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ORIGINAL PAPER

Farid Regad · Christine Hervé · Olivier Marinx
Catherine Bergounioux · Dominique Tremousaygue
Bernard Lescure

The *tef1* box, a ubiquitous *cis*-acting element involved in the activation of plant genes that are highly expressed in cycling cells

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Abstract In *Arabidopsis thaliana*, the *tef1* box is a *cis*-acting promoter element of the EF-1α A1 gene involved in the activation of transcription in meristematic tissues. The initiation of root *calli* in transgenic *Arabidopsis* by 2,4-D shows that the *tef1*-dependent expression of the *GUS* reporter gene is not restricted to meristematic regions but involves all of the cycling cells. Hybridization experiments conducted using *Arabidopsis* cDNA clones organized in a dense array on filters, and cDNA probes prepared from cells in various states of growth, or blocked at different steps of the cell cycle, indicate that the enhanced expression of EF-1α genes occurs in cycling cells at the point of entry into the cell cycle and remains constant during transit through the cycle. The analysis of several promoters of genes, other than EF-1α, which are overexpressed in growing cells and involved in the processes of translation or redox regulation, reveals the presence of sequences showing partial homologies with the *tef1* box. The *Arabidopsis* ribosomal gene *srp18* and the tobacco gene *thioh2*, encoding a thioredoxin h, contain such sequences. Gel retardation experiments suggest that these sequences are targets for the same proteins as those that interact with the *tef1* box of the *Arabidopsis* EF-1α *A1* gene. In transfected *Arabidopsis* protoplasts, the putative *tef1* sequence *thioh2* partially restores the activity of a *tef1* box-less EF-1α A1 promoter. These data demonstrate that the *tef1* box is a ubiquitous *cis*-acting element involved in the transcriptional activation of plant genes that are overexpressed in cycling cells. The deduced consensus sequence of the *tef1* box is arGGRYAnnnnnGTaa. The key role that this regulatory element may play in the cell cycle, by pleiotropic control of the expression of genes encoding components of the translational apparatus or involved in regulating the redox state of the cell is discussed.

Key words EF-1α genes · Transcription · *Cis*-acting element · Cell cycle · *Arabidopsis thaliana*

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F. Regad · C. Hervé · D. Tremousaygue · B. Lescure (✉)
Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes, CNRS-INRA, BP 27, 31326 Castanet-Tolosan, France

O. Marinx
SANOFI, Labège Innopole, Voie 1, BP 137, 31328 Labège Cedex, France

C. Bergounioux
Institut de Biotechnologie des Plantes, Bât 630, Laboratoire de Physiologie Végétale Moléculaire, Faculté des Sciences, 91405 Orsay Cedex, France

Introduction

A central feature of the totipotency of plant cells is the ability of differentiated, quiescent cells to reinitiate cell division. The study of molecular processes governing the passage of differentiated cells blocked in G0 phase back into the cell cycle is important for the understanding of plant development, as well as for the improvement of micropropagation, regeneration and other plant cell manipulations. One way of studying the transition from quiescence to division cells has been to identify molecular markers which are specifically expressed during this transition (see, for example, Takahashi et al. 1989; Koning et al. 1991; Medford et al. 1991; Pri-Hadash et al. 1992; Marty et al. 1993). Many of these genes are specifically expressed or show increased expression in the meristems and in other regions of active cell division. By itself, the characterization of such genes does not provide much new information about the molecular processes involved in the control of gene expression during the return to growth or during the cell cycle. However, the study of their promoters should afford insight into the general regulatory mechanisms.

Among plant growth-related genes, the genes encoding the translation elongation factor EF-1α were found

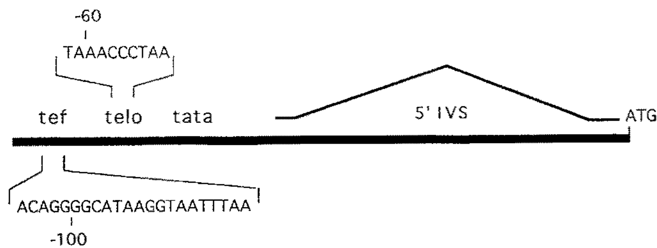


Fig. 1 Schematic representation of the *Arabidopsis* EF-1α A1 promoter (Curie et al. 1991). tef, *tef1* box; telo, *telo* box; tata, *tata* box; 5′IVS, 5′ intervening sequence containing downstream regulatory elements. The locations of the *tef1* and *telo* boxes are given relative to the transcription initiation site. The indicated *tef1* sequence corresponds to the residues protected in OP-Cu footprinting experiments (Curie et al. 1991)

to be highly expressed in young tissues (Ursin et al. 1991), in freshly isolated protoplasts (Marty et al. 1993) and in cell suspensions in growth phase (Liboz 1991; Regad et al. 1993). In *Arabidopsis thaliana*, the protein EF-1α is encoded by a small multigene family of four members (A1, A2, A3 and A4), which are all constitutively transcribed and overexpressed in cycling cells (Axelos et al. 1989; Liboz et al. 1990; Liboz 1991). The A1 promoter exhibits a modular organization. Sequences located both upstream and downstream of the transcription initiation site are involved in regulating the level and tissue specificity of expression during vegetative growth (Curie et al. 1993). Moreover, this activation process appears to be conserved among angiosperms (Curie et al. 1992). One cis-acting element, the *tef1* box (Fig. 1), located 100 bp upstream of the transcription initiation site, is sufficient specifically to activate expression in meristematic tissues (Curie et al. 1993).

In this paper we report data showing that the *tef1*-dependent activation is not restricted to meristematic tissues but is observed in all proliferating cells and probably occurs at the entry into the cell cycle. By in vitro and in vivo approaches, we demonstrate that the *tef1* box is a ubiquitous cis-acting element required for the expression, under conditions of active growth, of classes of genes involved in translation and in regulating redox processes.

Materials and methods

Histochemical analysis of GUS activity

Histochemical GUS staining was performed essentially according to Jefferson (1987). Whole young seedlings were immersed in a 0.5 mg/ml 5-bromo-4-chloro-indolyl-β-D-glucuronic acid solution and infiltrated under vacuum for 15 min. After 16 h incubation at 37° C, the plant material was cleared in hot 70% (v/v) ethanol.

Construction of the cDNA library and preparation of high density filters

RNA was isolated (Chomczynski and Sacchi 1987) from *Arabidopsis thaliana* cell suspension cultures, 3, 6, 9, 12 and 14 days after transfer to fresh medium (Axelos et al. 1992). mRNA was prepared using Dynabeads oligo(dT)25 according to the method recommended by the supplier (Dynal, Oslo, Norway). A cDNA library, containing equimolecular amounts of cDNA samples from the five growing states, was produced using the vector pTZ18R (Pharmacia) according to the method described by Caput et al. (1986). Bacteria (*Escherichia coli* strain MC1061) were transformed and 2×10^4 clones were distributed by a fluorescence-activated cell sorter into 1536-well plates. Clones were picked robotically (Biomeck 1000) and spotted onto multiple membranes.

Hybridization experiments on high density filters

cDNA probes were prepared by using poly(A)$^+$ RNAs prepared from exponentially growing or stationary phase suspension cultures (3 and 12 days after transfer to fresh medium, respectively) and from cells blocked at G1 or G2 phases of the cycle. To block the cells at G1/S, aphidicolin (Sigma) was added for 16 h at a final concentration of 30 μM to 10 ml of a 2-day cell suspension. The cells were blocked at metaphase in a similar way by adding the spindle toxinoryzalin (3,5-dinitro-N,N-dipropylsulfamide, a gift from Dow Elanco Belgium) for 18 h to a final concentration of 3 μM. Cytometric analysis was performed as follows. The pellet of cells treated with aphidicolin or oryzalin was digested in 5 ml of 2% cellulase RS (Onozuka), 0.1% pectolyase (Sigma) and 0.66 M sorbitol for 1 h at 35° C. The freshly isolated *Arabidopsis* protoplasts were washed twice in MS medium supplemented with 45 g/l mannitol and 18 g/l glucose. Nuclei were released from the protoplast pellet in buffer (Galbraith et al. 1983). After 1 min the remaining membranes were mechanically disrupted by repeated passage of the suspension through a Pasteur pipette. Finally, 1% formaldehyde (37%) was added and nuclei were filtered through nylon (pore size, 30 μm). The *Arabidopsis* nuclei in the filtrate were stained directly with 2 mg/ml (final concentration) of bisbenzimide (Hoechst 33342; Sigma). Cytometric analysis was done on 2×10^4 nuclei with an EPICS V flow cytometer (Coulter). Histograms were processed with Multicycle (Phoenix Flow Systems, San Diego).

Gel retardation experiments

Crude nuclear extracts were prepared as previously reported (Curie et al. 1991). Binding assays were done in a volume of 25 μl containing 10 mM TRIS-HCl pH 8, 50 mM NaCl, 7 mM mercaptoethanol, 10% glycerol, 1 μg of poly(dI-dC), 1000–5000 cpm (0.1–0.5 ng) of ^{32}P-labeled probe and 2–5 μg of crude extract proteins. In competition experiments, 10 ng of double-stranded oligonucleotide competitor was added as indicated. After 20 min at 25° C, the free and bound DNA were separated on 6% polyacrylamide gels in 0.5 × TBE at a constant 150 V.

Transient expression experiments

Protoplasts isolated from cell suspension cultures were prepared and transfected as previously reported (Axelos et al. 1992). The plasmid constructions used to transfect protoplasts are shown in Fig. 7 and are derived from the plasmid p-43GUS (Curie et al. 1991).

Results

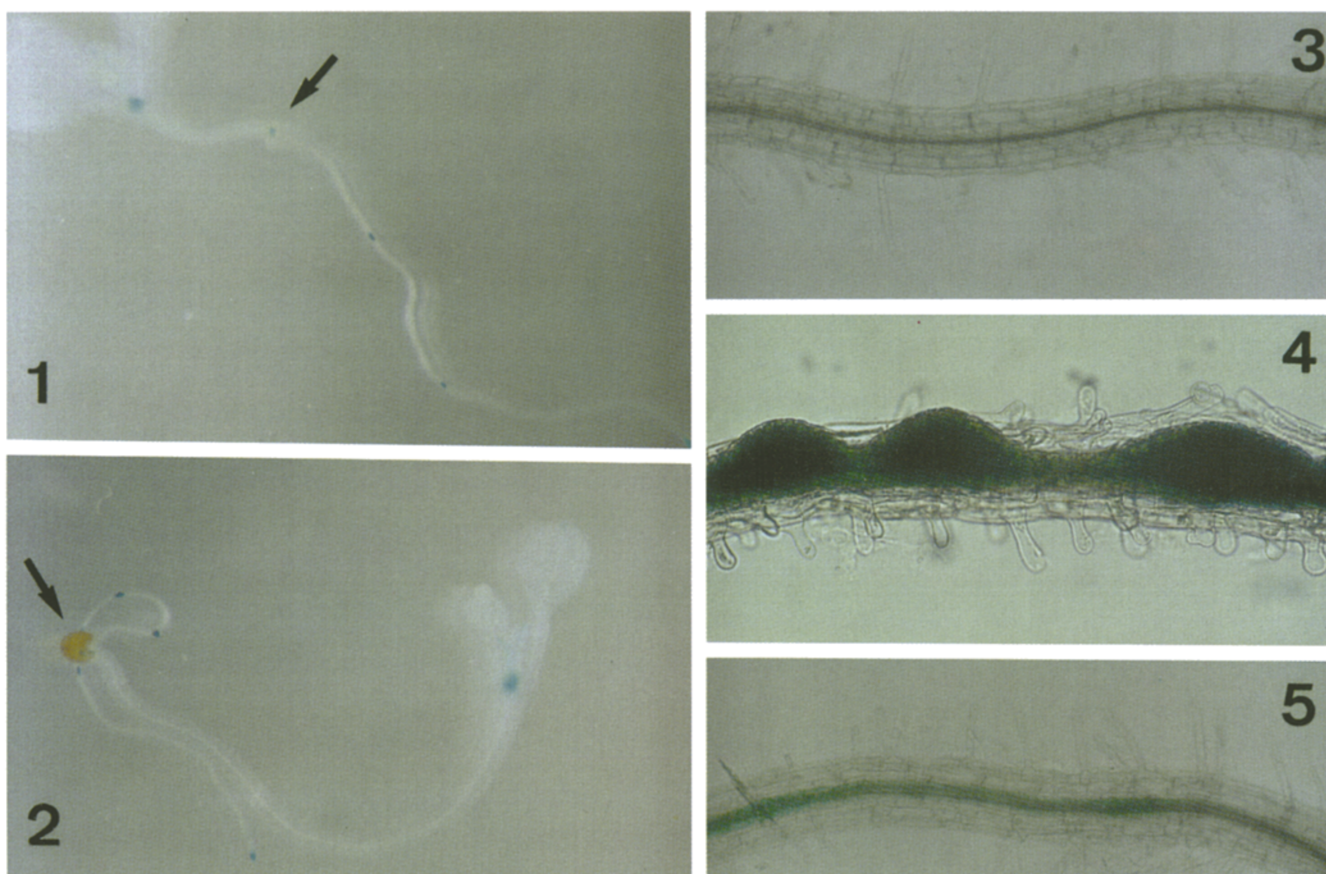
tef1-dependent gene expression in cycling cells

When placed under the control of an *Arabidopsis* EF-1α A1 promoter which was deleted of its downstream regulating elements, the expression of the GUS reporter gene in transgenic *Arabidopsis* is restricted to meristematic regions (Fig. 2-1). A histochemical analysis in etiolated seedlings shows that the GUS expression is not observed in growing tissues in the absence of cell division (Fig. 2-2). In addition to the *telo* box and the *tata* box, the promoter used in these experiments only contained the *tef1* cis-acting element (see Fig. 1). Since deletion of the *telo* box affected neither transient expression nor expression in transgenic plants (Curie et al. 1993), the *tef1* box alone appeared to be sufficient to direct the specific expression observed in meristematic tissues. To evaluate whether this expression was specific for meristems or for cycling cells in general, we analyzed the activity of the same promoter construct in transgenic plants after callus induction by 2,4-D. Under the experimental conditions used, induction of root calli is observed after 30 h. GUS activity was analyzed histochemically 30 h after transfer to 2,4-D medium. The results reported in Fig. 2-4 show a good correlation between the activity of the promoter construct used and the proliferation of cells that have been induced by 2,4-D. Nevertheless, a weak irregular GUS activity was also observed in the central part of roots, probably in the pericycle, in non-proliferating regions. This observation was confirmed by using aphidicolin, an inhibitor of DNA synthesis, in the callus induction experiments. Under these conditions, in the absence of cell division, GUS activity was observed in the pericycle (Fig. 2-5), suggesting that the activation of the expression observed in cycling cells occurs before DNA synthesis during the transition from quiescent to dividing cells.

Expression of EF-1α genes during the cell cycle

In order to evaluate the expression of EF-1α genes during the cell cycle, a cDNA library was constructed

Fig. 2 Histochemical analysis of GUS activity of a *tef1*-dependent A1 minimal promoter in cycling cells. GUS activity was analyzed in transgenic plants containing the ΔIVS A1 promoter (Curie et al. 1993). **1** GUS activity in a young seedling. **2** GUS activity in an etiolated seedling. In **1** and **2** the *arrow* indicates the collar. **3** Negative control of GUS activity in the root of a young seedling. **4** GUS activity in roots 30 h after callus induction by 2,4-D at a final concentration of 4.5 μM, and **5** after callus induction in the presence of aphidicolin (final concentration 3 μM)



from *Arabidopsis* cell suspension cultures as described in Materials and methods. A sample of this library was distributed in microtiter plates and the cDNA clones were robotically spotted onto membranes for hybridization experiments. Figure 3 shows the results obtained in a region of such a membrane containing a cDNA clone encoding the translation elongation factor EF-1α. A comparative analysis of the results of hybridization experiments conducted using labeled cDNA prepared from exponentially growing cells and stationary cells as probes confirms our previous results showing a strong activation of EF-1α expression in cycling cells (Liboz 1991; Regad et al. 1993). The data reported in Fig. 3 also reveal a dramatic change in gene expression between the two growing states analyzed (C. Hervé et al., in preparation). In contrast, the results obtained using labeled cDNA prepared from exponentially growing cells blocked in G1/S or metaphase, by aphidicolin or oryzalin respectively, did not show significant variations in the level of expression of EF-1α genes. These results indicate that the activation of expression occurs at entry into the cell cycle – during the transition from quiescence cells to division, and continues during the transit through the cycle.

Structural conservation of the *tef1* box in the promoters of genes for products related to the translational apparatus and regulation of redox states

We have already reported that sequences partially homologous to the *tef1* box of the *Arabidopsis* EF-1α A1 gene are observed within the promoters of all known plant genes encoding the translation elongation factor EF-1α (Liboz et al. 1990; Curie et al. 1992). Here, we extend this observation to other genes encoding components of the translational apparatus or involved in regulation of redox processes (Fig. 4). All of these genes are highly expressed in rapidly growing cells. Strikingly, most of these putative *tef1* boxes are, as in the case of EF-1α genes, associated with a *telo* box, a sequence homologous to plant telomere repeats (Richards and Ausubel 1988).

Functional conservation of *Arabidopsis srp18* and tobacco *thioh2 tef* boxes

The genomic sequences of the *Arabidopsis srp18* and of the *Nicotiana tabacum thioh2* genes have been recently reported (Van Lijsebettens et al. 1994; Bruguidou et al. 1993). The promoters of these genes, which are expressed mostly in organs or tissues which contain cycling cells, both contain sequences homologous to the *tef1* box (see Fig. 4). We have analyzed in vitro and in vivo whether these putative *tef1* boxes are functional equivalents of the *Arabidopsis* EF-1α A1 *tef1* box using gel retardation and transient expression experiments.

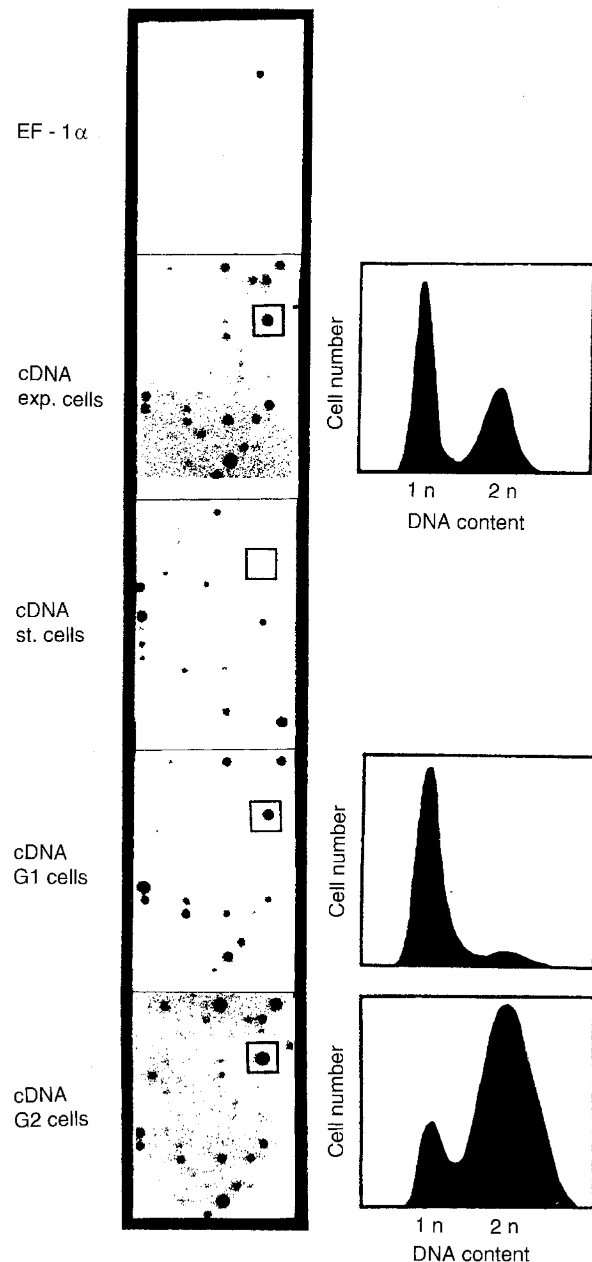


Fig. 3 Expression of EF-1α genes during the cell cycle. High density filters of cDNA clones were prepared as described in Materials and methods. The Figure shows the results of hybridization experiments in a region of a filter containing a cDNA clone encoding the translation elongation factor EF-1α. The probes used are indicated. EF-1α, EF-1α probe; cDNA exp. cells, labelled cDNA prepared from poly(A) of a 3-day cell suspension; cDNA st. cells cDNA synthesized from poly$(A)^+$ RNA of a 12-day cell suspension; cDNA G1 and G2 cells, cDNA prepared from poly$(A)^+$ RNA from a 2-day cell suspension treated with aphidicolin and oryzalin, respectively, as described in Materials and methods. The cytofluorometric data obtained from cycling cells and cells blocked in G1 or G2 are shown. After aphicolin treatment, 79.6%, 18.5% and 1.9% of cells are in the G1, S and G2 phases, respectively. After oryzalin treatment, 15%, 9% and 76% are at G1, S and G2, respectively

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              TEF1 box                       Telo box

At A1         aaacaggggcataatggtaatt..19bp..taaaccctaa
At A2         acataagggtaaaattgtcatt..14bp..taaaccctaa
At A3         ggataggggtacgtttgtaatt..14bp..taaaccctaa
At A4         gcgtaagggcaaattagtaaaa..18bp..aaaaccctag
Le EF-1       tgtaaggggcatttacgtaaat..21bp..tgaaccctaa

At srp 18     attaaagggtaaattagtaatt..26bp..aaaaccctaa
At srp 15     ggactagggttcccaagtatcc..94bp..ttagggtttt
At lrh        aaatttgggtaaccgagtttgg..23bp..ttagggtttt
Sy srp 11     ttctagggataagtaagtaatg..109bp.attagggttt

Nt thio h2    gagtagggacaaatctgtaaat..14bp..tccaccctaa
Os thio       acgcaagggcactccggtaatt
Nt parA       taagaaggatattttagtaatt..13bp..tttagg.ttt
              taagaaggatattttagtaatt..12bp..tttagggttt

consensus         arGGRYA.....GTaa
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Fig. 4 Conservation of *tef1* and *telo* boxes within the promoter of plant genes related to the translational apparatus and to the redox regulation processes. At A1, A2, A3 and A4, *Arabidopsis* EF-1α genes (Axelos et al. 1989; Liboz et al. 1990); Le EF-1, *Lycopersicon esculentum* EF-1α gene (Shewmaker et al. 1990); At srp18, *Arabidopsis* ribosomal gene *srp18* (Van Lijsebettens et al. 1994); At srp 15, *Arabidopsis* ribosomal gene *srp15* (Sangwan et al. 1993); At lrh, *Arabidopsis lrh* gene homologous to animal laminin receptors (unpublished results); SY srp 11, soybean ribosomal gene *srp11* (GeneBank accession no. L28831); Nt thioh2, *N. tabacum* thioredoxin h gene (Bruguidou et al. 1993); Os thio, *Oryza sativa* gene encoding a thioredoxin h (GeneBank accession no. D26547) Nt parA, *N. tabacum parA* gene showing homology with gluthathione S-transferase (Takahashi et al. 1990; Droog et al. 1993)

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(1) EF-1 A1 tef1   ctagaggctaaACAGGGGCATAATGGTAATTtaaagaatcga
(2) EF-1 A4 tef1   ctagaggctaaGTAAGGGCAAATTAGTAAAAtaaagaatcga
(3) srp18 tef1     ctagaggctaaTAAAGGGTAAATTAGTAATTtaaagaatcga
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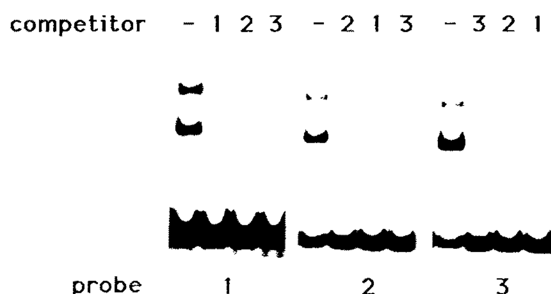


Fig. 5 Gel retardation experiments showing functional conservation between the A1 *tef1* box and the putative A4 *tef1* and *srp18 tef1* boxes. The preparation of *Arabidopsis* nuclear extracts and the binding conditions are reported in Materials and methods. The sequences of synthetic double-stranded oligonucleotides used as probes or as competitors are indicated. All oligonucleotides are flanked by the same sequences corresponding to cloning sites (*lower-case letters*). The sequences of *tef1* boxes found within the indicated promoters are in *capital letters*. EF-1 A1 tef1: *tef1* sequence of the *Arabidopsis* EF-1α A1 gene corresponding to the residues protected in OP-Cu footprinting experiments (Curie et al. 1991). EF-1 A4 tef1: putative *tef1* box of the *Arabidopsis* EF-1α A4 gene (see Fig. 4). srp18 tef1: putative *tef1* box of the *Arabidopsis* gene encoding the ribosomal protein srp18 (see Fig. 4)

Figure 5 shows the results of gel retardation experiments using as probes double-stranded synthetic oligonucleotides corresponding to the *Arabidopsis* EF-1α A1 *tef1* box and to the putative *Arabidopsis* EF-1α A4 *tef1* and *srp18 tef1* boxes. As already reported, a labeled double-stranded synthetic oligonucleotide corresponding to the EF-1α A1 *tef1* box is sufficient to form the two specific retarded complexes C1 and C2 (Curie et al. 1991). The formation of these complexes was prevented by the addition of an excess of unlabeled EF-1α A1 *tef1* sequences and also by the addition of unlabeled sequences corresponding to the putative *tef1* boxes found within the promoters of the *Arabidopsis* EF-1α A4 and *srp18* genes. In contrast, the formation of these complexes was unaffected by competition with a sequence corresponding to a motif found within the first intron of the EF-1α A1 gene (IVS1) showing a strong homology with the 3′ end of the A1 *tef1* box (see Fig. 6). These results suggested that the three sequences are recognized by the same *trans*-acting elements. This was confirmed by using as labeled probes the EF-1α A4 and *srp18* putative *tef1* sequences. Two retarded complexes showing migration properties identical to that observed using the EF-1α A1 *tef1* box sequence as probe were obtained (Fig. 5). In identical experiments, similar conclusions were reached using the putative tobacco *thioh2 tef1* sequence (Fig. 6).

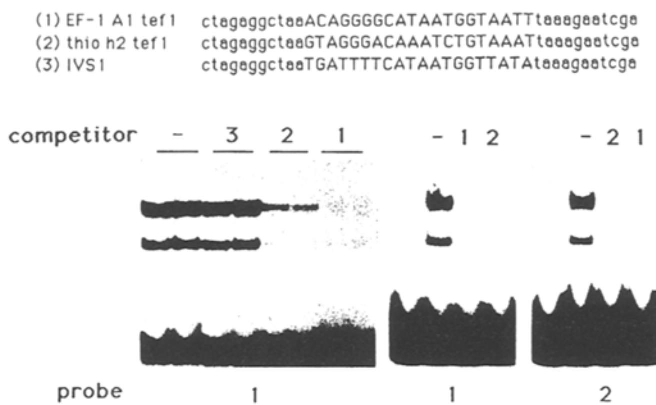
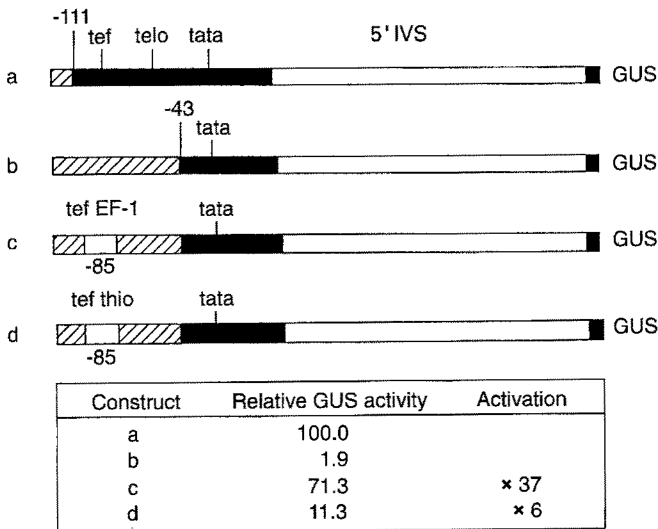


Fig. 6 Gel retardation experiments showing the functional conservation between the putative tobacco *thioh2 tef1* box and the *Arabidopsis* EF-1α A1 *tef1* box. The sequences of synthetic double-stranded oligonucleotides used as probes or as competitors are indicated (*lowercase* and *capital letters* are used as described in the legend of Fig. 5). EF-1 A1 tef1, as in Fig. 5. thioh2 tef1, putative *tef1* box of a tobacco gene encoding a thioredoxin *h* (see Fig. 4). IVS1, unrelated sequence used as negative control in the competition experiments. This sequence, found within the 5′ intervening sequence of the A1 gene (Axelos et al. 1989, see Fig. 1) shows a strong homology with the 3′ end of the A1 *tef1* box



Construct	Relative GUS activity	Activation
a	100.0	
b	1.9	
c	71.3	× 37
d	11.3	× 6

Fig. 7 Transient expression experiments showing the functional conservation between the putative *thioh2 tef1* box and the *Arabidopsis* EF-1α A1 *tef1* box. A schematic representation of the plasmids used in transient expression assays is shown. The A1 sequences are represented by *filled boxes* (promoter) or *open boxes* (5′IVS). The SK polylinker sequences are represented by striped boxes. The Positions are indicated relative to the transcription initiation site. The relative GUS activities are normalized to p-111 (nmol MU/min/mg protein) and each is the mean of three independent transfections

The activity of the EF-1α A1 promoter could be easily analyzed in transient expression experiments (Curie et al. 1991) to evaluate to what extent the putative *thioh2 tef1* box was able to restore the activity of a *tef1* box-less A1 promoter. 5′ deletions of the A1 promoter from position − 111 to position − 100 (relative to the transcription initiation site), disrupting the *tef1* box, and more extensive 5′ deletions strongly affect the expression of the *GUS* reporter gene in transfected *Arabidopsis* protoplasts (Curie et al. 1991). Synthetic oligonucleotides corresponding to the A1 *tef1* box or to the *thioh2 tef1* box sequences were fused upstream a 5′ − 43 deletion of the A1 promoter. SK polylinker sequences were placed between the synthetic *tef1* boxes and position − 43 in order to keep the distance between them the same as that found within the A1 promoter. In these constructs, the *telo* box of the A1 promoter (see Figs. 1 and 4) was replaced by SK polylinker sequences. These chimaeric promoters, fused to the GUS reporter gene, were used to transfect *Arabidopsis* protoplasts. The results reported in Fig. 7 show that the two synthetic *tef1* sequences are able to activate the expression of the *tef1* box-less promoter. The *thioh2 tef1* sequence appears to be less efficient than the A1 *tef1* box, although it does show a strong effect. In agreement with this observation, this sequence also shows a lower affinity for the protein that interacts with the *tef1* A1 sequence (data not shown).

Discussion

The data reported in this paper support the notion that the *tef1* box is a ubiquitous *cis*-acting element involved in the transcriptional activation of a class of genes related to cell division. Plant EF-1α genes from several species are highly expressed in young tissues, in freshly isolated protoplasts and in cell suspensions in growth phase (Ursin et al. 1991; Liboz 1991; Marty et al. 1993; Regad et al. 1993). The *tef1* box of the *Arabidopsis* EF-1α A1 gene is involved in the activation of expression in meristems (Curie et al. 1993). The expression of the *Arabidopsis* ribosomal *srp18* gene appears to be restricted to meristematic tissues (Van Lijsebettens et al. 1994). The *N. tabacum thioh2* gene, encoding a thioredoxin *h*, is highly expressed in growing tissues of tobacco plants (Bruguidou et al. 1993). All these genes contain, at similar locations within their promoters, sequences homologous to the *tef1* box of the *Arabidopsis* EF-1α A1 gene (Fig. 4). Here, we show that the putative *tef1* boxes of both the *Arabidopsis* EF-1α A4 and the *srp18* genes, as well as that of the tobacco *thioh2* gene are recognized by the same proteins as those interacting with the *tef1* box of the *Arabidopsis* EF-1α A1 gene (Figs. 5 and 6). The *tef1* box of a tomato EF-1α gene and that of the *Arabidopsis* EF-1α A3 gene have the same properties (Curie et al. 1992, and our unpublished results). The *thioh2 tef1* box was able partially to restore the activity of a *tef1* box-less A1 promoter (Fig. 7). These data clearly indicate that, despite the weak homology observed between the *tef1*

box-like sequences reported in Fig. 4, all play similar functional roles. The deduced consensus sequence is arGGRYAnnnnnGTaa. The weak conservation between the *tef1* primary sequences explains why, in the absence of functional studies, this regulatory element has not been characterized before. Interestingly, the GG and the GT residues, which are conserved in all these *tef1* boxes, are separated by one turn in the DNA helix, suggesting that the corresponding trans-acting elements interact with the template at two sites located on the same side of the DNA molecule.

Besides the EF-1α genes, it was interesting to find *tef1* boxes within the promoters of other genes encoding proteins of the translation apparatus (Fig. 4). This is the case for the *Arabidopsis srp18* and *srp15* genes, the soybean *srp11* gene and for the *Arabidopsis lrh* gene encoding a protein homologous to animal laminin receptors (Axelos et al. 1993). The *lrh* gene is also highly expressed in exponentially growing *Arabidopsis* cells (our unpublished results) and this protein may be a component of the translation machinery (Garcia-Hernandez et al. 1994). In plants, as in animals, the demand for new ribosomes varies dramatically during different developmental periods and the rates of accumulation of their individual components must be regulated accordingly. Very little information is available on the mechanisms involved in this process. In plants, a good illustration of this kind of regulation is provided by the coordinated expression of ribosomal protein mRNAs observed in soybean hypocotyls after auxin treatment (Gantt and Key 1985). The *tef1* box could play an important role in this coordinated expression.

The results reported in Fig. 2 show that the *tef1*-dependent expression in transgenic *Arabidopsis* is mainly observed in proliferating tissues, in root *primordia* or in calli induced by 2,4D, confirming the role of the *tef1* box in the elevated expression of EF-1α genes in growing tissues. The results shown in Fig. 3 suggest that this activation occurs during the transition from quiescence to active division and remains constant during the transit through the cell cycle. Despite the considerable progress that has been made in the understanding of the regulation of the cell cycle, few data are available concerning the relationship between the cell cycle controls and controls on cell growth and protein synthesis (for reviews see Cross et al. 1989; Kirschner 1992; Norbury and Nurse 1992). Translation has long been recognized as a critical step for both entry into, and transit through, the cell cycle (Brooks 1976). The requirement for a rapid rate of protein synthesis in G1 is important in the transition through START in yeast (Hartwell and Unger 1977; Shilo et al. 1979; Moore 1988), as well as in transition through the restriction control in mammalian cells (Rossow et al. 1979). Mutations in yeast genes required for protein synthesis have also been shown to cause a START-I phenotype (Bedard et al. 1981; Hanic-Joyce et al. 1987). A clear demonstration of the key role that translation plays in the regulation of cell growth comes from studies showing that products implicated in the control of the rate of protein synthesis have characteristics of proto-oncogene products (Rhoads 1991; Sonenberg 1993). The proteins that interact with the *tef1* box could be involved in early events in the regulation of gene expression at the transition from quiescent to cycling cells. Putative *tef1* boxes are also observed within the 5′ sequences flanking the tobacco *parA* gene which include a cis-acting auxin-responsive element (Takahashi et al. 1990, and Fig. 4). This gene, as in the case of soybean genes encoding ribosomal proteins, is induced by auxin prior to DNA synthesis (Gantt and Key 1985; Takahashi et al. 1989). Studies are in progress to evaluate to what extent the *tef1* box may be an auxin-responsive element.

In addition to genes encoding components of the translational apparatus, *tef1* boxes were found within the promoters of several plant genes involved in redox processes or in protection against the damaging effects of oxidative reactions. We have shown that the tobacco *thioh2 tef1* box plays the same regulatory role as that of the EF-1α A1 promoter (Figs. 6 and 7). A rice gene encoding a thioredoxin h and the tobacco *parA* gene mentioned above, encoding a protein homologous to a glutathione-S-transferase (Droog et al. 1993), also contain one or several putative *tef1* boxes (Fig. 4). Regulation of the intracellular redox state is critical for both cell viability and proliferation. Recent work has clearly shown that, in mammals, thioredoxin stimulates both cellular proliferation and DNA synthesis (Oblong et al. 1994). How this stimulation is achieved at the level of gene expression remains an open question. One attractive possibility could be regulation by a direct or an indirect redox control mechanism, of the activity of transcriptional factors, as observed for the Fos subunit of AP-1, NF-κB, BZLF1 and steroid receptors (Peleg et al. 1989; Abate et al. 1990; Bannister et al. 1991; Matthews et al. 1992; Okuno et al. 1993). Thus, besides the control of protein synthesis activity, a *tef1*-dependent regulation of genes encoding proteins involved in the redox control could be a very early event occurring in cells at the transition from quiescence to growth. The two processes could be related, as suggested by the observation that NADPH, thioredoxin and thioredoxin reductase are required for the maintenance of high protein synthesis activity in rabbit reticulocyte lysates (Hunt et al. 1983).

It is striking that all known plant EF-1α promoters, as well as the *Arabidopsis srp18* and *srp15* promoters, the tobacco *thioh2* and *parA* promoters and the soybean *srp11* promoter contain the conserved sequence AAACCCTAAA in association with a *tef1* box (Fig. 4). This element, the *telo* box, is homologous to the repeat motif AAACCCT of plant telomeres (Richard and Ausubel 1988), is over-represented within the *Arabidopsis* genome and is a specific target for

a nuclear protein (Regad et al. 1994). Despite this strong structural conservation and the specific interaction with a nuclear factor, our previous work (Curie et al. 1993) and the results reported in this work (Fig. 7) do not favour a role of this element in the activation of gene expression. We cannot exclude the possibility that the *telo* box could act as an antagonist of the *tef1* box and thereby participate in the repression of gene expression in quiescent and in senescent cells. In *Saccharomyces cerevisiae*, discrete motifs or internal tracts of telomeric DNA are known to act as silencers (Shore and Nasmyth 1987; Stavenhagen and Zakian 1994; Aparicio and Gottschiling 1994).

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