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Genetic aspects of the hsp70 multigene family in vertebrates

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Abstract. The family of genes encoding heat shock proteins of about 70 kDa (hsp70) in vertebrates is reviewed under genetic aspects. After a detailed description of the various hsp70 genes more general characteristics of the organization and evolution of the multigene family are discussed.
Key words. Hsp70; evolution; gene duplication; gene homology.

Introduction

The heat shock response was detected by the observation of a new phenomenon: formation of chromosome puffs in *Drosophila* cells after heat stress[63]. Later, the synthesis of distinct proteins, called heat shock proteins (HSP)*, was described as a consequence of heat shock[74]. Heat shock response and HSP were then also observed in all other organisms including vertebrates. Different HSP could be distinguished electrophoretically by apparent molecular mass and also by isoelectric point. The group of HSP of about 70 kDa constitutes the hsp70 family. Corresponding genes have been reported in the past years; however, not all of them are yet identified or characterized in sufficient detail. Sequence data confirmed that hsp70 genes of vertebrates constitute a multigene family. In invertebrate eukaryotic species, hsp70 genes also form multigene families, for example comprising 9 members in *Saccharomyces cerevisiae*[7] and about 9 members in *Drosophila melanogaster*[59]. In contrast, a single copy gene (dnaK) is found in the prokaryote *E. coli*. It was soon realized that expression of at least some hsp70 genes occurred constitutively (hsp70 cognate or hsc70) and that other inductors besides heat shock exist, e.g., glucose starvation. The main function of hsp70 molecules is presently seen in their role as molecular chaperones under physiological and stress conditions[19,52].

The distinction of individual hsp70 proteins on the basis of electrophoretic properties gave rise to inconsistent designations. The heat-inducible protein, for example, is variably called hsp68, hsp70, hsp71 or hsp72. Furthermore the designation of the genes is not standardized. Officially hsp70 genes in man are designated HSPA[45]. Mostly the designation introduced by the first describer is used, and human nomenclature is often adopted in other species.

* The abbreviations of HSP for heat shock protein in general and hsp for distinct heat shock proteins follows the use throughout this multi-author review. In the case of individual genes the nomenclature rules for man (capital letters) and mouse and rat (first letter capital, following in lower case) are applied.

Since among vertebrates most hsp70 data are reported in man, this review is orientated towards the human hsp70 genes (except when human homologs are not yet known). The review is mainly based on those hsp70 genes for which extensive and published sequence information is available. Thus data from the EMBL/GenBank data base are only referred to if published.

The hsp70 genes which have been clearly identified and characterized up to now are listed in table 1 and described in more detail below. The table includes the EMBL/GenBank accession number of the gene sequence and information about chromosomal localization, expression and orthologous genes (i.e. homologous gene in homologous chromosomal position in different species). Orthologous genes in various species will be distinguished by the prefixes b (bovine), h (human), ha (Chinese hamster), r (rat), m (mouse) and p (porcine).

The description of hsp70 genes starts with the three hsp70 genes, which map inside the major histocompatibility complex (Mhc). Their organization (and nomenclature) is illustrated in figure 1. Further heat-inducible hsp70 genes will then be described as well as the testis-specific non-Mhc linked hsp70 gene and the constitutively expressed *HSC70* and *GRP78* genes. The *PBP74*, *Mot-1*, *HSP70RY* and *STCH* genes present some additional features, which define new subsets of the hsp70 multigene family.

The deduced amino acid sequences of the genes discussed are summarized in figures 2 to 6. In figure 2 the homologous relationship between the human hsp70 genes is shown, and figures 3 to 6 demonstrate the similarity between genes of the same type in various species, i.e. presumably mostly the orthologous genes. Tables 2 to 5 correspond to the figures by presenting quantitative data about sequence similarity. Sequence comparisons were performed by AALIGN (DNAstar) using the default parameters.

HSP70-1 (HSPA1)

On the basis of a cosmid clone which carried three hsp70 genes and mapped into the class III region of the

Table 1. List of hsp70 genes for which detailed sequence information is available.

Gene	Species[a]	Accession no.	Chromosome	Expression[b]			Ref.	Orthologous genes				Ref.
				Tissue	Site	Mode		Designation	Species	Accession no.	Chromosome	
HSP70-1 (HSPA1)	h	M59828, *M34267*	6p21	ub	cyt, nuc	const, *hs*	31, 48	*Hsp70-2*	*r*	*X77208*	20	78
								Hsp70-3	m		17	42
HSP70-2	h	M34269	6p21	ub	cyt, nuc	hs	48	*Hsp70-1*	r	X77207[c]	20	78
								Hsp70-1	m	M35021	17	30
								HSP70	p	M69100	7	56, 60
HSP70-Hom	h	M59829, M34268	6p21	testis		const	17, 48	*Hsp70-3*	r	X77209	20	78
								Hsc70t	m	M20567	17	43, 68
HSPA6	h	X51757	1q	ub		hs	36		p	X68213		10
HSPA7	h	M11236	1q	ub		hs	36, 76					
HSC70	h	M12119		ub	cyt, nuc	const	14	*Hsc70*	r	Y0054		57, 69
								Hsc70	m			20
								HSC70	b	X53872		8
								Hsc70	ha	M34561		1
GRP78(BiP)	h	M19645	9q	ub	ER	const, gluc$^-$	72	*Grp78*	r	M14050		55
								Grp78	m		2	25, 26, 32
								Grp78	ha	N17169		73
Hsp70.2	m	M20567	12	testis		const	84	*Hst70*	r	X15705		81
Mot-1	m	D11089		ub		const	77					
PBP74	h	L11066			cyt	const	11	*Pbp74*	m	L06896		11
HSP70RY	h	L12723	5q31		cyt	const	15					
STCH	h	U04735		ub	cyt (micros)	const	58					

[a]b, bovine; h, human; ha, chinese hamster; m, mouse; p, pig; r, rat.
[b]ub, ubiquitous; cyt, cytosol; nuc, nucleus; micros, microsomal fraction; hs, heat-shock induced; const, constitutive; gluc$^-$, glucose deprivation.
[c]Slightly variant sequences are X74271, L16764, X75357 (refs 38, 41, 46).

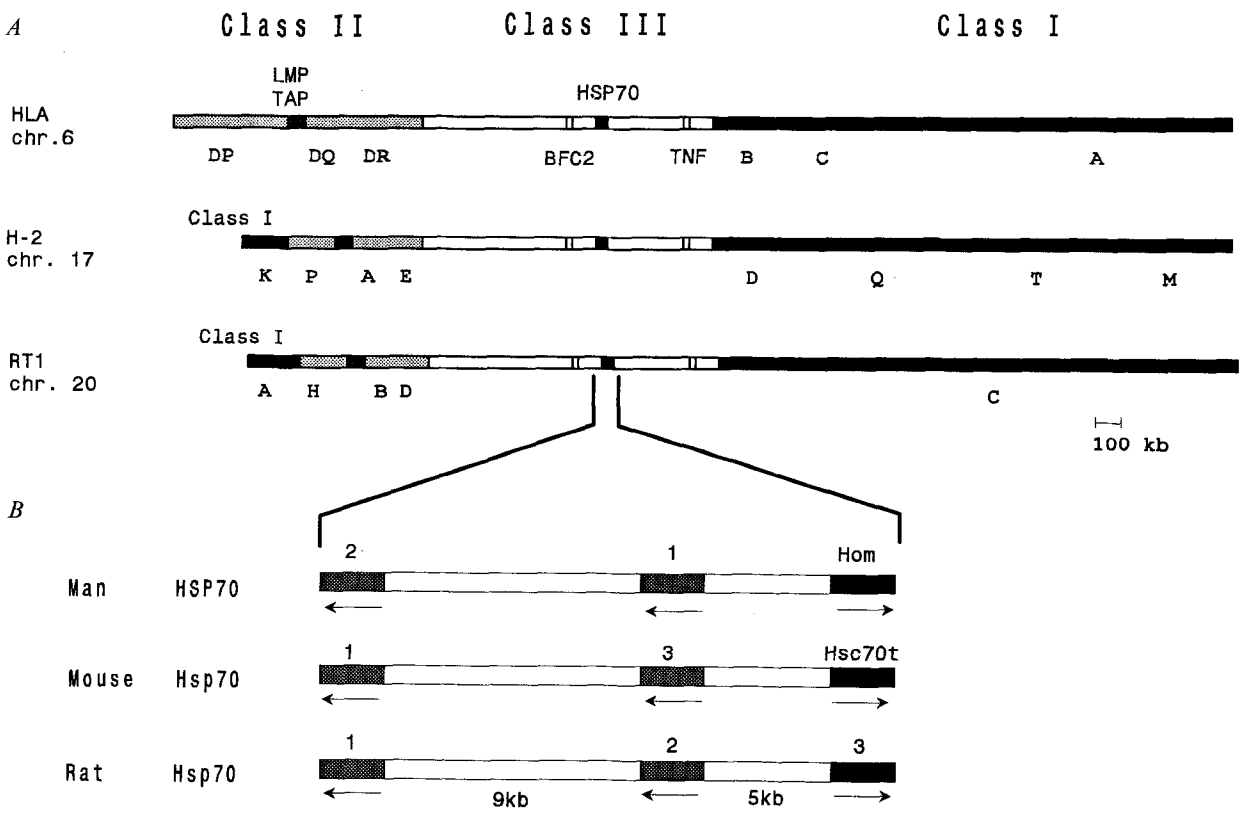

Figure 1. Localization of hsp70 genes inside the Mhc (*A*) and organization of the three Mhc-linked hsp70 genes in man, mouse and rat (*B*). Letters in (*A*) designate various Mhc genes[6,23]. (*B*) is based on references 32, 67, 78.

human Mhc, the localization of genes of the hsp70 family inside the Mhc, the HLA-system on chromosome 6p21, was detected in man[48,66]. The cosmid library was derived from cells with the *HLA-A2, B7, SC30, DR2* haplotype. The genes *HSP70-1, HSP70-2* and *HSP70-Hom* do not contain introns and encode proteins of 641 amino acids. Analysis of the *HSP70-1* gene in various cell lines revealed nucleotide polymorphisms in the 5′ untranslated region (utr) and a silent exchange in the coding region[49]. The *HSP70-1* gene is heat inducible and is found expressed at a low basal level in nonstressed HeLa cells[48]. In normal peripheral blood mononuclear cells (PBMC) constitutive expression could not be detected by in situ hybridization and northern blot analysis (Dressel and Günther, own unpubl. data).

Coding and noncoding sequences of *HSP70-1* are nearly identical to the hsp70 gene described by Hunt and Morimoto[31]. Both sequences differ by 11 nucleotides; in the coding region two amino acid exchanges can be observed and an additional codon (Arg at position 469) is present in *HSP70-1*. It is assumed that both sequences represent alleles of the same gene, which has been registered officially as *HSPA1* (ref. 45). It should be mentioned that a very similar sequence has been published also by Drabent et al.[12]. Already before the report of Sargent et al.[66] analysis of somatic cell hybrids had revealed that heat-inducible hsp70 genes, among them the gene described by Hunt and Morimoto[31], mapped to human chromosome 6p21 (refs 21, 27).

Studies performed with the hsp70 gene described by Hunt and Morimoto[31] showed the heat-inducibility of the gene(s) detectable with the respective probe[31] and demonstrated in HeLa cells that expression occurs in a cell-cycle dependent fashion during the S phase[47]. It is, however, not clear whether the gene(s) affected is *HSP70-1, HSP70-2* (see below) or both.

Mapping of a hsp70 gene inside the mouse Mhc was shown by Gaskins et al.[18]. The orthologous gene of *hHSP70-1* has been identified in the Mhc of the mouse (H-2 system, chromosome 17), and the rat (RT1-system, chromosome 20). In the mouse a previously known gene corresponding to clone pMHS213 of Lowe and Moran[42], now designated *Hsp70-3* (fig. 2), was localized to the Mhc[32,67]. The sequence is only partially known[42].

In the rat, hsp70 genes were mapped to the Mhc by restriction fragment length polymorphism (RFLP) analysis[83]. The gene orthologous to *hHSP70-1* is called *Hsp70-2* (ref. 78) (fig. 2) and has been sequenced for the haplotype $RT1^u$ (ref. 78). In rat lymphocytes *Hsp70-2* is expressed only after heat shock as determined at the RNA level by in situ hybridization and northern blot analysis (Dressel and Günther, own unpubl. data).

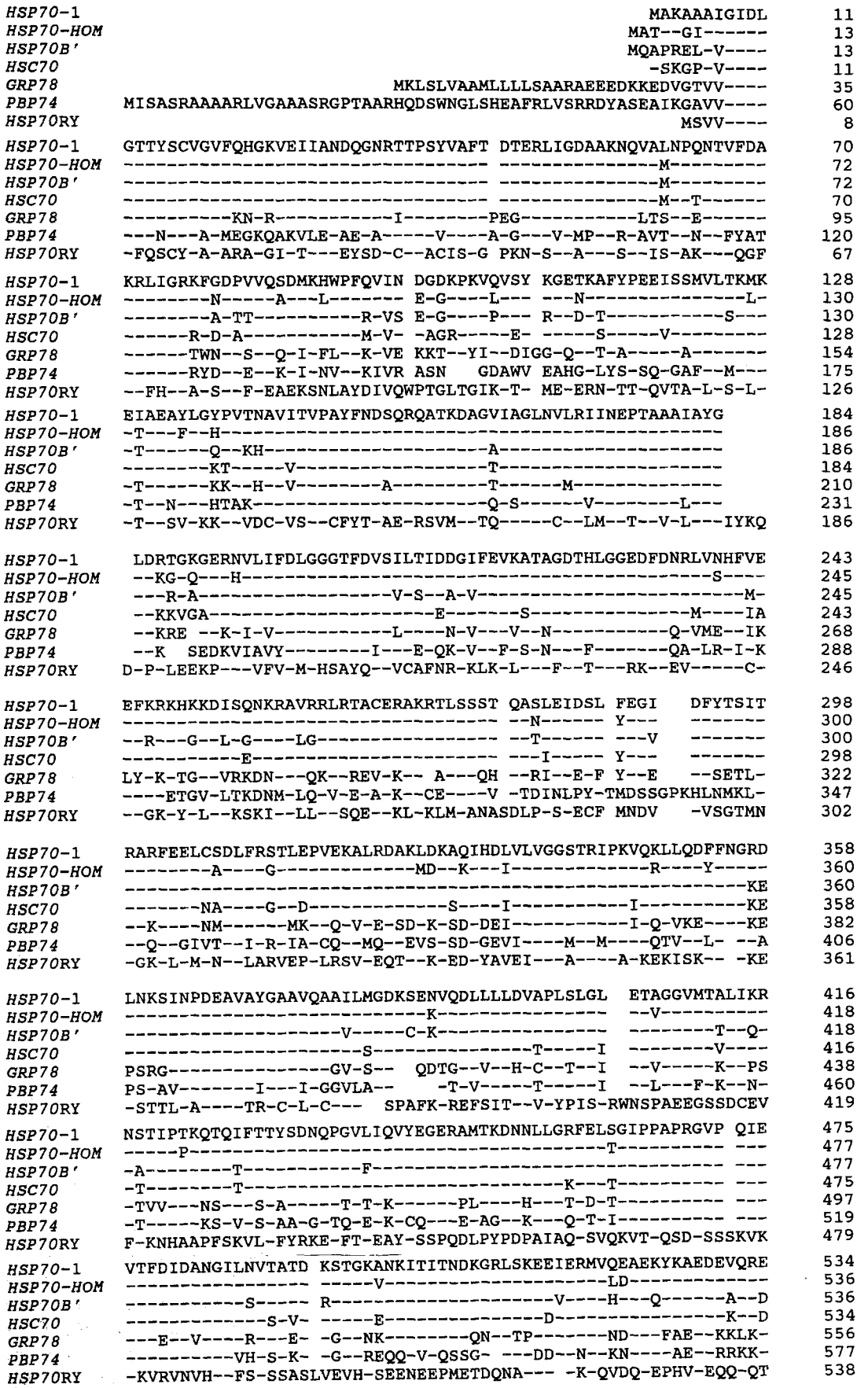

Figure 2. (continued on page 991)

```
HSP70-1     RVSAKNALESYAFNMKSAVED EGLKGKISEADKKKVLDKCQEVISWLDANTLAEKDEFE      593
HSP70-HOM   KIA--------------V-S- ---------S--N-I----N-LL---EV-QL------D      595
HSP70B'     --A---S--AHV-HV-GSLQE -S-RD--P-E-RR-MQ---R--LA--EH-Q----E-Y-      595
HSC70       K--S--S---------AT--- -K-Q---NDE--Q-I----N-I-N---K-QT---E---      593
GRP78       -IDTR-E-----YSL-NQIG-K-K-G--L-SE--ETMEKAVE-K-E--ESHQD-DIED-K      616
PBP74       --E-V-MA-GIIHDTETKM-   EF-DQLPADECN-LKEEISKMREL-ARKDSETGENIR      634
HSP70RY     PAEN-AES-EMETSQAGSKDK KMDQPPQCQEG-SEDQYCGPANRESAIWQIDR-MLNLY      597

HSP70-1     HKRKELEQVCNPIISGLYQGAGG  PG PGGF  GAQGPKGGSGSGPTIEEVD            641
HSP70-HOM   --------M-----TK---- -C  T- -ACG  TGYV- -RPAT--------            641
HSP70B'     -QKR----I-R--F-R--G-P-V  -- GSSC  -T-ARQ-DPST--I-----            643
HSC70       -QQ----K------TK---S---MPG-M----PG-GAP-S--AS---------            646
GRP78       A-K----EIVQ----K--GS--P  -P T-EE  DTAEKDEL                      653
PBP74       QAASS-Q-ASLKLFEMA-KKMAS  ER E-SG  SSGTGEQKEDQKEEKQ              679
HSP70RY     IENEGKMIMQDKLEKERNDAKNA  VE EYVY  EMRDKLS-EYEKFVS-DDRNSFTLKL    652
HSP70RY     EDTENWLYEDGEDQPKQVYVDKLAELKNLGQPIKIRFQESEERPNYLKN              701
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Figure 2 (continued). Comparison of deduced amino acid sequences of the human hsp70 multigene family. For references see table 1. In the case of *HSP70-1* the sequence reported in reference 48 is used.

```
hHSP70-1/2  MAKAAAIGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVALNPQNTVFDA   70
rHsp70-1/2  ---KT-------------------------------------------------------------   70
mHsp70-1    ---NT-------------------------------------------------------------   70
pHSP70      ---SV-----------------------------------S-------------------------   70

hHSP70-1/2  KRLIGRKFGDPVVQSDMKHWPFQVINDGDKPKVQVSYKGETKAFYPEEISSMVLTKMKEIAEAYLGYPVT  140
rHsp70-1/2  ----------------------V----------N----NRS----------------------H---  140
mHsp70-1    ----------A----------V----------N----SRS-F--------------------H---  140
pHSP70      --------------G-------R------------------G------------------G---H--S  140

hHSP70-1/2  NAVITVPAYFNDSQRQATKDAGVIAGLNVLRIINEPTAAAIAYGLDRTGKGERNVLIFDLGGGTFDVSIL  210
rHsp70-1/2  -------------------------------------------------------------------  210
mHsp70-1    ----R--------------------------------------------------------------  210
pHSP70      -------------------------------------------------------------------  210

hHSP70-1/2  TIDDGIFEVKATAGDTHLGGEDFDNRLVNHFVEEFKRKHKKDISQNKRAVRRLRTACERAKRTLSSSTQA  280
rHsp70-1/2  ----------------------------S---------------------------------------  280
mHsp70-1    ----------------------------S---------------------------------------  280
pHSP70      ------------------------------------Y-------------------------------  280

hHSP70-1/2  SLEIDSLFEGIDFYTSITRARFEELCSDLFRSTLEPVEKALRDAKLDKAQIHDLVLVGGSTRIPKVQKLL  350
rHsp70-1/2  ------------------------------G-------------------------------------  350
mHsp70-1    ------------------------------G------------M-----------------------  350
pHSP70      -------------------------------------------------------------------  350

hHSP70-1/2  QDFFNGRDLNKSINPDEAVAYGAAVQAAILMGDKSENVQDLLLLDVAPLSLGLETAGGVMTALIKRNSTI  420
rHsp70-1/2  -------------------------------------------------------------------  420
mHsp70-1    -------------------------------------------------------------------  420
pHSP70      -------------------------------------------------------------------  420

hHSP70-1/2  PTKQTQIFTTYSDNQPGVLIQVYEGERAMTKDNNLLGRFELSGIPPAPRGVPQIEVTFDIDANGILNVTA  490
rHsp70-1/2  ------T-----------------------R-------------------------------------  490
mHsp70-1    ------T-----------------------R-----------------------L----K-------  490
pHSP70      ----------------------------R-------------------------------------  490

hHSP70-1/2  TDKSTGKANKITITNDKGRLSKEEIERMVQEAEKYKAEDEVQRERVSAKNALESYAFNMKSAVEDEGLKG  560
rHsp70-1/2  ---------------------------------R-----------A----------------------  560
mHsp70-1    ---------------------------------R---------D--A----------------------  560
pHSP70      ---------------------------------------I-----G--------------V--------  560

hHSP70-1/2  KISEADKKKVLDKCQEVISWLDANTLAEKDEFEHKRKELEQVCNPIISGLYQGAGGPGPGGFGAQGP KG  629
rHsp70-1/2  ----------------------S------E--V---E---R--------------A--A------A- --  629
mHsp70-1    -L--------------------SN---D-E--V---DD--R--S-----------A--A------A-P--  630
pHSP70      ------------------------------------------------------------PDL --  629

hHSP70-1/2  GSGSGPTIEEVD                                                          641
rHsp70-1/2  ------------                                                          641
mHsp70-1    A-----------                                                          642
pHSP70      ------------                                                          641
```

Figure 3. Comparison of deduced amino acid sequences of orthologous heat-inducible hsp70 genes of the Mhc. For references see table 1. In the case of *rHsp70-1* the sequence reported in reference 78 is used.

```
hHSP70-HOM MATAKGIAIGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVAMNPQNTVF   70
rHsp70-3   --AN--M-------------------------------------------------------------   70
mHsc70t    --AN--M-------------------------------------------------------------   70

hHSP70-HOM DAKRLIGRKFNDPVVQADMKLWPFQVINEGGKPKVLVSYKGENKAFYPEEISSMVLTKLKETAEAFLGHP  140
rHsp70-3   ----------------S-----------A-------------------------M----------S  140
mHsc70t    ----------------S-----------A-----M------K---------------M----------N  140

hHSP70-HOM VTNAVITVPAYFNDSQRQATKDAGVIAGLNVLRIINEPTAAAIAYGLDKGGQGERHVLIFDLGGGTFDVS  210
rHsp70-3   ------------------------------------------------SH------------------  210
mHsc70t    ------------------------------------------------SH------------------  210

hHSP70-HOM ILTIDDGIFEVKATAGDTHLGGEDFDNRLVSHFVEEFKRKHKKDISQNKRAVRRLRTACERAKRTLSSST  280
rHsp70-3   ----------------------------------------------------A---------------  280
mHsc70t    -------------------------------------------------------------------  280

hHSP70-HOM QANLEIDSLYEGIDFYTSITRARFEELCADLFRGTLEPVEKALRDAKMDKAKIHDIVLVGGSTRIPKVQR  350
rHsp70-3   ------------------------------------S-------------------------K  350
mHsc70t    ------------------------------------S-------------------------K  350

hHSP70-HOM LLQDYFNGRDLNKSINPDEAVAYGAAVQAAILMGDKSEKVQDLLLLDVAPLSLGLETVGGVMTALIKRNS  420
rHsp70-3   ----------------------------------------------------A-----V------  420
mHsc70t    ----------------------------------------------------A-----V------  420

hHSP70-HOM TIPPKQTQIFTTYSDNQPGVLIQVYEGERAMTKDNNLLGRFDLTGIPPAPRGVPQIEVTFDIDANGILNV  490
rHsp70-3   ---T--------------------------R-----------------------------------  490
mHsc70t    ---T--------------------------R-----------------------------------  490

hHSP70-HOM TATDKSTGKVNKITITNDKGRLSKEEIERMVLDAEKYKAEDEVQREKIAAKNALESYAFNMKSVVSDEGL  560
rHsp70-3   --M------A-------------------QE--R------G-------------------A-G----  560
mHsc70t    --M------A-------------------QE--R------G-------------------A-G----  560

hHSP70-HOM KGKISESDKNKILDKCNELLSWLEVNQLAEKDEFDHKRKELEQMCNPIITKLYQGGCTGPACGTGYVPGR  630
rHsp70-3   -D-------K------S-V-----A------E----------N----------S-----T-AP--T---  630
mHsc70t    -D-------K--------V-----A-----------------N----------S-----T-TP--T---  630

hHSP70-HOM PATGPTIEEVD                                                           641
rHsp70-3   AR---------                                                           641
mHsc70t    A----------                                                           641
```

Figure 4. Comparison of deduced amino acid sequences from orthologous testis-specific hsp70 genes of the Mhc. For references see table 1. In case of *mHsc70t* the sequence reported in reference 68 is used.

Mhc-linked hsp70 genes have been described also in cattle[22], pig[56], goat[5] and frog[65]. Thus an orthologous gene of human *HSP70-1* is likely to exist in these species.

HSP70-2

The second hsp70 gene identified by Sargent et al.[66], *HSP70-2*, is separated from *HSP70-1* by about 8 kb (fig. 2). *HSP70-2* also lacks introns and its deduced amino acid sequence is identical to that of *HSP70-1* (ref. 48). These genes, however, differ from each other, most clearly in the 3'utr. *HSP70-2*, in contrast to *HSP70-1*, carries a PstI RFLP in the coding region, which does not result in an amino acid exchange[21,49]. Furthermore, a 5-bp-duplication polymorphism can be found in its 3'utr (ref. 13). Nucleotide exchanges occur also in the 5'utr (ref. 49).

HSP70-2 is expressed in HeLa cells only after heat shock and not constitutively[48]. Expression is also detectable in normal PBMC by northern blot analysis and in situ hybridization only after heat shock (Dressel and Günther, own unpubl. data).

In the mouse, the orthologous gene, *Hsp70-1*, which corresponds to clone pMHS214 (ref. 42), is separated from the *hHSP70-2* homolog, *Hsp70-3*, by about 9 kb (ref. 32) (fig. 2). Its sequence has been described[30]. It carries a TA-microsatellite polymorphism in the 3'utr (ref. 33). The mouse HSP70-1 protein contains one amino acid (position 628) more than the products of the orthologous rat and human genes.

In the rat (*RT1*^{u} haplotype), the orthologous gene, called *Hsp70-1*, is separated from *Hsp70-2* by about 9 kb (ref. 78) (fig. 2). The deduced amino acid sequences of rat *Hsp70-1* and rat *Hsp70-2* are identical[78], as is found for the orthologous genes in man. It is noteworthy that slightly variant sequences have been published[38,41,46], which most likely are alleles of the *rHsp70-1* gene. No basal expression of *Hsp70-1* is found in rat (ref. 38, Dressel and Günther, own unpubl. data) and mouse[30]. In bovine[22], pig[56] and frog[65] the occurrence of at least two Mhc-linked hsp70 genes indicates that genes orthologous to human *HSP70-2* could also exist in these species. The porcine hsp70 gene described by Peelman et al.[60] appears to be Mhc linked, because its restriction map fits that of hsp70-carrying genomic clones which map to

```
 hHSC70 MSKGPAVGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVAMNPTNTVFDA   70
 rHsc70 ----------------------------------------------------------------------   70
 mHsc70 ----------------------------------------------------------------------   70
 bHSC70 ----------------------------------------------------------------------   70
haHsc70 ----------------------------------------------------------------------   70

 hHSC70 KRLIGRRFDDAVVQSDMKHWPFMVVNDAGRPKVQVEYKGETKSFYPEEVSSMVLTKMKEIAEAYLGKTVT  140
 rHsc70 ----------------------------------------------------------------------  140
 mHsc70 ----------------------------------------------------------------------  140
 bHSC70 ----------------------------------------------------------------------  140
haHsc70 ----------------------------------------A-----------------------------  140

 hHSC70 NAVVTVPAYFNDSQRQATKDAGTIAGLNVLRIINEPTAAAIAYGLDKKVGAERNVLIFDLGGGTFDVSIL  210
 rHsc70 ----------------------------------------------------------------------  210
 mHsc70 ----------------------------------------------------------------------  210
 bHSC70 ----------------------------------------------------------------------  210
haHsc70 ----------------------------------------------------------------------  210

 hHSC70 TIEDGIFEVKSTAGDTHLGGEDFDNRMVNHFIAEFKRKHKKDISENKRAVRRLRTACERAKRTLSSSTQA  280
 rHsc70 ----------------------------------------------------------------------  280
 mHsc70 ----------------------------------------------------------------------  280
 bHSC70 ----------------------------------------------------------------------  280
haHsc70 ------------------------------------ND----------------------------------  280

 hHSC70 SIEIDSLYEGIDFYTSITRARFEELNADLFRGTLDPVEKALRDAKLDKSQIHDIVLVGGSTRIPKIQKLL  350
 rHsc70 ----------------------------------------------------------------------  350
 mHsc70 ----------------------------------------------------------------------  350
 bHSC70 ----------------------------------------------------------------------  350
haHsc70 ----------------------------------------------------------------------  350

 hHSC70 QDFFNGKELNKSINPDEAVAYGAAVQAAILSGDKSENVQDLLLLDVTPLSLGIETAGGVMTVLIKRNTTI  420
 rHsc70 ----------------------------------------------------------------------  420
 mHsc70 ----------------------------------------------------------------------  420
 bHSC70 ----------------------------------------------------------------------  420
haHsc70 ----------------------------------------------------------------------  420

 hHSC70 PTKQTQTFTTYSDNQPGVLIQVYEGERAMTKDNNLLGKFELTGIPPAPRGVPQIEVTFDIDANGILNVSA  490
 rHsc70 ----------------------------------------------------------------------  490
 mHsc70 -------L--------------------------------------------------------------  490
 bHSC70 ----------------------------------------------------------------------  490
haHsc70 ----------------------------------------------------------------------  490

 hHSC70 VDKSTGKENKITITNDKGRLSKEDIERMVQEAEKYKAEDEKQRDKVSSKNSLESYAFNMKATVEDEKLQG  560
 rHsc70 ----------------------------------------------------------------------  560
 mHsc70 ----------------------------------------------------------------------  560
 bHSC70 ------------------------------------------------------K---------------  560
haHsc70 ----------------------------------------------------------------------  560

 hHSC70 KINDEDKQKILDKCNEIINWLDKNQTAEKEEFEHQQKELEKVCNPIITKLYQSA    GGMPGGMPGGFP  626
 rHsc70 ------------------S-----------------------------------    ------------  626
 mHsc70 ------------------S-----------------------------------    ------------  626
 bHSC70 ----------------------------------------------------GGMP------------  630
haHsc70 ------------------S-----------------------------------    ------------  626

 hHSC70 GGGAPPSGGASSGPTIEEVD                                                    646
 rHsc70 --------------------                                                    646
 mHsc70 --------------------                                                    646
 bHSC70 --------------------                                                    650
haHsc70 --------------------                                                    646
```

Figure 5. Comparison of deduced amino acid sequences of *hsc70* genes from various species. For references see table 1.

the Mhc (ref. 56). On the basis of 3′utr sequence similarity this gene is most likely the *hHSP70-2* ortholog.

HSP70-Hom (HSPA1L)

The HLA cosmid clone containing the *HSP70-1* and *HSP70-2* genes carries a third hsp70 gene, *HSP70-Hom*, about 4 kb distant from *HSP70-1*[66]. The complete sequence of this intronless gene has been published[48]. The same gene has also been mapped and sequenced by Fujimoto et al.[17], who designated it *hum70t*. For the *HSP70-Hom* gene two nucleotide exchange polymorphisms have been described[49], and one of the nucleotide differences leads to a NcoI RFLP and an amino acid exchange at position 493.

HSP70-Hom is expressed constitutively in HeLa cells[48], but not in PBMC (Dressel and Günther, own unpubl. data).

```
hGRP78   MKLSLVAAMLLLLSAARAEEEDKKEDVGTVVGIDLGTTYSCVGVFKNGRVEIIANDQGNRITPSYVAFTP   70
rGrp78   --FTV---A----C-V-------------------------------------------------------   70
haGrp78  --FPM---A----C-V-------------------------------------------------------   70

hGRP78   EGERLIGDAAKNQLTSNPENTVFDAKRLIGRTWNDPSVQQDIKFLPFKVVEKKTKPYIQVDIGGGQTKTF   140
rGrp78   -------------------------------------------------------------------   140
haGrp78  -------------------------------------------------------------------   140

hGRP78   APEEISAMVLTKMKETAEAYLGKKVTHAVVTVPAYFNDAQRQATKDAGTIAGLNVMRIINEPTAAAIAYG   210
rGrp78   -------------------------------------------------------------------   210
haGrp78  -------------------------------------------------------------------   210

hGRP78   LDKREGEKNILVFDLGGGTFDVSLLTIDNGVFEVVATNGDTHLGGEDFDQRVMEHFIKLYKKKTGKDVRK   280
rGrp78   -------------------------------------------------------------------   280
haGrp78  -------------------------------------------------------------------   280

hGRP78   DNRAVQKLRREVEKAK ALSSQHQARIEIESFYEGEDFSETLTRAKFEELNMDLFRSTMKPVQKVLEDSD   349
rGrp78   ----------------R---------------F-----------------------------------   350
haGrp78  ----------------R---------------F-----------------------------------   350

hGRP78   LKKSDIDEIVLVGGSTRIPKIQQLVKEFFNGKEPSRGINPDEAVAYGAAVQAGVLSGDQDTGDLVLLHVC   419
rGrp78   -------------------------------------------------------------------   420
haGrp78  -------------------------------------------------------------------   420

hGRP78   PLTLGIETVGGVMTKLIPSNTVVPTKNSQIFSTASDNQPTVTIKVYEGERPLTKDNHLLGTFDLTGIPPA   489
rGrp78   ------------------R-------K-------------------------------------------   490
haGrp78  ------------------R-------K-------------------------------------------   490

hGRP78   PRGVPQIEVTFEIDVNGILRVTAEDKGTGNKNKITITNDQNRLTPEEIERMVNDAEKFAEEDKKLKERID   559
rGrp78   -------------------------------------------------------------------   560
haGrp78  -------------------------------------------------------------------   560

hGRP78   TRNELESYAYSLKNQIGDKEKLGGKLSSEDKETMEKAVEEKIEWLESHQDADIEDFKAKKKELEEIVQPI   629
rGrp78   ---------------------------P---------------------------------------   630
haGrp78  -------------------------------------------------------------------   630

hGRP78   ISKLYGSAGPPPTGEEDTAEKDEL                                               653
rGrp78   -------G----------S-----                                               654
haGrp78  ----------------S-----                                               654
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Figure 6. Comparison of deduced amino acid sequences of *grp78* genes from various species. For references see table 1.

Orthologous genes have been described in the mouse (*Hsc70t*)[32,43,68] and in the rat (*Hsp70-3*), the latter being separated from *rHsp70-2* by about 5 kb (ref. 78) (fig. 1). In the mouse, sequences for *Hsc70t* of the *H-2*^{b} and *H-2*^{d} haplotypes have been reported, which are identical[68], but differ from the sequence reported by Matsumoto and Fujimoto[43,78]. The *mHsc70t* gene is expressed specifically in the testis; expression is constitutive and restricted to germ cells of mainly postmeiotic stages[44]. Transcripts of the orthologous rat gene *Hsp70-3* are not detectable in lymphocytes, also not after heat stress, but are constitutively present in the testis during postmeiotic spermatogenesis (Dressel and Günther, own unpubl. data). Thus the orthologous genes of *HSP70-Hom* in mouse and rat are under developmental control and expressed in a tissue specific manner.

Table 3. Comparison (% identity) of the deduced amino acid sequences[a] between the orthologous Mhc-encoded hsp70 genes of man, rat and mouse.

	rHsp70-1/2	*mHsp70-1*
hHSP70-1/2	96.6	94.5
rHsp70-1/2		97.5
rHsp70-3		*mHsc70t*
hHSP70-Hom	93.6	94.4
rHsp70-3		98.9

[a]For sequences see figures 3 and 4 and for references see table 1.

Table 2. Comparison (% identity) of the deduced amino acid sequences[a] between different members of the human hsp70 gene family.

	HSP70-Hom	*HSP70B'*	*HSC70*	*GRP78*	*PBP74*	*HSP70RY*
HSP70-1/2	89	82	85	64	49	33
HSP70-Hom		79	82	63	49	33
HSP70B'			78	62	49	32
HSC70				66	50	32
GRP78					49	33
PBP74						27

[a]For sequences see figure 2 and for references see table 1.

Table 4. Comparison (% identity) of the deduced amino acid sequences[a] between hsc70 genes of man, rat, mouse, hamster and cattle.

	rHsc70	*mHsc70*	*haHsc70*	*bHSC70*
hHSC70	99.8	99.7	99.4	99.2
rHsc70		99.8	99.5	99.1
mHsc70			99.4	98.9
haHsc70				98.6

[a]For sequences see figure 5 and for references see table 1.

Table 5. Comparison (% identity) of the deduced amino acid sequences[a] between the grp78 genes of man, hamster and rat.

	haGrp78	*rGrp78*
hGRP78	98.2	97.9
haGrp78		99.4

[a]For sequences see figure 6 and for references see table 1.

HSP70B' (HSPA6)

The human *HSP70B'* gene[37], later designated *HSPA6* (ref. 36), is intronless and maps to chromosome 1q (ref. 36). The deduced protein of 643 amino acids is more basic than the *HSP70-1* gene product. The gene appears to be strictly heat inducible, since no basal expression was detected at the RNA level by northern blot analysis[36]. The sequence of the corresponding gene in pig has been reported[10]. This assignment is based on sequence similarity but not yet on mapping data. The porcine gene carries PstI and PvuII RFLP. Its expression is not constitutive, but heat inducible[10].

HSP70B (HSPA7)

The *HSP70B* gene[76], also designated *HSPA7* (ref. 36), has been sequenced only partially. The sequence shows 94% similarity to *Hsp70B'* (*HSPA6*), and has been mapped to chromosome 1q in close linkage with *HSPA6* (ref. 36). *HSPA7* carries a BamHI RFLP[36]. *HSPA7* mRNA is found by northern blot analysis only after heat shock. The pattern of inducibility in fibroblasts and various cell lines (HeLa, Daudi) is different from *HSPA6*[36]. Analysis by polymerase chain reaction (PCR), however, revealed specific transcripts in non-heat shocked cells[50].

A gene orthologous to human *HSPA6* or *HSPA7* has been identified in cattle and mapped to synteny group U6 (ref. 22).

HSC70

In contrast to the hsp70 genes mentioned so far, a gene (or genes) exists in the hsp70 family, which is expressed in each cell constitutively at high abundance, amounting to 1% of cellular protein[69]. Its expression is slightly enhanced after heat shock and is cell growth dependent[69]. The protein is often called heat shock cognate protein 70 (hsc70). The mRNA of the *HSC70* gene is shorter than that of heat-inducible hsp70 genes, so that the transcripts can be distinguished in northern blots. At the protein level hsc70 can be clearly separated electrophoretically from the faster-migrating heat-inducible hsp70 protein. Therefore hsc70 is also called hsp72 or hsp73 in distinction to heat-inducible hsp70, which is also designated hsp68, hsp70 or hsp72 in this context. The prp73 molecule is identical to hsc70[70].

In contrast to the heat-inducible members of the hsp70 family, the *HSC70* gene contains introns. Its genomic structure, characterized by eight introns, and its sequence are known in rat[69] and man[14]. The cDNA sequence has been reported for man[14], rat[57], mouse[20], Chinese hamster[1] and cattle[8]. Introns 1 and 3 of the human gene contain Alu-like repeats[14]. Interestingly U14 snRNA genes have been detected in introns 5, 6 and 8 of the *Hsc70* gene from mouse, rat and man[40].

The exact number of functioning hsc70 genes present in the genome is not known, because many processed hsc70 pseudogenes occur. This has been shown in man[54], mouse[20] and rat (ref. 69, Rothermel and Günther, own unpubl. data). The number of cross-hybridizing fragments in Southern blot analysis with *Hsc70* probes amounts to about 10 in the mouse and more than 20 in the rat (Rothermel and Günther, own unpubl. data). Genomic sequences of some pseudogenes have been determined. They revealed typical features of processed pseudogenes: absence of introns, occurrence of stop codons and deletions in the coding part, presence of a poly(A) tail and of short direct repeats at both ends of the insertion[54,69]. In the rat, a processed hsc70 pseudogene, *Hsc70-ps1*, has been isolated which still shows the open reading frame of the original gene (Rothermel and Günther, own unpubl. data).

A few chromosomal mapping data of hsc70 genes have been reported. A human hsc70 pseudogene is supposed to map to the X-chromosome[54], and in the rat pseudogene *Hsc70-ps1* has been localized to chromosome 2 (own unpubl. data).

GRP78

The glucose-regulated protein of about 78 kDa (grp78) is identical to the binding protein (BiP)[55], which had been detected by its function of binding immunoglobulin heavy chains in pre-B cells[25]. Grp78/BiP is assigned to the hsp70 family on the basis of the high degree of sequence similarity. The *GRP78* gene contains 7 introns. It maps to chromosome 9q34 in man[28,72] and chromosome 2 in the mouse[26,32]. In cattle it is assigned to syntenic group U16 (ref. 71). The respective syntenic groups are conserved. The sequence has been described in man[72], rat[55], Chinese hamster[73] and partially in the mouse[25]. The coding part of the gene begins with a

leader sequence and ends with a KDEL motif, identifying grp78 as an endoplasmic reticulum (ER)-resident protein.
The gene product is indeed found in the ER. Expression is constitutive and enhanced by glucose deprivation, inhibitors of glycosylation, SH-reducing agents, the Ca ionophore A23187 and hypoxia, but not by heat shock.
A processed pseudogene of *GRP78* has been described in man[72].

Hsp70.2 and *Hst70*

The *Hsp70.2* gene has been identified in the mouse because of its testis-specific expression, which is restricted to premeiotic germ cells[84]. The gene maps to chromosome 12 (ref. 32). The cDNA sequence has been reported[84]. The gene product is identical to the p70 protein described by Allen et al.[2].
The *Hst70* gene of the rat[35,81] shows the same expression pattern as *Hsp70.2*, and its sequence presents a high degree of similarity to *Hsp70.2*, which is 99.4% at the protein level. *Hsp70.2* and *Hst70* are assumed to represent orthologous genes. The rat gene has been shown to lack introns and to be not heat inducible.
The orthologous human gene has not yet been identified. According to Hunt et al.[32] it is supposed to map to chromosome 14.

PBP74 and *Mot-1*

In the course of studies on antigen presentation proteins of 72 to 74 kDa have been found which reacted with anti-hsp70 antibodies[9]. The human gene encoding the 74 kDa protein, *PBP74*, has been isolated and sequenced[11]. The cDNA includes a 'presequence' of 46 codons.
PBP74 encodes a protein of 679 amino acids. The gene product, which is found in cytoplasmic vesicles, is expressed in a constitutive manner and is not heat inducible[11]. Its role in antigen presentation has not been shown.
The deduced coding sequence of the orthologous mouse gene[11] shows 99% similarity to the human gene.
A mouse gene has been identified, which controls mortality of cells and belongs to the hsp70 family on the basis of cDNA sequence similarity[77]. The similarity of the deduced amino acid sequence was higher to the yeast mitochondrial HSP SSC1 than to mouse hsc70. The gene, *Mot-1*, is not heat inducible. The gene product of 679 amino acids, $p66^{mot\text{-}1}$ or mortalin, is found in the cytosol. Injection of anti-mortalin antibodies into senescent fibroblasts prevented death and enabled the cell to resume division. Mortalin does not appear to bind ATP as other hsp70 molecules do.
The nucleotide sequence of the mortalin gene shows very high similarity to mouse *Pbp74* (ref. 11); only 2 exchanges are found in the 5'utr and none in the 3'utr. Similarity in the coding part of *Pbp74* and *Mot-1* is 99.6% for the nucleotide and deduced amino acid sequences. Thus the sequences are very closely related and could represent alleles of the same gene.

HSP70RY (HSPA4)

The *HSP70RY* gene[15], identified in a human cDNA library, shows relatively low sequence similarity to *hHSP70-1* (fig. 2, table 2). It maps to chromosome 5q31. The gene product is a cytosolic protein of 701 amino acids, which is not heat inducible.

STCH

The stress/chaperone gene, *STCH*[58], has been identified in a human cDNA library. The sequence contains a hydrophobic leader and a 50-codon insertion in the ATP-binding domain; it lacks a large part of the carboxy terminus typical of hsc70 genes, and the protein resembles a truncated hsc70/grp78 protein. Sequence similarity is about 33% to the *hHSP70-1* and 43% to the *hGRP78* encoded proteins and is restricted to the ATP-binding domain. (The sequence is therefore not included in fig. 2 and table 2). The protein has ATPase activity and is found in the microsomal cell fraction. It is constitutively expressed and inducible by the calcium ionophore A23187, but not by heat shock.

Structure of the multigene family

The structure of hsp70 proteins has been mainly extrapolated from data obtained by biochemical, crystallographic and molecular modeling analysis. A general picture has emerged for hsp70 and hsc70. A N-terminal, proteolytically resistant ATP-binding domain of about 44 kDa is followed by a peptide-binding domain and a 10 kDa domain at the C-terminus[52,79]. The peptide-binding area resembles the peptide-binding domain of Mhc class I molecules[16,62]. It is to be expected that some of the newly detected members of the hsp70 family deviate from this general picture.
The promoter of most hsp70 genes is compact and contains the essential elements in a region of about 200 nucleotides upstream of the 5'utr, which itself usually encompasses about 200 nucleotides. The characteristic element of the promoter is the often repeatedly occurring heat shock element (HSE), which is essential for heat inducibility[52].
An interdependent regulation of hsp70 genes is provided by the common heat inducible transcripton factor HSF which binds to HSE. A few instances of apparent inverse regulation of expression are known, e.g. for *HSP70* and *GRP78* (ref. 52) and for *Hsp70.2* and *Hsc70t* in the testis (see above).

The hsp70 multigene family was defined originally by the size of its gene products of about 70 kDa, and, more stringently, by significant sequence similarities between the genes and proteins. The hsp70 genes are dispersed in the genome, but some are found as groups of two to three closely linked genes.

The members of the hsp70 multigene family can be distinguished by several parameters.

1) Ubiquitous vs tissue-specific expression. Most hsp70 genes can be or are expressed in each cell, but some, like *mHsp70.2* and *mHsc70t*, are detectable only in certain tissues.
2) Constitutive vs stress-induced expression. At least three types of expression can be distinguished: a) strictly heat-inducible hsp70, e.g. *hHSP70-2*, *hHSPA6*, *hHSPA7*; b) cell-cycle dependent and also heat-inducible hsp70 ('hsp70×' [ref. 61]), e.g. *hHSPA1*; c) constitutively expressed and less stress-regulated hsp70 genes, e.g. *HSC70*. The distinction of strongly heat-inducible and mainly constitutively expressed hsp70 genes is also reflected by the genomic organization, since the heat-inducible hsp70 genes do not contain introns. Further subdivision is possible on the basis of diversity of hsp70 inductors, e.g. heat shock versus glucose starvation.
3) Subcellular expression. The hsp70 genes differ with respect to the subcellular localization of their products. They can be expressed in a) the cytosol, nucleus and nucleolus (*HSP70-1*, *HSP70-2*, *HSC70*), b) the ER (*GRP78*) or c) mitochondria (*GRP75*, *P71*, see below). The subgroups of cytosolic and ER-resident hsp70 proteins differ in their coding sequences by characteristic motifs, which can be traced back phylogenetically to bacteria[24].
4) Hsp70 proteins might also turn out to become discernible by their peptide binding preference or functional specificity[4,19,70].

For practical purposes distinction of individual genes is possible on the basis of gene-specific sequences, e.g. in the 3'utr, which allow the construction of specific probes and primers. In several cases hsp70 genes can be distinguished by the different length of the mRNA. At the protein level SDS gel electrophoresis or 2-dimensional analysis are used to separate the proteins. Some monoclonal antibodies appear to be specific for a given hsp70, others cross-react with different hsp70.

Size of the gene family

The human hsp70 family encompasses at least ten functioning genes, for which detailed sequence information is available (table 1, fig. 2). In addition, two mitochondrial proteins, GRP75 (ref. 51) and P71 (ref. 39), have been assigned to the hsp70 family on the basis of their reactivity with anti-hsp70 and anti-DnaK antibodies, respectively; sequence data are lacking.

The size of the hsp70 multigene family has been studied by different approaches.

1) Analysis of somatic cell hybrids. Somatic cell hybrids, in which human chromosomes segregate, have been tested for induction of hsp70 protein after heat shock. Heat-inducible hsp70 genes were found to be present on chromosomes 6p21, 14q22-24, 21 and at least one further chromosome[21,27]. Southern blot analysis with the same somatic cell hybrids confirmed the presence of hsp70 genes on chromosomes 6, 14, one further chromosome, but hybridization with chromosome 21 failed under the stringency conditions chosen[27]. The genes on chromosome 6 have been identified in the meantime as *HSP70-1* (*HSPA1*), *HSP70-2* and *HSP70-Hom*[66], the other genes, e.g. on chromosome 14q22-24 (*HSPA2* according to ref. 45), are not yet further characterized. A gene possibly orthologous to the hsp70 gene on chromosome 14q has been described in cattle (*HSP70-3* on bovine chromosome 10)[22].
2) Southern blot analysis. On the basis of hsp70-cross-hybridizing fragments the existence of at least 10 genes or pseudogenes has been postulated in man[54]. In the rat 25 to 30 hsp70-like sequences were detected with a *Hsp70-1* probe[82]. Similar analyses with an *Hsc70* probe identified about 25 cross-hybridizing fragments in the rat[57]. The interpretation of these data is highly dependent upon the stringency conditions applied.
3) Screening of a library. In the rat at least 20 different hsp70 clones were isolated from a genomic library with a *Hsp70-1* probe[82], and 24 independent clones were obtained from a genomic library with a *Hsc70* probe, a large fraction of them being processed pseudogenes[69].

In pig a hsp70 gene in addition to the Mhc-linked hsp70 genes has been mapped to chromosome 14q by fluorescent in situ hybridization[56].

Evolutionary aspects of hsp70 genes

The hsp70 coding sequences have been highly conserved during evolution[24,29,31]. Homologs can be traced back to bacteria (dnaK)[31]. The degree of sequence similarity between the different members of the hsp70 family of vertebrates is shown in figure 2 and table 2 for the hsp70 genes of man. Interspecies comparisons of deduced amino acid sequences are summarized in figures 3 to 6 and tables 3 to 5 for hsp70 family genes, for which sequences of the orthologous genes or genes of the same type (hsc70, grp78) are known in more than two species. The high degree of sequence conservation extends also to hsp70 genes of trout[34], chicken[53] and Xenopus[3], for which only limited data are available.

Sequence similarity is higher in the N-terminal domain than in the C-terminal part, being least in the final

10 kDa region of the hsp70 molecule[52,79]. Sequence similarity between the various members of the hsp70 family in a given species (fig. 2) is less than found for orthologous or putative orthologous genes at the interspecies level (figs 3–6).

The extent of sequence conservation of the coding region is similar at the amino acid and nucleotide levels. This implies that there is a constraint on 3rd position diversity of codons[31]. It has also been noted that codon usage in hsp70 genes is biased[42].

Screening for polymorphisms of given hsp70 genes confirms the high degree of sequence conservation. Only few exchanges and RFLP are found in the Mhc-linked hsp70 genes, which have been studied most intensively in this respect[49,64,68].

Conservation of the hsp70 family is also observed at the level of chromosomal organization, since similar synteny groups are found in various species. The best studied group are the Mhc-linked hsp70 genes (fig. 1).

The occurrence of three genes of the hsp70 multigene family in the class III region of the Mhc between the genes for complement component C2 and cytokine TNFα has been reported for man, rat and mouse. In the goat at least one hsp70 gene has been localized into this region[5]. In pig three closely linked hsp70 genes map to the Mhc on chromosome 7 (ref. 56), and in cattle at least two hsp70 genes have been identified on the Mhc-bearing chromosome 23 (ref. 22). In the frog, two to three hsp70 genes are linked to the Mhc[65]. Thus the presence of hsp70 genes inside the Mhc is detectable in various species belonging to different classes of animals – mammals and amphibia, which diverged about 350 million years ago. The three hsp70 genes in the Mhc have arisen by gene duplications, which in the case of man and rat had presumably occurred before the separation of primates and rodents about 70 million years ago[78]. Two of the genes, *HSP70-1* and *HSP70-2*, are identical at the protein level in man[48] and rat[78], indicating the occurrence of gene homogenization events in both species or their progenitors. Interestingly, the orientation of the Mhc-linked testis-specific gene is opposite to the neighboring, ubiquitously expressed and heat-inducible hsp70 genes.

From the amino acid sequence of the hsc70 peptide-binding domain a three dimensional structure has been constructed, which is similar to the peptide-binding groove of Mhc class I molecules[16,62]. This relationship gave rise to the hypothesis that the peptide-binding domain of class I Mhc molecules is derived phylogenetically from hsp70 genes (for discussion see 16).

It remains to be established whether the presence of hsp70 genes inside the Mhc indeed reflects common descent, is conserved because of functional interaction or represents a 'frozen' accident of evolution.

A further example of two closely linked hsp70 genes are *HSPA6* and *HSPA7* in man[36], which most likely arose by tandem gene duplication or translocation of tandem genes. An orthologous cluster appears to be present in cattle[22].

A characteristic of the hsc70 subfamily is the occurrence of many processed pseudogenes. It is assumed that transcripts expressed during early embryonic development are, in rare cases, reverse-transcribed and integrated into the genome. Processed pseudogenes have also been described for grp78, but not for heat-inducible hsp70 genes.

In poikilothermic species variability of the expression of heat-inducible hsp70 genes has been observed, which could reflect natural selection processes. In various lizard species the degree of activation of hsp70 genes appears to be related to the environment[75]. In certain species of desert fishes (Poeciliopsis) different isoforms of heat-inducible hsp70 protein can be detected by two-dimensional analysis[80]. This diversity appears to be due to different genes and not to modifications of the gene products.

A comparison of hsp70 protein sequences by Gupta et al.[24] has led to the detection of characteristic sequence 'signatures' in the hsp70 multigene family. Thus, all conventional hsp70 proteins contain a GIDLGT-TYSCV-like sequence in the beginning of the coding part. Particular motifs distinguish the cytosolic hsp70 from the ER-resident hsp70, which are supposed to have arisen by gene duplication early during evolution of eukaryotic cells[24]. Recently genes have been isolated (*PBP74*, *HSP70RY*, *STCH*), which are homologous to hsp70, but contain or lack certain sequences and present characteristics uncommon for the so far known hsp70 genes of vertebrates. One could speculate that further, 'new' members or subfamilies of the hsp70 multigene family might exist.

Note added in proof

Two publications have appeared since submission of the review, which add information to *HSP70RY* and *Hsp70.2/HSPA2*.

1) Dyer, K. D., and Rosenberg H. F., HSP70RY: Further characterization of a novel member of the hsp70 protein family. Biochem. biophys. Res. Commun. *203* (1994) 577–581.

The expression of the *HSP70RY* gene in several human cell lines and the existence of a homologous gene in the mouse are shown.

2) Bonnycastle, L. L. C., Yu, C.-E., Hunt, C. R., Trask, B. J., Clancy, K. P., Weber, J. L., Patterson, D., and Schellenberg G. D., Cloning, sequencing, and mapping of the human chromosome 14 heat shock protein gene (HSPA2). Genomics *23* (1994) 85–93.

The sequence of an intronless human hsp70 gene (accession number L26336) is reported, which is constitutively expressed in most tissues, particularly in the testis, maps to chromosome 14q24.1 and shows strong sequence similarity (98% at the amino acid level) to the mouse *Hsp70.2* and rat *Hst70* genes. It is interpreted to be the human homolog of these genes and to correspond to the hsp70 gene identified previously on chromosome 14 (*HSPA2*, refs. 27, 45). The expression pattern of the mouse *Hsp70.2* gene is reported to be not restricted to testis as assumed before (see ref. 84).

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