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HEAT SHOCK PROTEINS GENES: A REVIEW

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Abstract

A large part of the data published on human Hsp70 family deals with the major stress-inducible members of the family,Hsp70-1a and 1b (called Hsp70-1). Hsp70-1a and-1b are encoded by closely linked, stress-inducible and intronless genes, HSPA1A and HSPA1B, that reside in the major histocompatibility complex (MHC) class III genes are located on chromosome 6 (6p21.3). It covers 700 kb and contains 61 genes. The gene cluster is the most gene-dense region of the human genome. They are basically similar with those of other animals. The functions of many genes are yet unknown. Many retro elements such as human endogenous retrovirus (HERV) and Alu elements are located in the cluster. The region containing genes G11/C4/Z/CYP21/X/Y, varying in size from 142 to 214 kb, is known as the most complex gene cluster in the human genome.

Key words: Heat, shock, proteins genes

Introduction

The genes responsible for producing heat shock proteins are called the major histocompatibility complex (MHC) is a set of genes that code for cell surface proteins essential for the acquired immune system to recognize foreign molecules in vertebrates, which in turn determines histocompatibility. The MHC gene family is divided into three subgroups: class I, class II (the structure and functions of which are well defined in immune response), and class III.

The first descriptions of the MHC were made by British immunologist Peter Gorer in 1936 (Klein, 1986).

MHC gene families are found in all vertebrates, though they vary widely. In humans, the MHC region occurs on chromosome 6, between the flanking genetic markers MOG and COL11A2 (from 6p22.1 to 6p21.3 about 29Mb to 33Mb on the hg38 assembly), and contains 224 genes spanning 3.6 megabase pairs (3 600 000 bases), about half have known immune functions (MHC, 1999). The gene cluster was discovered when genes (specifically those of complement components C2, C4, and factor B) were found in between class I and class II genes on the short (p) arm of human chromosome 6. It was later found that it contains many genes for different signaling molecules such as tumor necrosis factors (TNFs) and heat shock proteins (Wu *et al.*, 1985).

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More than 60 MHC class III genes are described, which is about 28% of the total MHC genes (224) (HMC, 1999).

Class III is a group of proteins belonging the class of major histocompatibility complex (MHC), molecules have physiologic roles unlike classes I and II, but are encoded between them in the short arm of human chromosome 6. Class III molecules include several secreted proteins with immune functions: components of the complement system (such as C2, C4, and B factor), cytokines (such as TNF-α, LTA, and LTB), and heat shock proteins.

Unlike other MHC types such as MHC class I and MHC class II, the structure and functions of which are well defined in immune response, MHC class III are poorly defined structurally and functionally.

Only few of them are actually involved in immunity while many are signaling molecules in other cell communications. They are mainly known from their genes because their gene cluster is present between those of class I and class II (Gruen, and Weissman, 2001).

Heat shock proteins (HSP) are considered as molecular chaperone proteins, which have the ability to promote the right folding of some proteins reversibly, the formation, the trans-membrane transport of these proteins, and prevent the formation of non-specific protein aggregates (Castro *et al.*, 2013, Singh *et al.*, 2017).

Increased expression of stress proteins is one of the most conserved stress response mechanisms. Elevated synthesis of a few proteins following exposure to heat or other stresses occurs in all organisms studied, ranging from prokaryotic bacteria to mammals (Latchman, 1999).

Many stress proteins are encoded by genes encoding proteins with similar but special features. Molecular chaperones belong to several highly conserved ubiquitously distributed families of proteins, role of which is to control protein quality and regulate protein structures in cells, as ATPase (Macario *et al.,* 2004).

All chaperones share the ability to check different protein structures in the packed cellular environment (Ellis, 2006). They recognize of unfolded and misfolded proteins. Stress proteins belong to a multigene family and range in molecular size from 8 to 150 kd (David *et al.,* 1999).

When an animalsare exposed to any stress condition (may be toxins, low oxygen levels, physical, physiological, nutritional, chemical, psychological and environmental), the metabolism tends to synthesize more of HSP, to maintain normal cellular function within the body and help the cell to recognize the damaged protein for it repair (Ashley, 2018). And the HSP has an essential role in the conservation of cellular life as they inhibit apoptosis (Mullins *et al.,* 2016).

Heat stress occurs when animals are exposed to temperature beyond the upper critical level causing an increase in heat production in the animal body (Smruti and Tapan, 2014).

When stress occur, increased body temperature, can affect cattle by raising cortisol levels. Cortisol, which is the main stress hormon in cattle. At stress, a cell will stop or slow down some of its functions, which includes DNA, RNA, and protein synthesis (Bhat *et al.,* 2016). Because of this stress, HSP are synthesized and released into the body and their role in maintaining normal cellular function in the body. There three most common of the HSP family are Hsp60, Hsp70, and Hsp90 (Lindquist and Craig, 1988).

Because of these induced responses to stress, understanding the relationship between cortisol and HSP could help in predicting animal stress response. The HSP family has several functions, one of which is to "regulate protein folding, transport, translocation and assembly" as well as refolding proteins (Wang *et al.,* 2014).

Literature review

According to the published sequences, Hsp70-1a (NM_005345) and Hsp70-1b (NM_005346) share all but two (E110D, N499S) of their 641amino acids being more than 99% identical (Mads et al., 2007). During various stress conditions, both Hsp70-1 genes are activated by binding of a stress-inducible transcription factor, heat shock factor 1 (HSF1), to heat shock elements (HSE) found in multiple copies in the upstream regulatory regions of the genes (Milner and Campbell, 1990; Anckar and Sistonen, 2007). During normal conditions, Hsp70-1 proteins are expressed in a cell type and cell cycle dependent manner accumulating in G1- and S-phase (Milarski and Morimoto, 1986; Taira *et al.,* 1997). Accordingly, Hsp70 promoters also contain several binding sites for basal transcription factors such as TATA factors, CCAAT-box-binding transcription factor and SP1 (Greene *et al.,* 1987). The basal expression of HSPA1A and HSPA1B mRNAs differs lightly in most tissues, with somewhat higher expression of HSPA1A in most tissues and cell types.

Supporting the major role for Hsp70-1in protection against external stresses, mice deficient of the equivalent murine proteins, Hsp70.1 or Hsp70.3, are viable and fertile, but Hsp70.1 deficient mice display increased sensitivity to pancreatitis, UV light (epidermis), osmotic stress (renal medulla) and ischemia (brain), and reduced capacity to acquire resistance to TNF-induced liver toxicity and inflammatory shock after preconditioning with heat (Lee *et al.,* 2001). Furthermore, cells lacking either Hsp70.1 or Hsp70.3 display increased sensitivity to heat (Huang *et al.,* 2001). HSP70-1is an intronless gene located on chromosome 23 of bovine (BTA 23) and has 1926 nucleotides (Nitin *et al.,* 2010).

Hsp70-1

The molecular characterization of HSP70-1 gene in goat revealed that at nucleotide level, there was 96–99% similarity with that of sheep, cattle, and buffalo whereas 95-100% similarity at amino acid level (Gade *et al.,* 2010). Sequence analysis in the same study reported that there is1926-bp-long open reading frame of HSP 70-1 gene encoding 641amino acids in goat, as reported in cattle. The 5' flanking region of HSP 70 gene in Zebu cattle of Hariana breed was characterized for cisacting sites which, when compared with that of Taurus cattle revealed that promoter variation may not be the source of the difference in expression level of HSP70 (Archana *et al.,* 2017).

The amino acid sequence analysis of HSP70 in buffalo lymphocytes showed 98% identity with *Bos taurus*, *Bos indicus*, Yak, *Capra hircus* and 90–95% identity with *Camelus dromedaries*, *Felis catus*, *Canis familiaris*, *Sus scrofa*, and *Homo sapiens* and it reported 1,926bp long open reading frame of HSP70 gene

encoding 641 amino acids in buffalo (Pawar *et al.,* 2014).

Hsp70-1t

The gene encoding Hsp70-1t (HSPA1L) is intronless and located in the same MHC class III region as HSPA1A and HSPA1B (Goate *et al.,* 1987). The protein is 91% identical to Hsp70-1a, the major variation being in the C-terminal end. The HSPA1L gene contains no HSE in its promoter region and it is constitutively expressed at high levels in testis and at very low levels in other tissues. The function and transcriptional regulation of Hsp70-1t are currently unknown.

Heat shock proteins also provide support for steroid nuclear receptors and aide in steroid response (Kovacs *et al.,* 2005). Nuclear receptors are needed for normal function of cortisol, estrogen, progesterone, and testosterone. Those steroids affect stress response and reproductive success in mammals. Therefore, improving steroid function could improve cattle efficiency and the HSP family is integral in this process of steroid response.

Single nucleotide polymorphisms in the heat shock protein 70 (Hsp70) gene have been associated with calving percentage, and Julian calving date in spring-calving crossbred Brahman cows (Rosenkrans, *et al.,* 2010).

In the previous studies, polymorphisms in the Hsp70 gene are related to milk quality and calving percentage (Ashley, 2018).in a study focused on SNPs in the bovine cytochrome p450 region and its effects on cow productivity, four SNPs for cytochrome p450 were related to cow productivity.

Evaluated of significant differences in birth weight and weaning size and lower lifetime calving rate. Their results suggested that SNP in cytochrome P450 gene were associated with cattle productivity (Sales *et al.,* 2013).

HSPs are classified into several families, including *hsp100, hsp90, hsp70, hsp60* and other HSPs with low molecular masses (Stensløkken *et al.,* 2010).

Hsp70 and hsp90 family was widely studied in eukaryotes. Hsp90 family has two major cytosolic subtypes such as hsp90-alpha and hsp90-beta. Well-recognized members of the hsp70 multigene family are two closed cytosolic forms: cognate hsc70 and inducible hsp70 (Lindquist and Craig, 1988).

Diversity

MHC class III genes are similar in humans, mouse, frog (*Xenopus tropicalis*) and gray short-tailed opossum. But not all genes are common. For example, human NCR3, MIC and MCCD1 are absent in mouse. Human NCR3 and LST1 are absent in opossum (Deakin *et al.,* 2006). But birds (chicken and quail) have only a single gene, which codes for a complement component gene (C4) (Shiina *et al.,* 2004). In fishes, the genes are distributed in different chromosomes (Sambrook *et al.,* 2005).

Heat Shock Protein 60

Members of the HSP60 group are large oligomeric complexes that are essential for growth, and are induced by forms of cellular stress that cause protein denaturation (Frydman and Hartl, 1994).HSP60 family is important in protein stability. Although gene expression of HSP60 in cattle has been reported as higher in the summer months than in winter months, genetic variants in the bovine HSP60 have not been related to cattle productivity (Bhat *et al.,* 2016). In the human reproductive tract, concentrations of the protein were related to genotype at Hsp60 SNP sites and have been related to fertility success (Lev-Sagie *et al.,* 2009). Because of this evidence, this particular heat shock protein might be of interest moving forward.

Heat Shock Protein 70

The most abundant member of the heat shock protein family, Hsp70, is essential for cell survival at high temperatures and in normal cellular physiology (Lindquist and Craig, 1988). The HSP70 family is necessary for protein synthesis, translocation, and folding. Its size is 68-73 kDa (Pockley *et al.,* 2008). It is encoded by a single exon of the Hsp70 gene, the open reading frame to this gene is 1926 bp approximately. The protein contains 641 amino acids (Gade *et al.,* 2010). Functions of HSP70 include acting as molecular chaperones when an organism is exposed to stressful pathological or environmental conditions, like viral infection, fever, hypoxia, etc. (Ohtsuka and Hata, 2000). A molecular chaperone will protect cells against exposure to lethal heat shock, which can denature proteins, by binding to the denaturing protein and stabilizing it, which preserves its activity. In a study conducted on Tharparkar cattle to discover possible differences in thermo-tolerance, it was discovered that the allelic variants of HSP70 gene were associated to heat tolerability (Bhat *et al.,* 2016). Results from a study conducted on crossbred Brahman-influenced cows, where a deletion of cytosine was detected at base 895, discovered that cows that were homozygous for the deletion had a significantly lesser calving percentage when compared with heterozygous or homozygous cytosine cows (Rosenkrans *et al.,* 2010). These studies support that the gene is important in thermo-tolerance in cattle of different breeds, and suggest that cattle managed under stressful conditions such as heat due to climate change would likely have altered HSP expression. Although

HSP70 is heavily induced by stress, "non-stressed" cows were found to have circulating concentrations of plasma HSP70, which might indicate that it is produced in preparation of combating increased stress (Kristensen *et al.,* 2004).

Heat Shock Protein 90

The HSP90 family are important in the formation of the steroid receptor complex. Highly conserved members of the heat shock protein 90 family are important in carrying out biological functions in the cytosol and endoplasmic reticulum of eukaryotes (Frydman and Hartl, 1994). Polymorphisms in Hsp90 of sheep has been related to heat stress response (Marcos-Carcavilla *et al.,* 2010). Since cellular HSP90 is required for steroid biological response, it could be useful to study polymorphisms in the bovine Hsp90 gene since steroid function is critical for cattle reproduction, and response to stress.

Synthesis of heat shock proteins

In eukaryotic organisms the expression of heat shock protein messenger RNA-s is mediated by a family of transcription factors, called heat shock factors. Heat shock factor I (HSF-I) plays a major role in heat shock response, while other members of the family are activated after prolonged stress, or participate in processes such as embryonic development, or cell differentiation. In resting cells HSF-I is complexed with various heat shock proteins, such as with Hsp70, or with Hsp90. After stress, damaged proteins become abundant and liberate the heat shock factor from its Hsp70/Hsp90 complexes. This process sets the stage for the trimerization, nuclear translocation and phosphorylation of HSF-I, which are all pre requisites for its binding to the special nucleotide segments, called heat shock elements, in the promoter region of heat shock protein genes. All these steps are modulated by numerous co-chaperones of the major heat shock proteins, Hsp70 and Hsp90, and most probably by other proteins as well (Morimoto, 1999). The nucleosomal structure of the DNA-segment containing the heat shock element is reorganized by a special protein-machine, called the GAGA-factor (Tsukiyama *et al.,* 1994). Interestingly, many heat shock protein genes recruit an active DNA-dependent RNA polymerase II even in the absence of heat shock factor. This "pausing polymerase" transcribes a small segment of the gene, but becomes arrested by its binding to the initial complex of TATA-binding general transcription factors. Binding of the heat shock factor-trimer to the heat shock element sets the polymerase free, which can proceed to complete the transcription of the heat shock RNA. During stress, all the subsequent steps of protein synthesis (RNA splicing, nuclear export and translation itself) are blocked. Heat shock RNA-s developed various strategies to circumvent these problems. Primary transcripts (such as Hsp70 RNA) usually do not contain introns, or the open reading frame encoding the protein itself begins after the intron and the initialization may proceed from the intron as well (*e.g.* Hsp90). Recognition of heat shock RNA-s also utilizes special routes avoiding those translational initialization factors, which became inactivated during stress.

Chaperones as general helper of cell survival

Molecular chaperones are responsible for the "conformational homeostasis" of cellular proteins. When the homeostasis of the host organism is perturbed, an increased capacity of the chaperones is highly advantageous. Many of the perturbations (such as alcohol, other poisons, sunburn, anxiety, etc.) may induce the synthesis of these chaperone proteins per se, but in case of bacterial and viral infections the developing fever also helps this process. Several common drugs, such as aspirin, also promote the induction of heat shock proteins (Jurivich *et al.,* 1992). Ischemia and the consecutive oxidative damage of reperfusion are also common perturbations in higher organisms. Since Currie *et al.,* (1988) have shown that the induction of molecular chaperones, most notably Hsp70, may prevent damage to cardiac muscle by both ischemia and reperfusion, molecular chaperones are actively being investigated as possible tools in the treatment of heart attack or stroke. Indeed, transgenic animals, where Hsp70 has been constitutively expressed either in heart or brain, are much less likely to develop heart attack or stroke. Induction of heat shock proteins is also beneficial in transplanted organs, where moderate heat treatment reduces transfer-damage and the risk of organ rejection (Perdrizet *et al.,* 1993).

Chaperones in aging and in neurodegenerative diseases

Aging is frequently described as a consequence of an impaired function of repair processes (immune system, DNA-repair, elimination of free radicals, etc.). Molecular chaperone catalyzed refolding of damaged proteins may well be one of these crucial repair processes. In agreement with this hypothesis, aged organisms contain an increased amount of misfolded proteins, and the induction of Hsp70 is impaired in both aged rats and humans. On the contrary, better induction of heat shock proteins leads to an increased life expectancy in yeast, Drosophila or *C. elegans* (Tatar *et al.,* 1997). Protein damage becomes especially dangerous when it affects neuronal cells, which, generally, cannot renew themselves

by multiple mitotic events. In most of the neurodegenerative diseases, such as in Alzheimer's disease, in Parkinson's disease, in Huntington's disease, in Wilson's disease, in Alexander's disease and in prion-related human syndromes, nerve cells develop massive protein aggregates. These inclusion bodies usually contain various heat shock proteins, such as ubiquitin-tags, the small heat shock protein, Hsp27, Hsp70 and Hsp90 (Mayer *et al.,* 1991). Heat shock protein co-aggregation may reflect the fight of these chaperones against the aggregation process. In accordance with this view, over expression of Hsp70, or other heat shock proteins protects Drosophila-s from neuro degeneration in Huntington disease-like polyglutamine-induced aggregation.

Chaperones and the immune response

Molecular chaperones are one of the most conserved proteins in living organisms (Lindquist, 1986). Invading bacteria experience major changes in their environment when entering their host. These changes and the activation of defense mechanisms (depletion of nutrients, pH changes, osmotic changes, digestive enzymes, peroxides, superoxides and an increase in temperature) induce numerous heat shock proteins in bacteria, among which some are also expressed on the bacterial surface. Because of their conservative structure, these bacterial heat shock proteins, especially the bacterial homologue of Hsp70 become a common recognition signal, and therefore provoke a general, high-capacity immune response (van Eden and Young, 1996). There are at least two dozen infectious diseases in which immune responses to heat shock proteins have been reported, including tuberculosis, leprosy, legionnaire's disease, Chagas's disease, lyme disease, chlamidyal infections and Q fever. In some unfortunate cases (such as in rheumatoid arthritis, in lupus erythematosus, in multiple sclerosis and in insulin dependent diabetes mellitus, IDDM) certain proteins of the host organism resemble some epitopes of these bacterial heat shock proteins. In these patients the common, antibacterial immune response attacks the cells bearing these host-proteins, and a severe autoimmune response develops. Vaccination with modified epitopes of a bacterial Hsp70 homologue diminish, and in some cases prevent the development of the disease (van Eden and Young, 1996). Some recent reports raise the possibility that expression of human Hsp60 on the surface of epithelial cells may be one of the initial events of arterial plaque development.

Chaperones as anti-cancer or anti-viral vaccines. As we have discussed before, tumor cells experience a lot of stress (hypoxia, nutrient deprivation, etc.). Stressful conditions induce heat shock protein synthesis, and several types of cancer cells expose heat shock proteins on their surface (Multhoff and Hightower, 1996). These cells are recognized and killed by a special class of T lymphocytes, the -cells. There are ongoing clinical trials, where tumor cells are exposed to various types of stresses to prime them for a subsequent immune attack. At the beginning of the eighties, several research groups isolated molecular chaperones (Grp94 and Hsp90) as tumor specific transplantation antigens, *i.e.* surface proteins, which provoked a highly specific immune response against a certain type of tumor. When painstaking research efforts made it clear that neither the primary structure, nor major posttranslational modifications were different in chaperones coming from normal versus malignant tissue, researchers in the field were puzzled. Pramod Srivastava first suggested that the differences may come from the peptides, which are carried by the chaperones. In a 1994 hypothesis paper he proposed the existence of a relay-type mechanism, where various cytoplasmic chaperones, such as Hsp70 and Hsp90 as well as their counterparts in the endoplasmic reticulum, such as Grp94, give each other the peptides and help their presentation to the MHC-I complex, specialized to present the "self " antigens to the immune system. MHC-I molecules induce a cytotoxic T-cell response, which is a fast and local immune attack, against a cell expressing "false" self-antigens (e.g. after a viral infection). This may significantly enhance the efficiency of the MHC-II immune response, which is based on helper T lymphocytes, and generates a slow and general immune attack. According to Srivastava's hypothesis, chaperones carrying tumor-peptides may

Others of the chaperons are summarized in the table below:

Name	Molecular size (kd)	Location	Remarks
Ubiquitin	8	Cytosol/nucleus	Facilitates targeting and removal of proteins denatured by stress
Hsp10	10	Mitochondria	Cofactor for Hsp60
Low (small)–	20 to 30	Cytosol/nucleus	Some may be responsible for regulating the cellular
molecular weight hsps			cytoskeleton and migration, and others regulate vascular
			tone and vessel wall remodeling
Hsp56	56	Cytosol	Binds and stabilizes the steroid hormone receptor complex

enter the MHC-I pathway of macrophages and dendritic cells and activate a cytotoxic immune response against a "foreign" antigen. Later studies proved that each major step of the original hypothesis was correct (Srivastava *et al.*, 1998). Chaperone-induced "cross-priming" (channeling of the MHC-II peptides to the MHC-I presenting pathway) is an interesting phenomenon by itself, but its real importance lies in the clinical application. Srivastava's discovery made it possible to induce an anti-tumor immune response by vaccination. Isolation of peptide-chaperone complexes from the primary tumor and administration of the complex to the dendritic cells as a vaccine provokes an efficient anti-tumor immune response and is the subject of ongoing clinical trials (Srivastava *et al.*, 1998). In the future the establishment of common important peptide-antigens in certain type of tumors, such as prostate cancer, may alleviate the need for time consuming and costly "personalized vaccines". Similar approaches may be used to provoke an anti-viral immune response by the help of viral-specific peptide-chaperone complexes.

The relationship of heat shock proteins to semen quality

Semen quality is of traits importance to the bovine industry as sub-fertile bulls can be economically expensive (Kastelic and Thundathil 2008). Temperature is the most important parameters affecting semen quality (Kunavongkrit *et al.*, 2005). Thus, the high temperature of the scrotum in males leads to deterioration of sperm characteristics (Taylor and Bogart 1988).

Heat shock proteins family found in seminal plasma with complex mixture of secretions, which the cell protecting it from harmful effects of high temperature (Pockley 2001; Rajoriya *et al.*, 2014).

The best known of the HSP families is the HSP 70 family, so it is present in the reproductive tissues and has important roles (Rynkowska *et al.*, 2011).

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