(cryo-EM)[9]. Cryo-EM obtains molecular structures by analysing thousands of images of individual protein molecules that have been frozen randomly throughout a thin layer of non-crystalline ice. It has proved particularly powerful for solving the structures of large membrane proteins, such as ion channels, that do not readily form the crystals required for X-ray crystallography.

A model of the target protein is often required as a template for cryo-EM analysis. Wang *et al.* used the previously reported structures of Piezo1 as the model for Piezo2. The resulting enormous structure of Piezo2 can therefore be viewed as the culmination of multi-year efforts from multiple labs.

These efforts have revealed that Piezo channels have four key features, which have been given helpfully descriptive names: the propeller, the cap, the pore and the nano-bowl (sometimes also called the dome). The propeller has three blades, one from each of the constituent proteins, equally spaced around the centre and each casting an arc roughly 200 ångströms in length. Each blade contains 36 α-helices, which have the correct lengths to fit across a lipid bilayer, and the correct hydrophobicity to be embedded in one. The string of helices therefore probably weaves back and forth across the membrane. Together, the three spring-like blades define an area of membrane of about 600 square nanometres — a sound arrangement for a sensor of membrane stretch (Fig. 1a).

The cap is a large, extracellular domain that sits outside the pore, similar to the caps that have been implicated in the gating of other trimeric ion channels[10]. The pore region is composed of six α-helices (two from each protein), and it has the internal surface charge expected of channels that allow the passage of positive ions rather than negative ones[6]. The pores of Piezo1 and Piezo2 are both clamped shut in the solved structures, with one constriction site in Piezo1 and two in Piezo2.

So, what is the transduction mechanism by which force opens the pore? There are two general ways in which a membrane protein might detect an external force: it could simply sense the stretch of the lipid membrane itself, or it might be pulled or pushed by other proteins that are attached to it in the regions either side of the membrane (the extracellular matrix or the cytoplasm). Both types of Piezo channel have a curved geometry, like a bowl open to the outside of the cell, and this unusual shape suggests a simple mechanism for force transduction[8]: if the curve of the protein causes local distortion of the membrane, then a lateral stretch could flatten both the membrane and the protein. If this flattening of the protein structure is somehow linked to the gates that open the channel's pore, no accessory proteins would be needed for transduction.

Lin *et al.* tested this proposal in two ways. First, they studied Piezo1 channels in lipid vesicles. This was crucial because previously reported Piezo structures were obtained in aggregates of detergents (detergent micelles), rather than in lipids, so it has been uncertain how Piezos sit within, and interact with, lipid bilayers. The authors found that Piezo1 does indeed distort the vesicle membrane (Fig. 1b), clearly puckering the vesicles into non-spherical shapes. In the second test, the authors applied force to the Piezo channel using the probe of an atomic force microscope (AFM), and found that the bowl underwent reversible flattening at biologically relevant pressures.

It should be noted that the AFM applies force perpendicular to the membrane, rather than laterally through it, but the experiment still demonstrates the springiness of the bowl at appropriate forces — a crucial requirement of the proposal. However, the experiment does not address whether biological stimuli directly stretch cell membranes or act through an accessory protein (akin to the action of the AFM probe). Also not addressed is how flattening of the bowl would open the pore. The extracellular cap might be important for pore opening because, as Wang *et al.* show[3], mutations that eliminate most of it render the channel insensitive to membrane deformation. However, there are many ways to explain the loss of function of a protein when a large part of it is deleted.

Further effort is now required to verify which transduction mechanism — membrane stretch or protein–protein interaction — operates in cells. The first task is to solve the atomic structure of both Piezo channels isolated in artificial lipid membranes, rather than in detergent micelles. Ideally, this would be achieved using lipid vesicles that have been frozen under different osmotic conditions. Osmotic forces will stretch the membrane and, according to the membrane-stretch model, should bias Piezo towards different gating states. Such states might be detectable using cryo-EM or by measuring ion flux through the pores, thereby demonstrating that Piezo can open without aid from other proteins. The two Piezo channels might behave differently in such experiments.

The protein–protein interaction model could be explored further by electron tomography, a method that can distinguish between and locate different types of membrane protein in nerve cells and identify cytoplasmic proteins with which they interact[11]. The observation of force transduction in real time will also be essential. The activity of ion channels, and conformational changes in them, have been measured simultaneously in real time in live cells[12]. This would be a powerful assay for detecting whether protein–protein interactions affect Piezo transduction, and for investigating how channel flattening might link to gates in the pore. Each of these lines of research presents formidable technical challenges. Nevertheless, such high-risk research seems worth the effort, because our sense of touch is so fundamental to our sense of being. ■

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This article was published online on 21 August 2019.

PALAEOANTHROPOLOGY

Elusive cranium of early hominin found

A 3.8-million-year-old hominin fossil reveals what the cranium of the oldest known *Australopithecus* species looked like, casting doubt on assumptions about how these ancient relatives of humans evolved. SEE ARTICLES P.214 & P.220

FRED SPOOR

Human evolution often captures the imagination, not least because some of our extinct hominin relatives are personified by well-preserved fossils with catchy nicknames. For example, a partial skeleton of *Australopithecus afarensis* is named Lucy, and an *Australopithecus africanus* cranium (the skull without its lower jaw) is called Mrs Ples. However, the oldest known species that is unambiguously part of the human evolutionary tree[1], *Australopithecus anamensis*, has mainly languished away from the limelight because of its small and not particularly glamorous fossil record. Until now,

ADAPTED FROM YOHANNES HAILE-SELASSIE BY FRED SPOOR

A. anamensis was known only from partial upper and lower jaw bones, isolated teeth, a small part of the braincase and a few limb bones. These specimens were found in Kenya and Ethiopia and are between 4.2 million and 3.9 million years old[2].

Haile-Selassie *et al.*[3] (page 214) and Saylor *et al.*[4] (page 220) report the discovery of a mostly complete 3.8-million-year-old cranium found in the Woranso-Mille area of Ethiopia. The fossil is of an adult, probably male, and was identified as *A. anamensis* mainly on the basis of the characteristics of its jaw and canine teeth. This cranium looks set to become another celebrated icon of human evolution.

A complete skull is not essential for a good understanding of the morphology of an extinct species. For example, *A. afarensis* had already been well documented from a large collection of fragmentary remains when the first skull from an adult of this species was found[5]. However, the newly discovered cranium of *A. anamensis*, casually named MRD after its collection number, MRD-VP-1/1, provides a wealth of information about *A. anamensis* by revealing for the first time what its full face and braincase looked like (Fig. 1).

MRD offers insight into the shape of hominin skulls at an early stage of the better understood part of human evolution, from about 4.2 million years ago to the present. The new information will help scientists to determine which skull features are primitive (ancestral) and which are derived (evolved — that is, different from the corresponding feature in an ancestor); this, in turn, will affect inferences about the evolutionary relationships between species. The discovery will also trigger a re-evaluation of the sparse hominin fossil record from before 4.2 million years ago. Whether previously discovered fossils assigned to species of *Ardipithecus*, *Orrorin* and *Sahelanthropus* are all indeed part of the human evolutionary tree or are extinct apes is controversial[1,6]. MRD provides information that will advance this debate.

By comparing *A. anamensis* with other species, and including their new evidence, the authors generated evolutionary family trees in which *A. anamensis* was consistently placed as the most ancestral of all *Australopithecus* species and later hominins. This result confirms previous findings[6], and reflects the fact that the cranium shows predominantly primitive features — including some in parts never documented before in *A. anamensis* fossils. MRD has a distinctly protruding face (Fig. 1) and a notably long and narrow braincase. The latter feature is remarkably similar in this respect to that of the 7-million-year-old cranium of *Sahelanthropus*[7], and these two species both had a small brain. The new fossil has several features that are assumed by the authors to be derived rather than primitive. Most striking is the forward projection of the cheek bones, which creates a facial appearance reminiscent of much younger *Paranthropus* hominin species, particularly the 2.5-million-year-old *Paranthropus aethiopicus*[8]. The authors conclude that this facial characteristic evolved independently in *A. anamensis* and later species, but the resemblance might inspire alternative interpretations.

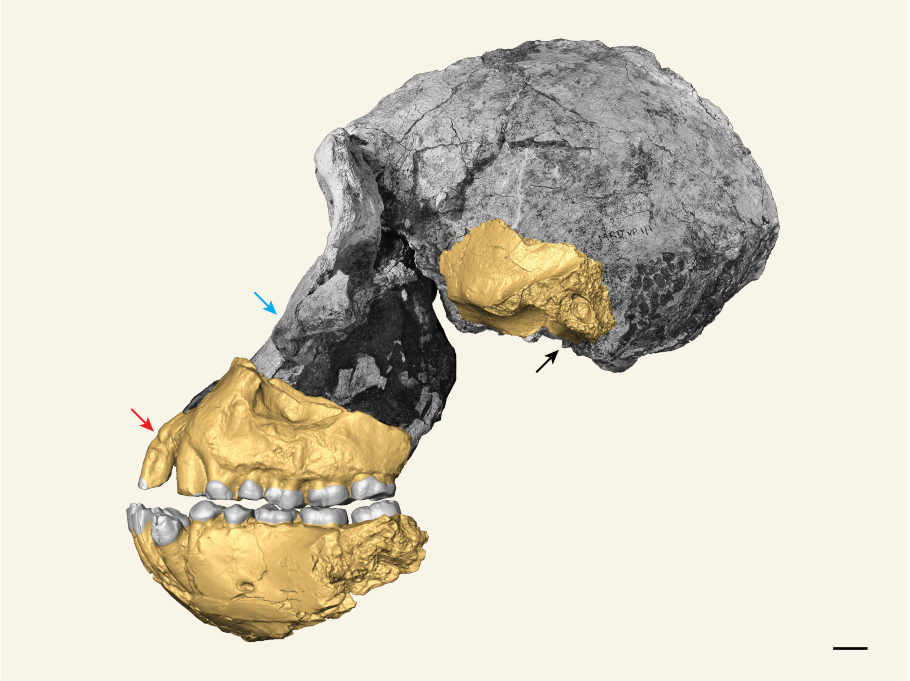

Figure 1 | The skull of the hominin species *Australopithecus anamensis*. Haile-Selassie *et al.*[3] and Saylor *et al.*[4] report the discovery and dating of a 3.8-million-year-old cranium (grey) of *A. anamensis*, found in Ethiopia (collection number MRD-VP-1/1). Surface reconstructions (yellow) of fossils representing the only previously known parts of the skull region of this species are superimposed on the cranium. These 4.2-million-year-old fossils from Kenya[2] are an upper jaw bone (collection number KNM-KP29283) and a lower jaw and ear bone (KNM-KP29281). The projecting cheekbones (blue arrow) create an apparent facial similarity to a 2.5-million-year-old specimen of the hominin *Paranthropus aethiopicus*[8]; however, the authors propose that this feature evolved independently in *Paranthropus* and *A. anamensis*. Both the Kenyan and Ethiopian fossils of *A. anamensis* are characterized, among other features, by protruding jaws (red arrow) and a small earhole (black arrow). Scale bar, 1 centimetre. The Kenyan fossils are presented at 97% of their original size to match the cranium size.

"MRD is a great addition to the fossil record of human evolution."

On the basis of previous comparisons in which only information about jaws and teeth was available for *A. anamensis*, it has been widely accepted that *A. anamensis* and *A. afarensis* were successively part of a single evolving lineage through time, and were represented in the fossil record, respectively, from 4.2 million to 3.9 million years ago, and from 3.8 million to 3.0 million years ago[2,9]. Thus, it has been argued that *A. anamensis* and *A. afarensis* should be considered a single evolutionary species[9].

The MRD cranium now increases the number of *A. anamensis* features that can be compared with those of the other species to explore this issue further, and the authors present evidence that is not consistent with the two species being part of a single evolving lineage. First, they identify a number of features that are derived in *A. anamensis* but are primitive in *A. afarensis*. Second, with the shape of MRD as a basis, the authors conclude that a 3.9-million-year-old frontal bone (part of the forehead) from Ethiopia represents *A. afarensis* rather than *A. anamensis*. This attribution, along with the discovery of the 3.8-million-year-old MRD cranium of *A. anamensis* (dating evidence reported by Saylor *et al.*), provides a revised timeframe indicating that *A. anamensis* existed from at least 4.2 million to 3.8 million years ago, and *A. afarensis* from at least 3.9 million to 3.0 million years ago — so the temporal overlap between the two species was at least 100,000 years.

The model of a single, evolving lineage is certainly challenged by this new evidence, but more aspects will need to be considered. The isolated frontal bone attributed to *A. afarensis* might instead belong to *Kenyanthropus platyops* or *Australopithecus deyiremeda*, other broadly contemporary hominin species from eastern Africa[10]. Moreover, little is known about the face of early *A. afarensis*[2,9], and in particular, whether it showed more similarities to the face of the MRD cranium than does the face of later *A. afarensis*.

One way in which Haile-Selassie and

colleagues' analysis of the fossil specimen stands out is in their use of wide-ranging digital reconstruction that corrects distortions of the fossil's shape, and estimates missing parts. These digital methods are readily available and offer unique opportunities for research. However, many more shapes can be morphed and matched this way than would be possible with conventional methods, and care is needed to generate only the most realistic options. It is therefore essential that any digital reconstruction is carried out with detailed, first-hand knowledge of the original fossil, including how it is preserved and distorted.

This point is particularly relevant with respect to the forward-projecting cheekbones of the newly discovered fossil. After reconstruction, this area looks smoothed, with hardly any sign of the original bone surface. One prominent aspect of MRD where the reconstruction could be improved is the front of the upper jaw. Here, digital processing resulted in a less accurate representation of what the characteristic, strongly projecting subnasal area would have looked like before the fossil was broken.

MRD is a great addition to the fossil record of human evolution. Its discovery will substantially affect our thinking on the origin of the genus *Australopithecus* specifically, and on the evolutionary family tree of early hominins more broadly. This work demonstrates the importance that a single fossil can have in palaeontology, something we should remember when we get puzzled looks and sighs from our colleagues in the experimental biosciences regarding excitement about a sample size of $n = 1$. ■

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This article was published online on 28 August 2019.

METROLOGY

One tick closer to a nuclear clock

Clocks that are based on the nucleus of a single thorium atom could be more precise than existing timekeepers. Such clocks have not yet been realized, but two experiments provide keys steps towards this goal. SEE LETTERS P.238 & P.243

JASON T. BURKE

Atomic clocks are currently the gold standard of timekeeping. These devices measure time on the basis of transitions between two states of an atom. On pages 238 and 243, respectively, Masuda *et al.*[1] and Seiferle *et al.*[2] report progress towards a clock that instead uses transitions between two states of an atomic nucleus. Such a nuclear clock could outperform existing atomic timekeepers, and have applications in both fundamental and applied physics.

Humans have been trying to measure the passage of time for thousands of years. From the sundial, to the hourglass, to the pocket watch, we have continually tried to improve our ability to quantify and standardize time. In the early 1900s, scientists struggled to define time consistently, and put forth various standards to help synchronize humanity. What was missing was a natural reference point that could be used, regardless of its location on Earth. We needed to define what a second truly meant: something fundamental that remains accurate and precise across all space, for all millennia.

Scientists realized that the properties of atomic transitions are independent of location in space or time. This recognition led to the idea of using a known transition between two atomic states as a means to define time. If a standardized second could be defined as a specific and agreed-on number of atomic transitions, time could be quantified. Researchers set out to do this in the 1930s, and by the end of the 1940s the world had its first atomic clock[3].

Over the past 70 years, atomic clocks have been continually improved and currently have a precision[4] of about 1 part in 10^{18}. But what if we could do better than these devices? What if we could make a clock that was 100,000 times smaller, was less susceptible to its environment and possibly had a precision of 1 part in 10^{19}? An atomic nucleus, which is about 100,000 times smaller than an atom, could provide such a device[5].

Since 2003, researchers around the world have been trying to make a nuclear clock using the nucleus of a thorium-229 atom[6]. This nucleus, unlike all others that are known, has an excited state (called an isomeric state) that is only a few electronvolts (eV) in energy above its ground state[7]. As a result, the transition between these two states is accessible using specialized lasers. The problem is that the exact energy of the isomeric state is currently unknown. Masuda *et al.* and Seiferle *et al.* have made progress towards understanding the exact character of the thorium-229 isomeric transition, by carrying out experiments that extend previous work[7].

In Masuda and colleagues' experiment, a high-intensity X-ray beam was passed through a pair of silicon crystals that narrowed the energy range of the X-rays to 0.1 eV. These X-rays were then used to irradiate a thorium-229 nucleus that was in the ground state (Fig. 1). The nucleus transitioned to a second excited state that has an energy much higher than that of the isomeric state. The narrow X-ray energy range allowed the authors to determine the exact energy of

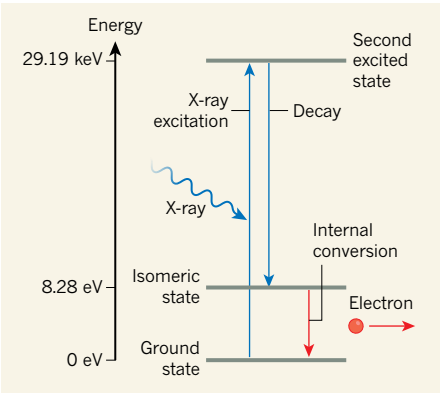

Figure 1 | Low-energy states and transitions of the thorium-229 nucleus. Masuda *et al.*[1] report a technique to produce nuclei of thorium-229 atoms in an excited state called an isomeric state. The authors irradiated a thorium-229 nucleus in the ground state with X-rays, which caused the nucleus to transition to a second excited state that has an energy of 29.19 keV (eV; electronvolts). The nucleus then decayed to the isomeric state. Seiferle *et al.*[2] observed a process known as internal conversion, in which a thorium-229 nucleus in the isomeric state decayed to the ground state and the neutral atom emitted an electron. By studying the energy of emitted electrons, the authors estimated the energy of the isomeric state to be about 8.28 eV. These two studies could lead to ultraprecise clocks that are based on thorium-229 nuclei.