Splice variants in hsp70 cDNAs from the marine copepod *Calanus finmarchicus*

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The copepod *Calanus finmarchicus* is an abundant crustacean in the North Atlantic, providing a major food source for many teleosts and some elasmobranchs. Its oceanic distribution depends at least in part on water temperatures, which are increasing measurably as a result of global warming. In a 2003 study, we found that the expression of heat shock protein 70 (hsp70) increases following either an acute (30-minute) temperature shock or an extended (48-hour) exposure to a warmer environment (transfer from 8 to 18° C)[2]. In a follow-up study, we have analyzed in greater detail the hsp70 cDNAs prepared from *C. finmarchicus* and have found evidence for at least two hsp70-encoding genes in the Gulf of Maine population, with one of them showing apparent splice variants.

Total RNA was prepared from whole copepods preserved in RNA*later* (Ambion). Analysis of total RNA with an Agilent Bioanalyzer revealed a single sharp peak of rRNA, the crustacean 28S rRNA fragmenting to two smaller products, both of which coincide with the 18S rRNA peak. cDNA was reverse transcribed from total RNA using oligo-dT as primer and a partial hsp70 sequence was amplified using *Calanus*-specific primers and Red*taq* DNA polymerase (Sigma) at an annealing temperature of 55° C. Following electrophoresis on agarose gels, single bands were excised and the DNA prepared for sequencing (Qiagen). Raw sequence traces, analyzed by Chromas software, revealed numerous instances of multiple overlapping peaks, particularly in the third codon position.

To isolate individual cDNAs, the PCR product was ligated into the TA cloning vector (Invitrogen) and seven individual clones were isolated for plasmid extraction. Following a subsequent round of sequencing, we obtained evidence for the existence at least two hsp70 genes (or alleles) encoding nearly the same amino acid sequence (Fig. 1). mRNAs transcribed from one of these genes, however, were clearly the result of alternative splicing, producing proteins that differed in length by 26 amino acids (Fig. 1, clones 5R and 6R).

Numerous instances of third-position sequence variations were noted. Of 23 variations, 20 were in the third codon position, and only 3 of these represented amino acid substitutions. Some of these variants may have resulted from the lack of proofreading of the *taq* polymerase used to generate the amplification products, notable in clone 8R that contained several unique variants, including one not in the third position. Most of the variations, however, were exhibited by two or more of the clones, suggesting that they existed in the organism and were not artifacts of amplification. Our data suggest that a family of hsp70 genes exists in Gulf of Maine *C. finmarchicus*, similar to the phenomenon observed in other species[1]. Variant-specific probes and primers will be required to sort out the expression pattern of these heat shock proteins in response to environmental perturbation.

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1. **Feder, M.E., and G.E. Hofmann.** Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Ann. Rev. Physiol.* 61:243-282, 1999.

2. Voznesensky, M., P.H. Lenz, C. Spanings-Pierrot, and D.W. Towle. Genomic approaches to detecting thermal stress in *Calanus finmarchicus* (Copepoda: Calanoida). *J. Exp. Mar. Biol. Ecol.* 311: 37-46, 2004.

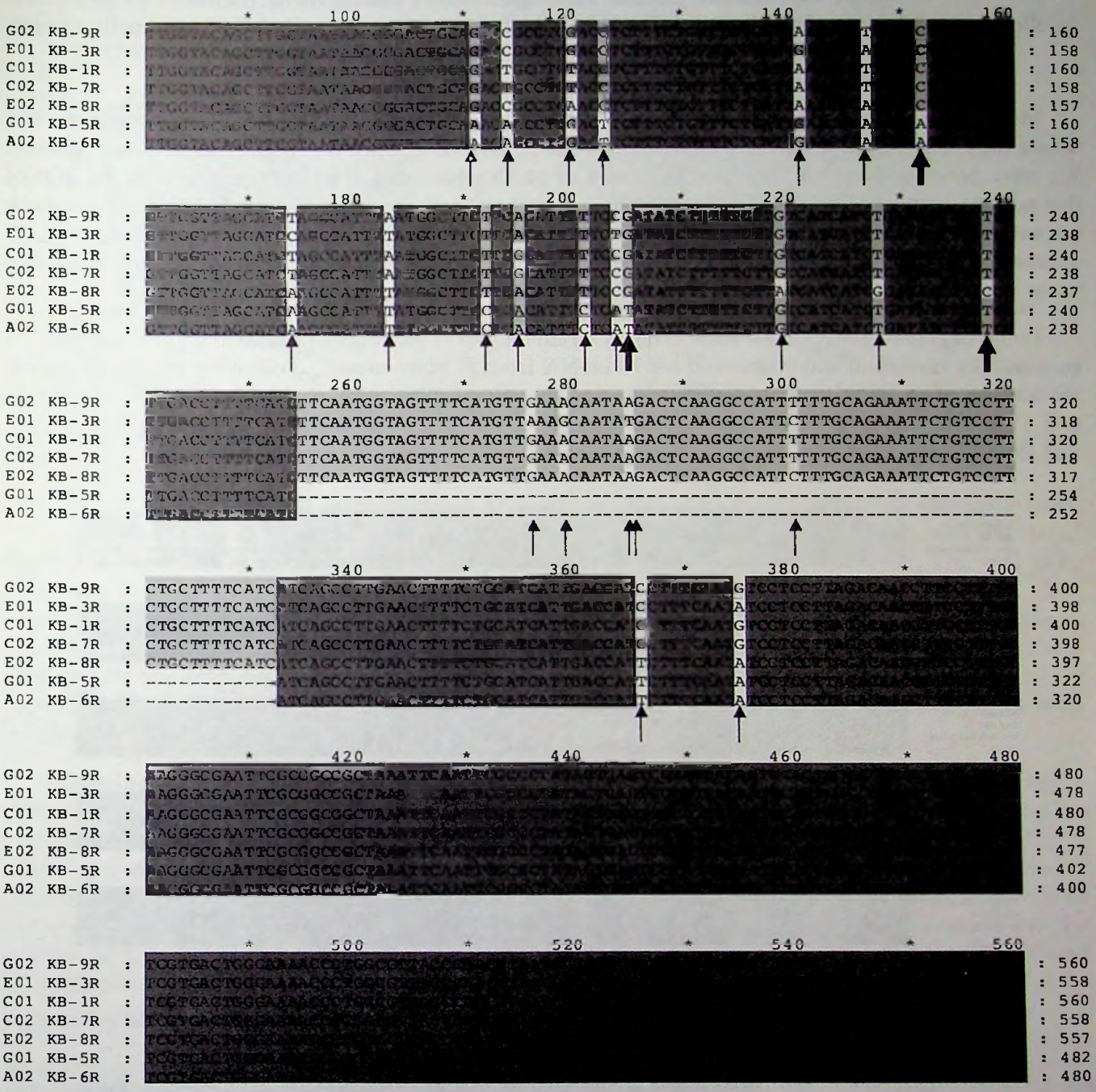

Fig. 1. Multiple nucleotide alignment of partial hsp70 cDNA sequences of individual recombinant plasmids obtained by PCR with *Calanus*-specific hsp70 primers. Clones 5R and 6R are clearly splice variants lacking a 78-nucleotide region that is present in the remaining 5 clones. Third-position variations, indicated by thin arrows, are particularly notable in the region upstream of the splice site. Several variations occur in other codon positions as well (heavy arrows). An in-frame stop codon appears in the insert within the extended sequence of clones 1R, 7R, 8R, and 9R (double arrow), indicating that these mRNAs may yield an incomplete protein product.