

Auxin/nitrogen interaction in root development

Auxin transport as a key regulator of root developmental responses to nitrogen in *Arabidopsis thaliana* and *Casuarina glauca*

ABSTRACT

The aim of this project was to investigate how nitrogen availability modulates local auxin gradients within the root system, and how this results in changes in root or nodule development. Two biological models were used: i) *Arabidopsis thaliana*, where the aim is to determine how nitrate (NO₃⁻) sensing by the AtNRT1.1 NO₃⁻ transporter regulates the root system architecture through altered auxin transport, and ii) *Casuarina glauca*, where the aim is to determine the role of auxin transport in the formation of nitrogen-fixing nodules triggered by nitrogen deficiency.

In *Arabidopsis*, we previously found that the nitrate transporter NRT1.1 modulates the growth rate of lateral roots because it mediates a nitrate-regulated auxin transport (briefly, NRT1.1 transports auxin in the absence of nitrate but not when nitrate is available as a substrate). NRT1.1 slows down growth of the lateral roots at low external nitrate concentration because it lowers auxin accumulation in these roots. Thus, auxin accumulates at a high level only in lateral roots in contact with a high nitrate concentration. Because auxin enhances lateral root growth, this allows the plant to specifically promote this growth in the nitrate-rich patches of the soil. However, the mechanism by which NRT1.1 prevents auxin accumulation in lateral roots was unknown. The work done by F. Perrine-Walker allowed to characterize this mechanism. The localization of the NRT1.1 protein (generation and investigation of NRT1.1-GFP transformants) showed that it is specifically expressed in tissues involved in the basipetal transport of auxin out of the lateral root toward the inner tissues of the primary root. Two additional findings were made thereafter: (1) The expression of the NRT1.1 protein is repressed by nitrogen, and (2) nitrate affects the expression of several other auxin carriers in the lateral roots (LAX3, PIN1 and PIN2). In more detail, nitrogen starvation results in an earlier expression of NRT1.1 during the initial stages of lateral root primordia development. This earlier expression of NRT1.1 seems to be associated with primordia abortion, suggesting that in addition to regulating lateral root growth rate, NRT1.1 also controls a key step in the initial development of lateral root primordia. On the other hand, the effect of nitrate on the expression of LAX3 (stimulation) and PIN1-2 (repression) shows that nitrogen exerts a general control on the overall auxin traffic in lateral roots, beyond the specific action of NRT1.1. These new data are original and raise additional hypotheses. Complementary experiments are currently performed to strengthen these findings.

In *Casuarina*, actinorhizal nodule formation in conditions of nitrogen deprivation and in symbiosis with the bacteria *Frankia* is associated with the expression of the CgAUX1 auxin influx carrier gene in infected plant cells. Using biochemical techniques, we were able to show that nodules contain more auxin (both indole acetic acid (IAA) and phenyl acetic acid (PAA)) than non infected roots. Immunolocalization of IAA and PAA in nodules showed the preferential accumulation of auxin in *Frankia*-infected cells. Moreover, auxin efflux carriers of the PIN family were immunolocalised in *C. glauca* nodules and were found to be specifically expressed in non-infected cortical cells. Modelling auxin fluxes within nodules showed that this specific expression of auxin transporters (both CgAUX1 and PIN-like transporters) restrict auxin accumulation to *Frankia*-infected cells. Furthermore, *Frankia* genomes mining and gene expression studies indicate that *Frankia* produces auxin (both IAA and PAA) in planta. Altogether, our results indicate a link between auxin and symbiotic infection.

In summary, the experimental work has been successful. This was due to the high efficiency of F. Perrine-Walker, who could only devote one-year work to each part of the project. The tight connection between the two parts of the project was ensured by the fact that the same questions (local auxin accumulation,

expression of AUX1 and PIN carriers) were addressed in both species.

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PERSPECTIVES

In Arabidopsis, additional work is scheduled to determine whether NRT1.1 governs initial stages of lateral root development, in addition to modulating the growth rate of these roots as a function of nitrate availability (time-lapse studies of NRT1.1 expression in lateral root primordia, coupled with determination of abortion rate). The effect of nitrate on the expression of the other auxin carriers will be carried out in a NRT1.1 mutant genetic background, to determine whether this effect is dependent on NRT1.1.

In Casuarina, further work is planned to analyse the role of auxin during the infection process. We will use a 15K Casuarina microarray to study changes in gene expression in nodules treated with an inhibitor of auxin influx (NOA) versus non treated nodules. This will help us identify symbiotic genes regulated by auxin. Moreover, we recently generated a dominant negative version of an auxin response regulator (CgIAA7-DN). We will express this gene in Frankia infected cells to inhibit auxin responses specifically in those cells and study the effects on the symbiotic interaction.