Plant CDC2 is not only targeted to the pre-prophase band, but also co-localizes with the spindle, phragmoplast, and chromosomes

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Abstract A polyclonal antiserum against the p34^{cdc2} homologue of *Arabidopsis thaliana*, CDC2aAt, was used in parallel with a polyclonal antiserum against the PSTAIRE motif to study the subcellular localization of CDC2 during the cell cycle of isolated root tip cells of *Medicago sativa*. During interphase, CDC2 was located in the nucleus and in the cytoplasm. The cytoplasmic localization persisted during the complete cell cycle, whereas the nuclear signal disappeared at nuclear envelope breakdown. At the beginning of anaphase, the anti-CDC2aAt antibody transiently co-localized with condensed chromosomes. The chromosomal co-localization disappeared as anaphase continued and remained excluded from the separated chromosomes until cytokinesis, when CDC2 re-located to the newly forming nuclei. We also observed a co-localization of CDC2 with three microtubular structures, the pre-prophase band, the spindle, and the phragmoplast.

Key words: *Arabidopsis thaliana*; Cell cycle; Immunolocalization; *Medicago sativa*; Mitosis; p34^{cdc2} kinase

1. Introduction

Cyclin-dependent kinases (CDKs) play a central role in cell cycle control of all eukaryotes [1]. Cloning of cell cycle regulators from different plant species (for a review see [2]) has shown that the basic machinery of cell cycle control is conserved in higher plants.

The cell cycle-dependent complex formation of CDKs with their regulatory subunits, the cyclins, is the first requirement to activate the kinase. Furthermore, the CDK/cyclin kinase complex is regulated by phosphorylation and dephosphorylation of the kinase subunit and through binding with inhibitory proteins (for a review see [3]). In addition to intramolecular modifications of CDK/cyclin complexes, regulation of the in vivo kinase activity involves both spatial and temporal control of the cyclin and CDK component. In mammalian cells, part of the CDC2 protein is associated with centrosomes and with the mitotic apparatus during mitosis [4–7]. In fission yeast, CDC2 co-localizes with its regulatory subunit, cyclin B, to the spindle pole bodies [8,9]. During meiosis re-initiation of starfish oocytes, the CDC2/cyclin B complex re-locates, after activation, to the nucleus and accumulates in the nucleolus and on the chromosomes, whereas another fraction associates with meiotic asters and spindle microtubules [10].

The subcellular localization of CDC2 during interphase has been the subject of some debate. In fission yeast, it is localized only in the nucleus [9]; in animal cells, p34^{cdc2} is localized only in the cytoplasm [10], only in the nucleus [7], or in both [4,6]. In root tip cells of maize, the CDC2 homologue is predominantly localized in the nucleus, with the exception of the nucleolus [11]; in alfalfa cell suspension cells, CDC2MsA is exclusively localized in the nucleus of S phase- and G2 phase-arrested cells [12]; in onion root tip cells the PSTAIRE-containing CDK homologue is mainly located in the cytoplasm [13].

Division of plant cells not only involves karyokinesis and cytokinesis, but also the control to form daughter cells with the correct relative position to their surrounding cells. The acquisition of cell identity is determined by the relative position in a meristem [14]. To ensure that the relative position of the cell wall and, subsequently, of the cells to each other is correct, immobile plant cells have a unique structure which determines the position of the future cell wall, namely the pre-prophase band (PPB) [15]. An *Arabidopsis* mutant lacking PPBs underlined the importance of this positional information by the formation of distorted cells and tissues [16]. Immunocytochemical staining of onion root tip cells with an anti-PSTAIRE antibody and of maize cells with an anti-C-terminal CDC2 antibody revealed that the plant CDC2 homologue co-localizes with the PPB microtubules [11,13]. Therefore, it has been suggested that the p34^{cdc2} homologue of plants has a function in PPB assembly [11].

Here, we use an anti-PSTAIRE and an anti-CDC2aAt antibody to document the cell cycle-dependent immunofluorescent localization of CDC2 in root tip cells of *Medicago sativa*. During interphase, CDC2 was localized both in the cytoplasm and in the nucleus, but was excluded from the nucleolus. During mitosis, we could show that CDC2 was localized at the position of the PPB, the spindle, the phragmoplast, and of the condensed chromosomes. Our data suggest that plant CDC2/cyclin complexes not only are involved in processes common to yeast and vertebrates (nuclear envelope breakdown, chromosome condensation, and spindle formation), but probably also play a role in the regulation of plant-spe-

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Abbreviations: BSA, bovine serum albumin; CDC, cell division cycle; CDK, cyclin-dependent kinase; CLSM, confocal laser scanning microscopy; DAPI, 4′,6-diamidino-2-phenylindole; EGTA, ethylene glycol-bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid; MAP, microtubule-associated protein; MTSB, microtubule-stabilizing buffer; PIPES, piperazine-*N,N′*-bis(ethanesulfonic acid); PPB, pre-prophase band; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; STBI, soybean trypsin inhibitor; Tween, polyoxyethylenesorbitan

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cific mitotic events, such as the disassembly of PPB and the formation of phragmoplast.

2. Materials and methods

2.1. Plant material

Arabidopsis thaliana cell suspension culture (ecotype Colombia, gift of Dr. M. Axelos, Toulouse) was maintained as described previously [17]. Sterile *A. thaliana* seeds (ecotype C24 and the 35S-cdc2a$^+$ transgenic line [18]) were germinated at 22°C on K1 medium in a 16-h light/8-h dark interval. Seeds of alfalfa were germinated at room temperature in the dark on moist filter paper. Root tips were harvested 3 days after germination.

2.2. Protein blot analysis

Protein extracts of plant tissues were prepared as follows. Tissue was harvested and frozen in liquid N_2. The proteins were extracted by grinding cells with quartz sand in extraction buffer (25 mM Tris-HCl, pH 7.6, 15 mM $MgCl_2$, 15 mM EGTA, 75 mM NaCl, 15 mM *para*-nitrophenylphosphate, 60 mM β-glycerophosphate, 1 mM dithiothreitol, 0.1% Nonidet NP-40, 0.1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride; 10 μg/ml leupeptin, 10 μg/ml aprotinin, 5 μg/ml antipain, 5 μg/ml chymostatin, 5 μg/ml pepstatin, 10 μg/ml STBI, 0.1 mM benzamidine). The crude homogenate was centrifuged at 40 000×*g*, 1 h, 4°C and then at 200 000×*g*, 1 h, 4°C. Total protein was separated by 12% SDS-PAGE, electroblotted onto nitrocellulose membrane (Hybond C-super; Amersham, Aylesbury, UK), and further processed as described earlier [18].

2.3. Immunofluorescence

Root tips were fixed and prepared following standard procedures [19]. Squashed root tip cells were incubated overnight at 4°C with a mixture of anti-α-tubulin (1/100, Amersham) and anti-CDC2aAt (1/200) or anti-PSTAIRE (1/100) antibody in 5% BSA, microtubule-stabilizing buffer (MTSB; 50 mM PIPES, 5 mM EGTA, 5 mM $MgSO_4$). After washing with MTSB for 1 h, cells were incubated with secondary antibody. The secondary antibodies against rabbit anti-CDC2aAt and anti-PSTAIRE antibodies consisted of a 1/200 dilution of biotinylated goat anti-rabbit antibody (Amersham). The secondary antibody was detected either with a fluorescein-conjugated extra avidin (1/100; Sigma, St. Louis, MO) or with rhodamine-conjugated avidin D (1/100; Vector Laboratories, Burlingame, CA, USA). Secondary antibodies used to detect the anti-α-tubulin antibody were an anti-mouse IgG Texas red-linked whole antibody from sheep (1/100; Amersham) or an anti-mouse IgG fluorescein-linked F_{ab} fragment from sheep (1/50; Sigma), depending upon the fluorochrome used for the anti-CDC2aAt or anti-PSTAIRE antibody. The incubation time for the secondary antibody was 2 h at 37°C. Fluorochrome-conjugated avidin was incubated for 30 min at 37°C. Finally, the slides were washed with MTSB for 1 h, stained with 1 μg/ml DAPI for 2 min, rinsed, dried, and mounted in Vectashield (Vector Laboratories). Control experiments of single labeling, either with anti-CDC2aAt or with anti-PSTAIRE antibody, gave results comparable to a double labeling regime. Changing the fluorochrome label from Texas red to fluorescein and vice versa on the secondary antibody or on avidin had no effect on the results. The use of the K1/K2 filter block eliminated a possible leakage of the Texas red-labeled microtubules, specifically excited with the 568 nm line of the krypton-argon laser, into the image of fluorescein-labeled CDC2.

Arabidopsis leaf protoplasts (ecotype C24 wild-type and a transgenic *Arabidopsis* line overproducing CDC2a, 35S-cdc2a$^+$ [18]) were prepared by standard procedures [20] and immunolabeled as follows. Protoplasts were fixed in 8% paraformaldehyde in 100 mM PIPES, 10 mM EGTA, 10 mM $MgSO_4$, 0.4 M mannitol for 1 h, washed once in MTSB, 0.4 M mannitol, and incubated with MTSB, 0.5% Tween-20, 5% BSA for 30 min. After centrifugation, the protoplasts were incubated overnight at 4°C with anti-CDC2aAt (1/200) or anti-PSTAIRE (1/100) or anti-α-tubulin (1/100) antibody diluted in MTSB, 0.5% Tween-20, 5% BSA, washed for 1 h in MTSB and incubated with the secondary antibody for 1.5 h at room temperature. The secondary antibodies were used as for the root tip immunofluorescence. The protoplasts were after washing stained with DAPI (1 μg/ml) for 5 min and mounted in Vectashield (Vector Laboratories) on poly-L-lysine-coated (10 mg/ml) slides.

2.4. Fluorescence microscopy

Images were recorded with a MRC-600 Confocal Laser Scanning Microscope (CLSM) system (Bio-Rad, Hercules, CA, USA). Fluorescein and Texas red fluorescence were recorded with the K1/K2 filter block combination for excitation with the 488 nm or 568 nm lines, respectively, from a krypton-argon laser (Ion Laser Technology, Salt Lake City, UT). Fluorescence from DAPI-counterstained chromatin was recorded with the IN1/IN2 filter block combination for excitation with the 363 nm line of an Ar^+ ion laser (Innova Technology, Palo Alto, CA). Optical sections were recorded at intervals of 0.5 μm. Black and white photographs were taken from a high-resolution video display with T-max 100 ASA film (Eastman Kodak, Richmond, NJ).

The mesophyll cells and alfalfa root tip cells of Fig. 2 were examined and photographed with an Axioskop microscope (Zeiss, Oberkochen, Germany) using the filter sets 9 and 10 for fluorescein and 01 for DAPI. Color slides were taken with Fujichrome 400 ASA film.

3. Results

3.1. Immunospecificity of the anti-CDC2aAt antibody

In a total protein extract of *A. thaliana* cell suspension and of alfalfa roots, the anti-CDC2aAt antibody as well as the anti-PSTAIRE antiserum detected one protein band of 34 kDa (Fig. 1a, lanes 1 and 2, lane 3 and 4). Because both CDC2 homologues of alfalfa, CDC2MsA and CDC2MsB, are highly identical at the amino acid level (89%) and because both contain the PSTAIRE region and have the same molecular weight, it was impossible to determine which CDC2 homologue was recognized by the anti-CDC2aAt or by the anti-PSTAIRE antibody. For this reason, the 34-kDa protein of *Arabidopsis* and of alfalfa recognized by the anti-CDC2aAt and by the anti-PSTAIRE antibody will be referred to here-

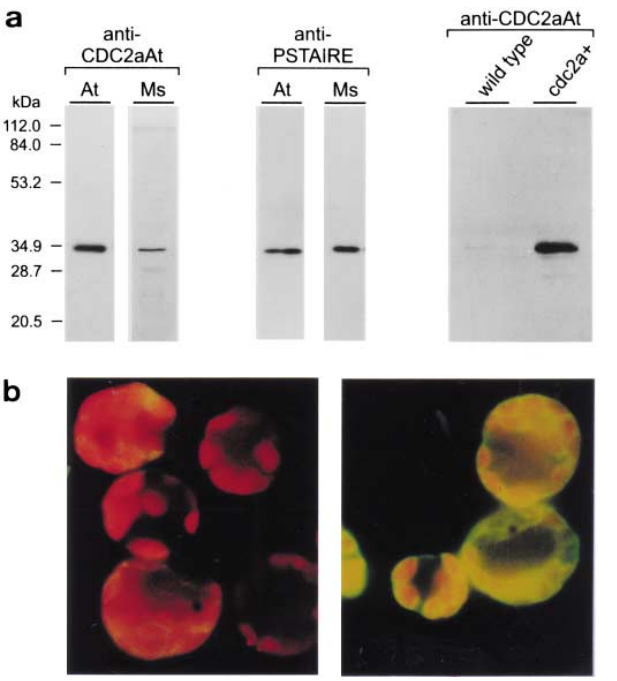

Fig. 1. a: Immunoblot of total protein extracts of *A. thaliana* cell suspension culture (At), root tissue of 3-day-old alfalfa seedlings (Ms), and leaf tissue of 4-week-old wild-type (wild type) and 35S-cdc2a$^+$ transgenic (cdc2a$^+$) *Arabidopsis* plants. Lanes 1, 2, 5, and 6 were probed with polyclonal anti-CDC2aAt antiserum, lanes 3 and 4 with polyclonal anti-PSTAIRE antibody. b: Immunofluorescence labeling of *A. thaliana* mesophyll protoplasts with anti-CDC2aAt antiserum. Protoplasts were isolated from wild-type (left panel) or from 35S-cdc2a$^+$ transgenic plants (right panel). Magnification = 1050×.

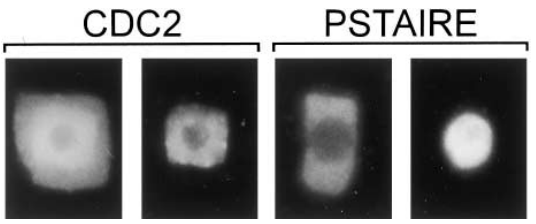

Fig. 2. Anti-CDC2 and anti-PSTAIRE signals together with DAPI chromosomal staining during interphase. Depending on the antiserum used, a stronger CDC2 nuclear or cytoplasmic signal was observed during interphase. Magnification = 540×.

after as CDC2. Further evidence for the immunospecificity of the used antibodies was obtained by immunological analysis of CDC2 in a transgenic *Arabidopsis* line (35S-cdc2a$^+$), which ectopically overproduced wild-type CDC2aAt. In adult leaves of untransformed plants, the protein levels of CDC2aAt were very low and only detectable after prolonged exposure (Fig. 1a, lane 5), whereas in the 35S-cdc2a$^+$ transgenic line, CDC2aAt was highly abundant (Fig. 1a, lane 6). The difference in CDC2aAt protein content between wild-type and 35S-cdc2a$^+$ transgenic plants could also be observed after immunofluorescence microscopy of mesophyll cells derived from these plants (Fig. 1b). In wild-type cells, a very weak green fluorescent CDC2aAt signal was localized in the cytoplasm, between the red autofluorescence of the chloroplasts (Fig. 1b, left panel). In 35S-cdc2a$^+$ protoplasts, the high levels of CDC2aAt protein resulted in a strong cytoplasmic signal partially masking the red autofluorescence (Fig. 1b, right panel). Similar observations were made using the anti-PSTAIRE antibody (data not shown).

3.2. *CDC2 localization in meristematic root tip cells during interphase*

Initially immunofluorescent labeling experiments of CDC2 were performed simultaneously in root tip cells of *A. thaliana* and *Medicago sativa*, the two most advanced models for cell cycle research in plants. We could observe similar subcellular localization patterns in cells of both plants, albeit with a higher quality for alfalfa because the cells were larger. Furthermore, it was technically easier to reproducibly generate separated intact squashed alfalfa cells. Therefore, we focused the immunolocalization to alfalfa rather than to *Arabidopsis*. Consequently, the data shown are of alfalfa cells. We are currently adapting the immunolocalization protocol for *A. thaliana*.

During interphase, CDC2 was localized both in the cytoplasm and in the nucleus, but was excluded from the nucleolus (Figs. 2 and 3a). Immunofluorescence staining with the anti-CDC2aAt antibody resulted in a stronger nuclear than cytoplasmic signal (CDC2; Fig. 2). Neither in the nucleus nor in the cytoplasm could any specific subcellular structures be distinguished with which CDC2 was associated, nor could any association with the cortical microtubules be observed (Fig. 3a). Using the anti-PSTAIRE antibody, a strong cytoplasmic and a very weak nuclear signal (PSTAIRE; Fig. 2) were detected.

3.3. *CDC2 co-localizes with the PPB, the spindle, the phragmoplast, and the condensed chromosomes*

The cytoplasmic CDC2 signal persisted during the entire cell cycle. During PPB formation, when the microtubular ring is broad, CDC2 was localized both in the cytoplasm and in the nucleus and did not co-localize with the PPB (Fig. 3b). In late prophase cells, a co-localization of CDC2 with the narrowing PPB was observed in approximately 35% (Fig. 3c). At that stage, the CDC2aAt signal became consistently weaker in the center of the nucleus and appeared to surround the nucleus and associate with the nuclear envelope (Fig. 3c). Using the anti-PSTAIRE antiserum, we observed similar localization patterns during prophase, no co-localization with the broad PPB (Fig. 5a), and strong association with the narrow PPB (Fig. 5b).

At the end of prophase, when chromosomes are lining up at the metaphase plate, CDC2 was excluded from the chromosomal material and became distributed at the position of the spindle (Fig. 4a); this signal, however, was weaker than that of the cytoplasm. As metaphase progressed, a prominent association of CDC2 with the microtubules of the spindle microtubules was observed (Fig. 4b). The anti-PSTAIRE signal strongly co-localized with the spindle during the entire metaphase (Fig. 5c).

At anaphase, when the spindle poles became broader and the lined-up chromosomes were starting to segregate into two chromatids, the anti-CDC2aAt antibody localized strongly at the chromosomes (Fig. 4c). This co-localization was found in 36% of all anaphase cells. Association with the spindle was not seen during this stage. During later stages of the anaphase, CDC2 localization to the chromosomes was absent. Instead CDC2 became concentrated at the anaphase spindle. The signal was, however, not as strongly associated with the microtubules as the metaphase spindle (Fig. 4d). By using the anti-PSTAIRE antiserum during anaphase, a strong signal was seen between the two chromosome pairs at the future position of the cell wall, before the phragmoplast was visible

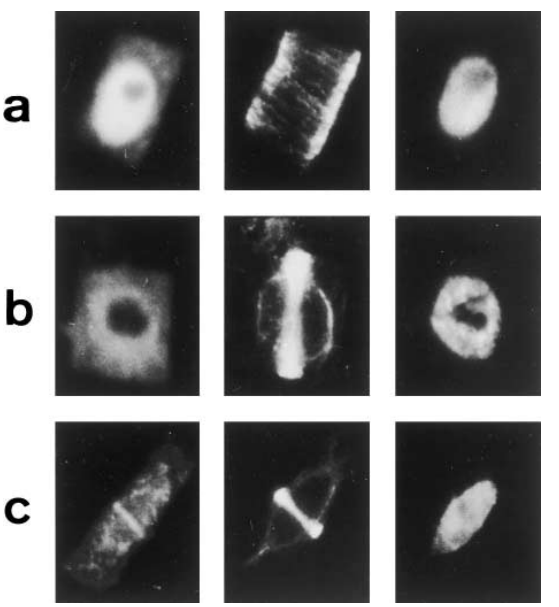

Fig. 3. CDC2 localization during interphase and prophase. Cells were stained with anti-CDC2aAt antiserum (left column), anti-α-tubulin antibody (central column), and DAPI (right column). a, b, and c show a root tip cell during interphase, early prophase, and late prophase, respectively. In late prophase, a narrower PPB (c) is observed than in the pre-prophase cell (b), the nucleolus is no longer visible, and CDC2 co-localizes with the PPB and the periphery of the nucleus. Magnification = 900×.

(Fig. 5d). We could not assign the organellic structure to which this signal might co-localize.

During telophase, CDC2 was seen to co-localize with microtubules of the phragmoplast when detected either with the anti-CDC2aAt (Fig. 4e) or with the anti-PSTAIRE antiserum (Fig. 5e). Co-localization of the anti-CDC2aAt antibody with the phragmoplast was more evident during later stages of the telophase (Fig. 4f). The anti-PSTAIRE antiserum showed, besides an association with the phragmoplast, a strong cytoplasmic signal, mainly at the position of the newly formed cell wall (Fig. 5e).

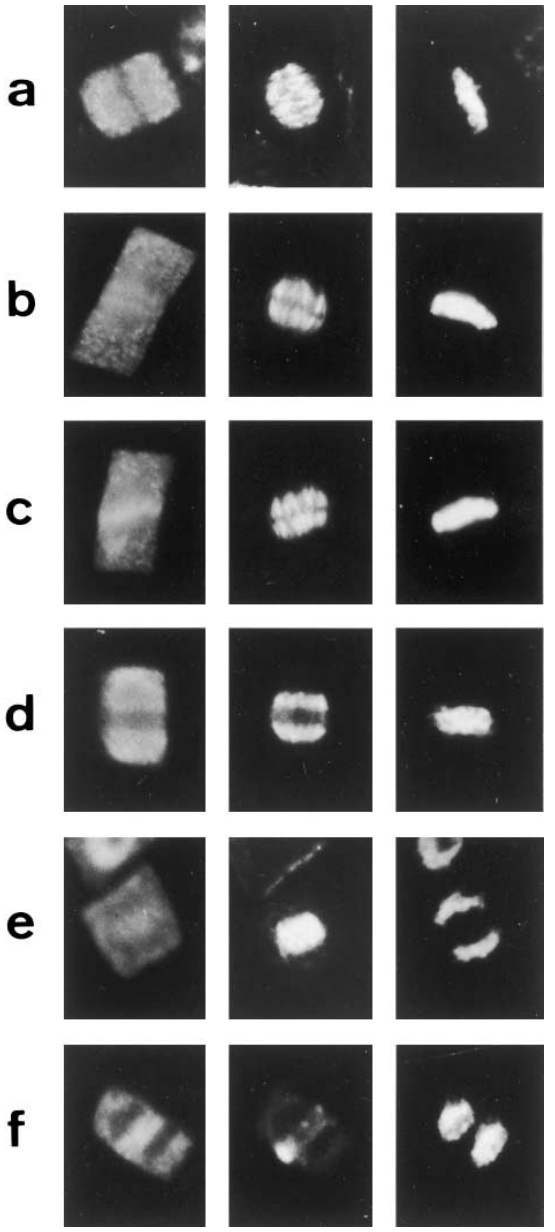

Fig. 4. CDC2 localization during meta-, ana-, and telophase. Cells were stained with anti-CDC2aAt antiserum (left column), anti-α-tubulin antibody (central column) and DAPI (right column). a, d: Root tip cell during meta- and anaphase, respectively, in which CDC2 is distributed at the position of the spindle; b, e, f: strong co-localization of CDC2 with the microtubules of the spindle, early, and late phragmoplast, respectively; c: strong co-localization with condensed chromosomes. Magnification = 900×.

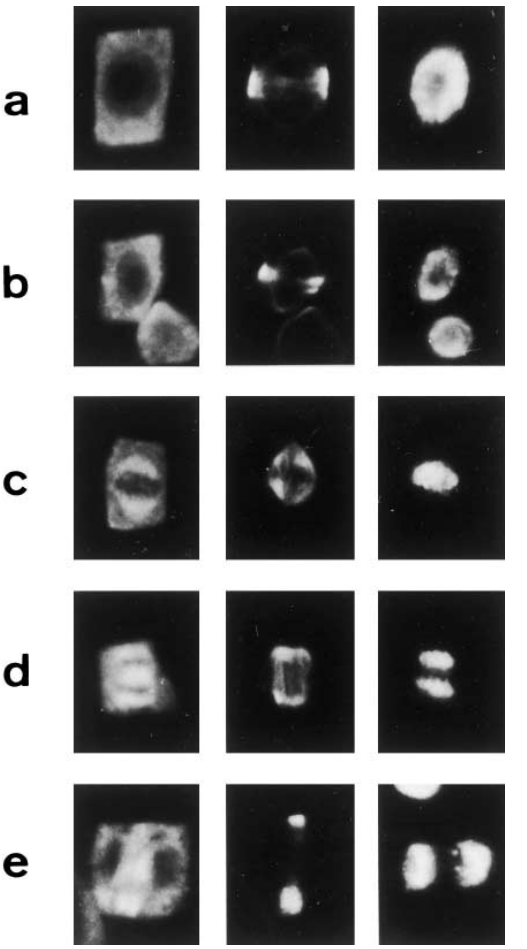

Fig. 5. Distribution of the anti-PSTAIRE signal during the cell cycle. Cells were stained with anti-PSTAIRE antiserum (left column), anti-α-tubulin antibody (central column), and DAPI (right column). Root tip cell during early prophase (a), late prophase (b), metaphase (c), anaphase (d), and late telophase (e). Magnification = 900×.

4. Discussion

4.1. CDC2 localization during interphase

We found that in interphase cells of *Arabidopsis* and of alfalfa, CDC2 was localized in both the nucleus and the cytoplasm. Previously, CDC2 was reported to be mainly concentrated in the nucleus of maize and in alfalfa cells [11,12], whereas in onion root cells it was mainly localized in the cytoplasm [13]. Similar contradictory observations about the cellular distribution of CDC2 during interphase were made in yeast and mammalian cells. Nevertheless, biochemical data obtained after subcellular fractionation of mammalian cells confirmed that CDC2 was present in both cytoplasmic and nuclear fractions [4].

Depending on the antibody used, anti-CDC2aAt or anti-PSTAIRE, we observed a stronger nuclear or a stronger cytoplasmic immunofluorescent signal, respectively. Crystal structure analysis of the human CDK2/cyclin A/ATP complex revealed that the PSTAIRE helix of CDKs is central to the interaction with cyclins [21]. Interaction of CDC2 with a cyclin could be an explanation for the weaker nuclear PSTAIRE signal during interphase. Such a mechanism, however, makes

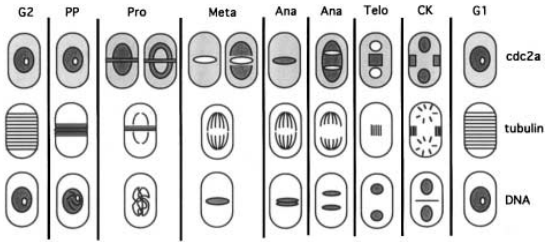

Fig. 6. Schematic representation of the cell cycle-dependent localization patterns of CDC2. The top, middle, and bottom rows show the cell cycle-dependent fluorescent labeling of CDC2, microtubules, and chromosomal material, respectively. During prophase, two patterns of nuclear CDC2 localization were observed: a strong localization at the nucleus and a concentration at the periphery of the nucleus either during early or later stages of the prophase (Pro). Depending on the stage of the metaphase, a stronger or a weaker co-distribution of CDC2 with the spindle microtubules was found (Meta). As the condensed chromosomes were separating, CDC2 co-localized strongly with them (Ana). At that moment, a weaker signal than that of later stages was observed at the position of the anaphase spindle. During telophase and cytokinesis, CDC2 co-localized with the phragmoplast. Abbreviations: Ana, anaphase and anaphase transition; CK, cytokinesis; G1, G1 interphase; G2, G2 interphase; Meta, metaphase; PP, pre-prophase; Pro, prophase; Telo, telophase.

it difficult to explain why the PSTAIRE signal during mitosis co-localizes so strongly with microtubular structures.

4.2. *CDC2 co-localizes with the PPB, the spindle, and the phragmoplast during mitosis*

During late pre-prophase, plant cells form a PPB. This band of closely packed microtubules at the periphery of the cell encircles the nucleus and predicts the position of the future cell wall by a yet unknown mechanism [15]. Immunostaining of PSTAIRE-containing protein in onion root [13] and an antibody specific for the maize $p34^{cdc2}$ co-localized with the PPB [11], suggesting a role of the CDC2 plant homologue in the assembly of the PPB [11]. Conversely, microinjection of a plant mitotic CDK preparation into early prophase *Tradescantia* cells caused, besides acceleration of chromatin condensation and nuclear envelope breakdown, a rapid disassembly of the microtubules of the PPB but not of interphase cortical, spindle, or phragmoplast microtubules [22]. Co-localization of the plant CDC2 homologue with the microtubules of the PPB and with the periphery of the nucleus during the G2/M transition, detected either with the anti-CDC2aAt or with the anti-PSTAIRE antibody, could reflect a function of CDC2 in the disassembly of these structures and, hence, in the re-organization of the microtubule cytoskeleton at the onset of M phase. Consistent with our data, a similar association with the late PPB and the nuclear envelope only just prior to their breakdown was reported for cyclin ZmIb, a cyclin B1 homologue from maize [23]. As such, the plant CDC2/cyclin B kinase could be involved in the processes as nuclear envelope and PPB breakdown.

At prophase, plant cells form de novo the mitotic spindle on the outer surface of the nuclear envelope [24]. CDC2 co-localized with the spindle microtubules, as metaphase progressed. In yeast and animals a biochemical and cellular analysis showed a strong association of the CDC2/cyclin B complex with the mitotic spindle [4,6,8,10,25–27]. The interaction with the microtubules is, in vertebrates, mediated through binding of cyclin B with microtubule-associated protein 4 (MAP4) [10,28]. In vitro phosphorylation of MAP4 by CDC2 kinase alters its microtubule-polymerizing ability [28]. A MAP protein fraction, isolated from mitotic tobacco BY2 cells, more efficiently induced microtubule assembly in isolated tobacco nuclei than a MAP fraction isolated from interphase cells [29]. Because maize cyclin ZmII has been reported to be also localized at the spindle [23], an active CDC2/cyclin complex associated with the spindle could regulate the activity of the plant MAPs and hence regulate the microtubule dynamics. Moreover, preliminary data showed that an α-tubulin fraction co-purified with CDC2 on a $p9^{CKShs1}$-Sepharose column, suggesting an association of the plant CDC2 kinase with microtubules (data not shown).

Association of CDC2 with the microtubule arrays persisted during anaphase and telophase. Interpretation of an apparent co-localization to the phragmoplast must be made with caution because, due to simple exclusion of organelles by the palisade of microtubules, a more accessible volume for soluble proteins is created within the phragmoplast [30]. However, we noted a difference in immunofluorescence intensities using the anti-PSTAIRE or the anti-CDC2aAt antibody, suggesting a specific localization of the different antibodies. Moreover, during late anaphase the PSTAIRE-containing protein localized at the region where the phragmoplast would be formed, before any microtubules were visible (Fig. 5d). These data, together with the persistence of plant cyclins through telophase and their strong association with the microtubules of the phragmoplast [23], support the idea that in plants a new kind of CDK/cyclin kinase complex would be involved in regulation of the phragmoplast function, hence, in the formation of the cell plate.

4.3. *CDC2 strongly co-localizes with chromosomes at anaphase transition*

CDC2 was strongly, transiently concentrated at the chromosomal material, when the lined-up chromosomes started to segregate. Association with condensed chromosomes has previously been reported for cyclin B in *Drosophila* embryos [31], in HeLa cells [25,27], for the active CDC2/cyclin B complex in starfish oocytes [10] and for cyclin ZmIb in maize cells [23].

Our data on immunolocalization of CDC2 (summarized in Fig. 6), together with the data on the localization on maize cyclins [23], suggest that active plant CDC2/cyclin complexes are involved in processes common to yeast and vertebrates, such as nuclear envelope breakdown, chromosome condensation, and spindle formation. But they also indicate that in higher plants the cell cycle proteins are associated with plant-specific microtubular arrays, such as the PPB and phragmoplast.

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