Оригинальные работы

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EFFECTS OF TYROSINE KINASE AND PHOSPHATASE INHIBITORS ON MITOSIS PROGRESSION IN SYNCHRONIZED TOBACCO BY-2 CELLS

To test whether reversible tubulin phosphorylation plays any role in the process of plant mitosis the effects of inhibitors of tyrosine kinases, herbimycin A, genistein and tyrphostin AG 18, and of an inhibitor of tyrosine phosphatases, sodium orthovanadate, on microtubule organization and mitosis progression in a synchronized BY-2 culture has been investigated. It was found that treatment with inhibitors of tyrosine kinases of BY-2 cells at the G_2/M transition did not lead to visible disturbances of mitotic microtubule structures, while it did reduce the frequency of their appearance. We assume that a decreased tyrosine phosphorylation level could alter the microtubule dynamic instability parameters during interphase/prophase transition. All types of tyrosine kinase inhibitors used caused a prophase delay: herbimycin A and genistein for 2 h, and tyrphostin AG18 for 1 h. Thereafter the peak of mitosis was displaced for 1 h by herbimycin A or genistein exposure, but after tyrphostin AG18 treatment the timing of the mitosis-peak was comparable to that in control cells. Enhancement of tyrosine phosphorylation induced by the tyrosine phosphatase inhibitor resulted in the opposite effect on BY-2 mitosis transition. Culture treatment with sodium orthovanadate during 1 h resulted in an accelerated start of the prophase and did not lead to the alteration in time of the mitotic index peak formation, as compared to control cells. We suppose that the reversible tyrosine phosphorylation can be involved in the regulation of interphase to M phase transition possibly through regulation of microtubule dynamics in plant cells.

Introduction. Reversible phosphorylation is one of the most common post-translational protein modifications, which regulates numerous biological processes in eukaryotic cells, namely it plays an important role in cellular proliferation, growth and development [1]. It is known that combined activity of protein kinases, which catalyze the phosphorylation of serine/threonine or tyrosine residues, and protein phosphatases, that remove the phosphate groups from appropriate residues, determines the level of substrate protein phosphorylation [2, 3].

Tyrosine phosphorylation, despite its overwhelming importance in animals, has been largely neglected in plants simply because typical tyrosine kinases were not found in higher plants. Recently with the development of mass spectrometry, it has been reported that tyrosine phosphorylation is as important in plants as in animals [4]. By searching for tyrosine kinase-specific sequence motifs, a bioinformatic screen for tyrosine kinases in plants predicted two tyrosine kinases in the *Arabidopsis thaliana* genome [5], 34 putative tyrosine kinases and five dual-specificity proteins, indicating that nearly 4 % of all *A. thaliana* kinases can phosphorylate tyrosine residues [6]. Moreover putative tyrosine kinases are found in several plants: six tyrosine kinases in *Oryza sativa* ssp. Indica and seven in *Oryza sativa* ssp. Japonica [5]. Using bioinformatics methods on the basis of homology to the *Mus musculus* Zap70 tyrosine kinase catalytic domain, the existence of 494 protein kinase genes in *A. thaliana* was predicted. Moreover non-receptor Syk-like tyrosine kinase was identified in the green algal species, which could have a critical role in algal growth and development [7].

A. thaliana has few protein tyrosine-specific phosphatases. Recent studies have shown that specific tyrosine phosphatases exist in higher plants [3] and play a key role in some physiological processes, such as the bending of touch-sensitive petioles in *Mimosa pudica* [8], regulation of stomata movement in *Commelina communis* leaves [9], in pollen germination and pollen tube growth [10].

Tyrosine phosphorylated proteins have been detected in different plant species [8, 11–13]. It is known that protein tyrosine phosphorylation is involved in the control of specific steps in plant development [11, 14] and in plant signalling processes [15–17].

As it was revealed in higher plants one of the cytoskeleton components, microtubules, can be intensively regulated by post-translational modifications of tubulin, including phosphorylation [18, 19]. Recently it was established that both α- and β-tubulins undergo phosphorylation on tyrosine residues [19]. Immunofluorescence microscopy revealed that tyrosine phosphorylation of β-tubulin could be one of the targets for tyrosine kinases [20]. In our previous paper we have provided data that tyrosine kinase inhibitors as herbimycin A, genistein and tyrphostin AG18 as well as an inhibitor of tyrosine phosphatases, sodium orthovanadate, affect microtubule organization in *Arabidopsis thaliana* root cells. As a result root growth and development are altered, leading to the interpretation that tubulin phosphorylation/dephosphorylation events are involved in these regulating cascades [21]. However, at present no evidences exist for possible roles of these modifications in the plant's microtubular organization, nor for its functional impact on the cell-cycle progression.

To investigate the potential involvement of tyrosine phosphorylation of plant proteins in regulation of mitosis, the effects of tyrosine kinase inhibitors (herbimycin A, genistein and tyrphostin AG18) and a tyrosine phosphatase inhibitor (sodium orthovanadate) on microtubule organization and mitotic progression in synchronized suspension *Nicotiana tabacum* BY-2 cell culture were investigated. It was found that at G_2/M transition treatment of BY-2 cells with inhibitors of tyrosine kinases caused no visible disturbances of microtubular organization, but that the frequency of the mitotic figures was reduced. It is suggested that the decrease of tyrosine phosphorylation level could cause the slowing down of the interphase/prophase transition. Probably this is the result of alterations in dynamic parameters of plant microtubules in dividing cells via regulation of phosphorylation levels of microtubular proteins during cell cycle-specific stages transition.

Materials and methods. *Cell culture and synchronization.* The tobacco BY-2 (*Nicotiana tabacum* L. cv. Bright Yellow-2) suspension cell line expressed GFP-MBD [22] was maintained as described by Nagata et al. [23] with modifications. The culture samples was refreshed weekly by transfer of 2,5 ml of 7-day-old culture into 50 ml of fresh Murashige and Skoog medium [24], («Duchefa», Netherlands) pH 5.8, containing 3 % (w/v) sucrose («Duchefa», Netherlands), 0,2 g/L KH_2PO_4 («Merck», Germany), 10 mg/L myo-inositol («Sigma», USA), 1 mg/L thiamin hydrochloride («Sigma», USA), and 0,2 mg/L 2,4-D («Serva», Germany). The culture was kept at +28 °C at constant darkness and 130 rpm.

Cell culture synchronization protocol was based on the method of Nagata et al. [23]. The stationary culture was transferred in a proportion 15:100 to the fresh Murashige and Skoog medium, supplied with 5 μg/ml aphidicolin (ICN Biomedicals, Belgium). After 20 h incubation, cells were released by extensive washing (5 L per 115 ml of blocked culture). After the aphidicolin release the main population of cells was at the early S-phase [23]. Afterwards, cells were transferred into fresh medium and divided equally into five subcultures, which were subsequently treated with inhibitors (Fig. 1, see color plate). Untreated culture was used as a control. For experiments we used previously defined effective concentrations of herbimycin A, genistein, tyrphostin AG18 and sodium orthovanadate [21]. The synchronization of BY-2 culture by aphidicolin at the beginning of S-phase, a drug that inhibits DNA polymerase α [23], enables to investigate effects of tyrosine kinase and tyrosine phosphatase inhibititors on both G_2/M and mitosis transitions.

Confocal laser scanning microscopy. Effects of tyrosine kinase and tyrosine phosphatase inhibitors on microtubule organization in synchronized BY-2 cells were visualized after 1–8 h of treatment. GFP-labeled microtubules in tobacco BY-2 (GFP-MBD) cells were visualized *in vivo* using confocal laser scanning microscopes C1 («Nikon», Japan) and LSM 510 META («Carl Zeiss», Germany) with 60× water-immersion and 63× oil-immersion objectives respectively, using the 488 line of the Ar-lazer.

Mitotic index calculation. For calculation of the mitotic index cells were sampled during 10 h at 1 h intervals (0,5 ml per sample), and fixed in a ethanol/acetic acid 3:1 (v/v) solution. Cells were washed in phosphate-buffered saline, stained with 4',6-diamino-phenylindole (DAPI, 5 mg/ml) and counted (Nikon fluorescent microscope, Nikon Europe, Badhoevedorp, The Netherlands). The

mitotic index was established as the sum of the percentages of cells in pro-, meta-, ana- and telophases. The percentage of pro-, meta-, ana- and telophases was assessed as a number of cells in correspondence phase of mitosis to the percentage of the total number of cells. For each determination more than one thousand cells were examined. All results were presented as a mean ± standard error (SE) of at least three replicates.

Chemicals. The inhibitor of non-receptor tyrosine kinase, herbimycin A («Sigma», USA) [25], was dissolved in DMSO as 1 mM stock solution and used in 30 μM concentration. The inhibitor of receptor tyrosine kinases, genistein («Sigma», USA) [26] was dissolved in DMSO at 10 mM stock solution and used in 10 μM concentration. The inhibitor of receptor tyrosine kinase, tyrphostin AG18 («Calbiochem», Germany) [27] was dissolved in DMSO at 5 mM stock solution and used in 50 μM concentration. Stock solutions of tyrosine kinase inhibitors were stored at −20 °C. The concentration of DMSO in all experiments did not exceed 0,5 %. The inhibitor of protein tyrosine phosphatases, sodium orthovanadate («Sigma», USA) [28] was dissolved immediately before use in H_2O at 250 μM concentration.

Results. *Effects of tyrosine kinase and tyrosine phosphatase inhibitors on the mitotic microtubules organization in synchronized BY-2 cells.* In control BY-2 cells all microtubule arrays typical for higher plant cells were observed. The interphase microtubule network in non-treated cells consists of cortical microtubules oriented mostly transverse to the cell elongation axis (Fig. 2, *a*, see color plate). In prometaphase, cortical microtubules were substituted by the preprophase band (Fig. 2, *b*). Dissolution of the preprophase band and nuclear envelope coincides with formation of the mitotic spindle (Fig. 2, *c*). As mitosis proceeds, the phragmoplast (Fig. 2, *d*) forms in the midzone of the late anaphase spindle, precisely at the site landmarked by the preprophase band. The phragmoplast, like the preprophase band, is a structure unique to plant cells. In conclusion, in higher-plant cells, successful cell division requires the temporal and spatial coordination of the assembly of highly organized microtubule arrays, where these microtubule reorganizations are closely linked to the acquisition of specialized properties and functions [29].

Control synchronized BY-2 culture entered into mitosis after 4 h following aphidicolin release as far as at this time first PPBs were observed. It was estimated that all used tyrosine kinase inhibitors reduced the frequency of mitotic figure appearance in treated synchronized BY-2 cells but that it did not cause the alteration of microtubular organization. It was found that after 3 h treatment with inhibitors of tyrosine kinases in synchronized BY-2 culture all typical mitotic microtubular arrays were present without any visible disturbances (Fig. 3, see color plate). The interphase microtubules also maintain the normal organization (Fig. 3, *a*, *e*, *i*). Long-term (6 h) incubation of cells with tyrosine kinase inhibitors did not lead to any dramatic alteration of mitotic microtubules.

The same results were obtained after tyrosine phosphatase inhibitor treatment. It was shown that BY-2 treatment with sodium orthovanadate did not lead to the alteration in organization of both mitotic and cortical microtubular arrays (Fig. 4, *a*–*d*, see color plate), as compared to non-treated cells (Fig. 2, *a*–*d*). Short- as well as long-term incubation of cells with sodium orthovanadate did not disturb organization, nor orientation of the preprophase band, spindle and phragmoplast in treated cells.

Inhibitors of tyrosine kinases and tyrosine phosphatases influence the mitotic index of BY-2 cells. In a second set of experiments the effects of treatment with tyrosine kinase and tyrosine phosphatase inhibitors on the mitotic index of the synchronized BY-2 culture were investigated. It was established that treatment with all tested tyrosine kinase inhibitors as well as with one tyrosine phophatase inhibitor caused alterations in mitotic progression as compared to control cells (Fig. 5). The peak of mitotic index was reached at 35 ± 1,9 % in control culture after 7 h following aphidicolin release (Fig. 5). The most obvious alterations in mitotic progression were found after application of herbimycin A and genistein. In the synchronized BY-2 culture treated with 30 μM herbimycin A and 10 μM genistein the cells reached a maximum mitotic index of 16 ± ± 2,2 and 17 ± 1,4 %, correspondingly (1 h later than in control cells) (Fig. 5). Both inhibitors caused similar changes in mitotic progression of synchronized BY-2 cells, namely, dividing cells

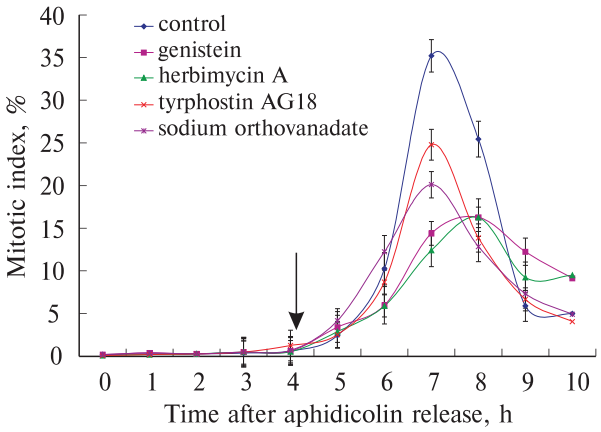

Fig. 5. The effects of different types of tyrosine kinase and tyrosine phosphatase inhibitors on mitotic activity in synchronized BY-2 cells. Inhibitors were applied 4 h after aphidicolin release (arrow)

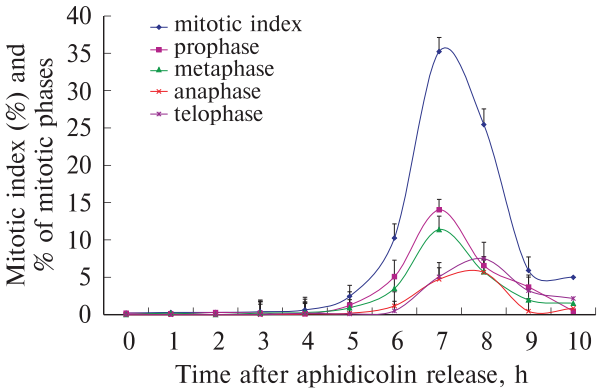

Fig. 6. The mitotic index and percentage of cells in different phases of mitosis in control synchronized BY-2 culture. The peak of cells in pro-, meta-, ana- and telophases are indicated an arrow

entered mitosis 1 h later and finish the process with 2 h delay. On the other hand, BY-2 cell treatment with another inhibitor, tyrphostin AG18, did not cause changes in cells exiting mitosis. Besides the reduction of the mitotic index level to 24,8 ± 1,4 %, there were no differences at the time of mitotic index peak after tyrphostin AG18 treatment in comparison to control cells (Fig. 5).

Culture treatment with the tyrosine phosphatase inhibitor sodium orthovanadate resulted in a reduction of the mitotic index peak (20 ± 1,9 %), but it did not lead to the alteration in the timing of the mitotic index peak formation, as compared to control cells (Fig. 5).

Effects of tyrosine kinases and tyrosine phosphatase inhibitors on the progression of mitotic phases in synchronized BY-2 cells. In the next step the effects of tyrosine kinase and tyrosine phosphatase inhibitors on different mitotic phases' progression of synchronized BY-2 culture were determined. In the control culture the percentage of cells in pro-/metaphases was maximal at 7 h after aphidicolin release, in ana- and telophases this occurred at 8 h (Fig. 6). Treatment with tyrosine kinase inhibitors of a synchronized BY-2 cell culture led to obvious changes in prophase progression. Both herbimycin A (Fig. 7) and genistein (Fig. 8) treatments led to a 2 h delay in prophase entry; tyrphostin AG18 caused a 1 h lagging into prophase entry (Fig. 9), as compared to the control (Fig. 6). Moreover, the amount of inhibitor-treated cells entering the prophase was significantly lower, probably due to the further decrease in the level of mitotic index (Fig. 7–9). It was found that the observed delay into mitosis entry after herbimycin A and genistein treatment led to the lagging of mitotic index peak and a subsequent delayed exit of cells from mitosis (Fig. 5), as compared to control cells.

On the other hand, BY-2 treatment with an inhibitor of tyrosine phosphatases caused the opposite effect on mitotic phase's progression, as compared to influence of tyrosine kinases inhibitors. It was found that 1 h treatment with sodium orthovanadate causes an acceleration of cells entering prophase (Fig. 10). However the total amount of dividing cells after sodium orthovanadate treatment was significantly lower as a result of the obvious reduction of the number of cells entering prophase (Fig. 10), as compared to the control.

Discussion. It was found that BY-2 treatment with inhibitors of tyrosine kinases at the point of G_2/M transition did not lead to visible disturbances of mitotic microtubular structures, while it significantly reduced the frequency of the typical mitotic figure appearances. The total amount of the cells with a preprophase band after 1–2 h tyrosine kinases inhibitors treatment was significantly reduced without disruption in preprophase band architecture, in comparison with control. Moreover cell treatment with any tyrosine kinases inhibitor did not affect mitotic microtubules (spindle and phragmoplast), nor did

it affect the cortical microtubule network. Thus it was demonstrated that all used tyrosine kinase inhibitors at tested concentrations did not cause mitotic arrest in G_2/M, but that they did lead to slower prophase entry. As argument for this finding we can note that all types of used tyrosine kinases inhibitors caused prophase delay: herbimycin A and genistein – for 2 h, and tyrphostin AG18 – for 1 h. Thereafter the peak of mitosis was displaced 1 h later after herbimycin A or genistein exposure, whereas after tyrphostin AG18 treatment it was comparable to this in control cells. Moreover the total amount of mitotic cells was significantly reduced.

These results demonstrate that herbimycin A and genistein have a more potent action on mitotic progression in synchronized BY-2 cell culture than tyrphostin AG18. It is known that herbimycin A is a benzoquinoid ansamycin antibiotic that acts as a very specific inhibitor of non-receptor tyrosine kinases of the src family, binding directly to the kinase domain (probably to thiol groups) and preventing ATP binding [30]. Other types of tyrosine kinase inhibitors, like genistein and tyrphostin, are potent receptor-type broad range tyrosine kinase inhibitors that interact with ATP binding sites [26] or act through binding the substrate binding site [31], subsequently. Possibly, the observed distinction in the effects of tyrosine kinase inhibitors could be a result of the different substrate specificity and mechanisms of action of the tested inhibitors.

Previously, effects of different tyrosine kinase inhibitors on cell cycle progression were investigated only on animal cells. It was shown that herbimycin A and genistein were able to inhibit the proliferation of some cancer cells lines in a dose-dependent manner [32, 33]. Several cancer cell studies have shown that genistein can trigger G_2/M cell cycle arrest and inhibit cell growth [34, 35]. Our data show for the first time that inhibition of receptor and non-receptor tyrosine kinases lead to delay of G_2/M cell cycle transition in synchronized BY-2 cells as a result of 2 h prophase lagging after herbimycin A and genistein treatment, as compared to mitotic progression in control cells.

Enhancement of tyrosine phosphorylation induced by the tyrosine phosphatase inhibitor did not cause the alteration in organization of both

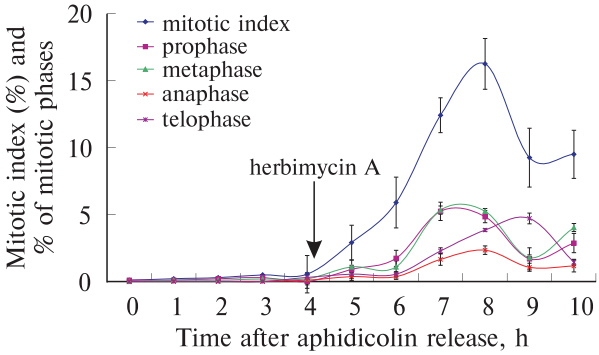

Fig. 7. The mitotic index and percentage of cells in different phases of mitosis in synchronized BY-2 culture after herbimycin A treatment. Inhibitor application is indicated by the arrow

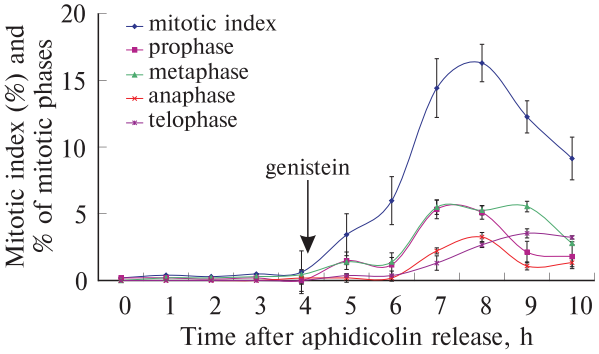

Fig. 8. The mitotic index and percentage of cells in different phases of mitosis in synchronized BY-2 culture after genestein treatment. Inhibitor application is indicated by the arrow

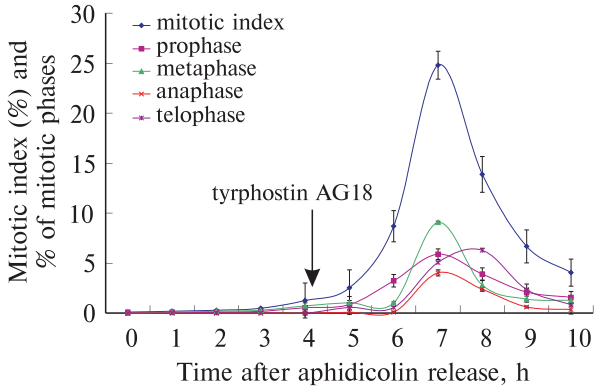

Fig. 9. The mitotic index and percentage of cells in different phases of mitosis in synchronized BY-2 culture after tyrphostin AG18 treatment. Inhibitor application is indicated by the arrow

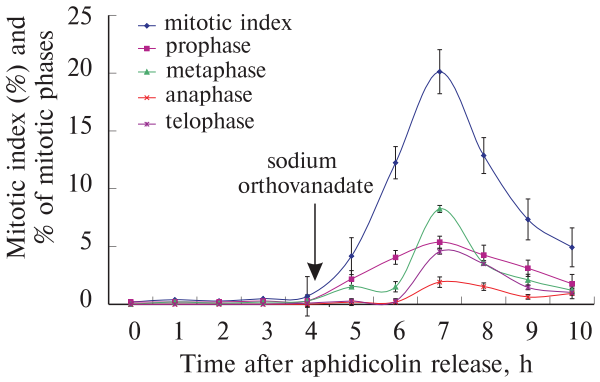

Fig. 10. The mitotic index and percentage of cells in different phases of mitosis in synchronized BY-2 culture after sodium orthovanadate treatment. Inhibitor application is indicated by the arrow

cortical and mitotic microtubule arrays but resulted in the opposite effect on BY-2 mitotic transition in comparison with effects of tyrosine kinases inhibitors. One hour treatment with sodium orthovanadate slightly promotes cells entering into prophase; the total amount of the cells that entered mitosis was higher than in the control. Though, in cells incubated with sodium orthovanadate the mitotic index was reduced, which is in accordance with results obtained for animal cells [36]. It was shown for different animal cells that sodium orthovanatade protects cells from apoptosis but that in certain situations it can indeed induce growth inhibition and apoptosis [37, 38]. Downregulation of several tyrosine phosphatases resulted in a significantly reduced mitotic index [39]. Up to now, effects of sodium orthovanadate on mitotic microtubule organization as well as mitosis transition in higher plant cells were not investigated. Only in animal cells it was shown that sodium orthovanadate increases phosphorylation on tyrosine residues of cellular proteins, including α-tubulin [40]. Sodium orthovanadate plus H_2O_2 decreases the degree of microtubule polymerization in hepatocyte cultures and antagonizes the effect of herbimycin A. The authors proposed that the degree of tyrosine phosphorylation of α-tubulin after the cells' exposure to inhibitors of tyrosine kinases and tyrosine phosphatases is an important factor to determine the state of assembly of microtubules in animal cells [40].

Recently using double-label staining experiments on *A. thaliana* microtubules with a polyclonal anti-phosphotyrosine antibody (P-Tyr), a monoclonal anti-α-tubulin (TU-01) and an anti-β-tubulin antibody (TUB 2.1) it was shown that phosphotyrosine labeling correlated with cortical microtubules in interphase cells as well as with all types of mitotic microtubules [20]. It was also estimated that the signal detected with the anti-phosphotyrosine antibody was markedly enhanced after pretreating the cells with sodium orthovanadate, as compared with control (untreated) cells. Thereby immunofluorescence microscopy revealed tyrosine phosphorylation on polymerized microtubules in plant cells, implying that α- and β-tubulins could be targets for tyrosine kinases [20].

Taken together, our observations revealed that inhibitors of tyrosine kinases could slow down the interphase/prophase transition, probably as a result of alterations in dynamic parameters of plant microtubules in dividing cells by regulating phosphorylation levels of microtubule proteins during specific stages of cell cycle transition. Dynamic instability of microtubules is characterized by four parameters: growth rate, shrinkage rate, frequency of transition from growth to shrinkage (catastrophe frequency), and frequency of transition from shrinkage to growth (rescue frequency) [41]. It is known that the dynamic instability parameters of plant microtubules are important determinants for the transition from interphase microtubules to the preprophase band [42]. During the transition from interphase to preprophase band formation the microtubule growth rate and catastrophe frequency doubles, but the shrinkage rate and rescue frequency remains constant, which makes microtubules shorter and more dynamic [43]. Earlier it was estimated in animal cells that α-tubulin phosphorylation on C-terminal tyrosine residues by non-receptor tyrosine kinases effects microtubules polymerization [44] since phosphorylated α-tubulin fails to polymerize into microtubules [44].

Comparison of our results with previously published data [21] suggests that the role of tyrosine phosphorylation/dephosphorylation processes in dividing and differentiated plant cells can be different. Earlier we found that the most sensitive microtubules to the action of tyrosine kinases in *A. thaliana* primary roots were those in differentiated cells. Cortical microtubules in cells

of the roots differentiation zone (trichoblasts and atrichoblasts) and root hairs are more sensitive to tyrosine kinases inhibitors than microtubules in meristematic cells [21]. We suggested that the tyrosine phosphorylation/dephosphorylation process is involved in regulating the development and differentiation of *Arabidopsis* root cells. This assumption is in accordance with previous data on carrot and *A. thaliana* cells were protein tyrosine phosphorylation involvement in the control of specific steps in plant development was estimated [11].

Using specific inhibitors of non-receptor and receptor tyrosine kinases and inhibitors of tyrosine phosphatases we found that tyrosine phosphorylation/dephosphorylation processes participate in mitosis progression in synchronized BY-2 culture. We suppose that inhibition of tyrosine phosphorylation causes the slow down in the transition from the interphase to the prophase in mitotic cells through regulation of phosphorylation levels of microtubule proteins. We proposed that cells respond to stimuli, and that the regulation of cellular morphogenesis and control of cell division can be partly interpreted in terms of the microtubule protein phosphorylation/dephosphorylation status.

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ВЛИЯНИЕ ИНГИБИТОРОВ ТИРОЗИНКИНАЗ И ТОРОЗИНФОСФАТАЗ НА ПРОХОЖДЕНИЕ МИТОЗА В СИНХРОНИЗИРОВАННОЙ КУЛЬТУРЕ ТАБАКА BY-2

Для изучения участия обратимого фосфорилирования белков в прохождении митоза растительной клеткой исследовано влияние на этот процесс и организацию микротрубочек в синхронизированной культуре BY-2 ингибиторов тирозинкиназ – гербимицина А, генистеина и тирфостина AG18, а также ингибитора тирозинфосфатаз – ортованадата натрия. Обнаружено, что обработка клеток BY-2 ингибиторами тирозинкиназ при прохождении G_2/M фазы не приводила к видимым нарушениям митотических структур микротрубочек, однако вызывала уменьшение их количества. Возможно, снижение уровня фосфорилирования белков микротрубочек по остаткам тирозина может нарушать динамические свойства микротрубочек при прохождении интерфазы/профазы. Все использованные ингибиторы тирозинкиназ приводили к задержке вступления клеток в профазу: гербимицин А и генистеин – на 2 ч, тирфостин AG18 – на 1 ч. После обработки гербимицином А или генистеином отмечали задержку в появлении пика митоза на 1 ч по сравнению с контролем, а после обработки тирфостином AG18 изменений не было. Обработка культуры клеток ингибитором тирозинфосфатаз ортованадатом натрия на протяжении 1 ч оказывала противоположное воздействие на прохождение митоза: вступление клеток в профазу ускорялось, но без изменений во времени формирования митотического пика по сравнению с контролем. Можно предположить, что фосфорилирование белков по остаткам тирозина является важным звеном в регуляции перехода клеток из интерфазы в М-фазу посредством регуляции динамических свойств микротрубочек в растительных клетках.

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ВПЛИВ ІНГІБІТОРІВ ТИРОЗИНКІНАЗ І ТИРОЗИНФОСФАТАЗ НА ПРОХОДЖЕННЯ МІТОЗУ В СИНХРОНІЗОВАНІЙ КУЛЬТУРІ ТЮТЮНУ BY-2

Щоб дослідити участь зворотного фосфорилювання білків у проходженні мітозу рослиною клітини, вивчено вплив на цей процес і організацію мікротрубочок в синхронізованій культурі BY-2 інгібіторів тирозинкіназ – гербіміцину А, геністеїну та тирфостину AG18, а також інгібітора тирозинфосфатаз – ортованадату натрію. Виявлено, що обробка клітин BY-2 інгібіторами тирозинкіназ при проходженні G_2/M не призводила до очевидних порушень мітотичних структур, проте викликала зменшення їхньої кількості. Можливо, що зниження рівня тирозинфосфорилювання білків мікротрубочок по залишках тирозину може порушувати динамічні властивості мікротрубочок впродовж інтерфази/профази. Всі використані нами інгібітори тирозинкіназ призводили до затримки входження клітин у профазу: гербіміцин А і геністеїн – на 2 год, тирфостин AG18 – на 1 год. Після обробки гербіміцином А або геністеїном виявлено затримку в прояві піку мітозу на 1 год у порівнянні з контролем, а після обробки тирфостином AG18 змін не було. Обробка культури клітин інгібітором тирозинфосфатаз, ортованадатом натрію впродовж 1 год призводила до протилежного впливу на проходження мітозу: вступ клітин в профазу прискорювався, проте без змін в часі

формування мітотичного піку в порівнянні із контролем. Можна зробити припущення про те, що зворотне тирозинфосфорилювання білків по залишках тирозину є важливою ланкою в регуляції переходу із інтерфази в М-фазу за рахунок регуляції динамічних властивостей мікротрубочок в рослинних клітинах.

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