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combine desirable properties (e.g., coated droplets). At the same time, exploration of alternative compartment types probes the chemical limits of life and may well have unexpected relevance to our own biology.

Protocells at the intersection

Protocells occupy an interesting intersection of two intellectual approaches in biology. The spirit of bottom-up synthetic biology is famously captured by Richard Feynman's final blackboard writing "What I cannot create, I do not understand". The practical process of creating a protocell requires grappling with fundamental chemical and biophysical realities in pursuit of understanding the cell as a unit of life. On the other hand, systems biology is broadly concerned with interactions among parts of complex biological systems, to understand the 'whole' as more than the sum of its parts. Systems biology is usually applied to extant biology, i.e., thousands or millions of parts requiring high-throughput techniques and computational models with many parameters. For the protocell, even though there are many fewer parts, a deep level of mechanistic understanding of interactions among parts is integral to its practical construction, particularly for developing and recognizing emergent behaviors. The study of protocells thus merges ideas from systems biology and bottom-up synthetic biology.

The journey toward a chemical system capable of open-ended Darwinian evolution faces many challenges. Yet progress is evident in several directions. For example, while it is not yet clear how to couple the rates of various processes to achieve a self-sustaining life cycle, the discovery of several interesting mechanisms (e.g., the coupling of growth and division, the coupling of membrane stress and growth) suggests that this may be achievable with relatively few interacting components. Another area of interest is the first synergy between ribozymes with different functions, leading toward greater complexity. While natural selection could favor cooperating ribozymes through multilevel selection or other mechanisms, how exactly such a system would arise in practice represents an important subject for detailed study. Finally, the environment plays a pivotal role in natural selection. Which environment would present selection pressures on the protocell that would stimulate the emergence of new functions and greater complexity without destroying the system? Like the hydrogen atom in physical chemistry and E. coli in molecular biology, protocells are simple experimental models that serve as a focal point while moving toward a greater understanding of cells themselves.

FURTHER READING

Bansho, Y., Furubayashi, T., Ichihashi N., and Yomo, T. (2016). Host–parasite RNA oscillation in a compartment. Proc. Natl. Acad. Sci. USA *113*, 4045–4050.
Blain, J.C., and Szostak, J.W. (2014). Progress toward synthetic cells. Annu. Rev. Biochem. *83*, 615–640.
Deamer, D.W. (1985). Boundary structures are formed by organic components of the Murchison carbonaceous chondrite. Nature *317*, 792–794.
Joyce, G.F., and Szostak, J.W. (2018). Protocells and RNA self-replication. Cold Spring Harb. Perspect. Biol. *10*, a034801.
Joyce, G.F. (2012). Bit by bit: The Darwinian basis of life. PLoS Biol. *10*, e1001323.
Li, M., Huang, X., Tang, T.-Y.D., and Mann, S. (2014). Synthetic cellularity based on non-lipid micro-compartments and protocell models. Curr. Opin. Chem. Biol. *22*, 1–11.
Mills, D.R., Peterson, R.L., and Spiegelman, S. (1967). An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule. Proc. Natl. Acad. Sci. USA *58*, 217–224.
Nowak, M.A. (2006). Five rules for the evolution of cooperation. Science *314*, 1560–1563.
Pressman, A., Blanco, C., and Chen, I.A. (2015). The RNA world as a model system to study the origin of life. Curr. Biol. *25*, R953–R963.
Strulson, C.A., Molden, R.C., Keating, C.D., and Bevilacqua, P.C. (2012). RNA catalysis through compartmentalization. Nat. Chem. *4*, 941–946.
Sutherland, J.D. (2016). The origin of life - out of the blue. Angew. Chemie Int. Ed. *55*, 104–121.
Szostak, J.W., Bartel, D.P., and Luisi, P.L. (2001). Synthesizing life. Nature *409*, 387–390.
Zwicker, D., Seyboldt, R., Weber, C.A., Hyman, A.A., and Jülicher, F. (2017). Growth and division of active droplets provides a model for protocells. Nat. Phys. *13*, 408–413.

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Primer
Fossil cells

Philip C.J. Donoghue

Although the fossil record may be commonly perceived as a trove of old bones and shells, for most of Earth's history — its first four billion years — it is comprised almost exclusively of cells, their cell walls or resting cyst stages. Even in more recent history, those old bones preserve a record of the cells that precipitated the mineralised tissues in which they are now petrified. Hence, fossils have the potential to provide unique insights into the evolution of cells — from the emergence of cellular life itself, through many of the major evolutionary transitions including eukaryogenesis, multicellularity, sexual reproduction and cellular differentiation, and even cellular insights into genome evolution through deep time.

Life in a cell

Divining the very origin of cellular life on Earth is a tricky business, even assuming that the last universal common ancestor of extant life (LUCA) was a cellular organism rather than an unappetisingly thin genetic soup. While there are truly ancient (4.1–3.8 billion years ago) geochemical records of enrichment of the light carbon isotope that life prefers, this could have resulted from abiotic processes. Conclusive evidence for the oldest cellular life requires either biomolecular fossils of cell membranes, isotopic evidence of metabolism that evolved after LUCA or fossil remains of the cells themselves.

The 3.46 billion year old Apex Chert microbiota of the Pilbara Craton, Western Australia holds perhaps the oldest coherent claim for life. Originally described as an unanticipatedly early diversity of microbial organisms including possible photoautotrophs, the Apex microbiota appeared to evidence an early emergence of LUCA and its subsequent diversification. Some of the fossil remains (Figure 1A,B)

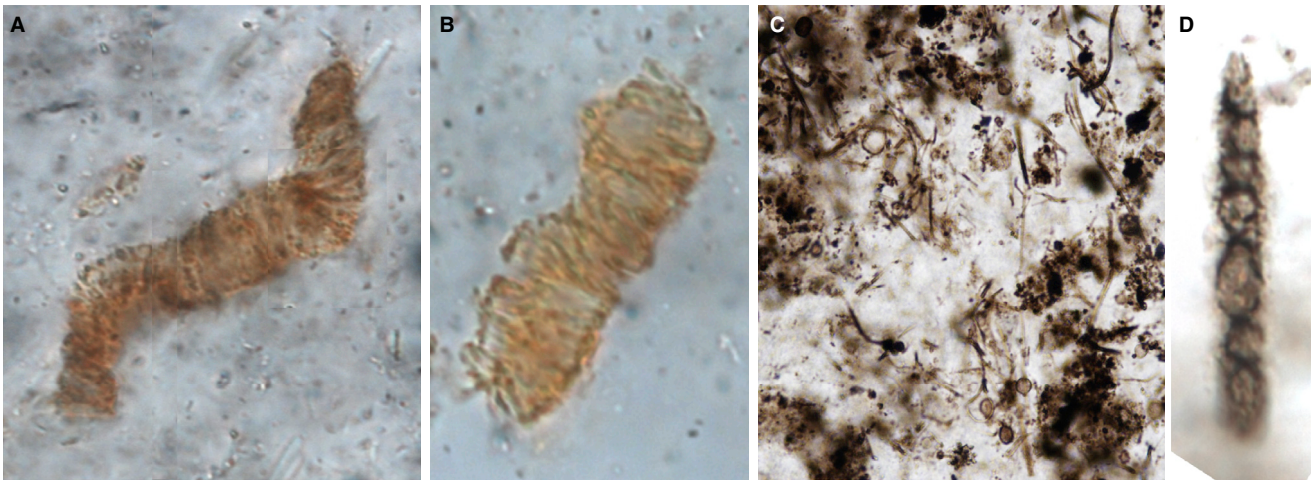

Figure 1. Fossil cells.
(A) *Primaevifilum conicoterminatum* ('cells' 4–6 µm wide) and (B) *Archaeoscillatoriopsis grandis* ('cells' 8–11.5 µm wide), probable pseudofossils from the Archaean Apex Chert of Western Australia. (C,D) Fossil cells from the Palaeoproterozoic Gunflint Chert of northern Lake Superior; (C) an assemblage of filaments and coccoid cells (frame 85 µm high); (D) individual trichome of a probable cyanobacterium (61 µm length). Images courtesy of David Wacey, University of Western Australia.

strongly resemble living and fossil cyanobacterial trichomes (filament-like arrangements of cells). However, reexamination has shown these structures to intergrade with dispersed graphite crystals, undermining claims that they are derived from living cells. They have been reinterpreted convincingly as products of a complex geological history of transformation from the silicate mineral mica, through to vermiculite (the hydrophilic mineral used for mopping up oil spills and cat wee). The vermiculite phase is envisaged as having absorbed younger kerogen as it migrated through the rock. It is this much younger organic matter that was eventually thermally altered to form the graphite trichome-like pseudofossils (Figure 1A,B). This interpretation is not uncontested, but the debate is symptomatic of a more general malaise in the search for early life — structures as simple in shape as cell membranes can be generated by abiotic processes, hence every claim for fossils of life on early Earth has been subject to challenge.

The oldest widely accepted record is from the 3.43–3.35 billion year old Strelley Pool Formation, in the same Pilbara Craton sequence, where there is a convergence of isotopic, sedimentary and fossil evidence of microbial life. This is a minimum age constraint on the emergence of cellular life on Earth, but older records cannot be dismissed out of hand. Molecular clock methodology provides a means of integrating this evidence within an explicitly probabilistic framework that attempts to calibrate molecular evolution — recorded in the genome of all living organisms — to geological time using these fossil constraints. This combined molecular and palaeontological approach suggests that LUCA existed about as soon as Earth became habitable 4.52–4.47 billion years ago (Figure 2).

The origin of eukaryotes — life goes nuclear

The same molecular clock analyses estimate the next great leap in cellular evolution — the origin of eukaryotes — to have occurred two billion years or more after LUCA, though there is a lot of uncertainty in timing (Figure 2). In large part this uncertainty is due to the challenge of distinguishing fossil eukaryotes from bacterial-grade organisms. Eukaryotes can be diagnosed by their internal organisation — they have a cytoskeleton that allows them to change the shape of their cells, and a membrane-bound nucleus and mitochondria, among other traits. All living eukaryotes, their last common ancestor and all of its descendants are 'crown-eukaryotes', while fossil species more closely related to living eukaryotes than to their archaeal and alpha-proteobacterial relatives are stem-eukaryotes. Together, stem- and crown-eukaryotes comprise a eukaryote total group. Discriminating fossil bacterial-grade microbes from crown eukaryotes is a challenge; distinguishing between fossil stem and crown unicellular eukaryotes may be truly impossible. Generally, palaeontologists have separated fossil eukaryote and prokaryote cells based on size, but this distinction is probabilistic not definitive. Alternatively, fossil eukaryotes have been recognised on circumstantial evidence of an actin cytoskeleton, manifest as cyst wall processes (Figure 3A) or excystment structures that required the cell to change shape in cyst-formation or escape. However, some archaea are now known to possess such a cytoskeleton, leaving cell or cyst wall differentiation (first convincingly seen in rocks of 1.62 billion years of age from North China) as the only good criterion for identifying fossil eukaryotes, though even this distinction is rooted in the faith that prokaryotes have been incapable of such complexity. Much more convincing fossil evidence of eukaryote affinity would be cells with nuclei or organelles, and there are reports of fossil nuclei in cells

from the 0.83 billion year old Bitter Springs Formation of central Australia (Figure 3B). Indeed, nucleus-like structures are not uncommon in fossil cysts from much older strata, including *Dictyosphaera* (Figure 3C,D) and *Shuiyousphaeridium*, appearing to provide definitive evidence for total group eukaryotes older than 1.75 billion years. They are not accepted as such because of fossilization experiments which have shown that, after death, bacterial cytoplasm shrinks within the cell to produce a small dense body resembling putative fossil nuclei from the Proterozoic. These classic experiments urged caution over the identification of fossil organelles but, over time, they have been misinterpreted as evidencing that eukaryote nuclei and organelles cannot be fossilised. Chloroplasts, nuclei and even mitochondria have been described from much younger Eocene and Miocene age leaves, and nuclei with possible chromosomes from a Jurassic fern (Figure 3E,F). No one will have doubted that Jurassic ferns had nuclei or that Cenozoic angiosperms had chloroplasts, but these fossils provide unequivocal evidence that the nuclei and organelles of eukaryotes can be fossilised. So perhaps, older Proterozoic records should be considered more seriously. At least, complementary fossilization experiments should be conducted on unicellular eukaryotes to help establish robust criteria for identifying eukaryote-grade fossils.

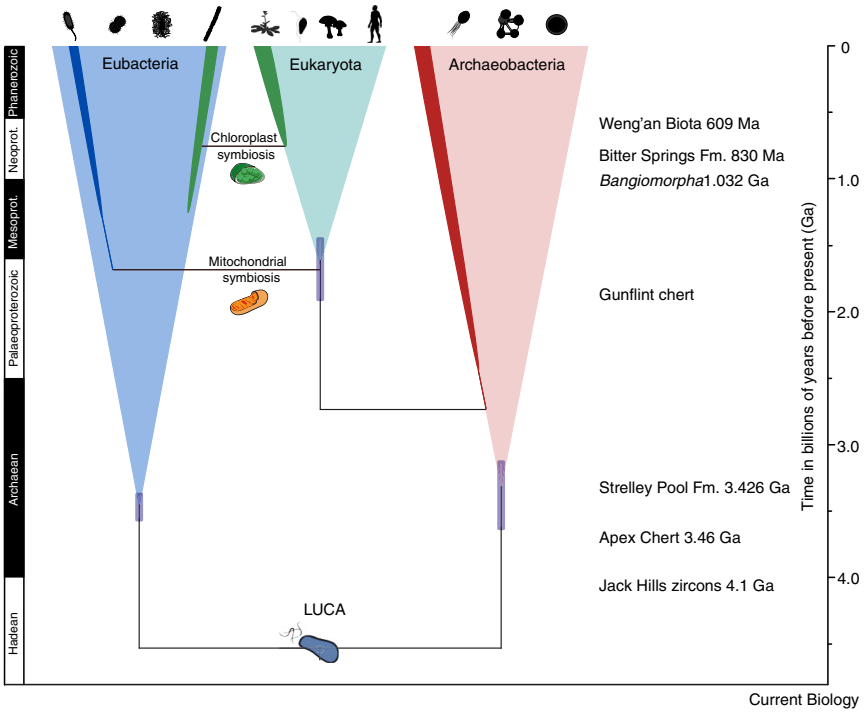

Figure 2. Timeline.
The evolutionary timescale for early life on Earth and the attendant evidence of fossil cells, based on Betts *et al.* (2018).

LECA and the emergence of eukaryote multicellularity

The oldest definitive fossil eukaryotes are crown-eukaryotes. The oldest possible records, *Rafatazmia chitrakootensis* and *Ramathallus lobatus* (~1.6 billion years old), are attributed to red algae. Ramathallus strongly resembles the late Neoproterozoic *Paramecia* and *Wengania*, unequivocally accepted as members of the living floridiophyte group of red algae. The red algal affinity of the latest Mesoproterozoic *Bangiomorpha pubsecens* (Figure 3G–I) is more widely accepted because it is known from distinct developmental stages including possible sexual dimorphs, strongly resembling living bangiophyte red algae. However, many of its characteristics are general to red algae and so it may be best interpreted as a total-group red alga. No matter, *Bangiomorpha* provides a minimum constraint on the timing of origin of red algae, crown eukaryotes and the last eukaryote common ancestor (LECA) itself (Figure 2). It also age-constrains minimally the primary plastid symbiosis of the plant kingdom (Archaeplastida) and maximally the secondary and tertiary endosymbioses of red algal plastids with diverse photosynthetic eukaryote lineages.

There are many records of aggregative multicellularity in the fossil record, appearing at least by the late Archaean (>2 billion years ago). Disparate records occur also through the Palaeoproterozoic and Mesoproterozoic, but their eukaryotic affinity is contested. *Bangiomorpha* is as good an early record of a multicellular eukaryote as one may hope to find; records of other extant multicellular clades are considerably younger, though their lineages may be considerably older. Nevertheless, they record a switch from temporal to spatial cell differentiation. There may be evidence of this switch preserved in the 0.61 billion year old Weng'an Biota of South China, which preserves in three dimensions and subcellular resolution the remains of diverse organisms with coordinated multicellularity facilitated by functional cell adhesion. Currently, the most likely interpretation is that they represent diverse non-metazoan holozoans, the grade of organisms from which extant ichthyosporeans, filastereans, choanoflagellates and animals ultimately emerged. Multicellular land plants emerged at a similar time in Earth history, during the late Neoproterozoic, with the living clade arising about half a billion years ago — though, of course, they emerged from within broader clades that may have achieved multicellularity earlier and independently.

A metazoan explosion of cell-type diversity

It is perhaps unsurprising that fossils provide a poor record of the transition to multicellularity, but the

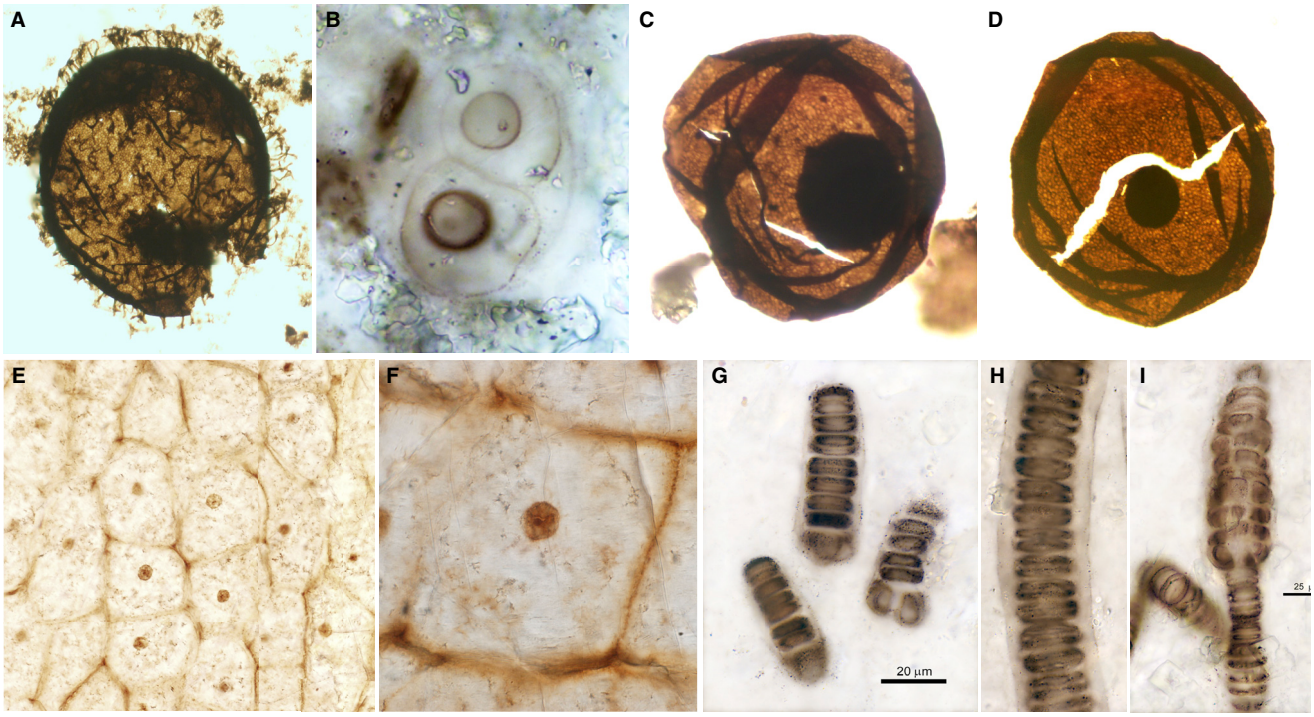

Figure 3. Early fossil eukaryotes and eukaryote-like cells.
(A) *Shuiyousphaeridium macroreticulatum*, one of the earliest records of eukaryotes, from the Paleoproterozoic Ruyang Group of North China (120 µm diameter). (B) Possible fossil nuclei within a pair of cells enclosed within a membrane, from the early Neoproterozoic Bitter Springs Formation of central Australia (64 µm diameter). (C,D) *Dictyosphaera deliculata*, another of the earliest records of eukaryotes, which preserves intracellular nucleus-like bodies, from the Paleoproterozoic Ruyang Group of North China; (both ~110 µm diameter). (E,F) Cells preserving nuclei in the permineralised stem of the fern *Osmundastrum pulchellum* from the early Jurassic of Scania (nuclei 23 µm diameter). (G–I) *Bangiomorpha pubescens*, a red alga from the latest Mesoproterozoic of Somerset Island, arctic Canada; (G) longitudinal intercalary growth; (H) early morphogenesis showing development of the holdfast; (I) development of spheroidal spores (cells 14–18 µm wide). (A,C,D) courtesy of Ke Pang, NIGPAS Nanjing and Shuhai Xiao Virginia Tech, (B) courtesy of David Wacey, University of Western Australia, (E,F) courtesy of Benjamin Bomfleur, University of Muenster, (G–I) courtesy of Nicholas Butterfield, University of Cambridge.

consequences of this transition are written large on the rock record, with plants and their fungal symbioses transforming global biogeochemical cycles and even the nature of the sediments in which fossils are found. The consequences for biological diversity are also clear, with fossils demonstrating the rapid emergence of diverse animal and plant body plans through the 'Cambrian explosion'. Without doubt this dramatic episode of diversification is paralleled by a concomitant, significant increase in the cell type diversity manifest as the disparate tissues and organs preserved in early Cambrian fossil *Lagerstätten*. Generally, little remains of the cells themselves which once comprised these organisms, aside from the tissues and organs that the fossilised remains are originally composed of. However, in one lineage at least, there is a good record of cell phenotype diversity and its diversification — in the evolution of the vertebrate skeleton where the cells that created the mineralised tissues are, more often than not, entombed. Much of this evolution is preserved in bones from the earliest skeleton-forming vertebrates which preserve the phenotypic diversity of the secreting cells, from osteoblasts and osteoclasts to chondroblasts, odontoblasts and ameloblasts, recorded as cell lacunae and canaliculi (Figure 4A). These reveal a rapid expansion of cell diversity. Odontoblasts and ameloblasts are manifest in the earliest mineralised vertebrate skeletons. The earliest bone was acellular but it is nevertheless possible to resolve the distortion tracks of fibroblasts that migrated through bone collagen fibre matrices more than four hundred million years ago. Chondroblasts and perichondral ossification appear later in the fossil record, associated with the origin of a mineralised brain case, though cartilage has a deeper evolutionary origin associated with the origin of the cartilaginous rods that support the pharyngeal 'gill slits' and oral cirri of invertebrate chordates.

This fossil record of cellular phenotypes is informative, evidencing a second rapid expansion of neural crest cell fates, as well as the emergence of the canonical skeletal cell phenotypes from among a greater initial diversity. In particular, odontoblasts have long been recognised to exhibit a broad diversity in early fossil skeleton-forming jawless ostracoderms (Figure 4B). Odontoblasts in living vertebrates are generally unipolar but in early skeleton-forming vertebrates these cells have processes that range

all the way to being unpolarised and thus are difficult to distinguish from osteoblasts on phenotype alone. Indeed, it has been argued that 'mesodentine' (Figure 4B) was an intermediate of bone and dentine, or that dentine evolved from bone, or that the distinction between these cells and their aggregative tissues was not originally as clear as it is in living jawed vertebrates. However, the reason that these cells and tissues can be distinguished as dentine is because of their topology — their positional and developmental relationship with the surrounding tissues — which appears to bear out their distinction. The canonical skeletal cell types seem to have been distinct from their earliest manifestation, but it is the development of the component tissues and so the behaviour of the cells that has evolved.

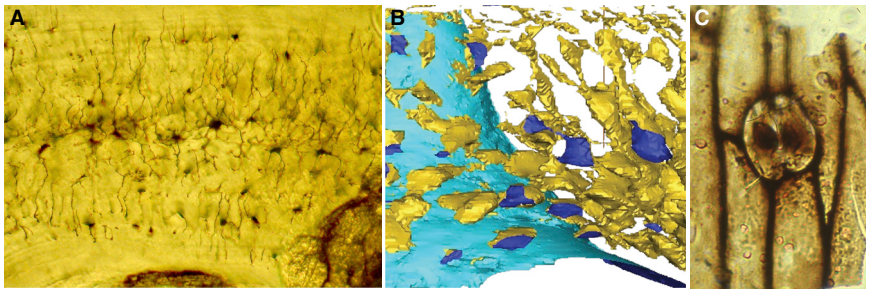

Figure 4. Fossil cells in vertebrates and plants.
(A) Histological section of fossilized dermal bone from a Permian sarcopterygian fish showing the osteocyte lacunae and canaliculi (cells ~10 μm diameter). (B) Computed tomographic model of the odontoblast and osteoblast cell lacunae surrounding the vascular system in the dermal skeleton of the jawless ostracoderm *Trememataspis mammilata* from the early Silurian of Saaremaa, Estonia (osteoblasts ~11 μm diameter). (C) Unidentified land plant cuticle fragment showing a stoma and its pair of guard cells (36 μm across) from the early Devonian of Shropshire. (C) Courtesy of Dianne Edwards, Cardiff University.

Cellular palaeogenomics

The fossil record of skeletal cells has also been exploited as a proxy for genome size, as in some clades at least, there is a linear relationship between cell size and genome size. Thus, it has been possible to infer genome size in entirely extinct lineages, such as the sauropod dinosaurs, which seem to have maintained comparatively small genomes despite their frequently gigantic body size. Analysis of bones from the extinct pterosaurs have revealed that these ancient archosaurs miniaturised their genomes relative to their ground dwelling kin, just as in birds and bats. Indeed, reduction in genome size has been tracked through analysis of bone cells in the skeletons of dinosaurs — the bird stem lineage. All three lineages evolved flight independently, demonstrating that coincidence in reduction in genome size in birds and bats must reflect a real correlation with the evolution of flight.

Many of these insights have been gleaned from thin sections of fossil bones which are tricky to make. The increasingly routine application of X-ray microtomography in palaeontology is set to change all of this (Figure 4B), facilitating high-throughput volumetric measurements of cell size. Hence, it may soon be possible to pinpoint the timing of genome duplication events that have been invoked as influential in vertebrate macroevolution in the stem-lineages of vertebrates, jawed vertebrates and teleost fishes. These are exceedingly long evolutionary branches on which numerous phenotypic innovations accrue, but which are represented only by fossils. If the phylogenetic order of genome duplication events and phenotypic innovations can be established in these fossil species, it will be possible to discriminate between deterministic and permissive roles for genome duplication in effecting the evolutionary success of vertebrates.

Palaeobotanists have stolen a march on their zoological colleagues, tracking the evolution of genome size through genome duplication events which are more prevalent in land plant evolutionary history. This is especially important because although many plant lineages have undergone recurrent rounds of genome duplication, many have subsequently slimmed their genomes. Consequently, it can be difficult to determine which plant lineages experienced genome doubling even though it is possible to obtain genome size data directly for extant species and even track genome evolution through comparative analysis of gene family evolution. Thankfully, there is a rich fossil record of plant cell sizes representative of much of their evolutionary history.

Palaeobotanists have seized upon the guard cells of stomata, the paired cells that regulate the little gas valves embedded in stems and leaves (Figure 4C), as their preferred proxy for genome size. This has revealed that ancestral land plants had comparatively large genomes — in contrast to analyses of living species which have inferred genome size increasing through land plant evolution. There is certainly much greater scope for investigating land plant genome size evolution, tracking patterns of genome reduction and tests of hypotheses such as the role of differential loss and regulation of duplicate genes, which may be the real motor of macroevolutionary change arising from genome duplication events.

Clearly, there are limits to the fossil record of cell evolution, in terms of biochemistry and resolution, as well as biases that favour only a small subset of evolutionary lineages and their developmental stages, and what is preserved is not always easy to interpret. Fossil cells are invariably just ghosts of their once living counterparts but, nevertheless, they provide unique insights into some of the most fundamental of episodes in cell evolution, insights that may not be possible, and would certainly not be so refined, in the absence of fossil evidence. Indeed, fossil evidence may provide even further insight and data that might help arbitrate in heated debates, such as on the evolutionary roles of genome duplication or the

sequence of evolutionary steps in the process of eukaryogenesis. The key challenge is perhaps in learning how to decode the fossil record of cells. Further experimental research into the patterns and processes of the decay and fossilization of cells and their organelles is likely to uncover the necessary cipher.

FURTHER READING

Betts, H.C., Puttick, M.N., Clark, J.W., Williams, T.A., Donoghue, P.C.J., and Pisani, D. (2018). Integrated genomic and fossil evidence illuminates life's early evolution and eukaryote origin. Nat. Ecol. Evol. *2*, 1556–1562.

Bomfleur, B., McLoughlin, S., and Vajda, V. (2014). Fossilized nuclei and chromosomes reveal 180 million years of genomic stasis in royal ferns. Science *343*, 1376–1377.

Brasier, M.D., Antcliffe, J., Saunders, M., and Wacey, D. (2015). Changing the picture of Earth's earliest fossils (3.5–1.9 Ga) with new approaches and new discoveries. Proc. Natl. Acad. Sci. USA *112*, 4859–4864.

Butterfield, N.J. (2009). Modes of pre-Ediacaran multicellularity. Precambrian Res. *173*, 201–211.

Butterfield, N.J. (2015). Early evolution of the Eukaryota. Palaeontology *58*, 5–17.

Davesne, D., Schmitt, A.D., Fernandez, V., Benson, R.B.J., and Sanchez, S. (2020). Three-dimensional characterisation of osteocyte volumes at multiple scales, and its relationship with bone biology and genome evolution in ray-finned fishes. J. Evol. Biol. http://doi.org/10.1111/jeb.13612.

Donoghue, P.C.J., Sansom, I.J., and Downs, J.P. (2006). Early evolution of vertebrate skeletal tissues and cellular interactions, and the canalization of skeletal development. J. Exp. Zool. B Mol. Dev. Evol. *306B*, 278–294.

Javaux, E.J. (2019). Challenges in evidencing the earliest traces of life. Nature *572*, 451–460.

Keating, J.N., Marquart, C.L., Marone, F., and Donoghue, P.C.J. (2018). The nature of aspidin and the evolutionary origin of bone. Nat. Ecol. Evol. *2*, 1501–1506.

Lomax, B.H., Hilton, J., Bateman, R.M., Upchurch, G.R., Lake, J.A., Leitch, I.J., Cromwell, A., and Knight, C.A. (2014). Reconstructing relative genome size of vascular plants through geological time. New Phytol. *201*, 636–644.

McElwain, J.C., and Steinthorsdottir, M. (2017). Paleoecology, ploidy, paleoatmospheric composition, and developmental biology: a review of the multiple uses of fossil stomata. Plant Physiol. *174*, 650–664.

Organ, C., Struble, M., Canoville, A., de Buffrénil, V., and Laurin, M. (2016). Macroevolution of genome size in sarcopterygians during the water–land transition. C. R. Palevol. *15*, 65–73.

Organ, C.L., and Shedlock, A.M. (2009). Palaeogenomics of pterosaurs and the evolution of small genome size in flying vertebrates. Biol. Lett. *5*, 47–50.

Organ, C.L., Shedlock, A.M., Meade, A., Pagel, M., and Edwards, S.V. (2007). Origin of avian genome size and structure in non-avian dinosaurs. Nature *446*, 180–184.

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Primer

Cell biology of photosynthesis over geologic time

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Land plants, algae, and cyanobacteria are collectively responsible for nearly all global primary production of organic carbon. As such, photosynthesis provides energy and reduced carbon (i.e. food) for the vast majority of Earth's ecosystems. Although chlorophyll-based photosynthesis is a very complex process depending on many proteins, pigments, and multiple membrane systems, it is also ancient and arose at least three billion years ago. Earth's environment has changed in dramatic ways over geologic time (Figure 1). Indeed, photosynthesis is responsible for some of the biggest transformations in the atmosphere and biosphere. Since photosynthesis uses light energy to convert CO_2 + H_2O into sugars and molecular oxygen (O_2), photosynthesis is partly responsible for our contemporary high-O_2 atmosphere. This, in turn, enabled the explosive diversification of complex, multicellular life we find on Earth today. Over the same time period, the atmospheric CO_2 concentration dropped precipitously (Figure 1). The focus of this primer is to describe various mechanisms that photosynthetic organisms evolved to compensate for sweeping changes in the planetary environment by concentrating CO_2 inside their bodies.

So photosynthetic organisms are ancient and have adapted to sweeping changes in the global environment through the evolution of new enzymatic functions, regulatory systems, metabolic modes and physiologies. Here we focus on the cellular adaptations that evolved in response to one particular global change: the rise of oxygen in our atmosphere. Free O_2 was exceedingly rare on early Earth (<1 ppm) and is now quite abundant (roughly 21% of the present-day atmosphere). The first major oxygenation, the so-called Great Oxygenation Event (GOE) occurred about 2.5 billion years ago. The second, the Neoproterozoic

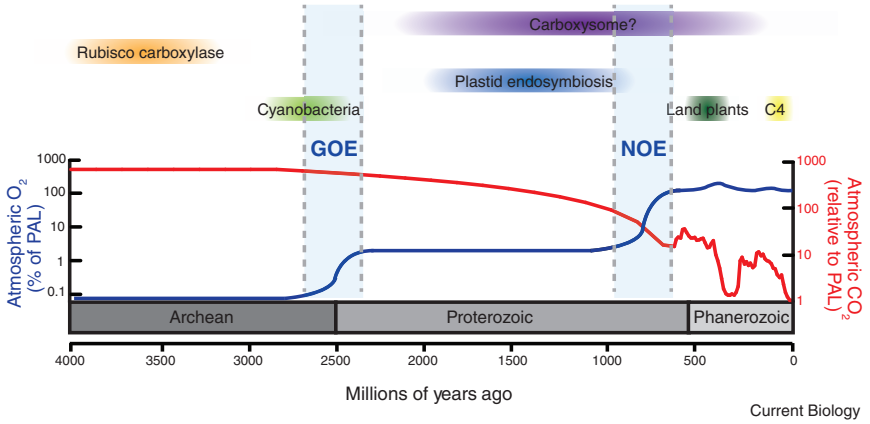

Figure 1. Timeline of major transitions in atmospheric chemistry during the evolution of photosynthesis.

Approximate atmospheric CO_2 and O_2 levels have changed dramatically over geologic time. The first major increase in atmospheric O_2 is called the Great Oxygenation Event (GOE), followed by the Neoproterozoic Oxygenation Event (NOE), which brought dioxygen levels to present atmospheric levels (PAL). The approximate origin of biological events is marked on the timeline; wide ranges are used to convey substantial uncertainty about exact timing. Carbon isotope data from Archean carbonates suggest the early evolution of rubisco carboxylase activity and rubisco-based autotrophy, followed by the rise of oxygenic photosynthesis in the cyanobacteria, which lead to the GOE. The plastid endosymbiosis that gave rise to Archaeplastida (i.e., algae and plants) occurred during the Proterozoic Eon, though the timing of this event is poorly constrained. Plants began to colonize land after the NOE. C_4 and CAM photosynthesis arose after the NOE, within the last 30 My. The NOE and a contemporaneous drop in CO_2 levels may have promoted CCM evolution, though other factors (e.g. growth temperature and aridity) were likely involved. The timing of carboxysome and pyrenoid evolution remains uncertain, but both biophysical CCMs likely evolved sometime during the Proterozoic Eon.