

Article Addendum

A Role for auxin during actinorhizal symbioses formation?

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The symbiotic interaction between the soil bacteria Frankia and actinorhizal plants leads to the formation of nitrogen-fixing nodules resembling modified lateral roots. Little is known about the signals exchanged between the two partners during the establishment of these endosymbioses. However, a role for plant hormones has been suggested.

Recently, we studied the role of auxin influx activity during actinorhizal symbioses. An inhibitor of auxin influx was shown to perturb nodule formation. Moreover we identified a functional auxin influx carrier that is produced specifically in Frankia-infected cells. These results together with previous data showing auxin production by Frankia lead us to propose a model of auxin action during the symbiotic infection process.

Actinorhizal symbioses result from the interaction between the soil actinomycete Frankia and plants belonging to eight angiosperm families collectively called actinorhizal plants.¹ This symbiotic interaction leads to the formation of a new organ on the root system, the actinorhizal nodule, where the bacteria are hosted and fix nitrogen.² Unlike legume nodules, actinorhizal nodules are structurally and developmentally related to lateral roots.³ Little is known about the signals exchanged between the two partners during the establishment of the symbiosis.² Diffusible signals are emitted by Frankia at early stages of the interaction resulting in root hair deformation.² The chemical nature of these signals remains unknown, however, detailed studies revealed that they are different from rhizobial Nod factors.⁴ Phytohormones are chemicals that control many developmental processes⁵ and have been linked to many plant-microbe interactions. Recently, we studied the role of auxin influx in actinorhizal nodule formation in the tropical tree *Casuarina glauca*.⁶

Auxin Influx Activity is Specifically Associated with Frankia Infection

Inhibition of auxin influx using the competitive inhibitor naphthoxyacetic acid (1-NOA) perturbs actinorhizal nodule formation in *C. glauca*.⁶ Two genes encoding putative auxin influx carriers from *C. glauca* were cloned and characterized. One of these genes named *CgAUX1* was shown to encode a functional auxin influx carrier by complementation of the Arabidopsis *aux1* mutant. Interestingly, *CgAUX1* is expressed in Frankia-infected cells during actinorhizal nodule formation.⁶ This, together with the negative effect of 1-NOA on nodulation, suggests a role for auxin influx during the infection process.

A role for auxin was also speculated during the colonization of plant root by arbuscular mycorrhizal fungi (AMF).^{7,8} In order to check whether auxin influx mediated by *CgAUX1* is associated with the infection by AMF, *CgAUX1* expression was studied during the symbiotic interaction between *C. glauca* and the AMF *Glomus intraradices*. *C. glauca* seedlings were inoculated in vitro using a previously described system (ref. 9). A slight but significant increase in *CgAUX1* expression level was detected by qRT-PCR in *G. intraradices* colonized roots compared to non-inoculated roots (Fig. 1). However using transgenic *C. glauca* plants containing a *ProCgAUX1::GUS* construct we did not observe any GUS activity in plant cells colonized by the symbiotic fungus at any stage of the interaction nor did we detect any change in the expression pattern compared to non inoculated plants. This indicates that *CgAUX1* expression during plant cell infection by the microsymbiont is not a general feature of endosymbioses but a specific response to Frankia.

Frankia Produces Auxin

Frankia like many soil bacteria has been known for a long time to produce phytohormones including auxins. For instance, indole-3-acetic acid (IAA) and phenylacetic acid (PAA) are found at relatively high concentration (10^{-5} to 10^{-6} M) in the supernatant of various Frankia strains in pure culture.^{10,11} However, it remains to be elucidated whether or not Frankia produces auxins in planta during the infection process. This could explain the higher levels of auxins found in actinorhizal nodules compared to uninfected roots.¹⁰ Interestingly, an auxin inducible gene called *EuNOD-ARPI* is expressed in Frankia-infected cells in actinorhizal nodules of *Eleagnus umbellata*¹² thus suggesting auxin accumulation in those cells.

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A Role for Auxin Signalling During the Infection Process?

Taken together all the available data suggest a role for auxin during plant cell infection by *Frankia* (Fig. 2). The functional auxin influx carrier *CgAUX1* is produced in plant cells upon infection in response to a bacterial signal. The nature of this signal remains to be determined but it is not an auxin or any diffusible signal found in *Frankia* supernatant. *CgAUX1* expression makes the infected plant cells more permeable to auxin and allows the perception of auxins produced by *Frankia*. A specific auxin response might occur in infected cells allowing the infection to proceed. The infection threads are surrounded by the plant cell membrane and a new cell wall-like structure composed mainly of pectin derivatives.¹³ Auxins are known to regulate genes involved in cell wall remodelling and pectin biosynthesis and methylation.¹⁴ Auxin perception in infected plant cells might therefore be necessary to allow the growth of infection threads. Our future work will focus on testing this model.

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References

- Benson DR, Silvester WB. Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. *Microbiol Rev* 1993; 57:293-319.
- Obertello M, Sy MO, Laplace L, Santi C, Svistoonoff S, Auguy F, Bogusz D, Franche C. Actinorhizal nitrogen fixing nodules: Infection process, molecular biology and genomics. *Afr J Biotechnol* 2003; 2:528-38.
- Pawlowski K, Bisseling T. Rhizobial and actinorhizal symbioses: What are the shared features? *Plant Cell* 1996; 8:1899-913.
- Cérémonie H, Debellé F, Fernandez MP. Structural and functional comparison of *Frankia* root hair deforming factor and rhizobia Nod factor. *Can J Bot* 1999; 77:1293-301.
- Teale WD, Paponov IA, Palme K. Auxin in action: Signalling, transport and the control of plant growth and development. *Nat Rev Mol Cell Biol* 2006; 7:847-59.
- Péret B, Swarup R, Jansen L, Devos G, Auguy F, Collin M, Santi C, Hocher V, Franche C, Bogusz D, Bennett M, Laplace L. Auxin influx activity is associated with *Frankia* infection during actinorhizal nodule formation in *Casuarina glauca*. *Plant Physiol* 2007; 144: 1852-62.
- Jentschel K, Thiel D, Rehn F, Ludwig-Muller J. Arbuscular mycorrhiza enhances auxin levels and alters auxin biosynthesis in *Tropaeolum majus* during early stages of colonization. *Physiol Plant* 2007; 129:320-33.
- Barker SJ, Tagu D. The roles of auxins and cytokinins in mycorrhizal symbioses. *J Plant Growth Regul* 2000; 19:144-54.
- St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA. Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an in vitro system in the absence of host roots. *Mycol Res* 1996; 100:328-32.
- Wheeler CT, Henson IE, MacLaughlin ME. Hormones in plants bearing actinomycete nodules. *Bot Gaz* 1979; 140:52-7.
- Hammad Y, Nalin R, Marechal J, Fiasson K, Pepin R, Berry AM, Normand P, Domenach AM. A possible role for phenyl acetic acid (PAA) on *Alnus glutinosa* nodulation by *Frankia*. *Plant Soil* 2003; 254:193-205.
- Kim HB, Lee H, Oh CJ, Lee NH, An CS. Expression of *EuNOD-ARF1* encoding auxin-repressed protein homolog is upregulated by auxin and localized to the fixation zone in root nodules of *Elaeagnus umbellata*. *Mol Cells* 2007; 23:115-21.
- Lalonde M, Knowles R. Ultrastructure, composition, and biogenesis of the encapsulation material surrounding the endophyte in *Alnus crispa* var. *mollis* root nodules. *Can J Bot* 1975; 53:1951-71.
- Lerouxel O, Cavalier DM, Liepman AH, Keegstra K. Biosynthesis of plant cell wall polysaccharides - A complex process. *Curr Opin Plant Biol* 2006; 9:621-30.

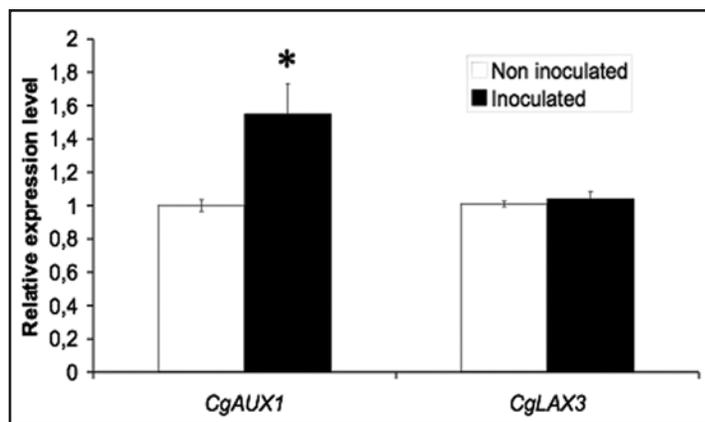


Figure 1. *CgAUX1* and *CgLAX3* expression levels in *C. glauca* root colonized by *G. intraradices* versus non-inoculated roots. Seedlings were grown together with the fungus using a two compartment system previously described (ref. 9). RNA extraction and qRT-PCR were performed as previously described (ref. 6). Data presented are means \pm SD. *indicates a significant difference from control (Student's t test $p < 0.05$).

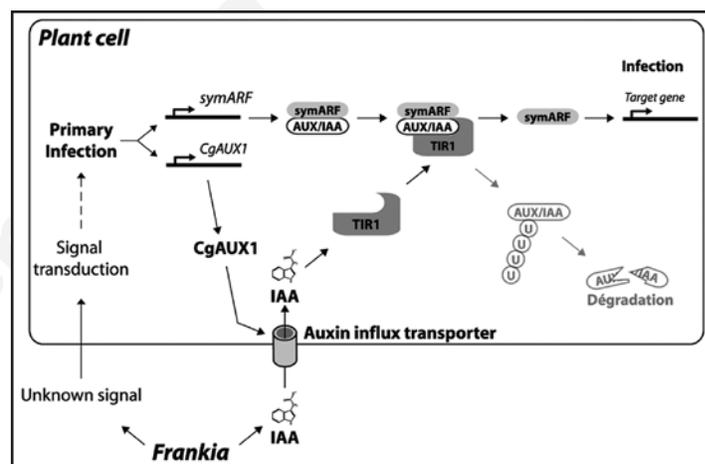


Figure 2. A model for auxin action during plant cell infection by *Frankia*. Auxin produced by *Frankia* enters infected plant cells through the action of the auxin influx transporter *CgAUX1*. Inside the cell, auxin is probably perceived through the SCF^{TIR1} complex leading to AUX/IAA degradation and allowing the fixation of some symbiotic ARF (*symARF*) to the promoter of downstream target genes. These genes remain to be identified but could be involved in infection thread formation.