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Cell Division and Subsequent Radicle Protrusion in Tomato Seeds Are Inhibited by Osmotic Stress But DNA Synthesis and Formation of Microtubular Cytoskeleton Are Not[1]

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We studied cell cycle events in embryos of tomato (*Lycopersicon esculentum* Mill. cv Moneymaker) seeds during imbibition in water and during osmoconditioning ("priming") using both quantitative and cytological analysis of DNA synthesis and β-tubulin accumulation. Most embryonic nuclei of dry, untreated control seeds were arrested in the G_1 phase of the cell cycle. This indicated the absence of DNA synthesis (the S-phase), as confirmed by the absence of bromodeoxyuridine incorporation. In addition, β-tubulin was not detected on western blots and microtubules were not present. During imbibition in water, DNA synthesis was activated in the radicle tip and then spread toward the cotyledons, resulting in an increase in the number of nuclei in G_2. Concomitantly, β-tubulin accumulated and was assembled into microtubular cytoskeleton networks. Both of these cell cycle events preceded cell expansion and division and subsequent growth of the radicle through the seed coat. The activation of DNA synthesis and the formation of microtubular cytoskeleton networks were also observed throughout the embryo when seeds were osmoconditioned. However, this pre-activation of the cell cycle appeared to become arrested in the G_2 phase since no mitosis was observed. The pre-activation of cell cycle events in osmoconditioned seeds appeared to be correlated with enhanced germination performance during re-imbibition in water.

Embryos of maturing seeds exhibit a programmed transition from cell proliferation of quiescence (Buddles et al., 1993). In maturing tomato (*Lycopersicon esculentum*) seeds, this transition is characterized by the arrest of most embryonic radicle cells in the G_1 phase of the cell cycle (Liu et al., 1997). The transition from quiescence to that of cell proliferation occurs during imbibition. In tomato seeds it is characterized by increasing numbers of radicle tip cells that are in the G_2 phase of the cell cycle (Bino et al., 1992; de Castro et al., 1995). This increase is accompanied by an accumulation of β-tubulin, not only during seed imbibition (i.e. prior to radicle protrusion), but also during incubation in a solution of polyethylene glycol (M_r 6,000) that prevents radicle protrusion (de Castro et al., 1995, 1998). Both the relative number of cells in G_2 and the level of β-tubulin are correlated with enhanced seed performance after osmotic treatment ("priming effect") (de Castro et al., 1995).

The relationship between DNA replication and β-tubulin accumulation during seed germination is not yet understood. The general consensus is that, prior to radicle protrusion, radicle cells may either contain 2C DNA only or a portion of the cells may contain 4C DNA (Bewley and Black, 1994). Furthermore, it is generally accepted that mitosis only occurs after radicle protrusion, i.e. at the onset of seedling growth (Coolbear and Grierson 1979; Haigh, 1988). From recent studies it is known that during imbibition of tomato seeds, DNA replication and β-tubulin accumulation are concentrated in the embryonic radicle tip, suggesting an intimate interplay in the preparation for radicle growth (de Castro et al., 1998). However, in cabbage it was shown that the increase in 4C DNA during imbibition could be inhibited by hydroxyurea, whereas β-tubulin accumulation and radicle protrusion were unaffected (Górnik et al., 1997). Thus, questions as to whether the increase in 4C DNA is causally related to the accumulation of β-tubulin, whether it is restricted to the radicle tip, whether it leads to cell division only after radicle protrusion, and whether this is species dependent remain to be clarified. We address these questions by using an immunohistochemical analysis of DNA synthesis activity and organization of the microtubular cytoskeleton. These cytological data are compared with data obtained from a quantitative analysis of DNA replication and β-tubulin accumulation that was executed in parallel.

MATERIALS AND METHODS

Seed Material and Imbibition Conditions

Seeds of tomato (*Lycopersicon esculentum* Mill. cv Moneymaker) with a moisture content of 6.0% ± 0.1% (on a fresh weight basis) were used in the present study. Seed cleaning, drying, and storage were as previously described (de Castro et al., 1995). Dry seeds were imbibed in water or

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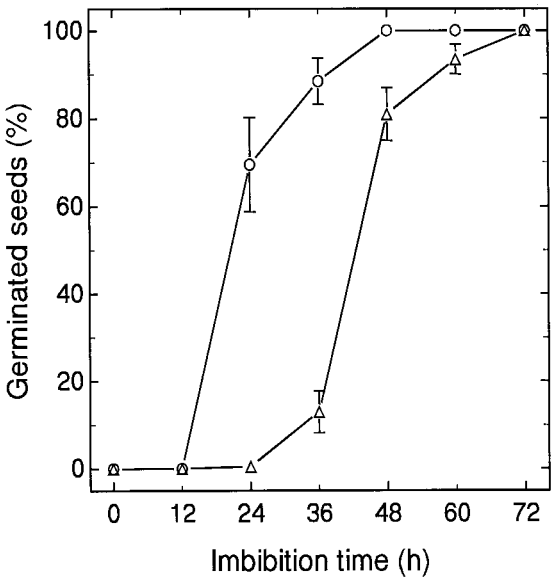

Figure 1. Germination of control (△) and osmoconditioned (○) tomato seeds (±SE), cv Moneymaker, upon imbibition in water. During osmoconditioning in −1 MPa PEG no germination occurred.

were osmoconditioned in −1.0 MPa PEG-6000 (Serva, Heidelberg) for 7 d at 25°C (de Castro et al., 1998), re-dried, and then re-imbibed in water.

Germination

Germination analysis was conducted on four replicates of 50 seeds placed on top of two layers of filter paper soaked with 6 mL of distilled water or −1.0 MPa PEG-6000 at 25°C ± 1°C in darkness for 7 d. Germination was expressed as the percentage of seeds that exhibited 1-mm radicle protrusion.

Flow Cytometry and Detection of β-Tubulin

Two replicates of five whole embryos were used for flow cytometric analysis of nDNA contents according to the method of Sacandé et al. (1997). With all samples, at least 10,000 nuclei were analyzed. Extraction and detection of β-tubulin by western blotting were conducted as described previously (de Castro et al., 1995, 1998).

Immunohistochemical Detection of Bromodeoxyuridine (BrdU) and β-Tubulin

Seeds were imbibed in the PEG-6000 solution or in water, and subsequently immersed in a 1:500 (v/v) BrdU solution (Amersham, Buckinghamshire, UK) at 25°C in the dark, either as longitudinally cut dry seeds or as isolated embryos from imbibed seeds. From each of the studied stages, at least five embryos were randomly selected, except when a distinction was made between germinated (radicle protruded) and ungerminated seeds. Ten to 20 sections on the same slide were observed for each embryo. One of the median sections was selected as representative for the whole population. Independent repetitions using this protocol yielded essentially similar results.

The cytotoxicity of the BrdU solution (Rös and Wernicke, 1991) was assessed at various pulse lengths by comparing the pattern of the flow cytometric profiles and the microtubular cytoskeleton with the patterns observed in the absence of BrdU. A 3-h pulse length was found to be optimal because it allowed detection of BrdU incorporation without cytotoxic effects. Furthermore, the microtubular cytoskeleton was investigated in material that was not incubated with BrdU in order to avoid any negative effect of immersion in BrdU-containing solutions. The 3-h BrdU labeling time is indicated between brackets after the times of imbibition. Embryos were fixed in 4% (w/v) paraformaldehyde, dehydrated, and embedded in butylmethylmetacrylate according to the method of Baskin et al. (1992). Samples were sectioned, affixed on slides, and processed either for the detection of incorporated BrdU or for microtubular cytoskeleton.

Labeling of β-tubulin and BrdU was according to the method of Xu et al. (1998). An anti-β-tubulin monoclonal antibody (Amersham) was diluted 1:200 (v/v) and anti-BrdU (Amersham) was diluted 1:1 (v/v). Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse (1:200, v/v) was the second antibody (Amersham). nDNA was counterstained with 1 mg mL^{-1} propidium iodide (Molecular Probes, Eugene, OR). Omission of the first antibody and application of preimmune serum served as controls and showed no fluorescence. Confocal laser scanning microscopy and photography were as described by Xu et al. (1998).

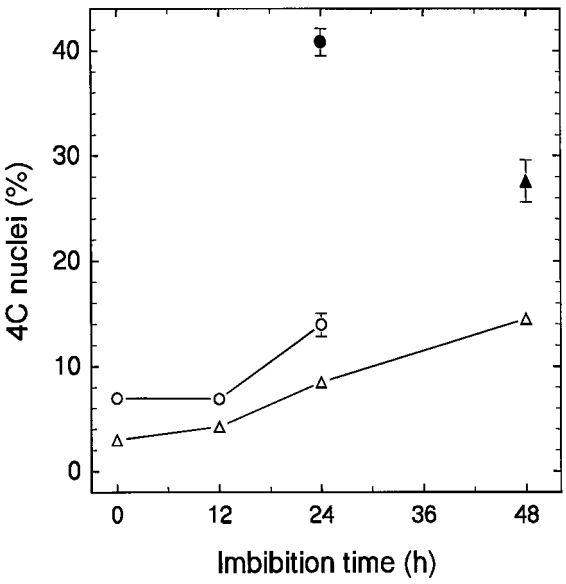

Figure 2. Frequency of nuclei with 4C DNA contents (±SE) expressed as percentage of the total number of nuclei (2C + 4C) from embryos of control (triangles) or osmoconditioned (circles) seeds during imbibition (white symbols) and from seedlings after completion of germination (black symbols) (i.e. radicle protrusion of approximately 1 mm).

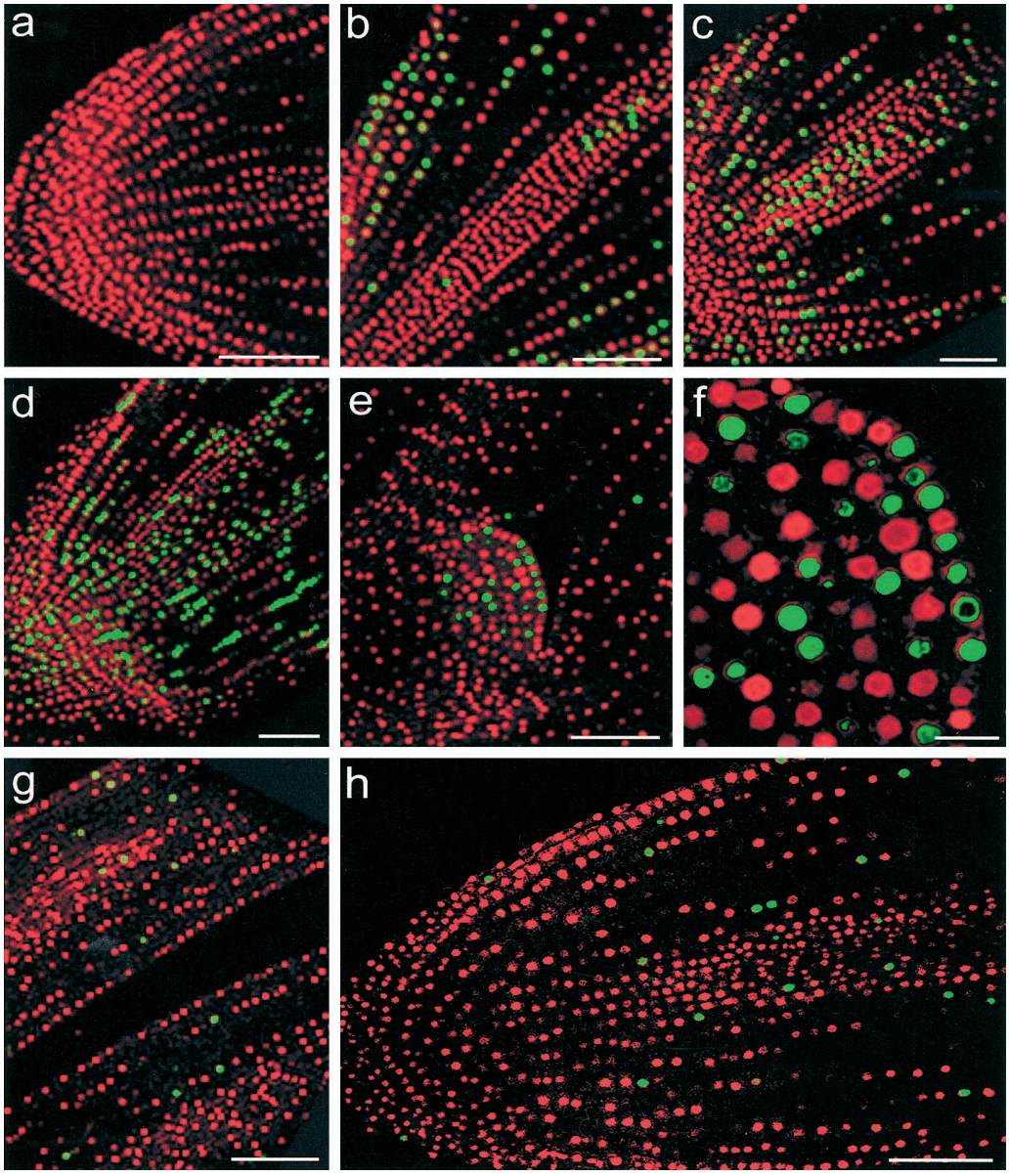

Figure 3. Development of DNA synthesis in tomato embryos during seed germination. Shown are fluorescent micrographs of longitudinal sections of embryos from untreated control seeds during germination (a–g) and embryos from dried osmoconditioned seeds (h). Nuclei show red fluorescence as a result of staining with propidium iodide. Nuclei showing green fluorescence are labeled with FITC, which indicates BrdU incorporation into actively replicating DNA (S-phase). Bars indicate 100 μm (a–e, g, and h) or 25 μm (f). a, Radicle tip region of dry control seeds showing the absence of BrdU incorporation after a 3-h pulse labeling, indicating the absence of DNA synthesis. b, Radicle tip of control seeds showing nuclei labeled with BrdU after a 3-h pulse labeling at 12 h of imbibition, indicating the initiation of nDNA synthesis. c, BrdU labeling in the radicle tip of control seeds imbibed for 24 h. Note that there are more nuclei labeled with BrdU than at 12 h (b), indicating higher DNA synthesis activity at this stage. d to g, BrdU labeling in the radicle tip (d), shoot meristem (e and f), and cotyledons (g) of germinated control seeds at 48 h of imbibition. At this stage, DNA synthesis activity in the radicle tip was highest and had also started in the shoot meristem and cotyledons. In the close-up view of the shoot meristem (f), unsynchronized cells containing nuclei with various levels of BrdU labeling showing early and late stages of S-phase can be seen. h, Radicle tip region of re-dried osmoconditioned seeds.

RESULTS

Germination

Germination of control and osmoconditioned seeds was determined to assess its relationship with embryonic nDNA replication and β-tubulin accumulation. Osmoconditioned seeds attained 100% germination within 48 h after transfer to water, and control seeds within 72 h (Fig. 1). Thus, osmoconditioned seeds germinated approximately 1 d earlier than the control seeds and had a time to 50%

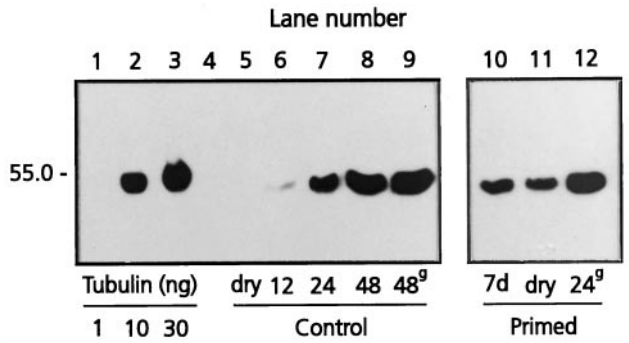

Figure 4. β-Tubulin accumulation in embryos of tomato seed during germination. β-Tubulin levels are shown for embryos of untreated control seeds during imbibition in water (12–48 h, lanes 5–9), as well as for those of seeds after 7 d of osmoconditioning, after re-drying, and during subsequent imbibition in water (lanes 10–12). Total protein loaded per lane was 30 μg. Lanes 1 to 3 were loaded with 1, 10, and 30 ng of pure bovine brain tubulin, respectively. The films were exposed for a maximum of 1 min. [g], Embryos of seeds that had germinated (i.e. with 1-mm radicle protrusion).

germination (t_{50}) of 22 h, compared with 44 h for control seeds.

Amounts and Distribution of nDNA Synthesis

Flow cytometric histograms from embryonic nuclei of dry control seeds showed one large peak, corresponding to the 2C DNA content (G_1 phase of the cell cycle), and a second smaller peak with about twice the amount of fluorescence, corresponding to nuclei with replicated 4C DNA content (G_2 phase) (not shown). During imbibition in water, the relative portion of 4C nuclei significantly increased, indicating nDNA replication activity (Fig. 2). An increase in the frequency of embryonic 4C nuclei was also observed after 7 d of osmoconditioning in PEG-6000. The frequency of 4C nuclei in osmoconditioned and dried-back seeds was significantly higher than that of control seeds (Fig. 2, 7% versus 3%, $P < 0.05$). Upon imbibition in water, the frequency of 4C nuclei steadily increased in control seeds during the 48 h of measurement. However, in osmoconditioned seeds the number of 4C nuclei started to increase only after 12 h, but was comparable to that of 48-h-imbibed control seeds after 24 h (Fig. 2). Furthermore, the frequency of 4C nuclei in the osmoconditioned seeds with protruded radicles after 24 h was significantly higher than in the control seeds that had germinated after 48 h.

DNA replication, as detected by flow cytometry, was compared with the analysis of DNA synthesis visualized by immunohistochemical detection of BrdU incorporated into actively replicating DNA (Gratzner, 1982; Ellward and Dörmer, 1985) (Fig. 3). BrdU incorporation was not observed in embryonic nuclei from dry control seeds after 3 h of labeling (Fig. 3a), but was observed in increasing levels from 12 h (plus 3 h of BrdU labeling) onward (Fig. 3, b–d). Initially, most of the BrdU labeling occurred in the radicle tip; however, by 48 h (plus 3 h of BrdU labeling) BrdU labeling was also observed in the hypocotyl (not shown), shoot meristem, and cotyledons (Fig. 3, e–g). BrdU labeling was also detected in embryonic nuclei of osmoconditioned seeds at levels that were similar before and after re-drying (Fig. 3h). As in control seeds, most BrdU labeling in embryos of osmoconditioned, dried-back seeds occurred in nuclei of the radicle tip region, but in lower numbers than in embryo radicle tips of 12-h-imbibed (plus 3 h of BrdU labeling) control seeds (Fig. 3, b and h). Upon renewed imbibition in water, the number of labeled nuclei in osmoconditioned embryos increased until completion of germination, in a pattern similar to that observed in embryos of control seeds (not shown).

β-Tubulin and Microtubule Arrays

The level of soluble β-tubulin in embryos of control seeds increased during imbibition. β-tubulin was not detected in embryos of dry control seeds, but increasing levels were detected from 12 h of imbibition onward, being highest at 48 h in embryos of germinating seeds (Fig. 4). β-Tubulin accumulated in embryos also after the osmotic conditioning of the seeds. However, the level of soluble β-tubulin in osmoconditioned embryos after seed re-drying appeared lower than before drying. During renewed imbibition of the osmoconditioned seeds in water, β-tubulin levels increased further, reaching maximum levels at 24 h of imbibition in embryos of germinating seeds (Fig. 4).

The pattern of β-tubulin accumulation detectable on western blots was compared with the pattern of microtubules detected by immunohistochemistry (Fig. 5). Analysis of sectioned embryos from dry or imbibed seeds showed that labeling of β-tubulin was found either in the form of fluorescent granules or assembled in microtubular cytoskeletal arrays (Fig. 5). Embryos of dry control seeds did not contain a microtubular cytoskeleton but did contain fluorescent fragments or granules in cells of the stele in the hypocotyl (not shown), radicle tip, shoot meristem, and meristele in the cotyledons (Fig. 5, a–c). However, during seed imbibition, β-tubulin labeling showed an increasing presence of microtubules, while the fluorescent granules became less prominent. Microtubules appeared at 12 h of imbibition, most prominently in the radicle tip region, where the formation of an integrated cortical microtubular cytoskeleton was observed (Fig. 5d).

From 12 h of imbibition onward, the appearance of the cortical microtubular configurations advanced toward the hypocotyl, the shoot meristem, and finally toward the cotyledons (Fig. 5, e–l). The accumulation of microtubules in the hypocotyl and cotyledons was initiated in the cells of the central cylinder (stele) and meristele, respectively, where fluorescent granules were initially observed (Fig. 5, c, f, i, and l). Mitotic microtubular arrays were also observed. They first appeared in the radicle tip region after 24 h of imbibition, and functioned in cell division before the radicle protruded (Fig. 5g). As a control, cell divisions were confirmed by counterstaining of the nDNA with propidium iodide (not shown). When the radicle protruded at 48 h of imbibition, cortical microtubules were apparent in

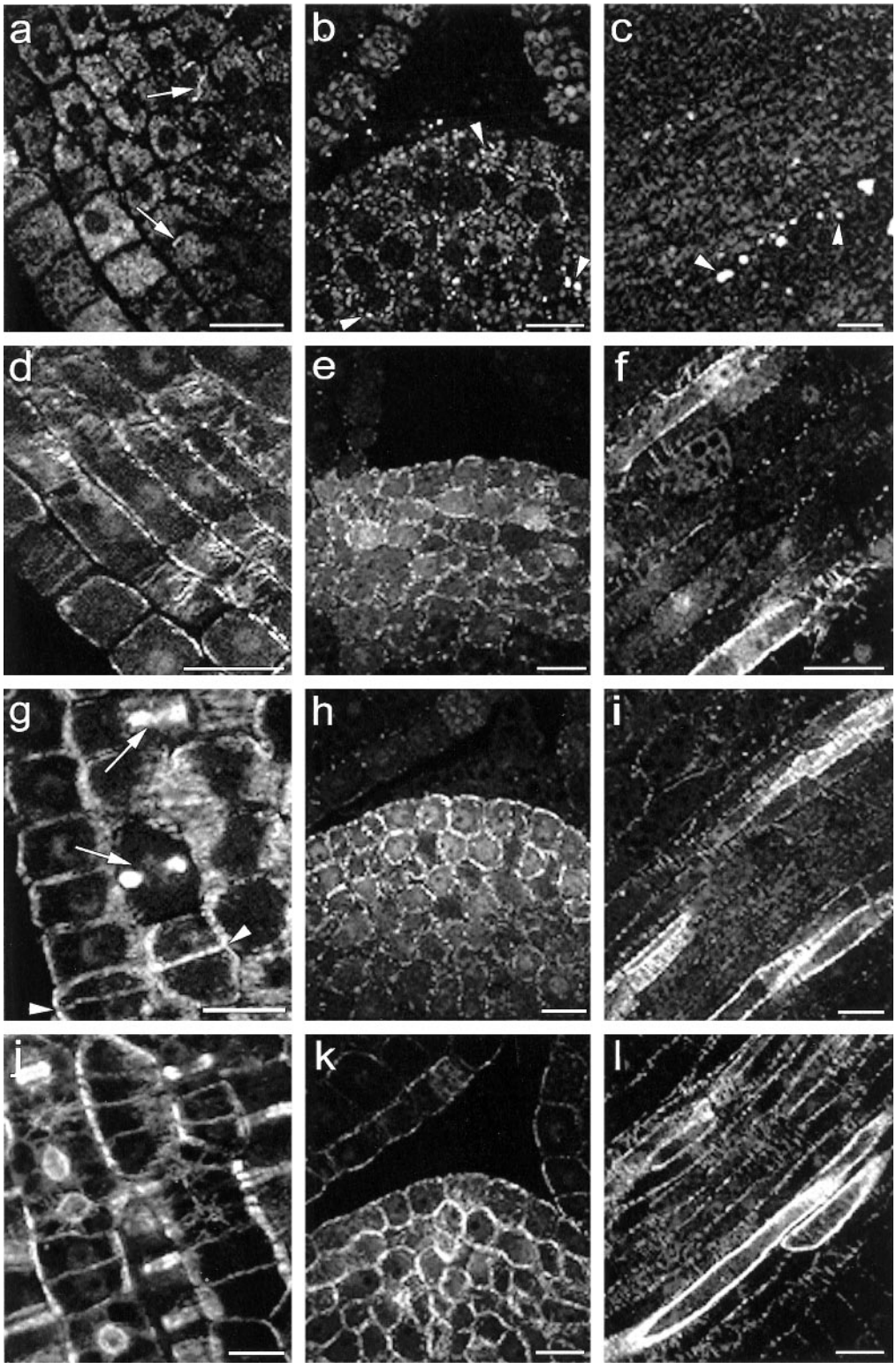

Figure 5. (Continues on next page.)

cells throughout the embryonic tissues, while at the same moment the number of mitotic arrays and divisions had increased in the radicle tip (Fig. 5j) and appeared to progress toward the hypocotyl (not shown).

Microtubules accumulated in embryos also during seed osmoconditioning. After 7 d, the radicle tip, hypocotyl, and shoot meristem contained cells with clear and well-established cortical microtubular networks, whereas in the

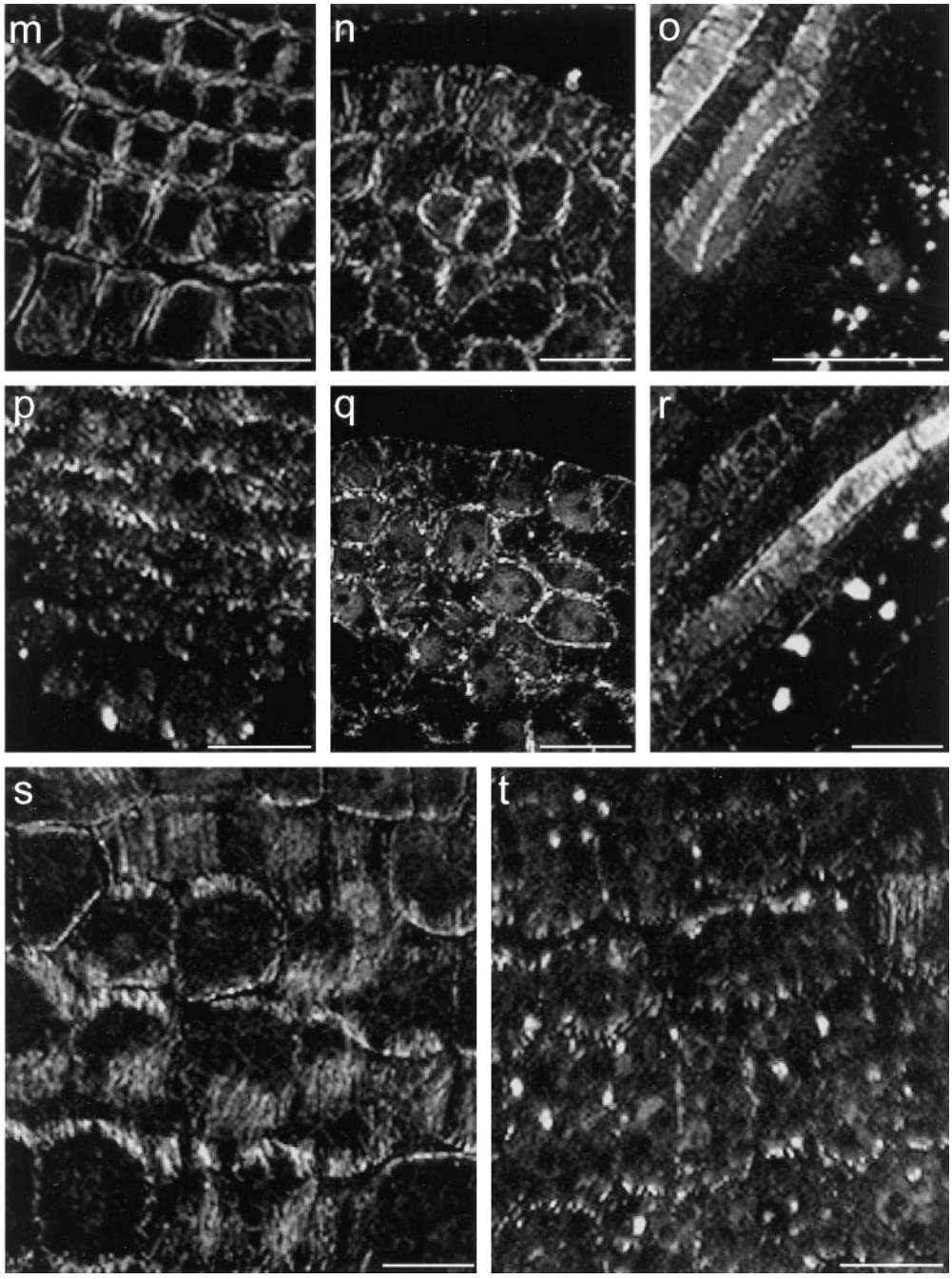

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Figure 5. Development of the microtubular cytoskeleton in embryos during tomato seed germination. Fluorescent micrographs of longitudinal sections of embryos from untreated control seeds during germination (a–l), and from osmoconditioned seeds before and after re-drying and during renewed imbibition in water (m–t) labeled with anti-β-tubulin/FITC are shown. The latter images ("primed") are all in "confocal Z-series" projections to enhance the visualization of β-tubulin either in microtubules or in granules. Bars indicate 20 μm. Because the sections are relatively thin (4 μm) with respect to the diameter of the cells, only a few cells have their cortical cytoplasm with microtubules in the plane of the section. a to c, Radicle tip (a), shoot meristem (b), and cotyledon (c) of embryos from untreated dry seeds. Note the absence of microtubules. There were only remnants of microtubules in the radicle tips (arrows) and fluorescent granules (arrowheads) in the shoot meristem and cotyledons. d to f, Radicle tip (d), shoot meristem (e), and cotyledon (f) of embryos from untreated seeds imbibed for 12 h showing β-tubulin labeling in microtubules. Note that an integrated cortical microtubular cytoskeleton was formed in the radicle tip. Microtubules accumulated in the shoot meristem and meristele of the cotyledons concomitantly with the disappearance of the tubulin granules. g to i, Radicle tip (g), shoot meristem (h), and cotyledon (i) of embryos from untreated

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cotyledons most microtubules were found in the meristele cells (Fig. 5, m–o). Mitotic arrays were not observed in the osmoconditioned embryos; the distribution of cortical microtubules in these embryos was comparable before and after re-drying. However, a significant number of small fluorescent granules were observed after re-drying in the radicle tip region and in cells of the cortex, hypocotyl, and shoot meristem. As opposed to the cotyledons (Fig. 5, c, o, and r), the granules in the radicle appeared only when seeds were re-dried after osmoconditioning (Fig. 5, p and t). During imbibition of the osmoconditioned, dried-back seeds in water, the granules disappeared, while the microtubular cytoskeleton reconstituted and appeared throughout all embryonic regions (not shown), in a pattern comparable to control seeds. However, mitotic arrays started to appear 12 h earlier, also resulting in cell divisions prior to radicle protrusion. At 24 h of imbibition, the protruded radicles of the osmoconditioned seeds contained a larger number of mitotic arrays compared with those from control seeds at 48 h of imbibition (not shown).

DISCUSSION

DNA Synthesis and Appearance of Microtubule Arrays Is Correlated with Cell Division Prior to Radicle Protrusion and Progresses from the Embryonic Radicle Tip Region toward the Cotyledons

As was previously observed (Bino et al., 1992, 1993; Liu et al., 1994, 1997; de Castro et al., 1995, 1998), the levels of 4C DNA and β-tubulin were low in embryos of dry control tomato seeds. In the dry state most metabolic activities in the seed are suppressed (Roberts and Ellis, 1989), which may contribute to the arrest of the cell cycle in the G_1 phase. In this report the absence of BrdU incorporation into embryonic nDNA from dry control seeds showed a lack of DNA synthesis activity, whereas the absence of a microtubular cytoskeleton network reflected the absence of β-tubulin, probably resulting from the process of seed dehydration during maturation.

At 12 h of imbibition, the initial accumulation of β-tubulin upon re-hydration occurred concomitantly with the initial assembly of the cortical microtubular arrays and DNA synthesis in the radicle. Evidently, rearrangement of microtubules and DNA synthesis are required for cell division (Gunning and Sammut, 1990; Gunning and Steer, 1996); however, there seems to be no relationship between cell expansion and DNA synthesis. First, as was shown for germination of cabbage (*Brassica oleraceae* L.) seeds (Górnik et al., 1997), DNA replication can also be inhibited in tomato by hydroxyurea without affecting cell expansion (Y. Liu and S.P.C. Groot, personal communication). Second, during osmoconditioning, DNA synthesis was observed but cell expansion was restricted by the low osmotic potential of the PEG-6000 solution, and cell division did not occur (Figs. 2 and 3h). The initiation of cell cycle events in maize roots requires the formation of the microtubular cytoskeleton (Baluška and Barlow, 1993). Furthermore, the synthesis of β-tubulin and assembly into cortical microtubules in meristematic cells might be a prerequisite for the formation of pre-prophase bands, as observed in wheat root tips (Gunning and Sammut, 1990).

A further 12-h lag was required for the completion of DNA replication, as a significant increase in the number of 4C nuclei was detected only at 24 h of imbibition. Evidently, this gap comprised the S-phase in the imbibing embryo, which may have been required both for replicative DNA synthesis and for DNA repair (Davidson and Bray, 1991; Osborne and Boubriak, 1997).

The increase in the number of 4C nuclei in control seeds from 24 h of imbibition onward was coincident with the occurrence of mitotic events and divisions. This may indicate that the interphase between G_2 and mitosis is short in tomato embryos and that cells in G_2 immediately enter mitosis when seeds are imbibed in water. Rearrangements of microtubules involved in establishing cell division planes, i.e. pre-prophase bands, start immediately after DNA synthesis, during G_2 (Gunning and Sammut, 1990). So far, cell division has not been visualized in the embryos of tomato seeds before the start of radicle protrusion through the endosperm and seed coat. Indeed, cell division has been considered to occur in tomato embryos only after completion of germination (Coolbear and Grierson, 1979; Haigh, 1988). However, this was based on quantitative analysis of nucleic acids only. Evidently, the immunocytological approach we used is substantially more sensitive.

Figure 5. (Legend continued from facing page.)
seeds imbibed for 24 h. Both early and later mitotic phragmoplasts (cytokinesis, arrows), and divided cells (arrowheads) can be observed in the radicle tip. j to l, Radicle tip (j), shoot meristem (k), and cotyledon (l) of embryos from germinated seeds imbibed for 48 h. At this stage the microtubular cytoskeleton was abundant throughout the embryo. More mitotic arrays and divisions were observed in the radicle tip, and could also be observed in the hypocotyl (not shown). A well-established cytoskeleton was then observed in the shoot meristem and in the cotyledons (l). m to o, Radicle tip (m), shoot meristem (n), and cotyledon (o) of embryos from osmoconditioned seeds before re-drying. A cortical microtubular cytoskeleton had formed during osmoconditioning throughout the radicle tip, hypocotyl (shown in "s") and shoot meristem. In the cotyledons, microtubules were only observed in cells of the meristele, whereas the tubulin granules were still (detected also in a) present in the mesophyll. Mitotic arrays were not detected in embryos of osmoconditioned seeds. p to r, Radicle tip (p), shoot meristem (q), and cotyledon (r) of embryos from osmoconditioned seeds after re-drying. Note in the radicle tip, hypocotyl (shown in t), and shoot meristem the presence of a large number of tubulin granules resulting from degradation of the microtubular cytoskeleton accumulated during osmoconditioning. s and t, Hypocotyls of osmoconditioned seed before (s) and after re-drying (t). As in the radicle tip (m and p), the microtubular cytoskeleton, which was well formed after osmoconditioning, degraded after re-drying as a result of depolymerization of microtubules.

Although embryonic DNA replication in tomato can be blocked by hydroxyurea without affecting the accumulation of β-tubulin and radicle protrusion, subsequent seedling development is hampered (Y. Liu, personal communication). Similar observations were made in cabbage seeds (Górnik et al., 1997). This implies that cell division is not a prerequisite for radicle protrusion in tomato. However, the retarded completion of germination in the presence of hydroxyurea suggests that, simultaneously with cell expansion, mitotic divisions are required for normal seed germination and seedling growth.

The progression of DNA synthesis activity and the appearance of the cortical microtubular cytoskeleton toward the hypocotyl, shoot meristem, and cotyledons prior to radicle protrusion clearly shows that the occurrence of both events is not restricted to the embryonic radicle tip, as was previously suggested (Bino et al., 1992; de Castro et al., 1995, 1998). Thus, we may conclude that the increase in DNA synthesis, as measured by flow cytometry, is not only the result of increased activity in the radicle tip region, but also in other parts of the embryo. Similarly, the accumulation of β-tubulin reflects the spreading of the microtubular cytoskeleton throughout all embryonic tissue while seeds advance toward the completion of germination.

Enhanced Germination Performance of Osmoconditioned Seeds Is Correlated to Pre-Existing DNA Synthesis Activity and Microtubular Cytoskeleton

The immunohistochemical labeling of BrdU and β-tubulin from embryos during seed osmoconditioning confirmed the presence of cells synthesizing DNA (Bino et al., 1992), and showed the accumulation of β-tubulin (de Castro et al., 1995) as the building of a microtubular cytoskeleton. Furthermore, it showed the occurrence of both events during osmoconditioning in embryonic tissues other than tissues of the radicle tip. The actively replicating DNA appeared tolerant to drying, as incorporation of BrdU was detected in embryo nuclei before and after osmoconditioned seeds were re-dried. Irrespective of the conformational state of the DNA, embryonic cells in the S-phase may be desiccation tolerant when a continuous and functional DNA repair process has occurred to ensure integrity of the genome (Osborne and Boubriak, 1994, 1997). In the present study, this may have occurred during osmoconditioning. Microtubules, however, were likely to be sensitive to dehydration, as they were partly depolymerized after re-drying, i.e. depolymerization characterized by the presence of granules or clusters of tubulin (Bartolo and Carter, 1991a). Depolymerization of microtubules has been claimed as a characteristic response to dehydration with the ability for recovery upon re-hydration (Bartolo and Carter, 1991a), as was observed here in embryos of osmoconditioned tomato seeds upon subsequent imbibition in water. The fact that the amount of soluble β-tubulin detected after re-drying was still relatively high may be explained by the fact that microtubules are dynamic structures and may exist in an equilibrium between soluble tubulin subunits and the polymerized microtubules (Bartolo and Carter, 1991b).

Although the frequency of 4C nuclei after the osmoconditioning treatment was higher than that of untreated seeds imbibed in water for 24 h, lower numbers of BrdU-labeled nuclei were detected in osmoconditioned embryos. This may be the result of a slower process of DNA synthesis under osmotic conditions that required 7 d to result in a comparable number of 4C nuclei. DNA replication is known to be retarded when seed hydration is limited (Bino et al., 1993; Saracco et al., 1995). Unlike imbibition in water, imbibition in −1.0 MPa PEG-6000 did not lead to mitosis. This implies that during osmoconditioning the cell cycle was arrested, allowing the synchronization of cells in G_2. Apparently, this is a checkpoint controlled by the osmoticum and explains why the number of 4C or G_2 nuclei becomes invariable after 7 d of osmoconditioning (van Pijlen et al., 1996). Furthermore, mitotic events and cell divisions occurred earlier in embryos of primed seeds upon subsequent imbibition in water and in higher numbers than in the control seeds. The pre-activation of the cell cycle was related to the higher frequency of 4C nuclei and mitotic divisions in embryos of osmoconditioned seeds relative to those of untreated seeds. This may explain why pretreated tomato seeds exhibit superior germination performance relative to untreated seeds (Heydecker and Coolbear, 1977; Argerich and Bradford, 1989; Argerich et al., 1989).

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