We isolated and analyzed two genomic DNAs that encode the heat-shock protein Hsp30 from *Coriolus versicolor*. The amino acid sequences substitute only three amino acid substitutions. The promoter regions contain the consensus heat-shock element, a xenobiotic-response element, a stress-response element, and a metal-response element. The levels of mRNAs for Hsp30 increased markedly after exposure of *C. versicolor* to pentachlorophenol and levels were higher than those after heat shock.

**Key words:** white-rot fungi; heat shock protein; HSE; XRE

The white-rot fungi *Phanerochaete chrysosporium* and *Coriolus versicolor* are typical lignin-degrading microorganisms. Since they can degrade lignin, it has been proposed that these fungi might be useful for bioremediation. Indeed, the ligninolytic systems of these fungi can degrade a wide variety of aromatic pollutants. A full understanding of the degradation of hazardous chemicals by white-rot fungi at the molecular level might be useful for future applications of these microorganisms to bioremediation. Recently, we identified a cDNA, designated Fdd123, in *C. versicolor* (IFO30340) the expression of which is increased by the hazardous chemical pentachlorophenol (PCP). The gene encodes a highly hydrophobic transmembrane protein of 283 amino acids that is homologous to Hsp30 and Yro2 of *Saccharomyces cerevisiae*. In this report, we describe the structure of two genomic genes for Hsp30 from *C. versicolor* and their promoter regions.

The genomic library was screened with a fragment of Fdd123. Seven positive clones were analyzed by Southern blotting. Two different types of genomic clone which we designated cvhsp30/W1 and cvhsp30/W2, were recognized. The nucleotide sequences reported herein will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases under the accession numbers AB018405 (Fdd123b), AB018406 (cvhsp30/W1), and AB018407 (cvhsp30/W2). The sequences of the genomic clone cvhsp30/W1 corresponded to Fdd123 cDNA and cvhsp30/W2 corresponded to Fdd123b. Both genomic clones consisted of four exons (Fig. 1). The cvhsp30/W1 and cvhsp30/W2 genes each contained three introns, which were found at the same respective positions in both genes. We found 5′ consensus splice sites (GTANGT) in all of the introns but the 3′ splice sites of some introns did not strictly match the consensus sequence (YAG). The consensus lariat sequence found in fungal introns (PuCTPuAC) was conserved in only one of these introns. Many of the base changes were found at the third position of codons within the open reading frames. The 37 nucleotide differences within the open reading frames resulted in only three amino acid substitutions: Ile185 to Leu; Ala264 to Thr; and Gln265 to His. The genes both encoded proteins of 283 amino acids and the proteins were 98.9% homologous at the amino acid level. The restriction maps of the two cDNAs and two genomic clones that we isolated differed as shown in Fig. 1. Genomic Southern blotting analysis suggests that the isolated genes each represent the allele of a single hsp30 gene (data not shown).

No significant homology was detected when the Hsp30s of *C. versicolor* were compared with Hsp30s from *Coriolus versicolor* and the increase of their expression by heat shock and exposure to a hazardous chemical...
Fig. 2. Nucleotide Sequence of the 5′ Untranslated Regions for Hsp30.

The promoter elements are underlined and they include sequences that match the heat-shock element (HSE; nGAAnnTTCnnGAAn), the stress-response element (STRE; CCCCT and AGGGG), the xenobiotic-response element (XRE; CACGCW), the metal-response element (MRE; TGCGCAC), the TATA box and the CAAT box. Blocks of GAA and TTC referred to in the text are boxed. Identical nucleotides are indicated by asterisks. Missing nucleotides are indicated by hyphens.

from *Neurospora crassa*,9) *Aspergillus nidulans*,10) and *Drosophila melanogaster*.11) Alignment of the amino acid sequence of Hsp30s from C. *versicolor* with those of Hsp30 and Yro2 from *S. cerevisiae* revealed strong conservation of domains that are conserved among the small Hsps of *S. cerevisiae*. It seems that small Hsps form a heterogeneous group.

Within the 5′ flanking regions of *cvhsp30/1* and *cvhsp30/2*, we found a putative TATA box- and a CAAT box-like sequence. In the promoters of genes of filamentous fungi, conserved nucleotides are found near the site of initiation of translation that agree fairly well with those found in higher eukary-
otes, RNNATGG. In filamentous fungi, the −3 positions are adenine or a purine 90% of the time. The −3 positions were adenine in both cvhsp30/1 and cvhsp30/2.

As shown in Fig. 2, the promoter regions also contained a consensus heat-shock element (HSE), nGAAAnnTTTcnGAAAn; a xenobiotic-response element (XRE), CACGGC; a stress-response element (STRE), CCCCT, and AGGGG; and a metal-response element (MRE), TGCGCAC upstream of the TATA box-like sequence. Maximum induction of the gene for Hsp70 in Drosophila requires two distinct TATA-proximal HSEs, to which a heat-shock transcription factor binds cooperatively. Furthermore, this HSE is essential for transcription and binds a heat-shock transcriptional factor. Amin et al. showed that the promoter elements of this gene might be strengthened further by periodic blocks of GAA. In each promoter region of genes for Hsp30 from C. versicolor, we found one strictly conserved HSE and adjacent periodic blocks of GAA and TTC. The promoter of the Hsp30 gene of S. cerevisiae has no good agreement to the HSE and the STRE consensus sequences. S. cerevisiae Hsp30 has recently been shown to be under a novel stress control, not the HSE or STRE. On the other hand, examination of the promoter for Hsp30 genes found perfect matches to the HSE and the STRE consensus sequences. We suppose that either the HSE or the STRE will control the expression of the Hsp30 genes from C. versicolor.

To find whether the cvhsp30/1 and cvhsp30/2 genes might be responsive to heat shock, as expected from their sequences, we examined the expression of cvhsp30/1 and cvhsp30/2 by Northern blotting (Fig. 3). Agarose gel electrophoresis, followed by staining with SYBR GREEN II (Molecular Probes) to see 28S and 18S rRNA, confirmed that samples from different preparations contained equal amounts of intact total RNA. The intensities of rRNA signals were scanned with a FluorImager 595 (Amersham Pharmacia Biotech) and measured with an ImageQuant software (Amersham Pharmacia Biotech) and measured with an ImageQuant software (Amersham Pharmacia Biotech). A single band of mRNA of about 1.2 kb hybridized with the antisense Fdd123 RNA probe. Northern blotting revealed the presence of transcripts of cvhsp30/1 and cvhsp30/2 at 28°C, a normal culture temperature, and the signal is two-fold higher intensity in (Fig 3, lane 4) within 1 h at 37°C. The heat-induced accumulation of the mRNAs reached a maximum within 1 h and then decreased slightly. Northern blotting analysis revealed that expression of the genes for Hsp30 was increased by heat shock. It also appeared that the genes were expressed constitutively at normal growth temperatures, as is the case for many major heat-shock proteins, and such proteins are often essential for cell growth. We also examined the effects of chemical stress by expos-


