The Goodwin oscillator and its legacy

Didier Gonze [(1)] and Peter Ruoff [(2)]

(1) Unité de Chronobiologie Théorique, Service de Chimie Physique CP 231,
Université Libre de Bruxelles, Bvd du Triomphe, 1050 Bruxelles, Belgium

(2) Centre for Organelle Research, University of Stavanger,
Richard Johnsens gate 4, 4021 Stavanger, Norway

Abstract

In the 1960's Brian Goodwin published a couple of mathematical models showing how feedback inhibition can lead to oscillations and discussed possible implications of this behaviour for the physiology of the cell. He also presented key ideas about the rich dynamics that may result from the coupling between such biochemical oscillators. Goodwin's work motivated a series of theoretical investigations aiming at identifying minimal mechanisms to generate limit cycle oscillations and deciphering design principles of biological oscillators. The three-variable Goodwin model (adapted by Griffith) can be seen as a core model for a large class of biological systems, ranging from ultradian to circadian clocks. We summarize here main ideas and results brought by Goodwin and review a couple of modeling works directly or indirectly inspired by Goodwin's findings.

Keywords: Goodwin model, limit cycle oscillations, feedback inhibition, circadian rhythms

The Goodwin oscillator: feedback inhibition, non-linearity, and limit cycle

The development and adaptation of living organisms rely on the temporal ordering and spatial self-organization of cellular events. Regulatory mechanisms play a central role in various aspects of cell physiology, including the control of tissue size (proliferation/apoptosis), the metabolic balance (e.g. glucose homeostasis), as well as the response to environmental stress (immunity) and to periodic environmental changes (day/night cycle). These mechanisms originate at the cellular level, through the regulation of enzyme activity and of gene expression. Feedback inhibition, whereby enzyme activity is repressed by one of the metabolic products constitutes one of the major regulatory mechanism. While several examples of feedback inhibition were reported in the sixties (Jacob and Monod, 1961, 1962; Umbarger, 1961), the dynamical implication of this mechanism was not yet formally investigated until Brian Goodwin entered the scene.

Goodwin's first publications on this subject were a seminal book based on his 1959 PhD thesis and a follow-up paper where he expounded a couple of thoughtful ideas, supported by mathematical models and results of numerical simulations (Goodwin, 1963, 1965). His motivation stems from several well documented examples of feedback inhibition. For example, cytidine triphosphate (CTP) was known to inhibit aspartate transcarbamoylase (ATCase), the first enzyme in the biosynthesis of pyrimidines in *B. subtilis* (Masters and Donachie 1966). Similarly, it was shown that tryptophan inhibits anthranilate synthetase, an enzyme involved in its biosynthesis pathway, in *E. coli* (Cohen and Jacob 1959). To describe such a mechanism, Goodwin devised a mathematical model describing the time evolution of two variables, which can be interpreted as follows: a given gene is transcribed into mRNA (variable X) which is then translated into protein (variable Y). The latter acts as a repressor: it inhibits mRNA synthesis (Fig. 1A). This repression is described by a nonlinear, hyperbolic function: $f = K/(K + Y)$, which decreases with increasing inhibitor concentration (Y) and determines the transcription rate. The other processes follow zero-order or first-order kinetics (Fig. 1B). Numerical integration of these equations produces self-sustained oscillations of X and Y (Fig. 1D). This first observation challenges the commonly accepted idea that concentrations would eventually stabilize at equilibrium. This minimal model also highlights the critical role of nonlinear negative feedback in the generation of oscillatory behaviour and serves as a basis to investigate the physiological consequences of such oscillations.

Anticipating that this type of regulatory circuit would not be found as an isolated system in the cell, Goodwin (1963, 1965) explored possible dynamical behaviours resulting from the interaction between two oscillators. He considered the case of two repressors that can repress both the genes coding for itself and the gene coding for the other repressor. Through numerical simulations, performed for various sets of parameter values, he observed several types of non-trivial behaviours: entrainment (obtained when one oscillator drives the second one), beating (quasi-periodicity), synchronization with various phase relationships, subharmonic resonance (associated to frequency demultiplication) or asynchronous quenching (oscillation death). Importantly, Goodwin remarked that the type and stability of the resulting behaviour depends on the amplitude of the free oscillators (itself related to the non-linearity of the repression) and on their relative frequencies (Goodwin, 1963). Goodwin commented on the possible physiological roles that such complex regulatory networks may have. For example, anti-phase oscillations may underlie the circadian organization in the algae *Gonyaulax* where certain processes (such as photosynthesis) show a peak of activity during the day while other processes (such as luminescence) are maximal at night.

An important caveat of the two-variable model should be underlined. Due to the zero-order kinetics of the degradation rates, the variables, supposed to represent chemical concentrations, may become negative (Fig. 1D), questioning the validity of the model to describe biological observations. This limitation can however be circumvented by using the Michaelis-Menten type of degradation kinetics (Fig. 1C). This guarantees that the variables remain positive and practically undamped oscillations are observed when the Michaelis constants K_1 and K_2 become negligible in comparison with, respectively, X and Y (Fig. 1E). However, with increasing K_1 and K_2 values, the oscillations become more and more damped. When K_1 and K_2 values are negligible Goodwin's two-dimensional oscillator shows robust homeostasis due to integral control in X, where X oscillates around the set-point $X_{set} = \delta_2/\alpha_2$ (Thorsen et al, 2014). As an alternative to a linearization of the equations, the period can be approximated by writing the equations in form of a harmonic oscillator (Box. 1).

It should also be stressed, as demonstrated by Goodwin, that his two-variable model from 1963 is conservative (Box 2). It thus lies in the class of harmonic oscillators, idealized by the pendulum or the spring (under ideal conditions), much like the Lotka-Volterra predator-prey or autocatalytic chemical oscillator models (Lotka, 1920; Volterra 1926). The oscillations depend on the initial conditions. As a consequence, there is no damping (no energy dissipation) and, after a perturbation, the amplitude of the oscillations does not recover its initial value (Fig. 1D). Recognizing that this type of oscillators does not account for the presumably robust nature of biological clocks, Goodwin then devised a three-variable extension of the previous model (Goodwin 1963, 1965; Fig. 2A,B). Using first-order degradation rates, he ensured that the variables remain positive, and that adding a delay (through the third variable) would favor the occurrence of self-sustained oscillations. This type of oscillations, referred to as limit cycle oscillations, are robust in the sense that, after an instantaneous perturbation in concentrations, the system would eventually recover its defined amplitude and period (Fig. 2C).

Repression in this three-variable model is described by a sigmoidal, Hill function, $f = K^n/(K^n + Z^n)$ (Fig. 3A). A condition, derived by Griffith (1968), to obtain limit cycle oscillations in this system is that the Hill exponent (sometimes referred to as the cooperativity degree, and often named, arguably, Hill coefficient), n, must be larger than 8. The oscillations reported by Goodwin for n=1, obtained with an analog computer, obviously resulted from numerical artifacts. Nevertheless, today, the idea that biological rhythms may be viewed as limit cycle oscillations is taken for granted (Goldbeter, 1996; Forger, 2017). Phenomena such as periodic metabolic processes, neuronal activity, calcium signalling, or circadian rhythms are indeed commonly modeled by limit cycle oscillators (Goldbeter, 1996) and their analysis benefits from a well-established theoretical framework (Forger, 2017).

The emergence of oscillations in negative feedback systems led Brian Goodwin to explain the observation that certain enzymes show a periodic increase in their activity, once per division cycle, as reported in several studies on *E. coli* and *B. subtilis* (Masters and Donachie, 1966, and references therein). Such a temporal ordering of events during the cell cycle cannot be explained by the sequential doubling of the gene copies during the DNA replication cycle as such model would predict that genes could only be transcribed according to a fixed sequence (determined by their position in the genome) and that it would be impossible to induce an enzyme at any point in the cell cycle or to reverse the order in which enzyme steps occur during the cell cycle. An oscillatory system, entrained by periodic cell division, can however account for this flexibility provided that the autonomous period of the oscillator is close to the cell division time (Goodwin, 1966, 1967). Later, Tyson showed that this condition implies that the half-life of the enzyme must be much shorter than the cell division time (Tyson, 1979).

Theoretical developments: in search of ingredients to promote oscillations

The three-dimensional Goodwin model, due to its apparent simplicity and due to its rich dynamics, rapidly attracted the attention of mathematical biologists. Their work not only led to analytical developments and theorems but also provided important insights on the design of biological oscillators, and their behaviour in presence of noise or external periodic stimulation.

Early theoretical works on the Goodwin model were devoted to prove the existence of limit cycle oscillations (Griffith, 1968; Allwright, 1977) or to analytically solve the two-variable model (Singh, 1977). In numerous subsequent articles, authors have proposed variants and extensions of Goodwin's oscillator in search of processes favoring the emergence of limit cycle oscillations. Those extensions include nonlinear reaction rates (Walter, 1974; Palsson and Groshans, 1988), time delay through multiple reaction steps (Tyson and Othmer, 1978; Invernizzi and Treu, 1991), incorporation of an explicit time delay (MacDonald 1977; Bliss et al, 1982), and multi-loop negative feedbacks (Mees and Rapp, 1978).

In a short paper, Tiwari and Fraser (1973) reported the first stochastic simulations of the Goodwin model. They assumed that the synthesis of mRNA obeys a Poisson process and that the kinetic constants (delay in translation, degradation rates, etc) are random variables, exponentially distributed. By screening different parameter values, while keeping the Hill exponent to n=1, they found that, in general, the variables displayed irregular but non-damping oscillations (Tiwari and Fraser, 1973, Tiwari et al, 1974). This highlights the possible constructive role that noise can have in the emergence of self-sustained oscillations.

Fraser and Tiwari (1974) recognized that stochastic oscillations resulting from a single feedback loop would not constitute a reliable clock but they predicted that a number of such circuits, operating independently or coupled to each other, could produce a more precise rhythm. They developed a computer program to efficiently simulate networks of Goodwin oscillators cyclically connected: the repressor of each circuit represses the gene of the next circuits, and the repressor of the last circuit represses the gene in the first circuit. Their numerical simulations showed that a network with an odd number of genes cyclically connected can oscillate over a wide range of conditions and that stochasticity favors the emergence of oscillations. Remarkably, 25 years later, a very similar design was experimentally implemented in *E. coli* to demonstrate that oscillations can readily be generated through a network of three cyclically repressing genes, a system named the *Repressilator* (Elowitz and Leibler, 2000).

Periodic entrainment, already tackled by Goodwin, was reinvestigated by Woller et al (2014). Combining analytical and numerical results, they showed that very different dynamics are obtained, depending on whether the Goodwin oscillator exhibits damped or limit cycle oscillations. More specifically they found that the entrainment region as a function of the modulating period is much larger when the free oscillator is in the damped regime. In contrast, when the free oscillator is in the limit cycle region, it exhibits, upon entrainment, a much richer dynamics: quasi-periodicity, birhythmicity, or coexistence of a limit cycle and quasi-periodicity. The fact that damped oscillators are more easily entrained over large domains of periods may be an advantage for circadian clocks, which, in natural conditions, run under light-dark cycles and must then be efficiently entrained, presumably without exhibiting complex dynamics (e.g. chaos or quasi-periodicity). Similarly, simulations of coupled Goodwin-like oscillators suggested that damped oscillators are more efficiently synchronized to each other (Gonze et al, 2005; Komin et al, 2001), which may explain why circadian oscillations recorded

at the single cell level in the mammalian pacemaker appear damped in many cells in isolated cell cultures (Webb et al, 2012).

The Hill function and its alternatives

Hill functions are commonly used to model genetic and biochemical systems. There are several ways to derive such a function from detailed molecular mechanisms (Fig. 3B). In enzyme kinetics, the Hill function may result from the cooperative binding of multiple substrate or ligand molecules to an enzyme or a receptor (Segel, 1975). At the transcriptional level, using the Hill function can be motivated by the formation of repressor protein complexes or by the cooperative binding of the repressor to the gene promoter (Keller, 1995; Alon, 2006). A large Hill exponent promotes oscillations (Griffith, 1968) and allows analytical studies (Painter and Bliss, 1981; Woller et al, 2013). However, the molecular processes mentioned here rarely yield Hill exponent values higher than 3 or 4. This led several authors to question the validity of this function for $n>8$ and to propose alternatives (Fig. 4).

Some works cited in the previous section already provide mechanisms allowing a reduction of the minimum value of the Hill exponent required to obtain limit cycle oscillations. These mechanisms include delay via additional intermediary variables (Fig. 4B) or additional sources of non-linearity (Fig. 4C) (Walter, 1974; Bliss et al, 1982). It should be noted that replacing a linear degradation step by one with Michaelian kinetics not only enables limit cycle oscillations with $n=1$, but also relaxes the condition that all degradation rates must be nearly equal (Bliss et al, 1982, summarized in Fall et al, 2002 (see chapter 9)).

Motivated by observations from the *Neurospora* circadian clock, Cheng et al (2009) considered that the total concentration of the compound Z is constant but that Z can reversibly switch between an active state (e.g. phosphorylated form) and an inactive state (e.g. dephosphorylated form). They further assume that Z is a transcriptional activator and that protein Y induces the deactivation of Z (Fig. 4D). The phosphorylation / dephosphorylation kinetics obeys Michaelis-Menten kinetics and displays zero-order ultrasensitivity (ZOU) (Goldbeter & Koshland, 1981). This adaptation of the model allows to generate oscillations with a reduced value for the Hill exponent, characterizing, here, the transcriptional activation.

In the context of circadian clocks in mammals and *Drosophila*, Kim & Forger (2012) considered a mechanism based on the sequestration of a transcriptional activator (A) by the repressor (Z) as another alternative to Hill function (Fig. 4E). In this model, a nonlinear threshold is obtained when the dissociation constant between A and Z is small, and limit cycle oscillations occur when a stoichiometric balance between A and Z is achieved. The difference between the Hill and the sequestration mechanisms was further investigated by Kim (2016). Besides other differences, the sequestration mechanism was shown to enable a better control of the period when a population of oscillators are coupled.

Finally, it should be stressed that a high Hill exponent can be reached by mechanisms such as multi-site phosphorylation of a protein (Gunawardena, 2005). A model that explicitly describes such a process agrees well with the original Goodwin model provided that the phosphorylation/dephosphorylation kinetics is fast enough (Fig. 4F) (Gonze and Abou-Jaoudé 2013).

Period control and temperature compensation

A fundamental characteristic of any oscillatory behaviour is the period. It is important to understand how kinetic parameters affect the period because the function of a cellular oscillator critically depends on it. Goodwin already showed that, in his three-variable model, protein stability is the major determinant of the period of the oscillations. When discussing properties of coupled oscillators, he also noted that relatively long-period rhythms such as circadian or monthly rhythms, can be explained in terms of nonlinear interactions and do not necessary require slow kinetic processes (Goodwin, 1965).

As mentioned earlier, some oscillatory enzymes exhibit a periodic increase in their activity which matches the cell division cycle. The enzyme oscillator is actually entrained by the cell cycle (Goodwin, 1966). This entrainment is only possible if the period of the oscillator is close to the cell division time. Tyson (1979) analyzed a generalized Goodwin model (with additional intermediary components) to understand how such a long period can be generated. He showed that if the cooperativity in the negative feedback is not too large (compensated by the additional steps in the loop), then the period of the oscillations will be much longer than the half-life of the most stable component in the loop. On the other hand, if the cooperativity is very large, then the period of the oscillations can approach the longest half-life.

It is however not straightforward to intuitively predict the relationship between the half-life of a protein and the resulting period for arbitrary oscillator models. Intriguingly, even in closely related minimal models, the profile of the period as a function of a clock protein degradation rate can be markedly different. Whereas in the original three-variable Goodwin oscillator, the period decreases with the degradation rate of variable Z, an opposite tendency is observed in a variant of the Goodwin model that incorporates additional intermediate variables, reversible processes (phosphorylation/dephosphorylation), and Michaelis-Menten kinetics (Goldbeter, 1995). A systematic analysis of the two models revealed that the degree of saturation of various processes such as mRNA and protein degradation, as well as the kinetic order and velocity of the phosphorylation steps, have crucial effects on the period profile (Gérard et al, 2009).

Biochemical reaction rates depend on temperature. Kinetic rates generally increase with temperature, although, at high temperature, some enzyme reaction may slow down (due to the denaturation of enzymes). Such changes in kinetic rates are expected to influence the dynamics of the oscillator. Sensitivity to temperature may impair the function of the oscillator and thereby be harmful for the cell. The Goodwin model was used to establish the conditions that allow keeping the period relatively constant with respect to temperature, a property called temperature compensation (see more below).

How to make the oscillations robust and the period tunable?

The Goodwin oscillator demonstrates that with a minimum number of ingredients, it is possible to generate limit cycle oscillations. In biological systems, however, the mechanisms underlying oscillations are often more complex. They typically involve multiple sources of non-linearity and interlocked feedback loops. It is therefore interesting to study the possible advantages of such designs (Novak and Tyson, 2008). Inversely, we may also wonder how to construct a robust oscillator with a tunable period.

The role of saturable kinetics was already pointed out above. Kurosawa and colleagues (Kurosawa et al 2002; Kurosawa and Iwasa 2002) re-investigated this question more systematically. They distinguished "in-loop" reaction steps (such as synthesis and phosphorylation) from "branch" reaction steps (degradation and back transport of the protein from the nucleus to the cytosol). They proved mathematically that kinetic saturation in the "in-loop" reaction steps tends to suppress the oscillations, whereas saturated "branch" reaction kinetics rather favors the occurence of the oscillations.

Tsai et al (2008) showed, through extensive computational simulations, that tunability of the period (while keeping the amplitude nearly constant) can be easily achieved when the negative circuit is coupled to a positive circuit. This tunability makes the interlocked feedback loop design suitable for biological rhythms like heartbeats, cell cycle, or frequency-coding systems, which need to produce a constant output over a range of frequencies. Positive-plus-negative oscillators also appear to be more robust towards changes in parameter values, explaining why they are also found in contexts where an adjustable frequency is not needed, but where robustness to fluctuations in the environment is required, as in circadian clocks.

In the same line, Saithong et al (2010) showed that interlocked multi-loop structures reinforces robustness by enhancing the response to external and internal variations. Interestingly, they also found that reducing the degree of nonlinearity could sometimes increase the robustness of models, implying that ad hoc incorporation of nonlinearity could be detrimental to a model's performance.

Ananthasubramaniam and Herzel (2014) showed that the addition of positive feedbacks to the Goodwin model promotes oscillations at lower degrees of cooperativity, and highlighted kinetic mechanisms that may facilitate the emergence of oscillations, such as self-activation and Michaelis-Menten degradation. The positive feedback loops are most beneficial when acting on the shortest-lived component, where they function by balancing the lifetimes of the different components of the oscillator. This benefit is measured by the reduction in the cooperativity degree required to generate limit cycle oscillations. Interestingly, these authors also showed that the benefit of multiple positive feedbacks is cumulative. Moreover, these positive feedback motifs allow oscillations with longer periods than that determined by the lifetimes of the components alone.

Baum et al (2016) studied the robustness of period and amplitude of various prototypical systems, which can be viewed as extensions of the Goodwin model. To this end, they adopted a comprehensive computational approach and focused on three features: negative vs positive feedback, Michaelis-Menten vs mass action kinetics in degradation and conversion reactions, and reactions vs regulatory processes. This work, as well as the previously cited studies, highlight the importance of reaction kinetics and feedback types for the variability of period and amplitude and therefore for the development of predictive models.

Applications of the Goodwin model: From metabolic regulation to circadian and ultradian rhythms

The differential equations that bear today Goodwin's name were, as commented by him (Goodwin, 1997), inspired by the discoveries of Jacob and Monod (1961a,b) on repressing and de-repressing gene regulatory mechanisms. Although Goodwin (1963, pages 20-21) described the potential importance of negative feedback regulation of genes for circadian oscillations,

there was, however, no immediate attempt to apply the Goodwin equations to circadian rhythms. In early works, his so-called "epigenetic system" was mainly applied to the regulation of biosynthetic/metabolic pathways (Goodwin, 1963, 1965, 1976), and protein synthesis (Maynard Smith, 1968), and served as a generic model to investigate the coupling between biochemical oscillators (Goodwin, 1963, 1976).

The first negative feedback modeling of a circadian rhythm (including phase resetting behaviors) appears to be due to Johnsson and Karlsson (1972) when studying the petal movements in *Kalanchoë* plants (Karlsson and Johnsson, 1972). The approach taken there is based on a control-engineering aspect, where a circadian variable $c(t)$ oscillates around a reference point c_{ref} due to a certain time delay and nonlinear elements imposed on a negative feedback system. This control systems approach, which has been further developed and applied by Lewis (1999), can be considered to be analogous to Goodwin's, but instead of focusing on the molecular repressing and de-repressing reactions as in the Goodwin oscillator, the Johnsson-Karlsson model gives a description of the different biophysical subsystems within a negative feedback loop. Thus, the Goodwin oscillator can be considered as a molecular representation of Johnsson and Karlsson's control systems approach.

Ludger Rensing's lab was probably the first to apply the Goodwin oscillator to circadian rhythms (Drescher et al, 1982). Among other experiments, they perturbed the circadian glow rhythm of *Gonyaulax* with single or double pulses of anisomycin and modelled the ensuing response/resetting kinetics with the Goodwin equations. The similarity of the response to chemical pulses observed in the experiments and simulated by the Goodwin model led Rensing and Schill (1985) to conclude that "*the functional structure of the model may represent a possible structure of the circadian oscillator*."

A few years later, experimental studies on *Drosophila* (Hardin et al, 1990) and *Neurospora* (Aronson et al, 1994) confirmed the negative feedback structure of circadian rhythms predicted by Rensing and Schill, implemented as transcriptional-translational negative feedback loops (Dunlap, 1999). The X, Y, and Z variables of the Goodwin oscillator (Fig. 2A,B) could then be directly linked to the clock-mRNA, clock-protein, and the repressor, forming the core of the circadian clock.

This correspondence allowed to predict the dynamics of circadian clock components. The degradation terms in the Goodwin oscillator were found to have a significant effect on the period length indicating that mutations in a clock gene leading to either shorter or longer period length may relate to more rapid or slower degradation rates for this gene's mRNA or protein. This relationship between stability of clock components and the circadian oscillator's period length has been used to account for the influence of temperature on *Neurospora* and *Drosophila* clock mutants (Ruoff et al, 1996, 1999a). For *Neurospora*, the Goodwin model-based predictions were confirmed by showing that the half-life of the clock-protein FREQUENCY (FRQ) largely determines the circadian period (Ruoff et al. 2005), but, interestingly, only as long as the proteasomal complex which degrades FRQ remains intact (Larrando et al. 2015). The Goodwin model was also tested by using perturbation pulses of heat and cycloheximide (Ruoff et al, 1999b), as well as single and double perturbation pulses of light on the conidiation rhythms of different *Neurospora* clock mutants (Ruoff et al. 2001). In all cases, predictions with the Goodwin oscillator agreed well with experiments.

Temperature compensation, one of the defining properties of circadian clocks (Pittendrigh, 1954; Dunlap et al, 2004), has probably been the most important single factor that sparked the

interest of scientists in biological rhythms (Sweeney and Hastings, 1960). Temperature compensation means that for different but constant temperatures the period length is kept within a relatively narrow range. There have been many different approaches to explore how homeostasis of the period may be achieved (Ruoff et al, 1997). Among the first proposals, Hastings and Sweeney (1957) suggested that the underlying chemical reactions of a circadian oscillator should oppose each other, and thereby damping the influence of the temperature on the period. By applying the Arrhenius equation to each rate constant of the clock component processes, it can be shown that all biochemical oscillator models, including the Goodwin oscillator, should be capable of temperature compensation. In general, a temperature insensitive period is achieved due to a balance between temperature-induced period increasing and period decreasing reactions, where the activation energies of the individual component processes serve as scaling factors for the individual reactions' contributions to the period. In the Goodwin model the degradation reactions of the clock components X, Y, Z, oppose their synthesis reactions (Ruoff et al, 2005).

In parallel with the works by Rensing and Ruoff, Goldbeter extended the Goodwin oscillator by including enzymatic degradation reactions, reversible phosphorylation/dephosphorylation steps, and nuclear transport (Goldbeter, 1995). This model accounts for the circadian oscillations of PER mRNA and protein in *Drosophila*. The model has been tested with respect to temperature compensation (Leloup and Goldbeter, 1997), where it was found that changes in the parameter values have opposing effects on the period in agreement with the "opposing balancing" concept described above.

Over the years the discovery of additional clock genes and multiple interlocked feedback loops led to the development of detailed molecular models (e.g. Leloup and Goldbeter, 2003; Forger and Peskin, 2003; Becker-Weimann et al, 2004; Mirsky et al, 2009; Relogio et al, 2011), but, interestingly, despite the presence of additional positive and negative feedback loops which may also contribute to the generation of stable oscillations, their core structure always relies on a Goodwin-like negative feedback loop. It is noteworthy that, despite its simplicity, the Goodwin model properly reproduces core features of the circadian clock, notably its response to short light pulses, jetlag and seasonal phase shifts (Ananthasubramaniam et al, 2020).

Besides circadian rhythms, a variety of ultradian rhythms, characterized by a period significantly smaller that 24h, have also been identified, among which many originate from a feedback inhibition mechanism. Thus, the pulsatile secretion of hormones, the oscillatory behaviour of the NF-κB signalling transcription factor, or the oscillations of the Notch effector gene Hes1 are examples of ultradian rhythms that have been modeled by Goodwin-like equations (Smith, 1980; Krishna et al, 2006; Zeiser et al, 2007). Recently, Santorelli et al (2018) build a hybrid "Hes1" oscillator combining synthetic and natural parts and, remarkably, the oscillations of Hes1 expression, recorded at the level of single cells, are in good agreement with the prediction of a (slighly adapted) Goodwin model.

A delayed negative feedback loop, possibly augmented with additional feedback loops, thus appears at the core of various biological oscillators. It is however worth to note that not all biological oscillators are appropriately described by the Goodwin model. Glycolytic oscillations for example result from a positive feedback loop exerted by the product of a reaction on the enzyme that catalyzes its own production (Goldbeter 1996). Calcium oscillations are generated by a mechanism called calcium-induced calcium release (CICR), whereby calcium triggers the release of calcium from intracellular Ca^{2+} stores (e.g. endoplasmic reticulum) into the cytosol (Goldbeter 1996). These examples, as many others, fall in the class

of "amplified negative-feedback loops" oscillators, for which the positive loop is an essential component of the oscillator (Novak and Tyson 2008).

Concluding remarks: Towards a theoretical physiology of behavior

Goodwin concluded his 1965 paper by stating that "*the ultimate goal of these studies is a theoretical physiology of behavior, which will allow one to use the knowledge of elementary control processes such as those governing enzymatic synthesis and activity as the basis for a comprehensive, predictive theory of biological organization*" (Goodwin, 1965). The number of theoretical papers on oscillatory systems in biology, inspired directly or indirectly by Goodwin, is continuously growing. Today detailed predictive models for circadian and ultradian rhythms are available. These models, constructed in close relationship with experimental data and sometimes parameterized by fitting to experimental time series, still fully exploit the notions of feedback, limit cycle, and synchronization evoked by Goodwin, and are of great help to understand biological organization.

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Figures captions

Figure 1: (A) Scheme and (B) equations of the two-variable conservative Goodwin model. (C) Equations of the modified two-variable Goodwin model with Michaelis-Menten degradation kinetics. (D) Oscillations obtained by numerical integration of the two-variable model given in panel B, for the following parameter values: α_1=2, α_2=1, δ_1=δ_2=1, K=0.5. At time t=30 the value of variable X is increased. (E) Damped oscillations obtained by numerical integration of the modified model given in panel C for the same parameter values as in panel D and K_1=K_2=0.001.

Figure 2: (A) Scheme of the three-variable Goodwin model. (B) Equations of the Goodwin-Griffith three-variable model. (C) Limit cycle oscillations obtained by numerical integration of the model given in B, for the following parameter values: α_1=5, α_2= α_3=5, γ_1=γ_2=γ_3=0.5, n=10, K=1. At time t=30 the value of variable X is increased.

Figure 3: (A) Linear vs non-linear response curves. In biomolecular systems, non-linearity typically originates from saturable enzyme reaction (Michaelis-Menten kinetics, $f(S) = K/(K+S)$) and cooperative processes (Hill kinetics, $f(X) = K^n/(K^n + S^n)$, here shown for a low Hill exponent (grey curve) and a large Hill exponent, black curve). (B) Various mechanisms that can lead to a Hill kinetics: (a) Positive cooperative binding of the substrate S to multiple catalytic sites of an enzyme. (b) Multi-site phosphorylation. (c) Complex formation of a transcriptional factor prior to the binding to the promoter of a gene. (d) Cooperative binding of a transcription factor to multiple binding sites in the promoter of a gene.

Figure 4: Variants and extensions of the Goodwin model. (A) Original Goodwin model. (B) Delay introduced through additional intermediary variables (Tyson and Othmer, 1978). (C) Non-linear degradation kinetics (Michaelis-Menten) (Gonze et al, 2005). (D) Y-mediated, reversible activation/deactivation (e.g. via phosphorylation/dephosphorylation) of variable Z, standing here for a transcriptional activator (Cheng et al, 2009). (E) Sequestration of a transcriptional activator A by protein Z (Kim and Forger, 2012). (F) Multi-site phosphorylation (Gonze and Abou Jaoudé, 2013).

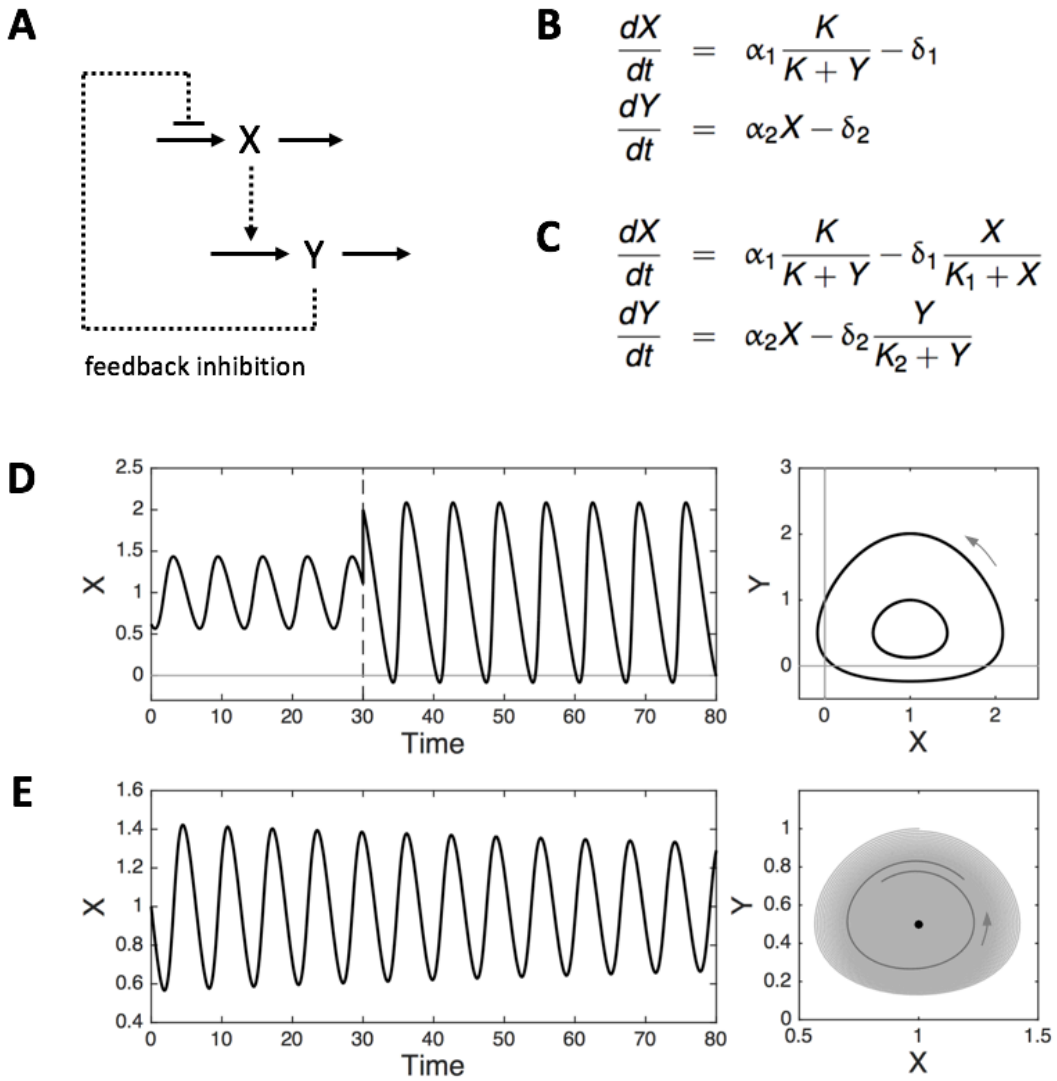
A
X
Y
feedback inhibition
B
$\frac{dX}{dt} = \alpha_1 \frac{K}{K+Y} - \delta_1$
$\frac{dY}{dt} = \alpha_2 X - \delta_2$
C
$\frac{dX}{dt} = \alpha_1 \frac{K}{K+Y} - \delta_1 \frac{X}{K_1+X}$
$\frac{dY}{dt} = \alpha_2 X - \delta_2 \frac{Y}{K_2+Y}$
D
X
Time
Y
X
E
X
Time
Y
X

Figure 1

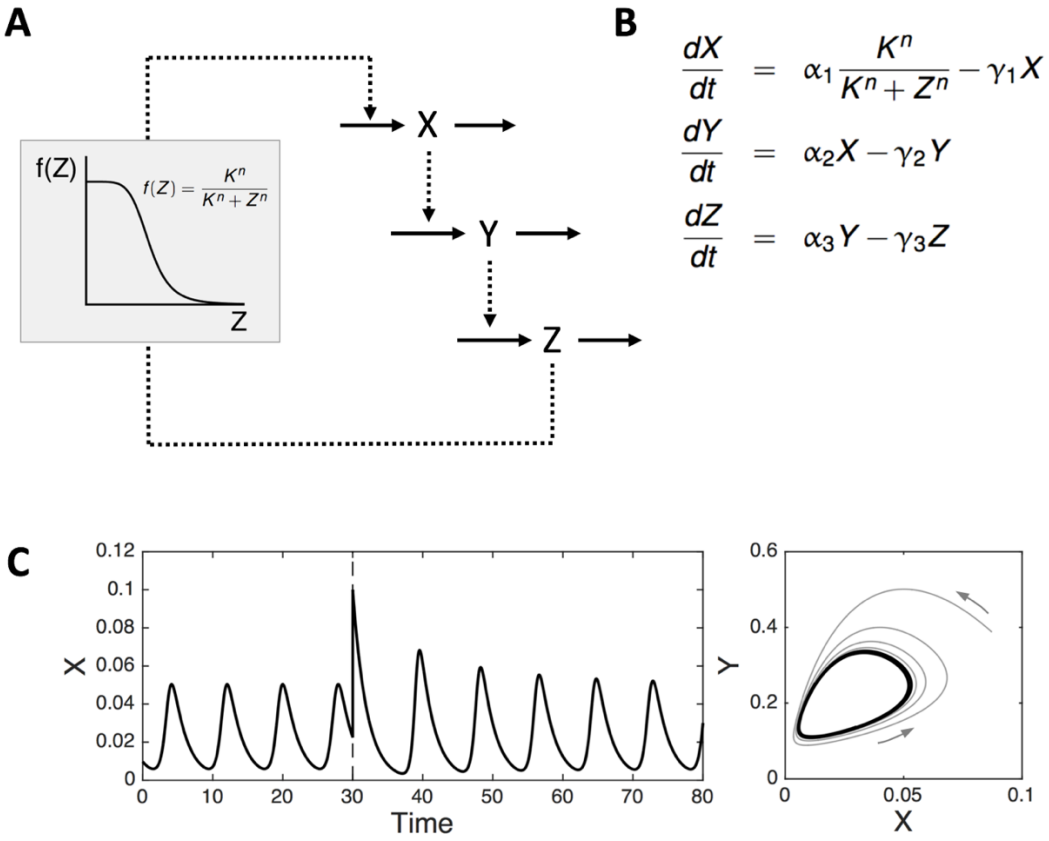
A
B
f(Z)
$f(Z) = \frac{K^n}{K^n + Z^n}$
Z
X
Y
Z
$\frac{dX}{dt} = \alpha_1 \frac{K^n}{K^n + Z^n} - \gamma_1 X$
$\frac{dY}{dt} = \alpha_2 X - \gamma_2 Y$
$\frac{dZ}{dt} = \alpha_3 Y - \gamma_3 Z$
C
X
Time
Y
X

Figure 2

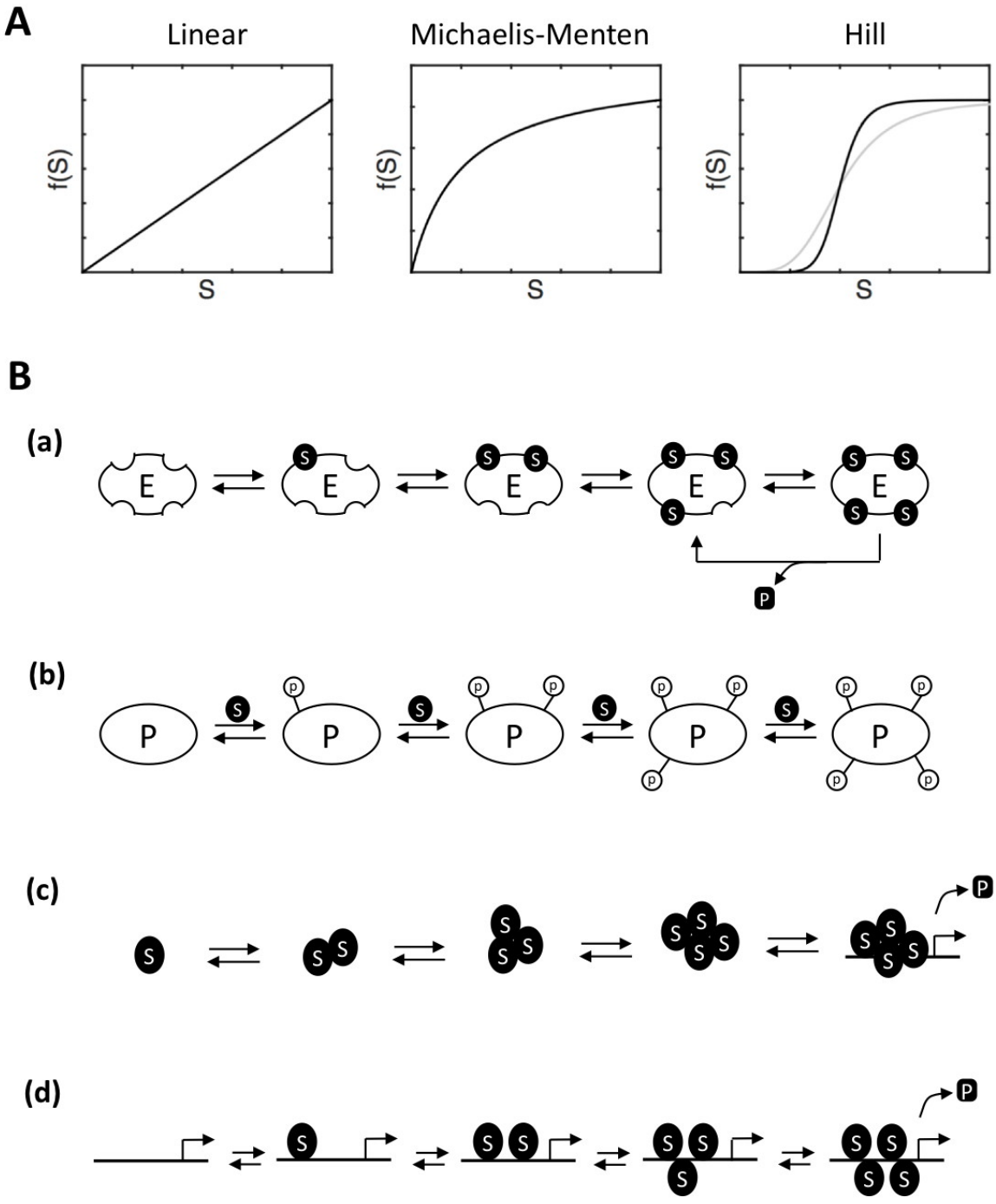

Figure 3

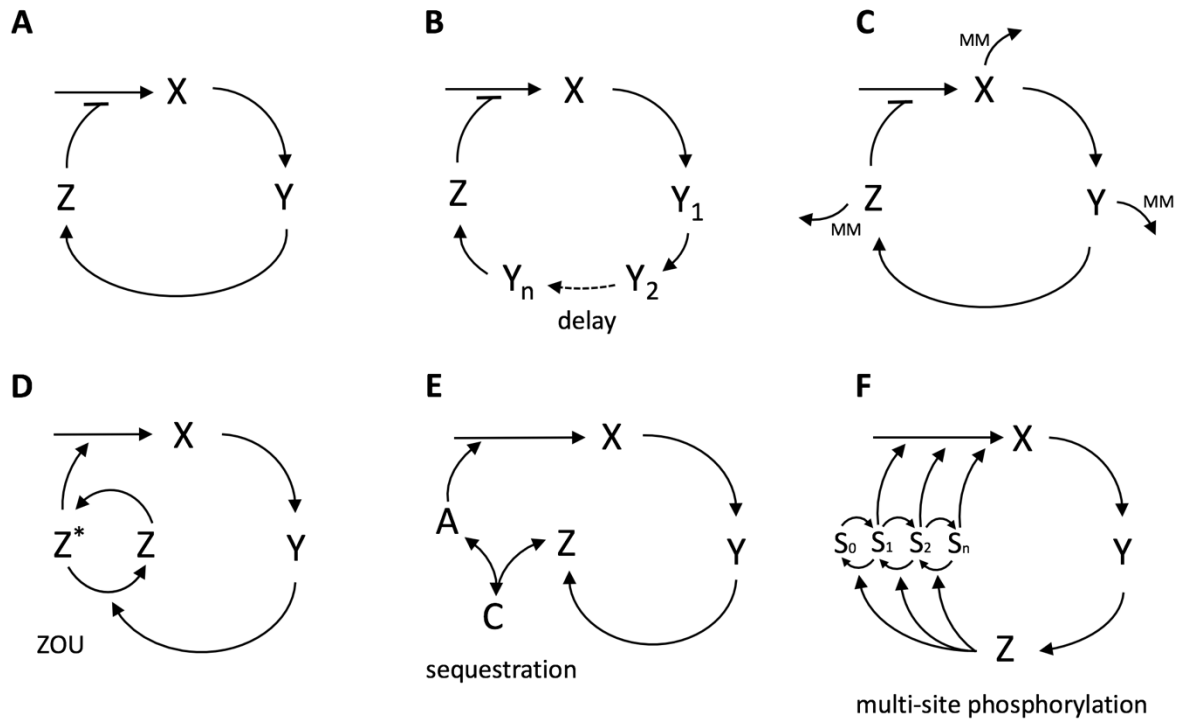
A
X
Z
Y
B
X
Z
Y_1
Y_n
Y_2
delay
C
MM
X
Z
MM
Y
MM
D
X
Z^*
Z
Y
ZOU
E
X
A
Z
Y
C
sequestration
F
X
S_0
S_1
S_2
S_n
Y
Z
multi-site phosphorylation

Figure 4

Box 1: Estimation of the period for the two-variable Goodwin model.

Assuming that the Michaelian constants K_1 and K_2 are small, then Eqs. in Fig. 1C reduce to the Eqs. in Fig. 1B. In this case, the model can be approximated by an harmonic, homeostatic oscillator and the period can be estimated as follows.

We can rewrite the first order ODE system as a single second order equation:

$$\ddot{X} = -\frac{\alpha_1 K}{(K+Y)^2}\dot{Y} = -\frac{\alpha_1 K}{(K+Y)^2}(\alpha_2 X - \delta_2)$$

$$\frac{\ddot{X}}{\omega^2} + X = \frac{\delta_2}{\alpha_2} = X_{set}$$

Thus, X oscillates around its "set" value, $X_{set} = \frac{\delta_2}{\alpha_2}$ at a frequency given by $\omega^2 = \frac{\alpha_1 \alpha_2}{(K+Y)^2}$.

Note that X_{set} does not depend on α_1 or δ_1 (homeostatic oscillations).

At steady state, $\dot{X} = \dot{Y} = 0$, $X_{ss} = X_{set} = \frac{\delta_2}{\alpha_2}$, and $Y_{ss} = K\frac{\alpha_1 - \delta_1}{\delta_1}$. The period is thus estimated as

$$T = \frac{2\pi}{\omega} = \frac{2\pi}{\delta_1}\sqrt{\frac{\alpha_1}{\alpha_2}K}$$

For the parameter values used in Fig. 1, the estimated period is $T = 2\pi$.

Box 2: The two-variable Goodwin model is a conservative system

Goodwin's two-variable oscillator is an example of a *conservative system*, which means that, in analogy to classical mechanics, an "energy function" H (or Hamiltonian) can be found of the form

$$H(X,Y) = -\int \dot{X}dY + \int \dot{Y}dX$$

which satisfies the Hamilton-Jacobi equations of motion, i.e.

$$\frac{\partial H}{\partial Y} = -\dot{X} \quad ; \quad \frac{\partial H}{\partial X} = -\dot{Y}$$

Assuming that K_1 and K_2 (Fig. 1C) are negligible, we insert the expressions of $\dot{X}$ and $\dot{Y}$ into the above Eq. for H, integrate, and obtain (by setting the integration constant to zero):

$$H(X,Y) = -\alpha_1 \, K \, ln(K+Y) + \delta_1 Y + \frac{\alpha_2}{2} X^2 - \delta_2 X$$

The oscillations of Goodwin's two-variable model can be described as closed trajectories on the $H(X,Y)$ surface. The figure below shows the H-function for the parameters used in Fig. 1 (α_1=2.0, α_2=1.0, δ_1=δ_2=1.0, K=0.5), as well as its projection onto the (X,Y) plane. The close curve is the trajectory obtained for H=0.3.