Microtubular Organization in Tobacco Cells: Heat-Shock Protein 90 can Bind to Tubulin *in Vitro*

A. Freudenreich and P. Nick

Institut für Biologie II, Freiburg, Germany

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Abstract: **The heat-shock protein 90 (HSP90) from tobacco VBI-O cells specifically binds to nitrocellulose that had been coated with polymerized microtubules or tubulin dimers. HSP90 is expressed preferentially during cell division and becomes downregulated during cell elongation. HSP90 cofractionates with tubulin dimers during affinity chromatography with sepharose coupled to the tubulin-binding drug ethyl N-phenylcarbamate (EPC). Binding of HSP90 to EPC-sepharose depends on the presence of tubulin. Antibodies against tubulin and HSP90 immunoadsorb HSP90 and tubulin, respectively. These results demonstrate that HSP90 behaves as a microtubule-binding protein *in vitro*.**

Key words: **Chaperones, heat-shock protein 90 (HSP90), microtubules, tobacco cells.**

Abbreviations:
HSP90 heat-shock protein 90

Introduction

The heat-shock protein 90 (HSP90) family of proteins has been discovered originally because these proteins are upregulated in response to temperature stress (reviewed in Georgopoulos and Welch, 1993). Lately, some members of this family have been found to be constitutively expressed, and in addition to their role in the heat-shock response additional functions have been ascribed to this type of proteins. They seem to function as molecular chaperones (Wiech et al., 1992), to be involved in the hormone–ligand interaction of the glucocorticoid receptor (Kimura et al., 1995), and to interact with both actin microfilaments (Koyasu et al., 1986) and the microtubule system (Czar et al., 1996).

In plants the microtubular cytoskeleton is involved in the spatial control of cell division and cell expansion (reviewed in Goddard et al., 1994) and thus in the flexible response by changes of cell shape to environmental and developmental signals. In maize coleoptiles, for instance, a reorientation of cortical microtubules accompanies the growth responses triggered by blue or red light (Nick et al., 1990). The control of microtubular dynamics by environmental and developmental signals must be regarded as a key step in the morphogenetic response to the environment. However, the molecular mechanism of this control is far from being understood.

In maize coleoptiles, subunits of the cytosolic chaperone CCT have been found recently to be regulated by phytochrome and to be localized at the nuclear envelope and along cortical microtubules (Himmelspach et al., 1997). A second phytochrome-regulated microtubule-binding protein has been isolated from the same system (Nick et al., 1995) and could be identified by peptide sequencing as a member of the HSP90 family of heat-shock proteins (Nick et al., manuscript in preparation). However, in the non-cycling epidermal cells of maize coleoptiles, this protein was not associated with cortical microtubules, but with the nuclear envelope (Nick et al., 1995). This raises the question whether the interaction between HSP90 and microtubules depends on the state of the cell (cycling versus non-cycling).

In the present work we investigate the relationship between HSP90 and microtubules in the cycling tobacco cell culture VBI-O (Zažímalová et al., 1995). In this cell culture a cycle of axial cell division and cell expansion can be induced by addition of auxin. Using extracts from cycling tobacco cells, the interaction of HSP90 and microtubules was tested *in vitro*. In a forthcoming paper we will describe the results from a immunofluorescence study on HSP90 localization in this cell culture. In the present work we demonstrate the developmental regulation of HSP90 in this cell culture and the specific binding of this protein to microtubule-coated nitrocellulose. We show further that HSP90 cofractionates with tubulin dimers during affinity chromatography based on the tubulin-binding drug ethyl N-phenylcarbamate (EPC). The binding of HSP90 to EPC-sepharose depends on the presence of tubulin. Finally, we present evidence from coimmunoprecipitation experiments that supports an association of tobacco HSP90 and tubulin *in vitro*.

Materials and Methods

Plant material

The tobacco cell culture VBI-O (*Nicotiana tabacum* L. cv. Virginia Bright Italia) was derived in 1967 from stem pith tissue and has been propagated from a stock callus culture on slightly modified Hellers medium, supplemented with 1-naphthylacetic acid (5 μM) and 2,4-dichlorophenoxyacetic

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acid (5 μM) Zažímalová et al., 1995). Batch cell suspension cultures were initiated by suspending the stationary phase inoculum in a liquid medium of the same composition. This primary cell culture was subcultivated every three weeks in 100 ml Erlenmeyer flasks with a density of $5 \cdot 10^4$ cells $\cdot$ ml^{-1}. The cell suspensions were maintained in darkness at 25 °C on a horizontal shaker at 150 rotations per min. Soluble extracts were obtained at day 6 after inoculation, when the cell files passed through the first and second division cycles and had reached a density of about $2 \cdot 10^5$ cells $\cdot$ ml^{-1}.

Assay for neurotubule affinity of HSP90

Cells were harvested, the culture medium removed, and the cells homogenized in a French press (at 640 psi pressure) with half a volume of ice cold extraction buffer (300 mM Mes, 30 mM EGTA, 15 mM $MgCl_2$, 3 M glycerol, 3 mM GTP, 3 mM DTT, 3 mM PMSF, 10 μg $\cdot$ ml^{-1} aprotinin, 30 μg $\cdot$ ml^{-1} leupeptin, 30 μg $\cdot$ ml^{-1} pepstatin, pH 6.8). The homogenate was clarified by ultracentrifugation (60 000 g, 20 min, 4 °C), the pellet discarded, and the supernatant centrifuged a second time (100 000 g, 30 min, 4 °C). The resulting supernatant, containing 1–2 mg $\cdot$ ml^{-1} of total protein, was defined as soluble extract and either analysed directly or used for the various assays.

Neurotubulin was purified from fresh pig brain by two cycles of cold-induced disassembly and warm-induced assembly (Shelanski et al., 1973). Copurified neural microtubule-associated proteins (MAPs) were removed by cation exchange perfusion chromatography (Fractogel EMDSO^{3-}-650 (M), Merck; Darmstadt, Germany) on an FPLC sytem (Pharmacia; Freiburg, Germany). Upon loading of the sample and subsequent washing (50 mM PIPES, 1 mM EGTA, 0.2 mM $MgCl_2$, 0.1 mM GTP, pH 6.8) at a flow rate of 1 ml $\cdot$ s^{-1}, the neural MAPs remained quantitatively on the column, whereas the pure neurotubulin passed unbound in the flowthrough (Fig. **1 B**, lane NT). After silver staining (Ansorge et al., 1982) barely any traces of MAPs were detectable together with the neurotubulin (data not shown).

Binding of HSP90 to polymerized neurotubules was initially assayed by a cosedimentation assay (Vantard et al., 1991). Although the results of this assay suggested binding of HSP90 to microtubules (data not shown), interpretation was hampered by the tendency of HSP90 to form sedimentable complexes under the conditions of this assay even in the absence of brain tubulin. To circumvent this problem, a new assay had to be developed that was not based upon sedimentation. For this purpose the following affinity assay had been established (Fig. **1 A**):

To obtain a neurotubule-coated nitrocellulose, neurotubulin (> 1 mg $\cdot$ ml^{-1}) was prepolymerized by addition of 20 μM taxol (Fluka; Neu-Ulm, Germany) and 1 mM GTP for 20 min at 30 °C. 1.5 ml of these prestabilized microtubules were then added to 10 cm^2 patches of nitrocellulose (Sartorius; Göttingen; Germany) for 90 min at 30 °C to saturate the nitrocellulose with microtubules. 100 mg of tubulin were added per 10 cm^2 of nitrocellulose. The membrane was washed twice for 5 min with 1.5 ml of incubation buffer (one part of extraction buffer on two parts of water) to remove unbound microtubules. To block membrane areas that had not been coated by neurotubules, the membrane patches were subsequently incubated with 2% w/v of bovine serum albumin in incubation buffer for 1 hour at 30 °C.

In parallel, two additional sets of membrane patches were coated with unpolymerized neurotubulin (designated as tubulin control) or with bovine serum albumin alone as negative control (designated as BSA control). The membrane patches were then incubated for 20 min at 4 °C with 4 ml of soluble cell extract (complemented with 1 mM GTP, 20 μM taxol, and 100 mM NaCl to reduce unspecific binding). Extracts from cells in division phase were assayed and compared to extracts from cells in elongation phase. Unbound proteins were then washed off twice 10 min by 1.5 ml of incubation buffer. Bound proteins were then detached from each patch by addition of 1 M NaCl in 3 ml of incubation buffer (complemented with 20 μM taxol) for 10 min at 4 °C. This salt fraction, termed the MAP fraction, was then precipitated by 7.2% w/v of trichloroacetic acid, the sediment washed with 80% of ice cold acetone and then analyzed on conventional SDS-polyacrylamide slab gels (10% w/v acrylamide and 0.2% w/v bisacrylamide) and subjected to Western blotting as described in (Nick et al., 1995). Protein gels were stained with silver according to Ansorge (1982).

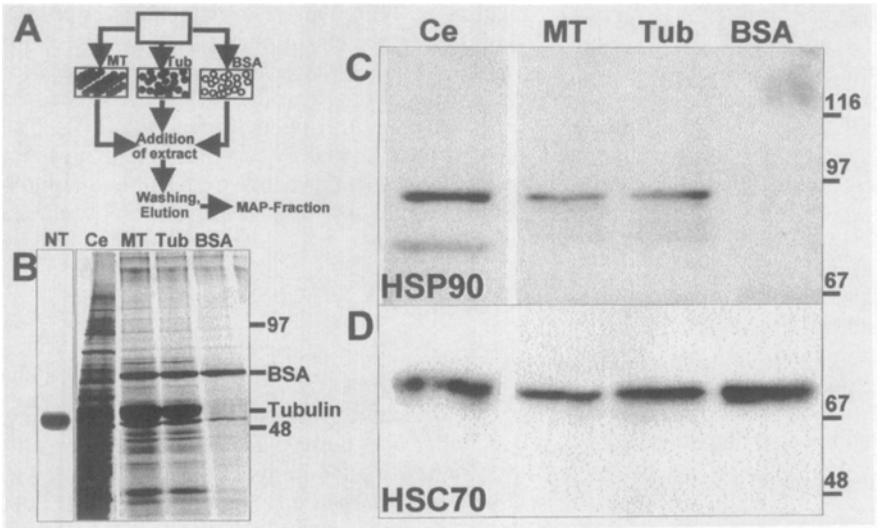

Fig. 1 Affinity of plant HSP90 from dividing tobacco cells to nitrocellulose coated with microtubules (MT) or tubulin (Tub). **A** Principle of the experiment. Nitrocellulose was coated with microtubules, with tubulin or with BSA (as a negative control), incubated with soluble extract, washed and eluted with salt. **B** Silver stain of the eluted fractions after separation by SDS-PAGE, Ce cytosolic extract used for the binding assay, NT silver stain of the purified neurotubulin used for the assay. **C** Western blot of the eluted fractions probed with anti-HSP90 antibodies. **D** Reprobing of the blot shown in **C** with anti-HSC70 antibodies.

EPC-affinity chromatography

Sepharose 4 B was coupled to the microbutule polymerization blocker, ethyl N-phenylcarbamate, according to Mizuno et al. (1981). The soluble protein extract (typically 2 ml) was ultrafiltrated through a 0.22 μm membrane (Schleicher and Schüll), and mixed with the same volume of EPC sepharose that had been preequilibrated with extraction buffer. After slow rotation topover for 1 h at 4 °C unbound proteins were removed by filtration through Whatman filter paper in a 10 ml syringe. Bound proteins were subsequently detached from the EPC sepharose by stepwise increasing concentrations of KCl in extraction buffer. For each elution step, one volume of buffer containing the respective concentration of salt was thoroughly mixed with the EPC sepharose with a spatula and removed again through filtration. The fraction was then precipitated by trichloroacetic acid (Bensadoun and Weinstein, 1976), and the column washed with a second volume of the same salt concentration before the next elution step.

Thermostable extraction

Soluble extract was adjusted to pH 6.9 and complemented with 0.75 M NaCl. The extract was then boiled for 7 min and immediately cooled on ice. Precipitated proteins were removed by ultracentrifugation (100 000 g, 45 min, 4 °C), and the supernatant dialyzed three times for 3 h against 1 l of 10-fold diluted extraction buffer containing 10% polyethyleneglycol 4000 to concentrate and desalt the sample simultaneously.

Coimmune precipitation experiments

A volume of soluble extract corresponding to 100 μg total protein (typically between 100 μl and 200 μl) was complemented with the primary antibodies (diluted 1 : 10) and rotated topover overnight at 4 °C. Then, 10% v/v protein-A coated *Staphylococcus aureus* cells (Pansorbin, Calbiochem; San Diego, USA) were added and the incubation continued for 2 h at 4 °C. The unbound fraction was removed by centrifugation (15 300 g, 10 min, 4 °C), and the sediment thoroughly washed five times for 5 min with the same volume of water. Eventually, the sediment was boiled for 5 min in sample buffer containing 8 M urea and analyzed.

Protein analysis

All samples were boiled for 7 min in the same volume of sample buffer containing 8 M urea (Nick et al., 1995). Concentration of total proteins was determined directly in the denatured samples by staining with amido black (Popov et al., 1975). Proteins were separated by SDS-PAGE on 10% acrylamide gels and analyzed by Western blotting as described in Nick et al. (1995). For reprobing of membranes for different epitopes the antibodies were stripped off the membrane by incubation in stripping buffer (62.5 mM Tris/HCl, pH 6.7, 2% v/v mercaptoethanol, 1% w/v SDS).

Antibodies

Mouse monoclonal anti-α-tubulin and anti-β-tubulin (Amersham; Little Chalfont, U.K.) was diluted 1 : 3000, and rabbit anti-heat-shock protein 90 raised against HSP90 from *Catharanthus roseus* (Schröder et al., 1993) 1 : 1000. Some of the experiments were repeated using rabbit anti-heat-shock protein 90 raised against HSP90 from mouse (Koyasu et al., 1986) with essentially the same results (data not shown). This antibody was used in the same concentration as that against HSP90 from *Catharanthus roseus*. A rabbit polyclonal antibody against tomato HSC70, a kind gift from Prof. L. Nover, Frankfurt, was used as a negative control for the specificity of the affinity assay (Fig. **1 D**). It was diluted 1 : 1000 for Western blotting. Peroxidase-conjugated antibodies against rabbit and mouse IgG (Sigma; Neu-Ulm, Germany) were diluted 1 : 3000.

Results

The antibody raised against HSP90 from *Catharanthus roseus* (Schröder et al., 1993) recognized a band of 95 kDa in soluble extracts of dividing tobacco VBI-O cells (Fig. **1 C**, Ce). In addition, a weaker band at 75 kDa was labeled by this antibody. Nitrocellulose patches that had been coated with polymerized microtubules (Fig. **1 C**, MT), with unpolymerized tubulin dimers (Fig. **1 C**, Tub) or with BSA (Fig. **1 C**, BSA), respectively, were incubated with soluble extract, washed thoroughly, and subsequently treated with salt (Fig. **1 A**). Equal amounts of protein were loaded on each lane and analyzed by SDS-PAGE and Western blotting using anti-HSP90 antibodies. HSP90 bound to nitrocellulose that had been coated with microtubules (Fig. **1 C**, MT) or with unpolymerized tubulin dimers (Fig. **1 C**, Tub), but not to nitrocellulose that had been coated with BSA (Fig. **1 C**, BSA). The minor band at 75 kDa did not bind to nitrocellulose at all. If the fractions were probed for the presence of a different heat-shock protein, HSC 70, that protein was detected not only in the cytosolic extract, but in eluates from all three experiments, i.e. it bound also to the BSA-coated nitrocellulose (Fig. **1 D**). This suggests that the binding of HSC70 was unspecific, in contrast to the binding of HSP90 that was observed only in the presence of microtubules or tubulin dimers.

The abundance of HSP90 was followed by Western blotting in total extracts over the culture cycle (Fig. **2**). The protein was most abundant during cell division (Fig. **2 B**, 6 d and 9 d) and disappeared gradually with the transition of the cell culture from cell division to cell elongation (Fig. **2 B**, 12 d and 18 d). A second minor band was observed during the second phase of the culture cycle, usually from the second week of cultivation (Fig. **2 B**, 12 d and 18 d).

In the next experiments, soluble extracts from dividing VBI-O cells were subjected to EPC-affinity chromatography and the fractions were probed for the presence of HSP90 (Fig. **3 A**). HSP90 was found to be attached (along with the 75 kDa protein) tightly to EPC sepharose and to be detached by KCl in a concentration of 0.4 M or higher. If the same membranes were reprobed for the presence of tubulin (Fig. **3 B**), tubulin was detected in those fractions, where HSP90 had been observed.

To test whether the binding of HSP90 to EPC sepharose depends on the presence of tubulin, a preparation of HSP90 had to be prepared that was free of tubulin. To obtain such a tubulin-free fraction, a thermostable extract was obtained from dividing VBI-O cells. Tubulin is not thermostable (Fig. **4 A**), in contrast to HSP90 that can be enriched by thermostable extraction (Fig. **4 B, C**). When the thermostable extract was subjected to EPC affinity chromatography, most of the

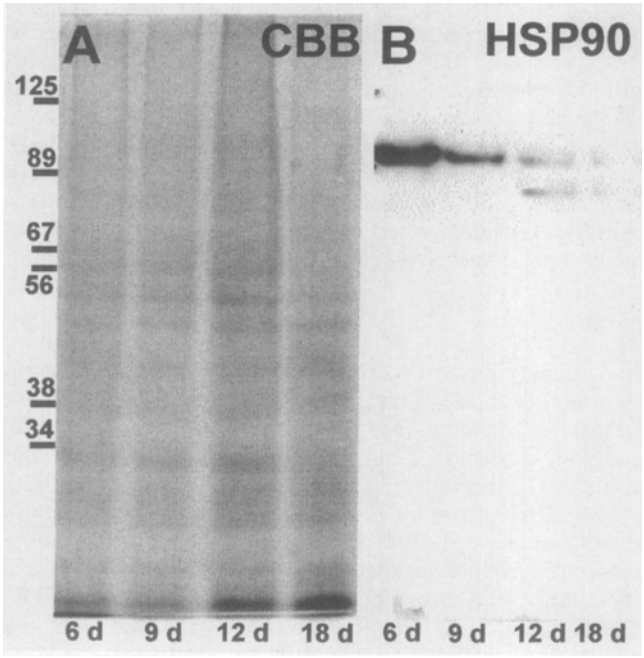

Fig. 2 Developmental regulation of HSP90 abundance during the cultivation cycle. **A** Total extracts from a VBI-O cell culture, 6, 9, 12, and 18 days after inoculation, stained with Coomassie Brilliant Blue. Cell division is maximal between 6 and 9 days, at 12 and 18 days cells pass through elongation and differentiation. 10 μg loaded per lane. **B** Western blot of the extracts shown in **A** probed with anti-HSP90 antibodies.

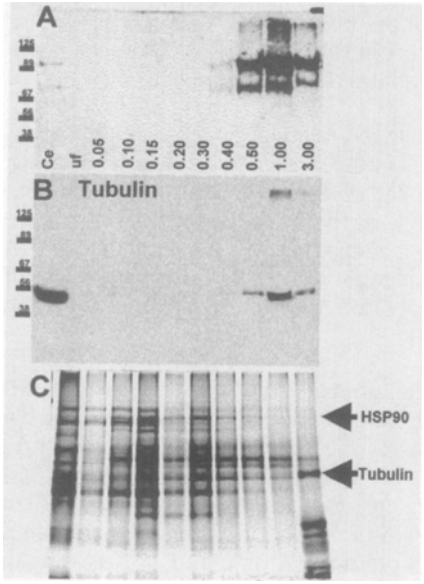

Fig. 3 Affinity-chromatography of soluble extracts from dividing tobacco cells using EPC sepharose. The extract was mixed with EPC sepharose and the sepharose then eluted by stepwise increasing concentrations of KCl (given in M), Ce cytosolic extract, of unbound fraction. **A** Western blot of the different fractions obtained by EPC affinity chromatography probed with anti-HSP90 antibodies. **B** Reprobing of the blot shown in **A** with antibodies against α-tubulin. **C** SDS-PAGE of fractions used for Western blotting stained with silver.

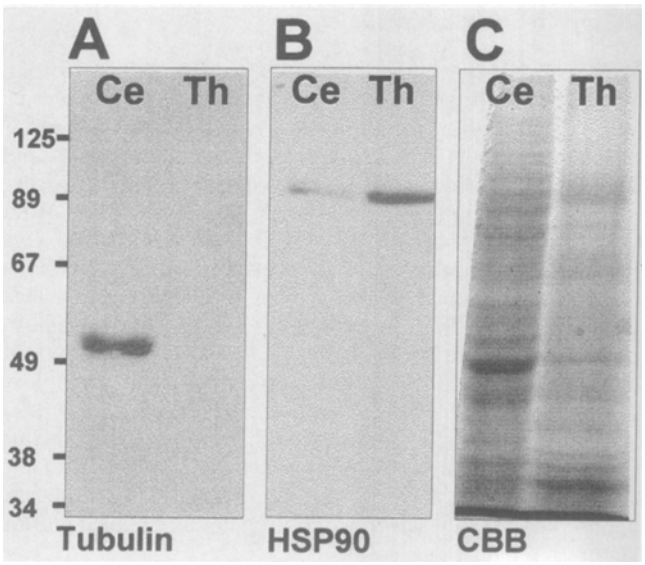

Fig. 4 Thermostable extraction of soluble extracts from dividing tobacco cells. The soluble extract (Ce) was boiled in the presence of salt and ultracentrifuged. The supernatant was desalted and concentrated to obtain the thermostable fraction (Th). **A** Western blot of soluble extract and thermostable fraction probed with antibodies against α-tubulin. **B** Reprobing of the blot shown in **A** with anti-HSP90 antibodies. **C** Silver stain of soluble extract and thermostable fraction after separation by SDS-PAGE.

HSP90 was found in the unbound fraction, very little in the first fraction obtained by elution with 0.05 M KCl (Fig. **5 A**). However, when the thermostable fraction was complemented by a small amount of tubulin dimers (Fig. **5 C**), the binding of HSP90 to EPC sepharose could be restored and 1 to 3 M KCl were required to detach HSP90 from the column (Fig. **5 B**).

When soluble extracts from dividing VBI-O cells are subjected to immunoprocipitation with anti-tubulin antibodies, a part of HSP90 cosediments together with the precipitated tubulin (Fig. **6 A**, lane Sed+anti-Tub). When the experiment is repeated replacing the anti-tubulin antibodies by a serum from unchallenged mice, HSP90 remains completely soluble (Fig. **6 A**, lane Sup+Mouse-Ns). When soluble extracts are immunoprecipitated with anti-HSP90 antibodies, tubulin cosediments with the precipitated HSP90 (Fig. **6 B**, lane Sed+anti-HSP90). Again, this cosedimentation is not observed, if the anti-HSP90 antibodies are replaced by a rabbit normal serum (Fig. **6 B**, lane Sup+Rabbit-Ns). There is no mutual cross-reactivity of the antisera (Fig. **6 A**, lane anti-Tubulin, Fig. **6 B**, lane anti-HSP90).

Discussion

The binding of HSP90 to nitrocellulose membranes that have been coated with microtubules or with unpolymerized tubulin dimers, is specific (Fig. **1 B, C**). The specificity of this HSP90-binding is emphasized by the observation that the binding of a second heat-shock protein, HSC70, is unspecific (Fig. **1 D**). The binding of HSP90 to membranes that were coated with tubulin dimers suggests that the target for binding is tubulin itself rather than the polymerized array of tubulin dimers. The observation that a part of HSP90 remains in the unbound fraction (data not shown) indicates that only

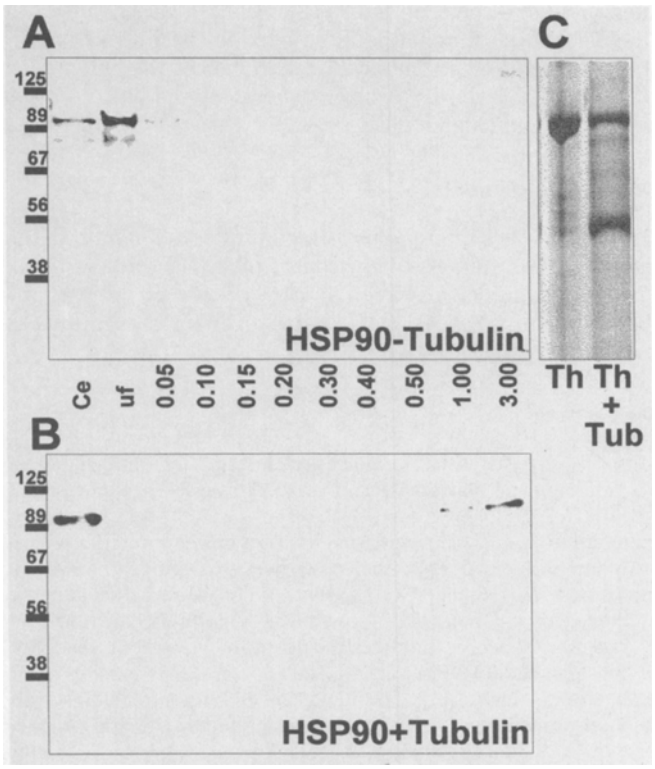

Fig. 5 Affinity chromatography of thermostable extracts from dividing tobacco cells using EPC sepharose, for details refer to Figure **3**. **A** Western blot of the different fractions obtained by EPC affinity chromatography of a tubulin-free thermostable fraction probed with anti-HSP90 antibodies. **B** as **A** but using a thermostable fraction that was complemented with neurotubulin. **C** SDS-PAGE of the tubulin-free thermostable fraction used for the experiment shown in **A** (left lane, Th) and of the thermostable fraction complemented with neurotubulin used for the experiment shown in **B** (right lane, Th+Tub).

Table 1 Increase in cell density over time during cultivation of the VBI-O tobacco cell culture.

Day after subcultivation	average density [cells·ml^{-1}]
1	$60 \cdot 10^3 \pm 18 \cdot 10^3$
3	$70 \cdot 10^3 \pm 20 \cdot 10^3$
5	$140 \cdot 10^3 \pm 32 \cdot 10^3$
7	$220 \cdot 10^3 \pm 45 \cdot 10^3$
9	$260 \cdot 10^3 \pm 48 \cdot 10^3$
11	$290 \cdot 10^3 \pm 39 \cdot 10^3$
15	$310 \cdot 10^3 \pm 29 \cdot 10^3$
19	$314 \cdot 10^3 \pm 24 \cdot 10^3$

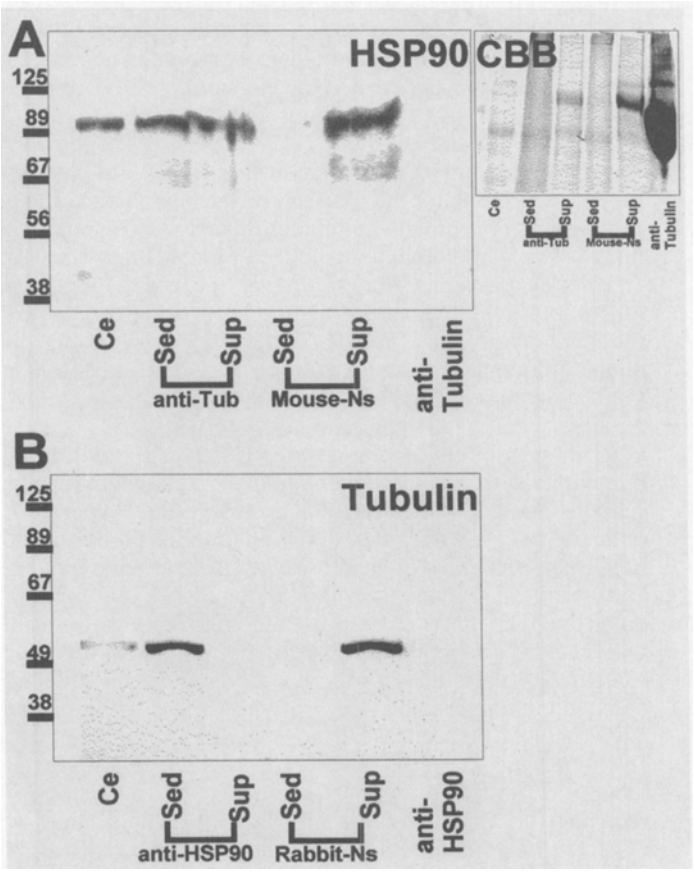

Fig. 6 Coimmune precipitation of HSP90 and tubulin from tobacco. **A** Western blot probed with anti-HSP90 of fractions that were obtained from a precipitation experiment with antibodies against α-tubulin. Soluble extract from dividing tobacco cells (Ce) was complemented with antibodies against α-tubulin (anti-tub) and Pansorbin, HSP90 was detected in the sediment (Sed) and in the supernatant (Sup). As a negative control, the experiment was repeated replacing the antibodies against α-tubulin by a mouse normal serum. In the last lane the antibody against α-tubulin was loaded. Inset: SDS-PAGE of the fractions used for Western blotting stained by Coomassie Brilliant Blue (CBB). **B** Western blot probed with antibodies against α-tubulin of fractions that were obtained from a precipitation with anti-HSP90. Soluble extract from dividing tobacco cells (Ce) was complemented with anti-HSP90 and Pansorbin, α-tubulin was detected in the sediment (Sed) but not in the supernatant (Sup). As a negative control, the experiment was repeated replacing the antibodies against HSP90 by a rabbit normal serum. In the last lane the antibody against HSP90 was loaded.

certain members of the HSP90 family are able to interact with microtubules. Alternatively, the number of binding sites on the membrane could be limiting – this is unlikely, however, because the natural concentration of tubulin in plant cells is orders of magnitudes lower.

This microtubule-affinity assay tests for the ability of HSP90 to interact with animal (neural) tubulin. It does not reveal whether HSP90 can bind to endogenous (plant) tubulin as well. It is very hard to obtain sufficient amounts of functional plant tubulin necessary for the affinity assay. Therefore, the cofractionation of HSP90 with endogenous tubulin was investigated using EPC sepharose affinity chromatography (Fig. **3**). Ethyl N-phenylcarbamate binds to tubulin thus preventing tubulin polymerization (Mizuno and Suzuki, 1990).

HSP90 binds to EPC sepharose and cofrationates with endogenous plant tubulin (Fig. **3**). This observation could be explained in two ways: (1) HSP90 binds directly to EPC, (2) HSP90 is bound to tubulin dimers and remains attached to the column due to the affinity of tubulin to EPC. The loss of HSP90-EPC sepharose binding in tubulin-free thermostable extracts (Figs. **4, 5 A**) demonstrates that the binding of HSP90 to EPC sepharose requires the presence of tubulin. The

restoration of HSP90-binding to EPC sepharose upon addition of neurotubulin to the thermostable extract (Figs. **5 B, C**) shows that the lack of binding observed in the thermostable extract was not caused by damage to the HSP90 protein that might have occurred during the boiling procedure.

The principal result from the EPC affinity assays is confirmed by the coimmune precipitation experiments (Fig. **6**). Anti-tubulin antibodies can coprecipitate HSP90 (Fig. **6 A**) and anti-HSP90 antibodies can coprecipitate tubulin (Fig. **6 B**). The specificity of HSP90-tubulin coimmune precipitation is demonstrated by the failure to coprecipitate HSP90 if the tubulin antibody is replaced by a mouse normal serum (Fig. **6 A**, lanes Sed and Sup, Mouse-Ns), and by the failure to observe coprecipitation of tubulin if the anti-HSP90 antibody is replaced by a rabbit normal serum (Fig. **6 B**, lanes Sed and Sup, Rabbit-Ns). Similarily, if anti-HSP90 is replaced by anti-chalcone synthase antibodies (there is no indication, so far, for an interaction between chalcone synthase and tubulin) tubulin remains in the supernatant (data not shown).

These experiments strongly suggest that HSP90 can bind not only to neurotubulin, but also to endogenous plant tubulin. The binding of a protein to tubulin *in vitro* is no proof for a function as a microtubule-associated protein (MAP) *in vivo*. The decisive criterion for a MAP is the association with microtubules in the cell (Solomon et al., 1979). We will therefore address this point in a forthcoming paper by double-immunofluorescence staining of tubulin and HSP90 in VBI-O tobacco cells.

It is interesting to ask whether HSP90 can bind to all microtubular arrays or whether binding is confined to specific subpopulations of the microtubular cytoskeleton. When cells leave the cell cycle and enter differentiation the abundance of HSP90 decreases (Fig. **2**). However, microtubule-binding assays with matured cells where microtubules are preferentially found in form of cortical microtubules, demonstrate that the binding of HSP90 to neurotubules *in vitro* is comparable (data not shown) to that observed for dividing cells, where microtubules are organized in a different set of microtubules (preprophase band, spindles, phragmoplasts). This might indicate that the interaction between HSP90 and tubulin is regulated by the abundance of HSP90 or by changing the binding properties of tubulin. The binding of HSP90 to microtubules is not complete indicating that only specific members of the HSP90 family interact with tubulin.

What is the functional significance of HSP90 binding to tubulin? The regulation pattern (Fig. **2**) indicates a correlation between HSP90 and high microtubular dynamics when microtubules reorganize rapidly between cortical microtubules, preprophase band, spindle and phragmoplast. Similarily, HSP90 transcription is induced in response to cold when microtubules depolymerize and reorganize during cold acclimation (Krishna et al., 1995). This suggests that the role of HSP90 is not confined to a mere chaperone function, but participates in the organization of the microtubular cytoskeleton. A similar conclusion has been reached for the chaperone CCT in maize (Himmelspach et al., 1997). Association of HSP90 with microtubules *in situ* has also been demonstrated for animal cells (Sanchez et al., 1988; Czar et al., 1996) and is discussed in terms of a role in cytoskeletal organization (reviewed in Liang and MacRae, 1997). Future work will be dedicated to resolve, on the biochemical as well as on the cell biological level, how HSP90 is targeted to specific microtubular arrays and what functional consequences this interaction may have for the dynamic plasticity of the microtubular cytoskeleton in higher plant cells.

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P. Nick

Institut für Biologie II
Schänzlestr. 1,
D-79104 Freiburg
Germany
e-mail: pnick@sun2.ruf.uni-freiburg.de
Fax + 49 (761) 2032612

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