Advances in Zoology and Botany 11(4): 246-256, 2023 http://www.hrpub.org
DOI: 10.13189/azb.2023.110402

Small Heat Shock Protein and *Drosophila melanogaster* Development

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Received August 3, 2022; Revised November 30, 2022; Accepted December 22, 2022

Cite This Paper in the Following Citation Styles

(a): *[1] Shreya Tripathi, Sangeeta Sinha , "Small Heat Shock Protein and Drosophila melanogaster Development," Advances in Zoology and Botany, Vol. 11, No. 4, pp. 246 - 256, 2023. DOI: 10.13189/azb.2023.110402.*

(b): *Shreya Tripathi, Sangeeta Sinha (2023). Small Heat Shock Protein and Drosophila melanogaster Development. Advances in Zoology and Botany, 11(4), 246 - 256. DOI: 10.13189/azb.2023.110402.*

Abstract To understand the regulation and function of the heat shock response, *Drosophila* model system has been used for the past few decades. *Drosophila* heat shock protein is a family of proteins which show large changes in their expression pattern upon deviation from optimal temperature of organism in either direction, thereby preventing cells from potential damages. Small heat shock protein (sHsp) is a subfamily under this family and is known for its role in a wide variety of cellular functions (ageing, immunity, proteotoxicity, apoptosis, etc.) apart from maintaining homeostasis. In all domains of drosophila life cycle, ATP-independent stress proteins i.e. small heat shock proteins (sHsps) are found. The review highlights the various roles played by different members of sHsp in *Drosophila melanogaster* and their contribution to ageing and autophagy. Some members of the subfamily also show differential localization in different organelles and tissues, at different developmental stages as well as in adults owing to their functions. For understanding protein function, determination and localization of cellular proteins was studied. Determining the cellular localization of proteins is important for understanding protein functions. Some of the Hsps are up-regulated in organ and tissue-specific manner which help to understand life span and different biomarkers during the course of *Drosophila* life cycle. Some of the members of small heat shock protein in *Drosophila melanogaster* are orthologs of human sHsp and hence can serve as a good model for studying diseases in humans associated with members of this subfamily. Different parameters and tools can be used experimentally which will help in manipulating gene function and determining health span in *Drosophila*. This provides an unparalleled opportunity to further study the role of sHsps.

Keywords *Drosophila melanogaster*, Small Heat Shock Proteins, Proteotoxicity, Aging, Homeostasis

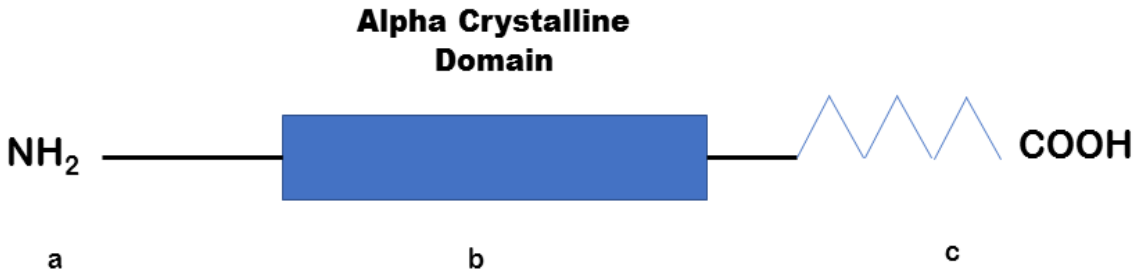
1. Introduction

Heat shock proteins (Hsp) are a family of evolutionary conserved molecular chaperones that helps in maintaining protein homeostasis in the cells and protects cells from stress conditions. Based on sequence homology and molecular weight, Hsps can be divided into six subfamilies namely Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and small heat shock proteins (sHsp) (Table 1). Among these six subfamilies, sHsps are the ones most upregulated following stress.

Different families of Hsp proteins maintain proteostasis by regulating different mechanisms like chaperone binding, misfolded protein binding etc. This table summarizes the cellular function, in which different Hsps are involved, comprising of 12 sHsp proteins. Their molecular weight lies in the range of 12- 42 kDa and these proteins share a common structural framework (Figure 1) comprising of a conserved Carboxy terminal domain homologous to mammalian alpha crystalline domain and a variable N-terminal domain lying upstream of alpha crystallin domain. At the N-terminus, there is a hydrophobic region of 15 amino acids common to Hsp23, Hsp26, Hsp27 but absent in Hsp22.3) a flexible short C-terminal sequence [1,2,3].

Table 1. Function of other subfamilies of heat shock proteins. Data is taken from https://flybase.org/

Name of the subfamilies	Function the subfamilies
Hsp100	• Unfolded protein binding
Hsp90	• Insulin receptor binding • Protein binding • ATP binding • Unfolded protein binding
Hsp70	• ATP binding • Misfolded protein binding • Unfolded protein binding
Hsp60	• ATP binding • Chaperone binding • Involved in mitochondrion organization. • Response to heat • Involved in mitochondrial UPR (Unfolded protein response.) • Protein refolding.
Hsp40	• Unfolded protein binding • Chaperone binding • Transcription factor binding • Protein binding
sHsp	• Protein folding • Aging • Autophagy • Involved in mitochondrial UPR • Involved in synapse formation • Plays a role in immune pathways



(a) N terminal region is related to oligomer formation, (b) alpha crystallin domain helps in dimer formation, (c) short flexible C-terminal sequence helps in mediating oligomer stability.

Figure 1. Basic organization of sHsp

Table 2. Role of sHsps in cellular functions

Cellular Responses	Role played by sHsp	sHsps involved
Immunity	p38 MAPK pathway	Hsp26, Hsp27
	in vitro antiviral immunity	Hsp23
	Involved in phagosome protein network	Hsp26
Aging	Downregulation in head	Hsp27
	Upregulation in mitochondria	Hsp22
	Dnmt2 downstream target	Hsp22, Hsp23, Hsp26
	miRNA 279 downstream target	Hsp27
Disease	Protection against proteotoxicity.	Hsp67Bc, CG7409, CG14207, Hsp26, Hsp27, l(2)efl.
	Protection against contractile dysfunction	Hsp23

Different roles played by various sHsps at cellular level.

Apart from their regular function, sHsps are reportedly shown to be associated with a wide variety of cellular structures and functions like ageing, immunity, apoptosis, cargo movement, polyglutamine aggregation, etc. showing their importance at cellular level (Table 2). Due to their structural similarity with mammalian sHsps [4,5], they are of prime importance in study of human diseases and protein interactions. This review is focused on the role of sHsp in some of the major biochemical pathways associated with cell survival in *Drosophila melanogaster*.

***Spatio-temporal distribution of sHsps*:** The expression and abundance of small heat shock proteins varies with the developmental stage, tissue and cellular organelles under normal conditions. Prior to the expression of zygotic genes, some of the sHsps are transported from nurse cells to zygote, where they are translated creating an initial concentration difference of different sHsps [6].

Distribution in developing Drosophila: The developing *Drosophila* displays difference in the concentration and expression of sHsp throughout life cycle [7,8]. This difference is created by upregulation of certain sHsps with the developmental stage in response to their requirement. The mechanism of their regulation in this stress-independent condition, however, is still not clear. Two factors have been proposed so far which can possibly regulate sHsp stress-independent expression:

Ecdysone dependent regulation: The regulation by ecdysone can be primary or secondary depending on the sHsp. Many of the sHsp contain Ecdysone response element (EcRE) in their promoter region. The activated ecdysone receptors bind to this response element and promotes the expression of sHsp. In the absence of activated ecdysone receptor, the EcRE can act as repressor of promoter activity. The repressor effect is, however, dependent on the distance between the EcRE and the TATAA box (Figure 2). Thus, this ecdysone dependent regulation provides two ways, in which sHsp expression can be affected one by stage dependent secretion of ecdysone hormone and the other by physical proximity between EcRE and TATAA box. While this effect is pronounced for some sHsps (hsp 23 and hsp27), the extent of expression is yet to be determined in other sHsps [8].

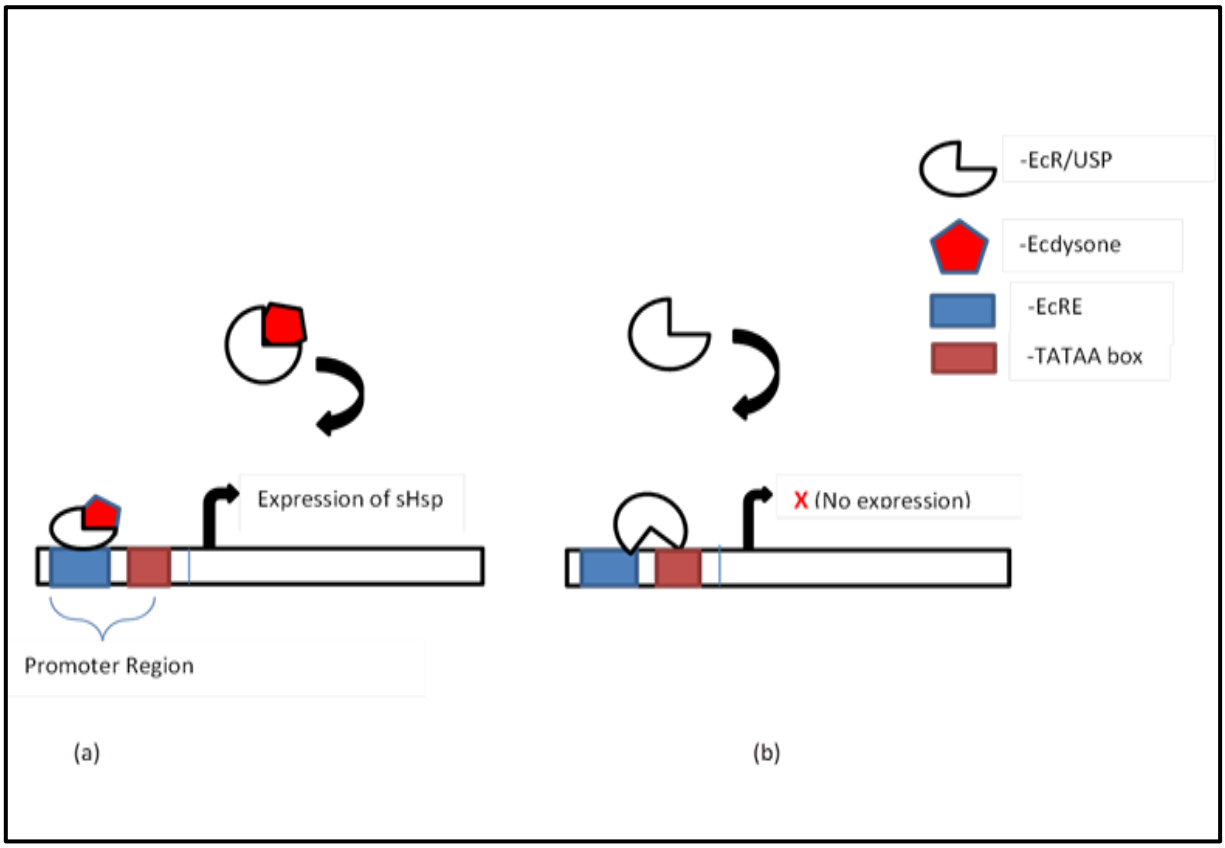


Figure 2. Arbitrary diagram to explain possible interaction between EcR/USP and EcRE based on the information provided (a) in presence of ecdysone, the activated EcR/USP dimer binds to EcRE in the promoter region to initiate transcription. (b)in absence of ecdysone EcR shows inhibitory effect

Table 3. Intracellular localization of *Drosophila* sHsps and their post translational modification

CG Number	Name	Intracellular Localization	Post translational modifications observed
CG14207	-	Nucleoplasm Cytoplasm [7,14,16,21,22]	Phosphorylation [7,53]
CG13133	-	Cytoplasm [7,16]	NR
CG4533	l(2)efl	Cytoplasm Perinuclear space [7,15,16]	NR
CG7409	-	Cytoplasm [7,16]	NR
CG4461	-	Cytoplasm [7,16]	NR
CG4167	Hsp67Ba	Cytoplasm [7,16]	NR
CG4183	Hsp26	Cytoplasm Nucleus [1,7,12,16]	Phosphorylation [7,53]
CG4190	Hsp67Bc	Cytoplasm [7,16,20]	NR
CG4460	Hsp22	Mitochondria [7,8]	Phosphorylation [7,54]
CG4463	Hsp23	Cytoplasm [1,7,16]	NR
CG4466	Hsp27	Nucleus Endoplasmic reticulum Chaperone complex Cytoplasm [7,11,12]	Phosphorylation [53,54]

Different sHsp localize in different organelles where they participate in organelle specific functions. NR: Not Reported.

Post translational modification: The phosphorylation state of different sHsps varies with the developmental stage of the *drosophila*. However, the functional implication of this variation remains to be assessed [8].

Intracellular localization of sHsps: Small heat shock proteins are localized to different organelles following their synthesis/translation (Table 3). This localization is sometimes specifically associated with the functions it performs within the organelle. For example, Hsp22 is a resident of mitochondria and associates with ATP synthase and proteins in ETC chain regulating these pathways [9,10].

Similarly, Hsp27 has been found to localize typically in nucleus but its expression is also detected in cytoplasm and chaperone complex in Endoplasmic reticulum where it is shown to interact with Xport [11,12] Whether this atypical distribution is restricted to certain cells is not determined. Hsp26 and Hsp23 are located in cytosol but a numat preparation from *Drosophila* S2 cells has also shown nuclear localization of Hsp26 [7,13]. CG14207 is located in nucleoplasm and cytoplasm [7,14] while l(2)efl localises around nuclei (perinuclear space) and cytoplasm [15]. The localization for rest of sHsps (CG13133, CG7409, CG4461, Hsp67Ba) is cytosolic as detected so far [16]. This normal distribution of sHsps is also influenced by phosphorylation state sometimes as seen for Hsp 27 whose serine 75 phosphorylation has been linked to its presence in *Drosophila* Kc cells and early embryos [7,17].

Tissue specific localization of sHsps: The distinct pattern of sHsp expression is not only limited to organelles but also varies with tissue (Table 4). This distribution can be blamed on the specific functions sHsp regulate in the tissues. For example, Hsp26 loss of function results in adverse effects on presynaptic cytoskeleton and is responsible for reduced number of synaptic buttons at neuromuscular junction [18,19], Hsp67Bc and CG14207 localizes at Z-band suggesting their possible role in cytoskeleton modulation [20,21,22]. l(2)efl also localizes at Z disk where it maintains muscular integrity, Z-band patterning and other associated functions [5,20]. Tissue specific distribution has been reviewed previously for gonads, muscle and developing nervous system [5].

Table 4. Expression of sHsps throughout *Drosophila* life cycle. The coloured boxes represent that expression has been detected in these tissues. (Adapted from Expression data presented in https://flybase.org/)

	Egg/Oocyte stem cell	Primordium	Sensory system	Nervous system	Circulatory system	Digestive system	Reproductive system	Integumentary system	Muscle system
CG14207		■			■				■
CG43851									
CG13133			■					■	
CG4533					■				■
CG7409*									
CG4461Ɨ									
CG4167							■		
CG4183		■		■	■	■			
CG4190		■	■	■					
CG4460		■	■	■					
CG4463	■	■	■	■	■				
CG4466	■	■		■					

* Expression detected in other anatomical entities

Ɨ Expression not curated

sHsp and their role in immunity: Immunity against pathogens bacteria, fungi and viruses is generally imparted through Toll, Imd, JNK, JAK-STAT and p38 MAPK pathways. Findings have suggested that sHsp plays a role in downstream signalling of some of these pathways. One such pathway is p38 MAPK pathway. p38 genes are part of the host defence mechanism and are activated in response to microbial invasion. Studies on the mutants of p38 have shown downregulation of Hsp 26, Hsp 27, Hsp 60D, Hsp70Bc [23]. However, the precise role of sHsp i.e., how it imparts immunity to *Drosophila* remains undefined. Upregulation of sHsps are also reported in other experiments but their involvement in immune response is not proved [24,25].

The sHsp also has antiviral properties and is experimentally proved to reduce viral load both in vivo and in vitro. An interesting fact to note is the significant difference in the expression pattern of hsp in *in vitro Drosophila* S2 cells and *in vivo Drosophila* adult flies i.e, increase in hsp 26,hsp23,hsp27 and hsp70 transcripts are seen when cells are infected with DCV, CrPV and IIV-6 virus in *in vitro* experiment but *in vivo* experiment only hsp 23 and hsp70 transcripts increases (relative to basal level) upon infection with same viruses (Figure 3) [26]. An increase in Hsp 23 and Hsp 70 is also reported in other studies suggesting their definite role in antiviral immunity. Apart from these, Hsp26 is detected in the phagosome protein network, suggesting a new route by which sHsp might play a role in immune response [7,27].

sHsp and aging: Aging is characterized by various degenerative changes occurring at molecular and tissue level [28]. These changes include aggregation of misfolded proteins, increased oxidative stress, reduced ATP synthesis, etc. small heat shock proteins are associated with at least half of these changes which is quite evident from their age dependent expression change. For example, Hsp27 expression goes down in the head with increase in age [29] while a dramatic increase in Hsp22 is seen in mitochondria of cells [30,31,32].

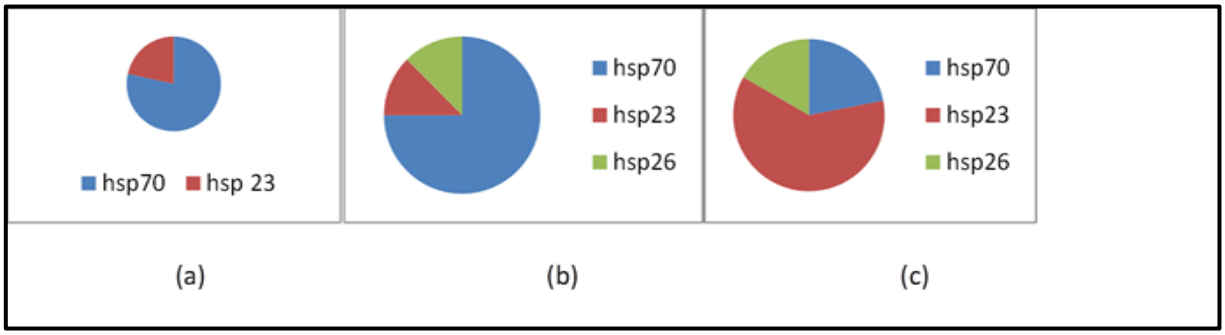
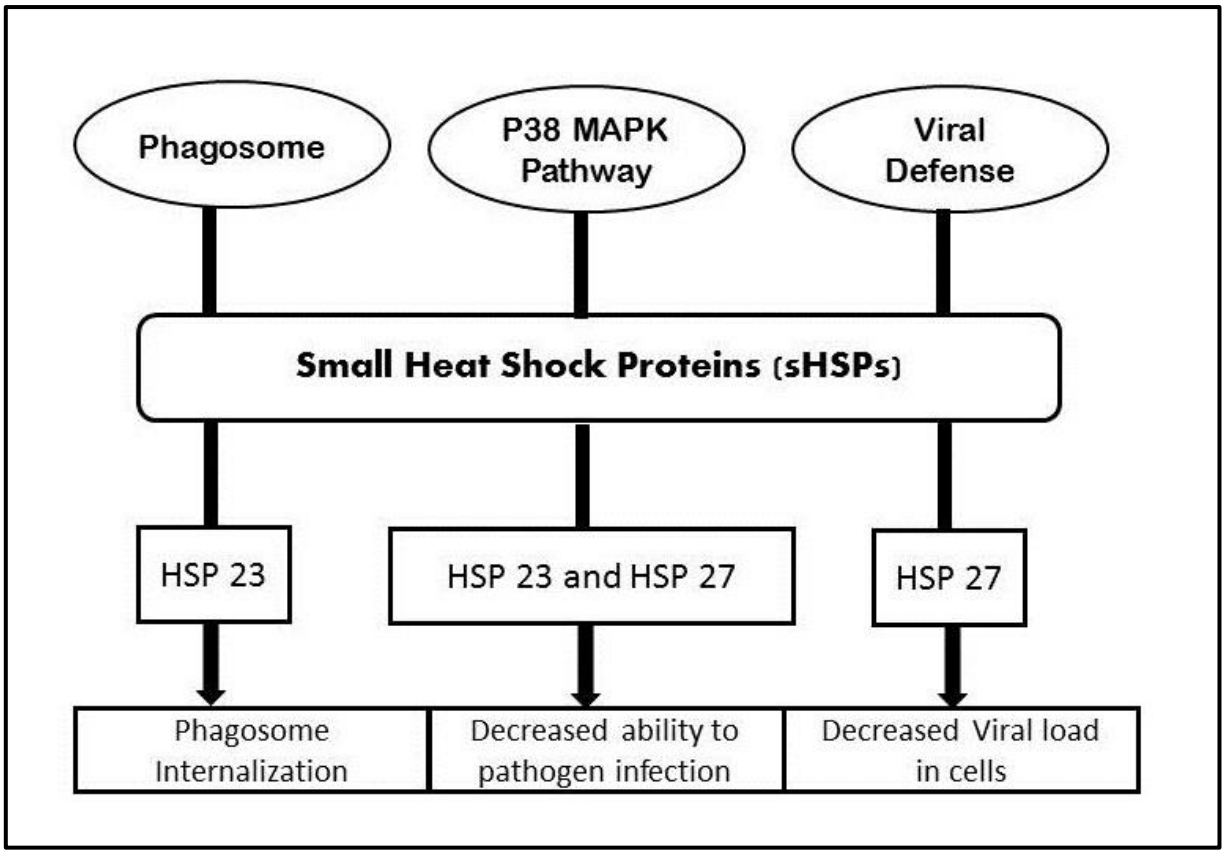
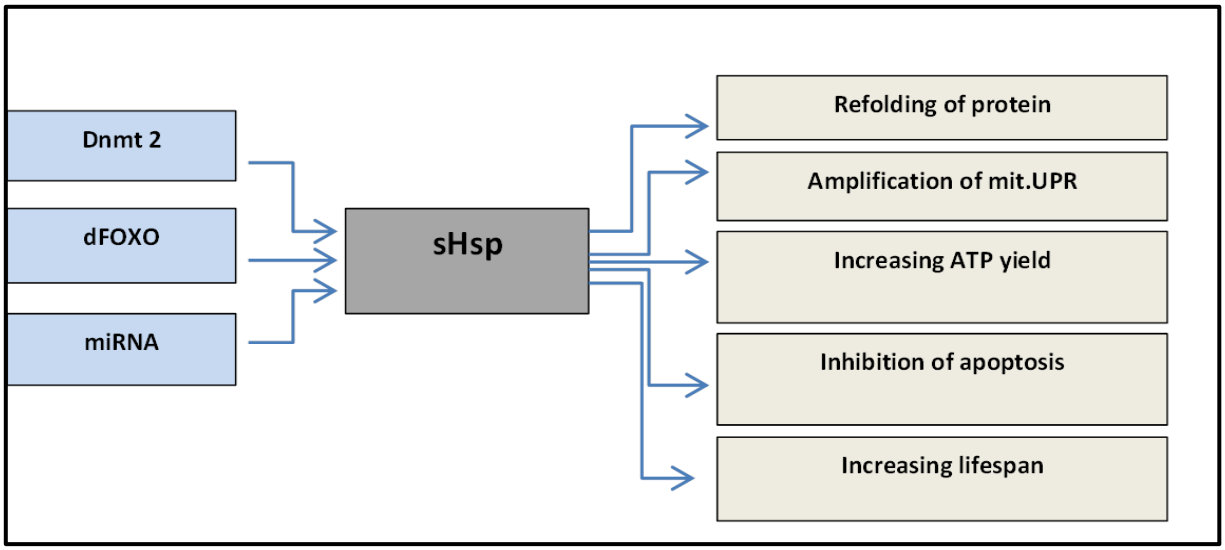


Figure 3. Pie chart distribution of expression pattern of sHsp based on experimental data (a) hsp transcripts found in vivo in adult flies upon infection with DCV and CrPV virus, (b) *In vitro* expression pattern for infection with DCV and IIV-6 virus, (c) in vitro expression upon infection with CrPV



Small heat shock proteins are downstream targets of p38 MAPK pathway, Viral defense and phagosome and help by increasing defense against pathogens.

Figure 4. Summarization of pathways used by sHsps in immune response



Small heat shock proteins are upregulated in response to transcription factor dFOXO, Dnmt 2 and miRNA and act by increasing factors (like ATP yield, protein refolding) which delays aging.

Figure 5. Regulatory mechanisms of sHsps and their mode of action

Expression of sHsps is regulated by many pathways that lead to aging. Dnmt 2 is a methyltransferase that is known to increase lifespan whose downstream target are Hsp22, Hsp23, Hsp26 [33,34]. dFOXO, which is known to increase lifespan and stress tolerance, has been shown to regulate the expression of Hsp22, CG14207, Hsp23. Hsp27,l(2)efl [32,35,36]. Hsp27 is also a target gene of miRNA279 which alters its expression after aging and is a potential target of H3K4me1 indicating a slight possibility of epigenetic regulation of Hsp27 (Figure 5) [37] Moreover, Menin protein plays an active role in expression of sHsps during heat stress and has an active role in their regulation but there is no report on whether it influences their expression in aging or not [38,39].

Overexpression of many sHsps has been shown to extend the lifespan of *Drosophila melanogaster* but the central spot is occupied by mitochondrial Hsp22. Hsp22 associates extensively with different components of respiratory chain like ATP synthase, Complex I of ETC, TCA cycle and influences their efficiency. Overexpression of Hsp22 has been shown to increase ATP yield which significantly decreases during aging [10]. Its overexpression has also shown to upregulate Hsp70 levels in mitochondria and has a suggested role in amplifying mitochondrial unfolding protein response (UPR) [32,36,40,41,42]. It also plays a role in mitochondria to nuclear signaling in response to increased ROS production [32,40].

Role of sHsp in diseases: Proteotoxicity is caused by aggregation of misfolded proteins which disturbs proteostasis of the cell as seen in polyglutamine diseases. sHsps can reduce this aggregation but at different levels like CG7409 offers very little protection against protein aggregation while Hsp67Bc reduces aggregation to a significant level whereas the resistance offered by CG14207 lies in between these two [7,43]. CG14207 has also shown to provide mild protection against Ataxia-3-mediated degeneration [44] but failed to provide protection against both soluble or aggregated insoluble forms of Htt128Q as reported by Carra et al., 2010. Hsp67Bc and L(2)efl however, has shown to reduce the soluble levels of Htt128Q significantly by inducing eIF2α pathway which leads to inhibition of protein synthesis. In addition to this, Hsp67Bc has also shown to reduce the amount of aggregated high molecular weight Htt128Q [7,20]. This capacity of Hsp67Bc is attributed to its role in stimulating autophagy

Moreover, Hsp67Bc has also shown to provide protection against TDP-43 aggregates found in several pathologies like Amyloid Lateral Sclerosis, IBMPFD, Frontotemporal dementia [45]. Hsp67Bc co expression with the mutated form of TDP-43 which lacks nuclear localization signal (also called TDP-43-NLS) has a protective effect on eye degeneration while its downregulation increases the accumulation and toxicity caused by TDP-43-NLS. Pupae lethality induced by TDP-35 can be rescued by coexpression of Hsp67Bc [46,47].

Apart from these sHsps, hsp23, hsp26, hsp27 also play a protective role against some diseases. Hsp23 protects against contractile dysfunction induced by tachypacing [16,48]. DmHsp23 also has a protective role against heat shock stress induced by degeneration of muscle neuron and glia in cell autonomous and cell non-autonomous fashion respectively [49]. Hsp23 along with Hsp26 is suggested to form a complex that promotes synapse formation in presynaptic neurons wherein modification in any direction of sHsp26 results in incorrect establishment of synapse number during development. sHsp23 also has a role to play in synapse formation and its overexpression is detrimental for the neuron development [19]. Hsp27 overexpression is reported to reduce the mild toxicity caused by a short polyQ but is ineffective against long polyQ. It also reduces lethality induced by hid, a gene promoting apoptosis [50,51]. Hsp27 also has Atg7 as its downstream target which helps in reducing polyQ toxicity [51].

Unlike other sHsps of *Drosophila melanogaster* which have a positive impact against diseases, DmHsp22 impacts Human Breast Cancer cells negatively. Human breast cancer cells show an increase in malignant properties (like anchorage independent growth, migration and tumor formation.) when infected with DmHsp22. Upon immunoprecipitation it was shown that DmHsp22 interacts with p53 and inhibits its nuclear transport, suggesting one way in which it could enhance the malignant properties of cells [52].

Hsp67Bc and autophagy: Autophagy is a mechanism by which cell removes toxic or dysfunctional components. Autophagy plays a role in degradation of several aggregated or mutated forms of protein which otherwise may lead to proteotoxicity. Hsp67Bc is suggested to play a role in this pathway. DmHsp67Bc is an ortholog of human HSPB8 which plays an essential role in preventing protein aggregation generated by severe diseases. Carra et al., 2010 suggested a possible pathway, through which Hsp67Bc might regulate autophagy based on the following observations: a)Hsp67Bc interacts with starvin, the only BAG protein present in *Drosophila melanogaster* and their coexpression increases autophagic vacuole turnover number. b) Hsp67Bc increased both LC3I and LC3II levels along with an increase in LC3II/LC3I ratio c) Reduction in Hsp67Bc ability to prevent aggregation seen in the presence of elF2a inhibitor suggesting that Hsp67Bc acts through elF2a to activate both inhibition of protein synthesis and autophagy (Figure 6) [7,20].

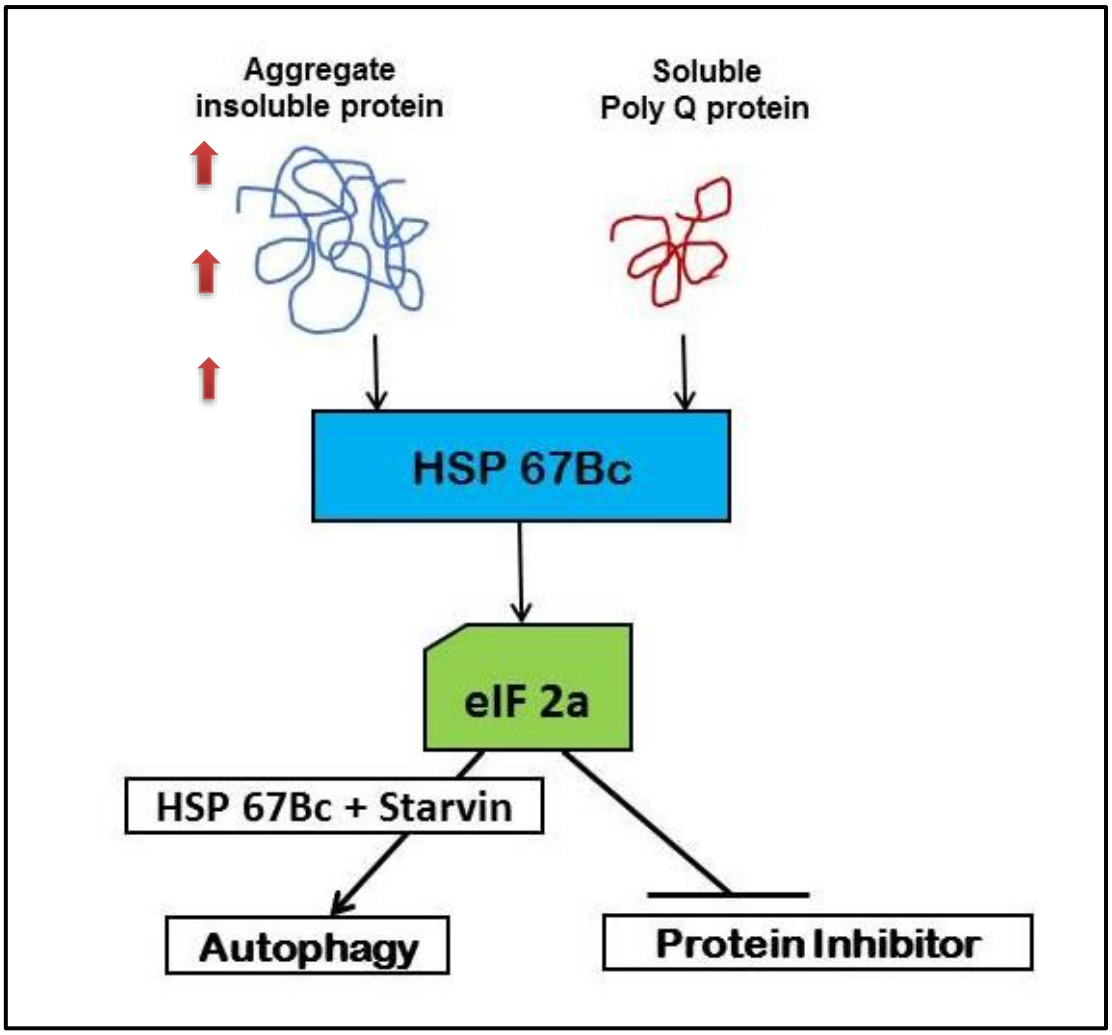


Figure 6. Schematic representation of hsp67Bc mode of action in preventing proteotoxicity

2. Conclusions

SHsp is a family of Heat shock proteins which takes an active part in cellular processes. Their expression varies with the developmental stage [6,7,8] which suggests their useful role in pathways associated with development of an adult fly. One possible way through which they exert their importance could be by changing their phosphorylation states [8]. However, this needs to be further explored by investigating the precise role of varying phosphorylation states observed for sHsps. sHsps also play a major role in preventing proteotoxicity by reducing the volume of insoluble and soluble protein aggregates observed in various diseases and hence sHsp mutants of fly can be used to study diseases associated with proteotoxicity [7,20,43,44]. Recent work has shown their involvement in neuron development and synapse formation [19]. Many sHsps show pleotropic activity which helps in understanding both beneficial and deleterious effects with respect to health and diseases by annotating gene functions. Some of the Dm sHsp are orthologous with human sHsp [7, 20] which makes *Drosophila* a good model for studying upstream and downstream molecules/complexes/proteins interacting with sHsp.

Abbreviations

sHsps: Small heat shock Proteins
Dm: *Drosophila melanogaster*
EcRE: Ecdysone Response Element
USP: Ultraspiracle
Imd pathway: Immune deficiency pathway
JNK pathway: Jun N-Terminal Kinase pathway
JAK-STAT: Janus kinase and Signal Transducer and activator of Transcription
MAPK: Mitogen activated protein kinase
DCV: *Drosophila* C virus
CrPV: Cricket paralysis virus
IIV-6: Invertebrate iridescent virus -6
miRNA: MicroRNA
TDP-43: TAR DNA-binding protein 43
Poly Q: Polyglutamine

Acknowledgements

Authors acknowledge the Department of Zoology, Nowrosjee Wadia College, Savitribai Phule Pune University, Pune, India for the support.

Competing Interests

The authors declare no conflict of interest.

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