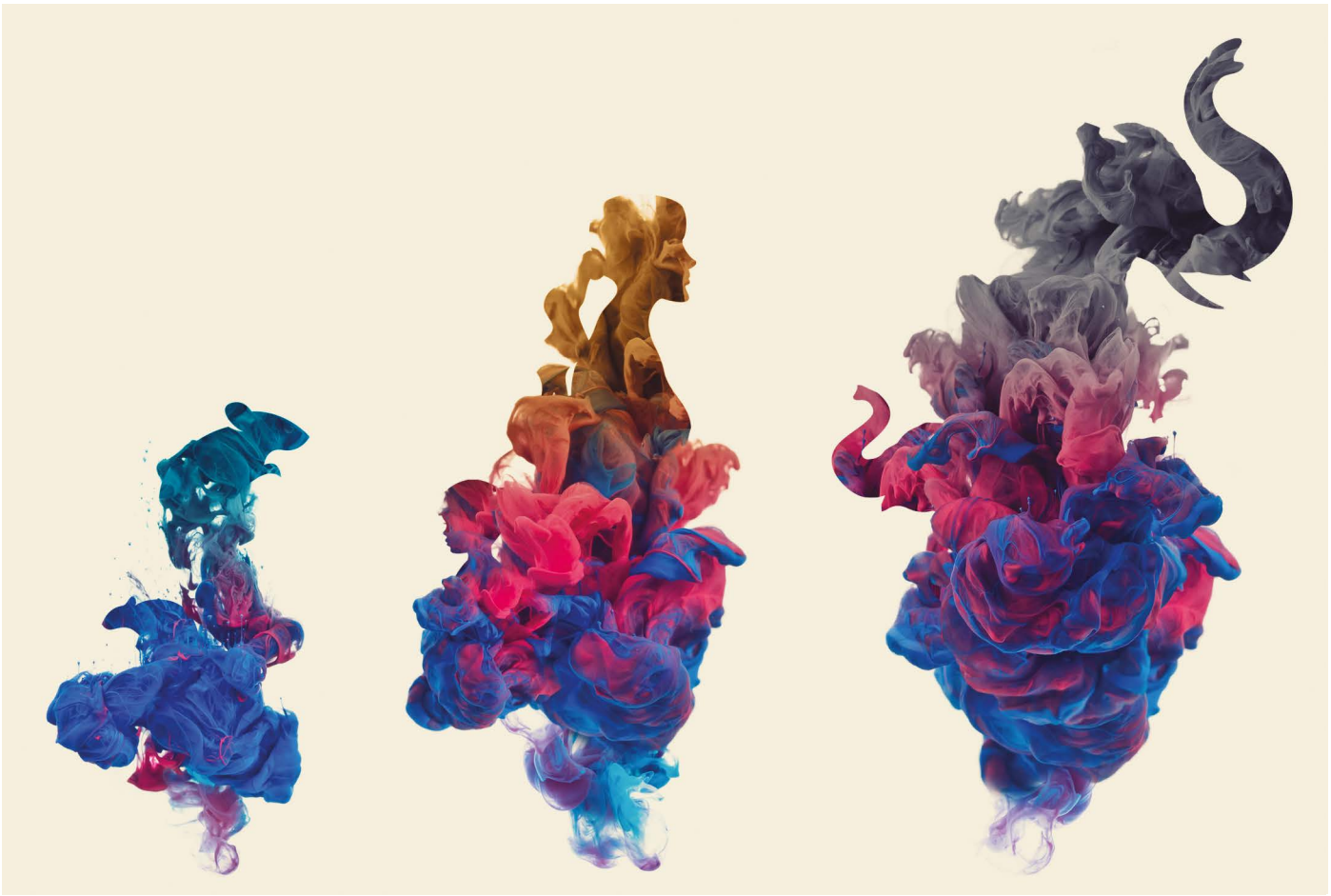


ILLUSTRATION BY ALBERTO SEVESO

THE PACE OF DEVELOPMENT

Researchers are starting to work out why animals develop at different speeds. The key could be tiny timekeepers inside cells. **By Michael Marshall**

In her laboratory in Barcelona, Spain, Miki Ebisuya has built a clock without cogs, springs or numbers. This clock doesn't tick. It is made of genes and proteins, and it keeps time in a layer of cells that Ebisuya's team has grown in its lab. This biological clock is tiny, but it could help to explain some of the most conspicuous differences between animal species.

Animal cells bustle with activity, and the pace varies between species. In all observed instances, mouse cells run faster than human cells, which tick faster than whale cells. These differences affect how big an animal gets, how its parts are arranged and perhaps even how long it will live. But biologists have long wondered what cellular timekeepers control these speeds, and why they vary.

A wave of research is starting to yield answers for one of the many clocks that control the workings of cells. There is a clock in early embryos that beats out a regular rhythm by activating and deactivating genes. This 'segmentation clock' creates repeating body segments such as the vertebrae in our spines. This is the timepiece that Ebisuya has made in her lab.

"I'm interested in biological time," says Ebisuya, a developmental biologist at the European Molecular Biology Laboratory Barcelona. "But lifespan or gestation period, they are too long for me to study." The swift speed of the segmentation clock makes it an ideal model system, she says.

Biologists have been studying the segmentation clock since the 1990s, and they know that it runs about twice as fast in mouse embryos as it does in human embryos. The speed at which an embryo develops, or at which different parts of it develop, has an important influence on the adult body. Ebisuya and others want to understand how differences in developmental pace give rise to organisms with such different bodies and behaviours.

In the past three years, answers have begun to emerge. This is mostly because biologists can now grow the tissue that generates the segmentation clock *in vitro*, from human stem cells, and observe its activity in detail.

"What's truly exciting here is that you can watch it in human [tissue]," says stem-cell biologist Helen Blau at Stanford University in California. "It's a major advance."

The findings are already overturning some long-held assumptions about how different animals develop. So far, there is no sign of a master gene controlling the speed of the segmentation clock. Instead, its speed seems to be controlled by the differing rates at which proteins are broken down. Scientists had assumed the speed was mostly constant for each protein across animals, so the discovery might require them to revise some molecular-biology textbooks.

These differences in cellular speed might even help to explain unique features of human development, such as our oversized brains, protracted childhoods and long lives, relative to many other species.

If results from studies of the segmentation clock are true, this tiny, fleeting timepiece could help to reveal the existence of deeper, biochemical principles that shape all our lives.

Haeckel and heterochrony

Speed matters when it comes to building species. Evolution didn't give giraffes long necks by adding extra bones; they have the same number of vertebrae as their stubby-necked okapi relatives. Rather, neck vertebrae in giraffes grow over longer periods of time, which allows them to reach bigger sizes.

This variation in the speed at which different body parts develop is called heterochrony, a concept described by Ernst Haeckel, a German zoologist noted for his work on embryo development. Modern developmental biologists regard heterochrony as a key concept that helps to explain a core mystery: at the earliest stages of development, all vertebrate embryos look alike, yet as the embryos develop, they become easily recognizable. How do the cells of a human embryo develop into a human baby, and not into an infant chimpanzee or juvenile fish?

A big part of the answer is that the speed at which the parts of the body develop makes a big difference to what the final animal looks like. But what controls the speed of development?

Like Haeckel, modern biologists have found vertebrae and other repeating body segments useful as case studies in how the speed of development shapes animals. Decades ago, they began to investigate how body segments arise during embryogenesis.

As an embryo develops, one of its compartments splits itself into repeating segments known as somites, which run from head to tail. Each somite gives rise to a single vertebra and its associated tissue.

In 1976, two researchers proposed that cells in this compartment might each contain an oscillating mechanism of some kind, which turns itself on and off on a repeating cycle, controlling the production of somites[1]. "That remained as a curiosity for some time," says Olivier Pourquié, a developmental biologist at Harvard Medical School in Boston, Massachusetts. "And then, in the late 1990s, we identified a gene that showed a rhythmic behaviour in the tissue that's going to form the somite."

Pourquié's team studied developing chick embryos and found that a gene called *c-hairy1* flicked on and off every 90 minutes[2] — the time it takes for a somite to form. Waves of *c-hairy1* expression moved along the embryo from tail to head, oscillating in synchrony with the development of somites. Similar segmentation clocks have since been found in mice and other species.

Ever since, Pourquié and other biologists have been trying to take the segmentation clock apart and understand how it works, building a long list of genes and proteins that help the clock to keep time. One key gene is *Hes7*, the mammalian equivalent of the bird gene *c-hairy1*. *Hes7* can repeatedly turn itself on and off, as can several other genes involved in the clock. That makes it "a key pacemaker for the segmentation clock", says Ryoichiro Kageyama, a developmental biologist at Kyoto University in Japan who has studied the gene for almost two decades.

But it is still unclear why *Hes7* turns on and off at different speeds in different species, and thus how the speed of the segmentation clock is ultimately controlled. A series of studies over the past three years point to an answer.

Unpicking the clock

In 2019 and 2020, several labs showed that they can recreate the human segmentation clock *in vitro*, by culturing stem cells so that they develop into somite-forming tissue[3–5]. This was the first hard evidence that humans have a segmentation clock — although this was widely expected. More importantly, creating the clock *in vitro* meant that it could be studied in human tissue for the first time, and allowed much more fine-grained analysis of its mechanism.

These studies were made possible by advances in the culturing of stem cells to persuade them to grow into specific tissues, says Pierre Vanderhaeghen, a developmental neurobiologist at Leuven University in Belgium. In human embryos, the segmentation clock is active only between about the third and fourth weeks of development. "That's even before women know they're pregnant," says Pourquié. "So we know nothing about it. This *in vitro* system provides a proxy for us to study."

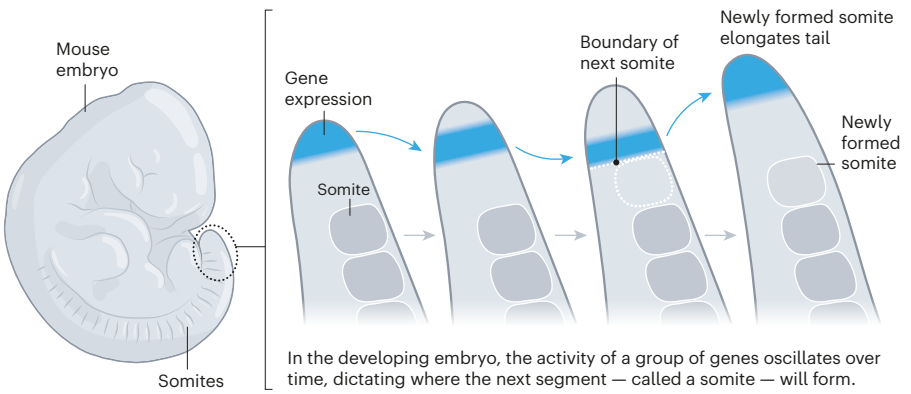
In 2018, Kageyama's team showed that it could take mouse embryonic stem cells and grow them into somite-forming tissue, complete with oscillating *Hes7* gene expression[6]. In 2019 and 2020, three independent groups, led by Pourquié, Ebisuya and stem-cell biologist James Thomson at the Morgridge Institute for Research in Madison, Wisconsin, showed that the same trick could be achieved with human stem cells[3–5].

These studies revealed many similarities between the segmentation clock of humans and those of other animals. Analogues of the same genes and proteins are involved in mice and humans, for instance.

But there was one striking difference. The human segmentation clock is slow. Each oscillation takes 5–6 hours, twice as long as the 2–3 hours it takes in mouse embryos: a clear

UNLOCKING THE SEGMENTATION CLOCK

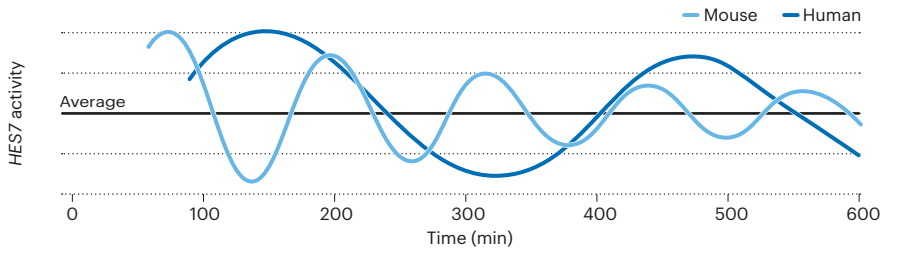
For decades, researchers have studied a clock that helps developing embryos to form repeating body segments such as vertebrae. The clock keeps time by turning various genes on and off in waves of expression.



In the developing embryo, the activity of a group of genes oscillates over time, dictating where the next segment — called a somite — will form.

Human versus mouse

Scientists have recreated the human segmentation clock *in vitro*, and followed the activity of a key gene called *Hes7*. When compared with that of the mouse, the human clock 'ticks' half as fast.



SOURCE: REF. 7. GRAPHIC: NIK SPENCER/*NATURE*

example of heterochrony (see 'Unlocking the segmentation clock'). But why is the human segmentation clock so slow, and what is controlling it?

Running slow

Two papers published together in *Science* last September[7,8] offered a possible answer.

Ebisuya's team focused on the *Hes7* gene, which she calls "the core of the segmentation clock". To check whether the human and mouse versions of *Hes7* were controlling the cells' different speeds, they placed human *Hes7* into mouse cells and mouse *Hes7* into human cells, then watched to see whether the human cells started oscillating at mouse speed and vice versa. But the speed of the oscillations hardly changed at all[7]. Something else was influencing *Hes7*.

To explore this, the team considered how *Hes7* actually works. When the gene is active, it produces the Hes7 protein, and when enough of the protein builds up, this deactivates the gene. Then, once the Hes7 proteins have been broken down, the gene can reactivate. In this way *Hes7* keeps turning itself on and off.

Ebisuya's team wondered whether the Hes7 protein might be broken down more slowly in human cells than in mouse cells, and whether this would account for the slower oscillations in *Hes7* activity — and thus the slower segmentation clock. In further experiments, they found that the Hes7 protein and its RNA template were indeed degraded much more slowly in human cells[7].

It's not clear exactly why this is. Hes7 proteins are degraded by structures called proteasomes, after first being tagged for destruction. "But we don't know which part of this degradation process is slower," says Ebisuya.

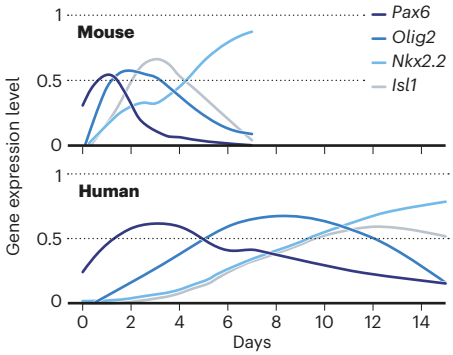
What is intriguing is that this slow degradation of human proteins is not limited to Hes7, or even the segmentation clock. This was borne out by the second study[8], which was led by James Briscoe, a developmental biologist at the Francis Crick Institute in London. His team studied the differentiation of motor neurons in the spinal cords of mouse and human embryos (see 'Speedy genes'). This takes place in a different part of the embryo from the formation of vertebrae, and does not involve the segmentation clock. Yet the process is still slow in humans, taking about 2 weeks, compared with 3–4 days in mice.

The team found that the human proteins took about twice as long to break down as the mouse proteins did — which seemed to be determining the speed at which motor neurons develop. This is strikingly similar to what Ebisuya found, says Briscoe. "We found exactly the same time difference in the spinal cord that she was finding in segmentation."

The mouse and human proteins are close to identical, adds Briscoe's team member Teresa Rayon. That means it is unlikely that the mouse proteins are inherently less stable. "We suspect it's something to do with how proteins are degraded."

SPEEDY GENES

A study from 2020 found that as human and mouse stem cells differentiate into motor neurons, they express the same genes in the same sequence but the process is twice as fast in mice — a possible reason why the two creatures develop at different rates.



Nevertheless, Blau and Vanderhaeghen are cautious about the idea that the rate of protein degradation is the key to the segmentation clock's variable speed. "I don't know that we know that," says Blau. The studies have ruled out some explanations for the difference in speed, she says, but they don't yet prove that the rate of protein degradation is responsible.

Reaction speed

Meanwhile, in unpublished experiments, Ebisuya is exploring whether all human proteins are degraded more slowly than mouse proteins. "We think there's a general trend that degradation rates become slower in human cells", she says, but it might not apply to all proteins. Her team also has evidence that as well as being degraded at more leisurely rates, proteins are produced more slowly in human cells than in other species. Vanderhaeghen says that some other component of the cell, such as metabolic cycles or mitochondrial processes, might also be running faster or slower in different species.

The researchers are all uncertain why biochemical reactions would be systematically slower in human cells — both how the difference arises mechanistically, and why it arose in evolutionary history.

The relative sluggishness of human cells could be a product of their composition or complexity, says J. Kim Dale, a developmental biologist at the University of Dundee, UK. For example, the degradation machinery might find itself struggling to keep up with demand, slowing reactions down. "With the information that we have, it would suggest it's the cell environment," she says.

The *in vitro* segmentation clock studies could well resolve this question, but also suggest a broader mystery: do human cells run slower than those of other species, not just during specific periods of development, but throughout our lives? If so, that could help to explain why our lifespan is extended compared with that of other species.

It is too early to be sure, but a January study[9] suggests that these variations in biochemical reaction speeds run deep in biology. A team led by Sina Ghaemmaghami, who studies proteomics at the University of Rochester in New York, compared how rapidly proteins were created and destroyed in the skin cells of 12 mammals, ranging from golden hamsters that barely live 4 years, to humans, to bowhead whales that can live 200 years.

"I thought there was no way we were going to see much difference," says Ghaemmaghami. Proteomics textbooks often argue that the half-life of a protein is inherent in its structure, he says, so these highly conserved proteins — which vary little between species — should last about as long in all animals. But in fact the team found a strong inverse correlation with lifespan: longer-lived species had slower turnover of proteins.

Are longer-lived animals simply running slower at the biochemical level? "That's the million-dollar question," says Ghaemmaghami. "Is it that the slow turnover is in some way causing long lifespan, or is it that these organisms have long lifespans for a completely independent reason and then can adjust their turnover rate? It's really hard to know."

For now, Ghaemmaghami's working hypothesis is that the slowness is a consequence of long lifespan. He points out that making and breaking proteins rapidly is a good thing, because it ensures the cells are using high-quality proteins — but all that activity releases harmful waste products that can damage the cell. "If you're a long-lived organism, you can't just rapidly turn your proteins over, because you damage everything else," he says. Instead, his team thinks that long-lived animals reduce the overall turnover and pinpoint only damaged proteins for degradation.

Ebisuya thinks that the speed of chemical reactions could be key to these differences, but she wants to work out the mechanism before she is ready to generalize. "I'm still not sure whether similar mechanisms can explain other biological processes," she says.

It might take time, but the clocks Ebisuya and others have built promise to reveal much more about how animals tick.

Michael Marshall is a science writer based in Devon, UK.

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SOURCE: REF. 8