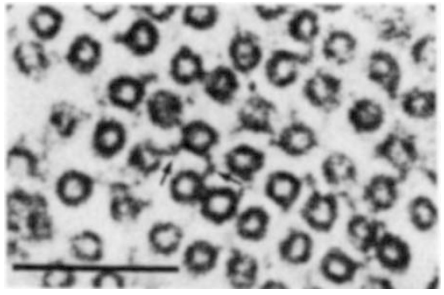
# Microtubule bundling in cells

SIR—Lewis and Cowan have amended[1] their hypothesis[2] that a small hydrophobic segment of neuron-specific microtubule-associated protein 2 (MAP2) is responsible for bundling microtubules in cells transfected with MAP2. The authors now suggest[1] that MAP2 is directly involved in bundling without using its hydrophobic carboxy terminus for this purpose. We suggest, instead, that the main effect of high-level expression of MAP2 in cultured fibroblasts is to stabilize dynamic microtubules, that the bundling that results is a direct consequence of this stabilization, and that bundling is mediated by an endogenous (as yet unidentified) bundling protein, rather than by the exogenously introduced MAP2. There is evidence for this two-step bundling process of stabilization followed by bundling and against the idea that bundling is mediated directly by MAP2.



Electron micrograph showing a cross section through a microtubule bundle in a fibroblast or presumptive myoblast treated with 10 μM taxol for 3 days before fixation. Arrows, cross-bridges resembling those seen in cells transfected with MAP2 (see text). Scale bar, 0.1 μm. (Reproduced from Fig. 15 of ref. 10 by permission of the Rockefeller Press; provided by Howard Holtzer and Camille DiLullo.)

Three experimental treatments can cause bundling of microtubules in cultured cells. First, when fibroblasts overexpress transfected MAP2 or tau[3] (neuronal-specific MAPs known to stabilize microtubules *in vitro* and *in vivo*), microtubule bundles are formed.

Second, taxol treatment causes almost immediate stabilization of microtubules *in vivo*[4] and, with longer treatments, results in bundling in many cell types, including 3T3 and HeLa, the cell lines used in the studies by Lewis and colleagues[5]. Although at the light microscope level, some differences in the appearance and distribution of MT bundles may be observed between taxol-treated and MAP2- (or tau-) transfected cells, at the electron microscope level, one observes structural characteristics of the bundles that point to a common mode of bundling. Regardless of the origin of the bundles, the spacing between microtubules is 25 nm (compare our figure with fig. 6*c* in ref. 2). Similar spacing within taxol-induced bundles has also been observed by other authors. Moreover, both MAP2- and taxol-induced bundles exhibit the same crossbridge structure; namely, a fine filament with a central thickening. The similarity in microtubule spacing and crossbridge structure in MAP2- and taxol-induced bundles strongly suggests a common mode of bundling independent of MAP2, as MAP2 is absent from cells exhibiting taxol-induced bundles. Taxol-treated 3T3 cells containing microtubule bundles do not contain MAP2 or tau (G. G. G., unpublished results).

Third, microinjection of a nonhydrolysable GTP analogue[6], which stabilizes microtubules *in vitro*[7], into cells containing neither tau nor MAP2 induces microtubule bundles, further evidence for a bundling mechanism dependent on microtubule stabilization but independent of MAP2 and tau.

Thus, three independent treatments result in microtubule stabilization and bundling in cultured cells. Additional evidence against the hypothesis that MAP2 or tau directly mediates bundling is that both proteins behave as monomeric species in solution (ref. 8; J. C. B., unpublished data). The identity of the endogenous factor that is responsible for bundling is unknown, although dynamin, a recently identified bundling protein[9], is a possible candidate. Stabilization of microtubules may stimulate bundling by increasing the longevity or concentration of microtubules, the abundance of non-centrosomal microtubules or biosynthesis of tubulin.

Given the relationship between stabilization and bundle formation, our interpretation of the deletion analysis of MAP2 described by Lewis and Cowan[1] is that those MAP2 constructs that did not induce bundling failed to do so because the transfected MAP2 did not stabilize microtubules adequately to allow endogenous bundling factors to act. MAP2 was co-localized with microtubules in some of these instances, in these cases we would expect that MAP2 stabilizes microtubules only slightly. Alterations in MAP2 synthesis, steady-state level or binding affinity could explain the less dramatic effects of these constructs on the microtubule cytoskeleton. Thus, Lewis and Cowan have not demonstrated a direct function for MAP2 in bundling, but they have raised the interesting question of the role of stabilization in generating microtubule bundles.

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# Name dropping

SIR—Dryja in News and Views[1] discussed several examples of inherited retinal degradation caused by mutations in the genes encoding known photoreceptor-specific proteins. He said in passing in his article that the gene that is mutant in the *rds* (retinal degeneration slow) mouse encodes the rod disk membrane protein peripherin. This statement misleadingly suggests that peripherin is a well-characterized retinal protein.

The *rds* gene was originally cloned by myself and co-workers[2] by using a 'reverse genetics' approach, and we determined the sequence of the wild-type *rds* messenger RNA. A sequence database search showed that the *rds* protein was novel. We next showed biochemically that the *rds* product is a glycoprotein associated with disk membranes whose distribution is confined to photoreceptor outer segments[3]. Molday *et al.* raised monoclonal antibodies against rod outer-segment disks and observed an antigen of unknown identity in the disk margins[4]. They named this protein 'peripherin' because of this distribution. They later showed[5] that this antigen is the bovine homologue of the *rds* protein.

To what extent does giving a name to an otherwise unknown protein based on a relatively superficial characteristic, such as its position within an organelle, amount to a functional characterization? I believe that the 'claiming' of novel proteins of unknown function by naming them adds little to our understanding. I suggest that we leave 'peripherin' for the unrelated neurofilament protein that already has that name, particularly as our work[3] on the *rds* protein does not support its distribution as confined to the disk periphery.

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