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Research Article

Polymorphism in the regulatory sequence of the human *hsp70-1* gene does not affect heat shock factor binding or heat shock protein synthesis

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Abstract. A bi-allelic polymorphism found in the regulatory region of the human heat shock (HS) protein (HSP) *hsp70-1* gene, which comprises an A→C transversion, 3 bp upstream of the HS element (HSE), has been associated with extended HLA haplotypes. In view of the chaperoning and protective functions of Hsp70, we investigated whether this *hsp70-1* bi-allelic polymorphism could modulate the stress response, which may relate to enhanced resistance or susceptibility to certain diseases. We compared the basal and HS-induced HS factor (HSF)-binding activity of the two polymorphic HSEs, *hsp70-1* mRNA accumulation and HSP expression in two human Epstein–Barr virus (EBV)-transformed B cell lines typed for *hsp70-1* promoter alleles. Our results suggest that *hsp70-1* promoter polymorphism does not influence HSF-binding activity, *hsp70* mRNA accumulation or synthesis in human EBV-transformed B cell lines.

Key words. Heat shock element; promoter; allelles; binding-activity.

Heat shock (HS) proteins (HSPs) are essential for cellular homeostasis and adaptative functions. The first function to be attributed to these extaordinarily conserved proteins was thermotolerance, i.e. protection from further exposure to HS [1]. The protective role of HSPs has been extended to other stresses and generally correlates with their properties as molecular chaperones controlling protein folding, translocation or degradation [2, 3]. HSPs also play major roles in the processes of adaptation to a number of cellular stresses, in particular those associated with oxidative stress [4–6].

Polymorphism in *hsp* promoters and quantitative modifications in Hsp70 expression have been correlated with variation in thermotolerance in fish, insects and cloned mammalian cell lines, with differences in adaptation to environmental conditions in plants and animals and with susceptibility or resistance to disease in humans [7]. Differential HSP expression in human diseases is supported by several studies [8–10]. We have proposed that centenarians, along with their peculiar immune response [11], might benefit from specific genetically determined mechanisms for stress resistance, possibly determined by *hsp* gene polymorphism and differential HSP expression [6].

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Indeed, in humans, three members of the *hsp70* gene family map within the class III region of the highly polymorphic major histocompatibility complex (MHC): *hsp70-1*, *hsp70-2* and *hsp-HOM* [12–14]. In other species, *hsp* genes are also located in the MHC region [15–17]. A bi-allelic polymorphism in the cis regulatory sequence of the human *hsp70-1* gene lies within the HS element (HSE). HSEs consist of arrays of inverted NGAAN repeats. The polymorphism at nucleotide −110 of *hsp70-1* which involves a nucleotide A→C transversion was originally described as lying 3 bp upstream of the HSE, defined as CTGGAATATTCCCG [18]. Later work by Abravaya et al. [19] showed the HSE to be more extensive than this, including five NGAAN repeats and encompassing nucleotide −110 [14]. The study by Cascino et al. [20] showed an association of the *hsp70-1C* allele with the *HLA-DR3* allele. In *HLA*-homozygous cell lines, Milner et al. [21] revealed an association of the *hsp70-1C* allele with *HLA-B8*, *DR3* and *HLA-B18*, *DR3* haplotypes, and between *hsp70-1A* and most *B7*, *DR2* haplotypes. A second study by Cascino et al. [22] corroborated these results with respect to the *DR3* haplotype and revealed an association of *hsp70-1A* with most *DR4*-positive haplotypes (fig. 1).

On the one hand, differential HSP expression may modulate stress or disease susceptibility or resistance [4, 23–25], while on the other hand, nucleotide substitutions, deletions and insertions have been shown to modulate promoter strength and transcriptional activity [26–28]. This prompted us to investigate a possible relationship between *hsp70-1* gene promoter region polymorphism and differential HSF-binding activity. Two Epstein–Barr virus (EBV)-transformed human B cell lines typed for *hsp70-1* alleles (BM14 and MOU, homozygous for *hsp70-1C* and *hsp70-1A*, respectively) were compared for (i) the affinity of HSF for the two distinct HSEs, (ii) *hsp70-1* mRNA accumulation and (iii) HS-induced HSP expression.

Material and methods

Cells and HS treatment. Two EBV-transformed lymphoblastoid homozygous B cell lines from the 10th International Histocompatibility Workshop were used: MOU 9050, homozygous for allele *hsp70-1A*, and BM14 9033, homozygous for *hsp70-1C*. The human pre-monocytic line U937 in which HSP expression and regulation have been well characterized was used as a control [29, 30]. Cells were maintained in RPMI 1640 medium (GIBCO, Paisley, Scotland) supplemented with 10% v/v fetal calf serum, 0.03% glutamine, 25 mM HEPES at 37 °C in a humidified atmosphere (95% air/5% CO_2). For HS, cells were incubated in a waterbath at 44 °C for 30 min followed by 2.5 h recovery at 37 °C.

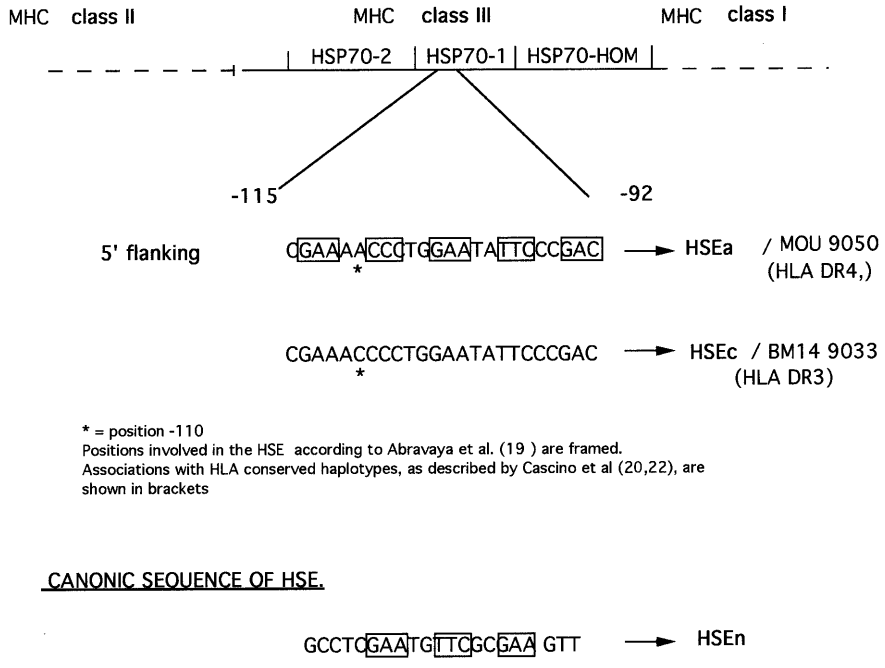

Figure 1. Polymorphic variants at position −110 in the promoter region of the *hsp70-1* gene and their associations with *HLA* conserved haplotypes. The upper part of the figure shows a schematic representation of the position of the *hsp70-1* gene within the human MHC. The lower part of the figure displays the canonic HSE sequence.

Preparation of nuclear extracts and gel mobility-shift assays. Preparation of nuclear-protein extracts was adapted from the method described by Dignam et al. [31]. Samples were analysed for HSE-binding activity by gel mobility-shift assays. Oligonucleotides were synthesized and purchased from Genset (Paris, France). Binding reactions were performed for 30 min at 25 °C by adding 5 μg of nuclear proteins to a mixture containing 10^6 cpm of γ[^{32}P]-ATP end-labelled, double-stranded canonic (HSEn) oligonucleotide, 5′-GCCTCGAATGTTCGCGAAGTT-3′ and the two following polymorphic double-stranded oligonucleotides, 5′-GGAGGCGAAACCCCTGGAATATTCCCGACCTGGC-3′ (HSEc) and 5′-GGAGGCGAAAACCCTGGAATATTCCCGACCTGGC-3′ (HSEa), in 15 μl of buffer containing 2 μg of poly(dI-dC) and 10 μg BSA. Homologous and heterologous competition experiments were performed with one- to three-fold molar excess of each non-radioactive HSE. Samples were electrophoresed on a non-denaturating 4% polyacrylamide gel, dried and autoradiographed.

Isolation of RNA and Northern blot analysis. Total RNA was prepared from *HLA*-homozygous EBV-transformed B lymphoblastoid cell lines, using a single-step procedure [32]. Samples of total RNA (10 μg) were fractionated in 0.8% agarose-formaldehyde denaturing gels, then transferred to a nylon membrane (Hybond N+, Amersham, UK) and hybridized at 65 °C under standard conditions with 10^8 cpm/ml of random-primed (Boehringer Mannheim, Mannheim, Germany) α[^{32}P]-dCTP-labelled probe. The probe was obtained by polymerase chain reaction from genomic DNA using sequence-specific oligonucleotide primers: 5′-GGGCATCACTTGAATTTT-3′ (nucleotide position 2412) and 5′-TCCAAGATTGCTGTTTTTGT-3′ (nucleotide position 2149) from the 3′ untranslated region of *hsp70-1*, a region which is heterologous in the three MHC-linked *hsp70* genes.

Analysis of protein synthesis. In these experiments, cells were maintained in RPMI 1640 medium without methionine. Labelling was performed with [^{35}S]-methionine (6 μCi/ml, specific activity > 1000 Ci/mmol, Amersham) for 90 min at 37 °C. After a recovery period, proteins were separated by SDS-PAGE on a 10% (w/v) acrylamide gel according to Laëmmli [33]. Protein concentration was determined by a micro-BCA assay (Pierce, Rockford, IL, USA). Approximately 100,000 cpm of labelled protein was loaded in each lane.

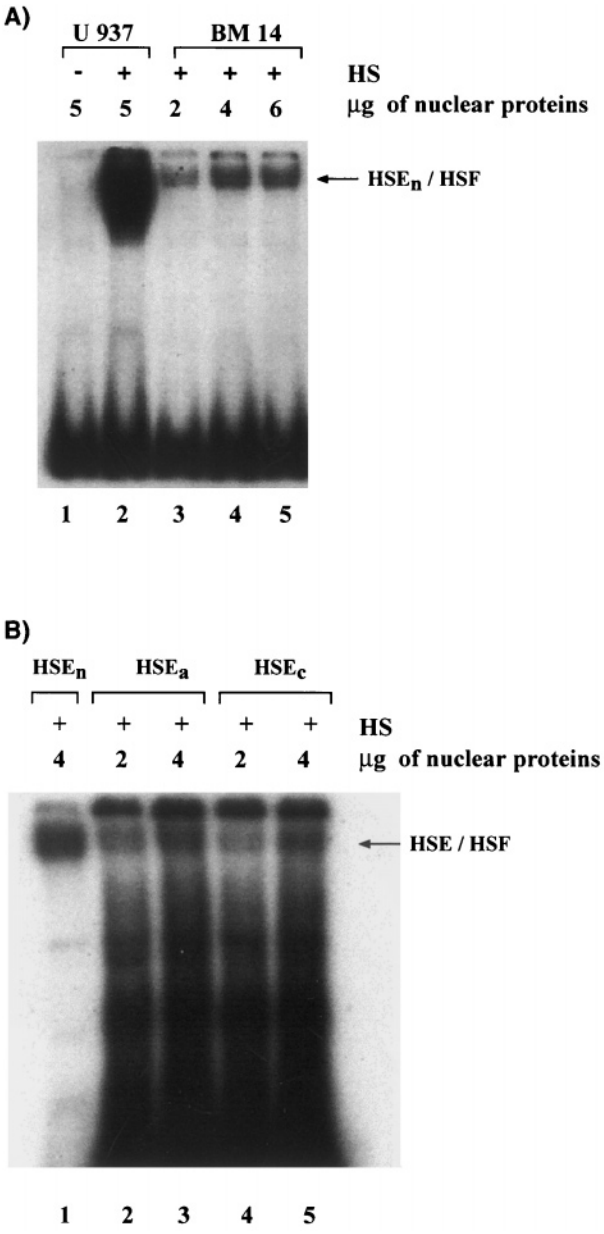

Figure 2. HSF DNA-binding activity. (*A*) U937 cells and the lymphoblastoid cell line BM14 were exposed to HS (44 °C, 30 min) or maintained at 37 °C and nuclear proteins submitted to gel mobility shift assay. HS induced binding of HSF to the canonic HSE (HSEn) in both cell lines. (*B*) BM14 cells were exposed to HS (44 °C, 30 min) and binding of HSF to the different HSEs (HSEn, HSEa, HSEc) was tested. Concentration dependence on the amount of nuclear extracts is apparent for all HSEs tested.

Results

Comparison of HSF DNA-binding activities of the polymorphic HSE from *hsp70-1*. A double-stranded oligonucleotide representing the canonic consensus HSF-binding site (HSEn) was first used to compare the translocation of HSF into the nuclei of heat-shocked U937 (control) and BM14 cells (fig. 2A). Control U937 nuclear extract (5 μg) did not contain HSF-HSEn binding activity (lane 1), while proteins (5 μg) obtained from U937 cells exposed to HS showed significant binding to

HSEn (lane 2), indicating that HSF translocation had occurred. Quantitative experiments performed with variable amounts of nuclear protein extracts from heat-shocked BM14 cells indicated that HSF-HSEn binding activity was detectable with just 2 μg of nuclear proteins, and increased to a maximum with 4 μg of nuclear extract (lanes 3–5). Furthermore, HSF translocation and the resulting HSEn-binding activity were much weaker in BM14 cells than in U937 when similar amounts of nuclear extract were used (compare lane 2 to lanes 4 and 5). According to the *HLA* typing of the U937 cell line (*DR2*, *DR6*), negative for *HLA-DR3*, and according to the studies of Cascino et al. [20, 22] and Milner et al. [21], it appears that the *HLA* haplotype of this cell line might be associated with *hsp70-1A*.

To establish whether HSE polymorphism has any effect on HSF-binding affinity, nuclear extracts from heat-shocked BM14 cells were incubated with the three HSE sequences: HSEn (canonic oligonucleotide, lane 1), HSEa (oligonucleotide allele A, lanes 2 and 3) and HSEc (oligonucleotide allele C, lanes 4 and 5) (fig. 2B). No significant differences in HSF-binding activity were observed between the three HSEs, when equivalent amounts of nuclear protein were used.

Comparative affinity of HSF for HSEa and HSEc. To evaluate more precisely the relative affinities of HSF for HSEa and HSEc, we performed homologous and heterologous competition experiments with excess cold oligonucleotides. In nuclear extracts from U937 cells (fig. 3A), HSF bound efficiently both HSEa (lanes 1 and

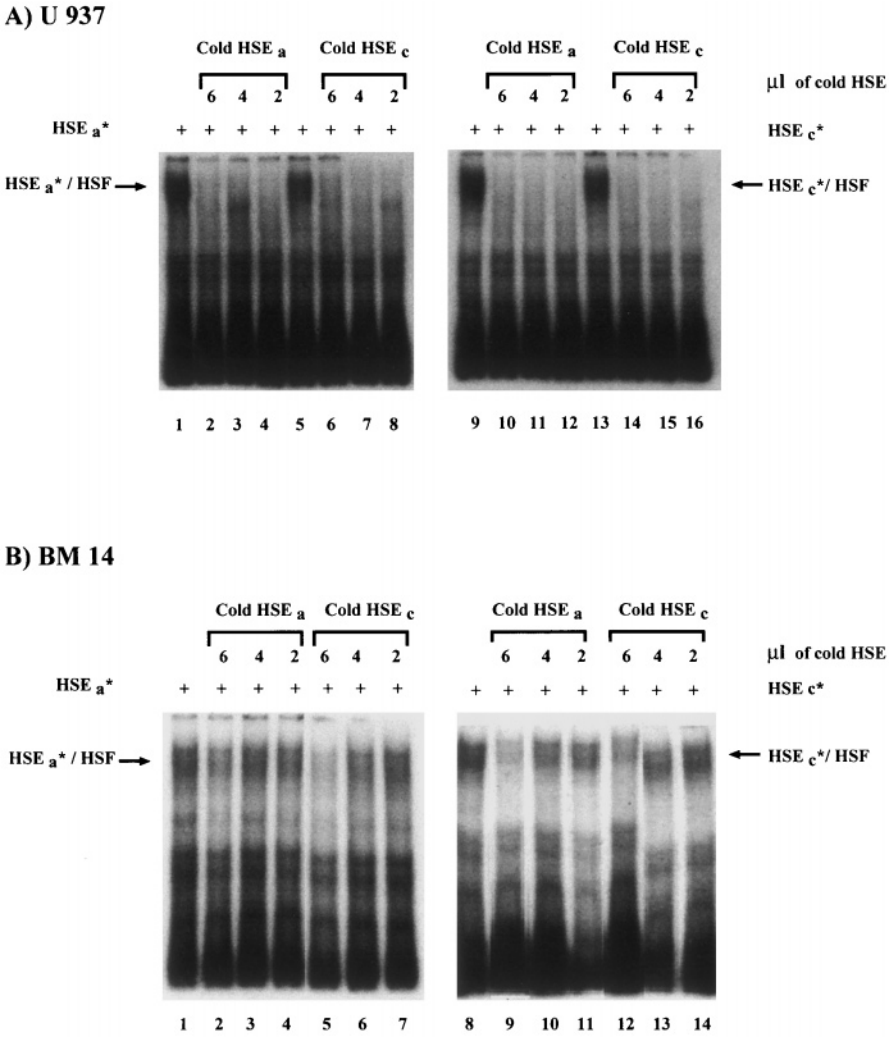

Figure 3. Comparative HSF binding affinity by homologous and heterologous competition. The specificities of HSF to labelled HSEs (HSE*) were tested using U937 (*A*) or BM14 (*B*) nuclear extracts in the presence of increasing molar excess of unlabelled HSEs.

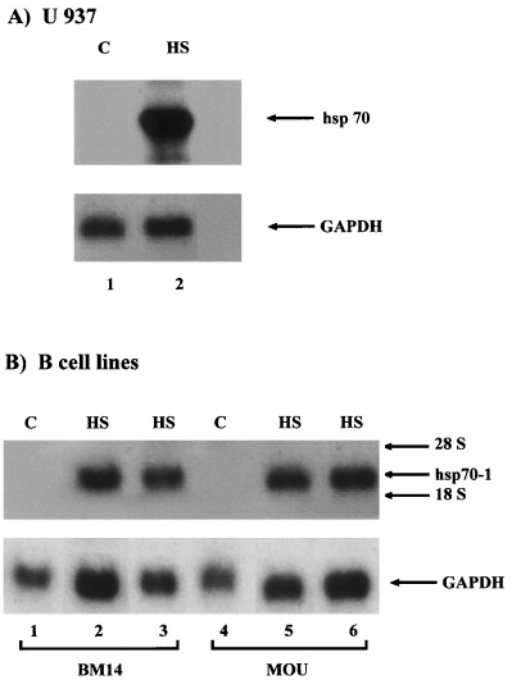

Figure 4. HS-induced *hsp70-1* mRNA expression. Control (C) and HS-induced (HS) levels for *hsp70-1* mRNA were tested in U937 cells (*A*), BM14 and MOU cells (*B*). The *hsp70-1* probe detected a transcript of $\approx$2.4 kb. *hsp70* mRNA levels were higher in U937 cells, but no difference was detected between the two homozygous cell lines tested. The position of migration of 28S and 18S RNA is shown, as is the 1.27-kb control GAPDH transcript.

5) and HSEc (lanes 9 and 13). Two microlitres (corresponding to 5 $\times$ the concentration of labelled probe) of unlabelled HSEa or HSEc were sufficient to inhibit formation of both HSF-HSEa* complexes (lanes 2–4 and 6–8) and HSF-HSEc* complexes (lanes 10–12 and 14–16). Using nuclear extracts from BM14 cells (fig. 3B), the amounts of HSF-HSEa complexes (lane 1) and HSF-HSEc complexes (lane 8) formed were much lower than in U937 cells. Furthermore, cold HSEa and HSEc effectively inhibited formation of both HSF-HSEa* and HSF-HSEc* complexes: the effect was discernible with 6 µl of cold HSE. Taken together, these results indicate that the different HSE sequences are interchangeable and exhibit a similar affinity for HSF.

Comparison of *hsp70-1* mRNA expression in BM14 and MOU cells. The lack of significant difference in HSF affinity for HSEa and HSEc was not sufficient to exclude a differential regulation in *hsp70-1* gene transcription. We thus also compared *hsp70-1* mRNA expression in the two homozygous cell lines. Northern blots were probed with a PCR fragment specific for the 3′ untranslated region of *hsp70-1* and 10 µg of total RNA was loaded on each lane (fig. 4). While HS increased the accumulation of *hsp70-1* mRNA in U937 as well as in the two B cell lines, the levels of *hsp70-1* mRNA were greater in the U937 cells (compare fig. 4A, B). No significant differences in *hsp70-1* mRNA accumulation appeared between the two B cell lines (fig. 4B). The intensity of the 1270-bp signal detected by a probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a standard for *hsp70-1* mRNA quantification, in order to take into account variations in the initial amounts of mRNA present in each sample. Hybridization signals were quantified using the image analysis computer program 2D Scan, (Pharmacia, Uppsala, Sweden) and *hsp70-1* signals were corrected to take into account variations in the corresponding GAPDH signals. Quantification indicated similar levels of *hsp70-1* mRNA in heat-shocked BM14 and MOU cells in duplicate experiments. We thus conclude that the polymorphism in the HSE sequence did not influence *hsp70-1* mRNA expression.

HS-induced HSP synthesis in BM14 and MOU cells. Finally, we investigated the influence of HSE polymorphism on HSP synthesis by metabolic labelling of cells (fig. 5). We found that, as in U937 cells, HS induced Hsp70 and HsP90 in both MOU and BM14 cell lines (compare lane 2 to lanes 4 and 6). For both MOU and BM14, $\approx$100,000 cpm, corresponding to 70 µg protein, was loaded in each lane; for U937 cells, $\approx$100,000 cpm, but six-fold less protein, was loaded, relating to the distinct metabolic activity of these cells. The levels of Hsp70 and Hsp90 were not different in MOU and BM14. Furthemore, HS did not affect overall protein synthesis, as actin synthesis was equivalent in control and heat-shocked BM14 and MOU cells.

Discussion

In this study, we found that the described bi-allelic polymorphism in the 5′ regulatory region of the *hsp70-1* gene did not generate differential gene expression. Using 5′-labelled synthetic oligonucleotides featuring the A to C mutation within the HSE, we found no differences in HSF affinity for HSEa or HSEc. We also observed no significant differences in *hsp70-1* mRNA accumulation and HSP expression between *hsp70-1C* and *hsp70-1A* homozygous cell lines. Our results thus suggest that the *hsp70-1A*/*hsp70-1C* bi-allelic promoter polymorphism does not affect HSP expression. The strong signals observed for U937 cells in gel shift assays (figs 2, 3) as well as in Northern blots (fig. 4) is probably cell specific and may relate to the high metabolic activity of these cells (fig. 5). Indeed, U937 is a premonocytic cell line, while MOU and BM14 are EBV-transformed lymphocytes. We have previously established that the

expression of HSPs is higher in monocytes than in EBV-transformed lymphocytes or any other cell type tested [34–36].

Other authors have reported a polymorphism in the coding region of the *hsp70-2* gene and its association with diseases such as insulin-dependent diabetes mellitus has been investigated [37]. This polymorphism consists of an A→G transition at position 1267 which generates a *Pst*I restriction site. Furthermore, a significant linkage disequilibrium ($p \leq 7 \times 10^{-7}$) between the *hsp70-1C* allele and the *hsp70-2* allele containing a *Pst*I restriction site (*Pst*I$^+$) has been previously observed [21, 38]. These two *hsp70* genes also share a similar HSE box, with *hsp70-2* having a C at nucleotide −110 [14], which suggests that they will have similar affinities for HSF. A functional implication of this polymorphism was analysed by Pociot et al, [37], studying *hsp70-2* mRNA expression in heat-shocked peripheral blood mononuclear cells from individuals with different *hsp70-2* genotypes. Their data showed that *Pst*I$^+$-homozygous individuals have slightly lower *hsp70-2* mRNA expression than heterozygous and *Pst*I$^-$-homozygous individuals. Thus, inter-individual and interracial differences in Hsp70 expression could relate to regulatory mechanisms distinct from transcriptional regulation, as also suggested by Lyashko et al. [39]. These authors compared the HS response in ethnically and ecologically different human populations. They showed that heat-shocked skin fibroblasts isolated from Turkish men exhibited intensive synthesis of HSP while only trace synthesis of HSP was observed in the second group (Russians), although the mRNA levels were similar in the two groups. The difference in HSP synthesis observed by Lyashko et al. [39] might either relate to post-translational modifications, or involve a distinct as yet undefined polymorphism.

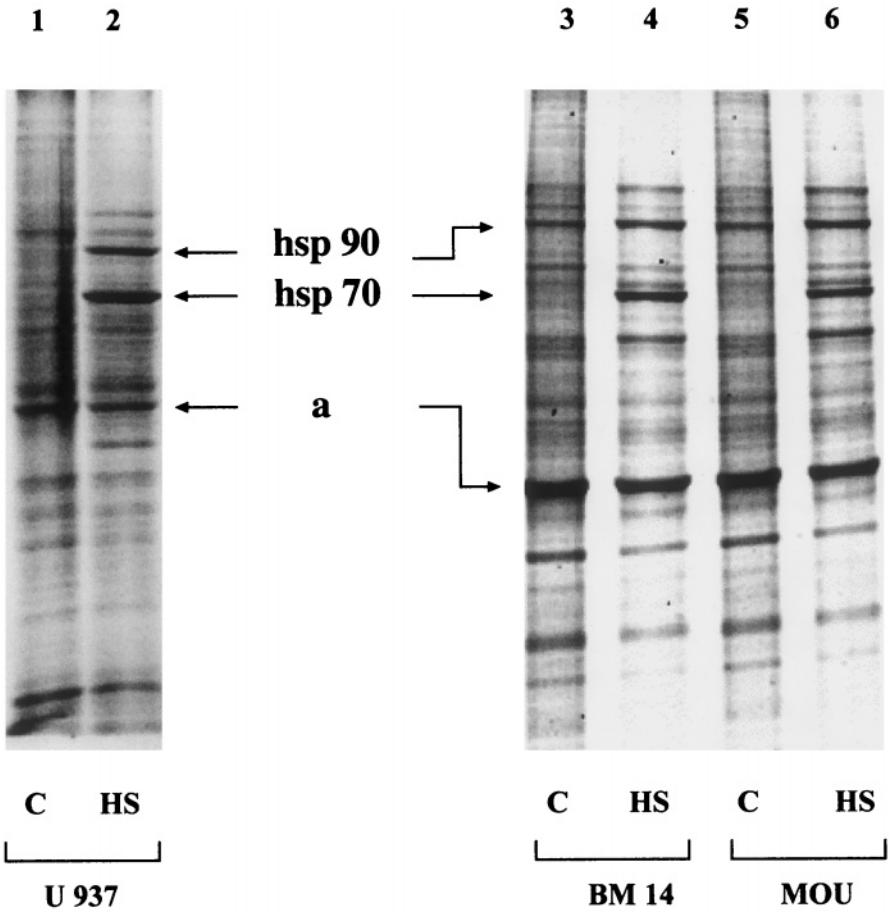

Figure 5. HS-induced HSP expression. Cells maintained at 37 °C (C) or exposed to HS (44 °C, 30 min) were labelled as described and proteins analysed by one-dimensional SDS-PAGE. Similar amounts of protein (≈ 100,000 cpm) were loaded on each lane, corresponding to 70 μg for BM14 and MOU cells and 12 μg for U937 cells. No differences were observed in the rate of HSP and overall protein synthesis in BM14 and MOU. The positions of Hsp70, Hsp90 and actin (a) are indicated by arrows.

Finally, our study suggests that differences in *hsp* gene regulation and HSP expression more likely relate to distinct cell types and specific metabolic activities than to promoter polymorphism, since the natural polymorphism of the promoter region of the *hsp70-1* gene was found to have no effect on Hsp70 levels, at least in the cell types studied. However, we cannot exclude that unknown polymorphisms in other *hsp* genes, including *hsp70* genes, might provide individual variability in protection against environmental changes, ageing or disease.

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1 Barbe M., Tytell M., Gower D. J. and Welch W. J. (1988) Hyperthermia protects against light damage in the rat retina. Science **241:** 1817–1820
2 Gething M. J. and Sambrook J. (1992) Protein folding in the cell. Nature **355:** 33–45
3 Craig E. A., Weissman J. S. and Horwich A. L. (1994) Heat shock proteins and molecular chaperones: mediators of protein conformation and turnover in the cell. Cell **78:** 365–372
4 Plumier J. C. L., Ross B. M., Currie L. W., Angelidis C. E., Kazlaris A. H., Kollias G. et al. (1995) Transgenic mice expressing the human heat shock protein 70 have improved post ischemic myocardial recovery. J. Clin. Invest. **95:** 1854–1860
5 Jacquier-Sarlin M. R., Fuller K., Dinh-Xuan A. T., Richard M. J. and Polla B. S (1994) Protective effects of hsp70 in inflammation. Experientia **50:** 1031–1038
6 Favatier F. and Polla B. S. (1996) Proteines de stress et vieillissement. Ann. Gérontol. **10:** 217–224
7 Favatier F., Bornman L., Hightower L. E., Günther E. and Polla B. S. (1997) Variation in hsp gene expression and Hsp polymorphism: do they contribute to differential disease susceptibility and stress tolerance? Cell Stress Chaperones **2:** 141–155
8 Deguchi Y. and Kishimoto S. (1990) Enhanced expression of the heat shock protein gene in peripheral blood mononuclear cells of patients with active systemic lupus erythematosus. Ann. Rheum. Dis. **49:** 893–895
9 Dhillon V. B., McCallum S., Norton P., Twomey B. M., Erkeller-Yuksel F., Lydyard P. et al. (1993) Differential heat shock protein overexpression and its clinical relevance in systemic lupus erythematosus. Ann. Rheum. Dis. **52:** 436–442
10 Shimizu S., Nomura K., Ujihara M., Sakamoto K., Shibata H., Susuki T. et al. (1996) An allele-specific abnormal transcript of the heat shock protein 70 gene in patients with major depression. Biochem. Biophys. Res. Commun. **219:** 745–752
11 Franceschi C., Monti D., Sansoni P. and Cossarizza A. (1995) The immunology of exceptional individuals: the lesson of centenarians. Immunol. Today **16:** 12–16
12 Sargent C. A., Dunham I., Trousdale J. and Campbell R. D. (1989) Human major histocompatibility complex contains genes for the major heat shock protein 70. Proc. Natl. Acad. Sci. USA **86:** 1968–1972
13 Sargent C. A., Dunham I. and Campbell R. D. (1989) Identification of multiple HTF-island associated genes in the human major histocompatibility complex class III region. EMBO J. **8:** 2305–2312
14 Milner C. M. and Campbell R. D. (1990) Structure and expression of the three MHC-linked Hsp70 genes. Immunogenetics **32:** 242–251
15 Wurst W., Benesch C., Drabent B., Rothermel E., Benecke E. and Günther E. (1989) Localization of heat shock protein 70 genes inside the rat major histocompatibility complex close to class III genes. Immunogenetics **30:** 46–49
16 Romano J. W., Seldin M. F. and Apella E. (1989) Linkage of the mouse hsp84 heat shock protein structural gene to the H-2 complex. Immunogenetics **29:** 42–44
17 Cameron P. U., Tabarias H. A., Pulendran B., Robinson W. and Dawkins R. L. (1990) Conservation of the central MHC genome: PFGE mapping and RFLP analysis of complement, HSP70, and TNF genes in the goat. Immunogenetics **31:** 253–264
18 Hunt C. and Morimoto R. I. (1985) Conserved features of eukaryotic hsp70 genes revealed by comparison with the nucleotide sequence of human hsp70. Proc. Natl. Acad. Sci. USA **82:** 6455–6459
19 Abravaya K., Philips B. and Morimoto R. I. (1991) Heat shock induced interactions of heat shock transcription factors and the human hsp70 promoter examined by in vivo footprinting. Mol. Cell Biol. **11:** 586–592
20 Cascino I., Sorrentino R. and Tosi R. (1993) Strong genetic association between HLA DR3 and a polymorphic variation in the regulatory region of the hsp70-1 gene. Immunogenetics **37:** 177–182
21 Milner C. M. and Campbell R. D. (1992) Polymorphic analysis of three MHC-linked Hsp70 genes. Immunogenetics **36:** 357–362
22 Cascino I., D'Alfonso S., Capello N., Giordano M., Pugliese A., Awdeh Z. et al. (1993) Gametic association of hsp70-1 promoter region alleles and their inclusion in extended HLA haplotypes. Tissue Antigens **42:** 62–66
23 Corzo D., Yunis J. J., Salazar M., Lieberman J. A., Howard A., Awdeh Z. et al. (1995) The major histocompatibility complex region marked by hsp70-1 and hsp70-2 variants is associated with clozapine-induced agranulocytosis in two different ethnic groups. Blood **86:** 3835–3840
24 Angelidis C. E., Lazaridis I. and Pagoulatos G. N. (1991) Constitutive expression of heat shock protein 70 in mammalian cells confers thermoresistance. Eur. J. Biochem. **199:** 35–39
25 Seo J. S., Park Y. M., Kim J. I., Shim E. H., Kim C. W., Jang J. J. et al. (1996) T cell lymphoma in transgenic mice expressing the human Hsp70 gene. Biochem. Biophys. Res. Commun. **218:** 582–587
26 Anderson L. C., Beaty J. S., Nettles J. W., Seyfried C.E., Nepom G. T. and Nepom B. S. (1991) Allelic polymorphism in transcriptional regulatory regions of HLA-DQB genes. J. Exp. Med. **173:** 181–192
27 Beaty J. S., West K. A. and Nepom G. T. (1995) Functional effects of a natural polymorphism in the transcriptional regulatory sequence of HLA-DQB1. Mol. Cell. Biol. **15:** 4771–4782
28 Song Z., Casolaro V., Chen R., Georas S. N., Monos D. and Ono S. J. (1996) Polymorphic nucleotides within the human IL4 promoter that mediate overexpression of the gene. J. Immunol. **156:** 424–429
29 Polla B. S., Kantengwa S., François D., Salvioli S., Francheschi C., Marsac C. et al. (1996) Mitochondria are selective targets for the protective effects of heat shock against oxidative injury. Proc. Natl. Acad. Sci. USA **93:** 6458–6463
30 Vayssier M., Banzet N., François D., Bellmann K. and Polla B. S. (1998) Tobacco-smoke induces both apoptosis and necrosis in mammalian cells: differential effects of HSP70. Am. J. Physiol. **275:** L771–L779
31 Dignam J. P., Lebovitz R. M. and Roeder R. G. (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11:** 1475–1489
32 Chomcynski P. and Sacchi N. (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:** 156–159

33 Laëmmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
34 Kantengwa S., Donati Y. R. A., Clerget M., Maridonneau-Parini I., Sinclair F., Mariéthoz E. et al. (1991) Heat shock proteins: an autoprotective mechanism for inflammatory cells? Sem. Immunol. **3:** 49–56
35 Bachelet M., Adrie C. and Polla B. S. (1998) Macrophages and heat shock proteins. Res. Immunol. **149:** 727–732
36 Bachelet M., Multhoff G., Vignola M., Himeno K. and Polla B. S. (1999) Heat shock proteins in inflammation and immunity: handbook of experimental pharmacology. Vaccines **136:** 281–303
37 Pociot F., Ronningen K. S. and Nerup J. (1993) Polymorphic analysis of the human MHC-linked heat shock protein 70 (HSP70-2) and HSP70-Hom genes in insulin-dependent diabetes mellitus (IDDM). Scand. J. Immunol. **338:** 491–495
38 Aron Y., Busson M., Polla B. S., Dusser D., Lockhart A., Swierczewski E. et al. (1999) Analysis of hsp70-1 gene polymorphism in allergic asthma. Allergy **54:** 165–170
39 Lyashko V. N., Vikulova V. K., Chernikov V. G., Ivanov V. I., Ulmasov K. A., Zatsepina O. G. et al. (1994) Comparison of the heat shock response in ethnically and ecologically different human populations. Proc. Natl. Acad. Sci. USA **91:** 12492–12495