sheets is necessarily a slow process, limited by the transfer of moisture through the atmosphere, and it appears likely that this process initially limited the rate of climatic cooling. Then, approximately 114,000 years ago, with temperatures having dropped less than halfway to typical full glacial values, the first rapid climate changes began — as documented here for the first time. The timing and characteristics of these events offer an invaluable subject for climate modellers; the mechanisms underlying rapid climate change are still being debated, and climate models have not yet convincingly predicted them.

There is much work yet to be done on the NGRIP core, especially examining the high-resolution characteristics of the record, quantifying the temperature history, and investigating the biogeochemical changes that accompanied the transition to glacial climate. The overview presented in this issue[1] is sufficient to demonstrate that it is a valuable and remarkable core. Yet the NGRIP project has not achieved its primary goal: a reasonably complete record of climate during the last interglacial. How warm did this period get? Were any parts of it climatically unstable? Such information is crucial for evaluating climate models of a warmer world, and for understanding sea-level changes induced by melting of the Greenland ice sheet. Analysis of basal ices gives direct and compelling evidence that the ice sheet retreated significantly during this period[9].

There is only one way to fill this gap. A new ice core will have to be extracted from the dry regions of north-central Greenland, but at a safe distance from the heat-flow anomaly discovered at the NGRIP site. The cost and effort of such a project are trivial compared with the possible impact of a rise in sea level, and maybe even rapid climate change, induced by warming of the Arctic region. ■

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Evolutionary biology

Early evolution comes full circle

William Martin and T. Martin Embley

Biologists use phylogenetic trees to depict the history of life. But according to a new and roundabout view, such trees are not the best way to summarize life's deepest evolutionary relationships.

Charles Darwin described the evolutionary process in terms of trees, with natural variation producing diversity among progeny and natural selection shaping that diversity along a series of branches over time. But in the microbial world things are different, and various schemes have been devised to take both traditional and molecular approaches to microbial evolution into account. Rivera and Lake (page 152 of this issue[1]) provide the latest such scheme, based on analysing whole-genome sequences, and they call for a radical departure from conventional thinking.

Unknown to Darwin, microbes use two mechanisms of natural variation that disobey the rules of tree-like evolution: lateral gene transfer and endosymbiosis. Lateral gene transfer involves the passage of genes among distantly related groups, causing branches in the tree of life to exchange bits of their fabric. Endosymbiosis — one cell living within another — gave rise to the double-membrane-bounded organelles of eukaryotic cells: mitochondria (the powerhouses of the cell) and chloroplasts (of no further importance here). At the endosymbiotic origin of mitochondria, a free-living proteobacterium came to reside within an archaebacterially related host — see Fig. 1 for terminology. This event involved the genetic union of two highly divergent cell lineages, causing two deep branches in the tree of life to merge outright. To this day, biologists cannot agree on how often lateral gene transfer and endosymbiosis have occurred in life's history; how significant either is for genome evolution; or how to deal with them mathematically in the process of reconstructing evolutionary trees. The report by Rivera and Lake[1] bears on all three issues. And instead of a tree linking life's three deepest branches (eubacteria, archaebacteria and eukaryotes), they uncover a ring.

The ring comes to rest on evolution's sorest spot — the origin of eukaryotes. Biologists fiercely debate the relationships between eukaryotes (complex cells that have a nucleus and organelles) and prokaryotes (cells that lack both). For a decade, the dominant approach has involved another intracellular structure called the ribosome, which consists of complexes of RNA and protein, and is present in all living organisms. The genes encoding an organism's ribosomal RNA (rRNA) are sequenced, and the results compared with those for rRNAs from other organisms. The ensuing tree[2] divides life into three groups called domains (Fig. 2a). The usefulness of rRNA in exploring biodiversity within the three domains is unparalleled, but the proposal for a natural system of all life based on rRNA alone has come increasingly under fire.

Ernst Mayr[3], for example, argued forcefully that the rRNA tree errs by showing eukaryotes as sisters to archaebacteria, thereby obscuring the obvious natural division between eukaryotes and prokaryotes at the level of cell organization (Fig. 2b). A central concept here is that of a tree's 'root', which defines its most ancient branch and hence the relationships among the deepest-diverging

Prokaryotes Cells lacking a true nucleus. Gene transcription occurs in the cytoplasm.

Archaebacteria Prokaryotes with a plasma membrane of isoprene ether lipids. Protein synthesis occurs on distinctive, archaebacterial-type ribosomes. Synonymous with Archaea.

Eubacteria Prokaryotes with a plasma membrane of fatty acid ester lipids. Protein synthesis occurs on distinctive, eubacterial-type ribosomes. Synonymous with Bacteria.

Eukaryotes Cells possessing a true nucleus (lacking in prokaryotes), separated from the cytoplasm by a membrane contiguous with the endoplasmic reticulum (also lacking in prokaryotes). Include double-membrane-bounded cytoplasmic organelles derived from eubacterial endosymbionts[11–13]. The plasma membrane consists of fatty acid ester lipids. Protein synthesis occurs on ribosomes related to the archaebacterial type. Synonymous with Eucarya.

Proteobacteria A name introduced for the group that includes the purple bacteria and relatives[18]. The endosymbiotic ancestor of mitochondria was a member of the proteobacteria as they existed more than 1.4 billion years ago.

Figure 1 **Who's who among microbes. In 1938, Edouard Chatton coined the terms prokaryotes and eukaryotes for the organisms that biologists still recognize as such[3]. In 1977 came the report of a deep dichotomy among prokaryotes[19] and designation of the newly discovered groups as eubacteria and archaebacteria. In 1990, it was proposed[2] to rename the eukaryotes, eubacteria and archaebacteria as eucarya, bacteria and archaea. Although widely used, the latter names left the memberships of these groups unchanged, so the older terms have priority.**

lineages. The eukaryote–archaebacteria sister-grouping in the rRNA tree hinges on the position of the root (the short vertical line at the bottom of Fig. 2a). The root was placed on the eubacterial branch of the rRNA tree based on phylogenetic studies of genes that were duplicated in the common ancestor of all life[2]. But the studies that advocated this placement of the root on the rRNA tree used, by today's standards, overly simple mathematical models and lacked rigorous tests for alternative positions[4].

One discrepancy is already apparent in analyses of a key data set used to place the root, an ancient pair of related proteins, called elongation factors, that are essential for protein synthesis[5]. Although this data set places the root on the eubacterial branch, it also places eukaryotes within the archaebacteria, not as their sisters[5]. Given the uncertainties of deep phylogenetic trees based on single genes[4], a more realistic view is that we still don't know where the root on the rRNA tree lies and how its deeper branches should be connected.

A different problem with the rRNA tree, as Ford Doolittle[6] has argued, is that lateral gene transfer pervades prokaryotic evolution. In that view, there is no single tree of genomes to begin with, and the concept of a natural system with bifurcating genome lineages should be abandoned (Fig. 2c). Added to that are genome-wide sequence comparisons showing eukaryotes to possess far more eubacteria-like genes than archaebacteria-like genes[7,8], in diametric opposition to the rooted rRNA tree, which accounts for only one gene. Despite much dissent, the rRNA tree has nonetheless dominated biologists' thinking on early evolution because of the lack of better alternatives.

Rivera and Lake's ring of life[1] (Fig. 2d) includes the analysis of hundreds of genes, not just one. It puts prokaryotes in one bin and eukaryotes in another[3]; it allows lateral gene transfer to be used in assessing genome-based phylogeny[7]; and it recovers the connections between prokaryote and eukaryote genomes as no single gene tree possibly could. Their method — 'conditioned reconstruction' — uses shared genes as a measure of genome similarity but does not discriminate between vertically or horizontally inherited genes. This method does not uncover all lateral gene transfer in all genomes. But it does uncover the dual nature of eukaryotic genomes[7,8], which in the new scheme sit simultaneously on a eubacterial branch and an archaebacterial branch. This is what seals the ring.

As the simplest interpretation of the ring, Rivera and Lake[1] propose that eukaryotic chromosomes arose from a union of archaebacterial and eubacterial genomes. They suggest that the biological mechanism behind that union was an endosymbiotic association between two prokaryotes. The ring is thus at odds with the view of eukaryote origins by simple Darwinian divergence[9,10], but is consistent with symbiotic models of eukaryote origins, variants of which abound[11]. Some symbiotic models suggest that an archaebacterium–eubacterium symbiosis was followed by the endosymbiotic origin of mitochondria; others suggest that the host cell in which mitochondria settled was an archaebacterium outright.

Rivera and Lake's findings do not reveal whether a symbiotic event preceded the mitochondrion. But — importantly — they cannot reject the mitochondrial endosymbiont as the source of the eubacterial genes in eukaryotes. The persistence of the mitochondrial compartment, especially in anaerobic eukaryotic lineages[12,13], among which the most ancient eukaryote lineages have traditionally been sought, provides phylogeny-independent evidence that the endosymbiotic origin of mitochondria occurred in the eukaryotic common ancestor. Phylogeny-independent evidence for any earlier symbiosis is lacking. So the simpler, hence preferable, null hypothesis is that eubacterial genes in eukaryotes stem from the mitochondrial endosymbiont.

Rejecting that null hypothesis will require improved mathematical tools for probing deep phylogeny. Indeed, it is not clear if conditioned reconstruction alone is sensitive enough to do this — analyses of individual genes are still needed. But eukaryotes are more than 1.4 billion years old[14] and such time-spans push current tree-building methods to, and perhaps well beyond, their limits[15].

Looking into the past with genes is like gazing at the stars with telescopes: it involves a lot of mathematics[16], most of which the stargazers never see. With better telescopes we can see more details further back in time, but nobody knows for sure how good today's gene-telescopes really are. Mathematicians have a well-developed theory for building trees from recently diverged gene sequences[17], but mathematical methods for recovering ancient mergers in the history of life are still rare. Rivera and Lake's ring depicts the eukaryotic genome for what it is — a mix of genes with archaebacterial and eubacterial origins. ■

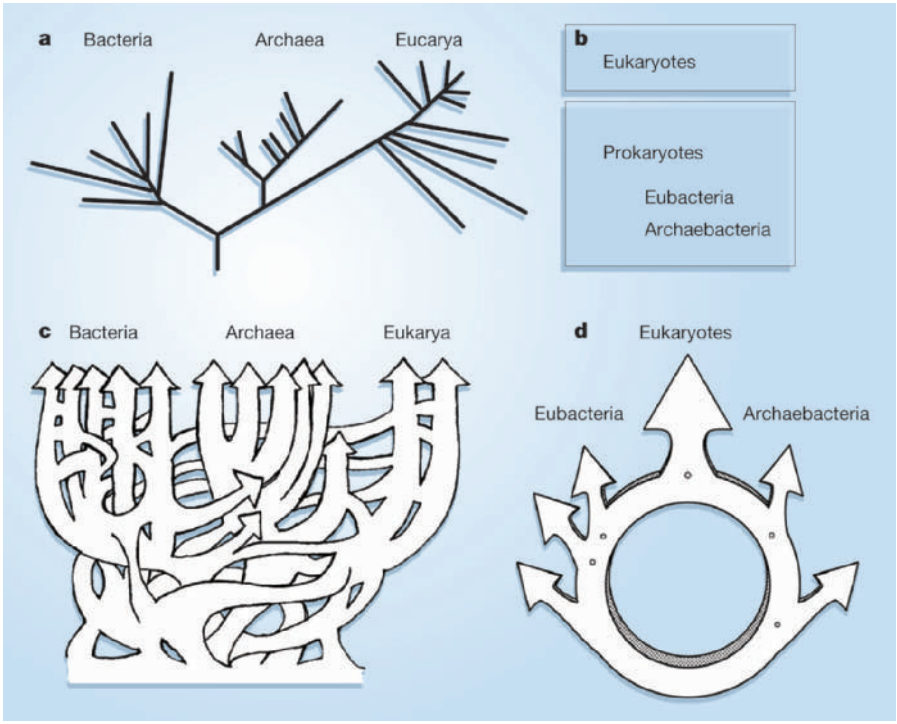

Figure 2 **Four schemes of natural order in the microbial world.** a, **The three-domain proposal based on the ribosomal RNA tree, as rooted with data from anciently duplicated protein genes.** b, **The two-empire proposal, separating eukaryotes from prokaryotes and eubacteria from archaebacteria.** c, **The three-domain proposal, with continuous lateral gene transfer among domains.** d, **The ring of life, incorporating lateral gene transfer but preserving the prokaryote–eukaryote divide. (Redrawn from refs 2, 3, 6 and 1, respectively.)**

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Neurobiology

Feeding the brain

Claire Peppiatt and David Attwell

In computationally active areas of the brain, the blood flow is increased to provide more energy to nerve cells. New data fuel the controversy over how this energy supply is regulated.

Like all tissues, our brains need energy to function, and this comes in the form of oxygen and glucose, carried in the blood. The brain's information-processing capacity is limited by the amount of energy available[1], so, as has been recognized for more than a century, blood flow is increased to brain areas where nerve cells are active[2]. This increase in flow provides the basis for functional magnetic resonance imaging of brain activity[2], but exactly how the flow is increased is uncertain. On page 195 of this issue, Mulligan and MacVicar[3] reveal a previously unknown role for non-neuronal brain cells called astrocytes in controlling the brain's blood flow. Intriguingly, the new data contradict a previous suggestion for how astrocytes regulate flow.

Figure 1 shows recent developments in our understanding of how the blood flow in the brain is controlled. Glucose and oxygen are provided to neurons through the walls of capillaries, the blood flow through which is controlled by the smooth muscle surrounding precapillary arterioles. Dedicated neuronal networks in the brain signal to the smooth muscle to constrict or dilate arterioles and thus decrease or increase blood flow[2]; for example, neurons that release the neurotransmitter molecule noradrenaline constrict arterioles. In addition, the neuronal activity associated with information processing increases local blood flow. This is in part due to neurons that release the transmitter glutamate, which raises the intracellular concentration of Ca^{2+} ions in other neurons, thereby activating the enzyme nitric oxide (NO) synthase and leading to the release of NO. This in turn dilates arterioles[4].

A radical addition to this scheme came with the claim of Zonta *et al.*[5] that glutamate also works through astrocytes in the brain to dilate arterioles. Glutamate raises the Ca^{2+} concentration in astrocytes, and thus activates the enzyme phospholipase A_2, which produces a fatty acid, arachidonic acid. This is converted by the enzyme cyclooxygenase into prostaglandin derivatives, which dilate arterioles. An attractive aspect of a role for astrocytes in controlling blood flow is that, although most of their cell membrane surrounds neurons and so can sense neuronal glutamate release, they also send out an extension, called an endfoot, close to blood vessels: thus, astrocyte anatomy is ideal for regulating blood flow in response to local neuronal activity[6]. In this scheme, a rise in the Ca^{2+} levels in astrocytes, just like in neurons, would dilate arterioles and increase local blood flow.

The new data contradict these results. Mulligan and MacVicar[3] inserted a 'caged' form of Ca^{2+} into astrocytes in brain slices taken from rats and mice. By using light to suddenly uncage the Ca^{2+}, they found that an increase in the available Ca^{2+} concentration within astrocytes produces a constriction of nearby arterioles that could powerfully decrease local blood flow (the 23% decrease in diameter seen would increase the local resistance to blood flow threefold, by Poiseuille's law).

They show that this constriction results from Ca^{2+} activating phospholipase A_2 to generate arachidonic acid, as above; the twist is that this arachidonic acid is then processed by a cytochrome P450 enzyme (CYP) into a constricting derivative. The authors propose that this derivative is 20-hydroxyeicosatetraenoic acid (20-HETE), formed by CYP4A in the arteriole smooth muscle[7] (but the high concentration of CYP4A blocker used to deduce this might also block other enzymes[8]). The authors also found that noradrenaline evoked a rise in astrocyte Ca^{2+} concentration and arteriole constriction. Unexpectedly, therefore, it seems that rather than noradrenaline-producing neurons signalling directly to smooth muscle, as is conventionally assumed, much of their constricting action may be mediated indirectly by astrocytes. In fact this is consistent with the finding that many noradrenaline-release sites on neurons are located near astrocytes[9].

Is it possible to reconcile the new data[3] (a rise in astrocyte Ca^{2+} levels constricts arterioles) with those of Zonta *et al.*[5] (a rise in Ca^{2+} dilates arterioles)? A likely solution is that the increased concentration of Ca^{2+} in astrocytes leads to the production of both constricting

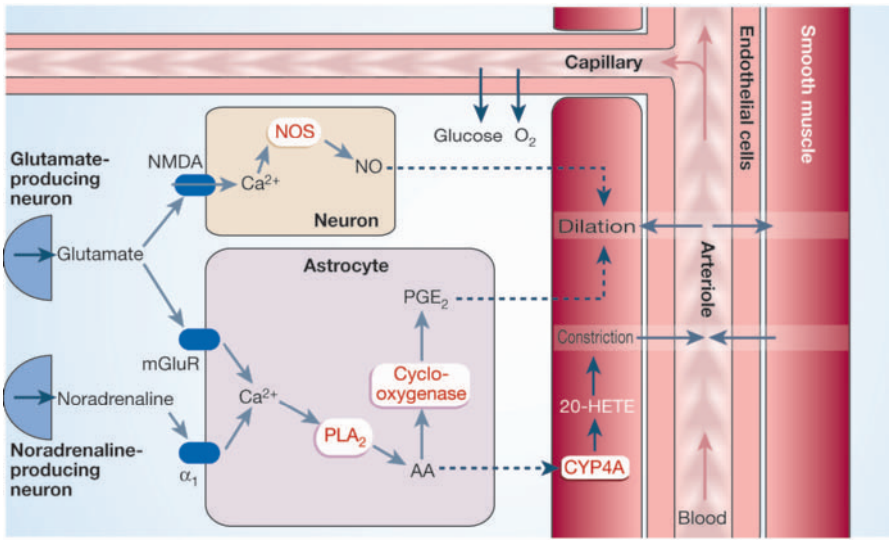

Figure 1 **Controlling blood flow in the brain. Computationally active neurons release glutamate (top left). This activates neuronal NMDA-type receptors, Ca^{2+} influx through which leads to nitric oxide synthase (NOS) releasing NO, which works on smooth muscle to dilate arterioles. This increases the supply of oxygen and glucose to the brain. Glutamate also spills over to astrocyte receptors (mGluRs), which raise the Ca^{2+} levels in astrocytes and generate arachidonic acid (AA) via phospholipase A_2 (PLA_2). Cyclooxygenase-generated derivatives of AA (PGE_2) dilate arterioles[5], whereas, as Mulligan and MacVicar show[3], the CYP4A-generated derivative 20-HETE constricts them. Astrocyte Ca^{2+} levels can also be raised by noradrenaline — released from dedicated neurons that control the circulation — which works through α_1 receptors (bottom left). Dotted lines show messengers diffusing between cells. The detailed anatomy of synapses and astrocytes is not portrayed.**