Protoplasma (2000) 211: 234–244

PROTOPLASMA
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Printed in Austria

Plant chaperonins: a role in microtubule-dependent wall formation?

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Received June 21, 1999
Accepted September 15, 1999

Summary. The cytosolic chaperonin containing t-complex peptide-1 (CCT) is involved in the correct folding of newly synthetized actin and tubulin molecules. To get insight into potential additional functions of plant CCT, the localization of the subunit CCTε was followed throughout cell cycle, cell elongation, and cell differentiation in the tobacco cell culture VBI-O with relation to the microtubular cytoskeleton by double-immunofluorescence and confocal microscopy. The CCTε subunit was found to colocalize with sites of microtubule nucleation such as nuclear envelope and preprophase band. In addition, CCTε was associated with tubulin in sites of elevated wall synthesis such as phragmoplast or along secondary-wall thickenings. CCTε and its substrate tubulin were found to be soluble during periods of cytoskeletal dynamics, whereas sedimentable, vesicle-bound forms of CCTε and tubulin prevailed during cell differentiation. The sedimentability of CCTε was increased by calcium, whereas it was detached from microsomes by ATP. CCTε can bind to both polymerized microtubules and tubulin dimers. These data suggest an additional function of plant CCT in microtubule-driven transport of vesicles that contain cell-wall material.

Keywords: Chaperone; Confocal microscopy; Microtubules; Vesicle traffic; Tobacco.

Abbreviations: CCT cytosolic chaperonin containing t-complex polypeptide 1.

Introduction

The cytosolic chaperonin containing TCP-1 (CCT) specifically folds the cytoskeletal proteins tubulin and actin (reviewed in Lewis et al. 1997) and is upregulated in cells that synthetize large amounts of tubulin such as mammalian testes (Willison et al. 1990). In bovine testes and rabbit reticulocyte lysate, the complex has been found to contain at least eight subunits, and for mouse nine sequences of different subunits have been published (Kubota et al. 1995a, b, 1997). The activity of CCT in centrosomes of mammalian cells (Brown et al. 1996) suggests a role of this chaperone complex in the nucleation of new microtubules and the organization of the microtubular cytoskeleton.

Higher plants do not possess centrosomes (reviewed in Lambert 1993) and organize their cytoskeleton into completely different arrays that are not known from animal cells such as cortical array, preprophase band, and phragmoplast (reviewed in Nick 1998). These differences provide an interesting framework to address the role of CCT for the organization of microtubules. CCT is present in plants and subunit-specific sequences have been published from *Arabidopsis thaliana* (CCTα; Mori et al. 1992), oat (CCTε; Ehmann et al. 1993), cucumber (CCTε; Ahnert et al. 1996), and soybean (CCTδ; EMBL/GenBank Database, accession nr. AJ012318).

An analysis in maize coleoptiles, a tissue that consists exclusively of noncycling, but nevertheless growing cells (Himmelspach et al. 1997), suggested a colocalization of CCT subunits with cortical microtubules, a cytoskeletal array that is responsible for the correct deposition of cellulose microfibrils (reviewed in Williamson 1991). This colocalization was especially pronounced in cells of the protoxylem that are characterized by conspicuous secondary-wall thickenings (Himmelspach et al. 1997). Tubulin and actin, the folding substrates of CCT, cofractionated with CCT subunits upon sucrose-gradient centrifugation or anion-exchange chromatography. Both, the chaperone complex and its folding substrates actin and tubulin became sedimentable upon irradiation of the tissue with far-red light, triggered via the plant photorecep-

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tor phytochrome. These data suggested a role for the CCT complex in either organization or function of cortical microtubules.

So far, the role of CCT has not been analyzed in cycling plant cells. Such approaches would be worthwhile, though, because the transitions between cortical microtubules, preprophase band, spindle, and phragmoplast are characterized by dramatic transitions that are likely to involve drastic changes in tubulin synthesis and nucleation. The preprophase band, for example, disappears virtually in that instant when the spindle is formed, and the formation of the phragmoplast, a structure that controls the growth of the new cell plate, is heralded by the dissolution of the spindle. These dynamic transitions should be mirrored in the localization of plant CCT if this chaperone complex is involved in microtubule nucleation as should be expected from work with mammalian cells (Brown et al. 1996).

Such considerations stimulated the present work that follows the localization of the CCTε subunit in relation to microtubules through cell cycle, cell growth, and cell differentiation by double-immunofluorescence analysis and confocal laser-scanning microscopy. In order to facilitate the interpretation of the observed localization patterns, a tobacco cell line was used, where cell growth and cell division are aligned along an axis that is maintained by transport of the plant hormone auxin (Petrášek et al. 1998). The results suggest an additional function of plant CCT in the spatial control of cell-wall deposition.

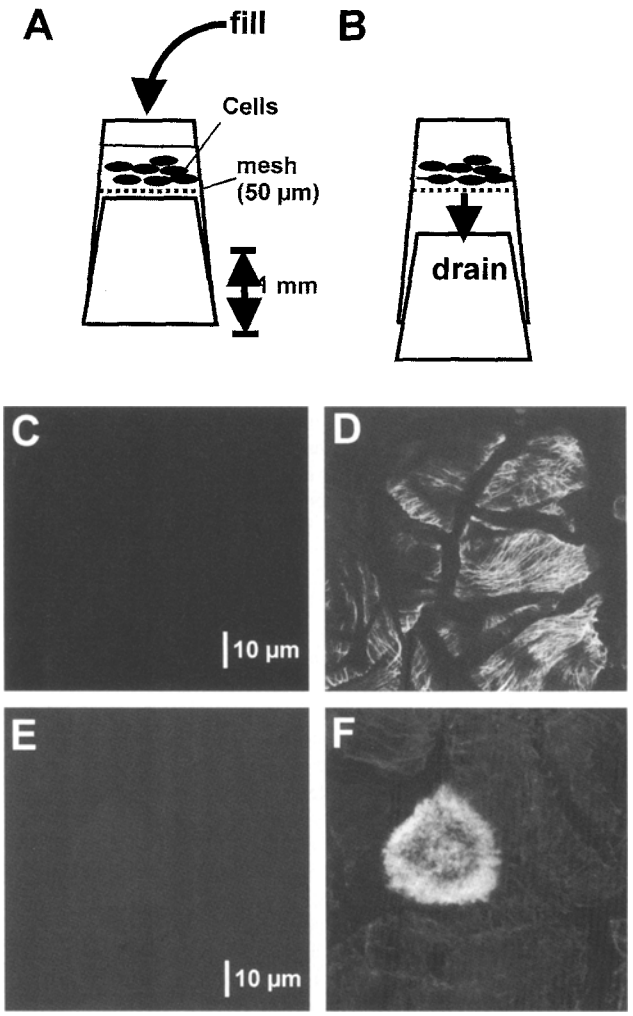

Fig. 1 A–F. Removal of unspecific background signals in double immunofluorescence of plant cells by an improved washing protocol. The miniaturized filter holder is shown in the closed (**A**) and in the open configuration (**B**). **C–F** Results for interphase (**C** and **D**) and telophase (**E** and **F**) VBI-O tobacco cells stained with anti-tubulin antibodies, visualized by TRITC (**D** and **F**) and with rabbit preimmune serum, visualized by FITC (**C** and **E**)

Material and methods

Cell culture

The tobacco cell line VBI-O (*Nicotiana tabacum* L. cv. Virginia Bright Italia) was cultivated in fresh modified Heller medium (Heller 1953) supplemented with 5 µM 1-naphthylacetic acid and 5 µM 2,4-dichlorophenoxyacetic acid. The culture was subcultivated every three weeks at an inoculation density of $5 \cdot 10^4$ cells per ml. Under these conditions maximal division activity was reached between 6 and 8 days after inoculation (Petrášek et al. 1998), producing pluricellular cell files. During the second week of the cultivation cycle, cells elongated parallel to the file axis, and during the third week of the cycle, the files gradually disintegrated into individual cells that begin to differentiate and to produce secondary-cell-wall thickenings. Details of the developmental parameters are described for this cell line in Petrašek et al. (1998).

Double-immunofluorescence staining and confocal laser-scanning microscopy

Aliquots of the culture were sampled every two days during a culture cycle and processed for double immunofluorescence in a miniaturized incubation chamber with a fine polyamid mesh (mesh size, 50 µm; PA-69/35 Nybolt; Franz Eckert GmbH, Waldkirch, Federal Republic of Germany) on top of a mobile stopper in a miniaturized filter holder (Fig. 1 A, B). The cells were allowed to sediment on the mesh, and the various solutions were added from the top (Fig. 1 A) and could be removed easily and efficiently by pulling the stopper (Fig. 1 B). This approach improved the efficiency of fixation and washing considerably, reducing unspecific background signals to undetectable levels (Fig. 1 C–E). Cells were fixed for 30 min in paraformaldehyde, 3.7% (w/v) freshly dissolved from a frozen stock solution into warm (25 °C) microtubule-stabilizing buffer (Toyomasu et al. 1994) and washed twice for 5 min in microtubule-stabilizing buffer. Prior to antibody incubation, the cells were incubated with a mixture of 1% mazerozyme (Yakuruto, Kyoto, Japan) and 0.1% pectolyase (Yakuruto) in microtubule-stabilizing buffer for 10 min at room temperature. They were then

blocked with 5% (v/v) horse normal serum (Sigma) in Tris-buffered saline (20 mM Tris-HCl, 150 mM NaCl, 0.25% [v/v] Triton X-100, pH 7.4) at 25 °C and incubated with the primary antibodies for 1 h at 37 °C. They were subsequently washed three times for 5 min in Tris-buffered saline and incubated with the secondary antibodies for 1 h at 37 °C, washed five times for 5 min, and mounted in Tris-buffered saline. The slide glass was sealed with nail-polish and the specimen viewed by confocal microscopy. The specificity of the obtained signals was checked in parallel series of negative controls, where the primary antibodies were replaced by the respective preimmune sera as described in detail in Petrášek et al. (1998). These controls confirmed the specificity of the obtained signals for tubulin and CCTε (see Figs. 1C–D and 2F, F′ as examples). The cells were visualized under a confocal laser microscope (DM RBE; Leica, Bensheim, Federal Republic of Germany) in a two-channel scan with an argon-krypton laser at 488 nm and 568 nm excitation wavelength, a beam splitter at 575 nm wavelength, and barrier filters at 580 nm and 590 nm wavelength, using a line averaging algorithm based on 32 individual scans per image. The immunofluorescence study over the culture cycle was repeated in six independent series with different culture batches.

Antibodies

Mouse monoclonal anti-α-tubulin and anti-β-tubulin (Amersham, Little Chalfont, U.K.) were used at a dilution of 1 : 100 dilution in Tris-buffered saline for immunofluorescence and at 1 : 300 for Western-blot analysis. The rabbit polyclonal serum against oat CCTε (Ehmann et al. 1993) was purified against bacterially overexpressed oat CCTε that had been coupled to a matrix (Eurocell ONB-Carbonat P; Knauer, Berlin, Federal Republic of Germany) as described in detail in Ehmann et al. (1993) and was used at a 1 : 30 dilution in Tris-buffered saline for immunofluorescence and at 1 : 300 for Western-blot analysis. To check for specificity of the signal, preimmune sera of the unchallenged animals were used at the same dilution as the primary antibodies. To visualize the CCTε-signal, a secondary anti-rabbit IgG antibody (Sigma, Neu-Ulm, Federal Republic of Germany) conjugated with tetramethylrhodamine isothiocyanate (TRITC) was used, whereas the tubulin signal was visualized by means of a secondary anti-mouse IgG antibody (Sigma) conjugated with fluorescein isothiocyanate (FITC). Both secondary antibodies were diluted 1 : 25 in Tris-buffered saline.

Solubility assays, microtubule affinity assays, and Western analysis

To detect potential changes in solubility of CCTε and tubulin, extracts were prepared from dividing and from differentiating VBI-O cells as described in Freudenreich and Nick (1998). Cells were harvested at different time points from 0 to 35 days after subcultivation. The culture medium was removed and the cells homogenized in a French press (at 640 lb/in^2 pressure) with one volume of ice cold extraction buffer (100 mM morpholineethanesulfonic acid, 5 mM $MgCl_2$, 1 M glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl, 10 μg of aprotinin, 10 μg of leupeptin, and 10 μg of pepstatin per ml, pH 6.8). The extraction buffer was administered in four parallel variants: (1) with 5 mM EGTA, (2) with 5 mM $CaCl_2$, (3) with 5 mM EGTA plus 5 mM ATP, and (4) with 5 mM $CaCl_2$ plus 5 mM ATP. In the time-course experiments shown in Fig. 6A, the extraction buffer of type 3 (containing EGTA and ATP) was used. The homogenate was first spun down with 5000 **g** for 10 min at 4 °C to remove nuclei and cell fragments that remained in the sediment. The supernatant was then subjected to ultracentrifugation at 100,000 **g** for 30 min at 4 °C yielding a soluble and a sedimentable (microsomal) fraction. Multisubunit granules of CCT remain soluble under these conditions (Himmelspach et al. 1997). The soluble fractions were subjected to a microtubule affinity assay following the protocol described in detail in Freudenreich and Nick (1998) and based on binding of proteins on nitrocellulose patches that had been coated either with polymerized microtubules, with tubulin dimers, or with bovine serum albumin (BSA). The patches were washed with small volumes of extraction buffer (type 3) containing increasing concentrations of KCl thus yielding fractions of weakly, intermediate, and strongly bound proteins. The fractions were then precipitated by 7.2% (w/v) trichloroacetic acid, as described in Freudenreich and Nick (1998), and then analyzed. Polyacrylamide gel electrophoresis, protein quantification and staining, Western blotting, and immunodetection by bioluminescence followed the protocol described in Nick et al. (1995).

Results

Localization of CCTε during mitosis

When a tobacco cell prepares for division, this is heralded by a displacement of the nucleus from the cell periphery towards the prospective division plane in the center of the cell. This event is accompanied by the appearance of radial microtubules that emerge from the nuclear envelope (Fig. 2B′) and tether the nucleus to the cortical cytoskeleton (Fig. 2C′). The CCTε epitope is detected along these radial microtubules and at those sites of the nuclear envelope where the radial microtubules initiate (Fig. 2B). In addition, the signal can be observed in the nucleolus (Fig. 2A).

In cells that have somewhat advanced in their cycle, the cortical microtubules that had coexisted with the radial microtubules for some period (Fig. 2A′–C′) suddenly disappear, such that only the radial microtubules remain (Fig. 2D′). During that stage, CCTε is found to form characteristic clusters consisting of small dots on the nuclear surface (Fig. 2D–G). These clusters can be observed as well when the cells are stained for CCTε alone without the addition of anti-tubulin antibodies (Fig. 2F, F′, G).

During the formation of the preprophase band, just prior to mitosis, the CCTε epitope is concentrated along the preprophase band and decorates interconnections between nuclear envelope and preprophase band (Fig. 3A, B). Moreover, it can be seen in those sites where the preprophase band aligns with the cell wall (Fig. 3B, B′).

Although CCTε can be detected in division spindles (Fig. 3C, C′), the signal is relatively weak as compared to the massive tubulin signal. Moreover, the spindle microtubules are not evenly decorated with CCTε, but there seems to be a preference for the periphery of the spindle, mainly the spindle poles (Fig. 3C, C′).

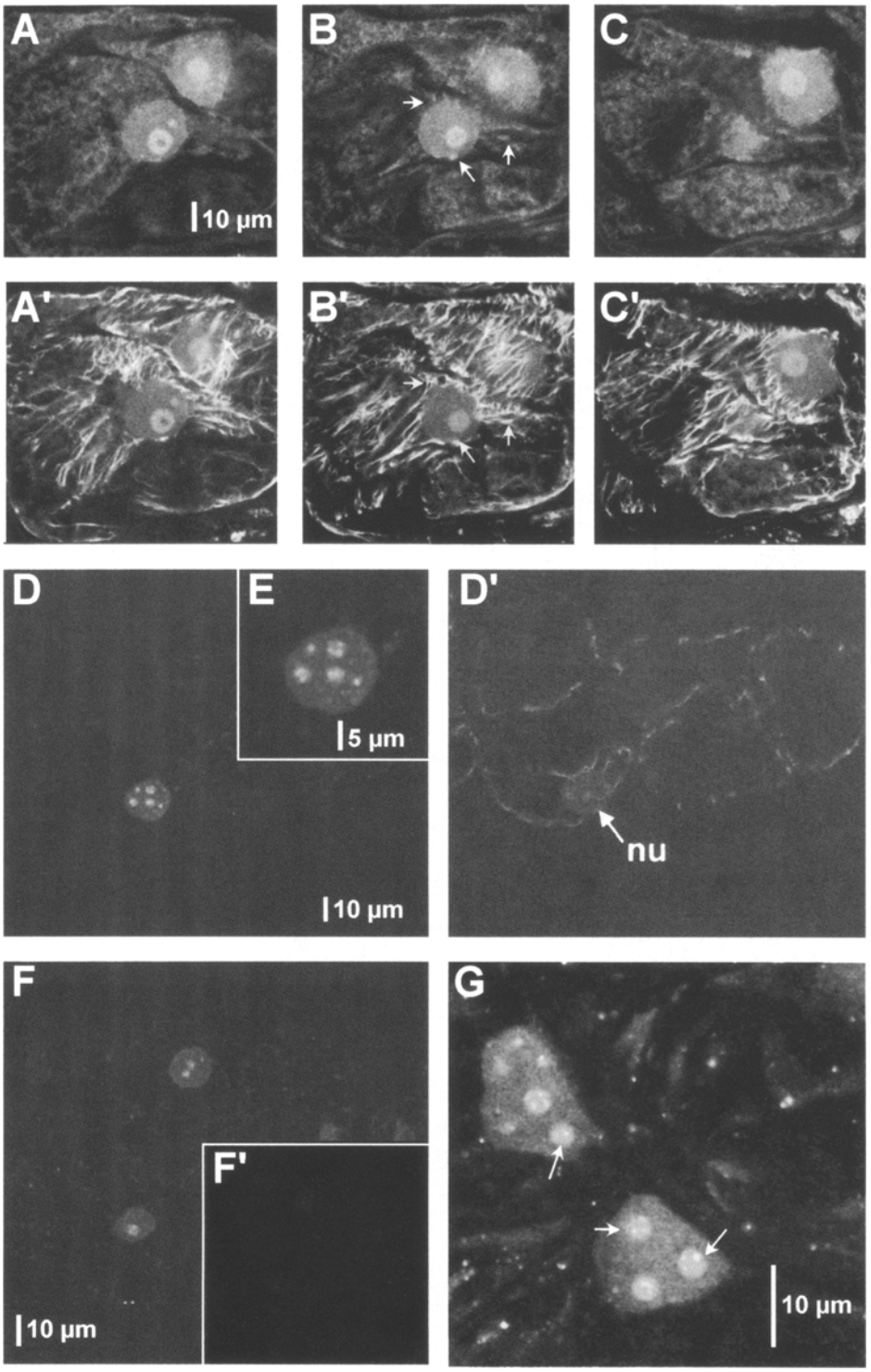

Fig. 2 A–G. Localization of CCTε in premitotic VBI-O tobacco cells. **A–C** and **A′–C′** Confocal sections of two adjacent cells stained for CCTε (**A–C**) and tubulin (**A′–C′**). The white arrows indicate the junctions of radial microtubules with the nuclear envelope. **D** and **D′** Premitotic cell in the interval preceding the formation of a preprophase band stained for CCTε (**D**) and tubulin (**D′**). **E** At higher magnification, the CCTε-clusters shown in the section in **D** focussing on the nuclear periphery. **F** and **G** Visualization of the nuclear clusters (white arrows in **G**) by single staining with CCTε, when the antitubulin antibody is replaced by a mouse preimmune serum. **F′** TRITC signal of the cell in **F**

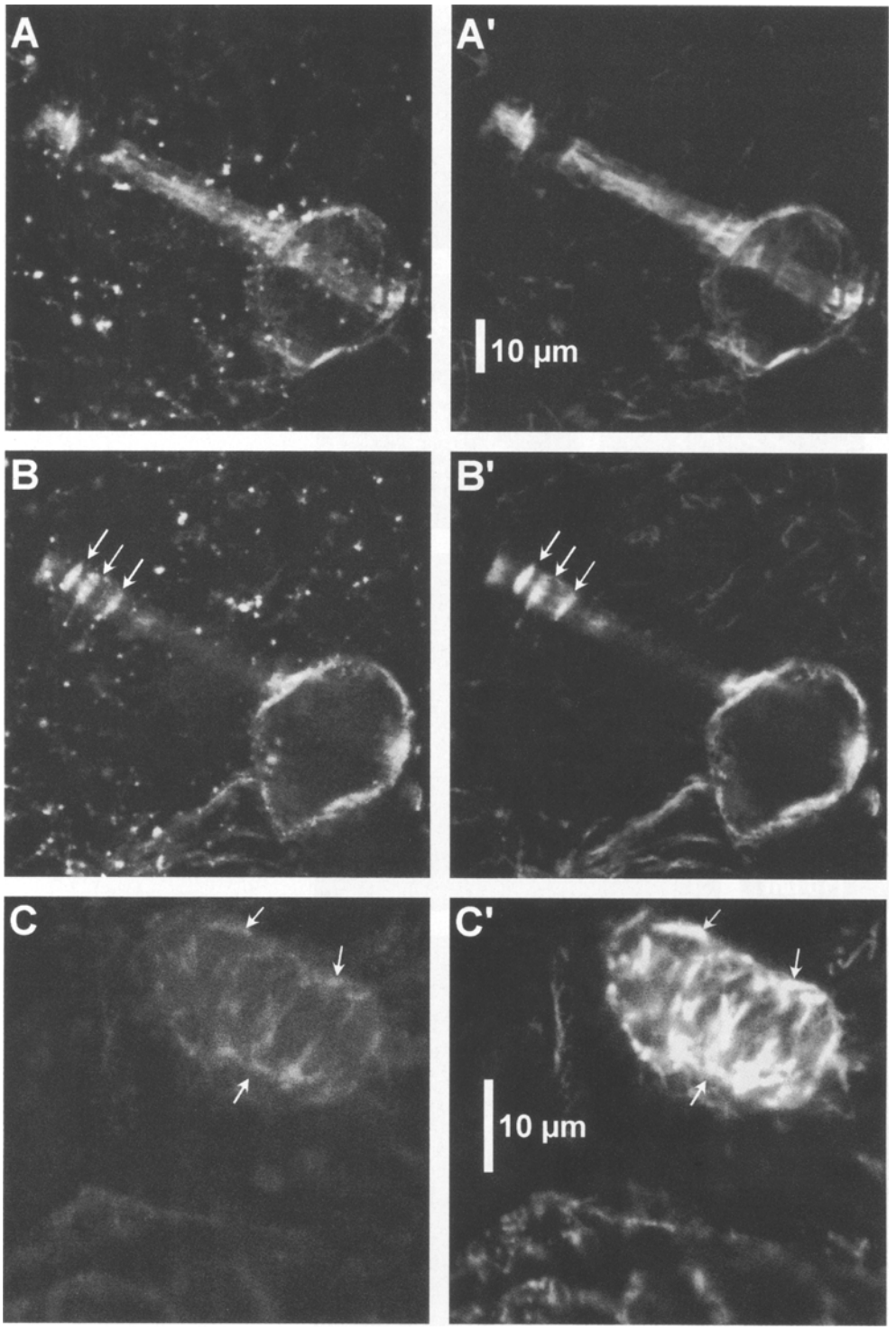

Fig. 3. Localization of CCTε in the preprophase band (**A**, **B** and **A′**, **B′**) and in the periphery of the division spindle (**C** and **C′**). **A–C** CCTε signals, **A′–C′** tubulin signals. The white arrows in **B** indicate locations where the preprophase band undulates along the cell wall

Association of CCTε with sites of cell-wall synthesis

Following mitosis, the CCTε epitope is strongly concentrated in the phragmoplast, a structure consisting of microtubules and vesicles that control the formation of the new cell wall between the daughter cells (Fig. 4). Interestingly, the CCTε signal appears to be vesiculate and aligned along the microtubules that converge towards the expanding edge of the growing cell plate (Fig. 4 A–C). In phragmoplasts that are seen laterally, the CCTε signal mirrors the typical double-ring structure of the microtubular phragmoplast (Fig. 4 D, D′).

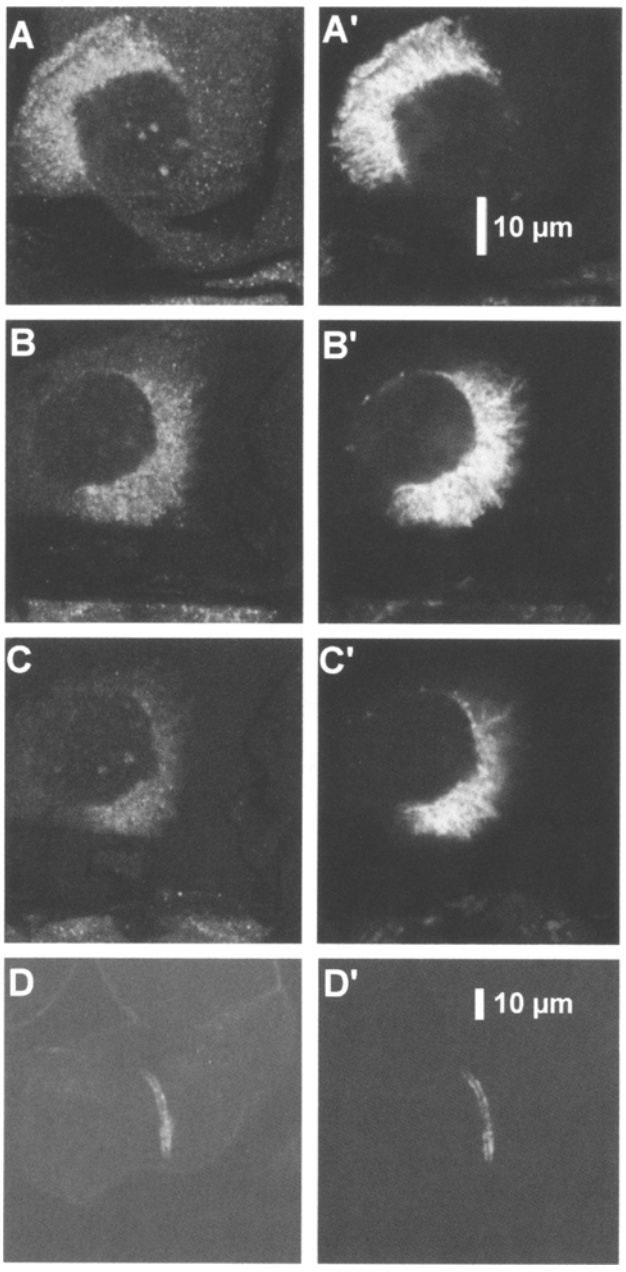

Fig. 4 A–D. Localization of CCTε in the phragmoplast. Three confocal sections of a phragmoplast seen from above (cell plate oriented in the image plane) are shown for the CCTε signal (**A–C**) and for the tubulin signal (**A′–C′**). **D** and **D′** Phragmoplast in side view; **D** CCTε signal, **D′** tubulin signal

Interestingly, CCTε is barely detectable in young interphase cells that initiate elongation (Fig. 5 A, A′). Thus, cortical microtubules in those cells seem scarcely decorated by CCTε. This situation changes dramatically, when cells begin to form secondary-wall thickenings in the late phases of the cultivation cycle. At this stage, the cortical microtubules are bundled into thick cables that accompany the wall thickenings that protrude into the cytoplasm and are visible in confocal sections as elongated, black gaps between the microtubules (Fig. 5 B′). These microtubular bundles are discussed as directional matrix for the localized deposition of cellulose along these cell-wall thickenings (Fukuda and Kobayashi 1989). These microtubule bundles are clearly decorated by CCTε (Fig. 5 B–D, B′–D′). However, the CCTε epitope is not localized continuously along the entire microtubule, but observed as a punctate pattern with the CCTε foci aligned along the microtubule (Fig. 5B, B′).

The solubility of CCTε changes, its binding to tubulin is maintained

The relative abundance of CCTε and its folding substrates tubulin and actin was followed by Western blotting over the culture cycle in total extracts and in soluble and sedimentable fractions (Fig. 6 A). The anti-CCTε antibody recognized a protein with a molecular mass of 65 kDa, whose abundance changed depending on the phase of the culture cycle (Fig. 6 A, upper panel): Whereas only low quantities of CCTε were detected in total extracts of dividing cells (between days 2 and 8 after subcultivation) and even less in elongating cells (between days 8 and 12), the amount of CCTε increased dramatically during the late stage of the culture cycle (from day 18), i.e., during the time when the cells begin to form secondary-wall thickenings. A comparison of the respective soluble (Fig. 6 A, middle panel) and sedimentable (Fig. 6 A, lower panel) fractions reveals fundamental changes in solubility. Whereas most of CCTε is found in the soluble fraction in dividing cells, this protein is completely shifted into the sedimentable fraction with the onset of secondary-wall formation. The presumptive folding

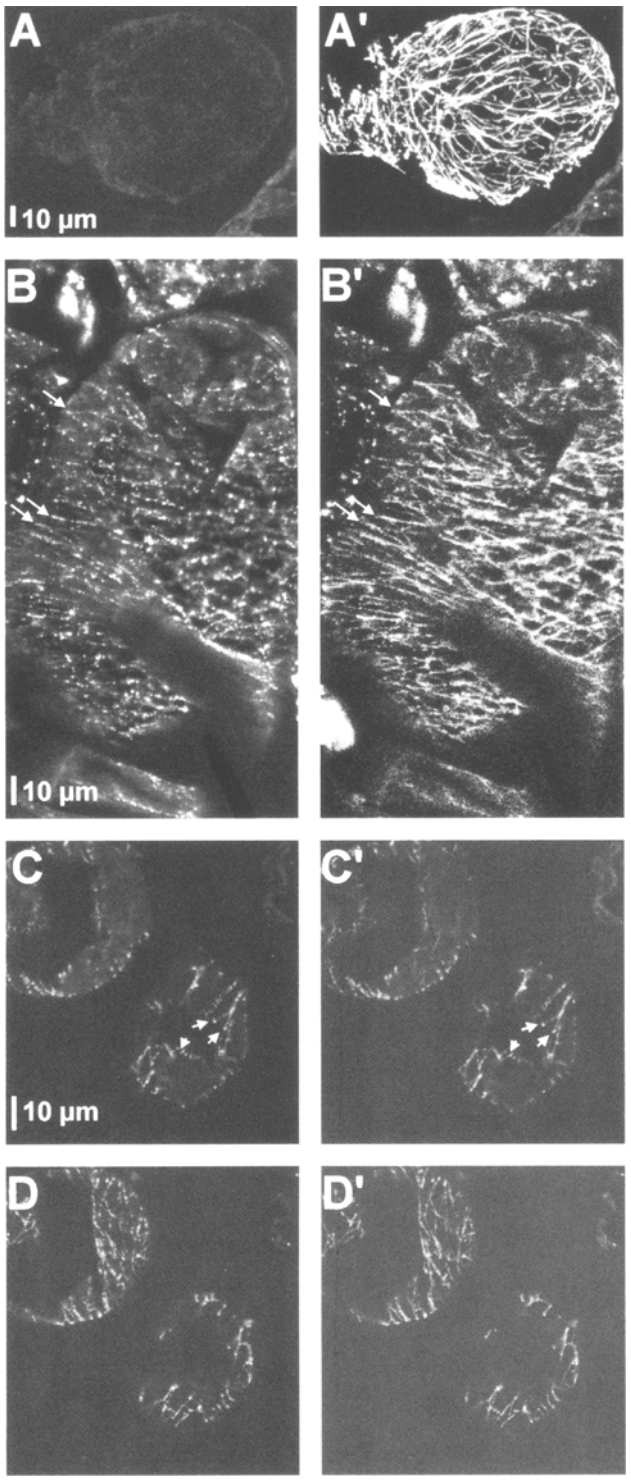

Fig. 5 A–D. Localization of CCTε with respect to cortical microtubules. **A** and **A′** CCTε (**A**) and tubulin (**A′**) signals for a young cell that is in the process of initiating elongation growth. **B–D** and **B′–D′** Localization of CCTε (**B–D**) along microtubule bundles (**B′–D′**) in differentiating cells that form secondary-wall thickenings. The white arrows in **B** and **B′** indicate examples for the alignment of CCTε with individual microtubule bundles

substrates α- and β-tubulin are increased during cell division, vanish during cell elongation, and reappear during cell-wall thickening (Fig. 6 A, central and lower panel). In contrast, the total amount of actin appears to be more constant. Irrespective of these differences in the total amount of the three substrates, they all show the same solubility shift as found for CCTε with high solubility in dividing cells and low solubility during wall thickening. This is observed for actin, α-tubulin (Fig. 6 A), and β-tubulin (data not shown).

The influence of calcium and ATP on the solubility of CCTε and its folding substrate α-tubulin was investigated in dividing (7 days after subcultivation; Fig. 6 B, left-hand panel) and in differentiating cells that develop pronounced secondary-wall thickenings (28 days after subcultivation; Fig. 6 B, right-hand panel). A comparison of soluble and sedimentable fractions revealed that CCTε and tubulin are soluble in dividing cells, independently of calcium or ATP (Fig. 6 B, left-hand panel). In contrast, in differentiating cells, CCTε becomes sedimentable in the presence of calcium but is solubilized in the presence of ATP (Fig. 6 B, right-hand panel). Tubulin, on the other hand, is solubilized by both factors, calcium and ATP (Fig. 6 B, right-hand panel). This means that tubulin, which otherwise cofractionates with CCTε (Fig. 6 A), can be separated from the chaperone by calcium in differentiating cells, and that dividing and differentiating cells differ with respect to the calcium response of CCTε.

The ability of CCTε to bind to polymerized microtubules and/or to tubulin dimers was assayed by a microtubule affinity assay (Freudenreich and Nick 1998). In this assay, nitrocellulose patches that are coated either with polymerized microtubules, with unpolymerized tubulin dimers, or with BSA were incubated with soluble extracts containing CCTε, and the bound proteins were subsequently detached from the membrane by the application of increasing ionic strength. These experiments (Fig. 6 C) show that, in dividing cells, CCTε is moderately bound to nitrocellulose coated with polymerized microtubules or with tubulin dimers, whereas binding to BSA-coated membranes is barely detectable. The situation in differentiating cells seems to be similar with moderately or strong binding of CCTε to membranes coated with polymerized microtubules or with unpolymerized tubulin dimers. Again, binding to BSA-coated membranes was barely detectable.

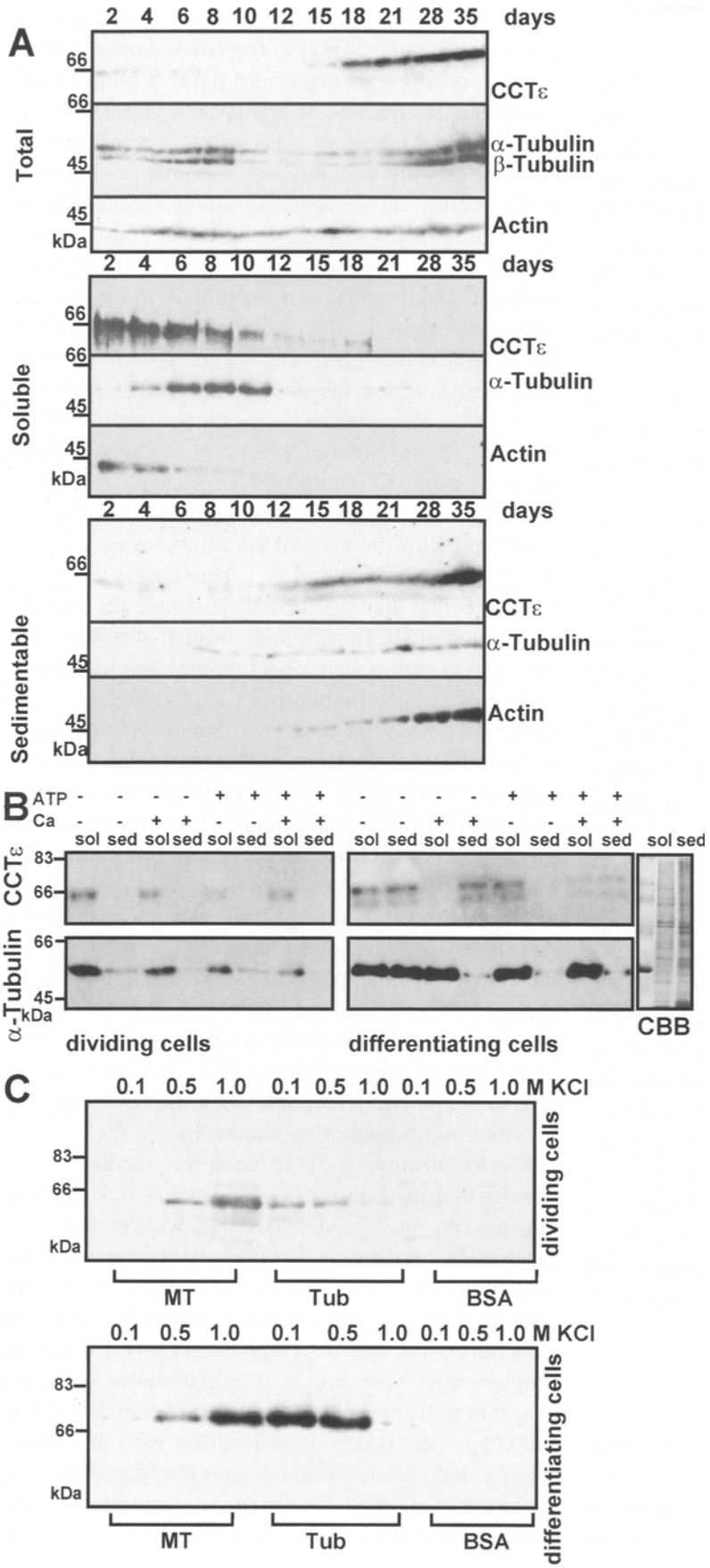

Fig. 6 A–C. Biochemical characterization of CCTε abundance and behavior. **A** Developmental regulation in abundance and solubility of CCTε, tubulin, and actin. Samples were collected during the whole culture cycle and fractionated into soluble and sedimentable fractions that were probed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting. 10 μg of total protein were loaded per lane and the loading was verified by staining replicate gels with Coomassie Brilliant Blue (data not shown). **B** Changes in solubility of CCTε and tubulin in the presence of calcium and ATP. Extracts from dividing (7 days after subcultivation) and differentiating (28 days after subcultivation) cells were fractionated in presence or absence of calcium and ATP and the fractions probed by Western blotting for the distribution of CCTε and tubulin. 10 μg of protein were loaded per lane. **C** Microtubule affinity assay for CCTε from dividing and differentiating cells. Nitrocellulose patches of equal size were coated with either assembled microtubules (*MT*), disassembled tubulin dimers (*Tub*), or BSA and incubated with soluble extracts from dividing or differentiating cells. Bound CCTε was detached from the membranes by subsequent washes with 0.1, 0.5, and 1 M KCl. The fractions were concentrated by trichloroacetic acid precipitation and analyzed by Western blotting for the abundance of CCTε that was bound to microtubules or tubulin dimers, respectively. The amount of protein that could be detached from 1 cm^2 of coated nitrocellulose was loaded per lane

Discussion

CCTε is associated with specific microtubular arrays

In dividing cells, the CCTε epitope was associated with radial microtubules (Fig. 2A–C), with the preprophase band (Fig. 3A, B), and with certain areas of the division spindle (Fig. 3C). In addition, it was observed on the nuclear envelope of premitotic cells (Figs. 2B and 3B) and is organized into characteristic cluste1rs on the nuclear surface (Fig. 2D–G) during the transition between the disappearance of cortical microtubules and the formation of the preprophase band.

The nuclear envelope seems to be the major microtubule-organizing center in dividing cells of higher plants (Lambert 1993, Stoppin et al. 1994), whereas the preprophase band marks the site where after completed mitosis new microtubules will be nucleated (Lloyd 1991). The radial microtubules are newly formed prior to mitosis and emerge from the nuclear envelope connecting to the cortical cytoskeleton. The CCTε epitope is thus observed in those sites where the nucleation of new microtubules is taking place. This is congruent with the result from the microtubule affinity assay that CCTε can bind to nitrocellulose membranes coated with polymerized microtubules as well as with unpolymerized tubulin dimers (Fig. 6C). Interestingly, virtually all of the CCTε is soluble during the early cultivation cycle (Fig. 6A, B), when microtubular turnover is high. A similar pattern had been observed, in the same cell line, for the chaperone HSP90 (Wiech et al. 1992), which was found to bind to tubulin dimers (Freudenreich and Nick 1998) and to decorate radial microtubules, preprophase band, and the nuclear envelope (Petràšek et al. 1998).

The localization of CCTε in potential microtubule nucleation sites and its ability to bind tubulin dimers are thus consistent with a cytoskeletal chaperone function of CCTε during cell division, in agreement with the findings in mammalian cells, where CCT has been found to fold cytoskeletal proteins in vivo (Sternlicht et al. 1993) and to be involved in the polymerization of microtubules at the centrosomes (Brown et al. 1996).

CCTε is present in sites of wall synthesis

The situation in differentiating cells seems to be somewhat different. These cells develop massive protrusions of the cell wall by apposition of cellulose responsible for the dark gaps that are occasionally seen in confocal sections of fluorescently labelled cells (for instance in Fig. 4B, B′). The cortical microtubules in these cells are arranged into parallel bundles perpendicular to the axis of cell growth and parallel to these wall thickenings (Fig. 4B′). These bundled microtubules are certainly less dynamic as compared to the cortical microtubules of younger cells that are still in the process of vivid cell growth. Thus, a microtubular chaperone would be expected to decorate preferentially the cortical microtubules in the younger cells rather than the microtubular bundles in the differentiating cells. In fact, this has been observed in the same cell line, for the chaperone HSP90 (Petràšek et al. 1998). However, the opposite is true for CCTε: Whereas the microtubular bundles are clearly decorated with the CCTε epitope in a punctate pattern (Fig. 4B–D, B′–D′), CCTε is barely visible and not associated with the cortical microtubules of younger, elongating cells (Fig. 4A, A′). This observation confirms previous findings in maize, where the clearest association of CCTε with microtubular structures was observed in protoxylem cells that undergo secondary-wall thickening (Himmelspach et al. 1997). In those cells, CCTε was aligned along the massive microtubular bundles that accompany the helicoidal wall protrusions. Interestingly, CCTε and its substrates tubulin and actin become increasingly sedimentable during the late phase of the culture cycle, i.e., during the period when, after completed elongation, cells initiate differentiation and undergo wall thickening (Fig. 6A). Although CCTε retains the ability to bind tubulin dimers during that period (Fig. 6C), it seems to be preferentially associated with polymerized microtubules rather than with the (soluble) tubulin dimers. This behavior suggests that CCTε, in addition to its role as a chaperone (see above), must have a second function in differentiating plant cells.

The localization of CCTε along the bundled microtubules that mark the sites of secondary-wall thickening has to be discussed in connection with the prominent localization of this chaperone with the phragmoplast, a complex structure involved in the formation of the cell plate which separates the daughter cells during the mitotic telophase (Fig. 4). The phragmoplast edge can act as a microtubule-organizing center in wall-free plant cells (Vantard et al. 1990), and the chaperone HSP90 is colocalized with the microtubules that radiate from the outer phragmoplast edge (Petràšek et al. 1998). It might be thus conceivable that these microtubules act as organizers for the cortical

interphase cytoskeleton. On the other hand, transitions between the phragmoplast and the new cortical cytoskeleton have not been reported so far for walled plant cells. Moreover, there is evidence for disperse nucleation sites in the cortical cytoplasm giving rise to the cortical microtubule array (Marc and Palevitz 1990, Cleary and Hardham 1990). Thus, the function of phragmoplast microtubules might be similar to that of the cortical microtubule bundles in differentiating cells: to guide vesicles containing cell-wall precursors to those sites where wall synthesis takes place. In fact, electron microscopical data support a model in which the microtubules emerging from the outer edge of the phragmoplast pull at tubular-vesicular protrusions emanating from the endoplasmic reticulum (Samuels et al. 1995). The localization of CCTε in the phragmoplast and along the sites of secondary-wall thickening might thus point to an additional function of this protein. It seems to be more than just a chaperonin for tubulin.

CCTε – a link between microtubules and vesicle transport?

A conspicuous part of CCTε and its substrates tubulin and actin is associated with the microsomal cell fraction in differentiating cells (Fig. 6B). Calcium can solubilize a part of this microsomal tubulin, whereas the microsomal association of CCTε is increased (Fig. 6B). This means that the otherwise close association of CCTε and tubulin is interrupted by calcium. Plant microtubules depolymerize in response to calcium (Bartolo and Carter 1992), what might explain the repartitioning of tubulin into the soluble fraction. ATP, on the other hand, causes both CCTε and tubulin to become soluble. When ATP and calcium are combined, tubulin remains soluble, whereas for CCTε the calcium effect appears to be somewhat ameliorated. This solubility shift of CCTε in response to calcium or ATP is confined to differentiating cells that are characterized by intensive cell-wall thickening. This shift recalls previous publications on the so-called chromobindin A complex from chromaffin granule membranes (Martin and Creutz 1987). Chromobindin A was later identified as a membrane-bound form of CCT (Creutz et al. 1994) and has originally been isolated by virtue of its ability to bind to membranes in the presence of calcium. Whereas this binding was stimulated by calcium, strontium, and barium, the release from the membrane was stimulated by ATP and other nucleotides. The chromobindin A complex was suggested to be involved in exocytosis (Martin and Creutz 1987). This means that in animal cells as well, CCT fulfills a dual function: as a chaperone for cytoskeletal protein and as a mediator of exocytosis; although most emphasis so far has been posed upon the chaperone function.

How does this compare with the situation in plant cells? Whereas CCT as a chaperone seems to be important during the cell cycle, when the microtubular dynamics is high, in interphase cells it seems to be mainly associated with polymerized, relatively stable microtubules on the one hand and with sites of cellulose synthesis on the other hand. Cellulose synthesis involves transport of cell-wall material across the plasma membrane, i.e., localized exocytosis. This event is closely associated with and spatially organized by microtubules (for a review, see Giddings and Staehelin 1991). CCTε retains its ability to bind to microtubules in differentiating cells (Fig. 6C), and it can bind to the microsomal fraction in the presence of calcium (Fig. 6B).

The most straightforward interpretation of these data is a model where CCTε is involved in the guided transport of vesicles containing cell material along microtubules towards those sites where wall synthesis actually occurs. This would explain the localization of CCTε along phragmoplast microtubules (Fig. 4) as well as its association with the cortical bundles that accompany secondary-wall thickenings (Fig. 5). It thus seems that the dual role of the CCT complex in tubulin folding and vesicle trafficking is mirrored in plants, however with a shift in the weight of the two functions.

Acknowledgments

This work has been supported by funds of the Deutsche Forschungsgemeinschaft to B.E. Prof. E. Schäfer is thanked for reading the manuscript.

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