Telling time while dividing

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→ The oldest watchmaker on Earth is not a man but a cyanobacterium – a tiny, single-celled organism that lives in the water and needs sunlight to grow. For billions of years, it has used its internal biological clock to predict the daily rising and setting of the sun. This clock is remarkably robust: even though the bacterium can divide up to four times a day, the clock keeps a 24-hour rhythm. Research in the biochemical networks group at AMOLF shows that it requires a very special clock to keep a precise rhythm inside a dividing cell.

Circadian clocks (from the Latin words *circa*, around, and *diem*, day) exist in all branches of life, ranging from bacteria to humans. In our body, these clocks help to coordinate the activity of many organs throughout the day. As it turns out, it is quite difficult to maintain a 24-hour rhythm inside a living cell. This year, the Nobel Prize for Physiology and Medicine was awarded to researchers who have discovered the mechanism of the biological clock in fruit flies.

Across a wide variety of different organisms, all biological clocks a very similar. We study the clock of the cyanobacterium because of its simplicity. At the heart of this clock lies the rhythmic production and degradation of so-called 'clock proteins'. These proteins regulate their own production through negative feedback: when the protein concentration in the cell reaches a certain level, production stops. When the levels drop again due to protein degradation, production restarts. Because there is a considerable delay between the moment when the protein concentration crosses the critical threshold level and the actual change in production, the concentration will start to oscillate (i.e. move up and down in a recurrent fashion) with a rhythm of 24 hours. The cell knows the time of the day from the concentration of the different clock proteins.

However, cyanobacteria do not only live by the rhythm of their biological clock. Key to all life are perpetual cycles of growth and division. During the cell cycle, the bacterium copies all the molecules it is made of and divides them over two daughter cells. Importantly, the DNA, including the genes that encode for the clock proteins, is copied as well. The moment this gene is copied, the production of the clock protein will also double. This means that during each cell cycle the clock receives a little 'push' due to the sudden jump in clock protein production.

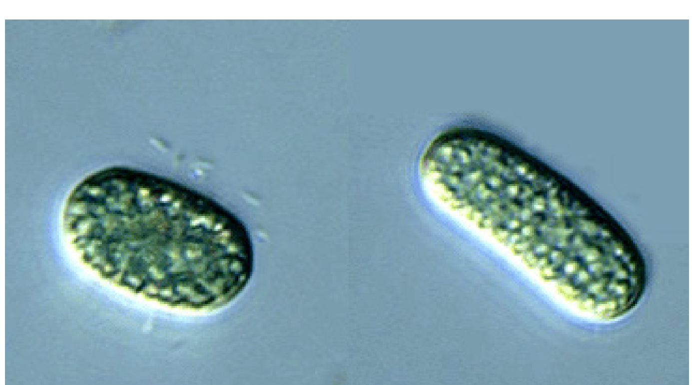
These little rhythmic pushes driven by the cell cycle could have a dramatic effect on the biological clock. Due to a phenomenon called resonance, the rhythm of one oscillator (here: the clock) is extremely sensitive to the motion of another oscillator (here: the cell cycle). Imagine two metronomes ticking out of sync. When you place them on a skateboard, the movement of their rods causes the board to start rocking back and forth. As a result, the metronomes can essentially 'feel' each other. Due to resonance, they will eventually move in synchrony.

Using computer simulations, we found that resonance can indeed occur at the molecular level when we consider the biological clock and the cell cycle. When the cell divides, say, every 28 hours, the clock receives a small push every time the clock protein production doubles. The clock starts lagging behind and will have a 28-hour period. This is harmful to the bacterium, since it cannot predict sunrise anymore. Experimental evidence however, demonstrates that the cyanobacterial clock always retains its 24-hour period, independent of how fast the bacterium grows. How can cyanobacteria achieve this?

It turns out that the clock consists of two oscillators: one production-degradation oscillator described above and one in which the clock proteins are modified with a 24-hour rhythm. It turns out that somehow, a phosphate group is rhythmically added onto and removed from certain sites on the clock protein. The cell can now tell time by measuring the concentration of occupied phosphorylation sites. Because this concentration does not depend on the number of clock proteins inside the cell, it provides a robust read-out as it is much less sensitive to sudden jumps in protein production.

"The oldest watchmaker on Earth is a microbe."

When we incorporate this extra rhythm (the presence or absence of a phosphate group) in our model, the exact length of the cell cycle has a much smaller effect on the rhythm of the biological clock. This explains why the cyanobacterial clock is more complex than the simpler model we started with. Our research has also proved useful to the field of synthetic biology, in which cells are altered to give them new beneficial properties such as the production of new materials and medicine. A clock is essential to properly coordinate production in these engineered cells. Our research shows how to design a robust synthetic clock.Ω



→ Reference
J. Paijmans, M. Bosman, P. R. ten Wolde, & D. K. Lubensk. Discrete Gene Replication Events Drive Coupling between the Cell Cycle and Circadian Clocks. *Proceedings of the National Academy of Sciences*, 113 (15), 4063-4068 (2016).

← Figure
We studied the circadian clock of the cyanobacterium Synechococcus elongatus, which is known to exhibit stable rhythms over a wide range of growth rates.