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Identification of a novel microtubule-binding domain in microtubule-associated protein 1A (MAP1A)

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SUMMARY

Several microtubule-associated proteins (MAPs) have been shown to bind to microtubules via short sequences with repeated amino acids motifs. A microtubule-binding domain has hitherto not been defined for the adult brain microtubule-associated protein 1A (MAP1A). We have searched for a microtubule-binding domain by expressing different protein regions of MAP1A in cultured cell lines using cDNA constructs. One construct included an area with homology to the microtubule-binding domain of MAP1B (Noble et al. (1989) *J. Cell Biol.* 109, 437-448), but this did not bind to microtubules in transfected cells. Further investigation of other areas of MAP1A revealed a protein domain, capable of autonomously binding to microtubules, which bears no homology to any previously described microtubule-binding sequence. This MAP1A domain is rich in charged amino acids, as are other mammalian microtubule-binding domains, but unlike them has no identifiable sequence repeats. Whereas all previously described mammalian microtubule-binding domains are basic, this novel microtubule-binding domain of MAP1A is acidic. The expression of this polypeptide in cultured cell lines led to a rearrangement of the microtubules in a pattern distinct from that produced by MAP2 or tau, and increased their resistance to treatment with the microtubule depolymerising agent nocodazole. When the MAP1A microtubule-binding domain was co-expressed in cultured cell lines together with MAP2c, the MAP1A microtubule-binding domain was able to bind to the MAP2c-induced microtubule bundles. These results suggest that different microtubule-binding sequences have a common ability to stabilise microtubules but differ in their influence on microtubule arrangement in the cell. This may be significant in neurons, where microtubule-associated proteins with different microtubule-binding sequences are expressed in different cell compartments and at different times during development.

Key words: MAP1A, MAP1B, microtubule-associated protein, microtubule, neuronal morphology, development, cytoskeleton stabilisation

INTRODUCTION

Microtubule-associated protein 1A (MAP1A) is the largest brain microtubule-associated protein with an apparent molecular mass of 350 kDa. In the fetal rat brain MAP1A is barely detectable, but after birth its level of expression increases steadily up to postnatal day 20, after which it remains expressed at high levels through adult life (Matus and Riederer, 1986; Riederer and Matus, 1985; Schoenfeld et al., 1989). On the basis of this pattern of expression it has been suggested that MAP1A may be important for the stabilisation of the adult neuronal cytoskeleton (Garner et al., 1990; Matus, 1988). MAP1A is related to another structural microtubule-associated protein, MAP1B, by sequence similarities in the amino- and carboxyl-terminal ends of the molecules (Langkopf et al., 1992). In addition, MAP1A and MAP1B co-purify with three light chains LC1, LC2 and LC3 of molecular masses of 34, 28 and 18 kDa, respectively (Kuznetsov et al., 1986; Vallee and Davis, 1983). Recently it was shown that LC1 and LC2 are encoded within the 3′-terminal regions of the MAP1B and MAP1A mRNAs, respectively (Hammarback et al., 1991; Langkopf et al., 1992). These similarities, together with the fact that MAP1B expression is higher in the embryonic than in the adult brain, suggest that they are related gene products conferring different properties on microtubules that are appropriate to either growing (MAP1B) or stable (MAP1A) neuronal processes (Garner et al., 1990; Matus, 1988; Schoenfeld et al., 1989).

MAP1B binds to microtubules via a basic domain close to the N terminus of the molecule, containing 19 repeats of the motif KKE, and this region alone appears to be responsible for MAP1B microtubule-binding in vivo and in vitro (Noble et al., 1989). This indicates that in MAP1B the light chain, contained at the C terminus (Hammarback et al., 1991) is not essential for binding to microtubules and its functional role in MAP1A and in MAP1B remains unclear. The mechanism by which MAP1A binds to microtubules is not known, but it has been suggested that a basic region in MAP1A containing 3 repeats of the KKE motif could be the MAP1A microtubule-binding domain (Langkopf et al., 1992). To investigate further these questions we have expressed MAP1A cDNA constructs in vivo by transfecting cultured non-neuronal cells. Initially we

examined the MAP1A region containing the three KKE repeats that shows similarity to the MAP1B microtubule-binding domain. However, expressed protein from this region did not bind to cellular microtubules. Further experiments implicated another region of the molecule that has no sequence homology to the other mammalian microtubule-binding domains described to date. Like other known microtubule-binding domains it is rich in charged amino acids, however it contains no identifiable repeated motifs. Moreover, the MAP1A microtubule-binding region is acidic, unlike other previously described mammalian binding domains, all of which are basic.

MATERIALS AND METHODS

cDNA cloning and sequencing

Poly(A)$^+$ RNA from three adult rat brains was extracted using the guanidinium isothiocyanate/caesium chloride method (Glisin et al., 1974; Sambrook et al., 1989) followed by oligo dT-cellulose column chromatography. Double-strand cDNA synthesis was primed with oligo-dT linker-primer. The cDNA was inserted in *Eco*RI- and *Xho*I-digested λZAPII vector (Stratagene, La Jolla, CA, USA). This adult rat brain oligo-dT-primed cDNA library was probed with the overlap region of the previously described MAP1A clones 37b and 9a (Garner et al., 1990). The MAP1A cDNA clones obtained were used to screen a commercial adult rat brain oligo-dT + random primed cDNA library (Stratagene no. 936515). This resulted in the isolation of a series of overlapping MAP1A cDNA clones, which were sequenced in both strands using the dideoxynucleotide chain termination method (Sanger et al., 1977). The sequence information obtained agrees with that recently reported by Langkopf et al. (1992).

Expression constructs

A 4 kb MAP1A cDNA clone was used to produce expression construct N-termSS1. Other expression constructs were made by PCR amplification with *Pfu* DNA polymerase (Stratagene), which produces high-fidelity sequence copies (Lundberg et al., 1991). The primers used for the amplification added an *Eco*RI site at the 5′ end and a *Cla*I site at the 3′ end for directional cloning. The selected cDNA sequences were inserted into pBact-myc, a eukaryotic expression vector that adds an 11 amino acid epitope tag in-frame at the N terminus of the synthesised polypeptide, expressed under the control of the chicken β-actin promoter (Cravchik and Matus, 1993). The PCR amplifications were performed in 100 μl reactions, using 100 ng of template DNA and 20 pmols of each primer. After 5 minutes of denaturation at 95°C followed by 5 minutes of hybridisation at 60°C, 2.5 units of *Pfu* DNA polymerase were added to the reaction to avoid primer DNA degradation by the exonuclease activity of *Pfu* DNA polymerase. The extension was then performed at 75°C for 2 minutes, followed by 24 cycles of 30 seconds denaturation at 95°C, 30 seconds hybridisation at 60°C and 2 minutes extension at 75°C. The reaction was carried out in Stratagene's incubation buffer 1. The amplified fragments were blunt-end ligated into pBluescript (Stratagene), excised with *Eco*RI and *Cla*I and directionally inserted into pBact-myc. The complete coding sequence of rat MAP2c was subcloned into the pBact-16 vector, which contains the chicken β-actin promoter as previously described (Meichsner et al., 1993).

Cell transfection and immunofluorescence

HeLa cells were cultured in Dulbecco medium supplemented with 5% fetal calf serum, in a 5% CO_2 humidified atmosphere. Cells were transfected using the calcium phosphate method (Chen and Okayama, 1987), and fixed 48 hours after transfection by immersion into a methanol-dry ice bath at −75°C for 3 minutes. Detergent extraction was performed by washing the cells with 0.1% Triton X-100 in microtubule stabilising buffer prior to fixation (Solomon et al., 1979). Microtubule stability was tested by adding nocodazole (Janssen Chimica, Brüggen, Germany) to a final concentration of 2.5 μg per ml and incubating the samples for 30 minutes to 2 hours. After fixation the cultures were washed with PBS and blocked with 5% bovine serum albumin in PBS. The cultures were immunostained by incubation with GE10, a monoclonal antibody against the human c-myc epitope (Evan et al., 1985) obtained from the European Collection of Cell Cultures (Porton Down, UK). Double-labelling was performed with rabbit polyclonal antibodies against tubulin (a gift from Dr C. Bulinski) or with a rabbit polyclonal serum raised against a synthetic peptide sequence from the 14 C-terminal amino acids of MAP2 for cultures co-transfected with MAP2c (kindly supplied by B. Ludin). Rhodamine-labelled goat anti-mouse and fluorescein-conjugated goat anti-rabbit IgG antibodies (Jackson ImmunoResearch, Milan Analytica, La Roche, Switzerland) were used to detect the primary antibodies by immunofluorescence. Photography was carried out with short exposure times to avoid bleed-through fluorescence channels.

Confocal and electron microscopy

Confocal microscopy was performed with a Bio-Rad MRC-600 laser scanning system. The 2-D laser scanning images were analysed with the CoMOS software package and 3-D reconstruction images were obtained with Thru-View software. For whole-mount electron microscopy the cells were plated and transfected on carbon/Formvar-coated gold 'finder' grids. Prior to fixation the cells were permeabilised with 0.125% glutaraldehyde and 2% Octyl-POE (Bachem, Bubendorf, Switzerland) in microtubule stabilising buffer for 5 minutes. Fixation was performed in 1% glutaraldehyde in stabilising buffer for 15 minutes, followed by thorough washing with PBS and quenching the glutaraldehyde with 0.5 mg/ml $NaBH_4$ for 10 minutes. The cultures were immunostained by incubation with anti-myc monoclonal antibody, followed by rhodamine-conjugated goat anti-mouse secondary antibody. Transfected cells were identified by immunofluorescence and localised with the grid built-in orientation marks. The cell cultures were negatively stained for electron microscopy with 1% uranyl formate (Small, 1988) and transfected cells were located using the grid orientation marks.

RESULTS

MAP1A potential functional domains

MAP1A has been shown to bind directly to microtubules in neuronal cells (Shiomura and Hirokawa, 1987) and in vitro (Pedrotti et al., 1993), therefore the MAP1A deduced amino acid sequence was searched for potential tubulin-binding domains. This search was based on previous observations that in all other mammalian MAPs the tubulin-binding domains are basic regions containing repeated motifs rich in charged amino acids. In the case of MAP2, tau and MAP4 isoforms, the microtubule-binding domain consists of three or four 31 or 32 amino acid repeats (Aizawa et al., 1991; Chapin and Bulinski, 1991; Lee et al., 1988; Lewis et al., 1988; West et al., 1991) with an isoelectric point of 10. The MAP1B microtubule-binding domain contains 19 repeats of the KKE motif (Noble et al., 1989) and a pI of 9.65.

Comparing the MAP1A amino acid sequence against itself revealed two regions with self-similarity (Fig. 1), because of which we refer to them as self-similar regions 1 and 2 (SS1 and SS2). Neither of these two regions contains any highly conserved sequence motif, but both are rich in charged residues. This analysis of the MAP1A heavy chain sequence and its comparison with the protein sequence database allowed

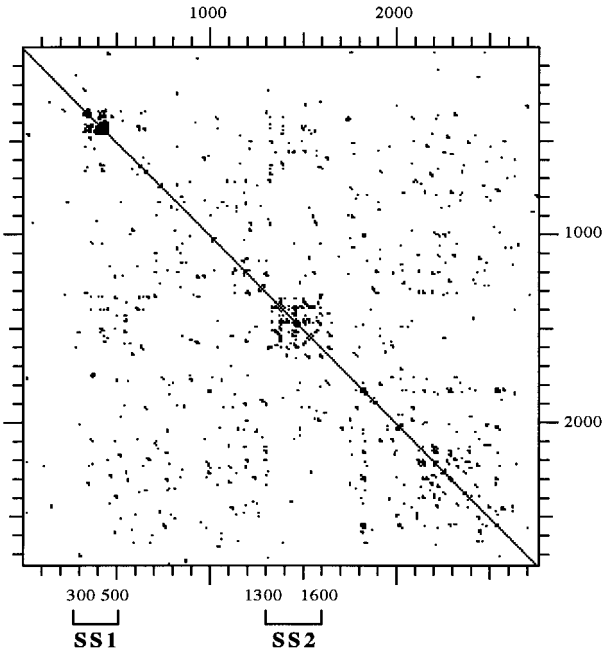

Fig. 1. Analysis of the MAP1A amino acid sequence. A dotplot matrix comparison of MAP1A with itself was performed using DNA Strider software (window, 19 amino acids, stringency, 7 amino acids). No conserved repeated sequences were found, but two protein regions with self-similarity could be identified. These regions were named self-similar 1 (SS1), between residues 300 and 500, and self-similar 2 (SS2) from amino acids 1300 to 1600.

the identification of three putative domains: the conserved N-terminal domain and the self-similar SS1 and SS2 domains (Fig. 2). The MAP1A N-terminal domain is highly conserved in the N terminus of MAP1B, the electromotor neuron-associated proteins 1 and 2 from *Torpedo californica* (Ngsee and Scheller, 1989) and in the human partial sequence EST00368 (Adams et al., 1991). The sequence conservation of the N-terminal region observed between these different genes indicates that it could have a functional significance. The conserved N-terminal domain has not been implicated in microtubule-binding (Noble et al., 1989) but it has been suggested that it could have a possible role in light chain binding (Langkopf et al., 1992). The SS1 region contains three repeats of the motif KKE, which is present in the MAP1B tubulin-binding domain and it has been recently suggested that it could represent the MAP1A tubulin-binding domain (Langkopf et al., 1992). The SS1 domain is a basic protein region with an isoelectric point of 9.61 and an electric charge at pH 7 of 18.81. The SS2 protein region of MAP1A is also rich in charged residues and broadly repetitive, but it differs dramatically from SS1 in its properties. The SS2 domain is acidic, with an isoelectric point of 4.51 and a charge at pH 7 of −29.64. The charged amino acids do not form identifiable consensus motifs but they are clustered in groups of three, four or five residues separated by irregular intervals (Fig. 3).

Expression of MAP1A cDNA constructs in vivo

To study the biological effect of the expression of these putative protein domains in vivo, we transfected the MAP1A expression constructs (Fig. 2) into cultured HeLa cells. Their expression in transfected cells was detected with a monoclonal antibody against the myc epitope tag. The c-myc epitope tag has been extensively used to localise proteins expressed from cDNA constructs (Cravchik and Matus, 1993; Meichsner et al., 1993; Munro and Pelham, 1987; Pelham, 1993) and has not been found to interfere with either the protein function or localisation.

The cDNA construct N-termSS1 (Fig. 2), containing the conserved N-terminal domain and the SS1 domain was trans-

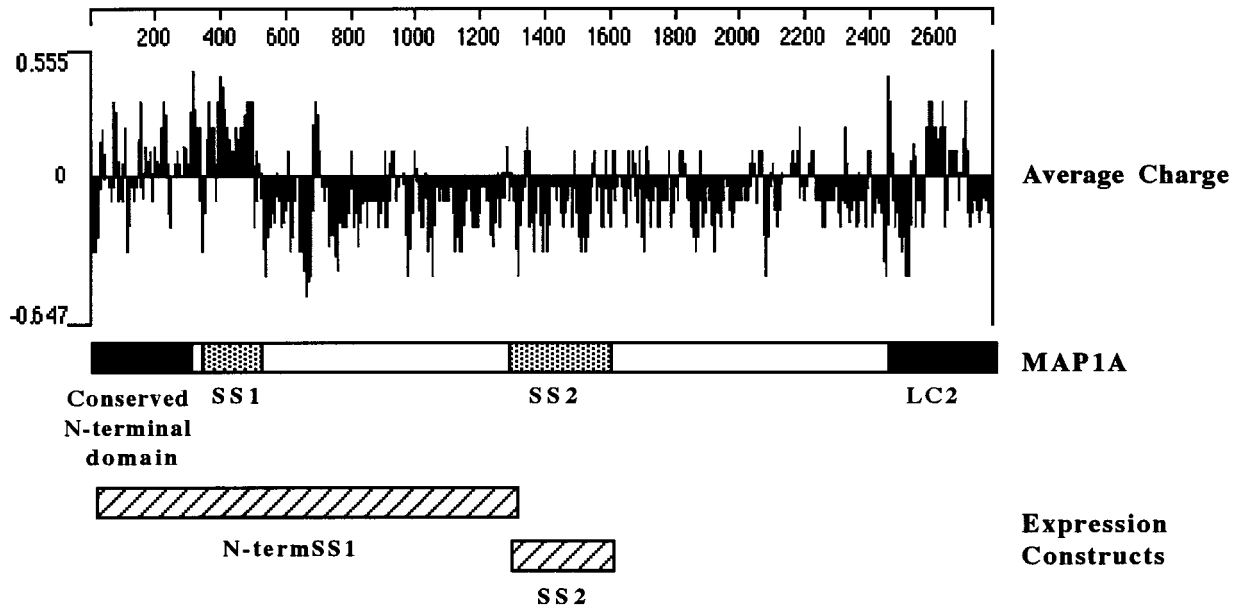

Fig. 2. MAP1A potential functional domains. Representation of the MAP1A protein domains and their average charge distribution aligned with the MAP1A expression constructs. Construct N-termSS1 included the conserved N-terminal domain and SS1 domain, and the SS2 construct contained the SS2 domain.

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1307  SPAMEDLAVEWEGKAPGKEKEPELKSETRQQKGQILPEKVAVVEQDLIIH
      QKDGALDEENKPGRQQDKTPEQKGRDLDEKDTAAELDKGPEPKEKDLDRE
      DQGQRAGPPAEKDKASEQRDTDLQQTQATEPRDRAQERRDSEEKDKSLEL
      RDRTPEEKDRILVQEDRAPEHSIPEPTQTDRAPDRKGTDDKEQKEEASEE
      KEQVLEQKDWALGKEGETLDQEARTAEQKDETLKEDKTQGQKSSFVEDKT
      TTSKETVLDQKSAEKADSVEQQDGAALEKTRALGLEESPAEGSKAREQEK   1606
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Fig. 3. Amino acid sequence of the self-similar 2 (SS2) domain, the clusters of charged residues are underlined.

fected into cultured cells but in no case could any binding to microtubules be detected (Fig. 4). On the other hand, protein synthesised from the cDNA construct SS2 bound to microtubules and rearranged them in a characteristic pattern (Fig. 5). The pattern of microtubule rearrangement induced by the MAP1A-SS2 domain is characteristic and quite different to that described for other neuronal MAPs transfected into non-neuronal cells (Goedert et al., 1991; Kanai et al., 1992; Kanai

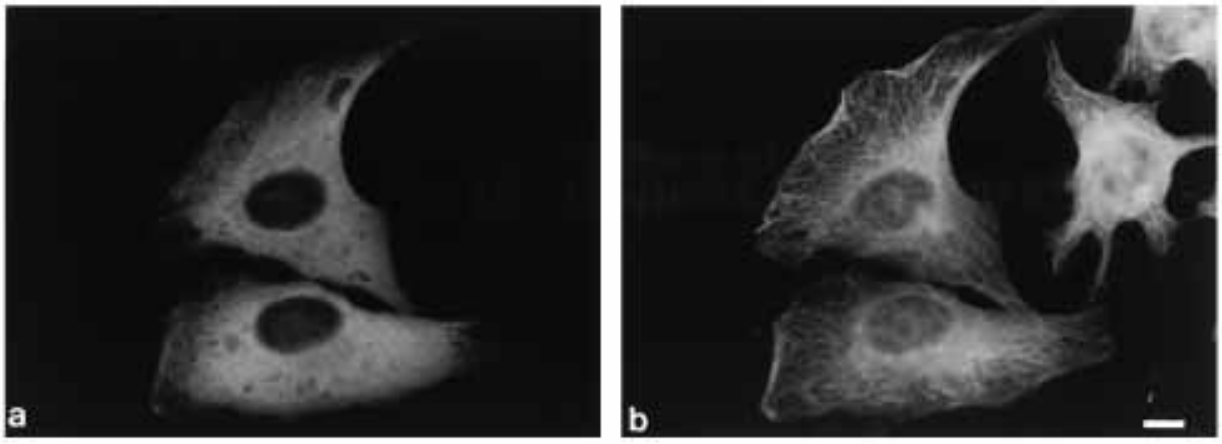

Fig. 4. The conserved N-terminal and SS1 domains do not confer microtubule-binding ability. HeLa cells were transfected with construct N-termSS1 containing the N-terminal and SS1 domains: (a) localisation of the N-termSS1 protein fragment; (b) anti-tubulin staining. No binding to cellular microtubules can be observed. Bar, 5 μm.

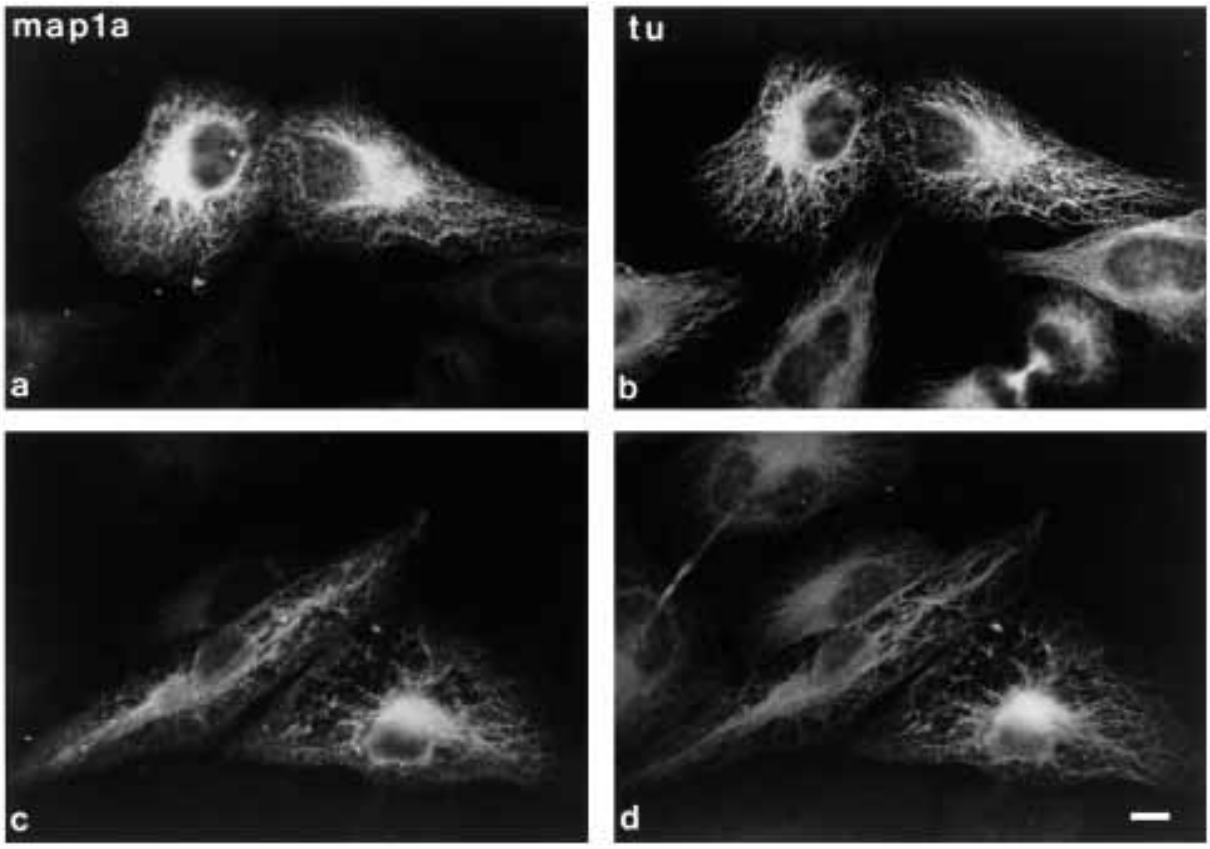

Fig. 5. The MAP1A-SS2 domain binds to microtubules. In HeLa cells transfected with construct SS2, the synthesised SS2 polypeptide co-localises with cellular microtubules and induces a minimal (a,b) or intermediate (c,d) microtubule rearrangement. (a and c) Immunolocalisation of the MAP1-SS2 protein fragment bound to microtubules; (b and d) the same cells stained with anti-tubulin antibody. Bar, 5 μm.

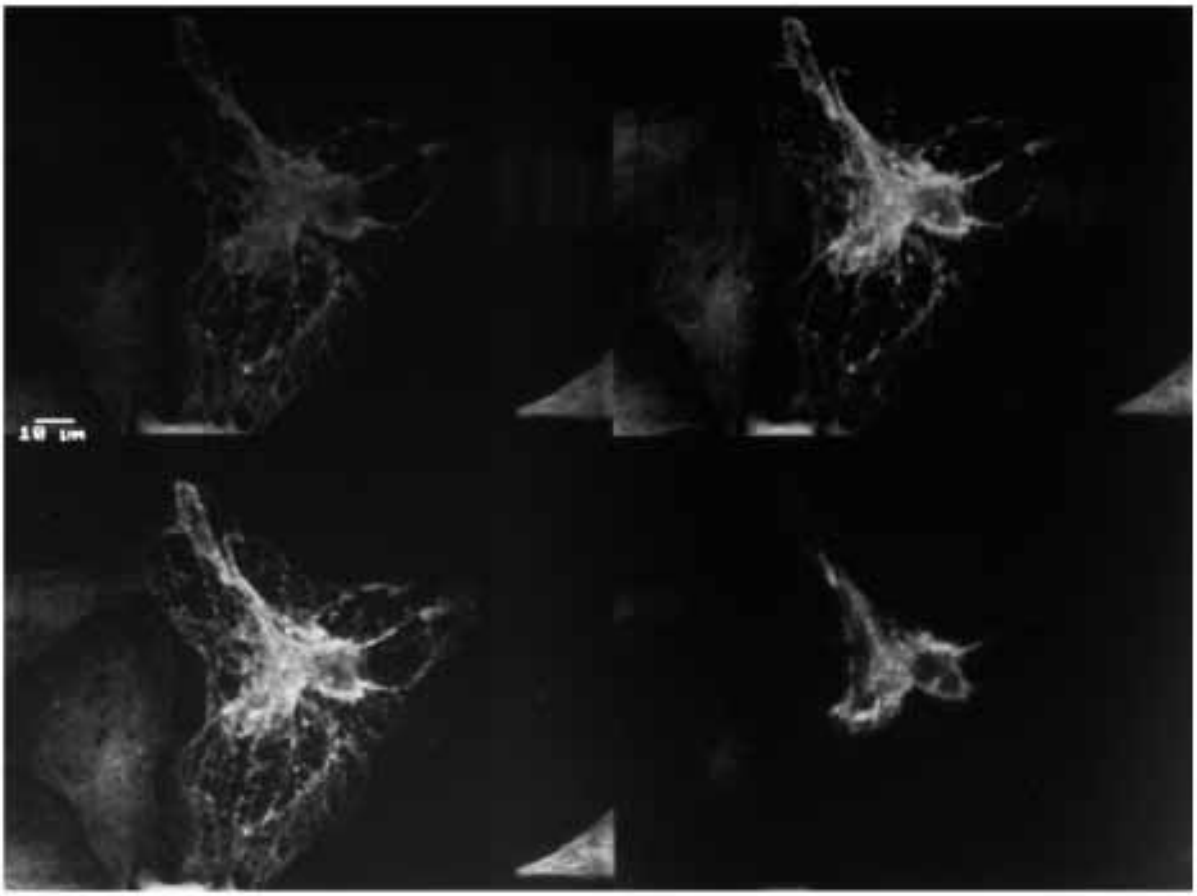

Fig. 6. Maximal microtubule rearrangement induced by MAP1A-SS2 expression. Optical sections taken by confocal laser scanning microscopy of anti-SS2 stained microtubules in SS2 transfected cells. The depth of section progresses from bottom (top left image) to top (image at bottom right) of the cell in steps of 1.8 μm. Bar, 10 μm.

et al., 1989; Lewis et al., 1989; Noble et al., 1989; Umeyama et al., 1993; Weisshaar et al., 1992) (see also Discussion). The degree of microtubular rearrangement in MAP1A-SS2-expressing cells ranged from very little (Fig. 5a) to a complete rearrangement in which the microtubules were concentrated in the perinuclear region from where short and thick bundles were extended (Figs 6, 7). The spatial conformation of the cytoskeleton of transfected HeLa cells with a maximal microtubule

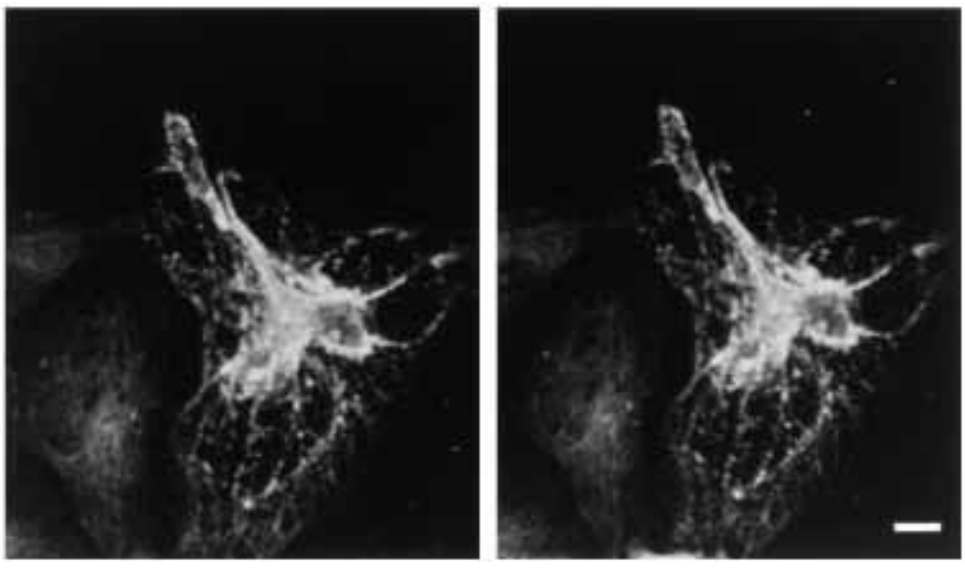

Fig. 7. Microtubule rearrangement induced by MAP1A-SS2 expression. Confocal stereo reconstruction of a SS2-transfected cell, microtubules were stained with anti-SS2 antibody. Microtubules are concentrated close to the cell nucleus from where bundles of different lengths are extended. Bar, 10 μm.

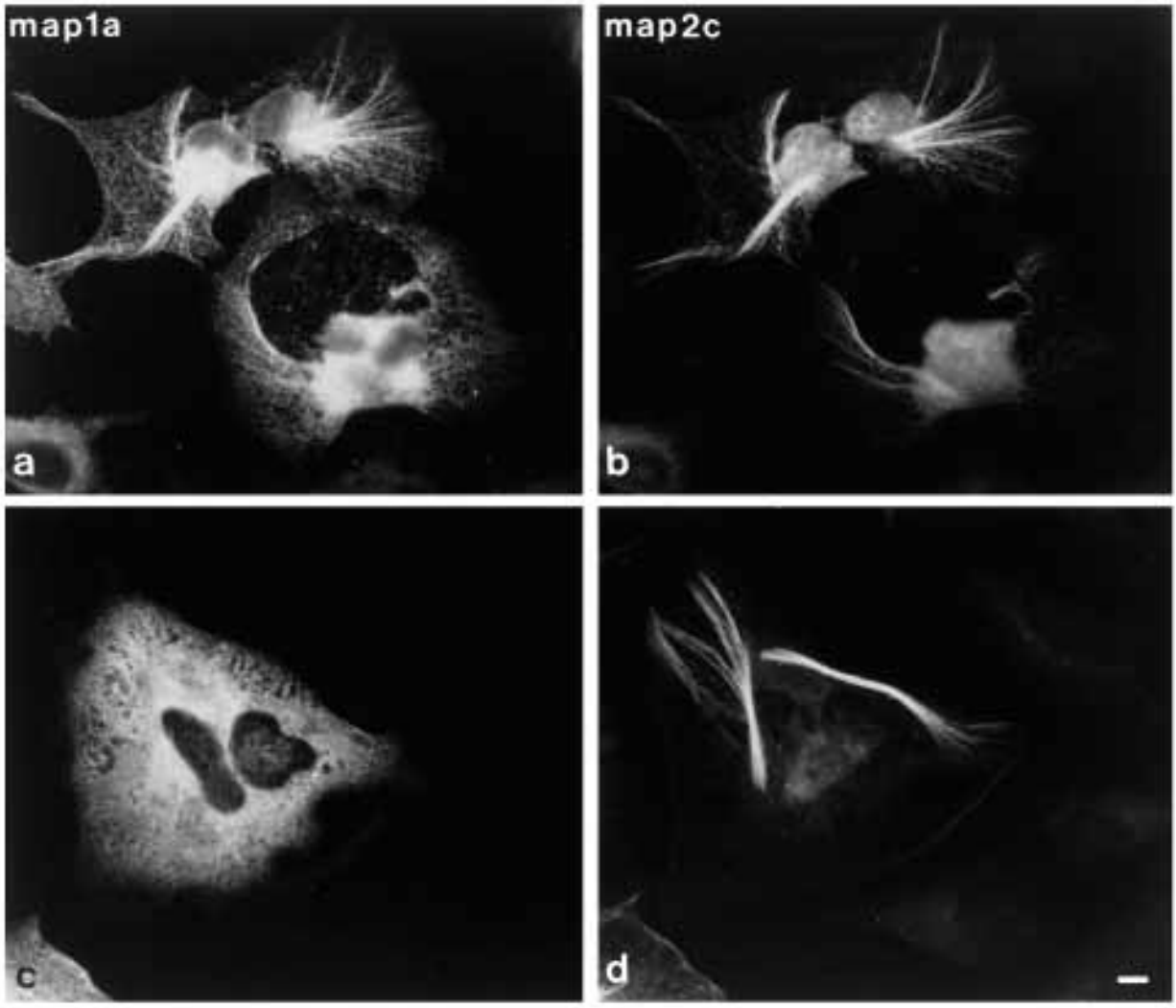

Fig. 8. Co-transfection of MAP1A fragments together with MAP2c into non-neuronal cells. The MAP1A-SS2 domain binds to MAP2c-induced microtubule bundles. Top panels show HeLa cells co-transfected with the SS2 construct and MAP2c: (a) SS2 localisation; (b) MAP2 staining. Bottom panels show cells co-transfected with N-termSS1 construct and MAP2c, no co-localisation can be observed between the N-termSS1 polypeptide and MAP2c: (c) N-termSS1 localisation; (d) MAP2 staining. Bar, 5 μm.

rearrangement induced by MAP1A-SS2 was analysed by laser scanning confocal microscopy. Optical sections from MAP1A-SS2 transfected cells taken at different depths showed that the rearranged microtubules were present throughout all the cell volume but concentrated at the perinuclear region (Fig. 6). From this perinuclear concentration of microtubules, bundles of various lengths and thicknesses were extended (Fig. 7).

To find out whether different microtubular rearranging effects of different MAPs compete with each other, we co-transfected MAP1A-SS2 and MAP2c into cultured HeLa cells (Fig. 8). The MAP2c rearrangement effect clearly predominated over the MAP1A-SS2 effect. The co-transfected cells contained long microtubule bundles typical of those produced by MAP2c, to which both MAP2c and MAP1A-SS2 were bound (Fig. 8a,b). Whereas all the MAP2c synthesised by the cell appeared bound to microtubules, some of the SS2 polypeptide remained cytoplasmic. This observation could be explained by a difference between MAP1A-SS2 and MAP2c in their ability to bind to cellular microtubules. Furthermore, when MAP1A-SS2 transfected cells were detergent-extracted in microtubule-stabilising buffer prior to fixation (Solomon et al., 1979), very little of the SS2 domain remained bound to cellular microtubules (results not shown). This was in contrast to MAP2c, which remained bound after detergent extraction, as has been shown for high molecular mass MAP2 (Lewis et al., 1988). This suggests that the binding of the MAP1A-SS2 domain to microtubules is weaker than that of MAP2c. MAP1B binding to microtubules has also been suggested to be weaker than MAP2 binding, based on their different sensitivity to detergent extraction (Noble et al., 1989). On the other hand, the cDNA construct N-termSS1, containing the conserved N-terminal domain and SS1 domain, was also co-transfected with MAP2c into HeLa cells and it was never found to bind to the MAP2c-induced microtubule bundles (Fig. 8c,d).

The stability of microtubules with MAP1A-SS2 domain bound to them was tested by treatment with the microtubule depolymerising agent nocodazole (Jacobs and Stevens, 1986; Lee et al., 1980). Despite its binding to microtubules being

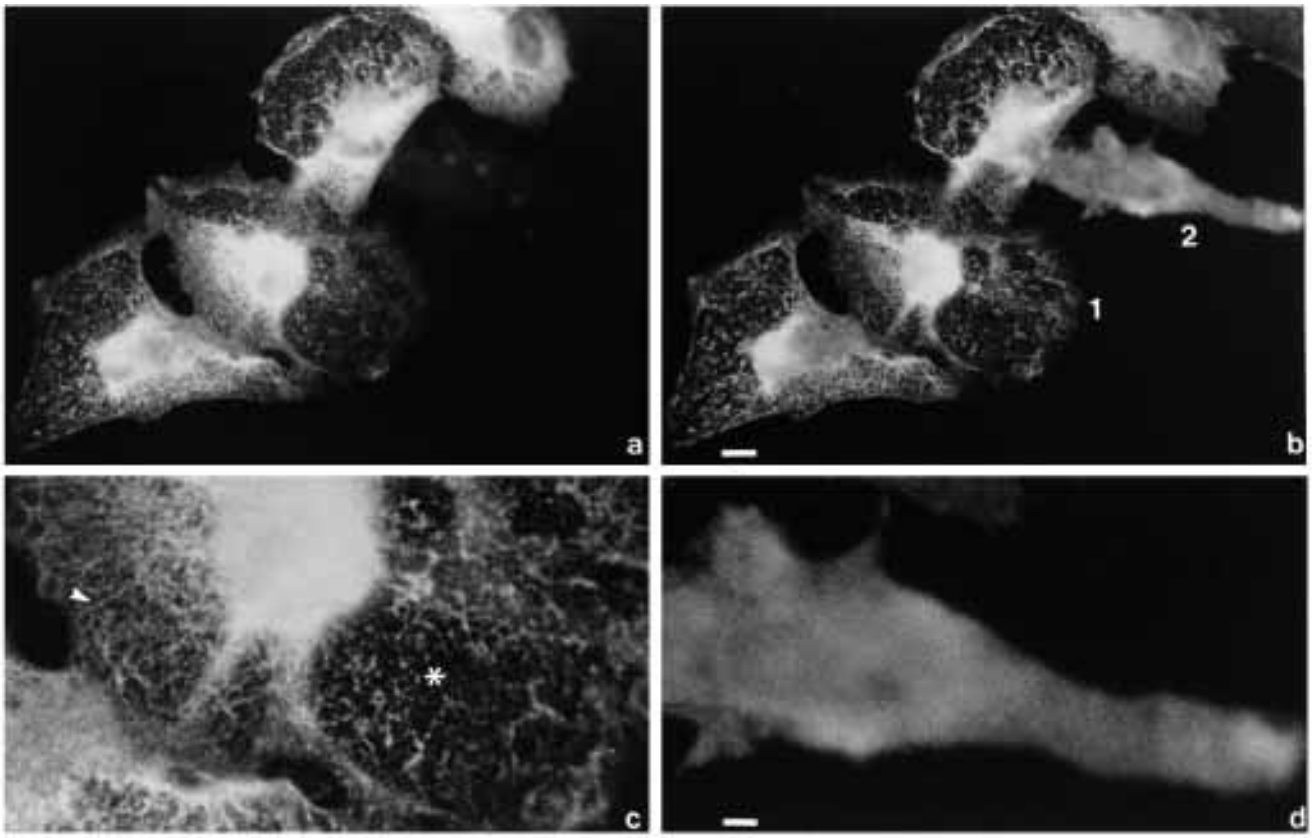

Fig. 9. MAP1A-SS2 expression increases resistance to nocodazole. HeLa cells were transfected with SS2 domain of MAP1A and treated with the microtubule depolymerising agent nocodazole (2.5 μg/ml) for 2 hours: (a) SS2 staining; (b,c,d) tubulin staining. (a,b) Differential sensitivity to nocodazole can be detected between MAP1A-SS2 transfected cells (cell 1) and non transfected cells (cell 2). (c) An enlargement of cell 1 in (a) and (b), showing long microtubules in one region of the cytoplasm (arrowhead) and fragmented tubulin filaments in another cytoplasm region (asterisk), the latter giving a punctuate staining pattern. (d) Cell 2, non-transfected, contains no detectable tubulin polymer. Bars: top panels, 5 μm; bottom panels, 13 μm.

weaker than MAP2c, the MAP1A-SS2 domain was able to stabilise the microtubules upon binding to them, as assessed by their increased resistance to depolymerisation induced by nocodazole treatment (Fig. 9). After 2 hours of nocodazole treatment, cells transfected with MAP1A-SS2 still contained microtubules decorated with the SS2 polypeptide (Fig. 9a,b), whereas non-transfected cells contained no detectable tubulin polymers (Fig. 9d). In MAP1A-SS2 transfected cells two classes of tubulin filaments could be observed (Fig. 9c): certain regions of the cytoplasm contained long microtubules (Fig. 9c arrowhead), whereas other regions contained short tubulin filaments (Fig. 9c, asterisk). This could be the result of the existence of different populations of microtubules in HeLa cells with different sensitivity to the microtubule depolymerising drug nocodazole, as it has been found in axons of cultured neurons (Baas and Black, 1990). Indeed, non-neuronal cells have been shown to contain different microtubule populations with different stability, subunit exchange rate and post-translational modifications (Schulze et al., 1987; Schulze and Kirschner, 1987). The correspondence between the residual tubulin filaments and the SS2 polypeptide in the cytoplasm indicates that the SS2-binding domain of MAP1A stabilises fragments of polymerised tubulin.

In order to obtain more information on the effect of MAP1A-SS2 over-expression on the cytoskeleton of non-neuronal cells, whole-mount electron microscopy was performed on MAP1A-SS2 transfected cells showing a maximal degree of microtubular rearrangement identified by immunofluorescence. Only microtubules close to the cell periphery could be analysed because resolution is compromised where the cytoplasm is thicker. In the thick microtubule bundles running close to the periphery of the transfected cells, the microtubules were observed to run in tight parallel arrays (Fig. 10). Parallel microtubule arrays are not normally present in non-neuronal cells, but are a characteristic of the cytoskeleton of neuronal axons and dendrites. Thus, expression of a fragment of neuronal MAP1A appears to confer some neuronal characteristics to the microtubules of HeLa cells.

DISCUSSION

A novel microtubule-binding domain in MAP1A

We have identified a protein domain in MAP1A that is able to bind to, rearrange, and stabilise microtubules when transfected into cultured cells. This protein domain is rich in charged

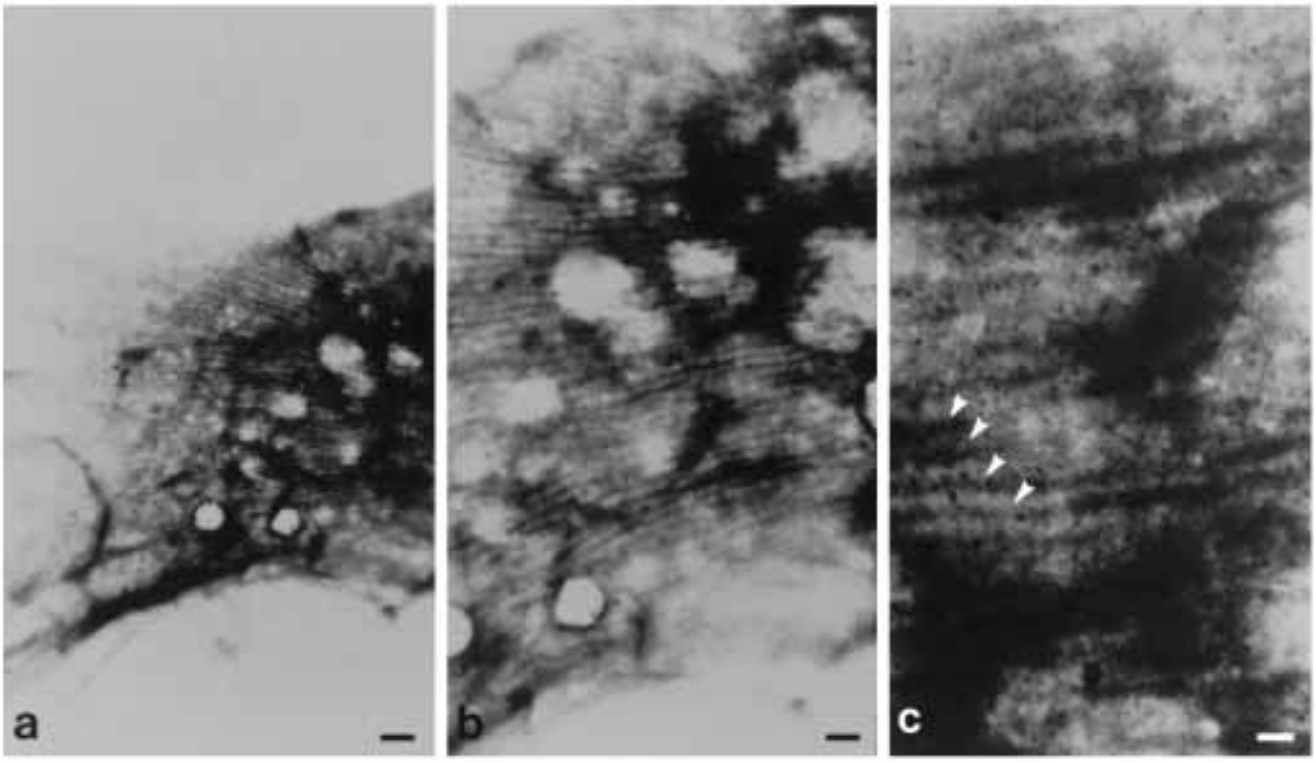

Fig. 10. Microtubules running in a tight parallel array can be observed in a bundle at the periphery of a MAP1A-SS2 transfected cell. HeLa cells were grown on gold electron microscopy grids and transfected with the SS2 construct. Transfected cells were localised by immunofluorescence, prepared for whole-mount electron microscopy and negatively stained with uranyl formate. Arrowheads in (c) point to single microtubules in the parallel array. Bars: (a) 0.55 μm; (b) 0.275 μm; (c) 0.15 μm.

amino acid residues like other identified tubulin-binding domains, but unlike them it does not contain any identifiable repeated motif. Whereas the other mammalian microtubule-binding domains described to date are basic, the MAP1A-SS2 domain is acidic. The microtubule-binding domains of MAP2, tau and MAP4 all have a net positive charge (Aizawa et al., 1990; Lee et al., 1988; Lewis et al., 1988) as does the binding domains of MAP1B (Noble et al., 1989) and a *Drosophila* MAP of molecular mass 205 kDa (Goldstein et al., 1986; Irminger-Finger et al., 1990). The only acidic microtubule-binding domain identified to date occurs in the high molecular mass microtubule-associated protein from *Trypanosoma brucei*, MARP-1 (Hemphill et al., 1992). This acidic microtubule-binding domain consists of a large number of 38 amino acid repeats, and shares no homology with other binding domains (Schneider et al., 1988). Interestingly, the microtubular cytoskeleton of *T. brucei*, where MARP-1 is one of the more prominent MAPs, consists of a regular array of tightly packed microtubules (Hemphill et al., 1991; Sherwin and Gull, 1989), resembling the array observed in MAP1A-SS2 transfected cells at the electron microscopy level (Fig. 10). In the array of closely spaced *T. brucei* microtubules, new polymers do not originate from microtubule-organising centres but are inserted into the existing array, and it has been suggested that tubulin polymerisation is nucleated by lateral interactions with MAPs of neighbouring microtubules.

The charged amino acids in the MAP1A-SS2 domain are clustered in groups of three, four or five residues separated by irregular intervals (Fig. 3). These charged residues occur in zones with high probability of forming α-helices according to the Chou-Fasman and the Garnier-Osguthorpe-Robson structure prediction algorithms (Chou and Fasman, 1978; Garnier et al., 1978). The predicted α-helices containing the charged amino acid clusters have a negative charge and are separated by short positive regions (Fig. 11a). Some of these acidic α-helices contain an alignment of acidic and basic residues on different sides of the α-helical loop (Fig. 11b). Such a structure could represent a domain involved in protein-protein interaction, like binding to cellular microtubules. An arrangement of this kind has been described for the actin-binding protein villin where the actin-binding domain is predicted to form α-helices in which basic residues are present on both sides of the helix (Friederich et al., 1992). In this case amino acid substitutions in one side of the helix were found to have a more drastic effect on villin function than substitutions on the opposite side (Friederich et al., 1992). A requirement for clusters of basic and acidic amino acid residues for binding between structural proteins is not infrequent. In addition to the MAP1A domain identified here and the KKE repeats in the tubulin-binding domain of MAP1B (Noble et al., 1989) other protein-protein binding domains rich in charged residues include the KKEK motif responsible for the actin-binding activity of villin (Friederich et al., 1992), the DAIKKK motif in actin depolymerisation factor (ADF), cofilin and tropomyosin (Yonezawa et al., 1989), the KKGGKKKG cluster of myosin (Yamamoto, 1991) and the KSKKLKKT

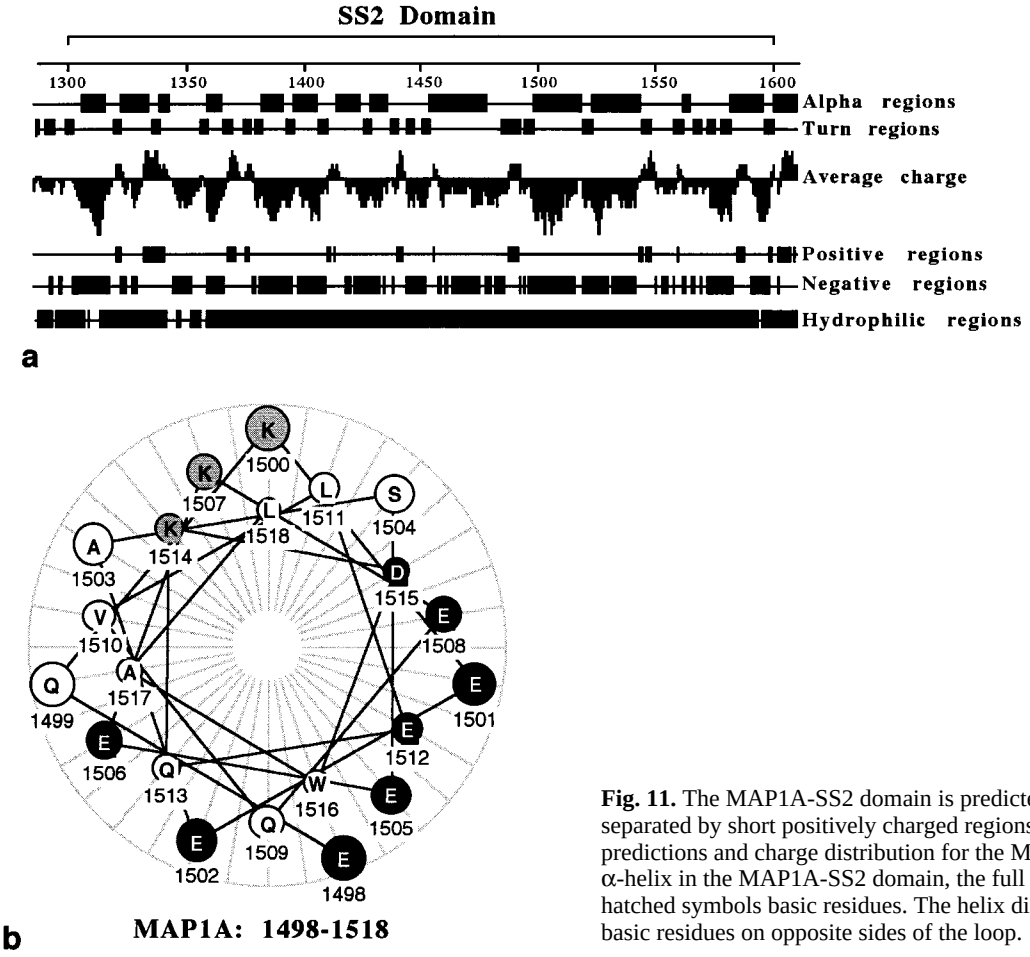

Fig. 11. The MAP1A-SS2 domain is predicted to contain acidic α-helices separated by short positively charged regions. (a) Secondary structure predictions and charge distribution for the MAP1A-SS2 domain; (b) predicted α-helix in the MAP1A-SS2 domain, the full symbols represent acidic and the hatched symbols basic residues. The helix displays an alignment of acidic and basic residues on opposite sides of the loop.

sequence in the actin-binding region of thymosin β4 (Safer et al., 1991).

The mechanism of binding of MAPs to microtubules is believed to involve an ionic interaction between the basic tubulin-binding domains of MAP2 and tau, and the acidic C terminus of tubulin (Littauer et al., 1986; Paschal et al., 1989; Serrano et al., 1984a,b; Vallee, 1982). The high molecular mass microtubule-associated protein MARP-1 from *T. brucei*, which contains an acidic binding domain, has been shown to bind to an area of tubulin distinct from the C terminus (Hemphill et al., 1992). That this could also be the case for the acidic microtubule-binding domain in MAP1A is suggested by in vitro studies, which indicate that MAP1A and MAP2 do not compete for binding to microtubules (B. Pedrotti and K. Islam, personal communication).

Microtubule rearrangement by the MAP1A binding domain

The pattern of microtubular rearrangement induced by SS2 expression is quite different to that induced by other neuronal microtubule-associated proteins that have been expressed in non-neuronal cells so far. On the other hand, the property of stabilising microtubules, measured by enhanced resistance to nocodazole treatment seems to be shared by all these neuronal MAPs. Members of the MAP2-tau family stabilise microtubules and induce the formation of long microtubule bundles (Lee and Rook, 1992; Lewis et al., 1989; Weisshaar et al., 1992). MAP1B, which produces no change in the microtubule arrangement (Noble et al., 1989) can stabilise microtubules although to a lower extent than MAP2 and tau (Takemura et al., 1992). The mechanism by which different microtubule-associated proteins or deletion cDNA constructs containing MAP tubulin-binding domains rearrange the microtubular network of the cell is still the subject of debate. For tau and MAP2, it has been proposed that bundling occurs as the result of active cross-linking of the microtubules (Hirokawa et al., 1988; Lewis et al., 1989), either directly or through a third, as yet unidentified, cross-bridging molecule (Lewis and Cowan, 1990). On the other hand, it has been proposed that bundling results from the enhanced stabilisation of microtubules induced by tau binding (Lee and Rook, 1992). In this view, microtubules stabilised by MAPs may have an intrinsic affinity for one another and form parallel bundles without the involvement of an active cross-linking molecule. This could also be the case for microtubules stabilised by MAP1A-SS2, which run in parallel arrays. Cellular microtubules stabilised by MAP2 can exist in the cytoplasm independently of the microtubule-organising centre (MTOC) (Weisshaar et al., 1992). In *T. brucei*, microtubules also occur in a peripheral cytoplasmic location that appears to arise via a mechanism called lateral nucleation (Sherwin and Gull, 1989), in which new tubulin polymers are nucleated in-between the existing microtubular parallel array. Cells transfected with the MAP1A-SS2 microtubule-binding domain also show parallel arrays of microtubules at the

electron microscopy level (Fig. 10), suggesting that a mechanism similar to lateral nucleation may be operating.

Axons of cultured neurons contain two classes of microtubules with different sensitivity to nocodazole (Baas and Black, 1990). More stable microtubules are abundant in proximal region of axons whereas more labile microtubules are abundant in the distal regions of axons (Ahmad et al., 1993; Baas et al., 1993). This arrangement is thought to produce a more dynamic cytoskeleton to the advancing axon tip. In this organisation, the plus ends of the stable microtubules have been shown to be the exclusive nucleating structures in the axon (Baas and Ahmad, 1992). At the growth cone, microtubules are assembled from a pool of soluble tubulin and elongated as the growth cone advances (Gordon-Weeks, 1991a,b) and direct application of agents that decrease or enhance microtubule stability onto the growth cone has been shown to prevent neurite growth (Bamburg et al., 1986). Thus, control of microtubule stability plays a critical role in determining neuronal morphology (Gordon-Weeks, 1991a; Mitchison and Kirschner, 1988).

A surprising variety of agents can stabilise microtubules. In addition to the MAPs discussed here, chemicals such as taxol and DMSO also stabilise microtubules in living cells (Weisshaar et al., 1992). However, these various molecules differ strikingly in their influence on microtubule arrangement. MAP2 and tau, whose basic microtubule-binding domains are closely related, produce similar microtubule arrangements. Taxol, which binds to a site on tubulin distinct from that of MAP2 or tau (Vallee, 1982), produces a different arrangement. Both MARP-1 in *T. brucei* cytoskeleton and MAP1A-SS2-binding domain, which are acidic, produce a third distinctive arrangement. Thus, the site on tubulin to which a stabilising molecule binds may be an important determinant of its effect on microtubule arrangement. This provides one possible explanation for the co-existence of several different structural MAPs within the same area of neuronal cytoplasm. The variability in microtubular stability in different neuronal compartments may be the result of the existence of different microtubule-binding proteins with different microtubule stabilising and arranging effects.

Adult microtubules are more stable and assemble more efficiently than embryonic microtubules (Faivre et al., 1985; Fellous et al., 1979; Lennon et al., 1980), furthermore, the polymerisation of embryonic brain microtubules has been shown to be markedly stimulated by the addition of adult brain MAPs (Francon et al., 1982). MAP1A is expressed in the adult, rather than the embryonic brain. What contribution might it make to a mature, rather than a developing neuron? One possible answer may lie in the lateral arrays of microtubules observed by electron microscopy in MAP1A-SS2 transfected cells. If those arrangements are produced by lateral nucleation, then the appearance of MAP1A during neuronal development could result in the ability of microtubules in maturing neuronal processes to nucleate the assembly of additional microtubules in the same stretch of process, further enhancing its structural stability.

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