supported by the earlier histological studies of Probst *et al.*¹⁰ from which they deduce that the amyloid plaque is directly associated with trophic factors that stimulate the abnormal growth of neurites into the plaque.

Taken together, all these observations provide a working hypothesis that plaque formation in Alzheimer's disease is a consequence of proteolysis of the amyloid

precursor protein; self-aggregation of the cleaved A4 peptides explains the precipitated amyloid; and release of a trophic inhibitory domain explains the interwoven neuritic outgrowths. Speculative? True, but it will doubtless be a convincing start for grant applications! □

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Cell biology

Chromosome movement *in vitro*

Roy Burns

THE article by Koshland, Mitchison and Kirschner on page 499 of this issue¹ provides evidence about how chromosomes move towards the spindle poles of the nuclei of cells during the anaphase stage of mitosis. The authors constructed microtubule-chromosome complexes *in vitro* which have the same geometry as one component of the anaphase spindle, and have been able to use them to mimic chromosome movement.

The early anaphase spindle (see figure) consists of two, usually interspersed microtubule systems. One is formed of kinetochore microtubules, running from the two poles to the chromosomal kinetochores: shortening of these kinetochore microtubules results in movement of the chromosomes towards the spindle poles. The second system consists of two sets of interdigitating pole-to-pole microtubules, and continued assembly and sliding in the region of overlap results in the increased separation of the two poles. The first process is called anaphase A, the second anaphase B.

Microtubules have an intrinsic polarity, best defined by their relative rates of assembly at the two ends with the 'plus' end growing about threefold faster than the 'minus' end. All the microtubules in each half-spindle have the same polarity, with the minus ends embedded within the polar centrosomal material, and the plus

ends attached to the kinetochores or lying towards the mid-plane (see figure).

This polarity is central to understanding the microtubule-chromosome complexes constructed by Koshland *et al.* The authors prepared biotinylated brain microtubules from tubulin, which they then lightly crosslinked and sheared. They used these fragments to nucleate brain tubulin, selecting conditions which restricted assembly to the plus end of the seeds, and then captured isolated chromosomes onto the plus ends of the newly assembled microtubules. The construct therefore resembles the spatial arrangement of the anaphase A system: a microtubule linked to the chromosomal kinetochore by its plus end and with a stable 'cap' at its minus end (see figure).

Microtubule assembly and disassembly is governed by the relative rates of addition and loss of tubulin subunits to the microtubule end. A concentration of free tubulin subunits, the critical concentration, remains when assembly has attained steady state, with pre-assembled microtubules dissociating on dilution until this critical concentration is reached.

Koshland *et al.* diluted their microtubule-chromosome complexes into different concentrations of free tubulin, and then, using immunofluorescence, looked at the position of the chromosomes, the biotinylated stable cap and the inter-

connecting microtubule. They report a remarkable observation: the length of the interconnecting microtubule shortens while remaining attached both to the stable cap and to the chromosome. They go on to show, by using interconnecting microtubules formed in part of partially biotinylated tubulin, that the disassembly occurs at the point of the microtubule-kinetochore attachment. The rate of this disassembly depends, as predicted, on the exogenous tubulin concentration, and can achieve rates of up to $12 \mu\text{m min}^{-1}$.

These *in vitro* results closely mirror *in vivo* observations: for example, photobleaching studies after microinjection of fluorescently labelled tubulin show that the kinetochore microtubules shorten from their plus ends². The *in vitro* experiments do, though, provide one real surprise: the movement of the chromosome along the shortening microtubule does not require either GTP or ATP. This movement towards the minus end does not therefore involve a 'motor', a mechanochemical enzyme such as dynein, kinesin or MAP 1C which effect microtubule sliding or transport of particles along microtubules in various cells (see the recent News and Views article by Ian Gibbons³). Instead, chromosome movement towards the minus end either appears to be driven by energy derived from a conformational change in the disassembling tubulin subunits, or it results from biased diffusion of the kinetochore along the microtubule.

The new results from Kirschner's laboratory provide a further twist. When microtubule-chromosome complexes are diluted into tubulin at concentrations above critical, the distance between the kinetochore and the biotinylated cap remains constant. By contrast, studies in the same laboratory with an earlier model system had shown chromosomal movement at $2.3 \mu\text{m min}^{-1}$ towards the plus end under such conditions, but only when ATP was included⁴. Consequently, a motor is needed for chromosomal movement along a microtubule towards the plus end while disassembly drives it towards the minus end.

Use of microtubule-chromosome constructs is helping to identify the precise molecular basis of one of the most important biological machines. Attention is now likely to focus on the composition and structure of the kinetochore, on how microtubule disassembly induces polewards movement, on the identity of the unidirectional motor, and on the control mechanisms governing the polarity of chromosomal movement. □

1. Koshland, D.E. *et al.* *Nature* **331**, 499–504 (1988).

2. Gorbsky, G.J. *et al.* *J. Cell Biol.* **104**, 9–18 (1987).

3. Gibbons, I.R. *Nature* **330**, 600 (1987).

4. Mitchison, T. & Kirschner, M.W. *J. Cell Biol.* **101**, 766–777 (1985).

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a, The arrangement of microtubules in the early anaphase spindle. The two systems are shown separately, showing the intrinsic polarity of the microtubules. Left, kinetochore microtubules, whose shortening results in anaphase A or the movement of the chromosomes towards the spindle poles. Right, pole-to-pole microtubules, whose continued assembly and antiparallel sliding results in anaphase B or the increasing separation of the spindle poles. b, A microtubule-chromosome construct consisting of a microtubule interconnecting a stable, biotinylated cap and an isolated chromosome and having the same polarity as a kinetochore microtubule. On dilution, the length of the interconnecting microtubule shortens, due to loss of tubulin subunits from the site of chromosomal attachment.