are involved in transcriptional regulation. Many developmentally important genes in *Drosophila* and other species encompassing many phyla, have been found to encode homoeodomains. The interesting feature of the *rough* protein is that it appears to control, at the transcriptional level, the expression of signals from one cell type that are received and interpreted by others.

By comparison with research on *Drosophila* embryos, eye development has been less intensely studied. But it has two distinct features in its favour. First, the pattern formation is more simple and easy to follow than in the embryo, and less complex developmental questions that focus on the behaviour of single cells can be asked. Second, the entire visual system can be removed without affecting the viability of the fly, and extensive manipulations of the system are possible. This latter point becomes even more important when it is realized that flies are still viable in the absence of function of *glass*, *sevenless* and *rough*. This will allow extensive

analyses of the various protein functions to be performed in a way that is not possible for lethally mutant genes. For example, it will be possible to modify any of the three genes *in vitro*, reintroduce them into a mutant fly and then assay the effect on eye development. In this way the various operational domains of the proteins can be biochemically dissected in a systematic way and further insights into their function gained. □

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CELL DIVISION

Photoactive microtubule flux

Roy Burns

THE mitotic spindle, a complex structure that organizes the separation of the chromosomes during cell division, consists of two kinds of microtubule fibres. The pole-to-pole microtubules slide relative to each other in an antiparallel fashion to effect anaphase B (see figure). The kinetochore microtubules link the chromosomes to the poles of the spindle. In a new paper¹ in the *Journal of Cell Biology*, Tim Mitchison reports a novel and elegant approach to demonstrate the flux, *in vivo*, of tubulin subunits along the kinetochore microtubules during metaphase, which complements earlier work showing that these microtubules shorten during anaphase A.

Mitchison has turned on its head the widely used technique of fluorescence recovery after photobleaching. He has prepared a 'caged' derivative of the molecule FITC, which becomes fluorescent only on photoactivation, and coupled it to tubulin. This complex becomes incorporated into the spindle microtubules upon microinjection into cells at metaphase, where the chromosomes are at the equator of the spindle. Using light at a wavelength of 365 nm, Mitchison irradiated a narrow bar across the spindle between the chromosomes and the poles; the bar fluoresces at a longer wavelength and its fate can be monitored as the cells move through metaphase to anaphase.

Mitchison observed an initial rapid decrease in the intensity of the photo-

activated bar and a general increase in the background fluorescence, which he ascribes to the turnover of the more labile pole-to-pole microtubules. The residual fluorescence of the bar appeared to be restricted to the bundles of kinetochore microtubules, and this fluorescence slowly migrated towards the spindle poles while the cells remained at metaphase. As the chromosome-to-pole distance remained approximately constant, this movement of the residual fluorescence clearly demonstrates continued addition of tubulin subunits at the kinetochores and loss of subunits at the poles.

This flux resembles the treadmilling, or head-to-tail polymerization, of microtubules assembled *in vitro* to steady state^{2–4}, and which results from a difference in the critical concentration at the two ends of the microtubule (see figure). Although the observed flux is in the direction predicted from the polarity of the microtubules, Mitchison notes that treadmilling is only one possible mechanism, as it could also be effected by one of the various mechanochemical motors (see ref. 5).

It has long been unclear whether treadmilling is of any relevance *in vivo*, as most microtubules terminate at their slow-growing or minus ends in specialized structures called microtubule-organizing centres, such as the centrosomes. Indeed, the kinetic difference between the two ends has been postulated to be a mechanism for restricting microtubule assembly

to such organizing centres⁵, so establishing the specific spatial organization characteristic of the microtubule cytoskeleton. The close association of the minus ends with such structures might be expected to prevent subunit addition and loss: treadmilling would simply be an artefact resulting from the availability of the two ends *in vitro*.

This argument against treadmilling occurring *in vivo* has looked rather flimsy since Mitchison and collaborators demonstrated, using *in vitro* microtubule-chromosome constructs, that microtubules could assemble⁷ or disassemble⁸ at the kinetochore-microtubule junction without transitory detachment. However, both ends of the microtubule must be kinetically active if subunit flux is to occur: Mitchison's new studies¹ show that this condition is satisfied, at least for the kinetochore microtubules during metaphase.

Although treadmilling has been considered in terms of subunit flux along the microtubule, the evidence has relied heavily upon the kinetics of incorporation of radiolabelled GTP in pulse-chase experiments. Interpretation of these kinetics was complicated by the subsequent demonstration that microtubules can exhibit dynamic instability (see ref. 9) resulting from the simultaneous elongation and shortening of distinct subpopulations. Both behaviours require microtubules to respond to two different critical concentrations, either at the two ends or between the microtubule subpopulations, and at first sight the GTP data could be interpreted by either alternative.

Further detailed studies, however, confirm that treadmilling and dynamic instability are not mutually exclusive, and that the presence or absence of microtubule-associated proteins can push

100 years ago

DO CATS COUNT?

ABOUT two weeks ago, the cat of a dairyman in this neighbourhood gave birth to three kittens. Next day, one of them was removed, during the mother's absence, and drowned. On returning from a foraging expedition, and discovering her loss, puss immediately set out in search of the missing one. All her efforts in this direction, of course, proved fruitless; but, evidently determined to at least make up the right number, she did so by carrying off, from its nest close by, a young hare, not more than a week old. This she is at present suckling side by side with her own kittens. In view of these facts the above question very naturally suggests itself.

J.T. WALKER

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