Proteolysis in the Escherichia coli heat shock response: a player at many levels

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Proteolysis in the *Escherichia coli* heat shock response: A player at many levels

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Abstract

Proteolysis is a fundamental process used by all forms of life to maintain homeostasis, as well as to remodel the proteome following environmental changes. Here, we explore recent advances in understanding the role of proteolysis during the heat shock response of *Escherichia coli*. Proteolysis both regulates and contributes directly to and the heat shock response at multiple different levels, from adjusting the levels of the master heat shock response regulator (\(\sigma^{32}\)), to eliminating damaged cellular proteins, to altering the activity of chaperones that refold heat-denatured proteins. Recent results illustrate the complexity of the heat shock response and the pervasive role that proteolysis plays in both the cellular response to heat shock and the subsequent limiting of the response, as cells return to a more “normal” physiological state.

Introduction

The heat shock response in *Escherichia coli* is a complex program of cellular changes activated by an increase in temperature. The major regulatory player in the heat shock response is the transcription factor \(\sigma^{32}\), which upregulates expression of a suite of cellular factors that assist in restoring cellular homeostasis. Numerous chaperones and proteases are members of this heat shock regulon; these enzymes act to stabilize, refold, or eliminate cellular proteins that have been denatured by the high temperatures. In this review, we focus on several recent findings regarding the role that proteolysis plays in the *E. coli* heat shock response, highlighting the emerging insight that proteolysis is key to the regulation of the heat shock response and that it participates at many different levels.

The heat shock response: general roles for proteases

Temperatures of 37°C or higher endanger bacterial homeostasis largely due to thermal denaturation of folded proteins [1]. In reaction to an upshift in temperature, bacteria employ the heat shock response. Elevated temperatures lead to increased activity of transcription directed by sigma factor \(\sigma^{32}\) (RpoH), inducing the upregulation of over 120 regulon products [2-4]. Among these induced genes are those encoding molecular chaperones that contribute to the maintenance of protein homeostasis, DNA repair components, additional transcription factors that broaden the effect of \(\sigma^{32}\) activation, and...
metabolic enzymes that permit adaptation to heat stress. Eleven distinct factors with direct roles in protein degradation are also upregulated, including most of the bacterial AAA+ proteases and several of their regulators.

Heat shock regulon proteases presumably contribute to the heat shock response by removing damaged or unfolded cellular proteins, although completely non-specific degradation of unfolded proteins is unlikely. Substrate selection by AAA+ proteases is typically tightly controlled, mediated through the recognition of specific displayed sequences or motifs. These “degradation tags” have been most extensively characterized for ClpXP [5-9], and studies of other AAA+ proteases suggest a common strategy of recognizing distinct residues within exposed peptides [10-15]. A recent study reveals that the Lon protease preferentially interacts with a subset of amino acids and that these “interaction signatures” are enriched in aromatic residues [16]. Lon may then specifically engage this class of hydrophobic degradation tags exposed on substrate proteins after heat-induced unfolding. This recognition mechanism may underlie the major role of Lon in clearing the cell of damaged proteins during heat shock [17].

**Stability of σ^{32}**

In addition to a likely general role of Lon in eliminating damaged proteins, specific examples of proteases contributing to the heat shock response have also been established. The activity of σ^{32} during heat shock is intricately controlled, with regulation at the levels of translation, protein activity, as well as protein stability. The degradation feedback loop functions as follows. The σ^{32} protein is quite unstable during steady-state growth at moderate temperatures, with a half-life of ~1 min [18-19]. Following temperature up-shift, σ^{32} degradation is transiently slowed for 5-10 minutes during the induction phase of the heat shock response [19]; this phase is then followed by the resumption of degradation at an extremely fast rate (half-life of ~20 sec) as cells adapt to the elevated temperature and reach a new steady-state [18, 20]. σ^{32} is a substrate for multiple bacterial proteases. Deletions of the genes encoding the HslUV, Lon, and Clp proteases stabilize σ^{32} to a limited degree, but an absence of FtsH results in almost complete σ^{32} stabilization. Thus, FtsH is thought to be the major protease responsible for σ^{32} degradation [21-23].

Multiple studies have explored the molecular determinants of σ^{32} recognition and degradation by FtsH. Genetic analyses from three different groups each identified point mutations within a small section of conserved region 2.1 of σ^{32} that decrease stability in vivo [24-26]. Molecular modeling of the structure of σ^{32} suggests that these residues may all align on the same face of an α-helix and form an interaction surface [25]. In vitro analysis of hybrid proteins constructed between *Escherichia coli* σ^{32} and *Bradyrhizobium japonicum* σ^{32}, which is stable in *E. coli*, however indicate that region 2.1 is not sufficient for proteolysis by FtsH [27], and thus suggest that an additional element is required for turnover.

Previous in vivo results from fusion proteins [28] as well as in vitro FtsH degradation of σ^{32}-derived peptides [29] suggested that region C of σ^{32} may contain a FtsH recognition sequence, although several specific point mutations generated within this region had no significant effect on degradation [30]. Recent work from the Narberhaus laboratory defined two additional point mutations in σ^{32} that provide significant stabilization against FtsH degradation in vivo when both are present [31]. These mutations are located at the very start of the RpoH box that lies within region C. This RpoH box sequence element is unique to σ^{32} (among sigma factors) and contributes to interactions with RNA polymerase. The mutated amino acids in region C that stabilize σ^{32} are predicted to extend and re-orient an α-
helix within \( \sigma^{32} \) [31] ••, albeit one located on the opposite side of the folded protein from the \( \alpha \)-helix in region 2.1 also implicated in \( \sigma^{32} \) stability. Residues from both regions face the same side of the \( \sigma^{32} \) protein and could therefore potentially comprise an extended binding surface for an interacting protein.

Although \( \sigma^{32} \) contains two distinct elements required for its degradation, the molecular contribution of these sequences to the process of \( \sigma^{32} \) proteolysis remains unclear. Critical residues in region 2.1 and C could bind directly to the protease FtsH to mediate recognition, acting as degradation tags. Neither of the turnover elements is located on an extended peptide sequence or adjacent to the N- or C-terminus of \( \sigma^{32} \), as is the case for many of the best-characterized degradation tags for AAA+ proteases [32]. However, recent experiments have indicated that the Lon protease may recognize conserved elements within a folded domain of its substrates Ibpa and Ibpb [33] •• (see below), suggesting a novel mode of interactions between AAA+ proteases and secondary or tertiary structure elements of their substrates.

Many AAA+ proteases also utilize an additional mode of substrate recognition in which adaptor proteins modulate the degradation of specific substrates. No adaptor has currently been identified for FtsH. However, \textit{in vitro} \( \sigma^{32} \) degradation by FtsH is typically an order of magnitude slower than measured rates of degradation \textit{in vivo} [18-19, 21, 23], raising the possibility that assistance by an adaptor protein may facilitate intracellular degradation of \( \sigma^{32} \). Intriguingly, degradation of the closely related sigma factor \( \sigma^{9} \) by ClpXP requires binding of the RssB adaptor protein both \textit{in vitro} and \textit{in vivo}; mutational studies indicate that this interaction involves an \( \alpha \)-helix located in region 2.5 of \( \sigma^{9} \) [34-35]. One of the turnover sequences in \( \sigma^{32} \) may therefore contribute to degradation by binding to an adaptor protein, in a manner analogous to the adaptor-mediated degradation of \( \sigma^{9} \).

Additional factors that influence the degradation of \( \sigma^{32} \) include the molecular chaperones DnaK/J/GrpE (often referred to as the DnaK system) and GroEL/S. Inactivation of either set of chaperones results in stabilization of \( \sigma^{32} \) \textit{in vivo} [36-37], although introduction of DnaK/J and GrpE do not alter the rate of degradation by FtsH \textit{in vitro} [38]. The role of molecular chaperones in \( \sigma^{32} \) degradation could potentially explain the temporary stabilization of \( \sigma^{32} \) immediately following a shift to higher temperatures. The resulting increase in unfolded proteins could create a large substrate load for the cellular protein-folding machinery, titrating chaperones away from their role in assisting in the degradation of \( \sigma^{32} \) and slowing the reaction. Interestingly, a recent study by Rodriguez and colleagues identified the specific binding sites on \( \sigma^{32} \) for DnaK and DnaJ. DnaK and its cochaperone DnaJ interact with separate distinct regions of \( \sigma^{32} \), and the DnaJ interaction site lies adjacent to the turnover element in region 2.1 of \( \sigma^{32} \). Binding of DnaJ and DnaK each result in significant destabilization of the folded N-terminal structure of \( \sigma^{32} \) as measured by hydrogen-deuterium exchange [39] ••. Earlier work indicated that FtsH is a poor protein-unfoldase and therefore the rate of degradation of a substrate protein depends on the stability of that protein’s fold [40]. Perhaps molecular chaperones assist in \( \sigma^{32} \) degradation by promoting a conformational change in the N-terminal domain of \( \sigma^{32} \) (such as partial unfolding), thereby allowing \( \sigma^{32} \) to be engaged by FtsH; this model is consistent with fluorescence polarization analyses suggesting that degradation of \( \sigma^{32} \) may proceed from the N- to the C-terminus [41]. The mutations in region 2.1 residues that stabilize \( \sigma^{32} \) may therefore act by interfering with its destabilizing interaction with DnaJ.

**Regulation of CbpA through degradation of CbpM**

As described above, the DnaK molecular chaperone system crucially influences the activity of \( \sigma^{32} \) during heat shock. DnaK also contributes to the heat shock response as a member of

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the cellular protein-folding machinery. DnaK is assisted by its cochaperone DnaJ in the refolding and remodeling of many client proteins through its ability to deliver substrates. *E. coli* encodes five additional DnaJ homologs that share a conserved J-domain that mediates interactions with binding partners. The DnaJ homolog CbpA is essential for growth at temperatures above 37°C and is required for efficient resolubilization of protein aggregates at 42°C [42]. Unlike other DnaJ homologs, CbpA exhibits DNA-binding activity, with no sequence specificity but with a tighter affinity for curved DNA, and localizes to the nucleoid during certain stress conditions, including nutrient limitation.

The activity of CbpA is modulated by CbpM, which is encoded in the same operon as CbpA and can specifically inhibit both its DNA-binding and its chaperone activity *in vitro* and *in vivo* [43-44]. A recent study explored the regulation of the CbpAM operon, revealing control at the levels of both transcription and protein stability [45]. CbpA and CbpM were found to be stable proteins when coexpressed. However, CbpM is unstable in the absence of CbpA, being degraded by both Lon and ClpP proteases [45]. These results suggest that free CbpM is a good substrate for proteolysis, whereas the formation of a complex with CbpA may result in inhibition of CbpM degradation. CbpA and CbpM are transcribed from the same operon and accumulate to similar levels in the cell [45]. However, environmentally specific changes in the propensity of these two proteins to interact, leading to changes in the stability of CbpM, potentially a powerful method for regulating the “J-protein” chaperone activity of CbpA during heat shock.

**Degradation control of Ibps**

Along with the proteins in the DnaK system, other crucial molecular chaperones and their cofactors are upregulated during by heat shock [2-4]. The *E. coli* small heat shock proteins (members of the sHsp family) IbpA and IbpB are encoded in the same operon and are the most highly upregulated heat shock genes in the σ32 regulon [46]. *In vitro*, IbpA and IbpB appear to co-associate at elevated temperatures and cooperate with each other to stabilize thermally aggregated client proteins [47]. Heat-damaged proteins that have interacted with IbpA and IbpB and thereby avoided aggregation can then be transferred to members of the refolding machinery (ClpB and the DnaK system) for reactivation [48-49]. Recent work has described the complex temperature-dependent regulation of the IbpA and IbpB proteins; these molecular chaperones are subject to regulation not only at the levels of σ32-activated transcription but also by effects on RNA processing, translation, and protein stability [33••, 50].

*E. coli* IbpA and IbpB have recently been identified as substrates of the AAA+ protease Lon, itself a critical protease during the heat shock response [17]. The two Ibp proteins share substantial sequence similarity as well as a conserved central α-crystallin domain flanked by both N- and C-terminal extended tails [51]. Motifs found in extended peptide sequences located at the ends of proteins are often used as recognition determinants for AAA+ proteases [32]. Unexpectedly, the Ibp tails were not required for Lon recognition but rather served to adjust the maximal rate of Lon-mediated degradation [33••]. Investigation of human α-crystallin variants revealed that they are also Lon substrates recognized with similar affinities (although somewhat weaker) as the Ibp proteins, and the central α-crystallin domain alone is required for Lon degradation. These results suggested a model in which the Lon protease may recognize folded structural elements within the conserved α-crystallin domain of Ibp proteins rather than utilizing the strategy of interacting with unstructured peptide elements at the N- or C-termini of substrates [33••]. This model is being actively tested using several approaches.
Comparison of the two small heat shock proteins revealed that Lon degrades IbpB with a 14-fold higher maximal rate than observed for IbpA degradation. This finding, along with certain aspects of Ibp transcriptional control [50], may underlie the greater accumulation of IbpA over IbpB found at elevated temperatures [52]. Interestingly, IbpB also stimulates the rate of IbpA degradation both in vivo and in vitro [33] ••. The two small heat shock proteins therefore cooperate both in refolding client proteins and controlling their own inactivation and removal through degradation. Robust degradation of IbpA by Lon was observed under heat-shock conditions, when the small heat shock proteins would presumably be associated with heat damaged/misfolded substrates which may be beneficial to refold [33] ••. These results suggest that there exists a previously undiscovered link between the degradation and refolding pathways of the protein quality control network during the heat shock response. Perhaps IbpA and IbpB deliver their client proteins to Lon for degradation and are themselves degraded in the process; alternately, degradation of the Ibp chaperones may release their bound substrates back into the milieu for refolding.

Conclusion

Recent findings highlight the powerful influence that proteolysis has over many aspects of the *E. coli* heat shock response. Proteases take an active role in removing damaged proteins from the cell, modulate the activity of the master heat shock transcription regulator σ32, and alter the levels of molecular chaperones involved in restoring protein homeostasis. In turn, these proteases and their activity are themselves regulated by such factors as the conformation and oligomeric state of their substrates, which in several cases may serve as read-outs for the degree of cellular recovery from heat stress. Past research has done much to uncover the pervasive and varied roles that proteases play during heat shock. However, many facets of proteolysis and its regulation remain unexplored, leaving important challenges and opportunities for future researchers.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest


50. Gäubig LC, Waldminghaus T, Narberhaus F. Multiple layers of control govern expression of the Escherichia coli ibpAB heat shock operon. Microbiology. 2010


1.

A. Recognition, unfolding, and degradation of substrates by a AAA+ protease. AAA+ proteases consist of a hexameric AAA+ unfoldase ring stacked on top of a peptidase chamber. Substrates are often initially recognized through binding of exposed peptide degradation tags to loops located within the central pore of the AAA+ unfoldase ring. Cycles of ATP binding and hydrolysis by the AAA+ ring result in unfolding of the bound substrate during translocation into the proteolytic chamber. Protease active sites then degrade the substrate into small peptide fragments that are released.

B. Regulation of $\sigma^{32}$ in *E. coli*. Elevated temperatures lead to misfolded proteins as well as increased $\sigma^{32}$ activity, activating the heat shock response. Chaperones and proteases are among the upregulated members of the heat shock regulon, and their activity serves both to alleviate misfolded proteins and to downregulate $\sigma^{32}$ activity, thereby limiting the heat shock response.