COMMUNICATIONS ARISING

Cell biology

Tubulin acetylation and cell motility

Although the protein tubulin is known to undergo several post-translational modifications that accumulate in stable but not dynamic microtubules inside cells, the function of these modifications is unknown. Hubbert *et al.*[1] have shown that the enzyme HDAC6 (for histone deacetylase 6) reverses the post-translational acetylation of tubulin, and provide evidence that reducing tubulin acetylation enhances cell motility. They also suggest that decreasing tubulin acetylation reduces microtubule stability. However, we find that microtubule stabilization is not promoted by tubulin acetylation. We conclude that the alteration in cell motility observed by Hubbert *et al.* in cells overexpressing HDAC6 results not from changes in the formation of stable microtubules, but from alterations in the degree of tubulin acetylation.

Most mammalian cells possess two subsets of microtubules: dynamic microtubules with a half-life of 5–10 min, and stable microtubules that have a half-life of hours, and which contain one or more types of post-translationally modified tubulin[2]. One of these modifications, detyrosination, accumulates in stable microtubules but does not cause microtubule stabilization[3–7]. For other tubulin modifications, however, the case is less clear.

Hubbert *et al.*[1] did not investigate whether changes in tubulin acetylation alter microtubule stability. To test this, we treated wound-edge, serum-starved NIH 3T3 fibroblasts, which have few stable microtubules[6,8], with inhibitors of HDAC6 and used resistance to depolymerization by nocodazole and accumulation of detyrosinated tubulin as assays for increased stable microtubules[6,8]. Cells treated with trichostatin A (TSA), an inhibitor of HDAC6, showed an increase in microtubule acetylation[1] (Fig. 1a, b, insets), but not in the detyrosination of microtubules compared with untreated cells (Fig. 1a, b). Cells treated with sodium butyrate, a deacetylase inhibitor that does not affect HDAC6 activity[1], did not increase either acetylation or detyrosination of microtubules (results not shown).

Serum-starved cells treated with TSA did not contain nocodazole-resistant microtubules either (Fig. 1c), in contrast to cells treated with a physiological stimulator of stable microtubules, lysophosphatidic acid (LPA)[6,8] (Fig. 1d). LPA-treated cells had more acetylated microtubules (results not shown).

These results indicate that increased tubulin acetylation does not increase levels of stable microtubules; rather, microtubules must be stabilized by other mechanisms (such as capping[7]) and then these stable microtubules accumulate acetylated tubulin, just as they accumulate detyrosinated tubulin. This is consistent with results showing that tubulin acetylation has no effect on microtubule assembly *in vitro*[9] and that acetylated tubulin is only detectable in long-lived stable microtubules *in vivo*[10].

Hubbert *et al.* found that HDAC6 overexpression enhances cell motility[1]. Our results imply that this increase in cell motility is not caused by changes in levels of stable microtubules, but by changes in the acetylation of tubulin (or of an as-yet-unidentified protein). Migrating wound-edge fibroblasts contain stable, post-translationally modified microtubules that are orientated towards the cell's leading edge[2,6,8] (Fig. 1d), and these may direct organelles and other important cellular components to the leading edge.

Detyrosinated tubulin seems to have an enhanced affinity for kinesin *in vitro*[11], and could be involved in kinesin-dependent recruitment of intermediate filaments to microtubules[12] and in the recycling of endocytic vesicles[13]. Perhaps acetylation will also turn out to affect the activity of microtubule-associated proteins or motors on microtubules.

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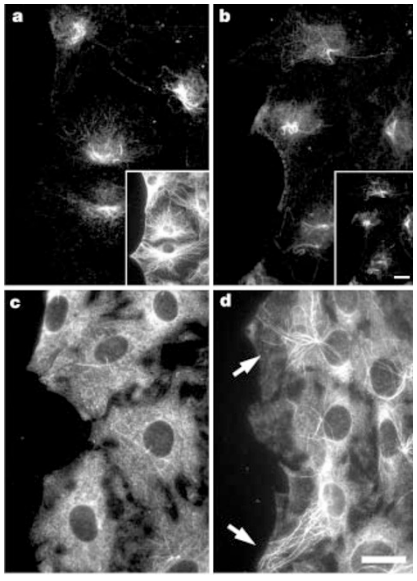

Figure 1 Immunofluorescent images of serum-starved, wounded NIH 3T3 fibroblasts, showing that increased acetylation of tubulin does not stabilize microtubules in these cells. Cells were incubated with trichostatin A (TSA; 5 μM; 4 h; **a**, **c**) or without TSA (**b**, **d**); cells in **d** were treated with 10 μM lysophosphatidic acid (LPA). Cells were fixed and immunostained for detyrosinated tubulin[6,8] (**a**, **b**), acetylated tubulin[10] (insets) or bulk tubulin (**c**, **d**). **a, b,** TSA increases microtubule acetylation (insets) but does not increase microtubule detyrosination compared with untreated controls (**a**, **b**). **c, d,** TSA does not increase the number of microtubules that are resistant to nocodazole (**c**; 10 μM; 30 min), whereas cells treated with LPA have nocodazole-resistant microtubules (**d**). Arrows show stable, modified microtubules orientated towards the leading edge. Scale bars, 15 μm.

1. Hubbert, C. *et al. Nature* **417,** 455–458 (2002).
2. Bulinski, J. C. & Gundersen, G. G. *Bioessays* **13,** 285–293 (1991).
3. Webster, D. R., Gundersen, G. G., Bulinski, J. C. & Borisy, G. G. *Proc. Natl Acad. Sci. USA* **84,** 9040–9044 (1987).
4. Khawaja, S., Gundersen, G. G. & Bulinski, J. C. *J. Cell Biol.* **106,** 141–149 (1988).
5. Webster, D. R., Wehland, J., Weber, K. & Borisy, G. G. *J. Cell Biol.* **111,** 113–122 (1990); erratum *ibid.* 1325–1326 (1990).
6. Cook, T. A., Nagasaki, T. & Gundersen, G. G. *J. Cell Biol.* **141,** 175–185 (1998).
7. Infante, A. S., Stein, M., Zhai, Y., Borisy, G. & Gundersen, G. G. *J. Cell Sci.* **113,** 3907–3919 (2000).
8. Palazzo, A. F., Cook, T. A., Alberts, A. S. & Gundersen, G. G. *Nature Cell Biol.* **3,** 723–729 (2001).
9. Maruta, H., Greer, K. & Rosenbaum, J. L. *J. Cell Biol.* **103,** 571–579 (1986).
10. Webster, D. R. & Borisy, G. G. *J. Cell Sci.* **92,** 57–65 (1989).
11. Liao, G. & Gundersen, G. G. *J. Biol. Chem.* **273,** 9797–9803 (1998).
12. Kreitzer, G., Liao, G. & Gundersen, G. G. *Mol. Biol. Cell* **10,** 1105–1118 (1999).
13. Lin, S. X., Gundersen, G. G. & Maxfield, F. R. *Mol. Biol. Cell* **13,** 96–109 (2002).

correction

Visual structure of a Japanese Zen garden

G. J. Van Tonder, M. J. Lyons, Y. Ejima

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In the legend for Fig. 2, the date AD 1681 is incorrect: in fact, the plan of the garden and temple indicates their likely layout before the building was destroyed by fire in AD 1797 and is based on ref. 4 of our communication. This error does not affect our conclusions.

addendum

Magnetic shape-memory effects in a crystal

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It has been drawn to our attention that the magnetic shape-memory effects we reported in $La_{2-x}Sr_xCuO_4$ (LSCO) crystals bear similarities to the conventional magnetostriction associated with antiferromagnetic domain structures. Indeed, in the Néel state, static antiferromagnetic domains may generate in LSCO crystals a pattern of structural distortions that can be modified by magnetic fields. However, we find that the magnetic shape memory in LSCO is a distinct phenomenon whereby magnetic fields affect genuine orthorhombic domains in both antiferromagnetic and paramagnetic states of LSCO, regardless of the existence of a magnetic order. This was not made sufficiently clear in our communication.

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