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An overview of heat-stress response regulation in Gram-negative bacteria considering *Escherichia coli* as a model organism

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ABSTRACT

Response to heat stress (HSR) is a key stress response for endurance in *Escherichia coli* mediated by transcriptional factor σ-32. Even though there has been extensive investigation on the contribution of proteins and chaperones in retaining protein stability in cells under stress conditions, limited information is available regarding the dynamic nature of mechanisms regulating the activity of the highly conserved heat shock proteins (Hsps). Several gene expression-based studies suggest the pivotal role of Hsp70 (DnaK) in the regulation of the expression of heat shock genes (Hsg). Direct interaction of Hsp70 with σ-32 may regulate this function in *E. coli*. Recent studies revealed that localization of σ-32 to the membrane interior by SRP-dependent pathway enables them to function appropriately in their role as regulators. The contributions of different cellular components including cell membrane remain unknown. Other cellular components or σ-32 interfere with polypeptides which could play a crucial role in cell survival. Sigma factor monitors and preserves outer membrane integrity of *E. coli* by stimulating the genes regulating outer membrane proteins (OMPs) and lipopolysaccharide (LPS) assemblage as well as through expression of small RNAs to down-regulate surplus unassembled OMPs. σ-E activity is regulated by the rate at which its membrane-encompassing anti-sigma factor, RseA is degraded. Mutations in *rseA* are reported to constitutively increase the sigma (E) activity that is validated at both genetic and biochemical levels. In this review, the basic mechanism of heat stress regulation in gram-negative bacteria has been elaborated using *E. coli* as a model organism.

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1 Introduction

1.1 Bacterial stress response

Bacteria and other microorganisms have evolved to endure in inconsistent environmental conditions that may sometimes be adverse to their existence. A multitude of stress response programs empowers the bacteria to engender appropriate responses to diverse environmental challenges like heat shock, oxidative stress, anti-microbial agents, and nutritional restrains. A well-coordinated and effective response is generated by a complex network of regulatory systems at a global scale that helps them to preserve a stable cellular equilibrium under multiple stresses simultaneously (Scott et al. 2010). These pathways recruit suites of transcriptional regulators to remodel the cellular proteome that facilitates adaptive changes in microorganisms in variable and extreme environmental conditions (Giuliodori et al. 2007; Fiebig et al. 2015). Transcriptional regulation in response to environmental change can be deduced using three models of signal transduction (Figure 1): (i) One component system: One-component regulators are simple and abundant; consisting of a sensory input domain controlling its adjoining output domain that functions as the DNA binding domain (DBD) (Ulrich et al. 2005), (ii) Two-component system: The two-component systems comprises of a sensory protein with histidine kinase activity along with a response regulatory receptor. The response regulator stereotypically consists of a phosphoryl group-transfer domain and an output domain that interacts with the DNA. Translocation of a phosphoryl group from a His residue on the kinase to an Asp residue on the receiver domain is mediated by the sensory region of histidine kinase that in turn controls the DBD output domain (Ulrich et al. 2005), and (iii) Use of alternative sigma factors: Binding of σ-factors to core RNA polymerase (RNAP) impart promoter selectivity to regulon of gene-expression. Adaptation to non-optimal conditions by the implementation of compensatory physiological changes can be attributed to the induction of new σ-factors or by regulating its activity (Helmann 2010).

2 Types of the bacterial stress response

Stress response systems not only help in the survival of the microorganisms but can also play a vital part in the disease-causing ability of virulent microbes. Some of the most significant stress response systems of bacterial origin include: (i) Heat shock response which is primarily controlled by σ-32 that protect at elevated temperature, (ii) Envelope stress response regulates cellular integrity with the help of sigma factor σ -E together with Cpx dual-component system, (iii) Cold shock response is generated in stress condition induced by cold temperature aided by expression of RNA chaperones and ribosomal factors, (iv) General stress response modulates gene expression globally depending on the σ factor S and enables cell growth under a variety of adverse situations, (v) (p)ppGpp-dependent stringent response controls the overall physiological response of the cell when there is a limitation of nutrients through the reduction in protein synthesis capacity of the cells (Yura and Nakahigashi 1999).

3 Heat shock response (HSR)

Ritossa (1962) first recorded the event of heat shock response in a strongly amplified form of interphase chromosomes isolated from salivary glands of *Drosophila melanogaster* flies when they were

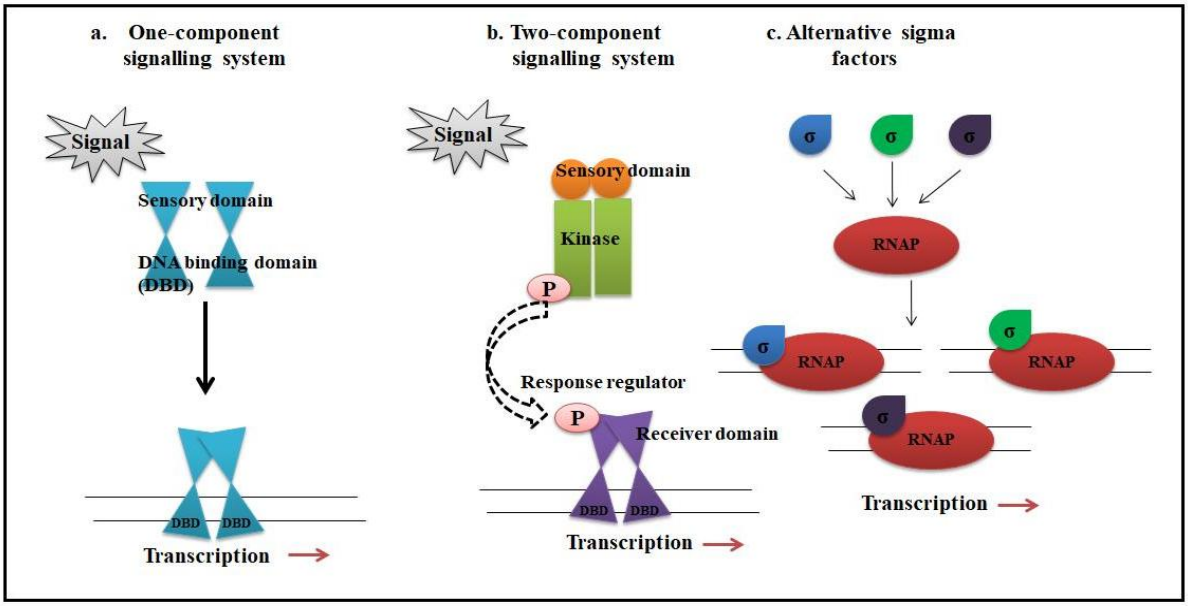

Figure 1 Modes of transcriptional regulation in response to environmental stress a. one-component signalling system, b. two—component signalling system and c. alternative sigma factor (Adapted from Fiebif et al. 2015)

shifted from their usual growth temperature to 37°C. The next step of HSP activity was detected by Tissières et al. (1974) followed by the invention of the σ-32 (σ-H) as a substitution sigma factor that is in charge of HSG expression in *E. coli* (Feng et al. 2019).

All microorganisms are equipped with well-coordinated genetic programs that allow them to survive under stressful situations. A harmonization of expression of genes that orchestrate several vital cellular processes as well as structures like those involved in DNA metabolism, determination of cell membrane composition, and house-keeping genes play a pivotal role in the improvement of bacterial stress tolerances. In the majority of the cases, cells get acclimatized to stress conditions due to elevated expression of a particular class of proteins that is dedicated to deal with the stress factor. A panel of stress proteins known as heat shock proteins (HSPs) exhibit significantly enhanced expression following a drastic elevation in temperature in course of HSR (Schumann 2016). HSR is a cytoprotective response induced by heat shock and other stressful conditions, represented by a specific program of gene expression that empowers the cells to survive and pull through from otherwise fatal circumstances. It constitutes increased production of a panel of molecular chaperones (HSPs) from the family of heat shock genes (HSGs) (Wierstra 2013). The HSPs function in regulating precise folding and localization of proteins, decreasing protein denaturation and inhibiting the clumping of oxidized proteins (Vabulas et al. 2010).

A dedicated panel of sensory biomolecules (DNA, RNA, proteins) of bacterial origin called Thermo sensors can perceive temperature variations and transduce signals from one cell to another that direct gene expression outputs. As soon as stresses signal is recognized, it acts as a stimulus to trigger specific regulatory mechanisms controlling the expression of HSPs: Positive as well as negative regulations. While positive regulation readdresses the RNA polymerase to a subgroup of designated promoters with help of specific substitute sigma factors, the negative regulation is facilitated by repressors of transcription (Roncarati and Scarlato 2017). It is mention-worthy that while several species of bacteria implement either positive or negative mechanisms solely, there are some species exhibiting coexistence of these two differing strategies. Therefore, HSG expression can be regulated by two major mechanisms: recruitment of (a) alternative sigma factors and (b) transcriptional repressors (Schumann 2012, 2016).

3.1 Principal mediators of HSR

It is crucial to maintain proteostasis in a normal cell as conservation of protein folding is essential for preserving their functionality which in turn controls the balance of cellular homeostasis. Perturbation in the programming of protein homeostasis can induce protein dysfunction that may lead to lethal consequences as severe as cell death. Critical physiological processes of the bacteria could be adversely affected by the alteration of protein structures that ultimately lead to cell damage or death (Díaz-Villanueva et al. 2015). Therefore, heat shock response confers protection against hyperthermia by immediate induction of several families of HSPs acting as molecular chaperones that impart protection from cellular stress and damage induced by protein misfolding (Vabulas et al. 2010).

Interestingly, some HSPs have copious prevalence in all metabolic conditions as they are also essential when the bacteria are growing normally. GroEL (also called Cpn60) along with DnaKem bodies two chief chaperone families Hsp60 and Hsp70, respectively in bacteria. Both of them are reported to contribute significantly to protein assembly even during the growth phase of microorganisms devoid of any stress. However, their action becomes more domineering during HSR. Accompanied by their co-chaperones GroES (also called Cpn 10) and DnaJ-GrpE, together with ATP hydrolysis, they can interact with hydrophobic moieties of unstructured proteins and promote proper folding. Another group of HSPs of bacterial origin constitutes a multi-component system of proteases that are dedicated for the clearance of non-functional polypeptides from stressed cells. The proteases comprise of subunits like ClpA/ClpX and HslU with substrate recognition attribute that when associated with respective catalytic subunits ClpP and HslV respectively remodel substrate polypeptides. The altered polypeptides are then subjected to deterioration by proteolysis (Missiakas et al.1996; Wawrzynow et al.1996). HSP family also comprises of members such as Lon, FtsH, and DegPthat conglomerate chaperone activity with protease activity on a single polypeptide. Small HSPs are a diverse panel of proteins that are committed to provide protection to proteins in unfolded conformation by binding with them until they will be re-assembled to functional form by ATP-dependent chaperones (Matuszewska et al. 2005). Also, the HSPs are directly or indirectly associated with microbial pathogenesis. In this context, there are many instances where molecular chaperones serve miscellaneous purposes other than mere protein folding. For instance, a GroEL paralog of *Mycobacterium smegmatis*, named GroEL1, exhibit no activities related to heat shock but is associated with the establishment of biofilm and synthesis of mycolic acid (Ojha et al. 2005). Also, human gastric pathogen *Helicobacter pylori* possess a GroES homolog that along with its co-chaperonin function, contributes to its pathogenicity by participating in the storage and trafficking of one of its virulence factors Ni^{2+} ions (de Reuse et al. 2013). There are also instances where molecular chaperones might act as virulence factors directly. It is anticipated that during host-microbe interaction, numerous species of bacteria utilize cell surface GroEL and DnaK chaperones for adhesional though this concept demands experimental validation. The chaperones also contribute to cell-to-cell communication, induction of pro-inflammatory cytokine production as well as in apoptosis (Henderson and Martin 2011).

3.2 Regulatory mechanism of HSR

In physiological temperatures, the conformation of a macromolecule permits either only the basal level of HSG expression or is maintained in an inactive form until it receives the stimulus of heat shock. HSR is regulated by RNA, DNA, and proteins with thermo-sensory properties (Schumann 2012). Nucleic acid thermo-sensors can acquire two different temperature-dependent secondary structures that allow either a ground-level or elevated level of gene expression at mRNA as well as protein levels. The chaperones sequester functional σ factors rendering them unavailable or maintaining transcriptional repressors in an active form at physiological temperatures. Following the shock due to elevated temperature, as the inactivated proteins get titrated by chaperons the transcription factors will prevail in its non-functional state allowing the σ factors to interact with the core enzyme of RNA polymerase. With the propensity of denatured proteins being subjected to refolding or protease-mediated degradation, the chaperones will have greater scope to regulate protein turnover with help of transcriptional regulators.

4 Heat-shock transcription factors (HSF)

A group of proteins functioning as HSFs is responsible for regulating HSP expression at transcriptional levels. Hsf1 is the most well-studied transcription factor of all the reported ones that is crucial for HSR (Pirkkala et al. 2001). Hsf1 mostly has cytoplasmic localization as a non-functional monomer, in association with Hsp70 and Hsp90 under normal physiological conditions (Vabulas et al. 2010). Hsf1 dissociates from this complex when exposed to the selective pressure of thermal stress. Hsf1 then forms a trimeric complex followed by phosphorylation and the phosphorylated protein then enters into the nucleus promptly to activate the expression of HSP genes. Hsf1 activation is associated with its detachment from the chaperone complexes which is proposed to be triggered by the interaction of the accumulated unfolded proteins with Hsp70 and Hsp90 (Figure 2) (Jacobs and Marnett 2010). Likewise, electrophiles like HNE, nitroalkenes, sulforaphane, and 15d-PGJ2 can react with target cysteine residues on Hsp90 and Hsp70 covalently, prompting them to dissociate from Hsf1. This is followed by elevated accumulation

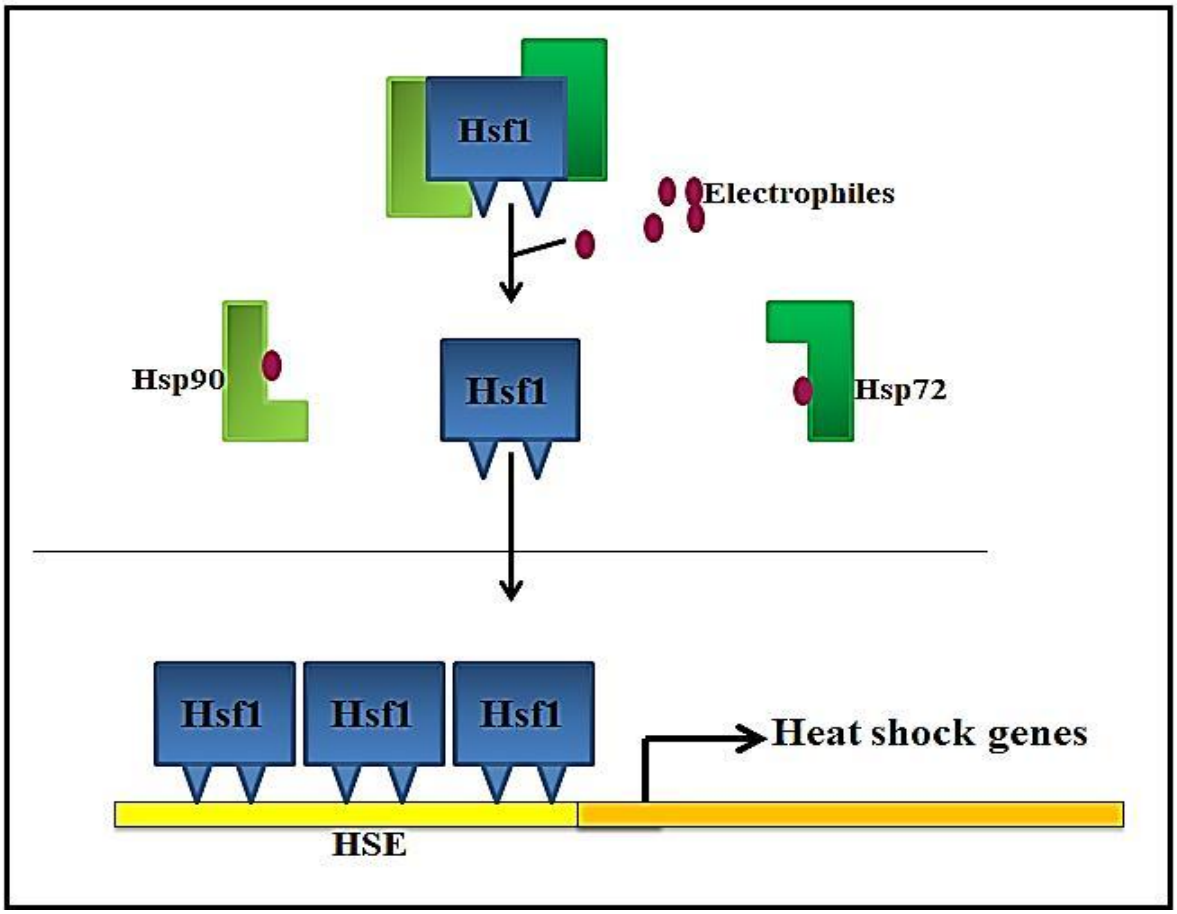

Figure 2 Pictorial representation of mode of action of HSF: Under the selective pressure of thermal stress and accumulation of unfolded proteins, in presence of electrophiles inactive monomer of Hsf1 is detached from Hsp70 and Hsp90 which then forms a trimeric complex followed by phosphorylation. This is followed by entry of the phosphorylated protein into nucleus to activate HSP gene expression.

of Hsf1in the nucleus and subsequent HSR activation (Vabulas et al. 2010; Kansanen et al. 2011).

5 Heat-shock regulations in *E. coli*

Escherichia coli K-12 strain was the first candidate microorganism subjected to heat-shock response related studies in bacteria where the operons dedicated for HSR are equipped with defined promoters regions identified by σ-32 that perform the role of a transcriptional activator. The half-life of σ-32 is short as the proteolytic activity of the gene product of *hflB*(*ftsH*) leads to its degradation (Yura 2019)**.** Every bacterium possesses one primary sigma factor (*E. coli*: σ-70) with multiple alternative σ factors. While the housekeeping σ factors control the expression of genes essential for bacterial growth and propagation, the alternative counterparts are activated only when cells are subjected to certain stressful events affecting their cellular physiology. There are reports of the existence of six, sixteen, and even sixty-two varieties of substitute sigma factors in *E. coli* K12 strains, *B. subtilis*, and *Streptomyces coelicolor* respectively. In *E.coli* two diverse forms of regulators of heat shock σ-32 and σ-E have been reported till date. Following a heat shock (for instance, an abrupt elevation in temperature from 30°C to 42°C) buildup of unfolded polypeptides in the cytoplasm prompts σ-32 activation. In ambient temperature σ-32 is produced in nominal concentration with a half-life <1 min. On the other hand, amassing of denatured proteins in the periplasm triggers activation of σ-E.

5.1 σ-32 heat shock regulon

Three different mechanisms function in consortia to endure stress due to elevated temperature and to preserve protein stability and functionality in viable cells by regulating σ-32 activity (i) at 30°C a partial secondary structure formation causes sequestration of the Shine-Dalgarno sequence of rpoH mRNA coding for σ-32. So at this temperature translation is witnessed only at a minimal level. However, following a temperature change to 42°C, RNA strand separation leads to an elevated level of protein expression (Yura 2019), (ii) when the temperature is low then either DnaK/DnaJ/GrpE or GroEL/ES chaperone system promotes majority of σ-32 molecule sequestering. Once a heat shock is triggered, the chaperones detach from σ-32 allowing them to bind to the denatured proteins through the process of chaperone titration (Gamer et al.1992). Overexpression of any one of these chaperone systems rapidly inhibits σ-32 activity while overexpression or depletion of chaperon substrate increase or decrease σ-32 activity, and (iii) at 30°C, majority of σ-32 molecules in free conformation are directed to FtsH protease or ClpXP protease either through association with signal recognition particle or modification by ubiquitin-like protein respectively for denaturation (Kanemori et al. 1997; Xu et al. 2015; Miyazaki et al. 2016).

Once subjected to thermal stress, there is a fast rise in the concentration and functionality of σ-32 due to increased expression of rpoH. Concurrent to this, the buildup of unfolded proteins in the cytoplasm causes momentary sequestration of the chaperones (5–10 min) that in turn leads to stabilizes σ-32. Both mechanisms lead to a prompt 12- to 15-fold surge in σ-32 content with a10 minute half-life ensuing the induction phase (Schumann 2016).

5.2 RpoH or σ-32: Controller of HSR

The sigma factor RpoH (also known as σ-32) is the prime regulator of expression of a majority of the heat-shock genes such as proteases and chaperones in *E. coli* as a component of the heat-shock regulon (Yura and Nakahigashi 1999). Transcriptional regulation of *rpoH* during translation is attributed to secondary structure formation in mRNA and post-translationally by FtsHand other proteases. The secondary structure of the RNA thermometer (RNAT) encompassing the 5'-UTR to a portion of the coding region of the *rpoH* transcript controls translation efficiency. At normal temperature, the closed conformation of RNAT prevents ribosome binding to the transcript while elevated temperature prompts melting of the secondary structure allowing ribosome to initiate the process of protein synthesis (Nagai et al. 1991; Yuzawa et al. 1993). At physiological temperatures, DnaK/DnaJ/GrpE and GroEL/GroES chaperone systems associate with RpoH and guide it for FtsH-mediated degradation. Stress induced by heat shock enhances intracellular concentration of misfolded proteins and dissociates the chaperone systems from RpoH and permits it to accompany the core RNA polymerase (RNAP) inducing transcription of HSGs many of which translate for chaperones and proteases (Gamer et al. 1996; Horikoshi et al. 2004). These proteases as well as chaperone took part in recovery from the heat-inflicted impairment of activity. As a result HSR is turned down rendering sufficient concentration of existing DnaK/DnaJ to sequester RpoH again (Blaszczak et al. 1995; Gamer et al. 1996) (Figure 3). Studies reveal that amino acid residues L^{47}, A^{50}, and I^{54} located on a superficial α-helix in the 2.1 region of amino-terminal domain of RpoH protein is associated with protein stability and this is the segment that binds with core-RNA polymerase. However, this area 2.1 of RpoH is vital although not adequate enough for denaturation by FtsH. In C region of RpoH that is located in the center of the sigma factor another region has been identified that binds with the RNA polymerase involving amino acid residues A^{131} and K^{134} which has also been reported to be linked with RpoH decay (Obrist et al. 2009). This location in the C region is identified as the turnover component for proteolytic degradation by FtsH protease. An RpoH fragment encompassing 37–147 amino acids consisting of regions 2.1 and C is found to be degraded by FtsH. This observation signifies that these two sites are adequate for precise identification of RpoH by FtsH and subsequent degradation (Obrist and Narberhaus 2005; Obrist et al.

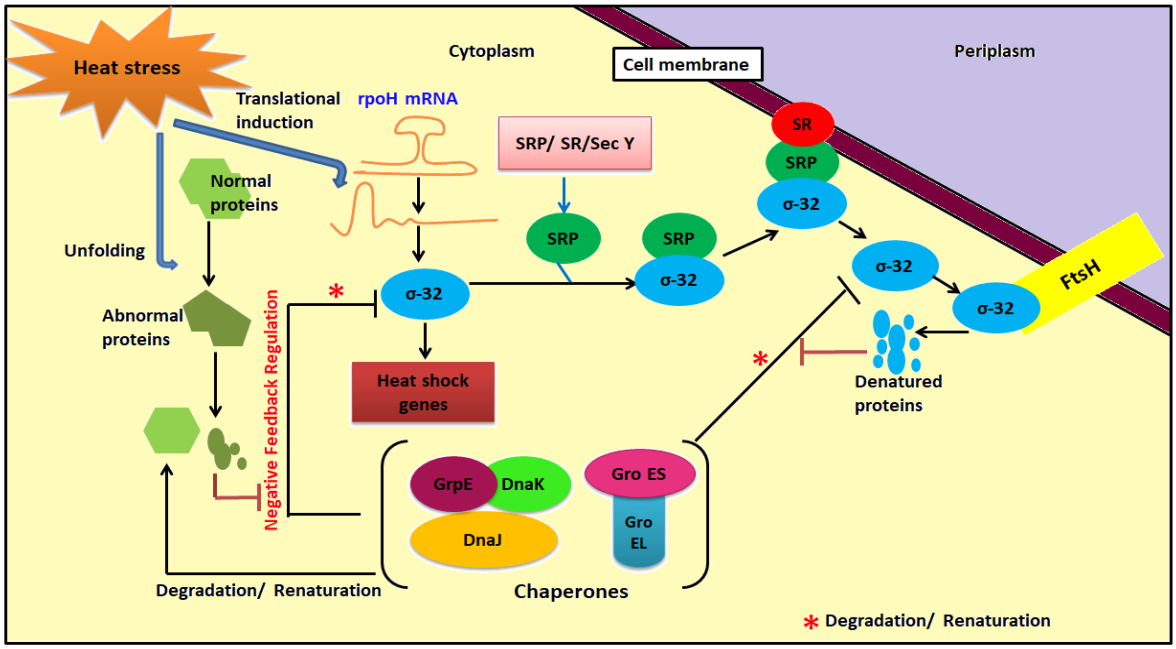

Figure 3 Pictorial representation of regulation of σ-32 mediated heat-shock response in *Escherichia coli:* σ-32 is the master regulator of transcription of most of the heat-shock genes in *E. coli*. σ-32 expression is regulated at translational level by secondary structure formation in mRNA and post-translationally by FtsH and other proteases. Rise in temperature allows melting of the secondary structure followed by protein synthesis.At normal temperatures, chaperone systems DnaK/DnaJ/GrpE and GroEL/GroES associate with σ-32 and guide it to FtsH-mediated degradation. Thermal stress elevates intracellular concentration of misfolded proteins, detaching the chaperone systems from σ-32 and allowing it to induce expression of heat-shock genes. Also, SRP/FtsY-dependent pathway recruits σ-32 first to the inner membrane followed by transfer to the chaperone-dependent system to induce FtsH-induced degradation.

2007, 2009). There is direct interaction of DnaJ with region 2.1triggering conformational changes that are further presumed to induce DnaK-attachment to the site 3.2 of RpoH (Rodriguez et al. 2008; Suzuki et al. 2012; Noguchi et al. 2014; Miyazaki et al. 2016).

Apart from *dnaK/dnaJ/grpE*, *groEL/groES*, and *ftsH*, the *ffh* gene is a component of the σ-32 regulon (Nonaka et al. 2006). Ffh protein coupled with the 4.5S RNA constitutes the signal recognition particle (SRP) which along with its membrane-bound receptor FtsY, directs RpoH degradation. Independent of DnaK/DnaJ binding, the signal peptide binding site of Ffh binds associates with the hydrophobic residues in the amphipathic helix of site 2.1 of RpoH assigned with the function of homeostasis control. Thus, the SRP/FtsY-dependent pathway recruits RpoH first to the inner membrane followed by handover to the chaperone-based system to prompt FtsH-induced lysis (Lim et al. 2013; Miyazaki et al. 2016) (Figure 3).

5.3 FtsH and regulation of σ-32-mediated HSR

FtsH protease is a membrane-anchored AAAmetallo protease that is crucial for maintaining intracellular stability of σ-32 along with other proteases like HslVU (Herman et al. 1995; Tomoyasu et al. 1995). However proteolytic enzymes like HslVU may also cause σ-32 denaturation significantly (Kanemori et al. 1997). Three of the well-characterized substrates of FtsH include LpxC, the main biocatalyst of LPS biosynthesis; RpoH/σ-32, the alternative heat-shock σ-factor, and YfgM, a membrane-bound protein with a dual role, concerned with cytosolic stress adaptation and periplasmic chaperone activities. Through degradation of LpxC this universal protease regulates the ratio of phospholipid and LPS in the outer membrane. Thus, the concentration of lipid and sugar residues of lipopolysaccharides together with free forms of SecY protein is regulated not only by σ-32 but also by FtsH. Therefore both of them contribute to the regulation of membrane protein transport (Ito and Akiyama 2005; Bittner et al. 2016). During HSR, the growth rate of the cells is slow, so a lesser amount of LPS is required and hence LpxC level is adjusted by FtsH-dependent degradation.

In ambient growth conditions under normal temperature σ-32is subjected to FtsH-mediated degradation through DnaK/DnaJ chaperones. Under heat stress, the chaperones are released from σ-32by non-functional proteins leading toσ-32 stabilization. σ-32 in free form the associated with RNA polymerase to trigger heat-shock regulon expression.

6 Heat shock regulon of σ-E

σ-E or σ-24 belongs to the family of extra-cytoplasmic function σ-factors that have the potential to respond to a diverse form of stress

stimuli like cell envelope stress and oxidative stress (Helmann 2002). In *E. coli*, the *rpoE* gene of operon rpoE-rseA-rseB-rseC encodes for σ-E. An antagonist sigma factor of σ-E, named RseA, is a transmembrane protein that prevents the collaboration of σ-E with core RNAP by entrapping it to the membrane (Missiakas et al. 1997). A periplasmic protein named RseB interacts with RseA functioning as a co-anti-sigma factor (Cezairliyan and Sauer 2007). RseC protein, located in the inner-membrane, positively regulates σ-E activity through a mechanism yet to be deciphered. In course of a cell envelope stress, the outer membrane proteins (OMPs) together with lipopolysaccharides mediate the detachment of σ-E from its anti-σ factor through proteolytic cleavage. The C-terminal domain of unfolded/ denatured OMPs is identified by DegS protease that causes detachment of RseB from RseA along with concomitant inactivation of the latter anti-σ factor in its periplasmic milieu (Walsh et al. 2003; Grigorova et al. 2004; Mecsas et al. 1993; Lima et al. 2013). Then, within or in close vicinity to the transmembrane region RseP proteolytically activates RseA releasing a shortened version of the latter in the cytoplasm (Kanehara et al. 2002, 2003). In the end, the remaining fraction of RseA will undergo complete degradation by one of the several cytoplasmic proteases (Chaba et al. 2011). Finally, upon cytoplasmic release σ-E will bind to RNAP directing it to σ-E-dependent promoters to activate σ-Eregulons comprised of a total of 89 transcriptional units.

Research in the last few years has demonstrated that the thermal stress-related response of *E. coli* forms the basis of the heat-shock response characteristic of other bacteria. *E. coli*, *P. aeruginosa*, *V. cholera*, and other members of γ2 and γ3 purple bacteria are reported to be unique as the sole regulator of their HSGs appear to be σ-32 with no additional explicit control element being identified. In other eubacterial groups manifold regulatory mechanisms dictating HSG transcription have been recorded. For instance, in *Bacillus subtilis*, a minimum of three clusters of heat-shock genes have been identified, among which only one is triggered byσ-B (Yura et al. 1993).

7 Insight from ribosomal profiling

Zhang et al (2017) performed ribosome profiling to excavate translational regulation in *E. coli* under heat stress. Alteration in ribosomal footprints was found to coincide with changes in transcript level upon thermal stress. Concerning transcript level and translational efficiency, expression profiling revealed upregulation of 58 genes with simultaneous down-regulation of 57 genes under thermal stress compared to normal conditions. Gene ontology and KEGG pathway-based analysis of the functional implications of this altered gene expression profile revealed a significant correlation of the two-component system pathway with heat stress in terms of 5 genes, namely, *rstA, frd, dcuB, phoB* and *pstS*. RstA is a translational controller of effector proteins of the two-component system that responds to environmental stimulus together with RstB. On the contrary, genes associated with cellular growth, amino acid biosynthetic pathways (*metE, asd, serA, mtn*), and ribosomal assembly (*rps K* and *rps Q*) along with translational efficiency (*infA* and *inf C*)are down-regulated (Zhang et al. 2017).

8 Possible application of the *E.coli* HSR

The HSR is instrumental in conferring the cell with protection against adverse conditions that elevate the levels of denatured proteins, namely, viral infection, heat shock, high alcohol concentrations, UV irradiation, oxidative stress, heavy metals, and recombinant protein production (Zhao et al. 2005; Li et al 2007). These stress factors can be detrimental to the cells altering their biological activities (Carroni et al. 2014). In the confrontation with such stressful situations, cellular responses are manifested in form of synthesis of HSPs like proteases and chaperones. If there is misfolding or unfolding of proteins, chaperones assist in protein renaturation (Müller et al. 2013). The HSR mechanism, though very complex, has a great possibility of being utilized in different approaches to synthetic biology. The creation of a library of biological components that are large in number, with predictable activities as well as can be designed, are suitable for easy integration into complex genetic systems (Seo et al. 2013; HoynesO'Connor and Moon 2016). For instance, enhanced complexities of pathways necessitate the expansion of the array of inducible promoters and other regulatory components that are available. Many of the members comprising the HSR mechanism of *E.coli* are suitable for becoming parts of a toolkit of well-studied biological components that in turn can be implemented for the construction of devices/systems, like biosensors and microbial cell factories. Such devices have a wide range of applications in environmental remediation, health care, or industrial sectors (Rodriguez et al. 2008). The sensing unit can be composed of a heat-shock promoter region stimulated by triggering the *E.coli* HSR by the diverse form of stress conditions like heat shock or elevated levels of chemicals. Heat shock promoter activation mediates the expression of a reporter gene. Such biosensors are highly sensitive to diverse applications. Moreover, this strategy can also be employed to translate a protein of interest producing a compound of therapeutic significance in the industry such as antibiotics (Rodriguez et al. 2008; Carroni et al. 2014).

9 Discussions

In this review, we have emphasized on the significance of an abrupt reaction of the bacterial population to temperature alterations and appraised the main mechanisms are adopted to counteract impending cellular damage. There are a plethora of diverse strategies availed by bacterial cells to regulate the highly conserved defense strategy of HSR. Regulation of HSG gene expression is proficiently realized by proteins with specialized

regulatory potentials which exert their beneficial or adverse effect on the gene expression by the RNAP. For reprogramming of gene expression in response to stress perception, both positive as well as negative regulatory modes, alone or in combination, orchestrate with posttranscriptional machinery to be utilized by bacteria. The model organism *E. coli* has been used for a long as the central archetype for elucidating the basis of stress-instigated HSG transcription through the usage of alternative σ-factors. After extensive research over several years, σ-32 homeostasis is achieved when chaperone-mediated modulation of σ-32 stability is complemented by regulation at the RNA and protein level. Also, the recent identification of SRP-SR co-translational targeting system-mediated localization of the heat-shock sigma factor in the inner membrane augments the understanding of the intricate mechanism of heat shock response regulation. Incorporation of Tn5 transposon upstream of *ftsY* gene encoding SR, the receptor for SRP significantly increased σ-32 concentration in presence of excess chaperones. This observation confirmed that for the proper functioning of heat shock regulation it is essential for σ-32 to be transmitted to membrane interior by SRP-SR-SecY pathway (Lim et al. 2013; Miyazaki et al. 2016). Nevertheless, further experimentations are required to understand other mechanistic facets of the HSR driven by σ-32 and other molecules in *E. coli*.

Conclusion

HSR is a highly preserved defense strategy in bacteria against environmental stress that is regulated by a plethora of diverse strategies. There is a panel of dedicated regulatory proteins to reprogram the expression of heat-shock proteins through positive and/or negative modes of controlling transcription as well as posttranscriptional regulatory mechanisms.

The model organism *E. coli* is an archetype to elucidate the mode of stress-induced activation of HSG expression based on the usage of alternative σ factors. Several studies have revealed the existence of a multifaceted regulatory cascade of gene expression control in combination with the regulation of σ32 stability and homeostasis driven by chaperons. Recent studies on the localization of heat-shock σ-factor internal to the plasma membrane through SRP-SR co-translational targeting system has further enhanced the understanding of the mechanism of HSR in bacterial cells. In *E. coli* HSG expression is controlled solely by the positive mechanism of regulation. σ-32 or σ-E/ σ-24 take part in heat-shock regulation with σ-32 playing an important part in recognizing signal from both cytoplasm and inner membrane, while σ-E/ σ-24 is devoted to stress response beyond the cytoplasm.

Several studies have shown that HSR in *E.coli* involves complex interactions where chaperones and proteases regulate functionality and stability of σ-32 respectively involving feedback loop. With the help of such feedback loops, the system becomes capable of functioning if there is any variation in its physical parameters, particularly in their permissible range. Further elaborate studies will be vital for exploring diverse aspects of the multifaceted HSR schemes.

Conflict of Interest

Nil

Authors' Contribution

Conception: Deborupa Paul and Sanmitra Ghosh; Literature review: Deborupa Paul and Sanmitra Ghosh; Data interpretation: Deborupa Paul and Sanmitra Ghosh; Drafting the manuscript: Deborupa Paul and Sanmitra Ghosh; Supervision: Sanmitra Ghosh

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