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REVIEW

Mycoplasma Heat Shock Proteins and Their Genes

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Abstract—Mycoplasmas (class *Mollicutes*) are the simplest prokaryotic organisms capable of independent reproduction and are therefore considered a natural model of a "minimal" cell. The systems retained by mycoplasmas in the course of their reductive evolution may be fundamental for all cells. This is the first review to summarize and systematize available information concerning the genes encoding the heat shock proteins (HSP) in mycoplasma. An attempt is made to analyze the presence or absence of the mycoplasma analogues of the major bacterial chaperones and proteases, which determine cell resistance to stresses, as well as protein homeostasis under optimal growth conditions. The data on the mechanisms for the regulation of transcription of the HSP genes in mycoplasma are presented. The properties and functions of the best-characterized mycoplasma HSP, namely, DnaK, DnaJ-like proteins, the GroEL/GroES system, ClpB, and small heat shock proteins (sHSP), are discussed.

Keywords: mycoplasmas, "minimal" cell, stress, genomes, genes, transcription regulation, chaperones, proteases

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At sublethal temperatures both prokaryotic and eukaryotic cells stop the synthesis of most proteins but increase the rate of the heat shock proteins (HSP) synthesis. The HSP have been found in all studied eukaryotes and bacteria. Even mycoplasmas (class *Mollicutes*), the smallest prokaryotic organisms capable of independent reproduction, were not an exception [1, 2]. From the evolutionary point of view, HSP are among the most conservative proteins. While HSP differ in their properties and functions, their major role in the cell is the maintenance of homeostasis and formation of the adaptive responses under stress conditions.

Many HSP are chaperones and co-chaperones. These proteins participate in the folding of the peptide chain of newly synthesized proteins and play a protective role: they prevent protein denaturation and aggregation under stress conditions. Another major group of the HSP are proteases. They are responsible for the degradation of the polypeptides irreversibly damaged by stress factors. The third group of the HSP are regulatory proteins that control transcription, translation, and modification of the polypeptides synthesized under heat shock conditions. The synthesis of some HSP may be induced not only by elevated temperature but also by other stress factors such as a decrease of temperature and addition of oxidants or heavy metal salts. Such proteins belong to the group of general stress proteins [3]. Moreover, the cells of most organisms synthesize small amounts of the HSP under normal conditions [4]. Such HSP include the chaperones that affect protein folding, as well as some proteases.

Classification of the HSP is based on their size. According to their molecular mass (kDa), HSP are divided into several families: HSP100, 90, 70, 60, 40, 33, and small HSP (sHSP). The sizes of the polypeptide chains within these families vary widely; however, their functions in the cells of different organisms are similar [5, 6]. For example, both eukaryotic Hsp70 chaperones including 13 human HSPA proteins [7] with molecular masses from 52 (HSPA13) to 76 kDa (HSPA12B), and prokaryotic Hsp70 homologues including *Escherichia coli* DnaK-like proteins [8] with molecular masses from 62 to 71 kDa belong to the well-studied HSP70 family. The sHSP demonstrate high size diversity. Representatives of this big and heterogenous family (from 10 to 43 kDa) play a variety of functions in the cells [9]. The presence of the alpha-crystallin domain is the structural feature typical of both eukaryotic and prokaryotic sHSP [6, 10].

Mycoplasmas differ from other eubacteria by the absence of the cell wall, by small genome size, and by the low DNA C+G content. Almost all mycoplasmas are obligate parasites. Despite the seeming genome "incompleteness," mycoplasmas are successful pathogens of mammals, birds, insects, and plants [11, 12]. It is suggested that in the course of evolution mycoplasmas lost a significant amount of genetic material of their ancestors (common with lactobacilli) with a resulting decrease in the genome size up to the theoretically calculated minimum sufficient to provide independent reproduction [13]. It was therefore suggested to consider mycoplasmas as a natural model of a "minimal" cell [14]. However, the number of genes

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in this case must depend directly on the environmental conditions. Thus, the absence of the genes coding for the enzymes that are necessary for nucleotide or amino acid synthesis may be explained by the fact that mycoplasmas obtain these compounds directly from the host cell [15, 16].

The scientists who began studies regarding the mycoplasmal HSP were interested in whether these organisms retained a universal system for the heat shock response or it has been deleted as a useless one. The mycoplasmal HSP were discovered in the Institute of Cytology, Russian Academy of Sciences [1]. Using pulse label incorporation technique, the synthesis of nine polypeptides in *Acholeplasma laidlawii* cells was shown to be increased while synthesis of others was suppressed when the temperature increased from 32 to 44°C for 2 h. The maximal increase was observed in case of the 72, 65, 17, and 10 kDa polypeptides. According to the densitometry results, in *A. laidlawii* PG8 heat shock resulted in the concentration of main HSP increasing from 0.8 to 4.2% (72 kDa protein) and from 0.05 up to 4.8% (65 kDa protein). Accumulation of the low-molecular HSP p17 (Hsp20, IbpA) reached 7.2% from the total cell protein. Enhanced concentrations of the HSP in the mycoplasmas maintained for 4 h after the temperature returned to normal values. In the same year, Dascher et al. [2] demonstrated the heat shock response in *A. laidlawii* (strains JA1 and K2) and *Mycoplasma capricolum*. Thus, by 1990 two independent research groups have shown mycoplasmas, as other known microorganisms, to synthesize the HSP in response to heat shock. Since the genomes of mycoplasmas are significantly reduced, the presence of the HSP in their cells demonstrates a fundamental role of these proteins for all cells including the "minimal" cells.

What has been done for mycoplasmal HSP investigation since their discovery? First, more than 40 complete mycoplasma genomes have been sequenced so far, and more than 30 full-size sequences from these genomes have been deposited (not including the genomes that belong to different strains within one species). This made it possible to predict the presence of the ORFs in mycoplasma genomes that corresponded to the genes encoding the putative products with high homology to the known bacterial HSP. An interest to mycoplasma genomes sequencing was due to the fact that their cells are relatively simple and thus may be considered a convenient model for the study of the vitally important cell processes. Second, application of such integrated approaches as the studies of a transcription answer and proteomic analysis of the mycoplasma cells under heat shock made it possible to reconstruct a general picture of the changes that happen in the cells. However, many details of these processes still remain unclear. Third, a set of studies on the interactions of mycoplasma and eukaryotes in the "parasite–host" system revealed many mycoplasma HSP to be strong antigens that induce a response of the host immune system. These data are used for serological investigations [17] and for the development of novel efficient vaccines [18]. Moreover, the structure, properties, and functions of some specific mycoplasma HSP also have been reported. However, not many works in this area appeared in the last 20 years. Among them one could mention the studies on the DnaK [19], TopJ [20], DnaJ [21], ClpB [22], and IbpA [23] proteins. In two of these works [19, 23] electron microscopy was used to determine HSP localization in mycoplasma cells: for the HspA1 protein from *Mycoplasma suis* and for the IbpA (Hsp20) from *A. laidlawii*, respectively.

In this review, all available information regarding the genes encoding the mycoplasma HSP, their transcription regulation, as well as the information regarding the properties and functions of the well-characterized HSP is systematized and discussed.

THE HSP GENES FROM THE FULLY SEQUENCED MYCOPLASMA GENOMES

By early 2013, the genomes of 35 mycoplasma species have been completely sequences and deposited, and the projects for sequencing of several more tens of mycoplasma genomes were declared. These studies made it possible to carry out a detailed comparative analysis of the mycoplasma genes including those encoding the HSP.

The so-called multichaperone network responsible for the state of proteins is crucial for the cell viability both under optimal and stress conditions [9]. The more components this network includes, the more interactions between them take place, and, therefore, the "buffer capacity" of the network becomes higher, allowing the cells to adapt to unfavorable changes in environmental conditions [24]. The HSP are the main components of multichaperone network. Among bacteria, mycoplasmas have the minimal set of the genes encoding the chaperones and proteases [25]. However, the HSP are well represented in the reduced mycoplasma genomes (table).

The number of the genes coding for the analogues of the known bacterial HSP is different in the genomes of different mycoplasma species. Excluding the paralogous genes, only 6 genes for the HSP were found in *Mycoplasma crocodyli, M. haemominutum, M. wenyonii*, and *M. suis*. This is an absolute minimum among the studied species of the class *Mollicutes*. Moreover, the genome of *M. crocodyli* does not contain the gene encoding the homologue of one of the most important bacterial chaperones, the DnaK protein, and the genomes of *M. haemominutum, M. wenyonii*, and *M. suis* do not contain the genes for another important chaperone, Tig trigger-factor, which was found in almost all other mycoplasma species. *Mycoplasma penetrans, M. gallisepticum, M. genitalium, M. pneumoniae, Candidatus* Phytoplasma asteris, and *Candidatus* Phytoplasma australiense each contain 10 genes cod-

The genes encoding the proteins of mycoplasma multichaperone network: chaperones, co-chaperones, proteases, and regulatory proteins participating in the heat shock response

Mycoplasmas/HSP	ClpB	ClpA	ClpX	HtpG	DnaK	DnaJ	GrpE	GroEL	GroES	FtsH (HflB)	DegP (HtrA)	Protease La (Lon)	Hsp33 (HslO)	sHsp (lbpAB)	HrcA (repressor)	Trigger factor
Mesoplasma florum (AE017263.1)	–	–	–	±	+	3±	+	–	–	–	–	+	±	–	±	+
Ureaplasma urealyticum (CP001184.1)	+	–	–	–	+	+	+	–	–	–	–	+	–	–	+	+
Ureaplasma parvum (AF222894.1)	+	–	–	–	+	+	+	–	–	+	–	+	–	–	+	+
Mycoplasma agalactiae (CU179680.1)	+	–	–	–	+	+	+	–	–	+	–	+	–	–	+	+
Mycoplasma hominis (FP236530.1)	+	–	–	–	+	+	+	–	–	+	–	+	–	–	+	+
Mycoplasma hyorhinis (CP002170.1)	+	–	–	–	+	+	+	–	–	+	–	+	–	–	+	+
Mycoplasma hyopneumoniae (AE017332.1)	+	–	–	–	+	+	+	–	–	+	–	+	–	–	+	+
Mycoplasma bovis (CP002188.1)	+	–	–	–	+	+	±	–	–	+	–	+	–	–	+	+
Mycoplasma fermentans (CP002458.1)	+	–	–	–	+	+	+	–	–	+	–	+	–	–	+	2+
Mycoplasma arthritidis (CP001047.1)	+	–	–	–	+	+	+	–	–	+	–	+	–	–	+	+
Mycoplasma cynos (HF559394.1)	+	–	–	–	+	+	+	–	–	+	–	+	–	–	+	+
Mycoplasma mycoides (BX293980.2)	+	–	–	–	+	+	+	–	–	+	–	+	±	–	+	–
Mycoplasma conjunctivae (FM864216.2)	+	–	–	–	+	+	+	–	–	+	–	+	+	–	+	+
Mycoplasma capricolum (CP000123.1)	+	–	–	–	+	+	+	–	–	+	–	+	+	–	+	+
Mycoplasma leachii (CP002108.1)	+	–	–	–	+	+	+	–	–	+	–	+	+	–	+	+
Mycoplasma mobile (AE017308.1)	+	–	–	–	+	+	+	–	–	+	–	+	+	–	+	+
Mycoplasma putrefaciens (CP003021.1)	+	–	–	–	+	+	+	–	–	+	–	+	+	–	+	+
Mycoplasma crocodyli (CP001991.1)	–	–	–	–	–	+	+	–	–	+	–	+	–	–	+	+
Mycoplasma haemofelis (FR773153.2)	–	–	–	–	+	+	+	–	–	+	–	+	–	–	+	+
Mycoplasma pulmonis (AL445566.1)	–	–	–	–	+	+	+	–	–	±	–	+	–	–	+	+
Mycoplasma synoviae (AE017245.1)	–	–	–	–	+	+	+	–	–	+	–	+	–	–	+	±
Mycoplasma haemocanis (CP003199.1)	–	–	–	–	+	+	1+, 1±	–	–	+	–	+	–	–	+	+
Ca. Mycoplasma haemominutum (HE613254.1)	–	–	–	–	+	+	+	–	–	+	–	+	–	–	+	–
Mycoplasma suis (FQ790233.1)	–	–	–	–	+	+	+	–	–	+	–	+	–	–	+	–
Mycoplasma wenyonii (CP003703.1)	–	–	–	–	+	+	+	–	–	±	–	+	–	–	+	–
Ca. Mycoplasma haemolamae (CP003731.1)	–	–	–	–	+	+	+	1+, 1±	–	±	–	+	–	–	+	–
Mycoplasma genitalium (L43967.2)	+	–	–	–	+	3+	+	+	+	+	–	+	–	–	+	+
Mycoplasma pneumoniae (U00089.2)	+	–	–	–	+	3+	+	+	+	+	–	+	–	–	+	+
Mycoplasma penetrans (BA000026.2)	+	–	–	–	+	+	+	+	+	+	–	+	–	–	+	+
Mycoplasma gallisepticum (AE015450.2)	+	–	–	–	+	8±	+	+	+	+	–	+	–	–	+	+
Ca. Phytoplasma mali (CU469464.1)	–	–	–	–	+	+	+	+	+	12+	–	+	–	–	+	+
Ca. Phytoplasma asteris OY-M (AP006628.2)	–	–	±	–	+	+	+	+	+	+	–	+	–	+	+	–
Ca. Phytoplasma asteris AY-WB (CP000061.1)	–	–	–	–	+	+	+	+	+	+	–	+	–	+	+	+
Ca. Phytoplasma australiense (AM422018.1)	–	–	–	–	+	+	+	+	+	+	–	+	–	+	+	+
Acholeplasma laidlawii (CP000896.1)	+	–	–	–	+	+	+	+	+	+	–	+	+	+	+	+

"–" indicates that the genes coding for the HSP analogues are absent in the completely sequenced genome. "±" indicates that the genome contains the ORFs with predicted hypothetical products possessing some homology with the known HSP. "+" indicates that the genes have been found in the genome possessing a high level of homology with the known HSP, or that the presence of the HSP in the mycoplasma has been experimentally confirmed.

Accession numbers of complete genome sequences in the GenBank database are shown in parenthesis.

ing for the HSP. Notably, among mycoplasma species whose genomes have been completely sequenced, *M. genitalium* possesses the smallest genome. The *A. laidlawii* genome contains 12 genes coding for the analogues of the known HSP. *A. laidlawii* is the only mycoplasma occurring in the natural environment out of the host organism. This "ubiquitous" mycoplasma was isolated from soil, compost, wastes, as well as from the tissues of humans, plants, and animals [11]. The HSP genes homologous to those found in *A. laidlawii* genome individually are present in the genomes of other 34 mycoplasma species. Probably, higher capacity of *A. laidlawii* for survival under unfavorable conditions compared with other mycoplasma is associated with the fact that this organism possesses a more complete set of the genes encoding the HSP.

In almost all mycoplasma with sequenced genomes, the genes for the DnaK/DnaJ/GrpE chaperone complex have been found. The proteins of this complex provide co-translational and post-translational protein folding in a bacterial cell [26]. The only exception is *M. crocodyli*, the genome of which does not contain the *dnaK* gene, while retaining the genes coding for the co-chaperones (*dnaJ* and *grpE*). Both for gram-negative *Escherichia coli* and for gram-positive *Bacillus subtilis* the *groEL* gene has been previously shown to be essential for cell viability, while the *dnaK* gene was shown to be not essential [27, 28]. In both cases the cells carrying mutations in the *dnaK* exhibited seriously impaired growth, up to loss of viability, only at elevated temperature. However, in *M. crocodyli* the genes *groEL/groES* were not detected, and the only predicted protein with chaperone function was the Tig indicating its unique role in the folding.

In *A. laidlawii* the genes *dnaK, dnaJ*, and *grpE* are located as a cluster and are organized in the operon: probably, inducible HSP synthesis in *A. laidlawii* is mediated by retaining of the functional *dnaKJ* operon [29]. The *dnaKJ* operon was also found in *M. capricolum* [30]. In the chromosome of *Ureaplasma urealyticum* the *dnaK* gene is located separately and is flanked by two ORFs coding for the putative products with unknown functions. The residue of the *dnaKJ* operon in this mycoplasma is represented by the *grpE* and *dnaJ* cluster [31]. In the chromosomes of *M. genitalium* and *M. pneumoniae* the genes are organized in the same way: the *grpE* and *dnaJ* form a cluster, and the *dnaK* is located separately [32]. In addition, these mycoplasmas contain two more putative ORFs homologous to the *dnaJ*.

Loss of the genes for the GroEL/GroES chaperone complex by many mycoplasma species was surprising for the investigators. For many years it has been considered that the GroEL was necessary for the viability of any bacterial cell. For example, in *E. coli* the GroEL/GroES system is the only chaperone system necessary for viability of the cell, both under normal and heat shock conditions [33]. The GroEL and GroES proteins mediate the folding of approximately 10% of the newly synthesized proteins [34]. The GroEL homologues are important for the viability of *B. subtilis* cells [35]. However, even weak homologues of the *groEL* have not been found in many mycoplasma genomes. Moreover, the GroEL proteins of *M. pneumoniae* and *M. genitalium* were shown to be inessential for cell viability [36]. Under heat shock conditions, the level of the *groEL* expression did not increase in *M. genitalium* [37] and increased insignificantly in *M. pneumoniae* [22]. In mycoplasmas certain functions of the GroEL could be fulfilled by the Tig and DnaK/DnaJ/GrpE proteins. However, substrate specificity of the Tig and DnaK is significantly different from that of the GroEL. The Tig and DnaK bind to the proteins in an opened conformation at early folding stages, while the GroEL protein recognizes compact intermediates. The GroEL/GroES remains to be the only bacterial chaperone system where polypeptide folding is going in the cavity formed by the chaperone complex [34]. That is why other mycoplasma chaperones can hardly substitute the GroEL protein completely. The mechanisms providing protein homeostasis in mycoplasma may differ significantly from those of other bacteria. It was suggested that a fundamental shift towards the processes providing degradation of the defective proteins rather than their folding occurred in mycoplasmas in the course of evolution [25]. The genomes of mycoplasmas with the minimal number of the HSP contain only one gene encoding for the protein with chaperone functions (Tig or DnaK), although they retain the genes for two different proteases (FtsH and Lon). Notably, the genes coding for the Clp protease complex and the DegP (HtrA) proteinase were absent from all the sequenced mycoplasma genomes. Thus, the mycoplasma multichaperone network contains both less chaperones and less proteases than that of other bacteria.

The genome of *Candidatus* Mycoplasma haemolamae does not contain the genes with homology to the genes for bacterial GroES co-chaperones, while it contains two ORFs encoding the products homologous to the second component of the GroEL/GroES complex, the GroEL chaperone. The *groEL* gene is considered to be lost by the common mycoplasma ancestor; the *groEL* in the genome of the highly invasive species *M. penetrans* could appear as a result of a horizontal transfer from the unrelated invasive bacteria *Helicobacter pylori* [38]. Among other mycoplasma that contain the GroEL/ES system, invasive species are also present: *M. gallisepticum, M. pneumoniae*, and *M. genitalium*. In these mycoplasma, the GroEL is suggested to act as an adhesin–invasin. However, the mechanism of GroEL-mediated invasion has not been studied yet [39]. The same may be the case of the *groEL* gene of *Candidatus* M. haemolamae. Nevertheless, the absence in this mycoplasma genome of the gene coding for the homologue of the GroES co-chaperone still remains a mystery.

As for the proteins whose folding depends on the GroEL, a significant number of the genes encoding the homologues of these proteins could be lost in the course of evolution due to the high level of mycoplasma genome reduction. For example, in the genome of *M. capricolum* only 61 homologues from 252 proteins of *E. coli* GroEL substrate proteins were found, and only 3 of them are obligate substrates for the GroEL [40]. Using computer modeling, in 11 mycoplasma species the homologues of the known bacterial proteins being obligate substrates for the GroEL/ES complex were predicted to lose their dependence on the GroEL [38]. This loss was confirmed experimentally [41].

What could be an evolutionary significance of this loss of the most important chaperone system (GroEL/ES)? Recognition of certain phenotypes of a parasite by the host could stimulate the loss of specific genes that maintained these phenotypes [25]. This may be an explanation for the loss of some chaperones that are not significant for the slowly growing mycoplasma cells [11, 15, 36] but are rather immunogenic [42, 43]. Mycoplasmas are known to use different ways to minimize the host immune response [11]. Mutations resulting in a loss of the GroEL protein may therefore be considered an adaptation to the protective reactions of the host organism. The absence of the GroEL/GroES chaperone system in 25 of 35 mycoplasma genomes sequenced so far suggests a unique role of the DnaK/DnaJ/DnaE system and of the Tig chaperone in the protein folding and re-folding.

Another protein complex within the pool of bacterial HSP, Clp, is an ATP-dependent heterogenous protease complex, which contains several subunits. It contains the HSP100 that are closely related to the HSP70 and also have the protective function. HSP100 form ring-shaped complexes [44]. The *E. coli* HSP100 include ClpA, ClpB, ClpX, ClpP, ClpY (HslU), and ClpQ (HslV). One of the distinguishing features of the mycoplasma genomes is the absence of the genes coding for these proteins. The only exception is the predicted product of the PAM_667 ORF in *Ca.* P. asteris str. OY-M genome, which is homologous to bacterial ClpX proteases. The authors who sequenced *Ca.* P. asteris str. OY-M genome didn't comment the described "anomaly" [45]. The gene coding for the putative ClpX homologue could probably get into the mycoplasma genome by horizontal transfer of the genes. The genes coding for the CtsR (negative regulators of the *clp* genes expression in many gram-positive bacteria), ClpS (modulators of the ClpAP substrates recognition), and SspB (stimulators of the ClpXP proteolytic activity) proteins also have not been found in mycoplasmas [46]. All studied mycoplasmas also don't have the *clpA* genes. Despite the importance of the Clp proteins for bacterial cells under stress conditions, mycoplasmas don't have the genes coding for the HSP100 except the *clpB*. It was found in 14 of 35 sequenced mycoplasma genomes. The ClpB protein, unlike other Clp proteins that form protease complex, is a molecular chaperone and participates in re-folding of the aggregated proteins by interaction with the DnaK/DnaJ/GrpE chaperone system. In *M. capricolum* the genes *clpB, dnaK, dnaJ*, and *grpE* are organized in an operon [30], and in *M. pneumoniae* and *M. genitalium* the *clpB* and *dnaK* genes are not connected spatially but are expressed under control of the common regulatory element [37, 47, 48].

The molecular chaperone Tig (trigger factor) which participates in co-translational folding of the polypeptides [26] is expressed by all mycoplasma cells except *Mycoplasma mycoides* subsp. *mycoides* SC, *M. suis, M. haemominutum, M. wenyonii, Ca.* M. haemolamae, and *Ca.* P. asteris OY-M. In the cells of *Mycoplasma hyopneumoniae* the Tig protein was suggested to replace the function of the absent SecB protein that retains secreted proteins in the state competent for translocation, as the Tig is involved in secretion of some proteins [49]. The genes or ORFs encoding molecular chaperones HtpG (Hsp90) relating to the Hsp90 family are absent in all sequenced mycoplasma genomes, except the genome of *Mesoplasma florum*. The level of similarity of amino acid sequences of *M. florum* Mfl628 ORF putative product to those of bacterial HSP90 proteins made it possible to designate the Mfl628 product as a Hsp90. The Hsp90 is known to be necessary for the yeasts and for *Drosophila melanogaster*, although it is not significant for *E. coli* or other bacteria [50]. As for the genes encoding the chaperones of Hsp33 family, they are predicted for eight mycoplasma: *Mycoplasma conjunctivae, M. leachii, M. mobile, M. florum, M. mycoides, M. capricolum*, and *A. laidlawii*. Chaperone activity of these proteins is redox-dependent, and they efficiently prevent the aggregation of both thermally damaged proteins and the proteins damaged by oxidative stress [51]. The function of the mycoplasma analogues of the Tig, HtpG, and Hsp33 proteins was not confirmed experimentally.

The genes for the sHSP were revealed only in four species of the *Mollicutes* and are apparently not present in other mycoplasma studied. Except for the *A. laidlawii ibpA* gene product, whose chaperone functions were recently shown [23, 52], the genes for the sHSP were predicted for three phytoplasmas: *Ca.* P. asteris AYWB, *Ca.* P. asteris OY-M, and *Ca.* P. australiense. These mycoplasmas belong to the family *Acholeplasmataceae* and are plant pathogens. The role of these proteins in the phytoplasma cells was not experimentally confirmed. Not all the sHSP function as molecular chaperones [53, 54]. However, amino acid sequence of the IbpA (Hsp20) from *A. laidlawii* demonstrated high level of similarity to the predicted sequences of the phytoplasma *ibpA* (*hsp20*) genes putative products, thus indicating their similar functions.

Why do some representatives of the class *Mollicutes* carry the genes for the sHSP while others do not? One

could suggest that in most mycoplasma the sHSP are absent due to significant reduction of their genomes. However, the genes for the sHSP were absent not only in *M. genitalium*, which is known to have the minimal genome (0.58 Mb) among the organisms capable of independent reproduction [47], but also in *M. mycoides* subsp. *mycoides* SC str. PG1 [55] and *M. penetrans* HF-2 [56] with genome sizes of 1.2 and 1.36 Mb, respectively. In the genome (1 Mb) of an intracellular parasite *Chlamydia trachomatis* [57] the sHSP are also absent. At the same time, the genes for sHSP were found in small genomes of the intracellular parasites *Buchnera* sp. APS (0.64 Mb) and *Rickettsia prowazekii* (1.1 Mb), which spend most of their life cycle in poikilothermic insects [58, 59]. Probably, the presence or absence of the sHSP depends on the temperature regimen of the environment, rather than on the size of the genome [60]. Thus, most mycoplasmas not carrying the genes for sHSP are human and animal parasites and inhabit isothermal niches. Unlike other mycoplasma, representatives of the family *Acholeplasmataceae* inhabit mainly the plant tissues. However, the genome of the phytoplasma *Candidatus* Phytoplasma mali [61] has been sequenced recently. This mycoplasma causes the diseases of apple-trees and is currently related to the family *Acholeplasmataceae*. In its genome no one ORF was found corresponding to the gene encoding the sHSP homologue. Notably, the features of *Ca.* P. mali differ significantly from both those of the phytoplasmas *Ca.* P. asteris and *Ca.* P. australiense and from all known species of the *Mollicutes*. In *Ca.* P. mali the nucleoid is organized in a linear chromosome, while in all other mycoplasmas it is a circular DNA. Moreover, the *Ca.* P. mali genome is smaller (0.6 Mb) than that of the phytoplasmas *Ca.* P. asteris OY-M (0.86 Mb) [45], *Ca.* P. asteris AY-WB (0.71 Mb) [62], *Ca.* P. australiense (0.88 Mb) [63], and of the mycoplasma *A. laidlawii* (1.5 Mb) [64]. The DNA C+G content in *Ca.* P. mali is very low (21.4%), and the metabolic capabilities of this phytoplasma are highly limited compared to those of other mycoplasmas. Nevertheless, the phytopathogenic organism *Ca.* P. mali may survive within a wide range of temperatures. The question of why the genes for sHSP are present or absent in different mycoplasma genomes still has no answer.

It is suggested that since mycoplasmas lost the HslUV (ClpYQ) protease system, the Lon (La) or FtsH proteases may replace its function [25]. For example, mutations in the *lon* gene in *E. coli* could be suppressed by overproduction of the HslUV [65]. Moreover, the Lon and HslUV proteases possess cross substrate specificity. The genes coding for the Lon protease, which provides for the degradation of incorrectly folded and inactivated proteins, have been found in all mycoplasma genomes without exceptions. Any analogues of the other protease, *ftsH*, were not found in *M. florum* and *U. urealyticum*. It must be noted that none of the mycoplasma genomes encodes bacterial DegP (HtrA) protease.

In some mycoplasma genomes, paralogous genes were found whose putative products demonstrated high level of similarity with the known bacterial HSP. For example, in *M. fermentans* genome two putative genes encoding for the *tig* and *tig-2* homologues of the trigger factor were found. In *M. genitalium, M. pneumoniae*, and *M. florum* three ORFs corresponding to the paralogous genes for the DnaJ-like proteins were predicted, while in *M. gallisepticum* genome eight of such genes were found. The genome of the phytoplasma *Ca.* P. mali is a good example of the presence of several copies of the same gene within one genome. It contains 12 ORFs that correspond to the *ftsH* paralogous genes. In the genomes of *Mycoplasma haemocanis* and *Ca.* M. haemolamae two ORFs were found coding for the proteins that possess different levels of similarity to the GrpE and GroEL chaperones, respectively. The functional role of duplication of the genes coding for the HSP in small mycoplasma genomes with limited encoding capability is not clear.

The studies on mycoplasmas provoked a discussion regarding the size and content of the so-called "minimal genome." The criterion for the introduction of the gene into the list of "necessary" genes was the fact that its product, together with the products of other candidate genes, could provide the viability of the putative "minimal" cell. The list of the genes coding for the chaperones and proteases of such a cell was suggested by Gil et al. [16]: *dnaJ, dnaK, groEL, groES, grpE, hflB* (*ftsH*), and *lon*. Although the authors named the *groEL* and *groES* genes as significant for the "minimal" cell, impaired *groEL*, while negatively affecting *M. pneumoniae* and *M. genitalium* cells, was not shown to result in the loss of their viability [36]. It is presently known that the *groELS* operon is absent in most mycoplasmas. The DnaK/DnaJ/GrpE complex also causes some questions. It is involved in many cellular processes, such as protein folding and translocation through biological membranes, the assembly of the oligomeric proteins, and their degradation. The DnaK protein is insignificant for some free-living bacteria [27, 28], although it is necessary for the *M. genitalium* cells viability [36]. In other mycoplasma, *M. crocodyli*, the *dnaK* gene is absent; among all genes encoding the chaperones, only *tig* was retained in this organism. The trigger factor (Tig) is also not necessary for all mycoplasmas: the *tig* gene was not found in 6 of 35 sequenced genomes. In 2006 under laboratory conditions using rich culture media and mutagenesis technique, Glass et al. carried out a big study of *M. genitalium* aiming to detect the genes insignificant for the mycoplasma survival [66]. According to their results, the *clpB* and *tig* genes were included into the list of 100 insignificant genes. Thus, the discussion regarding the complete set of the HSP in a "minimal" cell is not closed.

The genome of a natural "minimal" cell, the smallest cell among all known mycoplasmas, deserves special consideration. Among the genes for the HSP, *M. genitalium* genome (0.58 Mb) contains the *hrcA* (encodes the HrcA regulatory protein), *lon, dnaK*, and *clpB* genes [47]. Promoters of the *lon, dnaK*, and *clpB* genes contain the CIRCE regulatory element (**c**ontrol **i**nverted **r**epeat **c**haperone **e**xpression), which binds to the HrcA repressor. The *groEL* and *groES* genes, as well as *tig* and *ftsH* were also found in *M. genitalium* genome, while they were absent in the larger genomes. *M. pneumoniae, M. gallisepticum*, and *M. penetrans* have an analogous set of the HSP genes. However, in *M. mycoides, M. capricolum*, and *M. mobile* the genes encoding the Hsp33 were found, while in *M. genitalium* the gene encoding for this chaperone homologue was absent. Moreover, in the genome of *M. genitalium* several genes-paralogues of the *dnaJ* were found. Except this mycoplasma, multiple copies of the *dnaJ* were found only in *M. pneumoniae, M. gallisepticum*, and *M. florum*. Thus, the HSP genes are well represented even in the smallest mycoplasma genome and some of them even have several copies.

EXPRESSION OF MYCOPLASMA HSP GENES

To understand an adaptation of the cell to unfavorable environmental changes, it is necessary to study the regulatory mechanisms that control the numerous genes and operons with the subsequent interaction of these genes products. Expression of the HSP in prokaryotes occurs at transcriptional, posttranscriptional, and posttranslational levels.

Transcription-regulatory factors have been studied in detail for *E. coli* [67] and *B. subtilis* [68]. Typical for *E. coli* cells is a positive regulation involving the σ^{32} factor. For *B. subtilis* three regulatory mechanisms have been described: negative control involving the HrcA repressor; positive regulation which depends on the alternative σ^{B} factor; and negative regulation mediated by the CtsR protein (**c**lass **t**hree **s**tress response **r**egulator). Mycoplasmas have no alternative sigma factors (σ^{32}, σ^{B}) that are typical of bacteria and are involved into the heat shock response. Only one sigma factor was identified in mycoplasma: this is a homologue of *B. subtilis* σ^{A}. The mycoplasma genes are considered to be regulated by a limited set of transcriptional regulators typical for the "minimal" cell [46]. One of these regulators (if not the only) is the HrcA protein which was identified in all sequenced mycoplasma genomes.

In gram-positive bacteria, negative regulation of the HSP genes under heat shock is performed by the mechanism involving the CIRCE element and the HrcA repressor. HrcA binds to the CIRCE inverted repeat TTAGCACTC-N_9-GAGTGCTAA (where N may be represented by any base) and prevents transcription of the downstream genes [68]. Conservatism of the nucleotides within the CIRCE determines an efficiency of repression. In many gram-positive bacteria, the inverted repeat within the CIRCE element was found upstream the *dnaKJ* and *groESL* operons, but it never was laying upstream the other HSP genes [69]. In most mycoplasmas the genes that organize the *groESL* operon were not found. However, upstream the *dnaK, clpB*, and *lon* genes the CIRCE-like repeat was identified [25]. General expression regulation of these genes in mycoplasmas suggested the overlapping functions of their products, the ClpB, DnaK, Lon, and GroEL proteins. However, upstream the *ftsH* gene the CIRCE element was absent.

The first detailed study of the transcriptional profile changes in response to heat shock was carried out for *M. pneumoniae* [48]. During the study, 47 genes were identified whose expression increased with an increase in temperature from 32 to 42°C, and 30 genes whose expression decreased under the same conditions. The genes that demonstrated an induced expression under heat shock conditions included conservative HSP genes, the genes for several ribosomal proteins, and also 10 genes with unknown functions. Among the genes encoding the classical HSP, an increase in the level of expression was observed for the *dnaK, lonA, groES*, and *mpn002* (encodes the predicted product containing the DnaJ j-domain), as well as *clpB*. Maximal increase in the level of expression was shown for the *clpB*, presupposing that the ClpB protein is very important for this mycoplasma under heat shock conditions. The details of transcriptional regulation of gene expression in *M. pneumoniae* under heat shock still remain unclear. Since CIRCE is lying upstream the *dnaJ, lonA, dnaK*, and *clpB* genes, their transcription presumably depends on the HrcA repressor. However, analysis of the promoters of other genes whose expression was induced under heat shock did not reveal any regulatory sequence typical for the promoters of the HSP genes.

Analysis of *M. hyopneumoniae* transcriptome under heat shock conditions was carried out [70]. Under stress conditions the level of the *dnaK* gene increased almost 14-fold, of the *dnaJ*, twofold, and of the *clpB*, 6.3-fold. In promoters of the *dnaK* and *dnaJ*, as in case of *M. pneumoniae*, the inverted CIRCE repeats were identified. While the *parC* and *ffh* are the genes not belonging to the typical HSP genes, they demonstrated increased expression at elevated temperature. As for the *ffh* gene, this is the only described case of its expression regulation in response to heat shock. For example, the level of transcription of this gene analogue in *M. pneumoniae* under heat shock conditions remains unchanged [48]. Therefore, mycoplasmas are capable of different gene control in response to stresses. In general, in *M. hyopneumoniae* the level of expression of 50 genes increased in response to heat shock, and that of 41 other genes decreased significantly [70].

Despite its small genome size, *M. genitalium* possesses a set of the genes associated with heat shock response. The analogues of most genes for bacterial regulatory proteins, including the two-component transduction systems, were not found in its genome, as in the genomes of other mycoplasmas; however, the putative gene encoding the HrcA repressor was identified [47]. In *M. genitalium* genome three copies of the CIRCE sequence were also found in the promoters of the *dnaK, clpB*, and *lon* [37]. This suggests the functional activity of the HrcA-CIRCE regulatory mechanism. Musatovova et al. demonstrated that induced expression of the HSP genes in *M. genitalium* under heat shock was selective and reversible. Among the genes with an induced expression appeared to be the *lon, dnaK, clpB, dnaJ3*, and *grpE*. The *dnaJ1, dnaJ2, groES, groEL, tig, ftsH, hrcA, rpoD*, and *tuf* genes demonstrated the same level of expression or its decrease. The most significant increase was observed in the expression of the genes encoding the DnaK, Lon, and ClpB proteins. After heat shock, the level of the HSP genes transcription returned to the control values after 1 h of incubation at 37°C, thus illustrating the temporary character of transcriptional response to the stress. None of the known regulatory sequences was found in the promoter of the *dnaJ3* gene, although its expression increased under heat shock. The CIRCE-like sequence different from the consensus one by several nucleotide substitutions was identified lying upstream the *dnaJ2* gene. However, expression of the *dnaJ2* did not change in response to heat shock. In *M. genitalium* combined transcription of the *tig* and *lon* genes was shown, although the *lon* has its own promoter. Notably, expression of the *groELS* operon did not change at elevated temperature. In the promoter zone of this operon, the CIRCE sequence typical for the *groELS* bacterial genes was identified. This may be considered an experimental confirmation of the hypothesis regarding a fundamental shift in the mycoplasma protein homeostasis towards degradation.

Identification in mycoplasma genomes of the only sigma factor, the HrcA repressor, and of the sequences homologous to the CIRCE indicates that all mycoplasma probably use the HrcA-mediated mechanism of negative regulation in response to heat shock. This may be the only regulatory mechanism in mycoplasma cells [46]. In this case, it could be an example of the simplest and the most economical way for the HSP genes control. However, the changes in the level of transcription of a number of genes with their promoters containing no known bacterial regulatory sequences indicate that mycoplasmas presumably have different, as yet unstudied regulatory mechanisms. Interesting to note, some mycoplasmas are capable of inducible synthesis of the HSP (*A. laidlawii, M. pneumoniae*), while others synthesize them only constitutively (*M. genitalium, M. fermentas*) [71].

The regulation of mycoplasma adaptive responses to heat shock performed at posttranscriptional and posttranslational levels still remains unclear. Proteomic analysis of the *A. laidlawii* PG8 cells subjected to different stresses has been performed recently [72, 73]. Under the cold shock and oxidative stress the synthesis of the IbpA (Hsp20) protein decreased; however, when the main substrate in the culture medium was changed from glucose to trehalose, the IbpA synthesis increased as well as the synthesis of such stress proteins as GroEL, ClpB, and Lon. Production of the GroEL, GroES, Lon, Hsp20, GrpE, and Tig proteins by *A. laidlawii* cells increased after the combined effect of several stress factors. The Hsp20, Lon, and 21 more proteins are general stress-reactive proteins in case of short-term action of the stress factors, and four more proteins including the Lon protease are general stress-reactive proteins whose expression changes under all stress conditions. Transcriptional analysis, as in case of *M. genitalium, M. pneumoniae*, and *M. hyopneumoniae*, was not performed for *A. laidlawii*. Probably, a combination of transcriptomic and proteomic approaches could elucidate the mechanisms of mycoplasma stress response, including the heat shock response.

In many microorganisms, modification of the chaperone proteins is known to occur under stress conditions, including temperature changes, starvation, and changes in pH. In *M. pneumoniae* and *M. genitalium* cells the DnaK, DnaJ, GroEL, and Tig proteins were shown to be phosphorylated [74]. During the stationary phase of growth of both mycoplasmas the levels of DnaK and GroEL synthesis increased, as well as the degree of their phosphorylation. In *A. laidlawii* cells the IbpA (Hsp20) protein is probably also modified under different stress conditions: three forms of this protein were detected using two-dimensional PAGE; they were registered in different combinations depending on the stress factors [72, 73]. The modified (phosphorylated) HSP could act as the sensors and modulated the mycoplasma stress response [74].

PROPERTIES AND FUNCTIONS OF THE MYCOPLASMA HSP

Are specific HSP functionally active in mycoplasma cells, or are their functions weakened, changed, or lost? In the discussion regarding the components of a hypothetical "minimal" cell [16] many proteins were considered as necessary for the cell survival, including the GroEL/GroES system and the key protein for prokaryotic cell division, FtsZ. However, the homologues of these proteins in mycoplasma cells have been subsequently shown not to be significant for their viability [36, 75]. Moreover, in most mycoplasmas the genes encoding the CroEL/GroES chaperone complex were shown to be absent [39], and in 8 of 35 mycoplasma species with completely sequenced genomes (*Ureaplasma urealyticum, U. parvum, Mycoplasma cynos, M. suis, M. haemocanis, M. haemominu-*

tum, *Ca.* M. haemolamae, and *M. wenyonii)* the gene for the FtsZ protein was also not found. More recently, this protein was considered as the necessary component of the septation groove in all prokaryotes. Genome sequencing and identification of ORFs encoding the proteins possessing high levels of similarity with the known proteins can not guarantee the presence in mycoplasmas of functionally active products acting in the same mode as in bacterial or eukaryotic cells. It is necessary to support the theoretical discussion regarding the role of certain proteins in mycoplasma cell homeostasis by experimental data.

Up to now only a small part of the HSP from mycoplasmas belonging to different families has been characterized, and their properties and functions are incompletely studied. A little more is known concerning the immune properties of mycoplasma chaperones. In case of mycoplasmosis, as in case of any infectious diseases caused by pathogenic microorganisms, an immune response of the host organism occurs. Among the HSP the strongest antigens are the HSP70 (DnaK, Hsp70) and HSP60 (GroEL). They are considered as potential targets for the development of diagnostic tests and vaccines.

The DnaK (HSP70) proteins. The properties of several DnaK-like mycoplasma proteins were described: DnaK from *A. laidlawii*, HspA1 from *M. suis*, and DnaK from *Mycoplasma haemofelis* [17, 19, 29]. Western blotting revealed that heat shock resulted in an increase of the DnaK amount in the cells of *A. laidlawii* and *M. pneumoniae* [71]. However, the cells of *M. genitalium* and *M. fermentans* under normal conditions contained considerable amounts of the DnaK, which did not increase after heat shock. Probably, the HSP synthesis regulation differs significantly in different species of mycoplasmas. For the efficient functioning of the DnaK/DnaJ/GrpE chaperone complex, the proteins must be present in the cell in a certain ratio [76]. The ratio of the number of DnaK and DnaJ molecules in *B. subtilis* is 3 : 1 [77]. In the case of *A. laidlawii*, the level of DnaJ expression was shown to be lower than that of the DnaK [78]. Presumably, the DnaK/DnaJ/GrpE complex in *A. laidlawii* functions in a similar way to that of *B. subtilis*.

The gene encoding *A. laidlawii* DnaK has been sequenced long before the complete sequencing of its genome was performed [29, 78]. This gene encodes the DnaK protein of 65721 Da consisting of 610 amino acid residues. It contains all three motives typical for the bacterial HSP70 (DnaK). These motives are located between 9–17, 169–183, and 310–325 amino acid residues. Similar to the DnaK protein from *B. subtilis*, the DnaK from *A. laidlawii* contains an additional amino acid at 81 position of the consensus sequence. The frequencies of the charged, basic, polar, and other types of amino acid residues in the DnaK proteins of these two bacteria are also the same. The *A. laidlawii* DnaK at 394–424 positions contains the motif of a leucine zipper. It may be responsible for dimerization or multimerization of the DnaK molecules. Compared with the DnaK from *B. subtilis*, that from *A. laidlawii* contains three additional N-terminal amino acids but shows significantly lower frequencies of glycine (by 18.67%) and proline (by 20.7%). At the same time, the frequencies of alanine (by 7.5%) and arginine (by 6.19%) are higher, while the frequency of lysine (by 2.62%) is unexpectedly lower. The DnaK from *A. laidlawii* contains all conservative glycine doublets and triplets that may participate in the formation of a frame for the tertiary protein structure. The biochemical properties of the *A. laidlawii* DnaK have not been reported.

The HspA1 is present in the *M. suis* cells in the cytoplasmic and membrane-bound form [19]. Its localization on the surface of the *M. suis* cells was revealed by immune electron microscopy using specific antibodies to the HspA1. The amino acid sequence of this protein has 58–63% identity with the DnaK proteins from other mycoplasmas. The recombinant HspA1 possesses ATPase activity typical for the HSP70. Apart from its chaperone function, the HspA1 protein is suggested to be involved in adhesion of the *M. suis* cells to the surface of erythrocytes. Numerous functions of the proteins belonging to the DnaK family in mycoplasmas are biologically understandable, since these microorganisms have small genomes and therefore limited encoding capability.

The DnaK protein from *M. haemofelis* is well characterized [17]. Among all mycoplasma DnaK proteins it has the highest percent of similarity (72%) with the HspA1 (DnaK) from *M. suis*. Molecular mass of the DnaK protein from *M. haemofelis* is 66.5 kDa. The chaperone activity of *M. haemofelis* recombinant DnaK produced under heat shock conditions by the *E. coli* cells deficient in the *dnaK* was confirmed by complementation analysis. Recombinant DnaK had typically low ATPase activity in the absence of the DnaJ and GrpE co-chaperones. During thermal denaturation of the DnaK, two transitions were observed. The first one was presumably associated with denaturation of the N-terminal nucleotide-binding domain, and the second, with denaturation of the C-terminal substrate-binding domain. ATP stabilized the DnaK molecule and enhanced the temperature threshold of the first transition. K^+ and Mg^{2+} ions also had a stabilizing effect on the chaperone molecule. The data on the secondary structure, thermal denaturation, and ATPase activity of the DnaK from *M. haemofelis* indicate this protein to be the typical representative of the DnaK (Hsp70) family.

Immunogenic properties of the mycoplasma HSP70 are well studied. The Hsp70 from *M. mycoides* subsp. *mycoides* is one of the antigens able to cause an immune response in cattle in case of pleuropneumonia [79]. Among the most immunogenic proteins of *M. synoviae* are the trigger factor Tig and the chaperone DnaK [80]. The *M. hyopneumoniae* Hsp70 is a highly immunogenic protein: monospecific antibodies

to this protein may block the growth of mycoplasma cells, and the DNA vaccine on the base of the P65 gene encoding the Hsp70 causes induction of both humoral and cell immunity of the infected organism [18]. The *M. suis* HspA1 protein (Hsp70) is localized on the surface of the cell and acts as an immunodominant antigen in case of the porcine eperytrozoonosis [19]. The biological role of HspA1 in the pathogenesis of this infection is unclear, as well as the putative participation of this protein in the interaction of the host and pathogen. The *M. haemofelis* DnaK protein also possesses immunogenic properties: the serum obtained from the cats infected with *M. haemofelis, M. haemominutum*, and *Candidatus* Mycoplasma turicensis reacted with the DnaK from *M. haemofelis* while the non-immune cat serum did not [17]. The antibodies to the human Hsp70 were shown to interact with the DnaK proteins of different mycoplasma species: *A. laidlawii, M. genitalium, M. pneumoniae*, and *M. fermentans* [78].

DnaJ-like proteins (Hsp40). Scarce information exists regarding the DnaJ-like proteins from *M. genitalium* and *M. pneumoniae* [20, 21]. The *M. genitalium* genome contains three genes coding for the DnaJ-like proteins [47]. The DnaJ1 and DnaJ3 proteins have one J-domain each and belong to the III type of J-proteins that stimulate ATP hydrolysis by the chaperones of the Hsp70 (DnaK) family [21]. J-Domains of all three *M. genitalium* DnaJ-like proteins have common properties. Analysis of the secondary structure of these domains made it possible to predict four α helix (I–IV): the II helix was rich in basic amino acid residues, the loops between the II and III helix contained a conservative HPD motif, while the hydrophobic residues that were necessary for the structure stabilization were conservative. Apart from the J-domain, DnaJ2 contained two additional conservative domains similar to those from *E. coli* DnaJ co-chaperone (J-protein of the I type). Central domain, a "zink finger," of the *M. genitalium* DnaJ2 protein contains three complete and one incomplete CXXCXGXG motives. This domain is involved in substrate binding. Similar to *E. coli*, the region between the J-domain and the "zink finger" is rich in glycine residues. In addition, the *M. genitalium* DnaJ2 contains also the C-terminal domain homologous to that of *E. coli* DnaJ co-chaperone.

The DnaJ-like protein from *M. pneumoniae* is named TopJ. It is a co-chaperone containing the so-called J-domain typical for the DnaJ proteins and the unique APR and C-terminal domains that were found in the terminal organelle proteins [20]. *M. pneumoniae* terminal organelle participates in adhesion, gliding motility, and division of the mycoplasma cells. The TopJ mediates the functions of the terminal organelle and stabilizes the P24 protein, which is necessary for initiation of the organelle formation. The J-domain provides the co-chaperone function of the TopJ.

The GroEL/GroES (Hsp60/10) proteins. Despite an interest of the scientists to the genes and proteins of the mycoplasma GroEL/GroES chaperone complex associated with the discussion regarding the content of a "minimal" cell [25, 36, 38, 39], no experimental data exist so far confirming the properties and functions of these proteins in mycoplasma cells. However, immunogenic properties of these proteins during the interaction of mycoplasma with macroorganisms in the "parasite–host" system were proved.

Since the antigenic structure is highly conservative, bacterial GroEL protein (Hsp60) is frequently referred to as a general bacterial antigen. Its homologues with molecular masses of 58–65 kDa are present in the cells of most bacteria and archaea [43]. Antigenic structure of the GroEL family proteins in mycoplasmas is variable and does not contain a set of conservative epitopes that are typical for most bacterial HSP60. Nevertheless, cross interaction of the GroEL-specific antibodies with different GroEL mycoplasma proteins was shown repeatedly. For example, the 62-kDa proteins from *M. pneumoniae, M. genitalium*, and *M. fermentans* reacted with the antibodies to the GroEL from *Legionella micdadei* [43]. Production by mycoplasmas of the HSP with molecular mass of 60 kDa was detected in *M. salivarium* and *M. orale*; antibodies to the *H. pylori* Hsp60 were used for immunoblotting [81]. Western blotting with antibodies to the *Pseudomonas aeruginosa* GroEL revealed its expression and intracellular accumulation under heat shock in *A. laidlawii* and *M. pneumoniae* cells [71].

Some patients infected with *M. pneumoniae* demonstrated a strong immune reaction to the mycoplasmal GroEL. The immune serum appeared to be specific to *M. pneumoniae* and did not react with the *M. genitalium* GroEL protein, which is most close to the *M. pneumoniae* GroEL in its antigenic properties: amino acid sequences of the Hsp60 chaperones of these mycoplasmas are identical by 85% [42]. Although the GroEL is considered insignificant for the viability of *M. pneumoniae* and *M. genitalium*, in *M. pneumoniae* cells its expression is high [36]. At the same time, antibodies to the GroEL from *M. pneumoniae* were found in the serum of less than 20% of the patients infected with this mycoplasma: the GroEL protein appeared to be unusable as the marker for serological studies [42].

The ClpB protein (Hsp100). Only one mycoplasma ClpB protein has been described in detail so far: it was isolated from *M. pneumoniae* [22]. The size of *M. pneumoniae* ClpB molecule differs from that of *E. coli* ClpB. The mycoplasma protein consists of 715 amino acid residues, the *E. coli* protein, of 857. In *E. coli* ClpB is synthesized in two forms—of 93 ($ClpB_{EC}$) and 79 kDa ($ClpB'_{EC}$) [82]. Two sites of translation initiation were identified in the transcript of the *M. pneumoniae clpB* [22]. Recombinant *M. pneumoniae* ClpB is produced by *E. coli* cells also in

two forms: of 85 and 65 kDa [82], while the native ClpB in mycoplasma cells is present in only one form [22]. *M. pneumoniae* ClpB contains two conservative AAA-domains (ATP-1 and ATP-2) consisting of 234 and 192 amino acid residues, respectively. Both ATP-binding domains of *M. pneumoniae* ClpB have the core region with a nucleotide-binding pocket where the "central pore" responsible for substrate binding is located. *M. pneumoniae* ClpB is highly variable in the middle of the molecule and its C-terminal region is homologous to the ClpB proteins of other bacteria, although its amino-terminal region is shortened. The "coiled–coil" sequence in the middle of the molecule is involved in the protein–protein interactions associated with the DnaK-dependent re-folding of the polypeptides. Recombinant *M. pneumoniae* ClpB possesses ATPase activity [22]. In the presence of the functional DnaK/DnaJ/GrpE system and of the DnaK protein without the co-chaperones, the recombinant ClpB promoted disaggregation and reactivation of luciferase. Casein and lysine slightly stimulated the ATPase activity of *M. pneumoniae* ClpB as compared with other bacteria. This may be due to the too short N-terminal sequence of this chaperone. The ClpB protein is probably the key chaperone participating in solubilization of the protein aggregates in mycoplasma cells. Deletion of the *clpB* gene affected the growth of *M. pneumoniae* cells that were unable to compensate for the loss of the ClpB protein. The ClpB protein caused also a strong immune response in the patients infected by *M. pneumoniae*.

Small heat shock proteins (sHSP). Only one sHSP from mycoplasmas has been identified and characterized: the IbpA from *A. laidlawii* [23, 52, 60]. This protein attracted attention due to the fact that its synthesis increased dramatically under heat shock. Its amount increased up to 7.2% of the total protein amount, indicating its unique role in mycoplasma cells under stress conditions. All other HSP accumulated in *A. laidlawii* cells in smaller quantities [71]. *A. laidlawii* IbpA was identified as the sHSP since it possessed the properties typical for this family: the presence of a conservative α-crystallin core domain; capability for spontaneous in vitro oligomerization; and chaperone activity against the model substrates [23]. The IbpA amino acid sequence flanking the conservative α-crystallin core domain at the N-terminus has low homology with the prokaryotic sHSP, while the C-terminal sequence demonstrates high similarity with analogous sequences of the predicted sHSP from *Ca.* P. asteris strains AYWB and OY-M as well as from *Ca.* P. australiense. The degree of similarity between the sHSP from various bacteria is known to be lower than that for other chaperones [83]. Nevertheless, *A. laidlawii* IbpA demonstrates significant similarity with the sHSP from phytoplasmas [23]. The capability of the IbpA to form oligomers is presumably determined by the presence at the N-terminus of the protein of a relatively conservative sequence FDDFFEDF similar to the double WDPF motif, which is responsible for spontaneous multimerization of the sHSP [84]. IbpA is an efficient chaperone only at elevated temperatures [23], similar to the Hsp26 from *Saccharomyces cerevisiae* [10]. Moreover, recombinant IbpA from *A. laidlawii* protects some proteins of *E. coli* cell extract from denaturation and aggregation at high temperature and enhances the resistance of *E. coli* cells to heat shock [52]. In the mycoplasma cells, the IbpA probably also has a chaperone-like function. Under heat shock a significant amount of the IbpA molecules in *A. laidlawii* cell is associated with the cellular structural elements (membrane, cytoskeleton-like elements, "granular bodies," and the site of septation) [23]. The sHSP may have multiple targets in mycoplasma cells and participate in stabilization of various structures under heat shock. The targets for the IbpA have not been identified as yet.

CONCLUSIONS

Taking into account that in the course of evolution mycoplasmas lost a significant amount of the genetic material of their ancestors, the presence of the HSP in mycoplasma cells indicates a fundamental role of these proteins for the viability of the cell. The HSP form one of the most conservative families and play an important role for the viability of the cell under both normal and stress conditions. However, among bacteria capable of independent reproduction mycoplasmas have the minimal set of the genes encoding the chaperones and proteases, and their expression regulation may also be simplified. The protein homeostasis mechanisms in mycoplasmas probably differ essentially from those of other bacteria. This suggestion is supported by the fact that transcription of many mycoplasma genes is changed in the absence of universal bacterial regulatory sequences in the relevant promoter regions. This indicates the presence of other, unknown regulatory mechanisms in mycoplasmas. It is likely that in mycoplasma metabolism a notable shift occurred towards intensification of the processes, providing for the degradation of incorrectly folded or damaged proteins and, on the contrary, towards the weakening of the processes providing for protein structural reparation.

Mycoplasmas appear to be the leaders among other microorganisms with regard to the number of species with completely sequenced genomes. This fact favors comparative studies of their genes, including the genes for the HSP, and promotes the investigations of specific proteins, although the HSP remained insufficiently studied. Only a small number of various mycoplasma HSP has been characterized so far, and the available data were reported in the section "Properties and functions of the mycoplasma HSP" of this review. The absence in mycoplasma genomes of the genes that were considered necessary for the viability of any bacterial cell (for example, the genes for the GroEL and

Clp) and the unique fact of the absence of the DnaK protein in *M. crocodyli* genome have not been explained yet. The functions of these chaperones and proteases in mycoplasma cells may be fulfilled by the other HSP.

The chaperones from the DnaK, DnaJ, and GrpE families, as well as some others, may have plural functions in mycoplasma cells. This seems to be biologically reasonable for the organisms with small genomes due to their limited encoding capability. For example, the HspA1 protein from *M. suis* (the DnaK analogue), apart from its chaperone function, is involved in the adhesion of the mycoplasma cells to the surface of erythrocytes. The TopJ from *M. pneumoniae* contains the J-domain typical for the DnaJ proteins and is found in the protein from the terminal organelle, which participates in adhesion, gliding motility, and cell division of this mycoplasma. The sHSP integrated into the multichaperone network which controls the state of the proteins in a cell may simultaneously play the role of regulators of the fluidity of the cell membrane, stabilize the liquid crystal bilayer state, and maintain the membrane integrity during thermal fluctuations. The sHSP are universal protectors of the proteins and the cell structures together with other chaperones possessing ATPase activity, including the DnaK, GroEL, and ClpB proteins. Some patterns of the IbpA localization in *A. laidlawii* cells indirectly confirm the plural functions of this protein.

Mycoplasma HSP are rather immunogenic and could be considered as potential targets for development of the novel diagnostic tests and vaccines. The loss of the important GroEL/GroES chaperone system (these proteins are strongly immunogenic) by many mycoplasma species may be partially explained by the pressure from the immune system of the host. For slowly-growing mycoplasma cells, this chaperone system could be of less importance. If it is so, the retaining of mutations resulting in the loss of the GroEL could be considered a counteraction to the protective mechanisms of the host. Then the role of the DnaK/DnaJ/GrpE system and of the Tig in the protein folding and re-folding in mycoplasmas becomes of a special significance.

Investigation of the simplest cell capable of independent reproduction (as mycoplasma cells) is very important for the understanding of the regulatory mechanisms in all cells. Detailed understanding of the role which the HSP play in mycoplasmas and other prokaryotic cells may result in the development of new strategies for the treatment of the diseases caused by persisting pathogenic microorganisms.

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