bones. Although palaeontologists often like the idea of being swashbuckling excavating explorers, many of the best discoveries can be made in existing museum collections.

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Regenerative biology

Dual stem-cell populations interact in the skull

Andrei S. Chagin & Dana Trompet

The discovery that the skull has two groups of stem cell that produce similar types of descendant cell has big implications for the field of stem-cell research — and casts light on a developmental disorder that affects many children. **See p.804**

During adult life, the differentiated cells required to maintain the function of a tissue are generated from stem cells located within that tissue, generally from a single population of stem cells. But on page 804, Bok *et al.*[1] report that the skull harbours two distinct populations of stem cells that have complementary functions under physiological conditions, but might work in opposing directions under conditions that cause disorders.

The cranium houses and protects the brain, and comprises eight bones interconnected by bands of cells. These bands, known as sutures, have a crucial role in enabling growth of the cranium to accommodate the developing brain. Such development is pronounced in newborn babies and gradually diminishes with age, but nonetheless persists well into adulthood. Craniosynostosis is a common condition (affecting 4 to 5 children in 10,000)[2,3] caused by premature fusion of these sutures, which restricts the expansion of the cranium, leading to increased pressure on the brain that results in cognitive and learning difficulties.

Numerous studies have revealed that sutures house stem cells — often referred to as calvarial stem cells (CSCs) — and their progeny, which contribute to the formation of cranial bone by giving rise to cells known as osteoblasts. CSCs have been identified by various markers, including expression of the genes *Gli1* (ref. 4), *Axin2* (ref. 5) and *Prrx1* (ref. 6). However, many of the findings of these studies have been inconsistent, and key questions remain unresolved because of differences in the selectivity and rigorousness of the approaches used to identify CSCs.

It has generally been assumed that depletion of CSCs is the main cause of craniosynostosis[4,7], and that repopulating the sutures with these cells would therefore restore their function and allow cranial expansion[7,8]. Bok *et al.* question this assumption in their study. They argue that, because fusion of sutures requires the formation of new bone, depletion of CSCs would actually result in sutures remaining open.

To test this hypothesis, the authors generated mice in which CSCs, identified on the basis that they express the *Ctsk* gene[9], were genetically modified. More specifically, these cells (known as CTSK$^+$ CSCs) were altered so that they did not express the *Twist1* gene; loss of function of the TWIST1 protein in humans is associated with Saethre–Chotzen syndrome, a condition that results in craniosynostosis. The authors observed that these CTSK$^+$ CSCs underwent a form of programmed cell death,

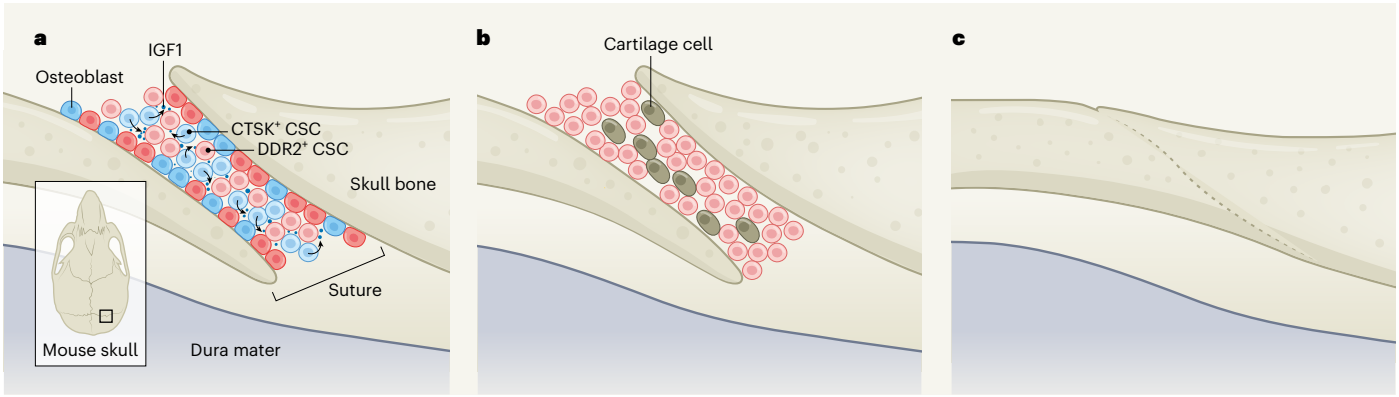

Figure 1 | The behaviour of distinct stem-cell populations in skull sutures under different conditions. a, The bones of the skull are connected by sutures (bands of cells), which include calvarial stem cells (CSCs). Bok *et al.*[1] report the unexpected finding in mice that sutures contain two distinct pools of CSCs: those that express the *Ctsk* gene (CTSK$^+$ CSCs, shown in light blue) and those that express the *Ddr2* gene (DDR2$^+$ CSCs, shown in pink). Both types of CSC self-renew and differentiate to produce bone cells (osteoblasts; those shown in red derive from DDR2$^+$ CSCs, those in darker blue from CTSK$^+$ CSCs). The CTSK$^+$ CSCs secrete the protein IGF1, which signals to DDR2$^+$ CSCs and promotes their normal osteoblast-forming activity. The dura mater is the membrane that underlies the skull. **b**, However, in the absence of CTSK$^+$ CSCs (and therefore of IGF1), the DDR2$^+$ CSCs differentiate to produce cartilage cells. **c**, The cartilage is then converted into bone through a process known as endochondral ossification, thereby causing fusion of the suture. The findings cast light on the mechanism of a disorder called craniosynostosis — a common condition in humans, caused by the premature fusion of sutures in children.

a process that correlated with fusion of the sutures in the mice. These findings prompted the authors to look for cells other than CTSK$^+$ CSCs that could build bone.

Bok and colleagues' thorough analysis revealed that the observed fusion occurs through a process called endochondral ossification, which involves the initial formation of cartilage and its subsequent substitution by bone. This raised the question of where the cartilage-producing cells (chondrocytes) come from. Under normal conditions, there are no chondrocytes in sutures (with the exception of one type of cranial suture, which has a different developmental origin from that of the other types[10] and therefore is not relevant to the current findings).

Further investigation revealed the presence of another population of CSCs characterized by the expression of the *Ddr2* gene (DDR2$^+$ CSCs), which normally generate cell types similar to those produced by CTSK$^+$ CSCs (Fig. 1). However, the authors found that, in the absence of CTSK$^+$ CSCs, DDR2$^+$ CSCs differentiate into chondrocytes that contribute to the observed fusion of the sutures. Notably, an independent study published this year also identified DDR2-expressing cells as potential CSCs in sutures[11].

From a scientific perspective, it is tremendously exciting to discover a case in which two distinct populations of stem cells with the same developmental origin are located in the same tissue and generate similar types of cell. The finding underscores the complexity of stem-cell biology and, if similar arrangements occur in other organs, could have important implications for our understanding of how stem cells are involved in tissue regeneration more broadly.

Nevertheless, several questions still need to be answered. The observation that altering the size of one population of CSCs affects the behaviour of another indicates an interaction between these populations, the underlying mechanism (or mechanisms) of which remains to be explored. Bok *et al.* identify one mechanism, which involves secretion of the hormone IGF1 by CTSK$^+$ CSCs; binding of this hormone by receptors on DDR2$^+$ CSCs prevents cartilage formation. However, the interaction is probably considerably more complex than this, and might well involve other proteins[7,12] known to influence stem cells.

Another issue that requires further investigation is a partial discrepancy between different findings. Genetic ablation of *Gli1*-expressing cells, which probably include both the CTSK$^+$ and the DDR2$^+$ CSCs, leads to abrupt fusion of sutures[4], contradicting the conclusion that DDR2$^+$ CSCs are required for fusion. By contrast, the ablation of *Prrx1*-expressing cells, which also probably include both populations of CSCs, does not cause craniosynostosis[6]. And although Bok *et al.* found that ablation of the *Twist1* gene in CTSK$^+$ CSCs leads to suture fusion, ablation of the same gene in *Gli1*-expressing cells does not[8] – suggesting that *Twist1* in DDR2$^+$ CSCs might have a role in promoting the differentiation of these cells into chondrocytes. These discrepancies and possibilities remain to be explored.

From a clinical perspective, Bok and co-workers' remarkable discovery greatly improves our understanding of the processes underlying craniosynostosis. The conventional treatment involves surgically opening the fused sutures, but refusion often occurs[13], a phenomenon that clearly requires investigation. In this context, further characterization of the relationship between the two populations of CSCs, as well as of other cell sources that potentially underlie refusion, is of considerable interest. This would build on the finding that cells of the dura mater (a fibrous membrane underlying the skull) can prevent refusion[8]. Perhaps Bok and colleagues' discovery will open up fresh avenues of research aimed at developing new therapies for craniosynostosis.

"The finding could have implications for our understanding of how stem cells are involved in tissue regeneration."

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Particle physics

Antimatter falls

Anna Soter

A test performed on antihydrogen atoms has shown that gravity acts on matter and antimatter in a similar way. The experimental feat is the latest in efforts to probe the crossover between theories of relativity and particle physics. **See p.716**

Isaac Newton's now-famous revelation in an apple orchard makes the nature of gravity seem obvious. But would an apple made of antimatter also fall to the ground? On page 716, Anderson *et al.*[1] (members of the ALPHA collaboration) answer in the affirmative. Although there was already some theoretical and indirect experimental[2,3] evidence to suggest that antimatter is subject to the same gravitational pull as matter, the authors have made the first direct observation of free-falling antimatter. Whether the fall is completely indistinguishable from that of a normal apple has yet to be determined.

The underlying physical principle behind the ALPHA collaboration's experiment is the universality of free fall. This idea was first formulated in the sixteenth century by Galileo Galilei, who reportedly observed that spheres that were dropped from the Leaning Tower of Pisa hit the ground at the same time, irrespective of their size and composition. The first precise measurements proving this universality came around the turn of the twentieth century, when Hungarian physicist Loránd Eötvös compared objects made from different materials suspended on a pendulum[4]. A century later, a satellite-borne microgravity experiment showed that titanium and platinum are subject to the same gravitational acceleration as each other, within 15 digits of precision[5]. The universality of free fall has also been tested on very small scales using atom interferometry[6], and on large scales by investigating the Moon's orbit[7].

Why are such measurements so intriguing? Simply because it cannot be assumed that an object's inertial mass, which measures its resistance to acceleration, is the same fundamental property as its gravitational mass,