Evolutionary biology

Ancient bacteria liked it hot

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Proteins from ancestral bacteria have been modelled and their genes chemically synthesised. Strikingly, protein heat-stability parallels ocean temperatures through the ages, as determined from the geological record.

The study of the environment in which early life evolved has long been the domain of the physical sciences. For example, analyses of the chemical and isotopic compositions of rocks formed during the Archean period (from 3,800 to 2,500 million years ago, Mya) allow the precise dating and reconstruction of environmental parameters at that time, such as seawater temperature and atmospheric composition. But the natural sciences have recently discovered other ways to study the habitats of early life. On page XXX of this issue, Gaucher *et al.*¹ describe one such approach. They have ‘resurrected’ proteins from bacteria of bygone ages, providing clues on the temperatures at which these organisms lived.

The protein targeted by Gaucher *et al.*¹ is elongation factor thermo unstable (EF-Tu), a crucial player in protein synthesis. Every extant cell contains variants of the genes that encode EF-Tu, and so the factor is expected to have existed in all cells throughout evolutionary history. In modern-day bacteria, the temperature above which EF-Tu loses its precise three-dimensional structure is highly correlated to the temperature of each species’ habitat — EF-Tu proteins of hyperthermophilic bacteria (which thrive at temperatures of 80 °C or more) maintain their structures at high temperatures, whereas the analogous proteins of mesophilic

bacteria (which prefer temperatures between 25 and 40 °C) unfold at much lower temperatures.

The sequences of many extant bacterial genomes are known, providing EF-Tu sequences from species representing various groups and habitats. In contrast, ancient protein sequences can only be estimated, using methods based on statistical modelling of the molecular evolutionary process. Gaucher *et al.*¹ have made such estimations for a dozen organisms in the bacterial phylogenetic tree, starting from the last common ancestor and ending with present-day bacteria. The corresponding gene sequences were synthesized and cloned into *Escherichia coli* cells, which then produced ancestral EF-Tu proteins so that their thermostability could be measured.

The results are striking. In bacteria, there is a clear correlation between age and thermostability for ancestral EF-Tu sequences: proteins from older bacteria are more heat-stable. This trend parallels the changes in temperature proposed for Precambrian oceans (which existed between 3,800 Mya and 542 Mya), based on analyses of the isotopic composition of Precambrian cherts. These cherts are some of the best-preserved sedimentary rocks from the period. It has been shown that the ¹⁸O and ³⁰Si isotopic contents of Precambrian cherts are best explained by a progressive cooling of the oceans — from temperatures of about 70 °C some 3,500 Mya, to 20 °C just 800 Mya [refs 2,3].

But ancestral protein-sequence estimation is dogged by several uncertainties. The first problem is that such estimates are based on a phylogenetic tree that models evolutionary

relationships between organisms — but relationships between large bacterial groups remain uncertain. The second issue is that statistical models of protein-sequence evolution depend on simplifying hypotheses: the probabilities of replacement of each amino acid by each other are supposed to have remained invariant along time. Finally, for any given tree and evolutionary model, billions of ancestral sequences are possible. But Gaucher and colleagues' results¹ are robust. They performed a series of control experiments that convincingly show their results to be qualitatively insensitive to the intrinsic uncertainties of their technique: they have used two bacterial phylogenies, two replacement probability sets, and synthesised five of the possible EF-Tu proteins of the bacterial ancestor.

Other procedures for estimating ancient environmental temperatures from extant molecular-sequence data are known (Fig. 1). For example, the amounts of the nucleic-acid bases guanine and cytosine found in certain RNA sequences of modern-day bacteria and archaea (a group of microorganisms that is similar to bacteria, but which evolved separately) strongly correlate with environmental temperatures. By determining these RNA sequences for ancestral microorganisms, the temperatures of ancient habitats can therefore be estimated. But this method has yielded conflicting results, suggesting that very ancient lifeforms were hyperthermophilic⁴ but also that the last universal common ancestor (LUCA) of all modern life was mesophilic⁵. Previous statistical estimations of protein-sequences support the idea that LUCA was hyperthermophilic⁶.

Some of these conflicts might result from the inadequacy of the hypotheses used in evolutionary models. Emerging statistical methods that account for the variation with time of replacement probabilities in DNA and protein sequences⁷ should help in accurately estimating

the features of ancestral protein sequences. The recently discovered correlation between environmental temperatures and the average content of seven specific amino acids in bacterial and archaeal proteins⁸ also provides a promising opportunity for future research.

Gaucher *et al.*¹ envisage two possible scenarios to explain the striking agreement between the thermostability of bacterial EF-Tus and ocean temperatures in the Precambrian period. The first is that bacteria from the Archean period lived in hot oceans, and progressively adapted to cooler oceans. Alternatively, bacteria could have started out in hot springs or thermal vents, before expanding their territory into the cooler, global environment.

Sadly, geological evidence that might discriminate between these scenarios is scarce — Archean microorganisms are poorly preserved, compared with those of the late Precambrian period (the Proterozoic eon, 2,500 Mya to 542 Mya). Geologists have, however, found laminated, sedimentary structures that are 3,430 million years old in the Pilbara craton of Western Australia. By comparison with modern analogues, these ‘stromatolite’ structures are interpreted as reefs constructed by cyanobacteria^{9,10}. In addition, carbonaceous microstructures of a similar age (3,460 million years) have been found in bedded cherts from the Apex formation of Western Australia¹¹. These are most probably fossils of bacteria or archaea, although the evidence for this is still debated. Nevertheless, the existence of these stromatolites and of fossil-bearing cherts, and their enrichment in ¹⁸O and ³⁰Si, favour the idea of bacteria living in hot oceans.

Matching the phylogenetic tree of life to the geological record remains a fundamentally difficult problem, because this tree is built from molecules whose early rates of evolution are unknown. Gaucher and colleagues' contribution¹ might open a new way to overcome this difficulty: molecular data contain information about environmental features that could allow dating ancestral organisms through the geological record.

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Figure 1 Environmental temperatures at pivotal points in early evolutionary history.

The tree of life stems from the last universal common ancestor (LUCA), which evolved into bacteria, archaea (the two groups of organisms with non-nucleated cells), and eukaryota (with nucleus-containing cells). Modern-day bacteria are mostly mesophilic (they thrive at temperatures of about 25–40 °C), but some are thermophilic (preferring temperatures ≥ 60 °C). Many extant archaea are thermophilic, but no such eukaryota exist. Gaucher *et al.*¹ modelled and reconstructed proteins from ancestral bacteria. From the heat-stability of the proteins, they infer the temperature of the bacteria's habitats; the results agree with evidence from the geological record. The authors conclude that the earliest bacteria lived in oceans at temperatures of 65–73 °C. Previous studies^{4,5} based on models of ancient RNA suggest that LUCA is either mesophilic or thermophilic. The temperature at which the earliest archaea flourished has not been estimated.