamics of a simplified model.

It remains to identify the parameters associated with PC. Four situations have to be studied :

1. When G is off and light is on, there is no transcription of PC and existing complexes PER-CRY are kept outside the nucleus by light. Then PC (in the nucleus) cannot increase nor stay at 1. This property exactly means $K_{PC,\{L\}} = 0$.

2. When G is on and light is on, there is transcription of PER-CRY in the cytosol but light prevents these complexes to enter the nucleus. In other words $K_{PC,\{G,L\}} = 0$.

3. When G is off and light is off, there is no transcription of PER-CRY complexes in the cytosol and *a fortiori* they do not enter the nucleus, leading to $K_{PC,\{\}} = 0$.

4. When G is on and light is off, then PER-CRY complexes accumulate in the cytosol and in the absence of light, they can enter the nucleus. So, $K_{PC,\{G\}} = 1$.

The parameters being identified and following section 5.1, we build the state graph of Figure 7. Green transitions stand for alternation of days and nights whereas blue ones represent the evolution of genes G and complexes PC. Thick transitions sketch the common path for a regular day-night alternation; let us start from the state $(L, G, PC) = (1, 0, 1)$:

- During the day, complexes PC (in the nucleus) are first degradated (transition $(1, 0, 1) \rightarrow (1, 0, 0)$) and genes are transcripted (transition $(1, 0, 0) \rightarrow (1, 1, 0)$).

- Then night falls (transition $(1,1,0) \rightarrow (0,1,0)$).
- During the night, the concentration of complexes PC grows and becomes sufficient to inhibit G (transitions $(0,1,0) \rightarrow (0,1,1)$ and $(0,1,1) \rightarrow (0,0,1)$).
- At dawn, the transition $(0,0,1) \rightarrow (1,0,1)$ closes the circadian cycle.

Notice that, whatever the state, one can switch light *ad libitum*. This results from the negative auto-inhibition of L that, within the standard logical approach of René Thomas, cannot be temporized (all vertical transitions of Fig. 7).

6 Discrete model with delays

Clearly, a valuable model of the circadian clock needs to integrate chronometric information to predict behaviours which could usefully be confronted with experimental data. The idea developed in this section is to manually associate with each transition of the qualitative state graph, a delay which represents the time spent to go from a state to a neighbour state along a transition. This simple solution is made possible by the small number of transitions in our state graph. For bigger models more elaborated *hybrid* frameworks are required: Siebert and Bockmayr proposed an automaton product which allows to handle time [28], Batt and co-workers used verification tools on timed automata to learn about the possible qualitative behaviors of the network under a whole range of uncertain delay parameters [29]. We also proposed such sophisticated frameworks [30, 31, 32] where one of our motivations was to take into consideration small successive accumulations.

6.1 Labelling the state graph with delays

In Figure 8 we label each transition of the state graph with a delay. For example, if we consider that days and nights last the same duration, each of vertical transitions are labelled with 12 hours (others transitions require some more elaborated reasoning).

Let us first remark that in the setting of René Thomas, at any state and consequently at any time, it is impossible to have two opposite transitions. If a variable can evolve, then it can *either* increase or decrease. We consequently introduce a set of clocks, one clock per variable. When active, the clock of a given variable measures the time elapsed since that variable can increase (resp. decrease). Within one state, if there are several outgoing transitions, the next triggered transition will be the one which is associated with the variable whose clock reaches its delay.

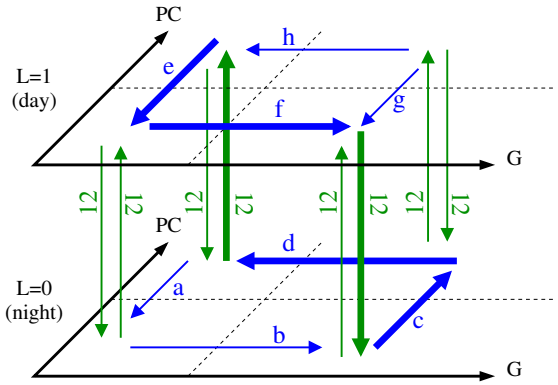

Figure 8: A qualitative model with delays remaining unknown

When a transition is fired, the clock of the involved variable, say x, is of course reset to 0. But some other clocks have also to be reset: When a variable is directly influenced by x, its possibility of variation may change, in which case the corresponding clock has also to be reset to 0.

6.2 Identification of delays for the mammalian circadian clock

The goal of this subsection is to identify the parameters a to g of Figure 8 using standard knowledge about the circadian cycle:

- During the night, the time necessary for firing transitions associated with delays c and d is equal to 12:

$$c + d = 12 \tag{13}$$

  A shorter sum would lead to trigger the transition labelled by a before the up light transition of the figure. Conversely a longer sum would lead to trigger the up light transition before the end of the transition labelled by d.

- The trajectory from $(L = 1, G = 0, PC = 1)$ to $(L = 1, G = 1, PC = 0)$ is performed during the day. Thus,

$$e + f \leq 12 \tag{14}$$

  A longer sum would lead to trigger the down light transition before the end of the transition labelled by f. On the contrary, a sum shorter than 12 hours will lead to the state $(l = 1, G = 1, PC = 0)$, which is stable in the plane $L = 1$ and consequently the trajectory will stay there, waiting

for the down light transition without modifying the discrete trajectory in the state graph.

- In constant night, the mammalian circadian cycle stabilizes around 24 hours, see [33, 34]. Thus,

$$a + b + c + d = 24 \tag{15}$$

- The transcription of genes G does not depend directly on the light L. Then

$$b = f \tag{16}$$

$$d = h \tag{17}$$

- When light is on, PC is inhibited. Thus a is notably smaller than e:

$$a > e \tag{18}$$

The resulting constraints (13-18) admit an infinity of solutions. The following delays satisfy these constraints and seem sensible with respect to current knowledge:

a	=	7	b	=	5	c	=	7	d	=	5
e	=	1	f	=	5	g	=	?	h	=	5

The delay g is not valuated. Indeed, for the purpose of this chapter, the interest of the transition labelled by g is only that it induces a stable state in the plane $L = 1$, whatever the actual value g.

6.3 Robustness to day length

The parameters values proposed in the previous subsection are remarkably robust to day length variations. The previous model imposes a constant and equal durations of nights and days. Indeed, it reflects the durations of nights and days during the periods of equinoxes. In the northern hemisphere at the latitude 50 degrees north, the duration of night increases up to 16 hours in the winter, see Figure 9-left, and it decreases down to 8 hours in the summer, see Figure 9-right.

To illustrate the clock manipulation in our dynamics, let us consider the winter model (left part of the figure).

1. Let us start from the beginning of the night with a state where $(L, G, PC) = (0, 1, 0)$ and all clocks are set to 0. Because $c = 7$ is smaller than 16, the next state is $(L, G, PC) = (0, 1, 1)$. The clock of PC is of course reset to 0 and the clock of G is also reset to 0 because PC now inhibits G. And it remains 9 hours for light to go up.

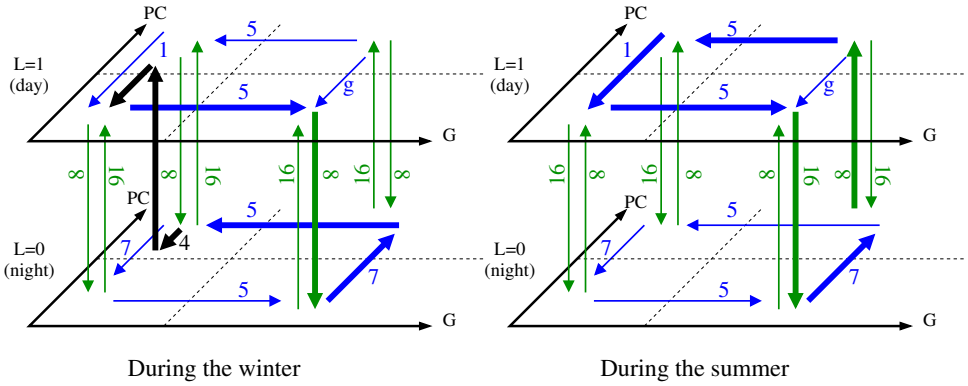

Figure 9: Influence of seasons on the qualitative model of the mammalian circadian cycle. **(left)** during the winter. **(right)** during the summer.

2. From $(0, 1, 1)$, because $d = 5$ is smaller than 9, the next state is $(0, 0, 1)$. The clock of G is reset to 0 and the clock of PC is also reset to 0 because G does not activate PC anymore. And it remains 4 hours for light to go up.

3. From $(0, 0, 1)$, because $a = 7$ is greater than 4, the next state is $(1, 0, 1)$. The clock of L is reset to 0 and the clock of PC is not reset to 0 because it continues to decrease. *However*, $e = 1$ and $a = 7$ and in first approximation we can consider that $\frac{4}{7}$ of PC have been degradated. Consequently, the clock for PC becomes $\frac{4 \times e}{a} = \frac{4}{7}$. It remains $\frac{3}{7}$ of an hour for PC to become equal to 0.

4. From $(1, 0, 1)$, because $\frac{3}{7}$ is smaller than 8, the next state is $(1, 0, 0)$. The clock of PC is reset to 0 and the clock of G is also reset to 0 because PC now activates G. And it remains $8 - \frac{3}{7} = 7 + \frac{4}{7}$ hours for light to go down.

5. From $(1, 0, 0)$, because $f = 5$ is smaller than $7 + \frac{4}{7}$, the next state is $(1, 1, 0)$. The clock of G is reset to 0 and the clock of PC is also reset to 0 because $(G, PC) = (1, 0)$ is a stable state in the plane $L = 1$ and consequently, PC does not decrease anymore. And it remains $2 + \frac{4}{7}$ hours for light to go down.

6. After $2 + \frac{4}{7}$ hours, we leave the state $(1, 1, 0)$ to go back to $(0, 1, 0)$. The clock of L is reset to 0, the clock of PC is also reset to 0, and the clock of G is equal to $2 + \frac{4}{7}$.

7. From $(0, 1, 0)$, because $c = 7$ is smaller than 16, the next state is $(0, 1, 1)$. The clock of PC is reset to 0 and the clock of G is also reset to 0; it remains 9 hours for light to go up and the circadian cycle is phased again.

The summer model behaves similarly (right part of the figure) leading to the observation that our model is robust to the alternance of seasons.

7 Discussion

The designer of a model in the context of genomics is often torn between two options: to elaborate a rich model reflecting as much biological knowledge as possible in a consistent way, or to design a simplified model dedicated to a given family of questions in order to study the main causalities at a coarse-grained scale. This chapter was inspired by the second philosophy.

This approach may induce drastic simplifications, however it provides chains of causalities in the broad outlines, which make it possible to apprehend a problem in its entirety. For example, it is frequently observed that a jet lag from East to West induces less disorders than travels from West to East. Indeed:

- When one travels from East to West, one increases the awakening time, thus one increases the time of exposure to light. In the simplified model of Figure 7, we observe that a longer stay in the upper states (where $L = 1$) does not change the trajectories because they reach the locally stable state $(L, G, PC) = (1, 1, 0)$, and when night comes, the cycle is immediately synchronised again.

- When one travels from West to East, one does not feel like sleeping, the model continuing its trajectory until the locally stable state $(L, G, PC) = (1, 1, 0)$. Since the full day duration is shorter, the night length ($L = 0$) is reduced. At night ($(L, G, PC) = (0, 1, 0)$), for a sufficiently significative jet lag, the system does not reach the state $(L, G, PC) = (0, 1, 1)$ before light switches on again. And when light switches on, the system is "prisoner" of the transition $(1, 1, 1) \to (1, 1, 0)$, unable to cross the states $(1, 0, 1)$ and $(1, 0, 0)$ and the circadian cycle is consequently strongly disturbed.

Of course, this explanation is crude. It nevertheless gives a good intuitive idea of the main causality mechanisms involved in jet lag.

More generally, reducing the number of variables may help establishing mathematical results, as illustrated in Section 4 where we have established the existence of a limit cycle. Many other global properties can be established, and, afterwards, studying how far the properties established on a simple model

can be propagated on a more detailed model, belongs to the scope of multilevel modelling.

References

[1] De Maria, E., Fages, F., Rizk, A., Soliman, S.: Design, optimization and predictions of a coupled model of the cell cycle, circadian clock, DNA repair system, irinotecan metabolism and exposure control under temporal logic constraints. Theor. Comput. Sci. **412**(21) (2011) 2108–2127

[2] Murray, J.: Mathematical Biology, I. An introduction. Springer (2002 (third edition))

[3] Thomas, R., d'Ari, R.: Biological Feedback. CRC Press (1990)

[4] Chabrier, N., Fages, F.: Symbolic model checking of biochemical networks. In: CMSB'03: Proceedings of the first workshop on Computational Methods in Systems Biology. Volume 2602 of Lecture Notes in Computer Science., Springer-Verlag (2003) 149–162

[5] Gillespie, D.: A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. J. Comput. Physics **22**(4) (1976) 403–434

[6] Leloup, J.C., Goldbeter, A.: Modeling the mammalian circadian clock: Sensitivity analysis and multiplicity of oscillatory mechanisms. J.T.B. **230** (2004) 541–562

[7] Forger, D., Peskin, C.: Stochastic simulation of the mammalian circadian clock. PNAS **102**(2) (2005) 321–324

[8] Reghunandanan, V., Reghunandanan, R.: Neurotransmitters of the sprachiasmatic nuclei. J. of Circadian Rhythms **4** (2006) 2

[9] Bell-Pedersen, D., Cassone, V., Earnest, D., Golden, S., Hardin, P., Thomas, T., Zoran, M.: Circadian rhythms for multiple oscillators: lessons from diverse organisms. Nat. Rev. Genet. **6**(7) (2005) 544–556

[10] Klein, D., Moore, R., Reppert, S.: Suprachiasmatic nucleus, the mind's clock. Oxford University Press (1991)

[11] Lesauter, J., Silver, R.: Output signals of the SCN. Chronobiol. Int. **15** (1998) 535–550

[12] Weaver, D.: The suprachiasmatic nucleus: a 25-year retrospective. J.Biol.Rhythms **13** (1998) 100–112

[13] Johnson, R., Moore, R., Morin, L.: Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. Brain Res. **460** (1988) 297–313

[14] Maywood, E., O'Neill, J., Reddy, A., Chesham, J., Prosser, H., Kyriacou, C., Godinho, S., Nolan, P., Hastings, M.: Genetic and molecular analysis of the central and peripheral circadian clockwork of mice. In: Cold Spring Harb Symp Quant Biol. Volume 72. (2007) 85–94

[15] Gallego, M., Virshup, D.: Post-transcriptional modifications regulate the ticking of the circadian clock. Nature Reviews Molecular Cell Biology **8** (2007) 139–148

[16] Sato, T., Panda, S., Miragia, L., Reyes, T., Rudic, R., McNamara, P., Naik, K., Fitzgerald, G., Kay, S., Hogenesch, J.: A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. Neuron. **43**(4) (2004) 527–37

[17] Belden, W., Loros, J., Dunlap, J.: CLOCK leaves its mark on histones. Trends Biochem Sci. **31**(11) (2006) 610–3

[18] Eide, E., Vielhaber, E., Hinz, W., Virshup, D.: The circadian regulatory proteins BMAL1 and cryptochromes are substrates of casein kinase Iε. J. Biol. Chem. **277**(19) (2002) 17248–54

[19] Froy, O., Miskin, E.: Effect of feeding regimens on circadian rhythms: implications for aging and longevity. Aging **2**(1) (2010) 7–27

[20] Albrecht, U. ande Bordon, A., Schmutz, I., Ripperger, J.: The multiple facets of Per2. Cold Spring Harb. Symp. Quant. Biol. **72** (2007) 95–104

[21] Maury, E., Moynihan Ramsey, K., Bass, J.: Circadian rhythms and metabolic syndrome: from experimental genetics to human disease. Circ. Res. **106** (2010) 447–462

[22] Vanselow, K., Kramer, A.: Role of phosphorylation in the mammalian circadian clock. Cold Spring Harb. Symp. Quant. Biol. **72** (2007) 167–76

[23] Lyapunov, A.: The general problem of the stability of motion. Taylor & Francis, London, UK (1992)

[24] Gradshteyn, I., Ryzhik, I.: Table of Integrals, Series and Products. Translation edited and with a preface by A.Jeffrey and D.Zwillinger, Academic Press, San Diego, Calif, USA, 6th edition (2000)

[25] Gérard, C., Gonze, D., Goldbeter, A.: Dependence of the period on the rate of protein degradation in minimal models for circadian oscillations. Phil. Trans. R. Soc. A **367** (2009) 4665–4683

[26] Thomas, R.: Logical analysis of systems comprising feedback loops. J. Theor. Biol. **73**(4) (1978) 631–56

[27] Snoussi, E.: Qualitative dynamics of a piecewise-linear differential equations : a discrete mapping approach. Dynamics and stability of Systems **4** (1989) 189–207

[28] Siebert, H., Bockmayr, A.: Incorporating time delays into the logical analysis of gene regulatory networks. In: CMSB. Volume 4210 of LNCS. (2006) 169–183

[29] Batt, G., Ben Salah, R., Maler, O.: On timed models of gene networks. In: FORMATS. Volume 4763 of LNCS., Springer (2007) 38–52

[30] Ahmad, J., Bernot, G., Comet, J.P., Lime, D., Roux, O.: Hybrid modelling and dynamical analysis of gene regulatory networks with delays. ComPlexUs **3**(4) (2007) 231–251

[31] Comet, J.P., Bernot, G.: Introducing continuous time in discrete models of gene regulatory networks. In: Proc. of the Nice Spring school on Modelling and simulation of biological processes in the context of genomics. EDP Sciences, ISBN : 978-2-7598-0545-7 (2010) 61–94

[32] Comet, J.P., Fromentin, J., Bernot, G., Roux, O.: A formal model for gene regulatory networks with time delays. In: CSBio'2010. Volume 115 of CCIS., Springer (2010) 1–13

[33] Oster, H., Yasui, A., van der Horst, G., Albrecht, U.: Disruption of mCry2 restores circadian rhythmicity in mPer2 mutant mice. Genes Dev. **16** (2002) 2633–2638

[34] Van der Horst, G., Muijtjens, M., Kobayashi, K., Takano, R., Kanno, S., Takao, M., de Wit, J., Verkerk, A., Eker, A., van Leenen, D., Buijs, R., Bootsma, D., Hoeijmakers, J., Yasui, A.: Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. Nature **398** (1999) 627–630

[35] Ahmad, J., Roux, O., Bernot, G., Comet, J.P., Richard, A.: Analysing formal models of genetic regulatory networks with delays: Applications to lambda phage and T-cell activation systems. Int. J. Bioinformatics Research and Applications **4**(3) (2008) 240–262

[36] Ahmad, J., Bernot, G., Comet, J.P., Lime, D., Roux, O.: Hybrid modelling and dynamical analysis of gene regulatory networks with delays. ComPlexUs **3**(4) (2006, Cover Date: November 2007) 231–251

Appendix

Theorem: Let $x = x^*$ be an equilibrium point of a nonlinear system:

$$\dot{x} = f(x),$$

where $f : D \rightarrow \mathcal{R}^n$ is continuously differentiable and $D \subset \mathcal{R}^n$ is the neighbourhood of the equilibrium point x^*. Let λ_i denote the eigenvalues of the Jacobian matrix $A = \frac{\delta f}{\delta x} \mid_{x^*}$ then the following are considered.

- If $Re\lambda_i < 0$ for all i, then $x = x^*$ is asymptotically stable.
- If $Re\lambda_i > 0$ for one or more i, then $x = x^*$ is unstable.
- If $Re\lambda_i \leqslant 0$ for all i and at least one $Re\lambda_j = 0$, then $x = x^*$ may be either stable, asymptotically stable, or unstable.

Since A is only defined at x^*, stability determined by the indirect method is restricted to infinitesimal neighborhoods of x^*.

To study the signs of the real parts of eigenvalues, we have the following criterion [24].

Routh-Hurwitz Criterion:
Given the polynomial $P(\lambda) = \lambda^n + a_1\lambda^{n-1} + \ldots + a_{n-1}\lambda + a_n$, where the

coefficients a_i, $i = 1, 2, \ldots, n$ are real constants, define the n Hurwitz matrices

$$
\begin{aligned}
H_1 &= \begin{bmatrix} a_1 \end{bmatrix}, \\
H_2 &= \begin{bmatrix} a_1 & 1 \\ a_3 & a_2 \end{bmatrix} \\
&\vdots \\
H_n &= \begin{bmatrix} a_1 & 1 & 0 & 0 & \cdots & 0 \\ a_3 & a_2 & a_1 & 1 & \cdots & 0 \\ a_5 & a_4 & a_3 & a_2 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \cdots & \vdots \\ 0 & 0 & 0 & 0 & 0 & a_n \end{bmatrix},
\end{aligned}
$$

where $a_i = 0$ if $i > n$.

All of the roots of the polynomial have negative real part if and only if the determinats of all Hurwitz matrices are positive: $detH_i > 0, i = 1, 2, \ldots, n$.

Routh-Hurwitz criteria for $n = 3$ are $a_1 > 0, a_3 > 0$ and $a_1 a_2 - a_3 > 0$ and for $n = 4$ are $a_1 > 0, a_2 > 0, a_3 > 0, a_4 > 0$ and $a_1 a_2 a_3 a_4 - a_1^2 a_4^2 - a_3^2 a_4 > 0$.