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Mini-review

Heat shock proteins and metal ions – Reaction or interaction?

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ABSTRACT

Heat shock proteins (HSPs) are part of the cell's molecular chaperone system responsible for the proper folding (or refolding) of proteins. They are expressed in cells of a wide variety of organisms, from bacteria and fungi to humans. While some HSPs require metal ions for proper functioning, others are expressed as a response of the organism to either essential or toxic metal ions. Their presence can influence the occurrence of cellular processes, even those as significant as programmed cell death. The development of research methods and structural modeling has enabled increasingly accurate recognition of new HSP functions, including their role in maintaining metal ion homeostasis. Current investigations on the expression of HSPs in response to heavy metal ions include not only the direct effect of these ions on the cell but also analysis of reactive oxygen species (ROS) and the increased production of HSPs with increasing ROS concentration. This minireview contains information about the direct and indirect interactions of heat shock proteins with metal ions, both those of biological importance and heavy metals.

1. Introduction

In 1962, Ritossa reported gene activity that resulted from the rapid heating of *Drosophila* glands from 25 to 42 degrees Celsius [1]. Since then, HSPs have been found in all cellular organisms and are highly conserved across all species [2]. In addition to their protective function during sudden temperature changes, they are expressed in response to a wide variety of other factors, including growth factor expression, infections, inflammation, ROS, or the presence of metal ions. HSPs are generally a part of the cell's molecular chaperone system, which is responsible for the proper folding (or refolding) of proteins.

Classically, HSPs have been segregated according to their mass. The heavy chaperone family includes HSPs with a mass greater than 100 kDa. The other families include HSP90, HSP70, HSP60, and HSP40, while those with a mass of less than 34 kDa are referred to as small HSPs [3]. In 2009, new guidelines for the nomenclature of HSP families were proposed based on the gene symbols assigned to HSPs by the HUGO Gene Nomenclature Committee (HGNC) [4]. In this system, HSPs are named after the genes encoding them, e.g., HSPA1A instead of HSP72 or HSP70–1, DNAJB1 instead of HSP40, HSPB1 instead of HSP27, etc. However, the classical family names are still in use, especially for the common characterization of the group, and in this review, we will refer to them as such.

The HSPs, usually in the form of oligomers, are required for the proper folding of their substrate protein. In the case of the "larger" HSPs, HSP-organizing protein (HOP) links HSP70 and HSP90 so that the client protein can be passed from HSP70 to HSP90 to obtain its active form. The co-chaperone HOP is bound to the C-termini of HSP70 and HSP90 via two tetratricopeptide (TPR) domains, namely TPR1 and TPR2. As shown in studies by Kajander et al., the electrostatic interaction of HSP70 with TPR1 and HSP90 with TPR2 is the preferred arrangement [5]. The use of electrostatic Poisson-Boltzmann continuum calculations, free energy perturbation, molecular dynamics simulations, and site-directed mutagenesis has enabled the determination of the binding pockets of HSP proteins as well as the influence of charge residues, on the affinity of the interaction.

2. HSPs and the utilization of metal ions

2.1. Calcium and its homeostasis

Glucose-regulated protein 78 (GRP78, HSPA5) is a member of the HSP70 family located in the membrane of the endoplasmic reticulum (ER). In a normal, healthy cell, it functions as a molecular chaperone, being able to bind misfolded proteins and initiate ER-associated degradation (ERAD). This process regulates the Unfolded Protein Response (UPR), which can trigger apoptosis if homeostasis

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cannot be restored via ER [6]. The non-chaperone function of HSPA5 is to maintain the balance of Ca^{2+} in the lumen, in which HSPA5 binds Ca^{2+} via paired anionic residues, not via a specific domain. Although its Ca^{2+} binding capacity is lower than that of other ER proteins, such as protein disulfide isomerase (PDI) or calreticulin, HSPA5 plays an important role in maintaining Ca^{2+} homeostasis [7]. HSPA5 may prevent Ca^{2+}-induced apoptosis, and Ca^{2+} binding may have implications for its cytoprotective role. However, it should be emphasized that the regulation of Ca^{2+} by HSPA5 is not only based on direct binding. Cell surface HSPA5 was found to interact with autoantibodies, which occur in some pathologies such as prostate or ovarian cancer. This interaction triggers a signaling cascade which results in phospholipase C (PLC) activation and inositol 1,4,5-trisphosphate (IP3) production. IP3 diffuses through the cytosol and binds to a receptor on ER Ca^{2+} channels, which increases cytosolic Ca^{2+} [8].

Another HSP involved in Ca^{2+} homeostasis is HSP90. This protein is predominantly expressed in the neck, midpiece, and tail of human sperm, and these regions drive sperm motility. The expression of HSP90 increased during capacitation, the biochemical event that occurs during sperm maturation and is required for further fertilization of the oocyte. Normally, the Ca^{2+} concentration also increases during capacitation, but the aim of the study was to analyze whether HSP90 regulates the Ca^{2+} signal. Geldanamycin, a specific inhibitor of HSP90 was used, and the Ca^{2+} concentration in sperm was determined by measuring fluorescence intensity after the addition of a Ca^{2+} probe. This research demonstrated that HSP90 is involved in Ca^{2+} homeostasis within human sperm, although it is an indirect interaction [9].

Further involvement of Ca^{2+} ions in the interaction with HSP has been presented in studies on S100A4 and HSP47 [10]. Using a co-immunoprecipitation assay, the authors demonstrated that Ca^{2+}-bound S100A4 interacts with HSP47 in deer antler periosteal cells. Interestingly, further molecular dynamics simulations using ZDOCK software to construct the complexes showed that this process is not Ca^{2+}-dependent for S100A4. Nevertheless, the interactions of Ca^{2+} and HSP47 influence the conformational change of both Ca^{2+}-dependent and Ca^{2+}-independent patterns of S100A4 (Fig. 1). However, it has been suggested that the interaction of HSP47 with the Ca^{2+}-independent S100A4 can improve the microenvironment for antlerogenic periosteum cell differentiation by remodeling the extracellular matrix and basement membranes.

The involvement of Ca^{2+} ions and misfolded proteins is associated with amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by the loss of motor neurons. The activity of the chaperone protein Grp94 (a member of the HSP90 family, found at the ER) is regulated by Ca^{2+} binding (Fig. 1). Fluctuations in Ca^{2+} concentration disrupt the proper function of Grp94 and lead to a UPR from the cell [11]. During the progression of ALS, the heat shock response (HSR) is also likely to be impaired, leaving cells unable to remove unfolded or misfolded proteins, leading to their aggregation and cytotoxicity [12].

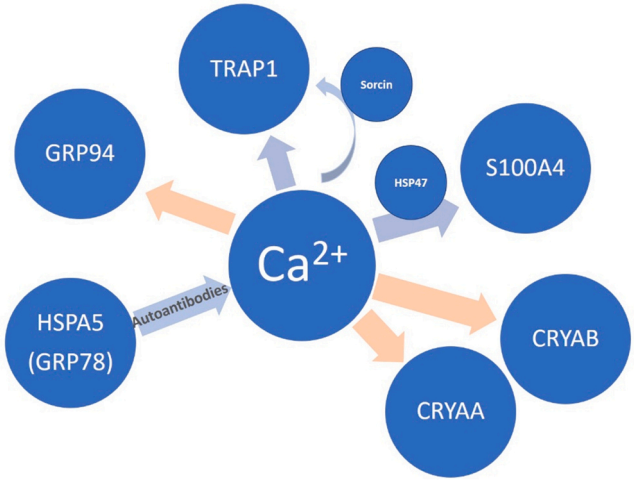

Fig. 1. The involvement of various proteins in either direct (orange arrows) or indirect (blue arrows) Ca^{2+} interactions.

2.2. TRAP1

A member of the HSP90 family, Tumor Necrosis Factor Receptor-Associated Protein 1 (TRAP1), is a mitochondrial chaperone found in large concentrations in cancer cells, such as adenocarcinomas of the breast, pancreas, colon, and lung [13]. The gene, formerly known as Hsp75, is encoded by the TRAP1 gene and is involved in Ca^{2+} homeostasis by interacting with Sorcin, a member of the penta EF-hand Ca^{2+}-binding protein family [14]. Sorcin plays a role in maintaining Ca^{2+} homeostasis by binding Ca^{2+} ions followed by translocation from the cytoplasm to the cell membrane, and the possible interaction with other proteins, e.g., Ca^{2+} channels and exchangers [15].

Previous studies by Landriscina et al. revealed a reciprocal regulation between TRAP1 and Sorcin with probable involvement of TRAP1 in the transport of the 18 kDa isoform of Sorcin into mitochondria, a non-canonical localization for this protein. However, the Ca^{2+}-dependent interaction between the Sorcin 18-kDa isoform and TRAP1 appears to be important for the protective role of this HSP.

Besides indirect participation in Ca^{2+} homeostasis, TRAP1 needs metal ions for proper functioning as an enzyme (Fig. 1). For example, its ATPase activity requires an Mg^{2+} ion, and, as for other HSP90s, such ATP hydrolysis is non-cooperative. However, when a Ca^{2+} ion is bound to TRAP1, the hydrolysis is cooperative by the two protomers within the Hsp90 dimer [16]. As proposed by Elnatan and Agard, the presence of Ca^{2+} ions allow the transition from the closed to open state of TRAP1 in one step, omitting or skipping the asymmetry flip of the protein. Furthermore, at a low ATP concentration, TRAP1 presents a higher affinity for magnesium ions compared to Ca^{2+}, but both metal ions bind preferably to protomers with one ATP molecule bound. Conversely, at high ATP concentrations, it binds to Ca^{2+} ions with higher affinity. Moreover, for Mg^{2+} ions, the Hills coefficient n remains near 1.0 at low and high ATP concentration, which indicates non-cooperative hydrolysis, but for the Ca^{2+} ion, high ATP concentration increases n from 1.0 (at low ATP concentration) to 1.7, indicating significant cooperativity, i.e., increasing affinity for ligand molecule after previous binding of one ligand molecule.

2.3. Magnesium

The Hsp70 family consists of proteins that are highly conserved across species [17–19], containing an N-terminal nucleotide-binding domain (NBD) and a C-terminal substrate binding domain. Four subdomains of the NBD, namely IA, IB, IIA and IIB, surround the ATP/ADP binding pocket. Using isothermal calorimetry (ITC), Mg^{2+} ions were shown to dramatically increase the affinity of Hsp70 for ADP [20]. Interestingly, inorganic phosphate increases this affinity in the presence of Mg^{2+} compared to Mg-free buffer. Thus, additional and indirect interaction between NBD and ADP was discovered by crystallography, including the interactions of Mg^{2+} ions with the β-phosphate group of ADP. Magnesium is also thought to be required by human Hsp90 for the ATP hydrolysis reaction [21]. Interestingly, the Mg^{2+} ion is believed to stabilize the intermediate state of the catalytic reaction. Chaurasia et al. used bioinformatics tools to investigate the structure of HSP60, HSP70, and HSP90 in *Macrobrachium rosenbergii* and confirmed 11 ATP/Mg^{2+} binding sites in

MrHSP60 and 1 Mg^{2+} binding sites in MrHSP90 while pointing out the need for deeper analysis [22].

3. HSPs response to the presence of metal ions in humans and vertebrates

The small HSPs (sHSPs) consist of 150–250 amino acid residues and have a highly conserved α-crystallin domain in their structure [23,24]. This domain is involved in dimer formation, with either two identical or different sHSP monomers [25–28]. Interestingly, in the case of yeast HSP26, the middle domain was identified as a client-independent interface, while the α-crystallin domain and the C-terminus determined client specificity [29]. Cross-linking mass spectrometry (XL-MS) used in this study, a method that is gaining more attention nowadays, enables the determination of the three-dimensional structure of proteins and protein-protein interaction [30]. In general, to apply this method, a crosslinker (a chemical compound with a specific mass and length) must be inserted between two side chains of amino acid residues. Because of the covalent bond, the cross-linked amino acids and the distance between them can be identified by MS. Since the amino acids occupy specific positions in the protein along with the length of the linker, distance constraints arise in the 3D structure of the protein or complex [29,30]. However, protein binding is not the only crucial factor. Proper reversible association and dissociation of oligomeric subunits are critical for the proper function of HSPB1 [31]. Mutations in genes that encode regions of the protein responsible for oligomer formation are likely to be associated with neurodegenerative diseases [32–34]. Such mutations lead to changes in the quaternary structure, incomplete dissociation of large oligomers after phosphorylation by MAPKAP kinase 2, and other disturbances in reversible association and dissociation of oligomers and their stability, ultimately affecting interactions with other proteins. A consequence of this phenomenon is always a decrease in HSPB1 chaperone-like activity [27,31,35,36]. The formation of oligomeric complexes can be checked by SDS PAGE, while the quaternary structure of oligomers can be studied by dynamic light scattering (DLS) or size exclusion chromatography [36].

3.1. HSPB1

Iron is an essential metal ion for both prokaryotic and eukaryotic organisms. It is involved in many cellular processes and can be found in the center of enzymes responsible for redox reactions [37].

The iron transport mechanism in humans centers around two proteins, namely transferrin (Tf) and transferrin receptor 1 (TfR1). Circulating iron is bound to Tf and remains in the Fe^{3+} form. The membrane protein TfR1 transports two Tfs (each is bound with two iron ions) through the cell membrane and facilitates localization within the endosome. Iron release from endosomes is followed by hydrolysis of the Tf-TfR1 complex, after which TfR1 returns to the cell membrane [38–40]. The process of iron release via TfR1 endocytosis and recycling was found to be down-regulated by HSPB1 [41]. The mechanism of this down-regulation includes stabilization of the cortical actin cytoskeleton, which acts as a barrier that blocks off the movement of both the TfR1-Tf-containing cytoplasmic vesicles from the cell surface and the recycling of the vesicles. Co-localization of actin and HSPB1 was revealed by immunofluorescence imaging. Labeled antibodies against Hsp27 (also known as HSPB1) were used to visualize HSPB1 and rhodamine-phalloidin staining was used to visualize actin filaments. The involvement of ferroportin-1, an iron exporter, was tested in the same studies, but its expression was HSPB1-independent.

Interestingly, the studies of Sun et al. have shown that overexpression of HSPB1 is not only a negative regulator of intracellular accumulation and uptake of iron, but that the protein itself also inhibits erastin-induced iron uptake and ferroptosis, an iron-dependent programmed cell death [42,43].

3.2. Crystallin

The effect of metal ions varies not only from organism to organism, but also from tissue to tissue and molecules that interact with the ions. In the case of HSPs, the interaction can lead to an increase in the thermodynamic stability of the oligomers, as in the case of α-crystallin and Zn^{2+} ions [44]. α-Crystallin is the common name of oligomers formed by two sHSPs, namely CRYAA (HSPB4, also αA-crystallin) and CRYAB (HSPB5, αB-crystallin). The structure of α-crystallin oligomers, with an average mass of 800 kDa, is closely related to their function as a chaperone in the vertebrate lens. It protects the lens from protein aggregation during thermal stress, and it may play a protective role against cataract formation [45]. Since its proper function is required throughout life, α-crystallin must remain stable indefinitely. The Zn^{2+}-dependent inter-subunit bridge was found to increase the thermodynamic stability of α-crystallin oligomers, making them resistant to dissociation in 6 M urea solutions, while the zinc ions themselves could be removed by dialysis [44]. In further studies, it was found that the Zn^{2+} binding residues in α-crystallin are His residues, i.e., H79, H107, and H115 of CRYAA and H104, H111, and H119 of CRYAB [46]. Therefore, a deficiency of Zn^{2+} ions associated with age may lead to a decrease in the stability of α-crystallin oligomers and may be a risk factor for the development of cataracts.

During cataract formation, the interaction of α-crystallin with Ca^{2+} ions also plays an important role (Fig. 1). The chaperone activity of α-crystallin in the presence of Ca^{2+} is decreased, likely due to the conformational changes that lead to aggregation of the protein and exposure of the Tyr and Trp residues (together with the remainder of the hydrophobic domain) [47]. Such conformational changes impair the ability of α-crystallin to bind to other proteins due to the loss of stability of the secondary, tertiary, and quaternary structures. In addition, this process leads to an accumulation of partially misfolded α-crystallin, reducing the natural protection of the lens. Increased Ca^{2+} levels in the eyeball were observed in patients with cataracts, aging, and chronic hyperglycemia, and such disturbances in Ca^{2+} homeostasis could be considered a risk factor for the development of cataracts in diabetic patients [48–50]. Interestingly, glycated β- and γ-crystallins have been shown to partially resist Ca^{2+}-induced aggregation [51]. However, these sHSPs are important potential reservoirs for Ca^{2+} sequestration and changes in their structure that occur in diabetes may lead to a decrease in intracellular Ca^{2+} buffering capacity. A dangerous accumulation of Ca^{2+} under hyperglycemic conditions may lead to the activation of various pathogenic pathways and result in lens opacification and cataract formation.

The binding of Cu^{2+} by CRYAB has also been investigated [52,53]. Residues of the N-terminus and middle domain, namely His101, His119, Lys121, His18 and Glu99 (Lys and Glu complementing the coordination sphere), have been suggested as being able to anchor and bind Cu^{2+}. The studies have been done by spectroscopic investigations and molecular modeling [53]. Despite His residues being common for Cu^{2+} complex formation in many proteins [54–56], the importance of identifying these His residues was not confirmed through His-to-Ala modifications [57]. However, seven peptide fragments with potential Cu^{2+}-binding histidine residues were identified by on-column trypsinization followed by MALDI-TOF mass spectrometry, finding 3 residues at the N-terminal domain and 4 at the C-terminal domain. Later, it was found by NMR investigations that His and Asp residues, namely H83, H104, H111, and D109 at the dimer interface, are involved in the formation of the copper complex with picomolar binding affinity [52]. This model showed similarity to that of the Zn^{2+}-binding model. Moreover, the sequence

of amino acids present in the binding loop is also conserved in human CRYAA and HSPB1.

The researchers suggest that HSP expression does not occur solely in direct response to the unwanted presence of metal ions. In HeLa cells, the heat shock protein response can be triggered by metal ion-induced oxidative stress [58]. In HeLa cells transformed with the HSE-SEAP (heat shock element-secreted alkaline phosphatase) reporter gene, the expression of HSPs was examined in correlation with ROS production after the cells were incubated with different metal ions. This data suggested that HSP expression following metal ion exposure is due to an increase in cellular superoxide anion levels. Interestingly, among the many metal ions tested, the strongest HSP inducer was Cd^{2+}, followed by Hg^{2+} and Pb^{2+}, while Cu^{2+}, Al^{3+}, and Ni^{2+} all exerted relatively weak effects on HSPs expression. The two last metal ions, Zn^{2+} and Mn^{2+}, did not affect the HSPs even at the highest studied concentration (of 200 μM). As stated by the authors, the increase in HSP expression is proportional to the increased concentration of superoxide, and therefore HSP may be an indicator for superoxide anion elevation, or the HSP-responding reporter genes may be used to track the dynamic level of superoxide anions or metal-induced oxidative stress.

4. HSPs response to the presence of metal ions in invertebrates and microorganisms

HSPs are found in many organisms, from bacteria to copepods to humans and play a mainly defensive role. Their expression is not only correlated to temperature-related stress, but they are often expressed in response to variable and adverse environmental conditions, such as the presence of heavy metals in the environment (Table 1). Some metals are important for the proper functioning of an organism, but some of them adversely affect its function. HSPs can protect proteins in the body from abnormal folding caused by heavy metals [59]. For example, increased expression of HSPs has been observed in earthworm immune cells (coelomocytes) after skin exposure to metal ions [60].

The nematode *Caenorhabditis elegans* (*C. elegans*) has been widely used as a model organism for many years. In *C. elegans,* there are small HSPs (sHSPs), such as CeHSP17, whose expression can be induced by heavy metal ions such as Cd^{2+} and Zn^{2+}. The increased concentration of cadmium had a greater effect on the level of the heat shock protein tested than that of zinc [61]. Moreover, Ezemaduka et al. demonstrated that the use of two stress factors, temperature and heavy metals, increased the expression level of CeHSP17, with zinc having a much stronger effect than cadmium. Furthermore, in another study, the exposure of *C. elegans* to copper, arsenic and glyphosate (GPS) was tested for their effect on the HSPs response [62]. Interestingly, GPS alone did not cause any significant increase in HSPs synthesis. However, Cu and As ions caused increased expression of Hsp70, both individually and as a mixture. Concurrently, the expression of Hsp90 was not significantly different from those of the control. The authors concluded that Hsp70 could be a sensitive biomarker of copper and arsenic individually or as a mixture of components. Therefore, another method for assessing Cd and Zn ions could be CeHSP17, the sHSP from *C. elegans* [61]. Cytotoxic levels of these metal ions were found to moderately increase the expression of CeHSP17 in *C. elegans* after an 8-h exposure.

Table 1
List of HSPs involved in response to a specific metal ion in different species.

Species	HSP	Metal ion
Caenorhabditis elegans	CeHSP17	Zn
Tigriopus japonicus	Hsp20	Cu
Musca domestica	MdomHsps10	Cr
Sinonovacula constricta	ScsHsp	Pb
Artemia franciscana	HSP70	Cd
Candida albicans	CaHSP12p	Cd
Saccharomyces cerevisiae	ScHSP12P	Cd
Helicobacter pylori	HspA	Ni
Crassostrea gigas	Hsp	Pb
Mytilus galloprovincialis	MgHSPA12A	Cd

Another organism that has been studied for the influence of heavy metal-induced environmental stress is the copepod *Tigriopus japonicus*. Many HSPs are expressed in this model organism. In their research, Kim et al. checked the impact of heavy metals on the formation of ROS, as well as their influence on the expression of HSPs. It was shown that among the metals examined (Cu, Zn, Ag, As, Cd), copper ions had the greatest influence on increasing the expression of Hsp20 and Hsp70, and this research confirms that the mentioned proteins could be treated as biomarkers. In addition, most of the heavy metals tested influenced ROS formation, except for Cd^{2+} and Zn^{2+}, that, despite testing different concentrations, did not produce a detectable effect [63].

sHSPs, characterized by lower molecular weight (12–43 kDa), are found in many organisms. They are also responsible for protection against heat shock, starvation, and heavy metals. One of the organisms in which sHSPs are found is the housefly *Musca domestica*; from the name of the housefly, the proteins are called MdomHSPs. Tian et al. investigated the effect of heavy metals (Pb, Cr, and Cd) on the expression of MdomHSPs27, MdomHSPs27.1, MdomHSPs27.2, and MdomHSPs10 proteins in 4-day-old larvae [64]. As it turns out, the presence of metals had the greatest effect on MdomHSPs10, a noticeable effect on MdomHSPs27. MdomHSPs10 was most expressed in the presence of chromium.

The investigation by Zhang et al. examined whether cadmium and lead ions had any influence on the expression of sHSPs in *Sinonovacula constricta* (razor clam). The expression of ScsHSP is related to the concentration, and exposure duration to the heavy metals. It was found that lead had a stronger effect than cadmium in both gills and hemocytes. Notably, after 12 h of exposure, the expression levels of ScsHsp mRNA were 100-fold higher when Pb^{2+} was used in comparison to Cd^{2+}, at the same concentration [59].

As the action of various stress factors can influence the production of HSPs and modify their levels, previous authors examined the influence of metals (Cd^{2+} and Zn^{2+}) on the production of HSPs after exposure to non-lethal heat shock (NLHS) in the organism of *Artemia franciscana* (shrimp). Prior treatment of the shrimp with NLHS induced cross-tolerance to both Cd^{2+} and Zn^{2+}, although HSP70 expression was induced by Cd^{2+}, not Zn^{2+} treatment [65].

Many researchers point to the influence of heavy metals on the production of HSPs in plants. To this end, Heckathorn et al. investigated the effect of four metals, namely Cu, Pb, Ni, and Zn, on sHsp production and showed that the level of chloroplast sHsp was similar for all heavy metals and generally increased with increasing metal concentration [66,67]. The protective role of chloroplast sHSPs on photosynthetic electron transport in the presence of Pb^{2+} has been shown previously. In addition, an increase in sHSP production was observed to protect photosynthesis from the presence of Pb and Ni ions.

It has also been demonstrated that the sHSP OsMSR3 is involved in multiple mechanisms aimed at overcoming the negative effects of Cu stress in *Arabidopsis thaliana*, that can result from soil pollution, among others [68].

Candida albicans, which is a dimorphic fungus belonging to the human microflora, also produce HSPs in response to the increased concentration of metals. Man-Shun Fu et al. demonstrated that *C. albicans,* in the presence of 0.5 mM cadmium ions, increased CaHSP12p expression. Similar observations were made with *Saccharomyces cerevisiae,* for which a Cd^{2+} concentration of 0.1 mM was sufficient for the expression of the sHSP ScHSP12p [69].

HspA is an sHSP found in *Helicobacter pylori* and has been shown to bind Ni^{2+} and Bi^{3+} in both the apoprotein and native protein

forms. Cun et al. discovered that HspA binds bismuth and nickel in irreversible and reversible manners, respectively [70]. The authors suggested a possible effect of reversible nickel binding on homeostasis and pointed out the need for further research in the case of bismuth.

The oyster *Crassostrea gigas*, which lives in the Pacific Ocean and is known as a bioindicator of heavy metal pollution, is representative of mollusks that have adapted to survive in highly stressful conditions. Next-generation sequencing (NGS) and hierarchical assembly were used to determine gene expression changes under different stressors [71]. The researchers found that the largest response of genes, apart from air exposure (4420 differentially expressed genes), concerns stress caused by the presence of heavy metals such as zinc, cadmium, copper, lead and mercury.

Studies conducted on *Mytilus galloprovincialis* demonstrate that, at certain concentrations, cadmium has a significant effect on the increased expression of MgHSPA12A, a member of the HSP70 family [72], most likely related to the production of ROS. However, in both *Mytilus galloprovincialis* and other organisms (e.g., *Venerupis philippinarum* [73]), high concentrations of heavy metals have been found to reduce the organism's ability to respond to ROS, resulting in decreased expression of antioxidant enzymes.

The situation is slightly different in the case of long-term (up to 15 days) exposure of *Patinopecten yessoensis* scallops to the presence of toxic dinoflagellate *Alexandrium catenella*, which caused a decline phase and re-expression of HSP70 genes after a period of adaptation and return to homeostasis [74]. As shown above, invertebrates and microorganisms can alter the level of HSP expression depending on the presence of toxins and heavy metals in their environment and the timing of exposure.

5. Conclusions

HSPs are expressed by all living species and act as chaperones that protect cells from the accumulation of misfolded proteins. However, they may also be a line of defense against metal ion poisoning that results from environmental pollution. While some HSPs require metal ions for their function, some of them are involved in processes that are metal ion-dependent, and their expression may influence this progression. Nevertheless, the question remains whether HSPs are expressed in response to the presence of metal ions or to metal-induced oxidative stress in all species and how the metal-protein interactions may be exploited?

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