

consumption, and which have been measured in mussel communities at the surface of methane seeps<sup>6</sup>. 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<sup>7</sup>. 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<sup>8</sup> has shown that distinctive limestones, the so-called 'calcarei *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 <sup>13</sup>C, with intracrystalline bio-

marker lipids for sulphate-reducing bacteria having even less <sup>13</sup>C content. Moreover, biomarkers for archaea are among the most <sup>13</sup>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.*<sup>8</sup> 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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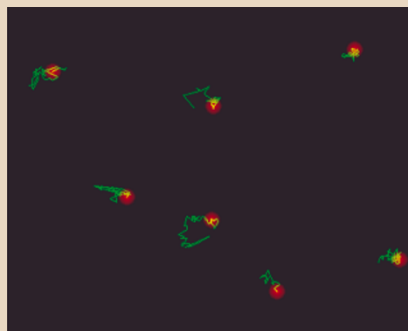
## Cell biology

### 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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