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Effects of tyrosine kinase and phosphatase inhibitors on microtubules in Arabidopsis root cells

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Abstract

To investigate the role of tyrosine phosphorylation/dephosphorylation processes in plant cells the morphology of *Arabidopsis thaliana* primary roots and the organization of cortical microtubules (MTs) were studied after inhibition of protein tyrosine kinases (PTKs) and tyrosine phosphatases (PTPs). It was found that all tested types of PTKs inhibitors (herbimycin A, genistein and tyrphostin AG 18) altered root hair growth and development, probably as a result of their significant influences on MTs organization in root hairs. The treatment also led to MTs reorientation and disruption in epidermis and cortex cells of both elongation and differentiation zones of primary roots. Enhanced tyrosine phosphorylation after treatment with a PTPs inhibitor (sodium orthovanadate) resulted in intense induction of root hair development and growth and caused a significant shortening of the elongation zone. It also led to changes of MTs orientation from transverse to longitudinal in epidermis and cortex cells of the elongation and differentiation zones of the root. From the data obtained we can suppose that tyrosine phosphorylation can be involved in the dynamics and organization of MTs in different types of plant cells.

Keywords: *Arabidopsis thaliana*; Plant microtubules; Tyrosine phosphorylation; Inhibitors

1. Introduction

Microtubules (MTs) behaviour in animal cells is known to be sensitive to protein phosphorylation on tyrosine residues where the level of tyrosine phosphorylation is determined by the balanced activity of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). In animals, tyrosine phosphorylation has been found on α- (Akiyama et al., 1986; Ishibashi et al., 1999), β- (Akiyama et al., 1986; Matten et al., 1990) and γ-tubulin (Kukharskyy et al., 2004), where it is known to play a key role in cellular growth and differentiation.

In plant cells tyrosine phosphorylation plays also an important role in different cellular processes such as cell cycle control (Huang et al., 2003), MAP kinase cascade pathways (Huang et al., 2000), plant cell signaling (Luan, 2002) and plant development (Barizza et al., 1999). Available information indicates that tyrosine-specific phosphatases do exist in plants, whereas till now no typical PTKs have been characterized. Only protein kinases with dual-specificity have been reported in plant species (Wang et al., 2007). Using different biochemical approaches it was demonstrated by us also that both α- and β-subunits of plant tubulin undergo phosphorylation on tyrosine residues (Blume et al., submitted for publication). However, a possible functional role of this post-translational tubulin modification, with impact on MTs organization and stability in plant cells remains an open issue to date. Here we analyzed the MTs organization in *Arabidopsis* root cells by visualizing MTs labeled with GFP-MAP4 after treatment

Abbreviations: MTs, microtubules; HA, Herbimycin A; SO, sodium orthovanadate; PTKs, protein tyrosine kinases; PTPs, protein tyrosine phosphatases.

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by different inhibitors of PTKs and PTPs. Taking into account that two types of PTKs (transmembrane receptor PTKs and non-receptor PTKs) do exist in animal cells, we used herbimycin A (HA) as inhibitor of non-receptor PTKs (Uehara and Fukazawa, 1991; Fukazawa et al., 1994), genistein and tyrphostin as inhibitors of receptor PTKs (Rudrabhatla and Rajasekharan, 2004) in our study. Sodium orthovanadate (SO) was used as inhibitor of PTPs.

2. Materials and methods

2.1. Plant material

A line of *Arabidopsis thaliana* ecotype Landsberg erecta (Ler) expressing GFP-MAP4 (Mathur and Chua, 2000) was used in this research. *A. thaliana* seeds were surface sterilized in 6% (v:v) commercial bleach for 15 min and rinsed five times with distilled water. All seeds were placed on a half-strength MS (1/2MS) medium including vitamins (Duchefa, Haarlem, the Netherlands), supplemented with 10 g L^{-1} sucrose and solidified with 4 g L^{-1} Gelrite (Duchefa) at pH 5.7. After overnight incubation at 4 °C, the dishes were placed vertically in a growth chamber at 22 °C under 16 h light/8 h dark photoperiod. Four-days-old seedlings were treated with inhibitors of PTKs and inhibitor of PTPs during 3, 6, 12, 24 and 48 h.

2.2. Chemicals

Herbimycin A (*HA*) (Sigma, USA) (an inhibitor of non-receptor PTK) was dissolved in dimethylsulfoxide (DMSO) as 1 mM stock solution, stored at −20 °C and used in 1, 30, 50 μM concentrations. *Genistein* (Sigma, USA) (inhibitor of PTKs) was dissolved in DMSO as 10 mM stock solution, stored at −20 °C and used in 0.1, 1, 10 μM concentrations. *Tyrphostin AG 18* (Calbiochem, Merck, Germany) (a broad-spectrum PTKs inhibitor) was dissolved in DMSO as 50 mM stock solution, stored in the dark at −20 °C and used in 10, 50 μM concentrations. Because of the photolability of tyrphostin, the experiments were performed in the dark. *Sodium orthovanadate* (*SO*) (Sigma, USA) (inhibitor of PTPs) was dissolved immediately before use in H_2O as 250 mM stock solution and used in 250, 500 μM concentrations.

2.3. Microscopy

GFP-labelled MTs in *A. thaliana* root cells were visualized *in vivo* by confocal laser scanning microscopy (CLSM Nikon C1 and LSM 510 META, Carl Zeiss) with 60x water-immersion and 63× oil-immersion objectives respectively, using the 488 line of the Ar-lazer.

2.4. Measurement of roots growth

Time-lapse series of images of growing roots were obtained using a Nikon DXM 1200 digital camera. The increase in root length was measured on the images with Image J (version 1. 33 u). Influences of PTKs and PTPs inhibitors on *A. thaliana* root growth were determined as percentage ratio between roots length (in mm) at the beginning of experiments (0 h) to the roots length (in mm) after treatment with inhibitors (during 6, 24, 42 and 72 h). Root growth rates (Δ) were calculated using the following equation: $(L_{ev} - L_0/L_0) \times 100\%$, where L_0 is the meaning of initial root length (no treatment); L_{ev} is the meaning length of the roots after inhibitor treatment. All results were presented as a mean ± standard error (SE) of at least three replicates.

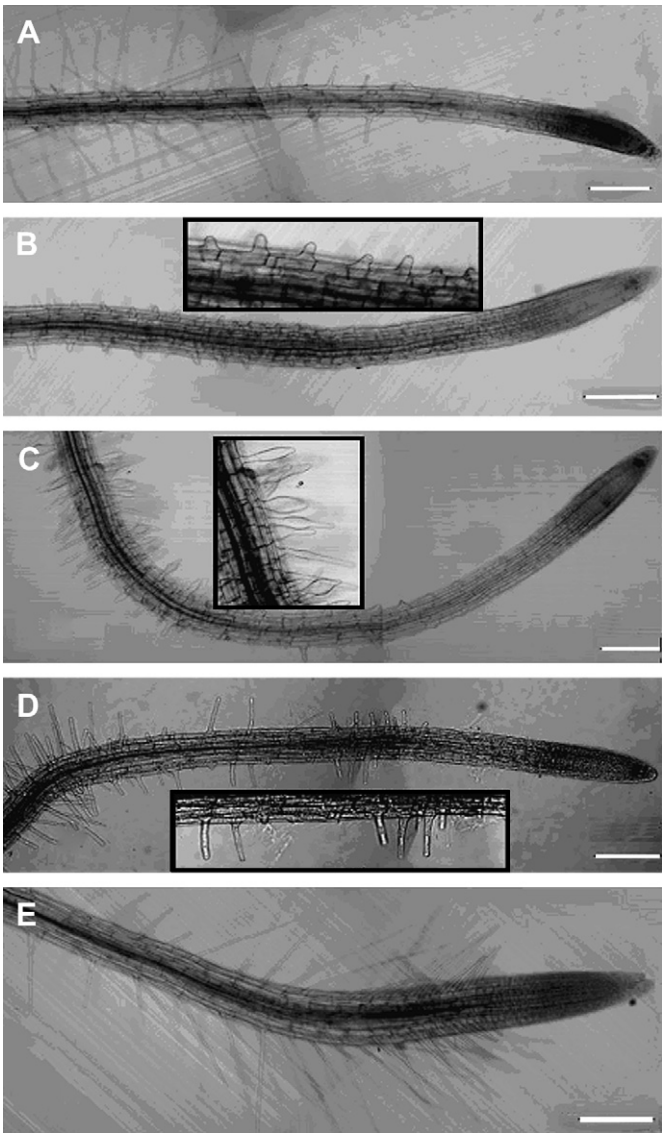

Fig. 1. Effects of inhibitors of tyrosine kinases and inhibitor of tyrosine phosphatases on *Arabidopsis* root morphology and root hair development and growth. Pictures taken of 4-days-old seedlings treated for 24 h. A: Untreated control root; B: 30 μM HA; C: 50 μM tyrphostin AG 18; D: 10 μM genistein; E: 250 μM SO. Bar: 0.1 mm.

3. Results and discussion

To estimate the effects of PTKs inhibitors and an inhibitor of PTPs on *A. thaliana* root morphology, cell elongation and