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Developmental Analysis of Elongation Factor-1α Expression in Transgenic Tobacco

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The developmental regulation of the translational elongation factor EF-1α has been analyzed in tobacco. A gene fusion was constructed consisting of the 5′ and 3′ regions of the tomato genomic clone LeEF-A from the EF-1α gene family and the β-glucuronidase coding region. Analysis of the transgenic plants containing this chimeric gene demonstrated that the tomato LeEF-A flanking sequences were sufficient to confer expression patterns similar to those of the endogenous tobacco EF-1α gene. The patterns of β-glucuronidase activity in this system indicated that during plant growth and development EF-1α is regulated with increased expression corresponding to regions of high protein synthesis, including meristems, rapidly growing tissues, and developing gametophytes. In addition, EF-1α expression responds rapidly to changes in growth patterns induced by hormone treatment. Our results are in agreement with studies in animals indicating that EF-1α expression may be rate limiting for protein synthesis and demonstrate that the analysis of EF-1α is of value for studying interrelationships between protein synthesis and developmental control.

INTRODUCTION

Eukaryotic polypeptide elongation factor EF-1α is an essential enzyme for protein synthesis, promoting the GTP-dependent binding of aminoacyl tRNA to ribosomes during the elongation phase of translation and participating in the proofreading of codon-anticodon accuracy (Song et al., 1989). EF-1α is one of the few proteins directly required for the synthesis of all cellular proteins, and the presence of at least one active gene encoding EF-1α is necessary for cell viability (Cottrelle et al., 1985).

Evidence from a number of eukaryotes indicates that the activity and expression of EF-1α are regulated developmentally and that this regulation occurs at several levels. For example, in some fungi and *Drosophila*, transcriptional regulation of EF-1α has been reported (Linz and Sypherd, 1987; Hovemann et al., 1988, respectively). In addition, post-transcriptional and post-translational modifications have been reported to regulate EF-1α activity in fungi (Hiatt et al., 1982; Fonzi et al., 1985) and mammals (Tuhackova et al., 1985; Merrick et al., 1990). There is also compelling evidence to suggest that EF-1α activity affects cellular protein synthesis rates and possibly development. In fungal sporangiospores, in which protein synthesis rates are markedly higher than in hyphae, levels of mRNA corresponding to one EF-1α gene family member increase twofold (Linz and Sypherd, 1987). In plants, steady-state levels of EF-1α mRNA are higher in the meristematic regions of the plant, which are characterized by high rates of protein synthesis, than in older, less metabolically active tissues (Pokalsky et al., 1989). The most convincing evidence, however, for the role of EF-1α in regulating development comes from studies on the aging process, where decreasing EF-1α activity has been shown to be an important cause in the characteristic decline in protein synthesis in cultured mammalian cell lines and in some invertebrate and vertebrate species (Webster, 1985; Cavallius et al., 1986; Shepard et al., 1989). In cultured human fibroblasts, EF-1α levels remain constant during the first 80% to 85% of the culture life span, but beyond this point, EF-1α levels and activity decline dramatically. EF-1α activity also decreases after low-serum-associated arrest in G_1 of the cell cycle. In contrast, immortalized (transformed) cell lines exhibit no decrease in EF-1α levels during culture or drop in activity after arrest in G_1 (Cavallius et al., 1986). Similarly, in *Drosophila*, it was demonstrated that levels of translatable EF-1α mRNA decline in aging flies and that overexpression of EF-1α slowed the aging process (Shepard et al., 1989).

We investigated the expression of EF-1α in plants in relationship to rates of protein synthesis and stages of plant development. By integration of a tomato EF-1α promoter-β-glucuronidase (GUS) fusion into the tobacco genome by way of *Agrobacterium*, we were able to investigate the regulation of EF-1α expression during vegetative and reproductive phases of plant growth and after growth-modifying treatments. Our results demonstrated that EF-1α is regulated at the level of either transcription or

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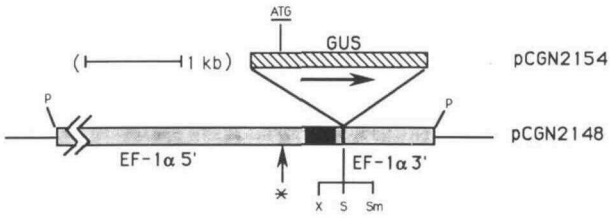

Figure 1. Structure of EF-1α Expression Cassette pCGN2148 and Transcriptional Fusion Plasmid pCGN2154.

Schematic representations of expression cassette pCGN2148, showing the 5′ and 3′ flanking regions from genomic clone LeEF-A (shaded areas), the intron (black area), transcription initiation signal (*), and the multiple cloning site. P, PstI; X, XhoI; S, SstI; Sm, SmaI. The GUS coding region, including an initiation methionine, was cloned into the multiple cloning site to form the transcriptional fusion plasmid pCGN2154.

mRNA stability during plant development and that this regulation is correlated with specific changes in patterns of growth and development.

RESULTS

Vector Construction and Tobacco Transformation

An EF-1α-GUS chimeric gene, pCGN2154, was constructed by introducing the GUS reporter gene (Jefferson et al., 1987) into the EF-1α expression cassette pCGN2148, composed of sequences from the genomic clone LeEF-A, the most highly expressed tomato EF-1α gene family member (Shewmaker et al., 1990). In the resulting transcriptional fusion, pCGN2154, the GUS coding sequence is flanked by 6.5 kb of 5′ DNA, which includes the EF-1α promoter region and 5′ untranslated leader containing a 750-bp intron, and by a 1.0-kb 3′ region, containing 0.4 kb of coding sequence, the transcriptional stop signal, and polyadenylation sites (Shewmaker et al., 1990). Maps of pCGN2148 and pCGN2154 are presented in Figure 1.

Transgenic tobacco plants were generated by using pCGN2158 (the EF-1α-GUS chimera in the binary vector pCGN1558 [McBride and Summerfelt, 1990]) in an *Agrobacterium*-mediated transformation system. Individual transformed plants, representing 22 independent transformation events, were analyzed for GUS activity. A 10-fold range in GUS specific activity was observed, varying from approximately 250 pmol to 2500 pmol of 4-methylumbelliferone/min/mg of protein. Five individual EF-1α-GUS transformed lines were selected based on moderate to high GUS expression levels for the analyses presented herein.

Expression of EF-1α during Vegetative and Reproductive Growth

Cell expansion, maturation, and senescence typify the development of most vegetative organs after their differentiation. Endogenous EF-1α and EF-1α-GUS transcript levels and GUS activity were analyzed during these stages in a series of leaves from transgenic tobacco plants to determine the validity of using GUS activity as a measure of EF-1α expression.

mRNA levels for the EF-1α-GUS chimeric gene and the endogenous gene were analyzed in three leaf stages representing rapidly growing, mature, and senescing tissues. These results are shown in Figure 2. For both the chimeric gene (Figure 2A) and the endogenous EF-1α gene (Figure 2B), steady-state levels of mRNA were highest in the youngest leaves analyzed, accounting for approximately 0.025% and 0.5% of the poly(A)$^+$ RNA in these leaves, respectively. These levels decreased progressively with leaf age. The steady-state level of EF-1α-GUS mRNA

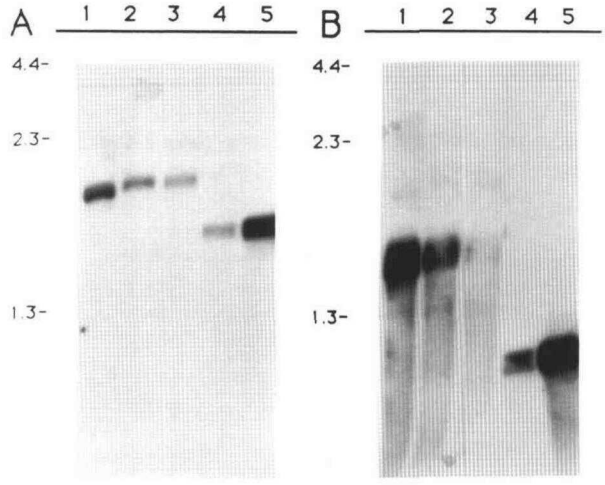

Figure 2. Comparison between EF-1α-GUS and Endogenous EF-1α Transcript Levels during Leaf Development.

(A) EF-1α-GUS (line 2158-9) transcript levels. Lanes 1 to 3 contain 2 μg of poly(A)$^+$ RNA from: lane 1, 1-cm to 4-cm leaves from the uppermost nodes of plants at the 20-leaf stage; lane 2, 12-cm to 14-cm mature leaves taken from the eighth node; lane 3, 12-cm to 14-cm senescing leaves taken from the eighteenth to twentieth nodes of the plant; lanes 4 and 5, 0.01 ng and 0.1 ng, respectively, of in vitro synthesized GUS RNA from plasmid pCGN7004. The blot was hybridized with a GUS cDNA probe from an isolated insert of pCGN7004.

(B) Endogenous EF-1α transcript levels. Lanes 1 to 3, same gel as in **(A)**; lanes 4 and 5, 0.1 ng and 1.0 ng, respectively, of in vitro synthesized EF-1α RNA from plasmid pCGN680. The blot was hybridized with a tobacco EF-1α cDNA probe from an isolated insert of pCGN680.

decreased approximately fivefold from youngest to oldest leaf sample (Figure 2A), whereas that of the endogenous EF-1α mRNA decreased 10-fold to 20-fold in the same samples (Figure 2B).

GUS activity was analyzed in leaves spanning the developmental spectrum, including the leaf stages comparable to those from which EF-1α and EF-1α-GUS mRNA levels were determined (samples in Figure 2, lanes 1, 2, and 3 are equivalent to leaf samples 1, 9, and 17, respectively, in Figure 3). Uniform discs were collected from leaves at every other node down the axis of the plant, beginning with a 4-cm leaf closest to the plant apex, and the GUS activity per leaf disc was measured; the results are shown in Figure 3. GUS activity was detected in all of the leaves sampled but was significantly higher in the youngest leaves and decreased steadily in leaves of increasing age (Figure 3). The approximate fivefold decrease in GUS activity from the youngest to the oldest leaves assayed correlates with the change in mRNA levels seen in comparable leaves. These results suggest that GUS activity provides an accurate indication of EF-1α-GUS mRNA levels, and this, in turn, is a valid measure of endogenous EF-1α expression.

Histochemical localization of GUS expression was analyzed qualitatively during various stages of vegetative and reproductive development to observe the cell type-specific and tissue-specific expression patterns of EF-1α. The results are presented in Figure 4. In intact, 6-day-old seedlings, GUS activity was highest in the apical and root tip meristems, and after longer incubation with substrate, staining was also prevalent along most of the root axis and in the petioles. Activity was conspicuously absent from the seedling hypocotyl (Figure 4A). The shoots and roots from 6-week-old plants were also analyzed for GUS activity. In thin longitudinal sections of shoot tips, GUS activity exhibited a high degree of cell specificity, with staining most prevalent in the apical and lateral meristematic regions (Figure 4B), as well as in the provascular tissues and leaf primordia (Figure 4B). In the intact roots from these plants, GUS activity was also most prevalent in the root tip and lateral meristems (Figure 4C).

EF-1α-GUS expression was analyzed in gametophytes from three stages in flower development: flower buds less than 5 mm in length, flower buds approximately 2 cm in length, and flowers at anthesis. Flowers were hand-sectioned either longitudinally through the ovary, or in cross-section through the anthers, and stained histochemically for GUS activity. At the earliest bud stage analyzed, pronounced GUS activity was apparent throughout most of the ovary and pistil, but staining was most intense within the ovary locule containing the developing ovules (Figure 4D). In cross-sections of 2-cm buds, GUS activity appeared similarly localized to the ovary, primarily to developing ovules (Figure 4E). At anthesis, GUS activity was detected almost exclusively in the ovules within the ovary (Figure 4F). When dissected away from the ovary wall, the intense staining of the mature female gametes was evident (Figure 4G).

In cross-sections of the youngest flowers, GUS activity was detected in all cell layers of the anther. However, highest activity levels were detected within the anther locule containing the pollen mother cells and the tapetum, and in the vascular tissues (Figure 4H). In anthers from the 2-cm buds, GUS activity appeared primarily localized to the anther locule, most notably to the tapetal cell layer surrounding the developing microspores and to the vascular tissue between the anther lobes (Figure 4I). At anthesis, GUS activity was, again, most notable in the anther locule, but was also present in the anther endothecial cell layers between the locules and the vascular tissues (Figure 4J). When isolated pollen grains were analyzed for GUS activity, strong staining was observed, although individual grains appeared to segregate for intensity of staining (Figure 4K).

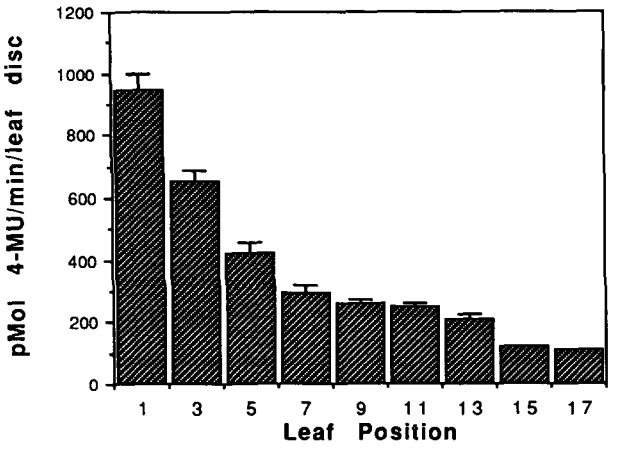

Figure 3. GUS Expression Levels in Developing Leaves of EF-1α-GUS Transgenic Tobacco, Line 2158-9.

Leaf discs from growth chamber-grown transgenic plants at the 20-leaf to 22-leaf stage were analyzed for GUS activity, determined as picomoles of 4-methylumbelliferone leaf disc^{-1} min^{-1}. Each data point represents the average of four replicate samples per leaf assayed. Error bars represent the SE.

Hormonal Influence on EF-1α-GUS Activity during Seedling Growth

To determine whether the patterns of GUS expression would be altered qualitatively after a distinct change in plant growth patterns, seedlings were treated with the growth-modifying synthetic plant hormone 2,4-D and stained for GUS activity. Results are shown in Figure 5. When 4-day-old seedlings were transferred onto nutrient

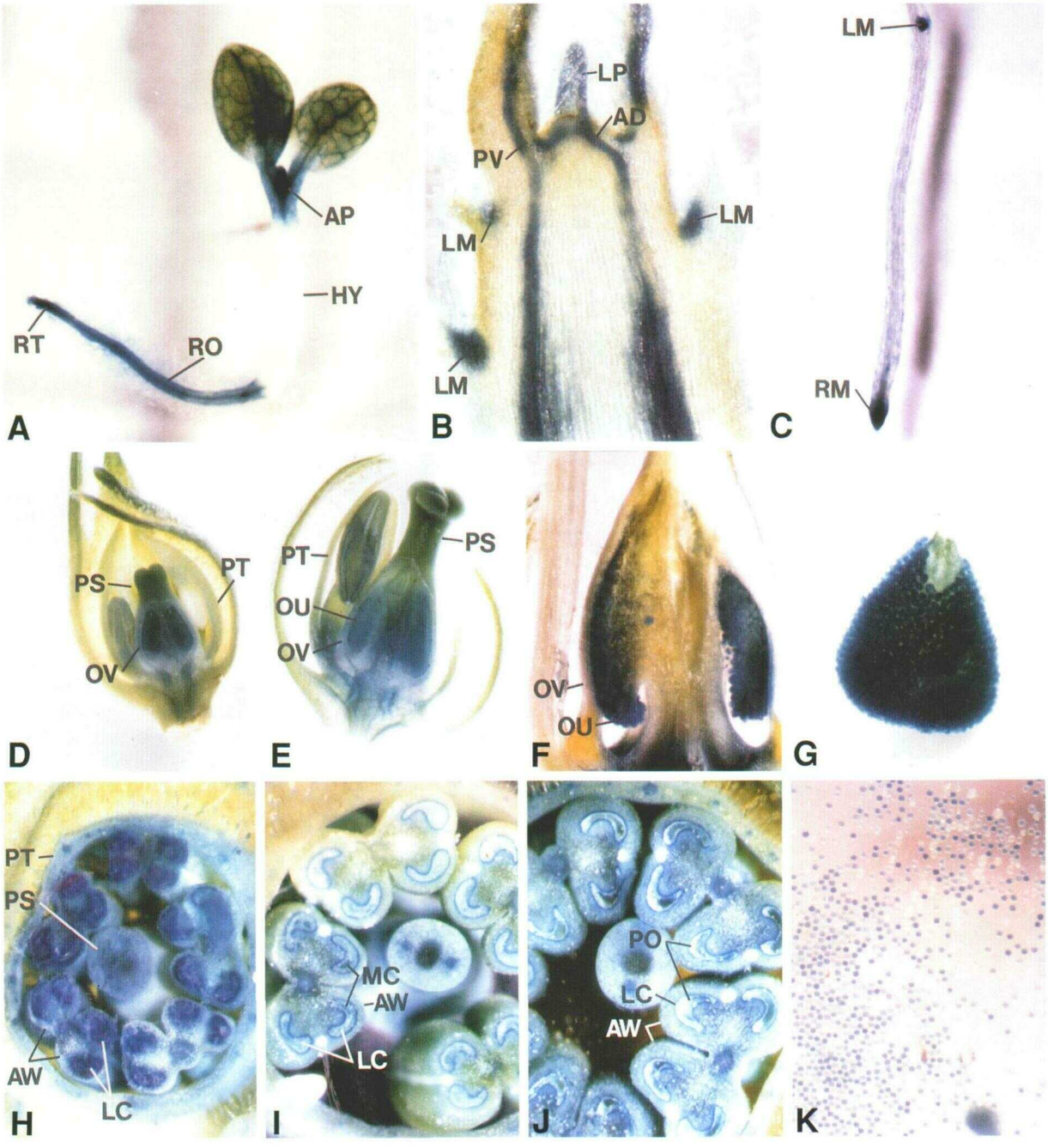

Figure 4. Histochemical Localization of GUS Activity in Vegetative and Reproductive Tissues of EF-1α-GUS Transgenic Tobacco, Line 2158-9.

GUS activity in intact and hand-sectioned tissues was visualized by overnight incubation in the indigogenic substrate X-gluc.

(A) A 6-day-old seedling from self-pollinated T_1 transgenic. AP, apical meristem; HY, hypocotyl; RO, root; RT, root tip.

(B) An approximately 100-μm-thick longitudinal section of a shoot tip from a 6-week-old tobacco plant. AD, apical dome; PV, provascular tissue; LP, leaf primordium; LM, lateral meristems.

(C) Intact root from a 6-week-old tobacco plant. RM, root meristem; LM, lateral meristem.

(D) Longitudinal section through a 3-mm flower bud. OV, ovary; PS, pistil; PT, petal.

(E) Longitudinal section through a 2-cm flower bud. OV, ovary; OU, ovules; PS, pistil; PT, petal.

(F) Longitudinal section through an anthesis-stage flower. OV, ovary; OU, ovules.

(G) Ovules isolated away from the ovary wall in an anthesis-stage flower.

(H) Cross-section through the ring of anthers in a 3-mm flower bud. AW, anther wall; LC, locule; PS, pistil; PT, petal.

(I) Cross-section through an anther from a 2-cm flower. AW, anther wall; LC, locule; MC, microspores.

(J) Cross-section through anthers from an anthesis-stage flower, just before anther dehiscence. AW, anther wall; LC, locule; PO, pollen.

(K) Mature pollen from a dehiscent anther.

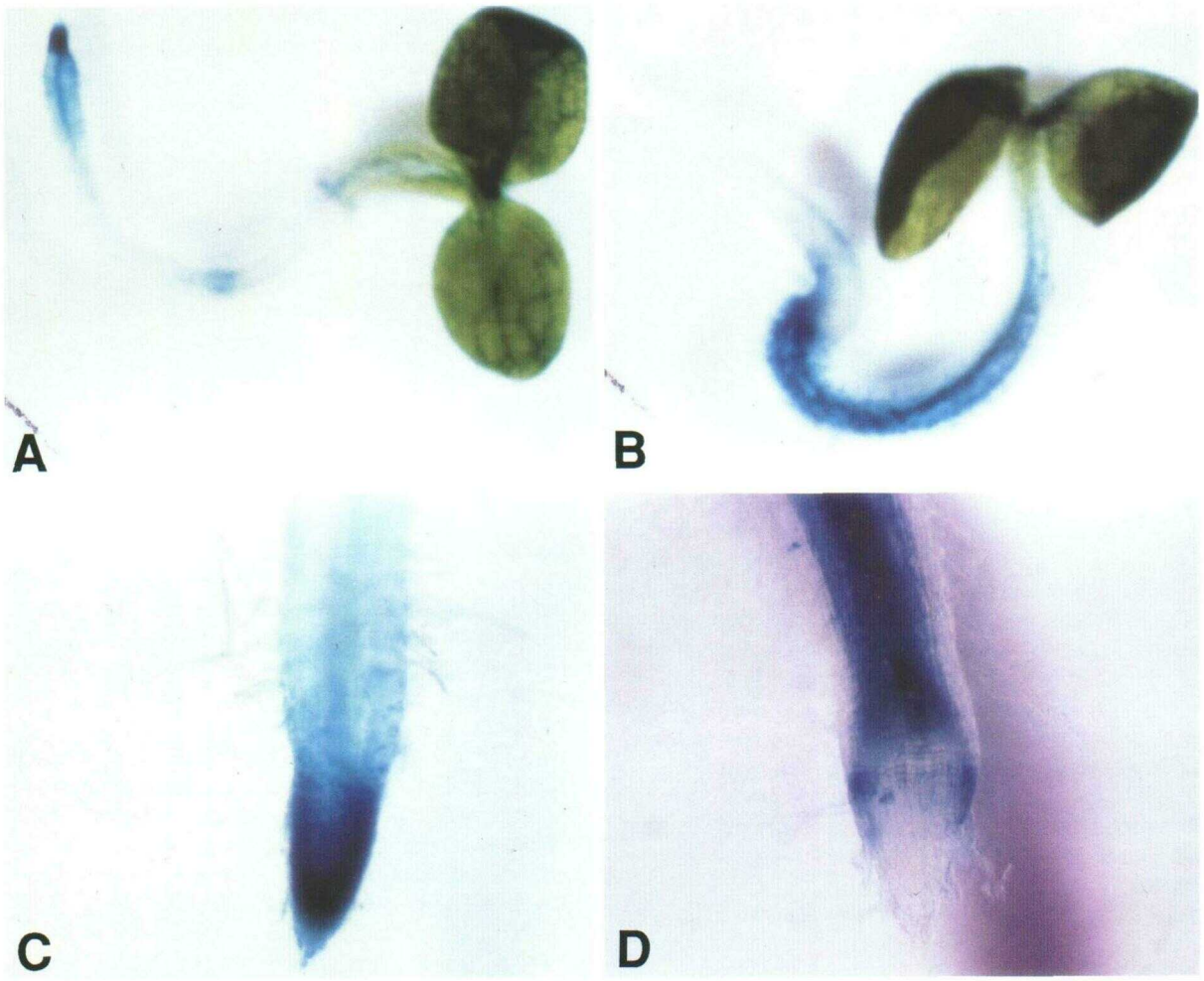

Figure 5. Effect of 2,4-D Treatment on the Localization of GUS Activity in 5-Day-Old EF-1α-GUS Seedlings from Self-Pollinated T_1 Transgenic Line 2158-9.

Kanamycin-resistant seedlings from self-pollinated EF-1α-GUS plants were incubated with and without 10 mg/L 2,4-D for 24 hr and stained for GUS activity overnight in X-gluc.
(A) Control seedling.
(B) A 2,4-D-treated seedling.
(C) Close-up of root tip from control seedling.
(D) Close-up of root tip from 2,4-D-treated seedling.

media containing 10 mg/L of the synthetic auxin for 24 hr, typical auxin effects on growth were observed, such as hypocotyl curvature, root-tip swelling, and petiole epinasty (Crocker et al., 1935; Evans, 1984). When stained for GUS activity, the untreated seedlings exhibited the predicted pattern of localization primarily to the apical and root meristems (Figure 5A). In the auxin-treated seedlings, however, a striking change in the staining pattern was observed (Figure 5B). Staining was no longer evident at the root tips, but instead, intense staining was observed at the root-hypocotyl junction and in the hypocotyl (Figure 5B). Where the hormone had induced curvature of the hypocotyl, staining was most evident at the abaxial side of the curve. In a closer examination of the root tips where the meristem is darkly stained in control seedlings (Figure 5C), the dramatic redistribution of GUS activity away from the meristem in the treated seedlings can be observed (Figure 5D).

DISCUSSION

There is now a substantial body of data supporting the contention that the elongation phase of translation is rate limiting for protein synthesis (Webster, 1985; Riis et al., 1990) and, in some cases, development (Shepard et al., 1989). Plants offer unique advantages over animal systems for investigating the role of EF-1α in development because of the range of developmental stages of most organ systems present on a single plant and the relative ease with which plant growth and development can be manipulated.

The transcriptional fusion between the 6.5-kb 5′ and 1.0-kb 3′ flanking regions isolated from the most transcriptionally active tomato EF-1α gene and the GUS coding region conferred measurable and often high levels of expression in every tissue analyzed from developing transgenic plants. Although there was considerable variation in the levels of expression in the 22 transformants analyzed, within the five lines selected for detailed analysis, no qualitative differences in expression patterns were observed. Where compared, the GUS activity in individual EF-1α-GUS transgenic lines often exceeded the activity measured in plants containing a double cauliflower mosaic virus 35S promoter-GUS fusion (Comai et al., 1990). These relatively high EF-1α-GUS expression levels were consistent with previous reports of strong EF-1α promoter activity measured in vitro (Axelos et al., 1989; Uetsuki et al., 1989).

The validity of using GUS as an indicator of EF-1α expression levels in transgenic tobacco was established by a series of observations. First, when steady-state levels of mRNA for the endogenous and the chimeric EF-1α genes were compared in a developmental series of leaves, we observed that, although differing quantitatively in expression levels, both the endogenous and the chimeric genes followed the same basic expression pattern of declining RNA levels with increased leaf age. This strongly suggested that the introduced tomato promoter was being regulated transcriptionally in a manner similar to the endogenous gene, although regulation at the level of mRNA stability could not be ruled out. Second, when GUS activity was analyzed in the same developmental series of leaves, the change in activity levels during leaf aging was found to parallel that of the EF-1α-GUS mRNA. Finally, the tissue specificity of GUS staining in shoot and root-tip sections was found to mirror closely the pattern of EF-1α mRNA hybridized in situ to shoot and root sections of tomato (Pokalsky et al., 1989).

Using this assay system to analyze EF-1α expression levels and patterns in different developmental contexts allowed us to confirm some previous assertions and expand our understanding of EF-1α regulation during plant development. Our data strongly suggest that regulation of EF-1α mRNA levels is in large part responsible for the developmental regulation of EF-1α expression, as was previously suggested (Linz and Sypherd, 1987; Pokalsky et al., 1989). In addition, the drop in EF-1α expression during leaf aging and senescence in tobacco, which is characterized by precipitous drops in cellular protein content (Brady, 1988; V.M. Ursin, unpublished data), is consistent with a number of reports on declining EF-1α activity during cellular aging and senescence in animals (see Webster, 1985). The demonstration of this in plants suggests that aspects of aging and senescence are shared between animal and plant systems at the cellular level.

The microsporangium and megasporangium are formed within the developing flowers during the reproductive phase of plant growth. These organs are characterized by multiple cell layers and specialized tissues that give rise to the male and female gametophytes. The examination of EF-1α-GUS activity during gametophyte development allowed us to correlate EF-1α expression with both cell division and cell growth, as well as with high cellular metabolism. GUS activity was detected in the ovary during periods of growth (Figures 4D and 4E), but when the ovary had attained full size, activity appeared localized entirely to the ovules within the locule. The developing ovules, on the other hand, stained intensely at all stages analyzed, coincident with meristematic activity and cell expansion during integument formation and meiotic and mitotic activity within the egg sac (Foster and Gifford, 1974). Although the intense staining of the ovules at maturity suggests high EF-1α promoter activity at this stage, it cannot be ruled out that relatively low GUS turnover rates in the mature ovules resulted in the observed high GUS levels.

In developing anthers, high levels of GUS activity were observed throughout development, but especially conspicuous was the localization of activity to the tapetum and pollen mother cells during early and mid-development and, later in development, the localization of activity to the maturing pollen grains. This is particularly interesting in light of the metabolic events occurring in these cell types during anther development. In young anthers, development of the tapetum, which functions in a nutritive role during microspore development, is characterized by ribosome proliferation indicative of high levels of protein synthesis activity (Chapman, 1987). The tapetum degenerates shortly after microspore meiosis, depositing its contents into the locular cavity. The dark staining observed in the tapetal cell layer in the youngest anther stage analyzed (Figure 4H) suggests high EF-1α-GUS promoter activity in the tapetum during the period of high protein synthesis activity, whereas later in anther development the staining at the locular wall is probably residual GUS protein from the degenerated tapetal cells (Figures 4I and 4J). In addition, pollen is characterized by high levels of RNA and protein synthesis at various points during its development (Mascarenhas, 1975; Porter et al., 1984), with increases in cytoplasmic protein levels of up to 72% noted in the later stages of pollen development in some species (Mascarenhas, 1975).

It has long been recognized that plant growth can be modified rapidly by the growth regulator auxin and by synthetic auxin analogs such as 2,4-D (Evans, 1984). Specifically, in seedlings, auxin can inhibit root elongation and induce root and hypocotyl swelling and epinasty of the cotyledons. We took advantage of these developmental effects of 2,4-D to determine whether EF-1α expression patterns are coordinately regulated with externally induced changes in growth within a short time frame. Our observation that the tissue specificity of EF-1α-GUS expression is modified rapidly in parallel with the morphological effects of the hormone treatment provides strong evidence that the patterns of EF-1α expression are, on the one hand, not necessarily predetermined and that changes in EF-1α expression can occur very quickly in response to either endogenous or exogenous cues during plant development. More significantly, the change in expression patterns involves what appears to be both the cessation of transcription in tissues whose growth is inhibited by the hormone (e.g., root tips), and promotion of transcription in tissues where cell growth is induced (e.g., regions of root swelling and hypocotyl curvature). Again, it cannot be ruled out that changes in EF-1α mRNA stability are at least partly responsible for these different expression patterns.

In this study, we examined the expression of EF-1α at the level of mRNA accumulation; there is evidence from several systems that EF-1α activity is also extensively regulated by modification of the protein. Our results suggested that the expression of EF-1α is an important control point during plant development and are consistent with the emerging view that EF-1α may play a critical role in regulating rates of protein synthesis. This assertion can be tested further by manipulating EF-1α levels in transgenic plants. In addition, the EF-1α-GUS plants that we described may be extremely useful for analyzing the effects of various perturbations, such as other hormone treatments and pathogenesis, on patterns of protein synthesis at the cellular level.

METHODS

Constructs

EF-1α Expression Vectors pCGN2148, pCGN2154, and pCGN2158

An 11-kb genomic clone LeEF-A, corresponding to the tomato EF-1α cDNA pCGN666 (Pokalsky et al., 1989), was isolated from a tomato (*Lycopersicon esculentum* cv VFNT cherry) partial Sau3A, Charon 35 library (kindly provided by Robert L. Fischer, University of California, Berkeley). Standard restriction mapping indicated that LeEF-A contained approximately 9 kb 5′ to the coding region of EF-1α and approximately 0.7 kb 3′ to the gene. Approximately 4.5 kb of the genomic clone was sequenced and determined to contain the entire coding region of tomato EF-1α, including an intron in the 5′ untranslated sequence and an intron within the coding portion of the EF-1α gene (Shewmaker et al., 1990).

An EF-1α expression cassette pCGN2148 was constructed from LeEF-A using standard cloning techniques. This construct contained approximately 6.5 kb of the 5′ flanking sequence, terminating 3 bp from the translational initiation codon (Shewmaker et al., 1990), multiple unique cloning sites (XhoI, SstI, and SmaI), and approximately 1 kb of coding and 3′ flanking DNA (nucleotides 3619 to 4540 of LeEF-A) (Shewmaker et al., 1990). PstI sites at either end allow easy excision.

To allow insertion of GUS into pCGN2148, a 2-kb BamHI-SstI fragment from pBI221 containing the *Escherichia coli* β-GUS gene (Jefferson et al., 1987) was subcloned into pUC119. The resulting plasmid pCGN1804 was linearized with EcoRI, and XhoI linkers were added. The GUS coding region in the resulting plasmid pCGN1805 was excised as a XhoI-SalI fragment and ligated into the unique XhoI site of pCGN2148. A plasmid with the GUS gene in the correct orientation for transcription from the EF-1α promoter was selected and designated pCGN2154. The approximately 9-kb PstI fragment from pCGN2154 was cloned into binary vector pCGN1558 (McBride and Summerfelt, 1990), such that the GUS gene was transcribed in the same direction as the 35S-*kan-tml* gene, included to serve as a selectable marker for transformants. The resulting clone was designated pCGN2158.

Tobacco EF-1α cDNA, pCGN680

Plasmid pCGN680 contained 900 bp of tobacco EF-1α cDNA and was obtained by using polymerase chain reaction (PCR) amplification of a cDNA first-strand synthesis reaction. Oligonucleotide primers homologous to the tomato EF-1α cDNA were synthesized with 5′ end restriction sites to facilitate cloning of the PCR products. For the first-strand synthesis reaction, total cellular RNA was purified from mature tobacco shoot tips as described previously (Ursin et al., 1989). A cDNA first-strand synthesis reaction was run using the Bethesda Research Laboratories' cDNA synthesis system per the manufacturer's recommendation, including 4 μg of tobacco RNA and 200 units of M-MLV reverse transcriptase. For the PCR reaction, a GeneAmp kit (Perkin Elmer Cetus) was used according to the manufacturer's recommendations and included 1.0 μM primer EFp19-RI (5′CTGCAGGAATTCGGTTTTGAAGC-TGGTATCTCCAAA3′), 1.0 μM primer EFp2-RI (5′GAGCTCGAATTCACCAACAGCAA-CAGTCTGCCTCAT3′), 10 μL of first-strand cDNA synthesis reaction, and 2.5 units of Amplitaq DNA polymerase (Perkin Elmer Cetus). After 25 cycles (1 min at 94°C, 2 min at 55°C, 3 min at 72°C per cycle), the PCR products were digested with EcoRI and ligated into the EcoRI site of pGEM4Z (Promega). The identity of the cloned fragment was determined by sequence analysis and DNA gel blot hybridization.

Tobacco Transformation

Agrobacterium tumefaciens strain 2760 (LBA4404) was transformed with the desired binary vectors by using the method of Holsters et al. (1978). Tobacco leaf disc explants were transformed by using cocultivation with the transformed *Agrobacterium*

strain as follows: explants, approximately 5 mm to 10 mm by 5 mm to 10 mm, were cut from the third to sixth leaf from the apex of tobacco plants grown on solid Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 3% sucrose, 0.7% Phytagar (GIBCO BRL), 1.0 mg/L indoleacetic acid (IAA), and 0.15 mg/L kinetin and then plated on solid MS medium containing 3% sucrose, 0.7% Phytagar, 40 mg/L adenine sulfate, 2 mg/L IAA, and 2 mg/L kinetin, on the surface of which was placed a No. 1 Whatman filter. Explants were incubated for 24 hr in the dark at 24°C. *Agrobacterium* strains were grown overnight in MG/L broth (50% Luria broth and 50% mannitol-glutamate salts; Garfinkel and Nester, 1980) at 30°C on a shaker at 180 rpm. Explants were dipped into a bacterial suspension of approximately 3.3×10^8 cells/mL for 5 min, blotted on sterile paper towels, and replated onto the same plates. After 48 hr, explants were placed on selection medium containing the same components as above plus 350 mg/L cefotaxime and 100 mg/L kanamycin. Cocultivated control tissue was placed onto the same medium without kanamycin. The explants were transferred to fresh media every 2 weeks. Shoots were harvested 4 weeks to 12 weeks after cocultivation, placed into 50-mL culture tubes containing 25 mL of solid MS medium containing 3% sucrose, 0.7% Phytagar, 1 mg/L indolebutyric acid, 350 mg/L cefotaxime, and 100 mg/L kanamycin. All tissues were grown under a regime of 24°C to 28°C, 12 hr light/dark, and 80 μE to 100 μE m^{-2} sec^{-1} light intensity. Shoots rooted in 1 week to 2 weeks and were then either maintained in culture on solid MS media containing 0.7% Phytagar, 3% sucrose, 1 mg/L IAA, and 0.15 mg/L kinetin without selection, or transplanted into soil and placed in growth chambers under a regime of 16-hr days, constant day/night temperature of 28°C, 0.2 μE m^{-2} sec^{-1}, at 50% to 60% relative humidity. Under these conditions, plants begin flowering at approximately the 22-leaf stage. All putative transformants were assayed for GUS activity as described below.

RNA Gel Blot Analysis

Total RNA was isolated from 1 g to 4 g of leaf tissue as previously described (Ursin et al., 1989). Polyadenylated RNA was purified by oligo(dT)-cellulose chromatography essentially as described by Maniatis et al. (1982). In vitro synthesized transcripts were made from linearized plasmids pCGN680 and pCGN7004 (a pBI221 derivative [Jefferson et al., 1987] in which the GUS BamHI-SstI fragment has been cloned into pBluescript KS– [Stratagene], downstream from the T7 promoter; L. Comai, unpublished data), using the Riboprobe Gemini System II kit (Promega). Reactions were carried out essentially as recommended by Promega, and contained 1 × transcription buffer (Promega) 10 mM DTT; 1 unit/μL RNasin; 125 μM rATP, rUTP, rGTP, and rCTP; 5 μg of linearized plasmid; and 5 units/μL SP6 or T7 RNA polymerase. Reactions were incubated for 60 min at 37°C.

RNA gel blot analysis was performed as described previously (Ursin et al., 1989). Radioactively labeled oligonucleotide probes were synthesized from the 0.9-kb EcoRI fragment of pCGN680 and the 2.0-kb EcoRI/BamHI fragment from pCGN7004, by random-primer labeling using the Random Primed DNA labeling kit (Boehringer Mannheim) following the manufacturer's recommendations. RNA blots were prehybridized, hybridized, and washed as described previously (Pokalsky et al., 1989).

Analysis of GUS Activity in Transgenic Plants

Fluorometric analysis of GUS activity was performed essentially as described by Jefferson et al. (1987). Uniform leaf discs were taken from attached leaves of growth chamber-grown plants and frozen immediately on dry ice. Samples were ground in microcentrifuge tubes with a Teflon grinding rod in 50 mM $NaPO_4$ (pH 7.0), 10 mM DTT, 0.05% Sarkosyl, 0.05% Triton X-100, centrifuged, and the supernatant was assayed for GUS activity in the above buffer without detergents and including 1 mM 4-methylumbelliferyl glucuronide (Molecular Probes, Eugene, OR). Fluorescence was determined by using a Farrand System 3 scanning spectrofluorometer. Protein content was assayed with the Bio-Rad Protein Assay kit (Bio-Rad), and by using BSA as a standard. All experiments were replicated at least twice, and data from representative experiments are shown.

Histochemical localization of GUS activity was performed on hand sections or intact organs from in vitro grown plants, flowers from growth chamber-grown plants, or germinated seedlings, as described by Jefferson et al. (1987), with the exception that, for localization of GUS activity in reproductive structures, 0.2% PVP and 10 mM DTT were included in the incubation medium to prevent browning of these tissues.

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REFERENCES

Axelos, M., Bardet, C., Lizbo, T., Thai, A.L.V., Curie, C., and Lescure, B. (1989). The gene family encoding the *Arabidopsis thaliana* translation factor EF-1α: Molecular cloning, characterization and expression. Mol. Gen. Genet. **219,** 106–112.

Brady, C.J. (1988). Nucleic acid and protein synthesis. In Senescence and Aging in Plants, L.D. Nooden and A.C. Leopold, eds (New York: Academic Press), pp. 147–179.

Cavallius, J., Rattan, S.I.S., and Clark, B.F.C. (1986). Changes in activity and amount of active elongation factor 1α in aging and immortal human fibroblast cultures. Exp. Gerentol. **21,** 149–157.

Chapman, G.P. (1987). The tapetum. Int. Rev. Cytol. **107,** 111–125.

Comai, L., Moran, P., and Maslyar, D. (1990). Novel and useful properties of a chimeric plant promoter combining CaMV and MAS elements. Plant Mol. Biol. **15,** 373–381.

Cottrelle, P., Cool, M., Thuriaux, P., Price, V., Theile, D., Buhler, J., and Fromageot, P. (1985). Either one of the two yeast EF-1α genes is required for cell viability. Curr. Genet. **9,** 693–697.

Crocker, W., Hitchcock, A.E., and Zimmerman, P.W. (1935). Similarities in the effects of ethylene and the plant auxins. Contrib. Boyce Thompson Inst. **7,** 177–218.

Evans, M.L. (1984). Functions of hormones at the level of the cell to the whole plant. In Encyclopedia of Plant Physiology, Vol. 10, T.S. Scott, ed (Berlin: Springer-Verlag), pp. 23–62.

Fonzi, W.A., Katatama, C., Leathers, T., and Sypherd, P.S. (1985). Regulation of protein synthesis factor EF-1α in *Mucor racemosus*. Mol. Cell. Biol. **5,** 1100–1103.

Foster, A.S., and Gifford, E.M. (1974). Comparative Morphology of Vascular Plants. 2nd ed. (San Francisco: W.H. Freeman and Co.).

Garfinkel, D.J., and Nester, E.W. (1980). *Agrobacterium tumefaciens* mutants affected in crown gall tumorigenesis and octopine catabolism. J. Bacteriol. **144,** 732–743.

Hiatt, W.R., Garcia, R., Merrick, W.C., and Sypherd, P.S. (1982). Methylation of elongation factor 1α from the fungus *Mucor*. Proc. Natl. Acad. Sci. USA **79,** 3433–3437.

Holsters, M., DeWaele, D., Depicker, A., Messens, E., Van Montagu, M., and Schell, J. (1978). Transfection and transformation of *Agrobacterium tumefaciens*. Mol. Gen. Genet. **163,** 181–187.

Hovemann, B., Richter, S., Walldorf, U., and Cziepluch, C. (1988). Two genes encode related cytoplasmic elongation factors 1α (EF-1α) in *Drosophila melanogaster* with continuous and stage specific expression. Nucl. Acids Res. **16,** 3175–3194.

Jefferson, R.A., Kavanagh, T.A., and Bevan, M.W. (1987). GUS fusions: β-Glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. **6,** 3901–3907.

Linz, J.E., and Sypherd, P.S. (1987). Expression of three genes for elongation factor 1α during morphogenesis of *Mucor racemosus*. Mol. Cell. Biol. **7,** 1925–1932.

Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory).

Mascarenhas, J.P. (1975). The biochemistry of angiosperm pollen development. Bot. Rev. **41,** 259–314.

McBride, K.E., and Summerfelt, K.R. (1990). Improved binary vectors for *Agrobacterium* mediated plant transformation. Plant Mol. Biol. **14,** 269–276.

Merrick, W.C., Dever, T.E., Kinzy, T.G., Conroy, S.C., Cavallius, J., and Owens, C.L. (1990). Characterization of protein synthesis factors from rabbit reticulocites. Biochim. Biophys. Acta **1050,** 235–240.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. **15,** 473–497.

Pokalsky, A.R., Hiatt, W.R., Ridge, N., Rasmussen, R., Houck, C.M., and Shewmaker, C.K. (1989). Structure and expression of elongation factor 1α in tomato. Nucl. Acids Res. **17,** 4661–4673.

Porter, E.K., Parry, D., Bird, J., and Dickinson, H.G. (1984). Nucleic acid metabolism in the nucleus and cytoplasm of angiosperm meiocytes. J. Cell Sci. **62,** 177–186.

Riis, B., Rattan, S.I.S., Clark, B.F.C., and Merrick, W.C. (1990). Eukaryotic protein elongation factors. Trends Biochem. Sci. **15,** 420–424.

Shepard, J.C.W., Walldorf, U.P.H., and Gehring, W.J. (1989). Fruit flies with additional expression of the elongation factor EF-1α live longer. Proc. Natl. Acad. Sci. USA **86,** 7520–7521.

Shewmaker, C.K., Ridge, N.P., Pokalsky, A.R., Rose, R.E., and Hiatt, W.R. (1990). Nucleotide sequence of an EF-1α genomic clone from tomato. Nucl. Acids Res. **18,** 4276.

Song, J.M., Picologlou, S., Grant, C.M., Firoozan, M., Tuite, M.F., and Liebman, S. (1989). Elongation factor EF-1α gene dosage alters translational fidelity in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9,** 4571–4575.

Tuhackova, Z., Ullrichova, J., and Hradec, J. (1985). Regulation of the activity of eukaryotic peptide elongation factor 1 by autocatalytic phosphorylation. Eur. J. Biochem. **146,** 161–166.

Uetsuki, T., Naito, A., Nagata, S., and Kaziro, Y. (1989). Isolation and characterization of the human chromosomal gene for polypeptide chain elongation factor-1α. J. Biol. Chem. **264,** 5791–5798.

Ursin, V.M., Yamaguchi, J., and McCormick, S. (1989). Gametophytic and sporophytic expression of anther-specific genes in developing tomato anthers. Plant Cell **1,** 727–736.

Webster, G.C. (1985). Protein synthesis in aging organisms. In Molecular Biology of Aging: Gene Stability and Gene Expression, R.S. Sohal, ed (New York: Raven Press), pp. 263–289.