

Definition of constitutive gene expression in plants: the translation initiation factor 4A gene as a model

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Abstract

The NeIF-4A10 gene belongs to a family of at least ten genes, all of which encode closely related isoforms of translation initiation factor 4A. The promoter region of NeIF-4A10 was sequenced, and four mRNA 5' ends were determined. Deletions containing 2750, 689 and 188 bp of untranscribed upstream DNA were fused to the GUS reporter gene and introduced into transgenic tobacco. The three constructs mediated GUS expression in all cells of the leaf, stem and shoot apical meristem. Control experiments using *in situ* hybridization and tissue printing indicated that the observed GUS expression matches the expression patterns of NeIF-4A mRNA and protein. This detailed analysis at the level of mRNA, protein and reporter gene expression shows that NeIF-4A10 is an ideal constitutively expressed control gene. We argue that inclusion of such a control gene in experiments dealing with specifically expressed genes is in many cases essential for the correct interpretation of observed expression patterns.

Introduction

In recent years the techniques of *in situ* hybridization and reporter gene analysis have provided a wealth of data on the organ, tissue and cell specificity of gene expression in plants [e.g. 2, 5, 10, 24, 30]. However, there are relatively few reports on the expression of constitutively expressed genes. Constitutive genes may be defined as genes that are equally expressed in all cells of an organism, and under most if not all environmental con-

ditions. From this definition it follows that a constitutive gene will not give an equally strong signal in all cells but, rather, that a lower apparent signal is to be expected in large vacuolated cells than in small densely cytoplasmic cells. This phenomenon is particularly evident in the phloem and the shoot apical meristem, which are both surrounded by much larger cells. In such cases, high signal density in the smaller cells may simply reflect the difference in cell size, and not at all be evidence for cell-specific expression of the gene under

The nucleotide sequence data reported will appear in the GenBank, EMBL and DDBJ Nucleotide Sequence Databases under the accession number X79008.

study. In a number of cases genes have been reported as being specifically expressed in the shoot apical meristem [15, 23, 32]. However, it would have been preferable if such data had been presented in conjunction with the expression pattern of a constitutively expressed gene.

The cauliflower mosaic virus (CaMV) 35S promoter has often been used as a constitutive promoter. However, when fused to the GUS reporter gene it gives rise to intense staining of the vascular bundles relative to the adjacent parenchyma cells [4, 13]. This certainly is caused, at least in part, by the differences in cell size, but it could also indicate a preferential expression of CaMV in the vascular bundles. Whether any plant housekeeping genes are really expressed in each and every cell of an organ is not known with certainty. There are relatively few publications on the spatial expression of housekeeping genes, and in these studies the tissues are analyzed by hand sections of variable thickness and resolution. Moreover, the available evidence tends to suggest that housekeeping genes are not expressed to identical levels in all cells. For example, in our work, we have studied gene expression in the shoot apical meristem by *in situ* hybridization [11]. We found that all genes expressed in the meristem gave a much higher signal in the meristem than in the subtending tissue. Control experiments showed that a probe for ribosomal RNA and a stain for general nucleic acid gave similar staining patterns, indicating that such patterns are indicative of constitutive expression. Among the genes studied were two housekeeping genes, one coding for ribosomal protein L2 (*rpl2*), the other one encoding ribosomal protein L38 (*rpl38*). The *rpl2* gene was uniformly expressed in all cells of the meristem, whereas *rpl38* was preferentially expressed in the peripheral zone. This is a clear example of differential expression of housekeeping genes.

Genes coding for components of the translational apparatus are likely to be expressed in all metabolically active cells, and may potentially serve as ideal constitutive genes. We previously performed a detailed characterization of the genes coding for translation initiation factor eIF-4A [5, 6, 21, 22]. eIF-4A is an RNA helicase which is

thought to unwind secondary structure in the 5'-untranslated leader, and thereby enable the 40S subunit of the ribosome to proceed from the cap to the initiator AUG [20]. In tobacco, eIF-4A is encoded by a large multigene family [21, 22]. Based on northern blot analysis, most genes are expressed in all tissues, but there are subtle differences in the quantitative expression levels of individual genes. One gene was found to be exclusively expressed in the male gametophyte [5].

Here we report on the cell specificity of eIF-4A expression using a combination of *in situ* hybridization, tissue printing, and high-resolution histochemical analysis of promoter-GUS fusions.

Materials and methods

Genomic cloning and constructions

A genomic *Bgl* II fragment containing sequences matching the cDNA NeIF-4A was previously isolated [6]. This fragment extended only 25 bp into the 5'-untranslated region, and did not contain any promoter sequences. Therefore, an overlapping *Asp*718 fragment was isolated with an additional 5 kb of upstream DNA. Numbering of the sequence is such that the longest transcript begins at +1 (see Fig. 1). Three fragments containing various amounts of 5'-untranscribed DNA were fused to a GUS-nos 3' end reporter gene. Naturally occurring *Cla* I (−2750), *Eco* RV (−989), and *Bgl* I (−188) sites formed the 5' ends of the constructs. At the 3' end, an *Nco* I site in the second exon was fused in frame to the *Nco* I site at the ATG of the GUS gene, which was derived from pMOGEN18, and is followed by a nos terminator [26]. The constructs, depicted in Fig. 2, were recloned into the broad-host-range vector pMON505, and introduced into *Nicotiana tabacum* cv. Samsun, as previously described [5]. A fragment (−410 to +15) from the pea *rbcS-3A* gene 16 was similarly fused to the GUS reporter gene. The 35S-GUS construct has been described before [5]. Second-generation plants were used for all analyses.

Determination of transcription start site

Primer extension analysis was carried out essentially according to Sambrook *et al.* [25]. An end-labelled primer (GGAAAAGATCTGAG-GAATAG, nt +143 to +124 in Fig.1) was hybridized to 10 µg leaf poly(A)⁺ RNA, and extended using AMV reverse transcriptase. The reverse transcripts were run on a 6% urea gel next to a sequencing ladder obtained with the same primer.

Nuclease S1 protection was described previously [10]. A uniformly ³²P-labelled probe (nt +143 to -188), prepared using the same primer as above, was hybridized to 50 µg total RNA in 80% formamide at 37 °C, digested with 60 units nuclease S1 (Boehringer, Mannheim). The protected fragments were run on a 6% urea gel, and sized as above.

In situ localization and reporter gene analysis

The fluorometric GUS assay was performed essentially as described [5, 13]. For *in situ* localization of GUS activity, thin hand sections were incubated in a buffer containing 0.5 mg/ml X-gluc in 100 mM sodium phosphate buffer pH 7.5, 10 mM EDTA, 0.1% (v/v) Triton X-100, 5 µM ferrocyanide, 5 µM ferricyanide at 37 °C for 16–72 h. Samples were then washed in sodium phosphate buffer before fixation for 1 h in 4% (w/v) formaldehyde, 0.25% (w/v) glutaraldehyde in 50 mM sodium phosphate buffer (pH 7.2). Samples were then dehydrated through ethanol before embedding in Technovit 7100 according to the manufacturer's instructions (Kulzer, Wehrheim, Germany). Sections (10 µm) were mounted on slides and counterstained with 0.05% safranin red. The indole precipitate was visualized either under bright field (GUS activity appears blue), or under dark field (GUS activity appears red to purple).

In situ hybridization was performed as previously described [10, 11]. The probes used contained the entire coding region of the NeIF-4A10 cDNA. For quantitation, silver grains were

counted on magnified prints. Background seen in the sense controls was subtracted. It must be pointed out that we do not know to what extent the response of the photographic emulsion to the radioactive signal is linear, and that consequently such quantitation can only give rough estimates of the relative expression levels.

Tissue printing was performed as follows. Leaf and stem sections (0.5–1 mm) were placed onto a piece of nitrocellulose and, for a few seconds, gently pressed onto the paper with a glass slide. Endogenous peroxidase was inactivated by incubation in 20mM NaN₃ for 15 min. The paper was blocked with TBS/5% skim milk for 30 min at room temperature, and subsequently incubated overnight with a 1000-fold dilution of an antiserum prepared against the C-terminal two thirds of a recombinant NeIF-4A1 protein [22]. After washing with TBS, TBS/0.05% Tween 20, TBS for 5 min each, the paper was incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase. Color was developed with 4-chloronaphthol in the presence of 0.01% H₂O₂.

Results

Characterization of the NeIF-4A10 genomic clone

A λ clone was isolated from a tobacco library which contained a sequence exactly matching the NeIF-4A10 cDNA [6]. Sequence analysis and comparison with the cDNA sequence indicated that this gene contains four introns, one of which is situated in the 5' UTR, two nucleotides upstream of the initiator ATG (Fig.1A). Based on the sequence of the cDNA, we infer that the 5' leader must be at least 100 nt long. A reliable determination of the transcription start site is complicated by the presence in tobacco of at least ten closely related eIF-4A genes [5, 6, 22]. Primer extension analysis was performed using a 20 nt primer complementary to a sequence in the 5' UTR. Six major and several minor bands were detected (Fig. 1B). Some or most of these bands may not represent NeIF-4A10 transcripts, but may be due to reverse transcription of other

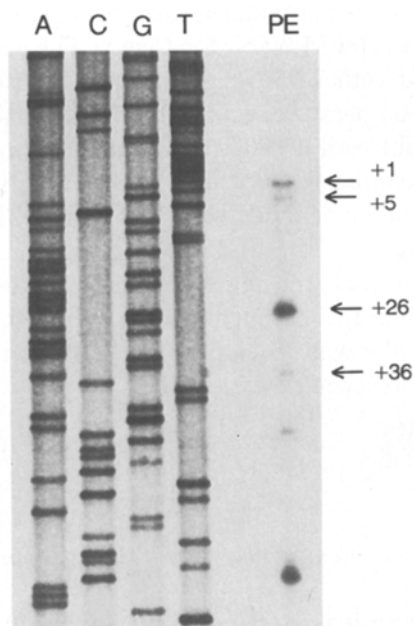


Fig. 1. Determination of the transcription start sites of the *NeIF-4A10* gene. A (top). Sequence of the upstream region from the *Bgl*II site to the initiator ATG. The four major start sites are indicated by arrows. The first base of the longest transcript is designated +1. B (bottom). Lanes A, C, G, T: sequencing ladder using a primer complementary to nt 143–124. PE: primer extension products obtained with the same primer. Sites matching those obtained by nuclease S1 protection analysis are indicated by arrows.

obtained, four of which matched primer extension products. These four sites, indicated in Fig. 1A, are likely to represent the 5' ends of four mRNAs transcribed from the NeIF-4A10 gene. Upstream of these four sites no sequences resembling TATA boxes are present.

Construction and activities of promoter-GUS fusions

Translational fusions were constructed between the GUS reporter gene and portions of the NeIF-4A10 upstream region (Fig. 2A). Transgenic tobacco plants were raised and GUS activity was

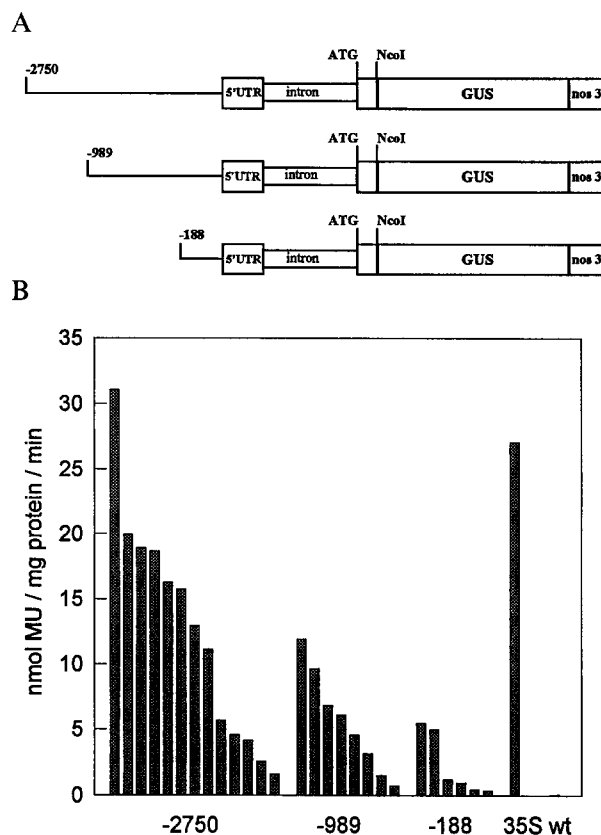


Fig. 2. Fluorometric determination of GUS activity in individual transgenic tobacco plants containing NeIF-4A10 promoter-GUS constructs. **A** (top). Schematic drawing of the constructs used. The drawing is not to scale. **B** (bottom). GUS activity in extracts of leaves of individual plants, containing either the three NeIF-4A10 promoter deletions fused to the GUS reporter gene (-2750, -989, -188), a 35S-GUS construct (35S), or a wild-type control (wt).

measured in leaves using the standard fluorometric assay (Fig. 2B). All three constructs were active, although the activity varied between individual transgenic plants. Such variation in activity has often before been observed, and is thought to reflect the influence of the position of integration of the transgene [14, 17]. All three constructs were active in all tissues tested (data not shown). Even the shortest 5' deletion, containing only 188 bp of non-transcribed sequence, displayed a similar pattern of expression as the -2750 construct in both fluorometric and histochemical (see below) GUS assays, although the absolute level of expression tended to be lower. These results show that sequences downstream of -188 are sufficient for correct expression of the NeIF-4A10 gene. The minimal sequence is AT-rich and devoid of potential Sp1-binding sites.

Histochemical analysis of NeIF-4A10-GUS expression

The promoter-GUS system allows for a high-resolution cytological analysis of gene expression. In particular, we wanted to address the following questions: (1) Is the NeIF-4A10-GUS construct expressed in all cells derived from the shoot apical meristem? (2) What are the relative expression levels of the GUS enzyme in different cells? (3) Does the activity of the GUS enzyme correlate with the expression levels of the NeIF-4A mRNA and protein? (4) Can NeIF-4A10-GUS serve as a model constitutive control gene for our studies on gene expression in the shoot apical meristem?

Transverse sections through leaves and stems of transgenic plants show that GUS activity is

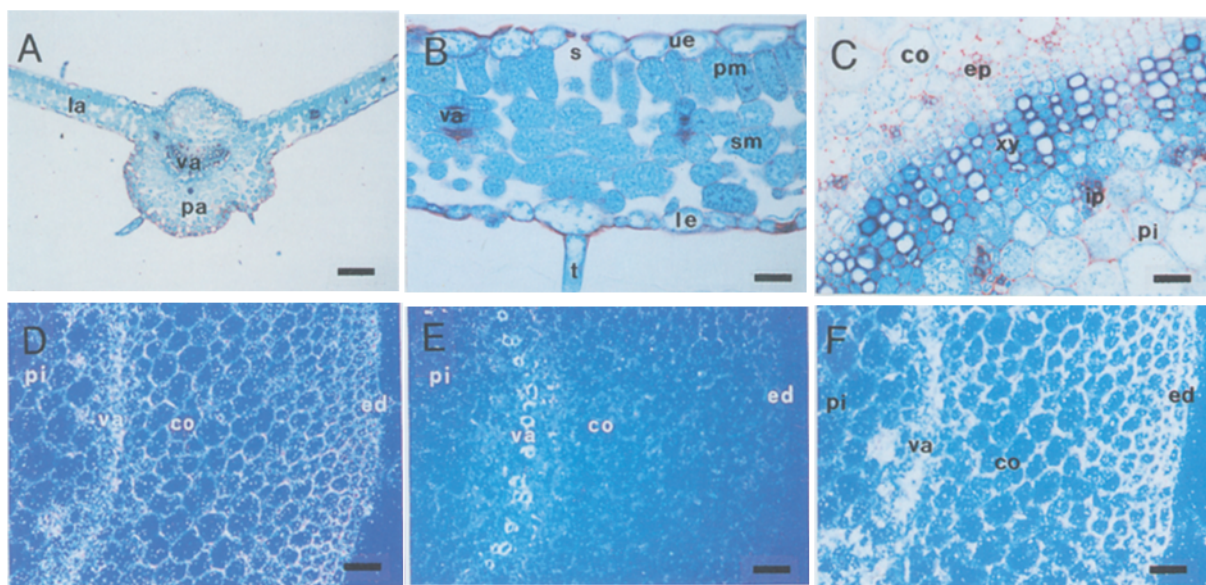


Fig. 3. NeIF-4A gene expression in tobacco leaves and stems. A. Cross section through a leaf of a transgenic tobacco plant expressing the -2750 NeIF-4A10-GUS construct, stained with X-Gluc. Scale bar is 250 μ m. B. Cross section through a transgenic leaf blade, stained as in A. Scale bar is 50 μ m. C. Cross section through a transgenic stem, stained as in A. Scale bar is 50 μ m. The GUS expressing cells appear blue under the bright-field illumination. D. *In situ* hybridization of a wild type stem section probed with the antisense strand of a NeIF-4A cDNA. E. NeIF-4A sense control. F. Stem section hybridized with a rRNA probe. The signal in D and F is visible as fine silver dots, not to be confused with the light reflection in the xylem, which is also seen in the sense control in E. Scale bar is 100 μ m. Abbreviations: co, cortex; ed, epidermis; ep, external phloem; ip, internal phloem; la, lamina; le, lower epidermis; pa, parenchyma; pi, pith; pm, palisade mesophyll; s, stoma; sm, spongy mesophyll; t, trichome; ue, upper epidermis; va, vasculature; x, xylem.

present in all cells: lower and upper epidermis, trichomes, stomatal cells, cortex, pith, xylem parenchyma, various cells of the internal and external phloem, spongy and palisade mesophyll cells of the leaf (Fig. 3A-C). In general, small cells appear more intensely blue than large cells. The large parenchyma cells in the stem often stain in

the periphery, reflecting the fact that the enzyme is targeted to the cytoplasm, and not to the large central vacuole. Most xylem vessels do not stain, as expected of dead cells. We do not know why occasional xylem elements display rather prominent blue coloration.

The GUS mRNA and GUS protein are quite

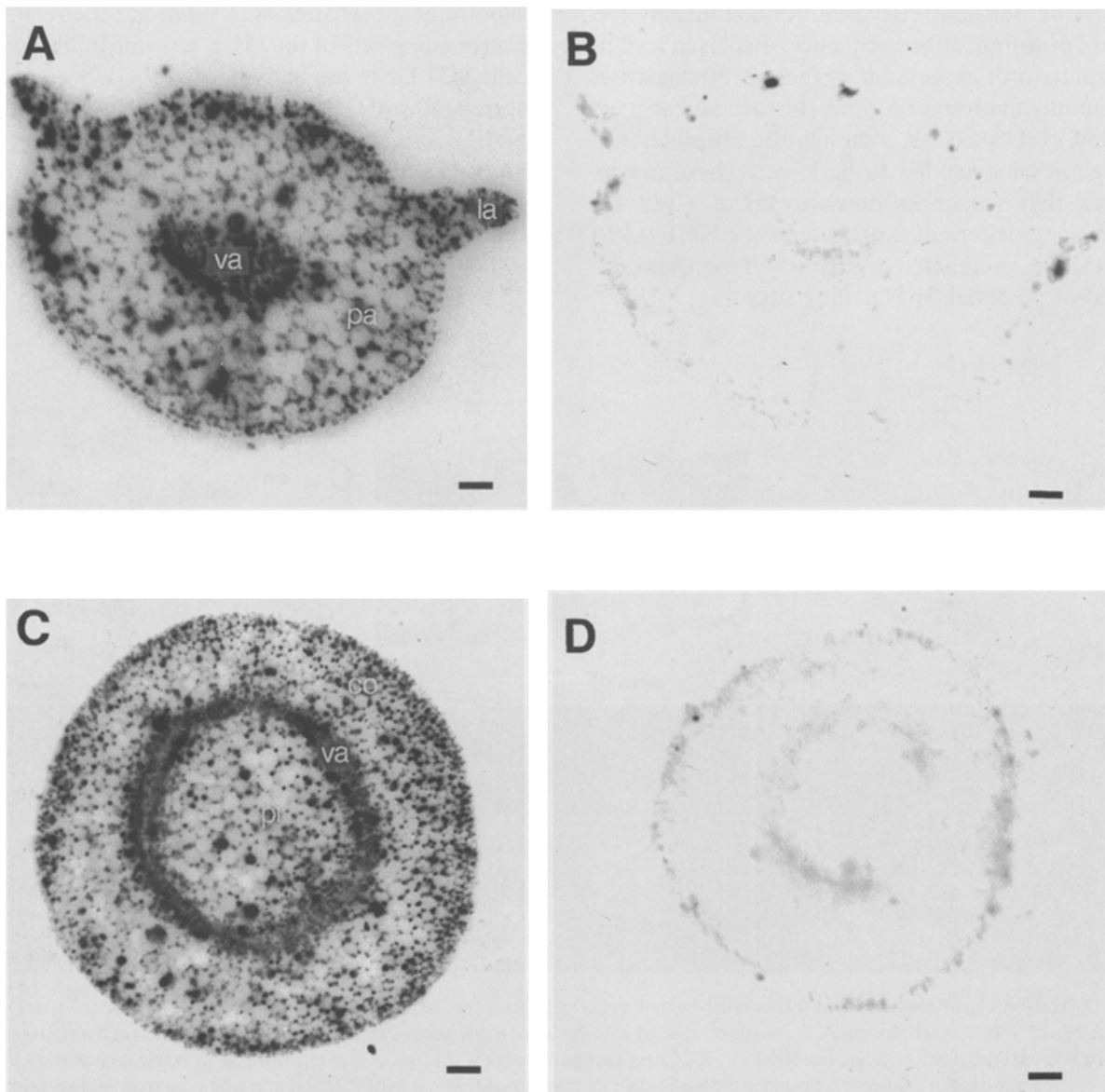


Fig. 4. Visualization of the NeIF-4A protein by tissue printing. A. Cross section through a leaf, incubated with anti-NeIF-4A antibody. B. Cross section through a leaf, incubated with preimmune serum. C. Cross section through a stem, incubated with anti-NeIF-4A antibody. D. Cross section through a stem, incubated with preimmune serum. Scale bars are 200 μ m. Abbreviations: co, cortex; la, lamina; pa, parenchyma; pi, pith; va, vasculature.

stable, and consequently the image obtained with the GUS-reporter gene system only reflects the expression of the gene under study if this gene also codes for stable mRNA and protein. Discrepancies between the expression pattern obtained with the GUS reporter gene system and the patterns of mRNA and protein have been observed before, and it is generally assumed that the GUS system is not immune to artefacts [e.g. 30, 31]. In order to determine whether the GUS staining pattern is a true reflection of NeIF-4A gene expression, we determined the distribution of eIF-4A transcript and protein directly. Transverse sections of wild-type tobacco stems were prepared for *in situ* hybridization and probed with the antisense and sense strands of an eIF-4A cDNA (Fig. 3D, E). As a positive control, a stem section was hybridized with a probe for rRNA (Fig. 3F). Although resolution is not as high as in the histochemical GUS assays, it can be seen that the silver grains are present over all cells with the smaller cells of the vascular tissue having stronger apparent signals than the larger cells of the cortex. However, when the silver grains of the NeIF-4A signal were counted, the outer cortex cells had 33 grains, the inner cortex cells had 60 grains and the small cells in the vascular region had 40 grains per cell. Thus, on a per cell basis the expression levels vary no more than twofold between different cell types. Tissue prints incubated with an antibody raised against recombinant NeIF-4A revealed staining of all cells in a pattern comparable to that seen in the *in situ* hybridization (Fig. 4). Therefore, the enzymatic activity conferred by the NeIF-4A10-GUS construct, reflects the levels of NeIF-4A mRNA and protein in stems and leaves.

Gene expression in the shoot apex

The vegetative shoot apical meristem consists of small, thin-walled cells with many cytoplasmic connections. In most reported experiments, GUS expression in the meristem was measured in hand sections, or even in whole uncut apices [4, 19]. In order to improve substrate penetration, but avoid

influences of potentially very active young leaves, shoot tips were dissected, and apices containing five or six primordia were incubated with the X-Gluc substrate, plastic-embedded, and the blue precipitate visualized in thin sections. It can be seen that the NeIF-4A10 promoter drives uniform expression in all cells of the shoot apical meristem (Fig. 5A). In a control experiment (Fig. 5B), the pea *rbcS-3A* promoter was found not to mediate GUS expression in the meristem, substantiating our previous data from *in situ* hybridization experiments [11]. Hybridization of apex sections with NeIF-4A probes (data not shown) gave a strong apparent signal in the small cells of the meristem and a less dense signal in the larger cells below, in agreement with the distribution of the GUS signal seen in Fig. 5A. The small

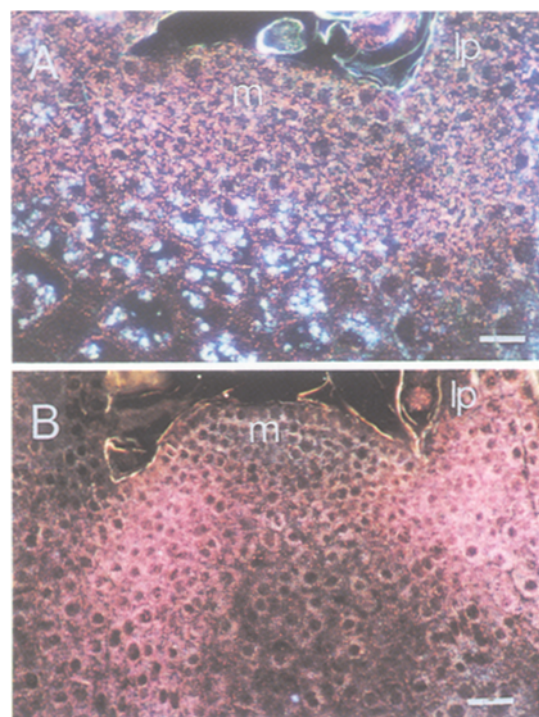


Fig. 5. Gene expression in the shoot apical meristem. A. Longitudinal section through the shoot apex of a transgenic tobacco plant expressing the -2750 NeIF-4A10-GUS construct. The GUS expressing cells appear purple under the dark field illumination. B. As A except that a transgenic tobacco plant expressing the pea *rbcS-3A*-GUS construct was employed. Scale bars are 20 μ m. Abbreviations: lp, leaf primordium; m, meristem.

meristem cells had on average 15 silver grains per cells, the larger cells below had 11 grains per cells. Thus, as in the stem sections (Fig. 3), the mRNA expression level on a per cell basis is fairly constant.

Discussion

The upstream region of the NeIF-4A10 gene is somewhat unusual for several reasons. First, it contains an intron in the 5' UTR. Second, no TATA-box sequence is present. Third, primer extension and nuclease S1 protection analysis define at least four mRNA 5' ends. Finally, the 5' UTR of NeIF-4A10 and other tobacco eIF-4A genes [22] is deficient in Gs, and rich in polypyrimidine stretches. Introns have occasionally been found in the 5' UTRs of other plant genes, among them genes coding for other components of the translational apparatus [1, 8, 9]. TATA-box-less genes with multiple start sites are not well characterized in plants, but have been described for a number of animal genes [18, 27]. It is not known, however, what the functional significance of this alternative mode of transcription is. Polypyrimidine stretches also have precedents in non-plant genes, where it is thought that they regulate the turning off of translation when cells enter the quiescent state [3, 12, 29]. Thus, the organization around the transcription start site may be related to the function of NeIF-4A and other genes coding for components of the translational machinery.

One practical goal of this study was to generate a promoter-GUS fusion which could be used as a constitutive control in our experiments on cell and tissue-specific gene expression. We chose NeIF-4A because previous experiments had shown that the NeIF-4A genes are expressed in all tissues [21, 22], and that expression is not influenced by any environmental factors so far tested [7, 28, our unpublished data]. There is a large body of high-quality data on the spatial expression of genes expressed in specific cells or tissues, but histological analysis of constitutive gene expression is far less advanced, with virtu-

ally no studies providing single-cell resolution. We feel that it is often essential to relate observed specific expression patterns to the pattern obtained with a constitutive control gene. In many cases authors might have reached very different conclusions had they included such a control. The data presented here show that the NeIF-4A10-GUS construct is active in all cells tested, and that the signal generally appears stronger in small cells than in large cells. When silver grains were counted and mRNA levels were calculated on a per cell basis, the expression levels appeared fairly constant. However, it is difficult to quantitate *in situ* signals, whether mRNA, protein or GUS. In the absence of precise quantitation methods, we cannot argue that NeIF-4A is expressed to identical levels in all cells.

The localization of the GUS protein in leaf, stem and meristem sections is in good agreement with the data on the expression of the NeIF-4A mRNA and protein, as determined by *in situ* hybridization and tissue printing. This shows that stabilities of the eIF-4A mRNA and protein are not radically different from the stability of the GUS mRNA and protein. Furthermore, it indicates that the NeIF-4A10 gene is expressed in the same manner as the sum of the NeIF-4A gene family members. The small cells of the vascular tissue stain darker than the surrounding large cells. However, the differences in staining intensity are not as striking as generally seen with 35S-GUS fusions [4, 13]. Perhaps the 35S mRNA is preferentially expressed in the vasculature. It would obviously be advantageous for the CaMV virus to synthesize its genomic RNA at high levels in the conducting system of the plant.

NeIF-4A appears to be uniformly expressed in the meristem, as judged both by the GUS staining and by the *in situ* hybridization experiments. In contrast, the same techniques show that *rbcS* is not expressed in the meristem [11, Fig. 5]. This shows that the GUS technique can be used for studies on differential meristematic gene expression. Taken together, the even distribution of the mRNA, protein and GUS reporter gene signal in all cells of the aerial organs makes the NeIF4A10 gene an ideal constitutive control gene, useful for

many studies on cell-specific gene expression. Such a control becomes especially important when dealing with vascular- and meristem-specific gene expression. Finally, we intend to define regulatory sequences required for expression in the meristem by adding fragments of the NeIF-4A10 upstream region to the very well defined pea *rbcS-3A* promoter. The fact that 188 bp of untranscribed sequence are sufficient for correct expression provides us with an attractive starting point for such experiments.

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References

1. Aguilar F, Montandon P-E, Stutz E: Two genes encoding the soybean translation elongation factor eEF-1 α are transcribed in seedling leaves. *Plant Mol Biol* 17: 351–360 (1991).
2. Angenent GC, Franken J, Busscher M, Colombo L, van Tunen AJ: Petal and stamen formation in petunia is regulated by the homeotic gene *fpl*. *Plant J* 4: 101–112 (1993).
3. Avni D, Shama S, Loreni F, Meyuhas O: Vertebrate mRNAs with a 5' terminal pyrimidine tract are candidates for translational repression in quiescent cells: characterization of the translational *cis*-regulatory element. *Mol Cell Biol* 14: 3822–3833 (1994).
4. Benfey PN, Ren L, Chua N-H: Combinatorial and synergistic properties of CaMV 35S enhancer domains. *EMBO J* 9: 1677–1696 (1990).
5. Brander KA, Kuhlemeier C: A pollen-specific DEAD-box protein related to translation initiation factor eIF-4A. *Plant Mol Biol* 27: 637–649 (1995).
6. Brander KA, Mandel T, Owttrim GW, Kuhlemeier C: Highly conserved genes coding for eukaryotic translation initiation factor eIF-4A of tobacco have specific alterations in functional motifs. *Biochim Biophys Acta* 1261: 442–444 (1995).
7. Bucher M, Kuhlemeier C: Long term anoxia tolerance: multi-level regulation of gene expression in the amphibious plant *Acorus calamus* L. *Plant Physiol* 103: 441–448 (1993).
8. Curie C, Liboz T, Bardet C, Gander E, Medal C, Axelos M, Lescure B: *Cis* and *trans*-acting elements involved in the activation of *Arabidopsis thaliana* A1 gene encoding the translation elongation factor EF-1. *Nucl Acids Res* 19: 1305–1310 (1991).
9. Curie C, Axelos M, Bardet C, Atanassova R, Chaubet N, Lescure B: Modular organization and developmental activity of an *Arabidopsis thaliana* EF-1 gene promoter. *Mol Gen. Genet* 238: 428–436 (1993).
10. Fleming AJ, Mandel T, Hofmann S, Sterk P, de Vries SC, Kuhlemeier C: Expression pattern of a tobacco lipid transfer protein gene within the shoot apex. *Plant J* 2: 855–862 (1992).
11. Fleming AJ, Mandel T, Roth I, Kuhlemeier C: The patterns of gene expression in the tomato shoot apical meristem. *Plant Cell* 5: 297–309 (1993).
12. Jefferies HBJ, Reinhard C, Kozma SC, Thomas G: Rapamycin selectively represses translation of the 'polypyrimidine tract' mRNA family. *Proc Natl Acad Sci USA* 91: 4441–4445 (1994).
13. Jefferson RA, Kavanagh T, Bevan MW: GUS fusions: β -glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J* 6: 3901–3907 (1987).
14. Jorgensen RA: Cosuppression, flower color patterns, and metastable gene expression states. *Science* 268: 686–691 (1995).
15. Kohler S, Coraggio I, Becker D, Salamini F: Pattern of expression of meristem-specific cDNA clones of barley (*Hordeum vulgare* L). *Planta* 186: 227–235 (1992).
16. Kuhlemeier C, Fluhr R, Green PJ, Chua NH: Sequences in the pea *rbcS-3A* gene have homology to constitutive mammalian enhancers but function as negative regulatory elements. *Genes Devel* 1:247–255 (1987).
17. Kuhlemeier C, Green PJ, Chua N-H: Regulation of gene expression in plants. *Annu Rev Plant Physiol* 38: 221–257 (1987).
18. Lu J, Lee W Jiang C, Keller EB: Start site selection by Sp1 in the TATA-less human Ha-ras promoter. *J Biol Chem* 269: 5391–5402 (1994).
19. Medford JI, Elmer JS, Klee HJ: Molecular cloning and characterization of genes expressed in shoot apical meristems. *Plant Cell* 3: 359–370 (1991).
20. Merrick WC: Mechanisms and regulation of eukaryotic protein synthesis. *Microbiol Rev* 56: 291–315 (1992).
21. Owttrim GW, Hofmann S, Kuhlemeier C: Divergent genes for translation initiation factor eIF-4A are coordinately expressed in tobacco. *Nucl Acids Res* 19: 5491–5496 (1991).
22. Owttrim GW, Mandel T, Trachsel H, Thomas, AAM, Kuhlemeier, C: Characterizaion of the tobacco eIF-4A gene family. *Plant Mol Biol* 26: 1747–1757 (1994).
23. Pokalski AR, Hiatt WR, Ridge N, Rasmussen R, Houck CM, Shewmaker CK: Structure and expression of elongation factor 1 α in tomato. *Nucl Acids Res* 17: 4661–4673 (1989).
24. Ryser U, Keller B: Ultrastructural localization of a bean

- glycine-rich protein in unlignified primary walls of protoxylem cells. *Plant Cell* 4: 773–783 (1992).
25. Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).
 26. Sijmons PC, Dekker BMM, Schrammeijer B, Verwoerd TC, Van den Elzen PJM, Hockema A: Production of correctly processed human serum albumin in transgenic plants. *Bio/technology* 8: 217–221 (1990).
 27. Smale ST, Baltimore D: The 'initiator' as a transcription control element. *Cell* 57: 103–113 (1989).
 28. Taylor CB, Bariola PA, DelCardayre SB, Raines RT, Green PJ: RNS2: a senescence-associated RNase of *Arabidopsis* that diverged from the S-RNases before speciation. *Proc Natl Acad Sci USA* 90: 5118–5122 (1993).
 29. Terada N, Patel HR, Takase K, Kohno K, Nairn A, Gelfand EW: Rapamycin selectively inhibits translation of mRNAs encoding elongation factors and ribosomal proteins. *Proc Natl Acad Sci USA* 91: 11477–11481 (1994).
 30. Thoma S, Hecht U, Kippers A, Botella J, de Vries S, Somerville C: Tissue-specific expression of a gene encoding a cell wall-localized lipid transfer protein. *Plant Physiol* 105: 35–45 (1994).
 31. Uknes S, Dincher S, Friedrich L, Negrotto D, Williams S, Thompson-Taylor H, Potter S, Ward E, Ryals J: Regulation of pathogenesis-related protein 1a expression in tobacco. *Plant Cell* 5: 159–169 (1993).
 32. Van Lijsebettens M, Vanderhaeghen R, De Block M, Bauw G, Villaroel R, Van Montagu M: An S18 ribosomal protein gene copy at the *Arabidopsis* *PFL* locus affects plant development by its specific expression in meristems. *EMBO J* 13: 3378–3388 (1994).