Figure 1 | Four biological groups that achieved macroscopic size and complex morphology during the Ediacaran. The numbers are the age of the oldest confirmed examples (Myr, millions of years). The scale bars represent 1 centimetre, and † denotes groups that have no extant descendants. The algal example (**a**) is a representative of the Lantian biota described by Yuan *et al.*[3]. It is reproduced from their paper, where other algae are depicted, as well as fossils of uncertain affinities. (Images **b** and **c** are field photographs by G. M. Narbonne; image **d** is from J. G. Gehling, SAM P40137.)

age (the geological period that follows the Ediacaran). Fossils of the Avalon assemblage were typically preserved as impressions beneath beds of volcanic ash or sandstone. Both sets of fossils are typically centimetre-scale in size, but the largest examples of the Avalon assemblage are one to two orders of magnitude bigger than the largest Lantian fossils.

The environmental contexts are also different. The Lantian algae inhabited quiet, shallow-water marine environments within the euphotic (sunlit) zone[3], implying that they carried out photosynthesis. By contrast, the Avalon organisms lived in deeper water, up to abyssal depths, and probably acquired energy from the osmotic intake of dissolved organic compounds[2,7]. Later, shallow-water assemblages of the Ediacara biota include other extinct groups such as the erniettomorphs, alongside probable ancestors of radial and bilaterian animals. But the differences in preservation between the carbon compressions of the Lantian/Miaohe biotas and the sandstone impressions of the Ediacara biota allow minimal taxonomic overlap in their resultant fossil assemblages[8]. These different preservational and ecological windows reveal different evolutionary pathways in the Ediacaran development of complex multicellularity among algae, animals and extinct groups such as rangeomorphs and erniettomorphs (Fig. 1).

With the exception of highly derived forms, large eukaryotes, including animals and even algae, strictly require oxygen for their metabolism, and numerous studies have shown a strong link[6] between glaciation, oxidation and the development of complex life during the Neoproterozoic (the era between about 1,000 million and 542 million years ago). The Lantian biota probably predates the oxidation of the deep sea in the Avalon locations[5] and northwest Canada[9], which occurred 582 million years ago, but the Lantian deposits were laid down at much shallower (euphotic) depths that may well have been oxygenated by this time.

Geochemical studies have shown that the Lantian[9] and equivalent strata in central China[10] were deposited under anoxic conditions. Yuan and colleagues[3] regard the Lantian fossils as having been preserved in their original life positions on the sea floor, however, and thus that they are indicative of brief oxygenation events that may have gone unnoticed and unsampled in the geochemical studies[9,10]. This hypothesis should be easy to test, and will help to elucidate the complex but crucial role of ocean oxidation in the Ediacaran emergence of complex multicellular life. ■

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BIOPHYSICS

Push it, pull it

During migration, cells interact with their environment by exerting mechanical forces on it. A combination of two techniques shows that they do so in all three dimensions by a push–pull mechanism.

PASCAL HERSEN & BENOÎT LADOUX

Mechanobiology is an emerging field that investigates how living cells sense and respond to the mechanical cues of their surroundings. In contrast to passive objects such as water droplets, living cells actively probe their environment by exerting forces on it as they migrate[1]. Such forces not only drive mechanical events such as cell deformation but also trigger cellular processes such as cell–environment adhesion signalling and cytoskeletal reorganization. In this context, mechanical forces have been shown[1–3] to have a key role in many biological functions, including cell migration, cancer progression and stem-cell differentiation. But the precise characterization of these forces in space and time has remained elusive. Writing in *Physical Review Letters*, Delanoë-Ayari and colleagues[4] describe a microscopic technique that does just that.

In the early 1980s, seminal work by Harris *et al.*[5] demonstrated that cells can exert forces on and deform compliant two-dimensional (2D) substrates. Since then, various techniques have been developed to map the deformation induced by traction forces exerted by cells on elastic substrates[1]. These traction-force microscopy techniques have led to a greater understanding[6–8] of the processes that regulate cell–substrate interactions, from the molecular to the multicellular level. However, until recently, the techniques have been used only to compute in-plane (horizontal) forces, thus assuming that the cellular biomechanical components responsible for the establishment of forces within cells were mostly oriented parallel to the surface. In other

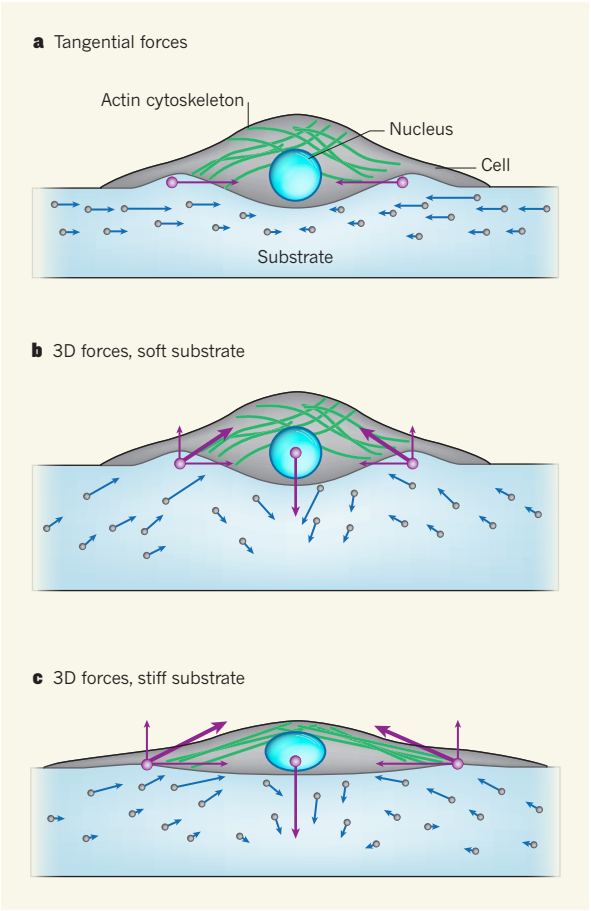

Figure 1 | Mechanical forces involved in cell adhesion. **a**, Classic representation of the technique of traction-force microscopy, which involves measuring the tangential traction forces (purple arrows) exerted by a cell on a deformable substrate on the basis of the displacements (blue arrows) that the cell induces on fluorescent beads (grey dots) embedded in a substrate. **b**, Delanoë-Ayari and colleagues[4] extend the technique to allow the traction forces exerted by a cell on a soft substrate to be precisely determined in three dimensions. They find that cell–substrate interactions are regulated by a push–pull force mechanism: the substrate is pushed vertically in the region underneath the cell nucleus but pulled obliquely towards the cell centre at the cell's edges. Thin purple arrows represent the tangential and vertical components of the oblique forces. **c**, On stiffer substrates, because cells are spread more thinly across the substrate surface, the nucleus may be subject to greater stress induced by tensions in the cell's actin cytoskeleton (green filaments).

words, they presumed that the component of the forces that is perpendicular to the substrate was negligible (Fig. 1a).

However, we have come to learn that cells act in all dimensions as they probe and respond to the three-dimensional (3D) geometry of their environment[9,10]. In their study, Delanoë-Ayari *et al.*[4] devise a method that can accurately map the 3D force pattern generated by adherent cells. The method is essentially an extension of the traction-force microscopy technique developed by Dembo and Wang[11]. It consists of measuring a cell's traction forces, and so a substrate's deformation, on the basis of the substrate's elastic properties and the displacement that the cell induces on fluorescent beads embedded near the substrate's surface. Combined with a technique[12] that permits 3D tracking of the beads' dynamics, the method enables the spatial and temporal distributions of the cell's traction forces to be precisely determined in all directions.

The authors apply their method to cells of the soil-living amoeba *Dictyostelium discoideum* on a soft-gel substrate with easily controlled mechanical properties. Surprisingly, although the fluorescent beads are randomly distributed inside the gel, when focusing light on the gel's upper surface, the researchers observe a 'black hole' in the fluorescent signal just where the cell is located. This happens because the cell pushes the beads towards the gel's interior, causing them to go out of focus. The fluorescent signal re-emerges when the cell is removed from the substrate, because the beads recover their equilibrium position. What's more, the observed 3D force pattern clearly indicates that *D. discoideum* cells regulate their interactions with the soft substrate through a push–pull force mechanism: the cells push the gel vertically in the region underneath the cell nucleus but pull it obliquely towards the cell centre at the cell's edges (Fig. 1b). Because the overall force has to be zero, the pulling forces in the vertical direction exactly balance the pushing forces.

Delanoë-Ayari *et al.*[4] demonstrate not only that vertical forces exist, but also that they are of the same order of magnitude as horizontal forces, thus highlighting the need to consider vertical forces in studies that examine the role of cell–substrate interactions in biological functions. Taken together with previous studies[13,14] on mammalian tissue cells that showed that they deform their environment in much the same way as *D. discoideum* does, the authors' findings not only highlight the importance of taking into account 3D forces for all adherent cell types, but also give a new and clear description of the mechanical balance between the pushing and pulling forces.

Because substrate elasticity can govern cell fate[3], one of the main issues in the field of mechanobiology concerns understanding the interplay between gene expression and mechanical forces exerted by cells on the environment. The observed force pattern raises questions about the physical coupling between the nucleus and the elastic components of the cytoplasm[15]. The authors cultured cells on substrates that are softer than the cells' nuclei, so the implication is that, on contact with the substrate, the cells deformed the substrate more than their nuclei were deformed. The question of whether, on stiffer substrates, the pushing forces could lead to nuclear deformation and cell-fate reprogramming requires investigation.

It is well known that on stiffer substrates mammalian tissue cells exert larger forces and are more spread out across the substrate surface, thus leading to a higher nuclear compression (Fig. 1c). Increasing substrate rigidity may therefore result in an increase in the horizontal forces and a relative decrease in the vertical ones. To what extent the authors' technique can be applied over a broad range of substrate stiffness remains an open question.

Although we are still far from a complete understanding of the mutual interaction between cell function and mechanical cues, Delanoë-Ayari *et al.* have shown that cellular traction forces in all three dimensions matter, and should be taken into account to fully understand cell–substrate interactions. ■

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