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Short communication

Molecular cloning, characterization and expression of an elongation factor 1α gene in maize

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Abstract

A cDNA (*zmEF1*A) and the corresponding genomic clone (*zmgEF1*A) of a member of the gene family encoding the α subunit of translation elongation factor 1 (EF-1α) have been isolated from maize. The deduced amino acid sequence is 447 residues long interrupted by one intron. Southern blot analysis reveals that the cloned EF-1α gene is one member out of a family consisting of at least six genes. As shown by northern hybridizations in leaves the mRNA level increases at low temperature whereas time-course experiments over 24 h at 5 °C show that in roots the overall mRNA level of EF-1α is transiently decreased. These results indicate that the expression of EF-1α is differently regulated in leaves and roots under cold stress.

In eukaryotes the translation elongation factor 1 contains four different subunits (α, β, γ, δ). The α-subunit (EF-1α) is functionally homologous to the bacterial factor EF-Tu and catalyzes the binding of aminoacyl tRNA to the acceptor site on the ribosome under the hydrolysis of GTP [16]. Besides this function in protein synthesis where the expression of EF-1α is the major limiting step [16] EF-1α is involved in various other cellular processes and has to be regarded as a multifunctional protein [7, for review]. In *Dictyostelium discoideum* EF-1α was found to bind actin [20]. In *Xenopus* eggs it has been identified as a microtubule severing protein [18] that may participate in the rearrangement of microtubules during the cell cycle since it is part of the spindle organizing center in mammalian cells [13]. Furthermore, EF-1α binds calmodulin [6, 10] and is essential for the degradation of certain proteins via the ubiquitin pathway [9]. It has been shown that a vitronectin-like adhesion protein isolated from salt-adapted tobacco cells in nearly identical with EF-1α [21].

Recently, a low-temperature-inducible EF-1α

The nucleotide sequence data reported will appear in the GSDB, DDBJ, EMBL and NCBI Nucleotide Sequence Databases under the accession numbers D45407 (cDNA *zmEF1*A) and D45408 (genomic clone *zmgEF1*A).

clone has been isolated by differential screening of a barley cDNA library [5]. Since we have cloned the maize cDNA, m*lip* 15, which encodes a low-temperature-inducible DNA-binding protein of the bZIP type [12], we are interested in genes that might be transcriptionally activated by this protein during cold stress. Therefore, we tried to isolate the cDNA and genomic clone corresponding to the low-temperature-inducible EF-1α homologue from maize.

Total leaf RNA was prepared from 14-day old maize seedlings (*Zea mays*, edible variety Honey Bantum) incubated at 5 °C for 24 h prior to isolation, as described previously [1]. Poly(A^+) RNA was prepared using the FAST TRACK mRNA isolation kit (Invitrogen) and cDNA was synthesized using a cDNA synthesis kit (Pharmacia). The library constructed in λgt10 phage vector was screened with a ^{32}P-labeled yeast EF-1α cDNA-fragment. An about 1.5 kb *Eco* RI cDNA fragment was excised from the λ phage, subcloned into pUC18 and sequenced in both directions (*Bca* BEST Dideoxy Sequencing Kit, TaKaRa). This cDNA was named *zmEF*1A and used to screen a maize genomic library constructed by cloning *Sau* 3A partially digested, size-fractionated (9.7–22 kb) fragments into the *Bam* HI site of phage λEMBL3 (Stratagene). One genomic clone (*zmgEF*1A) was subcloned and sequenced. The nucleotide sequence and the deduced amino acid sequence are shown in Fig. 1. The coding region is interrupted by one 756 bp long intron from 462 to 1218. The deduced amino acid sequence shows 94.2% homology to the barley sequence [5] and 96.4% homology to the tomato sequence [15], respectively.

In genomic Southern experiments (Fig. 2), a *Sph* I fragment of the cDNA containing 390 bp of a region that is highly conserved among EF-1α genes hybridized to at least six fragments in each lane of *Bam* HI-, *Eco* RI- and *Hin*d III-digested maize DNA. This indicates that in maize the EF-1α gene family consists of at least six members which is in accord with findings in other plant species. In *Arabidopsis thaliana* four EF-1α genes have been characterized [2], in tomato plants EF-1α was found to be encoded by four to

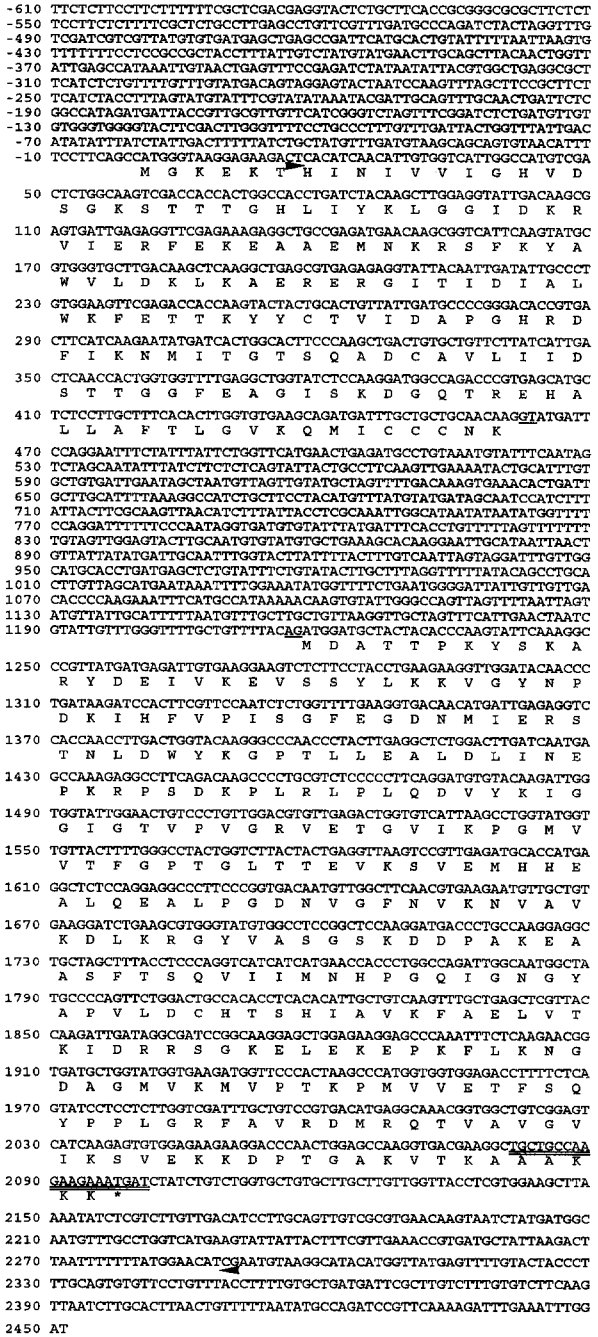

Fig. 1. Nucleotide sequence of *zmgEF1*A and deduced amino acid sequence. The start and the end of the cDNA clone *zmEF1*A are marked by arrowheads under nucleotide 17 and 2290, respectively. The intron/exon boundaries are underlined and the sequence to which the primer for the PCR amplification of the gene-specific probe has been designed is double underlined.

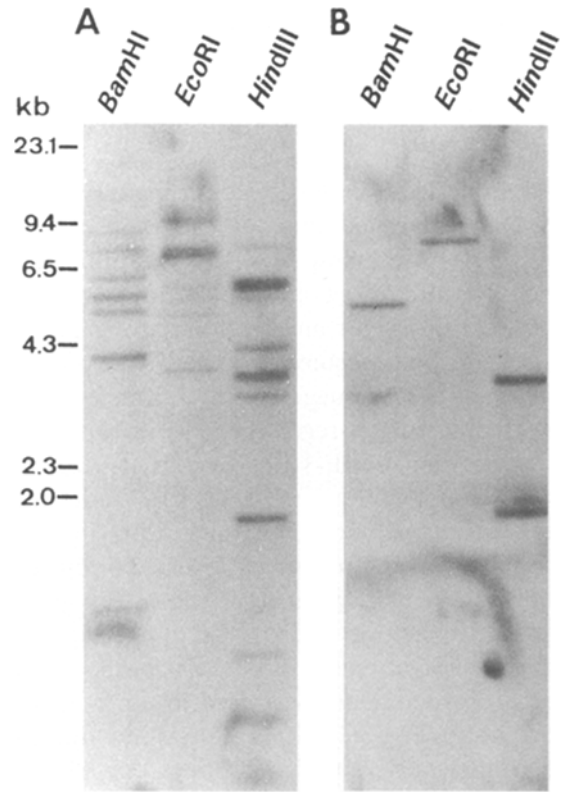

Fig. 2. Southern blot of maize genomic DNA. A. The membrane was hybridized with a *Sph* I cDNA-fragment of the conserved 5′ region covering nucleotides 17 to 406 of the sequence shown in Fig. 1. B. Hybridization was with a *zmEF1*A 3′-specific probe generated by PCR using the specific primer sequence marked in Fig. 1 and a primer corresponding to the multiple cloning site of the plasmid vector. The DNA (5 μg per lane) was cut with various restriction enzymes, electrophoresed in a 0.7% agarose gel and transferred onto Hybond-N$^+$ membrane (Amersham) with 0.4 M NaOH. The DNA fragments were labeled by the random primer method using the T7 QuickPrime kit (Pharmacia) and hybridization was performed in Rapid hybridization buffer (Amersham) at 65 °C for 4 h. The filter was washed in 0.1 × SSC, 0.1% SDS for 20 min at 65 °C and exposed to X-ray film. After stripping off the probe with 0.4 M NaOH for 30 min at 45 °C the membrane was rehybridized. The restriction enzymes used and the positions of molecular weight marker bands in kb are indicated.

eight genes [17] and in barley EF-1α is also organized as a gene family [5]. The Southern data further reveal that the gene described here corresponds to the 5.4 kb *Bam* HI fragment and to the 7.8 kb *Eco* RI fragment as shown by hybridization of the same blot with a specific PCR-amplified 215 bp DNA fragment of the 3′-noncoding region of the cDNA. In the lane of *Hin*d III-digested DNA two bands of 3.6 kb and 1.7 kb were detected. This confirms one *Hin*d III site at nucleotide position 2146 in the genomic sequence that is located within the amplified hybridization probe.

The expression of EF-1α in maize was studied by northern hybridizations. Total RNA was extracted from root and leaf tissue as described previously [1]. In leaves the overall mRNA level of EF-1α is increased after 24 h at 5 °C (Fig. 3A). This response to low-temperature of EF-1α as a component of the translational apparatus has been described for barley shoot meristem and has been related to the observation that in frost hardened plants the soluble protein levels are increased [5]. However, in roots the EF-1α mRNA is highly abundant even at normal temperature and the level is unchanged after 24 h cold treatment (Fig. 3A). Time-course experiments display a transient decrease in the overall EF-1α mRNA level in roots (Fig. 3B). After 12 h at 5 °C the mRNA level increases again and reaches the same level after 24 h as before the cold treatment. Prolonged incubation at 5 °C does not change the mRNA level (data not shown). In order to compare these mRNA levels with these of the cold-induced gene m*lip* 15 (maize low-temperature-induced protein of 15 kDa [12]) the same blot was hybridized with that cDNA as a probe. In contrast to the EF-1α mRNA, the m*lip* 15 mRNA level increases steadily in response to the cold treatment.

It has been reported that increases in protein synthesis are accompanied by an accumulation of EF-1α protein and transcript and that the expression of EF-1α may serve as an indicator of translational stimulation [14, 19]. Interestingly, in potato tubers wounding first induces the expression of EF-1α peaking 2 or 4 h after wounding followed by a repression and a second peak 24 to 36 h after wounding [14]. This biphasic induction of EF-1α on both protein and transcript level was shown to reflect changes in the translational activity during the wound response [14]. Employing these results to our findings we can conclude

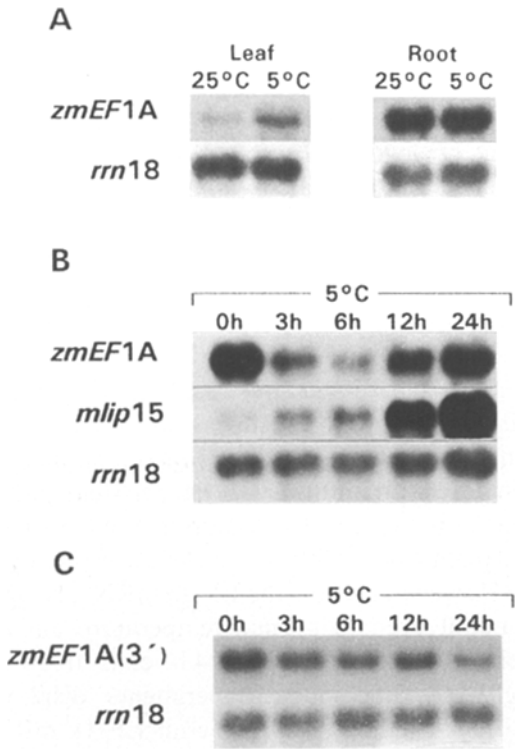

Fig. 3. Northern blot analysis of total RNA isolated from roots and leaves (A) or roots only (B, C) of maize plants grown under continuous light at 25 °C and kept at 5 °C for the times indicated. RNA (10 μg per lane) was separated in formaldehyde-1.2%-agarose gels and transferred to Hybond-N membrane (Amersham) with 20× SSC. The DNA probes were labeled as described in Fig. 2 and hybridization was in 5× SSPE and 50% formamide for 16 h at 42 °C. The filters were washed as described for the Southern hybridization and exposed to X-ray film. In A and B the filters were hybridized with the total *zmEF1*A cDNA and in C the PCR-amplified 3′-specific DNA fragment was used as the probe. In B the same blot was rehybridized with the low-temperature-inducible *mlip*15 cDNA [12]. To confirm equal loading of RNA samples all blots were rehybridized with a 18S rRNA probe [8].

that in maize roots the translational activity may be inhibited during the first hours of cold treatment followed by a recovery to the same extend as under normal growth temperature corresponding to the changes in EF-1α transcript levels. It cannot be said whether the transient decrease in the overall EF-1α mRNA level in roots corresponds to the transcript of one single EF-1α gene or if it represents an overlap of decreasing and increasing transcript levels of different members of the gene family. One or more genes might be especially responsible for restoring the protein synthesis activity at low temperature and then showing a parallel transcript accumulation as m*lip* 15.

An analysis of the A1 BF-1α gene promoter in *Arabidopsis* revealed that for the expression in roots one region corresponding to an intron within the 5′-noncoding region, is important and that the same region plus an additional upstream sequence are both required for the expression in leaves [3, 4]. This suggests that a concerted action of different *cis*-regulatory elements control the expression of EF-1α in various tissues and also developmental stages [4, 11, 19]. Under cold stress distinct EF-1α genes might contribute to the changes in the transcript levels. To find out whether the EF-1α gene described in this paper plays a role in this finding, the cDNA specific fragment that has been used as a probe in the Southern experiments was used in northern hybridizations (Fig. 3C). The results show that the level of the transcript of this gene is only slightly decreasing during the low temperature treatment but does not show the transient repression as it could be demonstrated for the overall EF-1α transcript level.

Zhu *et al.* isolated a vitronectin-like protein, PVN1, from NaCl-adapted tobacco cells that is almost identical with EF-1α. The protein was detected by the anti-PVN1 antibody in different plant species including maize [21]. With this knowledge we used the 3′-specific DNA-probe in northern experiments with root-RNA from salt-treated maize plants. The steady state mRNA level of *zmEF*1A was not increased after 24 h salt treatments with 200 mM or 400 mM NaCl (data not shown). In total the *zmEF*1A-gene is neither upregulated by low temperature nor by salt stress although some members of the EF-1α gene family seem to play a role in both cases [5, 21].

The results presented here show that EF-1α is differently expressed in leaves and roots in response to cold stress. Furthermore, in roots the transient repression of EF-1α may exhibit a transient repression of translational activity in the cold-treated tissue in analogy to the findings in

wounded potato tubers [14]. It would be of great interest to isolate the EF-1α genes that are activated or inactivated by stresses like wounding, salt and low temperature and to search for putative responsive elements in the promoter regions. A main question is also which of the various functions the EF-1α proteins accomplish during these stresses.

Acknowledgements

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