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Posttranscriptional control in the expression of the genes coding for high-light-regulated HL#2 proteins

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Abstract. An antibody was raised against the protein HL#2 which is a nuclear-encoded light-stress-induced protein of barley (*Hordeum vulgare* L.). The expression of the mRNA and the protein of HL#2 was determined under the influence of high light and methyl jasmonate. The mRNA of HL#2 was induced by high light (1800 μmol m^{-2} s^{-1} and 25 °C) and the steady-state levels remained elevated for up to 48 h of exposure to high-light stress. In contrast, using an antibody against HL#2 there was no observable change in the level of HL#2 proteins of 18 kDa and 15.5 kDa during the same treatment. These data indicate a pronounced stress-induced control of HL#2 expression at a post-transcriptional level. In the presence of methyl jasmonate (45 μM), the induction of HL#2 occurred together with that of the two most closely related jasmonate-inducible proteins (JIPs) of 32.6 and 32.7 kDa, as judged by their cross-reactivity with the antibody against HL#2. In contrast to the mRNA and protein levels of early light-inducible proteins (ELIPs) in green barley, those of HL#2 appeared not to be influenced by low temperatures. Therefore, the control of ELIPs and HL#2 by high light fluxes may be measured via the same photoreceptor but must, at least partially, be under the control of two divergent signal transduction chains.

Key words: Abscisic acid – Early light-inducible protein – *Hordeum* (light stress) – Jasmonate – Lectin – Light stress

Introduction

Under light conditions that activate photosystems I and II in such a way that the capacity of the electron transport chain exceeds the consumption of reduction equivalents delivered to the stroma side of the thylakoid membranes, a situation is created which is described as light stress. This will be compensated by short-term regulations such as changes in the xanthophyll cycle or spillover of energy (Niyogi et al. 1997). Long-lasting light stress will be harmful to plants and will activate stress genes within hours or days. Previously, we have shown that the mRNA for a light-inducible protein, designated HL#2, is induced by high light fluxes that are experienced as light stress by barley cells (Pötter et al. 1996). The expression of the mRNA was regulated in a way very similar to those coding for early light-inducible proteins (ELIPs; Adamska et al. 1992; Pötter and Kloppstech 1993). The function of ELIPs is not known; however, the characteristic properties of their regulation have suggested that ELIPs might confer protection of chloroplast membranes against light stress. A similar protective function could thus be assumed in the cell for HL#2. Interestingly, another light-stress-enhanced mRNA which codes for subunit P of glycine decarboxylase (Turner et al. 1992) was upregulated by high light but in a rather distinct manner. In contrast to the ELIP and HL#2 mRNAs, its level was highest in the apical part of the leaf, indicating an expression which might be positively correlated with the development or function of plastids and the photosynthetic capacity of thylakoid membranes (Pötter et al. 1996).

The deduced amino acid sequence of HL#2 (Pötter et al. 1996) shows similarities to the deduced amino acid sequences of two proteins from rice (Claes et al. 1990; de Pater et al. 1992) again of unknown function, but apparently also involved in the stress response. A recent repeat of the search in the Swiss protein bank revealed quite a number of other proteins with homology to HL#2 protein; the most remarkable similarities were to lectins (Skea et al. 1988; Geshi and Brandt 1998) and to two jasmonate-inducible proteins (Lee et al. 1996).

Abbreviations: ELIP = early light-inducible protein; GST = glutathione-*S*-transferase; jasmonic acid-ME = jasmonic acid-methyl ester; JIP = jasmonate-inducible protein;

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These similarities raise the possibility that HL#2 might not be a plastidic protein. This, in turn, raises the interesting question of where in the cell the light stress that activates HL#2 expression is perceived. If, as we assume, it is the chloroplast the problem arises of how the different compartments are connected in terms of signal transfer during light stress.

The protein HL#2 is a good candidate with which to investigate this signal-transfer problem experimentally. The fact that previously we could analyse the expression of HL#2 only at the level of mRNA limited the value of our work considerably as it is well known that in many cases the levels of mRNAs and their corresponding proteins can differ to a great extent. It was shown, for instance, that the mRNA and the protein levels for a nuclear-encoded plastid heat-shock protein have an inverse relationship within the barley leaf (Kruse et al. 1993). Similarly, in plants which expressed the antisense RNA to LHC II it was found that a reduction in the mRNA levels by a factor of 100 did not visibly affect the amount of the accumulated protein (Flachmann and Kühlbrandt 1995). To circumvent this problem we decided to develop an antibody against HL#2. The results obtained with this antibody in light-stressed and control plants are described in the present publication.

Materials and methods

Plant growth and evaluation of data. Barley (*Hordeum vulgare* L. cv. Apex; v. Lochow-Petkus, Bergen, Germany) was grown for 6 d on Vermiculite at 25 °C at a constant irradiance of 100 μmol m^{-2} s^{-1} in a 12 h-light/12 h-dark cycle. All represented data have been obtained from experiments carried out at least three times. The SD values in Fig. 5 were smaller than 15%.

High-light treatment. High-light stress treatments were performed on isolated leaves of equal length floating on water of 25 °C and under a water filter of 3 cm height at an irradiance of 1800 μmol m^{-2} s^{-1} in white light (Pötter and Kloppstech 1993). At the end of the experiment, the leaves were divided into three segments of equal length (apical, middle and basal parts), immediately frozen in liquid nitrogen and stored at −70 °C for further use.

Jasmonic acid (jasmonate-methyl ester) treatment. Leaves of one-week-old barley seedlings were floated on water containing jasmonic acid-methyl ester (jasmonic acid-ME; 45 μM) under the culture conditions described above. The leaves of control seedlings were kept under identical conditions but floating on normal tap water.

Analysis of proteins. For the extraction of total soluble protein, barley leaves were homogenized under liquid nitrogen in a pre-cooled mortar, extracted with 10 vol of extraction buffer [50 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$, 0.24 M NaCl and 1 mM PMSF, 1 mM benzamidine and 5 mM caproic acid] and filtered through a 45-μm Nylon gauze. Cellular debris was removed by centrifugation for 10 min at 15 000 *g* at 4 °C. The supernatant was mixed with 0.7 vol of 3× concentrated lithium dodecyl sulfate (LDS)-medium [6% LDS, 150 mM Tris-HCl (pH 8.0), 30% glycerol and 78 mM DTT]. For the isolation of the membrane fraction the 15 000 *g* pellet was washed twice with the extraction buffer, suspended in 1 × LDS and used as the membrane fraction.

Total proteins were extracted from leaf material that was homogenized under liquid nitrogen and extracted in 10 vol of 1 × LDS medium, incubated for 20 min at 65 °C and centrifuged at 12 000 *g* for 5 min at room temperature, and the supernatant was used as total protein. Protein concentration was determined by the method of Lowry et al. (1951) after precipitation with trichloroacetic acid.

Electrophoresis and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed according to Laemmli (1970) using the Hoefer minigel system as described by Pötter et al. (1996). For immunoblots the proteins were transferred according the procedure of Towbin et al. (1979) but using poly(vinylidenedifluoride) membranes (Westran, Schleicher and Schüll). Blots were incubated with HL#2 primary antibody raised in goat against a fusion protein between HL#2 and a glutathione-binding domain of glutathione-*S*-transferase (GST). For this purpose, the DNA sequence coding for HL#2 (Pötter et al. 1996) was ligated in-frame into the vector pGEX4T-3 (Pharmacia) to obtain a fusion protein with GST. The fusion protein was expressed in *E. coli* SG13009 and purified by affinity chromatography using glutathione-Sepharose (Pharmacia). The correctness of the construct was verified by restriction analysis (Menhaj 1998).

The immunoreactive bands were visualized using an alkaline-phosphatase-conjugated anti- (goat) serum (A4187, Sigma) with the 5 bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium colour reaction (Harlow and Lane 1988).

Isolation of total RNA. RNA isolation was done according to the modified procedure of Barlow et al. (1963) and von Gromoff et al. (1989), first solubilizing the homogenised leaf tissue in PCl (phenol:chloroform:*iso*-amyl alcohol 25:24:1, by vol) and mixing with an equal volume of lysis buffer [0.6 M NaCl, 10 mM EDTA, 100 mM Tris-HCl (pH 8.0), 4% SDS]. The upper phase collected after the centrifugation was precipitated with 8 M LiCl at 4 °C and later washed and precipitated with ethanol in the presence of 3 M Na-acetate (pH 5.2). The pellet was washed with 70% ethanol, vacuum-dried after centrifugation and dissolved in sterile double-distilled water. Quantification was done with a spectrophotometer at 260 and 280 nm. Total RNA (10 μg per lane) was separated by electrophoresis on agarose gels containing formaldehyde and transferred onto Biodyne-B transfer membrane (Pall, Dreieich Germany) as described by Pötter et al. (1996).

Northern blotting. Northern blots were performed using a modification of the method of Kroczek and Siebert (1990). The membranes were hybridized with ^{32}P-labelled random-primed homologous HL#2 cDNA. The autoradiograms were scanned using an image analysis program (Gel Doc 1000; BioRad).

Isolation of organelles. Intact chloroplasts were isolated as described by Grossman et al. (1982). The chloroplasts were ruptured with distilled water in the presence of protease inhibitors and the stroma fraction of the chloroplast was obtained by centrifugation at 10 000 rpm for 10 min at 4 °C in an HB4 rotor (Sorvall).

Mitochondria were isolated according the procedure of Hause and Wasternack (1988). Freshly harvested leaves were homogenized in buffer [100 mM Tris-HCl (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$, 6 mM EDTA, 300 mM sucrose, 0.1% BSA and 10 mM ascorbic acid]. The homogenate was passed through 45-μM Nylon gauze and centrifuged at 1000 *g* for 10 min. The pellets obtained were used for the isolation of nuclei. The supernatant was loaded onto a gradient consisting of 25, 35 and 50% sucrose cushions in 0.1 M Tris (pH 7.8), 50 mM KCl and 10 mM $MgCl_2$ and centrifuged at 19 000 rpm for 45 min at 4 °C in a SW27 rotor (Beckman). Mitochondria were collected from the interphase between 35 and 50% sucrose, washed with the homogenization buffer and centrifuged at 10 000 *g* for 20 min at 4 °C. Pellets were resuspended in homogenization buffer and centrifuged at 1500 *g*. The supernatant was collected and centrifuged at 12 000 *g* for

20 min in the SS34 rotor (Sorvall), and the pellet was suspended in native extraction buffer, broken down using three freeze-and thaw-cycles and resuspended with 1 × LDS for further use.

For isolation of nuclei (Cox and Goldberg 1988), pellets obtained after the first centrifugation as described above (isolation of mitochondria) were loaded onto a discontinuous Percoll gradient (onto a cushion of 13 ml of 3 M sucrose, 3-ml fractions were layered each consisting of 76, 60, 40, or 0% Percoll in 25 mM Tris-HCl, pH 8.5; 10 mM $MgCl_2$) and centrifuged for 30 min at 4 °C at 5000 rpm in an HB-4 rotor. Nuclei were collected as a white pellet at the bottom of the tube. The pellets were washed four times with homogenization buffer, resuspended in native extraction buffer and broken down with three freeze-thaw cycles and again centrifuged briefly at 12 000 *g* at 4 °C. Supernatants and pellets were treated with 1 × LDS buffer for further use.

Results

Expression of HL#2 in the barley leaf. Cloning of high-light-expressed mRNAs using differential display resulted in a clone HL#2 whose sequence showed homology to proteins of rice (Gos 9 and SalT oryza: Pötter et al. 1996) whose functions, however, are not known. The cloned sequence was fused to a glutathione-binding domain of GST and expressed in *E. coli* after confirmation of the correct construct by restriction analysis. The isolated fusion protein had the predicted size; it was purified by affinity chromatography and used for the immunization of goats and rabbits. The resulting antibody from goat, which was used throughout this study, recognized a protein of apparent molecular mass 44 kDa in *E. coli*. This is the predicted size of the fusion protein. Using thrombin, the protein was cleaved into fragments of 26 kDa and 18 kDa which both reacted with the antisera (data not shown). The molecular masses of the cleavage products were very close to those of the individual proteins GST and HL#2 of barley.

As outlined previously (Pötter et al. 1996) the remarkable properties, of HL#2 mRNA are the induction by high light fluxes, the increase in mRNA levels relative to total RNA towards the base of the leaf, and finally a short half-life of the mRNA under recovery conditions in low light after return from light-stress conditions. These properties of HL#2 are shared with the ELIP mRNAs (Adamska et al. 1992; Pötter and Kloppstech 1993). To our surprise, the level of the HL#2 protein as detected with the antibody was hardly influenced by light between light fluxes of 70 (low light) and 1800 μmol m^{-2} s^{-1} (high light) (data not shown). This finding raised the question as to whether the antibody would correctly detect the HL#2 proteins. Since sequence comparison had shown that HL#2 should belong to the group of jasmonate-inducible proteins (JIPs) we decided to check this property of the gene using the antibody for quantification of the protein.

HL#2 induced by jasmonic acid-ME but not by abscisic acid. When cut leaves were exposed to 45 μm jasmonic acid-ME for 48 and 72 h, the two JIPs of 32.6 and 32.7 (Lee et al. 1996) most closely related to HL#2 were induced de novo as judged from the calculated apparent molecular mass of corresponding bands on the immunoblots. These two bands could not be detected in a silver-stained gel (data not shown), they were however, recognized by the antibody against HL#2 (Fig. 1A). This fact already indicates that the specificity of the antibody should be directed against HL#2 as this protein possesses similarity to JIPs. More important is the finding that the antibody also detected a protein of the calculated molecular mass of HL#2. The 18-kDA band of HL#2 is already present in etiolated leaves (data not shown) and in green leaves grown under low-light conditions (Fig. 1A); however, jasmonic acid-ME raises the level of this protein by about 5-fold above the level of the controls. We take this finding as additional confirmation for the specificity of the antibody against HL#2.

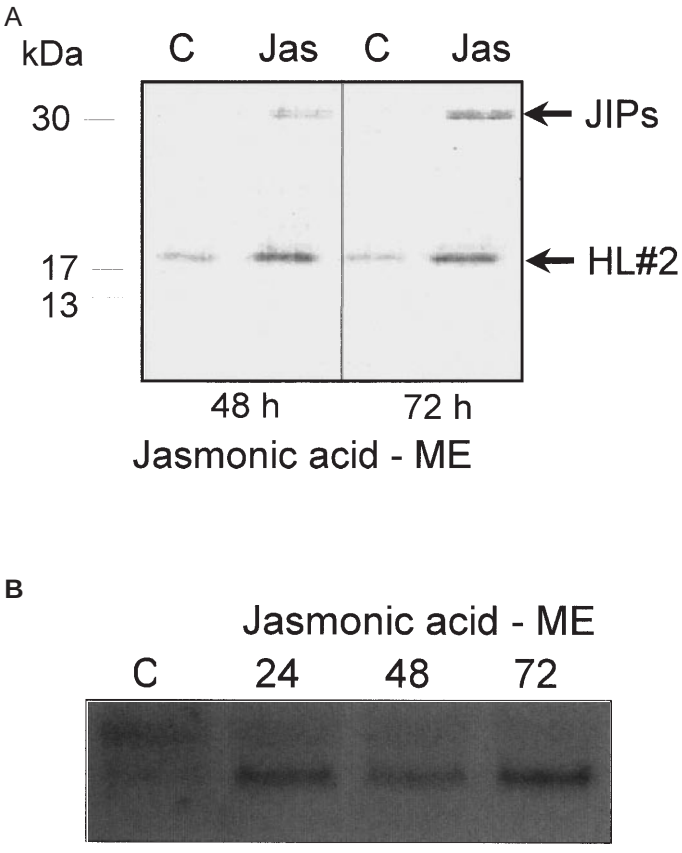

Fig. 1. **A** Immunoblot showing induction of JIPs in detached green barley leaves by jasmonic acid-ME. Leaves detached from 6-d-old plants grown in the standard light/dark regime were exposed to jasmonic acid-ME (45 μM) for the indicated times in low light (70 μmol m^{-2} s^{-1}). During incubation with jasmonic acid-ME (*Jas*), two JIPs (32.6 and 32.7 kDa) accumulate. These JIPs cross-react with the antibody to HL#2 and are not seen in the controls (*C*) without jasmonic acid-ME. The protein of >17 kDa represents HL#2 (*arrow*). **B** Transcript levels of HL#2 after application of jasmonic acid-ME to detached leaves for the indicated times (hours) under low light (70 μmol m^{-2} s^{-1})

Figure 1B shows the effect of treatment with jasmonic acid-ME on the level of the HL#2 mRNA in barley leaves. The levels of HL#2 mRNA are upregulated in the presence of jasmonic acid-ME within 24 h while the proteins accumulate only after 48 h. The protein blot did not detect a band after 24 h of incubation (data not shown).

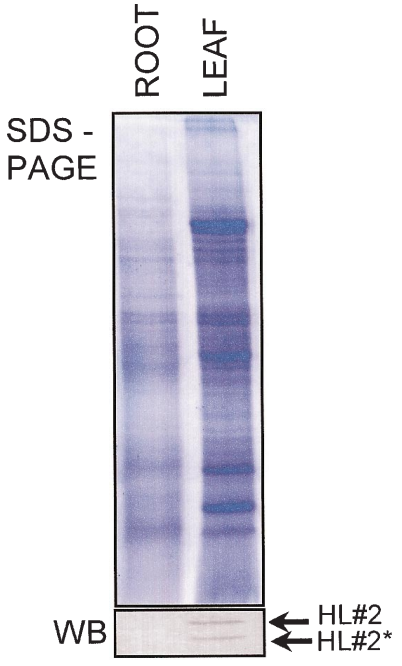

Fig. 2. Organ-specific expression of HL#2 in barley. Six-day-old light/dark-grown seedlings were harvested. Total protein was extracted from roots and primary leaves and subjected to SDS-PAGE (*upper panel*) and immunodetection by western blotting (*WB*). HL#2 and HL#2* were found in the primary leaves as indicated by the arrows

The application of abscisic acid (ABA) at concentrations of 100 to 200 μmol/L for up to 72 h did not influence the level of either HL#2 or the two JIPs (data not shown). The lack of accumulation of the HL#2 protein in the presence of ABA is in accordance with the observation that the mRNA for the leaf form of SalT, but not the corresponding protein, responds to the application of ABA (Moons et al. 1997).

Localization of HL#2 in barley cells. Initially, roots and leaves were analyzed for the presence of HL#2. Figure 3 shows that the protein was present in the leaves but could not be detected in the roots. At first glance this finding is surprising as in rice, SalT has preferentially been demonstrated in roots (Claes et al. 1990). Also more recently, the presence of SalT was observed in roots as well as in shoots (Moons et al. 1997). It is therefore possible that the antibody against HL#2 might not recognize a homologous protein in the roots or, alternatively, that such a protein might not be expressed in barley under our conditions. Moreover, we observed in addition to the HL#2 protein of 18 kDa a protein of 15.5 kDa, HL#2*, which reacted specifically with the antibody (Fig. 2). The specificity is based on the fact that no signal could be obtained with the antibody raised against the overexpressed GST (Pötter and Kloppstech 1993). However, it should be mentioned, that a band of approximately 32 kDa was found in the membrane fraction (see Fig. 3A). In addition to the data shown in Fig. 2, we found considerable amounts of both the 15.5- and the 18-kDa protein in dark-grown seedlings 5 d after imbibition of the seeds (data not shown).

Figure 3A shows that HL#2 is exclusively found in the soluble fraction from leaf cells. There are some proteins in the membrane fraction which are detected by the antibody but they are different from HL#2 and their identity is not known. In differential-centrifugation experiments, the HL#2 protein could not be detected in the soluble fractions from mitochondria, the nuclei (Fig. 3B) or plastids (Fig. 3C). We thus may assume that the HL#2 protein prevails in the cytosol or in the apoplast as the analysis of the derived amino acid sequence by G. V. Heijne (University of Stockholm, Sweden) gave no conclusive evidence for the existence of a clear-cut transit sequence for chloroplasts or mitochondria. The definitive answer, however, would require transport experiments or immunodetection by microscopic analyses.

The effect of high light fluxes on the protein and RNA levels in the barley leaf. The analysis of the distribution of the HL#2 proteins in the barley leaf is shown in Fig. 4. Green leaves of 6-d-old plants were cut at the base and exposed for various times at a photon flux density of 1800 μmol m^{-2} s^{-1} for up to 8 h. Immediately at the end of the exposure the leaves were cut into three segments of equal length and total proteins extracted. The analysis of total proteins (Fig. 3C) showed that chloroplast proteins are accumulated in the apical part of the leaf. In contrast, HL#2 is distributed over the entire leaf with a preference towards the base. The latter observation is in agreement with the distribution of the mRNA for HL#2 as determined by dot-blot hybridization (Pötter et al. 1996). In addition, the antibody detects the second band of apparent molecular mass 15.5 kDa, which is exclusively located in the basal part of the leaf. Although the apparent molecular masses of the two immunoreactive proteins are indicative of a precursor-product relationship the unequal distribution of the two immunoreactive proteins within the leaf gradient does not support such a relationship as it is the higher-molecular-mass form which prevails in the older, i.e. more mature, cells. In addition, the higher-molecular-mass form of HL#2 appears somewhat more concentrated in the basal part of the leaf. We have to consider, however, that although equal amounts of protein have been applied to the gel the protein concentration in the basal part of the leaf is lower on a per-segment basis as well as on a fresh-weight basis than in the other, more apical segments; this aspect is visualized by the schematic representation of a leaf below the graph of Fig. 4. It is thus more realistic to state that HL#2 is more or less equally distributed throughout the leaf in contrast, for example, to proteins of the chloroplast such as the major LHC II protein or the subunits of the Rubisco which accumulate preferentially in the apical part of the leaf. This distribution of HL#2 agrees rather well with that of its mRNA as the amounts of HL#2 mRNA have been found to be elevated in the base of the leaf, again on a per unit of

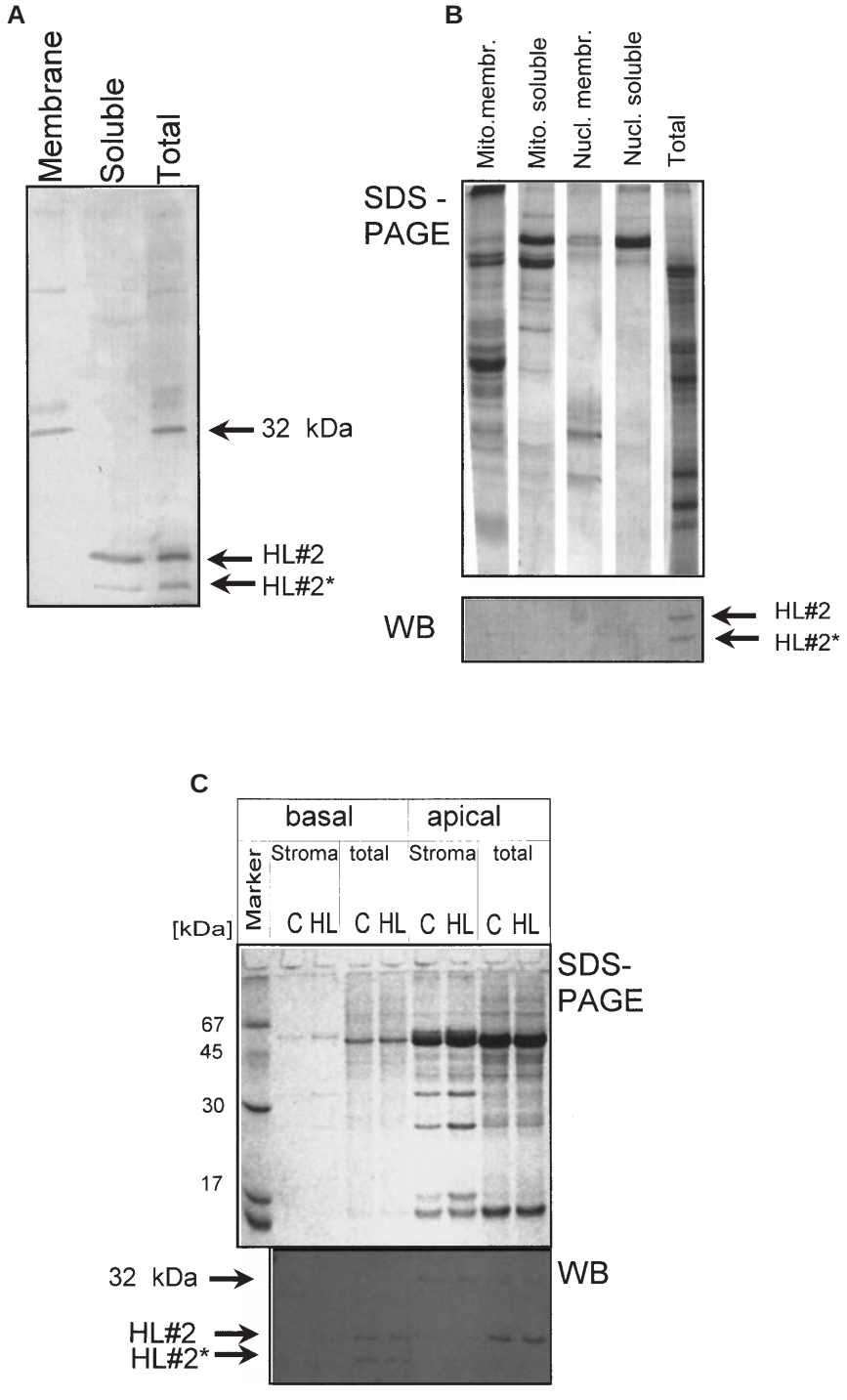

Fig. 3A–C. Localization of HL#2 within the barley leaf. Analyses by SDS-PAGE (*A–C*) and western blotting (*WB*; **B,C**). Six-day-old light/dark-grown seedlings were harvested and fractionated. **A** Separation of the total soluble and membrane proteins. The HL#2 proteins are found in the soluble fraction. The identity of the immunoreactive band at 32 kDa is not known. **B** The HL#2 proteins are not found in mitochondria or nuclei from 4-h high-light-treated (*HL*) or control (*C*) plants. **C** HL#2 is present in total extracts from apical and basal segments of primary leaves but not in extracts from isolated, purified chloroplasts (*Stroma*)

total RNA basis; this analysis per unit of RNA does not take into consideration that mature cells in the apical part of the leaf, including their chloroplasts, accumulate considerable amounts of ribosomal RNA which dominates the total RNA. The 18- and 15.5-kDa HL#2 proteins occur in almost the same concentration per unit of applied protein if we assume that the reactivity of the two proteins towards the antibody might be similar. At present there is no possibility of further discriminating between these two proteins as only evidence for a single gene containing one intron has been obtained.

Since the RNA data (Pötter et al. 1996) were not in agreement with the protein data (Fig. 4) as far as induction by high light is concerned we decided to repeat the quantification of the RNA for HL#2 by using northern blotting. This method is superior to dot-blot analyses at least as far as the determination of the size of the RNA and the specificity of the hybridization signal is concerned. The data from this analysis are summarized in Fig. 5, which shows a number of additional interesting properties of the RNA. First, it is confirmed that the mRNA levels are influenced by the duration of

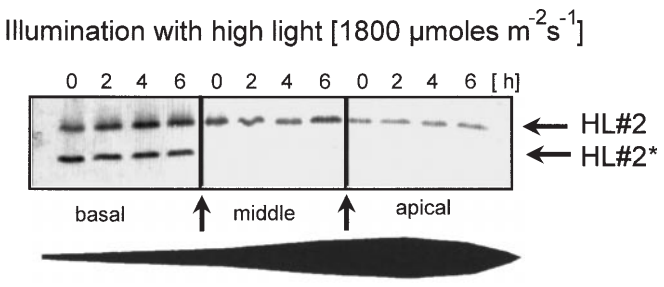

Fig. 4. The effect of high light on the expression of the HL#2 proteins in different segments of the barley leaf. HL#2* appears only in the basal segment. There is hardly any effect of the duration of the high-light treatment on the appearance of the two proteins under these conditions

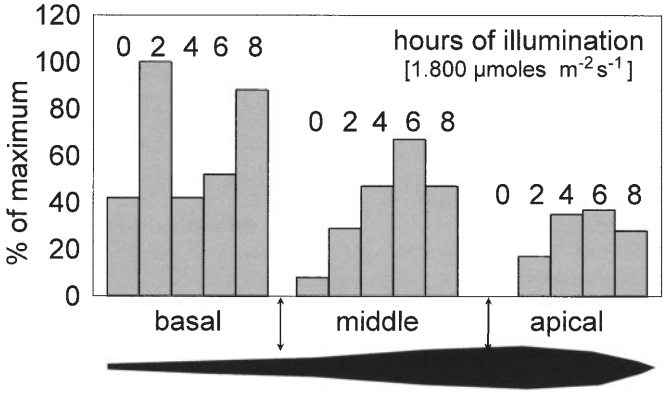

Fig. 5. The influence of the duration of the light treatment on the expression of HL#2 mRNA. Barley leaves were illuminated for the indicated times with high light of 1800 µmol m^{-2} s^{-1} at room temperature. The leaves were harvested and fractionated into three segments. The total RNA (10 µg per lane) was separated by gel electrophoresis and blotted to nitrocellulose membranes. The signals were quantified after autoradiography. The linearity of the signal with exposure time was ascertained

high-light treatment (Pötter et al. 1996) as there was an increase for up to 8 h throughout the entire leaf. Furthermore, there was a decline in the HL#2 mRNA levels of the low-light controls (0 h HL) towards the apical part of the leaf, such that this RNA could no longer be detected in the apical segment. This behaviour may explain why the total levels of HL#2 mRNA appeared to decline towards the tip of the leaf (Fig. 5). Finally, the mRNA levels which prevail in the apical part of the leaf depend to a higher extent on the duration of high-light treatment than those of the basal part. It should also be mentioned that the mRNA which is confined to the basal segments responds more quickly to light than the mRNA which is found throughout the leaf thus explaining the double peak in the response to light stress in the basal segments. However, so far there is no further evidence which favours the existence of two mRNA species coding for the HL#2 proteins.

The effect of high light and low temperature on the induction of HL#2. It is well known that low temperature increases the damaging effect of high-light fluxes. One explanation is that the absorption of light is temperature-independent while chemical reactions are influenced by the temperature. The levels of ELIP mRNA (Adamska and Kloppstech 1994) and protein have been found to be enhanced under the combination of cold and light stress (Montané et al. 1996, 1997). A similar observation does not hold true in the case of HL#2 proteins. Despite the similarity in the induction of ELIP and HL#2 by high light that has been described so far, we did not observe an increase in the mRNA and protein levels of HL#2 as a result of up to 36 h of exposure of green leaves to the combination of high light and low temperature (data not shown). This indicates that these two groups of light-stress proteins, which have been shown to be under light-control at the level of their mRNA, should be induced by at least partially divergent signal transduction chains.

Discussion

The antibody raised against the fusion protein obtained in *E. coli* HL#2 proteins in goat recognized two related proteins in barley. Of these the 18-kDa protein (HL#2) had approximately the same apparent molecular mass as was calculated from the cloned cDNA sequence. Thus we assume that the determination of the size of the smaller protein of 15.5 kDa (HL#2*), for which so far no sequence data exist, should also be correct. Although these data indicate a precursor-product relationship between the two bands, such a relationship appears unlikely on the basis of the available information. Firstly, the lower-molecular-mass form appears to exist only in the basal segments of the leaf while the other form is distributed throughout the entire leaf and appears to be the exclusive form in the apical part. If we take into consideration that the younger cells are at the base of the leaf we prefer the interpretation that these bands represent two independent but closely related proteins. At least one of these two proteins (HL#2) might possess an aminoterminal sequence which could represent a transit peptide. The evidence for the existence of such transit peptide was suggested by de Pater et al. (1992) who claimed that a plastid transit peptide might be contained in the sequence of a shoot-specific protein and by the similarity that was observed between this protein and HL#2. It has never been shown that this peptide has a plastid-targeting function.

Our data favour a localization of both proteins exclusively in the shoot in contrast to rice where a protein (SalT) with high similarity has also been found in the roots (Claes et al. 1990). Presently there is no explanation available for this difference between the otherwise rather closely related plant species. The argument that a related HL#2 in the roots might be entirely dependent on the induction by salt does also not hold true. Exposure of roots to 175 mM salt for 3 d does not induce the mRNA for HL#2 (data not shown). This is in contrast to the behaviour of the related SalT.

One important observation during this study was that the mRNA levels for at least one form of HL#2 respond

to high light fluxes in a manner similar to those of ELIPs. This observation seems to limit the number of similarities as the levels of both proteins showed almost no change in response to varying light intensity during short-term exposure. This observation can easily be explained by the assumption of posttranscriptional control. The controlling mechanism is not known. The HL#2 protein is also different from ELIPs with respect to control by low temperature. In the green leaves investigated, the quantities of the two HL#2 proteins did not change in response to a lowering of the environmental temperature. We have previously suggested a control of ELIP levels by the redox poise of the photosynthetic chain (Montané et al. 1997). The same type of regulation may hold true for HL#2 but eventually only for the accumulation of mRNAs. This indicates the existence of at least two different signal chains which are connected to the high-light receptor which is proposed to exist within the chloroplast. Indeed, blue and red light have differential effects on gene expression in etiolated plants (Anni and Akoyunoglou 1981; Chory 1992) and it has been shown that in addition to the red-light-sensitive phytochromes at least two types of photoreceptor exist in the blue region of the spectrum (Lin et al. 1998).

Another remarkable observation is the finding that HL#2 belongs to the group of JIPs. Jasmonic acid and its methyl ester are ubiquitously occurring plant growth regulators (Meyer et al. 1984) apparently involved in the regulation of biotic and abiotic stress responses. Jasmonates are able to inhibit, promote or induce various events of plant growth and development (Sembdner and Parthier 1993). Several genes are known to be expressed specifically after application of jasmonates. In leaves of the monocotyledonous plant barley, a thionin of 6 kDa and functionally unknown proteins of different molecular masses, such as 10/12 kDa, 23 kDa, 37 kDa and 100 kDa, have been observed after jasmonate treatment (Weidhase et al. 1987; Andresen et al. 1992). The diversity of jasmonate-responsive genes indicates that this plant growth regulator might be a signal molecule for a wide range of growth and developmental processes (Wasternack et al. 1995), perhaps including light-stress regulation.

The evidence of a similarity to JIPs is threefold. Firstly, the sequence comparison favours this view. Sequence comparisons using the Swiss protein data bank revealed a remarkable similarity to the two JIPs. Both derived amino-acid sequences share similarities of 65% (50% identity) with HL#2 in their carboxyterminal ends (Lee et al. 1996). Secondly, the antibody prepared against HL#2 recognizes rather specifically the two jasmonate-inducible proteins of 32.6 and 32.7 kDa found in the database (Lee et al. 1996). Finally, the mRNA levels of HL#2 are enhanced after the application of jasmonic acid-ME. Our observations led us to the conclusion that the difference between JIPs and HL#2 lies in the fact that the two JIPs are exclusively inducible by jasmonates while HL#2 can not only be induced by high-light stress but also enhanced by jasmonate. So far, nothing is known about the effect of light on the mRNA levels of the two mentioned JIPs which do not show cross-hybridization with the probe of HL#2.

While at present the function of HL#2 proteins remains obscure, a repeat search in the Swiss protein data bank has confirmed that the similarities between SalT, Gos9 and HL#2 concern the entire protein. Similarities were also observed with jacalin (Skea et al. 1988) and ipomoelin (Imanishi et al. 1997), indicating the existence of sugar-binding domains at least in the C-terminal part of HL#2. Interestingly, ipomoelin of sweet potato is inducible in leaves by jasmonic acid-ME and is not detected in roots. The domains between these two groups of proteins, salt-stress-regulated proteins and lectins (SalT and ipomoelin), overlap to some extent. Ipomoelin has only a limited functional similarity to the mentioned lectins as it can be enriched on an affinity glucose column but the affinity was found to be considerably lower than that of a proper lectin. Considering the rather high similarity of 65% the same observation may hold true for HL#2. The analyses indicate that this group of proteins is generated from two protein domains by gene fusion. Such an interpretation might also explain the rather high variability in the molecular size and organ specificity, as well as in their hormone and light responses. According to our data, HL#2 should be close to the branching point of the group of proteins which include for instance ipomoelin and jacalin. The function of these proteins remains to be elucidated.

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