

**Walking the Devonian walk.** Tetrapods exist in a range of different forms (tetrapodomorphs). Here they are arranged in a cladistic sequence, each with its left forelimb or forefin in dorsal view. The new humerus (ANSP 23150) from a Devonian tetrapod discovered in Pennsylvania by Shubin *et al.* (2) is shown at the node of the cladogram (question mark) suggested

by the authors. Sources: rhizodont based on (9) and (10); *Eusthenopteron* compiled from (11); *Panderichthys* based on (12) and (13); *Acanthostega* from (6); *Ichthyostega* based on provisional reconstructions in (1) and (4); *Tulerpeton* from (1) and (14).

precursor to the evolution of weight-supporting limbs in tetrapods. However, the three more completely known Devonian tetrapod limbs were polydactylous (see the figure), and given the phylogenetic position proposed for the owner of this new humerus, more than five digits seem likely, together with an ulna that was shorter than the radius. There is evidence that digits arose before wrists or ankles (6), and polydactylous limbs with an array of digits could well have assisted in spreading the load on a prop-like appendage, before stable load-bearing joints at wrists and ankles had evolved.

Recent discoveries of Devonian tetrapods have demonstrated not only a worldwide distribution, but also a range of specializations hitherto unsuspected. These are not the conservative, clumsy creatures envisaged by popularists. Rather, they show a range of morphologies and adaptations consistent with innovators exploiting new and previously vacant niches. *Ichthyostega*, for example, had a highly specialized ear adapted for underwater audition (7). These animals probably did not walk efficiently, but their modes of locomotion certainly varied, as they adapted skeletons and sensory organs for the challenges posed by emergence from the water.

The new humerus comes from a site in Pennsylvania that recently has yielded two other new taxa of Devonian tetrapod. If this is really a third form, it hints at a wide diversity of tetrapods existing in close proximity, in what is emerging as one of the richest and most varied of any late Devonian vertebrate site [for a faunal and floral list, see (8)]. North America is relatively unexplored for Late Devonian and Early Carboniferous vertebrates, and it is to be hoped that these new discoveries will stimulate further exploration in this potentially highly rewarding area of study.

#### References

1. J. A. Clack, *Gaining Ground: The Origin and Early Evolution of Tetrapods* (Indiana Univ. Press, Bloomington, IN, 2002).
2. N. H. Shubin, E. B. Daeschler, M. I. Coates, *Science* **304**, 90 (2004).
3. E. Jarvik, *Fossils Strata* **40**, 1 (1996).
4. J. A. Clack, H. Blom, P. E. Ahlberg, *J. Vertebr. Paleontol.* **23**, 41A (2003).
5. J. E. Jeffery, *Biol. J. Linn. Soc.* **74**, 217 (2001).
6. M. I. Coates, *Trans. R. Soc. Edinburgh Earth Sci.* **87**, 363 (1996).
7. J. A. Clack *et al.*, *Nature* **425**, 65 (2003).
8. E. B. Daeschler, A. C. Frumes, F. Mullison, *Rec. Aust. Mus.* **55**, 45 (2003).
9. S. M. Andrews, *Trans. R. Soc. Edinburgh Earth Sci.* **76**, 67 (1985).
10. E. B. Daeschler, N. Shubin, *Nature* **391**, 133 (1997).
11. S. M. Andrews, T. S. Westoll, *Trans. R. Soc. Edinburgh Earth Sci.* **68**, 207 (1970).
12. E. I. Vorobyeva, *Paleontol. J.* **34**, 632 (2000).
13. E. I. Vorobyeva, *Neues Jahrb. Geol. Palaeontol. Monatsh.* **1975**, 315 (1975).
14. O. I. Lebedev, *Recherche* **21**, 1274 (1990).

#### GENOMICS AND EVOLUTION

## Shotgun Sequencing in the Sea: A Blast from the Past?

Paul G. Falkowski and Colomban de Vargas

**O**ur evolutionary heritage is imprinted in the genes of microbes that live in the oceans, yet that genomic information is barely understood, let alone written in biological textbooks. A research article by Venter and colleagues (1) on page 66 of this issue harnesses the power of high-throughput DNA sequencing and computational genomics to produce a massive data set of large DNA fragments from total microbial genomes extracted from the subtropical North Atlantic Ocean off the Bermuda coast. Their study identifies more than 1.2 million new genes recovered from the DNA extracted from ~1500 liters of surface seawater. Such an enormous num-

P. G. Falkowski is with the Environmental Biophysics and Molecular Ecology Program and the Department of Geological Sciences. C. de Vargas is with Molecular Ecology and Evolution of Open Ocean Plankton, Institute of Marine and Coastal Science, Rutgers University, New Brunswick, NJ 08901, USA. E-mail: falko@imcs.rutgers.edu, vargas@imcs.rutgers.edu

ber of new genes from so few samples obtained in one of the world's most nutrient-impooverished bodies of water poses significant challenges to the emerging field of marine molecular microbial ecology and evolutionary biology.

The biological and geochemical history of Earth can be separated into two supereons (see the figure). The first, beginning ~3.8 billion years ago and lasting until ~2.3 billion years ago when oxygen in the atmosphere and oceans increased substantially (2), was characterized by metabolic experimentation and innovation. During this 1.5-billion-year interval, life consisted of aquatic microbes. These microbes evolved redox-based metabolic pathways, which led to nitrogen fixation, photosynthesis, sulfate reduction, methanogenesis, and numerous other processes that would ultimately alter the chemistry of our planet. The evolution of oxygen-producing photosynthesis, and the subsequent oxidation of the atmosphere and

oceans (3), required microbes to become adapted to an aerobic environment. This accommodation has been manifested over the past ~2 billion years as biological adaptations that strive to protect nature's investment in the old, anaerobic biological machinery. On a macroscopic scale, these adaptations include the evolution of secondary metabolic pathways, behaviors, morphologies, diversification, and species redundancy that ensures the survival of geochemically critical biological processes. The ensemble of these adaptations depended on mutations in genes, gene complexes, and genome landscapes that are recorded in patterns of genetic diversity within contemporary microbial communities. Arguably, nowhere on Earth is this microbial diversity—poorly understood as it is—more apparent than in the contemporary oceans.

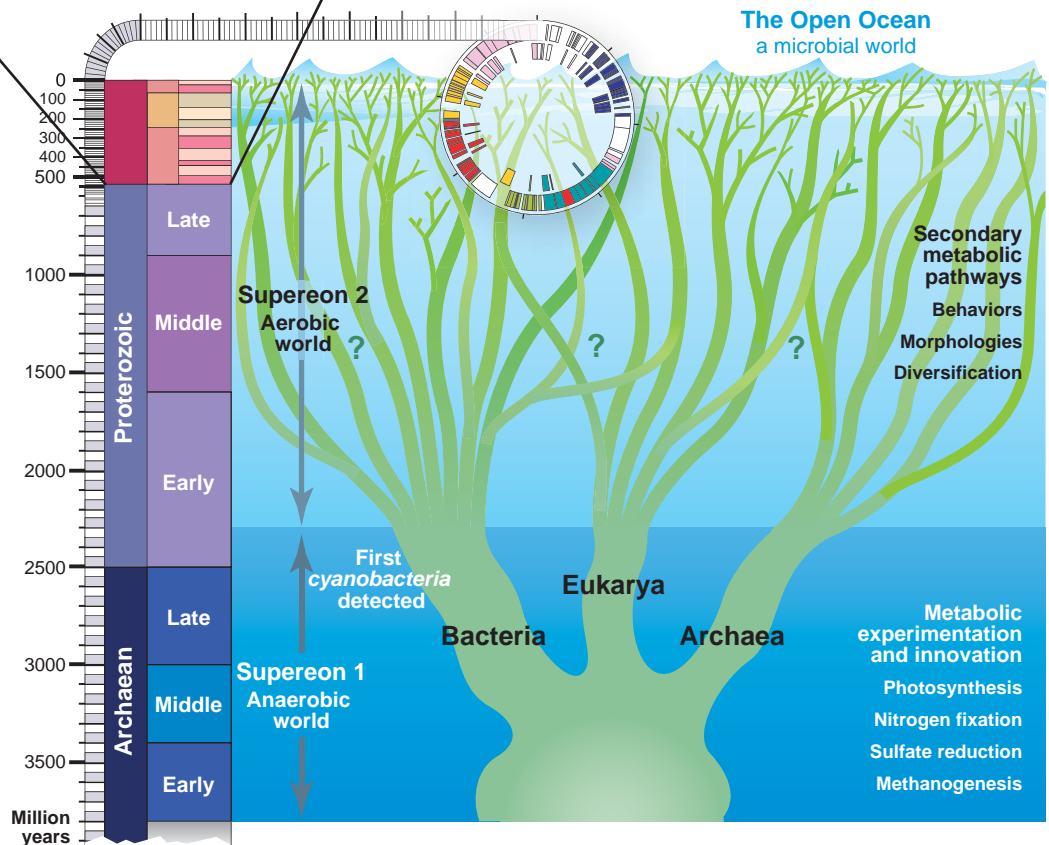
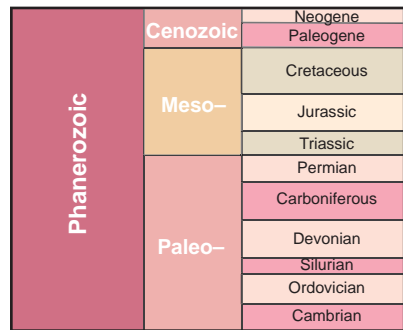
During the past decade, biological oceanographers have assessed microbial diversity primarily by sequencing ribosomal genes obtained by polymerase chain reaction (PCR) amplification of DNA extracted from organisms filtered from seawater (4). PCR-based approaches have revealed that the large majority of marine microbes cannot be cultured *ex situ*. Such approaches have identified simultaneously at least 20 major phyla in the Bacteria and Archaea, in addition to thousands of new phylotypes (the microbiological analog of “species”). When applied to the smallest marine unicellular Eukarya, PCR analyses unveil the tip of an iceberg of hidden biodiversity (5). The larger oceanic eukaryotic microbes, which can reach millimeter sizes and have been classified into ~5000 autotrophic and ~1500 heterotrophic “species” based on morphological criteria, have been largely ignored in molecular genetic surveys of the marine microbial community. However, PCR-based approaches have two major limitations: They undersample the total number of genotypes, and they access only a very small subsample of the

millions of nucleotides that are present in the genome of even the smallest microbes.

To circumvent these limitations, bacterial artificial chromosome (BAC) libraries have been constructed to directly isolate and clone large pieces of oceanic microbial DNA. Gene sequencing has identified undiscovered proteins that imply new metabolic strategies, such as rhodopsin-based photosynthesis in bacteria (6). Venter and colleagues have taken this basic strategy to a new, quasi-industrial level by randomly sequencing ~2 million cloned DNA fragments, 2 to 6 kb in size. Their approach reveals the presence of 1164 different 16S ribosomal DNA (rDNA) genes among the 1.66 million clones they analyzed from the first 900 liters of filtered seawater. They estimate that ~80% of the total microbial biodiversity—

which could reach 47,700 “species”—is represented by rare organisms that are not detected in their study. More than an order of magnitude more sequence would be needed to obtain 95% coverage of these rare microbes! However, some of the results reported by Venter *et al.* may reflect problems with their method of sample collection. For example, the highly redundant ~340,000 clones that make up more than 50% of their library #1 were assembled into only two bacteria typically found in terrestrial and aquatic nutrient-rich environments. Moreover, marine microbes associated with organic particles, dead bodies, zooplankton feces, etc., can create hotspots of bacterial growth that bias estimations of diversity. Retrospective analyses of diversity in the original samples—using microscopy or molecular probes such as fluorescence *in situ* hybridization—should be performed in future studies.

Furthermore, despite their huge sequencing effort, Venter and collaborators were able to reconstruct only two, almost-complete



**Oceans apart.** The diversity of microbes in the world's oceans is the outcome of over 3.8 billion years of evolution. We suggest that for the first ~1.5 billion years of Earth's history, critical metabolic processes were selected primarily in prokaryotes. These processes culminated in the evolution of oxygenic photosynthesis in cyanobacteria, which led to the oxidation of the oceans and atmosphere ~2.3 billion years ago. From that time to the present, marine microbes have evolved numerous biochemical, physiological, morphological, and behavioral adaptations that have served to protect key microbial metabolic processes. These adaptations appear to have given rise to the extremely large molecular diversity observed in the contemporary oceans and potentially could allow reconstruction of the metabolic pathways and evolutionary history of past, extinct microbial worlds.

genomes, and this was with the help of fully sequenced templates existing in the ever-expanding microbial genome database. The Venter *et al.* study concentrates on the smaller size range of oceanic microbes. The challenge of assembling millions of DNA fragments into contigs and scaffolds will be increased by orders of magnitude in the larger, eukaryotic microbial world. The average size of a prokaryote genome is ~2 to 3 Mb; however, the few genomes sequenced in eukaryotic plankton are much larger. For example, the genome of a marine diatom is 30 Mb, that of a coccolithophore exceeds 200 Mb, and the genome of dinoflagellates can exceed even 2000 Mb (7). The last group alone comprises ~2000 morphologically distinct species (8). The size of the dinoflagellate nuclear genome is comparable to that of humans, each morphospecies potentially representing an assemblage of tens or even hundreds of different ribotypes (genetically distinct taxonomic units that might be considered different species) (9). To illustrate the size of the problem, of the ~91,000 clones sequenced by Venter and co-workers obtained from 200 liters of seawater in a size fraction between 0.8  $\mu\text{m}$  and 3  $\mu\text{m}$ , only five unique 18S rDNA (that is, ribosomes from picoeukaryotic cells) were obtained. In contrast, a simple PCR analysis and minimum sequencing effort targeting 18S rDNA of the same size fraction from 1 liter of tropical Pacific seawater revealed more than 20 totally new and divergent phylotypes (10).

The strategy of Venter and colleagues is clearly dependent on technological capabilities that are not presently accessible to

most marine microbiologists. However, their approach certainly increases the awareness of the vast genetic diversity and complexity present in contemporary oceans. The huge panoply of new functional genes unveiled by this first shotgun sequencing of the oceans begs fundamental questions in marine microbial ecology. For example, what ecological and evolutionary processes maintain such high microbial diversity in the oceans? How many new functional components are there? Have we been missing major players, or is the apparent diversity the expression of an extreme redundancy? What is the tempo of evolution in marine microbes? Is their diversity the outcome of Darwinian selection through vertical inheritance, or is it due to nearly neutral modes of evolution in which the hundreds of millions of viral and bacteriophage particles in any milliliter of seawater act as major agents of horizontal gene transfer and genome scrambling?

This list of questions merely suggests that the approach described by Venter *et al.* is neither a beginning nor an end to understanding marine microbial ecology. Rather, it is a clear signpost on a longer journey that will occupy a broad spectrum of the scientific community for decades. One of the major problems in marine microbial ecology is that organisms in the water column are transported by the ocean currents. Therefore, it is simply impossible to understand patterns of community structure from random sampling of the world's oceans. However, by taking the Venter *et al.* strategy into a global oceanographic context, it

will be possible to reconstruct the evolution and consequences of microbial metabolic pathways that have so successfully permeated this planet (11, 12). Most marine microbes are not preserved in the fossil record; hence, their evolutionary pathways can best be inferred from genetically heritable molecules. Understanding the ecological ramifications of microbial biological chemistry will require substantial investments in new technologies, including biophysical and physiological techniques that can help to reveal the functions of new microbial proteins (13). These efforts are critical to understanding how life evolved.

#### References and Notes

1. J. C. Venter *et al.*, *Science* **304**, 66 (2004); published online 4 March 2004 (10.1126/science.1093857).
2. A. Bekker *et al.*, *Nature* **427**, 117 (2004).
3. A. D. Anbar, A. H. Knoll, *Science* **297**, 1137 (2002).
4. S. J. Giovannoni, T. B. Britschgi, C. L. Moyer, K. G. Field, *Nature* **345**, 60 (1990).
5. D. Moreira, P. Lopez-Garcia, *Trends Microbiol.* **10**, 31 (2002).
6. O. Beja *et al.*, *Science* **289**, 1902 (2000).
7. M. Veldhuis, T. Cucci, M. E. Sieracki, *J. Phycol.* **33**, 527 (1997).
8. P. G. Falkowski, J. A. Raven, *Aquatic Photosynthesis* (Blackwell Scientific, Oxford, 1997).
9. X. Pochon, L. Zaninetti, R. Rowan, J. Pawlowski, *Mar. Biol.* **139**, 1069 (2001).
10. S. Y. Moon-van der Staay, R. De Wachter, D. Vaulot, *Nature* **409**, 607 (2001).
11. A. H. Knoll, *Life on a Young Planet* (Princeton Univ. Press, Princeton, NJ, 2003).
12. P. Falkowski, *Nature* **387**, 272 (1997).
13. Z. S. Kolber *et al.*, *Science* **292**, 2492 (2001).
14. We thank the National Science Foundation Bio-complexity Program and grant OCE-0083415 for support.

Published online 4 March 2004;  
10.1126/science.1097146

Include this information when citing this paper.

#### CELL BIOLOGY

## Telomere Wedding Ends in Divorce

Claus M. Azzalin and Joachim Lingner

The faithful duplication and segregation of DNA are fundamental to life. Inaccurate chromosome segregation is observed frequently both in solid tumors and in spontaneously aborted embryos. In diploid human cells, the 46 DNA molecules are replicated in S phase of the cell cycle and are compacted 1000-fold into chromosomes that segregate between the two daughter cells during mitosis (see the figure). To accomplish this cumbersome task, newly replicated sister chromatids are held together af-

ter replication by topological DNA catenations and proteinaceous bridges called cohesins until the cell is ready to segregate them into daughter cells during mitosis (1). Whereas most cohesin molecules dissociate from chromosomes during prophase in response to polo-like kinase-dependent phosphorylation, a smaller fraction of cohesins persists at centromeres (see the figure). At the onset of anaphase, the anaphase-promoting complex (APC) activates a specialized protease called separase by targeting the separase inhibitor, securin, for ubiquitin-mediated proteolysis. Activated separase, in turn, cleaves the cohesin complexes remaining at the centromeres. The sister chromatids are

then pulled toward opposite poles of the mitotic spindle. On page 97 of this issue, Dynek and Smith (2) describe the remarkable discovery of telomere-specific cohesion, which is resolved not by separase but by the enzyme tankyrase 1.

Tankyrase 1 was first described as a protein localized at the ends of human chromosomes (telomeres) by interaction with the telomeric protein TRF1, a negative regulator of telomere length (3). Biochemically, tankyrase 1 is a poly(adenosine diphosphate-ribose) polymerase (PARP). Overexpression of tankyrase 1 promotes ADP-ribosylation of TRF1, leading to its release from telomeres, after which it is ubiquitinated and degraded by the proteasome (4). TRF1 release facilitates telomere elongation by the telomeric DNA-synthesizing enzyme telomerase. Tankyrase 1 has therefore been implicated in the control of telomere length.

In the new study, Dynek and Smith use small interfering RNAs (siRNAs) to shut down tankyrase 1 expression in human cells and show that this enzyme is important for

The authors are at the Swiss Institute for Experimental Cancer Research (ISREC), CH-1066 Epalinges, Lausanne, Switzerland. E-mail: joachim.lingner@isrec.unil.ch