

Year of CfP: 2007

Project No: 07024 Completed

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

Unit managing the project: BPMP (Plant Molecular Physiology and Biochemistry) (CNRS, INRA, SupAgro, UMII)

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Country involved in the project: UK

Research units from the Foundation's scientific network involved: AGAP, DIADE

Subthematic axes: IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*)

Objectives:

One main characteristic of plants is their ability to react to a highly fluctuating environment by initiating profound developmental changes. This is particularly true in response to nutrient stress, one of the most frequent abiotic constraints plants have to face. Nutrient stress has profound effects on root system architecture, which are triggered by specific signaling pathways. In the case of nitrate (NO₃⁻) and phosphate (PO₄), these signaling pathways have been shown to involve auxin, a key phytohormone in root development. Auxin plays a particularly central role in lateral root (LR) formation and growth, which depend on the establishment of local auxin gradients at the site of LR initiation, and within the young emerging LR.

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

The common question addressed with both species is: how does nitrogen modulate auxin transport in the root system to regulate LR growth (in *Arabidopsis*) or the formation of a modified LR (the actinorhizal nodule) in *Casuarina*? This will extend the knowledge obtained in the model species *A. thaliana* to a tropical plant that plays an important role as a pioneer species for the rehabilitation of poor or degraded sites in tropical and subtropical countries.

The investigation of auxin gradients in root tissues is challenging because of the difficulty to determine local auxin concentrations and fluxes. This project aims at overcoming this difficulty through the development of complementary approaches

Action carried-out and results obtained:

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.

Publications:

Gojon A, Krouk G, Perrine-Walker F, Laugier E. 2011. Nitrate transceptor(s) in plants. *J Exp Bot* 62: 2299-2308.

Krouk G, Ruffel S, Gutiérrez RA, Gojon A, Crawford NM, Coruzzi GM, Lacombe B. 2011. A framework integrating plant growth with hormones and nutrients. *Trends Plant Sci* 16: 178-182.

Perrine-Walker F, Doumas P, Lucas M, Vaissayre V, Beauchemin NJ, Band LR, Chopard J, Crabos A, Conejero G, Péret B, King JR, Verdeil JL, Hocher V, Franche C, Bennett MJ, Tisa LS, Laplace L. 2010. Auxin carriers localization drives auxin accumulation in plant cells infected by Frankia in *Casuarina glauca* actinorhizal nodules. *Plant Physiol* 154: 1372-1380.

Gabriel Krouk, Benoît Lacombe, Agnieszka Bielach, Francine Perrine-Walker, Katerina Malinska, Emmanuelle Mounier, Klara Hoyerova, Pascal Tillard, Sarah Leon, Karin Ljung, Eva Zazimalova, Eva Benkova, Philippe Nacry, Alain Gojon (Jun 2010) Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. *Dev. Cell* 18(6):927-937

Péret B, De Rybel B, Casimiro I, Benková E, Swarup R, Laplace L, Beeckman T, Bennett MJ. 2009 *Arabidopsis* lateral root development: an emerging story. *Trends Plant Sci.* 14: 399-408.

Prospects for the future:

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.

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Funding categorie(s): Agropolis Fondation post-doctoral fellowship

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