Plant Physiol. (1992) 100, 1764–1771
0032-0889/92/100/1764/08/$01.00/0

Received for publication March 26, 1992
Accepted July 21, 1992

Structure and Expression of a Heat-Shock Protein 83 Gene of *Pharbitis nil*[1]

Roderick F. Felsheim and Anath Das*

Department of Biochemistry (R.F.F., A.D.) and Plant Molecular Genetics Institute (A.D.), University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT

Four cDNA clones representing mRNAs whose levels were affected by a photoperiod that induces flowering in *Pharbitis nil* were isolated by a differential hybridization screening procedure. The level of mRNAs represented by three clones (12L, 15L, and 17L) increased following a photoperiod that induces flowering and that represented by the fourth clone (clone 27) increased under conditions in which flowering was inhibited. DNA sequence analysis showed that one cDNA, clone 17L, is homologous to members of the 83- to 90-kD heat-shock protein (hsp) gene family. The corresponding gene, *hsp83A*, was isolated and its DNA sequence was determined. *hsp83A* encodes a protein that exhibits 70% amino acid identity with *Drosophila melanogaster* HSP83. The *P. nil hsp83A* gene contains two introns within the coding region. *hsp83A* mRNA was not detectable in cotyledons of plants grown in continuous light, but its level increased transiently following a 14-h dark period and reached a maximum 2 h after the lights were turned on. A dramatic increase in the level of *hsp83A* mRNA was also found 2 h after an end-of-day dark treatment. Genomic Southern blot analysis demonstrated that the *P. nil hsp83-90* gene family consists of at least six members, one of which appears to be constitutively expressed in the light.

Flowering in plants is dependent on several environmental factors including photoperiod. *Pharbitis nil* (Japanese morning glory) is a qualitative SD plant whose seedlings can be induced to flower following exposure to a single dark period of sufficient length (≥10 h) (reviewed in refs. 6 and 29). Flowering can be inhibited with a night break at the 8th h after the beginning of the dark period. The primary sensor of the photoperiod in seedlings is the cotyledons. Physiological studies indicate that in response to the critical night length the cotyledons produce a transmissible substance that is rapidly transported to the apical meristem, the site of stable changes, to initiate processes leading to the transition from vegetative to reproductive growth (15, 16). Biochemical studies indicated that flowering in *P. nil* is suppressed by application of actinomycin D, an RNA synthesis inhibitor, to the cotyledons before a dark treatment (2). In a similar study, cyclohexamide, an inhibitor of protein synthesis, was found to inhibit flowering in another SD plant, *Xanthium strumarium* (27). Results of these studies suggest the involvement of new RNA and protein synthesis in floral induction.

To investigate the molecular changes associated with the photoperiodic inductive process we used differential hybridization screening to isolate genes whose expression is altered upon exposure of plants to an inductive photoperiod. In this paper, we report the isolation of four cDNA clones that represent mRNAs whose steady-state levels are affected by photoperiod. DNA sequence analyses of two of the cDNA clones indicate that they can encode proteins homologous to two families of known hsps[2]. We report the structure, sequence, and expression of one of these genes that is a member of the eukaryotic *hsp83–90* gene family.

MATERIALS AND METHODS

Plant Material

Pharbitis nil, strain Violet, seeds were purchased from Marutane Co., Ltd., Japan. Seeds were treated with concentrated sulfuric acid for 40 min and rinsed in running tap water at 28 to 30°C for 30 min. The seeds were allowed to imbibe in tap water for about 10 h at 30°C and germinated between moist Whatman 3MM filter paper overnight at 30°C. The germinated seeds were selected for uniformity, sown in Gibco professional soil mix, and grown in a growth chamber for 5 d at 23°C in continuous fluorescent white light (110 μE m^{-2} s^{-1}). At this time, plants were subjected to an inductive dark period of 14 h. For control experiments, plants received a 20-min red light interruption in the middle of the dark period. Red light was obtained by wrapping the fluorescent bulbs with red cellophane. After the dark period, plants were exposed to light as indicated in the figure legends. The cotyledons were excised, frozen in liquid nitrogen, and stored at −70°C. Plants were scored for flowering about 4 weeks following the dark treatment.

For heat-shock experiments plants were grown in continuous light. The cotyledons were excised and submerged in a solution containing 1 mM potassium phosphate, pH 6.0, and 1% sucrose for 2 h at 25°C (control) or at 40°C (heat shock).

For the experiment shown in Figure 7, seeds were germinated and grown in darkness for 2½ d, at which point they had emerged from the soil. Plants were then subjected to 12 or 24 h of cool-white fluorescent light (145 μE m^{-2} s^{-1}) before

[1] This work was supported by grants from the National Institute of Health (GM42557) and the Agricultural Experiment Station of the University of Minnesota.

[2] Abbreviations: hsp, heat-shock protein; SSC, standard sodium citrate; GuSCN solution, 6.25 M guanidinium isothiocyanate, 0.05 M Tris (pH 7.5), and 0.01 M EDTA.

light treatments as described in the figure legend. Where indicated, 650- and 750-nm plastic filters (Carolina Biological Supply) on boxes were placed over the plants. The amount of light reaching the plants under the 650- and 750-nm filters was 5 and 0.05 $\mu E\ m^{-2}\ s^{-1}$, respectively.

RNA Isolation

Total RNA from *P. nil* cotyledons was isolated by a phenol/SDS extraction procedure described by Ausubel et al. (3). Contaminating polysaccharide material was removed by adsorption onto cellulose (21). Poly(A)$^+$ RNA was purified by chromatography on an oligo(dT)-cellulose column (4).

Construction and Screening of *P. nil* Libraries

Poly(A)$^+$ RNA, isolated from induced *P. nil* cotyledons harvested 2 h after a 14-h dark period, was used to construct a cDNA library in the bacteriophage vector λgt10 (13). Approximately 200,000 recombinants were plated, and duplicate filters were screened with radiolabeled cDNA probes prepared against poly(A)$^+$ RNA from induced and control cotyledons. Probes were generated with Maloney murine leukemia virus reverse transcriptase (BRL) using conditions recommended by the manufacturer. Clones representing mRNA whose abundance is affected by an inductive photoperiod were isolated by three rounds of screening. The hybridization for the differential screen was carried out with 9 × 10^6 cpm of probe DNA per mL in 50% formamide, 3× SSC, 5× Denhardt's solution, 50 mM sodium phosphate (pH 6.5), 2 mM EDTA, 0.1% SDS, and 100 μg/mL of denatured salmon sperm DNA at 50°C for 24 h and at 42°C for an additional 12 h. The filters were washed at 65°C in 2× SSC and 0.1% SDS at 65°C and in 0.2× SSC and 0.1% SDS at 65°C. Each wash was for 30 min.

To isolate genomic clones corresponding to the cDNA clones of interest, a *P. nil* genomic library constructed in bacteriophage λ vector EMBL3 was screened with the appropriate cDNA clones as probes. The genomic library was constructed by cloning *Sau*3AI fragments of *P. nil* genomic DNA into the *Bam*HI site of EMBL3. A single clone homologous to the *hsp83A* cDNA clone was isolated by screening 400,000 recombinant plaques under high stringency. Four additional overlapping *hsp83A* clones and the *hsp83B* genomic clones were isolated by low-stringency hybridization to a *P. nil* genomic library created similarly in Stratagene's λ DASH vector by Dr. John Larkin.

Nucleic Acid Hybridization

Low-stringency Southern hybridization analysis was performed in 30% formamide, 3× SSC, 50 mM sodium phosphate (pH 6.5), 5× Denhardt's solution, 0.1% SDS, 2 mM EDTA, and 100 μg/mL of denatured salmon sperm DNA. Hybridization was for 16 h at 45°C. Following hybridization, blots were washed at room temperature twice with 5× SSC and 0.1% SDS, once with 0.2× SSC and 0.1% SDS, and finally with 0.2× SSC and 0.1% SDS at 50°C. Each wash was for 30 min.

Northern hybridizations were performed using Nytran membranes as directed by Schleicher and Schuell except that the hybridization solution was identical with that described for the differential screen hybridization. DNA probes for hybridizations were prepared by the random primer method.

DNA Sequence Analysis

The DNA sequences of the cDNA clones and the *hsp83A* gene were determined by the dideoxy-chain termination method using Sequenase (28, United States Biochemical). Template DNA, prepared by the alkaline lysis procedure, was passed through a Sepharose CL-6B spun column following sodium hydroxide denaturation (22). For sequence analysis of the *hsp83A* genomic clone, overlapping segments of the gene were subcloned into the plasmid vector pBluescript KS(+/−) (Stratagene, Inc.). Overlapping deletions were constructed by exonuclease III-mung bean nuclease treatment (Stratagene, Inc.). A segment of the gene, residues 1586 to 1924 (Fig. 3), could not be cloned into a plasmid vector. The DNA sequence of this region was determined using synthetic deoxyoligonucleotides as primers and the recombinant phage DNA as the template.

RNase Protection

The RNase protection analysis (Fig. 6) was performed according to the method of Maniatis et al. (20). A plasmid RF117 was constructed by cloning a 3.36-kb *Eco*RI fragment from the genomic clone (residues −2095 to +1266) into the plasmid vector pBluescript KS(−) (Stratagene, Inc.). pRF117 DNA was linearized at an *Hpa*I site at position −536 and was used as a template for in vitro transcription by T7 RNA polymerase in the presence of radiolabeled CTP. After purification, the radiolabeled RNA (100,000 cpm), complementary to the *hsp83A* mRNA, was hybridized overnight to 20 μg of total RNA at 55°C. The hybridization mixture was diluted with 300 μL of solution containing 300 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl (pH 7.5), 50 μg/mL of RNase A, and 2 μg/mL of RNase T1. The reaction was incubated at 37°C for 1 h. Proteinase K and SDS were added to a final concentration of 0.2 mg/mL and 0.5%, respectively, and the incubation was continued for an additional 30 min at 37°C. The samples were extracted with 1 volume of a phenol/chloroform mixture, and the nucleic acids in the aqueous layer were precipitated by the addition of 2 volumes of ethyl alcohol. The pellet was resuspended in 20 μL of gel-loading buffer (80% formamide, 10 mM EDTA) and heated for 3 min at 100°C, and a 5-μL aliquot was loaded on a urea-6% polyacrylamide sequencing gel.

The RNase protection analysis (Fig. 7) was performed using a procedure modified from that described by Firestein et al. (10). Cotyledons of 10 plants per treatment were frozen, ground in liquid nitrogen, and homogenized in the presence of 10 mL of phenol and 8 mL of 6.25 M GuSCN solution. Chloroform (10 mL) was added, and the sample was mixed and centrifuged for 20 min at 3500 rpm in a table-top centrifuge. The aqueous layer was precipitated with ethanol, and the pellet was washed with 5 mL of 3 M sodium acetate, pH 5.2, and centrifuged at 10,000*g* for 10 min. The mRNA-containing pellet was dried and resuspended in 100 μL of 5

M GuSCN solution per gram of tissue. Ten microliters of this RNA was mixed with 1.5 μL of antisense RNA probe in 5 M GuSCN solution, heated to 65°C for 3 min, and hybridized for 2 h at room temperature. The samples were then diluted with 400 μL of RNase solution (0.3 M NaCl, 0.03 M Tris [pH 7.5], 0.01 M EDTA, 50 μg/mL of RNase A, and 1 μg/mL of RNase T1) and incubated at 30°C for 30 min. Proteinase K was added (0.2 mg/mL), and the samples were incubated at 37°C for 30 min. After the sample was extracted with phenol/chloroform, the aqueous layer was precipitated with ethanol and glycogen. The pellet was resuspended in 6 μL of 80% formamide and 0.01 M EDTA and run on a urea-polyacrylamide sequencing gel. RNA probes for *hsp83A* and *hsp83B* were synthesized by in vitro transcription of linearized pRF110S and pRF256SA, respectively. Plasmid RF110S contains residues −2095 to +231 as an *Eco*RI-*Sac*I fragment in plasmid vector pBluescript KS. Plasmid RF256SA contains the homologous region of the *hsp83B* gene.

Primer Extension

The transcription start site of *hsp83A* was determined by primer extension as described by Ausubel et al. (3). An end-labeled synthetic deoxyoligonucleotide, dCTCGGCCATCTGAACATCCG, complementary to residues 116 to 135 (Fig. 2), was hybridized to 25 μg of total RNA isolated from heat-shocked *P. nil* cotyledons. Hybridization was performed overnight at different temperatures, as indicated in the legend to Figure 7.

RESULTS

To determine the time at which the maximum difference in the mRNA population of induced and uninduced plants could be found, two-dimensional gel electrophoretic analyses of in vitro translation products were performed. Plants were subjected to an uninterrupted dark period of 14 h (induced plants) or to a similar treatment with a 20-min red light night break in the middle of the dark period (uninduced plants). The cotyledons were harvested at various time points relative to the end of the dark period. Poly(A)$^+$ RNA was isolated from the cotyledons and translated in vitro in a rabbit reticulocyte lysate system in the presence of [^{35}S]methionine, and the translation products were analyzed by two-dimensional gel electrophoresis (23). Significant differences in the polypeptide pattern of induced and uninduced samples were observed in poly(A)$^+$ RNA isolated from cotyledons harvested 2 h after the dark period (data not shown). This time was chosen for the construction and screening of the cDNA library.

A cDNA library representing poly(A)$^+$ RNA from induced cotyledons was constructed in bacteriophage vector λgt10 and screened by differential hybridization with radiolabeled cDNA probe prepared against cotyledon RNA isolated from induced or uninduced (treatment with a night break) plants (3, 13). Four unique clones that represented mRNA whose level is altered by an inductive photoperiod were isolated by this approach. These four cDNA clones were used to probe poly(A)$^+$ RNA from cotyledons of induced (I) and night break control (C) plants in a northern blot hybridization assay (Fig. 1). The level of mRNA corresponding to clones 12L, 15L, and 17L increased following an inductive photoperiod (lanes 3–8) and that corresponding to the fourth clone, clone 27, decreased under these conditions (lanes 1 and 2). Time-course analysis by RNA dot blots showed that the level of RNA corresponding to two of the clones (12L and 17L) peaked 2 h after the lights were turned on and rapidly decreased thereafter. These two clones were chosen for further analysis of the light dependence and the inhibitory effect of the night break on the expression of the corresponding genes.

The DNA sequences of the cDNA clones were determined, and comparison of the polypeptides encoded by the cDNA clones with sequences in the Genbank data base showed that two of the gene products were homologous to hsps. The gene product encoded by clone 12L was homologous to members of a small hsp gene family of plants, and that encoded by clone 17L was homologous to 83-kD hsps found in animals. No sequence homologous to the polypeptides encoded by clones 27 and 15L was found in the data base. In this paper, we report the structure and expression of a *P. nil hsp83* gene.

Isolation of *hsp83* Genomic Clone and Structural Features of the Gene

A *P. nil* genomic library was constructed in the bacteriophage λ EMBL3 vector, and a clone containing the *hsp83* gene was identified by screening 400,000 recombinants with

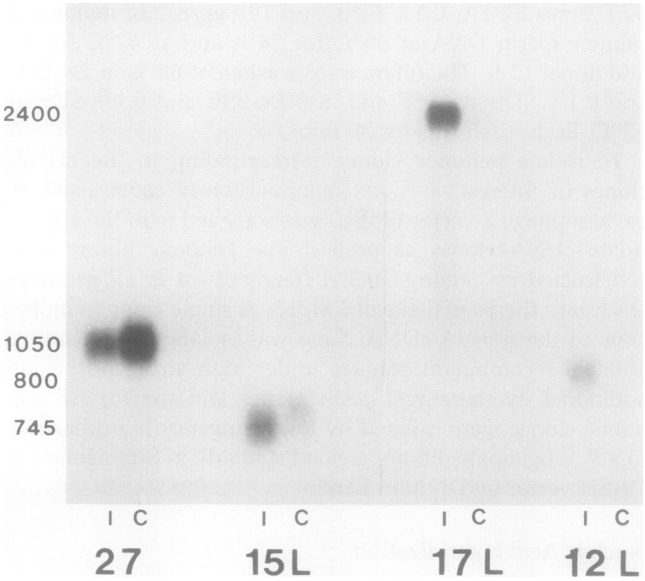

Figure 1. Effect of photoperiod on the level of 27, 12L, 15L, and 17L mRNA accumulation. Poly(A)$^+$ RNA (0.4 μg) was subjected to electrophoresis on a 1% agarose-formaldehyde gel, blotted onto a Nytran membrane, and hybridized with ^{32}P-labeled cDNA probes as indicated. Lanes I, RNA isolated from cotyledons of induced plants 2 h after the dark period; lanes C, RNA isolated from cotyledons of night break control plants 2 h after the dark period. Numbers on the left indicate the approximate size (nucleotides) of the transcripts.

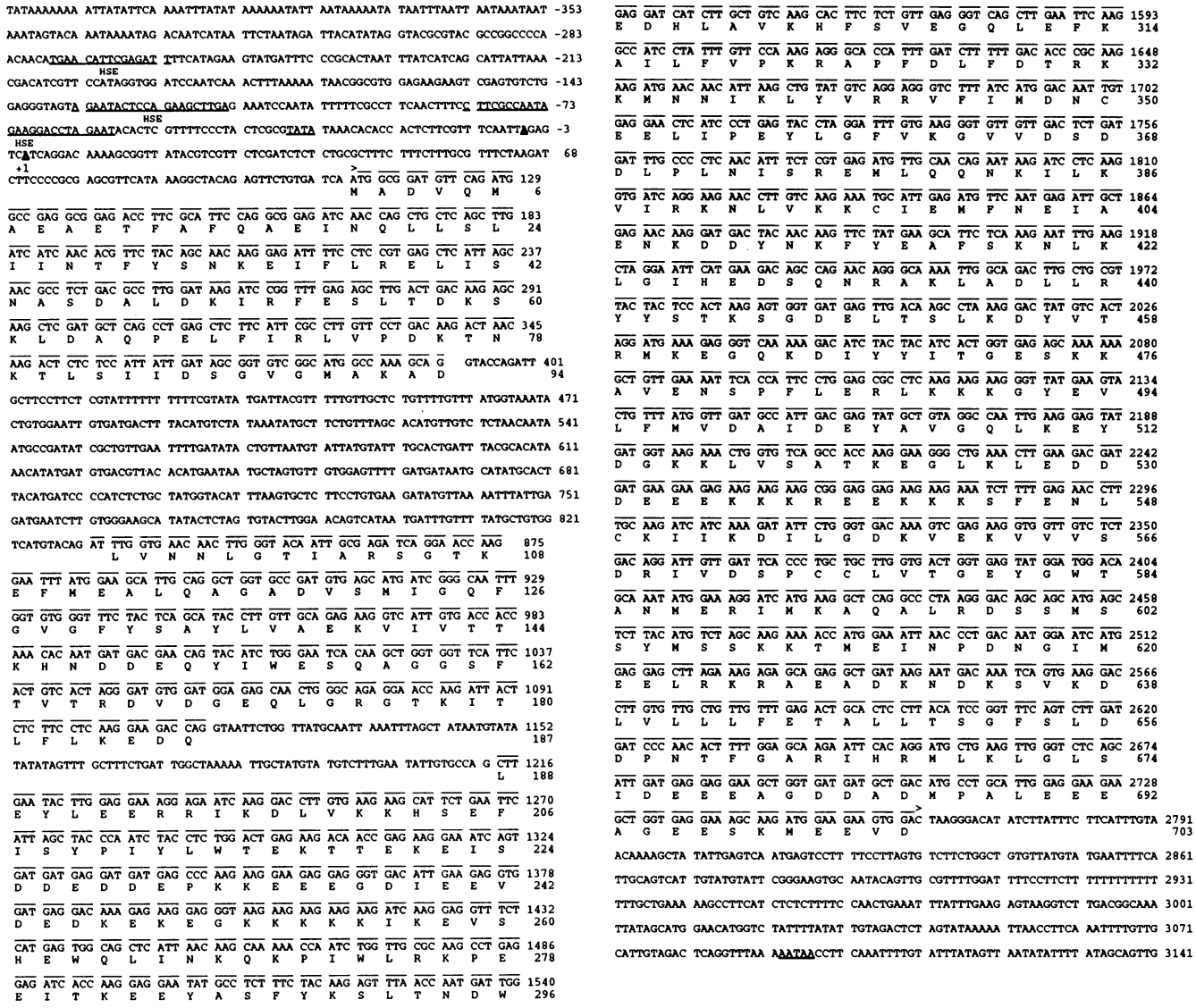

Figure 2. Nucleotide sequence of the *P. nil hsp83A* gene. The complete DNA sequence and the encoded polypeptide sequence are shown. The transcription start sites are shown in bold and underlined. The potential TATA element, heat-shock elements, and polyadenylation signal are underlined. (GenBank accession No. M99431.)

the cDNA clone 17L as a probe (20). Restriction fragments encompassing the *hsp83A* gene were subcloned into plasmid pBluescript KS(+/−) (Stratagene, Inc.) for DNA sequence analysis. A region of the gene (residues 1586–1924, Fig. 2) could not be cloned into this vector. The DNA sequence for this region was obtained directly from the λ clone using synthetic deoxyoligonucleotide primers and the phage DNA as a template. The complete sequence of the *P.nil hsp83A* gene is presented in Figure 2. In the 5′-nontranscribed region, several copies of the heat-shock element, nGAAn, are found arranged in alternating orientation. These sequences are known to control heat-induced gene expression (1, 25). Upstream of the transcription start site, a putative TATA element is located at position −36 to −33, and a putative CAAT element is found at position −77 to −74.

The coding region of *hsp83A* is interrupted by two introns of 439 and 103 bp. The position of the introns was initially assigned on the basis of intron-exon junction consensus sequences and amino acid sequence comparison with its homologs and was subsequently confirmed experimentally by RNase protection analysis (see Fig. 6). The *P. nil hsp83* open-reading frame has two methionine codons that could act as the initiation codon. The sequence context of the first methionine codon has only one mismatch when compared to the consensus sequence (AACAAUGGC) found around initiation codons in plants (18), whereas the sequence around the second methionine codon (amino acid 6) has four mismatches; therefore, the first codon was used as the putative initiation codon (Fig. 2). The polypeptide encoded by the *hsp83A* gene is predicted to be 703 amino acid residues in

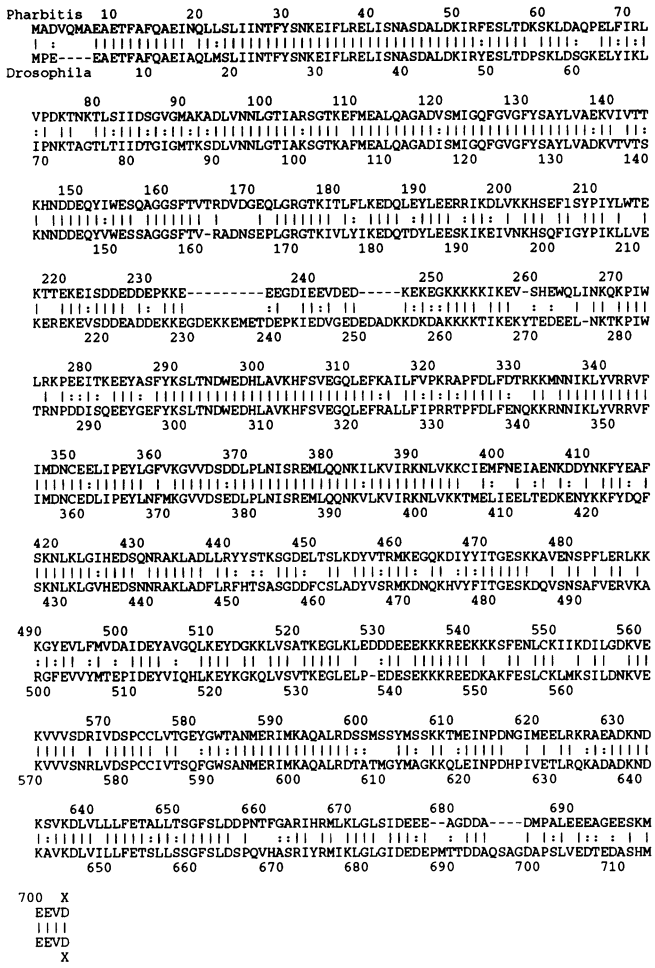

Figure 3. Comparison of polypeptides encoded by the *P. nil hsp83* and *Drosophila hsp83* genes. Sequence comparison was performed using the Intelligenetics Fastdb program. Identities and conservative changes are indicated by vertical lines and dots, respectively. Gaps were introduced to maximize homology.

length with a molecular mass of 80.8 kD. Comparison of this sequence with that in the Swiss-Prot Protein data base showed that the *P. nil hsp83* is most homologous to the *Drosophila hsp83* gene product (Fig. 3). The two gene products share 70% identity and 11% conservative changes.

The transcriptional start site of *hsp83A* under heat-shock conditions was determined by primer extension (Fig. 4). Two major extension products (indicated by arrows) were observed when the primer was hybridized at various stringency conditions. An increase in hybridization stringency had no effect on the pattern of primer extension products, suggesting that all products were derived from the *hsp83A* mRNA. The start site of the most abundant transcript was designated +1 (see Fig. 2). The start site of the next abundant transcript maps to residue −6.

Our preliminary studies of *hsp83* expression suggested that there may be additional expressed copies of the *hsp83* gene in *P. nil*. To determine the copy number of this gene, Southern blot analysis was carried out at low stringency (Fig. 5). *P. nil* genomic DNA was digested with *Eco*RI or *Hin*dIII and analyzed by Southern blot hybridization. In both cases, six to seven restriction fragments hybridized to a 328-bp *hsp83A* gene probe (which contains exon I sequences), indicating that *hsp83A* is a member of a multigene family. A *P. nil* genomic library was then screened at a low stringency to identify other members of the gene family. DNA sequence analysis of these clones (data not shown) and results of the low-stringency Southern hybridization studies indicate that there are at least six *hsp83* genes in *P. nil*. Recently a cDNA clone representing an *Arabidopsis hsp83* gene was isolated (9). The derived protein sequence of the *Arabidopsis hsp83* is 93% identical with that of the *P. nil hsp83A* gene product. The *Arabidopsis hsp83*, like its *P. nil* homolog, is also a member of a multigene family (9).

Mapping of Introns and Expression of *hsp83* in *P. nil*

RNase protection assays were used to confirm the location of the introns and to study the expression of the *hsp83A* gene. Total RNA isolated from plants subjected to different environmental conditions was hybridized to in vitro synthesized radiolabeled cRNA. The cRNA includes sequences (residues −536 to +1270) that span exons 1 and 2, a small segment of exon 3, and both introns (Fig. 6B). The use of a cRNA containing both introns allowed us to identify the location of the introns experimentally as well as to monitor expression of the *hsp83A* gene. It is expected, based on our intron location assignments, that in RNase protection assays three fragments of 391, 281, and 57 nucleotides corresponding to exon 1, exon 2, and the segment of exon 3, respectively, will be protected. Results presented in Figure 6 demonstrate that two fragments of 391 and 281 nucleotides, corresponding to exons 1 and 2, respectively, are protected from RNase digestion. The third expected fragment of 57 nucleotides was also detected but is not shown in this figure. The sizes of the protected fragments from the heat-shock sample (lane 1) and the light-induced sample (lane 5) are identical, indicating that the precursor RNA is processed properly and efficiently under both conditions.

Expression of *hsp83A* is strongly induced upon heat-shock (lane 1). No expression of this gene was observed in cotyledons when plants were grown in continuous light (lane 2). When plants were exposed to an uninterrupted dark period of 14 h and cotyledons were harvested at times following the dark period, expression of *hsp83A* was maximum 2 h after light treatment (lane 5). Expression declined thereafter, and no expression was detectable at or after 8 h of light exposure (lanes 8 and 9). Expression of *hsp83A* requires exposure of plants to light following the dark period because no *hsp83A* expression was observed in plants collected immediately after the dark period (lane 3).

In an attempt to reveal other aspects of the light-regulated abundance of *hsp83A* mRNA, we observed that the level of *hsp83A* mRNA increased dramatically soon after the transition to darkness (Fig. 7). The level of *hsp83A* mRNA was found to be very low in the cotyledons of plants grown in white light (lanes 1 and 9). When light below 650 nm was removed with a filter, no effect on the level of *hsp83A* mRNA was detected (lanes 2 and 10). A large increase in the level of *hsp83A* mRNA was observed when plants were transferred

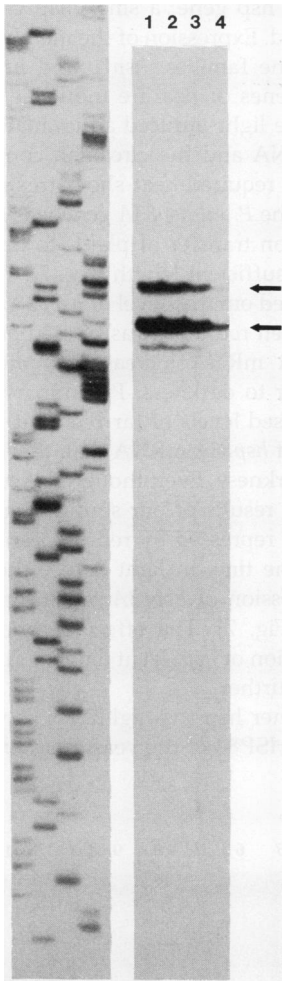

Figure 4. Identification of transcription start site of *P. nil hsp83A* gene. Primer extension analysis was used to identify the *hsp83A* transcription initiation site. Total RNA isolated from heat-shocked cotyledons was hybridized with a synthetic primer at 50, 55, 60, or 65°C, lanes 1 to 4, respectively. The major primer extension products are indicated by arrows. To determine the size of the products, a DNA-sequencing reaction (A,C,G,T) was performed using the same primer.

to darkness following 24 h of light (lane 12), whereas no increase was seen after transfer to darkness following 12 h of light (lane 4). When the red light was removed with a 750-nm filter, the level of *hsp83A* mRNA increased (lane 11) as it did following transfer to darkness. Thus, it seems the transition from light to dark as well as from dark to light leads to an increase in *hsp83A* mRNA in the cotyledons. In contrast, the level of *hsp83B* mRNA, corresponding to a second member of the gene family, was high in light and was not significantly affected by the various treatments (lanes 5–8 and 13–16).

DISCUSSION

Flowering in *P. nil* is controlled by photoperiod, and young seedlings are competent to respond to an inductive photoperiod. The cotyledons, in particular, respond by transmitting a substance(s) to the apical meristem to initiate the transition of a vegetative meristem to a floral meristem. To isolate genes whose expression in *P. nil* cotyledons is affected by an inductive photoperiod, a screening procedure utilizing differential hybridization was used. Four cDNA clones that represent mRNAs whose abundance was affected by an inductive photoperiod were isolated by this approach (Fig. 1). Two of the genes represented by these clones encode hsps—one homologous to plant small hsp genes and the other homologous to animal *hsp83* genes. The *hsp83* gene of *P. nil* is a member of a multigene family and is present in six to seven copies in the genome (Fig. 5). In contrast, there appears to be only one *hsp83* gene in *Drosophila* and two genes in yeast (7, 8). *hsp83A* is the most heat-inducible gene in cotyledons, whereas the others exhibit a low level of heat induction in cotyledons (our unpublished results). One member of the family (*hsp83B*) is constitutively expressed in the cotyledons of plants grown in continuous light (Fig. 7).

DNA sequence analysis showed that the *P. nil hsp83A* gene is interrupted by two introns within its coding region. Other genes of the *hsp83–90* family contain introns, including a *Drosophila hsp83* gene, but the processing of the *Drosophila* transcript is impaired under heat-shock conditions (30). Our studies demonstrate that the *P. nil hsp83* introns are processed properly and efficiently under heat-shock conditions (Fig. 6). The protein encoded by *hsp83A* is homologous to animal 83- to 90-kD hsps. As in eukaryotic HSP83s, the carboxy-terminal four residues, GluGluValAsp, are conserved in the plant gene product. The *P. nil* HSP83, like its eukaryotic homologs,

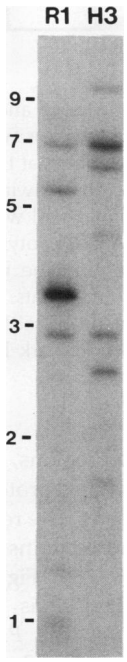

Figure 5. Determination of *hsp83* gene copy number. The number of *hsp83* genes in the *P. nil* genome was determined by low-stringency Southern blot hybridization. A 328-bp fragment (residues 68–395, Fig. 2) was used as a hybridization probe. Lane R1, *Eco*RI; lane H3, *Hin*dIII. Numbers on the left indicate size, in kb, of the molecular mass marker DNA.

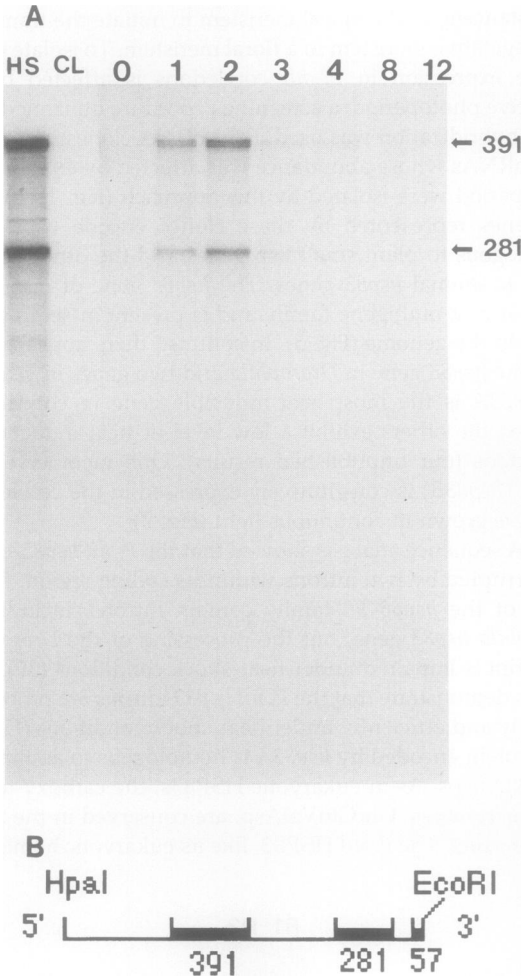

Figure 6. Expression of *hsp83A* gene and determination of intron locations. A, RNase protection analysis was used to monitor accumulation of *hsp83A* mRNA. Numbers at the top indicate time (h) at which cotyledons were harvested following exposure to a 14-h dark period. Other RNA samples analyzed were: HS, RNA from heat-shocked cotyledons; CL, RNA from cotyledons of plants grown in continuous light. The numbers on the right indicate the size (in nucleotides) of the protected fragments. B, Map of the DNA fragment used to synthesize the probe cRNA for RNase protection analysis. The exons are shown as dark bars. Numbers at bottom indicate size in bp.

contains two highly charged regions, A and B. These regions are speculated to be involved in protein-protein interactions with other cellular proteins (5). The region A of *P. nil* HSP83 is 45 residues in length and contains 21 acidic and 12 basic residues (amino acids 216–260, Fig. 2). The homologous region in the *Drosophila* HSP83 is 14 residues longer. In region B (residues 520–571), the *P. nil* HSP83 is highly homologous to other known HSP83s.

Expression of the *hsp83A* gene is regulated by both heat shock and light. Light-inducible expression of *hsp83A* requires exposure of plants to a dark period before exposure to light (Fig. 6). Under these conditions, expression of *hsp83A* is transient in nature, and maximum accumulation of *hsp83A* mRNA was observed 2 h after light treatment. Similar regulation of another hsp gene, a small hsp gene of *P. nil*, has also been observed. Expression of the members of two *Chlamydomonas* hsp gene families, *hsp70* and *hsp22*, and that of four small hsp genes of pea are inducible by light (11, 14, 24). However, the light-induced accumulation of *Chlamydomonas hsp22* mRNA and the circadian control of pea small hsp mRNA levels required heat-shock treatment as well.

Expression of the *P. nil hsp83A* gene was found to increase in cotyledons upon transfer of plants to darkness following a light period of sufficient length (Fig. 7). When light below 650 nm was filtered out, the level of *hsp83A* mRNA remained very low, but when red light was filtered out as well (below 750 nm), *hsp83A* mRNA increased to the level observed following transfer to darkness. Preliminary experiments indicate that increased levels of far-red light result in an even greater increase of *hsp83A* mRNA than that observed following transfer to darkness. Even though the cut-off filters used were suboptimal, results of our studies suggest that *hsp83A* mRNA levels are repressed by red light and *hsp83B* mRNA levels are not. The time in light before the dark treatment also affects expression of *hsp83A* much more strongly than that of *hsp83B* (Fig. 7). The effect of photoperiod on the transitory expression of *hsp83A* at dawn and dusk is currently being examined further.

HSP83, like other hsps, is highly conserved in all eukaryotes. Studies of HSP83 of the yeast *Saccharomyces cerevisiae*

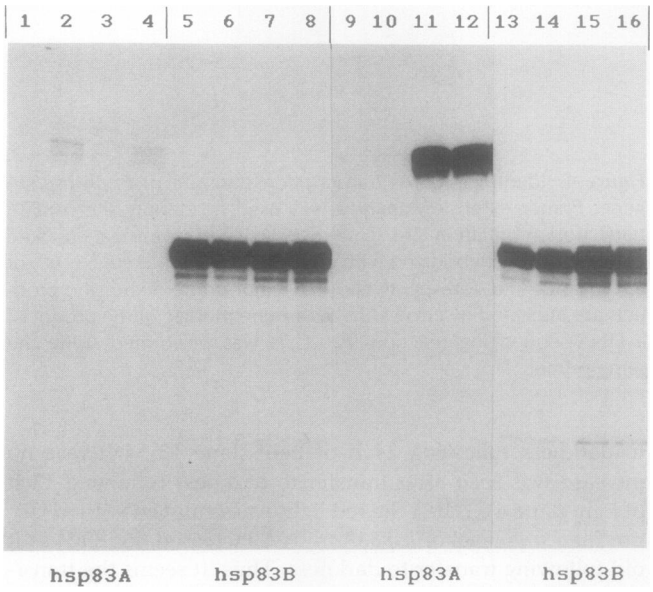

Figure 7. Dark-induced expression of *hsp83A* gene. RNase protection analysis was used to monitor accumulation of *hsp83A* and *hsp83B* mRNA. Plants were grown for 2½ d in the dark, at which time they had emerged from the soil. Following 12 h (lanes 1–8) or 24 h (lanes 9–16) in cool-white fluorescent light (145 μE m^{-2} s^{-1}), plants were subjected to one of four 2-h treatments: lanes 1, 5, 9, and 13, white light; lanes 2, 6, 10, and 14, light above 650 nm; lanes 3, 7, 11, and 15, light above 750 nm; lanes 4, 8, 12, and 16, dark. Cotyledons were harvested immediately following each treatment, and the steady-state level of *hsp83A* and *hsp83B* mRNA was determined using gene-specific RNA probes (indicated at the bottom).

and animals provide insight into its function. There are two *hsp83* homologs in yeast, *Hsp82* and *Hsc82* (8). Disruption of either gene impairs growth at elevated temperatures, and simultaneous inactivation of both genes by gene disruption is lethal, indicating that *hsp83* is an essential gene in yeast. Biochemical studies of HSP83 showed that it is found complexed with several proteins, including members of the steroid hormone receptor superfamily, casein kinase II, eukaryotic initiation factor-2 α-kinase, actin, and tubulin (reviewed in ref. 17). The most investigated of these is the interaction of HSP83 and steroid hormone receptors. These studies indicate that HSP83 functions as a special type of chaperon to maintain the receptor in a preinduction complex (reviewed in ref. 12). In response to a specific trigger molecule, the HSP83-receptor complex dissociates to form a hormone-receptor complex, which regulates gene expression. The requirement of HSP83 for proper signal transduction by steroid receptors was demonstrated experimentally (26). A similar function for HSP83 in plants is not unlikely in view of the fact that plants contain estrogens and a number of steroids, known as brassinosteroids, that affect plant growth (19). How HSP83 affects floral initiation, if at all, is not known at present.

ACKNOWLEDGMENTS

We thank Eric Eckwall and Diana Mercado-Meléndez for technical assistance and Dr. John Larkin and Ann Vogel for a critical reading of the manuscript.

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