What makes a biological clock efficient?

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Abstract

Biological clocks regulate the proper periodicity of several processes at the cellular and organismal level. The cell cycle and circadian rhythm are the best characterized among these but several other biological clocks function in cells at widely variable periodicity. The underlying molecular networks are controlled by delayed negative feedbacks, but the role of positive feedbacks and substrate-depletion has been also proposed to play crucial roles in the regulation of these processes. Here we will investigate which features of biological clocks might be important for their efficient timekeeping.

Evolution of biological clocks

The ability of organisms to temporally co-ordinate their physiology is evolutionarily advantageous, and is therefore ubiquitous in nature. Organisms have evolved numerous so-called biological clocks to optimise their fitness, by co-ordinating their physiology with the availability of resources. Simple experiments monitoring the growth of bacteria against varying nutrient availability illustrate an enormous flexibility in deciding how frequently cells choose to divide. Yet, the developmental programs that lead to the replication of entire organisms (mammals) are relatively inflexible. The cell cycle, which culminates in cell division, is controlled by regulatory networks that have numerous conserved features and components across the eukaryotic kingdom (Harashima *et al.*, 2013).

Circadian clocks allow organisms to co-ordinate their physiology with the external time of day, enabling anticipation of changes in temperature, light availability, predator activity, etc. In contrast to the cell cycle, circadian clocks have evolved multiple times and have many different features (though some shared components) across the eukaryotic kingdom (Dalchau and Webb, 2011). The overall structure of circadian networks involves input pathways, a core oscillator, and output pathways (Dunlap, 1999). The core oscillator comprises multiple feedback loops that sustain circadian rhythms with a period of approximately 24 h. Input pathways enable the

oscillator to maintain synchrony with external time, while output pathways provide the biochemical means of the oscillator to regulate downstream physiology, including gene expression, metabolism and signalling.

Synthesizing biological clocks

In recent years, there has been a large rise in the number of attempts to engineer biological systems. The field of *synthetic biology* seeks to improve understanding of biological functions by attempting to re-create specific systems and their behaviours, using existing cells and their housekeeping components (RNA polymerase, ribosomes, proteasomes, etc.) as a chassis. The creation of *biological devices* is beginning to open new opportunities in industry, for example using bacteria to produce biofuels and medicines. A seminal work in this field was the construction of a biological clock, termed the *repressilator*, in which three transcriptional repressors were taken from non-oscillatory networks and inserted into *Escherichia coli* on a plasmid, but arranged as a cycle of repression (Elowitz and Leibler, 2000). Briefly, TetR was placed under the control of a LacI-repressible promoter, LacI was placed under the control of a CI-repressible promoter, and CI under the control of a TetR-repressible promoter. It was demonstrated that oscillations in the abundance of the constituent proteins could be generated when the strengths of the interactions between the repressor proteins and their cognate DNA-binding domains were tuned to appropriate levels.

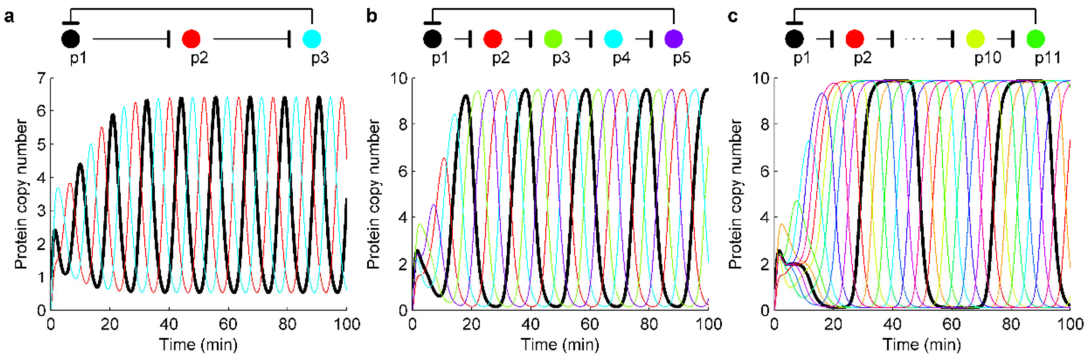

Figure 1. Deterministic simulation of ring oscillators. Sequential inhibition of transcriptional repressors around a single feedback loop produces oscillations. **a.** Simulation of the repressilator (3-component ring oscillator) model in Elowitz & Leibler. **b,c.** Extending the model to 5 and 11 components also yields oscillations in protein copy number. Protein 1 is plotted with a thickened black line to emphasize differences in oscillation waveform between different degree ring oscillators.

The repressilator network is an example of a 3-stage *ring oscillator*. Ring oscillators are often used in electrical engineering for generating oscillations. However, only rings with an odd number of components can give oscillatory dynamics ((Sprinzak and Elowitz, 2005); examples in Figure 1). This is because each regulator inverts the gradient of the following regulator, which for an even number of components would result in an equilibrium ON-OFF-ON-OFF-…-ON-OFF. Using an odd number of

components breaks this pattern, and can yield oscillations. Increasing the length of the feedback loop with additional components leads to more square-like waveforms (Figure 1). It has recently been established that a repressilator motif also exists in nature, lying at the heart of the circadian clock in plants (Pokhilko *et al.*, 2012) and in the core of transcriptional regulation of the cell cycle (Sriram *et al.*, 2007).

Oscillators have also been created in mammals (Tigges *et al.*, 2009) as well as in cell-free conditions, mixing chemical compounds in such a way as to recapitulate interaction networks that can exhibit oscillatory behaviour, for instance using negative feedback. In various works dating back to the 1950s, the famous Belousov-Zhabotinsky reaction was shown to both oscillate in time and propagate over excitable media (Field *et al.*, 1972). More recently, the construction of chemical oscillators made from DNA has been demonstrated, inspired by predator-prey (PP) cycles (Fujii and Rondelez, 2012). A system of 3 reactions is sufficient to generate PP cycles: i. an autocatalytic growth of the prey species, ii. an autocatalytic predation of the prey, and iii. decay of the predator species. The DNA-based PP network relies on DNA polymerization-depolymerization reactions to recapitulate these reactions, and is capable of sustaining many (>20) cycles before eventual depletion of necessary cofactors.

Mathematical analysis of biological clock architectures

There is a long history on the mathematical analysis of biological clocks (Goldbeter, 1997, 2002), still it is not fully understood what makes such a periodic system efficient. Biological clocks can run with a period of seconds (neural, cardiac, calcium rhythms) to months and years (ovarian, annual and ecological rhythms) and are regulated by delayed negative feedback loops that cause oscillations in the activities of system components (Goldbeter, 2008). Direct negative feedback loops lead to stabilization of steady states but delay in the loop and non-linearity in the interactions can induce oscillations (Goodwin, 1965; Griffith, 1968). This generic rule that delayed negative feedback loops form the basis of biological oscillations is now very well established for many biological clocks (Fig. 2A). The daily rhythms of the circadian clock might be the best example, where it was established that the existence of a direct time delay caused by a transcriptional-translational loop is driving the periodic appearance of a transcriptional repressor (Dunlap, 1999). Interestingly it was recently revealed that even in the absence of the delay caused by transcription-translation the circadian clock is robustly ticking (Nakajima *et al.*, 2005; O'Neill *et al.*, 2011). Later it was proposed that a positive feedback loop might play a crucial role in the control of this reduced system (Mehra *et al.*, 2006). Indeed the importance of positive feedback loops in the robustness of circadian clock regulation was proposed at other places as well (Tyson *et al.*, 1999; Becker-Weimann *et al.*, 2004; Hong *et al.*, 2009). These led to the conclusion that the circadian clock is controlled by interactions of positive and negative feedback loops.

Another highly investigated biological clock is driven by the cell cycle regulatory network. The controlled timing of DNA replication and cell division is determined by this clock and again earliest models considered a delayed negative feedback loop to drive this system (Goldbeter, 1991) and later results revealed the importance of positive feedback loops as well (Pomerening *et al.*, 2005; Tsai *et al.*, 2014). Thus it is a reoccurring pattern that crucial biological clocks are regulated by interlinked positive and negative feedback loops (Tsai *et al.*, 2008; Ferrell Jr *et al.*, 2011).

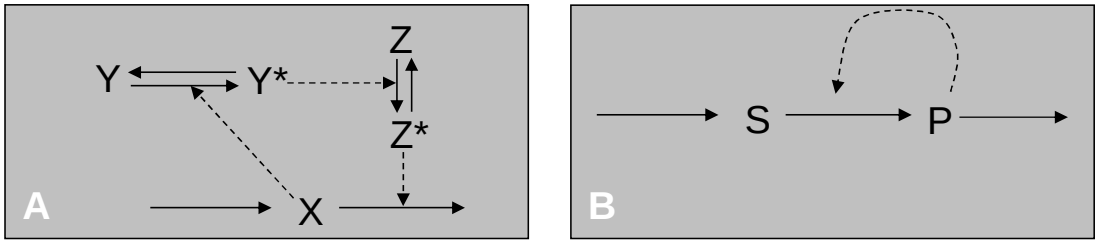

Figure 2. Feedback loops leading to oscillations. A, negative feedback loop, where protein X activates Y, which activates Z which is eventually inducing the degradation of X. **B,** Substrate-depletion, where a substrate S is produced and piling up in this form until the product P cannot turn on its autocatalytic loop converting most S into P. As P is less stable than S, the system runs out of both S and P, thus S will pile up again and oscillations emerge.

In the case of glycolytic oscillations of the metabolic system it was proposed very early that a positive feedback loop has a crucial role in controlling this biological clock and the oscillations appear as a result of the depletion of the substrate of an autocatalytic process (Higgins, 1964; Sel'kov, 1968). In this system a stable substrate is produced and converted into an unstable product in an autocatalytic manner (Fig. 2B) leading to oscillations where S is slowly increasing until P reaches a threshold and quickly converts all S into P (Fig. 3). The requirements for this system to oscillate are: i, non-linear autocatalysis on the S→P transition, ii, a background S→P conversion independent of P to allow P reaching the threshold and iii, removal rate of P has to be much higher than that of S. Note that this system shows high resemblance to the above mentioned predator-prey cycles. In both cases the pile up of one species is followed by the conversion of this species to another species by an autocatalytic step and eventual removal of the second species.

Interestingly one of the earliest cell cycle models was also working as a substrate-depletion oscillator (Tyson, 1991) and since then it was further established that the kinetics of the substrate-depletion model resemble that of the negative feedback with positive feedback model (Fall *et al.*, 2002). Indeed one can see the delayed negative feedback in the substrate-depletion model as P removes its activator S (by converting it to P). Thus we could state again that interlocked positive and negative feedbacks regulate glycolytic oscillations. It is also important to mention that the substrate-depletion mechanism that leads to oscillations in time can drive spatial biological clocks such as pattern formation and emergence of travelling waves (Meinhardt, 1982).

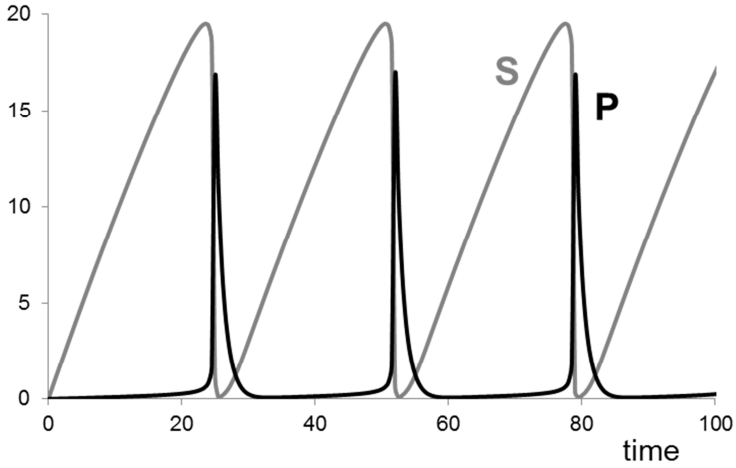

Figure 3. Deterministic simulation of a substrate-depletion oscillator. The substrate S is produced and first slowly converted into P. When P reaches a threshold it converts all S to P, which gets quickly destroyed. Leading to a bursting-like pattern in P oscillations.

Efficiency of biological clocks

Going back to the original question in the title: what makes biological clocks efficient? In fact, how to measure the efficiency of biological clocks? The robustness of the periodicity of biological clocks were investigated in the context of the circadian rhythm (Barkai and Leibler, 2000; Gonze *et al.*, 2002) and the cell cycle (Steuer, 2004; Mura and Csikász-Nagy, 2008). Both were found to be quite robust to parameter perturbations and also to intrinsic noise resulting from the low molecular numbers present in the system. So far we have seen many parallels between the circadian clock and cell cycle regulatory systems. There is one major point where they differ. The period of the circadian clock is quite insensitive for temperature changes whereas the cell cycle time can be greatly influenced by alterations in temperature (Klevecz and King, 1982). This result might suggest that the circadian rhythm regulatory network is a more efficient time keeper, while the cell cycle regulatory systems is more efficient in adjusting its period to adapt to environmental changes (Zámborszky *et al.*, 2007; Hong *et al.*, 2014). Changes in temperature affect chemical reactions exponentially, following the Arrhenius equation. How such changes in reaction rates do not influence the period of the circadian clock is a debated question (Tyson *et al.*, 2008). Several models have been worked out to understand what causes the temperature compensation in the circadian clock (Ruoff and Rensing, 1996; Leloup and Goldbeter, 1997; Gould *et al.*, 2006; Hong *et al.*, 2007; François *et al.*, 2012) and some more generic models of temperature compensation in biochemical reaction networks have also been proposed (Ruoff *et al.*, 1997; Hatakeyama and Kaneko, 2012). Recently even a synthetic temperature compensated oscillator was created (Hussain *et al.*, 2014), interestingly containing both a positive and a negative feedback loop. Furthermore non-biological ring oscillators on semiconductors were also designed to be temperature compensated

(Hayashi and Kondoh, 1993). Despite all of these results and theoretical ideas we still lack a coherent generic picture of what makes biological oscillators temperature compensated and in general robust in proper periodicity.

Conclusions

Recently it was established by Cardelli and Csikász-Nagy that a class of biological switches follow the dynamical features of an efficient computational algorithm (Cardelli and Csikász-Nagy, 2012). The Approximate Majority (AM) algorithm is used in distributed computing as a population protocol computing the majority of two finite populations by converting the minority population into the majority population (Angluin *et al.*, 2008). It was shown that AM can mimic the dynamics of the cell cycle switch regulatory network that induces the transition between stable cell cycle states. It was also postulated that the cell cycle switch efficiency is maximal only when its dynamics fully captures that of the AM algorithm (Cardelli and Csikász-Nagy, 2012) and later this prediction was experimentally verified (Hara *et al.*, 2012). We have seen that it is very well established that reliable biological time keeping mechanisms are regulated by the interconnection of such switch generating positive feedback loops with oscillation inducing negative feedback loops. The existence of negative feedback is essential and in almost all highly investigated systems the role of the positive feedback is important for the robust behaviour of the biological clock. It was established that the positive feedback module of the cell cycle regulatory network behaves like an efficient algorithm (Cardelli and Csikász-Nagy, 2012). Later, Cardelli (2014) established a theory to identify kinetically identically behaving regulatory networks. A future challenge will be the elucidation of which aspects of real life biological oscillators are important for their proper ticking and how far their kinetics could be associated to a minimalistic oscillator model.

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