Review

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Revival of the *Escherichia coli* heat shock response after two decades with a small Hsp in a critical but distinct act

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Abstract: The heat stress response is an essential defense mechanism in all organisms. Heat shock proteins (Hsps) are produced in response to thermal stress, with their expression levels regulated by heat shock transcription factors. In *Escherichia coli,* the key transcription factor σ^{32} positively regulates Hsp expression. Studies from over two decades ago revealed that σ^{32} abundance is negatively controlled under normal conditions, mainly through degradation mechanisms involving DnaK, GroEL, and FtsH. Beyond this established mechanism, recent findings indicate that a small heat shock protein IbpA also plays a role in the translational regulation of σ^{32}, adding a new layer to the established model. This review highlights the role of a new actor, IbpA, which strongly suppresses σ^{32} expression under non-stress conditions and markedly increases it during heat shock.

Keywords: heat shock response; σ^{32}; heat shock protein; chaperone; small Hsp

1 Introduction

The heat stress response is an essential protective mechanism for all organisms. Heat shock proteins (Hsps) are specialized to counteract stress-induced protein denaturation and aggregation, with their expression primarily triggered by heat shock transcription factors such as HSF1 in eukaryotes and σ^{32} in prokaryotes (Hipp et al. 2019). These Hsps function mainly as molecular chaperones, assisting in the folding of nascent polypeptides, refolding denatured proteins, and preventing the aggregation of misfolded proteins. Hsps play a key role in maintaining protein homeostasis, or proteostasis, by responding to environmental stresses like heat shock, oxidative stress, and heavy metal exposure (Bukau 1993; Hipp et al. 2019; Richter et al. 2010). This review summarizes the updated regulatory mechanism of the σ^{32} subunit of RNA polymerase, which plays a central role in the heat shock response of *Escherichia coli*. Beyond the well-established regulation involving DnaK, GroEL, and FtsH at the degradation level (Bittner et al. 2017; Guisbert et al. 2004, 2008; Guo and Gross 2014; Meyer and Baker 2011), IbpA, a small Hsp (sHsp) in *E. coli*, has a critical role in suppressing σ^{32} expression at the translation level.

2 Heat shock response *in E. coli*

Proteostasis has become a diverse field in recent years, but its research roots trace back to studies on heat shock responses. A key focus is the heat shock response in *E. coli*. Upon heat shock, such as at 42 °C, *E. coli* rapidly increases the expression of many Hsps (Bukau 1993; Hipp et al. 2019; Richter et al. 2010). The major Hsps in *E. coli* include DnaK, GroEL, ClpB, and IbpA-IbpB, each with specialized roles in protein refolding, stabilization, and disaggregation (Arsène et al. 2000; Dahiya and Buchner 2019; Hartl et al. 2011). DnaK, a versatile chaperone in the Hsp70 family, maintains proteostasis with ATP and the cofactors DnaJ and GrpE (Arsène et al. 2000; Dahiya and Buchner 2019; Hartl et al. 2011). GroEL, a barrel-shaped chaperone, binds nonnative proteins and encapsulates them within its cavity with the aid of the co-chaperonin GroES in an ATP-dependent manner (Arsène et al. 2000; Dahiya and Buchner 2019; Hartl et al. 2011). Unlike DnaK and GroEL, IbpA and IbpB (IbpA/B) function independently of ATP, coaggregating with partially denatured or misfolded proteins to prevent irreversible aggregation (Arsène et al. 2000; Dahiya and Buchner 2019; Hartl et al. 2011; Mogk et al. 2019). Under normal conditions, IbpA/B expression is tightly suppressed by

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the binding of IbpA, not IbpB, to *ibpA/B* mRNAs (Cheng et al. 2023; Miwa et al. 2021), preventing the mild toxicity associated with their high abundance. IbpA exhibits a stronger binding affinity for denatured proteins compared to IbpB, whereas IbpB shows a higher affinity for DnaK and is more prone to degradation by Lon protease (Bissonnette et al. 2010; Obuchowski et al. 2019). These distinctions are pivotal for the functional difference between IbpA and IbpB as chaperones. Furthermore, although *ibpA* and *ibpB* are encoded within the same operon, the downstream region containing *ibpB* undergoes degradation by RNaseE, likely resulting in different expression levels of IbpA and IbpB (Gaubig et al. 2011). These variations may underlie IbpA's specialized role as a translational repressor. Due to this self-repression mechanism at the translational level and transcriptional regulation via σ^{32} (see below), IbpA/B expression increases rapidly and substantially in response to heat shock, rising 10- to 50-fold compared to normal conditions (Calloni et al. 2012; Zhao et al. 2019).

3 Regulation of the heat shock response in *E. coli*: established mechanism

The RNA polymerase subunit σ^{32}, the product of the *rpoH* gene, serves as a key regulator of the heat shock response in prokaryotes (Arsène et al. 2000). σ^{32} was identified in *E. coli* in the early 1980s when Yura and Neidhardt independently showed that Hsp induction is controlled by a genetic factor (Neidhardt and VanBogelen 1981; Yamamori and Yura 1980, 1982). In 1984, Gross and colleagues discovered that σ^{32}, a minor sigma factor in *E. coli*, regulates Hsp transcription (Grossman et al. 1984). It was also found that the synthesis of σ^{32} is significantly inhibited when excess Hsps accumulate in cells, revealing a feedback mechanism for controlling the heat shock response (Straus et al. 1987, 1990; Tilly et al. 1983). Throughout the 1990s further research revealed that σ^{32} is tightly regulated at multiple levels, including synthesis, activity, and degradation (Figure 1A) (Grossman et al. 1987; Kamath-Loeb and Gross 1991; Straus et al. 1990). This multilayered regulation ensures a precise and efficient heat stress response in *E. coli*. Under non-stress conditions, σ^{32} is subject to feedback regulation by the chaperones GroEL and DnaK (Guisbert et al. 2004, 2008; Tilly et al. 1983). These chaperones inhibit σ^{32} activity and destabilize it through direct binding. During stress, these chaperones are recruited by heat-denatured proteins, releasing σ^{32} from repression (Guisbert et al. 2004, 2008; Tilly et al. 1983). Moreover, deletion of the inner membrane protease FtsH stabilizes σ^{32}, indicating that FtsH is involved in σ^{32} degradation (Bittner et al. 2017; Guisbert et al. 2008; Guo and Gross 2014; Meyer and Baker 2011; Mogk et al. 2011). Even after FtsH's role in degrading σ^{32} was established, *in vitro* studies suggested the involvement of other factors. Analysis of mutants showed

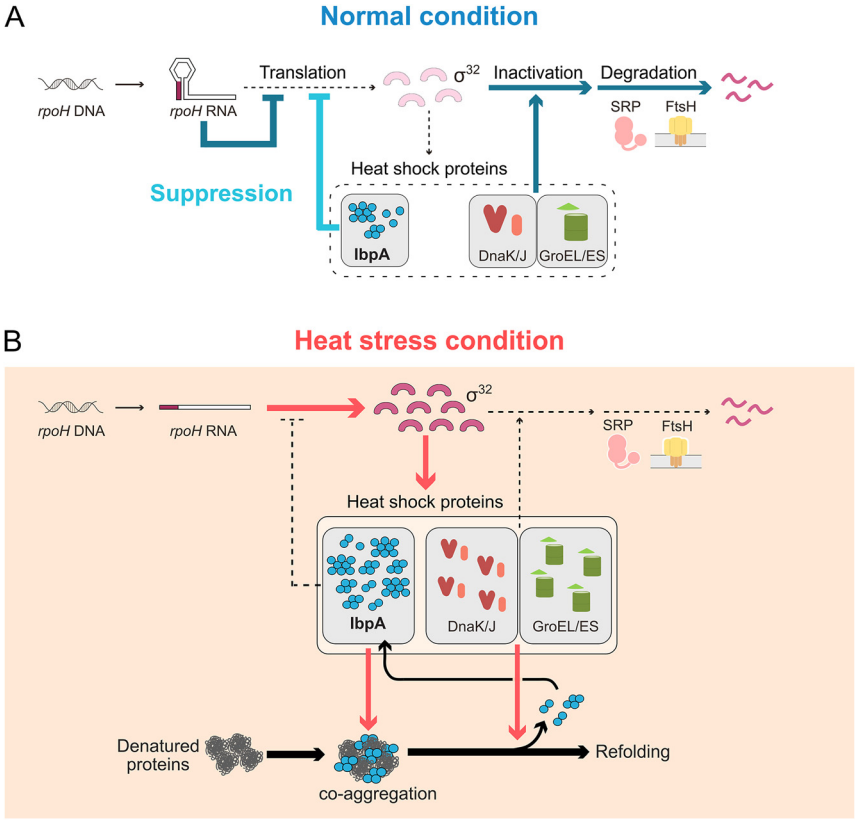

Figure 1: Schematic diagrams of heat shock response control in *E. coli*. (A) Under normal conditions, σ^{32}, the *rpoH* gene product, is inactivated by DnaK and GroEL, which promotes its degradation by FtsH. Additionally, the translation of *rpoH* mRNA is repressed by IbpA and RNAT. (B) Under heat stress conditions, chaperones are recruited to manage protein aggregation, releasing σ^{32} from repression. IbpA, dissociated from coaggregates by DnaK, again represses σ^{32} translation.

that σ^{32} localization to the inner membrane, crucial for its degradation, requires a signal recognition particle and its adaptor (Lim et al. 2013; Miyazaki et al. 2016). This finding shows that σ^{32} not only responds to cytoplasmic conditions but also monitors proteostasis in the inner membrane, underscoring its broader role in maintaining cellular stability under stress.

In addition to regulating degradation and activity, σ^{32} has a *cis*-regulatory element, an RNA thermometer (RNAT), which controls translation (Guisbert et al. 2008; Kortmann and Narberhaus 2012; Morita et al. 1999b; Nagai et al. 1991). RNATs are temperature-sensitive regulatory elements located in the 5′ untranslated regions (UTRs) of certain mRNAs (Kortmann and Narberhaus 2012). The secondary structures within the RNATs modulate translation of the downstream coding region by altering their conformation in response to temperature fluctuations, thereby controlling gene expression based on environmental conditions (Kortmann and Narberhaus 2012). In *rpoH* mRNA, this element includes the region from the 5′ UTR to the mid-ORF, forming a secondary structure that masks the Shine–Dalgarno sequence and initiation codon, preventing translation (Figure 1A) (Morita et al. 1999a, 1999b; Nagai et al. 1991). This secondary structure unfolds at high temperatures, enabling temperature-dependent production of σ^{32} (Morita et al. 1999a, 1999b; Nagai et al. 1991). These regulatory mechanisms were believed to control the intracellular abundance of σ^{32}.

4 Regulation of σ^{32} translation by a new cast, IbpA

In addition to the established mechanism regulating σ^{32}, regulatory pathway involving feedback translation by IbpA was recently discovered by Miwa et al. adding another layer for the tight and rapid control of σ^{32} abundance (Figure 1A) (Miwa and Taguchi 2023).

IbpA functions as a chaperone that binds to denatured proteins (Haslbeck et al. 2019; Mogk et al. 2019). When denatured proteins accumulate in the cell, IbpA binds to them in an ATP-independent manner, forming coaggregates (Haslbeck et al. 2019; Mogk et al. 2019). These coaggregates facilitate the efficient processing of denatured proteins by improving access for DnaK, ClpB, and proteases (Haslbeck et al. 2019; Mogk et al. 2019; Żwirowski et al. 2017). The role of sHsps, including IbpA, as “sequestrase” explains why sHsps are often referred to as the first line of defense in the cellular response to aggregation stress (Haslbeck et al. 2019; Mogk et al. 2019). In addition to its well-known role in managing denatured proteins, IbpA has recently been found to play a crucial role in regulating the heat shock response by repressing the translation of *rpoH* mRNA (Miwa and Taguchi 2023). This repression occurs in a 5′ UTR-dependent manner, illustrating IbpA’s additional function in fine-tuning the heat shock response by controlling σ^{32} production at the translational level (Miwa and Taguchi 2023). Overexpression of IbpA reduces *rpoH* translation by approximately 50 %, while loss of IbpA increases *rpoH* translation by 1.5-fold. This effect can partially be recapitulated *in vitro* using a reconstituted cell-free translation system (PURE system), indicating that IbpA represses translation independently of other intracellular factors (Miwa and Taguchi 2023). Furthermore, this translational regulation is distinct from the degradation control by DnaK, GroEL and FtsH and is independent of known mutations that disrupt σ^{32} and DnaK-mediated degradation pathways (Miwa and Taguchi 2023). A close relationship between RNAT and IbpA-mediated translational repression has also been proposed. Since the 5′ UTR, which contains the RNAT region, is essential for IbpA-dependent translational repression, it is plausible that IbpA recognizes RNA secondary structures functioning as RNATs to exert its regulatory effects (Miwa and Taguchi 2023). Notably, other regulatory targets of IbpA, such as *ibpA* mRNA itself and *ibpB* mRNA, also contain an RNAT region in their 5′ UTRs (Miwa et al. 2021). Mutations in the structural elements of the RNAT abolish IbpA-mediated translational repression of *ibpA*, indicating that the RNAT structure is crucial for this suppression (Miwa et al. 2021). RNAT can be partially derepressed even at normal temperatures, suggesting that IbpA may act as a “safety catch” to strictly enforce translational suppression. A similar role is likely attributed to the RNAT in *rpoH* mRNA.

Since the oligomer formation motif is essential for the translational repression activity of IbpA, its oligomeric state likely functions as a translational repressor (Miwa et al. 2021). High-molecular-weight oligomers of sHsps are referred to as the storage form, and in this state, sHsps exhibit low chaperone activity (Haslbeck et al. 2019; Miwa and Taguchi 2021; Mogk et al. 2019). It is probable that IbpA acts as a translational repressor when it is not needed as a chaperone – under non-stress conditions – by functioning in its storage oligomeric state.

5 σ^{32} shut-off mechanism during heat shock recovery

The σ^{32} level is known to rise rapidly upon heat stress, peaking within 5 min (Guisbert et al. 2008; Meyer and Baker 2011; Straus et al. 1987). However, its abundance decreases over time, returning to pre-stress levels about 10 min after

the onset of heat stress (Guisbert et al. 2008; Meyer and Baker 2011; Straus et al. 1987). Previous studies identified degradation control mechanisms as responsible for this shut-off phase of σ^{32} (Guisbert et al. 2008; Meyer and Baker 2011; Straus et al. 1987). Recent findings also show that the abundance of IbpA under stress conditions affects the duration of this phase (Miwa and Taguchi 2023). Specifically, *E. coli* recovery from heat stress is delayed by either excess or absence of IbpA, indicating that an optimal amount of IbpA is required for proper σ^{32} shut-off (Miwa and Taguchi 2023). The observation that shut-off still occurs 30 min after heat shock onset, even without IbpA (Miwa and Taguchi 2023), suggests that the early phase of shut-off depends on IbpA-mediated translational suppression, while the later phase is likely governed by degradation processes.

6 Advantages of sHsp in controlling the heat stress response

The abundance of σ^{32} is regulated by IbpA-mediated translational control and by degradation control involving other Hsps, including DnaK and GroEL. IbpA differs significantly from DnaK and GroEL as it sequesters heat-damaged proteins by coaggregating with them during stress. This sequestration of IbpA leads to an apparent depletion of free IbpA in the cytosol, temporarily reducing its inhibitory effects on σ^{32} translation. This mechanism enables rapid translation of already transcribed *rpoH* mRNA when needed. The pronounced propensity of IbpA to engage in aggregation likely facilitates the release of *rpoH* from rapid translational repression. Additionally, when IbpA coaggregates with denatured proteins, it facilitates the recruitment of DnaK, which subsequently releases IbpA from the coaggregate (Mogk et al. 2019; Żwirowski et al. 2017). Thus, IbpA is freed once other chaperones take over the management of aggregation (Figure 1B). This mechanism supports IbpA's critical role in initiating the shut-off of σ^{32} during heat stress. The rapid sequestration and release of IbpA reflect the dynamic state of protein aggregation management, allowing IbpA to tightly regulate σ^{32}. Unlike DnaK and GroEL, which are constitutively expressed to maintain proteostasis, IbpA is exclusively regulated by σ^{32}, enabling it to more precisely to the repression of σ^{32}.

7 Future perspectives

Although IbpA-mediated translation repression of certain mRNAs is conserved in other γ-proteobacteria (Cheng et al. 2023), the detailed mechanism of σ^{32} regulation by IbpA remains elusive. It is still unclear which features of *rpoH* mRNA are recognized by IbpA for translational repression. The requirement for the 5′ UTR suggests that IbpA targets RNATs, which are complex secondary structures with multiple stem-loop (Miwa and Taguchi 2023). However, the RNAT in *rpoH* does not share structural or sequence similarities with RNATs in other translation control targets, making it difficult to identify the elements essential for regulation (Kortmann and Narberhaus 2012; Miwa and Taguchi 2023; Miwa et al. 2021). Additionally, the RNA binding site of IbpA remains unidentified, as no known nucleic acid-binding motifs are present within this chaperone protein. Thus, the mechanism by which IbpA regulates σ^{32} requires further investigation.

The characteristics of IbpA, such as the presence of positively charged amino acids critical for RNA binding and regulation (Cheng et al. 2023), along with its ability to form oligomers of various sizes, may resemble liquid-liquid phase separation (LLPS). Notably, HspB2, an eukaryotic sHsp, undergoes LLPS, with its behavior modulated by another sHsp, HspB3, in the cell (Morelli et al. 2017). Given these similarities, it is plausible that LLPS may also occur in bacterial sHsps like IbpA and that its unique role in translational regulation could result from such phase separation dynamics.

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