news and views

Stops and starts in microtubules

from Dennis Bray

THE conventional wisdom that microtubules run the entire length of nerve axons and dendrites probably comes from a paper by Weiss and Mayr (*Acta Neuropath.* supp V, 198; 1971). On page 200 these authors conclude that "each tubule is a single unitary entity, extending in uninterrupted and undivided continuity from its base in the cell body to its peripheral tip". Clear enough one would think, but alas, the data are far less so. The evidence amounts to no more than the approximate constancy of tubule number in cross sections of six rat motor axons taken before and after bifurcations — a result that could obviously have arisen in several ways. Tubular continuity was presumably chosen as the explanation because it was consistent with the prevailing view of axonal dynamics. Tubules were believed to be synthesised at the soma and pushed out into the process; they were the continuous rails on which the fast transport ran. The whole story made such a neat package that it would have been churlish to worry about such corollaries as that the tubules in many parts of the mature nervous system would then be metres long. Best not to think of the recurrent laryngeal nerve of the giraffe.

Questions of continuity and point of origin also exist, although in a less extreme form, in non-neuronal cells. In fibroblasts, microtubules wander within the cytoplasm and can be seen by immunofluorescence as fine threads in tangled skeins. The method is sensitive enough to detect single tubules (Osborn *et al. J. Cell Biol.* 77, R27; 1978) but cannot resolve those in bundles. In consequence it is impossible to say in most preparations where the tubules begin and end. In 1976, however, three laboratories described a way to cut this Gordian knot. The tubules are depolymerised with colchicine or other antimitotic agent, the drug is washed out, and the cells fixed as the tubules begin to grow back again and then stained with antitubulin. When this is done the reforming tubules are first found in discrete star-like bodies in the cytoplasm, usually close to the nucleus (all of this applies to interphase cells; mitosis is different). The tubules lengthen from these centres until eventually the original network is regained. There is usually one, sometimes two, of these centres in a cell and it seemed most reasonable to suggest that they were the centrioles or cytocentres which have sometimes been seen by electron microscopy within arrays of microtubules in interphase cells. The implication was then that in such cells all tubules lead to the cytocentre which serves as a nucleation point for their growth. This was analogous to the role of the nuclear assembly point envisaged for the nerve cell.

The picture at this time was simple and satisfying. There were one or two structures in a fibroblast, epithelial cell or neurone which are responsible for the initiation and subsequent growth of all the microtubules in that cell. A simple picture, satisfying — and shortlived. A few months ago in *Cell* (16, 239; 1979) Spiegelman and colleagues showed that a minor variation in the staining procedure can produce not 1 or 2, but 5 to 10 centres in the average fibroblast, all close to the nucleus and each with 10–30 radiating microtubules. The main change in procedure was a previous extraction with non-ionic detergent and the new result was attributed to the loss of background staining — the new centres emerging from the background like stars at dusk, so to speak. Even worse, the same approach when used on human fibroblasts or epithelial cells showed one or two major centres together with many single points so small that they seemed little more than individual tubules growing in the cytoplasm. There are a number of imponderable factors, as always. Is it possible that the preparation of cytoskeletons might disrupt the organising centres and scatter the newly growing pieces through the gutted cell? Or, to go back a step, how do we know that the recovery from antimitotic agents will follow the same course as growth in a healthy cell? Within the limitations of the method, however, the picture obtained by immunofluorescence is of multiple nucleating centres, possibly of several distinct types.

Back at the nerve cell, a cultured neuroblastoma when treated by the above procedure shows a single organising centre at the base of its one or two neurites (Spiegelman *et al. op. cit*). Dividing neuroblastomas look more like a fibroblast in that they have an average of 12 smaller sites distributed around the nucleus. Obviously as the cell 'differentiates', the multiple centres coalesce to a single one and from a timed series of specimens Spiegelman *et al.* show that this occurs, on average, before the neurite grows out. The intriguing possibility is thereby raised that this coalescence in some way instigates neurite outgrowth. At any event, the tubules in this study are still firmly anchored to organising centres, small or large, in the soma.

The hint that even this tenet might be on shaky ground existed in an ultrastructural analysis of crayfish nerve (*J. Neurocytol.* 3, 73; 1974). Nadelhaft found that sections of the same axon at different points along its length could show a significant variation in microbutule number. His polite, but firm conclusion was that the hypothesis of microtubular continuity could not be universally valid. A conclusion that received a thumping validation in the July issue of the *Journal of Cell Biology* (82, 278; 1979). Here Chalfie and Thomson present a serial analysis of certain axons in the world's most photographed worm. These axons are notable for their distinct tubules which are larger than tubules elsewhere in the animal and very highly ordered. Serial sections show major fluctuations in tubule number along the length of these axons and frequent terminations. The tubules are in short lengths, between 6 and 27 μm, distributed in staggered arrays along the axon. Even more interesting is the observation that the two ends of each tubule are not the same. The distal end, furthest from the cell body, terminates in a cloud of light staining that is always right up against the plasma membrane. The proximal end can be anywhere in the cytoplasm but is characterised by central density in the tubule. Assuming that these features are not freaks of these specialised axons — and breaks can be found in other tubules in the animal — the Weiss and Mayr picture of continuity is roundly wrong. And the question of how microtubules grow, and from where, in neurones as in other cells, is back to the starting point. Perhaps the rabid reductionists are right and we will have to wait until the analysis of tubulin polymerisation *in vitro*, under controlled conditions and with defined components, provides the answer. There have been many such studies and they have revealed a rich and multifarious set of phenomena. It is true that they have yet to point to a single mechanism, but that is another story. □

Dennis Bray is in the MRC Unit of Cell Biophysics, King's College, London.

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