The EMBO Journal vol.11 no.8 pp.2771 – 2778, 1992

A desiccation-related *Elip*-like gene from the resurrection plant *Craterostigma plantagineum* is regulated by light and ABA

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Communicated by F.Salamini

The resurrection plant *Craterostigma plantagineum* tolerates an extreme loss of cellular water. Therefore this plant is being studied as model system to analyse desiccation tolerance at the molecular level. Upon dehydration, new transcripts are abundantly expressed in different tissues of the plant. One such desiccation-related nuclear gene (*dsp*-22 for desiccation stress protein) encodes a mature 21 kDa protein which accumulates in the chloroplasts. Sequence analysis indicates that *dsp*-22 is closely related to early light inducible genes (*Elip*) of higher plants and to a carotene biosynthesis related gene (*cbr*) isolated from the green alga *Dunaliella bardawil*. In contrast to other desiccation-related genes, light is an essential positive factor regulating the expression of *dsp*-22: ABA-mediated gene activation leads to the accumulation of the transcript only in the presence of light. During the desiccation process, light acts at the transcriptional and post-transcriptional levels. The implications of these different controls and the possible role of the dsp-22 protein in the desiccation/rehydration process are discussed.

Key words: ABA/desiccation tolerance/Elip gene family/light control/resurrection plant

Introduction

Adaptation to osmotic stress is an important biological process that protects organisms against the lethal effects of dehydration. Tolerance to protoplastic dehydration—or to the extreme form of it, desiccation—is common among lower plants; in higher plants it is strictly limited to seeds and to all tissues and organs of the plant body in some rare 'resurrection plants' (Bewley and Krochko, 1982; Gaff, 1971). These plants can recover uninjured from complete dryness within 24 h of contact with water. The tolerance to desiccation is induced during the drying process itself. Desiccation tolerance is a complex trait and involves the synthesis of a range of osmoprotective substances which are mainly low molecular weight substances such as polyhydric alcohols, e.g. glycerol or sucrose, free amino acids, amino acid derivatives and methylamines (Yancey *et al.*, 1982).

To understand the molecular biology of desiccation tolerance, a representative of the resurrection plants, *Craterostigma plantagineum*, is under investigation. Upon drying this plant changes its metabolism: a high concentration of sucrose accumulates (Bianchi *et al.*, 1991) and a set of specific transcripts is induced (Bartels *et al.*, 1990; Piatkowski *et al.*, 1990). These transcripts accumulate rapidly in leaves and other tissues as a response to water loss. Several of them have been cloned as cDNAs and have been characterized in terms of their nucleotide sequences, their induction kinetics, their tissue specificity and their subcellular localization. Several of the desiccation-induced gene products have been found to share sequence homologies with genes expressed during late embryogenesis in seeds of many higher plants (Piatkowski *et al.*, 1990). From this observation the important conclusion was drawn that at least in part the gene products involved in the desiccation-induced pathways are common to resurrection plants and embryos of higher plants. Based on structural features of these proteins, an osmoprotective role for some of them has been suggested, but their functions still remain to be demonstrated (Dure *et al.*, 1989).

Although the chloroplasts including the photosynthetic structures undergo severe ultrastructural changes during desiccation, this process is reversible and the rehydrated plants rapidly resume full photosynthetic activities. This observation suggests that the photosynthetic structures are protected during the resurrection process. Recently we discovered that some of the desiccation-induced gene products were localized in chloroplasts of desiccated leaves (K.Schneider, B.Wells, E.Schmelzer, F.Salamini and D.Bartels, in preparation). In this study we report the identification of one such desiccation-induced, chloroplast associated gene product (*dsp*-22), which could be involved in protective mechanisms of the photosynthesis apparatus. *Dsp*-22 is homologous to the genes that encode Elips (early light inducible proteins) which accumulate in plants during the transition from dark to light (Grimm *et al.*, 1989) and to *cbr*, a carotene biosynthesis-related gene isolated from the alga *Dunaliella bardawil* (Lers *et al.*, 1991a).

Current evidence suggests that the plant hormone abscisic acid (ABA) is involved in the signal transduction mechanism between sensing turgor changes and gene expression (Skriver and Mundy, 1990). Increased ABA concentrations lead to the expression of stress-related genes, and ABA responsive promoter elements have been identified in some of these genes (Guiltinan *et al.*, 1990; Skriver *et al.*, 1991). In contrast to ABA inductions described so far, we demonstrate that, for the gene discussed here, ABA is essential but not sufficient for the accumulation of the gene product; light is a second stimulus which has to be present during ABA incubation. Not only the ABA induction but also the desiccation process requires illumination and evidence is provided for transcriptional and post-transcriptional light regulation of the *dsp*-22 gene product.

Results

The clone dsp-22 encodes an Elip-like protein

In order to identify genes which are related to desiccation tolerance, a cDNA library was constructed from poly(A)$^+$ RNA derived from desiccated leaves of the resurrection plant *C.plantagineum*. Differential hybridization resulted in the identification of a number of clones encoding transcripts whose accumulation correlated with the desiccation phenomenon (Bartels *et al.*, 1990). The molecular analysis of one such cDNA clone, *dsp*-22, which represents a class of the most abundant desiccation-induced transcripts, is reported here.

The nucleotide sequence of the *dsp*-22 insert contains only one long unbroken possible open reading frame, encoding a protein with a predicted molecular mass of 22 kDa and an isoelectric point of 8.7 (Figure 1). The predicted amino acid sequence of the protein encoded by *dsp*-22 shows significant sequence homology to Elips from pea and barley (Kolanus *et al.*, 1987; Grimm *et al.*, 1989) as well as to a *cbr* gene from the unicellular green alga *D.bardawil* (Lers *et al.*, 1991a) (Figure 2): 48% and 41% respectively of the amino acids are identical with the Elip proteins SO1056 from pea (Kolanus *et al.*, 1987) and HV90 from barley (Grimm *et al.*, 1989) and 31% of the residues match those of the cbr protein from *D.bardawil* (Lers *et al.*, 1991a). The structural similarity between this class of proteins is further substantiated by a comparison of their hydrophilicity plots which show a conserved domain structure consisting of alternating hydrophilic and hydrophobic regions with three characteristic hydrophobic domains (I–III). By analogy to the Elips, the hydrophobic N-terminal part of the dsp-22 sequence possesses a putative signal sequence followed by a possible transit peptide (Keegstra *et al.*, 1989). A feature

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   1  TTCGTCAAATTACGACATACAAATAAGCAGGAAAGATCAACCATGGCTTCTTCAACTTGC    60
   1                                                M  A  S  S  T  C     6
  61  TACGCAACGATCCCAGCAATGTCTTGCCGAGGACAAAGCACTATTACGAGGTTTGGCCCT   120
   7  Y  A  T  I  P  A  M  S  C  R  G  Q  S  T  I  T  R  F  G  P     26
 121  AACAACCTATTCTTGGGAAAGCAAAGCTATGAATTACCATTGATGAGGAGGAATGCTAAG   180
  27  N  N  L  F  L  G  K  Q  S  Y  E  L  P  L  M  R  R  N  A  K    46
 181  TTTACTGTCCGAAGCATGAGAGAGGACAATGAGAAGGAGGAGCAGCAGCAGCAAAAACAA   240
  47  F  T  V  R  S  M  R  E  D  N  E  K  E  E  Q  Q  Q  Q  K  Q    66
 241  CAACAGACGCATGACGGAGGGCCCGACTTGACACCCAACAGAACTGAGGTGACGACAAAG   300
  67  Q  Q  T  H  D  G  G  P  D  L  T  P  N  R  T  E  V  T  T  K    86
 301  AGGACAGTCGATTTGTTCTCCTTCGACGGACTTGCGCCCGAGAGGATCAACGGTCGGTCG   360
  87  R  T  V  D  L  F  S  F  D  G  L  A  P  E  R  I  N  G  R  S   106
 361  GCAATGATAGGATTCGTGGCGGCCGTAGGAGTGGAATTAGCGACTGGGCGGGACGTATTT   420
 107  A  M  I  G  F  V  A  A  V  G  V  E  L  A  T  G  R  D  V  F   126
 421  TCTCAAGTGTTCAATGGAGGCGTTATGTGGTTCCTTTTGACGAGCGCAGTGTTGGTGCTC   480
 127  S  Q  V  F  N  G  G  V  M  W  F  L  L  T  S  A  V  L  V  L   146
 481  GCCACTCTGATTCCTATCTATAGAGGGCTTTCTCCCGAGGCGAAGAACAATGGGTTTTGG   540
 147  A  T  L  I  P  I  Y  R  G  L  S  P  E  A  K  N  N  G  F  W   166
 541  AATTCAGACGCCGAGATATGGAATGGGAGGTTCGCCATGATTGGGCTCGTGGCTCTCGCC   600
 167  N  S  D  A  E  I  W  N  G  R  F  A  M  I  G  L  V  A  L  A   186
 601  TTTACGGAGTACGTCAAAGGTGGCCCGCTAATCAATGTGTGAGGAAGTGATAGATTAGGG   660
 187  F  T  E  Y  V  K  G  G  P  L  I  N  V  *                     206
 661  TTTGGTTGTTATTGCAGATAGTTGTAAGTAGATGGTTGTGCGTGATGTATATTGAA       716
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Fig. 1. Nucleotide sequence (mRNA strand) and predicted amino acid sequence of *dsp*-22.

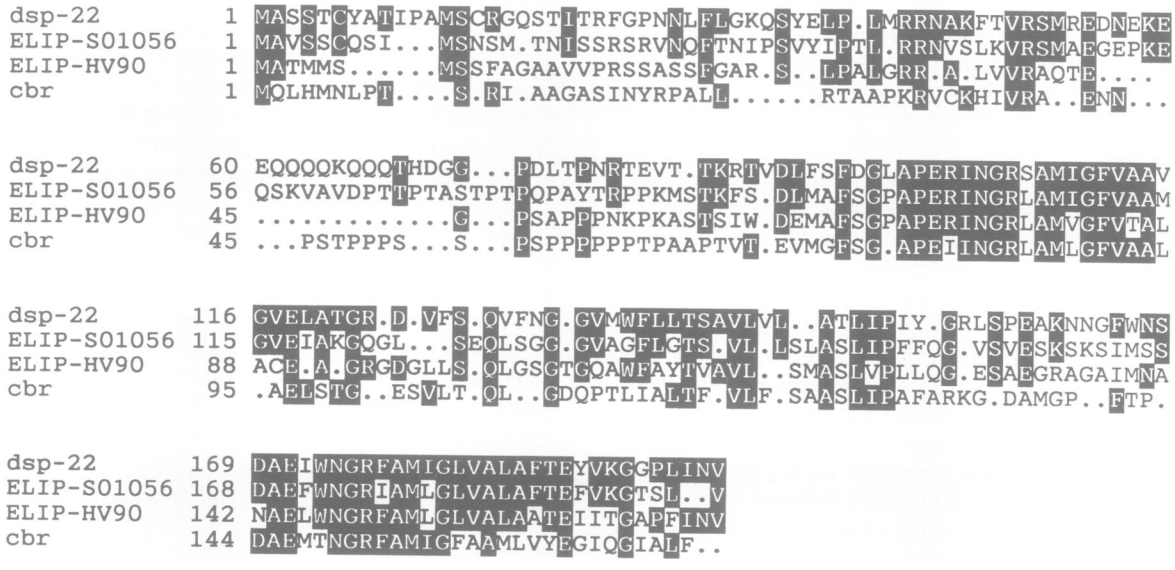

Fig. 2. Alignment of the dsp-22 deduced protein sequence with other members of the Elip family. Optimized alignment of the protein sequences of *Craterostigma* dsp-22, the pea Elip SO1056 (Kolanus *et al.*, 1987), the barley Elip HV90 (Grimm *et al.*, 1989) and the *D.bardawil* cbr protein (Lers *et al.*, 1991a). Gaps were introduced to optimize matches. Amino acids identical between dsp-22 and any of the other sequences are indicated in black.

of the dsp-22 protein noted is the extremely hydrophilic motif between amino acid positions 57 and 68 which is composed entirely of glutamine or glutamic acid residues interspersed with two lysine residues.

Genomic organization

Southern blot hybridization analysis of genomic DNA revealed a complex pattern under high stringency conditions (Figure 3), indicating that the *dsp*-22 sequence is a representative of a multigene family. This observation was supported by the finding that ~15% of the cDNA clones initially isolated by differential hybridization were related to *dsp*-22.

Tissue specific expression and subcellular localization of the dsp-22 protein

Polyclonal antibodies raised against a glutathione-*S*-transferase–dsp-22 fusion protein were used to analyse the tissue distribution of the dsp-22 protein in *Craterostigma* (Figure 4). A mature protein of 21 kDa was detected in extracts of desiccated leaves and a faint protein band of 19 kDa in seeds, but no signal was obtained in roots or untreated leaves (Figure 4). *In situ* immunolocalizations using light microscopy showed that, in cross-sections of dried leaves, the dsp-22 protein was preferentially localized in the chloroplast-rich palisade parenchyma cells (Figure 5). In differential centrifugation of subcellular components, the dsp-22 protein was not detectable in mitochondria or microsomal fractions, but was detectable in chloroplasts isolated from desiccated leaves (Figure 6). When chloroplasts

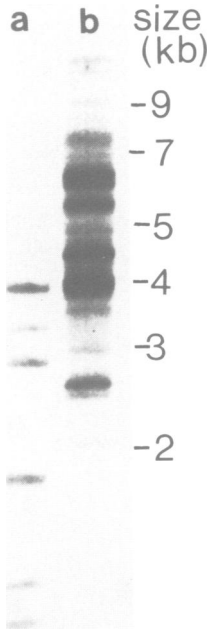

Fig. 3. Southern analysis of genomic DNA from *Craterostigma* DNA cut with *Eco*RI (lane a) and *Hin*dIII (lane b) was probed with the ^{32}P-labelled insert of *dsp*-22.

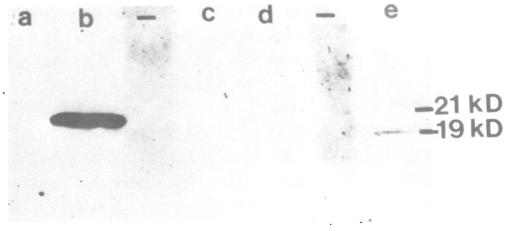

Fig. 4. Western blot analysis of *Craterostigma* tissues. The following samples were analysed via immunoblotting for the presence of dsp-22 proteins: (a) untreated leaves, (b) desiccated leaves, (c) untreated roots, (d) dried roots and (e) seeds.

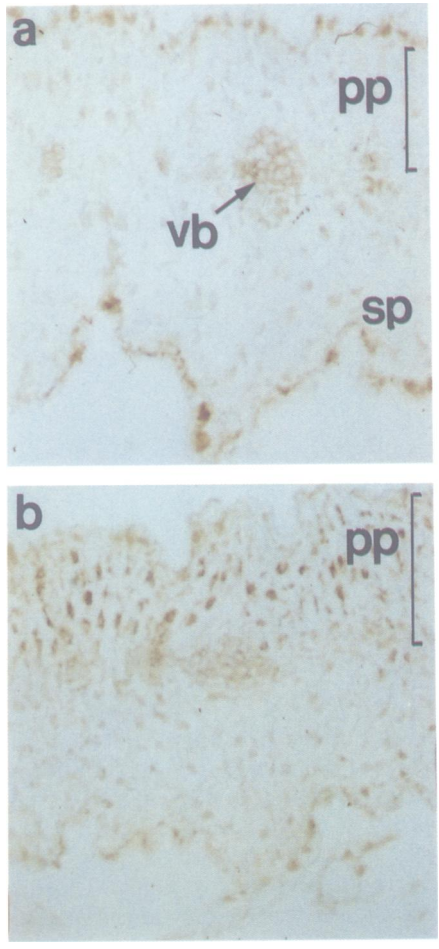

Fig. 5. Immunolocalization of the dsp-22 protein on cross-sections through desiccated *Craterostigma* leaves: (**a**) preimmune control, (**b**) treatment with dsp-22 antiserum (magnification 125×) (pp = palisade parenchyma cells; sp = spongy parenchyma cells; vb = vascular bundle).

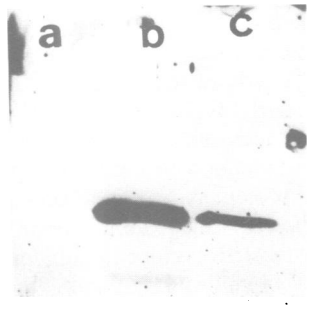

Fig. 6. Protein extracts of chloroplasts from dried leaves were separated on a 12% polyacrylamide–SDS gel and analysed by immunoblotting for the presence of dsp-22 proteins. Protein extracts were from (a) soluble chloroplast fraction, (b) chloroplast membrane fraction and (c) total chloroplast preparation.

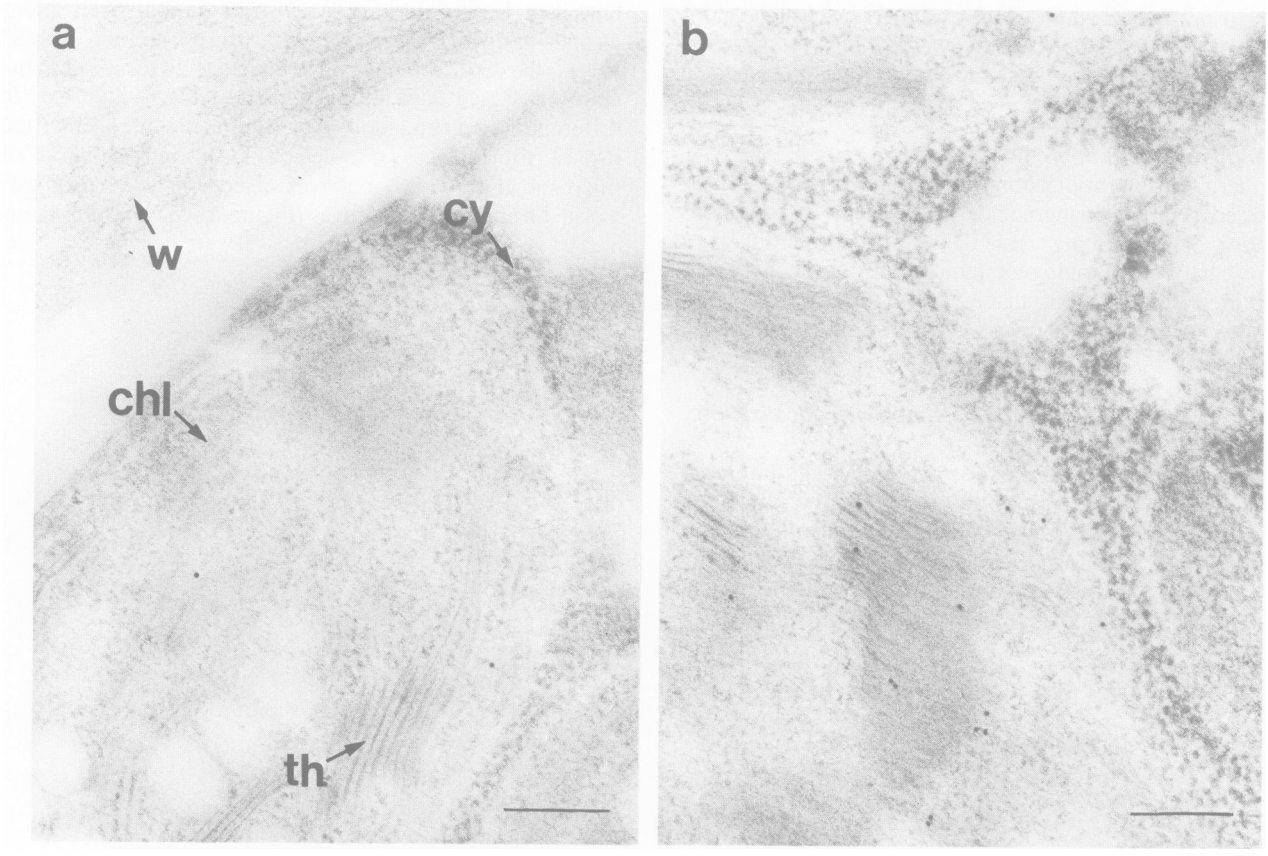

Fig. 7. Subcellular localization of the dsp-22 protein in dry *Craterostigma* leaves by immunogold electron microscopy. (**a**) Preimmune control, (**b**) incubation with dsp-22 antiserum. The bar corresponds to 200 nm; chl: chloroplast, cy: cytosol, th: thylakoid, w: wall.

were further fractionated into stroma and membranes, the dsp-22 protein was found to be retained in the chloroplast membrane fractions (Figure 6). To substantiate the chloroplastic localization of the dsp-22 protein, an electron microscopic analysis was performed using immunogold labelling. The result, presented in Figure 7, indicates that the dsp-22 protein is detectable only in chloroplasts, and is associated with thylakoid membranes.

Transcription of dsp-22 is regulated by ABA in the light

The level of the *dsp*-22 gene transcript was studied by Northern blot analysis in plants grown with a daily light/dark regime (Figure 8, part 1). The transcript could be induced in fully hydrated leaves by treatment with the plant hormone ABA. The presence of light was essential for ABA inducibility: ABA did not lead to the accumulation of the transcript in the dark (Figure 8, compare lanes b and c). No transcript was detectable in untreated leaves (Figure 8, lane a). The full induction of the transcript by ABA also requires *de novo* protein synthesis, as only a faint signal is obtained when the ABA incubation is done in the presence of chloramphenicol and cycloheximide, protein synthesis inhibitors that affect chloroplastic and cytoplasmic protein synthesis (Figure 8, lane e). When in a control hybridization the same Northern blot filter was probed with a different type of ABA-inducible *Craterostigma* cDNA clone, pcC6-19 (Piatkowski *et al.*, 1990), the ABA induction of the transcript was not dependent on light, and it was only partially blocked by protein synthesis inhibitors (Figure 8, part 2).

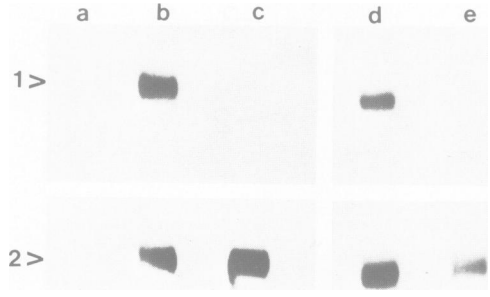

Fig. 8. ABA-regulated expression. Hybridization of the cDNA clone *dsp*-22 to Northern blots of poly(A)$^+$ RNAs. 2 μg of RNAs were separated on 1.5% denaturing formaldehyde–agarose gels and transferred to nylon filters. RNAs were isolated from *Craterostigma* leaf tissue treated as follows: (a) untreated fully hydrated leaves, (b) 24 h ABA treatment with illumination, (c) 24 h ABA treatment in the dark, (d) 24 h ABA treatment with illumination, (e) ABA treatment with illumination in the presence of protein synthesis inhibitors (cycloheximide and chloramphenicol). The filter was first hybridized with the *dsp*-22 insert (part 1); this was followed by hybridization with pcC-6-19, another ABA inducible, desiccation-related clone isolated from *Craterostigma*. Afterwards the filter was hybridized with a ribosomal probe (part 2) to monitor equal loading of RNAs (data not shown).

dsp-22 synthesis is also regulated at the translational level by desiccation and light

During desiccation of leaves, light regulates the expression of the *dsp*-22 transcript at the transcriptional and translational

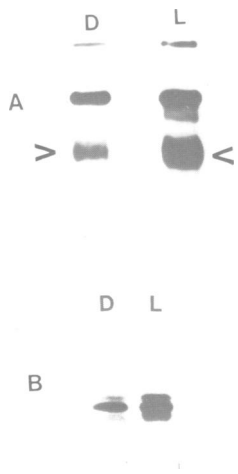

Fig. 9. Effect of light during desiccation on RNA transcripts. **A**: Northern blot hybridization of poly(A)$^+$ RNAs from (D) leaves dried in the dark and (L) leaves dried with illumination. The band indicated by the arrowhead is the RNA hybridizing to the *dsp*-22 insert. The filter was hybridized with a ribosomal probe to monitor equal loadings of RNAs; the two upper bands are the ribosomal RNAs. **B**: Immunoprecipitations of *in vitro* synthesized proteins. Equal amounts of poly(A)$^+$ RNAs were translated in a wheat germ system and subsequently immunoprecipitated with an antiserum against the dsp-22 protein; lane D, poly(A)$^+$ RNA from leaves dried in the dark and lane L, from leaves dried in the light.

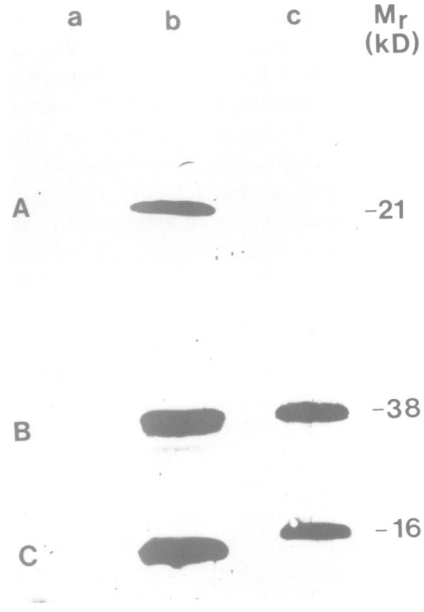

Fig. 10. Influence of light on the expression of desiccation-induced *Craterostigma* proteins. Protein extracts were prepared from leaf tissues (a) untreated, (b) dried in the presence of light (16 h per day) and (c) dried in the dark. The proteins were separated, blotted and then analysed for the presence of the dsp-22 protein (**A**). The same filter was treated with Tween-20 and then incubated with the pcC13-62 antiserum (**B**), then the filter was stripped of signal and the filter was incubated with the pcC27-45 antiserum (**C**). On the right hand side the relative molecular mass of the proteins detected is given. [The proteins pcC27-45 and pcC13-62 and the respective antisera have been described by K.Schneider, B.Wells, E.Schmelzer, F.Salamini and D.Bartels, (in preparation).]

levels. When leaves were dried in the dark, the level of the transcripts is slightly lower than that for leaves dried during light (Figure 9A). The result of the Northern analysis is confirmed by *in vitro* translation data. When poly(A)$^+$ RNA from leaves dried either under light or in the dark was translated in a wheat germ system, less dsp-22 protein was immunoprecipitated from RNA of leaves dried in the dark (Figure 9B). However, when the dsp-22 protein level was compared between dark and light grown plants, the protein was absent in leaves dried in the dark and was present in leaves dried in the light (Figure 10), i.e. the reduced level of dsp-22 mRNA was either not translated or the protein was degraded in leaves dried in the dark. This observation demonstrates that light during the drying treatment is an essential factor for the *in vivo* accumulation of the dsp-22 protein. The light control is a particular feature of the dsp-22 desiccation-related protein. Two other desiccation or ABA inducible *Craterostigma* genes (pcC27-45 and pcC13-62, Piatkowski *et al.*, 1990), for example, do not require light during desiccation for their expression (Figure 10).

Discussion

During the dehydration process in the resurrection plant *C.plantagineum*, a number of new transcripts are observed, several of which have been characterized molecularly (Bartels *et al.*, 1990; Piatkowski *et al.*, 1990). The cDNA clone *dsp*-22 described here represents a different type of gene product. It is structurally related to nuclear-encoded chloroplast proteins (Elips and cbr proteins) and accumulates upon dehydration in chloroplasts where it is associated with thylakoid membranes and possibly exerts a protective role during water loss. In contrast to other desiccation-induced genes, the ABA-mediated gene activation is strictly light dependent and during desiccation light is necessary both for transcription and for the accumulation of the gene product.

Structural homologies of the dsp-22 protein

In addition to the tissue specific and subcellular localization of the dsp-22 protein, its sequence similarity to Elip proteins (Grimm *et al.*, 1989; Kolanus *et al.*, 1987) and to a cbr protein of the alga *D.bardawil* (Lers *et al.*, 1991a) point to a role related to photosynthesis. The most conserved protein sequence motifs of all these groups of proteins are three hydrophobic domains which are potential membrane spanning α-helices and are structurally related to those of the chlorophyll *a/b* binding (cab) proteins (Green *et al.*, 1991; Grimm *et al.*, 1989). This strict evolutionary conservation at the protein level suggests a selection for function. *Dsp*-22, like other plant Elip genes, is present as a small multigene family (Kolanus *et al.*, 1987; Grimm *et al.*, 1989) and the expression of their transcripts is light-regulated. Elip proteins appear transiently during greening of etiolated seedlings in higher plants and disappear before chloroplast development is completed (Meyer and Kloppstech, 1984; Scharnhorst *et al.*, 1985; Grimm and Kloppstech, 1987; Kolanus *et al.*, 1987; Grimm *et al.*, 1989). They are synthesized as precursor proteins and transported into chloroplasts, where they have been found associated with both photosystems I and II (Adamska and Kloppstech, 1991; Cronshagen and Herzfeld, 1990). A role in the assembly of the photosystem or in the integration of pigments into the mature pigment–protein complexes has been suggested. The latter possibility is favoured because

the Elip-like cbr protein from *D.bardawil* was shown to be associated with β-carotene (Lers *et al.*, 1991b). It has been proposed that the cbr protein may provide photo-protection of the assembly of antennae and reaction centre complexes (Lers *et al.*, 1991a). The dsp-22 protein may have a similar role. During desiccation the *Craterostigma* chloroplasts undergo morphological changes and lose part of their internal organization, while retaining their chlorophyll. By analogy to the cbr protein, the dsp-22 protein may bind pigments or help to maintain assembled photosynthetic structures essential for resuming active photosynthesis during resurrection.

Control of expression

The control of the dsp-22 protein expression is complex with regulation occurring at several levels. Light, the phytohormone ABA and desiccation are positive factors involved in the regulation. In leaves, water deficits result in higher ABA levels which are thought to mediate transcriptional gene activation (Bartels *et al.*, 1990). Both ABA and desiccation require the presence of light for the accumulation of the dsp-22 gene product.

Light and ABA control. Light regulation of plant processes is thought to be mediated by receptor systems among which the phytochrome and the blue light receptor have been best characterized (Quail, 1991). The role of phytohormones in the transmission of the light stimulus from illumination to gene activation is poorly understood. The ABA induction of the *dsp*-22 gene establishes a case where only both light and ABA together lead to an accumulation of the transcript. Only when illuminated leaves were treated with ABA was the *dsp*-22 mRNA detected. This could mean that light induces factors required either for the transcriptional activation of ABA or for the stability of the mRNA. The light-mediated gene activation of *dsp*-22 by ABA is dependent on *de novo* protein synthesis, unlike the ABA induction of a desiccation-related *Craterostigma* gene homologous to *Rab* 16 from rice and other plants (Mundy and Chua, 1988; Skriver and Mundy, 1990). This result suggests different molecular mechanisms for gene activation by ABA in *Craterostigma*. Interestingly, it was recently reported for photosynthesis genes that ABA supersedes the light activation and represses the *rbcS* and *cab* genes in tomato leaves (Bartholomew *et al.*, 1991). This observation, together with the results reported here for *dsp*-22, may indicate that under water stress conditions, light together with ABA leads to the activation of proteins required for stress survival but to the concomitant repression of photosynthesis-related genes.

Light and desiccation. The influence of light during desiccation on the expression of the *dsp*-22 gene product was analysed at the transcript and protein levels. When plants were dried in the dark, the mRNA level of *dsp*-22 was reduced but clearly detectable, whereas the protein was absent. This suggests a regulation by light at the transcriptional and at the post-transcriptional or translational level. Light regulation of gene expression at the transcriptional level has been demonstrated for many nuclear and chloroplast-encoded plant genes and a number of light responsive promoter elements have been identified (for a review see Simpson and Herrera-Estrella, 1990).

Post-transcriptional control of gene expression is a general mechanism for gene regulation in a variety of organisms including plants (for a review see Hershey, 1991). In the plant kingdom such a mechanism has been observed for chloroplast-encoded mRNAs related to photosynthesis: mRNAs accumulate to relatively high levels in dark-grown plants but the protein products cannot be detected (Berry *et al.*, 1985; Herrmann *et al.*, 1985; Inamine *et al.*, 1985; Mullet, 1988). A possible mechanism for light-regulated translational control was recently suggested by Danon and Mayfield (1991) who revealed a set of nuclear-encoded proteins binding specifically to the 5′ leaders of chloroplastic mRNAs of *Chlamydomonas*. Other mechanisms such as mRNA stability (Stern and Gruissem, 1987) and protein turnover (Mullet *et al.*, 1990) have also been described for chloroplastic genes. The main form of light regulation for the small subunit of ribulose bisphosphate carboxylase in *Amaranthus* is post-transcriptional and is regulated in two distinct ways: by preventing initiation on specific mRNAs (Berry *et al.*, 1990) and by blocking elongation (Berry *et al.*, 1988). Environmental factors besides light have been shown to exert translational control using two distinct mechanisms: wounding of potato tubers leads to degradation of specific mRNAs from polysomes, whereas hypoxic stress inhibits translation of pre-existing mRNAs without causing their dissociation from polysomes or degradation (Crosby and Vayda, 1991). Further experiments are required to determine which of these mechanisms is responsible for the post-transcriptional light control observed here.

A concerted action of control mechanisms is responsible for the rapid accumulation of the *dsp*-22 gene products during desiccation. The challenge ahead lies in defining in precise terms both the co-operation between light and ABA and light and desiccation.

Materials and methods

Plant material

The origin and propagation of *C.plantagineum* Hochst plants have been described previously (Bartels *et al.*, 1990). For nucleic acid and protein extractions, tissue samples were frozen in liquid nitrogen. For drying treatments, detached leaves or whole plants were air-dried under the same conditions as they were grown (60% humidity, 20−24°C in a daily light period of 14 h using a light intensity of 60−200 $\mu E/m^2 \times \cdot s$). Drying of plants or detached leaves without illumination was done using similar temperature and humidity conditions. All tissue samples were dried until 90% of the fresh weight was lost. For the treatment with abscisic acid (ABA-*cis*, *trans*, Sigma) detached leaves were floated on water containing 10^{-3} M ABA for the time indicated, or whole plants were sprayed with ABA (10^{-3} M) at regular intervals for 24 h. Protein synthesis inhibitors were applied as follows: detached leaves were incubated with 10 μM cycloheximide and 100 μM chloramphenicol for 2 h, followed by the addition of ABA for 4 or 16 h; in two parallel control experiments leaves were either tested with water alone or incubated with ABA.

Recombinant DNA techniques and nucleic acid extractions

Isolation of plasmid and phage DNA, preparation and ligation of DNA fragments and transformation of *Escherichia coli* cells were carried out essentially according to Maniatis *et al.* (1982). Extraction of poly(A)$^+$ RNA was described by Bartels *et al.* (1986), and isolation of plant DNA and Southern blot analysis as described by Piatkowski *et al.* (1990). DNA fragments were labelled with ^{32}P by the method of Feinberg and Vogelstein (1984).

In vitro translation and immunoprecipitation

Poly(A)$^+$ RNAs were translated *in vitro* using a commercial wheat germ system (Amersham Buchler, Braunschweig, Germany). Saturating amounts of RNA (0.5−1 μg) were incubated according to the suppliers' instructions using 3.77×10^5 Bq [^{35}S]methionine (3.02×10^{13} Bq/mmol; Amersham Buchler, Braunschweig, Germany). Immunoprecipitation was as described by Gatenby *et al.* (1981).

cDNA library screening, DNA sequencing and computer analysis

A cDNA library has been constructed using as a template poly(A)$^+$ RNA from dried *Craterostigma* leaves; construction and differential screening of this library are described in Bartels *et al.* (1990). The nucleotide sequence of the cDNA clones were determined on both strands by subcloning of suitable restriction fragments into plasmid (pUC18 and pUC19) or phagemid (M13mp18 and M13mp19) vectors (Messing and Vieira, 1982) followed by dideoxynucleotide sequencing (Sanger *et al.*, 1977) with the T7 polymerase kit (Pharmacia LKB, Freiburg, Germany). The program WISGEN (version 4.0) of the University of Wisconsin Genetic Computer Group was used for DNA and protein sequence analysis (Devereux *et al.*, 1984), the TFASTA program was used for amino acid comparisons (Pearson and Lipman, 1988).

Nomenclature of cDNA clones

The following nomenclature for desiccation stress-related cDNA clones isolated from *C.plantagineum* is used here. According to their inducibility the cDNA clones are termed dsp (desiccation stress protein) followed by the molecular weight predicted. The clone pcC37-31 (Bartels *et al.*, 1990) is henceforth termed *dsp*-22.

Overexpression in E.coli, preparation of antisera and immunodetection

The insert of *dsp*-22 was digested with *Sau*3A, resulting in a 276 bp fragment which was ligated into a *Bam*HI site of the expression vector pGEX 3X (Smith and Johnson, 1988) to yield a translational fusion with glutathione-*S*-transferase (GST). The *E.coli* strain TG2 was transformed with this construct and the expression of the fusion protein was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to 0.5 mM. The soluble fusion protein was affinity-purified on glutathione-coupled agarose beads and used for raising polyclonal antibodies in rabbits.

For immunodetection, protein samples were prepared as follows: 500 mg of hydrated plant tissue or 50 mg of dried tissue was ground and dissolved in 200 μl of Laemmli sample buffer (0.625 M Tris–HCl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.001% bromophenol blue). 20 μl were separated on 12% polyacrylamide–SDS gels (Laemmli, 1970). Electrophoretic transfer of proteins from gels to nitrocellulose membranes was carried out as described by Towbin *et al.* (1979). The membrane was probed with antiserum (1:500 dilution) raised against the fusion protein followed by an incubation with anti-rabbit IgG-conjugated horseradish peroxidase. The protein–antibody complex was detected using the chemiluminescence (ECL) Western blotting detection system from Amersham Buchler (Braunschweig, Germany) according to the manufacturer's recommendations. When the Western blots were probed with other antisera the membrane was washed for 2 × 10 min in Tris-buffered saline containing 0.1% Tween-20. To remove the signal from the filter prior to incubation with other antisera, the filter was incubated for 30 min at 50°C in a solution of 100 mM β-mercaptoethanol, 2% SDS, 62.5 mM Tris, pH 6.7.

Light microscopy

Tissue pieces of dry leaves were fixed, embedded in paraffin, sectioned and subsequently treated as described by Schmelzer *et al.* (1989). The antigen was detected on 8 μm thick sections by indirect immunoperoxidase staining (Jahnen and Hahlbrock, 1988). After 90 min incubation with a 1:50 dilution of the dsp-22 antiserum, a secondary peroxidase-conjugated antibody (Diagnostics Pasteur, Marne la Coquette, France) was incubated with the filter for 60 min. The protein was visualized by the enzyme reaction with 3,3-diaminobenzidine.

Electron microscopy

For immunogold electron microscopy the tissue was subjected to low temperature resin embedding (Wells, 1985) using LR white resin (Agar Scientific Ltd, Stansted, UK). 0.1 μm thick sections were fixed to carbon-coated gold grids. After blocking the proteins with 3% bovine serum albumin (BSA) in TBS (10 mM Tris, 150 mM NaCl, pH 7.4) the tissue was incubated with a 1:50 dilution of the antibody directed against the dsp-22 protein overnight at 4°C. Excess antibodies were washed off with distilled water before the sections were treated with a gold-labelled antibody (Janssen Life Sciences Products, Belgium). The tissue was stained with uranyl acetate and lead citrate prior to electron microscopic analysis.

Plant cell fractionation and chloroplast preparation

Cell fractionation was carried out using differential centrifugations according to Nagahashi and Hiraike (1982). Chloroplasts and thylakoid membrane fractions were essentially prepared according to Chua and Bennoun (1975) except that the chloroplasts were isolated on a 20–50% sucrose gradient in 5 mM HEPES, pH 7.5.

Acknowledgements

The authors are grateful to B.Eilts and M.Feck for technical assistance, to M.Pasemann for typing the manuscript and to R.Thompson for critically reading the manuscript. K.S. was the recipient of a fellowship from the Deutsche Studienstiftung. Part of this work was supported by a grant from the European Community (contract no. TS2-0030-D).

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Received on March 16, 1992; revised on April 15, 1992

Note added in proof

The sequence data reported here have been deposited in the EMBL nucleotide sequence database under the accession number X66598.