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Isolation and structural analysis of rat heat-inducible hsp70 gene

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Heat shock (hsp) genes are a group of genes activated in prokaryotic and eukaryotic cells in response to various environmental and endogenous stimuli [1]. The most prominent and of the highest evolutionary conservation is the hsp70 multigene family, the genes of which encode proteins of relative molecular mass of approx. 70000 [2]. In eukaryotes the hsp70 gene family, besides the heat inducible genes, contains also several related ones which are constitutively expressed [1–3].

HSP70 proteins belong to a class of proteins called molecular chaperons [3] the function of which is to control conformational changes of other cellular proteins during various physiological procesess. In cells subjected to environmental stress (e.g. heat shock) the HSP70 proteins seem to recognize misfolded proteins and help them to regain the native conformation.

While both heat inducible and constitutively expressed genes of human and mouse hsp70 family are relatively well characterized, none of the rat heat inducible genes have been sequenced so far. Rat hsp70 multigene family seems to contain at least two heat inducible genes, the expression of which gives rise to transcripts of 2.5 kb and 2.7 kb, respectively [4]. Recently, from rat DNA library we isolated a genomic clone (λ68, Fig. 1) containing hsp70 gene-related sequences, which under stringent conditions hybridized specifically with the 2.5 kb transcript [6].

To determine the nucleotide sequence of that part of the λ68 clone in which hsp70 sequences were identified, we used the strategy shown on Fig. 1. DNA sequencing was performed on double stranded templates by dideoxy-chain termination method [7] using T7 DNA polymerase or Klenow fragment of DNA polymerase I.

Analysis of the DNA sequence revealed an uninterrupted open reading frame of 1926 bp (Fig. 2). The identified frame codes for a protein of 641 amino acids with a relative molecular mass of approx. 70100. The predicted amino-acid sequence shows the highest similarity (98%) with the mouse hsp70.1 gene [8].

We established the nucleotide sequence of an approx. 3 kb DNA fragment which contains the entire transcription unit of the hsp70.1 gene (2487 bp from the transcription initiation site to the polyadenylation signal), and 5′ end and 3′ end flanking regions. The sequence of the gene has been registered in the EMBL Data Library under accession number X74271 *R. norvegicus* hsp70 gene.

The position of the transcription initiation start site (223 bp upstream the ATG codon) was determined by the RNase protection and primer extension methods (not shown). In the region which flanks the 5′ end of the gene several short DNA sequences of potential regulatory functions were identified (Fig. 3). Among them are heat shock elements (HSEs) which, in heat shock genes are recognized by a specific transcription factor (heat shock factor, HSF) [10]. Preliminary experiments in which rat hepatoma cells were transfected with constructs containing the chloramphenicol acetyl transferase (CAT) reporter gene ligated to DNA sequences flanking the 5′ end of the hsp70.1 gene demonstrated that the gene isolated by us was functional and heat inducible (not shown).

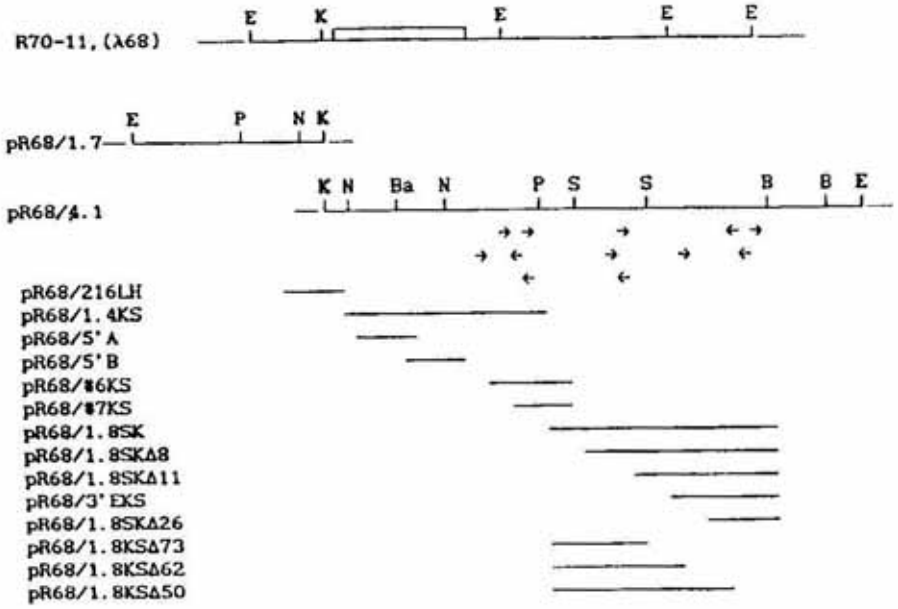

Fig. 1. Strategy of sequencing of the hsp70.1 gene.
The genomic fragment termed R70-11 which contains the heat inducible hsp70 gene (open bar) was isolated (as a λ68 clone) by screening rat DNA library [5]. Subclones pR68/4.1 and pR68/1.7 were constructed by inserting the indicated DNA fragments into *Kpn*I and *Eco*RI restriction sites of the pUC19 [6]. Subclone pR68/4.1 contains the entire transcription unit of the hsp70.1 gene. Main subclones and deletion clones (Δ) used for sequencing are shown below the restriction maps. Certain regions of the gene were sequenced using synthetic oligonucleotide primers (small arrows) complementary to already known DNA sequence. B, Ba, E, K, N, P, S correspond to restriction sites: *Bam*HI, *Bal*I, *Eco*RI, *Kpn*I, *Nco*I, *Pst*I and *Sma*I, respectively.

In conclusion, this is first report on cloning and determining the nucleotide sequence of the functional, heat inducible gene which belongs to the rat hsp70 gene family. This will facilitate investigations on differential expression of hsp70 genes in rat tissues. It opens also the possibility of establishing the chromosomal localization of the gene all the more so that Wurst *et al.* [11] has demonstrated that in rat, similarly as in the case of humans and mice an hsp70-related genes are localized within the major histocompatibility complex.

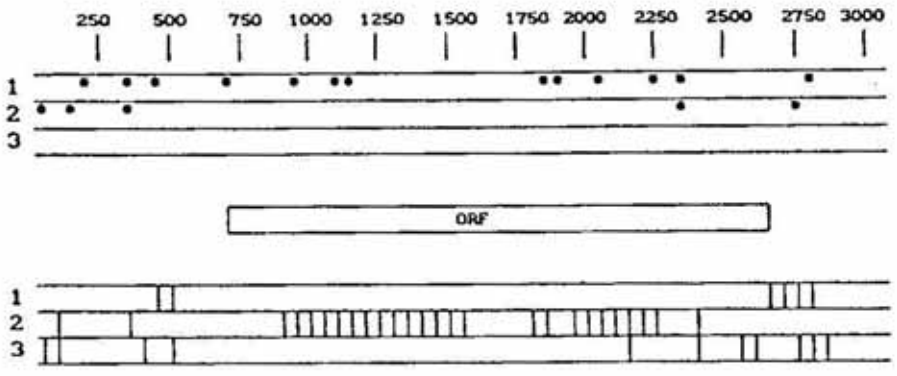

Fig. 2. Identification of the open reading frame (ORF) of the hsp70.1 gene.
The diagram shows the localization of ATG codons (✱) and stop codons (|) in three reading frames. The identified open reading frame (shown as a box) contains 1926 nucleotides and codes for a protein of 641 amino acids.

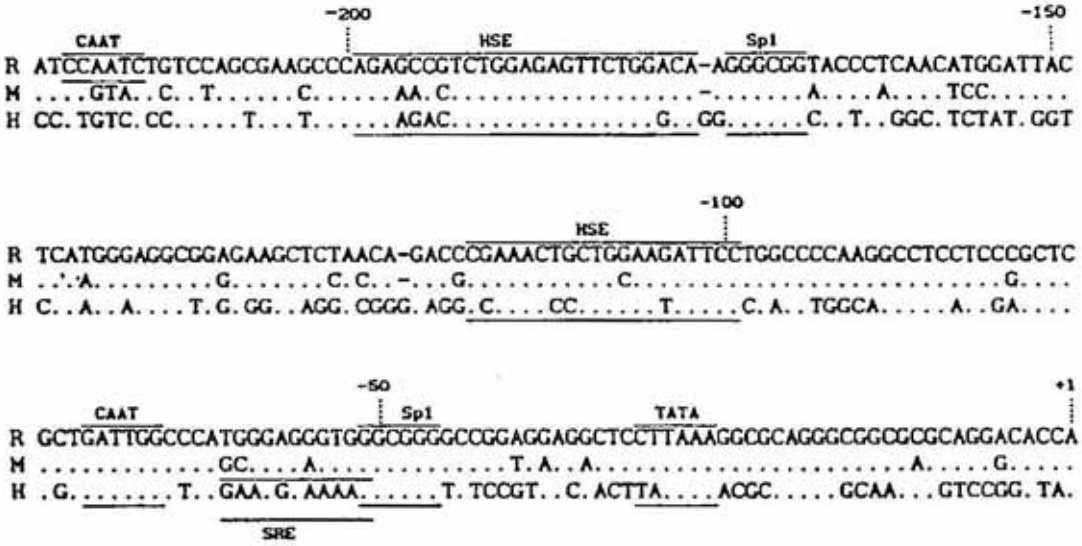

Fig. 3. Nucleotide alignment of the rat (R) hsp70.1 promoter to the mouse (M) hsp70.1 promoter [8] and human (H) hsp70/hsp70.1 promoter [9].

Nucleotide number 1 refers to the transcription initiation start site of the rat gene. This nucleotide corresponds to a nucleotide number 437 according to the DNA sequence deposited in the EMBL Data Library under accesion number X74271 *R. norvegicus* hsp70 gene. Sequences of possible regulatory functions: HSE (heat shock element); CAAT box; Sp1 binding sites; TATA box; and serum response element (SRE) are indicated. Dots represent nucleotides identical to corresponding ones in the rat hsp70.1 gene promoter.

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