100 YEARS AGO

Mr A. Hall, of Highbury, has designed an almanac with the object of eliminating the inconvenience consequent on the various days of the months falling on different week days, owing to the changing number of days in each month. His scheme is to make New Year's Day separate from the rest, calling it January 0, and then divide the remaining 364 days into thirteen months of twenty-eight days each. Following this plan, therefore, any particular day of the month will always fall on the same day of the week, and this would, of course, be convenient for many purposes. The extra month he proposes to denote by the name "Christember." The almanac sent us is printed on this principle, and a useful item included is the table of corresponding dates between the Gregorian, Julian, Jewish and Mohammedan calendars.

From *Nature* 27 April 1899.

50 YEARS AGO

During Easter 1947, an inspection was made of the seaweed cast up on the west mainland of Orkney. One heavy cast, 3 ft. thick at the water's edge, was found in a sandy bay north of the graveyard northwest of Stromness ... . There is little chance of anything remaining intact before being cast ashore as the Atlantic waters break on this coast. The cast weed on this occasion, composed mainly of the fronds of *Laminaria Cloustoni*, had acted as a buffer for several large shells, such as *Pecten*, which were found intact. Normally, such shells become so broken up that they merely add to the shell sand comprising the foreshore in this region. From among the cast weed a bivalve, 4 in. long, was picked up which when discovered had its two halves hinged; the contents were absent. It has now been identified as *Tellina magna*, a native of the East American coast, North Carolina to West Indies – and is, I am informed, the first recorded instance of a marine invertebrate from the American continent to be found in British waters. Subsequent visits to the same area have produced only limpet shells and one small bivalve. There can be no doubt the cast weed was responsible for bringing the shell ashore intact. How the animal (or its ancestors) crossed the Atlantic can only be speculation.

From *Nature* 30 April 1949.

insight into the molecules that define and distinguish the identity of fore- and hindlimbs. But many questions remain. How is restricted expression of the *Pitx* and *Tbx* genes controlled? How do these genes specify the distinct bone, tendon and muscle structure of each limb? What role did these genes or their ancestors play in the development of limb types during evolution? The rapid identification of these genes, their targets and their phylogenetic relatives suggests that these gaps in our understanding will be filled quickly. □

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Microbial ecology

Molecular probing of deep secrets

Roger Summons

Techniques from molecular biology and organic geochemistry have been combined to provide a new tool for microbial ecologists, as shown by ribosomal RNA surveys and carbon isotopic analysis of sedimentary lipids reported by Hinrichs *et al.* on page 802 of this issue[1]. Several lines of evidence suggest that methane gas seeping from unstable methane hydrates supports a newly discovered microbial community, which is unusual both in its genetic relationships and in its metabolism.

The concept of microbial diversity has been transformed by the growth in sequence data from ribosomal RNA (16S rRNA)[2]. The cloning and sequencing of RNA from microbes living in their natural environments has revealed a genetic diversity beyond the dreams of researchers whose tools were limited to microscopy and cell culturing[3,4]. RNA molecular probes have revealed previously unknown evolutionary lineages, as well as associations between the genetic structure of communities and their ecophysiology[5].

As Hinrichs and co-workers[1] have shown, 16S rRNA cloned from shallow sediment samples above unstable methane hydrate deposits in the Eel River basin, offshore California, is dominated by a variety of previously unknown genes from archaea, which along with bacteria and eukaryotes comprise the three domains of life on Earth. These were accompanied by sequences from known anaerobes, including sulphate-reducing and Gram-positive bacteria. There were two main types of archaea discovered in the seep sediments, with some clones closely related to known methanogens (anaerobic methane-producing bacteria) of the order Methanosarcinales. Most sequences, however, were from a new and closely similar group that was distinct from, but related to, the methanogenic orders Methanomicrobiales and Methanosarcinales. Besides being distinguishable from known methanogens, these new organisms also had sequences unlike any cloned from contemporary freshwater and marine environments.

As well as being new to science, these organisms have created an unusual sediment chemistry. Analyses of the membrane lipid and stable carbon isotope distributions of the samples tell us not only about their history, but also about their carbon metabolism. Methane is renowned for containing less ^{13}C than virtually any other biological product on Earth, and large amounts of it are produced where organic matter is buried. Organic matter in the Eel River basin samples had less ^{13}C than nearby controls, and methane gas bubbling into the water column had more ^{13}C than near-surface methane hydrates in the same region. This is a powerful indicator that some members of the microbial community were consuming part of the methane from the decomposing hydrate.

Confirmation came from the carbon isotope contents of individual lipids present in the samples. Archaea have a diagnostic membrane component, a glycerol ether lipid known as 'archaeol'. Some members of the Methanosarcinales also have a unique variant of this lipid, *Sn*-2-hydroxyarchaeol. Both of these compounds were present in the seep samples, but absent from controls, and so depleted in ^{13}C that they could only be the products of methane-consuming archaea. So, we have the enigma of organisms living on a diet of methane that have the same membrane lipids as organisms normally associated with methane production. Hinrichs *et al.*[1] hypothesize that cells containing the ^{13}C-depleted lipids and the newly discovered 16S rRNA genes are one and the same.

Little is known about the specific metabolic processes of the microbes found in the Eel River basin and other methane-rich sediments. Carbon isotope and biomarker lipid patterns are quite different from those that characterize 'conventional' aerobic methane

consumption, and which have been measured in mussel communities at the surface of methane seeps[6]. Given that the sediments are anaerobic, and that there are sulphate-reducing bacteria present, it is virtually certain that methane is being oxidized at the expense of sulphate. Independent studies using radiotracers, stable isotopes and mass-balance analyses, point to a consortium of methanogens (somehow operating in reverse) and sulphate-reducing bacteria, which together are responsible for anaerobic methane oxidation[7]. However, until now, specific information about their genetic relationships has been lacking. The hypothesis favoured by Hinrichs *et al.* is that the new archaea are not simply methanogens operating in reverse, but a new group for which methane consumption is the predominant, or even exclusive, metabolism. If this is proven, it will have far-reaching consequences for our understanding of the physiology and evolution of archaea as well as their role in the carbon cycle.

Research conducted independently by Volker Thiel and co-workers[8] has shown that distinctive limestones, the so-called 'calcari a *Lucina*' that are widely distributed in the Apennines, carry a similar geochemical signature for methane venting. These Miocene-age carbonates, in places packed with the remains of tubeworms, are highly depleted in ^{13}C, with intracrystalline biomarker lipids for sulphate-reducing bacteria having even less ^{13}C content. Moreover, biomarkers for archaea are among the most ^{13}C-depleted yet reported. So, there is a neat alignment of geochemical patterns for a modern seep site and a 20 million-year-old counterpart.

These papers are exciting in the way they chart a new direction for biogeochemists. Studies of biogeochemical processing in contemporary environments, and particularly within the deep biosphere, can now be undertaken with more certainty about the ecology and physiology of the microbes. The new molecular probes that Hinrichs and colleagues have developed may soon be applied in a more quantitative manner. This will greatly strengthen our understanding of lipid biomarkers and their relationships to precursor organisms. These relationships were previously established indirectly, and sometimes haphazardly, from lipid analyses of organisms in cultures and corresponding samples from natural environments. Because the majority of rRNA genes cloned from natural environments are new to science, there is an obvious knowledge gap. As this is rectified and linked to compound-specific isotopic analyses of characteristic biomarkers, not just with carbon, but also with nitrogen, hydrogen, oxygen and sulphur, we have a key to improved information about biogeochemical processing. The work of Thiel *et al.*[8] shows how this type of knowledge can be used to project back in time.

Through various lines of investigation, anaerobic methane oxidation has been demonstrated as a viable metabolism for the deep biosphere and can be added to the compendium of ways in which microbes manipulate the distribution of chemical elements on a global scale. These microbes may eventually be cultured and their metabolic processes opened to further study. The combined organic-geochemical and molecular-biological strategy used by Hinrichs *et al.* is an important development in the study of global biogeochemical cycles. □

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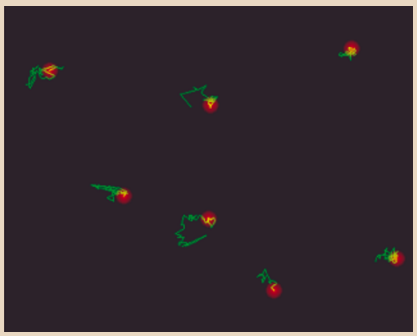
Cell biology

Coated-pit dynamics

Endocytosis — the process by which cell-surface receptors and other membrane proteins are taken up into the cell — occurs mainly through coated pits. These pits are specific sites on the plasma membrane where clathrin and the adapter protein AP-2 associate with the cargo. The pit invaginates, then a clathrin-coated vesicle is pinched off. Despite extensive characterization, we do not know whether these coated pits form randomly or at specific sites on the plasma membrane; how long they persist before detaching as coated vesicles; and how quickly coated vesicles uncoat.

Reporting in the first issue of *Nature Cell Biology* (1, 1–7; 1999), Gaidarov and colleagues now provide an insight into these questions. They looked at the formation and internalization of coated pits in living cells, using a fusion protein of green fluorescent protein (GFP) and the clathrin light-chain. Their work, which reveals an important relationship between the structural organization of clathrin-coated pits and the membrane cytoskeleton, has implications for organization of the plasma membrane.

The authors found that coated pits



labelled with GFP–clathrin appear gradually, persist for several seconds, then disappear abruptly without moving far from where they originated. That is, detached coated vesicles are stripped of their clathrin coat in the vicinity of the pit — they do not move through the cytoplasm first. Surprisingly, Gaidarov *et al.* saw that the coated pits and vesicles do not move outside regions of about 0.5–0.8 μm in diameter (see picture). But latrunculin B — which inhibits actin assembly — relaxes this restricted mobility, implying that an actin-based framework maintains the structural organization of clathrin-coated pits.

The authors found that the formation of coated pits also seems to be coordinated by an underlying membrane skeleton. Coated pits appeared and disappeared at defined, rather than random, sites on the plasma membrane. Indeed, once fluorescence at a coated pit disappeared, it usually reappeared at the same site. This meant that coated pits did not form at all over large areas of the membrane.

These observations are difficult to reconcile with the conventional view of how coated pits are formed. Diffusible transmembrane receptors or membrane-docking sites are thought to recruit AP-2 and clathrin to form a coated pit. But the new data indicate that coated-pit formation is coupled to events at the membrane skeleton, possibly through scaffold proteins found in specific (and limited) places. Future work with GFP fusion proteins should resolve this discrepancy, allowing us to see how coated pits form and work in a living cell.

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