The cell-cycle promoter cdc2aAt from *Arabidopsis thaliana* is induced in the lateral roots of the actinorhizal tree *Allocasuarina verticillata* during the early stages of the symbiotic interaction with *Frankia*

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The symbiosis between the actinorhizal tree *Allocasuarina verticillata* and the actinomycete *Frankia* leads to the formation of root nodules inside which bacteria fix atmospheric nitrogen. Actinorhizal nodule organogenesis starts with the induction of cell divisions in the root cortex and in the pericycle cells opposite protoxylem poles near *Frankia*-infected root hairs. To study the ability of *Frankia* to induce progression through the cell cycle, we monitored the expression of the β-glucuronidase (*gus*) gene driven by the promoter from cdc2aAt, an *Arabidopsis* cyclin-dependent kinase gene that displays competence for cell division, during plant growth and nodule ontogenesis. In non-symbiotic tissues, the *gus* gene was mainly expressed in primary and secondary meristems of roots and shoots. Auxins and cytokinins were found to induce reporter gene activity in the root system of whole plants, showing that the promoter cdc2aAt displayed the same regulation by hormones in *Allocasuarina* as that reported in *Arabidopsis*. In transgenic nodules, *gus* expression was found to be restricted to the phellogen. During the early stages of the interaction between *Frankia* and the plant root system, cdc2aAt was strongly induced in the lateral roots surrounded by hyphae of the actinomycete. Histochemical analysis of β-glucuronidase activity revealed that cells from the pericycle opposite protoxylem poles were very deeply stained. These data indicate that upon *Frankia* infection, cells from the lateral roots, and notably pericycle cells that can give rise to a nodule or a root primordium, prepare to re-enter the cell cycle.

**Introduction**

Actinorhizal plants have the ability to form a root symbiosis with the soil actinomycete *Frankia*, a nitrogen-fixing filamentous prokaryote (Benson and Silvester 1993, Pawlowski 2002). Actinorhizal hosts described to date are shrub and tree species from 25 genera of angiosperm plants belonging to eight phylogenetically diverse families. The establishment of the actinorhizal symbiosis is a complex process where both the host plant and *Frankia* undergo biochemical, physiological and molecular changes (Franche et al. 1998, Vessey et al. 2005). It is likely that these modifications involve...
compounds that are synthesized by the two symbiotic partners. However, compared with the progress made in elucidating the first steps of the interaction between Rhizobium and legumes (Geurts et al. 2005), little is known about the early stages of the actinorhizal symbiosis.

Actinorhizal nodules are perennial multilobed structures that resemble modified lateral roots with a central vascular system, and which originate from the pericycle (Berry and Sunell 1990). Depending on the host, nodules are initiated either via root hair infection by Frankia or by intercellular invasion. In the Casuarinaceae family, the infection process occurs intracellularly and starts with curling and branching of the root hair induced by unknown Frankia signals (Callaham et al. 1979). Frankia hyphae associated with deformed root hairs penetrate at the site of folding of deformed root hairs and become sheathed by host plasmalemma and host-derived wall material containing pectin, cellulose and hemicellulose (Berg 1999). This interfacial matrix is the equivalent of the infection thread wall in legume nodules. Upon infection, cell divisions occur in the root cortex near the infected hairs, leading to a small external protuberance called prenodule (Berry and Sunell 1990). Most of the prenodule cells are invaded by Frankia filaments, but the prenodule does not evolve into a nodule. At the same time, mitotic activity occurs in pericycle cells opposite a protoxylem pole close to the prenodule, giving rise to a lobe primordium that will grow and become infected by encapsulated hyphae of Frankia (Callaham and Torrey 1977). Each nodule lobe has an indeterminate growth pattern and four different zones: a persistent apical meristem (zone I), an infection zone (zone II), a nitrogen fixation zone (zone III) and in older portions of nodules, a senescent zone (zone IV).

Following nitrogen starvation and inoculation by Frankia, fully differentiated root cells of actinorhizal plants re-enter the cell cycle. Cell division in the cortex near the infected root hair results in the formation of a prenodule, while the division of pericycle cells located near these activated cortical cells will generate a nodule primordium. In eucharyotes, the progression through the cell cycle is regulated by cyclin-dependent kinases (CDKs) in association with regulatory subunits, called cyclins (Pines 1995). In plants, the CDK/cyclin complexes are influenced by a combination of different gene products and factors which ensure that the progression of the cell cycle is initiated as an integral part of the growth and development program or in response to the environment (Joube`s et al. 2000, Stals et al. 2000). In the model plant Arabidopsis thaliana, the family of CDK-related kinases is composed of 12 proteins grouped in six types (Wang et al. 2004). The P34cdc2 kinase, which is encoded by the gene cdc2a, belongs to the A-type CDKs and is a key component in the regulation of the G1 to S and G2 to M transitions (Ferreira et al. 1991, Hemerly et al. 1992). In Arabidopsis, cdc2aAt is highly expressed in all meristems and apparently throughout all phases of the cell cycle (Hemerly et al. 1992, 1993). It is also expressed in cells, which have not yet entered the mitotic cell cycle but have increased competence to do so.

When the promoter region of cdc2aAt was fused to the β-glucuronidase (GUS) gene and genetically transformed into Arabidopsis, the pattern of reporter gene activity was found to resemble the pattern of cdc2aAt mRNA accumulation observed by in situ hybridization (Hemerly et al. 1993). External signals such as light, wounding and hormones were found to regulate cdc2aAt-gus transcription. When introduced into the heterologous host tree Populus, cdc2aAt-gus was shown to retain the same specificity of expression as that observed in Arabidopsis (Rohde et al. 1997). It also appeared to be a useful indicator of growth activity in individual axillary buds of poplars subjected to different growth conditions and abiotic stress. cdc2aAt-gus expression can thus be used to monitor the cells that are progressing through the cell cycle or that acquire competence for cell division. However, more recent data established that cdc2aAt could also direct transcription during specific stages of cell morphogenesis. A deletion analysis of the promoter cdc2aAt showed that a 595-bp DNA fragment located upstream from the transcription start could direct transcription in a period of trichome development that did not include cell division (Imajuku et al. 2001).

In this paper, we obtained transgenic plants of the tropical actinorhizal tree Allocasuarina verticillata expressing the cdc2aAt-gus construct. We first determined the GUS expression pattern conferred by cdc2aAt in roots and shoots and compared the data we obtained to those reported for Arabidopsis (Hemerly et al. 1993). We then checked if hormones such as auxins and cytokinins could regulate the transcription of cdc2aAt in the root system of Allocasuarina. Finally, to study the ability of Frankia to induce the progression of root cells through the cell cycle, we performed a time course analysis of cdc2aAt-gus expression during actinorhizal nodule ontogenesis.

**Materials and methods**

**Plasmid and bacterial strains**

The chimeric fusion Pcdc2aAt-gus consisted of the 1.7-kb promoter and leader sequences of the A. thaliana (L.) Heynh. cdc2aAt gene (Hemerly et al. 1993) fused to the GUS (gus or uidA from Escherichia coli) reporter gene
A. verticillata used for inoculation and nodulation of supplemented with appropriate antibiotics. Allocasuarina verticillata seeds were collected in Plant material and culture media (Benoist et al. 1992). pionate medium with phosphatidyl choline and MES) plants was grown in a modified BAP-PCM (basic pro- medium (H medium) supplemented with 0.13 m calcium hypochlorite for 35 min, and rinsed three times under sterile conditions on a quarter-strength Hoagland with sterile distilled water. Seeds were germinated with 5% sulfuric acid for 2 min and washed for 30 min under run- ning tap water. They were then surface sterilized with 5% calcium hypochlorite for 35 min, and rinsed three times with sterile distilled water. Seeds were germinated under sterile conditions on a quarter-strength Hoagland medium (H medium) supplemented with 0.13 mM ammonium sulfate (Hoagland and Arnon 1938) at pH 5.6 and solidified with 8 g l⁻¹ Difco bacto-agar as described previously (Le et al. 1996). The plantlets were grown for 1–2 months at 28°C with a 16-h photoperiod and a light intensity of 50 µE m⁻² s⁻¹.

Plant material and culture media

Allocasuarina verticillata seeds were collected in Australia and provided by the Versepuy Company (le Puy-en Velay, France). Seeds were scarified with 95% sulfuric acid for 2 min and washed for 30 min under running tap water. The presence and the integrity of the chimeric gene fusion introduced into Agrobacterium were verified by Southern blot analy- sis of digested bacterial DNA (data not shown). Prior to genetic transformation of A. verticillata, the A. tumefaciens strain was grown at 28°C in Ag medium (Le et al. 1996) supplemented with appropriate antibiotics.

The Frankia strain Allo2 (Girgis and Schwencke 1993) used for inoculation and nodulation of A. verticillata plants was grown in a modified BAP-PCM (basic pro- pionate medium with phosphatidyl choline and MES) (Benoist et al. 1992).

Genetic transformation and regeneration of transgenic plants

Transgenic A. verticillata plants containing the chimeric gene construct Pcdc2aAt-gus were generated by A. tumefaciens mediated transformation as previously described (Franche et al. 1997). Transgenic calli were selected on nutrient Murashige and Skoog modified for Casuarina medium (Franche et al. 1997) supplemented with 6-benzylaminopurine (BA; 2.5 mM), α-naphthalen- acetic acid (NAA; 0.5 mM), kanamycin (100 mg l⁻¹) and ceftaxime (300 mg l⁻¹). Gene transfer was confirmed in regenerated rooted plants by polymerase chain reaction using nptII and gus primers (data not shown). Untrans- formed plants were regenerated from non-transgenic calli and used as negative controls.

Plant inoculation by Frankia

Transgenic and non-transformed A. verticillata plants with a main root at least 3 cm in length were transferred to tubes (Gibson 1963) containing H liquid medium with ammonium sulfate and placed in a controlled environment chamber as described above. When the root system reached at least 10 cm in length, plants were deprived of nitrogen and inoculated by Frankia Allo2 (Smouni et al. 2002). Nodule initiation was monitored weekly using a stereomicroscope.

Plant growth regulator treatments

At least five plants of each transgenic callus line were grown for 3 weeks in sterile conditions in H liquid medium. All the hormones tested were applied at a concentration ranging from 1 to 10 µM. After 1–3 days of induction, GUS activity was analyzed histochemically in the root system as described below.

Histochemical GUS assay

Excised shoots or roots were immersed overnight at 37°C into a staining solution containing 1 mM 5-bromo-4-chloro-3-indolyl β-D-glucuronic acid (X-Gluc) substrate and incubated at 37°C as recommended by Jefferson et al. (1987). To confine the localization of the blue staining, 0.5 mM potassium ferrocyanide and 0.5 mM potassium ferrocyanide were added as catalysts. Stained samples were then fixed for 1 h in a solution containing 5% formaldehyde, 5% acetic acid and 50% ethanol. Soluble pigments were removed by immersing the plant material in several baths of 70% ethanol until cleared.

To allow the penetration of the substrate X-Gluc, actinorhizal nodules were embedded in 3% agarose and sliced into 45–50-µm thick longitudinal or transverse sections on a vibratome (VT1000E; Leica, Wetzlar, Germany). After incubation in the X-Gluc solution, samples were mounted on glass slides with a drop of 50% glycerine solution and examined under a stereo- microscope.

Microscopy

Transgenic-stained shoots and roots were fixed and embedded in Histo-Technovit 7100 resin as described by Laplaze et al. (1999). Thin 4–8-µm sections were made with a microtome (Historexange 2218; LKB, Bromma, Sweden), immersed for 10 min in 5% ruthenium red and mounted on glass slides with Clearium Mountant (Surgipath Medical Instruments, Richmond, IL). Micrographs were taken with a light microscope (model DMR13; Leica).
Results

Analysis of $cdc2aAt$ promoter activity in shoots and roots of transgenic $A. verticillata$ plants

From two separate experiments involving the genetic transformation of 200 epicotyl fragments of $A. verticillata$, 38 kanamycin-resistant calli were obtained and further analyzed. Gene transfer was monitored by the expression of the GUS gene and by the amplification of the gus and nptII genes (data not shown). Five independent transgenic callus lines exhibiting representative GUS patterns and strong reporter gene activity were chosen for detailed cytological analyses and induction experiments. For each callus line, an average of five to seven plants were incubated with X-Gluc and histochemically analyzed. Non-transformed control plants did not exhibit any endogenous GUS activity (data not shown).

First, we checked whether the expression of the chimeric $cdc2aAt$-gus construct could be used as an indicator of cell division in $Allocasuarina$. All through the shoots, $cdc2aAt$ was found to drive reporter gene activity at the node level under the scale-like leaves of $A. verticillata$ (Fig. 1A). The intensity of blue staining was uniform in the nodes along the shoot and was observed in the apical region and near the base of the shoot. To further localize $cdc2aAt$-driven gus expression, longitudinal and transverse sections were made with a microtome in the gus-expressing region (Fig. 1B, C). In the longitudinal section (Fig. 1B), GUS activity was localized at the base of axillary buds and in proximal tissues of the stem and leaves. On the transgenic node cross section made just above the axillary buds (Fig. 1C), $cdc2aAt$ gene expression was observed in the internal part of the foliar sheath and in the epidermis of the stem. Blue staining was also observed in the epidermis and in the subepidermal cell layers on the adaxial face of the scales. $cdc2aAt$-gus was also expressed in the cells of the foliar mesophyll surrounding the conductive tissues, but here blue staining

Fig. 1. Histochemical localization of GUS activity in transgenic $cdc2aAt$-gus $Allocasuarina verticillata$ plants. (A) Shoot; (B) longitudinal section through a shoot at level of the node; (C) transverse section through a shoot just above a node; (D) apex of a primary root; (E) longitudinal section through the apex of a primary root; (F) meristematic dome of an emerging lateral root; (G) root treated with $10^{-6}$ M 2iP for 3 days; (H) root treated with $10^{-5}$ M BAP for 3 days; (I) root treated with $10^{-5}$ M kinetin for 3 days; (J) root treated with $10^{-5}$ M zeatin for 3 days; (K) root treated with $10^{-5}$ M 2,4-D for 3 days; (L) root treated with $10^{-5}$ M NAA for 3 days; (M) root treated with $10^{-5}$ M IAA for 3 days. Scale bars: 1 mm (A, D–M); 100 μm (B, C, E).
was less intense. There was no blue staining in the palisade parenchyma of the scales. In contrast, all the meristematic cells of the internal young axillary buds expressed the cdc2aAt-driven reporter gene. Positive GUS activity was confined to the phloem poles of the vascular bundles in the scales. In the internodes, no GUS activity was observed on the longitudinal and transverse sections (data not shown).

In the primary roots, cdc2aAt-gus expression was restricted to the apex (Fig. 1D). No expression was observed in either the elongation zone or in older parts of the root system. The root vascular system was completely devoid of indigo blue crystals. A longitudinal section made in a primary root tip showed blue staining in apical and basal meristem cells, in the quiescent center and in the root cap cells (Fig. 1E). In the lateral roots, strong reporter gene expression was observed in the meristematic domes of emerging lateral roots (Fig. 1F). GUS staining persisted in lateral root apical meristems throughout root development (data not shown).

**Control of cdc2aAt-gus expression by hormones**

We investigated if regulation of cdc2aAt by hormones observed in transgenic Arabidopsis (Hemerly et al. 1993) also occurred in the tropical actinorhizal tree A. verticillata. Two different concentrations of hormones (1 or 10 μM) were tested on the plant root system of five plants regenerated from each of the callus lines previously selected. The plant root system was incubated in X-Gluc after 1, 2 or 3 days of hormone treatment.

The cytokinins tested included 6-(-γ-γ-dimethylamino)purine (2iP), BAP, kinetin and zeatin. Reporter gene induction was detected 24 h after incubation with 1 μM of cytokinin (data not shown). After a 72-h treatment with 10 μM of cytokinin, the roots showed increased GUS activity in the primary root whatever the hormone tested (Fig. 1G–J). 2iP and BAP provided the best induction of the cdc2aAt promoter in the primary root and along the young lateral roots including the emerging meristematic domes.

We then analyzed cdc2aAt-gus induction by 1 or 10 μM of the auxins 2,4-dichlorophenoxyacetic acid (2,4-D), NAA or IAA. After 72 h of hormone treatment, GUS staining was observed in the transgenic A. verticillata root system. Intense blue staining was observed in the plant incubated with 2,4-D and NAA in both primary and lateral roots (Fig. 1K–L). With IAA, induction was mainly observed in the primary roots (Fig. 1M). These differences might be linked to the stability of the exogenously applied auxins, IAA being a less stable growth regulator than NAA and 2,4-D (Teale et al. 2006).

**cdc2aAt expression upon Frankia infection and nodule ontogenesis**

Twenty transgenic cdc2aAt-gus plants from each callus line were inoculated with Frankia Allo2 and three plants were tested for GUS activity at different stages of the nodulation process.

In the first stages of the infection process in A. verticillata, Frankia hyphae are embedded within a mucilage layer in the root hairs and in the epidermal layer. This mucilage layer is secreted by root hairs (Berry and Sunell 1990, Bhuvaneswari and Solheim 2000). In transgenic cdc2aAt-gus plants, lateral roots surrounded by Frankia hyphae were found to express strong reporter gene activity (Fig. 2A, B). In the primary root, staining was restricted to root hairs. A control experiment was conducted to check that Frankia Allo2 did not express any endogenous GUS activity (data not shown). A cross section made in gus-expressing lateral roots revealed that all cell types stained blue, although the intensity of staining varied (Fig. 2C). The pericycle cells opposite the xylem poles exhibited a very strong gus expression; intense blue staining was also observed in the root epidermis. However, because cells in the epidermis and in the cortex are not of the same size, it is difficult to conclude that the varying intensity of staining is the result of different GUS activities.

**Fig. 2.** Histochemical localization of GUS activity in transgenic cdc2aAt-gus Allocasuarina verticillata plants inoculated by Frankia. (A) Whole root system inoculated for 7 days with Frankia; (B) close-up of a lateral root; (C) transverse section through a lateral root inoculated for 7 days with Frankia; (D): vibratome section through a young nodule collected 4 weeks after inoculation; (E) longitudinal section through a 2-month-old nodule. Scale bars: 2 mm (A, B); 100 μm (C–E).
After about 3 weeks, the first nodules with one or two lobes were observed and GUS activity was analyzed on vibratome sections. Pale blue staining suggested that GUS activity was lower than in roots (Fig. 2D). cdc2aAt-gus expression was observed in a peripheral nodule layer corresponding to the phellogren. In more mature nodules exhibiting five to eight nodular lobes, a similar pattern of blue staining was observed (Fig. 2E). No gus expression was seen in the nodule vascular system or in the cortical region containing both Frankia-infected cells and uninected cells.

**Discussion**

We used GUS fusion and transgenic plants of the actinorhizal tree *A. verticillata* to monitor the expression of the cell-cycle marker *cdc2aAt* from *A. thaliana* during plant development and nodule ontogenesis. In *Arabidopsis*, the expression of *cdc2aAt* has been mainly associated with cell division and competence to divide (Hemerly et al. 1993). Additional roles during cell morphogenesis and cell elongation have been suggested more recently (Bursens et al. 2000; Imajuku et al. 2001).

In *Arabidopsis*, *cdc2aAt* is expressed without any specificity in all plant meristems and in dividing cells (Hemerly et al. 1993). The promoter displayed similar specificity of expression in *A. verticillata* and was found to be mainly expressed in both primary and secondary meristems of roots and shoots. However, in *Arabidopsis* roots, GUS staining was also observed throughout the pericycle and parenchyma of the vascular cylinder, suggesting that *cdc2aAt* expression reflects a state of competence for cell division (Hemerly et al. 1993). Whatever the transgenic line of *Allocasuarina*, we observed no GUS expression in the root vascular cylinder, suggesting that the expression of *cdc2aAt* is strictly meristem specific in the root system during the development of actinorhizal trees. In transgenic nodules, whatever their developmental stage, the gus expression conferred by *cdc2aAt* was only observed in the phellogren. The phellogen is a cortex cambium corresponding to a lateral ring of meristematic tissue which produces cork on the outside and phelloderm on the inside. *cdc2aAt* activity was not observed in Frankia-infected cells. In the same way as in the *Rhizobium-legume* symbiosis, the infected threads containing the *Frankia* hyphae do not penetrate the dividing cells. Penetration of the actinomycete hyphae in the nodule cortical cells is only followed by enlargement of host cells that become surrounded by a specialized cell layer with hydrophobic properties (Berry and Sunell 1990).

Hormones control cell division in plants (Francis and Sorrell 2001, del Pozo et al. 2005). In *Arabidopsis*, expression of *cdc2aAt* is regulated by various hormone treatments (Hemerly et al. 1993) and the promoter contains both abscisic acid and auxin response elements (Chung and Parish 1995). In *A. thaliana*, after 72 h of treatment either with cytokinins including BA and kinetin, or auxins such as IAA, NAA and 2,4-D, the transgenic roots *cdc2aAt-gus* showed increased reporter gene activity in the pericycle and parenchyma cells of the vascular cylinder. Our data showed that *cdc2aAt* retains its inducibility by auxins and cytokinins in the root system of whole plants of *A. verticillata*. In plants, auxins play a central role in triggering lateral root formation (Casimiro et al. 2003, Teale et al. 2006). With a local increase in auxin, pericycle cells in regions immediately adjacent to protoxylem poles re-enter the cell cycle and divide to form a primordium that eventually forms a lateral root.

Under conditions of limited combined nitrogen and active photosynthesis, a few cells close to the infected root hair acquire the competence to enter the nodule development program in *Allocasuarina*. In this zone, the cell cycle is activated in the cortical cells that subsequently give rise to the prenodule, and in pericycle cells opposite the protoxylem pole near the prenodule. We found that highest level of *cdc2aAt-gus* expression occurred during the initial phases of *Frankia* infection, before the nodules had developed. At the stage where *Frankia* is embedded in a mucilage layer excreted by the deformed root hairs (Bhuvaneswari and Solheim 2000), the young lateral roots exhibited strong and uniform *cdc2aAt-gus* activity from the root tip to the zone of attachment to the primary root. Interestingly, intense GUS staining was observed in the pericycle cells opposite the protoxylem poles, which can give rise either to a nodule primordium or to a root primordium. These data suggest that in response to *Frankia*, the lateral root cells are able to recover mitotic competence.

Modifications in endogenous phytohormone gradients probably mediate the activation of cell cycle during actinorhizal ontogenesis. High levels of auxin conjugates, cytokinin derivatives and gibberellins have been measured in early active actinorhizal nodule tissue (Wheeler et al. 1979). In addition, IAA and cytokinins have been reported in *Frankia* culture medium (Berry et al. 1989, Stevens and Berry 1988, Wheeler et al. 1984), and two *Alnus*-inf ective *Frankia* strains and two *Elaeagnus*-inf ective strains were recently found to produce auxin phenyl acetic acid at a concentration of $10^{-5}$ to $10^{-6}$ M (Hammad et al. 2003). It should also be noted that in the actinorhizal plant *Alnus*, the number of lateral root primordia initiated on roots following inoculation by *Frankia* is higher than that on uninoculated control roots, indicating that the actinomycete can stimulate lateral root initiation (Wheeler et al. 1979). Auxins and cytokinins
secreted by the actinomycete may also be involved in the hypertrophy of Frankia-infected cells.

In legumes, it has been established that Nod factors are involved in the activation of the cell cycle during the early stages of the interaction. After the addition of Nod factors to a microcallus cell suspension of *Medicago sativa*, an increase in *cdc2* expression was observed within 2 h, indicating that Nod factors stimulate re-entry of quiescent cells into the cell cycle (Savoure´ et al. 1997). This induction was followed by the expression of the S-phase-specific histone H3 gene and the expression of a mitotic cyclin. Reactivation of the cell cycle by Nod factors was also shown in *planta* in alfalfa and pea roots (Yang et al. 1994) and in *Medicago truncatula* (Foucher and Kondorosi 2000). Several studies suggest that Nod factors affect local distribution and concentration of auxins and cytokinins, which then appear to play a major role in controlling the plant symbiotic programs (Mulder et al. 2005). Using the auxin-sensitive promoter GH3 fused to the *gus* reporter gene, transient inhibition of auxin transport was observed in white clover, leading to the accumulation of auxin at the site of nodule initiation (Mathesius et al. 1998). With the cytokinin-sensitive promoter ARR5, upregulation of cytokinin levels was observed in root hairs undergoing deformation and in initially dividing cells in the root cortex (Lohar et al. 2004).

In actinorhizal plants, the deformation of root hairs is the first morphological indication of molecular signal exchange between the host plant and Frankia. These deformations can be induced by cell-free filtrates of Frankia, but the chemical nature of the deformation factors (DF) is still not known (Cérémonie et al. 1999). Attempts to purify the DF have failed because of technical difficulties associated with the slow rate of growth and biomass production in Frankia cultures and the absence of mutagenesis and transformation techniques. Recent sequencing of the genome of Frankia strains failed to locate any genes resembling rhizobial common *nod* genes, except for *NodB*, suggesting that rhizobia Nod factors and Frankia DF are structurally divergent (P. Normand, unpublished data). Postgenomic analyses of Frankia using DNA chips to monitor the modification of transcripts during the early stages of the interaction and proteomic analyses should provide valuable information on the signals produced by Frankia that induce plant root cells to progress through the cell cycle. Furthermore, the isolation and functional characterization of cyclin and CDK genes isolated from *Casuarinaceae* will be of great importance for a deep understanding of the cell cycle modifications and regulations linked to Frankia infection.

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