Cis and *trans*-acting elements involved in the activation of *Arabidopsis thaliana* A1 gene encoding the translation elongation factor EF-1α

Catherine Curie, Thierry Liboz, Claude Bardet, Eugen Gander, Claire Médale, Michèle Axelos and Bernard Lescure*
Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes, CNRS-INRA, BP 27, 31326 Castanet Tolosan, France

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ABSTRACT

In *A. thaliana* the translation elongation factor EF-1α is encoded by a small multigenic family of four members (A1-A4). The A1 gene promoter has been dissected and examined in a transient expression system using the GUS reporter gene. Deletion analysis has shown that several elements are involved in the activation process. One *cis*-acting domain, the TEF 1 box, has been accurately mapped 100 bp upstream of the transcription initiation site. This domain is the target for *trans*-acting factors identified in nuclear extracts prepared from *A. thaliana*. Homologies are found between the TEF 1 box and sequences present at the same location within the A2, A3 and A4 promoters. This observation, together with those obtained from gel retardation assays performed using DNA fragments from the A4 promoter, suggest that the activation process mediated by the TEF 1 element is conserved among the *A. thaliana* EF-1α genes. Analysis of nearly full length cDNA clones has shown that in addition to a single intron located within the coding region, the A1 gene contains a second intron located within the 5′ non coding region. Such an intron is also present within the A2, A3 and A4 genes. This 5′ intervening sequence appears to be essential to obtain a maximum GUS activity driven by the A1 gene promoter.

INTRODUCTION

With the aim of elucidating the molecular mechanisms underlying the activation of plant housekeeping genes, we have recently initiated studies using the genes of *A. thaliana* encoding the translation elongation factor EF-1α as a model system. The regulation of EF-1α expression is of interest for the following reasons: (A) the constituents of the translational apparatus are synthesized in a balanced way and therefore must depend on common regulation processes at transcriptional or post-transcriptional levels; thus, in yeast, EF-1α gene transcription depends on the *cis*-acting elements, the HOMOL1 and RPG boxes, which are found in the promoter region of several genes encoding ribosomal and housekeeping proteins (1,2); (B) recent data obtained from *Drosophila* germ line transformation experiments with the EF-1α gene have suggested a possible important control on the aging of the cells exerted at the transcriptional level by this gene (3); (C) Southern experiments carried out using cDNA or genomic probes have revealed that EF-1α is encoded by small multigenic families of at least two members (4−10); these genes have been shown to be differentially and developmentally regulated in *Mucor racemosus* (11) and in *Drosophila melanogaster* (9); (D) it has been demonstrated that the Cauliflower mosaic virus 35S promoter contains at least two *cis*-acting domains which can confer different tissue-specific expression (12); it is possible that cellular constitutive promoters are similarly organized and therefore could provide interesting information on molecular elements in the pathways that determine development; the EF-1α genes are an appropriate system with which to examine such a possibility.

In *Arabidopsis thaliana*, EF-1α is encoded by a multigenic family of four members (A1-A4) which are all actively transcribed in cell suspension cultures (13). Based upon both their physical separation and a comparison of their sequences, we have suggested that the A4 gene and the A1, A2 and A3 genes constitute two distinct subfamilies within the genome (14). We have also shown that a DNA fragment containing 2.3 Kb upsteam the coding region of A1 gene, is abble to direct a strong transient expression in Arabidopsis transfected protoplasts (14).

In this paper, we report results of studies on the structure of the four EF-1α mRNAs and on the characterization of *cis* and *trans*-acting elements involved in the activation of the A1 gene.

MATERIALS AND METHODS

Gene constructions and DNA manipulations

All DNA manipulations were carried out following the standard protocols given by Sambrook et al (15). Deletions from the unique XbaI site within the A1 promoter (figure1) were achieved using Bal31 exonuclease. Unidirectional deletions were created by

* To whom correspondence should be addressed

removing the remaining A1 sequences located upstream of the XbaI site. Sequencing and mapping of the extent of Bal31 promoter deletions were achieved using the double-stranded sequencing method (16).

Transient expression assays

Preparation of protoplasts from *Arabidopsis* cell suspension cultures has already been described (14). Transfections were performed according to the $Ca(NO_3)_2$-PEG procedure (17) using 10 μg of CsCl purified plasmid DNA and 10^6 protoplasts. After a 16 h expression period, GUS activity was assayed by fluorimetry (18).

Preparation of nuclear extracts

Leaves or the whole plants (Columbia ecotype) were immersed into ice-cold homogenization buffer (10 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 1.14 M sucrose) at a ratio of 5 ml of buffer *per* 1 g of tissue. After addition of Triton X-100 and β-mercaptoethanol to final concentrations of 0.15% and 7 mM respectively, the tissue was homogenized with four 15 sec pulses in a Waring blender at 4°C. The homogenate was filtered through two layers of Miracloth and centrifuged for 10 min at 4500×g, at 4°C. The pellets were resuspended in 20 ml of cold homogenization buffer containing Triton X-100 and β-mercaptoethanol and centrifuged as described above. After a second identical wash, this cellular fraction enriched in nuclei, was resuspended in nuclear extraction buffer (10 mM Tris-HCl pH 8.0, 140 mM NaCl, 1.5 mM $MgCl_2$, 0.25 mM EDTA, 25% glycerol) at a ratio of 50 μl buffer *per 1* g of starting tissue. Nuclear extraction was achieved by adding NaCl and spermidine to final concentrations of 500 mM and 5 mM respectively. The suspension was left on ice for 1 h with occasional shaking, then centrifuged for 45 min in an Eppendorf centrifuge (12,000 g) in the cold room. The supernatant was dialysed for 4 h against 'binding' buffer (10 mM Tris-HCl pH 8.0, 50 mM NaCl, 7 mM β-mercaptoethanol, 10% glycerol). The nuclear extracts were then stored at −80°C.

Binding assays and footprinting experiments

The binding assays were done in a volume of 30 μl of binding buffer containing 2−5 μg of nuclear proteins, 2 μg poly(dI-dC), 1000−5000 cpm (0.1−0.5 ng) of ^{32}P labelled probe. The reaction was started by adding the nuclear proteins. After 20 min at 25°C, the free and bound DNA were separated on 5% polyacrylamide gels in 0.5×TBE. The gels were then fixed, dried and autoradiographed.

Footprinting of DNA-protein complexes assays using 1,10-phenanthroline-copper ion were achieved as described by Kuwara and Sigman (19).

RESULTS

Characterization of an intron within the 5′ non-coding region

The complete sequence of the A1 gene and its flanking regions has been reported (14). The transcription initiation site was located 679 bp upsteam from the ATG initiation codon. We have suggested that a location far upstream of the transcription initiation site could be explained by the presence of one or several introns within the 5′ flanking region. In order to identify potential maturation sites at the 5′ ends of EF-1α mRNAs, we have screened a cDNA library prepared from mRNA of cell suspension cultures of *Arabidopsis* and we have isolated nearly full length cDNA clones. The sequences of such clones were compared to the genomic sequence (14). An intervening sequence was thus characterized within the 5′ non-coding region. It was located between the positions extending from +56 to +657 relative to the transcription initiation site (−23 to −624 relative to the translation initiation codon ATG). The location of this IVS within the 5′ flanking region of the A1 gene is shown in figure 1. Such an intron is also found at a similar position within the three other *Arabidopsis* EF-1α genes. For the A2, A3 and A4 genes, it is located between the positions extending from −24 to −671, −23 to −619 and −2 to −501 respectively, relative to the translation initiation codon ATG. In all the cases, these 5′ IVS are flanked by consensus splice donor and acceptor sequences.

Deletion analysis of the A1 promoter

As a first step towards the identification of DNA sequences required for expression, a series of promoter deletion mutants was created. Each construct was cloned in front of the GUS

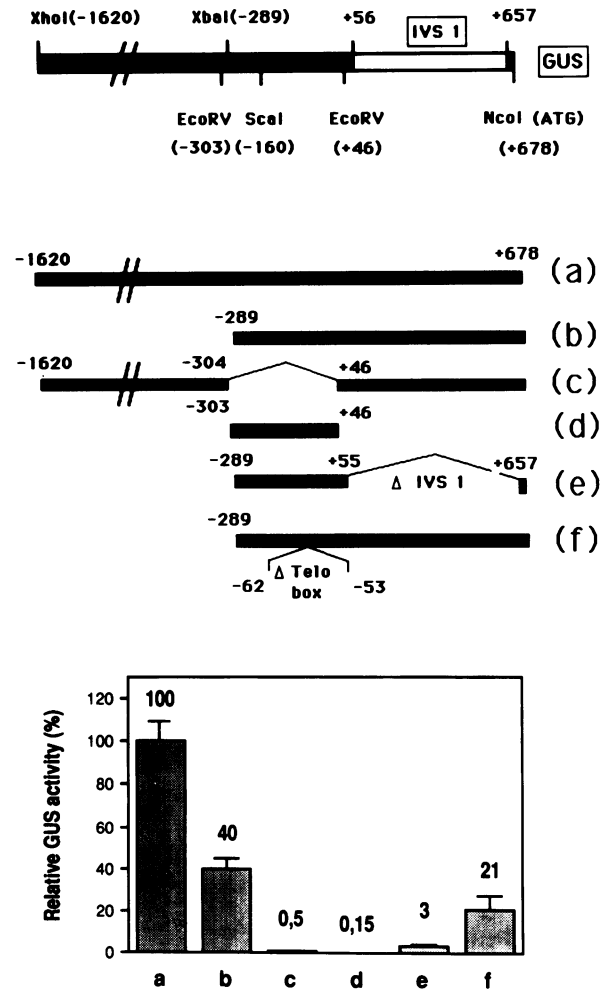

Figure 1. Transient expression analysis of GUS fusions of A1 promoter deletion mutants. In the upper part of the figure, a schematic representation of the full length promoter is shown. The position of the relevant restriction sites is indicated relative to the transcription initiation site. The location of the intron (IVS 1) within the 5′ non coding region is shown. The transfection experiments were carried out with GUS fusions of the indicated constructs (a−f). The relative GUS activities, normalized to the full-length construct (construct a: 150 nmol MU/min/mg protein), are the average of at least three independent transfection experiments.

reporter gene in a plasmid derived from pCCP1 GUS (14) and used to transfect protoplasts prepared from cell suspension cultures of *Arabidopsis thaliana*. Nomenclature for the mutants with an indication of the extent of the deletion is given in figure 1.

As already reported (14), a XhoI-NcoI fragment containing 2.3 Kbp upstream of the coding region (construct a, figure 1) is able to direct a strong transient expression, two-fold higher than that obtained using the CaMV 35S promoter. A deletion down to position −289 (XbaI site) relative to the transcription initiation site (construct b), retained 35−45% of the expression from the full length construct.

As expected, deletion of the EcoRV-EcoRV fragment, extending from positions −303 to +46 (construct c) and containing the TATA box and the cap site (see figure 3), reduced the GUS activity to a very low level (about 200-fold). When the same EcoRV-EcoRV fragment was fused in front of the GUS reporter gene (construct d), the transient expression observed was only slightly higher than the experimental background. These results suggested the presence of a *cis*-acting element located downstream of the transcription initiation site. However, in this latter construct, the efficiency of GUS expression could be affected by the modified translational initiation context. In construct e, the genomic DNA fragment of construct b, extending from the EcoRV site (+46) to the NcoI site (+678), was replaced by the corresponding EcoRV-NcoI fragment prepared from a cDNA clone. This chimaeric promoter lacking the 5′ IVS, mediated a GUS activity 10−20-fold lower than that obtained using construct b, demonstrating the requirement of the 5′ IVS for a maximum GUS activity.

We have recently focussed attention on a strikingly conserved sequence, the telo box AAACCCTAA. This element, homologous to the repeat motif of *Arabidopsis* telomeres (20), was found at approximately the same location within the A1, A2 and A4 promoters (14). Now, we have isolated genomic clones containing sufficient DNA sequences upstream of the A3 gene, to show that this telo box element is also present at the same location within the A3 promoter (figure 2). The telo box sequence

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-1820 GAATTCGAATGATTTCTTTAATAGGTTCATGCAAATCAGGGTTTAAATCAGATGTGAAAC
-1760 GTTTTGAATCTCTGCCAAAGTACACATATTTGGAATGTTTTTGAGTCTACCAAACATTCA
-1700 CTCATGATTCTCCAAAATCTAATTTTTACTGTTGTTTCAAAACTGGGTTGTTTTGTTAGC
-1640 TTATGTCCTCATTGTGTTTGTTGAATGATTTCTTTTAGGCATATGGATTAGGGAAGTTAC
-1580 ATAGGCCACTCAAGTGTTGTTGAAAGATTTCTTATGAGTCTCTGGAGTGTTGTTAACTTA
-1520 CTTCCTCACCAATGGTGGCAGGTTCAAGCAGCTGATTCTCTTTTGAAACTCGTATCAGAA
-1460 CTTAAGCAAACAGCGATATTTTCAGGATTTGCATCACTAAACGATCATGTAGAACAAAGA
-1400 ATGAAGAGTTTGATCAAGAGGCCGAGAAGACAAATCGATTGTTGGCTAGAATTGCTGATG
-1340 ATGCATCTGCCAATCTTAAAGAGCTCGAGTCTCACTATTACTCTTCTGCTCAGAGATTGA
-1280 CTCTAGACATCTGATATTAGAATGCCTGGAGACACCAGAGGAAACAGATAAAACAATTGG
-1220 TTCATAAACCAGAGGAAACGGCCATCGAAGGATATGCAGTTTGAAGTCATGGACAACGTT
-1160 TTGGACTATCCTTTCCCACATTGATCATATTTTCTCTCTCAATTCGCATATTACTTAGCA
-1100 ATCTTAAATGTTCCAGTCTGATGAACAAGCGTTTGATGTTTTCATCAATCATTAGTGATT
-1040 TTTTTGCTGTTATCCTCTGTATGTTTGATAATTACGTGAAATTACATTCTCTTAACCTGC
 -980 AAAGTGTATATCGTCCAAGTAAAACCTTCTTGATTTAATTCGCGTTGAACCAAATTTAAT
 -920 ATATTCCACTTTTGGATTGAACTATCAAATTCAAATTAGCTTGACTTATGAAACAAGAGC
 -860 GACCCCTCGAGGTTAGTGGACTTTTATGAAACGCGACCGACTCTTCAAGGCCCAAAGAAA
 -800 AAGAGAATATTCGGCGGGATAGGGGTACGTTTGTAATTTGGCAAACGATGTTATGTTAAA
 -740 CCCTAACTTATTATTTCTTTCGATTCACCACTATAAATAAACTCGAATCCCAAACATAGC
 -680 TTTATTCCTTGCTTCATTTTTCGTCACCCTAGCCGCTTTACTCTCTTGCGATATCTCTGA
 -620 GGTAAGCTTTTTCGTCACCATCTTCGATCTGCTTCTTTCTCTTCTGTTGATCTGTGTTGA
 -560 ATCTGTTTCGATCCTTCTCGTTTGTTTGTTGTAATCTCTTGGATTCGATTCTCTTTGTTT
 -500 ATAGATCGTTTGGAATTTCTGATCTGTGGTGTAAAGTTTT...
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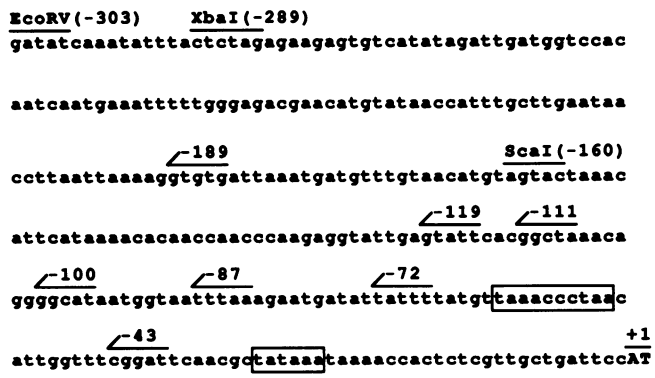
Figure 2. Nucleotide sequence of the 5′ flanking region of the A3 gene. The sequence shown corresponds to the region located upstream of that previously sequenced (13). The telo box and the TATA box and the putative cap site are underlined. The positions indicated are relative to the ATG translation initiation codon (13).



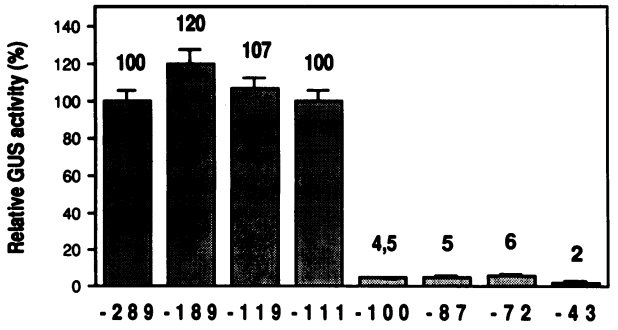


Figure 3. Accurate mapping of a *cis*-acting element of the A1 gene. The extent of 5′ Bal31 deletions is shown on the DNA sequence. The indicated positions are relative to the transcription initiation site. The telo box and the TATA box are boxed. The GUS activities are normalized to the −289 deletion (construct b in figure 1).

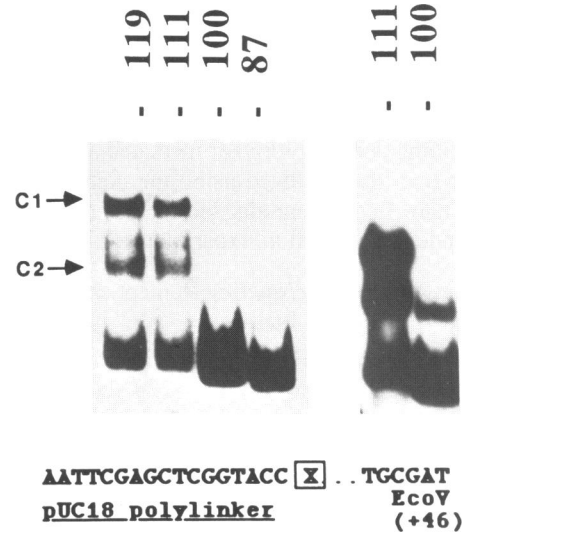


Figure 4. Identification by gel retardation assays of *trans*-acting factors interacting with the TEF 1 element. The extent of deleted fragments used as probes is indicated. A schematic representation of probes used is shown under the autoradiographs: 5′ deletions from the XbaI site of the A1 promoter (position −289, see figure 1 and 3) have been subcloned in pUC18; characterized recombinant DNA plasmids (x indicates the extent of the 5′ deletion) were cleaved by EcoRI, within the pUC18 polylinker, and by EcoRV (position +46 within the A1 promoter, see figure 1); the resulting EcoRI-EcoRV DNA fragments were 3′ end labeled and used as probes in gel retardation experiments. On the right panel is shown an over-exposure of the gel from an experiment driven using the −111 and −100 deletions. The indicated positions are relative to the transcription initiation site.

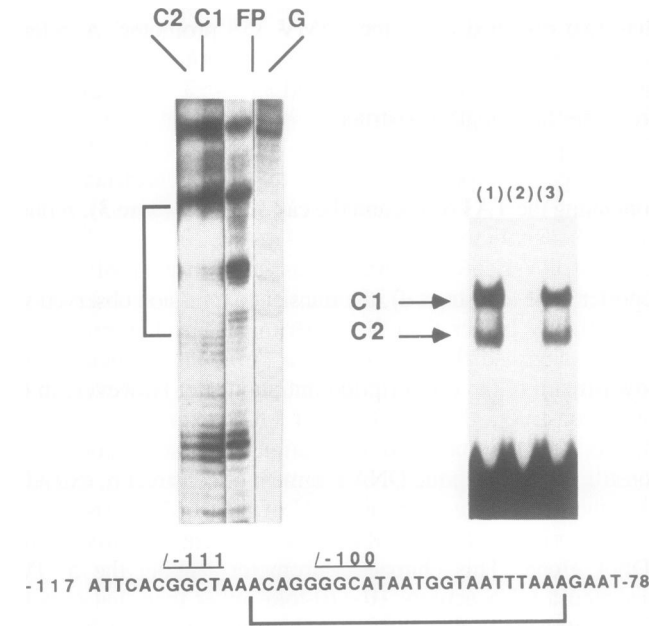


Figure 5. Characterization of the TEF 1 sequence involved in the formation of C1 and C2 complexes. On the left part of the figure is presented the results of an OP-Cu footprinting experiment. The protected region is shown by a square bracket. (C2): C2 complex; (C1): C1 complex; (FP): free probe; (G) Maxam and Gilbert G channel. The protected sequence is presented under the autoradiographs; the positions are indicated relative to the transcription initiation site. On the right part of the figure, is shown the results of a gel retardation experiment conducted using as probe the synthetic double-stranded oligonucleotide of the TEF 1 sequence 5'AATTCACGGCTAAACAGGGGCATAATGGTA-ATTTAAAGAATT3'. (1): gel retardation of the TEF 1 oligonucleotide; (2) as in (1) supplemented with 10 ng of unlabelled double stranded oligonucleotide; (3) as in (1) supplemented with 10 ng of unlabelled unrelated double stranded oligonucleotide corresponding to the sequence 5'TCGAATTCTATGTTAAA-CCCTAACATTGATATCGA3'.

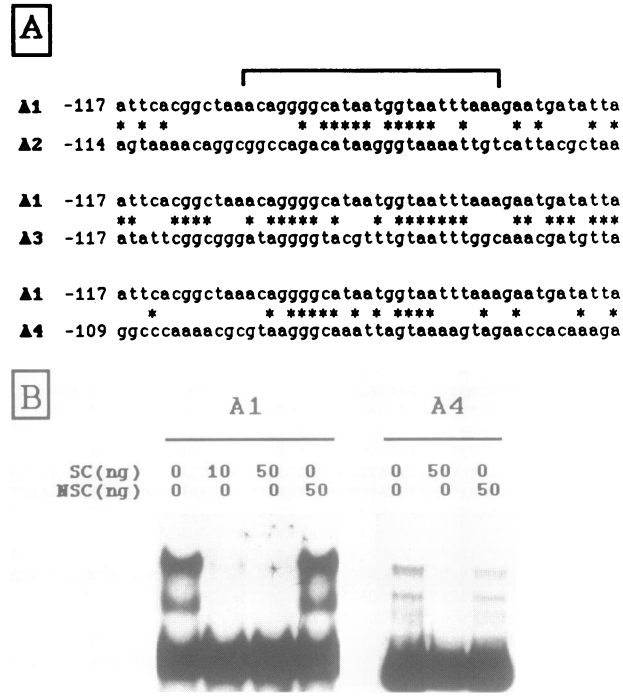


Figure 6. Conservation of the TEF 1 element among *Arabidopsis* EF-1α genes. (A) comparison of sequences surrounding the TEF 1 box of the A1 promoter with the corresponding regions within the promoters of the A2, A3 and A4 genes. The positions indicated are relative to the transcription initiation site for the A1, A2 and A4 genes and to the putative transcription initiation site for the A3 gene. Nucleotide identities are indicated by *asterisks* between the sequences. (B) Comparative gel retardation experiments using probes from the A1 and A4 promoter. The radioactive probes extend from positions −119 to +46 and −158 to −14 for the A1 and A4 genes respectively. SC and NSC: specific and non-specific competitors respectively, as described in the legend to figure 5.

AAACCCTAA, extending from positions −62 to −53 in the A1 promoter has been deleted from construct b to create construct f. The resulting deleted promoter mediated a GUS activity two-fold lower than that obtained using the construct b (figure 1). This moderate effect is reproducible and has been estimated from 16 independent transfection experiments.

Accurate mapping of a *cis*-acting element and characterization of related *trans*-acting factors

The experiments reported in the previous section have shown that a deletion down to position −289 (construct b) retained 35−45% of the expression from the full length promoter. In order to identify *cis*-elements involved in this expression, upstream the TATA box, a series of promoter deletions was generated from the XbaI site (−289) using Bal31 exonuclease. The data presented in figure 3 show the activity of each construct relative to the −289 construct (construct b in figure 1). Expression of the deleted constructs was not significantly affected down to position −111. Deletion of the next 11 bp (to position −100) produced a severe drop in GUS activity. All subsequent deletions gave the same low level of expression. These deletion experiments have therefore accurately mapped a *cis*-acting element about 100 bp upstream of the transcription initiation site that we have called the TEF 1 box (Transcription Elongation Factor 1 box).

We then undertook a search for specific protein-DNA interactions in the region of the TEF1 box. The gel retardation assay (21), modified for the search of eucaryotic regulatory proteins (22), was used for this approach. Previous experiments (data not shown) carried out with *Arabidopsis* nuclear extracts prepared from cellular suspensions or leaves, have shown that retarded complexes were formed using the ScaI-EcoRV fragment (−160 to +46, figure 1). No binding activity could be detected using the fragment EcoRV-ScaI (−303 to −160). To delimit more accurately the DNA elements responsible for the interaction of nuclear factors with the ScaI-EcoRV fragment, the Bal31 promoter deletions used in the transient expression experiments (figure 3), were tested for their ability to form retarded complexes. The results reported in figure 4 show that up to position −111, two major complexes (C1 and C2) were observed which then disappeared with subsequent deletions. Thus a perfect correlation was observed between the protein binding properties and the functional data obtained from transfected protoplasts. However, an overexposure of the gel (figure 4, right panel) shows that a complex with migration properties corresponding to C2 remained detectable using the −100 deletion, then disappeared with subsequent deletions (not shown). This point will be further discussed.

The precise location of the A1 promoter sequences recognized by the nuclear factors were determined by OP-Cu footprinting experiments of complexes C1 and C2 (19). Protection from OP-Cu cleavage was observed from positions −106 to −82 and was very similar for both complexes (figure 5). In order to confirm these data a double stranded synthetic oligonucleotide corresponding to the sequences extending from positions −117 to −78 was used in gel retardation assays. The results reported

in figure 5 show that this oligonucleotide is sufficient to form the C1 and C2 complexes.

Comparison of sequences surrounding the TEF 1 box with the corresponding regions within the promoter of A2, A3 and A4 genes have been undertaken. Figure 6A shows that at approximately the same location relative to the transcription initiation site, homology could be detected with the A2, A3 and A4 genes. A DNA fragment of the A4 promoter, extending from positions −158 to −14 upstream to the transcription initiation site of A4 gene and containing a putative TEF 1 box, is able to form complexes which are specifically competed by a synthetic oligonucleotide corresponding to the TEF 1 box (figure 6B). Experiments performed with a DNA fragment of the A2 promoter gave the same results (not shown). These data strongly suggest that the TEF 1 element is conserved among the *Arabidopsis* EF-1α genes.

DISCUSSION

The data reported here support the notion that the expression of the A1 EF-1α gene depends on several elements. Sequences located between −1620 and −289 bp relative to the transcription initiation site are required for a maximum activity. *Cis*-acting element(s) could be present within this region acting upon the expression of the designated transcription initiation site. However, at the moment, we cannot exclude the possibility that the effect mediated by these sequences could result from the presence of an alternative upstream transcription initiation site.

A *cis*-acting element, the TEF 1 box, has been accurately located between −106 and −82 bp from the transcription initiation site of the A1 gene. Homologous sequences occur in approximately the same location in the other three genes indicating that the functional role of this element may be conserved among the four EF-1α genes. Studies are however needed to confirm this point.

We have shown that nuclear factors interacted with the TEF 1 box. Figures 4, 5 and 6 show that two major protein-DNA complexes are invariably observed on retardation gels. These two complexes could be due to the interaction of two distinct nuclear proteins with TEF 1. Upsteam activating sequences are often composed of multiple adjacent or overlapping elements which can combine their effects in a synergistic or exclusive manner (for examples see 23−26). Alternatively, the C1 and C2 complexes could result from the interaction of TEF 1 with the DNA binding domain of the same protein, partially proteolysed during the preparation of nuclear extracts. While this possibility cannot be excluded, the presence of a unique complex in the experiments conducted using the −100 deletion (figure 4) is in favour of the interaction of TEF 1 with several distinct *trans*-acting elements.

The presence of a telo box AAACCCTA located at the same position within the four *Arabidopsis* EF-1α genes strongly suggests a role for these sequences. This element, homologous to the repeat motif of *Arabidopsis* telomeres (20), is reminiscent of the structure of the yeast RPG box (1) involved in the activation of several yeast ribosomal and housekeeping genes (2). The RPG box also shows structural similarities with the telomeric repeat of yeast (27). A two-fold decrease in GUS expression was observed when this element was deleted from the A1 promoter (figure 1). This moderate effect obtained using transient expression experiments, could not reveal a more fundamental role played by this element. It has recently been reported that an element from the chicken lysozyme gene, involved in the attachment of chromatin to the nuclear scaffold, mediates an elevated and position independent gene activity in stably transformed cells, while it has no significant effect in transient expression systems (28). In this context, it must be noted that more than 90% of TUF 1 (or RAP-1), the yeast protein interacting with the RPG box, is associated with the nuclear scaffold (29). Studies are in progress to test the effect of the deletion of the telo box in transgenic plants.

We have shown that the DNA sequences of the 5′ IVS were essential for good expression of GUS activity driven by the A1 promoter. Many reports have described the critical role played by introns in the expression of eucaryotic genes (see for examples 30−33). It is interesting to note that introns are very often found within the 5′ non coding region or near the 5′ end of genes encoding constituents of the translational apparatus. Thus, in yeast, it is remarkable in view of the paucity of intron containing genes, that most of the ribosomal genes are split by an intron near the 5′ end (34). In *Drosophila* the ribosomal protein S 31 gene and the two genes encoding EF-1α all contain an IVS within the 5′ non-coding region (8,35). At present we do not know whether the 5′ IVS of the A1 gene is involved in the stabilization of A1 mRNA, as suggested for the maize alcohol dehydrogenase gene (36) or whether *cis*-acting elements are present within the DNA sequences of this 5′ IVS as reported for the regulatory elements of the β3 tubulin gene in *Drosophila* (37). Studies are in progress to clarify this point.

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REFERENCES

1. Leer,R.J., Van Raamsdonk-Duin,M.C., Mager,W.H. and Planta,R.J. (1985) *Curr. Genet.* **9**, 273−277.
2. Huet,J., Cottrelle,P., Cool,M., Vignais,M.L., Thiele,D., Marck,C., Buhler,J.M., Sentenac,A.and Fromageot,P. (1985) *EMBO J.* **4**, 3539−3547.
3. Shepherd,J.C.W., Walldorf,U., Hug,P. and Gehring,W.J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7520−7521.
4. Cottrelle,P., Thiele,D., Price,V.L., Memet,S., Micouin,J-Y., Mark,C., Bulher,J.M., Sentenac,A. and Fromageot,P. (1985) *J. Biol. Chem.* 260, 3090−3096.
5. Linz,J.E., Katayama,C. and Sypherd,P.S. (1986) *Mol. Cell. Biol.* **6**, 593−600.
6. Lendstra,J.A., Van Vliet,A., Arnberg,A.C., Van Hemert,F.J. and Möller,W. (1986) *Eur. J. Biochem.* **155**, 475−483.
7. Brands,J.H.G., Maasen,J.A., Van Hemert,F.J., Amons,R. and Möller,W. (1986) *Eur. J. Biochem.* **155**, 167−171.
8. Rao,T.R. and Slobin,L.I. (1986) *Nucleic Acids Res.* **14**, 2409.
9. Hovemann,B, Richter,S., Waldorf,U. and Cziepluch,C. (1988) *Nucleic Acids Res.* **16**, 3175−3194.
10. Pokalsky,A.R., Hiatt,W.R., Ridge,N., Rasmussen,R., Houck,C.M. and Shewmaker,C.K. (1989) *Nucleic Acids Res.* **17**, 4661−4673.
11. Linz,J.E. and Sypherd,P.S. (1987) *Mol. Cell. Biol.* **7**, 1925−1932.
12. Benfey,P., Ren,L. and Chua,N.H. (1989) *EMBO J.* **8**, 2195−2202.
13. Liboz,T., Bardet,C., Le Van Thai,A., Axelos,M. and Lescure,B. (1989) *Plant Mol. Biol.* **14**, 107−110.
14. Axelos,M., Bardet,C., Liboz,T., Le Van Thai,A., Curie,C. and Lescure,B. (1989) *Mol. Gen. Genet.* **219**, 106−112.
15. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) Molecular Cloning : a laboratory manual. Cold Spring Harbor University Press, Cold Spring Harbor.
16. Murphy,G. and Kavanagh,T. (1988) *Nucleic Acids Res.* **16**, 5198.
17. Pröls,M., Töpfer,R., Schell,J. and Steinbiss,H.H. (1988) *Plant Cell Rep.* **7**, 221−224.

18. Jefferson,R.A., Kanavagh,T.A. and Bevan,M.W. (1987) *EMBO J.* **6**, 3901–3907.
19. Kuwara,M.D. and Sigman,D.S. (1987) *Biochemistry* **26**, 7234–7238.
20. Richards,E.J. and Ausubel,F.M. (1988) *Cell* **53**, 127–136.
21. Garner,M.M. and Revzin,A. (1981) *Nucleic Acids Res.* **9**, 3047–3060.
22. Arcangioli,B. and Lescure,B. (1985) *EMBO J.* **4**, 2627–2633.
23. Ardnt,K., Styles,C. and Fink,G. (1987) *Science* **237**, 874–880.
24. Pfeifer,K., Arcangioli,B. and Guarente,L. (1987) *Cell* **49**, 9–18.
25. Giniger,E. and Ptashne,M. (1987) *Nature* **330**, 670–672.
26. Monaci,P., Nicosia,A. and Cortese,R. (1988) *EMBO J.* **7**, 2075–2087.
27. Buchman,A.R., Kimmerly,W.J., Rine,J. and Kornberg,R.D. (1988) *Mol. Cell. Biol.* **8**, 210–225.
28. Stief,A., Winter,D.M., Strätling,W.H. and Sippel,A.E. (1989) *Nature* **341**, 343–345.
29. Hofmann,J.F.X., Laroche,T., Brand,A.H. and Gasser,S.M. (1989) *Cell* **57**, 725–737.
30. Gruss,P., Lai,C.J., Dhar,R. and Khoury,G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4317–4321.
31. Hamer,D.H. and Leder,P. (1979) *Cell* **18**, 1299–1302.
32. Gillies,S.D., Morrison,S.L., Oi,V.T. and Tonegawa,S. (1983) *Cell* **33**, 717–728.
33. Neuberger,M.S. and Williams,G.T. (1988) *Nucleic Acids Res.* **16**, 6713–6724.
34. Planta,R.J., Mager,W.H., Leer,R.J., Woudt,L.P., Rahoué, H.A. and El-Baradi,T.T.A.L. (1988) in Hardesty,B. and Kramer,G. (eds), Structure Function and Genetics of Ribosomes. Springer Verlag, N.Y., pp. 699–718.
35. Itoh,N., Ohta,K., Ohta,M., Kawasaki,T. and Yamashina,I. (1989) *Nucleic Acids Res.* **17**, 2121.
36. Callis,J., Fromm,M. and Walbot,V. (1987) *Genes and Development* **1**, 1183–1200.
37. Bruhat,A., Tourmente,S., Chapel,S., Sobrier,M.L., Couderc,J.L. and Dastugue,B. (1990) *Nucleic Acids Res.* **18**, 2861–2867.