Auxin influx inhibitors 1-NOA, 2-NOA, and CHPAA interfere with membrane dynamics in tobacco cells

Martina Laňková[1], Richard S. Smith[2], Bedřich Pešek[1], Martin Kubeš[1], Eva Zažímalová[1], Jan Petrášek[1] and Klára Hoyerová[1]

[1]Institute of Experimental Botany, the Academy of Sciences of the Czech Republic, Rozvojová 263, CZ-165 02 Prague 6, Czech Republic

[2]Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland

Supplementary material

Fig. S1 Partial colocalization of EYFP:AUX1 (green) with the endocytic tracer FM 4-64 (red) in BY-2 cells after 20 min. Detail section shows cutted area from merge. Scale bar 20 µm, in detail section scale bar 10 µm.

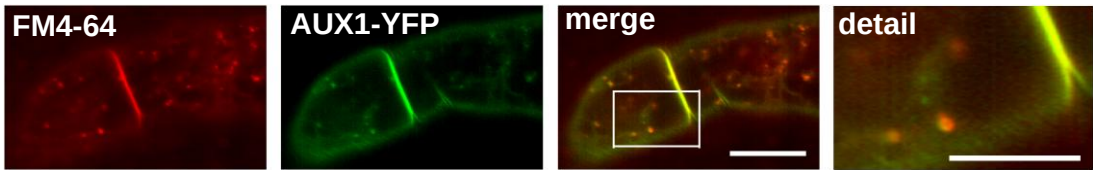


Fig. S2 The formation of cell plate in control cells or in the cells treated with 1-NOA. Cell plate formation in control EYFP:AUX1 BY-2 cells (upper panel) and in cells after 1-NOA treatment (20 μM, 24h) (lower panel). After 1-NOA treatment the cell plate is not formed in the regular centrifugal manner. Instead, oblique cell plates are formed and sometimes they are even not fusing with parental plasma membrane.

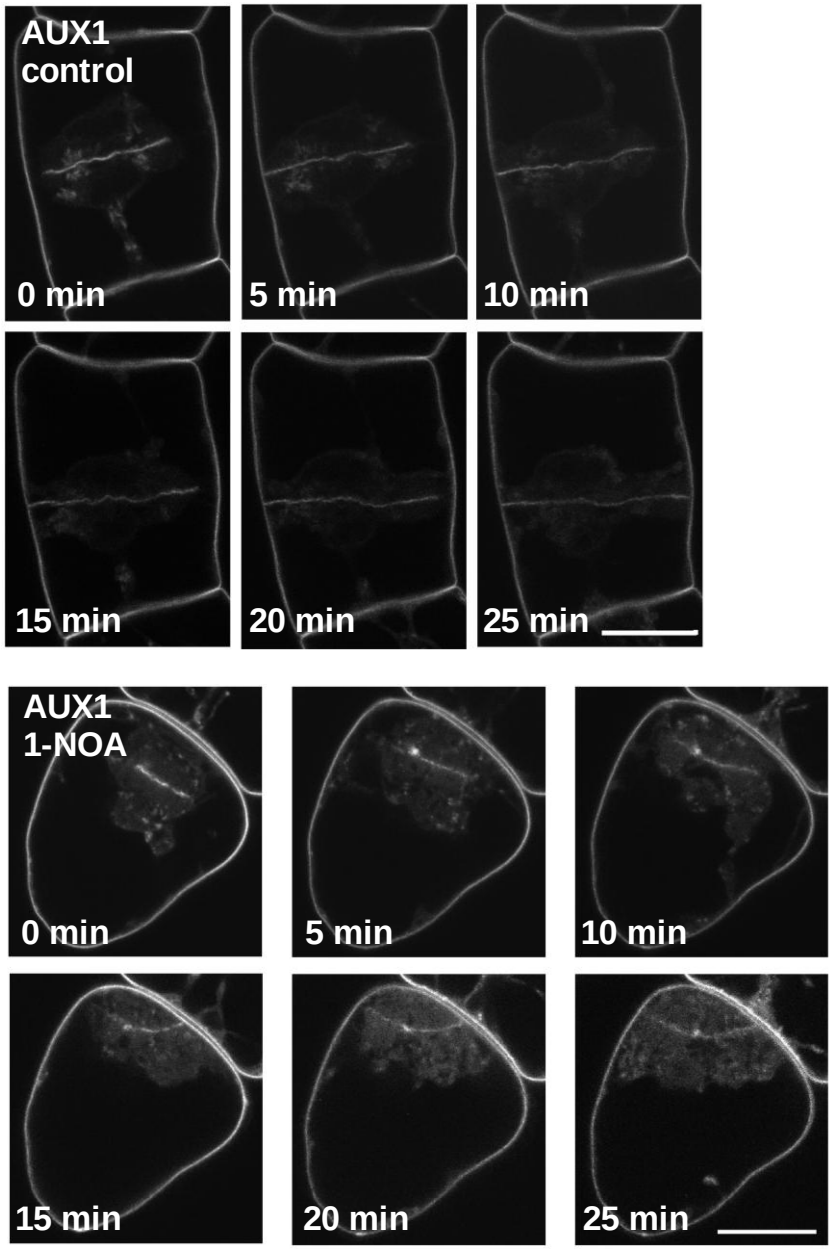


Fig. S3 Long-term treatments of EYFP:AUX1 or PIN1:GFP BY-2 cells with auxin influx inhibitors.

(A, E) non-treated controls. The effects of 1-NOA (50 μM, 48 h) (B), 2-NOA (50 μM, 48 h) (C) and CHPAA (50 μM, 48 h) (D) on membrane dynamics in BY-2 cells transformed with *EYFP:AUX1* construct. The effects of 1-NOA (50 μM, 48 h) (F), 2-NOA (50 μM, 48 h) (G) and CHPAA (50 μM, 84 h) (H) on membrane dynamics in BY-2 cells transformed with *PIN1:GFP* construct. Single confocal sections through the perinuclear region (C-Apochromat 40x/1.2 W water immersion objective with a 1 Airy Unit pinhole was used). Scale bars 20 μm.

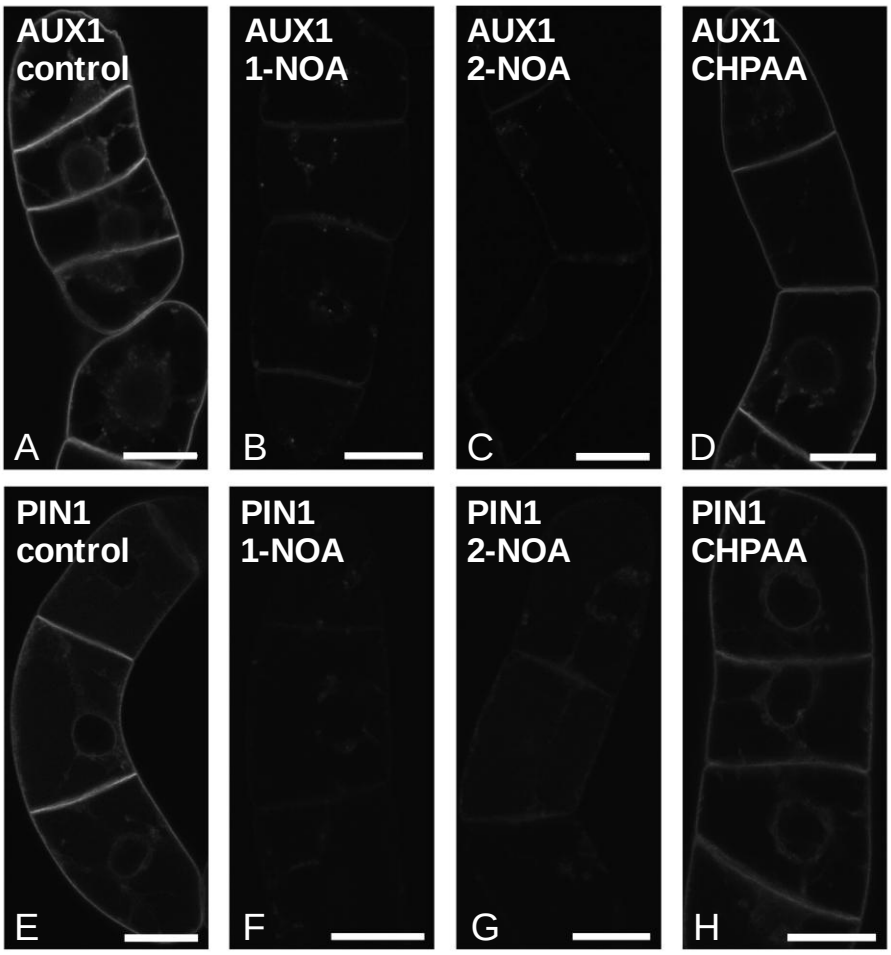


Fig. S4. The effects of the putative auxin influx inhibitors on subcellular distribution of EYFP:AUX1 and PIN1:GFP fusion proteins in tobacco BY-2 cells. (A, E) non-treated controls. The effects of 1-NOA (20 μM, 30 min) (B), 2-NOA (20 μM, 30 min) (C) and CHPAA (20 μM, 30 min) (D) on subcellular distribution of EYFP:AUX1. The effects of 1-NOA (20 μM, 30 min) (F), 2-NOA (20 μM, 30 min) (G) and CHPAA (20 μM, 30 min) (H) on subcellular distribution of PIN1:GFP. Single confocal sections through the cortical cytoplasm (C-Apochromat 40x/1.2 W water immersion objective with a 1 Airy Unit pinhole). Scale bars 10 μm.

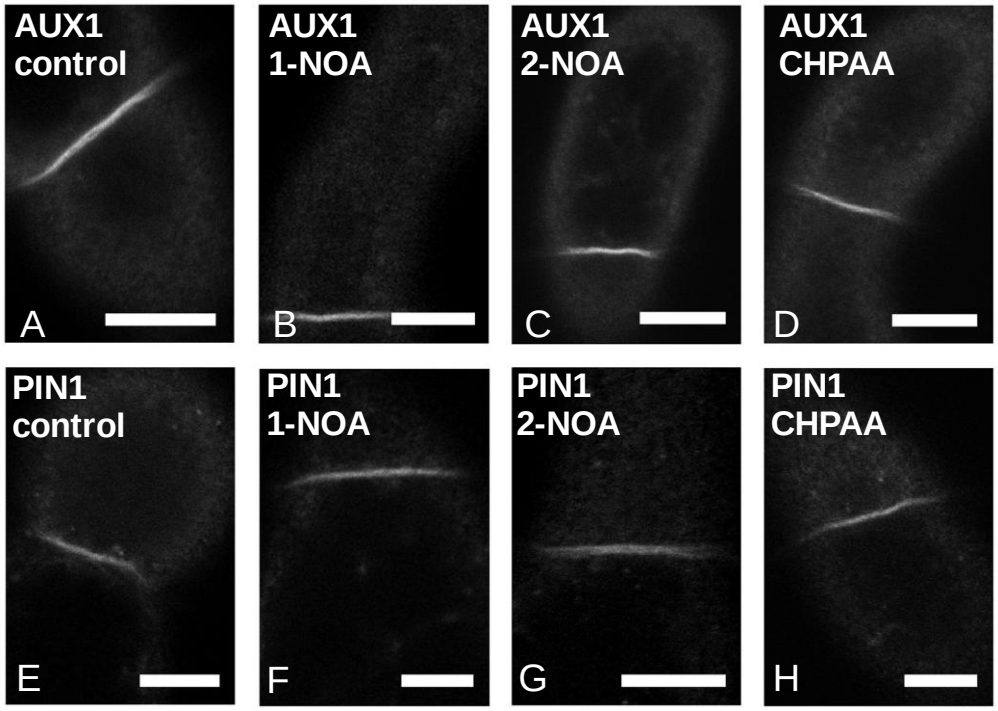
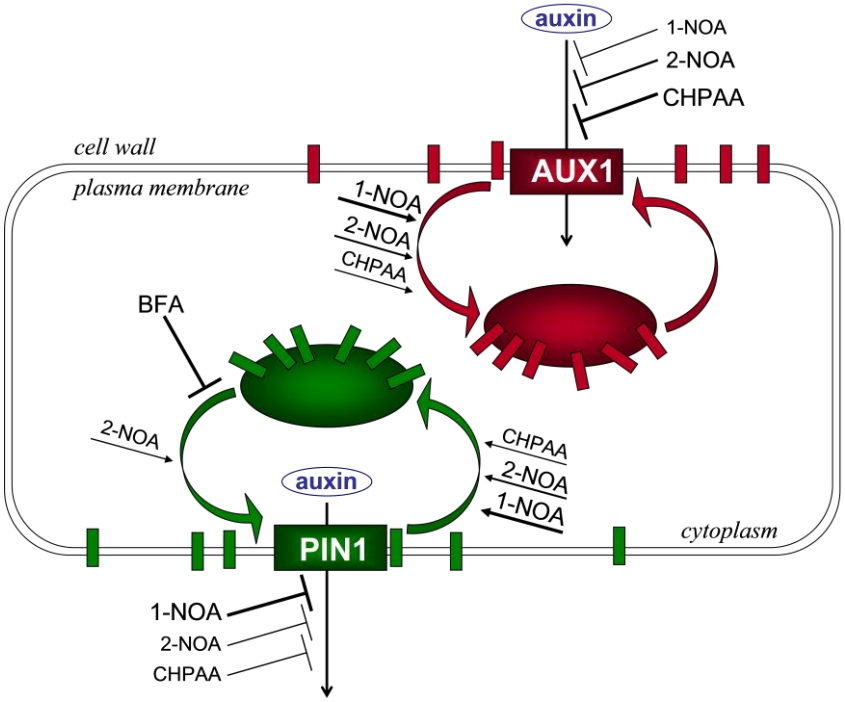


Fig. S5 Model of the effect of auxin influx inhibitors on membrane trafficking processes in plants cell.

The scheme summarizes our findings on the action of auxin transport inhibitors 1-NOA, 2-NOA and CHPAA in tobacco BY-2 cells. The findings are based on data from accumulation assays, BFA treatments and cytological experiments. Results from the accumulation assays are related to the effects of inhibitors on the auxin influx or efflux. CHPAA is the most potent in blocking auxin influx whereas 1-NOA, in contrast to 2-NOA or CHPAA, significantly blocks auxin efflux activity. On the basis of our data obtained from the BFA treatments (Fig. 3C) a possible effect of 2-NOA on the stimulation of exocytosis is implied. In this experiment 2-NOA has no additional effect on accumulation of NAA in tobacco BY-2 cells treated with BFA, while in BY-2 cells without BFA treatment the accumulation of NAA is slightly increased (Fig. 2B). Our model also depicts the unexpected effect of the putative auxin influx inhibitors on the dynamics of plasma membrane. Base on our microscopical observations and auxin accumulation data it seems that inhibitors can stimulate endocytosis and that 1-NOA is the most active in this respect.It could be speculated that 1-NOA is more effective probably due to its interaction with both faster processes of AUX1 cycling and slower cycling of PIN1. Less effective 2-NOA and CHPAA would interfere only with relatively faster processes of AUX1 recycling. Red rectangles - AUX1, green rectangles - PIN1.


auxin
1-NOA
2-NOA
CHPAA
cell wall
plasma membrane
AUX1
1-NOA
2-NOA
CHPAA
BFA
2-NOA
CHPAA
2-NOA
1-NOA
auxin
PIN1
cytoplasm
1-NOA
2-NOA
CHPAA

Video S1:

The movie shows cell plate formation in control BY-2 cells (3frames/sec for 83 frames).

Video S2:

The movie shows cell plate formation in cells after 1-NOA treatment (20 μM, 24h) (3frames/sec for 93 frames).