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Plant Gene Register

Two *Oryza sativa* Genomic DNA Clones Encoding 16.9-Kilodalton Heat-Shock Proteins[1]

Shyh-Shyun Tzeng, Kai-Wen Yeh, Yih-Ming Chen, and Chu-Yung Lin*

Department of Botany, National Taiwan University, Taipei, Taiwan, Republic of China

When all living organisms are exposed to elevated temperatures, they exhibit induced synthesis of a set of proteins known as HSPs.[2] Using rice heat-shock cDNA pTSS.1 (5) as a probe, we isolated two 16.9-kD HSP genes from a rice genomic library (Table I). The rice genomic library was constructed in a λgt10 vector using *Eco*RI-digested rice genomic DNA fragments (size between 2.0 and 4.0 kb) as inserts that were eluted from agarose gel pieces by a NA 45 DEAE membrane (Schleicher and Schuell). The heat-shock cDNA pTS1 hybridized to 2.5- and 3.4-kb *Eco*RI-digested rice genomic DNA fragments in Southern hybridization. The DNA sequences of the two rice genomic HSP genes, Oshsp16.9A and Oshsp16.9B, were determined and analyzed as shown in Figure 1. Oshsp16.9A contained the sequence of cDNA pTS1. Comparison of Oshsp16.9A and Oshsp16.9B genes reveals that they share 98.8% homology in terms of the nucleotide sequence in the coding regions and 99.3% homology at the level of the deduced amino acid sequence. Multiple copies of the HSE-like sequence, similar to the *Drosophila* heat-shock consensus sequence (4), can be identified upstream from the putative TATA box, TATAAATA. The initiation site of the transcription of Oshsp16.9A and Oshsp16.9B, which was identified by the primer extension method (1), is located 131 bases upstream from the coding region and 26 bases downstream from the TATA-like region. The deduced polypeptide sequence of the Oshsp16.9A gene exhibits 73 and 84% homology to Gmhsp17.5E from soybean (2) and c5-8 from wheat (3), respectively.

LITERATURE CITED

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[2] Abbreviations: HSP, heat-shock protein; HSE, heat-shock element.

Table I. *Characteristics of Two 16.9-kD Heat-Shock Genes from Oryza sativa*

Organism:
Oryza sativa L. (rice), cv Tainon 67.

Location on Chromosome:
Unknown.

Gene Designation:
Oshsp16.9A and Oshsp16.9B.

Source:
Obtained from a λgt10 subgenomic library using rice 16.9-kD heat shock cDNA pTS1 as a probe. Fragments subcloned in M13 mp18 vector and sequenced by dideoxy sequencing method.

Method of Identification:
Sequence comparison with cDNA pTS1. (100 and 98.8% nucleotide homology to coding region of pTS1 in Oshsp16.9A and Oshsp16.9B, respectively).

Regulation:
The putative regulatory sequences, HSE-like sequences, are located in the 5′-upstream regions of the genes and may possibly be recognition sites of heat-shock transcription factors.

Features of Gene Structure:
TATA box, polyadenylation signal, HSE, and transcriptional initiation site (Fig. 1).

Structural Features of Protein:
Open reading frame of 450 base pairs encoding a polypeptide of 150 amino acid residues of 16.9 kD. Isoelectric point 6.4. Belonging to low molecular mass HSPs.

Tissue Location:
Found in rice seedlings.

EMBL Accession Nos:
M80938 and M80939.

a) Oshsp16.9A

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                                                              8.
-1661 gtctcaaggattcatttccacatgtataaaggctagcttcggacggatcgttat
-1607 cactttagtgtttcaccttcatatatacttattgtaagccgccgttcggcgttg
-1553 taacctcaacccgttatagtgaagatatttgctgcctggcacccgtggcttttt
-1499 ttctcttgctttgagaggattttccacgttaaatcttatgccttatgtgattga
-1445 tctattgctatttatcgtttatttgtctatcgttttctaacccttcttactaac
-1391 tgaatttaatacggtcaatttaatcatgagtctcttaaccgaattaacattagc
-1337 taaaactcagataaataaatatttttttcgtcccaaaaaaaaaagtacgggatgg
-1283 tatatatgtatgagacaaaggaagtatcatgcttgaaaaaataaaattaaaacc
-1229 actatattggttacttggtgttatatataatttgttgttgtgattgttcacttt
-1175 tgaaatatgtcctaaaacaagatttattgttgattgcgtttcttttagtatcct
-1121 caaattttgatcatgacccaagaccgaaccgaatgtatgcccagttgcccaccc
-1067 ctaacgttattatggggtgaaaagatggttttgcccttaagagcaggtataata
-1013 gcaagctataagccagctataaacatattttaaaaagataaaagaagatagaga
 -959 agagcagcgggctacagatctgtagtcagctgcagcacggactccaatacgtaa
 -905 tgtatgtatgataggtggaaccaggtattaatagtacagtaagcaactattgta
 -851 tgaattgactattacattagctatagatgattttaagttagtagtgggctatac
 -797 tattaaacttgctctaattgttttatgccagagctataattttatcctttcttt
 -743 tgaggaataaattttatactacggagtaacttattccgcgattaacggttgaat
 -689 atctgttattaaactaacaatctcatctttttacttatacttatatttatcaat
 -635 caaaatttaaaatttcaactttaaatttgaagttgattttaggggttttgatca
 -581 aaattcatttttcagtctttactttaagatcactaaaaacacgtatatacaagt
 -527 tttattaataaattattcttttttttttggcaaatatgctgtttgcaaatgtgt
 -473 tataggcttgtagtaacggggtgtgatacaaaatcctttaaacgtccgtattta
 -419 tcatgctgaatgaataggttggcaagtgtttaaaaaaaaataggttggcaagca
 -365 gtgggtcgctgattaatcattcgtcgttgtgcagaagcatctcgtcttgcagct
 -311 acctgattaagtatatgtgattaacactggacaaatctggaacaaaaattaaat
 -257 ccggctggtctttctaaatactccagtttaatacagtacacatcaaatctaagt

                                                             9.
 -203 aggcaatcattgatttcatccagaccagcacttgggagtgtggagactagtctg
 -149 gactgggaagcaaagccttttctagaagggagcagatcaaatcaggagcaccga
  -95 aacaaaagtagatgactcgagacgcctcaagaacgtccattgcgcagctcccgg
  -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
  230 CGACAACGACACCGCCGCCTTCGCCAACGCCCGCATCGACTGGAAGGAGACGCC
       D  N  D  T  A  A  F  A  N  A  R  I  D  W  K  E  T  P  51
  284 GGAGTCGCACGTCTTCAAGGCCGACCTCCCCGGCGTCAAGAAGGAGGAGGTGAA
       E  S  H  V  F  K  A  D  L  P  G  V  K  K  E  E  V  K  69
  338 GGTGGAGGTGGAGGAAGGCAACGTGCTGGTGATCAGCGGGCAGCGCAGCAAGGA
       V  E  V  E  E  G  N  V  L  V  I  S  G  Q  R  S  K  E  87
  392 GAAGGAGGACAAGAACGACAAGTGGCACCGCGTGGAGCGCAGCAGCGGGCAGTT
       K  E  D  K  N  D  K  W  H  R  V  E  R  S  S  G  Q  F 105
  446 CATGCGGCGGTTCCGGCTGCCGGAGAACGCCAAGGTGGACCAGGTGAAGGCCGG
       M  R  R  F  R  L  P  E  N  A  K  V  D  Q  V  K  A  G 123
  500 ACTGGAGAACGGCGTGCTCACCGTCACCGTGCCCAAGGCCGAGGTCAAGAAGCC
       L  E  N  G  V  L  T  V  T  V  P  K  A  E  V  K  K  P 141
  554 TGAGGTGAAGGCCATTGAGATCTCCGGTTAAgctcctgaagatgtgatcggtga
       E  V  K  A  I  E  I  S  G  *                         150
  608 gggaagaagtcatgtttggtgtcagtaattcagtatttcagtgtgtgtttgttt
  662 ggtcgtgcaagtatggtctgctgctggtgtgtcgtacgcgttgggagtccgagt
  716 ggctgagtcggccggtttcatgtattcctttgtgagtacttgagtaatcgcctt
  770 cttagttcttgctcgttgaggaataaataaagagcttttcaggttgcaaacatc
  824 atgcaaacgttttgaattc
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b) Oshsp16.9B

```
                                                              10.
-1620 gtctcaaggattcatttccacatgtacaccttcatatatacttattgtaagccg
-1566 ccgttcggcgttgtaacctcaacccgttatagtgaagatatttgctgcctggca
-1512 cccgtggctttttttctcttgctttgagaggattttccacgttaaatcttatgc
-1458 cttatgtgattgatctattgctatttatcgtttatttgtctatcgttttctaac
-1404 ccttcttactaactgaatttaatacggtcaatttaatcatgagtctcttaaccg
-1350 aattaacattagctaaaactcagataaataaatatttttttcgtcccaaaaaaaa
-1296 aagtacgggatggtatatatgtatgagacaaaggaagtatcatgcttgaaaaaa
-1242 taaaattaaaaccactatattggttacttggtgttatatataatttgttgttgt
-1188 gattgttcacttttgaaatatgtcctaaaacaagatttattgttgattgcgttt
-1134 cttttagtatcctcaaattttgatcatgacccaagaccgaaccgaatgtatgcc
-1080 cagttgcccacccctaacgttattatggggtgaaaagatggttttgcccttaag
-1026 agcaggtataatagcaagctataagccagctataaacatattttaaaaagataa
 -972 aagaagatagagaagagcagcgggctacagatctgtagtcagctgcagcacgga
 -918 ctccaatacgtaatgtatgtatgataagtggaaccaggtattaatagtacagta
 -864 agcaactattgtatgaattgactattacattagctatagatgattttaagttag
 -810 tagtgggctatactattaaacttgctctaattgttttatgccagagctataatt
 -756 ttatcctttcttttgaggaataaattttatactacggagtaacttattccgcga
 -702 ttaacggttgaatatctgttattaaactaacaatctcatctttttacttatact
 -648 tatatttatcaatcaaaatttaaaatttcaactttaaatttgaagttgatttta
 -594 ggggttttgatcaaaattcatttttcagtctttactttaagatcactaaaaaca
 -540 cgtatatacaagttttattaataaattattcttttttttttggcaaatatgctg
 -486 tttgcaaatgtgttataggcttgtagtaacggggtgtgatacaaaatcctttaa
 -432 acgtccgtatttatcatgctgaatgaataggttggcaagtgtttaaaaaaaaat
 -378 aggttggcaagcagtgggtcgctgattaatcattcgtcgttgtgcagaagcatc
 -324 tcgtcttgcagctacctgattaagtatatgtgattaacactggacaaatctgga
 -270 acaaaaattaaatccggctggtctttctaaatactccagtttaatacagtacac
 -216 atcaaatctaagtaggcaatcattgatttcatccagaccagcacttgggagtgt

                                                              11.
 -162 ggagactagtctggactgggaagcaaagccttttctagaagggagcagatcaaa
 -108 tcaggagcaccgaaacaaaagtagatgactcgagacgcctcaagaacgtccatt
  -55 gcgcagctcccggacaattcctataaatatgtgcaccgctcttgagctgaaacc
    1 caccgaggaacacacaacaaacaagacttgcatacgtcccaaactcccaaatcg
   55 ccctctttgcaatcacttcttccgttccatttcagtagcccacaacttcgcatc
  109 agaaagcgaaagctagagcaaccATGTCGCTGGTGAGGCGCAGCAACGTGTTCG
                             M  S  L  V  R  R  S  N  V  F  D 11
  163 ACCCATTCTCCCTCGACCTCTGGGACCCCTTCGACAGCGTGTTCCGCTCCGTCG
        P  F  S  L  D  L  W  D  P  F  D  S  V  F  R  S  V  V 29
  217 TCCCGGCCACCTCCGACAACGACACCGCCGCCTTCGCCAACGCCCGCATCGACT
        P  A  T  S  D  N  D  T  A  A  F  A  N  A  R  I  D  W 47
  271 GGAAGGAGACGCCGGAGTCGCACGTCTTCAAGGCCGACCTCCCCGGCGTCAAGA
        K  E  T  P  E  S  H  V  F  K  A  D  L  P  G  V  K  K 65
  325 AGGAGGAGGTGAAGGTGGAGGTGGAGGAAGGCAACGTGCTGGTGATCAGCGGGC
        E  E  V  K  V  E  V  E  E  G  N  V  L  V  I  S  G  Q 83
  379 AGCGCAGCAAGGAGAAGGAGGACAAGAACGACAAGTGGCACCGCGTGGAGCGCA
        R  S  K  E  K  E  D  K  N  D  K  W  H  R  V  E  R  S101
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Figure 1. DNA sequence of rice heat shock genes, (a) Oshsp16.9A and (b) Oshsp16.9B, putative TATA boxes and polyadenylation signals are designated by dashed underlines. The sites of initiation of transcription are indicated by filled triangles. The HSE-like sequences are underlined.

```
433  GCAGCGGGCAGTTCATGCGGCGGTTCCGGCTGCCGGAGAACGCCAAGGTGGACC
        S  G  Q  F  M  R  R  F  R  L  P  E  N  A  K  V  D  Q119

487  AGGTGAAGGCCGGCATGGAGAACGGCGTGCTCACCGTCACCGTGCCCAAGGCCG
        V  K  A  G  M  E  N  G  V  L  T  V  T  V  P  K  A  E137

541  AGGTCAAGAAGCCCGAGGTGAAGGCCATTGAGATCTCTGGCTAAaatggtgaaa
        V  K  K  P  E  V  K  A  I  E  I  S  G  *            150

595  acgggagtttgtcgaggtcaaggcgtgtcatgcatgttcactctgaactgtttg

649  cggtgtttcactcgggtttgatggtttttactagtcctatgaactgtgtattct

703  tttgtactctgttgtgagcgcgtttgcacgaagcaataaataaaaataaaatca

757  gcttgtttgtatattgatcatgcatgtgtgctcatgtcgatcgcggcagccacg

811  caatgcgtgtgcacagctgcacaggtcgaataaatagagtgtacacgttgccgg

865  cgataactagctaaactccagtggtgcggttaatcttctctgcttgcggtttta

919  ttgatcgttgttctagtgcccttgaagcggaactgcttgcttgtaaggagggta

973  ttaatatggcgctccaatggaccttactccccattattgttgtggcggattgct

1027 tggtggctgtctctatgatcaggatgcaaaggaggaggcgccaggggcggagcc

                                                        12.
1081 aggattttagatcgatggaggatgcaaaggaggcgtcaaccaggggcagagcca

1135 ggatattagatcgatggtgtcactatcaatgataacaacttaagtaaaataaaa

1189 ttagtgctaattgataaagtaaaatagaattttcttttcaaaaataattcgact

1243 tgtattcatgccaactatctaattggtgaaattaaattataaaagactaaatta

1297 aaattgctagatatacatgtgcaagtgaataataatattctttctaggttaata

1351 atacttgccattttggataagaacattatctcagaaaacaactctgaccactaa

1405 ctgtgagttttaggctatcaaacaactttgaccacgagtcaaatattgtgagtt

1459 ttgggctatcaatagttaattaagctgctagctactgtctggctgtccatgctc

1513 acataggatggtgtcacaaagcaagttaatttggtgtcactcattgcaaagtaa

1567 aaggaataatgaagacaagacacaagccattggtgtcatgtgacaccaatggtt

1621 gtacactggctccgcccctggcgtcagcgttggctcatcttgtcagagacatcg

1675 atactctgttgagaggaagaagggagatcatactgaagaaaattcatcgttctc

1729 a
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