

a *baguette* of reindeer antler, 40 mm long, was stuck vertically into the sediment. Two flint bladelets, 14 and 22 mm long, were still attached, one at each side of the shaft, though the fixing-agent is now replaced by sediment. The object seems to have been placed there purposefully and to represent a weapon rather than a knife.

Moreover, a living-floor was uncovered in level IV-3 which contained large flint blades and nuclei, bigger than those of later occupations. Below, about 20 m² of a IV-4 floor were uncovered, featuring a superb habitation (see the figures) with a stone-filled hearth, 80 cm in diameter. Numerous flints (especially burins) lie on heavily ochred soil to the north and west of the hearth; to the south, ash and lithic/bone material stretch for several

square metres. The fauna consists principally of reindeer, but also includes horse, hare and even mammoth.

It looks as though Pincevent has not ceased to surprise us, and will continue to be one of the most fascinating and instructive archaeological sites in Europe for many years. □

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Cell motility

Particle transport

from Dennis Bray

ONE of the most astonishing facts about the intracellular movement of membranous organelles is that we still do not know what drives them. We cannot say, at present, whether the particle we see in the light microscope, moving steadily from one side of a cell to the other, is being driven by a microtubule-based mechanism, as in ciliary beating; by an actin-based mechanism, as in muscle contraction; or by an as-yet-unprecedented mechanism based on intermediate filaments. The reason for our ignorance is quite simple: it has so far not been possible to separate the motile machinery in a functional form from the rest of the cell. No one yet knows how to break open a cell and pull out a functional transport system, free of extraneous material, that continues to carry mitochondria and vesicles from place to place. But a start has been made, and four recent papers illustrate the progress and limits of contemporary efforts. A fifth paper raises the novel prospect of starting from the other direction: of building up the transport from scratch with purified molecules.

Organelle movements in the cytoplasm do not depend on ion flux across the plasma membrane, which is important only in that it maintains the internal *milieu* of ions and small molecules. Thus, it is possible to remove the cell membrane without interrupting organelle movements provided that a suitable buffer is supplied. A recent study of rapid axonal transport — one of the most accessible and extreme forms of organelle movement — illustrates the approach¹. Single large axons were dissected from the walking legs of the lobster (with edible parts suitably discarded) and mounted for observation in a buffer containing 0.02 per cent saponin, a soapy plant glycoside. If the buffer lacked ATP then all movement rapidly ceased and the organelles became totally immobilized, not

even showing brownian movement. Addition of ATP — but not a nonhydrolysable analogue of ATP — then restored movements which continued in an apparently normal fashion for up to an hour. From this we may conclude, first, that the normal permeability barrier of the axon had been destroyed; and second, that rapid organelle movements require ATP hydrolysis. In a separate study in which the plasma membrane of crab axons was disrupted by electrical discharge rather than with detergents², movements were similarly found to be dependent on ATP hydrolysis. Interestingly, they also seemed to be independent of Ca²⁺ concentration over a wide range, implying that organelles move through the cytoplasm 'flat out', or in an unregulated manner.

A more cathartic approach is to remove the cytoplasm entirely from the cell. Years ago, Baker, Hodgkin and Shaw³ found that axoplasm could be squeezed from a giant squid axon like toothpaste from a tube. Their interest was in the empty tube, which could be perfused with buffers and cannulated with electrodes and which eventually became the doyen of electrophysiological preparations. But the blob of expelled cytoplasmic jelly appeared inert under the microscope and was therefore ignored.

Then, a year ago at the Woods Hole Marine Biology Laboratory, a group of biologists looked again at extruded squid axoplasm using a newly developed optical system. This has the endearing acronym of AVEC-DIC (Allen video-enhanced contrast differential-interference contrast) and uses the background-subtract facility of some commercially available video cameras, usually in concert with a computer image-analysis system. Astounding results have been obtained with AVEC-DIC, which allows detection of structures

far smaller than the 0.2- μ m resolving power of light microscopes. In flattened keratinocytes, for example, organelles and vesicles have been observed moving closely along linear elements subsequently identified as microtubules⁴, and described in a paper that comes with its own video disc. Moreover the extruded axoplasm, thought hitherto to be so barren of activity, is with this technique revealed to be teeming with rapidly moving small particles⁵. These are submicroscopic vesicles 30–50 nm in diameter, which travel at steady speeds of up to 10 μ m s⁻¹. Movement continues unabated for several hours, in the absence, it should be remembered, of a plasma membrane or any other permeability barrier, thereby presenting unparalleled opportunities for future experimental intervention.

Exciting though these preparations are, however, it may not be possible to continue the dissection physically or pharmacologically down to the level of the elemental motile machine. In which case, some radically different approach is required. Considerable progress has been made in recent years in the construction of motile 'models', in which large-scale movements can be produced using actin and myosin, the two proteins that generate muscle contraction. Now a model very close to organelle movement has been described in which myosin-coated beads are made to crawl along aligned bundles of actin filaments⁶. The plastic beads, 0.7 μ m in diameter, were coated with heavy mero-myosin (HMM) — the active head region of the myosin molecule — while the actin filaments were those naturally present on the inner surface of the giant algal cell of *Nitella*. Beads applied to the exposed inner surface of the giant cell attached and moved at steady rates of up to 10 μ m s⁻¹, persistently and in one direction. Movement depended on ATP and on a functional myosin ATPase activity but was insensitive to variations in Ca²⁺ concentration. Calculations showed that a single myosin molecule could provide enough force to move a bead at a rate of 5 μ m s⁻¹, and that 25 or more functional myosin heads on the surface of a bead would be sufficient to give uninterrupted movement at this rate.

Clearly an actin-myosin interaction is one way to produce organelle movement. Could this be how the cell does it, with the myosin bound to the organelle and the actin filaments closely associated with microtubules? It looks as though we may soon know. □

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