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OsIAA1, an *Aux/IAA* cDNA from Rice, and Changes in Its Expression as Influenced by Auxin and Light

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Abstract

The *Aux/IAA* class of genes are rapidly induced by exogenous auxins and have been characterized extensively from many dicot species like *Arabidopsis*, *Glycine max* and *Pisum sativum*. We report here the isolation and characterization of rice (*Oryza sativa* L. subsp. *Indica*) *OsIAA1* cDNA as a monocot member of the *Aux/IAA* gene family. The predicted amino acid sequence of OsIAA1 corresponds to a protein of ca. 26 kDa, which harbors all four characteristic domains known to be conserved in Aux/IAA proteins. The conservation of these *Aux/IAA* genes indicates that auxins have essentially a similar mode of action in monocots and dicots. Northern blot analysis revealed that the *OsIAA1* transcript levels decrease in the excised coleoptile segments on auxin starvation, and the level is restored when auxin is supplemented; the increase in *OsIAA1* transcript level was apparent within 15 to 30 min of auxin application. Auxin-induced *OsIAA1* expression appears to be correlated with the elongation of excised coleoptile segments. In light-grown rice seedlings, *OsIAA1* is preferentially expressed in roots and basal segment of the seedling, whereas in the etiolated rice seedlings, the *OsIAA1* transcripts are most abundant in the coleoptile. A comparative analysis in light- and dark-grown seedling tissues indicates that the *OsIAA1* transcript levels decrease on illumination.

Key words: *Oryza sativa* (rice); *OsIAA1* (auxin-inducible gene); coleoptile elongation; gene-regulation

1. Introduction

The phytohormone auxin regulates a number of different responses including cell elongation, cell division, phototropism, gravitropism, root formation, apical dominance, xylem differentiation and ethylene biosynthesis.[1–3] Among these diverse responses, auxin-mediated cell elongation has been studied extensively, due to its rapidity. Auxin can induce elongation of stems and coleoptiles within 15 min of its application.[1,3,4] In the 1960s, studies with inhibitors of RNA and protein synthesis provided correlative evidence that the auxin-induced increase in these macromolecules is essential for elongation growth.[5,6] In the early 1970s, the acid growth theory was propounded,[7,8] which suggests that auxin-induced proton secretion into the apoplast initiates cell elongation through activation of wall-loosening processes. It is now believed that the auxin-induced elongation is biphasic. During the early phase, auxin induces proton secretion that makes the cell wall more extensible, and the second phase involves changes in auxin-induced gene expression.[6,9]

Considering the diversity of responses elicited by auxin, it is not surprising that the hormone has been found to regulate the expression of diverse genes. Many genes induced by auxin in elongating tissues have been reported from pea, soybean, *Arabidopsis*, mung bean, and more recently cucumber.[10–16] Based upon their kinetics of induction, responsiveness to cycloheximide and mRNA stability, and their auxin-response specificity, these genes have been broadly classified into four groups: *Aux/IAA*, *GST*, *SAUR*, and *GH*.[17] However, the biochemical functions of most of the encoded proteins are unknown.

The transcript levels of *Aux/IAA* genes are higher in elongating regions of etiolated hypocotyls and epicotyls than in the dividing and mature regions.[11,16,18] When excised sections of hypocotyls/epicotyls are incubated in the absence of auxin, the transcript levels of these genes decrease drastically but are restored rapidly on auxin application. The Aux/IAA proteins range from 20 to 30 kDa and share four conserved domains (I, II, III, and IV) consisting of 7 to about 40 amino acid residues.[17,19–21] As identified mainly by database com-

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parisons and in a few cases by functional tests, these proteins contain nuclear localization signals (NLS) at both ends.[22,23] Domain III is proposed to be a part of the amphipathic $\beta\alpha\alpha$ motif found in β-ribbon DNA binding domains of Arc and MetJ repressor proteins of prokaryotic origin.[22,24] The presence of NLS and DNA binding domain suggests that this Aux/IAA class of proteins represents transcriptional factors. The Aux/IAA proteins have in fact been shown to interact with one another and also with ARFs (auxin response factors), that bind AuxRE, the auxin-response element.[17,25]

Although the effect of auxin on protein synthesis (translatable mRNA) in maize coleoptile segments has been examined,[26] *Aux/IAA* or *SAUR* genes/cDNAs have not been characterized in detail from any monocot species studied so far. As part of an ongoing effort to decipher the mechanism of hormone-mediated cell division and elongation, at least one auxin-induced cDNA has been isolated and characterized from rice (*Oryza sativa* L. subsp. *Indica*). The deduced amino acid sequence shows similarity to other known Aux/IAA proteins. The cDNA corresponds to a gene whose transcript levels increase in the elongation zone of the coleoptile in response to the natural auxin, IAA, and the synthetic auxin, 2,4-D.

2. Materials and Methods

2.1. Plant material

All experiments were performed with *Oryza sativa* L. subsp. *Indica* (var. Pusa Basmati 1) obtained from the Regional Station of the Indian Agricultural Research Institute, Karnal. After thorough washing with water purified by reverse-osmosis (RO water) and disinfection with 0.1% $HgCl_2$ for 1 hr, the seeds were soaked overnight in RO water. Seedlings were grown on cotton saturated with RO water, at 28±1°C, either in dark or under constant illumination, as per the requirement. The light was provided from a bank of fluorescent tubes (Philips TL 40W/54, 6500°K), with a fluence rate of 70 μmol m^{-2} s^{-1}, as measured by a LI-189 radiometer (LI-COR, USA).

2.2. Coleoptile elongation assay

Coleoptile segments 5 mm long were cut from the apical portion of 3-day-old etiolated rice seedlings. The segments were incubated in KPSC buffer (10 mM potassium phosphate, pH 6.0, 2% sucrose, 50 μM chloramphenicol) for 8 hr to deplete endogenous auxins, and the buffer was changed every 2 hr.[13] Samples were then transferred to fresh buffer with different concentrations of IAA or 2,4-D. Controls were incubated in the same KPSC buffer continuously for 8 hr before the addition of auxins, if required. Auxins were dissolved in a minimum amount of ethanol before adding to KPSC buffer. Around 20 segments were placed in a Petri dish containing 20 ml of KPSC buffer and gently shaken at 60 rpm and 26±1°C. Coleoptile length was measured to the nearest millimeter. The whole experiment was performed in a dark room under green safe light. Each experiment was performed at least twice but the data of only a representative experiment are presented. The values plotted represent the mean±S.E.

2.3. cDNA isolation and sequencing

The light-grown rice root cDNA library made using the cDNA synthesis kit, ZAP Express™ and Gigapack III Gold (Stratagene Cloning Systems, USA) was screened with full-length *cyc1At* probe. Considering the heterologous nature of the probe, hybridization was carried out at 50°C in the buffer containing 6 × SSC, 5 × Denhardt's solution, 0.5% SDS, and 100 μg/ml denatured herring sperm DNA. Ten putative clones were selected after screening ca. 4×10^6 recombinant plaques. After further purification through three successive rounds of screening, the λZap recombinant clones were excised *in vivo* to obtain recombinant pBk-CMV phagemids according to the manufacturer's instructions. Clones were then sequenced using T3 and T7 primers. The sequence from the 3′ end of one clone showed significant homology to *Aux/IAA* cDNAs, and this clone was thus designated *OsIAA1*. The full-length *OsIAA1* cDNA was sequenced using an automated DNA sequencer (ABI Prism 377), with the Thermosequenase Dye Terminator cycle sequencing kit (Amersham, UK) as per the manufacturer's specifications.

2.4. DNA isolation and Southern analysis

Genomic DNA was extracted from shoots of dark-grown rice seedlings, according to the method of Dellaporta et al.[27] The tissue (3 g) was ground to a fine powder in liquid nitrogen with the help of a pestle and mortar and transferred to an SS-34 tube. It was followed by the addition of 15 ml of extraction buffer (100 mM Tris-Cl, pH 8.0; 50 mM EDTA, pH 8.0; 500 mM NaCl; 10 mM β-mercaptoethanol) and 2 ml of 20% SDS, before incubating at 65°C for 20 min. After incubation, 5 ml of 5 M potassium acetate was added and the tube kept on ice for 20 min. The sample was then centrifuged at 10,000 rpm for 10 min (SS 34 rotor, Sorvall RC 5B). To the supernatant, 10 ml of isopropanol was added and the tube was kept at −80°C for 2 hr. The DNA was pelleted at 10,000 rpm for 10 min and then dissolved in 1 ml of 50 mM Tris-Cl/10 mM EDTA (pH 8.0). Subsequently, a $1/10^{th}$ volume of 3 M sodium acetate (pH 5.2) and a $6/10^{th}$ volume of isopropanol were added. The precipitated DNA was pelleted at 10,000 rpm for 30 min, washed with 70% ethanol, dried in Speed Vac (Savant Instruments Inc., NY) and dissolved in 500 μl 1 × TE buffer (pH 8.0). An aliquot of

10 μg of rice DNA was separately digested with various restriction endonucleases (*Pvu* II, *Sac* I, *Hin*dIII, *Pst* I, *Not* I, and *Eco*RI), and the digested DNA was resolved on a large 1.0% agarose gel. The resolved DNA fragments were blotted onto a Hybond-N (Amersham, UK) membrane and non-stringent overnight prehybridization as well as hybridization for 24 hr were carried out in 5 × SSC, 5 × Denhardt's solution, 5% dextran sulphate and 250 μg/ml denatured herring sperm DNA, at 37°C. For stringent hybridization, 50% formamide was added to the above solution during hybridization. The full-length ^{32}P-labelled *OsIAA1* cDNA was used as a probe. Post-hybridization washing was performed thrice successively for 5, 15, and 30 min, in 2 × SSC/0.5% SDS, 2×SSC/0.1% SDS and 0.1×SSC/0.5% SDS, respectively, at 60°C for stringent conditions and at room temperature for non-stringent conditions. Membrane was wrapped in Saran Wrap and exposed to an X-ray film for autoradiography in a light-proof Hypercassette (Amersham, UK) with intensifying screen, for 72 hr, at −80°C.

2.5. RNA extraction and northern analysis

RNA was extracted according to Nagy et al.[28] with minor modifications. Tissue (1 g) was powdered in liquid nitrogen and incubated in 5 ml extraction buffer (300 mM NaCl; 50 mM Tris-HCl, pH 8.0; 5 mM EDTA, pH 8.0; 2% SDS; 10 mM β-mercaptoethanol) at 50°C for 5 min. To this, 0.7 ml of 3 M KCl was added and the sample was incubated on ice for 20 min. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was transferred to a fresh tube and 5 ml of 8 M LiCl added. RNA was precipitated overnight at 4°C and pelleted down at 12,000 rpm for 20 min. The pellet was dissolved in 500 μl Milli-Q (MQ) water and then 500 μl of buffer-saturated phenol was added. To the aqueous phase, 100 μl of 5 M NaCl and 900 μl of ethanol were added, and the tube was kept overnight at −20°C. RNA was pelleted at 10,000 rpm for 30 min and then washed twice with 70% ethanol before drying in the Speed Vac. The dried RNA pellet was dissolved in MQ water.

Total RNA was resolved on 1.2% agarose gel containing 1.1% formaldehyde, at 120 V. The resolved RNA was transferred onto a Hybond-N membrane (Amersham, UK) and fixed using UV-crosslinker (Amersham, UK). Hybridization was carried out in 5×SSC, 50% formamide, 5 × Denhardt's solution, 0.1 M sodium phosphate buffer (pH 6.5) and 250 μg/ml denatured herring sperm DNA, at 37°C. The membrane was then subjected to three successive washings for 5, 15, and 30 min, with 2×SSC/0.5% SDS, 2 × SSC/0.1% SDS, and 0.1 × SSC/0.5% SDS, respectively, at 26±1°C. Autoradiogram was developed after 72 hr of exposure of the membrane (wrapped in Cling film) to X-ray film in a light-proof Hypercassette with an intensifying screen. Ethidium bromide-stained rRNA or 25S rRNA probed with the *Lemna gibba* rRNA gene served as a control to depict the quantity and quality of total RNA employed.

3. Results

To understand the regulation of cell division in monocots, the rice root cDNA library was screened with a heterologous probe for a cyclin gene, *cyc1At*.[29] On sequence analysis of the putative clones obtained, one showed significant similarity with known *Aux/IAA* genes from dicot species like *Arabidopsis*, tobacco, mung bean, pea and soybean (see Introduction for details). This cDNA (accession no. AJ251791) has been designated as *OsIAA1* (*Oryza sativa IAA1*) and its characteristic features are described below.

3.1. Sequencing and characterization of OsIAA1 *cDNA clone*

The *OsIAA1* cDNA is 1068 bp long and contains an open reading frame of 711 bp (Fig. 1). A 153-bp-long 5′-untranslated region (UTR) precedes the ATG initiation codon. Bases surrounding the ATG codon, GGCTGCTGCGATGGCCGGCGCCG, are consistent with the initiator consensus sequence described for monocotyledons.[30] The 3′-UTR of 187 bp contains a poly(A) site and two potential polyadenylation signals at 28 bp and 78 bp upstream of the poly(A) site (Fig. 1). The cDNA encodes a protein of 236 amino acid residues with a predicted molecular mass of ca. 26 kDa. The predicted amino acid sequence of OsIAA1 shows significant identity (33% to 44%) with known Aux/IAA proteins (Fig. 2). The OsIAA1 protein contains all four domains (I to IV), varying from 7 to 40 amino acids, that are highly conserved in Aux/IAA proteins (Figs. 1, 2); in these regions, the amino acid identity reaches up to 77%. Moreover, the invariant amino acids of variant region[23] are also present in OsIAA1. The basic amino acids located in between Domains I and II (...KR.....RSFR...) may constitute a bipartite nuclear localization signal (NLS) (Fig. 2).[31,32] A basic cluster KRLRIMK, resembling SV40[33] and MATα2-like NLS,[34,35] is also present at the end of Domain IV. These putative bipartite NLS and basic cluster are conserved in other Aux/IAA proteins. Also, the conserved phosphorylation sites proximal to putative NLS are exhibited by OsIAA1 (Fig. 2). Domain III and five invariant hydrophobic amino acid residues at conserved positions resemble an amphipathic $\beta\alpha\alpha$ DNA-binding domain of prokaryotic repressor proteins such as Arc and MetJ.[24] The hydrophobic amino acids of OsIAA1, like that of other Aux/IAA proteins, closely match the hydrophobicity pattern in the prokaryotic $\beta\alpha\alpha$ domain (Fig. 2).[22]

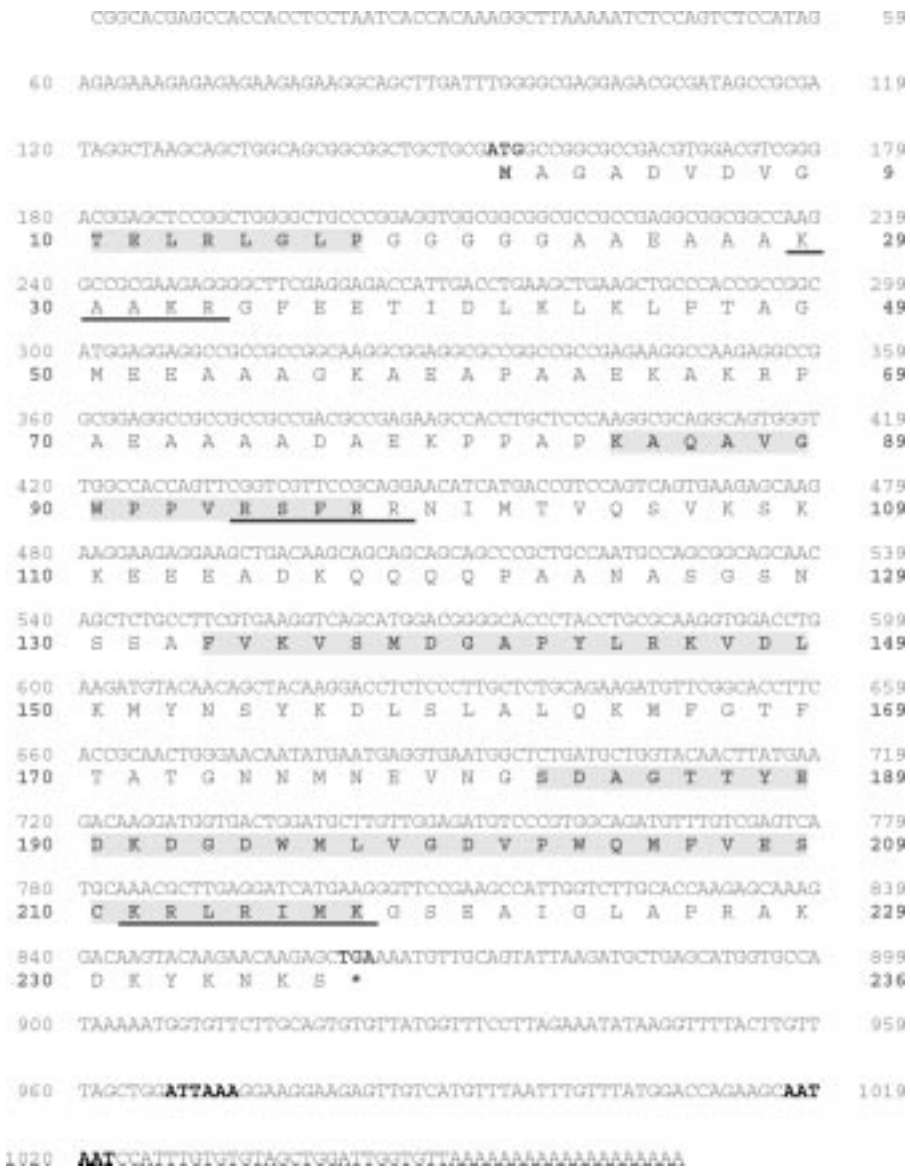

Figure 1. Nucleotide and deduced amino acid sequences of rice *OsIAA1* cDNA (accession no. AJ251791). Nucleotides and amino acids are given in numerals. Highlighted with grey boxes and typed in bold letters are amino acids of conserved Domains I, II, III, and IV. Possible polyadenylation signals are also shown in bold letters. Basic clusters resembling motifs of NLS are underlined. Invariant amino acids, in variable region, are shown in light grey letters. Start and stop codons are marked as bold letters.

3.2. Southern analysis

To determine whether the gene corresponding to *OsIAA1* cDNA belongs to a multigene family or is represented as single copy in the rice genome, rice genomic DNA was digested with different restriction enzymes and processed for Southern analysis. The autoradiogram in Fig. 3a shows that only one or two fragments hybridized after restriction digestion with *Pvu* II, *Sac* I, *Hin*dIII, *Pst* I, *Not* I, and *Eco*RI. However, under low-stringency conditions for hybridization, more bands could be detected in the autoradiogram (Fig. 3b). This observation indicates that *OsIAA1* belongs to a small multigene family, which is similar to the situation in most of the dicot species examined earlier.[17]

3.3. Auxin-regulated coleoptile elongation and changes in OsIAA1 *expression*

To determine whether *OsIAA1* is induced endogenously and performs a physiological role, first, the auxin-regulated elongation of coleoptile segments was studied. As is obvious from the data in Fig. 4, the endogenous auxin present in the apical segment of the dark-grown rice seedlings was nearly optimal for growth of the undepleted coleoptile segments. However, the de-

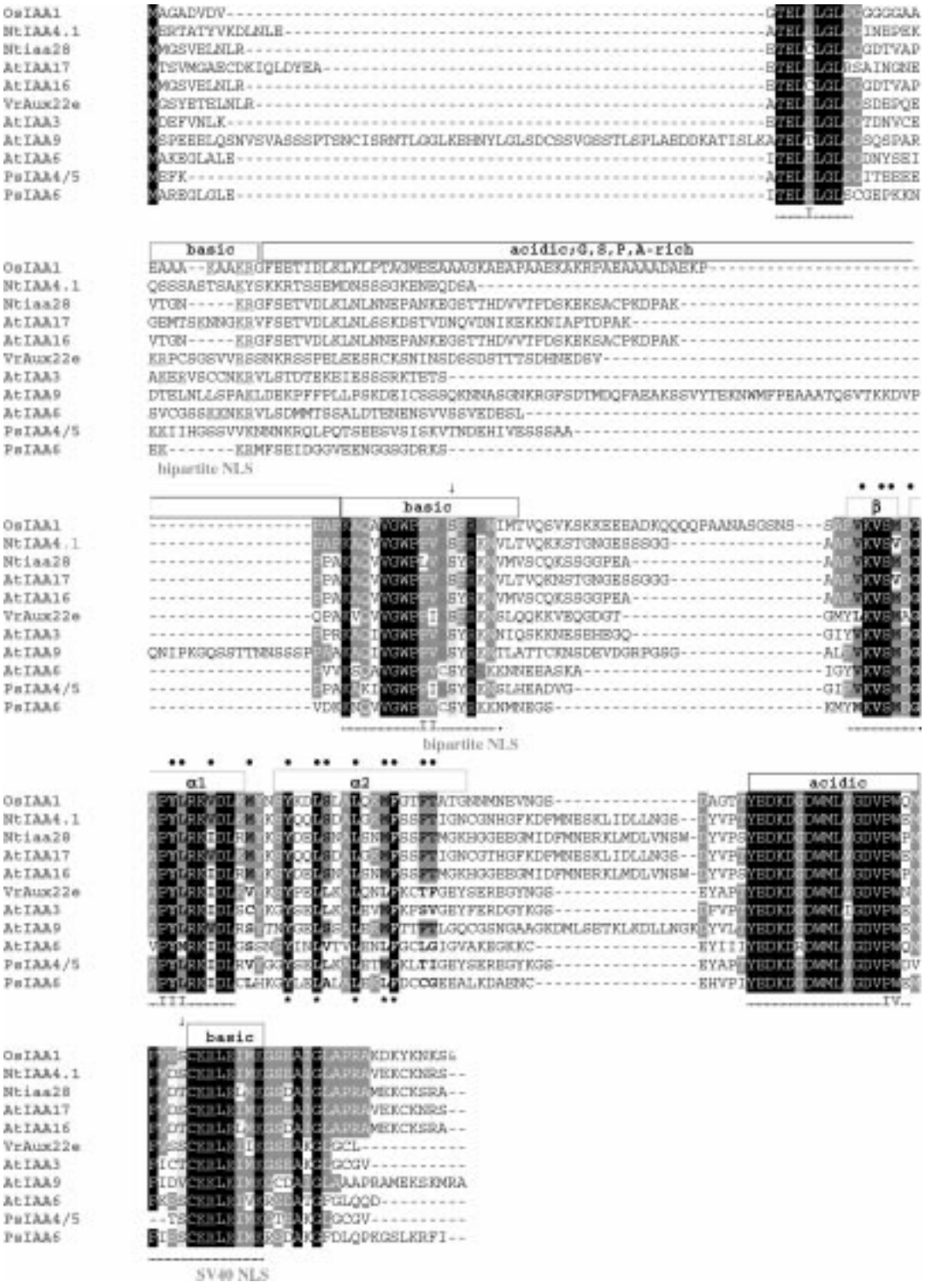

Figure 2. Alignment of deduced amino acid sequence of *OsIAA1* with deduced amino acid sequences of other Aux/IAA proteins of dicot plants. A possible domain structure of the proteins is also shown. Conserved Domains I to IV are underlined by dotted lines. Fully and partially conserved amino acid residues are highlighted with black and grey boxes, respectively. Basic residues that may contribute to possible NLS are underlined. Possible phosphorylation sites in the vicinity of putative NLS are shown by dark letters in light grey boxes and are marked by arrows. Amino acids that may constitute possible hydrophobic surfaces in the predicted amphipathic $\beta\alpha\alpha$ motif are highlighted in bold letters and black dots (·). Invariant amino acids in variant regions are indicated by an asterisk (*). Alignment was done using Gene Runner Version 3.04. The accession numbers are as follows: NtIAA4.1, AF123509; Ntiaa28, AF123508; AtIAA17, U49073; AtIAA16, AC011437; VrAux22e, AB004933; AtIAA3, U18406; AtIAA9, U18411; AtIAA6, U18408; PsIAA4/5, X68216.1; PsIAA6, X68218.

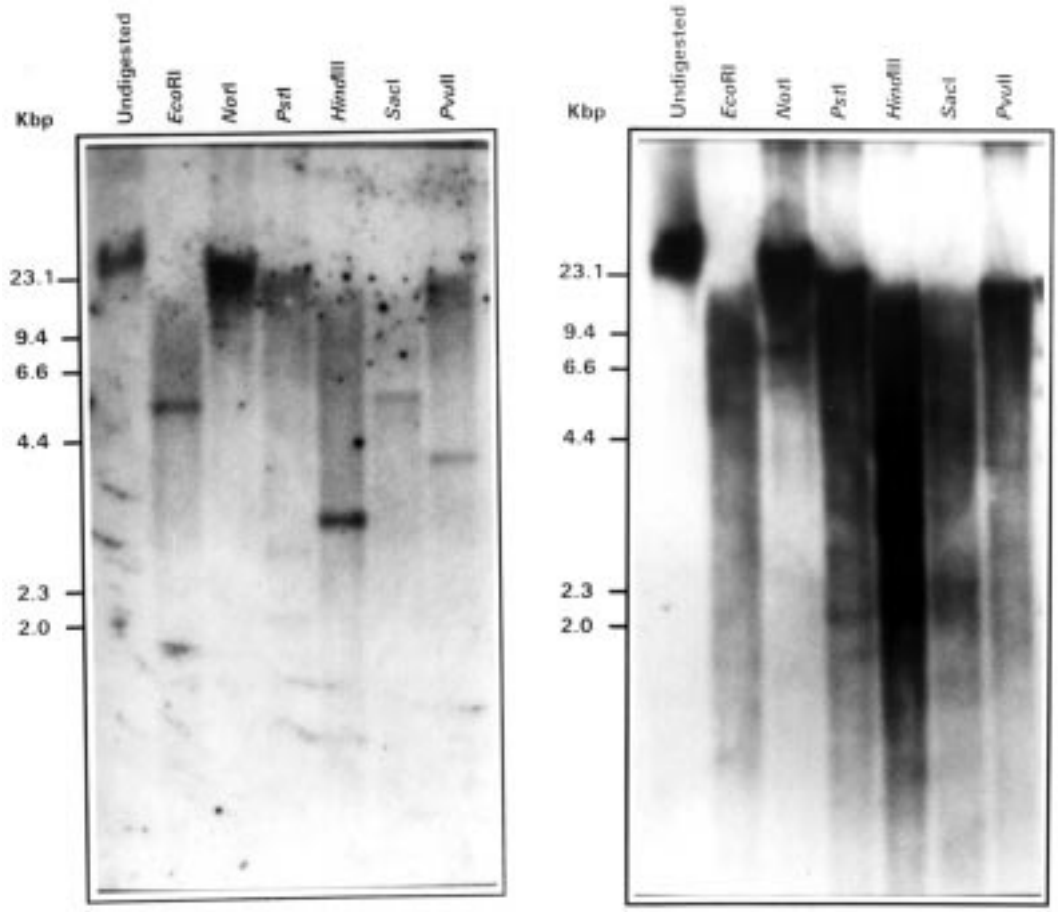

Figure 3. Genomic DNA gel blot analysis with *OsIAA1* probe. Rice DNA was separately digested with different restriction endonucleases, mentioned on top of the autoradiographic profile. Each lane contains 10 μg of the digested DNA. Southern hybridization was carried out in stringent (a) or non-stringent (b) conditions. Some signal at the top in a few lanes may represent partially digested DNA by a particular enzyme.

pletion of endogenous auxin virtually arrested the elongation growth of cut segments, which could be restored to near normal by the addition of exogenous IAA. For subsequent experiments, the endogenous auxin was routinely depleted by floating the coleoptile segments in KPSC buffer for 8 hr. The data presented in Fig. 5 show the effect of various concentrations (0 to 50 μM) of auxin (IAA or 2,4-D) applied to the 3-day-old etiolated rice coleoptile segments. Maximum elongation occurred with 30 μM IAA (or 2,4-D), where segment length increased from ca. 6 mm to 14 mm in 24 hr. The growth kinetics during IAA- or 2,4-D-induced elongation were essentially similar (Fig. 6).

To examine the changes in *OsIAA1* transcript abundance during hormone depletion, the total RNA was isolated from coleoptile segments floated in KPSC buffer after specified time intervals and subjected to northern analysis. In comparison to the undepleted control, a distinct decrease in steady-state transcript levels of *OsIAA1* was detected even within 2 hr (Fig. 7a) and it decreased drastically after 8 hr of auxin depletion. For induction studies, however, the coleoptile segments were floated in buffer for 16 hr to minimize the basal level of the *OsIAA1* transcript, and then treated with 30 μM IAA for various durations. The increase in the steady-state transcript levels of *OsIAA1* was apparent in the sample incubated with IAA even for 15 min and, thereafter, it registered a steady increase with the extended duration of IAA treatment (Fig. 7b). Essentially a similar pattern of increase in *OsIAA1* transcript was also observed with 30 μM 2,4-D (data not presented).

3.4. Light-sensitive and tissue-specific expression of OsIAA1

To study the changes in *OsIAA1* transcript abundance, northern analysis was performed with the total RNA extracted from leaves, coleoptiles and roots of light-grown rice seedlings, and from shoots and roots of dark-grown rice seedlings (Fig. 8a). In light-grown samples, the tran-

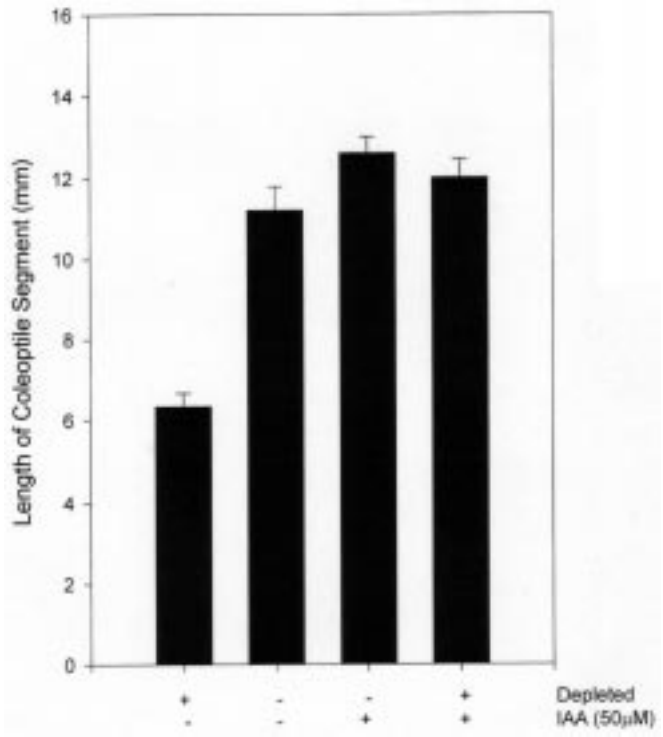

Figure 4. Elongation growth of the etiolated coleoptile segments (5 mm length) as affected by endogenous and exogenous IAA. On the X-axis, 'depleted' stands for the endogenous IAA depletion in KPSC buffer for 8 hr. The length of the coleoptile segments subjected to various treatments was measured after 20 hr. The error bars represent the mean±S.E.

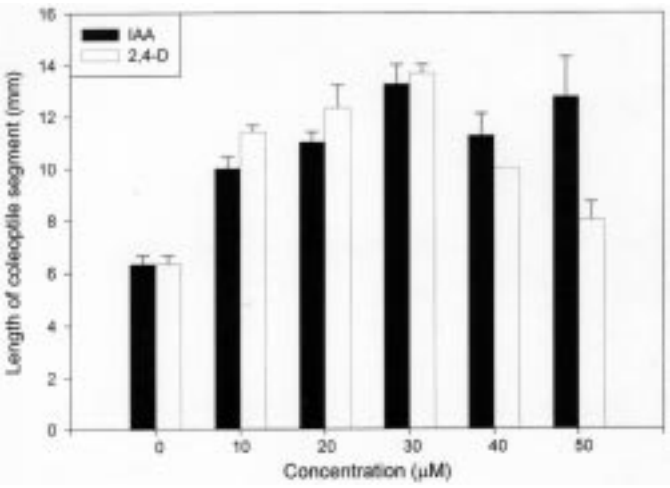

Figure 5. Effect of various concentrations of IAA or 2,4-D on elongation growth of 3-day-old etiolated rice coleoptile segments. The auxin treatment was given after 8 hr of endogenous auxin depletion in KPSC buffer. The length was measured after 20 hr of auxin treatment and is expressed as mean±S.E.

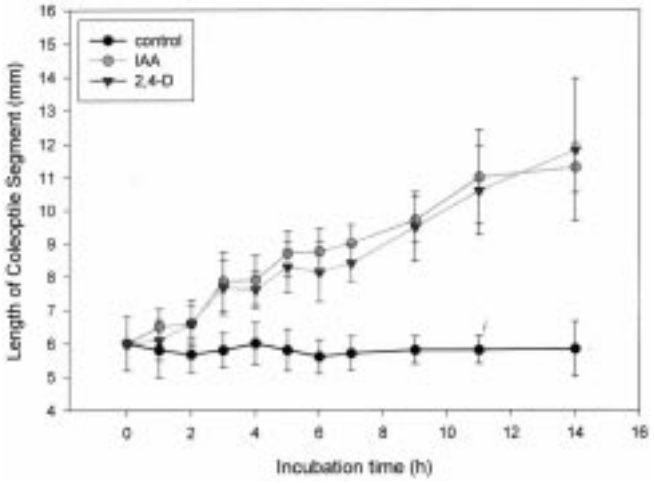

Figure 6. Kinetics of elongation of 3-day-old etiolated rice coleoptile segments treated with 30 μM IAA or 30 μM 2,4-D. Before giving hormone treatment, the endogenous auxin was depleted by floating the segments in KPSC buffer for 8 hr. The segment length was measured after specified durations and is expressed as mean±S.E.

script level of *OsIAA1* was found to be high in root but barely detectable in mature leaf. In contrast, in the dark-grown seedlings, the transcript level of *OsIAA1* in shoot (coleoptile plus leaf) was found to be higher than that in root. The transcript level of *OsIAA1* in roots of light-grown seedlings was low compared to the dark-grown seedling roots. To study the kinetics of accumulation of mRNA of *OsIAA1* in light, the total RNA was extracted from 5-day-old etiolated seedlings irradiated with white light for various durations. As shown in Fig. 8b, white light irradiation for 4 hr caused a distinct decrease in *OsIAA1* transcript level, and its effect was more pronounced after 8 hr of irradiation.

The distribution of *OsIAA1* transcripts along the vertical axes of dark-grown leaves and coleoptiles, and light-grown leaves was also examined (Fig. 8c). In dark-grown seedlings, from tip to the base, 10 mm segments of coleoptiles, and ca. 7 mm segments of leaves were pooled, separately. Since coleoptiles of 5-day-old light-grown rice seedlings were relatively small, the whole coleoptile was used for RNA isolation. The *OsIAA1* transcript level was distinctly high in etiolated coleoptiles, when compared to that detectable in dark-grown leaves and in light-grown leaves and coleoptiles. In 5-day-old etiolated coleoptile, the transcript level was highest in the top 1-cm segment and was lowest in the basal 1 cm, whereas, in leaf, the distribution was fairly uniform. In the leaf and the coleoptile of light-grown seedlings, a significant level of *OsIAA1* transcript could be detected only in the basal 1-cm region (Fig. 8c).

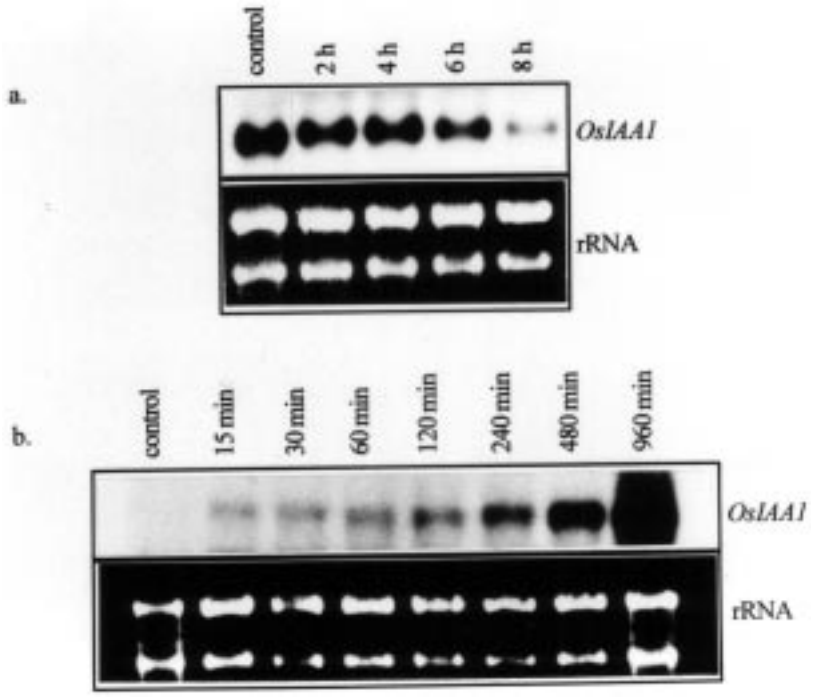

Figure 7. (a) Time-kinetics of decrease in *OsIAA1* transcript abundance during incubation of the excised coleoptile segments in KPSC buffer. RNA was extracted from the unincubated tissue (control) and tissues incubated in KPSC buffer for different durations. Each lane contained 25 μg of RNA. (b) Northern blot hybridization showing the kinetics of increase in *OsIAA1* transcript abundance in excised coleoptile segments of 3-day-old etiolated rice seedlings by 30 μM IAA. Each lane contains 10 μg of RNA. As a control, RNA was isolated from 16 hr auxin-depleted tissue. EtBr-stained rRNA represents the control.

4. Discussion

4.1. Comparison of OsIAA1 *cDNA with known* Aux/IAA *genes/cDNAs*

In this study, a rice cDNA was isolated and characterized that apparently represents an auxin-inducible gene, designated as *OsIAA1*. The *OsIAA1* cDNA has single site for restriction endonucleases *Pvu* II, *Sac* I and *Pst* I. Accordingly, under high stringency conditions (50% formamide), at least two fragments of the rice genomic DNA digested with *Pvu* II, *Sac* I, and *Pst* I hybridized with *OsIAA1* cDNA probe (Fig. 3a). Under similar conditions, one or more fragments hybridized with the probe in DNA digested with *Hin*dIII, *Eco*RI, or *Not* I. These sites are not present in the cDNA, but their presence in any intron of the gene was not ruled out. Thus, the overall pattern of the Southern autoradiogram suggests that *OsIAA1* is most likely present as single copy in the rice genome. However, under low stringency conditions (without formamide), the radiolabeled probe of *OsIAA1* hybridized to several DNA fragments (Fig. 3b), suggesting that many *OsIAA1*-like genes may be present in the rice genome. The database search also revealed the existence of several rice ESTs; at least four of these (AU029620, AU070571, AU032248, and AU032992) show 94% to 96% similarity with *OsIAA1* in regions spanning more than 300 nucleotides. This is not surprising because in all the dicot species examined so far, *Aux/IAA* genes constitute a multigene family, with *Arabidopsis* genome harboring at least 20 genes.[17,21]

The Aux/IAA proteins range from 20 to 35 kDa and the OsIAA1 protein also has a molecular mass of 26,000 Da. In addition, the predicted OsIAA1 protein harbors all four domains (I, II, III, and IV) typical of the class of Aux/IAA proteins.[17,36] The amino acid alignment of OsIAA1 and other known Aux/IAA proteins reveals some conserved clusters of basic amino acids. Sequences of these short basic clusters are in accordance with the requirement of two classes of NLS[35,37] viz., a MATα2-like NLS (KIPIK) at the end of domain IV,[34] and a bipartite NLS in between Domains I and II.[31,32] Although experimental proof is lacking, the presence of these putative NLSs in OsIAA1 indicates that it is probably localized in the nucleus. This is a property which OsIAA1 shares with many Aux/IAA proteins like PS-IAA4/6, AtIAA1/2 and AtAux2-11.[14,23,38,39] As demonstrated for several mammalian transcription factors, conserved phosphorylation sites in the vicinity of putative NLS of OsIAA1 (Fig. 2) and other Aux/IAA proteins may play a role in the regulation of their nuclear transport.[37] As discussed by Abel et al.[22] for some Aux/IAA proteins, Domain III, along with five invari-

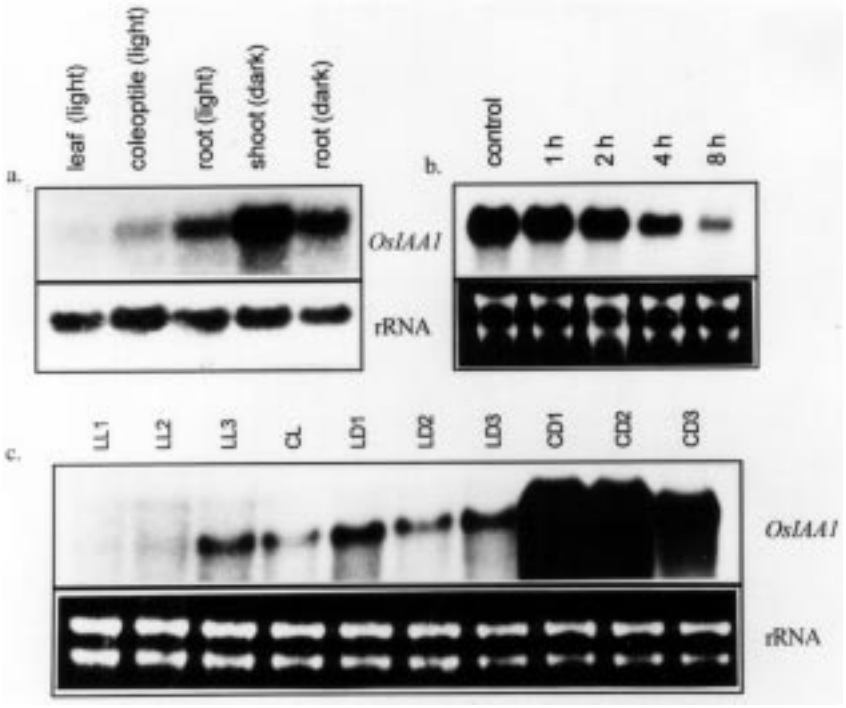

Figure 8. (a) The steady-state transcript levels of *OsIAA1* in various tissues of rice seedlings grown in dark or light for 5 days. Each lane was loaded with 10 μg of total RNA. (b) Down-regulation of *OsIAA1* by light. Total RNA was isolated from 5-day-old etiolated rice seedlings irradiated with light for different durations (1–8 hr) and subjected to northern analysis. Each lane was loaded with 25 μg of total RNA. (c) Northern blot hybridization showing the distribution of *OsIAA1* transcripts in different segments of various organs/tissues of 5-day-old seedlings. Each lane was loaded with 10 μg of total RNA. LL1 - Top 10 mm of leaf of light-grown seedlings; LL2 - Middle 10 mm of leaf of light-grown seedlings; LL3 - Basal 10 mm of leaf of light-grown seedlings; CL - Complete coleoptile of light-grown seedlings; LD1 - Top 7 mm of leaf of dark-grown seedlings; LD2 - Middle 7 mm of leaf of dark-grown seedlings; LD3 - Basal 7 mm of leaf of dark-grown seedlings; CD1 - Top 10 mm of coleoptile of dark-grown seedlings; CD2 - Middle 10 mm of coleoptile of dark-grown seedlings; CD3 - Basal 10 mm of coleoptile of dark-grown seedlings. rRNA shows the control panel representing EtBr-stained rRNA, except in 8a where it was probed with radiolabelled rDNA.

ant hydrophobic amino acids of OsIAA1, may form a $\beta\alpha\alpha$ structure that has significant similarity with the $\beta\alpha\alpha$ DNA-binding domain of prokaryotic repressors Arc and MetJ.[24] It can be speculated that OsIAA1 binds to DNA and functions as a transcriptional factor. The regulatory role of Aux/IAA proteins is also facilitated by Domains III and IV, which seem to be important for protein-protein interaction, amongst themselves or with ARFs.[25,40] The conservation in amino acid sequences of OsIAA1 and other known Aux/IAA proteins from several dicots, within and outside the four characteristic domains, suggests that these proteins may have similar functions in both monocots and dicots.

4.2. Auxin-induced changes in OsIAA1 *expression and its physiological relevance*

The level of endogenous auxin in the elongation zone of a seedling is nearly optimal for growth. When endogenous auxin is depleted, the growth rate decreases. The excised segments depleted of endogenous auxin respond dramatically to exogenously applied auxin and their growth rate increases.[1,41] Essentially similar results were obtained with excised coleoptile segments of 3-day-old etiolated rice seedlings. Elongation ceased in the absence of endogenous auxin and was restored by the addition of either natural auxin (IAA) or synthetic auxin (2,4-D). Besides a striking decrease in the elongation growth of dark-grown rice coleoptile segments kept in KPSC buffer, the level of *OsIAA1* transcript declined gradually and was undetectable after 16 hr (see Figs. 7a,b). Although a direct correlation between the decrease in *OsIAA1* transcript levels and the depletion of endogenous auxin remains to be established, a sharp decline recorded in the *OsIAA1* transcript level on depletion of auxin is consistent with most of the auxin-inducible genes viz., early *Aux/IAA* genes, *SAUR* genes and those encoding GSTs, that have a short half-life.[11–13,16,22]

Earlier studies have shown that *Aux/IAA* genes are rapidly induced by exogenous auxin in elongating regions of etiolated hypocotyls and epicotyls. On addition of auxin, some of the *Aux/IAA* mRNAs begin to accumulate within 20 to 30 min, whereas others take more

than 90 min.[12,36,38] In the case of *OsIAA1*, the increase in steady-state transcript level could be detected within 15 to 30 min of IAA or 2,4-D treatment. It is thus clear that *OsIAA1* is an auxin-inducible gene that belongs to the *Aux/IAA* family of genes that are induced in less than 30 min of auxin treatment. Although it remains to be established whether *OsIAA1* is regulated by auxin at the transcriptional level, earlier studies employing nuclear run-on transcription assays and metabolic inhibitors indicate that many *Aux/IAA* and *SAUR* genes are transcriptionally regulated.[12,38,42–44]

4.3. OsIAA1 *is expressed differentially in various organs/tissues*

Mostly, *Aux/IAA* mRNAs are expressed in elongating regions.[11–13,38] In light-grown rice seedlings, the transcript level of *OsIAA1* was highest in root, moderate in the coleoptile, and quite low in mature leaf. The finding that *OsIAA1* expression in the basal leaf portion is higher than in mature leaf blade is probably due to the abundance of meristematic cells in the former. The *OsIAA1* gene was also found to be expressed more in elongating rice coleoptile cells. Higher *Aux/IAA* transcript accumulation in the elongating hypocotyl and epicotyl has been described earlier for soybean gene *GmAux22* and, recently, in the cucumber *CS-IAA* gene.[11,13,16,18] Surprisingly, no noticeable change was observed in the steady-state transcript levels of *OsIAA1* in the top and the basal portion of the leaf of dark-grown seedlings, and its level in the coleoptile was considerably higher than that in the light-grown seedlings. The transcripts were more abundant in the apical and middle segments of the coleoptile compared to the lower segment. This may be directly related to the sensitivity of the tissue (coleoptile segments) to auxin for elongation growth, as has been shown earlier for elongating hypocotyls of soybean and cucumber.[11,16]

4.4. OsIAA1 *transcript level is affected by light*

Since light regulates auxin transport and most probably also its production,[45] auxin-inducible genes like *OsIAA1* may also be influenced by light, directly or indirectly. In light-grown rice seedlings, *OsIAA1* transcripts were present in root and almost undetectable in total leaf but, in dark-grown seedlings, the level was comparatively higher in both organs (Fig. 8a). Moreover, the transcript level was found to be significantly higher in shoot (that contained both leaf and coleoptile) than in the root of the dark-grown seedlings. When 5-day-old etiolated seedlings were transferred to white light, the steady-state transcript levels of *OsIAA1* in seedling shoots declined significantly (Fig. 8b). Although there are reports where promoters of some early-auxin-inducible genes like *SAUR* and *AtAux2-11* have been found to be more active in dark-grown than in light-grown seedlings,[18,46] it remains to be determined whether *OsIAA1* is also regulated in an identical manner.

In conclusion, the comparative analysis of the rice *OsIAA1* cDNA with analogous sequences in dicots like soybean and *Arabidopsis* indicates that the *Aux/IAA* family has remained conserved during evolution. This high degree of conservation between monocots and dicots probably reflects the strong structural constraints for the biological activity of Aux/IAA proteins. However, the high degree of divergence and duplication among dicots, especially *Arabidopsis*, needs a proper justification. We would also like to be able to provide evidence as to how extended the *Aux/IAA* family is in rice. It will be interesting to know the precise role of OsIAA1-like proteins in the auxin-response cascade. Transgenic plants for suppressing or over-expressing *OsIAA1* gene would provide some useful clues regarding its function.

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