REVIEW ARTICLE

Does overproduction of chaperone proteins favour the repair of DNA injuries induced by oxidative stress? (Mini review)

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Abstract

Genotype resistance to oxidative stress, induced by various physical/chemical stimuli has been the focus of scientists for the last decades, with several aspects – ecological (the formation of the genetic elite of population), agricultural and medical (radio-chemotherapy).

Genotype resistance to oxidative stress is regarded as the integration of different morphological, physiological, biochemical, metabolic and genetic characteristics. Currently, it is supposed that the mechanisms involved in the formation of genotype resistance to oxidative stress are inter-correlated and inter-dependent, comprising changes in genes, proteins, enzymes, different metabolic pathways and/or biological networks. According to the present state of knowledge, various cellular targets, resulting in genotoxic stress, induction of DNA damage, mutations, genomic instability or apoptosis can trigger different signal transduction pathways, activating DNA repair, antioxidant and chaperone defence systems.

Till now, a lot of experimental data have been accumulated concerning the contribution of DNA repair to the formation of genotype resistance to oxidative stress. At the same time, genotype resistance of organisms is largely determined by the ability of molecular chaperones to maintain conformational homeostasis of proteins (folding – misfolding – refolding or aggregation – degradation). The role of chaperones in protein homeostasis and cell death, especially in apoptosis, is well discussed in literature, but much less is known about their function in DNA repair. In this regard, here we addressed the question of whether the overproduction of chaperone proteins contributes to the repair of DNA damage caused by oxidative stress.

In this mini-review article, several items that we believe are of fundamental importance to the given topic have been highlighted.

The first one concerns the term genotype resistance - what exactly does this term mean?

The genotype resistance to oxidative stress is considered as an integration of different morphological (Lipiec et al. 2013; Rai and Agrawal 2017), physiological, biochemical, and metabolic (Badahur et al. 2011; Chankova and Yurina 2012; Lipiec et al. 2013; Marcińska et al. 2013; Wegener and Jansen 2013; Chankova et al. 2014; Rai and Agrawal 2017) and genetic characteristics (Dimova et al. 2008, 2009; Ahuja et al. 2010; Xu et al. 2011; Zinati et al. 2013; Dimitrova et. al. 2014; Todorova et al. 2015, 2019; Marinovska et al. 2022). Currently, it is believed that the mechanisms involved in the formation of genotype resistance to oxidative stress are inter-correlated and inter-dependent and include changes in genes, proteins, enzymes, different metabolic pathways or biological networks (Costantini et al. 2013; Gong and Miller 2019). According to the present state of knowledge, various cellular targets, resulting in genotoxic stress, induction of DNA damage, mutations, genomic instability or apoptosis can trigger different signal transduction pathways activating DNA repair, antioxidant and chaperone defence systems (Toulany 2019; Clementi et al. 2020; Kotob 2021).

The second one concerns the significance of genotype resistance for living organisms and their quality of life

sDuring the last decade, genotype resistance to oxidative stress, induced by various physical/chemical stimuli has been a focus of scientists in many aspects of science – ecological (the formation of the genetic elite of the population, adaptation in the target regions), medical (disease resistance, radio- chemotherapy), agronomics – tolerance/resistance to different abiotic and biotic environmental factors. The first ones who proposed the name "genetic elite" were Dobzhansky and Spassky in the far 1963 (Dobzhansky and Spassky 1963). They have understood "genetic elite" as "...genotypes whose fitness is greater than two standard deviations above the population mean. These elite lines have similar essential alleles for desirable end-use characteristics, agronomics, disease resistance and adaptation in the target region...".

The third one is focused on the possible mechanisms involved in the formation of genotype resistance

Over the years, much data concerning the contribution of DNA repair, chaperone and antioxidant repair systems for the formation of genotype and induced resistance have been collected. Additionally, the contribution of other factors, such as high levels of constitutive and induced levels of SOD, SH-groups, the presence of cell wall, stability of ultra-structural compartments of cells, phases of the mitotic cycle, the energy provision of cells and others has been clarified (Chankova et al. 2000; Goldberg and Lehnert 2002; Marnett et al. 2003; Ramotar and Wang 2003; Schaue and McBride 2005; Bao et al. 2006; Chalmers 2007; Chankova and Yurina 2012, 2016; Wu et al. 2017).

DNA permanently is the main target of different damaging endogenous factors as a result of the work of cells metabolism machinery and exogenous factors – climate changes, ionising (IR) and non-ionising (UV) radiation, as well as various chemicals, drugs etc. This fact results in:

• The induction of different types of DNA damage as single-strand breaks (SSBs), double-strand breaks (DSBs), base and nucleotide modifications, as well as cross-links and dimers. The type of induced injuries depends on many factors (specific action of physical or chemical agent, species, tissue, age, experimental design, cell cycle and physiological state etc.) (Chankova et al. 2000, 2007, 2009; Sottile and Nadin 2018; Miteva et al. 2020; Penninckx et al. 2021).

• The induction of a complex system for recognition and activation of multiple defence systems, named DNA Damage Response mechanism (DDR) has evolved evolutionarily. DDR involves the detection of DNA injuries, DNA repair or apoptosis (Guénolé et al. 2013; Sottile and Nadin 2018; Gong and Miller 2019).

For some time, the question concerning the relationships between genotype resistance and the contribution of DNA susceptibility and/or efficiency of DSBs repair has been under discussion. Why was our attention focused on induced DSBs and their recovery?

Here, it is necessary to mention that DSBs are believed to be the most lethal for living organisms (Kelm et al. 2022). It was found by Khozooei et al. (2022) that non-repaired DSBs can induce cell death in radio-sensitised triple-negative breast cancer cells.

As it was described by Sottile and Nadin (2018) and Li et al. (2021), DNA repair mechanisms could be split into five categories: direct reversion of DNA damage, base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER) and homologous recombination (HR) and non-homologous end joining (NHEJ) (Kciuk et al. 2020).

Quantification of radiation-induced DNA double-strand breaks is a good tool for the evaluation and prediction of cells/organisms' response to IR (Penninckx et al. 2021), especially in the case of occupational exposure (Kvitko et al. 2012), accidents and medical purposes.

In order to gain an insight into the mechanisms of genotype resistance, two main relationships should be clarified: the contribution of DNA susceptibility to this process and the contribution of DSBs repair capacity.

Currently, little is known about the possible role of DNA susceptibility, as well as DNA repair capacity in the formation of genotype resistance. Data in literature are very contradictory. Some of them have confirmed the crucial importance of DNA susceptibility for this process. For example, a significant correlation between initially induced levels of DSBs and cell radio-sensitivity of tumour cell lines has been reported by el Awady et al. (2003). Using constant-field gel electrophoresis (CFGE), the same correlation has been obtained by Saleh et al. (2012) concerning cells' sensitivity to cis-platin (CIS).

To clarify, the contribution of increased DNA repair capacity to the formation of genotype resistance is up-to-date because it relates to problems of radio-chemo-therapy (Ghahe et al. 2021; Kelm et al. 2022). Data have been gathered, identifying that several factors including upregulation of DNA repair, especially DSBs and activation of DDR can promote tumour resistance to therapy, inducing an adaptive response. It was described by Ghahe et al. (2021) that increased glioblastoma cells' resistance to photodynamic therapy (PDT) is a result of accelerated repair of BER and DNA breaks, as well as DNA damage signalling. The finding that accelerated DNA repair essentially can contribute to the elevation of tumour resistance to the treatment provides a new perspective on treatment using DSBs repair targeted inhibitors (Kelm et al. 2022).

As was pointed out at the beginning of this mini-review, genotype resistance is of great concern to agriculture and the environment. Víquez-Zamora et al. (2022), by characterising the DNA repair capacity of the US inbred lines B73 and Mo17, as well as Central American maize landraces from Guatemala and Costa Rica, have directed this molecular approach for the breeding of more tolerant to DNA damaging environmental factors plants.

Our own results, using mutant strains or extremophile species of unicellular green algae, as well as *Saccharomyces cerevisiae* strains, demonstrated that differences in DSB's repair capacity are probably one of the main mechanisms involved in the formation of genotype resistance to chemical and physical factors. In Fig. 1, the DSBs' repair capacity of *Chlamydomonas reinhardtii* strains – 137C WT, CW15 cell-wall less with WT radio-resistance and H-3 –highly radio-resistant hybrid strain obtained by mating CW15 × AK-9-9 (mutant strain constructed by us using chemical mutagenesis approach) (Chankova et al. 2005; Dimova et al. 2009) are compared. The variation in repair capacity is clear. The most radio-resistant hybrid strain H-3 expresses several-fold higher potential to repair DSBs by accelerating DSBs' rejoining.

A similar picture was obtained in *Saccharomyces cerevisiae* strains (Fig. 2) The curves in Fig. 2 illustrate the relationship between DNA susceptibility, measured as the level of primary Zeocin-induced DNAs and repair capacity. A strain BY4741 that exhibits less DNA susceptibility, i.e. has a more resistant genotype to the radiomimetic Zeocin, is characterised by more effective DVR repair.
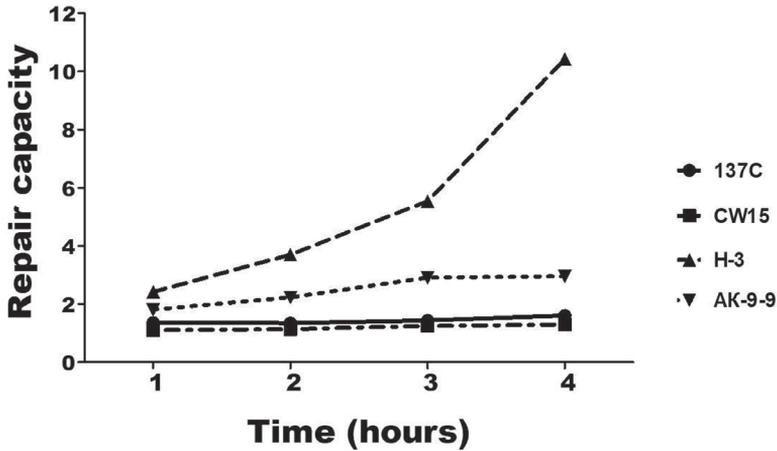
Figure 1. DSBs' repair capacity of WT 137C and CW15 and radio-resistant strains – H3 and AK-9-9 of *Chlamydomonas reinhardtii*.
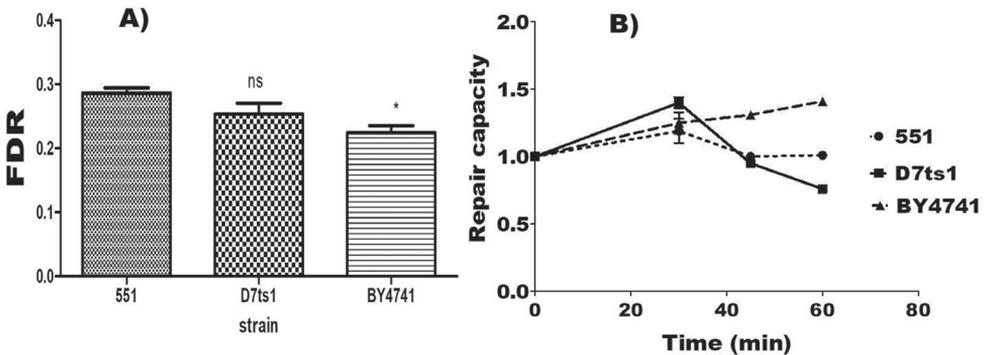
Figure 2. DNA susceptibility of *Saccharomyces cerevisiae* strains 551, D7ts1 and BY4741 measured as DSBs' induction (**A**) and DSBs' repair capacity (**B**). FDR – fraction damages released.

The differences between repair capacity of *Chlamydomonas reinhardtii* and *Saccharomyces cerevisiae* strains and extremophiles *Chlorella vulgaris* are probably amongst the mechanisms involved in the formation of cells' resistance to different inducers of oxidative stress through the acceleration of DSBs' repair rejoining (Dimitrova et al. 2014, 2022; Miteva et al. 2020; Marinovska et al. 2022). Using *Chlamydomonas reinhardtii* mutants – UVS-10 - *rec*- repair deficient and UVS-14 - mismatch repair deficient, the role of these two types of DNA repair for the formation of genotype resistance to different DNA damaging factors was confirmed (Dimitrova et al. 2022).

At the same time, high constitutive levels and overproduction of HSP70B were identified for more resistant *Chlamydomonas reinhardtii* strains and *Chlorella vulgaris* species after the induction of oxidative stress by various physical or chemical stressors (Chankova et al. 2013; Miteva et al. 2020)

About the contribution of the chaperone system in the formation of genotype resistance

Genotype stability of organisms is determined to a large extent by the ability of molecular chaperones to maintain conformational homeostasis of proteins (folding, improper folding, re-folding or aggregation - degradation). Heat shock proteins (HSPs) occupy one of the main places amongst biological protective reactions to oxidative stress (Chen et al. 2018). The role of chaperones in protein homeostasis and cell death, especially apoptosis, is widely described in literature, but much less is known about their function in DNA repair processes. In this regard, studies of the interdependence of the DNA double-strand break repair system and the activity of the chaperone system attract much attention (Sottile and Nadin 2018; Dubrez et al. 2020).

Heat shock proteins (HSPs) are found in all living organisms. Based on their molecular weight and cell functions, HSPs are classified into several families - small HSPs with mol. mass from 10 to 30 kDa and HSP40s, HSP60s, HSP70s, HSP90 and HSP100 (Al-Whaibi 2010; Ul Haq et al. 2019; Dubrez et al. 2020). It should be mentioned that the abbreviations of bacterial HSPs differ from those in eukaryotic cells as could be seen in Table 1.

Table 1. HSP of prokaryotic and eukaryotic cells.

Bacterial proteins	Eukaryotic proteins
Clp B	HSP100
Htp G	HSP90
Dna K	HSP70
GroEL	HSP60
Dna J	HSP40
Ibp A, Grp E	HSP20, HSP27
Gro ES	HSP10

Of particular interest are small sHSP, HSP70 and HSP90. Today, studies about their particular contribution to DNA damage sensing, signalling and repair are in a progress (Pennisi et al. 2015; Dubrez et al. 2020).

Below, HSP groups, related to the topic of the mini-review, are presented briefly.

Low-molecular-weight sHSP proteins are ancient proteins characterised by the presence of the main domain of α-crystalline. Under stressful conditions, sHSP prevents irreversible aggregation of unfolding proteins by integrating into the resulting protein aggregates. sHSP- containing aggregates have easier access to Hsp70 and ClpB/Hsp104 chaperones. These chaperones in ATP-dependent reactions secrete individual proteins from aggregates and contribute to their refolding into the native state (Rutgers et al. 2017).

The most numerous group of sHSP was found in higher plants and algae (19 in Arabidopsis, 23 in rice and 39 in poplar) than in Volvocales species (8 in *Chlamydomonas reinhardtii*, 7 in *Volvox carteri* and 6 in *Gonium pectorale*) (Rutgers et al. 2017). The more complex HSP system in plants compared to animals may be due to the sessile lifestyle that not allows them to avoid stressful conditions.

A comprehensive genome-wide analysis was used to identify and characterise the functional dynamics of the HSP20 gene family. Advances in whole genome sequencing have made it possible to detect all the suspected HSP genes, their duplication and their diversification. For example, this has allowed Hu et al (2021) to construct a phylogenetic tree of members of the HSP20 family showing by its example that a total of 33 HSP20 genes distributed across 13 chromosomes were identified from the genome.

The expression levels of HSP20 genes were differentially induced by heat stress. The transcript level of six proteins was down-regulated by heat stress, while twelve were up-regulated by heat stress. The last proteins are very interesting because they could be used as heat tolerance candidate genes (Hu et al. 2021).

HSPs are pleiotropic proteins involved in a variety of biochemical processes and perform many important functions in eukaryotes, as well as contribute to enhanced stress tolerance/resistance. HSP70 is the most universally induced chaperone in response to various cellular stressors, such as UV radiation, gamma radiation and chemicals. The HSP70 chaperone network implements diverse housekeeping- and stress-related activities. The HSP70 chaperones participate in a wide range of cellular housekeeping functions - the folding of newly-synthesised proteins, the translocation of polypeptides into mitochondria, chloroplasts and the endoplasmic reticulum, the assembly and disassembly of protein complexes, regulation of protein activity, assisting in the HSP90 folding machinery and chaperonins (Rosenzweig et al. 2019). Stress-related activities of HSP70 are associated with preventing the aggregation of proteins, solubilising aggregated proteins, promoting the refolding of misfolded or unfolded proteins, cooperating with cellular degradation machinery, such as the ubiquitin-proteasome system, to clear aberrant proteins and protein aggregates (Rosenzweig et al. 2019).

Representatives of another chaperone family, HSP90, are localised in the cytosol in the absence of stress. The main function of HSP90 is to regulate protein metabolism, ensure protein stability and participate in intracellular protein transport. Usually, the chaperone HSP90 acts in combination with other chaperones, such as HSP70 (Dubrez et al. 2020). Several years ago, it was reported that cancer cells are characterised by over-expression of HSP90 chaperone and this poses a new challenge for cancer treatment (Pennisi et al. 2015).

HSP70B is a good marker of oxidative stress and stress state of cells

The synthesis of chaperones is induced and depends on abiotic and biotic stresses and, thus, the content of HSP can be a useful indicator of stress and stress reactions in various organisms. Previously, we have compared the heat stress response of two extremophiles - *Chlorella vulgaris* strain Antarctic, isolated from the soil of the Antarctic and 8/1 – thermophile, isolated from the hot spring Rupite in Bulgaria with those of *Chlorella keslerii* – mesophilic strain. Both higher constitutive levels and well-marked overproduction of HSP70B were obtained for *C. vulgaris* Antarctic strain – Fig. 3 (Chankova et al. 2013). It was also shown that the overproduction of HSP70 in this strain correlates with the increased resistance to UV - B irradiation and well-expressed
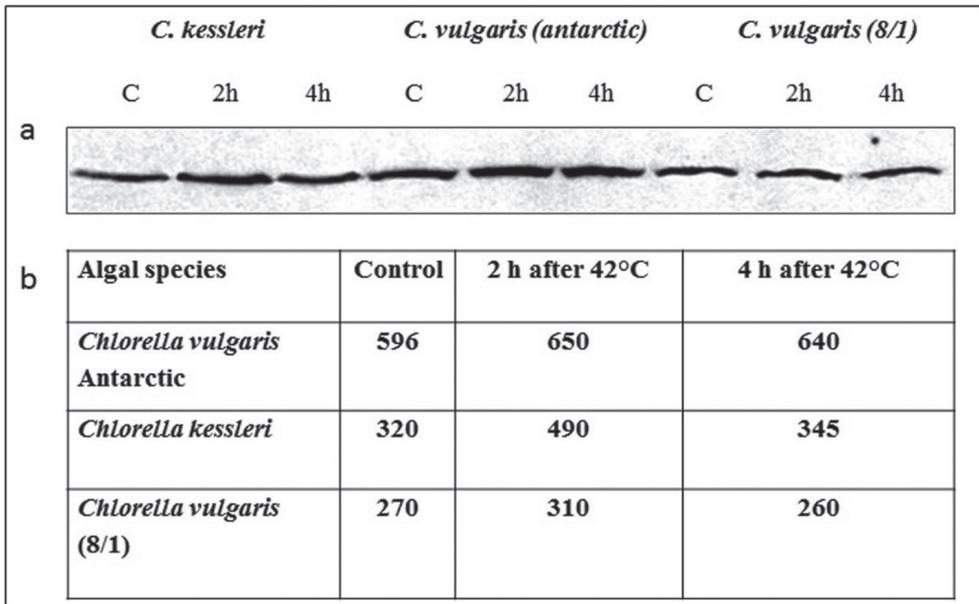
Figure 3. Comparison of the HSP70B level in *Chlorella* after heat stress (42 °C for 5 min) **a** western blot analysis of the HSP70B level in *Chlorella* cells incubated at heat-stress temperature **b** densitometry of the HSP70B contents; C - control sample.

photo-reactivation and dark repair (Miteva et al. 2020). Here, we can speculate that probably due to the higher constitutive level and well-marked overproduction of HSP70B, as well as effective DNA repair systems, this strain can survive in the extreme environment of Antarctica. Our finding contributes to the hypothesis of the conserved functional properties of HSP70B as a mechanism of thermo-tolerance in plants.

Heat Shock Transcription Factors (HSF) are the main regulators of HSPs

The expression of the HSP genes is mainly regulated by heat shock transcription factors (HSFs). HSFs are a group of evolutionarily conservative regulatory proteins present in all eukaryotes and regulating various responses to stress and biological processes in plants.

Plants have a more complex response to stress than yeast and animals, which may be due to their sessile nature. So, for example - the HSF family of plants contains 18-52 members, while in yeast and Drosophila, it is represented by single copies of HSF, in mammals - 4 HSFs (Andrasi et al. 2020; Tian et al. 2021). Despite significant variation in the number and sequence of HSFs, their structure and functions are highly conserved across plant species.

HSF contains a conserved DNA-binding domain at the N-end of the protein that recognises the DNA motif of 11 nucleotides: 5'-nGAAnnTTCn-3'. This motif is usually found in the promoter region of HSF-regulated genes (Tian et al. 2021).

Plants are simultaneously exposed to many types of stress (abiotic and biotic) that result in oxidative or secondary stress. Plants' response to heat stress is regulated by Heat shock transcription factors (HSFs), which bind to *cis*-acting elements known as HSE (heat shock elements).

Three domains have been identified in the HSFs structure: the DNA-binding domain, the oligomerisation domain and the C-terminal activation domain. Based on the differences in the composition of these domains, the HSFs of plants are divided into three classes: A, B and C which differ in their functions. Amongst HSFs, HSFA apparently plays a unique function as the main regulator of acquired thermal tolerance. Under normal conditions, HSFA activity is inactivated by HSP90. Under stress, this repression is reversed and HSF changes into the functional trimer state. This HSFA trimer then binds to heat shock elements (HSE) in the promoter region of the genes, transcription occurs and HSPs are synthesised (Fig. 4). HSFs regulate the down-stream HSPs, antioxidant enzyme genes, which help the plants to develop stress tolerance.

HSFs' class B and C factors have been scarcely studied and in fewer plant species. So, in contrast to the activity of class A HSFs, the class B HSFs factors lack the C-terminal activation domain and have a transcription repression domain at the C-terminus of the
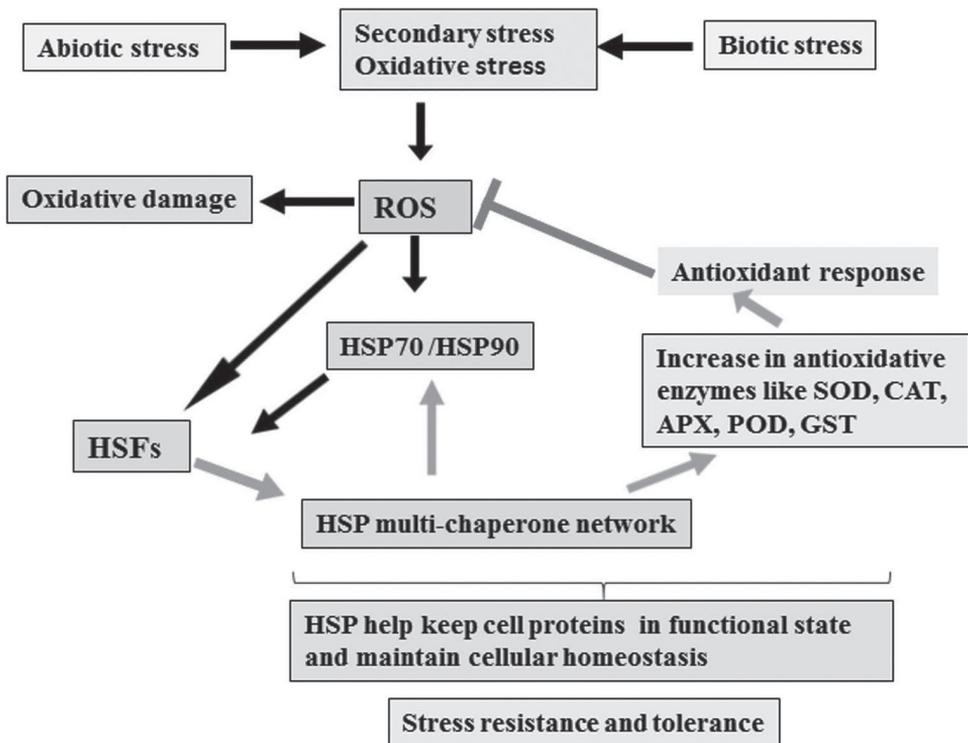
Figure 4. Scheme of the HSP transcriptional regulation, illustrating HSFs activation and their interaction with the other pathways to counter abiotic and biotic stress. ROS (Reactive oxygen species), HSF (Heat shock transcription factor), HSP (Heat shock protein), APX (Ascorbate peroxidase), GST (Glutathione-s-transferase), SOD (Superoxide dismutase), POD (Peroxidase), CAT (Catalase).

protein (Tian et al. 2021). The study of new HSF class B genes has shown that they play an important role in the response of plants to biotic and abiotic stresses (Peng et al. 2013).

Plants' heat shock proteins play a key role in ensuring plant resistance to stress through different mechanisms. They can use ROS as a signal to induce HSF and HSP biosynthesis (see Fig. 4), can increase the stability of membranes and can detoxify reactive oxygen species (ROS) and can positively regulate antioxidant enzyme systems (Ul Haq et al. 2019).

When plants are exposed to stress, the synthesis of normal proteins is decreased while the expression of stress genes is up-regulated and, as a result, the synthesis of HSPs is triggered. HSP gene expression positively regulates protective enzyme activities. So, for example, in Arabidopsis, over-expression of small HSP17.8 enhanced the SOD activity and, in tobacco, HSP16.9 increased the activities of peroxidase - POD, catalase – CAT and superoxide dismutase – SOD (Driedonks et al. 2015; Ul Haq et al. 2019).

HSF and HSP form a complex regulatory network in response to stress. With the rapid development of transcriptome sequencing technology and an increase in the volume of big data in publicly available databases, it has become possible to use networks of joint gene expression to study possible ways of regulating the stress response of the cell and protein-protein interactions (Tian et al. 2021).

On the possible relationship between HSP and DNA repair pathways

The potential role of heat-shock proteins in both cellular carcinogenesis and/or their contribution to DNA repair machinery has been under discussion over the last decade. This problem is closely related to mechanisms of carcinogenesis, as well as anti-cancer therapy and increased resistance of some tumours to medical treatment (Kang et al. 2015; Pennisi et al. 2015; Dubrez et al. 2020).

The HSP chaperoning system is associated with the reaction to DNA damage and can directly regulate the signalling pathways of DNA repair. In response to DNA damage, adaptive coordinated defence mechanisms are activated in cells. Depending on the nature of DNA damage, various DNA repair pathways will be involved. Damage affecting only one of the two DNA strands, such as single-stranded breaks (SSBs), is the most common type of damage. In mammals, there are several ways to repair single-stranded DNA breaks. The first pathway is base excision repair (BER). The second pathway is mismatch repair (MMR). The third pathway is the nucleotide excision repair system - NER (Sottile and Nadin 2018; Dubrez et al. 2020).

What is currently known about HSPs contribution to the regulation of SSB and DSBs repair? HSP70 cooperates with small HSP27 and HSP90 to reactivate misfolded substrates. Inducible HSP70 confers cell resistance against radiation and chemotherapeutic agents and facilitates DNA damage repair. HSP90 generally acts downstream of HSP70, during the later folding steps (Sottile and Nadin 2018; Dubrez et al. 2020). In the last years, HSP90 has been found in association with chromatin-bound proteins. Thus, HSP90 has emerged as a potent regulator of nuclear processes including DNA repair and transcription. Since HSP90 is overexpressed in a large variety of tumours, it is an attractive target for anti-cancer therapy.

Table 2. HSP chaperones regulate the repair of double-stranded DNA breaks.

Pathway	DNA lesions	DSB detection	DNA resection and exchange strands	DNA-polymerase/ Ligase
Non-homologous end-joining (NHEJ)	Ionising radiation, X-rays, chemicals	HSP27	HSP110, HSP90	DNA synthesis, incision and ligation
Homologous recombination (HR)	Ionising radiation, X-rays, chemicals	HSP27, HSP70, HSP90	HSP90	DNA synthesis, incision and ligation

As it was described previously, double-stranded DNA breaks (DSBs) could be repaired using two main repair mechanisms. The first one is named the non-homologous ends joining repair (NHEJ) and the second one is homologous recombination (HR) repair. As shown in Table 2, the non-homologous ends joining pathway can be regulated by the chaperones HSP27, HSP90 and HSP110, while the chaperones HSP27 and HSP90 regulate the homologous recombination pathway (Sottile and Nadin 2018; Dubrez et al. 2020). It has been shown that HSP90 is required for both repair mechanisms: NHEJ and HR (Steckleina et al. 2012).

It is assumed that the chaperone system is associated with the reaction of cells to DNA damage and can directly regulate the signalling pathways of DNA repair (Dubrez et al. 2020). It has been shown that HSP is rapidly induced when exposed to an agent that damages DNA. Additionally, it should be mentioned that HSPs were identified in DNA repair sites by confocal microscopy (Castro et al. 2015).

Recently, it has been shown that HSP110 can regulate DNA repair signalling pathways in mammals. It was found that, by blocking these chaperones, it is possible to elevate tumour cells' sensitivity to drugs. The HSP chaperoning system is associated with the reaction to DNA damage and can directly regulate the signalling pathways of DNA repair.

In conclusion, it could be summarised that several mechanisms are involved in the formation of genotype resistance:

• Up-regulation of DNA repair, especially DSBs and activation of DDR are of great importance for purposes of agriculture and medical treatment of cancer. The new finding that accelerated DNA repair essentially can contribute to the elevation of tumour resistance to medical treatment provides a new perspective on treatment using DSBs repair targeted inhibitors.

• Chaperones are not directly involved in DNA repair, but contribute to cell's/organisms' survival in stressful conditions due to their numerous interactions with proteins involved in DNA repair. Amongst the different HSPs, some of them - HSP27, HSP70, HSP90 and HSP110 are considered directly involved in the regulation of DNA repair.

• Having in mind the finding that hypersensitivity to anti-cancer therapy could be achieved by blocking the expression of HSP27, HSP70 or Hsp90 genes, new perspectives for cancer treatment are in progress.

• Over-expression of HSP70 genes in stressful conditions resulting in over-production of HSP70B content can be used as an indicator of oxidative stress and

organisms' stress response. This finding is closely related to problems that have given rise to climate change and anthropogenesis and could be used for purposes of agriculture as well as for environmental impact assessment.

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