

feeding and related behavioural strategies among hybodonts might have been as diverse as among living sharks and rays.

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## A pivotal Archaea group

Barns *et al.* have identified two novel Archaea sequence types in an analysis of organisms from a hot spring in Yellowstone National Park<sup>1</sup>. These types are different from all known archaeal sequences, and seem to represent a pivotal group, Korarchaeota, in the phylogenetic tree<sup>2</sup>. The ability to culture and to identify these organisms is critical for understanding the evolution of the Archaea and Eukarya.

To cultivate the organisms in the laboratory, we took anaerobic water and sediment samples (pH 5.5; 70–90 °C) from Obsidian Pool, a hot spring in the mud volcano area of Yellowstone National Park, Wyoming<sup>1</sup>. We set up a continuous culture at 85 °C in the laboratory (Fig. 1). After about 1 week,

we found roughly  $2 \times 10^8$  cells per ml of very complex community with heterogeneous morphologies (cocci, rods and filaments). We determined that the appearance of the community remained stable for more than a year, using phase-contrast microscopy. Whole-cell hybridization with fluorescently labelled oligonucleotide probes targeted to Archaea- and Bacteria-specific regions within the 16S ribosomal RNA<sup>3–5</sup> reveals that most of the organisms (more than 90%) belong to the Archaea.

Korarchaeota have, so far, been detected only by analyses of 16S rDNA sequences obtained directly from the environment<sup>1,2</sup>. To detect members of this new archaeal kingdom in the mixed culture, we isolated DNA twice monthly for more than a year, and used it in polymerase chain reactions with primers highly specific for the 'korarchaeal' 16S rRNA gene (Fig. 1). All isolated DNA produced sequences identical to the korarchaeal sequence type pJP27 (GenBank accession no. L25852).

We determined the morphology of the Korarchaeota by whole-cell hybridizations<sup>3,6,7</sup> using different fluorescently labelled oligonucleotide probes complementary to three regions of the korarchaeal 16S rRNA sequence (Fig. 1). We checked the sequence specificity of the probes by comparison with all 16S rRNA sequences available in databases, including the sequences obtained by *in situ* analyses of the Yellowstone hot spring<sup>1,2</sup>. We also evaluated the specificity of the probes by hybridizations of pure cultures from known hyper-

thermophilic Archaea and Bacteria. Only those cells reacting to two differently labelled korarchaeal probes in a single hybridization experiment were identified as Korarchaeota.

As a positive control for the other organisms present in the culture, we used a universal probe in hybridization experiments. Very few cells (about  $10^4$ – $10^5$  cells per ml) gave a positive hybridization signal with the korarchaeal probes. The organism corresponding to the sequence type pJP27 is rod-shaped, variable in length (between 5 and 10  $\mu\text{m}$ ) and most of the cells are slightly curved (Fig. 1). The diameter of the cells is about 0.5  $\mu\text{m}$ . We detected dividing, actively growing cells. The number of these cells within the 85 °C mixed culture was stable for more than a year, indicating that this member of the Korarchaeota is a hyperthermophile.

To characterize the Korarchaeota, it is crucial to grow these organisms in the laboratory. Here we have shown that they can be cultivated as a stable population in a mixed laboratory culture. Isolation of the Korarchaeota into a pure culture for study of their biochemical and physiological properties in detail should improve our understanding of phylogenetic relationships during the early stages in the evolution of life.

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Figure 1 Micrographs of the mixed culture at 85 °C, taken on a Nikon Microphot EPI-FL microscope. Scale bars, 5  $\mu\text{m}$ . a, Whole-cell hybridization with the fluorescently labelled probe 1135R (5'-GTTTG-CCCGGCCAGCCGTAA-3') specific for Korarchaeota. Similar results were obtained with the probes 604R (5'-TGTCTCAGGCGGATTTAAC-3') and 546R (5'-AGTATGCGTGGGAACCCCTC-3') and combinations of these probes labelled with different fluorescence dyes. b, Diaminophenylindole-stained cells, showing the different morphotypes present in the culture. The primers used in the polymerase chain reaction to amplify 'korarchaeal' 16S rDNA specifically were 5'-GAGCCCCAG-GRTGGGACCG-3' (236F) and 5'-GTTTGCCCGGCCAGCCGTAA-3' (1135R). For cultivation, we placed ~400 ml of anaerobic water and sediment sample and ~400 ml anaerobic original spring water, supplemented with 0.001% yeast extract, 0.005% peptone and 0.005%  $\text{Na}_2\text{S}_2\text{O}_8$ , into a two-walled glass vessel (chemostat). We heated the sample to 85 °C by pumping heated glycerol between the two walls of the chemostat. The chemostat had a total volume of ~800 ml and was 'aerated' with  $\text{N}_2$  and  $\text{CO}_2$  (80/20 v/v, 20 ml  $\text{min}^{-1}$ ). Later we substituted the original spring water for an anaerobic, low-salt medium (dilution rate, 20 ml  $\text{h}^{-1}$ ) containing the same nutrients<sup>8</sup>.

## Fullerene 'crop circles'

While examining laser-grown single-wall carbon nanotube (SWNT) material<sup>1,2</sup> by scanning force and transmission electron microscopy, we regularly observed circular formations of SWNT ropes (Fig. 1). These ropes consist of between 10 and 100 individual nanotubes aligned over their entire length, packed in a two-dimensional crystalline array. Sceptical that these objects might be perfectly seamless toroidal nanotubes, we named them 'crop circles', but we are now convinced that many, perhaps most, of the individual tubes in these circular ropes are indeed perfect tori.

We estimate that between 0.01 and 1%

of ropes are of this circular type. Diameters typically range between 300 and 500 nm, and rope widths, 5–15 nm, are similar to those of ordinary ropes. Transmission electron micrographs of ‘crop circles’ clearly show fringes corresponding to individual tubes (Fig. 2). The scanning force microscopy (SFM) image in Fig. 1 shows a particularly thin circle, with uniform height and width (apart from local variations arising from small quantities of amorphous carbon). The circle shown in Fig. 1 has a height of only 1.0–1.2 nm, and is almost certainly a single continuous toroidal nanotube, with no beginning or end. There is no evidence in the SFM image of a step that would result from the start or end of a coil.

The fact that typical cross-sections of circular ropes are in the same range as SWNT ropes further compels us to conclude that many, perhaps most, constituents of the circles are perfect tori. If the circles consisted only of coils, then they should be much thicker, because roughly 100 turns would be required to produce a total length similar to that observed in rope fibres<sup>1</sup>. The observed rope width of the circles is, however, consistent with the formation of an initial torus, which may serve as a template for subsequent circular growth from tube nuclei deposited from the vapour. Although we cannot rule out the existence of coiled tubes in these circles, it is likely that there are at least some continuous tubes with no ends.

To explain the formation of these toroidal fullerenes, we propose a Kekuléan image of a growing nanotube eating its own tail. The first step, in which a single tube bends around to touch near its ends, is not yet well understood (although one plausible mechanism has been proposed<sup>3</sup>), but subsequent steps in the formation of a torus can be explained more readily. After the ends of the tube have touched, they align to maximize van der Waals interactions, but slide over one another to alleviate bending strain. Usually they will slip out of contact, but occasionally the closed hemifullerene end will stick to a metal particle (added as a catalyst when making the nanotubes) that becomes attached to the growing end of the tubes. At the 1,500 K growth temperature, the two ends become welded together by the metal plug. They may be able to knit together seamlessly, annealing to the perfect toroidal tube structure, at which time the now useless metal particle is shunted either to the exterior or interior. With no edges, there is no further growth of the torus.

This picture of facile connection of ends may explain how ‘regular’ rope growth terminates, and why rope ends are almost never observed in high-yield SWNT material. During the growth phase, after nucleation, a rope end colliding with the side of another rope might align to maximize their van der

**Figure 1 Scanning force micrograph of a ‘crop circle’, imaged with a pyramidal SFM tip (the effect of a double tip can be seen at the top and bottom). The circle has an apparent height of 1.0–1.2 nm and width of 4–8 nm. This falls to the lower end of the distribution of tube diameters in this sample, but pyramidal tips produce artificially small circle heights<sup>6</sup>, a problem from which nanotube tips suffer little. Thus, the actual height of this tube is probably closer to 1.5 nm, a more typical SWNT diameter. SWNTs were prepared as described previously<sup>1</sup>. Transmission electron microscope and SFM samples were prepared from a drop of SWNT material sonicated in acetone. Scale bar, 100 nm.**

Waals interaction. As it continues to grow on its metal ‘chaperone’, the rope end may eventually encounter a third rope growing in the opposite direction, and by the mechanism described above, fuse into continuous tubes.

Single-wall carbon nanotubes are very promising new materials with unusual properties. The metallic subclass of ‘armchair’ SWNTs, such as the archetypal (10,10) tube<sup>1</sup>, constitute genuine quantum wires, owing to their nanometre-dimension diameters<sup>1,4,5</sup>, thus electron transport in these materials should prove interesting both for the fundamental understanding of quantum wires, and for the development of new devices.

**Figure 2 Transmission electron micrograph of a ‘crop circle’, showing fringes with spacings typical of SWNTs in normal ropes. Scale bars, 15 nm; inset, 5 nm.**

Transport behaviours (for example the Aharonov–Bohm effect) in these toroidal fullerenes should now be studied.

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## Plants combat infection by gene silencing

Plants with resistance genes can combat viruses by eliciting an incompatible interaction at the site of infection. Resistance can also occur in plants containing transgenes that share homology with an infecting virus<sup>1,2</sup>, by the silencing of gene expression<sup>3–5</sup>. Here we describe a natural resistance to the DNA pararetrovirus cauliflower mosaic virus (CaMV)<sup>6</sup>, in non-transgenic brassicas, which also involves post-transcriptional gene silencing.

Kohlrabi (*Brassica oleracea gongyloides*) when infected by CaMV initially develops systemic symptoms from which it completely recovers by loss of virus within a matter of weeks<sup>7</sup>. We assessed CaMV activity in leaves during the onset of host recovery by analysing viral replication products. Cells containing a replicating virus accumulate DNA intermediates generated by reverse transcription in the cytoplasm<sup>8</sup>, which migrate as heterogeneous molecules on gels. They also contain supercoiled DNA, a component of the viral minichromosome in the nucleus. Between 10 and 13 days after we had inoculated plants with the virus, this pattern of replication products remained relatively unchanged. At 14–15 days after infection, a few days before amelioration of plant symptoms, there was a sudden transition in the composition of viral DNAs. Reverse transcription products were lost whereas supercoiled DNA was amplified (Fig. 1a–c).

During this transition there was a ~50-fold amplification of the viral minichromosome transcription template, but the levels of both of the main viral polyadenylated RNAs, present in infected leaves before the viral DNA transition, declined rapidly (Fig. 1d). We also observed this decline in a