Watching larvae grow up – in real time

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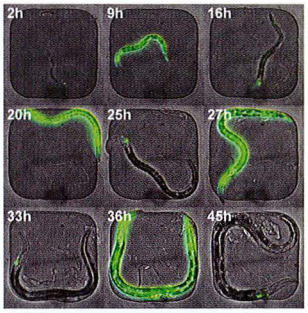

↗ Figure

A. Snapshots of a single C. *elegans* worm growing inside a microchamber (time since hatching indicated in hours). The nematode lights up green when a specific protein is synthesized. This protein is part of the biological clock that orchestrates the rhythm of the nematode's development.

B. Measurements of protein fluorescence vs. time in 15 individual nematodes, the black curve belonging to the worm pictured on the left. The length of the cycles varies, meaning some nematodes grow up faster than others.

→A developing embryo is a beehive of activity, with cells growing, dividing, migrating and deforming in exactly the right way, resulting in a much larger and incredibly complex adult organism. Time-lapse microscopy, with its ability to capture processes that take place very slowly, has become an indispensable tool to study embryonic development. For example, recent technological advances now allow researchers to follow thousands of cells over many hours during the development of zebrafish or fruit fly embryos.

However, development is not finished at birth. Even though the most dramatic transformations occur in the developing embryo, all animals continue to grow after birth. This often includes striking transitions, with puberty in humans as an example. What makes this period of post-embryonic development especially interesting is that, while the embryo develops in the protected environment of the egg or the uterus, development after birth is highly dependent on the environment, particularly on the amount and type of food available. Using microscopy to zoom in on development after birth is tricky for a number of reasons. For instance, whereas a zebrafish embryo is not only small, transparent, immobile and develops rapidly, the baby fish that emerges from its egg grows quickly in size, becomes opaque, moves around and takes a much longer time to develop into adulthood. We have developed a new microscopy approach that overcomes these challenges, allowing us to track the entire process of development from newborn organism to adult animal at the level of individual cells.

As a key first step to overcoming the challenges, we turned to the nematode *Caenorhabditis elegans*, a small soil worm that feeds on bacteria. Over the past decades, this roundworm has become an important model organism for developmental biologists. Two crucial advantages make C. *elegans* uniquely suited for studying post-embryonic development. First, it is small and remains fully transparent throughout its entire life. An adult worm is only a millimetre long - small enough for it to be viewed in its entirety under the microscope. Second, it develops quite rapidly – taking only two days to go from a single fertilised egg to an adult worm. However, one major challenge remained: C. *elegans* larvae, once hatched from their eggs, are highly motile, making them difficult beasties to track. Moreover, preventing their movement, for instance by holding them in place, also stops them from feeding, causing their development – the very thing we want to study - to halt immediately.

"We captured the ticking of a biological clock on film for the first time."

We solved this problem by placing C. *elegans* larvae inside small, purpose-built micro-fabricated chambers, no wider than the diameter of a human hair. These chambers keep the worms, that are otherwise moving normally, within the microscope's field of view for the entire duration of their development into adulthood. Using a microscope camera with a fast shutter speed (1-10 milliseconds), we were able to capture sharp images even when the larvae were moving rapidly inside their chambers. The result is a time-lapse film of a new-born larva – just one hundred micrometres long – developing into a one-millimetre-long adult nematode.

This new approach can be used to expand our knowledge of post-embryonic development in more complex animals, including humans. There is, for instance, the still unanswered question of how the body knows when exactly to initiate major transitions, such as the shedding of milk teeth or the onset of puberty. It is thought that a biological clock controls the precise timing of these and other developmental events, by regulating the production of specific proteins. A similar clock controls the timing of development in our worms. When we labelled one such clock protein fluorescently, we could show that it was produced every ten hours during post-embryonic development - the first time that the 'ticking' of this clock has been captured on film.

Apart from studying clock proteins, we are currently visualising how individual cells divide and communicate in feeding and moving C. *elegans* larvae. As a next step, we want to understand how the developmental clock impacts and controls the behaviour of cells and how this is influenced by environmental factors, such as food availability. Interestingly, the interaction between diet and development is mediated by the same signalling proteins (e.g. insulin) in both worms and humans. We hope that our tiny worms, neatly parked in their microchambers, might tell us more about how the human body deals with food abundance or scarcity. Ω

→ Reference

N. Gritti, S. Kienle, O. Filina and J.S. van Zon, Long-term time-lapse microscopy of C. *elegans* post-embryonic development. *Nature Commun.* 7, 12500: 1-5 (2016).