*Proc. Natl. Acad. Sci. USA*
Vol. 84, pp. 8986–8990, December 1987
Biochemistry

# Artificial combination of two cis-regulatory elements generates a unique pattern of expression in transgenic plants

(heat-shock elements/light induction/organ specificity/S1 nuclease mapping)

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*Communicated by J. E. Varner, September 10, 1987 (received for review July 2, 1987)*

ABSTRACT **We show that a 36-base-pair-long upstream fragment from the soybean *hsp17.3-B* gene comprising two partly overlapping heat-shock element (HSE)-like sequences can confer heat inducibility to a reporter gene in transgenic tobacco. The heat-shock response does not display organ specificity and is not affected by light. Insertion of these HSE-like elements into the pea *rbcS-3A* 5′ flanking fragment (position −410 to +15) either at position −410 (5′ to the enhancer) or at position −49 (between the enhancer and the "TATA" box) renders the transcript level of the reporter gene light-inducible and organ-specific under heat-shock conditions. These results demonstrate the possibility of generating a unique pattern of expression (e.g., light-dependent and organ-specific heat-shock response) by artificial combination of appropriate cis-acting regulatory elements. Moreover, by using the HSE-like sequences as a weak heat-inducible enhancer in the chimeric regulatory regions we uncover the function of negative elements within the pea *rbcS-3A* upstream region. These negative elements are responsible for a repressed transcript level in roots as well as in dark-adapted leaves. Therefore, the upstream fragment containing two HSE-like elements can be considered a useful tool to test the function of other cis-acting elements.**

In most cells, the heat-shock response is transcriptionally regulated, and mRNAs coding for heat-shock proteins (hsps) are induced 10- to 1000-fold at elevated temperatures (1). In plants the transcription of low molecular weight hsps (15–24 kDa) is predominantly induced upon heat treatment (2). All genes for hsps in eukaryotes investigated to date contain several copies of a 14-base-pair (bp)-long sequence element upstream of the "TATA" box that is homologous to a consensus heat-shock element (HSE) sequence described initially for the *Drosophila hsp70* gene (refs. 3 and 4; for review, see ref. 5). Two different laboratories have shown that a heat-shock transcription factor binds to such HSEs in *Drosophila* and specifically promotes the transcription of heat-shock genes *in vitro* (6–10). According to these observations HSE-like sequences represent positive cis-acting elements, specific for the heat induction of the genes containing them. A 5′ deletion analysis of the soybean *hsp17.5-E* and *hsp17.3-B* genes revealed that mutants with 95 bp and 78 bp, respectively, of the 5′ upstream region are still able to respond to high temperatures, but in a reduced manner, in transformed plant tissue (11, 12). Although these mutants contain sequences that include two partly overlapping HSEs it is not known whether the latter by themselves are sufficient to confer thermoinducibility on a heterologous promoter in transgenic plants.

The 5′ flanking sequences of several *Drosophila* low molecular weight *hsp* genes contain, in addition to HSEs, independent and remote regulatory elements that confer developmental and cell-cycle-specific activation without heat induction (13–16). A CCAAT box is responsible for oocyte-specific activation of the *Xenopus hsp70* gene (17). These *hsp* genes are independently induced by two different stimuli, demonstrating that the expression is regulated by a combination of positive elements.

We sought to investigate the interaction between plant regulatory elements by artificially combining two HSE-like elements from the soybean *hsp17.3-B* gene with a *rbcS-3A* upstream fragment (position −410 to +15) that contains elements responsible for light-inducible and organ-specific expression (18, 19). We show here that a fragment comprising two partly overlapping HSE-like sequences is sufficient to confer thermoinducibility to a reporter gene in transgenic tobacco. A previously undescribed pattern of expression (i.e., light-dependent and organ-specific heat-shock response) can be generated by the artificial combination of this fragment with the pea *rbcS-3A* upstream region. Our results indicate that light responsiveness and organ specificity are mediated by negative elements within the *rbcS-3A* upstream region.

## MATERIALS AND METHODS

**DNA Cloning.** All cloning procedures were carried out by using established methods. An oligonucleotide that contains the DNA sequence from −76 to −41 (with respect to the transcription start site as +1) of the soybean *hsp17.3-B* gene (Fig. 1*b*) was synthesized with an Applied Biosystems model 380A DNA synthesizer. This oligonucleotide fragment contains two HSE-like sequences that partly overlap (20). The hybridized strands were first cloned into a derivative pEMBL8(+) (21) by way of *Bam*HI and *Eco*RI sites and their sequence was verified by dideoxy sequence analysis. DNA fragments containing the HSEs were then cloned into the upstream region of chimeric rbcS-3A-chloramphenicol acetyltransferase (CAT) or cauliflower mosaic virus (CaMV) 35S-CAT genes within pMON200 derivatives described earlier (18) to give the constructs shown in Fig. 1*a*.

**Transfer of Genes into Plants.** pMON plasmids were mobilized by triparental mating into *Agrobacterium tumefaciens* GV3111SE, which contains a "disarmed" Ti plasmid (22). *A. tumefaciens* harboring the recombinants were used to inoculate sterile leaf discs of *Nicotiana tabacum* var. *xanthi* by using the procedure of Horsch *et al.* (23) slightly modified by Kuhlemeier *et al.* (19). Plantlets that formed roots were first vegetatively propagated on MS medium (24) and then transferred to soil for growth under a 16-hr light/8-hr dark regime at 25°C.

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Abbreviations: HSE(s), heat-shock element(s); hsp, heat-shock protein; nt, nucleotide(s); CAT, chloramphenicol acetyltransferase; CaMV, cauliflower mosaic virus.

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**Heat-Shock Treatment.** Transgenic plants grown for 6–8 weeks on soil (≈20 cm high) were used. At least four different transgenic plants were tested for each of the constructs shown in Fig. 1*a*. For heat-shock experiments in the light, leaves were first harvested from untreated plants grown at 25°C, as the control. Plants were then incubated at 40°C in the light and leaves were harvested after 15 min, 30 min, and 60 min, to analyze the time course of the heat-shock response, or only after 60 min. In time course experiments, leaves were also collected during the recovery period at 25°C after 1 hr, 2 hr, and 4 hr. For heat-shock experiments in the dark, plants were first transferred to the dark at 25°C for 4 days and afterwards the temperature was raised to 40°C for 60 min. In all cases representative samples of leaves were collected to minimize developmental discrepancies.

To test for organ specificity of the heat-shock response eight cuttings of a transgenic plant were rooted on solid MS medium with carbenicillin (0.5 mg/ml) and kanamycin (0.1 mg/ml) at 25°C under a 16-hr light/8-hr dark regime. As a control, leaves and roots were harvested from four of these plantlets. The remaining four plantlets were incubated at 40°C (temperature measured in the agar) in the light and after 60 min leaves and roots were collected separately.

**RNA Isolation and S1 Nuclease Analysis.** Total RNA was extracted according to Kirk and Kirk (25) except that 1 mM aurin tricarboxylic acid was used as a RNase inhibitor (26). Transcript levels were determined by 3′ S1 nuclease protection by using a *rbcS-E9* 3′ fragment as a probe (27). The single-stranded probe protects 230 nucleotides (nt) of the CAT transcripts containing the *rbcS-E9* 3′ end (28). Dilution series of RNA samples were carried out to compare the intensities of their S1 nuclease signals.

## RESULTS

**Construction of a Heat-Inducible Plant Promoter.** The oligonucleotide fragment shown in Fig. 1*b* was cloned at position −46 of the CaMV 35S promoter to give construct B (Fig. 1*a*). In this construct the HSE-like sequences and the TATA box are separated by 33 bp. *N. tabacum* var. *xanthi* was transformed with this construct and transgenic plants were regenerated. A pMON200 derivative containing only the truncated 35S promoter (position −46 to +8) linked to the coding sequence of the CAT (construct A in Fig. 1*a*) served as a control. Heat-shock experiments were carried out as described in *Materials and Methods* and the CAT-E9 transcript level was analyzed by 3′ S1 nuclease protection. In these and subsequent experiments at least four independent transgenic plants were assayed for each construct. Similar results were obtained among independent transgenic plants containing the same construct and only representative results are shown. Fig. 2*a* shows that transgenic plants containing construct A do not express detectable amounts of CAT-E9 RNA in the light or dark and at 25°C or 40°C. These results confirm previous reports (29) that the truncated 35S promoter is poorly expressed. Furthermore, they demonstrate that the 35S promoter from position −46 to +8 alone does not confer heat inducibility. Control experiments show that the endogenous *hsp70* RNA level remains heat-inducible at 40°C in the leaves in light or dark and in the roots of these transgenic plants, as revealed by slot blot hybridization using antisense RNA derived from a fragment of the maize *hsp70* coding sequence as a heterologous probe (data not shown).

In contrast, transgenic plants containing two partly overlapping HSE-like elements from soybean placed upstream of the truncated 35S promoter (construct B of Fig. 1*a*) exhibit heat induction of the CAT-E9 transcript level. At 25°C no detectable transcript is found in the leaves in light or dark or in roots of these transgenic plants (Fig. 2*b*, lanes 1, 3, and 5). A 60-min incubation at 40°C in the light results in at least a

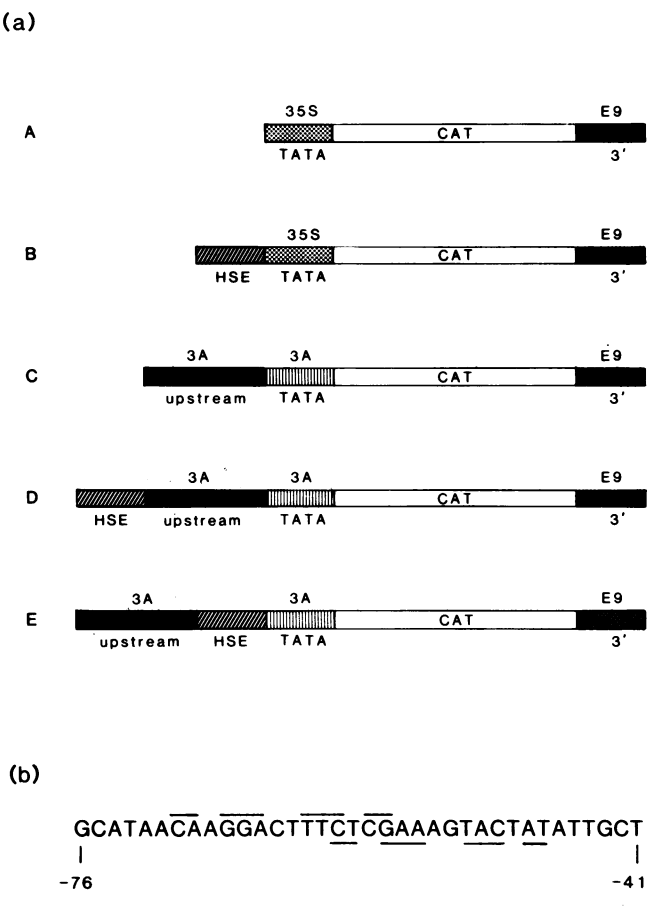


FIG. 1 (*a*). Schematic diagrams of the constructs inserted between the *Bgl* II and *Cla* I sites in the polylinker region of the intermediate cloning vector pMON200, which has been described in detail (22, 23). Construct A contains the CAT gene under control of the truncated 35S promoter (position −46 to +8; 35S TATA) (18). For the creation of construct B, an oligonucleotide fragment corresponding to position −76 to −41 of the upstream region of the soybean *hsp17.3-B* gene and thereby containing the HSE-like elements depicted in *b* was cloned at −46 of the truncated 35S promoter in construct A. In construct C the CAT gene is driven by the pea *rbcS-3A* upstream region (position −410 to −49; 3A upstream) and the *rbcS-3A* TATA box (position −48 to +15; 3A TATA) (18). Construct D contains the soybean HSE fragment inserted at position −410 of construct C. Construct E was created by cloning the soybean HSE fragment between the *rbcS-3A* upstream region and the *rbcS-3A* TATA box at position −49 of construct C. All constructs contain at their 3′ ends the poly(A) sequence of the pea *rbcS-E9* gene (E9 3′) (18, 19). (*b*) Nucleotide sequence of the *hsp17.3-B* gene upstream region from soybean (20) inserted at different locations upstream of the CAT reporter gene. Nucleotides corresponding to the HSE consensus sequence (3) are overlined or underlined; the sequence comprises two partly overlapping HSE-like elements.

5-fold increase of the transcript level (Fig. 2*b*, lane 2). The heat-shock response can also be detected in leaves of dark-adapted plants and in roots of light-grown plants, though in a slightly reduced manner (Fig. 2*b*, lanes 4 and 6, respectively). The lower transcript level in roots under these conditions could be due to the slower increase of the temperature in the solid MS medium.

5′ S1 nuclease mapping verifies that the CAT-E9 RNA synthesized under heat-shock conditions in transgenic plants containing construct B retains the correct 35S transcription start site (data not shown). These results show that an upstream fragment from the soybean *hsp17.3-B* gene, comprised of two partly overlapping HSE-like sequences, is sufficient to confer heat inducibility to a heterologous promoter. Thus, this HSE fragment acts as a conditional positive

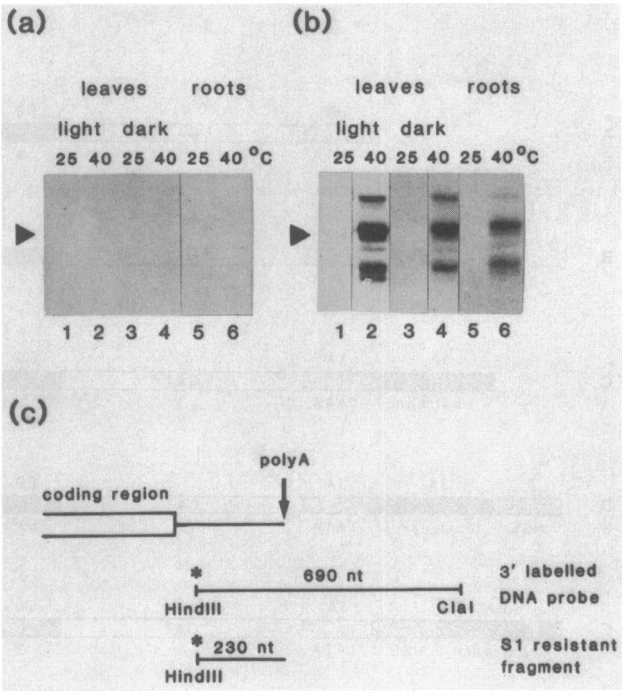


FIG. 2. An upstream fragment consisting of two partly overlapping HSE-like sequences is sufficient to confer heat inducibility to a heterologous promoter in transgenic tobacco. Construct A (*a*) and construct B (*b*) of Fig. 1*a* located on the intermediate vector pMON200 were used for the transformation of *N. tabacum* var. *xanthi*. Leaves were harvested from single transgenic plants grown at 25°C in light or dark (lanes 1 and 3 in each panel) and from the same plants after 60 min of heat shock at 40°C in the light or dark (lanes 2 and 4 in each panel). Roots were harvested from cuttings grown in the light at 25°C (lanes 5) or heat-shocked at 40°C for 60 min (lanes 6). Transcript levels were analyzed by 3′ S1 nuclease mapping. The main specific S1 nuclease signal is represented by a 230-nt-long protected DNA fragment (arrowheads). (*c*) Schematic diagram of probe and S1 nuclease-protected fragment. The 230-nt-long S1 nuclease-resistant fragment contains 82 nt from the labeled *Hin*dIII site to the original *rbcS-E9* termination codon and 148 nt of *rbcS-E9* 3′ terminal untranslated sequence to the poly(A) site.

element that potentiates transcription only at elevated temperatures. Moreover, the ability to respond to heat induction is neither light-dependent nor organ-specific.

**Combination of HSE-Like Sequences with the *rbcS-3A* Upstream Region Renders the Heat-Shock Response Light-Dependent.** We have previously reported that an ≈400-bp 5′ flanking fragment of the pea *rbcS-3A* gene can confer light-inducible and organ-specific expression on a reporter gene (18, 19). To examine the combinatorial effects of the soybean HSE-like sequences and the pea *rbcS-3A* cis-acting regulatory elements we inserted the former either upstream or downstream of the *rbcS-3A* enhancer-like element (18) to give constructs D and E, respectively (Fig. 1*a*). These constructs as well as a control construct containing only the *rbcS-3A* 5′ flanking fragment (position −410 to +15) linked to the CAT reporter gene (construct C of Fig. 1*a*) were transferred into *N. tabacum* var. *xanthi*. Transgenic plants containing construct C produce a high transcript level at 25°C in the light but not in the dark (Fig. 3*a*, lanes 1 and 3), confirming the light-inducible transcription conferred by the *rbcS-3A* upstream region as reported (18). The *rbcS-3A* 5′ flanking fragment appears to be inactive at high temperatures since no transcript is detected at 40°C (Fig. 3*a*, lanes 2 and 4). The endogenous *hsp70* RNA level is heat-inducible under the same conditions in the leaves of these transgenic plants, as shown by RNA transfer blot analyses (data not shown).

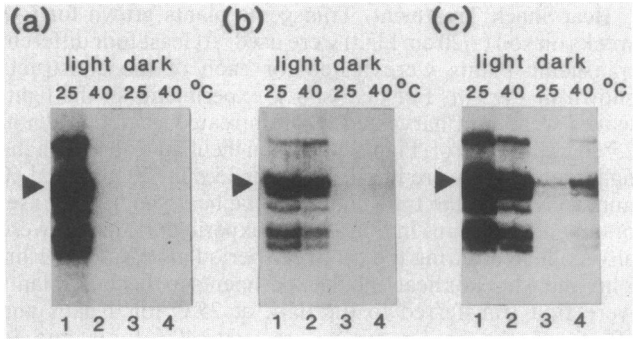


FIG. 3. Light-dependent heat-shock response. Construct C (*a*), construct D (*b*), and construct E (*c*) of Fig. 1*a* were used for the transformation of *N. tabacum* var. *xanthi*. Leaves were collected from single transgenic plants grown at 25°C in the light or dark (lanes 1 and 3, respectively) and from the same plants after heat treatment for 60 min at 40°C in the light or dark (lanes 2 and 4, respectively). Transcript levels were measured by 3′ S1 nuclease mapping. The signal of the 230-nt-long protected DNA fragment is indicated by the arrowheads.

Leaves of transgenic plants containing the soybean HSE fragment placed at −410 of the *rbcS-3A* upstream region (construct D of Fig. 1*a*) produce a high transcript level at 25°C in the light (Fig. 3*b*, lane 1). After a 60-min incubation at 40°C in the light, ≈80% of the transcript level at 25°C are reached (compare Fig. 3*b*, lanes 1 and 2). The time course of the heat-shock response (Fig. 4) reveals that the CAT-E9 RNA produced at 25°C in the light is very unstable at 40°C and is completely degraded within 15 min. The transcript level conferred by the HSE-like sequences reaches its maximum after 60 min at 40°C in the light and decreases during the first hour of the recovery period at 25°C. A 4-hr recovery at 25°C in the light again leads to a high CAT-E9 transcript level, which is always observed with the *rbcS-3A* upstream region under these conditions. These results demonstrate that the soybean HSE-like sequences can confer heat-inducible transcription on the *rbcS-3A* 5′ flanking fragment, which, by itself, is inactive at 40°C.

To determine if the heat-shock response conferred by the HSE fragment can be regulated by cis-acting elements in the *rbcS-3A* upstream region we repeated the heat-shock experiments in the dark with construct D. Fig. 3*b* shows that no

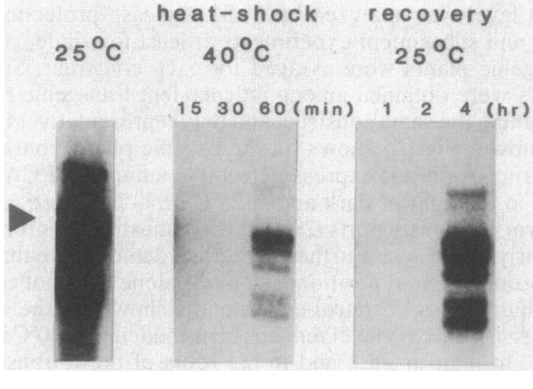


FIG. 4. Time course of heat-shock response and recovery. A transgenic *N. tabacum* var. *xanthi* plant containing construct D of Fig. 1*a* was used for heat-shock treatment in light. As a control, leaves were first harvested from this plant before the heat treatment (25°C). Then the plant was incubated at 40°C and leaves were collected after 15 min, 30 min, and 60 min. Afterwards the plant was again transferred to 25°C and leaves were harvested during the recovery period after 1 hr, 2 hr, and 4 hr. Transcript levels were analyzed by 3′ S1 nuclease mapping. The signal of the 230-nt-long S1 nuclease-protected DNA fragment is marked by an arrowhead.

CAT-E9 transcript can be detected either at 25°C (lane 3) or 40°C (lane 4). At 40°C, the light-induction ratio for this transcript is at least 5–10 (compare Fig. 3*b*, lanes 2 and 4).

Insertion of the soybean HSE-like elements between the *rbcS-3A* upstream regulatory region and the *rbcS-3A* TATA box (construct E in Fig. 1*a*) gives qualitatively similar results (Fig. 3*c*). At 25°C in the light the intensity of the S1 nuclease signal obtained with construct E (Fig. 3*c*, lane 1) is about two times higher than that obtained with construct D (Fig. 3*b*, lane 1). In the light, the transcript level obtained with construct E at 40°C reaches ≈80–90% of that at 25°C (compare lanes 1 and 2 of Fig. 3*c*). This heat-shock response is repressed by a factor of 5–10 when the plants are transferred to the dark (Fig. 3*c*, lane 4).

5′ S1 nuclease mapping experiments confirm that constructs C, D, and E use the same transcription start site (data not shown), indicating that the addition of the soybean HSE-like sequences does not change the 5′ terminus of the transcript either at 25°C or 40°C. Therefore, these results show that the combination of two HSE-like elements with the *rbcS-3A* upstream region can generate a previously undescribed expression pattern—i.e., light-dependent heat-shock response.

**Combination of HSE-Like Sequences with the *rbcS-3A* Upstream Region Renders the Heat-Shock Response Organ-Specific.** Transgenic plants of *N. tabacum* var. *xanthi* containing construct C, D, or E of Fig. 1*a* were tested for organ specificity of their heat-shock responses. To this end, cuttings grown on 1% agar containing MS medium were used to ensure illumination of the roots.

Transgenic plants containing construct C (Fig. 1*a*) grown at 25°C in the light show a high transcript level in leaves (Fig. 5*a*, lane 1). This RNA is completely absent in roots (Fig. 5*a*, lane 3), confirming the organ-specific expression conferred by the *rbcS-3A* upstream regulatory region (18). No transcript is found in leaves and roots of these plants exposed to 40°C (Fig. 5*a*, lanes 2 and 4).

Transgenic plants in which the soybean HSE fragment is located 5′ to the *rbcS-3A* upstream region (construct D of Fig. 1*a*) produce a high CAT-E9 RNA level in leaves (Fig. 5*b*, lane 1) but not in roots (Fig. 5*b*, lane 3) at 25°C in the light. In leaves ≈60% of this transcript level is observed at 40°C in light (Fig. 5*b*, lane 2) but no CAT-E9 RNA can be detected in roots of these plants under the same conditions (Fig. 5*b*, lane 4); the transcript level is reduced by a factor of at least 5–10 in the roots as compared to the leaves. Similar results are obtained when the soybean HSE fragment is inserted at position −49 of the *rbcS-3A* upstream region (construct E of Fig. 1*a*). Taken together, these results provide clear evidence that the heat-shock response conferred by the soybean HSE-like sequences can be modified by *rbcS-3A* regulatory elements to display organ specificity.

The results obtained with constructs A–E in transgenic *N. tabacum* var. *xanthi* have been confirmed with transgenic *Nicotiana plumbaginifolia* containing the same constructs.

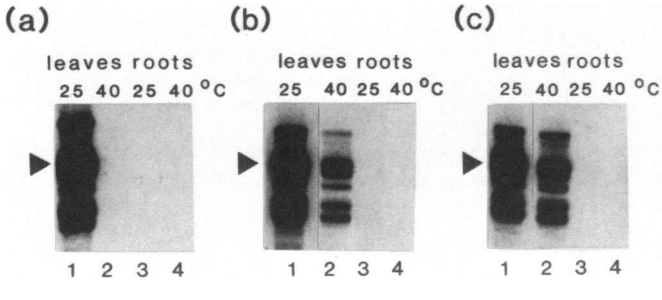


FIG. 5. Organ-specific heat-shock response. Construct C (*a*), construct D (*b*), and construct E (*c*) of Fig. 1*a* were used for the transformation of *N. tabacum* var. *xanthi*. Leaves and roots were separately collected from somatically propagated plantlets grown on MS medium in plastic containers at 25°C in the light (lanes 1 and 3 in each panel) or heat-shocked for 60 min at 40°C in the light (lanes 2 and 4 in each panel). Transcript levels were determined by 3′ S1 nuclease mapping. The main specific S1 nuclease signal is indicated by the arrowheads.

## DISCUSSION

**Heat Inducibility Conferred by Two HSE-Like Sequences.** We show here that a fragment comprising two partly overlapping HSE-like sequences from the soybean *hsp17.3-B* gene in combination with the 35S upstream region from position −46 to +8 is sufficient to confer heat inducibility to a test gene in transgenic plants. The ability to respond to heat induction is neither light-dependent nor organ-specific. In part, the apparently low induction rate is certainly caused by the instability of the CAT-E9 RNA at 40°C (see Fig. 4), as has been reported for many non-*hsp* mRNAs under heat-shock conditions (1, 2). On the other hand, it is possible that two HSE-like sequences alone are insufficient to account for the full heat-shock response in plants. For maximum heat-shock induction, more than two copies of HSEs in the upstream region, as found in the natural 5′ flanking region of heat-shock genes (1), may be necessary. The increase of heat-induced transcript level by raising the number of HSEs has been described earlier for the *hsp70* genes from *Drosophila* (30, 31) and *Xenopus* (32) as well as for several low molecular weight *hsp* genes from *Drosophila* (33, 34) and soybean (11, 12). Furthermore, a simian virus 40 enhancer core sequence and as yet unidentified enhancer-like upstream sequences that are found in a *hsp17.5* gene (11) and the *hsp17.3-B* gene (12), respectively, from soybean, may stimulate the basal and/or heat-induced transcript level.

The relative location of the soybean HSE-like sequences with respect to the transcription start site has no qualitative effect on the heat inducibility (compare Figs. 3 and 5). However, in most cases the basal and induced transcript levels are higher when the HSE fragment is located closer to the TATA-box (compare lanes 1 and 2 of Fig. 3*b* with lanes 1 and 2 of Fig. 3*c*). In the constructs tested here the HSE-like sequences are inserted 33 bp or 394 bp (E and D, respectively, of Fig. 1*a*) upstream of the TATA box. In both cases they are located further upstream than the most proximal heat-shock consensus-like sequence in most of the *hsp* genes described (1, 5). In this regard the soybean HSE-like elements function as a heat-inducible transcription enhancer, a property described for other HSEs (11, 14).

**Generating a Unique Pattern of Expression.** The combination of two soybean HSE-like sequences with the pea *rbcS-3A* 5′ flanking fragment (from position −410 to +15) renders the heat response light-inducible and organ-specific (Figs. 3–5), thus creating a pattern of expression that has not been observed before. In this regard our results are similar to the generation of a novel expression pattern in transformed *Drosophila* by combining regulatory elements from the *Drosophila Sgs-4* and *Adh* genes (35). A combination of different regulatory elements is also found in the 5′ flanking region of the human metallothionein-$II_A$ gene (36) and of several *hsp* genes (13–17), all of which respond to different external stimuli. In the constructs tested here (D and E of Fig. 1*a*) the function of the HSE-like sequences is dominated by the *rbcS-3A* upstream region (i.e., the heat-shock response is only possible in the leaves of light grown plants), reflecting the combination of positive cis-acting elements (HSEs) with negative cis-acting elements (domains within the *rbcS-3A* upstream region). Most likely, constructs composed of two positive cis-acting elements would maintain the regulatory pattern of each.

The generation of an unusual pattern of expression demonstrated here may prove a useful procedure for the genetic manipulation of plants.

**HSE-Like Sequences as Weak Heat-Inducible Test Enhancers.** The data presented here and investigations made by other groups (14, 32) show that HSEs function as weak heat-inducible transcriptional enhancers. Therefore we employed the soybean HSE fragment (Fig. 1*b*) as a test enhancer to probe the nature of the cis-acting elements responsible for light inducibility and organ specificity of the *rbcS-3A* gene. By using the HSE-like sequences for this purpose, instead of a strong constitutive enhancer like the CaMV 35S upstream regulatory region, we may avoid the possibility of overriding the expression of regulatory elements in the *rbcS-3A* upstream region.

From the expression pattern of the constructs containing a combination of the two soybean HSE-like sequences with the pea *rbcS-3A* upstream region (see above), we conclude that the latter contains negative elements that are responsive to light. These results confirm the work of Kuhlemeier *et al.* (19), who have demonstrated and identified negative light-responsive elements by another approach. In addition, we have shown here that organ specificity of the *rbcS-3A* gene is also mediated by negative elements within the sequence between position −410 and +15. Presumably, in dark-adapted leaves or in roots, one or more repressors bind to these negative elements and thereby block the transcription observed in illuminated leaves. Simpson *et al.* (37) have described an organ-specific silencer in the upstream region of a pea *lhcp* gene. The function of positive cis-acting elements stimulating expression of the *rbcS-3A* gene upon illumination has been demonstrated earlier (18). To account for leaf-specific expression, a positive cis-acting element that functions in leaf cells must also be postulated. Our results here do not exclude the possibility that an activator for this positive regulatory element is absent from roots.

The decrease of the transcript levels from constructs D and E in the dark and in roots under heat-shock conditions also indicates that the function of the putative repressor(s) is not inhibited by elevated temperature (40°C). In contrast, the function of positive trans-acting factors, which stimulate transcription from the *rbcS-3A* upstream region in leaves from light-grown plants at 25°C, is likely to be inhibited at 40°C, as demonstrated by the reduction of the transcript level from construct C in leaves at 40°C in light (Fig. 3*a*). Since the test for organ-specific heat-shock response was carried out with roots exposed to light our results strongly suggest that different repressors or one differently modified repressor blocks the transcription from the *rbcS-3A* upstream region in the dark and in roots. Clearly, the soybean HSE-like sequences can be used as a tool to investigate the mechanisms of other complex regulatory elements in transgenic plants.

We thank Cathy Ward for skilled technical assistance, Drs. Cris Kuhlemeier and Ferenc Nagy for helpful discussions, and Wendy Roine for assembling the manuscript. G.S. holds a Postdoctoral Fellowship from the Deutsche Forschungsgemeinschaft. This work was supported by a grant from Monsanto Company.

1. Lindquist, S. (1986) *Annu. Rev. Biochem.* **55,** 1151–1191.
2. Schoffl, F., Baumann, G., Raschke, E. & Bevan, M. (1986) *Philos. Trans. R. Soc. London Ser. B* **314,** 453–468.
3. Pelham, H. R. (1982) *Cell* **30,** 517–528.
4. Pelham, H. R. & Bienz, M. (1982) *EMBO J.* **1,** 1473–1477.
5. Pelham, H. R. (1985) *Trends Genet.* **1,** 31–35.
6. Wu, C. (1980) *Nature (London)* **286,** 854–860.
7. Parker, C. S. & Topol, J. (1984) *Cell* **36,** 357–369.
8. Parker, C. S. & Topol, J. (1984) *Cell* **37,** 253–262.
9. Wu, C. (1984) *Nature (London)* **309,** 229–241.
10. Wu, C. (1985) *Nature (London)* **317,** 84–87.
11. Gurley, W. B., Czarnecka, E., Nagao, R. & Key, J. L. (1986) *Mol. Cell. Biol.* **6,** 559–565.
12. Baumann, G., Raschke, E., Bevan, M. & Schoffl, F. (1987) *EMBO J.* **6,** 1167–1171.
13. Cohen, R. S. & Meselson, M. (1985) *Cell* **43,** 737–746.
14. Riddihough, G. & Pelham, H. R. B. (1986) *EMBO J.* **5,** 1653–1658.
15. Mestril, R., Schiller, P., Amin, J., Klapper, H., Amanthan, J. & Voellmy, R. (1986) *EMBO J.* **5,** 1667–1673.
16. Klemenz, R. & Gehring, W. J. (1986) *Mol. Cell. Biol.* **6,** 2011–2019.
17. Bienz, M. (1986) *Cell* **46,** 1037–1042.
18. Fluhr, R., Kuhlemeier, C., Nagy, F. & Chua, N.-H. (1986) *Science* **232,** 1106–1112.
19. Kuhlemeier, C., Fluhr, R., Green, P. J. & Chua, N.-H. (1987) *Genes Dev.* **1,** 247–255.
20. Schoffl, F., Raschke, E. & Nagao, R. T. (1984) *EMBO J.* **3,** 2491–2497.
21. Dente, L., Cesareni, G. & Cortese, R. (1983) *Nucleic Acids Res.* **11,** 1645–1655.
22. Fraley, R. T., Rogers, S. G., Horsch, R. B., Eichholtz, D. A., Flick, J. S., Fink, C. L., Hoffmann, N. L. & Sanders, P. R. (1985) *Biotechnology* **3,** 629–635.
23. Horsch, R. B., Fry, J. E., Hoffman, N. L., Eichholtz, D., Rogers, S. G. & Fraley, R. T. (1985) *Science* **227,** 1229–1231.
24. Murashige, T. & Skoog, F. (1962) *Physiol. Plant.* **15,** 473–497.
25. Kirk, M. M. & Kirk, D. L. (1985) *Cell* **41,** 419–428.
26. Nagy, F., Kay, S. A., Boutry, M., Hsu, M.-Y. & Chua, N.-H. (1986) *EMBO J.* **5,** 1119–1124.
27. Fluhr, R. & Chua, N.-H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2358–2362.
28. Fluhr, R., Moses, P., Morelli, G., Coruzzi, G. & Chua, N.-H. (1986) *EMBO J.* **5,** 2063–2071.
29. Odell, J. T., Nagy, F. & Chua, N.-H. (1985) *Nature (London)* **313,** 810–812.
30. Dudler, R. & Travers, A. A. (1984) *Cell* **38,** 391–398.
31. Amin, J., Mestril, R., Schiller, P., Dreano, M. & Voellmy, R. (1987) *Mol. Cell. Biol.* **7,** 1055–1062.
32. Bienz, M. & Pelham, H. R. B. (1986) *Cell* **45,** 753–760.
33. Pauli, D., Spierer, A. & Tissieres, A. (1986) *EMBO J.* **5,** 755–761.
34. Mestril, R., Rungger, D., Schiller, P. & Voellmy, R. (1985) *EMBO J.* **4,** 2971–2976.
35. Shermoen, A. W., Jongens, J., Barnett, S. W., Flynn, K. & Beckendorf, S. K. (1987) *EMBO J.* **6,** 207–214.
36. Karin, M., Haslinger, A., Holtgreve, H., Richards, R. J., Krauter, P., Westphal, H. M. & Beato, M. (1984) *Nature (London)* **308,** 513–519.
37. Simpson, J., Schell, J., Van Montagu, M. & Herrera-Estrella, L. (1986) *Nature (London)* **323,** 551–554.