J Plant Growth Regul (2011) 30:235–241
DOI 10.1007/s00344-010-9187-3

Molecular Characterization of an Aux/IAA of *Catharanthus roseus*

Pierre Poutrain · Grégory Guirimand · Gaëlle Glévarec · Vincent Courdavault · Olivier Pichon

Received: 7 May 2010 / Accepted: 27 October 2010 / Published online: 24 December 2010

Abstract In *Catharanthus roseus* cells, auxins are known to negatively regulate the biosynthesis of monoterpenoid indole alkaloids (MIA), a class of valuable secondary metabolites. Despite extensive studies of this regulation, no protein of the auxin signaling pathway has been isolated to date in this plant. We therefore decided to clone and characterize a *C. roseus* Aux/IAA protein that belongs to a family of gene expression repressors mediating auxin effects. Using PCR, a cDNA encoding the first *C. roseus* Aux/IAA was cloned and named *CrIAA1*. The deduced amino acid sequence has four highly conserved domains that are typical of the Aux/IAA protein family and has high homology to the Aux/IAA isoforms of *Arabidopsis* (>67%). The *CrIAA1* gene expression, monitored by real-time PCR, was found to be dramatically induced by auxin treatment in *C. roseus* cells. Using GFP imagery and a bimolecular fluorescence complementation assay, we found that CrIAA1 can form oligomers in the nucleus. We also found that CrIAA1 is quickly degraded following auxin treatments, suggesting that auxin regulates CrIAA1 availability via a feedback mechanism. These results should help to elucidate the molecular nature of the processes responsible for the auxin-mediated regulation of MIA biosynthesis in *C. roseus*.

Keywords IAA · Auxin signaling · *Catharanthus roseus* · Aux/IAA · GFP · Bimolecular fluorescence complementation

Introduction

Auxin regulates many aspects of plant development by controlling cell elongation, division, and differentiation through the regulation of the expression of specific gene subsets (Woodward and Bartel 2005). Such regulation is dependent on a rapid proteasome-mediated degradation of a large conserved family of gene expression repressors (Aux/IAA). Aux/IAA comprise short-lived nuclear proteins (Abel and others 1994) whose degradation is triggered by the binding of auxin to the $SCF^{TIR1/AFB}$ E3 ubiquitin-ligase complex which in turn allows the rapid expression of several families of early responsiveness genes, including the Aux/IAAs themselves (Teale and others 2006). Besides affecting the regulation of primary metabolism, auxin has also been implicated in the regulation of secondary metabolism. For instance, in *Catharanthus roseus*, several studies have shown that auxin has a role in the inhibition of monoterpenoid indole alkaloid (MIA) biosynthesis caused by the specific downregulation of MIA biosynthetic gene expression (Oudin and others 2007; Poutrain and others 2010). Furthermore, this downregulation is a consequence

Electronic supplementary material The online version of this article (doi:10.1007/s00344-010-9187-3) contains supplementary material, which is available to authorized users.

P. Poutrain (✉) · G. Guirimand · G. Glévarec · V. Courdavault · O. Pichon
"Biomolécules et Biotechnologies Végétales", Université François Rabelais de Tours, EA 2106, IFR 135 "Imagerie fonctionnelle", 37200 Tours, France
e-mail: pierre.poutrain@univ-tours.fr

G. Guirimand
e-mail: gregory.guirimand@etu.univ-tours.fr

G. Glévarec
e-mail: gaelle.glevarec@univ-tours.fr

V. Courdavault
e-mail: vincent.courdavault@univ-tours.fr

O. Pichon
e-mail: olivier.pichon@univ-tours.fr

of early events in auxin signaling because it could be monitored less than 1 h after the addition of auxin (Goddijn and others 1992). Although the inhibitory effects of auxin on MIA biosynthetic genes are well known, the exact nature of the auxin signaling pathway regulating MIA biosynthesis in *C. roseus* is unknown. This prompted us to undertake the isolation and the characterization of auxin signaling components in *C. roseus*. In the present work, we report on the first isolation of a *C. roseus* Aux/IAA (CrIAA1) and its molecular characterization. Our results show that CrIAA1 possesses the common property of Aux/IAA in terms of subcellular localization and organization as well as in response to auxin at the transcriptional and post-translational levels. Therefore, this Aux/IAA could be useful to study the auxin-mediated regulation of MIA biosynthesis in *C. roseus*.

Materials and Methods

Cell Culture Conditions and Treatments

C. roseus cells (line C20D) were maintained on a 7-day growth cycle in 50 ml Gamborg B5 medium containing 58 mM sucrose and 4.5 μM 2,4-D (Sigma, St Louis, MO, USA) as described previously (Poutrain and others 2010). For *CrIAA1* expression analysis, C20D cells were subcultured in 2,4-D-free medium for 3 days and treated thereafter with water (control), 2,4-D (4.5 μM), or IAA (40 μM) (Sigma). Cells were harvested and deep-frozen in liquid nitrogen 1 h after auxin treatment.

Isolation of *CrIAA1* cDNA

Based on the sequence of a *C. roseus* expressed sequence tag (EST) (GenBank FD425238), the 3′ and 5′ ends of the corresponding cDNA were amplified by PCR performed on an oriented *C. roseus* cell cDNA library (A. J. Simkin, unpublished data) cloned in the pBK-CMV phagemid vector system (Stratagene, La Jolla, CA, USA). For the 5′ end amplification, the T3 universal primer and the IAArev1 primer (5′-ATTAAACAAGCCAGACAATATTAGA-3′) were used. For the cloning of the 3′ end, a nested PCR was performed using the T7 universal primer and the IAAfor1 primer (5′-AGAGGATTTTCTGAGACCGT-3′) for the first PCR and a combination of the T7 and IAAfor2 primers (5′-CAAGGCTCAGGTGGTAGGTT-3′) for the second PCR. Full-length cDNA cloning was achieved via nested PCR amplifications using primers IAA-UTRfor1 (5′-GG CAAAGGAAAGCAAAATTGAAGG-3′) and IAA-UT Rrev1 (5′-AACAACGTAATATCTTCTTCTTCC-3′) for the first PCR and primers IAA-UTRfor2 (5′-AATTA GAGTGCCTTAGCCAGAGAT-3′) and IAA-UTRrev2 (5′-TTCAACTTTTAGCTATAATTTGAT-3′) for the second PCR. The resulting cDNA was cloned in the pGEM-T easy vector (Promega, Madison, WI, USA) and sequenced.

YFP-Fused IAA1 Expression Vectors

To construct the IAA-YFP expression vector, the full-length open reading frame (ORF) of *CrIAA1* (GenBank HM165 183) was amplified by PCR using primers IAA1-SpeI-for (5′-CGACTAGT ATGGAAGTACACGGTTTGAATCTA-3′) and IAA1-SpeI-rev (5′-CGACTAGT GCACCTGCTCTTG CATTTCTCCAT-3′) and subsequently cloned in pGEM-T easy vector. The pGEM-T/IAA1-SpeI plasmid was digested with *Spe*I restriction enzyme and the resulting *CrIAA1–Spe*I insert was cloned in frame into the *Spe*I restriction sites of pSCA-cassette YFPi (Guirimand and others 2009), with the 5′ extremity of the coding sequence of YFP driven by the 35SCaMV promoter to express the fusion protein. For bimolecular fluorescence complementation (BiFC) assays, the IAA1–*Spe*I insert was cloned into the pSPYNE(R)173 or pSPYCE(MR) vector in frame, with the 5′ end of the coding sequence of the N-terminal (YFP^N, residues 1–173) and C-terminal (YFP^C, residues 156–239) fragments of YFP, respectively (Waadt and others 2008). For positive control, the *Arabidopsis* leucine zipper bZIP63 fused to YFP^N and YFP^C was used (Walter and others 2004).

Biolistic-Mediated Transient Transformation of *C. roseus* Suspension Cells and GFP Imaging

Transient transformation of *C. roseus* cells by particle bombardment and GFP imaging was performed following the procedures described by Guirimand and others (2009). In addition, the CFP fluorescence was recorded with the CFP filter set (Chroma#31044v2, 426–446-nm excitation filter, 460–500-nm bandpass emission filter). The nucleus- and nucleocytosol-CFP markers used were described previously (Guirimand and others 2009) and correspond, respectively, to the CFP-GUS-nucleoplasmin NLS- and CFP-expressing vectors.

For the analysis of the auxin-induced degradation of CrIAA1, *C. roseus* cells were transiently cotransformed with the CrIAA1-YFP or CrIAA1-BiFC plasmids and the nucleocytosol-CFP expression vector. Sixteen hours after particle bombardment, cells were transferred to liquid Gamborg B5 medium supplemented with water (control), IAA (Sigma) (40 μM), or 2,4-D (4.5 μM) 30 min before treatment with cycloheximide (CHX, 700 nM). YFP and CFP fluorescence were monitored 3 or 30 min after CHX treatment using the same exposure time for both fluorescence channels in approximately 50 cells for each condition.

Real-Time PCR Analysis

Total RNA was obtained from deep-frozen C20D cells as described previously (Poutrain and others 2010), and first-strand cDNA was synthesized from 2 μg RNA following reverse transcription using M-MLV reverse transcriptase (Promega) and the oligo-dT primer. Protocols used for real-time PCR were described previously (Amini and others 2008) except that *CrIAA1* cDNA was amplified using the IAAfor1 and IAArev2 (5′-GTGGCCAACCTACCACCT-GAGCCTTG-3′) primers (amplicon 160 bp). The expression of *CrRPS9* (which encodes the 40S ribosomal protein S9) is unaffected by auxin treatments so this gene was used as a control to allow the normalization of the gene copy numbers in each sample. Results are representative of three technical replicates and statistical analysis was performed using ANOVA.

Results and Discussion

Isolation of *CrIAA1* cDNA

A careful analysis of *C. roseus* EST led to the identification of a sequence (GenBank FD425238) encoding a putative Aux/IAA that was used to design a set of specific primers. The full-length coding sequence of a *C. roseus* Aux/IAA, named *CrIAA1* (GenBank HM165183), was then amplified via a PCR strategy performed on an oriented *C. roseus* cDNA library (A. J. Simkin, unpublished data). A phylogenetic tree was constructed using the 29 Aux/IAAs from *Arabidopsis thaliana* (Fig. 1a) and the deduced amino acid sequence of CrIAA1 identified in this study. *CrIAA1* was shown to form a cluster with *AtIAA7* (GenBank Q38825), *AtIAA14* (GenBank Q38832), and *AtIAA17* (GenBank P93830) and sequence analysis revealed that *CrIAA1* was very similar to the three

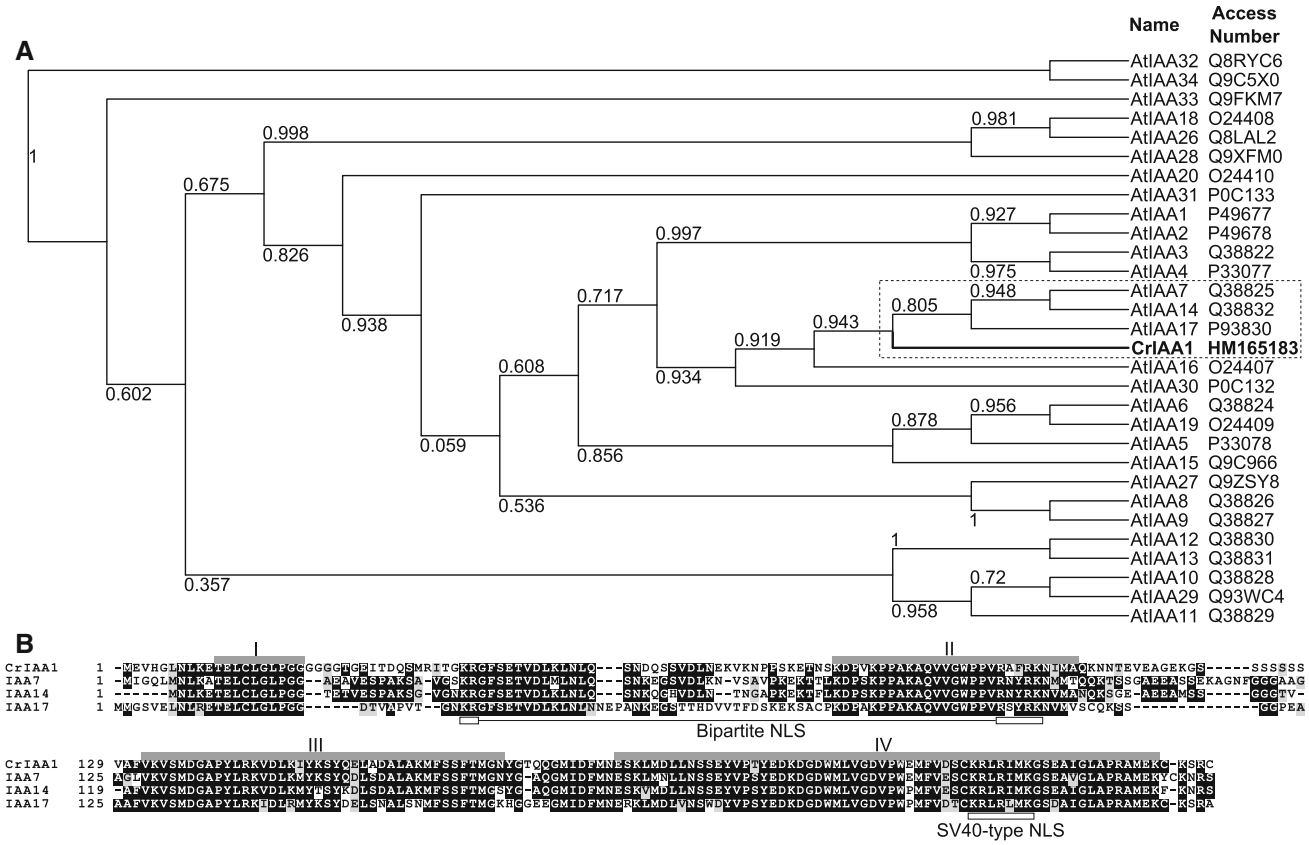

Fig. 1 Sequence analysis of the CrIAA1-deduced protein. **a** Unrooted tree of the 29 *Arabidopsis thaliana* Aux/IAAs and CrIAA1 amino acid sequences. The tree was constructed using phyML software. *Bootstraps* are indicated as ratio (1,000 replicates) and the cluster containing *CrIAA1* is shown within the *dotted box*. **b** The CrIAA1-deduced protein sequence was aligned with *AtIAA7* (GenBank Q38825), *AtIAA14* (GenBank Q38832), and *AtIAA17* (GenBank P93830) using ClustalX software. Conserved and similar residues are highlighted with *black* and *gray boxes*, respectively. The four conserved domains are presented with *gray lines* and *numbered with roman numbers* (I–IV). The bipartite and SV40-type NLSs are shown as *open boxes*

Aux/IAAs from the same cluster (*AtIAA14*, 72%; *AtIAA7*, 68%; *AtIAA16*, 64%; Fig. 1b). This analysis also revealed the presence of four highly conserved domains (I–IV) typical of the Aux/IAA family that are involved in the control of gene expression, protein stability, and protein interactions (Hagen and Guilfoyle 2002). Furthermore, the amino acid sequence contains both a bipartite-like and a SV40-type nuclear localization sequence (NLS) (Fig. 1b), which are generally found in members of the Aux/IAA family (Abel and Theologis 1995).

The Expression of CrIAA1 is Rapidly Induced by Auxins

To study the involvement of CrIAA1 in the auxin signaling pathway of *C. roseus*, the expression of the corresponding gene was monitored by real-time PCR. *CrIAA1* transcript levels were quantified in C20D cells after 3-day cultivation in a 2,4-D-depleted culture medium. These specific conditions allow the biosynthesis of MIA and thus reveal the role of auxin in the regulation of secondary metabolism. In these cells the addition of 2,4-D (4.5 µM) or IAA (40 µM) led to around a fivefold increase in *CrIAA1* transcript levels after 1 h as compared to mock-treated cells (Fig. 2). This result is in agreement with that of other authors who reported the rapid induction of Aux/IAA gene expression in response to auxin in *Pisum sativum* (Theologis and others 1985) and in *Oryza sativa* (Jain and others 2006). The result in *C. roseus* shows that *IAA1*, like its orthologs in other species, is an early auxin-related gene and that *CrIAA1* should be characterized further.

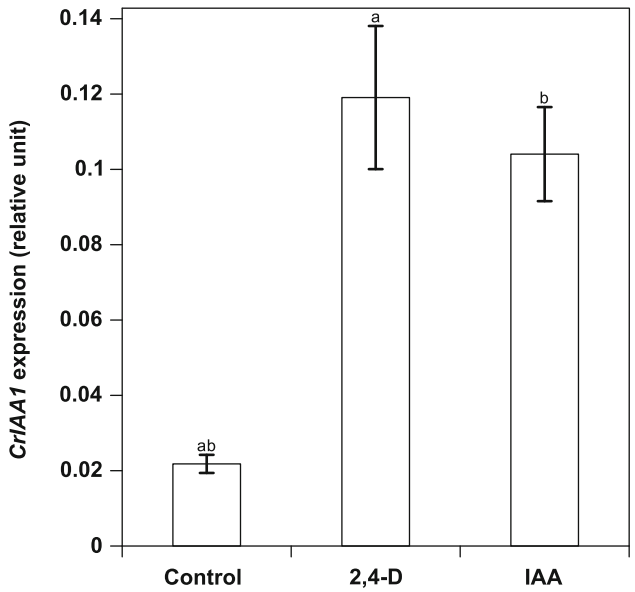

Fig. 2 CrIAA1 expression in *C. roseus* C20D cells in response to auxins. *C. roseus* cells were subcultured in 2,4-D-free medium for 3 days. Water (control), 2,4-D (4.5 µM) or IAA (40 µM) was added and cells were harvested 1 h after treatment. Total RNA was extracted and subjected to reverse transcription. Transcript levels for *CrIAA1* and *CrRPS9* were determined by real-time PCR using gene-specific primers. CrIAA1 expression levels were normalized using RPS9 and are representative of three technical replicates. Different *letters* indicate values that are significantly different ($p < 0.001$)

CrIAA1 is Located Within the Nucleus of *C. roseus* Cells

As Aux/IAAs act as regulators of nuclear gene expression, the consequence of the presence of the putative NLSs within the amino acid sequence was determined by studying the subcellular localization of CrIAA1 in *C. roseus* cells using GFP imaging. The full-length ORF of *CrIAA1* was cloned in the YFP-expressing vector pSCA-cassette YFPi and the resulting CrIAA1-YFP fusion proteins were transiently expressed using particle bombardment. In *C. roseus* transformed cells, CrIAA1-YFP displayed a fluorescence signal that coincided perfectly with the signal of the nucleus-CFP marker, suggesting that CrIAA1 is located within the nucleus (Fig. 3a). This localization is in agreement with previous Aux/IAA localizations (Abel and Theologis 1995; Song and others 2009) and with the classical role of Aux/IAAs as transcriptional regulators.

CrIAA1 Forms Homodimers in the Nucleus

To gain insight into subcellular organization of CrIAA1 within *C. roseus* cells, we studied the ability of the protein to interact with itself in vivo using bimolecular fluorescence complementation (BiFC) assays. The *CrIAA1* coding sequence was therefore cloned in the BiFC vectors upstream of the coding sequences of the two split-YFP fragments to generate the CrIAA1-YFP^N and CrIAA1-YFP^C fusion proteins. A positive control of protein-specific interaction in the nucleus of *C. roseus* cells was obtained using a bZIP63-YFP^N and bZIP63-YFP^C double transformation (Walter and others 2004), whereas the absence of nonspecific interaction was studied by combining bZIP63 and CrIAA1 BiFC vectors (Fig. 3b). Coexpression of CrIAA1-YFP^N and CrIAA1-YFP^C fusion proteins in *C. roseus* cells led to the formation of a BiFC complex visualized by a YFP fluorescence signal (Fig. 3b) that merged perfectly with the nucleus CFP marker (Supplementary Fig. S1). This result shows that CrIAA1 is able to self-interact in the nucleus of *C. roseus* cells. Based on previous studies of Aux/IAA interactions performed by yeast two-hybrid assays (Kim and others 1997; Ouellet and others 2001; Song and others 2009), it could be hypothesized that the CrIAA1 self-interaction corresponds to, at least, a homodimer. To our knowledge, this is the first report of an Aux/IAA self-interaction in the nucleus

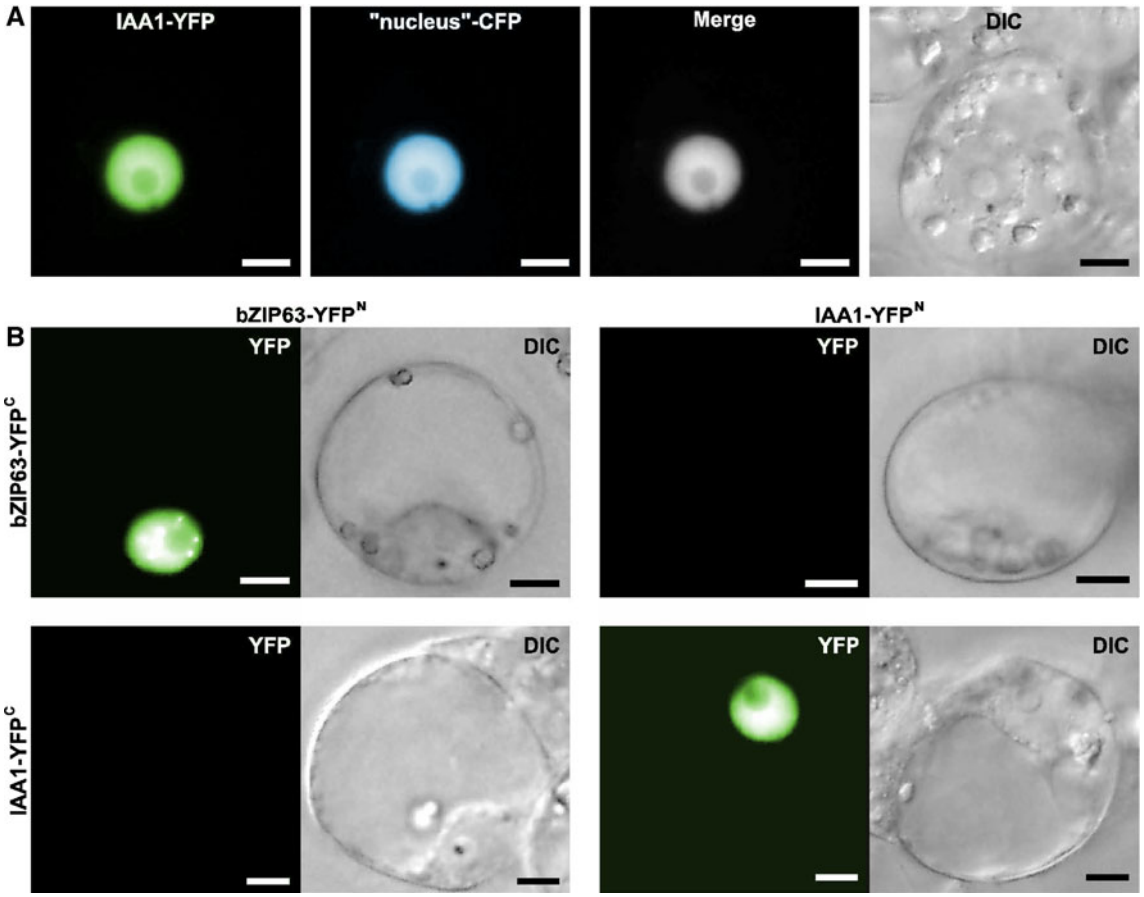

Fig. 3 Analysis of CrIAA1 subcellular localization (**a**) and self-interaction (**b**) in *C. roseus* transformed cells. **a** *C. roseus* cells were transiently transformed with the CrIAA1-YFP and CFP-GUS-nucleoplasmin NLS ("nucleus"-CFP)-expressing vectors. Colocalization of the two fluorescence signals is observed in the merged image. The morphology is shown using differential interference contrast (DIC). *Scale bar* = 10 μm. **b** *C. roseus* cells were cotransformed using a combination of plasmids (CrIAA1 or *Arabidopsis* bZIP63 transcription factor: positive control) as indicated on the *top* (fusion with the YFP^N fragment) and on the *left* (fusion with the YFP^C fragment). *Scale bar* = 10 μm

because no previous reports have provided information about the subcellular localization of the complex.

Auxins Promote the Degradation of CrIAA1

Finally, because the $SCF^{TIR/AFB}$-mediated ubiquitinylation/degradation of Aux/IAA constitutes a central event in auxin signaling (Dharmasiri and others 2005), we tested the ability of auxins to promote the degradation of CrIAA1-YFP proteins expressed using the pSCA-cassette YFPi plasmid. For such analysis, *C. roseus* C20D cells transiently expressing CrIAA1-YFP and the nucleocytosol-CFP marker were incubated for 30 min in liquid culture medium including IAA (40 μM) or 2,4-D (4.5 μM) and cycloheximide (CHX), which stops translation. The stability of the protein was then monitored by checking the fluorescence of the fusion proteins 3 or 30 min after CHX treatment. As observed in Fig. 4, a strong YFP fluorescence was detected exclusively in the nucleus of *C. roseus* cells 3 min after CHX treatment, for all the conditions tested (control, IAA, and 2,4-D). Following 30 min of CHX treatment, this fluorescence disappeared in cells incubated in the presence of IAA or 2,4-D, but did not disappear in the YFP control or in the case of the nucleocytosol-CFP marker, where the fluorescence was unaffected in the presence or absence of IAA or 2,4-D irrespective of the duration of CHX treatment (Fig. 4). Note that with very high exposure time (>5 s), the CrIAA1-YFP fluorescence was detected as a weak nucleocytoplasm fluorescence signal in IAA- or 2,4-D-treated cells, consistent with cells being cotransformed at a high frequency. These results suggest that both IAA and 2,4-D promote the rapid degradation of CrIAA1 in the absence of de novo protein synthesis. They are also in agreement with previous reports showing that auxins decrease Aux/IAA stability (Tiwari and others 2001) and induce SCF^{TIR1}-mediated degradation of AtIAA3 and AtIAA12 (Maraschin

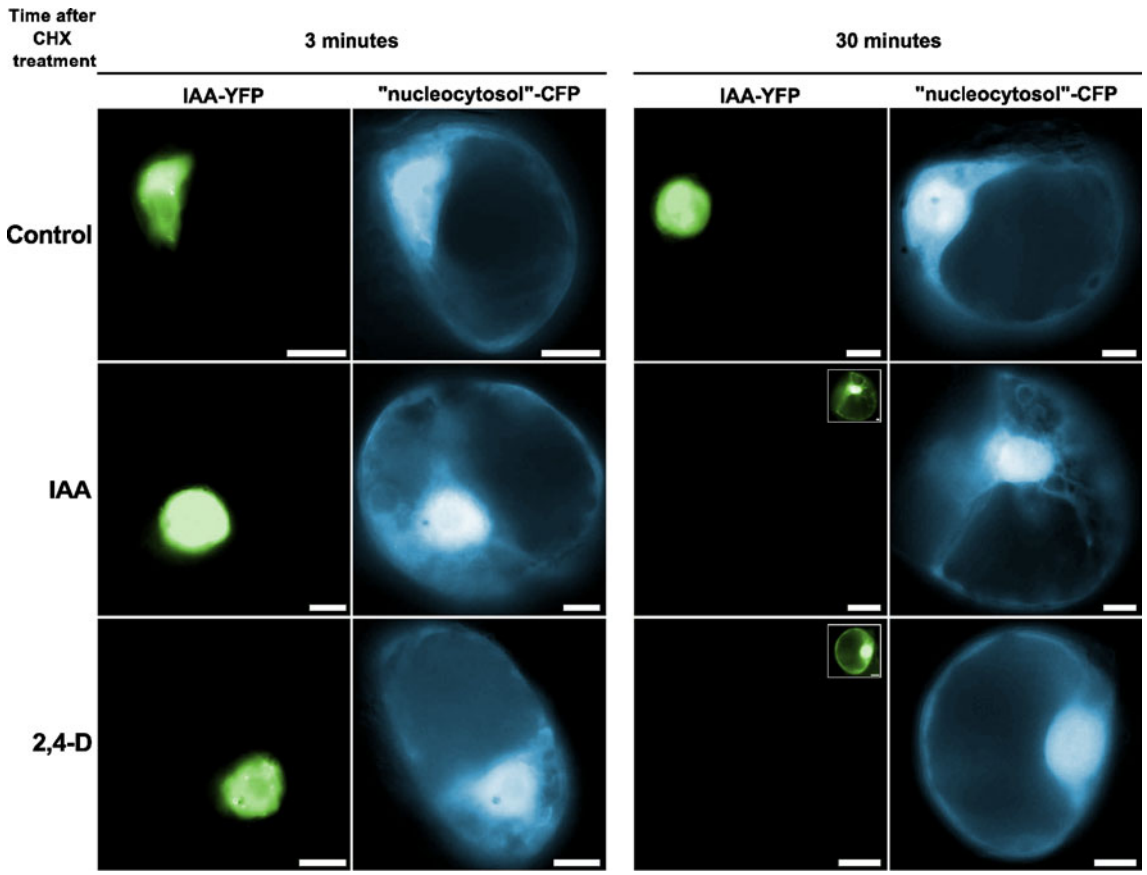

Fig. 4 Effect of auxins on the stability of CrIAA1-YFP in *C. roseus* transformed cells. *C. roseus* cells were transiently transformed with the CrIAA1-YFP and CFP ("nucleocytosol"-CFP)-expressing vectors and incubated in liquid B5 media supplemented with auxins (4.5 μM 2,4-D or 40 μM IAA) or with water (control) for 30 min. Cells were mounted on glass slides 3 and 30 min after cycloheximide (CHX) treatment (700 nM) and fluorescence imaging was performed on a representative cell. For each cell the exposure time was the same for the YFP and CFP images (50–100 ms). For IAA- and 2,4-D-treated cells, 30 min after CHX treatment an additional YFP image was acquired with an exposure time of 5 s (*square box*). *Scale bar* = 10 μm

and others 2009). The rapid disappearance of CrIAA1-YFP fluorescence is in accordance with the short *Pisum sativum* Aux/IAA half-life (<15 min) (Worley and others 2000). Finally, because Aux/IAAs are known to be ubiquitinylated by $SCF^{TIR1/AFB}$ E3 ubiquitin-ligase after auxin perception by TIR1 F-box protein (Dharmasiri and others 2005; Kepinski and Leyser 2005), the weak nucleocytosolic YFP signal observed in IAA- and 2,4-D-treated cells following 30 min of treatment could be the result of the nucleus-to-cytosol translocation of the ubiquitinylated form of IAA1-YFP.

Conclusion

In this study we report the first isolation and characterization of a *C. roseus* Aux/IAA, CrIAA1, a nuclear protein able to form oligomers in the nucleus. Furthermore, we show that auxins provided as IAA or 2,4-D promote a rapid degradation of CrIAA1 while strongly stimulating the expression of the *CrIAA1* gene, thereby revealing the existence of feedback regulation of Aux/IAA availability.

In other plant systems, Aux/IAAs are present as multigene families. In *Arabidopsis*, for example, 29 Aux/IAAs genes have been identified, and many of them have been shown to encode proteins that have regulatory activity mediated by auxins. CrIAA1 may be a member of a multigene family, and further work should reveal whether this Aux/IAA protein or other Aux/IAAs in *C. roseus* are responsible for the auxin-mediated downregulation of MIA biosynthesis.

Acknowledgments This research was financially supported by the Ministère de l'Enseignement Supérieur et de la Recherche. We thank Dr. A. J. Simkin, who kindly provided the *C. roseus* cDNA library, and Vic Norris for help with the English. G. Guirimand was financed by a MENRT fellowship.

References

Abel S, Theologis A (1995) A polymorphic bipartite motif signals nuclear targeting of early auxin-inducible proteins related to Ps-IAA4 from pea (*Pisum sativum*). Plant J 8:87–96

Abel S, Oeller PW, Theologis A (1994) Early auxin-induced genes encode short-lived nuclear proteins. Proc Natl Acad Sci USA 91:326–330

Amini A, Glévarec G, Andreu F, Reverdiau P, Rideau M, Crèche J (2008) Effects of phosphatidic acid on cytokinin signal transduction in perwinkle cells. J Plant Growth Regul 27:394–399

Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. Nature 435:441–445

Goddijn OJM, de Kam RJ, Zanetti A, Schilperoort RA, Hoge HC (1992) Auxin rapidly down-regulates transcription of the tryptophan decarboxylase gene from *Catharanthus roseus*. Plant Mol Biol 18:1113–1120

Guirimand G, Burlat V, Oudin A, Lanoue A, St-Pierre B, Courdavault V (2009) Optimization of the transient transformation of *Catharanthus roseus* cells by particle bombardment and its application to the subcellular localization of hydroxymethylbutenyl 4-diphosphate synthase and geraniol 10-hydroxylase. Plant Cell Rep 28:1215–1234

Hagen G, Guilfoyle T (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol 49:373–385

Jain M, Kaur N, Garg R, Thakur JK, Tyagi AK, Khurana JP (2006) Structure and expression analysis of early auxin-responsive Aux/IAA gene family in rice (*Oryza sativa*). Funct Integr Genomics 6:47–59

Kepinski S, Leyser O (2005) The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature 435:446–451

Kim J, Harter K, Theologis A (1997) Protein–protein interactions among the Aux/IAA proteins. Proc Natl Acad Sci USA 94:11786–11791

Maraschin FDS, Memelink J, Offringa R (2009) Auxin-induced, SCF^{TIR1}-mediated poly-ubiquitination marks Aux/IAA proteins for degradation. Plant J 59:100–109

Oudin A, Mahroug S, Courdavault V, Hervouet N, Zelwer C, Rodriguez-Concepcion M, St-Pierre B, Burlat V (2007) 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

Ouellet F, Overvoorde PJ, Theologis A (2001) IAA17/AXR3: biochemical insight into an auxin mutant phenotype. Plant Cell 13:829–841

Poutrain P, Guirimand G, Mahroug S, Burlat V, Melin C, Ginis O, Oudin A, Giglioli-Guivarc'h N, Pichon O, Courdavault V (2010) Molecular cloning and characterisation of two calmodulin isoforms of the Madagascar periwinkle *Catharanthus roseus*. Plant Biol. doi:10.1111/j.1438-8677.2010.00325.x

Song Y, You J, Xiong L (2009) Characterization of *OsIAA1* gene, a member of rice Aux/IAA family involved in auxin and brassinosteroid hormone responses and plant morphogenesis. Plant Mol Biol 70:297–309

Teale WD, Paponov IA, Palme K (2006) Auxin in action: signalling, transport and the control of plant growth and development. Nat Rev Mol Cell Biol 7:847–859

Theologis A, Huynh TV, Davis RW (1985) Rapid induction of specific mRNAs by auxin in pea epicotyl tissue. J Mol Biol 183:53–68

Tiwari SB, Wang XJ, Hagen G, Guilfoyle TJ (2001) Aux/IAA proteins are active repressors, and their stability and activity are modulated by auxin. Plant Cell 13:2809–2822

Waadt R, Schmidt LK, Lohse M, Hashimoto K, Bock R, Kudla J (2008) Multicolor bimolecular fluorescence complementation reveals simultaneous formation of alternative CBL/CIPK complexes in planta. Plant J 56:505–516

Walter M, Chaban C, Schütze K, Batistic O, Weckermann K, Näke C, Blazevic D, Grefen C, Schumacher K, Oecking C, Harter K, Kudla J (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. Plant J 40:428–438

Woodward AW, Bartel B (2005) Auxin: regulation, action, and interaction. Ann Bot (Lond) 95:707–735

Worley CK, Zenser N, Ramos J, Rouse D, Leyser O, Theologis A, Callis J (2000) Degradation of Aux/IAA proteins is essential for normal auxin signalling. Plant J 21:553–562