Mini-review

Cell Stress & Chaperones (1996) 1 (1), 23–28

A hitchhiker's guide to the human Hsp70 family

Michael Tavaria[1], Tim Gabriele[2], Ismail Kola[1], Robin L. Anderson[2]

[1]Molecular Genetics and Development Group, Institute of Reproduction and Development, Monash University, Clayton 3168, Australia.
[2]Peter MacCallum Cancer Institute, A' Beckett Street, East Melbourne, Victoria 3000, Australia.

Abstract The human Hsp70 family encompasses at least 11 genes which encode a group of highly related proteins. These proteins include both cognate and highly inducible members, at least some of which act as molecular chaperones. The location of cognate Hsp70s within all the major subcellular compartments is an indication of the importance of these proteins. The expression of several inducible Hsp70 genes is also an indication of the importance of these proteins in the stress response. The existence of multiple genes and protein isoforms has created confusion in the identification and naming of particular family members. We have compiled, from the literature, a list of genes and genetic loci and produced a two-dimensional protein map of the known human Hsp70 family members. This will enable researchers in the field to quickly and reliably identify human Hsp70s. We have also devised a more rational nomenclature for these genes and gene products which, subject to general acceptance, could be extended to Hsp70 families from other species.

INTRODUCTION

Organisms as diverse as bacteria and man express a family of highly related proteins in response to environmental stress. These proteins were originally identified in *Drosophila melanogaster* cells exposed to elevated temperatures and were, therefore, called heat shock proteins (Hsp). More recently, the term stress proteins has been invoked in recognition of the fact that many of these proteins are induced by a variety of other stimuli. A subset of the stress proteins, those which are expressed under normal physiological conditions, constitute the heat shock 'cognate' proteins. These proteins, which include members of the Hsp10 (Cpn10), Hsp60 (Cpn60) and Hsp70 families, bind to polypeptides to facilitate correct folding, transport and localization of the mature proteins (for review see Gething and Sambrook 1992). Stress proteins also associate with denatured or partially unfolded proteins, protecting them from further denaturation and assisting in their refolding. In fact, the ability to bind to other proteins in an energy dependent manner appears to be a common feature of stress proteins and has led to their inclusion in the molecular chaperone superfamily.

One of the most prominent and best characterized of stress proteins is the Hsp70 family. This family contains a number of highly-related protein isoforms ranging in size from 66 kDa to 78 kDa. These proteins have been shown to function early in the process of protein folding to stabilize the nascent polypeptide chain, preventing incorrect folding until sufficient protein has been synthesized to enable the proper conformation to be attained. Cognate members of the family are found within all the major intracellular compartments while the inducible isoforms appear to be predominantly cytoplasmic or nuclear in distribution. Although the precise function of each of the Hsp70 isoforms is still unclear, their co-localization at sites of protein synthesis and transport is an indication of their importance in these processes. The major inducible Hsp70 has also been implicated in the processes of protection and repair of stress-induced protein damage.

Naming of the Hsp70 genes has tended to reflect the

Editor's Note: The proposed system of nomenclature is a starting point for further discussion. The authors would like to receive feedback on this proposal. Comments and suggestions may be sent by E-mail to Robin L. Anderson.

Received 7 December 1995; Revised 10 January 1996; Accepted 16 January 1995

Correspondence to: Robin L. Anderson, Tel: +61 3 9656 1284; Fax: 61 3 9656 1411; E-mail: anderson@petermac.unimelb.edu.au

order of their discovery and the naming of the gene products has generally depended on characteristics such as estimated molecular weight, expression pattern or intracellular localization. These naming strategies have certain intrinsic problems:

1. Naming of proteins based on molecular weight estimated by electrophoretic mobility is dependent on a subjective comparison to molecular weight standards
2. Several members of the Hsp70 family are both constitutively expressed and inducible
3. Several Hsp70s are localized within more than one intracellular compartment.

Since none of these criteria have proven particularly reliable or consistent, there has been considerable confusion in the field over the identity of certain genes and/or proteins. We are interested in the organization of the human Hsp70 family and have, therefore, undertaken a review of the published literature and an analysis of the genome databases. The result is a complete list of human Hsp70 genes and the identification of the products of some of these genes using two-dimensional SDS polyacrylamide electrophoresis (2D-PAGE) and Western blot analysis. We also describe a nomenclature for the Hsp70 proteins which we have found particularly useful in distinguishing the various human Hsp70 isoforms. In this system, which does not rely on molecular weights or expression patterns, the protein is identified by a number corresponding to the gene locus number. We believe this may prove useful to others interested in stress proteins and would welcome any discussion on rationalization of the current nomenclature.

HUMAN *hsp70* GENES

The various Hsp70 isoforms are encoded by a multigene family consisting of at least 11 distinct genes in humans (Table 1). These genes are located at dispersed loci, although three Hsp70 genes are contained within the major histocompatibility class III (MHC class III) region on chromosome 6 (Goate et al 1987; Harrison et al 1987; Sargent et al 1989) and a pair of genes is located close together on the long arm of chromosome 1 (Voellmy et al 1985; Leung et al 1990). Other Hsp70 genes have been localized to chromosomes 5 (Fathallah et al 1993), 9 (Hendershot et al 1994), 11 (Tavaria et al 1995) and 14 (Bonnycastle et al 1994; Roux et al 1994). In addition to these genes, it has been proposed that an inducible Hsp70 protein is encoded by a gene on chromosome 21 (Harrison et al 1987) although analysis of chromosome 21 containing hybrids, Southern blot analysis and cloning attempts have not identified the gene involved (Goate et al 1987; Gabriele et al 1996). Human Hsp70 genes have been given the locus symbol HSPAx, where A defines members of the Hsp70 family and x designates the individual locus.

The first human Hsp70 gene to be cloned and characterized encoded the highly inducible cytoplasmic and nuclear localized Hsp70 (Wu et al 1985). This Hsp70 gene has been called a variety of names including *hsp70, hsp72, hsp70–1, hsp70i,* and *hsx70* and it appears in some gene databases as HSP70D. As with other inducible Hsp70s, this gene contains no introns. The chromosomal localization was determined in two separate studies using Southern blot analysis of somatic cell hybrids (Goate et al 1987; Harrison et al 1987) and later refined by cosmid mapping (Sargent et al 1989). This mapping localized the gene to chromosome 6p21.3, within the MHC class III region and identified two other nearby Hsp70 genes. Until recently, these three genes were all designated HSPA1. Detailed cloning and sequence analysis revealed that the second Hsp70 gene was almost identical to the first and encoded an identical protein. This gene, located 11 kb centromeric to *hsp70–1,* was then named *hsp70–2.* The third gene, which is located approximately 4 kb telomeric to *hsp70–1,* was named *hsp70-Hom* since it was not known whether it encoded a functional Hsp70 protein. *hsp70-Hom* has since been shown to be expressed at low levels both before and after heat shock (Milner and Campbell 1990). These genes now have the locus symbols HSPA1A, HSPA1B and HSPA1L and we propose the gene symbols *hsp70–1a, hsp70–1b* and *hsp70–1l.*

The analysis of somatic cell hybrids by 2D-PAGE also revealed the presence of 70 kDa heat shock proteins in hybrid cell lines containing human chromosomes 14 and 21 (Harrison et al 1987). Southern blot analysis confirmed the presence of an Hsp70-related sequence on chromosome 14 (also called *hsp70–3*) but not on chromosome 21 (Goate et al 1987). Two groups have recently reported the cloning of a chromosome 14 Hsp70 gene (Bonnycastle et al 1994; Roux et al 1994). Surprisingly, the two reports localize this gene, locus symbol HSPA2, to 14q22 (Roux et al 1994) and 14q24.1 (Bonnycastle et al 1994), a significantly different localization in cytogenetic terms. Close inspection of the published sequences reveals that the two are not, in fact, identical. The partial sequence published by Roux et al (1994) contains several differences in the 5′ untranslated region, including one which destroys the putative CAAT box identified by Bonnycastle et al (1994), and a three base-pair deletion in the open reading frame which results in a single amino acid deletion. It is unclear at this stage whether these differences are due to polymorphisms, sequencing, or cloning artifacts. It is also possible that these sequences represent different genes, particularly since the reported tissue expression patterns of the two differ considerably. This possibility is given added strength by the wide distribution of grains seen in the 14q22–24 region after in situ

Table 1 The human Hsp70 family

Locus symbol	Site	Ref	Alternative names	Intracellular localization	Cognate	Proposed names
HSPA1A	6p21.3	1,2	Hsp70,Hsx70,HSP72, Hsp70i, Hsp70-1	Nu/Cyto	Yes	Hsp70-1 *hsp70-1a*
HSPA1B	6p21.3	2	Hsp70-2	Nu/Cyto	Yes	Hsp70-1 *hsp70-1b*
HSPA1L	6p21.3	2	Hsp70-Hom, Hsp70t	?	Yes	Hsp70-1L *hsp70-1l*
HSPA2A	14q22	3	Hsp70-3	?	Yes	Hsp70-2A *hsp70-2a*
HSPA2B	14q24.1	4	Hsp70-3	?	Yes	Hsp70-2B *hsp70-2b*
HSPA3	21(?)	5	-	?	?	-
HSPA4	5q31.1	6	Hsp70RY	?	Yes	Hsp70-4 *hsp70-4*
HSPA5	9q34	7,8	BiP, GRP78	ER	Yes	Hsp70-5 *hsp70-5*
HSPA6	1q	9,10	Hsp70-6, Hsp70B'	?	No	Hsp70-6 *hsp70-6*
HSPA7	1q	10,11	Hsp70-7, Hsp70B	?	No	Hsp70-7 *hsp70-7*
HSPA8	11q24	12,13	HSC70, HSP73	Nu/Cyto	Yes	Hsp70-8 *hsp70-8*
HSPA9	?	14,15	GRP75, PBP74, mtHSP75	Mito	Yes	Hsp70-9 *hsp70-9*

Hsp70 locus symbols were obtained from Genome Data Base. Alternative names and intracellular localizations were obtained from published literature. Nu/Cyto = nucleus/cytoplasm, ER = endoplasmic reticulum, Mito = mitochondria. Yes = expression detectable in unstressed cells, No = no expression detected in unstressed cells. Upper line of last column represents protein nomenclature, lower line represents gene nomenclature.

Table 1 References:
1. Wu et al 1985
2. Milner and Campbell 1989
3. Roux et al 1994
4. Bonnycastle et al 1994
5. Harrison et al 1987
6. Fathallah et al 1993
7. Ting and Lee 1988
8. Hendershot et al 1994
9. Voellmy et al 1988
10. Leung et al 1992
11. Leung et al 1990
12. Dwornizcak et al 1987
13. Tavaria et al 1995
14. Domanico et al 1993
15. Bhattacharyya et al 1995

hybridization (Harrison et al 1987). We therefore propose to distinguish between the Bonnycastle et al (1994) and Roux et al (1994) sequences in the same way as the chromosome 6 Hsp70 genes, by using *hsp70-2a* and *hsp70-2b*, respectively, until such time as their identity (or lack thereof) is established.

The location of an Hsp70 gene on human chromosome 21 has been the subject of much speculation. Harrison et al (1987) used 2D-PAGE to identify an inducible Hsp70 in somatic cell hybrids containing chromosome 21 but could not show the presence of Hsp70-related gene sequences on this chromosome either by Southern blot analysis or by in situ hybridization. Similarly, by Southern blot analysis, Goate et al (1987) could not detect an Hsp70-related gene in a different set of hybrids containing human chromosome 21. Nevertheless, the Committee on the Constitution of Chromosome 21 gave a provisional assignment for an Hsp70 gene and this assignment was given the locus symbol HSPA3. Recently, we have analyzed, by 2D-PAGE, yet another group of somatic cell hybrids and have been unable to demonstrate the existence of a human chromosome 21 specific Hsp70 protein (Gabriele et al 1996).

An Hsp70 gene was recently cloned from a B-cell line and localized to 5q31.1–31.2 (Fathallah et al 1993). This gene, which has been called Hsp70RY, has been given the locus HSPA4 and under our nomenclature will be called *hsp70-4*. Interestingly, this localization is found when a member of the mouse Hsp70 family, $p66^{mot-1}$ (mortalin), is used for in situ hybridization to human chromosomes (Kaul et al 1995). A human mortalin has not yet been reported, although the amino acid sequence of mouse mortalin is most similar to human mitochondrial Hsp70, including the presence of a leader sequence.

The gene encoding the human mitochondrial Hsp70 (also called mtHSP75, GRP75 and PBP74) was cloned in two separate studies (Domanico et al 1993; Bhattacharyya et al 1995) but has not yet been localized. We have tentatively assigned the locus symbol HSPA9 (gene *hsp70–9*) for this gene.

The Hsp70 homologue located in the endoplasmic reticulum (ER) was first identified in association with immunoglobulin precursors and as a glucose regulated protein (GRP), hence the names BiP (immunoglobulin binding protein) or GRP78 (Haas and Wabl 1983; Lee 1987). The gene encoding BiP/GRP78 has been cloned (Ting and Lee 1988) and localized to human chromosome 9q34 (Hendershot et al 1994). The currently used locus symbol for this gene, GRP78, does not reflect the status of BiP/GRP78 as part of the Hsp70 family. In our nomenclature, this gene is represented by the locus symbol HSPA5 and the gene symbol *hsp70–5*.

Two highly related Hsp70 genes are located on the long arm of human chromosome 1 (Leung et al 1992). These genes, previously described as *hsp70B* or *hsp70–7* (Voellmy et al 1985) and *hsp70B′* or *hsp70–6* (Leung et al 1990), are both heat-inducible although the patterns of induction do differ slightly. The genes have been given the locus symbols HSPA7 (gene *hsp70–7*) and HSPA6 (gene *hsp70–6*), respectively.

The gene encoding the human major cytoplasmic cognate Hsp70 (also called HSC70 or HSP73) has recently been localized to 11q24 and given the locus symbol HSPA8 (Tavaria et al 1995). As for *hsp70–5*, the gene for the cognate ER protein, *hsp70–8* contains introns. The heat shock cognate protein is involved in stabilizing nascent polypeptides and is also the clathrin uncoating ATPase. It is abundantly expressed under normal conditions and is not significantly heat inducible.

HUMAN Hsp70 PROTEINS

Recent progress in cloning and characterization of genes encoding the Hsp70 family has not been accompanied by equivalent progress in the characterization of the heat shock proteins themselves. This may, in part, be due to difficulty in distinguishing between these highly related proteins. Using 2D-PAGE and Western blot analysis with characterized anti-Hsp70 antibodies, we can detect at least five members of the Hsp70 family. The five proteins are indicated on the autoradiographs and Western blots of control and heated human HT1080 cells (Fig. 1). The assignment of these Hsp70 gene products agrees with those published previously by Welch and colleagues (Mizzen et al 1989; Brown et al 1993). Three of these proteins, Hsp70–5, Hsp70–8 and Hsp70–9, are expressed constitutively and are not further inducible. Hsp70–1, the major inducible Hsp70, is expressed at relatively low levels in control cells and is markedly increased in heat shocked cells. The fifth protein, Hsp70–6, is more basic than the other Hsp70 family members and is only expressed in HT1080 after heat shock. Hsp70–5 exists in two forms, the more acidic variant of which is modified by mono-ADP-ribosylation and is the non-functional form of the protein (Leno and Ledford 1989). To the best of our knowledge, Hsp70–1L, –2A, –2B, –4 and –7 have not been identified on two-dimensional gels.

SUMMARY

Recent years have seen a rapid expansion in the size of the human Hsp70 family. We now believe that there are at least 11 genes which encode functional proteins, five of which we can identify by 2D-PAGE. In the process of identifying these genes and proteins, we have devised a new nomenclature that we have put forward as the starting point for further discussion. This nomenclature does not rely on estimated molecular masses, which vary between research groups, nor does it involve subcellular localization, which is complex for some of the proteins. For example, Hsp70–8 is localized predominantly in the cytosol before stress, but has a component in the nucleus which increases in amount immediately after stress. In addition, Hsp70–8 has been reported in association with the outer mitochondrial membrane (Lithgow et al 1993). Hsp70–1, while also predominantly localized in the cytosol and nucleus, is expressed on the surface of some cells (Multhoff et al 1995). A nomenclature based on expression characteristics would be equally confusing, since all but two of the proteins listed in Table 1 show some level of constitutive expression and the degree of inducible expression is variable depending on the gene and the nature of the inducing agent. Thus, we suggest a more logical nomenclature is one which does not rely on these characteristics and in which the cytogenetic loci, gene and protein symbols match. The nomenclature could easily be extended to other mammalian species for which Hsp70 homologues have been identified. Our goal in this report is to assist researchers in the field to rapidly and reliably identify particular Hsp70 family members and to encourage discussion on rationalizing the Hsp70 nomenclature.

ACKNOWLEDGEMENTS

The authors wish to thank Marilyn Smith for technical assistance, Dr W. Welch for the kind gift of the N6 antibody and Mrs Marian Huebner and Dr Phyllis McAlpine (Nomenclature Editor, Genome Data Base) for advice on Hsp70 nomenclature. This work was supported by an NH&MRC grant to IK and RLA.

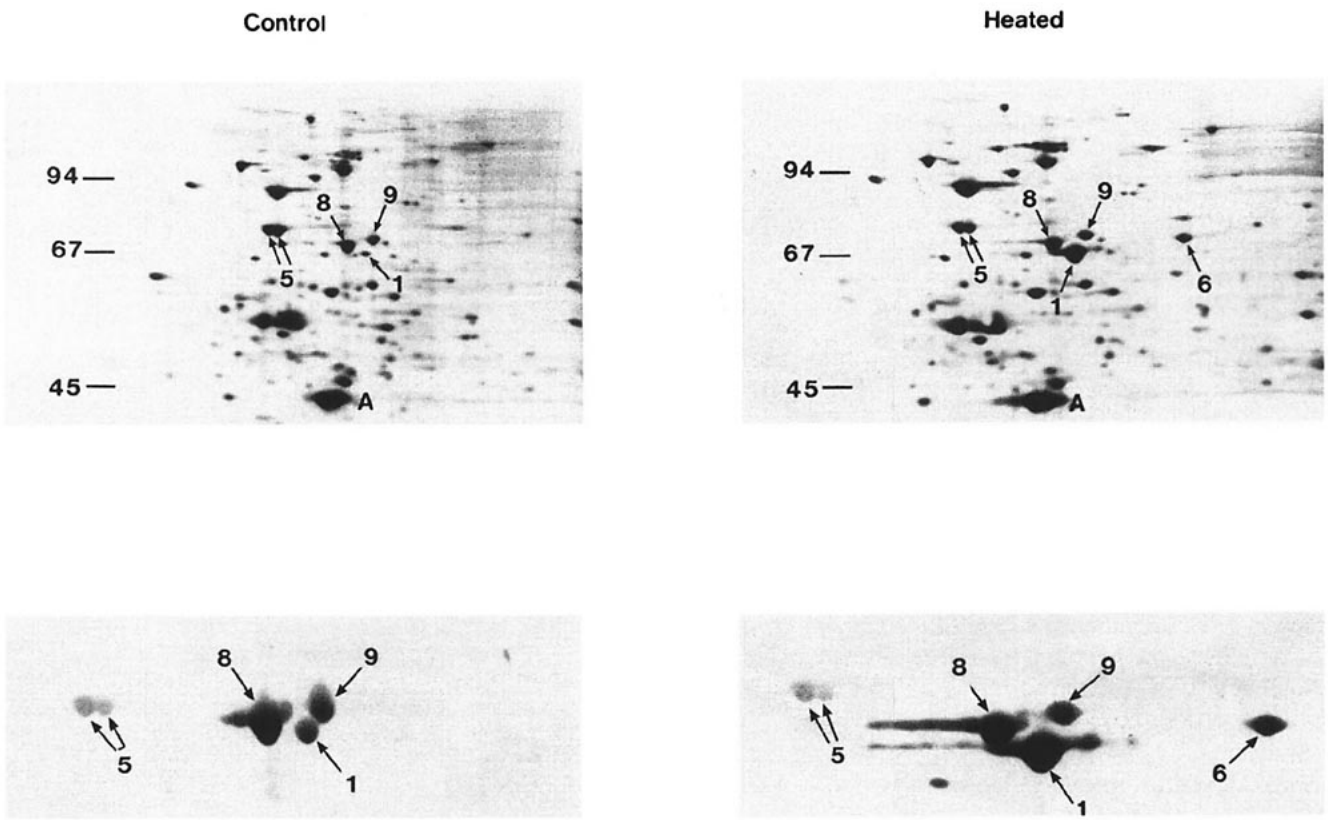

Fig. 1 Two-dimensional gels of human cells. The human fibrosarcoma cell line, HT1080, was incubated for 2 days in culture medium containing ^{35}S-methionine (10 μCi/ml) prior to changing the medium and heating the cells for 10 min at 45°C. Control cells were kept at 37°C. The cells were incubated for a further 6 h in radioactive medium prior to harvesting for gel analysis as described earlier (Anderson et al 1989). The proteins were separated on a pH 4–7 gradient in the first dimension and on a 10% reducing gel in the second dimension. The gels were dried and exposed to X-ray film. For Western blot analysis, the proteins were transferred to nitrocellulose and immunostained with a mixture of monoclonal antibodies that recognize various members of the Hsp70 family. The antibodies used were N6, a mouse antibody that recognizes Hsp70–1, −6 and −8 (a gift from Dr W. Welch); Clone 3a3 (Affinity BioReagents), a mouse antibody that recognizes Hsp70–9 in addition to the previously mentioned proteins and Clone 70.1 (also from Affinity BioReagents), a rat antibody that recognizes Hsp70–5 in addition to those recognized by N6. The weak immunostaining for Hsp70–5 is due to the use of a secondary goat anti-mouse IgG antibody which binds to rat IgG with lower affinity. Detection was achieved by alkaline phosphatase activity bound to the secondary antibody. The gels are presented with the acidic side on the left and only the top halves of the gels are shown. The various members of the Hsp70 family are indicated using the new nomenclature. A, actin. Molecular weight markers are shown on the left hand side of the two upper panels. The upper two panels are radiographs from control and heated cells, while the lower two panels show the Western analysis of the same samples.

REFERENCES

Anderson RL, van Kersen I, Kraft PE and Hahn GM (1989) Biochemical analysis of heat-resistant mouse tumour cell strains: a new member of the Hsp70 family. Mol. Cell. Biol. **9**: 3509–3516.

Bhattacharyya T, Karnezis AN, Murphy SP, Hoang T, Freeman BC, Phillips B and Morimoto RI (1995) Cloning and subcellular localization of human mitochondrial hsp70. J. Biol. Chem. **270**: 1705–1710.

Bonnycastle LL, Yu C-E, Hunt CR, et al (1994) Cloning, sequencing, and mapping of the human chromosome 14 heat shock protein gene (HSPA2). Genomics **23**: 85–93.

Brown CR, Martin RL, Hansen, WJ, Beckmann RP and Welch WJ (1993) The constitutive and stress inducible forms of hsp70 exhibit functional similarities and interact with one another in an ATP-dependent fashion. J. Cell Biol. **120**: 1101–1112.

Domanico SZ, DeNagel DC, Dahlseid JN, Greene AE and Mullivor RA (1993) Cloning of the gene encoding peptide-binding protein 74 shows that it is a new member of the heat shock protein 70 family. Mol. Cell. Biol. **13**: 3598–3610.

Fathallah DM, Cherif D, Dellagi K and Arnaout MA (1993) Molecular cloning of a novel human Hsp70 from a B. cell line and its assignment to chromosome 5. J. Immunol. **151**: 810–813.

Gabriele T, Tavaria M, Kola D and Anderson RL (1996) Analysis of heat shock protein 70 in human chromosome 21 containing hybrids. Int. J. Biochem. Cell Biol. (In press).

Gething M-J and Sambrook J (1992) Protein folding in the cell. Nature **355**: 33–45.

Goate AM, Cooper DN, Hall C, Leung TK, Solomon E and Lim L (1987) Localization of a human heat shock Hsp70 gene sequence to chromosome 6 and detection of two other loci by somatic-cell hybrid and restriction fragment length polymorphism analysis. Human Genet. **75**: 123–128.

Haas IG and Wabl M (1983) Immunoglogulin heavy chain binding protein. Nature **306**: 387–389.

Harrison GS, Drabkin HA, Kao FT, et al (1987) Chromosomal

location of human genes encoding major heat shock protein Hsp70. Somat. Cell Mol. Genet. **13**:119–130.

Hendershot LM, Valentine VA, Lee AS, Morris SW and Shapiro DN (1994) Localization of the gene encoding BiP/GRP78, the endoplasmic reticulum cognate of the Hsp70 family, to chromsome 9q34. Genomics **20**: 281–284.

Kaul SC, Wadhwa R, Matsuda Y, Hensler PJ, Pereira-Smith OM, Komatsu Y and Mitsui Y (1995) Mouse and human chromosomal assignments of mortalin, a novel member of the hsp70 family of proteins. FEBS Lett. **361**: 269–272.

Lee AS (1987) Coordinated regulation of a set of genes by glucose and calcium ionophores in mammalian cells. Trends Biochem. Sci. **12**: 20–23.

Leno GH and Ledford BE (1989) ADP-ribosylation of the 78-kDa glucose-regulated protein during nutritional stress. Eur. J. Biochem. **186**: 205–211.

Leung TK, Rajendran M, Monfries C, Hall C and Lim L (1990) The human heat shock protein family. Expression of a novel heat-inducible Hsp70 (Hsp70B') and isolation of its cDNA and genomic DNA. Biochem. J. **267**: 125–132.

Leung TK, Hall C, Rajendran M, Spurr NK and Lim L (1992) The human heat shock genes HSPA6 and HSPA7 are both expressed and localize to chromosome 1. Genomics **12**: 74–79.

Lithgow T, Ryan M, Anderson RL, Høj PB and Hoogenraad J (1993) A constitutive form of heat-shock protein 70 is located in the outer membranes of mitochondria from rat liver. FEBS Lett. **332**: 277–281.

Milner M and Campbell RD (1990) Structure and expression of the three MHC-linked Hsp70 genes. Immunogenetics **32**: 242–251.

Mizzen LA, Chang C, Garrels JI and Welch WJ (1989) Identification, charaterization and purification of two mammalian stress proteins present in mitochondria, grp75, a member of the hsp70 family and hsp58, a homolog of the bacterial groEL protein. J. Biol. Chem. **264**: 20664–20675.

Multhoff G, Botzler C, Wiesnet M, Müller E, Meier T, Wilmanns W and Issels RD (1995) A stress-inducible 72 kDa heat-shock protein (HSP72) is expressed on the surface of human tumour cells, but not on normal cells. Int. J. Cancer **61**: 1–8.

Roux A-F, Nguyen VTT, Squire JA and Cox DW (1994) A heat shock gene at 14q22: mapping and expression. Hum. Mol. Genet. **3**: 1819–1822.

Sargent CA, Dunham I, Trowsdale J and Campbell RD (1989) Human major histocompatibility complex contains genes for the major heat shock protein Hsp70. Proc. Natl. Acad. Sci. USA **86**: 1968–1972.

Tavaria M, Gabriele T, Anderson RL, Mirault M-E, Baker E, Sutherland G and Kola I (1995) Localization of the gene encoding the human heat shock cognate protein, HSP73, to chromosome 11. Genomics **29**: 266–268.

Ting J and Lee AS (1988) Human gene encoding the 78,000-dalton glucose-regulated protein and its pseudogene: structure, conservation and regulation. DNA **7**: 275–286.

Voellmy R, Ahmed A, Schiller P, Bromley P and Rungger D (1985) Isolation and functional analysis of a human 70,000-dalton heat shock protein gene segment. Proc. Natl. Acad. Sci. USA **82**: 4949–4953.

Wu B, Hunt C and Morimoto RI (1985) Structure and expression of the human gene encoding the major heat shock protein Hsp70. Mol. Cell. Biol. **5**: 330–341.