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Hypothesis

MAP2: a sensitive cross-linker and adjustable spacer in dendritic architecture

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Microtubule-associated protein 2 (MAP2), a long, filamentous molecule thought to cross-link dendritic cytoskeleton, is rich in PEST sequences, putative signals for rapid proteolytic degradation. It is suggested that MAP2 is indeed highly susceptible to protease, e.g. calpain, attack, which is needed for a plastic change, but actual breakdown depends on the regulation of protease(s). Phosphorylation is expected to make the molecule longer and rigid, similarly to what was observed with the related tau protein. Such a structural transition may provide a mechanism for the putative role of MAP2 in dendritic branching.

Microtubule-associated protein 2 (MAP2); Calpain; PEST-sequence; Protein phosphorylation; Neuronal plasticity; Dendritic branching

1. INTRODUCTION

Microtubule-associated protein 2 (MAP2) has been known for many years as a high-molecular weight ($M_{r,app}$ ~280 kDa), filamentous, heat-stable, neuron-specific protein, which along with other MAPs adheres to microtubules when they are prepared from mammalian brain extract by repeated assembly–disassembly of tubulin dimers. Early studies have distinguished two domains in MAP2: a short segment binding to microtubules through which MAP2 promotes tubulin polymerization, and a long projection arm, which harbours cAMP-dependent protein kinase (PKA) via binding the regulatory (R) subunit [1]. The projection arm was thought to mediate interactions with other cytoskeletal elements, such as neurofilaments [2] and actin [3]. Since high-M_r MAP2 is confined in neurons to the cell body and dendrites, it has become generally accepted that MAP2 is a cross-linker and thereby stabilizer of dendritic architecture [4,5]. Models of neuronal plasticity have invoked cytoskeletal rearrangements in which this cross-linking function of MAP2 is proposed to be transiently abolished [6,7].

A major advance toward understanding MAP2 and its function came in the late 1980s, when first the mouse MAP2 [8], then the rat MAP2b [9] was cloned and sequenced. (In the rat, high-M_r MAP2 has two closely related forms, *a* and *b*.) Partial sequences of human MAP2 have also been published [10]. The true M_r of these molecules proved to be much lower than estimated from SDS-PAGE: mouse MAP2 consists of 1828 amino acids and has M_r = 199 kDa. In addition to the high-M_r forms, a low-M_r form, MAP2c, has also been identified which earlier was referred to as 'young tau slow' [11] with $M_{r,app}$ = 70 kDa. MAP2c has a true M_r = 42.3 kDa, and is produced by altnernative splicing of the message from the single MAP2 gene [12]. MAP2c is a juvenile form abundant during embryonic life and disappears postnatally around the time of synaptic maturation, when in turn the synthesis of high-M_r MAP2a begins [4]. This switch-over may be rationalized by assuming that while neurite, and concomitant microtubule, growth is adequately promoted by the short MAP2c and other MAPs, for the dendritic (synaptic) structures to become mature the long MAP2 with its extensive cross-linking ability is needed [7,13]. With respect to function, it should also be considered that MAP2c is more ubiquitous than high-M_r MAP2, as it has been found in axons and glia as well (cf. [14]).

In the primary structure of high-M_r MAP2 several functional domains could be recognized (cf. Fig. 1). (i) Three imperfect repeats near the C-terminus, which would bind to three adjacent tubulin dimers within the microtubule [8]. (ii) A segment of about 30 amino acids near the N-terminus that binds the RII-subunit of PKA [15,16]. (iii) A putative calmodulin-binding domain [9]. (iv) A domain on the C-terminal side of the tubulin-binding segments, which seems responsible for the bundling of microtubules, i.e. the parallel stacking of microtubule arrays with a spacing of 20 nm mediated by a

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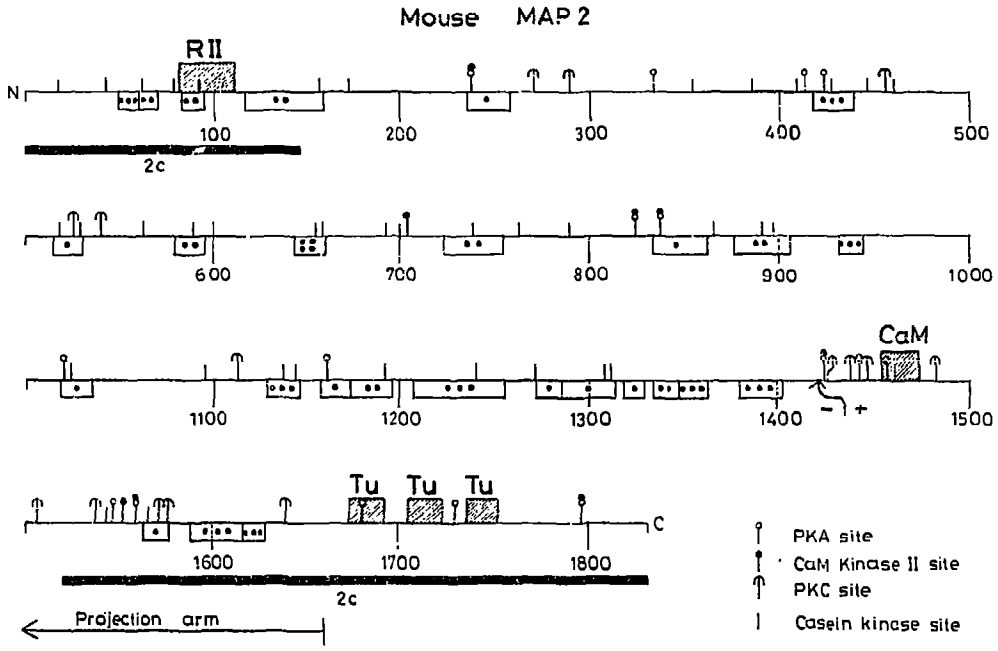

Fig. 1. Scheme of primary structure of mouse MAP2. The linear structure of the molecule is rendered in four rows, from the N-terminus (top left) to the C-terminus (bottom right), the residue numbers being periodically indicated; the overall length is 1828 amino acids. Heavy lines denote sequences constituting MAP2c. Hatched bars are binding domains for the regulatory subunit of PKA (RII), calmodulin (CaM) and tubulin (Tu). Open boxes underlie PEST sequences, the number of dots inside the boxes indicate strength (1, 2, 3 and 4 dots: correspond to PEST scores 0–5 (weak), 5–10 (medium), 10–20 (strong), and >20 (very strong), respectively. Putative protein kinase sites for the four major kinase classes are indicated by different symbols. The approximate position (residue ~1420) of transition from predominantly negative to positive net charge is marked by a curved arrow.

C-terminal part of two MAP2 molecules [17,18]. The developmental 'genetic engineering' that produces MAP2c is quite remarkable: MAP2c contains a short stretch from the N-terminal part of high-M_r MAP2 comprising the R-subunit binding site and the C-terminal end accommodating the microtubule-binding domains and the bundling segment, but lacks the major part of the projection arm.

The strong binding site for the R-subunit of PKA offers a mode for the cross-linking of two MAP2 molecules. Namely, the regulatory subunit exists in vivo as a stable dimer, R_2 (cf. [19]), which means two binding sites located in the 50-residue N-terminal segment of RIIβ [20] for MAP2 N-termini. This would create another way of cross-linking microtubules via the long projection arms, in addition to the close packing by bundling. Cross-linking with actin filaments may also occur via these interactions, since actin apparently binds to MAP2 at the tubulin-binding domains [21].

2. ABUNDANCE OF PEST-SEQUENCE IN MAP2 SUGGESTS HIGH PROTEOLYTIC SENSITIVITY

Rogers et al. [22] have put forward evidence that sequences in proteins, 8 or more residues long, rich in Pro (P), Glu (E) or Asp, Ser (S), and Thr (T), and flanked by basic residues are signals for proteolytic degradation. These PEST-sequences are negatively charged, which can be further enhanced by phosphorylation of the Ser and Thr side chains. The strength of a PEST-sequence is characterized by a PEST-score. Proteins with one or more strong PEST-sequences tend to have short half-lives in vivo.

Wang et al. [23] suggested that PEST-sequences are recognition sites for calpain, the Ca^{2+}-activated neutral protease. The negatively charged clusters may bind to a complementary area in calpain or may bind Ca^{2+}, which would locally activate calpain. According to this hypothesis substrate recognition by calpain would not be exercised by the active site of the enzyme, and indeed, attempts to characterize the substrate specificity of calpain in terms of the residues around the scissile bond have led to ambiguous results [24]. Nevertheless, the role of calpain in PEST-induced proteolysis has not been convincingly demonstrated, while the original PEST-hypothesis seems to be corroborated by accumulating evidence [25,26].

We analyzed the primary structures of MAP2 and other MAPs for PEST sequences (Table I). These proteins are extremely rich in such structural motifs. If the sum total of PEST-scores, distributed over several sequences, is taken, then MAP1b and MAP2 far exceed all other proteins analyzed so far. It is not clear, however, whether the sum total of PEST-scores is a meaningful quantity. It may well be that a single strong

PEST-sequence in a protein is sufficient to evoke rapid breakdown. Furthermore, the longer a polypeptide chain, the greater the chance to accommodate PEST-sequences and to attain higher sum-totals. The normalized PEST-score values in Table I show that, indeed, the 'density' of PEST in rat tau is about twice as great as in *Drosophila* MAP 205. Nevertheless, the various MAPs have significantly higher normalized PEST-scores than have other, highly unstable proteins, such as the proto-oncogene c-myc and c-fos proteins. Thus there is a relative enrichment of PEST sequences in MAPs. However, even without any relative enrichment, the multiplicity of PEST-sequences in a long polypeptide chain is expected to endow the molecule with enhanced proteolytic susceptibility: the longer the polypeptide, the greater the chance that it will be cleaved.

Following the above reasoning, high-M_r MAPs, and thus MAP2, are expected to be extremely sensitive to protease, possibly calpain, cleavage. The data from other proteins taken at face value [25], MAP2 would have a half-life in the order of minutes. Can such a degree of instability be reconciled with a structural stabilizing role?

Table I

PEST-scores of some proteins

Protein	Number of amino acids in protein	Number of PEST-sequences	PEST-score		
			Top-scored sequence	Sum total	Normalized
MAP1b (mouse brain)	2464	44	28.1	395.1	1.60
MAP2b (rat brain)	1830	32	20.3	267.2	1.46
MAP2 (mouse brain)	1828	27	20.3	233.9	1.28
MAP2-205 (*Drosophila*)	1163	20	22.5	120.5	1.04
MAP2c (rat brain)	467	7	17.2	68.1	1.46
tau (rat brain)	432	8	24.9	94.6	2.19
tau_1 (mouse)	364	7	16.8	61.1	1.68
Ca^{2+}-ATPase (human erythrocyte)	1220	9	21.3	94.5	0.77
RII, protein kinase A (bovine heart)	379	3	14.8	28.6	0.75
c-myc (human)	440	7	25.4	36.3	0.83
c-fos (human)	380	6	10.1	20.2	0.53
Tubulin (rat brain)	451	1		13.7	0.30
(human brain)	444	1		10.5	0.24

PEST-sequences were located and their scores calculated by the PEST-FIND algorithm [22]. PEST scores lower than 0 were disregarded, normalized scores are the sum total scores per 10 amino acid residues. Protein sequences were taken from the PIR database.

Before attempting to answer this question, some caveats must be made. The half-life data obtained experimentally [25] need not apply to neurons and to the dendritic compartment therein. In fact, MAP2 injected into cultured neurons could be detected over several days [27]. The proteolytic machinery recognizing PEST-sequences may not coexist with MAP2. However, if it is the calpain–calpastatin system as suggested [23], then dendritic coexistence can hardly be questioned [28,29]. Another consideration is that while MAP2 has little ordered structure [30], and therefore intramolecular steric hindrance to PEST-sequences is unlikely, many of its PEST-sequences may be masked by interacting other proteins. In vitro MAP2 is degraded rapidly by calpain [31,32].

We suggest that MAP2, by virtue of its high PEST-sequence content, is a protein highly sensitive to proteolytic attack: whenever a plastic change involving proteolysis in the dendrites occurs, MAP2 is one of the prime targets. A hypothetical – or genetically engineered – protease-resistant MAP2 might hinder the plastic transformation. The high proteolytic susceptibility is, however, only a potential: cleavage obviously requires an active protease around. It is probably the tight regulation of the proteolytic apparatus, as expected for the calpain–calpastatin system, that determines the fate of MAP2.

3. MAP2 AS A DENDRITIC SPACER ADJUSTABLE BY PHOSPHORYLATION

In the foregoing we have considered MAP2 as a cross-linker of dendritic cytoskeleton and implied that cross-links confer stability to the intracellular scaffolding. However, such a function is not trivial, as in the case of cross-links by disulfide bonds or isopeptide bonds formed by transglutaminase. The very length of MAP2 prompts the question: is it confined to such a passive function or does it play a more active role in morphogenetic and plastic processes?

The tau protein, one of a family of low-M_r, heat-stable MAPs, has recently been shown to undergo a major structural change upon phosphorylation. By analyzing the spacing in paracrystals of the protein Hagestedt et al. [33] found that phosphorylation may increase the length of the molecule by as much as threefold and renders the molecule rigid; dephosphorylation in turn shortens it and makes it flaccid. Apparently, the introduction of negative charges brings about these changes via electrostatic repulsion. In the net charge profile of rat tau (Fig. 2B) there is a major sharp transition from overall negative to overall positive at around residue 115 in the sequence. In the dephosphorylated state, when the illustrated charge conditions prevail,

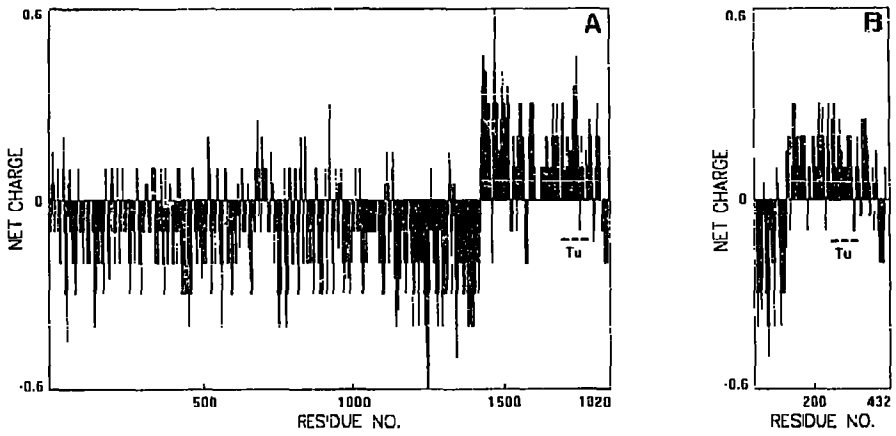

Fig. 2. Net charge profile of mouse MAP2 (A) and rat tau (B). The average charge per residue was calculated by using a scanning window of 10 residues. The tubulin-binding domains (Tu) are indicated by horizontal lines.

oppositely charged segments are likely to interact with each other. Phosphorylation in positively charged segments may open these folds extending the molecule. In the net charge profile of mouse MAP2 (Fig. 2A) there is also a sharp transition at around residue 1420, which is about the same position as in tau in relation to the tubulin-binding sites. There are several putative phosphorylation sites, in two major clusters, on the positive side of MAP2 (Fig. 1). In tau all sites for the four protein kinase classes considered in Table II are located in the positively charged C-terminal portion and apparently the phosphorylation of a single site by Cam-kinase II elicits the remarkable structural change [34]. By analogy to tau, we may assume that upon phosphorylation of some site(s), MAP2 also becomes longer and stiff.

Although the phosphorylation sites shown in Fig. 1 are putative, assigned on the basis of consensus sequences for substrate recognition by the four major types of kinases [35], the agreement between the number of sites predicted and found experimentally is satisfactory (Table II). Furthermore, there are many more Ser/Thr residues in MAP2 (altogether 326 Ser + Thr in the mouse protein), as well as there being more exotic kinases that have been reported to act on MAP2 (cf. [36]). Thus there is ample possibility for introducing phosphate groups, though a major part of these is likely to constitute a fairly stable, basal phosphorylation [37]. There is probably a concerted action of various kinases on MAP2, and its state of phosphorylation is poised such that stimuli bring about the appropriate structural transitions by minimal changes in phosphate groups.

Based on the foregoing, we can conceive MAP2 as a spacer whose length and rigidity can be changed in a graded manner by the orchestration of second messenger-regulated kinases, and also phosphatase(s) [38]. Such a scenario could be instrumental in morphogenetic/plastic phenomena. One may envisage that in a growing neurite, microtubule bundles divide if their co-assembled MAP2s 'stretch out', i.e. the flaccid projection arms become longer and stiff, pushing the bundles apart and initiating a dichotomy of the growing process (Fig. 3). Although this model is speculative, it is in line with several observations. (i) In rat cerebellar Purkinje cells high-M_r MAP2 accumulates at dendritic branchings [39]. (ii) The dendrites of mature olfactory receptor neurons contain MAP2c and tau instead of high-M_r MAP2, and these dendrites are unbranched and club-like [14,40]. (iii) Neuroblastoma cells induced to differentiate sprout thin, long, straight and unbranched pro-

Table II

High-M_r MAP2 phosphorylation sites

Enzyme	Number of sites		Ref.
	Predicted for mouse	Measured	
Protein kinase A	15	12–14	[42,43,44]
common	5	4	
CaM kinase II	8	7	[45]
Protein kinase C	16	15	
Casein kinases	47	40	[42]
	86		

References are related to the measured values obtained with MAP2s from various mammals. Prediction was based on the following kinase substrate site recognition motifs [35]:

Protein kinase A: [KR] (0,2) [KR] (1,2) S*
Protein kinase C: [ST]* (1,2) [KR] (0,2) [KR]
CaM-kinase II: RXX [ST]*
Casein kinases I and II: EXXS* and *SXXE, respectively.

Capital letters are standard one-letter amino acid codes, X denotes any amino acid. Amino acids within square brackets represent alternatives; numbers enclosed in brackets are the minimal and maximal numbers of intervening amino acids between two positions. An asterisk marks the phosphorylatable Ser or Thr.

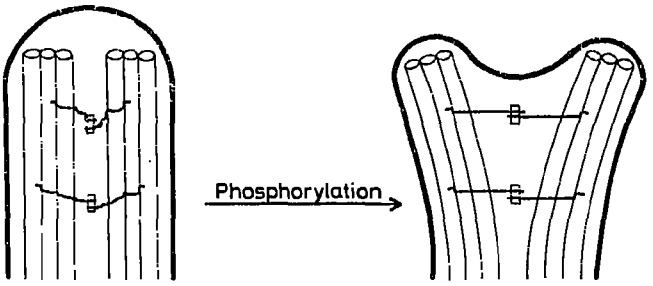

Fig. 3. Tentative mechanism for dendritic branching assisted by the extension of MAP2 upon phosphorylation. In the scheme microtubule bundles are held together by the C-terminal segments of MAP2 molecules. The N-termini of two MAP2s are assumed to be cross-linked by an intervening protein, e.g. the regulatory subunit dimer of PKA.

cesses when the inducer is NGF or retinoic acid, but produce curved, thick and heavily branched neurites when induced with dibutyryl-cAMP, an activator of PKA [41].

4. CONCLUSIONS

We have considered various functional aspects of MAP2 in light of its structural properties, adopting the PEST-hypothesis and suggesting mechanisms based on analogy with another MAP, the tau protein. Although the functions canvassed are conjectural, they are based on available evidence and, importantly, can be checked experimentally. MAP2 in its different phosphorylational states is expected to be an appropriate substrate to test whether PEST-sequences could serve as calpain substrate recognition motifs, by localizing cleavage points vis-à-vis PEST-sequences, an approach that would be cumbersome with globular proteins. The adjustable spacer function and its involvement in dendritic branching can be tested with engineered MAP2 constructs in transfected differentiating cells. Though MAP2 is devoid of a biological activity as ostentatious as, say, protein kinase activity, it would be mean to underestimate the potential of this developmentally and structurally versatile protein in serving a variety of functions in neuronal biology.

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