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Isolation and characterization of genes encoding 16.9 kD heat shock proteins in *Oryza sativa*[1]

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Abstract. Using rice heat shock cDNA pTS1 as a probe, two 16.9 kD HSP genes were isolated from a size-selected genomic library of rice. The DNA sequences of the two rice genomic HSP genes, designated as Oshsp 16.9A and Oshsp 16.9B, were determined and analyzed, Oshsp 16.9A containd the [illegible] of cDNA pTS1. Comparison of Oshsp 16.9A and 16.9B genes revealed that they shared 98.8% homology in terms of nucleotide sequence in the coding region, and 99.3% homlogy at the level of the deduced amino acid sequence. Multiple copies of heat shock-like promoter sequence (HSE) can be identified upstream from the putative TATA box, TATAAATA. The initiation site of transcription for the Oshsp 16.9A and 16.9B, was were identified by the primer extension method is located 131 bases upstream from the coding region and 26 bases downstream from the TATA box.

Key words: Heat shock; Heat shock protein gene; *Oryza.*

Introduction

When living organisms are exposed to elevated temperatures, a set of proteins known as heat shock proteins (HSPs) is induced. In eukaryotes, HSPs are divided into three major classes based on their molecular weights and degrees of homology: HSP 90 family (80-90 kD), HSP 70 family (70 kD), and low-molecular-[illegible] (LMW) HSPs that range in size from 15 to 30 in size and in amino acid sequence than the high-molecular-weight (HMW) HSPs, and can be subdivided into four multigene families (Vierling, 1991). The HMW HSPs of plants, in contrast to those of *Drosophila* (Ashburner and Bonner, 1979), represent a relatively small fraction of total HSPs accumulation (Key *et al.*, 1981). Following HS stress in soybean, some HSPs are reversibly enriched in subcellular fractions of nuclei, mitochondria and ribosomes (Lin *et al.*, 1984). The precise physiological role of HSPs is still unknown, although

genes sequenced contain multiple HSEs in the 5' flanking region of the gene (Nagao and Key, 1989). Further analysis indicates that the HSEs are best described as contiguous arrays of variable numbers of 5bp sequence nGAAn or nTTCn arranged in alternating orientation (Xiao and Lis, 1988; Amin *et al.*, 1988).

Genomic and cDNA clones of plant LMW HSPs genes have been isolated and characterized from a number of species including soybean (Czarnecka *et al.*, 1985; Nagao *et al.*, 1985; Raschke *et al.*, 1988; Schöffl *et al.*, 1984), maize (Goping *et al.*, 1991), *Arabidopsis* (Takahashi and Komeda, 1989), wheat (McElwain and Spiker, 1989) and carrot (Darwish *et al.*, 1991). Conservation of these sequences clearly suggests that LMW HSPs may play an important role in plants coping with HS.

Since rice is one of the most important food crops grown in Taiwan and the field temperature may approach heat shock conditions during the summer, heat shock responses of rice were studied. A LMW HS cDNA, pTS1, that encodes 16.9 kD HS protein has been isolated and sequenced in our laboratory (Tseng *et al.*, 1992). In this report, by use of pTS1 cDNA as a probe, two 16.9 kD HS genes from rice were isolated and characterized.

Materials and Methods

Plant Materials

Rice (*Oryza sativa* L. cv Tainong 67) seeds were germinated in rolls of moist paper towel at 28°C in a dark growth chamber for 3 days. The induction treatments of harvested seedlings without endosperms were performed in the incubation solution containing 5 mM potassium phosphate buffer (pH 6.0), 1% sucrose and 50 μg/ml chloramphenicol.

DNA Isolaiton and Southern Analysis

hours. Hybridization was performed at 42°C overnight in 50% formamide, 0.1% Ficoll, 5x SSC, 0.1% polyvinylpyrrolindone, 20 mM sodium phosphate (pH 6.5), 0.1% SDS, 250 μg/ml denatured salmon sperm DNA and ^{32}P-labeled pTS1 cDNA probe. Probe pTS1 DNA was labeled with (α-^{32}P) dCTP (3000 Ci/mmol, NEN) by the random primer method (Feinberg and Vogelstein, 1983). Then the filters were washed twice in 2x SSC, 0.1% SDS for 10 min at room temperature and twice in 0.1x SSC, 0.1% SDS for 30 min at 55°C.

Construction and Screening of Size-selected Genomic Library

Total rice genomic DNA was digested with EcoRI restriction enzyme, and the DNA sizes between 2.0 and 4.0 kb were eluted by NA-45 DEAE membrane (Schleicher & Schuell, Dassel, W. Germany) from the agarose gel pieces. The eluted DNA was then ligated into the EcoRI site of the cloning vector λgt10 to construct the genomic library. In order to isolate HSP genes from this genomic library, cDNA pTS1 was used as a probe. Approximately 20,000 plaques in 20 plates (85-mm) were screened. Filters containing plaque lifts were hybridized and washed as described in the Southern analysis. Positive plaques were identified by autoradiography.

Restriction Mapping of the Positive Clones

LambdaMap System (Promega, Madison, Wisconsin USA) was used to determine the restriction map of the positive clones. Included in the system were two synthetic 12-base oligonucleotides which are complementary to their respective cos sites of lambda DNA. Once kinased with (γ-^{32}P) ATP (3000Ci/mmol, NEN) and T4 polynucleotide kinase, these probes were hybridized to fragments of recombinant lambda DNA which were partially digested with a restriction enzyme. The hybrids were then separated by agarose

clease III as described by Henikoff (1984). DNA sequences were determined by the dideoxynucleotide chain-terminaiton method (Sanger *et al.*, 1977) using (α-^{35}S) dATP (1000 Ci/mmol, NEN) and the T7 DNA polymerase sequencing system (Promega).

Preparation of Poly (A)$^+$ RNA from Rice

Total RNA from the control (2 h at 28°C) and heat-shocked (2 h at 41°C) rice seedlings were prepared by the method of Lizardi and Engelberg (1979). Poly (A)$^+$ RNA was purified from total RNA by oligo (dT) cellulose chromatography (Aviv and Leder, 1972).

Primer Extension

200 ng of the oligonucleotide 5'GGACGTATG-CAAGTCTTGTTTGTTG3' (synthesized by DNA synthesizer, Applied Biosystems), which is complementary to bases 40–64 downstream from the TATA sequence of the Oshsp 16.9A gene, was labeled at the 5' terminus with (γ-^{32}P) ATP (3000 Ci/mmol, NEN) and T4 polynucleotide kinase. The labeled primer was allowed to anneal with 5 μg of poly (A)$^+$ RNA. Primer extension was carried out according to Sambrook *et al.* (1989). Primer extension products were electrophoresed in parallel with sequencing reaction which had been primed with the same oligonucleotide.

Results and Discussion

Southern Hybridization Analysis

Fig. 1 shows the results of Southern hybridization analysis at high stringency (42°C in 50% formamide condition). Multiple bands of homology to the pTS1 probe were observed when rice genomic DNA was digested with BamHI, EcoRI, HindIII or PstI. Three bands of 19.0, 14.0 and 9.4 kb were evident in the BamHI digest. Five prominent bands of 9.5, 5.9, 3.4, 2.5 and 1.7 kb were observed in the EcoRI digest. Five bands of 18.0, 4.3, 4.0, 3.3 and 0.5 kb were evident in the HindIII digest. Three prominent bands of approximately 18.5, 14.5 and 9.5 kb were observed in the PstI digest. The multiple fragment polymorphism clearly indicates that these LMW HSP genes belong to a multigene family. This is also known in soybean LMW HSP genes (Nagao and Key, 1989).

Screening and Isolation of Positive Clones

Among the five bands generated from the EcoRI

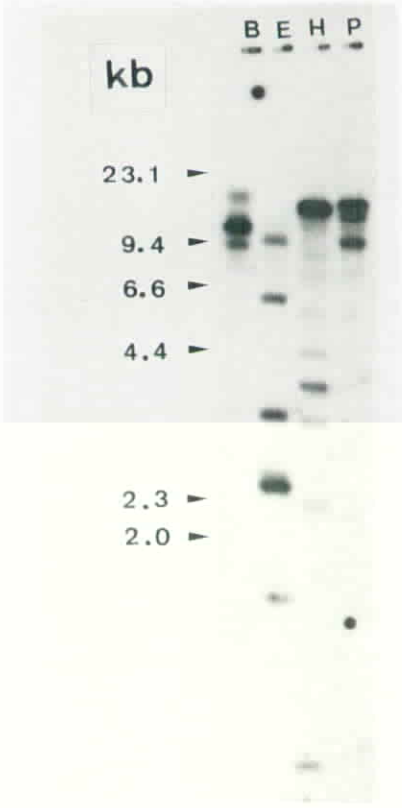

Fig. 1. Southern hybridization analysis of rice genomic DNA probed with cDNA pTS1. Genomic DNA was digested with BamHI (B), EcoRI (E), HindIII (H) or PstI (P).

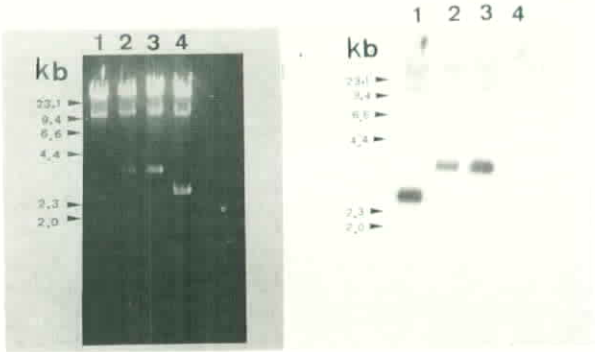

Fig. 2. Southern hybridization analysis of the recombinant phage DNA isolated from te four positive clones. pTS1 was used as a probe. left panel: ethidium bromide staining; right panel: Southern analysis; 1, 2, 3, 4: four positive clones.

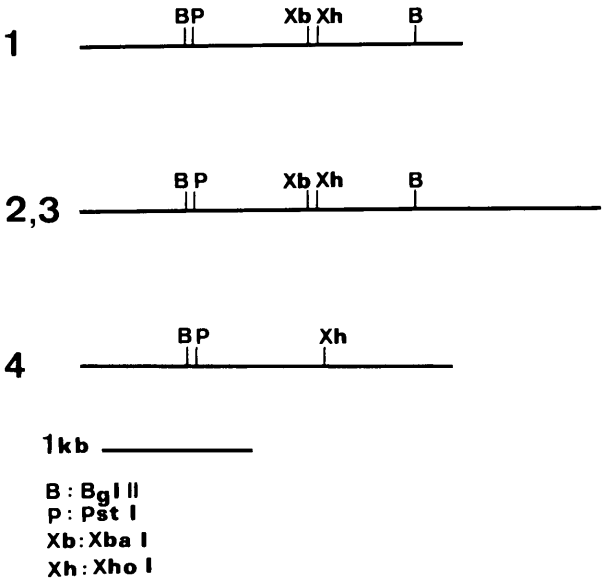

Fig. 3. Maps of restriction sites of the four positive clones. 1, 2, 3, 4: four positive clones.

digest (Fig. 1), the 3.4 and 2.5 kb bands showed strong positive signals. The DNA sizes between 2.0 and 4.0 kb were eluted and cloned into EcoRI site of λgt 10 vector to construct the size-selected genomic library. About 20,000 plaques (in 20 85 mm-plates) were screened with pTS1 probes. Four positive clones, namely, 1, 2, 3 and 4 were isolated. The recombinant phage DNA of the four positive clones was extracted and digested with EcoRI. Southern hybridization analysis of these positive clones is shown in Fig. 2. Clones 1 and 4 contained 2.5 kb fragment, while clones 2 and 3 contained 3.4 kb. The restriction sites of these four positive clones are shown in Fig. 3. Clones 2 and 3 exhibited the same restriction map, indicating that the two clones were identical or very similar. The restriction maps of clones 1 and 4 were different, and the hybridizing signal of clone 1 was stronger than that of the clone 4 (Fig. 2). Subsequently, clones 1 and 2 were subcloned into the EcoRI site of M13mp18 vector, and their DNA sequences were determined.

Nucleotide Sequences and the Deduced Amino Acid Sequences

The nucleotide sequence of the two genes is presented in Fig. 4 along with the derived amino acid sequences. The sequence has been published in the Plant Gene Register section of Plant Physiology (Tzeng *et al.*, 1992) but included here for discussion. The predicted open reading frames encoded polypeptides of 150 amino acids with calculated molecular weights of 16.9 kD for Oshsp 16.9A (clone 1) and Oshsp 16.9B (clone 2). The cDNA pTS1 used for screening these two genes had been sequenced (Tseng *et al.*, 1992) and found to match Oshsp 16.9A between nucleotides +15 and +819. There are no introns present in the Oshsp 16.9A gene. Comparison of Oshsp 16.9A and 16.9B genes revealed that they shared 98.8% homology in terms of the nucleotide sequence in the coding region, only 5 nucleotides were different. These were located at +500, +501, +554, +578 and +581. The deduced amino acid sequence of Oshsp 16.9A and Oshsp 16.9B was the same except for the 124th amino acid, i.e., Leu in Oshsp 16.9A and Met in Oshsp 16.9B. The amino acid sequence of these two genes exhibit 99.3% identity. The deduced amino acids of Oshsp 16.9A also exhibited striking homology to LMW HSPs of other species. The Oshsp 16.9A showed 71.9% and 68.7% homology to two class I HSPs of dicot, Gmhsp 17.5-E (Czarnecka *et al.*, 1985) and Athsp 17.6 (Helm and Vierling, 1989) respectively, and 83% to a monocot (wheat) class I HSP C5-8 (McElwain and Spiker, 1989). Analysis of LMW HSPs genes of plants indicates that most of the LMW HSPs belong to four multigene families: two of them most likely encode cytoplasmic proteins, one encodes a chloroplast-localized protein, and one appears to encode an endomembrane protein (Vierling, 1991). This classification is primarily based on the percentage of amino acid identity and similarity between LMW HSPs. Class I cytoplasmic LMW HSPs include soybean HSP17.5-E, *Arabidopsis* HSP17.6, pea HSP18.1, and wheat HSP C5-8. The homology among these genes ranges from 68.2 to 85.1% (Vierling, 1991). The amino acid homology of Oshsp 16.9A to class I LMW HSPs is high (68.7 to 83.0%), suggesting that rice HSP16.9A protein belongs to class I LMW HSPs. Within the class I LMW HSPs in general, there is a high degree of sequence conservation in the carboxyl-terminal portion of the proteins, while the amino-terminal shows significantly less identity (Vierling, 1991). The amino acid sequences alignment of class I cytoplasmic HSPs from four species are presented in Fig. 5. In the carboxyl-terminal, two thirds of the amino acids, 69 out of 106, were identical. In contrast, the amino-terminal showed a low degree of homology, with 16 out of 53 amino acids being identical.

(Oshsp16.9A)

-1661 gtctcaaggattcatttccacatgtataaaggctagcttcggacggatcgttat
-1607 cactttagtgtttcaccttcatatatacttattgtaagccgccgttcggcgttg
-1553 taacctcaacccgttatagtgaagatatttgctgcctggcacccgtggctttttt
-1499 ttctcttgctttgagaggattttccacgttaaatcttatgccttatgtgattga
-1445 tctattgctatttatcgtttatttgtctatcgttttctaacccttcttactaac
-1391 tgaatttaatacggtcaatttaatcatgagtctcttaaccgaattaacattagc
-1337 taaaactcagataaataaatatttttttcgtcccaaaaaaaaaaagtacgggatgg
10×a
-1283 tatatatgtatgagacaaaggaagtatcatgcttgaaaaaataaaattaaaacc
-1229 actatattggttacttggtgttatatataatttgttgttgtgattgtacacttt
-1175 tgaaatatgtcctaaaacaagatttattgttgattgcgtttctttttagtatcct
-1121 caaattttgatcatgacccaagaccgaaccgaatgtatgcccagttgcccaccc
-1067 ctaacgttattatggggtgaaaagatggttttgcccttaagagcaggtataata
-1011 gcaagctataagccacctattaaaatattttaaaaaa

716 ggctgagtcggccggtttcatgtattcctttgtgagtacttgagtaatcgcctt
770 cttagttcttgctcgttgaggaataaataaagagcttttcaggttgcaaacatc
824 atgcaaacgtttttgaattc

(Oshsp16.9B)

-1620 gtctcaaggattcatttccacatgtacaccttcatatatacttattgtaagccg
-1566 ccgttcggcgttgtaacctcaacccgttatagtgaagatatttgctgcctggca
-1512 cccgtggctttttttctcttgctttgagaggattttccacgttaaatcttatgc
-1458 cttatgtgattgatctattgctatttatcgtttatttgtctatcgttttctaac
-1404 ccttcttactaactgaatttaatacggtcaatttaatcatgagtctcttaaccg
-1350 aattaacattagctaaaactcagataaataaatatttttttcgtcccaaaaaaaaa
10×a

-959 agagcagcgggctacagatctgtagtcagctgcagcacggactccaatacgtaa
-905 tgtatgtatgataggtggaaccaggtattaatagtacagtaagcaactattgta
-851 tgaattgactattacattagctatagatgattttaagttagtagtgggctatac
-797 tattaaacttgctctaattgttttatgccagagctataatttttatccttttctt
-743 tgaggaataaatttttatactacggagtaacttattccgcgattaacggttgaat
-689 atctgttattaaactaacaatctcatctttttacttatacttatatttatcaat
-635 caaaatttaaaatttcaactttaaatttgaagttgattttaggggttttgatca
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-527 tttattaataaattattctttttttttttggcaaatatgctgtttgcaaatgtgt
10×t
-473 tataggcttgtagtaacggggtgtgatacaaaatcctttaaacgtccgtattta
-419 tcatgctgaatgaataggttggcaagtgtttaaaaaaaaaataggttggcaagca
9×a
-365 gtgggtcgctgattaatcattcgtcgttgtgcagaagcatctcgtcttgcagct
HSE HSE HSE
-311 acctgattaagtatatgtgattaacactggacaaatctggaacaaaaattaaat
HSE
-257 ccggctggtctttctaaatactccagtttaatacagtacacatcaaatctaaqt
-203 aggcaatcattgatttcatccagaccagcacttgggagtgtggagactagtctg
-149 gactgggaagcaaagccttttctagaagggagcagatcaaatcaggagcaccga
HSE HSE
-95 aacaaaagtagatgactcgagacgcctcaagaacgtccattgcgcagctcccgg
HSE HSE HSE HSE
-41 acaattcctataaatatgtgcaccgctcttgagctgaaacccaccgaggaacac
▲
14 acaacaaacaagacttgcatacgtcccaaactcccaaatcgccctctttgcaat
68 cacttcttccgttccatttcagtagcccacaacttcgcatcagaaagcgaaagc
122 tagagcaaccATGTCGCTGGTGAGGCGCAGCAACGTGTTCGACCCATTCTCCCT
M S L V R R S N V F D P F S L 15
176 CGACCTCTGGGACCCCTTCGACAGCGTGTTCCGCTCCGTCGTCCCGGCCACCTC
D L W D P F D S V F R S V V P A T S 33

-1242 taaaattaaaaccactatattggttacttggtgttatatataatttgttgttgt
-1188 gattgttcacttttgaaatatgtcctaaaacaagatttattgttgattgcgttt
-1134 cttttagtatcctcaaattttgatcatgacccaagaccgaaccgaatgtatgcc
-1080 cagttgcccacccctaacgttattatggggtgaaaagatggttttgcccttaag
-1026 agcaggtataatagcaagctataagccagctataaacatattttaaaaagataa
-972 aagaagatagagaagagcagcgggctacagatctgtagtcagctgcagcacgga
-918 ctccaatacgtaatgtatgtatgataagtggaaccaggtattaatagtacagta
-864 agcaactattgtatgaattgactattacattagctatagatgattttaagttag
-810 tagtgggctatactattaaacttgctctaattgttttatgccagagctataatt
-756 ttatcctttcttttgaggaataaatttttatactacggagtaacttattccgcga
-702 ttaacggttgaatatctgttattaaactaacaatctcatctttttacttatact
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-594 ggggttttgatcaaaattcatttttcagtctttacttttaagatcactaaaaaca
-540 cgtatatacaagttttattaataaattattctttttttttttggcaaatatgctg
10×t
-486 tttgcaaatgtgttatagqcttgtagtaacggggtgtgatacaaaatcctttaa
-432 acgtccgtatttatcatgctgaatgaataggttggcaagtgtttaaaaaaaaat
9×a
-378 aggttggcaagcagtgggtcgctgattaatcattcgtcgttgtgcagaagcatc
HSE
-324 tcgtcttgcagctacctgattaagtatatgtgattaacactggacaaatctgga
HSE HSE
-270 acaaaaattaaatccggctggtctttctaaatactccagtttaatacagtacac
-216 atcaaatctaagtaggcaatcattgatttcatccagaccagcacttgggagtgt
-162 ggagactagtctggactgggaagcaaagccttttctagaagggagcagatcaaa
HSE HSE
-108 tcaggagcaccgaaacaaaagtagatgactcgagacgcctcaagaacgtccatt
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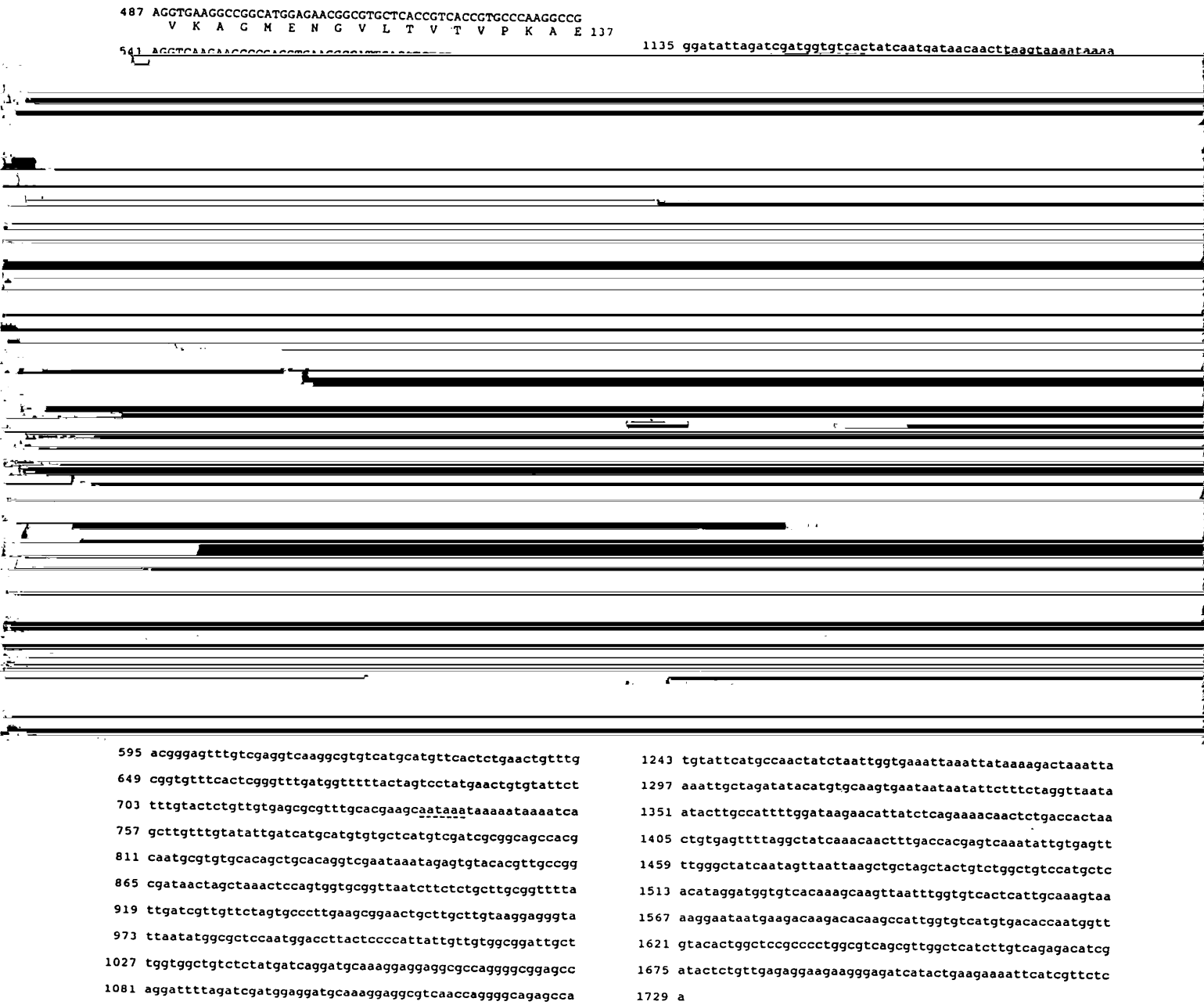

Fig. 4. DNA sequence of rice heat shock genes Oshsp 16.9A and Oshsp 16.9B. Putative TATA boxes and polyadenylation signals is designated by dashed underlines. The sites of transcriptional initiation are indicated by filled triangles. The HSE-like sequences and A/T-runs are underlined. EMBL accession numbers: M80938 (Oshsp 16.9A) and M80939 (Oshsp 16.9B). From Tzeng *et al.* (1992).

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                          10        20        30        40        50
1.Gmhsp17.5E      MSLIPGFFGGRRSNVFDPFSLDMWDPFKD-FHVPTSSVSA----ENSAFVS
2.Athsp17.6       MSLIPSIFGGRRTNVFDPFSLDVFDPFEG-FLTP-SGLANAPAMDVAAFTN
3.Ta C5-8         MSIV------RRSNVFDPFADLWADPFDT-FRSIVPAISGGSS-ETAAFAN
4.Oshsp16.9A      MSLV------RRSNVFDPFSLDLWDPFDSVFRSVVPA--TSDN-DTAAFAN
                  **          ** ******      ***    *                **

          60        70        80        90        100       110
1.     TRVDWKETPEAHVFKADIPGLKKEEVKVQIEDDRVLQISGERNVEKEDKNDTWHRVERSSGK
2.     AKVDWRETPEAHVFKADLPGLRKEEVKVEVEDGNILQISGERSNENEEKNDKWHRVERSSGK
3.     ARVDWKETPEAHVFKVDLPGVKKEEVKVEVEDGNVLVVSGERSREKEDKNDKWHRVERSSGK
4.     ARIDWKETPESHVFKADLPGVKKEEVKVEVEDGNVLVISGQRSKEKEDKNDKWHRVERSSGK
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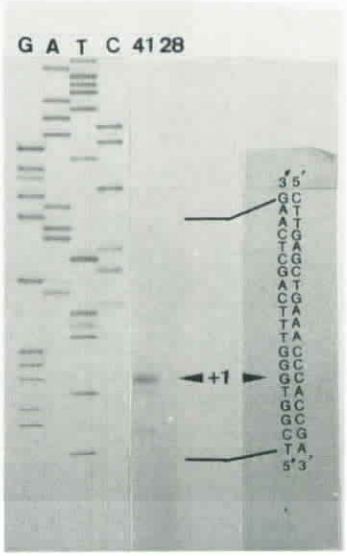

Fig. 6. Identification of the site of transcription initiation for Oshsp 16.9A and Oshsp 16.9B by the primer extension method. Lane 41 and lane 28 show the primer extension products of heat shock and control, respectively. The lanes designated G, A, T, C are from a DNA sequencing reaction in which the same primer was used. The transcription initiation site is indicated at +1.

cleotides located between the TATA box and the initiation of transcription is a common feature of many eukaryotic genes, which is related to accuracy of transcription (Schöffl *et al.*, 1984). Two weak bands were seen below the major band (lane 41 of Fig. 6), which might result from the cross-hybridization of RNA transcribed from closely related genes encoding HSPs in the same LMW class (Czarnecka *et al.*, 1985). There were no primer extension products in lane 28 (Fig. 6), indicating that the genes were heat-inducible. Once the transcription initiation site was determined, the 5' flanking sequences were searched for putative transcriptional regulatory elements, such as TATA-like motifs and HS consensus elements (HSEs). The TATA-like motif, TATAAATA, was present in the two genes from 26 to 33 bp upstream from the transcription start site. Multiple copies of HSE could be identified upstream from the TATA-like motif. There were tandem overlapping HSEs, (from -55 to -88) proximal to the TATA box, which is the typical feature of class I LMW HSP genes (Nagao and Key, 1989). Among these HSEs, 6 or 7 out of 10 nucleotides matched to the HS consensus, CTnGAAnnTTCnAG, as defined by Pelham (1985). Usually the HSEs of plant genes are not perfectly matched to the HS consensus (Czarnecka *et al.*, 1985; Nagao *et al.*, 1985); however, a perfect match is not required for functionality. The exact nature of the HSE is not clear. A deletion analysis of 5' upstream sequences of the soybean HS gene, Gmhsp 17.3-B, in transgenic tobacco plants indicated that two overlapping HSE-like sequences, located proximately to the TATA box, were required for heat inducible transcription of the native gene (Baumann *et al.*, 1987). Sequences other than the TATA-proximal HSEs have also been shown to be required for full expression of the promoter in plant HS genes (Czarnecka *et al.*, 1989). Further upstream from the putative promoter regions, Oshsp 16.9A and 16.9B genes contain DNA sequences very rich in A+T (69% compared to only 35% within the coding regions) starting with runs of "simple sequences" such as $(A)_9$ and $(T)_{10}$ at nucleotide position -388 and -509 (Fig. 4). Runs of "simple sequences", $(A)_n$, $(T)_n$, or $(AT)_n$ have been observed in most HS promoters in soybean (Raschke *et al.*, 1988). Czarnecka *et al.* (1990) demonstrated a binding of nuclear proteins to scattered AT-rich sequences of soybean Gmhsp 17.5 E gene promoter. It is possible that such sequences contribute to the transcriptional regulation of HS genes.

The significance of these upstream elements in gene regulation is being studied in our laboratory, using polymerase chain reaction (PCR) technique to amplify different DNA fragments.

Analysis of 3' Flanking Region

The 3' untranslated region (UTR) of Oshsp 16.9A and 16.9B shows a low degree of homology (44%). When the 259 nucleotides of 3' non-coding sequence of 16.9A were aligned to match that of 16.9B, only 116 nucleotides were homologous. Since the homology of 3'UTR in Oshsp 16.9A and 16.9B is low, we may determine the locations of these two gene in chromosomes by *in situ* hybridization using 3'UTR DNA fragments as probes. Localization of these genes in chromosomes may be helpful to resolve their role in HS response. The role of 3'UTR in affecting gene expression remains to be determined. However, the effect of the 3'UTR in two hsp70-adh cDNA gene chimeras were examined (Yost *et al.*, 1990). Both constructs were driven by the hsp70 promoter and contained the adh coding region. They differed in having either the adh

3'UTR or the hsp70 3'UTR. While transcripts with the adh 3'UTR were very stable during recovery from heat shock, transcripts with the hsp70 3'UTR were unstable, and decayed in a manner similar to the endogenous hsp70 transcripts. Thus, the 3'UTR of hsp70 message plays a critical role in regulating its degradation (Yost *et al.*, 1990). The putative polyadenylation signal, AATAAA, located between +791 and +796 in Oshsp 16.9A, and between +737 and +742 in 16.9B (Fig. 5). Oshsp 16.9A gene contained the sequence of pTS1 cDNA. In the pTS1 cDNA (Tseng *et al.*, 1992), the AATAAA sequence located in the region 20 nucleotides upstream of the site of poly (A) tail. A common feature of mRNA in higher eukaryotes is the presence of a sequence AAUAAA in the region from 11 to 30 nucleotides upstream of the site of the poly (A) addition. Since some plant HS genes do not have AATAAA polyadenylaiton signal at the end of the genes, it is possible that other sequences may act as signals for poly (A) addition in plants (Schöffl *et al.*, 1984).

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水稻 16.9 kD 熱休克蛋白質基因的分離和特性的研究

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本研究主要以已有的水稻熱休克蛋白質 cDNA pTS1 當探針，從基因庫中篩選到 2 個 16.9kD 的水稻熱休克蛋白質基因並加以定序，以爲日後研究熱休克蛋白質基因表現的調節機制之基礎。首先建立水稻次基因庫從中篩選到 2 個熱休克蛋白質基因定爲 Oshsp 16.9A 和 Oshsp 16.9B, pTS1 cDNA 完全包含在 Oshsp 16.9A 中。Oshsp 16.9A 和 Oshsp 16.9B 基因之 5'上游區域幾乎完全一樣而密碼區有 5 個核苷酸不同，但僅有一個胺基酸不一樣，即兩基因的密碼序列 98.8%相同，推導出的胺基酸 99.3%相同。在基因的 5'上游區域中，分別找出 TATA box, TATAAATA 和熱休克要素(HSEs)，與大豆的 class I 熱休克基因一樣，最接近 TATA box 的 HSEs 有部分並排重疊的現象，基因的轉錄起始點，以引子延伸方法定出，位於 TATA box 下游第 26 核苷酸 C 處，亦即蛋白質轉譯起始點上游第 131 核苷酸處。