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).

References

Shotgun Sequencing in the Sea: A Blast from the Past?
Paul G. Falkowski and Colomban de Vargas

Our 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 number of new genes from so few samples obtained in one of the world’s most nutrient-impoverished 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 superzones (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
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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 µ and 3 µ, 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 fraction of the oceans bogs 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 each 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).
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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. At the onset of mitosis, the telomere-specific cohesins are held together at centromeres (see the figure). At metaphase, a smaller fraction of cohesins persist at centromeres (see the figure). At anaphase, the telomeric DNA-synthesizing enzyme tankyrase 1 is degraded by the proteasome (4). TRF1 releases telomerase 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

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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 (1). Whereas most cohesins molecules dissociate from chromosomes during prophase in response to telomeric DNA-dependent phosphorylation, a smaller fraction of cohesins persists at centromeres (see the figure). At 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 opposite the mitotic spindle. On page 97 of this issue, Dynek and Smith (2) describe the remarkable discovery of telomere-specific cohesin, 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