Heat shock protein 70 (HSP 70) Genes of the Filarial nematode Setaria digitata

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Abstract:

Parasitic nematodes are a major cause of morbidity and mortality among humans and their domestic livestock. The filarial parasite Setaria digitata is the causative agent of cerebrospinal nematodiasis in its abnormal hosts such as sheep, goats and horses, and therefore is of significant veterinary importance. This study involved the isolation and characterization of heat shock protein 70 (hsp70) genes from S. digitata, in oredr to shed light on the molecular biology of this parasite. A genomic library of S. digitata was constructed and screened with a hsp70 cDNA fragment of Caenorhabditis elegans. The isolated clone, designated LESH 1, had an insert of 13.4 kb and contained sequences from two hsp70 genes. One gene, hsp70-2, was completely sequenced and found to contain nine introns ranging in size from 78 to 195 bp. The region upstream of the initiation codon contained a putative TATA box, two CCAAT box elements and three heat shock elements. A putative transcription initiation site was also identified. The 5' untranslated region contained a splice acceptor sequence. Preliminary PCR analyses of the 5' terminus of the hsp70-2 transcript suggested that the gene may be trans-spliced. The gene was typically AT-rich, having a GC content of 44.5 percent. The deduced amino acid sequence potentially encoded a cytosolic protein of 645 amino acids. Several conserved amino acids, involved in ATPase activity were identified in the amino terminal portion. A calmodulin binding site was also identified. Three consecutive repeats of a tetrapeptide motif GGMP, were present near the carboxyl end. The gene appeared to be constitutively transcribed, and was not significantly enhanced in response to heat shock in adult worms. Another hsp70 gene (hsp70-1) was located further upstream, arranged in direct tandem with hsp70-2. The amino terminal portion of hsp70-1 was however, absent in LESH 1. Partial sequencing of the hsp70-1 gene revealed that it was very similar in sequence, to the hsp70-2 gene, especially in the coding regions. Four introns were identified in hsp70-1, ranging in size from99 to 477bp. The relative positions of all but one of the introns, as well as the phase in which they interrupted the reading frame, were identical to hsp70-2. The 3' untranslated region of the hsp70-1 gene contained a putative polyadenylation signal, 59 bp downstream of the stop codon. A cDNA library was constructed from adult Setaria worms, and was screened with a subclone of lESH 1. An isolated clone designated pcHS 14, was partially sequenced and found to contain hsp70-1 gene sequences. The clone contained approximately half of the hsp70-1 gene from the carboxyl end. Southern blot analysis revealed the presence of two or three additional hsp70 related genes in the digitata genome.

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