

## Taxol-induced Flexibility of Microtubules and Its Reversal by MAP-2 and Tau\*

(Received for publication, January 21, 1993)

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When microtubules, ordinarily quite rigid structures, are treated *in vitro* with the anti-tumor drug taxol, they rapidly develop a wavy appearance and become strikingly flexible. A quantitative measure of their flexibility, the reciprocal statistical length,  $\lambda$ , increases by an order of magnitude when taxol is bound. Subsequent addition of either of the microtubule-associated proteins MAP-2 or tau causes the flexibility to disappear. It can be restored again by removing the microtubule-associated protein. These results show that taxol changes microtubular structure substantially, probably by weakening the interactions between protofilaments, and that microtubule-associated proteins reverse these effects, possibly by bridging protofilaments. This structural change and the accompanying flexibility may contribute importantly to taxol's cytotoxic activity.

Taxol, a small molecule isolated from the western yew, *Taxus brevifolia*, enhances tubulin's tendency to form microtubules by binding with high affinity, although reversibly and non-covalently, to the assembled structures (1-3). In cultured mammalian cells, taxol slows cellular motion and migration (2, 4, 5) and alters cytoskeletal morphology to produce an increase in lateral interaction between microtubules, which form "bundles" (2, 6-8). Interest in taxol's effects on microtubules has been heightened by recent confirmation of its promise as an anti-tumor agent (for reviews, see Refs. 9-11). Its cytotoxic activity in this context has been ascribed to the stabilization of assembled microtubules and to bundle formation (12).

Untreated microtubules have been observed to be quite inflexible, with Young's modulus near  $10^9$  dynes·cm<sup>-2</sup> (13, 14). Taxol-treated microtubules have often been assumed to be rigid as well, although their sinuousness in kinesin-motility assays has been noted (15). The direct investigation by light microscopy reported here reveals that taxol's major qualitative effect on single microtubules is a large increase in their flexibility. This phenomenon may underlie its pharmacological activity.

\* This work was supported by Grant GM-25638 from the National Institutes of Health and by the Vanderbilt University Research Council. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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## MATERIALS AND METHODS

Tubulin was prepared by assembly/disassembly followed by phosphocellulose chromatography (16, 17) and was equilibrated with PMD buffer (0.1 M Pipes,<sup>1</sup> pH 6.9, 1 mM MgSO<sub>4</sub>, 2 mM EGTA, 2 mM dithioerythritol, 0.1 mM GTP). MAP-2 was isolated from phosphocellulose-purified MAPs by heat treatment and gel filtration on Sephacryl S-300 (18) and equilibrated with PMD. Tau was prepared according to Baudier *et al.* (19). To test whether components other than MAPs could influence taxol-induced flexibility, a low molecular weight ultrafiltrate was prepared from a high speed supernatant of bovine brain (17) by collecting the material that passed through an ultrafiltration membrane (Centricon 30, type YM, Amicon Inc.) with an exclusion limit of approximately 30 kDa.

Assembly and observation of microtubules was carried out at 37 °C essentially as described in Ref. 20. To provide polymerization nuclei that anchor microtubules in a flow of buffer, axonemal pieces from sea urchin sperm-tails (21), suspended in PMD, were allowed to adhere to the glass coverslip of a microscope flow cell (22). Following removal of excess axonemes with a flow of PMD, microtubules were nucleated by introduction of 2.0 mg/ml tubulin in PMD and observed *via* video-enhanced differential interference contrast microscopy (23). To allow visual assessment of flexibility, the microtubules were then subjected to a flow of 2.0 mg/ml tubulin in PMD, followed by a flow of 10 or 50  $\mu$ M taxol in PMD. The velocity of flow was the same for all solutions in a given experiment and was measured by observation of small entrained particles in the same plane of focus as the microtubules. Free taxol was then removed by exchange of PMD. Microtubules thus grown and stabilized remained apparently unchanged for up to 2 h in 0.1 M Pipes, pH 6.9, 2 mM EGTA, 2 mM dithioerythritol in the presence or absence of Mg<sup>2+</sup> or GTP. In experiments to assess MAP effects, microtubules were grown from 0.8 mg/ml tubulin in PMD and stabilized with 10  $\mu$ M taxol in the same buffer. After microtubule growth had occurred, a solution of 0.1 mg/ml MAP-2 was introduced at a constant rate of flow of 0.67  $\mu$ l/s.

Quantitative assessment of flexibility was carried out by statistical measurement of the contour length and end-to-end distances of free microtubules (13, 24, 25). Microtubules were assembled, either from 0.1 mg/ml tubulin in PMD + 10  $\mu$ M taxol or from 2.5 mg/ml tubulin in PMD, in a chamber about 6  $\mu$ m thick. Video recordings were made of microtubules in the central region of the chamber, where contact with the walls did not occur. Approximately 60 measurements of the contour lengths and end-to-end distances of microtubules 10-15  $\mu$ m in length were made from single video frames. Only images in which a microtubule was clearly in focus throughout its length were used. The data were analyzed exactly as described by Mizushima-Sugano *et al.* (13). Because of the small effective focal depth of the optical system, the images chosen corresponded approximately to two-dimensional projections of the microtubule. Under those circumstances, the mean-squared end-to-end distance,  $\langle R^2 \rangle$ , can be related to the contour length,  $L$ , and to the flexibility parameter  $\lambda$  (the inverse of the statistical length of the microtubule) by the following equation.

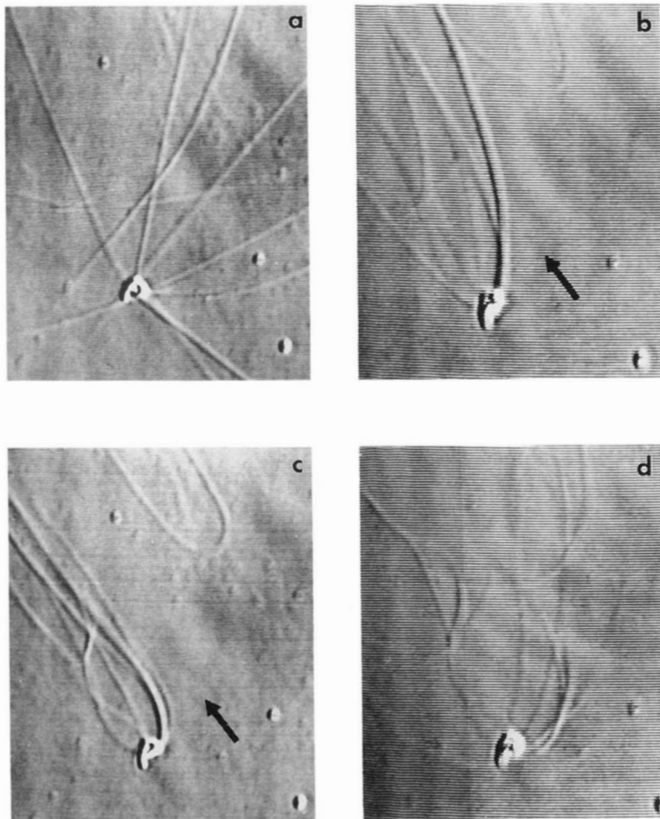
$$\langle R^2 \rangle = 2(\exp(-\lambda L) - 1 + \lambda L)/\lambda^2 \quad (\text{Eq. 1})$$

The best-fitting values of  $\lambda$  and the standard deviation of the estimate were obtained as described (13).

## RESULTS

Fig. 1a shows representative untreated microtubules in stationary buffer. Fig. 1b shows the small deformation observed when buffer was caused to flow past them at moderate velocity. When taxol was added to this steadily flowing buffer (Fig. 1c), microtubules abruptly (<2 s) became limp and flexible. The change in rigidity can be seen to be large. Movements of these microtubules, both in response to reversal of flow and due to Brownian motion, showed them to be

<sup>1</sup> The abbreviations used are: Pipes, 1,4-piperazinediethanesulfonic acid; MAP, microtubule-associated protein.



**FIG. 1. Taxol-induced flexibility of microtubules grown from pure tubulin.** Microtubules were nucleated from an axonemal piece anchored to the glass coverslip, and flexibility was qualitatively assayed by subjecting them to a flow of buffer. *Panel a* shows microtubules in stationary buffer, before initiation of flow. *Panel b* shows the same microtubules, subjected to a continuous flow of buffer with no taxol. The flow rate was constant at  $2.8 \times 10^{-2}$  cm/s in the direction shown by the arrow. *Panel c* shows the same microtubules approximately 3 s after  $50 \mu\text{M}$  taxol was introduced into the flow of buffer, which continued at the same rate. *Panel d* shows the same microtubules approximately 7 s after cessation of flow, as in *a*. (Elapsed time between *panels c* and *d* was 12 s.)

uniformly deformable along their whole length (Fig. 1*d*). Flexibility persisted for hours after taxol was removed from the surrounding buffer, reflecting its strong affinity for microtubules (2, 3).

Measurement of the flexibility parameter,  $\lambda$ , yielded  $0.0015 (\pm 0.0004) \mu\text{m}^{-1}$  for untreated microtubules and  $0.016 (\pm 0.004) \mu\text{m}^{-1}$  for taxol-treated microtubules, in qualitative agreement with the visual observation. Although, as might be expected, the value of  $\lambda$  for our MAP-free control microtubules differs somewhat from the value of  $0.0068 (\pm 0.0008)$  obtained for MAP-containing microtubules observed in glycerol-containing buffer by dark-field microscopy (13, 25), the comparison between untreated and taxol-treated microtubules appears reliable. It indicates that taxol causes approximately a 10-fold increase in flexibility.

Microtubule-associated proteins (MAPs) reverse taxol's effects. Fig. 2 shows the result of addition of purified MAP-2 to taxol-treated microtubules. Within some tens of seconds, the microtubules lost their flexibility and straightened, becoming visually indistinguishable from untreated microtubules (compare Fig. 2*e* to Fig. 1*a*). Evidently MAP-2, known to bind tightly to microtubules, either reverses or overcomes the flexibility-inducing effect of taxol. Tau ( $0.1 \text{ mg/ml}$ ) caused apparently identical effects, as did both the mixture of MAPs isolated from cycled microtubules by phosphocellulose chro-

matography (16) and a supernatant of bovine brain (17, 18). Straightening was not induced, however, by the addition of more tubulin or of the microtubule-stabilizing solvent glycerol ( $1.1 \text{ M}$ ), or by the low molecular weight components of bovine brain supernatant.

The effect of MAP-2 could itself be reversed, and flexibility caused to reappear, by application of a brief flow of PMD buffer supplemented with  $0.4$  or  $0.75 \text{ M}$  NaCl (known to release MAP-2 from microtubules; Ref. 3), followed by PMD buffer, all without additional taxol. This restoration of flexibility implies that taxol remained bound to microtubules when MAPs were present. MAPs must therefore *overcome* the effects of taxol, without causing it to dissociate from microtubules.

When microtubules assembled from pure tubulin were first treated with MAPs (high speed supernatant of brain, or whole MAPs), then with  $50 \mu\text{M}$  taxol, no taxol-induced flexibility was observed even after 20 min of exposure. When these microtubules were subsequently rinsed extensively with buffer to remove unbound taxol and with  $0.4 \text{ M}$  NaCl to remove MAPs, flexibility appeared. These findings imply that bound MAPs do not prevent the binding of taxol.

#### DISCUSSION

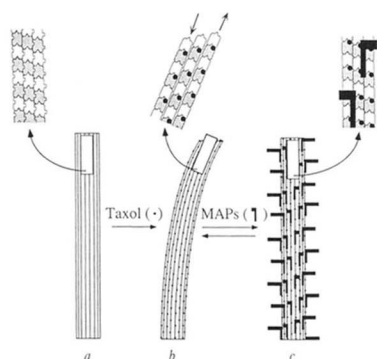
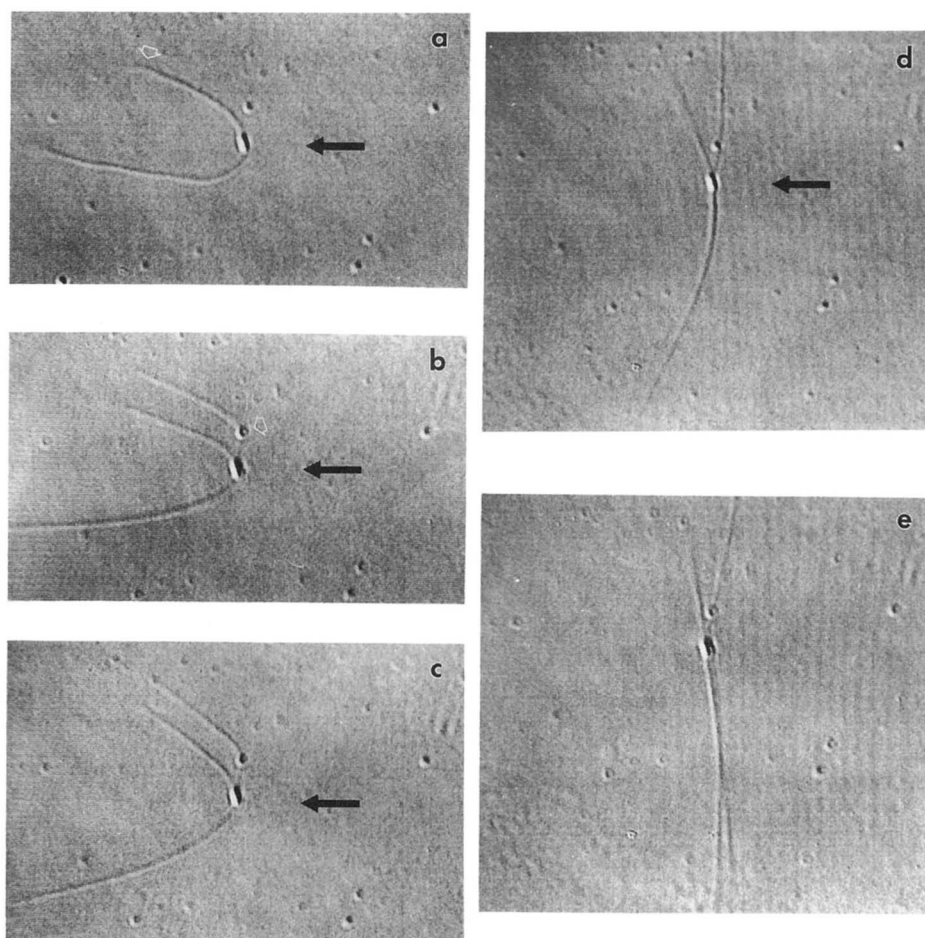
It is believed that microtubules are rigid because they are cylinders with shear-resistant walls (13). Because taxol-treated microtubules largely retain their tubular structure and do not become extensively ribbon-like or "C"-shaped (2, 15, 26), their loss of rigidity is unlikely to result from a loss of tubular cross-section. Instead, it seems likely that binding of taxol reduces rigidity by decreasing the strength of circumferential interactions between protofilaments, allowing them to slip relative to each other as indicated schematically in Fig. 3 (*a* and *b*). The extent of slippage in the axial direction (to be seen schematically by comparison of *panels a* and *b* of Fig. 3) can easily be estimated for a given length of microtubule and degree of bend. For  $10$ – $15\text{-}\mu\text{m}$  microtubules, the axial slippage (shear displacement) between two adjacent protofilaments corresponding to the tightest bends observed (radius of curvature about  $15 \mu\text{m}$ ) would be about  $6 \text{ nm}$ , on the order of magnitude of size of only a single tubulin dimer.

The mechanism by which MAPs reverse taxol-induced flexibility may involve their binding between protofilaments and acting to bridge them, as shown schematically in Fig. 3*c*. Such bridging could restore the strength of circumferential interactions between protofilaments. Furthermore, if the degree of axial displacement between neighboring protofilaments is less than the axial extent of a dimer, the bridging could act to draw dimers back into register, producing the observed "upstream" straightening of microtubules against a flow (Fig. 2). That MAPs may bridge protofilaments by binding between them has long been a part of hypothetical models (27, 28); such bridging has been observed in the binding of MAPs to  $\text{Zn}^{2+}$ -induced tubulin sheets (29).

Taxol-induced flexibility may, in part, underlie intracellular microtubule bundling. By reducing rigidity, taxol could allow microtubules to conform closely to each other in response to attractive forces (*e.g.* MAP-bridging) that are either pre-existing or caused by taxol-binding itself. The bundles thus formed would be expected to be flexible relative to those formed by untreated tubulin and to appear sinuous.

Some MAPs appear to induce microtubular rigidity *in vivo* (28, 30). One would expect the identities, concentrations, and distributions of MAPs to have major effects on the extent of flexibility induced by taxol in any particular cell. Because the degree of saturation of microtubules with MAPs in the cell is

**FIG. 2. Reversal of taxol-induced flexibility by MAP-2.** Taxol-treated microtubules were subjected to a continuous flow of taxol-free PMD buffer at  $3.1 \times 10^{-2}$  cm/s. MAP-2 was then added to the flow. Restoration of flexibility occurred more slowly than did its initial induction by taxol, requiring about 30 s for completion. In most cases, microtubules straightened upstream, in opposition to the flow. *Panel a* shows microtubules before addition of MAP-2. *Black arrow* indicates direction of flow. *White arrow* represents a point where microtubules adhered briefly to the substrate. As shown in the following frames, two pairs of microtubules have grown from the ends of the axoneme. *Panel b* shows the same microtubules about 15 s after the introduction of MAP-2 (final concentration 0.1 mg/ml) into the continuing flow of buffer. Note that the upper pair of microtubules have separated. *White arrow* shows a point where one microtubule has adhered to the substrate. *Panel c* shows the same field after 20 s, continuing flow. *Panel d* shows the same field after 30 s, continuing flow. *Panel e* shows the same microtubules after cessation of flow.



**FIG. 3. Schematic diagram showing that flexibility may be induced if protofilaments are allowed to slide relative to each other.** *a*, microtubule without taxol is shown. Subunits ( $\alpha$ , open;  $\beta$ , stippled) are shown in the three-start helical lattice. Longitudinal bonds are indicated by pointed projections; circumferential bonds are indicated by rectangular projections. Shear is prevented and bending is inhibited by relatively tight circumferential connections (symbolized by rectangular projections) between neighboring subunits in adjacent protofilaments. *b*, microtubule with taxol (filled circles) bound to tubulin subunits is shown. A bend is represented. The continued presence of longitudinal bonds, possibly strengthened by the presence of bound taxol (2), is indicated. The taxol-induced absence of circumferential bonds allows neighboring subunits to slip freely and to become displaced relative to each other, as symbolized by the short arrows. The extent of displacement is exaggerated for illustrative purposes. *c* shows microtubule with taxol and MAPs bound. MAP molecules are shown bridging the protofilaments, reinforcing the connections between neighboring subunits. The continued presence of bound taxol and the modification of circumferential tubulin-tubulin bonds are also represented.

likely to be only partial, only partial reversal of taxol-induced flexibility would be anticipated. The residual flexibility should have large consequences for the cell, reversing or inhibiting cellular functions, including shape changes, that require rigidity. Although little direct evidence exists to relate the rigidity of microtubules to their cellular function, strong general arguments support the notion that microtubules' cytoskeletal role involves support of compressive force (for review, see Ref. 31). More specifically, taxol has been found to inhibit a microtubule-dependent shape change in developing avian erythrocytes (32) and to promote neurite resorption in cultured neural hybrid cells (33). Tau, when overexpressed in cultured non-neuronal cells, leads to extensive microtubule polymerization and to bundle formation, and so does taxol, but only tau also causes production of long cellular processes (34). Thus, taxol in these three instances caused polymerization and bundling of microtubules but abolished effects attributable to their rigidity.

The existence of a drug that binds to and changes the flexibility of microtubules suggests that other ligands may be capable of modulating flexibility. The potential importance of microtubular rigidity in cellular function warrants a search for such ligands as well as further investigation of the possible correlation between microtubular flexibility and cytotoxic activity.

**Acknowledgments**—We thank Andrew Matus, for making available before publication findings about MAP-induced loss of flexibility, and Paula Flicker, for thoughtful reading of the manuscript.

#### REFERENCES

- Schiff, P. B., Fant, J. & Horwitz, S. B. (1979) *Nature* **277**, 665–667
- Parness, J. & Horwitz, S. B. (1981) *J. Cell Biol.* **91**, 479–487

3. Collins, C. A. & Vallee, R. B. (1987) *J. Cell Biol.* **105**, 2847-2854
4. Amin-Hanjani, S. & Wadsworth, P. (1991) *Cell Motil. Cytoskel.* **20**, 136-144
5. Roberts, R. L., Nath, J., Friedman, M. M. & Gallin, J. I. (1982) *J. Immunol.* **129**, 2134-2141
6. Schiff, P. B. & Horwitz, S. B. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77**, 1561-1565
7. De Brabander, M., Geuens, G., Nuydens, R., Willebrords, R. & De Mey, J. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78**, 5608-5612
8. Turner, P. F. & Margolis, R. L. (1984) *J. Cell Biol.* **99**, 940-946
9. Slichenmyer, W. J. & Von Hoff, D. D. (1990) *J. Clin. Pharmacol.* **30**, 770-788
10. Rowinsky, E. K., Cazenave, L. A. & Donehower, R. C. (1990) *J. Natl. Cancer Inst.* **82**, 1247-1259
11. Horwitz, S. B. (1992) *Trends Pharmacol. Sci.* **13**, 134-136
12. Rowinsky, E. K., Donehower, R. C., Jones, R. J. & Tucker, R. W. (1988) *Cancer Res.* **48**, 4093-4100
13. Mizushima-Sugano, J., Maeda, T. & Miki-Noumura, T. (1983) *Biochim. Biophys. Acta* **755**, 257-262
14. Cross, A. R. & Williams, R. C., Jr. (1991) *Cell Motil. Cytoskel.* **20**, 272-278
15. Amos, L. A. & Amos, W. B. (1991) *J. Cell Sci. Suppl.* **14**, 95-109
16. Williams, R. C., Jr. & Lee, J. C. (1985) *Methods Enzymol.* **85B**, 376-385
17. Correia, J. J., Baty, L. T. & Williams, R. C., Jr. (1987) *J. Biol. Chem.* **262**, 17278-17284
18. Herzog, W. & Weber, K. (1978) *Eur. J. Biochem.* **92**, 1-8
19. Baudier, J., Lee, S.-H. & Cole, R. D. (1987) *J. Biol. Chem.* **262**, 17584-17590
20. Williams, R. C., Jr. (1992) in *The Cytoskeleton: A Practical Approach* (Carraway, K. L., ed) pp. 151-166, Oxford University Press, Oxford
21. Walker, R. A., O'Brien, E. T., Pryer, N. K., Soboeiro, M. F., Voter, W. A., Erickson, H. P. & Salmon, E. D. (1988) *J. Cell Biol.* **107**, 1437-1448
22. Berg, H. C. & Block, S. M. (1984) *J. Gen. Microbiol.* **130**, 2915-2920
23. Inoue, S. (1986) *Video Microscopy*, Plenum Press, New York
24. Nagashima, H. & Asakura, S. (1980) *J. Mol. Biol.* **136**, 169-182
25. Yamazaki, S., Maeda, T. & Miki-Noumura (1982) in *Biological Functions of Microtubules and Related Structures* (Sakai, H., Mohri, H. & Borisy, G. G., eds) Academic Press, Tokyo
26. Ringel, I. & Horwitz, S. B. (1991) *J. Pharmacol. Exp. Ther.* **259**, 885-860
27. Amos, L. A. (1979) in *Microtubules* (Roberts, K. & Hyams, J. S., eds) pp. 1-64, Academic Press, London
28. Edson, K., Weisshaar, B. & Matus, A. (1993) *Development* **117**, in press
29. McEwen, B. F., Ceska, T. A., Crepeau, R. H. & Edelstein, S. J. (1983) *J. Mol. Biol.* **166**, 119-140
30. Weisshaar, B., Doll, T. & Matus, A. (1992) *Development* **116**, 1151-1161
31. Heidemann, S. R. (1990) *Cell Motil. Cytoskel.* **17**, 6-10
32. Winckler, B. & Solomon, F. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 6033-6037
33. Smalheiser, N. R. (1991) *Brain Res. Dev. Brain Res.* **58**, 271-282
34. Knops, J. Kosik, K. S., Lee, G., Pardee, J. D., Cohen-Gould, L. & McConlogue, L. (1991) *J. Cell Biol.* **114**, 725-733