with taxol-stabilized microtubules either in the presence or absence of endogenous MAPs.

These data, taken together, support the idea of Martz *et al.* [10] that the mitochondrial membrane is multivalent. That is, it contains multiple sites capable of interacting with the various components of the cytoskeletal filaments. There is controversy between our results and those of Lindén *et al.* [6] concerning the nature of the cytoskeletal components capable of binding to mitochondria. Our conclusion is that, under *in vitro* conditions, brain mitochondria have the capacity to interact not only with the microtubule-associated proteins MAP1 and MAP2 but also with the tau proteins, as well as with tubulin.

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How do microtubules interact *in vitro* with purified subcellular organelles?

In two recent papers, we described the interaction of brain mitochondria with microtubule (MT) proteins [1,2]. The conclusions drawn from these studies are: (a) specific high-affinity binding sites for MAP2 exist on the surface of the mitochondria, probably on the outer membrane, and (b) that MAP2 interacts with an outer membrane domain which contains porin, or that MAP2 binding influences the physical structure of the porin-containing domain. (c) We also pointed out in our discussion that several experiments are consistent with the idea that the projection domain (in contrast with the MT-binding domain) of MAP2 binds to the mitochondrial surface.

In a subsequent study, Jancsik *et al.* [3] confirmed point (a) above. However, in the preceding letter [4] they challenge our conclusions in point (c) concerning the MAP2-binding domain which recognizes the mitochondrial surface. In doing so, they have focused upon a minor point of discussion in our original paper [1] and have amplified it into an issue for debate. However, since this question is, in fact, an important one, we feel it worthwhile to reiterate our views.

We reported four experimental findings which led to our tentative conclusion that the projection domain on MAP2 might bind to mitochondria [1,2].

1. MAP1 and 2 were transferred from taxol-stabilized MT to mitochondria. Since MAPs are attached to MT via the MT-binding domain, we assumed that the free projection domain interacts with mitochondria.

2. Pure tubulin did not compete for MAP2 binding to mitochondria, suggesting that the projection domain, and not the MT-binding domain of MAP 2, interacts with mitochondria.

3. Upon addition of a mixture of MAP2 and Tau to mitochondria, only MAP2 was observed to bind. This result is not expected if the MT-binding domain on Tau and MAP2 interacts with the mitochondrial surface.

4. Electron microscopy disclosed cross-bridge structures which are consistent with MAP2 and MAP1 acting as the link between MT and mitochondria. If one assumes that the MT-binding domain of MAPs, is, in fact, bound to MT, then the projection domain must be bound to mitochondria.

Rendon *et al.* argue [4] that MAP2 bind to mitochondria via the MT-binding domain on the MAP2 molecule because (a) the IC_{50} for the replacement of radioactive MAP2 or tau by unlabelled MAP2 were similar [3], and (b) that the MT-binding domain isolated from MAP2 replaced 40 % of the bound MAP2 whereas the projection domain had no effect [3].

However, these arguments are not justified since, in (a) competition was done under conditions in which the Tau binding sites on mitochondria were saturated while the MAP2 sites were only 20 % saturated, and in (b) the authors have used isolated projection fragments consisting of several degradative products after proteolytic treatment. In fact, their demonstration that the number of binding sites for the MT-binding fragment of MAP2 is 3-fold higher than that of the intact molecule [3] suggests that the binding characteristics of both fragments (containing MT-binding and projection domains) have been altered during preparation. The invalidity of this latter argument was, in fact, recognized in their original paper [3]. Thus, the conclusion that MAP2 binds to mitochondria via the MT-binding domain is not rigidly proven by Jancsik *et al.* [3].

In the preceding letter [4], Rendon *et al.* take issue with some of our experiments that we suggest support binding of MAP2 via its projection domain (see points 1–4 above). For the first, they point out that pure Tau protein can bind to specific sites on mitochondria. This finding raises some questions about the validity of our ideas expressed in point 3 above. Their second objection concerns experiments in which we observed no effect of tubulin on MAP2 binding (point 2 above). Rendon *et al.* [4] express the opinion that since tubulin binds *in vitro* to liver mitochondria [5] it should also bind to brain mitochondria. Furthermore, Rendon *et al.* [4] argue that bound tubulin cannot compete for MAP2. The latter thinking is faulty, however, since even if tubulin binds to brain mitochondria there are three possible results depending on the nature of the interaction, i.e. tubulin could enhance, inhibit, or, as suggested by Rendon *et al.* [4], have no effect on, MAP2 binding. The only situation in which mitochondria-bound tubulin would not affect binding of MAP2 is if MAP2 does not interact with this membranous tubulin. The association of tubulin monomers with membranous compartments including mitochondria has been known for several years [5–7]. The physiological significance of this binding is not understood, but it is clear that the membranes do not promote the polymerization of tubulin [5,7].

Finally, Rendon *et al.* [4] present an experiment which they suggest counters our ideas presented in point 1 above. However, we do not feel that this experiment invalidates our suggestion since Rendon *et al.* [4] used more drastic centrifugation conditions than ours [1], which lead to considerable aggregation of taxol-stabilized MT (14.3 % and 8 % for MAPs–MT and pure tubulin–MT respectively). The values increased by 2.6 and 2.0-

Fig. 1. Polymerization of tubulin by NF saturated *in vitro* with MAP2 (a) and effect of increasing concentrations of purified NF H and M subunits on the binding of MAPs to rat brain mitochondria (b)

(c) NF from bovine spinal cord (1 mg/ml) prepared as described in Leterrier & Eyer [13] were saturated by incubation for 30 min at 37 °C with thermostable MAP2 (0.39 mg/ml) and further purified from unbound MAP2 according to Leterrier *et al.* [14]. Control was the same NF preparation processed under the same incubation and centrifugation steps in the absence of MAP2. The two NF samples were resuspended by homogenization in the microtubule reassembly buffer (RB) (Mes, 0.1 M, pH 6.8; $MgCl_2$, 1 mM; EGTA, 1 mM) supplemented with 2 M-glycerol and further incubated (at a final NF triplet concentration of 0.21 mg/ml) with assembly-competent phosphocellulose-purified tubulin (1.9 mg/ml) [15] at 37 °C in the presence of 1 mM-GTP. The polymerization of tubulin was monitored in a thermostatted spectrophotometer by the change of turbidity of the preparation at 350 nm. ■, MAPs-saturated NF; □, control NF; ○, tubulin alone. (b) Rat brain mitochondria (1.44 mg/ml) were incubated for 15 min at 37 °C with ^{32}P -labelled MAPs (0.2 mg/ml) (thermostable fraction, containing 80 % MAP2 and 20 % Tau) in the presence of increasing concentrations of cytochrome *c* (○), pure tubulin (◆), unlabelled MAPs (■) and each of the purified NF-H (●) or NF-M subunits (▲) purified from urea-solubilized bovine spinal cord NF and renatured by dialysis against RB as previously described [13]. The incubation buffer was RB supplemented with 0.32 M-sucrose and a mixture of protease inhibitors as described in [1,2]. After incubation, the samples were loaded onto 100 μ l of 0.5 M-sucrose in RB and centrifuged for 8 min at 12000 *g* (at 4 °C). The radioactivity of MAPs bound to the mitochondrial pellet was expressed as the percentage of the control value (mitochondria with labelled MAPs alone).

fold, respectively, after adding mitochondria, demonstrating a MAPs-dependent association of MT to mitochondria (some MAPs are already bound to purified organelles [1]). Thus, in order to assume that either pure tubulin or tubulin polymers bind mitochondria in a saturable fashion, Rendon *et al.* must provide controls showing the specific association of soluble, unpolymerized tubulin to mitochondrial membranes [5,6].

Rendon *et al.* [4] have raised an important point concerning the domain on MAPs which interacts with cellular organelles. This problem has been earlier discussed by Flynn *et al.* [8] but with regard to the interaction of MAP2 with neurofilaments (NF). These authors demonstrated the preferential binding of a 28 kDa MT-binding domain of MAP2 to NF 70 kDa subunits [8]. However, binding of the 240 kDa projection domain to the same NF was not discussed [8]. The possibility that the two domains of MAP2 (MT-binding and projection domains) can bind simultaneously to the same organelle (NF or mitochondria) is raised by the combination of results reported in [8] and in Fig. 1(a) where NF-bound MAP2 is shown to promote tubulin polymerization. This experiment (Fig. 1a) suggests that the tubulin-binding domain of MAP2 is not bound to the NF core. Furthermore, purified NF H and M subunits, which can induce the polymerization of tubulin [9], compete efficiently with MAPs for binding sites on brain mitochondria (Fig. 1b). This might result from a tubulin-like conformation of the mitochondrial MAP2 binding site (as is probably the case for the 70 kDa NF subunit [8] and for actin [10]) which could explain the observations from both groups [1–3]. However, putative MAPs binding proteins corresponding to tubulin could not be identified by Rendon *et al.* [11], or us [2].

In conclusion, our studies suggest the existence of an organelle-binding site on the projection domain in both MAP2 and the NF H and M subunits [9]. Studies similar to those recently reported by Lewis *et al.* [12] on the mechanism of MAP2 and Tau-mediated MT cross-bridging might decide this important issue.

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A common ancestor for mammalian 3β -hydroxysteroid dehydrogenase and plant dihydroflavonol reductase

The enzymic complex 3β -hydroxysteroid dehydrogenase (EC 1.1.1.145)/ Δ^5 - Δ^4 isomerase (EC 5.3.3.1), hereafter called 3β -hydroxysteroid dehydrogenase, catalyses the oxidative conversion of pregnenolone to progesterone (Fig. 1), which can be converted to various other steroids that function as glucocorticoids, mineralocorticoids, progestins, androgens, and oestrogens (Luu-The *et al.*, 1989b). As such, this enzyme is at a key branching point in the synthesis of steroids with central roles in mammalian metabolism and reproductive function. Recently, the sequences of two other steroid dehydrogenases, 17β -hydroxysteroid hydrogenase (Peltoketo *et al.*, 1988; Luu-The *et al.*, 1989a) and corticosteroid 11β -hydroxy dehydrogenase (Agarwal *et al.*, 1989), that are important in steroid metabolism, were determined and found to show similarity to each other (Agarwal *et al.*, 1989; Baker, 1990), even though they modify different classes of steroids and act as different positions in the steroid nucleus. Evidently, gene duplication and its subsequent divergence led to two proteins that are important in the synthesis and degradation of oestrogens, androgens and corticosteroids. Thus, when the sequences of human and bovine 3β -hydroxysteroid dehydrogenase were determined (Luu-The *et al.*, 1989b; Zhao *et al.*, 1989), it came as a surprise that there was no sequence similarity to either 17β -hydroxysteroid dehydrogenase or 11β -hydroxysteroid dehydrogenase, even at the most distant level. Moreover, 3β -hydroxysteroid dehydrogenase showed no similarity to other dehydrogenases, cytochrome P-450 enzymes, steroid isomerases, steroid binding proteins, or any other protein sequence, over 10000 of which are now in the protein sequence database. This left the ancestry of 3β -hydroxysteroid dehydrogenase unelucidated.

Recently, we uncovered a clue to resolving this puzzle, after completing a search of an updated version of the GenBank database for sequences with similarity to 3β -hydroxysteroid dehydrogenase. This search revealed a novel similarity between 3β -hydroxysteroid dehydrogenase and barley A1 protein, an NADPH-dependent reductase that converts dihydroquercetin to *cis*-leucocyanidin (Fig. 1) in barley (Rohde *et al.*, 1987). Homologous dihydroflavonol reductases in maize (Schwarz-Sommer *et al.*, 1987) and snapdragons (Coen *et al.*, 1986) are used to form pigments from flavonoid precursors (Reddy *et al.*, 1987). The sequence similarity between bovine β -hydroxysteroid dehydrogenase and residues 3–232 of barley A1 protein is sufficiently strong to suggest that these mammalian mammalian and plant enzymes are derived from a common ancestor.

Fig. 2 shows the alignment of residues 8–236 of bovine 3β -hydroxysteroid dehydrogenase and residues 3–232 of barley A1 protein. There are 55 matches out of a possible 227 matches (24%) and 29 conservative replacements (13%), with five gaps added by the computer to maximize the alignment between the two proteins. The ALIGN (Dayhoff *et al.*, 1983) comparison score between these proteins is 10.25 standard deviations higher than that of 1000 comparisons of randomized sequences of these

Fig. 1. (a) Conversion of pregnenolone to progesterone catalysed by human 3β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 isomerase, and (b) conversion of dihydroquercetin to *cis*-leucocyanidin catalysed by barley A1 protein; the C-4 ketone is reduced to an alcohol

protein segments. The probability of getting such a score by chance is 6×10^{-25} . Bovine 3β -hydroxysteroid dehydrogenase contains 372 residues. Most, but not all, of the sequence of barley (331 residues) and maize (367 residues) A1 proteins have been determined. The full maize A1 protein sequence contains an additional 12 residues at its N-terminus and 48 residues at its C-terminus (Rohde *et al.*, 1987). Barley A1 protein is likely to be about the same length as maize A1. Thus, the region of similarity between bovine 3β -hydroxysteroid dehydrogenase and barley dihydroflavonol reductase extends over more than half of each protein, which may be due to this area binding the nucleotide cofactor that is important in both proteins' enzymic activity.

The similarity between 3β -hydroxysteroid dehydrogenase and dihydroflavonol reductase could be due to either convergent evolution or to divergence from a common ancestor. We favour the latter possibility for several reasons. First, the proteins' ALIGN comparison score of 10.25 standard deviations has probability of occurring by chance of 6×10^{-25} . An event with such low probability usually is interpreted as indicating that the two proteins are derived from a common ancestor. This interpretation is supported by additional evidence of similarity between 3β -hydroxysteroid dehydrogenase and A1 protein: these proteins have similar oxido-reductase activity and there are similarities in the structure of their substrates (Fig. 1). Moreover, as described below, there are interesting functional similarities between their substrates, flavonoids and steroids, which may shed light on the origins of intercellular communication in vertebrates (Baker, 1990).

Although it would not appear, at first glance, that the flavonoid precursors of plant pigments have any biological function in common with steroids in vertebrates, in fact, like steroids, there is evidence that flavonoids can regulate gene transcription in a variety of organisms. The best characterized examples are in bacteria that interact with plants (Djordjevic *et al.*, 1987; Firmin *et al.*, 1986; Long, 1989; Peters *et al.*, 1986; Stachel *et al.*, 1985). The dependence of flavonoid biological activity in intercellular communication between plants and rhizobia on flavonoid structure has similarities to that of steroids in vertebrates. Thus, the addition or removal of a hydroxyl group from a flavonoid, or the oxidation of a hydroxyl group to a ketone, can have important effects on a flavonoid's (i) biological potency and (ii) selectivity for different rhizobia (Firmin *et al.*, 1986). Different plant