News & views

Developmental biology

Stretched skin cells divide without DNA replication

Aki Stubb & Sara A. Wickström

Analysis of zebrafish larvae reveals that epithelial cells in their skin undergo tension-driven division without DNA replication. This allows rapid expansion, enabling the cells to cover the fast-growing organism. **See p.119**

Our body and its organs are protected by a physical and biochemical barrier, in the form of an outer lining of epithelial tissue. Given its essential barrier function, it is crucial that this epithelial tissue can rapidly adapt to dynamic changes in the size of the organ or organism, without compromising the epithelial tissue's structural integrity. Chan *et al.*[1] report on page 119 that a population of surface epithelial cells (SECs) in the skin of zebrafish larvae responds to organismal growth through an atypical mechanism of cell division, which occurs without duplication of the genome.

Intuitively, it seems obvious that the expansion of epithelial tissue must perfectly match an organ or organism's growth rate, but the mechanisms underlying this process are only just beginning to emerge. Studies of the outer layer of mouse skin have shown that mechanical stretching triggers coordinated division of epithelial stem cells[2,3], whereas lateral compression of these cells through crowding promotes their progression towards fully differentiated cells that no longer divide[4] (such cells are described as being terminally differentiated). Together, these processes allow the epithelium to grow in a way that matches the needs of the underlying tissue. However, this process of mechanically controlled growth has not been quantitatively analysed in a live organism.

The surface epithelium of developing zebrafish larvae resembles that of mammalian embryos in many ways. Both have a multilayered structure consisting of basal progenitor cells (that is, progenitors of SECs located in the bottom layer of the tissue) that give rise to differentiated suprabasal cells. In mammals, however, the surface epithelium has a thick, opaque outermost layer of dead cell remnants called corneocytes, whereas in zebrafish this surface layer consists of differentiated, viable SECs covered by mucus[5]. This renders the skin of these tiny larvae transparent, providing an optimal setting for high-resolution light microscopy of the entire organism.

Chan *et al.* took advantage of this property of zebrafish skin, in combination with a clever, multicolour cell-tagging strategy called Palmskin, which they adapted from a technique named Brainbow[5]. In Palmskin, a combination of fluorescent proteins is specifically expressed in SECs, enabling the authors to analyse cell shapes and trace cell lineages to study their dynamic behaviour during body growth.

As zebrafish larvae grow, their surface-cover demands increase proportionally. Previous work has indicated that these demands are met purely through division of basal stem or progenitor cells and that terminally differentiated SECs can no longer divide[6,7]. However, Chan and colleagues observed substantial amounts of SEC division. These divisions occur in a directional manner, along the embryo's 'anterior–posterior' growth axis.

Consistent with previous work, the group observed no DNA replication. However, they found that SECs can still undergo two rounds of division without replicating their genome, generating up to four daughter cells. DNA content and cell size decline proportionally during these divisions. The authors call this mode of division asynthetic fission, because the synthesis of new cell building blocks seems to be minimized (Fig. 1).

This observation is striking because the phenomenon occurs in a healthy organism. A cell undergoing normal mitotic division has to pass through several stringent quality checkpoints to ensure that DNA is properly replicated and segregated; mitosis should therefore be blocked if DNA is not faithfully replicated[8]. Indeed, mitosis without replication has not been reported in mammals. It has been shown that cells in early embryos of certain species (such as frogs and fruit flies) can divide after treatment with an inhibitor of DNA replication[9], but asynthetic fission is unique in occurring in a physiologically relevant context, without external perturbations.

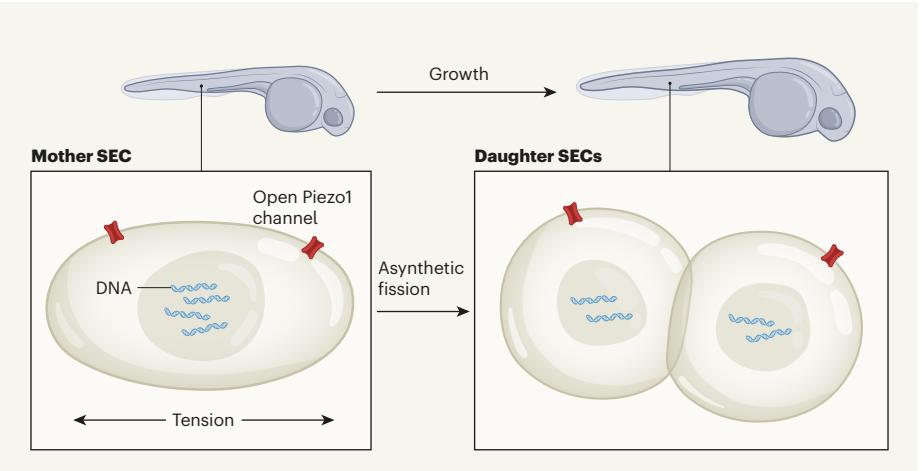

Figure 1 | Asynthetic fission. Zebrafish larvae undergo a period of rapid growth, when the number of surface epithelial cells (SECs) on their skin must increase rapidly to maintain a barrier between the organism and the outside world. Chan *et al.*[1] report that tension in the expanding skin leads them (through unknown mechanisms that involve the activity of open Piezo1 ion-channel proteins) to divide without replicating their DNA. The daughter cells produced through this 'asynthetic fission' process are flatter and smaller than their mother, and have less biomaterial, including DNA. However, they collectively have a larger total surface area, helping to maintain coverage of the growing larva.

Chan *et al.* showed that asynthetic fission peaks during the rapid expansion phase of larval development, when the need for increasing epithelial coverage is at its highest. In addition, they found that asynthetic fission can be triggered during tissue regeneration after a fin amputation. The authors also experimentally manipulated the growth rate of larvae by rearing them at different densities (the more fish there are in a given volume of water, the lower the growth rate). This manipulation led to changes in the division rate of SECs. Together, these data indicate that asynthetic fission can provide a flexible way to adjust the epithelial surface area.

The authors observed that the total volume of the four daughter cells matches that of the initial mother cell. Keeping this in mind, they predicted, using a simple mathematical model, that asynthetic divisions have the potential to efficiently increase SEC surface coverage. Surprisingly, however, one cell division takes longer than basal epidermal-cell mitosis. Thus, an intriguing question remains — what is the advantage of asynthetic fission compared with a mechanism by which non-dividing cells are stretched and flattened to increase their coverage area? One probable answer is that cell divisions that occur in the direction of stretch rapidly reduce mechanical tissue tension, as has been demonstrated in mammalian cell cultures[10] and early zebrafish embryonic development[11].

On the basis of previous work linking epithelial stretch and growth[2], the authors hypothesized that the build-up of tissue tension created by organismal growth could control asynthetic fission. One major group of tension sensors is mechanosensitive ion-channel proteins such as Piezo1 (ref. 12). Chan *et al.* found that experimental activation of Piezo1 led to a significant increase in asynthetic fission in SECs, whereas inhibition of *Piezo1* gene expression attenuated division. This indicates that the asynthetic fissions could be controlled by Piezo1-mediated stretch sensing. Interestingly, larvae in which *Piezo1* was inhibited showed normal body-growth rates despite the lack of asynthetic division. It would be interesting to know whether the division rate of basal progenitor cells is increased in these animals, as a compensatory mechanism to produce excess cells, or whether other adaptive growth mechanisms kick in.

It will be essential to understand the mechanisms of asynthetic fission in more detail. Chan *et al.* show that chemical inhibition of cell-cycle checkpoints, or of the enzymes that regulate them, does not strongly influence asynthetic fission. However, the negative systemic effects of these drugs prevented the authors from studying their effect on SECs for more than a few hours. In future, analyses involving genetic manipulation of cell-cycle regulators specifically in SECs could help to determine whether this mode of division is truly independent of cell-cycle regulation and checkpoints. In addition, the molecular mechanisms triggered by Piezo1 signalling in SECs remain to be determined. Finally, whether this mechanism is used in other tissues and organisms is a fascinating open question.

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The authors declare no competing interests.
This article was published online on 27 April 2022.

Atherosclerosis

Nerve remodelling at a distance

Courtney Clyburn & Susan J. Birren

Fatty structures called plaques can form in arteries, and are separated from nerves by the artery walls. But this is no barrier to communication — it seems that nerves interact with plaques and immune cells to drive cardiovascular disease. **See p.152**

The insidious accumulation of fatty structures called plaques on the inner walls of arteries wreaks havoc on the cardiovascular system, narrowing the vessels, reducing blood and oxygen delivery to crucial organs, and causing heart attacks and strokes[1]. Inflammation, triggered by immune cells, has been recognized as a major factor in plaque formation[2], bringing hope of new interventions against disease. Mohanta and colleagues[3] strengthen this hope even more on page 152, identifying tripartite interactions between nerves, immune cells and plaques as a key component of atherosclerosis — a life-threatening disease that affects millions of people[1]. When these interactions are disrupted, plaques shrink and plaque-associated immune structures are destabilized, suggesting that this unexpected crosstalk could be targeted to treat atherosclerosis.

Atherosclerotic plaques form when lipoproteins and immune cells accumulate on the inner surfaces of arteries. Immune cells in these plaques — such as macrophages, which are part of the innate immune system — initiate inflammatory signalling cascades, causing other immune cells, known as leukocytes, to infiltrate the outer arterial layer (the adventitia). Adventitial immune cells accumulate near the plaques, forming tertiary lymphoid organs that provide a platform for the amplification of inflammation. Strategies for treating atherosclerosis by dampening inflammation look promising, but progress is hampered by the complexity of the immune responses.

Until now, it was assumed that the nervous system was not involved in atherosclerosis, because the vessel wall physically separates plaques from nerve fibres. However, the peripheral nervous system (the nerves that communicate with, but are outside, the brain and spinal cord) uses the adventitia as a conduit for reaching peripheral targets, and there is evidence that fibres directly innervate the smooth muscle of blood vessels[4]. This led Mohanta and colleagues to an exciting realization: in atherosclerosis, aggregates of immune cells infiltrate the outer vessel layer that also contains peripheral fibres. This factor — together with accumulating evidence for inflammation-dependent plasticity of neurons of the peripheral and central nervous systems[5] — prompted the authors to investigate whether the peripheral nervous system interacts with plaque-associated adventitial immune cells, allowing plaques to drive neural remodelling from a distance.

The authors set out to identify differences in nerve fibres in plaque-laden and plaque-free arteries. Using extensive imaging, they evaluated the density and chemistry of neurons in plaque-burdened and plaque-free segments in a mouse model of atherosclerosis (mice lacking the *Apoe* gene), as well as in other rodent models of atherosclerosis and in human