ABSTRACT

The non-uniform distribution of the plant growth regulator (phytohormone) auxin is known to mediate many fundamental processes in plant development. Auxin is transported through the plant body either via vascular pathways or from cell to cell by specialized polar auxin transport machinery. This machinery consists of a balanced system of passive diffusion combined with the activities of auxin influx and efflux carriers. This work is focused on the processes that are involved in the uptake of auxin into plant cells.

On the basis of molecular-biological and biochemical characterization, the function as an auxin influx carrier was confirmed for *Pa*LAX1 protein from wild cherry (*Prunus avium*). The sequences of isolated cDNA of the Pa*LAX1* gene and of its protein product are highly similar to both the cDNAs and the corresponding protein products of the *AUX1/LAX*-type genes, coding for putative auxin influx carriers in model plant *A. thaliana*. On the level of organs and single cells, we have shown that the overproduction of *Pa*LAX1 in transgenic lines resulted in an increase of the content of native auxin indole-3-acetic acid as well as of the uptake of synthetic auxin, 2,4-dichlorophenoxyacetic acid.

Further, the mechanism of action of putative auxin influx inhibitors 1-naphthoxyacetic acid (1-NOA), 2-naphthoxyacetic acid (2-NOA), and 3-chloro-4-hydroxyphenylacetic acid (CHPAA) was examined by direct measurements of kinetics of auxin accumulation, cellular phenotypic analysis, as well as by localization studies of the *A. thaliana* auxin carriers heterologously expressed in tobacco (*Nicotiana tabacum)* suspension-cultured cells. The most potent inhibitor, 1-NOA, blocked the activities of both auxin influx and efflux carriers, whereas 2-NOA and CHPAA inhibited preferentially auxin influx. The mode of action of 1-NOA and 2-NOA has been shown to be linked with the dynamics of the plasma membrane while CHPAA has no obvious influence on membrane dynamics; therefore, CHPAA seems to be the most reliable auxin influx inhibitor.

We have also characterized the activity of auxin influx carriers in the cell-based system other than tobacco - i.e. in the *A. thaliana* cell suspension. Based on accumulation assays the active, carrier-driven transport of both 2,4-D and other synthetic auxin, naphthalene-1-acetic acid (NAA) into *Arabidopsis* cells was demonstrated. The AUX1 carrier had higher affinity to 2,4-D whereas NAA was better substrate for other influx carriers (of the LAX or ABCB type). Finally, quantitative data for 2,4-D transport at the cellular level has been provided; this data will serve as an input for mathematical modelling of processes involved in 2,4-D transport on the level of a single cell.