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KATARZYNA LISOWSKA, JAN WIŚNIEWSKI and ZDZISŁAW KRAWCZYK

ISOLATION OF A GENE CODING FOR A MAJOR HEAT SHOCK PROTEIN HSP71 IN RAT

Department of Tumour Biology. Institute of Oncology. Wybrzeże Armii Czerwonej 15; 44-100 Gliwice, Poland

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A rat gene closely related to a human heat- inducible/cell cycle-dependent hsx70 gene was cloned from a rat genomic library. Northern blot analysis showed that it encodes a 2.5 kb transcript, one of the two heat-inducible mRNAs (2.5 and 2.7 kb) detected in rat tissues. Comparison of the known expression pattern of rat major heat shock proteins and mRNAs suggests that the isolated rat gene most probably codes for HSP71 protein.

Hyperthermia and some other environmental stresses induce synthesis of several specific protein called heat shock proteins (HSPs) [1]. In all organisms the most abundant heat shock protein has molecular mass of about 70 000 (HSP70). In eukaryotes one or two HSP70 proteins of slightly different size or charge are encoded by a few closely related genes [1, 2] the activity of which can be separately regulated [3]. In particular, one of these heat-inducible genes called hsx70 is specifically expressed during the S phase of cell cycle [4]. Besides, under physiological conditions eukaryotic cells contain several HSP70-related proteins: constitutive HSC70 proteins [1], a glucose regulated GRP78 protein [5] and a spermatogenesis-specific P70 protein [6]. Genes encoding HSC73, GRP78 and P70 proteins have been cloned from rat genomic and cDNA libraries [5, 7, 8, 9]. Here we report on the isolation of the gene coding for a major heat shock protein in rat.

MATERIALS AND METHODS

Library screening and structure of the isolated hsp70-related clones were described earlier [10]. Restriction analyses and subcloning into pUC19 or pBluescript vectors were performed by standard methods [11]. DNA sequences was determined by dideoxy method using plasmid templates. [α-^{35}S]dATP and Klenow polymerase [12]. RNA was isolated from tissues of rats subjected to

a whole-body hyperthermia [13] by guanidine isothiocyanate-hot phenol method [11]. RNA was electrophoretically separated on 1.2% agarose--2.2 M formaldehyde gel [11] and electroblotted onto GeneScreen membrane [13]. Northern and Southern hybridizations were carried out as described earlier [10, 13] using nick-translated molecular probes (2×10^8cpm/μg) [11].

RESULTS AND DISCUSSION

Among 20 different hsp70-like sequences which we isolated from a rat genomic library, a R70-11 region (contained in phage λ68) hybridized under stringent conditions with a human hsx70 gene [10]. Its 4.3 kbp KpnI-EcoRI restriction fragment was subcloned into a pUC19 vector (Fig. 1A). Restriction mapping of the resulting plasmid p68/4.3 (not shown) revealed that its structure closely resembles that of a human hsx70 gene (Fig. 1A). Southern hybridization showed that probes derived from the specific fragments of a human hsx70 gene

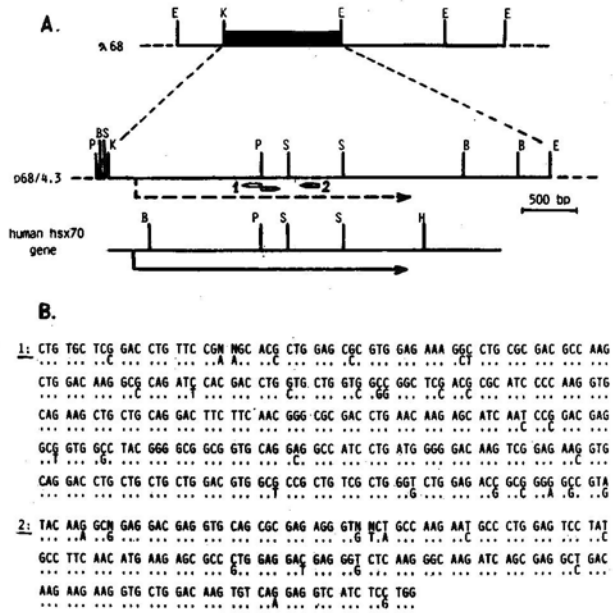

Fig. 1. Cloning and structure of a rat hsp71 gene. A) Comparison of a rat genomic fragment cloned in plasmid p68/4.3 with a human hsx70 gene. Filled box - hsp70-related sequence of λ68 DNA; broken and solid horizontal lines-vector and genomic sequences, respectively; long solid and broken arrows - 5′→3′ orientation of human and rat genes, respectively; short open arrows - sequenced regions of a rat hsp71 gene; B-BamHI, E-EcoRI, H-HindIII, K-KpnI, P-PstI and S-SmaI restriction sites. B) Nucleotide sequence of the two sequenced fragments of rat hsp71 gene (top line) as compared with corresponding regions of human hsx70 gene (bottom line). Only different bases are shown for the human gene.

recognize corresponding regions of the cloned rat gene (not shown). This suggested that the plasmid p68/4.3 contained an entire major heat shock gene and about 200 bp of its promoter region (Fig. 1A).

So far we sequenced about 500 nucleotides in two internal regions of the p68/4.3 insert which corresponds to about 20% of a typical hsp70 gene. Sequence comparison with a human hsx70 gene [14] showed only a few difference mainly in the third position of codons (Fig. 1B). Translation of sequenced DNA fragments into amino acids revealed that a rat protein has 93% of residues identical with the corresponding region of a human HSX70 protein [14], 89% with a mouse HSP68 protein [15], 78% with a rat HSC73 protein [7], 76% with a rat P70 protein [9], 54% with a rat GRP78 protein [5] and 47% with a bacterial DnaK protein [16].

Rat genome contains at least two hsp70 genes [10]. Under hyperthermia rat tissues synthesize two major heat shock proteins HSP70 and HSP71 [17] and Northern blot analysis revealed two hsp70 mRNAs of 2.7 and 2.5 kb [13]. To establish which transcript originates from a gene present in the plasmid p68/4.3 we performed Northern analyses using two kinds of molecular probes: i, an unspecific 5′ probe — the 1.6 kbp KpnI-SmaI fragment containing evolutionary conserved 5′ part of the gene: and ii, a specific 3′ probe — the 1.1 kbp SmaI-BamHI fragment containing evolutionary divergent 3′ end of the gene (homology between various hsp70 and related genes is >80% and <80% in these regions, respectively [1]. Figure 2 shows that only the 2.5 kb mRNA hybridized with the specific probe.

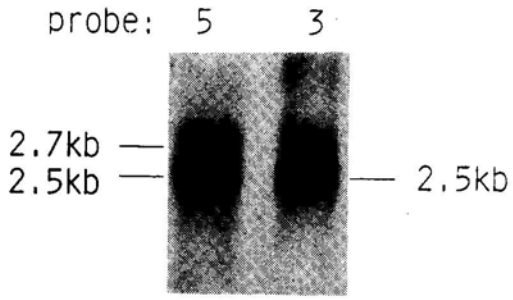

Fig. 2. Identification of RNA species encoded by a cloned rat hsp71 gene. Northern blots containing 15 μg of total RNA purified from liver of heat shocked rat were hybridized with unspecific 5′ or specific 3′ probes as described in the text.

This result together with available data on the expression pattern of rat major heat shock proteins and mRNAs [13, 17] suggests that the plasmid p68/4.3 contains a rat hsp71 gene. Its strong sequence similarity with a human hsx70 gene raises the possibility that the rat gene can also be expressed in the cell cycle-dependent manner: however so far we have no evidence to support

that supposition. Since hsx70 gene is regarded-as specific for primate genome [4] detection of similarily regulated gene in rat would change our view on evolution and function of the eukaryotic hsp70 multigene family.

REFERENCES

1. Craig, E. A. (1985) *CRC Crit. Rev. Biochem.*, **18**, 239-280.
2. Harrison, G. S., Drabkin, H. A., Kao, F.-T., Hartz, J., Hart, I. M., Chu, E. H. Y., Wu, B. J. & Morimoto, R. I. (1987) *Somatic Cell. Mol. Genet.*, **13**, 119-130.
3. Tanaka, K., Jay, G. & Isselbacher, K. J. (1988) *Biochim. Biophys. Acta*, **950**, 138-146.
4. Pelham, H. R. B. (1986) *Cell.*, **46**, 959-961.
5. Munro, S. & Pelham, H. R. B. (1986) *Cell.*, **46**, 291-300.
6. Allen, R. L., O'Brien, D. A. & Eddy, E. M. (1988) *Mol. Cell. Biol.*, **8**, 828-832.
7. O'Malley, K., Mauron, A., Barchas, J. D. & Kedes, L. (1985) *Mol. Cell. Biol.*, **5**, 3476-3483.
8. Sorger, P. K. & Pelham, H. R. B. (1987) *EMBO J.*, **6**, 993-998.
9. Wiśniewski, J., Kordula, T. & Krawczyk, Z. (1989) *Biochim. Biophys. Acta*, **1048**, 93-99.
10. Wiśniewski, J. & Krawczyk, Z. (1988) *Mol. Biol. Rep.*, **13**, 21-28.
11. Maniatis, S., Fritsch, E. F. & Sambrook, J. (1982) *Molecular cloning. A laboratory manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
12. Hattori, M. & Sakaki, Y. (1986) *Anal. Biochem.*, **152**, 232-238.
13. Krawczyk, Z., Wiśniewski, J. & Biesiada, E. (1988) *Acta Biochim. Polon.*, **35**, 377-385.
14. Hunt, C. & Morimoto, R. I. (1985) *Proc. Natl. Acad. Sci. U. S. A.*, **82**, 6455-66459.
15. Lowe, D. G. & Moran, L. A. (1986) *J. Biol. Chem.*, **261**, 2102-2112.
16. Bardwell, J. C. A. & Craig, E. A. (1984) *Proc. Natl. Acad. Sci. U. S. A.*, **81**, 848-852.
17. Fujio, N., Hatayama, T., Kinoshita, H. & Yukioka, M. (1987) *J. Biochem.*, **101**, 181-187.