Plant Cell Rep (2009) 28:83–93
DOI 10.1007/s00299-008-0610-1

PHYSIOLOGY AND BIOCHEMISTRY

Proteins prenylated by type I protein geranylgeranyltransferase act positively on the jasmonate signalling pathway triggering the biosynthesis of monoterpene indole alkaloids in *Catharanthus roseus*

Vincent Courdavault · Vincent Burlat · Benoit St-Pierre · Nathalie Giglioli-Guivarc'h

Received: 11 June 2008 / Revised: 3 September 2008 / Accepted: 7 September 2008 / Published online: 24 September 2008

Abstract In *Catharanthus roseus*, the first step of monoterpenoid indole alkaloids (MIA) biosynthesis results from the condensation of the indole precursor tryptamine with the terpenoid precursor secologanin. Secologanin biosynthesis requires two successive biosynthetic pathways, the plastidial methyl-D-erythritol 4-phosphate (MEP) pathway and the monoterpene secoiridoid pathway. In *C. roseus* cell culture, the expression of several genes encoding enzymes of these two pathways is dramatically down-regulated by auxin, while strongly enhanced by cytokinin and methyl-jasmonate. Furthermore, our previous studies have shown that protein prenylation events are also involved in the transcriptional activation of some of these genes. In the present work, we investigate the involvement of protein prenylation in the jasmonate signalling pathway leading to MIA biosynthesis. Inhibition of protein prenyltransferase down-regulates the methyl-jasmonate-induced expression of MEP and monoterpene secoiridoid pathway genes and thus abolishes MIA biosynthesis. Jointly, it also inhibits the methyl-jasmonate-induced expression of the AP2/ERF transcription factor ORCA3 that acts as a central regulator of MIA biosynthesis. Finally, a specific silencing of protein prenyltransferases mediated by RNA interference in *C. roseus* cells shows that inhibition of type I protein geranylgeranyltransferase (PGGT-I) down-regulates the methyl-jasmonate-induced expression of ORCA3, suggesting that PGGT-I prenylated proteins are part of the early steps of jasmonate signalling leading to MIA biosynthesis.

Keywords Protein prenylation · Jasmonate · *Catharanthus roseus* · Alkaloid · Type I protein geranylgeranyltransferase

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
C. roseus	*Catharanthus roseus*
CaaX-PTases	CaaX-prenyltransferases
CK	Cytokinins
CrRR3	Type-A response regulator 3
DXR	1-Deoxy-D-xylulose 5-phosphate reductoisomerase
DXS	1-Deoxy-D-xylulose 5-phosphate synthase
ESMB	Early stages of monoterpenoid biosynthetic pathway
ORCA	Octadecanoid-derivative responsive *Catharanthus* AP2 domain
G10H	Geraniol 10-hydroxylase
JA	Jasmonates
MeJA	Methyl-jasmonate
MEP	Methyl-D-erythritol 4-phosphate
MIA	Monoterpene indole alkaloids
PFT	Protein farnesyltransferase
PGGT-I	Type I protein geranylgeranyltransferase
RPS9	40S Ribosomal protein S9
SLS	Secologanin synthase
SP	S-perillyl alcohol
STR	Strictosidine synthase
TDC	Tryptophan decarboxylase
HDS	Hydroxymethylbutenyl 4-diphosphate synthase

Communicated by L. Jouanin.

V. Courdavault · V. Burlat · B. St-Pierre · N. Giglioli-Guivarc'h (✉)
Université François-Rabelais de Tours, UFR Sciences et Techniques, EA 2106 Biomolécules et Biotechnologies végétales, IFR 135 Imagerie Fonctionnelle, Parc de Grandmont, 37200 Tours, France
e-mail: nathalie.guivarch@univ-tours.fr

Introduction

The Madagascar periwinkle (*Catharanthus roseus*) is a pantropical Apocynaceae that synthesizes a wide range of monoterpene indole alkaloids (MIA) as part of its secondary metabolism. Among the 130 MIA characterized so far in the whole plant, several valuable therapeutic MIA have been identified including monomers such as ajmalicine and serpentine which are used in the treatment of circulatory diseases and heterodimers such as vinblastine and vincristine are known as powerful anticancer drugs (Levêque et al. 1996). MIA result from the condensation of the indole precursor tryptamine, with the terpenoid precursor secologanin (Fig. 1). This condensation is catalyzed by strictosidine synthase (STR; de Waal et al. 1995) and leads to the formation of strictosidine, the precursor of all MIA. Tryptamine derives from the decarboxylation of tryptophan catalyzed by tryptophan decarboxylase (TDC; De Luca et al. 1989) whereas secologanin biosynthesis requires two successive biosynthetic pathways, the plastidial methyl-D-erythritol 4-phosphate (MEP) pathway and the monoterpene secoiridoid pathway. The MEP pathway provides isopentenyl diphosphate and its isomer dimethylallyl diphosphate that are condensed to form geraniol through two enzymatic steps (Contin et al. 1998). The secoiridoid pathway is initiated by the hydroxylation of geraniol catalyzed by geraniol 10-hydroxylase (G10H; Collu et al. 2001) and ends with the formation of secologanin, catalyzed by secologanin synthase (SLS; Irmler et al. 2000).

Due to the complexity of the MIA biosynthetic pathway and considering the large number of enzymatic steps and their low biosynthetic rates in planta (Mahroug et al. 2007; Oudin et al. 2007a), simplified models of *C. roseus* such as suspension cells have emerged as powerful tools to study the regulation of MIA biosynthesis, including the role of hormonal signalling (Giglioli-Guivarc'h et al. 2006; Hedhili et al. 2007). In *C. roseus* C20D cell lines, auxin provided as 2,4- dichlorophenoxyacetic acid (2,4-D) inhibits MIA production (Arvy et al. 1994; Mahroug et al. 2006) as a consequence of the down-regulation of the expression of the genes encoding the 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), the first two MEP pathway genes (Chahed et al. 2000; Veau et al. 2000) and G10H (Papon et al. 2005). In contrast, cytokinins (CK) increase MIA biosynthesis (Décendit et al. 1992) and enhance at least DXS, DXR and G10H expression (Oudin et al. 2007b). Furthermore, synergistic interaction between CK and ethylene transduction pathways has been reported since the addition of these two hormones further enhance G10H expression (Papon et al. 2005).

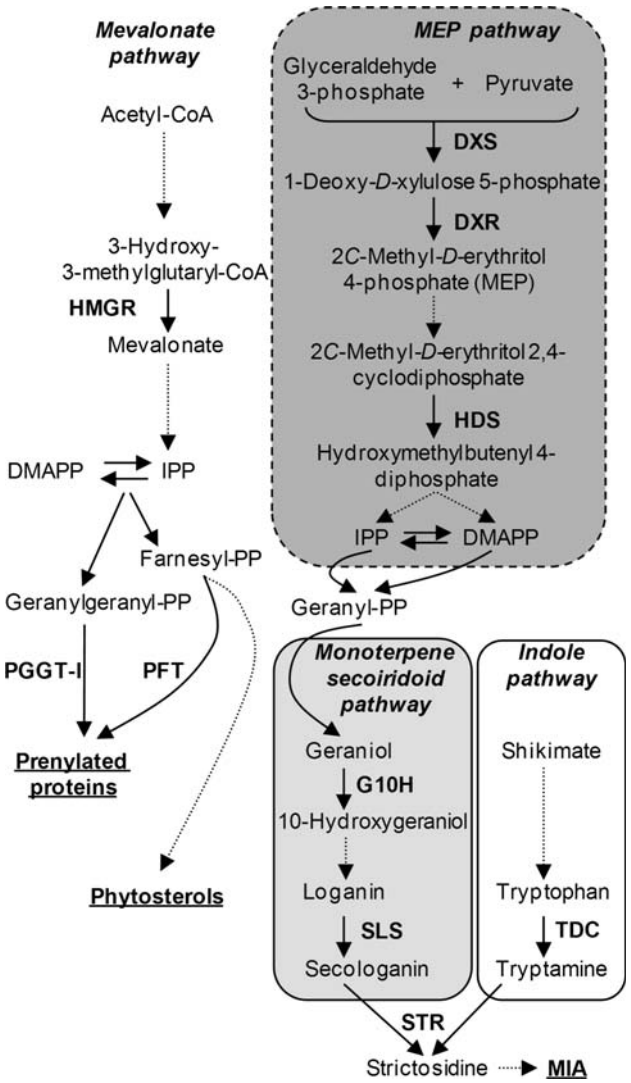

Fig. 1 Biosynthetic pathway of monoterpene indole alkaloids (MIA) and mevalonate metabolites in *C. roseus* cells. *Solid lines* represent a single enzymatic conversion whereas *dashed lines* indicate multiple enzymatic steps. *DXS* 1-deoxy-D-xylulose 5-phosphate synthase, *DXR* 1-deoxy-D-xylulose 5-phosphate reductoisomerase, *DMAPP* dimethylallyl diphosphate, *G10H* geraniol 10-hydroxylase, *HDS* hydroxymethylbutenyl 4-diphosphate synthase, *HMGR* 3-hydroxy-3-methylglutaryl-CoA reductase, *IPP* isopentenyl diphosphate, *PFT* protein farnesyltransferase, *PGGT-I* type I protein geranylgeranyltransferase, *PP* diphosphate; MIA, monoterpene indole alkaloids, *SLS* secologanin synthase, *STR* strictosidine synthase, *TDC* tryptophan decarboxylase

Jasmonate (JA) is an important plant stress hormone that induces various plant defense responses, including the biosynthesis of defensive secondary metabolites. JA or its methyl ester, methyl-jasmonate (MeJA) also appear as central regulators of MIA biosynthesis in *C. roseus*. It has previously been reported that both plants and seedlings of *C. roseus* respond to JA with an increased production of MIA (Aerts et al. 1994; El-sayed and Verpoorte 2005), whereas inhibition of JA biosynthesis in suspension cell

lines blocks MIA production (Gantet et al. 1998). Furthermore, exogenous treatment of *C. roseus* cell suspensions with MeJA leads to a coordinated up-regulation of all characterized genes associated with the biosynthesis of the terpenoid precursor of MIA (Oudin et al. 2007b), and a concomitant increase in MIA (Gantet et al. 1997).

It has recently been established that CaaX-prenyltransferases (CaaX-PTases) are required for MIA biosynthesis in the *C. roseus* C20D cell line as well as in planta (Courdavault et al. 2005b, c). CaaX-PTases catalyze the covalent addition to target proteins of two distinct prenyl moieties, the 15-carbon farnesyl or 20-carbon geranylgeranyl that are produced by the mevalonate pathway (Fig. 1). These post-translational prenylations are essential for the correct function of prenylated proteins by promoting their anchoring to membranes or mediating specific protein interactions (Schafer and Rine 1992; Sinensky 2000). CaaX-PTases include the protein farnesyltransferase (PFT, EC 2.5.1.58) and the type I protein geranylgeranyltransferase (PGGT-I, EC 2.5.1.59). The covalent addition of the prenyl moiety takes place at a conserved cystein residue belonging to a consensus CaaX motif located at the C-terminal extremity of target proteins. Within the CaaX motif "C" is the cystein residue,"a" are aliphatic amino acids and "X" is the residue conferring the specificity for PFT or PGGT-I. When "X" is a serine, methionine, cystein, alanine or a glutamine, the protein is recognized by the PFT whereas if "X" is a leucine, PGGT-I transfer a geranylgeranyl moiety to the protein substrate (Zhang and Casey 1996).

Using mutated *C. roseus* cell lines that have lost PFT or PGGT-I activities, we have previously shown that CaaX-PTase activities are required for the expression of *DXS*, *DXR* and *G10H*, three transcripts involved in the early stages of monoterpenoid biosynthetic pathway (ESMB), and thus MIA biosynthesis (Courdavault et al. 2005b, c). As JA acts as a central regulator of MIA biosynthesis in *C. roseus,* we examine in this work, the potential role of PFT and/or PGGT-I in JA signalling. Using different strategies of inhibition of CaaX-PTase activity, we show that PGGT-I prenylated proteins act in the early step of JA signalling pathway triggering MIA biosynthesis.

Materials and methods

Chemicals

S-perillyl alcohol (SP), an inhibitor of protein prenylation (Tamanoi 1993), pravastatin, an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase (HMGR-CoA) and methyljasmonate (MeJA) were purchased from Sigma (L'Isle d'Abeau, France). Cytokinins (CK) were provided as trans-zeatin obtained from Kalys Duchefa (France). ^{14}[C]-mevalonate was supplied as RS-[2-^{14}C] mevalonolactone, 50 mCi/mmol, (DuPont NEM, Brussel, Belgium). Either [^{3}-H] farnesyl diphosphate (FPP) (16 Ci/mmol) or [^{3}-H] geranylgeranyl diphosphate (GGPP) (23 Ci/mmol) were purchased from Perkin Elmer.

Cell growth and culture conditions of cell suspensions

Experiments were conducted on the C20D *C. roseus* cell line, which is auxin-dependent and cytokinin-independent. Cells were grown at 24°C under continuous shaking (100 rpm) for 7 days in 50 ml Gamborg B5 medium (Gamborg et al. 1968) containing 58 mM sucrose and 4.5 μM 2,4-D. MIA biosynthesis was induced by depleting 2,4-D from the culture medium during one cycle of culture as previously described (Imbault et al. 1996). PFT and PGGT-I interfered cell lines were previously generated (Courdavault et al. 2005c) and cultured in the same conditions as C20D cells. At the indicated time, cells were deep-frozen, freeze-dried and weighted, to determine dry weight and ajmalicin content or directly deep-frozen in liquid nitrogen for total RNA preparation.

Cell suspension treatments, cell growth and ajmalicine determination

Cell growth was estimated by measuring the dry weight contained in each flask (50 ml) at the end of the culture cycle (7 days). MIA biosynthesis was evaluated by measuring the ajmalicine content on 7-day-old cells. Aliquots of 60 mg freeze-dried cells were used for ajmalicine quantification according to Mérillon et al. (1986). For both cell growth and ajmalicine quantification, all measurements were done in triplicate. The addition of SP (1 mM final concentration), dissolved in 50% (v/v) ethanol, to C20D cells was performed at the end of the 3rd day of culture (around 80 h after subculture). For hormonal treatments, MeJA (100 μM) dissolved in ethanol and CK (1 μM) dissolved in 0.1 N HCl were added 24 h after SP supplementation. To test the ability of SP to inhibit protein prenylation in vivo, pravastatin dissolved in water was added at a final concentration of 20 μM, 8 h before SP treatment and 32 h before and ^{14}[C]-mevalonate supplementation.

In vivo inhibition of protein prenylation

Protein prenylation in C20D cells was estimated by visualising ^{14}[C]-labeled proteins in presence of ^{14}[C]-mevalonate. The radiolabeled mevalonate is metabolized into both ^{14}[C]-FPP and ^{14}[C]-GGPP prenyl substrates. Prior to the addition of ^{14}[C]-mevalonate (10 μCi), cells

were pre-treated during 24 h with pravastatin (20 μM) to reduce the pool of endogenous prenyl substrates of CaaX-PTases and, when indicated, 8 h with SP (1 mM) to demonstrate inhibition of protein prenylation. After 24 h, proteins were extracted as previously described (Courdavault et al. 2005a, b). Briefly, cells were grounded in liquid nitrogen, and proteins were extracted with 1 ml of cold lysis buffer containing 50 mM Hepes-KOH pH 7.5, 250 mM mannitol, 5 μM $ZnCl_2$, 1 mM DTT, 1 mM PMSF and protease inhibitors cocktail (Complete EDTA-free, Roche, Meylan, France). The cell lysate was centrifuged at 5,000*g* for 10 min at 4°C. The resulting supernatant was centrifuged at 29,000*g* for 40 min at 4°C. Total proteins were assayed with Bio-Rad reagent (Bio-Rad, France). After addition of loading buffer [50 mM tris-HCl, pH 6.8, 0.5% (v/v) *β*-mercaptoethanol, 5% (w/v) SDS, 10% (v/v) glycerol, 0.01% (w/v) bromophenol blue], 50 μg of total proteins were separated by 12% SDS-PAGE. Gels were stained with Coomassie blue and incubated in Amplify (Amersham-Biosciences) for 30 min before being dried and subjected to fluorography for 3 weeks at −80°C.

Protein prenylation assay

PFT and PGGT-I activities were monitored using a protein prenylation assay previously developed (Courdavault et al. 2005b). Recombinant protein glutathion S-transferase (GST) bearing a CAIM or CIIL C-terminal motif were used as protein substrate for PFT or PGGT-I, respectively. The enzymatic reaction mixtures contained 400 μg of total proteins from C20D, PFT- or PGGT-I- silenced cell lines, 2 μg of GST-CAIM or GST-CIIL, 20 mM $MgCl_2$ and 5 mM DTT. After pre-equilibration at 30°C for 5 min, 1 μCi of either [^{3}-H]-FPP or [^{3}-H]-GGPP was added and the reaction mixtures were incubated for 1 h 30 min at 30°C (200 μl total volume). At the end of the incubation, loading buffer was added and 50 μg of total proteins were separated by 12% SDS-PAGE. Gels were incubated in Amplify (Amersham-Biosciences) for 30 min before being dried and subjected to fluorography on sensitive film (Amersham hyperfilm^{TM} MP) for 1 week at −80°C. Films were manually developed during 3–5 min and fixed 10 min before being cleaned and dried.

RNA gel blot hybridization analysis

Total RNA were extracted from 4 to 5-day-old *C. roseus* suspension cells using RNeasy Plant Minikit extraction (Qiagen, courtaboeuf, France). For RNA gel blot hybridization, 10 μg of total RNA were fractionated by electrophoresis in a 1.2% (w/v) agarose gel containing 2.2 M formaldehyde according to Sambrook et al. (1989) and blotted onto a nylon N^{+} membrane (Qbiogene, Illkirch, France). For probe preparation, full-length cDNA were [^{32}P]-labelled with the 'prime-a-gene' labelling kit (Promega). Hybridizations were carried out for 15 h at 60°C in Church buffer [0.5 M $NaPO_4$ pH 7.2, 7% (w/v) SDS, 1 mM EDTA, 1% (w/v) BSA]. Washes were performed at 55°C for 45 min in 4× SSC (1× SSC is 0.15 M NaCl, 1.5 mM NaOAc) and 0.1% (w/v) SDS, for 45 min in 1× SSC and 0.1% (w/v) SDS and for 30 min in 0.2× SSC and 0.1% (w/v) SDS. Membranes were exposed to X-ray film (Fuji Xray RX) for 3–5 days, using amplifying screen at −80°C.

Results

Inhibition of in vivo protein prenylation in the *C. roseus* C20D cell line

To examine the putative involvement of CaaX-PTases in a JA-dependent mechanism regulating MIA biosynthesis, we first tested the ability of SP, a general inhibitor of protein prenylation (Tamanoi 1993; Gelb et al. 1995; Ren et al. 1997), to inhibit endogenous prenylations of proteins in C20D cells. Endogenous protein prenylation in C20D cells, following the incorporation of [^{14}C]-mevalonate-derived prenyl moieties, mainly allows the detection of three size classes of prenylated proteins: 55–60, 25 and less than 15 kD (Fig. 2). Similar sets of prenylated proteins have been observed in BY2 suspension-cultured tobacco cells with an apparent higher number of radiolabelled bands than those obtained in C20D cells (Morehead et al. 1995). Addition of SP in the culture medium during 24 h before ^{14}[C]-mevalonate supplementation, drastically inhibits the incorporation of mevalonate-derived prenyl moieties in proteins as compared to untreated cells (Fig. 2b). This result demonstrates the inhibitory effect of SP on endogenous protein prenylation in C20D cells.

Inhibition of protein prenylation disrupts the positive effect of MeJA on MIA biosynthesis

We subsequently evaluated, at the ajmalicine production level, the consequence of SP-mediated inhibition of protein prenylation on the positive effect of the two main hormonal regulators of MIA biosynthesis, MeJA and CK. In non-SP-treated cells, addition of CK or MeJA to cells cultured in a 2,4-D-free medium increase MIA biosynthesis by 93 and 140%, respectively, as compared with non-stimulated cells (Fig. 3), as already reported by Décendit et al. (1992) and Gantet et al. (1997). Inhibition of protein prenylation by addition of SP leads to a dramatic decrease of MIA biosynthesis without altering the cell growth. Addition of CK to SP pre-treated cells restores partially MIA biosynthesis

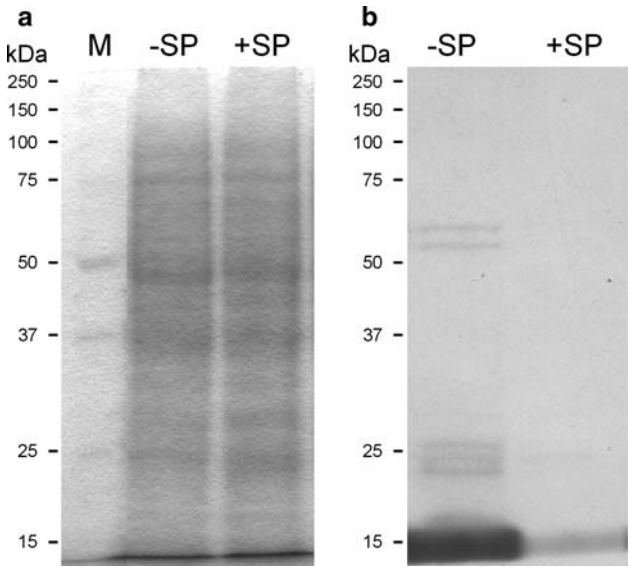

Fig. 2 Inhibition of protein prenylation by *s*-perillyl alcohol (*SP*) in *C. roseus* C20D cells. Inhibition of protein prenylation was estimated by visualising the incorporation of [^{14}C]-prenyl moieties in proteins of C20D cells that have been treated with pravastatine (an inhibitor of HMGR) to deplete the endogenous pool of CaaX-PTase prenyl substrates. After supplementing cells with SP (+*SP*) or not (−*SP*), the endogenous pool of prenyl substrates was restored by adding [^{14}C]-mevalonate to the cell culture medium. After 24 h of incubation, total proteins were extracted and separated on 12% SDS-PAGE stained with Coomassie blue (**a**) and autoradiographied to detect prenylated proteins (**b**). Molecular weight markers (*M*) are indicated on the left of the gels

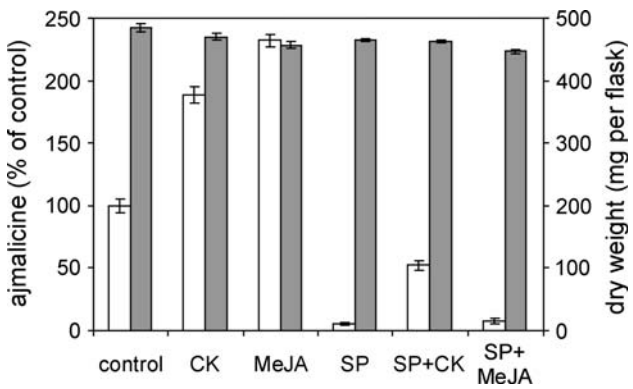

Fig. 3 Consequences of the inhibition of protein prenylation on C20D cell growth and CK-induced or MeJA-induced biosynthesis of MIA. Cells pre-treated or not with SP (1 mM) were stimulated with CK (1 μM) or MeJA (100 μM), or mock-treated (*control*) in auxin free medium. Ajmalicine content (*white bars*) and dry weight (*grey bars*) representing cell growth, of 50 mL suspension cells where then measured at the end of the culture cycle (7 days). Each *bars* represent the mean (±SE) of three experimental replicates

to around 25% of normal alkaloid levels. In contrast, addition of exogenous MeJA is not able to stimulate MIA production in SP pre-treated cells (Fig. 3). This suggests that MeJA-induced production of MIA is highly dependent on an active protein prenylation status.

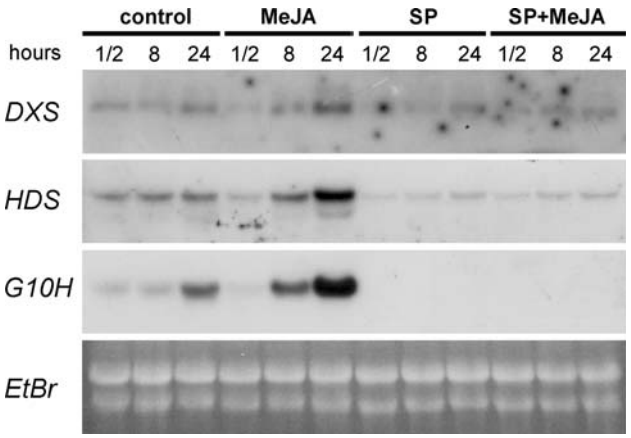

Fig. 4 Inhibition of protein prenylation causes the abolishment of the JA-induced expression of genes involved in the early stages of monoterpenoid biosynthetic pathway. Accumulation of *DXS*, *HDS* and *G10H* transcripts was monitored in C20D cells by RNA blot analysis. C20D cells were treated with methyljasmonate (100 μM) on the 4th day of culture (*MeJA*), with S-perillyl alcohol (1 mM) on the 3rd day (*SP*) or with both treatments (*SP* + *MeJA*). Total RNA was extracted from cells harvested ½, 8 or 24 h after MeJA addition. RNA blot analysis was performed using 10 μg of total RNA. Equal loading was checked by ethidium bromide (*EtBr*) staining

Protein prenylation is required for the MeJA-induced expression of MIA biosynthetic genes

Previous studies have shown that supplying C20D cells with exogenous MeJA stimulates MIA biosynthesis by inducing the expression of several key genes involved in the early stages of monoterpenoid biosynthetic pathway (ESMB) such as *DXS*, *HDS* and *G10H* (Oudin et al. 2007b). To investigate further the putative role of protein prenylation in MeJA signal transduction, the expression levels of ESMB genes were analyzed by RNA blot hybridization in C20D cells following the addition of 1 mM SP and/or 100 μM MeJA. The SP-mediated inhibition of protein prenylation leads to a down-regulation of *DXS* and *HDS* genes and a complete extinction of *G10H* expression (Fig. 4). Furthermore, the inhibition of protein prenylation in C20D cells leads to a complete abolishment of the MeJA-dependent induction of *DXS*, *G10H* and *HDS* gene expression, which could explain the decrease of MIA biosynthesis observed in Fig. 3. Thus, this result suggests that prenylated proteins may be involved in the JA signalling pathway leading to ESMB gene expression.

CK- and MeJA-induced genes show opposing responses to the inhibition of prenylated proteins

To gain insight into the involvement of prenylated proteins in the JA signalling, we further analyzed by RNA blot the expression of the octadecanoid-derivative responsive *Catharanthus* AP2 domain (ORCA) 3 transcription factor.

The expression of the gene encoding the 40S ribosomal protein S9 (*RPS9*) was used as a control (Menke et al. 1999). It has previously been shown that *ORCA 3* transcripts rapidly accumulate in response to exogenous MeJA in *C. roseus* cells (van der Fits and Memelink 2000; Memelink et al. 2001). We have shown here that *ORCA3* exhibits a similar pattern of expression in response to MeJA in C20D cells as well as in the *C. roseus* MP183 cell line depicted by van der Fits and Memelink (2001) (Fig. 5a). In the absence of MeJA, the *ORCA3* transcripts were not detected by RNA blot. Following the addition of MeJA, an induction in *ORCA3* expression levels was detected 30 min after treatment. This peak in transcript levels was followed by a decrease in expression levels until 6 h post treatment. When cells were pre-treated with 1 mM SP, *ORCA3* transcripts were barely detectable 30 min and 6 h after MeJA treatment. Since no prenylated proteins belong to the cascade of events beginning with the perception of the CK signal and ending with the expression of genes encoding type-A response regulator (Heyl and Schmulling 2003), the expression of the gene encoding the type-A CK response regulator *CrRR3* was also studied as a control. As expected, the induction of *CrRR3* expression 1 h after CK supply, described by Papon et al. (2003), is not affected by the prior inhibition of protein prenylation (Fig. 5b). This argues for a specific effect of SP on the JA signalling.

PGGT-I is specifically required for the JA signalling triggering ORCA3 expression

As SP is a general inhibitor of protein prenylation according to Gelb et al. (1995), we cannot decipher which kind of CaaX-PTase activity is involved in JA signalling. To circumvent this problem, we took advantages of the specific PFT or PGGT-I inhibited *C. roseus* cell lines that have been previously established by RNA interference, F18 and F21 for PFT and G4 and G5 for PGGT-I (Courdavault et al. 2005c). In these cell lines, the transcripts encoding the specific β-subunit of each CaaX-PTase αβ-heterodimer have been targeted by siRNA leading to a specific down-regulation of each CaaX-PTase activity (Fig. 6a). In parallel, *C. roseus* cell lines K9 and K11, transformed with a non-interfering construct (empty vector) have been used as a control, while no modification of PFT and PGGT-I activity has been observed compare to C20D cell line (Fig. 6a). In these control cell lines, the abundance of *ORCA3* transcripts following 30 min of MeJA treatment is similar to those observed in wild type C20D cells (Fig. 6b). Abolishment of PFT activity does not alter *ORCA3* expression indicating that farnesylated proteins are not likely to be part of JA signalling. On the contrary, the depletion of PGGT-I activity leads to a down-regulation of

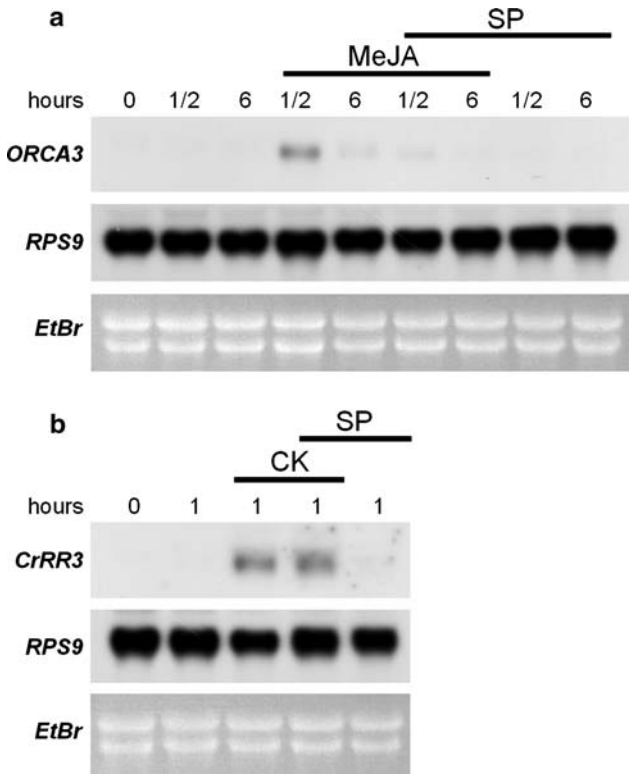

Fig. 5 Inhibition of protein prenylation blocks the MeJA-induced expression of *ORCA3* (**a**) but does not affect CK-induced *CrRR3* expression (**b**). The accumulation of *ORCA3* (**a**) and *CrRR3* (**b**) transcripts was monitored in C20D cells by RNA blot analysis using *RPS9* expression as a control. C20D cells were treated at the end of the 3rd day of the culture with SP (1 mM) or not and supplemented 24 h after with 100 µM MeJA (**a**) or 1 µM CK (**b**) when indicated. Total RNA were extracted from cells harvested at the indicated times following treatments. 10 µg of total RNA were subjected to RNA blot analysis and equal loading was checked by ethidium bromide (*EtBr*) staining

ORCA3 expression to a similar extend to those measured in SP treated cells. This partial inhibition of *ORCA3* expression argues for a positive and specific action of proteins prenylated by PGGT-I in the JA signalling.

CaaX-PTase activities are not modified by MeJA treatments

To assess the involvement of a modification of PGGT-I activity in the JA signalling, we finally analyzed the regulation of CaaX-PTase activities by MeJA. CaaX-PTases activities were measured in vitro in the crude protein extract from C20D cells using a specific protein substrate of PGGT-I (GST-CIIL) or PFT (GST-CAIM) as described previously (Courdavault et al. 2005b). As depicted in Fig. 7, supplying C20D with MeJA for 30 min or 6 h modified neither PGGT-I nor PFT activities as compared to non-stimulated cells. This suggests that the MeJA-induced expression of *ORCA3* is not a result of a modification of PGGT-I activity.

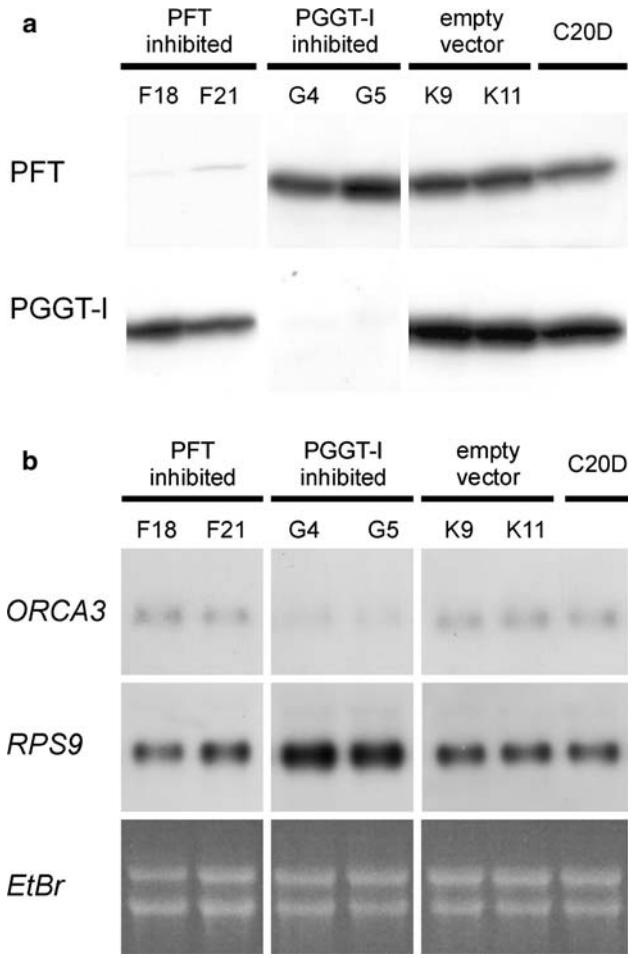

Fig. 6 PGGT-I silencing blocks the JA-induced expression of *ORCA3*. (**a**) PFT and PGGT-T activities were measured in silenced and C20D cell lines. Total soluble proteins were isolated from PFT-silenced cells (*F18*, *F21*), PGGT-I-silenced cells (*G4*, *G5*), cells transformed by a non-silencing vector (*empty vector*, *K9* and *K11*) and the C20D cells. PFT activity (*PFT*) and PGGT-I activity (*PGGT-I*) were assayed, respectively, in presence of [^{3}H]-FPP and GST-CAIM, or [^{3}H]-GGPP and GST-CIIL, as prenyl and peptide substrates, respectively. Prenyl incorporation was revealed by autoradiography after separation of proteins on a 12% SDS-PAGE (**b**) Accumulation of *ORCA3* transcripts was monitored by RNA blot analysis in PFT- and PGGT-I-silenced cell lines, cells transformed by a non-silencing vector and C20D cells as controls. Cells were treated at the 4th day of culture with MeJA (100 µM) and total RNA were extracted from cells harvested 30 min after treatment. 10 µg of total RNA were subjected to RNA blot analysis and equal loading was checked by ethidium bromide (*EtBr*) staining

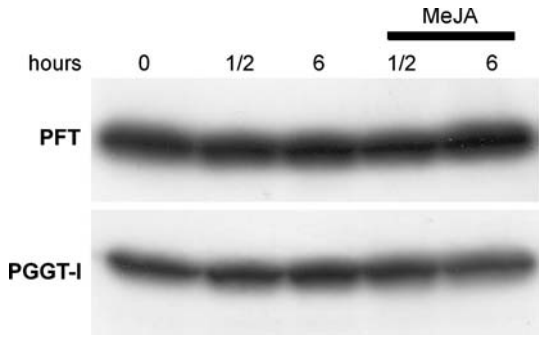

Fig. 7 CaaX-PTase activities are not affected by MeJA treatment. PFT and PGGT-I activities were monitored using protein prenylation assay performed on proteins extracted from C20D cells treated or not with MeJA (100 µM) during 30 min or 6 h

Discussion

In planta, numerous physiological functions have been assigned to CaaX-PTases and characterized using mutational analysis of *Arabidopsis thaliana*. In particular, PFT has been implicated in the initiation and the regulation of cell division (Morehead et al. 1995; Qian et al. 1996; Galichet and Gruissem 2006), in the regulation of cell differentiation in the meristem (Yalovsky et al. 2000; Running et al. 2004) and in the regulation of flower development (Ziegelhoffer et al. 2000). PFT has also been implicated in the transduction pathway of hormonal signals. Additionally, PFT is linked to the ABA signalling mechanisms controlling stomatal closure, drought tolerance (Cutler et al. 1996; Pei et al. 1998; Allen et al. 2002; Wang et al. 2005) and leaf senescence (Barth et al. 2004). Furthermore, PFT negatively modulates the expression of the transcription factor ABI3 that is also regulated by auxin, suggesting that PFT acts in the auxin-signalling pathway (Brady et al. 2003). In contrast, the physiological functions of PGGT-I remain less understood. Following an elegant biochemical characterization of PGGT-I (Caldelari et al. 2001), Johnson et al. (2005) have shown that PGGT-I negatively regulates the ABA signalling pathway in guard cells and the auxin signalling pathway leading to the lateral root initiation without affecting the other ABA or auxin responses.

In *C. roseus*, the biosynthesis of MIA is a highly regulated mechanism depending on various hormonal stimuli. Whereas auxin and CK exhibit strong and opposite effects on MIA biosynthesis, JA also appears as a central regulator of this process. For example, JA provided as MeJA is able to increase the production of MIA in *C. roseus* seedlings and cells (Aerts et al. 1994; Gantet et al. 1998) by triggering the production and activation of the transcription factors ORCA2 and ORCA3 (Menke et al. 1999; van der Fits and Memelink 2001; Memelink et al. 2001). ORCA3 exhibits the most pleitropic effects by enhancing the expression of genes involved in many steps of MIA biosynthesis, such as terpenoid and indole precursor biosynthesis, strictosidine synthesis and MIA modifications (van der Fits and Memelink 2000). Nevertheless, little data are available to explain the production and/or activation of ORCA by MeJA (Memelink et al. 2001). To date, the molecular details of the JA signalling pathway have only been partially characterized, notably with the recent discovery of jasmonate-

induced ZIM-domain-containing (JAZ) transcriptional repressors linking Coronative insensitive1 (COI1)-mediated jasmonate perception and jasmonate-induced genes (Chini et al. 2007; Thines et al. 2007) and their roles in the control of secondary metabolism (Shoji et al. 2008).

We have previously established that CaaX-PTases are essential for MIA biosynthesis in *C. roseus* (Courdavault et al. 2005b, c). It appears that proteins prenylated by both PFT and PGGT-I are involved in the induction of the expression of *DXS*, *DXR* and *G10H* following the depletion of 2,4-D from the cell culture medium. In an attempt to better understand the involvement of CaaX-PTases in the MIA biosynthesis, we have investigated the potential role of protein prenylation in the JA signalling cascade. In a first step, we have validated SP as a potent inhibitor of in vivo protein prenylation in *C. roseus* cells. Thus, addition of SP to the cell culture medium leads to the near total disappearance of proteins that could be detected following incorporation of [^{14}C]-mevalonate derivated prenyl moieties. Since the average half life of prenylated proteins was shown to be 20 h (Galichet and Gruissem 2003), our experimental conditions, in addition to block de novo synthesis of prenylated proteins (Fig. 2), should induce a partial depletion of this pool of modified proteins in cells. Nevertheless, inhibition of protein prenylation suppresses the basal level of MIA biosynthesis in control cells cultured in auxin-free medium, and totally blocks the increase of MIA biosynthesis induced by MeJA, while addition of exogenous CK partially reversed the SP-mediated inhibition of MIA biosynthesis (Fig. 3). The induction of MIA biosynthesis following transfer to auxin-free medium was shown to depend on active MeJA biosynthesis pathway (Gantet et al. 1997). The sensitivity of the MeJA signal transduction pathway to SP may explain both the inhibitory effect of this inhibitor on the basal level of MIA biosynthesis and on the MeJA-stimulation of MIA biosynthesis. The level of ajmalicine accumulation in response to CK is probably the cumulative effect of MeJA endogenous biosynthesis in response to auxin depletion in addition to the effect of CK per se. In agreement with these results, the MeJA-induced expression of *ORCA3* is strongly down-regulated by the inhibition of protein prenylation while the induction of *CrRR3* expression following CK treatment is not affected (Fig. 5a, b). Taken together, these results argue for a specific involvement of prenylated proteins in the JA signalling pathway and marginally or not in the CK signalling pathway that regulate the biosynthesis of MIA in *C. roseus*. Nevertheless, it was recently established in *Arabidopsis* that CK biosynthesis is dependent of the farnesylation status of AtIPT3, the adenoside phosphate-isopentenyl transferase involved in CK biosynthesis pathway (Galichet et al. 2008).

Using the PFT or PGGT-I silenced *C. roseus* cell lines, we also reported that the up-regulation of *ORCA3* induced by MeJA is specifically dependent upon prenylation events catalyzed by PGGT-I (Fig. 6b). In contrast to PFT, the abolishment of PGGT-I activity leads to a partial inhibition of MeJA-dependent *ORCA3* expression to a similar extend to the SP-inhibition of *ORCA3* expression (Fig. 5). Furthermore, the MeJA dependent up-regulation of *ORCA 3* does not seem to require a modification of PGGT-I activity since both CaaX-PTase activities remain constant following MeJA treatment (Fig. 7). This suggests that some proteins prenylated by PGGT-I and not PGGT-I *stricto sensu* act positively in the JA signal transduction. Thus, the availability of these proteins, or their ability to be prenylated could be the authentic factor involved in the JA signalling that induce MIA biosynthesis in *C. roseus*.

In agreement with this point of view, Trusov et al. (2006, 2007) have recently proposed that prenylated proteins function as part of the JA signalling pathway. Furthermore, the characterization of *Arabidopsis* mutants of the $\alpha\beta\gamma$ heterotrimeric G proteins led to the conclusion that the JA signalling is influenced positively by the G$\beta\gamma$ functional subunit but not by Gα. The two Gγ subunits of *Arabidopsis* are small proteins of around 11 kD that both bear a CaaX motif specific of PGGT-I (Mason and Botella 2000, 2001). Activity of heterotrimeric G proteins depends on the prenylation status of Gγ since *Arabidopsis* mutants, bearing a CaaX-truncated γ subunit, present altered responses to the physiological process involving heterotrimeric G proteins (Chakravorty and Botella 2007). This is directly related to the ability of the geranylgeranyl moity of Gγ to drive the anchoring of the G$\beta\gamma$ dimer to the plasma membrane or its interaction with receptors and effectors (Adjobo-Hermans et al. 2006). Similarly, in *C. roseus*, the inhibition of protein prenylation mediated by SP or RNA interference could impair the function of Gγ and the JA signalling leading to MIA biosynthesis.

Finally, we have also noticed that the inhibition of protein prenylation abolishes the MeJA-induced up-regulation of *DXS*, *HDS* and *G10H* that causes the decrease of MIA biosynthesis. *DXS* down-regulation correlates with the inhibition of *ORCA3* expression in SP-treated cells since *ORCA3* over-expression has been described to trigger *DXS* expression in *C. roseus* cells (van der Fits and Memelink 2000). However, previous works have shown that cycloheximide-mediated inhibition of de novo *ORCA3* expression did not affect the MeJA-induced expression of MIA biosynthesic gene that could be dependent of activation of pre-existing ORCA3 (van der fits and Memelink 2001). This could reflect the involvement of PGGT-I prenylated proteins in the early steps of JA signalling prior to ORCA3 activation and induction of *ORCA3* expression. Furthermore, *ORCA3* is not sufficient for the JA signalling

pathway that induced *G10H* expression suggesting that other mechanisms regulate the expression of MIA genes under the control of MeJA. Thus, it appears that PGGT-I prenylated proteins could act positively on early steps of JA signalling from which trigger an *ORCA3*-dependent and an *ORCA3*-independent regulation of MIA biosynthetic gene expression.

In conclusion, we provide evidences for a new physiological role of PGGT-I in the JA signalling. As previously described for the involvement of PFT in abscissic acid signalling (Cutler et al. 1996; Pei et al. 1998; Allen et al. 2002), PGGT-I may act indirectly in the signalling pathway by its specific protein targets. In *C. roseus* cells, these proteins act positively on the JA signalling triggering ESMB gene expression and MIA biosynthesis. Since JA is essential to MIA biosynthesis in *C. roseus* cells, such mechanism could also explain the requirement of PGGT-I for unaltered MIA biosynthesis.

Acknowledgments This work was financially supported by the "Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche" (France) and the "Ligue Nationale contre le cancer" (Comité Départemental du Loir et Cher). We thank Dr. J. Memelink (University of Leiden, the Netherlands) who kindly provided *C. roseus ORCA3* cDNA. We thank Pr J. Crèche who provided *C. roseus CrRR3* cDNA and Dr. M. Thiersault and M.A. Marquet for their technical assistance. We are grateful to Dr. Andrew Simkin for careful revision of the manuscript.

References

Adjobo-Hermans MJ, Goedhart J, Gadella TWJ Jr (2006) Plant G protein heterotrimers require dual lipidation motifs of Galpha and Ggamma and do not dissociate upon activation. J Cell Sci 119:5087–5097. doi:10.1242/jcs.03284

Aerts R, Gisi D, De Carolis E, De Luca V, Baumann TW (1994) Methyl jasmonate vapor increases the developmentally controlled synthesis of alkaloids in *Catharanthus* and *Cinchona* seedlings. Plant J 5:635–643

Allen GJ, Murata Y, Chu SP, Nafisi M, Schoeder JI (2002) Hypersensitivity of abscisic acid-induced cytosolic calcium increases in the *Arabidopsis* farnesyltransferase mutant era1-2. Plant Cell 14:1649–1662. doi:10.1105/tpc.010448

Arvy M-P, Imbault N, Naudascher F, Thiersault M, Doireau P (1994) 2, 4-D and alkaloid accumulation in periwinkle cell suspensions. Biochimie 76:410–416

Barth O, Zschiesche W, Siersleben S, Humbeck K (2004) Isolation of a novel cDNA encoding a nuclear protein involved in stress response and leaf senescence. Physiol Plant 121:282–293. doi:10.1111/j.0031-9317.2004.00325.x

Brady SM, Sarkar SF, Bonetta D, McCourt P (2003) The ABSCISIC ACID INSENSITIVE 3 (ABI3) gene is modulated by farnesylation and is involved in auxin signaling and lateral root development in *Arabidopsis*. Plant J 34:67–75. doi:10.1046/j.1365-313X.2003.01707.x

Caldelari D, Sternberg H, Rodriguez-Concepcion M, Gruissem W, Yalovsky S (2001) Efficient prenylation by a plant geranylgeranyltransferase-I requires a functional CaaL box motif and a proximal polybasic domain. Plant Physiol 126:1416–1429

Chahed K, Oudin A, Guivarc'h N, Hamdi S, Chénieux JC, Rideau M, Clastre M (2000) I-Deoxy-D-xylulose 5-phosphate synthase from periwinkle: cDNA identification and induced gene expression in terpenoid indole alkaloid-producing cells. Plant Physiol Biochem 38:559–566

Chakravorty D, Botella JR (2007) Over-expression of a truncated *Arabidopsis thaliana* heterotrimeric G protein gamma subunit results in a phenotype similar to α and β subunit knockouts. Gene 393:163–170. doi:10.1016/j.gene.2007.02.008

Chini A, Fonseca S, Fernandez G, Adie B, Chico JM, Lorenzo O, Garcia-Casado G, Lopez-Vidriero I, Lozano FM, Ponce MR, Micol JL, Solano R (2007) The JAZ family of repressors is the missing link in jasmonate signalling. Nature 448:666–671. doi:10.1038/nature06006

Collu G, Unver N, Peltenburg-Looman AM, van der Heijden R, Verpoort R, Memelink J (2001) Geraniol 10-hydroxylase, a cytochrome P450 enzyme involved in terpenoid indole alkaloid biosynthesis. FEBS Lett 508:215–220. doi:10.1016/S0014-5793(01)03045-9

Contin A, van der Heijden R, Lefebre AW, Verpoorte R (1998) The iridoid glucoside secologanin is derived from the novel triose phosphate/pyruvate pathway in a *Catharanthus roseus* cell culture. FEBS Lett 4:413–416

Courdavault V, Burlat V, Gantet P, St-Pierre B, Giglioli-Guivarc'h N (2005a) Isolation of a cDNA encoding the alpha subunit of CaaX-prenyltransferase from *Catharanthus roseus* and expression of an active recombinant protein farnesyltransferase. Cell Mol Biol Lett 10:649–657

Courdavault V, Burlat V, St-Pierre B, Giglioli-Guivarc'h (2005b) Characterisation of CaaX-prenyltransferases in *Catharanthus roseus*: relationships with the expression of genes involved in the early stages of monoterpenoid biosynthetic pathway. Plant Sci 168:1097–1107. doi:10.1016/j.plantsci.2004.12.010

Courdavault V, Thiersault M, Courtois M, Gantet P, Oudin A, Doireau P, St-Pierre B, Giglioli-Guivarc'h N (2005c) CaaX-prenyltransferases are essential for expression of genes involved in the early stages of monoterpenoid biosynthetic pathway in *Catharanthus roseus* cells. Plant Mol Biol 57:855–870. doi:10.1007/s11103-005-3095-0

Cutler S, Ghassemian M, Bonetta D, Cooney S, McCourt P (1996) A protein farnesyltransferase involved in abscisic acid signal transduction in *Arabidopsis*. Science 273:1239–1241

De Luca V, Marineau C, Brisson N (1989) Molecular cloning and analysis of cDNA encoding a plant tryptophan decarboxylase: comparison with animal dopa decarboxylases. Proc Natl Acad Sci USA 86:2582–2586

de Waal A, Meijer AH, Verpoorte R (1995) Strictosidine synthase from *Catharanthus roseus* : purification and characterization of multiple forms. Biochem J 306:571–580

Décendit A, Liu D, Ouelhazi L, Doireau P, Mérillon JM, Rideau M (1992) Cytokinin-enhanced accumulation of indole alkaloids in *Catharanthus roseus* cell cultures. The factors affecting the cytokinin response. Plant Cell Rep 11:400–403

El-Sayed M, Verpoorte R (2005) Methyljasmonate accelerates catabolism of monoterpenoid indole alkaloids in *Catharanthus roseus* during leaf processing. Fitoterapia 76:83–90. doi:10.1016/j.fitote.2004.10.019

Galichet A, Gruissem W (2003) Protein farnesylation in plants—conserved mechanisms but different targets. Curr Opin Plant Biol 6:530–535. doi:10.1016/j.pbi.2003.09.005

Galichet A, Gruissem W (2006) Developmentally controlled farnesylation modulates AtNAP1;1 function in cell proliferation and cell expansion during *Arabidopsis* leaf development. Plant Physiol 142:1412–1426

Galichet A, Hoyerova K, Kaminek M, Gruissem W (2008) Farnesylation directs AtIPT3 subcellular localization and modulates

cytokinin biosynthesis in Arabidopsis. Plant Physiol 146:1155–1164. doi:/10.1104/pp.107.107425
Gamborg OL, Miller RRA, Ojima K (1968) Nutriment requirements of suspension cultures of soybean root cells. Exp Cell Res 50:151–158
Gantet P, Imbault N, Thiersault M, Doireau P (1997) Inhibition of alkaloid accumulation by 2, 4-D in *Catharanthus roseus* cell suspension is overcome by methyljasmonate. Acta Bot Gallica 122:783–786
Gantet P, Imbault N, Thiersault M, Doireau P (1998) Necessity of a functional octadecanoic pathway for indole alkaloid synthesis by *Catharanthus roseus* cell suspensions cultured in an auxin-starved medium. Plant Cell Physiol 39:220–225
Gelb MH, Tamanoi F, Yokoyama K, Ghomashchi F, Esson K, Gould MN (1995) The inhibition of protein prenyltransferases by oxygenated metabolites of limonene and perillyl alcohol. Cancer Lett 91:169–175
Giglioli-Guivarc'h N, Courdavault V, Oudin A, Crèche J, St-Pierre B (2006) Madagascar periwinkle, an attractive model for studying the control of the biosynthesis of terpenoid derivative compounds. In: Jaime A Texeira da Silva (ed) Floriculture, ornamental and plant biotechnology: advances and topical issues, vol II, 1st edn. Global Science Books™, Isleworth, pp 496–506
Hedhili S, Courdavault V, Giglioli-Guivarc'h N, Gantet P (2007) Regulation of the terpene moiety biosynthesis of *Catharanthus roseus* terpene indole alkaloids. Phytochem Rev 6:341–351. doi:10.1007/s11101-006-9021-5
Heyl A, Schmulling T (2003) Cytokinin signal perception and transduction. Curr Opin Plant Biol 6:480–488
Imbault N, Thiersault M, Dupéron P, Benabdelmouna A, Doireau P (1996) Pravastatin: a tool for investigating the availability of mevalonate metabolites for primary and secondary metabolism in *Catharanthus roseus* cell suspension. Physiol Plant 98:803–809
Irmler S, Schroder G, St-Pierre B, Crouch NP, Hotze M, Schmidt J, Strack D, Matern U, Schroder J (2000) Indole alkaloid biosynthesis in *Catharanthus roseus*: new enzyme activities and identification of cytochrome P450 CYP72A1 as secologanin synthase. Plant J 24:797–804
Johnson CD, Chary SN, Chernoff EA, Zeng Q, Running MP, Crowell DN (2005) Protein geranylgeranyltransferase is involved in specific aspects of abscisic acid and auxin signaling in Arabidopsis. Plant Physiol 139:722–723. doi:101104/pp105065045
Levêque D, Wihlm J, Jehl F (1996) Pharmacology of *Catharanthus* alkaloids. Bull Cancer 83:176–186
Mahroug S, Courdavault V, Thiersault M, St-Pierre B, Burlat V (2006) Epidermis is a pivotal site of at least four secondary metabolic pathways in *Catharanthus roseus* aerial organs. Planta 223:1191–1200. doi:10.1007/s00425-005-0167-y
Mahroug S, Burlat V, St-Pierre B (2007) Cellular and sub-cellular organisation of the monoterpenoid indole alkaloid pathway in *Catharanthus roseus*. Phytochem Rev 6:363–381. doi:10.1007/s11101-006-9017-1
Mason MG, Botella JR (2000) Completing the heterotrimer: isolation and characterization of an *Arabidopsis thaliana* G protein gamma-subunit cDNA. Proc Natl Acad Sci USA 97:14784–14788
Mason MG, Botella JR (2001) Isolation of a novel G-protein gamma-subunit from *Arabidopsis thaliana* and its interaction with G beta. Biochim Biophys Acta 1520:147–153
Memelink J, Verpoorte R, Kijne JW (2001) ORCAnization of jasmonate-responsive gene expression in alkaloid metabolism. Trends Plant Sci 6:212–219
Menke FL, Champion A, Kjine JW, Memelink J (1999) A novel jasmonate- and elicitor-responsive element in the periwinkle secondary metabolite biosynthetic gene Str interacts with a jasmonate- and elicitor-inducible AP2-domain transcription factor ORCA2. EMBO J 18:4455–4463
Mérillon JM, Doireau P, Guillot A, Chénieux JC, Rideau M (1986) Indole alkaloid accumulation and tryptophan decarboxylase activity in *Catharanthus roseus* cells cultured in three media. Plant Cell Rep 5:23–26
Morehead TA, Biermann BJ, Crowell DN, Randall SK (1995) Changes in protein isoprenylation during the growth of suspension-cultured tobacco cells. Plant Physiol 109:277–284
Oudin A, Courtois M, Rideau M, Clastre M (2007a) The iridoid pathway in *Catharanthus roseus* alkaloid biosynthesis. Phytochem Rev 6:259–276. doi:10.1007/s11101-006-9054-9
Oudin A, Mahroug S, Courdavault V, Hervouet N, Zelwer C, Rodriguez-Concepcion M, St-Pierre B, Burlat V (2007b) Spatial distribution and hormonal regulation of gene products from methyl erythritol phosphate and monoterpene-secoiridoid pathways in *Catharanthus roseus*. Plant Mol Biol 65:13–30. doi:10.1007/s11103-007-9190-7
Papon N, Oudin A, Vansiri A, Rideau M, Chenieux JC, Crèche J (2003) Differential expression of two type-A response regulators in plants and cell cultures of *Catharanthus roseus* (L.) G. Don. J Exp Bot 54:1793–1795
Papon N, Bremer J, Vansiri A, Andreu F, Rideau M, Crèche J (2005) Cytokinin and ethylene control indole alkaloid production at the level of the MEP/terpenoid pathway in *Catharanthus roseus* suspension cells. Planta Med 71:572–574. doi:10.1055/s-2005-864163
Pei ZM, Ghassemian M, Kwak CM, McCourt P, Schroeder JI (1998) Role of farnesyltransferase in ABA regulation of guard cell anion channels and plant water loss. Science 282:287–290
Qian D, Zhou D, Ju R, Cramer CL, Yang Z (1996) Protein farnesyltransferase in plants: molecular characterization and involvement in cell cycle control. Plant Cell 8:2381–2394
Ren Z, Elson CE, Gould MN (1997) Inhibition of type I and type II geranylgeranyl-protein transferases by the monoterpene perillyl alcohol in NIH3T3 cells. Biochem Pharmacol 54:113–120
Running MP, Lavy M, Sternberg H, Galichet A, Gruissem W, Hake S, Ori N, Yalovsky S (2004) Enlarged meristems and delayed growth in *plp* mutants results from lack of CaaX-prenyltransferase. Proc Natl Acad Sci 101:7815–7820. doi:10.1073/pnas.0402385101
Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
Schafer WR, Rine J (1992) Protein prenylation: genes, enzymes, targets and functions. Annu Rev Genet 26:209–237
Shoji T, Ogawa T, Hashimoto T (2008) Jasmonate-induced nicotine formation in tobacco is mediated by tobacco COI1 and JAZ Genes. Plant Cell Physiol 49:1003–1012. doi:10.1093/pcp/pcn077
Sinensky M (2000) Recent advances in the study of prenylated proteins. Biochim Biophys Acta 1484:93–106
Tamanoi F (1993) Inhibitors of Ras farnesyltransferases. Trends Biochem Sci 18:349–353
Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He YS, Howe GA, Browse J (2007) JAZ repressor proteins are targets of the SCFCOI1 complex during jasmonate signalling. Nature 448:661–665. doi:10.1038/nature05960
Trusov Y, Rookes JE, Chakravorty D, Armour D, Schenk PM, Botella JR (2006) Heterotrimeric G proteins facilitate *Arabidopsis* resistance to necrotrophic pathogens and are involved in jasmonate signaling. Plant Physiol 140:210–220. doi:101104/pp105069625
Trusov Y, Rookes JE, Tilbrook K, Chakravorty D, Mason MG, Anderson D, Chen J-G, Jones AM, Botella JR (2007) Heterotrimeric G protein gamma subunits provide functional selectivity

in G$\beta\gamma$ dimer signaling in *Arabidopsis*. Plant Cell 19:1235–1250. doi:10.1105/tpc.107.050096

van der Fits L, Memelink J (2000) ORCA 3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. Science 289:295–297

van der Fits L, Memelink J (2001) The jasmonate-inducible AP2/ERF-domain transcrition factor ORCA3 activates gene expression via interaction with a jasmonate-responsive promoter element. Plant J 25:43–53. doi:10.1046/j.1365-313x.2001.00932.x

Veau B, Courtois M, Oudin A, Chenieux JC, Rideau M, Clastre M (2000) Cloning and expression of cDNAs encoding two enzymes of the MEP pathway in *Catharanthus roseus*. Biochim Biophys Acta 1517:159–163

Wang Y, Ying J, Kuzma M, Chalifoux M, Sample A, McArthur C, Uchacz T, Sarvas C, Wan J, Dennis DT, McCourt P, Huang Y (2005) Molecular tailoring of farnesylation for plant drought tolerance and yield protection. Plant J 43:413–424. doi:10.1111/j.1365-313x.2005.02463.x

Yalovsky S, Kulukian A, Rodriguez-Conception M, Youg CA, Gruissem W (2000) Functional requirement of plant farnesyltransferase during development in *Arabidospsis*. Plant Cell 12:1267–1278

Zhang FL, Casey PJ (1996) Protein prenylation: molecular mechanisms and functional consequences. Annu Rev Biochem 65:241–269

Ziegelhoffer EC, Medrano LJ, Meyerowitz EM (2000) Cloning of the *Arabidopsis* WIGGUM gene identifies a role for farnesylation in meristem development. Proc Natl Acad Sci USA 97:7633–7638