MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Hopf bifurcations and delayed feedback mechanisms in mathematical models of gene expression with periodic behaviour“

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Education (MEd)

Wien, 2023 / Vienna, 2023

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	UA 199 520 529 02
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Lehramt Sek (AB) Unterrichtsfach Mathematik Unterrichtsfach Spanisch
Betreut von / Supervisor:	Univ.-Prof. Dr. Christian Schmeiser

Acknowledgements

First, I want to thank my supervisor Christian Schmeiser. He allowed me the possibility to write about a topic I am really interested in and trusted in me that I could. During all these months, his door was always open when I needed him to help me out and I very much appreciated our conversations, which made me gain valuable new perspectives.

Furthermore, I want to wholeheartedly thank my family. My parents are the ones who make everything possible and I am deeply grateful that they support all my decisions, whatever it takes. I also want to thank my brothers: Florian, who shows me how to enjoy life while still mastering it, and Jakob, my reliable partner in crime. Moreover, I am genuinely grateful for my loving and cheering grandparents who always bring a smile on my face.

In addition to my family, I would not want to miss the amazing colleagues and friends who made these years so special. I want to highlight Jojo, as we have stood by each other's side for years, no matter the situation. Also, thanks to her and Kerstin, even bad days (and sentences) have turned into good ones lately. Furthermore, I am very grateful for the special connection with Julia and the conversations and moments we share. Last but not least, I want to thank Julian for everything I have learned and continue to learn with him.

Abstract

In this Master's thesis, two simple mathematical models of gene expression in the context of circular processes (circadian rhythms and the cell cycle) are examined. Both include delay differential equations and feedback mechanisms. The main questions addressed are whether the models reproduce periodic behaviour of the simplified biological system and which mathematical elements are decisive when they do. First, a model of a single periodically expressed gene which has been taken from the literature is analysed. Here, delayed feedback mechanisms as well as Hopf bifurcations at destabilised equilibrium points can be highlighted as key properties for periodic model behaviour. Second, a model of the alternation of two gene products during the cell cycle is constructed by adapting the telegraph model. In particular, delay parameters and a factor to limit the increase in gene product concentration are introduced. A mathematical analysis including linearisation about a nonzero equilibrium point, stability and bifurcation analysis indicates the occurrence of a Hopf bifurcation, but under conditions for parameters which cannot be fulfilled. After refinement, the model shows the expected periodic behaviour in numerical simulations carried out with Wolfram Mathematica.

Zusammenfassung

In der vorliegenden Masterarbeit werden zwei einfache Modelle von Genexpression im Kontext von zirkulären Prozessen (zirkadiane Rhythmen und Zellzyklus) untersucht. Beide Modelle beinhalten retardierte Differentialgleichungen sowie Feedbackmechanismen. Die behandelten Fragen sind, ob diese Modelle periodisches Verhalten der vereinfachten biologischen Systeme abbilden können und welche mathematischen Elemente dabei wesentlich sind. Zunächst wird ein Modell eines einzelnen periodisch exprimierten Gens aus der Literatur analysiert. Retardierte Feedbackmechanismen sowie Hopf-Bifurkationen bei destabilisierten Fixpunkten kristallisieren sich dabei als Schlüsselelemente für periodisches Verhalten heraus. In weiterer Folge wird ein Modell von der abwechselnden Exprimierung zweier Gene im Laufe des Zellzyklus aufgestellt und analysiert. Dabei wird ein Teil des Telegraph-Modells um Verzögerungsparameter und einen Faktor zum Limitieren der Genprodukt-Konzentrationen ergänzt. Die Analyse beinhaltet die Linearisierung um einen Gleichgewichtspunkt sowie eine Stabilitäts- und Bifurkationsanalyse. Die Ergebnisse deuten auf eine Hopf-Bifurkation hin, allerdings nur unter nicht erfüllbaren Bedingungen für die Parameter des Modells. Daher wurde das Modell weiterentwickelt, sodass es letztendlich das erwartete periodische Verhalten in den Simulationen, die mithilfe von Wolfram Mathematica erstellt werden, zeigt.

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Introduction

There are numerous aspects of gene expression in eukaryotic cells which are researched extensively. This does not come as a surprise, given that well-regulated gene expression is critical for any living organism. Malfunctions are regarded as causes of various diseases, among them cancer and neurodegenerative diseases (see e.g. [27], [14], [17] for reference). More detailed knowledge of the origins of diseases are, at best, concomitant with progress in therapy and drug development [27].

These major challenges require diverse perspectives, which is to say that different disciplines can complement each other in many beneficial ways. For instance, mathematical and experimental approaches can stimulate each other and, thereby, contribute to the generation of knowledge [11, p. 3]. This is what the present thesis aims to do, as it is located at the interface of biological and mathematical research. In particular, simple mathematical models of gene expression in the context of circular processes (i.e. circadian rhythms and the cell cycle) are the main objects of analysis. Both models of interest are intended to reproduce the (simplified) expression dynamics of periodically expressed genes. Consequently, the central questions addressed in this thesis are whether the periodic behaviour of the modelled system (i.e. the periodic expression of one or more genes) is reflected in the model behaviour and if so, why. Therefore, the aim of this thesis is to examine the mathematical elements which are substantial for periodic model behaviour in the sense of self-sustaining oscillations.

The models dealt with comprise one from the literature – the so-called *circadian pacemaker model* proposed in [23] – and one self-constructed. While the first model shows periodic behaviour and therefore reproduces the oscillating expression of a single gene, the newly developed one still requires further research so as to find out if and under which conditions its behaviour is periodic. Importantly, in the circadian

pacemaker model, the expression is regulated via some delayed negative feedback mechanism. The self-constructed model also includes delayed feedback mechanisms and an adaption of the gene expression model by Raj, Peskin, Tranchina et al. [32], i.e. the two-state telegraph model [28, p. 1281]. In a nutshell, the model demonstrates the succession of genes expressed in the course of the cell cycle.

In the first two chapters of this thesis, background information will be provided. Crucial terms and concepts for the subsequent considerations and analysis will be introduced with respect to both fields, biology and mathematics. Chapter 3 will be dedicated to the circadian pacemaker model as proposed by Lema, Golombek and Echave [23]. The analysis will be performed following the ideas of [29] and adaptions thereof in [35]. The aim of this chapter is to gain an insight into some of the determinant mathematical properties of periodic model behaviour. The conclusions drawn will further lead to the construction of a new model in chapter 4. Detailed analysis will be carried out analogously to the previously discussed model. Furthermore, Wolfram Mathematica will be used for numerical simulations. In the last chapter, the findings will be revisited and interpreted in view of the content discussed.

Chapter 1

Theoretical background

As a basis for the analysis and construction of mathematical models of gene expression, an introduction to the underlying biological matter is provided below.

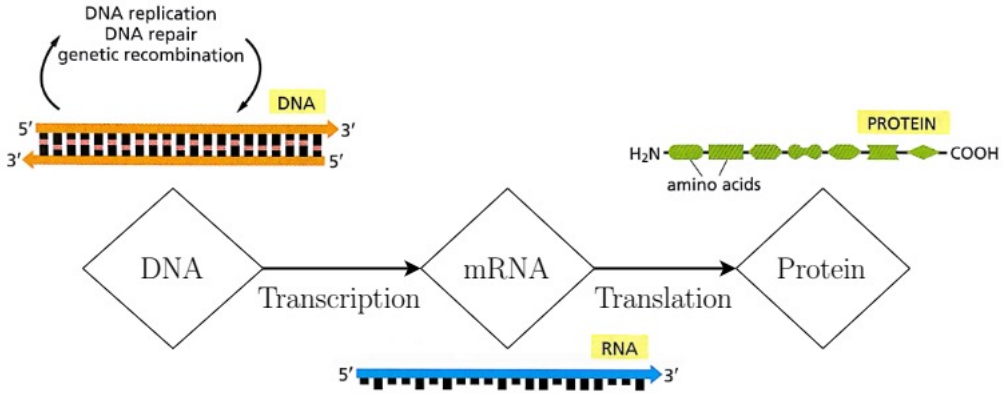

Figure 1.1. The central dogma of molecular biology. DNA fragments (genes) serve as templates for RNA synthesis, which is also called *transcription*. mRNAs (short for *messenger RNAs*) are RNA molecules which "code for proteins" [1, p. 305]. In the process of *translation*, proteins are synthesised based on the information contained in the corresponding mRNAs. (Fig. adapted from Fig 6-1 of [1])

1.1 Gene expression

First and foremost, what is gene expression? Genes are stretches of DNA (short for deoxyribonucleic acid) which contain the building plans for RNAs (ribonucleic acids) and further proteins. Gene expression refers to the realisation of the building plan, i.e. the synthesis of gene products (RNAs and proteins). This process, in

which numerous molecules and reactions are involved, is ubiquitous and crucial for all living organisms. [1, p. 4] Consequently, it is subject of the so-called *central dogma of molecular biology* (see Fig. 1.1). It must be emphasized that the central dogma reflects the fundamental structure common to all living cells. Nevertheless, there are multiple processes (e.g. diverse processing steps) which open up the possibility of modification. Therefore, gene expression varies in different organisms and cells. [1, p. 299] Another major cause for variation in gene expression – even in very similar cells – has been thoroughly investigated recently: *transcriptional bursting*.

1.2 Transcriptional bursting and regulation

In recent years, monitoring of transcription in single cells (instead of heterogeneous ensembles of cells) has become feasible [18, p. 21399]. Thanks to these new perspectives, it has become evident that transcription, i.e. RNA synthesis, usually does not occur in a continuous but rather in a *bursting* fashion. In other words, genes are expressed at a rather high rate for brief time spans (i.e. *in bursts*) and then not at all for a certain (longer) period. [25, p. 365]

The causes of transcriptional bursting are being investigated by various disciplines, involving diverse disciplines such as molecular biology and mathematics [28, p. 1281]. Some of the main factors which have been identified include:

- **Regulatory elements on the DNA strand:** The region around (especially before) the transcribed gene is crucial for transcription. Many regulatory elements (so-called *promoters* and *enhancers*) have been identified, not only in close proximity to the transcribed gene, but also surprisingly far away from it. There is a lot of evidence for the central role of these elements in transcription regulation and bursting dynamics, e.g. in [21].
- **Availability of essential proteins for transcription:** The transcription of a gene requires multiple proteins which do not only have to be present in the cell but also at the very location of transcription, i.e. in the nucleus and in close proximity to the transcribed gene. Additionally, the affinity of certain DNA regions to DNA-binding factors involved in transcription influences when and how fast transcription takes place. [28, p. 1283]
- **Storage of the DNA in a cell:** DNA is a very long molecule (human DNA reaches about 2 metres) and must be stored in the cell nucleus (diameter ap-

proximately $6 \cdot 10^{-6}$ metres). As a consequence, very efficient compaction and packaging is of utmost importance and requires specialised proteins (most notably, so-called *histones*). Despite the DNA being highly compacted, certain regions remain accessible for transcription and other essential processes like DNA replication and repair. [1, p. 179] The accessibility of a region as well as the composition of histones (which can both change rapidly) have an impact on transcription dynamics [28, p. 1283].

- **Other structural aspects:** Closely related to the previous factor are structural elements of the DNA such as DNA loops, which are required for transcription [28, p. 1283].

A detailed depiction of determining factors of transcriptional bursting as well as a review of the experimental support can be found in [28]. Another aspect which greatly influences transcription levels is the state of the cell cycle a cell is in.

1.3 The cell cycle

In order to *proliferate*, cells go through phases of growing and dividing, which has an impact on transcription and its regulation. For instance, the packing of the DNA is more compact in certain stages of the cell cycle and less compact in others. As mentioned in the previous section, this can be a decisive factor for transcriptional dynamics.

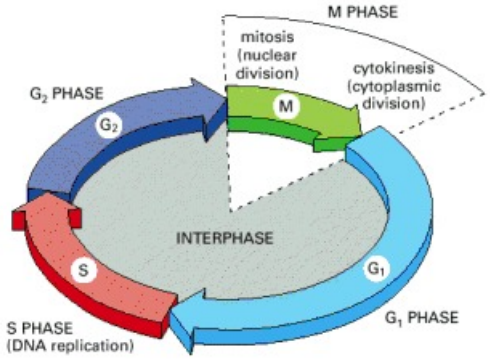

Figure 1.2. Schematic overview of the cell cycle. Mitosis (M) and S Phase (S) are separated by the gap phases G_1 and G_2. (Fig. 17-3 from [2])

Fig. 1.2 shows the phases of the cell cycle. What happens is that cells replicate, i.e.

duplicate, their DNA in S phase and divide in mitosis, forming two daughter cells with identical DNA. During the gap phases, cells can

a) grow (especially proteins and organelles must be provided sufficiently for both future daughter cells) and

b) monitor whether the necessary (internal and external) conditions for mitosis or DNA replication are met. [1, pp. 964-965]

A lot of specialised proteins are needed to ensure faultless duplication of DNA as well as precise cell division. Researchers are still in the process of uncovering how the transcription of the corresponding genes is regulated and at which point of the cell cycle it happens (see e.g. [13] for a review of recent findings). In general, the transgression through the cell cycle must be tightly regulated, since any irregularity might be detrimental to the cell affected. As in gene expression, it must be emphasised that deficiencies in cell-cycle regulation can have severe consequences for the organism. One of many examples is, again, cancer [13, p. 1009], [9, p. 947].

In this thesis, the cell cycle is considered a circular, recurrent process, in the context of which gene expression happens. We will simplify this tightly regulated biological system and assume that genes are repeatedly expressed whenever a cell reaches a (sometimes more and sometimes less exactly) determined point of the cell cycle. While some genes are expressed more often during a single cycle, others are expressed with lower frequency. In any case, a certain periodicity of a gene's expression will be assumed due to the recurrent cell cycles an actively proliferating cell goes through. Based on these ideas, we will construct a model in chapter 4. The following overview on mathematical modelling provides some background information.

1.4 Mathematical modelling of gene expression

The advantage of mathematical models is that even very complex networks can be represented precisely [8, pp. 68-69]. Therefore, it is not surprising that there is a plethora of mathematical models of gene expression, which differ considerably in their aims and objectives. A very basic distinction can be drawn between *forward* and *backward* modelling. In forward modelling, a model is designed based on experiments and observations, which could for example be observed reaction rates. In doing so, the principal objective is to obtain a model which can predict the behaviour of a

system based on observable quantities. Thus, the performance of these so-called *predictive* models is usually evaluated as to whether they reproduce observed behaviour. To name only one example, the model proposed by Cao and Grima predicts mRNA distributions, which are compared to experimental data [6]. When modelling backwards, modellers try to fine-tune a model which they know displays a certain desired behaviour. The aim of such *descriptive* models is to match them to observed behaviour. The approach in [19] is an example for this kind of modelling, as models used in queuing theory are fit to transcriptional bursting dynamics. Combinations of forward and backward modelling are, by all means, possible and can be beneficial. Furthermore, models of gene expression can vary considerably in the level of detail they contain [5, pp. 6-7] and with respect to their mathematical features. Fundamental distinctions include those of discrete (e.g. Boolean) and continuous (e.g. differential equation-based) models as well as deterministic and stochastic models. The latter aim to examine the effect of noise on the behaviour of modelled systems [4, p. 138], whereas deterministic models lack randomness and, therefore, have the same output unless the input changes [37].

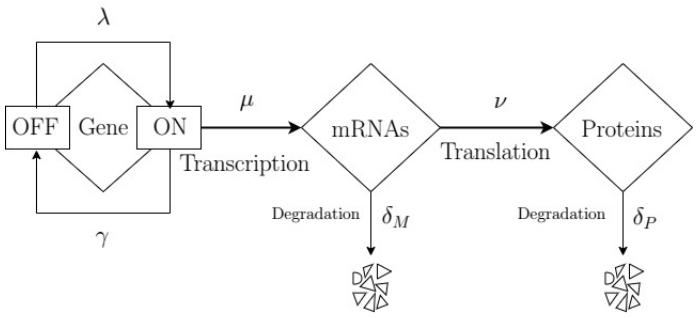

Figure 1.3. Schematic depiction of the telegraph model. Constant rates are indicated with Greek letters following [32] (δ_M adapted, ν and δ_P added). The expressed gene can switch from an active ("ON") to an inactive ("OFF") state and *vice versa* at the rates γ and λ, respectively. In case of the active state, mRNAs are produced at the rate μ. Furthermore, proteins are synthesised at the rate ν. mRNAs and proteins are degraded at the rates δ_M and δ_P, respectively.

As mentioned above, mathematical models of gene expression are extremely abundant. Various approaches with different focuses have been published, but many of them refer to the *telegraph model*. According to Cao and Grima, this model is the "standard model of stochastic mRNA dynamics in eukaryotic cells" [6, p.4682]. It comprises a gene which switches (stochastically) between an active ("ON") and an

inactive ("OFF") state and, in the former case, mRNA is synthesised at a constant rate. The mRNA molecules are further degraded at another constant rate. Moreover, protein synthesis (translation of the mRNA molecules) and protein degradation, with both of them being at constant rates are represented in the model (see Fig. 1.3) [28, p. 1281]. The telegraph model is also called *three-state model of gene expression* or *two-state model* if only mRNA synthesis and degradation are considered. Since we will focus on transcription in the present thesis, the model which is proposed in chapter 4 will be based on the two-state model of Raj, Peskin, Tranchina et al. [32].

The telegraph model clearly reflects the processes of the *central dogma of molecular biology* (see section 1.1), which makes it a general and adaptable structure for mathematical models. For example, the model from Cao and Grima mentioned above is an extension of the two-state model and aims at "time-dependent distributions of mRNA and protein numbers" [6, p.4682]. In general, the telegraph model yields what is called a *master equation*, which is a "set of stochastic differential equations". The solution to this system (in the case of the two-state model) is a probability distribution describing transcription in as much detail as for single molecules. [20, p. 635]

In this thesis, the objectives and assumptions require a different approach. Since we consider the moment of synthesis of a certain gene product a rather fixed moment in the cell cycle, we will not deal with stochastic differential equations. To compensate for the resulting loss of the stochastic element, which is closely linked to the bursting fashion of transcription, delay differential equations will be used. The time between the stimulus of transcription and its end product will be reflected in the delay introduced in the synthesis terms of the equations. Finally, the last section of this introduction is dedicated to another crucial element of the examined models: feedback mechanisms.

1.5 Negative feedback loops

Negative as well as positive feedback is essential for many regulatory mechanisms in cells. Negative feedback refers to any component inhibiting its own synthesis, possibly via the regulation of other components. For instance, protein A can enhance the synthesis of a repressor protein which then inhibits the synthesis of protein A. This

is a simple example of a so-called *negative feedback loop*. If the feedback becomes effective only after a certain delay – leading to a *delayed negative feedback loop* – this can result in periodic behaviour of the system. In fact, many oscillations observed in biological systems stem back to negative feedback loops. [1, pp. 515-516]

As we aim to observe periodic behaviour of models of periodically expressed genes, delayed negative feedback loops will be an important aspect. Before the analysis of the circadian pacemaker model will shed light on the mathematical consequences of delayed negative feedback, the tools needed will be provided in the following chapter.

Chapter 2

Preliminaries

In order to be able to properly examine the models of interest, it is necessary to discuss some central definitions and theorems. The introduction of key terms below follows the section "basic concepts" of [7]. With regard to notation, it must be mentioned that any variable (or spacial vector) with a dot on top refers to the first derivative of the variable (or vector) with respect to the time t.

First of all, let

$$\begin{aligned}
\dot{x}_1 &= f_1(x_1, x_2, \ldots, x_n, t) \\
\dot{x}_2 &= f_2(x_1, x_2, \ldots, x_n, t) \\
&\vdots \\
\dot{x}_n &= f_n(x_1, x_2, \ldots, x_n, t)
\end{aligned}$$

be a system of ordinary differential equations (ODEs). A special case thereof are *linear systems of ordinary differential equations* of the form

$$\begin{aligned}
\dot{x}_1 &= a_{10} + a_{11}x_1 + a_{12}x_2 + \cdots + a_{1n}x_n \\
\dot{x}_2 &= a_{20} + a_{21}x_1 + a_{22}x_2 + \cdots + a_{2n}x_n \\
&\vdots \\
\dot{x}_n &= a_{n0} + a_{n1}x_1 + a_{n2}x_2 + \cdots + a_{nn}x_n
\end{aligned}$$

with constant coefficients $a_{ij} \in \mathbb{R}$. In the case of $a_{i0} = 0$ for all $i \in \{1, 2, \ldots, n\}$, the linear system is *homogeneous* (else, *non-homogeneous*).

An equivalent notation of a linear homogeneous system is to be mentioned, namely, $\dot{X} = AX$ with

$$X := [1, x_1, x_2, \ldots, x_n]^{\mathrm{T}}$$

and the so-called coefficient matrix

$$A := \begin{pmatrix} a_{10} & a_{11} & a_{12} & \cdots & a_{1n} \\ a_{20} & a_{21} & a_{22} & \cdots & a_{2n} \\ \vdots & \vdots & \vdots & \cdots & \vdots \\ a_{n0} & a_{n1} & a_{n2} & \cdots & a_{nn} \end{pmatrix}.$$

The vectorised notation is also used for nonlinear systems of ODEs: $\dot{X} = f(X, t)$.

How a system changes (i.e. $\dot{X}$) depends on some *system state* X [7, p. 3]. More precisely, the changing of the system is determined by one particular system state and the time. This characteristic of ODEs can impose limitations on their applications, which is why other types of differential equations can be convenient in modelling as well. While partial differential equations (PDEs) are probably best known, the role of delay differential equations (DDEs) cannot be ignored in several fields of application such as the life sciences. DDEs can help to overcome the above-mentioned constraints, as $\dot{X}$ does not only depend on one single, current system state $X(t)$, but on any number $n \in \mathbb{N}$ of past states $X(t - \tau_i)$ for $i \in \{1, \ldots, n\}$. Hence, we obtain:

$$\dot{X} = f(X(t), X(t - \tau_1), \ldots, X(t - \tau_n), t),$$

a *a system of DDEs*, consisting of single DDEs in the form of

$$\dot{x} = f(x(t), x(t - \tau_1), \ldots, x(t - \tau_n), t).$$

DDEs have particular characteristics, which can differ markedly from those of ODEs. Nevertheless, solving differential equations analytically can be hard if not impossible for both. In addition, explicit solutions might not be meaningful when interpreted in the biological context. This can be particularly problematic for models based on "rough qualitative ideas about the biological laws" like those dealt with in this thesis. [5, p. 29] Therefore, we rather take interest in the qualitative behaviour of the model, which refers to properties like increasing, periodic or asymptotic. Various mathematical tools are available for the analysis of model behaviour, amongst them linearisation.

2.1 Equilibrium points and linearisation of nonlinear systems

An exact analytic solution of a system of differential equations contains all the information about a system's behaviour. For nonlinear systems, which we usually cannot solve explicitly, we can alternatively examine the local behaviour at different points of interest. Linearising a nonlinear system means approximating it through a linear system at a certain point. Thereby, a well-considered choice of these points is crucial. Usually, equilibrium points of systems are of particular interest. [5, p. 37]

Definition 2.1.1 (Equilibrium point of a nonlinear system)**.** Let $\dot{X} = f(X,t)$ be a nonlinear system of ordinary differential equations. A point $\bar{X} \in \mathbb{R}^n$ at $t = \bar{t}$ is an *equilibrium point* of the system, if $f(\bar{X}, \bar{t}\,) = 0$ [30, p. 102].

Remarks.

1. The definition of an equilibrium point is applicable to nonlinear systems with delay differential equations as well.
2. Besides *equilibrium point*, alternative terms like *fixed point* are commonly used as well.

In order to keep things simple and clear, we will hereafter consider a nonlinear system of two differential equations:

$$\begin{aligned} \dot{x} &= f_1(x,y) \\ \dot{y} &= f_2(x,y). \end{aligned} \tag{2.1}$$

Following [36], we will linearise system (2.1) about an equilibrium point $(\bar{x}, \bar{y})$ of the system. Note that this implies $f_1(\bar{x}, \bar{y}) = f_2(\bar{x}, \bar{y}) = 0$. Now, let

$$u(t) := x(t) - \bar{x} \text{ and} \tag{2.2}$$

$$v(t) := y(t) - \bar{y} \tag{2.3}$$

be components of small disturbances from the equilibrium point $(\bar{x}, \bar{y})$. As we examine the close proximity of $(\bar{x}, \bar{y})$ only, x and y are considered to be reasonably close to $\bar{x}$ and $\bar{y}$, respectively. Thus, u and v are small. In order to analyse the behaviour of system (2.1) around the equilibrium point, we can examine the behaviour of the perturbations u and v.

Note that $\bar{x}$ and $\bar{y}$ are constants, hence,

$$\dot{u} = \dot{x} = f_1(\bar{x} + u, \bar{y} + v) \tag{2.4}$$

$$\dot{v} = \dot{y} = f_2(\bar{x} + u, \bar{y} + v). \tag{2.5}$$

Taylor series expansion around the equilibrium point $(\bar{x}, \bar{y})$ yields

$$\dot{u} = f_1(\bar{x}, \bar{y}) + \frac{\partial f_1}{\partial x}(\bar{x}, \bar{y})\ u + \frac{\partial f_1}{\partial y}(\bar{x}, \bar{y})\ v + O(u^2, v^2, uv). \tag{2.6}$$

Since u and v are small, the quadratic terms are negligibly small. Furthermore, $f_1(\bar{x}, \bar{y}) = 0$ by definition. Thus, we can simplify:

$$\dot{u} \approx \frac{\partial f_1}{\partial x}(\bar{x}, \bar{y})\ u + \frac{\partial f_1}{\partial y}(\bar{x}, \bar{y})\ v. \tag{2.7}$$

Analogously,

$$\dot{v} \approx \frac{\partial f_2}{\partial x}(\bar{x}, \bar{y})\ u + \frac{\partial f_2}{\partial y}(\bar{x}, \bar{y})\ v. \tag{2.8}$$

We can, therefore, describe the behaviour of the disturbances u and v as follows:

$$\begin{pmatrix} \dot{u} \\ \dot{v} \end{pmatrix} \approx \begin{pmatrix} \frac{\partial f_1}{\partial x} & \frac{\partial f_1}{\partial y} \\ \frac{\partial f_2}{\partial x} & \frac{\partial f_2}{\partial y} \end{pmatrix} \begin{pmatrix} u \\ v \end{pmatrix} \tag{2.9}$$

It is important to note that we evaluate the derivatives at the equilibrium point $(\bar{x}, \bar{y})$. Thus, the components of the 2×2 matrix in (2.9) are real numbers. The matrix is called *Jacobian matrix* and is analogous to the derivative of functions with only one variable. [36, p. 151-152]

In conclusion, we can define:

Definition 2.1.2 (Linearisation of a nonlinear system about an equilibrium point)**.** Let $\dot{X} = f(X, t)$ be a nonlinear system of two differential equations as depicted in system (2.1) and let $(\bar{x}, \bar{y})$ be an equilibrium point of this system. Furthermore, let u, v be small disturbances from $(\bar{x}, \bar{y})$ as defined in equations (2.2) and (2.3). Then,

$$\begin{pmatrix} \dot{x} \\ \dot{y} \end{pmatrix} := \begin{pmatrix} \frac{\partial f}{\partial x} & \frac{\partial f}{\partial y} \\ \frac{\partial g}{\partial x} & \frac{\partial g}{\partial y} \end{pmatrix} (\bar{u}, \bar{v}) \ \begin{pmatrix} u \\ v \end{pmatrix} \tag{2.10}$$

is called a *linearisation of the system about the equilibrium point* $(\bar{x}, \bar{y})$.

Remarks.

1. Basically, a system of DDEs can be linearised likewise by replacing t by $t - \tau$ wherever necessary.

2. Note that (2.10) is a homogeneous linear system.

At this point, a general property of homogeneous linear systems shall be mentioned.

Lemma 2.1.1. Let

$$\begin{pmatrix} y_1 \\ y_2 \end{pmatrix} := \begin{pmatrix} a & b \\ c & d \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix}$$

be a linear system with coefficient matrix A. If the determinant of A is zero, i.e. $\det(A) = ac - bd = 0$, the system has infinitely many solutions. If $\det(A) \neq 0$, the system has only one solution.

Proof. According to [15, p. 30], a homogeneous linear system has exactly one solution iff its coefficient matrix A is nonsingular, and it has infinitely many solutions iff A is singular. Further, A is nonsingular iff $\det A \neq 0$, which yields the claim [15, p. 326]. □

2.2 Equilibrium point, equilibrium, steady state

Because of their frequent use in various disciplines and contexts, the terms *equilibrium point*, *equilibrium* and *steady state* are likely to be confused. Therefore, we need to be aware of differences in meaning and use the terms accordingly. We have already defined an equilibrium point of a nonlinear system in Definition 2.1.1. An equilibrium point in the mathematical sense is not necessarily an *equilibrium* in a biological or chemical system. In physicochemical terms,

> "a thermodynamic system is in thermodynamic equilibrium when all its state variables remain constant with time: there is no net flow of matter or energy, no phase changes, and no unbalanced potentials (or driving forces) within the system" [10, p. 2].

Thus, if a biological or chemical system is in equilibrium at a time t_{eq}, the system will remain in this exact state for any $t \geq t_{eq}$. Clearly, equilibrium conditions are highly unlikely to be found in living biological systems [31, p. 15063]. Consequently,

the term *steady state* seems to be more appropriate for a biological context. Strict and, therefore, unrealistic conditions for a steady state can be formulated as follows:

> "In the steady state, the concentrations of intermediates stay the same while the concentrations of starting materials and products are changing" [24, p. 1].

In other words, in a steady state, reactions are taking place consistently, while some conditions in the system might change. As a result, the system could either evolve towards another state or stay in one steady state. Thus, a steady state can be an equilibrium (if the system stays in one steady state forever), but not vice versa. An equilibrium point as defined mathematically can be stable or unstable in quite the same sense: The nonlinear system can either stay in the state forever (if the equilibrium point is stable) or its state might change at some point (if the equilibrium point is unstable). Therefore, we can connect and define:

Definition 2.2.1 (Steady state)**.** Consider a biological system modelled using a nonlinear system of differential equations. We assume a *steady state* of the biological system at an equilibrium point of the nonlinear system.

2.3 Stability of equilibrium points

Linking back to what has been discussed before, an equilibrium point of a nonlinear system can either be stable or unstable. The former can be interpreted as the system reaching a steady state and staying there, while the latter describes a temporarily limited steady-state behaviour of the system followed by some different (possibly as well steady-state) behaviour. More precisely, particular types of stability can be distinguished. One example is *asymptotic stability*, which corresponds to an equilibrium behaviour: "every solution whose initial value is sufficiently close to the equilibrium remains close to the equilibrium and approaches it as $k \to \infty$" [5, p. 39]. There is a variety of conditions for (asymptotic) stability, which could be discussed at this point. However, only one exemplary condition shall be mentioned, as it will be applied later:

Lemma 2.3.1. Let $\dot{X} := AX$ be a linear system with coefficient matrix A. If every eigenvalue of A has a negative real part, the system is asymptotically stable.

Proof. This lemma is commonly used and often stated without proof. However, Ramponi provides one based on Lyapunov's theorem [33, p. 5], and an illustrative proof by Lebovitz can be found in [22, p. 10]. □

This lemma allows us to draw conclusions about the stability of an equilibrium point by means of the eigenvalues of the linearisation about this equilibrium point. In particular, we can infer that at least one eigenvalue must be positive in order for a steady state to be unstable. A more detailed case differentiation for eigenvalues and stability can be found in [12, pp. 390-392].

2.4 Bifurcations

Given a stable steady state, the long-term behaviour of the system is quite certain. If a steady state is unstable, however, numerous different continuations are imaginable. Due to their relevance in a nature-related context, *bifurcations* must be mentioned here as a mathematical as well as a contextually interpretable concept. A very general characterisation of bifurcations can be found in [5]: bifurcations are "such qualitative changes, which involve the appearance, disappearance, or change in stability of the equation's equilibria" (meaning equilibrium points) [p. 242]. More precisely, the so-called *bifurcation theory* deals with "the changes in the qualitative nature of solutions to differential equations as a parameter varies" [12, p. 395]. In this respect, the variation of the delay parameter τ and its implications on the stability of a steady state will be of particular interest for this thesis.

It has become evident from Lemma 2.3.1 that eigenvalues of a linearised system reveal a lot about the stability of the system in a steady state. Thus, bifurcations at equilibrium points of nonlinear systems can be determined by an analysis of the eigenvalues of the linearisation. According to [12], there are two different scenarios as to how stability can change upon parameter variation:

(i) Negative eigenvalues $\lambda \in \mathbb{R}$ can first become zero and then positive or,

(ii) in the case of a pair of complex conjugate eigenvalues with negative real parts, these can cross the imaginary axis, which makes their real parts become positive [p. 395].

In both cases, qualitative changes of the system's behaviour, hence *bifurcations*, occur.

2.5 Hopf bifurcation

The second scenario describes a particular kind of bifurcation: the Andronov-Hopf bifurcation, often simply called *Hopf-bifurcation*. The originally postulated *Hopf theorem* (in $\mathbb{R}^2$ as well as generally for $\mathbb{R}^n$) is mathematically more intricate than is required for the applications in the present thesis and can be found in [26, pp. 65, 81]. Therefore, we will work with the formulation from [12]. Before we postulate the theorem, some additional definitions are necessary:

Definition 2.5.1. An equilibrium point $\bar{X} \in \mathbb{R}^n$ of a nonlinear system is *isolated* if one can find a neighbourhood of $\bar{X}$ which contains no further equilibrium points [16, p. 1].

Definition 2.5.2 (Trajectory, phase space)**.** Consider a system $\dot{X} = f(X,t)$ with solutions $\tilde{X}(t) \in \mathbb{R}^n$. The abstract space with coordinates $\tilde{x_1}, \ldots, \tilde{x_n}$ is called *phase space* of the system. Solutions $\tilde{X}(t)$ correspond to points in this phase space "moving along a curve in this space" upon variation of t, which is called *trajectory*. [36, p. 7]

Definition 2.5.3. A trajectory is *isolated* if trajectories in its neighbourhood are not closed [36, p. 198], i.e. their starting and end points do not coincide.

Definition 2.5.4 (Limit cycle)**.** An isolated closed trajectory is called *limit cycle*. A limit cycle is *stable* (or *attracting*) if its neighbouring (hence not closed) trajectories spiral towards the limit cycle. Otherwise, if they spiral away, the limit cycle is said to be *unstable*. Sometimes, a limit cycle can also be *half-stable* [36, p. 198].

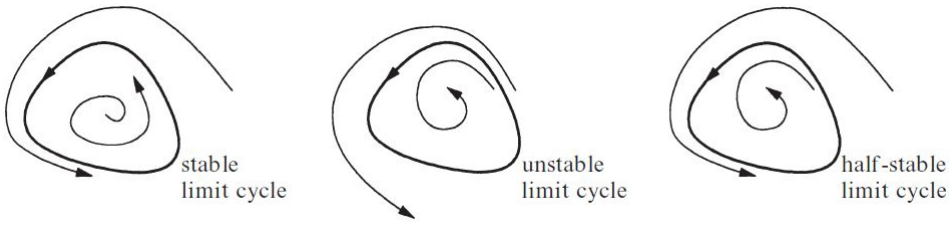

Figure 2.1. Stable, unstable and half-stable limit cycles (Fig. 7.0.1 of [36])

Definition 2.5.5. A limit cycle is *isolated* if no other limit cycles can be found in its neighbourhood.

Having defined these terms, we can finally formulate the following theorem:

Theorem 2.5.1 (Hopf bifurcation theorem)**.** Let $\dot{X} = f(X, \mu, t)$ with $\dot{X}, X \in \mathbb{R}^n$ be a nonlinear system of differential equations with a parameter $\mu \in \mathbb{R}^n$. Furthermore, let $\bar{X}$ be an isolated equilibrium point of this system and $A(\mu)$ the coefficient matrix of the linearisation about this equilibrium point. Suppose that A has eigenvalues

$$\lambda_{1,2} = \alpha(\mu) \pm i\omega(\mu),$$

i.e. "a pair of complex conjugate eigenvalues" with real part $\alpha(\mu)$ and imaginary part $\omega(\mu)$. Presume that the following conditions are satisfied for some μ_0:

(i) $\alpha(\mu_0) = 0$,

(ii) $\omega_0 := \omega(\mu_0), \omega_0 > 0$,

(iii) $\frac{d\alpha(\mu)}{d\mu}(\mu_0) \neq 0$, and

(iv) there are no other eigenvalues λ of $A(\mu_0)$ with $Re(\lambda) = 0$.

Then, we say that a *Hopf bifurcation* occurs when $\mu = \mu_0$. Furthermore, given $|\mu - \mu_0|$ is small, there is an isolated limit cycle of the system for either $\mu > \mu_0$ or $\mu < \mu_0$. The limit cycle is stable if

a) it exists for $\mu > \mu_0$ and $\frac{d\alpha(\mu)}{d\mu}(\mu_0) > 0$ holds, or

b) it exists for $\mu < \mu_0$ and $\frac{d\alpha(\mu)}{d\mu}(\mu_0) < 0$.

Otherwise, the limit cycle is unstable. [12, p. 399]

Remark. Note that Theorem 2.5.1 implies that either the steady state or the limit cycle is stable.

In conclusion, Hopf bifurcations are a cause for limit cycles. These are of paramount importance for models of biological systems as they indicate the occurrence of self-sustaining oscillations of the system, i.e. oscillations without any external stimuli. In other words, the existence of limit cycles indicates periodic behaviour of a system. Therefore,

> "[...] the best way to try to find periodic solutions in a system of differential equations is to look for parameter values where the stability of an equilibrium is lost as a complex conjugate pair of eigenvalues crosses the imaginary axis." [12, p. 399]

2.6 Computational approaches

Apart from rigorous mathematics, computational approaches can be convenient for the analysis of (non)linear models including systems of differential equations. The combination of powerful computational methods and numerical approaches allows us to compute solutions by iteration for systems with set parameters. More detailed mathematical and computational foundations would exceed the scope of this thesis, which is why existing tools will be applied without further theoretic depiction.

In this thesis, Wolfram Mathematica (version 13.1) will be used for calculations and simulations, since it offers in-built solvers for diverse kinds of (differential) equations. In particular, many DDEs can be solved numerically with the function `NDSolve`, as stated explicitly in the documentation [40]. Furthermore, different plotting options are available for obtained solutions, of which we will use `Plot` [41].

Chapter 3

The circadian pacemaker model

One of the central questions of this thesis concerns those elements or properties of mathematical models which are decisive for periodic behaviour (i.e. self-sustaining oscillations). Furthermore, we will later aim to construct a simple model of gene expression in the context of the cell cycle. Thereby, we will aim at a model which reproduces the periodic behaviour of the simplified biological system. As a consequence, we will try to identify crucial elements in the following analysis of the *circadian pacemaker* model developed by Lema, Golombek and Echave in [23]. The authors claim that, given an appropriate choice of parameters, their model can show periodic behaviour despite its simplicity.

The circadian pacemaker model reproduces the periodic (more precisely, circadian[1]) fluctuations of the expression of one single clock gene. Importantly, the gene's expression is regulated by a delayed negative feedback mechanism (see section 1.5). The model consists of the following delay differential equation:

$$\frac{dE(t)}{dt} = K_e\, G(t-\delta) - K_d\, E(t) \tag{3.1}$$

with

$$G(t-\delta) = \frac{1}{1+[E(t-\delta)/K_i]^n}, \tag{3.2}$$

[1] *Circadian* rhythms are a fascinating phenomenon common to all eukaryotes. They are characterised by repeating processes with an approximate duration of 24 hours, hence, *circa* a day. Importantly, so-called *molecular clocks* drive these rhythms from within the cell as they oscillate. Thus, these clocks must be *endogenous* as well as *self-running*. Furthermore, it must be possible to reset these clocks as a result of external influences, so-called *zeitgebers*. Best known among zeitgebers is the alternation of darkness and light during night and day which not only has an effect on the *sleep-wake cycle* of humans and other animals but also on endocrine systems and many other cycles in different organisms. [25, pp. 978-979]

where $E(t)$ stands for the amount of functional protein at time t [23, Equations (1) and (2)]. The production term $K_e\ G(t-\delta)$ contains the constant K_e, which corresponds to the rate of gene expression, as well as the activation term $G(t-\delta)$. The latter regulates the expression of the gene due to the time delayed activation/inhibitory effect it produces. Importantly, the time delay reflects the fact that there is a time lag between the activation of a gene and the actual presence of the gene product in a cell due to the gene expression process (including transcription, translation, modifications, transport, etc.). The constant K_i is the inhibition rate and n the "Hill coefficient of inhibition". [23, p. 2] Hill coefficients are used when interactions of molecules (usually, such as ligands and receptors) are considered to influence the reaction dynamics. It can, therefore, be considered an "*interaction coefficient*, reflecting cooperativity". [34, p. 86] Lastly, the degradation of the produced protein is determined by the degradation rate K_d [23, p. 2].

Combining equations (3.1) and (3.2) and applying the notation conventions established in this thesis, we arrive at

$$\dot{E} = K_e\ \frac{1}{1+[E(t-\tau)/K_i]^n} - K_d\ E(t). \tag{3.3}$$

Firstly, we can solve this DDE numerically with Mathematica's `NDSolve` in order to illustrate its periodic behaviour. Parameter values are chosen in accordance with the criteria stated in [23, Table 3]. As a result, we can indeed observe self-sustaining oscillations:

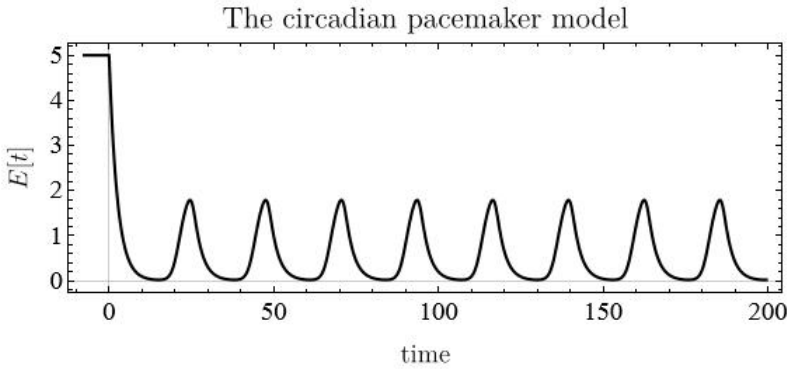

Figure 3.1. Simulation of the circadian pacemaker model given by the DDE (3.3) with parameters $K_e = 1$, $\delta = 8$, $K_i = 0.04$, $n = 2.5$, $K_d = 0.4$ and initial condition $E(0) = 5$. Mathematica's `NDSolve` and `Plot` were used for simulation and visualisation.

Secondly, we aim to analyse the foundations of the periodic model behaviour. For this purpose, we will apply some concepts of section 2. Furthermore, we will adopt ideas of [29, section 4] and [35, section 4.4]. To begin with, we are interested in steady states of the system and their stability.

3.1 Linearisation about an equilibrium point of the system

According to Definition 2.1.1, $\bar{E} \in \mathbb{R}$ is a steady state of the modelled system, i.e. an equilibrium point of the DDE, if

$$K_e \, \frac{1}{1 + (\bar{E}/K_i)^n} - K_d \, \bar{E} = 0,$$

which is equivalent to

$$\frac{K_e}{K_d} = \bar{E} \left(1 + \left(\frac{\bar{E}}{K_i} \right)^n \right).$$

An explicit expression for $\bar{E}$ cannot be obtained easily, not even computationally (Mathematica's `Solve` does not yield any result). However, we will in the following assume that there exists some equilibrium point $\bar{E} > 0$. In order to subsequently analyse its stability, a linearisation of equation (3.3) about $\bar{E}$ is undertaken.

Set $x := E(t)$ and $y := E(t - \tau)$. Furthermore, let $u(t) := x - \bar{E}$ and $v(t) := y - \bar{E}$ be small perturbations of the equilibrium point $\bar{E}$. Application on (3.3) results in

$$\begin{aligned} \dot{E} &= K_e \, \frac{1}{1 + [y/K_i]^n} - K_d \, x \\ &= K_e \, \frac{1}{1 + [(\bar{E} + v)/K_i]^n} - K_d \, (\bar{E} + u) =: f(\bar{E} + u, \bar{E} + v). \end{aligned}$$

With the aim of computing the linearisation as defined in Definition 2.1.2, we need to differentiate $f(\bar{E} + u, \bar{E} + v)$ with respect to x and y:

$$\begin{aligned} \frac{df}{dx} &= -K_d \\ \frac{df}{dy} &= -\frac{K_e \, n \, \left(\frac{y}{K_i} \right)^n}{y \, \left(\left(\frac{y}{K_i} \right)^n + 1 \right)^2}. \end{aligned}$$

Consequently, we obtain the following linearisation of $\dot{E}$ about the equilibrium point $\bar{E}$:

$$\dot{x} \approx -K_d\, u(t) - \underbrace{\frac{K_e\, n\, \left(\frac{\bar{E}}{K_i}\right)^n}{\bar{E}\, \left(\left(\frac{\bar{E}}{K_i}\right)^n + 1\right)^2}}_{C}\, v(t).$$

It follows directly from the definitions of $u(t)$ and $v(t)$ that $v(t) = u(t-\tau)$. Moreover, it is essential to note that $\dot{u} = \dot{E} = \dot{x}$ because $\bar{E}$ is a constant. Therefore, we finally get

$$\dot{E} = \dot{x} = \dot{u} \approx -K_d\, u(t) - C\, u(t-\tau). \tag{3.4}$$

By means of the linearisation obtained, we seek to evaluate the stability of $\bar{E}$, as the stability of equilibrium points is considered to be decisive for the (long-term) behaviour of a system.

3.2 Stability analysis of the equilibrium point

In section 2.3, the strong link between the stability of an equilibrium point and the eigenvalue(s) of the linearisation about this equilibrium point was highlighted. The aim of the following section is, therefore, to examine (in particular, the sign of) eigenvalues depending on the delay parameter τ and to draw conclusions about the (in)stability of the equilibrium point.

Following the idea in [35, p. 29], we make the ansatz

$$u(t) = e^{\lambda t} u_0. \tag{3.5}$$

Evidently, $\dot{u} = \lambda e^{\lambda t} u_0$. Respective substitution of $u(t)$ and $\dot{u}$ in the linearisation, i.e. equation (3.4), results in:

$$\dot{u} = \lambda e^{\lambda t} u_0 = -K_d e^{\lambda t} u_0 - C e^{\lambda (t-\tau)} u_0$$

which is equivalent to

$$\lambda = -K_d - C e^{-\lambda \tau}. \tag{3.6}$$

First of all, we consider the ODE case $\tau = 0$:

Proposition 3.2.1. If the delay parameter $\tau = 0$ and Hill coefficient $n \geq 0$, the equilibrium point $\bar{E}$ is stable, irrespective of the parameters K_e, K_i and K_d.

Proof. Since we consider an equilibrium point $\bar{E} > 0$ and Hill coefficient $n \geq 0$, the constant C is positive. K_d is positive by definition. Therefore,

$$\lambda = -K_d - C < 0. \tag{3.7}$$

According to Lemma 2.3.1, the steady state $\bar{E}$ is (asymptotically) stable, independent of the constant parameters. □

As a consequence of this result for $\tau = 0$, the question arises whether the delay parameter τ (if > 0) could interfere with the stability of the equilibrium point $\bar{E}$. Therefore, we aim to analyse the eigenvalue λ upon variation of τ.

3.2.1 Destabilisation of the equilibrium point upon variation of τ

In section 2.4, two scenarios for a change of stability depending on varying parameters were discussed:

(i) Either negative real eigenvalues become positive (and thereby cross 0) as the parameter changes or

(ii) the negative real part of a pair of complex conjugate eigenvalues becomes positive (upon crossing of the imaginary axis).

Scenario (i) can be ruled out as $\lambda = 0$ does not solve the characteristic equation (3.6). Therefore, $\bar{E}$ can only be destabilised in case a Hopf bifurcation occurs, hence, a pair of complex conjugate eigenvalues crosses the imaginary axis as their negative real parts become positive. In this case, there must be some τ_0 such that the eigenvalues are purely imaginary, i.e. in the form of $i\omega$ and $-i\omega$. To begin with, we aim to compute the corresponding τ as well as the imaginary part ω_0. Setting $\lambda = i\omega$ in the characteristic equation (3.6) gives:

$$i\omega + C\, e^{-i\omega\tau} + K_d = 0. \tag{3.8}$$

Then, we can provide evidence for the following:

Lemma 3.2.2. Set

$$\omega_0^{\pm} := \pm\sqrt{C^2 - K_d^2} \tag{3.9}$$

and

$$\tau_{0,n} := \frac{1}{\omega_0} \arctan\left(\frac{\omega_0}{-K_d}\right) + n\pi \tag{3.10}$$

for some $n \in \mathbb{N}$. Then, $\omega_0^{\pm} \in \mathbb{R}$ are solutions of the characteristic equation (3.8) if the delay parameter τ equals $\tau_{0,n}$ for some $n \in \mathbb{N}$ and $C > K_d$.

Proof. First, we will show how $\omega_0^{\pm}$ are obtained. Separation of the real and imaginary part of (3.8) leads to

$$C\ \cos(\omega\tau) = -K_d \tag{3.11}$$

$$C\ \sin(\omega\tau) = \omega \tag{3.12}$$

because $e^{-i\omega\tau} = \cos(\omega\tau) - i\sin(\omega\tau)$. Squaring of both equations gives

$$C^2\ \cos^2(\omega\tau) = K_d^2$$
$$C^2\ \sin^2(\omega\tau) = \omega^2$$

and adding yields

$$C^2\ \underbrace{(\cos^2(\omega\tau) + \sin^2(\omega\tau))}_{=1} = K_d^2 + \omega^2.$$

Thus,

$$\omega_0^{\pm} = \pm\sqrt{C^2 - K_d^2}.$$

Since $C^2 > K_d^2$ is assumed to hold, both values for ω_0 are real. Therefore, there exists a pair of purely imaginary eigenvalues $i\omega_0$ and $-i\omega_0$, provided $C^2 > K_d^2$. Subsequently, we divide (3.12) by (3.11) in order to obtain the corresponding value τ_0:

$$\tau_0 = \frac{1}{\omega_0}\ \arctan\left(\frac{\omega_0}{-K_d}\right).$$

More precisely, due to the periodicity of tan:

$$\tau_{0,n} = \frac{1}{\omega_0}\ \arctan\left(\frac{\omega_0}{-K_d}\right) + n\pi\ (\text{for } n \in \mathbb{N}).$$

In conclusion, we have shown that $\pm i\omega_0^{\pm}$ are solutions of equation(3.8) with $\tau = \tau_{0,n}$ (for some $n \in \mathbb{N}$). □

In order to finally prove that a Hopf bifurcation occurs as the equilibrium point is destabilised, it remains to be shown that the pair of complex conjugate eigenvalues $\pm i\omega_0^{+}$ indeed crosses the imaginary axis with changing τ, i.e. that the sign of their real parts changes as τ becomes larger than $\tau_{0,n}$.

3.2.2 Evidence for the occurrence of a Hopf bifurcation

Since we aim to understand how eigenvalues λ of the linearisation change upon variation of delay parameter τ, set $\lambda := \lambda(\tau) = \alpha(\tau) + i\omega(\tau)$. The characteristic equation then becomes

$$\lambda(\tau) + C\, e^{-\lambda(\tau)\tau} + K_d = 0. \tag{3.13}$$

Now, we can prove the following:

Theorem 3.2.3. Assume the constants $K_e, K_i, K_d > 0$ and

$$C = \frac{K_e\, n\, (\frac{\bar{E}}{K_i})^n}{\bar{E}\, ((\frac{\bar{E}}{K_i})^n) + 1)^2} > K_d. \tag{3.14}$$

Furthermore, let ω_0^{+} and $\tau_{0,n}$ be as defined in Lemma 3.2.2. Then, at $\tau = \tau_{0,n}$ a Hopf bifurcation occurs.

Proof. The formal conditions for a Hopf bifurcation are depicted in Theorem 2.5.1. We have already determined a unique pair of purely imaginary eigenvalues $i\omega_0^{+}$ and $-i\omega_0^{+}$ which occur at $\tau = \tau_{0,n}$. Therefore, $\alpha(\tau_{0,n}) = 0$. Moreover, we have established the condition $C > K_d$, which guarantees that $\omega_0^{+} > 0$ and $\omega_0^{+} \in \mathbb{R}$. Hence, conditions (i), (ii) and (iv) are fulfilled. Condition (iii) requires that

$$\frac{d\alpha(\omega_0)}{d\tau}(\tau_{0,n}) \neq 0.$$

To show that this holds true, we can examine

$$\operatorname{sgn}\left(\left[\frac{d(Re(\lambda))}{d\tau}\right]_{\tau=\tau_{0,n}}\right) = \operatorname{sgn}\left(Re\left(\left[\frac{d\lambda(\tau)}{d\tau}\right]_{\tau=\tau_{0,n}}\right)\right), \tag{3.15}$$

following [29, pp. 6-7].

Therefore, we differentiate both sides of (3.13) with respect to τ to obtain

$$\frac{d\lambda(\tau)}{d\tau}\left(1 - C\ e^{-\lambda(\tau)\tau}\tau\right) = C\ e^{-\lambda(\tau)\tau}\lambda(\tau), \tag{3.16}$$

which is equivalent to

$$\frac{d\lambda(\tau)}{d\tau} = \frac{C\ e^{-\lambda(\tau)\tau}\lambda(\tau)}{1 - C\ e^{-\lambda(\tau)\tau}\tau}. \tag{3.17}$$

As we are interested in the sign of the above expression, we can likewise work with

$$\left(\frac{d\lambda(\tau)}{d\tau}\right)^{-1} = \frac{1}{C\ e^{-\lambda(\tau)\tau}\lambda(\tau)} - \frac{\tau}{\lambda(\tau)}. \tag{3.18}$$

The second term is purely imaginary at $\tau = \tau_{0,n}$, because

$$\frac{\tau}{\lambda(\tau_{0,n})} = \frac{\tau}{i\omega_0} = -\frac{i\tau}{\omega_0}.$$

As a consequence,

$$\operatorname{sgn}\left(Re\left(\left[\left(\frac{d\lambda(\tau)}{d\tau}\right)^{-1}\right]_{\tau=\tau_{0,n}}\right)\right) = \operatorname{sgn}\left(Re\left(\left[\frac{1}{C\ e^{-\lambda(\tau)\tau}\lambda(\tau)}\right]_{\tau=\tau_{0,n}}\right)\right). \tag{3.19}$$

Since for $\tau_{0,n}$ the eigenvalues are purely imaginary, $\alpha(\tau_{0,n}) = 0$. Moreover, $\omega(\tau_{0,n}) = \omega_0$ and from equation (3.12) follows that $\sin(\omega\tau) = \frac{\omega}{C}$. As a result, we finally get

$$\begin{aligned}
Re\left(\left[\frac{1}{C\ e^{-\lambda(\tau)\tau}\lambda(\tau)}\right]_{\tau=\tau_{0,n}}\right) &= Re\left(\frac{1}{C\ e^{-i\omega_0\tau_{0,n}}\ i\omega_0}\right) \\
&= Re\left(\frac{1}{C\ \omega_0\ [\sin(\omega_0\tau_{0,n}) + i\cos(\omega_0\tau_{0,n})]}\right) \\
&= Re\left(\frac{[\sin(\omega_0\tau_{0,n}) - i\cos(\omega_0\tau_{0,n})]}{C\ \omega_0}\right) \\
&= \frac{\sin(\omega_0\tau_{0,n})}{C\ \omega_0} \\
&= \frac{1}{C^2} > 0.
\end{aligned}$$

In conclusion,

$$\operatorname{sgn}\left(\left[\frac{d(Re(\lambda))}{d\tau}\right]_{\tau=\tau_{0,n}}\right) = \operatorname{sgn}\left(\frac{1}{C^2}\right) > 0. \tag{3.20}$$

We can infer from the result obtained that condition (iii) is met as well and, thus, a Hopf bifurcation occurs at $\tau = \tau_{0,n}$ ($n \in \mathbb{N}$). This result also shows that the real parts of the pair of complex eigenvalues, which were negative for $\tau = 0$, become positive at the crossing of the imaginary axis. □

According to the Hopf bifurcation theorem (Theorem 2.5.1), a stable limit cycle exists for $\tau > \tau_{0,n}$, which explains the observed periodic behaviour of the system.

3.3 Conclusions

In short, the undertaken mathematical analysis of the circadian pacemaker model showed that a stable limit cycle originates from a destabilised equilibrium point. As a consequence, self-sustaining oscillations characterise the behaviour of the model. Importantly, the equilibrium point became unstable due to the incorporation of a delay $\tau > 0$. The core elements of the simple model with periodic behaviour are, therefore, the delay parameter τ and its effects. These are the destabilisation of the equilibrium point and the Hopf bifurcation as the origin of a stable limit cycle.

Chapter 4

A model of transcription in the context of the cell cycle

In this chapter, we will finally construct a simple model of gene expression, which covers the succession of synthesised mRNAs in the context of the cell cycle. We aim to examine whether the model reproduces the periodic behaviour of the biological system, which is to say that it shows self-sustaining oscillations. With this intention, we will analyse the model analogously to the circadian pacemaker model and run simulations in Wolfram Mathematica. In addition to the question as to whether the model shows the expected behaviour, determining mathematical elements will be addressed as well.

4.1 The model

To begin with, the constructed model is based on the fundamental model of Raj, Peskin, Tranchina et al., which reflects the core processes of gene expression or, more precisely, transcription. As discussed in section 1.4, this model comprises the synthesis of mRNA (if the gene is in an active state) as well as its degradation at constant rates [32]. Our model also focuses on transcription, which is a biologically reasonable restriction since a considerable number of mRNA molecules can have proper functions, for instance in gene expression regulation. In addition, mRNA molecules are not necessarily translated, i.e. used for protein synthesis. [1, p. 299] Consequently, we mean mRNA molecules when referring to *gene products*.

Furthermore, as stated in section 1.3, the recurrent nature of the cell cycle in a pro-

liferating cell implies that gene products appear periodically – either every time the cell cycle reaches a certain point or even at a (much) higher frequency than once per cycle. Of course, this is a very simplified approximation of the actual biological system, which will definitely not hold true for all genes[1]. Nevertheless, we will assume periodic synthesis of gene products as the cells (recurrently) go through the cell cycle.

As a consequence of the previous assumption, we presume that due to a complex regulatory system, gene products appear at a more or less determined time in the cell cycle. The exact time of synthesis, nevertheless, depends on various factors which trigger transcriptional bursts (see chapter 1.2), hence, the actual synthesis of the gene product. The response to a stimulus for mRNA synthesis can, therefore, be evoked with a certain delay. Furthermore, the time passing from the start of transcription to the gene product's functional activity contributes to this time lag as well.

With respect to transcriptional stimuli, the model includes a rather simple feedback mechanism. We assume the appearance of an antecedent gene product A to be the stimulus for the synthesis of the following gene product B. Thus, one gene product activates the synthesis of the next (positive feedback). At the same time, the synthesis of gene product B is limited, depending on the level of gene product A (negative feedback), and both products are degraded at constant rates.

Starting from these assumptions, a mathematical model can be constructed. Only the simplest case of two alternating gene products will be examined. So, consider $g_1, g_2 \in \mathbb{R}$ the concentrations of two gene products, resulting in $g_1, g_2 \geq 0$. The rates $\alpha_1, \alpha_2 \geq 0$ and $\gamma_1, \gamma_2 \geq 0$ are the gene products' synthesis and decay rates, respectively. The factors $\left(1 - \frac{g_1(t)}{\bar{g_1}}\right)$ and $\left(1 - \frac{g_2(t)}{\bar{g_2}}\right)$ are added multiplicatively to the first parts of the synthesis term in order to limit the increase in gene product concentrations, as the amount of a gene product usually cannot exceed a certain threshold in actual biological systems. These considerations and assumptions result in the following system of ordinary differential equations:

$$\begin{aligned} \dot{g}_1(t) &= \alpha_1 g_2(t) \left(1 - \frac{g_1(t)}{\bar{g_1}}\right) - \gamma_1 g_1(t) \\ \dot{g}_2(t) &= \alpha_2 g_1(t) \left(1 - \frac{g_2(t)}{\bar{g_2}}\right) - \gamma_2 g_2(t). \end{aligned} \tag{4.1}$$

[1] To name only one counterexample, some genes are not expressed regularly but only in case of life-threatening conditions.

Linearisation of this system is indicative of a stable steady state (see Appendix for details), thus, the system does definitely not reproduce the periodic characteristics of the modelled process. Therefore, it makes sense to develop the model further by taking the mentioned aspect of time lags into consideration. To this end, we introduce delays τ_1, τ_2, resulting in a system of delay differential equations:

$$\begin{aligned} \dot{g}_1(t) &= \alpha_1 g_2(t-\tau_1) \left(1 - \frac{g_1(t)}{\bar{g_1}}\right) - \gamma_1 g_1(t) \\ \dot{g}_2(t) &= \alpha_2 g_1(t-\tau_2) \left(1 - \frac{g_2(t)}{\bar{g_2}}\right) - \gamma_2 g_2(t). \end{aligned} \tag{4.2}$$

As explicit solutions of the nonlinear system of DDEs are clearly out of reach, we will first aim for linearisation about an equilibrium point of the system, which will further be used to examine the stability of the respective equilibrium point. We take particular interest in the role of the delay parameter τ, since it might be decisive for periodic solutions.

In the following, the concepts presented in the preliminaries section 2 will be applied to the constructed model. Moreover, we will proceed following section 4 of [29] and section 4.4 of [35].

4.2 Equilibrium points and linearisation of the nonlinear system

In order to linearise the system we first aim to determine equilibrium points of the system and choose a suitable one to linearise about.

4.2.1 Equilibrium points

First of all, we can reduce the complexity of our system by means of a simple substitution: Set $x(t) := g_1(t)$ and $y(t) := g_2(t-\tau_1)$, which results in:

$$\begin{aligned} \dot{x} &= \alpha_1 y(t) \left(1 - \frac{x(t)}{\bar{g_1}}\right) - \gamma_1 x(t) \\ \dot{y} &= \alpha_2 x(t-\tau) \left(1 - \frac{y(t)}{\bar{g_2}}\right) - \gamma_2 y(t) \end{aligned} \tag{4.3}$$

with the second equation being computed at $(t-\tau_1)$ and setting $\tau := \tau_1 + \tau_2$. [35, p. 28]

If $(\bar{u}, \bar{v})$ is an equilibrium point as defined in Definition 2.1.1, the following holds:

$$\alpha_1 \bar{v} \left(1 - \frac{\bar{u}}{\bar{g_1}}\right) = \gamma_1 \bar{u} \tag{4.4}$$

$$\alpha_2 \bar{u} \left(1 - \frac{\bar{v}}{\bar{g_2}}\right) = \gamma_2 \bar{v}. \tag{4.5}$$

Clearly, $(\bar{u}, \bar{v}) = (0, 0)$ is such an equilibrium point, hence, indicates a steady state of the biological system. This corresponds to the case of neither gene product being present in the cell. However, we take considerably more interest in an equilibrium point with $\bar{u}, \bar{v} > 0$ and will provide evidence for its existence under certain conditions.

Proposition 4.2.1. Assume $\alpha_1\alpha_2 > \gamma_1\gamma_2$. Then, the system (4.3) has an equilibrium point $(\bar{u}, \bar{v})$ with $\bar{u}, \bar{v} > 0$, namely

$$\bar{u} = \frac{\bar{g_1}\bar{g_2}(\alpha_1\alpha_2 - \gamma_1\gamma_2)}{\alpha_2(\alpha_1\bar{g_2} + \bar{g_1}\gamma_1)} \tag{4.6}$$

$$\bar{v} = \frac{\bar{g_1}\bar{g_2}(\alpha_1\alpha_2 - \gamma_1\gamma_2)}{\alpha_1(\alpha_2\bar{g_1} + \bar{g_2}\gamma_2)}. \tag{4.7}$$

Additionally, $\bar{u} < \bar{g_1}$ and $\bar{v} < \bar{g_2}$.

Proof. First, we compute $(\bar{u}, \bar{v})$ applying `Solve` on the equations (4.4) and (4.5) in Mathematica. We obtain (4.6) and (4.7) as well as the trivial case $\bar{u} = \bar{v} = 0$. With respect to the nonzero solutions, it is clear that $\bar{u}, \bar{v} > 0$ from the assumption $\alpha_1\alpha_2 > \gamma_1\gamma_2$. Furthermore, we prove that $\bar{u} < \bar{g_1}$ (and analogously $\bar{v} < \bar{g_2}$):
Assume the opposite holds, i.e.

$$\bar{u} = \frac{\bar{g_1}\bar{g_2}(\alpha_1\alpha_2 - \gamma_1\gamma_2)}{\alpha_2(\alpha_1\bar{g_2} + \bar{g_1}\gamma_1)} \geq \bar{g_1}.$$

Then,

$$\bar{g_1}\ \bar{g_2}(\alpha_1\alpha_2 - \gamma_1\gamma_2) \geq \bar{g_1}\ \alpha_2(\alpha_1\bar{g_2} + \bar{g_1}\gamma_1),$$

which is equivalent to

$$\bar{g_2}\alpha_1\alpha_2 - \bar{g_2}\gamma_1\gamma_2 \geq \bar{g_2}\alpha_1\alpha_2 + \bar{g_1}\gamma_1.$$

Since all of the included variables are positive, this is a contradiction and, therefore, $\bar{u} < \bar{g_1}$. Analogously, $\bar{v} < \bar{g_2}$. As a result, we can conclude that if $\alpha_1\alpha_2 > \gamma_1\gamma_2$ holds, there exists some $\bar{u} \in \mathbb{R}$ with $0 < \bar{u} < \bar{g_1}$ as well as some $\bar{v} \in \mathbb{R}$ with $0 < \bar{v} < \bar{g_2}$ yielding a nonzero equilibrium point $(\bar{u}, \bar{v})$. □

Now that we have found an equilibrium point of interest, we can examine its stability. For this purpose, we will linearise the system (4.3) about $(\bar{u}, \bar{v})$.

4.2.2 Linearisation

To begin with, set $u(t) := x(t) - \bar{u}$ and $v(t) := y(t) - \bar{v}$ in system (4.3) with u, v being small perturbations and $(\bar{u}, \bar{v})$ the equilibrium point (or steady state):

$$\dot{u} = \alpha_1 \, (\bar{v} + v) \, \left(1 - \frac{\bar{u} + u}{\bar{g_1}}\right) - \gamma_1(\bar{u} + u) =: f_1(\bar{u} + u, \bar{v} + v) \tag{4.8}$$

$$\dot{v} = \alpha_2 \, (\bar{u} + u(t - \tau)) \, \left(1 - \frac{\bar{v} + v}{\bar{g_2}}\right) - \gamma_2(\bar{v} + v) =: f_2(\bar{u} + u, \bar{v} + v). \tag{4.9}$$

Proceeding according to section 2.1, we compute the derivatives of f_1 and f_2 with respect to x and y:

$$\begin{aligned}
\frac{df_1}{dx}(\bar{u}, \bar{v}) &= -\alpha_1 \, \frac{\bar{v}}{\bar{g_1}} - \gamma_1 \\
\frac{df_1}{dy}(\bar{u}, \bar{v}) &= \alpha_1 \, \left(1 - \frac{\bar{u}}{\bar{g_1}}\right) \\
\frac{df_2}{dx}(\bar{u}, \bar{v}) &= \alpha_2 \, \left(1 - \frac{\bar{v}}{\bar{g_2}}\right) \\
\frac{df_2}{dy}(\bar{u}, \bar{v}) &= -\alpha_2 \, \frac{\bar{u}}{\bar{g_2}} - \gamma_2.
\end{aligned}$$

Applying Definition 2.1.2, we get the following linearisation:

$$\dot{u} \approx \alpha_1 \, v \left(1 - \frac{\bar{u}}{\bar{g_1}}\right) - \alpha_1 \, \frac{\bar{v}}{\bar{g_1}} \, u - \gamma_1 u \tag{4.10}$$

$$\dot{v} \approx \alpha_2 \, u(t - \tau) \left(1 - \frac{\bar{v}}{\bar{g_2}}\right) - \alpha_2 \, \frac{\bar{u}}{\bar{g_2}} \, v - \gamma_2 v. \tag{4.11}$$

By means of the linearisation, we can now examine the stability of the equilibrium point $(\bar{u}, \bar{v})$.

4.3 Stability analysis of the equilibrium point

As a first step of the stability analysis, we make the following ansatz (following [35, p. 29]):

$$u(t) = e^{\lambda t}\, u_0 \quad \text{and} \quad v(t) = e^{\lambda t}\, v_0$$

with $\lambda \in \mathbb{R}$. Application on the linearisation (4.10) and (4.11) leads to

$$\dot{u} = e^{\lambda t}\, v_0\, \alpha_1 \left(1 - \frac{\bar{u}}{\bar{g}_1}\right) - e^{\lambda t}\, u_0 \left(\alpha_1 \frac{\bar{v}}{\bar{g}_1} + \gamma_1\right) \tag{4.12}$$

$$\dot{v} = e^{\lambda (t-\tau)}\, u_0\, \alpha_2 \left(1 - \frac{\bar{v}}{\bar{g}_2}\right) - e^{\lambda t}\, v_0 \left(\alpha_2 \frac{\bar{u}}{\bar{g}_2} + \gamma_2\right). \tag{4.13}$$

Using $\dot{u} = \lambda e^{\lambda t} u_0$, we get

$$u_0 \left(\lambda + \alpha_1 \frac{\bar{v}}{\bar{g}_1} + \gamma_1\right) = v_0\, \alpha_1 \left(1 - \frac{\bar{u}}{\bar{g}_1}\right) \tag{4.14}$$

and analogously

$$v_0 \left(\lambda + \alpha_2 \frac{\bar{u}}{\bar{g}_2} + \gamma_2\right) = e^{-\lambda \tau}\, u_0\, \alpha_2 \left(1 - \frac{\bar{v}}{\bar{g}_2}\right). \tag{4.15}$$

Importantly, the term $e^{-\lambda \tau}$ stays in the second equation as a remainder of $u(t-\tau)$. So we are dealing with the following system, which resembles an eigenvalue problem for ODEs:

$$\begin{pmatrix} \lambda + \alpha_1 \frac{\bar{v}}{\bar{g}_1} + \gamma_1 & -\alpha_1 \left(1 - \frac{\bar{u}}{\bar{g}_1}\right) \\ e^{-\lambda \tau} \alpha_2 \left(1 - \frac{\bar{v}}{\bar{g}_2}\right) & -\left(\lambda + \alpha_2 \frac{\bar{u}}{\bar{g}_2} + \gamma_2\right) \end{pmatrix} \begin{pmatrix} u_0 \\ v_0 \end{pmatrix} = 0. \tag{4.16}$$

For some fixed τ, we can (carefully) resort to a statement for linear systems: A homogeneous linear system has infinitely many solutions if the determinant of the coefficient matrix equals zero (see Lemma 2.1.1). Otherwise, it has one unique solution – the trivial solution $u_0 = v_0 = 0$. Thus, the linear system has nontrivial solutions if

$$\left[\lambda + \alpha_1 \frac{\bar{v}}{\bar{g}_1} + \gamma_1\right] \left[-\lambda - \alpha_2 \frac{\bar{u}}{\bar{g}_2} - \gamma_2\right] + \left[e^{-\lambda \tau} \alpha_2 \left(1 - \frac{\bar{v}}{\bar{g}_2}\right)\right] \left[\alpha_1 \left(1 - \frac{\bar{u}}{\bar{g}_1}\right)\right] = 0. \tag{4.17}$$

Setting

$$b := \alpha_2 \frac{\bar{u}}{\bar{g_2}} + \alpha_1 \frac{\bar{v}}{\bar{g_1}} + \gamma_1 + \gamma_2 \tag{4.18}$$

$$c := \alpha_1 \alpha_2 \frac{\bar{v}}{\bar{g_1}} \frac{\bar{u}}{\bar{g_2}} + \alpha_1 \frac{\bar{v}}{\bar{g_1}} \gamma_2 + \alpha_2 \frac{\bar{u}}{\bar{g_2}} \gamma_1 + \gamma_1 \gamma_2 \tag{4.19}$$

$$D := \alpha_1 \alpha_2 \left(1 - \frac{\bar{u}}{\bar{g_1}}\right) \left(1 - \frac{\bar{v}}{\bar{g_2}}\right), \tag{4.20}$$

(4.17) is equivalent to

$$\lambda^2 + \lambda b + c - De^{-\lambda\tau} = 0. \tag{4.21}$$

Note that b and c are both sums of positive terms (as all included variables are positive), while D can become negative if $\bar{u} > \bar{g_1}$ or $\bar{v} > \bar{g_2}$. According to Proposition 4.2.1, however, we are considering $\bar{u} \in \mathbb{R}$ with $0 < \bar{u} < \bar{g_1}$ and $\bar{v} \in \mathbb{R}$ with $0 < \bar{v} < \bar{g_2}$ for the equilibrium point $(\bar{u}, \bar{v})$. Therefore, we can assume $D > 0$ as well.

Equation (4.21) resembles a characteristic equation, which can be used to detect eigenvalues. As highlighted in section 2.3, eigenvalues of the linearisation about an equilibrium point are bound with the stability of the equilibrium point. Therefore, we seek to find and analyse eigenvalues. If there was no delay, i.e. $\tau = 0$, we could simply solve the quadratic equation to obtain eigenvalues

$$\lambda_{1,2} = -\frac{b}{2} \pm \sqrt{\frac{b^2}{4} - c + D}. \tag{4.22}$$

We can show that under certain conditions, any eigenvalue λ satisfying (4.21) has a negative real part:

Proposition 4.3.1. Let b, c, D and λ be as given in equations (4.18), (4.19), (4.20) and (4.22), respectively. If $c > D$, then the equilibrium point $(\bar{u}, \bar{v})$ is stable.

Proof. We aim to show that the real part of λ is negative on condition that $c > D$. To begin with, set $\Delta := \frac{b^2}{4} - c + D$. We can then distinguish the cases $\Delta \leq 0$ and $\Delta > 0$.

(i) If $\Delta \leq 0$ holds, the real part of λ equals $-\frac{b}{2}$ and is therefore negative in either case ($+$ or $-$).

(ii) If $\Delta > 0$, λ is real (hence, $Re(\lambda) = \lambda$) and it is clear that λ has negative real

part if $\lambda = -\frac{b}{2} - \sqrt{\Delta}$. In case $\lambda = -\frac{b}{2} + \sqrt{\Delta}$, it remains to be shown that $-\frac{b}{2} + \sqrt{\Delta} < 0$:

The assumption $c > D$ is equivalent to $0 > -c + D$ and addition of $\frac{b^2}{4}$ on both sides of the equation yields

$$\frac{b^2}{4} > \frac{b^2}{4} - c + D = \Delta > 0.$$

As both sides are positive, we can take roots, which leads to

$$\frac{b}{2} - \sqrt{\Delta} > 0.$$

Hence,

$$Re(\lambda) = -\frac{b}{2} + \sqrt{\Delta} < 0.$$

To conclude, the real part of λ is negative, provided that $c > D$. As a consequence of Lemma 2.3.1, the equilibrium point $(\bar{u}, \bar{v})$ is stable on condition that $c > D$. □

Since eigenvalues λ depend on the delay τ, we are intrigued to find out, whether τ can be chosen in a way that the equilibrium point is destabilised and that a bifurcation occurs.

4.3.1 Variation of the delay τ to destabilise the equilibrium point

Consider again the "eigenvalue" problem (4.21):

$$\lambda^2 + \lambda b + c - De^{-\lambda\tau} = 0.$$

As became evident in Proposition 4.3.1, the equilibrium point is stable for the limit case $\tau = 0$ on condition that $c > D$, as there are eigenvalues with negative real parts only. Since $\lambda = 0$ does not solve the characteristic equation (4.21), the occurrence of a bifurcation with negative real eigenvalues becoming first zero and then positive is impossible (this would correspond to scenario (i) discussed in section 2.4). Therefore, the equilibrium point can only be destabilised when complex eigenvalues occur, i.e. under scenario (ii). This would again indicate the occurrence of a Hopf bifurcation and periodic behaviour of the system (see section 2.5). As a consequence, we aim to find parameters of the system (including τ) such that the equilibrium point is destabilised, and provide evidence for a Hopf bifurcation.

First, assume $\lambda = i\omega$ with $\omega \in \mathbb{R}^+$ is a purely imaginary eigenvalue, hence a solution to (4.21). Substituting λ by $i\omega$ in equation (4.21) results in

$$-\omega^2 + bi\omega + c - De^{-i\omega\tau} = 0. \tag{4.23}$$

It must be emphasized that we require $\omega \in \mathbb{R}^+$ to hold. With this in mind, we can infer essential constraints for parameters of the model.

Lemma 4.3.2. Set

$$\omega_0 := \sqrt{\frac{-b^2 + 2c}{2} + \sqrt{\frac{(b^2 - 2c)^2}{4} - c^2 + D^2}}. \tag{4.24}$$

and b,c and D as given in equations (4.18), (4.19) and (4.20), respectively.
Then ω_0 is a positive, real solution to (4.23) iff

$$\frac{-b^2 + 2c}{2} + \sqrt{\frac{(b^2 - 2c)^2}{4} - c^2 + D^2} > 0 \tag{4.25}$$

and

$$\frac{(b^2 - 2c)^2}{4} - c^2 + D^2 \geq 0. \tag{4.26}$$

Proof. First, we need to confirm $\pm i\omega_0$ with the above expression for ω_0 as solutions of equation (4.23). Separating the real and imaginary parts of (4.23) leads to

$$-\omega^2 + c = D\cos(\omega\tau) \tag{4.27}$$

$$b\,\omega = -D\sin(\omega\tau). \tag{4.28}$$

We can then eliminate τ by squaring and adding the equations (4.27) and (4.28):

$$\omega^4 + (b^2 - 2c)\omega^2 + c^2 = D^2. \tag{4.29}$$

Solving this quadratic equation with respect to ω^2 yields the critical value ω_0^2 for ω^2:

$$\omega_0^2 = \frac{-b^2 + 2c}{2} \pm \sqrt{\frac{(b^2 - 2c)^2}{4} - c^2 + D^2}. \tag{4.30}$$

From this expression for ω_0^2 follows the initial statement. □

Assuming $\omega_0 \in \mathbb{R}$, we can compute a critical value τ_0 from equations (4.27) and (4.28):

$$\tau_0 = \frac{1}{\omega_0} \arctan\left(\frac{-\omega_0 b}{-\omega_0^2 + c}\right).$$

More precisely, any

$$\tau_{0,n} = \frac{1}{\omega_0} \left[\arctan\left(\frac{-\omega_0 b}{-\omega_0^2 + c}\right) + n\pi\right] \tag{4.31}$$

for $n \in \mathbb{N}$ is a critical value for τ due to the periodicity of tan. As a result, we expect a pair of purely imaginary, complex conjugate eigenvalues if $\tau = \tau_{0,n}$ for some $n \in \mathbb{N}$. If we can show that $i\omega_0$ and $-i\omega_0$ indeed cross the imaginary axis, we can provide evidence for the destabilisation of the equilibrium point and, moreover, the occurrence of a Hopf bifurcation.

4.3.2 Hopf bifurcation

Finally, we aim to show that all requirements for a Hopf bifurcation are fulfilled. According to the Hopf bifurcation theorem (Theorem 2.5.1), we must prove the following for eigenvalues $\lambda(\tau) = \alpha(\tau) + i\omega(\tau)$:

(i) $\alpha(\tau_{0,n}) = 0$,

(ii) $\omega_0 := \omega(\tau_{0,n}), \omega_0 > 0$,

(iii) $\frac{d\alpha(\tau)}{d\tau}(\tau_{0,n}) \neq 0$, and

(iv) there are no other eigenvalues λ of $A(\tau_{0,n})$ with $Re(\lambda) = 0$.

Furthermore, we must consider the conditions which must be satisfied. All in all, we can formulate and prove the following theorem:

Theorem 4.3.3. Let b, c and D be as defined in (4.18), (4.19) and (4.20), respectively:

$$\begin{aligned} b &:= \alpha_2 \frac{\bar{u}}{\bar{g_2}} + \alpha_1 \frac{\bar{v}}{\bar{g_1}} + \gamma_1 + \gamma_2 \\ c &:= \alpha_1 \alpha_2 \frac{\bar{v}}{\bar{g_1}} \frac{\bar{u}}{\bar{g_2}} + \alpha_1 \frac{\bar{v}}{\bar{g_1}} \gamma_2 + \alpha_2 \frac{\bar{u}}{\bar{g_2}} \gamma_1 + \gamma_1 \gamma_2 \\ D &:= \alpha_1 \alpha_2 \left(1 - \frac{\bar{u}}{\bar{g_1}}\right)\left(1 - \frac{\bar{v}}{\bar{g_2}}\right). \end{aligned}$$

Further, set

$$\omega_0 = \sqrt{\frac{-b^2+2c}{2} + \sqrt{\frac{(b^2-2c)^2}{4} - c^2 + D^2}}.$$

Presume the following conditions hold true:

(C1) $\alpha_1\alpha_2 > \gamma_1\gamma_2$

(C2) $\frac{-b^2+2c}{2} + \sqrt{\frac{(b^2-2c)^2}{4} - c^2 + D^2} > 0$

(C3) $\frac{(b^2-2c)^2}{4} - c^2 + D^2 \geq 0$

(C4) $b^2 + 2(\omega_0^2 - c) \neq 0.$

Then, a Hopf bifurcation occurs at $\tau = \tau_{0,n}$ as given in (4.31) for some $n \in \mathbb{N}$. In other words, there is a pair of purely imaginary eigenvalues, $i\omega_0$ and $-i\omega_0$, which cross the imaginary axis when $\tau = \tau_{0,n}$.

Proof. In Lemma 4.3.2, we have already shown that $i\omega_0$ and $-i\omega_0$ are eigenvalues with $\omega_0 = \omega(\tau_{0,n})$ and $\omega_0 > 0$ if conditions (C2) and (C3) are satisfied. The corresponding values $\tau_{0,n}$ have been determined (see equation 4.31). Furthermore, $i\omega_0$ and $-i\omega_0$ are the only purely imaginary eigenvalues, and $\alpha(\tau_{0,n}) = 0$. As a result, only the third requirement for a Hopf bifurcation remains to be checked.

Let $\lambda(\tau) := \alpha(\tau) + i\omega(\tau)$ be an eigenvalue in the proximity of some $\tau_{0,n}$. Substitution of λ by $\lambda(\tau)$ in the characteristic equation 4.21 yields

$$\lambda(\tau)^2 + \lambda(\tau)\, b + c - De^{-\lambda(\tau)\tau} = 0. \tag{4.32}$$

We can now proceed analogously to the respective section of the circadian pacemaker model analysis, again following [29, pp. 6-7]. Differentiating both sides of (4.32) with respect to τ leads to

$$2\,\frac{d\lambda}{d\tau}\,\lambda(\tau) + \frac{d\lambda}{d\tau}\,b = -De^{-\lambda(\tau)\tau}\left(\frac{d\lambda}{d\tau}\tau + \lambda(\tau)\right)$$

and further

$$\frac{d\lambda}{d\tau} = \frac{-De^{-\lambda(\tau)\tau}\,\lambda(\tau)}{2\,\lambda(\tau) + b + De^{-\lambda(\tau)\tau}\tau}. \tag{4.33}$$

To determine the sign of $\left[\frac{d(Re(\lambda))}{d\tau}\right]_{\tau=\tau_{0,n}}$ we will again consider the sign of $Re\left(\left[\left(\frac{d\lambda(\tau)}{d\tau}\right)^{-1}\right]_{\tau=\tau_{0,n}}\right)$, which is more convenient for the following calculations:

$$\left(\frac{d\lambda(\tau)}{d\tau}\right)^{-1} = \frac{2}{-De^{-\lambda(\tau)\tau}} + \frac{b}{-De^{-\lambda(\tau)\tau}\ \lambda(\tau)} - \frac{\tau}{\lambda(\tau)}. \tag{4.34}$$

Because $\frac{\tau}{\lambda(\tau)}$ is purely imaginary for $\tau = \tau_{0,n}$, we aim to compute

$$\begin{aligned}\operatorname{sgn}\left(\left[\frac{d(Re(\lambda))}{d\tau}\right]_{\tau=\tau_{0,n}}\right) &= \operatorname{sgn}\left(Re\left(\left[\left(\frac{d\lambda(\tau)}{d\tau}\right)^{-1}\right]_{\tau=\tau_{0,n}}\right)\right)\\ &= \operatorname{sgn}\left(Re\left(\left[\frac{2}{-De^{-\lambda(\tau)\tau}} + \frac{b}{-De^{-\lambda(\tau)\tau}\ \lambda(\tau)}\right]_{\tau=\tau_{0,n}}\right)\right).\end{aligned}$$

Since $e^{-i\omega_0\tau_{0,n}} = \cos(\omega_0\tau_{0,n}) - i\sin(\omega_0\tau_{0,n})$ and $\lambda(\tau_{0,n}) = \underbrace{\alpha(\tau_{0,n})}_{=0} + i\omega_0$,

$$\begin{aligned}Re\left(\left[\frac{2}{-De^{-\lambda(\tau)\tau}}\right]_{\tau=\tau_{0,n}}\right) &= Re\left(\frac{2}{-D[\cos(\omega_0\tau_{0,n}) - i\sin(\omega_0\tau_{0,n})]}\right)\\ &= Re\left(\frac{2\ [\cos(\omega_0\tau_{0,n}) + i\sin(\omega_0\tau_{0,n})]}{-D}\right)\\ &= \frac{2\ \cos(\omega_0\tau_{0,n})}{-D}\end{aligned}$$

and

$$\begin{aligned}Re\left(\left[\frac{b}{-D\ e^{-\lambda(\tau)\tau}\ \lambda(\tau)}\right]_{\tau=\tau_{0,n}}\right) &= Re\left(\frac{b}{-D\ \omega_0\ [\sin(\omega_0\tau_{0,n}) + i\cos(\omega_0\tau_{0,n})]}\right)\\ &= Re\left(\frac{b\ [\sin(\omega_0\tau_{0,n}) - i\cos(\omega_0\tau_{0,n})]}{-D\ \omega_0}\right)\\ &= \frac{b\ \sin(\omega_0\tau_{0,n})}{-D\ \omega_0}.\end{aligned}$$

From equations (4.27) and (4.28), we can further infer expressions equivalent to $\cos(\omega\tau)$ and $\sin(\omega\tau)$:

$$\cos(\omega\tau) = \frac{-\omega^2 + c}{D} \tag{4.35}$$

$$\sin(\omega\tau) = \frac{b\,\omega}{-D}. \tag{4.36}$$

As a result, we can conclude:

$$\operatorname{sgn}\left(\left[\frac{d(Re(\lambda))}{d\tau}\right]_{\tau=\tau_{0,n}}\right) = \operatorname{sgn}\left(Re\left(\left[\frac{2}{-De^{-\lambda(\tau)\tau}} + \frac{b}{-De^{-\lambda(\tau)\tau}\ \lambda(\tau)}\right]_{\tau=\tau_{0,n}}\right)\right)$$

$$= \operatorname{sgn}\left(\frac{2\ (c-\omega_0^2)}{-D^2} + \frac{b^2}{D^2}\right).$$

Hence,

$$\operatorname{sgn}\left(\left[\frac{d(Re(\lambda))}{d\tau}\right]_{\tau=\tau_{0,n}}\right) \neq 0$$

if $b^2 + 2(\omega_0^2 - c) \neq 0$, i.e. (C4) holds.

In summary, a Hopf bifurcation occurs at $\tau = \tau_{0,n}$ if conditions (C1) - (C4) are satisfied. □

According to the Hopf bifurcation theorem (Theorem 2.5.1), a limit cycle originates at the unstable equilibrium point $(\bar{u}, \bar{v})$ and is stable for $\tau > \tau_{0,n}$ if $b^2 - 2(c - \omega_0^2) > 0$ or stable for $\tau < \tau_{0,n}$ if $b^2 - 2(c - \omega_0^2) < 0$. Since the existence of limit cycles indicates periodic behaviour of the system, we seek to observe oscillations in computational simulations. For this purpose, we must first find parameter values which abide by conditions (C1) - (C4).

4.4 Setting the parameters of the model

Let us first recall the model we aim to simulate numerically:

$$\begin{aligned} \dot{g}_1(t) &= \alpha_1 g_2(t-\tau_1)\ \left(1 - \frac{g_1(t)}{\bar{g_1}}\right) - \gamma_1 g_1(t) \\ \dot{g}_2(t) &= \alpha_2 g_1(t-\tau_2)\ \left(1 - \frac{g_2(t)}{\bar{g_2}}\right) - \gamma_2 g_2(t). \end{aligned} \tag{4.37}$$

In the simulations, we take interest in solutions of the model varying with time t. Furthermore, we intend to investigate the role of the delay parameters τ_1 and τ_2. Therefore, we consider all other parameters, namely the production rates α_1 and α_2, the decay rates γ_1 and γ_2 as well as the maximal concentrations $\bar{g_1}$ and $\bar{g_2}$ to be constant. Importantly, a Hopf bifurcation, hence self-sustainingly oscillating behaviour, can only be expected on condition that the conditions (C1) - (C4) stated above are satisfied for the model parameters. Moreover, all parameters must be positive, which

results in six additional constraints. Mathematica provides a powerful tool to determine parameter values so that given conditions hold, namely `FindInstance` [38]. Applying the tool on the included parameters and conditions yielded that there exists no such set of parameters (see Appendix for Mathematica code). Although (C4) was disregarded for running time reduction, no set of parameters could be identified. What can be concluded from this result is that the conditions are too restraining and no parameters can be chosen so that a Hopf bifurcation, i.e. self-sustaining oscillations, could be observed. Evidently, the resulting question is which changes need to be made in the model for it to produce periodic behaviour.

4.5 An improved model

A promising idea to improve the model with the objective to obtain periodic solutions is to introduce additional delays to the model, since delays were shown to have an impact on the characteristics of solutions in section 3. Moreover, there is a strong link between self-sustaining oscillations and delayed negative feedback, which was highlighted in section 1.5. Therefore, further delays were added to the production terms of both equations. More precisely, delays were introduced in those terms which limit the synthesis of a gene product depending on the levels of the respective other gene product. Hence, the negative feedback mechanism became delayed as well (the positive feedback mechanism had already been delayed before). As a consequence, the following system seemed to be promising with respect to periodic solutions:

$$\begin{aligned} \dot{g}_1(t) &= \alpha_1 g_2(t-\tau_1)\left(1-\frac{g_1(t-\tau_3)}{\bar{g_1}}\right)-\gamma_1 g_1(t) \\ \dot{g}_2(t) &= \alpha_2 g_1(t-\tau_2)\left(1-\frac{g_2(t-\tau_4)}{\bar{g_2}}\right)-\gamma_2 g_2(t). \end{aligned} \tag{4.38}$$

These changes made the model, and thus the mathematical analysis, significantly more complex. Consequently, we reverted to numerical simulations in order to analyse the behaviour of the system. First of all, parameters of the model had to be set as a prerequisite for any simulation. Since we had not determined any conditions mathematically, `FindInstance` was not an option. Therefore, a set of parameters was found out by trial and error. Numerical restrictions must be emphasized here, as they significantly limited the choice of parameters. In more detail, precision was lost, as very small numbers appeared according to Mathematica's warning message ("General::munfl", [39]). Importantly, these limitations had an impact on the choice

of delay parameters as well. In particular, they required $\tau_1 \neq \tau_3$ and $\tau_2 \neq \tau_4$. Nevertheless, a set of parameters could be found so that the above system of delay differential equations yields periodic solutions. Corresponding parameter values can be found in Table 4.1 and the simulation in Fig. 4.1.

Table 4.1. Parameter values for system (4.38).

α_1	α_2	γ_1	γ_2	$\bar{g_1}$	$\bar{g_2}$	τ_1	τ_2	τ_3	τ_4
2.1	2.1	1.32	1.32	80	75	3	3	5	5

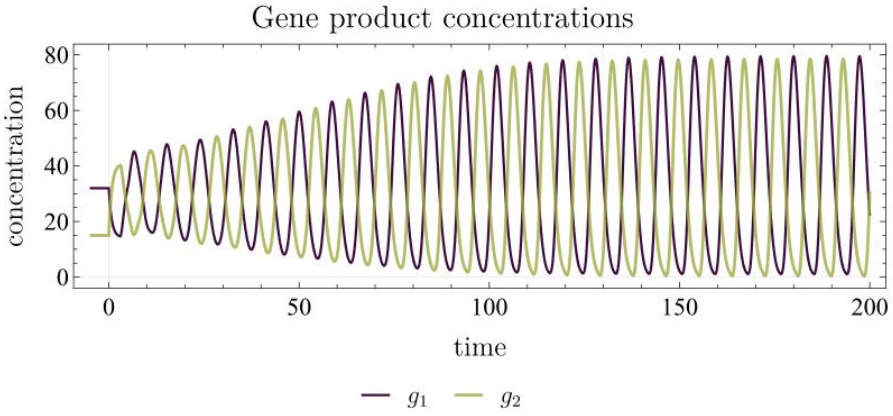

Figure 4.1. Simulation of system (4.38) with parameter values given in Table 4.1. The simulation was carried out using Mathematica's `NDSolve` and the resulting functions were visualised by means of Mathematica's `Plot` tool. As initial conditions, we set $g_1(0) = 32$ and $g_2(0) = 15$.

To conclude, we have finally found a system of delay differential equations resulting in periodic solutions for a set of parameters. We could further approximately determine bifurcation points in numerical experiments. Thereby, we proceeded analogously to the mathematical analysis in chapters 3 and 4 and examined solutions of the model upon variation of the delay parameters. The results are presented in the following section.

4.6 Bifurcation analysis by means of simulations

A combination of numerical simulations and visualisations allows for a very straightforward analysis of solutions to systems of (delay) differential equations. In the following, we will analyse some exemplary cases of how solutions of the system change upon variation of the delay parameters τ_1, τ_2, τ_3 and τ_4.

Example 4.6.1 (Variation of $\tau_1 = \tau_2$)**.** Set $\tau_3 = \tau_4 = 5$ in system (4.38). Fig. 4.2 shows that periodic behaviour appears at approximately $\tau_1 = \tau_2 = 2.85$, which is where we can assume the occurrence of a Hopf bifurcation.

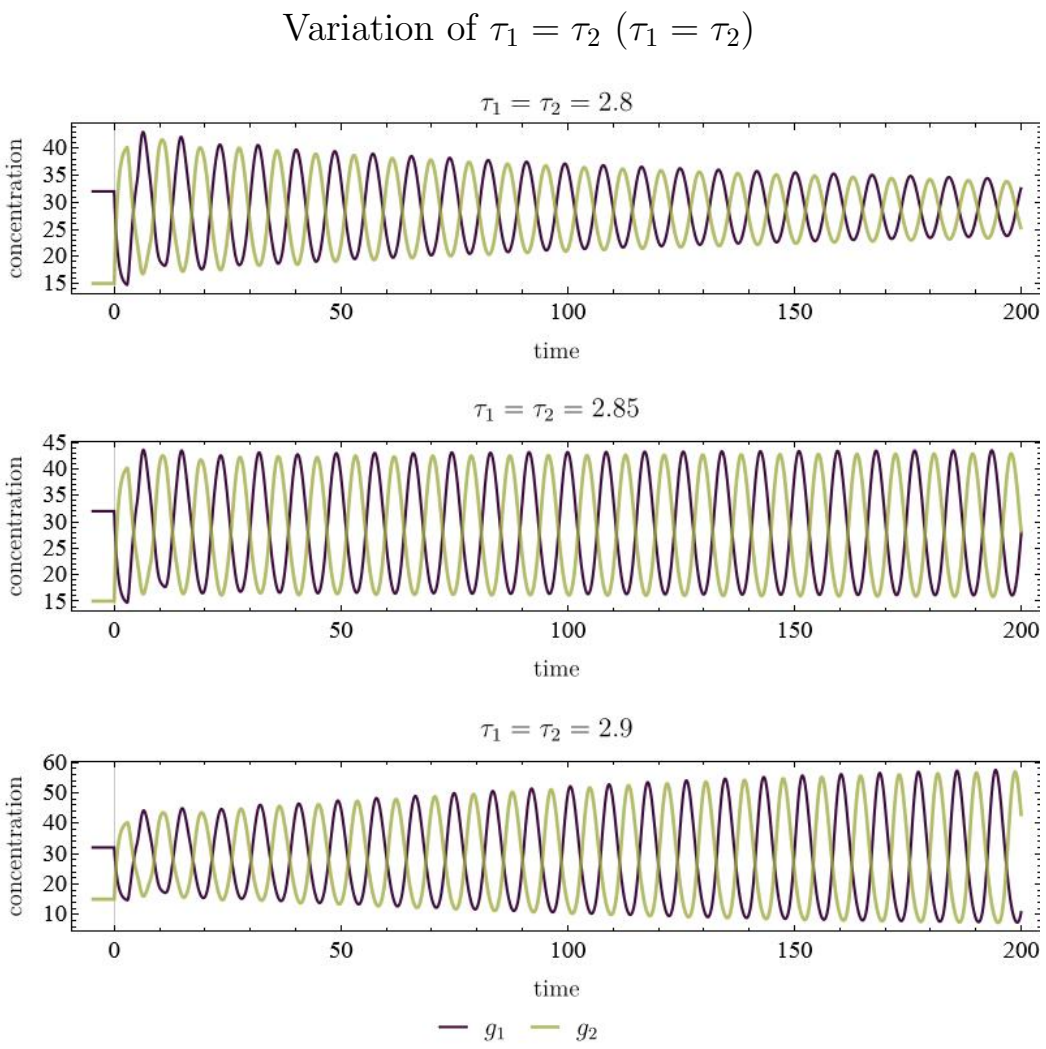

Figure 4.2. Variation of τ_1 and τ_2 with $\tau_1 = \tau_2$. For the remaining parameters see Table 4.1 and the initial conditions are $g_1(0) = 32$, $g_2(0) = 15$. The simulations and visualisations were carried out with Mathematica's `NDSolve` and `Plot`.

A stable limit cycle seems to exist for $\tau_1 = \tau_2 > 2.85$, since self-sustaining oscillations can be observed in this case. For $\tau_1 = \tau_2 < 2.85$, however, the amplitude of the oscillations decreases with time indicating convergence towards an equilibrium. Figures which cover longer time spans provide further evidence for periodic or decreasing behaviour and can be found in the Appendix.

Example 4.6.2 (Variation of $\tau_3 = \tau_4$)**.** Variation of τ_3 and τ_4 (with fixed $\tau_1 = \tau_2 = 3$) indicates a bifurcation point at approximately $\tau_3 = \tau_4 = 5.28$ (see Fig. 4.3). In contrast to example 4.6.1, the limit cycle appears to be stable for $\tau_3 = \tau_4 < 5.28$ and unstable for $\tau_3 = \tau_4 > 5.28$. In the Appendix, Figures which cover longer time spans are provided for this example as well.

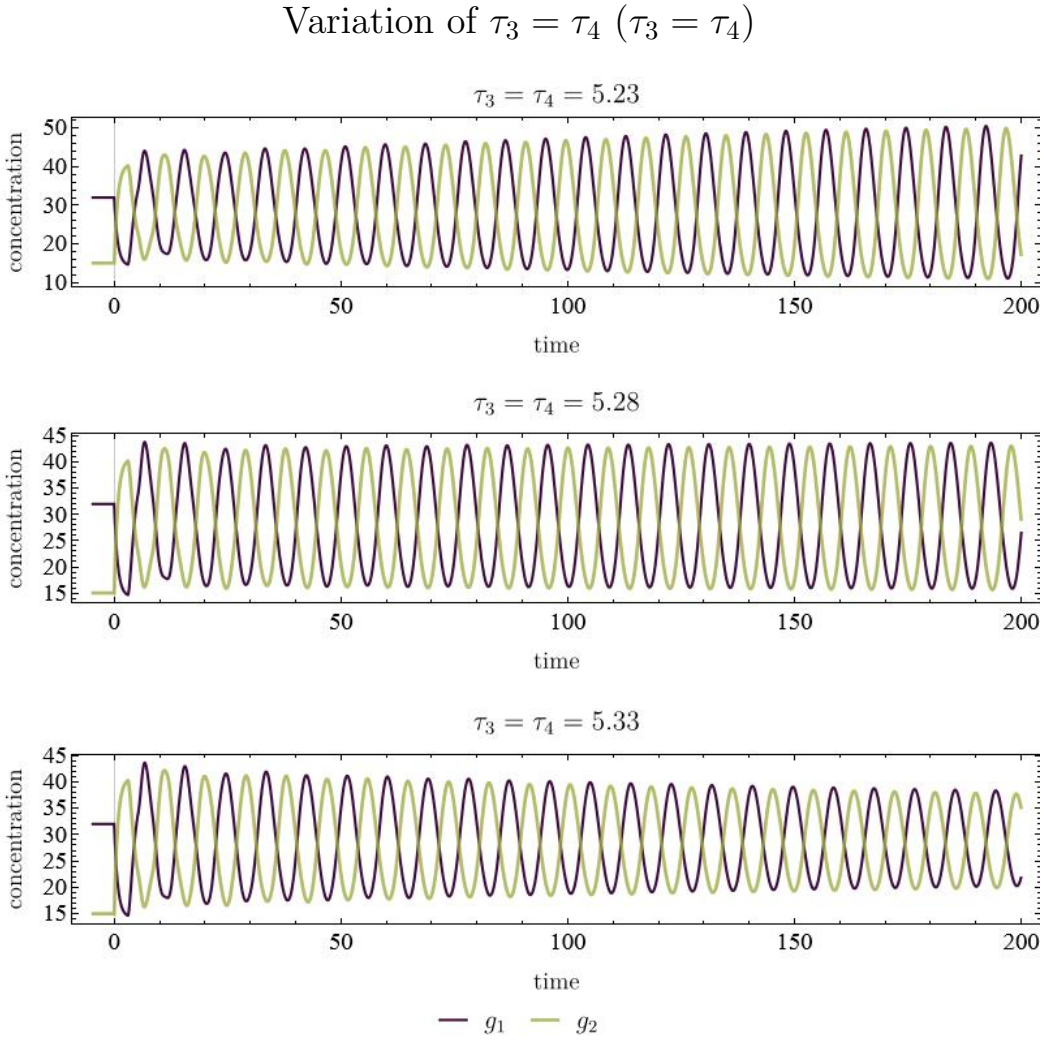

Figure 4.3. Variation of τ_3 and τ_4 with $\tau_3 = \tau_4$. For other parameter values see Table 4.1, the initial conditions are given by $g_1(0) = 32$ and $g_2(0) = 15$. The simulations were run using `NDSolve` and visualised with `Plot`.

So far, we have examined examples with $\tau_1 = \tau_2$ and $\tau_3 = \tau_4$ in system (4.38):

$$\dot{g}_1(t) = \alpha_1 g_2(t-\tau_1)\left(1 - \frac{g_1(t-\tau_3)}{\bar{g}_1}\right) - \gamma_1 g_1(t)$$
$$\dot{g}_2(t) = \alpha_2 g_1(t-\tau_2)\left(1 - \frac{g_2(t-\tau_4)}{\bar{g}_2}\right) - \gamma_2 g_2(t).$$

In Example 4.6.3, a case of variation of τ_1 and τ_2 (with $\tau_1 \neq \tau_2$) is shown. Furthermore, we can not only vary one delay per equation but also both delays within one equation of the system which is done in Example 4.6.4.

Example 4.6.3 (Variation of τ_1 and τ_2)**.** Fig. 4.4 shows exemplary cases for $\tau_1 \neq \tau_2$. If τ_1 is below the critical value, i.e. $\tau_1 < 2.85$, while $\tau_2 = 3$, which is slightly above the approximate bifurcation point, the amplitude gradually decreases. If τ_2, however, is increased further above the bifurcation point, in this example to $\tau_2 = 3.35$, self-sustaining oscillations are recovered.

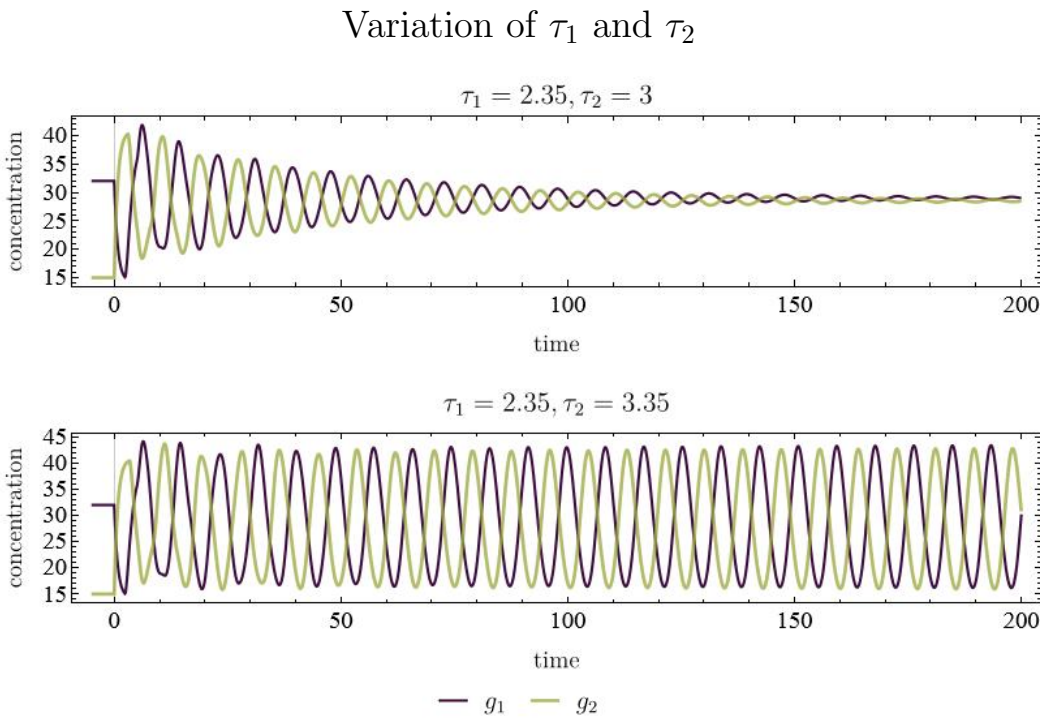

Figure 4.4. Variation of τ_1 and τ_2, while $\tau_3 = \tau_4 = 5$. Other parameter values can be found in Table 4.1 and $g_1(0) = 32$ and $g_2(0) = 15$ are set as initial conditions. (Simulation again by means of `NDSolve` and visualisation with `Plot`.)

Example 4.6.4 (Variation of τ_1 and τ_3)**.** In Fig. 4.5, an example is given for the case that a larger value of τ_3 compensates for τ_1 being below the critical value. Thus, the delay parameters within one production term can, apparently, recover self-sustained oscillations as well.

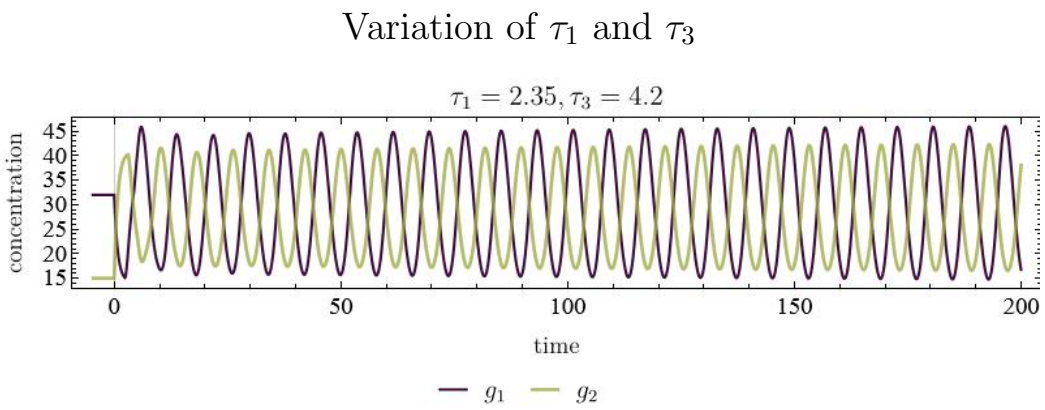

Figure 4.5. Variation of τ_1 and τ_3, while $\tau_2 = 3, \tau_4 = 5$. Other parameters are set as given in Table 4.1, the initial conditions are $g_1(0) = 32$ and $g_2(0) = 15$. The simulation was run with `NDSolve` and visualised with `Plot`.

In conclusion, the examples given convey an impression of (periodic) model behaviour and of how it is effected by the choice of delay parameters. The figures illustrate the self-sustaining oscillations, which are probably caused by the appearance of limit cycles at Hopf bifurcations. In more detail, we can observe periodic behaviour in Example 4.6.1, but only if the varying delay parameters exceed a certain critical value. In contrast, τ_3 and τ_4 must be set below a certain value in order to obtain periodic behaviour in Example 4.6.2. Example 4.6.3 and Example 4.6.4 show that if τ_1 is below a critical value, the periodic behaviour can be recovered by a sufficiently large value of either τ_2 or τ_3.

Chapter 5

Discussion

The main objectives of this thesis were a) the construction of a mathematical model of gene expression which reproduces the periodic behaviour of the modelled system and b) the analysis of core elements of mathematical models with periodic behaviour. Having refined the first proposed DDE model by adding additional delay parameters, periodic behaviour of the model was observed. With respect to b), delayed (negative) feedback and Hopf bifurcations at destabilised equilibrium points turned out to be decisive elements for self-sustaining oscillations.

Periodic behaviour results from delayed feedback

Rigorous mathematical examination, including linearisation about nonzero equilibrium points and subsequent stability and bifurcation analysis, shed light on the mathematical properties of two simple models of gene expression. The first, proposed by Lema, Golombek and Echave, consists of only one delay differential equation and models one self-inhibitory gene. Importantly, the inhibitory effect exerts with a certain time delay, hence the DDE. [23] The analysis showed that the delay facilitates the destabilisation of the equilibrium point, thereby indicating periodic behaviour. The latter is in line with discussed effects of delayed negative feedback in 1.5. On a side note, the mathematical analysis brought about a condition for model parameters: $C > K_d$ (see Lemma 3.2.2 and Theorem 3.2.3). Interestingly, this corresponds to the necessity that the derivative of the production term is greater than the derivative of the degeneration term. Similarly, the condition $\alpha_1\alpha_2 > \gamma_1\gamma_2$ (i.e. the product of synthesis rates must be greater than the product of degeneration rates) is imposed upon the self-constructed model. Since this model has the same equilibrium point

before and after the refinement, we can assume that the condition is equally relevant for the improved model. Both conditions are not surprising when considered in the biological context: If the degeneration of the molecules exceeds their synthesis, the system will sooner or later reach an equilibrium. More precisely, the gene product concentration(s) will level off zero. Therefore, the mentioned conditions must be met if nonzero equilibrium points are examined.

Parameter settings

With the crucial roles of feedback mechanisms and delay parameters in mind, we adapted a fundamental model of gene expression to the context of the cell cycle and included delay parameters. In the course of the analysis, several conditions for parameters arose and no suitable parameter set could be found. Thus, the conditions were too restrictive and probably mutually exclusive, all of which gave reason to improve the model.

More delay parameters finally allow for periodic behaviour

In the first attempt, only the first part of the production terms contained a delay parameter in both equations of the system. We then added delay parameters to the second parts, which limit the increase of the gene product concentrations. Thus, the negative feedback became delayed in addition to the already delayed positive feedback. Considering the biological context, it makes sense that the negative feedback becomes effective with some time lag as well, since this kind of feedback is likely to require regulatory mechanisms and additional components as well. As a result of the refinement, the model became considerably more complex. Nevertheless, numerical simulations in Wolfram Mathematica facilitated the analysis of the system and, finally, the expected periodic behaviour could be observed. Therefore, the proposed model reproduces the alternating expression of two gene products regulated by feedback mechanisms.

It is important to highlight that both parts of the production term had to include delay parameters for self-oscillating behaviour to occur. More precisely, delayed positive feedback was not sufficient for periodic solutions of the proposed model. Subsequently, bifurcation points, i.e. critical values for the delay parameters, could be approximated. Interestingly, variation of the delay parameters (with a constant

set of values for the other model parameters) showed tendencies of balancing: If one delay parameter was set below its critical value, it could be balanced out by another delay parameter and periodic behaviour could still be observed. Even though the model does not allow for conclusions on biological systems due to its simplicity, it can be said that such a balancing effect could be useful in real biological systems. If one step in a succession of (production) steps takes longer and delays the whole process, the delay can be balanced out by another step being faster.

Furthermore, balanced variation of τ_1 and τ_3 (Fig. 4.5) showed slightly different oscillation patterns compared to preceding figures. The concentration of the second gene product seems to be further limited in its maximal and minimal values. Thus, apart from other model parameters like production and decay rates, the delay parameters can, apparently, influence properties of the oscillations (like the amplitude). Unfortunately, numerical limitations significantly restricted further possibilities to examine different effects, since they limited the choice of (delay) parameters.

Limitations of the undertaken analysis

Although Wolfram Mathematica simplified the analysis considerably, the simulation possibilities were limited by numerical issues mentioned before. For instance, the delays in the first and the second part of the productive term could not be set to equal values due to loss of precision. Closely linked to this issue, some interesting properties of the oscillations observed could not be examined in detail. For example, it was not possible to determine in how far the (delay) parameter values influence the amplitude, period and frequency of the oscillations. This would have been of particular interest since gene products are assumed to be synthesised periodically (at least as the cell goes trough multiple consecutive cell cycles), but at varying frequencies. By variation of the model parameters, the model could be fit to different gene expression dynamics of different gene products.

In terms of significance, the model would need more complexity to reproduce the real biological system. Since the focus of this thesis was mainly on the mathematical properties of periodic model behaviour, biological details were secondary. As a result, the model could be properly analysed with mathematical tools, but lacks significance for biological research. However, some crucial aspects of mathematical models of circular processes could be emphasised, which might also be applicable to more

complex models.

Conclusion and outlook

In this thesis, the relevance of systems of delay differential equations for models of periodic behaviour was highlighted based on two different models of gene expression. With mathematical rigour, the very foundations of this model behaviour could be examined: Hopf bifurcations occur at equilibrium points which are destabilised by an adequate choice of delay parameters. In general, parameter settings as well as (delayed) feedback mechanisms showed to be decisive for the model's behaviour.

Based on the refined model, it would be interesting to further investigate the mathematical foundations of the observed self-sustaining oscillations. Furthermore, the question arises, which of the four delay parameters are sufficient and necessary for the periodic behaviour. Further research might then investigate more complex models including some more biological details. Here, Mathematica can be a very useful tool if the limits of manual calculations are reached. Furthermore, it offers powerful numerical tools for DDEs whose applications exceed the methods applied in this thesis. Comparable python modules like `JiTCDDE` (see [3] for details) have still resulted in more limitations in this respect. In general, it is likely that computational tools for systems of DDEs will be further improved in the future, since DDEs are promising elements for mathematical modelling.

In order to increase the significance for biological research, a multi-disciplinary approach including experimental data could prove useful. The analysis performed in this thesis have certainly demonstrated the potential of research at the interface of scientific computing, mathematics and molecular biology.

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Appendix

Stability of the equilibrium point of the delay-free system

In section 4.1, more precisely in system 4.1, the following preliminary ODE model is briefly mentioned:

$$\dot{g}_1(t) = \alpha_1 g_2(t) \left(1 - \frac{g_1(t)}{\bar{g_1}}\right) - \gamma_1 g_1(t)$$
$$\dot{g}_2(t) = \alpha_2 g_1(t) \left(1 - \frac{g_2(t)}{\bar{g_2}}\right) - \gamma_2 g_2(t).$$

It can be determined quickly that the system reaches a constant equilibrium by substitution of

$$g_2 = \frac{\dot{g_1} + \gamma_1 g_1}{\alpha_1 \left(1 - \frac{g_1}{\bar{g_1}}\right)}$$

into the second equation of the system. Since we aim to find equilibrium points of the system (as defined in Definition 2.1.1), set $\dot{g_1} = \dot{g_2} = 0$, which yields

$$g_1 = \frac{\bar{g_1}\bar{g_2}(\alpha_1\alpha_2 - \gamma_1\gamma_2)}{\alpha_2(\alpha_1\bar{g_2} + \gamma_1\bar{g_1})},$$

and analogously

$$g_2 = \frac{\bar{g_1}\bar{g_2}(\alpha_1\alpha_2 - \gamma_1\gamma_2)}{\alpha_1(\alpha_2\bar{g_1} + \gamma_2\bar{g_2})}.$$

This result does not come as a surprise, since the computation is quite similar to the determination of an equilibrium point of the model with delay differential equations, given in system 4.2. In contrast to the DDE system, there are no delay parameters τ_1, τ_2 which could destabilise the equilibrium point. Therefore, the system will at some point reach the equilibrium

$$G = (\frac{\bar{g_1}\bar{g_2}(\alpha_1\alpha_2 - \gamma_1\gamma_2)}{\alpha_2(\alpha_1\bar{g_2} + \gamma_1\bar{g_1})}, \frac{\bar{g_1}\bar{g_2}(\alpha_1\alpha_2 - \gamma_1\gamma_2)}{\alpha_1(\alpha_2\bar{g_1} + \gamma_2\bar{g_2})}).$$

Extensions to bifurcation analysis

In section 4.6, bifurcation points were approximated by means of numerical simulations. Fig. 5.1 and Fig. 5.2 (Fig. 5.3 and Fig. 5.4) show the first and the third panel of Fig. 4.2 (Fig. 4.3) for longer time spans, which better illustrates the described long-term behaviour.

Example 4.6.1

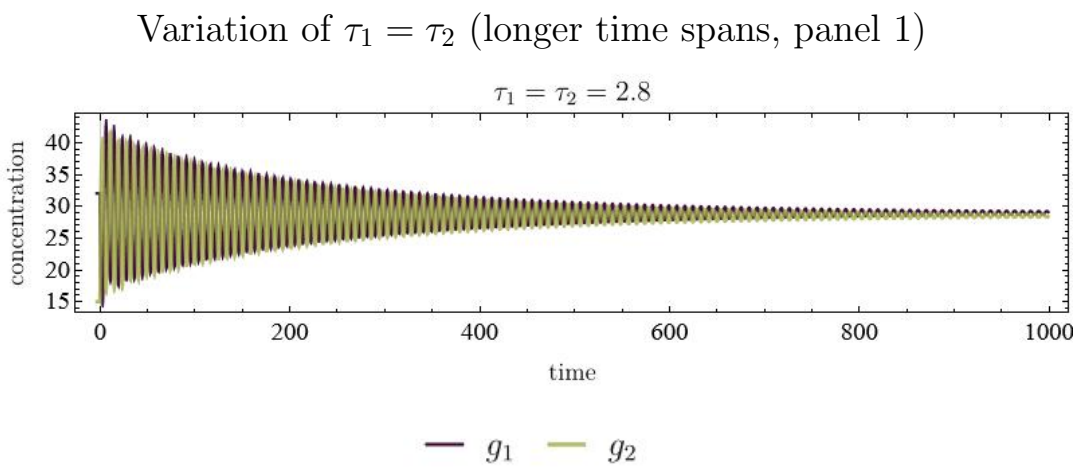


Figure 5.1. Variation of τ_1 and τ_2, $\tau_1 = \tau_2$ with longer time spans. For further details see Fig. 5.5.

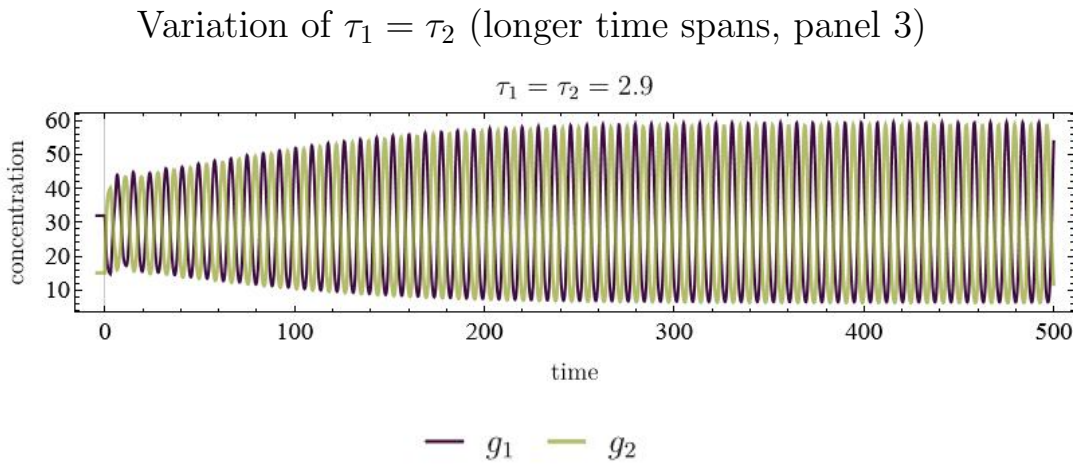


Figure 5.2. Variation of τ_1 and τ_2, $\tau_1 = \tau_2$ with longer time spans. For further details see Fig. 5.5.

Example 4.6.2

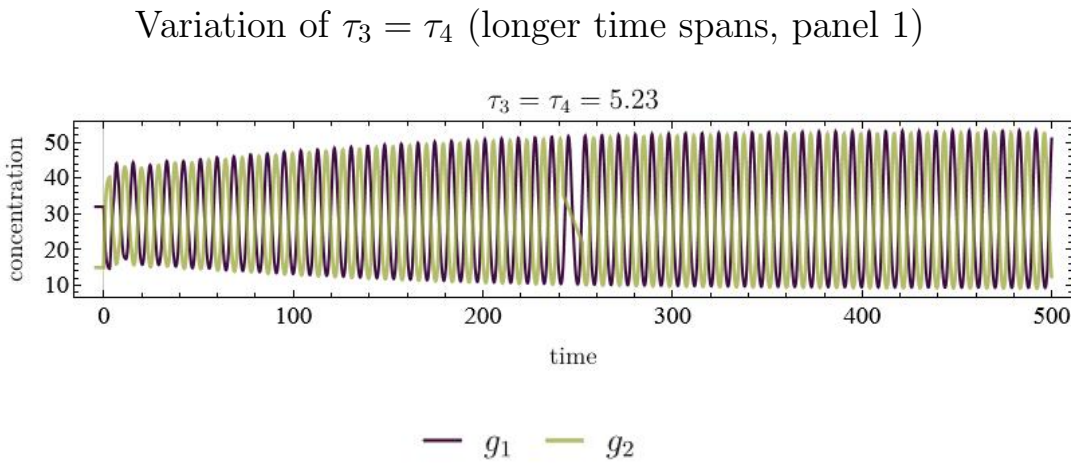


Figure 5.3. Variation of τ_3 and τ_4, $\tau_3 = \tau_4$ with longer time spans. For further details see Fig. 5.6.

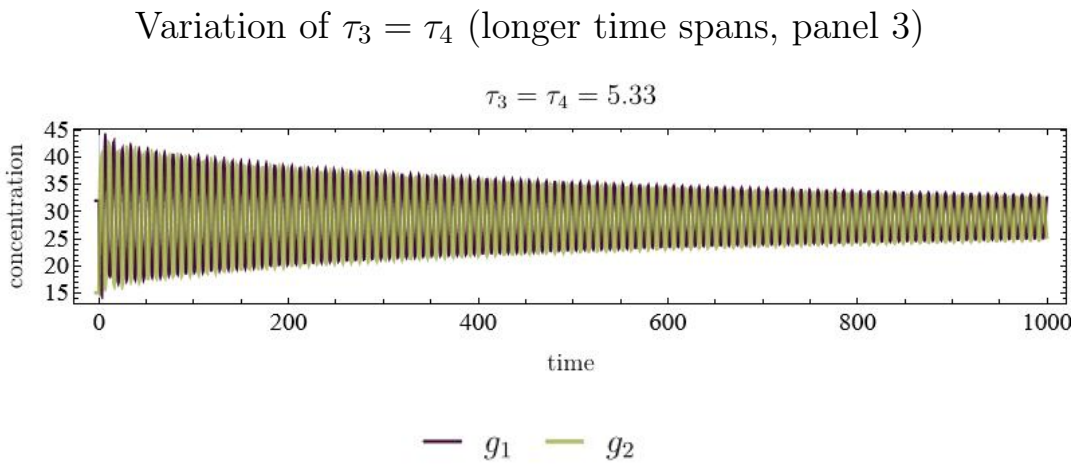


Figure 5.4. Variation of τ_3 and τ_4, $\tau_3 = \tau_4$ with longer time spans. For further details see Fig. 5.6.

Furthermore, Fig. 4.2 and 4.3 show quite clearly what the model behaviour looks like before and after the bifurcation point thanks to rather large steps (0.05). In Fig. 5.5 and Fig. 5.6, a more detailed depiction is provided.

Example 4.6.1

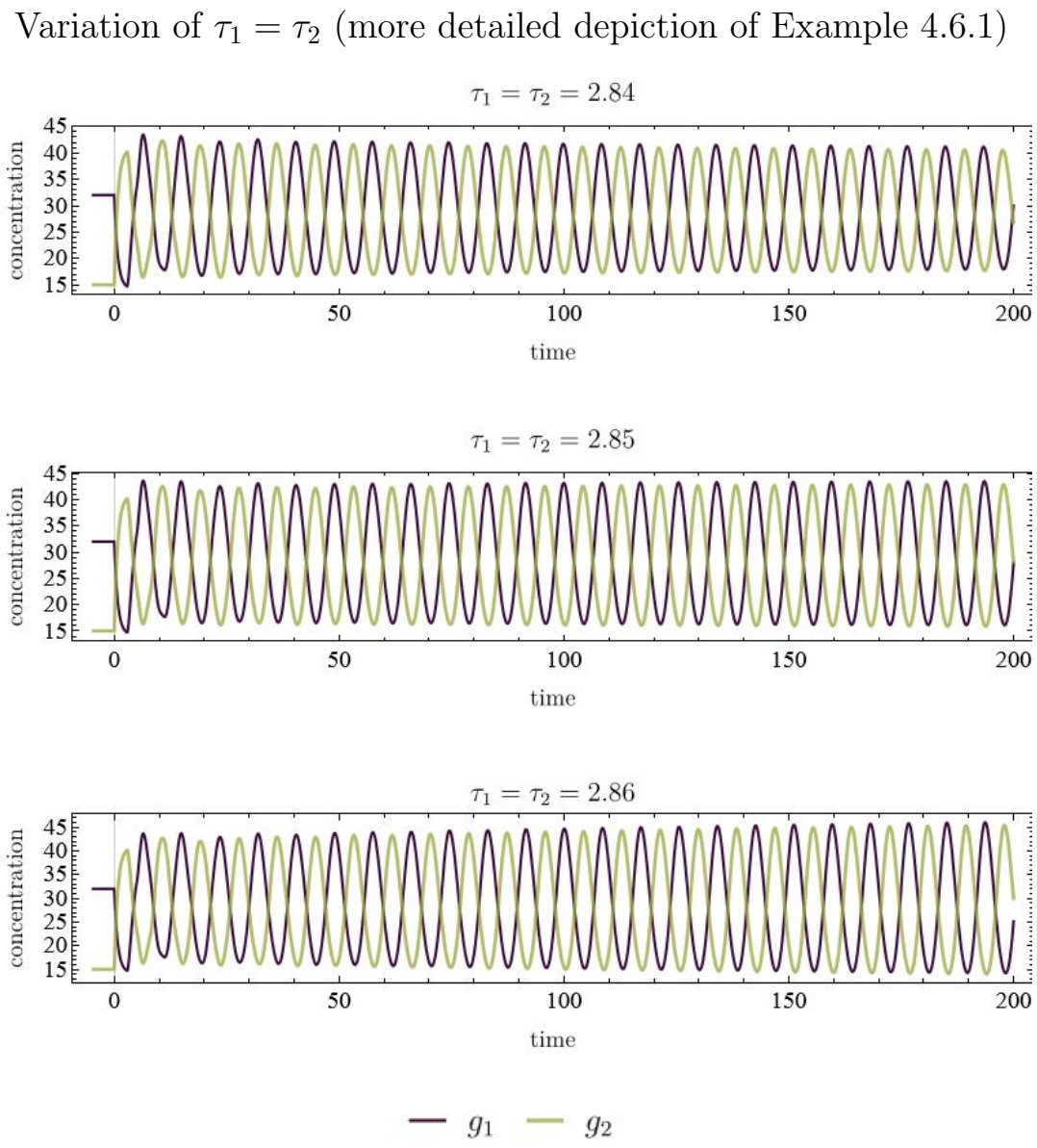


Figure 5.5. Variation of τ_1 and τ_2, $\tau_1 = \tau_2$ with smaller step sizes (0.01). The remaining parameters are as given in Table 4.1 and the initial conditions are $g_1(0) = 32$ and $g_2(0) = 15$. The simulations were carried out by means of Mathematica's `NDSolve` and for the visualisation, `Plot` was used.

Example 4.6.2

Variation of $\tau_3 = \tau_4$ (more detailed depiction of Example 4.6.2)

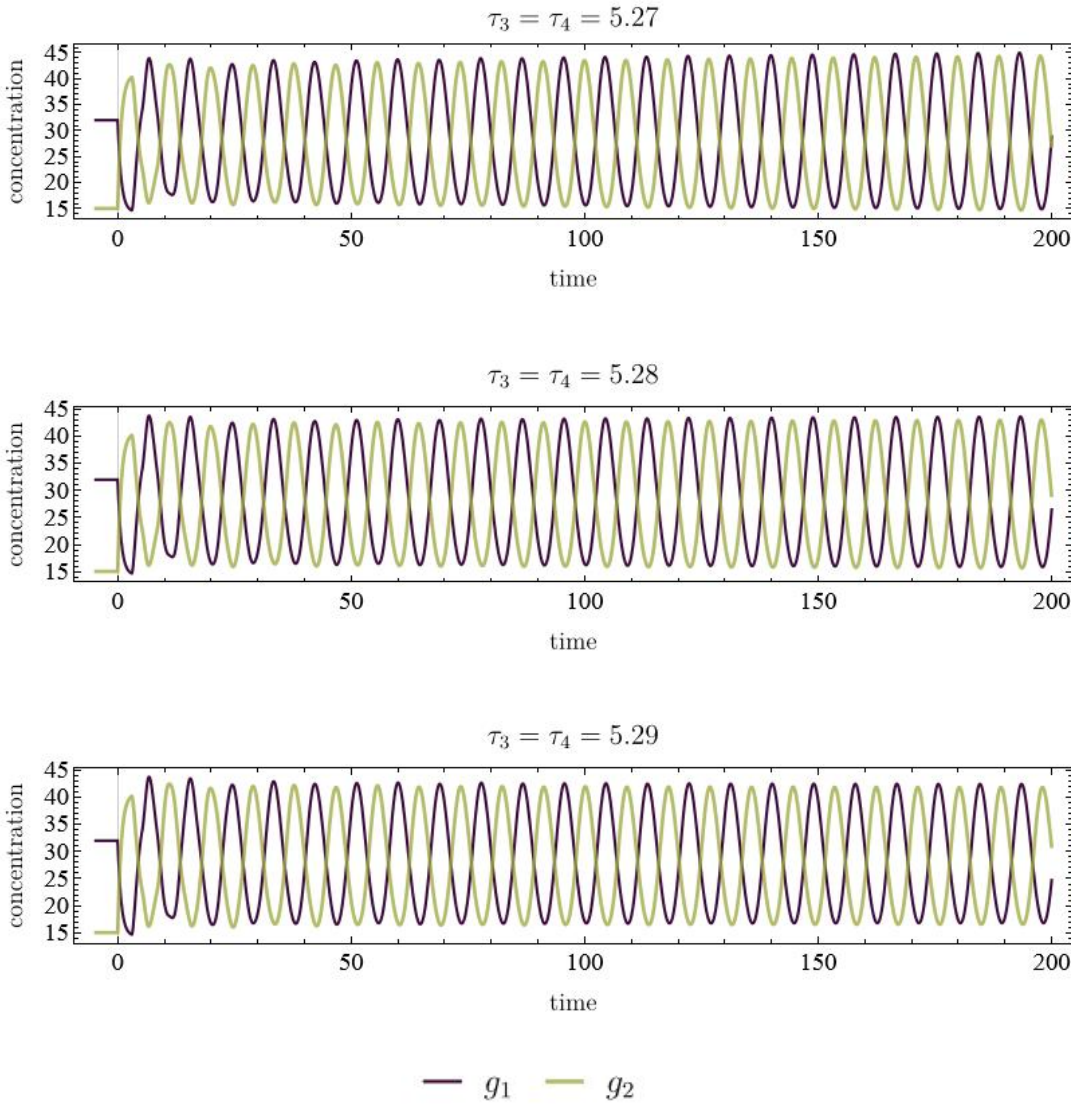


Figure 5.6. Variation of τ_3 and τ_4, $\tau_3 = \tau_4$ with smaller step sizes (0.01). For other parameter values see Table 4.1, initial conditions are given by $g_1(0) = 32$ and $g_2(0) = 15$. The simulations were run using `NDSolve` and visualised with `Plot`.

Exemplar Mathematica Code

For better reproducibility and transparency, exemplar snippets of Mathematica code are provided in this section.

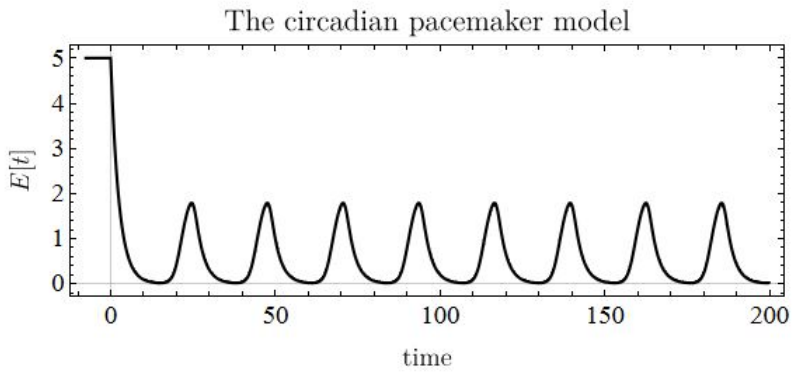
Circadian pacemaker model – simulation

```
In[193]:= solution = NDSolve[{x'[t] == 1 / (1 + (x[t - 8] / 0.04)^2.5) - 0.4 * x[t],
                              x[t /; t ≤ 0] == 5}, x, {t, -8, 200}]

Out[193]= {{x → InterpolatingFunction[ Domain: {{-8., 200.}}
                                       Output: scalar ]}}
```

```
In[194]:= plt = Plot[Evaluate[{x[t]} /. First[solution]], {t, -8, 200},
          PlotRange → All,
          PlotTheme → "Scientific", PlotStyle → {Black},
          ImageSize → {600, 200}, AspectRatio → 7 / 20,
          FrameLabel → (MaTeX[#, Magnification → 14 / 12] &) /@ {time, "E[t]"},
          FrameStyle → BlackFrame,
          BaseStyle → {FontFamily → "Latin Modern Roman", FontSize → 14},
          PlotLabel → MaTeX[Text["The circadian pacemaker model"],
            Magnification → 16 / 12], PlotRange → Full]
```

Out[194]=



FindInstance yielding no suitable parameter set

```
In[203]:= FindInstance[a1 * a2 > ga1 * ga2 && osq > 0 && 0 < a1 && 0 < a2 &&
          0 < g1 && 0 < g2 && 0 < ga1 && 0 < ga2, {a1, a2, ga1, ga2, g1, g2}]

Out[203]= {}
```

Simulations of the proposed model and visualisation

In[204]:=
```
sol2 =
 NDSolve[{x'[t] == 2.1 * y[t - 2.9] (1 - x[t - 5] / 80) - 1.32 * x[t],
   y'[t] == 2.1 * x[t - 2.9] (1 - y[t - 5] / 75) - 1.32 * y[t],
   x[0] == 32, y[0] == 15}, {x, y}, {t, -5, 200}]
```

NDSolve: Conditions given at t = 0.` will be interpreted as initial history functions for t /; t ≤ 0..

Out[204]=
```
{{x → InterpolatingFunction[ <Domain: {{-5., 200.}} Output: scalar> ],
  y → InterpolatingFunction[ <Domain: {{-5., 200.}} Output: scalar> ]}}
```

In[205]:=
```
p1 = Plot[Evaluate[{x[t], y[t]} /. sol2], {t, -5, 200},
  PlotRange → All,
  PlotTheme → "Scientific",
  PlotStyle → {Directive[ColorData["GreenPinkTones", 1]],
    Directive[ColorData["DarkRainbow", 0.5], Thick, Opacity[0.8]]},
  ImageSize → {600, 200}, AspectRatio → 3.5 / 20,
  FrameLabel → (MaTeX[#, Magnification → 14 / 12] &) /@
    {time, concentration}, FrameStyle → BlackFrame,
  BaseStyle → {FontFamily → "Latin Modern Roman", FontSize → 14},
  PlotLabel → MaTeX["\\tau_1 = \\tau_2 = 2.9", Magnification → 16 / 12],
  PlotRange → Full]
```

Out[205]=