Heat Shock Proteins: innate immune stimulators of *C. elegans*

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Recognition of microbial pathogens is an important strategy for a host to produce an immune response. These responses cause recruitment of neutrophils, activation of macrophages, and induction of IFN-stimulated genes or certain antimicrobial peptides resulting in direct killing or inhibition of the pathogens. Innate immune system being the first line of defense mounts the immune response needed and when the response is not sufficient it can initiate antigen specific adaptive immunity. Since adaptive immune system fails to differentiate between self and non-self-antigens and requires signals to perform that activity, the evolutionarily conserved innate immune system will be able to provide the instructions and transmit signals to adaptive immune system. Albeit the adaptive immunity is a prolonged process and can store the information of the previous pathogen encounter, boosting the innate immune system by certain adjuvants would easily minimize the role of adaptive immunity in a host system. Adaptive immune response to a particular antigen can be provided by the adjuvants. These adjuvants signal adaptive immune system through the aid of pathogen recognition receptors on innate immune cells.

Heat shock proteins (HSP's) can also act as adjuvants while it involves in both innate and adaptive immunity. In addition to their well-described role in pathogen sensing and host defense, HSP's play a major role in maintaining tissue homeostasis. These proteins maintain a balance between immune stimulation and immunoregulation. HSP's are essential molecular chaperone required for the maintenance and stabilization of housekeeping proteins. Many of these proteins are involved in signal transduction pathways that are critical for survival of the host. HSP's are chaperonic molecules that were originally described to be synthesized in response to heat shock, but their expression also increases following microbial infections to act as chaperones preventing protein denaturation and loss of function.

Caenorhabditis elegans a versatile invertebrate model has a well-defined innate immune system and is a preferable host to study the microbial infections. *C. elegans* lifespan is influenced by insulin signaling (IIS) pathway and a part of this process involves the HSP's too. HSP overexpression significantly prolongs the aging of the nematode. The regulation of HSP is dependent on the expression of Heat shock factor (HSF) which is a transcription factor that acts parallel to the *daf-16* that is directly involved in IIS pathway. HSF signals the chaperonic molecule when there is protein damage due to prolonged heat stress or a bacterial exposure. HSP's eventually prevent protein aggregation in folding and unfolding proteins. Inducing the HSP's will proportionally ameliorate the immunity and thereby extends the lifespan of a host system. In addition to it, the activation of the HSF pathways and their function in the host appear to strengthen innate immune system against bacterial infections.

Keywords: *C. elegans*; Heat shock proteins; Innate immunity; Insulin signaling pathway; Microbial resistance.

1. Introduction

Until recently, the immune system was thought to be solely responsible for the elimination of pathogenic microbial invaders. However, the significance of immune system has broadened effectively in maintaining and restoring the homeostasis of cells following tissue damage **[1]**. In the past decade, scientists directed much of their attention on mechanism and development of adaptive immune system. Despite the fact that adaptive immune system is capable of storing the information of the previous infection, they mount the immune response slow; which probably take more time to respond specifically. In contrast, innate immunity which is in-born provides immediate and essential response to the foreign particle invading a host system. There is very little information about how adaptive immune responses get activated via innate immune signals and the role of innate immunity was probably neglected **[2]**. The fitness and the capability of adaptive immune response primarily rely on the signals received from the innate immune system.

Innate immune system is dynamic and it is difficult to predict the end of its function or the start of the adaptive responses. Moreover, studies are yet to reveal whether adaptive immunity replaces innate immunity or works in parallel **[3]**. In essence, the innate immune response recognizes the host to the arrival of the pathogen; the major question here is how they limit infection and what kind of signals they pass to the adaptive immune system to produce a more specific response. Fever is an old mechanism adapted by the metazoans to get rid of the microbial infections **[4]**. The increase in internal body temperature aids in eliminating the microbial invaders. The raise in the heat can be contributed to several cellular molecules specifically heat shock proteins (HSP's). HSP's serve as molecular chaperones which bind to polypeptide chains or partially folded protein intermediates, preventing misfolding and aggregation **[5].** With an extensively studied nematode model like *Caenorhabditis elegans*, the immune response against the bacterial infections can be effortlessly deduced. Still, the effect of heat on the *C. elegans* and how the HSP's respond during a bacterial infection is less clear. This chapter describes the role of HSP's in promoting the innate immune system of *C. elegans* and the response produced in return to combat against the pathogens.

2. HSP's history and function

The first seminal discovery of HSP's was made by Ritossa in 1960's in the salivary glands of *Drosophila melanogaster* **[6]**. It was shown to produce puffing pattern in the polytene chromosome through robust activation of HSP's when temperature is induced **[7].** The heat shock is implied to respond as a consequence of various proteotoxic insults. The responses produced due to heat shock are highly conserved in all organisms beginning from yeast to humans making it indispensable for survival in a stressful environment **[8]**. HSP's are assigned into 5 families based on the sequence homology and molecular weight; HSP-100, HSP-90, HSP-70, HSP-60 and small HSPs (sHSPs) families **(Table 1)**.

HSP-100 and HSP-40 was reported to depolymerize amyloid fibrils and can assist in nucleotide exchange factor for HSP-70 but also displays chaperone activity **[9, 10, 11].** HSP-100 also performs disaggregase activity that comes under superfamily of ATPases. They afford a variety of other functions such as cell cycle regulation, intracellular trafficking, DNA replication, and protein degradation **[12, 13, 14].** HSP-90 is crucial for maintaining cellular homeostasis by regulating the conformation, activation, and consequent function of over 100 client proteins **[15]**. In addition to it, HSP-90 also regulates unfolded protein response (UPR) by accumulating misfolded proteins in endoplasmic reticulum (ER). This eventually up regulates genes coding the ER chaperone for the proteasome-mediated degradation of misfolded proteins **[16]**. HSP-60 is majorly involved in xenobiotic detoxification and pathogen responsive pathways [**17**]. The members of the HSP-12 family were identified as sHSP's which are expressed mostly during oxidative stress **[18].**

Table.1. List of HSP's and their function in *C. elegans*.

S. No	HSP Family	HSP family members	Localization	Function	Reference
1	HSP-100	HSP-78/clp	Cytosol	Stress tolerance, protein aggregation, disaggregation	19, 20
2	HSP-90	GRP-94, HSP90AB1, TRAP-1	Cytosol/ER	Signal transduction, Stabilize misfolded proteins	21, 22
3	HSP-70	HSP-40 chaperone	Cytosol/ER/Mitochondria	Folding of actin	19, 23
4	HSP-60	HSPD1	Cytosol/ Mitochondria	Refolds and prevents aggregation	23
5	sHSP	HSP-12, HSP-16	Cytosol	Suppresses aggregation, heat inactivation	24, 25

3. HSP's in stress response

C. elegans experience multiple type of stresses namely heat shock, oxidative, hypoxia and osmotic stress during their life span. Among these heat shock and oxidative stress are those that are studied widely **[26]**. Temperature affects the cellular process and requires a repair mechanism that can maintain the cellular homeostasis. In the nematode *C. elegans,* the growth rates vary between 16°C and 26°C. However, the nematodes those are viable at 26°C cannot withstand 27°C and most of them are perishable below 15°C. A slight change from the maintenance temperature can trigger the HSP's response **[27]**. On the other hand, there are various reports that state exposure of nematodes to 35°C would elicit stress response **[28, 21].** Upon increased tolerance against heat stress, the nematode switches between normal to the dauer development, where in, the metabolic rate of nematode alters and providing a steep increase in survival **[29]**. The transition to dauer stage is visualized by the crowding of nematodes in the nematode growth medium **(Fig 1).**

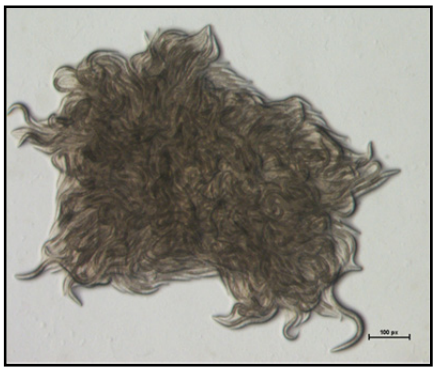

Fig. 1 The picture represents the crowding of nematodes after heat shock at 35°C for 2 hours.

The reactive oxygen species (ROS) damage to nucleic acids, proteins, lipids can be caused exogenously or endogenously. The endogenous ROS damage is due to formation of free radical formation when superoxide reacts with iron sulphur clusters **[30]**. Some of the compounds that cause exogenous ROS damage are tert-butylhydroperoxide, arsenite, paraquat, and juglone **[31, 32, 33].** sHSP such as *hsp-16.1* and *hsp-16.2* were involved in increased oxidative resistance and are considered as well-known biomarkers for oxidative stress **[34].** Exposure to UV stress has also been extensively studied recently and was reported to regulate heat shock factor (HSF-1) **[35].** HSF-1 is a transcriptional regulator that becomes the primary receiver of any heat stress which prompts HSP's. HSF-1 activation results in trimerization and bind to the heat shock element to provoke the HSP's chaperonic activity conferring longevity to the nematode **[21**].

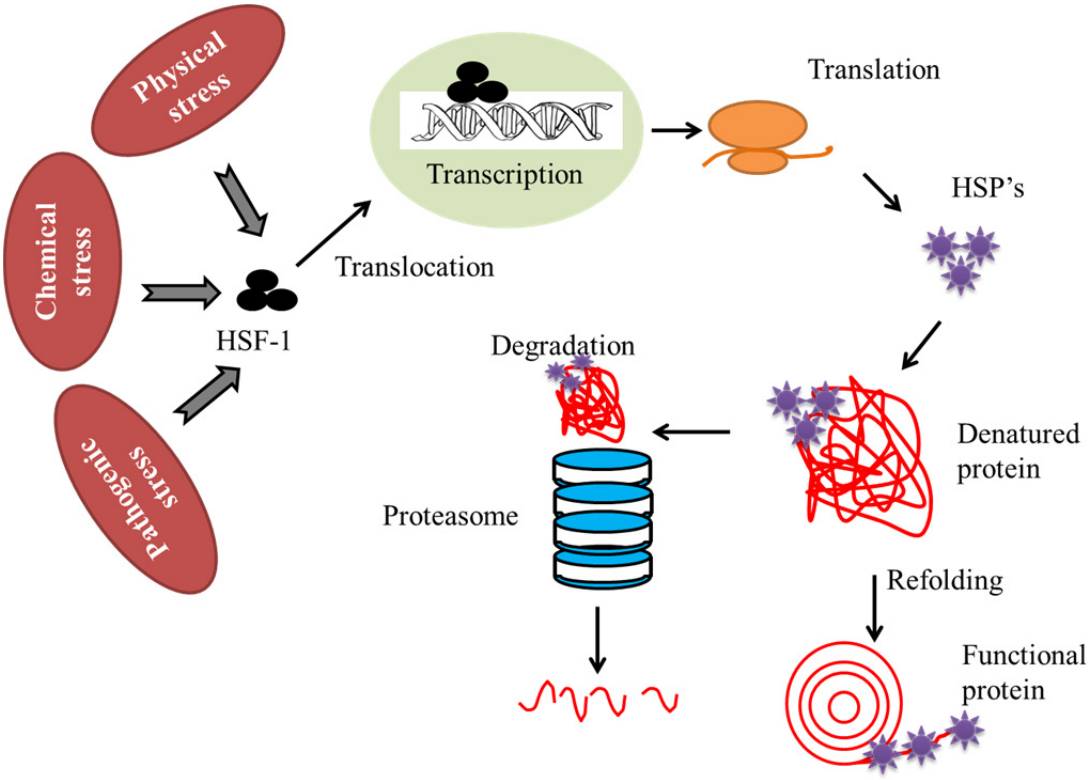

Fig. 2 Schematic representation of the mechanism of HSP'S and HSF-1 during various stress conditions. Figure modified from [36].

4. HSP's in pathogen response

The nematode's immune response depends on its survival in a hostile environment. *C. elegans* as a soil nematode encounters multitude of bacteria and produces specific immune response to fight against each of them. HSP's have been of great importance since its discovery and the research has markedly increased in *C. elegans*. The mitochondrial chaperone HSP-60 has been implicated to protect the nematode by bestowing resistance against *Pseudomonas*

aeruginosa 14 (PA14). It was also found that *hsp-60* inhibition down regulates the evolutionarily conserved pathway mitogen activated protein kinase (MAPK) **[17]**. The expression of *hsp-16.2* was prominent and appeared to influence the adult nematodes during exposure of different *Escherichia coli* pathogenic strain. In addition to it, increase in expression of *hsp-16.2* has been also shown to regulate the transcription factors HSF-1 and DAF-16 **[37]**. The study by **Jebamercy et al 2016** also comprehends the role of HSP-90 in the resistance of the nematode against the opportunistic pathogen *Proteus mirabilis* **[22]**. Any mutation in the HSP's or the related transcription factor would alleviate the immune system making it feasible for the pathogens to colonize the nematode **[21, 22]**. Pathogen infection is often related to the modification of proteins either by unfolding or misfolding them inside the host system. PA14 causes UPR in *C. elegans* that eventually activates *hsp-4, hsp-6* and *hsp-60* that guides the unfolded/misfolded proteins for degradation **[38]**. HSP's influences the host survival by coordinating immune response, development and longevity.

5. HSP's in aging

Induction of HSP's following pre-treatments with heat or other sources is a conventional paradigm to increase survival. The insulin signaling pathway of *C. elegans* modulate resistance by targeting the *daf-16* **[39]**. *daf-16* is one of major transcriptional regulators that benefit in longevity of the nematodes. Even though there are several other factors that can prolong the survival of the nematode, inducing the HSP's is still considered an exceptional method to lengthen its existence. HSF-1 involves multistep process; initially they oligomerize then get post translationally modified and finally undergo nuclear translocation **[40]**. *hsp-16* and *hsp-70* are targets of HSF-1that promote longevity in *C. elegans* **[41]**. Among the cell organelles present inside the system, mitochondria are considered as an important regulator of aging **[42].** The perturbation in the functioning of electron transport chain affects the lifespan of *C. elegans*. This disturbance results in the induction of UPR and increases the chaperonin activity (HSP-60 and HSP-70) **[43, 44]**. **Lechler et al 2014** have also reported that HSF-1 is a main regulator of RNA binding protein aggregation in young and aged nematodes by decreasing the build-up of other misfolded proteins **[45]**. These findings highlight the importance of HSP's and HSF-1 in longevity associated mechanism.

6. Conclusion

The influence of HSP's to adapt to diverse biological niche is inadequately understood in the terms of cross talk between innate and adaptive immune system in the higher vertebrates. This also raises obvious questions on the stoichiometry between the mechanisms by which they interact with discrete complexes located in different cellular compartments. The inter- and intra-relationship between HSP's could spawn new opportunities in finding the drug targets and designing therapeutics for various diseases and disorders.

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