Circadian Rhythms: A Mathematician's Error Notes

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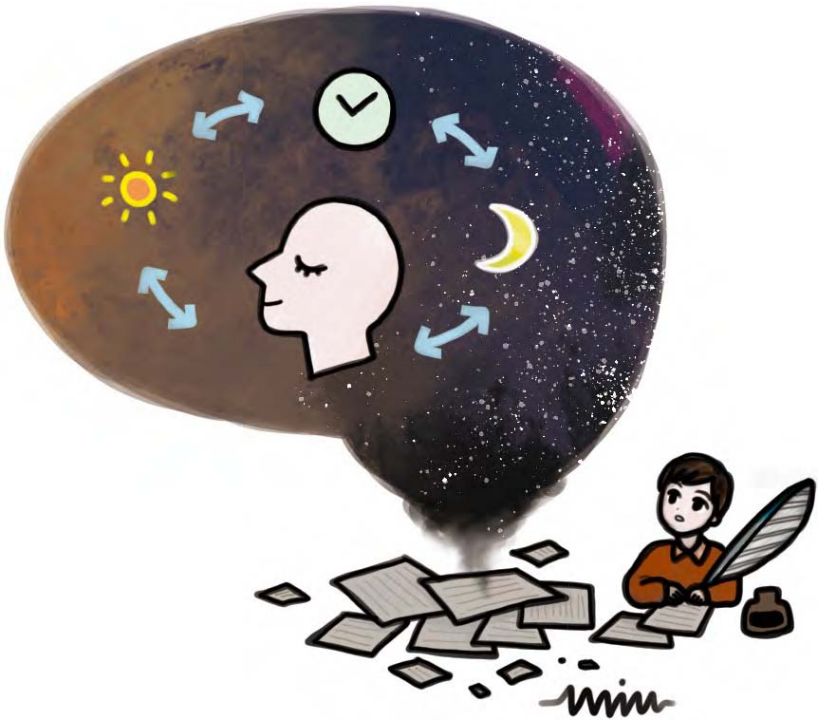


(Illustration by Kim Min-jeong)

As a rule, our bodies undergo changes in 24-hour cycles. Around 9 p.m. each night, we become drowsy as the hormone melatonin is secreted; around 7 a.m., the secretion stops and we wake up. Many other things besides our sleep patterns also change according to 24-hour cycles, including our body temperature, blood pressure, and metabolism. These are what are known as "circadian rhythms."

When we first hear about "circadian rhythms," we might think of our body simply following along with day-night cycle. But if we recall the agony of traveling overseas and being unable to sleep at night because our circadian rhythms are still stuck in original time, we can recognize that our circadian rhythms do not simply follow along with day-night cycle. In 1962, the French speleologist Michel Siffre spent two months in a pitch-black cave, observing that his circadian rhythms still persisted and realizing that our bodies contain their own timekeeping mechanism. How do our bodies generate these regular rhythms? Back in 1965, when experimental technology was not enough for research at the molecular level, Brian Goodwin of the University of Edinburgh used differential equations to identify molecular mechanisms generating regular rhythms [1]. He used differential equations to describe the negative feedback loop of proteins suppressing their own synthesis. Then he solved the equation by computer and found that the level of protein oscillates (Figure 1A). When the level of protein (R) is high, it inhibits the production of mRNA (M), so that the level of proteins decreases, which results in the increase of mRNA production and the level of proteins rising once again —that is, a repeated cycle of increasing and decreasing level of proteins. To describe how the rate of mRNA production

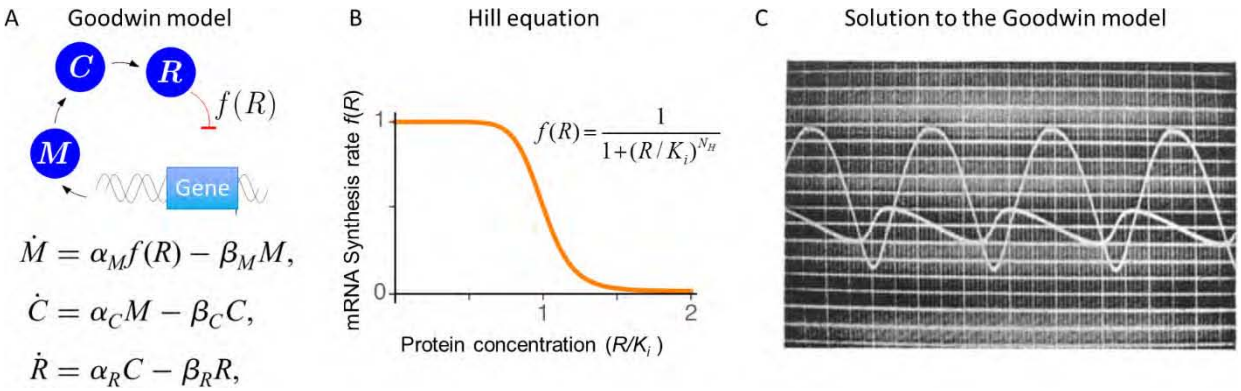
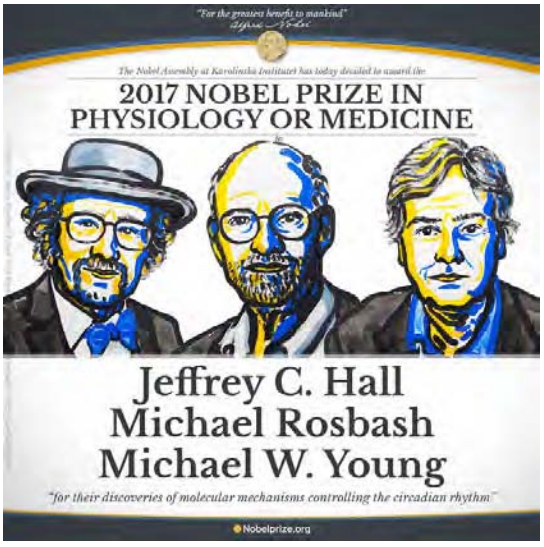


Fig. 1: Goodwin model. A. The negative feedback loop where mRNA (M), after transcription into protein form (C), enters the cell nucleus (R) to block mRNA transcription, with the accompanying differential equations. B. Hill function describes how the rate of mrNA transcription (f(R)) declines as protein concentrations within the nucleus (R) increase. C. The solution of the Goodwin model that Goodwin calculated using a computer in 1965. Proteins and mRNA undergo repeated cycles of increasing and decreasing [1].

declines as the level of proteins increases, Goodwin used a Hill function (Figure 1B), which has been widely used to describe biochemical reactions. Goodwin used a value of 1 for the Hill-exponent (N_H), which adjusts how rapidly the Hill function declines; three years later, Griffith [2] showed that in fact, this resulted in the cycle going away after a few cycles, concluding from this that an N_H value of 8 or higher was necessary for the sustained oscillation (Figure 1C). All this happened because the calculation was not a simple feat with the computers at the time. In any event, Goodwin's discovery that a regular rhythm could be generated through a negative feedback loop was still valid.

Later surprisingly enough, the protein PERIOD (PER) actually does generates circadian rhythms through a negative feedback loop that inhibits its own synthesis; the scientists initiated this discovery—Jeffrey C. Hall, Michael Rosbash, and Michael W. Young—were awarded the 2017 Nobel Prize in Physiology or Medicine for their efforts.



(Image from NobelPrize.org)

Since the discovery of the PER negative feedback loop, the revolution in molecular biology has led to the identification of more complex molecular mechanisms underlying circadian rhythms. This leads to the expansion of the Goodwin model to a more complex mathematical model consisting of dozens of equations.

While mathematical models have gotten more complex and sophisticated, many of the key experimental findings have unfortunately not been reproduced. For example, the circadian clock is composed of over 20,000 individual cells that generate their own rhythms. They communicate through neurotransmitters to generate synchronized circadian rhythm. Yet the models had a problem: when individual cells are coupled, the period of synchronized rhythms is a long way off from 24 hours. The more experimental data obtained, the more numerous the model's problems have been identified. More complex models have been developed to address this issue, but the results have been not fully satisfactory.

When I was in graduate school, I also made various attempts at cracking the problem with the established models with my adviser Daniel Forger, but the results were not satisfactory. It took quite a long time for us to suspect that the issue might lie in using the Hill function to describe negative feedback. Once we realized that the Hill function

was not necessary to describe the negative feedback loop, we searched alternative way. The most attactive mechanism was one in which repressor proteins inhibits their own synthesis by sequestering activator proteins. Once we used another equation to describe this instead of the Hill function, a lot of the problems with the previous models were immediately solved (Figure 2) [3, 4, 5]. In particular, the prediction could only be made with the new model were later confirmed through experiments [6].

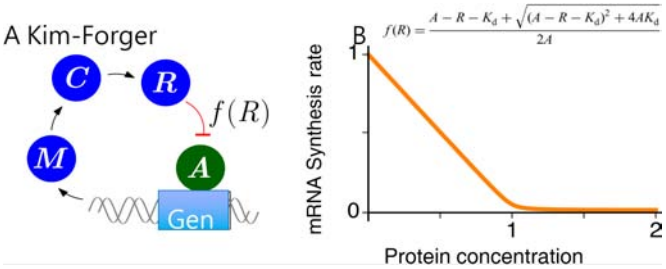


Fig. 2: The Kim-Forger model. A. The protein within the nucleus (R) inhibits an active protein (A) that activates the gene rather than inhibiting the gene directly [3]. B. An equation describing this. It shares the same principle as the Hill equation in that the rate of synthesis decreases where more proteins are present, but the shape is different [5].

But the new model was unable to replicate all of the experimental findings, a failure that made us realize there was something we were missing. This led to an expansion of the previously simple model [7, 8, 9, 10, 11]; the most recently developed model by my graduate student Dae-Wook Kim consists of over 200 equations [12]. The model is precise enough to be used by the global pharmaceutical company Pfizer in its development of new clock-modulating drug. For example, the model has been used to identify the cause of important problems delaying the drug development: different efficacy between mice and monkeys, different efficacy among different people, and so forth. Before carrying out expensive and risky clinical trial, performing virtual experiments with the model have been able to reduce the costs and likelihood of failure; the model has also been used to develop ways of finding personalized drug administration conditions for different individuals [8].

So is it perfect, this complicated model with its 200+ equations that we've spent the past three years to create? Will it be possible to replicate all the numerous experimental findings that keep appearing in dozens of new papers every day? There's little chance of that. But with each failure, more and more data are added to the error notes, which will lead in the end to a new discovery.

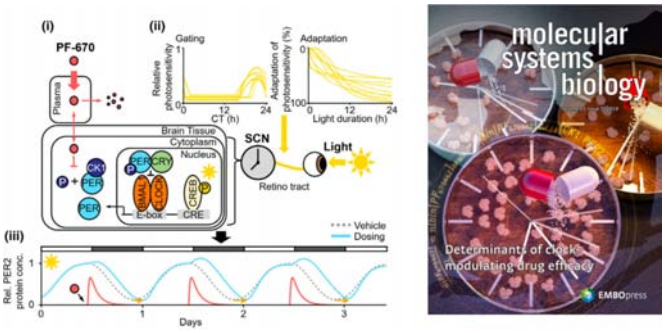


Fig. 3: A recently developed model consisting of over 200 differential equations has been used in new drug development to simulate the effects of medications on circadian rhythms [7]. (Image courtesy of embopress.org)

To learn, we must first make mistakes.

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